Mini-review

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Strategies for preparing fluorescently labelled polymer nanoparticles

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Abstract

There is great interest in the use of fluorescent polymer nanoparticles as optical imaging agents. When designing and synthesising a fluorescent polymer nanoparticle imaging agent there is a large variety in both the particle formation and dye attachment strategies that can be pursued. In this mini-review we detail this range of possibilities, illustrating with examples from the literature, and highlighting particular advantages in each case.

Keywords: polymer nanoparticle; fluorescence labelling; imaging agent; self-assembly; conjugated polymers

INTRODUCTION

Fluorescence spectroscopy and microscopy is a widely used technique for the detection and visualisation of compounds containing fluorophores, which is highly useful for optical diagnostics in biomarker analysis, immunoassays and diagnostic imaging including cancer diagnosis.[1,2] Fluorescently labelled nanoparticles possess several features that make them more valuable for this purpose than classical fluorescent organic dyes.[3] Organic dyes often have low absorption coefficients, which reduces detection sensitivity, and are susceptible to photobleaching, thus limiting their use to short-term measurements. Furthermore, organic fluorophores often have short emission lifetimes, which can prohibit use in time-resolved measurements, while the toxicity of many organic fluorophores impedes their application to *in vitro* and *in vivo* analysis.[4] In contrast, polymer nanoparticles with fluorescent functionality often contain multiple fluorophores leading to brighter emission, while the encapsulation of fluorophores in a macromolecular structure can improve dye stability, reduce photobleaching, impart biocompatibility and improve retention time for the fluorescently labelled agent.[2,4] A further advantage of fluorescently labelled nanoparticles over small molecules is their potential to combine imaging and drug delivery, as theranostic agents.[5] Covalent attachment of dye molecules to polymer nanoparticles is more efficient and stoichiometrically precise than physical absorption of fluorophores, can eliminate particle-to-particle variation and uncertainties about labelling location, and also reduces leaching of the dye from the nanoparticle.[3,6,7]

In this mini-review we highlight a number of ways in which fluorescently labelled polymer nanoparticles with covalently attached fluorophores can be prepared. Depending on the labelling strategy used, the location of the fluorophore within the polymer nanoparticle can be varied, while additional particle properties can be selected based on the synthetic particle formation strategy employed. We have selected examples which demonstrate the most common routes for nanoparticle synthesis: block copolymer (BCP) self-assembly, nanogelation, conjugated polymer nanoparticle preparation, single-chain polymer collapse, nanoprecipitation, and dendrimer synthesis. For each category of nanoparticle synthesis we also illustrate the various methods for dye incorporation: use of a fluorescent monomer or initiator (pre-polymerisation functionalisation), reaction of polymer or particle with a dye-functional reagent (post-polymerisation or post-particle formation functionalisation) and the use of emissive polymers. This range of strategies for preparing fluorescently labelled polymer nanoparticles is summarised in Fig. 1. The choice of dye can be motivated by desired optical properties (such as excitation/emission wavelength) or by ready availability of functional derivatives. The chosen examples attempt to illustrate the range of fluorophores (Fig. 2) and functionalisation chemistry available, while readers are also directed to reviews that focus extensively on these individual aspects.[2,8,9]

BCP SELF-ASSEMBLY

Self-assembly of BCP amphiphiles occurs in selective solvents – solvents in which (at least) one block is soluble and (at least) one block is insoluble. This self-assembly is driven by the solvophobic attraction between associating blocks, which is counterbalanced by steric repulsions between solvophilic blocks (and electrostatic repulsions where these solvophilic blocks are ionised).[10] A number of different solution-state self-assembled microdomain morphologies have been observed, including spherical micelles, vesicles (polymersomes), cylinders/rods, lamellae, bicontinuous structures, large compound micelles and disc-like micelles,[11] with particle sizes on the nanometre scale.[12] BCP self-assemblies can display a dynamic response to external stimuli

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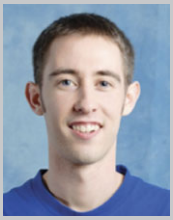

Rachel K O'Reilly is currently a professor in the Chemistry Department at the University of Warwick. In 2012 she won the IUPAC-Samsung young polymer scientist award and the RSC Hickinbottom medal. In 2013 she was awarded the ACS Mark Young Scholar award and an ERC consolidator grant. Her research focuses on bridging the interface between creative synthetic, polymer and catalysis chemistry, to allow for the development of materials that are of significant importance in medical, materials and nanoscience applications.

(e.g. temperature, pH),[13] and can possess diverse surface chemistry allowing both stealth or cell-surface receptor targeting.[7]

The polymerisation of monomers containing fluorescent functional groups has been utilised to produce amphiphilic BCPs that can self-assemble to form fluorescent micelles. The range of fluorescent monomers that have been synthesised is extensive,[8] and encompasses all commonly utilised classes of dyes – including polycyclic aromatic hydrocarbons, azo-dyes, coumarins, fluorescein, rhodamines – and compatibility with all commonly utilised polymerisation techniques – free radical polymerisation, atom transfer radical polymerisation,[14] reversible addition–fragmentation chain transfer (RAFT) polymerisation,[15] nitroxide-mediated polymerisation,[16] living anionic and cationic polymerisation and ring opening metathesis polymerisation.[17]

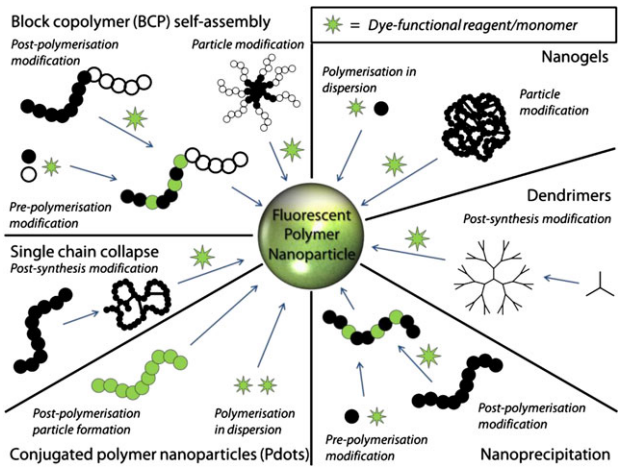

Figure 1. Overview of the general strategies for preparing fluorescently labelled polymer nanoparticles discussed in this review.

Furthermore there are also several commercially available vinyl monomers with fluorophore functionality, which further expand the versatility of this approach. This allows the simple design of complex systems, for example the use of two different fluorescent monomers, whose fluorophores comprise a Förster resonance energy transfer (FRET) pair. For FRET, a pair of fluorophores (the donor and acceptor) are coupled by a dipole–dipole interaction, so that excitation of the donor is followed by energy transfer to the acceptor, resulting in acceptor emission.[18] Using these FRET pair monomers Liu and co-workers prepared BCP spherical micelles of polystyrene-*block*-poly(*N*-isopropylacrylamide) labelled with an oxadiazole FRET donor in the styrene core and rhodamine FRET acceptor in the *N*-isopropylacrylamide corona (Fig. 3).[19] Initially the rhodamine was in the non-emissive spirolactam form, so oxadiazole (green) emission was observed. However, an increase of pH to >6 or the presence of Hg^{2+} ions led to formation of the acyclic emissive rhodamine, leading to FRET and therefore rhodamine emission. Subsequent increase of the temperature above the poly(*N*-isopropylacrylamide) lower critical solution temperature caused dehydration of the coronal chains, causing them to collapse onto the core, which decreased the donor–acceptor distance leading to a FRET enhancement.

One of the strengths of the BCP self-assembly approach is the diversity of nanoparticle structures that can be obtained, and the often dynamic nature of these assemblies.[13] For example Zhu and co-workers utilised a pH-responsive azobenzene-containing monomer to synthesise amphiphilic BCPs that self-assembled to form vesicles. Protonation of the membrane-forming azobenzene block led to both a change in particle morphology (vesicle swelling) and fluorescence quenching, reminiscent of the breathing process of jellyfish.[20]

Clickable monomers have also been used to produce fluorescent amphiphilic BCPs for the self-assembly of fluorescent micelles. This strategy is ideal where the polymerisation reaction is not tolerant to dye functionality, but is necessary to obtain polymers with a desired property.[9] An excellent example of this approach is the recent work of Manners and colleagues. Poly(ferrocenyldimethylsilane)-*block*-poly(dimethylsiloxane-*co*-methylvinylsiloxane) BCPs were synthesised by anionic ring-opening polymerisation. The vinyl groups of the methylvinylsiloxane units were subsequently functionalised by the thiol–ene 'click' reaction to produce three different BCPs containing BODIPY dyes with red, green or blue emission (Fig. 4(a)). Crystallisation-driven self-assembly (CDSA)[21] led to the formation of cylindrical micelles with uniform contour lengths of *ca* 2 μm whose emission wavelength could be tuned based on the ratio of red:green:blue BCPs in the CDSA reaction mixture. A full spectrum of emission was achieved, including white light, with emission visualised in solution and by confocal microscopy (Fig. 4(b)). Both symmetric (Fig. 4(c)) and asymmetric (Fig. 4(d)) multi-block cylindrical micelles could be fabricated by sequential BCP addition due to the living nature of CDSA, leading to the formation of 'nanoscale RGB pixels'.[22] Subsequent work demonstrated that lenticular platelets with concentric rings of red-, green- and blue-emitting dyes could also be fabricated via two-dimensional CDSA. Non-centrosymmetric single-headed spear-like micelles could also be produced, where spear head and shaft contained red- and green-emitting BODIPY fluorophores, respectively.[23]

Fluorescent micelles have been formed by the self-assembly of site-specifically labelled BCPs, which can be achieved by the use of fluorophore functional initiators in polymer

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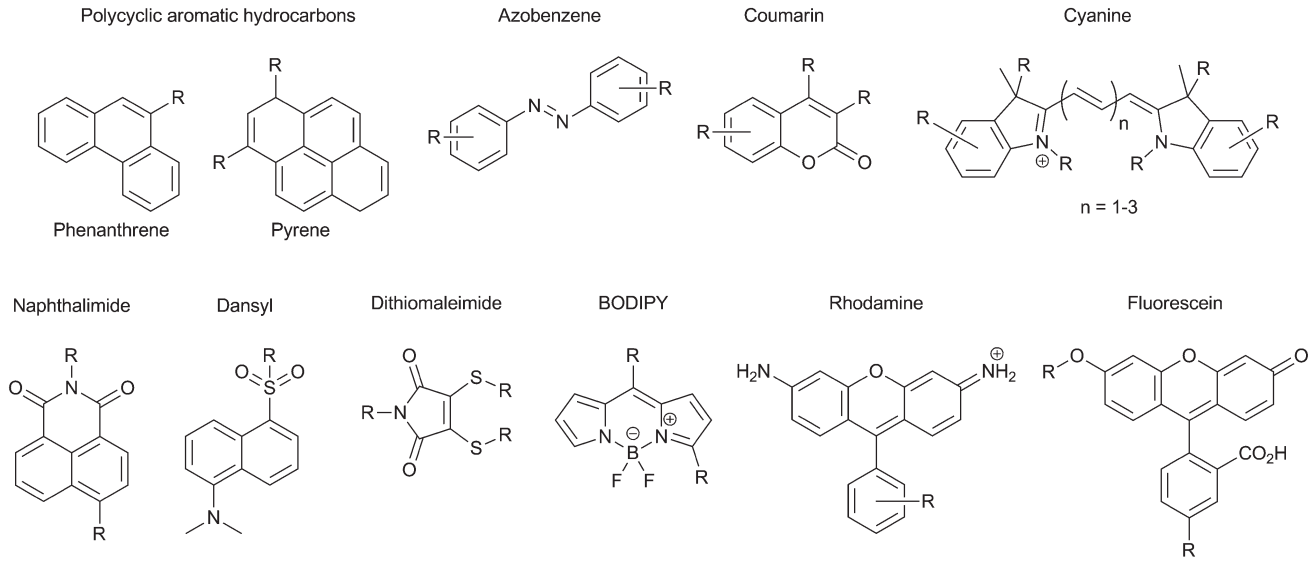

Figure 2. Generic chemical structures of commonly used fluorophores mentioned in this review. R-groups indicate positions used for covalent attachment to polymers and polymer nanoparticles.

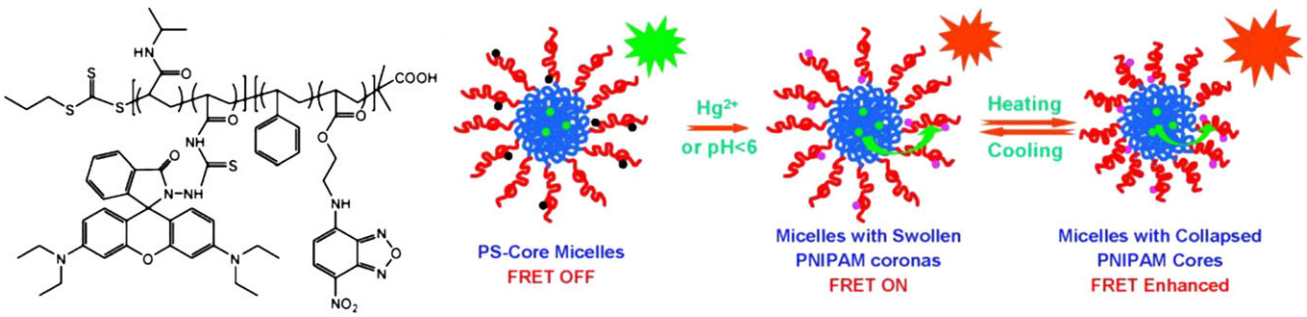

Figure 3. Synthesis of pH-, temperature- and Hg^{2+}-responsive core/corona dye-labelled spherical micelles using oxadiazole and rhodamine monomers. PS, polystyrene; PNIPAM, poly(*N*-isopropylacrylamide). (Reprinted with permission from Hu *et al.*[19] Copyright 2011 American Chemical Society.)

synthesis.[24] For example Farinha and co-workers utilised a phenanthrene-functional RAFT agent in the synthesis of poly(*N*-decylacrylamide)-*block*-poly(*N*,*N*-diethylacrylamide) amphiphilic BCPs. By using an R-group labelled RAFT agent, and polymerising the hydrophobic poly(*N*-decylacrylamide) block first, spherical micelles with the phenanthrene fluorophore in their core were formed upon aqueous solution-state self-assembly. By loading the micelles with anthracene and studying the effect of FRET with anthracene on micelle emission lifetime, the authors were able to calculate micelle core radii from the measured Förster critical radius.[25] A site-specific post-polymerisation functionalisation of BCP end-groups with a fluorophore has also been employed in fluorescent micelle fabrication. Kataoka and co-workers synthesised a poly(ethylene glycol)-*block*-poly[D,L-(lactic acid)] (PEG-*block*-P(D,L-LA)) diblock copolymer by successive anionic ring-opening polymerisation, and subsequently attached pyrene to the hydroxyl chain end of the P(D,L-LA) block via an esterification reaction. The high sensitivity of fluorescence detection was then utilised in the study of micelle–unimer exchange dynamics (by measuring exchange between labelled and non-labelled micelles), which demonstrated that micelles were dynamic at 40 °C (above the core block T_g) and frozen at 25 °C (below the core block T_g).[26] The fluorophore can also be located at the junction between core- and corona-forming blocks, through the use of a dual initiator with the dye located between orthogonal polymerisation sites. For example O'Reilly and co-workers used a ring-opening polymerisation/RAFT initiator to form a Y-shaped poly(triethyleneglycol acrylate)-*block*-P(D,L-LA) amphiphilic BCP, which self-assembled in water to form spherical micelles with a dithiomaleimide[27] fluorophore at the core–corona interface. As the precise fluorophore location within the micelles was known, changes in emission (fluorescence and anisotropy lifetime as well as steady-state emission and anisotropy) could be directly assigned to changes in the polymer assembly (disassembly to unimers), both in solution and *in vitro*.[28] O'Reilly and co-workers also showed that thiol exchange at the dithiomaleimide fluorophore could lead to BCP cleavage for this system. This resulted in both a micelle-to-vesicle morphology transition as well as an on-to-off switch in fluorescence.[29]

Post-polymerisation modifications for the introduction of fluorescent labels have also been performed on assembled BCP micelles. For example the use of monomers with reactive (or latently reactive) functional groups in BCP synthesis can afford micelles with reactive functional groups in the core and corona. This was demonstrated by Wooley and colleagues who produced poly(acrylic acid)-*block*-polystyrene and poly(acrylic acid)-*block*-poly(styrene-*co*-vinylbenzyl chloride) BCPs by nitroxide-mediated polymerisation. Self-assembly afforded spherical micelles which were shell crosslinked using a bisamine. The poly(acrylic acid) shells were further functionalised

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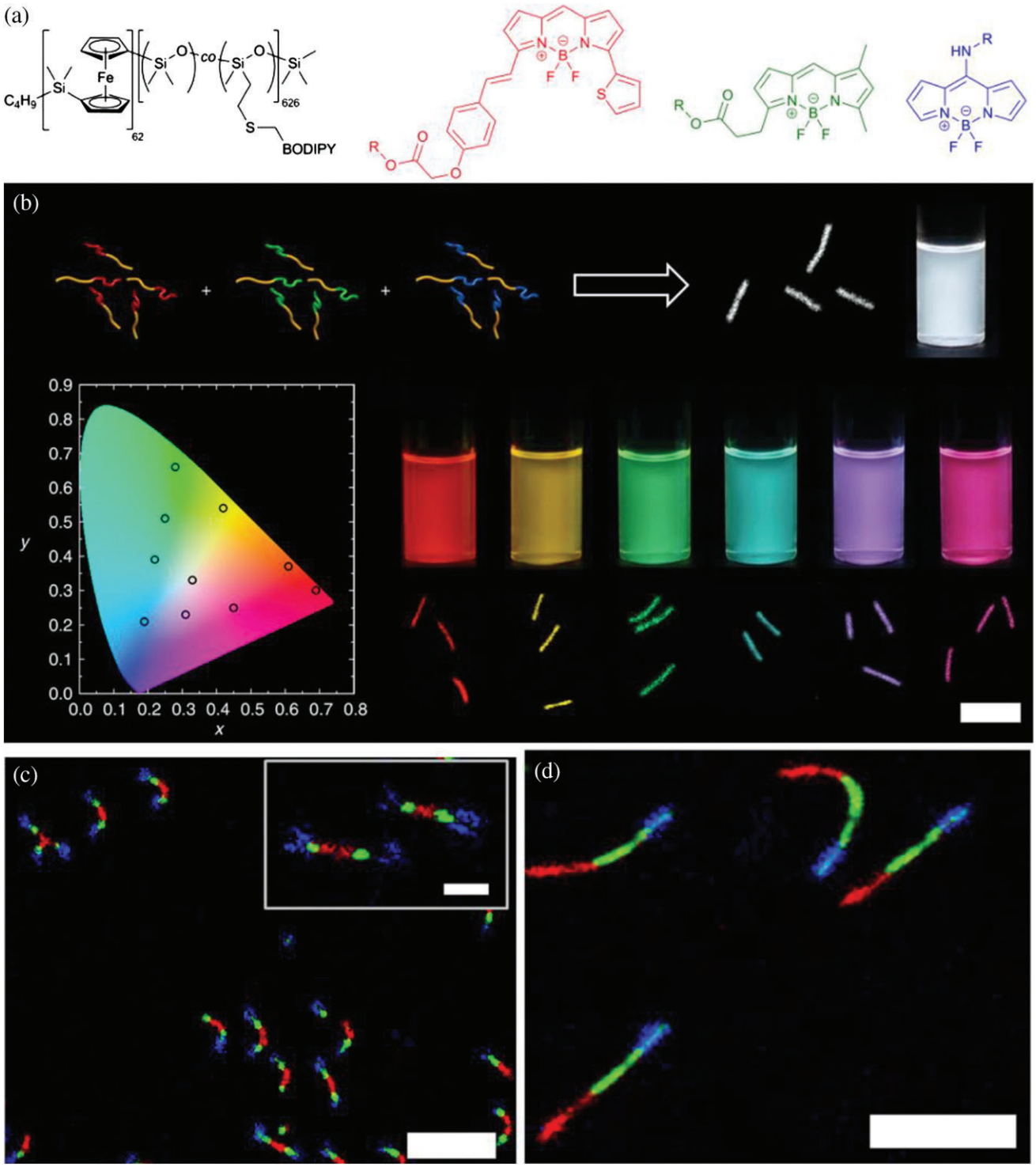

Figure 4. (a) Synthesis of red-, green- and blue-emissive BODIPY-labelled BCPs. (b) CDSA of mixtures of these BCPs into cylindrical micelles with tuneable emission wavelength; schematic representation of the self-assembly, confocal fluorescence micrographs (3 μm scale bar) and raw photographic images of micelle solutions under UV irradiation at 365 nm. (c) Confocal fluorescent micrographs of symmetric multi-block micelles (5 μm scale bar, 1 μm inset scale bar). (d) Confocal fluorescent micrographs of asymmetric multi-block micelles (5 μm scale bar). (Reprinted by permission from Macmillan Publishers Ltd: *Nature Commun.*[22] Copyright 2013.)

with alkyne and azide groups by amidation with propargylamine or azidopropylamine, respectively, allowing copper-catalysed azide–alkyne cycloaddition (CuAAC) reaction with azide- or alkyne-functional hydrophilic fluorescein dye. Azide groups were introduced into the core by reaction of vinylbenzyl chloride units with sodium azide. Attempts to label the core with the hydrophilic alkyne–fluorescein in aqueous media were unsuccessful, suggesting poor access to the core. Instead, by swelling the core with an organic solvent, reaction with a hydrophobic alkynyl–dansyl dye was successfully demonstrated.[30] The same group also

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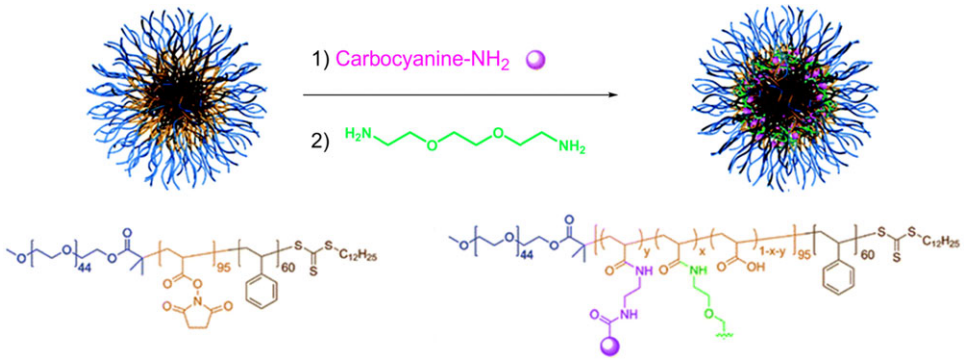

Figure 5. Post self-assembly functionalisation of triblock copolymer spherical micelles with near-infrared fluorescent dyes. (Adapted from Sun *et al.*[31] with permission of the Royal Society of Chemistry.)

demonstrated the potential to fluorescently label a BCP micelle at the interface between core and corona by self-assembly and subsequent functionalisation of a triblock copolymer with a reactive midblock. A PEG-functional RAFT agent was used in the synthesis of PEG-*block*-poly(*N*-acryloxysuccinimide)-*block*-polystyrene BCPs with aqueous self-assembly affording spherical micelles (Fig. 5). The midblock was labelled via amidation reaction of the activated ester groups with amine-functional near-infrared carbocyanine fluorophores. The subsequent reaction of residual succinimide groups with bisamine, or hydrolysis by water afforded a crosslinked poly(acrylic acid) midblock.[31]

Post self-assembly fluorescent labelling has also been achieved where an initiator with reactive functionality was used for BCP synthesis. Using a symmetric bifunctional RAFT agent, Davis and co-workers synthesised poly(PEG acrylate)-*block*-polystyrene-*block*-poly(PEG acrylate) triblock copolymers with a pyridyl disulfide group at the α- and ω-chain ends.[32] Addition of rhodamine–thiol to self-assembled spherical micelles resulted in a disulfide exchange reaction to give a rhodamine-functionalised corona. The authors calculated that *ca* 80% of the pyridyl disulfide units were available for dye conjugation (by comparison with the reaction of the non-assembled polymer in organic solvent), suggesting that the remaining *ca* 20% were embedded in the polystyrene core.

NANOGELS

Nanogel particles are highly branched or crosslinked polymer networks with diameters in the range 1–100 nm.[33] They can be synthesised by a variety of top-down and bottom-up approaches, however, the simplest and most popular method is free radical crosslinking copolymerisation in emulsion or mini-emulsion.[34] While perhaps the main advantage of this approach is its simplicity, more complex core–shell nanogels which demonstrate responsive swelling have received attention both theoretically and for practical applications.[35]

While fluorescent labels can be incorporated by encapsulation of dye molecules during the (mini-)emulsion polymerisation,[36] the use of functional monomers allows for the covalent attachment of fluorophores. For example Zeng and colleagues performed a mini-emulsion copolymerisation of methyl methacrylate with two fluorescent monomers that formed a FRET donor–acceptor pair: a naphthalimide donor and a merocyanine acceptor. The photo-response of merocyanine (which forms a non-emissive spiropyran upon irradiation with UV light and reverts to the emissive merocyanine form under visible light) was used as a trigger for FRET emission.[37] O'Reilly and co-workers also used a functional monomer to produce fluorescently labelled poly(methyl methacrylate) nanogels. In this case a free radical crosslinking oil-in-water emulsion polymerisation was performed with a high concentration of surfactant (sodium dodecylsulfate) to produce particles with hydrodynamic radii in the range 12–20 nm. By using the dithiomaleimide fluorophore which has a strong disinclination to self-quench when incorporated into a macromolecular architecture, particles were synthesised with a concentration-independent molar emission up to 1 wt%, and average fluorescent lifetimes in excess of 20 ns, with potential use as quantitative imaging agents.[38] Post-synthesis functionalisation of nanogel particles using 'click' chemistry has also been demonstrated for nanogels synthesised via a 'click-in-emulsion' approach. Anderson and colleagues produced bone-targeted nanogels by performing the CuAAC reaction between azide-functional dextran and alkyne-functional dextran in an inverse emulsion. After particle formation, hydroxyapatite binding bisphosphonate groups were introduced by CuAAC reaction with unreacted dextran alkyne groups on the particle surface, while an alkyne-functional cyanine dye (Alexa Fluor 647) could also be attached by CuAAC reaction with unreacted dextran azide groups. Fluorescent labelling allowed the bio-distribution of these nanogels after injection into mice to be analysed using confocal microscopy.[39]

CONJUGATED POLYMER NANOPARTICLES

Another strategy for the synthesis of fluorescent polymer nanoparticles is to form the particles from fluorescent π-conjugated polymers, which results in semiconducting polymer dots (Pdots).[40] These nanoparticles generally show high brightness and quantum yields, while diverse surface functionality (biocompatibility, targeting ligands) can be generated by covalent and non-covalent functionalisation.

One synthetic route to generate Pdots is to perform a step-growth polymerisation – which forms the π-conjugated polymers – in dispersion, as demonstrated by Mecking and co-workers. They employed a mini-emulsion Glaser coupling polymerisation to produce poly(arylene diethynylene) particles with diameters of 20–30 nm. Introducing perylene diimide or fluorenone units through copolymerisation allowed the emission maxima of the nanoparticles to be adjusted (Fig. 6).[41]

In addition to their fabrication by dispersion polymerisation, highly emissive conjugated polymer (CP) nanoparticles can also be prepared by one of three post-polymerisation routes (Fig. 7).[42]

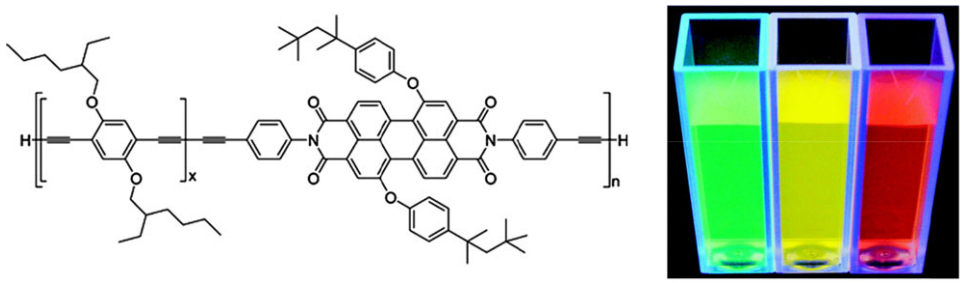

Figure 6. Arylene diethynylene/perylene diimide copolymers formed by step-growth mini-emulsion polymerisation (left) and solutions of the resultant conjugated nanoparticle dispersions (under 366 nm light) for various monomer loadings (right). (Reprinted with permission from Baier *et al.*[41] Copyright 2009 American Chemical Society.)

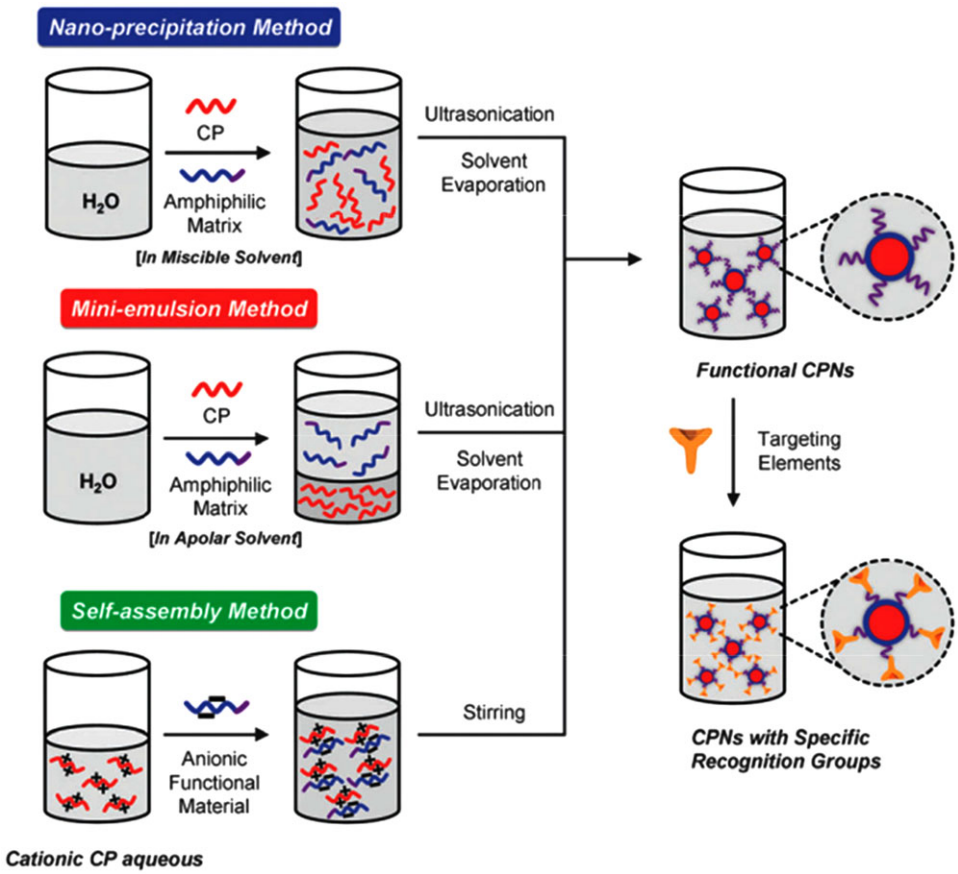

Figure 7. Three post-polymerisation routes for conjugated polymer nanoparticle (CPN) preparation. (Reproduced from Feng *et al.*[42] with permission of the Royal Society of Chemistry.)

By separating polymer and particle formation into two steps, these post-polymerisation methods eliminate the difficulties of conducting step-growth metal-catalysed polymerisations in dispersion.[40] Nanoparticles can be prepared from CPs by nanoprecipitation, where the CP is dissolved in a good solvent and rapidly added to an excess of a poor solvent, often under ultrasonic dispersion conditions.[42] By performing coprecipitation of a heterogeneous polymer mixture, the ability to surface functionalise fluorescent Pdots can be introduced. For example Chiu and colleagues used coprecipitation with polystyrene-based copolymers to provide Pdots with carboxylic acid surface functionality, allowing the particles to be decorated with antibodies, cell membrane glycoproteins and streptavidin.[43,44]

Alternatively, in the mini-emulsion method the CP is dissolved in a good solvent (e.g. dichloromethane) and then dispersed in an immiscible solvent (e.g. water) by ultrasonication. Landfester *et al.* demonstrated CP nanoparticle formation by the mini-emulsion approach using poly(*p*-phenylene), polyfluorene or polycyclopentadithiophene. Chloroform solutions of the pre-synthesised polymers were mini-emulsified in water using sodium dodecylsulfate as surfactant, with the resultant fluorescent particles having diameters between 75 and 250 nm.[45] In the self-assembly method a positively or negatively charged CP is dissolved or dispersed, then this solution/dispersion is blended together with an oppositely charged polymer or co-assembly reagent, causing aggregation and particle formation. For example the groups of Liu and Wang prepared nanoparticles from anionic polythiophene and a cationic

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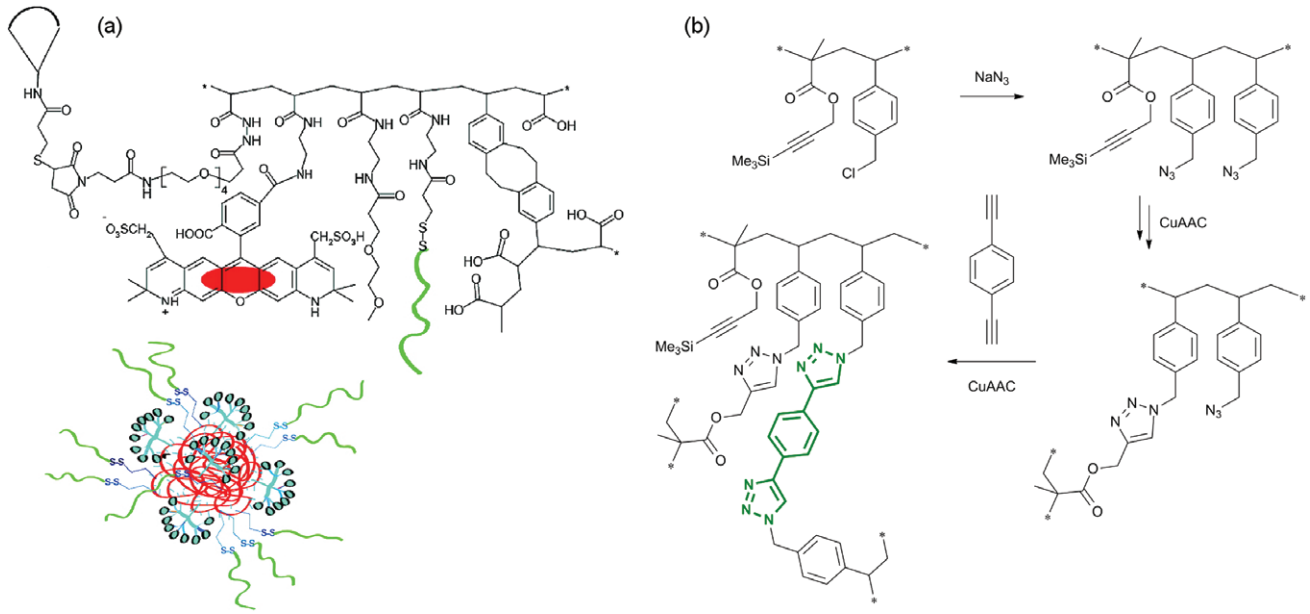

Figure 8. Fluorescently labelled single-chain polymer nanoparticles prepared by (a) Hamilton and Harth (reprinted with permission from Hamilton and Harth,[49] copyright 2009 American Chemical Society) and (b) Pomposo and co-workers.[50]

porphyrin where upon white light irradiation energy transfer from polythiophene to the porphyrin (held in close proximity within the nanoparticle) results in singlet oxygen formation, such that the nanoparticles demonstrated both imaging and antibacterial activities when incubated with *E. coli*.[46]

SINGLE-CHAIN POLYMER NANOPARTICLES

Although a relatively new field in nanoparticle design, there have been several reports of fluorescent particle synthesis using single-chain polymer collapse, where particles are prepared by the controlled folding or collapse of single polymer chains by intrachain crosslinking.[47] This approach allows the synthesis of particles with diameters of less than 15 nm, and has the potential to produce synthetic structures that mimic the globular structure of proteins.

Hamilton and co-workers demonstrated a post-synthesis modification of poly(acrylic acid) single-chain nanoparticles, to produce fluorescently labelled unimolecular nano-objects (Fig. 8(a)). Chain collapse was achieved by copolymerisation of a vinylbenzosulfone, which will dimerise at high temperature to form dibenzocyclooctadiene crosslinks.[48] Amine groups were introduced into the poly(acrylic acid) particles by amidation with, and subsequent deprotection of, *N*-trifluoroacetylethylenediamine. These amine groups could then react with an *N*-hydroxysuccinimide-functional fluorophore (Alexa Fluor 568), to produce fluorescently labelled particles. Further conjugation to a peptide and a dendron produced molecular dendritic transporter nanoparticle vectors whose cellular uptake could be monitored using confocal microscopy.[49]

A simpler strategy for the synthesis of fluorescently labelled single-chain polymer nanoparticles was pursued by Pomposo and co-workers (Fig. 8(b)). Copolymers of a trimethylsilane-protected alkyne-functional methacrylate with an excess of 4-chloromethylstyrene were synthesised by RAFT polymerisation. The 4-chloromethylstyrene units were converted to azides using NaN_3, with the CuAAC 'click' reaction leading to chain collapse. Residual azide groups were crosslinked using 1,4-diethynylbenzene, leading to the formation of the triazole–benzene–triazole fluorophore which has an emission maximum of *ca* 400 nm.[50]

ALTERNATIVE STRATEGIES

Aside from the principal methods used for the synthesis of fluorescent polymer nanoparticles detailed above, there are two other strategies that merit mentioning. Nanoprecipitation of linear polymers provides a simple route to polymer nanoparticles, and therefore the use of fluorescent polymers can give the resultant nanoparticles emissive properties. Particles are prepared from linear polymers which are dissolved in a good solvent, before this solution is exposed to an excess of a miscible non-solvent, either by dialysis of the solution against the non-solvent, or by dropwise addition of the solution to the non-solvent.[51,52] Nanoparticles form instantly and typically have narrow size distributions with diameters of *ca* 200 nm. Fluorescent nanoparticles were fabricated using this approach by Horisawa *et al.*, who modified a commercially available lactide/glycolide copolymer (PLGA) by amidation of the acid chain ends with fluoresceinamine. Nanoprecipitation of the modified PLGA from methanol–acetone gave fluorescent polymer particles with diameters of *ca* 250 nm.[53] The groups of Kataoka and Schubert also utilised the simplicity of the nanoprecipitation approach to systematically prepare fluorescent nanoparticles with a range of diameters (80–400 nm). Poly(methyl methacrylate)-*co*-poly(methacrylic acid) statistical copolymers were prepared by RAFT polymerisation, and then functionalised with one of three amino-functional fluorophores (fluorescein or cyanine) via amidation. Particle size control could be achieved by control over nanoprecipitation conditions, allowing the effect of diameter on cellular internalisation to be studied.[54]

Dendrimers are branched (tree-like) macromolecules with precise structure and topology.[55] Consisting of several generations of radially homocentric layers emanating from a central core, dendrimers have a high density and well-defined surface functionality which provides an ideal target for fluorophore incorporation.

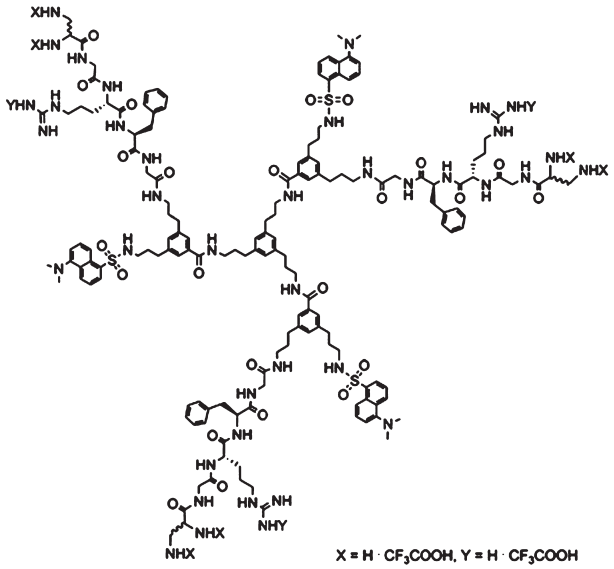

Figure 9. Polyamidoamine-type dendrimer, with fluorescent dansyl functionality. (Adapted from Fuchs *et al.*[56] with permission of the Royal Society of Chemistry.)

This is demonstrated in work of Schlüter and colleagues who functionalised a first-generation polyamidoamine variant dendrimer (Fig. 9). Three peripheral amine groups were conjugated to a dansyl dye, with the remaining three amines used as points of attachment for an enzymatically cleavable oligopeptide spacer. Cellular uptake and distribution in human HeLa cells were investigated using confocal microscopy, with the peptide spacer offering the potential for attachment of active agents (e.g. a drug) which could be released by the action of an intracellular protease.[56]

CONCLUSIONS

The theme of this mini-review has been the control over polymer structure that is possible using the tools of modern synthetic chemistry. Control can be imparted over the nature of single macromolecules, as well as the fabrication of colloidal polymer nanoparticles. One way in which control over single polymer molecules manifests is the functional groups that can be incorporated. This can be achieved either before polymerisation through the use of functional initiators and monomers, or after polymerisation through the use of efficient post-polymerisation modification reactions. An often encountered motive for functional group incorporation is the desire to fluorescently label polymers and polymer nanoparticles for use in delivery and tracking and a number of routes can be employed to achieve this target.

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