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ARTICLE TYPE

Fluorescent nanoparticles from PEGylated polyfluorenes

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Polyfluorenes with both alkyl and poly(ethylene glycol) (PEG) side-chains have been synthesised and processed into fluorescent nanoparticles via nanoprecipitation. The PEG/alkyl ratio is found to exert a significant influence over the size, polymer microstructure and optical properties of the resultant nanoparticles. This paves the way for a deeper understanding of how polymer structure can be manipulated to provide greater control over the nanoprecipitation process.

Conjugated polymer nanoparticles (CPNs) show tremendous potential for use in applications ranging from biological imaging to consumer electronics.¹⁻⁷ CPNs can be considered as alternatives to the use of Quantum Dots (QDs) as they are small (ca. 10 – 200 nm), bright, exhibit photostable fluorescence that can be tuned across the visible spectrum and can be isolated as stable dispersions in water. QDs have been employed in both *in vitro* and *in vivo* bioimaging applications, but the potential for oxidative degradation of QDs *in vivo* and the toxic heavy metal species (e.g. Cd, Pb) that these processes release will preclude their eventual use in humans or for long-term cell tracking experiments.⁸ Given that CPNs appear to exhibit many of the desirable properties of QDs but avoid some of the drawbacks it is important that limiting factors in the more widespread use of CPNs are addressed, including improved methods for their production and strategies for their surface modification.

CPNs are generally prepared from linear conjugated polymers by nanoprecipitation.⁹⁻¹⁰ This process involves the rapid injection of a solution of the polymer in a good solvent into a miscible non-solvent, leading to spontaneous nanoparticle formation. This process gives small particles (ca. 10–200 nm) with narrow size distributions. However, extremely low initial polymer concentrations (20 ppm) are required to form nanoparticles at the lower end of this size range, which provides a significant barrier towards scale-up of CPN production.¹ For cellular delivery in particular, access to particles in the sub 100 nm size regime is desirable in order to facilitate their delivery into cells by active uptake mechanisms (e.g. endocytosis).¹¹ An important limiting factor for the widespread use of CPNs is a lack of surface functionality. In the vast majority of reports, CPNs are prepared from conjugated polymers that contain simple alkyl side-chains. These side-chains are important to provide solubility for the linear polymers in organic solvents and to control their

microstructure in bulk, but beyond this they do not provide any opportunity to improve biological compatibility or enable further surface modification post-nanoparticle formation.

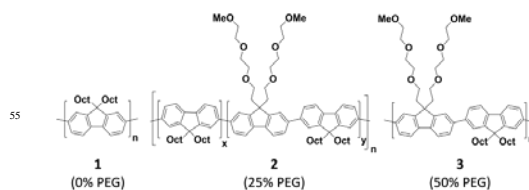


Figure 1 General structure of polyfluorenes 1-3

Strategies for introducing useful surface functionality into CPNs for use in bioimaging have involved the co-precipitation of an appropriately functionalised, saturated polymer that can partition to the surface of the nanoparticles during particle formation. PEG groups have been incorporated to reduce non-specific binding of biomolecules and carboxy groups have been utilised for attachment of biomolecules using this approach.^{3,4} Given that the factors that control the size and microstructure of CPNs formed by nanoprecipitation are not well understood, we feel that development of a more straightforward and reproducible approach to providing surface functionality is desirable. Our chosen approach is to synthesise bespoke conjugated polymers with the desired functional groups covalently attached to the polymer backbone and to develop an understanding of how these modifications influence the nanoprecipitation process and the properties of the resultant nanoparticles.

In this contribution we report polymers based on poly(9,9'-dioctylfluorene) (PFO), the fluorescent polymer that has been most widely used in CPN formation, in which some of the octyl side chains have been replaced with short PEG chains. The general structure of the polymers is shown in figure 1: PFO homopolymer 1 (as a control), random copolymer 2, with 25 mol% of PEGylated repeat units, and co-polymer 3 with alternating PEGylated and alkylated repeat units. These polymers were synthesised using well established Suzuki polymerisation techniques.[‡] All three polymers displayed identical UV/vis and photoluminescence (PL) emission spectra in solution in THF, indicating that the side groups have no influence upon their

solution phase optical properties. For nanoparticle formation from these polymers, we employed the nanoprecipitation method that has been reported by McNeill and co-workers in a number of reports, wherein a solution of the polymer in THF is rapidly injected into water. Within these reports, the authors suggest that for certain conjugated polymers, including polyfluorenes, the size of the particles formed is dependent on the initial polymer concentration.

Table 1 Volume mean diameters of CPNs formed from polyfluorenes **1-3** at varying initial polymer concentrations

Polymer (PEG content)	Initial Conc. (in THF)	Particle Diameter D_p^a (nm)
1 (0%)	500	118±6
	1000	114±3
	2000	155±22
2 (25%)	500	106±10
	1000	108±23
3 (50%)	2000	85±2
	500	90±25
	1000	63±1
	2000	94±6

^a Each value given for particle diameter is an average of 3 experiments with errors representing batch to batch variability (measured by DLS)

In order to provide a picture of the batch to batch reproducibility of the nanoprecipitation method for polymers **1-3**, as well as to clearly establish whether the initial polymer concentration and/or the alkyl/PEG ratio of our co-polymers had any influence over the size of the resultant nanoparticles, we repeated each nanoparticle formation experiment in triplicate and the DLS sizing data presented is an average of these three values (table 1).

For each polymer, nanoparticle formation was carried out at initial polymer concentrations of either 500, 1000 or 2000 ppm in tetrahydrofuran. The relative amount of polymer solution and non-solvent (water) was fixed in a ratio of 1 : 4 and for each experiment residual tetrahydrofuran was stripped from the solution by blowing a constant stream of argon over the surface of the nanoparticle suspension for 3 hours. McNeill *et al.* report a small amount of aggregate formation, which they removed by filtration through a 0.2 µM filter. However, we found that this procedure was unnecessary for polymers **2** and **3**, which showed no trace of aggregates following nanoparticles formation. Polymer **1** samples did partially aggregate; however the aggregates floated to the surface of the nanoparticle suspension and clean samples of nanoparticle suspension could easily be removed for analysis without resorting to filtration. The sizing analysis of the nanoparticles obtained by dynamic light scattering (DLS) (table 1) is therefore a true reflection of the as-formed samples at the defined polymer concentration. The data shows no correlation between initial polymer concentration and the diameter of the nanoparticles formed within experimental error. However the composition of the polymer exerts a significant influence over the size of particles formed by nanoprecipitation. Most notably, every batch of nanoparticles formed from the non-PEGylated control polymer **1** had a volume mean diameter above 110 nm, whereas both PEGylated polymers **2** and **3** formed significantly smaller particles (60-90 nm) under the same

conditions. In all cases the initial polymer concentration does influence the batch to batch reproducibility of the nanoparticles produced. For example, an initial polymer concentration of 1000 ppm for polymer **3** reproducibly provided sub-100 nm particles (mean diameter for the three batches = 63 ± 1 nm), whereas with an initial polymer concentration of 500 ppm there was a significant variation in particle diameter between the three batches (90 ± 25 nm).

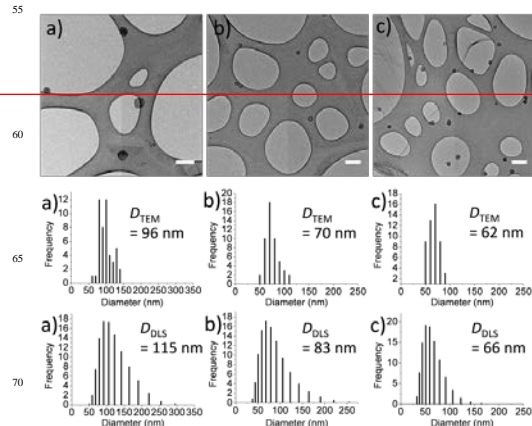


Figure 2 (top row) TEM images of nanoparticles prepared with a) polyfluorene **1** (x 12000 objective), b) polyfluorene **2** (x 10000 objective) c) polyfluorene **3** (x 10000 objective), scale bars = 200 nm; (middle row) histograms comparing TEM particle counting size distributions and (bottom row) DLS size distributions of the corresponding nanoparticles

Samples of nanoparticles, formed from polymers **1, 2** and **3**, with volume mean diameters of 115, 83 and 62 nm respectively (by DLS), were imaged by TEM (figure 2) on graphene oxide, a very thin support that is almost electron transparent. Use of graphene oxide enables the imaging of samples without staining, allowing for more accurate measurement of particle size.¹² The mean particle diameter, determined by particle counting from the TEM images corresponded closely to the values obtained by the DLS measurements (figure 2). Samples of the nanoparticles were also visualised by optical fluorescence microscopy (see supporting information), which, as expected, indicated that fluorescence was observed from discrete particles.

The absorption and PL emission spectra of polyfluorene based-CPNPs have previously been shown to provide useful information about the microstructure of the polymer chains within the nanoparticles.¹³ PFO is well known to adopt a thermodynamically favoured β-phase conformation in poor solvents and in solvent annealed thin films, which can be observed via a strong absorption band at 437 nm and an accompanying red shift in fluorescence.¹⁴ McNeill and co-workers have observed this β-phase conformation in the absorption and emission spectra of PFO nanoparticles, in both the as-formed nanoparticles (dependent on formation conditions) or by addition of a “good solvent” to glassy-phase PFO nanoparticles.¹³ For PEG functionalised polymers **2** and **3**, the

PEG content of the polymer has a significant effect upon the amount of the β -phase conformation observed in the corresponding CPNs. Figure 3 shows the absorption and emission spectra of CPNs formed from **1**, **2**, and **3** using an initial polymer concentration of 500 ppm. CPNs formed from **3**, with the highest PEG content, have an absorption spectrum that closely resembles that of linear PFO molecularly dissolved in THF (also shown in Figure 3). This spectrum is characteristic of polyfluorenes in the disordered glassy phase conformation. As the PEG content decreases to 25% for polymer **2**, absorptions arising from polymer chains in the β -phase are more apparent in the UV/vis spectrum and these signals have an even greater intensity in the CPNs formed from **1** (PFO).

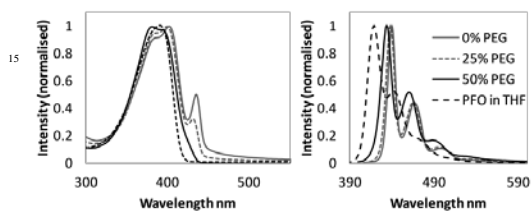


Figure 3 Absorption (left) and photoluminescence emission (right) spectra of nanoparticles formed from polymers **1-3** (with initial polymer conc. of 500 ppm) compared to those of polymer **1** (PFO) dissolved in THF.

A similar trend is observed for the emission maxima: CPNs of **3** (50% PEG) have the least red shifted fluorescence compared with PFO in THF, and the red shift increases as the PEG content decreases from 25% (CPNPs **2**) to 0% (CPNPs of **1**). As with McNeill and co-workers, we have found that the conditions under which the CPNs are formed can exert some influence over the degree of β -phase observed. For example, for CPNs of polymer **2**, with decreasing initial polymer concentration (2000-500 ppm), absorptions arising from the β -phase slightly increase and the corresponding emission spectra are slightly red-shifted (see supporting information). This is far less profound than the influence of the PEG content however. Our results demonstrate that the influence of the side chains upon microstructure is a key consideration for tailoring the optical properties of polyfluorene-based CPNs.

In conclusion, we have demonstrated that replacing a proportion of the alkyl side chains present on polyfluorene copolymers with a more hydrophilic side chain (triethylene glycol for our model system) has a significant influence on the size and optical properties of the CPNs formed by nanoprecipitation of these polymers. Both random and alternating copolymers with a mixture of alkyl and PEG side chains have been shown to produce smaller nanoparticles than the PFO homopolymer at initial polymer concentrations ranging from 500-2000 ppm. Furthermore, the proportion of polymer chains that adopt the more ordered β -phase conformation within these nanoparticles has been shown to be inversely proportional to the amount of PEG side chains incorporated into the polymers, allowing for control over the optical properties of the nanoparticles. Our ongoing research is focussed upon introducing surface functionality into such CPNs through side-chain modification of

conjugated polymers. These results indicate that such structural modification must be carefully controlled in order to obtain CPNs with optimal properties for their specific applications.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental methods for the synthesis of polymers **1-3** and accompanying spectral data. Optical microscope images of CPNs. Comparative absorption and emission spectra of CPNs formed with varying initial polymer concentrations. See DOI: 10.1039/b000000x/

‡ Polymer **1**: M_n 20,600 Da; M_w 44,800 Da; PDI 2.2.
 § Polymer **2**: M_n 20,300 Da; M_w 47,600 Da; PDI 2.3. Polymer **3**: M_n 33,500; M_w 96,500 Da; PDI 2.9.

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