SUPPORTING INFORMATION

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

*D. Mazzier, M. Favaro, S. Agnoli, S. Silvestrini, G. Granozzi, M. Maggini and Alessandro Moretto**

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy

## CONTENTS

## GENERAL METHODS

**NMR:** $^1$H spectra were recorded at room temperature on a Bruker AC-200 (200MHz) instrument using TMS as internal reference. The multiplicity of a signal is indicated as: s-singlet, d-doublet, m-multiplet. Chemical shifts ($\delta$) are expressed in ppm and coupling constants ($J$) in Hertz.

**FT-IR absorption:** FT-IR absorption spectra were recorded with a Perkin-Elmer 1720X spectrophotometer; $\nu_{max}$ is given for the main absorption bands.

**UV-Vis absorption:** The UV-Vis absorption spectra were recorded using a Shimadzu model UV-2501 PC spectrophotometer. A 1-cm path length quartz cell was used.

**Fluorescence:** The fluorescence spectra were measured upon excitation at different wavelengths using a Perkin–Elmer model LS-50B spectrofluorimeter. A 1 cm path length quartz cell was used. The samples prepared for UV-Vis were used to collect the fluorescence data.

**CD:** Circular dichroism measurements were carried out at room temperature using a Jasco J-715 spectropolarimeter. A fused quartz cell of 0.2-mm path length (Hellma) was used.

**XPS:** The *X-ray photoemission spectroscopy* (XPS) measurements were carried out on dialysed and lyophilised CQD powders. Core level photoemission spectra were taken on a VG ESCALAB MKII spectrometer using Mg anode of a conventional non-monochromatized X-ray source (K$\alpha$=1253.6 eV). The electron analyser pass energy was set to 50 eV for the survey wide scans and to 20 eV for the single spectral region. The measurements were taken at RT with a detection direction perpendicular to the sample surface. The calibration of the binding energy (BE) scale was carried out using Au 4f as reference. In order to characterize the chemical states of carbon and nitrogen, the C 1s and N 1s peaks were de-convoluted into individual components (after Shirley background removal) using a Doniach-Šunjić shape for the C $sp^2$ component and symmetrical Voigt functions for the fitting of the molecular-like components. The $\chi^2$ minimization was ensured by the use of the nonlinear least squares routines.

The micro- and nano-scale morphology of the materials here reported were performed using *Scanning Electron Microscopy* (SEM). The instrument used in this work was a field emission SEM (Zeiss Supra VP35), equipped with a GEMINI column. Micrographs were taken with an acceleration voltage of the primary beam of 5 kV and using the in-lens high resolution detector.

**TEM**: Samples were analyzed on a Jeol 300PX instrument. A small drop of solutions was floated on a glow discharged carbon coated grid and excess was removed by #50 hardened Whatman filter paper. For the samples with negative staining, the grid was then floated on 2% uranyl acetate solution for 10 seconds, and the excess was removed by #50 hardened Whatman filter paper.

**SEM:** A Carl Zeiss Merlin field emission scanning electron microscope operating at 5 kV accelerating voltage was used. A small drop of the milk-like aqueous suspension was placed on a microscope glass cover slip and allowed to dry overnight. The dry material was coated with platinum.

**MALDI-TOF:** Mass spectra were recorded with a AB SCIEX 4800 MALDI TOF/TOF Analyzer (AB SIEX Pte Ltd, Massachusetts, USA), using reflector mirror and positive ion detection. A dual microchannel plate reflector detector was used. LDI was performed with the 355nm (3-7ns) pulses of a Nd:YAG laser at a repetition rate of 200Hz. We used 70% of maximum laser power for all measurements. Each spectra was averaged over 400 laser shots and analyzed with the AB SCIEX 4000 Series Explorer Software (AB SCIEX Pte Ltd, Massachusetts, USA). Matrix: 2,5-dihydroxybenzoic acid 1 M in 1:1 methanol/water solution.

**SEC analysis:** measurements were done on a Agilent 1260 Infinity system equipped with 1260 isopump, 1260 TCC, 1260 VWD VL, 1260 RID, Phenogel 5u linear/mixed guard column (30 x 4.6 mm), followed by Phenomenex Phenogel 5u $10^4$ Å (300 x 4.6 mm)column working at 60 °C. DMF was used as eluent at a flow rate of 1 ml/min. Before SEC analysis is performed, the samples were filtered through a 0.2 μm PTFE filter (15 mm, Phenomenex).The molecular weights were calculated using polystyrene standards.

## SYNTHESIS AND CHARACTERIZATION

### General

Glutamic acid γ–benzyl ester and succinic anhydride were purchased from Fluka. Triphosgene, α-pinene, TEA and DPPA were obtained from Sigma-Aldrich. All other chemicals and solvents were Sigma-Aldrich, Fluka or Acros products and used as provided without further purifications.
Dialysis tubes with molecular weight cutoff 1 KD were obtained from Spectrum Labs and 12 KD cutoff from Sigma-Aldrich

### Synthesis of γ–benzyl glutamate N-carboxyanhydride (BLG-NCA)

Glutamic acid γ–benzyl ester (5.05 g, 21.3 mmol) and α-pinene (6.64 g, 48.7 mmol) were dissolved with 70 ml of ethylacetate in a three-neck flask and heated under reflux. Triphosgene (4.24 g, 14.2 mmol) was dissolved in 25 ml ethylacetate and added slowly with a dropping funnel once the reflux started. After 4 hrs of reaction, the heating was interrupted and 3/4 of the solvent was evaporated at reduced pressure. The compound was precipitated by addition of petroleum ether and the recovered solid was recrystallized twice, subsequently filtered with gooch and washed with petroleum ether. The NCA was recovered as a white solid (4.6 g, yield 84%).

$^1$H-NMR (200 MHz, $CDCl_3$): δ 7.35 (s, 5H, ArH), 6.61 (s, 1H, NH), 5.14 (s, 2H, Ar-$CH_2$), 4.37 (t, 1H, αCH), 2.59 (t, 2H, γ$CH_2$), 2.36-2.02 (m, 2H, β $CH_2$).

***Reference:*** G. J. M. Habraken, M. Peeters, C. H. J. T. Dietz, C. E. Koning; A. Heise *Polym. Chem.*, **2010**, *1*, 514–524.

### Synthesis of CQDs

Argine·HCl (5.6 g, 26.6 mmol) and 1,2-ethylendiamine (1.78 mL, 26.6 mmol) were dissolved in 13.3 ml of ultrapure water. The solution was placed in a domestic microwave oven and heated at 700 W for 180 sec. The brown-burned resulting solid was suspended in 50 ml of ultrapure water and centrifuged several times. The water solution was placed in a dialysis sack (1 KD cutoff) and dialyzed against ultrapure water for 24 hrs. Finally the aqueous solution was lyophilized giving solid CQDs 1.2 g.

**Synthesis of poli γ–benzyl-L-glutamate (PBLG) derivatives**

**Polymer 1**

BLG-NCA (450 mg, 1.7 mmol) was dissolved in 1 ml of dry DMSO under $N_2$ atmosphere. Then CQDs (20 mg) dissolved in 2 ml of DMSO were added to the NCA solution. The reaction was left to stir at room temperature for 96 hrs. The polymer was precipitated by adding MeOH to the solution. The polymer was filtered, washed several times with MeOH and $Et_2O$, and dried in vacuo. The polymer was recovered as a brown solid (350 mg, yield 78 %).

**COOH-PBLG-CQD (intermediate)**

Polymer **1** (160 mg) was dissolved in 2 ml of dry DMF. Succinic anhydride (100 mg, 1 mmol) was dissolved in 1 ml of dry DMF and added to the solution of the polymer together with TEA (50 µl, 0.36 mmol). After 24 hrs under stirring at 40 °C, aqueous acetic acid (1 ml) was added to the reaction mixture. Then the polymer was precipitated by adding MeOH and washed several times with MeOH and $Et_2O$. The polymer was recovered as a brown solid (140 mg).

**Polymer 2**

COOH-PBLG-CQD (100 mg) was dissolved in dry DMF and activated with DPPA (200 µl, 0.93 mmol) and TEA (250 µl, 1.8 mmol). CQDs (50 mg) were added to this mixture and maintained at 40 °C under stirring for 24 hrs. The polymer was precipitated from the mixture by adding acidic water and MeOH, washed several times and obtained as a solid (80 mg).

**Functionalization with silver nanoparticles**

**Synthesis of 3**: A CQDs and $AgNO_3$ blend solution (1:4, w/w) in ethanol was prepared at a concentration of 2 mg/ml and placed in a UV quartz cuvette (1×1×5 cm). This solution was exposed to UV light with wavelength of 254 nm for 30 min using a 6W UV (mineralight lamp, Model UVGL-54) . The cell was kept 3 cm apart from the light source. After UV irradiation for few minutes, the color of solution start to change from light yellow to dark brown, implying that the reduction of Ag ions to Ag nanoparticles.

Synthesis of free Ag NPs: $AgNO_3$ was dissolved in N-methyl-2-pyrrolidone at a concentration of 4 mg/ml. This solution was treated with same procedure as CD-Ag NPs, except UV irradiation was carried out for 1 h. Free Ag NPs prepared in this manner had an average diameter of 3 nm, equal to the diameters of Ag NPs synthesized using CD's.

***Reference:*** H. Choi, S-J. Ko, Y. Choi, P. Joo, T. Kim, B. R. Lee,J.-W. Jung, H. J. Choi, M. Cha, J-R. Jeong, I-W. Hwang, M. H. Song, B-S. Kim, J. Y. Kim, *Nat. Photonics* **2013**, *7*, 732–738.

**Microstructure Preparation**

Typically, 10 mg of polymer were dissolved in 5 ml of a solvent mixture DMF/THF 3:7. This solution was put into a dialysis tube with a molecular weight cutoff of 12 KD. The dialysis process was carried out against water for 48 hrs.

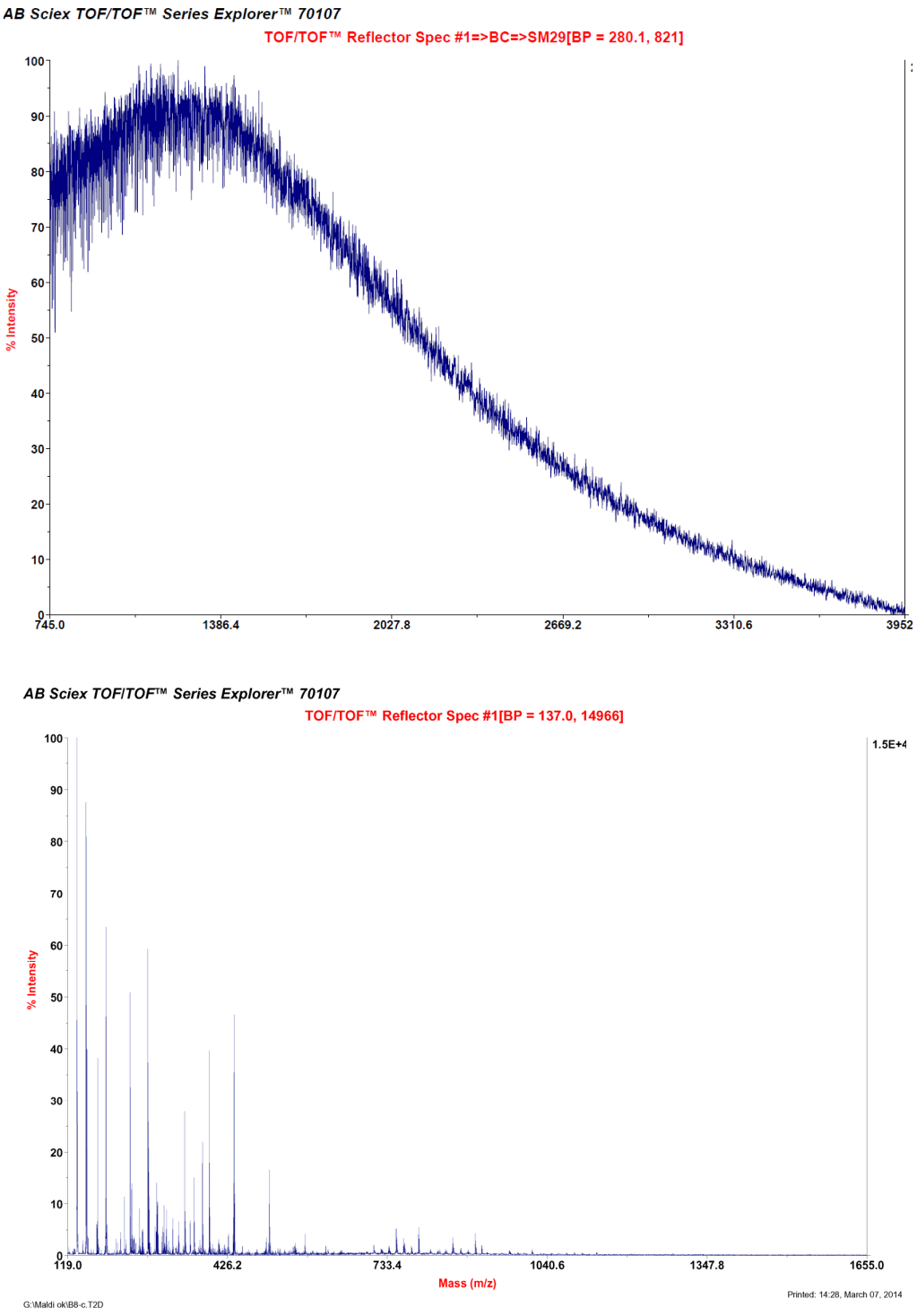


**Figure S1. Upper part:** MALDI spectrum of **CQDs**. **Bottom part**: MALDI spectrum of the matrix.

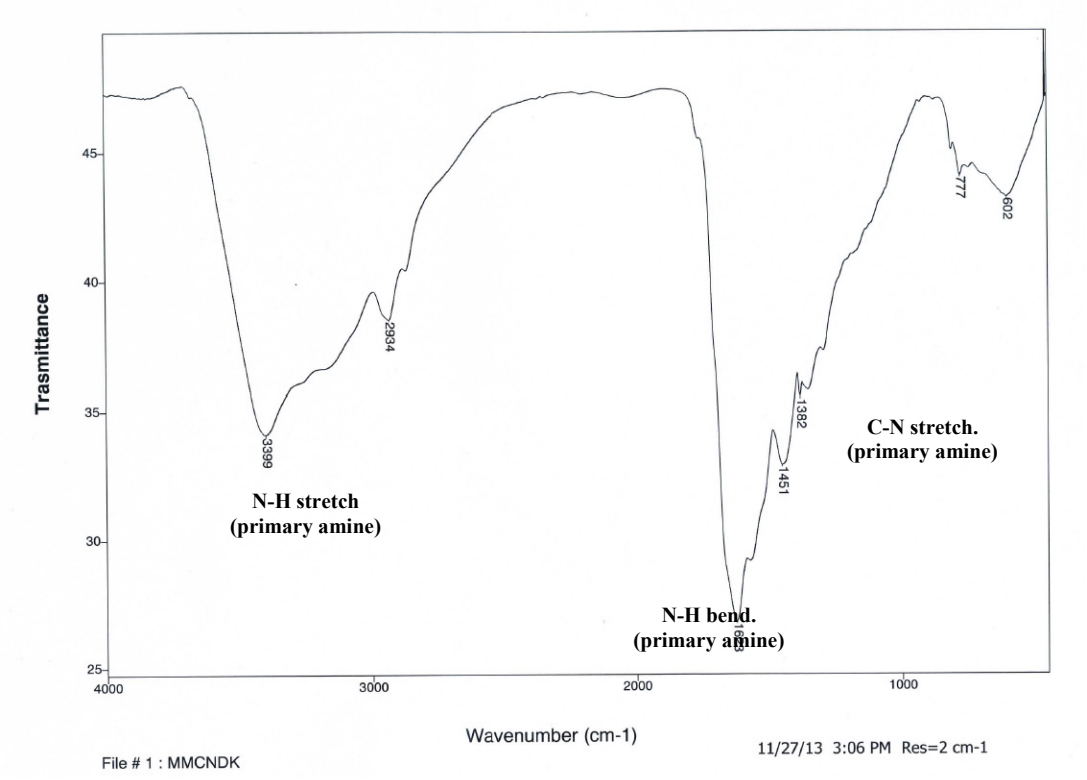


**Figure S2.** KBr FT-IR spectrum of **CQDs**.

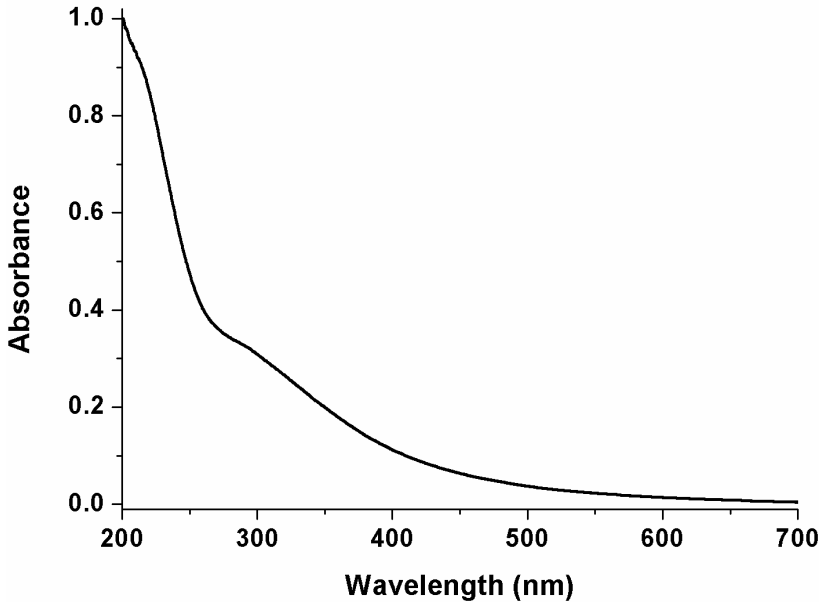


**Figure S3.** UV-Vis spectrum of **CQDs** in water.

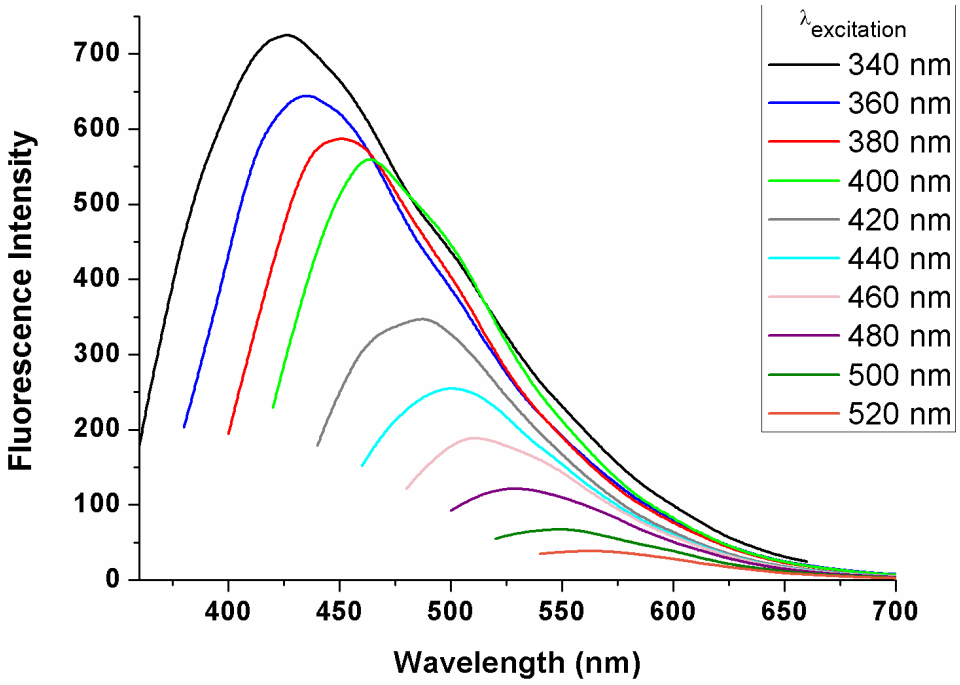


**Figure S4.** Fluorescence emission spectra of **CQDs** in water.

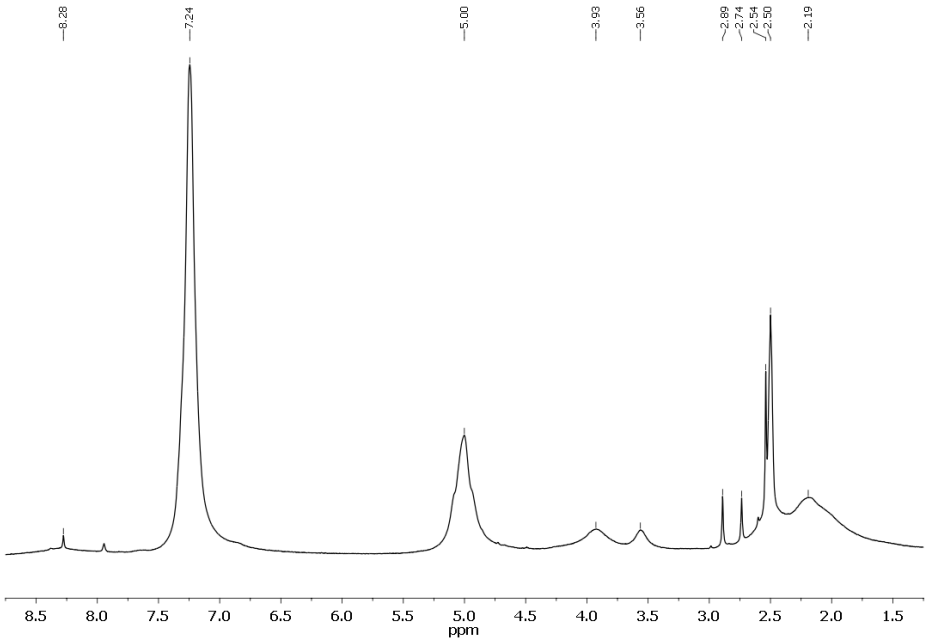


**Figure S5.** NMR spectrum of polymer **1** in DMSO solution.

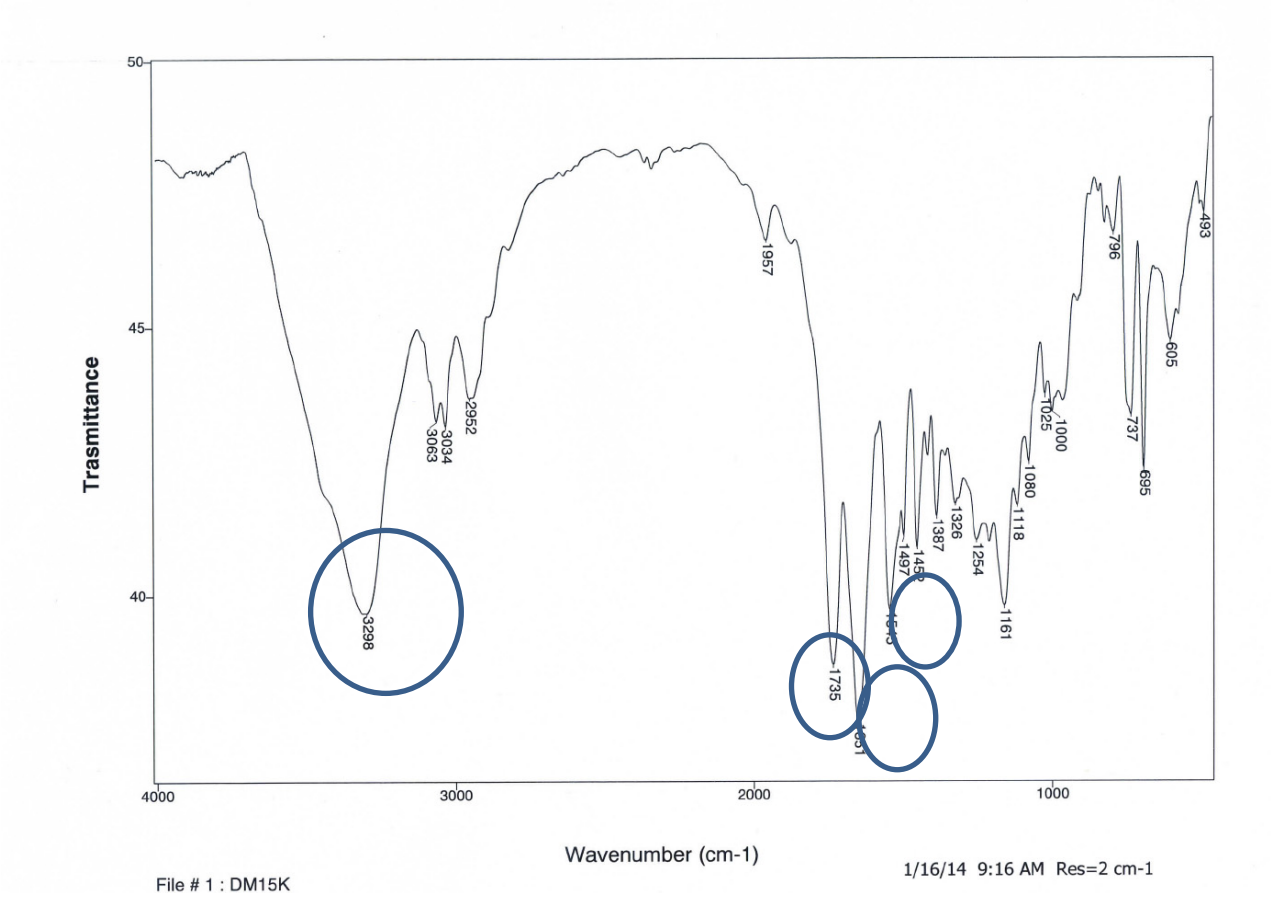


**Figure S6.** KBr FT-IR spectrum of polymer **1**. Highlighted, from left to right, the peaks relative to: amide A, ester, amide I and II respectively.

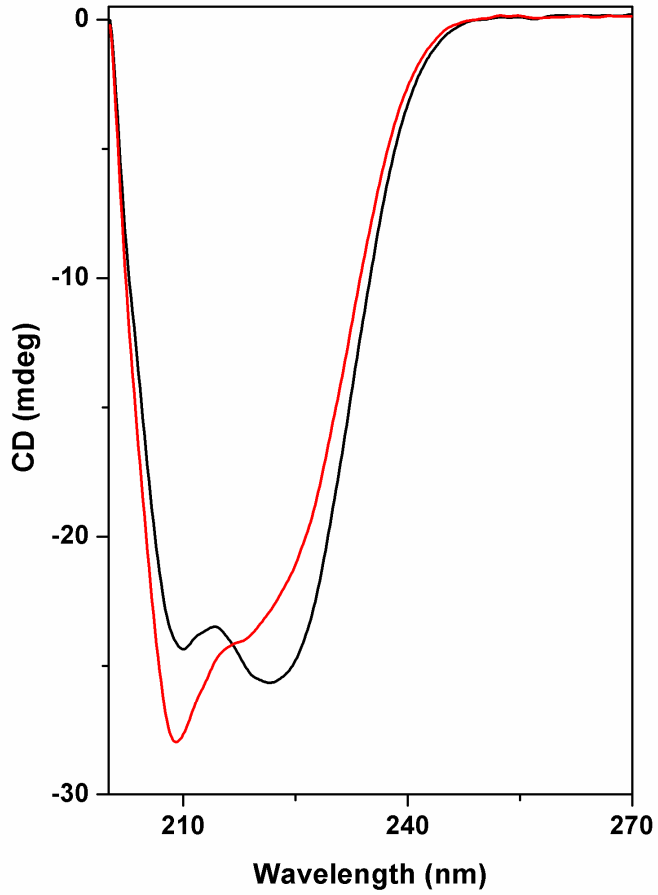


**Figure S7.** CD spectra of polymers **1** (red line) and **2** (black line) in HFIP solution (concentration 2 mg/ml).

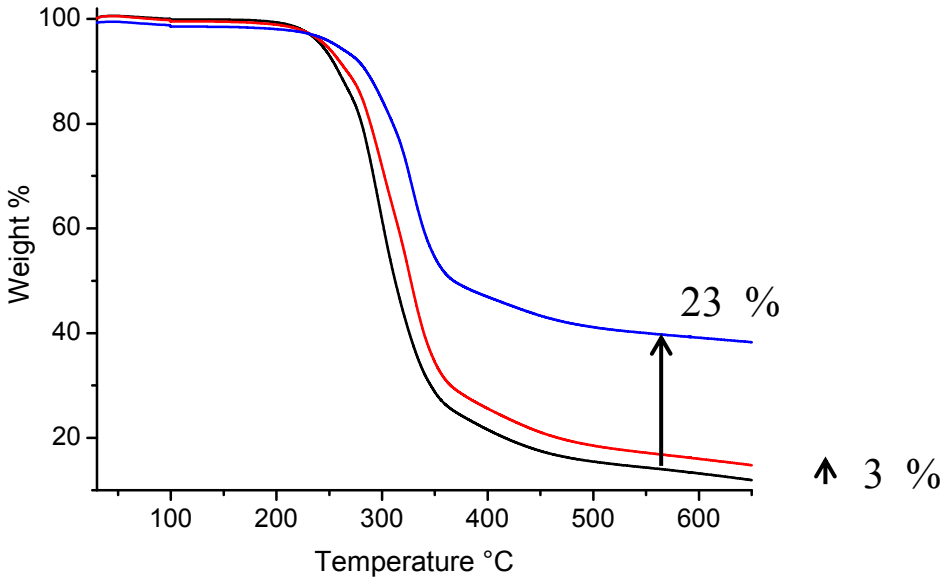


**Figure S8.** Thermogramivetric analysis comparison of a reference PBLG (black line), polymer **1** (red line) and polymer **2** (blue line).

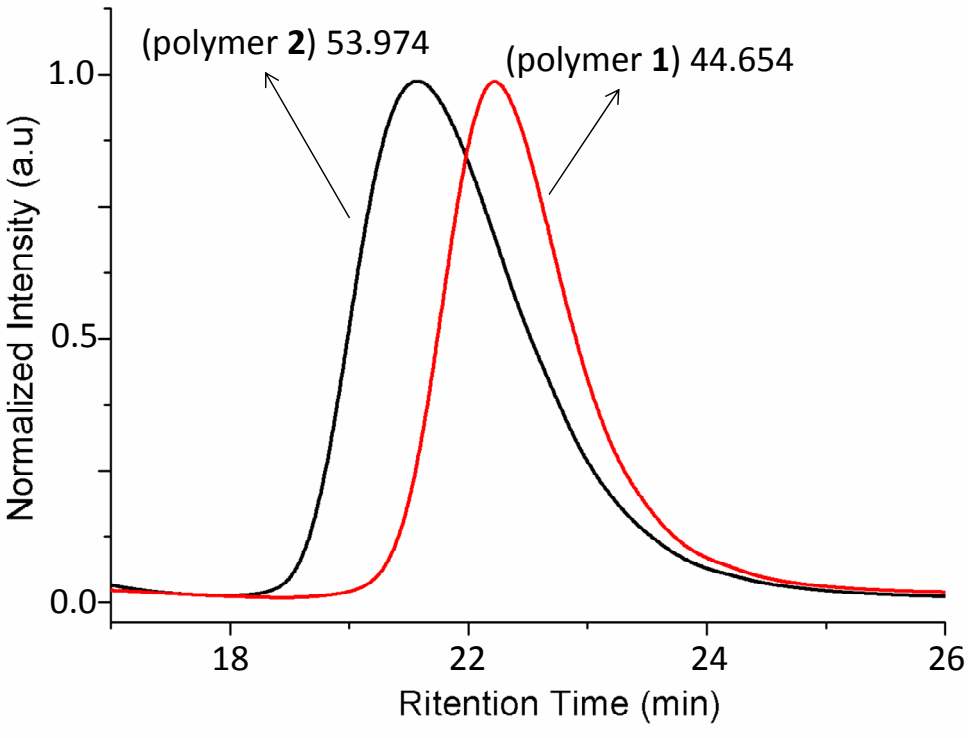


**Figure S9.** SEC traces of polymers **1** and **2**.

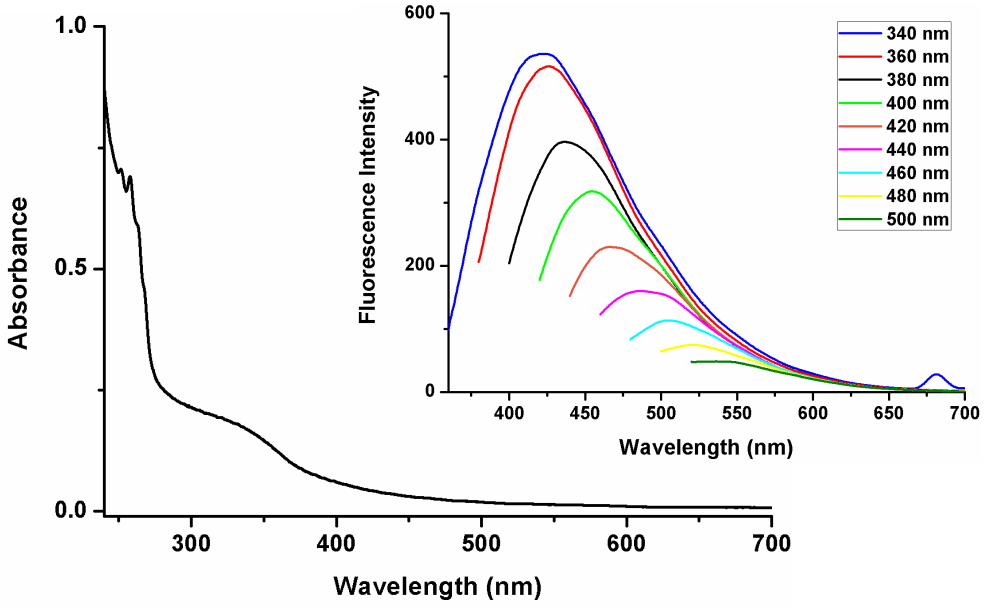


**Figure S10.** UV-absorption (left) and fluorescence emission (right) spectra of **1** in THF solution

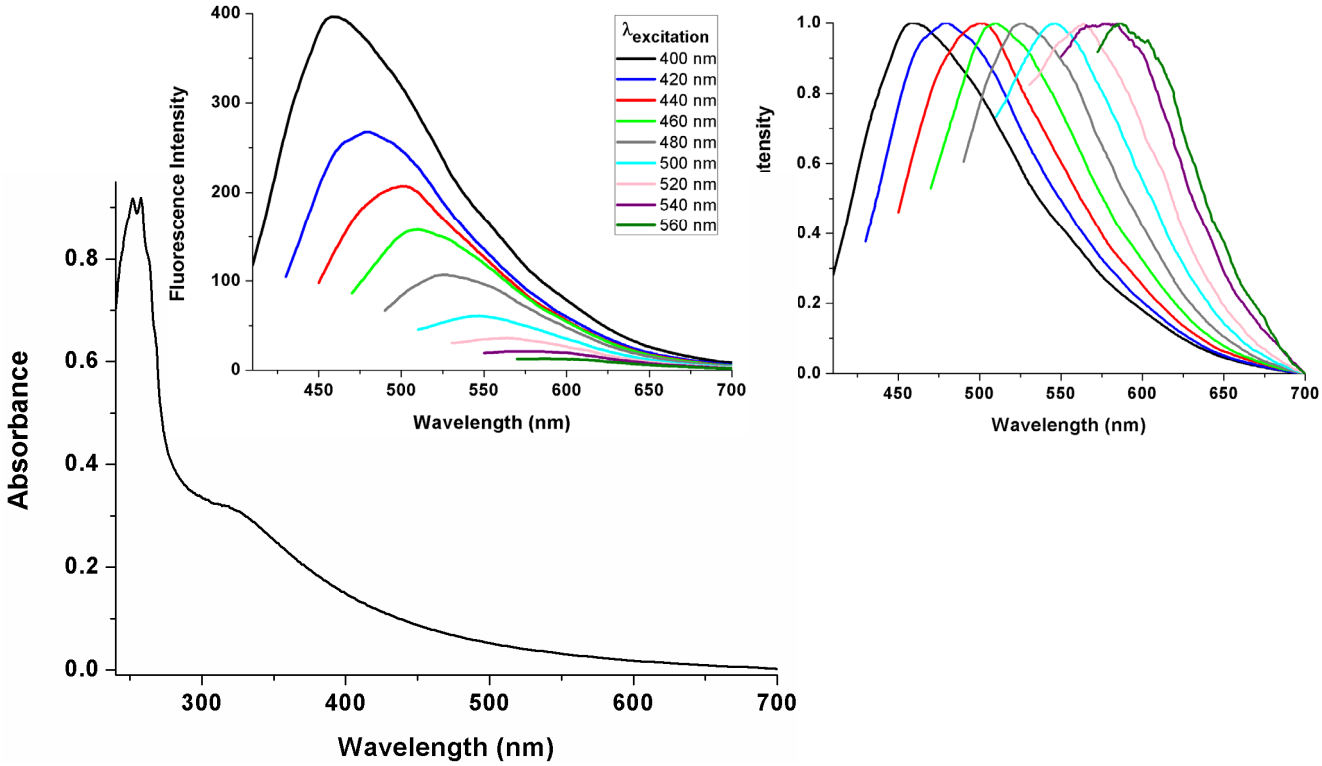


**Figure S11.** UV-absorption (left), fluorescence emission (center) and normalized fluorescence emission spectra of **2** in THF solution.

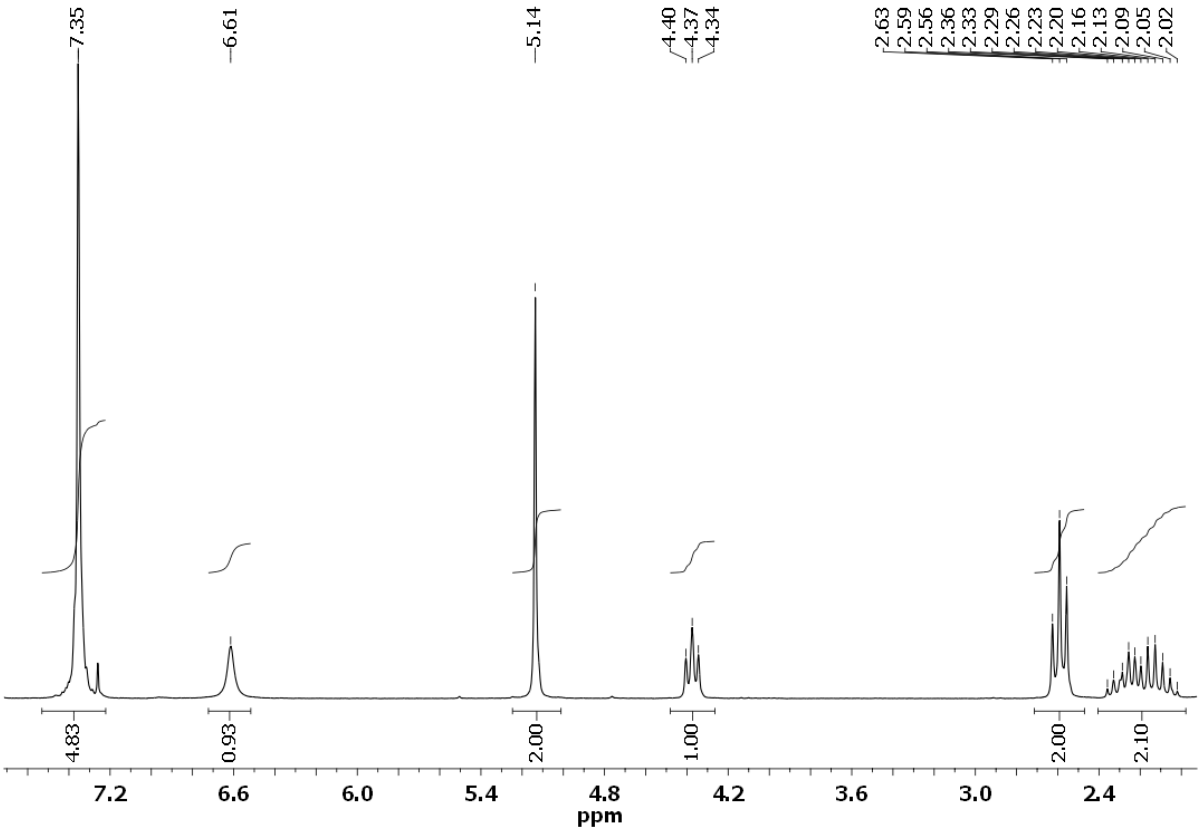


**Figure S12.** $^{1}$H NMR spectrum of BLG-NCA in $CDCl_3$.