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Meizhen Yin *et al.*

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Water-soluble perylenediimides: design concepts and biological applications

Mengmeng Sun,^a Klaus Müllen^b and Meizhen Yin^{*a}

Water-soluble perylenediimides (PDIs) with high fluorescence intensity, photostability and biocompatibility have been successfully prepared and applied in the biological field. In this tutorial review, we briefly focus on the synthetic strategies for the preparation of water-soluble PDIs by incorporating ionic or non-ionic substituents with multiple polar groups into the bay-region, imide- or ortho-positions of PDIs. These ionic/non-ionic substituents can suppress π - π aggregation and shield the inner perylene chromophores, thus contributing to the water solubility which is essential for biological applications. The optical properties, absorption and emission maxima above 500 nm, minimize the autofluorescence background of cells and provide access to imaging in living cells. The biological applications of water-soluble PDIs are discussed from simple (basic) to complex (advanced) processes, including biosensing *in vitro* studies, imaging and gene/drug delivering in living cells, tissues and the whole body. The promising future of designed multi-functional water-soluble PDIs will be highlighted in this review.

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Key learning points

- (1) The chemical structure affects the physical and optical characteristics.
- (2) Hydrophilic substituents (cationic, anionic or non-ionic polar groups) improve the water solubility.
- (3) Fluorescence-based techniques for biosensing and bioimaging.
- (4) Nanocarriers for gene/drug delivery.
- (5) *In vitro* and *in vivo* assays (living cells, tissues and the whole body).

1. Introduction

Fluorescence-based techniques have been widely applied in the visualization of biological components and processes due to their high selectivity and sensitivity. Successful fluorophores for biosensing or bioimaging should possess some unique properties, such as high water-solubility, fluorescence intensity, photostability and biocompatibility. As an attractive class of chromophores, perylene-3,4,9,10-tetracarboxylic acid diimides (perylenediimides, PDIs) have been widely used as active components of field-effect transistors (OFETs)¹ and organic light-emitting diodes (OLEDs),² and as dye sensitizers in solar cells^{3,4} because of their high extinction coefficients, high fluorescence quantum yields (FQYs), a broad color range, as well as outstanding chemical, thermal, and photochemical stability in

organic solvents. In particular, the absorption and emission maxima of PDIs are above 500 nm, which minimize the autofluorescence background of living cells. However, PDIs exhibit poor water solubility, very weak fluorescence in aqueous solution, and the tendency to form aggregates due to intrinsic π - π stacking interactions between perylene backbones.^{5,6} This creates a major challenge for the application of PDIs in the biological and medicinal fields. Great efforts have been made to increase the water solubility of PDIs by introducing hydrophilic groups in the bay-region or at the imide- and ortho-positions.

Ionic groups such as cationic ammonium salts,^{7–9} anionic carboxylic acids,¹⁰ sulfonic acids¹¹ and phosphonic acids¹² have been directly incorporated into perylene chromophores to generate water-soluble PDIs. Another powerful strategy to enhance water solubility is the attachment of non-ionic substituents with multiple polar groups, such as poly(ethylene glycol) (PEG), polyglycerol (PG) dendrons, and dendritic carbohydrate derivatives, in the bay region or at the imide positions of PDIs. These approaches effectively obstruct the aggregation of perylene cores, and the functionalized PDIs exhibit strong fluorescence and high FQYs in aqueous solution.

^a State Key Laboratory of Chemical Resource Engineering, Key Laboratory of Carbon Fiber and Functional Polymers of Ministry of Education, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, 100029, Beijing, China. E-mail: yinmz@mail.buct.edu.cn

^b Max-Planck Institut für Polymerforschung, Ackermannweg 10, 55128, Mainz, Germany



In this tutorial review, we briefly present synthetic strategies for the preparation of water-soluble PDIs by incorporating ionic or non-ionic substituents with multiple polar groups into the bay-region, imide- or ortho-positions of PDIs. These ionic/non-ionic substituents can suppress the π - π stacking of inner perylene chromophores, and also contribute to the biocompatibility. The optical properties and biological applications of water-soluble PDIs are discussed with a special focus on their biosensing and bioimaging applications in living cells, tissues, and the whole body. Moreover, benefiting from the positive charges or cavities provided by dendrimers, PDIs can be used as nanocarriers for gene/drug delivery and achieve gene therapy or chemotherapy.

2. Design of water-soluble perylenediimide chromophores

In general, PDIs are chemically modified in the bay-region, imide- or ortho-positions of PDIs (Fig. 1). The substituents at

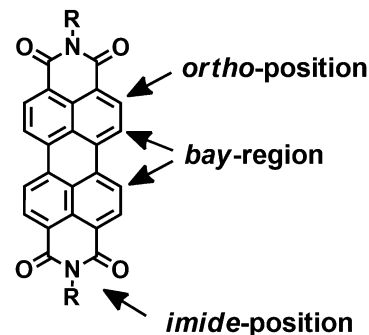


Fig. 1 General structure of PDIs.

the imide-positions are primarily used to tune the solubility of PDIs in various solvents while minimally altering the optical and electronic properties. The resulting derivatives retain the green-yellow emission of PDIs and their high FQYs. In contrast, modifications in the bay-region of PDIs result in a twist of the two naphthalene rings, which can tune the spectroscopic properties and yield a significant red shift in the absorption maximum with an increased Stokes shift. This has attracted great interest in biological and medicinal research because it can minimize phototoxicity and interference from biological auto-fluorescence. Selective functionalization in the ortho-positions can successfully tune the optical and electronic properties without affecting the planarity of the perylene core. Moreover, the solid state emission and solubility in organic solvents are significantly enhanced compared to the unsubstituted PDIs.^{13–16} Typically, ionic or non-ionic functions with multiple polar groups such as $-\text{SO}_3\text{H}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{NHR}$, $-\text{COOH}$, $-\text{O}-$ are introduced into modified PDIs to enhance the water-solubility, because these groups can form hydrogen bonds between the polar pendant groups and the water molecules. In addition, the polar substituents attenuate intermolecular π - π aggregation of PDIs due to steric hindrance or electrostatic repulsion forces.⁹



Mengmeng Sun

Mengmeng Sun obtained her BE degree in Polymer Materials and Engineering from Hebei University in 2011. Then she started her graduate studies under the supervision of Professor Meizhen Yin in Beijing University of Chemical Technology. Her current PhD thesis focuses on synthesis and bio-application of fluorescent phosphoester copolymers.



Klaus Müllen

Klaus Müllen received his PhD in 1972 at the University of Basel (Professor F. Garson) after completing his Diploma in Chemistry at the University of Cologne (Professor E. Vogel) in 1969. He pursued postdoctoral research in the group of J. F. M. Oth at ETH Zurich, where he received his habilitation in 1977 and was appointed as a Privatdozent. In 1979, he became a Professor in Organic Chemistry at the University of Cologne. In 1989, he became a scientific member of

the Max Planck Society and has been the director of the Synthetic Chemistry Department at the Max Planck Institute for Polymer Research. His current research focuses on synthetic macromolecular chemistry, supramolecular chemistry, and materials science.



Meizhen Yin

Meizhen Yin got her PhD in Organic Chemistry under the supervision of Prof. Brigitte Voit and Prof. Wolf D. Habicher from Dresden University of Technology (TUD), Germany in 2004. Afterwards, she worked as a postdoctor in Prof. Klaus Müllen group in Max Planck Institute for Polymer Research, Mainz, Germany. Since 2009, she is a Professor in Beijing University of Chemical Technology (BUCT), China. She was awarded "New

Century Excellent Talents" of the Education Ministry in 2010. Currently, she focuses on the design and synthesis of functional fluorescent macromolecules, multifunctional organic/inorganic nanoparticles and their biological applications.



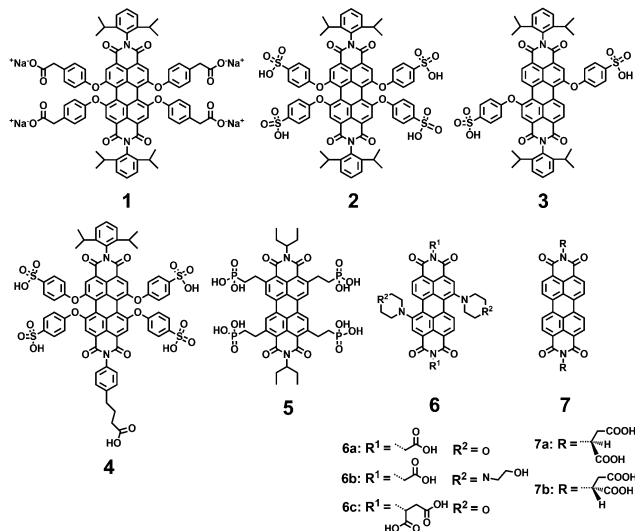


Fig. 2 PDIs 1–7 with small negatively charged substituents.

2.1 Anionic substituents

Anionic substituents, such as carboxylic, sulfonic and phosphonic acids and their corresponding salts, are the most widely used to improve the water-solubility of PDIs. Müllen and coworkers^{9,12,17} reported a series of water-soluble PDIs with anionic substituents in the bay-regions and imide-positions (Fig. 2). Compound 1, containing four carboxylic acid sodium salts attached in the bay-region, was prepared by reacting the parent acid with sodium hydroxide. The carboxylates improve the water solubility, while the chromophore with four carboxylic acid substituents is not soluble in water. Compound 2, containing four sulfonic acids in the bay-region, had a water solubility of $8.0 \times 10^{-2} \text{ mol L}^{-1}$. With a decreasing number of hydrophilic groups, from four down to two, the water solubility and FQY of 3 decreased (FQY = 12%). The monofunctionalized PDI 4 showed similar water solubility to the symmetric ionic PDIs 2. In addition, 4 can be further functionalized due to the presence of a carboxylic acid handle at the imide-positions of PDI. The FQY of 2 is 58%, displaying a reduction of 42% compared to PDI chromophores in organic solvents (FQY \approx 100%). The reduction of the FQY in polar solvents was explained by aggregation, vibrational relaxation and photo-induced electron-transfer (PET). Compound 1 bearing carboxylate groups at the terminus of a short alkyl spacer displays a low FQY of 7%, due to lipophilic and vibrational relaxation resulting from the alkyl spacers. The FQY of 4 is slightly lower (49%) than that of 2, because imide substitution affects the fluorescence of PDIs in water. Compound 5 with phosphonic acids in ortho-positions is capable of carrying negative charges at biologically relevant pH values, with enhanced resistance to aggregation and fluorescence quenching. The FQY of 5 is 77% in water. Because of the water-solubility and high FQY, 5 was impregnated into HeLa cells for successful labeling. Akkaya and coworkers synthesized several water-soluble green PDI dyes 6(a–c) by introducing dialkyl-amino groups into the bay-region.¹⁸ Under red light excitation, 6c was shown to be efficient generators of singlet oxygen and displayed significant light-induced cytotoxic effects on living cells.

A pair of chiral PDIs 7(a–b) with aspartic acid (L or D) at the imide-positions was prepared by Malik and coworkers.¹⁹ After the pH was decreased to 4.00, these two enantiomers formed a reddish-brown hydrogel with reddish color under UV light. Dried 7a or 7b gel formed well-defined left- or right-handed helical fibers of 20 nm diameter with an average pitch length of 20 nm. The perylene core forced 7a or 7b to coil until thick filaments were formed. These fibers maintained the original helical of the subunit and were stabilized by π - π stacking of the perylene core and hydrogen bonding interactions of the carboxyl groups.

Newkome and coworkers described the synthesis of 1 \rightarrow 3 C-branched polyamide dendrons (named Newkome-type dendrons) firstly.²⁰ The terminal carboxylic acids were easily hydrolyzed starting from peripheral esters, and consequently enhanced solubility of hydrophobic groups in aqueous media. Moreover, the peripheral carboxylic acids served as active sites for growing dendrons or coupling with biologically active species for medical applications. Hirsch and coworkers^{21–23} synthesized a series of water-soluble symmetric and asymmetric, chiral and achiral PDIs by incorporating first-generation (1G) and second-generation (2G) Newkome-type dendrons at the imide-positions (8–12) or in the bay-regions (13) (Fig. 3). The polyamide dendrons act as hydrophilic substituents to enhance water solubility and also exert an aggregation-inhibiting effect because of their bulkiness and anionic character, which can be easily generated in a buffer solution at pH 7.2. The symmetric 1G dendronized dye 8a forms aggregates more easily than its 2G-counterpart 8b. The perylene core in 8b is effectively shielded due to the increased steric

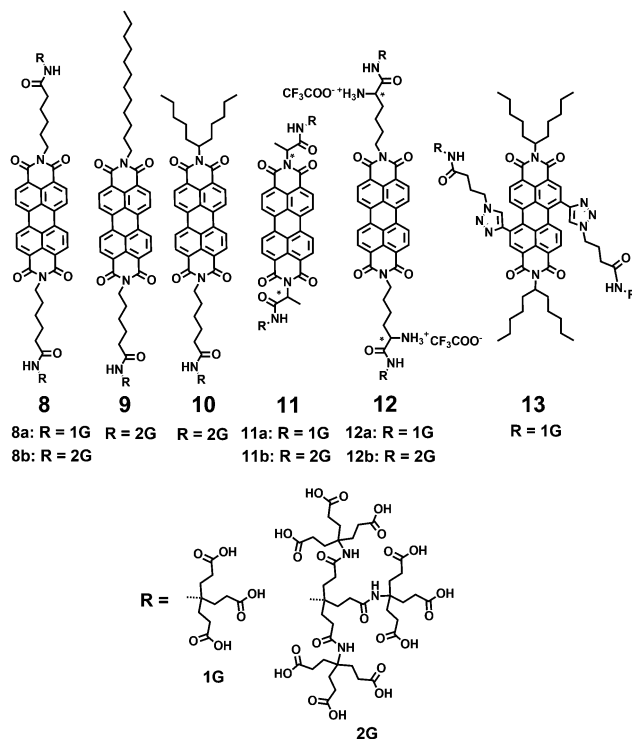


Fig. 3 PDIs 8–13 with Newkome-type carboxylates.

demand of the dendron units and electrostatic repulsion, as most of the 18 carboxy groups are deprotonated at neutral pH. The asymmetric derivative **9** reveals pronounced aggregation properties due to the presence of both a 2G-dendron and a flexible and non-bulky dodecyl substituent, and forms regularly shaped micelles in water (pH = 7.2) with a mean diameter of 16 nm. However, its 1G-analogue is not soluble in water, because the presence of just one 1G-dendron does not provide sufficient overall hydrophilicity. Asymmetric PDIs **10** with 2G-dendron are water soluble; however, their 1G-counterpart, bearing only three acid groups, is insoluble in water. The symmetric and chiral PDIs **11** and **12** are connected with 1G or 2G Newkome-type dendrimers by the amino acids alanine and lysine, respectively. The formation of chiral superstructures of **11a** was found to be pH- and concentration-dependent. Compound **13** is amphiphilic PDIs with 1G-dendron in the bay-regions. The symmetric 1G-dendronized dyes **8a**, **11a** and **12a** form aggregates more easily than their 2G-counterparts **8b**, **11b** and **12b**, due to the bulkiness of dendritic carboxylates.

Müllen and coworkers described a method for the rapid construction of branched polyphenylene dendrimers by Diels–Alder cycloaddition in 1997.²⁴ Three-dimensional polyphenylene dendrimers are shape-persistent and out-of-plane twisted phenyl components. Yin *et al.*^{25–27} reported types of negatively charged fluorescent core–shell macromolecules (**14**, **15** and **16**) consisting of a central PDI chromophore, surrounded by hydrophobic polyphenylene dendrimers and outer flexible polymers (Fig. 4). Compared with the above polyamide dendrons, the polyphenylene dendrimers are bulkier and more rigid, which can more effectively suppress aggregation and shield the inner perylene chromophores. The outer polymer shells with multiple carboxylic acids, introduced by atom transfer radical polymerization (ATRP), contribute to the water solubility and charge densities of the core–shell

macromolecules. PDIs **14** and **15** are 1G polyphenylene dendrimers, bearing eight arms with multiple carboxylic acids and corresponding sodium salts. **16** is the 2G analogue, bearing sixteen arms with multiple carboxylic acids. PDIs **14**, **15** and **16** exhibit water solubilities of $>10\text{ g L}^{-1}$ in aqueous media. The FQYs of **14** and **16** are 13% and 22% in water, respectively. PDI **14** ($n = 55$) forms unimolecular fluorescent polymeric micelles (UFPM) in aqueous solution.²⁸ The size and the fluorescence intensity of UFPM are pH-sensitive due to the polyelectrolytic nature of the outer polymer shell.

2.2 Cationic substituents

Hydrophobic dyes can be generally converted into water-soluble dyes by adding positively charged substituents. Nitrogen-containing functional groups often carry positive charges, and for this reason, cationic protonated amines (primary, secondary, tertiary and quaternary ammonium salts) are widely used to improve the water-solubility of PDIs.

A cationic PDI **17** with two positive charges was reported as a fluorescent probe by Yu (Fig. 5).²⁹ Its considerable water solubility ($>30\text{ mM}$) was provided by the two positive charges. Due to the repulsive interactions between the electrostatic charges, the tendency of PDI to aggregate decreased. Thus, **17** existed in both an aggregated form and a free monomeric form in aqueous solution. Yin and coworkers³⁰ reported a series of water-soluble symmetric cationic PDIs **18(a–f)**, which inhibit

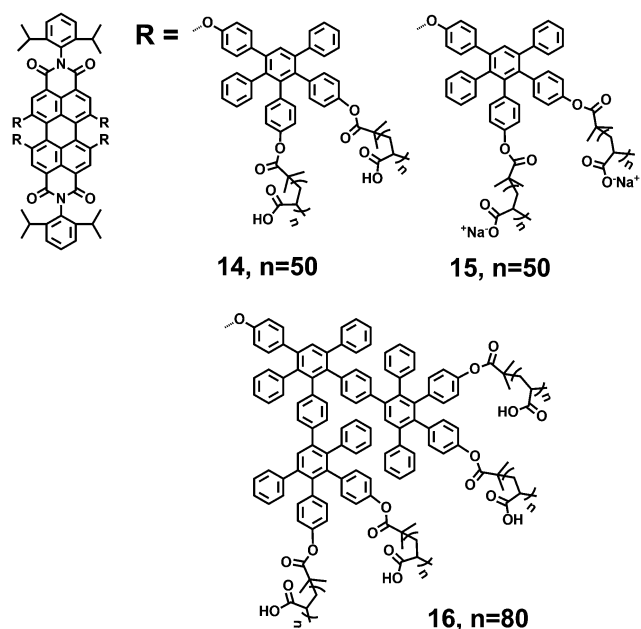


Fig. 4 PDIs **14–16** with polymeric carboxylates.

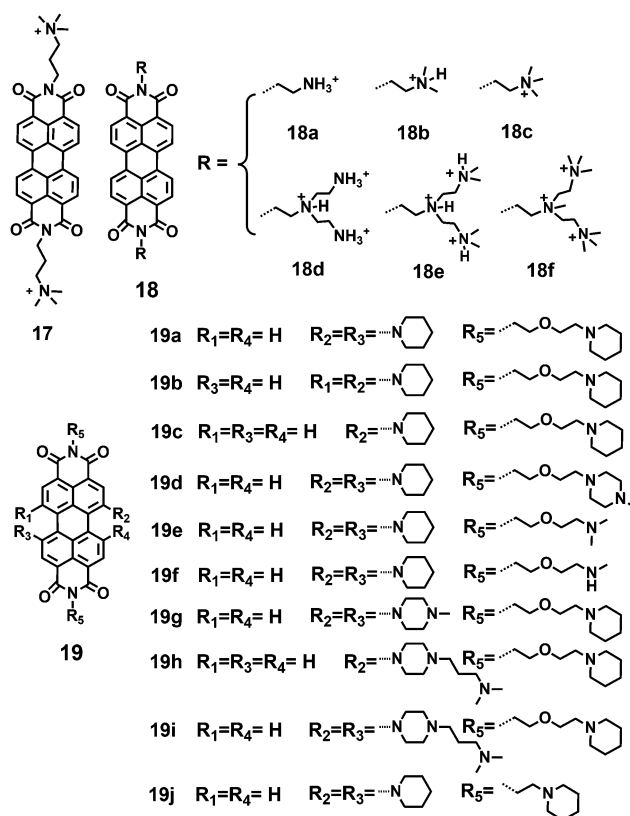


Fig. 5 Imide or bay substituted-PDIs **17**, **18(a–f)** and **19(a–j)** with primary, secondary, tertiary or quaternized ammonium salts and tertiary amines.

cancer cell growth as DNA intercalators. These PDIs contain a fluorescent perylene core and various protonated amines at the imide positions. Their nano-size and planar structure allow intercalation into double-stranded DNA base pairs. The water solubility is greatly increased due to the positive charges afforded by these protonated amines. PDIs **18c** and **18f** containing quaternary ammonium cations show higher water solubility ($>10 \text{ g L}^{-1}$ in water) than **18a** and **18d** carrying primary ammonium salts ($\leq 2 \text{ g L}^{-1}$ in water). The FQYs of **18(a-f)** are 1.1%, 22.8%, 26.2%, 4.7%, 5.6% and 25.3%, respectively. PDI **18b** exhibits low cytotoxicity with a cell viability of 90% to non-cancer cells. PDIs **18b**, **18c**, and **18e** can specifically accumulate in the cell nuclei of animal tissues by intercalation and electrostatic interaction with DNA. A series of PDIs **19** with different *N*-cyclic substituents in the bay-region and different side chains at the imide-position have been reported by Franceschin and coworkers.³¹ Their respective water-soluble hydrochlorides were prepared by precipitation with diethyl ether from an acidic (HCl) solution of methanol.

Müllen and coworkers^{9,17} also reported a series of water-soluble positively charged PDIs (**20–25**) by quaternization of dimethylamino or pyridinium-substituents in the bay-regions of PDI (Fig. 6). Chromophore **20** possesses the highest solubility in water ($8.0 \times 10^{-2} \text{ mol L}^{-1}$), while **25** with two hydrophilic groups exhibits low water solubility. The hydrophilic groups directly located on the phenyl ring (**20**) lead to a high FQY (66%), while those located at the terminus of a short alkyl chain (**21**) lead to a low FQY of 14%. The introduction of additional phenylene rings (**22** and **23**) produces highly lipophilic compounds with very limited water solubility and results in low FQYs in water (11%, 23%). The bulky and rigid dendritic polyphenylene dendrimer branches can effectively suppress aggregation and shield the inner chromophores. However, polyphenylene dendrimer **24** displays only weak FQY (15%), which is mainly attributed to energy loss through vibrational relaxation.³² PDI **25b** with two positively charged pyridinium substituents exhibits sufficient water solubility

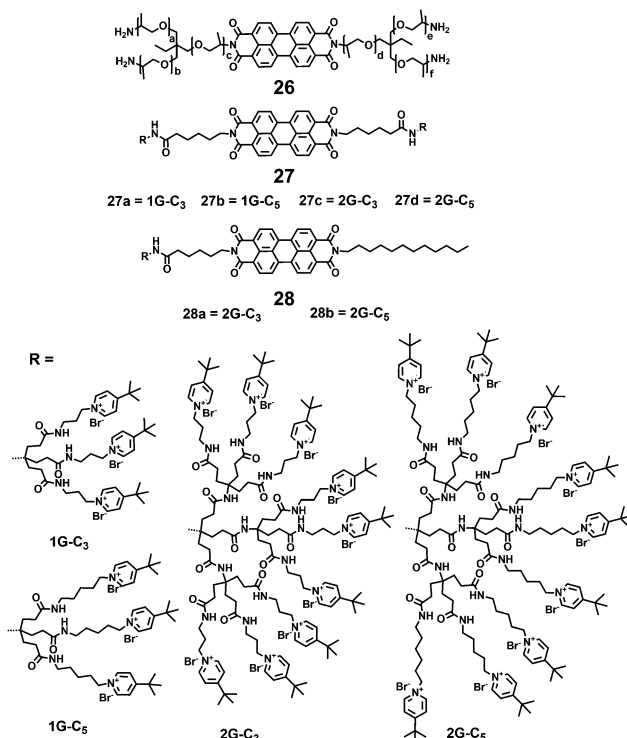


Fig. 7 Fluorescent polyetheramine **26** and PDI-cored cationic pyridinium dendrimers **27–28**.

and high FQY (98%), due to the suppressing of PET processes by a minimum number of functional groups.

Montalti and coworkers synthesized a fluorescent polyetheramine **26** by a microwave assisted reaction (Fig. 7).³³ PDI **26** can disperse in water and self-assemble into non-fluorescent nanoparticles. Hirsch and coworkers³⁴ synthesized a series of symmetric and asymmetric dendronized polycationic PDIs, **27** and **28**, by using amide coupling reactions of pyridinium salt head groups with terminal carboxylic groups of PDIs **8** and **9**. The water solubility of **27** and **28** is provided by the positively charged pyridinium salts at the periphery of these PDIs and is independent of pH. Compared with **27a** and **27b** containing small 1G dendrons, all the 2G dendronized PDIs (**27c**, **27d**, **28a** and **28b**) exhibit a pronounced increase in water-solubility, owing to the steric hindrance of larger dendrons in perylene stacking and aggregation. The FQYs of **27c**, **27d**, **28a** and **28b** are 63%, 79%, 68% and 69% in water, respectively. In contrast, the fluorescence of **27a** and **27b** is clearly quenched in water with low FQYs (7% and 0.11%). This observation is explained by a radiation free decay from the excited state due to the close proximity of aggregated molecules.³⁴

A class of highly photostable and water-soluble guanidinium-dendronized PDIs (**29–30**) were synthesized by utilizing the copper-catalyzed azide-alkyne cycloaddition (“click”) reaction (Fig. 8).³⁵ These dendronized guanidinium moieties were introduced to suppress the aggregation of the central PDI chromophore in water. Also the electrostatic repulsions of positive guanidinium moieties will further minimize the aggregation. Due to the planar conformation and high basicity of guanidine

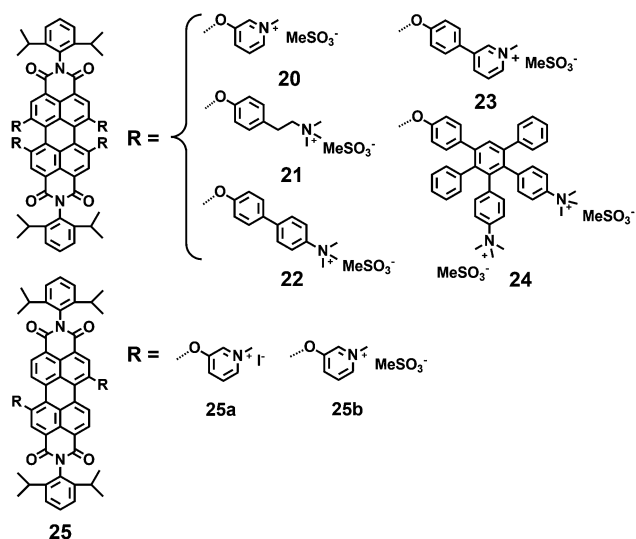
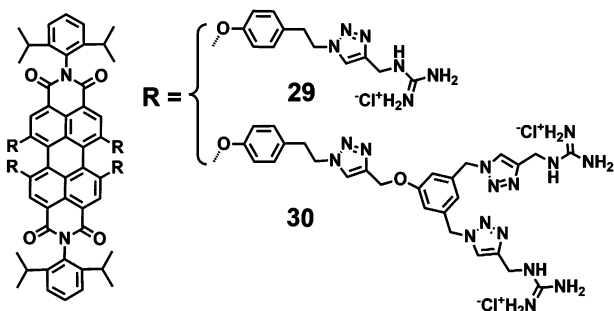
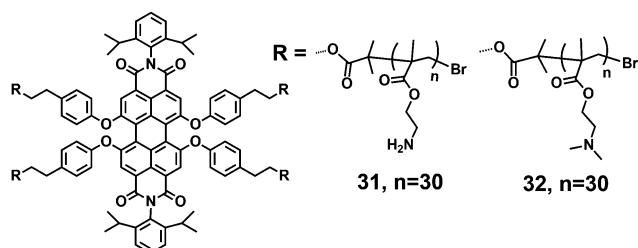
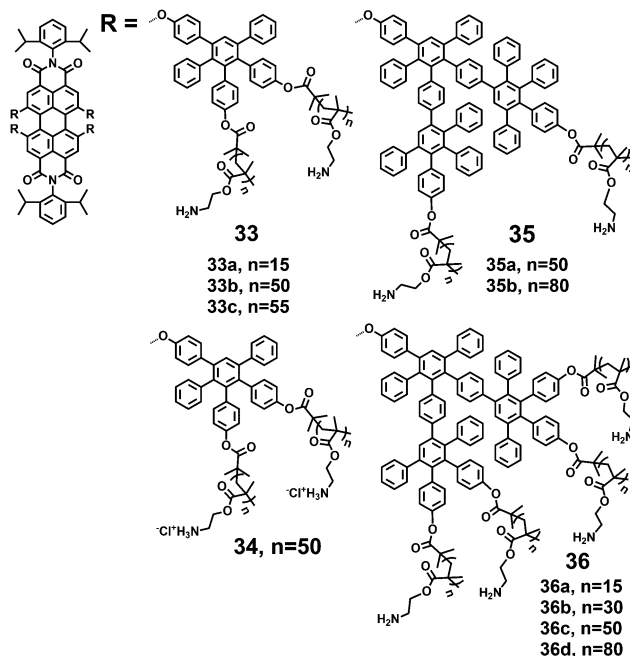


Fig. 6 Bay substituted-PDIs **20–25** with quaternized ammonium salts.

Fig. 8 Guanidinium-dendronized PDIs **29–30**.

($pK_a = 13.5$), the guanidinium moieties can form strong ion pairs with oxoanions, ensuring protonation and positive charge over a wide pH range. PDI **29**, bearing four guanidinium end groups, only dissolved in hot water and exhibited nearly no fluorescence. In contrast, **30**, containing eight analogues, showed increased water solubility with a FQY of 39%. Such differences are ascribed mainly to the lower number of guanidinium moieties, which cannot provide sufficient inhibition of aggregation and overall hydrophilicity. The moderate FQY for **30** is mainly due to substituents in the bay-regions of PDIs, which decreases the planarity of the aromatic scaffold. The cell viability of **30** treated cells was higher than 90% at concentrations of 5 mM, indicating the low toxicity of **30**.

Two water-soluble PDI-cored star polycations, **31** and **32**, with two different amines in the bay region of PDI, were synthesized by ATRP (Fig. 9).³⁶ The star polycations consist of a fluorescent central PDI and outer polymer arms of poly-(2-aminoethyl methacrylate) (PAEMA, **31**) or poly-(2-(dimethyl-amino)ethyl methacrylate) (PDMAEMA, **32**). PAEMA or PDMAEMA provides water solubility, due to the presence of polar groups such as $-NH_2$, $-OCO-$ and $-N(R)_2$, which can form hydrogen bonds with water molecules. The linear concentration-dependent absorption of **31** and **32** is observed in aqueous solutions, suggesting that both **31** and **32** remain in a single-molecule state over the whole concentration range studied (7.26–28.9 μM). In addition, the maximum emission peak is located at 622 nm, thus **31** and **32** with good FQYs (14% and 6%) are well-suited for single-molecule fluorescence detection and fluorescence tracing and imaging in live cell experiments. The high zeta potentials of **31** and **32** (37.8 and 56.5) provide the possibility to complex with DNA *via* electrostatic interactions.

Fig. 9 PDI-cored star polycations **31–32**.Fig. 10 PDIs **33–36** with polymeric amines.

Yin *et al.*^{25,27,37} synthesized several water soluble fluorescent core-shell macromolecules (**33–36**) surrounded by polyphenylenes dendrimer grafted with positively charged polymer chains (Fig. 10). The architectures consist of a fluorescent central PDI surrounded by 1G and 2G polyphenylene dendrimers and an outer polymer shell with multiple amine groups. The rigid polyphenylene dendrimer shell prevents the PDI chromophore from aggregation in water, and the outer polymer shell with multiple amine groups provides water solubility ($> 10 \text{ g L}^{-1}$) with FQYs varying from 7.6% to 16.8%. The absorption maxima exhibit bathochromic shifts for those macromolecules with a higher number of amino groups (**36b**, **36c**, **36d**). This trend is also observed in emission spectra: emission intensities increase and emission maxima shift bathochromically with increasing numbers of repeating units.

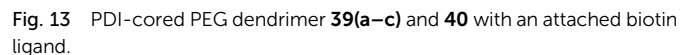
A series of water-soluble PDI-cored cationic dendrimers, **37(a–c)**, with three generations synthesized *via* a “click” reaction were reported by Yin and coworkers (Fig. 11).³⁸ The central fluorescent PDI chromophore allows the tracing of cell uptake *via* fluorescence microscopy. The peripheral primary amines provide positive charges upon a final treatment with hydrochloric acid. The outer cationic dendrimers noticeably suppress the aggregation of the encapsulated PDI chromophores due to steric hindrance and electrostatic repulsion forces. The peripheral primary amines and multiple polar groups ($-OCO-$ and $-S-$) enhance the water solubility. All the dendrimers show a water solubility of above 10 g L^{-1} and high photostability. The absorbance, fluorescence intensities and FQYs of **37(a–c)** (9%, 12% and 25%) in water increase with higher dendron generation. The cell viabilities of **37(a–c)** were all above 90% at concentrations of 6 mM, showing that the dendrimers exhibit low cytotoxicity.



2.3 Non-ionic substituents

Compared with the ionic hydrophilic groups, the non-ionic hydrophilic groups possess the unique advantages of pH and ionic strength insensitivities in biological buffer systems. The non-ionic hydrophilic groups also minimize undesired non-specific ionic interactions that may occur with membranes and other charged biomolecules. Widely used non-ionic substituents, which were introduced to improve the water-solubility of PDIs, are poly(ethylene glycol) (PEG), polyglycerol (PG) dendrons, and dendritic carbohydrate derivatives. These non-ionic bulky substituents provide an effective steric hindrance that weakens the π - π interactions between the PDI backbones. Furthermore, the non-ionic substituents consist of multiple polar groups such as ether and hydroxyl, which can form hydrogen bonds with water molecules and lead to water solubility.

A series of PDI-cored PEG dendrimers **39(a-c)** were synthesized (Fig. 13).⁴⁰ These PDIs consist of a PDI fluorescent core, branched oligo(glutamic acid)s, and PEG chains. The branched oligo (glutamic acid)s function as scaffolds to suppress the aggregation of the central PDI. Dye **39a** bearing 0G dendrons exists in an aggregated state over the whole accessible concentration range (1×10^{-6} to 1×10^{-4} M) evidenced by a broad absorption spectrum with a hypochromically shifted absorption maximum and weak fluorescence (4%). With higher dendron generation (**39b** and **39c**), the steric hindrance increases, leading to improved hydrophilicity of PDIs. Moreover, the FQYs of **39b** and **39c** also increase in water (65% and 93%), because the PDI fluorophores are efficient emitters in water as their aggregation



is completely suppressed. PDIs **39(a–c)** show very low cytotoxicity (over 90% cell viability) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell-viability assay, because PEG chains prevent the dyes from interacting nonspecifically with the extracellular proteins. In further studies, the authors prepared a bay substituted PDI **40** with a biotin ligand.⁴¹ Compound **40** can form self-assembled nanostructures with high stability in aqueous solution, leading to complete quenching of fluorescence.

Haag and coworkers^{42–44} synthesized a series of neutral, water-soluble PG-dendronized PDIs with high FQYs (Fig. 14). The 1G dendronized PDI **41a** exhibits the typical absorption spectra of the aggregated form over the whole accessible concentration range (1×10^{-6} to 1×10^{-4} M), as the 1G dendron at the imide position is apparently too small to shield the perylene core and suppress aggregation. With increased PG dendron generation, aggregation is inhibited. The observed absorption spectra of **41c** and **41d** bearing 3G and 4G dendrons arise from PDI monomers over the whole concentration range because these dendrons are sufficiently large to shield the π surfaces of the PDI. The FQYs of **41(a–d)** also reveal a continuous increase from 33% for 1G substituted **41a** up to 99% for 4G substituted **41d** with increasing dendron generation, due to the strong dendritic effect. Optical studies and dynamic light scattering reveal that **42** forms spherical micelles with a narrow size distribution and very low FQY (1%) in water, due to the aggregation of the PDI core. MTT assay shows that **42** is completely nontoxic to cells even at concentrations of 10^{-5} M. Upon gradually increasing the temperature from 298 K to 368 K, **43a** displays a transition from an aggregated state into monomeric species, as proven by the typical characteristics in temperature-dependent fluorescence measurements and ^1H NMR spectroscopy. Notably, the FQY of **43a** is still low (1.1%) even at high temperatures. The quenched

fluorescence can be explained by the PET mechanism from the phenoxy groups to the PDI core. The 3G-dendronized **43b** maintains monomeric species over the whole accessible concentration, but is almost nonfluorescent in water (FQY = 0.2%). The low FQY is again related to PET from the electron-rich pyrrolidine to the electron-poor PDI.

The introduction of functional groups in the bay-region of PDIs results in a significant red shift of the absorption maximum and an increased Stokes shift, minimizing phototoxicity and interference from biological auto-fluorescence. Zimmerman and coworkers³⁹ developed several neutral water-soluble PDIs (**44**, **45** and **46**) that are bay-substituted by amide coupling of the PDI cores with amine-functionalized PG dendrons (Fig. 15).⁴⁵ The PG dendrons provide steric bulkiness necessary to minimize π - π interactions of the perylene cores. Compounds **46b** and **46c** were obtained by the click reactions of monofunctional **46a** with alkyne-functionalized biotin and maleimide. All these neutral PDIs are water-soluble and highly fluorescent in water with FQYs ranging from 57% to 83%. The difference can be attributed to the varying degrees of encapsulation and solubilization of PDIs within the PG dendritic shell. The bulkier and more substituted **45** exhibits the highest FQY of 83%, due to the more effective encapsulation and solubilization of the PDI core by eight dendrons carrying a total of 128 peripheral hydroxyl groups. In further studies, intramolecular crosslinking of the dendronized and hyper-branched PG, which is substituted in the bay-region of PDI, was reported.^{46,47} The crosslinking results in a significant reduction in the FQY, due to the decrease of hydroxyl end-groups causing lower aqueous solubility.

Water soluble PDIs (**47–49**) modified by glycodendrimers at the imide-positions or in the bay-region have been synthesized through the click reaction and subsequent removal of protecting groups (Fig. 16).^{48–50} Upon a gradual increase of concentration and a decrease of temperature, the UV-vis spectra of **47** and **48** display the typical spectral characteristics of monomeric to aggregated state, indicating that the π - π aggregation of these PDIs is concentration- and temperature-dependent. The concentration-dependent aggregation of **46** and **48** also affects the FQYs: at a low concentration of 5.0×10^{-6} M, the FQYs of

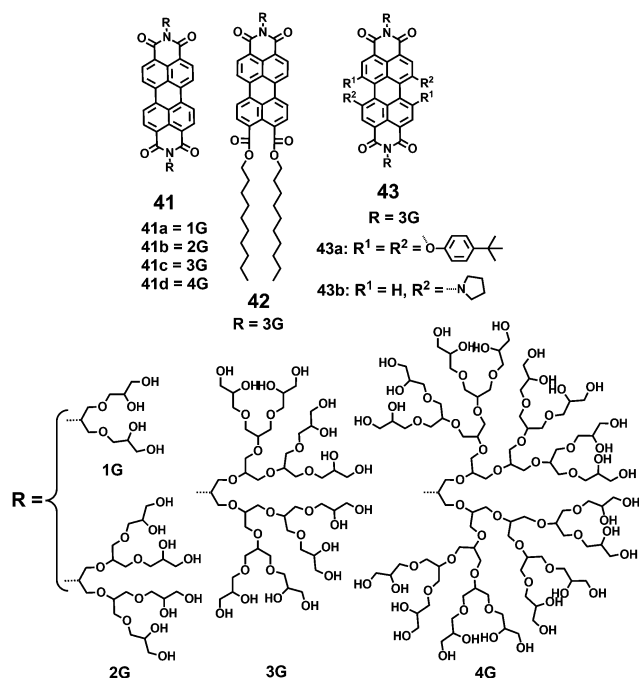


Fig. 14 PDI-cored PG dendrimers **41–43**.

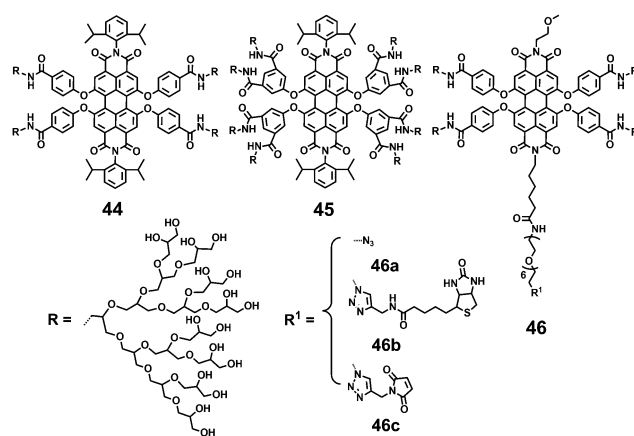


Fig. 15 PDI-cored PG dendrimers **44–46**.



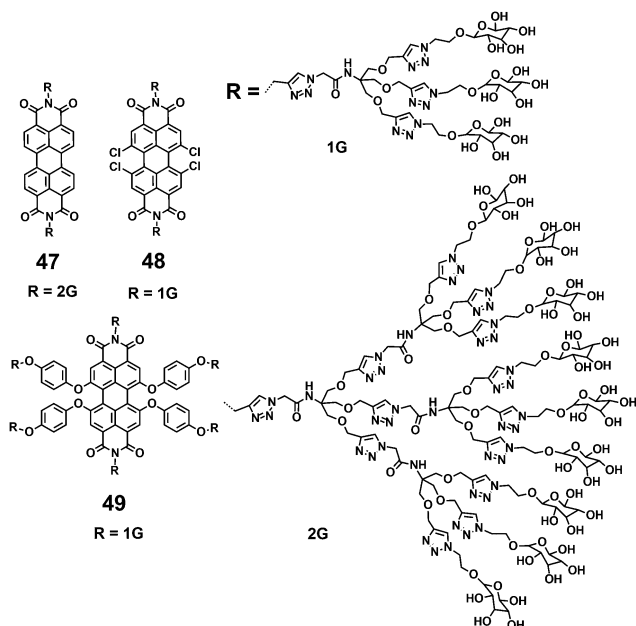


Fig. 16 PDI-cored glycodendrimer **47–49**.

47 and **48** are 59.9% and 54%, respectively. With increasing concentration, the fluorescence emission is gradually quenched, accompanied by a bathochromic shift because of aggregation. Notable, **49** bearing glycodendrimers both at the imide-positions and in bay-regions was barely detectable by fluorescence because of the intramolecular electronic transfer process from the electron-rich substituents (triazole group) to the PDI backbone. The MTT experiment reveals that the cell viability of **47** treated cells is 110% at the concentration of $50 \mu\text{g mL}^{-1}$, indicating that **47** is non-cytotoxic to living cells.

3. Biological applications of water-soluble perylenediimides

Water-soluble PDIs have been successfully applied in biological studies, because of their high fluorescence intensity and photostability, biocompatibility, and absorption and emission maxima above 500 nm to minimize the autofluorescence background of cells. Charged components are widely present in cells and tissues. For example, negatively charged extracellular matrices support and connect the organization structure, and regulate the physiological activity of the organization and cell; negatively charged membranes provide a relatively stable internal environment for cell activities and regulate the cellular uptake and efflux of molecules. In addition, positively charged histone proteins and negatively charged DNA form a DNA–protein complex for storing the genetic material. The ionic PDIs allow interactions with natural polyelectrolytes such as DNA or nuclear proteins through electrostatic interactions.

Non-ionic PEG, PG dendrons and dendritic carbohydrate derivatives have been widely used in biological studies. PEG is highly water soluble, biocompatible and nontoxic, and it shows no immunogenicity and possesses antifouling properties. In addition,

PEG can prolong the circulation time in blood due to the resistance of nonspecific protein adhesion/denaturing. Therefore, it has been approved for medical use by the Food and Drug Administration (FDA). Hydrophilic PG dendrons show similar properties, such as highly biocompatible and minimal nonspecific interaction. Glycodendrimers exhibit the potential to recognize carbohydrate–protein (lectin) interactions involved in key biological processes, such as cell recognition, differentiation and adhesion.⁴⁸ The biological applications of water-soluble PDIs are summarized below from simple (basic) to complex (advanced) processes, including imaging studies of living cells, tissues and the whole body.

3.1 In vitro studies

Due to the intrinsic fluorescence properties, the cationic or glycodendronized PDIs can be used as reporters for the specific and sensitive detection of biological molecules. The interactions between functional PDIs and biomolecules provide the foundation for further studies in living cells, tissues and the whole body.

Fluorescent nanotubes fabricated by oppositely charged **34** and **15** were reported as DNA biosensors by Yin *et al.*²⁷ Positively charged **34** was deposited at surfaces and interacted with negatively charged DNA through electrostatic forces (Fig. 17A). Compared with the nanotube alone, the nanotube/DNA complex showed an increase in normalized fluorescence intensity because the hydrophilic DNA structures improved the protection of the central PDI chromophore in the aqueous solution. Subsequently, Yin and coworkers developed a time-resolved, ultrasensitive and selective DNA sensor by combining fluorescence detection with energy transfer using the negatively charged **16** as the donor molecule and Cy5 labeled DNA targets as the acceptor molecule (Fig. 17B).⁵¹ Upon excitation at 543 nm, excitation energy was transferred from the donor to the acceptor. Due to the high molar ratio of the donor and the acceptor, the fluorescence signal was greatly increased, achieving the ultrasensitive and selective detection of DNA.

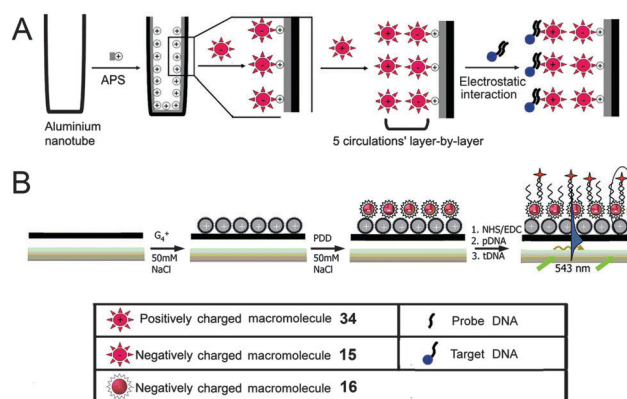


Fig. 17 (A) Schematic diagram describing the formation of fluorescent nanotubes by layer-by-layer deposition of **15** and **34**. Reproduced with permission from ref. 27. Copyright 2011, John Wiley and Sons. (B) Energy transfer between the **16** donor and the Cy5 acceptor. Reproduced with permission from ref. 51. Copyright 2011, John Wiley and Sons.

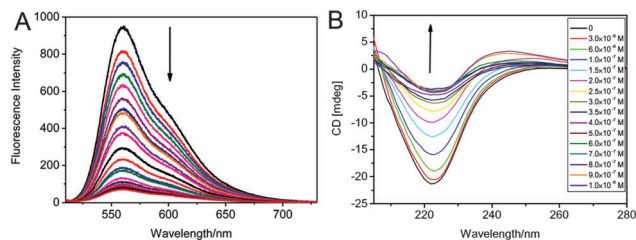


Fig. 18 (A) Fluorescence spectra of **48** upon the addition of Con A in PBS. Reproduced with permission from ref. 49. Copyright 2013, Royal Society of Chemistry. (B) CD spectra of Con A in PBS upon the addition of **49**. Reproduced with permission from ref. 48. Copyright 2013, Elsevier.

PDI-cored glycodendrimers **48** and **49** showed selective binding to Concanavalin A (Con A) due to the carbohydrate-lectin interaction of the peripheral mannose moieties and Con A (a model lectin).^{48,49} Fluorescence spectroscopy and circular dichroism (CD) measurements were performed to investigate the nature of binding. As described in Fig. 18A, with increasing concentrations of Con A, the fluorescence emission of **48** exhibits a progressive decrease. The PDI backbones enter the hydrophobic region of Con A, *via* hydrogen bonds and hydrophobic interactions, leading to progressive quenching of fluorescence. Upon the addition of **49**, the CD signal of the Con A, generated from the secondary-structure of β -sheet rich proteins, exhibits a progressive decrease and a bathochromic shift (Fig. 18B). The loss of the secondary structure due to the formation of cross-linked complexes induces the decreased intensity of the CD signal. The observed decrease of fluorescence emission intensity and CD signal intensity demonstrated the interactions of Con A with **48** or **49**.

A fluorescence turn-on approach for the sensitive and selective detection of proteins based on a nucleic acid aptamer and probe **17** is reported.²⁹ In aqueous solution, PDI **17** exists in both aggregated and monomeric forms, thus strong fluorescence can be detected. When a negatively charged nucleic acid aptamer is added to the aqueous solution, PDI **17** rapidly binds to the aptamer *via* strong electrostatic interactions between the dye monomer/aggregates and the aptamer. When the monomer of **17** aggregates, a significant decrease in the fluorescence intensity is observed. The lysozyme shows high affinity toward the aptamer. Upon the addition of lysozyme, **17** aggregates are replaced by the lysozyme. As a result, the monomer of **17** is released, and the fluorescence signal is turn-on (Fig. 19).

3.2 Live cell studies

Thanks to high selectivity and sensitivity, fluorescence bioimaging has been widely used in biological research because it offers a unique approach to visualize and manipulate the morphological details of cells and biological processes. Water soluble PDIs with high fluorescence intensity and biocompatibility have thus attracted great attention in bioimaging. Applications of water-soluble PDIs in live cell studies fall into three broad categories: the dye (i) target-specific label receptors produced by bacteria, (ii) are taken up into living cells *via* endocytic routes and accumulated in the cellular cytoplasm,

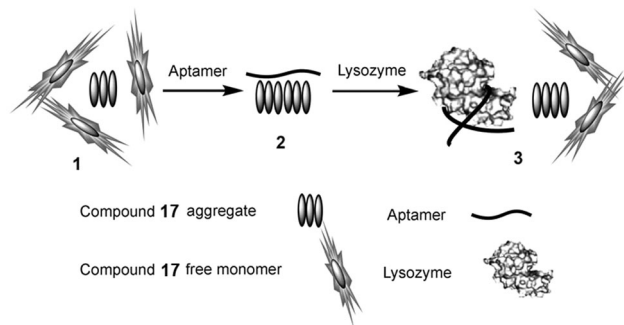


Fig. 19 Schematic diagram for the selective detection of lysozyme. (1) Coexistence of the aggregates and monomer of **17** in aqueous solution; (2) binding of the aptamer to **17** aggregates; (3) **17** is replaced by lysozyme, thus its monomer is released and the fluorescence is turn-on. Reproduced with permission from ref. 29. Copyright 2010, John Wiley and Sons.

plasma membrane, and small membrane-bound structures, or (iii) carry DNA into the cell through electrostatic forces, which can be applied to gene delivery and bioimaging.

Zimmerman's group⁴⁵ reported a PG-dendronized PDI **46b** containing a single biotin used in target-specific fluorescence bioimaging. Biotinylated PDI **46b** could be immobilized on a PEG-passivated surface through biotin-neutravidin linkages with bright fluorescence, while only negligible binding of **46b** was observed without pre-incubated neutravidin, indicating that immobilization was indeed highly specific. The target-specific fluorescence bioimaging was performed in living bacterial cells. *Escherichia coli* (*E. coli*) produced biotinylated λ receptors on the extracellular surface. The cells were pre-incubated with streptavidin. Fig. 20 shows that the bright fluorescent spots produced by **46b** appear only at the surfaces of the cells. Furthermore, the spots present a helical pattern along the long axis of the cell in some bacteria, which is consistent with the arrangement of the proteins on the bacterial surface. The above observation demonstrates that **46b** can be used as a highly target-specific label in bioimaging. This approach provides a new design for biolabeling by coupling a monofunctional fluorescent core to a multivalent periphery containing cellular receptors, therapeutic agents, targeting groups, or ligands.

A guanidinium-dendronized PDI **30** was reported for the cellular cytoplasm imaging of HeLa cells (Fig. 21A).³⁵ The acquired images indicated that **30** was efficiently taken up into HeLa cells and accumulated in the cellular cytoplasm. Fluorescent, positively charged dendritic star polymers (**33**, **35** and **36**)

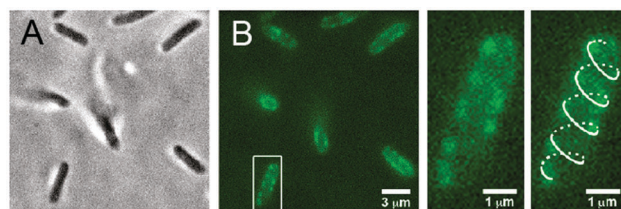


Fig. 20 (A) Bright-field and (B) fluorescence images of *E. coli* cells labeled with **46b**. Reproduced with permission from ref. 45. Copyright 2011, American Chemical Society.



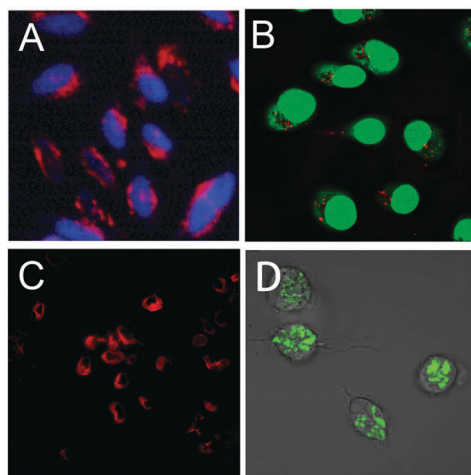


Fig. 21 (A) Fluorescent microscopy images of HeLa cells stained with **30** (red) and cell nuclei stained with DAPI (blue). Reproduced with permission from ref. 35. Copyright 2013, Royal Society of Chemistry. (B) Penetration of **33a** (red) into ECV-304 cells stained using a green fluorescence cell tracker. Reproduced with permission from ref. 25. Copyright 2008, American Chemical Society. (C) Fluorescence images of HeLa cells stained with **39c**. Reproduced with permission from ref. 40. Copyright 2011, Royal Society of Chemistry. (D) Confocal microscopic images of murine macrophage cells after incubation with **47**. Reproduced with permission from ref. 50. Copyright 2014, John Wiley and Sons.

were able to cross the cell membrane and enter living cells, whereas negatively charged dendritic star polymers (**14** and **16**) bearing carboxylic acids did not show any tendency to enter the cells.²⁵ For example, a confocal microscopy image of **33a** (red), which demonstrates penetration into the cells, is shown in Fig. 21B. The authors reported that the PDIs bearing a higher density of polymer chains with fewer amino groups underwent faster cell entry; however, PDIs with high numbers of amino groups led to considerable cytotoxicity.

Besides the above cationic PDI, two non-ionic PDIs, **39c** and **47**, were also used for live-cell imaging.^{40,50} Fig. 21C reveals that **39c** is efficiently internalized by live-cells and accumulated in the cytoplasm. Based on the successful demonstration of binding interactions between **48** and **49** with Con A, the interaction was further investigated *via* confocal microscopy in macrophage cells. These cellular uptakes are mannose receptor-mediated and the cells possess selective binding with mannosylated derivatives. The green fluorescence of **47** inside the cell (Fig. 21D) suggested that the endocytosis mechanism led to vesicular (endosomal) localization. Control experiments indicated no obvious fluorescence by using another water-soluble PDI with cyclodextrin conjugates, suggesting that **47** possessed selective binding interactions with the surface mannose receptor of the macrophage cells.

An amphiphilic PG-dendronized PDI **42** was reported as a membrane marker.⁴⁴ For highly specific membrane labeling, two alkyl chains were used as membrane anchoring groups and the length of the aliphatic chains was chosen to ensure that the overall length of the hydrophobic part matches the thickness of one lipid bilayer leaflet. In water, chromophore **42** formed

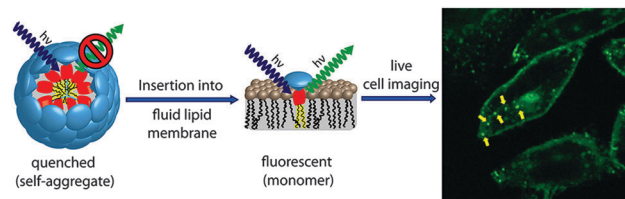


Fig. 22 Schematic diagram of the self-quenched amphiphilic dye **42** lighting up in the fluid lipid membrane and the confocal microscopy image of cells incubated with **42**. Reproduced with permission from ref. 44. Copyright 2013, American Chemical Society.

micelleular aggregates and was nearly nonfluorescent. However, when **42** was incorporated into a lipophilic environment, such as inside biological membranes, the micelles disaggregated and the fluorescence of the perylene core was restored (Fig. 22). Cellular uptake studies documented that **42** was accumulated in the plasma membrane and in small vesicular structures such as Golgi, suggesting that it is taken up into living cells *via* endocytosis. Furthermore, the stained intracellular structures arose from a passive diffusion of **42** through the plasma membrane, as no colocalization of **42** and BODIPY TR methyl ester, which efficiently stains membranes of intracellular organelles, was shown with the exception of Golgi. PG-dendronized PDI **42** is thus an ideal label to anchor and track PG bound bioactive compounds in cellular membranes.

PDI-cored cationic dendrimers **37(a-c)** and star polymers **31-32** were used for gene delivery and bioimaging by Yin and coworkers.^{36,38} By using fluorescence microscopy, the cellular uptake experiments demonstrated that all the cationic PDIs were internalized into cells. The cell viabilities of **37(a-c)** and **31-32** are all above 90%, indicating that the cationic PDIs exhibit low cytotoxicity. All the positively charged **37(a-c)**, **31** and **32** could act as DNA carriers because of the electrostatic forces with negatively charged DNA. The fluorescence images of **37c**/DNA and **32**/DNA complexes internalized into cells upon incubation (Fig. 23) displayed that **37c** (A-A') and **32** (B-B') were able to deliver DNA into cells. The gene transfection efficacies of **37(a-c)** decrease in the following order: **37a** > **37b** > **37c**. This observation can be attributed to larger outer branches and more positive charges. The cationic dendrimers and star polymers are explored as a general tool for *in vivo* gene transfection.

Some water-soluble PDIs have unique self-assembly properties, which have been used in biological research. PDI **40** with a biotin ligand in the bay-region was used as an "off-on" fluorescent probe for detecting and tracking proteins, which was driven by assembly-disassembly behavior.⁴¹ In aqueous solution, compound **40** self-assembles into nanostructures with complete quenching of fluorescence. After the addition of the target protein, the self-assembled nanostructures are disassembled since the probes bind to the targeted protein, resulting in a strong fluorescence signal. In the presence of avidin, **40** can be internalized by living cells (Fig. 24A and A'). Under physiological conditions, **26** behaves as a fluorogenic probe in biological cells, yielding a dosage-dependent green or red fluorescence.³³



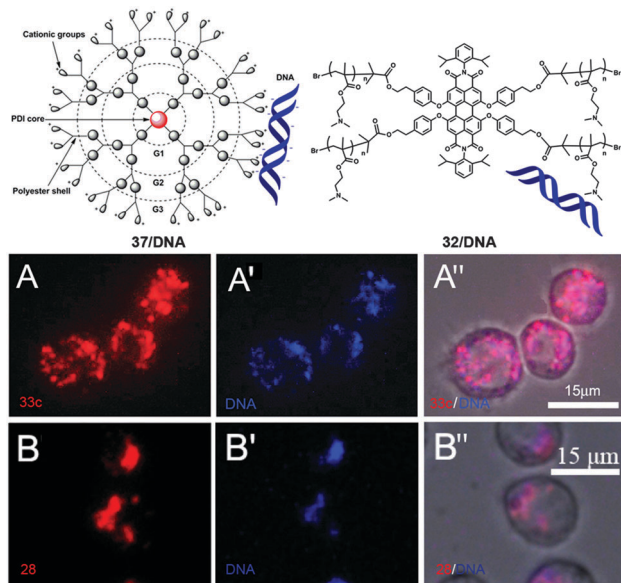


Fig. 23 Fluorescence images of the **37c**/DNA complex (A–A'') and **32**/DNA (B–B'') internalizing into cells after incubation. A and B are fluorescence images of **37c** and **32** (red), respectively. A' and B' are fluorescence images of CXR Reference Dye labeled DNA (blue). Reproduced with permission from ref. 38. Copyright 2013, Royal Society of Chemistry. Reproduced with permission from ref. 36. Copyright 2014, American Chemical Society.

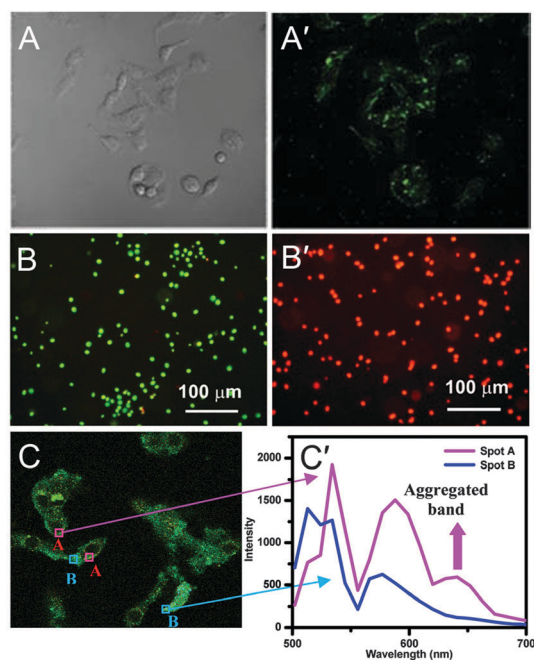


Fig. 24 (A) Bright-field and (A') fluorescence images of HeLa cells after co-incubation with **40** and avidin. Reproduced with permission from ref. 41. Copyright 2013, Royal Society of Chemistry. Fluorescence images of yeast suspensions for the concentration of **26** (B) 1×10^{-6} M and (B') 1×10^{-5} M. Reproduced with permission from ref. 33. Copyright 2013, Royal Society of Chemistry. (C) Lambda scan image and (C') the corresponding emission intensity vs. wavelength plots. Reproduced with permission from ref. 19. Copyright 2012, Royal Society of Chemistry.

Saccharomyces cerevisiae yeast cells were incubated with two concentrations of **26** in PBS (1×10^{-6} M and 1×10^{-5} M). The fluorescence microscopy images of the two samples are clearly different. Green emission can be detected in the yeast cells at the low concentration of **26** (1×10^{-6} M, Fig. 24B), while a red emission centered at about 630 nm was detected at the high concentration (1×10^{-5} M, Fig. 24B'). Particularly, under strong visible light, the fluorescence of some specific cellular compartments can be turned from red to yellow and then to green, due to the photo-tuning process of **26**. The chiral PDI **7a** can be internalized by living cells, since fluorescence is observed in the cytoplasm.¹⁹ Taking advantage of the aggregation of **7a** *in vitro*, the pH of different regions of the cellular environment can be estimated. In the lambda scanning image (excitation wavelength = 488 nm, Fig. 24C), the co-existence of aggregated (640 nm) and monomeric emissions (550 nm and 590 nm) is observed in spot A, indicating that the pHs of these areas are below 4.5. In contrast, only monomeric emissions (580 nm) are observed in spot B, indicating that the pH is above 4.5.

N-Cyclic bay-substituted PDIs **19(a–j)** were reported as G-quadruplex ligands, causing DNA damage in transformed cells.³¹ Modifications of the ending groups or the length of side chains at the imide-positions did not result in major changes of the affinity to the G-quadruplex formed by the human telomeric sequence. In contrast, the number or the nature of the groups in the bay-region has a large influence on the affinity. Transformed human fibroblasts (BJ-EHLT) were incubated with **19c**. Deconvolution microscopy images revealed that some of the damaged foci containing phosphorylated 53BP1 (a DNA-damage response factor) were induced by **19c** co-localized with TRF1 (an effective interphase telomeres marker), forming the so-called telomere-dysfunction induced foci (TIFs) (Fig. 25). The G-quadruplex binding compound **19c** can act as a telomere inhibitor and exhibit its potential as a selective antineoplastic drug.

3.3 Tissue imaging and body studies

Tissue imaging is usually achieved by fluorescence labeling, which is the process of covalently or non-covalently attaching a reactive derivative of the fluorophore to a functional group in the target molecule. The most commonly labeled objects are antibodies, proteins, amino acids, and peptides. Electrostatic

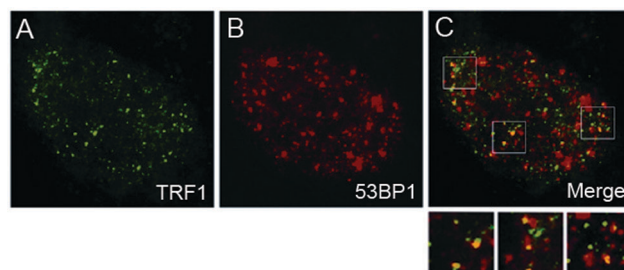


Fig. 25 Transformed (BJ-EHLT) human fibroblasts were treated with **19c**, fixed, and processed for IF by using antibodies against 53BP1. Representative images of IF using a Leica Deconvolution microscope. Enlarged views are below the merged images. Reproduced with permission from ref. 31. Copyright 2011, American Chemical Society.



interaction is very common in tissue imaging because there are many electrolytes in biological bodies.

Although the negatively charged dendritic star polymer **14** did not enter the cells,²⁵ it could specifically label the cell nucleus by binding to positively charged nuclear proteins in fixed tissues.²⁶ Fixed *Drosophila* larval tissues were used to demonstrate the binding interaction of **14** and the nuclear proteins. As described in Fig. 26, **14** staining (red) shows no overlap with CD8-GFP (cell-membrane marker, green, Fig. 26A) or with anti- α -Tubulin antibodies (cytoskeletal marker, green, Fig. 26B). In Fig. 26C and D, **14** staining is in accordance with DAPI (DNA dye, blue, Fig. 26C'') and demonstrates a significant co-localization with histone H4 (green, Fig. 26D''). Compound **14**, bearing multiple $-\text{COOH}$ groups, was demonstrated to bind specifically and directly with positively charged histones, resulting in the labeling of the cell nucleus. Compound **14** offers an attractive alternative to conventional expensive antibodies or chromophores displaying broad emission spectra such as DAPI.

The positively charged dendritic star polymer **33b** was able to enter and label living cells.²⁵ In 2008, Yin *et al.*³⁷ proposed a new application of **33b** to directly stain the extracellular matrix (ECM) (Fig. 27A) network at high resolution and achieve ECM network visualization firstly. The positively charged chromophore **33b** was strongly bound to the negatively charged constituents of ECM in both fixed and living tissue *via* electrostatic forces. The confocal images of ECM staining with **33b** (red, Fig. 27C) and Vkg-GFP (green, Fig. 27B) in living trachea epithelium are presented in Fig. 27. By using high resolution confocal microscopy, **33b** staining almost completely overlapped with Vkg-GFP (Fig. 27D), indicating that **33b** gave the same specificity as Vkg-GFP for the ECM micronetwork structure. The ECM staining with **33b** was much faster than antibody staining

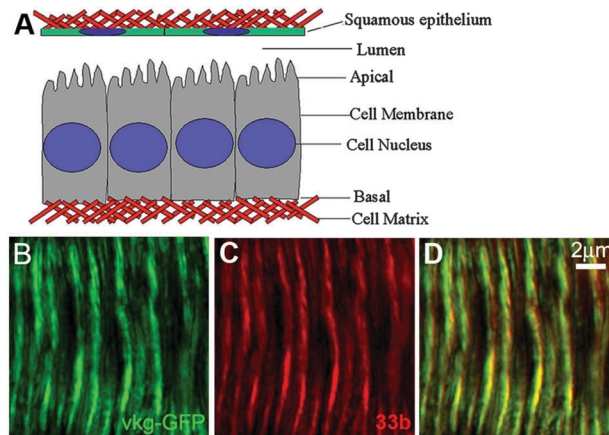


Fig. 27 Schematic illustration of the extracellular matrix in a small central section of the *Drosophila* wing disc epithelium (A). Confocal images of ECM staining with **33b** (red, C), Vkg-GFP (green, B) in living tracheal epithelium and merged channels (D). Reproduced with permission from ref. 37. Copyright 2008, American Chemical Society.

procedures, which may require several days to complete. Spectral analysis reveals a significantly increased emission intensity of **33b** after binding to ECM components, supporting the direct binding between negatively charged ECM components and **33b**. All these observations support that **33b** is an efficient and specific ECM labeling agent.

In addition to the applications in the detection and imaging of biological molecules, some water-soluble PDIs can be used as gene/drug carriers or DNA intercalators together with simultaneous imaging in plants, insects and mice. Thus, these PDIs can be applied in the fields of diagnosis and therapy, which have attracted great attention in recent years. PDI-cored cationic dendrimers **37(a-c)** have been successfully applied to deliver DNA into animal cells *in vitro* by Yin and coworkers.³⁸

Apart from the above application, the authors have applied **37b** as non-viral gene-vectors in plants using *Arabidopsis* as a model plant.⁵² Fluorescence images displayed that **37b** was able to pass through the cell wall and deliver DNA efficiently throughout the root tissues, because **37b** and DNA were detected in both the root cap and hair. The 10-day-old seedlings were treated with **37b**/dsRNA by dropping the complex solution to the seedling root. Two genes, *Shoot Meristemless (STM)* and *WEREWOLF (WER)*, which are essential for shoot tip and root development respectively, were chosen as target genes. **37b**/dsSTM-treated plants exhibit a smaller shoot tip compared with the control, which is treated with H_2O /dsRNA (Fig. 28(A and B)). The root morphology after H_2O /dsWER and **37b**/dsWER-treatment is given in Fig. 28(C and D). **37b**/dsWER-treated plants produced more lateral roots compared with the control. These observations reveal that **37b** can efficiently deliver dsRNA into plant cells and cause gene silencing with systemic phenotypes. Dendrimer **37b** provides a new platform for gene transfection and systemic gene silencing of important developmental genes in plants.

Yin and coworkers⁵³ first reported that the cationic and PDI-cored dendritic star polymer **33a** was able to deliver dsRNA to kill insect pests in oral feeding experiments. To kill insect pests,

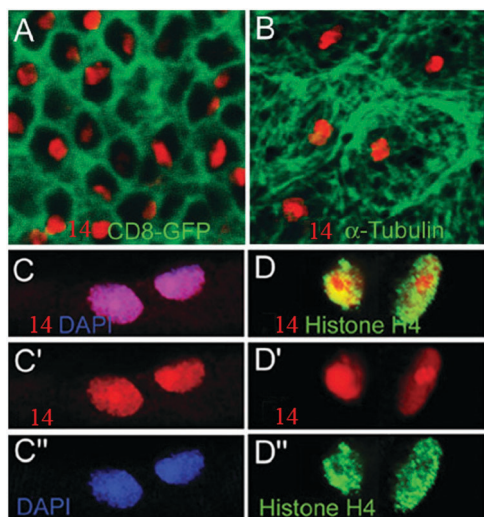


Fig. 26 Confocal images of *Drosophila* larval tissues stained by **14**. Double staining of **14** (red) with (A) CD8-GFP (green), (B) anti- α -Tubulin (green). (C–C'') DAPI (blue), and (D–D'') histone H4 (green). C', C'' and D', D'' are single channels. C and D are merged channels. Reproduced with permission from ref. 26. Copyright 2008, John Wiley and Sons.



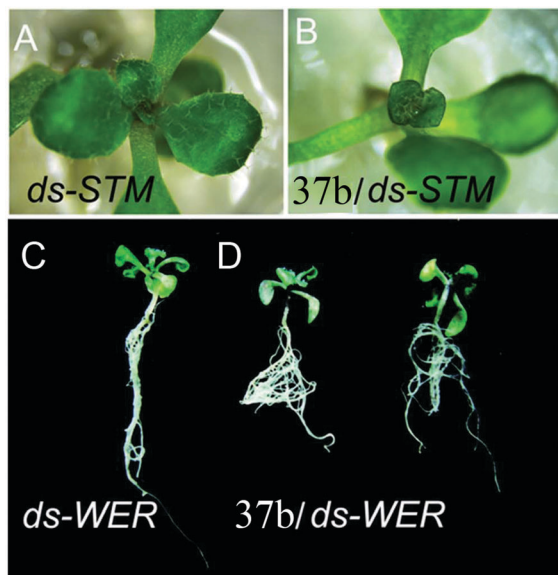


Fig. 28 Shoot morphological images of (A) $\text{H}_2\text{O}/\text{dsSTM}$ -treated (control) and (B) **37b**/ dsSTM -treated plants. Root morphology of (C) $\text{H}_2\text{O}/\text{dsWER}$ -treated (control) and (D) **37b**/ dsWER -treated roots. Reproduced with permission from ref. 52. Copyright 2014, Royal Society of Chemistry.

such as the corn borer, CHT10-dsRNA was synthesized to silence a key developmental gene and GFP-dsRNA was used as control. Fig. 29A presents a schematic diagram of a longitudinal

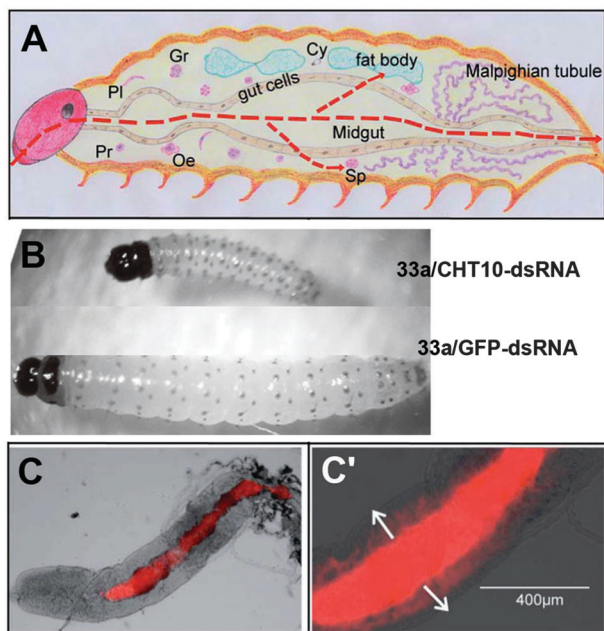


Fig. 29 (A) Schematic diagram for longitudinal section of an insect larva. Reproduced with permission from ref. 54. Copyright 2014, Royal Society of Chemistry. (B) Corn borer larvae fed with **33a**/CHT10-dsRNA and **33a**/GFP-dsRNA. Reproduced with permission from ref. 53. Copyright 2013, John Wiley and Sons. (C) Fluorescence images of dissected larval gut after three days oral feeding of **37b**. (C') part of enlarged (C), red: **37b**. Reproduced with permission from ref. 54. Copyright 2014, Royal Society of Chemistry.

section of the insect with red lines indicating the spreading directions of the gene-vectors. As demonstrated in Fig. 29B, **33a**/CHT10-dsRNA fed larvae are clearly smaller than **33a**/GFP-dsRNA fed ones. Moreover, all the **33a**/CHT10-dsRNA-fed larvae died due to defective growth and molting. The *in vivo* experiments provided firm evidence that dsRNA was delivered into insect cells and efficiently silenced the target gene. Subsequently, the authors reported another gene vectors **37b**, which also exhibited high gene transfection and gene interference performance *in vivo* via oral feeding.⁵⁴ After three days of oral feeding, **37b** (red) is observed in the mid-gut and penetrated into the gut cells (Fig. 29C and C'). This non-viral gene delivery system opens a new avenue for the control of insect pests without using chemical insecticides, which could cause environmental pollution.

Besides the above applications in gene delivery, **37b** was also used as a hydrophobic drug carrier by Yin *et al.* due to the cavities provided by outer dendritic polyester units.⁵⁵ The dendrimer-based nanocarriers could efficiently deliver the drug into the living cells and largely increase the cytotoxicity of the drug. The larvae fed with **37b**/thiamethoxam complexes possess a smaller size than that of the controls fed with **37b**/ H_2O (Fig. 30). Furthermore, after five day feeding with **37b**/thiamethoxam complexes, more than 90% larvae died. The *in vivo* experiments indicate that the drug toxicity was greatly enhanced with the help of **37b**-mediated cellular internalization.

Yin and coworkers⁸ reported a perylene-based DNA intercalator **18b** that could efficiently localize at the cell nucleus and inhibit cancer cell and tumor growth. The small cationic groups are incorporated in the imide-positions of PDIs to generate water solubility and maintain the planar structure of **18b**, which provides the potential for use as a DNA intercalator. Spectral analyses show that **18b** specifically accumulates in the cell nuclei by strong interaction with DNA. Chromophore **18b** shows a stronger suppression effect to cancer cells, which is attributed to the effective DNA intercalation and nuclear accumulation that leads to the inhibition of cancer cells. However, **18b** possesses a lower cytotoxicity to non-cancer cells, because of the much higher DNA replication rate of cancer cells than that of normal cells. Furthermore, with **18b** intercalation,

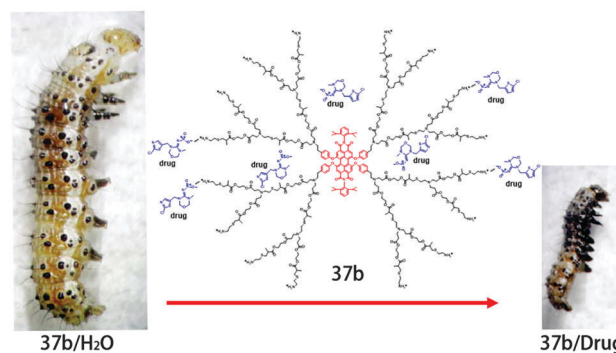


Fig. 30 The structure of **37b**/drug complexes and the digital photographs of larvae fed with diet containing **37b**/ H_2O and **37b**/drug complexes. Reproduced with permission from ref. 55. Copyright 2014, Royal Society of Chemistry.



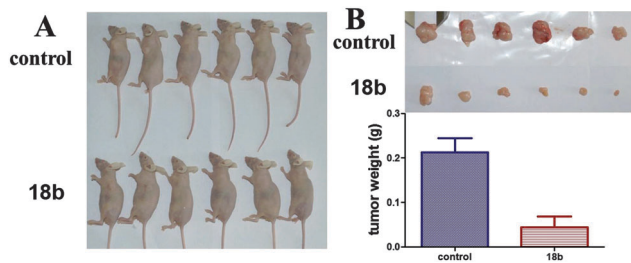


Fig. 31 Antitumor activity of PBDI was evaluated *in vivo*. (A) Mice injected with **18b** (15 mg kg⁻¹ per injection). In the control group, mice were injected with the same volume of stroke-physiological saline solution (SPSS). (B) Tumor size and weight of dissected tumors from (A). Reproduced with permission from ref. 8. Copyright 2014, John Wiley and Sons.

DNA replication of cancer cells is inhibited more strongly than that of normal cells, which leads to the higher toxicity to cancer cells. Mice with HCT116 tumors were used as a model to investigate the antitumor activity of **18b**. The body weights of the mice were not changed after 10 days of injection with **18b**. In addition, the tumor sizes are significantly smaller than those of the control (Fig. 31). The DNA intercalator **18b** with low toxicity is a potential anti-cancer drug for the suppression of cancer cells and tumors. Because of DNA intercalation, these types of PDIs could be developed as potential drugs for suppressing cancer cells and tumors.

4. Conclusions and outlook

This review summarizes the design and synthesis of water-soluble PDIs as well as their biological applications. The synthetic strategies leading to water-soluble PDIs include the introduction of ionic or non-ionic substituents with multiple polar groups in the bay-region, imide- or ortho-positions of PDIs. The biological applications include simple to complex studies: *in vitro* studies, live cell studies, tissue and body imaging. Due to the fluorescent nature of the central PDI fluorophore, which allows for the tracing of cellular uptake *via* fluorescence microscopy, biological applications can be visualized using optical spectroscopy, energy transfer imaging, and other bioimaging techniques. Nevertheless, due to undesirable self-aggregation, the low FQY of PDI fluorophores in aqueous solution and low cytotoxic selectivity toward cancer cells, the synthesis of multi-functional water-soluble PDIs with useful applications in biological fields is still a topic of ongoing research. Despite these challenges, a promising future of water-soluble PDIs can still be predicted. Looking ahead, water-soluble PDIs with multiple functions will find a wide range of applications such as simultaneous imaging with high specificity, diagnosis with high accuracy, and therapy with a few side effects. The combination of fluorescence techniques and nanotechnology in biological applications will remain an active research area in the future, which can be supported by facile synthetic routes to multi-functional and water-soluble PDIs with high biological specificity.

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