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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 polymers[5] or dendrimers to the PDI core. Dendrimers represent a class of perfectly branched polymers with a well-defined three-dimensional 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 polyesters[8] 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 ^{1}H NMR and MALDI-TOF MS spectroscopy. Fig. S1 (ESI†) shows that the characteristic peaks of the methylene group (CH_2=) 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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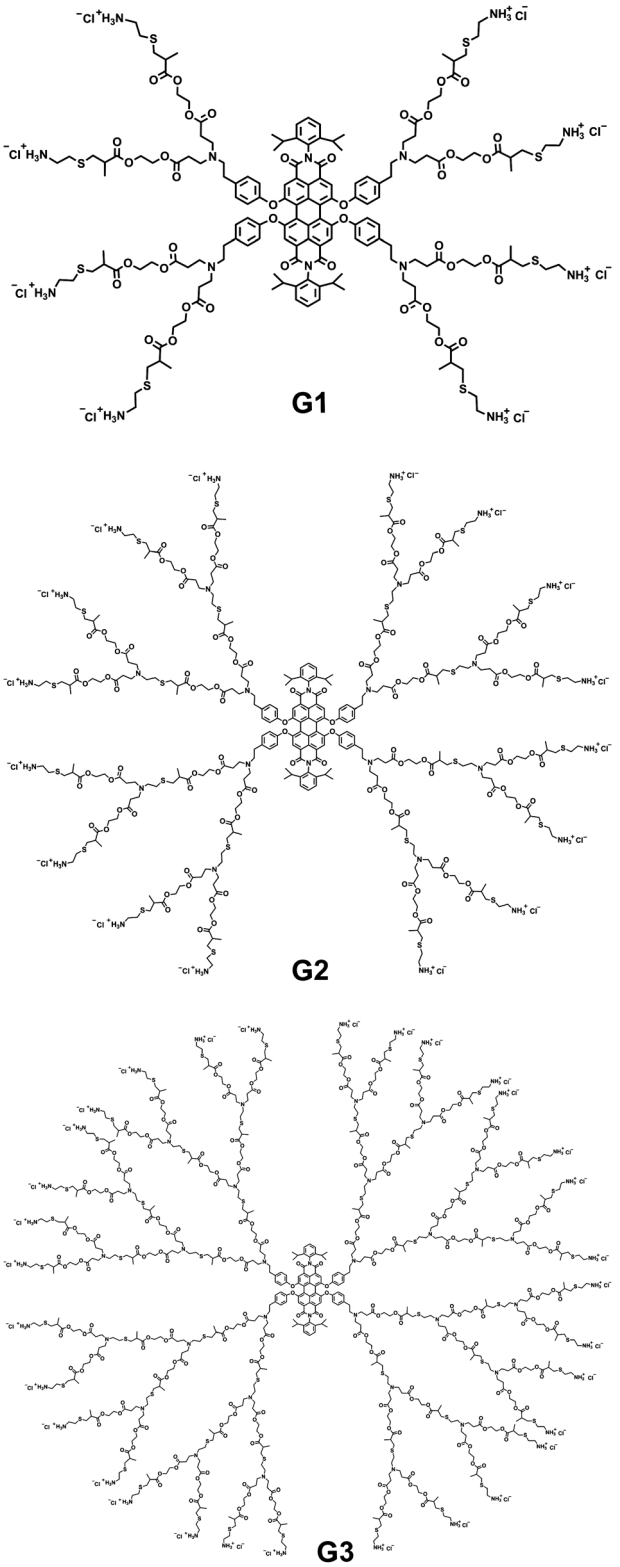

Scheme 1 Chemical structures of three cationic dendrimers.

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

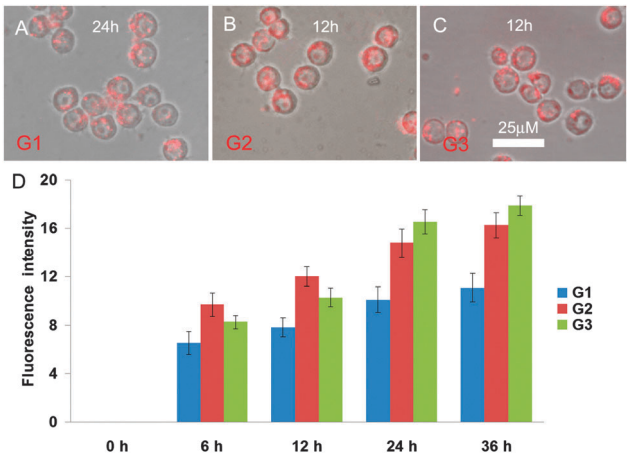

Fig. 1 The fluorescence images and intensities of **G1**, **G2**, and **G3** internalizing into cells.

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 literature[13] 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

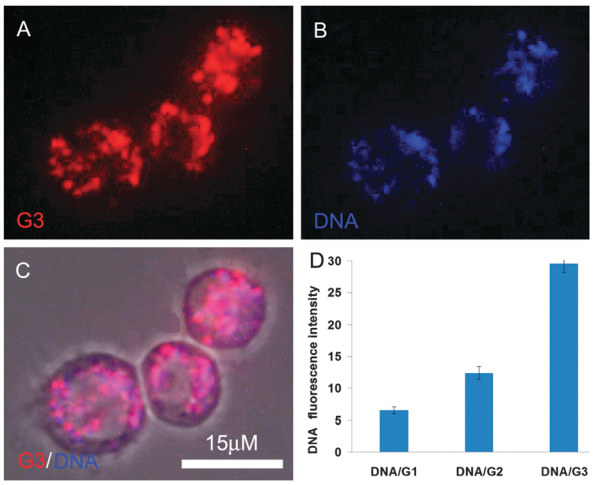

Fig. 2 Gene transfection assay of dendrimers. Fluorescence images (A–C) of the **G3**–DNA complex internalizing into cells after 48 h incubation (2 μM **G3**, 100 μM DNA, N/P = 8 : 1). (A) **G3** fluorescence image (red). (B) CXR Reference Dye labeled DNA (blue). (C) Merged channels of (A) and (B). (D) Fluorescence intensities of dendrimer–DNA complexes internalizing into cells after 48 h incubation (2 μM dendrimer, 100 μM DNA, N/P = 8 : 1). The data were mean ± 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 (Fig. 2A–C). **G2** showed intermediate efficacy of 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 Tali™ 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 Tali™ kit. **G1**, **G2**, and **G3** all showed low cytotoxicity at concentrations of 2 μM, 4 μM and 6 μM after 48 h (Fig. S4B, ESI†). The cell viability of **G3** was 94.3%, 93.7%, 92.2% at concentrations of 2 μM, 4 μM, and 6 μM, 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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Notes and references

1 (*a*) M. D. Yilmaz, O. A. Bozdemir and E. U. Akkaya, *Org. Lett.*, 2006, **8**, 2871; (*b*) O. A. Bozdemir, M. D. Yilmaz, O. Buyukcakir, A. Siemiarczuk, M. Tutas and E. U. Akkaya, *New J. Chem.*, 2010, **34**, 151; (*c*) L. Zhu, W. Wu, M. Q. Zhu, J. J. Han, J. K. Hurst and D. Q. Li, *J. Am. Chem. Soc.*, 2007, **129**, 3524.
2 (*a*) G. Schnurpfeil, J. Stark and D. Wlhrle, *Dyes Pigm.*, 1995, **27**, 339; (*b*) H. Langhals, W. Jona, F. Einsiedl and S. Wohnlich, *Adv. Mater.*, 1998, **10**, 1022.
3 (*a*) C. Kohl, T. Weil, J. Qu and K. Müllen, *Chem.–Eur. J.*, 2004, **10**, 5297; (*b*) J. Qu, C. Kohl, M. Pottek and K. Müllen, *Angew. Chem., Int. Ed.*, 2004, **43**, 1528; (*c*) S. Rehm, V. Stepanenko, X. Zhang, T. H. Rehm and F. Würthner, *Chem.–Eur. J.*, 2010, **16**, 3372; (*d*) C. D. Schmidt, C. Böttcher and A. Hirsch, *Eur. J. Org. Chem.*, 2007, 5497; (*e*) C. Backes, C. D. Schmidt, F. Hauke, C. Bottcher and A. Hirsch, *J. Am. Chem. Soc.*, 2009, **131**, 2172.
4 (*a*) S. Demmig and H. Langhals, *Chem. Ber.*, 1988, **121**, 225; (*b*) H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke and Y. Urano, *Chem. Rev.*, 2010, **110**, 2620.
5 (*a*) M. Yin, J. Shen, G. O. Pflugfelder and K. Müllen, *J. Am. Chem. Soc.*, 2008, **130**, 7806; (*b*) M. Yin, C. R. W. Kuhlmann, K. Sorokina, C. Li, G. Mihov, E. Pietrowski, K. Koynov, M. Klapper, H. J. Luhmann, K. Müllen and T. Weil, *Biomacromolecules*, 2008, **9**, 1381; (*c*) M. Yin, J. Shen, R. Gropeanu, G. O. Pflugfelder, T. Weil and K. Müllen, *Small*, 2008, **4**, 894.
6 F. Wurm and H. Frey, *Prog. Polym. Sci.*, 2011, **36**, 12.
7 (*a*) T. Heek, C. Fasting, C. Rest, X. Zhang, F. Würthner and R. Haag, *Chem. Commun.*, 2010, **46**, 1884; (*b*) B. Gao, H. Li, H. Liu, L. Zhang, Q. Bai and X. Ba, *Chem. Commun.*, 2011, **47**, 3894; (*c*) S. K. Yang, X. Shi, S. Park, S. Doganay, T. Ha and S. C. Zimmerman, *J. Am. Chem. Soc.*, 2011, **133**, 9964.
8 (*a*) D. J. Welsh, S. P. Jones and D. K. Smith, *Angew. Chem., Int. Ed.*, 2009, **48**, 4047; (*b*) M. Ye, Y. Qian, Y. Shen, H. Hu, M. Sui and J. Tang, *J. Mater. Chem.*, 2012, **22**, 14369.
9 H. A. Klok, J. R. Hernánedz, S. Becker and K. Müllen, *J. Polym. Sci., Part A*, 2001, **39**, 1572.
10 X. Ma, J. Tang, Y. Shen, M. Fan, H. Tang and M. Radosz, *J. Am. Chem. Soc.*, 2009, **131**, 14795.
11 (*a*) A. Akinc, D. M. Lynn, D. G. Anderson and R. Langer, *J. Am. Chem. Soc.*, 2003, **125**, 5316; (*b*) N. Wang, A. Dong, M. Radosz and Y. Shen, *J. Biomed. Mater. Res., Part A*, 2007, **84**, 148.
12 N. Wang, A. J. Dong, H. D. Tang, K. E. A. Van, P. A. Johnson, W. J. Murdoch, M. Radoaz and Y. Shen, *Macromol. Biosci.*, 2007, **7**, 1187.
13 (*a*) F. P. Seib, A. T. Jones and R. Duncan, *J. Controlled Release*, 2007, **117**, 291; (*b*) S. Rathgeber, M. Monkenbusch, M. Kreitschmann, V. Urban and A. Brulet, *Chem. Phys.*, 2002, **117**, 4047; (*c*) D. A. Tomalia, A. M. Naylor and W. A. Goddard III, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 138.
14 (*a*) M. Yin, C. Feng, J. Shen, Y. Yu, Z. Xu, W. Yang, W. Knoll and K. Müllen, *Small*, 2011, **7**, 1629; (*b*) Y. Yu, M. Yin, K. Müllen and W. Knoll, *J. Mater. Chem.*, 2012, **22**, 7880; (*c*) C. Feng, M. Yin, D. Zhang, S. Zhu, A. M. Caminade, J. P. Majoral and K. Müllen, *Macromo. Rapid Comm.*, 2011, **32**, 679.
15 H. Liu, H. Wang, W. Yang and Y. Cheng, *J. Am. Chem. Soc.*, 2012, **134**, 17680.

