A Novel 'Prebinding' Strategy Dramatically Enhances Sortase-mediated Coupling of Proteins to Liposomes

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Supporting Information

Table S1

Primers used for PCR reactions

(NcoI)-SrtAΔ1-59-GGSENLYFQS-(HindIII) for pET28a-Srt-His_6
5'-ccatggctaaacctcaaattccgaaagataaatc-3',
5'-aagcttgctctggaaatacaggttttcggatccacctttgacttctgtagctacaaag-3'

(NcoI)-GFP-LPETGR-(BamHI) for pET28a-GFP_{GR}-His_6
5'-ccatggtgagcaagggcgaggag-3',
5'-ggatccacgaccagtttcaggaagcttgtacagctcgtccatgcc-3'

(NcoI)-GFP-LPETG-(BamHI) – for pET28a-GFP_{GG}-His_6
5'-ccatggtgagcaagggcgaggag-3',
5'-ggatccaccagtttcaggaagcttgtacagctcgtccatgccg-3'

(NcoI)-GFP-LPETAA-(BamHI) – for pET28a-GFP_{AA}-His_6
5'-ccatggtgagcaagggcgaggag-3',
5'-ggatccggccgcagtttcaggcagcttgtacagctc-3'

(NdeI)-GFP-LPETGGSENLYFQSKLA_3H_8-(HindIII) for pET42a-GFP_{GG}-His_8
5'-catatggtgagcaagggcgaggagc-3',
5'-aagcttttagtggtgatgatggtgatggtggtgggccgccgccagcttgctctggaaatacaggttttc-3'

(NdeI)-GFP-LPETGGSENLYFQSKLA_3H_{10}-(HindIII) for pET42a-GFP_{GG}-His_{10}
5'-catatggtgagcaagggcgaggagc-3',
5'-aagcttttaatggtggtggtgatgatggtgatggtggtgggccgccgccagcttgctctggaaatacaggttttc-3'

(NcoI)-MGSS**H_6**SSGLVPRGSH-(O-AGT)-ENLYFQSR<u>LPETGK</u>S-(BamHI) for pET28($SNAP_{GK}$-His_6)
First primer set:
5'-ctcgagtggtctggtgccgaggggcagtcacatggacaaagactgcgaaatgaag-3',
5'-tctagactggaagtacaggttttcacccagcccaggcttgccc
Second primer set:
5'-catatgtaaggtaccatgggcagtagccatcaccatcatcaccactcgagtggtctggtgccgag-3',
5-ggatccttagctcttaccagtctcaggaagtctagactggaagtacaggttttcaccc-3'

(NcoI)-GFP-GGTSGGGHHHG GTGLPETGGS GGSHHH-(HindIII) for pET28-GFP-His_{3GG3}
First primer set:
5'-ccatggtgagcaagggcgaggagctgttc-3,
5'-ggtaccgccatggtggtgaccgccaccactagtaccgcccttgtacagctcgtccatgcc
Second primer set:
5'-ccatggtgagcaagggcgaggagctgttc-3,
5'-aagcttttaatggtggtgggaaccgccggatccaccagtttcaggaaggccggtaccgccatggtggtgac-3'

(NcoI)-GFP-GGTSGGGHHHGGTGLPETGGSGGSHHH-(HindIII) for pET28-GFP-His_{6GG}

First primer set -

5'-ccatggtgagcaagggcgaggagctgttc-3,

5'-ggtaccgccgtgatggtgatggtggtgaccgccaccactagtaccgcccttgtacagctcgtccatgcc-3'

Second primer set:

5'-ccatggtgagcaagggcgaggagctgttc-3,

5'-aagcttttaggaaccgccggatccaccagtttcaggaaggccggtaccgccgtgatggtgatg-3'

Supplemental Figures

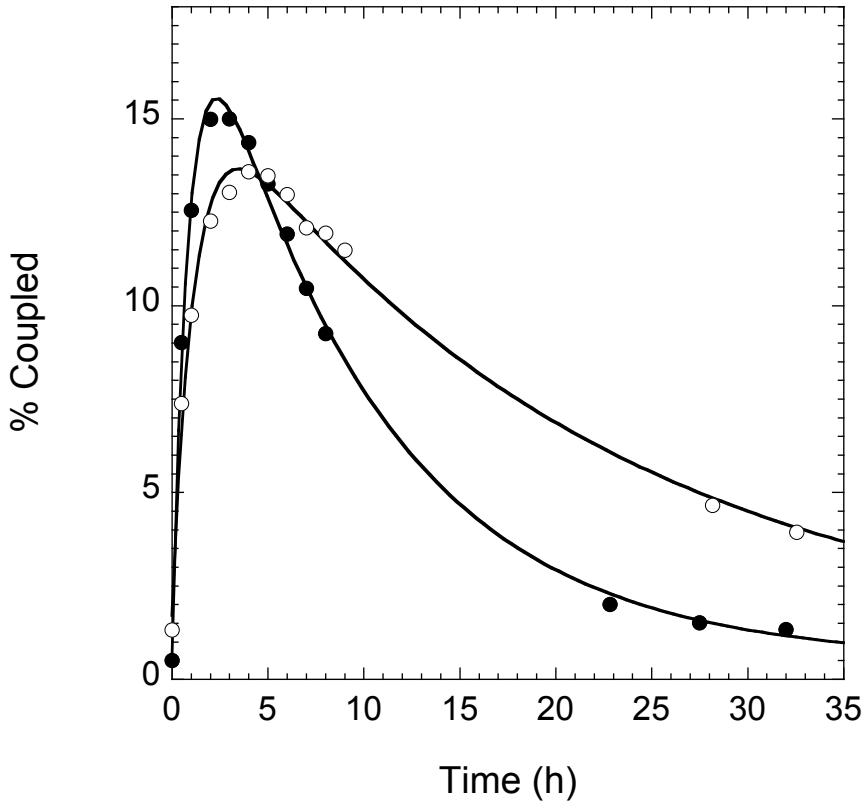

Figure S1. Time courses of coupling of (●) GFP_{GG}-His_6 (10 μM) to liposomes incorporating 2 mol% GG-PEG_3-DPPE in the presence of the Ca^{2+}-independent variant of *S. aureus* sortase A (2 μM) or (○) GFP_{AA}-His_6 (10 μM) to liposomes incorporating 2 mol% AAG-PEG_3-DPPE in the presence of *Str. pyogenes* sortase A (15 μM). Reactions were carried out at 23ºC using 3.5 mM liposomes; time courses shown are representative of the results obtained in two independent experiments.

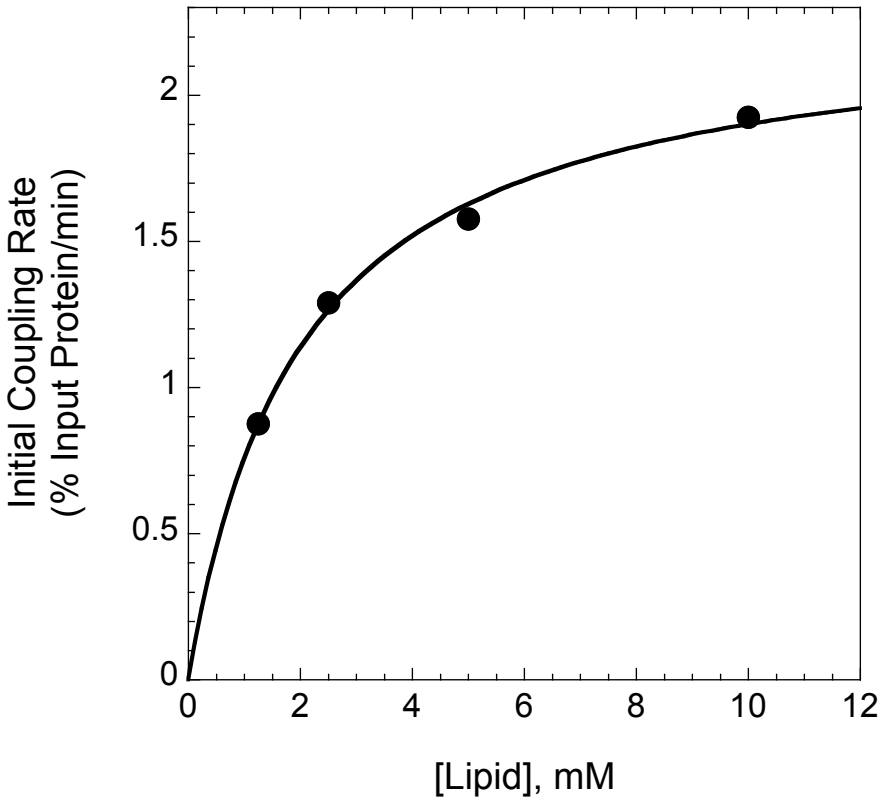

Figure S2. Initial rates of coupling of GFP_{GG}-His_6 at 23ºC to liposomes (at varying concentrations) incorporating 2 mol% Gly_2-PEG_3-DPPE in the presence of $SrtHis_6$ (50 μM). Initial coupling rates were determined by fitting experimental time courses to equation [1], then calculating the initial slope as $(\%Coupling)_{max} \cdot k_{cpl}$. Results shown are representative of the results obtained in two independent experiments.

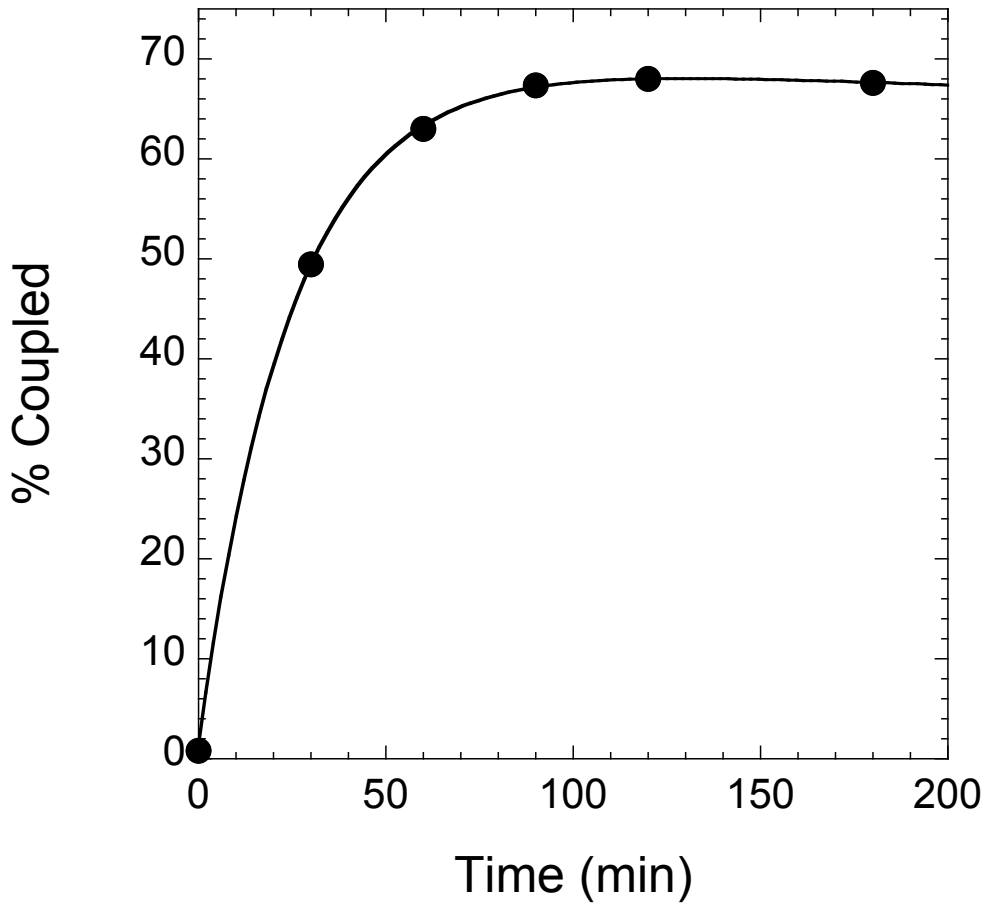

Figure S3. Time course of coupling of carboxyfluorescein-labeled His_6SNAP_{GK} (5 μM) to liposomes incorporating 2 mol% each Gly_2-PEG_3-DPPE and DOGS-NTA·Ni in the presence of $SrtHis_6$ (10 μM). Reactions were carried out at 23ºC using 3.5 mM liposomes; time course shown is representative of the results obtained in two independent experiments.

^{1}H-NMR Spectral Data for Gly_4-DPPE, Gly_3-PEG_3-DPPE, Gly_3-PEG_3-DPPE and Ala_2Gly-PEG_3-DPPE

All ^{1}H NMR spectra were collected using a Bruker AVIIIHD 500 MHz NMR spectrometer; samples were prepared in $CDCl_3/CD_3OD$ 2:1.

Gly_2-PEG_3-DPPE: δ 5.23 (m, 1H), 4.38 (m, 1H), 4.18 (m, 1H), 3.99 (t, 2H), 3.94 (s, 2H), 3.90 (m, 2H), 3.72 (s, 2H), 3.62 (m, 4H), 3.57 (t, 2H), 3.56 (t, 2H), 3.42 (m, 4H), 3.38 (t, 2H), 2.51 (m, 4 H), 2.32 (m, 4H), 1.61 (m, 4 H), 1.27 (m, 48H), 0.89 (t, 6H)

Gly_3-PEG_3-DPPE: δ 5.23 (m, 1 H), 4.38 (m, 1 H), 4.17 (m, 1 H), 3.99 (t, 2H), 3.92 (s, 2H), 3.91 (m, 2H), 3.89 (s, 2H), 3.76 (s, 2H), 3.63 (t, 2H), 3.61 (s, 4H), 3.54 (t, 2H), 3.42 (m, 4H), 3.37 (t, 2H), 2.51 (s, 4H), 2.32 (m, 4H), 1.60 (m, 4H), 1.27 (m, 48H), 0.89 (t, 6H)

Ala_2Gly-PEG_3-DPPE: δ 5.23 (m, 1H), 4.38 (m, 1H), 4.18 (t, 1H), 4.17 (q, 1H), 4.02 (q, 1H), 3.99 (t, 2H), 3.96 (d, 1H), 3.91 (m, 2H), 3.77 (d, 1H), 3.65 (m, 2H), 3.61 (m, 4H), 3.53 (t, 2H), 3.42 (m, 4H), 3.37 (t, 2H), 2.50 (m, 4H), 2.32 (m, 4H), 1.60 (m, 4H), 1.54 (d, 3H), 1.43 (d, 3H), 1.27 (m, 48 H), 0.89 (t, 6H).

DPPE-Gly_4 – δ 5.24 (s, 1H), 4.37 (m, 1H), 4.18 (m, 1 H), 4.06 (t, 2H), 4.02 (m, 2H), 3.96 (s, 2H), 3.94 (s, 2H), 3.90 (s, 2H), 3.76 (s, 2H), 3.48 (m, 2H), 2.33 (m, 4H), 1.60 (m, 4H), 1.28 (m, 48 H), 0.89 (t, 6H)