Supramolecular Polymer Dot Ensemble for Ratiometric Detection of Lectins and Targeted Delivery of Imaging Agents

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ABSTRACT: A supramolecular, polymer dot based ensemble has been developed for the ratiometric detection of lectins and targeted delivery of glycoprobes. Self-assembly between a blue-emitting polymer dot and a red-emitting glycoprobe, results in an ensemble that shows red emission upon excitation of the polymer dot because of Förster Resonance Energy Transfer. Resulting in ratiometric detection of lectins in buffer solution as well as targeted delivery of the glycoprobe to cells that highly express a sugar receptor. Unlike conventional systems where both the agent and vector are co-delivered intracellularly, our ensemble developed here shows a receptor-controlled dissociation on the cell membrane.

KEYWORDS: Polymer dot, Glycoprobe, Cell imaging, Ratiometric, Probe

Receptor proteins are transmembrane proteins that modulate a number of cellular recognition events through the selective interaction with ligand molecules. In addition to their role in activating cellular pathways, receptor proteins can endocytose specific ligands into the intracellular milieu. The asialoglycoprotein receptor (ASGPr) that is highly expressed on hepatocyte is responsible for the clearance of asialoglycoproteins in the blood. However, ASGPr has been identified as an invasion site for hepatotropic viruses and is overly expressed in liver inflammation. Therefore, receptor proteins can be exploited as a promising target for imaging-guided disease diagnosis and therapy.

Polymer dots have attracted a great deal of interest due to their excellent optical properties and biocompatibility. The polymer dots are typically formed by collapsing and folding of hydrophobic polymer chains, and the hydrophobic matrix formed provides an ideal scaffold on which to load hydrophobic compounds. This improves the efficacy of poorly water-soluble compounds. The strong fluorescence of the semiconductor polymer enables researchers to optically track and investigate drug transport and pharmacological effect in real time.

Here we develop a supramolecular polymer dot based ensemble for the ratiometric detection of lectins and receptor-targeting delivery of imaging agents. The self-assembly between a blue-emitting polymer dot and a red-emitting glycoprobe (sugar-decorated fluorescent probe) produces the ensemble with a red emission upon excitation of the polymer dot (Fig. 1). The ensemble has proven suitable for the ratiometric (emission color from
The polymer dot based on poly(9,9-dioctyfluorenyl-2,7-diyl) (PFO) (Fig. 1a) was prepared by a reprecipitation method, by mixing PFO with poly(styrene-co-maleic anhydride) (PSMA), as previously described. The addition of PSMA (20% w/w) helps improve the water solubility and stabilize the PFO-based polymer dots formed. The polymer dots are negatively charged and colloidally stable, allowing long-term storage. A red-emitting dicyanomethylene-4H-pyran (DCM) was used to label galactose and mannose, producing the glycoprobos CZ1 (Gal) and CZ2 (Man), respectively, for assembly with PFO. The assembly was achieved by mixing the polymer dots with the glycoligands (CZ1 or CZ2) in pure water, forming the supramolecular CZ/PFO polymer system. We believe that hydrophobic interactions between the DCM moiety of CZs and the aromatic core of PFO-based polymer dots are the main driving force by which the supramolecular ensembles are formed. The PFO polymer is in a folded state, thus leaving hydrophobic cavities to accommodate aromatic compounds. This is supported by previous reports on the formation of hydrophobic-drug-PFO ensembles. Likewse, due to the amphiphilic feature of CZs, the DCM moiety can dock into the hydrophobic cavities of PFO based polymer dots while the hydrophilic sugar parts are exposed to the aqueous solution.

A variety of techniques were used to characterise the supramolecular polymer system. A fluorescence titration assay indicated that the fluorescence of PFO gradually decreased in the presence of increasing CZ1 (Fig. 2a) and CZ2 (Fig. 2d). This suggests that the attachment of CZ to the surface of PFO dots can result in Förster Resonance Energy Transfer (FRET) upon excitation of the latter. This was corroborated by the overlapping absorbance band of CZ and emission band of PFO (Fig. S1). This observation is in agreement with similar reports on the use of polymer dots as FRET donors.

To test their biospecificity, we used lectins (proteins that recognise glycoligands) to interact with the supramolecular polymer system. We observed concentration-dependent fluorescence increase and decrease of PFO and CZ, respectively, in the presence of a selective lectin for the supramolecular polymer system (i.e. galactose-selective peanut agglutinin (PNA) and mannose-selective concanavalin A (Con A) for CZ1/PFO (Fig. 2b) and CZ2/PFO (Fig. 2e), respectively). This suggests the detachment of CZ from the surface of PFO as a result of lectin recognition, compromising the FRET mechanism. In addition, the ratiometric fluorescence change (intensity ratio of IPFO/ICZ2) was determined to be specific for selective lectins over a range of unselective proteins (Fig. 2c and 2f). This evidence suggests that the supramolecular polymer-dot based systems could dissociate selectively upon interaction with a selective glycoligand receptor.
surface (Fig. 2h), the zeta potential of the negatively charged polymer increased after assembly with CZ1 (Fig. 2i). TEM indicated that assembly with the CZs did not change the morphology of the PFO nanoparticles (Fig. S3), and confocal microscopy indicated that the blue PFO and red CZ1 emissions were colocalised well on identical dots and clusters (Fig. S4).

With the supramolecular polymer system in hand, we set out to examine its cell imaging ability. The CZ1/PFO ensemble was used since the galactoligand of CZ1 can be recognised by ASGPr. A human hepatoma cell line, Hep-G2, that expresses ASGPr and a human cervical cell line (HeLa) without ASGPr expression were used. Fig. 3 shows the imaging results for CZ1/PFO with the two cell lines obtained by confocal laser scanning microscopy. We observed that the red fluorescence of CZ1 was produced in Hep-G2 rather than in HeLa. Interestingly, the blue-emitting PFO dots were observed to be localised on the cell membrane surface of Hep-G2 (for more fields, see Fig. S5), whereas no such blue dots were detected on HeLa.

A set of control assays were also carried out. We determined that knockdown of ASGPr in Hep-G2 significantly reduced the imaging effect of the ensemble (Fig. S6a). Meanwhile, pre-incubation of Hep-G2 with increasing free galactose also competitively diminished the fluorescence (Fig. S6b). These control experiments suggest that the imaging (release of CZ1 to Hep-G2 cells) is receptor-targeting. Incubation of PFO alone with the cells resulted in the unselective adhesion of the polymer to the membrane of both Hep-G2 and HeLa (Fig. S7). This is contrary to our observation that treatment with the CZ1/PFO ensemble did not result in an extensive membrane adhesion of PFO to HeLa cells (Fig. 3). These results imply that the presence of the CZ1 targeting agent could reduce the unselective interaction of PFO with the control cells.

Figure 3. Confocal laser scanning microscopic imaging of Hep-G2 (human hepatoma) and HeLa (human cervical) cells with the CZ1/PFO ensemble (14.82 μM/20 ppm) 405 nm (emission: 435-475 nm) and 559 nm (emission: 590-690 nm) for PFO and CZ1, respectively (Scale bar: 40 μm; Bright field/Merge means fluorescence merged with bright field; arrows indicate some membrane-adhered PFO dots).

To evaluate the interaction between the polymer ensemble and Hep-G2, stimulated emission depletion (STED) super-resolution microscopy capable of producing high-resolution, spatiotemporal cell imaging was employed. With STED we carried out a time-dependent imaging assay of CZ1/PFO with Hep-G2 (Fig. 4a). The result indicates that the CZ1 fluorescence was gradually produced with time in a cluster of Hep-G2 cells (the frames indicate three cellular regions that gradually show CZ1 fluorescence with time; the images that merge fluorescence and bright fields are shown). (b) Enlarged areas of cells to elaborate dynamic localisation of PFOs (each set of images contains an initial field and the same field recorded after 2 seconds) (Scale bars: 2 μm).

Figure 4. (a) Time-dependent stimulated emission depletion super-resolution microscopy imaging of Hep-G2 (human hepatoma) cells with CZ1/PFO ensemble (40 μM/40 ppm) with excitation of 405 nm (emission channel: 418-485 nm) and 552 nm (emission channel: 590-665 nm) for PFO and CZ1, respectively (Scale bar: 20 μm; the frames indicate three evident regions that gradually show CZ1 fluorescence with time; the images that merge fluorescence and bright fields are shown). (b) Enlarged areas of cells to elaborate dynamic localisation of PFOs (each set of images contains an initial field and the same field recorded after 2 seconds) (Scale bars: 2 μm).
These imaging results support our hypothesis that the CZ/PFO ensemble dissociates on the membrane surface in a receptor-controlled manner, leading to 1) endocytosis of the fluorescent CZ ligands and 2) the adhesion and then diffusion of PFO to the extracellular milieu. The inability of PFO to penetrate cell membrane might be attributed to its negatively charged nature that is repelled by the phospholipid bilayer of cells. In addition, a preliminary cell viability assay showed that the ensemble was not toxic to Hep-G2 and a human embryonic kidney cell line (HEK293) with increasing concentrations (Fig. 5).

In summary, we have demonstrated the supramolecular assembly of a polymer dot based system for ratiometric detection of lectins and targeted delivery of imaging agents. Besides its ability to detect lectins in a ratiometric fashion, microscopic techniques used suggest that the ensemble could be dissociated on the membrane of a cell that highly expresses a sugar receptor. The dissociation leads to selective, quick endocytosis of the imaging agent and subsequent diffusion of the polymer dot vector to the extracellular milieu. This research has implications for the construction of delivery systems using membrane-impermeable materials as the vector for the targeted delivery of therapeutic agents controlled by receptor-ligand recognition.33-35

ASSOCIATED CONTENT
Supporting Information. Additional figures (Fig. S5-S7), experimental section and a video file of STED microscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES


TOC only

Unloading of Imaging agents

Receptor

Dissociation

Diffusion

Initial

STED

2.5