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Communication

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Friction-Mediated Dynamic Disordering of Phospholipid Membrane by Mechanical Motions of Photoresponsive Molecular Glue: Activation of Ion Permeation

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Supporting Information

ABSTRACT: A water-soluble photoresponsive molecular glue, Azo-^{18}Glue, consisting of a photochromic azobenzene core and two adhesive dendritic wedges with a total of 18 peripheral guanidinium ion (Gu^+) pendants tightly adheres to the surface of a phospholipid membrane, even in buffer, via a multivalent salt-bridge formation with phosphate anions. A photomechanical motion of adhering Azo-^{18}Glue possibly gives rise to dynamic structural disordering of the phospholipid membrane and activates transmembrane ion permeation. In sharp contrast, no activation of ion permeation results when poorly adhesive Azo-^{6}Glue carrying only six Gu^+ pendants is used in place of Azo-^{18}Glue.

Friction is a force resisting the relative motion of two surfaces in contact sliding against each other. Not only in macroscopic but also molecular-scale events, friction is often crucial for mechanical information transfer between two objects. Examples of friction-mediated interfacial phenomena in biology include muscle contraction,[1] selectin-mediated internalization of a flowing leukocyte,[2] and operation of biomolecular machines.[3] We envisioned if such a molecular-scale friction, intentionally generated by a tailored adhesive motif, might noncovalently give rise to a structural perturbation of cell membranes and therefore alter cellular activities. As a proof of concept, we designed Azo-^{18}Glue (Figure 1) that consists of a photochromic azobenzene core and two sticky dendritic wedges carrying 18 peripheral guanidinium ion (Gu^+) pendants, and explored the possibility that its "photomechanical motion" might dynamically alter physical properties of a natural phospholipid membrane.

Recently, we developed water-soluble dendritic molecular glues having multiple peripheral Gu^+ pendants.[4] Gu^+ is known to bind oxyanions by the formation of a salt bridge via hydrogen-bonding and electrostatic interactions.[5] The Gu^+-appended dendritic molecular glues were designed with such an expectation that a multivalency, given to the salt-bridge formation by the dendritic scaffold, could amplify the adhesivity toward target objects.[4a] In fact, the dendritic molecular glues, in aqueous media, can tightly adhere to the surfaces of proteins (CO_2^-)[4a,b] and clay nanosheets (SiO^-).[4c] In the present work, Azo-^{18}Glue (Figure 1) was designed in such a way that its two adhesive dendritic wedges, upon photoisomerization of the azobenzene core, can change their relative locations (Figure 2).

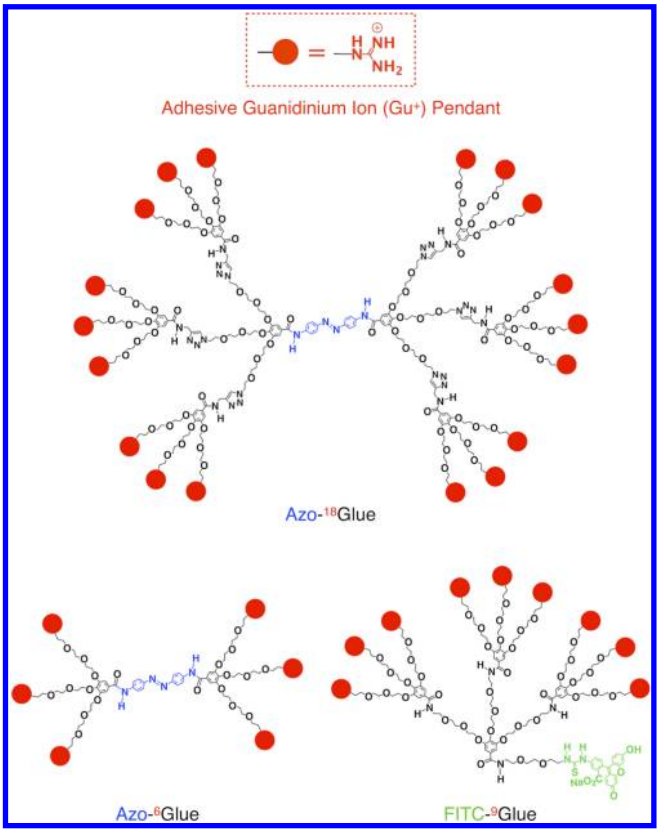

Figure 1. Schematic structures of photoresponsive molecular glues Azo-^{6}Glue and Azo-^{18}Glue together with that of fluorescently labeled molecular glue FITC-^{9}Glue. Red circles represent adhesive guanidinium ion (Gu^+) pendants.

Photoisomerization of azobenzene, at a single-molecule level, is known to occur almost instantaneously in a subpicosecond range.[6] Although such an abruptness in motion might be much tempered at the adhesive points due to a conformational flexibility of the dendritic architecture, we found that the photomechanical motion of Azo-^{18}Glue possibly activates ion permeation across a phospholipid membrane.

Photoresponsive Azo-^{18}Glue (Figure 1) was synthesized by condensing azodianiline with a dendron carrying azide groups,

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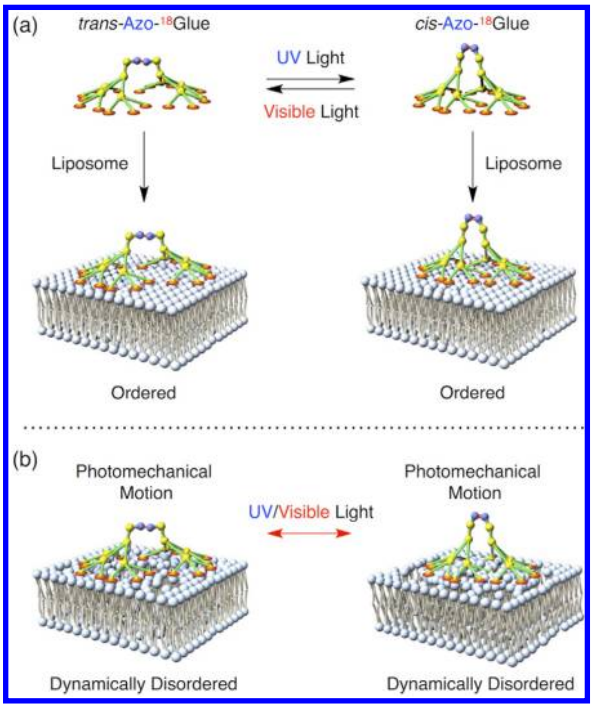

Figure 2. Schematic representations of possible effects of photoresponsive molecular glue Azo-^{18}Glue on a phospholipid membrane. (a) Static adhesion effects of *trans*-Azo-^{18}Glue (left) and *cis*-Azo-^{18}Glue (right). In either case, structural disorder is unlikely for the phospholipid membrane due to a thermodynamic equilibration. (b) Dynamic effects of *trans*-to-*cis* (left) and *cis*-to-*trans* (right) photoisomerization events of adhering Azo-^{18}Glue. In either case, a dynamic structural disorder, allowing for transmembrane ion permeation (see Figure 4a), can be expected for the phospholipid membrane by an abrupt photomechanical motion of Azo-^{18}Glue.

followed by deprotection of a 'click' reaction product between the resultant dendritic azide with a dendron carrying an alkyne focal core and Boc-protected Gu^+ pendants.[7] Fluorescently labeled FITC-^{9}Glue (Figure 1) carrying nine Gu^+ pendants was synthesized by the isothiocyanate-mediated addition of FITC (fluorescein isothiocyanate) onto a dendron carrying a focal amino group and Boc-protected Gu^+ pendants.[7] An aqueous dispersion of egg-yolk phosphatidylcholine (EYPC) liposomes in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer ([HEPES] = 10 mM, [NaCl] = 100 mM)[8] was prepared according to a literature method[9] by subjecting a HEPES buffer dispersion of crude EYPC liposomes to freeze–thaw cycles followed by repeated extrusion processes.[7] For fluorescence microscopy and flow cytometry, additional freeze–thaw cycles were conducted subsequently to obtain giant EYPC liposomes.[9c] When mixed with FITC-^{9}Glue (Figure 1; 25 nM) in HEPES buffer, the EYPC liposomes (140 μM), as observed by fluorescence microscopy, turned fluorescent upon photoexcitation at 488 nm (Figure 3a). In flow cytometry (λ_{ext} = 488 nm; Figure 3b), the EYPC liposomes treated with FITC-^{9}Glue displayed a monodisperse histogram (green) that shifted completely from that of the original EYPC liposomes (blue). From this histogram along with a fluorescence spectral feature of the mixture, roughly 17% of added FITC-^{9}Glue molecules were estimated to attach to the EYPC liposomes.[7] Therefore, it is most likely that a multivalent salt-bridge formation operates properly even in buffer. Now one can expect that Azo-^{18}Glue having a larger number (18) of

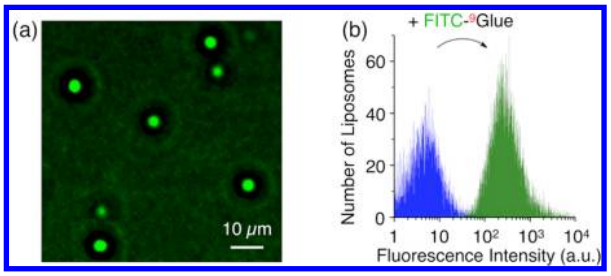

Figure 3. Adhesion of fluorescently labeled molecular glue FITC-^{9}Glue onto a phospholipid membrane in HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 7.0). (a) Fluorescence microscopy (λ_{ext} = 488 nm) of giant EYPC liposomes (140 μM) containing FITC-^{9}Glue (25 nM). (b) Flow cytometry histograms (λ_{ext} = 488 nm) of giant EYPC liposomes (140 μM) before (blue) and after (green) mixing with FITC-^{9}Glue (25 nM).

the Gu^+ pendants could adhere more tightly than FITC-^{9}Glue to the EYPC liposomes.

Photoisomerization of the azobenzene core of Azo-^{18}Glue was confirmed by electronic absorption spectroscopy (Figure S3, Supporting Information [SI]).[7] When a HEPES buffer solution of Azo-^{18}Glue was irradiated at 365 nm for 2 min in the presence of EYPC liposomes (~100 nm), an absorbance at 385 nm due to the *trans*-form of the azobenzene core[10] decreased (Figure S3b, SI, green curve).[7] Successive photoirradiation at 546 nm allowed resultant Azo-^{18}Glue to recover the 385-nm absorbance almost completely (Figure S3b, SI, red curve).[7] The spectral change profiles thus observed were virtually identical to those of Azo-^{18}Glue without EYPC liposomes (Figure S3a, SI,),[7] indicating that adhesion onto the liposomal surface hardly affects the isomerization profile of Azo-^{18}Glue.

Of particular interest, the photoisomerization of Azo-^{18}Glue is accompanied by ion permeation across the liposomal membrane (Figure 4a). The transmembrane ion permeation was traced by using EYPC liposomes (35 μM) including pyranine in their interior and Azo-^{18}Glue (1.5 μM) in both the interior and exterior liposomal environments. Pyranine is a pH-responsive fluorescent dye, which emits fluorescence at 510 nm upon photoexcitation at 460 and 405 nm.[11] The fluorescence intensity upon 460-nm excitation (I_{460}) is known to change with pH, whereas that excited at 405 nm (I_{405}) remains intact to a pH variation.[11] Thus, the pH change at the liposomal interior can be evaluated from I_{460}/I_{405}.[11] By using NaOH, the initial pH values of the inner and outer environments of the liposomes were adjusted to 7.0 and 8.4, respectively, in order to generate a gradient of $[OH^-]$. The resultant dispersion, located in a fluorescence spectrometer, was continuously exposed over a period of 5 min to an external UV light (100-W xenon light source) at 365 nm for executing the *trans*-to-*cis* isomerization of Azo-^{18}Glue. Then, the sample was subjected to fluorescence spectroscopy as for the internal pyranine dye by photoexcitation at λ_{ext} = 460 and 405 nm to record I_{460}/I_{405} for obtaining a time-dependent pH-change profile at the liposomal interior. As shown in Figure 4a (red circles), the ratio I_{460}/I_{405} after the 5-min photoirradiation of Azo-^{18}Glue at 365 nm was greater than that before the irradiation, indicating an increment of pH at the liposomal interior (blue-shaded areas). Such a light-driven internal pH jump could be repeated multiple times. Dynamic light scattering (DLS) analysis (Figure 4b) revealed that the liposomes, even after 15 min photoirradiation at 365 nm, maintain their morphological and dimensional integrities

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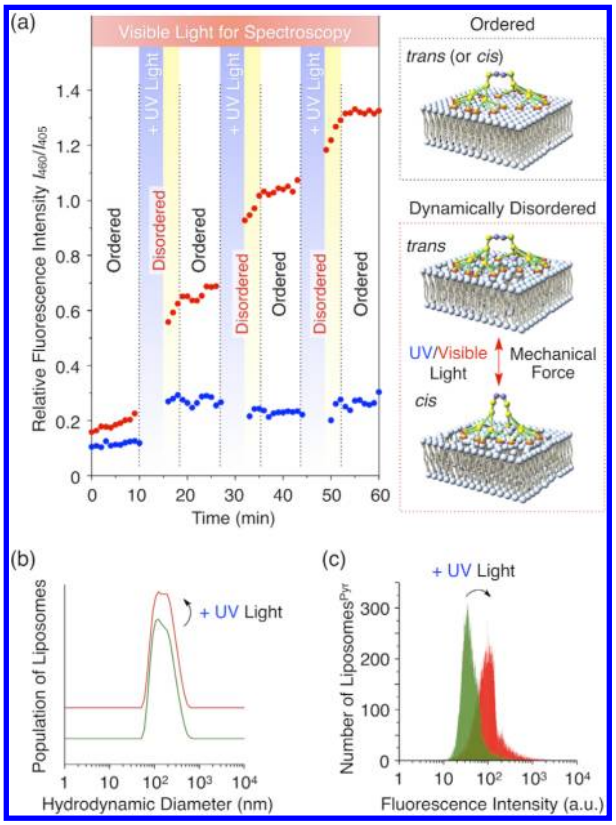

Figure 4. Transmembrane ion permeation profiles of pyranine-containing EYPC liposomes (35 μM; initial inner pH = 7.0) at 25 °C in HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 8.4) containing Azo-^{18}Glue (1.5 μM) or Azo-^{6}Glue (4.5 μM) ([Gu^+] = 27 μM). (a) Changes in relative fluorescence intensity I_{460}/I_{405} at 510 nm (λ_{ext} = 460 and 405 nm for I_{460} and I_{405}, respectively) upon on−off photoirradiation of Azo-^{18}Glue (red circles) and Azo-^{6}Glue (blue circles) with a 365-nm external UV light (blue-shaded areas). Enhanced I_{460}/I_{405} is the consequence of an increment of the inner pH by OH^- permeation, possibly by photomechanically induced disordering of the liposomal membrane (Figure 2b). Note that the sample in yellow-shaded areas was exposed to visible excitation lights for fluorescence spectroscopy, so that *cis*-Azo-^{18}Glue likely isomerized back to its *trans*-form. This isomerization may also give rise to disordering of the liposomal membrane (Figure 2b). (b) DLS histograms of EYPC liposomes before (green) and after (red) 15 min photoirradiation of Azo-^{18}Glue with a 365-nm UV light. (c) Flow cytometry histograms (λ_{ext} = 488 nm) of pyranine-containing giant EYPC liposomes before (green) and after (red) 10-min photoirradiation of Azo-^{18}Glue with a 365-nm UV light.

(green histogram; before photoirradiation, red histogram; after photoirradiation). By flow cytometry (Figure 4c, λ_{ext} = 488 nm), we confirmed that the individual liposomes, after continuous UV irradiation for 10 min, display an enhanced fluorescence emission due to the interior pyranine dye (green histogram; before photoirradiation, red histogram; after photoirradiation), again indicating the occurrence of light-driven transmembrane permeation of OH^- into the liposomal interior. Lower-generation Azo-^{6}Glue (Figure 1) presumably bears a smaller conformational freedom than Azo-^{18}Glue, thereby a mechanical force may transmit more efficiently from the azobenzene core to the adhesive points. However, photoisomerization of Azo-^{6}Glue (4.5 μM) led to little activation of transmembrane ion permeation (Figure 4a, blue circles). This is most likely due to poor adhesivity of Azo-^{6}Glue onto the liposomal surface.

We consider that the light-driven transmembrane ion permeation, thus observed in Figure 4a (red circles), is most likely due to dynamic structural disordering of the phospholipid membrane by a photomechanical motion of adhering Azo-^{18}Glue (Figure 2b). Another possibility to consider is that the adhesion of *cis*-Azo-^{18}Glue just statically deteriorates the phospholipid membrane for inducing the ion permeation. However, this possibility was excluded by means of fluorescence depolarization.[13] Thus, by using DPH-embedded EYPC liposomes (DPH: 1,6-diphenyl-1,3,5-hexatriene), we investigated if the liposomal membrane might alter its fluidity by the interaction with *cis*-Azo-^{18}Glue.[14] Photoexcited DPH at 336 nm is known to fluoresce at 450 nm. If the membrane turns more fluid upon interaction with *cis*-Azo-^{18}Glue, embedded DPH could rotate more vigorously. As a possible consequence of this, its polarized fluorescence, observable upon photoexcitation with a 336-nm plane-polarized light,[14] may be depolarized to some extent. However, as shown in Figure 5,

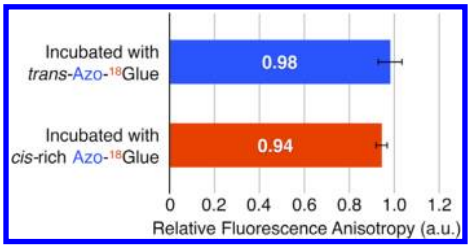

Figure 5. Static adhesion effects of Azo-^{18}Glue on the fluidity of a phospholipid membrane. Fluorescence depolarization profiles at 25 °C of DPH (70 nM), embedded in an Azo-^{18}Glue-attached EYPC liposomal membrane (35 μM), relative to that without Azo-^{18}Glue, in HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 7.0) containing 1.5 μM *trans*- (blue bar) or *cis*-rich[12] Azo-^{18}Glue (red bar).

neither *trans*- nor *cis*-Azo-^{18}Glue on the EYPC liposomes altered the fluorescence anisotropy[15] of embedded DPH. Regardless of its configuration, adhering Azo-^{18}Glue does not statically deteriorate the phospholipid membrane (Figure 2a).

Finally, we point out that Figure 4a, upon closer look, shows a continuous increase in internal pH for a short period (yellow-shaded areas) after stopping the 365-nm UV irradiation. This observation seems a bit intriguing but is consistent with our claim, considering that the sample at this stage was kept exposed to visible excitation lights for fluorescence spectroscopy, wherein *cis*-Azo-^{18}Glue may be tempted to isomerize backward. As discussed in Figure 2b, this isomerization can also generate a photomechanical force for disordering the phospholipid membrane.

In conclusion, we demonstrated that Azo-^{18}Glue (Figure 1), upon photoisomerization, can activate ion permeation across a natural phospholipid membrane by photoisomerization. Through detailed investigations, the activated ion permeation is most likely due to dynamic structural disordering of the phospholipid membrane, induced by a "photomechanical motion" of adhering Azo-^{18}Glue (Figure 2). This mechanism is essentially different from that reported for an assembly of a lipid molecule covalently attached to a photochromic module,[16] where a photoisomerized lipid with a poor molecular packing nature likely deteriorates the lipid membrane statically. The concept of "photoresponsive molecular glues" is universal and

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potentially applicable to live cells, because neither genetic nor chemical modification of native cells is required.[17]

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of Azo-^{18}Glue, Azo-^{6}Glue, and FITC-^{9}Glue; NMR and MALDI-TOF mass spectral data, flow cytometry, fluorescence microscopy, electronic absorption spectroscopy, and related experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(15) $r = (I_{VV} - G \times I_{VH})/(I_{VV} + 2G \times I_{VH})$, where I represents fluorescence intensity, subscripts V and H denote vertical and horizontal orientations of the excitation and emission polarizers, respectively, and G is given by I_{HV}/I_{HH}, which accounts for the relative instrument sensitivity toward vertically and horizontally polarized lights.

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