Microscopic investigation of the emission efficiency of nanostructures

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Carbon dots

Carbon dots (CDs) are carbon-based fluorescent nanoparticles, a versatile material which comes in many different compositions and forms. CDs can exhibit excitation-dependent PL ‘tunable’ throughout the entire visible range, interesting for optoelectronic and imaging applications. The mechanism underlying this tunable emission is highly dependent on the exact structure of the material and hence remains debated, most prominently being ascribed to dot-to-dot variations that ultimately lead to excitation-dependent ensemble properties. Here we use single-dot spectroscopy to elucidate the origin of the excitation-dependent PL of CDs, produced by a facile one-step synthesis from chloroform and diethylamine. We show that the single dot spectra show several characteristic peaks differing in emission peak position, spectral width and shape, indicating the presence of distinct emission sites. These emission spectra slightly vary from dot to dot, but always retain their basic characteristics. By excitation-dependent single-CD measurement, we furthermore provide evidence that already individual CDs exhibit excitation-dependent emission spectra, indicating the presence of distinct emission sites within one and the same single CD. Based on our previous work, we relate these emission sites to the sp$^2$ sub-regions in the carbon core, coupled to specific functional groups on the surface. Since these CDs are produced via a facile single-step bottom-up synthesis, these results indicate that a straightforward synthesis route can lead to the integration of multiple emission sites within individual CDs.

5.1 Introduction

Fluorescent carbon dots (CDs) have, like Si-QDs, many advantageous properties such as bio-compatibility [44–47] and resource abundance. Moreover, CDs can be produced using relatively facile synthetic routes [43]. The emission mechanism is less well understood, however, owing to the large structural diversity and complexity of CDs [62]. Moreover, in literature the majority of studies employs ensemble spectroscopy techniques, which are unable to distinguish the individual microscopic contributions to the optical properties of the poly-disperse CD ensembles. Here we employ single-dot spectroscopy, to investigate
the PL of carbon dots emitting between 400 and 700 nm. Single-dot spectroscopy avoids ensemble averaging effects and enables the investigation of the individual single-dot spectra that underlie the ensemble spectrum.

5.2 Ensemble characterization

5.2.1 Size evaluation

CDs were prepared via a mild one-pot process by refluxing of chloroform and diethy-lamine\textsuperscript{1}, as described in detail in Nie et al. [50]. TEM and Raman spectroscopy data reveal that the resulting CDs are composed of a nano-crystalline core (graphitic sp\textsuperscript{2} carbon atoms with sp\textsuperscript{3} carbon defects) [50]. We evaluated the size of CDs using AFM, yielding an average height of 2.0 ± 0.7 nm (Figure 5.1). When compared to the 2-4 nm range determined for this material previously [50] by TEM, this is a lower average value. The difference could be due to a lower sensitivity of TEM to smaller particles, or more likely, due to a slightly anisotropic, disk-like shape of the nanoparticles, leading to a different size in lateral dimension and height when measured with TEM and AFM (e.g. as observed for graphene QDs [156]).

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{afm_scan.png}
\caption{AFM topography scan of CDs on a mica substrate; the inset shows the height distribution histogram obtained from ∼1200 particles.}
\end{figure}

5.2.2 Ensemble spectroscopy

The PL of the ensemble of CDs is continuously tunable with excitation wavelength, extending through the whole visible range, as shown for a low concentration (∼0.1 mg/mL) dispersion of CDs in ethanol in Figure 5.2a. The corresponding spectra for various excitation wavelengths are shown in Figure 5.2b, showing a narrow peak at 560 nm for

\textsuperscript{1}Samples courtesy of Dr. H. Nie and MSc. J. Bo (Jilin University, China)
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530 nm wavelength excitation. The PL QY, determined following the procedure described in Chapter 2, is between 1 and 2.5% with the highest value of 2.5% for excitation at 530 nm, corresponding to a narrow spectral band with maximum at around 560 nm (Figure 5.2b). Absorption and PL excitation (PLE) spectra in Figure 5.2c and d provide further insights into the energy structure of the system. The emission wavelength changes with excitation wavelength, switching between a broad blue-green band emitting at 490 nm with excitation maximum at around 380 nm, and a narrow green band at 560 nm with excitation maximum at around 530 nm. In addition, in previous work [50] a red band, with emission at ~660 nm and the excitation maximum at around ~630 nm was observed, which is not included here as the internal correction function of our current system ends at 600 nm excitation.

The PLE spectral map (Figure 5.2c) shows narrow Stokes shifts of only 89 meV and 130 meV for the red [50] and green bands, respectively, as opposed to ~730 meV for the blue-green band. The dissimilar spectral characteristics of the blue, green and red bands suggest that those are related to different emission mechanisms. In previous work, Nie et al. [50] showed that these three emission bands are robust features of the synthesized CDs and related their origin to the core and oxygen (-C=O) and nitrogen (-C=N) related surface moieties respectively.

**Figure 5.2:** Emission and absorption from a low concentration (~0.1 mg/mL) dispersion of CDs in ethanol: (a) Real-color photo of PL under pulsed ns laser excitation at specified wavelengths in nm. (b) PL intensity and absolute PL QY (inset) excited by a Xe lamp. The PL intensity is plotted normalized to the number of absorbed photons at respective excitation wavelengths. (c) PLE spectral map and (d) PLE spectra for specified detection wavelengths and absorption coefficient. Black dots in PLE maps indicate PL maxima for each excitation wavelength.
5.3 Single CD spectroscopy

5.3.1 Single CD vs ensemble spectra

To obtain insight into the microscopic distribution of the blue, green and red emission within the CDs, we employ single-dot spectroscopy to circumvent ensemble averaging effects and enable investigation of the PL from individual CDs. To do so, we use an inverted microscope equipped with a spectrometer coupled to an EMCCD camera (Figure 5.3), where either a grating for spectrally resolved measurements is selected, or a mirror to record PL images enabling spatial localization of emissive CDs (for more details, see the Materials & methods section). We use highly diluted dispersions of CDs in ethanol, which we drop cast onto quartz substrates, to achieve spatially separated CDs at a particle density below 0.1 µm$^{-2}$. The low particle density ensures that on average, we should observe at most a single CD within a diffraction limited detection spot of radius $\sim$300 nm.

![Figure 5.3: (a) Schematic of the wide-field single-dot spectroscopy experimental setup. (b) Wide-field PL image under 488 nm excitation with the projected position of the slit (projected width $\sim$ 1µm) selecting part of the image for spectrally resolved measurements.](image)

To verify that we measure with a large probability individual CDs, we record PL time-traces of individual bright spots in the FOV. The vast majority of observed bright spots displays intensity fluctuations between two distinct levels (Figure 5.4): a bright ON state and a dark OFF state. Such two-step blinking is characteristic for single quantum emitters (Chapter 4) [15] and was also previously observed for single CDs [55, 57, 64, 65]. To obtain further insight into the excitation-dependent PL of the ensemble, we selectively study the blue (490), green (560 nm) and red (660 nm) emission bands, by using excitation wavelengths close to their excitation maxima, i.e. 405, 488 and 638 nm respectively, in combinations with suitable detection filters. We select individual CDs using a slit to narrow down the FOV (Figure 5.3) and record $\sim$500 single-dot spectra. Several representative examples taken under 405, 488 and 638 nm wavelength excitations are shown in Figure 5.5 (top), while the superposition of all $\sim$500 spectra compared to the ensemble measurements are shown below.

As expected for single dots, most of the PL spectra are narrower than the PL spectrum of the ensemble. To link the single CD properties to the ensemble, we superimpose the single-dot spectra and compare the resulting spectrum to that of the ensemble. The
5.3. Single CD spectroscopy

Figure 5.4: Blinking of CDs under 488 nm (∼50 W/cm²) excitation: (a) The number of CDs in emissive ON state as a function of time. The red line is a single exponential fit yielding an average time of ∼4 minutes. (b,c) Examples of blinking time-traces of CDs; acquisition time per frame is 2 s.

Superimposed single-dot spectra show good agreement with the ensemble spectra, although there is significant variation in the single-dot spectra, indicating the polydispersity of the sample (bottom panels in Figure 5.5). For the green emission band for example, studied under 488 nm wavelength excitation (Figure 5.5b), the single CD spectra vary between 500 and 750 nm, clearly giving rise to the extended width of the ensemble spectrum, while the individual CD spectra are much narrower (∼100-400 meV) as plotted in Figure 5.6. This is also observed for the red emission band, showing a FWHM of 50-200 meV (Figure 5.5c). For the blue emission band excited at 405 nm (Figure 5.5a), the single-CD spectra are significantly broader (∼300-700 meV), indicating a different emission origin (Figure 5.6).

Interestingly, ∼17% of the green single-dot spectra display multiple peaks (such as the bottom spectra in Figure 5.5b) or a peak that is clearly asymmetric towards longer wavelengths. The blue and red single-dot emission spectra do not show clear replicas, again suggesting an origin different from the green emission band. These side peaks are typically ascribed to energy replicas due to optical energy losses to local vibrational states (bulk or surface phonons) [64, 157], trion formation [158] and/or vibrational coupling to the substrate defect centers (in case of quartz, ∼170 meV has been reported [159]). To quantify the replica’s data, the single CD spectra were fitted on the energy scale (corrected for the wavelength to energy conversion) by the sum of equally spaced normal distributions \( G(\mu, \sigma) \): \( \sum_i A_i G(\mu - (i - 1)\Delta\mu, \sigma) \), with \( A \) the amplitude, \( \mu \) the main peak position, \( \Delta\mu \) the splitting energy, \( \sigma \) the standard deviation and \( i \leq 3 \). The average FWHM of the spectra with clear side-peaks structure is around 100 meV, which is very common for room temperature measured single dot spectra and is typically related to inhomogeneous broadening caused by local environment field fluctuations; this is an important factor especially for relatively long detection times as used here (∼5 minutes).
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Figure 5.5: (a-c) Single CD spectra under 405 nm (a, ~40 W/cm$^2$), 488 nm (b, ~50 W/cm$^2$) and 638 nm (c, ~125 W/cm$^2$) wavelength excitation, single-dot spectra (top) and superposition of single-dot spectra compared to the ensemble spectrum of a low concentration (0.1 mg/mL) dispersion of CDs in ethanol (bottom). Solid lines are fits to the data. The superimposed spectra show good agreement with the ensemble spectrum; the deviation for green excitation in the red (>600nm) might arise from the low emission intensity of red emitting particles being too weak to be efficiently detected by single-dot spectroscopy, or from the previously reported local environment dependence of the CD PL [56, 160].

Between ~500-600 nm, all structured single CD spectra reveal similar (average) phonon replica energy of around 160 meV (Figure 5.7a), independent of the emission peak energy, i.e. independent of the size of CD core and/or origin of PL. The replica energy is high in comparison to values reported previously for this type of material by Ghosh et al. (70-150 meV) [64] and could be related to a vibration from sp$^3$ C defect in the graphene flakes in the CD core active in Raman spectroscopy at 1300 cm$^{-1}$ (160 meV) [50]. Other possible sources of losses with similar vibrational energy are surface vibrations of the C-O stretching mode (~150-160 meV [161]), contributing to the Fourier-transform IR (FTIR) absorption spectrum (Figure 5.7b). Possible relation of the replica energy to sometimes suggested trion formation [158], however, is unlikely, as the replica energy shows very weak (if any) spectral dependence (Figure 5.7). To evaluate the possible role of the quartz substrate [159], we have compared measurements from partially deposited
sample (part empty, part deposited with low CD concentration). On the clean area, we found under the same experimental conditions only very few non-blinking spots, with low brightness, in strong contrast with the side containing CDs, which shows multiple blinking bright luminescent spots. We thus expect that direct excitation of SiO$_2$ defects in the substrate does not significantly contribute to the detected emission. This does not mean, however, that the SiO$_2$ defects could not contribute to the energy losses from the CDs, i.e. into the phonon replicas. Still, both the average replica energy and PL peak energy is slightly lower than that reported for silica defects [159] ($\sim$170 meV at 600 nm).

![Distribution of the full width at half maximum (FWHM) (top), and its relation to the emission peak wavelength of all recorded single-CD PL spectra (bottom). Colors indicate the different excitation wavelengths.](image)

**Figure 5.6:** Distribution of the full width at half maximum (FWHM) (top), and its relation to the emission peak wavelength of all recorded single-CD PL spectra (bottom). Colors indicate the different excitation wavelengths.

To further investigate the microscopic origin of the energy replicas, we analyzed the spectral dependence of the Huang-Rhys factor, $S$, (Figure 5.7c), which gives the mean number of emitted phonons during radiative recombination. The Huang-Rhys factor can be obtained from the amplitudes $I$ of the energy replicas $n = 0, 1, 2, \ldots : I(n) \approx e^{-S} S^n / n!$ [162]. Analysis reveals weak exciton-phonon coupling with in average of 0.2-0.3 phonons emitted per emitted photon (Figure 5.7c), which is roughly constant over the whole spectral range. Lack of significant spectral dependence of both replica's energy and Huang-Rhys factor in the green spectral region indicates that the vibrational structure (i.e. the energy losses) is independent of the origin of the emission site.

### 5.3.2 Excitation-dependent single-dot spectroscopy

In the following section, we also record the change in the PL of the spatially localized single CDs, by consecutive excitation at 638, 488 and 405 nm wavelength. Several selected spectra recorded at the three excitation wavelengths are shown in Figure 5.8. In some
cases, we observe the simultaneous presence of blue and green, and eventually blue and red emission bands. These emission bands differ in their spectral features with red and green spectra being narrower than the blue spectrum, similar to the spectra depicted in Figure 5.5.

We note that the majority of spots only show a single spectrum (such as the top spectrum in Figure 5.8), which could either be the result of photo-degradation during the measurement (Figure 5.4a) or due to only a single emissive site on the respective CD. To reduce the effect of photo-degradation, we record the spectrally unresolved PL intensity under the different excitation wavelengths, detecting the emission through band-pass filters to selectively study the three emission bands. Examples of such filtered images are depicted in Figure 5.9. Their comparison reveals that many blue and green emitting spots show spatial overlap (arrows). By comparing the location of spots in detail, we find roughly 45% overlap between the CDs excited at 405 and 488 nm, suggesting that they exhibit both blue and green emission. Overlap with red emitting CDs is observed as well: while only a small fraction of the dots emit in the red (~5%), ~24% of those CDs emitting in red display overlap with the ones emitting in green, and ~30% with the ones emitting in blue. Furthermore, ~15% of them appear under both blue and green emissions.
5.3. Single CD spectroscopy

excitation, indicating emission of all three colors.

\[ \text{Figure 5.8: Excitation-dependent single CD PL spectra measured under subsequent excitation} \]
\[ \text{of 638 (\sim 125 W/cm}^2\text{), 488 nm (\sim 50 W/cm}^2\text{) and 405 nm (\sim 40 W/cm}^2\text{) CW laser excitation.} \]
\[ \text{Smoothed PL spectra are shown in colored lines.} \]

\[ \text{Figure 5.9: False-color wide-field PL images of highly dilute drop-casted samples of CDs. The} \]
\[ \text{three panels show identical fields of view, taken at 405 (a) 488 (b) and 638 (c) nm wavelength} \]
\[ \text{excitation, detecting blue-green (480-520 nm), yellow (580-620 nm) and red (680-720 nm) emission, respectively. Arrows indicate examples of the same CDs luminescing under different} \]
\[ \text{excitation wavelengths. The exposure time per image was kept at 30 seconds to limit the effect} \]
\[ \text{of photo-degradation.} \]

Such excitation-dependent PL of single CDs is surprising, as excitation-dependent PL spectra are commonly not observed for molecules or single quantum dots, indicating the presence of multiple independent emission sites within one and the same CD. This direct observation of excitation-dependent single-dot PL spectra thus substantiate suggestions of excitation-dependent PL from single CDs [51, 61, 163]. In contrast, Ghosh et al. [64] did not observe such shifts in the PL spectra when exciting individual CDs at different excitation wavelengths. However, the 467 and 488 nm wavelength excitation in their experiment might have been too close for efficient excitation of the separate emission centers. Also, the large degree of overlap observed in Figures 5.9 is clearly different from the observations made by Das et al. [65], where no overlap was found between green and
red emission. Our results are, however, in good quantitative agreement with a single CD study on the very same CDs, where the CDs were immobilized in a Poly(vinyl alcohol) film [163]. This shows that the excitation-dependent spectra do not arise from clusters which form upon drop-casting the CDs on a substrate, which is further supported by the spatial separation observed in the AFM topography (Figure 5.1).

5.4 Discussion

Regarding the emission mechanism behind the excitation-dependent single dot spectra, in previous work by Nie et al. [50] it was proposed that the blue, green and red emission sites in these CDs are related to the core and to oxygen and nitrogen containing surface functional groups respectively. Based on chemical characterization combined with ensemble spectroscopy, PL was shown to change dramatically after selective reduction of C=O and C=N bonds on the surface [50], in line with studies available in literature that show that the PL spectral position and width strongly depend on the type of surface functionalization [52, 54, 164]. The differences in the width, peak position and structure of the spectra that we observe here for single CDs, support the view of different origins of the emission bands. Hence, we arrive at the following mechanism underlying the excitation-dependent single CD emission (Figure 5.10): the green and red emission originates from C=O and C=N containing surface groups respectively [50, 163], while the blue emission originates from the core. Indeed, FTIR and X-ray photoelectron spectroscopy (XPS) measurements on our dots reveal the presence of O and N containing functional groups (such as –OH, C=O, and C=N) [50]. This is in line with the evidence for a common origin of the green emission in carboxyl/carbonyl (O-related) surface functional groups [52, 54, 64, 65, 165]. The core-related emission originates most likely from smaller sp² domains within the CD, as graphene QDs with a pure sp² structure and comparable size to our CDs (2.0 nm) are expected to emit in the red spectral region (>600 nm) and not in the blue-green [166], like in our case. This is reasonable as our synthesis does not yield graphene-like cores, but rather CDs with mixed sp² and sp³ phase as schematically shown in Figure 5.10.

Figure 5.10: Schematic of the proposed CD structure giving rise to the three emission bands.
5.5. Conclusion

Using excitation-dependent single-dot spectroscopy, we identified characteristic single-dot PL spectra with different emission peak positions, spectral widths and spectral shapes, indicating multiple active and independently excitable emission sites that can be present within a single carbon dot. Superposition revealed that these single-dot spectra are at the origin of the excitation-dependent PL of the ensemble. By quantifying the number of CDs that emit multiple colors, our findings suggest that while not all individual CDs exhibit excitation-tunable emission, it is possible to integrate and engineer different types of electron transitions in nanoscopic dimensions through facile chemical synthesis, making these CDs even more versatile than organic dyes or inorganic QDs. This makes this particular type of CDs interesting for optoelectronic applications as an excitation-tunable broad-band phosphor and for single-nanoparticle multi-color imaging.

5.6 Materials & methods

Synthesis

CDs were synthesized by refluxing of diethylamine (DEA) and chloroform (volume ratio of 1:10) at 62 °C for 60 h as described by Nie et al. [50]. The products were separated from the solution with a rotary vacuum evaporator and re-dissolved in ethanol. The CDs were purified by dialysis (weight cut-off: 3500) in water and then ethanol to remove any molecular byproducts. FTIR and XPS measurements reveal the presence of O and N containing functional groups (such as –OH, C=O, C=N) on the CDs [50]. Oxygen-containing functional groups are presumably introduced from water and oxygen, and nitrogen-containing functional groups are presumably introduced from DEA.

Atomic force microscopy

The size of the CDs has been evaluated from their height, measured from a drop-casted dilute solution of CDs on a freshly cleaved mica substrate using an AFM (JPK Instruments) in tapping mode. The driving frequency was set slightly lower than the cantilever’s resonance frequency in air in order to minimize the effect of substrate-tip interactions on the determined height [167]. For reference, an empty area was imaged, containing less than ∼10 particles per 25 µm². From z-scan dimension data analysis of ∼1200 particles we obtained an average CD size of 2.0 ± 0.7 nm (inset in Figure 5.1).

Ensemble spectroscopy

CDs dispersed in UV-grade ethanol were studied in a quartz cuvette (Hellma Analytics) excited either by pulsed laser excitation provided by a Nd:YAG laser (Solar LS) or by continuous wave (cw) excitation provided by a Xe lamp. PL was detected in a spectrofluorometer (Horiba, Fluorolog3-22) equipped with a double grating monochromator.
coupled to a photomultiplier tube (PMT; amamatsu, R36-10). PL spectra were measured at room temperature and are corrected for the spectral sensitivity of the setup.

Quantum yield
Absolute QY at 405 and 430 nm excited by a Xe lamp was determined inside an integrating sphere (for more details, see Chapter 2). Relative QY values were scaled to these values to obtain absolute QY estimates for longer excitation wavelengths.

Single-dot spectroscopy
Single-dot PL was detected using an inverted microscope (Zeiss, AxioObserver Z1) coupled to a spectrometer (Princeton Instruments, Acton SP2300) and a CCD (Princeton instruments, Pylon 400B) in a wide-field scheme. For this, a dilute dispersion of CDs in ethanol was drop-casted on a quartz cover slip (Structure Probe Inc.). The cover slips were cleaned by sonication in an alkaline cleaning solution (Hellma Analytics, Hellmanex III) for 60 minutes, followed by sonication in and rinsing with demineralized water (30 minutes), drying by nitrogen flow and an ozone dry-cleaning procedure (UVP, PR-100) for ~20 minutes. Cw excitation is provided by laser diodes with 405 nm (ThorLabs, ML320G2-11), 488 nm (Lasos, BLD-488-SMN) and 638 nm (Lasos, RLD-XT) wavelength or by the 488 nm Ar+ laser line (Spectra-Physics, Stabillite 2017). Emitted light is collected using a 100x objective (Zeiss, Epiplan-Neofluar NA 0.75) and is filtered by a long pass filter to remove scattered excitation light. All spectra are measured at room temperature and are corrected for spectral sensitivity of the setup.

Data analysis
For localization of the CD positions, bright domains with significant intensity compared to the background were identified as CDs. The position of the center of this domain was compared between different images of the same field of view. A tolerance of 1 pixel between center coordinates was allowed to count as overlapping CDs. The different band-pass filters used in our experiments result in a slight displacement of the recorded images, for which we correct by quantifying the shift from transmission images taken by white-light illumination on a reference sample.