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Reversible photo-gated transmembrane channel assembled from an acylhydrazone-containing crown ether triad†

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We have prepared a crown ether triad containing acylhydrazone units. In solution, the triad can self-assemble linearly to form an organogel. UV light-induced *E*/*Z* isomerization of the C=N bond of the acylhydrazone unit endows the assembly with photo-sensitivity. The triad was able to insert into the lipid bilayer to form a supramolecular transmembrane channel which showed transport selectivity for NH_4^+ over K^+. The channel exhibited photo-gating properties under microscopic and macroscopic conditions. The transport of the channel could be reversibly switched off and on by irradiation with alternating 320 and 365 nm UV light as supported by the conductance measurements.

Natural ion channels are proteins that form pores spanning through the cell membrane to mediate the transport of ions.[1] Many of these channels are able to respond to an environmental pulse, which could induce variation of the channel conformation, and thus cause the transmembrane transport to switch on and off – a behavior called gating.[2] The gating behavior is important for the metabolic and signalling functions in biological systems.[3] During the last three decades, a large number of synthetic channels have been developed in the lipid bilayer, which mimic the transmembrane transport behavior of the natural channels well.[4] In this context, channels that are sensitive to an environmental pulse, such as the membrane potential,[5] chemical ligands,[6] and light,[7] are also available. However, channels with reversible gating behavior are very limited because of the lack of synthetic architecture that can quickly respond to the pulse in the bilayer.[5d,8] We herein describe the self-assembly of a synthetic channel containing acylhydrazone units. The channel can respond to UV light, which leads to its gating behavior.

It has been established that the C=N bond of the acylhydrazone unit could undergo quick, reversible configurational *E*/*Z* isomerization activated by UV light.[9] Crown ethers, a class of important macrocyclic molecules,[10] have been broadly employed for the construction of synthetic ion channels due to their high binding affinity for ions.[11] We thus designed the crown ether triad **1** containing acylhydrazone (Fig. 1a). The triad was prepared from trimethyl *cis*-1,3,5-cyclohexanetricarboxylate by reaction with hydrazine in methanol, followed by condensation with 24-crown-8 ether aldehyde in the presence of HCl (see ESI†). The *E* and *Z* configurations of the C=N bond could produce two isomers, namely $\mathbf{1}_E$ and $\mathbf{1}_Z$. At room temperature without irradiation, $\mathbf{1}_E$ should be stable and the predominant isomer in solution. The hydrophobic outer surface of the crown ether would endow $\mathbf{1}_E$ with high membrane-incorporation capability.[12] The N–H bond of the acylhydrazone could form intermolecular hydrogen

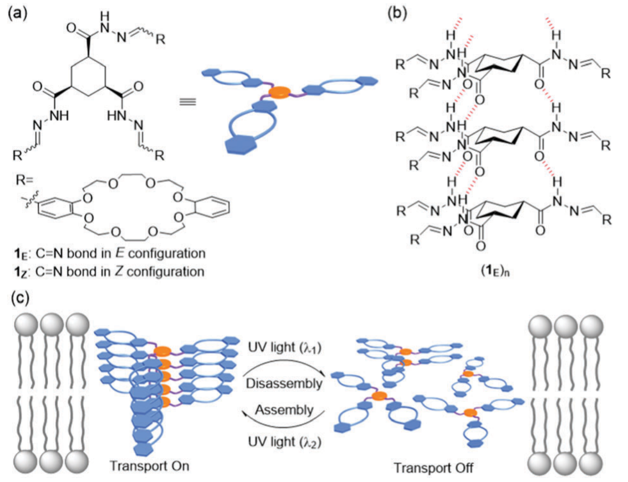

Fig. 1 (a) The structure of $\mathbf{1}_E$ and $\mathbf{1}_Z$. (b) Schematic representation for the assembly of $\mathbf{1}_E$ induced by the intermolecular hydrogen bonding. (c) Schematic representation for the mechanism of photo-gated transmembrane transport.

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bonds with the carbonyl group of the adjacent molecules, and induce the formation of a linear assembly with triple cavities to facilitate the transmembrane transport of ions (Fig. 1b).[13] We envisioned that the C═N bonds of the acylhydrazone units would isomerize from the *E* to *Z* configuration to yield $\mathbf{1}_Z$ under UV light. This isomerization might lead to the disassembly of the transmembrane channel and thus switch off the ion transport (Fig. 1c). After irradiation of $\mathbf{1}_Z$ with longer wavelength light, $\mathbf{1}_E$ might be produced and reassemble to form the transmembrane channel again and switch on the ion transport.

The possibility of the isomerization of **1** was first investigated using ^{1}H NMR in DMSO-d$_6$, from which high resolution spectra were produced. It was found that the ^{1}H NMR spectrum of $\mathbf{1}_E$ showed two sets of amide proton signals at 11.25 and 11.17 ppm (Fig. 2a). These two sets of signals were the result of rotation of the amide single C–N bond. After irradiation of the solution of $\mathbf{1}_E$ with 320 nm UV light for 5 min, two new signals were observed at 10.59 and 10.16 ppm (Fig. 2b), which were assigned to the amide signals of $\mathbf{1}_Z$. The ratio of $[\mathbf{1}_E]/[\mathbf{1}_Z]$ was determined to be 1 : 1. After irradiation of the solution of $\mathbf{1}_Z$ with 365 nm UV light for 5 min, the *Z* configuration could isomerize back to the *E* configuration, indicated by the absence of the proton signals of the amide N–H of $\mathbf{1}_Z$ (Fig. 2c), demonstrating that the acylhydrazone *E*/*Z* isomerization was reversible by irradiation of the solution with alternating 320 nm and 365 nm UV light.

The assembly of the triad was then investigated in solution. It was found that $\mathbf{1}_E$ could form the stable organogel in CHCl$_3$ with a minimal gelation concentration of 8.5 mM (Fig. 3). TEM and SEM images were recorded for the sample obtained from a diluted suspension of the native gel in CHCl$_3$, which showed the formation of a ramified network of inter-winded fibers. These results indicated that $\mathbf{1}_E$ self-assembled to form a linear assembly, which should be driven by the intermolecular hydrogen bonding between the acylhydrazone N–H bonds and the adjacent carbonyl groups. After irradiation of the gel with 320 nm UV light for 5 min, a clear solution was produced due to the isomerization of the C═N bond of the acylhydrazone units to form $\mathbf{1}_Z$. The TEM and SEM images were also recorded by coating the solution on the carbon membrane of a copper grid, which showed the formation of amorphous structures. These results suggested that, in the solution, $\mathbf{1}_Z$ was unable to form regular structures. After irradiation of the solution of $\mathbf{1}_Z$ with 365 nm UV light for 5 min, the formation of the organogel was observed again, indicating that the assembly–disassembly of **1** was reversible by applying alternating 320 nm and 365 nm UV light.

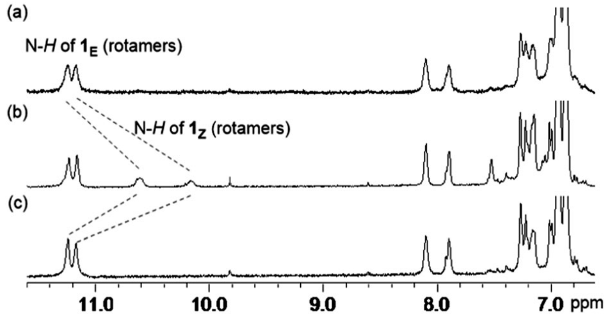

Fig. 2 Partial ^{1}H NMR spectra of $\mathbf{1}_E$ after irradiation with alternating 320 and 365 nm UV light in DMSO-d$_6$ at 298 K. (a) $\mathbf{1}_E$; (b) $\mathbf{1}_E$ after irradiation with 320 nm UV light; (c) the solution of (b) after irradiation with 365 nm UV light.

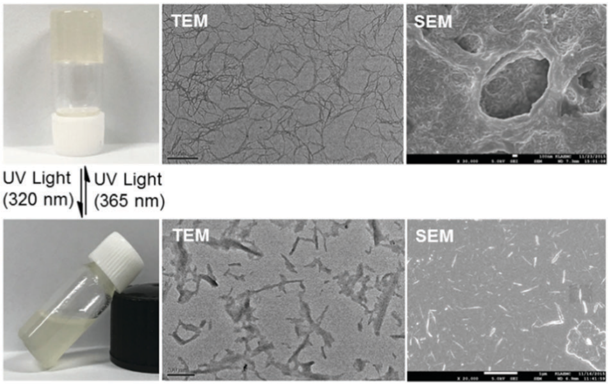

Fig. 3 Photographs and TEM and SEM images of $\mathbf{1}_E$ after irradiation with alternating 320 nm and 365 nm UV light.

The self-assembly behavior of $\mathbf{1}_E$ prompted us to investigate its possibility for the formation of a transmembrane channel in the lipid bilayer using conductance measurement experiments.[14] In a typical experiment, two compartments containing KCl solution (1.0 M) were separated by a planar lipid bilayer composed of diphytanoylphosphatidylcholine (diPhyPC). $\mathbf{1}_E$ (1.0 μM) was added to the *cis* compartment which was grounded. A clamped voltage of −100 mV was applied across the bilayer, and the conductance traces were recorded. From the trace, square-like ionic conductance signals were observed (Fig. 4a), strongly supporting the formation of a transmembrane channel in the lipid bilayer.[15] The opening of the channel could last up to 10 s, indicating that the conformation of the channel was stable in the bilayer. Although the traces showed multi-levels of currents, the current at each level was identical. This result clearly indicated that the self-assembled transmembrane

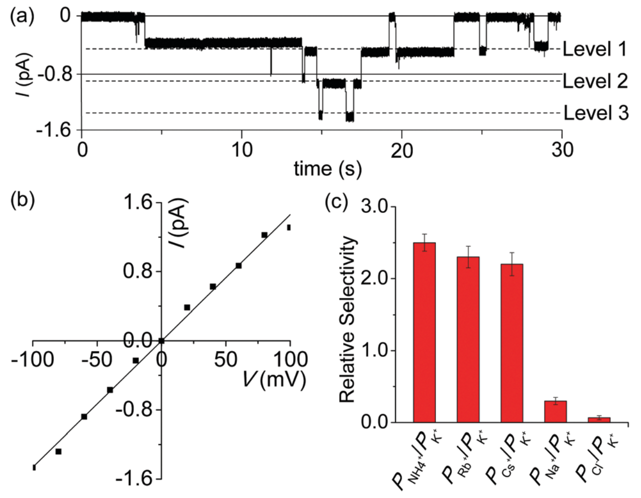

Fig. 4 Microscopic conductance measurements of the channel from $\mathbf{1}_E$ (1.0 μM) in the planar lipid bilayer composed of diPhyPC. (a) Current trace at −100 mV; (b) *I*–*V* plot; (c) permeability (*P*) ratios of the ions through the channel.

channel had a uniform channel length which should match well with the thickness of the bilayer. The recording of the current at various voltages showed a linear current–voltage (*I*–*V*) relationship in the range of −100 to +100 mV (Fig. 4b). The channel conductance (γ) for K^+ was calculated to be 15 pS using the current values of one level. This γ value was close to the corresponding value of the reported single-channel containing the 21-crown-7 unit,[16] suggesting that each conductance event was ascribed to the formation of the single-channel. Thus, the first, second and third level of conductance indicated the formation of single, double and triple-channels from the single linear assembly of $\mathbf{1}_{E}$ (Fig. 4a).

The selectivity of the channel from $\mathbf{1}_{E}$ for Na^+, K^+, Rb^+, Cs^+, NH_4^+, and Cl^- was further measured using unsymmetrical salt solution on the two sides of the bilayer (Fig. S14, ESI†). The permeability (*P*) ratios of the ions through the channel could be determined using the Goldman–Hodgkin–Katz equation (Fig. 4c).[17] The channel showed transport selectivity in the order of $NH_4^+ \approx Rb^+ \approx Cs^+ > K^+ > Na^+ > Cl^-$, demonstrating that the channel was cation selective. The ion transport selectivity order was slightly different from the complexation selectivity sequence of the 24-crown-8 unit for the cations in solution.[18] This difference should be a result of the deformation of the 24-crown-8 unit in the bilayer caused by the squeezing effect of the surrounding lipid molecules.[19] The deformation of the crown ether unit led to its complexation selectivity in the bilayer being different from that in solution.

To exploit the photo-gated behavior of the channel, further conductance experiments were performed by applying 320 and 365 nm UV light alternately to the bilayer containing the channel of $\mathbf{1}_{E}$ (Fig. 5). It was found that, in KCl solution (1.0 M), after irradiation of the bilayer with 320 nm UV light for 5 min, conductance signals were absent from the current traces under +100 mV potential. This is not unexpected, because of the isomerization of $\mathbf{1}_{E}$ to $\mathbf{1}_{Z}$ after irradiation, which led to the disassembly of the channel and the switch-off of the transport of K^+. Although only 50% of the molecules of $\mathbf{1}_{E}$ underwent isomerization, the amount of molecules that were un-isomerized was insufficient for the formation of the channel spanning through the bilayer. Further irradiation of this silent bilayer with 365 nm light for 5 min could switch on the transport of K^+ again. The switch off–on cycle could be run up to three times by applying 320 and 365 nm UV light alternately, which indicated the photo-gated property of the channel. Further cycles would lead to the breaking of the bilayer.

To further investigate the photo-gated behavior of the channel at the multi-channel (macroscopic) level, conductance measurements were also performed by premixing $\mathbf{1}_{E}$ with diPhyPC (0.01% molar ratio). The macroscopic currents were recorded by applying +100 mV potential while the bilayer was irradiated with alternating 320 nm and 365 nm UV light (Fig. 6a). The current trace is shown in Fig. 6b. When the bilayer was irradiated with 320 nm UV light, the current decreased quickly and reached a stable value of around 100 pA in 30 s. The decrease of the current reflected the decrease of the channel number, which should be a result of the disassembly of the channel caused by the isomerization of $\mathbf{1}_{E}$ to $\mathbf{1}_{Z}$. The current value decreased by 60% after irradiation, suggesting that 60% of the channels disassembled in the bilayer. It was expected that self-assembly would stabilize the $\mathbf{1}_{E}$ form in the bilayer. However, the obvious isomerization after irradiation demonstrated that self-assembly had little effect on the stabilization of $\mathbf{1}_{E}$. Different from the disruption of the channel structure after irradiation under microscopic conditions, under macroscopic conditions, part of the channels could survive the irradiation. Thus, a stable current could be observed when the isomerization reached equilibrium. After switching the 320 nm light to 365 nm light, the current value was observed to increase gradually and reached the initial current value, indicating the reassembly of the channels. Although this switch off–on cycle could only be run

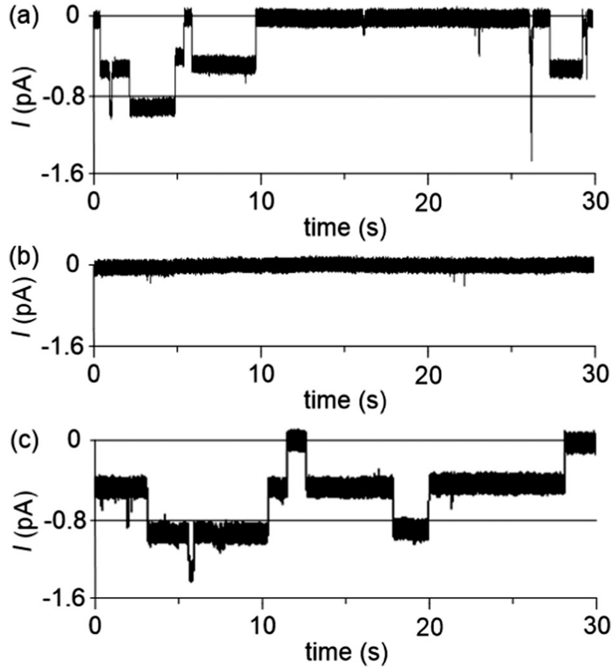

Fig. 5 Current traces of $\mathbf{1}_{E}$ at −100 mV by irradiation with alternating 320 and 365 nm UV light. (a) Without irradiation; (b) irradiation of the bilayer in (a) with 320 nm UV light; (c) irradiation of the bilayer in (b) with 365 nm UV light. Permeability (*P*) ratios of the ions through the channel.

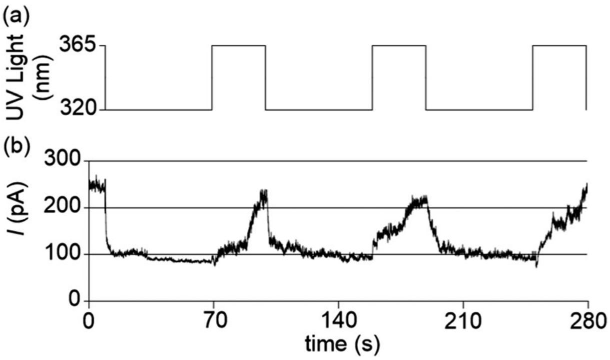

Fig. 6 (a) The alternating 320 nm (60 s) and 365 nm (30 s) UV light irradiation protocol. (b) The macroscopic current trace of $\mathbf{1}_{E}$ at +100 mV by applying the pulse in (a). Permeability (*P*) ratios of the ions through the channel.

three times, the results strongly supported that the transport behavior of the channel was photo-gatable.

In conclusion, we have developed a transmembrane channel with photo-gated behavior from the self-assembly of a crown ether triad containing the photo-sensitive acylhydrazone unit. The photo-induced E/Z isomerization of the C=N bond of the acylhydrazone unit drives the channel to disassemble and assemble in the bilayer, which leads to the gating property of the channel. Importantly, the gating process is reversible by irradiation of the channel with alternating 320 and 365 nm UV light. This finding provides a simple and efficient strategy to control mass transport across the bilayer.

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