## Supporting Information

# Grafting synthetic transmembrane units to the engineered low-toxicity α-hemolysin to restore its hemolytic activity

Mihoko Ui,*[a] Kousuke Harima,[a] Toshiaki Takei,[b] Kouhei Tsumoto,[b,c,d] Kazuhito V. Tabata,[e] Hiroyuki Noji,[e] Sumire Endo,[a] Kimio Akiyama,[a] Takahiro Muraoka,[a] and Kazushi Kinbara*[a]

[a] *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1, Katahira, Aoba-ku, Sendai 980-8577, Japan.*

[b] *Department of Medical Genome Sciences, Graduate School of Engineering, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa 277-8562, Japan.*

[c] *Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.*

[d] *Department of Bioengineering and Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan.*

[e] *Department of Applied Chemistry, School of Engineering, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8656, Japan.*

E-mail: kinbara@tagen.tohoku.ac.jp

**Contents**

## 1. Size-exclusion chromatography of the stem-truncated mutants (STM1-6)

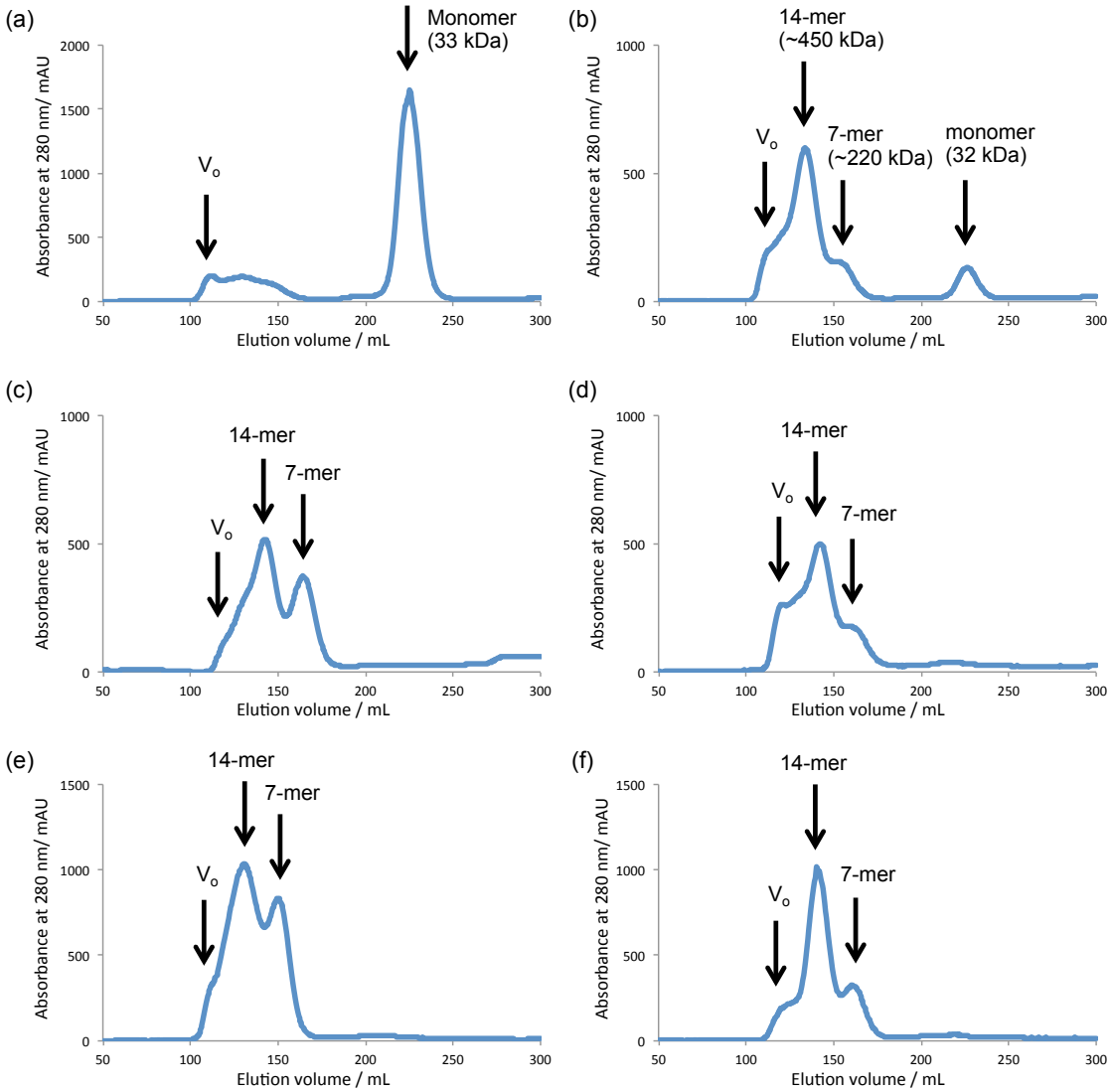



***Figure S1***. Size-exclusion chromatograms of (a) STM1, (b) STM2, (c) STM3, (d) STM4, (e) STM5, and (f) STM6. The proteins in Tris-HCl buffer (pH 8.0; 50 mM; containing 200 mM NaCl, 200 mM arginine hydrochloride, and 5 mM dithiothreitol) were loaded onto a HiLoad 26/60 Superdex 200-pg column pre-equilibrated with the same buffer. Each elution peak is identified as monomer, 7-mer, or 14-mer based on the elution volumes of molecular weight standards, respectively.

**2. Size-exclusion chromatography of STM6 with multiangle light scattering (SEC-MALS) analysis**

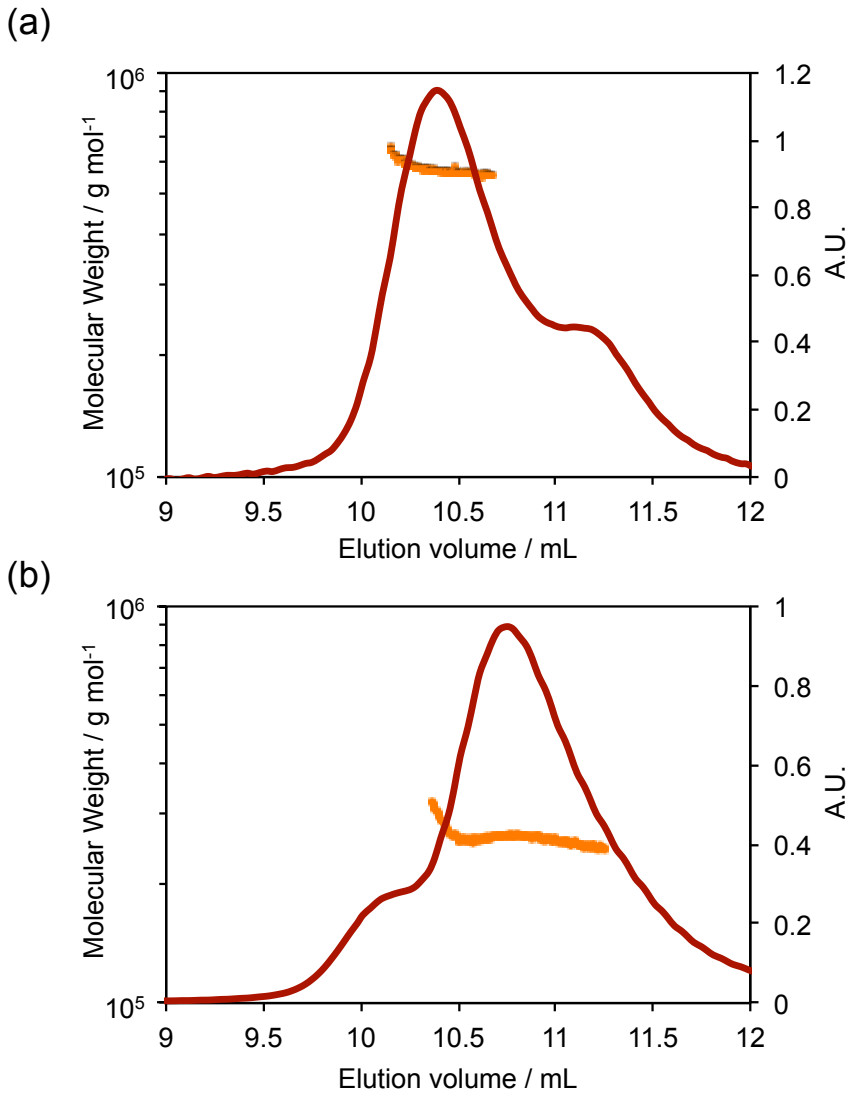



***Figure S2***. Size-exclusion chromatogram of STM6 (red) for (a) the 14-meric and (b) 7-meric forms in Tris-HCl buffer (pH 8.0; 50 mM; containing 200 mM NaCl and 200 mM arginine hydrochloride) detected by refractive index (RI) signals. The orange dots represent the molecular weight evaluated by MALS analysis.

### 3. Western blot analysis of STM5 and STM6 binding to SRBCs

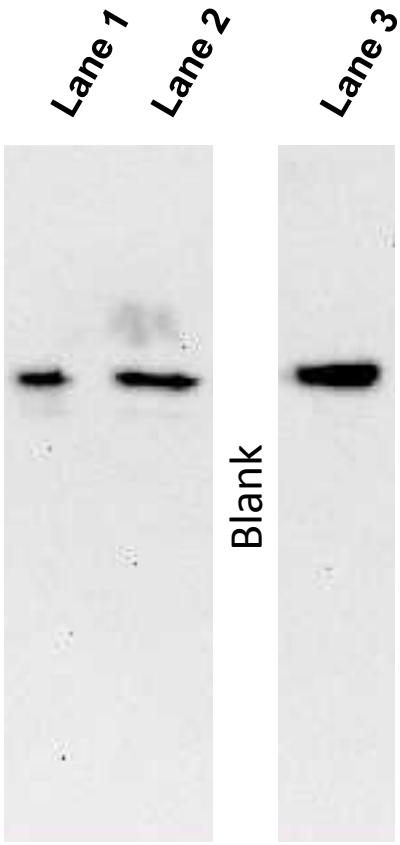



***Figure S3***. Western blot analysis of STM5 and STM6 binding to sheep red blood cells (SRBCs). The stem-truncated mutants STM5 and STM6 (each 0.2 mg/mL) were incubated with SRBCs (50 mL, $OD_{700}$ = 0.5) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) for a definite period of time at 25 °C. Then, the supernatant and pellet fractions were separated by centrifugation at 1,500 ×g for 5 min, and the collected pellets were washed with 500 μL of the same buffer for three times. The mutant proteins attached to SRBCs were detected by western blotting using anti-$His_6$ antibody. Lane 1: STM6 (incubated for 30 min), Lane 2: STM6 (incubated over night), Lane 3: STM5 (incubated for 30 min).

## 4. Hemolytic activity of 14-meric and 7-meric forms of STM5

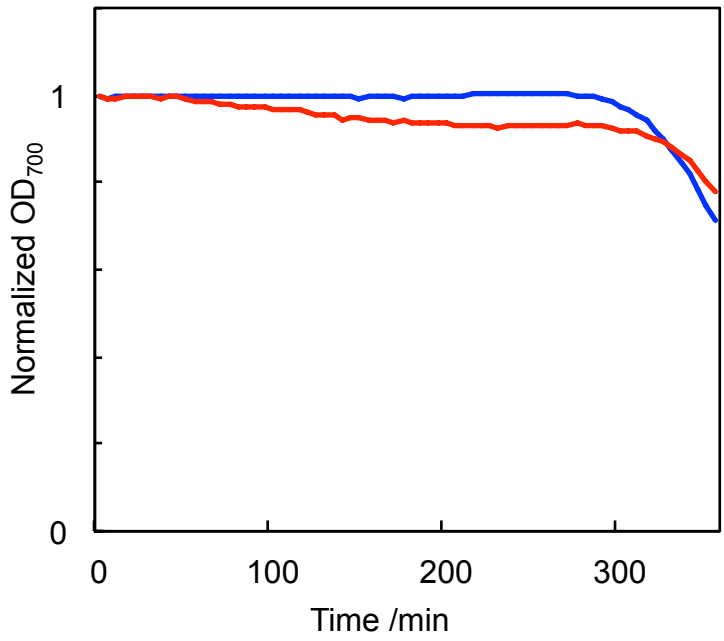



***Figure S4.*** Time-course curves of the hemolysis of SRBCs by STM5 in 7-meric (red line) and 14-meric (blue line) forms (each 0.02 mg/mL) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) at 25 °C, as monitored by optional density at 700 nm.

## 5. Transmission electron microscopy of STM5 with 6.25 mM sodium deoxycholate

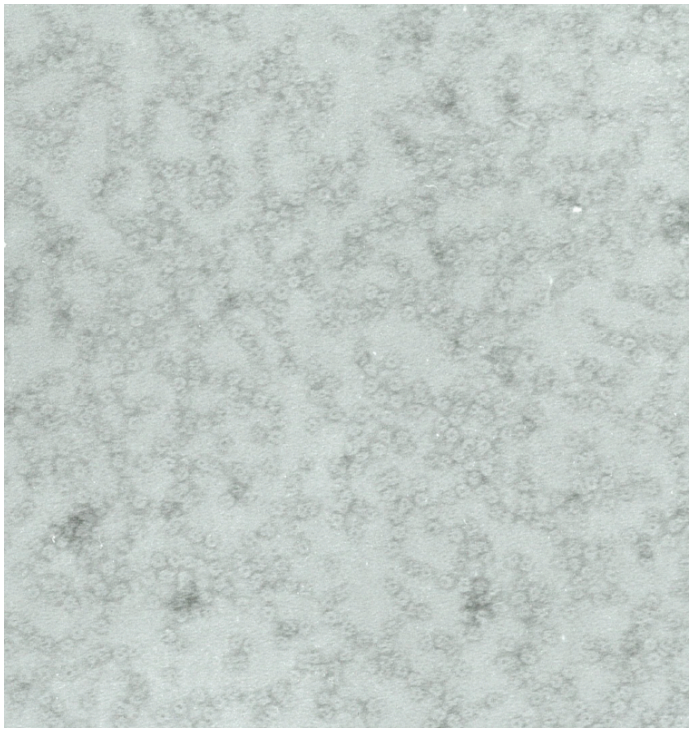
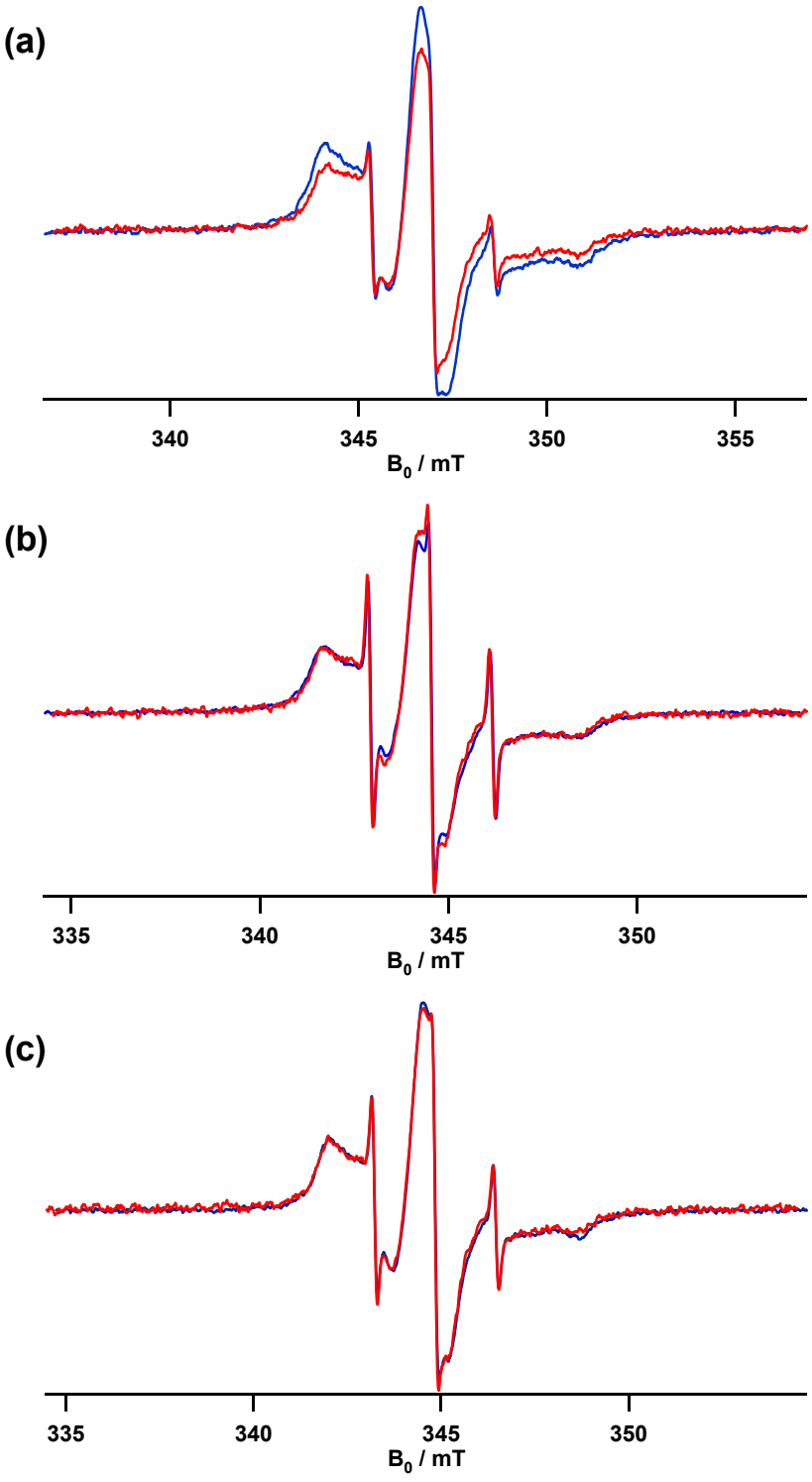


***Figure S5.*** Transmission electron micrograph of STM5 in Tris-HCl bufer (pH 8.0; 50 mM; containing 200 mM NaCl, 200 mM arginine hydrochloride, and 6.25 mM sodium deoxycholate) negatively stained with uranyl acetate.

## 6. Electron paramagnetic resonance (EPR) spectroscopy of spin-labeled STM5

(A)



(B)

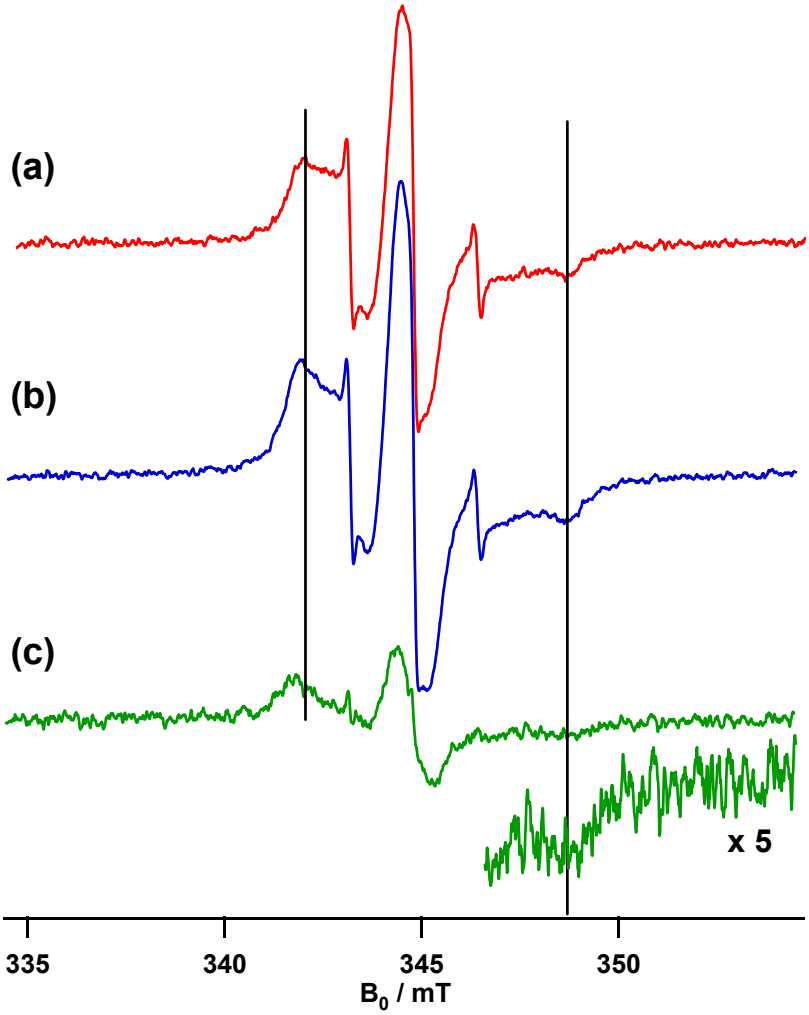



***Figure S6***. (A) EPR spectra of spin-labeled STM5 (a) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride), (b) in suspension of SRBCs ($OD_{700}$ = 0.5), and (c) in PBS buffer (pH 7.4; containing 6.25 mM sodium deoxycholate). Red line is the buffer solution containing spin-labeled STM5 in 7-meric form and blue line is in 14-meric form, respectively. (B) EPR spectra of spin-labeled STM5 in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) containing (a) 7-meric form, (b) 14-meric form, and (c) spectrum (a) subtracted from (b). Black vertical lines were drawn for easy comparison of the peaks

**7. Electron paramagnetic resonance (EPR) spectroscopy of spin-labeled STM2 in a monomeric form**

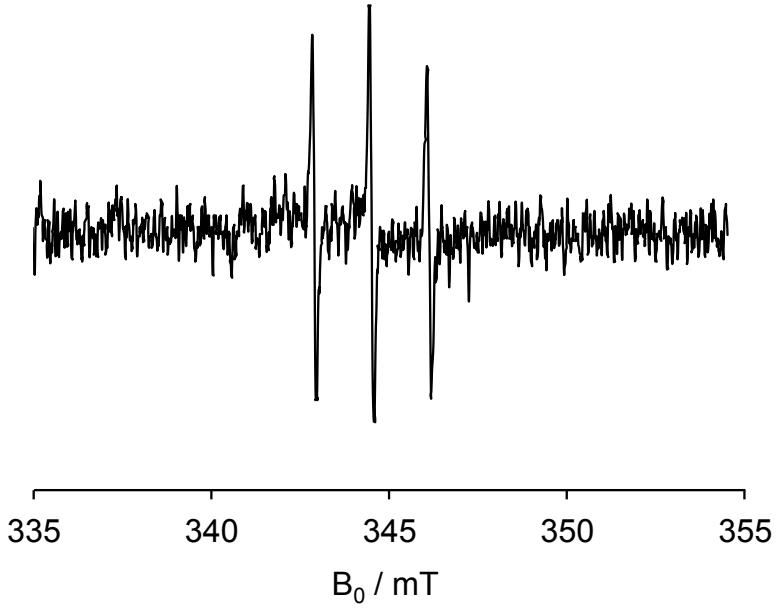



***Figure S7***. EPR spectra of spin-labeled STM2 monomer in Tris-HCl buffer (pH 8.0; 50 mM; containing 200 mM NaCl).

## 8. Western blot analysis of STM5, STM5-PhM, STM5-PyM, and STM5-FlM

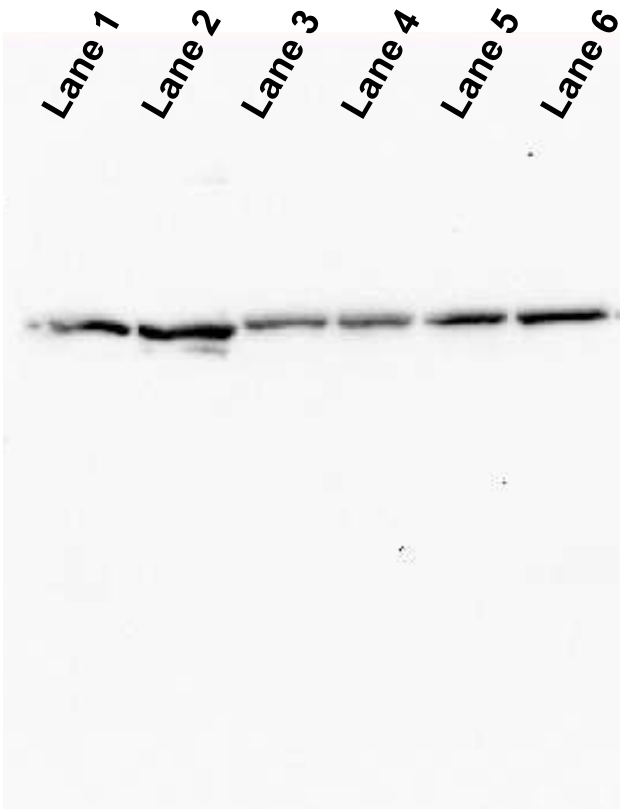
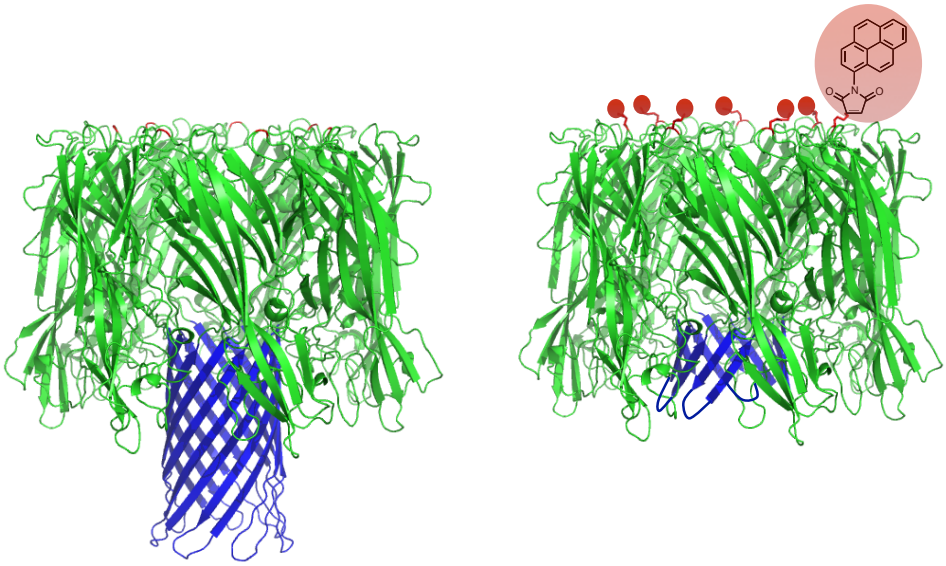



***Figure S8***. Western blot analysis of STM5 derivatives binding to SRBCs. Each protein (0.2 mg/mL) was incubated with SRBCs (50 mL, $OD_{700}$ = 0.5) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) for a definite period of time at 25 °C. Then, the supernatant and pellet fractions were separated by centrifugation at 1,500 ×g for 5 min, and the collected pellets were washed with 500 μL of the same buffer for three times. The mutant proteins attached to SRBCs were detected by western blotting using anti-$His_6$ antibody. Lane 1: STM5 without chemical modification (incubated for 30 min), Lane 2: STM5 without chemical modification (incubated for 180 min), Lane 3: STM5-FlM (incubated for 30 min), Lane 4: STM5-FlM (incubated for 180 min), Lane 5: STM5-PhM (incubated for 30 min), Lane 6: STM5-PyM (incubated for 30 min).

**9. Hemolytic activity of PyM-modified stem-truncated Hla mutant K46C**

**(a)**



**(b)**

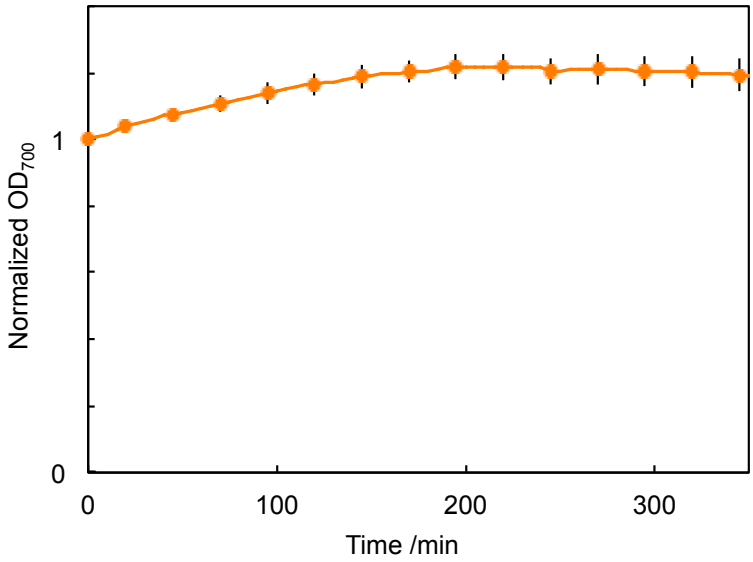



***Figure S9.*** (a) The crystal structure of wild-type Hla (left) and the schematic image of PyM-modified stem-truncated Hla mutant K46C (right). The red-colored residues indicate the site of K46 and the red spheres show the pyreneyl-maleimide moiety. (b) Time-course curves of the hemolysis of SRBCs by PyM-modified stem-truncated Hla mutant K46C (each 0.2 mg/mL) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) at 25 °C, as monitored by optional density at 700 nm.

## 10. The conductance of membranes containing STM5, STM5-PyM, and STM5-FlM

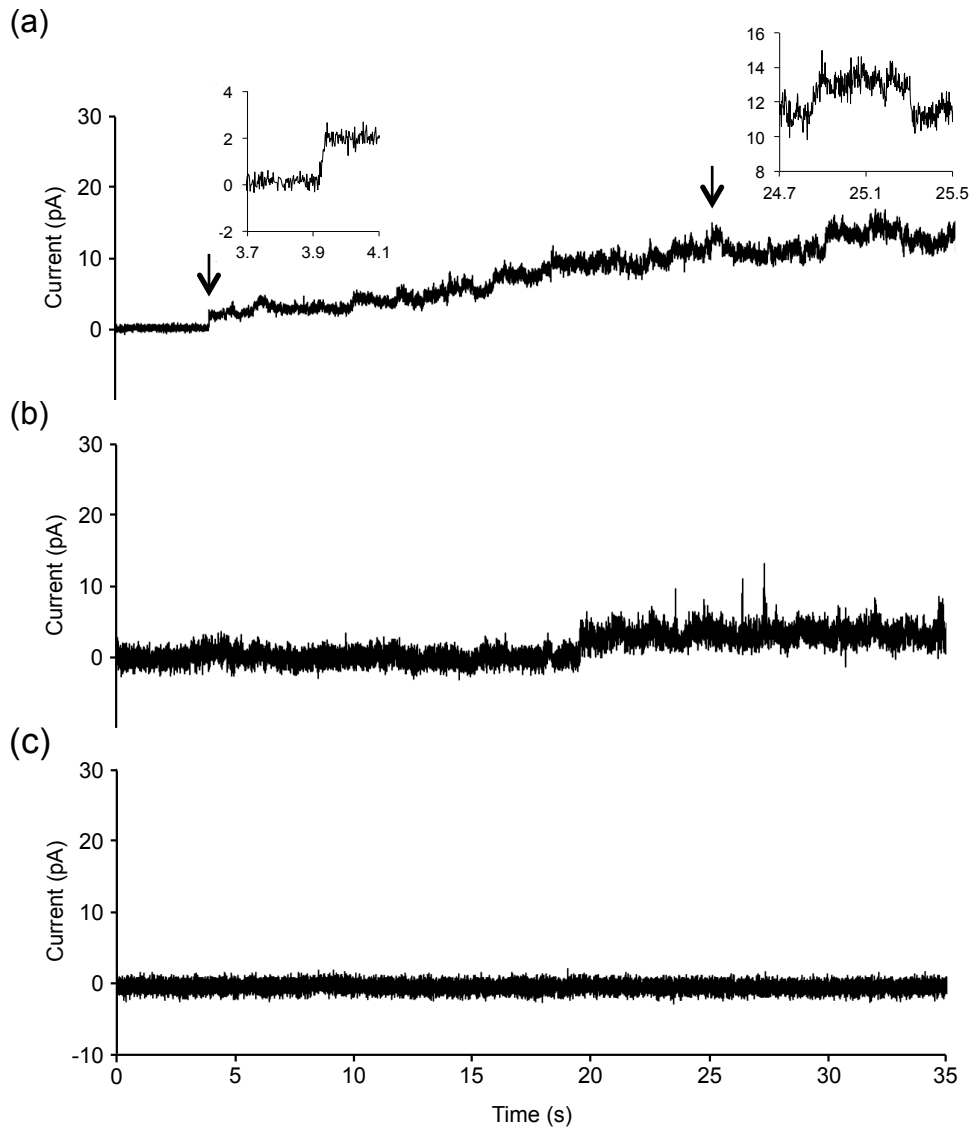
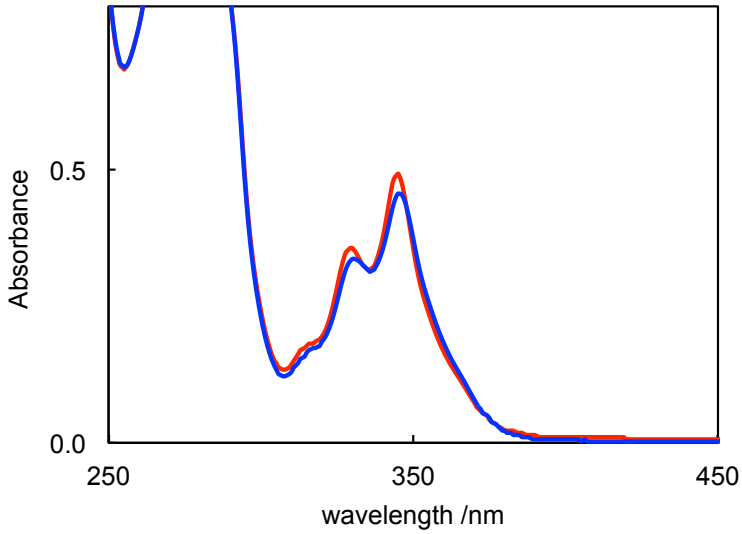



***Figure S10***. Current recording for the planar lipid bilayer containing (a) STM5-PyM, (b) STM5, and (c) STM5-FlM (each 0.13 mg/mL) at the applied voltage of +50 mV in HEPES buffer (pH 7.0, 10 mM, containing 100 mM KCl) at 20 °C. The insets show the enlarged views of the current recordings at the time periods denoted by arrows in (a).

## 11. Absorption and fluorescence spectroscopy of STM5-PyM

(a)




(b)

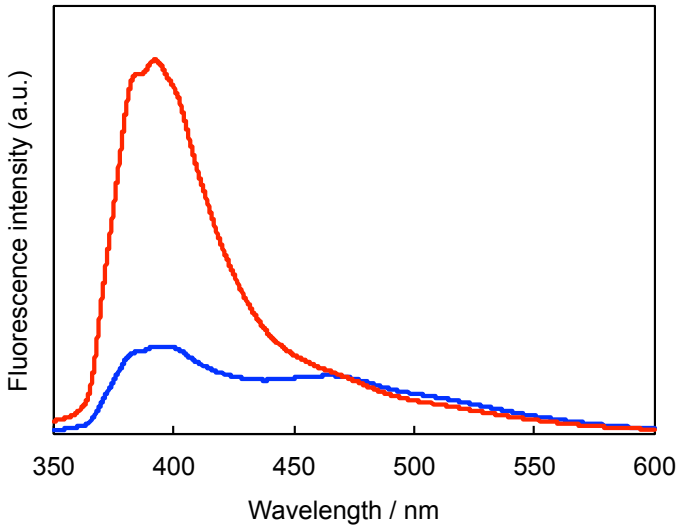



***Figure S11.*** (a) UV-vis absorption spectra of STM5-PyM (2 mg/mL) in Tris-HCl buffer (pH 8.0; 50 mM; containing 200 mM NaCl and 200 mM arginine hydrochloride) without (blue line) and with (red line) 6.25 mM sodium deoxycholate at 25 °C. (b) Fluorescence spectra of STM5-PyM (2 mg/mL) in Tris-HCl buffer (pH 8.0; 50 mM; containing 200 mM NaCl and 200 mM arginine hydrochloride) without (blue line) and with (red line) 6.25 mM sodium deoxycholate at 25 °C.

## 12. Hemolytic activity of chemically-modified STM6

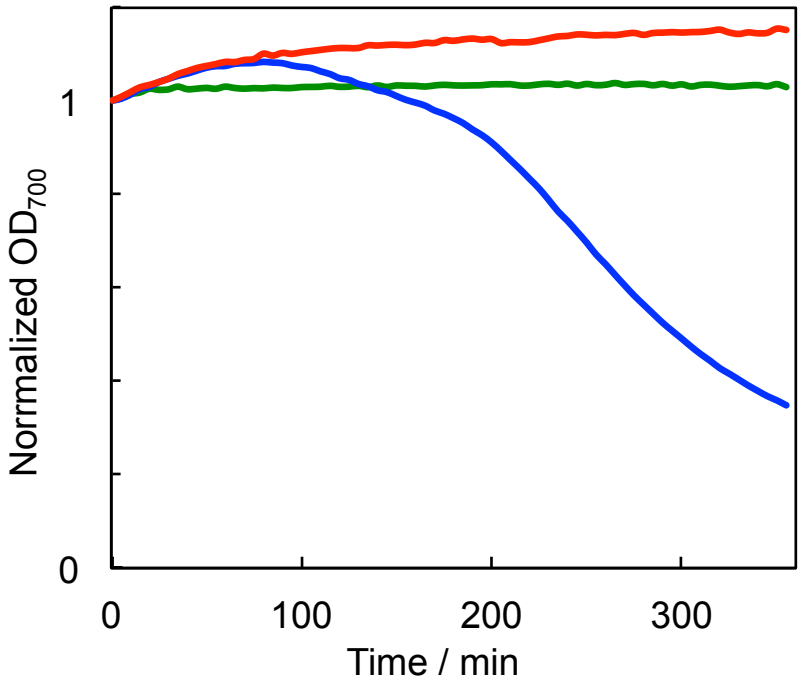



***Figure S12.*** Time-course curves of the hemolysis of SRBCs by STM6 without chemical modification (green line), modified with *N*-(1-pyrenyl)maleimide (blue line) and modified with fluorescein-5-maleimide (red line) (each 0.02 mg/mL) in PBS buffer (pH 7.4; containing 200 mM arginine hydrochloride) at 25 °C, as monitored by optical density at 700 nm.

## 13. The far-UV CD spectra of STM5 and STM-PyM

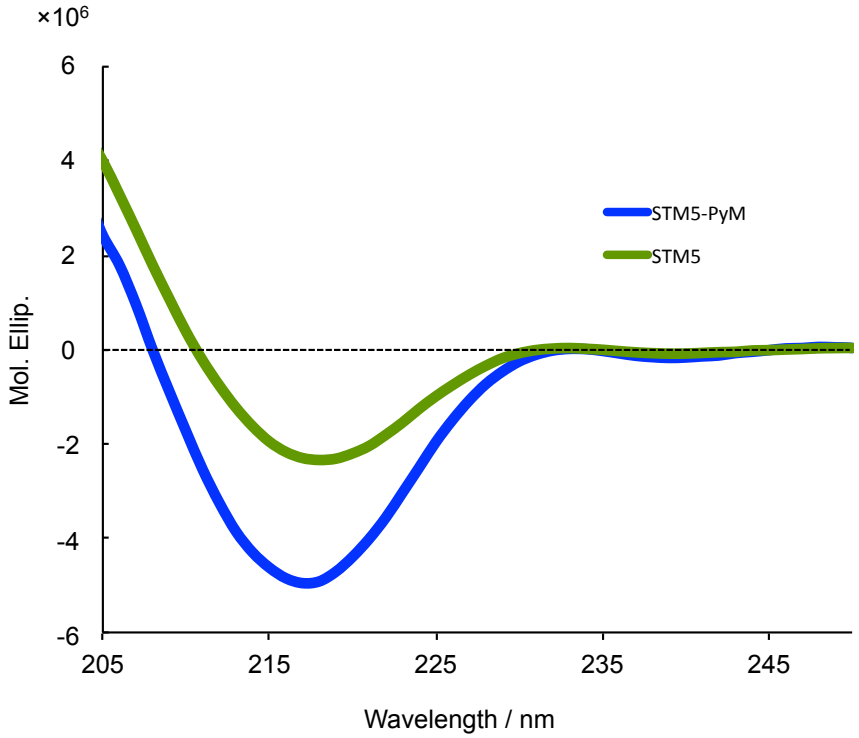



***Figure S13.*** The far-UV CD spectra of STM5 (green line) in phosphate buffer (pH 7.0; containing 150 mM NaCl) and STM-PyM (blue line) in phosphate buffer (pH 7.0; containing 150 mM NaCl and 5 mM dithiothreitol) (each 0.1 mg/mL) at 25 °C.