Substrate relay in an Hsp70-cochaperone cascade safeguards tail-anchored membrane protein targeting

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Appendix

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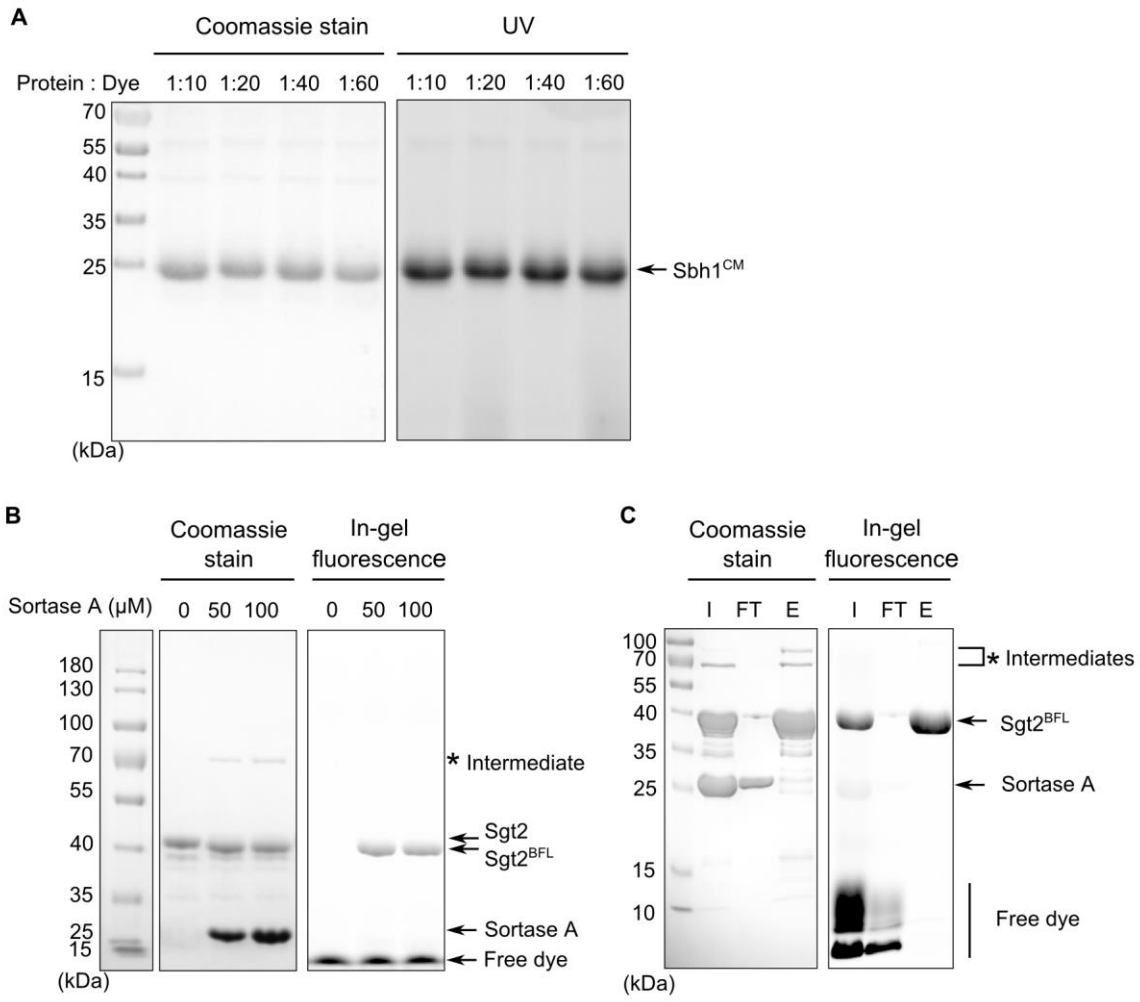
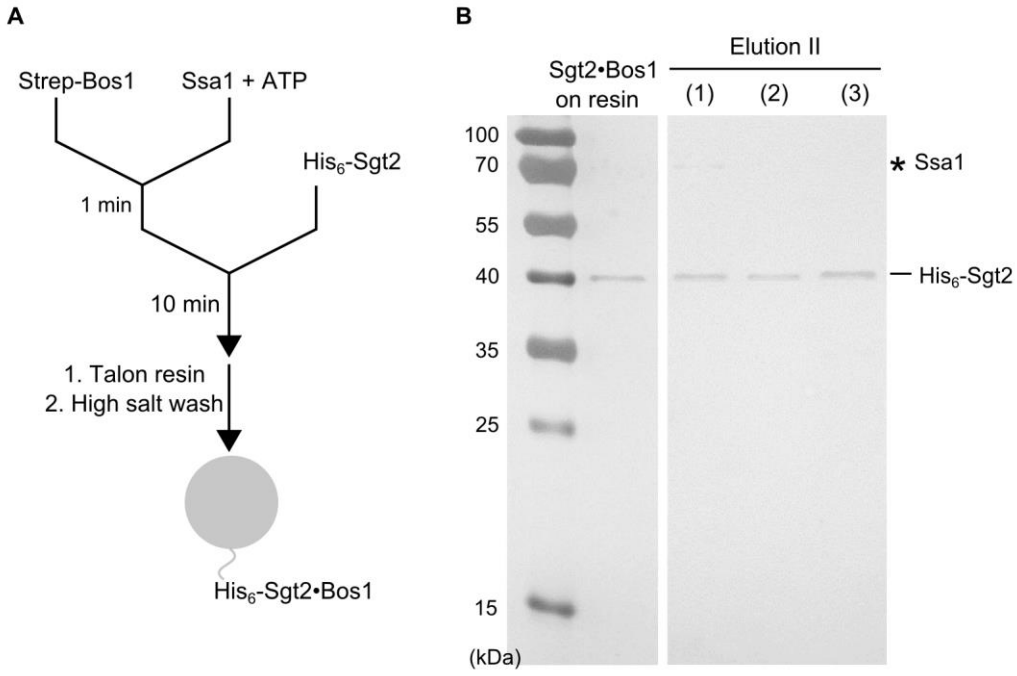


Appendix Figure S1. Fluorescence labeling of TA and Sgt2.

A. Coumarin-maleimide labeling of Sbh1. Sbh1 immobilized on Strep-tactin resin was incubated with CM at the indicated protein to dye molar ratios at 4 °C overnight. A 10-fold molar excess of CM was sufficient to label Sbh1. Labeling efficiency was estimated to be ≥70% based on the extinction coefficient of CM (ε_{383} = 27,000 $M^{-1}cm^{-1}$).

B, C. C-terminal labeling of Sgt2 using Soratase-mediated ligation. Part B shows the SDS-PAGE analysis of a small-scale labeling reaction. Reactions were carried out as described in the Methods at indicated concentrations of Sortase A. After measurement of in-gel fluorescence on the Typhoon Scanner (right), proteins were visualized by Coomassie stain (left). Labeling efficiency was estimated to be >90% based on quantification of the relative intensities of the Sgt2 and $Sgt2^{BFL}$ bands on SDS-PAGE. * denotes a covalent intermediate, Sortase-conjugated Sgt2, formed during the reaction. Part C shows the purification of His_6-$Sgt2^{BFL}$ over Talon resin. I, FT, and E denote the input, flowthrough, and elution fractions, respectively. Hisless sortase and free GGGG-BODIPY-FL were removed in the FT.



Appendix Figure S2. Preparation of immobilized Sgt2•Bos1 complex.
A. Work flow for reconstitution and immobilization of the Sgt2•Bos1 complex on Talon resin. TA transfer reactions were carried out as outlined in Figure 3B using 1.5 µM Strep-tagged Bos1, 3 µM Ssa1 and 2 mM ATP during the pre-incubation, and 3 µM of His_6-tagged Sgt2 during the second incubation. The mixture was incubated with the resin for 15 min at 4 °C, and the resin was washed with high-salt buffer containing 500 mM NaCl. The resin mix containing immobilized Sgt2•Bos1 complex was exchanged into assay buffer prior to additional experiments.
B. The immobilized Sgt2•Bos1 complex and elution II from the indicated reactions in Figure 5 were analyzed by SDS-PAGE and silver stain. The amount of Bos1 associated with Sgt2 was detected by western blot as shown in Figure 5B. A trace amount of Ssa1 (denoted as *) was detected in Elution II of reaction (1).