A multifunctional chemical toolbox to engineer carbon dots for biomedical and energy applications

Luka Đorđević,*[1,4,5] Francesca Arcudi,*[1,4,5] Michele Cacioppo,[1,2] and Maurizio Prato*[1,2,3]

[1]Department of Chemical and Pharmaceutical Sciences, INSTM UdR Trieste, University of Trieste, Trieste, Italy
[2]Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia San Sebastián, Spain
[3]Basque Foundation for Science, Ikerbasque, Bilbao, Spain
[4]Present address: Department of Chemistry, Northwestern University, Evanston, Illinois, United States
[5]These authors contributed equally: Luka Đorđević, Francesca Arcudi.
e-mail: dordevic.luka@gmail.com; francescarcudi@gmail.com; prato@units.it

Photoluminescent carbon nanomaterials, or carbon dots (CDs), are an emerging class of materials that has recently attracted considerable attention for biomedical and energy applications. They are defined by characteristic sizes of <10 nm, a carbon-based core, and the possibility to add various functional groups at their surface for targeted applications. These nanomaterials possess many interesting physicochemical and optical properties, including tuneable light emission, dispersibility and low toxicity. In this Review, we categorize how chemical tools impact the properties of CDs. We look for pre- and post-synthetic approaches for the preparation of CDs and their derivatives or composites. We then showcase examples to correlate structure, composition, and function and use them to discuss the future development for this class of nanomaterials.

Carbon dots (CD) is a generic term used for nano-sized photoluminescent carbon nanomaterials, an emerging subclass of nanoparticles with characteristic size of <10 nm and quasi-spherical morphology. From both fundamental and applied perspectives, the fluorescence (FL) of CDs is one of their most appealing characteristics. It was their FL that allowed the serendipitous discovery of CDs in 2004, during the electrophoretic purification of arc-synthesized single-walled carbon nanotubes.[1] In 2006, the FL was found to increase when the surface of CDs, obtained by laser ablation of carbon targets, was passivated.[2] Attaching simple organic species to the surface of CDs, such as diamine-terminated oligomeric poly(ethylene glycol), was the first example of a post-functionalization reaction. In 2008, another breakthrough was the reported synthesis of small and fluorescent carbogenic nanoparticles through the thermal decomposition of organic precursors (such as citrate salts).[3] In 2010, highly crystalline CDs were synthesized and purified from graphite, showing size-dependent blue, yellow, and red FL.[4] These pioneering studies set the stage for the next era of research, and for what is the basis of this Review: chemical tools currently available for the synthesis and post-functionalization of CDs.

In the last decade, CDs have been explored extensively, and considerable knowledge gained has placed this class of carbon-based nanoparticles as a prominent photoluminescent material (see Box 1 for comparison of their properties with molecular dyes, inorganic quantum dots and other carbon nanomaterials).[5] Substantial progress has been achieved in elucidating the factors contributing to their physicochemical properties, including their fluorescence. Currently, four kinds of FL mechanisms have been reported: (*i*) quantum confinement effects, (*ii*) defect states, (*iii*) molecular (fluorophore) states, and (*iv*) crosslink-enhanced emission states. These mechanisms have led to a classification of CDs as graphene or carbon quantum dots, carbon nanodots, and carbonized polymer dots (**Box 1**). However, these names have regularly been used inaccurately or interchangeably, and even today, discrimination between CDs remains challenging given that the contribution from the structure and the FL can be difficult to resolve. This classification is especially challenging since current synthetic methods yield carbonaceous cores functionalized with various surface groups. In this Review, therefore, we will use the term CDs to describe all quasi-spherical carbon nanomaterials, which include those with molecular-like behaviour or delocalized electronic structure and all those that range between them.[6] The classification can be based on the characteristic structural elements rather than on the FL mechanisms, i.e. amorphous (*a*-CDs), graphitic (*g*-CDs), or single-layer graphitic (*sg*-CDs). Finally, heteroatom doping information will also be included when denoting CDs by an addition prefix, e.g. nitrogen- and sulfur-doped graphitic carbon dots are denoted as *g*-N,S-CDs.

There are two main synthetic approaches for preparing CDs: top-down and bottom-up (Fig. 1a). The former uses carbon materials precursors (such as graphite), whereas the latter employs organic

monomer/polymer as starting materials. Historically, the top-down strategy was first exploited and consisted mainly of electrochemical or chemical oxidation of graphite.[7] While these approaches can yield relatively large quantities of CDs, they usually employ harsh conditions (in terms of voltage applied or chemical oxidant used), long synthetic times, and still need post-synthetic procedures to tune the optoelectronic properties. From the fluorescence perspective, oxidative cutting of carbon sources leads to more structural defects, resulting in less appealing photoluminescence properties.

Currently, the bottom-up syntheses are more popular and will be the focus of this Review. In addition to the multitude of molecular precursors available, other benefits include multiple choices of thermal treatments, quicker reaction times, and more uniform properties in the final material. The choice of precursors and synthetic procedures (i.e. pre-synthetic control) affects the physicochemical properties of CDs in terms of size, graphitization degree, surface functional groups, and doping. Nevertheless, the structural features of the precursors can be retained in the nanoparticles, allowing for some degree of predictability. Single-component, to a certain extent, and multi-component reactions enable the use of straightforward doping strategies. These include heteroatoms (examples here include boron, nitrogen, sulfur, selenium, or a combination of them) and metals (such as lanthanides).

Besides pre-synthetic control, engineering the surface composition via post-synthetic approaches is a promising way to optimize and expand the utilization of CDs (Fig. 1b). Post-synthetic strategies usually affect the surface functional groups of the CDs since they are generally inefficient in changing properties and chemical composition of the core. Exploiting their surface chemistry also prompted the development of multifunctional CD-based materials (Fig. 1c).

There are many excellent sources of information about the intricacies of CDs properties[7–11] and their applications,[12–16] as well as their progress in comparison to traditional inorganic quantum dots.[17] At first, emphasis grew very rapidly on applications that rely on their FL. However, the set of available CD properties progressively expanded, and consequently, their applications did as well. In parallel, translating the advances in surface chemistry into more sophisticated design schemes played a crucial role in improved performances.

In this Review, we focus on the available chemical strategies to prepare CDs with a particular structure or property, highlighting the relationship between chemistry and performance. Since CD research has attracted the attention of multiple biological and energy-related scientific fields, we focus on the critical advancements from the applications point of view. In this way, we aim to demonstrate that mastering the chemistry of CDs is a crucial step for the further development of the field. We hope to promote the available chemical tools that can be used to prepare CDs with a (relatively) defined molecular structure and with many more tuneable parameters than just the composition, size, and shape. Moreover, understanding and embracing the palette of available chemical procedures is critical to access applications. Specifically, we place emphasis on the composite (core and surface) chemical structure of CDs to achieve various functions. We work from the "inside out", considering first the pre-synthetic designs to prepare the CD core (and their surface), followed by post-functionalization reactions that tune the interface with which they interact with other (bio)molecules, (bio)materials, and organisms.

Pre- and post- synthetic control of emission properties

Before discussing the chemistry behind biomedical and energy-related applications, we discuss the tunability of CDs through representative examples that allowed tuning of their most intriguing property, fluorescence.

Bottom-up preparation of multicolour carbon dots. The field has progressed immensely since the bottom-up synthesis of blue-emitting CDs with low FL quantum yields (FLQY <10%).[7] Two main bottom-up procedures can be used to obtain nanoparticles that emit from the blue to the near-infrared region (Fig. 2a): (*i*) thermal decomposition of starting materials in a solvent-free condition (pyrolysis) and (*ii*) closed vessel heating in the presence of a solvent at a temperature higher than its boiling point (solvothermal procedure, or hydrothermal when the solvent is water). Operationally, these reactions are performed in a round-bottomed flask, autoclave, or microwave vessel. The autoclave (in an oven) is commonly used since it allows to reach high pressures; however, extra caution needs to be taken as these vessels contain a massive amount of potential energy.

Of particular interest are approaches that allow the rational preparation of CDs that absorb and emit in the red and near-infrared (NIR) part of the spectrum (Fig. 2): (*i*) using electron-rich arenes, polycyclic aromatic hydrocarbons, or conjugated polymers as precursors and (*ii*) solvothermal treatment using solvents such as dimethylformamide (DMF), which can decompose and participate in the formation of CDs. In Figure 2b-e we summarize the most promising approaches towards the preparation of CDs with controlled physicochemical and emission properties. It is evident that the latter can change due to

a variety of reasons that include size, core graphitization degree, presence, and content of heteroatoms (either in the core and/or at the surface), and the degree of the oxidation of the surface. Citric acid is a commonly used precursor to afford multicolour CDs, especially in multi-component systems, and can be used as a model to evaluate how synthetic conditions and procedures affect the CDs properties (Box 2). Ideally, a synthetic method should be able to control only one of the properties of CDs mentioned above, but such examples are rare, and at least two of these features are usually affected.

Only a few remarkable examples demonstrated that the CD optical and electronic properties are affected only by the size of the nanoparticle (Fig. 2b).[18,19] In these cases, the prepared nanomaterials show a quantum confinement effect, with their emission being red-shifted as the size of the particles increases. The key is finding the arene single-source precursor that can undergo a two-dimensional polymerization, under the utilized synthetic conditions. The said polymerization (under solvo- or hydrothermal conditions) is then controlled by parameters like reaction time, temperature or addition of catalysts (e.g. sulfuric acid). The functional groups that are not being used in the polymerization forming the core end up at the surface of the nanoparticle and are needed to aid the dispersibility of the graphitic planes. The formation of a distinctive core and a small amount of surface defects are responsible for their exceptional photophysical properties and high stability. With only few examples available right now, there are still many opportunities to develop procedures for the preparation of CDs with size-dependent optical properties. One avenue could be moving from single-source to multi-component synthesis, however this can lead to increased complexity and special care should be placed in the purification and characterization of such nanomaterials (Box 3).[20]

In other cases, multicolour CDs have optical properties affected not only by size but also by the content of graphitic nitrogen (Fig. 2c). This observation was reported either by using different (regio)isomers of the same precursor (o-, m-, or p-phenylenediamine) under the same conditions,[21] or using the same starting materials under different solvothermal conditions (changing the solvent).[22,23] In addition to these approaches, introducing additives[24,25] will also influence the polymerization and graphitization processes, as well as alter the content of heteroatoms in the final nanoparticle. For example, the addition of nitric acid in the hydrothermal conditions yielded red FL from phenylenediamine-derived g-N-CDs, which otherwise had green to yellow emission.[26] Red-emitting CDs were not obtained by using other acids (sulfuric, phosphonic, or hydrochloric acid), demonstrating that nitric acid plays a crucial role in tuning the g-N-CD properties. The amount of added nitric acid controls the degree of pyridinic and pyrrolic nitrogen, which helps the formation of the conjugated π-system. Nitric acid acts non only as a catalyst but can also participate in electrophilic aromatic substitution reactions on phenylenediamine or electron-rich π-surfaces. Interestingly, some recent work provides versatile approaches to tune the CD properties regardless of the employed precursors. Strong Lewis acids, such as zinc chloride, were reported as a pyrolysis-promoting agent and also lead to highly crystalline carbon core from a diverse array of organic precursors.[27] Similarly, aluminium chloride was found to help with the carbonization of o-phenylenediamine under solvent-free conditions into g-N-CDs with red/near-infrared emission and relatively high FL quantum yields.[28] When working with electron-rich arene precursors (such as o-phenylenediamine) special care should be taken since red-emitting molecules have been reported to form in the same synthetic conditions.[20,29]

The introduction of heteroatoms, their location, as well as the choice of heteroatom are also known approaches to tune the emission wavelength of CDs (Fig. 2d). Fractions of CDs, prepared by solvothermal treatment of citric acid and urea, were found to have a red-shift in emission as graphitic nitrogen (core) content increased, with amidic nitrogen content decreased.[30] It was also noted that doping of the graphitic plane with electron-donating elements (such as nitrogen) is responsible for the creation of mid-band energy states, causing the shrinking of the bandgap. Further doping or co-doping with other elements can also affect the emission of CDs.[31] Accordingly, doping with nitrogen and sulfur is reported to cause a red-shift emission due to the introduction of intermediate n-orbitals between the graphitic π and π* orbitals,[32] which ultimately have beneficial implications in composite materials and lead to improved photoelectrochemical performance.[31]

The oxidation degree of surface groups can also alter the emission of CDs (Fig. 2e).[33,34] Fractions of CDs, prepared by hydrothermal treatment of p-phenylenediamine and urea, were reported to have a multicolour emission.[33] Although nanoparticles with the same size (and similar size distribution) were isolated, an increase in the number of carboxylates was attributed to an increased degree of surface oxidation and related surface defects, finally resulting in a red-shifted emission. In other cases, the red-shift in emission could be ascribed not only to an increased surface oxidation but also to a higher graphitization degree.[35]

In most of the reported examples, the observation of multicolour emission is empirical. Although some degree of pre-synthetic design is originating from the precursor's choice and synthetic procedure

(including reaction time and temperature), these should be used as examples for the future design of CDs. The proper purification and characterization of CDs (Box 3) is imperative and necessary for obtaining useful information for future synthetic approaches. However, purification of multicolour mixtures is not an ideal approach and inevitably leads to decreased yield of pure-colour nanoparticles.

Post-synthetic tuning of emission. Following synthesis, purification and characterization, CDs can undergo further chemical (i.e. post-synthetic) treatments to tune their properties. The choice of the procedure will depend on the functional groups present on CDs after the synthesis. Most examples of post-synthetic tuning of the CDs colour emission have focused on top-down produced samples, since bottom-up syntheses already allow a fair window of tunability (vide supra). The procedures discussed in this section could, however, be adapted also to bottom-up produced CDs and employed for the modification of physicochemical properties. It is worth mentioning that already established chemical protocols for other carbon nanoforms[36,37] could be adapted to CDs as well.

A graphitized carbon nanoparticle can be functionalized in multiple ways (Fig. 3a).[38] For example, the carbons can react with strong electrophiles (such as aryl diazonium salts), epoxides groups can be opened with nucleophiles (such as ammonia) and the other oxygenated functional groups can be functionalized also. For example, tuning the energy levels can be accomplished by reacting the oxygenated g-CDs with o-aryldiamines, under solvothermal conditions (Fig. 3b).[39] Presumably, the α-dicarbonyls and o-aryldiamines undergo cyclocondensation reactions that lead to the formation of 1,4-diazine (pyrazine) rings, resulting in an enlarged π-conjugated system and nitrogen-doped g-N-CDs.

The abundant edge carboxylate groups can also be transformed into aromatic anhydrides, followed by imidisation (or cyclocondensation) reactions to tune their emission properties.[40,41] The aromatic anhydride edges can be obtained by refluxing oxidized graphite with oxalyl chloride (in DMF).[40] In one example, the intermediate anhydrides were first subjected to imidisation, with a benzylamine carrying an alkyne group, followed by a copper-catalysed Huisgen cycloaddition with dendrons possessing azide groups.[40] The introduction of dendrons around nanographenes greatly improves their solubility in organic solvents, and their fluorescence shows a red-shift in emission maxima and white-light emission. An alternative functionalization strategy of edge carboxylates is to react them with aniline derivatives (or other aromatic amines) to alter the bandgap by introducing n-orbitals between the graphitic π and π* orbitals (Fig. 3c).[42]

Tuning the bandgap of the nanoparticles obviously affects their emission properties, and this strategy has been successfully exploited for several applications: by tuning the conduction and valence bands, g-N-CDs could be exploited in perovskites solar cells, graphene phototransistors, photocatalytic water splitting, and CO_2 reduction reaction.[38,39] Especially in the latter case, the existence of an intramolecular Z-scheme structure (oxygenated p-type groups and n-type cyclocondensed groups separated by sp^2-hybridized carbons ohmic contact) was found responsible for their good performance.[39]

Towards energy applications

Prominent applications include CDs being used to enhance the power conversion efficiency in solar cells,[43,44] to photo-sensitize or -catalyse the conversion of solar energy into fuels (vide infra) or employing their fluorescence for the preparation of light-emitting diodes (vide infra). CDs have shown promising chemical inertness, photostability, and are considered environmentally benign. However, for their successful utilization, it is important to master how to tune their core and surface properties by improving control and characterization at the atomic level, and how these features influence their catalytic and optoelectronic properties. Post-synthetic modifications of CDs have focused mostly on the functionalization chemistry of their surface and/or on implementing them in composite materials, which has allowed their fine-tuning towards applications or has endowed them with new functionalities. Besides their fluorescence, CDs were found to possess other properties that could be exploited, such as conductivity, fast electron transfer, and electron reservoir ability. These properties make them candidates in the preparation of composite materials to enhance even further the catalytic activities of the original materials.

Graphitization and nitrogen-doping. Controlling the amorphous versus graphitic nature of the core, as well as nitrogen doping, has profound implications in the photoreduction properties of CDs, especially when their energy levels can be coupled with redox mediators[45–47] or catalysts for proton reduction or water oxidation reactions.[48,49] Photosensitizers that include carboxy-coated undoped a-CDs or g-CDs (obtained by thermal decomposition of citric acid) and g-N-CDs (prepared by pyrolysis

of aspartic acid) were compared (Fig. 4a).[48,50] Hydrogen evolution showed the following trend: a-CDs < g-CDs < g-N-CDs. This trend was attributed to the enhanced light absorption of g-CDs, due to the higher content of C=C bonds, and to the rapidly quenched holes and a higher concentration of long-lived photogenerated electrons of g-N-CDs than the undoped CDs.

Moving from single-source precursor to a multi-component approach for nitrogen doping of CDs was found to be beneficial for their photoreduction properties. Nitrogen-doped amorphous CDs (a-N-CDs), prepared by hydrothermal treatment of arginine and ethylenediamine, have shown better photoreduction properties (using methyl viologen acceptor probe) compared to the g-N-CDs mentioned above. This behaviour is presumably due to the presence of molecular fluorophores that lead to higher FLQY and longer FL lifetimes.[51,52] Further benefits of the multi-component synthesis were shown by introducing redox-active precursors, such as quinones, together with arginine and ethylenediamine precursors.[51] The insertion of quinone-based motifs in the CD structure resulted in enhanced light absorption and tuneable electrochemical levels. Furthermore, these quinone-based a-N-CDs were shown to possess improved photoreduction properties compared to either a-N-CDs and g-N-CDs, probably due to a combination of the presence of molecular fluorophores and more efficient electron transfer events mediated by the quinone derivatives.

Usually, CDs are used as photosensitizers, so it is remarkable when they can function as all-in-one photocatalysts in water.[53,54] Nitrogen-doping, including the type of nitrogen present, is critical (Fig. 4b).[55,56] One remarkable example is a series of g-N-CDs prepared by the pyrolysis (microwave-assisted) of citric acid and variable amounts of branched polyethyleneimine.[54] The highest H_2 generation was observed for the g-N-CDs with the highest nitrogen content. For the intrinsic capacity of reaction centres in the g-N-CDs to be responsible for the H_2 evolution reaction, without any additional co-catalysts, the presence of various types of amines is crucial. Pyrrolic and pyridinic nitrogen fragments are believed to enable efficient electron transfer and act as catalytically actives sites, respectively. In addition, the alkylamines, which originate from branched polyethyleneimine and are present on the surface, act as hole traps that facilitate charge separation and hole transfer. Another example, in which simultaneous H_2 and O_2 production was reported, consists on g-N-CDs photocatalysts for water splitting in pure water (Fig. 4c).[57] These g-N-CDs were obtained via top-down chemical oxidation of nitrogen-doped graphene and their water-splitting photocatalytic activity was ascribed to the combination of n-type and p-type domains, originating from N-doped graphene and oxygenated surface groups, respectively. Finally, the C(sp^2) planes are necessary junctions between the two domains and act as recombination sites for most of the carriers.

Heteroatom- and metal-doped carbon dots. Introducing heteroatoms, other than nitrogen, can be beneficial for preparing CDs as metal-free alternatives for catalysing the oxygen reduction reaction, which plays an important role as renewable energy technology from fuel cells to metal−air batteries.[58–60] One example of preparing new CDs as electrocatalysts is the modified electrochemical etching of graphite rods, by employing different amounts of phosphoric acid, which resulted in a series of g-P-CDs.[58] The oxygen reduction reaction activity was shown to increase with higher contents of phosphorus in g-P-CDs and to proceed through the direct four-electron pathway. Similarly, boron doping in g-CDs could be easily introduced by adding boric acid in the hydrothermal reaction mixture.[61] The presence of boron atoms could lead to adsorption and subsequent reduction of O_2 and therefore catalyse the oxygen reduction reaction.

Metal atoms can also be rationally introduced in the CDs structure, and their presence can be taken advantage in photocatalysis, for example.[62,63] By the one-step pyrolysis of the ethylenediaminetetraacetate-CuNa$_2$ complex, it was possible to obtain g-Cu,N-CDs.[62] The photocatalytic abilities, in terms of a photooxidation reaction, showed higher reaction yields for the g-Cu,N-CDs compared to the g-N-CDs without copper. Finally, experiments were performed to confirm the good electron acceptor and donor abilities, as well as strong conductivity of g-Cu,N-CDs.

Surface functional groups for catalysis. In catalysis, especially organic (photo)catalysis, the surface functional groups and their chemistry play an important role. Graphitic CDs, obtained by chemical etching of graphite rods, were exploited as heterogeneous (nano)catalysts for chemical reactions under visible light irradiation.[64,65] Electrochemical oxidation in ultrapure water yielded graphitic nanoparticles with a surface rich in hydroxyl, carbonyl, and carboxyl groups.[65] Visible light irradiation of g-CDs was found responsible for the generation of protons, which was exploited for a series of reactions that undergo acid catalysis (esterification, Beckman, and aldol condensation). Control experiments with chemically reduced g-CDs confirmed the importance of surface groups for their photocatalytic properties.

Understandably, CDs have been mostly exploited for their photocatalytic properties, although their use in chemical reactions has also been investigated.[66] However, very few studies have shown the benefits of using CDs over molecular, polymeric, or heterogeneous organocatalysts, as they should bring together homogeneous catalysis, heterogeneous separation, and CD recycling.[67] In one such example, the surface chemistry of CDs was used for studying their reversible phase transfer and catalysis of the Knoevenagel reaction (Fig. 4d).[68] A series of amine-terminated g-N-CDs were prepared by pyrolysis of citric acid and various alkyl diamines. After the CD-catalysed reaction between malononitrile and 4-bromobenzaldeyde in organic solvent is completed, the CDs could be transferred to the aqueous phase by bubbling CO_2, and the precipitated product (now suspended in the organic phase) could be filtered out. Furthermore, after the addition of new reactants to the organic phase and the removal of CO_2, the same reaction could be repeated.

Multicolour carbon dots in catalysis and devices. Decreasing the bandgap in CDs is important for enhancing their light absorption and harvest as many visible light photons as possible. Multicolour CDs for photocatalysis have been prepared either by top-down or bottom-up methods. Top-down synthesis usually involves electrochemical oxidative cutting of graphite rods or chemical treatment of graphene oxide, followed by fractionation using silica gel column chromatography[4] or membranes with different molecular cut-offs.[69] The obtained graphitic CDs are reported to have optical properties affected by their size, ascribed to the quantum confinement effect. These can be used either to form electron/hole pairs with TiO_2 (or SiO_2) nanoparticles for the generation of active oxygen radicals under light or to photosensitize proton reduction catalysts for H_2 generation.[4,69]

The development of synthetic protocols for the preparation of multicolour nanomaterials has been an active area of research for the preparation of light-emitting diodes (LEDs), with CDs employed either as phosphors or as active emission layers. As discussed in Box 2, citric acid is a common precursor in the multi-component solvothermal synthesis of multicolour CDs. Solvothermal treatment of citric acid and urea in DMF was found to afford g-N-CDs with orange emission, which could be combined with starch (to decrease aggregation-induced quenching of the g-N-CDs in the solid-state) and used to prepare warm white LEDs.[70] Multicolour emitting g-N-CDs could also be prepared by adjusting the ratio of citric acid and urea while changing the temperature of the solvothermal treatment in DMF, with higher temperatures and higher ratios of urea leading to red-shifted emission.[35] In this way, blue, green, and red, as well as white LEDs, could be prepared by dispersing the g-N-CDs in an epoxy matrix and using them as phosphors.

Replacing urea with diaminonaphthalene (either the 2,3- or 1,5-regioisomer) in the citric acid-based bottom-up synthesis was found to afford g-N-CDs with multicolour bandgap emission under different solvothermal conditions.[71] Diaminonaphthalene, considered one of the smallest $C(sp^2)$ domains and a nitrogen-doping source, was critical for obtaining CDs with high surface passivation, a high degree of crystallinity, uniform, and narrow size distribution, along with red-shifted emission arising from quantum confinement effects. These high-quality nanoparticles were exploited for the preparation of monochromatic LEDs from blue to red, using CDs as the active emission layer.

Employing single-source aromatic precursors was also shown to be an effective strategy for preparing g-CDs with red-shifted emission. Solvothermal treatment of N,N-dialkyl-p-phenylenediamine in DMF was found to afford red-emitting g-N-CD with high FLQYs.[72] During the solvothermal synthesis, the deamination of −NH$_2$ functional groups led to the formation of C−C bonds, presumably followed by dehydrogenation and dealkylation reactions, resulting in the formation of a nitrogen-doped π-conjugated structure and −NR$_2$ passivated surface. These g-N-CDs further showed good solubility in organic solvents for electroluminescent warm white LEDs. Another example of single aromatic precursors was discussed earlier, with 1,3,5-trihydroxybenzene heated in ethanol (200 °C) for different reaction times and with(out) addition of sulfuric acid.[19] The resulting g-CDs were triangular in shape, with their size determining the emission wavelength, exhibited extremely narrow absorption and emission peaks, with ultrasmall Stokes shifts and high FLQYs.

Chemical transformation of surface functional groups. The carboxylate functional groups present on the edges of nanoparticles can undergo esterification or amidation reactions through established protocols. These carboxylates can be activated by using carbodiimide chemistry or transformed into acyl chlorides (by oxalyl or thionyl chloride), or thermally treated in the presence of amines or anilines. The introduction of new molecules on the CD surface usually leads to changes in physicochemical properties determined by the former, such as switching the surface charge, inducing a change of solubility, adding polymerizable monomers, or introducing n-orbitals (thus reducing the bandgap). Examples of such changes, as well as their relative importance, are outlined below.

Amidation reaction from carboxylate-coated to ammonium-terminated g-CDs did not change the optical properties of the nanoparticles and was used to switch their surface charge, which was shown to greatly enhance their interaction with enzymes and made the photoelectron transfer more efficient (Fig. 4e).[45] Carboxylate coated g-CDs, obtained by pyrolysis of citric acid, were converted to acid chlorides by heating in neat thionyl chloride, and finally allowed to react with neat N,N-dimethylethylenediamine. Hydrophilic to hydrophobic switch of the nanoparticles can be achieved by the reaction between the activated carboxylates and alkylamines. Surface carboxylates usually impart water-solubility/dispersibility to CDs and, to avoid their aggregation in non-polar solvents and allow fabrication of devices, they were decorated with long alkyl chains.[73,74]

Esterification, or even amidation reactions, with molecules capable of polymerization is an interesting approach, especially if a conjugated polymer is obtained.[75] One example involved the use of oxidized sg-CDs,[40] the microwave-assisted conversion of the carboxylic acids to acyl chlorides by oxalyl chloride, followed by esterification with hydroxymethyl-derivative of 3,4-ethylenedioxythiophene (Fig. 4f).[75] The successful functionalization was followed by electrochemical polymerization, and the resulting films showed that the capacitance of the hybrid is higher compared to the electropolymerized thiophene alone, leading to a promising material for energy storage applications.

Other oxygenated surface groups, like aldehydes, can be used to form dynamic covalent imine bonds and can be exploited for the formation of fluorescent self-healing gels.[76] Multicolour g-CDs were cross-linked through the Schiff base reaction with primary amine residues of the polyethyleneimine polymer, and the resulting gel did not show aggregation-induced fluorescence quenching of the CDs.

Carbon nanoparticles that have surfaces rich in amino-groups[10] can follow similar post-functionalization schemes, e.g. with moieties carrying carboxylic acids. In addition to the carbodiimide coupling for amidation reactions (Fig. 4g),[77,78] alkylation[79] and imidization[80] reactions were also employed. Functionalization of the CD surface with electron-donors or -acceptors enables formation and study of charge-transfer processes.[78,80,81]

Surface oxidation and reduction modification reactions. Oxidation and reduction post-synthetic modifications were found to greatly alter the surface structure of CDs, which ultimately impacted their fluorescence and applications.[82,83] For instance, refluxing g-N-CDs in nitric acid can lead to an oxygen-containing amorphous structure around the carbon core.[84] As discussed earlier, this leads to more surface traps that shift the emission at longer wavelengths and decrease FLQY. Oxidation, on the other hand, was found to increase the electrochemiluminescence (ECL) intensity of CDs, presumably because the shell can now produce radicals, with their annihilation leading to the formation of excited states. Furthermore, the presence of carboxylates on the surface can be used to bind and detect Cu(II) ions through the decrease of the ECL emission intensity. If oxidation reactions lead to more surface traps, lower and red-shifted emission, reduction reactions should have the opposite effect. Indeed, g-N-CDs (prepared through the solvothermal treatment of citric acid and 1,5-diaminonaphthalene) that underwent surface reduction (in ammonia and hydrazine hydrate) were found to have narrower absorption and emission peaks, with higher FLQY and blue-shifted emission.[85] These g-N-CDs were then used to prepare deep-blue LED devices and showed to outperform even devices based on Cd(II)/Pb(II)-comprising materials.

Incorporation of carbon dots in (in)organic matrices. Embedding or immobilizing CDs into polymeric or porous matrices has become an established strategy to prepare composite materials,[86–88] especially for their use in optics, catalysis, and energy storage. It is important for CDs to act not only as fillers but to participate, and possibly cooperate, with the matrix to achieve improved performances.

Graphitized CDs that possess good thermal and chemical stabilities could be mixed with (in)organic precursors to embed them in a composite material.[89,90] In a pioneering report, g-N-CDs were mixed with urea and thermally treated to prepare a composite with carbon nitride (C_3N_4), a known photocatalyst for the generation of H_2 and H_2O_2 from water.[91] With the g-N-CDs present, the composite could decompose the H_2O_2 produced by C_3N_4, and the final result was a production of H_2 and O_2 (in 2:1 ratio). Notably, O_2 was not detected when irradiating a simple physical mixture of g-N-CDs and C_3N_4, showcasing the importance of the composite material. When implemented in a composite material comprising an electrocatalyst for the hydrogen evolution reaction (e.g. Co_3O_4) and an electrocatalyst for CO_2 reduction to CO (e.g. C_3N_4), g-CDs triggered both reaction channels by generating active hydrogen. The resulting three component electrocatalytic system provided tuneable, stable, selective and efficient production of syngas.[92] In another demonstration, g-CDs improve the activity of a metal-free composite catalyst for photocatalytic oxygen reduction to produce H_2O_2 in seawater.[93] Interestingly, the functional groups of CDs are ionized in presence of metal ions in seawater, which enhances the electron sink effect of CDs on electrons.

Procedures for the preparation of composite materials can take advantage of the CD chemical stability. Graphitic CDs could be incorporated into organic polymers, through radical polymerization of methacrylate monomers, and were found to be helpful in self-healing and improving the anticorrosion properties of the polymer matrix.[94]

Carbon nanoparticles can also be mixed with metal precursors for the preparation of organic-inorganic matrices. Among the various possible inorganic matrices, silica gel and organically modified silicates were used to embed CDs (due to their optical properties and stabilities), which allow their easy application in the fields of photonics and optoelectronics.[95] Additionally, the inorganic matrix is one of the most used options to avoid the aggregation-induced FL quenching of CDs.[96] Embedding the nanoparticles in silica-based matrices can be accomplished through the hydrothermal treatment of g-CDs in the presence of silica or by synthesizing silane functionalized CDs for the preparation of gels to be used as phosphors.[97,98]

Various other inorganic components (such as metals oxides, mixed transition metal oxides, ultrathin two-dimensional materials, or colloidal nanoparticles) were combined with CDs to improve the functionality of the former. Nitrogen-doped graphitic CDs and $RuCl_3$ (as a less expensive alternative to platinum) were combined and hydrothermally treated in an autoclave, followed by thermal treatment under inert gas.[99] The corresponding composite, made of g-N-CDs and ruthenium nanoparticles, showed an electrocatalytic hydrogen evolution activity superior and more durable than that of commercial Pt/C in alkaline solutions. Similarly, g-CDs could also be combined with nickel acetate and ferrous nitrate to afford a composite with nickel-iron layered double hydroxides after solvothermal treatment.[100] The electrochemical oxidation of water showed highest current and earliest onset for the composite when compared to the two components alone. While the electrocatalytic activity is primarily attributed to the inorganic component, it is further enhanced by g-CDs due to their conductivity, fast electron transfer, and electron reservoir properties. Likewise, hydrothermal treatment of g-N-CDs, nickel nitrate, and cobalt nitrate could afford (CDs/$NiCo_2O_4$) composites used as supercapacitors.[101] Excellent capacity performances and cycling stabilities were attributed to the CDs, possibly facilitating ion diffusion and electron transport while suppressing the dissolution and agglomeration of the inorganic component.

The oxygen-rich surface of g-CDs was also used to complex various metal ions, the resulting metal complex was then assembled with carbon nanotubes (through π-π interactions) and finally thermally treated under ammonia/argon.[102] This procedure was found to yield single-atom catalysts on carbon support that exhibit high and selective CO_2 to CO electroreduction.

Preparation of composites through hydrothermal treatment. Another common approach for the preparation of composites is the thermal treatment of g-CD in the presence of other organic or inorganic components, used to establish covalent bonds between the two components of the composite material.[103] In the case of g-CDs and colloidal silica, the formation of covalent bonds by a hydrothermal treatment was responsible for a long afterglow lifetime.[104] At room temperature, the major component of the photoluminescence was found to be from room temperature phosphorescence and was ascribed to the insensitivity of the composite to water and oxygen. Room temperature phosphorescent materials could find applications in safety encryption applications,[105–107] where the combination of CDs with (in)organic materials is one successful approach.[108–111]

Another example of a common inorganic matrix used with CDs is titanium oxide, with the former used to improve some drawbacks of the latter, like the large bandgap (UV light needed) and fast recombination of photoexcited electrons and holes (Fig. 4h). The hydrothermal treatment between g-CDs and TiO_2 nanoparticles was found to greatly improve the photo-response and photocatalytic properties of the single materials, also compared to the samples made of a physical mixture of the two components.[112]

After the hydrothermal treatment, needed for the formation of covalent bonds, sometimes it is still necessary to anneal the composite to improve the conductivity and connectivity between the two components. This approach was exploited for their electrocatalytic activity in the oxygen reduction reaction, either to improve the activity of sulfur-doped C_3N_4 with g-CDs[113] or to introduce catalytic sites with boron-doped CDs in composites with graphene oxide.[61]

Towards biomedical applications

Low toxicity, hydrophilicity, stability, and biocompatibility play a pivotal role in the biomedical utilization of CDs, but there is still the need for systematic studies at the nano-bio interface. Other influential properties for bio-related application are the CD absorption and fluorescence. In the early stages, nearly all CDs exhibited blue FL emission with FLQY <10%. During the last years, tremendous efforts have

been directed towards improving the optical properties within the therapeutic window. Advances in controlling surface oxidation, core graphitization, and doping promoted bioimaging capability and therapeutic applications of CDs. The substantial progress in producing CDs with specific features and tailorable surface composition has stimulated research for post-synthetic strategies targeting sophisticated architectures. Composite materials can be engineered to tailor CDs features for desirable light-absorbing applications and to access therapeutic properties absent in the individual units.

Approaches towards red- and NIR-light-responsive carbon dots. High absorption and strong fluorescence in the red-to-NIR region are important to fulfil the requirements for efficient in vivo bioimaging and phototherapy (Fig. 5a). Extending the conjugation of C(sp^2) domains boosts the efficiency of CDs in photothermal therapy (PTT) and introduces multiple imaging capabilities. Using polycyclic aromatic hydrocarbons as precursors is a viable strategy to achieve CDs with extended C(sp^2) conjugated structure. However, pursuing favourable reaction pathways is key because polycyclic aromatic hydrocarbons are highly stable and their fusion into nanomaterials usually requires high temperatures. g-N-CDs with emission at ca. 590 nm have been prepared via the radical reaction between acetonitrile and sodium naphthalene at room temperature and they have been reported as in vitro and in vivo FL bioimaging agents.[114] To prepare CDs at accessible temperatures (around 200 °C), the polycyclic aromatic hydrocarbon precursor can be designed to be more reactive toward the condensation reaction. In the case of the pyrene, this can be achieved with the nitration at the 1,3,6 positions to promote substitution reactions.[115] g-N-CDs were prepared by the hydrothermal treatment (at 220 °C) of 1,3,6-trinitropyrene and a branched polyethyleneimine polymer. g-N-CDs showed a graphitic-like structure with abundant graphitic nitrogen functionalities, which contributed to their broad optical absorption from visible light to NIR and PTT ability in vivo.

Polymers are alternative precursors for obtaining large conjugated C(sp^2) clusters. Photostable and water-dispersible red-emitting g-S-CDs were prepared via the hydrothermal treatment of polythiophene phenylpropionic acid and were used for imaging-guided PTT in vivo.[116] Combining polymers with doping agents in a two-components synthesis creates opportunities to access CDs with NIR optical properties. For example, N-doped poly(3-alkylthiophene) and diphenyl diselenide were used as precursors for g-S,N,Se-CDs with NIR emission in one- and two-photon excitation modes and PTT ability.[117] Another strategy to alter the CD imaging properties is to exploit precursors with NIR-emissive properties. For example, introducing Nd and Tm doping elements in the crystalline graphene lattice[118] or using an organic NIR chromophore as precursor endowed the CDs with NIR fluorescence.[119]

From a clinical imaging and therapeutic viewpoint, CDs also require rapid excretion from the body via renal filtration, the ability to accumulate within the tumour after intravenous injection to prevent the spreading of cancer cells to other parts of the body, and the use of relatively low irradiating powers to prevent tissue damage. An illustrative example are g-N,S-CDs prepared by the solvothermal treatment of citric acid and urea in dimethyl sulfoxide, which exhibited all the properties described above, efficient NIR imaging and PTT ability in vivo.[120]

While there is a growing awareness that the graphitization of the core promotes CD properties in the NIR window, complementary surface engineering strategies can have a critical impact. For example, a high degree of surface oxidation provided CDs for efficient FL bioimaging in the NIR-II region and in vivo thermal ablation of cancer.[121] Alternatively, introducing electron-donor or electron-acceptor moieties on the CD surface can amplify the bioimaging ability and extend the functional window to the NIR region via multiphoton excitation optical properties. Recent work showed that CDs with donor-π-acceptor architectures, within a C(sp^2) conjugated aromatic system, have large multiphoton absorption cross-section and multiphoton excitation upconversion fluorescence properties. In order to do this, red emissive g-N,F-CDs were prepared via a solvothermal approach where citric acid was employed as the precursor for the carbon core, while urea and ammonium fluoride were used as the N and F sources.[122]

Similar efforts have been pursued towards CD photosensitizers that undergo type I (electron transfer) or type II (energy transfer) reactions to produce radical and radical anion species or singlet oxygen, respectively, for photodynamic therapy (PDT). In one of the earlier work in the field, red-emissive polythiophene-derived CDs (g-N,S-CDs) were reported as photosensitizer for PDT with excellent performances.[123] However, CDs that function as NIR light-triggered photosensitizers can afford higher penetration depths and lower phototoxicity to normal tissues. A possible strategy to perform PDT upon NIR irradiation is by using the two-photon excitation technique, in which a NIR-pulsed laser is used as the excitation light source.[124] It was demonstrated that g-CDs with large two-photon absorption efficiently generate $^1O_2$ under the two-photon excitation mode,[125] but more work is expected to catch up with other photosensitizers.

Further advancements would also require an improved understanding of how synthetic strategies translate to structural features that promote preferential PDT pathway (either type I or type II), in addition

to high PDT efficiency. This is especially important because most existing PDT systems based on CDs operate with a type II pathway, in which the production of $^1O_2$ is oxygen-dependent. The PDT efficiency in type II is undermined by the reduced partial oxygen pressure in tumour tissues and by the limited diffusion of reactive oxygen species (ROS) inside the cells. Therefore, a strategy to minimize the oxygen dependence of the PDT efficiency is to control the composition and structure of CDs to work under the type I pathway. For example, a recent work correlated the efficient production of $O_2^{·-}$ to the highly crystalline structure of g-N,Cl,Zn-CDs.[27] g-N,Cl,Zn-CDs were prepared by introducing $ZnCl_2$ in the reaction mixture and exhibited sufficient conduction band energy, following light irradiation, to transfer the excited electron to molecular oxygen and produce $O_2^{·-}$.

An alternative approach to boosting the therapeutic efficiency involves the combination of PDT with PTT, with the latter being oxygen-independent phototherapy. Red-emissive g-S-CDs that integrate PDT and PTT functions were prepared by the hydrothermal treatment of polythiophene phenylpropionic acid.[126] A possible strategy to improve the NIR absorption feature of CDs for NIR dual-mode theranostic applications is doping with transition metals. For example, the doping with copper can enhance the NIR absorption of CDs and promote NIR-mediated PTT/PDT.[127] Alternatively, metal-free g-N,S-CDs for synergistic PDT and PTT were prepared through the hydrothermal method by using 1,3,6-trinitropyrene and sodium sulfite as precursors.[128]

Carbon dots for magnetic resonance imaging. Doping is a practical strategy to impart magnetic resonance imaging (MRI) capabilities to CDs. The most used approach consists of incorporating paramagnetic Gd(III) ions in the CD structure. Synthetic approaches that span from hydrothermal synthesis (with common precursors such as citric acid, amines, and, eventually, the aid of small chelating agent) to calcination or template-assisted synthesis have been explored.[11,129,130] The major limitation of the earlier work was the short emission wavelength of CDs. However, the field has seen rapid advances that include developing CDs with multimodal imaging functions and, eventually, their combination with therapeutic functions for an all-in-one theranostic tool or preparing metal-free CDs.

Co-doped CDs with multiple imaging capabilities aim at coupling the strengths of single modalities in terms of resolution, sensitivity, and information provided. For example, Gd,Yb-CDs exhibited simultaneous MR and computed tomography contrast properties by combining the spatial resolution of the former and the sensitivity to electron-dense areas of the latter.[131]

The combination of MRI imaging components with therapy functionalities is an appealing target for the clinical translation of CDs. In a representative example, g-N,Gd-CDs, prepared by solvothermal reaction using p-phenylenediamine and $Gd(NO_3)_3$ as precursors, performed as FL/MRI imaging probe for in vivo imaging-guided PDT with a larger r_1 relaxivity value than clinical Gd-diethylenetriamine pentaacetic acid (Magnetovist®) contrast agent.[132] Besides the MRI imaging ability, g-N,Gd-CDs efficiently produced $^1O_2$ inside the cells under photoirradiation, presumably because the strong spin-orbit coupling at the Gd centre enhanced the intersystem crossing, thereby improving the ROS production.

More recent efforts have been directed towards the synthesis of metal-free CDs with MRI contrast properties. One strategy is to introduce magnetic properties with vacancies and boron doping. For example, g-B-CDs with magnetic properties were prepared using 4-vinylphenylboronic acid and boric acid as precursors and served as efficient FL/MRI imaging contrast agent in vivo with a larger r_1 relaxivity value than Gd-diethylenetriamine pentaacetic acid.[133]

Another emerging strategy to impart MRI capabilities in metal-free CDs is to exploit natural chemical exchange saturation transfer (CEST) MR contrast properties (Fig. 5b). Recent work demonstrated that the abundant exchangeable protons from the surface functional groups of g-N-CDs are inherently CEST-MRI-detectable.[134] g-N-CDs exhibited appreciable CEST-MRI contrast, which was attributed to the abundant exchangeable hydroxyl and guanidinium protons on the CD surface derived from the glucose and arginine precursors. This example points to further advances of CDs as CEST-MRI contrast agents because the variety of functional groups commonly found on the CD surface usually contains exchangeable protons, and the nature of these groups can also be easily controlled through pre-synthetic design.

Control of surface functional groups. The functional groups on the CD surface can also be designed to trigger selective biological interactions or impart specific properties (Fig. 5c). The choice of the precursors is crucial to develop CDs with structural motifs capable of stimulating specific biological responses. For example, CDs with abundant carboxylate and amino groups were prepared from ascorbic acid and polyethyleneimine.[135] These surface functional groups camouflage CDs as cystine knots, which are hallmarks of ligands able to recognize the receptors of the transforming growth factor-β. As a result of this targeting ability, CDs promoted wound healing. In another example, large amino

acid mimicking *g*-N-CDs were prepared through the hydrothermal treatment of 1,4,5,8-tetraminoanthraquinone and citric acid.[136] About eight amino acid groups decorated the edge of each CD, which displayed a crystalline core with a large π-conjugated structure. The multiple paired α-carboxyl and amino groups on the CDs surface triggered multivalent interactions with the large neutral amino acid transporter 1, which is highly expressed in a wide variety of tumours. *g*-N-CDs showed an unprecedented high degree of tumour specificity and penetrability in vivo that is affected by CD precursors.

The surface of CDs can be tailored to afford chirality, a property that broadens their bio-related application. A common method to prepare chiral CDs is to use a chiral molecule as precursor, which is often an amino acid. L- or D-CDs obtained from L- or D-cysteine, respectively, were found to exhibit interesting properties, such as two-photon absorption[137] or enzymatic enantioselectivity,[138] and their chirality was also found to influence cellular glycolysis.[139] The synthetic conditions are especially important because the chiral information can be lost at the high temperatures usually employed to synthetize CDs. For example, the hydrothermal synthesis at 180 °C using L-arginine as precursor afforded achiral CDs, and chiral CDs can be prepared in a multi-component approach when using a chiral diamine ((*R*,*R*)- or (*S*,*S*)-1,2-cyclohexanediamine) that retains chirality at the reaction temperature.[140]

Surface functional groups are known to affect the ECL properties of CDs, which are relevant when exploring biosensing applications. CDs can serve as either co-reactants or luminophores. Alkylation reactions, in particular, were able to transform the primary amino groups on the CD surface into tertiary ones, without notable changes in their optical and physicochemical properties.[79] However, the modified tertiary amino-capped *a*-N-CDs were able to increase the ECL response, by a factor of ten, when tested as co-reactants to generate the excited state of the $Ru(bpy)_3^{2+}$ luminophore. Interestingly, recent work showed that both pre- and post-synthetic approaches enhance the ECL emission from CDs.[141] The surface-emitting states correlate with the ECL efficiency, but the states of the core influence the ECL emission, thus suggesting that electron and hole wavefunctions can interact strongly with the surface.

CDs inspired also research for their use as antibacterial nanomaterials. In the quest for nanoparticle-based antibacterial agents, which have the potential to overcome the clinical issues associated with antibiotic-resistant bacteria, the high biocompatibility and the metal-free nature of CDs are especially appealing when compared to silver, copper, iron, zinc, and titanium oxide nanoparticles. The control of the surface chemistry of CDs is key to enhance interactions with bacterial membranes. For example, CDs with a positively charged surface caused the collapse of the cell membrane without the need for external activation.[142]

The fine tuning of the CDs surface charge, together with other properties, controls both their interactions at the nano-bio interface and their biological fate. Understandably, CDs conform to most of the rules that have been established for other nanoparticles, such as inorganic quantum dots, in the last decades.[143,144] One direction in which the field has progressed more rapidly is the selective labelling of subcellular organelles, and dedicated sources of information have started to become available.[145] Interestingly, the synergy between organelle-targeting and therapy could be exploited for more effective treatments. In a recent report, CDs prepared using citric acid and ethylenediamine were found to preferentially localize in the nucleolus due to selective RNA targeting ability, which was attributed mainly to the presence of both positive and negative charges on their surface.[146] The nucleolus-targeting capability of CDs enhanced their PDT effect. In addition, the small sizes of CDs enabled their rapid renal clearance. Nevertheless, engineering CDs for specific kidney accumulation is desirable for designing therapeutic applications for renal diseases. For example, *g*-N,Se-CDs prepared using selenocysteine as the precursor exhibited sheet-like morphology and negative surface charge, which collectively contributed to their primary kidney retention and antioxidant properties because of the selenium doping.[147] The combination of renal uptake and intrinsic antioxidant properties in *g*-N,Se-CDs have been exploited for the attenuation of acute kidney injury in murine models.

Amidation reactions. Post-synthetic functionalization of the CD surface chemistry with photosensitizers could be used to prepare covalent hybrid materials with improved and/or synergistic therapeutic efficiencies (Fig. 5d). For example, the surface reactivity of CDs rich in amino groups, obtained through a solvothermal reaction of citric acid and polyethyleneimine in formamide, was exploited for the amidation reaction with the carboxyl functionalities of the photosensitizer chlorin e6, affording a hybrid material for simultaneous PDT and PTT with higher cancer phototherapy efficacy than the two separate components.[148]

Amidation reactions were also used to afford hybrid materials with improved mitochondrial accumulation and tumour-targeting ability, and that can be further modified to provide combined phototherapy.[149] Recently, the surface functionalization of CDs with targeting agents was exploited to

develop an anti-amyloidogenic agent.[150] CDs, able to generate ROS, were used as photosensitizers to induce a photomodulation effect that irreversibly denatures β-amyloid peptides, thereby alleviating the formation of neurotoxic β-amyloid aggregates. This was accomplished through covalent functionalization of carboxylate-capped g-N-CDs, prepared by solvothermal reaction of citric acid and urea in DMF, via an amidation reaction with amine-modified β-amyloid-targeting aptamers. Importantly, the functionalized material exhibited inhibitory efficacies that can be spatiotemporally controlled by illumination time, site, and dose of CDs.

Hollow nanocomposites. Core-shell hollow nanocomposites are known for the high loading of phototherapy agents or anticancer drugs. Recent work showed that hollow-structured CuS/CD nanocomposites, prepared by a solvothermal reaction, allow an efficient electron transfer between the two materials.[151] The CuS/CD nanocomposite exhibited a PTT capability almost two times higher than that of CuS alone, and that was coupled with efficient drug loading and tumour-targeting units for enhancing the antitumor efficacy. In another example, g-N-CDs@hollow mesoporous silica nanospheres were coated with a C_3N_4 layer to afford a laser-activable water-splitting material, which is a promising strategy to improve the PDT efficacy under hypoxic conditions.[152]

Carbon dot-based assemblies. Another strategy to enhance the NIR absorption properties and the therapeutic efficiency is by engineering CDs through supramolecular interactions (Fig. 5e). The assembly of g-N-CDs, obtained by hydrothermal reaction of citric acid and urea, through electrostatic interactions and hydrogen-bonding, resulted in the formation of supramolecular aggregates with broad absorption that were used for PTT.[153,154]

Surface-driven assembly is a strategy to afford nanocomposite material with improved imaging ability[155] or to implement CDs in heterojunction architectures in which the PTT ability of the single units is improved through enhanced separation and migration of photoexcited charges. The assembly of positively charged g-N-CDs, prepared via hydrothermal reaction of 1,3,6-trinitropyrene and branched polyethyleneimine, with negatively charged MoS_2 nanosheets yielded a sandwich heterojunction.[156] The hybrid showed absorbance extending from the visible to the NIR region and PTT efficiency higher than those of the single components. Accordingly, a complete tumour growth inhibition was observed only when mice were treated with the hybrid material and it was not observed when they were treated with either CDs or MoS_2.

Assembled materials can also provide boosted PDT efficiency and take advantage of accumulation in tumour sites through the enhanced permeability and retention effect when injected intravenously. For example, the ionic self-assembly of positively charged g-N,S-CDs with a negatively charged amphipathic sodium dodecyl benzene sulfonate, provided a noncovalent route for the synthesis of assembled CD-based nanospheres.[157] The nanospheres exhibited NIR-light photosensitization ability that was absent in the individual CDs, leading to efficient $^1O_2$ generation.

The assembly of CDs with building blocks able to impart specific functions could also provide nanostructures with additional functionalities. For example, a CD-gadolinium metallofullerene complex assembled via electrostatic interaction exhibited higher MRI properties and PDT efficacy than those of the single components.[158]

Stimuli-responsive hybrid materials. Hybrid materials that integrate multiple functions, via the rational choice of each building block, offer a convenient platform for preparing stimulus-responsive theranostics (Fig. 5f). Both endogenous and exogenous activations can be used as stimuli.

CDs can be combined with stimulus-responsive units by employing either supramolecular or covalent strategies. For example, hydrogen bonding interactions were exploited for the preparation of responsive hydrogels via in situ immobilization of g-CDs in a polymer network.[159] In this case, the synergistic effect of the pH-responsive swelling/deswelling transitions of the polymer and the NIR properties of the g-CDs afforded pH/NIR-light dual triggered drug release. Recently, the coordination ability of the CD surface functional groups for Cu(II) ions was exploited to drive stimuli-responsive CD assembly with therapeutic properties.[160] The peculiarity of this design was the assembly/disassembly process, regulated by the tumour microenvironment that could activate the PDT function, while neutral conditions resulted in quenching.

The covalent functionalization of the CD surface with pH-responsive units is a straightforward strategy to prepare imaging-guided drug nanocarriers with enhanced therapeutic efficiency. In a recent example, mesoporous nano-assemblies were prepared through the amidation reaction of g-N-CDs with a peptide sensitive to proteins overexpressed in cancer-associated fibroblasts.[161] These honeycomb-like nano-assemblies of CDs allowed the loading of multiple drugs via encapsulation strategies or π-π stacking interactions. Once the nano-assemblies dissociated in response to cancer-associated

fibroblasts, the individual CDs were capable of the sequential and spatiotemporal release of the therapeutic agents for efficient chemoimmunotherapy of cancer.

CDs have also emerged as supporting material for atomically dispersed metals to deplete mitochondrial glutathione.[162] *g*-N-CDs, prepared through a pyrolysis route using citric acid and a polyamine as precursors, were used as a support material for the synthesis of Au nanoparticle. The surface of the Au-CDs was decorated with mitochondria-targeting phosphonium salts and ROS-generating cinnamaldehydes. Individual Au atoms were highly active sites for conjugating glutathione via the formation of Au−S bonds. The efficient and selective glutathione-depletion ability, coupled with the amplification of the cinnamaldehyde-induced ROS damage in mitochondria, altered the mitochondrial membrane potential and reduced the oxygen uptake, finally causing tumour growth inhibition in vivo.

Another strategy to afford responsive systems is by engineering the CD composition. For example, *g*-N,S-CDs, prepared through hydrothermal microwave-assisted synthesis from citric acid and polyethyleneimine-poly(amido amine) modified with disulphide bonds, exhibited glutathione-triggered degradation and efficient gene delivery because the disulphide bonds of the precursor were retained in the CDs structure.[163] Following a similar design strategy, a Mn-containing CDs (*g*-N,Mn-CDs) assembly with H_2O_2-responsive properties was used for in situ oxygen generation to enhance the PDT efficiency in hypoxic tumours and simultaneously provided MRI imaging ability.[164]

Perspectives

We have outlined the chemical toolbox to engineer tuneable and tailored carbon dots through pre- and post-synthetic approaches. Since our goal was to demonstrate the tremendous progress in this area of research, we selected examples from diverse areas, ranging from biomedical to energy applications. However, the facile preparation of CDs through a multitude of precursors and methodologies has brought both advantages and disadvantages. On one hand, the apparent ease of synthesis and low-cost production has generated a flurry of research activities. On the other hand, most of the research groups have pursued investigations only on a particular set (or subset) of carbon dots. This diversity continues to lead to apparent FL mechanism controversies and purification issues (with red-emissive CDs being recently referred as "mythical",[29] due to presence of molecular dyes in the mixture),[20,165] and there continues to be a lack of deep understanding of how operating conditions can be translated into versatile methodologies towards tailorable properties.

In the last years, there have been a few remarkable examples of bottom-up procedures (single-source or multi-component) that allow the preparation of CDs with tuneable properties. These are influenced by both core (amorphous or graphitic) and surface (usually rich in surface functional groups, and hydrophilic or hydrophobic) affording a set of CDs with different structures. While the applications of CDs continue to grow, both in energy and biomedicine, many of them continues to rely on the fluorescence properties. From the examples detailed in this Review, it is evident that the choice of the precursor(s) and of the synthetic condition have become powerful tools to afford specific structures/properties for further applications. Undoubtedly, there is further necessity to expand on these examples, improve control over the chemical structure and the structure-property relationship, and report methods that are ready to be widely adopted by the field. Ideally, for their widespread acceptance, the experimental procedures should also include details that have been frequently overlooked, report reaction scales, their yields, and include reproducible purification procedures. Afterwards, large-scale productions of CDs can be addressed. A fully automated method combining machine learning and high-throughput synthesis/characterization might aid in the discovery of CDs with targeted properties.[166,167] For the moment, the production of carbon dots from biomass precursors could bring variability and confusion, although potentially is significant as a renewable, inexpensive, and green source.[168] Then, further post-functionalization reactions can be applied to the widely adopted CDs, with some of them already explored, but further research is needed to expand on the examples described in the present Review. Therefore, the future of this field, and the widespread application, will be to work inside-out: establish reproducible synthetic procedures that can achieve general acceptance and then institute post-functionalization protocols.

Besides establishing these protocols, there will always be a need for further research into the synthesis of CDs, such as reaching FLQY of unity, tuning the photoluminescent lifetimes, reaching size distribution <10%, and narrow emission. For other luminescent nanomaterials, like inorganic quantum dots, progress has allowed the control of such properties, and carbon dots research is therefore still considered in its early stages. It will be fascinating to witness if further improvements of the CD photophysical properties will fully disclose their advantages over other nanoparticles. This becomes evident if we consider that CDs have not (yet) found an irreplaceable role in biomedical and energy-

related fields, and their consumerization compared to other carbon nanomaterials[169] has a long way forward.

Finally, carbon dots are finding their place in between organic dyes, carbon nanomaterials, and inorganic quantum dots. Their molecular-like characteristic increases the difficulty of their synthesis[170] but, at the same time, gives them the tunability that is difficult to achieve in other nanomaterials. The continuous review, improvement, and learning from mistakes made with other nanomaterials should provide guidance on the conceptual and applicative level for carbon dots. Future efforts should focus on fundamental understanding of the formation process,[171] for example via in situ characterization, so that we can gain general design principles and expand the chemical toolbox for specific structure and/or application. Although CDs have already permeated many different fields, it is apparent that any application will encounter the challenge of expanding knowledge on the structure-activity relationship of CDs. Further development in controlled synthesis, along with development of innovative methods,[172] will surely lead to rapid progression and new unforeseen applications. There is great scope and potential for absolute control over function and application in bottom-up approach. However, thermal carbonization is generally considered as random and chaotic so that systematic control of the process is considerably challenging. Ideally, we will access rationally designed and "atomically precise" CDs on demand to unlock their full potential and, eventually, to witness a transformative impact in material science.

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Acknowledgments

Competing interests

The authors declare no competing interests.

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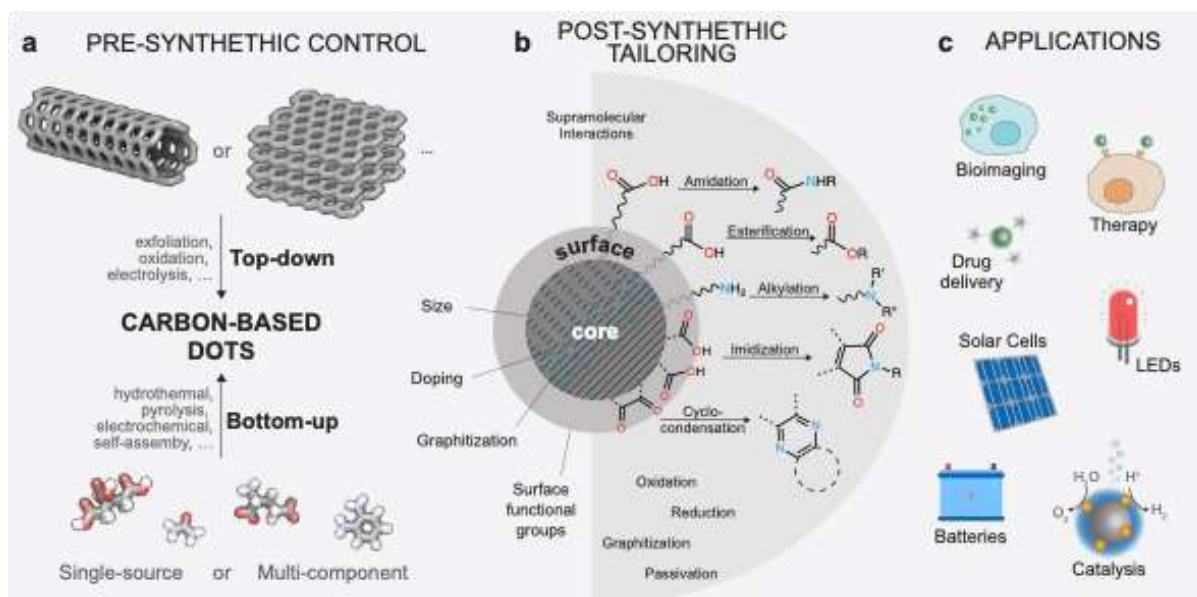
Fig. 1: **Overview of synthetic approaches and applications of carbon dots**. Pre-synthetic control includes top-down cutting of larger carbon structures and bottom-up thermal treatment of either single-source or multi-component organic precursors. Post-synthetic tailoring encompasses reactions for the transformation of surface functional groups. Both the pre- and post-synthetic reactions impact their physicochemical properties and applications.
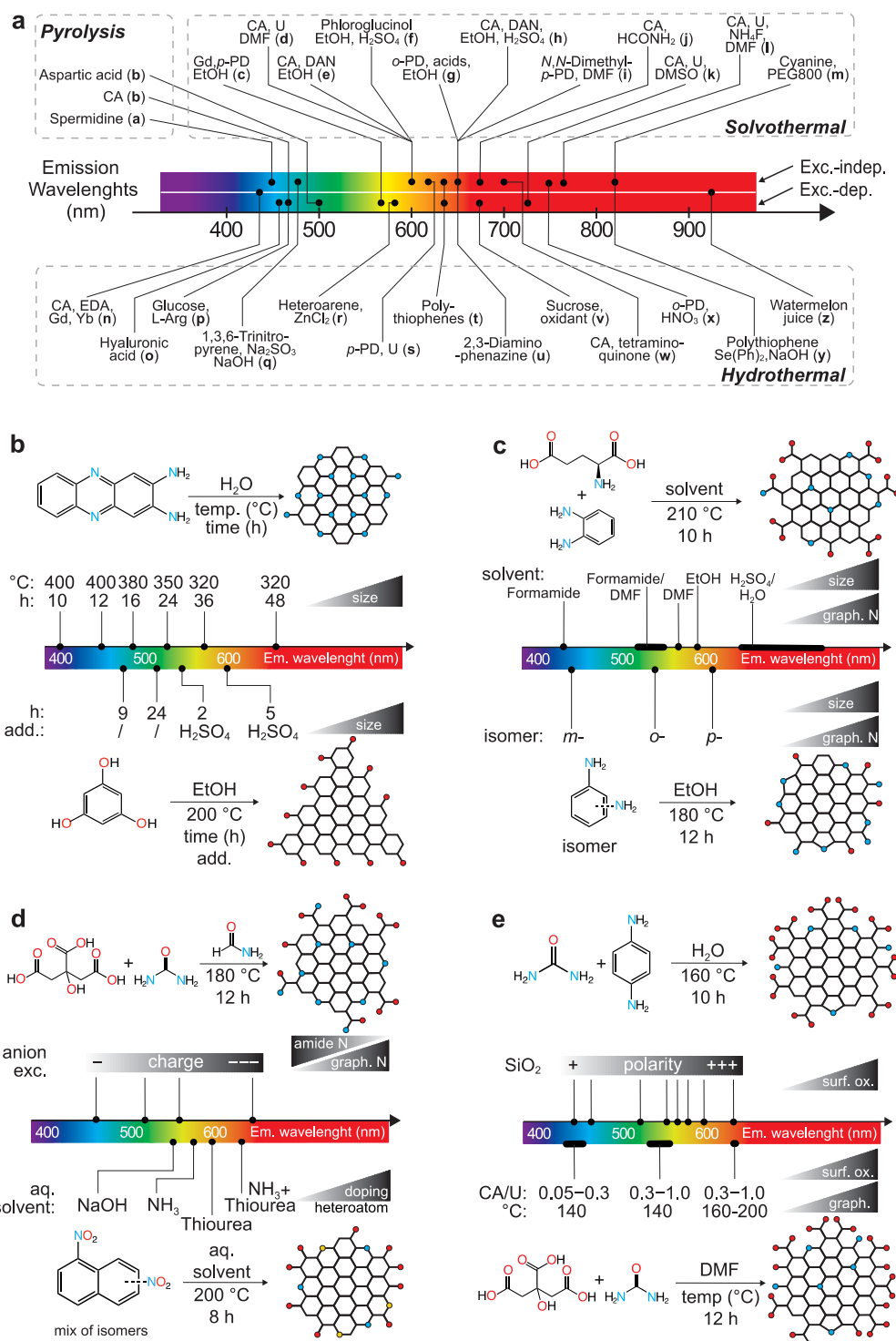
Fig. 2: Overview of multicolour fluorescence of carbon dots from bottom-up syntheses. **a**, Reported CDs fluorescence emission using common synthetic approaches. The varied fluorescence wavelength (nm) emission and excitation-(in)dependence are as reported in literature and are due to the diverse nature of CDs. Fluorescence properties refer to the following references: ref.[142] (a), ref.[48] (b), ref.[132] (c), ref.[35,70] (d), ref.[71] (e), ref.[19] (f), ref.[24] (g), ref.[71] (h), ref.[72] (i), ref.[173] (j), ref.[120] (k), ref.[122] (l), ref.[119] (m), ref.[131] (n), ref.[174] (o), ref.[134] (p), ref.[128] (q), ref.[27] (r), ref.[33] (s), ref.[116,175] (t), ref.[18] (u), ref.[176] (v), ref.[136] (w), ref.[26] (x), ref.[117] (y), and ref.[121] (z). References (n) and (z) do not report on the excitation-(in)dependence. **b**, Synthesis of nanoparticles with size-dependent emission properties through the single-source solvothermal approach.[18,19] **c**, Preparation of size- and graphitic nitrogen-dependent emission CDs through solvent engineering and regio-isomers.[21,22] **d**, Synthesis of CDs with heteroatom-dependent emission properties.[30,35] **e**, Synthesis of multicolour emission CDs with different surface oxidation degree.[33,35] Abbreviations: citric acid (CA), *ortho-* or *para*-phenylenediamine (*o-* or *p*-PD), ethanol (EtOH), urea (U), *N,N*-dimethylformamide (DMF), diaminonaphthalene (DAN), dimethyl sulfoxide (DMSO), ethylenediamine (EDA), L-arginine (L-Arg).
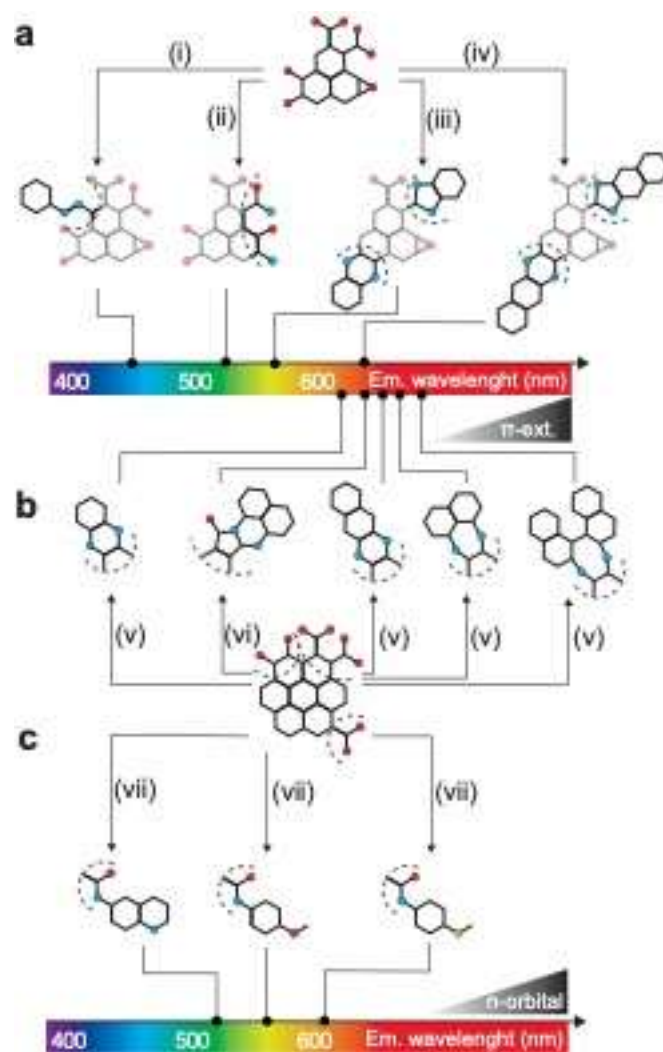
Fig. 3: Overview of post-synthetic approaches for tuning multicolour fluorescence of carbon dots. Oxidized *g*-CDs can present C(sp^2) domains and multiple oxygen-containing functional groups, which can be functionalized through a variety of synthetic protocols. **a**, Tailored emission of a (schematized oxidized fragment) *g*-CDs can be achieved by reaction with (i) benzenediazonium chloride to form azo-functionalized CDs; (ii) thermal ammonia treatment; (iii) cyclocondensation reaction with *o*-phenylenediamine or (iv) diaminonaphthalene.[38] **b**, the cyclocondensation approach has been expanded to other diaminoarenes like (v) *o*-phenylenediamine, 2,3-diaminonaphthalene, 1,8-diaminonaphthalene, 1,1′-bi(2-naphthalene) or preparation of (vi) perimidine edge groups.[39,41] **c**, surface functionalization through (vii) amidation between edge carboxylates and aniline derivatives.[42]
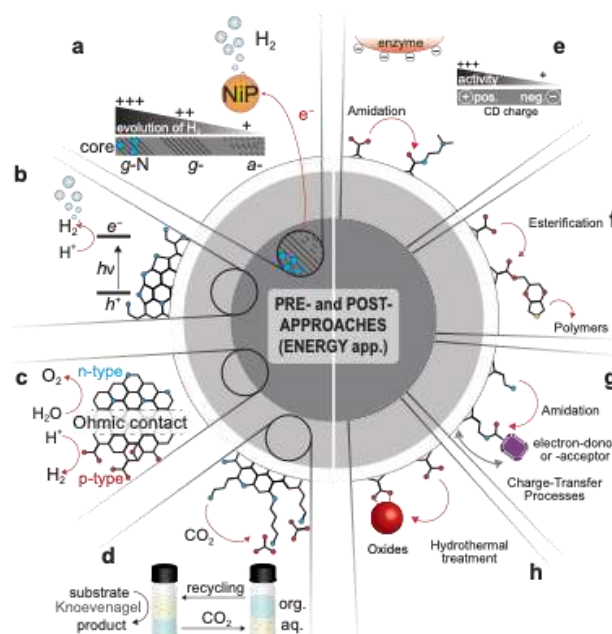
Fig. 4: Overview of pre- and post-synthetic strategies for enhancing carbon dots applicability in energy applications. **a**, Graphitization and heteroatom-doping of the core control photosensitization ability of catalysts.[48,50] **b**, Degree of heteroatom doping and inclusion mode determine the photocatalytic behavior.[54] **c**, Nitrogen-doping and oxygen functional groups create n- and p-type domains, separated by $C(sp^2)$ clusters, for photocatalytic water splitting.[57] **d**, Surface groups can be used to switch the CD hydrophobicity and thus enable their recycling in organocatalytic reactions.[68] **e**, Amidation reactions can switch the charge of the CDs and thus pair electrostatic interaction for more efficient photocatalysis.[45] **f**, Esterification reactions with molecules (or monomers) can be used for subsequent polymerization reactions to form composite materials.[75] **g**, Amidation reactions can introduce donor or acceptor systems, which allow investigation of charge-transfer processes.[10] **h**, Hydrothermal treatment of CD and (metal) oxides leads *to* formation of covalent bond in the composite materials.[112]
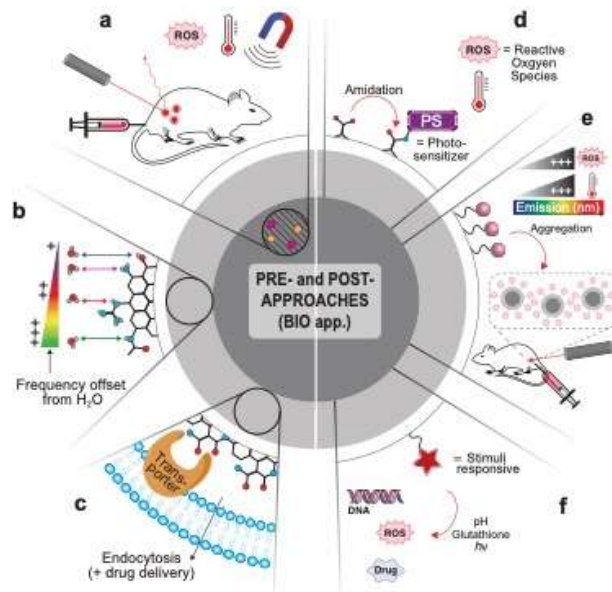
Fig. 5: Overview of pre- and post-synthetic strategies for enhancing carbon dots applicability in biomedical applications. **a**, Graphitization and doping promote bioimaging and phototherapy (e.g. reactive oxygen species, ROS, generation).[118,128,131] **b,** Exchangeable protons of the surface functional groups give chemical exchange saturation transfer magnetic resonance imaging properties.[134] **c**, Surface functional groups trigger selective biological interactions or impart biological properties.[135,136,142] **d**, Amidation reactions can be used to achieve improved and/or synergistic phototherapies.[148] **e**, Assembly strategies enhance the NIR absorption properties and the therapeutic efficiency.[154,157] **f**, CD-based materials can be designed in stimulus-responsive theranostics.[159–163]

Box 1 Summary of CDs properties and comparison to other luminescent molecular materials

Apart from molecular dyes, there is a diverse group of alternative fluorescent materials that includes carbonaceous, biological, organic and inorganic materials.[5] Their physicochemical (size, structure, shape, solubility, toxicity, …) and photophysical properties (excitation/emission ranges, quantum yield, photobleaching, …) are also very varied. The table below provides a brief comparison of the properties of carbon-based dots, organic dyes, inorganic quantum dots and carbon nanotubes. In general, each class will have their advantages and disadvantages depending on the application. A practical consideration is the advantage of readily soluble and functionalisable CDs after their synthesis, while carbon nanotubes (or graphene) require post-synthetic modifications to make them dispersible, for instance, in aqueous media. Another practical consideration is the multitude of simple and inexpensive preparations of CDs, which eventually provide an advantage over inorganic quantum dots. To date, however, despite few notable advances, the photophysical properties of CDs are generally less attractive than those of inorganic quantum dots, but still relatively unexplored. For a more extensive overview of carbon dots properties compared to inorganic quantum dots and other luminescent materials the reader is refereed to recent articles.[5,17]

Property	Material			
	Carbon-based dots	Organic dyes	Inorganic quantum dots	Carbon nanotubes
Size (nm)	1−10	<2	2−10	10^2−10^3
Structure	Amorphous−crystalline	Molecular	Crystalline	Amorphous−crystalline
Shape	Discoidal/quasi-spherical	Molecular	Spherical	Tubular
Solubility	High	High	Low−High	Low
Toxicity	Low	Low	Low−High	Low−High
exc. range	UV−Vis	UV−NIR-II	UV−NIR-II	Vis−NIR
exc. fwhm (nm)	50−200	30−70 (Vis) 50−150 (NIR)	broad	Diameter- and chirality-dep.
em. range	Vis	Vis−NIR-II	Vis−NIR-II	NIR
em. fwhm (nm)	30−200	25−70 (Vis) 50−150 (NIR)	20−200	25−50
PL QY	0.05−0.9	0.05−0.9	0.1−1	0.001−0.01
τ (s)	10^{-9}−10^{-8}	10^{-10}−10^{-8}	10^{-9}−10^{-6}	10^{-11}−10^{-10}

Carbon dots are a diverse set of small carbon-based nanoparticles with varied physicochemical (shape and crystallinity) and photophysical properties (quantum confinement effect, QCE) as depicted in the figure.[6] Based on these properties, another classification has been used to describe CDs, as mentioned in the main text. Graphene quantum dots (GQDs) are discoidal-shape graphene sheets, which possess graphene lattices, surface functional groups and exhibit quantum confinement effect from their size (or the size of the emissive π-domains). Carbon quantum dots (CQDs) are quasi-spherical in shape, possess crystallinity arising both from the graphene lattice and the interplane stacking, functional groups on the surface, and show quantum confinement effect depending on their size. Carbon nanodots (CNDs) are quasi-spherical in shape, possess a carbonized structure (no obvious crystallinity) with surface functional groups. Finally, carbonized polymer dots (CPDs) are quasi-spherical in shape, possess a hybrid polymer/carbon-based structure with a carbon-based core and functional groups/polymers on the surface. The degree of carbonization and graphitization of the carbon core can be regulated by synthetic conditions such as the reaction temperature, to afford more, e.g. GQDs or CQDs or CNDs, or less, e.g. CPDs, carbonized carbon-based dots. The employed precursors also affect the structure, and therefore the type, of the final CDs. For instance, CDs prepared via the incomplete carbonization of small molecules and polymer precursors might retain a polymeric structure on the surface, but CDs with large C(sp^2) conjugated clusters can be afforded using polymers or aromatic precursors. Fluorescence properties will depend on the carbon-based core (carbonization similar to either CQDs or CNDs) but can also arise from crosslink enhanced emission. While it is not uncommon that different types of CDs share similar fluorescence features, fluorescence mechanisms can also be distinctive for each of those types, e.g. quantum confinement effects or conjugated π-domains for GQDs and CQDs, defect states for CNDs, molecular (fluorophore) states and crosslink-

enhanced emission states for CPDs. A more comprehensive comparison of different types of CDs has been reviewed elsewhere.[6,177,178]
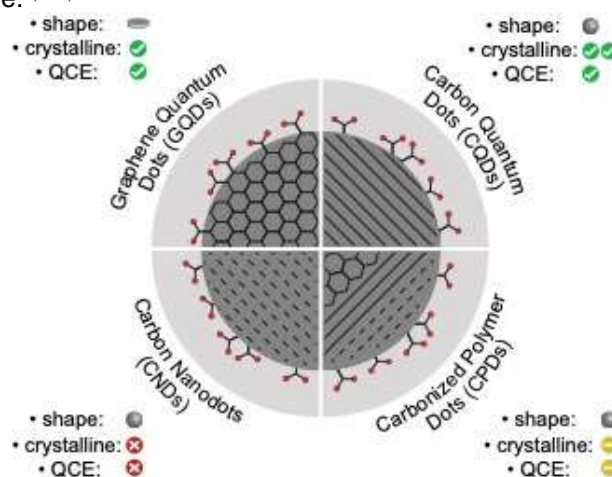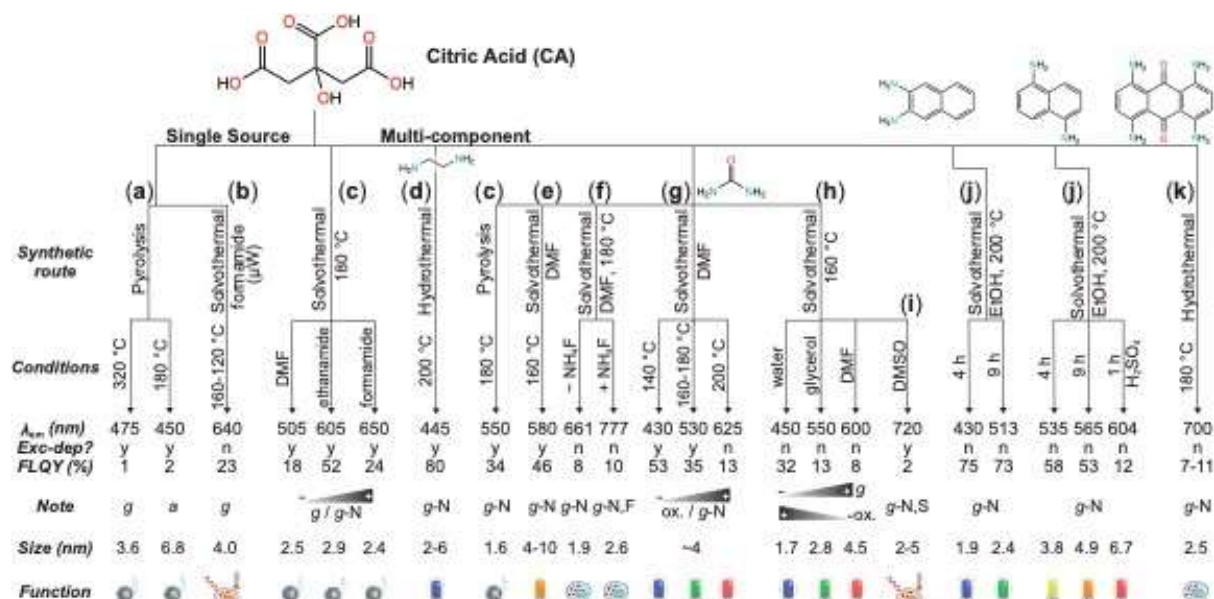

Box 2 Citric acid as model precursor in the synthesis of CDs

To get further design principles for future preparations of fluorescent functional nanoparticles, this Box is focused on one of the most utilized precursor for the synthesis of CDs, citric acid (CA).[179] CA has been used as single-source or in multi-component reactions under various thermal treatments. Pyrolysis of only citric acid yields blue FL nanoparticles, with higher temperatures leading to more graphitization.[48] Both amorphous and graphitic core structures usually exhibit excitation-dependent FL properties and low FLQY due to many mid-band energy states.

To red-shift the CD optical properties, CA can either undergo solvothermal treatment or can be used in a multi-component approach, with both approaches aiming at the heteroatom doping and/or at the extension of the C(sp^2) core. Solvothermal treatment of formamide (or DMF) causes its thermal decomposition, which leads to the generation of ammonia (or dimethylamine) that reacts with CA.[173] Changing the solvent from DMF to formamide is reported to promote dehydration reactions, resulting in higher graphitic nitrogen content and larger C(sp^2) conjugated domains.[180] Numerous sources were employed to achieve nitrogen doping of CA-derived CDs in the multi-component synthesis, most common being ethylenediamine[181] and urea. CA and urea, under hydrothermal conditions, form molecular fluorophores of the pyridine family (and other heterocyclisation products) that usually dominate the CD optical properties. Under these conditions, the extent of dehydration and carbonization is limited, so solvothermal conditions are preferred to obtain CDs with red-shifted emission.[70,97] Thermal treatment of CA and urea (in DMF) is a popular way to obtain nanoparticles that emit orange/red light and possess nitrogen-doped graphitic planes. Furthermore, adjusting the ratio of CA to urea, as well as tuning the reaction temperature, are other approaches to alter the emission properties of the g-N-CDs.[35] Increasing the urea content and the temperature was found to give nanoparticles of similar size, but with red-shifted emission, higher graphitization, and extended surface oxidation.

Remarkably, the emission can be further shifted to the NIR region by using a co-doping strategy or using π-extended precursors. In one example, this was achieved by adding ammonium fluoride as dopant source to CA and urea (in DMF), with the formation of donor-π-acceptor domains presumably responsible for the red-shifted emission.[122] Another approach would be using a solvent like dimethyl sulfoxide (instead of DMF),[120] in which the sulfur doping plausibly introduces lower energy levels, thus reducing the optical bandgap. Combining CA with phenylenediamines[25] and diamino- (or tetraamino-) substituted polycyclic aromatic hydrocarbons[71,136] under solvothermal conditions has yielded CDs with NIR emission. In this way, the obtained nitrogen-doped graphitic CDs have size-dependent optical properties and show excitation-independent behaviour.

Figure data (bottom-up approaches to multicolour citric acid-based carbon dots):

Panel	Synthetic route	Conditions	λ_{em} (nm)	Exc-dep?	FLQY (%)	Note	Size (nm)
(a) Single Source	Pyrolysis	320 °C / 180 °C	475 / 450	y / y	1 / 2	g / a	3.6 / 6.8
(b)	Solvothermal formamide (µW)	160–120 °C	640	n	23	g	4.0
(c) Multi-component	Solvothermal 180 °C	DMF / ethanamine / formamide	505 / 605 / 650	y / y / y	18 / 52 / 24	g / g-N	2.5 / 2.9 / 2.4
(d)	Hydrothermal	200 °C	445	y	80	g-N	2–6
(c)	Pyrolysis	180 °C	550	y	34	g-N	1.6
(e)	Solvothermal DMF	160 °C	580	y	46	g-N	4–10
(f)	Solvothermal DMF, 180 °C	–NH$_4$F / +NH$_4$F	661 / 777	y / y	8 / 10	g-N / g-N,F	1.9 / 2.6
(g)	Solvothermal DMF	140 °C / 160–180 °C / 200 °C	430 / 530 / 625	y / y / y	53 / 35 / 13	ox. / g-N	~4
(h)	Solvothermal 160 °C	water / glycerol / DMF / DMSO (i)	450 / 550 / 600 / 720	n / n / n / y	32 / 13 / 8 / 2	g ... ox.	1.7 / 2.8 / 4.5 / 2–5
(j)	Solvothermal EtOH, 200 °C	4 h / 9 h	430 / 513	n / n	75 / 73	g-N,S	1.9 / 2.4
(j)	Solvothermal EtOH, 200 °C	4 h / 9 h / 1 h H$_2$SO$_4$	535 / 565 / 604	n / n / n	58 / 53 / 12	g-N	3.8 / 4.9 / 6.7
(k)	Hydrothermal	180 °C	700	n	7–11	g-N	2.5

Overview of bottom-up approaches to multicolour citric acid-based carbon dots. Synthetic procedures refer to the following references: ref.[48] (a), ref.[173] (b), ref.[180] (c), ref.[181] (d), ref.[70] (e), ref.[122] (f), ref.[35] (g), ref.[97] (h), ref.[120] (i), ref.[71] (j) and ref.[136] (k). Abbreviations: "λ_{em}" stands for the lowest energy maximum emission peak; "Exc-dep?" indicates the excitation-dependent emission behaviour ("y" is excitation-dependent, "n" is excitation-independent; "FLQY" indicates the fluorescence quantum yield as reported in the references; "g" and "a" stand for the graphitized or amorphous nature of CD core, e.g. "g-N,F" stands for nitrogen- and fluorine-doped graphitized CDs and so on; "ox." stands for the presence of oxygen functional groups on the CD surface; "Size" is reported as the mean value extracted from the references or, if the average size or distribution is not reported, as the size range; "Function" is schematized in the following order: photocatalytic (●), photothermal (), light-emitting diodes (▮), and bioimaging ().

Box 3 Purification and characterization

Since this Review is focused on the chemical tools used in the CD research area, we do not discuss how these CDs were purified and characterized in each specific case but state their graphitisation and surface composition based on the reported characterization. This Box summarises the purification procedures and characterization techniques commonly used for CDs.

Purification. The reaction mixture contains unreacted precursors, intermediates, or side products. They can be in the form of small polymers, molecular fluorophores intermediates, or large organic fragments formed along with the CDs when precursors undergo condensation reactions. The traditional purification method is dialysis against water or organic solvents. The use of dialysis membranes with appropriate pore sizes frequently provides reliable methods for separating CDs from smaller undesired products. Ultrafiltration and/or centrifugation are usually complementary procedures to remove larger aggregates. Chromatography based on size (size exclusion chromatography) or polarity (silica gel column) is the preferred option[182] and is particularly useful in separating CDs with narrow emission bands.

Optical Properties. Some characterization techniques are commonly used, but others have also to be tailored to the specific application.[183,184] Given the widespread use of CDs for their optical properties, recording their absorption and fluorescence spectra is fundamental. Absorption spectra should start being reported in mass absorption coefficients, which would give information of how much these nanoparticles absorb light and could also indicate their solubilities, aggregation, and presence of impurities. Fluorescence should be reported only together with the corresponding quantum yield (FLQY). A combination of absorption and emission spectroscopies can give valuable information on the type of CDs, but it should not be used as the only way to classify them. For example, narrow absorption and excitation-independent emission peaks can reveal the presence of quantum confinement effects and lack of defects. On the other hand, broad peaks accompanied by excitation-dependent fluorescence are dominated by surface defects. Nonetheless, these must be complemented by physical characterization techniques (vide infra).

Photoluminescence lifetimes have been useful in studying the time-resolved decay profiles of the excited states, distinguish between fluorescence (short-lived emission) and phosphorescence (long-lived emission), and mainly to probe charge separation or interactions for photocatalytic purposes. Other techniques, like ultrafast photophysics, are still rare and not widely available but are essential to

elucidate the exciton dynamics. Further optical characterization should be tailored to the nanomaterials. For example, CDs can exhibit chirality and recording their electronic and vibrational circular dichroism spectra is needed.[140]

Other Characterization Techniques. Physical characterization techniques for assigning amorphous or graphitic nomenclature to CDs are mostly based on transmission electron microscopy (TEM) and X-ray diffraction (XRD). Especially high-resolution TEM can give information about the presence of graphitic planes (lattice spacing of 0.21 nm corresponding to the (100) facet of graphitic carbon) and the lateral size. XRD patterns can reveal the presence of stacked graphene layers (25° corresponds to 0.34 nm interlayer spacing). For amorphous CDs, although it is possible to use TEM, it can be time-consuming because of low-contrast and their propensity to aggregate on the grid. Atomic force microscopy (AFM), on the other hand, can be used to determine the height of CDs, and therefore both should be used to define the CD morphology. The phase and type of carbon can be further probed by X-ray photoelectron spectroscopy (XPS), nuclear magnetic resonance (NMR), and Raman spectroscopy. Out of these, XPS has been particularly useful in determining the elemental composition and the type of bonds present. While ^{13}C-NMR can be a great option for studying the surface functionalities, as well as the extent of the carbon sp^2/sp^3 hybridization, it usually requires long acquisition times, high amounts of material, and/or ^{13}C-enriched starting material. Other less used techniques, such as Raman spectroscopy, can further confirm the amorphous or graphitic morphologies. A simple but particularly useful technique for studying surface functionalities and following post-synthetic reactions is infrared spectrophotometry.

In the examples reported through pre- and post-synthetic approaches for CDs in this Review, sometimes the use of specific techniques is required. One example is when the charge of CDs needs to be determined and, in this case, zeta potential (ζ) measurements or potentiometric-conductometric titrations are useful. Another example is the case of metal-doping in CDs, and the use of inductively coupled plasma mass spectrometry is particularly valuable. Other physical characterization techniques can have (sometimes limited) usefulness in studying CDs but can be of great use when characterizing their derivatives or presence in composite materials. One example is dynamic light scattering, which can probe the hydrodynamic size of CDs derivatives and aggregates rather than CDs alone due to their small size, fluorescence, and low density. In composite materials, HR-TEM can confirm the presence of g-CDs graphitic planes, while scanning transmission electron microscope, especially with elemental mapping, can also give useful information about the composition. The formation of new bonds in composite materials is most often probed by using infrared spectrophotometry and XPS. Thermogravimetric analysis, although not commonly used, can be performed on the organic precursor, and thus determine the temperature used for synthesis. In addition, it can be used to discern the presence of organic impurities in the sample and used to study hybrid materials.

As the functional groups on the surface can be used for post-functionalization reactions, they can also be probed through chemical reactivity experiments. For example, Kaiser and Purpald tests can be used to estimate the presence of primary amine and aldehyde groups, respectively.

Energy levels determination. For catalytic purposes, as well as for optoelectronic devices, the determination of the energy levels is essential. Sometimes these can be probed by using known electron acceptors like, for example, methyl viologen that can show if the CDs have sufficient reducing potential (for proton reduction in this case). The energy levels and the respective bandgaps can be determined through several methods: a combination of ultraviolet photoelectron spectroscopy and optical absorption spectra (Tauc plot), cyclic (or differential pulse) voltammetry for determining the redox potentials, linear sweep voltammetry, and electrochemical Mott−Schottky analysis of impedance data. Recently, a semiempirical method for the determination of the conduction and the valence bands was proposed for understanding the optical and photoelectrochemical properties.[185]