Galactose functionalized diketopyrrolopyrrole as NIR fluorescent probes for lectin detection and HepG2 cell targeting based on aggregation-induced emission mechanism

Yandi Hang^{1,2†}, Xiaolei Cai^{2,3†}, Jian Wang¹, Tao Jiang¹, Jianli Hua^{1*} & Bin Liu^{2*}¹Key Laboratory for Advanced Materials and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, School of Chemistry and Molecular Engineering, East China University of Science & Technology, Shanghai 200237, China;²Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore 117585, Singapore;³NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117456, Singapore

Received March 10, 2018; accepted April 16, 2018; published online June 19, 2018

Since the elucidation that sugar-lectin interactions contribute to the understanding of “Glycomics”, how to construct glyco-sensors with rapid response, excellent sensitivity and selectivity is of intense research interest. Herein, we report the design of three NIR emissive glyco-probes based on diketopyrrolopyrrole (DPPs) conjugated with two (DPPG), four (DPPF-G) and six (DPPS-G) galactose groups. All three molecules could probe lectins with excellent sensitivity and selectivity. The increase of glyco-DPP emission in NIR region upon interaction with lectin is due to the aggregates formation induced by sugar-lectin interactions, which have been verified by dynamic light scattering (DLS) and scanning electronic microscope (SEM) analysis. Due to the multiple glyco-ligands on DPPS-G, it has been successfully used to stain HepG2 cells through interactions between galactose and asialoglycoprotein (ASGP-R), which are overexpressed on the surface of HepG2 cells.

NIR emissive, glyco-probes, diketopyrrolopyrrole, sugar-lectin interactions

Citation: Hang Y, Cai X, Wang J, Jiang T, Hua J, Liu B. Galactose functionalized diketopyrrolopyrrole as NIR fluorescent probes for lectin detection and HepG2 cell targeting based on aggregation-induced emission mechanism. *Sci China Chem*, 2018, 61: 898–908, <https://doi.org/10.1007/s11426-018-9259-3>

1 Introduction

Carbohydrate-mediated biological interactions play a crucial role in different cellular events [1,2]. Specific intercellular recognitions between carbohydrate and protein receptors (e.g., lectins) are found on cell surfaces [3]. The elucidation of these interactions may contribute to the understanding of the “Glycomics” and facilitate early-state disease diagnosis, as well as sugar-based drug and vaccine development [4]. Galactins and the asialoglycoprotein receptors (ASGPr) are

galactoside-recognition lectins encoded by the human genome. While these lectins play a key role in maintaining normal cellular functions, furthermore, recent investigations demonstrated that some human fatal diseases (such as cardiopathy, inflammation, and cancer) are associated with them [5,6]. The currently established methods such as western blotting and immune-cytochemistry techniques generally require complex operation [7]. Synthetic glyco-sensors have been developed and demonstrated to be a rapid, simple, sensitive, and selective tool for investigating carbohydrate-based biological events.

Recently, a number of optical probes for lectin detection have been reported [8–10]. Conventional dyes that possess

[†]These authors contributed equally to this work.*Corresponding authors (email: jlhua@ecust.edu.cn; cheliub@nus.edu.sg)

planar and well-conjugated structures usually work in fluorescence-quenching mode when they are dispersed in aqueous media or upon interaction with biological analytes, which results in negative effects on practical applications. Since the intriguing discovery of the aggregation-induced emission (AIE) by Tang *et al.* [11], it has received intense research interest as it is opposite to aggregation-caused quenching mechanism. Luminogens with AIE characteristics can fluoresce strongly in the aggregate state, which opens a new avenue towards efficient solid-state light emitters [12,13], bioprobes [14–18], and chemosensors [19–22]. However, the current AIE systems are mainly focused on silole, tetraphenylethene (TPE) and cyanostilbene derivatives, which show short-wavelength fluorescence in the range of 400–600 nm.

Diketopyrrolopyrrole (DPP) and their derivatives have attracted considerable research interests over the last few decades due to their relatively simple synthesis, intense color and excellent photo-stability. Currently, DPPs have been extensively applied in construction of materials for two-photon absorption [23,24], fluorescent probes [25–27] and imaging [28,29]. DPP derivatives functionalized with electron donor groups usually exhibit red to near-infrared (NIR) emission and large Stokes shifts that are advantageous in biological applications [30–33].

Considering the polymeric feature of lectins that renders the formation of sugar-lectin complexes with high avidities [34], to evaluate the effect of the number of sugar groups on the probe performance, we developed three glyco-DPPs with two (DPPG), four (DPPF-G) and six (DPPS-G) sugar groups, respectively (Figure 1). The increasing number of sugars can improve both water solubility and binding efficiency. Furthermore, galactose is chosen to functionalize DPP to target HepG2 cells with overexpressed galactose receptor asialoglycoprotein (ASGP-R) on their surface, which has been suggested to evolve as a new class of biomarker for liver cancer [35,36].

2 Experimental

2.1 General experimental section

N,N-dimethylformamide (DMF) was refluxed with calcium hydride and distilled before use. Tetrahydrofuran (THF) was pre-dried over 4 Å molecular sieves and distilled under an argon atmosphere from sodium benzophenone ketyl immediately before use. 4,4'-(4-Bromophenyl)azanediyldiphenol (compound 1) [37], 3,6-bis(4-bromophenyl)-2,5-dihexyl-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (compound 4) [38], 2,5-bis(6-bromohexyl)-3,6-bis(4-bromophenyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (compound 5) [39], and galactose alkyne [40] were prepared according to previous literature protocols. All other reagents and pro-

Figure 1 Structures of glyco-DPPs (color online).

teins were purchased from Sigma-Aldrich (USA) and used as received. The solutions for analytical studies were prepared with deionized water treated with a Milli-Q System (Billerica, USA).

^1H and ^{13}C NMR spectra were recorded on a Bruker AM 400 MHz spectrometer with tetramethyl silane (TMS) as the internal reference (Germany). Electrospray ionization and time-of-flight analyzer (ESI-TOF) mass spectra were recorded with a Waters Micromass LCT mass spectrometer (USA). Matrix assisted laser desorption ionization and time-of-flight analyzer (MALDI-TOF) were recorded with an Applied Biosystems 4700 Proteomics Analyzer.

2.1.1 Synthesis of 4-bromo-*N,N*-bis(4-((6-bromohexyl)oxy)phenyl)aniline (2)

A mixture of **1** (1.0 g, 2.81 mmol) and aqueous potassium carbonate (2.33 g, 16.84 mmol) was dissolved in 20 mL acetone under argon atmosphere. Then 1,6-dibromohexane (2.6 mL, 16.84 mmol) was added dropwise to the reaction solution and stirred at 80 °C for 12 h. After cooling to room temperature, the mixture was filtered and washed with acetone. After removal of the filtrate solvent, the crude product was purified by column chromatography on silica gel (eluent: dichloromethane/petroleum ether (1:4, *v/v*) to obtain **2** (1.6 g, 83.5% yield) as colorless liquid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.23 (d, $J=8.5$ Hz, 2H), 7.01 (d, $J=7.3$ Hz, 4H), 6.80 (d, $J=8.8$ Hz, 6H), 3.93 (t, $J=5.8$ Hz, 4H), 3.43 (t, $J=6.8$ Hz, 4H), 1.90 (p, $J=6.9$ Hz, 4H), 1.79 (p, $J=6.5$ Hz, 4H), 1.55–1.45 (m, 8H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 158.15, 155.53, 147.96, 140.45, 133.39, 131.75, 127.69, 126.57, 121.94, 115.34, 114.75, 112.27,

67.97, 33.85, 32.70, 29.17, 27.95, 25.35. LRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{30}H_{37}^{81}Br_2^{79}BrNO_2^+$ 684.0333, found 684.0386.

2.1.2 Synthesis of 4-(bis(4-((6-bromohexyl)oxy)phenyl)amino)phenylboronic acid (**3**)

A solution of **2** (1.36 g, 2.0 mmol) in 20 mL THF was stirred at -78°C for 0.5 h under argon atmosphere. Then $n\text{-BuLi}$ in $n\text{-hexane}$ solution (2.5 M, 1.6 mL) was added dropwise to the solution. After stirred at for another 1 h, triisopropyl borate (0.6 mL, 2.4 mmol) was added to the reaction mixture. The mixture was stirred at -78°C for 2 h, then warmed to room temperature for another 2 h. After completion of the reaction, the mixture was adjusted to weak acidic with hydrochloric acid (1 M), then adjusted to neutral with a saturated solution of potassium carbonate. The solvent was removed off to give crude product (0.8 g, 62.0%) as yellowish liquid. This crude product was used directly for the next step without further purification.

2.1.3 Synthesis of 3,6-bis(4'-(bis(4-((6-bromohexyl)oxy)phenyl)amino)-[1,1'-bihenyl]-4-yl)-2,5-dihexyl-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**6**)

A mixture of **4** (307 mg, 0.5 mmol), **3** (1.29 g, 2.0 mmol), and $\text{Pd}(\text{PPh}_3)_4$ (10.5 mg, 0.02 mmol) was dissolved in 15 mL THF under argon atmosphere. Aqueous potassium carbonate solution (2 M, 5 mL) was added to the reaction solution and stirred at 60°C for 12 h. After cooling to room temperature, the reaction mixture was extracted by dichloromethane (3×15 mL). The combined organic layer was washed with water and dried over Na_2SO_4 . After removal of the solvent, the crude product was purified by column chromatography on silica gel (eluent: dichloromethane/petroleum ether (3:1, v/v) to obtain **4** (338 mg, 41.3% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.89 (d, $J=8.2$ Hz, 4H), 7.70 (d, $J=8.2$ Hz, 4H), 7.47 (d, $J=8.5$ Hz, 4H), 7.09 (d, $J=8.7$ Hz, 8H), 6.99 (d, $J=8.4$ Hz, 4H), 6.84 (d, $J=8.8$ Hz, 8H), 3.95 (t, $J=6.3$ Hz, 8H), 3.86–3.74 (t, $J=8.0$ Hz, 4H), 3.43 (t, $J=6.8$ Hz, 8H), 1.91 (p, $J=6.6$ Hz, 8H), 1.80 (p, $J=6.2$, 5.8 Hz, 8H), 1.57–1.47 (m, 16H), 1.25–1.27 (m, 16H), 0.83 (t, $J=5.3$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 162.91, 155.61, 148.00, 143.39, 140.36, 131.13, 129.20, 127.53, 126.93, 126.49, 126.05, 120.07, 115.29, 109.66, 67.94, 42.15, 33.86, 32.68, 31.24, 29.46, 29.15, 27.93, 26.44, 25.33, 22.49, 13.99. LRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{90}H_{107}^{81}Br_2^{79}Br_2N_4O_6^+$ 1659.4883, found 1659.4557.

2.1.4 Synthesis of 3,6-bis(4'-(bis(4-((6-bromohexyl)oxy)phenyl)amino)-[1,1'-bihenyl]-4-yl)-2,5-bis(6-bromohexyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**7**)

The synthesis was performed following the same procedure of **6** starting with **5** (386 mg, 0.5 mmol) and **3** (1.29 g, 2.0 mmol). The crude product was purified by column

chromatography on silica gel (eluent: dichloromethane/petroleum ether (3:1, v/v) to obtain **4** (322 mg, 35.7% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.88 (d, $J=8.4$ Hz, 4H), 7.71 (d, $J=8.5$ Hz, 4H), 7.47 (d, $J=8.8$ Hz, 4H), 7.10 (m, 8H), 6.99 (d, $J=8.7$ Hz, 4H), 6.85 (m, 8H), 3.95 (t, $J=6.3$ Hz, 8H), 3.83 (m, 4H), 3.44 (t, $J=6.8$ Hz, 8H), 3.34 (t, $J=6.8$ Hz, 4H), 1.91 (m, 8H), 1.80 (m, 12H), 1.66 (dd, $J=14.4$, 7.2 Hz, 5H), 1.52 (m, 15H), 1.39 (m, 8H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 162.89, 155.67, 149.00, 147.99, 140.38, 132.31, 131.03, 130.07, 129.20, 128.91, 127.57, 126.98, 126.56, 125.96, 120.07, 115.34, 109.69, 67.98, 41.88, 33.88, 33.75, 32.71, 32.54, 29.18, 27.96, 27.64, 25.93, 25.36. LRMS (ESI, m/z): $[M+H]^+$ calcd. for $C_{90}H_{104}^{81}Br_3^{79}Br_3N_4O_6^+$ 1817.3073, found 1817.2965.

2.1.5 Synthesis of 3,6-bis(4'-(bis(4-((6-azidohexyl)oxy)phenyl)amino)-[1,1'-biphenyl]-4-yl)-2,5-dihexyl-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**8**)

A mixture of **6** (238 mg, 0.14 mmol) and sodium azide (111 mg, 1.7 mmol) was dissolved in 10 mL dry DMF. The mixture was stirred at 80°C under argon atmosphere for 20 h. After cooling to room temperature, the reaction mixture was poured into water and filtered. The resulting filter cake was washed with water and dried in vacuum. The crude product was purified by column chromatography on silica gel (eluent: dichloromethane/petroleum ether (3:1, v/v) to obtain **8** (177 mg, 81.8% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.89 (d, $J=8.0$ Hz, 4H), 7.70 (d, $J=8.1$ Hz, 4H), 7.47 (d, $J=8.6$ Hz, 4H), 7.10 (d, $J=8.7$ Hz, 8H), 6.99 (d, $J=8.3$ Hz, 4H), 6.84 (d, $J=8.8$ Hz, 4H), 3.95 (t, $J=6.2$ Hz, 8H), 3.88–3.72 (m, 4H), 3.29 (t, $J=6.8$ Hz, 8H), 1.80 (p, $J=6.2$ Hz, 8H), 1.65 (p, $J=6.9$ Hz, 12H), 1.55–1.41 (m, 16H), 1.36–1.25 (m, 12H), 0.82 (d, $J=6.8$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 162.91, 155.61, 148.00, 143.39, 140.36, 131.13, 129.20, 127.53, 126.93, 126.49, 126.05, 120.07, 115.29, 109.66, 67.94, 42.15, 33.86, 32.68, 31.24, 29.46, 29.15, 27.93, 26.44, 25.33, 22.49, 13.99. Human resource management system (HRMS) (ESI, m/z): $[M+H]^+$ calcd. for $C_{90}H_{107}N_{16}O_6^+$ 1507.8560, found 1507.8525.

2.1.6 Synthesis of 2,5-bis(6-azidohexyl)-3,6-bis(4'-(bis(4-((6-azidohexyl)oxy)phenyl)amino)-[1,1'-biphenyl]-4-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**9**)

The synthesis was performed following the same procedure of **8** starting with **7** (280 mg, 0.15 mmol) and sodium azide (120 mg, 1.8 mmol). The crude product was purified by column chromatography on silica gel (eluent: dichloromethane/petroleum ether (3:1, v/v) to obtain **9** (227 mg, 92.6% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.88 (d, $J=8.5$ Hz, 4H), 7.71 (d, $J=8.5$ Hz, 4H), 7.47 (d, $J=8.8$ Hz, 4H), 7.10 (dd, $J=7.2$, 2.4 Hz, 8H), 6.99 (d, $J=8.7$ Hz, 4H), 6.85 (dd, $J=6.8$, 1.6 Hz, 8H), 3.95 (t, $J=6.3$ Hz, 8H), 3.82 (t, $J=6.3$ Hz, 4H), 3.29 (t, $J=6.9$ Hz,

8H), 3.20 (t, $J=6.9$ Hz, 4H), 2.00–2.03 (m, 6H), 1.77–1.83 (m, 8H), 1.61–1.68 (m, 16H), 1.46–1.54 (m, 18H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 162.89, 155.67, 149.01, 147.99, 143.52, 140.38, 132.29, 131.02, 130.07, 129.20, 127.56, 126.98, 126.55, 125.97, 120.07, 115.34, 109.69, 67.97, 51.41, 51.31, 29.34, 29.23, 28.83, 28.70, 26.54, 26.32, 26.23, 25.75. HRMS (ESI, m/z): $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{90}\text{H}_{105}\text{N}_{22}\text{O}_6^+$ 1589.8587, found 1589.8573.

General procedure for the click reaction. To a vigorously stirred solution of **8** (177 mg, 0.06 mmol) and sugar alkyne (177 mg, 0.48 mmol) in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}/\text{tert-butanol}$ (21 mL, 10:10:1, v/v/v) was added $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (120 mg, 0.48 mmol) and sodium ascorbate (180 mg, 0.96 mmol). The reaction mixture was stirred at 50 °C for 12 h, and then an aqueous solution of EDTA (5 mL) was added dropwise after the reaction was cooled to room temperature. Then the mixture was extracted by dichloromethane (3×15 mL). The combined organic layer was washed with saturated brine and dried over Na_2SO_4 . After removal of the solvent, the crude product was purified by column chromatography on silica gel.

2.1.7 Synthesis of compound **10**

From **8** (186 mg, 0.12 mmol) and galactose alkyne (177 mg, 0.48 mmol), column chromatography (eluent: dichloromethane/methanol (20:1, v/v) gave **10** (204 mg, 64.9% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.89 (dd, $J=8.0$, 4.0 Hz, 4H), 7.70 (dd, $J=8.0$, 0.8 Hz, 4H), 7.52 (s, 4H), 7.47 (dd, $J=7.2$, 1.8 Hz, 4H), 7.09 (d, $J=8.7$ Hz, 8H), 7.02–6.94 (m, 4H), 6.89–6.73 (m, 8H), 5.40 (d, $J=3.2$ Hz, 4H), 5.23 (t, $J=8.7$ Hz, 4H), 5.06–4.97 (m, 4H), 4.88–4.76 (m, 4H), 4.67 (t, $J=7.7$ Hz, 4H), 4.44–4.31 (m, 8H), 4.24–4.05 (m, 12H), 4.00–3.89 (m, 12H), 3.86–3.75 (m, 4H), 2.25–2.18 (m, 4H), 2.15 (s, 12H), 2.05 (d, $J=5.5$ Hz, 20H), 2.00 (s, 12H), 1.98 (s, 12H), 1.96–1.90 (m, 4H), 1.83–1.74 (m, 16H), 1.48–1.38 (m, 16H), 0.87 (t, $J=4.0$ Hz, 6H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 170.54, 170.45, 170.34, 170.25, 170.12, 170.04, 169.55, 162.93, 155.59, 148.91, 148.01, 144.23, 140.45, 131.24, 129.22, 127.61, 127.57, 126.92, 126.52, 122.48, 120.14, 115.31, 109.71, 100.49, 95.60, 70.84, 68.81, 67.99, 67.86, 67.52, 67.06, 63.10, 61.59, 61.43, 61.29, 50.31, 31.25, 30.29, 29.48, 29.14, 26.44, 26.31, 25.63, 22.49, 20.80, 20.72, 20.68, 20.59, 13.98. HRMS (MALDI, m/z): $[\text{M}]$ calcd. for $\text{C}_{158}\text{H}_{194}\text{N}_{16}\text{O}_{46}$: 3501.3333, found: 3051.3374.

2.1.8 Synthesis of compound **11**

From **9** (113 mg, 0.03 mmol) and galactose alkyne (170 mg, 0.42 mmol), column chromatography (eluent: dichloromethane/methanol (20:1, v/v) gave **11** (143 mg, 51.6% yield) as a red solid. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.86 (d, $J=8.1$ Hz, 4H), 7.71 (d, $J=8.2$ Hz, 4H), 7.55 (s, 4H), 7.52 (s, 2H), 7.48 (d, $J=2.5$ Hz, 4H), 7.09 (d, $J=8.8$ Hz, 8H),

6.98 (dd, $J=8.8$, 1.5 Hz, 4H), 6.84 (d, $J=8.9$ Hz, 8H), 5.42–5.32 (m, 24H), 5.26–5.19 (m, 10H), 5.18–5.09 (m, 6H), 4.83 (t, $J=12.9$ Hz, 6H), 4.71–4.63 (m, 6H), 4.39 (t, $J=7.4$ Hz, 10H), 3.94 (t, $J=5.4$ Hz, 12H), 3.81 (t, $J=4.0$ Hz, 4H), 2.07–2.03 (m, 40H), 2.01 (dd, $J=5.8$, 4.0 Hz, 25H), 1.97 (dd, $J=7.0$, 2.2 Hz, 35H), 1.84–1.74 (m, 10H), 1.47–1.40 (m, 10H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 170.55, 170.35, 170.25, 170.06, 170.01, 155.65, 140.38, 129.18, 129.12, 127.54, 126.98, 126.82, 126.56, 125.90, 122.54, 115.31, 95.60, 70.84, 67.99, 67.93, 67.87, 67.51, 67.06, 67.01, 66.46, 61.59, 61.43, 61.27, 54.45, 53.45, 50.34, 50.24, 30.30, 30.10, 29.15, 26.34, 26.19, 26.02, 25.63, 20.80, 20.77, 20.67, 20.60. HRMS (MALDI, m/z): $[\text{M}]$ calcd. for $\text{C}_{192}\text{H}_{236}\text{N}_{22}\text{O}_{66}$: 3905.5787, found: 3905.5884.

General procedure for the deacylation. To a solution of the acetyl intermediates in a mixed solvent of methanol (1.6 mL) and H_2O (0.18 mL) was added triethylamine (0.18 mL, 0.65 mmol), and the reaction mixture was stirred at rt. for 24 h. After removal of the solvent, the crude product was purified by column chromatography on silica gel.

2.1.9 Synthesis of compound **DPPF-G**

From **10** (204 mg, 0.05 mmol), column chromatography (eluent: dichloromethane/methanol (4:1, v/v) gave **DPPF-G** (44 mg, 26.7% yield) as a dark-red solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.12 (s, 4H), 7.90 (d, $J=8.3$ Hz, 4H), 7.82 (d, $J=8.5$ Hz, 4H), 7.64 (d, $J=8.6$ Hz, 4H), 7.08 (d, $J=8.9$ Hz, 8H), 6.93 (d, $J=9.0$ Hz, 8H), 6.83 (d, $J=9.1$ Hz, 4H), 4.86–4.71 (m, 4H), 4.61 (d, $J=12.1$ Hz, 4H), 4.35 (t, $J=7.0$ Hz, 10H), 4.20 (d, $J=7.2$ Hz, 4H), 3.94 (t, $J=6.1$ Hz, 10H), 3.77 (dd, $J=9.5$, 3.9 Hz, 4H), 3.70 (d, $J=2.8$ Hz, 3H), 3.66–3.59 (m, 9H), 3.57–3.49 (m, 8H), 2.00 (q, $J=7.0$ Hz, 4H), 1.84 (p, $J=7.0$, 6.6 Hz, 8H), 1.78–1.63 (m, 8H), 1.45 (p, $J=8.5$, 7.5 Hz, 16H), 1.30 (dd, $J=16.1$, 8.0 Hz, 12H), 0.75 (t, $J=6.4$ Hz, 6H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$), δ (ppm): 161.63, 155.73, 155.54, 143.79, 139.44, 130.14, 129.20, 127.47, 127.17, 125.79, 124.01, 118.67, 115.52, 108.73, 108.63, 102.70, 99.73, 75.30, 73.89, 73.40, 71.40, 70.46, 68.13, 67.50, 61.50, 61.43, 60.47, 49.21, 48.44, 30.47, 29.66, 28.53, 28.46, 25.62, 24.97, 21.80, 13.75. HRMS (MALDI, m/z): $[\text{M}]$ calcd. for $\text{C}_{126}\text{H}_{162}\text{N}_{16}\text{O}_{30}$: 2379.1643, found: 2379.1619.

2.1.10 Synthesis of compound **DPPS-G**

From **11** (120 mg, 0.03 mmol), column chromatography (eluent: dichloromethane/methanol (1:1, v/v) gave **DPPS-G** (60 mg, 69.7% yield) as a dark-red solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm): 8.13 (s, 4H), 8.05 (s, 2H), 7.91 (d, $J=7.8$ Hz, 4H), 7.83 (d, $J=7.8$ Hz, 4H), 7.65 (d, $J=8.9$ Hz, 4H), 7.09 (d, $J=8.7$ Hz, 8H), 6.94 (d, $J=8.9$ Hz, 8H), 6.83 (d, $J=8.2$ Hz, 4H), 4.82–4.71 (m, 8H), 4.67–4.46 (m, 25H), 4.42–4.32 (m, 15H), 4.22 (dt, $J=21.2$, 5.8 Hz, 6H), 3.94 (t, $J=6.2$ Hz, 8H), 3.70 (q, $J=5.2$, 4.3 Hz, 5H), 3.67–

3.56 (m, 11H), 1.91–1.79 (m, 9H), 1.75–1.62 (m, 13H), 1.45 (dt, $J=14.3$, 8.0 Hz, 13H), 1.37–1.26 (m, 13H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$), δ (ppm): 155.54, 143.84, 139.44, 129.62, 129.50, 129.23, 127.50, 127.20, 126.19, 125.83, 125.50, 124.01, 123.75, 116.92, 115.52, 108.61, 102.69, 98.32, 75.30, 73.39, 71.39, 70.45, 70.43, 69.51, 68.85, 68.26, 68.15, 67.50, 60.63, 60.50, 60.01, 49.23, 49.10, 29.64, 29.45, 28.54, 25.63, 25.45, 25.28, 24.97. HRMS (MALDI, m/z): [M] calcd. for $\text{C}_{144}\text{H}_{188}\text{N}_{22}\text{O}_{42}$: 2897.3251, found: 2897.3906.

2.2 UV-Vis and fluorescent (FL) spectra

The absorption spectra were measured with a Varian Cary 500 UV-Vis spectrophotometer (USA). Fluorescent spectra were measured with a Horiba Fluoromax-4 Fluorescence spectrometer (Japan). Stock solutions of glycol-DPPs were prepared in DMSO (1 mM). Aliquots of the stock solution were transferred to 1.0 mL volumetric flasks. After addition of appropriate amounts of DMSO, water was added dropwise under vigorous stirring to furnish 1.0×10^{-5} M solutions with different water fractions (0–99 vol%). The UV and FL measurements of the resulting solutions were then performed immediately.

2.3 Fluorescence titration measurements

Stock solutions of glyco-DPPs were prepared in DMSO (0.1 mM). Selective lectin PNA (for **DPPG**, **DPPF-G** and **DPPS-G**) was dissolved in 0.1 mM, pH 7.2 Tris-HCl buffer (50 mM Tris). Fluorescence titrations were carried out by sequential addition of 0–200 μL aliquots of lectins solution to a 10 μL stock solution of glycol-DPPs followed by the addition of Tris-HCl buffer to acquire a solution of 1.0 mL. The final concentration of glyco-DPPs was 1.0×10^{-6} M. The FL measurements of the resulting solutions were then performed immediately.

2.4 Calculation of LOD

By plotting the ratio of fluorescence intensity as a function of lectin concentration, the limit of detection (LOD) was calculated by the equation $3\delta/\text{slope}$ (δ is a standard deviation of the measured value of the emission maxima of blank solution for seven times).

2.5 Selectivity measurements

Equal concentrations of other proteins (10 μM) including LcA, ConA, PNA, trypsin (Try), cytochrome C (CyC), bovine serum albumin (BSA), and RNase A (RNA) and physiological ions (100 μM) including K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Cl^- , Br^- , I^- , CO_3^{2-} , AcO^- were added to glyco-DPP solutions (1 μM). The FL measurements of the resulting

solutions were performed immediately.

2.6 Dynamic light scattering analysis and scanning electron microscope

Dynamic light scattering analysis (DLS) was measured by a Zetasizer Nano ZS90 (UK). Glyco-DPPs were prepared as 1.0×10^{-6} M, and then different aliquots of lectins solutions were added. The DLS measurements of the resulting solutions were then performed immediately. The SEM images were recorded with a JEOL JSM-6360 scanning electron microscope (Japan). The samples used were prepared by dispersion on quartz plate, and then dried at room temperature prior to measurement.

2.7 Cell culture

HepG2 and HeLa cells were cultured in T-25 flasks with Dulbecco's Modified Eagle's Medium (DMEM, Gibco), 10% heat-inactivated fetal bovine serum (FBS, Gibco), 100 U mL^{-1} penicillin (Gibco), and 100 $\mu\text{g mL}^{-1}$ streptomycin (Gibco). Cells were incubated at 37 $^{\circ}\text{C}$ in an ambient air and 5% CO_2 atmosphere until 80% confluence was reached prior to use.

2.8 Cytotoxicity studies

Methylthiazolyldiphenyltetrazolium bromide (MTT) assays were used to assess the viability of HepG2 cells after incubation with the probe. The cells in 96-well plates (Costar, IL, USA) were first incubated with the probe for designated time in the dark. The cells were further incubated in fresh medium for 24 h and washed with phosphate buffer saline (PBS). Then MTT in PBS solution (100 μL , 0.5 mg mL^{-1}) was added into each well. After incubation for 3 h, the supernatant was discarded and the precipitate was dissolved in DMSO (100 μL) with gentle shaking. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan).

3 Results and discussion

3.1 Synthesis of DPPG, DPPF-G and DPPS-G

The synthesis of **DPPG** follows the previous report [34]. The target compounds were synthesized through coupling between alkyl substituted-triphenylamine and alkyl-DPP cores, which was followed by the reaction with alkynyl galactoside via the Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition (Scheme 1). Firstly, 1,6-dibromohexane was introduced to 4,4'-((4-bromophenyl)azanediyl)diphenol (**1**) by an electrophilic substitution reaction to form compound **2**, which was treated with triisopropyl borate to yield **3**. In the second step, two different alkyl-DPPs were synthesized according to

Scheme 1 Synthesis of DPPF-G and DPPS-G.

literature protocols. Palladium-mediated Suzuki cross-coupling between **3** and alkyl-DPPs gave bromides **6** and **7**, which were reacted with an azide in DMF to produce the azido DPP intermediates **8** and **9** (Scheme 1). Copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction between **8** or **9** and galactose alkyne led to the formation of glycosyl **10** and **11** in good yields. Finally, deacetylation of **10** and **11** yielded the desired divalent galactosyl DPPF-G and DPPS-G (Scheme 1). All the new compounds were well characterized by ^1H NMR, ^{13}C NMR, and HRMS (see Supporting Information online).

3.2 Optical property measurements

The UV-Visible absorption and fluorescent spectra of DPPS-G and DPPF-G were measured in DMSO/H₂O mixtures. The absorption maxima (λ_{abs}) of DPPS-G and DPPF-G in pure DMSO solution are located at 519 and 525 nm, respectively (Figure 1(a, d)). With the increased water fraction (f_w , the volume percentage of water in DMSO/water mixtures), their UV absorption spectra become broader, which indicate the slight aggregates formation of DPPS-G and DPPF-G in DMSO/water. However, the change in the absorption spectrum of DPPS-G is less significant than that of DPPF-G, due to the better water solubility of the former. DPPS-G is nearly non-emissive in pure DMSO and low water fraction mixtures ($f_w \leq 30\%$), but exhibits distinct fluorescence in NIR region (located around 721 nm) when f_w is $\geq 40\%$ and continues to increase until f_w is 99% (Figure 2(a–c)). The emission properties of DPPF-G are

slightly different from those of DPPS-G (Figure 2(d–f)). The fluorescence enhancement of DPPF-G is observed when f_w is 30% and saturates at $f_w = 60\%$ (Figure 2(f)). Meanwhile, its maximum emission wavelength is located at 679 nm, which is relatively shorter than DPPS-G. Therefore, both DPPS-G and DPPF-G show typical AIE characteristics. Additionally, the UV and PL spectra of DPPG were also plotted to compare with those for DPPS-G and DPPF-G. As shown in Figure 2(g–i), in DMSO, DPPG is nearly non-emissive and its λ_{abs} is centered at 521 nm. With low water fractions ($f_w < 30\%$), the FL and absorption maxima remain unchanged. In contrast, when f_w is higher than 30%, evident FL enhancement in the NIR region is observed, which saturates at $f_w = 50\%$. However, the FL intensity of the DPPG decreases with further increase of f_w (50%–90%). Upon addition of water, at the low f_w , the DPP molecules tend to aggregate which limits the intramolecular rotations to enhance its fluorescence by AIE. However, while in a mixture with higher water ratio, the hydrophobic DPPs quickly aggregate in a random way to form less emissive and smaller particles, which lead to the decrease of FL [41]. However, this phenomenon was not observed for DPPS-G since only very small aggregates could form even in the 99% Tris-HCl buffer due to the presence of six galactoses in each molecule, which was also confirmed by DLS experiments (Table S1, Figure S1, Supporting Information online).

3.3 Detection of lectin

As the galactose is specifically recognized by peanut ag-

Figure 2 Normalized UV-absorption spectra of (a) **DPPS-G**, (d) **DPPF-G** and (g) **DPPG**. Fluorescence (FL) emission spectra of (b) **DPPS-G**, (e) **DPPF-G** and (h) **DPPG** in DMSO with increasing water fractions (0–99 vol%). Plotting the ratio of I/I_0 (I_0 refers to value of the emission maxima in pure DMSO) change of (c) **DPPS-G**, (f) **DPPF-G** and (i) **DPPG** in DMSO as a function of increasing water fractions. Emission maxima: 721, 679 and 681 nm, respectively. Figure (g)–(i) reprinted with permission from Ref. [34], Copyright 2015, Elsevier (color online).

glutinin (PNA), the plant lectin was used to perform bio-sensing. As shown in Figure 3(a), when addition of PNA to **DPPS-G** solutions, **DPPS-G** was induced to aggregate due to the interaction of galactoside with the sugar-recognized region of PNA, thus led to a sharp increase of the FL intensity. Although **DPPF-G** has a relatively strong emission in Tris-HCl buffer (containing 50 mM Tris, 1% DMSO, pH 7.2), the fluorescence intensity could still enhance gradually when PNA is added (shown in Figure 3(d)). Similarly, addition of PNA into **DPPG** also leads to sharp and concentration-dependent increase of the FL in the NIR region. However, the fluorescence enhancement has level off when 8–10 eq. PNA was added, and the emission maximum of **DPPS-G** is blue-shift by about 52 nm (Figure 3(a)), while only slight blue-shift is observed for both **DPPF-G** and

DPPG (Figure 3(d, g)). As DPP with more sugar groups have higher affinities with lectins, the difference in the blue-shift is due to much more distinct aggregates of DPP molecules in the presence of lectins as indicated by SEM images (Figure 4(a–f)). The aggregates formation provides better protection of DPP molecules from polar environment to limit the intramolecular charge transfer (ICT) effect arising from the D-A-D (D represents donor and A represents acceptor) structure and eventually leads to more obvious blue shift in the FL spectra for **DPPS-G**. In addition, the plots of FL of glyco-DPPs at their own emission maximum were found to be linear against the concentration of lectins at low concentration range (0.0–6.0 μM). The limits of detection (LOD) of **DPPS-G**, **DPPF-G** and **DPPG** for PNA were determined to be 18, 12 and 11 nM, respectively

Figure 3 Fluorescence (FL) titration spectra of (a) **DPPS-G** (1 μM), (d) **DPPF-G** (1 μM) and (g) **DPPG** (1 μM) in the presence of increasing peanut agglutinin (PNA). Plotting the FL as a function of lectin concentration for determination of the limit of detection (LOD) of (b) **DPPS-G**, (e) **DPPF-G** and (h) **DPPG** for PNA. FL spectra of (c) **DPPS-G** (1 μM), (f) **DPPF-G** (1 μM) and (i) **DPPG** (1 μM) in the absence and presence of PNA (10 μM) or pretreated PNA with *D*-galactose (100 μM). FLs were measured in 1% DMSO/99% Tris-HCl buffer. $\lambda_{\text{ex}}=520$ nm for **DPPF-G**, $\lambda_{\text{ex}}=525$ nm for **DPPG** and **DPPS-G**. Figure (g)–(i) reprinted with permission from Ref. [34], Copyright 2015, Elsevier (color online).

Figure 4 Scanning electronic microscope imaging of (a) **DPPS-G**, (c) **DPPF-G**, (e) **DPPG**, (b) **DPPS-G**+PNA, (d) **DPPF-G**+PNA and (f) **DPPG**+PNA. Figure (e,f) reprinted with permission from Ref. [34], Copyright 2015, Elsevier.

(Figure 3(b, e, h)).

3.4 Dynamic light scattering and SEM images

To understand the sensing mechanism of DPP-based probes, the morphology variation of the three probes' aggregates was visualized by SEM images. From the SEM images (Figure 4), the sizes of the aggregates of DPPs are much larger in the presence of lectins. The results further indicate that the enhancement of fluorescence of DPPs is attributed to the formation of aggregates based on selective sugar-lectin interactions (Scheme 2). The **DPPS-G** and **DPPF-G** functionalized with more galactose have shown better binding efficiency, leading to larger aggregates in the presence of PNA. Furthermore, DLS analysis was used to investigate the aggregation process of **DPPS-G** and **DPPF-G** in the pre-

Scheme 2 The illustrate of the AIE mechanism (color online).

sence of PNA. The data were summarized in Table S2. As shown in Figure S3(a), the hydrodynamic diameters of **DPPS-G** increase gradually from 8.5 to 462.1 nm after PNA addition. Under the same conditions, the size of **DPPF-G** also increases from 23.4 to 503 nm (Figure S3(b)). It is worth noting that **DPPS-G** with more galactose dissolves better in water, resulting in smaller particles than **DPPF-G**.

3.5 Targeting the HepG2 cells and fluorescence imaging

At the molecular level, all three probes can detect PNA with good sensitivity and selectivity by sugar-glycoprotein interaction. Subsequently, their ability in probing specific recognitions between sugar ligands and glycoprotein receptors on cancer cells was evaluated. Cell experiments with **DPPG**, **DPPF-G** and **DPPS-G** were carried out to evaluate the up-

take ability of the three probes using HepG2 cells as an example, which express a substantial amount of galactose receptor (ASGP-Rs) on the surface. After incubation with **DPPG**, **DPPF-G** or **DPPS-G** solutions (10 μ M in PBS buffer, pH 7.4) for 30 min at 37 $^{\circ}$ C in the culture medium, HepG2 cells were subsequently imaged by CLSM with 530 nm laser excitation and the fluorescent signals were collected from 600 to 800 nm. The HeLa cells were used as a negative control at the same time. As shown in Figure 5(a2, a3), red fluorescence is observed in HepG2 cells incubated with **DPPF-G** and **DPPS-G**. The fluorescence of **DPPS-G** is distributed evenly in cytoplasm region and is stronger than **DPPF-G**. However, only very weak red fluorescence could be observed from HepG2 cells incubated with **DPPG** under the same condition (Figure 5(a1)). Additionally, almost no red fluorescence could be observed in HeLa cells (Figure 5(d1–d3)). These results suggest that galactose functionalized DPP dyes have the ability to target HepG2 cells and glycol-DPPs with more galactose groups have more binding sites so that they are more efficient in interacting with the target cells.

As low toxicity is essential for probes applied in biosystems, the cytotoxicity of these three probes was also evaluated by MTT assay. As shown in Figure 6, cell viability was over 90% after 24 h incubation of the cells with probes at a concentration of 20 μ M, which reveals that the glyco-DPP dyes have very low toxicity to cells.

4 Conclusions

In summary, three glyco-DPPs fabricated with two (**DPPG**), four (**DPPF-G**) and six (**DPPS-G**) galactose groups have been developed. All three molecules could probe lectins with

Figure 5 Confocal laser scan imaging of HepG2 cells incubated with **DPPG** (a1–c1), **DPPF-G** (a2–c2) and **DPPS-G** (a3–c3) in PBS, pH 7.4, 37 $^{\circ}$ C. Confocal laser scan imaging of HeLa cells incubated with **DPPG** (d1–f1), **DPPF-G** (d2–f2) and **DPPS-G** (d3–f3) in PBS, pH 7.4, 37 $^{\circ}$ C. Dark field: a1–a3 and d1–d3. Bright field: b1–b3 and e1–e3. Merged: c1–c3 and f1–f3. λ_{ex} =530 nm, λ_{em} collected: 600–800 nm (color online).

Figure 6 Cytotoxicity of (a) DPPG-incubated cells, (b) DPPF-G-incubated cells and (c) DPPS-G-incubated cells for 24 h.

excellent sensitivity and selectivity by using AIE mechanism. The increases of glyco-DPPs emission in NIR region with the addition of lectins were attributed to formation of dye aggregates induced by sugar-glycoprotein interactions, which were determined by DLS and SEM analyses. More importantly, compared with DPPG and DPPF-G, DPPS-G with more sugar groups exhibits higher water solubility, better biocompatibility, longer emission and more significant fluorescence changes in the presence of lectins, suggesting that it is more suitable for intracellular imaging. In fact, DPPS-G has the ability to target HepG2 cells with very low toxicity. These AIE probes are easy-to-construct, biospecific and economic, which provide a reliable and widely applicable strategy for sensing both intercellular sugar-receptor interactions and targeted cancer cells. It is expected that this assay could form a generic platform for advanced molecular sensing with a high promise in practical applications.

Acknowledgements This work was supported by the National Natural Science Foundation of China (21772040, 21421004, 21372082, 21572062), the Fundamental Research Funds for the Central Universities (222201717003), the Programme of Introducing Talents of Discipline to Universities (B16017), a Visiting Program at NUS of China Scholarship Council (CSC), the Singapore Ministry of Education (R279-000-391-112), Singapore NRF Investigatorship (R279-000-444-281) and the National University of Singapore (R279-000-482-133).

Conflict of interest The authors declare that they have no conflict of interest.

Supporting information The supporting information is available online at <http://chem.scichina.com> and <http://link.springer.com/journal/11426>. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

- Zeng X, Andrade CAS, Oliveira MDL, Sun XL. *Anal Bioanal Chem*, 2012, 402: 3161–3176
- Wang KR, An HW, Rong RX, Cao ZR, Li XL. *Biosens Bioelectron*, 2014, 58: 27–32
- Xue C, Jog SP, Murthy P, Liu H. *Biomacromolecules*, 2006, 7: 2470–2474
- Li Z, Deng SS, Zang Y, Gu Z, He XP, Chen GR, Chen K, James TD, Li J, Long YT. *Sci Rep*, 2013, 3: 2293–2299
- Jain K, Kesharwani P, Gupta U, Jain NK. *Biomaterials*, 2012, 33:

- 4166–4186
- Liu FT, Rabinovich GA. *Nat Rev Cancer*, 2005, 5: 29–41
- Zhang HL, Wei XL, Zang Y, Cao JY, Liu S, He XP, Chen Q, Long YT, Li J, Chen GR, Chen K. *Adv Mater*, 2013, 25: 4097–4101
- Sansone F, Casnati A. *Chem Soc Rev*, 2013, 42: 4623
- Gorityala BK, Lu Z, Leow ML, Ma J, Liu XW. *J Am Chem Soc*, 2012, 134: 15229–15232
- Martin Rodriguez E, Bogdan N, Capobianco JA, Orlandi S, Cavazzini M, Scalera C, Quici S. *Dalton Trans*, 2013, 42: 9453
- Mei J, Leung NLC, Kwok RTK, Lam JWY, Tang BZ. *Chem Rev*, 2015, 115: 11718–11940
- Zhu Q, Zhang Y, Nie H, Zhao Z, Liu S, Wong KS, Tang BZ. *Chem Sci*, 2015, 6: 4690–4697
- Zhang CJ, Feng G, Xu S, Zhu Z, Lu X, Wu J, Liu B. *Angew Chem Int Ed*, 2016, 55: 6192–6196
- Chang ZF, Jing LM, Chen B, Zhang M, Cai X, Liu JJ, Ye YC, Lou X, Zhao Z, Liu B, Wang JL, Tang BZ. *Chem Sci*, 2016, 7: 4527–4536
- Zhao E, Chen Y, Chen S, Deng H, Gui C, Leung CWT, Hong Y, Lam JWY, Tang BZ. *Adv Mater*, 2015, 27: 4931–4937
- Yuan Y, Xu S, Cheng X, Cai X, Liu B. *Angew Chem Int Ed*, 2016, 55: 6457–6461
- Yuan Y, Zhang CJ, Xu S, Liu B. *Chem Sci*, 2016, 7: 1862–1866
- Yuan Y, Zhang CJ, Liu B. *Angew Chem Int Ed*, 2015, 54: 11419–11423
- Feng G, Yuan Y, Fang H, Zhang R, Xing B, Zhang G, Zhang D, Liu B. *Chem Commun*, 2015, 51: 12490–12493
- Zhao N, Lam JWY, Sung HHY, Su HM, Williams ID, Wong KS, Tang BZ. *Chem Eur J*, 2014, 20: 133–138
- Sanji T, Shiraiishi K, Nakamura M, Tanaka M. *Chem Asian J*, 2010, 5: 817–824
- Wang JX, Chen Q, Bian N, Yang F, Sun J, Qi AD, Yan CG, Han BH. *Org Biomol Chem*, 2011, 9: 2219–2226
- Purc A, Sobczyk K, Sakagami Y, Ando A, Kamada K, Gryko DT. *J Mater Chem C*, 2015, 3: 742–749
- Gao Y, Feng G, Jiang T, Goh C, Ng L, Liu B, Li B, Yang L, Hua J, Tian H. *Adv Funct Mater*, 2015, 25: 2857–2866
- Zhang X, Hang Y, Qu W, Yan Y, Zhao P, Hua J. *RSC Adv*, 2016, 6: 20014–20020
- Qu W, Yang L, Hang Y, Zhang X, Qu Y, Hua J. *Sens Actuat B-Chem*, 2015, 211: 275–282
- Qu Y, Wu Y, Gao Y, Qu S, Yang L, Hua J. *Sens Actuat B-Chem*, 2014, 197: 13–19
- Huang S, Liu S, Wang K, Yang C, Luo Y, Zhang Y, Cao B, Kang Y, Wang M. *Nanoscale*, 2015, 7: 889–895
- Aigner D, Ungerböck B, Mayr T, Saf R, Klimant I, Borisov SM. *J Mater Chem C*, 2013, 1: 5685–5693
- Shen XY, Wang YJ, Zhang H, Qin A, Sun JZ, Tang BZ. *Chem Commun*, 2014, 50: 8747–8750
- Wang L, Yang L, Cao D. *Sens Actuat B-Chem*, 2015, 221: 155–166
- Hang Y, Wang J, Jiang T, Lu N, Hua J. *Anal Chem*, 2016, 88: 1696–1703
- Jiang T, Li D, Hang Y, Gao Y, Zhang H, Zhao X, Li X, Li B, Qian J,

- Hua J. *Dyes Pigments*, 2016, 133: 201–213
- 34 Hang Y, He XP, Yang L, Hua J. *Biosens Bioelectron*, 2015, 65: 420–426
- 35 Lai CH, Lin CY, Wu HT, Chan HS, Chuang YJ, Chen CT, Lin CC. *Adv Funct Mater*, 2010, 20: 3948–3958
- 36 Lai CH, Chang TC, Chuang YJ, Tzou DL, Lin CC. *Bioconjugate Chem*, 2013, 24: 1698–1709
- 37 Maniam S, Holmes AB, Leeke GA, Bilic A, Collis GE. *Org Lett*, 2015, 17: 4022–4025
- 38 Zhang F, Jiang KJ, Huang JH, Yu CC, Li SG, Chen MG, Yang LM, Song YL. *J Mater Chem A*, 2013, 1: 4858–4863
- 39 He F, Liu L, Li L. *Adv Funct Mater*, 2011, 21: 3143–3149
- 40 Hasegawa T, Numata M, Okumura S, Kimura T, Sakurai K, Shinkai S. *Org Biomol Chem*, 2007, 5: 2404–2412
- 41 Tong H, Dong Y, Häussler M, Lam JWY, Sung HHY, Williams ID, Sun J, Tang BZ. *Chem Commun*, 2006, 127: 1133–1135