Supporting information:

Trans-membrane Fluorescence Enhancement by Carbon Dots: Ionic Interactions and Energy Transfer

Stefanie D. Pritzl[†,§], Fernando Pschunder[‡], Florian Ehrat [†,§], Santanu Bhattacharyya[†,§,],*

Theobald Lohmüller [†,§], Maria Ana Huergo[†,‡], Jochen Feldmann [†,§]

[†] Chair for Photonics and Optoelectronics, Nano-Institute Munich, Department of Physics, Ludwig-Maximilians-Universität (LMU), Königinstraße 10, 80539 Munich, Germany

[§] Nanosystems Initiative Munich (NIM) and Center for Nanoscience (CeNS), Schellingstraße 4, 80799 Munich, Germany

[‡]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata—CONICET, Sucursal 4 Casilla de Correo 16, 1900 La Plata, Argentina

*Present address: Department of Chemical Sciences, IISER Berhampur, Transit campus (Govt. ITI), Eng. School Road, Berhampur, Odisha 760010, India

1. Preparation of large vesicles (LVs) and blue-emitting carbon dots (CDs). 5 mg of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids) were dissolved in 1 ml chloroform (≥99% stabilized with amylenes, Sigma Aldrich) and mixed with varying amounts of PE CF (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(carboxyfluorescein), Avanti Polar Lipids) and/or 30µl of the solution with blue emitting carbon dots (CDs). CDs were synthesized according to the protocol from Cheng *et al.*[1] and stored at room temperature. The solvent was removed by evaporation over the course of several hours. The addition of 1 ml of a HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (10mM) based NaCl-solution (0.9%) followed by a sonication step led to the formation of large vesicles. For measurements with free fluorescein (5-carboxyfluorescein, 1mg/ml in HEPES + 0.9% NaCl) 10µl of the dye solution was added to vesicles with only CDs.

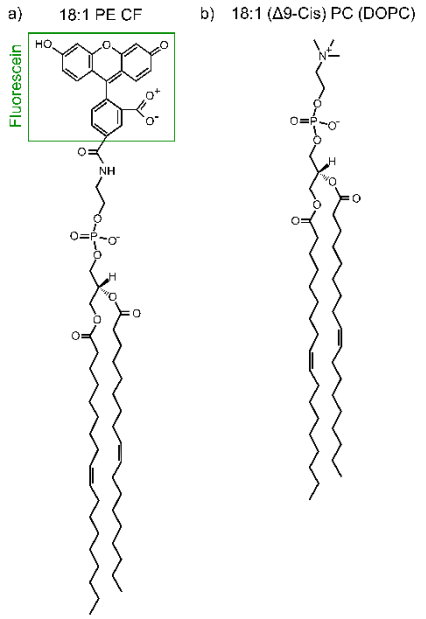

Figure S1: Chemical structure of a) PE CF and b) DOPC. The fluorescein dye is covalently bound to the hydrophilic side of the amphiphilic lipid molecule.

We also performed photoluminescence (PL) measurements of free CDs in HEPES buffer and of vesicles with only CDs in ddH_20 and buffer solution to investigate how the emission properties are affected in different environments. As shown in Figure S2, all CD samples show a maximum fluorescence at 450 nm and no change in the spectral shape. This observation demonstrates that the CD emission is not affected by the environmental conditions or due to aggregation.

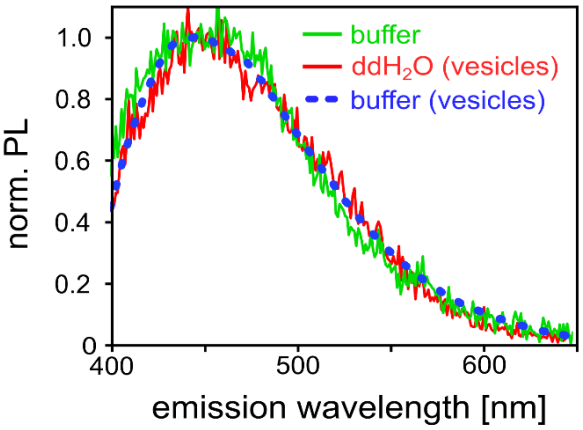

Figure S2: PL spectra of CDs in different environments: PL spectra of free CDs in HEPES buffer + 0.9% NaCl (green line), CD-vesicles in ddH20 (red line) and CD-vesicles in HEPES buffer + 0.9% NaCl (blue dotted line) display a similar spectrum.

2. Carbon dot size. The size and morphology of the CDs were analysed by atomic force microscopy (AFM) and dynamic light scattering (DLS). AFM measurements in intermittent mode were performed in air with a multimode microscope operated by a Nanoscope V control unit (Veeco Instruments) using silicon AFM tips (f = 285 kHz; k = 42 N/m). Samples were prepared by drop-casting a solution of CDs in tetrahydrofuran onto mica substrates and letting the solvent evaporate. Image analysis was done with the Nanoscope 7.30 software. DLS measurements were performed with a Zetasizer Nano ZS device (Malvern Panalytics).

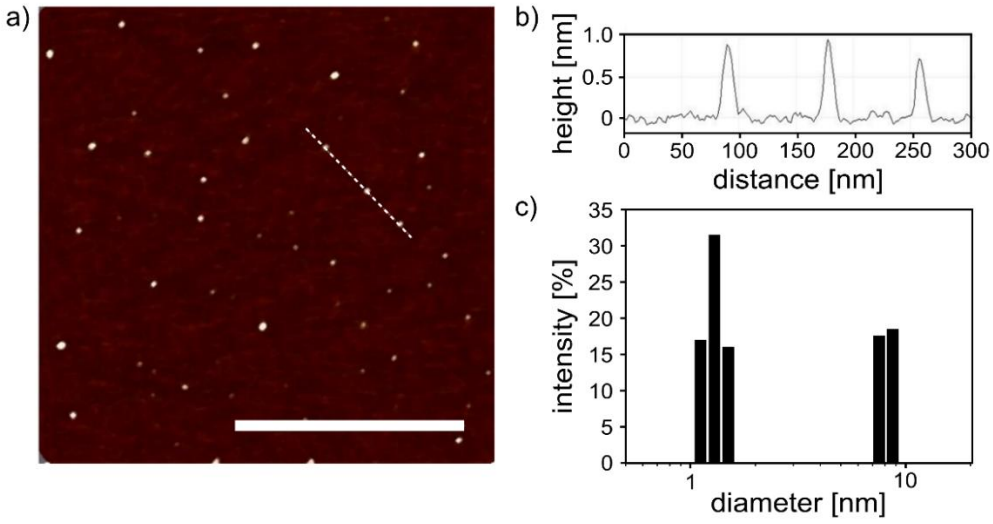

Figure S3: Atomic force microscopy (AFM) and Dynamic light scattering (DLS) measurements of CDs. a) AFM image of single CDs on a mica substrate, scale bar: 500 nm. b) AFM linescan of three particles illustrating the particle height of ~1 nm. c) DLS measurements of CDs show that the individual particles have an average size of (1.3 ± 0.2) nm. Also, ~35% of the particles form small aggregates with an average diameter of (8.1 ± 0.6) in solution.

3. Fluorescence microscopy. LVs were imaged with a 20x UPlanSApo oil immersion objective (Olympus, NA 0.85) on an IX81 inverted microscope (Olympus). The LV dispersion was mixed with deionized water to immobilize the vesicles between two cleaned glass slides. Images were acquired with either a colour EOS 550D CMOS camera (Canon) or a monochrome iXon Ultra 897 CCD camera (Andor). Suitable filter sets were used to excite the specific sample fluorescence. The CD emission was imaged with a U-MWU2 UV filter set (Olympus) and the PE FC fluorescence with a U-MNB2 blue filter set (Olympus).

4. Time-correlated single photon counting (TCSPC). The fluorescence excitation of the different LV samples was performed with a SuperK EXTREME EXR-20 pulsed white light laser (NKT Photonics) equipped with an EXTEND-UV box running at 360 nm for the C-dot excitation and 450 nm for the dye excitation at a repetition rate of 5.56 MHz. A monochromator (Princeton Instruments) connected to a low-noise Avalanche Photodiode (Excelitas) and a TimeHarp 260 board (PicoQuant) was used for the time-correlated signal detection.

5. Optical spectroscopy: Absorption spectra of vesicles with CDs and/or dyes in HEPES buffer were measured with a Cary 60 UV/Vis Spectrophotometer (Agilent Technologies). PL and PL excitation (PLE) spectra were recorded with a Fluorolog-3 FL3-22 spectrometer (Horiba Scientific) equipped with a 450W Xenon lamp, double monochromators for both excitation and emission and a water-cooled R928 photomultiplier tube. The obtained spectra were corrected with respect to the spectral sensitivity of the detector and the excitation intensities.

6. Effect of CD/fluorescein ratio. PLE measurements of vesicles with only CDs and CD-vesicles with increasing concentration of lipid dyes (0.14 mol%, 0.37 mol% and 0.56 mol% in the DOPC membrane) were recorded to investigate the impact of the CD-dye ratio. The fluorescence emission of the carbon dots at 450 nm decreases for higher amounts of fluorescein. The largest emission decrease is observed for a fluorescein concentration of 0.56 mol%.

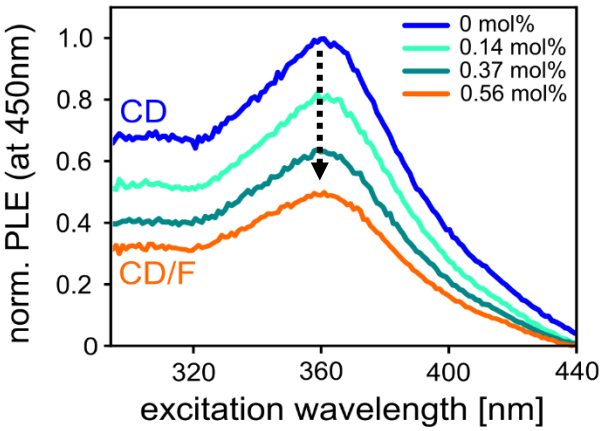

Figure S4: Normalized photoluminescence excitation (PLE) spectra: Normalized PLE spectra of the CD (blue line) and CD/F vesicles with varying amounts of fluorescein. By increasing the amount of the dye in the bilayer membrane, the CD emission at 450 nm decreases accordingly, as illustrated by the dotted black arrow.

8. Comparison between lipid bound fluorescein and fluorescein in solution. We performed PL and TCSPC measurements with free fluorescein dyes (5-carboxyfluorescein) to investigate the distance dependence of FRET across the membrane in more detail. Measurements with free fluorescein were recorded 15 min after dye addition. The PL of the CD/fF at 450 nm decreases compared to the emission of vesicles with only CDs. At the same time, the emission at ~510 nm of the CD/fF complex is enhanced compared to only free fluorescein. However, no spectral shift is observed and we therefore did not find an indication for ionic interactions between the fluorescein and the CDs. In PL lifetime measurements, we find that the amplitude averaged lifetime of the CD sample $\langle\tau_{CD}\rangle$ = 4.1 ns is reduced to $\langle\tau_{CD/fF}\rangle$ = 3.4 ns in presence of the dye. The FRET efficiency is accordingly only 17.1% (compared to 51.3% for membrane bound fluorescein) while the FRET-rate is only k_{ET} = 0.05 ns^{-1}. This shows that the distance between membrane embedded CDs and the free dye is indeed larger compared to the distance for lipid bound fluorescein.

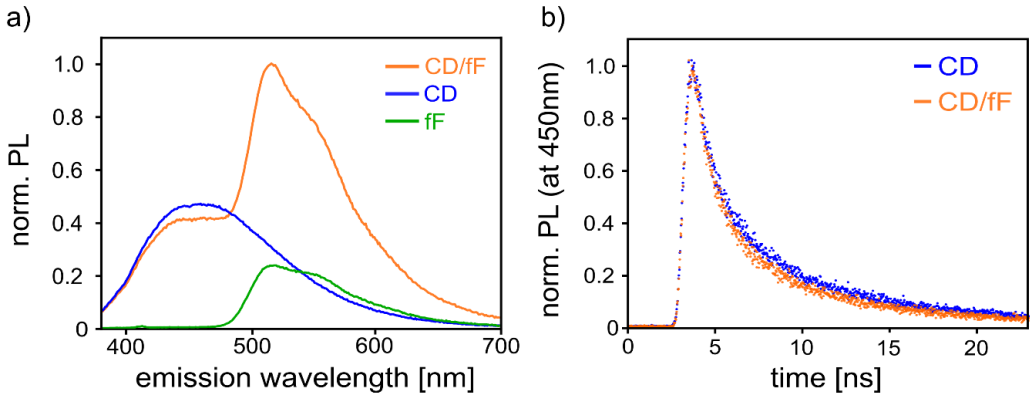

Figure S5: Normalized PL spectra of CD vesicles and free Fluorescein (fF) and time-resolved PL decays: (a) The PL spectrum of the CD/fF system (orange) after excitation with 360 nm. A decrease of the CD fluorescence emission at 450 nm is observed, while the fluorescein fluorescence emission shows an increase at the same time, thus indicating the occurrence of FRET. b) Time-resolved donor fluorescence decay traces of the CD (blue) and the CD/fF conjugate (orange). For the CD sample, we obtain a short decay component of 1.6 ns and a long decay time component of 8.7 ns. For the CD/fF conjugate, the decay lifetimes are reduced to 1.4 ns and 7 ns, respectively. By using the amplitude-weighted average decay times $\langle\tau_{CD}\rangle$ = 4.1 ns and $\langle\tau_{CD/fF}\rangle$ = 3.4 ns, we obtain an energy transfer efficiency of 17.1 % and a transfer rate of k_{ET} = 0.05 ns^{-1}.

9. Zeta-Potential measurements: The surface charge of LVs containing CDs or fluorescein lipids was obtained with Zeta-Potential measurements in a Zetasizer Nano ZS device (Malvern Panalytics). The samples were analysed in appropriate folded capillary Zeta Cells (Malvern Panalytics).

References

(1) Cheng, F *et al.*; *Green synthesis of fluorescent hydrophobic carbon quantum dots and their use for 2,4,6-trinitrophenol detection*, RSC Advances, 5 (113) 93360-93363 (2015)