



Functional Nanocarbon Hybrids for Optoelectronic Applications

Funktionale Nanokohlenstoff-Hybride für optoelektronische Anwendungen

Kumulative Dissertation

zur Erlangung des akademischen Grades

„Doctor rerum naturalium“

- Dr. rer. nat. -

Eingereicht am Fachbereich

Biologie und Chemie

der

Justus-Liebig-Universität Gießen

von

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Gießen, Mai 2025

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There are the things that are out in the open, and there are the things that are hidden.

The real world has more to do with what is hidden.

Saul Leiter

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Die vorliegende Arbeit und die zugrundeliegenden Experimente wurden in der Zeit von Februar 2022 – Mai 2025 am Physikalisch-Chemischen Institut der Justus-Liebig-Universität Gießen in der Arbeitsgruppe von Prof. Dr. Teresa Gatti angefertigt.

Acknowledgements – Danksagung

Zunächst möchte ich mich ganz herzlich bei **Prof. Dr. Teresa Gatti** bedanken, die mir die Möglichkeit gegeben hat, in ihrer Arbeitsgruppe zu promovieren. Ihre engagierte Betreuung, der anregende wissenschaftliche Austausch und die zahlreichen Gelegenheiten, an Konferenzen teilzunehmen, haben meine Entwicklung als Wissenschaftler und als Person maßgeblich geprägt.

Mein besonderer Dank gilt **Prof. Dr. Bernd M. Smarsly**, der mir die Arbeit in seinen Laboren ermöglichte und mich von Anfang an voll in seine Arbeitsgruppe integrierte. Er stand mir stets mit wertvollen Ratschlägen zur Seite und war bei Fragen und Unklarheiten jederzeit ansprechbar, wofür ich ihm herzlich danke.

Mein Dank gilt auch **Dr. Jaime Gallego**, dessen Mitarbeit an meinen Projekten und fundierte wissenschaftliche Expertise meine Arbeit inhaltlich entscheidend bereichert hat.

Für die hervorragende Zusammenarbeit danke ich **Dominic Schatz, Michal Langer** und **Yagmur Aydogan** sowie allen weiteren Kooperationspartner:innen, mit denen ich im Laufe der Zeit zusammenarbeiten durfte - es sind zu viele, um sie alle namentlich zu nennen.

Mein besonderer Dank gilt **Dr. Heike Hausmann** und dem gesamten **Team der OC-Analytik** für die zahlreichen durchgeführten Messungen sowie **Pascal Schweitzer** für die Unterstützung bei den AFM-Messungen.

Außerdem danke ich allen Arbeitsgruppen des **Zentrums für Materialforschung** für die stets hilfreichen Diskussionen und die Ausleihe von Geräten und Chemikalien.

Melanie Pagel, Simeon Muntean und **Paula Poldrack** danke ich für ihre engagierte und zuverlässige Mitarbeit im Labor im Rahmen ihrer Abschlussarbeiten.

Ein besonderer Dank geht auch an die „Fischfreitagsrunde“: **Alex, Florian, Julian, Lorena, Lysander** und **Moritz**, sowie an die „Schnitzel-Oma“-Gefährten **Christian N.** und **Michael** - für die gemeinsamen Mittag- und Abendessen, Gespräche und den Humor, der den Arbeitsalltag so oft aufheitert hat.

Sehr dankbar bin ich für die bereichernde Zeit und Unterstützung meiner Kolleg:innen und Freund:innen aus der AG Smarsly und der AG Gatti: **Felix, Aline, Chantal, Christian B., Fabian, Luca, Matteo, Roberto, Rafael, Usman, Freddi, Eric, Sara** und **Micaela**. Natürlich

auch **Antonella D'Ambrosio**, **Kerstin Rose** und **Gabi Scheller** für die tatkräftige Unterstützung bei administrativen Dingen und Reiseangelegenheiten.

Ich danke **Raoul**, **Melanie**, **Chantal** und **Lysander** für das Korrekturlesen dieser Arbeit.

Ich möchte mich auch ganz herzlich beim **JungesChemieForum (JCF)** und allen, die mich in dieser Zeit begleitet haben, für die unvergesslichen Erlebnisse bei der Planung und Durchführung des Frühjahrssymposiums sowie für die inspirierende Zeit im Bundesvorstand bedanken.

Mein besonderer Dank gilt **Jürgen Glaab**, der mir den Weg geebnet hat, den ich bis heute gegangen bin.

Ich möchte mich auch für die Unterstützung bedanken, die ich von meinen **Freunden** und meiner **Familie** in allen Phasen meiner Promotion erhalten habe.

Von Herzen danken möchte ich meinem Papa **Ralf** und meiner Mama **Margarete** für ihre ständige und liebevolle Unterstützung in allen erdenklichen Hinsichten und ihr grenzenloses Vertrauen in mich.

Schließlich danke ich meiner Freundin **Debby** - für ihre Liebe, Geduld und Unterstützung in allen Lebenslagen. Ich liebe dich.

Abstract

Carbon dots (CDs) are carbon nanomaterials with remarkable photoluminescence, tunable surface chemistry, and high biocompatibility, making them attractive for applications in catalysis, sensing, and biomedicine. This work presents a comprehensive investigation of the synthesis, surface functionalization, characterization, and application of CDs, with a particular focus on carbon nanodots (CNDs). The broad objective was to understand how precise surface modifications and advanced analytical techniques could reveal structure-property relationships and unlock application potentials. CNDs were synthesized using bottom-up methods that allow precise control over particle morphology and surface functionalities. A major focus was the quantification of surface functional groups for covalent modification strategies.

In **Publication 1**, the question of whether the total amount of primary amines on the surface correlates with the efficiency of amide coupling reactions was addressed. Using a combination of the Kaiser test and quantitative ^{19}F -NMR spectroscopy, it was found that coupling efficiency does not scale directly with the apparent amine content. Instead, steric accessibility and reactivity were identified as critical factors governing surface functionalization. These findings provide a basis for the rational selection of linkers and optimization of reaction conditions in covalent surface chemistry.

Publication 2 focused on whether covalent attachment of organic dyes to CNDs could improve their stability and integration into photocatalytic systems. The study demonstrated that covalent dye-CND hybrids, when integrated into TiO_2 -based hybrid photocatalysts, exhibited reduced dye leaching and consumption, thus advancing the development of more sustainable and long-lasting photocatalytic systems.

In **Publication 3**, the focus was on how the photophysical behavior of CND-azobenzene hybrids can be modulated by structural design, including a spacer and connectivity. It was shown that the molecular spacer significantly influences the electronic coupling and fluorescence quenching behavior and enables reversible photoisomerization. These results underline the potential of these hybrid materials for light-controlled applications such as optostimulation and targeted biotechnological processes.

The results highlight the importance of surface engineering in tailoring CNDs-hybrids. Combining surface analysis, targeted covalent modification, and application-driven hybrid design provides the foundation for solving challenges in photocatalysis, bioimaging, and stimuli-responsive nanotechnology.

Zusammenfassung

Kohlenstoffdots (CDs) sind Kohlenstoffnanomaterialien mit bemerkenswerter Photolumineszenz, anpassbarer Oberflächenchemie und hoher Biokompatibilität, was sie für Anwendungen in der Katalyse, Sensorik und Biomedizin attraktiv macht. Diese Arbeit präsentiert eine umfassende Untersuchung der Synthese, Oberflächenfunktionalisierung, Charakterisierung und Anwendung von CDs mit besonderem Schwerpunkt auf Kohlenstoffnanodots (CNDs). Das übergeordnete Ziel bestand darin, zu verstehen, wie präzise Oberflächenmodifikationen und fortschrittliche analytische Techniken Struktur-Eigenschafts-Beziehungen aufdecken und Anwendungspotenziale erschließen können. Die CNDs wurden mit Bottom-up-Methoden synthetisiert, die eine präzise Kontrolle der Partikelmorphologie und der Oberflächenfunktionalitäten ermöglichen. Ein besonderer Fokus lag auf der Quantifizierung von Oberflächengruppen für kovalente Modifikationsstrategien.

In **Publikation 1** wurde der Frage nachgegangen, ob die Gesamtmenge primärer Amine auf der Oberfläche mit der Kupplungseffizienz von Amid-Kupplungen korreliert. Mit Hilfe einer Kombination aus dem Kaiser-Test und quantitativer ^{19}F -NMR-Spektroskopie konnte gezeigt werden, dass die Kupplungseffizienz nicht direkt mit dem scheinbaren Amingehalt skaliert. Stattdessen wurden die sterische Zugänglichkeit und die Reaktivität als entscheidende Faktoren identifiziert, die die Funktionalisierung der Oberfläche bestimmen. Diese Ergebnisse bilden die Grundlage für die gezielte Auswahl von Linkern und die Optimierung von Reaktionsbedingungen in der kovalenten Oberflächenchemie.

Publikation 2 konzentrierte sich auf die Frage, ob die kovalente Bindung organischer Farbstoffe an CNDs deren Stabilität und Integration in photokatalytische Systeme verbessern kann. Die Studie zeigte, dass kovalente Farbstoff-CND-Hybride, wenn sie in TiO_2 -basierte Hybrid-Photokatalysatoren integriert werden, eine verringerte Farbstoffauswaschung und einen verringerten Farbstoffverbrauch aufweisen und somit die Entwicklung von nachhaltigeren und langlebigeren photokatalytischen Systemen fördern.

In **Publikation 3** wurde untersucht, wie das photophysikalische Verhalten von CND-Azobenzol-Hybriden durch strukturelles Design, einschließlich Spacer und Konnektivität, moduliert werden kann. Es konnte gezeigt werden, dass der molekulare Spacer einen signifikanten Einfluss auf die elektronische Kopplung und das Fluoreszenzquenching hat und eine reversible Photoisomerisierung ermöglicht. Diese Ergebnisse unterstreichen das Potential dieser Hybridmaterialien für lichtgesteuerte Anwendungen wie Optostimulation und gezielte biotechnologische Prozesse.

Die Ergebnisse verdeutlichen die Bedeutung der Oberflächenmodifizierung bei der Entwicklung maßgeschneiderter CND-Hybride. Die Kombination von Oberflächenanalyse, gezielter kovalenter Modifikation und anwendungsorientiertem Hybriddesign bildet die Grundlage für die Bewältigung von Herausforderungen in den Bereichen der Photokatalyse, des Bioimagings und der anregungsabhängigen Nanotechnologie.

List of Publications

Publication 1:

Debes, P. P.; Langer, M.; Pagel, M.; Menna, E.; Smarsly, B.; Osella, S.; Gallego, J.; Gatti, T. Functional Groups Accessibility and the Origin of Photoluminescence in N/O-Containing Bottom-up Carbon Nanodots. *ChemNanoMat* **2024**, *10* (2), e202300471. <https://doi.org/10.1002/cnma.202300471>.

Publication 2:

Debes, P. P.; Pagel, M.; Muntean, S.; Hessling, J.; Smarsly, B. M.; Schönhoff, M.; Gatti, T. Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis. *Photochem* **2025**, *5* (1), 1. <https://doi.org/10.3390/photochem5010001>.

Publication 3:

Debes, P. P.; Schatz, D.; Aydogan-Sun, Y.; Martínez, J. P.; Langer, M.; Hessling, J.; Gallego, J.; Menna, E.; Smarsly, B. M.; Schönhoff, M.; Osella, S.; Wachtveitl, J.; Wegner, H. A.; Gatti, T. Covalent Carbon Nanodot-Azobenzene Hybrid Photoswitches: The Role of *Meta/Para* Connectivity and sp³ Spacer in Photophysical Properties. *J. Mater. Chem. C* **2025**, accepted. DOI: [10.1039/D5TC00116A](https://doi.org/10.1039/D5TC00116A).

Further publications as co-author

Wilming, F. M.; Marazzi, B.; Debes, P. P.; Becker, J.; Schreiner, P. R. Probing the Size Limit of Dispersion Energy Donors with a Bifluorenylidene Balance: Magic Cyclohexyl. *J. Org. Chem.* **2023**, *88* (2), 1024–1035. <https://doi.org/10.1021/acs.joc.2c02444>.

List of Contributions

Poster and Flash Presentation

Debes, P. P.; Maggini, M.; Schreiner, P. R., *Molecular Rectifiers: Diamantane-Fullerene Adducts*, **ChemOnTubes 2022, San Sebastian, Spain. 2022**

Poster Presentation

Debes, P. P.; Gatti, T., *Photoactive Functional Nanocarbon Hybrids*, **Bunsentagung, Giessen, Germany. 2022**

Oral Presentation

Debes, P. P.; Pagel, M.; Gallego, J.; Gatti, T., *Surface Accesible Functional Group Quantification of Carbon Nanodots*, **MATSUS 2023, Valencia, Spain. 2023**

Oral Presentation

Debes, P. P.; Langer, M.; Pagel, M.; Osella, S.; Gallego, J.; Gatti, T., *Experimental and Theoretical Insights into the Accessibility of Functional Groups in Bottom-up Carbon Nanodots*, **FisMat 2023, Milan, Italy. 2023**

Poster Presentation

Debes, P. P.; Langer, M.; Pagel, M.; Menna, E.; Smarsly, B. M.; Osella, S.; Gallego, J.; Gatti, T., *Functional Groups Accessibility and the Origin of Photoluminescence in N/O-Containing Bottom-up Carbon Nanodots*, **Spring Symposium, Ulm, Germany. 2024**

Oral Presentation

Debes, P. P.; Langer, M.; Pagel, M.; Menna, E.; Smarsly, B. M.; Osella, S.; Gallego, J.; Gatti, T., *Functional Groups Accessibility and the Origin of Photoluminescence in N/O-Containing Bottom-up Carbon Nanodots*, **ChemOnTubes 2024, Strasbourg, France. 2024**

Poster and Flash Presentation

Debes, P. P.; Schatz, D.; Hesseling, J.; Schönhoff, M.; Gallego, J.; Wegner, H. A.; Gatti, T., *Understanding Structure-to-Property Relationships in Photo Switchable Carbon Nanodot-Azobenzene Covalent Hybrids*, **International Symposium on Nano & Supramolecular Chemistry ISNSC2024, Santa Margherita di Pula, Italy. 2024** – Awarded with a poster prize

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Introduction

The discovery and development of nanometer-sized, zero-dimensional semiconductor quantum dots (QDs) with size-dependent optical and electronic properties was recognized with the 2023 Nobel Prize in Chemistry.¹ When engineered appropriately, QDs exhibit exceptional optoelectronic behavior, including size-tunable emission, high quantum yields, and robust photostability. These characteristics have been applied in various technologies, including light-emitting diodes (LEDs),² photovoltaic applications,³ ultrasensitive chemical and biological sensors,⁴ and high-resolution imaging platforms.⁵ Despite their outstanding properties, the practical use of semiconductor QDs is constrained by their typical composition, which frequently includes toxic heavy metals (e.g., cadmium or lead) and scarce elements such as indium, tellurium, or gallium.⁶ These components raise significant concerns in terms of environmental toxicity, biocompatibility, and resource sustainability, particularly in light of their limited natural abundance and growing industrial demand.^{7,8}

In this context, carbon-based quantum dots, collectively referred to as CDs have emerged as a promising class of carbon-based nanomaterials to overcome the aforementioned limitations. These quasi-spherical, sub-10 nm carbonaceous nanomaterials exhibit notable photoluminescence (PL), chemical stability, water solubility, and most importantly, low toxicity. Consequently, CDs are regarded as environmentally safe alternatives to their inorganic counterparts, with a rapidly expanding role in fields such as optoelectronics, catalysis, sensing, and nanomedicine.^{9,10} CDs can be categorized into four main subclasses: graphene quantum dots (GQDs), carbon quantum dots (CQDs), CNDs, and carbonized polymer dots (CPDs). These materials exhibit important differences in crystallinity, chemical composition, surface functionalities, and both electronic and optical properties.^{11,12} Unfortunately, the classification and nomenclature of CDs remain inconsistently applied in the literature, contributing to ambiguity and hindering the development of systematic structure-property relationships.¹³ This complexity is further amplified by the wide range of synthetic methods used, which are generally categorized into top-down approaches (e.g., oxidative fragmentation of carbon-rich materials) and bottom-up strategies (e.g., hydrothermal carbonization of organic precursors).¹⁴ Among these, bottom-up syntheses using biomass or bio-derived chemicals have gained attention due to their environmentally benign nature and tunable functionalization potential.⁸ A key feature underlying the versatility of CDs is their chemically modifiable surface. The

presence of abundant functional groups such as hydroxyls, carboxyls, and amines provides numerous opportunities for surface engineering, which is essential for the tailoring of solubility, reactivity, optical properties, and surface interactions.⁹

The implementation of surface functionality can be achieved through two distinct strategies. Pre-synthetic strategies involve the introduction of desired functionalities via molecular precursors containing the desired functional groups, although other functionalities may emerge during the synthesis. In contrast, post-synthetic modifications allow for covalent or non-covalent attachment of specific molecules after CD formation. Pre-synthetic methods often result in uniform surface coverage, while post-synthetic techniques offer enhanced selectivity and adaptability.^{15–17} However, due to the complexity of CD surfaces, the quantification and characterization of these functional groups remains a challenging task, especially when assessing their accessibility and reactivity. The group of Prato et al. used an imine reaction with a fluorinated cinnamaldehyde derivative and subsequent quantitative ¹⁹F-NMR measurements to quantify the amount of primary and secondary amines on CNDs.¹⁸ The alkene moiety of the cinnamaldehyde provides low steric hindrance and the imine reaction is highly reactive. This enables the detection of the majority of primary and secondary amines on the surface of CNDs. Meanwhile, the most widely used approach for post-synthetic functionalization of CNDs is the amide coupling, which involves the reaction of amines with carboxylic acids.^{19,20} Unlike the imine reaction, the amide coupling is less reactive and often involves sterically more hindered groups. Consequently, the reliability of protocols for targeted surface functionalization is questionable in scenarios where not all primary and secondary amines are functionalized. This discrepancy leads to functionalization values that do not match the actual ones and therefore complicates the targeted design of such materials. When it comes to applications of these hybrid materials, the degree of functionalization determines the resulting properties of the material.

This work focuses on the synthesis and characterization of CNDs and their rational post-functionalization for targeted applications. In **Publication 1**, particular emphasis is placed on detailed quantitative characterization of functional groups on CND surfaces. Sophisticated analytical techniques, including the Kaiser test, which allows for selective quantification of primary amines, and quantitative ¹⁹F-NMR spectroscopy, which enables precise monitoring of functionalization through fluorinated molecular tags, are used. This approach provides insight into the processes of functionalization, accessibility, and reactivity. It also offers a suitable protocol for the quantification of primary and secondary amines, which are accessible for amide

coupling in CNDs. Beyond characterization, the surface engineering of CNDs has been strategically applied in photocatalytic applications. **Publication 2** explores the construction of a visible light responsive hybrid material by covalently attaching an organic dye molecule to CNDs. These functionalized CNDs serve as light sensitizers when combined with wide-bandgap semiconductors, such as TiO_2 . The resulting hybrid exhibits improved adsorption on TiO_2 , enhanced stability, reduced dye consumption, and promising pollutant degradation performance. These results highlight the synergistic role of functionalized CNDs in modulating the photophysical properties of semiconductor systems. In biomedical applications, CNDs are particularly attractive due to their low toxicity, aqueous solubility, and robust photostability. Their PL behavior enables high-resolution imaging, while their surface chemistry supports functionalization with targeting ligands, drugs, and biomolecules. In **Publication 3**, CNDs were covalently modified with azobenzene derivatives, which are photoresponsive molecules capable of undergoing reversible isomerization under light irradiation. The resulting nanohybrids exhibited controllable structural transitions, rendering them suitable candidates for optostimulation platforms in biomedical contexts. These light-responsive systems have the potential to create new avenues for externally controlled membrane-targeted therapies.

This study will demonstrate how the integration of advanced techniques for the quantification of surface functional groups can significantly deepen our understanding of structure-function relationships in CNDs. This analytical framework is essential for the rational design and functional optimization of CNDs, thereby laying the groundwork for their broader implementation across a wide range of scientific and technological fields (see **Figure 1**).

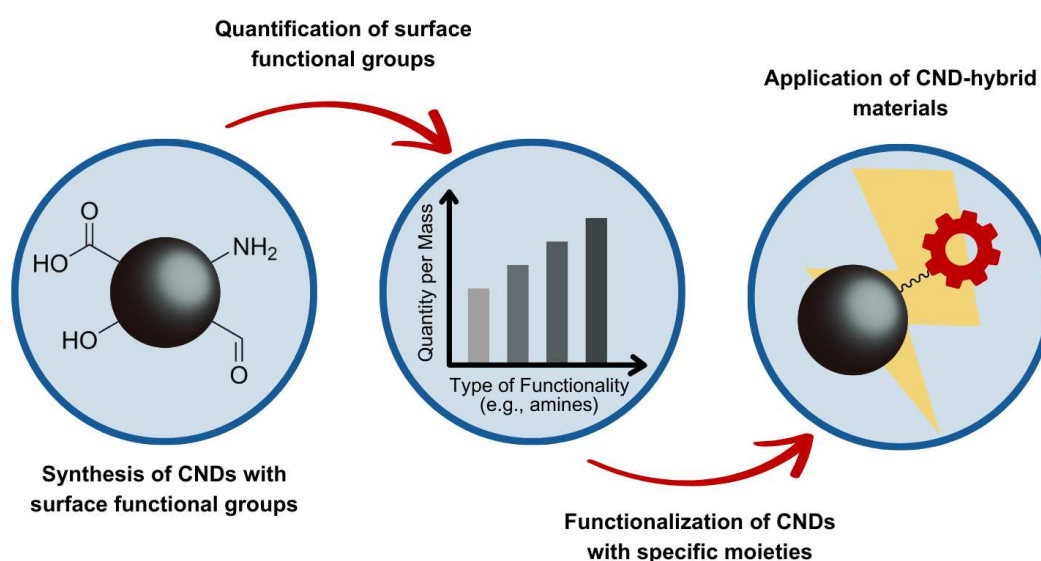


Figure 1. Illustration of the importance of surface functional group quantification for the understanding of structure-function relationships in carbon nanodots (CNDs). Accurate quantification of functional groups enables rational design and optimization of CND hybrids for specific applications.

1. Background

1.1. Carbon Dots – Classification and Properties

Carbon atoms can bond in various configurations, resulting in different allotropes, like diamond and graphite.²¹ These allotropes can have very different physical properties: Within these allotropes, graphite is one of the softest materials, and diamond is the hardest natural material. Carbon's nine possible oxidation states form many stable covalent bonds with other atoms, enabling the formation of numerous compounds.²² In recent decades, significant efforts have been dedicated to the study of carbon nanomaterials. Nanomaterials are defined by their morphological properties and are restricted in size from one to one hundred nanometers in at least one dimension.²³ After discovering fullerenes, carbon nanofoams, and graphenes, carbon materials have been used in nanotechnology.²⁴ This use has led to significant advancements in the field of nanotechnology, resulting in the discovery of novel structures such as carbon nanotubes (CNTs), carbon nanobuds, and nanodiamonds.^{25,26} CNTs, for example, possess a number of useful properties, such as easy surface functionalization, strong tendency to form π - π interactions, relatively economic, excellent conductivity, thermally and mechanically very stable, large surface-to-volume ratio.²⁷ These properties can be implemented in a variety of applications, including batteries^{28,29}, biocompatible hydrogels³⁰, supercapacitors³¹, and sensors.³² Advances like these demonstrate the versatility and importance of carbon-based nanomaterials in scientific research and practical applications in various scientific disciplines.³³ Carbon nanomaterials, like nanomaterials in general, can be classified according to their dimensionality, ranging from 0D fullerenes²⁴ and CDs³⁴, with all three dimensions in the nanoscale (below 100 nm)²³, to 1D CNTs,^{35,36} with one dimension above 100 nm, as well as 2D graphene³⁷ and its derivatives graphene oxide and reduced graphene oxide³⁸ with two dimensions above 100 nm (see **Figure 2**).

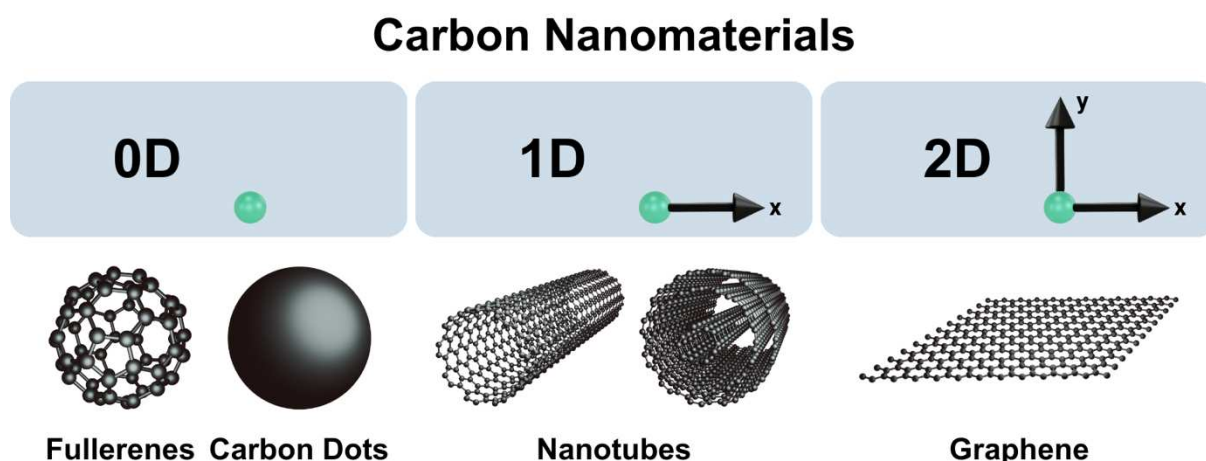


Figure 2. Classification of carbon nanomaterials based on dimensionality. The scheme categorizes carbon nanomaterials into three distinct classes: zero-dimensional (0D) materials, including fullerenes and CDs, which have all dimensions at the nanoscale (below 100 nm); one-dimensional (1D) materials, such as CNTs, which are characterized by one dimension greater than 100 nm; and two-dimensional (2D) materials, represented by graphene - which has two dimensions greater than 100 nm.³⁹

Sometimes, 3D carbon nanomaterials are reported in literature which exhibit no dimension within the nanoscale range.^{40–43} Such examples include graphite⁴¹, carbon-nanofiber bundles⁴⁴, and carbon nanotube networks⁴⁵, but are formally no nanomaterials according to the definition above. In a broader sense, a material is referred to as a nanomaterial as soon as it features a size-dependent property, which is different from the bulk material. The transition is rarely instantaneous, and the properties evolve continuously from bulk to nanoscale; therefore, the definition of a threshold size is debatable. The resulting materials combine the properties of each dimension and, depending on the morphology, exhibit both bulk and nanoscale properties.⁴⁶ In 2004, the research group of Scrivens identified a fluorescent material within the soot of single-walled carbon nanotubes (SWCNTs) during purification processes.⁴⁷ This fluorescent material was approximately one nanometer in size, was water-soluble, and was composed of carbon, hydrogen, nitrogen, and oxygen. This discovery marked the identification of a new class of materials known as CDs. Since their discovery, CDs have attracted significant scientific interest due to their luminescent properties.³⁴ CDs can be synthesized through various methods and starting materials, leading to a wide range of structures, sizes, and properties.^{11,48–50} The International Union of Pure and Applied Chemistry (IUPAC) defines a "carbon material" as a solid consisting of a high proportion of the element carbon in a non-graphitic state. However, CDs do not meet this definition, as they may exhibit low carbon content and a lack of a long-range crystalline order in all three spatial dimensions.⁵¹ Given that CDs typically have diameters smaller than 10 nm, they are commonly classified as "carbon nanomaterials." This term is frequently used and accepted in the current literature to describe CDs. Nonetheless, it is worth noting that "carbon nanomaterial" remains an ambiguous term, as it has not been

explicitly defined by IUPAC. Additionally, the IUPAC does not supply a particular definition for the category of CDs, which contributes to the complexity of their classification. To address the structural variability and potential confusion with semiconductor QDs, Valcárcel's group proposed a nomenclature in 2016, that categorizes CDs into three subcategories: GQDs, CQDs, and CNDs.¹² This classification primarily focus on structural differences and quantum confinement effects (QCEs). GQDs and CQDs possess extended π -conjugated structures, while CNDs feature a mix of amorphous and graphitic domains without QCE. The fluorescence emission is significantly influenced by the level of π -conjugation present in GQDs and CQDs; conversely, in CNDs, it primarily arises from molecular fluorophores (MFs).⁵² Although this structural classification has helped to clarify inconsistencies in CD taxonomy, it has proven insufficient in certain respects. GQDs are individual discoidal graphene sheets, their edges often contain structural defects such as oxygen-containing functional groups. Furthermore, CQDs and CNDs are classified as quasi-spherical nanoparticles, they can comprise varying mixtures of sp^2 and sp^3 carbons along with differing amounts of oxygen functional groups ($-COOH$, $-CHO$, $-OH$). However, a key difference is the presence of an amorphous carbon core in CNDs, which contains a higher proportion of oxygen atoms. In 2017, the group of Reisner redefined the nomenclature of these nanoparticles by considering the main structural features of the nanoparticles.¹⁴ They defined CDs as graphitic (g-CDs), or amorphous (a-CDs). In 2019, the group of Naccache expanded the classification to include surface polarity, identifying CDs as hydrophobic (hb-CDs), hydrophilic (hp-CDs), and amphiphilic (a-CDs).⁵³ Recent advancements have further diversified the classification with the identification of a novel class of CDs on particles exhibiting polymeric features.¹¹ These are referred to as CPDs, which are formed through the partial carbonization of polymeric precursors. Notably, these nanoparticles possess a carbonized core that is encircled by polymeric chains, which contain a multitude of functional groups.^{54,55} Based on this discovery, the following categorization can be deduced: GQDs, CQDs, CNDs, and CPDs (see **Figure 3**).

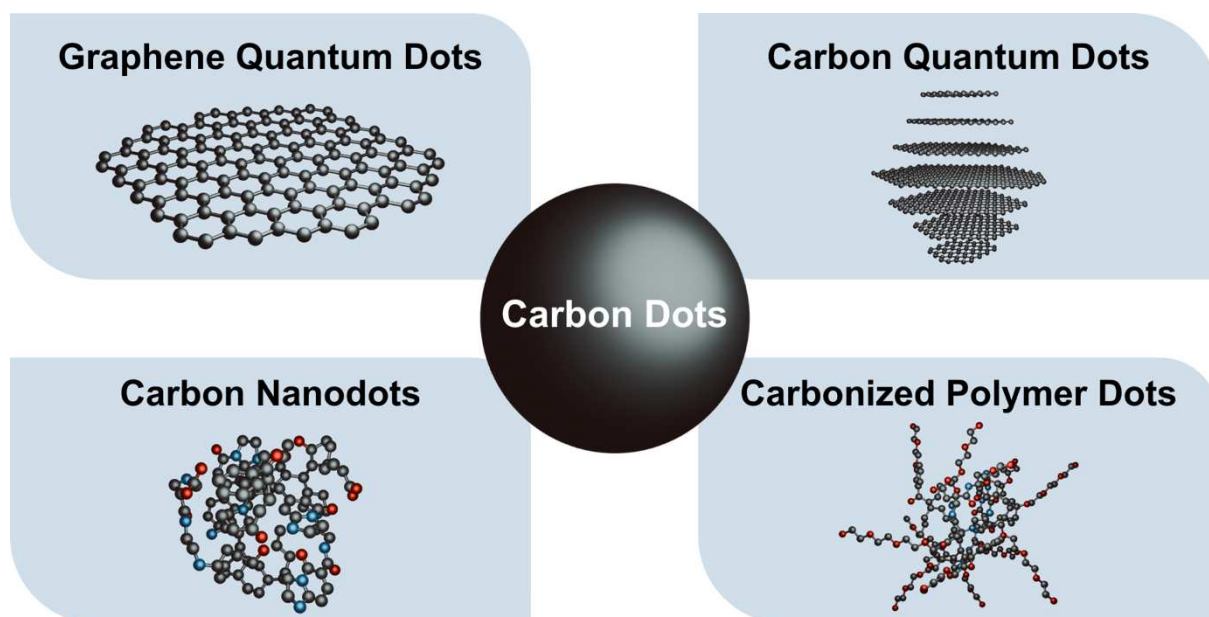


Figure 3. Schematic representation of the different categories of CDs: graphene quantum dots (GQDs), carbon quantum dots (CQDs), carbon nanodots (CNDs), and carbonized polymer dots (CPDs). Black spheres represent carbon atoms, blue spheres oxygen, and red spheres nitrogen. The presence of oxygen and nitrogen in the CNDs and CPDs should emphasize their amorphous character, although defects and functional groups can also be found in GQDs and CQDs.

Carbon dots share several properties, including dispersibility across a wide range of solvents, low toxicity, biocompatibility, biodegradability, low synthesis costs, the potential for facile and scalable syntheses, and significant light absorption and emission characteristics. PL mechanisms are closely linked to the structural characteristics of the CDs. To date, the mechanisms and processes governing the PL of CDs remain poorly understood. The diversity of CD structures and the ambiguous classification systems used in literature further complicate the complexity of PL properties. This variability hinders the establishment of a unified understanding of the underlying principles governing their PL behavior.^{13,56} The most accepted luminescence mechanisms include surface state effects, QCEs, and MFs.⁵² The physical and chemical properties of different CD types are discussed in more detail in the following sections.

Graphene Quantum Dots (GQDs)

GQDs are composed of one to a few graphene-like layers, featuring nitrogen or oxygen functional groups covalently bonded to their edges.⁵⁷ The GQD is rarely a pure monolayer, although it was originally defined like this. Their lateral dimensions typically range from 2 nm to 40 nm, while their overall thickness, corresponding to the number of stacked layers, varies between 1.3 nm to 8 nm.^{58–60} The core structure consists of sp^2 -hybridized carbon atoms or heteroatoms. GQDs exhibit a X-ray diffraction (XRD) reflex around $2\theta = 26^\circ$, corresponding to the (002) reflection, caused by the stacking of graphene layers (interplanar spacing

~ 0.34 nm).^{58,61,62} The PL behavior of GQDs is primarily controlled by the band gap of the core states. As the size of GQDs increases, the band gap narrows, resulting in a red shift of the emission spectrum. Conversely, as the size of the GQD decreases, the band gap increases, resulting in a blue shift in the emission spectrum.⁶³ This so-called QCE occurs when the quantum dots are smaller than their exciton Bohr radius.⁶⁴ Thus, the PL emission of GQDs can be tuned by adjusting the size of the conjugated π -domains.^{65,66} The intrinsic emission originates from the GQD core, while surface states contribute significantly to the PL dynamics.⁶⁷ Following excitation, intrinsic PL may diminish due to non-radiative decay into these surface states, resulting in observable red shifts in emission wavelengths. The PL of GQDs can range from the ultraviolet (UV) to the near-infrared region due to their size diversity, potential heteroatom doping, surface functionalization, and the presence of various defects.⁶⁸

Carbon Quantum Dots (CQDs)

CQDs are quasi-spherical with a diameter of up to 10 nm.⁶⁹ The carbon core possesses non-graphitic microstructure, and the surface contains oxygen/nitrogen groups, which show intrinsic PL and QCE. They consist of a mixture of non-graphitic to amorphous sp^2 - and sp^3 -carbon and typically show broad reflections around $2\theta = 20-24^\circ$ in the XRD pattern.^{70,71} The PL wavelength can be tuned by the size of CQDs and the functionalization of the surface, which is similar to the GQDs with the main difference in the structure.⁷²⁻⁷⁴ The QCE distinguishes CQDs and GQDs from CNDs and CPDs, which do not exhibit this effect.

Carbon Nanodots (CNDs)

CNDs are quasi-spherical nanoparticles with a diameter smaller than 10 nm, comprising an electron-dense carbonaceous core and a less-dense shell.^{75,76} These particles exhibit no ordered (amorphous) structure.⁷⁷ In the XRD, hardly any defined reflections appear, instead a very flat and diffuse diffractogram.⁷⁸ Experimental evidence has indicated the presence of MFs in some CNDs, which resemble common organic fluorescent dyes.⁷⁹⁻⁸² Under mild reaction conditions (180 °C), an improvement in PL was observed, which is consistent with low molecular mass structures. Photobleaching of the PL in the CNDs synthesized under mild conditions after irradiation with UV radiation further confirmed the presence of MFs. According to the mechanism proposed in literature, the condensation phase of the precursors is followed by carbonization. With shorter reaction times and/or lower temperatures, carbonization can be suppressed. While it is tempting to attribute the PL signal of the MFs solution to individual (solvated) molecules, several studies in past years supported the idea of MFs closely interacting

with the CD structure, possibly directly incorporated inside.⁸⁰ In such a scenario, the absence of QCE is notable. The CNDs, which are typically obtained at low temperatures, emit via this mechanism and exhibit a very broad emission band as a consequence of the superposition of several emissions (i.e., diverse emitter centers). The formation of a carbonaceous core only occurs at high temperatures ($>230\text{ }^{\circ}\text{C}$) and the resulting PL is attributed to the presence of both MFs and the carbonaceous core. In such instances, the boundaries between CNDs and CQDs become fluid. At elevated temperatures ($300\text{ }^{\circ}\text{C}$ and $400\text{ }^{\circ}\text{C}$, respectively), the predominance of CQDs becomes evident.^{75,83}

Carbonized Polymer Dots (CPDs)

CPDs are hybrid nanostructures consisting mainly of organic polymer chains with a carbonaceous core.⁸⁴ They can be classified as either low carbonization carbon dots or fluorescent non-conjugated polymer nanoparticles. By condensation of small molecules or polymer precursors, they can be synthesized.^{79,85} CPDs exhibit polymer-like properties, such as an abundance of functional groups and a highly cross-linked network structure. Their PL is influenced by the carbonaceous core and surrounding polymer chains; potential PL centers within the polymers exhibit weak fluorescence when unconfined.⁸⁶ When they are spatially confined in CPDs, vibrational relaxation is suppressed, significantly increasing the PL intensity, which is different from MFs, which exhibit PL even without spatial confinement.⁸⁷

However, these nomenclatures tend to be used inaccurately or interchangeably, and even today, distinguishing between CD types remains a challenge because the contributions of structure and PL can be difficult to resolve. A more thorough understanding of how these different types of materials can be produced is discussed in the next section.

1.2. Synthesis Methods of CDs

In recent years, a plethora of synthetic methods for CDs has been investigated. A broad classification of CD synthesis methods based on precursor typology divides them into two main categories: top-down and bottom-up (see **Figure 4**).¹⁴ The former approach is based on the degradation of larger carbon structures, whereas the latter approach is based on the formation of CDs from small molecular precursors, polymers, or even biomass. Biomass derived from diverse sources such as perennial grasses, domestic waste, agricultural residues, and fishery by-products represent abundant and biodegradable organic materials composed of cellulose, hemicellulose, lignin, proteins, and other substances.^{88,89} Utilization of biowaste is a sustainable

approach that is readily available.⁹⁰ The selection of precursors and reaction conditions are crucial to result in a particular topology. It is noteworthy that GQDs are primarily synthesized using top-down methods, while CNDs and CPDs are typically produced through bottom-up approaches.^{91–93} This distinction can be attributed to the substantial structural differences between the precursors utilized and the resultant carbon dots. In contrast, CQDs can be effectively synthesized using both approaches, demonstrating comparable efficiency in their production.^{94–96} Furthermore, in the case of CDs, the PL behavior is highly dependent on the synthesis methods, reaction conditions, and subsequent post-functionalization or passivation strategies employed to modify their surface chemistry.

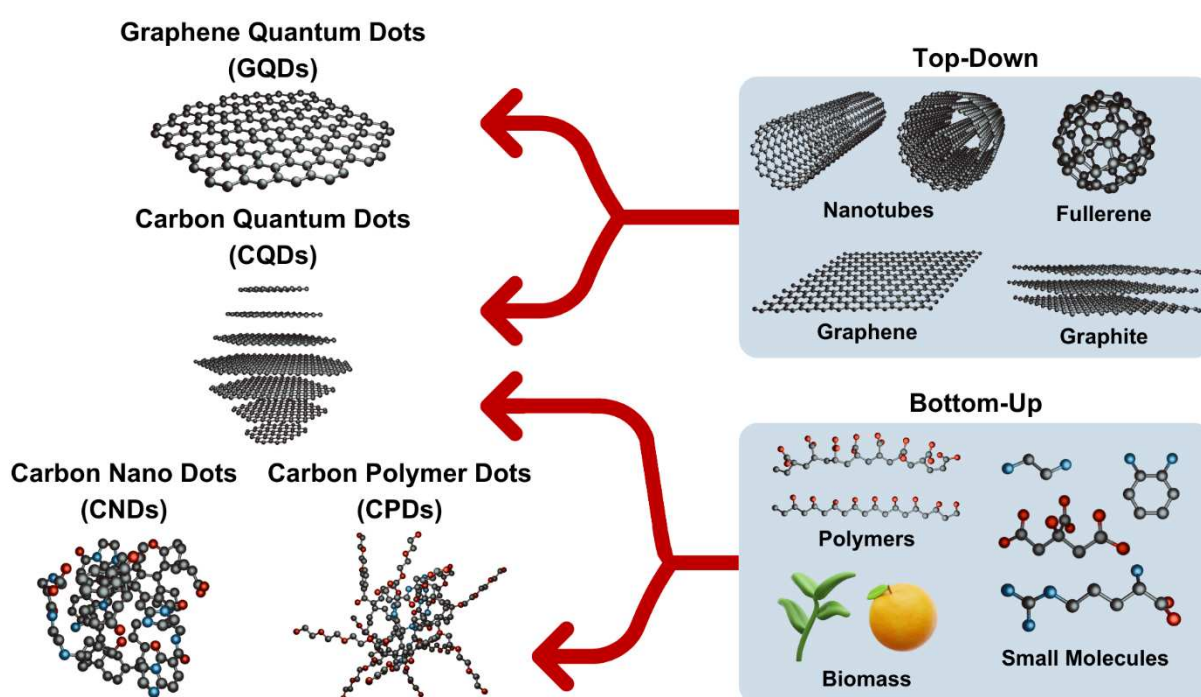


Figure 4. The classification of CDs synthesis methods according to precursor typologies into two main categories: top-down and bottom-up. This categorization encompasses the inclusion of graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbonized polymer dots (CPDs), along with their primary preparation approaches.

Top-down Synthesis Methods

In a top-down approach, CNTs, graphite, and soot are built down to CDs using for example arc discharge, laser ablation, sonochemical methods, or chemical oxidation.⁹⁷ The arc discharge technique involves the generation of an electric arc between two graphite electrodes in an inert gas atmosphere, with the addition of small amounts of water vapor to facilitate the formation of oxidized groups. As a result, the carbon precursors sublime and travel towards the cathode,

where they deposit as nanostructures. CDs were firstly discovered in this way.⁴⁷ Conversely, laser ablation effectively abrades a solid carbon source using a combination of laser wavelengths to produce small CDs that can be isolated from the bulk material.⁵⁵ In the chemical oxidation process, the utilization of potent oxidants can effectively disrupt the covalent bond in the carbon source, thereby facilitating its gradual fragmentation into small molecules. The progression of this reaction is predominantly dependent on the oxidizing strength of the oxidant. Common oxidants used in this procedure includes sulfuric acid and nitric acid, or a mixture of both.^{98,99} From the perspective of oxidation, the process can be classified into two categories: chemical oxidation and electrochemical oxidation. Electrochemical oxidation of CNTs or graphite benefits from simple procedures and mild reaction conditions, typically using water or ionic liquids as solvents.⁹¹ In general, the materials used in top-down approaches already provide perfect sp^2 carbon structures, but lack efficient band gaps to exhibit PL. To achieve PL, both the size and the surface chemistry of these carbon sources need to be modulated. Although the top-down approaches are favored for their convenience and scalability, they often involve multi-step reactions and complex mechanisms that hinder the precise control of the chemical structure of the resulting CDs.^{17,100}

Bottom-up Synthesis Methods

The bottom-up approach has been demonstrated to be a more accessible and less expensive method for producing CDs than the top-down approach.^{101–103} A variety of strategies are employed, including stepwise organic syntheses,¹⁰⁴ hydrothermal/solvothermal syntheses, chemical vapor deposition, and microwave(MW)-assisted techniques. These approaches typically utilize small molecular or polymeric precursors and follow a three-step mechanism: (1) precursor reactions, such as condensation between small organic molecules;^{105–107} (2) reactive species begin to assemble into extended, polymer-like intermediates; and (3) carbonization, which includes aromatization to yield the final nanoparticles.^{108,109} Solvothermal or hydrothermal methods involve the use of partially or fully dissolved precursors, employing conventional heating near the boiling point of solvents or utilizing autoclaves to achieve elevated temperatures and pressures within sealed Teflon® vessels.¹¹⁰ MW-assisted solvothermal techniques provide a rapid and uniform heating by exciting solvent molecules by resonant MW frequencies throughout the entire volume.^{111,112} Absorption of the radiation leads to excitation to higher rotational states, which release heat upon relaxation. While MW ovens are commonly used to synthesize CDs, the use of a MW reactor offers enhanced control and modulation of reaction parameters, including temperature, pressure, duration, and power

output.¹¹³ The disadvantage of the laboratory-scale MW reactor is the limited quantity of starting materials that can be used for synthesis, which means that only small amounts of CDs can be produced. In several studies, well-crystalline CDs with homogeneous structures have been successfully synthesized through solvothermal or hydrothermal reactions.⁵³ The final state of CNDs, characterized by an amorphous core containing sp^2 -hybridized domains, appears to serve as an intermediate stage in the formation of CQDs, preceding the graphitization process that leads to an ordered crystalline lattice.⁷⁵ However, there are cases where bottom-up syntheses has been successfully used to produce GQDs.^{58,114}

Purification Methods

The purification of CDs after their synthesis presents considerable challenges due to their small size and low density, which make conventional isolation methods such as centrifugation less effective.¹¹⁵ Dialysis, a frequently employed purification technique, necessitates the utilization of a small dialysis membrane (e.g., with a molecular weight cut-off < 1000 Da) to minimize material loss.^{79,116} Other methods, such as size exclusion chromatography, facilitate size-based separation of CDs, while silica gel chromatography can assist in identifying fluorescent molecular species present in the samples.^{117,118} Despite the efficacy of these techniques, the effective isolation and differentiation of CDs from other molecular species remains a considerable challenge in the field.⁷⁹ In cases of significant carbonization leading to precipitation, the resulting CDs are isolated by centrifugation or filtration.¹¹⁹ Highly volatile by-products and excess solvent are then removed by evaporation under reduced pressure or freeze-drying techniques.

Tuning the Optical Properties

The pre-synthetic design of CDs has emerged as a valuable strategy for tailoring the structural features of CDs by selecting specific precursors. The synthesis temperature, precursor concentrations, incorporation of dopants, selection of solvent, and pH during synthesis are among the critical factors influencing the optical properties of CDs.^{120–123} In the case of GQDs and CQDs, particles smaller than 10 nm tend to exhibit a red shift in PL emission with increasing π -structure size. However, controlling the size of the π -domains poses a challenge for modulating color emission via QCEs. The presence of different functional groups can lead to the formation of distinct surface defect states, thereby resulting in the emergence of multiple energy level band gaps that regulate the color emission process.¹⁶ Elemental doping with nitrogen, sulfur, and other heteroatoms or metal ions allows effective tuning of optical

properties. These dopants can be incorporated both in the core and on the surface, especially via N-rich functional groups.^{15,124–126}

The design of CNDs and CPDs poses significant challenges due to their amorphous nature. The extensive library of molecular and polymeric precursors available enables the creation of a diverse database of structures, each with distinctive properties, which complicates the design process. Additionally, the incorporation of MFs into the carbon structure to exhibit direct fluorescence is a viable option; however, the existing literature on the use of molecular state mechanisms for color modulation is limited due to the difficulties in controlling the reactions.^{15,118,127}

A substantial body of research has focused on the role of surface passivation and heteroatom doping in modulating the optical properties of CDs. The incorporation of functional groups through dopant-containing precursors has proven to be a more effective and reproducible method. Specifically, the use of ethylenediamine (EDA) demonstrated a selective accumulation on the surface of the CDs, serving as both a nitrogen dopant and an in-situ passivating agent. Consequently, the quantity of amine groups on the surface is modifiable, with the potential to enhance PL efficiency. For instance, the synthesis of highly passivated nitrogen-doped CNDs (hp-N-CNDs) was achieved via MW-assisted hydrothermal carbonization of citric acid (CA) in the presence of various amines, including EDA, diethylamine (DEA), triethylamine (TEA), and 1,4-butanediamine (BDA). The variation in amine type, concentration, and reaction time resulted in distinct doping/passivation levels and PL responses.^{118,128} In **Publication 1**, an amorphous hp-N-CND system was synthesized via a MW-assisted hydrothermal method using either CA or D-L-arginine (Arg) as carbon precursors in combination with varying concentrations of EDA. We also observed that different amounts of EDA influenced the amount of amines on the surface of the CNDs. Subsequent functionalization and quantification showed that the different amounts of EDA also dictated the degree of subsequent functionalization, reactivity, and accessibility.^{118,129}

Metal doping is another well-established strategy for tuning CD properties. This method is typically accomplished by reacting metal salts or hydroxides with organic precursors, which act as ligands, thereby facilitating the integration of metal ions into the carbon framework.^{130,131}

Discrepancies in the available literature on CDs may be due to inadequate synthetic protocols, as well as unsatisfactory purification and characterization procedures.¹³² To ensure a comprehensive and precise classification of CDs, it is essential to undertake a detailed structural characterization, taking into account the presence of amorphous or crystalline structures. The approach employed will have a direct impact on the functional groups present on the synthesized CDs.¹²⁸ As outlined in **Publication 1**, the analysis of the functional groups should be conducted in both qualitative and quantitative approaches.

1.3. Characterization of Carbon Dots

Physical and chemical characterization techniques are essential for elucidating the structural and functional properties of CDs. Among these techniques, ¹³C-NMR spectroscopy is particularly valuable for investigating surface functionalities and determining the extent of sp²/sp³ hybridized carbon atoms. Furthermore, the combination of ¹H-NMR and ¹³C-NMR can serve as a vital tool for the determination of the reaction progress and for the confirmation of the complete decomposition of precursor compounds.^{133,134} In the case of heteroatom-doped CDs, other NMR-active nuclei, such as ¹⁵N, ³¹P, and ¹¹B, can provide further insights into structural characteristics.^{14,83,135–137}

XRD is employed to evaluate the crystallinity of CDs, while transmission electron microscopy (TEM) offers detailed morphological insights.^{138–141} However, it should be noted that TEM sample preparation can be challenging due to the tendency of CDs to aggregate on the grids or exhibit low contrast.¹¹⁵ Alternatively, atomic force microscopy (AFM) offers measuring CD height (corresponding to their diameter assuming spherical entities), typically using silicon wafers or mica silicate minerals as substrates.¹¹⁸ The integration of both AFM and TEM techniques facilitates a more comprehensive understanding of CD morphology than either method alone.¹⁴² Fourier-transform infrared (FT-IR) spectroscopy is a valuable tool for assessing surface functionalization by tracking characteristic shifts in carbonyl (C=O) environments on the CD surface.¹⁴³ Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) complement this analysis by providing information about the types of carbon environments present and their respective types.^{138–141} Dynamic light scattering (DLS) is employed to determine the hydrodynamic size of CDs; however, this technique can be complicated by the small size, low density, and particular their intrinsic fluorescence of CDs.¹⁴ UV-Vis absorption and fluorescence spectroscopy are two valuable techniques for characterizing the optical properties of CDs. The energy levels of CDs can be determined using

various techniques, including ultraviolet photoelectron spectroscopy (UPS) combined with optical absorption spectra (Tauc plot), cyclic or differential pulse voltammetry for redox potential estimation, linear sweep voltammetry, and electrochemical Mott-Schottky analysis of impedance data.⁹

In **Publication 1**, the characterization and quantification of surface functional groups on the synthesized CNDs are essential for understanding their suitability for possible applications (such as optoelectronics or bioapplications). The composition of the outer shell and the nature of terminal functional groups substantially influence the interaction of the CNDs with their environment. These surface properties govern recognition, binding, reactivity, solvation, and processability. Employing a combination of techniques for detailed analysis of the nature, quantity, and accessibility of these terminal groups represents the fundament for effective engineering of the properties of these nanosized materials. A particular emphasis was placed on primary and secondary amines, which serve as key reactive sites for covalent functionalization.

Kaiser Test

Amines represent a widely used class of functional groups in the functionalization of CDs, for instance via an amide coupling. One reason for this is that they are easily quantifiable. The Kaiser test has been established as a reliable method for the qualitative and quantitative analysis of primary amines, amino acids (excluding proline), and ammonia.^{144–146} A main application of this assay is the testing of residual amino groups of amino acids in solid-phase-supported peptide synthesis. Due to the colorimetric nature of this assay, its implementation is remarkably convenient. In this study, the Kaiser test is used in **Publication 1** to characterize the functional groups present on the surface of CNDs. The identification and quantification of terminal groups is crucial as they significantly influence the interactions of CDs with their environment and enable post-functionalization. The reaction of ninhydrin within Kaiser test solutions with primary amines on the surface of CDs results in the formation of the characteristic dye

Ruhemann's purple, whose absorption maxima at 570 nm can be accurately measured using UV-Vis spectroscopy (see **Figure 5**).^{147–149}

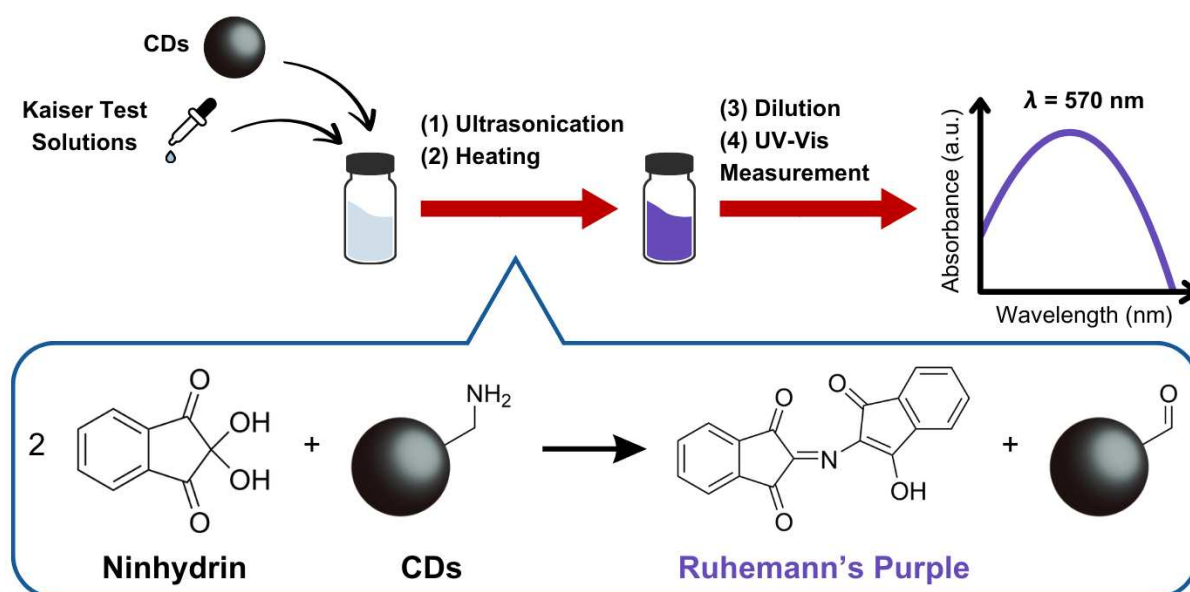


Figure 5. Schematic representation of the colorimetric Kaiser test approach for CDs. The inset illustrates the formation of Ruhemann's purple resulting from the reaction of ninhydrine with primary amines on the surface of the CDs.

This approach allows not only a qualitative assessment of the surface chemistry of CDs, but also a precise quantitative analysis, which is essential for the targeted engineering of their properties.¹⁸ The quantification of primary amine content is accomplished through the application of Beer-Lambert's law, followed by the conversion of molar concentration to molality, as outlined in **Equation 1**.¹⁵⁰ In this equation, A represents the measured absorbance at 570 nm, ε signifies the molar absorption coefficient of Ruhemann's purple ($\varepsilon = 15000 \text{ L mol}^{-1} \text{ cm}^{-1}$), V denotes the volume of the test solution, m denotes the added mass of the CDs, and L indicates the path length of the utilized cuvette.¹⁵¹ The molality b of primary amines on the CDs surface can be calculated from this relationship as follows.

$$b = \frac{A V}{L \varepsilon m} \quad (1)$$

Beyond the Kaiser test, acid-base back titrations are also frequently employed to determine the total amount of acid-base-active functional groups. Furthermore, the Kaiser test has shown to underestimate the number of primary amines. Consequently, procedures based on quantitative ¹⁹F-NMR measurements have become a common practice.^{18,152,153}

Quantitative ^{19}F -NMR

In the characterization of CDs, usual ^1H -NMR spectroscopy is inadequate due to the broad spectral region, over which proton resonances are distributed, making the analysis of surface functionalities difficult.¹⁸ To address this limitation and obtain reliable information regarding the functional groups present on the surface of CDs, a covalent fluorine-labeling strategy combined with quantitative ^{19}F -NMR analysis can be employed. In this approach, for example, *trans*-4-fluorocinnamaldehyde can serve as a molecular fluorinated probe whose ability to condense with primary amines is utilized. The addition of 4,4'-difluorobenzophenone or other suitable internal standards (IS) allows for the quantification of the covalently bound labeling molecules by integration of their ^{19}F -NMR signals in comparison to the integral of the IS (see **Figure 6**).^{18,152,154} The molality b , in this example, indicates the amount of primary amines on the CD surface and can be calculated using **Equation 2**. This calculation involves the integral of the signals from the IS and the functionalized CD, n_{IS} represents the amount of IS, and m_{CD} represents the mass of the functionalized CDs.

$$b = \frac{\text{Integral}_{\text{CD}} n_{\text{IS}}}{\text{Integral}_{\text{IS}} m_{\text{CD}}} \quad (2)$$

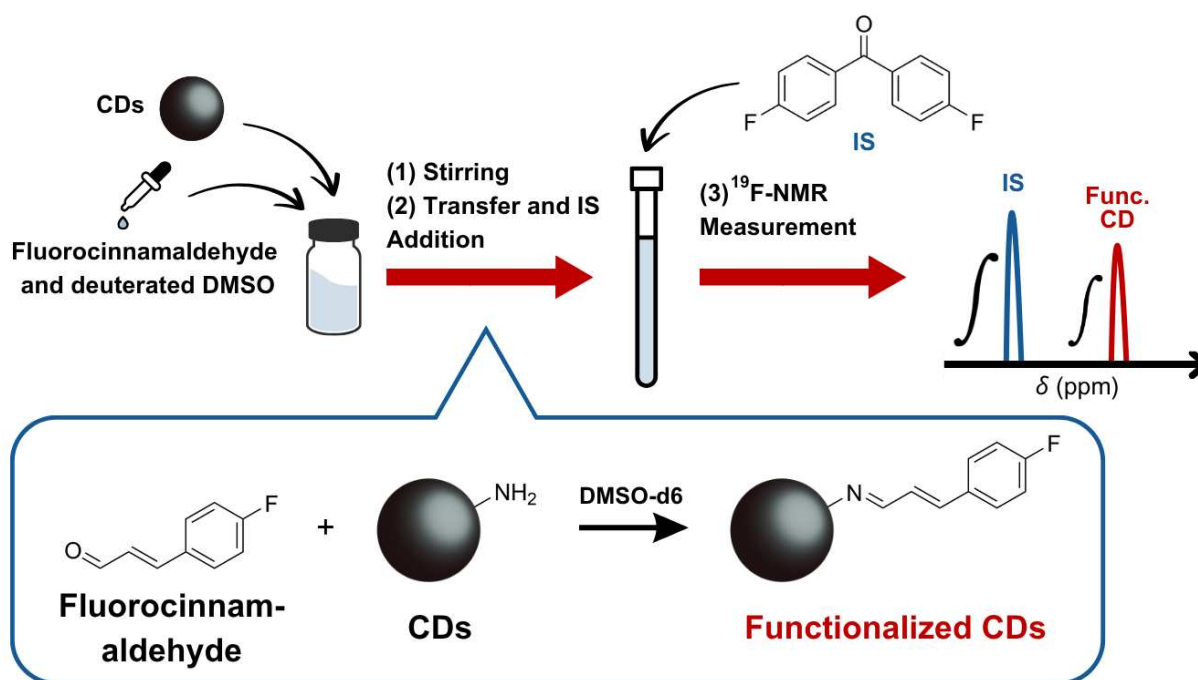


Figure 6. Schematic representation of the fluorine-labeling ^{19}F -NMR analysis. In this approach, *trans*-4-fluorocinnamaldehyde as a labeling molecule was coupled by an imine reaction with the CDs. 4,4'-difluorobenzophenone is added right before the ^{19}F -NMR measurement as an internal standard. The integral values necessary for the calculation of the degree of functionalization from the ^{19}F -NMR spectrum of the functionalized CDs and the IS are illustrated.

The number of imines formed upon condensation with the fluorine-labeled probe, which corresponds to the number of available primary amines on the CD surface, is significantly higher when assessed using the ^{19}F -NMR technique than the result of the Kaiser test.¹⁸ The bulky groups involved during the Kaiser test and the higher reactivity of imine coupling are suggested to be responsible for this difference. This underlines the effectiveness of ^{19}F -NMR analysis with a fluorinated probe as a method for the determination of surface primary amines.¹⁸ Furthermore, the versatility of this approach is evident in its capacity to expand the detection of functional groups beyond primary amines. This can be achieved by replacing the molecular fluorinated probe with another fluorinated molecule that reacts selectively with, for example, alcohols, ketones, or carboxylic acids. The detection capability of the method enables researchers to investigate the extent of functionalization of CDs following an array of post-functionalization approaches.¹⁵⁵ In **Publication 1**, a fluorinated model compound with a carboxylic acid moiety was used to compare the degree of functionalization by amide coupling with the primary and secondary amines of different CNDs. The results demonstrated a considerably lower degree of functionalization in comparison to the primary amine content that was measured by the imine-specific fluorine labeling technique.

1.4. Post-synthetic Functionalization and Applications of Carbon Dots

CDs demonstrate tunable PL properties that are considerably influenced by surface defects, including functional groups and heteroatoms. Even without further modification, their intrinsic optical and electronic characteristics enable applications in fields such as sensing, imaging, and catalysis. The surfaces of CDs commonly feature hydroxyl, epoxy, carbonyl, and amino groups, as well as nitrogen dopants (e.g., pyridinic or pyrrolic nitrogen) which can contribute a red shift of the emitted light through defect-related states.^{156,157} To achieve precise control over these properties, post-synthetic surface functionalization has become a widely utilized strategy.¹⁷ This approach enables the systematic tuning of PL and redox behavior and serves as a foundation for the design of CD-based hybrid materials with enhanced or targeted functionalities.⁹

Common functionalization strategies include covalent modifications, such as esterification¹⁵⁸, amidation¹⁹, alkylation¹⁵⁹, sulfonylation¹⁶⁰, imidization¹⁶¹, and cyclocondensation^{162,163}, as well as non-covalent methods, including supramolecular interactions and surface passivation.^{55,164,165} These modifications tailor the photophysical properties and enhance solubility, dispersibility, and stability, thereby expanding their utility as electron donors or

acceptors in hybrid materials (see **Figure 7**).^{76,118,166–170} For example, the covalent attachment of hydrophobic compounds to the hydrophilic surfaces of CDs can significantly improve solubility and stability in solution.¹⁷¹ In another case, hydrothermally synthesized CDs with a negative surface charge were modified by electrostatic interactions with polyethyleneimine and Nile blue chloride to create nanocomposite CDs with two-photon fluorescence capable of detecting S^{2-} ions in living organisms.¹⁷²

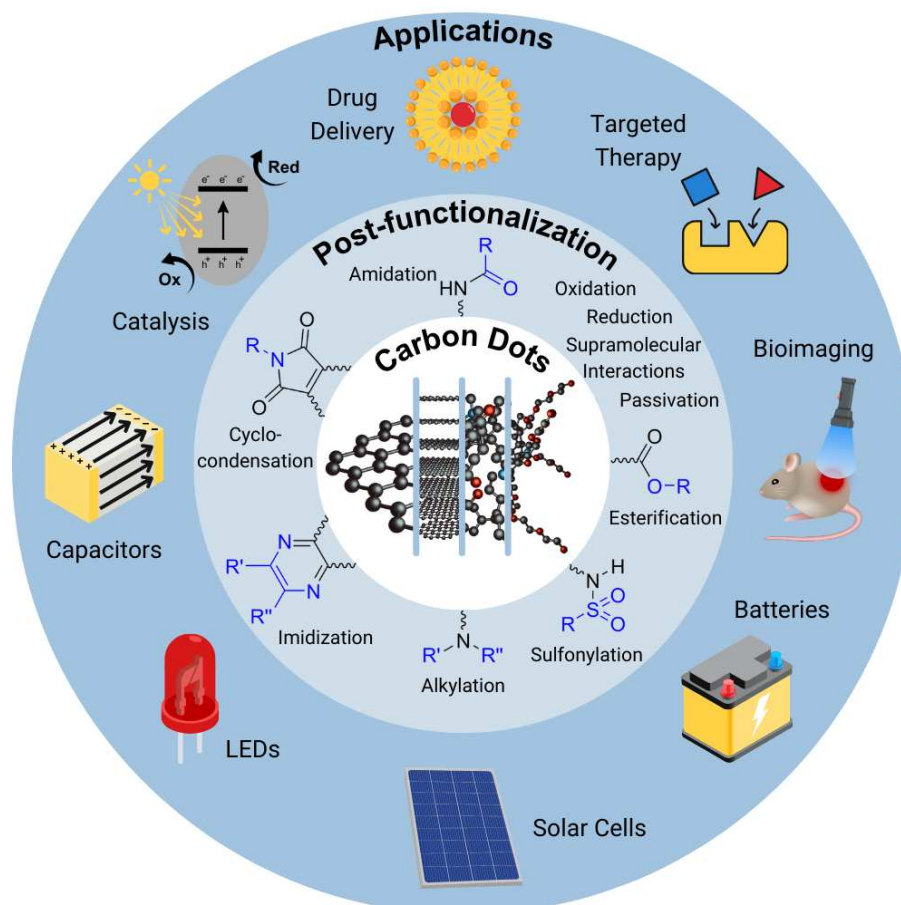


Figure 7. Overview of post-synthetic approaches and applications of CDs. The scheme illustrates the importance of post-synthetic tailoring through covalent and non-covalent approaches that modify surface functional groups. These modifications play a crucial role in influencing the physicochemical properties of CDs, thereby expanding their potential applications across diverse fields such as optoelectronics, bioapplications, and catalysis.

The integration of such customized CDs in diverse applications such as sensing^{88,164,173,174}, bioapplications^{173,175,176}, optoelectronics^{177,178}, photocatalysis^{137,179}, and electrochemiluminescence^{180–182}, as well as in drug delivery systems¹⁸³, in which enhanced imaging and targeted transport of bioactive molecules are desirable, becomes increasingly important (see **Figure 7**).^{184,185} These advancements not only broaden the scope of applications for carbon dots but also contribute to the ongoing development of innovative nanomaterials capable of addressing complex challenges in diverse scientific domains.

Photocatalysis

The use of metal oxides and metal chalcogenides in photocatalysis has been a subject of extensive research. TiO_2 remains one of the most studied semiconductors for photocatalytic applications, owing to its high oxidative power, environmental compatibility, and photochemical stability.^{186,187} However, the intrinsic wide bandgap of TiO_2 (3.2 eV for anatase), corresponding to an absorption edge at 390 nm, confines its photoactivity to the UV region, which constitutes less than 5% of the solar spectrum.^{188–191} In order to address this limitation, heterostructure engineering strategies have been developed.¹⁹² These strategies include the incorporation of co-catalysts, organic dyes, or narrow bandgap semiconductor dopants.^{193–199} The main goal of these approaches is to spatially separate photoexcited electrons and holes at different reaction sites, thereby suppressing charge recombination and increasing redox efficiency.¹⁹⁸ Furthermore, the integration of visible-light-responsive components serves to broaden the absorption spectrum, thereby, enabling enhanced photocatalytic activity under natural sunlight.¹⁹²

In the context of wastewater treatment, dye-sensitized TiO_2 systems have shown great promise in degrading a wide variety of pollutants, particularly synthetic dyes, which are commonly released by textile, printing, and cosmetic industries.²⁰⁰ These compounds are chemically stable and often resistant to biodegradation. Photocatalytic degradation proceeds via generation of reactive oxygen species (ROS), primarily superoxide ($\bullet\text{O}_2^-$) and hydroxyl radicals ($\bullet\text{OH}$), which oxidize and ultimately mineralize organic pollutants to CO_2 and water.²⁰¹

In conventional dye-sensitized systems, organic chromophores are anchored to the TiO_2 surface. Upon absorption of visible light, the dye is photoexcited, and an electron is injected from its LUMO into the conduction band (CB) of TiO_2 (**Figure 8**). This process effectively initiates the photocatalytic cycle and the oxidized dye can subsequently be regenerated via redox interactions in the surrounding medium or directly engage in oxidation reactions. The alignment of frontier energy levels is a critical requirement for this mechanism: the dye's LUMO must lie above the CB of TiO_2 to allow favorable electron transfer described above. Thus, the sensitizer's HOMO must remain sufficiently low to maintain oxidative potential and stability, while the TiO_2 surface must exhibit high surface area to ensure optimal dye adsorption and interfacial contact.¹⁹⁹

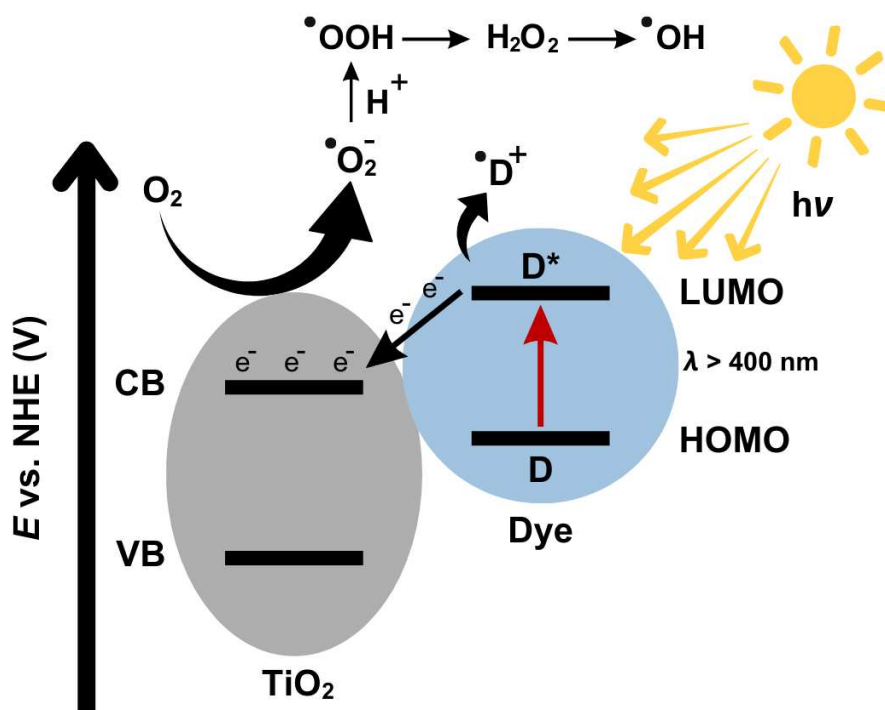


Figure 8. Mechanism of carrier transfer in dye-sensitized TiO₂ and the generation of ROS under visible light irradiation.

Typical dye sensitizers include noble metal complexes (e.g., Ru-based dyes)²⁰², semiconductor QDs (e.g., CdS, CdSe)^{203,204}, and organic dyes²⁰⁵. However, concerns about toxicity, cost, and photostability limit their practical use. In contrast, CDs offer a cost-effective and environmentally compatible alternative. They can be synthesized inexpensively and exhibit broad optical absorption across the UV-Vis spectrum, rendering them attractive candidates for sensitizing TiO₂.^{192,206–209} The photocatalytic role of CDs remains incompletely understood, with evidence supporting their participation in both direct photocatalytic and photosensitization-driven degradation pathways; the latter is illustrated in **Figure 8**.^{210–214} Their function is highly dependent on structural and surface chemical properties. Despite recent progress, further mechanistic insights are needed. Especially, property tuning through doping, surface functionalization, and integration with magnetic materials represent promising approaches for future research to improve recyclability, facilitate recovery, and enhance catalytic stability over multiple cycles.⁹

Co-sensitization demonstrated to enhance the overall efficiency of dye-based systems by employing two or more dyes to enhance attributes of the resulting dye.²¹⁵ This strategy is also applicable to CDs, where functionalization with organic dyes results in the formation of hybrid nanostructures that exhibit the desirable properties of both components.^{163,216,217} In **Publication 2**, CNDs were functionalized with dye moieties serving as primary visible-light

absorbers. This hybrid system offered advantages such as reduced dye loading, minimized photobleaching, and improved control over dye dispersion and anchoring.

Bioapplications

The tunable optical properties of CDs, particularly PL, combined with their low toxicity, represent significant advantages over conventional fluorescent dyes and semiconductor QDs for bioimaging applications. CDs are widely employed as imaging probes for cellular and tissue visualization, benefiting from their excellent aqueous dispersibility and photostability.^{218–222}

Beyond imaging, CDs have demonstrated considerable promise in the fields of drug delivery²²³, cancer therapy²²⁴, gene delivery²²⁵, and biosensing.^{226,227} Their advantageous properties, including small size, high surface-area-to-volume ratio, and hydrophilic nature, facilitate efficient cellular uptake and interaction with biological systems.²²⁸ Notably, CDs demonstrate low toxicity at moderate concentrations and are generally biodegradable or excretable, which supports their safe use in physiological environments.²²¹ To optimize CDs for specific bioapplications, pre- and post-synthetic modification strategies have been employed.²²⁹ Covalent functionalization is particularly advantageous for introducing bioactive or responsive moieties with targeting ligands and biomolecules. This process enables precise modulation of biological activity, including enzyme regulation, membrane permeability, and gene expression.^{230–232}

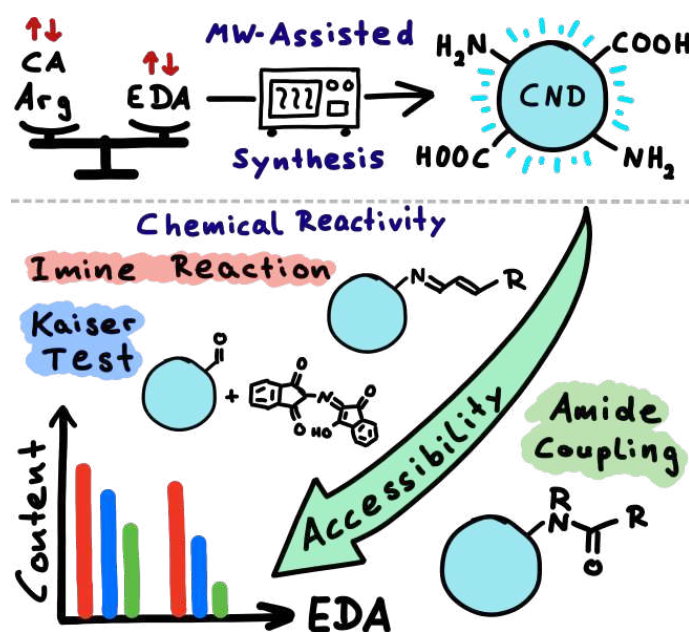
While water-soluble CDs predominate in current biomedical research, the potential for water-insoluble or lipophilic CDs in nanomedicine remains unexplored. Their compatibility with lipid membranes suggests potential in drug delivery and membrane-targeted therapies, though further investigation is needed to fully harness their biointerface capabilities.²³³

In **Publication 3**, the covalent functionalization of CNDs with azobenzene moieties was investigated for potential applications in optostimulation. The study centers on the impact of *meta* and *para* substitution patterns, as well as the incorporation of a glycine spacer, on the photophysical and structural behavior of the resulting hybrid materials. The resultant azobenzene-functionalized CNDs demonstrated photoresponsive characteristics, enabling structural transitions in response to light irradiation. This functionality may be exploited for the development of light-controlled nanoplateforms capable of membrane penetration, stimuli-responsiveness, and biomolecular sensing, offering novel opportunities in optostimulation.

2. Publications

2.1. Publication 1

Functional Groups Accessibility and the Origin of Photoluminescence in N/O-containing Bottom-up Carbon Nanodots



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Debes, P. P.; Langer, M.; Pagel, M.; Menna, E.; Smarsly, B.; Osella, S.; Gallego, J.; Gatti, T. Functional Groups Accessibility and the Origin of Photoluminescence in N/O-Containing Bottom-up Carbon Nanodots. *ChemNanoMat* **2024**, *10* (2), e202300471.

DOI: [10.1002/cnma.202300471](https://doi.org/10.1002/cnma.202300471)



doi.org/10.1002/cnma.202300471

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Functional Groups Accessibility and the Origin of Photoluminescence in N/O-containing Bottom-up Carbon Nanodots

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Chemical surface functionalization of carbon nanodots (CNDs) offers a valuable opportunity to tailor multifunctionality in these nanocarbons, by engineering the composition of their molecular surface. Therefore, it is important to elucidate the type and amount of CNDs surface functionalization to be able to design their properties accurately. CNDs are often functionalized through amide coupling without validating the degree of surface functionalization. As a measure of surface functionalization, the amounts of primary amines via Kaiser test (KT) or imine reactions of the bare CNDs is often considered. However, this may lead to overestimating the degree of surface functionalization obtained by the pure amide coupling due to different

reaction mechanisms and involved intermediates. Herein, four different CNDs prepared by microwave-assisted synthesis from arginine or citric acid with varying amounts of ethylenediamine are presented. We resorted to combining physicochemical methods to provide elemental, structural, and optical information. By that, we developed a method to quantify the degree of surface functionalization by amide coupling and show that the surface functionalization is lower than anticipated. Comparing experimental optical features of the CNDs with different computed model systems enables us to provide a more advanced vision of structure-property relationships in these still elusive nanocarbons.

Introduction

Due to their valuable electronic, thermal, optical, chemical, and mechanical properties, 0D carbon dots (CDs) have attracted great interest of the chemistry and materials science communities in the last two decades.^[1] In more detail, there are three different subclasses of CDs concerning the proportion of sp²/sp³ carbons,^[2,3] namely carbonized polymer dots (CPDs), graphene quantum dots (GQDs), and carbon nanodots (CNDs).^[4] All these species are the focus of different research efforts, ranging from purely synthetic/structural to more application-oriented ones. CPDs are obtained by carbonizing polymer clusters, and the degree of carbonization plays an important role in forming their

polymer/carbon hybrid structure, which defines their properties.^[3] In addition to high photoluminescence quantum yields (PLQY), good aqueous solubility, and high oxygen/nitrogen contents, CPDs are unique in having short polymer chains, abundance of functional groups, and highly cross-linked network structures.^[5–7] GQDs consist of a graphene/graphite core, and edges that can be modified by functional groups, resulting in good solubility.^[8,9] Due to the quantum confinement effect, the fluorescence properties are unrivalled and can be adjusted for various applications.^[10] CNDs are quasi-spherical shaped nanoparticles, and their size is less than 10 nm.^[11] Furthermore, they show remarkable PL, solubility in aqueous solutions, and the toxicity has been reported to be low.^[12,13] A

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Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cnma.202300471>

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facile, fast, and inexpensive synthesis is the most significant benefit of CNDs.^[14] Different procedures to synthesize CNDs in a bottom-up fashion, starting from variable small molecule-based precursors, are well-studied.^[15–17] The bottom-up approach is commonly based on thermal,^[18] microwave(MW)-assisted,^[19] and hydro-/solvothermal treatments^[20] of smaller molecules. Bottom-up approaches make it easier to modulate the amount and variety of functional groups by using a wide range of small molecules as starting materials.^[14] Citric acid (CA), for example, predominantly resolves in oxygen-containing groups like carboxylic acids, aldehydes, hydroxyls, carbonyls, and epoxides.^[21–23] While small nitrogen-containing molecules, such as urea, arginine (Arg), or other amino acids, have been used to introduce amine groups into the CNDs.^[24,25] One of the reagents used to increase the amine content in the CNDs is also ethylenediamine (EDA).^[15,26] Further surface functionalization of the aforementioned functional groups with small molecules, polymers,^[27] ions, metals, DNA, or proteins is used to tune the physicochemical properties of CNDs and their potential applications, including sensing,^[28,29] catalysis,^[30,31] and optoelectronics.^[11,32,33] Due to their photobleaching resistance, chemical inertness, and variety of functional groups, CNDs fulfill the prerequisites for their use in optoelectronic applications.^[34] Since the degree of functionalization affects the optoelectronic properties of nanocarbon hybrid materials in general, e.g. fullerenes, graphene, and carbon nanotubes, it is expected that the amount of small molecules coupled to the CNDs changes the optoelectronic properties as well.^[35,36] Therefore, it is important to elucidate the type and amount of CND surface functionalization to accurately design their optoelectronic properties. In 2020, Prato et al. used the Kaiser test (KT) and imine functionalization with a fluorine-containing cinnamaldehyde derivative and subsequent quantitative ¹⁹F-NMR measurements to quantify primary amines on the surface of CNDs.^[30,37] The same authors compared the amounts of primary amines between the imine functionalization and KT, showing that the primary amine content is higher with the imine method. Bulkier reactants of KT are thought to result in lower accessibility of the primary amines. A common approach to functionalize primary and secondary amines of CNDs comprises the amide coupling due to the high bond strength of amides.^[38,39] Since bulky intermediates are present during the amide coupling of small molecules with CNDs, a low amine group accessibility needs to be considered.^[40] Other reactions, such as reductive amination, have recently been reported in a quantitative manner to determine the number of reactive amines.^[41] So far, suitable methods to quantify the primary and secondary amines accessible for amide coupling within CNDs are missing.

In this contribution, we propose a method to quantify accessible pendant primary and secondary amine groups in four different CNDs by amide coupling using fluorine-containing model systems. Through different stoichiometries of EDA as starting material, two previously unreported CNDs with varying amounts of primary amines are prepared. Nitrogen-rich CNDs are obtained through a MW-assisted carbonization treatment with two different ratios of Arg and EDA. At the same time, oxygen-rich CNDs were prepared similarly by employing

variable ratios of CA and EDA. This selection provides a broad distribution of cases, going from amine rich to amine poor CNDs. Our comprehensive investigation of the elemental composition and amine quantification provides valuable information about the reactivity and accessibility of the pendant functional groups in the bottom-up CNDs. In addition, from density functional theory (DFT) modeling, we propose that the source of PL in our CNDs is very likely due to small aggregates of molecular fluorophores (MFs).

Results and Discussion

Four different CNDs were synthesized from small molecules by MW-assisted reactions and purified through dialysis against Milli-Q water. Arg and EDA were used as precursors for one type of CNDs (Figure 1A), where two different molar ratios of Arg:EDA (1:1.2) (**Arg-1**) and Arg:EDA (1:2) (**Arg-2**) were considered. For the second type of CNDs, we used CA and EDA with molar ratios of CA:EDA (1:1) (**CA-1**) and CA:EDA (2:1) (**CA-2**), respectively. The starting molecules' nature and ratios were chosen to vary the amounts of surface functional groups, especially the accessible amines, in the prepared CNDs.

After MW-assisted synthesis, the actual presence and properties of the CNDs in the obtained products were investigated by dynamic light scattering (DLS), atomic force microscopy (AFM), UV-Vis absorbance, PL, infrared (IR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The synthesized CNDs' size ranged from 1 nm to 5 nm, as indicated by DLS (Figure S1) and AFM measurements (Figure 2, Figure S2–5). DLS and AFM characterization showed comparable results, with AFM being likely more reliable due to the difficulty in measuring DLS, as the intrinsic absorption and emission properties of the CNDs were too strong for this technique. Furthermore, for a valid measurement, the actual refractive index of the CNDs must be known, which was only assumed in our case.^[42] The size within the DLS is indeed underestimated, and the **CA-2** and **Arg-2** showed a more distinct size distribution, as it could be observed from AFM images. **CA-2** are the smallest CNDs obtained, with an AFM size of about 1.9 nm, whereas the **CA-1** CNDs were the biggest ones, with a size of about 4.4 nm. In addition, larger particles with higher amounts of EDA were observed in both cases. Transmission electron microscopy was not possible to measure with our CNDs, due to the lack of uniformity.^[17]

These CNDs absorbed light in the ultraviolet region of the electromagnetic spectra and emitted light in the visible blue range (Figure 1), with PLQYs between 3% and 12% (Figure S6). Table 1 shows the UV-Vis absorbance maxima, PL maxima, Stokes shift, PLQY, and mean size values of the four CNDs. A good reproducibility was found by comparing the measured absorbance and PL maxima values of **Arg-1** and **CA-1** with the literature.^[15,16] These two species are already reported in the literature, making a comparison feasible. In contrast, the **Arg-2** and **CA-2** species have been synthesized using MW-assisted carbonization and characterized here for the first time.^[43,44] These optical data demonstrated no significant

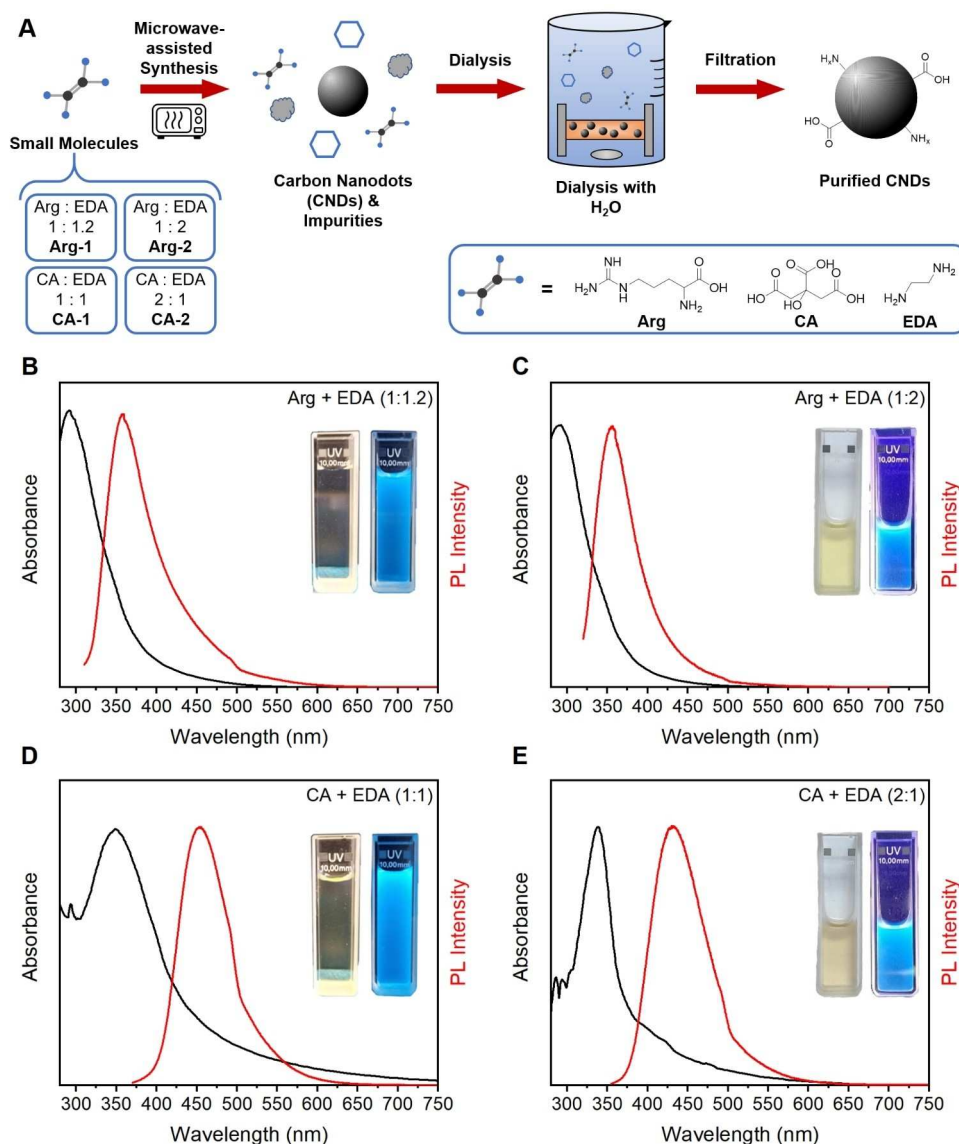


Figure 1. (A) The schematic process to obtain and purify CNDs using MW-assisted treatment of Arg, CA, and EDA. For clarity, four different CNDs were synthesized: one type with two ratios of Arg and EDA, and another with two ratios of CA and EDA. UV-Vis absorbance (black line) and photoluminescence (red line, $\lambda_{exc} = 290$ nm for Arg and $\lambda_{exc} = 340$ nm for CA) of (B) Arg-1 CNDs, (C) Arg-2 CNDs, (D) CA-1 CNDs, and (E) CA-2 CNDs measured in Milli-Q water. Insets: The left and right picture of the cuvette show the CND dispersion under day light and UV light (366 nm), respectively.

change in absorption and emission properties for the two Arg-derived samples. Nevertheless, the two CA-derived CNDs show a considerably different PLQY, with the newly proposed system (CA-2) having a four-fold increase in this value, accompanied by

a 10 nm hypsochromic shift of the absorption and a higher (~20 nm) analogous shift in the PL maximum. Due to their smaller size, a blue shift goes along and reasonably causes the higher PLQY in the CA-2 sample.

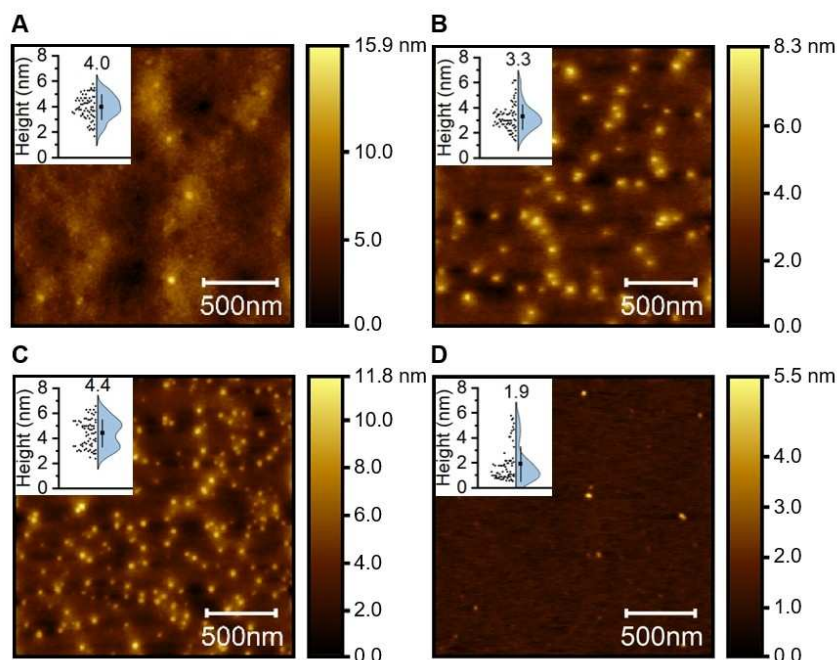


Figure 2. 2 $\mu\text{m} \times 2 \mu\text{m}$ AFM images of (A) Arg-2 (B) Arg-1 (C) CA-1 (D) CA-2 CNDs on a mica substrate. The insets in each figure show the height distribution for each sample, with the mean height and the standard deviation obtained from ~ 70 CNDs.

Table 1. Measured UV-Vis absorbance (λ_{Abs}) and PL (λ_{Em}) maxima compared to literature values (where available), together with the according Stokes shifts, PLQY, and mean size with standard deviation obtained from AFM of the four different CNDs. ^{15,17}							
CNDs	$\lambda_{\text{Abs}}/\text{nm}$	Lit. ($\lambda_{\text{Abs}})/\text{nm}$	$\lambda_{\text{Em}}/\text{nm}$	Lit. ($\lambda_{\text{Em}})/\text{nm}$	Stokes shift/nm	PLQY/%	Size/nm
Arg-2	290		356		66	8	4.0 ± 1.0
Arg-1	290	285	358	356	68	7	3.3 ± 1.0
CA-1	348	340	453	450	105	3	4.4 ± 1.1
CA-2	338		431		93	12	1.9 ± 1.4

Elemental composition, quantity, and type of functional groups on the CNDs were first analyzed by XPS and Fourier-transform infrared spectroscopy (FT-IR). From the detailed C1s XPS spectra of the CNDs (Figure 3A), the contribution of oxygenated carbon species (highlighted in red) was lower compared to the starting materials, most likely due to the loss of CO_x , H_2O , and N-based compounds (i.e., NH_3 and NO_x) during the MW heating process (carbonization process).^[45] In addition, the formation of amide bonds was observed with the addition of EDA. The binding energies of the carboxylic acids shifted to slightly lower values of about 288.7 eV (highlighted in red) compared to the starting molecules. Due to the variety of functional groups, a clear denotation and the exact specification of the proportions was too difficult. At the binding energy of 287.7 eV carbonyl, epoxy, and guanidine-like moieties (as in

arginine), can be observed. Between the binding energies of 286.5 eV and 285.7 eV the single-bonded C-N and C-O species, like ether, alcohols, and amines, were found. In the N1s spectra, a shift towards primary amine features with lower binding energies was observed in samples containing higher EDA amounts. In the O1s region of the spectra, a higher amount of EDA resulted in broader signals due to a more diverse chemical environment of the oxygen-containing functional groups.

Primary amines were identified by comparing the IR spectra of the CNDs in the regions around 2900 cm^{-1} with the ones of the precursors (Figure S7). Upon addition of EDA, the increase in the intensity of the amide I (1645 cm^{-1}) and amide II (1545 cm^{-1}) regions indicated a possible reaction pathway between amine from EDA and carboxylic acids from CA or Arg.^[46,47] The amide regions are associated with the CO and CN

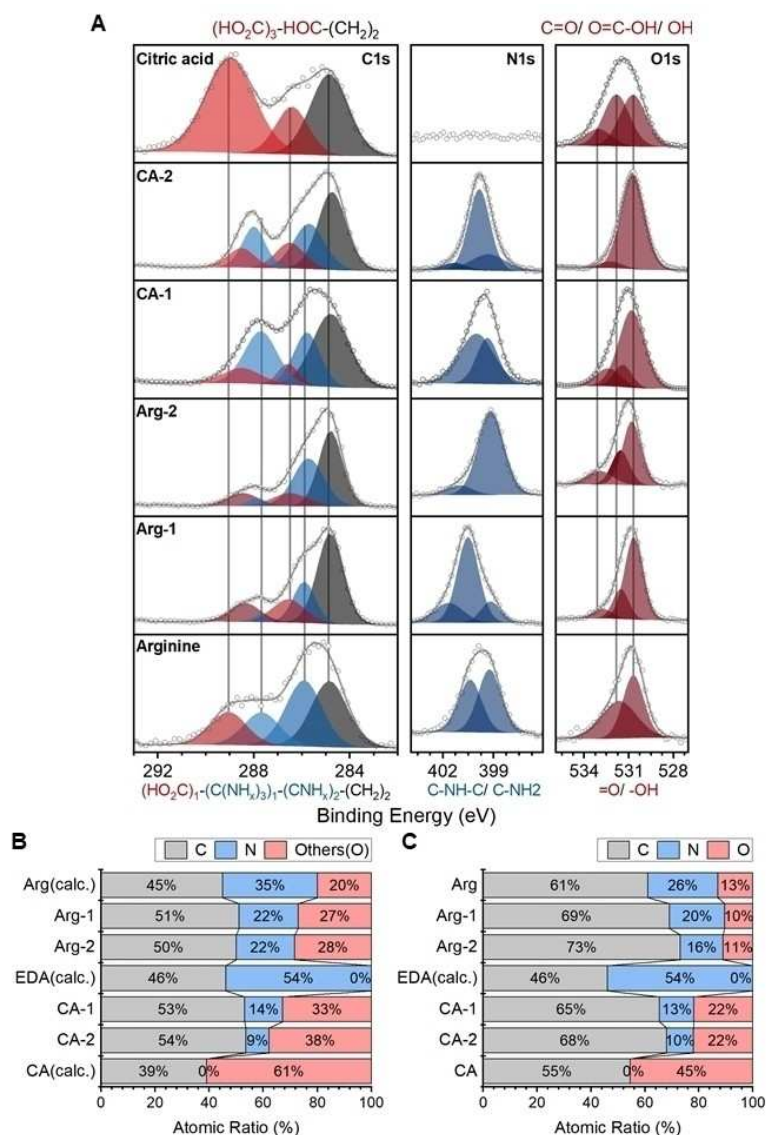


Figure 3. (A) Detailed C1s, N1s, and O1s XPS spectra of the four synthesized CNDs in comparison with pristine CA and Arg, and their mathematical decomposition. The underlying contributions to the spectra of precursors are labelled with the chemical formula below and above, respectively. Aliphatic carbon features are highlighted in black, nitrogen-related in blue and oxygen-related in red, for the sake of clarity. (B) EA data for the four synthesized CNDs compared with the theoretical atomic ratios of the starting materials. (C) Atomic composition of the CNDs, CA, and Arg derived by XPS spectra compared with the theoretical atomic ratios of EDA.

stretching vibration and NH in-plane bending displacement in different proportions. Higher amounts of CA resulted in a more pronounced $\nu(\text{C=O})$ stretching vibration at around 1700 cm^{-1} ,

indicating a higher carboxylic acid content. The $\nu(\text{N-H})$ stretching vibration (2900 cm^{-1}) increased with the addition of EDA, whereas at the same time, the $\nu(\text{C=O})$ stretching vibration

decreased. This suggests a higher fraction of amine functional groups with increasing EDA amounts and a higher fraction of carboxylic acids with higher amounts of CA, which is in accordance with the XPS detailed spectra.

An augmented amount of CA resulted in a lower amount of nitrogen and a higher amount of oxygen. This was observed in the atomic compositions obtained from elemental analysis (EA) and XPS (Figure 3). With increasing EDA amount, the atomic composition was not significantly changed, even though the physical methods like XPS and IR suggested different relative quantities of functional groups. The EA analysis showed that the amounts of hydrogen and oxygen are higher than what could be predicted (Figure S8). Since the CNDs are hygroscopic and moisture during the measurement cannot be ruled out, the discussed values are high, most likely due to the presence of residual water, although a careful vacuum drying of the samples was carried out prior to the analysis. In the elemental quantitative information derived from XPS, it was noticeable that the carbon ratio is higher than that derived from classical EA, likely due to adventitious carbon present in the former case (as it is typical during XPS experiments).^[48] Since XPS detected no elements other than oxygen, carbon, and nitrogen, we assumed that the percentages of other elements in the sample correspond primarily to oxygen in the EA. Nevertheless, the elemental trends in the EA and XPS chemical characterization as a function of the precursors' ratio variation are comparable and show similar behavior. By comparing the starting materials with the CNDs, it was possible to detect a clear change and reduced heteroatom contents after the MW-assisted reaction.

Acid-base back titrations were carried out to estimate the total amount of pH-sensitive functional groups present and accessible in the CNDs. First, all functional groups with acidic character were basified with sodium hydroxide, and back titration with hydrochloric acid was performed, thus getting access to all basic and acidic functionalities. By applying the Gran plot,^[49,50] it was possible to identify the precise values of the equivalent points, which are directly proportional to the number of functional groups. The number of acid-base sites was quantified by back titration and using the Gran plot analysis (Figure S9–S12).^[50] The highest acid-base site quantities were observed within the **Arg-2** CNDs, followed by the **CA-2** CNDs (Figure 4A). Considering the isoelectric point (pI) of **CA-2** at pH 4.0, the sample must have a high amount of carboxylic acids or other acidic moieties (Figure S13). The higher pI at pH 9.4 for the **Arg-1** CNDs indicated predominantly basic moieties. **Arg-2** instead showed the pI at pH 7.6, suggesting nitrogen heterocycles with typically low pKa values. For comparison, the pI of arginine is at pH 10.76 and for proline at pH 6.3.^[51] This correlates well with the lower proportion of basic amine functional groups in this species found by the other chemical analysis methods.

Thermogravimetric analysis (TGA) was performed for all samples in air and nitrogen up to 900 °C (Figure S14). The thermograms can be divided into three general regions: (I) From 100 °C to 300 °C, an initial thermal decomposition of thermolabile functional groups was observed, (II) the mass loss from 300 °C to 600 °C (in N₂) indicated the decomposition of

stable functional groups present in the bulk or multiple C-hetero atom bonds, (III) the decay from 450 °C to 650 °C (in air) is related to the combustion of condensed carbonaceous structures. **CA-2** showed a higher mass loss in the first region than the **CA-1** CNDs, indicating a larger amount of functional groups. For Arg CNDs, the loss of thermolabile groups started earlier than for CA CNDs, and in general, the loss in this region was more significant. **Arg-2** showed the highest loss among all four samples. Thermolabile functional groups decomposing between 100 °C and 300 °C followed the same tendency of the acid-base functional group content obtained through back titration. Regarding the morphology, one can observe higher degrees of carbonized residues for the CA CNDs in the TGA analysis measured in an inert atmosphere. This reflects the visual appearance of the dark brown CA CNDs compared to the orange-to-brownish Arg CNDs (Figure S15). In addition, the Arg CNDs showed lower decomposition temperatures. Thus, they are less thermally stable than the CA CNDs.

Taking the ¹H-NMR spectra (Figure S16) of the CNDs into account, one can observe higher amount of amine-related protons (1 ppm–2.5 ppm) in the Arg CNDs, which is less with decreasing EDA amount. Aliphatic protons (2 ppm–4.5 ppm) are the main contributors in both the CA and Arg CNDs. Aromatic protons are barely visible in both CND species, confirmed by the ¹³C{¹H}-NMR, DEPT-90, and DEPT-135 (Figure S17–19). The CA CNDs show only quaternary carbon signals in the regions 70 ppm–80 ppm and 150 ppm–190 ppm. In the first region are allocated resonances due to carbon-heteroatom bonds, while in the second one those due to aromatic carbons and highly oxidized carbon atoms near heteroatoms. Since both carbon species are most likely present in our CNDs, it is difficult to address the carbon signals accurately. DEPT-135 and DEPT-90 (Figure S18) show that the aliphatic carbons (40 ppm–50 ppm) are tertiary and shift slightly downfield, possibly due to adjacent heteroatoms. In comparison, **Arg-1** CNDs predominantly consist of secondary carbon atoms in the aliphatic region (20 ppm–50 ppm), which corresponds with the differences in the chemical nature of the starting materials (Figure S19). In DEPT-90, the carbon signals in the 20 ppm–50 ppm range are artifacts, since they exhibit the same shape but lower intensity as in the DEPT-135. These signals are observed in the opposite phase because of their high intensity.

Arg contains secondary carbon atoms, while CA does not. Only EDA contributes secondary carbons to the CA-based CNDs, and considering the higher degree of carbonization, it is reasonable that we cannot observe secondary carbons in those. In the region 50 ppm–70 ppm of the **Arg-1** CNDs, one can observe tertiary carbons in close proximity to heteroatoms. In the aromatic/highly oxidized carbon region, a few tertiary carbons are observable. Still, most of them are quaternary as well, which is again in good agreement with the lower degree of carbonization of the Arg-based CNDs.

To probe the presence and amount of accessible primary amines, KT was employed. When the results of KT were compared with the EA, it appeared evident that, even with a similar amount of nitrogen, the **Arg-2** CNDs show the highest accessible primary amine content of 1900 μmol·g^{−1} (Figure 4).

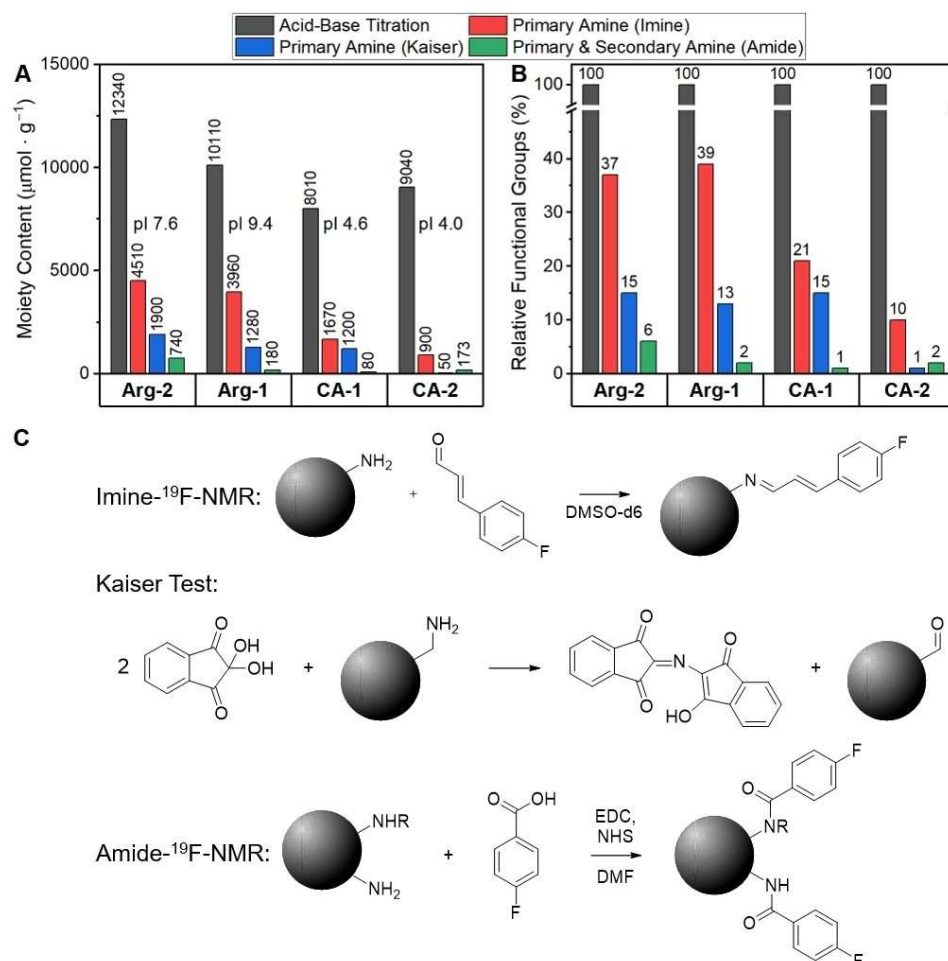


Figure 4. (A) Accessible surface functional groups determined through different methodologies. Total acid-base groups by back-titration, primary amine by KT, primary amine by quantitative ^{19}F -NMR of imine functionalized, and primary and secondary amine by quantitative ^{19}F NMR after the amide functionalization. (B) Functional group content relative to the total amount of acid-base groups. (C) The reaction schemes of KT, imine, and amide couplings are depicted below for the sake of clarity.

Consistent with this behavior, it has been reported in the literature, using carbon isotope-labeled starting materials and subsequent ^{13}C -NMR studies, that EDA is the major contributor to the shell of the CNDs, while Arg is mainly responsible for the core.^[15] Therefore, the amount of accessible functional groups will change with the amount of EDA added, even though the overall amount of nitrogen remains similar. This resulted in 33% (in molar fraction) more primary amines in **Arg-2** CNDs than in the **Arg-1** CNDs. The amount of EDA precursor employed is 39% more in **Arg-2** than in **Arg-1**, consistent with the detected higher primary amine content of the former

compared to the latter. The obtained accessible primary amine amount of the **Arg-1** CNDs was almost identical to the reported literature value of $1350 \mu\text{mol} \cdot \text{g}^{-1}$.^[30] The EDA precursor amount used for the synthesis of the **CA-1** CNDs in comparison to the **Arg-1** CNDs is 23% lower but leads to only 7% lower content of primary amines ($1200 \mu\text{mol} \cdot \text{g}^{-1}$). This suggests that either more primary amine groups can be found on the **CA-1** CNDs, or they are more accessible for the KT reagents than in the **Arg-1** CNDs. However, when the amount of EDA was halved, the amine content dropped to $50 \mu\text{mol} \cdot \text{g}^{-1}$ for the **CA-2** CNDs, which was 96% lower than the **CA-1** CNDs. As a result, EDA

induced different product formations for the resulting CNDs when changing the ratio of the starting materials. On the other hand, when more EDA was used to prepare the Arg-based CNDs, the quantity of primary amines increased rather linearly compared to the CA-based CNDs.

Since it was shown that KT cannot access the total amount of primary amines due to a possible hindrance effect, the CNDs were chemically functionalized with 4-fluorocinnamaldehyde to form an imine.^[30] Followed by quantitative ^{19}F -NMR measurements with an internal standard, this method enabled fluorine functionalized primary amine groups to be quantified. A value of $3960\ \mu\text{mol}\cdot\text{g}^{-1}$, for **Arg-1**, showed good agreement with the literature value of $4100\ \mu\text{mol}\cdot\text{g}^{-1}$. In our work, we also found higher values for the imine functionalization compared to KT and a similar trend from high to low EDA contents (Figure 4A). In the case of the CA CNDs, the quantity of primary amines obtained from the KT and the imine functionalization showed a lower deviation than the other CNDs, suggesting a higher accessibility of the moieties. The primary amine content of the CA-2 CNDs from the imine functionalization was over ten times higher than that of the KT result. This indicates that, although the precursors used are the same (only the relative ratio varies), different products are likely to be formed during the synthesis of CA-CNDs. In agreement with the literature, EDA changed the surface composition of the CNDs and, therefore, the content of accessible functional groups as well.^[15]

Amide bonds were chosen for the future surface functionalization of the CNDs due to their higher bond strength compared to imines, and the utilization of amide coupling was initially studied by quantitative ^{19}F NMR. The primary and secondary amine groups were functionalized through a peptide coupling reaction with 4-fluorobenzoic acid in the presence of EDC and NHS (see details in the experimental section) (Figure 4C). To prevent self-cross-linking of the CNDs, they were added to the reaction mixture after activation of the 4-fluorobenzoic acid with EDC. After extracting the coupling product, the ^{19}F -NMR was measured in DMSO- d_6 with 4,4'-difluorobenzophenone as an internal standard. Contrary to expectation, the primary and secondary amine content followed a similar trend to the back titration. The highest degree of surface functionalization was obtained with the **Arg-2** CNDs, probably due to a higher reactivity and accessibility of amine groups in the Arg CNDs. Considering the back-titration access to the total acid-base moieties, the CNDs can be functionalized in the 1% to 6% range by amide coupling, under the conditions studied herein. Assuming that the surface functionalization of the primary amines is fully effective during the imine reaction, the maximum degree of surface functionalization of the acid-base sites is 39% for the **Arg-1** and 21% for the **CA-1** CNDs. In this comparison, it is necessary to consider the different starting materials of the coupling reactions since 4-fluorocinnamaldehyde has an alkene chain with three carbons between the reacting aldehyde and the ring. On the other hand, the reacting carboxylic acid is located directly on the benzene ring of the 4-fluorobenzoic acid, and the acid is activated by EDC, which is a bulky molecule.^[40] It seems to make no difference that the secondary amines can be additionally functionalized with the

amide coupling. Most likely, this is due to the lower activity and accessibility of secondary amines compared to primary amines. Nevertheless, the amide coupling with 4-fluorobenzoic acid better reflects the surface functionalization that could be carried out with other bulky molecules, for example, to produce dye-CND hybrids useful for optoelectronic applications, and one can expect similar degrees of surface functionalization in such cases.

To understand the relationship between the unique structure and optical properties of our CNDs, (time-dependent) density functional theory ((TD)-DFT) calculations were performed to study the electronic structure and to compute absorption/emission spectra of CNDs. Unfortunately, the structure-photoluminescence relationship of CNDs is yet to be established, thus, it is challenging to unambiguously report the source of PL in any CDs synthesized by hydrothermal treatment. Therefore, our modelling was based on *ad-hoc* structures which could be possibly formed based on the synthetic protocols/reports in the literature. These model structures have compositions as close as possible to the experimental XPS data. However, the models do not perfectly correspond to the experimental composition, due to the inherent complexity of the studied systems. Thus, with the selected models we aim to separate the complex CNDs composition into different parts, considering the system's size, its nitrogen/oxygen content and the possibility of molecular fluorophore assembly as reliable building blocks. Starting with **Arg-1** CNDs, three families of model systems (Figure 5) were designed based on: I) available experimental information ('large' model), II) data in the literature ('medium' model),^[52] and III) possible formation of small organic molecular fluorophores (MFs) during the synthesis of CDs ('MF' model).^[53,54]

Our calculations demonstrated that among the three selected models, only those based on MFs were in good agreement with the experimental data, while the other two led to a strong red shift in the absorption spectra (Figure 5, Table S1), implying that the PL would be in the IR region. In particular, the calculated vertical excitations of the 'large' model demonstrated that the first excited state S_1 , lying at 941 nm, is strongly red shifted compared to experiments ($\Delta\lambda = 651\ \text{nm}$, $f = 0.021$), implying that the experimentally measured absorption at 290 nm does not originate from large N, O-functionalized N-doped polycyclic aromatic hydrocarbons. It shall be noted that besides surface functional groups, graphitic nitrogens were also considered to achieve higher N-doping in our model. Although it has been reported that graphitic nitrogen red-shifts the absorption spectra^[55], we observed that the 'large' model with no N-doping of the aromatic core also exhibits the red-shift in the absorption spectrum compared to the experimental data (Figure S20). Similarly, a large red-shift of 305 nm was observed for the 'medium' model, with $S_0 \rightarrow S_1$ transition peaking at 595 nm, even though this exact model structure was suggested in the literature as the source of PL of Arg-CNDs.^[52] On the other hand, when the arbitrary model of Arg-MF, constructed based on the possibility of a synthetic reaction to occur under high temperature conditions in the complex, is considered as a constituent for CNDs, the $S_0 \rightarrow S_1$ excitonic

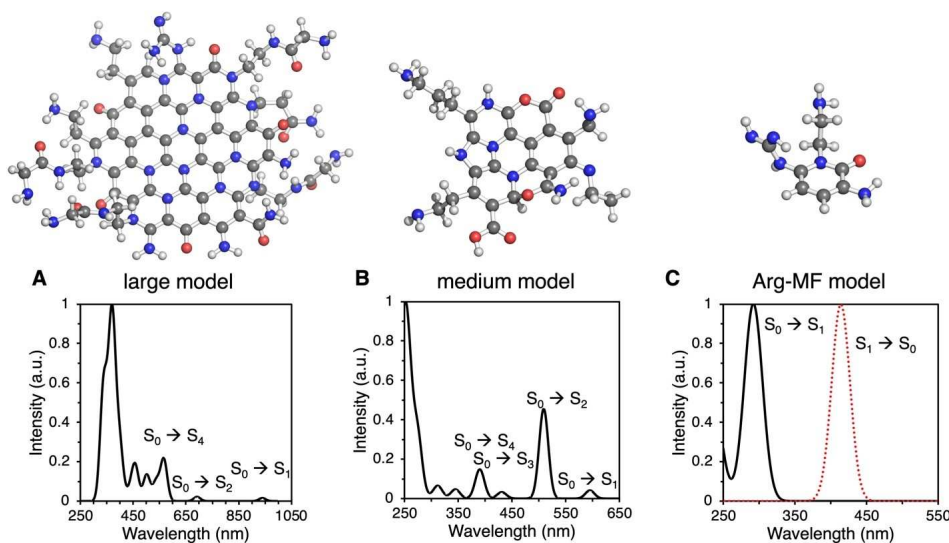


Figure 5. Top panel: structures of the different families of model systems considered for the (TD-)DFT calculations. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white. Bottom panel: Calculated absorption (black) and emission (red) spectra of (A) large, (B) medium, (C) Arg-MF models. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm.

transition (Figure S22) has been found to have the first bright absorption peak at 292 nm (Figure 5, Table S1), which differs only by 2 nm from the experimental data. In addition, its emission is red-shifted by 56 nm compared to experimental data (414 nm vs. measured 358 nm). Comparing these three families of models, it could be rationalized that MFs can be used to also obtain CNDs synthesized in the condition of high temperature, but not high enough to ensure complete carbonization, which may not be the general rule in CNDs, but we believe it occurs in our synthesized CNDs.^[3,56]

Nevertheless, this Arg-MF model is too small to account for the size distribution of CNDs reported in Figure 2 (Figure S1–S5). Therefore, additional computations were performed considering an extended model in which one (Arg-1-MF-a) or two (Arg-1-MF-b) more molecules of Arg and EDA were covalently linked together with a short aliphatic chain (Figure 6). Interestingly, increasing the size of the Arg-MF model has a negligible effect on their absorption and emission spectra (Figure 6A, B, Table S2), as the bright absorption peak corresponding to the first (second) excited state lies at 308 nm (293 nm) for Arg-1-MF-a (Arg-1-MF-b). This is due to the fact that these transitions can be described as $\pi\text{-}\pi^*$, both localized on the aromatic cores (Figure S23), which are in turn separated by a short aliphatic chain. To further strengthen our idea of CNDs made from MFs, MD simulations with five non-covalently bound Arg-1-MF-b molecules were carried out.

Our results demonstrated that these molecules formed a stable assembly (Figure S24), where the average Coulomb and Lennard-Jones interaction between these fluorophores is

−41.8 kcal·mol^{−1} and −42.7 kcal·mol^{−1}, respectively. The calculated radius of gyration averaged over the entire simulation trajectory has a value of 1.1 nm, which agrees well with the measured hydrodynamic diameter (Figure S1). The bimodal broad distribution of this plot suggests that our assemblies may be highly dynamic.

Additionally, the calculated excitation and emission values for Arg-2-MF model showed no significant shifts compared to the above considered Arg-1-MF models (Figure 6C).

This arbitrary model is used to rationalize both absorption and emission spectra of the Arg-2 CNDs, as it contains an additional EDA molecule covalently bound to the part connecting two aromatic arginine cycles, thus representing the higher 1:2 ratio of Arg:EDA present in Arg-2. Adding the EDA molecule in the Arg-2-MF model increases the number of amine groups in the Arg-CNDs, which agrees with the KT experiments (Figure 4A).

Considering that out of three different families the small MF models performed the best in reproducing the experimental optical data of the Arg:EDA CNDs, the MF based models were also applied to the CA:EDA CNDs. Moreover, it has been accepted in the literature that a MF called 5-oxo-1,2,3,5-tetrahydroimidazo-[1,2-*a*]-pyridine-7-carboxylic acid (IPCA) (Figure 7) is formed during the high-temperature synthesis used for CNDs of about 200 °C.^[57–59] Our theoretical calculations of IPCA agreed well with literature,^[60] showing that the first absorption peak lies at 346 nm and emission at 432 nm, i.e., 2 nm blue-shift and 21 nm blue-shift, respectively, in comparison with the experimental value of CA-1 CNDs (Figure 7A,

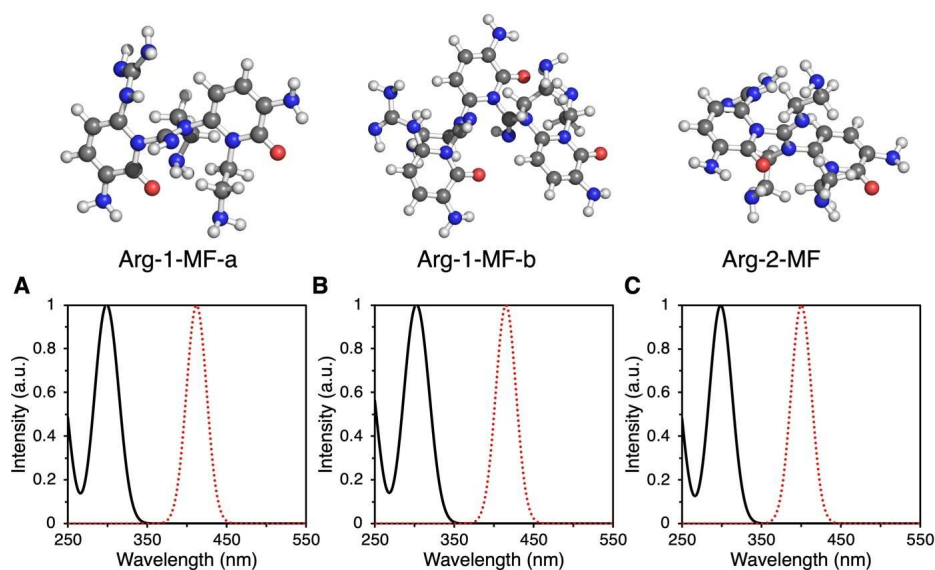


Figure 6. Top panel: Three different structure models based on small organic molecules, which explain the optical properties of Arg:EDA CNDs. The Arg-MF model can be further extended to dimers (Arg-1-MF-a) and trimers (Arg-1-MF-b), with similar absorption/emission agreeing with the measured experimental spectra. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white. Bottom panel: Calculated absorption (black) and emission (red) spectra of (A) Arg-1-MF-a, (B) Arg-1-MF-b, (C) Arg-2-MF models. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm.

Table S3). Thus, for the synthesized **CA-1** CNDs, the origin behind the PL could be explained by the presence of aggregates of MF IPCA in the sample.^[61,62] On the other hand, computational models based on covalently bonded IPCAs (CA-2-MF-a) and a model composed of short oligomers (CA-2-MF-b) were considered for the **CA-2** CND, as we have there is more CA than EDA groups. Both these **CA-2** models demonstrated excellent agreement with experiments, where the absorption (emission) peak of the CA-2-MF-a model at 336 nm (430 nm) exhibited a negligible 2 nm (1 nm) blue shift compared to the experimental data (Figure 7B, Table S3, Figure S26). Moreover, our computations with the CA-2-MF-b model showed that **CA-2** CNDs could be composed of small oligomers with covalently attached IPCAs (Figure S27), being the source of PL, as the first absorption peak corresponding to $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ vertical transitions is blue shifted from the experimental data by 19 nm. These transitions are excitations localized on the IPCA part of the oligomer (Figure 7C, Table S3, Figure S28).

The explanation of optical properties based on MFs is supported by the fact that during the dialysis purification of the CNDs, high fluorescence of the dialysate under UV irradiation was observed (Figure S30); additionally, in the publication by Baker et al.^[63] it was shown that the quantum yield decreases with further purification of the CNDs, which is attributed to highly fluorescent small molecules entering the dialysate during purification. Nevertheless, we would like to once again stress

that the source of PL in CNDs is a difficult task to tackle, and the theoretical models shall be considered as a simplification of these complex systems.

Conclusions

We report the synthesis, detailed characterization, and quantification of accessible functional groups of two novel and two previously reported bottom-up CNDs obtained by MW-assisted synthesis. Through the variation of the starting material and the addition of different amounts of EDA, we were able to tune the quantity and type of functional groups. IR, XPS, and EA indicate changes of the functional group amount with varying EDA quantities. The functional groups were in depth quantified by KT, acid-base back titrations, and surface functionalization with fluorine containing model systems with subsequent quantitative ^{19}F -NMR measurements.

We used a modified method to determine the degree of surface functionalization after the amide coupling of small molecules with CNDs. We show that a functionalization from 1% to 6% can be achieved with the amide coupling, even though the amine content is probed to be higher. Moreover, the CNDs do not follow the expected trend from the KT or imine functionalization for the functionalization by amide coupling. This demonstrates that the amount of amine groups

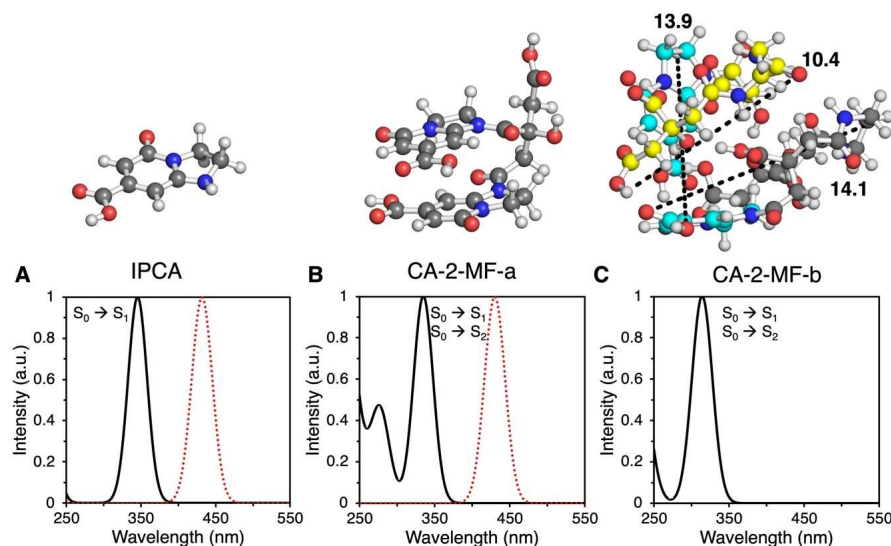


Figure 7. Top panel: Structure models based on small organic molecules, which explain the optical properties of CA:EDA CNDs. Coloring: carbons-grey, cyan, yellow; nitrogens-blue; oxygens-red; hydrogens-white. For CA-2-MF-b model, carbons of two non-bonded short oligomers are colored grey and yellow. The carbons in cyan represent IPCA covalently bonded to the oligomer. Distances are displayed in Å. Bottom panel: Calculated absorption (black) and emission (red) spectra of (A) IPCA, (B) CA-2-MF-a, (C) CA-2-MF-b models. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm. Due to the size of the system, def2 SVP basis set was used for CA-2-MF-b model, which could lead to a blue shift of around 10 nm (see the footnote in Table S3).

cannot be used as a direct measure for determining the potential for covalent surface functionalization of CNDs. Determining the degree of accessibility serves as a tool for targeted surface functionalization to produce precisely tailored optoelectronic materials. CNDs' accessibility and reactivity, the coupling reaction, and the molecule used to functionalize the CNDs, strongly influence the degree of surface functionalization.

In addition, we used different model systems and computed their optical properties. The calculated spectra based on simplified models of MFs and the related oligomers show great agreement with the experimental data, while for larger aromatic systems the calculations result in a strong red shifted absorption and emission. This indirectly validates the assumption that the formation of small molecules during the synthesis of the CNDs is the main cause of the photophysical properties in these still elusive nanocarbons.

Materials and Methods

D/L-Arginine (Arg, $\geq 98.5\%$), citric acid (CA, $\geq 99.5\%$), 3-(((ethylimino)methylidene)amino)-N, N-dimethylpropan-1-amine hydrochloride (EDC, $\geq 99\%$), and ethylenediamine (EDA, $\geq 99.5\%$) were purchased from Carl-Roth. 4,4'-difluorobenzophenone (99%), N-hydroxysuccinimide (NHS, $\geq 99\%$) were purchased from Sigma-Aldrich. 4-fluorobenzonitrile (99.0%) were purchased from Fluorochem. Deuterated dimethyl sulfoxide (DMSO- d_6 , 99.8%) were purchased from Deutero. Anhydrous N, N-dimethylformamide (DMF, 99.8%, AcroSeal) was purchased from Thermo Scientific. Dimethylsulfoxide (DMSO, 99.5%) was purchased from Gruessing. Ultrapure water was obtained with the Milli-Q Direct Water Purification System. Unless otherwise stated, the reagents were used without further purification. The MW-assisted reactions were performed in a Silvia homeline and CEM Discover-SP MW set-ups. The purification of CNDs was performed with Spectra/Por Biotech-Grade Cellulose Ester (CE) dialysis tubing with a molecular weight cut-off (MWCO) of 500 Da–1000 Da. NMR-spectra were recorded with the Bruker Avance II 400 MHz, Bruker Avance III 400 MHz HD, Bruker Avance III HD 600 MHz, and Bruker Avance NEO 700 MHz. ^{13}C -NMR spectra in D_2O were calibrated to the external standard trimethylsilylpropionic acid. XPS measurements were conducted with a PHI 5000 VersaProbe IV Scanning ESCA Microprobe (Physical Electronics) with monochromatized Al K_{α} 1486.6 eV X-ray source (beam diameter 100 μm , X-ray power: 25 W, X-ray Voltage: 15 kV). Samples were prepared by filling a sample cap made of Teflon and

attaching them to the XPS sample holder using an isolating tape. Time steps of 100 ms, a step size of 0.2 eV, and an analyzer pass energy of 27 eV were used for measuring the detail spectra. For every detailed region 30 sweeps or a P/N ratio equal to 180, were set. For the general survey a time per step of 50 ms, analyzer pass energy of 224 eV, and a step size of 0.8 eV, were used. The sample surface was charge neutralized with slow electrons and argon ions, and the pressure was in the range from 10^{-7} Pa to 10^{-6} Pa during the measurement. Data analysis was performed using the CasaXPS software.^[64] All of the spectra were calibrated using C1s signal located at 284.8 eV, associated to C–C bond and the signals were decomposed using GL(30) function. DLS and zeta potential measurements were conducted with a Malvern Zetasizer Nano-ZS device (Malvern, U.K.) at 20 °C. The system was operated by the Zetasizer software from Malvern Panlytical (Malvern, U.K.). UV-Vis measurements were conducted with the Bio-Tek Uvikon XS Spectrophotometer and the system was operated with the software UV VISION Lite. Fluorescence measurements were conducted with a Jasco FP-8300 Fluorescence Spectrometer. EA (CHN) was performed with the Thermo FlashEA-1112 Series equipment. Back titrations were conducted with the TitroLine 7000 from SI Analytics. ATR-IR was performed on a VERTEX70/ Platinum ATR (Fa. Bruker Optics) with a minimal resolution of 0.2 cm^{-1} . AFM was performed with the AIST-NT SmartSPM 1000 under ambient conditions in AC mode. AFM probe (SHR150 probe, BudgetSensors', single hydrophobic diamond-like carbon extratip at the apex of Gold coated silicon etched probe, 150 kHz, 5 N m^{-1}) Data analysis was performed with Gwyddion software.^[65] Thermogravimetric measurements were carried out on a TGA Q5000IR (TA instruments, New Castle, DE, USA). The analyses were performed using a 100 μL Platinum-HT TAG pan. In order to remove adsorbed moisture, each sample was subjected to a sequence of isotherms under N_2 (at 60 °C for 10 minutes, then at 80 °C for 3 minutes and then at 100 °C for 2 minutes), immediately before the measurement ramp (10 °C $\cdot$ min $^{-1}$ from 100 °C to 900 °C) either under N_2 atmosphere or under air atmosphere.

CNDs Synthesis

Arg + EDA

For the sake of clarity, the details of the already reported and slightly modified method reported by Prato et al.^[52] are given here. In a MW vessel, Arg (87.4 mg, 0.502 mmol, 1 eq) was dispersed in 100 μL Milli-Q water and EDA (Arg-1: 33 μL , 0.61 mmol, 1.2 eq; (Arg-2: 67 μL , 1.0 mmol, 2 eq) was added. With an added stirring bar, the reaction mixture was stirred for 30 s at 300 rpm on a magnetic stir plate. The vessel was placed in the MW reactor (CEM Discover SP) with 12 power cycles including a power interval of 200 W for 15 s and a cooling interval of 5 s. Overall reaction time was 180 s. The temperature was limited between 240 °C and 250 °C. The obtained brown resin was dispersed in 3 mL Milli-Q water and filtered over a 0.2 μm PTFE syringe filter.

CA + EDA

For the sake of clarity, the details of the already reported and slightly modified method reported by Pham et al.^[16] are given here. CA (1.4410 g, 7.5004 mmol, 1 eq) was dissolved in 5 mL Milli-Q water and was stirred until everything was dissolved. EDA (CA-1: 0.50 mL, 7.5 mmol, 1 eq; (CA-2: 0.25 mL, 3.8 mmol, 0.5 eq) was added and the mixture was stirred for 20 minutes. The reaction mixture was heated in a domestic MW oven for five minutes at 400 W (50 % power). The obtained brown resin was dispersed in

10 mL Milli-Q water and the brown dispersion was filtered over a 0.2 μm PTFE syringe filter.

The filtered dispersions of all the CNDs were filled up to a volume of 10 mL and dialyzed in a 0.5 kDa–1 kDa dialysis membrane against Milli-Q water for 48 hours with water changed every two hours during the day. The obtained brown dispersion was lyophilized and an orange/ brown solid was obtained (Figure S15).

^1H -NMR for each CND species showed broad signals to ensure the presence of CNDs (Figure S16).^[66] $^{13}\text{C}\{^1\text{H}\}$ -NMR together with DEPT-90 and DEPT-135 was conducted in D_2O -d2 for the CA-1 and Arg-1 CNDs (Figure S17–S19). Due to the highly demanding measurement of the inverse gated $^{13}\text{C}\{^1\text{H}\}$ -NMR spectra we refrained from measuring the Arg-2 and CA-2.

UV-Vis Absorbance and Photoluminescence

A concentration of 10 $\text{mg} \cdot \text{L}^{-1}$ in Milli-Q water was used for the UV-Vis absorbance and photoluminescence measurements. The respective absorption maximum was selected as the excitation wavelength for the PL measurement for each CND species.

Amide Coupling of CNDs with 4-fluorobenzoic acid

4-Fluorobenzoic acid (41.3 mg, 0.295 mmol, 1 eq) was dissolved in 20 mL anhydrous DMF. EDC (111.3 mg, 0.5806 mmol, 2 eq) and NHS (65.4 mg, 0.568 mmol, 2 eq) were added and the mixture was stirred for 15 minutes at room temperature. CNDs (25.2 mg) were finally added and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the obtained crude was dispersed in 10 mL aqueous hydrochloric acid (0.1 M). The mixture was washed three times with chloroform and the obtained aqueous phase was dialyzed in a 0.5 kDa–1 kDa MWCO dialysis tube against Milli-Q water for 24 hours with water changed every two hours during the day. The obtained brown dispersion was lyophilized and an orange/brown solid was obtained.

Imine Formation of CNDs with trans-4-fluorocinnamaldehyde

CNDs (10.3 mg) were dispersed in 0.6 mL deuterated DMSO and trans-4-fluorocinnamaldehyde (18.3 mL, 0.140 mmol) was added. The reaction mixture was stirred overnight at room temperature and directly analyzed.^[30]

Kaiser Test

Four different solutions were prepared for the modified Kaiser test.^[67]

1. Ninhydrine solution: 2.5 g of ninhydrine is dissolved in 50 mL of absolute ethanol.
2. KCN solution: 2 mL of 0.03 M aqueous KCN solution is diluted to 100 mL with freshly distilled pyridine.
3. Phenol solution: 40 g of phenol is dissolved in 10 mL ethanol by gently heating the solution until it is dissolved.
4. Ethanol solution: 60 mL ethanol are diluted with 40 mL of Milli-Q water.

In a 4 mL screw cap vial around 1 mg of CNDs were placed together with 75 μL of the phenol solution, 100 μL of the KCN solution, and the mixture was sonicated for two minutes. Then 75 μL of the ninhydrine solution was added and the mixture was stirred at 120 °C for 10 minutes. After cooling to room temperature, the reaction mixture was diluted with 32 mL of the ethanol solution

and a UV-Vis absorbance spectrum was measured. As a reference for the absorbance measurement, the same procedure was performed without the addition of CNDs to the solutions. Each CNDs sample underwent this preparation at least three times and the average value was calculated.

Acid/base Back Titration

An HCl (0.10987 M) and NaOH (0.6024 M) solutions were prepared, and the titer was determined with a 0.1 M NaHCO₃ solution. For quantification, around 9 mg of the CNDs was weighed, dissolved in 7 mL Milli-Q water, and 1 mL of the NaOH solution was added to the mixture. Back titration was performed with the prepared HCl solution (0.10987 M). The pH was measured throughout the titration. A Gran plot was performed with the obtained values of the added volume of HCl solution and the pH to determine the two equivalence points through linear fitting.^[50] Here, the equivalence points are within a small $\pm 15\%$ volume interval around pH 7. By subtracting the two equivalence points, the number of active sites within the CNDs are obtained. The first equivalence point results from the neutralization of the excess hydroxide ions and the second from the complete protonation of the functional groups of the CNDs.

Computational Methods

To examine the possible models explaining the optical properties of the experimentally synthesized Arg:EDA and CA:EDA CNDs, the lowest excited states were analyzed in terms of the vertical (de-)excitation energies and the corresponding oscillator strengths as well as in terms of natural transition orbitals (NTOs). All calculations were performed for hydrated systems by employing the implicit universal solvation model based on solute electron density (SMD)^[68] and using the Gaussian16 package.^[69] The geometries of all the models were fully optimized without any symmetry constraints, within the wB97X-D/def2-SVP level of theory.^[61,70] Genuine minima at the potential energy surface were confirmed by the absence of imaginary frequencies in the harmonic vibrational analysis. The electronic excited levels were computed at the time-dependent density functional theory (TD-DFT)^[71] framework with the range-separated hybrid wB97X-D exchange-correlation functional and def2-TZVP^[61] basis set on all atoms. The electronic vertical excitation energies (VEEs) were calculated using the linear response (LR) with the first twenty singlet states taken into consideration.^[72–74] The vertical emission energies were obtained by first optimizing the S₁ geometry with the def2-SVP basis set, followed by the calculation of emission energies with the def2-TZVP basis set applying the equilibrium solvation regime for the excited state calculations and nonequilibrium solvation for the subsequent calculation of the ground state. NTOs were rendered with the Chemcraft software (version 1.8).^[75]

In molecular dynamics (MD) simulations, all models were parametrized in the Generalized Amber Force Field (GAFF)^[76] RESP^[77] partial charges were assigned by the Antechamber tool^[78] from the Amber software package^[79] after the geometry optimization of single molecule (Arg-1-MF-b) at the B3LYP/6-31G* level,^[80,81] and calculation of the electrostatic potential at this geometry at the HF/6-31G* level in gas. Five molecules were randomly placed in a cubic simulation box of 3.5×3.5×3.5 nm³ and solvated with TIP3P^[82] explicit water molecules (1242 in total). After minimization, a two-step equilibration was carried out. First, the system was thermalized from 0 K to 300 K for 1 ns with the V-rescale^[83] thermostat with a 0.1 ps scaling constant. Then, 2 ns equilibration of pressure was performed using the isotropic Berendsen barostat^[84] to keep the pressure at 1 bar during the simulation, the time constant for

pressure relaxation was set to 2 ps, and the temperature was kept at 300 K. 100 ns long production MD simulations were carried out in the canonical (NPT) ensemble with a 2 fs time step. The bonds involving hydrogen were constrained using the LINCS^[85] algorithm. The temperature was kept at 300 K with the V-rescale thermostat with a 0.1 ps scaling constant, the pressure was kept at 1 bar using the isotropic Parrinello–Rahman barostat^[86] and the time constant for pressure relaxation was 1 ps. The electrostatic interactions were treated by means of the particle-mesh Ewald (PME)^[87] method with a real-space cut-off of 1 nm; the same cut-off was applied for van der Waals interactions. Periodic boundary conditions were applied in all three dimensions. All MD simulations were performed in Gromacs 5.0.^[88] Figures from the MD simulations were rendered in PyMol.^[89]

To assess the pH effect over the absorption properties on the investigated systems, different protonation states for the guanidine/amine groups have been considered for the Arg:EDA CNDs, as well as for the carboxylic groups for the CA:EDA CNDs. The absorption spectra of the models with protonated guanidium groups showed the same absorption position as in the spectra of neutral structures (Figure S25), due to the localization of the excitonic transitions on the aromatic parts of the molecular fluorophores for both holes and electrons. On the other hand, for the CA:EDA systems, it is known from literature that the deprotonation of the carboxylic group of IPCA causes the blue-shift of the absorption spectra.^[60] The calculations with different number of deprotonated carboxylic groups in IPCA and CA-2-MF-a models display a blue-shift in the absorption spectra in comparison with the experimental data only when all carboxylic groups are deprotonated (Figure S29). At the experimental pH condition, we can expect either one or two deprotonated carboxylic groups. For this situation, a negligible shift in the absorption spectra has been observed. We can thus conclude that i) the CA:EDA molecules have either protonated groups due the embedding of IPCA molecules within the CNDs framework while not being exposed to the solvent, or ii) different protonation states co-exist, leading to a broader absorption peak centered around 330 nm (green curve in the Figure S2F).

Supporting Information

The authors have cited additional references within the Supporting Information.^[30,50,51,65,67,90–95]

Acknowledgements

We thank the financial support of the DFG and of the National Science Center, Poland through the joint project “Low-Dimensional Nano-Architectures for Light Emission and Light-to-Electricity Conversion” (LOW-LIGHT, grant no. UMO/2020/39/I/ST4/01446). S.O. also acknowledges the financial support of the “Excellence Initiative – Research University” (IDUB) Program, Action I.3.3 – “Establishment of the Institute for Advanced Studies (IAS)” for funding (grant no. UW/IDUB/2020/25). T.G. also acknowledges the financial support of the European Research Council through the ERC StG project JANUS BI (grant agreement No. [101041229]) and of the European Commission through the H2020 FET-PROACTIVE-EIC-07-2020 project LIGHT-CAP (grant agreement No. [101017821]). This research was carried out with the support of the Interdisciplinary Center for



Mathematical and Computational Modeling at the University of Warsaw (ICM UW) under grants no. G83-28 and GB80-24. E.M. would like to acknowledge Centro Studi di Economia e Tecnica dell'Energia Giorgio Levi Cases (project PRINTERS) and University of Padova (P-DiSC#05BIRD2021-UNIPD) for funding. The authors thank Heike Hausmann (Giessen) for performing the NMR experiments, Pascal Schweitzer (Giessen) for the support with the AFM measurements, and Samuel Pressi (Padua) for technical assistance with the TGA experiments. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Carbon Nanodots · Amide coupling · Quantitative ¹⁹F-NMR · Isoelectric point · Density functional theory

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Manuscript received: October 4, 2023

Revised manuscript received: November 29, 2023

Accepted manuscript online: November 29, 2023

Version of record online: December 8, 2023

ChemNanoMat

Supporting Information

Functional Groups Accessibility and the Origin of Photoluminescence in N/O-containing Bottom-up Carbon Nanodots

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Supporting Information

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Dynamic Light Scattering

The CNDs were measured in Milli-Q water with a concentration of $0.1 \text{ mg}\cdot\text{mL}^{-1}$ in a plastic cuvette after 10 minutes sonication and filtering over a PTFE syringe filter ($0.2 \mu\text{m}$).

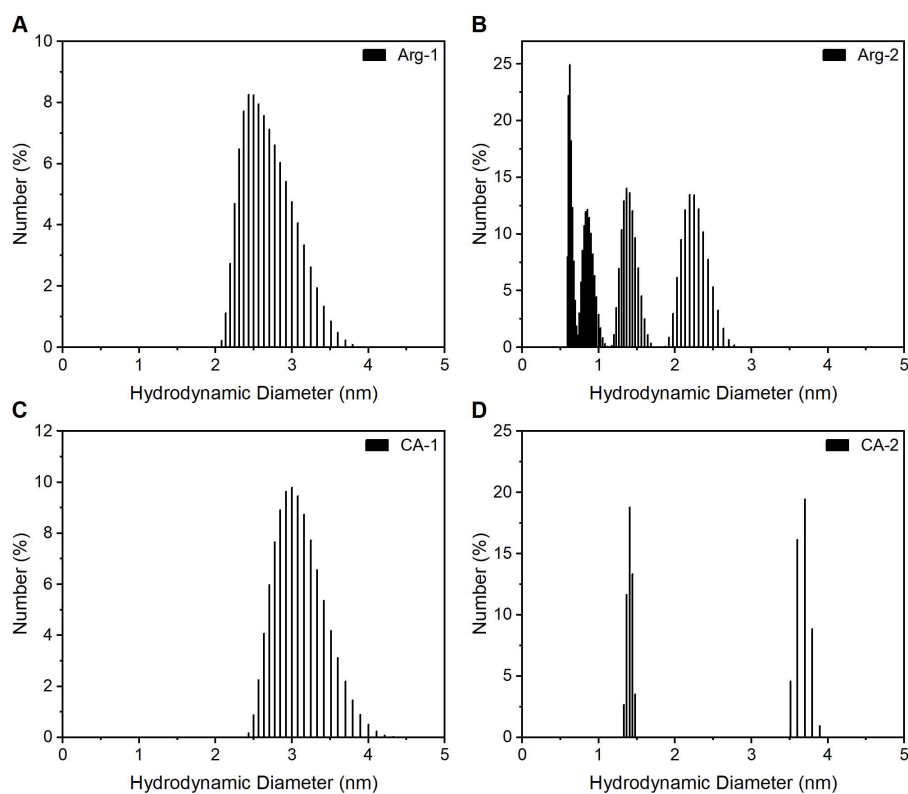


Figure S1. Hydrodynamic diameter distribution measured by dynamic light scattering (DLS) of (A) Arg-1 CNDs, (B) Arg-2 CNDs, (C) CA-1 CNDs, and (D) CA-2 CNDs in water.

Atomic Force Microscopy

0.2 mg·mL⁻¹ dispersions of CNDs in Milli-Q water were drop casted on freshly cleaved V1 grade Mica discs (10 mm diameter, 0.15–0.21 mm thickness). After keeping the prepared samples overnight under reduced pressure, they were measured in AFM tapping mode with a SHR150 probe (150 kHz, 5 N·m⁻¹). The obtained images are analyzed with Gwyddion 2.62^[1] and the size of ~70 nanoparticles was measured for a size histogram.

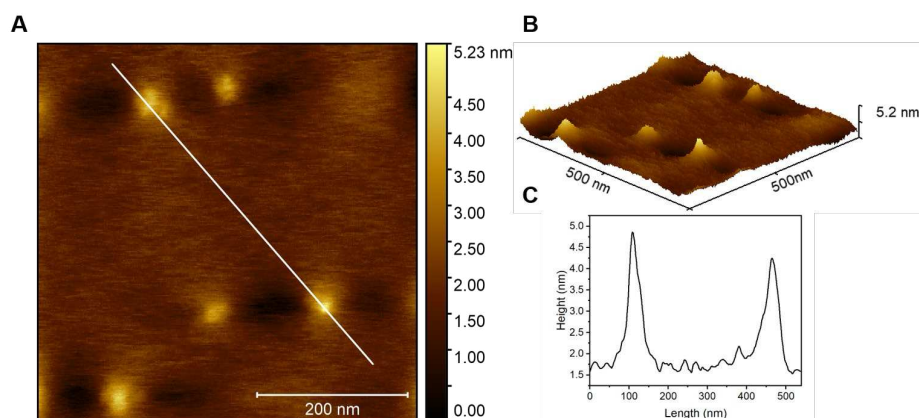


Figure S2. (A) 2D AFM image (500×500 nm) of **Arg-2** CNDs. (B) 3D AFM image (500×500 nm) of **Arg-2** CNDs and height profile along the white line in the 2D image.

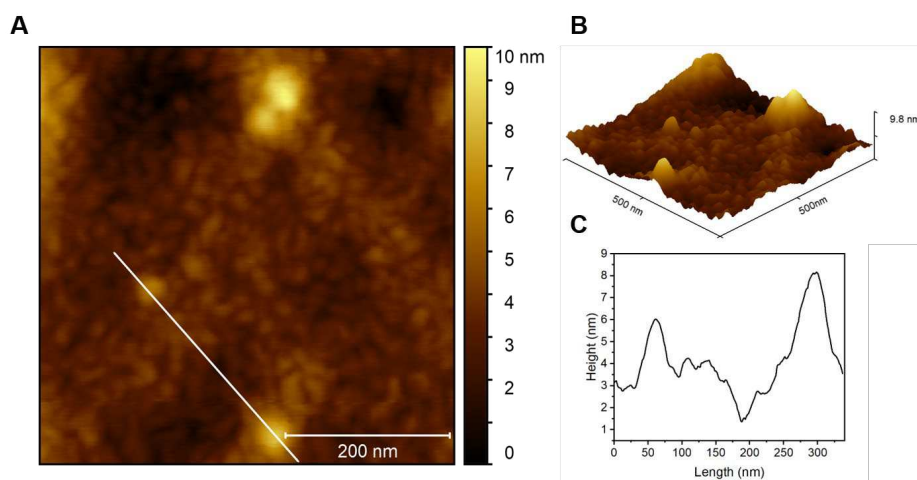


Figure S3. (A) 2D AFM image (500×500 nm) of **Arg-1** CNDs. (B) 3D AFM image (500×500 nm) of **Arg-1** CNDs. (C) Height profile along the white line in the 2D image.

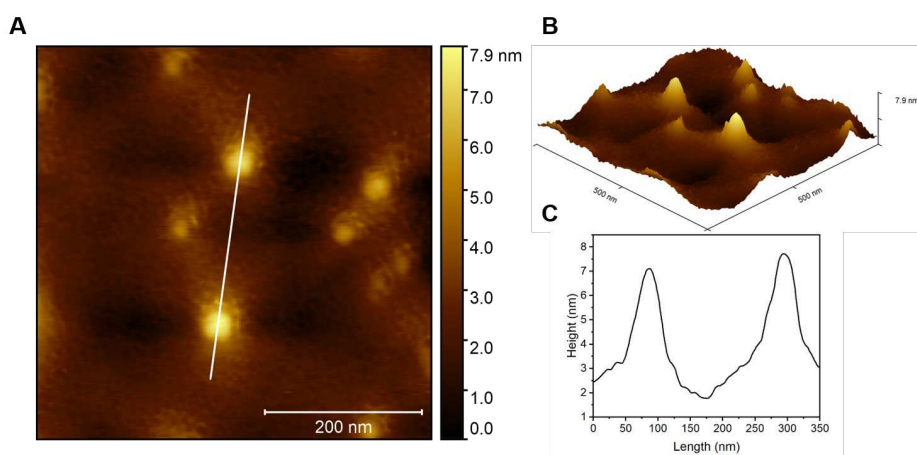


Figure S4. (A) 2D AFM image (500×500 nm) of **CA-1** CNDs. (B) 3D AFM image (500×500 nm) of **CA-1** CNDs. (C) Height profile along the white line in the 2D image.

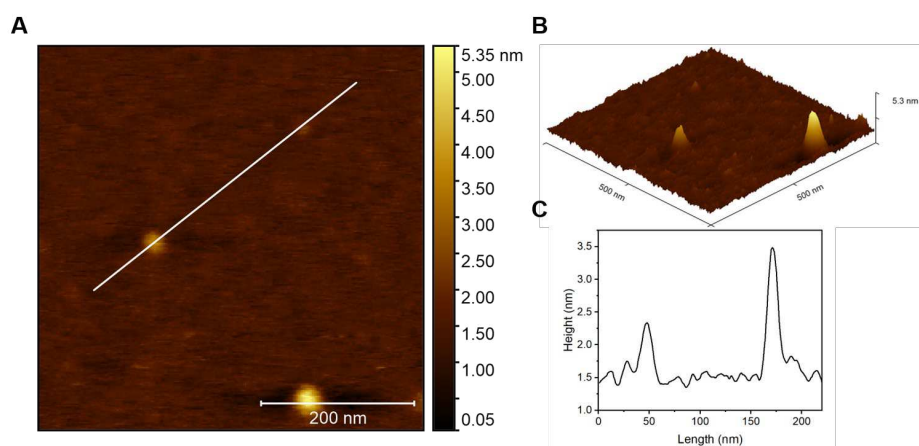


Figure S5. (A) 2D AFM image (500×500 nm) of CA-2 CNDs. (B) 3D AFM image (500×500 nm) of CA-2 CNDs. (C) Height profile along the white line in the 2D image.

Quantum Yield

The relative PL quantum yield method with quinine sulfate as a reference compound was used.^[2] For five different concentrations of each sample and the reference sample, the UV-Vis absorbance and PL were measured. The maximum values of each absorbance measurement with the respective integrated PL intensity are plotted against each other. From the obtained slopes and the following equation one can calculate the PL quantum yield. Since the similar solvent is used, the refractive index can be neglected in the equation.

$$\Phi_S = \frac{slope_S}{slope_R} \cdot \frac{n_S^2}{n_R^2} \cdot \Phi_R \cdot 100$$

Φ_S = quantum yield of the sample [%]

Φ_R = quantum yield of the reference compound (quinine sulfate = 0.51)

$slope_S$ = slope from plotted absorbance against integrated intensity of the sample

$slope_R$ = slope from plotted absorbance against integrated intensity of the reference compound

n_S = refractive index of sample solvent (0.1 M H₂SO₄)

n_R = refractive index of reference solvent (0.1 M H₂SO₄)

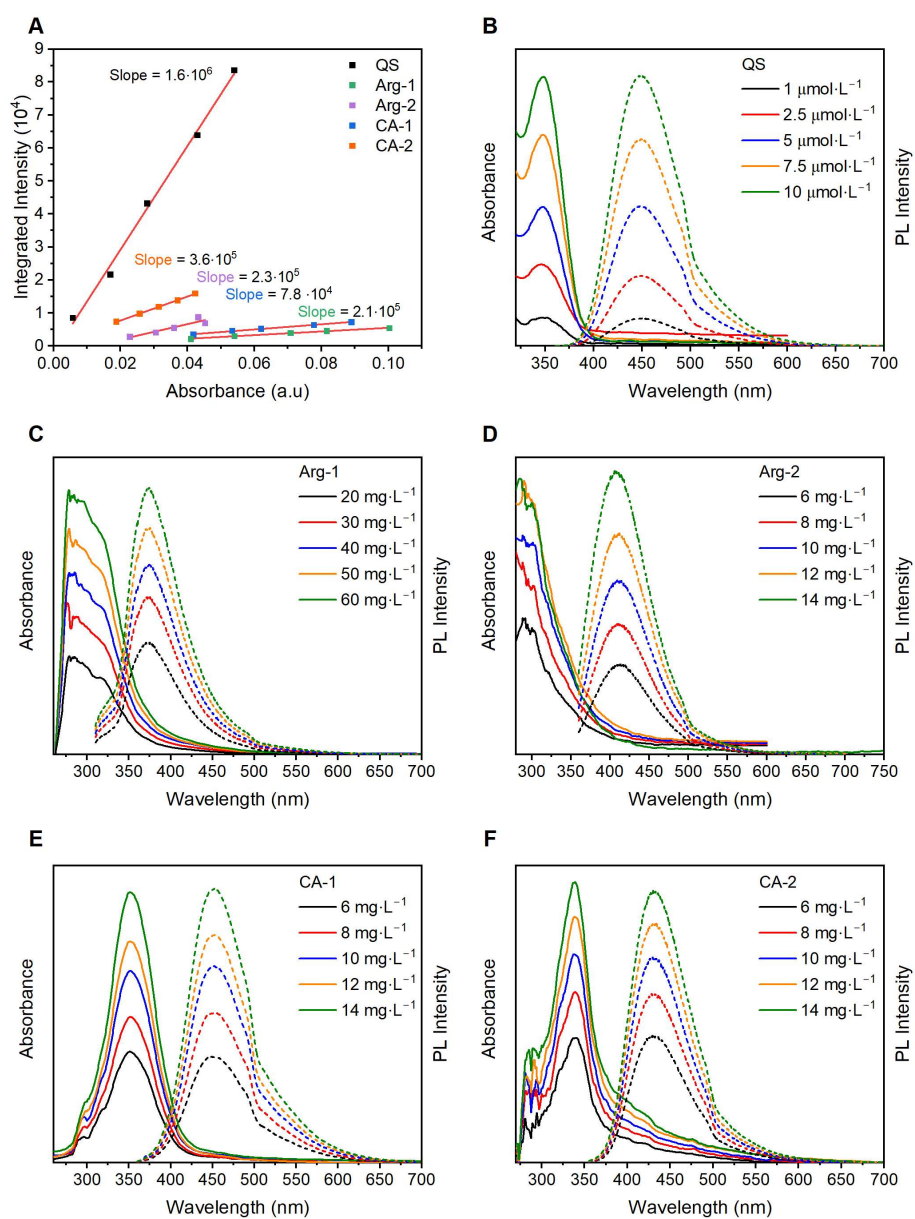


Figure S6. (A) Absorbance maxima plotted against the integrated PL intensity of the five different concentrations and the respective slopes derived from a linear fit. (B) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350 \text{ nm}$) of five different concentrated quinine sulfate (QS) solutions. (C) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 290 \text{ nm}$) of five different concentrated **Arg-1** dispersions. (D) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 290 \text{ nm}$) of five different concentrated **Arg-2** dispersions. (E) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 340 \text{ nm}$) of five different concentrated **CA-1** dispersions. (F) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 340 \text{ nm}$) of five different concentrated **CA-2** dispersions.

Infrared Spectroscopy

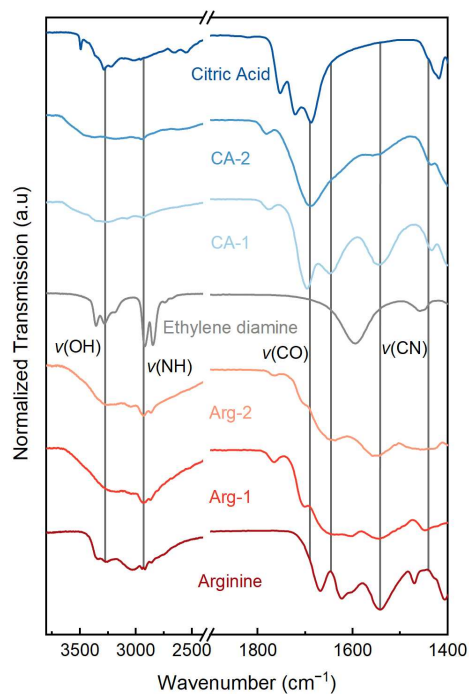


Figure S7. Normalized IR spectra of the pure starting materials citric acid, ethylenediamine, and arginine in comparison with the four different types of CNDs **CA-1**, **CA-2**, **Arg-1**, and **Arg-2**.

Elemental Analysis

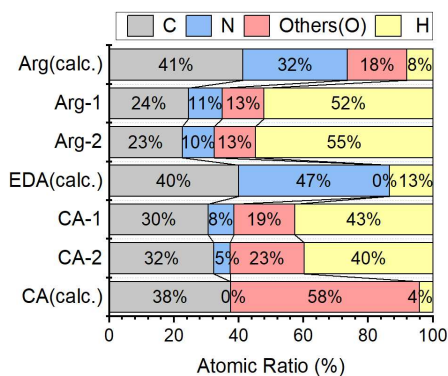


Figure S8. CNH elemental analysis of the four CNDs (CA-1, CA-2, Arg-1, Arg-2) in comparison with the calculated compositions of arginine (Arg), ethylenediamine (EDA), and citric acid (CA). The main contribution of other elements was estimated to be oxygen.

Kaiser Test

Primary amines react with ninhydrine to form Ruhemann's blue^[3], a dye whose extinction coefficient is well-known.^[4] Therefore, the primary amine content (molality) can be calculated from the maximum absorbance of Ruhemann's blue at 570 nm after the absorbance measurement from the Kaiser test solutions.^[5]

$$c = \frac{A}{L \cdot \varepsilon}$$

c = concentration [$\text{mol} \cdot \text{L}^{-1}$]

A = absorbance

L = cuvette thickness [cm]

$\varepsilon = 15000$ = molar extinction coefficient of Ruhemann's blue [$\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]

$$b = \frac{\left(\frac{c}{V}\right)}{m}$$

$$b = \text{molality} [\text{mol} \cdot \text{g}^{-1}]$$

$$V = 0.03225 = \text{volume} [\text{L}]$$

$$m = \text{mass of the CNDs} [\text{g}]$$

Acid-base Back Titration

The Gran plot was obtained from calculating n_{HCl} and n_{NaOH} from the hydrochloric acid volume V_{HCl} in the range of 15% above and below the pH value of 7 together with the added volume of sodium hydroxide V_{NaOH} which was used to basify the CNDs.^[6] Following equations were used:

$$n_{\text{HCl}} = c_{\text{HCl}} \cdot V_{\text{HCl}}$$

$$n_{\text{NaOH}} = c_{\text{NaOH}} \cdot V_{\text{NaOH}} - n_{\text{HCl}}$$

$$n_{\text{NaOH}} \cdot 10^{\text{pH}}$$

$$n_{\text{HCl}} \cdot 10^{-\text{pH}}$$

$$n = \text{molarity} [\text{mol}]$$

$$c = \text{concentration} [\text{mol} \cdot \text{L}^{-1}]$$

$$V = \text{volume} [\text{L}]$$

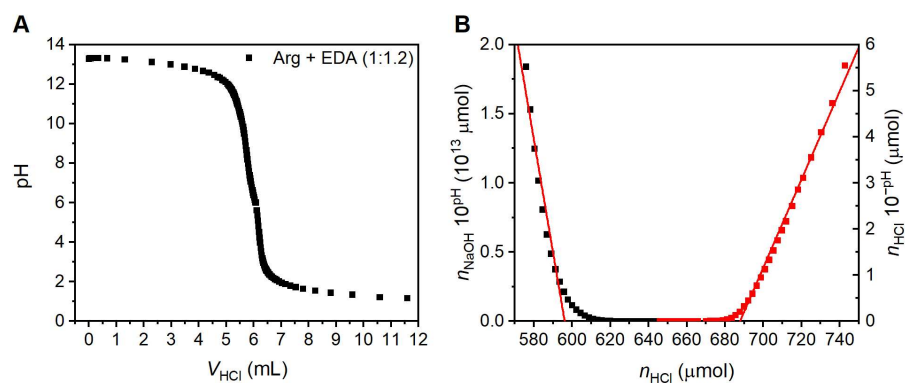


Figure S9. pH titration curve (A) obtained from the acid-base back titration of the **Arg-1** CNDs with the respective Gran plot (B).

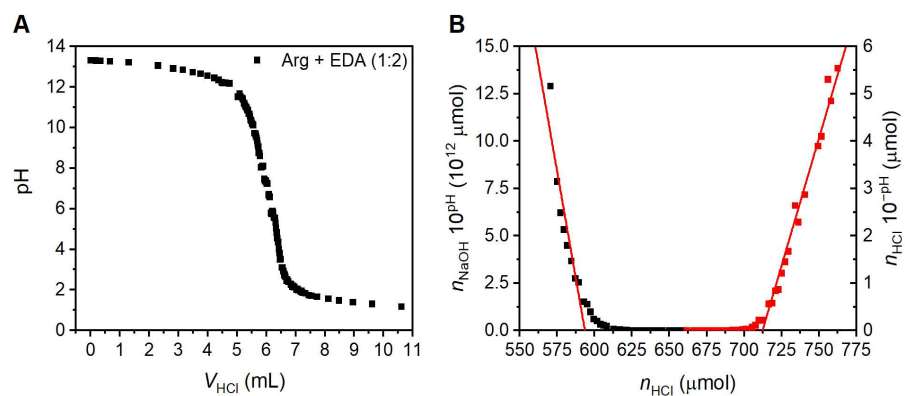


Figure S10. pH titration curve (A) obtained from the acid-base back titration of the **Arg-2** CNDs with the respective Gran plot (B).

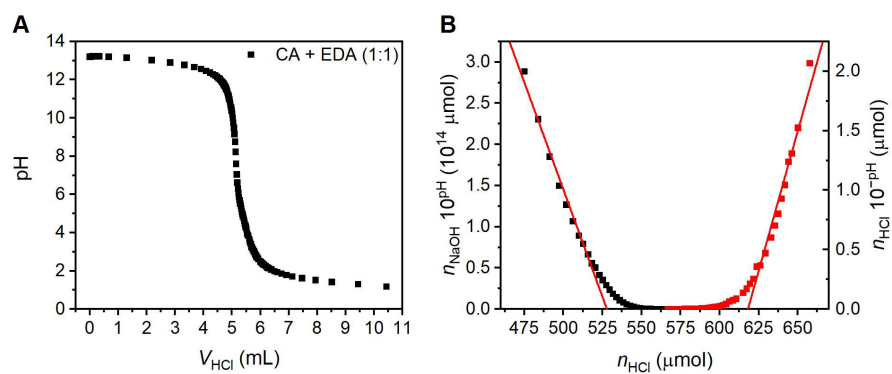


Figure S11. pH titration curve (A) obtained from the acid-base back titration of the **CA-1** CNDs with the respective Gran plot (B).

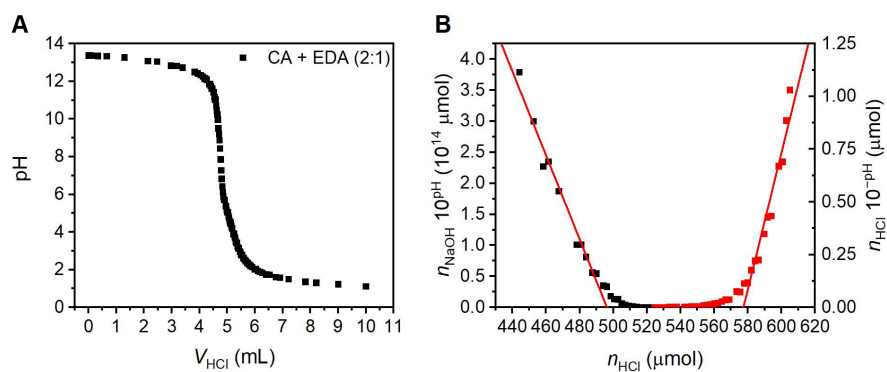


Figure S12. pH titration curve (A) obtained from the acid-base back titration of the **CA-2** CNDs with the respective Gran plot (B).

Isoelectric Point

The isoelectric points were determined by zeta potential measurements at different pH values. The pH value where the current is zero and no surface charge can be observed is the isoelectric point.^[7] 8 mg of CNDs are dispersed in 8 mL Milli-Q water and the pH value was changed by dropping slowly 0.1 M aqueous HCl or NaOH into the dispersion.

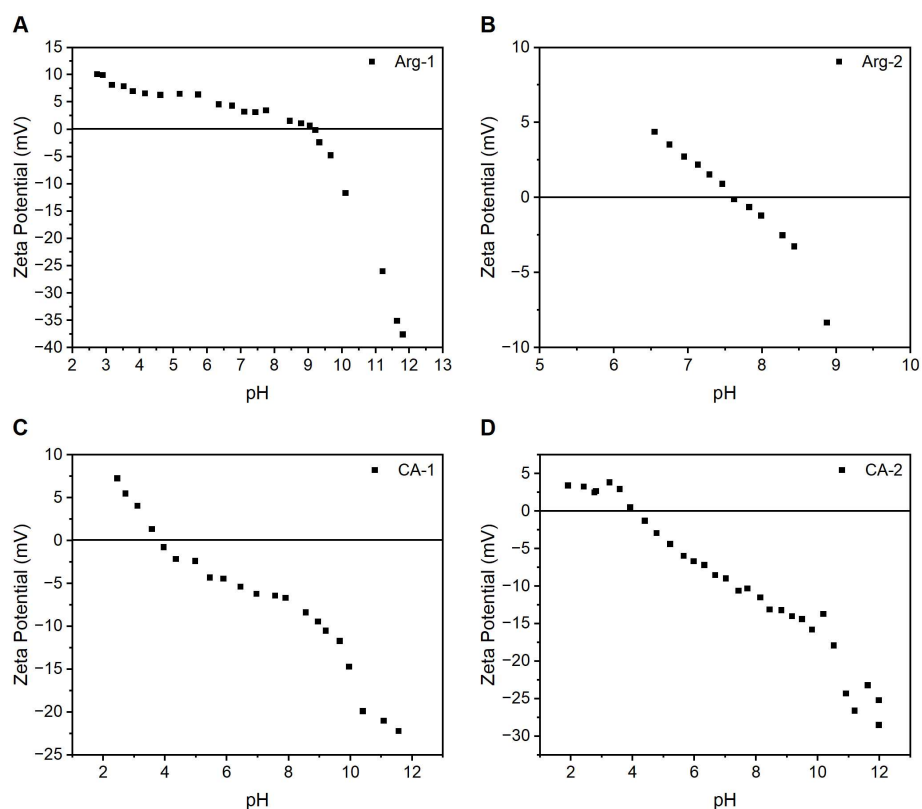


Figure S13. Isoelectric point determination by zeta potential measurements depending on the pH values performed in water of the four different CNDs. (A) Arg-1, (B) Arg-2, (C) CA-1, (D) CA-2.

Quantitative ^{19}F -NMR

For the quantitative ^{19}F -NMR 10 mg of the amide-coupled CNDs were dispersed in 0.7 mL of deuterated DMSO and 4 mg of 4,4'-difluorobenzophenone were added to the mixture as an internal standard (IS).^[8] 1 mg of the IS were added to the reaction mixture of the imine functionalized CNDs for the quantitative ^{19}F -NMR. IS: 4,4'-difluorobenzophenone (^{19}F -NMR: $\delta = -106.5$ ppm in DMSO- d_6)

$$b_{\text{amine}} = \frac{\text{Integral}_{\text{CND}}}{\text{Integral}_{\text{IS}}} \cdot \frac{2 \cdot n_{\text{IS}}}{m_{\text{CND}}}$$

$$b_{\text{amine}} = \text{molality} [\text{mol} \cdot \text{g}^{-1}]$$

$$n_{\text{IS}} = \text{amount of internal standard} [\text{mol}]$$

$$m_{\text{CND}} = \text{mass of the CNDs} [\text{g}]$$

Thermogravimetric Analysis

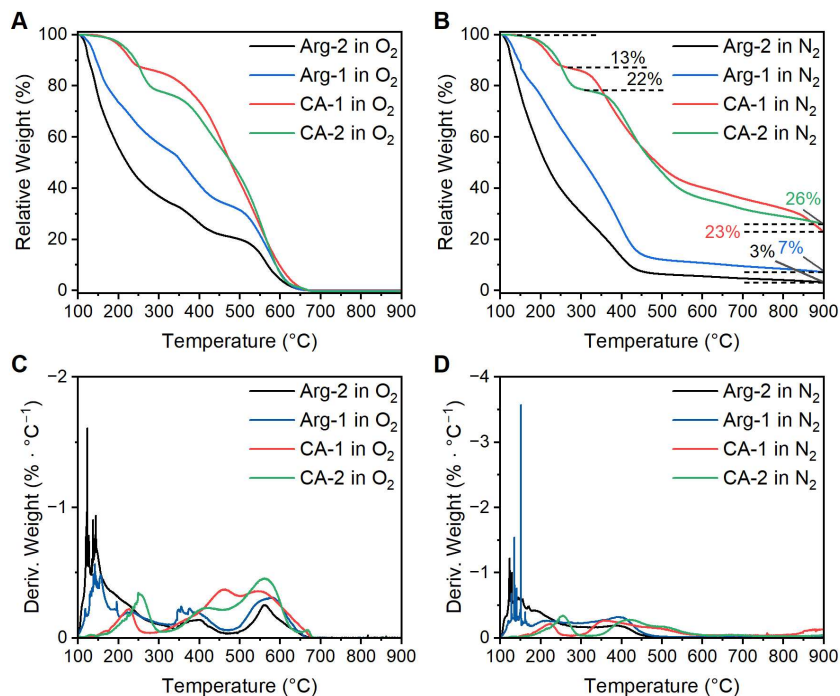


Figure S14. TGA analysis of the CNDs (A) measured in air (B) measured in nitrogen with a carbonized residue at 900 °C of 3% for **Arg-2**, 7% for **Arg-1**, 23% for **CA-1**, and 26% of **CA-2**. In all cases, the first significant mass loss was observed between 100 °C and 300 °C. Decomposition started at around 500 °C in air and around 350 °C under nitrogen atmosphere.

Visual Appearance of CNDs



Figure S15. Visual appearance of the different CNDs (**Arg-1**, **Arg-2**, **CA-1**, and **CA-2**) after purification and freeze-drying.

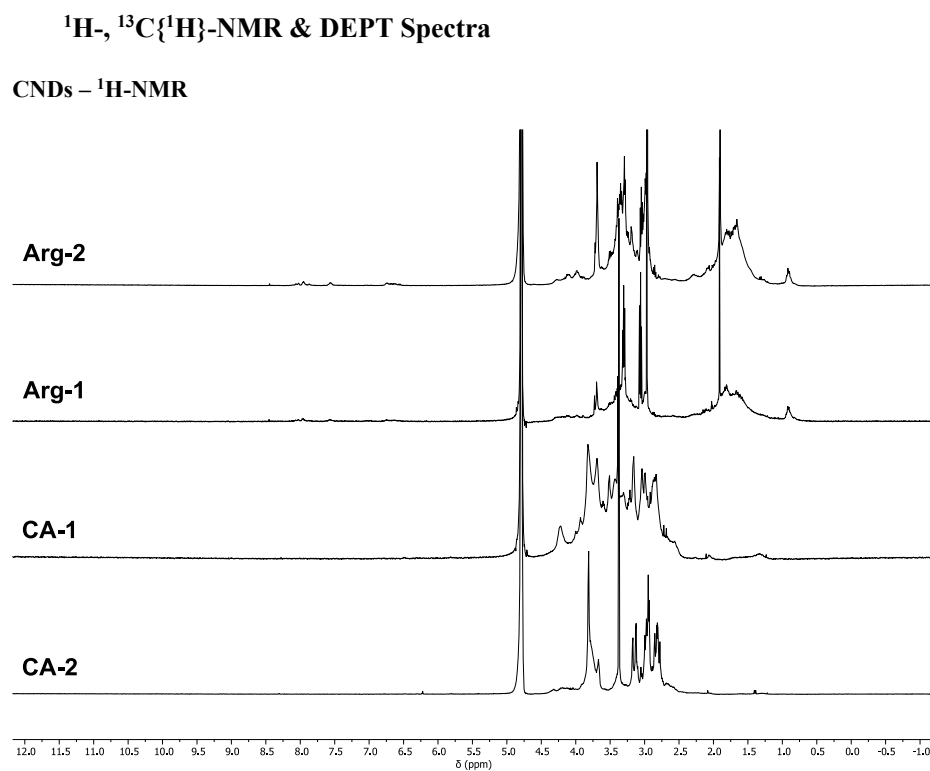


Figure S16. Stacked ^1H -NMR spectra of **Arg-2**, **Arg-1**, **CA-1**, and **CA-2** CNDs measured in D_2O - d_2 .

CA+EDA (1:1) – $^{13}\text{C}\{^1\text{H}\}$ -NMR & DEPT-135

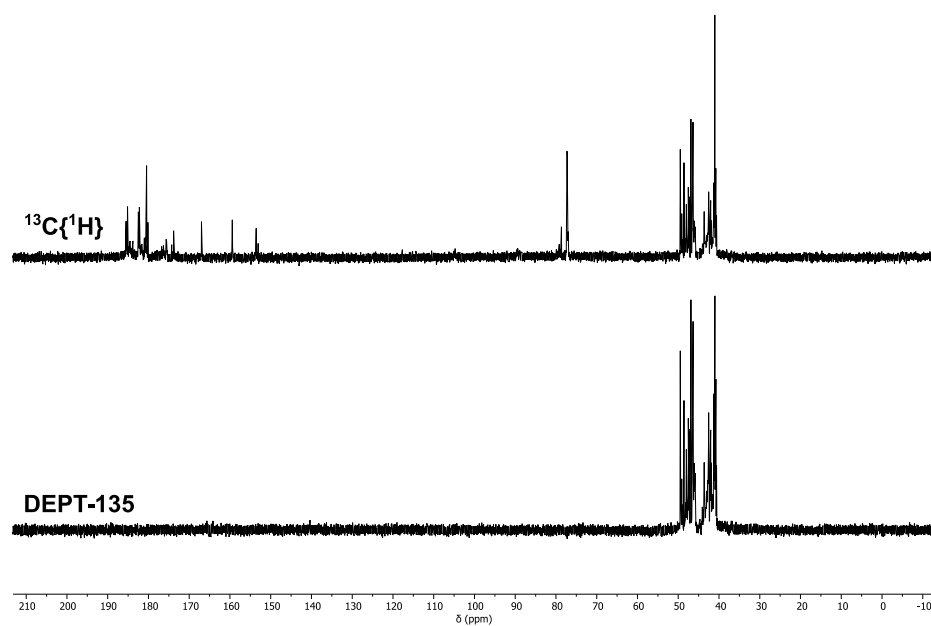


Figure S17. Stacked $^{13}\text{C}\{^1\text{H}\}$ -NMR and DEPT-135 spectra of CA-1 CNDs measured in $\text{D}_2\text{O-d}_2$.

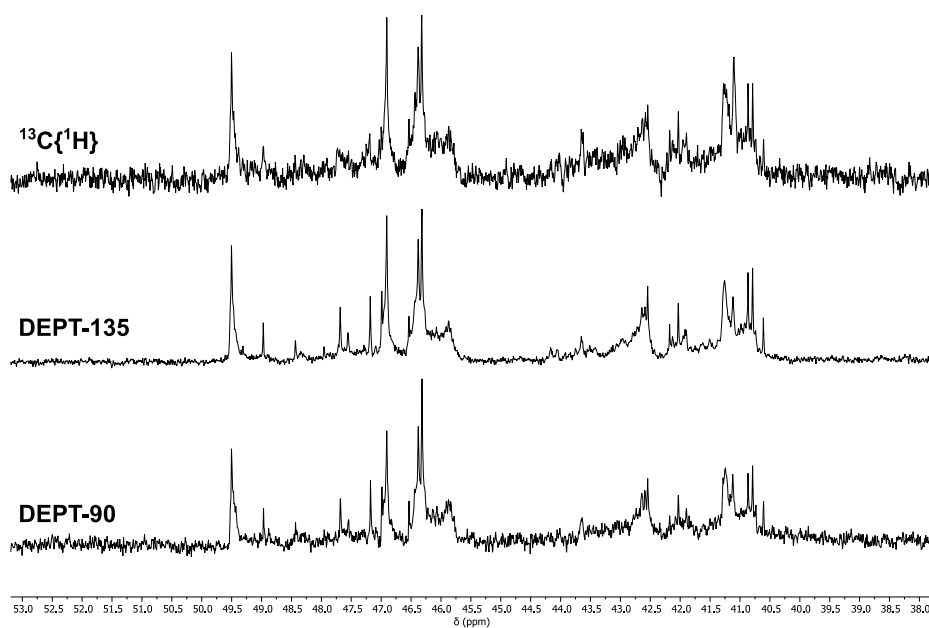
CA+EDA (1:1) – $^{13}\text{C}\{^1\text{H}\}$ -NMR, DEPT-90, and DEPT-135

Figure S18. Stacked $^{13}\text{C}\{^1\text{H}\}$ -NMR, DEPT-90, and DEPT-90 spectra of CA-1 CNDs measured in $\text{D}_2\text{O-d}_2$.

Arg+EDA (1:1.2) – $^{13}\text{C}\{^1\text{H}\}$ -NMR, DEPT-90, and DEPT-135

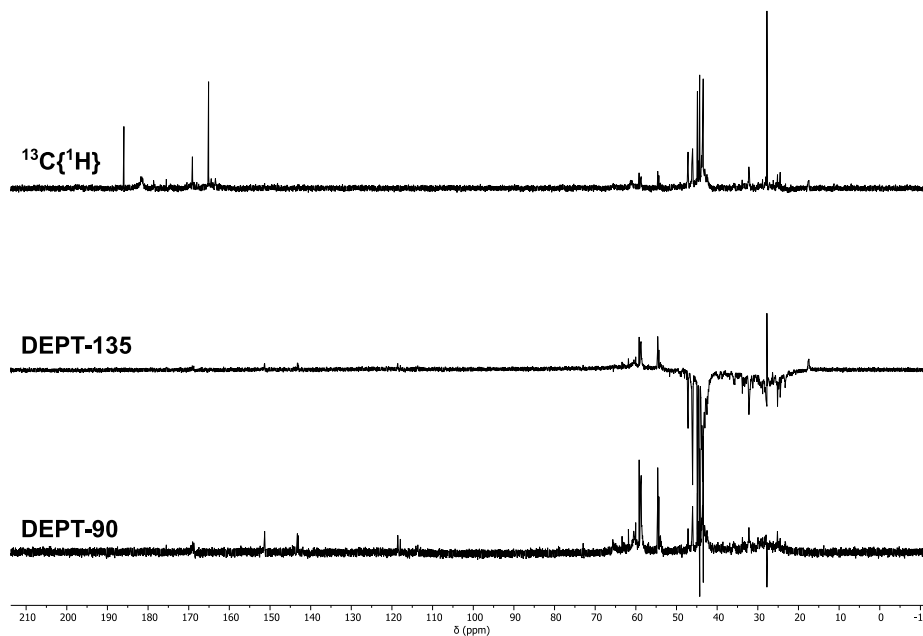


Figure S19. Stacked $^{13}\text{C}\{^1\text{H}\}$ -NMR, DEPT-135, and DEPT-90 spectra of **Arg-1** CNDs measured in $\text{D}_2\text{O-d}_2$.

Computational Analysis

Models based on large polyaromatic hydrocarbons

The ‘large’ model was constructed based on the available experimental data,^[9] demonstrating that one kind of CNDs formed during microwave-assisted hydrothermal synthesis at a maximum temperature of 250 °C from Arg and EDA have core radius around 0.54 nm and shell thickness 0.55 nm. The calculated excitation energy levels of our ‘large’ model are displayed in Table S1.

Table S1. First five calculated vertical excitation and emission wavelengths of different models of Arg:EDA CNDs, and the corresponding oscillatory strengths for $S_0 \rightarrow S_n$ transition.

	Large model*		Medium model		Arg-MF model	
	ΔE (nm)	f	ΔE (nm)	f	ΔE (nm)	f
S ₁	941	0.021	595	0.037	292	0.290
S ₂	693	0.030	509	0.410	234	0.163
S ₃	580	0.003	431	0.030	221	0.005
S ₄	567	0.287	390	0.134	211	0.005
S ₅	538	0.135	345	0.042	202	0.210
Emission						
S ₁					414 nm	

* Due to the size of the system, 6-31+G(d) basis set was used for ‘large’ model.

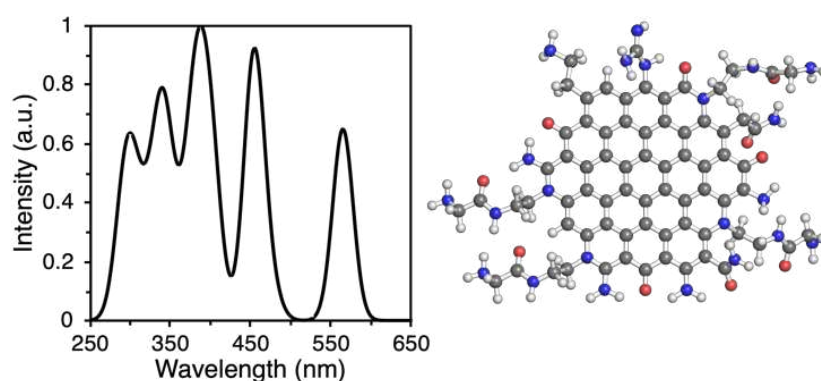
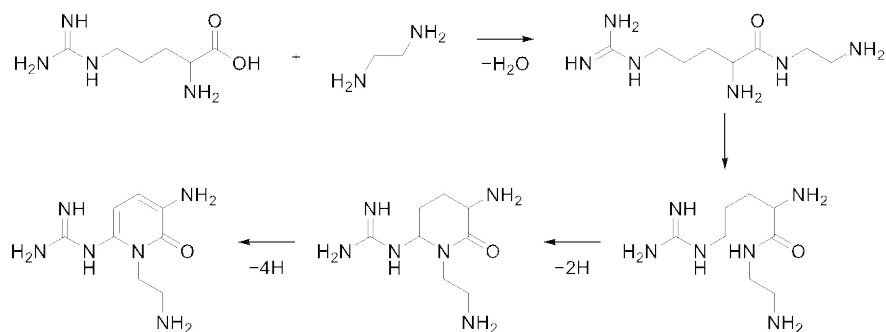
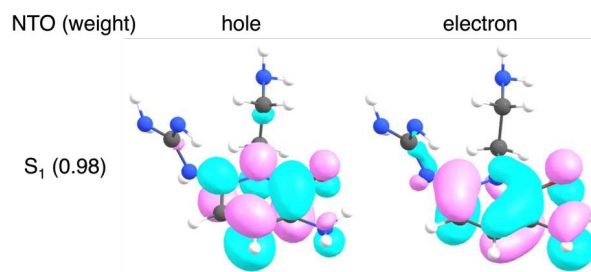


Figure S20. Left panel: Calculated absorption spectrum of a ‘large’ model with no graphitic N-doping in the aromatic core. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm. Right panel: The corresponding structure considered for the (TD-)DFT calculations. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white.

Models based on small organic fluorophores

The presence of molecular fluorophores in our obtained CNDs is suggested by the observation of PL of the dialysate after the purification processes in our synthesis, which was also reported in the literature for other MFs.^[10,11]

Molecular fluorophore (MF) formed from arginine (Arg) and ethylenediamine (EDA)**Figure S21.** The reaction route showing a possible formation of Arg-1-MF.**Figure S22.** NTO orbitals of Arg-1-MF model, which could explain the source of PL of Arg:EDA

CNDs (isovalue = 0.04 a.u.).

Table S2. First five calculated vertical excitation and emission wavelengths of different MF models of Arg:EDA CNDs, and the corresponding oscillatory strengths for $S_0 \rightarrow S_n$ transition.

	Arg-1-MF-a		Arg-1-MF-b		Arg-2-MF	
	ΔE (nm)	f	ΔE (nm)	f	ΔE (nm)	f
S ₁	308	0.253	314	0.280	306	0.232
S ₂	293	0.345	304	0.290	294	0.346
S ₃	239	0.148	294	0.363	239	0.082
S ₄	238	0.228	242	0.234	238	0.300
S ₅	227	0.010	240	0.085	228	0.014
Emission						
S ₁	412 nm		415 nm		400 nm	

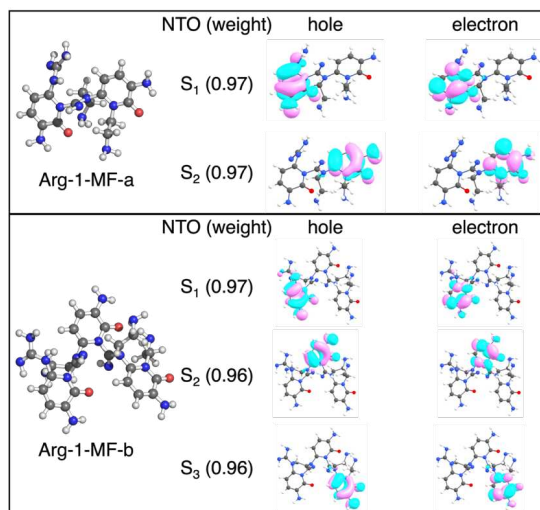


Figure S23. Structure and NTO orbitals of Arg-1-MF-a and Arg-1-MF-b models (isovalue = 0.04 a.u.), which could be the source of PL of Arg:EDA CNDs. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white.

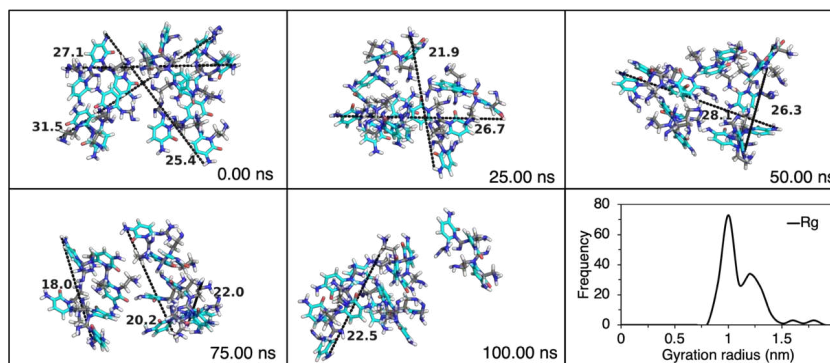


Figure S24. Evolution of the assembly process of the Arg:EDA CNDs model starting from the five Arg-1-MF-b randomly distributed in the simulation box. Last panel displays distribution of the gyration radius of the simulated system. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white. Water is omitted for clarity. Distances are displayed in Å.

MF formed from citric acid (CA) and ethylenediamine (EDA)**Table S3:** First five calculated vertical excitation and emission wavelengths of IPCA and CA-2-MF-a and CA-2-MF-b models of CA:EDA CNDs, and the corresponding oscillatory strengths for $S_0 \rightarrow S_n$ transition.

	IPCA		CA-2-MF-a		CA-2-MF-b*	
	ΔE (nm)	f	ΔE (nm)	f	ΔE (nm)	f
S ₁	346	0.159	336	0.218	319	0.188
S ₂	241	0.000	331	0.070	310	0.193
S ₃	231	0.004	280	0.058	252	0.005
S ₄	225	0.005	274	0.078	239	0.003
S ₅	215	0.311	246	0.000	237	0.046
Emission						
S ₁	432 nm		430 nm			

* Due to the size of the system, def2-SVP basis set was used for CA-2-MF-b model. It was estimated by the benchmarking calculation on IPCA molecule that the use of this basis set blue-shifts the $S_0 \rightarrow S_1$ transition by 12 nm (334 nm, $f = 0.171$), meaning that for CA-2-MF-b model, the S_1 level could be shifted to 331 nm.

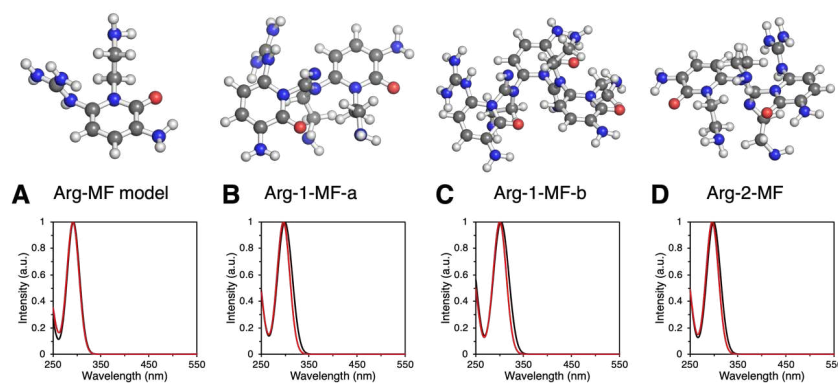


Figure S25. Top panel: Protonated models based on small organic molecules, which explain the optical properties of Arg:EDA CNDs. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white. Bottom panel: Calculated normalized absorption spectra of neutral (black) and protonated (red) (A) Arg-MF-model, (B) Arg-1-MF-a, (C) Arg-1-MF-b, (D) Arg-2-MF models. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm.

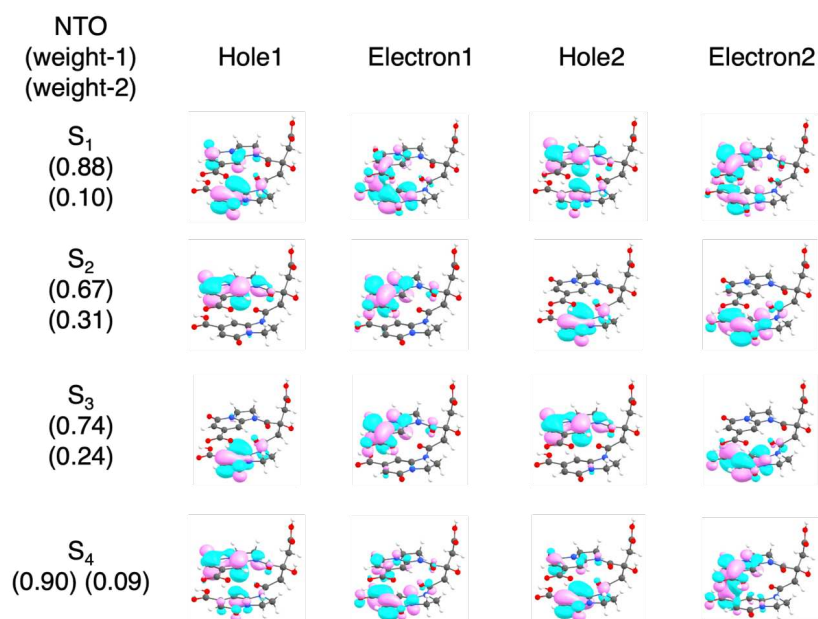


Figure S26. NTO orbitals of CA-2-MF-a (isovalue = 0.04 a.u.).

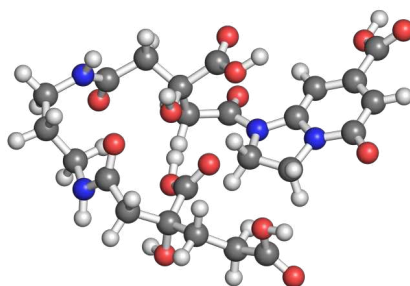


Figure S27. An oligomer, whose two copies form CA-2-MF-b model by their non-covalent interaction. Coloring: carbons-grey; nitrogens-blue; oxygens-red; hydrogens-white.

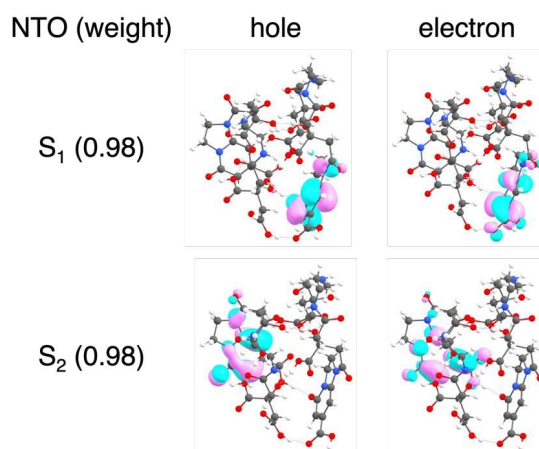


Figure S28. NTO orbitals of CA-2-MF-b (isovalue = 0.04 a.u.).

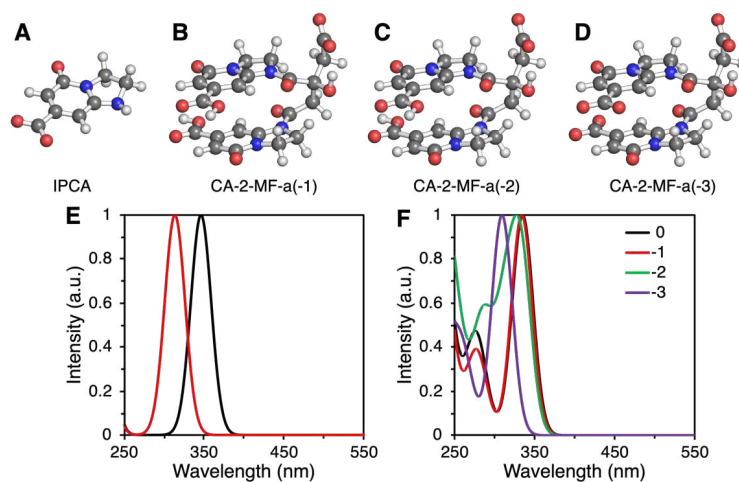


Figure S29. (A)-(D) Models based on small organic molecules with deprotonated carboxylic groups, which explain the optical properties of CA:EDA CNDs. Coloring: carbons-grey, cyan, yellow; nitrogens-blue; oxygens-red; hydrogens-white. (E)-(F) Calculated normalized absorption spectra of neutral (black) and deprotonated (red, green, purple) spectra of (E) IPCA(-1), (F)

different deprotonated models of CA-2-MF-a. Line spectra were convoluted by a Gaussian function assuming the inhomogeneous broadening of peaks with $\sigma = 30$ nm.

Fluorescent Dialysate



Figure S30. Fluorescent dialysate under UV irradiation (365 nm) of **CA-1** CNDs after 12 hours of dialysis.

CA+EDA (1:1) – q-¹⁹F-NMR - Amide

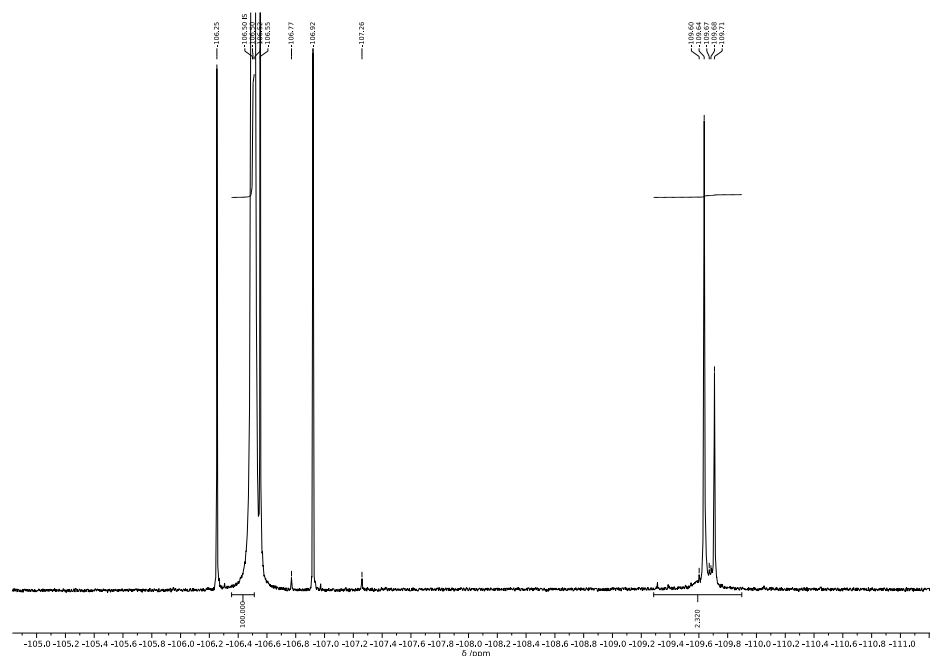


Figure S31. Quantitative ^{19}F -NMR of the CA-1 CNDs amide coupled with 4-fluorobenzoic acid and measured in DMSO- d_6 and 4 mg of the IS.

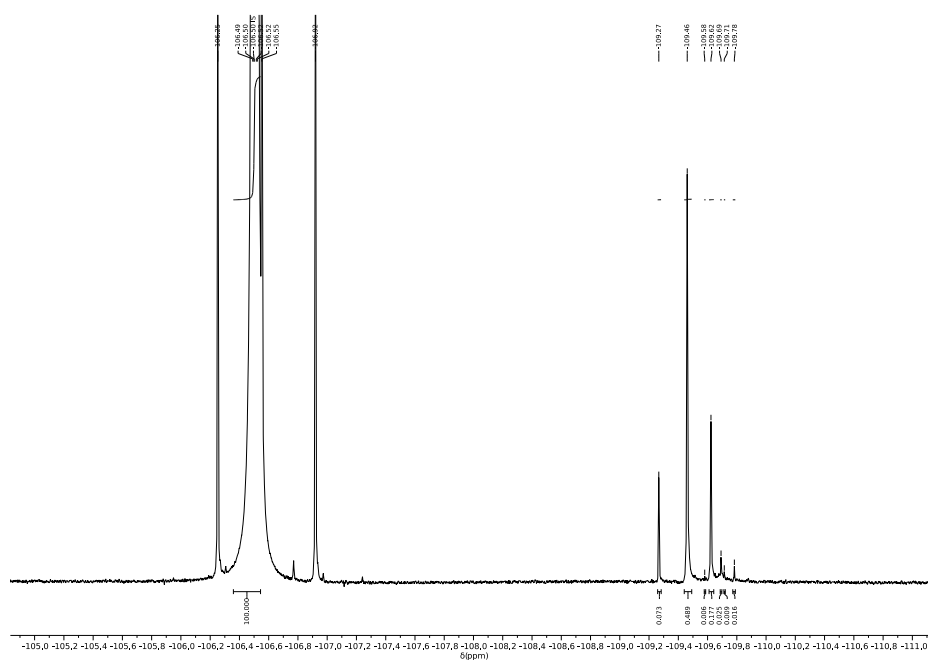
CA+EDA (2:1) – q-¹⁹F-NMR – Amide

Figure S32. Quantitative ¹⁹F-NMR of the CA-2 CNDs amide coupled with 4-fluorobenzoic acid and measured in DMSO-d₆ and 4 mg of the IS.

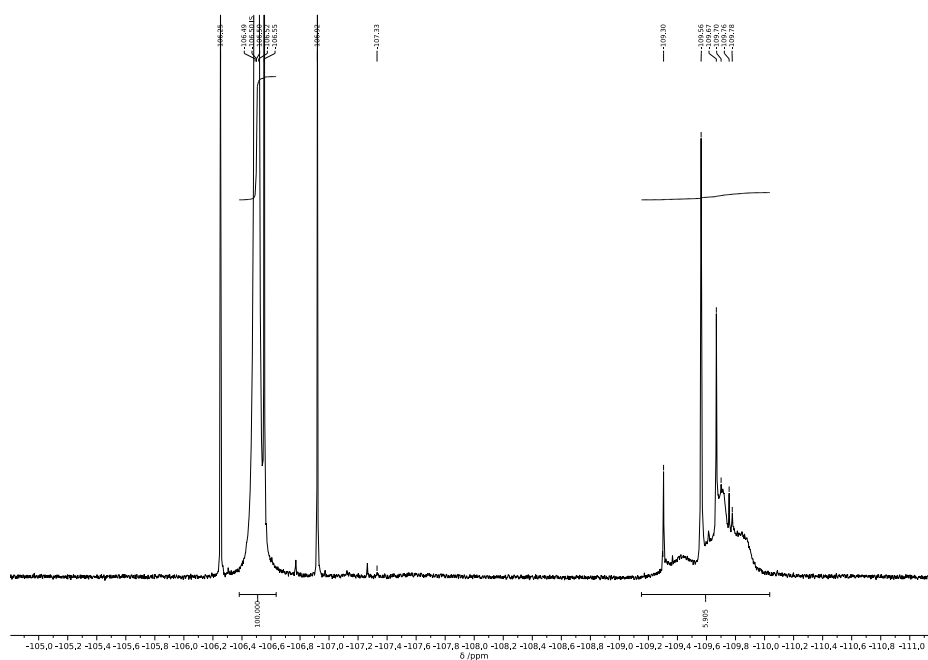
Arg+EDA (1:1.2) – q-¹⁹F-NMR – Amide

Figure S33. Quantitative ¹⁹F-NMR of the **Arg-1** CNDs amide coupled with 4-fluorobenzoic acid and measured in DMSO-d₆ and 4 mg of the IS.

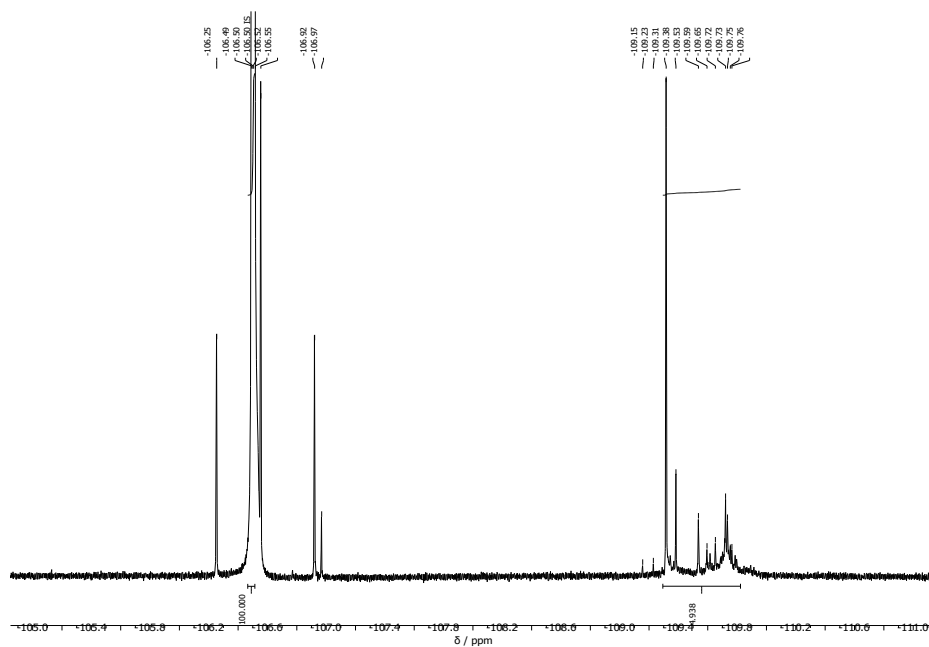
Arg+EDA (1:2) – q- ^{19}F -NMR – Amide

Figure S34. Quantitative ^{19}F -NMR of the **Arg-2** CNDs amide coupled with 4-fluorobenzoic acid and measured in DMSO- d_6 and 4 mg of the IS.

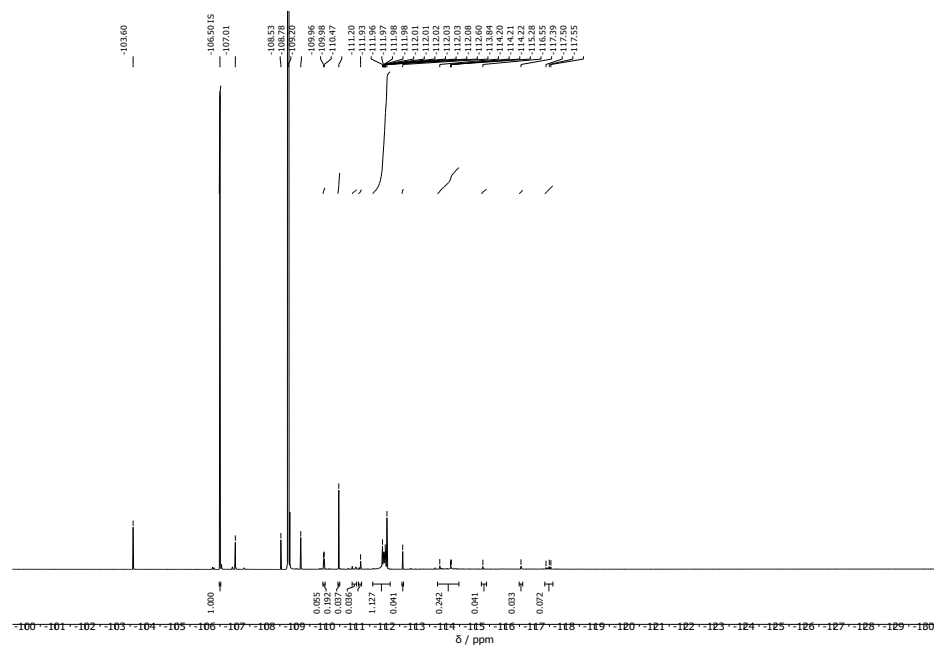
CA+EDA (1:1) – q-¹⁹F-NMR – Imine

Figure S35. Quantitative ¹⁹F-NMR of the CA-1 CNDs after the imine reaction with 4-fluorocinnamaldehyde and measured in DMSO-d₆ and 1 mg of the IS.

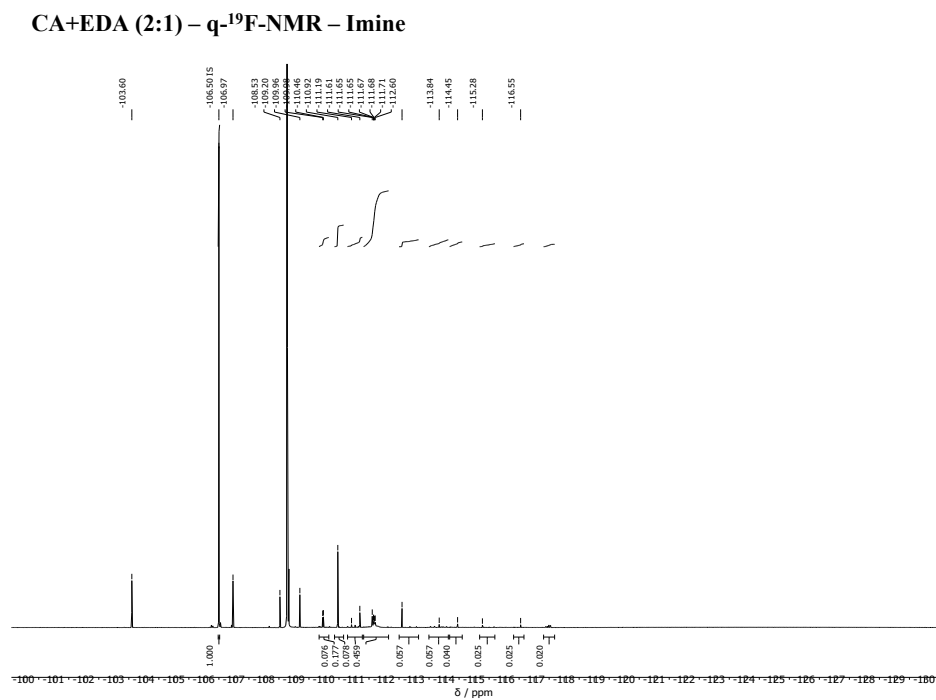


Figure S36. Quantitative ¹⁹F-NMR of the **CA-2** CNDs after the imine reaction with 4-fluorocinnamaldehyde and measured in DMSO-d₆ and 1 mg of the IS.

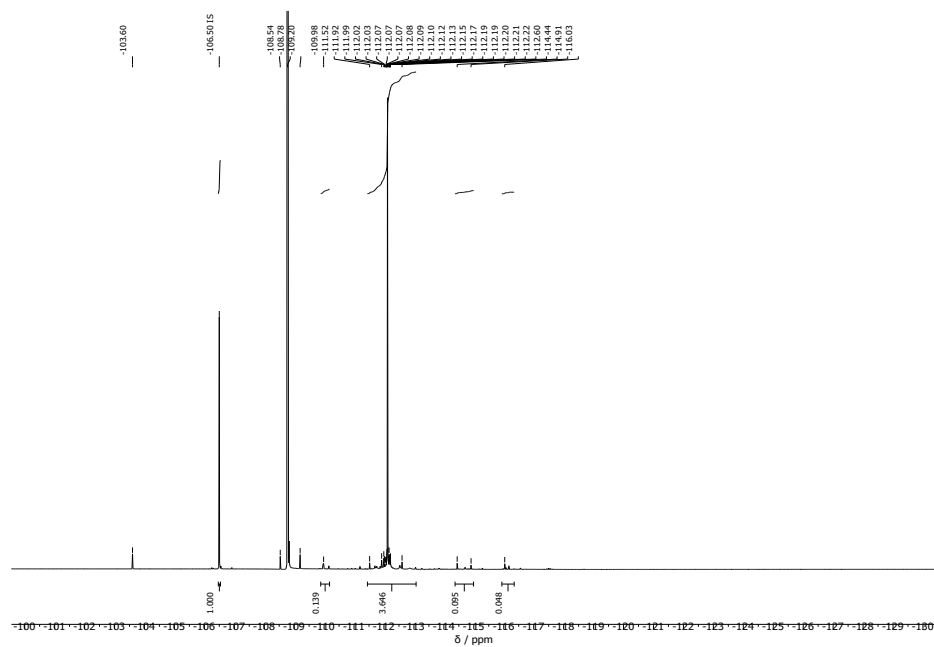
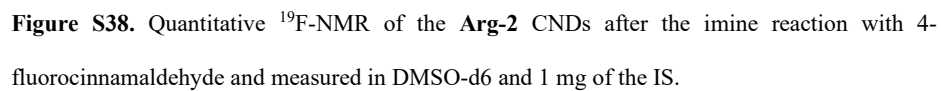
Arg+EDA (1:1.2) – q-¹⁹F-NMR – Imine

Figure S37. Quantitative ¹⁹F-NMR of the **Arg-1** CNDs after the imine reaction with 4-fluorocinnamaldehyde and measured in DMSO-d₆ and 1 mg of the IS.

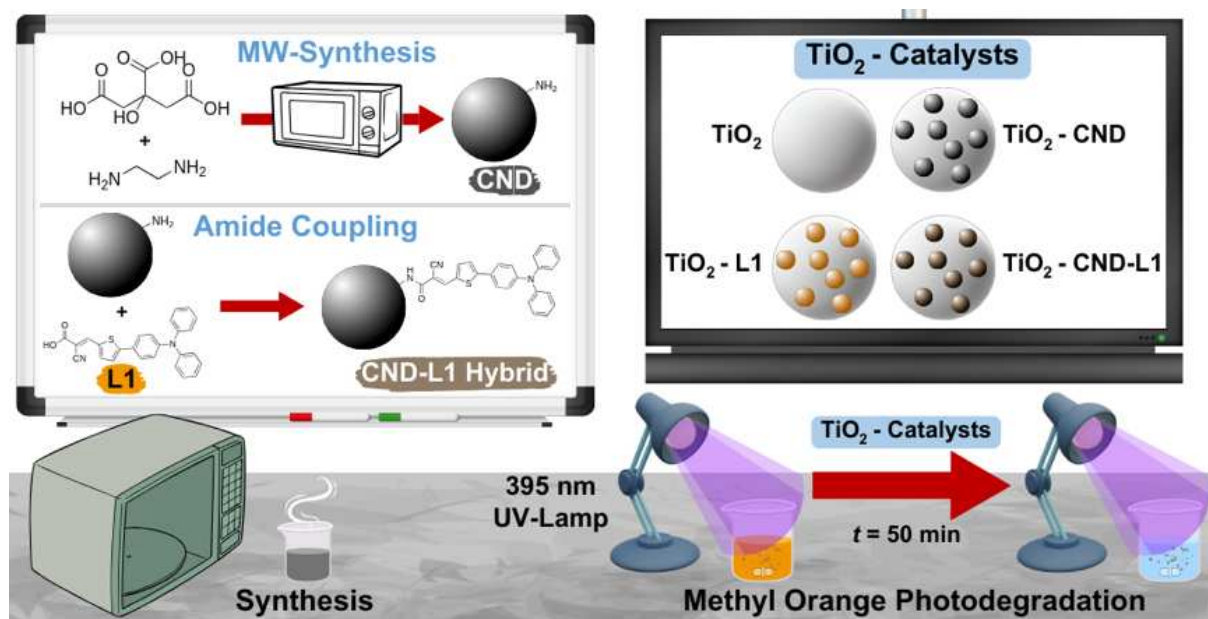


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2.2. Publication 2

Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis



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Debes, P. P.; Pagel, M.; Muntean, S.; Hessling, J.; Smarsly, B. M.; Schönhoff, M.; Gatti, T. Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis. *Photochem* **2025**, 5 (1), 1.

DOI: [10.3390/photochem5010001](https://doi.org/10.3390/photochem5010001)



Article

Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis

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Abstract: The textile industry is a major contributor to environmental pollution, primarily through the discharge of wastewater loaded with dyes and contaminants that disrupt natural ecosystems. This study aims to develop a hybrid material by functionalizing carbon nanodots (CNDs) with the donor- π -acceptor organic dye L1 via amide coupling. By chemically modifying the surface of CNDs, we can enhance their multifunctionality and tailor their molecular composition. This innovative approach seeks to replace expensive dyes with cost-effective CNDs synthesized from citric acid and ethylenediamine using a domestic microwave oven, potentially improving the stability of the resulting hybrid. Additionally, TiO₂ anatase particles were synthesized as a metal oxide platform and sensitized with both pristine materials and the CND-L1 hybrid. A range of physicochemical methods was employed to analyze the elemental, structural, and optical properties of these materials. In photocatalytic degradation tests of methyl orange, the sensitized catalysts demonstrated significantly improved efficiency compared to TiO₂ alone. While CNDs exhibited good stability and enhanced L1's stability, scavenger experiments revealed that holes and hydroxyl radicals play crucial roles in the degradation mechanism. This research underscores the promise of CND hybrids in advancing pollutant degradation technologies while reducing reliance on costly photocatalysts.

Keywords: carbon nanodots; photocatalysis; sensitization; dye degradation; reusability



Academic Editor: Vincenzo Vaiano

Received: 12 December 2024

Revised: 23 December 2024

Accepted: 25 December 2024

Published: 30 December 2024

Citation: Debes, P.P.; Pagel, M.; Muntean, S.; Hessling, J.; Smarsly, B.M.; Schönhoff, M.; Gatti, T. Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis. *Photochem* **2025**, *5*, 1. <https://doi.org/10.3390/photochem5010001>

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1. Introduction

A significant contributor to environmental contamination is the discharge of wastewater from the textile industry, which is often loaded with dyes and other pollutants. This wastewater ultimately reaches natural systems, causing further damage to the environment [1]. The rise of fast fashion and overconsumption has led to a corresponding increase in the production of inexpensive textiles, which are often produced without adequate consideration of the waste they generate. The multitude of chemicals utilized in these processes has the potential to cause significant health and safety issues for humans and the environment [2]. Additionally, the primary route of release into the natural ecosystem occurs via aqueous media, resulting in rapid and continuous contamination of marine and ground water resources. Given that textile production is predominantly concentrated in

countries with inadequate infrastructure for waste management and regulatory oversight, the resulting pollution directly poses a considerable health risk to the local ecosystem and population [1,3]. In order to address this issue, a number of methods are currently being explored and have already been deployed with the objective of reducing the quantity of pollutants released. The majority of methods employed to reduce the concentration of harmful dyes in aqueous solutions are based on biological and physicochemical approaches. These approaches are highly effective in removing pollutants. However, the high sensitivity of biological systems to external conditions makes them difficult to apply in a versatile manner [4–6]. In the case of physicochemical approaches, the removal of pollutants is usually accompanied by the formation of secondary waste products, which only serve to distort the problem rather than solving the pollution issue [7,8].

Since the discovery of the properties of titanium dioxide (TiO_2) as a photocatalyst a few decades ago, research on photoinduced reactions has increased considerably [9]. In particular, photocatalysis offers a promising approach to the remediation of pollution, with a considerable number of photocatalytic systems being researched [10,11]. The products of these catalytically driven reactions are reactive oxygen species (ROS), such as $\bullet\text{OH}$ and $\bullet\text{O}_2^-$. These can further react with organic, inorganic or pathogenic contaminants, including viruses, leading to oxidative decomposition of the pollutants [12]. When considering the energy cost, solar light provides a simple and sustainable energy source for the procedure. This, in combination with the complete degradation of the pollutants, without the formation of by-products, offers a highly promising alternative compared to commonly used wastewater treatment methods [13]. TiO_2 is one of the most extensively researched materials in the field of photocatalysis, given its high abundance, low toxicity and chemical stability. A significant drawback is its high band gap energy of approximately 3.0–3.2 eV, which only permits light absorption below a wavelength of 390 nm, thereby accessing approximately 5% of the solar energy spectrum [14–16]. To address this limitation, a plethora of modifications can be implemented to enhance the degradation rate. These include heteroatom doping, morphological modification, and post-synthetic surface modification [17,18]. Dye sensitization represents a process that has been the subject of extensive research with the objective of increasing the absorption of light by a material. Research in this field has been particularly focused on dye-sensitized solar cells (DSSCs), where the enhancement of solar light absorption represents one of the key objectives in the advancement of energy production technologies [19,20]. Beyond DSSCs, this method offers improvement for various approaches of photoinduced processes, including photocatalysis [21]. The dye-sensitization process has been observed to enhance photocatalytic activity in comparison to alternative methods, such as doping, thereby underscoring its potential as a viable approach in visible-light photocatalysis [22–24]. The efficiency of dye-sensitized catalysts in facilitating charge separation through rapid electron injection from the excited state of the dye to the conduction band of TiO_2 has led to their emergence. The prospect of utilizing a class of catalysts capable of functioning under visible light irradiation, in conjunction with the potential for the recovery and reuse of photosensitizers, renders this process both environmentally and economically viable. However, the synthesis and cost of structurally complex dye molecules present challenges that must be considered when exploring dye-sensitization strategies. It is crucial to optimize the utilization of these dyes in order to facilitate broader applications of this technique in a more accessible and cost-effective manner.

The term “carbon dots” (CDs) collectively refers to a group of nanoscale carbonaceous particles that have been the subject of scientific interest since their initial discovery in 2004 [25]. Based on the synthetic approach employed, CDs can be classified into subcategories, including carbon nanodots (CNDs), carbon quantum dots (CQDs), and graphene

quantum dots (QDs). Classification according to the presence of sp^2 -carbon, size, heteroatom content, or photoluminescent (PL) properties may be employed as a means of differentiating between the various types of CDs [26]. CNDs are amorphous carbonaceous particles, whereas QDs contain a sp^2 -hybridized carbon core. QDs exhibit a layered structure and a composition analogous to that of graphene and graphite [27]. The bottom-up synthetic approach of CNDs, which employs simple organic molecules such as citric acid (CA), amino acids, and ethylene diamine (EDA), allows for significant variation in the surface functional group composition, rendering it a promising candidate for further modification [28–31]. In particular, the functionalization of CNDs with organic dye molecules has the potential to enhance their applications in hydrogen evolution and bio-imaging by extending their light-absorbing capabilities [32,33].

The objective of the present study is to synthesize a hybrid species by functionalizing CNDs derived from CA and EDA with an organic dye via amide coupling. The hybrid is then combined with TiO_2 particles for the purpose of assessing its photocatalytic performance in dye degradation reactions under light irradiation. Characterization is conducted using UV-Vis spectroscopy, X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), and scanning electron microscopy (SEM) to evaluate the optical properties, crystal phase, atomic composition, and size. In order to gain insight into the sensitization process, the characterization includes elemental analysis (EA) and thermogravimetric analysis (TGA). The catalytic degradation of methyl orange (MO) under UV irradiation can be monitored through absorbance measurements for the four catalysts comprising TiO_2 , TiO_2 -CND, TiO_2 -dye, and TiO_2 -hybrid. Finally, the degradation efficiency of the catalysts is evaluated, with special regard to determining catalytic activity relative to dye content, with the objective of minimizing the use of costly sensitizers. Moreover, reusability, and scavenger experiments are conducted to further examine the catalysts' stability and the varying involvement of reactive species. Scavengers are compounds that trap an oxidizing species or electron holes in order to diminish the respective participation in the degradation [34]. Commonly used scavengers include ethylenediaminetetraacetic acid (EDTA) and isopropyl alcohol (IPA). The use of scavengers to suppress the degradation of target molecules, such as MO, facilitates the identification of the most active ROS or charge carriers in the photocatalytic process. This technique offers valuable insight into the contribution and behavior of different reactive species in the overall degradation mechanism.

2. Results & Discussion

The synthesis of CNDs involved a microwave (MW)-assisted reaction between CA and EDA at a 1:1 ratio, followed by purification through filtration and size-exclusion chromatography [35]. To verify their presence and assess their properties, the synthesized CNDs were subjected to thorough characterization using atomic force microscopy (AFM), ultraviolet-visible (UV-Vis) absorbance, PL, and nuclear magnetic resonance (NMR) spectroscopy. The dimensions of the CNDs were characterized using AFM. As illustrated in Figure S1, the CNDs exhibit an average size of 2.0 ± 0.4 nm. In a previous study, the presence of CNDs featuring surface-exposed primary amine groups was reported [28]. These groups are capable of undergoing further functionalization via an amide coupling reaction. The synthesis and characterization of the donor- π -acceptor dye L1, which was synthesized for subsequent functionalization of the CNDs, is described herein (Figure 1). L1 is a commercially available dye that is utilized in the fabrication of DSSCs and in a multitude of applications within low-cost light-energy harvesting and conversion [36,37]. The CND-L1 hybrid underwent purification via filtration using a syringe filter and size exclusion gel chromatography.

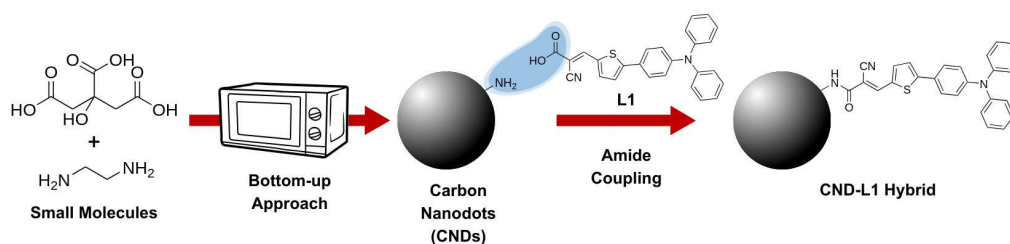


Figure 1. Schematic representation depicting the synthesis of CND from CA and EDA (1:1) via a MW-assisted reaction in water. This is followed by the amide coupling between the CND and the dye L1, which yields the CND-L1 hybrid.

To validate the successful functionalization of the CND with L1, diffusion-ordered spectroscopy (DOSY) NMR was employed. A comparable methodology was utilized by Prato and colleagues in 2018 to illustrate the covalent functionalization of CNDs [38]. Bare L1, pristine CND, a physical mixture of CND and L1, and the CND-L1 hybrid were subjected to 2D DOSY NMR as well as diffusion decay analysis. In all cases an integral over the chemical shift region from 8.9 to 6.4 ppm was evaluated, since in mixed and hybrid samples no separate L1 resonances could be identified. The results demonstrated a single diffusion/distribution profile for the L1, CND, and CND-L1 hybrid samples, indicating that the constituents of these samples are present in a mono-dispersed state. In contrast, the physical mixture of CND and L1 demonstrates two discrete diffusion distributions, indicating that the two moieties exhibit independent movement in the mixture (Figures S2–S5). In order to obtain more detailed quantitative data, the diffusion coefficients were derived from the diffusion echo decays (see Figure 2A) by applying the Stejskal-Tanner equation (see Supplementary Materials, Equation (S1)) [39]. Assuming a log-normal distribution of the diffusion coefficients (see Supplementary Materials, Equations (S2) and (S3)) resulted in the distributions in Figure 2B. The diffusion coefficient of pure L1 is depicted as a dotted red line at $1.94 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, as it does not possess a size distribution, in contrast to the CND or the CND-L1 hybrid. The pristine CND sample exhibits the maximum value for the particle diffusion coefficient (D), with a value of $1.07 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. The diffusion coefficient of the CND-L1 hybrid was found to be 33% lower than that of the pure CND, with a value of $0.72 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. As the particle size increases, the diffusion coefficients decrease, which is consistent with the successful functionalization of the CND. In contrast, the physical mixture of CND with L1 exhibits two distinct peaks in the diffusion domain of the 2D spectrum (Figure S4), which can be viewed as separate peaks in the distribution of diffusion coefficients in Figure 2B. The fast component exhibits a diffusion coefficient similar to that of bare L1. Moreover, the diffusion coefficient of the CNDs in the mixture, represented by the shoulder with the lower diffusion coefficient, agrees to that of bare CND. This shows that the physical mixture consists of L1 and CND as separate species. In contrast, the sample with the hybrid lacks any component with a fast diffusion coefficient, indicating the absence of single L1 molecules, while the diffusion of the CND particles is slowed down, reflecting the increase of radius due to functionalization.

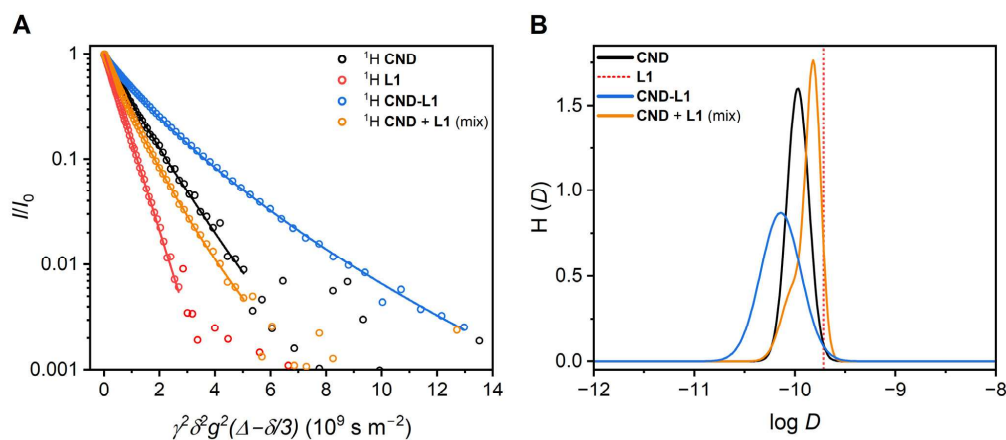


Figure 2. (A) The ^1H diffusion decay measurements, with shifts ranging from 8.9 ppm to 6.4 ppm, were conducted for CND, L1, as well as for a physical mixture of CND and L1 in a 10:1 weight ratio, and the CND-L1 hybrid. (B) The diffusion coefficients were analyzed using the method described by Lenocho et al. (2023) [40], which illustrates the diffusion coefficient distributions of CND, L1 (represented as a straight dotted line due to its lack of size distribution), the physical mixture of CND and L1 (10:1), and the CND-L1 hybrid.

In applying the Stokes–Einstein equation (see SI, Equation (S4)), the hydrodynamic diameter can be determined from the maximum diffusion coefficient. In this way the dimensions of the CND have been determined to be 1.9 nm, while CND-L1 exhibits a diameter of 2.8 nm (Table 1). The dimensions of the CND are consistent with the findings of the AFM analysis.

Table 1. Maximum of the diffusion coefficient distribution obtained from fitting of echo decays, along with the hydrodynamic diameter calculated using the Stokes–Einstein equation.

Sample	$D/\text{m}^2 \text{ s}^{-1}$	Hydrodynamic Diameter/nm
CND	1.07×10^{-10}	1.90
L1	1.94×10^{-10}	1.00
CND + L1 (physical mix) [†]	0.98×10^{-10}	2.00
CND-L1	1.53×10^{-10}	1.30
	0.72×10^{-10}	2.80

[†] The physical mixture corresponds to a weight ratio of 10:1 of CND to L1.

The UV-Vis and PL spectra of pure CND, L1, and the CND-L1 hybrid are presented in Figure 3. CND displays an absorption maximum at 357 nm, accompanied by a corresponding emission maximum at 443 nm. L1 displays absorption at both 302 nm and 412 nm, with the latter being the more prominent of the two. An emission maximum was observed at 550 nm ($\lambda_{\text{Ex, L1}} = 412 \text{ nm}$). A comparison of the spectra of pure CND and L1 with those of CND-L1 reveals the presence of a combination of both initial materials, with absorption at 302 nm, 357 nm, and 454 nm. However, while the absorption originating from the CND molecule is identical, the absorption originating from L1 at 412 nm exhibits a red shift, with a maximum at 454 nm. No notable shift in the emission spectra was observed for the CND-L1 hybrid.

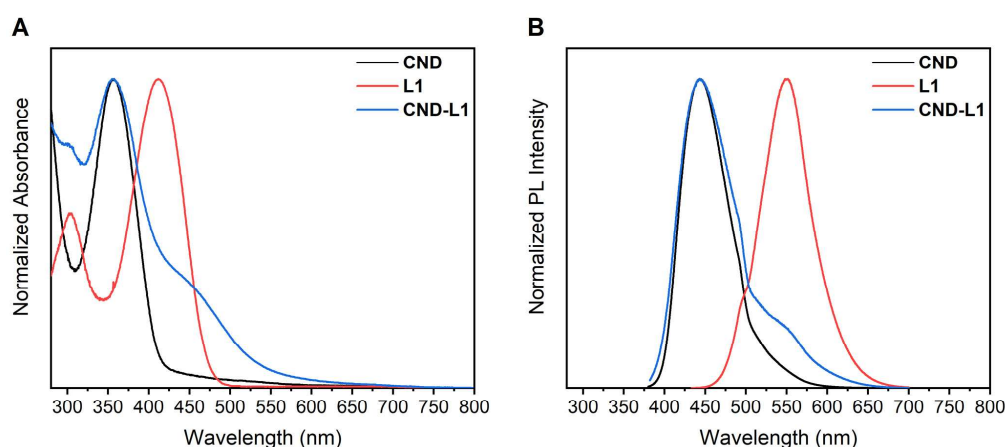


Figure 3. Normalized (A) absorbance and (B) PL of CND (black lines), L1 dye (red lines), and CND-L1 hybrid (blue lines).

The photoluminescence quantum yield (PLQY) of CND was found to be 39.0%, that of L1 6.8%, and of the CND-L1 hybrid 9.6% (Figure S6), as determined by the relative method with quinine sulfate as the reference. The functionalization of CND resulted in a notable decrease in PLQY, indicating that the surface amines of CND may have undergone functionalization, potentially leading to a non-radiative de-excitation pathway [41,42]. This is because surface states are a significant source of PL in CNDs [29]. Furthermore, a through-space mechanism, such as Förster resonance energy transfer (FRET) or electron transfer, is a viable option, given the close proximity of the units [43–46]. FRET is based on a dipole-dipole interaction, where the resonance between the donor's emission and the acceptor's absorbance and the relative orientation of their transition dipole moments are crucial [47]. In contrast, electron transfer necessitates a substantial overlap between the donor and acceptor orbitals, a prerequisite that is plausible. As demonstrated in Figure 3, the CND emission strongly overlaps with the dyes absorbance, rendering a FRET from CND to the L1 dye feasible [48,49]. Both FRET and electron transfer generally result in a reduced PLQY. This outcome indicates the potential for both FRET and the process of charge transfer to influence the overall efficiency of quenching. It is noteworthy that the efficiency of charge transfer is significantly dependent on the concentration of acceptor molecules in the system. The absence of a well-defined structural configuration in the synthesized hybrid and the imprecise determination of the number of dye molecules residing on the surface of a single CND hinders the precise quantification of the respective contributions of charge and electron transfer processes [45]. The data presented in Table 2 provides a comprehensive overview of the absorption and emission characteristics, as well as the PLQY, and absorption coefficient, of the pristine and functionalized samples (Figures S6 and S7). In addition, the excitation–emission maps and the excitation spectra are in agreement with the previously mentioned results (Figures S8 and S9).

Table 2. UV-Vis absorption maxima (λ_{Abs}), PL maxima (λ_{Em}), Stokes shift, PLQY, and absorption coefficient (ϵ at 412 nm) of the CND-L1 hybrid compared to pristine CND, and L1.

Sample	$\lambda_{\text{Abs}}/\text{nm}$	$\lambda_{\text{Em}}/\text{nm}$	Stokes Shift/nm	PLQY/%	$\epsilon_{412\text{nm}}/\text{L g}^{-1} \text{ cm}^{-1}$
CND	357	443	86	39.0	0.79
L1	412	550	138	6.8	92.7
CND-L1	357	443	86	9.6	3.29

TGA reveals a significant weight loss for CND, L1, and the CND-L1 hybrid between 100 °C and 300 °C, as illustrated in Figure S10. Both CND and L1 exhibit decomposition within a similar temperature range. Notably, the TGA profiles of the CND-L1 hybrid demonstrate a greater mass loss compared to that of pure CND, suggesting the occurrence of functionalization. The residues at 900 °C during the TGA measurement would correspond to approximately a 50% mass fraction of dye L1 in the CND-L1 hybrid. However, the XPS and EA measurements do not indicate this 50% ratio; instead, they show a strong similarity to pure CND, suggesting a significantly lower proportion of dye in the hybrid (Figure S11). Nonetheless, the concurrent degradation of both components and the considerable changes in the morphology of the CND limit the ability to accurately quantify the extent of functionalization. For this reason, a deconvolution of the absorption spectrum of the CND-L1 hybrid was performed, with the aim of separating it into its two components, L1, and CND. This was achieved by utilizing four Gaussian functions (Figure S12). By employing the absorbance of the dye L1 at 454 nm, derived from the deconvolution contribution, and assuming that the experimental absorption coefficient of the pure dye L1 at 412 nm can be applied, we can calculate the corresponding concentration using Beer-Lambert's law. The concentration of the CND-L1 hybrid is known, consequently from the absorption spectrum we can estimate the weight percentage of L1 dye to be 2.2%. In our previous study, we demonstrated that the CND under consideration could be functionalized up to 80 $\mu\text{mol g}^{-1}$ via an amide coupling, with the 2.2% calculated here corresponding to approximately 50 $\mu\text{mol g}^{-1}$ [28]. The larger size of the L1 dye in comparison to the moiety utilized in our previous study led to a slight reduction in the degree of functionalization. We therefore assume the 2.2% of L1 dye on the CND-L1 with a reasonable degree of approximation.

TiO₂ particles were synthesized at a calcination temperature of 350 °C with a heating ramp of 2 °C min^{−1} [50]. The selection of a lower calcination temperature was intended to produce TiO₂ particles with an increased surface area, which may result in a higher density of surface hydroxyl groups. It is anticipated that this enhancement will facilitate greater degrees of sensitization and improved photocatalytic activity [18]. A nitrogen physisorption (77 K) isotherm was used to determine the surface area of the TiO₂ particles using the BET approach, resulting in a value of 82 m² g^{−1} (Figure S13). In the low-pressure regime of the isotherm, the presence of microporosity is negligible, while the majority of the pore volume is comprised of mesopores in the form of interstitial particle space, with a mode diameter of 12 nm. The SEM images indicate a particle size of 31 ± 5 nm, in addition to the presence of aggregated particles in an aqueous solution, with an average diameter of 685 ± 160 nm, as determined by dynamic light scattering (DLS) measurements (Figure S14). The XRD measurements indicate that the synthesized TiO₂ particles consist entirely of anatase, as only reflections corresponding to the anatase phase are observed (Figure S15). In the following step, the synthesized TiO₂ particles were sensitized with CNDs, L1 dye, and CND-L1 hybrid in acetonitrile. The resulting mixture was then subjected to centrifugation, and the particles were washed multiple times with water through sonication and centrifugation. This process yielded three distinct sensitized photocatalysts, namely: TiO₂-CND, TiO₂-L1, and TiO₂-CND-L1 (Figure 4).

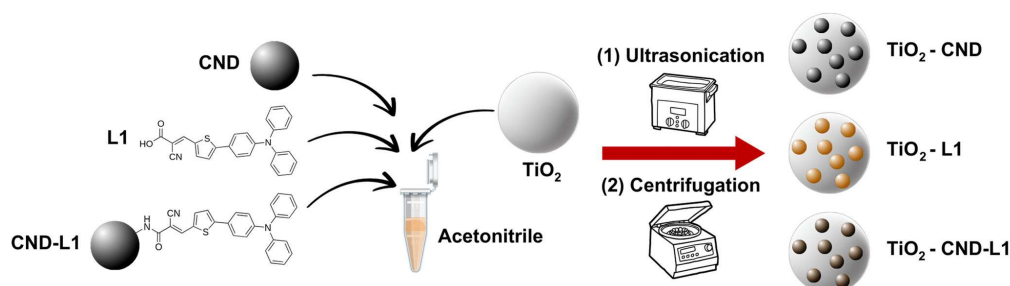


Figure 4. Schematics of the TiO₂ nanoparticles sensitization process with CNDs, L1 dye, and a CND-L1 hybrid. After this initial step, the mixture was treated with ultrasonication followed by centrifugation. The particles were subsequently washed several times with water using a combination of sonication and centrifugation. This procedure resulted in the formation of three unique sensitized photocatalysts: TiO₂-CND, TiO₂-L1, and TiO₂-CND-L1.

A visible color change from white to yellow, red, and brown was observed for TiO₂-CND, TiO₂-L1, and TiO₂-CND-L1, respectively. This prompted us to perform diffuse reflectance spectroscopy on both the TiO₂ and sensitized catalysts (Figure 5). The spectra of the sensitized samples unambiguously demonstrate that the absorbance range of pristine TiO₂ is extended in the region between 400 nm and 600 nm, which is indicative of a successful sensitization process. The indirect band gap of TiO₂ anatase particles was determined to be 3.19 eV through the conversion of diffuse reflectance data to the Kubelka-Munk function [51] and the subsequent application of Tauc plot [52] analysis (Figure S16).

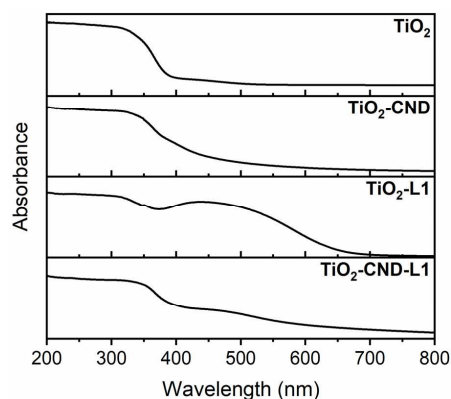


Figure 5. Diffuse reflectance spectra of TiO₂ nanoparticles compared to the three sensitized TiO₂ samples: TiO₂-CND, TiO₂-L1, and TiO₂-CND-L1.

Furthermore, XPS measurements were conducted, the results of which are presented in Figure S17. As previously demonstrated in Figure S11, the XPS analysis of the pure L1 dye and CND-L1 hybrid exhibited a negligible sulfur content, rendering its detection on the nanocomposite challenging. XPS is a highly surface-sensitive method that does not consider the entire particle, but rather a limited area on the surface. Considering the total amount of 12 mg sensitizer added to the 500 mg TiO₂, the theoretically calculated mass fraction of all three sensitizers on TiO₂ is 2.34%, but it should be noted that the mass fraction might differ due to the washing steps. Accordingly, the resulting mass fractions were determined by TGA measurements and are presented in Figure S18. The

actual loading of CND, L1, and CND-L1 on the TiO₂ particles was determined at 900 °C, resulting in a weight loss of 0.73%, 1.27%, and 2.70%, respectively. It is assumed that all organic matter on the catalyst will be decomposed at 900 °C. The observed increase in weight at temperatures above 500 °C is thought to result from the possible phase transition from amorphous powder to anatase phase [53]. The different adsorption capabilities of the sensitizers may be attributed to interactions between the TiO₂ surface and the surface moieties of CND, L1, and CND-L1. The CND exhibits a plethora of oxygen- and nitrogen-based functional surface groups, including carboxylic acids, which are capable of coordinating with surface Ti in a monodentate or bidentate manner [54]. The L1 dye exhibits hydrophobic characteristics due to its apolar structure, which results in reduced absorbability onto TiO₂. In the hybrid, the accessible amine groups are functionalized with dye molecules, whereby the number of carboxylic acid moieties remains unaltered and can interact with TiO₂. Given that the preparation of the catalysts entails washing steps with water, the resulting catalyst loading can be explained by the fact that the CND will be washed off the most. The L1 dye will likely adsorb less due to a low number of moieties for coordination. By combining the hydrophobic properties of the L1 dye with the large number of coordinating functional groups of CND, the CND-L1 hybrid enables the highest amount of adsorbed species. A rapid decline in mass of 0.3% can be observed at about 580 °C in the TGA measurement of the TiO₂-CND-L1 (Figure S18). This indicates that the mass fraction of the CND-L1 hybrid on the TiO₂ nanoparticles is approximately 2.4%. Given potential deviations, this value is reasonable and aligns with the maximum theoretical value of 2.34%.

In this study, pure TiO₂, CND-, L1-, and CND-L1-sensitized TiO₂ catalysts are employed to investigate the impact of sensitization on the catalytic performance. The photocatalytic degradation process was conducted at a temperature of 22.5 °C. 10 mg of the catalyst were added to 10 mL of an aqueous MO solution (50 µM), and the mixture was stirred for 30 min in the absence of light. The pH of the MO solution was 6.5. In order to perform the catalysis, a beaker with continuous cooling at 22.5 °C and a 395 nm UV LED (Figure S19) has been employed. Each catalysis cycle was conducted for a period of 50 min, with 250 µL aliquots being sampled at 10-min intervals. The extent of the degradation process was determined by measuring the UV-Vis transmission of the solution of degraded MO, which exhibited a maximum absorbance at 464 nm. In order to quantify the degradation activity, the degradation efficiency and first-order kinetics were calculated using the following Equations (1) and (2), respectively.

$$\ln\left(\frac{A_0}{A_t}\right) = kt \quad (1)$$

$$\text{degradation efficiency}(\%) = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad (2)$$

In this case, A_0 represents the initial absorbance following adsorption equilibrium, k is the rate constant, t is time, and A_t is the absorbance at a given time t . Figure 6A–C illustrates the catalytic results in terms of normalized absorbance, first-order kinetics, and degradation efficiency. Moreover, the same catalysis procedure was conducted in the absence of irradiation to eliminate any significant effects of MO adsorption onto the catalyst's surface. This was done to exclude any non-irradiation-based degradation that could distort the results (Figure 6D). All sensitized TiO₂ catalysts exhibited a higher rate constant than the unfunctionalized TiO₂ nanoparticles. The rate constant of TiO₂-L1 is approximately five times higher than that of TiO₂-CND-L1. In comparison to TiO₂-CND,

however, the rate constant could be increased by 73% for the TiO₂-CND-L1 catalysts with dye functionalization (Table 3).

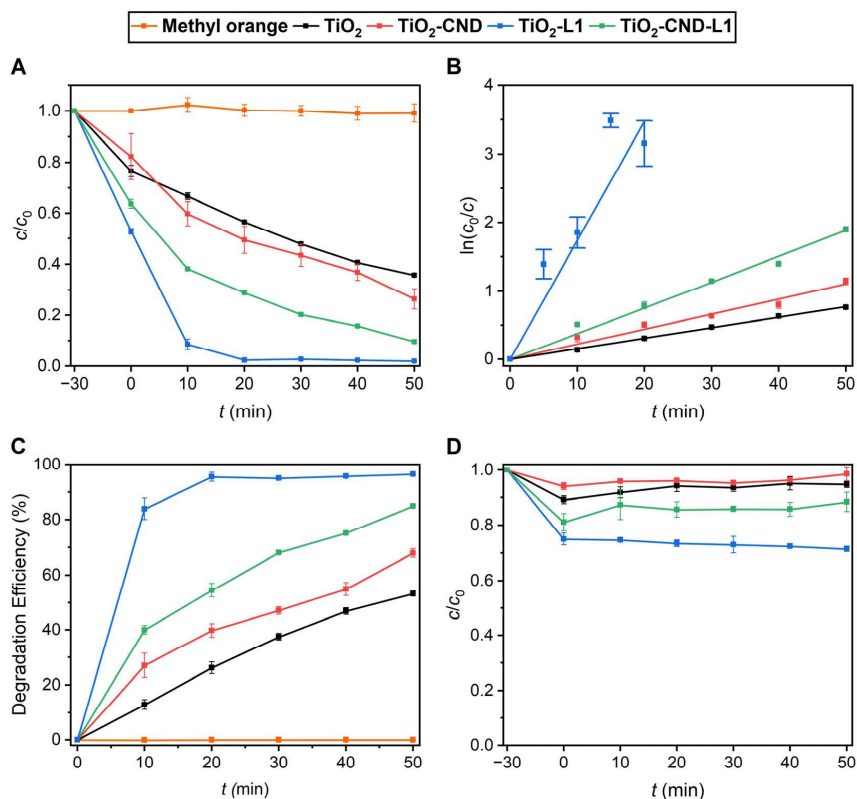


Figure 6. The results of the catalytic degradation are presented in three ways: (A) normalized absorbance measurements, (B) first-order kinetics analysis, and (C) degradation efficiency. These results demonstrate that all sensitized catalysts exhibit superior performance compared to bare TiO₂ and catalyst-free photolysis of MO. In the absence of irradiation, no significant degradation was observed (D), indicating that any potential adsorption or degradation occurring without irradiation is effectively negligible.

Table 3. First order kinetics rate constants k of the respective catalysts.

Sample	k/s^{-1}
TiO ₂	0.16
TiO ₂ -CND	0.22
TiO ₂ -L1	1.74
TiO ₂ -CND-L1	0.38

In comparison to the photolysis of MO, a notable degree of degradation was observed for all TiO₂ materials. With regard to the composite catalysts, all three were found to enhance the overall catalytic performance. The pure TiO₂ underwent degradation of up to 50% of MO under UV irradiation, as a consequence of exciton formation. These excitons contribute to the degradation of MO either directly or indirectly through the production

of ROS, which further promote the decomposition process [55]. Among the composite materials, TiO₂-CND exhibited the lowest degradation efficiency, with a rate of 57%. The TiO₂-CND-L1 composite demonstrated up to 83% degradation over a 50-min irradiation period, whereas TiO₂-L1 exhibited complete degradation after 20 min. The elevated degradation rate observed in the composite materials relative to the pure TiO₂ can be attributed to two factors: firstly, the enhanced capability and range of light absorption. Secondly, the effective prevention of exciton recombination is achieved through the improved facilitation of excitons reaching the surface to react with the medium. The loading of the dye L1 for the catalysts, TiO₂-L1, and TiO₂-CND-L1, was calculated based on the amount of sensitizer present on the TiO₂ component via TGA (Figure S18). A total of 2.4% of the hybrid CND-L1 was identified on the surface of TiO₂-CND-L1. Based on the deconvolution of the UV-Vis spectrum of CND-L1 (Figure S12), it was determined that 2.2% of the hybrid can be attributed to the L1 dye. Consequently, the amount of L1 dye present in 10 mg of the TiO₂-CND-L1 catalyst has been determined to be 0.00538 mg. In contrast, the TiO₂-L1 catalyst contains 0.127 mg of the L1 dye in 10 mg of catalyst. This corresponds to a 24-fold increase in the amount of L1 dye present on the TiO₂-L1 catalyst surface. The functionalization of CND increases the ability to adsorb in combination with the dye, which contributes to efficient light absorption and electron transfer. The CND-L1 hybrid indicates a significant improvement in catalytic degradation depending on the amount of dye on the catalyst. In order to investigate the reusability of the catalysts, a reaction mixture consisting of 10 mg of the respective catalyst in the corresponding volume (1 mL MO solution per 1 mg catalyst) was irradiated for 50 min. The degradation efficiency of each catalyst is represented by gray bars following each catalytic cycle in Figure 7. The red bars additionally indicate the amount of catalyst that could be recovered for subsequent runs following the completion of each catalytic cycle. The values presented refer to the quantities weighted prior to the initiation of the first cycle.

The results of the TiO₂ measurements (Figure 7A) indicate an increase in the degradation efficiency of the catalyst, rising from an average degradation efficiency of 57.9% in the first cycle to 80.2% in the third cycle. This can be attributed to a post-synthesis aging process of the utilized TiO₂ particles. Some photocatalysts are known to require a period of use to reach their maximum efficiency due to changes in their structure [56]. Furthermore, we employed ultrasonication between each catalytic cycle, which has the potential to enhance the efficiency. In contrast, the other three modified catalysts, TiO₂-CND, TiO₂-CND-L1, and TiO₂-L1, exhibited a reversed trend, demonstrating a decline in efficiency during catalysis. The TiO₂-CND (Figure 7B) catalyst exhibited the lowest increase in degradation efficiency, while demonstrating the highest stability among the sensitized catalysts. In the initial cycle, TiO₂-CND achieved an efficiency of 73.5%, which decreased by 5.9% to 67.6% by the third cycle. The TiO₂-L1 catalyst (Figure 7C) demonstrated the highest degradation efficiency of 94.9% in the initial cycle, which subsequently decreased by 8.7% to 86.2% by the third cycle. In comparison, the TiO₂-CND-L1 catalyst (Figure 7D) exhibited a similarly high initial efficiency of 94.4%. However, this efficiency declined significantly, reaching 73.3% in the third cycle, which represents a 21.1% decrease. This observation indicates that, despite the TiO₂-CND-L1 catalyst having 24 times less dye on its surface, it maintains a comparable high efficiency to the TiO₂-L1 catalyst during the first cycle.

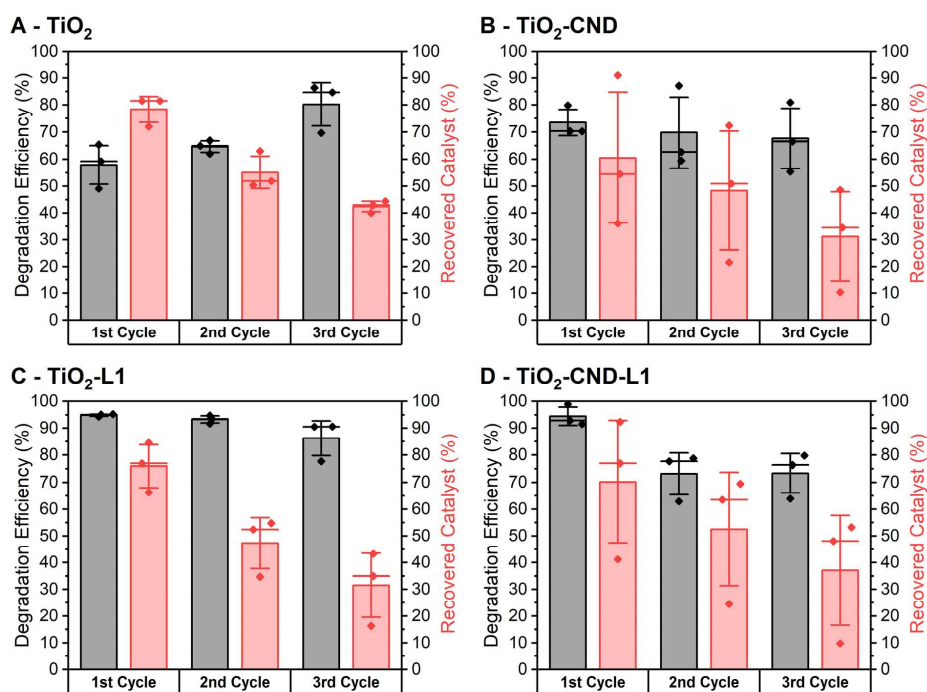


Figure 7. Degradation efficiency of the different catalysts (depicted in grey) and fraction of recovered catalyst after each cycle (depicted in red).

As illustrated in Figure 7C, the L1 dye does not demonstrate permanent photostability on the TiO_2 surface. It appears that the L1 dye was primarily degraded in the $\text{TiO}_2\text{-CND-L1}$ catalyst, as the efficiency in the second cycle decreased to 73.1%, which is comparable to the efficiency of the $\text{TiO}_2\text{-CND}$ catalyst. This suggests that the CND was the sole active component in the $\text{TiO}_2\text{-CND-L1}$ catalyst from the second cycle onwards, while the L1 dye appears to have lost its activity. The markedly lower concentration of L1 dye in the $\text{TiO}_2\text{-CND-L1}$ catalyst compared to the $\text{TiO}_2\text{-L1}$ catalyst results in a more rapid depletion of the dye during photocatalytic activity. It can be concluded that covalent binding with CNDs does not result in a notable enhancement in the activity of the L1 dye. However, the dye appears to exhibit enhanced stability when bound to the CND.

In order to facilitate a more accurate comparison of the stability of the L1 dye within the $\text{TiO}_2\text{-L1}$ catalyst with that of the $\text{TiO}_2\text{-CND-L1}$ catalyst, we calculated the turnover number (TON). The TON is a metric for comparing the efficiency of different catalysts. It indicates the number of molecules of a reactant converted per active catalyst center until the catalyst is inactive. In this analysis, we assume that the L1 dye is the active center in the respective catalysts. In this context, a higher TON indicates that the L1 dye on the respective catalyst is more effective and can decompose a greater quantity of MO, thereby demonstrating superior catalytic conversion. In the case of the $\text{TiO}_2\text{-CND-L1}$ catalyst, it was assumed that the L1 dye would remain active only during the first cycle. Consequently, the amount of MO degraded during this first cycle was used as the basis for calculation. In the case of the $\text{TiO}_2\text{-L1}$ catalyst, the amount of MO degraded was taken from the total of three cycles. It is noteworthy that the L1 dye in the $\text{TiO}_2\text{-L1}$ catalyst retains partial activity following three cycles. The TON for the $\text{TiO}_2\text{-L1}$ catalyst was found

to be 1.57, while the $\text{TiO}_2\text{-CND-L1}$ catalyst exhibited a TON of 16.8 (see SI for details). Consequently, the TON for the $\text{TiO}_2\text{-CND-L1}$ catalyst is 10.7 times greater than that of the $\text{TiO}_2\text{-L1}$ catalyst (Figure 8). This suggests that, in order to achieve the same level of conversion as the $\text{TiO}_2\text{-CND-L1}$ catalyst, the L1 dye on the $\text{TiO}_2\text{-L1}$ catalyst would need to decompose 10.7 times more MO than it has done so far. In light of the observed decline in the efficiency of the $\text{TiO}_2\text{-L1}$ catalyst by 8.7% following three cycles and the fact that only 32% of the catalyst amount could be recovered after these three cycles, this result appears implausible. These findings indicate that the $\text{TiO}_2\text{-L1}$ catalyst exhibits notable limitations in sustainability when compared to its CND-modified counterpart. It is important to note that the presented catalysts herein are not run through an optimization process of the conditions influencing the catalytic performance yet, namely dye-concentration, mass of catalyst, pH value, temperature, wavelength, and radiant flux. This would require an in depth study to figure out the optimal catalytic efficiencies of each catalyst.

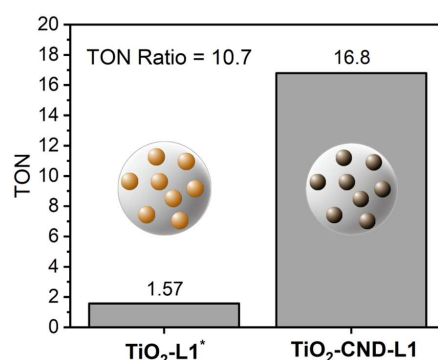


Figure 8. A comparison of the TON of the $\text{TiO}_2\text{-CND-L1}$ catalyst with that of the $\text{TiO}_2\text{-L1}$ catalyst, with relation to the quantity of L1 dye present on each catalyst. * The L1 dye in the $\text{TiO}_2\text{-L1}$ catalyst exhibits residual activity following three cycles.

In order to identify the reactive species involved in the degradation process of MO, a variety of scavenger reagents were employed. Scavengers are capable of trapping specific radicals that may form during the reaction. Consequently, a reduction in the degradation efficiency resulting from the presence of one of the scavenger compounds can be employed to evaluate the impact of the corresponding radical on the degradation mechanism. Specifically, *p*-benzoquinone (*p*-BQ) was employed as a scavenger for superoxide radicals ($\bullet\text{O}_2^-$), IPA was used to scavenge hydroxyl radicals ($\bullet\text{OH}$), and EDTA was applied to capture holes (h^+).

p-BQ was observed to react with TiO_2 and the reaction mixture turned black (Figure S20), even in the absence of an MO solution. The change in color resulted in an inability to measure the UV-Vis absorbance of the MO. Accordingly, an alternative trapping agent for $\bullet\text{O}_2^-$ was selected. Mondol et al. conducted the scavenger experiment with ascorbic acid (AA) to investigate the presence of $\bullet\text{O}_2^-$ species [57].

The results indicate that the addition of EDTA significantly reduces the degradation efficiency of all catalysts (Figure 9). The presence of EDTA resulted in a notable decline in the degradation efficiency of the TiO_2 catalyst, with a reduction of the degradation efficiency of 53.6%. This was followed by the most pronounced decrease in the $\text{TiO}_2\text{-CND}$ catalyst, with a reduction of 89.4%. The $\text{TiO}_2\text{-CND-L1}$ catalyst exhibited a moderate decline of 78.3%, while the $\text{TiO}_2\text{-L1}$ catalyst demonstrated the least significant reduction, of 47.7%. The role of the holes formed in the mechanism is of significant weight in each of the reactions. CNDs are capable of preventing the electron-hole recombination, which

increases the amount of electrons and holes available for catalysis, a phenomenon that can be observed in this experiment [58]. The addition of IPA resulted in a 23.3% reduction in the efficiency of the process for TiO_2 , 23.4% for $\text{TiO}_2\text{-CND}$, 13.4% for $\text{TiO}_2\text{-CND-L1}$, and 15.0% for $\text{TiO}_2\text{-L1}$. Additionally, $\bullet\text{OH}$ radicals are present in the MO degradation reaction with each catalyst, though they exert less influence on the subsequent reaction than h^+ . In contrast to the observed decrease in degradation efficiency for the other catalysts, the addition of AA resulted in an increase of the degradation efficiency for all four catalysts. For TiO_2 , the observed increase was 69.5%, while for $\text{TiO}_2\text{-CND}$, it was 32.0%. The remaining two catalysts had already nearly completely degraded the MO in the absence of scavenger molecules. However, the reference efficiencies were determined at a pH value above 4.4 at a wavelength of 464 nm, while the degradation efficiency with AA was calculated using the results of the UV-Vis measurement at 517 nm at pH 3.0. To eliminate the possibility that the acidic environment is responsible for the improvement in the reaction, reference measurements were performed without ascorbic acid, but at a pH value of 3.0. To prevent the introduction of other molecules into the solution that could act as scavengers, the solution was acidified with dilute HCl (Figure S21). A comparison with the aforementioned reference values also demonstrates that the reaction exhibits enhanced degradation efficiencies when AA is present in the reaction mixture. It has been demonstrated that AA can form a complex with TiO_2 , which serves to mediate the formation of $\bullet\text{O}^{2-}$. This enhances the photocatalytic decomposition of MO under UV light [59].

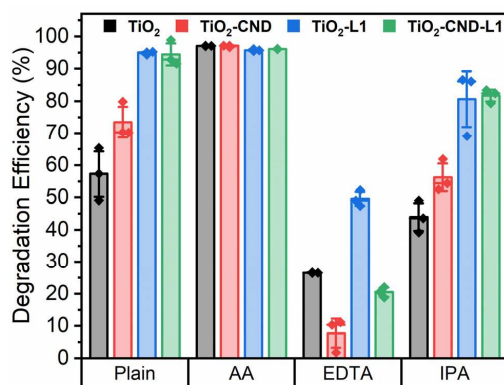


Figure 9. Effects of different radical scavengers on the MO degradation efficiency of the respective catalysts. Plain = no scavenger added; AA = ascorbic acid; EDTA = ethylenediaminetetraacetic acid; IPA = isopropyl alcohol.

The application of EDTA demonstrates the most significant scavenging effect, indicating that the predominant occurrence is the formation of holes in the mechanism. In general, heterojunction photocatalysts are designed to create junctions between the different materials. The implementation of this approach offers several advantages, including improved charge separation, enhanced catalytic activity, and broader light absorption [55]. Given that the energy levels of the various materials typically differ from one another, a resulting band offset is created at the interface. Following irradiation with light in both materials, electron–hole pairs are generated. Due to the band offset, electrons migrate from the conduction band (CB) of one material to the CB of the other material, while holes migrate in the opposite direction. This separation prevents recombination, thereby increasing the availability of electrons and holes for photocatalytic reactions [55].

In the absence of a scavenger or available trap states, the recombination of the electron and the hole is prevented. In response, the holes can act as powerful oxidants directly or indirectly, while the electron acts as a reductant [60]. The holes may also undergo further reactions with adsorbed water, hydroxides, and surface titanol groups, resulting in the formation of hydroxyl radical species. These species have the potential to promote the mineralization of MO. The presence of IPA was also found to result in the generation of hydroxyl radicals. It can thus be concluded that the main active species from the presented catalysts for MO degradation are holes, h^+ , and hydroxyl radicals.

The degradation mechanism of MO is dependent on several parameters, including dye concentration, catalyst mass, wavelength, radiant flux, and the addition of oxidants or the presence of natural substances such as inorganic ions. A comprehensive analysis, including the detection and quantification of the various photoproducts, is essential for a detailed understanding of the degradation process [61]. Given the unconfirmed structural and energetic attributes of the CNDs, a comprehensive examination of the electron transfer and hole generation processes within the developed heterojunctions is not within the scope of this study.

3. Conclusions

In this study, MW-assisted synthesized CNDs derived from CA and EDA were employed to develop a novel hybrid material through amide coupling with the dye L1, which is recognized for its application in DSSCs. The optical characteristics of this hybrid were meticulously characterized using UV-Vis and PL spectroscopy, while its compositional analysis was conducted via XPS, EA, and TGA. The formation of covalent bonds between the CND and L1, along with the dimensions of the hybrid, was confirmed through DOSY NMR.

Subsequently, TiO_2 particles were synthesized and characterized employing SEM, DLS, physisorption techniques, and XRD. By adsorbing CNDs, L1, and the CND-L1 hybrid onto TiO_2 , three distinct sensitized catalysts were generated and subjected to further examination using XPS, TGA, and diffuse reflectance spectroscopy. Notably, CND-L1 hybrid exhibited near-complete adsorption on the TiO_2 surface, in contrast to the less effective attachment of the pure L1 dye and CNDs.

The photocatalytic degradation of MO was evaluated across all three sensitized catalysts in comparison to TiO_2 alone. Each sensitized catalyst exhibited enhanced degradation efficiency relative to TiO_2 . Reusability studies demonstrated that while the CND exhibited the greatest stability of the sensitizers over time, the activity of the catalysts containing L1 dye diminished. Despite containing 24 times more L1 in the TiO_2 -L1 catalyst than in the TiO_2 -CND-L1 variant, the latter appeared to exhibit a higher TON.

The results of scavenger experiments highlighted the crucial roles of holes and hydroxyl radicals in the MO degradation mechanisms. Despite the fact that efforts to quantify $\bullet O_2^-$ radical levels using *p*-BQ and AA as scavengers yielded inconclusive results, thereby leaving open the possibility that superoxide anions play a pivotal role in the catalysis, it was evident that the addition of AA improved degradation efficiency for both TiO_2 and TiO_2 -CND catalysts. In contrast, TiO_2 -L1 and TiO_2 -CND-L1 approached nearly 100% degradation without AA addition.

In summary, the synthesis of CND hybrid materials presents a promising strategy for enhancing dye adsorption on TiO_2 while minimizing dye usage. This approach not only enhances catalyst stability during catalysis but also offers potential cost savings by reducing reliance on expensive dyes due to the economical synthesis of CNDs. Furthermore, the presence of CNDs facilitates hole generation within both pristine and functionalized states in catalysts. This study provides a firm basis for future research into additional

hybrid materials with diverse dyes and optimization of reaction conditions with the aim of achieving more efficient pollutant degradation.

4. Materials and Methods

Citric acid (CA, $\geq 99.5\%$), 3-[(ethyylimino)methylidene]amino)-*N,N*-dimethylpropan-1-amine hydrochloride (EDC, $\geq 99\%$), tetrakis(triphenylphosphin)palladium(0) ($\geq 98\%$), and ethylenediamine (EDA, $\geq 99.5\%$) were purchased from Carl-Roth (Karlsruhe, Germany). *N*-hydroxysuccinimide (NHS, $\geq 99\%$), ammonium acetate ($\geq 98\%$), and cesium carbonate (99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bromothiophene-2-carbaldehyde ($>97\%$) was purchased from TCI (Zwijndrecht, Belgium). Triphenylamino boronic acid (98%) was purchased from BLDpharm (Shanghai, China). Cyanoacetic acid (97%), and titanium(IV) chloride (99.9%) were purchased from Thermo Scientific (Waltham, MA, USA). Glacial acetic acid (99–100%) was purchased from VWR Chemicals (Radnor, PA, USA). Deuterated dimethyl sulfoxide (DMSO- d_6 , 99.8%), chloroform ($CDCl_3$ - d , 99.8%), and acetone- d_6 (99.8%) were purchased from Deutero (Sejong-si, Republic of Korea). Anhydrous *N,N*-dimethylformamide (DMF, 99.8%, AcroSeal) and tetrahydrofuran (THF, 99.5%, AcroSeal) were purchased from Thermo Scientific (Waltham, MA, USA). Dimethylsulfoxide (DMSO, 99.5%) was purchased from Gruessing (Filsim, Germany). Solvents for UV-Vis and PL spectroscopy was purchased from Merck (Darmstadt, Germany) (Uvasol® quality). Ultrapure water was obtained with the Milli-Q Direct Water Purification System. Unless otherwise stated, the reagents were used without further purification. The MW-assisted reactions were performed in a Silvia homeline set-up. The purification of CNDs was performed with Cytiva (Marlborough, MA, USA) Sephadex G-10 and LH-20 gel filtration media for size-exclusion chromatography. NMR-spectra were recorded with a Avance II 400 MHz, and Avance III 400 MHz HD (both Bruker, Ettlingen, Germany). Diffusion measurements were performed using an AVANCE III HD 400 MHz NMR spectrometer equipped with a gradient probe head (“Diff50”, Bruker, Ettlingen, Germany) with a selective 1H insert, providing a magnetic field gradient strength of up to $28\ T\ m^{-1}$. The samples were measured at $25\ ^\circ C$. To calibrate the temperature a PT 100 thermocouple inserted in an NMR tube, filled with oil, was used. UV-Vis measurements were carried out with a SPECORD® 200 PLUS spectrophotometer equipped with two automatic eight-fold cell changers and a Peltier element thermostat system ($0.1\ ^\circ C$ accuracy) by Analytik Jena (Jena, Germany). The system was operated with the ASpect UV 2.0 software by Analytik Jena. Fluorescence measurements were conducted with a Jasco (Heckmondwike, UK) FP-8300 Fluorescence Spectrometer. The sample solutions were measured in QS High Precision Cells made of Quartz Suprasil® with a light path of 10 mm by Hellma Analytics (Muellheim, Germany). Experiments were carried out in concentrations of $6\text{--}14\ mg\ L^{-1}$. Stock solutions ($1\ mg\ mL^{-1}$) were prepared from freshly purified samples, and then diluted prior to every UV/Vis and PL experiment. The spectra of the L1 dye was obtained using $0.5\text{--}2.5\ \mu mol\ L^{-1}$ solutions in DMSO at $25\ ^\circ C$. UV-Vis spectra of catalysis were recorded using a Bio-Tek Uvikon XS Spectrophotometer by Agilent (Santa Clara, CA, USA), controlled by UV VISION 1.0 Lite software. The sample solutions were measured in QS High Precision Cells made of Quartz Suprasil® with a light path of 1.0 mm by Hellma Analytics (Muellheim, Germany). Diffuse reflectance spectra were recorded using a Cary 5000 UV-Vis-NIR spectrophotometer by Agilent (Santa Clara, CA, USA) in conjunction with the Cary WinUV Scan Application software (ver. 6.3.0.1595) from Agilent Technologies (Santa Clara, CA, USA). The spectrophotometer was equipped with a Praying Mantis™ Diffuse Reflectance Accessory from Harrick Scientific Products (Pleasantville, NY, USA) and operated in double beam mode, with a reduced slit for white samples and full slit for colored samples. The data was collected in reflectance units, as this method is relative, hence Polytetrafluoroethylene (PTFE)

was selected as the reference material. Both the reference and the samples were ground and loaded into a DRP-SAP microsample-cup (Harrick Scientific Products (Pleasantville, NY, USA)) and flattened with a spatula. Elemental analysis (CHNS) was performed using the Organic elemental analyzer UNICUBE (Langensfeld, Germany). The residual percentage in EA was assumed to be oxygen for pristine materials and TiO_2 for the nanocomposites. XRD was performed on a XRDynamic 500 diffractometer by Anton Paar (Graz, Austria) in the range of $20\text{--}90^\circ$ with a step size of 0.01° and 100.364 s per step. XPS measurements were conducted using a PHI 5000 VersaProbe IV Scanning ESCA Microprobe by Physical Electronics (Chanhassen, MN, USA) with a monochromatized $\text{Al K}\alpha$ X-ray source (1486.6 eV, 100 μm beam diameter, 25 W power, and 15 kV voltage). The samples were prepared in PTFE sample caps and attached to the XPS holder using insulating tape. During the measurements, charge neutralization was achieved using slow electrons and argon ions, and pressure was maintained between 10^{-7} and 10^{-6} Pa. Spectral data were analyzed using CasaXPS software (Version 2.3.25PR 1.0). Dynamic light scattering (DLS) measurements were performed at 20°C using a Malvern Zetasizer Nano-ZS device (Malvern, UK), operated with Malvern Panalytical's Zetasizer software 7.13. SEM images were recorded using a GeminiSEM 560 microscope by Zeiss (Oberkochen, Germany) with an InLens detector. For working distance, a range of 2.4 to 2.6 mm and an acceleration voltage of 1 kV was used. All samples were sputter-coated with platinum using a Leica EM ACE600 sputter coater (Wetzlar, Germany) in order to increase the conductivity of the specimens. Fiji ImageJ 1.54 software was used for image evaluation. Nitrogen physisorption experiments were performed using a Quadrasorb evo instrument by Quantachrome Instruments (Graz, Austria) at a temperature of 77 K. Pore size distributions were calculated using an NLDFT kernel (N_2 at 77 K, silica, cylindr. pores, adsorption branch). Atomic force microscopy (AFM) was performed in AC mode using an AIST-NT (Novato, CA, USA) SmartSPM 1000 under ambient conditions. 0.02 mg mL^{-1} dispersions of CNDs in DMSO were drop casted on freshly cleaved V1 grade Mica discs (10 mm diameter, 0.15–0.21 mm thickness). The AFM utilized a SSS-FMR probe by NANOSENSORS (Neuchatel, Switzerland), a super sharp silicon tip apex, and an aluminum-coated detector side (75 kHz, 2.8 N m^{-1}). Data analysis was carried out using Gwyddion 2.62 software. Thermogravimetric analysis (TGA) was conducted using a Luxx STA 409 PC Simultaneous thermal analyzer (Netzsch, Selb, Germany) with coupled mass spectrometry QMS 403 C Aeolos (Netzsch, Selb, Germany). Prior to the measurement ramp ($10^\circ\text{C min}^{-1}$ from 100°C to 900°C) under argon or synthetic air atmospheres, pristine samples underwent isothermal desorption of adsorbed moisture under Ar (60°C for 10 min, followed by 80°C for 3 min and 100°C for 2 min). For titania-based materials, measurements were taken without any prior treatment.

4.1. CND Synthesis

For the sake of clarity, the details of the already reported and slightly modified method reported by Pham et al. [35] are given here. CA (1.4410 g, 7.50 mmol, 1 eq.) was dissolved in 5 mL Milli-Q water and was stirred until the substrate was completely dissolved. EDA (0.50 mL, 7.5 mmol, 1 eq.) was added and the mixture was stirred for 20 min. The reaction mixture was heated in a domestic MW oven for five minutes at 400 W (50% power). The obtained brown resin was dispersed in 4 mL Milli-Q water, filtered through a $0.2\text{ }\mu\text{m}$ PTFE syringe filter, and purified using a Sephadex LH-20 gel column with Milli-Q water as eluent. The obtained brown dispersion was lyophilized and an orange/brown solid was obtained. Yield: 0.3734 g, 20 wt%.

$^1\text{H-NMR}$ of the CND species showed broad signals to ensure the presence of CNDs (Figure S38) [62].

4.2. 5-(4-(Diphenylamino)phenyl)thiophene-2-Carbaldehyde

Bromothiophene-2-carbaldehyde (1.200 g, 6.28 mmol, 1.0 eq.), triphenylamino boronic acid (2.0017 g, 6.92 mmol, 1.1 eq.), cesium carbonate (4.1120 g, 12.6 mmol, 2.0 eq.), and Tetrakis(triphenylphosphine)palladium(0) (0.3625 g, 0.31 mmol, 0.05 eq.) were dissolved in 40 mL dry THF and 6.3 mL water, and refluxed overnight under an argon atmosphere. The reaction mixture was cooled to room temperature, diluted with DCM and water. The mixture was extracted three times with DCM and the organic phase was combined and dried over MgSO_4 , filtered, and the solvent was removed under reduced pressure. The resulting yellow crude product was purified by column chromatography using DCM: n-hexane (1:1) as an eluent, which was subsequently changed to DCM after the elution of the starting material. A yellow crystalline solid was obtained as the final product.

Yield: 1.9043 g, 85%.

$^1\text{H-NMR}$ (400 MHz, Chloroform- d): δ = 9.86 (s, 1H), 7.71 (d, J = 4.0 Hz, 1H), 7.55–7.49 (m, 2H), 7.34–7.27 (m, 5H), 7.14 (d, J = 7.5 Hz, 4H), 7.10 (d, J = 7.3 Hz, 2H), 7.06 (d, J = 8.7 Hz, 2H) ppm.

Analytical data corresponded to the literature [63].

4.3. 2-Cyano-3-(5-(4-(diphenylamino)phenyl)thiophen-2-yl)acrylic Acid (L1)

5-(4-(Diphenylamino)phenyl)thiophene-2-carbaldehyde (0.6012 g, 1.69 mmol, 1.0 eq.), cyanoacetic acid (0.7195 g, 8.46 mmol, 5.0 eq.), and ammonium acetate (0.3315 g, 4.30 mmol, 2.5 eq.) were dissolved in 105.5 mL glacial acetic acid, and refluxed overnight. The reaction mixture was cooled to room temperature and the solvent was removed by distillation under reduced pressure. The resulting purple crude product was purified by column chromatography using DCM: methanol (10:1) as an eluent. A red solid was obtained as the final product.

Yield: 0.6508 g, 84%.

$^1\text{H-NMR}$ (400 MHz, Acetone- d_6): δ = 8.43 (s, 1H), 7.96 (d, J = 4.1 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 4.0 Hz, 1H), 7.37 (dd, J = 8.8, 7.0 Hz, 4H), 7.16 (d, J = 7.7 Hz, 6H), 7.06 (d, J = 8.8 Hz, 2H) ppm.

Analytical data corresponded to the literature [64].

4.4. Amide Coupling of CND with L1

L1 (0.6670 g, 1.54 mmol, 1.0 eq.), *N*-hydroxysuccinimide (0.3642 g, 3.16 mmol, 2 eq.), and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.6061 g, 3.16 mmol, 2.0 eq.) were dissolved in 130 mL dry DMF in a 250 mL Schlenk round bottom flask and stirred at room temperature for 30 min under argon atmosphere. CND (0.3790 g) was added and the reaction mixture was stirred at room temperature for two days under argon atmosphere. The solvent was removed under reduced pressure. The dark-red resin was dispersed in DCM: methanol (10:1) and packed on a silica gel glass frit. Then it was washed with DCM: methanol (10:1) to remove any side products and precursors until the solvent had little to no color after washing. The product adsorbed on the silica was washed off with DMSO and then freeze-dried. Further purification was carried out using a Sephadex G-10 gel column with DMSO as eluent. The obtained red/brown dispersion was lyophilized and a red/brown solid was obtained.

4.5. TiO_2 -Particle Synthesis

The synthesis proceeded with modification after Yeh et al. [50]. Under Schlenk conditions titanium tetrachloride (12 mL, 21 g, 110 mmol) were added into a three neck round bottom flask and 240 mL Milli-Q water were added dropwise, stirred, and cooled with a cooling bath of acetonitrile and liquid nitrogen. Formed HCl gas was captured by using

NaOH solution in a washing flask. Following the absence of a reaction upon the further addition of Milli-Q water, the reaction mixture was stirred for one hour. By addition of aqueous ammonia (25%) the pH of the solution was adjusted to 7. The white precipitate was washed with copious amounts of Milli-Q water to remove any soluble residues and dried at 40 °C overnight. The obtained colorless powder was then calcinated for 10 h at 350 °C with a heating rate of 2 °C min⁻¹.

4.6. TiO₂-Sensitized Catalyst

TiO₂ (500 mg, 6.26 mmol) was dispersed with 2 mg sensitizer (CND, L1 dye, CND-L1 hybrid) in 1 mL of acetonitrile, followed by sonication for 45 min. After this, an additional 10 mg of sensitizer was added, and sonication for another 45 min to ensure complete adsorption of the sensitizer onto TiO₂. The sonication process was conducted while cooling the sonication bath with ice to maintain room temperature. After adsorption, the dispersions were centrifuged for five minutes to remove acetonitrile. The catalyst underwent a three-stage wash process using water and centrifugation to remove water-soluble residues. The catalyst was dried overnight under reduced pressure.

4.7. Photocatalytic Procedure

Photocatalytic degradation was measured at 22.5 °C. 10 mg of catalyst was added to a 10 mL aqueous methyl orange (MO) solution (50 µM) and stirred for 30 min in dark. The pH of MO-solution was 6.5. For the catalysis a beaker with continuous cooling at 22.5 °C and an UV LED (Figure S19) has been used. The light intensity on the surface of the solution is 270 µW cm⁻² at a distance of 16 cm. A single catalysis cycle was conducted over a period of 50 min, with 250 µL aliquots being sampled at 10-min intervals and subsequently subjected to centrifugation for 15 min. The extent of the degradation process was determined by measuring the UV-Vis absorbance of the solution of degraded MO in the wavelength range of 300–700 nm, with the maximum absorbance observed at 464 nm.

4.8. Reusability Procedure

Photocatalytic degradation was measured at 22.5 °C. 10 mg of catalyst was added to a 10 mL aqueous MO solution (50 µM) and stirred for 30 min in dark. The pH of MO-solution was 6.5. For the catalysis a beaker with continuous cooling at 22.5 °C and a UV LED (Figure S19) has been used. The light intensity on the surface of the solution is 270 µW cm⁻² at a distance of 16 cm. A single catalysis cycle was conducted over a period of 50 min, with 250 µL aliquots being sampled at t_0 and t_{50} and subsequently subjected to centrifugation for 15 min. The extent of the degradation process was determined by measuring the UV-Vis absorbance of the solution in a 1.0 mm Hellma QS cuvette of degraded MO in the wavelength range of 300–700 nm, with the maximum absorbance observed at 464 nm. The extent of colorant degradation was determined through the use of UV-Vis spectrometry. Following irradiation, the suspension was transferred into a centrifuge tube and subjected to centrifugation. The supernatant was removed, and the catalyst was washed with water. After centrifuging and removing the water, the catalyst was dried under reduced pressure overnight. The residual amount of catalyst was then reused with 1 mL MO solution per 1 mg catalyst, and the procedure was repeated three times in total to determine the change in degradation efficiency after each catalytic cycle.

4.9. Scavenger Procedure

A total of 10 mg of the respective photocatalyst were dispersed in 10 mL MO solution (50 µM in milliQ®). Then, 10 mg of one of the scavenger substances was added (IPA, *p*-BQ, AA, EDTA). The respective mixtures exhibited the following pH values: 5.7 for IPA, 4.8 for EDTA, and 3.0 for AA. The dispersion was stirred in the dark for 30 min at 22.5 °C before

irradiation with the LED for 50 min. Before and after irradiation an aliquot of 250 μL was taken, centrifuged for 15 min and measured with the UV-Vis spectrometer. For analysis the absorbance [a.u.] of $\lambda_{\text{MO,max}} = 464 \text{ nm}$ ($\text{pH} < 4.4$) [65] and $\lambda_{\text{MO,max}} = 517 \text{ nm}$ ($\text{pH} > 4.4$) [66] was used.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/photochem5010001/s1>. Figure S1. (A) The 2D AFM image ($1000 \times 1000 \text{ nm}$) of CND is presented, with an inset showing the mean height and standard deviation of approximately 70 CNDs. (B) 2D AFM image ($500 \times 500 \text{ nm}$) of CND. (C) Height profile along the white line in the $500 \times 500 \text{ nm}$ 2D image. (D) 3D AFM image ($500 \times 500 \text{ nm}$) of CND. Figure S2. 2D DOSY NMR spectra of L1 dye measured in DMSO- d_6 . Figure S3. 2D DOSY NMR spectra of CND measured in DMSO- d_6 . Figure S4. 2D DOSY NMR spectra of a physical mixture of CND ($10 \text{ mg} \cdot \text{mL}^{-1}$) and L1 ($1 \text{ mg} \cdot \text{mL}^{-1}$) measured in DMSO- d_6 . Figure S5. 2D DOSY NMR spectra of CND-L1 hybrid measured in DMSO- d_6 .; Figure S6. (A) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 360 \text{ nm}$) of five different CND dispersions. (B) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 412 \text{ nm}$) of five different concentrated L1 solutions. (C) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 362 \text{ nm}$) of five different concentrated CND-L1 dispersions. (D) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 340 \text{ nm}$) of five different concentrated QS solutions. (E) Absorbance maxima plotted against the integrated PL intensity of the five different concentrations and the respective slopes derived from a linear fit. Figure S7. UV-Vis absorbance at 412 nm of five different concentrated CND, L1, and CND-L1 solutions and the respective slopes which correspond to the absorption coefficient derived from a linear fit. The UV-Vis absorbance at 412 nm of five different concentrated CND, L1, and CND-L1 solutions/dispersions, along with the corresponding slopes, were obtained from a linear fit and correspond to the absorption coefficient ϵ_{λ} . Figure S8. Excitation-emission maps recorded in an excitation wavelength range of 260 nm to 650 nm and an emission wavelength range of 280 nm to 720 nm . (A) CND with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO. (B) L1 with a concentration of $1.0 \mu\text{M}$ in DMSO. (C) CND-L1 with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO. Figure S9. Excitation spectra recorded in the excitation wavelength range of 260 nm to 700 nm . (A) CND with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO at an emission wavelength of 440 nm . (B) L1 with a concentration of $1.0 \mu\text{M}$ in DMSO at an emission wavelength of 559 nm . (C) CND-L1 with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO at an emission wavelength of 445 nm . Figure S10. TGA analysis of the CNDs measured in argon (A) CND in comparison to L1 and CND-L1. (B) Derived weight loss of CND in comparison to L1 and CND-L1. In all cases, the first significant weight loss was observed between 100°C and 300°C . Furthermore, the CND-L1 hybrid showed higher weight loss in this range. Decomposition started at around 350°C . Figure S11. XPS Survey-spectra for (A) CND, (B) L1 dye, and (C) CND-L1 hybrid respectively. (D) The atomic ratios were obtained from XPS measurements. (E) Elemental composition determined through elemental analysis (EA), highlighting the overall composition of the materials. (F) The element composition (from EA) is given without hydrogen, so that a direct comparison with the XPS atomic ratios is possible. Figure S12. Deconvoluted absorbance spectrum of CND-L1 ($14 \text{ mg} \cdot \text{L}^{-1}$ in DMSO) with four Gaussian-shaped functions. For Peak 1 (CND), the wavelength was fixed at 265 nm . For Peak 2 (L1), the wavelength was fixed at 302 nm . For Peak 3 (CND), the wavelength was fixed at 357 nm . For Peak 4 (L1), the wavelength was fixed at 454 nm . A maximum absorbance of 0.0291 was obtained for Peak 4 (L1). This indicates that 2.2% of the CND-L1 hybrid represents the L1 dye component. Figure S13. (A) Nitrogen (77 K) isotherm of TiO_2 particles and (B) their pore size distribution obtained by applying a non-local density functional theory (NLDFT) kernel for cylindrical pore geometries on the adsorption branch. Figure S14. (A+B) SEM images showing particles of $(31 \pm 5) \text{ nm}$ and agglomerated particles. (B) Insert: aggregated particles in an aqueous solution, with an average diameter of $(685 \pm 160) \text{ nm}$, as determined by dynamic light scattering (DLS). Figure S15. The XRD pattern of the TiO_2 particles is in accordance with the standard data file (ICSD Card No. 98-018-4368) for pure anatase TiO_2 . No additional reflections are present other than those illustrated in the reference card. Figure S16. $(F(R_\infty)h\nu)^{1/2}$ plotted against $h\nu$ for the pristine TiO_2 particles with linear extrapolation for band gap energy E_g determination. Figure S17. XPS Survey-spectra for

(A) TiO₂, (B) TiO₂-CND, (C) TiO₂-L1, and (D) TiO₂-CND-L1 respectively. (E) The atomic ratios were obtained from XPS measurements and compared with the previously determined values of the materials without TiO₂. Figure S18. TGA analysis was conducted on both the TiO₂ particles and the sensitized ones, which were measured in synthetic air. Figure S19. Emission spectra of the employed 395 nm LED Chip. YXO YUXINOU COB Chip, 220 V AC voltage, and 50 W power. The light intensity on the surface of the solution is 270 $\mu\text{W}\cdot\text{cm}^{-2}$ at a distance of 16 cm. Table S1: TiO₂-CND-L1 values from reusability experiments for TON/TOF calculation. Table S2: TiO₂-L1 values from reusability experiments for TON/TOF calculation. Figure S20. The appearance of 10 mg of the TiO₂ catalyst in 10 mL of a MO solution (aqueous, 50 μM) following irradiation is illustrated. Figure S21. Degradation efficiency of each catalyst for MO photodegradation at pH 3.0, acidified with HCl, compared to degradation efficiency with AA as scavenger at pH 3.0. Figure S22. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂ catalyst. Figure S23. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-CND catalyst. Figure S24. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-L1 catalyst. Figure S25. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-CND-L1 catalyst. Figure S26. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂ catalyst without light irradiation. Figure S27. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-CND catalyst without light irradiation. Figure S28. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-L1 catalyst without light irradiation. Figure S29. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO₂-CND-L1 catalyst without light irradiation. Figure S30. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with TiO₂ catalyst. (A) First measurement; (B) second measurement; (C) third measurement. Figure S31. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with TiO₂-CND catalyst. (A) First measurement; (B) second measurement; (C) third measurement. Figure S32. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with TiO₂-L1 catalyst. (A) First measurement; (B) second measurement; (C) third measurement. Figure S33. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with TiO₂-CND-L1 catalyst. (A) First measurement; (B) second measurement; (C) third measurement. Figure S34. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with AA acting as the scavenger. (A) TiO₂; (B) TiO₂-CND; (C) TiO₂-L1; (D) TiO₂-CND-L1. Figure S35. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with EDTA acting as the scavenger. (A) TiO₂; (B) TiO₂-CND; (C) TiO₂-L1; (D) TiO₂-CND-L1. Figure S36. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with IPA acting as the scavenger. (A) TiO₂; (B) TiO₂-CND; (C) TiO₂-L1; (D) TiO₂-CND-L1. Figure S37. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle without a scavenger at pH 3.0. (A) TiO₂; (B) TiO₂-CND; (C) TiO₂-L1; (D) TiO₂-CND-L1. Figure S38. ¹H-NMR spectra of CND measured in DMSO-d₆. Figure S39. ¹H-NMR spectra of 5-(4-(Diphenylamino)phenyl)thiophene-2-carbaldehyde measured in Chloroform-d₁. Figure S40. ¹H-NMR spectra of 2-Cyano-3-(5-(4-(diphenylamino)phenyl)thiophen-2-yl)acrylic acid measured in Acetone-d₆. Figure S41. ¹H-NMR spectra of CND-L1 Hybrid measured in DMSO-d₆. References [39,40,51,52,65,67–71] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, P.P.D. and T.G.; methodology, P.P.D., J.H. and T.G.; formal analysis, P.P.D., M.P., S.M. and J.H.; investigation, P.P.D., M.P., S.M. and J.H.; resources, B.M.S., M.S. and T.G.; data curation, P.P.D., M.P., S.M. and J.H.; writing—original draft preparation, P.P.D.; writing—review and editing, J.H. and T.G.; visualization, P.P.D.; supervision, B.M.S., M.S. and T.G.; project administration, T.G.; funding acquisition, B.M.S. and T.G. All authors have read and agreed to the published version of the manuscript.

Funding: We thank the financial support of the DFG through the joint project “Low-Dimensional Nano-Architectures for Light Emission and Light-to-Electricity Conversion” (LOW-LIGHT, grant no.

UMO/2020/39/I/ST4/01446). T.G. acknowledges the European Research Council for the project JANUS BI (grant agreement no. [101041229]).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank Heike Hausmann (JLU, Giessen) for performing the NMR experiments and Pascal Schweitzer (JLU, Giessen) for the support with the AFM measurements.

Conflicts of Interest: The authors declare no conflicts of interest.

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Supporting Information

Functional Nanocarbon Hybrids in Metal Oxide Nanocomposites for Photocatalysis

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Atomic Force Microscopy

0.02 mg·mL⁻¹ dispersions of CNDs in DMSO were drop casted on freshly cleaved V1 grade Mica discs (10 mm diameter, 0.15–0.21 mm thickness). After keeping the prepared samples overnight under reduced pressure, they were measured in AFM tapping mode with a SSS-FMR probe (75 kHz, 2.8 N·m⁻¹). The obtained images were analyzed with Gwyddion 2.62 [67] and the size of ~70 nanoparticles was measured for a size histogram.

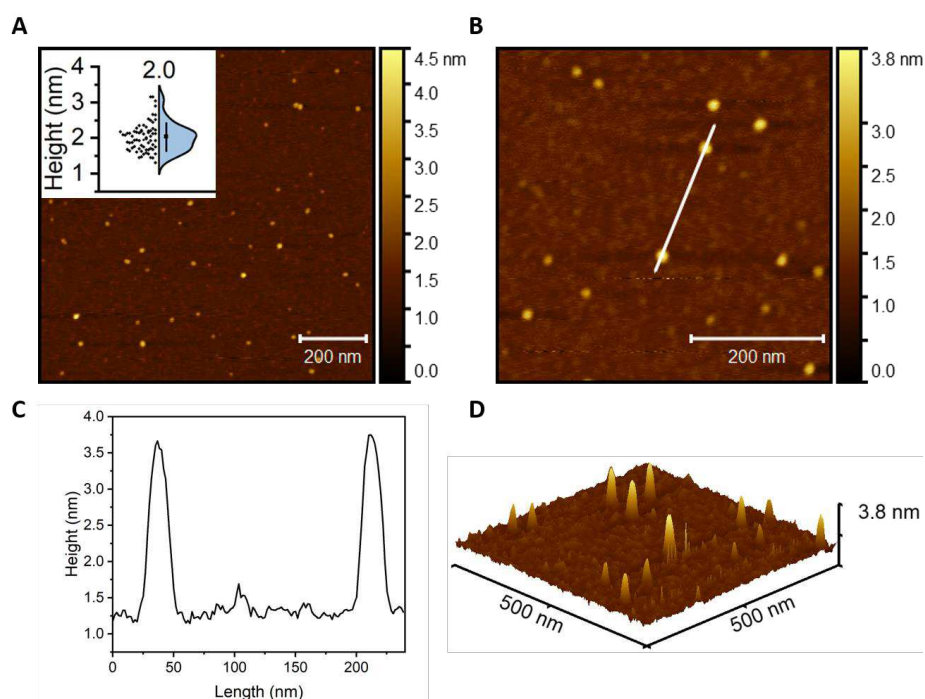


Figure S1. (A) The 2D AFM image (1000×1000 nm) of CND is presented, with an inset showing the mean height and standard deviation of approximately 70 CNDs. (B) 2D AFM image (500×500 nm) of CND. (C) Height profile along the white line in the 500x500 nm 2D image. (D) 3D AFM image (500×500 nm) of CND.

Diffusion Measurement

For ^1H -NMR analyses the samples were dissolved in DMSO- d_6 and sealed in NMR tubes with Parafilm. The respective concentrations of the samples were, $10 \text{ mg}\cdot\text{mL}^{-1}$ **CND**, $1 \text{ mg}\cdot\text{mL}^{-1}$ for **L1**, $10 \text{ mg}\cdot\text{mL}^{-1}$ of **CND** and $1 \text{ mg}\cdot\text{mL}^{-1}$ of **L1** for the physical mixture, and $11 \text{ mg}\cdot\text{mL}^{-1}$ for the **CND-L1** hybrid.

The diffusion measurements were carried out using the stimulated echo experiment with a variation of the gradient pulse strength g . The gradient pulse duration δ was 1 ms and the observation time $\Delta = 20$ ms. The resulting echo decays were evaluated by the Stejskal-Tanner equation [39]:

$$\frac{I}{I_0} = \exp\left(-\gamma^2 \delta^2 g^2 \left(\Delta - \frac{\delta}{3}\right) \cdot D\right) \quad (\text{S1})$$

in which γ denotes the gyromagnetic ratio of the ^1H nucleus.

Due to the polydispersity of the CNDs, a single self-diffusion coefficient cannot describe the echo decay sufficiently. Therefore, following a method described by Lenocho et al. [40], a distribution of diffusion coefficients $P(D)$ has to be considered. This is done in the form of an integral equation:

$$\frac{I}{I_0} = \int_{-\infty}^{\infty} P^*(D) \exp\left(-\gamma^2 \delta^2 g^2 \left(\Delta - \frac{\delta}{3}\right) \cdot D\right) d\ln(D) \quad (\text{S2})$$

Here, $P^*(D)$ is a modified distribution with $P^*(D) = D \cdot P(D)$. A commonly used function for $P(D)$ in the case of polymer diffusion [39], but also applicable here, is the log-normal distribution:

$$P(D) = \frac{1}{\sqrt{2\pi}\sigma_D D} \exp\left(-\frac{[\ln D - \ln\langle D \rangle]^2}{2\sigma_D^2}\right) \quad (\text{S3})$$

In combination with Equation (S2), $\langle D \rangle$ corresponds here now to the maximum of the distribution.

Using the Stokes-Einstein Equation (S4) the diameter of the diffusing species can be estimated from the diffusion coefficients. The temperature T during the measurement was 298.4 K, and the viscosity of DMSO-d₆ [68] at 298 K of 2.195 mPa·s was used for the viscosity η .

$$r = \frac{k_B T}{6\pi\eta\langle D \rangle} \quad (\text{S4})$$

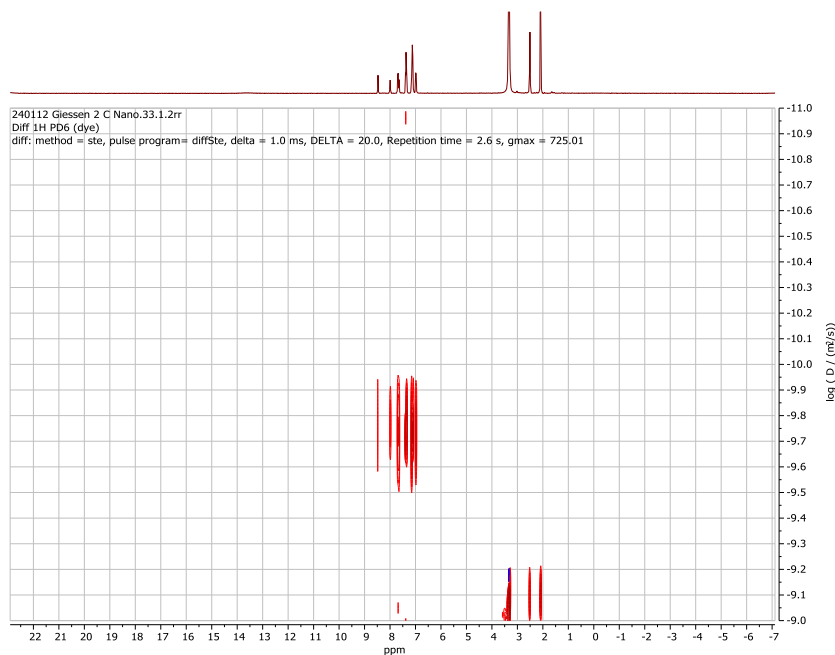


Figure S2. 2D DOSY NMR spectra of **L1** dye measured in DMSO-d₆.

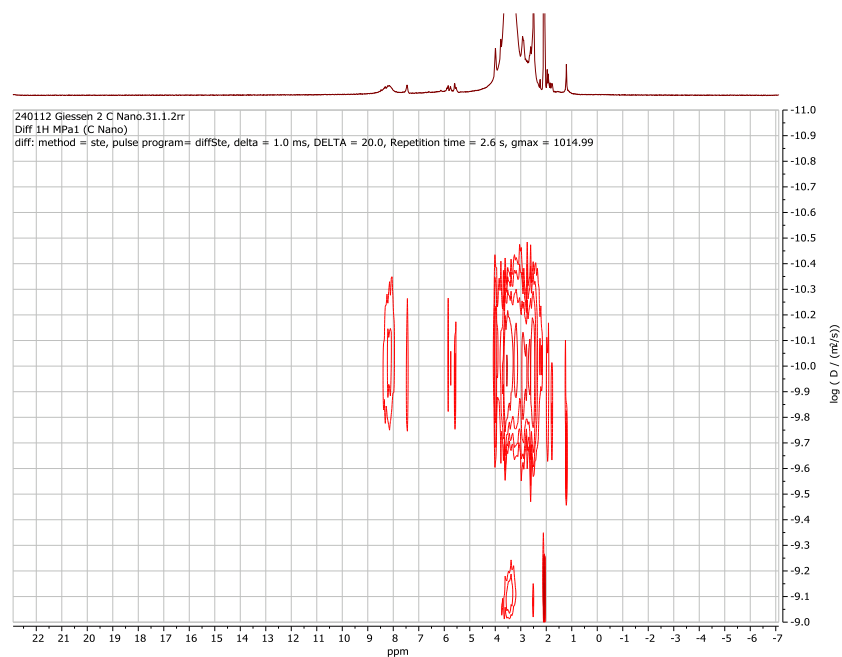


Figure S3. 2D DOSY NMR spectra of **CND** measured in DMSO-d₆.

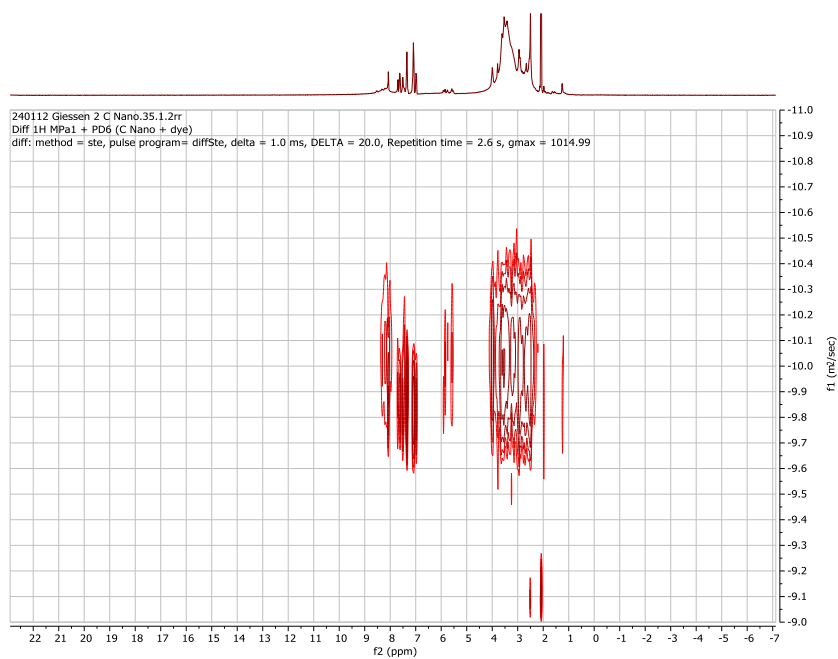


Figure S4. 2D DOSY NMR spectra of a physical mixture of **CND** ($10 \text{ mg}\cdot\text{mL}^{-1}$) and **L1** ($1 \text{ mg}\cdot\text{mL}^{-1}$) measured in DMSO- d_6 .

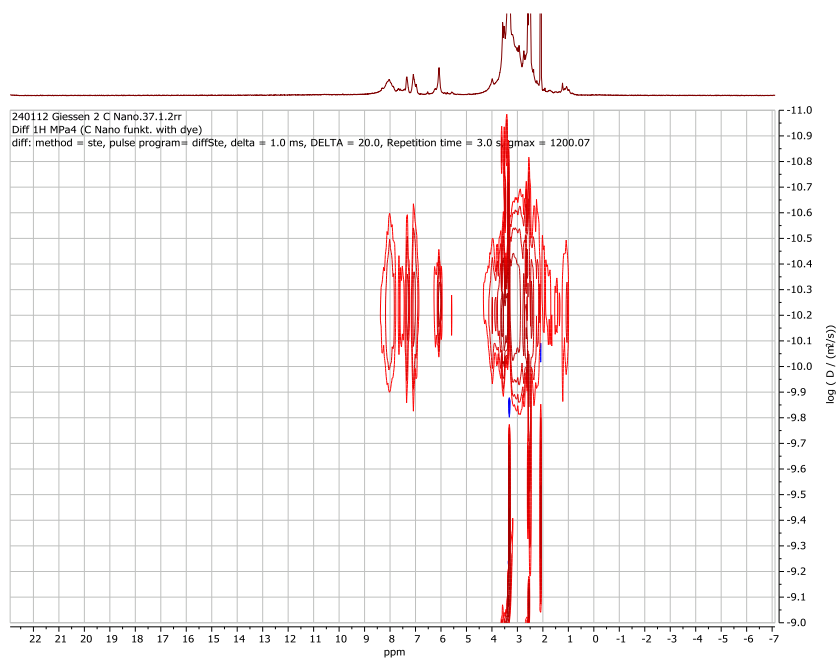


Figure S5. 2D DOSY NMR spectra of **CND-L1** hybrid measured in DMSO-d₆.

Photoluminescence Quantum Yield

The relative PL quantum yield method with quinine sulfate (QS) as a reference compound was used [69]. For five different concentrations of each sample and the reference sample, the UV-Vis absorbance and PL were measured. The maximum values of each absorbance measurement with the respective integrated PL intensity are plotted against each other. From the obtained slopes and the following equation one can calculate the PL quantum yield.

$$\Phi_S = \frac{m_S}{m_R} \cdot \frac{n_S^2}{n_R^2} \cdot \Phi_R \cdot 100$$

$$\Phi_S = \text{quantum yield of the sample [\%]}$$

$$\Phi_R = \text{quantum yield of the reference compound (QS = 0.51)}$$

$$m_S = \text{slope from plotted absorbance against integrated intensity of the sample}$$

$$m_R = \text{slope from plotted absorbance against integrated intensity of the reference compound}$$

$$n_S = 1.4785 \text{ refractive index of sample solvent (DMSO)}$$

$$n_R = 1.33415 \text{ refractive index of reference solvent (0.1 M H}_2\text{SO}_4\text{)}$$

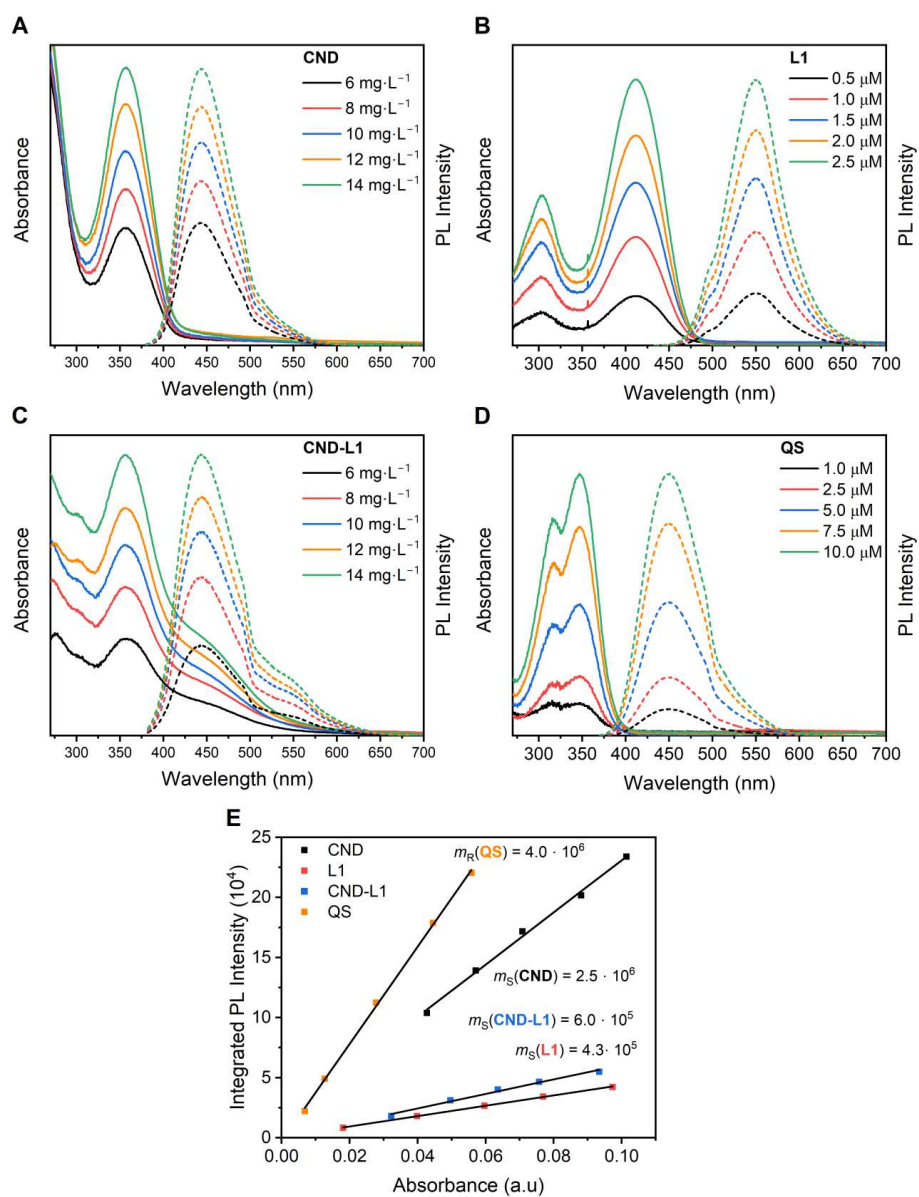


Figure S6. (A) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 360$ nm) of five different **CND** dispersions. (B) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 412$ nm) of five different concentrated **L1** solutions. (C) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 362$ nm) of five different concentrated **CND-L1** dispersions. (D) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 340$ nm) of five different concentrated **QS** solutions. (E) Absorbance maxima plotted against the integrated PL intensity of the five different concentrations and the respective slopes derived from a linear fit.

Absorption Coefficients

The UV-Vis absorbance maxima at five different concentrations for the respective concentration were plotted against the concentration. From the obtained slopes and the Law of Lambert-Beer in the following equation, it is possible to determine the absorption coefficient directly [70].

$$A = \varepsilon_{\lambda} \cdot c \cdot l$$

A = absorbance [a.u]

ε_{λ} = absorption coefficient at the respective wavelength [$\text{L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$]

c = concentration [$\text{mg} \cdot \text{L}^{-1}$]

l = layer thickness of the irradiated body (1 cm)

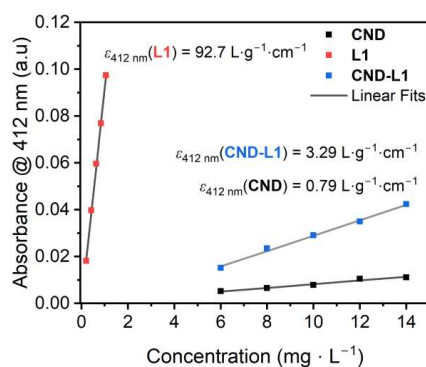


Figure S7. UV-Vis absorbance at 412 nm of five different concentrated **CND**, **L1**, and **CND-L1** solutions and the respective slopes which correspond to the absorption coefficient derived from a linear fit. The UV-Vis absorbance at 412 nm of five different concentrated **CND**, **L1**, and **CND-L1** solutions/dispersions, along with the corresponding slopes, were obtained from a linear fit and correspond to the absorption coefficient ε_{λ} .

Excitation-Emission Maps

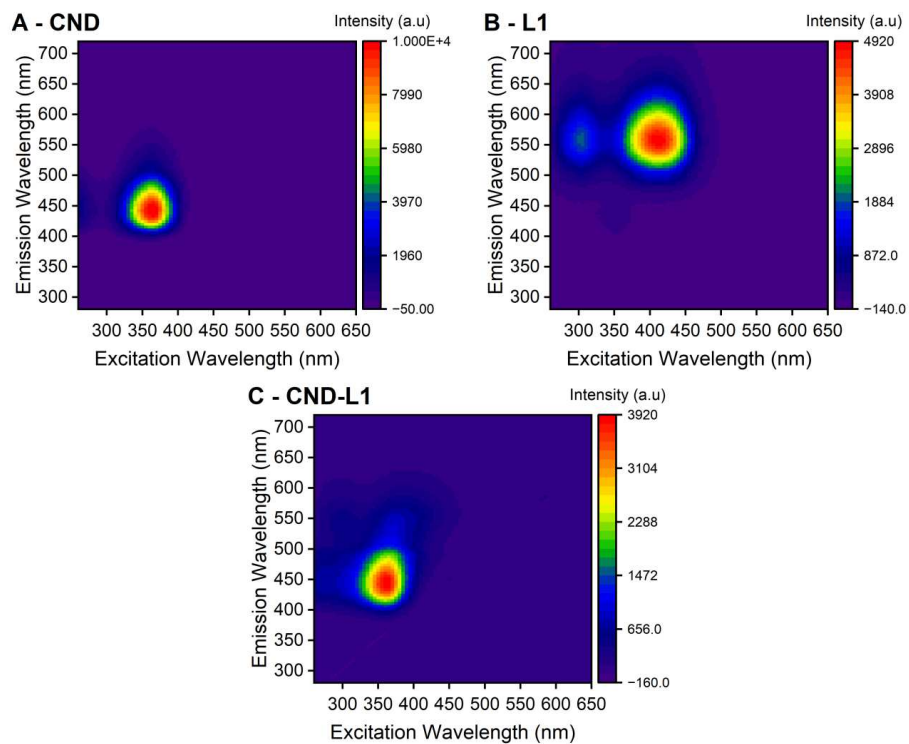


Figure S8. Excitation-emission maps recorded in an excitation wavelength range of 260 nm to 650 nm and an emission wavelength range of 280 nm to 720 nm. **(A)** CND with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO. **(B)** L1 with a concentration of $1.0 \text{ } \mu\text{M}$ in DMSO. **(C)** CND-L1 with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO.

Excitation Spectra

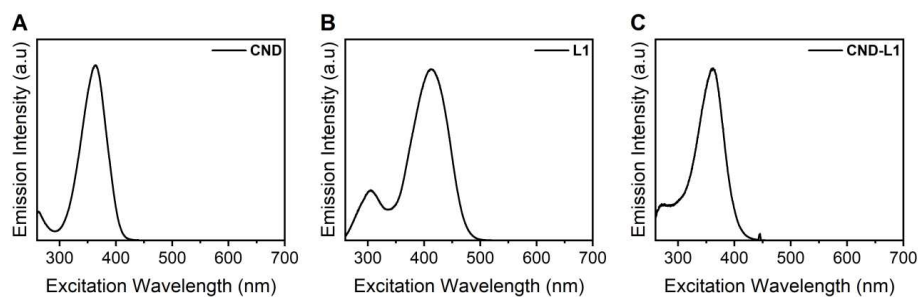


Figure S9. Excitation spectra recorded in the excitation wavelength range of 260 nm to 700 nm.

(A) CND with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO at an emission wavelength of 440 nm. (B) L1 with a concentration of $1.0 \text{ } \mu\text{M}$ in DMSO at an emission wavelength of 559 nm. (C) CND-L1 with a concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO at an emission wavelength of 445 nm.

Thermogravimetric Analysis - Sensitizers

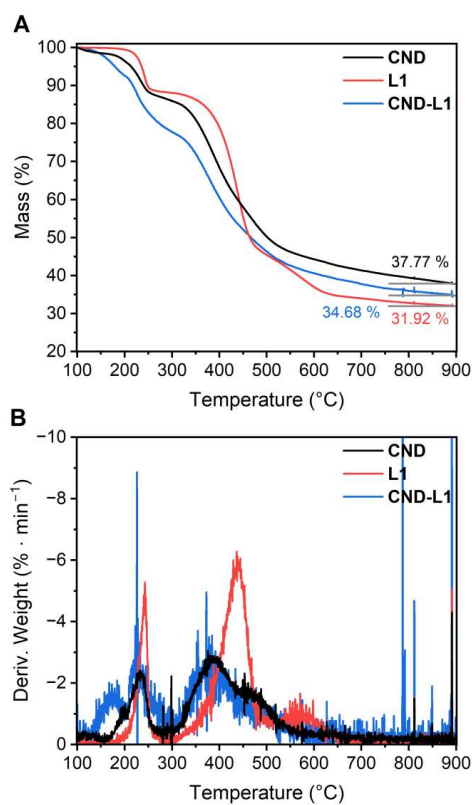


Figure S10. TGA analysis of the CNDs measured in argon (A) CND in comparison to L1 and CND-L1. (B) Derived weight loss of CND in comparison to L1 and CND-L1. In all cases, the first significant weight loss was observed between 100 °C and 300 °C. Furthermore, the CND-L1 hybrid showed higher weight loss in this range. Decomposition started at around 350 °C.

X-Ray Photoelectron Spectroscopy & Elemental Analysis - Sensitizers

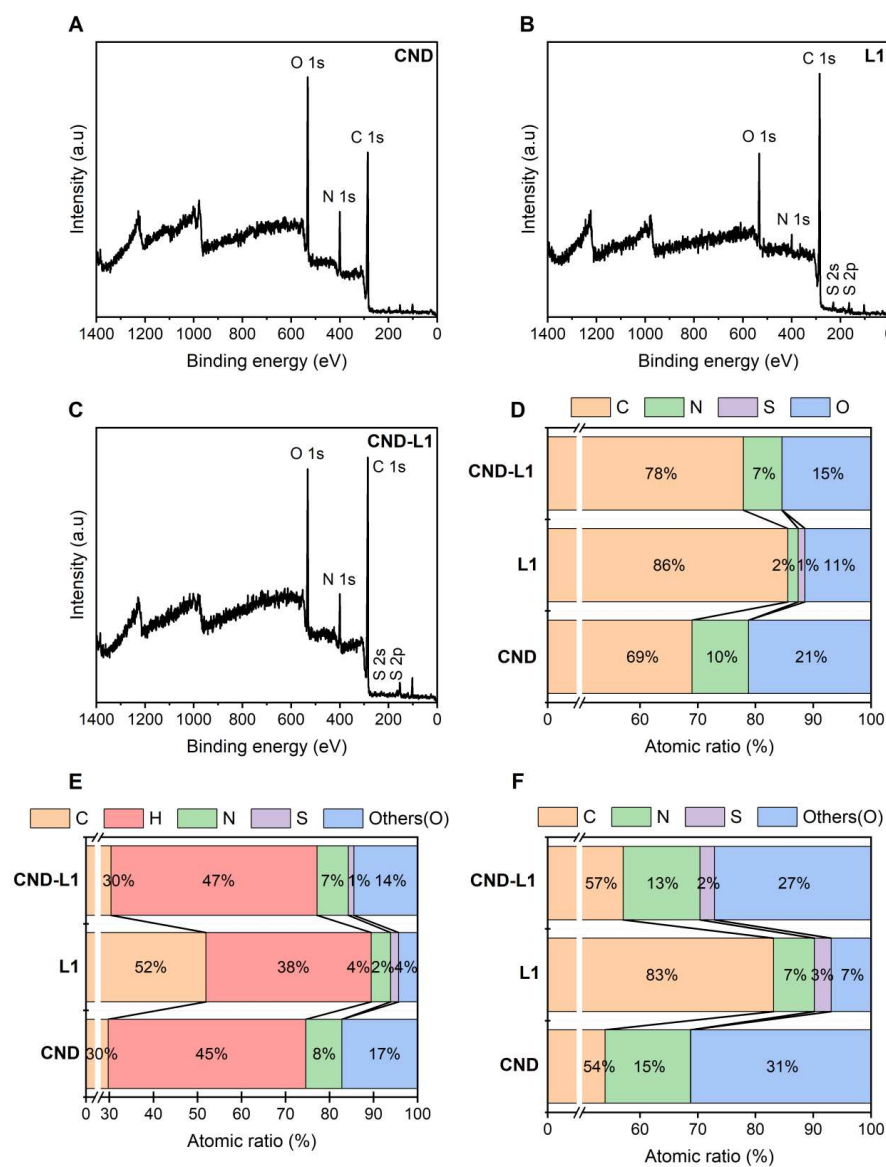


Figure S11. XPS Survey-spectra for (A) CND, (B) L1 dye, and (C) CND-L1 hybrid respectively. (D) The atomic ratios were obtained from XPS measurements. (E) Elemental composition determined through elemental analysis (EA), highlighting the overall composition of the materials. (F) The element composition (from EA) is given without hydrogen, so that a direct comparison with the XPS atomic ratios is possible.

Degree of Dye Functionalization

The UV-Vis absorption spectrum of the **CND-L1** hybrid was deconvoluted with the help of four Gaussian-shaped functions. The wavelength of the **L1** dye and the **CND** components was set to the following values: 265 nm for Peak 1, 302 nm for Peak 2, 357 nm for Peak 3, and 454 nm for Peak 4 as the maximum values for the Gaussian functions. The maximum absorbance value for the obtained deconvolution of the **L1** compartment was used for the calculation of the concentration of **L1** in the **CND-L1** hybrid with the Law of Lambert-Beer and the previously determined absorption coefficient of the **L1** dye at 412 nm. It is assumed that the absorption coefficient at the original wavelength of 412 nm can be applied to the corresponding peak in the **CND-L1** hybrid at 454 nm. The percentage of **L1** dye was calculated in accordance with the concentration of $14 \text{ mg}\cdot\text{L}^{-1}$ of the **CND-L1** hybrid utilized for deconvolution.

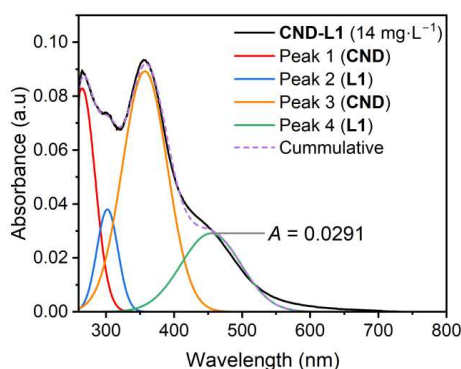


Figure S12. Deconvoluted absorbance spectrum of **CND-L1** ($14 \text{ mg}\cdot\text{L}^{-1}$ in DMSO) with four Gaussian-shaped functions. For Peak 1 (**CND**), the wavelength was fixed at 265 nm. For Peak 2 (**L1**), the wavelength was fixed at 302 nm. For Peak 3 (**CND**), the wavelength was fixed at 357 nm. For Peak 4 (**L1**), the wavelength was fixed at 454 nm. A maximum absorbance of 0.0291 was

obtained for Peak 4 (L1). This indicates that 2.2% of the CND-L1 hybrid represents the L1 dye component.

Physisorption Isotherm – TiO₂

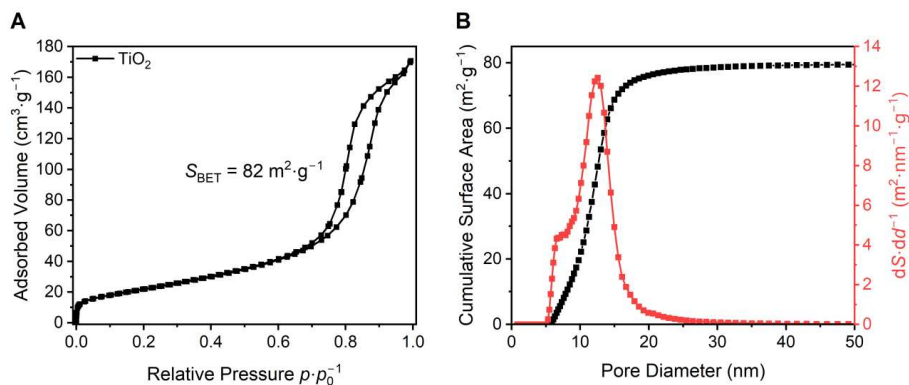


Figure S13. (A) Nitrogen (77 K) isotherm of TiO₂ particles and (B) their pore size distribution obtained by applying a non-local density functional theory (NLDFT) kernel for cylindrical pore geometries on the adsorption branch.

Scanning Electron Microscopy & Dynamic Light Scattering

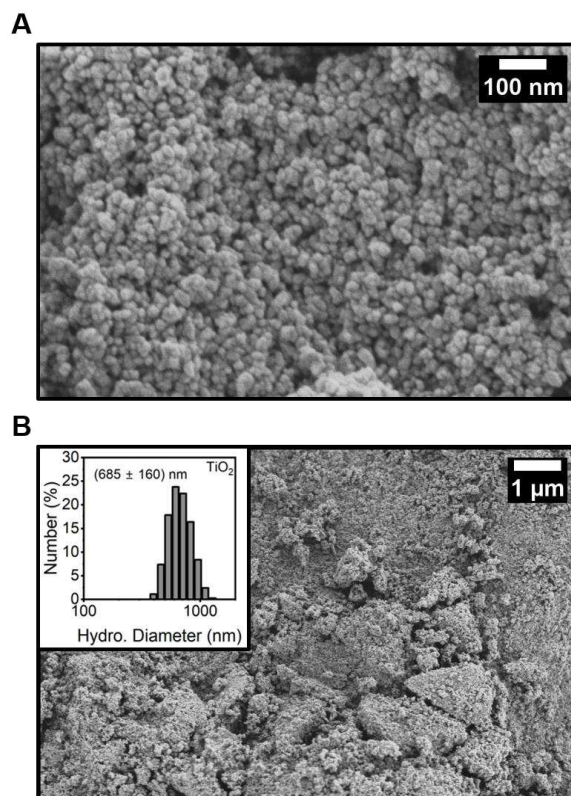


Figure S14. (A+B) SEM images showing particles of (31 ± 5) nm and agglomerated particles. (B)

Insert: aggregated particles in an aqueous solution, with an average diameter of (685 ± 160) nm, as determined by dynamic light scattering (DLS).

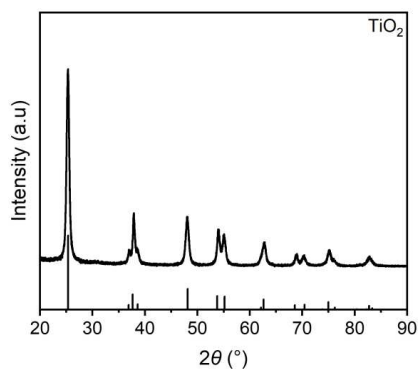
X-ray Diffraction

Figure S15. The XRD pattern of the TiO_2 particles is in accordance with the standard data file (ICSD Card No. 98-018-4368) for pure anatase TiO_2 . No additional reflections are present other than those illustrated in the reference card.

Tauc Plot

Following the transformation of reflectance R to the corresponding Kubelka-Munk function [51] $F(R_\infty)$ (Equation S1), the use of $F(R_\infty)$ permits the calculation of the band gap energy E_g through the application of the Tauc plot (Equation S2) [52].

$$F(R_\infty) = \frac{(1-R_\infty)^2}{2R_\infty} \quad \text{with } R_\infty = \frac{R_{\text{sample}}}{R_{\text{standard}}} \quad (\text{S1})$$

$$(F(R_\infty) \cdot h\nu)^{1/\gamma} = B(h\nu - E_g) \quad (\text{S2})$$

R_∞ : reflectance of an infinitely thick specimen

R_x : reflectance of x

ν : photon frequency

h : Planck constant

B : constant

E_g : band gap energy

Band gap energy values are obtained by extrapolation of the linear section of the plot to $y=0$. The factor γ refers to direct and indirect allowed transition with $\gamma = 0.5$ and 2 respectively. The TiO_2 anatase phase is classified as an indirect band gap semiconductor, and thus $\gamma = 2$ was selected as the appropriate value.

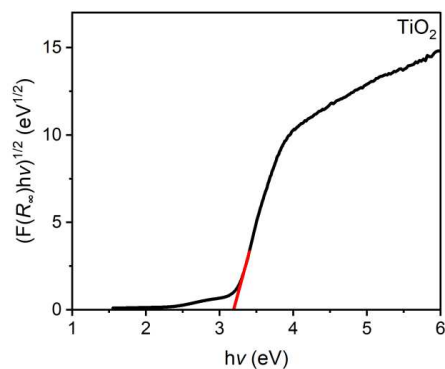


Figure S16. $(F(R_{\infty})hv)^{1/2}$ plotted against $h\nu$ for the pristine TiO_2 particles with linear extrapolation for band gap energy E_g determination.

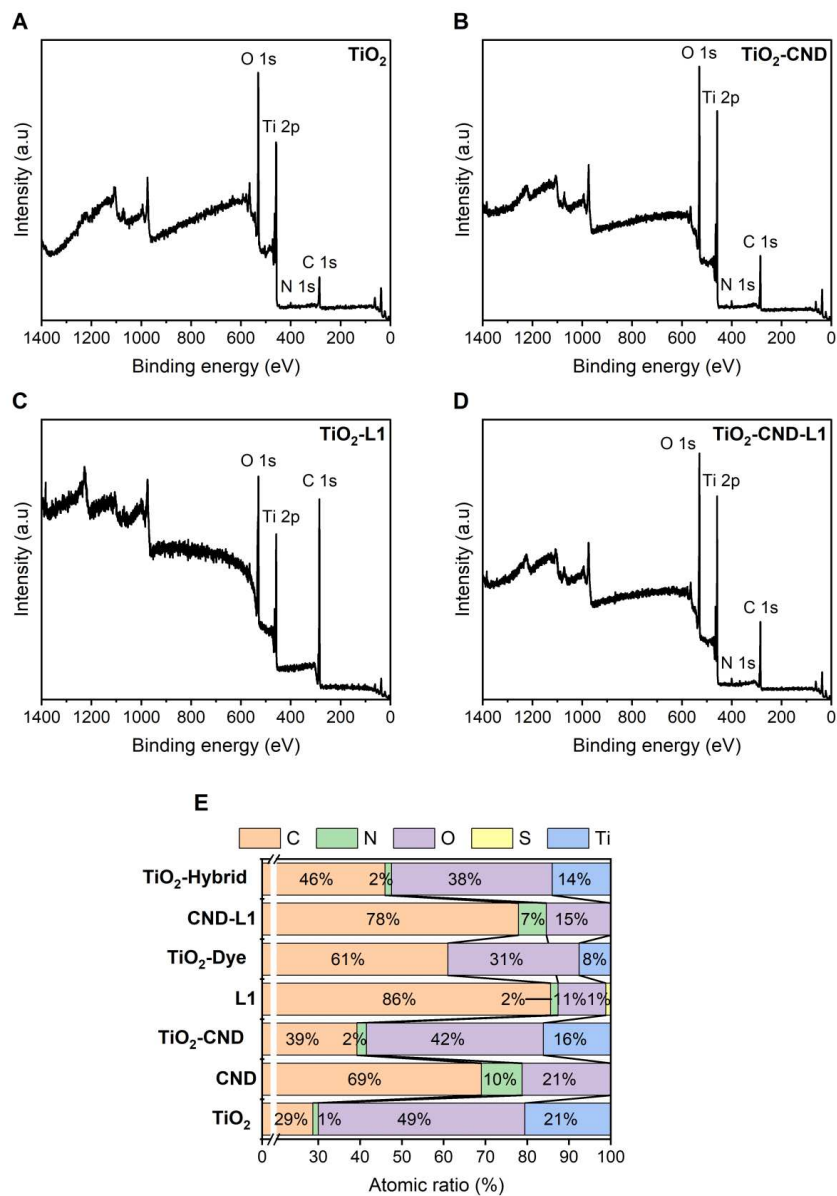
X-Ray Photoelectron Spectroscopy – Sensitized TiO₂

Figure S17. XPS Survey-spectra for (A) TiO_2 , (B) $\text{TiO}_2\text{-CND}$, (C) $\text{TiO}_2\text{-L1}$, and (D) $\text{TiO}_2\text{-CND-L1}$ respectively. (E) The atomic ratios were obtained from XPS measurements and compared with the previously determined values of the materials without TiO_2 .

Thermogravimetric Analysis – Sensitized TiO_2

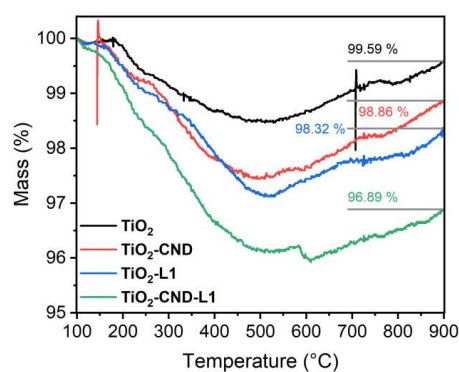


Figure S18. TGA analysis was conducted on both the TiO_2 particles and the sensitized ones, which were measured in synthetic air.

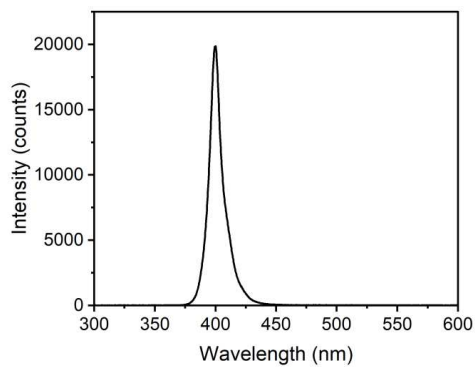
LED for Photocatalysis

Figure S19. Emission spectra of the employed 395 nm LED Chip. YXO YUXINOU COB Chip, 220 V AC voltage, and 50 W power. The light intensity on the surface of the solution is $270 \mu\text{W}\cdot\text{cm}^{-2}$ at a distance of 16 cm.

TON/TOF Calculation

Table S1: TiO₂-CND-L1 values from reusability experiments for TON/TOF calculation.

	a	a	a	b	b	b	c	c	c
	1st cycle	2nd cycle	3rd cycle	1st cycle	2nd cycle	3rd cycle	1st cycle	2nd cycle	3rd cycle
A_0	0.0500	0.0648	0.0624	0.0403	0.0621	0.0652	0.0520	0.0625	0.0635
A_{50}	0.0036	0.0241	0.0148	0.0004	0.0138	0.0235	0.0044	0.0132	0.0131
$c_0 /$ mol/L	0.00002 32	0.00003 00	0.00002 89	0.00001 86	0.00002 87	0.00003 02	0.00002 41	0.00002 89	0.00002 94
$c_{50} /$ mol/L	0.00000 166	0.00001 114	0.00000 683	0.00000 020	0.00000 641	0.00001 090	0.00000 204	0.00000 613	0.00000 605
$\Delta c /$ mol/L	0.00002 15	0.00001 89	0.00002 20	0.00001 84	0.00002 23	0.00001 93	0.00002 20	0.00002 28	0.00002 34
V / L	10.2	4.2	2.5	10.5	9.7	7.3	10.4	8.0	6.6
$n /$ mmol	0.00021 9	0.00007 9	0.00005 5	0.00019 4	0.00021 7	0.00014 1	0.00022 9	0.00018 2	0.00015 4

Table S2: TiO₂-L1 values from reusability experiments for TON/TOF calculation.

	a	a	a	b	b	b	c	c	c
	1st cycle	2nd cycle	3rd cycle	1st cycle	2nd cycle	3rd cycle	1st cycle	2nd cycle	3rd cycle
A_0	0,0375	0,0412	0,0671	0,0364	0,0562	0,0637	0,0410	0,0570	0,0623
A_{50}	0,00186	0,00269	0,01500	0,00207	0,00298	0,00601	0,00198	0,00475	0,00593
$c_0 /$ mol/L	0,00001 74	0,00001 91	0,00003 11	0,00001 69	0,00002 60	0,00002 95	0,00001 90	0,00002 64	0,00002 88
$c_{50} /$ mol/L	0,00000 086	0,00000 125	0,00000 694	0,00000 096	0,00000 138	0,00000 278	0,00000 092	0,00000 220	0,00000 274
$\Delta c /$ mol/L	0,00001 65	0,00001 78	0,00002 41	0,00001 59	0,00002 46	0,00002 67	0,00001 81	0,00002 42	0,00002 61
V / L	9,8	6,5	3,4	10,4	8	5,7	10,3	8,7	5,4
$n /$ mmol	0,00016 2	0,00011 6	0,00008 2	0,00016 5	0,00019 7	0,00015 2	0,00018 6	0,00021 0	0,00014 1

The concentration c was calculated from the absorbance A at 464 nm with the absorption coefficient of MO $\varepsilon_{464\text{ nm}} = 21600\text{ L mol}^{-1}\text{ cm}^{-1}$ and a cell thickness of $l=0.1\text{ nm}$ and the previously reported law of Labert-Beer [65]. For the **TiO₂-CND-L1** the mean value of the first cycle was used as $n(\text{MO}; \text{TiO}_2\text{-CND-L1}) = 0.000214\text{ mmol}$ and $t = 3000\text{ s}$. For the **TiO₂-L1** the mean values of the three cycles were added together as $n(\text{MO}; \text{TiO}_2\text{-L1}) = 0.000471\text{ mmol}$ and $t = 9000\text{ s}$. For the **TiO₂-CND-L1** the amount of L1 dye was $n(\text{dye}; \text{TiO}_2\text{-CND-L1}) = 0.0000127\text{ mmol}$. For the **TiO₂-L1** the amount of L1 dye was $n(\text{dye}; \text{TiO}_2\text{-L1}) = 0.000301\text{ mmol}$. From **Table S1** and **S2** the values for or the calculation of the turn-over number (TON) was calculated with the following equation (1) and the turn-over frequency (TOF) with equation (2):

$$\text{TON} = \frac{n(\text{MO})}{n(\text{dye})} \quad (3)$$

$$\text{TOF} = \frac{n(\text{MO})}{n(\text{dye}) \cdot t} \quad (2)$$

This results in a TON of 16.8 and a TOF of 0.00561 for **TiO₂-CND-L1** and a TON of 1.57 and a TOF of 0.000174 for **TiO₂-L1**.

Since the reaction rates are not constant over the catalysis time, the reported TOF is not reliable [71].

p-BQ Scavenger

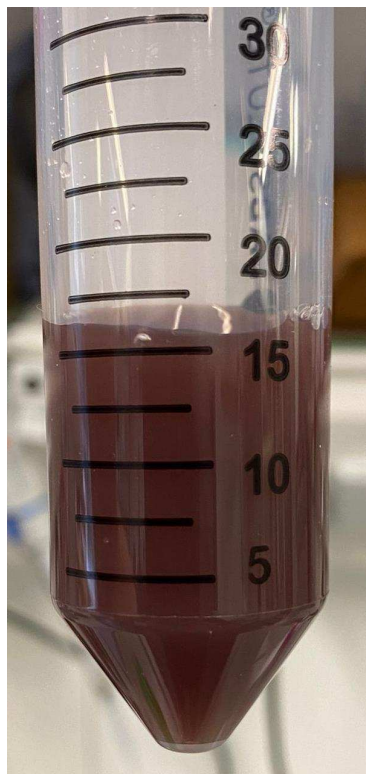


Figure S20. The appearance of 10 mg of the TiO_2 catalyst in 10 mL of a MO solution (aqueous, 50 μM) following irradiation is illustrated.

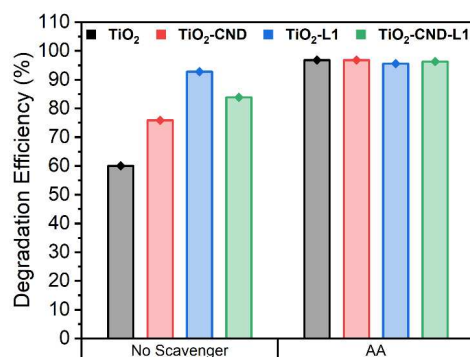
Photodegradation of MO at pH 3

Figure S21. Degradation efficiency of each catalyst for MO photodegradation at pH 3.0, acidified with HCl, compared to degradation efficiency with AA as scavenger at pH 3.0.

Photodegradation Spectra

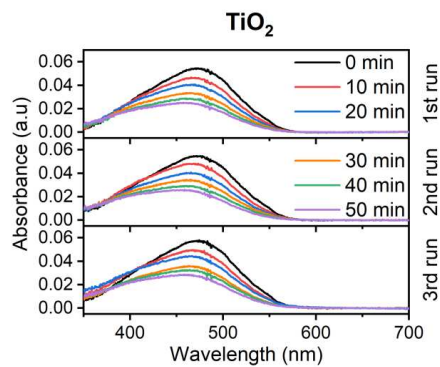


Figure S22. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO_2 catalyst.

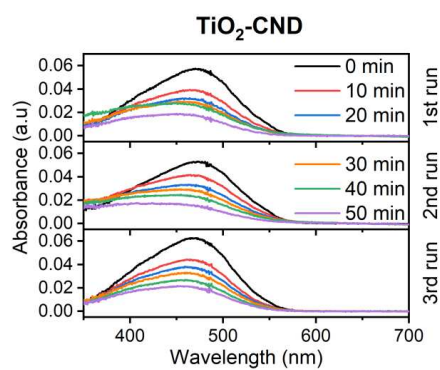


Figure S23. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-CND}$ catalyst.

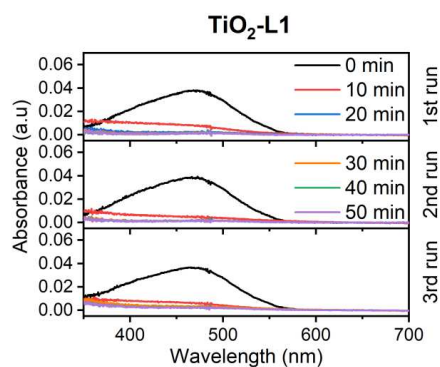


Figure S24. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-L1}$ catalyst.

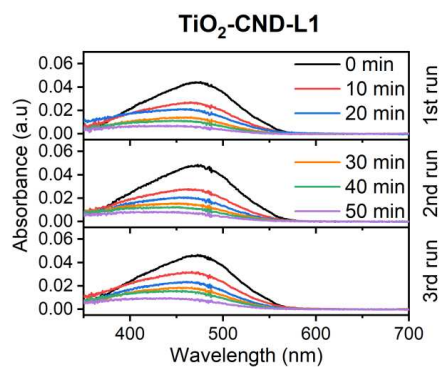


Figure S25. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-CND-L1}$ catalyst.

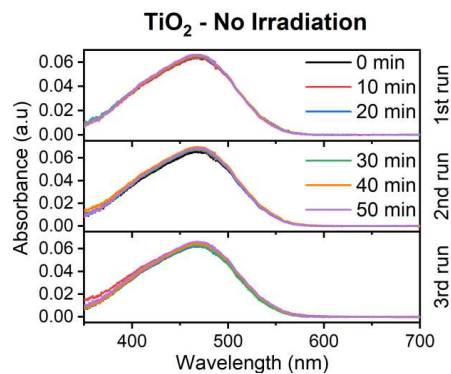
Photodegradation Spectra – No Irradiation

Figure S26. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with TiO_2 catalyst without light irradiation.

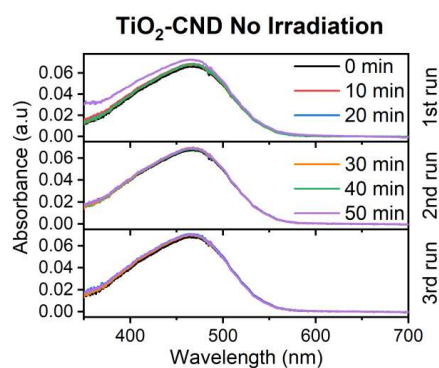


Figure S27. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-CND}$ catalyst without light irradiation.

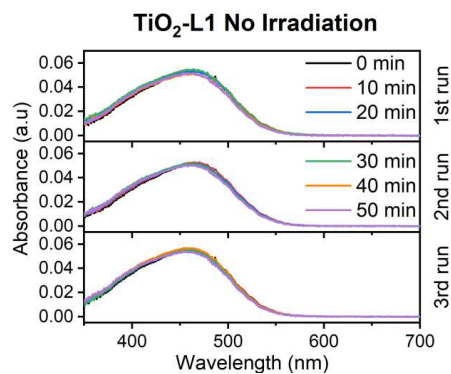


Figure S28. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-L1}$ catalyst without light irradiation.

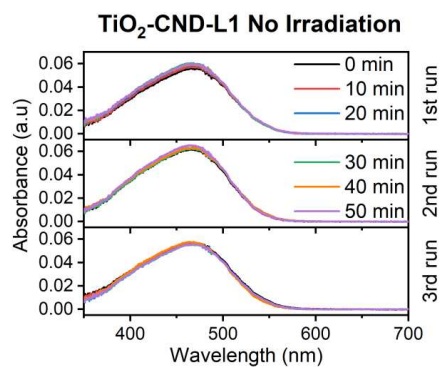


Figure S29. UV-Vis absorbance spectra for the three runs of catalytic MO-degradation over a period of 50 minutes with $\text{TiO}_2\text{-CND-L1}$ catalyst without light irradiation.

Photodegradation Spectra – Reusability

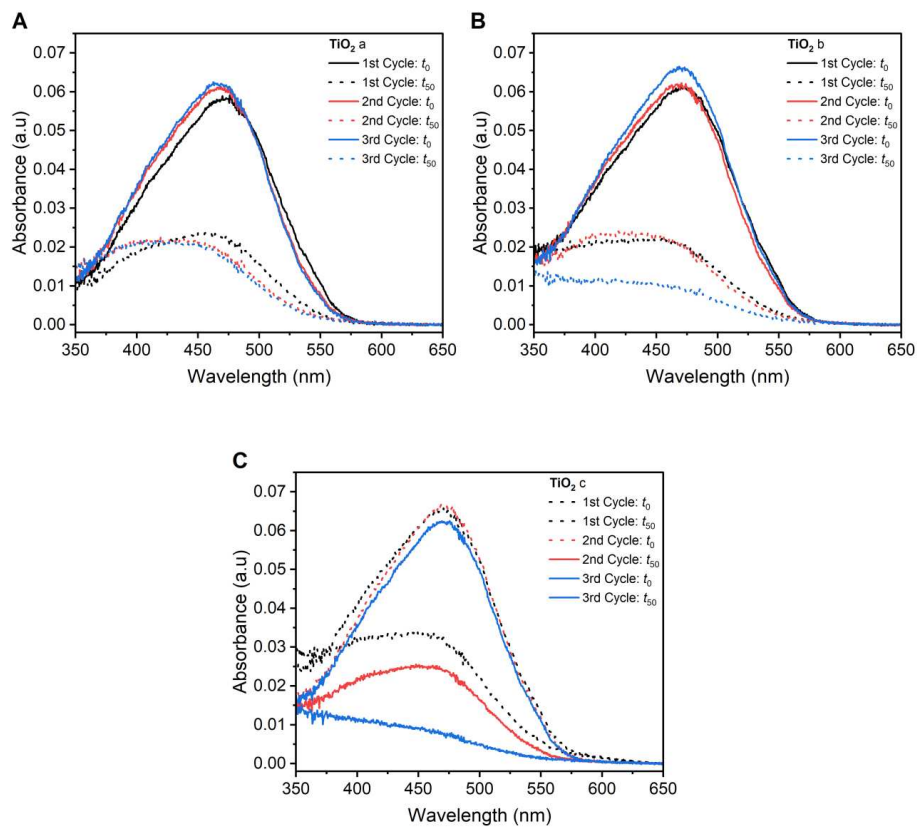


Figure S30. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with TiO_2 catalyst. (A) First measurement; (B) second measurement; (C) third measurement.

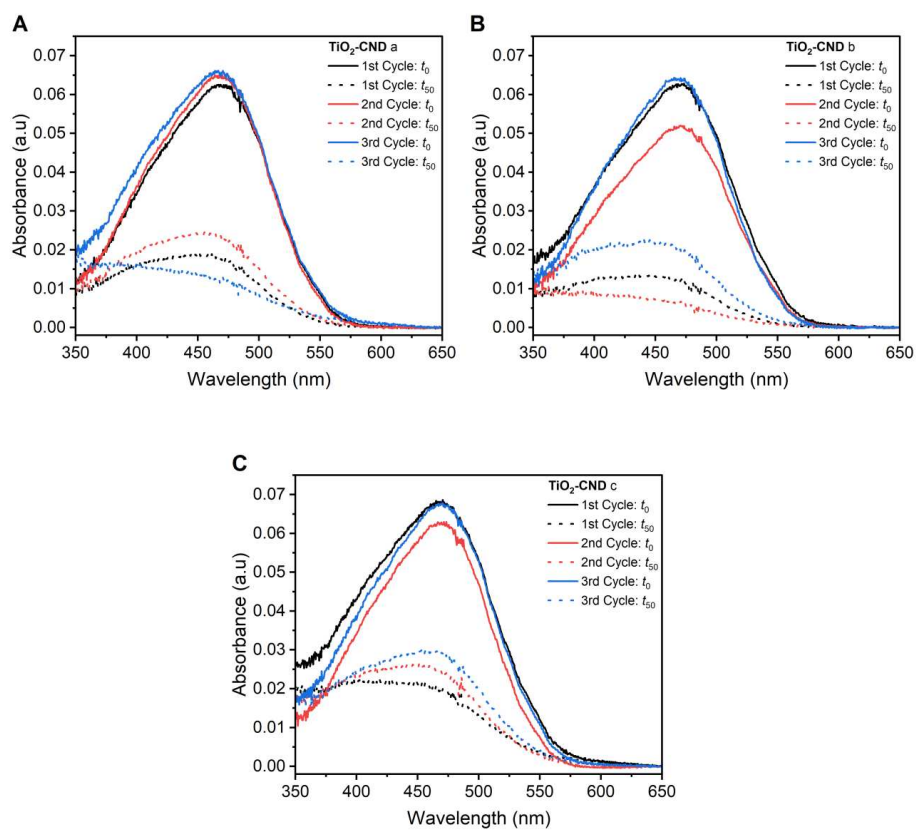


Figure S31. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with $\text{TiO}_2\text{-CND}$ catalyst. (A) First measurement; (B) second measurement; (C) third measurement.

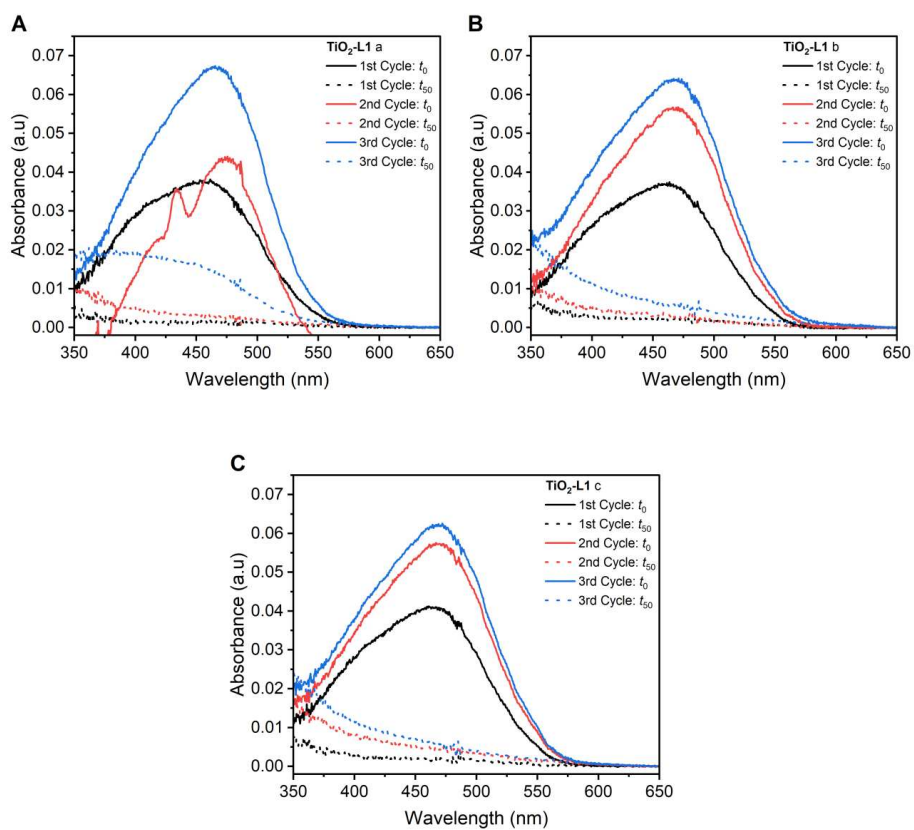


Figure S32. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with $\text{TiO}_2\text{-L1}$ catalyst. (A) First measurement; (B) second measurement; (C) third measurement.

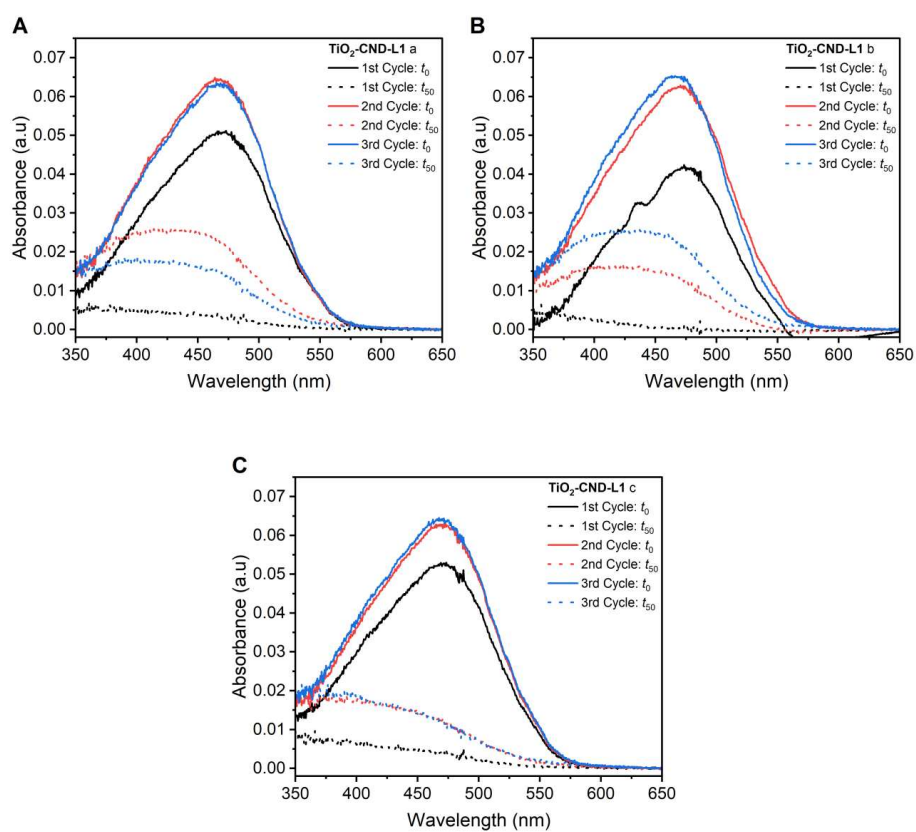


Figure S33. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with $\text{TiO}_2\text{-CND-L1}$ catalyst. **(A)** First measurement; **(B)** second measurement; **(C)** third measurement.

Photodegradation Spectra – Scavenger Experiments

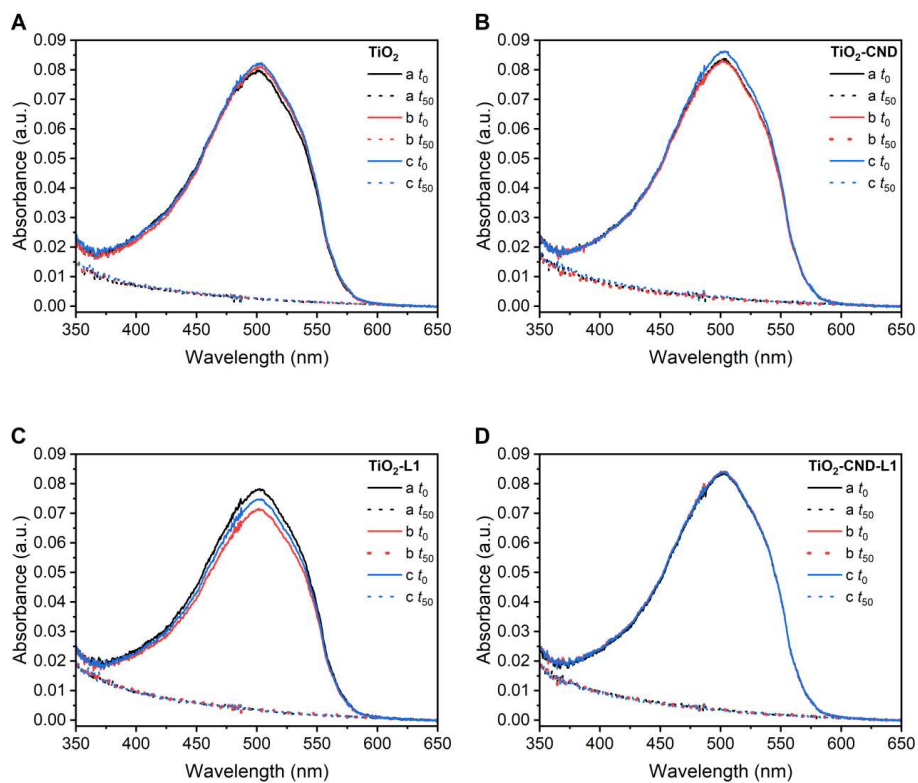


Figure S34. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with AA acting as the scavenger. (A) TiO_2 ; (B) $\text{TiO}_2\text{-CND}$; (C) $\text{TiO}_2\text{-L1}$; (D) $\text{TiO}_2\text{-CND-L1}$.

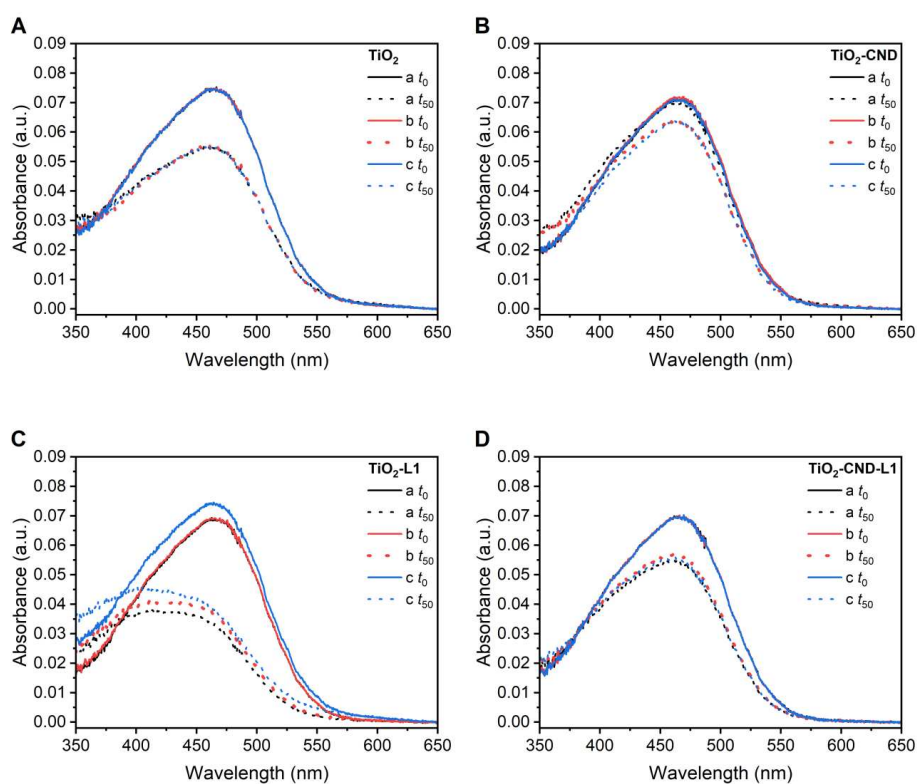


Figure S35. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with EDTA acting as the scavenger. (A) TiO_2 ; (B) $\text{TiO}_2\text{-CND}$; (C) $\text{TiO}_2\text{-L1}$; (D) $\text{TiO}_2\text{-CND-L1}$.

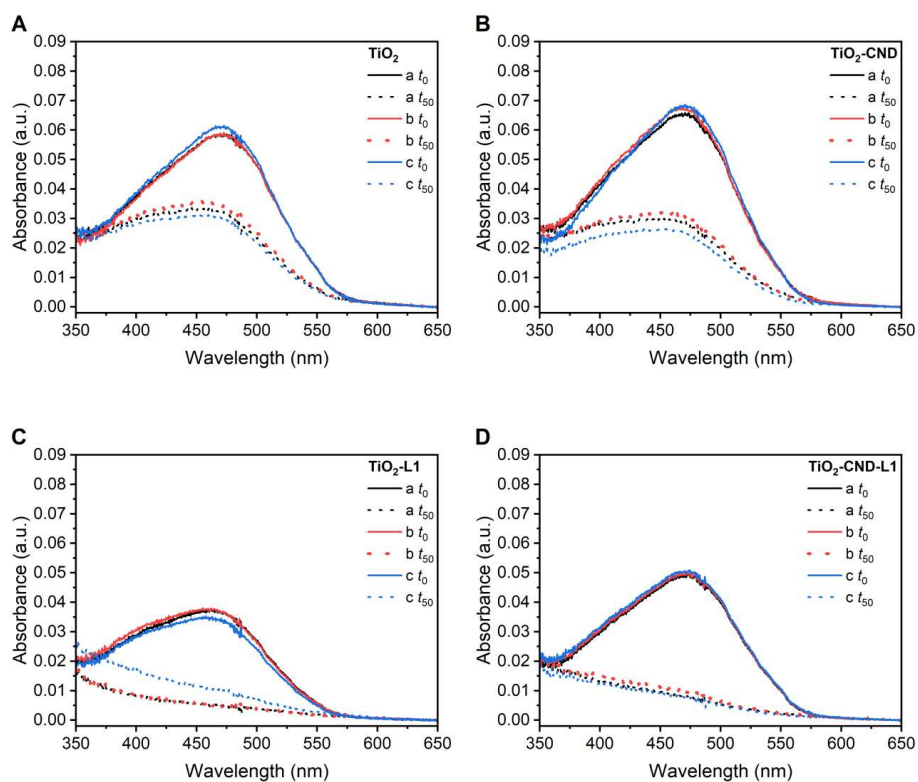


Figure S36. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle with IPA acting as the scavenger. (A) TiO_2 ; (B) $\text{TiO}_2\text{-CND}$; (C) $\text{TiO}_2\text{-L1}$; (D) $\text{TiO}_2\text{-CND-L1}$.

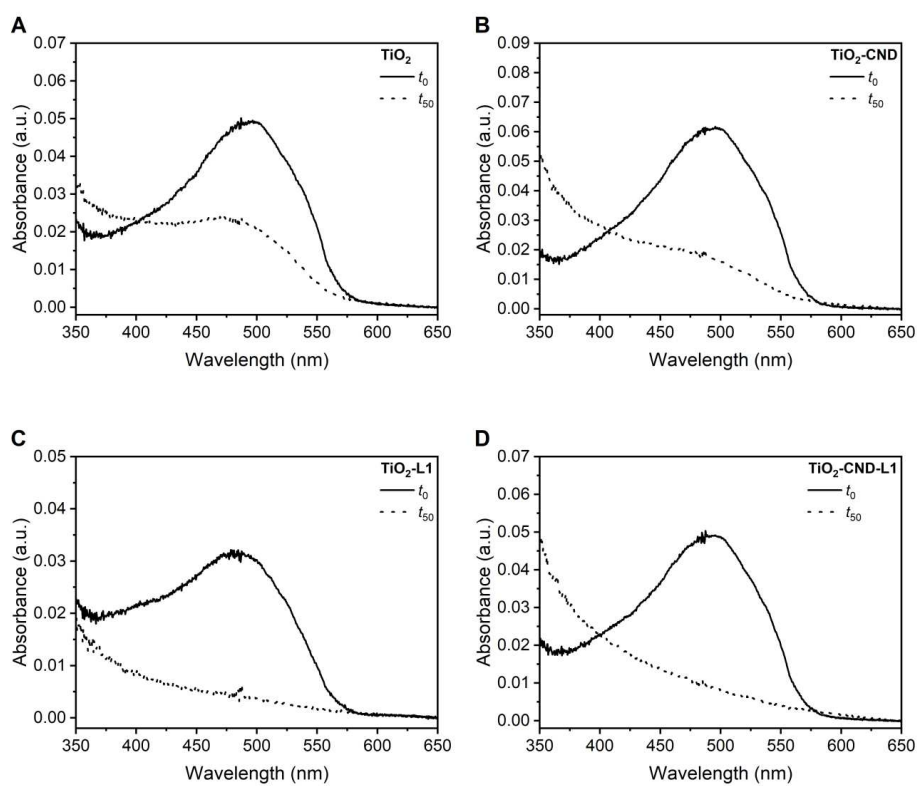


Figure S37. UV-Vis absorbance spectra of catalytic MO-degradation over a period of 50 minutes per cycle without a scavenger at pH 3.0. (A) TiO_2 ; (B) $\text{TiO}_2\text{-CND}$; (C) $\text{TiO}_2\text{-L1}$; (D) $\text{TiO}_2\text{-CND-L1}$.

NMR Spectra

CND

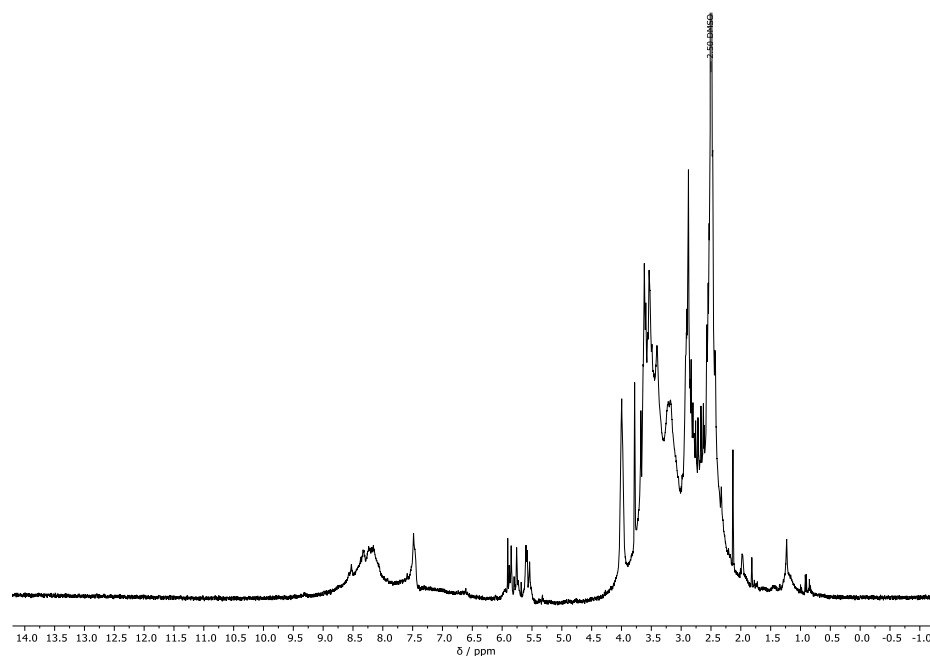


Figure S38. ¹H-NMR spectra of CND measured in DMSO-d₆.

5-(4-(Diphenylamino)phenyl)thiophene-2-carbaldehyde

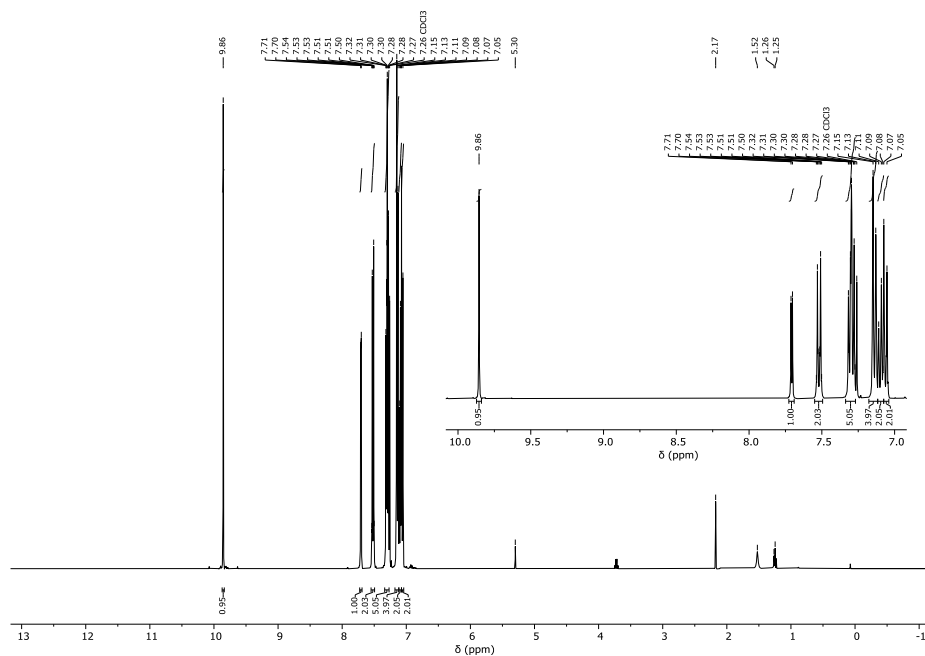


Figure S39. ^1H -NMR spectra of 5-(4-(Diphenylamino)phenyl)thiophene-2-carbaldehyde measured in CDCl_3 .

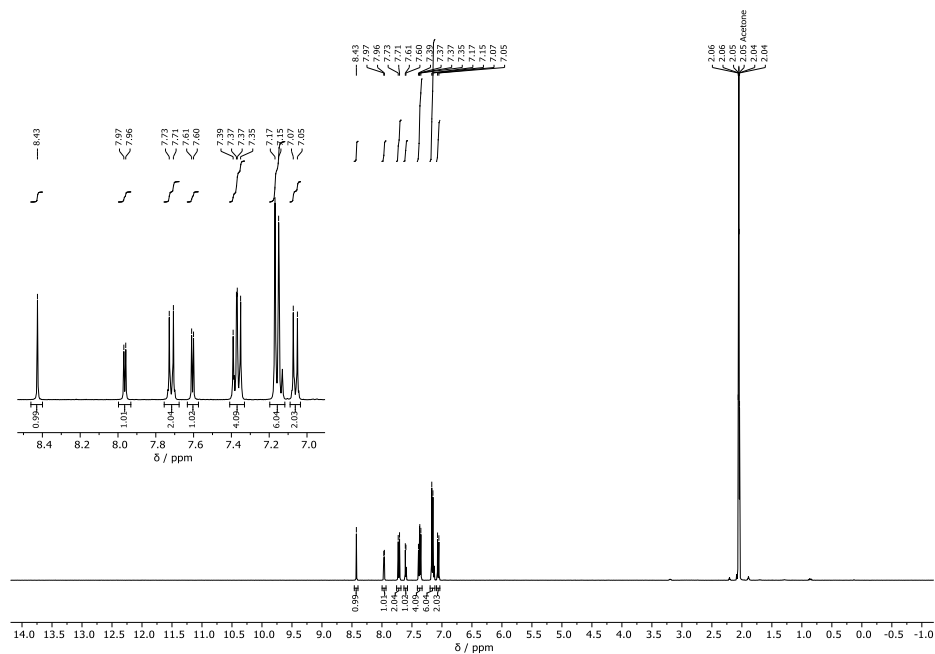
2-Cyano-3-(5-(4-(diphenylamino)phenyl)thiophen-2-yl)acrylic acid (L1)

Figure S40. ^1H -NMR spectra of 2-Cyano-3-(5-(4-(diphenylamino)phenyl)thiophen-2-yl)acrylic acid measured in Acetone-d_6 .

CND-L1 Hybrid

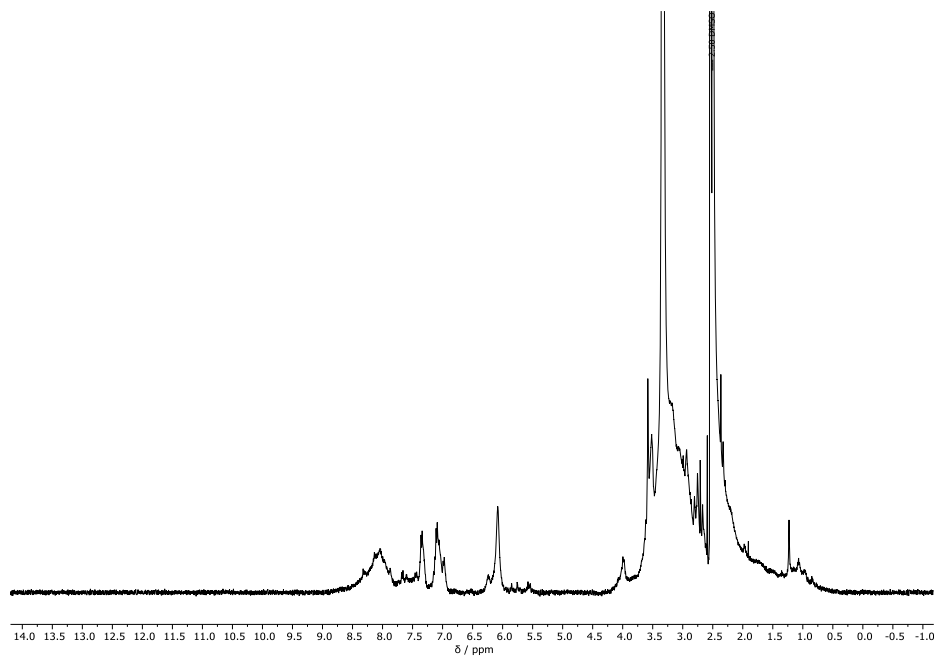
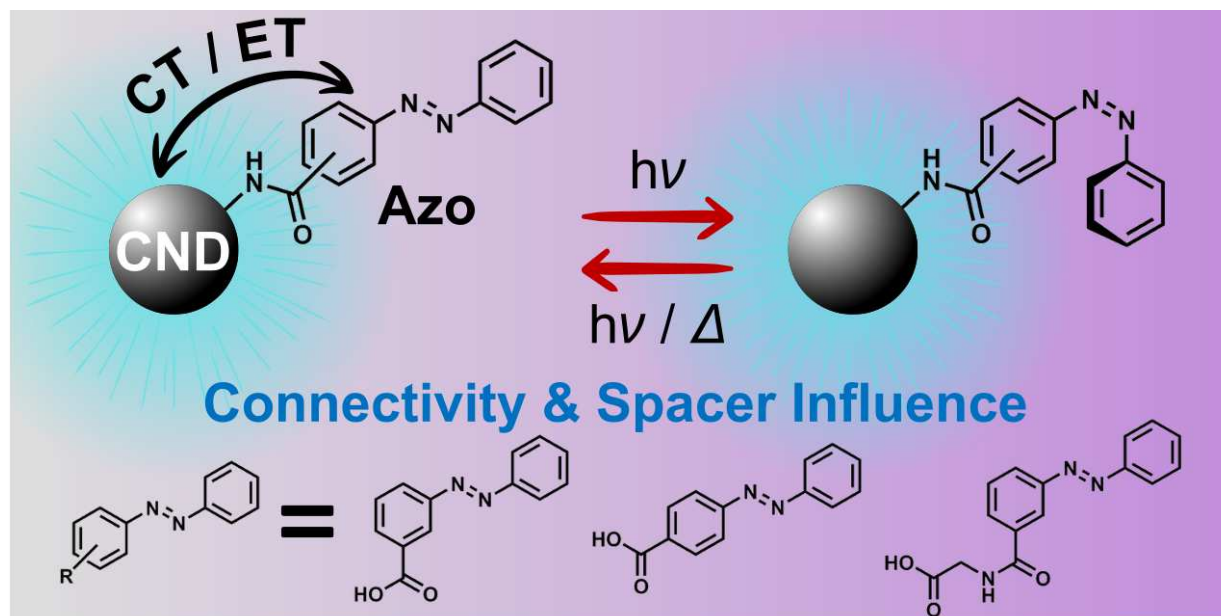


Figure S41. ^1H -NMR spectra of CND-L1 Hybrid measured in DMSO- d_6 .

2.3. Publication 3

Covalent Carbon Nanodot-Azobenzene Hybrid Photoswitches: The Role of *Meta/Para* Connectivity and sp^3 Spacer in Photophysical Properties



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Debes, P. P.; Schatz, D.; Aydogan-Sun, Y.; Martínez, J. P.; Langer, M.; Hessling, J.; Gallego, J.; Menna, E.; Smarsly, B. M.; Schönhoff, M.; Osella, S.; Wachtveitl, J.; Wegner, H. A.; Gatti, T. Covalent Carbon Nanodot-Azobenzene Hybrid Photoswitches: The Role of *Meta/Para* Connectivity and sp^3 Spacer in Photophysical Properties. *J. Mater. Chem. C* **2025**, accepted.

DOI: [10.1039/D5TC00116A](https://doi.org/10.1039/D5TC00116A)

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Cite this: DOI: 10.1039/d5tc00116a

Covalent carbon nanodot-azobenzene hybrid photoswitches: the impact of *meta/para* connectivity and sp^3 spacer on photophysical properties†

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The covalent surface functionalization of carbon nanodots (CNDs) can facilitate the design and development of nanocarbon hybrids with photoswitching properties, which can be applied in a wide range of applications, including sensing, optoelectronics, and even bio-applications. This study underscores the potential utilization of these hybrids as photoresponsive materials, for potential application in optostimulation. In this study, we examine the characteristics of covalent azobenzene-functionalized CNDs, with a particular emphasis on the impact of *meta* and *para* connectivity and the additional introduction of a glycine spacer. The CND synthesis process comprises a bottom-up microwave condensation of ethylenediamine and citric acid. Amide coupling to azobenzenes is confirmed through NMR diffusion-ordered spectroscopy and diffusion decay analysis. A comprehensive investigation is conducted into the size and optical properties of the resulting hybrids. Moreover, time-dependent density functional theory computations are employed to understand absorption spectra and charge transfer events. Furthermore, advanced optical characterisation is utilised to examine energy/charge transfer between the constituents. Finally, the switching properties, fatigue resistance, and half-life of the hybrids are studied to evaluate their performance for prospective applications like in optostimulation.

Received 10th January 2025,
Accepted 6th May 2025

DOI: 10.1039/d5tc00116a

rsc.li/materials-c

Introduction

The field of optostimulation is receiving increased attention due to its potential to enable remote monitoring of cellular signaling with high precision.¹ Furthermore, the utilization of nanoparticles for biomedical applications is an expanding area of research, with the primary objectives of enhancing imaging techniques and improving drug delivery systems.² Both approaches serve as valuable tools for advancing our understanding of bacterial cell physiology in both fundamental and applied research contexts. In 2023, the group of Paternò demonstrated that an amphiphilic azobenzene (azo) unit can non-covalently attach to the plasma membrane of *Bacillus subtilis* bacteria.³ Upon irradiation and azo isomerization, the membrane structure relaxes and expands. It has been demonstrated that carbon nanodots (CNDs) are capable of localizing within biomembranes. Consequently, covalent functionalization of CNDs with azos could enable the development of a hybrid material capable of entering biomembranes, being sensed, and undergoing structural changes in response to light

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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5tc00116a>

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irradiation.^{4,5} Ultimately, this research aims to provide new tools for effective optostimulation and imaging applications in biomedical research.

Combining different materials not only improves functionality, but also results in a wide range of properties that can be tailored for specific applications in microbial systems and beyond. Over the past decade, the field of carbon nanomaterials has witnessed a growing body of research. Fullerenes, **CNDs**, carbon nanotubes, and graphene are prominent examples of these carbon nanomaterials.^{6–10} Notably, **CNDs** have attracted attention due to their facile synthesis, low cost, excellent biocompatibility, easy functionalization, water solubility, and bright luminescence.¹¹ Bottom-up **CNDs** are synthesized using small molecules as starting materials.¹² Commonly utilized molecules for synthesis include citric acid (CA), urea, amino acids, and ethylene diamine (EDA). The resulting nanoparticles exhibit a quasi-spherical shape and are typically smaller than 10 nm in size.¹³ These nanoparticles consist of carbon, hydrogen, oxygen, and often nitrogen.¹⁴ **CNDs** are composed of a carbon core surrounded by functional groups.¹⁵ The type and quantity of functional groups on the surface of **CNDs** are influenced by the selected starting materials and their ratios.¹⁶ Alcohols, amines, and carboxylic acids are common functional groups that allow covalent functionalization of a wide range of materials.^{17,18} Among the innumerable possibilities, functionalization with photochromic molecules enables the production of carbon nanomaterials with the ability to respond to light irradiation.¹⁹ Photoswitchable molecules have the capability to undergo a light-induced reversible transformation from one species to another. Depending on the molecule, there are at least two states which are stable or metastable.²⁰

One of the widely used photochromic motifs is the *trans/cis* isomerization of azos,^{21,22} stilbenes,²³ hydrazones,^{24–26} and Schiff bases.^{27,28} Azos undergo a significant change in length and geometry through isomerization, making them useful in energy or information storage,^{29–34} photobiology,^{35,36} host-guest systems,³⁷ as molecular wind-up meters,³⁸ and molecular machines.^{39,40} The *trans* to *cis* isomer switch is usually induced by irradiation in the π – π^* band, typically between 300 nm and 360 nm. Reversion to the thermodynamically favored *trans* isomer can be induced thermally or by n – π^* excitation around 440 nm,^{40,41} or by various catalytic processes.^{42,43} The linkage of two azo units, either *via* the *para* or the *meta* position, has demonstrated that the resulting combinations exhibit distinct properties. Compared to the π -conjugation in the *para*-connected azo, the *meta*-connection resulted in a more independent behavior of the two entities.^{44–46} Even if the systems are separated through space and are not in conjugation with each other, they influence one another.^{47,48} For a better understanding of the connectivity and through-space interaction between azos and nanocarbons such as **CNDs**, we report in this work three different systems with either *meta*- or *para*-connectivity, and considering a sp^3 spacer between the two moieties in one of the systems. The impact of functionalization type and inter-unit distance on physicochemical characteristics—specifically absorbance, photoluminescence (PL), quantum yield (QY), half-life, and fatigue resistance—has been

comprehensively investigated. Moreover, time-correlated single photon counting (TCSPC) is utilized to investigate the energy/charge transfer occurring between these components. Additionally, theoretical analysis of absorbance properties was conducted using time-dependent density functional theory (TDDFT) computations to provide deeper insights.

These different **CND-azo** hybrids could serve as valuable platform for functional species in several light-triggered applications.

Results and discussion

CNDs were synthesized *via* a microwave (MW)-assisted reaction between CA and EDA in a 1 : 1 ratio, followed by purification through filtration and dialysis. The synthesized **CNDs** were thoroughly characterized using atomic force microscopy (AFM), UV-vis absorbance, PL, and NMR spectroscopy to confirm their presence and gain insight into their properties. In a previous study, we reported the presence of **CNDs** featuring surface-exposed primary amine groups, which are available for further functionalization *via* an amide coupling reaction.¹⁶ Three distinct azo derivatives were synthesized utilizing the Baeyer–Mills reaction, each containing a carboxylic acid functional group. The first derivative has *meta* (**m**) connectivity, the second has *para* (**p**) connectivity, and the third has a glycine spacer in the *meta* position (**m-Gly-Azo**). Subsequently, the azo compounds were covalently bonded to the **CNDs** *via* an amide coupling, as illustrated in Fig. 1. To provide comprehensive evidence of the successful functionalization of the **CND** with the respective azo diffusion-ordered spectroscopy (DOSY) NMR was used. A similar approach was employed in 2018 by Prato and co-workers to demonstrate covalent functionalization of **CNDs**.⁴⁹ Bare **m-Azo**, pristine **CND**, a physical mixture of **CND** and **m-Azo**, and the three hybrids were subjected to 2D DOSY NMR (see Fig. S1 to S6, ESI†) as well as an analysis of the diffusion decays (see Fig. 2). In the DOSY spectra **m-Azo**, **CND**, and the hybrids exhibit a single monomodal distribution, indicating that the constituents of these samples are present in a monodispersed state. In contrast, the physical mixture of **CND** and azo shows two separate diffusion distributions, which proves that the two moieties move separately in the mixture. For a more precise quantification, the diffusion coefficients were derived from the diffusion echo decays (see Fig. 2A) by applying the Stejskal–Tanner equation,⁵⁰ and assuming a log-normal distribution of the diffusion coefficients (see ESI,† eqn (S2) and (S3)).

Since no separate **m-Azo** resonances could be identified in the hybrid samples (see Fig. S1–S6, ESI†), the integral over the chemical shift region from 8.5 to 7.3 ppm was evaluated in all cases. **m-Azo** was used as a representative Azo moiety to compare the molecular diffusion coefficients to those of **CND** and the hybrids. The diffusion coefficient of **m-Azo** in solution is depicted as a dotted red line at $2.93 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, as it does not possess a size distribution, in contrast to the **CND** or the hybrids (Fig. 2B). The diffusion coefficient distribution for pristine **CND** exhibits a maximum at $D = 1.05 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.



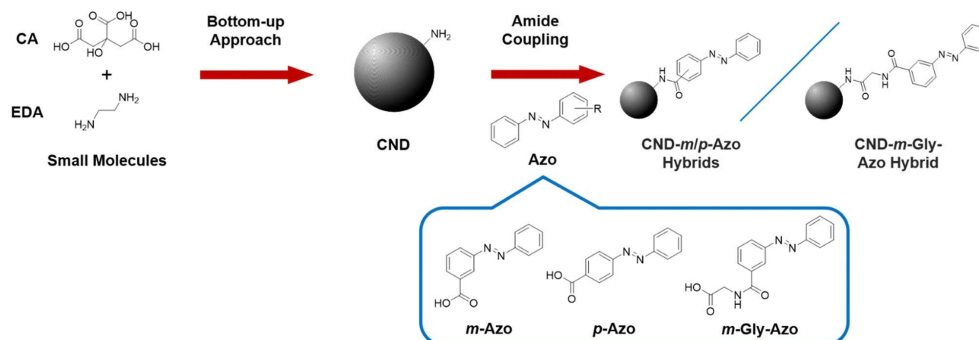


Fig. 1 Schematic representation of the microwave-assisted bottom-up synthesis of the CND from CA and EDA (1:1) in aqueous solution, followed by the covalent functionalization via amide coupling (EDC, NHS in DMF) with three different azos (*m*-Azo, *p*-Azo, and *m*-Gly-Azo) forming three distinct CND-azo hybrids, namely: CND-*m*-Azo, CND-*p*-Azo, and CND-*m*-Gly-Azo.

Measurements of the hybrids revealed a 20% reduction in the diffusion coefficient, with values of $0.84 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ observed for both the CND-*m*-Azo and the CND-*m*-Gly-Azo hybrids. The CND-*p*-Azo hybrid exhibited a diffusion coefficient of $0.93 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, which corresponds to a decrease of 11% in comparison to pristine CND. A decrease of the diffusion coefficients accompanies an increase in particle size, which is consistent with successful functionalization of the CNDs. In contrast, the physical mixture of CNDs with *m*-Azo shows two independent peaks in the diffusion domain of the 2D spectrum (Fig. S3, ESI†), which can be viewed as separate peaks in the distribution of diffusion coefficients in Fig. 2B. Notably, the fast-diffusing component, attributed to *m*-Azo, exhibits a slightly slower diffusion in the presence of CNDs as compared to bare

m-Azo solution, but generally agrees with the diffusion coefficient of bare *m*-Azo. Furthermore, the diffusion coefficient of the CNDs in the mixture, represented by the shoulder with the lower diffusion coefficient, agrees very well with that of bare CND. This indicates that the physical mixture consists of *m*-Azo and CND as distinct species. In contrast, the hybrid samples do not exhibit any component with a fast diffusion coefficient, suggesting the absence of individual *m*-Azo molecules. Additionally, the reduced diffusion of the hybrid CND particles reflects an increased radius, consistent with functionalization. In consideration of the Stokes-Einstein equation (see ESI†, eqn (S4)), the hydrodynamic diameter can be determined from the diffusion coefficient at the maximum. The size of the CND is at around 1.9 nm and the CND-*m*-Azo at around 2.4 nm, which is in good

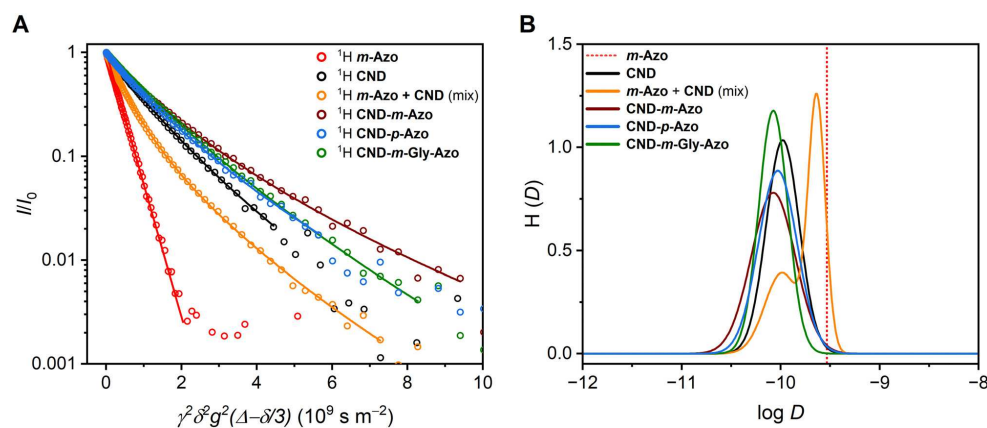


Fig. 2 (A) ^1H diffusion decay signal, integrated from 8.5 ppm to 7.3 ppm for the *m*-Azo, CND, a physical mixture of *m*-Azo and CND (1:10), CND-*m*-Azo, CND-*p*-Azo, and CND-*m*-Gly-Azo hybrids. (B) Distribution of diffusion coefficients according to the method of Leno et al.²⁷ (see description in ESI†) for *m*-Azo, CND, a physical mixture of *m*-Azo and CND (1:10), CND-*m*-Azo, CND-*p*-Azo, and the CND-*m*-Gly-Azo. The diffusion coefficient of *m*-Azo is depicted as straight dotted line due to lack of a size distribution.

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Table 1 The maximum of the diffusion coefficient distribution, derived from fitting of echo decays. Moreover, the hydrodynamic diameter, calculated using the Stokes–Einstein equation

Sample	$D/m^2\ s^{-1}$	Hydrodynamic diameter/nm
<i>m</i> -Azo	2.93×10^{-10}	0.68
CND	1.05×10^{-10}	1.90
<i>m</i> -Azo + CND (physical mix) ^a	2.32×10^{-10}	0.86
	1.03×10^{-10}	1.90
CND- <i>m</i> -Azo	0.84×10^{-10}	2.40
CND- <i>p</i> -Azo	0.93×10^{-10}	2.10
CND- <i>m</i> -Gly-Azo	0.84×10^{-10}	2.40

^a The physical mixture corresponds to a weight ratio of 1 : 10 of *m*-Azo to CND.

Table 2 UV-vis absorption maxima (λ_{Abs}), PL maxima (λ_{Em}), Stokes shift, and PLQY of the three CND-azo hybrids compared to pristine CNDs, pristine azos, and physical mixtures of the azos and the CNDs

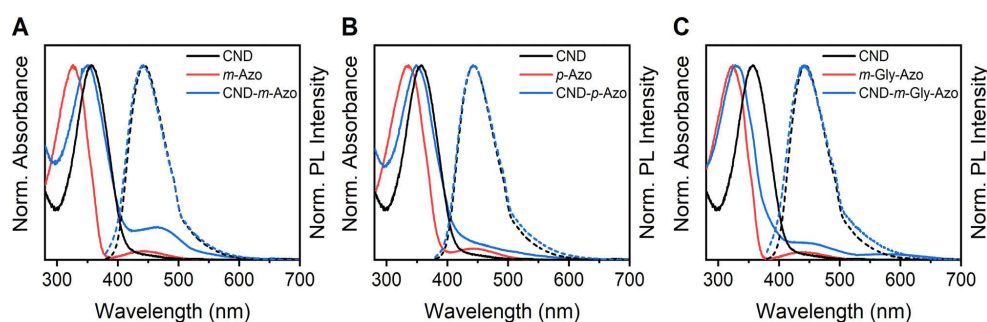
Sample	$\lambda_{\text{Abs}}/\text{nm}$	$\lambda_{\text{Em}}/\text{nm}$	Stokes shift/nm	PLQY/%
<i>m</i> -Azo	326			
<i>p</i> -Azo	335			
<i>m</i> -Gly-Azo	323			
CND	357	443	86	37
CND- <i>m</i> -Azo	350	440	90	18
CND- <i>p</i> -Azo	349	443	94	11
CND- <i>m</i> -Gly-Azo	329	441	112	1
CND + <i>m</i> -Azo (mix)	349	443	94	31
CND + <i>p</i> -Azo (mix)	343	443	100	18
CND + <i>m</i> -Gly-Azo (mix)	338	443	105	23

agreement with the measured AFM size (see Fig. S7, S8, and S11A and B, ESI†). Accordingly, the size of the remaining hybrids can be given by the hydrodynamic diameter as well. CND-*p*-Azo possess a size of 2.1 nm, while the CND-*m*-Gly-Azo measures 2.4 nm (Table 1). Whereas AFM measurements indicate a size of 4.9 nm for the CND-*p*-Azo and 4.8 nm for the CND-*m*-Gly-Azo, which can be attributed to potential agglomerates on the mica surface (see Fig. S9, S10, and S11C and D, ESI†).⁵¹

Thermogravimetric analysis (TGA) suggests the occurrence of functionalization; however, the CND morphology precludes the possibility of making a precise statement regarding the degree of functionalization based on TGA results (Fig. S12, ESI†). As illustrated in Fig. 3, the absorption and emission spectra of the three hybrids are displayed in comparison to the pristine CND and the absorption of the corresponding azo compound (which are non-emissive). Table 2 presents the individual maxima of the absorption λ_{Abs} and emission λ_{Em} , the Stokes shift, as well as the PLQY (Fig. S13 and S14, ESI†).

A comparative analysis of the absorption spectra of the three hybrids revealed that CND-*m*-Azo exhibited the smallest blue shift compared to the CND, with an absorption maximum at 350 nm versus 357 nm for CND. In contrast, CND-*m*-Gly-Azo displayed the largest blue shift (maximum at 329 nm), while CND-*p*-Azo (maximum at 349 nm) showed a shift similar to that of CND-*m*-Azo. Qualitative analysis of the ¹H NMR spectra

indicates that CND-*m*-Gly-Azo has a greater quantity of *m*-Gly-Azo on its surface, as evidenced by more pronounced azo group peaks compared to CND-*m*-Azo and CND-*p*-Azo (Fig. S57–S59, ESI†). This finding suggests that the extent of functionalization significantly influences the observed shifts of the absorption maxima. Although CND-*p*-Azo and CND-*m*-Azo exhibit comparable blue shifts relative to pure CND, *p*-Azo shows a larger red shift (335 nm) than *m*-Azo (323 nm), contributing to the minor blue shift in CND-*p*-Azo. To quantify surface functionalization, UV-vis spectra were deconvoluted using two Lorentzian functions, allowing for calculation of azo concentrations via Beer-Lambert law.⁵² The absorbance coefficients of the azo groups were considered (Fig. S15, ESI†), along with the maximum absorbance values derived from the deconvolution, which represent the respective azo species (Fig. S16, ESI†). This methodology has already been applied in a similar way to functionalized CNDs in one of our previous studies.⁵³ The azo content was found to be 3.4% by mass for CND-*m*-Azo and 4.5% for CND-*p*-Azo, while CND-*m*-Gly-Azo exhibited a significantly higher value of 24%. Corresponding primary amine amounts were calculated as 150 $\mu\text{mol g}^{-1}$ for CND-*m*-Azo, 200 $\mu\text{mol g}^{-1}$ for CND-*p*-Azo, and 850 $\mu\text{mol g}^{-1}$ for CND-*m*-Gly-Azo, consistent with previous findings on amine functionalization on these CNDs. In a previous study, the maximum amount of primary amines measured on the surface of the CND

**Fig. 3** Normalized absorbance (red solid lines) of (A) *m*-Azo, (B) *p*-Azo, (C) *m*-Gly-Azo, normalized absorbance (blue solid lines) and PL (blue dotted lines) of (A) CND-*m*-Azo, (B) CND-*p*-Azo, (C) CND-*m*-Gly-Azo compared to pristine CNDs.

was measured to be $1670 \mu\text{mol g}^{-1}$.¹⁶ It is a well-known phenomenon that complete functionalization of primary amines on the CND's surface is rarely achieved. However, to increase the degree of functionalization, it is possible to change the starting materials of the CND to more nitrogen-rich precursors, such as arginine, or to increase the EDA content to obtain a larger amount of primary amines on the surface of the CND.⁵⁴

The degree of azo functionality on the surface of the CNDs may be influenced by the nature of the established connectivity. Preliminary observations suggest that *m*-Azo may exhibit greater steric demand compared to *p*-Azo; however, this assertion requires further investigation. In contrast, the introduction of a glycine spacer appears to reduce this steric demand, thereby facilitating a higher degree of functionalization within the *meta*-connected hybrids. This indicates that the connectivity and spacer design can modulate the optical properties. *Meta*-Functionalization is expected to lead to a more independent behavior of the CND and azo, while *para* connectivity may allow conjugation between the two systems.^{15,44} As a matter of fact, the hybrids exhibited frontier molecular orbitals localized on either the CND or azo fragments, but the conjugation is not extended on both (Fig. 4). Although *para*-functionalization should enable conjugation and thus strong intramolecular interaction, this is not evident in the absorption spectra. This implies that the azo moiety remains spatially separated from the fluorophores of the CND and only engages through space.

Computational investigations were further employed to understand the UV-vis spectra presented in Fig. 3. First, molecular dynamics (MD) simulations were performed to develop a thermally stable model representing the molecular structure of the CND (Fig. S17 and S20, ESI†). Under high-temperature conditions, precursor molecules undergo polymerization and dehydration, forming complex polymer/carbon hybrid structure of CDs, where molecular fluorophores may be covalently attached to CDs.¹⁰ Unfortunately, the structural complexity of CDs makes it difficult to build exact model structures, thus forcing researchers to reach for *ad hoc* models.^{55–59} Our *ad hoc* model represents the polymeric CND model synthesized in the conditions before reaching full-carbonization, where small

fluorophores may still dominate the optical properties of CNDs.^{57,60,61} The MD simulations revealed that the system self-assembled into the CND shaped particle within the simulation time (Fig. S17 Sections b–f, ESI†), with the average radius of gyration 1.0 nm (Fig. S18a, ESI†), thus the estimated hydrodynamic radius of this model shall be around 2.5 nm⁶² (see the note in the ESI†). This CND model was observed to be stabilized not only by hydrogen-bond formation (Fig. S18b, ESI†), but especially through the stacking of the 12 IPCA molecules (5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*x*]pyridine-7-carboxylic acid), which are covalently embedded within the CND (Fig. S19, ESI†).

Subsequently, a quantum mechanics/molecular mechanics (QM/MM) approach based on tight-binding DFT^{63,64} and TD- ω B97XD/6-31G(d) methods were benchmarked to calculate UV-vis spectra in DMSO (Table S1 and Fig. S21 and S22, ESI†). The absorption spectra for CND shows that the most intense and lowest-energy transition S_1 (357 nm, $f = 0.27$, Fig. 5) is in excellent agreement with the experimental $\lambda_{\text{Abs}} = 357$ nm value reported in Table 2. Motivated by these results, the CND model was chemically functionalized with one azo derivative at different sites at the surface of the CND (Table S2, ESI†). The most thermodynamically stable constitutional isomer of CND-azo was selected for TDDFT computations (Fig. S24, ESI†).

Specifically, CND-*p*-Azo was selected as a representative example for the analysis of the TDDFT results, while calculated spectra and electron transition schemes for both *para* and *meta* conformers of *cis/trans* azo compounds, and the respective CND-azo hybrids, are presented in Fig. S25–S30 (ESI†). To confirm that the ω B97XD/6-31G(d) functional and basis set combination used is a good choice to reproduce the experimental spectra, we performed benchmark experiments (see ESI† for details). The lowest-energy electronic transitions in CND-*p*-Azo incorporating the *trans* isomer of the azo fragment are illustrated in Fig. 5. The first strong peak obtained is attributed to the S_1 $n-\pi^*$ transition at 513 nm, localized on the azo fragment, CND-*p*-Azo* (where the * indicates the moiety that is excited). The second peak/shoulder relates to the S_2 $\pi-\pi^*$ transition at 397 nm, exhibiting a strong CT character, from the CND to the Azo moiety (CNDs+*p*-Azo[−]). Furthermore, the calculated maximum of absorption intensity at 336 nm ($f = 1.08$) is in very good agreement with the experimental value of 349 nm. This peak is assigned to the $\pi-\pi^*$ transition S_3 localized on the azo moiety, CND-*p*-Azo*, which is analogous to S_2 , although they differ in their orbital contributions. The $\pi-\pi^*$ transitions S_4 and S_5 are localized excitations on the nanodot fragment, CND*-*p*-Azo, which are blue shifted by at least 26 nm compared with the peak of 357 nm in pristine CND. Furthermore, the system is prone to CT events, since either S_4 or S_5 may undergo internal conversion to CT S_2 state. The corresponding CT reactions CND*-*p*-Azo $\rightarrow$ CND*-*p*-Azo[−] are associated with favorable driving forces (0.6 eV for $S_4 \rightarrow S_2$ and 0.8 eV for $S_5 \rightarrow S_2$, see Fig. 5), assuming neglecting entropic effects, which is valid under the Franck-Condon approximation. In summary, considering CND-azo incorporating the azo fragment in *para/meta* and *cis/trans* configurations, electronic transitions in the UV region

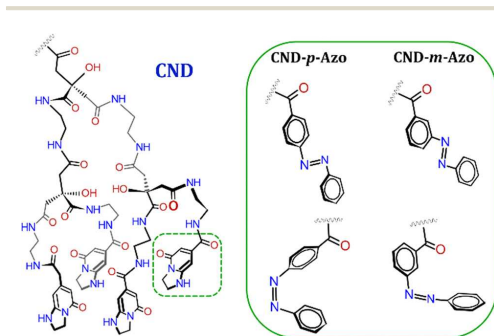


Fig. 4 Structures of the CND-azo hybrids used in the computation.

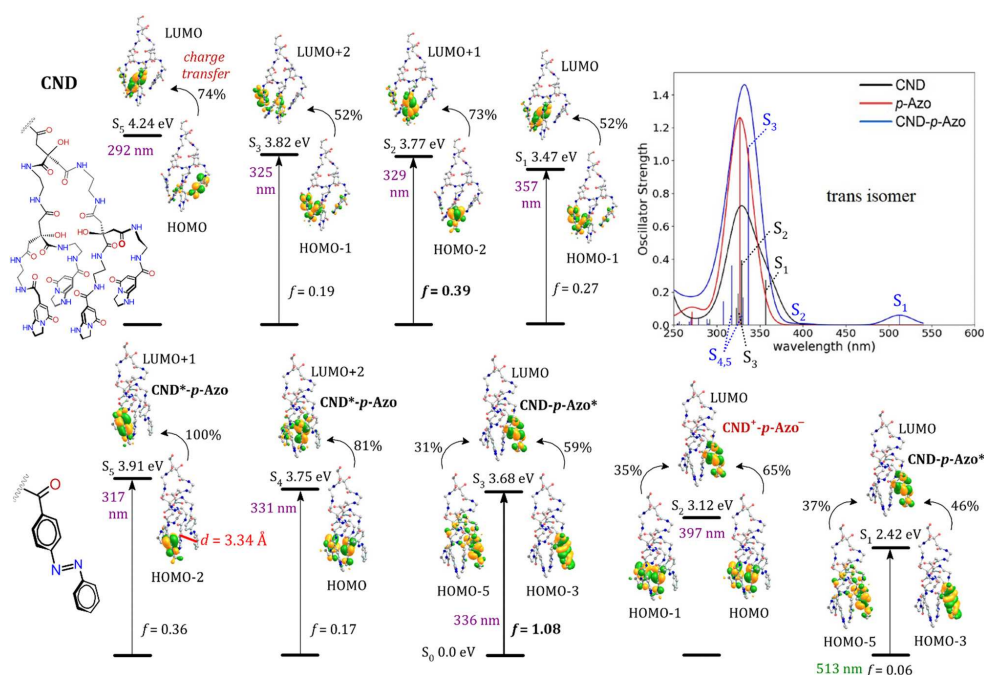


Fig. 5 The lowest-energy electron transitions in the **CND** (top) and **CND-p-Azo** (bottom, f = oscillator strength, % of molecular-orbital contributions). The azo moiety is in the *trans* configuration. Only the fragment simulated at the TDDFT level is illustrated for the sake of clarity. The calculated spectrum is also illustrated.

occur in both fragments (**CND-azo*** and **CND*-Azo**) for both *para* and *meta* conformers (see Fig. 5 and Fig. S30, respectively, ESI†) in the *trans* azo isomer. Conversely, for the *cis* isomer, excitations are primarily characterized by **CND*-Azo** in the UV region (Fig. S26 and S27, ESI†). We observed that the spectra of the hybrids incorporating the *trans* configuration of the azo moiety exhibit closer alignment with the experimental evidence (Fig. S28, ESI†). These findings suggest that the *trans* isomer of azo in the **CND-azo** hybrid is predominant in the electron transitions. In this regard, experiments determined that a

wavelength of 340 nm induces the conversion to the *cis* isomer in the hybrids (Fig. 7). We additionally examined CT excitation energies (ω) in the hybrids, which shifted by 72 nm and 65 nm for *trans* **CND-p-Azo** and **CND-m-Azo** compared with the *cis* conformer, respectively (see Table 3). Nonetheless, CT excited states can be also accessed in the hybrids containing the *cis* isomer, albeit with smaller values. In this context, this *cis/trans* CT activity may be envisioned for use in photoswitching (on/off), data storage (0, 1), or molecular transistors (*gate* open/close).

Similar trends are observed for the incorporation of a molecular spacer, **CND-m-Gly-Azo** (Fig. S31–S35, ESI†). Nonetheless, the flexibility provided by the molecular spacer either induces (when $d < 4$ Å) or inhibits (when $d > 6$ Å) the formation of CT states (presented in Table 3). However, the flexibility of the molecular spacer allows access to various structural configurations, indicating that CT states are not strictly inhibited.

Further, functionalization of the **CND** did not significantly change the emission maximum, as observed in the excitation–emission maps and excitation spectra of the **CND** and the hybrids (Fig. S37 and S38, ESI†). This aligns with the computational assumption put forth in this study and in our previous investigation, where an aggregated molecular fluorophore, IPCA, was identified as the primary PL source of the **CNDs**.^{10,16}

Table 3 Charge transfer excitation energies (ω), Hirshfeld charges (q) in azo and **CND** along with the distance (d)^a between these fragments

Hybrid	ω [nm (eV)]	q_{azo} (e)	q_{CND} (e)	d (Å)
<i>trans</i> CND-p-Azo	397 (3.12)	−0.72	+0.72	3.34
<i>trans</i> CND-m-Azo	375 (3.30)	−0.64	+0.64	3.44
<i>trans</i> CND-m-Gly-Azo	374 (3.31)	−0.76	+0.76	3.41
<i>trans</i> CND-m-Gly-Azo	Inhibited			7.95
<i>cis</i> CND-p-Azo	325 (3.81)	−0.74	+0.74	3.24
<i>cis</i> CND-m-Azo	310 (4.00)	−0.85	+0.85	3.90
<i>cis</i> CND-m-Gly-Azo	351 (3.53)	−0.85	+0.85	2.86
<i>cis</i> CND-m-Gly-Azo	Inhibited			17.02

^a d stands for the distance between the phenyl centroid in *trans*- or the N=N group in *cis*-azo and the closest centroid of IPCA in the **CND**.



It is notable that the molecular fluorophores present in the **CND** are not directly covalently linked with the azos; rather, they interact through space. Moreover, a through-bond mechanism appears to be unlikely for the lowest excited states, as the excitation energies are localized on the individual units, as illustrated in Fig. 5.

Accordingly, a through-space mechanism, such as Förster resonance energy transfer (FRET) or electron transfer, is a viable option, given the close proximity of the units (see Table 3).^{65–68} FRET relies on a dipole–dipole interaction, where the resonance between the donor's emission and the acceptor's absorbance and the relative orientation of their transition dipole moments plays a crucial role.⁶⁹ In contrast, electron transfer requires a substantial overlap between the donor and acceptor orbitals, a prerequisite that is plausible based on our computed model.^{70,71} The potential of our synthesized hybrids to undergo both charge transfer and FRET suggests that quenching efficiency may be influenced by both mechanisms. As shown in Fig. 3, the **CND** emission strongly overlaps with the $n\text{--}\pi^*$ absorbance of the azo moieties, making a FRET from **CND** to the azo moieties possible. This was further confirmed by overlap integral calculations, which were used to determine the critical Förster radius (R_0) (see ESI† for details). The R_0 values vary slightly among the hybrids, averaging around 2.1 nm. Using these values and the distances listed in Table 3, FRET efficiencies were calculated, revealing a near-complete (99.9%) transfer efficiency for all three hybrids. This high efficiency results from the short **CND**-to-azo distance, which at 0.33 nm is well below the Förster radius.

However, the PLQY measurements indicate that this high efficiency is valid only for **CND-*m*-Gly-Azo**, as the other hybrids still exhibit a significant amount of **CND** fluorescence. To account for these differences, the quenching efficiency was determined using PLQY, since both FRET and electron transfer typically reduce the fluorescence quantum yield and lifetime of the donor. The resulting quenching efficiencies were 51% for **CND-*m*-Azo**, 70% for **CND-*p*-Azo**, and 97% for **CND-*m*-Gly-Azo**. Notably, quenching efficiency depends on the acceptor concentration within the system. Due to the undefined structural configuration of the hybrids and the unknown number of azo moieties per **CND**, accurately quantifying the relative contributions of different quenching processes is not possible.⁶⁷

The elevated quenching efficiencies should also correspond to reduced fluorescence lifetimes of the **CND** moiety. To verify this, the fluorescence lifetimes of the pure **CND** and the hybrids were measured using TCSPC. All four systems exhibited three distinct fluorescence lifetimes, reflecting the complex surface structure of the **CNDs**. For the simplicity, these lifetimes were combined into an amplitude-weighted average lifetime. As expected, the pure **CND** holds with 5.70 ns the longest average fluorescence lifetime, followed by 3.60 ns and 3.45 ns for **CND-*p*-Azo** and **CND-*m*-Azo**, respectively. In contrast, with only 2.24 ns, the linker in **CND-*m*-Gly-Azo** clearly leads to the shortest fluorescence lifetime (Fig. 6 and Table S3, ESI†). While these lifetimes can also be used to estimate the quenching efficiencies of the hybrids, the obtained values are less reliable

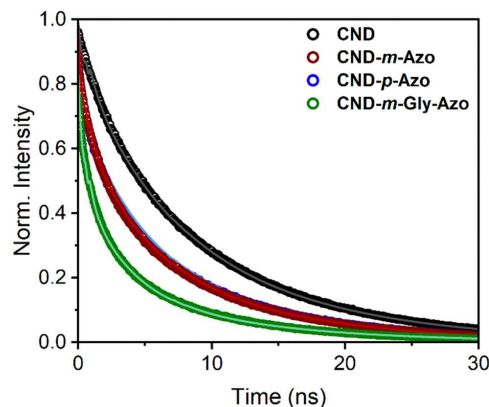


Fig. 6 Decay curves of the fluorescence transients recorded with TCSPC. While the raw data are shown as circles, the respective fits consist of solid curves in a slightly darker colour.

(see ESI† discussion for more details). The superior quenching efficiency of **CND-*m*-Gly-Azo**, in comparison to the hybrids that lack a linker, can be attributed to its higher density of functionalization. A greater density of azo moieties on the **CND** surface increases the probability of quenching. Additionally, our computational study demonstrated that the larger spacer in **CND-*m*-Gly-Azo** enhances flexibility and brings the azo units into closer proximity to the **CND**, further facilitating charge transfer processes.

To confirm the retained photoswitching properties of the azos after covalent bonding to **CNDs**, the hybrids and bare azos were irradiated with a 325 nm LED for two minutes, followed by a 448 nm LED irradiation for 30 seconds, followed by measurement of the absorption spectra before and after each illumination (Fig. S39, ESI†). Among the hybrids, **CND-*m*-Azo** exhibited the smallest absorbance change upon *trans*-*cis* isomerization, whereas **CND-*m*-Gly-Azo** showed the largest, consistent with their respective degrees of functionalization. Among the pristine azos, the *p*-Azo displayed the smallest decrease in absorbance when irradiated with the 325 nm LED (Fig. S39, ESI†). However, the **CND-*p*-Azo** demonstrated a greater change in absorbance than the **CND-*m*-Azo**, indicating that its 1.1% higher degree of functionalization outweighs the smaller change in absorbance observed in pure *p*-Azo. Additionally, pure azos exhibited greater changes in absorbance compared to hybrids, likely due to the significant contribution of the **CND** component to overall absorbance. The PL response to photoswitching indicated no significant reversible change in PLQY from the *trans* to the *cis*-isomers of the covalently linked azos. However, a slight decrease in PL intensity post-illumination prompted an investigation into fatigue resistance across multiple photoswitching cycles and associated PL properties (Fig. 7A–C). The selection of wavelengths was based on the absorption maxima of the hybrids, specifically 340 nm and 448 nm. The 340 nm wavelength was chosen to balance sufficient excitation energy while



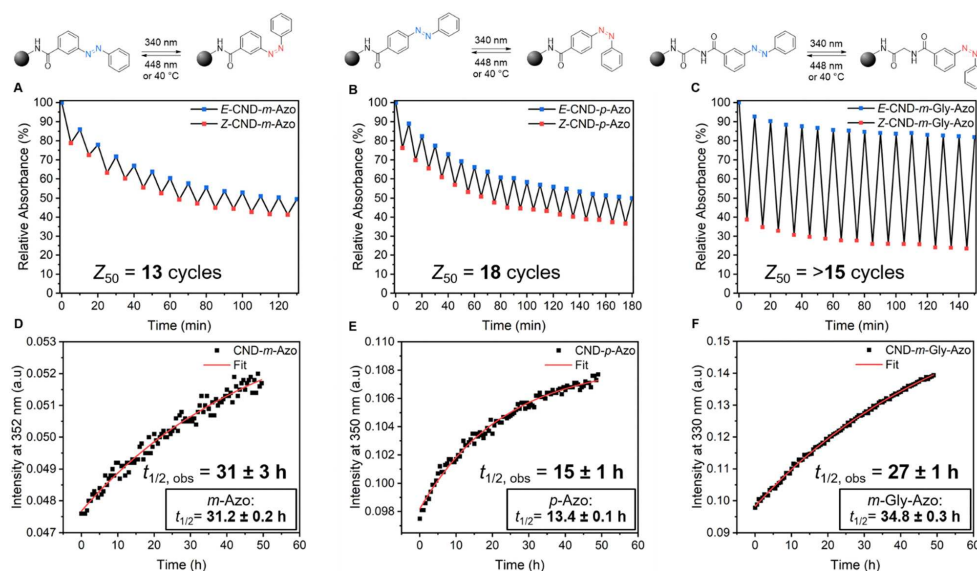


Fig. 7 Top panel: Schematic illustration of the azo-functionalized CNDs (black spheres), showing the photoinduced *trans*-to-*cis* conversion under 340 nm irradiation and subsequent reversion to the *trans*-isomer via 448 nm exposure or thermal isomerization at 40 °C. Middle panel: Fatigue resistance of CND-azo hybrids over multiple photoisomerization cycles using alternating 340 nm and 448 nm irradiation. (A) CND-*m*-Azo retains 50% of its initial absorbance at 350 nm for 13 *trans*-*cis*-*trans* cycles. (B) CND-*p*-Azo shows stability for 18 cycles at 349 nm. (C) CND-*m*-Gly-Azo maintains more than 50% absorbance at 329 nm for more than 15 cycles. Bottom panel: Thermal *cis*-to-*trans* isomerization kinetics of the hybrids at 40 °C over 50 h. Pristine azo half-lives are included for comparison (see ESI† for details). (D–F) Evolution of the absorbance at 352 nm (CND-*m*-Azo), 350 nm (CND-*p*-Azo), and 330 nm (CND-*m*-Gly-Azo) during thermal reversion. Single-exponential fits were used to determine half-lives. Note: Graphs D and E are normalized to their respective isosbestic points at 363 nm and 397 nm respectively, as agglomeration occurs over the time scale of the kinetic experiment. Due to the higher loading of azo in the CND-*m*-Gly-Azo hybrid, no spectra processing was necessary (see ESI† for details).

minimizing photodegradation. Each hybrid was irradiated for five minutes at these wavelengths to achieve the photostationary state (PSS) of the respective isomer (see Fig. S40, ESI†). CND-*m*-Azo underwent 13 *trans*-*cis*-*trans* cycles before reaching 50% of its original absorbance (Z_{50}).

In contrast, CND-*p*-Azo maintained 50% of its original absorbance over 18 cycles, demonstrating greater stability than CND-*m*-Azo. CND-*m*-Gly-Azo exhibited an 18% decrease in absorbance after 15 cycles, indicating it is the most durable hybrid produced. Fig. S41 (ESI†) shows the PL spectra following fatigue resistance tests, showing a similar decline in PL intensity corresponding to absorbance degradation. This suggests that the switching properties of the azos on the CND surface remain unaltered, while contributions from absorption and emission of the CNDs diminish. This hypothesis is supported by fatigue resistance measurements conducted under identical conditions with the pure Azos (Fig. S42, ESI†). After 120 minutes of light exposure, no significant drop in absorbance can be observed. Azos exhibit high fatigue resistance due to minimal occurrence of side reactions during photoisomerization. The increased fatigue resistance of the CND-*m*-Gly-Azo hybrid may be due to a higher azo coverage of the CND surface by the introduction of a glycine spacer. In classical organic

chemistry, undesired reactions involving a reactive intermediate often lead to the generation of side-products. By offering an inert environment for these intermediates, confinement can serve as a method to reduce side-product formation and enhance the fatigue resistance.⁷² Additionally, we assessed the thermal half-life of the *cis*-isomer of the hybrids at 40 °C in DMSO (Fig. 7D–F). A schematic representation of the azos on the CND surface, which is switched between *trans*- and *cis*-isomers via light or heat, is shown in the top panel of Fig. 7. Fig. S43–S48 (ESI†) display UV-vis spectra illustrating thermal conversion from *cis*- to *trans*-isomer alongside single exponential fits against time to determine the thermal half-lives (Table S6, ESI†). The hybrids tend to agglomerate during the 50-hour thermal *cis*-to-*trans* isomerization, which is potentially distorting the data. In order to compensate that, we normalized the absorption spectra of the CND-*m*-Azo and CND-*p*-Azo to the isosbestic points (see ESI† for details).

The CND-*m*-Azo variant exhibits minimal variation in half-life compared to pure *m*-Azo, while the CND-*m*-Gly-Azo hybrid displays a reduced half-life relative to *m*-Gly-Azo. For the *meta*-linked hybrids, it is assumed that they behave electronically independently from the CNDs. The observed differences can be attributed to steric effects and the degree of functionalization,



resulting in comparable or shorter half-lives than pure azos. Further systematic studies are required to quantify these contributions. In contrast, the **CND-*p*-Azo** demonstrates an increased half-life compared to ***p*-Azo**, suggesting that the electronic properties of the hybrid – modulated by the exchange of the carboxylic acid for an amide – can also play a role in altering the half-life. Furthermore, the precise chemical background of the functionalization employed, namely the amine on the **CND** side, has the potential to exert different electronic influences, given the lack of knowledge regarding the **CND** structure.

A comparison of the obtained results with other carbon allotropes functionalized with azos in the literature reveals that the influence of **CNDs** on the switching properties of the azos is relatively weak. In the case of fullerene, a publication by Shirai *et al.*⁷³ in 2008 demonstrated that direct conjugation of fullerene with an azo led to reduced photoisomerization yields, suggesting an efficient electronic energy transfer between the azo and fullerene. However, when fullerene and azo were electronically separated *via* a spacer, this effect was not observed. In our case, this further supports the hypothesis that the chemical nature of **CNDs** leads to electronic separation of the molecular fluorophores on **CNDs** and azo in all three of our investigated hybrids. Indeed, TDDFT results for **CND-*m*-Gly-Azo** revealed that the formation of CT states depends upon the distance between **CND** and azo, as previously concluded. In 2013, Feng *et al.*⁷⁴ introduced a reduced graphene oxide (RGO)-azo hybrid for long-term solar thermal storage. By achieving high packing densities and incorporating additional *ortho* and *para* functionalization of the azos with moieties that facilitate the formation of H-bonds, they successfully attained extended half-lives of the *cis*-isomer. The quasi-spherical and amorphous character of **CNDs** prevents the establishment of a repeating structure, allowing the azo units to orient in various directions without forming ordered configurations as seen on a RGO surface. Furthermore, the limited amount of azo present on the surface likely suppresses interactions between different azo units on a single **CND**.

Conclusions

In this study, three distinct **CND-azo** hybrids were synthesized, featuring either *meta*- or *para*-linkages as well as an additional glycine spacer. These hybrids exhibit both photoswitching capability and emissive properties, allowing them to be sensed and optically stimulated in the same system. The incorporation of the glycine spacer resulted in an increase in fatigue resistance, likely due to the formation of an inert environment around the **CND**. Furthermore, a reduction in the half-life of the *cis* isomer was observed in the case of the hybrid with the glycine spacer. In contrast, the two hybrids without the spacer exhibited similar half-lives compared to their pristine azo counterparts. This shorter half-life renders the system useful in optical switches due to their fast response to external stimuli. Furthermore, investigations using TDDFT methods

were performed to gain deeper insights into the experimental UV-vis absorption spectra and CT properties of the **CND-azo** hybrids. This approach enhanced our understanding of the influence of isomer configuration on electronic transitions, and it provided valuable guidance for the development of potential applications. The results demonstrate that *trans*-azo in **CND-azo** hybrids significantly stabilizes the LUMO energy, leading to enhanced CT activity compared to the *cis* counterpart. Specifically, *trans* isomers exhibit a lower CT excitation energy and more favorable driving forces for CT reactions, making them more effective for applications requiring efficient electron transfer. In contrast, *cis* isomers undergo CT processes but with reduced driving forces. This difference underscores the potential of functionalized **CND-azo** hybrids for use in photo-switches, data storage, and molecular transistors where CT is critical. The addition of molecular spacers, such as in **CND-*m*-Gly-Azo**, further illustrates that spacer flexibility influences the formation of CT states. Specifically, spacer distances greater than 6 Å inhibit CT states, while shorter distances can promote them, emphasizing the need for careful spacer design to optimize electronic properties. Lastly, the total emission quenching efficiencies for the hybrids were calculated from fluorescence lifetimes obtained with TCSPC measurements. The fluorescence lifetimes of the hybrids were observed to be shorter than those of the pristine **CND**, with the shortest lifetime noted for the **CND-*m*-Gly-Azo** hybrid. This is attributed to the higher concentration of azo groups present on the surface, as well as the higher flexibility of the spacer, which promotes CT processes. Overall, these findings highlight the potential of azo-functionalized **CND** hybrids in developing advanced light-responsive systems. This represents an intriguing point of departure for the design of novel scaffolds that can be integrated into devices, sensors/actuators, or living cells that are remotely controlled through light irradiation.

Author contributions

P. P. D.: writing original draft, conceptualization, investigation; D. S.: writing original draft, conceptualization, investigation; Y. A.-S.: investigation, conceptualization; J. P. M.: writing original draft, conceptualization, investigation in the computational section; M. L.: writing original draft, investigation, computations; J. H.: investigation; J. G.: writing – review & editing, conceptualization; E. M.: investigation, supervision, resources; B. M. S.: supervision, resources; M. S.: supervision, resources; S. O.: writing – review & editing, conceptualization, resources; J. W.: supervision, resources; H. A. W.: writing – review & editing, conceptualization, project administration; T. G.: writing – review & editing, conceptualization, project administration.

Data availability

The data supporting this article have been included as part of the ESI.†



Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the financial support of the DFG and of the National Science Center, Poland through the joint project “Low-Dimensional Nano-Architectures for Light Emission and Light-to-Electricity Conversion” (LOW-LIGHT, grant no. UMO/2020/39/1/ST4/01446). D. S. thanks the European and Hessian Government for funding the “Innovationslabor Prozessdiagnostik” (EFRE 21031934). J. P. M. acknowledges the funding of a post-doctoral contract by the LOW-LIGHT grant. T. G. acknowledges the European Research Council for the project JANUS BI (grant agreement no. [101041229]). M. L. acknowledges that this article has been produced with the financial support of the European Union under the REFRESH – Research Excellence For Region Sustainability and High-tech Industries project number CZ.10.03.01/00/22_003/0000048 via the Operational Programme Just Transition. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID: 90254). J. W. gratefully acknowledges the European Union for funding the TCSPC laser system through the European Regional Development Fund as part of the Union's response to the COVID-19 pandemic – REACT-EU, IWB-EFRE-Programm Hessen, #20008794. The authors thank Heike Hausmann (JLU, Giessen) for performing the NMR ^1H and ^{13}C spectra and Pascal Schweitzer (JLU, Giessen) for the support with the AFM measurements.

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Supplementary Information (SI) for Journal of Materials Chemistry C.
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Supporting Information

Covalent Carbon Nanodot-Azobenzene Hybrid Photoswitches: The Role of *Meta/Para* Connectivity and sp^3 Spacer in Photophysical Properties

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Experimental

Materials and Methods

Citric acid (CA, $\geq 99.5\%$), 3-[[[(ethylimino)methylidene]amino)-*N,N*-dimethylpropan-1-amine hydrochloride (EDC, $\geq 99\%$), and ethylenediamine (EDA, $\geq 99.5\%$) were purchased from Carl-Roth. *N*-hydroxysuccinimide (NHS, $\geq 99\%$) were purchased from Sigma-Aldrich. Deuterated dimethyl sulfoxide (DMSO- d_6 , 99.8%) was purchased from Deutero. Anhydrous *N,N*-dimethylformamide (DMF, 99.8%, AcroSeal) was purchased from Thermo Scientific. Dimethylsulfoxide (DMSO, 99.5%) was purchased from Gruessing. Solvents for UV-Vis and PL spectroscopy was purchased from Merck (Uvasol® quality). Ultrapure water was obtained with the Milli-Q Direct Water Purification System. Unless otherwise stated, the reagents were used without further purification. The MW-assisted reactions were performed in a Silvia homeline set-up. The purification of CNDs was performed with Spectra/Por Biotech-Grade Cellulose Ester (CE) dialysis tubing with a molecular weight cut-off (MWCO) of 500 Da–1000 Da. Sephadex® G-10 and LH-20 gel filtration media were used for size-exclusion chromatography. NMR-spectra were recorded with a Bruker Avance II 400 MHz, and Bruker Avance III 400 MHz HD. Diffusion measurements were performed using an AVANCE III HD 400 MHz NMR spectrometer equipped with a gradient probe head (Bruker “Diff50”) with a selective ^1H insert, providing a magnetic field

gradient strength of up to 28 T m^{-1} . The samples were measured at 25°C . To calibrate the temperature a PT 100 thermocouple inserted in an NMR tube, filled with oil, was used. DOSY spectra were acquired by a stimulated echo sequence, as detailed in SI. UV-Vis measurements were carried out with a SPECORD® 200 PLUS spectrophotometer equipped with two automatic eight-fold cell changers and a Peltier element thermostat system (0.1°C accuracy) by Analytik Jena. The system was operated with the ASpect UV software by Analytik Jena. Fluorescence measurements were conducted with a Jasco FP-8300 Fluorescence Spectrometer. The sample solutions were measured in QS High Precision Cells made of Quartz Suprasil® with a light path of 10 mm by Hellma Analytics. Experiments were carried out in concentrations of $1\text{--}14 \text{ mg L}^{-1}$. Stock solutions (1 mg mL^{-1}) were prepared from freshly purified samples, and then diluted prior to every UV/Vis and PL experiment. The absorption spectra of the respective azo were obtained using $5 \mu\text{mol L}^{-1}$ solutions in DMSO at 25°C . **CND + *m*-Azo** (mix): $5 \text{ mg L}^{-1} + 0.83 \mu\text{mol L}^{-1}$; **CND + *p*-Azo** (mix) and **CND + *m*-Gly-Azo** (mix): $5 \text{ mg L}^{-1} + 1.79 \mu\text{mol L}^{-1}$ in DMSO at 25°C . Irradiation of UV/Vis samples was conducted in an in-house built box using LEDs by Lumitronix or Nichia purchased from leds.de, or by mounted LEDs from Thorlabs. AFM was performed with the AIST-NT SmartSPM 1000 under ambient conditions in AC mode. Thermogravimetric measurements were carried out on a TGA Q5000IR (TA instruments, New Castle, DE, USA). The analyses were performed using a $100 \mu\text{L}$ Platinum-HT TAG pan. In order to remove adsorbed moisture, each sample was subjected to a sequence of isotherms under N_2 (at 60°C for 10 minutes, then at 80°C for 3 minutes and then at 100°C for 2 minutes), immediately before the measurement ramp ($10^\circ\text{C min}^{-1}$ from 100°C to 900°C) under N_2 atmosphere.

CND Synthesis

For the sake of clarity, the details of the already reported and slightly modified method reported by Pham et al.¹ are given here. CA (1.44 g, 7.50 mmol, 1.00 eq) was dissolved in 5 mL Milli-Q water and was stirred until the substrate was completely dissolved. EDA (0.50 mL, 7.5 mmol, 1.0 eq) was added and the mixture was stirred for 20 min. The reaction mixture was heated in a domestic MW oven for 5 min at 400 W (50% power). The obtained brown resin was dispersed in 10 mL Milli-Q water and the brown dispersion was filtered over a 0.2 μ m PTFE syringe filter. The filtered dispersion of the CNDs were filled up to a volume of 10 mL and dialyzed in a 0.5 kDa – 1 kDa dialysis membrane against Milli-Q water for 48 h, with water changed every 2 h during the day. The obtained brown dispersion was lyophilized and an orange/ brown solid was obtained. ¹H-NMR of the CND species showed broad signals to ensure the presence of CNDs (**Figure S50**).²

Azobenzene Synthesis

3-(Phenylazo)benzoic acid (*m*-Azo):

3-Aminobenzoic acid (2.77 g, 20.0 mmol, 1.00 eq) and nitrosobenzene (2.79 g, 26.0 mmol, 1.30 eq) were stirred in degassed AcOH (40 mL) at rt overnight. The formed suspension was added to water (600 mL), the solid was filtered off and thoroughly washed with water. After drying, the crude compound was recrystallized from toluene (approx. 50 mL) to yield the product as an orange/golden solid.

Yield: 2.17 g, 48%.

¹H-NMR (400 MHz, DMSO-*d*₆) δ = 13.33 (s, 1H), 8.38 (t, *J* = 1.8 Hz, 1H), 8.19 – 8.09 (m, 2H), 7.94 (dd, *J* = 7.8, 1.9 Hz, 2H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.67 – 7.57 (m, 3H) ppm.

Analytical data corresponded to the literature.³

4-(Phenylazo)benzoic acid (*p*-Azo):

4-Aminobenzoic acid (277 mg, 2.00 mmol, 1.00 eq) and nitrosobenzene (279 mg, 2.60 mmol, 1.30 eq) were stirred in degassed AcOH (5 mL) at rt overnight. The solvent was removed under reduced pressure and the product was purified by column chromatography (80 g SiO₂, Cyclohexane:EtOAc = 10:1 → 4:1) to yield the product as an orange, crystalline solid.

Yield: 110 mg, 24%.

¹H-NMR (400 MHz, DMSO-d₆) δ = 13.25 (s, 1H), 8.15 (d, *J* = 8.3 Hz, 2H), 8.04 – 7.86 (m, 4H), 7.63 (dd, *J* = 5.2, 2.1 Hz, 3H) ppm.

Analytical data corresponded to the literature.³

Glycine methyl ester:

Glycine (1.88 g, 25.0 mmol, 1.00 eq) was suspended in MeOH (250 mL). The suspension was cooled to 0 °C and SOCl₂ (3.00 mL, 41.1 mmol, 1.00 eq) was added dropwise. The suspension was stirred at rt overnight. Then, the clear solution was concentrated into dryness under reduced pressure to yield the product as a white, crystalline solid. It was used as yielded for next steps.

3-(Phenylazo)benzoyl chloride:

3-(Phenylazo)benzoic acid (226 mg, 1.00 mmol, 1.00 eq) was suspended in CH₂Cl₂ (dry, 4 mL) with one drop DMF (dry). Oxalyl chloride (103 mL, 1.20 mmol, 1.20 eq) was added dropwise. Immediately formation of gas was observed and the orange suspension turned to a red solution. Stirring continued at rt for 2 h. Solvent was removed under reduced pressure and dried thoroughly under high vacuum to yield the product as a dark red solid. It was used as yielded for next steps.

***N*-[3-(Phenylazo)benzoyl]glycine methyl ester:**

3-(Phenylazo)benzoyl chloride (245 mg, 1.00 mmol, 1.00 eq) and glycine methyl ester (126 mg, 1.00 mmol, 1.00 eq) were suspended in CH₂Cl₂ (dry, 3 mL). DIPEA (400 μ L, 2.32 mmol, 2.32 eq)

was added dropwise and the reaction mixture was stirred at rt overnight. SiO₂ (2 g) was added and the solvent was removed under reduced pressure. Crude product was purified by column chromatography (30 g SiO₂, cyclohexane:EtOAc 2:1) to yield the product as an orange, crystalline solid.

Yield: 211 mg, 71%.

¹H-NMR (400 MHz, DMSO-d₆) δ = 9.22 (t, J = 5.8 Hz, 1H), 8.38 (t, J = 1.8 Hz, 1H), 8.11 – 8.04 (m, 2H), 7.96 – 7.91 (m, 2H), 7.74 (t, J = 7.8 Hz, 1 H), 7.68 – 7.57 (m, 3H), 4.06 (d, J = 5.9 Hz, 2H), 3.68 (s, 3H) ppm.

¹³C-NMR (101 MHz, DMSO-d₆): 170.3, 165.9, 151.9, 134.9, 131.9, 130.1, 129.8, 129.6, 125.7, 122.7, 121.0, 51.2, 41.3 ppm.

HRMS (ESI) m/z : [MNa]⁺ calculated: 320.1005, found: 320.1005

***N*-[3-(phenylazo)benzoyl]glycine (*m*-Gly-Azo):**

N-[3-(Phenylazo)benzoyl]glycine methyl ester (211 mg, 710 μ mol, 1.00 eq.) and LiOH (51 mg, 2.13 mmol, 3.00 eq) were stirred in THF (4 mL) and H₂O (16 mL) at rt overnight. The mixture was acidified with 1 M HCl and shortly placed on the rotavap to evaporate some of the THF. The reaction mixture was extracted with CH₂Cl₂ (5x 20 mL). Organic phases were combined and silica (2 g) was added and thoroughly dried under reduced pressure. Crude product was purified by a short silica plug (15 g SiO₂, cyclohexane:EtOAc 1:1) to yield the product as an orange solid.

Yield: 110 mg, 55%.

¹H-NMR (400 MHz, DMSO-d₆) δ = 12.7 (b, 1H), 9.10 (t, J = 5.9 Hz, 1H), 8.38 (t, J = 1.8 Hz, 1H), 8.11 – 8.03 (m, 2H), 7.99 – 7.89 (m, 2H), 7.73 (t, J = 7.8 Hz, 1H), 7.67 – 7.57 (m, 3H), 3.97 (d, J = 5.8 Hz, 2H) ppm.

^{13}C -NMR (101 MHz, DMSO- d_6): 171.2, 165.8, 151.9, 135.2, 131.9, 130.1, 129.7, 129.6, 125.6, 122.7, 121.0, 41.3 ppm.

HRMS (ESI) m/z : [M-H] calculated: 280.0884, found: 282.0884

Amide Coupling of CNDs with Azobenzene Derivatives

Azobenzene (1 eq) was dissolved in 90 mL mmol^{-1} anhydrous DMF. EDC (2 eq) and NHS (2 eq) were added, and the mixture was stirred for 15 min at rt. CNDs (112 mg mmol^{-1}) were added and the reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure. The obtained crude was packed on a Sephadex G-10 gel column with DMSO as eluent. The obtained brown dispersion was lyophilized and an orange/brown solid was obtained.

Computational Details

A model of the **CND** was initially derived from molecular dynamics simulations (**Figure S20**). In the molecular dynamics (MD) simulations, the ad-hoc model of polymeric CD was first designed by the condensation of citric acid (CA) and ethylenediamine (EDA) molecules, and all the formed chains were capped with a prototypical molecular fluorophore IPCA. The initially stretched polymer (**Figure S17a**) This model was set up with parameters from the Generalized Amber Force Field (GAFF).⁴ RESP partial charges were determined using the Antechamber tool⁵ from the Amber software package⁶ by fitting of the electrostatic potential computed at the HF/6-31G* level in a gaseous state. After this parametrization, the CD was added to the center of a cubic simulation box measuring $8.0 \times 8.0 \times 8.0 \text{ nm}^3$ and solvated with 16804 TIP3P⁷ explicit water molecules. After an initial minimization, a two-step equilibration process was employed. The first step involved heating the system from 10 K to 300 K over 1 ns using a V-rescale⁸ thermostat with

a 0.1 ps time constant. This was followed by a 2 ns equilibration of pressure using the isotropic Berendsen⁹ barostat, keeping the pressure at 1 bar and maintaining the temperature at 300 K with a pressure relaxation time constant of 2 ps. Subsequently, the MD simulations was conducted for 100 ns in the NPT ensemble with a 2 fs time step. The bonds with hydrogens were constrained using the LINCS¹⁰ algorithm. Temperature control was maintained at 300 K via the V-rescale thermostat with a 0.1 ps time constant, while pressure was regulated at 1 bar using the isotropic Parrinello–Rahman barostat¹¹ with a 2 ps relaxation time. Electrostatic interactions were managed using the particle-mesh Ewald (PME)¹² method, applying a real-space cut-off of 1 nm, which was also used for van der Waals interactions. Periodic boundary conditions were applied in all directions. These MD simulations were executed in Gromacs 5.0.¹³

The resulting geometry of the **CND** and subsequent chemical modifications were optimized at the GFN2-xTB level of theory (as implemented in the xtb ver. 6.4.1 software).^{14,15} The analytical linearized Poisson-Boltzmann (ALPB) model was utilized to incorporate solvent effects due to DMSO. For all optimized structures, UV-Vis absorption spectra were calculated via ONIOM (Our own N-layered Integrated molecular Orbital and molecular Mechanics)^{16,17} including time-dependent density-functional-theory (TDDFT) under the linear response formalism. For the high layer (TDDFT level), the long-range hybrid functional ω B97X-D^{18–21} was utilized to calculate 30 singlet-to-singlet transitions. The electronic configuration of the molecular systems was described by the gaussian 6-31G(d) basis set.²² The polarizable continuum model (PCM)^{23–25} was used to account for the effect of DMSO in the electronic transitions. The medium layer was simulated by means of the semi empirical PM6²⁶ method in the ground state. In this context, the reaction field corresponding to DMSO was computed separately in each sub-calculation, using the cavity of the real system in all instances.^{27,28} All ONIOM TDDFT:PM6 calculations were completed using the

quantum-chemistry code Gaussian 16.²⁹ Finally, for the isolated azo compounds, geometry optimizations were confirmed as local minima via analytical-frequency calculations and UV-Vis spectra were calculated at the (TD) (PCM:DMSO)-wB97X-D/6-31G(d) level of theory.

Diffusion Measurement

For ^1H NMR analyses the samples were dissolved in DMSO- d_6 and sealed in NMR tubes with Parafilm. The respective concentrations of the samples were (i) $10 \text{ mg}\cdot\text{mL}^{-1}$ **CND**, (ii) $1 \text{ mg}\cdot\text{mL}^{-1}$ ***m*-Azo**, (iii) $10 \text{ mg}\cdot\text{mL}^{-1}$ of **CND** and $1 \text{ mg}\cdot\text{mL}^{-1}$ of ***m*-Azo** for the physical mixture, and (iv) $11 \text{ mg}\cdot\text{mL}^{-1}$ for the hybrids.

The diffusion measurements were carried out using the stimulated echo experiment with a variation of the gradient pulse strength g . The gradient pulse duration δ was 1 ms and the observation time $\Delta=20$ ms. The resulting echo decays were evaluated by the Stejskal-Tanner equation³²:

$$\frac{I}{I_0} = \exp\left(-\gamma^2 \delta^2 g^2 \left(\Delta - \frac{\delta}{3}\right) \cdot D\right) \quad (1)$$

in which γ denotes the gyromagnetic ratio of the ^1H nucleus.

Due to the polydispersity of the CNDs, a single self-diffusion coefficient cannot describe the echo decay sufficiently. To perform an analysis of the echo decays we therefore followed a method described by Lenocho et al.³³, considering a distribution of diffusion coefficients $P(D)$ with a resulting echo decay of the form:

$$\frac{I}{I_0} = \int_{-\infty}^{\infty} P^*(D) \exp\left(-\gamma^2 \delta^2 g^2 \left(\Delta - \frac{\delta}{3}\right) \cdot D\right) d\ln(D) \quad (2)$$

Here, $P^*(D)$ is a modified distribution with $P^*(D) = D \cdot P(D)$. A commonly used function for $P(D)$ in the case of polymer diffusion³², but also applicable here, is the log-normal distribution:

$$P(D) = \frac{1}{\sqrt{2\pi}\sigma_D D} \exp\left(-\frac{[\ln D - \ln\langle D \rangle]^2}{2\sigma_D^2}\right) \quad (3)$$

In combination with Equation (2), $\langle D \rangle$ corresponds to the value at the maximum of the distribution. Using the Stokes-Einstein Equation (4) the diameter of the respective diffusing species was estimated from $\langle D \rangle$. The temperature T during the measurement was 298.4 K, and the viscosity of DMSO- d_6 ³⁴ at 298 K of $2.195 \text{ mPa}\cdot\text{s}$ was used for the viscosity η .

$$r = \frac{k_B T}{6\pi\eta(D)} \quad (4)$$

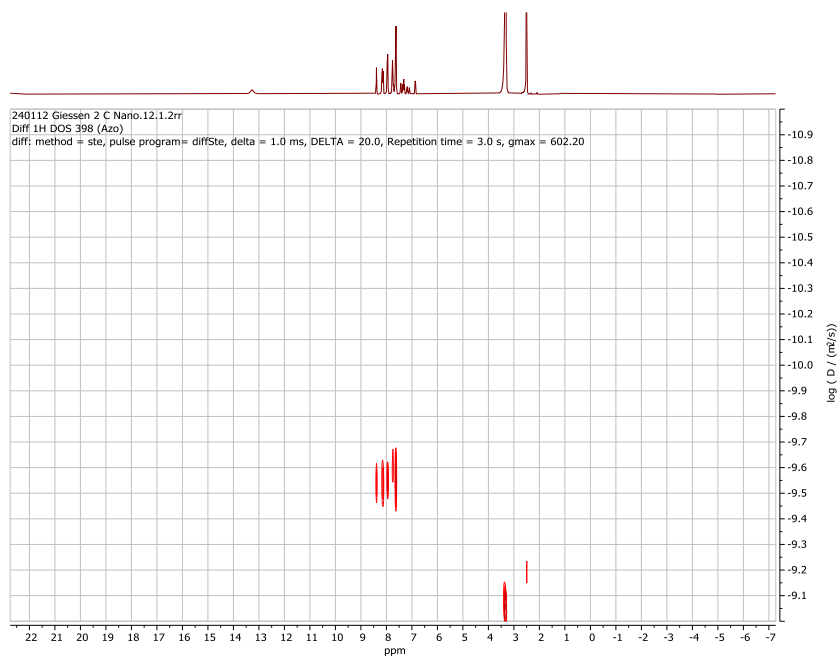


Figure S1. 2D DOSY NMR spectra of *m*-Azo measured in DMSO-d₆.



Figure S2. 2D DOSY NMR spectra of **CND** measured in DMSO-d₆.

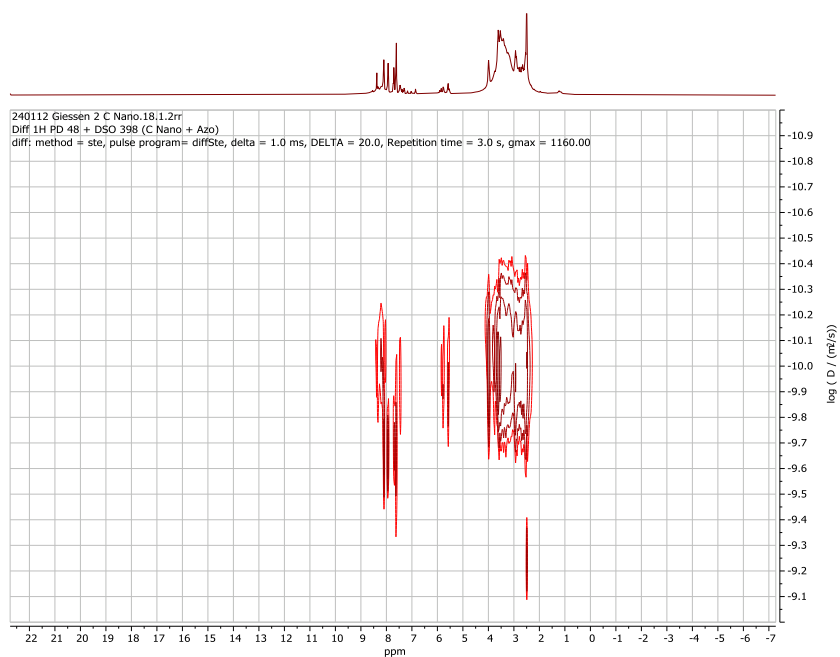


Figure S3. 2D DOSY NMR spectra of a physical mixture of CND (10 mg) and *m*-Azo (1 mg) measured in DMSO-*d*₆.

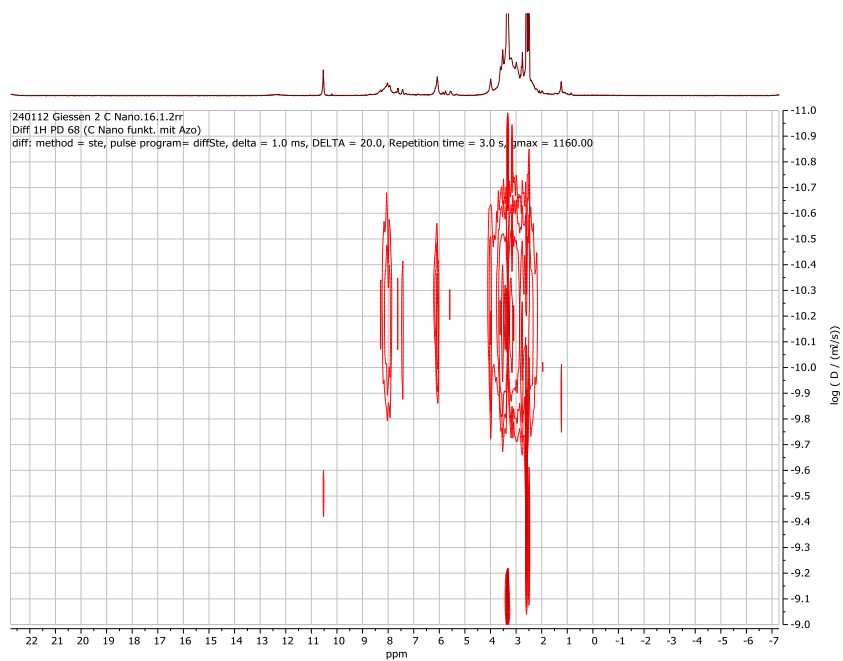


Figure S4. 2D DOSY NMR spectra of **CND-*m*-Azo** CNDs measured in DMSO-d₆.

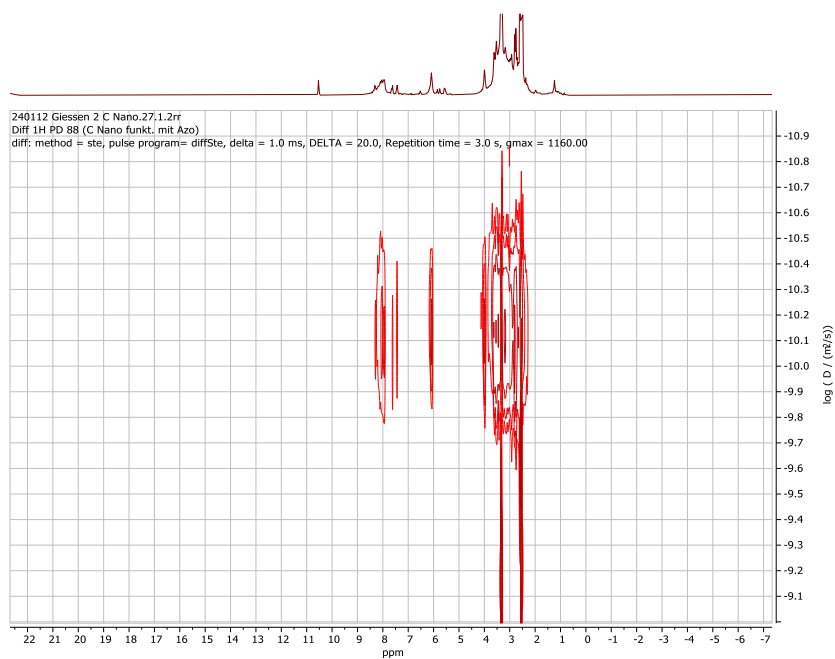


Figure S5. 2D DOSY NMR spectra of **CND-*p*-Azo** CNDs measured in DMSO-d₆.

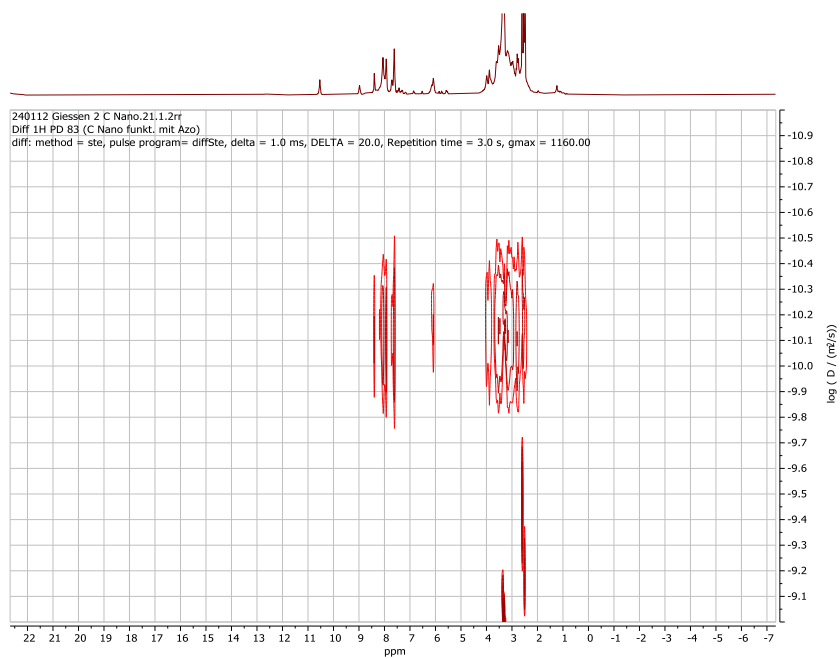


Figure S6. 2D DOSY NMR spectra of **CND-*m*-Gly-Azo** CNDs measured in DMSO- d_6 .

Atomic Force Microscopy

0.02 mg·mL⁻¹ dispersions of CND and CND-azo hybrids in DMSO were drop casted on freshly cleaved V1 grade Mica discs (10 mm diameter, 0.15–0.21 mm thickness). After keeping the prepared samples overnight under reduced pressure, they were measured in AFM tapping mode with a SSS-FMR probe (75 kHz, 2.8 N·m⁻¹). The obtained images were analyzed with Gwyddion 2.62³⁰ and the size of ~70 nanoparticles was measured for a size histogram.

AFM directly images the surface of particles, capturing their actual physical size, shape, and surface structure. However, when particle agglomerates are present and appear as single larger particles in AFM measurements, there can be a discrepancy in the measured size.³¹ This aggregation can potentially be observed in **Figure S11C-D**, as CNDs are known to have a tendency to aggregate. However, it can be said that AFM is limited locally to a few micrometers, and thus the concentrations can vary locally, which can certainly influence the formation of agglomerates on the mica surface.

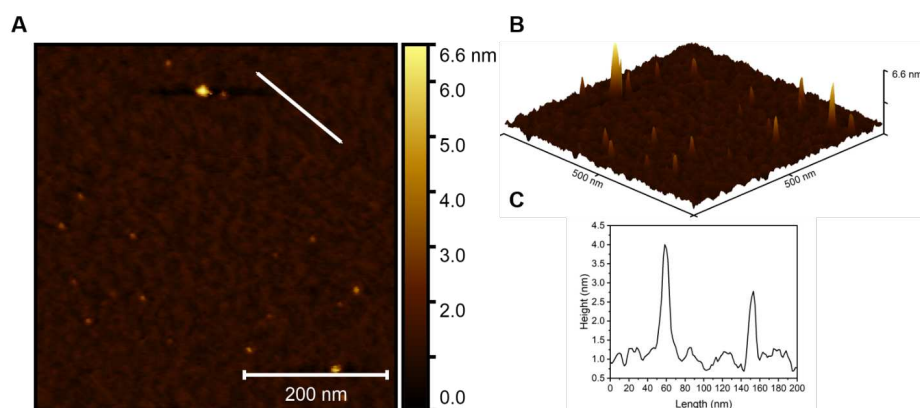


Figure S7. (A) 2D AFM image (500×500 nm) of CND. (B) 3D AFM image (500×500 nm) of CND and height profile along the white line in the 2D image.

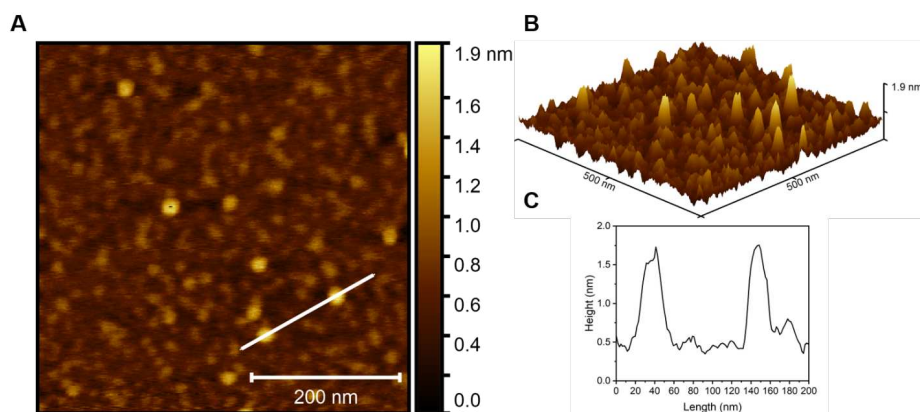


Figure S8. (A) 2D AFM image (500×500 nm) of **CND-*m*-Azo** CNDs. (B) 3D AFM image (500×500 nm) of **CND-*m*-Azo** CNDs. (C) Height profile along the white line in the 2D image.

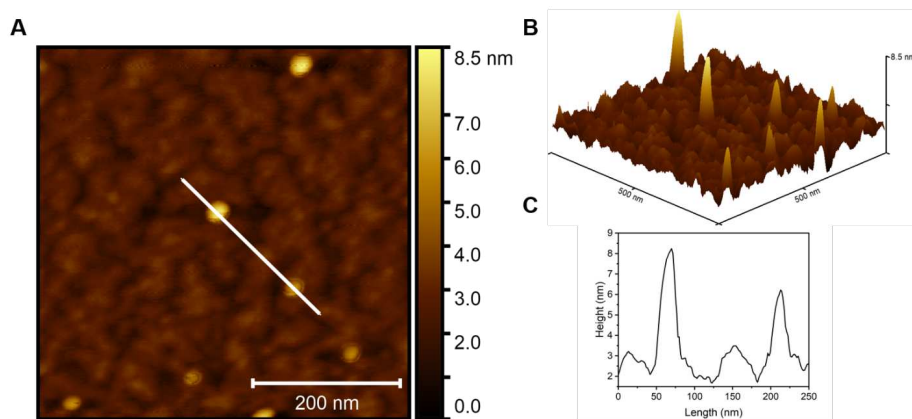


Figure S9. (A) 2D AFM image (500×500 nm) of **CND-*p*-Azo** CNDs. (B) 3D AFM image (500×500 nm) of **CND-*p*-Azo** CNDs. (C) Height profile along the white line in the 2D image.

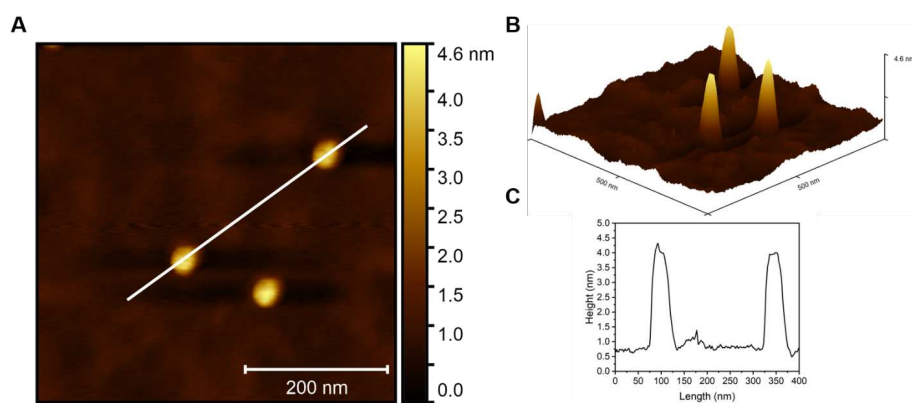


Figure S10. (A) 2D AFM image (500×500 nm) of CND-*m*-Gly-Azo CNDs. (B) 3D AFM image (500×500 nm) of CND-*m*-Gly-Azo CNDs. (C) Height profile along the white line in the 2D image.

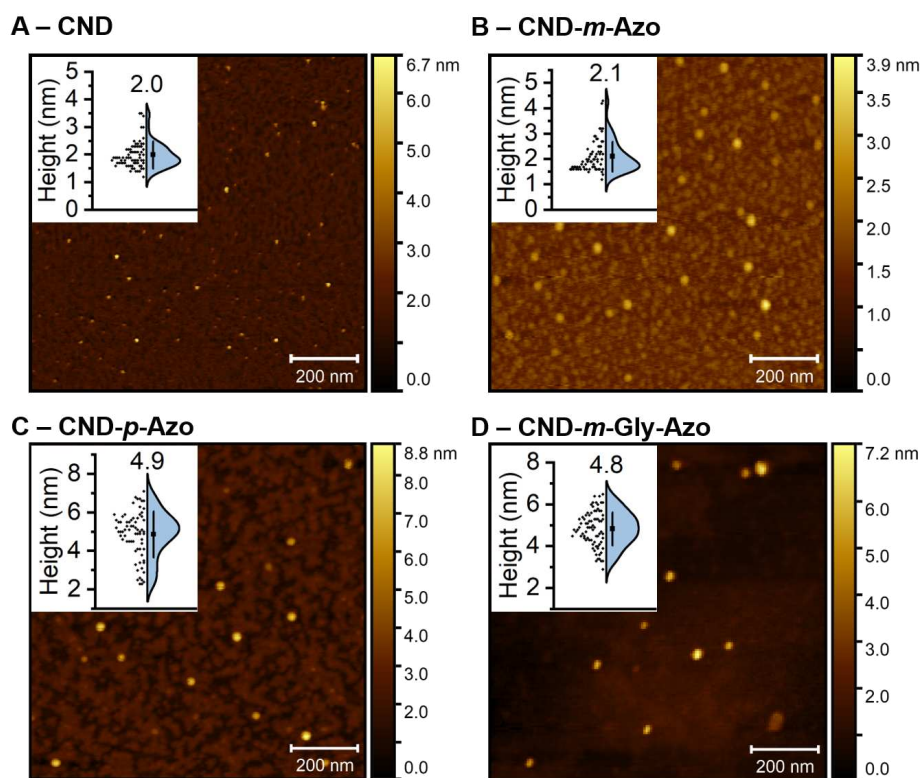


Figure S11. 1 $\mu\text{m} \times 1 \mu\text{m}$ AFM images of (A) CND, (B) CND-*m*-Azo (C) CND-*p*-Azo, and (D) CND-*m*-Gly-Azo on a mica substrate. The insets in each figure show the height distribution for each sample, with the mean height and the standard deviation obtained from ~ 70 CNDs.

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) reveals a greater weight loss between 100 °C and 300 °C for the **CND-*p*-Azo** and the **CND-*m*-Gly-Azo** in comparison to the **CND-*m*-Azo** (see **Figure S12**). As previously shown for azo compounds, they decompose within a comparable temperature range³⁵ as the pristine **CND**. The TGA curves of the hybrids thus exhibit a greater mass loss than the pure **CND**, indicating functionalization. However, the simultaneous pyrolysis of the two components and the substantial alteration of the **CND** morphology precludes the possibility of making a precise statement regarding the degree of functionalization by TGA.

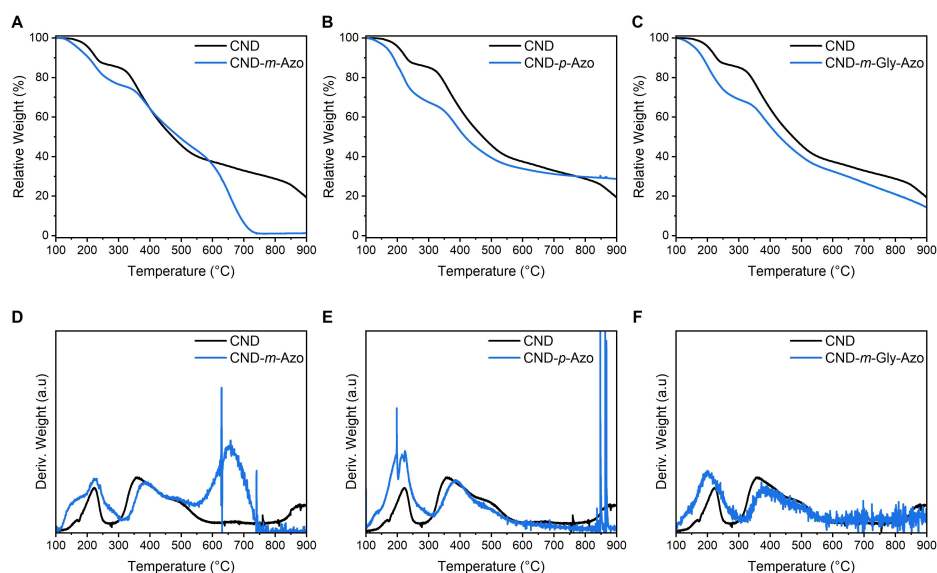


Figure S12. TGA analysis of the CNDs measured in nitrogen (A) **CND** in comparison to **CND-*m*-Azo** (The loss of weight that begins at 600 °C is most likely due to a residual amount of oxygen contamination.) (B) **CND** in comparison to **CND-*p*-Azo** (C) **CND** in comparison to **CND-*m*-Gly-Azo** (D) Derived weight loss of **CND-*m*-Azo** in comparison to **CND**. (E) Derived weight loss of

CND-*p*-Azo in comparison to **CND**. (F) Derived weight loss of **CND-*m*-Gly-Azo** in comparison to **CND**. In all cases, the first significant weight loss was observed between 100 °C and 300 °C. Furthermore, all functionalized CNDs showed higher weight loss in this range. Decomposition started at around 350 °C.

Photoluminescence Quantum Yield

The relative PL quantum yield method with quinine sulfate (QS) as a reference compound was used.³⁶ For five different concentrations of each sample and the reference sample, the UV-Vis absorbance and PL were measured. The maximum values of each absorbance measurement with the respective integrated PL intensity are plotted against each other. From the obtained slopes and the following equation one can calculate the PL quantum yield.

$$\Phi_S = \frac{m_S}{m_R} \cdot \frac{n_S^2}{n_R^2} \cdot \Phi_R \cdot 100$$

Φ_S = quantum yield of the sample [%]

Φ_R = quantum yield of the reference compound (QS = 0.51)

m_S = slope from plotted absorbance against integrated intensity of the sample

m_R = slope from plotted absorbance against integrated intensity of the reference compound

n_S = 1.4785 refractive index of sample solvent (DMSO)

n_R = 1.33415 refractive index of reference solvent (0.1 M H₂SO₄)

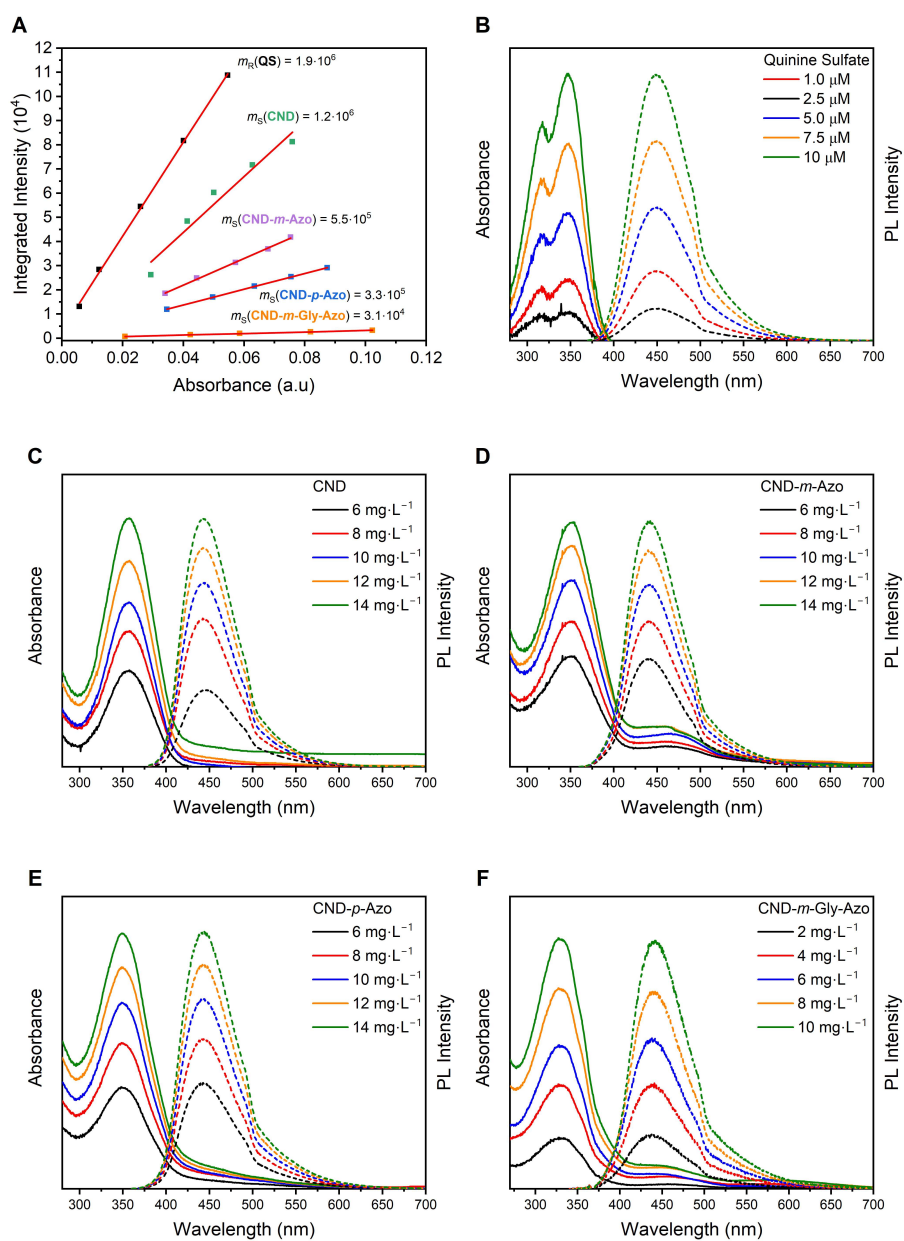


Figure S13. (A) Absorbance maxima plotted against the integrated PL intensity of the five different concentrations and the respective slopes derived from a linear fit. (B) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350 \text{ nm}$) of five different concentrated quinine sulfate (QS) solutions. (C) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 360 \text{ nm}$) of five different concentrated **CND** dispersions. (D) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350 \text{ nm}$) of five different concentrated **CND-*m*-Azo** dispersions. (E) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350 \text{ nm}$) of five different concentrated **CND-*p*-Azo** dispersions. (F) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 330 \text{ nm}$) of five different concentrated **CND-*m*-Gly-Azo** dispersions.

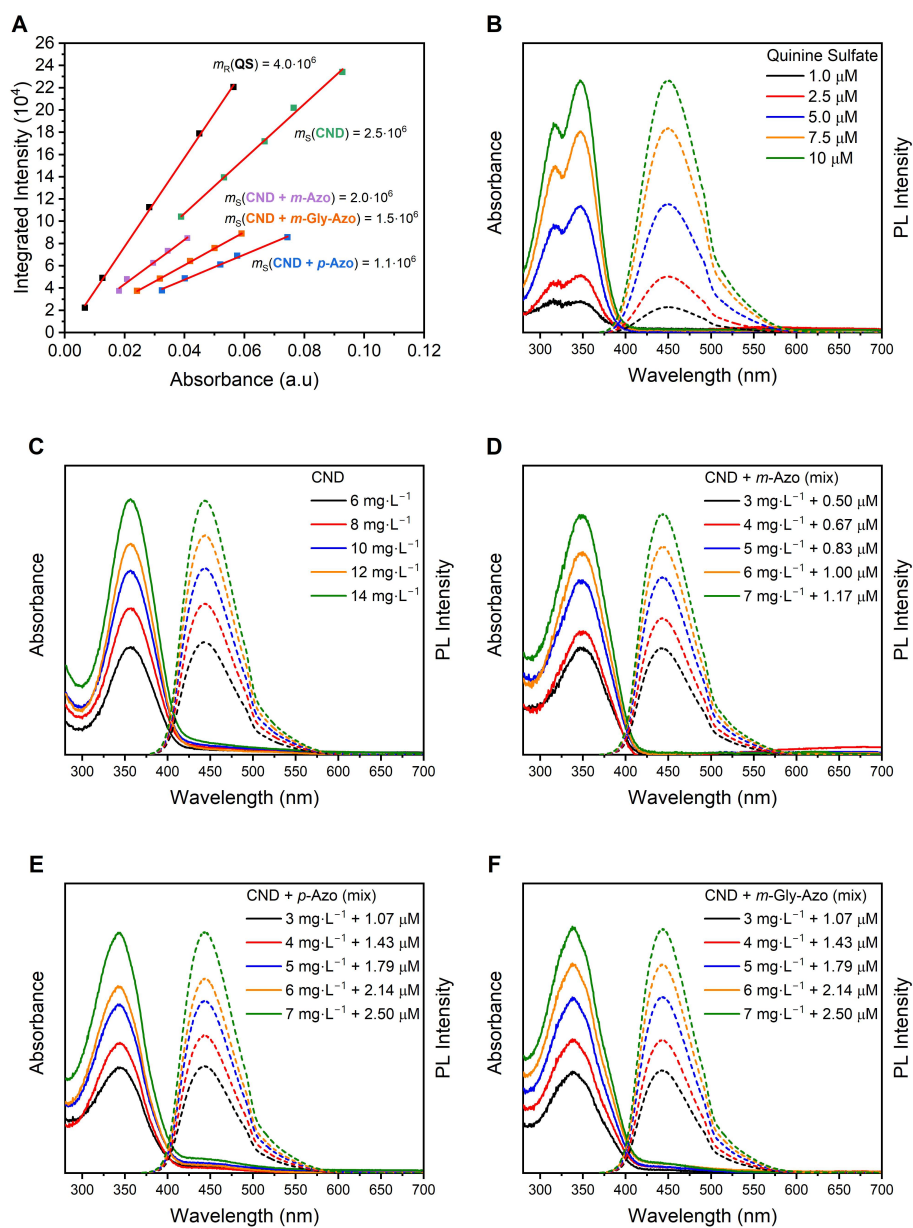


Figure S14. (A) Absorbance maxima plotted against the integrated PL intensity of the five different concentrations and the respective slopes derived from a linear fit. The CNDs and QS are reported twice because of a lamp change in the fluorescence device. (B) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350$ nm) of five different concentrated QS solutions. (C) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 360$ nm) of five different concentrated CND dispersions. (D) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350$ nm) of five different concentrated CND + *m-Azo* physically mixed dispersions. (E) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350$ nm) of five different concentrated CND + *p-Azo* physically mixed dispersions. (F) UV-Vis absorbance (solid lines) and PL intensity (dashed lines, $\lambda_{\text{Exc}} = 350$ nm) of five different concentrated CND + *m-Gly-Azo* physically mixed dispersions.

Absorption Coefficients and Degree of Azo Functionalization

The UV-Vis absorbance of each azobenzene sample was measured at five different concentrations. The maximum values of each absorbance measurement for the respective concentration were plotted against the concentration. From the obtained slopes and the Law of Lambert-Beer in the following equation, it is possible to determine the absorption coefficient directly.³⁷ In the next step, the respective absorption spectra of the hybrids were deconvoluted with the help of two Lorentzian-shaped functions. The wavelength and the full width at half maximum (FWHM) of the respective pristine azo and the CND were fixed for the Lorentzian functions. The maximum absorbance value for the obtained deconvolution of the azo compartment was used for the calculation of the concentration of azo in the respective hybrid with the depicted equation and the previously determined absorption coefficients. The percentage of azo was calculated in accordance with the concentration of the hybrid utilized for deconvolution.

$$A = \varepsilon_{\lambda} \cdot c \cdot l$$

$$A = \text{absorbance [a.u]}$$

$$\varepsilon_{\lambda} = \text{absorption coefficient at the respective wavelength [L} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}]$$

$$c = \text{concentration [mg} \cdot \text{L}^{-1}]$$

$$l = \text{layer thickness of the irradiated body (1 cm)}$$

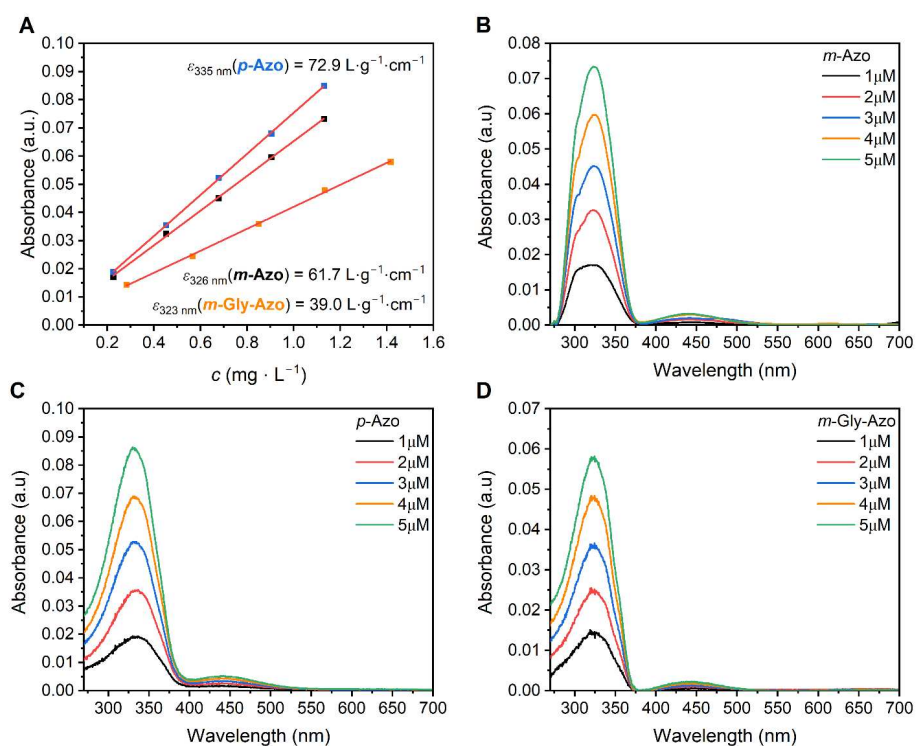


Figure S15. (A) Absorbance maxima of each azobenzene sample at five different concentrations plotted against the concentrations, and the respective slopes (absorption coefficients) derived from a linear fit. (B) UV-Vis absorbance of five different concentrated *m*-Azo solutions ($\lambda_{\text{max}} = 326 \text{ nm}$). (C) UV-Vis absorbance of five different concentrated *p*-Azo solutions ($\lambda_{\text{max}} = 335 \text{ nm}$). (D) UV-Vis absorbance of five different concentrated *m*-Gly-Azo solutions ($\lambda_{\text{max}} = 323 \text{ nm}$).

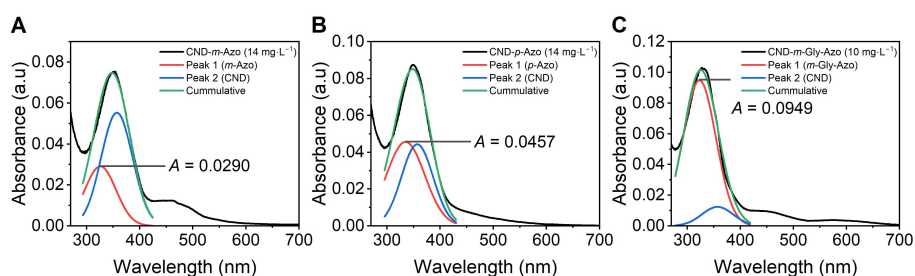


Figure S16. (A) Deconvoluted absorbance spectra of **CND-*m*-Azo** ($14 \text{ mg}\cdot\text{L}^{-1}$ in DMSO) with two Lorentzian-shaped functions. For Peak 1 (***m*-Azo**), the FWHM was fixed at 59 nm, while the position was fixed at 326 nm. A maximum absorbance of 0.0290 was obtained for Peak 1 (***m*-Azo**). This indicates that 3.4% of the hybrid represents the azo component. For Peak 2 (**CND**), the FWHM was fixed at 59 nm, while the position was fixed at 357 nm in all cases. (B) Deconvoluted absorbance spectra of **CND-*p*-Azo** ($14 \text{ mg}\cdot\text{L}^{-1}$ in DMSO) with two Lorentzian-shaped functions. For Peak 1 (***p*-Azo**), the FWHM was fixed at 72 nm, while the position was fixed at 335 nm. A maximum absorbance of 0.0457 was obtained for Peak 1 (***p*-Azo**). This indicates that 4.5% of the hybrid represents the azo component. (C) Deconvoluted absorbance spectra of **CND-*m*-Gly-Azo** ($10 \text{ mg}\cdot\text{L}^{-1}$ in DMSO) with two Lorentzian-shaped functions. For Peak 1 (***m*-Gly-Azo**), the FWHM was fixed at 62 nm, while the position was fixed at 323 nm. A maximum absorbance of 0.0949 was obtained for Peak 1 (***m*-Gly-Azo**). This indicates that 24% of the hybrid represents the azo component.

Computational Analysis

Model of the CND

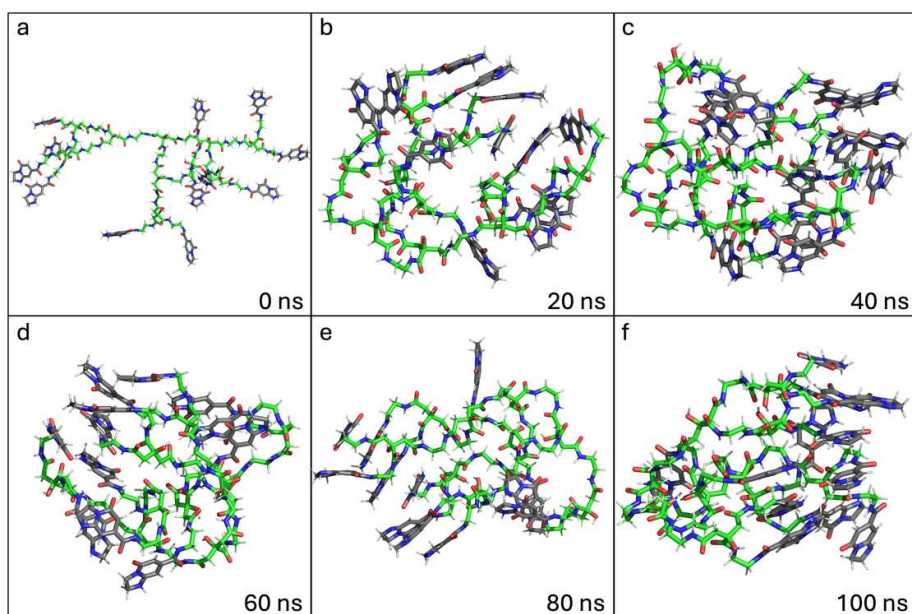


Figure S17. Evolution of polymeric CND model as modelled using MD simulations. Coloring scheme: green-carbon; red-oxygen; blue-nitrogen; white-hydrogens. Carbons of IPCA parts of the CND are displayed in grey. Water was omitted for clarity.

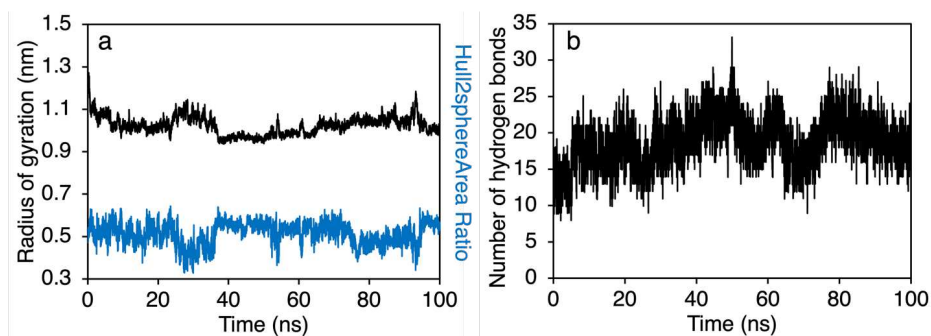


Figure S18. (a) Radius of gyration relaxed after 40 ns in stable self-assembly (black curve). (Please note that the gyration radius differs from the hydrodynamic radius by about 1.5 nm. The hydrodynamic radius reflects the apparent size of the solvated particle, while the gyration radius measures the average distance from the center of mass to the macromolecular surface); Hull2SphereArea ratio displays similar trend as radius of gyration, and it additionally suggests that **CND** is quasi-spherical. Hull2SphereArea³⁸ is the ratio between the surface area of an ideal sphere embedding the whole **CND** and the real **CND** surface area. (b) Evolution of the number of hydrogen bonds formed within **CND** model during the MD simulation.

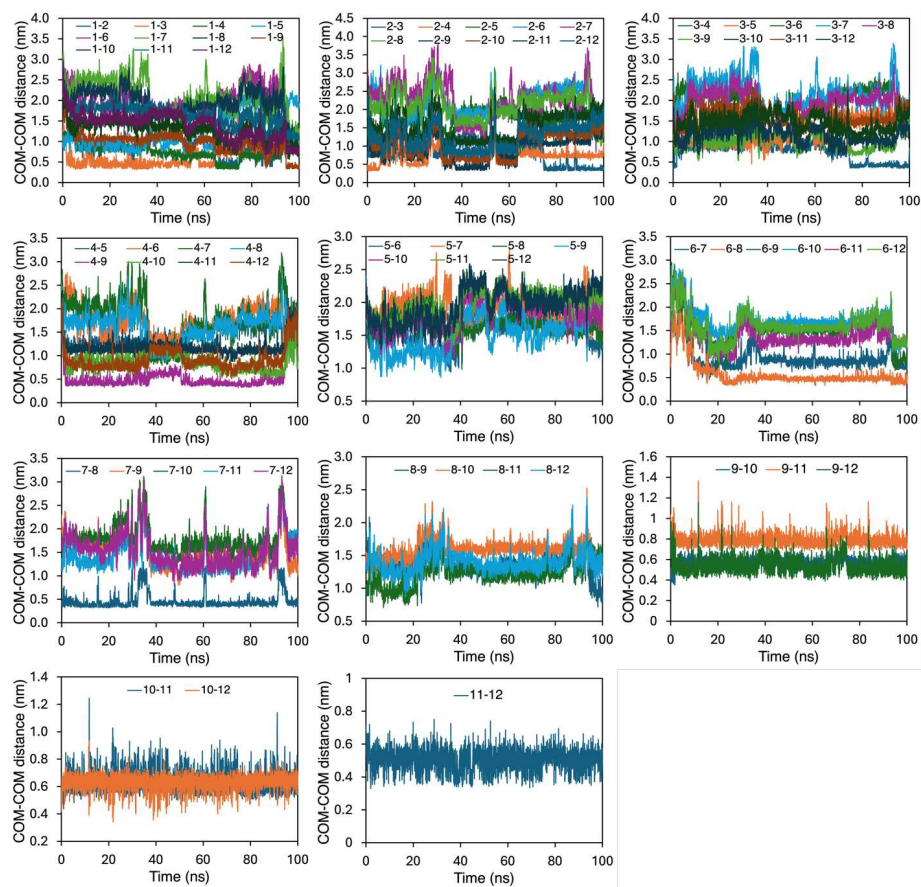


Figure S19. Time evolution of COM-COM distances of 12 IPCA parts of the CND, which were collected within 100 ns of the simulation time. Numbers in legends refer to the specific IPCA part of the CND.

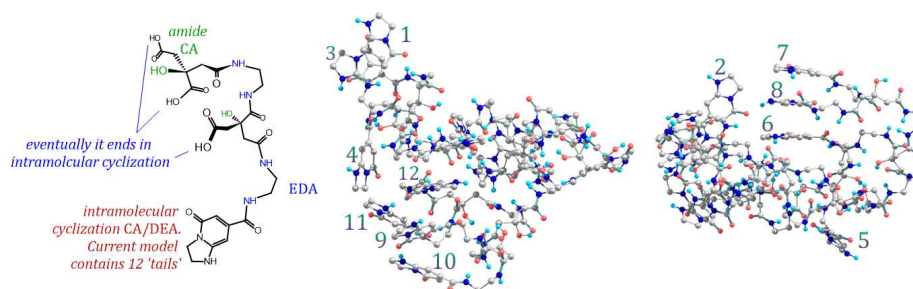
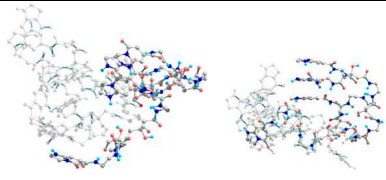
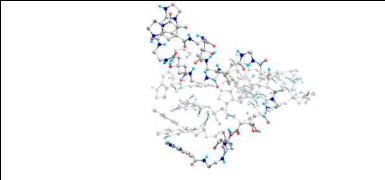
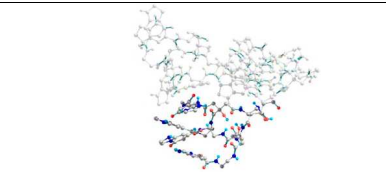
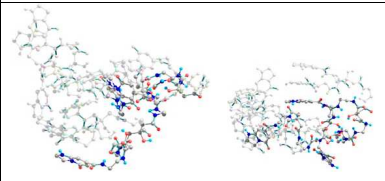
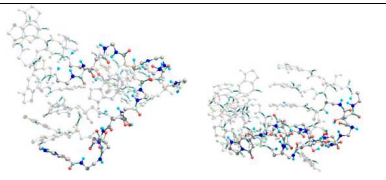
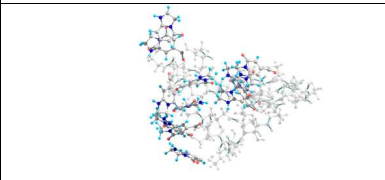


Figure S20. Profile views of the ad-hoc model of polymeric CND formed by condensation of citric acid (CA) and ethylene diamine (EDA) as thermally equilibrated via molecular dynamics simulations. Atoms C in grey, N in blue, O in red, and H in cyan.

The Truncated Model of the CND

The model of the CND (**Figure S20**) simulated via MD consists of 588 atoms and has the empirical formula $C_{198}N_{66}H_{260}O_{64}$. As a result, a series of truncated models (**trM**) was investigated to perform quantum-chemical calculations, which are reported in **Table S1**. Truncated structures **trM1** to **trM3** exhibit stacked IPCA heterocycles. In contrast, the ring stacking was eliminated in **trM4**, and a few heterocycles were replaced with amide terminals in **trM5**. The amide-alkyl chains were removed in **trM6**, thus preserving exclusively the heterocycles. Our preliminary investigations involved the complete removal of atoms not included in the **trM** models (represented as white-colored fragments in **Table S1**). The respective TDDFT spectra were subsequently calculated and are presented in **Figure S21**.

Table S1. Truncated models (**trM**) of the **CND**. White-colored atoms were either removed for TDDFT calculations or assigned to the medium layer of the ONIOM approach.

	
trM1 (260 atoms, <i>stacking</i>)	trM2 (232 atoms, <i>reduced stacking</i>)
	
trM3 (182 atoms, <i>displaced stacking</i>)	trM4 (206 atoms, <i>non-stacked rings</i>)
	
trM5 (200 atoms, <i>amide tails</i>)	trM6 (228 atoms, <i>only rings</i>)

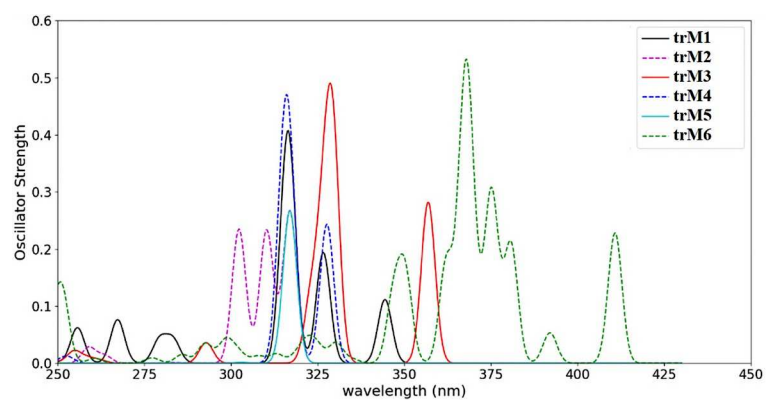


Figure S21. Calculated UV-Vis spectra for the truncated models of the **CND**.

The selection of the truncated model is primarily based on the comparison between calculated and experimental absorption maxima, with the latter observed at $\lambda_{\text{max}} = 357$ nm. The lowest-energy electronic transition (S_1) in model **trM3**, associated with $\lambda = 357$ nm ($f = 0.28$), accurately resembles the experimental λ_{max} . However, another calculated transition in **trM3** was noted as $\lambda_{\text{max}} = 329$ nm ($f = 0.40$). The absorption bands in models **trM1** to **trM5** are blue-shifted relative to those in **trM3**, with λ_{max} values below 325 nm. Model **trM6** exhibited a λ_{max} of 368 nm ($f = 0.20$) and several allowed transitions in the 340–425 nm range, which aligns with the experimental data. However, model **trM6** consists exclusively of IPCA heterocycles, as the amide-alkyl chains were removed. Therefore, we suggest that **trM3** serves as more accurate representation of the real system.

The UV-Vis spectra of model **trM3** were further examined by including the atomic background (represented by the white-colored fragment in **Table S1**) utilizing a lower level of theory. Specifically, TD- ω B97X-D/6-31G(d) was applied to the atoms in **trM3**, while the atomic background was described using either the 3-21G or STO-3G basis sets in the same TD calculation (see **Figure S22**). Additionally, ONIOM calculations were carried out for the system in both the gas phase and in DMSO solution. In these calculations, the atoms in **trM3** were treated as the high layer at the (PCM: DMSO) TD- ω B97X-D/6-31G(d) level, while the atomic background was modeled as the medium layer using the ground-state PM6 method. The absorptions at 357 nm and 329 nm were consistently reproduced across all approaches, with a blue shift and a decrease in intensity observed in the calculations considering the gas phase. The TD- ω B97X-D/6-31G(d)/3-21G method exhibited a greater number of peaks, which are attributed to the non-truncated IPCA heterocycles; although these are virtually missing when the basis set quality is reduced to STO-3G, or when these are excluded from the TDDFT calculation as in the ONIOM medium layer.

Nonetheless, since the main absorptions calculated using ONIOM align with those obtained using TD- ω B97X-D/6-31G(d)/3-21G, we conclude that ONIOM calculations incorporating a semiempirical method for the atomic background are as valid as those obtained via TDDFT.

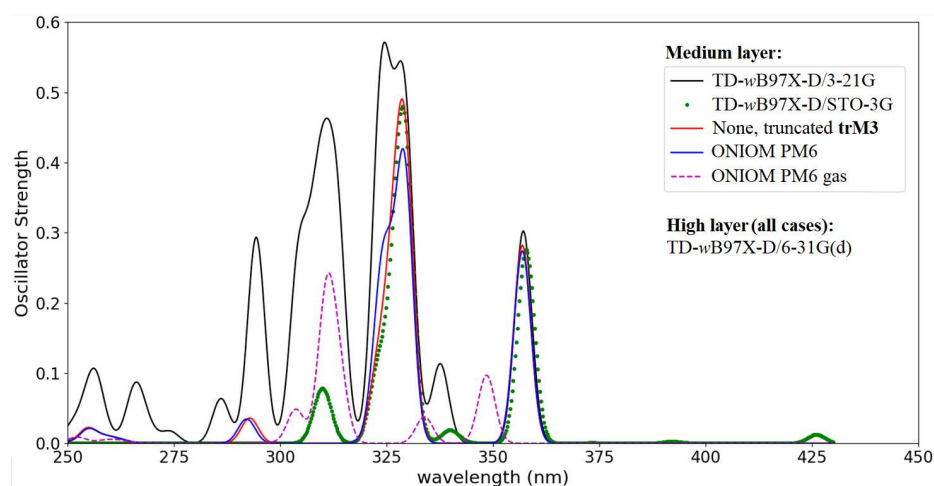


Figure S22. UV-Vis spectra for the truncated model **trM3** of the **CND** excluding and including atomic background calculated via different theoretical approaches. Implicit DMSO solvent was simulated via PCM, unless indicated otherwise.

CNDs Functionalized with Azobenzene

Electron transitions derived from the ONIOM approach are illustrated in **Figure S23** for the unmodified **CND**. We focus on electron transitions associated with excitation energies $\omega > 300$ nm. In addition, transitions are considered allowed when the oscillator strength criterion $f \geq 0.05$ is met (represented with a vertical arrow). Furthermore, a criterion of $\varphi_i \rightarrow \varphi_j > 35\%$ was selected to ensure that significant molecular orbital contributions were not omitted in each electron transition. The lowest-energy transitions S_1 and S_2 exhibited relatively large oscillator strengths.

A visual inspection indicates that S_1 and S_2 are primarily localized π - π^* transitions, although S_1 may be associated with partial charge-transfer (CT) character. These transitions were selected for comparison with azobenzene-modified hybrids, which are discussed in subsequent sections. Additionally, transition S_5 suggests that the system is prone to CT events.

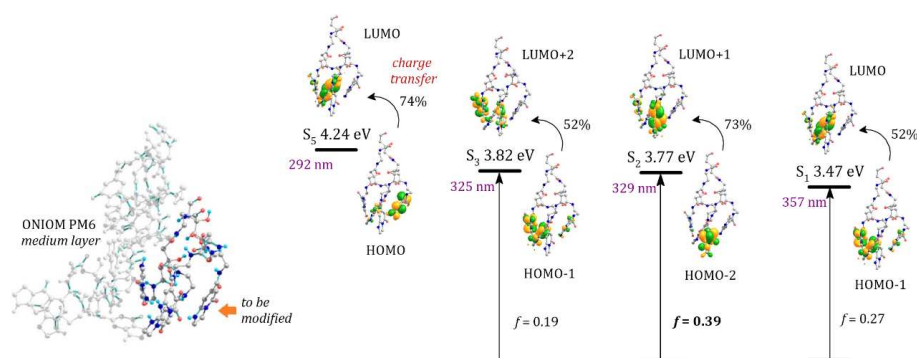


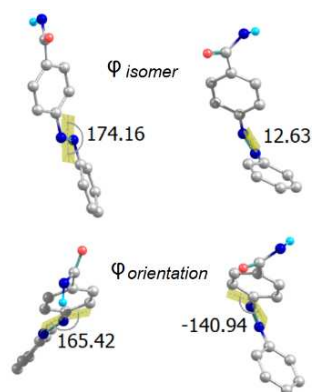
Figure S23. Structure of the CND exhibiting the ONIOM medium (in white) and high layers. The five lowest-energy electron transitions calculated in the high layer are also illustrated. (S_4 is omitted due to an artificial transition in the C=O fragment where the model is truncated).

The CND structure depicted in **Figure S20** contains 12 IPCA heterocycles. Initially, one heterocycle moiety was replaced with one equivalent of *para*-azobenzene, leading to 12 distinct conformers of CND-*p*-Azo (the labeling scheme was provided in **Figure S20**). Each structure was characterized by dihedral angles φ measured in the azo fragment. *Cis/trans* isomerism was reported by φ_{iso} , a dihedral angle defined by $\text{C}_{\text{ipso}}\text{-N}\equiv\text{N-C}_{\text{ipso}}$. Accordingly, the *cis* isomer was characterized as $\varphi_{\text{iso}} \rightarrow 0^\circ$, and the *trans* counterpart as $\varphi_{\text{iso}} > |120^\circ|$. The orientation of the terminal phenyl group of the azobenzene fragment was determined via φ_{ori} ($\text{C}_{\text{ortho}}\text{-C}_{\text{ipso}}\text{-N}\equiv\text{N}$), so that two *trans*-azo conformers were characterized, *trans*₀ when $\varphi_{\text{ori}} \rightarrow 0^\circ$ and *trans* for $\varphi_{\text{ori}} > |120^\circ|$. This dihedral

angle is less relevant for the *cis* counterpart. The relative thermodynamic stabilization of the **CND-*p*-Azo** conformers is reported in **Table S2**, with the replacement of heterocycle label 10 yielding the most stabilized functionalization (this ring is also indicated in **Figure S23**). Additionally, the other azobenzene derivatives were also incorporated at the position of heterocycle 10 of the **CND** (see **Figure S24**). In the case of isolated azobenzene derivatives (**Azo**), the initial structures were extracted from the equilibrated geometries of the **CND-Azo** hybrids, thus resulting in a root mean square deviation smaller than 0.6 Å after geometry optimization of **Azo**.

Table S2. Comparisons of electronic and Gibbs energies (in kcal/mol) for the conformers of **CND-*p*-Azo** calculated at the GFN2-xTB level. Dihedral angles φ (in degrees) are schematized for the most stabilized structure (highlighted in bold).

Replaced heterocycle	Azo isomer	φ_{iso}	φ_{ori}	ΔE_{rel}	ΔG_{rel}
1	<i>cis</i>	14.4	46.8	9.5	10.5
	<i>trans₀</i>	-178.2	29.5	19.4	16.9
	<i>trans</i>	178.6	-151.7	16.8	16.0
2	<i>cis</i>	13.1	-42.8	10.4	10.2
	<i>trans'₀</i>	171.5	-29.8	9.8	11.6
	<i>trans₀</i>	176.1	-27.1	12.9	14.4
	<i>trans</i>	178.8	158.3	13.3	14.0
3	<i>cis</i>	15.6	-140.9	15.6	13.2
	<i>trans₀</i>	177.5	-29.4	16.1	13.7
	<i>trans</i>	173.9	153.2	17.0	15.2
4	<i>cis</i>	13.1	53.0	13.3	12.0
	<i>trans₀</i>	174.6	-40.1	13.6	12.4
	<i>trans</i>	175.5	149.9	13.6	13.2
5	<i>cis</i>	13.1	45.2	13.5	12.8
	<i>trans₀</i>	172.3	-31.4	14.2	12.8
	<i>trans</i>	175.4	146.0	11.7	12.3
6	<i>cis</i>	-9.1	-50.3	14.2	15.1
	<i>trans</i>	-179.2	-156.8	14.1	14.0
7	<i>cis</i>	-13.5	138.2	14.7	13.3
	<i>trans₀</i>	176.2	22.6	19.5	17.1
	<i>trans</i>	-172.9	-152.4	17.5	18.3



8 [†]	<i>trans</i> ₀	170.2	-33.8	18.4	18.6
	<i>trans</i>	174.0	148.9	17.5	16.1
9	<i>cis</i>	12.9	-128.8	15.6	14.1
	<i>cis'</i>	13.4	48.7	14.4	15.3
	<i>trans</i> ₀	174.4	-29.4	16.8	16.4
	<i>trans</i>	-172.9	-151.9	16.8	15.9
10	<i>cis'</i>	13.2	-135.0	16.0	15.4
	<i>cis</i>	12.6	-140.9	0.0	0.0
	<i>trans</i> ₀	-177.1	-25.4	16.2	16.0
	<i>trans</i>	174.2	165.4	2.9	3.4
11	<i>cis</i>	12.1	-128.1	13.3	12.2
	<i>trans</i> ₀	-177.4	-26.2	17.5	15.6
	<i>trans</i>	172.2	153.7	17.7	17.5
12 [†]	<i>trans</i> ₀	174.4	14.4	17.6	17.9
	<i>trans</i>	-179.8	-165.5	14.6	15.0

[†] Cis isomer is not possible due to structural restrictions.

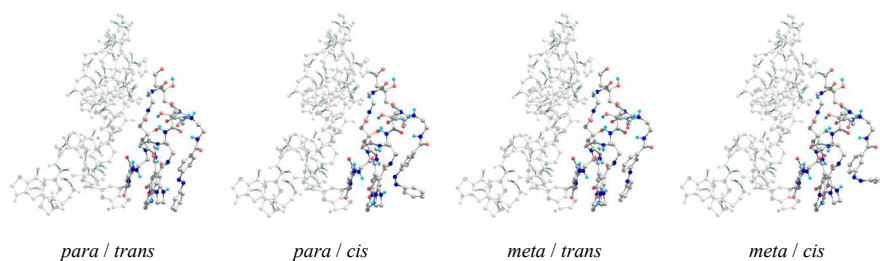


Figure S24. Structures of the CND functionalized with azo isomers, **CND-Azo**.

Isomer *Cis* of Azobenzene and Corresponding CND hybrids

The experimental spectra of the **CND**, *cis*-**Azo** and the respective **CND-Azo** hybrid are illustrated in **Figure S25**. In both *meta* and *para* azobenzene derivatives, it was observed that main peaks of *cis*-**Azo** and **CND-Azo** hybrids are shifted to smaller wavelengths ($\lambda < 300$ nm). However, the experimental spectra exhibited nearly overlapped peaks of **CND**, *cis*-**Azo** and **CND-Azo** in the 300–400 nm range.

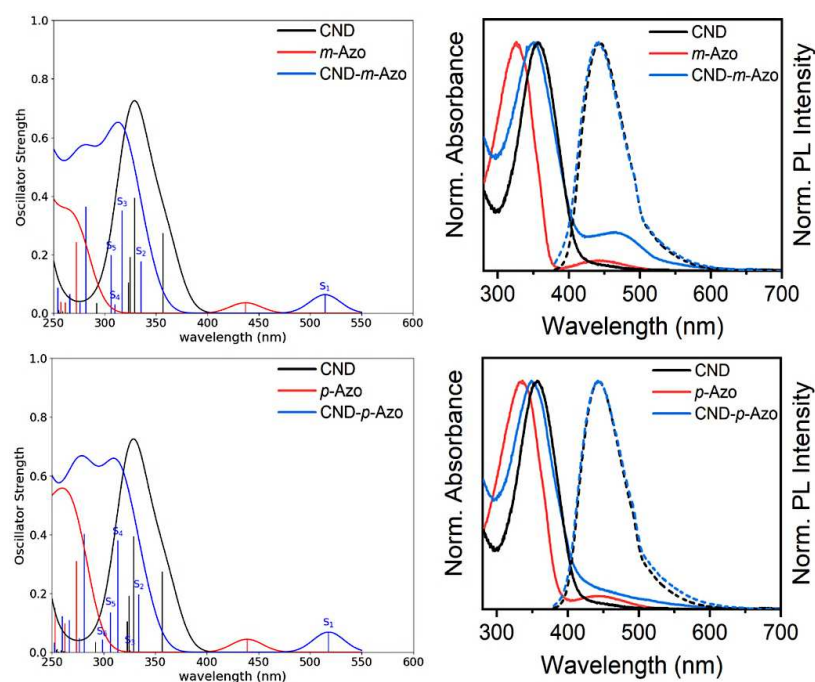


Figure S25. Comparisons between calculated and experimental spectra of the **CND**, the *cis* isomer of azobenzene, and the respective **CND-Azo** hybrids. For latter, the lowest-energy electron transitions S_n are also indicated.

The conversion of the isomer *cis* to *trans* was experimentally induced by light absorption utilizing a wavelength of 450 nm. The TDDFT results of *cis*-Azo exhibit a HOMO-LUMO transition at 439 nm for the *para* conformer and 437 nm for the *meta* counterpart, both weak due to $f = 0.04$ (see also **Figures S26** and **S27**). However, the equivalent transition in the CND-Azo hybrid (isomer *cis*) was calculated at 518 nm and 515 nm for the *para* and *meta* cases ($f = 0.07$), respectively. In this context, it was mentioned that the peaks for the isomer *cis* are displaced compared with the experimental evidence, therefore let us discuss the *trans* isomer in the next subsection.

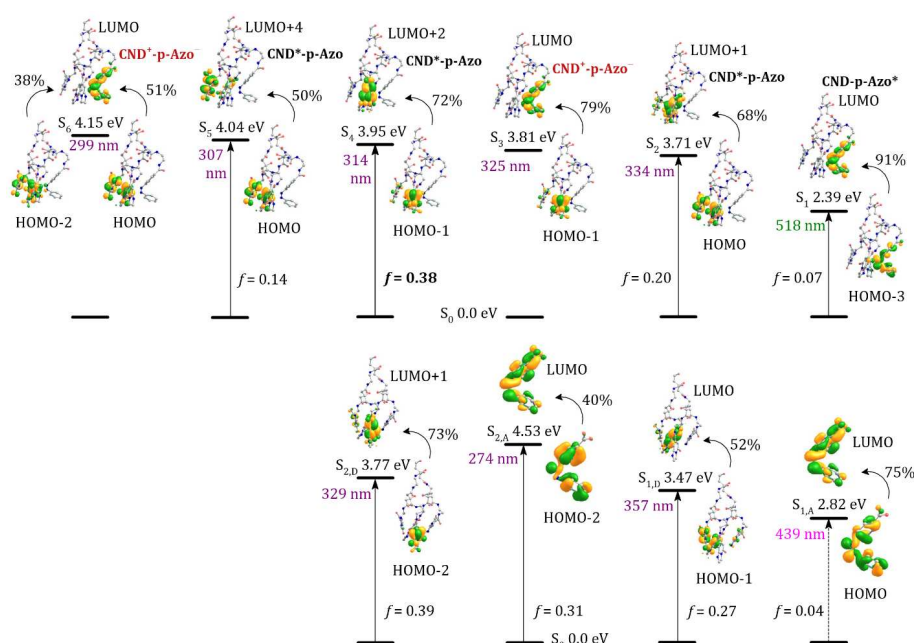


Figure S26. (Bottom) Lowest-energy electron transitions in the CND ($S_{n,D}$), in the isomer *cis* of *para*-azobenzene ($S_{n,A}$), (top) and in the CND-*p*-Azo. The transitions in the substrates (bottom) are aligned with those in the hybrid (top), if available.

Note: Henceforth, illustrations of transitions in the hybrid correspond to $\omega > 300$ nm, and those with $f \geq 0.05$ are represented with a vertical arrow. Orbital contributions are depicted under the criterion $\phi_i \rightarrow \phi_j > 35\%$. For **Azo** compounds, $S_{1,A}$ is depicted along with the transition with the largest f value ($S_{2,A}$ in most cases). For clarity, the ONIOM medium layer was omitted in all diagrams.

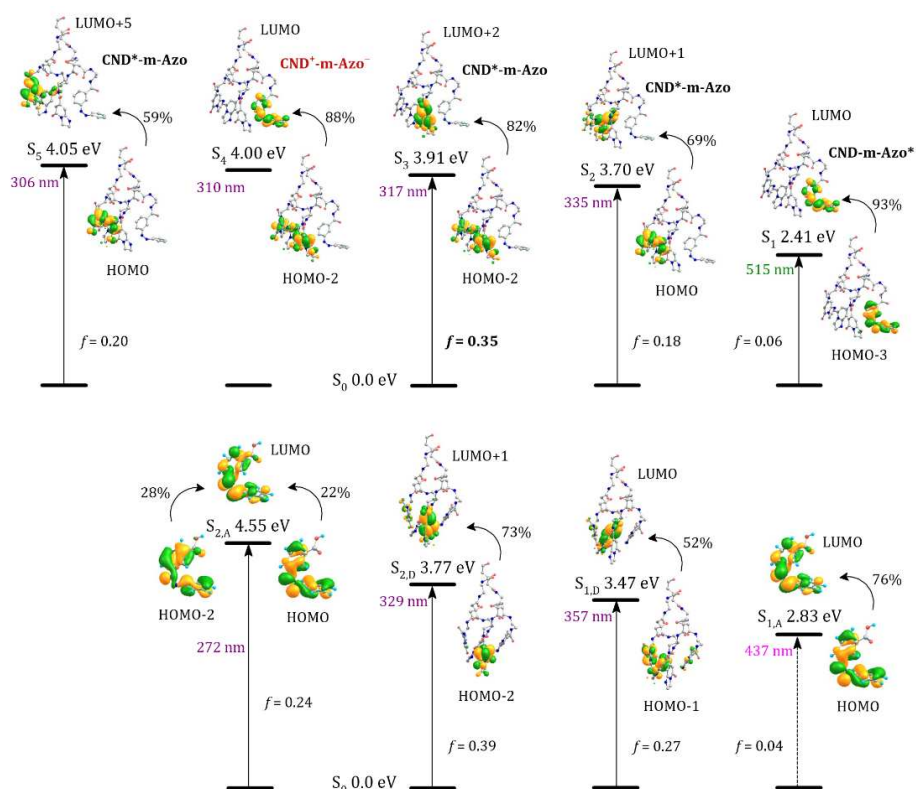


Figure S27. (Bottom) Lowest-energy electron transitions in the CND (S_{n,D}), in the isomer *cis* of *meta*-azobenzene (S_{n,A}), (top), and in the CND-*m*-Azo. The transitions in the substrates (bottom) are aligned with those in the hybrid (top), if available.

Isomer *Trans* of Azobenzene and Corresponding CND hybrids

In line with the description provided in the previous subsection, the calculated spectra for both the *para* and *meta* configurations of *trans*-Azo and their respective hybrids show a closer agreement with experimental data (see **Figure S28**). These findings suggest that the *trans* configuration of azobenzene in the CND-Azo hybrid is predominant in the electron transitions.

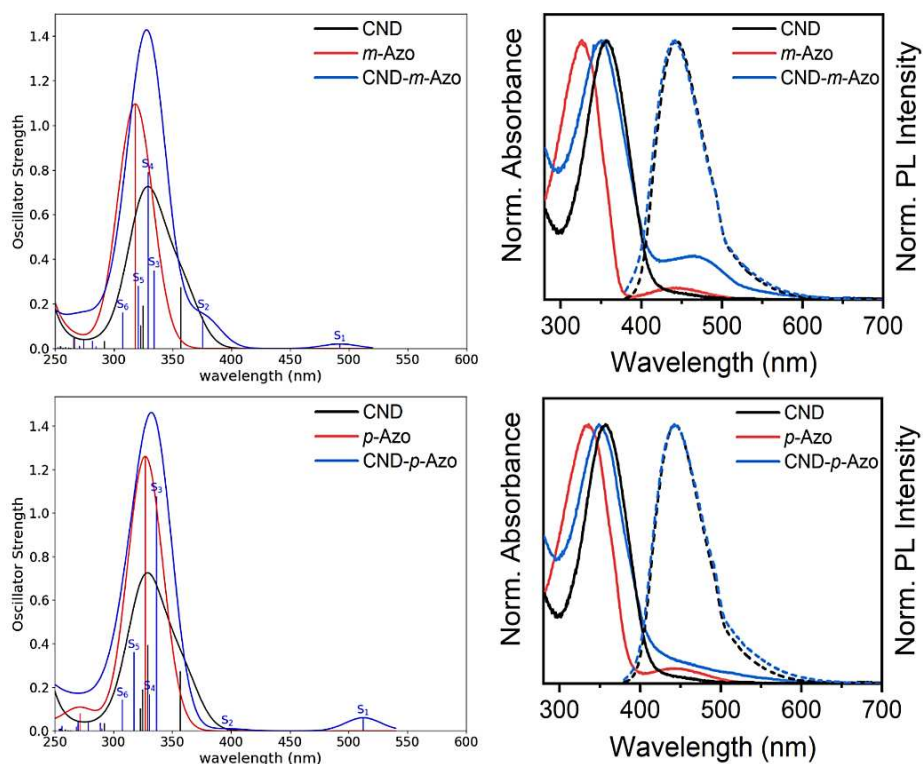


Figure S28. Comparisons between calculated and experimental spectra of the CND, the *trans* isomer of azobenzene, and the respective CND-Azo hybrids. For this latter, the lowest-energy electron transitions S_n are also indicated.

Experimental investigations determined that the conversion of the *trans* to *cis* isomer was achieved using light with a wavelength of 340 nm. TDDFT results for the *trans*-**Azo** isomer show a HOMO-1 to LUMO transition at 465 nm for the *para* conformer and at 453 nm for the *meta* conformer; these transitions are forbidden with an oscillator strength of $f=0.00$ (see also **Figures S29** and **S30**). This result confirms that the weak peak observed at 450 nm in the experimental spectrum of the **Azo** compound (red line) corresponds to the *cis* isomer in both the *para* and *meta* configurations. However, for the **CND-Azo** hybrid, we observed weak peaks in the visible region for all cases: 518 nm for *para/cis*, 515 nm for *meta/cis*, 513 nm for *para/trans*, and 492 nm for *meta/trans*. The blue line in the experimental spectra of the **CND-Azo** hybrid extends from 450 nm to 600 nm. We infer that the 450 nm irradiation used for converting the *cis*-**Azo** to the *trans* isomer in **CND-Azo** may overlap with electronic transitions occurring within the hybrid.

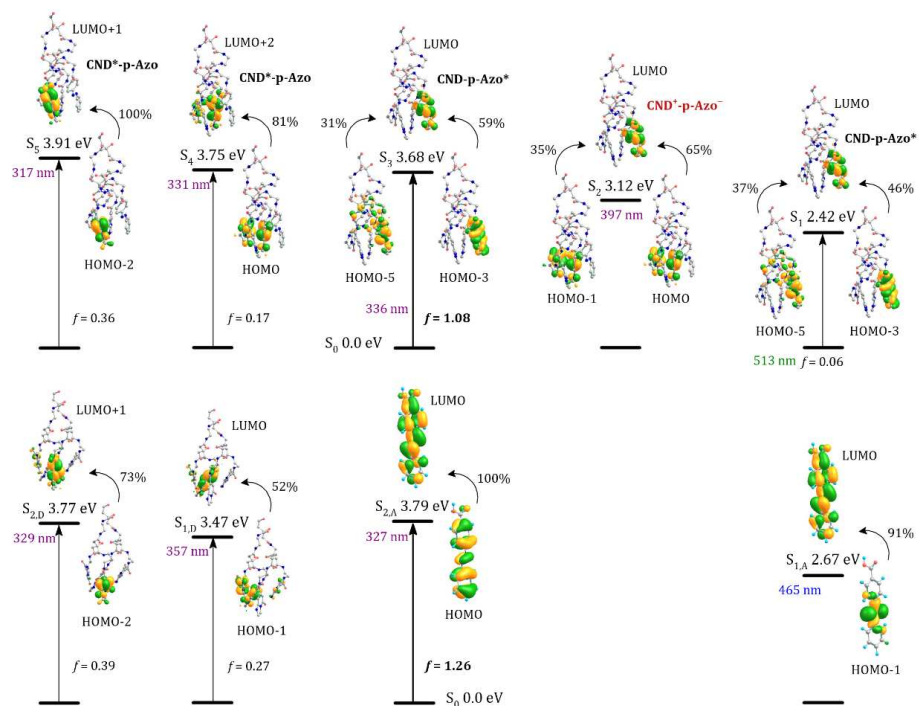


Figure S29. (Bottom) Lowest-energy electron transitions in the CND ($S_{n,D}$), in the isomer *trans* of *para*-azobenzene ($S_{n,A}$), (top) and in the CND-*p*-Azo. The transitions in the substrates (bottom) are aligned with those in the hybrid (top), if available.

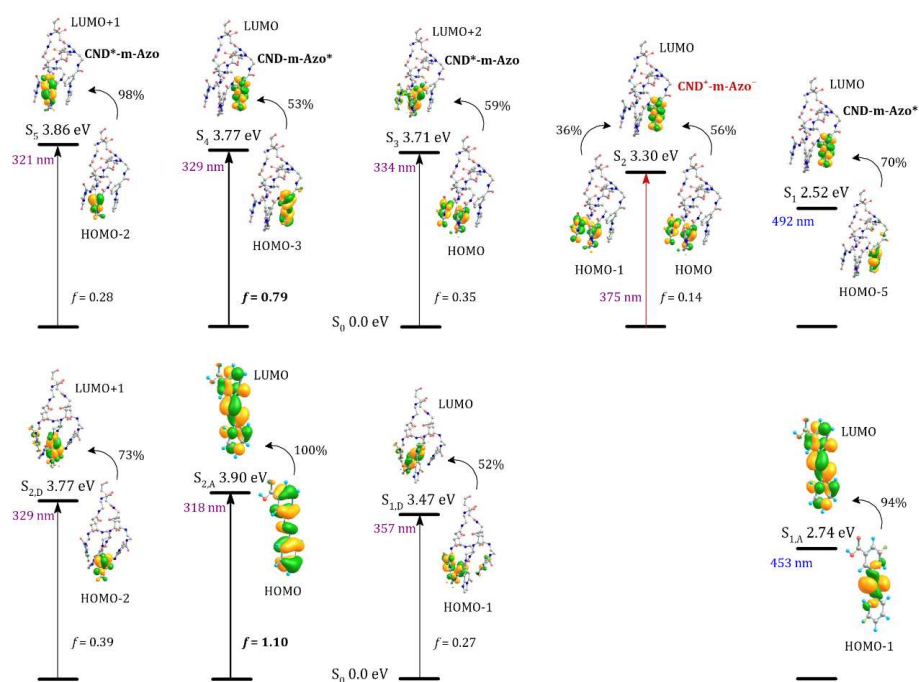


Figure S30. (Bottom) Lowest-energy electron transitions in the CND ($S_{n,D}$), in the isomer *trans* of *meta*-azobenzene ($S_{n,A}$), (top) and in the CND-*m*-Azo. The transitions in the substrates (bottom) are aligned with those in the hybrid (top), if available.

Meta-Glycine-Azobenzene and Corresponding CND hybrids

Azobenzene was additionally functionalized by incorporating a glycine unit at the *meta* position of the **Azo** moiety. The GFN2-xTB optimized structures are depicted in **Figure S31**. The HOMO and LUMO levels and energy gap E_g exhibited a similar trend to those reported in the previous subsection:

- The HOMO level remains unaltered (7.0 eV) through **CND** and hybrids. Therefore, E_g is primarily altered by the LUMO level.
- LUMO is more stabilized for the **CND-*m*-Gly-Azo** hybrids, as compared with the unmodified **CND**.
- LUMO is more stabilized for the *trans* isomers, as compared with the *cis* counterparts.

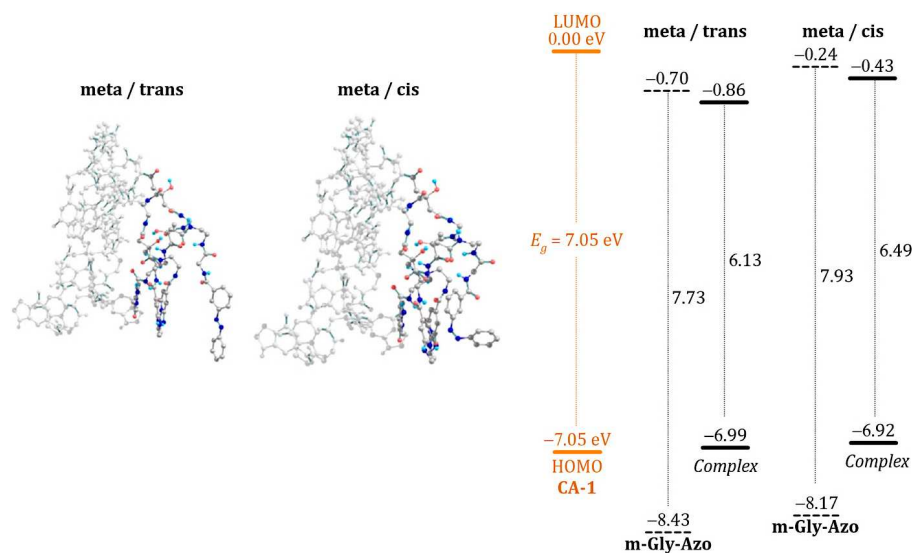


Figure S31. Structures of the **CND** functionalized with *m*-Gly-Azo. HOMO and LUMO levels and energy gaps in eV for the **CND** (in orange), *m*-Gly-Azo compounds, and the respective **CND-*m*-Gly-Azo**.

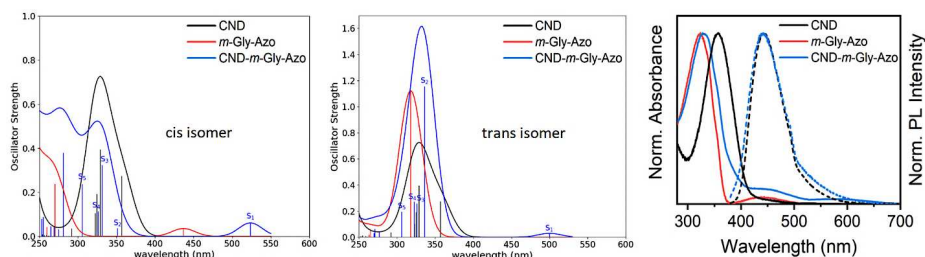


Figure S32. Comparisons between calculated and experimental spectra of the **CND**, the *meta* conformer of azobenzene attached to a glycine unit, and the respective **CND-*m*-Gly-Azo** hybrids. For this latter, the lowest-energy electron transitions S_n are also indicated.

The electronic spectra of the compound and hybrids incorporating glycine at the *meta* position exhibited a similar trend to those reported in previous subsections (see **Figure S32**). Specifically, the main peaks of the **CND-*m*-Gly-Azo** hybrid containing the *cis* isomer of the azobenzene fragment are shifted to shorter wavelengths (<300 nm). Therefore, the *trans* configuration is considered the predominant isomer of the azobenzene moiety, as evidenced by the nearly overlapping peaks, consistent with our previous conclusion.

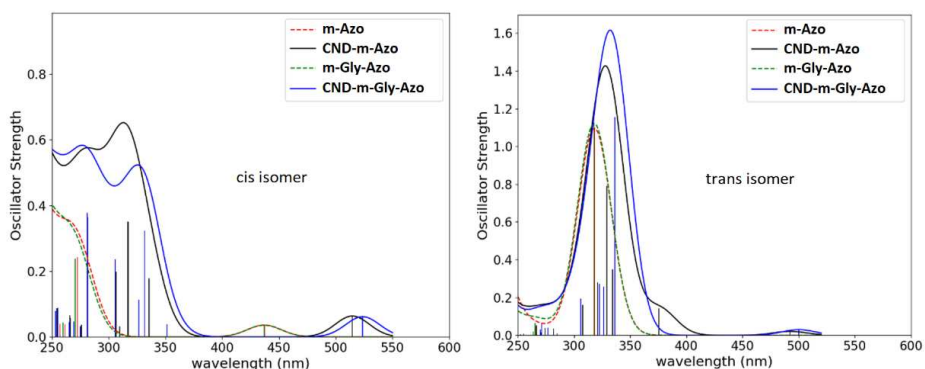


Figure S33. Calculated spectra of azobenzene and hybrids modified with a glycine spacer compared with non-modified counterparts.

Finally, in **Figure S33**, the calculated spectra for *m*-Gly-Azo and the CND-*m*-Gly-Azo hybrids are compared with those for *m*-Azo and CND-*m*-Azo. The spectra for the *cis* isomer of azobenzene exhibit similar features, with slight variations in oscillator strength values for certain peaks in the respective hybrids. This trend is also observed in the *trans* isomer, although the CND-*m*-Azo hybrid displays a shoulder around 375 nm. However, it should be noted that the *trans*-azo fragment in CND-*m*-Gly-Azo is located at $d = 7.95 \text{ \AA}$, where d stands for the molecular distance between CND structure and the azo fragment (see **Figure S34**, bottom section, for a graphical representation of parameter d). For the *trans*-azo fragment, the localized transitions in CND*-*m*-Azo (S_3 and S_5) and CND-*m*-Azo* (S_1 and S_4) are nearly identical to those in the hybrids with the molecular spacer (see dashed lines in **Figure S34**, bottom): $\Delta\omega < 10 \text{ nm}$, $\Delta f < 0.1$ (except for S_4 CND-*m*-Azo*), same or similar major $\varphi_i \rightarrow \varphi_j$ orbital contributions. A similar trend was observed for the hybrids containing the *cis* conformation of the azobenzene moiety (e.g., see dashed lines for S_5 of CND*-*m*-Azo in **Figure S35**, from the middle to the bottom sections). However, when $d < 4 \text{ \AA}$, the *cis* CND-*m*-Gly-Azo hybrid exhibited a more stabilized charge-transfer excited state than the corresponding state in *cis* CND-*m*-Azo (see dashed lines in **Figure S35**, from the top to the middle sections). To explore this, we investigated the variation of the electronic transitions as a function of the distance between the azobenzene fragment and the main structure of CND. The overall results reported in **Figures S34** and **S35** suggest that the flexibility provided by the glycine spacer either induces (when $d < 4 \text{ \AA}$) or precludes (when $d > 6 \text{ \AA}$) the formation of charge-transfer states.

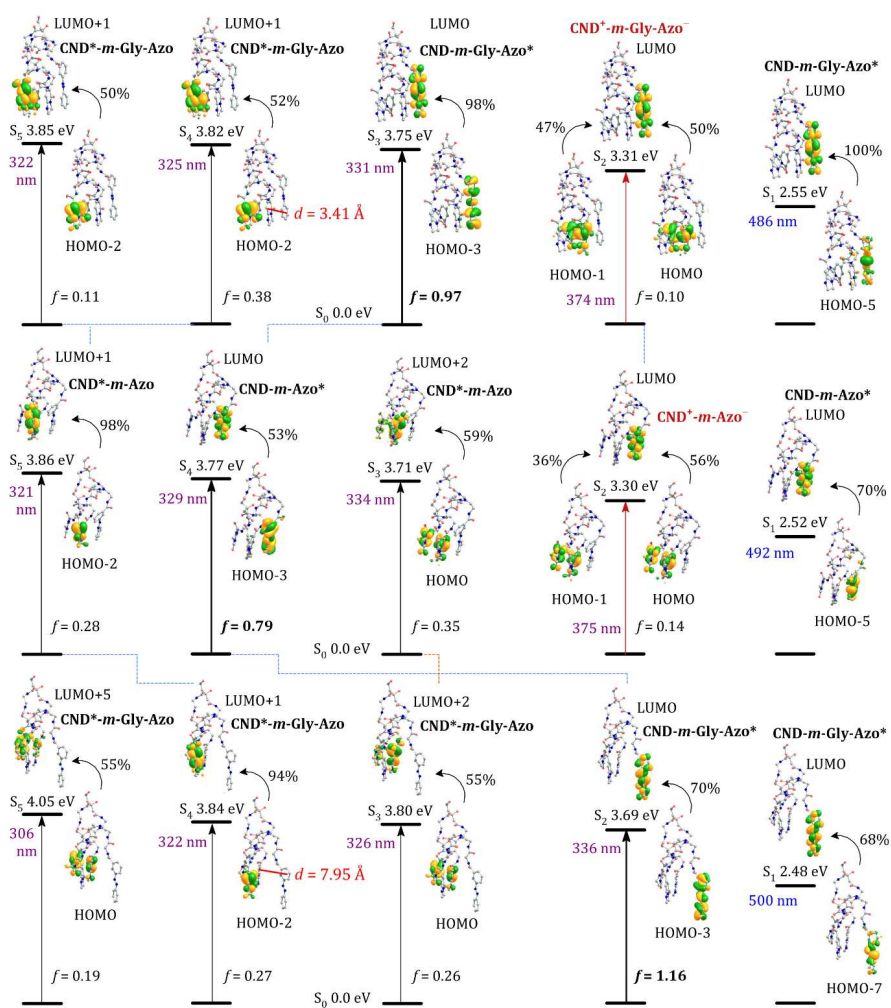


Figure S34. Lowest-energy electronic transitions in **CND-m-Azo** (middle) compared with **CND-m-Gly-Azo**. The hybrids incorporate the *trans* isomer of the azobenzene fragment, positioned either closer to (*top*) or farther from (*bottom*) the nearest IPCA centroid of the CND fragment.

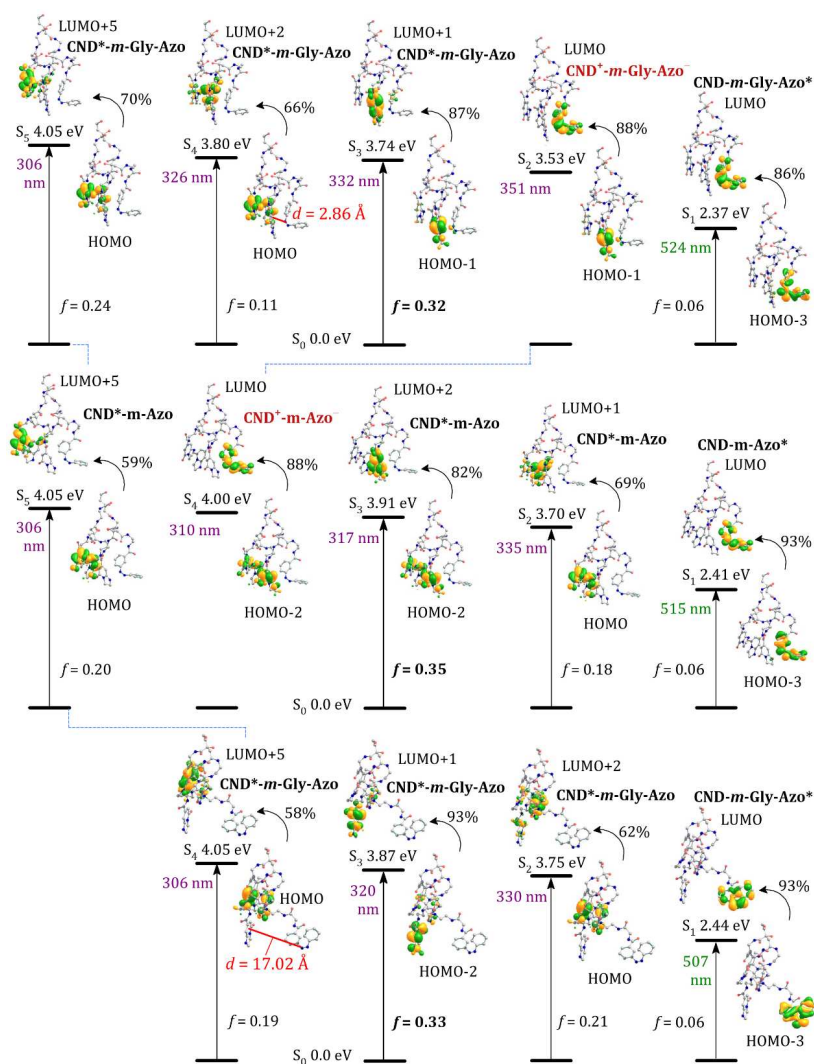


Figure S35. Lowest-energy electronic transitions in **CND-*m*-Azo** (*middle*) compared with **CND-*m*-Gly-Azo**. The hybrids incorporate the *cis* isomer of the azobenzene fragment, positioned either closer to (*top*) or farther from (*bottom*) the nearest IPCA centroid of the CND fragment.

Benchmarking Basis Sets and Functional

We performed a benchmarking considering different basis sets and different functional. For the basis set, we consider the wB97xD functional and apart from the original Pople's 6-31G(d), we consider a corresponsive cc-PVDZ and the more accurate cc-PVTZ basis sets. The absorption spectra are reported in **Figure S36**(left). From the spectra, we can conclude that there is little or no dependence on the used basis set. Next, we consider three different long-range corrected functionals (keeping the same cc-pVDZ basis set), which are essential to capture the van der Waals interactions present in the system. Apart from the wB97xD used throughout the study, we considered the HSE, CAM-B3LYP and HPBE functionals. As reported in **Figure S36**(right), there is a strong dependence of the absorption spectra on the used functional. While wB97xD and CAM-B3LYP leads to the same results, HSE tends to redshift the whole spectra, while HPBE blueshifts it. Comparing the obtained spectra with the experimental one (for the **CND-*p*-Azo**, reported in **Figure 4b** of the manuscript), we can exclude both HSE and HPBE, as their absorption is either too red or blue shifted compared to the experimental one. Thus, we can confirm that the wB97xD/6-31G(d) functional and basis set combination used is a good choice to reproduce the experimental spectra.

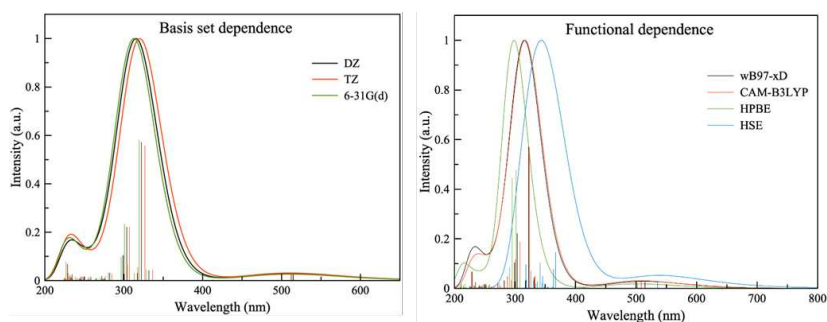


Figure S36. Absorption spectra of the CND-*p*-Azo obtained from computation while benchmarking different basis sets (left) and functionals (right).

Excitation-emission maps

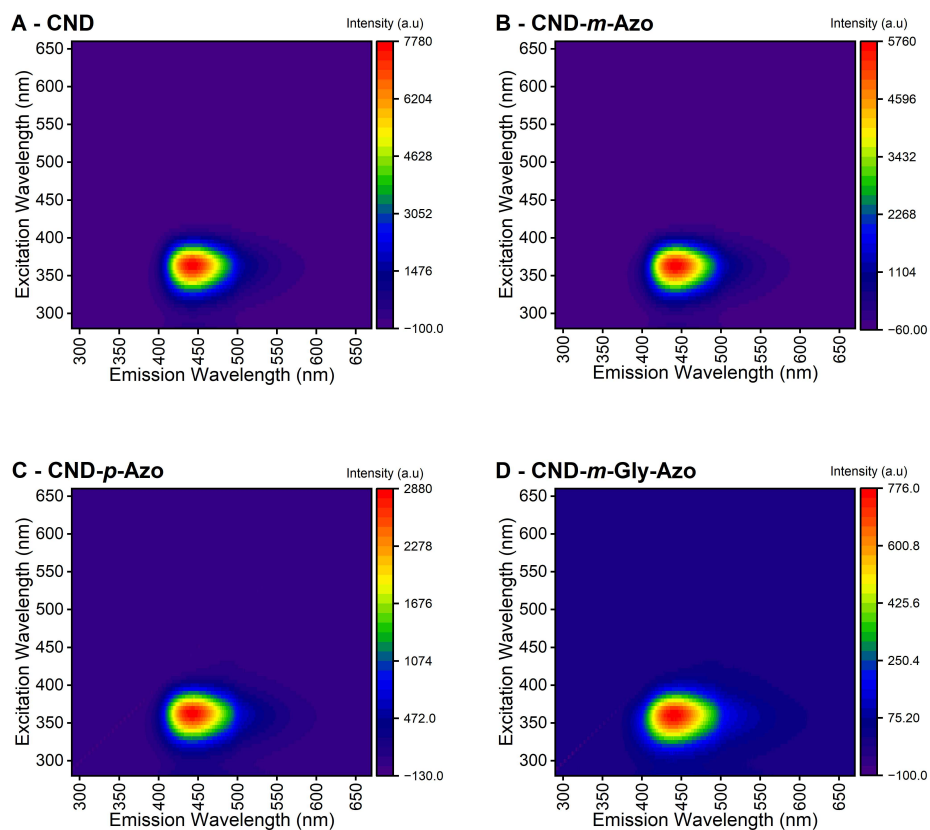


Figure S37. Excitation-emission maps recorded in an excitation wavelength range of 280 nm to 660 nm and an emission wavelength range of 290 nm to 670 nm. Concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO was used. (A) CND (B) CND-*m*-Azo (C) CND-*p*-Azo (D) CND-*m*-Gly-Azo.

Excitation Spectra

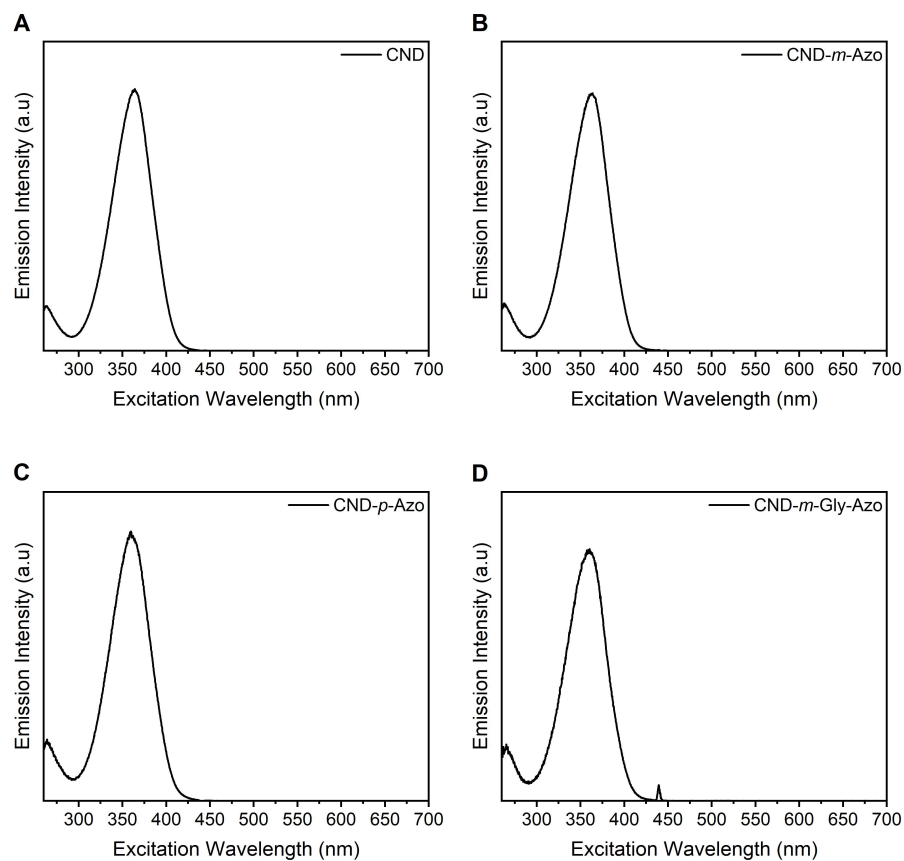


Figure S38. Excitation spectra recorded at an emission wavelength of 440 nm in the excitation wavelength range of 260 nm to 700 nm. Concentration of $6 \text{ mg} \cdot \text{L}^{-1}$ in DMSO was used. **(A) CND** **(B) CND-*m*-Azo** **(C) CND-*p*-Azo** **(D) CND-*m*-Gly-Azo** (the signal at 440 nm corresponds to the excitation wavelength which was not effectively subtracted from the background).

Time-Correlated Single Photon Counting (TCSPC)

For the determination of the fluorescence lifetimes, a homebuilt TCSPC setup was used. The excitation wavelength is generated with a tunable optical parametric amplifier (Orpheus-HP, Light Conversion) which is pumped by a 2 MHz carbide laser (CB3-40 W, Light Conversion). The excitation beam is tightly focused into the sample cuvette with a 75 mm lens and perpendicular to the excitation pathway, the fluorescence is collected by a second 75 mm lens. After separation from the excitation beam with a bandpass filter, the fluorescence was focused into a detector (PMA Hybrid 07, Picoquant) which is together with the laser's own trigger output connected to a time-tagging electronic (PicoHarp 330, Picoquant) for TCSPC measurements.

All samples were set to an optical density below 0.1 OD to prevent reabsorption effects. Since we were interested in the quenching efficiencies, an excitation wavelength of 385 nm was chosen, where predominantly the CND absorbs in the case of the hybrid systems. The obtained decay curves (see **Figure 6**) were analyzed using gaussian deconvolution in a commercial program (Fluofit, Picoquant). Each transient curve was fitted with three lifetimes (see **Table S3**).

Table S3. Obtained lifetimes τ and their respective amplitudes A from the fits of the TCSPC transients for the pure CND and the different hybrid systems. The average lifetime τ_{av} was used to calculate the quenching efficiencies and χ^2 describes the goodness of the fit.

Sample	τ_1 [ns]	A_1 [counts]	τ_2 [ns]	A_2 [counts]	τ_3 [ns]	A_3 [counts]	τ_{av} [ns]	χ^2 (red)
CND	0.52	28160	4.75	17596	11.25	28972	5.70	1.255
CND- <i>p</i> -Azo	0.37	41900	4.10	19085	10.92	16888	3.45	1.843
CND- <i>m</i> -Azo	0.40	43300	3.76	18453	10.53	17936	3.60	1.236
CND- <i>m</i> -Gly-Azo	0.37	50990	3.05	17545	10.70	9193	2.24	2.976

Determination of Transfer Rates and Emission Quenching Efficiencies

The three lifetimes were used to determine an amplitude-weighted average lifetime τ_{av} . This average lifetime was used to calculate the total emission quenching efficiencies according to following formula,

$$E_T(\tau) = 1 - \frac{\tau_{AzoCND}}{\tau_{CND}}$$

where τ_{AzoCND} and τ_{CND} are the average fluorescence lifetimes of the hybrid systems and pure CND, respectively.

Additionally, with the same formula, the total emission quenching efficiencies were calculated using the photoluminescence quantum yields (from **Table 2** of the main manuscript) instead of the fluorescence lifetimes. The results from both calculations are presented in the following **Table S4**. **Table S4.** The energy transfer efficiencies $E_T(F)$ and $E_T(\tau)$ calculated by the photoluminescence quantum yield and fluorescence lifetimes, respectively. The radiative k_r and non-radiative k_{nr} constants are also provided.

Construct	ϕ_F	$E_T(F)$ [%]	τ_{av} [ns]	$E_T(\tau)$ [%]	k_r [10^8 s^{-1}]	k_{nr} [10^8 s^{-1}]
CND	0.37	---	5.70	---	0.65	1.11
CND- <i>m</i> -Azo	0.18	51	3.60	39	0.50	2.27
CND- <i>p</i> -Azo	0.11	70	3.45	36	0.32	2.58
CND- <i>m</i> -Gly-Azo	0.01	97	2.24	61	0.045	4.42

Interestingly, the emission quenching efficiencies derived from fluorescence lifetimes, $E_T(\tau)$, are significantly lower than those obtained from fluorescence quantum yields, $E_T(F)$. This discrepancy

arises from fundamental differences between the measurement methods. In TCSPC measurements, only the emissive state is detected, meaning that emitted photons are counted while non-emissive (dark) states remain hidden. As a result, this creates a bias, making the emissive state appear more dominant than it actually is.

Furthermore, we determined FRET parameters according to the program³⁹ and publication⁴⁰ by Peter Nagy, which are based on the commonly used FRET equations such as the one for the overlap integral J :

$$J = \int F_D(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 d\lambda$$

where $F_D(\lambda)$ represents the fluorescence spectrum of the donor, normalized to an area of 1, $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor and λ^4 serves as a wavelength correction factor.

Using the calculated overlap integral J , along with the orientation factor κ^2 , the photoluminescence quantum yield of the donor ϕ_D and the refractive index n , we determined the Förster radius R_0 .

$$R_0 = \text{const.} \sqrt[6]{J \cdot \kappa^2 \cdot \phi_D \cdot n^{-4}}$$

It should be noted that no clear value could be determined for κ^2 due to the unknown distribution of the azo moieties on the CND surface. Thus, an average value of 2/3 was assumed for the orientation factor. With R_0 , the transfer efficiency E_{FRET} can be estimated for the actual distances R (given in **Table 3** of the main manuscript) between the CND and the azo moieties:

$$E_{\text{FRET}} = \frac{R_0^6}{R_0^6 + R^6}$$

Additionally, if the excited-state lifetime of the donor τ_D is known, the transfer rate k_T can be determined using following relationship.

$$k_T = \frac{1}{\tau_D} \cdot \left(\frac{R_0}{r} \right)^6$$

Table S5. Calculated FRET parameters including the overlap integrals J , the Förster radii R_0 , the FRET efficiencies E_{FRET} , the transfer rates k_T and the transfer lifetimes τ_T .

Construct	J	R_0 [nm]	E_{FRET} [%]	k_T [s ⁻¹]	τ_T [fs]
CND-p-Azo	2.96*10 ¹³	2.25	99.9	1.76*10 ¹³	56
CND-m-Azo	1.88*10 ¹³	2.09	99.9	9.47*10 ¹²	105
CND-Gly-m-Azo	1.28*10 ¹³	1.96	99.9	6.44*10 ¹²	155

As discussed in the main manuscript, these high efficiencies are not representative of all three hybrids. Only **CND-m-Gly-Azo** exhibits such a high emission quenching efficiency based on photoluminescence quantum yield measurements. The primary reason for this discrepancy is that the calculated distances and the averaged orientation factor may not be uniformly valid across the entire **CND** surface. The variations in the azo moiety distribution on the surface are not accounted for in the calculations, which could likely contribute to the observed differences.

Photo-Switching

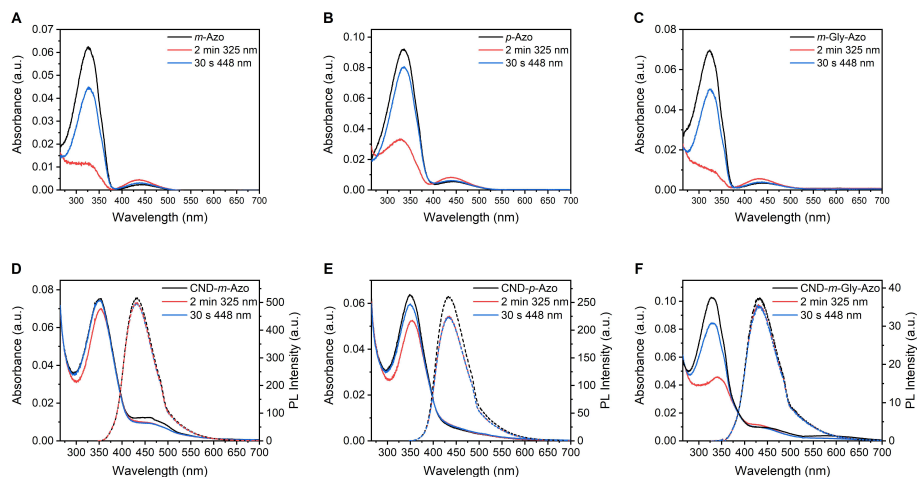


Figure S39. Absorption spectra of the (A) *m*-Azo, (B) *p*-Azo, and (C) *m*-Gly-Azo, as well as the absorption and emission spectra of the (D) CND-*m*-Azo, (E) CND-*p*-Azo, and (F) CND-*m*-Gly-Azo, were measured before and after light irradiation. The initial state (black, black dotted) was measured prior to illumination. The first illumination was conducted with a 325 nm LED for a duration of two minutes (red, red dotted). The second illumination was conducted with a 448 nm LED for a duration of 30 seconds. The measurements were performed with 5 $\mu\text{mol L}^{-1}$ solutions of the respective azo in DMSO at 25 $^{\circ}\text{C}$. The measurements were performed with 10 mg L^{-1} dispersions of the respective hybrid in DMSO at 25 $^{\circ}\text{C}$.

Fatigue Resistance

To ensure that the photostationary state (PSS) was reached during the fatigue resistance measurements, different times were tested with the 340 nm and 450 nm LEDs (see **Figure S40**). Based on this, five minutes was chosen. This was to ensure that the hybrids would also have enough time to reach the PSS.

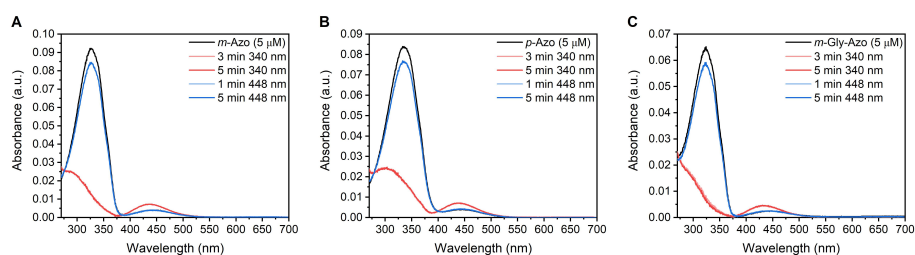


Figure S40. First, the non-irradiated samples were irradiated at 340 nm for three minutes and then for a further two minutes to check whether the PSS of the *Z* isomer had been reached. The samples were then irradiated at 450 nm for one minute and then for a further four minutes to check whether the PSS for the *E* isomer had also been reached. For the three pristine azos: (A) *m*-Azo (B) *p*-Azo (C) *m*-Gly-Azo. The measurements were performed with 5 $\mu\text{mol L}^{-1}$ solutions of the respective azo in DMSO at 25 $^{\circ}\text{C}$.

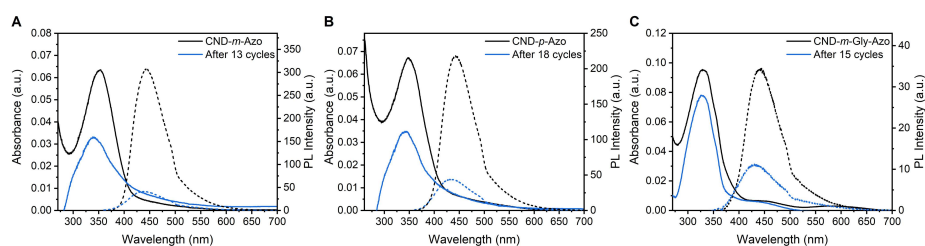


Figure S41. Absorbance and emission of the (A) CND-*m*-Azo (B) CND-*p*-Azo and (C) CND-*m*-Gly-Azo before and after several cycles of light irradiation. One cycle consisted of five minutes of illumination with a 340 nm LED and five minutes of illumination with a 448 nm LED. The measurements were performed with 10 mg L⁻¹ dispersions of the respective hybrid in DMSO at 25 °C.

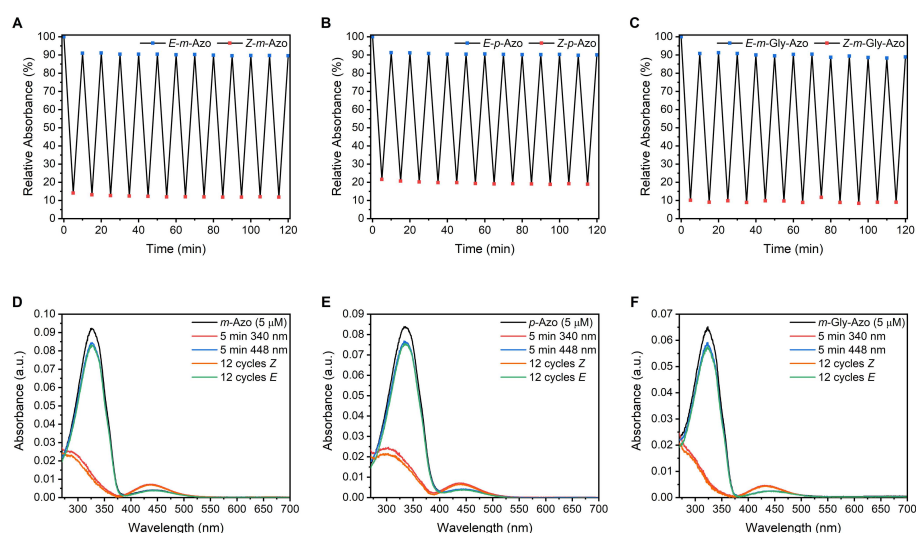


Figure S42. (A) The relative absorption intensity of *m*-Azo at 326 nm was not found to decrease significantly over time. (B) The relative absorption intensity of *p*-Azo at 335 nm was not found to

decrease significantly over time. (C) The relative absorption intensity of *m*-Gly-Azo at 323 nm was not found to decrease significantly over time. Absorbance of the (D) *m*-Azo (E) *p*-Azo and (F) *m*-Gly-Azo before and after 12 cycles of light irradiation for each isomer. One cycle consisted of five minutes of illumination with a 340 nm LED and five minutes of illumination with a 448 nm LED. The measurements were performed with 5 $\mu\text{mol L}^{-1}$ solutions of the respective azo in DMSO at 25 °C.

Kinetic Measurements

Kinetic experiments were done following an already published approach.⁴¹

For the kinetic measurements of the *cis* (*Z*) to *trans* (*E*) isomerization, samples were irradiated with an appropriate wavelength at 40 °C. A spectrum was recorded for each sample every 30 min from 290 nm to 700 nm. Due to scattering likely caused by agglomeration of the hybrids, **CND-*m*-Azo** and **CND-*p*-Azo** were normalized to their isosbestic points at 363 nm and 397 nm respectively. The azo loading on **CND-*m*-Gly-Azo** was high enough to abstain from this spectra processing. We want to highlight here that the obtained half-lives are flawed due to agglomeration, spectra processing and the handling of a multi-switch particle as a first order kinetic system. Nevertheless, it demonstrates the photoswitch properties and displays the general trend of the thermal properties. **Figures S46-S47** show spectra of **CND-*m*-Azo** and **CND-*p*-Azo** normalized to the isosbestic point for as accurate of an analysis as possible, while **Figures S49** show the raw spectra. **Figures S48** presents the unprocessed spectra of **CND-*m*-Gly-Azo** and the respective analysis. A low concentration was intentionally used to minimize aggregation effects. Three samples of each compound were measured.

All half-lives and rate constant were determined by a triple determination. Their calculation was conducted with OriginPro® by the OriginLab Corporation. For this procedure, the absorption at a certain wavelength was plotted against the time elapsed after irradiation. The measured points were fitted with the following exponential decay function 1 in Origin (Equation 5).

$$y = y_0 + A_1 e^{-x/t_1} \quad (5)$$

The exponential time constant t_1 obtained by this fitting was further used to calculate the kinetic constant k by Equation 6 and the half-life $t_{1/2}$ by Equation 7.

$$k = 1/t_1 \quad (6)$$

$$t_{1/2} = t_1 \times \ln(2) \quad (7)$$

As the hybrid materials contain more than a single azobenzene, we measured their t_{obs} following the same procedure as for the pristine azobenzenes.

Table S6. Half-lives at 40 °C for the pristine azos, as well as for the azo-hybrids.

Compound	$t_{1/2}$ or t_{obs} in h / Exp 1	$t_{1/2}$ or t_{obs} in h / Exp 2	$t_{1/2}$ or t_{obs} in h / Exp 3	$\bar{t}_{1/2}$ or t_{obs} in h	Standard deviation / h
<i>m</i>-Azo	31.3	30.9	31.3	31.2	0.2
CND-<i>m</i>-Azo	33	34	26	31	3
<i>p</i>-Azo	13.4	13.3	13.4	13.4	0.1
CND-<i>p</i>-Azo	15	15	16	15	1
<i>m</i>-Gly-Azo	34.7	35.2	34.6	34.8	0.3
CND-<i>m</i>-Gly-Azo	28	26	26	27	1

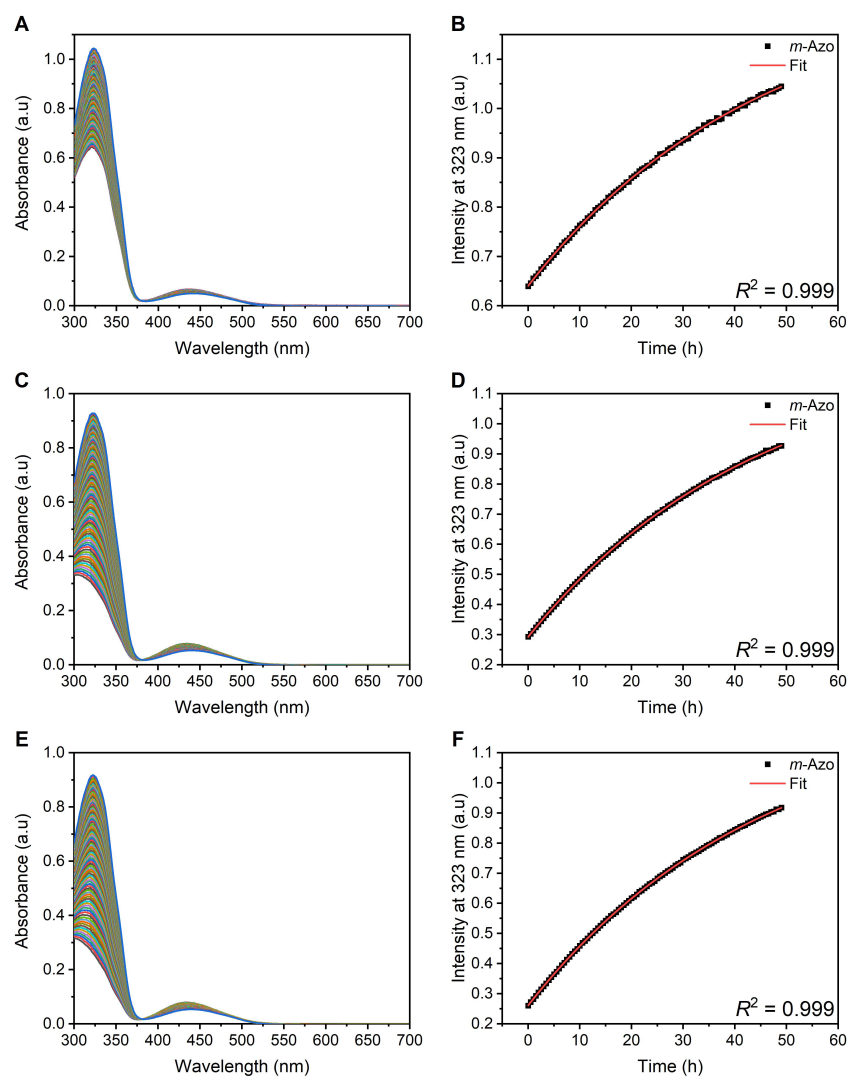


Figure S43. UV-vis spectra of thermal Z- to E-isomerization of *m*-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 323 nm plotted against time, with a single exponential fit to determine half-life.

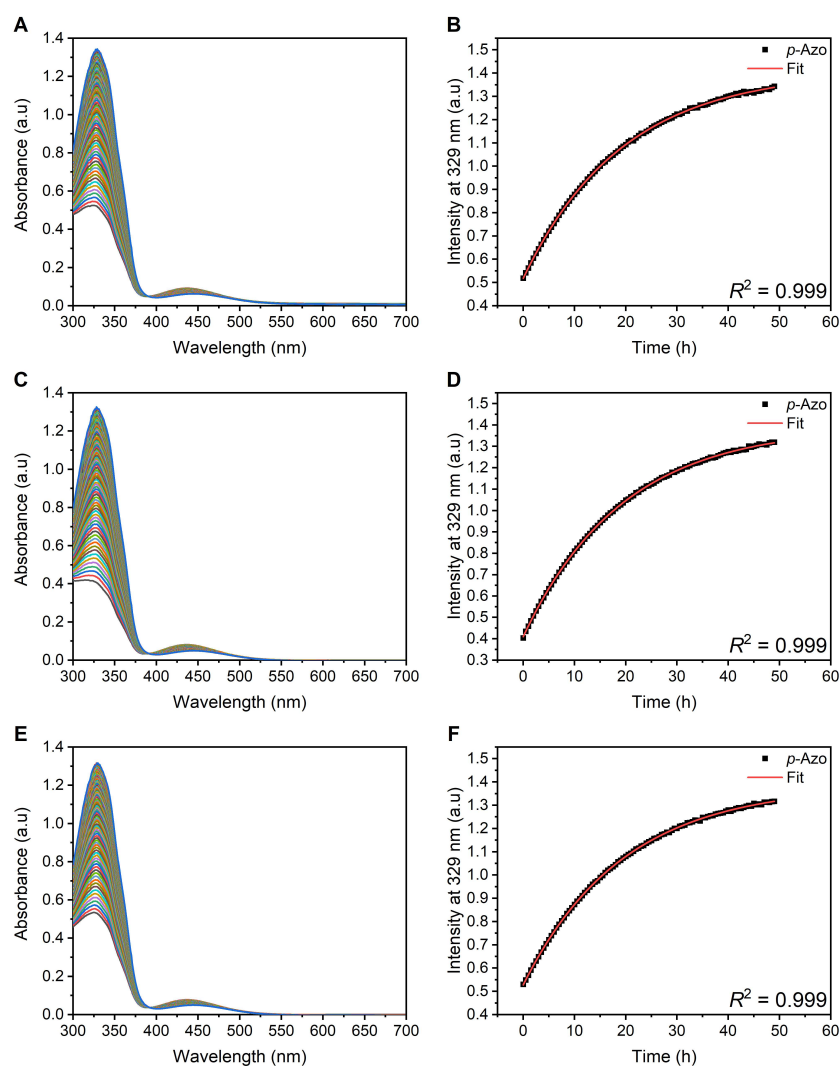


Figure S44. UV-vis spectra of thermal Z- to E-isomerization of *p*-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 329 nm plotted against time, with a single exponential fit to determine half-life.

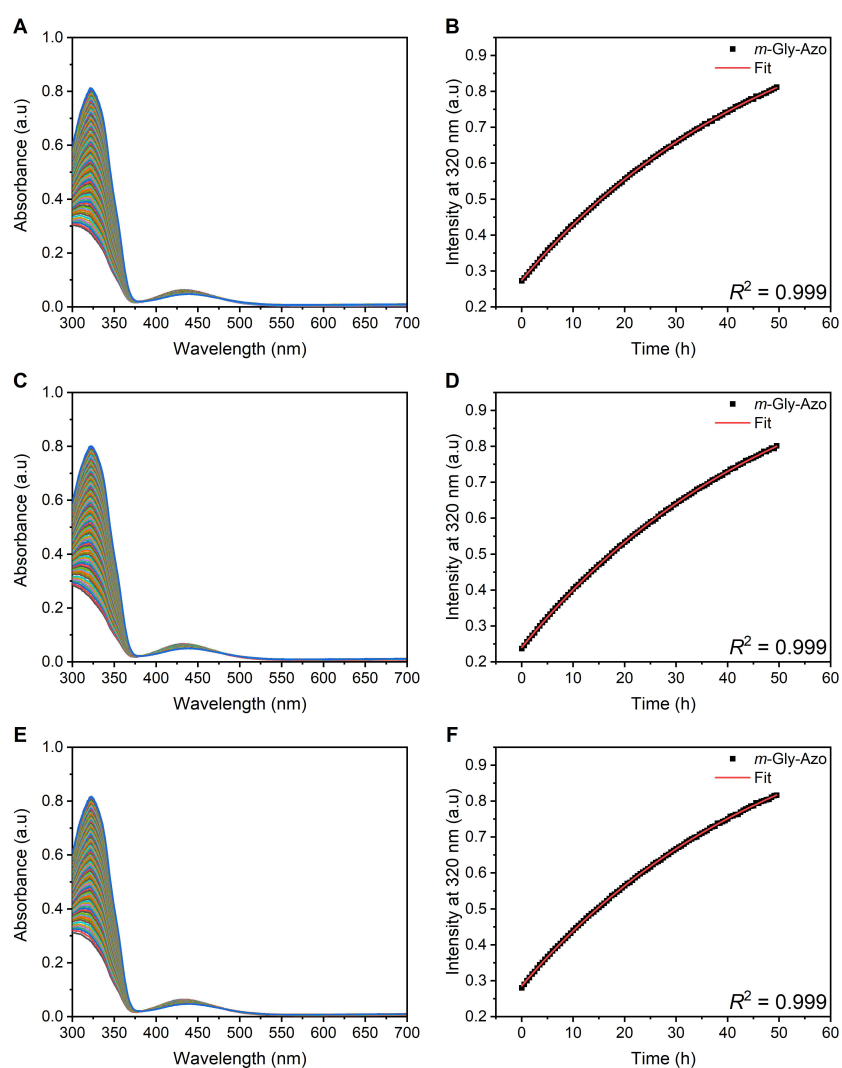


Figure S45. UV-vis spectra of thermal *Z*- to *E*-isomerization of *m*-Gly-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 320 nm plotted against time, with a single exponential fit to determine half-life.

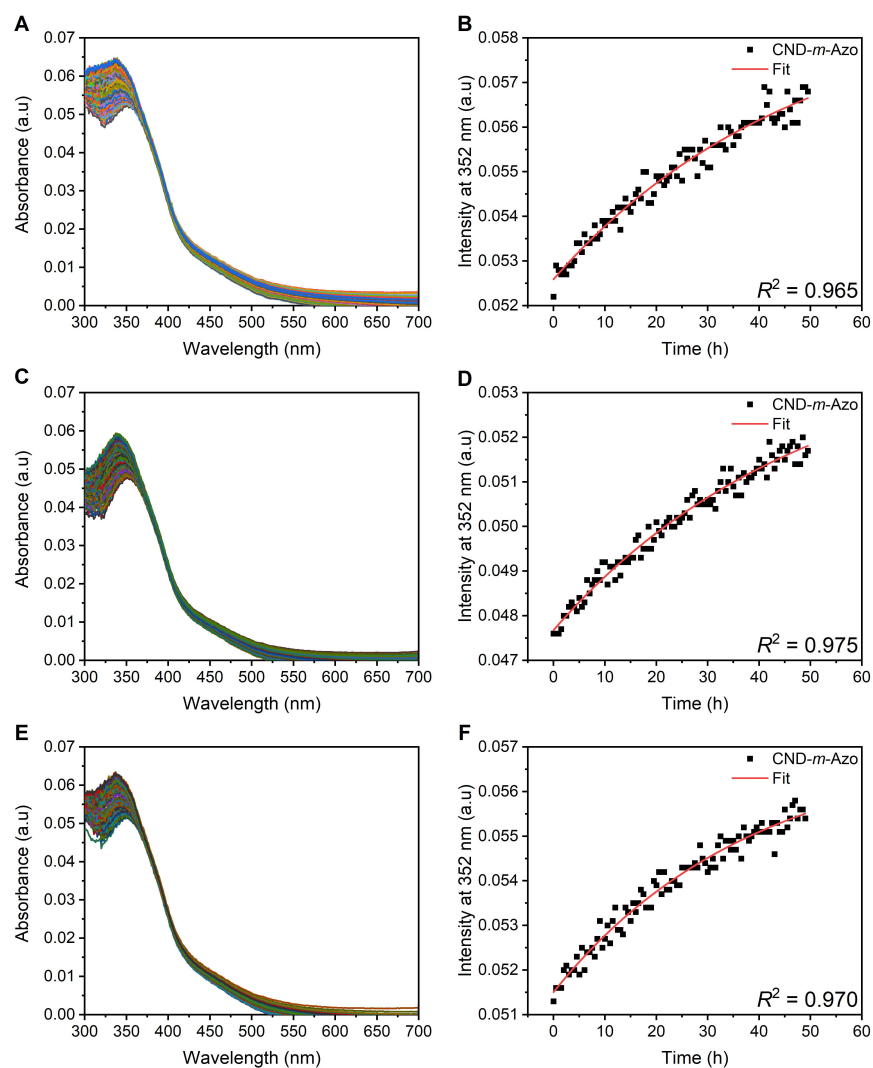


Figure S46. Normalized UV-vis spectra of thermal Z- to E-isomerization of CND-*m*-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 352 nm plotted against time, with a single exponential fit to determine half-life.

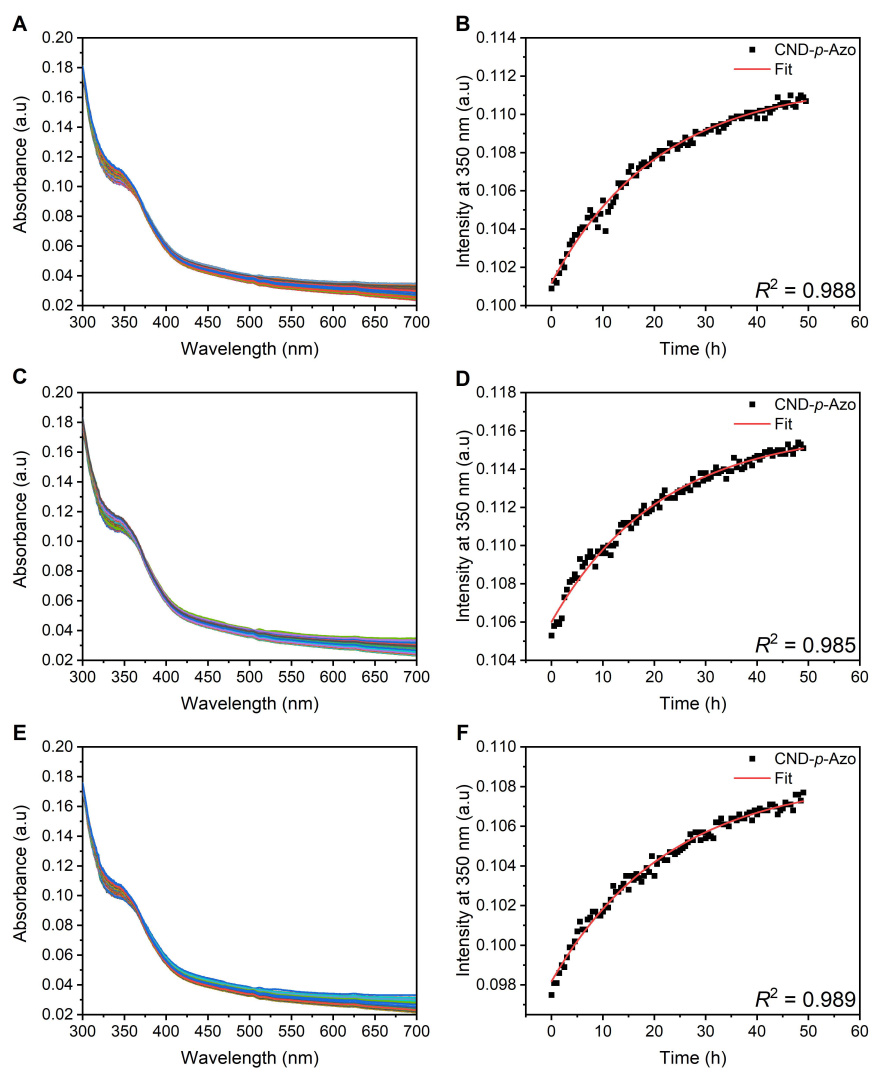


Figure S47. Normalized UV-vis spectra of thermal Z- to E-isomerization of CND-*p*-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 350 nm plotted against time, with a single exponential fit to determine half-life.

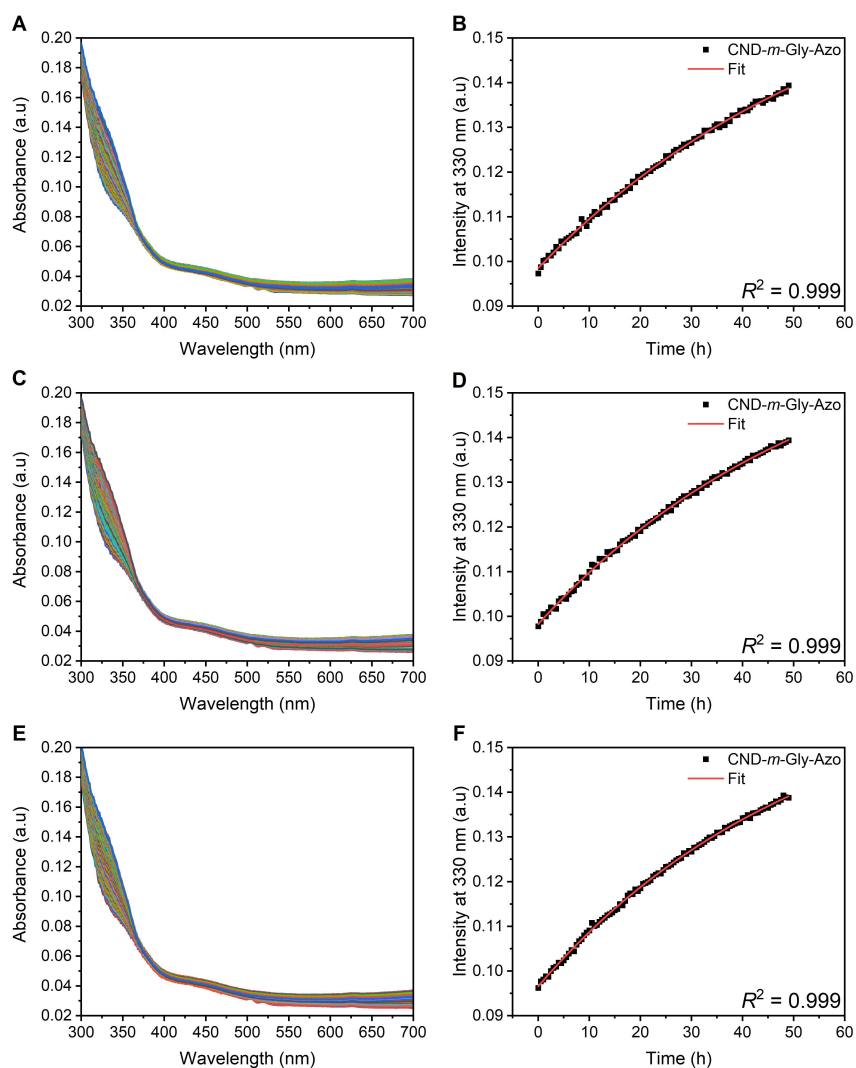


Figure S48. UV-vis spectra of thermal *Z*- to *E*-isomerization of CND-*m*-Gly-Azo (A) first, (C) second, and (E) third measured in DMSO at 40 °C. (B), (D), and (F) single absorption wavelength at 323 nm plotted against time, with a single exponential fit to determine half-life.

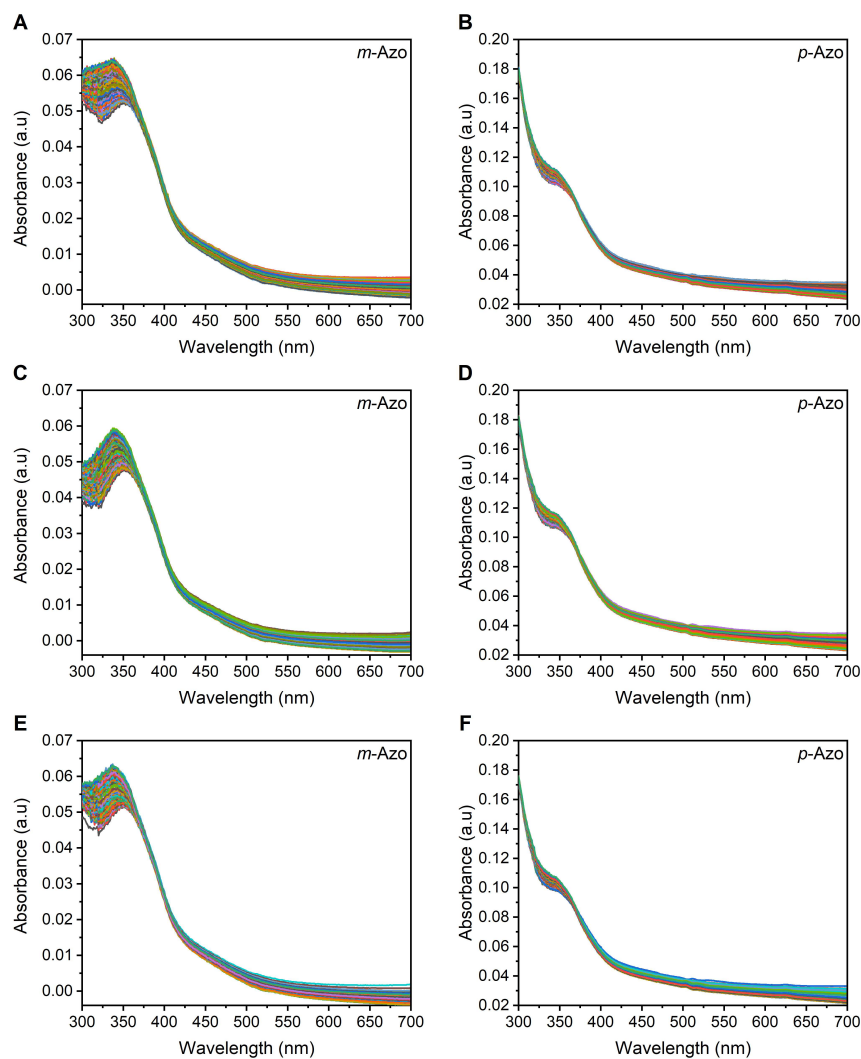


Figure S49. Non processed UV-vis spectra of thermal Z- to E-isomerization of CND-*m*-Azo (A) first, (C) second, and (E) third, and CND-*p*-Azo (B) first, (D) second, and (F) third were measured in DMSO at 40 °C.

NMR Spectra

CND

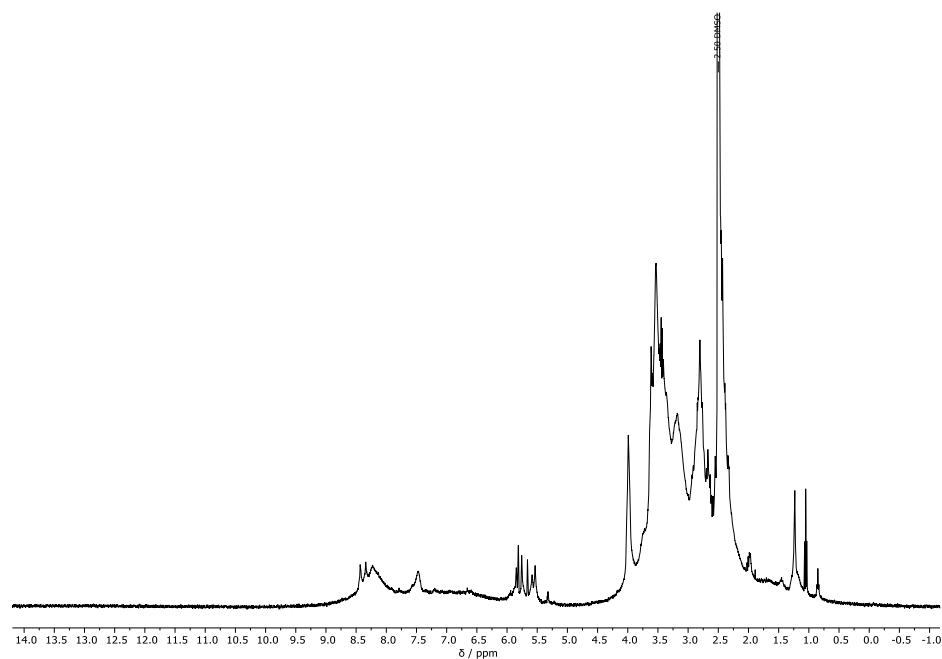


Figure S50. ¹H-NMR spectra of **CND** measured in DMSO-d₆.

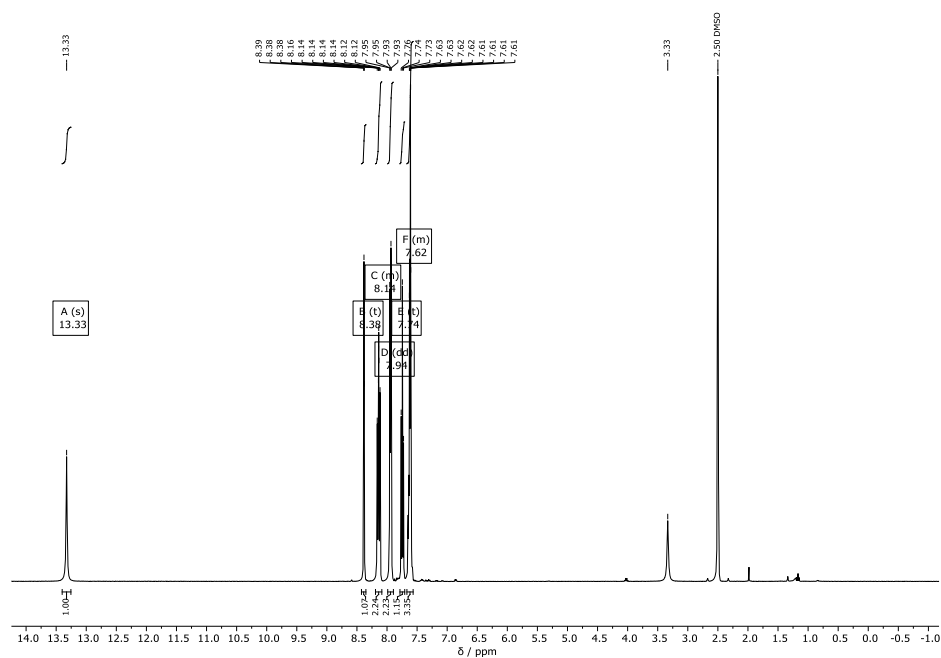
3-(Phenylazo)benzoic acid (*m*-Azo)

Figure S51. ¹H-NMR spectra of 3-(Phenylazo)benzoic acid (*m*-Azo) measured in DMSO-d₆.

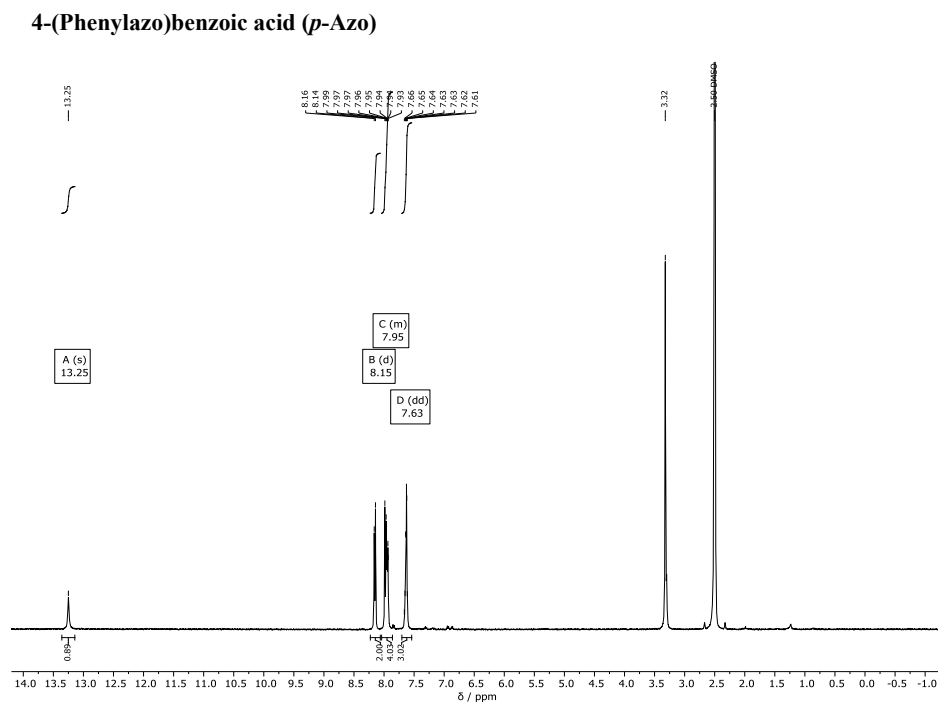


Figure S52. ^1H -NMR spectra of 4-(Phenylazo)benzoic acid (*p*-Azo) measured in DMSO- d_6 .

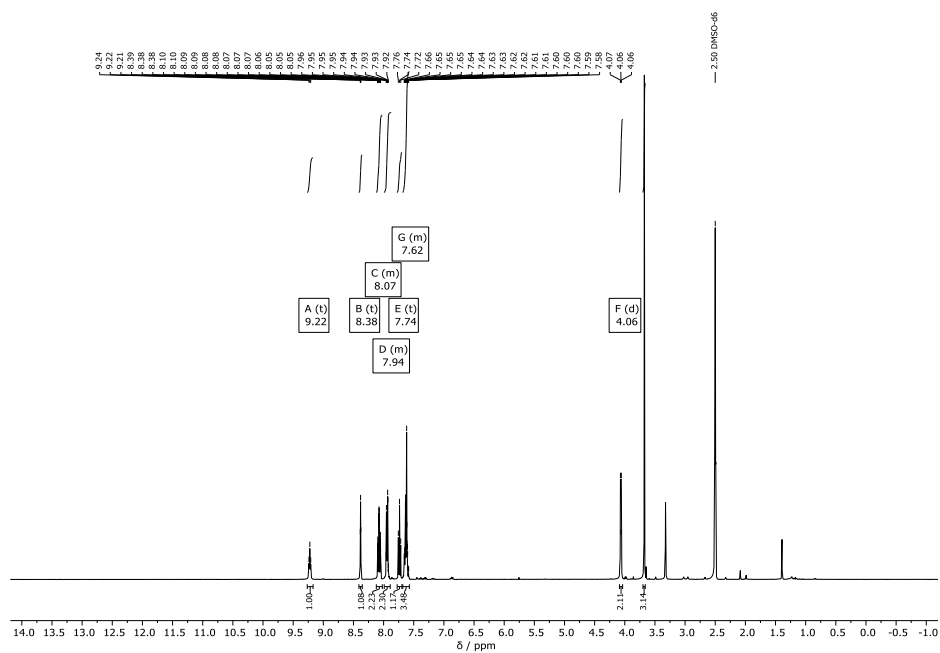
N-[3-(phenylazo)benzoyl]glycine methyl ester

Figure S53. ¹H-NMR spectra of *N*-[3-(phenylazo)benzoyl]glycine methyl ester measured in DMSO-d₆.

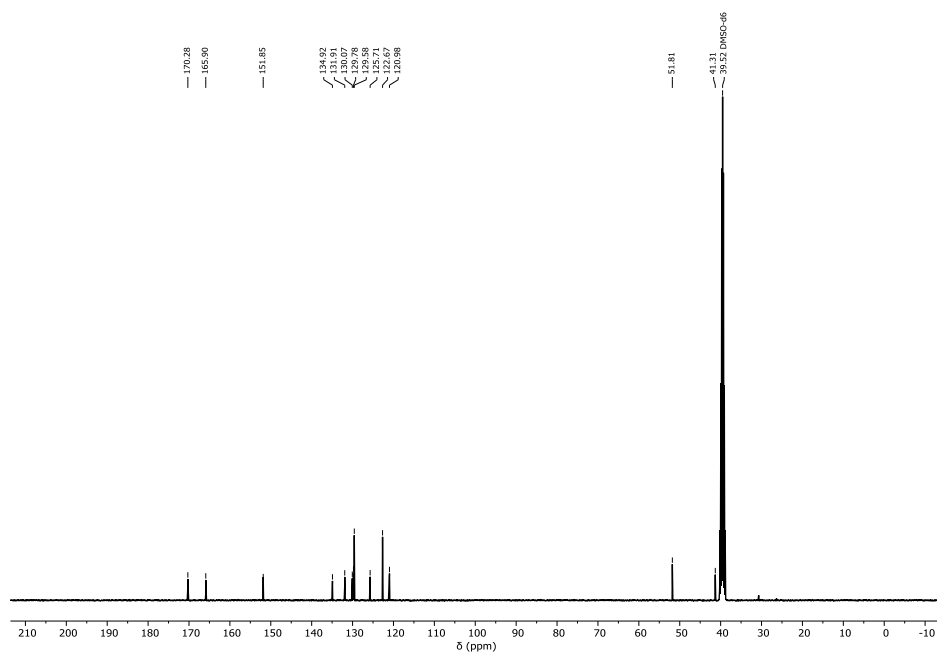


Figure S54. ^{13}C -NMR spectra of *N*-[3-(phenylazo)benzoyl]glycine methyl ester measured in DMSO-d_6 .

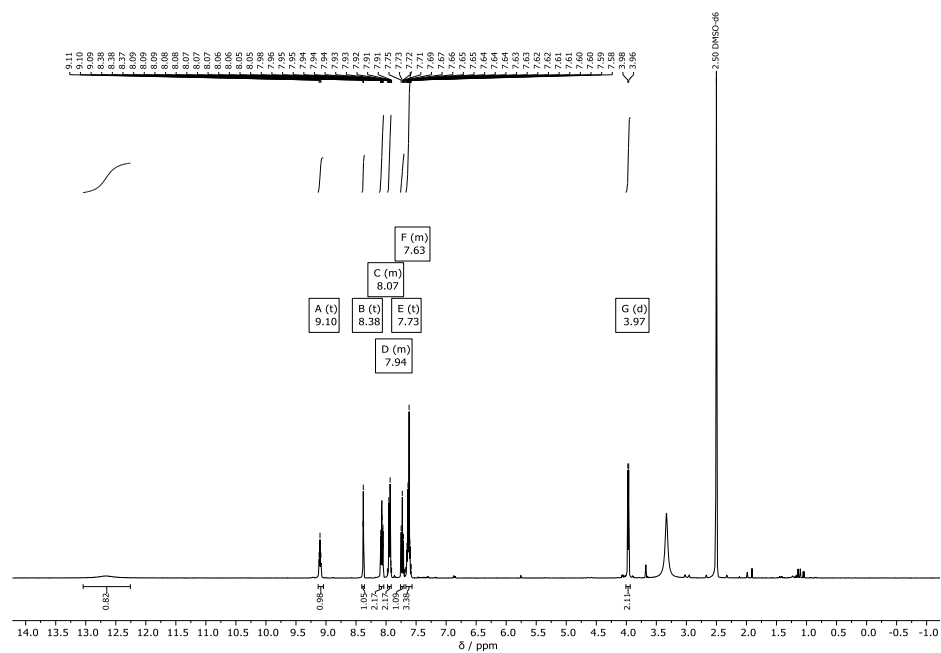
***N*-[3-(phenylazo)benzoyl]glycine (*m*-Gly-Azo)**

Figure S55. ¹H-NMR spectra of *N*-[3-(phenylazo)benzoyl]glycine (*m*-Gly-Azo) measured in DMSO-d₆.

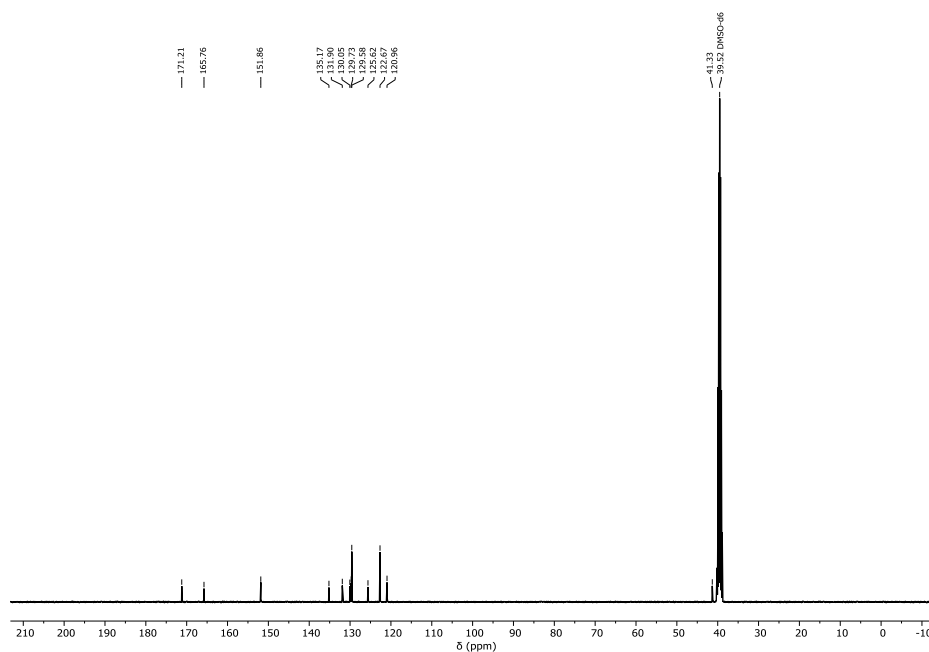


Figure S56. ^{13}C -NMR spectra of *N*-[3-(phenylazo)benzoyl]glycine (*m*-Gly-Azo) measured in DMSO- d_6 .

CND-*m*-Azo

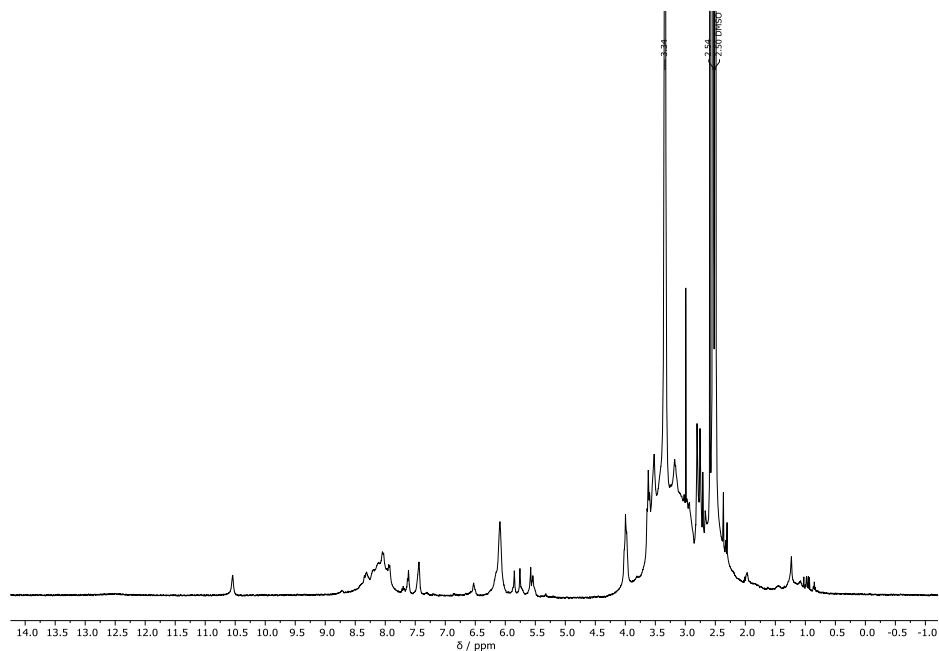


Figure S57. ^1H -NMR spectra of CND-*m*-Azo hybrid measured in DMSO- d_6 .

CND-*p*-Azo

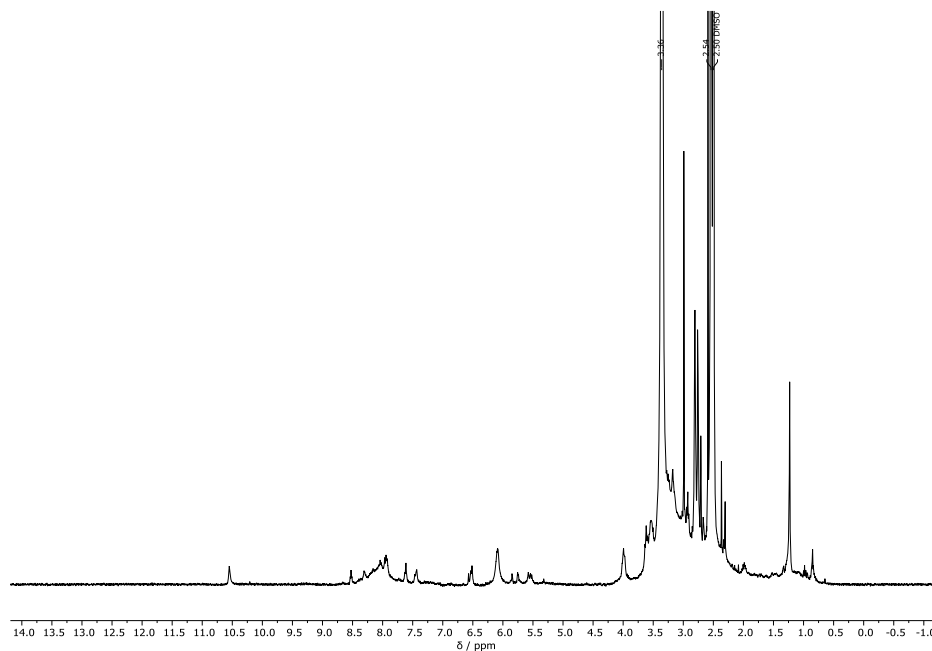


Figure S58. ^1H -NMR spectra of CND-*p*-Azo hybrid measured in DMSO- d_6 .

CND-*m*-Gly-Azo

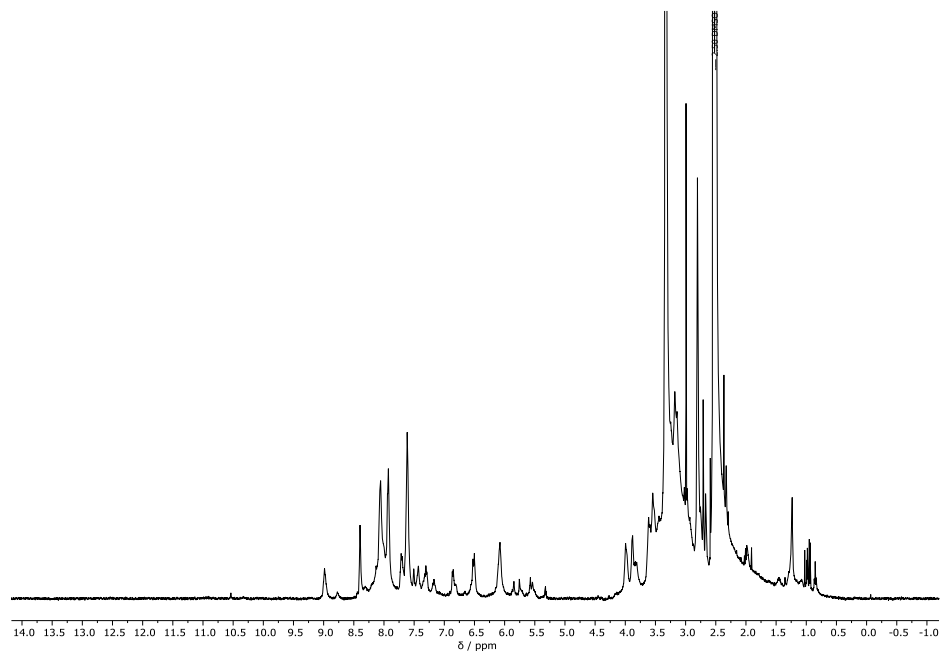


Figure S59. ¹H-NMR spectra of **CND-*m*-Gly-Azo** hybrid measured in DMSO-d₆.

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3. Conclusion and Outlook

This work presents a systematic investigation of the synthesis, surface functionalization, characterization, and application of CNDs. The investigation is supported by three representative studies that exemplify the functional versatility of CNDs. In general, CNDs represent a particularly promising class of carbon nanomaterials whose optical and chemical properties can be tailored through a combination of controlled bottom-up synthesis and targeted post-synthetic modifications. It has been shown that both the core structure and the surface chemistry significantly determine the photophysical behavior, reactivity, and integration potential in hybrid systems. A central part of this work was the quantification of surface functional groups, with particular emphasis on primary amines. A range of complementary techniques was employed in the evaluation of these groups, including the Kaiser test and quantitative ^{19}F -NMR spectroscopy. These methods provided essential insight into the chemical accessibility of the functional groups and enabled the rational design of surface modifications. The results showed that amide coupling efficiencies are not directly correlated with the apparent amine content, highlighting the importance of accessibility and molecular compatibility in surface covalent reactions. In the second study, the design of dye-functionalized CNDs and their integration into TiO_2 photocatalysts demonstrated how covalent hybrid formation could enhance dye adsorption, stability, and photocatalytic efficiency, while minimizing dye consumption. This approach provides a foundation for the development of cost-effective and durable photocatalytic systems. The third study introduced photoresponsive CND-azobenzene hybrids, which exhibit tunable charge transfer properties and photoswitching behavior, highlighting their potential for light-controlled applications in optoelectronics and biomedicine. The employment of molecular design strategies, incorporating spacers, has been demonstrated to provide enhanced control over electronic interactions and emission dynamics.

Nevertheless, challenges such as variability in composition, inadequate control over doping and surface states, and the absence of standardized protocols continue to limit broader application. Future studies should prioritize the development of robust, scalable synthesis protocols with precise control over structural parameters. These protocols should be integrated with advanced characterization techniques, such as multidimensional NMR, AFM/TEM, XRD, and quantitative surface analytics, to ensure comprehensive analysis. In the domain of applications, the design of CD-based hybrid materials stands to benefit from interdisciplinary approaches integrating materials science, photochemistry, and molecular biology. Specifically, the

incorporation of responsive or biological active moieties has the potential to unlock new capabilities in targeted therapeutics, dynamic sensing platforms, and smart interfaces. Moreover, the exploration of amphiphilic and water-insoluble CDs has the potential to reveal new applications in interfacial catalysis and membrane technologies. The long-term use of CDs as an alternative to conventional nanomaterials depends on a full evaluation of their production lifecycle. While CDs are free of toxic and scarce elements found in semiconductor QDs, current synthesis methods use energy-intensive processes, consume high amounts of solvent, and may contain harmful chemicals. To make fully sustainable nanotechnology a reality, future studies would need to report the material, solvent, and energy used in CD synthesis and purification under realistic conditions and evaluate the environmental impacts.

Overall, the results of this work demonstrate the potential of CNDs as highly functional and modular nanomaterials. Through a combination of precise surface engineering, advanced characterization, and application-driven design, CNDs are capable of making significant contributions to a variety of fields, including photocatalysis and bioresponsive nanotechnology. With continued interdisciplinary research and an increased focus on environmental responsibility, CNDs may evolve into a transformative platform for addressing global challenges in energy, health, and sustainability.

4. Literature

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