Nearly monodispersed, emission- tuneable conjugated polymer nanoparticles

Supporting information

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Experimental Methodology

Materials

Poly(2,5-di(hexyloxy)cyanoterephthalylidene) (CN-PPV) (MW=Unknown), pluronic F127 (MW=12.5 kDa avg), tetrahydrofuran (with 250 ppm BHT as inhibitor, 99.9%) (THF) and tetramethyl orthosilicate (99%) (TMOS) were purchased from Sigma-Aldrich (England, UK). Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), Keratinocyte-Serum Free Medium (SFM), bovine pituitary extract (BPE), human recombinant epidermal growth factor (EGF) were purchased from Thermofisher Scientific (England, UK). CellTiter-Glo® reagent was purchased from Promega (England, UK).

Preparation of conjugated polymer nanoparticles

In a typical example, CN-PPV was prepared in THF by dissolving 1 mg of polymer into 1 ml of THF to make a 1 mg/ml polymer solution. The solution was sonicated in a 35 kHz ultrasound bath for 5 minutes to ensure the polymer was completely dissolved. In separate vials, 1 mg, 2 mg, 5 mg, 10 mg, 25 mg, 50 mg, 75 mg and 100 mg of the pluronic F127 were added to 1.1 ml of THF and sonicated for 5 minutes. 100 µL of the 1 mg/ml CN-PPV polymer solution was added to the F127 solution and left stirring overnight at 45 °C. Upon cooling to room temperature, 87 µl of TMOS were added and the solution was injected into 13.5 ml of deionised water under sonication, and further sonicated for 15 minutes. The solution was then stirred continuously at 400 rpm, at room temperature, for four days to evaporate off THF and to ensure complete hydrolysis of TMOS at the interface between the core and corona of the F127 micelles. Loss of water was compensated by readjustment to 10 ml. The nano-suspension (10 µm/ml of CN-PPV) was then filtered through a 0.22 µm syringe filter. The filtrate was stored at room temperature.

CPNs Optical and Physical measurements

Absorption spectra were measured using a Hitachi U-4100 UV-Visible-NIR spectrometer using a 1 cm path length quartz cuvette. Photoluminescence spectra were measured using a Horiba Fluoromax-4 spectrofluorometer. Particle size distributions and zeta potentials were obtained using a Malvern Zetasizer (utilising dynamic light scattering). Transmission electron microscopy images were acquired Transmission electron microscopy images were acquired on a JEOL JEM 1400Plus with filament electron source at 120 kV; For selected systems, CPNs were stored at 37 °C in a dark incubator and the particle size measured at regular intervals over a time period of up to 60 days.

The QY were measured using the dye comparison method. Photoluminescent quantum yields (PLQYs) were estimated using indocyanine fluoroscein-5-isothiocyanate (FITC, PLQY = 0.79) in ethanol[23]; coumarin 6 (PLQY = 0.78) in ethanol[24]; and rhodamine 6G (PLQY = 0.95) in ethanol[25] as standards.

$$\Phi_{sample} = \Phi_{ref} \frac{I_{sample}\ OD_{ref}\ \eta_{sample}^{2}}{I_{ref}\ OD_{sample}\ \eta_{ref}^{2}}$$

Where the subscripts *sample* and *ref* note sample and reference fluorophore respectively, Φ is the fluorescence quantum yield, I is the integrated sample fluorescence, OD is the optical density at the chosen excitation wavelength and η is the refractive index of the solvent. To avoid self-absorption effects, the absorbances of the sample and reference fluorophore solutions at the excitation wavelength were below 0.1.

Cell Culture
HeLa cells were verified as HeLa by STR profiling from Eurofins MWG. HeLa cells were grown at 37 °C in complete DMEM (Invitrogen) supplemented with 10% foetal bovine serum (FBS - Sigma) and 1 % Penicillin-Streptomycin (PenStrep - Invitrogen) in T75 tissue culture flask (Helena TTP).

Nanoparticle treatment
HeLa cells were grown on 1.5 µ-plate 8 well plate (ibidi) overnight at 30,000 cells/ml. Cells were treated with NPs, negative and positive controls for 24h. The nanoparticle suspension was serially diluted in DMEM to have a polymer concentration of 5 µm/mL. 150 µl of the CN-FO-DPD nanoparticle suspension was added to 150 µL of the aforementioned media (for the 8 well plate). These were incubated for 24 hours prior to fixation for immunofluorescence or live cell imaging.

Cytotoxicity Experiment
HEK cells were cultured on a sterilised 96 well microplate. Cell cultures were kept at physiological temperature (~37°C), 5% CO2 in a humidified incubator. After 24 hours, to all HEK cells to attach to the microplate, conjugated polymer nanoparticles were added and imaged at the following time intervals of 1 hour, 24 hours and 48 hours. At these time points, 100 µL CellTiter-Glo® reagent was added and cells were left to lyse for 10 minutes before the luminescence was recorded.

Image acquisition
Confocal images were acquired with an inverted confocal microscope (Leica TCS SP2) and an internal analogue photomultiplier tube detector, whose detection wavelength range for the fluorescence emission was set to 500-650 nm. The samples were excited with a solid-state CW laser (CNI Laser MBL-III-100mW) at a wavelength of 473 nm and a power of ~5 mW after passing through a neutral density filter wheel. A RSP 500 excitation beam splitter and a 63X 1.2 N.A. water-immersion objective were used to acquire the images. The line scan speed was set to 400 Hz, the image size to 512x512 pixels with a pixel size of 470x470 nm2 and a pinhole of 2 Airy units. Besides, the transmitted light images were taken at the same time as the confocal fluorescence images.

Figures

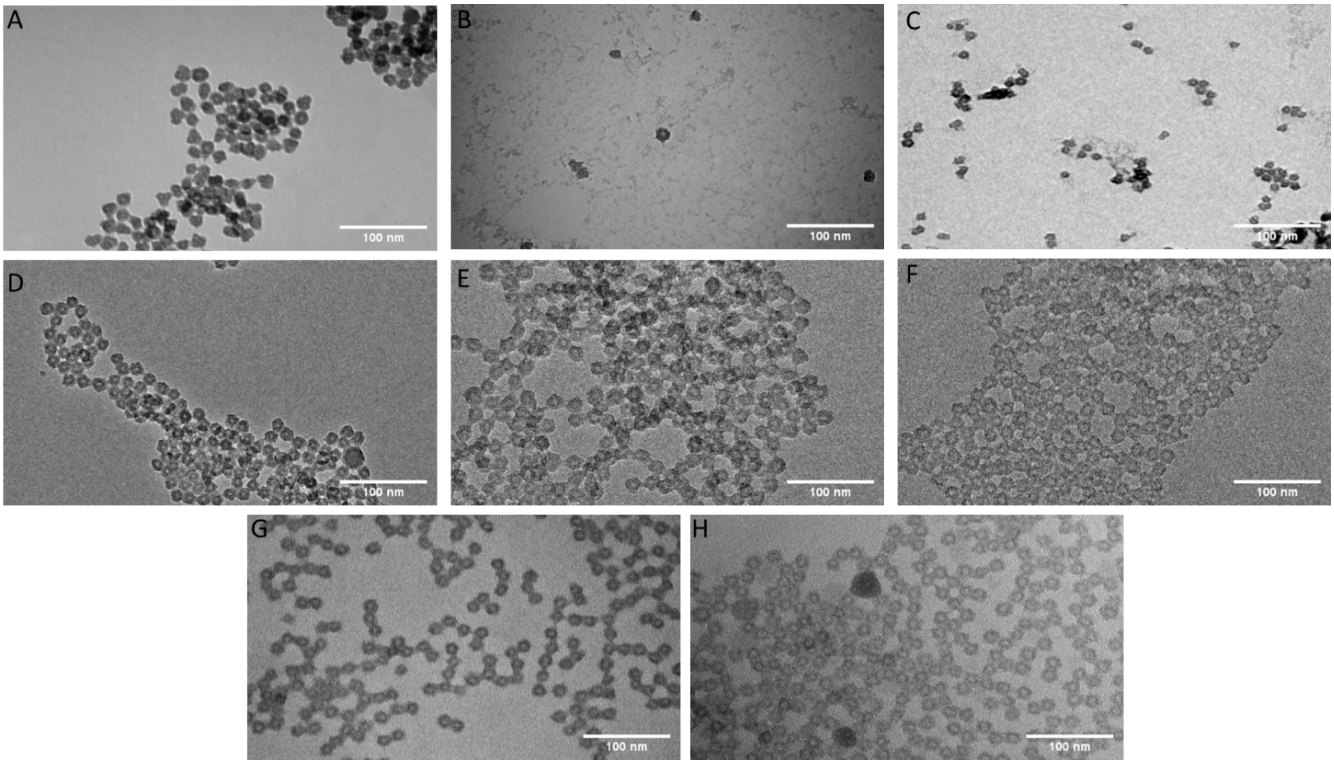

Figure S1 - TEM images of the CN-PPV NPs with silica shells consisting of different ratios of CP to F127. A) 1:10, B) 1:20, C) 1:50, D) 1:100, E) 1:250, F) 1:500, G) 1:750 and H) 1:1000. Taken at 120x resolution (scale bar = 100 nm).

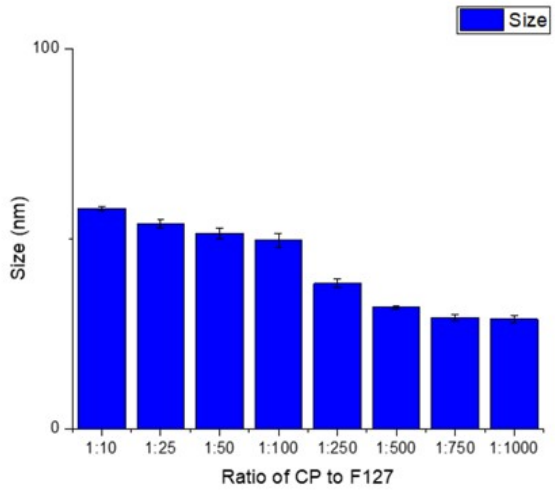

Figure S2 – Particle size variation (dynamic light scattering) with varying conjugated polymer to Pluronic F127 ratio.

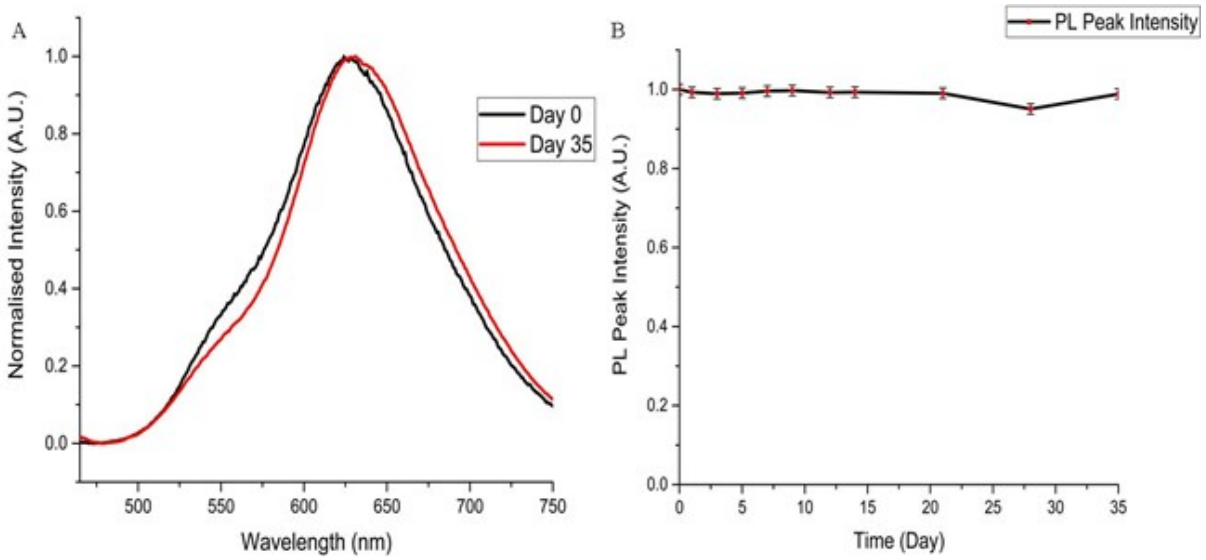

Figure S3 - A) normalised photoluminescence peak of sample at day 0 (black) vs Day 35 (red) (λ_{em} = 623). B) The PL peak intensity at different day intervals over the 35 day period. Error bars are standard deviation of the mean. All samples were excited at 450 nm.