Redirecting Pore Assembly of Staphylococcal α-Hemolysin by Protein Engineering

Supporting Information

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¶ This paper is dedicated to the memory of our wonderful colleague, Dr. Stephen Cheley, who passed away.

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Table S1. Binding efficacy toward RBCs, HL-60 cells and HT-1080 cells (%)

Cell type	rRBC		hRBC		HL-60		HT-1080	
Stage	UB	TB	UB	TB	UB	TB	UB	TB
αHL	67.12	32.88	99.94	0.06	90.40	9.59	87.18	12.82
αHLG1	24.38	75.62	37.45	62.55	57.05	42.95	58.43	41.57

UB: Unbound, OB: Oligomer Bound, TB: Total Bound protein (oligomer & monomer bound), UB + TB = 100 %

Band intensity was measured by ImageJ (NIH)

Table S2. Information of protease recognition domain

Name of construct	Sequence
RRHLG1	TRR**IGGL**G
MMPHLG1	**PLGL**AGGG

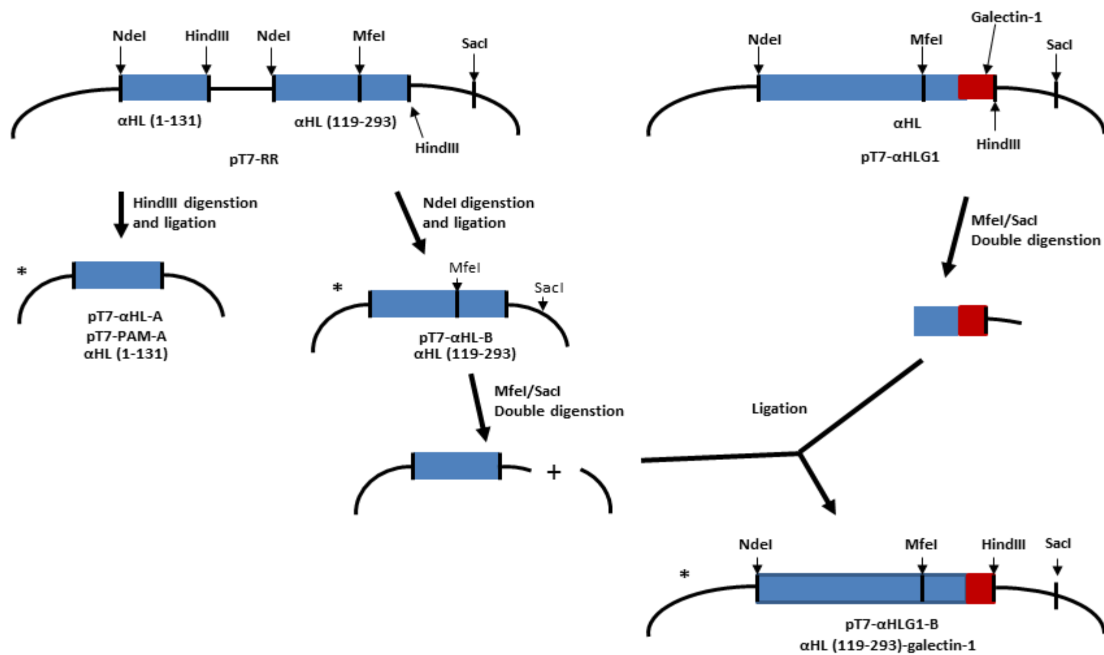

Figure. S1. Construction of PAMαHL and PAMαHLG1 (complimentary mutant of αHLG1: 1-131 and 119-293-galectin-1, pT7: pT7 vector). pT7-αHL-A (αHL, 1-131) and pT7-αHL-B (αHL, 119-293) were prepared from pT7-RR by *HindIII* digestion reaction and *NdeI* digestion reaction respectively. pT7-αHLG1-B (αHL 119-293 and galectin-1 chimera) was constructed by using oligos from pT7-αHLG1 and pT7-αHL-B. Products of double-digestion reaction of both vectors with *MfeI* and SacI were ligated to construct pT7-αHLG1-B.

*pT7-αHL-A: PAM-A, pT7-αHL-B: PAMαHL-B, pT7-αHLG1-B: PAMαHLG1-B

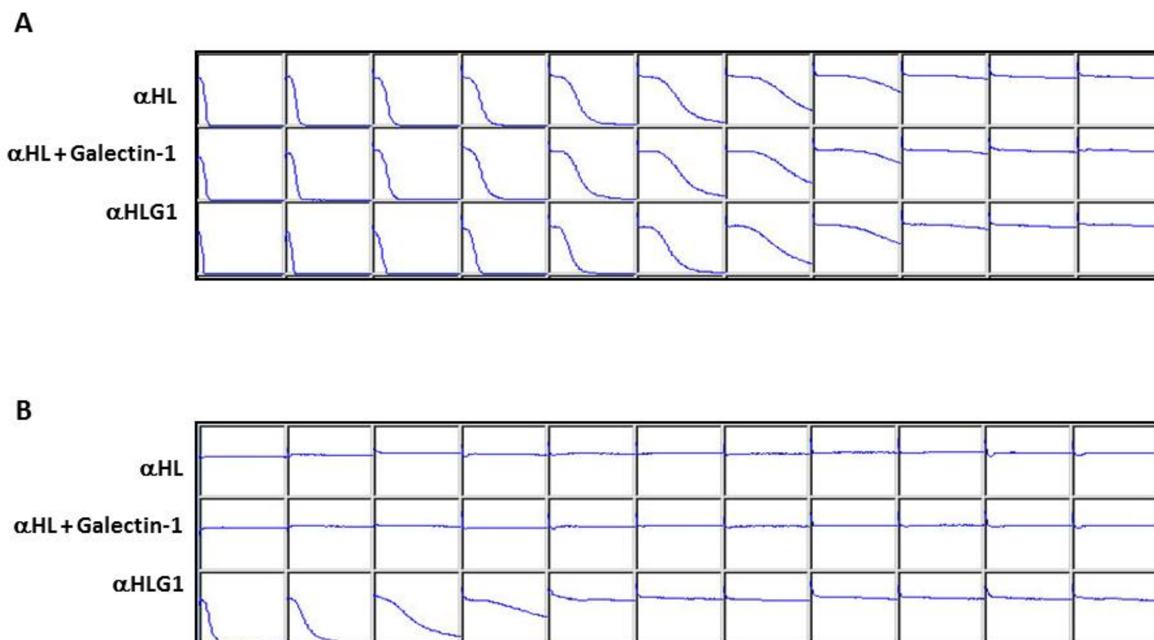

Figure. S2. Hemolysis assay of αHL, αHL + Galectin-1, and αHLG1 (29 nM) to investigate the osmofragility of RBCs by galectin-1. Galectin-1 was added to MBSA buffer to make MBSA-galectin-1 (galectin-1, 29 nM final). 1% rRBC (A) and 1% hRBC (B) were prepared with MBSA-galectin-1. αHL was diluted with MBSA buffer (29 nM final) in the first well of each row (100 μl final volume), and then subjected to 2-fold serial dilution with the same buffer across each row, leaving 50 μl in each well. The assay was initiated by the addition of 50 μl RBC mix to each well and monitored by observing the decrease in light scattering at 595 nm with microplate reader (TECAN).

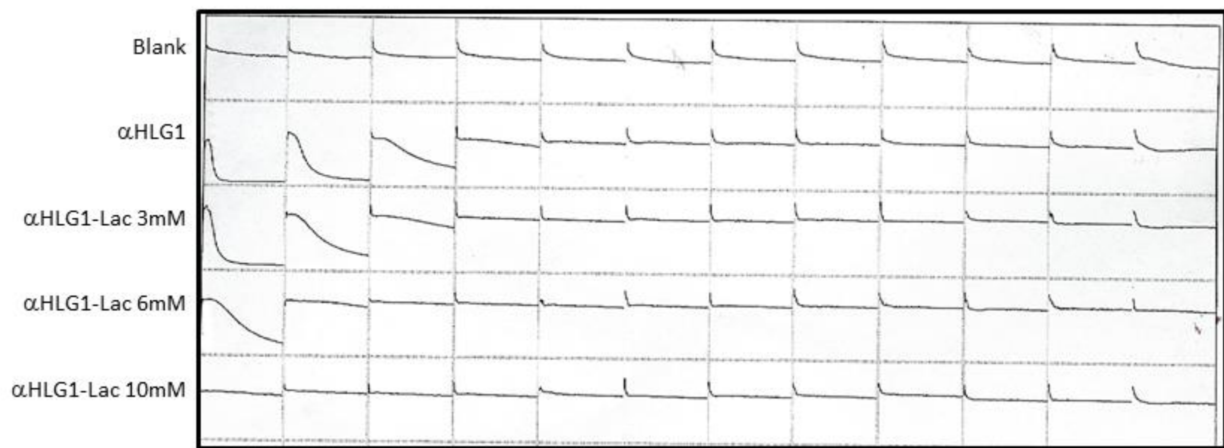

Figure. S3. Inhibition assay of αHLG1 with lactose. αHLG1 preincubated (20 min) with lactose (3 mM, 6 mM, and 10 mM) was added to the first well of each row and two-fold serially diluted across the row (50 μL final volume in each well). The hemolysis assay was initiated by adding 1% hRBC (50 μL to each well, 0.5% RBCs final).

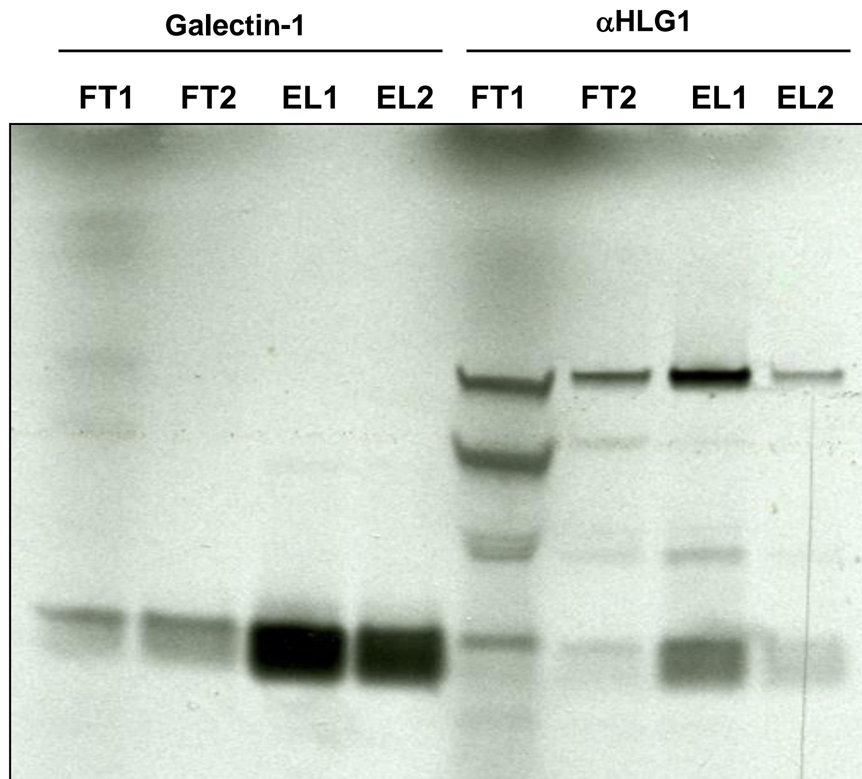

Figure. S4. Binding study of galectin-1 and αHLG1 to β-lactose-agarose column. Galectin-1 or αHLG1 synthesized by IVTT was incubated with the β-lactose-agarose resins in a small column (1.5 ml) for 6 h at 4°C. Then the column was centrifuged to obtain the first flow-through. The column was then washed with PBS buffer containing 2 mM of lactose to obtain the second flow-through. Elution buffer containing 10mM of lactose was then added to the column to obtain elution samples. All samples were loaded on 10% SDS-polyacrylamide gel: lane 1, galectin-1 FT-1; lane 2, galectin-1 FT-2; lane 3, galectin-1 EL-1; lane 4, galectin-1 EL-2; lane 5, αHLG1 FT-1; lane 6, αHLG1 FT-2; lane 7, αHLG1 EL-1; lane 8, αHLG1 EL-2. FT, flow through and EL, elution

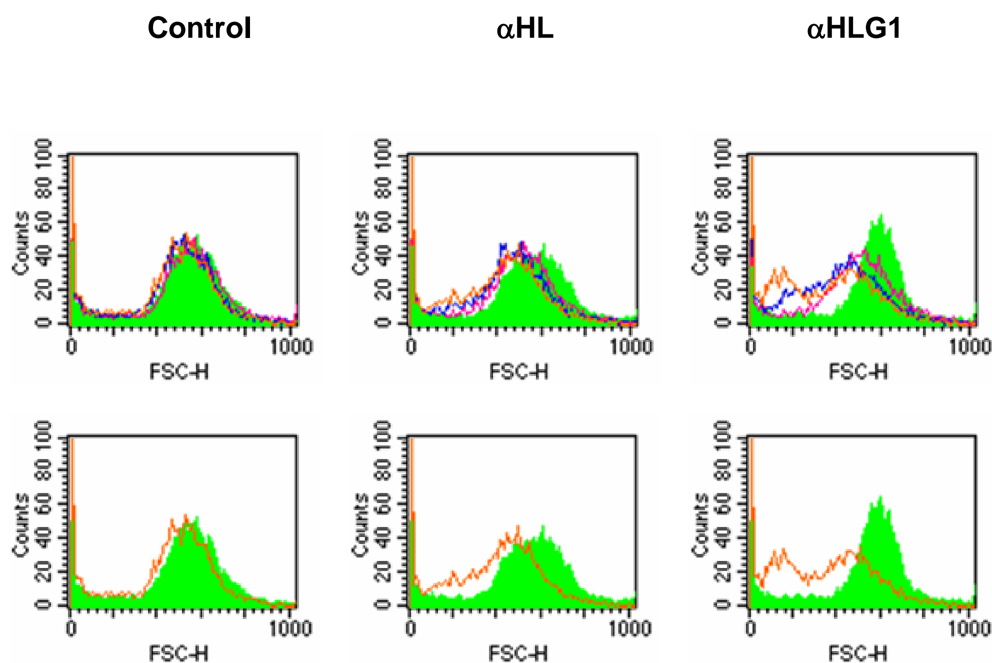

Figure. S5. Lysis assay with flow cytometer (law data of Fig. 4A). Using flow cytometer (parameters: FSC and SSC), cytotoxicity of toxin (αHL or αHLG1) toward HL-60 cells were monitored at every 5 min for 75 min. Top: Lytic activity are shown at the time points (green: 0 min; pink: 25 min; bule: 45 min; orange: 75 min.). Bottom: Lytic activities were shown at 0 min (green) and 75 min (orange). PBS-washed HL-60 cells were incubated with assay mix (αHL or αHLG1, 19 nM; IMDM containing 3% FBS) and very gently agitated before the reading on FACS.

CATATGGCAGATTCTGATATTAATATTAAAACCGGTACTACAGATATTGGAAGCAATACT

NdeI

ACAGTAAAAACAGGTGATTTAGTCACTTATGATAAAGAAAATGGCATGCACAAAAAAGTATTTTATA

GTTTTATCGATGATAAAAATCACAATAAAAAACTGCTAGTTATTAGAACAAAAGGTACCATTGCTGG

TCAATATAGAGTTTATAGCGAAGAAGGTGCTAACAAAAGTGGTTTAGCCTGGCCTTCAGCCTTTAAG

GTACAGTTGCAACTACCTGATAATGAAGTAGCTCAAATATCTGATTACTATCCGCGGAATTCGATTG

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TTCAAAACAATTTTAGAGAGCCCAACTGATAAAAAAGTAGGCTGGAAAGTGATATTTAACAATATGG

TGAATCAAAATTGGGGACCATACGATCGAGATTCTTGGAACCCGGTATATGGCAATCAACTTTTCAT

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GAAAAAGAAGAAATGACAAATACTAGTAGCGGATCGTCCAGAAGAGGTGGGCCCGCAGCGGCTCTTC

SpeI *EarI* *ApaI* *EarI*

CAGTTCGGGACACCACCATCACCACCATTGATAAGCTT

HindIII

Figure. S6. DNA sequence of αHL-CTEx-ESA to generate αHL-galectin-1 chimera.