Supplementary Information

An ATP-responsive smart gate fabricated with a graphene oxide-aptamer-nanochannel architecture

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I. Experimental section

Reagents and materials. PAAOM (diameter of the nanochannels is ca. 25 nm) was purchased from Whatman Co. Ltd. 6-Mercaptohexanol (MCH), tris-(2-carboxyethyl)-phosphine (TCEP), ethylenediaminetetraacetic acid (EDTA), adenosine triphosphate (ATP), bovine serum albumin(BSA), graphene oxide (GO), glucose, chloroauric acid ($HAuCl_4$), trisodium citrate, 2,2'-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS), glucose oxidase (GOx), and horse radish peroxidase (HRP) were purchased from Sigma-Aldrich. All the reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >18 MΩ cm. The thiolated ABA was supplied by Invitrogen with a sequence as follows: 5'-SH-C_6-AAA ACC TGG GGG AGT ATT GCG GAG GAA GGT-3'. The original concentration of the prepared AuNPs was estimated to be 14 nM, which was calculated from the quantity of starting material ($HAuCl_4$) and the size of the nanoparticles.

Synthesis of gold nanoparticles. Colloidal AuNPs with average diameter of 13 nm are synthesized using a citrate reduction method.[1] Briefly, trisodium citrate (5 mL, 38.8 mM) was added to a boiling solution of $HAuCl_4$ (50 mL, 1 mM) under reflux and rapid stirring. After further 30 min of boiling, the color of the solution changed from pale yellow to burgundy. Then, the solution was slowly cooled down to room temperature and filtered through a 0.8 μm membrane. Thus, citrate-stabilized colloidal AuNPs were prepared, and were kept at 4 °C while not in use.

Preparation of ABA-PAAOM Conjugate. A thin layer of homogeneous gold film (ca. 5 nm) was first sputtered onto one side of the PAAOM using a Q150R S Sputter Coater (Quorum, England). 200 μL of ABA solution (5 uM in a Tris-EDTA buffer containing 10 mM TCEP, pH 7.4) was then dropped onto the gold-plated PAAOM, and incubated at 4 °C for 18 h to form a stable self-assembled ABA layer through an "Au-S" bond. Subsequently, the PAAOM was immersed in a MCH solution (2 mM) at room temperature for 1 h to wipe off nonspecific adsorption of ABA. The prepared ABA-modified PAAOM was finally rinsed with doubly distilled water, and dried with nitrogen, and was ready for use. The surface conditions of the PAAOM were

characterized using a 5500 Atomic Force Microscopy (Agilent, America).

Fabrication of ATP-responsive gate. For the ON mode, the surface of the ABA-PAAOM was allowed to react with a certain concentration of ATP at room temperature for 1 h, while in the case of the OFF mode, this step was ignored. The ABA-PAAOM was then allowed to interact with a certain concentration of GO at room temperature for another 1 h. After being rinsed gently with doubly distilled water, the PAAOM (ATP-ABA-PAAOM in "ON" mode, or GO-ABA-PAAOM in "OFF" mode) was fixed in a detachable filter device that was connected to a syringe. 2 mL of different solutions (doubly distilled water only, glucose, BSA, or colloidal AuNPs) was injected into the syringe. Under an extra pressure of 0.1 atm, the solution flowed slowly through the PAAOM; and the effluent solutions were collected. For the flux of water, the flow velocity was measured by timing; while for the flux of glucose, BSA, and colloidal AuNPs, the concentrations after filtration were determined. In detail, in the case of glucose, colorimetric detection of glucose was achieved by using a cascade reaction catalyzed by GOx and HRP, which might turn glucose to gluconic acid and ABTS to green colored ABTS* (reaction equations shown in Eq. (1) and (2)). The absorption of ABTS* at 420 nm was linear to the concentration of glucose, and thus was adopted for the determination of the concentration of glucose. In the case of BSA and AuNPs, the direct absorptions at 280 nm and 520 nm were measured respectively for the determination of the concentrations. All the UV-vis measurements were carried out using a UV-2450 spectrophotometer (Shimadzu, Japan). To regenerate the gate, the GO-covered PAAOM (OFF mode) was sonicated in an ethanol solution containing 50 mM 1-pyrenecarboxylic acid for 5 min to destroy the π-π stacking between ABA and GO, followed by rinsing gently with doubly distilled water. While for the ATP-bound PAAOM (ON mode), it was sonicated in an aqueous solution containing 7 M urea for 5 min to destroy the hydrogen bonds between ATP and ABA.

$$\text{Glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2 \quad \text{(under the catalysis of GOx)} \quad (1)$$

$$H_2O_2 + \text{ABTS} \rightarrow H_2O + \text{ABTS*} \quad \text{(under the catalysis of HRP)} \quad (2)$$

II. Supporting Figures

Table S1 Detailed data of the flux of glucose, BSA, and AuNPs through the nanochannels in "ON" and "OFF" mode, respectively. Each group contains at least three parallel samples.

			C_{ori}	C_{eff}	*Interception*
Glucose	ON	Group I	1000 / μM	953.3±26.9 / μM	4.7±2.7%
		Group II	200	193.7±4.2	3.2±2.1%
		Group III	50	48.1±2.7	3.8±5.4%
		average	/	/	**3.9±3.4%**
	OFF	Group I	1000 / μM	516.3±17.2 / μM	48.4±1.7%
		Group II	200	87.8±8.6	56.1+4.3%
		Group III	50	27.9±3.1	44.2±6.2%
		average	/	/	**49.6±4.1%**
BSA	ON	Group I	20 / μM	18.94±0.32 / μM	5.3±1.6%
		Group II	10	9.77±0.18	2.3±1.8%
		Group III	5	4.66±0.19	6.8±3.8%
		average	/	/	**4.8±2.4%**
	OFF	Group I	20 / μM	4.50±0.61 / μM	77.5±3.1%
		Group II	10	2.36±0.74	76.4±7.4%
		Group III	5	1.02±0.43	79.6±8.6%
		average	/	/	**77.8±6.4%**
AuNPs	ON	Group I	14 / nM	13.43±0.25 / nM	4.1±1.8%
		Group II	3.5	3.42±0.06	2.3±1.7%
		Group III	1.4	1.32±0.07	5.7±5%
		average	/	/	**4.0±2.8%**
	OFF	Group I	14 / nM	0.26±0.18 / nM	98.1±1.3%
		Group II	3.5	0.08±0.06	97.7±1.7%
		Group III	1.4	0.04±0.04	97.1±2.9%
		average	/	/	**97.6±1.9%**

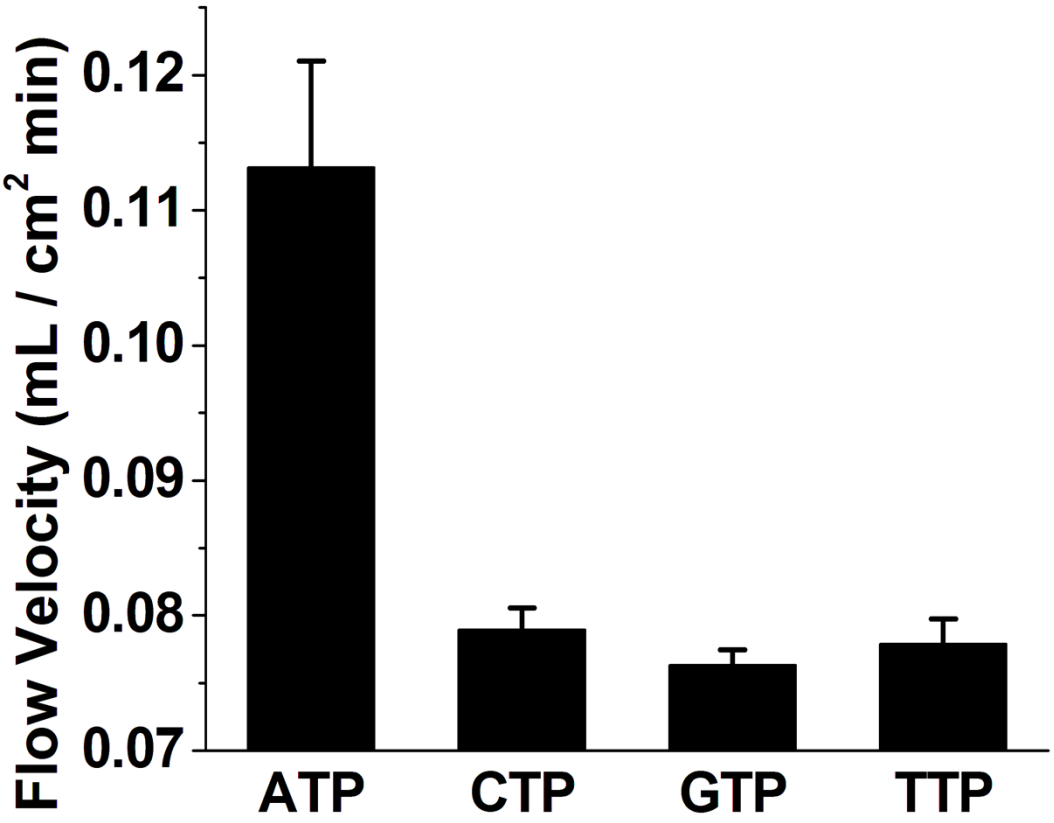

Fig. S1 Specificity of the smart gate in the "ON" mode towards different stimulus.

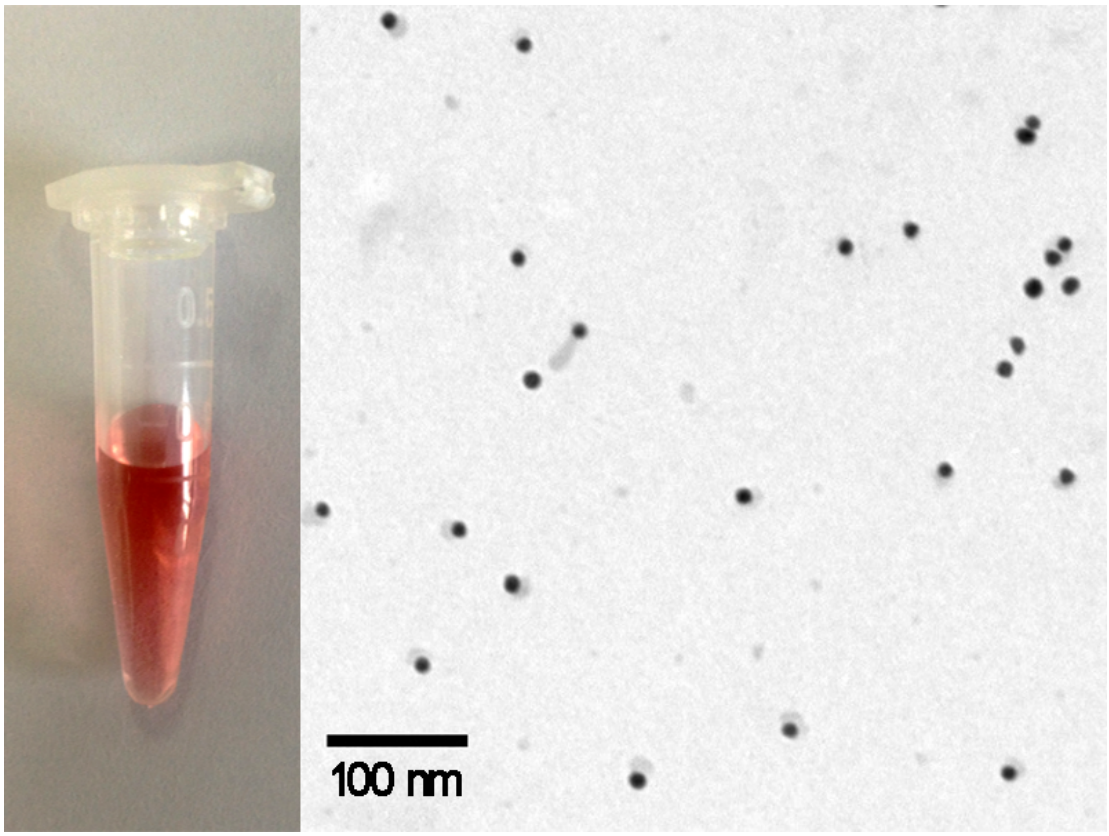

Fig. S2 Characterization of synthetized AuNPs under digital camera (left) and transmission electron microscope (right). Diameter of the AuNPs is measured to be ca. 13 nm.

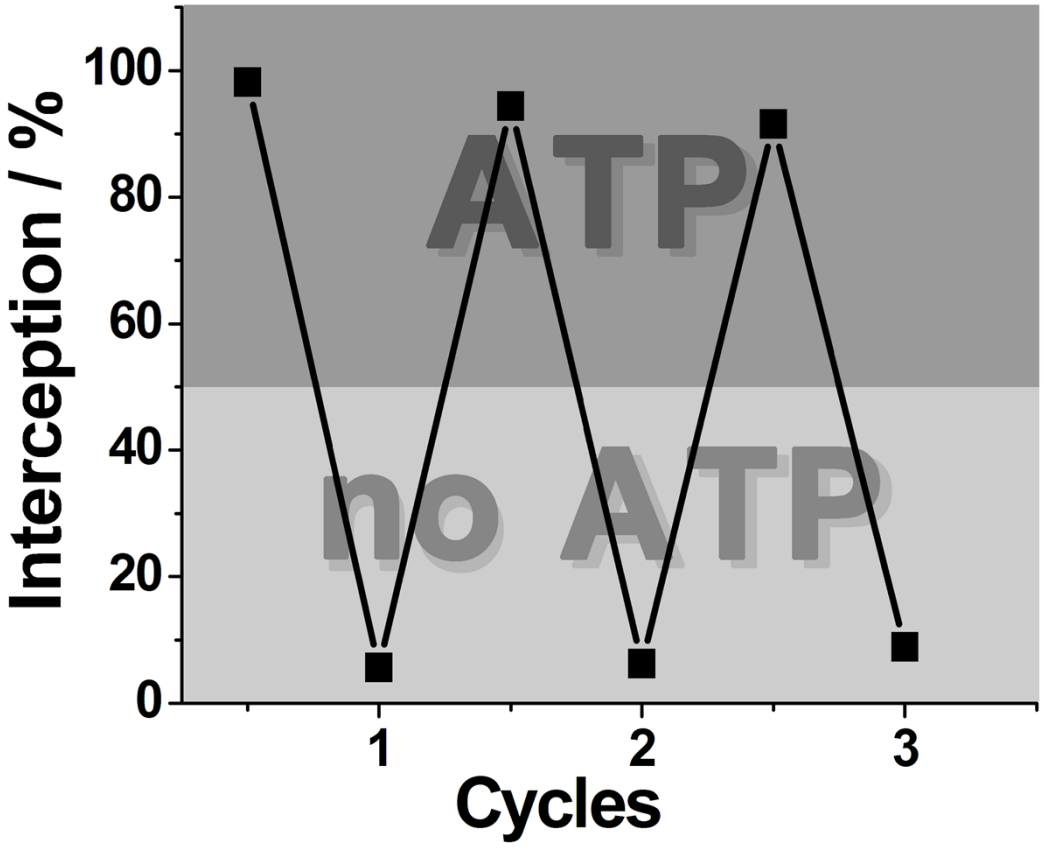

Fig. S3 Reversibility of the smart gate in three cycles.

References

1 X. L. Zhu, J. Zhao, Y. Wu, Z. M. Shen, G. X. Li, *Anal. Chem*., 2011, **83**, 4085–4089.