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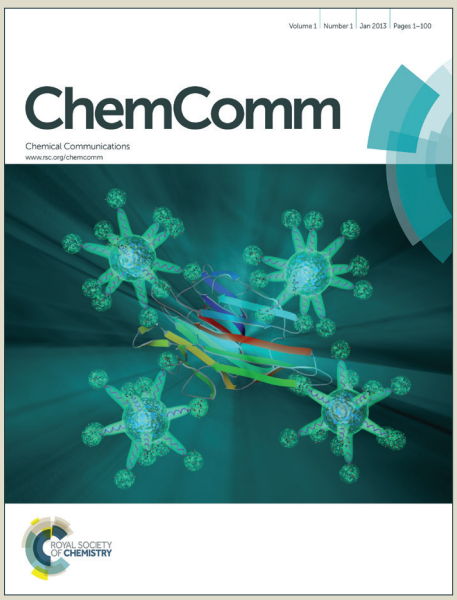

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An ATP-responsive smart gate fabricated with a graphene oxide-aptamer-nanochannel architecture

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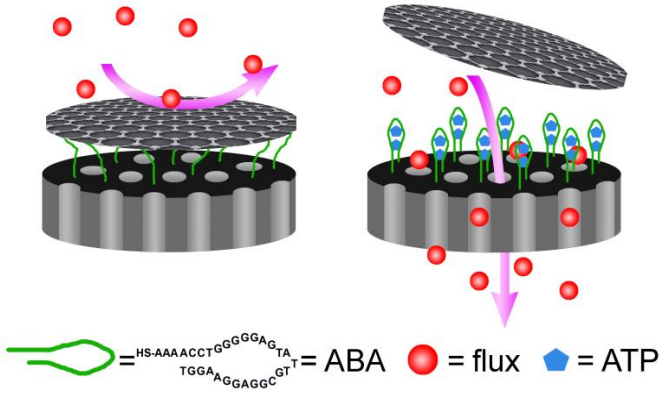
Here, we report a graphene oxide-aptamer-nanochannel architecture for the fabrication of a novel stimuli-responsive gate. The gate is OFF in the absence of ATP, and is switched to be ON when ATP is presented. The concept we proposed may contribute to a versatile platform for the development of stimuli-responsive gates.

Stimuli-responsive gates (SRG) also referred to as smart gates are nanoporous membranes that switch ON and OFF under the regulation of physicochemical triggers, including temperature,[1] pH,[2] ionic strength,[3] molecules,[4] etc.[5] In comparison with ordinary gates that always keep an ON state, the SRG make things controllable and thus have wide application prospect in drug delivery, separation, biosensing, etc.[6] Smart polymers are the most frequently used material to build the SRG;[7] and a series of stimuli-responsive polymers (e.g. poly(ethylene oxide)-block-poly((Namidino) dodecyl acrylamide), supramolecular diblock copolymer, and poly(ethylene glycol)-block-poly(lactic acid)) have been synthesized in recent years.[8] Zhou and his co-workers, for example, reported thermo-responsive gates based on a new polymer (poly(benzy-l-methacrylate)).[7c] When temperature rose to a lower critical solution temperature (LCST), the polymer would collapse, resulting in a random arrangement that might block open channels to present an OFF state.

These smart polymers, though work in a way that mimics nature biological channels embedded in cell membranes, have some intrinsic limitations. The biggest one is that the relatively simple chemical structure of these polymers does not allow them to recognize various biomolecules, thereby restricting their applications in biology. In addition, the performance of polymers, like response range, response speed, and reusability, is also unsatisfying. Still taking the thermo-responsive polymers for example, the LCST is difficult to be modulated, since one polymer corresponds to only one LCST. To overcome these limitations of polymers, researchers have been searching for alternatives.[9] Representatively, some DNA-regulated smart gates have been fabricated.[10]

Here, we successfully fabricate a novel biomolecule-responsive gate without using smart polymers. Aptamer, a flexible and versatile type of recognition molecules,[11] is adopted instead of smart polymers to respond to stimuli, while graphene oxide (GO) is adopted as a "universal cover" to control the flux through porous nanochannels in collaboration with the aptamer. ATP, the direct energy source of cells that also plays a key role in the regular of many biological channels *in vivo* (e.g. nuclear-pore-complex and potassium channel),[12] is selected as a typical biomolecular stimulus. The mimic of ATP-responsive biological channels *in vitro* may do great favor to the control of energy flux at the molecular level and the development of ATP-responsive drug delivery. Furthermore, this work also contributes to a novel versatile platform for the development of biomolecule-responsive gates.

The principle of our gating model, which is inspired from a pH-responsive gate we once fabricated,[13a] is shown in Scheme 1. A porous anodic aluminum oxide membrane (PAAOM) with regular array of cylindrical nanochannels (diameter of ca. 25 nm) is adopted as the gate. The upper side of the membrane is sputtered with a thin film of gold (thickness of ca. 5 nm) to facilitate further modification of 5'-end-thiolated ATP-binding aptamer (ABA) onto the surface of the membrane.[13] As has been widely reported, there is an interaction between GO and single-stranded DNA through π-π stacking.[14] So, here if GO is introduced, it may interact with the immobilized single-stranded aptamer, and thus cover the gate. Because GO is a faultless rigid sheet,[15] it is speculated that the coverage of GO will prevent the liquid flow through the nanochannels, making the gate OFF. Otherwise, if ATP working as the stimulus is presented, it will induce the self-folding of the aptamer and thus bind together. As a result, the bases of the aptamer are embedded, and they cannot interact with GO anymore, making the gate ON.



Scheme 1 Schematic illustration of the graphene oxide-aptamer-nanochannel architecture and the principle for gating.

To verify the feasibility, direct observation of the PAAOM surface using atomic force microscope (AFM) is first performed. As is shown in Fig. 1A & 1B, the gold-plated PAAOM and the ABA-modified PAAOM both show regular pores, which are similar to that for untreated PAAOM. So, the surface-sputtered gold film and the further molecular modification do little effect to the inner nanochannels of PAAOM. However, if the ABA-modified membrane is allowed to incubate with GO for a while, it is observed that GO adsorbs onto the membrane surface, hiding the pores (Fig. 1C). Otherwise, if the ABA-modified membrane is pretreated with ATP before the incubation with GO, the pores are still exposed and no GO on the membrane is observed anymore (Fig. 1D). The results validate that the coverage of GO can be control by ATP.

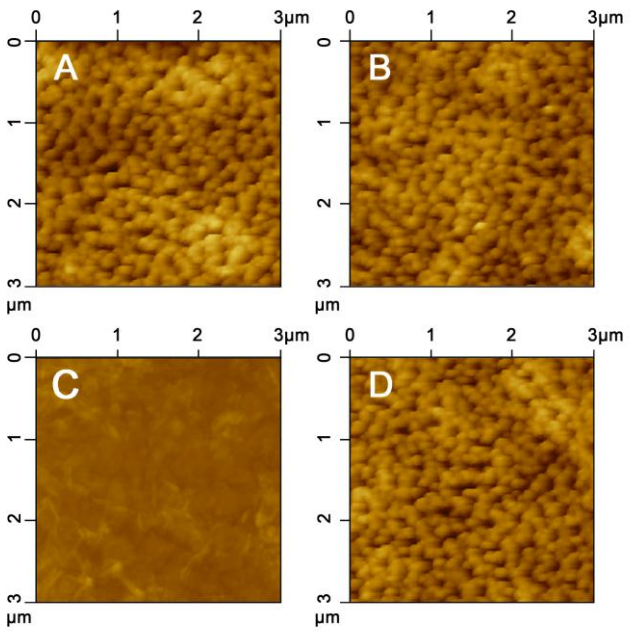


Fig. 1 AFM images of PAAOM in different conditions. (A) gold-plated PAAOM (Au/PAAOM), (B) gold-plated and ABA-modified PAAOM (ABA/Au/PAAOM), (C) GO-covered PAAOM (GO/ABA/Au/PAAOM), and (D) ATP treated before the GO-coverage (GO/ATP-ABA/Au/PAAOM).

The flux through the nanochannels is then measured to study the ON/OFF states of the gate during the whole process. As is shown in Fig. 2, water flows through untreated PAAOM with a velocity of 0.126 ml/cm^2·min under an extra pressure of 0.1 atm. After the successive treatment of gold-spurting and ABA modification, the velocity decreases a little bit maybe due to the acceptable system error or the narrowing of a small part of pores during the treatment. If GO is introduced further, a drastic fall of the velocity is observed. The result suggests that the rigid coverage of GO prevents the liquid flow as expected. Nevertheless, if the stimulus ATP is presented, the flow velocity keeps almost unchanged. Some ATP analogues (CTP, GTP and TTP) are also adopted to show if the gating response to ATP is specific. Results show that these analogues cannot switch the gate ON, suggesting that the gating is ATP-specific (Fig. S1 in ESI†). So, it can be concluded that the novel smart gate we have proposed here is feasible; and switching of the nanochannels between ON/OFF states can be achieved by using GO and ATP as the cover and stimulus, respectively.

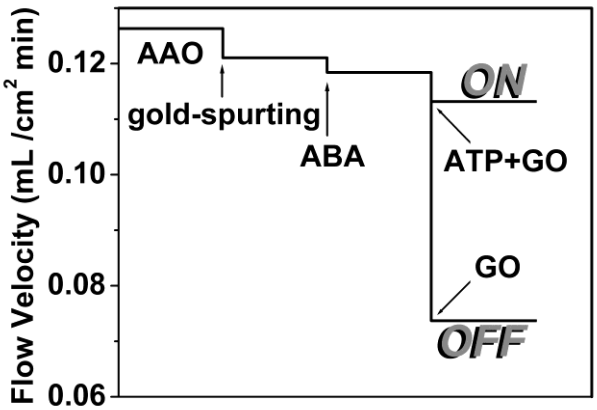


Fig. 2 Flow velocity of water through the PAAOM during the whole gating process from gold-spurting and ABA modification to ATP-controlled GO coverage. To accelerate the flow velocity, an extra pressure of 0.1 atm is added on the inpouring side of PAAOM.

Studies on the relationship between the flow velocity and the amount of GO as well as ATP are then conducted. Theoretically, it is believed that the flow velocity has a negative correlation with the amount of GO and a positive correlation with the amount of ATP. Indeed, the experimental results may support the speculation. As is shown in Fig. 3A, with the increase of GO concentration, the flow velocity decreases gradually. Because GO with higher concentration is not well dispersive and easy to deposit, it is a pity that the data under higher concentration is not available. Nevertheless, it seems from this figure that the velocity tends to be stable after 1 mg/mL. So, this concentration is settled as an optimized condition in our experiments. While ATP with different concentrations is presented, the GO-hindered flow velocity is observed to recover gradually and reach a plateau after 1.0 mM of ATP is introduced (Fig. 3B). 1.0 mM of ATP is then fixed in the following experiments as a critical concentration to switch the gate fully open.

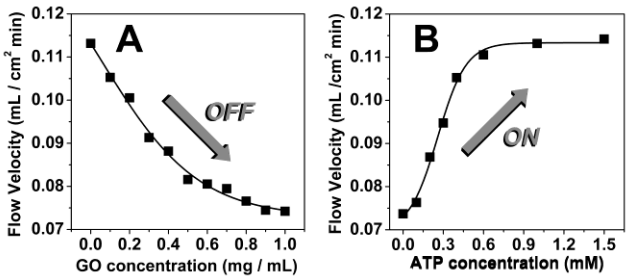


Fig. 3 (A) Influence of the concentration of GO to the flow velocity. No ATP is presented here; and an OFF mode is launched under the coverage of GO. (B) Influence of the concentration of ATP to the flow velocity. After the ATP treatment, 1 mg/mL GO was adopted to cover the nanochannels.

It is worth noting that the flux through the nanochannels is not blocked completely in the OFF state. The interception towards the flow velocity is abt 38% (the interception is defined as the ratio of $V_{ori}-V_{eff}$ to V_{ori}, where the V_{ori} and V_{eff} stand for the velocity before and after the coverage of GO). It may be ascribed to two possible scenarios. One is that gaps with a certain height (< 10 nm, calculated from the height of the aptamer) between the GO and the PAAOM exist during the coverage. Thus H_2O molecules with a diameter of only 4 Å may still pass through the gaps. Another scenario is that the GO cannot cover all the area of the PAAOM, leaving some area still exposed. These two possibilities are validated by investigating the ON/OFF switch towards the flux of different materials. Glucose, bovine serum albumin (BSA), and gold nanoparticles (AuNPs, TEM image shown in Fig. S2) are adopted to represent micromolecules (several Å), macromolecules (dozens Å), and larger nanoparticles (hundreds Å), respectively. As is shown in Fig. 4 and Table S1, in the ON mode, i.e., in the presence of ATP as the stimulus, the interception for all the cases is below 5%, suggesting all the materials can pass through the nanochannels without obstacle. The interception here has the same meaning as that for the flow velocity of H_2O, but is calculated from the ratio of $C_{ori}-C_{eff}$ to C_{ori}, where C_{ori} and C_{eff} stand for the concentration of the materials in the original and effluent solutions, respectively (detailed data shown in Table S1). The concentration of glucose is determined using a sophisticated enzyme-based colorimetric method (details shown in the experimental section in ESI†), while the concentrations of BSA and AuNPs are determined by measuring the absorbance at 280 and 520 nm, respectively. In the OFF mode otherwise, the interception differs a lot. A pattern is discovered that the bigger the material is, the higher the interception will be; and it seems the interception has no relationship with the original concentrations. Taking the AuNPs for instance, the interception reaches even close to 100% (exactly ca. 98%). This result suggests that no area of PAAOM is exposed under the coverage of GO, or else AuNPs may pass through the exposed area without obstacle, presenting an interception similar to that of H_2O. So, gaps between GO and PAAOM should be the main cause of the different interception. It is also consistent that AuNPs with a diameter of 13 nm cannot pass through the 10-nm gaps, while a small quantity of BSA with a diameter of ca. 7.3 nm can. As is shown in the bottom photos of Fig. 4, due to the large difference of interception towards AuNPs between ON and OFF mode, the effluent liquids show different color. In the OFF mode, the effluent liquid is colorless, whereas in the ON mode, ruby red is observed, representing the existence of AuNPs. Repeated switch of the gate between "ON" and "OFF" state towards the flux of AuNPs is also achieved simply by removing ATP or GO from the ABA-modified PAAOM under sonication (Fig. S3, experimental details shown in ESI†). Thus, the ATP-responsive gate provides an opportunity for controllable release, separation, and enrichment.

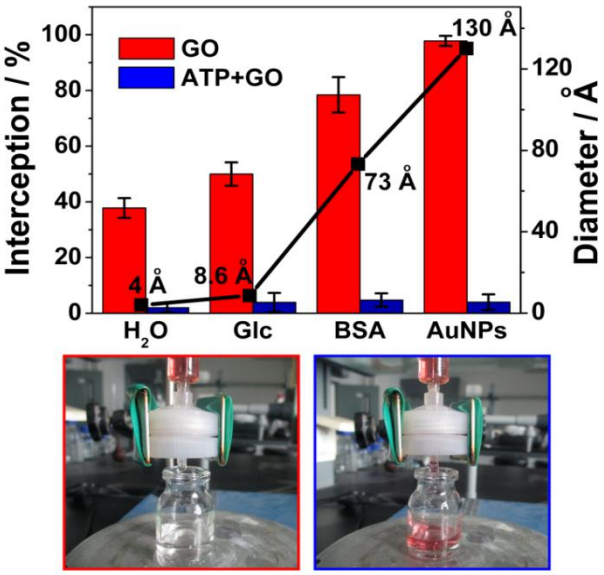


Fig. 4 The interception towards different materials (H_2O, glucose, BSA and AuNPs) in ON (blue columns) and OFF (red columns) mode, respectively. In the case of H_2O, the flow velocity is involved for the calculation of the interception; while for the other three materials, the concentrations in the original solution and the effluent solution are measured. The bottom photos show the appearance of the gating results towards AuNPs in ON (right) and OFF (left) mode, respectively.

Finally, we would like make a short discussion. Aptamers as a kind of recognition molecules have drastic conformational changes upon the binding with their targets. Thus, they may be recognized as an excellent responsive element towards specific molecular stimuli. The key point for the fabrication of aptamer-based smart gates is how to transfer the conformational changes to gating events. Usually, the conformational changes of aptamer, e.g. from an extended state to a folded state, also induce the changes of steric hindrance, which can be applied for gating directly.[16] However, the steric changes are not so remarkable and controllable, because the size of molecular stimuli with various molecular weights should also be taken into consideration. Here, we successfully transfer the conformational changes of aptamer to a remarkable gating event by using GO as a "universal cover". Similar to the ABA-ATP couple we have adopted, some other aptamer and their corresponding targets may also be employed, making this gating system a universal platform for the response to a variety of molecular stimuli. Furthermore, owing to the high flexibility of aptamer, the gap between GO and nanochannels in our model is tunable to achieve a "molecular sieve" for the separation of molecules with different critical molecular weights.

Conclusions

In this work, we have successfully developed a GO-aptamer-nanochannel architecture for the fabrication a novel smart gate, which may respond to ATP. In our method, GO may cover the nanochannels through the interaction with the surface-tethered aptamer, making the gate OFF. However, the presence of ATP molecules may induce the conformational changes of the aptamer, releasing GO from the surface of nanochannels to switch the gate to be ON. The gating efficiency has a positive relationship with the concentration of ATP. That is, the more ATP is, the more open the gate will be. Interestingly, the smart gate is also observed to have different interception towards the flux of different materials. Materials with bigger size are

proven to be more difficult to pass through the gate in the OFF mode. Thus, the ATP-responsive gate may work as a controllable "molecular sieve" for the potential application in smart release, separation, and enrichment. The concept of this work may also contribute to a novel versatile platform for the development of biomolecule-responsive gates.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, Table S1, and Fig. S1-S3. See DOI: 10.1039/c000000x/

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