SUPPORTING INFORMATION

Biomedical Nanomotors: Efficient Glucose-Mediated Insulin Release

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Supporting videos description

**Supporting Video S1.** Propulsion comparison between MS-Au nanomotors and Au nanomotors under an ultrasound field (US field: 6 V, 2.66 MHz).

**Supporting Video S2.** US-propulsion of an insulin-loaded MS-Au nanomotor into 10 mM glucose solution (US field: 6 V, 2.66 MHz).

**Supporting Video S3.** US-propulsion of an In-loaded MS-Au nanomotor: US On and Off (6 V, 2.66 MHz).

Materials and Methods

*Reagents and solutions*

Gold plating solution (Orotemp 24 RTU RACK) was acquired from Technic Inc. and phosphate buffered saline solution was purchased from Invitrogen. Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic P123, Mn = 5800), yeast expressed human recombinant insulin (Ins), glucose oxidase (GO, EC 1.1.3.4, from *Aspergillus niger*, Type VII, ≥100,000 units/g) and all other reagents (analytical grade) were purchased from Sigma-Aldrich. Ultrapure MilliQ water was employed in all experiments.

*Equipments and instruments*

Videos were captured by using a Cool SNAP HQ² camera provided with 20× and 40× objectives (unless mentioned otherwise) and acquired at the frame rate of 10 using the Metamorph 7.1 software (Molecular Devices, Sunnyvale, CA).
The ultrasound experiments were performed in an acoustic resonator setup, which consist in a piezoelectric transducer (Ferroperm PZ26 disk 10 mm diameter, 0.5 mm thickness), attached by conductive epoxy glue to the bottom center of a steel plate (50 mm × 50 mm × 0.94 mm). The top center of the steel plate had a sample reservoir (5 mm wide, 249 µm deep) made with Kapton tape protective layer. A glass slide was used to cover the reservoir, for ultrasound reflection and to protect the sample. The continuous ultrasound sine wave was applied via a piezoelectric transducer, through an Agilent 15 MHz arbitrary waveform generator, in connection to a home-made power amplifier. The applied continuous sine wave form had a frequency of 2.66 MHz and 6 V of voltage amplitude. All the experiments were carried out at room temperature.

Synthesis of insulin-Rhodamine B conjugate (In-RB):

To label insulin with the dye, Rhodamine B (10 mg, 20.9 µmol), N-hydroxysuccinimide (154 mg, 1.34 mmol), and ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (22 mg, 0.11 mmol) were dissolved in 2 mL of 50 mM sodium phosphate, pH 6.5, and stirred for 30 min at 4°C. Insulin (5 mg, 0.9 µmol) was further added, and the reaction mixture was stirred overnight at 4°C. The solution was then exhaustively dialyzed versus 50 mM sodium phosphate buffer, pH 7.5, using Amicon Ultra-05 centrifugal filter units with Ultracel-10 membranes (Millipore) and finally concentrated to an about 2.5 mg/mL protein concentration.

Preparation of Janus Au-mesoporous silica (MSF-Au) nanomotors:

Gold nanowires (AuNWs) were first prepared by a common template-directed electrodeposition protocol.[1] A thin gold film was first sputtered on one side of a
porous polycarbonate (PC) membrane template containing 100-nm diameter cylindrical nanopores (Whatman, Catalogue No. 7060-2501) to serve as a working electrode. The membrane was assembled in a Teflon plating cell with aluminum foil serving as an electrical contact for the subsequent electrodeposition. Then, Au was plated using a commercial gold plating solution at $-0.95 \text{ V (vs Ag/AgCl)}$, using a charge of 2 C.

Mesoporous silica thin films (MSF) were further electrodeposited on the exposed Au nanowire circular plane surface.$^{[2-5]}$ A solution mixture consisting of 4.4 mmol tetraethyl orthosilicate, 20 mL ethanol (95%), 20 mL of 0.1 M NaNO$_3$ aqueous solution, 1 mM HCl, and 1.4 mmol Pluronic P123 was prepared under magnetic stirring. The solution was aged for 2.5 h under stirring at 60°C temperature before use. The precursor solution was poured into the plating cell and the silica film electrodeposition was achieved under quiescent conditions by applying a cathodic potential of $-1.3 \text{ V vs Ag/AgCl}$ during 60 s. The membranes were then removed, rinsed with water to avoid undesirable deposition of nanoparticulated structures on the MSF, and then dried and aged overnight at 60°C in an oven.

The sputtered gold layer was removed from the PC membrane by mechanical polishing using cotton tip applicators soaked with alumina powder. The PC template was further dissolved in 2 sequential steps of dipping in methylene chloride for 30 min and 15 min, respectively, to complete release the MSF-Au nanomotors. The resulting nanomotors were purified by centrifugation at 7000 rpm for 30 s, sequentially washed with isopropanol, ethanol, and ultrapure water until a neutral pH was achieved. In each washing step, the nanomotors solution was briefly sonicated to ensure complete
dispersion of nanomotors, and the nanomotors were further separated by centrifugation. The resulting MS-Au nanomotors were stored in 1 mL of ultrapure water at room temperature until use.

Assembly of the enzyme-controlled based nanomachine (GOx/In/MS-Au)

The mesoporous silica surface film in the MS-Au nanomotors was first modified with phenylboronic acid (PBA) residues,\textsuperscript{[6]} by dispersion in a 45 mM ethanolic solution of (3-glycidyloxypropyl) trimethoxysilane (GPTMS) and kept under magnetic stirring at room temperature during 3 h. After washing with ethanol, the nanomotors were centrifuged, and further dispersed in a 45 mM ethanolic solution of 3-aminophenylboronic acid under shaking. The modified nanomotors were centrifuged, and exhaustively washed by stirring during 15 min in 0.1 M HCl in methanol. After another washing step with ultrapure water, the silica nanopores were loaded with the In-RB conjugate by stirring during 5 h at 4ºC in 0.1 M sodium phosphate buffer solution, pH 6.5, containing 1.25 mg/mL In-RB. The insulin-loaded nanomotors were further centrifuged, dispersed in a 5 mg/mL glucose oxidase (GOx) solution in 0.1 M sodium phosphate buffer solution, pH 8.0, and gently stirred at 4ºC overnight. The resulting enzyme-capped nanomotors were washed with 10 mM sodium phosphate buffer solution, pH 8.0, until not protein was detected in the washing solution. The enzyme-functionalized nanomotors were stored in 1 mL of 10 mM sodium phosphate buffer solution, pH 7.0, at 4ºC until use.

Glucose-dependent insulin delivery from MS-Au nanomotors

For the insulin delivery experiments, $7 \times 10^5$ MS-Au nanomotors were initially suspended in 500 µL of 10 mM sodium phosphate buffer (pH 7.5), and incubated at
25°C for 30 min. Different aliquots of this solution were taken, centrifuged to remove the nanomaterial, and the absorbance of the non-specific In-RB release (in absence of enzymatic substrate) was measured at 558 nm. After 30 min incubation in PBS, 10 mM D-glucose substrate was added to the MS-Au nanomotors suspension, and the mixture was treated with an US field (6 V, 2.66 MHz) for 5 min. The absorbance of the nanomotors solution was again measured at 558 nm.

For the insulin release experiment in response to different D-glucose concentrations, the MS-Au nanomotors were incubated in PBS, and, after 30 min incubation, the corresponding D-glucose concentration (0, 5, 10, 25, 50, or 200 nM) was added to the nanomotors solution and the US field was applied for 5 min; the absorbance of the solution was finally measured at 558 nm after 120 min of the D-glucose addition.

Supporting figures

Figure 1S. TEM images of MS-Au nanomotors.
Figure 2S. FT-IR analysis of MS-Au nanomotors before (a) and after (b) functionalization with phenylboronic acid residues. The peak at 1332 cm⁻¹ in the FT-IR spectrum of PBA-modified MS-Au nanomotors can be associated with the C-B vibrations, the peaks around 1435-1638 cm⁻¹ show the vibrations of phenyl groups, and the peak at 2895 cm⁻¹ can be ascribed to the C-H stretching in phenyl groups.

Figure 3S. UV-Vis spectra of insulin (a) and insulin-Rhodamine B conjugate (b).
References