Nanoscale

MINIREVIEW

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Cite this: *Nanoscale*, 2015, **7**, 1586

Recent advances in carbon nanodots: synthesis, properties and biomedical applications

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Received 30th September 2014,
Accepted 2nd December 2014

DOI: 10.1039/c4nr05712k

www.rsc.org/nanoscale

Herein, a mini review is presented concerning the most recent research progress of carbon nanodots, which have emerged as one of the most attractive photoluminescent materials. Different synthetic methodologies to achieve advanced functions and better photoluminescence performances are summarized, which are mainly divided into two classes: top-down and bottom-up. The inspiring properties, including photoluminescence emission, chemiluminescence, electrochemical luminescence, peroxidase-like activity and toxicity, are discussed. Moreover, the biomedical applications in biosensing, bioimaging and drug delivery are reviewed.

1. Introduction

Carbon is one of the most abundant natural elements, with applications in a huge number of useful materials including nanomaterials. Recently, carbonaceous nanomaterials have attracted extensive interest from various disciplines, such as carbon nanotubes,[1–3] graphene,[4–6] fullerene[7,8] and nanodiamond.[9–11] Nevertheless, they have certain shortcomings. For example, nanodiamond has expensive preparation and separation procedures; graphite and carbon nanotubes usually possess poor water solubility, and the inability to provide strong luminescence, particularly in the visible region, may limit their potential applications. Carbon-based dots, including graphene quantum dots (GQDs) and carbon nanodots (CDs) are a new form of zero-dimensional carbonaceous nanomaterial.[12–17] Up to now, much work has been carried out and significant progress has been achieved in the synthesis and applications of carbon-based dots.[18–22]

GQDs possess graphene lattices and usually have less than 10 layers of graphene.[23] They are excellent electron donors and acceptors.[24] Thus, they can be applied in photodetectors and solar cells. The feature of excellent conductivity also makes GQDs outstanding materials for the fabrication of electrochemical biosensors.[25] Few-layered GQDs exhibit photoluminescence (PL) behavior similar to that observed in CDs. In addition, controlling the layers of graphene may contribute to the transition from GQDs to CDs.[26] CDs are quasi-spherical carbon nanoparticles with a diameter of less than 10 nm. A typical structure of CDs is illustrated in Fig. 1. Nuclear magnetic resonance measurement results demonstrate that the inner part of CDs is mainly composed of sp^2 hybridized carbon atoms, while the outer part contains sp^3 hybridized carbon atoms.[27] In contrast with macroscopic carbon, a low water soluble and black material, CDs disperse well in water due to the abundant hydrophilic groups on their surface. One of the most intriguing properties of CDs is the PL emission. In addition, PL from CDs may be quenched by electron acceptors or donors, which promises the manipulation of photoinduced electron transfer.[28] Compared with commercial dyes and traditional semiconductor QDs, CDs have many merits, such as strong PL emission in the visible spectral range, excellent water solubility, low toxicity, fine resistance to photobleaching,

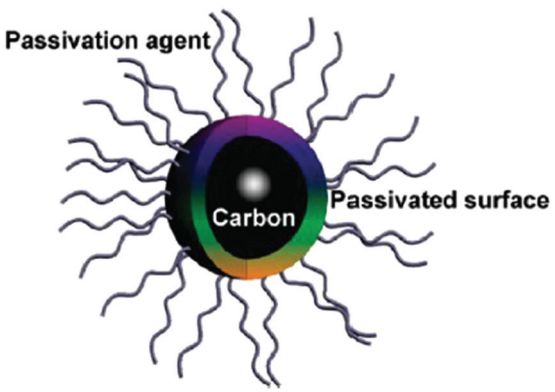

Fig. 1 Schematic illustration of the structure of CDs. (Reproduced from ref. 22 with permission from American Chemical Society.)

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ease of synthesis, surface modification, *etc.*[14,29–31] Therefore, CDs have been the star nanomaterials with potential uses[32–35] in biosensing,[36] bioimaging[37] and drug delivery.[38]

In this review, we introduce the synthesis and properties of carbon-based dots. CDs are extensively discussed. Some elegant biomedical applications are outlined. We hope to provide an insight into the mechanism of their excellent properties, and stimulate future research on potential biomedical applications.

2. Synthesis

At present, a variety of synthetic routes have been developed for the preparation of CDs. Most of the studies seek to pursue simple, cost-effective, size-controllable or large scale synthetic methods to obtain CDs of high quality. According to the properties of the changes in the carbon sources to the final products, the synthetic methods are divided into chemical and physical methods. Besides, considering the relationship between the sources and products, the two classes of top-down and bottom-up may summarize the current synthetic methods.

Synthetic conditions can be optimized to prepare specific CDs by methods such as capillary zone electrophoresis.[39] Further purification of the products may be included in the synthesis, which involves the techniques of centrifugation, electrophoresis, dialysis, *etc.* For example, Zhu *et al.* separated CDs from other reactants by three cycles of concentration/dilution.[40] Li *et al.* obtained CDs with different surface modifications by dialyzing against Milli-Q water using different cellulose ester membrane bags.[22] In addition, how to realize the surface modification and functionalization is still an obstacle in the use of CDs in biomedical applications.

2.1. Top-down

For this class, CDs are generated from the chemical or physical cutting processes of relatively macroscopic carbon structures,[41] such as carbon nanotubes, graphite column, graphene, suspended carbon powders, *etc.* Most of the cutting processes are nonselective. Electrochemistry, chemical oxidation, laser irradiation are the three methods mainly employed.

2.1.1. Electrochemistry. The essential advantages of electrochemical methods are low cost and easy manipulation.[42] Table 1 summarizes recent electrochemical approaches for the synthesis of CDs. Carbon nanotubes and graphite are the most frequently used carbon sources for electrochemical synthesis, since they are ideal materials for electrode preparation. Zhou *et al.* developed a method to convert multi-walled carbon nanotubes (MWCNTs) into nanodots.[43] A cyclic potential was applied in a degassed acetonitrile solution with 0.1 M tetrabutylammonium perchlorate. During the procedure, the colorless solution turned yellow and finally dark brown, which represented the formation of the CDs peeled from the nanotubes. Zhao *et al.* prepared CDs by the electrooxidation of a graphite column electrode at 3 V against a saturated calomel electrode (SCE).[44] The electrolyte was 0.1 M NaH_2PO_4. The solution changed from transparent color to yellow and then to dark brown as the oxidation proceeded. To obtain CDs with a narrow size distribution without any further separation procedures, Bao *et al.* developed a tuning system *via* the electrochemical etching of carbon fibers.[45] By adjusting the applied electrode potentials, CDs were obtained controllably and separation or surface passivation procedures were eliminated. Another research group also realized the size tuning by an electrochemical method.[41] The synthesis processing stages are shown in Fig. 2. This approach did not require any molecular capping agent and the reaction was in a non-aqueous solution. Taking this route, CDs with diameters of 3, 5, and 8.2 (±0.3) nm were synthesized electrochemically from MWCNTs in propylene carbonate using $LiClO_4$ at 90 °C.

2.1.2. Chemical oxidation. Oxidation of the substrate *via* treatment of an oxidative reagent is widely used for synthesis. Chemical oxidation is also a simple and effective approach for large scale production. Qiao *et al.* prepared biocompatible CDs with an effective direct chemical oxidation route.[47] Three typical commercially activated carbons were employed as the carbon sources, including coal, wood and coconut activated carbons. CDs could be easily etched from the amorphous structure of activated carbons in the presence of nitric acid. A passivation process using amine-terminated compounds was then carried out. The final products derived from the three carbon sources possessed a similar narrow size distribution, which also showed bright luminescence.

2.1.3. Laser irradiation. CD synthesis *via* laser irradiation mainly takes advantage of the high heat and high pressure produced by the laser.[48] Traditional synthesis *via* this method involves several complex steps and strict reaction conditions. Hu *et al.* creatively raised a one-step protocol for the synthesis of CDs by laser irradiation.[48] In this protocol, they employed a laser to irradiate the graphite powders suspended in an organic solvent. From the experiment results, they concluded that PL originated from the carboxylate groups on the surface

Table 1 Electrochemical synthesis of CDs

Working electrode	Counter electrode	Reference electrode	Size of CDs (nm)	Carbon source	Quantum yield	Ref.
MWCNT modified glassy carbon electrode	Platinum foil	Platinum wire	3, 5, 8.2, 23	MWCNTs	5.1–6.3%	41
MWCNT covered carbon paper	Platinum wire	$Ag/AgClO_4$ reference electrode	2.8	MWCNTs	6.4%	43
Graphite column electrode	Platinum wire	Calomel electrode	1.9, 3.2	Graphite	1.2%	44
Carbon fibers	Platinum sheet	Silver wire	2.2–3.3	Carbon fibers	1.12–1.47%	45
Platinum sheet	Platinum sheet	Calomel electrode	3–6	Alcohol	15.9%	46

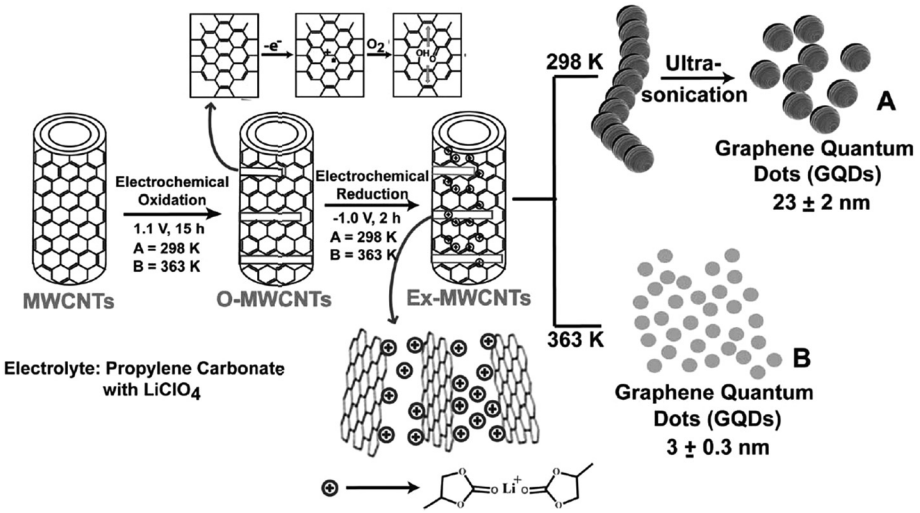

Fig. 2 Processing stages of the GQD synthesis by an electrochemical approach. (Reproduced from ref. 41 with permission from John Wiley and Sons Ltd.)

of the CDs. Moreover, through this route, once the CDs were synthesized, the surface modification of the nanodots was simultaneously accomplished. By selecting suitable solvents, nanomaterials with different luminescence properties could be prepared for potential use.

2.2. Bottom-up

For the bottom-up class, organic precursors are required as seeds to grow into CDs under certain conditions. Microwave, heat, and ultrasonic wave are the primary approaches used for energy aggregation and molecular structure transition. The presence of a precursor shows lower requirements of carbon sources. A diversity of carbon sources are chosen, such as sucrose, citric acid, amino acids, and even waste food.

2.2.1. Microwave assisted synthesis. Microwave is a type of electromagnetic wave with a large wavelength range from 1 mm to 1 m commonly used in daily life and scientific research. Similar to the laser, microwave is also able to provide intensive energy to break off the chemical bonds of the substrate. It is regarded that microwave is an energy efficient approach to synthesise CDs, and also, the reaction time may be largely shortened. Generally, microwave assisted synthesis involves the pyrolysis and surface functionalization of the substrate.[49,50]

Wang *et al.* first reported a synthetic method for the formation of CDs from egg shell membrane ashes with the assistance of microwave.[51] The schematic illustration is shown in Fig. 3. Egg shell membrane ashes are a kind of protein-rich waste and can be easily obtained at low cost. They were broken into fragments by microwave, since the electrons rotated and vibrated vigorously under the switching electronic field. After further polymerization, oxidization and surface passivation under basic conditions, CDs were prepared. The PL spectrum of the products indicated that the microwave assisted synthetic route was a cost-effective, eco-friendly and resource-saving one.

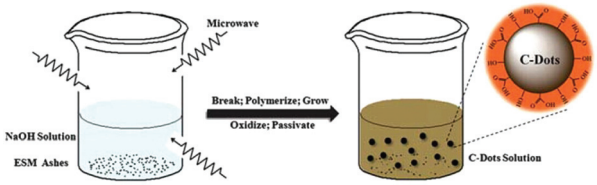

Fig. 3 Schematic way of a microwave-assisted process to form CDs. (Reproduced from ref. 51 with permission from The Royal Society of Chemistry.)

2.2.2. Thermal decomposition. Thermal decomposition has been used to synthesize different semiconductor and magnetic nanomaterials. Recent studies have found that external heat also contributes to the dehydration and carbonization of organics and turns them into CDs.[52]

Wang *et al.* injected carbon precursors into hot noncoordinating solvents and synthesized CDs by carbonizing precursors like citric acid.[53] The products could be stably used for at least two months. Liu *et al.* reported a combustion oxidation method to prepare CDs.[27] Soot was employed to be heated by a burning candle. Then, it was mixed with an oxidant to oxidize the particle surface. After further cooling, the products were purified by centrifugation or dialysis. Zhu *et al.* invented a hydrothermal method.[54] The reaction was conducted by condensing citric acid and ethylenediamine. The formed polymer was then carbonized to form CDs. The whole synthetic process involved ionization, condensation, polymerization and carbonization, which are shown in Fig. 4. Chen *et al.* developed another facile synthetic method for the formation of CDs in large scale quantities by heating sucrose and oleic acid at 215 °C under vigorous magnetic stirring and nitrogen protection.[55] Gram scale products were obtained.

2.2.3. Ultrasonic synthesis. Under certain frequencies of ultrasound irradiation, organic materials suffer from dehydration, polymerization, and carbonization sequentially, which

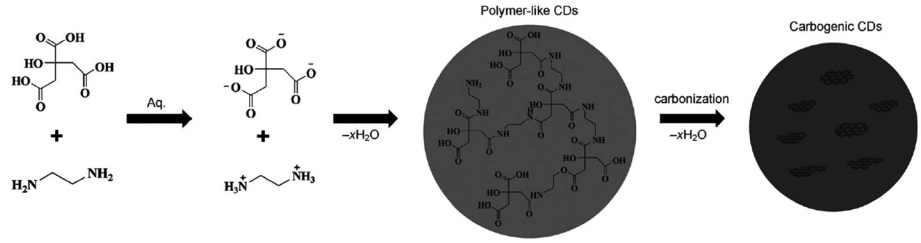

Fig. 4 The synthetic route of ionization, condensation, polymerization, and carbonization. (Reproduced from ref. 54 with permission from John Wiley and Sons Ltd.)

leads to the formation of a short single burst of nucleation. As a result, ultrasonic synthetic routes to prepare CDs are also raised. Li *et al.* introduced a one-step ultrasonic synthesis of CDs from glucose in 2011.[56] Park reported a large-scale green approach for the synthesis of CDs using waste food as carbon sources.[57] Ultrasound irradiation was performed at room temperature. Dehydration, polymerization, and carbonization sequentially occur on ultrasound at 40 kHz, forming nuclei, which then grow by the diffusion of solutes towards the carbon nanoparticle surface. Finally, 120 g of CDs were produced from 100 kg of food waste.

2.2.4. Others. More recently, Deng *et al.* synthesized CDs directly from alcohols.[46] Unlike the traditional electrochemical cutting-off mechanism, the CDs obtained *via* this method were more disordered and amorphous, which indicated that this method took place by a totally new route and the mechanism remains to be revealed. Low-molecular-weight alcohols were employed to be electrochemically carbonized under basic conditions. The synthesized CDs exhibit excellent excitation and size-dependent fluorescence properties.

2.3. Surface modification

Although surface passivation is not clearly understood, it has a significant impact on both the synthetic method and PL properties. Moreover, surface modification is the precondition for further biomedical application of CDs. The carboxyl groups on the surface of the CDs make them hydrophilic, which facilitates their applications in biological systems. In addition, by employing different deactivators, the solubility of CDs in a non-polar solvent is enhanced and the fluorescence characteristic is adjusted. The synthesis of CDs carrying different groups on the surface can also achieve different designed functions.[13] For example, suitable biodegradable polymeric precursors with different groups including amine, carboxylic acid and hydroxyl moieties can be selected for the surface functionalization. The surface of CDs can be deactivated by mixing with polyethylene glycol (PEG) containing amino groups.

3. Properties

CDs are usually composed of the elements C, H, N, O, in which C and O are the most abundant contents due to the presence of carboxylic acid moieties. These groups not only promise excellent water solubility, but also provide the possibility for further functionalization with different species. CDs can be analyzed by many different techniques, which could provide a diversity of information on the materials. TEM, XRD, Raman spectrum, FTIR spectrum of CDs prepared by a one-step microwave synthetic route are shown in Fig. 5.[58] The particle size could be easily observed from the TEM image. The XRD patterns displayed two broad peaks, which were attributed to highly disordered carbon atoms, similar to the graphite lattice spacing. From the Raman spectrum, the G band at 1575 cm^{-1} confirmed the in-plane vibration of the sp^2 carbon and the D band at 1365 cm^{-1} was related to the sp^3 defects. The relative intensity of the disordered D band and the crystalline G band (I_D/I_G) was calculated to be about 0.86, indicating that the structure of the prepared CDs was similar to that of graphite. The surface functional groups on CDs could be distinguished by the FTIR technique. Broad absorption bands at 1350–1460 cm^{-1}, 1600–1770 cm^{-1}, 3100–3500 cm^{-1} were separately assigned to $\delta(CH_2)$, $\nu(C{=}O)$ and $\nu(O–H)$, $\nu(N–H)$, respectively.

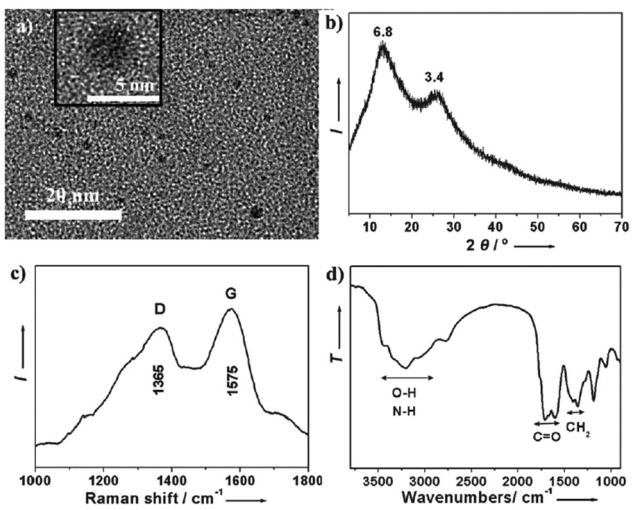

Fig. 5 (a) TEM image, (b) XRD patterns, (c) Raman spectrum, and (d) FTIR spectrum of the CDs. (Reproduced from ref. 58 with permission from John Wiley and Sons Ltd.)

3.1. Absorbance and PL properties

Organic dyes are usually employed as fluorescence signal sources.[59] However, the absorption spectrum of organic dyes is narrow and they do not have a sharp symmetric emission peak which may be further broadened by a red-tail.[60]

CDs usually show obvious optical absorption in the UV region and have an absorption band around 260–320 nm. After surface passivation, the wavelength may increase. PL emission, one of the most appealing features of CDs, includes λ_{ex}-dependent and independent PL, which are attributed to the core related and surface state related emissions.[27,45,61,62] The representative UV-vis absorption, photoluminescence excitation (PLE) and PL spectra of the CDs prepared by Liu *et al.* are shown in Fig. 6.[63] So far, the mechanisms of the PL of CDs are not fully understood. Zhao *et al.* claimed that the feature of λ_{ex}-dependence was due to the different sizes of CDs.[44] At certain excitation wavelengths, some emissive sites may be excited and fluoresce, which may also lead to λ_{ex}-dependent behaviours of the emission spectra.[15]

Quantum yield (QY) is a significant parameter to characterize PL. Naked CDs are reported to exhibit multicolor fluorescence emissions. However, the QY of such CDs is usually very low.[30,64] To increase the QY, several approaches have been attempted, such as passivation,[52] doping with other elements,[49] and purification procedures. Sun *et al.* functionalized CDs with diamine-terminated oligomeric PEG (PEG_{1500N}) or poly(propionylethyleneimine-*co*-ethyleneimine) (PPEI-EI). The products yielded bright emissions (QY: 4–10%).[62] Zheng proposed a reductive pathway to promote the QY from 2% to 24% *via* treating the CDs with $NaBH_4$ (Fig. 7).[65] Moreover, by adjusting the synthetic routes, the visible-to-near infrared (NIR) spectral range can also be achieved.[56] The NIR PL emission is particularly useful which makes CDs suitable for the imaging of biological samples with deeper depths in the NIR window. In addition, PL from CDs can be quenched by electron acceptors or donors. The photoinduced electron transfer

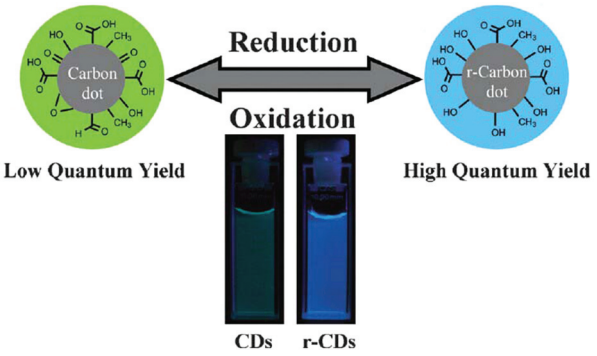

Fig. 7 CDs of the reduced state with high QY (Reproduced from ref. 65 with permission from The Royal Society of Chemistry.)

properties promise the materials new applications in energy conversion.

3.2. Chemiluminescence and electrochemical luminescence properties

Chemiluminescence (CL) is an important phenomenon and has been widely used in various analytical devices. CL properties of CDs have also been exploited recently.[66,67] Jiang *et al.* prepared CDs, which exhibit intense CL.[66] The schematic illustration of the $NaIO_4$–H_2O_2–CD CL system is depicted in Fig. 8. CL emission from the reaction between $NaIO_4$ and H_2O_2 could be remarkably enhanced in the presence of CDs, which was attributed to the various surface energy traps existing on the CDs. Besides, other CL systems like H_2O_2–HSO_3^- could also be enhanced by CDs.

Electrochemical luminescence (ECL) techniques were extensively used to study QDs due to their high sensitivity, simple equipment and wide linear range. Since the ECL behaviour of CDs is similar to that of QDs, the applications of ECL emission of CDs have been rising recently.[68] Ding's group prepared the Ag-CD composite by simply reducing the silver nitrate on the surface of CDs under basic conditions. This composite could be used for biosensing purposes, using ECL emission as the output signal. The Ag element on the surface of the nano-

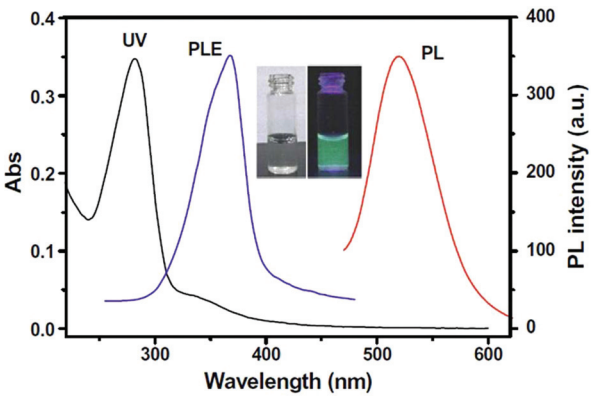

Fig. 6 UV-vis absorption, PLE (emission at 517 nm) and PL (excited at 360 nm) spectra of diethylene glycol stabilized CDs. Inset are the photographs under day light and UV lamp. (Reproduced from ref. 63 with permission from Elsevier.)

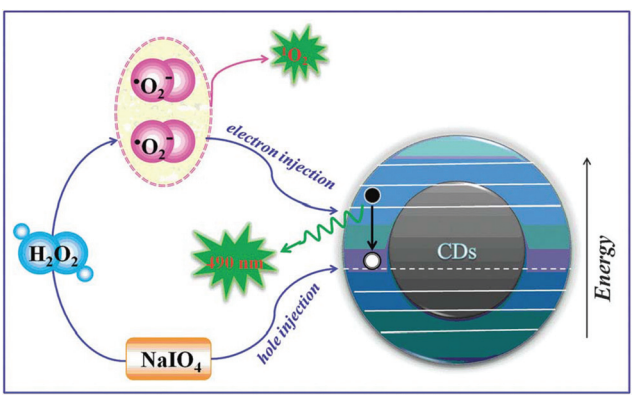

Fig. 8 Scheme of the $NaIO_4$–H_2O_2–CDs CL system. (Reproduced from ref. 66 with permission from The Royal Society of Chemistry.)

composites could selectively interact with the target S^{2-} ions, which then dramatically affected the ECL performance.

3.3. Peroxidase-like activity

Similar to platinum nanoparticles and Fe_3O_4 nanoparticles,[69] CDs are also found to have intrinsic peroxidase-like activity, which can be used to catalyse the oxidization of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2.[70] Shi *et al.* have taken advantage of this feature to generate color change in a colorimetric sensor for the detection of glucose.

3.4. Toxicity

Semiconductor QDs like CdSe may release Cd^{2+} and exhibit cytotoxicity. They may aggregate in biological systems and are difficult to be delivered out. They may also act as potential environmental hazard.[71] As a consequence of the environmental and health concerns, toxic metal-based QDs should be limited in many biomedical applications, and be replaced by nontoxic and eco-friendly materials.

CDs are excellent candidate materials. The toxicity of CDs has long been tested by various research groups. Ray *et al.* employed MTT and Trypan blue assays to assess the cell viability after the treatment of CDs.[30] 0.5 mg mL^{-1} of nanodots resulted in 75% cell survival rate, which suggested a limited toxicity effect. Moreover, Song *et al.* parallelly compared the toxic effects of unmodified CdTe QDs, gold nanoparticles and CDs on biological systems like cells and green gram sprouts.[72] The results clearly revealed that CDs were the most biocompatible materials among the three.

4. Biomedical applications

4.1. Biosensing

Due to the excellent PL properties of CDs, they have been widely used for fluorescence analysis of various targets including small molecules like ions,[73,74] H_2O_2 [75] and biomacromolecules like proteins.

Mercury ions (Hg^{2+}) are a kind of heavy metal ion, which cause serious contamination of the environment. The ions may accumulate in vital organs and tissues and lead to a diversity of diseases.[76] Qin *et al.* prepared CDs from flour, the PL emission of which could be selectively quenched by Hg^{2+} and a detection limit of 0.5 nM was achieved.[77] Tin ions (Sn^{2+}) are another heavy metal ion, which are also environmental pollutants and toxic to human health.[78] Yazid *et al.* reported a biosensor for Sn^{2+} *via* quenching of the fluorescence of CDs dehydrated from sago starch.[79] The concentration of Fe^{3+} could also be measured by the strong fluorescence quenching effect due to the formation of a complex of CDs and Fe^{3+}.[80] In contrast, Zhao *et al.* developed another biosensor for Fe^{3+} using CDs as the chemiluminescent probe.[81] The nanodots were functionalized by polyamine and the CL signals generated by the CDs were significantly improved by Fe^{3+}, which acted as an oxidant to inject holes into the CDs.

Hydrogen peroxide (H_2O_2) is one of the most stable reactive oxygen species (ROS) involved in cell proliferation, signal transduction, aging and death.[82] Nevertheless, excessive H_2O_2 may do harm to the central nervous system and cause a diversity of diseases.[83] Liu *et al.* realized a non-enzymatic H_2O_2 detection system by the use of CD-catalyzed Ag nanoparticles (AgNPs).[75] Nitrogen-doped CDs were found to possess the reductive activity that could catalytically reduce Ag^+ to form AgNPs, which showed better catalytic activity of reducing H_2O_2 compared with the ordinary AgNPs. The prepared nanocomposites could be used to sensitively monitor H_2O_2 levels with a detection limit of 0.5 μM.

Water soluble aliphatic primary amines are degraded mainly from amino acids and proteins, which widely exist in the environment. These hazardous products may cause harm to human health and to the skin, eyes, *etc* owing to their pungent and irritant odor.[84] Lin's group found that CDs are able to enhance the CL intensity of potassium peroxomonosulfate–sodiumsulfite–hydrochloric acid (PSHA) reactions dramatically.[67] This phenomenon originated from the energy transfer and electron-transfer annihilation effects of this system, which could be used as a detector for aliphatic primary amines (Fig. 9).

Aptamers are artificial single-stranded DNA or RNA that target different analytes with high affinity.[85,86] Xu *et al.* developed an aptasensor for the detection of thrombin (Fig. 10).[87] Thrombin is a specific serine endoprotease and acts as the key enzyme in pathological processes.[88] It contains multiple binding sites for aptamers. Two amino-modified thrombin aptamers were designed. They were modified on silica nanoparticles and CDs separately. Both of them could recognize thrombin by forming an intramolecular G-quadruplex. A linear relationship was established between 0 to 100 nM. The detection limit was evaluated to be 1 nM.

4.2. Bioimaging

The resolution, sensitivity and versatility of fluorescence microscopy mainly depend on the properties of the fluorescent

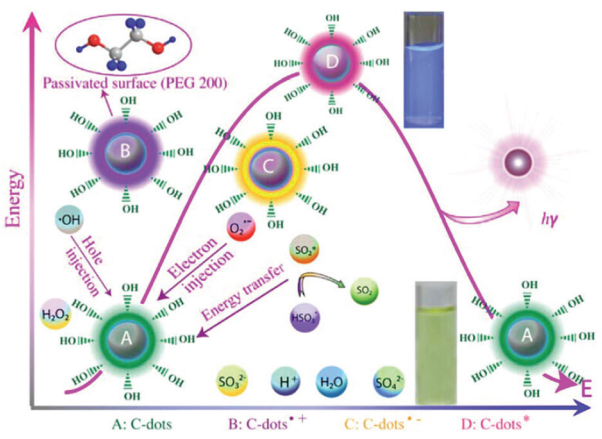

Fig. 9 Possible CL reaction mechanisms enhanced by CDs. (Reproduced from ref. 67 with permission from Elsevier.)

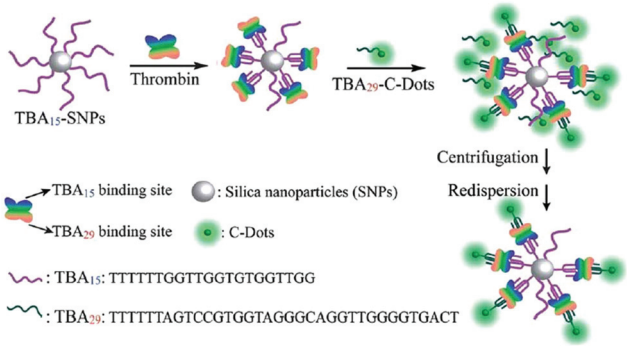

Fig. 10 Schematic representation of the sandwich-based thrombin detection using CDs. (Reproduced from ref. 87 with permission from The Royal Society of Chemistry.)

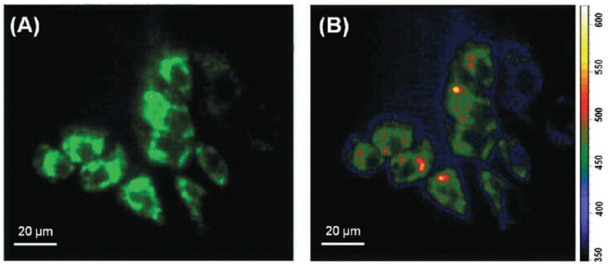

Fig. 11 Confocal PL images of LLC-PK1 cells after CD incubation. (Reproduced from ref. 12 with permission from The Royal Society of Chemistry.)

probes. Therefore, choosing suitable probes is significant for bioimaging purposes. CDs are promising candidates that have many advantages. First, CDs have low toxicity and side effects, which are suitable for biological systems. Second, the high stability and bright fluorescence promise results in the experiment and only a small number of CDs are needed to generate the signal. Third, excellent water solubility avoids complex surface modification. Fourth, CDs with NIR emission properties are promising candidates for the imaging of deeper tissue samples.

Na *et al.* prepared CDs that emit bright luminescence at 450 nm for the imaging of human serum proteins on gels.[89] After the polyacrylamide gel electrophoresis (PAGE) procedure, the proteins were stained by directly incubating with an acetic acid diluted CD solution. This method provided simple staining steps, low background and high resolution, which was a qualified protein imaging approach.

Zhu *et al.* prepared a two-photon "turn-on" fluorescent probe, which was then used for imaging hydrogen sulfide in live cells and tissues.[40] CDs were used as fluorophores due to their large two-photon absorption cross section. They were able to be quenched by the AE-TPEA–Cu^{2+} complex (AE-TPEA = *N*-(2-aminoethyl)-*N*,*N*,*N*′-tris(pyridine-2-ylmethyl)ethane-1,2-diamine). However, the interaction between H_2S and Cu^{2+} releases Cu^{2+} from the TPEA binding site, eliminating the quenching effect. The detection limit was as low as 0.7 μM and the "turn-on" two-photon fluorescence imaging of H_2S in live cells and tissues was achieved.

Hsu *et al.* tested the utility of CDs for the LLC-PK1 cell imaging.[12] The nanomaterials were likely internalized into the cells by endocytosis and green fluorescence was observed (Fig. 11). High cell viability also confirmed the low toxicity of the materials.

In order to further mitigate the cytotoxicity of nanomaterials in high concentration, Chandra *et al.* embedded GQDs in the PEG matrix to lower the ROS generated by the nanomaterials.[90] Moreover, the PEGylation process did not affect the size of GQDs and the size-dependent optical properties were maintained. After the internalization of PEG-GQDs into cells by incubating for 4 h, strong fluorescence from the cell cytoplasm could be observed.

For targeted imaging purposes, much work has been performed. Song *et al.* conjugated folic acid with CDs, which was then applied to distinguish folate-receptor-positive cancer cells from normal cells.[91] The receptor-mediated endocytosis of CDs promised a more accurate and selective cancer diagnostic approach.

4.3. Drug delivery

Concurrently, other theranostic attempts have been made using CDs as a multifunctional vehicle for drug loading and release.[92–94] The advantages include rapid cellular uptake, excellent biocompatibility, bright photoluminescence, high stability and limited influence of drug activity.[95,96]

He *et al.* successfully encapsulated CDs in zeolitic imidazolate frameworks, a subclass of metal–organic frameworks.[97] The PL and size of the nanocomposites were optimized by varying the concentration of CDs and precursors, which were then used carriers for a pH-responsive drug, 5-fluorouracil, to cancer cells. Different release efficiencies were observed in neutral and acidic environments. The pharmacological activity of the drug was also proved to be satisfactory, demonstrating the nanomaterials' potential use in a biomedical delivery platform.

Tang *et al.* developed a direct CD drug delivery system based on fluorescence resonance energy transfer (FRET).[32] CDs served as the donors of FRET pairs, which also carried drugs. Since the distance between the donor and the acceptor significantly affected the FRET signals,[98] the release of drugs like doxorubicin from CDs could be sensitively monitored in real time. Moreover, the results of the two-photon imaging of the FRET-CD drug delivery system in tumor tissues of 65–300 μm promised the improved imaging sensitivity and therapeutic efficacy.

5. Conclusion and outlook

In summary, we have reviewed the recent research progress of CDs, focusing on their synthesis, functionalization, typical properties, and biomedical applications. Huge effort by the research community has been made and great progress has

been achieved. However, further improvement and exploitation are still urgently desired. The low toxicity of CDs is supported by many references, but the resistance to degradation and accumulation of CDs may lead to certain metabolism turbulence. Therefore, *in vivo* applications may require a deeper investigation. In addition, the formation mechanisms and the key factors in the synthesis are not fully understood, and the production of CDs with controlled size and properties is still beyond reach. For example, common strategies to control the size of nanomaterials are not effective, such as temperature and concentrations of reactants.[99] An investigation of the properties, and the development of a novel synthesis and surface modification route may broaden the biomedical applications of CDs, which are of great value in biosensing, bioimaging, drug delivery and advanced therapy.

Acknowledgements

This work was supported by the National Key Instrument Developing Project of China (grant no. ZDYZ2013-1).

References

1 R. H. Baughman, A. A. Zakhidov and W. A. de Heer, *Science*, 2002, **297**, 787–792.
2 K. Y. Shi and I. Zhitomirsky, *ACS Appl. Mater. Interfaces*, 2013, **5**, 13161–13170.
3 A. D. Winter, E. Larios, F. M. Alamgir, C. Jaye, D. Fischer and E. M. Campo, *Langmuir*, 2013, **29**, 15822–15830.
4 A. K. Geim, *Science*, 2009, **324**, 1530–1534.
5 K. Han, P. Miao, H. Tong, T. Liu, W. B. Cheng, X. L. Zhu and Y. G. Tang, *Appl. Phys. Lett.*, 2014, **104**, 053101.
6 W. Chen, H. Chen, H. P. Lan, P. Cui, T. P. Schulze, W. G. Zhu and Z. Y. Zhang, *Phys. Rev. Lett.*, 2012, **109**, 265507.
7 T. Da Ros and M. Prato, *Chem. Commun.*, 1999, 663–669.
8 A. V. Savin and Y. S. Kivshar, *Sci. Rep.*, 2012, **2**, 1012.
9 S. Osswald, G. Yushin, V. Mochalin, S. O. Kucheyev and Y. Gogotsi, *J. Am. Chem. Soc.*, 2006, **128**, 11635–11642.
10 M. Aramesh, K. Fox, D. W. M. Lau, J. H. Fang, K. Ostrikov, S. Prawer and J. Cervenka, *Carbon*, 2014, **75**, 452–464.
11 J. Xiao, G. Ouyang, P. Liu, C. X. Wang and G. W. Yang, *Nano Lett.*, 2014, **14**, 3645–3652.
12 P. C. Hsu, Z. Y. Shih, C. H. Lee and H. T. Chang, *Green Chem.*, 2012, **14**, 917–920.
13 S. Chandra, S. H. Pathan, S. Mitra, B. H. Modha, A. Goswami and P. Pramanik, *RSC Adv.*, 2012, **2**, 3602–3606.
14 M. M. Liu and W. Chen, *Nanoscale*, 2013, **5**, 12558–12564.
15 S. N. Baker and G. A. Baker, *Angew. Chem., Int. Ed.*, 2010, **49**, 6726–6744.
16 X. Y. Xu, R. Ray, Y. L. Gu, H. J. Ploehn, L. Gearheart, K. Raker and W. A. Scrivens, *J. Am. Chem. Soc.*, 2004, **126**, 12736–12737.
17 L. P. Lin, M. C. Rong, F. Luo, D. M. Chen, Y. R. Wang and X. Chen, *TrAC, Trends Anal. Chem.*, 2014, **54**, 83–102.
18 H. T. Li, Z. H. Kang, Y. Liu and S. T. Lee, *J. Mater. Chem.*, 2012, **22**, 24230–24253.
19 A. Philippidis, D. Stefanakis, D. Anglos and D. Ghanotakis, *J. Nanopart. Res.*, 2013, **15**, 1414.
20 L. L. Zhu, Y. J. Yin, C. F. Wang and S. Chen, *J. Mater. Chem. C*, 2013, **1**, 4925–4932.
21 J. Tang, Y. Y. Zhang, B. Kong, Y. C. Wang, P. M. Da, J. Li, A. A. Elzatahry, D. Y. Zhao, X. G. Gong and G. F. Zheng, *Nano Lett.*, 2014, **14**, 2702–2708.
22 Q. Li, T. Y. Ohulchanskyy, R. L. Liu, K. Koynov, D. Q. Wu, A. Best, R. Kumar, A. Bonoiu and P. N. Prasad, *J. Phys. Chem. C*, 2010, **114**, 12062–12068.
23 Z. P. Zhang, J. Zhang, N. Chen and L. T. Qu, *Energy Environ. Sci.*, 2012, **5**, 8869–8890.
24 V. Gupta, N. Chaudhary, R. Srivastava, G. D. Sharma, R. Bhardwaj and S. Chand, *J. Am. Chem. Soc.*, 2011, **133**, 9960–9963.
25 J. Zhao, G. F. Chen, L. Zhu and G. X. Li, *Electrochem. Commun.*, 2011, **13**, 31–33.
26 P. Yu, X. M. Wen, Y. R. Toh and J. Tang, *J. Phys. Chem. C*, 2012, **116**, 25552–25557.
27 H. P. Liu, T. Ye and C. D. Mao, *Angew. Chem., Int. Ed.*, 2007, **46**, 6473–6475.
28 Y. Li, Y. Hu, Y. Zhao, G. Q. Shi, L. E. Deng, Y. B. Hou and L. T. Qu, *Adv. Mater.*, 2011, **23**, 776–780.
29 W. Kwon, G. Lee, S. Do, T. Joo and S. W. Rhee, *Small*, 2014, **10**, 506–513.
30 S. C. Ray, A. Saha, N. R. Jana and R. Sarkar, *J. Phys. Chem. C*, 2009, **113**, 18546–18551.
31 C. Yuan, B. H. Liu, F. Liu, M. Y. Han and Z. P. Zhang, *Anal. Chem.*, 2014, **86**, 1123–1130.
32 J. Tang, B. Kong, H. Wu, M. Xu, Y. C. Wang, Y. L. Wang, D. Y. Zhao and G. F. Zheng, *Adv. Mater.*, 2013, **25**, 6569–6574.
33 A. L. Antaris, J. T. Robinson, O. K. Yaghi, G. S. Hong, S. Diao, R. Luong and H. J. Dai, *ACS Nano*, 2013, **7**, 3644–3652.
34 J. H. Li, G. Wang, H. Q. Zhu, M. Zhang, X. H. Zheng, Z. F. Di, X. Y. Liu and X. Wang, *Sci. Rep.*, 2014, **4**, 4359.
35 A. Kleinauskas, S. Rocha, S. Sahu, Y. P. Sun and P. Juzenas, *Nanotechnology*, 2013, **24**, 325103.
36 Y. S. Liu, Y. A. Zhao and Y. Y. Zhang, *Sens. Actuator, B*, 2014, **196**, 647–652.
37 L. Cao, X. Wang, M. J. Meziani, F. S. Lu, H. F. Wang, P. J. G. Luo, Y. Lin, B. A. Harruff, L. M. Veca, D. Murray, S. Y. Xie and Y. P. Sun, *J. Am. Chem. Soc.*, 2007, **129**, 11318–11319.
38 S. Chandra, P. Das, S. Bag, D. Laha and P. Pramanik, *Nanoscale*, 2011, **3**, 1533–1540.
39 Q. Hu, M. C. Paau, Y. Zhang, W. Chan, X. J. Gong, L. Zhang and M. M. F. Choi, *J. Chromatogr. A*, 2013, **1304**, 234–240.
40 A. W. Zhu, Z. Q. Luo, C. Q. Ding, B. Li, S. Zhou, R. Wang and Y. Tian, *Analyst*, 2014, **139**, 1945–1952.

41 D. B. Shinde and V. K. Pillai, *Chem. – Eur. J.*, 2012, **18**, 12522–12528.
42 H. Ming, Z. Ma, Y. Liu, K. M. Pan, H. Yu, F. Wang and Z. H. Kang, *Dalton Trans.*, 2012, **41**, 9526–9531.
43 J. G. Zhou, C. Booker, R. Y. Li, X. T. Zhou, T. K. Sham, X. L. Sun and Z. F. Ding, *J. Am. Chem. Soc.*, 2007, **129**, 744–745.
44 Q. L. Zhao, Z. L. Zhang, B. H. Huang, J. Peng, M. Zhang and D. W. Pang, *Chem. Commun.*, 2008, 5116–5118.
45 L. Bao, Z. L. Zhang, Z. Q. Tian, L. Zhang, C. Liu, Y. Lin, B. P. Qi and D. W. Pang, *Adv. Mater.*, 2011, **23**, 5801–5806.
46 J. H. Deng, Q. J. Lu, N. X. Mi, H. T. Li, M. L. Liu, M. C. Xu, L. Tan, Q. J. Xie, Y. Y. Zhang and S. Z. Yao, *Chem. – Eur. J.*, 2014, **20**, 4993–4999.
47 Z. A. Qiao, Y. F. Wang, Y. Gao, H. W. Li, T. Y. Dai, Y. L. Liu and Q. S. Huo, *Chem. Commun.*, 2010, **46**, 8812–8814.
48 S. L. Hu, K. Y. Niu, J. Sun, J. Yang, N. Q. Zhao and X. W. Du, *J. Mater. Chem.*, 2009, **19**, 484–488.
49 X. Y. Zhai, P. Zhang, C. J. Liu, T. Bai, W. C. Li, L. M. Dai and W. G. Liu, *Chem. Commun.*, 2012, **48**, 7955–7957.
50 P. Zhang, W. C. Li, X. Y. Zhai, C. J. Liu, L. M. Dai and W. G. Liu, *Chem. Commun.*, 2012, **48**, 10431–10433.
51 Q. Wang, X. Liu, L. C. Zhang and Y. Lv, *Analyst*, 2012, **137**, 5392–5397.
52 S. T. Yang, X. Wang, H. F. Wang, F. S. Lu, P. J. G. Luo, L. Cao, M. J. Meziani, J. H. Liu, Y. F. Liu, M. Chen, Y. P. Huang and Y. P. Sun, *J. Phys. Chem. C*, 2009, **113**, 18110–18114.
53 F. Wang, S. P. Pang, L. Wang, Q. Li, M. Kreiter and C. Y. Liu, *Chem. Mater.*, 2010, **22**, 4528–4530.
54 S. J. Zhu, Q. N. Meng, L. Wang, J. H. Zhang, Y. B. Song, H. Jin, K. Zhang, H. C. Sun, H. Y. Wang and B. Yang, *Angew. Chem., Int. Ed.*, 2013, **52**, 3953–3957.
55 B. S. Chen, F. M. Li, S. X. Li, W. Weng, H. X. Guo, T. Guo, X. Y. Zhang, Y. B. Chen, T. T. Huang, X. L. Hong, S. Y. You, Y. M. Lin, K. H. Zeng and S. Chen, *Nanoscale*, 2013, **5**, 1967–1971.
56 H. T. Li, X. D. He, Y. Liu, H. Huang, S. Y. Lian, S. T. Lee and Z. H. Kang, *Carbon*, 2011, **49**, 605–609.
57 S. Y. Park, H. U. Lee, E. S. Park, S. C. Lee, J. W. Lee, S. W. Jeong, C. H. Kim, Y. C. Lee, Y. S. Huh and J. Lee, *ACS Appl. Mater. Interfaces*, 2014, **6**, 3365–3370.
58 S. N. Qu, X. Y. Wang, Q. P. Lu, X. Y. Liu and L. J. Wang, *Angew. Chem., Int. Ed.*, 2012, **51**, 12215–12218.
59 I. Fischer, K. Petkau-Milroy, Y. L. Dorland, A. P. H. J. Schenning and L. Brunsveld, *Chem. – Eur. J.*, 2013, **19**, 16646–16650.
60 U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke and T. Nann, *Nat. Methods*, 2008, **5**, 763–775.
61 Y. X. Fang, S. J. Guo, D. Li, C. Z. Zhu, W. Ren, S. J. Dong and E. K. Wang, *ACS Nano*, 2012, **6**, 400–409.
62 Y. P. Sun, B. Zhou, Y. Lin, W. Wang, K. A. S. Fernando, P. Pathak, M. J. Meziani, B. A. Harruff, X. Wang, H. F. Wang, P. J. G. Luo, H. Yang, M. E. Kose, B. L. Chen, L. M. Veca and S. Y. Xie, *J. Am. Chem. Soc.*, 2006, **128**, 7756–7757.
63 Y. Liu, N. Xiao, N. Q. Gong, H. Wang, X. Shi, W. Gu and L. Ye, *Carbon*, 2014, **68**, 258–264.
64 R. Shen, K. Song, H. R. Liu, Y. S. Li and H. W. Liu, *ChemPhysChem*, 2012, **13**, 3549–3555.
65 H. Z. Zheng, Q. L. Wang, Y. J. Long, H. J. Zhang, X. X. Huang and R. Zhu, *Chem. Commun.*, 2011, **47**, 10650–10652.
66 J. Jiang, Y. He, S. Y. Li and H. Cui, *Chem. Commun.*, 2012, **48**, 9634–9636.
67 Y. Zhou, G. W. Xing, H. Chen, N. Ogawa and J. M. Lin, *Talanta*, 2012, **99**, 471–477.
68 Z. X. Wang, C. L. Zheng, Q. L. Li and S. N. Ding, *Analyst*, 2014, **139**, 1751–1755.
69 P. Miao, M. Shen, L. M. Ning, G. F. Chen and Y. M. Yin, *Anal. Bioanal. Chem.*, 2011, **399**, 2407–2411.
70 W. B. Shi, Q. L. Wang, Y. J. Long, Z. L. Cheng, S. H. Chen, H. Z. Zheng and Y. M. Huang, *Chem. Commun.*, 2011, **47**, 6695–6697.
71 A. M. Derfus, W. C. W. Chan and S. N. Bhatia, *Nano Lett.*, 2004, **4**, 11–18.
72 Y. C. Song, D. Feng, W. Shi, X. H. Li and H. M. Ma, *Talanta*, 2013, **116**, 237–244.
73 M. Vedamalai, A. P. Periasamy, C. W. Wang, Y. T. Tseng, L. C. Ho, C. C. Shih and H. T. Chang, *Nanoscale*, 2014, **6**, 13119–13125.
74 S. W. Zhang, J. X. Li, M. Y. Zeng, J. Z. Xu, X. K. Wang and W. P. Hu, *Nanoscale*, 2014, **6**, 4157–4162.
75 S. Liu, B. Yu and T. Zhang, *RSC Adv.*, 2014, **4**, 544–548.
76 P. Miao, L. Liu, Y. Li and G. X. Li, *Electrochem. Commun.*, 2009, **11**, 1904–1907.
77 X. Y. Qin, W. B. Lu, A. M. Asiri, A. O. Al-Youbi and X. P. Sun, *Sens. Actuator, B*, 2013, **184**, 156–162.
78 S. Ulusoy, H. I. Ulusoy, M. Akcay and R. Gurkan, *Food Chem.*, 2012, **134**, 419–426.
79 S. N. A. M. Yazid, S. F. Chin, S. C. Pang and S. M. Ng, *Microchim. Acta*, 2013, **180**, 137–143.
80 J. Y. Xu, Y. Zhou, S. X. Liu, M. T. Dong and C. B. Huang, *Anal. Methods*, 2014, **6**, 2086–2090.
81 L. X. Zhao, F. L. Geng, F. Di, L. H. Guo, B. Wan, Y. Yang, H. Zhang and G. Z. Sun, *RSC Adv.*, 2014, **4**, 45768–45771.
82 J. Zhao, Y. L. Yan, L. Zhu, X. X. Li and G. X. Li, *Biosens. Bioelectron.*, 2013, **41**, 815–819.
83 Y. P. Luo, H. Q. Liu, Q. Rui and Y. Tian, *Anal. Chem.*, 2009, **81**, 3035–3041.
84 R. Freeman, X. Q. Liu and I. Willner, *J. Am. Chem. Soc.*, 2011, **133**, 11597–11604.
85 J. J. Li, J. You, Y. P. Zhuang, C. P. Han, J. F. Hu, A. Wang, K. Xu and J. J. Zhu, *Chem. Commun.*, 2014, **50**, 7107–7110.
86 H. Yoo, H. Jung, S. A. Kim and H. Mok, *Chem. Commun.*, 2014, **50**, 6765–6767.
87 B. L. Xu, C. Q. Zhao, W. L. Wei, J. S. Ren, D. Miyoshi, N. Sugimoto and X. G. Qu, *Analyst*, 2012, **137**, 5483–5486.
88 Y. W. Zhang and X. P. Sun, *Chem. Commun.*, 2011, **47**, 3927–3929.
89 N. Na, T. T. Liu, S. H. Xu, Y. Zhang, D. C. He, L. Y. Huang and O. Y. Jin, *J. Mater. Chem. B*, 2013, **1**, 787–792.

90 A. Chandra, S. Deshpande, D. B. Shinde, V. K. Pillai and N. Singh, *ACS Macro Lett.*, 2014, **3**, 1064–1068.
91 Y. C. Song, W. Shi, W. Chen, X. H. Li and H. M. Ma, *J. Mater. Chem.*, 2012, **22**, 12568–12573.
92 Y. Choi, S. Kim, M. H. Choi, S. R. Ryoo, J. Park, D. H. Min and B. S. Kim, *Adv. Funct. Mater.*, 2014, **24**, 5781–5789.
93 Q. L. Wang, X. X. Huang, Y. J. Long, X. L. Wang, H. J. Zhang, R. Zhu, L. P. Liang, P. Teng and H. Z. Zheng, *Carbon*, 2013, **59**, 192–199.
94 S. Pandey, M. Thakur, A. Mewada, D. Anjarlekar, N. Mishra and M. Sharon, *J. Mater. Chem. B*, 2013, **1**, 4972–4982.
95 N. Gogoi and D. Chowdhury, *J. Mater. Chem. B*, 2014, **2**, 4089–4099.
96 S. Karthik, B. Saha, S. K. Ghosh and N. D. P. Singh, *Chem. Commun.*, 2013, **49**, 10471–10473.
97 L. He, T. T. Wang, J. P. An, X. M. Li, L. Y. Zhang, L. Li, G. Z. Li, X. T. Wu, Z. M. Su and C. G. Wang, *CrystEngComm*, 2014, **16**, 3259–3263.
98 J. Wang, Z. H. Zhang, S. Zha, Y. Y. Zhu, P. Y. Wu, B. Ehrenberg and J. Y. Chen, *Biomaterials*, 2014, **35**, 9372–9381.
99 L. Scarabelli, M. Grzelczak and L. M. Liz-Marzan, *Chem. Mater.*, 2013, **25**, 4232–4238.