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Cite this: Chem. Commun., 2013, 49, 3646 Received 15th January 2013, Accepted 13th March 2013 DOI: 10.1039/c3cc40330k www.rsc.org/chemcomm Fluorescent water-soluble perylenediimide-cored cationic dendrimers: synthesis, optical properties, and cell uptake[†]

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Different generations of perylenediimide-cored dendrimers with peripheral amine groups were synthesized. All these water-soluble dendrimers could rapidly internalize into live cells with high efficacy of gene transfection and low cytotoxicity. Increasing dendrimer generation increased their ability for gene transfection.

Perylenediimides (PDIs) display exceptional chemical, thermal, and photochemical stability with high fluorescence quantum yields in organic solvents. To date, PDI derivatives have been widely used in various fields, such as dye lasers, solar cells and photovoltaic devices.¹ However, due to the easy aggregation of perylene chromophores, PDIs exhibit poor water solubility and very weak fluorescence in aqueous solution.² This is the major challenge in the application of PDIs in biological areas. In order to increase their water solubility, modifications of perylene chromophores were achieved by directly incorporating hydrophilic moieties into the bay regions or imide positions.³ In general, the bay-substituted PDIs have an advantage of a significant red shift in the absorption maximum with a larger Stokes shift. This has attracted great interest as it minimizes the interference from cell auto-fluorescence.⁴ Another modification strategy is the attachment of water-soluble star polymers⁵ or dendrimers to the PDI core. Dendrimers represent a class of perfectly branched polymers with a well-defined threedimensional shape. A characteristic of dendrimers is that numerous peripheral chain ends surround a single core.⁶ The previously reported PDI-cored dendrimers were water-soluble, biocompatible, and neutral with a nonionic character.7 Therefore, it would be of high interest to incorporate ionic dendrons into the bay regions of PDIs and explore their biological applications.

In this study, we synthesized water-soluble PDI-cored cationic dendrimers with different generations. The dendrimers were designed with three characteristics. The central fluorescent PDI chromophore allows the tracing of cell uptake *via* fluorescence

microscopy. Degradable dendritic polyesters⁸ are quite attractive for biological applications. The peripheral primary amines serve as active sites for growing dendrons, and also provide positive charges upon a final treatment with dilute hydrochloric acid. The chemical structures of three cationic dendrimers (**G1**, **G2**, and **G3**) are shown in Scheme 1. Their optical properties in water were investigated. The cell-penetrating abilities of these dendrimers and the gene transfection efficacies were assayed using live cells by fluorescent tracing of the complexes of dendrimers and DNA.

The synthesis strategy was started from amine-functionalized PDI (1, Scheme S1 in the ESI⁺) that was prepared according to the literature procedure.⁹ The four primary amines in dye 1 selectively reacted with the acrylate group in 2-methacryloyloxyethyl acrylate (MAEA) resulting in the intermediate product 2a.10 It should be noted that a slight excess of MAEA was needed to ensure the completion of the reaction. Repeated washing with hexane yielded pure 2a. The methacrylates at the periphery of 2a selectively reacted with the thiol group in cysteamine (CA) resulting in 2b.11 The purification of 2b was done by simply washing with cold brine to remove the unreacted CA. In this reaction dimethyl sulfoxide (DMSO) could be added as an accelerator.¹² The completion of this reaction was monitored using ¹H NMR and MALDI-TOF MS spectroscopy. Fig. S1 (ESI[†]) shows that the characteristic peaks of the methylene group (CH2=) at 5.56 and 6.11 ppm disappeared completely, indicating the completion of the "click" reaction of 2a. The desired molecular weight of 3343.57 was observed using MALDI-TOF MS, confirming the successful synthesis of the first generation dendrimer 2b. Similarly, alternately adding MAEA and CA produced the second and third generation dendrimers (3b and 4b). In order to improve the water solubility and stability, the peripheral primary amines in the dendrimers (2b, 3b, and 4b) were treated with 2 M HCl which yielded their ammonium salts (G1, G2, and G3). The desired products G1, G2, and G3 show good water solubility (>10 mg mL⁻¹) and high photostability, which is essential for biological applications. The life-time and sizes of these water-soluble dendrimers increased with increasing generation (Tables S1 and S2, ESI⁺). The overall synthesis strategy is shown in Schemes S1-S5 (ESI⁺). These dendrimers were fully characterized using NMR spectroscopy and MALDI-TOF mass spectrometry or gel permeation

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Scheme 1 Chemical structures of three cationic dendrimers.

chromatography (GPC) analyses (Fig. S5-S22, ESI⁺). Detailed synthesis procedures and material characterizations can be found in ESI.[†]

The concentration-dependent absorption and emission spectra of dendrimers G1-G3 in aqueous solution are provided in Fig. S2 (ESI⁺). Both the absorbance and fluorescence intensities increased with

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increasing concentration. The detailed description and discussions are provided in ESI.[†] The UV/Vis and fluorescence spectra of dendrimers G1-G3 in aqueous solution showed that both the absorbance and fluorescence intensities increased with the increase in dendron generation (Fig. S3, ESI⁺). The maximum absorption and emission was around 590 and 615 nm, respectively. The fluorescence quantum yields (FQY) of these three desired dendrimers in water were determined. G1 showed a FQY of 9%. With increasing dendron generation, there was a continuous increase in FQYs from 12% for G2 and up to 25% for G3. This showed that chromophore PDI was encapsulated in the center and its aggregation in aqueous solution was noticeably suppressed by the increase in the dendron generation.

To investigate the ability of these dendrimers to enter into live cells, Drosophila S2 cells were incubated with these dendrimers. Because all dendrimers contained a central PDI chromophore, their distribution in live cells could be observed using fluorescence microscopy. All dendrimers (G1, G2, and G3) were able to internalize into cells (Fig. 1A-C). G2 and G3 were detectable inside the cells after 1 h incubation at a concentration of 5 µM, and G1 required 2 h incubation, demonstrating the efficient and rapid internalization. As shown in Fig. 1D, fluorescence intensities gradually increased in cells along with the incubation time. The dendrimers with higher generations were detected with stronger fluorescence intensities in cells. Similar results have been observed in the literature¹³ in which fluorescein labelled cationic PAMAM dendrimers were detected inside cells in an increasing trend along with higher generation. Therefore, for such macromolecular architectures, the dendrimers with higher generations have a stronger ability to be detected in cells. The greater fluorescence intensity of the dendrimer with higher generation could partially contribute to this. The molecular weight, shape and the higher number of cationic groups on the surface have been interpreted as the causes for such difference in the cell uptake.¹³

The above data demonstrated that all the three dendrimers could be rapidly internalized into cells within short time incubation. The positive charges are able to interact with negatively charged macromolecules such as DNA through electrostatic forces.14 In order to assess whether these dendrimers (G1, G2, and G3) could act as carriers binding DNA to internalize into cells, S2 live cells were incubated with buffer containing dendrimer-DNA complexes at N/P



Fig. 2 Gene transfection assay of dendrimers. Fluorescence images (A–C) of the **G3**–DNA complex internalizing into cells after 48 h incubation (2 μ M **G3**, 100 μ M DNA, N/P = 8 : 1). (A) **G3** fluorescence image (red). (B) CXR Reference Dye labeled DNA (blue). (C) Merged channels of (A) and (B). (D) Fluorescence intensities of dendrimer–DNA complexes internalizing into cells after 48 h incubation (2 μ M dendrimer, 100 μ M DNA, N/P = 8 : 1). The data were mean \pm SEM.

ratios of 1:1, 2:1, 4:1 and 8:1, respectively. Gene transfection efficacies of dendrimer–DNA complexes were visualized by fluorescent tracing of the cellular distribution of dendrimer and CXR Reference Dye labeled DNA. After 48 h incubation, G3 showed effective transfection activity at all N/P ratios. At a N/P ratio of 8:1, G3 showed the most efficient gene transfection. The gene transfection efficacies of G1, G2, and G3 have been proved by the quantified fluorescence intensity of CXR Reference Dye labeled DNA inside the cells (Fig. 2D). The results clearly highlighted remarkable transfection efficacies of G2 and G3. By comparing with previous neutral hydrophilic dendrimers,⁷ our cationic dendrimers could bind DNA and be internalized into cells, thus, serving as gene carriers.

The cytotoxicities of dendrimers were assessed by the TaliTM viability assay. As shown in Fig. S4A (ESI[†]), the white arrows denoted apoptotic cells which were stained by the dye, Dead Cell Green, in the TaliTM kit. **G1**, **G2**, and **G3** all showed low cytotoxicity at concentrations of 2 μ M, 4 μ M and 6 μ M after 48 h (Fig. S4B, ESI[†]). The cell viability of **G3** was 94.3%, 93.7%, 92.2% at concentrations of 2 μ M, 4 μ M, and 6 μ M, respectively. The cytotoxicity of **G3** was relatively higher than that of **G1** and **G2**, in accordance with high generation dendrimers usually possessing relatively high cytotoxicity to transfected cells.¹⁵ Although the complexation of dendrimer with DNA caused additional cytotoxicity to cells compared with cells treated by dendrimer only, the cell viability is still high (>93%) as seen in Fig. S4C (ESI[†]).

In conclusion, the synthesis and optical properties of novel water-soluble PDI-cored dendrimers bearing positive charges were reported. The aggregation of the encapsulated PDI chromophores was noticeably suppressed by outer cationic dendrimers, thus leading to the enhancement of optical performances in water. The fluorescence detection, water solubility, and low cytotoxicity, together with biodegradability of polyester units, are the prime attractions for bio-applications. All dendrimers could rapidly internalize into live cells with high efficacy of gene transfection. The highest cellular uptake ability and transfection efficacy of G3 was explained by bigger outer branches and more positive charges. These dendrimers had remarkable gene transfection efficiency with low cytotoxicity, and would be applied to live animals to explore the potential gene therapy in ongoing experiments.

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