Biomimetic Synthesis

Zn²⁺ and EDTA Cooperative Switchable Nanofluidic Diode Based on Asymmetric Modification of Single Nanochannel

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Abstract: Design and fabrication of smart switchable nanofluidic diodes remains a challenge in the life and materials sciences. Here, we present the first example of a novel $Zn^{2+}/EDTA$ switchable nanofluidic diode system based on the control of one-side of the modified hourglass-shaped nanochannel with salicylaldehyde Schiff base (SASB). The nanofluidic diode can be turned on in the response of Zn^{2+} and turned off in response to EDTA solution with good reversibility and recyclability.

The coherent regulation of ion channels in controlling ion flow across the cell membrane is attached with great importance to the various significant physiological functions in life processes.^[1] Recently, by mimicking biological ion channels, synthetic nanochannels have triggered interest because of their robust mechanical and chemical properties.^[2] Ion current rectification (ICR), as the most promising feature of these artificial ion channels, is characterized by a nonlinear diode-like current-voltage response.^[3] By various chemical modifications, these nanofluidic diodes can respond to external stimuli, such as specific ions, pH, temperature and molecules, which result in distinct changes in ICR.^[4] However, functionalities of these artificial nanochannels are mainly realized by modification the whole inner surface of nanochannels, and how to endow them with complex and multiple functions is a remaining challenging task. The components of most biological nanochannels are asymmetrically distributed between membrane surfaces to implement complex biological functions.^[5] Inspired by this natural asymmetrical design, Hou et al. utilized a symmetric hourglass shaped nanochannel (H-shaped) with asymmetric plasma modification approaches to develop a responsive rectifier nanochannel system.^[6] Indeed, the symmetrically shaped nanochannel system has several advantages. For example, the symmetric hourglass-shaped pore provides the nonhomogeneity of the distribution of a chemical introduced from one side of the pore along the pore centerline, once the system has achieved steady-state. Furthermore, the development of an asymmetric multiresponsive nanochannel system could be easily realized by asymmetric chemical modification approaches to functionalize diverse specific local areas with different functional molecules. Hence, the combination of the symmetrically shaped nanochannel with asymmetric modification could be realize to build a more complex system, which moves one step farther toward the development of smart nanochannel systems for real-world applications.^[7] However, the present research towards asymmetric modification approaches for building responsive nanochannel systems is still in its early stages.

Zn²⁺, the second most abundant transition metal ion in the human body after iron, is known to actively participate in biological processes such as regulation of enzymes, neural signal transmission and modulation of ion channels.^[8] For example, the Cys loop ligand gated ion channel can be activated by zinc binding induced conformational change of the channel protein.^[9] Considering the roles of Zn²⁺ in living systems, building biomimetic Zn²⁺-regulated ion channels in vitro will have great applications in the field of biotechnology, like biosensors, controlled nanofluids and drug delivery systems.^[10] Salicylaldehyde Schiff bases (SASB) with N and O as donor atoms are known to form strong complexes with Zn²⁺ and are widely used as ionophores in sensors for Zn²⁺.^[11] Moreover, decomplexation of Zn²⁺/Schiff base complexes could be realized by washing with EDTA solution.^[12] Inspired by these results, herein, we present the first example of a novel Zn²⁺/EDTA cooperative switchable nanofluidic diode system based on an asymmetric SASB-modified H-shaped nanochannel. By asymmetric modification with SASB, we can realize the shift from symmetric shape to the broken symmetry of the nanochannel, and obtain an obvious ion rectification change. Furthermore, the state of rectification of the SASB functional channel can be controlled over the external stimulus (Zn²⁺/EDTA). The results show that the rectification of nanofluidic diode can be turned on in response to Zn²⁺ and turned off in response to EDTA solution (Figure 1 and Scheme 1). After several cycles there was little decay in the rectification gating behavior, and this switchable nanofluidic diode shows excellent reproducibility and reversibility. Such a device could potentially spark further experiment and theoretical efforts to explore the rectification of bioinspired intelligent nanomachines. To our knowledge, studies on the rectification in a symmetric nanochannel to fabricate an artificial switchable nanofluidic diode, which is the focus of our current study, are rarely reported.

To produce the H-shaped nanochannel, etching of the polyethylene terephthalate (PET, 12 μ m thick) membrane contain-

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Figure 1. By mimicking natural asymmetry nanochannels, an artificial asymmetric nanochannel was fabricated by asymmetric modification.



 $\label{eq:Scheme 1.} Scheme 1. A switchable nanofludic diode based on Zn^{2+}/EDTA over a one-side modified H-shaped nanochannel with SASB.$

ing a single ion track in the center was performed from both sides at room temperature. The large opening (base) was about 420 nm wide, as observed by scanning electron microscopy (SEM, Figure S2 in the Supporting Information), and the tip was calculated to be 12 nm by an electrochemical methods.^[13] The interior surface of one side of the H-shaped nanochannel was modified with SASB by a two-step coupling reaction. During the modification process, the PET film was mounted between the two halves of the cell. One half of the cell was firstly tested with classical EDC/NHSS coupling reactions to produce amine-reactive NHSS ester molecules. The other half of the cell was filled with phosphate buffer of pH 10 as stopping medium during the activation process (Figure S3 in the Supporting Information).^[14] Subsequently the succinimidyl intermediate was covalently coupled with SASB through a stable amide linkage (Figure 2A). After modification, the PET film was washed and treated with deionized water, overnight, before further experiments.

The success of the surface one-side modification was evidenced by the corresponding current-voltage (I-V) curves measured using 0.5 \times KCI/Tris-HCI (pH 6.8) in both half-cells. As shown in Figure 2D, significantly different I-V characteristics of the H-shaped nanochannel before and after SASB immobilization were observed. This is attributed to the neutral SASB diminishing the negative charge of the nanochannel surface, which resulted in the loss of current behavior. In contrast, the

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Figure 2. A) SASB was immobilized on one side of the H-shaped nanochannel. B) The corresponding contact angles of the PET membrane before and after modification. C) LSCM observation before and after modification on one side of the nanochannel. D) Current–voltage (I-V) properties of the single nanochannel before SASB (black) and after SASB (red) modification on one side of the H-shaped nanochannel. Each data point is the average of four measurements. Error bars are not shown in the I-V curves because they are approximately equivalent to the width of the lines in the plot. These results showed that SASB was successfully immobilized on one side of the inner surface of the H-shaped nanochannel.

I-V curves of symmetric modification with SASB on both sides of the nanochannel exhibit a distinct behavior, which further confirms the successful of one-side modification procedure (Figure 2D vs. Figure S4 in the Supporting Information). Moreover, we examined the wettability (Figure 2B) before and after modification. As expected, modification of the PET membrane with SASB molecules leads to a remarkable change on the wettability of the surface (from 72.1 $^{\circ}\pm$ 3.0 $^{\circ}$ to 51.1 $^{\circ}\pm$ 1.2 $^{\circ})$ corresponding to a change of the chemical composition. The successful nanochannel surface modification with SASB was also verified by laser scanning confocal microscopy (LSCM). A strong fluorescent signal appeared after the modification and the fluorescent signal showed the membrane thickness to be about 6.0(\pm 0.5) µm which agrees with its half of actual thickness (Figure 2 C). Also, X-ray photoelectron analysis shows a new N peak at 406 eV (Table S2 in the Supporting Information), which further confirmed that we had successfully immobilized SASB on the inner surface of the H-shaped nanochannel.

After successful asymmetrical incorporation SASB as metalbinding site, we investigated whether this modified H-shaped nanochannel could respond to external metal ions. The experiment was carried out with 1 mm solutions of different metal ions including Mg^{2+} , Ca^{2+} , Cd^{2+} , Ba^{2+} and Zn^{2+} in electrolyte KCI/Tris-HCI (pH 6.8). When the bare nanochannel was exposed to an electrolyte (pH 6.8) containing 1 mm of ions tested, the ionic current stayed nearly unchanged (Figure S5 in the Supporting Information). However, it is worth mentioning that there was an obvious asymmetric ionic current change when the functional H-shaped nanochannel was exposed to 1 mm Zn^{2+} (Figure 3 A). The diode-like behavior of this functional nanochannel can be quantified by the rectification ratio (R). The values were calculated as the ratio of the currents recorded at +2 V to the absolute values of the currents recorded at -2 V; high rectification ratio (>2) was defined as the "on" state of rectifier switch and low rectification ratio (around 1) as

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the "off" state. Compared with other metal ions, only Zn²⁺ turned the rectification switch "on" (Figure 3B). Hence, it is evident that the asymmetrically modified H-shaped nanochannel displayed good responsiveness to Zn²⁺ ions. In general, the ionic transport properties of the single polymeric nanochannels are determined by three major factors: surface charge, effective pore size and wettability.^[15] Any asymmetric distribution of these three factors may bring about asymmetric ionic transport properties. Herein, the "on" state of rectifier switch was realized by Zn²⁺ specifically binding with SASB immobilized on the inner walls of H-shaped nanochannel, which can both change surface charge and wettability. These two factors contribute greatly to the ionic conductance.^[16] To provide further proof of the success of Zn²⁺ binding characteristics of SASB, XPS analysis was carried out. After soaking the SASB-modified membrane in 1 mm zinc chloride solution for 1 h, the XPS spectrum showed a new Zn(2p³/2) peak at 1034 eV (Table S3 in the Supporting Information). Previous studies have shown that SASB analogues act as good Zn²⁺ binding ligands.^[11] Having achieved open rectifying behavior of the nanofluidic diode, we further evaluate the effect of Zn²⁺ concentration on rectification (from 0.01 to 5 mm) in an electrolyte (pH 6.8). Figure S6 in the Supporting Information shows that a remarkable increase in the rectification could be observed with rising Zn²⁺ concentrations (ratio from 0.8 to 2.1). The maximum rectification ratio was seen in the present of 1 mm (ratio = 2.1). This can be explained in terms of the fact that by increasing Zn^{2+} concentration the interaction between SASB and Zn²⁺ leads to a continuous decrease in the negative charge on the single side of the channel surface. In addition, we exploited the rectification of symmetric modification in response to Zn²⁺, which is another evidence for the success of modification on one side (Figure S7 in the Supporting Information).

Encouraged by the above results, we set out to explore closure of the rectifying switch of this SASB functional nanofluidic diode. We chose EDTA as the second external stimulus. Figure 4A shows the fabrication and operating principle of this nanofluidic diode system. It is interesting that the rectification behavior vanishes after the Zn²⁺-binding nanofluidic diode was exposed to an EDTA solution for 20 min (Figure 4B). Therefore, the "on" state of this rectification switch was successfully realized by EDTA. When designing a rectifier switch, it is very important to demonstrate its reproducible and reversible characters. Therefore, the switchable behavior was investigated with repeated measurements of rectification ratio between Zn^{2+} and EDTA. As shown in Figure 4C, good switchable ability of the rectification property of this functional nanochannel was observed. Hence, the measurements confirmed that this nanofluidic diode system can be switched between the "on" state and "off" state in response to Zn^{2+} and EDTA.

Since the wettability plays a crucial role in the rectifying behavior, we further studied the wettability switching behavior of the SASB-modified H-shaped nanochannel upon alternate treatment with Zn²⁺ and EDTA. Figure S8 in the Supporting Information indicates a remarkable change of the wettability of the surface (from $72.1^{\circ} \pm 3.0^{\circ}$ to $51.1^{\circ} \pm 1.2^{\circ}$), every cycle in the experiments was completed within 20 min. The relationship



Figure 3. A) *I–V* curves of the SASB modification on a single side of the H-shaped nanochannel in 0.5 m KCI/Tris-HCI (pH 6.8) with the addition of 1 mm Mg²⁺, Ca²⁺, Cd²⁺, Ba²⁺ or Zn²⁺. Each data point is the average of four measurements. Error bars are not shown in the *I–V* curves because they are approximately equivalent to the width of the lines in the plot. B) The rectification change ratios are in the presence of different metal ions. Herein, the rectification change ratio was defined as $(R_{metal} - R_{Blank})/R_{Blank}$. The rectification of this functional material can be "turned on" in response to Zn²⁺.



Figure 4. A) The fabrication and operating principle of the rectifier switch single nanochannel system. B) I-V curves of the SASB modification on a single side of the nanochannel in the presence of Zn²⁺/EDTA. C) Cycling experiments of the rectification ratio behavior of the single nanochannel.

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between the wettability and ionic transport properties has been discussed previously.^[17] We further investigated the possible molecular recognition mechanism among SASB, Zn^{2+} and EDTA by analyzing the fluorescence spectra. Figure S9A in the Supporting Information shows that there is an obvious change after the addition of Zn^{2+} , which is attributed to the formation of the $Zn^{2+}/SASB$ complex. After washing with EDTA, the fluorescence of SASB recovered to the former state, which suggests the decomplexation of $Zn^{2+}/SASB$. A cycling experiment again indicates that in the present of EDTA, Zn^{2+} could reversibly interact with SASB (Figure S9B).

In summary, we have experimentally demonstrated a novel nanofluidic diode, which fully reveals the advanced feature of providing efficient and reversible control of "on/off" state recti-fying switching depending on Zn^{2+} and EDTA. Moreover, our simple approach to control of one-side modification could promote an alternative to asymmetric chemical modification with various complicated functional molecules. With simple small-molecule regulation of this rectification switch, we can further understand different ionic transport systems as well as enhance the functionality of nanofluidic diodes.

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