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Supramolecular glyco-poly-cyclodextrin functionalized thin-layer manganese dioxide for targeted stimulus-responsive bioimaging†

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We have developed a supramolecular imaging probe based on thin-layer manganese dioxide functionalized with a fluorescent, multivalent glyco-poly-cyclodextrin for the targeted, stimulus-responsive bioimaging of cancer cells.

This paper describes the supramolecular construction of a biocompatible thin-layer manganese dioxide-based probe for the targeted, biothiol-responsive fluorescence imaging of liver and triple-negative breast cancer cells. Target-specific imaging is crucial for improving the precision of diagnostics. Specificity can be achieved through the simultaneous targeting of a membrane receptor and the stimulus-responsive signal production upon interacting with an intracellular biomarker. However, the majority of the current small-molecular probes or polymeric materials for sensing the overexpression of an intracellular disease biomarker lack the ability to be actively internalized by living cells. To address this issue, recent literature has seen an active movement towards the introduction of a targeting agent such as aptamers, peptides and carbohydrates to the imaging material, increasing the accumulation, and thus the stimulus-responsiveness of the probes in a target cell or tissue.^{1–3}

There are two key issues that need to be carefully considered to achieve targeted bioimaging. First, multivalent display of the targeting ligands on a material scaffold is crucial for significantly increasing the binding avidity for cell-surface receptors; this is particularly important for carbohydrate-based materials.⁴

On a second front, the materials used should be biocompatible in order to achieve real-world applications. Despite the extensive development of oligo- and polymeric architectures to realize multivalency through covalent bonding chemistries,^{5,6} simple yet effective functional imaging probes formed through a non-covalent supramolecular approach remains much less explored.

Here we show the construction of an imaging probe based on 2D manganese dioxide (2D probe) using two supramolecular approaches. Host-guest interaction between an adamantane-grafted fluorescent glycoprobe and poly- β -cyclodextrins (poly-CD) with different CD-grafting ratios (Fig. 1a, where m and n are the equivalent of CD and that of acrylamide, respectively) forms a multivalent, fluorescence-enhanced glycodot, which can be subsequently self-assembled with a biocompatible 2D material (manganese dioxide – MnO₂), producing the stimulus-responsive, fluorogenic 2D probe (Fig. 1b) capable of target-specific imaging of liver cancer and triple-negative breast cancer cells (Fig. 1c).

Cyclodextrins (CDs) have been widely used as a macrocyclic host molecule by the supramolecular inclusion of hydrophobic guest compounds in aqueous solution. The supramolecular “host-guest” chemistry of CDs plays an important role in photochemistry, analytical science, materials science and chemical biology.⁷ They have also been used extensively for enhancing drug-delivering efficacy *in vivo*.^{8,9} With the rapid progress in the discovery of advanced new materials, a myriad of 2D materials have been synthesized and employed for biosensing and bioimaging.¹⁰ Of the 2D materials developed, thin-layer MnO₂ has proven to be rapidly degradable in the presence of reducing agents¹¹ such as the physiologically important glutathione (GSH) that exists at higher levels in cancer cells.^{12,13} However, efforts to incorporate the biocompatible poly-CD with 2D MnO₂ producing functional material architectures for biomedical applications remain rare.

To the best of our knowledge, we describe the first supramolecular ensemble formed between functionalized poly-CDs and 2D MnO₂ for targeted, biothiol-responsive fluorescence imaging. We began with the design and synthesis of a naphthalimide glycoprobe (Fig. 1a and Scheme S1, ESI†). In previous studies, we have shown that the introduction of carbohydrate

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Fig. 1 (a) Structure of the poly-CD host polymers and the adamantane-modified glycoprobes as guest molecules. Schematic illustration of (b) the two-step supramolecular assembly forming the fluorogenic 2D probe and (c) use of the 2D probe for the targeted, stimulus-responsive cell imaging (GSH = glutathione).

targeting agents to naphthalimide based probes significantly enhanced their cell-targeting ability and imaging of intracellular species¹⁴ with a lowered cytotoxicity. We synthesized two such glyco-naphthalimide probes with galactose and mannose as the targeting ligand for the asialoglycoprotein receptor (ASGPr) and mannose receptor (MR) that exist on the surface of different cancer cells, respectively. An additional adamantane group was introduced to the probes for host-guest assembly with CDs,^{15,16} producing the Man-probe and Gal-probe (Fig. 1a). The CD monomer (6-acrylamido- β -CD) was synthesized according to the literature,¹⁷ and a subsequent radical binary copolymerization between acrylamide and the CD-monomer with different loading concentrations afforded the poly-CDs with different CD-grafting ratios (poly-CD₆, poly-CD₁₀₀ and poly-CD₃₀₀, where the numbers refer to the equivalent of acrylamide in the co-polymer).

With the compounds and polymers ready, the self-assembly was carried out in a phosphate buffered saline (PBS, 0.01 M, pH 7.4) as solvent. We determined that the fluorescence of the two glycoprobes enhanced gradually with added poly-CDs (Fig. 2a and d for Man-probe with poly-CD₁₀₀ and Gal-probe with poly-CD₆, respectively; for fluorescence spectra of the glycoprobes with all the poly-CDs, see Fig. S1a, ESI[†]), suggesting that the host-guest insertion of adamantane into the CD cavities might enhance the hydrophobic environment and thus enhance

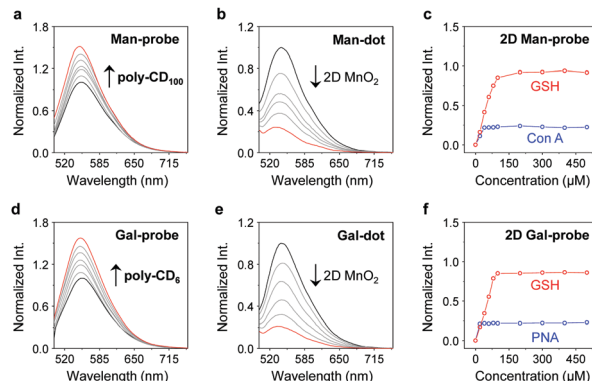


Fig. 2 Concentration-dependent fluorescence enhancement of (a) Man-probe (1 μ M) with increasing poly-CD₁₀₀ (0–90 μ g mL⁻¹; interval: 15 μ g mL⁻¹) and (d) Gal-probe (1 μ M) with increasing poly-CD₆ (0–90 μ g mL⁻¹; interval: 15 μ g mL⁻¹). Concentration-dependent fluorescence quenching of (b) Man-dot (Man-probe/poly-CD₁₀₀ = 1 μ M/80 μ g mL⁻¹) and (e) Gal-dot (Gal-probe/poly-CD₆ = 1 μ M/80 μ g mL⁻¹) with increasing 2D MnO₂ (0–5 μ g mL⁻¹; interval: 1 μ g mL⁻¹). Concentration-dependent fluorescence recovery of (c) 2D Man-probe (Man-probe/poly-CD₁₀₀/2D MnO₂ = 1 μ M/80 μ g mL⁻¹/5 μ g mL⁻¹) with increasing GSH and concanavalin A (Con A, a mannose selective lectin) and (f) 2D Gal-probe (Gal-probe/poly-CD₆/2D MnO₂ = 1 μ M/80 μ g mL⁻¹/5 μ g mL⁻¹) with increasing GSH and peanut agglutinin (PNA, a galactose selective lectin). The fluorescence spectra for glycoprobes and glycodots were obtained in phosphate buffered saline (PBS, 0.01 M, pH 7.4). The fluorescence spectra for 2D probes were obtained in Tris-HCl (0.01 M, pH 7.4). Excitation wavelength: 450 nm; slit widths ex = 5 nm and em = 5 nm.

the fluorescence emission.^{18,19} We then observed that the enhanced fluorescence intensity of the probes was proportional to the CD density of the polymers (poly-CD₆ > poly-CD₁₀₀ > poly-CD₃₀₀) (Fig. S1b, ESI[†]). This suggests that the polymers with a higher CD density can host more guest molecules to enhance the overall fluorescence emission. To corroborate that the fluorescence enhancement was the result of host-guest interactions, we used 1-bromonaphthalene as a competing molecule. The results indicated that the presence of 1-bromonaphthalene decreased the fluorescence of the glycoprobe/poly-CD ensembles (glycodots), suggesting the interruption of the adamantane-CD binding (Fig. S2, ESI[†]).

Having developed the glycodots, we set out to further construct the 2D probe. The glycodots were mixed with an aqueous dispersion of 2D MnO₂ (with a size distribution in the nanometer range – Fig. S3, ESI[†]) produced using an established method.²⁰ Then, the assembly was measured by fluorescence spectroscopy. We observed a concentration-dependent fluorescence decrease of the glycodots with 2D MnO₂ (Fig. 2b and e for a Man-dot and a Gal-dot, respectively), which is in agreement with the fluorescence quenching property of the 2D material for surface-bound fluorophores.^{21,22} The quenching plateau was reached with 5 μ g mL⁻¹ of the 2D material. The 2D probes were shown to be thermally stable over a temperature range of 0–200 °C as determined by thermogravimetric analysis. (Fig. S4, ESI[†]).

Next, we tested the stability of the 2D probes with both lectins (proteins that selectively recognize carbohydrates) and GSH. Interestingly, whereas the presence of selective lectins



interactions and self-assembly between fluorescent polymers and 2D materials. The simplicity in material construction yet effectiveness of the probes for stimulus-responsive fluorogenic imaging makes it possible to extend these systems for an even wider diversity of supramolecular, biocompatible imaging probes for functional bioimaging.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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