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Synthesis of luminescent 3D microstructures formed by carbon quantum dots and their self-assembly properties†

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We report in this communication the synthesis of star-shaped carbon quantum dots–(poly-γ-benzyl-L-glutamate) conjugates that self-assemble into microstructures and retain the characteristic emission properties of the native dots. Dots were used either as an initiator to give a daisy-like peptide–polymer structure or as capping agents towards more elaborated hybrid nanostructures.

Since their discovery,[1] carbon quantum dots (CQDs) have represented a fascinating class of water-soluble carbon nanostructures, which are characterized by a discrete, quasi circular shape with diameters of up to 10 nm.[2] CQDs are non-toxic[3] carbon nanostructures that show peculiar photoluminescence (PL) properties, such as multi-colour emission that varies with the excitation wavelength.[4] They have also been proposed as interesting candidates for sensing, bioimaging and, in general, for applications where the size, cost, and biocompatibility of the label are critical issues.[5] PL properties of CQDs have been attributed to the presence of surface energy traps that become emissive upon stabilization as a result of surface passivation.[4] This requirement, in order for CQDs to become PL active, is reportedly shared by silicon nanocrystals, for which a widely accepted PL mechanism is the radiative recombination of excitons.[6] Several procedures to prepare CQDs have been reported, according to both bottom-up[7] and top-down[8] approaches. Recently, nitrogen-rich organic molecules and natural precursors (*e.g.* carbohydrates, peanut skin, soy milk) have been used to prepare highly PL active CQDs by hydrothermal treatment at high temperature for several hours, without further surface passivation.[9] Also, microwave-assisted hydrothermal transformation of amino acids has been proposed as a direct route for the production of CQDs starting from abundant and inexpensive precursors.[10] CQDs have been used either in their pristine form or blended with inorganic materials. On the other hand, they have been studied rarely in combination with organic polymers.[11] In this context, we have explored the synthesis of CQDs–polypeptide hybrids, presenting here a novel CQD–poly-γ-benzyl-L-glutamate conjugated system (CQD–PBLG) where several polymer chains are grown in a star shaped fashion over a discrete CQD core. We further modified this structure by capping the loose end of the polymer with additional CQDs, thus obtaining functional nanostructures composed by the CQD-core, PBLG-spacers and an outer CQDs-shell. In this case, the morphology of the resulting material was assayed by transmission electron microscopy (TEM) after growing silver nanoparticles (AgNPs) directly on the CQD shell. Interestingly, the CQD–PBLG and CQD–PBLG–CQDs nanosystems self-assemble in solution to give spherical aggregates that retain the peculiar emission properties of the starting CQD material. We envisioned the use of *N*-carboxyanhydride ring opening polymerization (NCA-ROP) to grow polymeric structures starting from amino groups present on the surface of CQDs.

We therefore synthesized the starting CQDs by using arginine and 1,2-ethylendiamine precursors, as schematically reported in Fig. 1A, in order to maximize the number of available amine moieties. TEM analysis of the products (Fig. 1C) revealed round particles with uniform sizes and a diameter of 1.4 ± 0.2 nm. High resolution TEM analysis (Fig. 1D) and Fast Fourier Transform (FFT) (Fig. 1D, inset) highlight an interplanar distance of 0.21 nm, compatible with the (0001) graphite basal plane. The elemental analysis yield was 71.47% for C, 5.05% for H, 17.09% for N and 5.39% for residual elements, while MALDI analysis (ESI,† Fig. S1) displayed a MW dispersion, as expected from the random combination of different atoms, centred at 1150 Da.[5c] Solid state FT-IR data (ESI,† Fig. S2) are consistent with the presence of amines. XPS measurements were carried out to provide a deeper insight into the different chemical species introduced by the synthesis. The XPS survey spectrum reported in Fig. 2a shows the C 1s, N 1s and O 1s photoemission lines to be centred at binding energies (BEs) of 284.9 eV, 399.8 eV and 531.6 eV, respectively.

The observed elemental composition (C 74.3%, N 18.8% and O 6.9%) is in good agreement with that of the previous elemental analysis. The detailed scans of C 1s and N 1s lines were deconvoluted into single chemically-shifted components, as reported in

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† Electronic supplementary information (ESI) available: Synthetic details, SEC traces, UV-Vis absorption and emission spectra of polymers (**1** and **2**) are reported in the ESI. See DOI: 10.1039/c4cc02496f

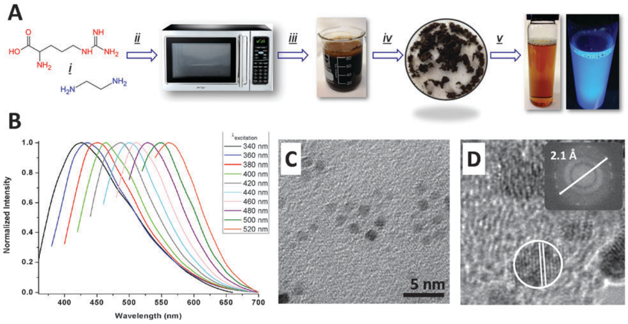

Fig. 1 (A) Schematic representation of CQDs synthesis. Reagents and conditions: (i) arginine and 1,2-ethylendiamine (1 : 1 mol mol^{-1}) in ultrapure water; (ii) domestic microwave, 700 watt; (iii) dissolution in ultrapure water and centrifugation; (iv) lyophilisation; (v) dissolution in ultrapure water (left: under irradiation, visible-light; right: under irradiation, UV-light at 365 nm). (B) Normalized emission spectra under different λ_{ex}. (C) and (D) CQDs TEM and HRTEM images, respectively.

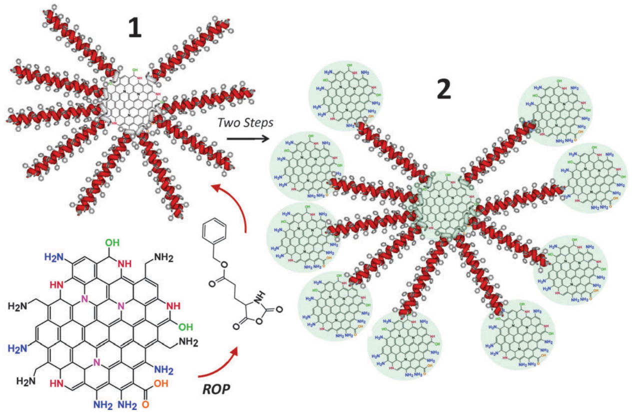

Fig. 3 Left part: hypothetic representation for the chemical structure of a single CQD, and ROP of BLG-NCA initiated by CQDs which yield **1**. Right part: schematic illustration of **2**.

Fig. 2b and c. The multicomponent analysis of the C 1s line confirms the presence of C–N bonds (285.2 eV) as well as that of several oxygen-bearing functional groups, such as C–O (286.5 eV), C=O (288.0 eV) and O–C=O (288.9 eV).[12] The weak component on the low BE slope of the main peak can be attributed to C atoms around vacancy sites (283.8 eV).[13] The fit of the N 1s line, on the other hand, shows five different chemical components centred at BEs of 398.0 eV, 399.1 eV, 400.2 eV, 401.3 eV and 402.2 eV, corresponding to pyridinic, amine, pyrrolic, graphitic and pyridine oxide groups, respectively.

In particular, the ratios between these last functional groups suggest that, on average, each CQD contains 8–10 –NH_2 groups (either aliphatic or aromatic, see Fig. 3 for a schematic representation). The UV-Vis absorption spectrum of the as-prepared CQDs (ESI,† Fig. S3) shows an absorption shoulder around 350 nm, typical of aromatic structures. Normalized emission spectra are shown in Fig. 1B and display the distinctive features of CQDs, such as the dependence of the emission maxima on the excitation wavelength (for non-normalized spectra, see Fig. S4, ESI†). We evaluated the emission quantum yield upon 360 nm excitation as 30.1% using quinine sulphate as a reference, in line with data reported earlier using similar synthetic methods.[2,11]

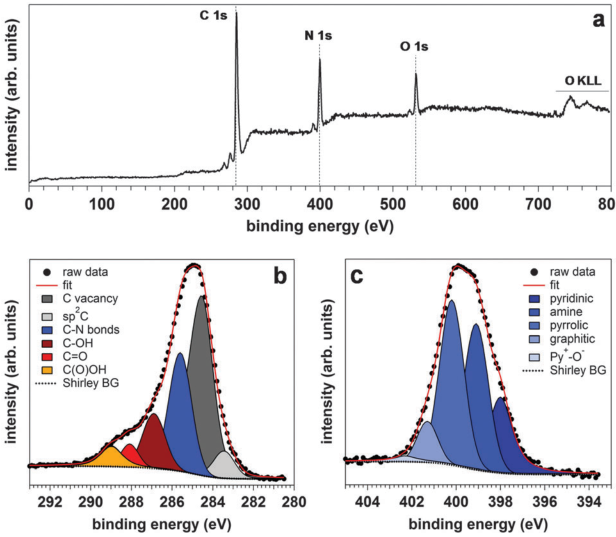

Fig. 2 (a) XPS survey scan. (b) Multipeak analysis for the carbon 1s and (c) nitrogen 1s photoemission lines.

Primary amines can be used to promote controlled NCA-ROP processes.[14] Thus, BLG-NCA was polymerized using CQDs as the amino-group-bearing initiator. NMR, FT-IR and circular dichroism (CD) spectra of the hybrid material **1** (schematically shown in Fig. 3) confirmed the formation of PBLG arms from the CQDs core (ESI,† Fig. S5–S7), the CD data being related to the secondary, rod-like, α-helical, structure of PBLG. Thermogravimetric analysis (TGA) yields a BLG:CQDs weight ratio of 97 : 3 (ESI,† Fig. S8). While we were not able to detect the MW distribution for **1** by MALDI experiments, size exclusion chromatography (SEC) showed an average MW of around 45 KDa (ESI,† Fig. S9).

Like pristine CQD, **1** shows a dependence of its emission spectra on λ_{ex} (ESI,† Fig. S10). The CQD–PBLG–NH_2 structure of **1** was further modified at its N-terminal position *via* reaction with a large excess of succinic anhydride in order to obtain the corresponding carboxylic derivative, CQD–PBLG–COOH. A negative Kaiser test (ninhydrin) confirmed the absence of primary amine groups upon reaction with the anhydride. Interestingly, CQD–PBLG–COOH did not display significant emission spectra, hinting at the possible modification of the amino groups of the CQD cores themselves, besides those capping PBLG chains. The carboxylic groups of this intermediate were further derivatized, by activation with diphenylphosphoryl azide and reaction with a large excess of CQDs. The SEC trace of the resulting material **2** confirmed the addition of several CQDs per core, by showing an increase of the MW, in comparison to **1**, of *ca.* 10 KDa (ESI,† Fig. S9). TGA analysis corroborated this finding, indicating a 22% increase of the CQD abundance with respect to PBLG in comparison to hybrid **1**. By assuming a MW of 1150 Da for CQDs, we estimate a covalent attachment of an average of nine peripheral CQDs per core for hybrid **2**. Another indirect confirmation of the attachment of new CQDs is the restoration of the typical CQD multicolour emission that had been shut off by the modification of the amino groups in **1** with succinic anhydride

(ESI,† Fig. S9). In order to observe the structure of **2**, we exploited the electron-donating capabilities of photoexcited CQDs, which enable the reduction of silver salts to the corresponding AgNPs on the surface of the CQDs themselves.[15] To this end, a THF solution of **2** was photoexcited using ultraviolet irradiation in the presence of silver nitrate (Experimental details in the ESI†). The formation of AgNPs brought about a change in hue from yellow to brown (Fig. 4, upper part). AgNPs are expected to form on the surface of the external layer of **2**, which is composed by CQDs. In Fig. 4 (lower part), the TEM analysis of the resulting organic–inorganic hybrid **3** shows the formation of star-shaped nanostructures with sizes in the 30–60 nm range, carrying several 5 nm AgNPs. According to previous studies,[15] the templating and clustering effect of the CQD supports has a dramatic effect on the plasmonic properties of the NPs. This is demonstrated by a broad surface plasmon resonance peak in the UV-Vis absorption spectrum of **3**, between 400 and 520 nm, reported in Fig. 4. The remarkable broadening of the UV-Vis spectrum of **3** compared to that of free-standing AgNPs in a similar size range (with a sharp absorption peak at 400 nm), is due to the compact arrangement of the AgNPs in **3**, which results in strong plasmonic coupling interactions.[16]

Finally, we made use of the self-aggregation properties of PBLG[17] to generate self-assembled microstructures from hybrids **1** and **2**. To this end, we solubilized **1** or **2** in a THF:DMF mixture and dialyzed against water for 48 h. The resulting milky, light-brown (**1**) and dark-brown (**2**) solutions were submitted to a size exclusion gel-filtration (water as the eluant) and the main fractions were collected and examined by TEM (without uranyl-acetate treatment) and scanning electron microscopy (SEM), as shown in Fig. 5. In both cases spherical aggregates with diameters ranging from 100 to 300 nm were observed. When **1** was used, the surfaces of the aggregates showed a smooth profile (Fig. 5A), whereas in the case of **2** the surfaces appeared rough, with limited flat areas (Fig. 5B). This is compatible with the exposition of the hydrophilic CQDs on the surface of the aggregate formed by **2**. Furthermore, the aggregated structures obtained in this last case, still displayed the multicolour emission characteristics described previously for CQDs and hybrids **1** and **2**. We were able to picture it directly in the fluorimeter by using a digital camera, as shown on the top of Fig. 5 (see also ESI,† Fig. S11, ESI†).

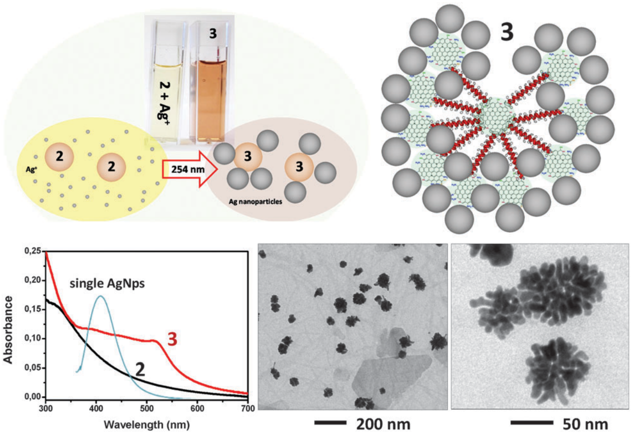

Fig. 4 Upper part: schematic representation of the photoreaction to generate **3**. Bottom part left: UV-Vis absorption spectra for **2**, **3** and single AgNPs. Bottom part right: two TEM images of single units of **3**.

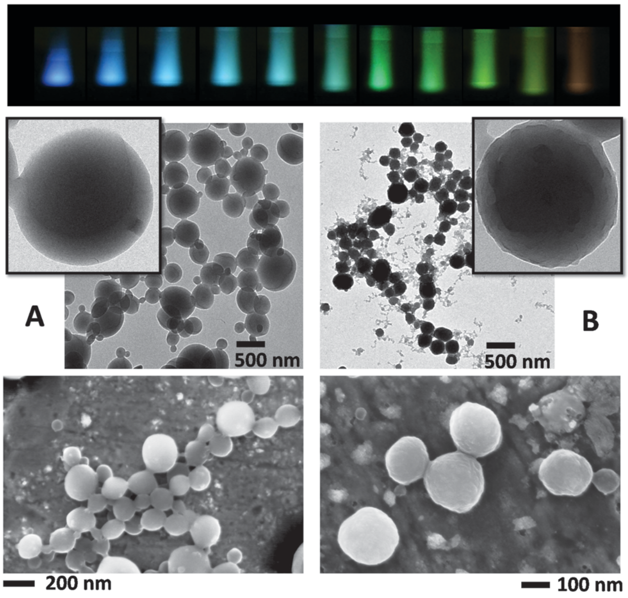

Fig. 5 (A) and (B) TEM and SEM images of microstructures generated by self-assembling of **1** and **2**, respectively. Upper part: emission colours occurring from **2** microstructures.

In conclusion, in this work we reported the synthesis and characterization of carbon quantum dots bearing amino groups at their edges, as demonstrated by elemental and XPS analyses. Functionalization of the dots with poly-γ-benzyl-L-glutamate gave the first reported CQD–polypeptide polymeric systems in a star shaped fashion that were further decorated with an outer shell of CQDs. We tracked the spectroscopic properties of the materials throughout the syntheses, and exploited the electron-donating capabilities of photoexcited CQDs to grow silver nanoparticles. These, in turn, allowed visualization of the structure of the resulting hybrid materials by TEM imaging, thanks to their contrasting effect. Finally, we assembled supramolecular aggregates of spherical shape with evidence of rough surface profiles in the case of CQD–capped hybrid materials, which may indicate the presence of aggregated areas of CQDs on the external layer. These microstructures are able to retain the characteristic emission properties of the native CQDs. The approach developed in this study is a facile, but highly efficient approach in the synthesis of fluorescent, peptide-based materials, which are attractive for many applications including optical sensing, biolabelling, imaging, targeted drug delivery/tracking and even UV protection for optical devices. We note that this synthetic approach is also highly versatile, since both α-amino acids and non-natural amino acids can be used, resulting in quantum dots with tailored surface chemistries that can be quantitatively assayed.

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