Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2019

Supramolecular Assembly of Carbon Nanoparticles as Messengers for Artificial Chemical Communication

Giuseppe Trusso Sfrazzetto,* Luca Fichera, Agatino Zammataro, Giovanni Li Destri and Nunzio Tuccitto*

SUPPORTING INFORMATION

Table of Content

General experimental methods. The NMR experiments were carried out at 27° C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H at 499.88 MHz, ^{13}C-NMR at 125.7 MHz) equipped with pulse field gradient module (Z axis) and a tuneable 5 mm Varian inverse detection probe (ID-PFG). A JASCO V-560 UV-Vis spectrophotometer equipped with a 1 cm path-length cell was used for the UV-Vis measurements. Luminescence measurements were carried out using a Cary Eclipse Fluorescence spectrophotometer with a 0.5 nm resolution, at room temperature. The emission was recorded at 90° with respect to the exciting line beam using 5:5 slit-widths for all measurements. All chemicals were reagent grade and were used without further purification. NDI was synthesized according to the literature.[1] Millipore water was used to prepare all aqueous solutions.

DOSY experiments. Diffusion-Ordered SpectroscopY (DOSY) NMR has been particularly used in host–guest chemistry to have information about the dimensions of supramolecular species. The DOSY technique provides information about the size of the molecular aggregate in solution. The samples for DOSY experiments were prepared by mixing an appropriate volume of **CDs-Triazine** stock solutions (3 mg/mL in DMSO-d_6) and **NDI** (7 mM in DMSO-d_6). D value of **CDs-Triazine** is (3.51±0.52) × 10^{-10} m^2s^{-1}, while D value of **CDs-Triazine@NDI** is (1.2±0.05) × 10^{-10} m^2s^{-1}, demonstrating the increase of the dimensions and the self-assembly process.

Artificial chemical communication system. The prototypal artificial molecular communication platform was assembled in-house. A peristaltic pump (ISMATECH) was used for carrier flowing. A 6-port valve (Valco-Vici) with a 20ul loop was used for the bit pulse injection. A Teflon-made tube was used as a closed environment transport system. An RF-535 Fluorescence HPLC monitor with an S1989 Xenon lamp (Shimadzu) was used as a signal receiver. A Xtralien X-100 (Ossila, UK) was used as an analog-to-digital converter. A Python-based GUI was developed for data acquisition and treatment.

Atomic Force Microscopy Characterization

Samples for atomic force microscopy (AFM) characterization were obtained by casting on mica 5 μL of the CDs solutions previously diluted 1000 times. Floc-containing samples were also obtained by casting a DMSO dispersion after 30 minutes sonication.

AFM images were obtained with a Nanoscope IIIa apparatus from Digital Instruments (Santa Barbara, CA) used in tapping mode in air. Tap 300G silicon probes from Budget Sensors, having a nominal resonance frequency of 300 kHz, were employed.

Synthesis of CDs. In a typical procedure of CDs synthesis, 21 g of citric acid (Aldrich, Milan, Italy) were put into a 500 mL beaker and heated at 215 °C. About 10 min later, salts were liquefied

obtaining a pale-yellow liquid, which turned into dark in 15 min, implying the formation of CDs. The obtained liquid was cooled down to room temperature and 200 ml of 0.25 M aqueous solution of NaOH (Aldrich, Milan, Italy) was added dropwise under vigorous stirring. Solution was centrifuged at 15000 RPM for 2 h at 3 °C and the supernatant solution was dialyzed for 24 h using a tube having 11'000 Da cut-off (Aldrich, Milan, Italy). Atomic force microscopy characterization confirmed the presence in the dialyzed solution of nanometric (about 2-3 nm) disc-shaped particles. Dialyzed aliquot was freeze dried and used as it for the subsequent covalent functionalization.

Synthesis of CDs-C6OH. 4 mg of native CDs were mixed with 2 mL of thionyl chloride (27.4 mmol) and 100 μL of pyridine under N_2. The reaction was heated at 140°C overnight. Then, 50 mg (0.427 mmol) of hexanolamine were added, temperature was regulated to 80°C and, after 3 days, rotovaporation of liquid fraction give crude product. Excess of hexanolamine was removed with CH_2Cl_2, thus obtaining 4.5 mg of **CDs-C6OH**. ^{1}H NMR (500 MHz, D_2O) δ 3.60 (t, *J* = 7 Hz, 2H), 3.00 (t, J = 7.5 Hz, 2H), 1.68 (m, 2H), 1.56 (m, 2H), 1.40 (m, 4H).

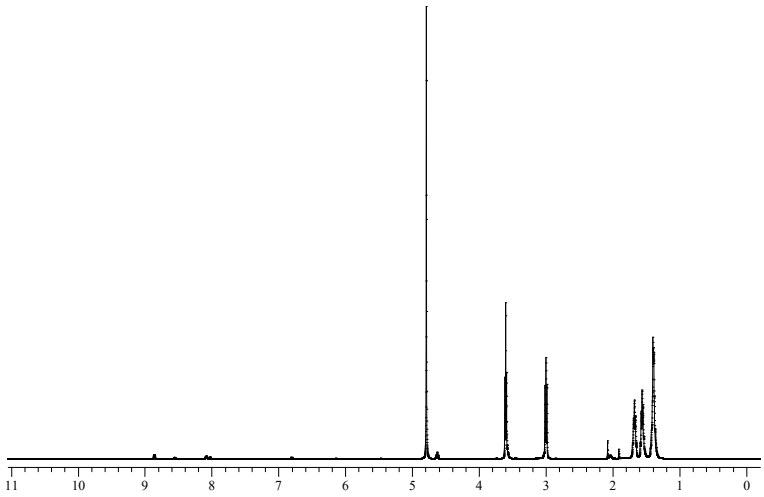

Figure S1. ^{1}H NMR spectrum of **CDs-C6OH** in D_2O.

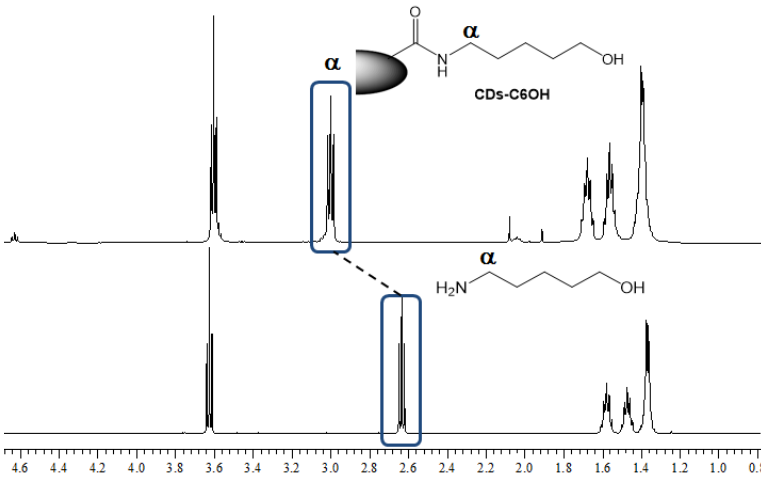

Figure S2. Comparison between ^{1}H NMR spectra of hexanolamine (down) and **CDs-C6OH** (up).

Synthesis of CDs-Ts. To 4.5 mg of **CDs-C6OH**, 50 mg (0.26 mmol) of tosyl chloride were added. The mixture was heated to 80°C under nitrogen and 50 mL of pyridine were added. Mixture was stirred overnight, then was cooled to room temperature and 5 mL of CH_2Cl_2 were added to solubilize CDs. The excess of tosyl chloride was removed by treatment with water. The resulting organic phase was evaporated, obtaining a brown solid which was further washed with diethyl ether, leading to 4.7 mg of **CDs-Ts**. **CDs-Ts**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 8 Hz, 2H), 7.14 (d, J = 8 Hz, 2H), 3.39 (t, J = 6.5 Hz, 2H), 2.76 (m, 2H), 2.34 (s, 3H), 1.58 (m, 2H), 1.49 (m, 2H), 1.23 (m, 2H), 1.15 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 145.3, 137.7, 128.0, 125.4, 45.2, 38.7, 31.7, 26.7, 25.6, 24.9, 20.7,

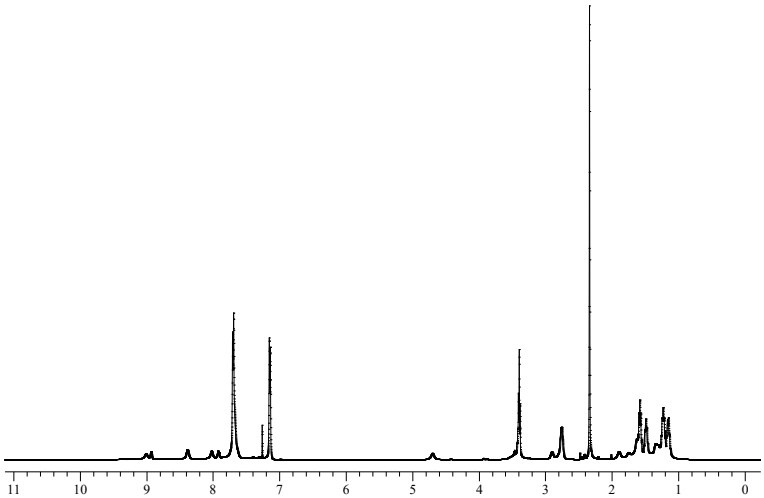

Figure S3. ^{1}H NMR spectrum of **CDs-Ts** in $CDCl_3$.

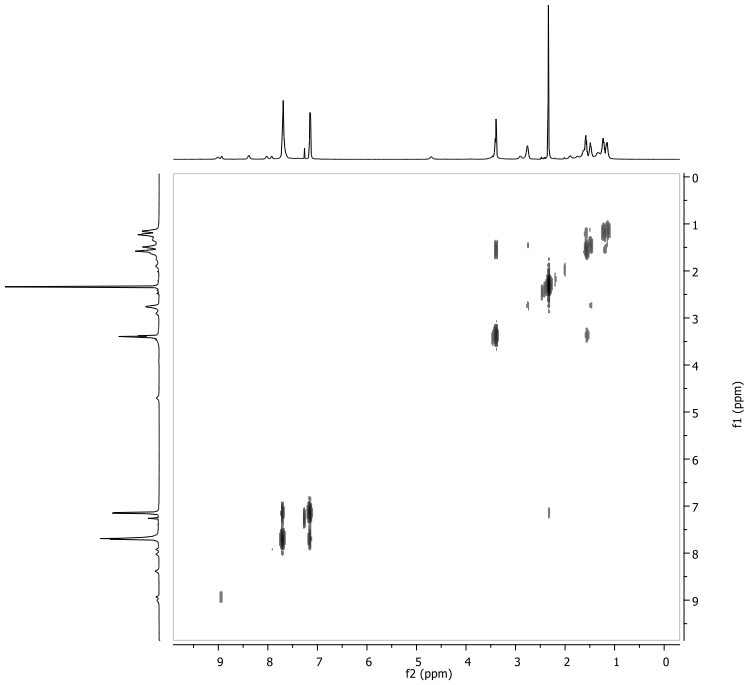

Figure S4. gCOSY spectrum of **CDs-Ts** in $CDCl_3$.

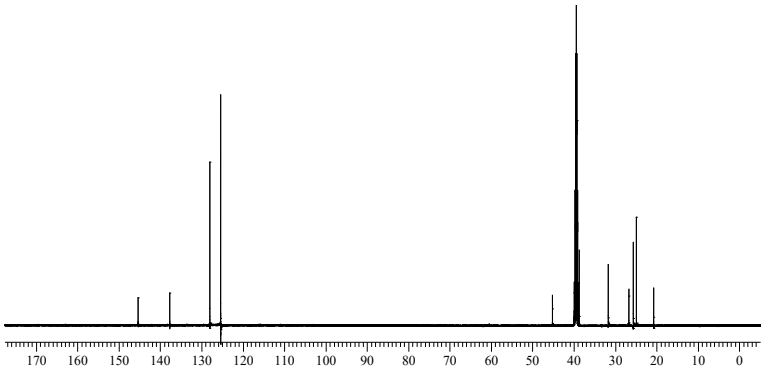

Figure S5. ^{13}C NMR spectrum of **CDs-Ts** in DMSO-d_6.

Synthetis of triazine ethylene-amine. To 12.6 g (0.21 mol) of ethylenedimine, saturated with N_2 for 1 h and heated at 120°C, were added 3 g (20.61 mmol) of 2-Chloro-4,6-diamino-1,3,5-triazine in 30 minutes. Mixture was stirred at 120°C for 1 h and at 80°C for 2 h. Then, excess of ethylendiamine unreacted was removed under reduced pressure and 15 mL of MeOH were added. By addition of NH_4OH, pH was regulated to 10. The organic phase was treated with hexane obtaining a yellow pale solid, which was filtered and washed with MeOH obtaining 2.24 g of

triazine ethylene-amine (yield 64%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 6.64 (t, J = 5.5 Hz, 1H), 2H), 6.05-6.30 (s, 4H), 3.35 (q, J = 5.5 Hz, 2H), 2.90 (t, J = 6 Hz, 2H).[2]

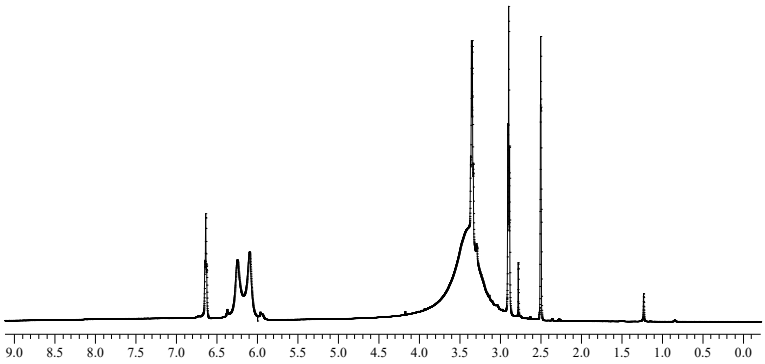

Figure S6. ^{1}H NMR spectrum of triazine ethylene-amine in DMSO-d_6.

Synthesis of CDs-Triazine. 4.7 mg of **CDs-Ts** and 146 mg (0.86 mmol) of triazine ethylene-amine were dissolved in 5 mL of dry DMF. Reaction was stirred at 120°C for 48h under nitrogen. Then, solvent was removed under reduced pressure. Crude product was dissolved in water (10 mL) and dialyzed with 1 L of water (3 times) using a tube having 11'000 Da cut-off. **CDs-Triazine** (3.8 mg) were obtained as brown solid after evaporation of water. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.79 (m, 1H), 6.22-6.55 (m, 4H), 3.37 (m, 4H), 3.28 (m, 2H), 3.21 (m, 2H), 2.95 (m, 2H), 2.93 (m, 1H), 1.72 (m, 2H), 1.39 (m, 2H), 1.28 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.6, 165.3, 164.6, 164.4, 161.3, 145.4, 137.7, 128.1, 125.5, 44.9, 38.2, 37.3, 36.5, 34.0, 26.1 , 25.9, 25.7, 24.4, 20.7.

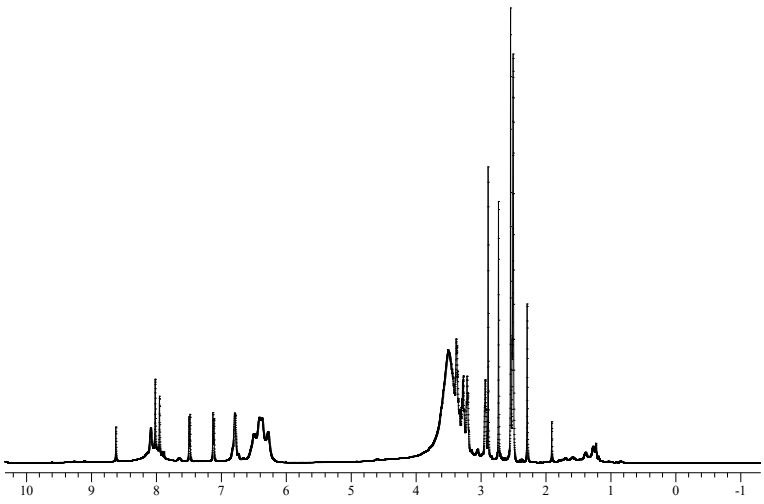

Figure S7. ^{1}H NMR spectrum of **CDs-Triazine** in DMSO-d_6.

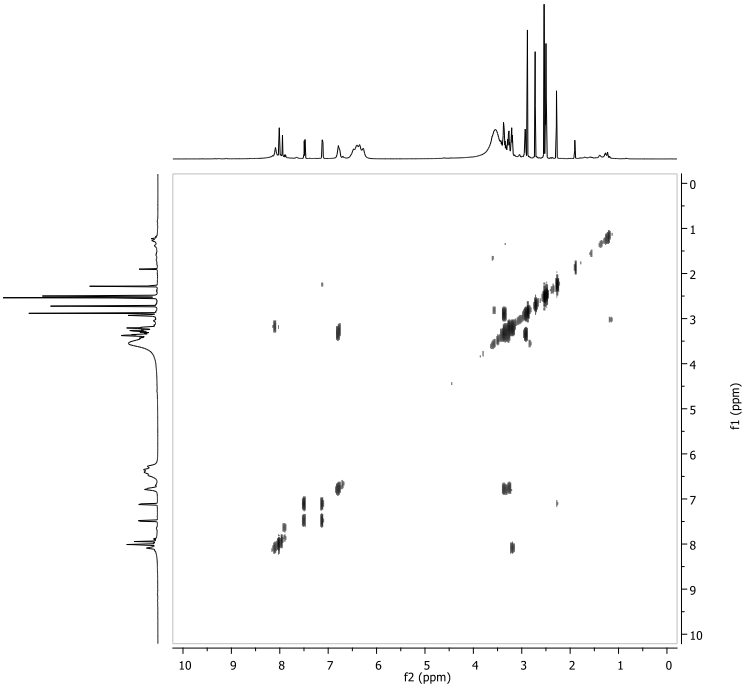

Figure S8. gCOSY spectrum of **CDs-Triazine** in DMSO-d_6.

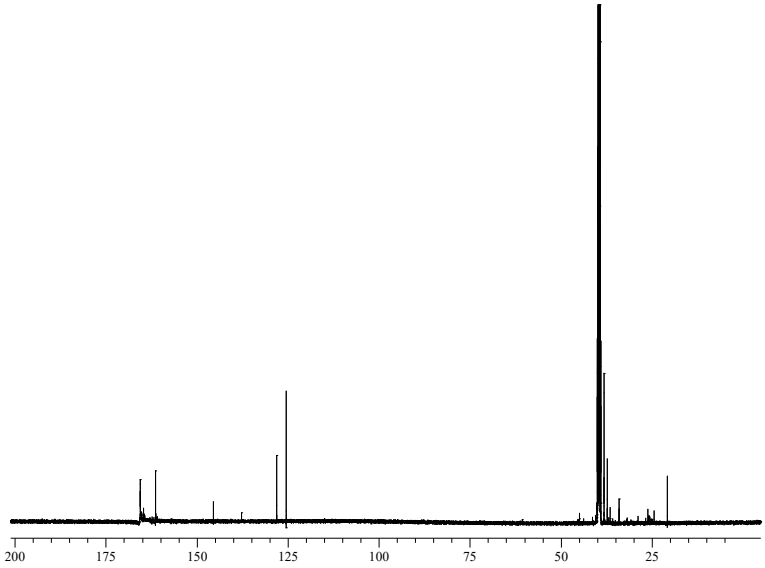

Figure S9. ^{13}C NMR spectrum of **CDs-Triazine** in DMSO-d_6.

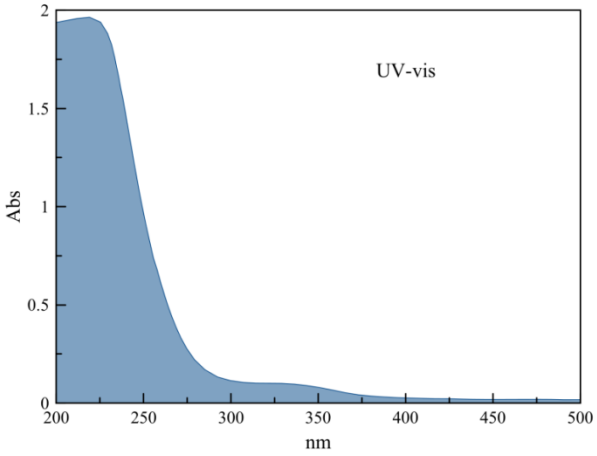

Figure S10. UV-vis spectrum of **CDs**

[1] T. Nakazato, T. Kamatsuka, J. Inoue, T. Sakurai, S. Seki, H. Shinokubo, Y. Miyake *Chem. Commun.*, **2018**, *54*, 5177-5180

[2] According to the literature: T.-H. Tsoi, W.-T. Wong *Anal. Methods*, **2015**, *7*, 5989–5995