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A glucose-responsive controlled release system using glucose oxidase-gated mesoporous silica nanocontainers†

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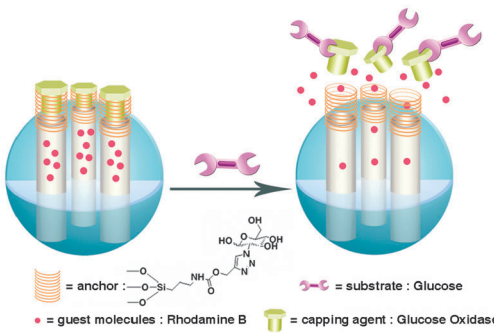
A glucose-responsive controlled-release system based on the competitive combination between glucose oxidase, glucosamine and glucose has been described, which exhibits perfect controlled release properties and high selectivity for glucose over other monosaccharides. This paved the way for a new generation of stimuli-responsive delivery systems.

An ideal delivery system should be able to transport the guest molecules to the targeted location and release them in a controlled manner. However, current polymer-based delivery strategies mainly rely on passive diffusion or degradation approaches.[1] Over the past decades there has been surging interest in mesoporous silica nanoparticles (MSNs) because the surface-functionalized, end-capped MSNs offer unique features, such as stable mesoporous structures, high surface areas (900–1500 $m^2\ g^{-1}$), tunable pore size, large accessible pore volumes (0.5–1.5 $cm^3\ g^{-1}$), less toxicity and biocompatability, which make them attractive vehicles for drug delivery.[2]

A number of functionalized mesoporous silica materials (FMSM)-based stimuli-responsive controlled-release systems using nanoparticles (*e.g.* CdS, Au or Fe_3O_4), polymers, dendrimers or large molecules (cyclodextrins or rotaxanes) as the capping agents[3] have been subjected to intensive studies. Moreover, a series of FMSM-based systems have also been reported using pH,[4] temperature,[3f] polarity,[5] redox potential,[6] light intensity,[7] or enzyme activity[8] as opening stimuli. A pH and NIR laser radiation triggered release using a saccharide derivative, which is capable of interacting with boronic acid functionalized gold nanoparticles as nanoscopic caps, has also been realized.[9] Based on displacement reaction between an antibody, an antigen and a hapten, antibody-gated mesoporous nanocontainers have also been demonstrated.[10] Despite the significant progress made, truly useful delivery systems with practical application are still in demand, and the use of biomolecules such as peptide sequences, DNA or collagen as "bio-gates" is still in its infancy.[11]



Scheme 1 Schematic illustration of enzyme inhibition mechanism-triggered release of guest molecules from the pores of functionalized mesoporous silica materials.

Herein, we report a glucose-responsive controlled-release system **(MSN-anchor-RB)@GOD**. The design strategy relies on the unique interaction between an inhibitor, an enzyme and a substrate (Scheme 1). Specifically, mesoporous silica materials are functionalized on the pore outlets with an inhibitor as an "anchor", and the "bio-gate" is built through the combination of the capping agent and the anchor. Opening the pore to release the entrapped guest molecules can be accomplished through effective competitive combination in the presence of a certain substrate.

To prove the working hypothesis, we employed MCM-41 as a carrier in this investigation. The external surface of MCM-41 was first functionalized with D-(+)-glucosamine, an effective inhibitor of GOD. As a proof-of-principle experiment, the pores were loaded with rhodamine B (RB), which was utilized as a model drug for convenient detection. GOD was selected as the capping agent because it can combine with D-(+)-glucosamine anchored outside the pores to form an enzyme–inhibitor (EI) complex, which acts as a "bio-gate", resulting in the closing of the mesopores. The opening event will occur by a highly effective competitive combination of glucose (substrate) and GOD, which forms the enzyme–substrate (ES) complex, then uncaps the pores and releases the entrapped guest molecules.

To prepare the glucose responsive controlled-release system **(MSN-anchor-RB)@GOD**, the mesoporous scaffold MCM-41

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was first synthesized by a base-catalyzed sol–gel procedure. Before the surfactant *n*-cetyltrimethylammonium bromide (CTAB) was removed from the mesopores of the nanoparticles, the compound prop-2-yn-1-yl(3-(triethoxysilyl)-propyl)carbamate was anchored to the outlets of MSNs to obtain the organic molecule functionalized MSNs (**MCM-41-alkynyl**, see detailed synthesis in the ESI†). The successful removal of CTAB and formation of the mesoporous structure under acidic conditions were confirmed by disappearance of peaks at 2940 cm^{-1}, 2830 cm^{-1} and 1510 cm^{-1}, which are characteristic peaks of CTAB in the FT-IR spectroscopy (**MSN-alkynyl**, Fig. S1, ESI†). Dynamic light scattering (DLS) of **MSN-alkynyl** showed that the particle size of **MSN-alkynyl** is about 100 nm (Fig. S2, ESI†). The amount of prop-2-yn-1-yl(3-(triethoxysilyl)propyl)carbamate in **MSN-alkynyl** was determined to be 1.21 mmol g^{-1} by elemental analysis. Then the inhibitor D-(+)-glucosamine was grafted onto the **MSN-alkynyl** through click reaction between the alkynyl moiety on **MSN-alkynyl** and the azido group formed by azide treatment of D-(+)-glucosamine. The resulting **MSN-anchor** was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM (Fig. S3a, ESI†) showed that as-synthesized **MSN-anchor** were all uniform and granular microspheres. And it could be seen from the TEM images (Fig. S3b–d, ESI†) that **MSN-anchor** have hexagonally arranged pores and the diameter was about 100 nm, which was consistent with DLS results. The amount of D-(+)-glucosamine in **MSN-anchor** was determined to be 0.56 mmol g^{-1} by elemental analysis.

After **MSN-anchor** was soaked in a PBS solution (20 mM, pH 7.4) containing RB, and then capped by GOD, we got the as-synthesized product **MSN-anchor-RB** and the target product **(MSN-anchor-RB)@GOD** respectively. Continuous decrease in intensity of powder X-ray diffraction (XRD) patterns of the **MSN-alkynyl**, **MSN-anchor**, **MSN-anchor-RB** and **(MSN-anchor-RB)@GOD** could be ascribed to surface modification and the pore-filling effect (Fig. S4, ESI†). Nitrogen adsorption–desorption isotherms of **MSN-alkynyl**, **MSN-anchor**, **MSN-anchor-RB** and **(MSN-anchor-RB)@GOD** showed typical type IV isotherms of mesoporous silica materials (Fig. S5–S8, ESI†). No remarkable differences in appearance and average particle size of **MSN-anchor-RB** and **(MSN-anchor-RB)@GOD** were observed compared with those of **MCM-41-alkynyl**, **MSN-alkynyl** and **MSN-anchor**. However, the meaningful reduction in surface area, average mesopore diameter and pore volume further confirmed that **MSN-anchor-RB** was loaded with a large number of RB (Table S1, ESI†). And the amount of RB loaded in **MSN-anchor-RB** was determined to be 33.81 mg g^{-1} (Fig. S9, Table S2, ESI†). The results confirmed the advantage of high load capacity of MSNs because of their large pore volumes. Therefore, a large number of molecules can also be loaded with fewer particles, such as drugs and biological macromolecules into the body, which can improve the efficiency of controlled release greatly.

The amount of GOD immobilized on the **(MSN-anchor-RB)@GOD** was determined to be 272 mg g^{-1}. And their activity was calculated to be 26.2 U mg^{-1}, which accounted for 52.5% of that (50 U mg^{-1}) of free GOD. It suggested that the enzyme immobilized on the **(MSN-anchor-RB)@GOD** was still active after immobilization (Fig. S10, ESI†).

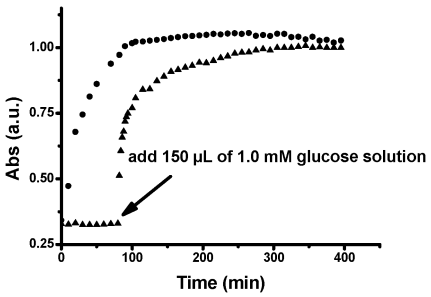

Fig. 1 The release curves of **MSN-anchor-RB** and **(MSN-anchor-RB)@GOD** in PBS solution (20 mM, pH 7.4) (●: uncontrolled release of rhodamine B from **MSN-anchor-RB**; ▲: controlled release of rhodamine B from **(MSN-anchor-RB)@GOD** by adding glucose solution (0–80 min: without glucose; 80–360 min: with 150 μL of glucose solution, 1.0 mM)).

In order to investigate the controlled delivery properties of the resulting GOD-capped FMSM system, 6 mg **(MSN-anchor-RB)@GOD** was placed in a centrifugal tube with 3 mL of phosphate-buffered saline (PBS) (20 mM, pH 7.4) in it. The centrifugal tube was centrifuged every 3 min, then some of the supernatant was drawn into a cuvette. The reversible enzyme inhibition mechanism-triggered release of cargo molecules was monitored using absorbance spectroscopy. The control experiment using **MSN-anchor-RB** was also performed in the same way.

As illustrated in Fig. 1, for **MSN-anchor-RB**, dye release was quickly completed within 100 min. In contrast, prior to the addition of glucose solution, a negligible dye release from **(MSN-anchor-RB)@GOD** could be detected. It indicated that **(MSN-anchor-RB)@GOD** can make "no premature release". Then 150 μL of glucose solution (1.0 mM) was added to the centrifugal tube to uncap the gates of **(MSN-anchor-RB)@GOD** by combining with GOD to form an ES complex. It could be observed from Fig. 1 that the absorbance intensity gradually increased after glucose solution was added until 360 min, which implied that the trapped RB molecules were released from **(MSN-anchor-RB)@GOD** progressively with the number of pores opened increasing.

The release profile shown in Fig. 2 also exhibited that there was nearly no leakage of RB if glucose was not added (Fig. 2, 0–80 min). When 1 μL of glucose solution (1.0 mM) was added to uncap the gates of **(MSN-anchor-RB)@GOD**, the absorbance

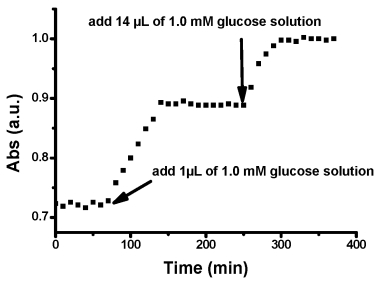

Fig. 2 Controlled release of rhodamine B from **(MSN-anchor-RB)@GOD** by adding different concentrations of glucose (0–80 min: without glucose; 80–260 min: with 1 μL of glucose solution, 1.0 mM; 260–360 min: with 14 μL of glucose solution, 1.0 mM).

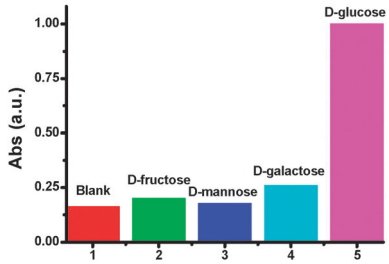

Fig. 3 The selectivity profiles of **(MSN-anchor-RB)@GOD** for different saccharides (glucose: 1.0 mM, others: 10.0 mM; 30 μL, PBS).

intensity increased gradually and reached a plateau (140–260 min), which could be ascribed to that the glucose has reacted adequately with GOD. The absorbance intensity increased continuously and reached a plateau again at 325 min when more 14 μL of glucose solution (1.0 mM) was added to the centrifugal tube at 260 min. The results indicated that the amount of RB molecules released was proportionally dependent on the amount of glucose added. This behavior can be ascribed to the different degree of ES complex formation along with the difference in the concentration of glucose, which implied that the drug delivery for the GOD-capped FMSM system was mediated by the glucose concentration, thus affording the enzyme inhibition mechanism-triggered controlled delivery.

To study the selectivity of **(MSN-anchor-RB)@GOD** for different monosaccharides, selective response experiments were carried out accordingly under the same conditions. As shown in Fig. 3, notably **(MSN-anchor-RB)@GOD** had high selectivity for glucose and almost no response for other monosaccharides such as D-(+)-fructose, D-(+)-mannose and D-(+)-galactose. This is ascribed to the specific binding between GOD and glucose.

In conclusion, we have developed a promising "intelligent" controlled delivery system based on the competitive combination between an inhibitor, an enzyme and a substrate for the first time. In the present design, we use D-(+)-glucosamine, glucose oxidase (GOD) and glucose as models to verify this hypothesis. D-(+)-Glucosamine, an inhibitor of glucose oxidase (GOD), is anchored outside the pores of MSNs. Then the mesopores of MSNs are capped by glucose oxidase (GOD) which can combine with D-(+)-glucosamine to form an EI complex. Due to the competitive combination between the substrate, enzyme and inhibitor, effective guest molecules release was triggered by introduction of glucose. In addition, **(MSN-anchor-RB)@GOD** had a perfect selectivity for glucose over other monosaccharides. Because of the wide use of the enzyme inhibition mechanism, this proof of principle might pave the way for a new generation of stimuli-responsive delivery systems, and could also provide a general route for the use of other enzymes as "bio-gates" in the field of versatile controlled delivery nanomaterials.

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