Glucose-Responsive Controlled Release System Using Glucose Oxidase-Gated Mesoporous Silica Nanocontainers

Mengjie Chen,a Chusen Huang,a Chunsheng He,a Weiping Zhu,*a Yufang Xu,a and Yunfeng Lu*b

a Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Meilong Road 130, Shanghai 200237, China. E-mail: wpzhu@ecust.edu.cn.

b Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California, 90095 (USA). E-mail: luucla@ucla.edu

1. Experimental Section

Materials. n-cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), and triethoxy(3-isocyanatopropyl)silane, propargyl alcohol, D-(+)-glucosamine hydrochloride, Rhodamine B, sodium ascorbate and glucose oxidase (GOD) were purchased from Aladdin and used without further purification. Toluene (PhMe) was distilled from sodium metal and indicated by benzophenone. Other chemicals are of analytical reagent grade, purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China) and used without further purification. PBS solution (20 mmol/L, pH 7.4) was prepared and used as the solvent for all the release experiments of Rhodamine B.

Methods. 1H NMR and 13C NMR spectra were obtained on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (TMS as internal standard). Melting points were recorded by a Büchi Melting Point B-540 micro-melting point apparatus and were uncorrected.
Electrospray ionization (ESI) mass spectrometry was performed in a HP 1100 LC-MS spectrometer. Absorption spectra were recorded by a Varian Cary 100 UV-vis spectrophotometer.

2. Synthesis of the compounds

Synthesis of the compound 1a

To a 250 mL cigar shaped flask 75 mL of anhydrous ethanol was added and cooled to 0 °C. AcCl (21.3 mL, 300.0 mmol) was added dropwise at 0 °C to this solution and the resulting mixture was stirred for 15 min at room temperature to get the HCl-EtOH solution.
Sulfuryl chloride (16.1 mL, 200.0 mmol) was added drop-wise to an ice-cooled white suspension of NaN₃ (13.00 g, 200.0 mmol) in MeCN (400 mL). The mixture was stirred overnight at room temperature and allowed to cool down by ice bath before imidazole (25.90 g, 380.0 mmol) was added. The resulting white suspension was stirred for 3 h at room temperature. The mixture was diluted with EtOAc (400 mL) and washed with H₂O (2×400 mL) and saturated aqueous NaHCO₃ (2×15 mL), then dried over MgSO₄ and filtered. The HCl-EtOH solution prepared above was added drop-wise to the ice-cooled filtrate with stirring, the mixture was chilled in an ice-bath, filtered and the filter cake was washed with EtOAc (3×100 mL) for 3 times to give compound 1a as colourless needles (25.27 g, 60.5%). m.p. 101.2-103.4 °C (Lit.,¹ 100-102 °C).

### 1H NMR (400 MHz, D₂O, TMS, 298K): δ 9.38 (d, J = 5.2 Hz, 1H), 7.96 (s, 1H), 7.55 (s, 1H); 13C NMR (100 MHz, D₂O, TMS, 298K): δ 137.64, 123.05, 120.13.

### Synthesis of the compound 1b ¹[1]

Compound 1a (1.00 g, 4.8 mmol) was added to D-(+)-glucosamine hydrochloride (864.00 mg, 4.0 mmol), CuSO₄•5H₂O (10.00 mg, 40.0 μmol) and K₂CO₃ (1.10 g, 8.0 mmol) in MeOH (20 mL) and the light blue suspension was stirred at room temperature for 2 h. The mixture was concentrated by vacuum distillation and co-evaporated with PhMe (2×40 mL) to give yellow residue. Acetic anhydride (3.0 mL, 32.0 mmol) was added to the residue in C₅H₅N (20 mL) and the mixture was stirred at room temperature for 3 h. The mixture was concentrated by vacuum distillation, diluted with H₂O (80 mL) and extracted with EtOAc (3×45 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated. Silica chromatography (PE/EtOAc = 3/1, v/v) gave the compound 1b as a colourless liquid (1.15 g, 76.4%).

### 1H NMR (400 MHz, CDCl₃, TMS, 293K): δ 5.56 (d, J = 8.4 Hz, 1H), 5.15-5.04 (m, 2H), 4.34-4.30 (m, 1H), 3.82 (d, J = 7.2 Hz, 1H), 3.68 (t, J = 8.4 Hz, 1H), 2.21 (s, 3H), 2.12-2.04 (m, 9H); 13C NMR (100 MHz, CDCl₃, TMS, 298K): δ 170.58, 169.82, 169.65, 168.60, 92.56, 72.71, 69.73, 67.70, 62.53, 61.36, 20.91, 20.72, 20.66, 20.58.

### MS (ESI): Calcd for [M+Na]+, 396.1; Found, 396.1.

### Synthesis of the compound 1c ²[2]

Compound 1b (187.00 mg, 0.5 mmol) was added to LiOH•H₂O (126.00 mg, 3.0 mmol) in the mixture of anhydrous methanol (2 mL), THF (2 mL) and H₂O (1 mL), and the resulting light yellow solution was stirred for 3 h at room temperature. Dowex® X8 (cation exchange resin, 50–100 mesh, pretreated by anhydrous methanol) was added to the mixture. Then the mixture was
stirred for 15 min at room temperature, and then filtered. The filtrate was concentrated by vacuum distillation. Silica chromatography (CHCl₃/MeOH = 8/1, v/v) gave the compound 1c as a light yellow oil liquid (57.00 mg, 69.5%, α/β mixture).

1H NMR (400 MHz, CD₃OD, TMS, 298K): δ 5.20 (s, 1H), 4.53 (d, J = 8.0 Hz, 1H), 3.91-3.79 (m, 4H), 3.74-3.66 (m, 2H), 3.38 (t, J = 9.2 Hz, 1H), 3.11 (t, J = 9.2 Hz, 2H);

13C NMR (100 MHz, CD₃OD, TMS, 298K): δ 95.86, 91.93, 76.60, 75.09, 71.74, 71.19, 70.83, 70.22, 68.09, 63.88, 61.27, 61.16.

MS (ESI): Calcd for [M-H]-, 204.1; Found, 204.3.

3. Synthesis of the mesoporous silica nanoparticles (MSNs)

Synthesis of the MSNs (with CTAB) [4]

n-Cetyltrimethylammoniumbromide (3.00 g, 8.2 mmol) was added to distilled water (1440 mL). To the resulting suspension was added 2 M NaOH (aq) (10.5 mL, 21.0 mmol). Then the temperature of the solution was adjusted to 80 °C before TEOS (15.0 mL, 67.3 mmol) was added dropwise over a period of 1 h to the surfactant solution. The reaction mixture was stirred vigorously at 80 °C for 2 h. The mixture was cooled down, filtered, washed extensively with deionized water and methanol, and dried in 90 °C for 8 h to give 4.85 g of MSNs (with CTAB) as a white power.

Synthesis of the MCM-41-Alkynyl (with CTAB) [5]

Compound 2a (4.08 g, 13.5 mmol) was dissolved in 150 mL of dry toluene before 3.00 g as-synthesized MSNs (with CTAB) was dispersed in the solution under a nitrogen atmosphere. The mixture was stirred vigorously and refluxed for 24 h. The mixture was filtered, the filter cake...
was washed extensively with hexane, dichloromethane, acetone, methanol and water and then dried in air to give 3.81 g of MCM-41-Alkynyl as a pale yellow powder.

**Synthesis of the MSN-Alkynyl (without CTAB)** [4]

To remove the surfactant template (CTAB), 3.00 g of as-synthesized MCM-41-Alkynyl (with CTAB) was refluxed for 24 h in a solution of 18.0 mL of HCl (37.4%) and 320 mL of methanol. The mixture was cooled, filtered, washed extensively with deionized water and methanol, and then dried in air to give 2.14 g of MSN-Alkynyl as a pale yellow powder. Elemental analysis result showed that the content of the compound 2a immobilized on the surface of MSN-Alkynyl is about 1.21 mmol g⁻¹ (Calculated according to C, N content: the C, N content in MSNs (without CTAB) are 2.56% and < 0.3% respectively, while the C, N content in MSN-Alkynyl are 12.74% and 2.10% respectively).

**Synthesis of the MSN-anchor** [6]

Compound 1c (60.00 mg, 0.3 mmol), 214.00 mg as-synthesized MSN-Alkynyl were added into 6 mL of water. To this light yellow suspension was added dropwise CuSO₄•5H₂O (150.00 mg, 0.6 mmol) in 4 mL of water and sodium ascorbate (1.19 g, 6.0 mmol) in 10 mL of water. The resulting mixture was stirred overnight at room temperature. The mixture was filtered, washed extensively with water and then dried in air to give 270.00 mg of MSN-anchor as a light yellow powder. Elemental analysis result showed that the content of the anchor immobilized on the surface of MSN-anchor is about 0.56 mmol g⁻¹ (Calculated according to C, N content: the C, N content in MSNs (with CTAB) are 2.56% and < 0.3% respectively, while the C, N content in MSN-anchor are 11.08% and 3.12% respectively).

**Synthesis of the MSN-anchor-RB** [7]

Rhodamine B (240.00 mg, 0.2 mmol) was dissolved in 25 mL water before 700.00 mg of as-synthesized MSN-anchor was dispersed in the solution under a nitrogen atmosphere. The reaction mixture was stirred vigorously at room temperature for 24 h. The resulted mixture was centrifuged, washed extensively with water, filtered, and then dried in air to give 720.00 mg of MSN-anchor-RB as a rose red powder.

**Synthesis of (MSN-anchor-RB)@GOD** [7]

210.00 mg of Glucose Oxidase (GOD) was dissolved in 15 mL of water, and then 400.00 mg of as-synthesized MSN-anchor-RB was added. The mixture was stirred vigorously at room temperature for 24 h under an Ar atmosphere. The mixture was centrifuged, washed extensively with water, filtered, and then dried with Vacuum Freeze-drying equipment to give 520.00 mg of...
(MSN-anchor-RB)@GOD as a rose red powder.

4. Characterization of the mesoporous silica nanoparticles (MSNs)

**Material characterization.** FT-IR spectra (4000-500 cm\(^{-1}\)) in KBr were collected on a Nicolet NEXUS 470 FT-IR spectrometer. SEM images of the particles were taken by Hitachi S-520 scanning electron microscopy to assess the particle size and shape. TEM was performed on a JEOL JEM2010 electron microscope, operating at an acceleration voltage of 200 kV. Dynamic light scattering (DLS) measurements were performed by an ALV/CGS-5022F from ALV Ltd. (German). XRD experiments were carried out on a Rigaku D/Max-RB diffractometer with Cu K\(\alpha\) radiation. N\(_2\) adsorption isotherms were measured at 77 K using a Quantachrome Nova 4000e analyzer, and the samples were measured after being outgassed at 423 K overnight. Pore size distributions were calculated using the BJH model. The specific surface areas (SBET) of samples were determined from the linear parts of BET plots.

![FT-IR spectra](image)

**Fig. S1.** FT-IR spectra of MCM-41-Alkynyl (with CTAB, dotted line) and MSN-Alkynyl (without CTAB, solid line) from KBr pellets.

As shown in Fig.S1, the C-H stretch peaks at 2830 cm\(^{-1}\), 2940 cm\(^{-1}\) and bending vibration peaks at 1510 cm\(^{-1}\) in the dotted line indicated that there were CTAB molecules in MCM-41-Alkynyl (with CTAB). While after extraction with acidic MeOH solution, the disappearance of C-H peaks above-mentioned demonstrated the successful removal of the surfactant CTAB (solid line). In addition, the C-H bending vibration peak of water molecules at 1630 cm\(^{-1}\) confirmed the result due to the increase of the hydrophilic silanol groups on the surface.
of MSN-Alkynyl (without CTAB) after removal of CTAB.

![Graph showing particle size distribution](image)

**Fig. S2.** DLS images of MSN-Alkynyl.

It can be seen from the Dynamic Light Scattering (DLS) pattern of MSN-Alkynyl (Fig. S2) that most of the particle size of MSN-Alkynyl distributed at about 100 nm, which was consistent with the SEM and TEM data (Fig. S3).

![SEM and TEM images](image)

**Fig. S3.** SEM (a, scale bar: 50nm) and TEM (b, scale bar: 20nm; c and d, scale bar: 10nm ) images of MSN-anchor.

SEM (Fig. S3a) showed that as-synthesized MSN-anchor were all uniform and granular microspheres. And it could be seen from the TEM (Fig. S3b, 3c, and 3d) that MSN-anchor have hexagonally arranged pores and the diameter was about 100 nm, which was consistent with the DLS results.
Fig. S4. Small Angel X-ray Diffraction patterns of MSNs (without CTAB), MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD.

Low angle diffraction with a 2θ range of 1 to 8° was used to investigate the long-range order of the materials. As shown in Fig. S4, the 2θ of MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD were all close to 2.0, which was typical small-angle X-ray diffraction peak of MSNs. It indicated that these particles retained the structural characteristics of mesoporous materials perfectly without change with the surface functionalization and the loading conditions in the mesopores of MSNs.

Fig. S5. Nitrogen adsorption-desorption isotherm for MSN-Alkynyl.
Fig. S6. Nitrogen adsorption-desorption isotherm for MSN-anchor.

Fig. S7. Nitrogen adsorption-desorption isotherm for MSN-anchor-RB.
The surface areas, average pore diameters and pore volumes of MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD were measured through N₂ adsorption/desorption measurement at 77K using a Quantachrome Nova 4000e analyzer. The samples were measured after being degassed at 423 K overnight. Pore size distributions, specific surface areas and pore volumes were calculated using the Barrett-Joyner-Halenda (BJH) methods, respectively. As shown in Fig.S5, S6, S7 and S8, MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD exhibited characteristic Type IV BET isotherms, which were typical isotherms for mesoporous materials. This was consistent with XRD results, which confirmed that these particles remained perfect mesoporous structure again. In addition, as outlined in Table S1, the average pore diameter of MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD were 3.921, 3.852, 2.369 and 2.082 nm, which were located in the pore diameter range (2~50 nm) divided by International Union of Pure and Applied Chemistry (IUPAC). This indicated that the mesoporous structure of these particles didn’t change with a series of functionalization and dye-loading. However, the average pore diameter and pore volume of MSN-anchor-RB were far lower than those of MSN-anchor (Table S1), it demonstrated that Rhodamine B molecules had successfully entered the mesopores of MSN-anchor-RB.
Table S1 BET surface areas, average pore diameters and pore volumes calculated from the N$_2$ Adsorption-Desorption Isotherms of MSN-Alkynyl, MSN-anchor, MSN-anchor-RB and (MSN-anchor-RB)@GOD.

<table>
<thead>
<tr>
<th>Material</th>
<th>BET Surface Area [m$^2$/g]</th>
<th>Average Pore Diameter [nm]</th>
<th>Pore Volume [cm$^3$/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSN-Alkynyl</td>
<td>1138</td>
<td>3.921</td>
<td>0.875</td>
</tr>
<tr>
<td>MSN-anchor</td>
<td>972</td>
<td>3.852</td>
<td>0.813</td>
</tr>
<tr>
<td>MSN-anchor-RB</td>
<td>508</td>
<td>2.369</td>
<td>0.303</td>
</tr>
<tr>
<td>(MSN-anchor-RB)@GOD</td>
<td>517</td>
<td>2.082</td>
<td>0.289</td>
</tr>
</tbody>
</table>

5. Estimation of the amount of Rhodamine B loaded in (MSN-anchor-RB)@GOD

In order to investigate the amount of Rhodamine B loaded in (MSN-anchor-RB)@GOD, 1 mg, 3 mg and 6 mg of (MSN-anchor-RB)@GOD were placed in centrifugal tubes which had 3 mL of phosphate-buffered saline (PBS) (20 mM, pH 7.4; 553 nm) in it, respectively. The suspensions were homogenized by ultrasonic vibration for 15 min and then were determined the absorption intensity by UV-visible spectrophotometer rapidly. The results were shown in Table S2. The standard curve for Rhodamine B was estimated by determination of the absorption intensity of different concentrations of Rhodamine B, and the UV absorption intensity-Rhodamine B concentration standard curve was shown as follows:

**Fig. S9.** Standard curve of UV absorption intensity of Rhodamine B as a function of its concentration (Rhodamine B : 0~25×10$^{-5}$ M; PBS: 20mM, pH 7.4; 553 nm)

In order to investigate the amount of Rhodamine B loaded in (MSN-anchor-RB)@GOD, 1 mg, 3 mg and 6 mg of (MSN-anchor-RB)@GOD were placed in centrifugal tubes which had 3 mL of phosphate-buffered saline (PBS) (20 mM, pH7.4) in it, respectively. The suspensions were homogenized by ultrasonic vibration for 15 min and then were determined the absorption intensity by UV-visible spectrophotometer rapidly. The results were shown in Table S2. The standard curve for Rhodamine B was estimated by determination of the absorption intensity of different concentrations of Rhodamine B, and the UV absorption intensity-Rhodamine B concentration standard curve was shown as follows:
\[ A = 0.02426 + 0.08066 \times 10^5 C \] (1)

The Rhodamine B molecules loaded in \((\text{MSN-anchor-RB})@\text{GOD}\) was calculated by the data in Table S2 according to the standard curve for Rhodamine B and the value was \(7.06 \times 10^{-2}\) mmol g\(^{-1}\), namely \(33.81\) mg g\(^{-1}\) of \((\text{MSN-anchor-RB})@\text{GOD}\). This confirmed the advantage of high load capacity of MSNs because of their structural properties like big pore volumes. Therefore, a large number of molecules, such as drugs and biological macromolecules can also be loaded with fewer particles into the body, which may improve the efficiency of controlled release greatly.

Table S2 Ultraviolet absorption intensity of \((\text{MSN-anchor-RB})@\text{GOD}\) with different masses

<table>
<thead>
<tr>
<th>Masses of (MSN-anchor-RB)@GOD (mg)</th>
<th>6.0</th>
<th>3.0</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV absorption intensity</td>
<td>1.394</td>
<td>0.703</td>
<td>0.253</td>
</tr>
</tbody>
</table>

6. Estimation of the amount and activity of the glucose oxidase immobilized on the \((\text{MSN-anchor-RB})@\text{GOD}\)

The amount of glucose oxidase immobilized on the \((\text{MSN-anchor-RB})@\text{GOD}\) was determined by comparing the absorbance of the \((\text{MSN-anchor-RB})@\text{GOD}\) solution and the free glucose oxidase solution on UV spectrophotometer at 280 nm.

To measure the activity of the glucose oxidase immobilized on \((\text{MSN-anchor-RB})@\text{GOD}\), 0.5 mL of \(\beta\)-D-glucose solution (0.55 M in PBS at pH 7.0), 0.1 mL of horse radish peroxidase (3.4 mg in 10 mL of H\(_2\)O with ice water cooling) and 2.4 mL of o-dianizidine solution (1.7 mg in 25 mL of PBS at pH 7.0) were mixed in a cuvette at 25 \(^\circ\)C. After 0.1 mL of the sample solution ((MSN-anchor-RB)@GOD in PBS at pH 7.0) was added to the mixture, the increase in absorbance was measured immediately at 436 nm on UV spectrophotometer for 15 min. The control experiment on free glucose oxidase solution was also performed with the same amount of glucose oxidase as that immobilized on \((\text{MSN-anchor-RB})@\text{GOD}\).

Fig. S10 Activity comparison between the glucose oxidase immobilized on the \((\text{MSN-anchor-RB})@\text{GOD}\) (●) and free glucose oxidase (▲).
7. Dye release

In order to investigate the delivery properties of the GOD-capped FMSM system, 6 mg of MSN-anchor-RB and (MSN-anchor-RB)@GOD was placed in a centrifugal tube which had 3mL phosphate-buffered saline (PBS) (20 mM, pH7.4) in it respectively. The centrifugal tube was centrifuged for every 3 min then some of the supernatant was drawn to a cuvette respectively. The release of Rhodamine B was measured by absorbance spectroscopy at different periods of time. For (MSN-anchor-RB)@GOD, 150 μL of glucose solution (1 mM) was added to the cuvette to release the trapped Rhodamine B at 80 min. The release of MSN-anchor-RB was measured under the same condition but without glucose.

For the release curve of (MSN-anchor-RB)@GOD with different concentrations of glucose, 6 mg of (MSN-anchor-RB)@GOD was placed in a centrifugal tube which was equipped with 3 mL of phosphate-buffered saline (PBS) (20 mM, pH7.4). The centrifugal tube was centrifuged for every 3 min then some of the supernatant was drawn to a cuvette respectively. The release of Rhodamine B was measured by absorbance spectroscopy at different periods of time. Firstly, 1 μL of glucose solution (1 mM) was added to the cuvette to release the trapped Rhodamine B at 80 min. When the absorbance intensity tended to be stable for over 60 min (at 265 min), more 14 μL of glucose solution (1 mM) was added to the cuvette. The release of Rhodamine B was measured by absorbance spectroscopy at different periods of time.

8. Selectivity

30 μL of 1 mM glucose solution, 10 mM D-(+)-fructose, D-(+)-mannose and D-(+)-galactose solution was added to four centrifugal tubes with 6 mg of (MSN-anchor-RB)@GOD and 3 mL of phosphate-buffered saline (PBS) (20 mM, pH7.4) in them, respectively. The centrifugal tube was centrifuged for every 3 min then some of the supernatant was drawn to a cuvette respectively. The release of Rhodamine B was measured by absorbance spectroscopy at different periods of time until the absorbance intensity tended to be stable for over 60 min.

9. References
