A Stimuli-Responsive Nanopore Based on a Photoresponsive Host-Guest System

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The open-close states of the ion channels in a living system are regulated by multiple stimuli such as ligand, pH, potential and light. Functionalizing natural channels by using synthetic chemistry would provide biological nanopores with novel properties and applications. Here we use para-sulfonato-calix[4]arene-based host-guest supramolecular system to develop artificial gating mechanisms aiming at regulating wild-type α-HL commanded by both ligand and light stimuli. Using the gating property of α-hemolysin, we studied the host-guest interactions between para-sulfonato-calix[4]arene and 4, 4’-dipyridinium-azobenzene at the single-molecule level. Subsequently, we have extended the application of this gating system to the real-time study of light-induced molecular shuttle based on para-sulfonato-calix[4]arene and 4, 4’-dipyridinium-azobenzene at the single-molecule level. These experiments provide a more efficient method to develop a general tool to analyze the individual motions of supramolecular systems by using commercially available α-HL nanopores.

Supramolecular chemistry examines the weak and reversible non-covalent interactions which are focused on assembled molecular subunits and components. Among all non-covalent interactions, the study of host-guest interaction is one of the most popular research fields which facilitate the understanding of the biological processes and functions. Researches of biomimetic architectures based on supramolecular host-guest interactions have been reported recently, such as protein assembly and immobilization, ion channels mimicking and bio-catalysis regulation. Sulfonato-calix[4]arene (SC4), a member of the host family of calixarenes, is shaped as a truncated cone with hydrophilic upper and lower rims connected by a hydrophobic mid-region. Strong complexes would be formed by binding SC4 with basic amino acid lysine and arginine as guest molecules. Previous studies showed that SC4 could inhibit the ion channel mainly through an electrostatic interaction. The apparent inhibition of SC4 toward ion channels promoted us to further explore its potential application in developing biological systems.

α-Hemolysin (α-HL) transporter system is responsible for the translocation of molecules and ions from the target cells to induce an ultimate rupture of the cell membrane. Seven monomers assemble into α-HL which inserts into the membrane (Fig. 1a). Realizing nature’s functions of α-HL, it became an ideal nanoscale material for probing molecular transport and recognition processes, e.g., the analysis of nucleic acids and the development of DNA sequencing methods. Various possible applications of α-HL nanopore-based biosensor have already been demonstrated, including the analysis of the structure of nucleic acids, the probing of peptide conformations, the monitoring of the interactions between biomolecules and binding targets and the detection of small molecules. Two synthetic host molecules, cyclodextrins and cucurbit[6]uril, have been studied by α-HL nanopore. Especially, the cyclodextrins integrated α-HL nanopore attracts intensive attention due to its potential application in DNA sequencing. Since the open-close states of the ion channels in a living system are regulated by multiple stimuli such as ligand, pH, potential and light, here we use supramolecular SC4-based host-guest interaction to develop artificial gating mechanisms aiming at regulating wild-type α-HL to command both ligand and light stimuli. Our results demonstrate that the open-close states of α-HL are being regulated by SC4. The inhibition of ion current flow through α-HL reveals a voltage as well as an orientational dependence. In the presence of SC4 at the trans side, it induces a long-term close-state of α-HL at the holding potential more negative than ~70 mV, probably due to a collapse of the stem. The close-states of α-HL recover to the open-state at a more positive repulsive potential suggesting the existence of the electrostatic interactions between SC4 and Lys41 (or Lys41-42) in the stem of the pore (Fig. 1a). Enlightened by this mechanism, light-sensitive 4, 4’-dipyridinium-azobenzene (V2”-Az)
was designed as a functional guest molecule to examine the effect of host-guest interaction with SC$_4$ on the open-closed state regulation (Fig. 1b). Moreover, light-induced association and dissociation of the respective photoisomers of V$^2$+-trans-Az (V$^2$+-trans-Az/ V$^2$+-cis-Az) to and from the SC$_4$ receptor, which would further modulate the open-close state of z-HL, were also studied by real-time probing the ion current through the pore channels (Fig. 1c). Different from previous studies$^{14-16}$, the construction of a light-regulated ion channels in our work has been achieved by non-covalent supramolecular interaction rather than the covalent modification of photoresponsive molecules inside the channel. Therefore, this would provide a more efficient approach to develop the wild-type ion channel into a general tool to analyze the individual motions of supramolecular systems.

Results

Sideneess of current inhibition by SC$_4$. Previous studies showed that z-HL exhibited uniform and stable open-channel states at both negative and positive holding potentials. The average diameter of SC$_4$ is about 0.2 nm which is 7 times smaller than the narrowest part of the stem region associated with z-HL. The insertion of SC$_4$ into either the cis or the trans side of the pore is anticipated to induce the blocking of the pore, and reduce the ion current by ca. 20% of the value of the z-HL open-channel current. Nonetheless, we find that SC$_4$ induces a substantially higher inhibition of the ion current and even produces a full blockage of the pore (Fig. 2). These blockages indicate that the negatively charged SC$_4$ stimulates a gating event on the z-HL that is amplified as anticipated from steric consideration only.

The inhibition of ion current flowing through z-HL reveals an orientational dependence (Fig. 2). The addition of SC$_4$ to the trans compartment induced both irreversible and long time reversible close-states of z-HL at holding potentials that are more negative than −70 mV (Fig. 2a–b). However, only the transient and reversible inhibitions were observed at positive holding potentials higher than +100 mV upon integration of SC$_4$ into the cis side of the pore (Fig. 2c–d). This behavior was retained even at an extreme holding potential of +140 mV and the high concentration of SC$_4$ ([SC$_4$] = 800.0 µM) as illustrated in Supplementary Fig. S1. The inter-event time-intervals of z-HL ($\tau_{\text{off}}$) upon the trans side inhibitions are 16 ~ 60 times shorter than those for the cis side inhibitions (Fig. 3 a–b, Supplementary Fig. S1–2 and Table S1–S2), indicating that SC$_4$ is easier integrated with z-HL from the trans side. The small volume of the stem increases the interaction probability between SC$_4$ and the binding residues of z-HL. These results demonstrate that the interactions of SC$_4$ with z-HL are controlled by the steric sideneess. SC$_4$ irreversibly binds to amino acid residues at the trans side of the z-HL, while it reveals reversible binding affinities upon interaction with the cis side of z-HL. In all the cis side inhibitions induced by SC$_4$, a Gaussian peak at the duration time ($\tau_{\text{off}}$) of 0.36 ms was observed and its position did not change significantly under various experimental conditions (Supplementary Fig. S1 and Table S1). As described in previous studies$^{14-16}$, the durations for the translocations of polynucleotide which carries negative charges decrease with the applied potential. Since the independence of the durations ($\tau_{\text{off}}$) for both applied potentials and concentrations of SC$_4$ in the cis side inhibitions, we ascribe the cis side inhibitions to the bumping rather than the translations of SC$_4$, e.g., a SC$_4$ interacts with the cis side of z-HL and then "bounces" off.

The inhibitions of z-HL by SC$_4$ from the trans side. We notice that the trans-inhibitions of z-HL exhibit strong voltage dependence. No blockages were observed at the holding potential more positive than −70 mV (Fig. 2b). The distributions of $\tau_{\text{off}}$ could be fitted by single-exponential distributions (Supplementary Fig. S2). The values of $\tau_{\text{on}}$ are inversely proportional to the applied holding potential from −70 mV to −140 mV (Fig. 3a), indicating that the probability to
sustain the full open-state of $\alpha$-HL is substantially lower with a more negative holding potential. This is attributed to the four negatively charged sulfonate groups associated with SC4 which facilitate its penetration into the channel of $\alpha$-HL at a more negative potential.

As the holding potential turns negative ($<-70$ mV), the currents for the close-states can be divided into three populations, labeled as PI, PII and PIII in Fig. 3c and Supplementary Fig. S3. For example, at the holding potential of $-100$ mV, the peak currents for the three populations are located at $i_{\text{PI}}/i_0 = 0.3$, $i_{\text{PII}}/i_0 = 0.65$ and $i_{\text{PIII}}/i_0 = 0.85$, respectively. As show in Supplementary Fig. S3–5 and Table S2, the current blockages in the population PI exhibit a substantially shorter duration time interval as compared to blockages in PII and PIII, indicating a lower association constant for the SC4-induced inhibitions of PI. The ratios of PI type events from the total events decreased significantly from 58% to 23% throughout the increased concentration of SC4 from 0.8 $\mu$M to 8.0 $\mu$M at the applied potential of $-100$ mV (Fig. 3c and Supplementary Fig. S6), revealing that lower concentration of SC4 favors the short inhibitions. After analyzing the events in PI for the three different concentrations of SC4 (0.8, 4.0 and 8.0 $\mu$M), we found that the fitted durations in PI ($\tau_{\text{off,PI}}$) were around 0.30 ms. The value of $\tau_{\text{off,PI}}$ did not change significantly with the concentration of SC4, even with the applied potential. Moreover, the values of $\tau_{\text{off,PI}}$ are similar to the durations for cis side inhibitions, confirming that the bumping events of SC4 might account for the assignment of PI. The affinity of SC4 is much lower to the neutral and acidic amino acids as compared to the basic amino acids$^{42}$. The weak interactions between SC4 and neutral and acidic amino acids may contribute to the bumping events.

The higher populations (PII and PIII) for the current inhibitions may be attributed to the two different close-states of $\alpha$-HL at pH 8.0. The plot of $t_{\text{off,PI}}$ and $t_{\text{off,PII}}$ represent linear relationship versus potential, the slopes of which show that neither the current of partial (PII) nor complete (PIII) close-states of $\alpha$-HL change significantly throughout the voltage range from $-70$ mV to $-140$ mV (Fig. 3d). The probability of the reversible inhibitions related to PI altered from 55% to 4% as the negative holding potential increased from $-70$ mV to $-140$ mV at [SC4] = 8 $\mu$M, whereas that of PII and PIII populations increased upon applying the negative potential (Supplementary Fig. S3). The voltage-dependent distributions of the blocking currents strongly support the suggestion that the positive-charged residues are involved in the inhibitions of $\alpha$-HL by SC4. The positively charged residues inside the stem of $\alpha$-HL may be distorted toward the stem end at a more negative potential$^{40}$, which poises the equilibrium process outlined in equation (1) in favor of $\alpha$-HL: SC4 and thereby increasing the probability of PII and PIII. As expected from equation (1), the value of $t_{\text{on}}$ decreased from 8.14 ± 0.75 s to 4.97 ± 0.24 s as the concentration of SC4 increased from 0.8 $\mu$M to 8.0 $\mu$M (Supplementary Table S2, Fig. S7). The ratios of the number of events in PII (N_{PII}) to that of PIII (N_{PIII}) show that the high potential favors the partial blocking of the pore (Supplementary Fig. S8). These results indicate that the free energies related to the partial and complete inhibition of $\alpha$-HL are voltage dependent. The above results together imply that two mechanisms are operative in the pore inhibition by SC4: i) the close-states of the $\alpha$-HL are prone to occur at the enhanced negative holding potential in the presence of SC4 at the trans compartment; ii) the positively charged binding sites are associated with SC4 in the channel closures.

**Figure 2** Models and current traces show the 8.0 $\mu$M SC4 binding with $\alpha$-HL. (a) SC4 were driven into the trans side of $\alpha$-HL and induced the close-states of $\alpha$-HL by binding with the positive-charged residues inside the stem. (b) The obtained raw data by the addition of SC4 to the trans chamber at the holding potential of $-60$ mV (top), $-70$ mV (middle) and $-80$ mV (bottom). (c) The illustration of the inhibition of an $\alpha$-HL in the presence of SC4 at the cis chamber. (d) The raw data for the current traces recorded after SC4 was driven into the cis side of an $\alpha$-HL at the holding potential of $+90$ mV (top), $+100$ mV (middle) and $+110$ mV (bottom).
In previous study, three different binding sites have been shown in the crystal structure of cytochrome-SC₄ complex. Each binding site involves one lysine side chain trapped inside the cavity of SC₄, which is mainly through electrostatic interactions. As illustrated in Fig. 1a, the red colour dots depict the positive-charged amino acids and they might act as the potential electrostatic binding sites for SC₄. Lys₁₁₀, Lys₁₁₆ and His₁₄₄ are located at the outer surface of the stem domain which faces to the bilayer. Lys₁₃₁ and Lys₁₄₇ are positioned at the inner surface which forms the interior of the stem. Due to the hydrophilicity of SC₄, its permeation into the hydrophobic bilayer is prohibited. In addition, the phospholipid head groups of bilayer would prevent the entrance of SC₄ which carries sulfonate groups at the upper-rim. Therefore, Lys₁₁₀, Lys₁₁₆ and His₁₄₄ are not likely to provide the binding sites for SC₄. In contrast, the other two lysine residues at the stem of α-HL, Lys₁₁₅ and Lys₁₄₅, facing to the interior of the stem, are crucial components for stabilizing the β-barrel of α-HL at the glycine-rich stem base. Previous studies indicated that di- and trivalent cations partially affect, or reduce completely, the conductance through α-HL. This channel blocking may originate from ion binding to Asp₁₂₇ and Asp₁₂₈ at the stem base, resulting in the collapse of the hydrophobic stem barrel. Similarly, SC₄ might form a complex with the collar of Lys₁₃₁ through electrostatic interactions, leading to the elimination of ion-pair interactions in the stem base. The crystal study demonstrated SC₄ could encapsulate L-lysine at the binding ratio of 1:5. Subsequently, the glycine-rich stem could undergo conformational changes leading to the partial, or complete, blocking of the pore (PII/PIII in Fig. 3c). Lys₁₄₇, another highly potential binding site for SC₄, is the vital residue for the assembly of α-HL. SC₄ induced inhibitions may involve the conformational changes of the stem top by binding to Lys₁₄₇. Alternatively, the ion pairs between Lys₁₄₇ and Glu₁₁₁ would be disrupted upon the binding of SC₄ to Lys₁₄₇, resulting in the enlargement of the pore neck by rearranging of Lys₁₄₇ and Glu₁₁₁. Our experiments show that the open pore current slightly increases with the probing time, suggesting that the pore neck is, indeed, enlarged. Apart from these, the hydrophobic effects and the cation-π interactions may also contribute to the interactions between α-HL and SC₄, leading to the conformational changes of β-barrel of α-HL.

By further exploring the irreversible inhibitions, we find that the open-close states of SC₄ integrated α-HL could be modulated by the holding potential. As shown in Fig. 4a and Supplementary Video,
SC₄ is repelled from the binding site by treating a repulsive potential across the bilayer. The repulsive potential is more positive than the holding potential for each irreversible inhibition. After measuring every individual irreversible inhibition, we find that −40 mV is the minimum repulsive potential which could shift the equation (1) toward the direction of dissociation (Fig. 4a). SC₄ would undergo a time-dependent repulsion process labeled as repulsion-time in Fig. 4a, prior to the dissociation of the complex at its specific value of repulsive potential. The repulsive potential will impede the access of cations into the channel and reduce the fraction of positive binding sites, resulting in the inhibitory action of the SC₄₋₋ (1). Compared the inhibitions in PIV with the ones in PV, we noted that the close-states with the larger inhibition currents exhibit more positive repulsive potentials and larger values of repulsion-time (Fig. 4b and Supplementary Fig. S10). These results might be attributed to the tight binding with SC₄ which affects the complete closure of α-HL. Thus, the collective results provide convincing evidence that the close-states of α-HL are mainly induced by strong host-guest interactions between the positive residues (probably Lys131 and Lys147) and SC₄.

Recognition of host-guest interactions through an α-HL. The next set of experiments demonstrates that the close-state of α-HL can be modulated by ligands which induce host-guest competition inside the channel. The experiment was carried out by driving the complex of SC₄₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋_-Az was formed by incubating equimolar ratios of SC₄ with trans₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋…..
other end (Fig. 1c). As a result, the complexation between V$^{2+}$-cis-Az guest and SC$_4$ host is weakened and this would be the origin for the lower affinity of V$^{2+}$-cis-Az to SC$_4$. It should be noted that the inhibition frequency of SC$_4$:V$^{2+}$-trans-Az after irradiation is about 26% of the value in SC$_4$ only, according to the slop values of the inhibition number in Fig. 5c. This result is similar to the photoisomerization efficiency of cis-azobenzene in SC$_4$:V$^{2+}$-trans-Az, which is about 33% calculated from $^1$H NMR (Supplementary Fig. S14–15), revealing the efficiency of the nanopore biosensor to study the photoresponsive host-guest system at the single-molecule level.

To achieve real-time monitoring open-close state of $\alpha$-HL induced by this photoresponsive host-guest system, the current traces were constantly recorded in the presence of the complex (SC$_4$:V$^{2+}$-trans-Az) along with irradiation under $\lambda = 356$ nm (Fig. 6a–c) at the potential of $-100$ mV. The structure of $\alpha$-HL kept stable under...
the UV irradiation in our experimental condition as shown in Supplementary Fig. S16. The number of blockages increased exponentially with the irradiation time during the initial 10 min resulting in a time constant of 316 s, and then gradually reached saturation as shown in Figure 6e. It should be noted that the saturated frequency obtained in real-time detection is comparable to the inhibition frequency of SC4;V2–trans Az after irradiation (0.32 s⁻¹). Therefore, this novel z-HL:SC4 system could real-time monitor the dynamic process for the photoisomerization of SC4;V2–Az at the single-molecule level.

Discussion

Our results demonstrate that the host compound SC4 could efficiently induce the voltage-dependent close-states of z-HL, probably by a collapse of the stem region at high negative potentials. We suggest that Lys⁵¹⁷ and Lys⁶⁴⁷ might be the most suitable binding sites by measuring the interactions of SC4 with z-HL over a wide range of holding potentials. Therefore, we developed artificial gating mechanisms of z-HL triggered by the SC4-based host-guest interactions. By virtue of this novel z-HL:SC4 system, the functionalized z-HL has achieved to be commanded by both ligand molecule (V²⁺–Az) and photo-stimulation at the single-molecule level for the first time. Subsequently, we have extended the application of this stimuli-responsive nanopore system to the real-time study of light-induced molecular machine based on SC4 and V²⁺–Az at the single-molecule level. The present study provides a general tool to probe dynamic processes of molecular machines in receptor-functionalized biomolecular nanoropes, and specifically the analysis of the interactions of a light-activated machine with the calixarene-modified z-HL nanopore was demonstrated. The various stimuli-responsive “on-off” host-guest systems could be integrated into the array of z-HL nanoropes to achieve the smart logical operations at the single-molecule level.

Methods

Materials. z-HL was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used without purification. Diphytanoyl-phosphatidyl-choline was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). All reagents and materials are of analytical grade, and solvents were purified by standard procedures. All solutions for analytical studies were prepared with deionized water obtained by a Milli-Q System (Billerica, MA, U.S.A.).

Prior to use, the SC4 and V²⁺–trans Az were dissolved in the buffer of Tris-EDTA (10 mM) at pH 8.0, respectively. 9.2 μM SC4 (0.88 mM) was incubated with 10.0 μM V²⁺–Az (0.81 mM) at the equal molar ratio before injection to the trans chamber. The UV irradiation of SC4;V2–Az was carried out by a 365 nm UV hand-held lamp (UVP Inc. 115 V, 0.16 A) with a home-made plexiglass window. Then, the phosphatidyl-choline in decane (Billerica, MA, U.S.A.) was applied to the chamber was 8.0 %, Sigma-Aldrich, St. Louis, MO, USA) to a 50-μm orifice in a 1 mL Delrin cup integrated into a lipid bilayer chamber (Warner Instruments, Hamden, CT, USA) with a home-made plexiglass window. Then, the chambers were filled with 1.0 M KCl and 10 mM Tris-EDTA (pH 8.0) buffer. The chamber with a plexiglass window is assigned to the trans chamber. The stability of the bilayer was determined by monitoring its resistance and capacitance. The two compartments of the bilayer cell are termed cis and trans, and the cis compartment was defined as the virtual ground. So that a positive potential indicates a higher potential in the trans chamber, and a positive current is the one in which cations flow from the trans to the cis side. The experiments were carried out under voltage-clamp conditions using a ChemClamp (Dagan Corporation, Minneapolis, MN, USA) instrument. The amplifier’s internal low-pass Bessel filter was set at 3 kHz. Data were required at a sampling rate of 10 kHz by using a DigiData 1440A converter and a PC running PClamp 10.2 (Axon Instruments, Forest City, CA, USA). The z-HL insertion was determined by a well-defined jump in current value. Once a stable single-pore insertion was detected, the analyte was added to the cis or trans chamber, proximal to the aperture.

In the real-time assay, the UV hand-held lamp (UVP Inc. 115 V, 0.16 A) was placed 15 cm away from trans chamber. In order to reduce the undesirable noise, the UV hand-held lamp was wrapped by the shielding cloth. The solution in trans chamber was irradiated under UV–VIS light through the plexiglass window. All of the experiments were carried out at room temperature.

Data analysis. Ionic current blockages that were larger than a threshold value of 10 pA were recorded. Data analysis was performed using home-designed software and OriginLab 8.0 (OriginLab Corporation, Northampton, MA, USA). The current blockages are described as i(t) = i(t) − i(t) with the ionic current for the empty nanopore, and i is the blockage current for the analyte partitioning into the nanopore. The values of i and i(t) were obtained by the fitted Gaussian distributions. tᵣᵢ(t) (the inter-event interval) and tₑ(t) (the event duration) were obtained from duration time histograms. The reported standard deviations are based on three separate experiments.

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