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ARTICLE

Facile fabrication of glycopolymer-based iron oxide nanoparticles and their applications in carbohydrate-lectin interaction and targeted cell imaging

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A novel method for facile fabrication of glycopolymer-based iron oxide nanoparticles (GIONs) was developed. Via perfluorophenylazide photochemically induced C-H insertion, alkynyl groups were introduced onto the polymer which was precoated on iron oxide nanoparticle surface. GIONs were then prepared by conjugating the azide-functionlized carbohydrate to the introduced alkynyl groups via click chemistry. Polyvinyl alchohol-coated and dextran-coated iron oxide NPs were chosen as scaffolds to attach two different carbohydrates, α-D-mannose and β-D-glucose, to fabricate multivalent GIONs, respectively. The multivalent GIONs demonstrated high binding affinities towards corresponding lectins in both protein and cell chips. As a proof of concept, fluorescent GIONs (Gal-RhB-IONPs) were fabricated, which showed selcetive and efficient internalization by ASGP-R overexpressing HepG2 cells targeted.

Introduction

Carbohydrates play crucial roles in biological processes as recognition sites for cells and various binding partners.[1-4] Efficient analysis and study of these important biological processes are, however, largely hampered by the weak affinities often associated with carbohydrate-protein interactions.[5-7] One promising way to overcome these obstacles is the development of glycopolymers with pendent carbohydrate moieties, where the binding capacity could be greatly enhanced by the multivalent effects of densely packed carbohydrate molecules, known as the "glycocluster effect".[8-10] In addition, recent research has shown that NPs with glycopolymers on the surface are one of the desirable bio-active particles to achieve superior sensitivity and selectivity,[11,12] which can effectively combine the glycocluster effect with the special chemico-physical properties of various nanoparticles, allowing them to have a wide range of potential applications in glycobiology and biomedicine.[13-16]

So far, gold and iron oxide nanoparticles (IONPs) are the most extensively used scaffolds for preparation of glyconanoparticles by grafting glycopolymers onto NP surfaces.[11,13,15] Owing to their unique magnetic properties that make them useful for magnetic resonance imaging, hyperthermia treatment, as well as sensing and drug delivery,[14,17-21] IONPs have attracted increasing attention in glyconanoparticle fabrication, where three main strategies have been developed. The first is the direct conjugation of previously prepared glycopolymers to IONPs.[14,22] For example, Haddleton and coworkers reported a one-pot synthesis of diblock PEG glycopolymers using a combination of Cu(0) mediated living radical polymerization and click chemistry to attach three different carbohydrates, α-D-mannose, α-D-glucose and β-D-glucose, to IONP surfaces.[14] The second is the combination of a polymer "grafting-from" approach with glycosylation on the surface of IONPs.[23] Tushar Borase fabricated glycopeptide-stabilized hybrid magnetic NPs by using N-carboxyanhydride ring-opening polymerization to graft an alkyne-functionalized peptide on the particle surface followed by glycosylation through click chemistry.[23] The third strategy is to introduce reactive groups onto polymers which were previously coated on IONP surface, followed by glycosylation.[24,25] In this strategy, polymer coating always endows NPs superior water solubility and biocompatibility. However, the coated polymers always lack reactive groups or simply have low-reactive functional group, which results in difficult functionalization and a time-consuming process. For example, Gillies group fabricated a series of GIONs based on dextran-coated IONPs. In their work, dextran coated on IONPs were first aminated by treating with epichlorohydrin followed by ammonium hydroxide, subsequent reaction with azide-functionalized carboxylic acid introduced azide; finally, the azide underwent click reaction with alkyne-functionalized carbohydrate to obtain GIONs.[25]

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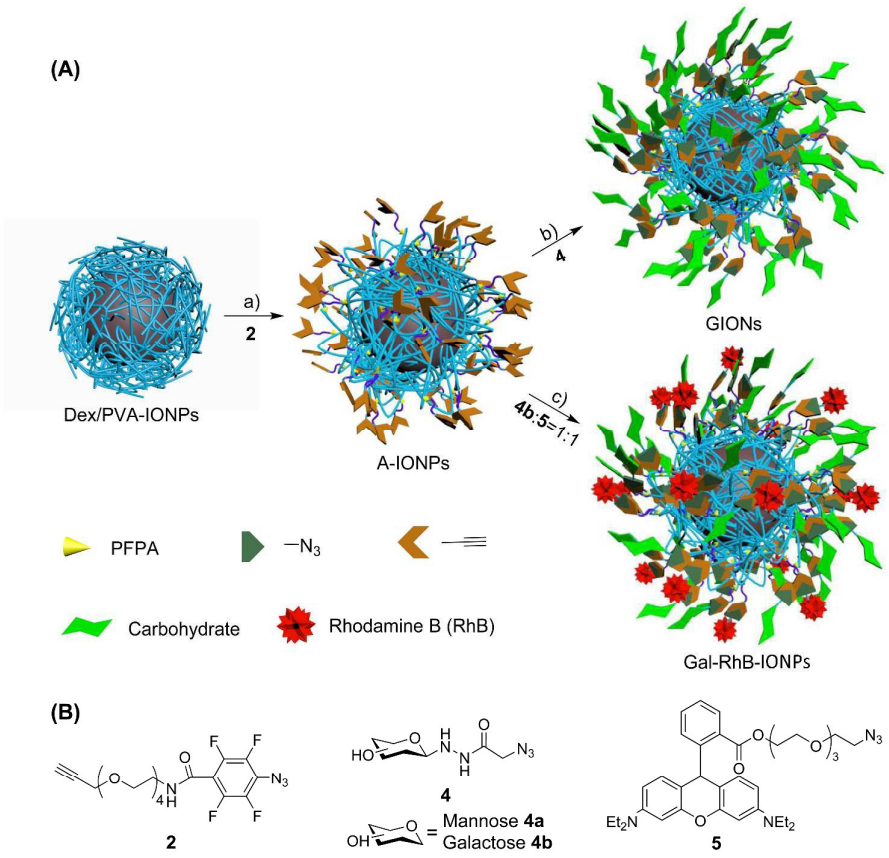

Fig. 1 (A) Schematic representation of GION/Gal-RhB-IONPs fabrication. (B) The compounds used in the GION/Gal-RhB-IONPs fabrication procedure. Reagents and conditions: a) UV, vigorous stirring, H_2O, 3 h; b) and c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMSO : H_2O = 4 : 1, v/v, r.t., 24 h.

Herein, we present a facile GION fabrication method where perfluorophenylazide (PFPA) photochemically induced C-H insertion reaction was used for the functionalization of polymers coated on IONP surface. Theoretically, PFPA photocoupling chemistry is applicable to all polymers with C-H bond.[26-28] In this work, two typical polymer-coated IONPs, dextran (Dex)-coated and polyvinyl alchohol (PVA)-coated IONPs (noted as Dex-IONPs and PVA-IONPs, respectively), were chosen as the scaffolds in GION fabrication to validate our method, where the polymer-coated IONPs can be easily synthesized via co-precipitation method.[29-31] To exemplify the potential of GIONs in biological applications, multivalent GIONs were prepared to measure GION-lectin and GION-cell interactions with QCM biosensor, while fluorescent GIONs were fabricated for targeted imaging human hepatoblastoma (HepG2) cells.

Results and discussion

The fabrication procedure of GIONs and the key compounds involved were illustrated in Fig. 1. The procedure included two steps:

1) Synthesis of alkynyl functionalized IONPs (A-IONPs). This was achieved by performing alkynyl-PFPA **2** photochemically induced C-H insertion reaction on the polymeric surface of dex/PVA-IONPs. Key compound **2** possesses an alkyne group, which was synthesized by coupling 4-azido-2,3,5,6-tetrafluorobenzoic acid (PFPA-COOH) with the amido-alkyne **1** (3,6,9,12-tetraoxapentadec-14-yn-1-amine) using N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Scheme S1). To synthesize A-IONPs, **2** was mixed with Dex-IONPs or PVA-IONPs in water and the mixture was subjected to sonication. The photoreaction was carried out in a quartz vessel under UV light (λ = 300 nm).

2) Synthesis of GIONs. This was done by connecting A-IONPs and **4** via azide-alkyne click reaction. **4** is a C-linked spacer (i.e. a spacer conjugated with carbohydrates and azide groups on respective ends), which was synthesized by reacting the desired carbohydrate with azide-modified hydrazide **3** via hydrazone reaction (Scheme S2). In the developed glyconanoparticle fabrication methods, the modification of carbohydrates is often a necessary and very laborious task prior to their linkage to spacers due to their structural complexity.[32,33] Though laborious modification of carbohydrates can be avoided by utilizing PFPA photochemically induced C-H insertion reaction,[34,35] the insertion site on carbohydrate structure is uncertain, which may result in the loss of the biological function of the carbohydrates.[36] In contrast, the easily performed hydrazone reaction between carbohydrate and hydrazide can effectively retain the biological function of carbohydrates.[37,38] In this work, the unmodified monosaccharides (mannose and galactose) reacted with 2-azidoacetohydrazide **3** at 80 °C in 3 : 1 mixed ethanol and water for 8 h to synthesize the C-linked spacer **4**. The conjugation of monosaccharides to A-IONP to fabricate GIONs employs azide-

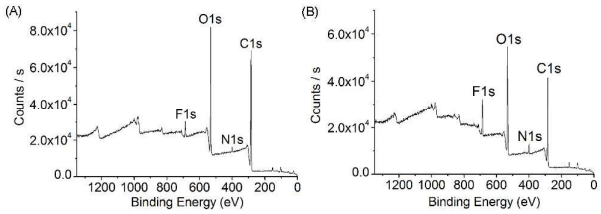

Fig. 2 XPS data of (A) alkynyl functionalized Dex-IONPs and (B) alkynyl functionalized PVA-IONPs.

alkyne click reaction, where azide-containing compound **4a** or **4b** was used as the specific C-linked spacer. Thus, three kinds of GIONs, galactose modified Dex-IONPs (Gal-Dex-IONPs), mannose modified PVA-IONPs (Man-PVA-IONPs), and galactose modified PVA-IONPs (Gal-PVA-IONPs) were prepared.

The alkyne modification on the polymer surfaces of the two scaffolds, Dex-IONPs and PVA-IONPs, was proven by XPS, as shown in Fig. 2, where the fluorine and nitrogen elements from compound **2** were found on A-IONPs, indicating that a successful photoreaction had taken place between **2** and polymer-coated IONPs. The analysis of HPLC showed that the amount of alkynyl groups on alkynyl functionalized Dex-IONPs and PVA-IONPs were 1.22 mmol/g NPs and 1.56 mmol/g NPs, respectively (Fig. S5), far more than the azide amount on the surfaces of DCNSs we synthesized previously.[39] To confirm the success of azide-alkyne click reaction in the GION fabrication process, an azide-functionalized Rhodamine B **5** was used instead of C-linked spacers **4** to synthesize Rhodamine B modified IONPs (RhB-IONPs) via azide-alkyne click reaction. As expected, the successful click reaction occurred between A-IONPs and **5**, which yielded RhB-IONPs with a wine-red color in water after thorough washing (Fig. 3). Furthermore, compared with the UV-Vis absorption spectra of IONPs and A-IONPs, a new peak was observed in RhB-IONPs, which was attributed to the absorption of Rhodamine B attached on the surface of A-IONPs (Fig. 3). The maximum absorption of Rhodamine B modified PVA-IONPs (RhB-PVA-IONPs) and Rhodamine B modified Dex-IONPs (RhB-Dex-IONPs) is 562 and 571 nm, respectively, a slight red shift compared to that of compound **5** (λ_{max} = 557 nm). These results fully demonstrated that the azide-alkyne click reaction was successful.

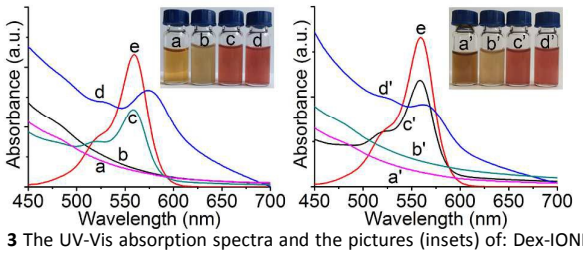

Fig. 3 The UV-Vis absorption spectra and the pictures (insets) of: Dex-IONPs (a), alkynyl functionalized Dex-IONPs (b), mixture of alkynyl functionalized Dex-IONPs and compound **5** before click reaction (c), RhB-Dex-IONPs (d), PVA-IONPs (a'), alkynyl functionalized PVA-IONPs (b'), mixture of alkynyl functionalized PVA-IONPs and compound **5** before click reaction (c'), and RhB-PVA-IONPs (d') in water. Red line is the UV-Vis absorption spectrum of compound **5** (e).

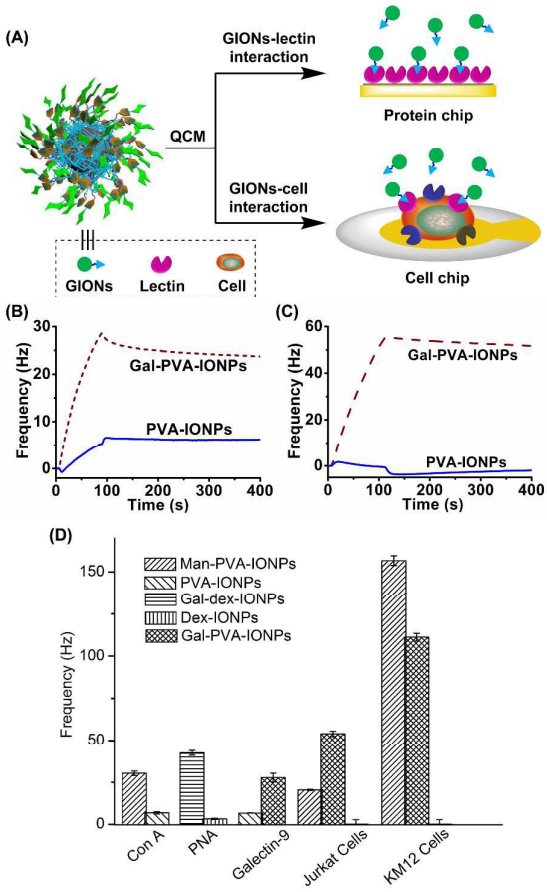

Fig. 4 (A) The routes of applying GIONs to QCM biosensor with protein chip and cell chip. (B) Frequency shifts caused by interaction of galectin-9 chip with Gal-PVA-IONPs (dotted line) and PVA-IONPs (solid line, control), respectively. (C) Frequency shifts caused by interaction of Jurkat cell chip with Gal-PVA-IONPs (dashed line) and PVA-IONPs (solid line, control), respectively. (D) QCM detected interactions (frequency shifts) of Dex/PVA-IONPs and GIONs with lectin/cell chips. The interactions of Dex/PVA-IONPs with lectins/cells were used as nonspecific controls. The flow rate of running buffer (PBS, pH = 7.2) was 20 μL/min; the concentration of injected NPs in PBS was 0.2 mg/mL; the binding time on chips was 84 s, and the dissociation was 316 s. All the experiments were performed at 22 °C.

The biological applications of GIONs synthesized in our experiments were first exemplified by measuring GION-lectin and GION-cell interactions with QCM biosensor, which is a label-free and real-time sensor technology for measuring biomolecular interactions.[40,41] However, the ability for light-mass molecule (Mw. less than 1000) detection by QCM has been a long-standing challenge due to the limitation of its mass-based principle.[42] In this work, GIONs were employed as signal amplifiers to measure carbohydrate-protein interactions by QCM with protein chips and cell chips, respectively (Fig. 4). Concanavalin A (Con A), peanut agglutinin (PNA) and galectin-9 were used for the fabrication of protein chips, respectively. Con A and PNA are phytolectins that selectively bind with mannose and galactose, respectively. Galectin-9 is an animal lectin that specifically recognizes galactose. The cell chips were fabricated by directly growing adherent cancer cells[43]

(colon cancer KM12 cells) or capturing suspension cancer cells[41] (human acute lymphocytic leukemia cell line, Jurkat cells) onto sensor surface.

Man-PVA-IONPs, Gal-Dex-IONPs, and Gal-PVA-IONPs were separately applied to detect the binding affinities towards Con A, PNA or galectin-9 protein chips. The detections on cell chips were carried out with Gal-PVA-IONPs and Man-PVA-IONPs. In all detection processes, Dex/PVA-IONPs (0.2 mg/mL in PBS) were used as control. The binding affinities were recorded as frequency shift of the quartz crystal (see Fig. 4B and 4C for examples), and the results are summarized as a histogram in Fig. 4D. The binding affinities of Man-PVA-IONPs, Gal-Dex-IONPs or Gal-PVA-IONPs towards Con A, PNA, or galectin-9 were recorded as 30.3, 42.5, and 27.9 Hz, respectively. The corresponding nonspecific interactions of PVA-IONPs or Dex-IONPs towards Con A, PNA, or galectin-9 were recorded as 6.7, 3.2, and 6.7 Hz, respectively. The QCM results showed excellent binding activity of GIONs for lectins using protein chip. The binding affinities of Gal-PVA-IONPs and Man-PVA-IONPs toward Jurkat cells were 54.4 and 20.7 Hz, respectively; that toward KM12 cells were 111.4 and 157.0 Hz, respectively. The nonspecific interactions of PVA-IONPs towards these two cell chips were both 0.4 Hz. The cell chip detection results indicate that, combined with QCM technology, GIONs can be employed to characterize and unlock the glycol-code of different cancer cells, which is of great significance for facilitating the study of the roles of carbohydrates and developing new diagnostic and therapeutic tools against cancer.[44]

To further exemplify the potential of GIONs in biological applications, experiments for targeted cell imaging were performed. First, galactose and Rhodamine B modified IONPs (Gal-RhB-IONPs) as targeted cell imaging nanoparticles were prepared by A-IONPs reacting with a mixture of compound **4b** and compound **5** (1:1) via click reaction according to the GION fabrication procedure mentioned above, which can be easily adapted to attach a fluorescent probe to construct a lectin-targeted cell imaging system. Notably, the biomolecules (compound **4b**) and fluorescent tags (compound **5**) can be conveniently attached onto a single nanocarrier platform, avoiding any direct labelling on the biomolecules. As shown in Fig. S3, Gal-RhB-IONPs displayed a wine-red color in water, with a maximum absorption on the UV-Vis absorption spectra of 562 nm. DLS showed that the average

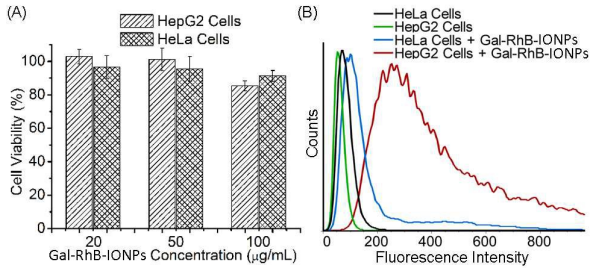

Fig. 5 (A) Cytotoxicity of Gal-RhB-IONPs to HeLa and HepG2 cells determined by MTT assays. The cells were incubated with Gal-RhB-IONPs for 24 h at 37 °C. Data are presented as the average ± standard deviation (n = 5). (B) Flow cytometry analysis of HeLa cells/HepG2 cells after incubation with Gal-RhB-IONPs (50 μg/mL) at 37 °C for 4 h, where HepG2 and HeLa cells without treated with Gal-RhB-IONPs were used as negative controls.

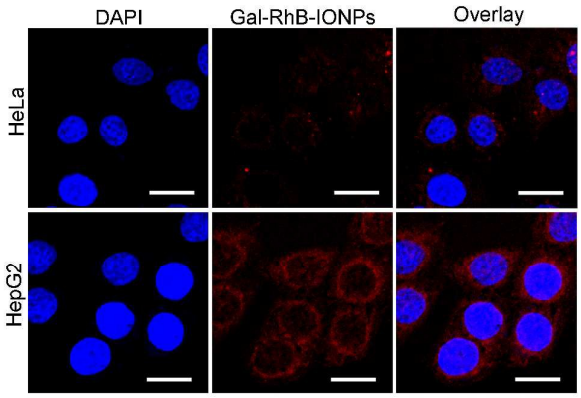

Fig. 6 CLSM images of HeLa cells/HepG2 cells incubated with Gal-RhB-IONPs (20 μg/mL) at 37 °C for 4 h. Scale bar: 20 μm.

diameter of Gal-RhB-IONPs was 531 nm (Fig. S4). The amount of galactose on the Gal-RhB-IONPs was determined as 1.128 mmol/g NPs by colorimetric assay of anthrone-sulfuric acid (Fig. S6). The amount of Rhodamine B on the Gal-RhB-IONPs was 0.082 mmol/g NPs, which was measured with UV-Vis absorbance (Fig. S7). In addition, to examine the potential toxicity of GIONs, HepG2 cells and HeLa cells were incubated with GIONs (20, 50, 100 μg/mL) for 24 h, respectively. No obvious apoptosis was observed (Fig. 5A), indicating that GIONs exhibit excellent biocompatibility.

To evaluate the targeting effect of Gal-RhB-IONPs, human hepatoblastoma cell line (HepG2 cells) and human cervical cell line (HeLa cells) were incubated with Gal-RhB-IONPs at 50 μg/mL for 4 h, respectively, where the efficiency of cellular uptake was analyzed by flow cytometry. The results showed that the uptake of Gal-RhB-IONPs by HepG2 cells was 2.4-fold higher than that of HeLa cells (Fig. 5B), resulting from the galactose residues on Gal-RhB-IONPs, which can act as the targeting ligands to overexpressing asialoglycoprotein receptors (ASGP-R) on HepG2 cells via carbohydrate-protein interaction.[45-47] In addition, the receptor-mediated target imaging of Gal-RhB-IONPs toward HepG2 cells was verified by confocal laser scanning microscope (CLSM). As can be seen in Fig. 6, strong red fluorescence was observed in HepG2 cells after incubating with 20 μg/mL Gal-RhB-IONPs for 4 h, whereas negligible red fluorescence was observed in HeLa cells after incubation under the same conditions, indicating that Gal-RhB-IONPs can effectively target to HepG2 cells via a receptor-mediated mechanism from the interaction of galactose and ASGP-R.

Conclusions

In conclusion, a facile and versatile GION fabrication method has been developed by employing the PFPA-photochemically induced C-H insertion reaction to introduce alkyne groups onto polymers which were previously coated on IONP surface; azide-functionlized carbohydrates were then conjugated to the introduced alkyne groups by click chemistry. The PFPA photocoupling chemistry is in theory applicable to all polymers with C-H bond. Three kinds of multivalent GIONs appeared to have high binding affinities towards corresponding lectins in both protein chips and cell chips by measuring carbohydrate-protein interactions with QCM biosensor.

One kind of fluorescent GIONs as targeted imaging system showed selective and efficient internalization by ASGP-R overexpressing tumor cells via receptor-mediated endocytosis. Furthermore, in this GION fabrication method, the scaffold, the coupled carbohydrate, and even the spacer linkage can be freely changed, thus allowing the potential to produce numerous desired glyconanoparticles for bioscience applications.

Experimental

Materials

All chemicals and solvents were of analytical reagent grade and used as received unless specified. PVA-coated IONPs were purchased from Gignano Biointerface. Dextran T40 were obtained from Pharmacia Uppsala, Sweden. Methyl pentafluorobenzoate was purchased from Matrix Scientific Trade Co.. EDC, rhodamine B, tetraethylene glycol, sodium ascorbate and chloroacetic acid were purchased from Aladdin. N-hydroxysulfosuccinimide (sulfo-NHS), EDC and ethanolamine (1 M, pH 8.5) for QCM experiments were obtained from Attana, Sweden. Column chromatography was performed using silica gel with a grain size of 40-63 μm (Qingdao Haiyang Chemical Co., Ltd.). Mannose, galactose, and Con A were purchased from Sigma. PNA was purchased from Vector Laboratories, Inc. Galectin-9 were obtained from Sino Biological Inc. Jurkat cells were kindly provided by Prof. Bin Gao at Institute of Microbiology, Chinese Academy of Sciences (IMCAS). KM12 cells were purchased from Guangzhou Jennio Biotech Co., Ltd. RPMI 1640 medium was obtained from Gibco BRL Co., Ltd. Fetal bovine serum was purchased from Yuanhengjinma Co., Ltd. Penicillin/streptomycin was purchased from Nanjing KeyGen Biotech. Co. Ltd. Photochemistry was carried out at ambient temperature with a Rayonet PRP-100 photochemical reactor. Ultrasonic irradiation was produced by an L-650Y ultrasonicator (Shanghai LNB Instrument Co., Ltd). A permanent magnet was used to collect and purify NPs in all processes.

Characterization

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 500 instrument in $CDCl_3$, D_2O or CD_3OD using the residual signals from $CHCl_3$ (^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.20 ppm), H_2O (^{1}H: δ = 4.79 ppm) or CH_3OH (^{1}H: δ = 3.31 ppm; ^{13}C: δ = 49.00 ppm) as internal standards. HRMS (High Resolution Mass Spectroscopy) analysis was performed on an Agilent 1290-6540 UHPLC Q-Tof-HRMS. The Dynamic Light Scattering (DLS) data was obtained by a Delsa Nano C analyzer (Beckman Coulter, Inc.). Transmission Electron Microscope (TEM) images were taken on a Hitachi H-600 instrument operated at 75 kV. X-ray photoelectron spectroscopy (XPS) data was obtained by Thermo Scientific K-Alpha. Ultrasonic irradiation was produced by an L-650Y ultrasonicator (Shanghai LNB Instrument Co., Ltd). UV-Vis absorption spectra were taken on a Shimadzu UV-1750 UV-Vis-NIR spectrometer. All the NPs in Fig. 3 were dispersed in water.

Synthesis of compound 2 and 4a/4b

Alkyne-PFPA (2) PFPA-COOH (489 mg, 2.07 mmol), amino-alkyne **1** (469 mg, 2.03 mmol) and EDC (794 mg, 4.14 mmol) were dissolved in DCM (15 mL) and stirred at r.t. overnight. Then the reaction mixture was washed with water (3 × 20 mL). The organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (DCM/MeOH, 100 : 1, v/v), yielding **2** as a pale yellow oil (690 mg, 76 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.95 (s, 1 H, -NH-), 4.14 (d, 2 H, J = 2.3 Hz, CHCCH_2O-), 3.67 - 3.61 (m, 16 H, -OCH_2CH_2O-), 2.41 (t, 1 H, J = 2.3 Hz, CHCCH$_2$O-) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 157.96, 145.12 - 143.08 (m, $J_{C\text{-}F}$ = 253 Hz), 141.56 - 139.42 (m, $J_{C\text{-}F}$ = 253 Hz), 121.72, 111.85, 79.55, 74.68, 70.62, 70.61, 70.51, 70.45, 70.41, 69.43, 69.10, 58.44, 40.22 ppm. ESI HRMS: calculated for $C_{18}H_{20}F_4N_4O_5Na^+$ $[M + Na]^+$ 471.1262; found 471.1268.

Azide-modified mannose/galactose (4a/4b) The monosaccharide (mannose or galactose, 180 mg, 1 mmol) and 2-azidoacetohydrazide **3** (173 mg, 1.5 mmol) were dissolved in a mixture of ethanol and water (8 mL, 3 : 1, v/v). The reaction mixture was stirred at 80 °C for 8 h. Then the solvents were evaporated under reduced pressure, and the crude was purified by column chromatography using DCM/MeOH (5 : 1 to 3 : 1, v/v) to give azide-modified mannose (**4a**, 222 mg, 80 %) or azide-modified galactose (**4b**, 216 mg, 78 %) as white solids. Both products were a mixture of cyclic form (major, α-anomeric and β-anomeric) and acyclic form (minor). The ^{1}H NMR data of **4a** and **4b** were showed in Fig. S16 and S16 (the deuterated D_2O was the solvent). The isomeric ratio of acyclic form, α-anomeric form, and β-anomeric form was determined by the peak areas of anomeric hydrogen in ^{1}H NMR spectra of their mixtures. For **4a**, the isomeric ratio is 1.00 : 2.29 : 9.85; for **4b**, the isomeric ratio is 1.00 : 1.54 : 4.92.

Preparation of Dex-IONPs

Under nitrogen atmosphere, dextran (2500 mg), $FeCl_2{\cdot}4H_2O$ (276 mg) and $FeCl_3{\cdot}6H_2O$ (225 mg) was dissolved in ultrapure water (5 mL) with vigorous stirring. Then the mixture was heated to 60 °C and ammonia solution (5 mL, 4 M) was added dropwise. 15 min later, the final suspension was washed thoroughly with ultrapure water by using a permanent magnet to remove the unbound dextran and other residual reactants. The size of new made Dex-IONPs was characterized by TEM and DLS, which was shown in Fig. S1.

Fabrication of A-IONPs

To an aqueous suspension of Dex- or PVA-coated IONPs (1 mL, 10 mg/mL) in a quartz vessel, alkyne-PFPA **2** (50 mg, 0.11 mmol) was added. The mixture was sonicated and then irradiated with 300 nm UV light under vigorous stirring for 3 h. The resulting nanoparticles were collected with a permanent magnet, washed 5 times with methanol and 3 times with water, and dried to get A-IONPs (10.8 mg). The final A-IONPs were characterized by XPS (Fig. 2). The amount of alkynyl groups introduced to the polymer coated on IONP surface was analyzed by HPLC (Fig. S5).

Fabrication of RhB-IONPs, GIONs and Gal-RhB-IONPs

Azide-functionalized Rhodamine B **5** (0.06 mmol), $CuSO_4{\cdot}5H_2O$ (2.5 mg, 0.01 mmol) and sodium ascorbate (8.0 mg, 0.04 mmol) were added to a suspension of A-IONPs (35 mg) in DMSO : H_2O (4 : 1, v/v, 7 mL). After 10 min sonication, the reaction mixture was stirred at room temperature for 24 h. The resulting NPs were then isolated

and washed with methanol for 5 times and PBS (pH = 7.2) for 3 times to get RhB-IONPs. RhB-IONPs were characterized by UV-Vis spectroscopy (Fig. 3).

GIONs and Gal-RhB-IONPs were fabricated in a similar procedure to that of RhB-IONPs. For GIONs, C-linked spacers **4** was used instead of **5**; For Gal-RhB-IONPs, **4b** : **5** = 1 : 1 (0.03 mmol : 0.03 mmol) was used in the azide-alkyne click reaction. The prepared GIONs or Gal-RhB-IONPs were stored in PBS (pH = 7.2) or ultrapure water for further use. The average size and distribution of GIONs and Gal-RhB-IONPs were characterized by DLS (Fig. S2).

Cell culture for QCM analysis

The cells (KM-12 or Jurkat cell line) were cultured in RPMI 1640 medium (Gibco) supplemented with 10 % (v/v) fetal bovine serum and 1 % (v/v) penicillin/streptomycin at 37 ˚C with 5 % CO_2 and 95 % humidity and were maintained by replacement of the medium every 2-3 days to keep the cell density between 1×10^5 and 1×10^6 cells/mL. To prepare the cell suspension for cell capture, the medium was removed by centrifugation and the cells were washed with 5 mL PBS. The cell pellets were then resuspended using appropriate volume of PBS to obtain the cell suspension at a concentration of 2×10^6 cells/mL.

QCM analysis of GION-lectin interactions and GION-cells interactions

The protein and cell chips were fabricated by following the published procedures, respectively.[41,43,48] The analysis of GION-lectin interaction was performed in Attana A100 QCM instrument, while the analysis of GION-cell interaction was performed in Attana Cell A200 QCM instrument. For measuring the interactions, each sensor chip was inserted into the instrument and allowed to stabilize (baseline drift < 0.2 Hz/min) under a continuous flow (20 µL/min) of running buffer (PBS, pH = 7.2). GIONs in PBS (0.2 mg/mL) were injected over the surface of the chip, allowed to bind for 84 s, and dissociate for 316 s. All the experiments were performed at 22 °C. The frequency shifts associated with the binding events were recorded with the Attester software (Attana, Sweden) in real time. All the experiments were repeated for three times.

MTT assay

HepG2/HeLa cells were seeded onto a 96-well plate at a density of 7000 cells per well in 100 µL of RPMI 1640/DMEM medium supplemented with 10 % (v/v) fetal bovine serum and 1 % (v/v) penicillin/streptomycin. After incubating for 24 h (37 °C, 5 % CO_2), the medium was replaced by 90 µL of fresh culture medium, and then 10 µL various concentrations of Gal-RhB-IONPs dispersed in PBS (10 mM, pH 7.4) were added. The cells were incubated for another 24 h (37 °C, 5 % CO_2), and then 10 µL of MTT solution (5 mg/mL) was added. The cells were incubated for 4 h, and the medium was replaced by 150 µL of DMSO to dissolve the resulting purple crystals. The optical densities at 570 nm were measured using a BioTek microplate reader. The cell viability (%) was determined by comparing the absorbance at 570 nm with control wells containing HepG2/HeLa cells without exposure to Gal-RhB-IONPs in incubation. Data were presented as average ± SD (n = 6)

Flow Cytometry Assay on cellular uptake

HepG2/HeLa cells were seeded onto 6-well plates at 1×10^5 cells per well using RPMI 1640/DMEM medium supplemented with 10 % (v/v) fetal bovine serum and 1 % (v/v) penicillin/streptomycin. After 24 h incubation (37 °C, 5 % CO_2), the medium was replaced by 0.9 mL of fresh RPMI 1640/DMEM and 0.1 mL of Gal-RhB-IONPs (50 µ g/mL). HepG2/HeLa Cells continue cultured in 1.0 mL fresh RPMI 1640/DMEM medium containing 10 % FBS and 1 % (v/v) penicillin/streptomycin (without exposure to Gal-RhB-IONPs) were used as controls. After incubation at 37 °C for 4 h, the medium was removed. The cells were washed twice with cold PBS, then digested by 0.25 w/v % trypsin/0.03 w/v % EDTA. The suspensions were centrifuged at 1000 rpm for 5 min at 25 °C, and the precipitate was then resuspended in 500 µL of PBS. Fluorescence histograms were recorded with a BD FACSCalibur (Beckton Dickinson) flow cytometer and analyzed using Flowjo 7.6 software. We analyzed 20000 gated events to generate each histogram. The gate was arbitrarily set for the detection of Rhodamine B fluorescence.

Targeted cell imaging by LSCM

HepG2/HeLa cells were seeded onto 35 mm confocal dishs (5×10^4 cells) using RPMI 1640/DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin. After 24 h incubation (37 °C, 5% CO_2), the medium was replaced by 0.9 mL of fresh RPMI 1640/DMEM and 0.1 mL of Gal-RhB-IONPs (20 µ g/mL). After incubation for 4 h, the culture medium was removed and the cells were washed three times with PBS, fixed with 3.7% formaldehyde for 10 min. The nuclei of the cells were stained with DAPI and the cells were rinsed with PBS. Fluorescence images of cells were obtained with Confocal Laser Scanning Microscope (Nikon, A1R).

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