Electronic Supporting Information (ESI)

Mechanically Strong, Fluorescent Hydrogels from Zwitterionic Fully **π**-Conjugated Polymers

Einat Elmalem, [a] Frank Biedermann, *[a,b] Maik R. J. Scherer, [a] Alexandros Koutsioubas, [c] Chris Toprakcioglu, [c] Giulia Biffi, [d] and Wilhelm T. S. Huck *[a,e]

[a] Melville Laboratory for Polymer Synthesis, Department of Chemistry, University of Cambridge, Cambridge, CB2 1EW, U.K.

[b] Institute of Nanotechnology, Karlsruhe Institute of Technology, 76021 Karlsruhe, Germany

[c] Department of Physics, University of Patras, Patras 26500, Greece.

[d] Cancer Research UK Cambridge Institute, Li Ka Shing Centre, Robinson way, Cambridge, CB2 0RE, UK.

[e] Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg, 135 6525 AJ Nijmegen, The Netherlands.

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Materials

1,4-phenylenediboronic acid, butane sultone, 2-bromo-ethanol, anhydrous toluene (sealed bottle), methyltrioctylammonium chloride, and Texas Red-labeled DNA oligonucleotides were purchased from Sigma-Aldrich used as received. DMSO and potassium carbonate were purchased from Acros and Pd(PPh3)4 was purchased from Strem. 2,7-dibromo-9,9-bis((N,N-dimethylamino)ethanyl) fluorene was received from Cambridge Display Technology (CDT) company. The synthesis is reported in SI-ref 1.

Synthesis of Poly[(2,2'-(2-methyl-7-(*p*-tolyl)-9H-fluorene-9,9-diyl)bis(N,N-dimethylethan-1-amine)] (1)[2]

The synthesis of this polymer was adopted from the literature (ref 2) with a few adaptations. To a mixture of 2,2'-(2,7-dibromo-9,9-bis((N,N-dimethylamino)ethanyl) fluorene (0.56 g, 1.2 mmol), 1,4-phenyldibronic acid (0.20 g, 1.2 mmol), 2 M K_2CO_3(1.32 g, 9.64 mmol, 4.8 mL degassed water), $Pd(PPh_3)_4$ catalyst (70 g, 0.06 mmol) and a few drops of methyltrioctylammonium chloride (phase transfer catalyst) was added 7 mL of anhydrous toluene. The mixture was stirred at 90 °C for 3 days under nitrogen atmosphere. The reaction was quenched when opening the flask to the air and the crude product was extracted with chloroform, filtered and concentrated under reduced pressure. The desired dark coloured polymer (1) was obtained upon precipitation from methanol (85% yield). ^1H NMR (500 MHz, $CDCl_3$): δ 1.63-1.77 (broad, 2H), 1.86-2.07 (m, 14H), 2.27-2.48 (broad, 4 H), 7.73 – 7.84 (m, 10H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.58, 45.38, 52.51, 54.11, 120.25, 121.21, 126.32, 127.43, 128.77, 131.93, 139.78, 139.98, 150.22 ppm. For GPC, see Fig. SII1.

Synthesis of Poly (Poly[4,4'-(((2-methyl-7-(p-tolyl)-9H-fluorene-9,9-diyl)bis(ethane-2,1-diyl))bis(dimethylammoniumdiyl))bis(butane-1-sulfonate)] (2a)[3]

The synthesis of this polymer was adopted from the literature (ref 3) with a few adaptations. 1,4-butane sultone (0.5 ml) was added to a solution of the neutral polymer (350 mg) in anhydrous DMSO. The mixture was stirred at 70 °C for 3 days under nitrogen. The product was precipitated from THF, filtered and washed with THF, and dried in a vacuum oven to give **2a** as a yellow powder (83% yield). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 1.41 (m, 4H), 1.56 (m, 4H), 2.60 (m, 8H), 3.31 (20H), 7.85-8.17 (m, 10H) ppm. ^{13}C NMR (125 MHz, DMSO-*d6*): δ 21.97, 31.75, 33.01, 42.88, 50.99, 51.70, 61.07, 121.95, 130.04, 128.08, 129.75, 132.41, 133.28, 140.09, 147.80, 175.57 ppm. For GPC, see Fig. SII1.

Synthesis of (N,N'-((9H-fluorene-9,9-diyl)bis(ethane-2,1-diyl))bis(2-hydroxy-N,N-dimethylethan-1-aminium) bromide (2b)

2-Bromoethanol (0.5 ml) was added to a solution of the neutral polymer (350 mg) in anhydrous DMSO. The mixture was stirred at 90 °C for 3 days under nitrogen. The product was precipitated from THF, filtered and washed with THF, and dried in a vacuum oven to give **2b** as a pale yellow-white powder (79% yield). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 1.65 (m, 4H), 3.40 (m, 20H), 3.60 (4H), 7.80-8.01 (m, 10H) ppm. GPC analysis was not possible because the polymer sticks on the columns.

Texas-Red-labeled DNA.

10 μM of each DNA oligonucleotide (28 bases) was annealed in 10 mM Tris HCl, pH 7.4, by slow cooling from 95 to 21 °C. Texas Red-labeled DNA oligonucleotides had the following sequence: 5’-GG(TTAGGG)4TTA-3’/3’-C(AATCCC)4AAT-5’ (with the G-rich DNA oligonucleotide labeled with Texas Red at the 5' end).

Typical procedure for hydrogel preparation

For 10.0 wt% hydrogel: 100 μL of distilled water was added to 10 mg of **2a** contained in a small vial. The mixture was slowly heated with a heat gun until everything was dissolved, and then let to cool down to room temperature, which results in the formation of a rigid hydrogel material.

Instrumentations.

Rheology. Characterization was performed at 20, 30 and 40 ^{o}C with an ARES strain-controlled rheometer using parallel plate geometry. Rheological analysis was performed using an ARES-LC controlled strain rheometer fitted with a water bath set to 25 ^{o}C. All measurements were performed using a 20 mm parallel plate geometry and analyzed using TA Instruments TA Orchestrator software.

NMR. ^{1}H NMR (500 MHz) spectra were recorded using a Bruker Avance QNP 500. Chemical shifts are recorded in ppm (δ) either in $CDCl_3$ or DMSO with the internal references set to δ = 7.25 or 2.50 ppm, respectively. ^{13}C NMR (125 MHz) spectra were recorded using a Bruker Avance Cryoprobe ATM TCI DRX 500 or a Bruker Avance 500 BB-ATM. Chemical shifts are recorded in ppm (δ) in $CDCl_3$ or DMSO with the internal reference set to δ = 77.230 and 40.45 ppm, respectively.

SAXS. Small-Angle X-ray Scattering (SAXS) experiments were performed at the SWING beamline of the French synchrotron facility SOLEIL. The x-ray wavelength ($\lambda = 1.23\text{Å}$) and sample-detector distance ($D = 6375\ mm$) corresponded to a scattering wavevector range of $0.00175\ \text{Å}^{-1} < q < 0.170\ \text{Å}^{-1}$. The gel was sandwiched between two thin sheets of mica so as to obtain a sample thickness of ca. 1 mm. The beam path was held under vacuum conditions, except for a path length of about 1 cm, around the sample. Then scattering data (40 frames of $1s$ duration for each sample) were collected with a $17 \times 17\ cm^2$ low-noise AVIEX CCD detector. The 2D scattering images were radially averaged, divided by the transmitted intensity and finally averaged for each sample. The scattering curves that correspond to the gel form and structure factor were obtained by subtracting the mica scattering signal.

SEM. Scanning electron microscopy (SEM) images were obtained using a variable pressure Leo1530 with an InLens detector. SEM samples were prepared by direct freezing of the supramolecular hydrogels in liquid nitrogen followed by lyophilization. The resulting cryo-dried materials were imaged after sputtering.

DMF-GPC. Gel permeation chromatography (GPC) was performed on two Jordi 5mm DVB-Glucose columns connected in series with a SPD-M20A prominence diode array detector and refractive index detector (both Shimadzu) calibrated in relation to polystyrene standards.

Samples were filtered over 0.45 mm nylon filters before injection using a 0.75 mL / min flow rate.

Absorption and Photoluminescence Spectroscopy. Absorption spectra were recorded with a Carry 400 UV/visible spectrometer, and photoluminescence spectra were measured with a Cary Eclipse fluorimeter.

Time Correlated Single Photon Counting. Photoluminescence decay kinetics were measured by time-correlated single photon counting (TCSPC). The excitation source was a pulsed 407 nm, 80 ps full width at half maximum, 10 MHz diode laser (Pico Quant LDH 400) and the luminescence was detected using a microchannel plate photomultiplier (Hamamatsu Photonics) coupled to a monochromator and TCSPC electronics (Lifespec-ps and VTC 900 PC card, Edinburgh Instruments).

FRET experiments: To a cuvette filled with 1.1 wt% hydrogel **2a**, ds-DNA-TR ([ds-DNA-TR] = 1.0×10^{-7} M) was successively added while stirring and maintain homogeneousness in the gel.

Supporting Figures:

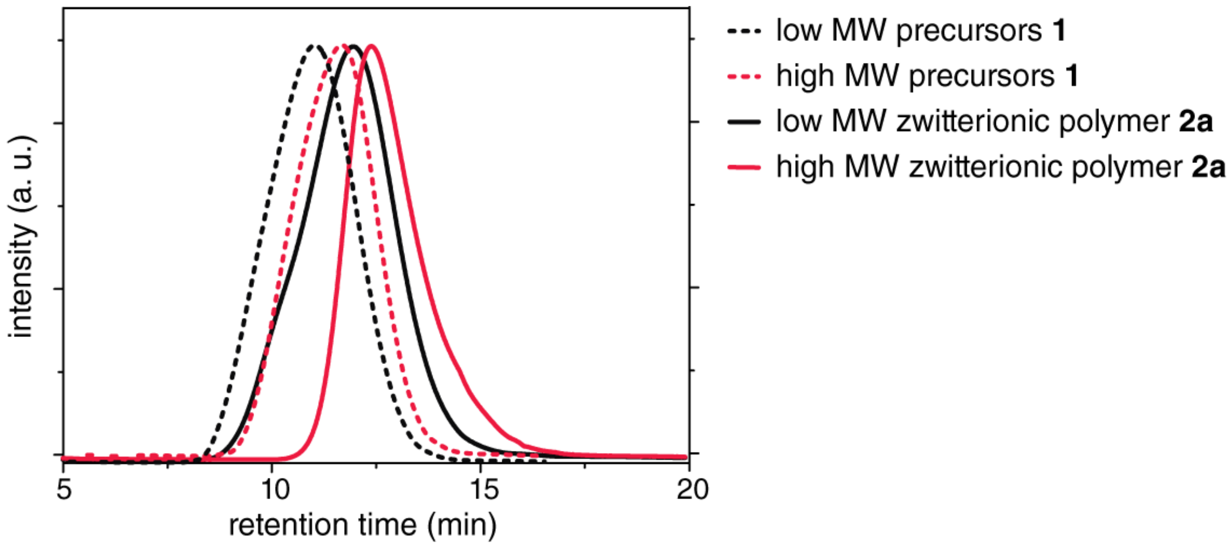

Fig. SI1. a) DMF gel permeation chromatography (GPC) for polymer **2a** with two different molecular weights, M_n = 60 and 100 kDa, based on polystyrene standards. Also shown are the GPC curves for the low and high MW precursor polymer **1**.

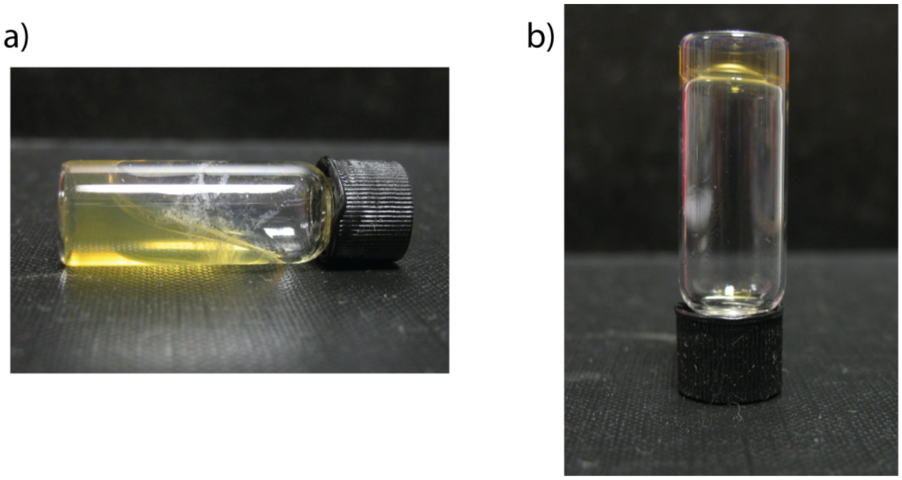

Fig. SI2. Photos of hydrogel **2a** presenting the different viscosity properties of different polymer loading at 1.1 wt% (a) and 10 wt% (b) in water at room temperature.

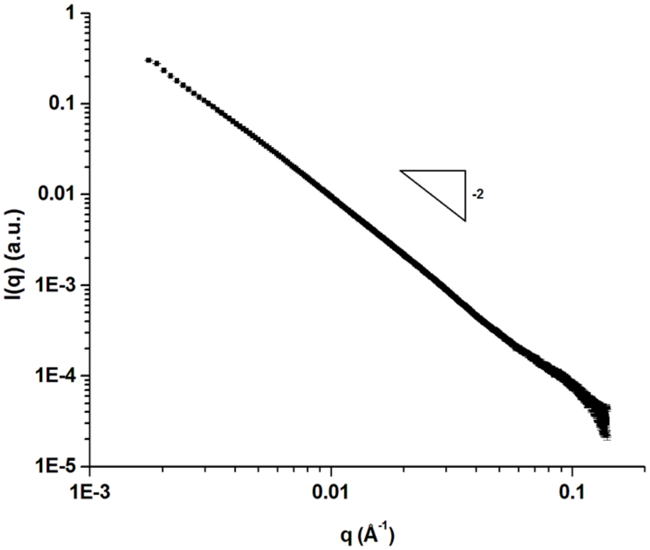

Fig. SI3: SAXS profile of a 1.1 wt% hydrogel of **2a** (100 kDa) at 25° C. The scattered intensity exhibits q^{-2} dependence over most of the measured q-range indicating that the gel structure is characterized by a network with a sheet-like arrangement

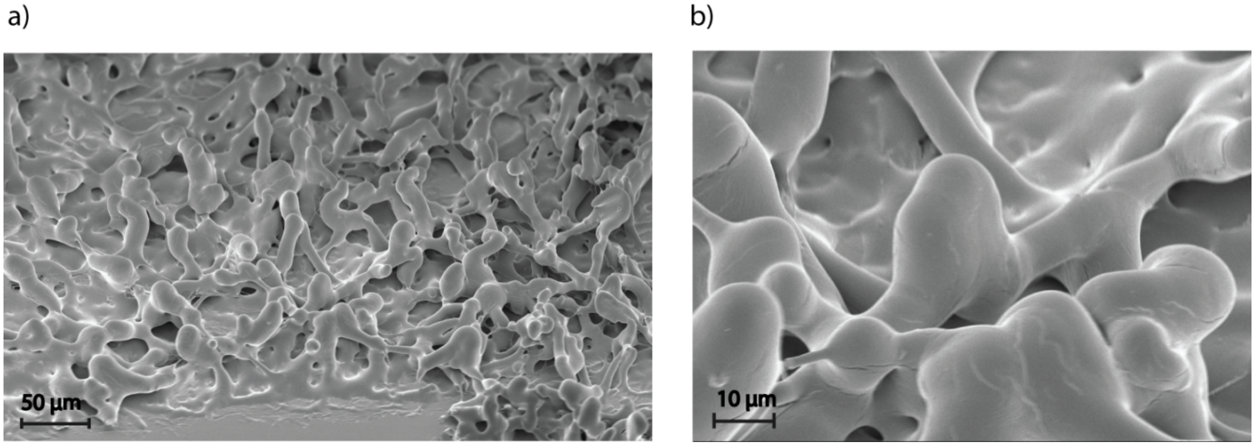

Fig. SI4. SEM images of cryo-dried and lyophilized sample of low molecular weight hydrogel **2a** at 2.5 wt% polymer loading (M_n = 60 kDa, estimated by DMF-GPC based on polystyrene standards), presenting a different porous structure compared to **2a** with higher molecular weight hydrogel (Figures 2c-d). Although the 60 kDa **2a** hydrogel possesses a porous microstructure, its edges are round and the structure has less network and linkage properties in comparison to the hydrogel obtained from the 100 kDa variant of **2a**. As a result, 60 kDa **2a** hydrogel has a smaller packaging volume than 100 kDa **2a** hydrogel and shows a reduced mechanical strength, which is shown in Fig. SI6 below.

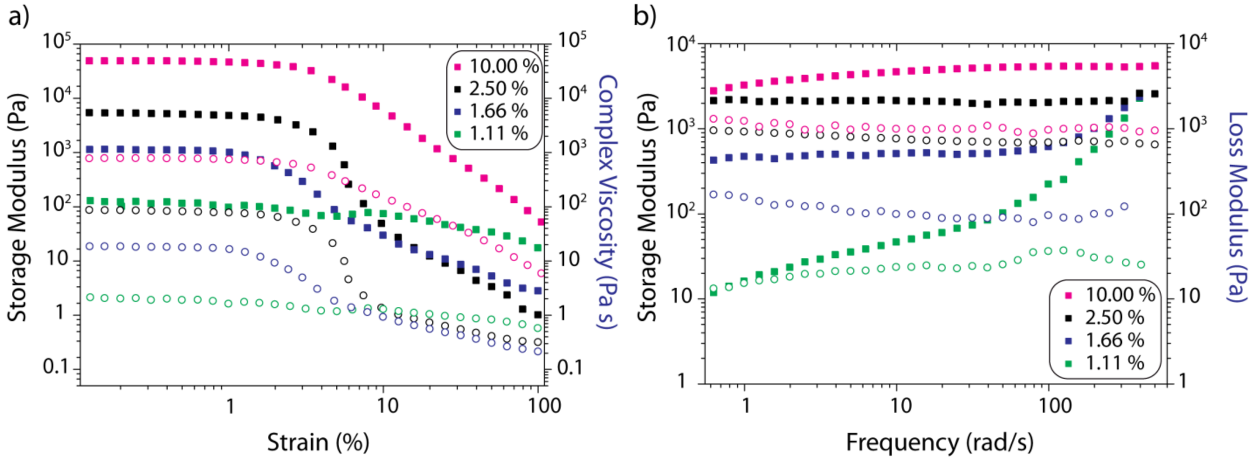

Fig. SI5. Oscillatory rheological analysis of **2a** zwitterionic hydrogels, demonstrating the effect of different polymer loading (10.0 - 1.1 wt %) on the mechanical properties of the hydrogel at 20 °C. a) Storage modulus and complex viscosity obtained from a strain-amplitude sweep preformed at 10 rad/s. b) Storage and loss modulus obtained from a frequency sweep preformed at 1% strain.

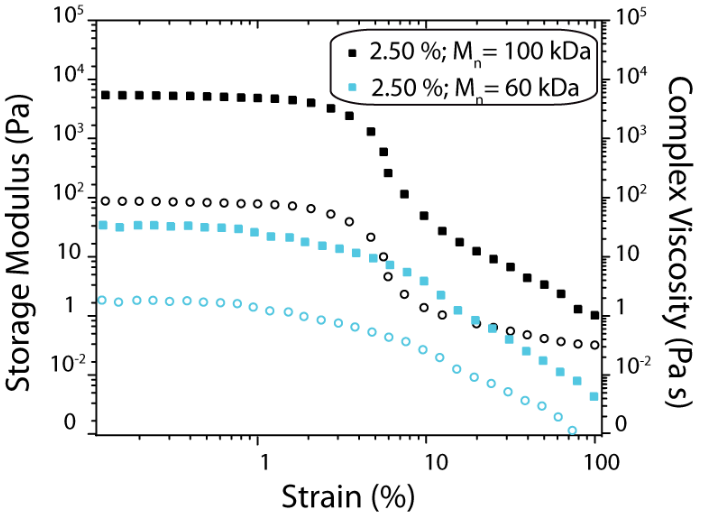

Fig. SI6: Oscillatory rheological analysis of **2a** zwitterionic hydrogels, demonstrating the effect of different molecular weights on the mechanical properties of the hydrogel at 20 °C. Storage and loss modulus obtained from a frequency sweep preformed at 1% strain. Lowering the molecular weight of polymer **2a** is accompanied with a reduction in the G’, G’’ and complex viscosity values.

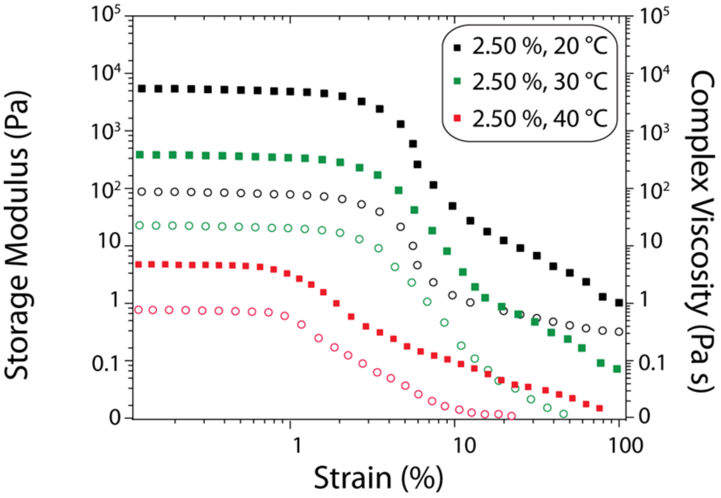

Fig. SI7. Oscillatory rheological analysis of **2a** zwitterionic hydrogel, demonstrating the effect of different temperatures on its mechanical properties. a) Storage modulus and complex viscosity obtained from a strain-amplitude sweep preformed at 10 rad/s. A large decrease in the G', G'' and complex viscosity values was observed upon heating, corroborating the fluorescence observations presented in Figure 3.

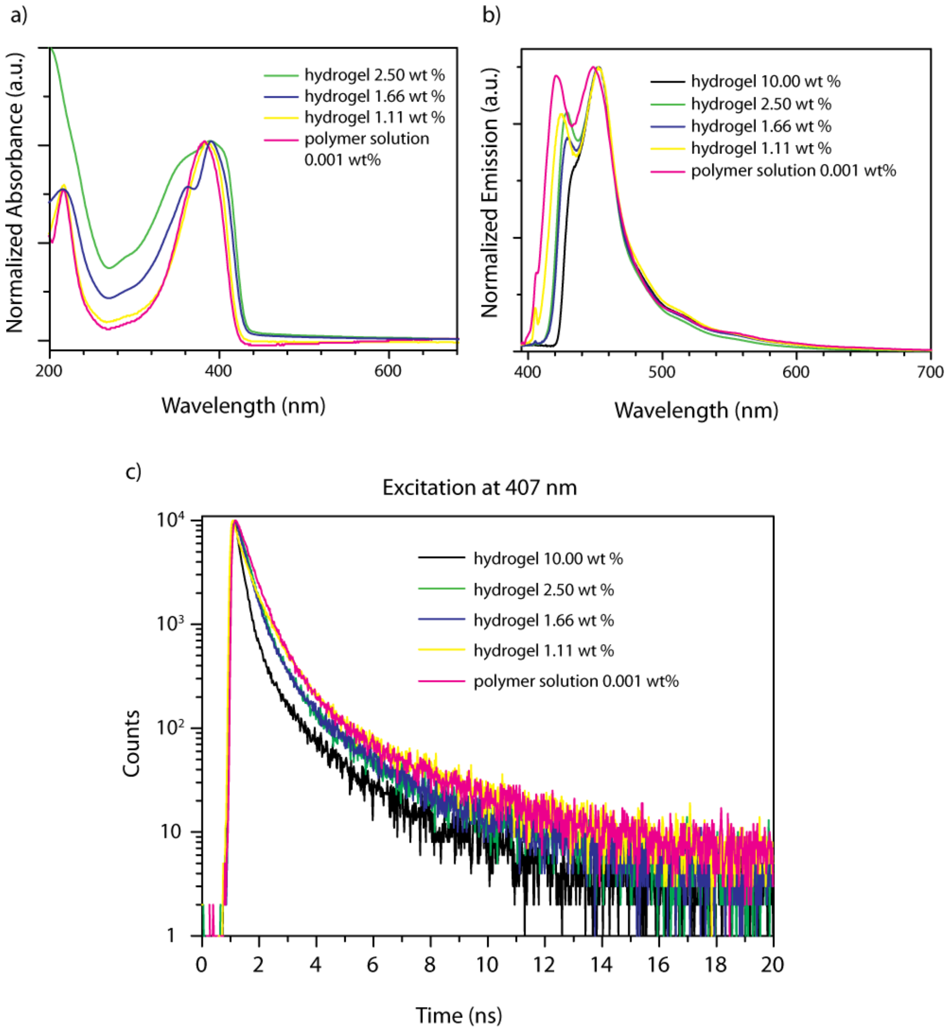

Fig. SI8. Photophysical characterization of the solution and the solid hydrogel of **2a** in different polymer loadings. a) Absorption and b) emission spectra of **2a**, showing no extra red-shifted bands in the hydrogel in comparison to the solution. In contrast, literature studies (ref 4) of π – aggregated fluorene polymers showed a peak broadness of approximately 50 nm in the absorption spectrum and an extra red peak at 500 nm. The apparent reduction in the emission intensity (b) of the fluorene band centered around 410 nm may result from self-absorption (inner-filter-effects) in the highly concentrated samples and from self-quenching between adjacent polymer chains. c) Emission lifetime spectra of **2a** shows a gradually not-monoexponential decrease in lifetime upon increasing in the polymer loading.

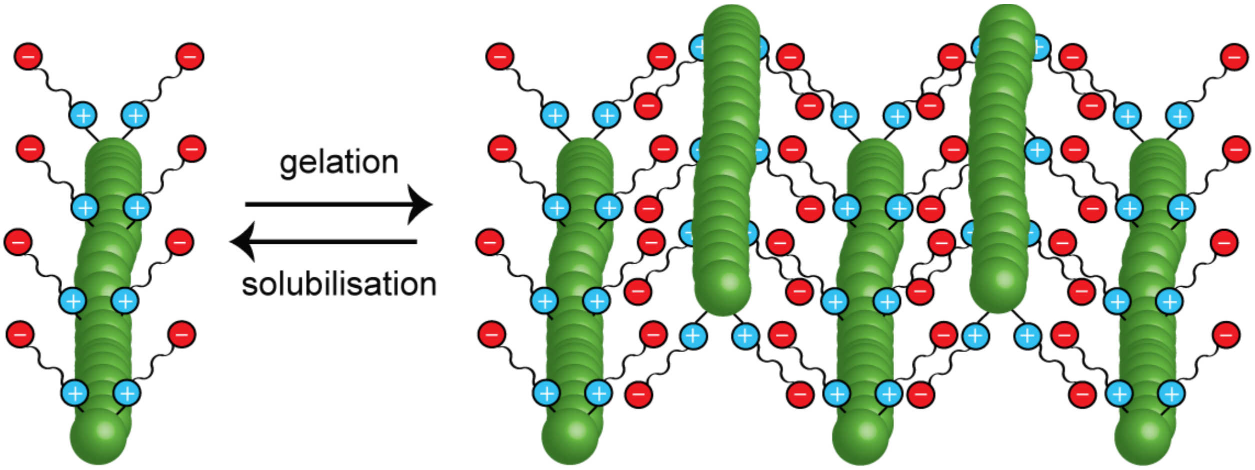

Fig. SI9: Schematic 3D representation of zwitterionic aggregation leading to a sheet-like structure. The green spheres represent the rigid poly(fluorene phenylene) back-bone. The model takes into account that side-chains point perpendicular to the main-chain in fluorene-type polymers. MMFF energy optimization studies showed that such an arrangement is indeed feasible for polymer **2a**.

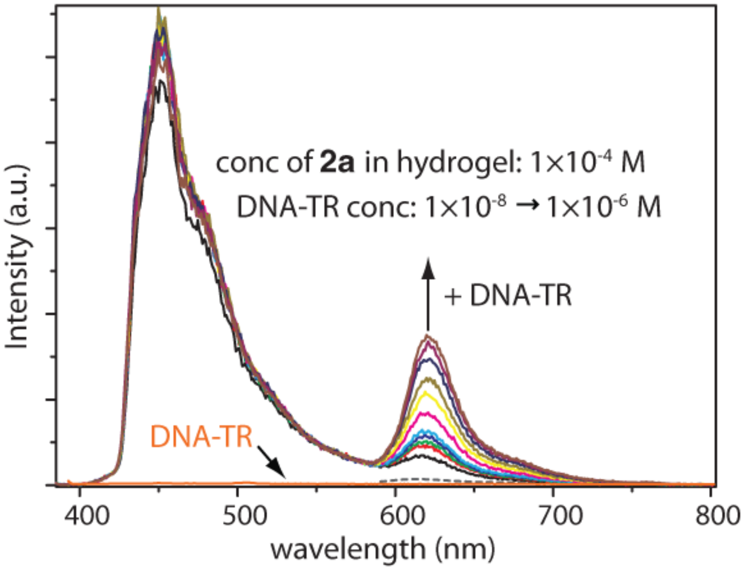

Fig. SI10: Emission spectra ($\lambda_{exc} = 380$ nm) of **2a** as a hydrogel (100 μM) upon addition of ds-DNA tagged with Texas Red (DNA-TR, 0 to 130 nM). The emission spectra of ds-DNA-TR alone (10 μM) for excitation at 380 nm (orange solid line) and 589 nm (grey dashed-line) are displayed for comparison.

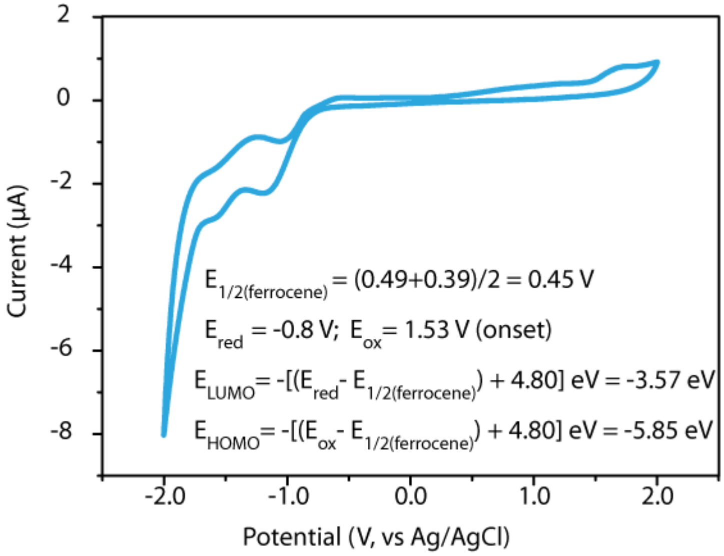

Fig. SI11. Cyclic voltammetry (CV) measurements, obtained with **2a** hydrogel modified glassy carbon as a working electrode (drop cast), platinum wire as a counter electrode and saturated calomel electrode (SCE) as a reference electrode (scan rate 100 mV/s). The CV displays the usual redox energy levels of fluorene-type polymers (against ferrocene as a reference).

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