DOI: 10.1002/chem.200902783

A pH-Responsive Gate Fabricated with Nanochannels and Nanoparticles

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Stimuli-responsive gates are usually porous membranes or microcapsules, the permeability of which can be regulated by stimuli such as temperature,[1–4] pH,[4–7] ionic strength,[8–10] electronic field,[11] light,[12] and biomolecules.[13,14] They have shown promising potential applications in the fields of drug delivery system, separation, sensors, and so on.[15–20]

Usually, synthetically prepared gates are always open, so they cannot control the transport properties by themselves. Therefore, an additional recognition system has to be involved to bring a stimuli-responsive gate into effect. Smart polymers are the most frequently used materials to construct the gating recognition system. They show a remarkable change in their properties, especially volume, as a response to slight changes in the surrounding environment.[1–14] Due to this unique property, gating membranes and microcapsules fabricated by using the stimuli-responsive polymers have been widely reported.

Though smart polymers may respond to stimuli sensitively, the gating efficiency and response speed are usually unsatisfactory due to the long time required for a volume change to occur.[21] Moreover, because there is a shortage of smart polymers available, and the development of new smart polymers is an arduous project, the application of smart polymers to the development of stimuli-responsive gates is greatly restricted. Taking thermoresponsive gates as examples, since no polymers except poly(*N*-isopropylacrylamide) (PNIPAM) together with its derivatives have been exploited, in most of the reported schemes of thermoresponsive gates, PNIPAM or its derivates have been employed as responsive components,[2,3,22–24] and the smart gates can only respond to a temperature of around 32 °C.

Herein, we report a novel pH-responsive gate fabricated without using smart polymers. Instead, amphoteric compounds, which are more easily available and cost-effective, are employed together with negatively charged nanoparticles as the recognition system. The results show that the smart gate we present herein is able to response to pH sensitively, taking an OPEN/CLOSE transition at a critical pH for the transport of biomolecules.

The mechanism of this pH-responsive gate fabricated with a porous anodic aluminum oxide membrane–gold nanoparticles (PAAOM–AuNPs) system has been illustrated in Scheme 1. It consists of three components. First, a synthetically prepared membrane with open pores is required. PAAOM (diameter of pores is ca. 25 nm, see Figure S1 in the Supporting Information), due to its regular array of cylindrical nanochannels and its accessibility for further modification, is a good choice.[25–27] Second, a pH-responsive element is required to construct the recognition system; we adopt an amino acid, cysteine. It has an isoelectric point (pI) of ≈6.2, and will be positively charged in acid and negatively charged in alkali. Third, a control element of the gate, which converts the pH-responsive information to an open/closed signal, is needed. In this work, we have employed negatively charged AuNPs for the control of the gate. As shown in the scheme, cysteine is immobilized on the surface of PAAOM and the negatively charged AuNPs are dispersed in the test solution. If the solution is alkaline, AuNPs will be repelled from the cysteine molecules modified on the PAAOM surface because both of them are negatively charged (Scheme 1A). Otherwise, if the solution is acidic, the AuNPs will be attracted to the surface of the PAAOM (Scheme 1B). In the former case, the nanochannels of PAAOM are kept in an open state and the gate is ready for the permeation of various species. However, in the latter case, the nanochannels of PAAOM are blocked by the AuNPs, so that the gate is closed.

AuNPs play a key role to this gate because the movement of the nanoparticles results in the open/closed state of the gate. So, the properties of the AuNPs should be well con-

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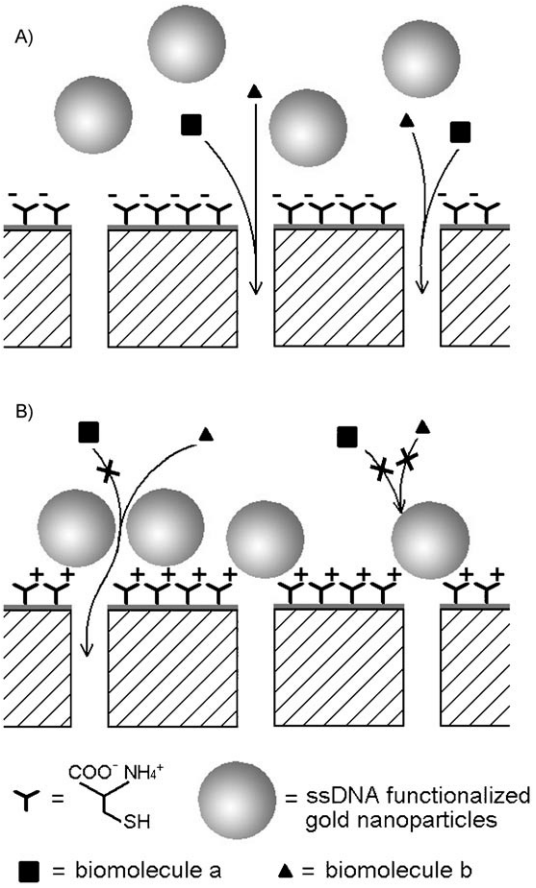
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902783.



Scheme 1. Schematic diagram of the gating mechanism of the PAAOM-AuNPs system in A) alkaline and B) acidic environment (■ and ▲ stand for macro- and micro-biomolecules, respectively).

trolled. First, AuNPs should be kept dispersed, stable, and negatively charged in the applied pH range. Freshly prepared AuNPs, though are negatively charged, are unstable in acid or alkaline. So, we have introduced single -stranded DNA (ssDNA) to functionalize the AuNPs. The ssDNA contains a thiol group in its 5′-end, which facilitates the combination of ssDNA to the surface of AuNPs (HS-ssDNA, sequence: 5′-HS-$(CH_2)_6$-$(G)_{12}$-3′). The HS-ssDNA is confirmed to not only provide rich negative charges, but also to protect AuNPs from aggregation in harsh environments.[28,29] In this work, the HS-ssDNA-functionalized AuNPs are observed to be stable in the pH range from 1.7 to 14.0 without any aggregation. Next, the size of the AuNPs should be well controlled. Ideal AuNPs should cover the nanochannels of PAAOM exactly, whereas smaller ones will pass through the nanochannels and bigger ones will leave gaps when they cover the nanochannels. To screen out AuNPs of an appropriate size, we have examined the filtration of the AuNPs with various sizes going through the PAAOM nanochannels. By measuring the concentrations of the AuNPs before and after filtration with UV/Vis spectroanalysis at their typical absorption peak position (520–533 nm, depending on their size), we found that AuNPs with a diameter $\geq(42\pm6)$ nm (Figures S2 and S3 in the Supporting Information) will be captured by PAAOM completely. So, we have chosen AuNPs with diameter of (42 ± 6) nm to control the gate.

To examine the pH response of the gate, the test solutions are adjusted to pH 9 or 5 to check the open/closed state of the gate. For the case of pH 9, if AuNPs are absent, the flow velocity of the solution through the PAAOM is 0.73 mL min^{-1}. In the presence of AuNPs, the flow velocity is the same, which is reasonable because the nanochannels are not blocked by the AuNPs (Scheme 1 A). Moreover, even if different kinds of biomolecules, such as bovine serum albumin (BSA), ssDNA (named ssDNA I to differentiate from the HS-ssDNA on AuNPs, sequence: 5′-CTG-GCC-GTC-GTT-TTA-CAA-CGT-CGT-G-3′), and adenosine triphosphate (ATP), are added to the solution, the flow velocity also keeps the same and these species may go thorough the nanochannels of the PAAOM freely. By measuring the concentration of these biomolecules with UV/Vis spectroscopy from their absorption peaks at 278, 262, and 260 nm, respectively, we have known that their concentrations keep almost unchanged as well after effusing from the PAAOM nanochannels.

To the case of pH 5, however, results are quiet different. The presence of AuNPs will result in the decrease of the flow velocity from 0.73 to 0.52 mL min^{-1}. The concentrations of the test biomolecules after effusing from the PAAOM are also decreased, the detailed results of which have been shown in Table S1 in the Supporting Information. So, we can conclude that AuNPs have indeed covered the nanochannels of the PAAOM, and consequently, blocked the passage of the biomolecules and the flow of the test solution.

A parameter "rejection", calculated from the ratio of $C_{ori}-C_{eff}$ to C_{ori}, can reflect the blocking efficiency of the gate to different biomolecules (C_{ori} and C_{eff} stand for the concentration of the biomolecules in the original and effluent solutions, respectively). Since the UV/Vis absorptions for all species are directly proportional to their concentrations, the rejection parameter can be obtained by calculating the ratio of $A_{ori}-A_{eff}$ to A_{ori} (A_{ori} and A_{eff} are the absorptions of the biomolecules in the original and effluent solutions, respectively). As is shown in Figure 1, it is found from the rejection data that the gate in the closed state rejects these molecules to different degrees. Additionally, the rejection has a positive correlation with molecular weight. The larger the molecular weight is, the higher the rejection of the gate will be. For comparison, the rejection values for the case of pH 9 have been also provided. From Figure 1, it is clearly known that the gate is pH responsive, with a quasi-full open state at pH 9 and a test species-dependent closed state at pH 5. And, the blocking efficiency of a closed gate depends on the molecular weight of the test species.

For the case of a theoretical condition, AuNPs with absolutely spherical bodies can cover the nanochannels completely and subsequently block the passage of all of the test molecules as well as the solvent, H_2O. However, it is impos-

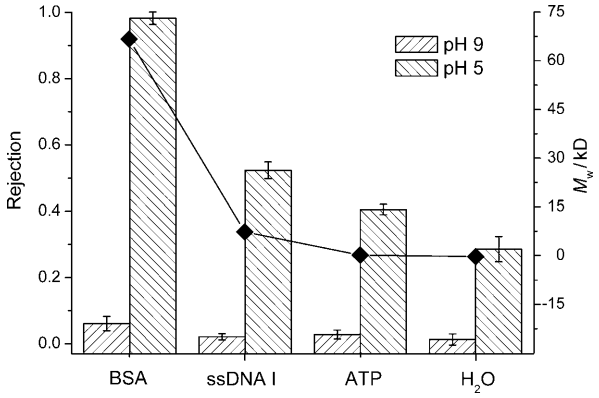


Figure 1. Rejections of the gate to the test species in acid or alkaline condition. ◆ corresponds to the molecular weight of these species.

sible for AuNPs to cover the nanochannels completely and exactly, so the solution can still flow through the gate from the uncovered nanochannels, presenting a relatively low flow velocity. We have monitored the rejection of the gate to H_2O, and found that the rejection value is about 29%, which is defined by the decrease of the flow velocity from 0.73 to 0.52 mL min^{-1}. Also, AuNPs are quasi-spherical and will leave small gaps when they cover the nanochannels of PAAOM. Clearly, it is more difficult for macromolecules to pass through these small gaps, so a higher rejection value than the micromolecules will be obtained, as shown in Figure 1.

The gate can also respond to pH in several cycles of pH changes, the experimental results of which are shown in Figure 2. The test solution was adjusted to pH 9 and then to pH 5, and the process was repeated three times. BSA and H_2O are chosen as the species to be monitored. The rejection parameter is employed to characterize the gating efficiency. As shown in Figure 2, the rejection values keep regular changes along with the pH cycles, presenting large values at pH 5 and small values at pH 9. The results suggest that the gate is able to regenerate after each cycle with only a little passivation. The slight increase in the rejection values for both BSA and the flow velocity after each cycle may be due to the irreversible adsorption of some AuNPs on the PAAOM surface.

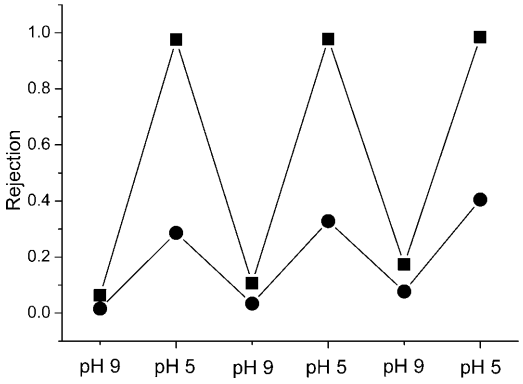


Figure 2. Rejections of the gate to BSA (■) and H_2O (●) (flow velocity) in cyclically changed pH.

In summary, we have developed a novel method to construct a pH-responsive membrane gate. The gate is open at pH 9 and closed at pH 5. Since cysteine can be substituted by other amphoteric compounds with different pI values (e.g., oligopeptides such as cys-glu or cys-lys), and positively charged AuNPs are also available, the pH-sensitive gate can be modulated for different pH purposes. Furthermore, amphoteric compounds are much more easily available than smart polymers and their pI values are more easily controlled to meet various demands. So, it will be very easy to construct pH-responsive gates with various critical pH sensitivities by utilizing different kinds of amphoteric compounds together with nanoparticles. The novel pH-responsive gate presented herein may pave a new way to the study of smart gates and may be also helpful for the development of drug delivery systems, separation apparatus, and sensors.

Experimental Section

Gating experiments: The gating behavior of the prepared PAAOM–AuNPs system was studied by measuring the transport of some biomolecules (BSA, ssDNA I, or ATP) through the nanochannels of the PAAOM. The PAAOM was fixed in a detachable filter device that was connected to a syringe. The syringe was filled with a solution (2 mL) containing 1 nM HS-ssDNA-functionalized AuNPs, and 25 μM ssDNA I or 1.34 μM ATP or 20 μM BSA. HCl or NaOH was used to adjust the pH of the solution. Extra pressure (10 kPa) was exerted on the top of the syringe to accelerate the flow velocity. The first two effluent drops (ca. 100 μL) were collected for UV/Vis analysis. To regenerate the AAO film, it was detached from the filter device and was washed by ultrasonicating in double-distilled H_2O for 1 min. Then it could be reused.

The information related to detailed experimental procedures and state analysis of AuNPs and PAAOM is given in the Supporting Information.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant Nos. 90406005 and 20575028) and the Program for New Century Excellent Talents in University (Grant NCET-04-0452), the Chinese Ministry of Education.

Keywords: membranes · nanochannels · nanoparticles · pH-responsive gates · zwitterions

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Received: October 9, 2009
Published online: December 28, 2009

