Supporting Information

Electrostatic Repulsion between Unique Arginine Residues Is Essential for the Efficient in Vitro Assembly of the Transmembrane Domain of a Trimeric Autotransporter
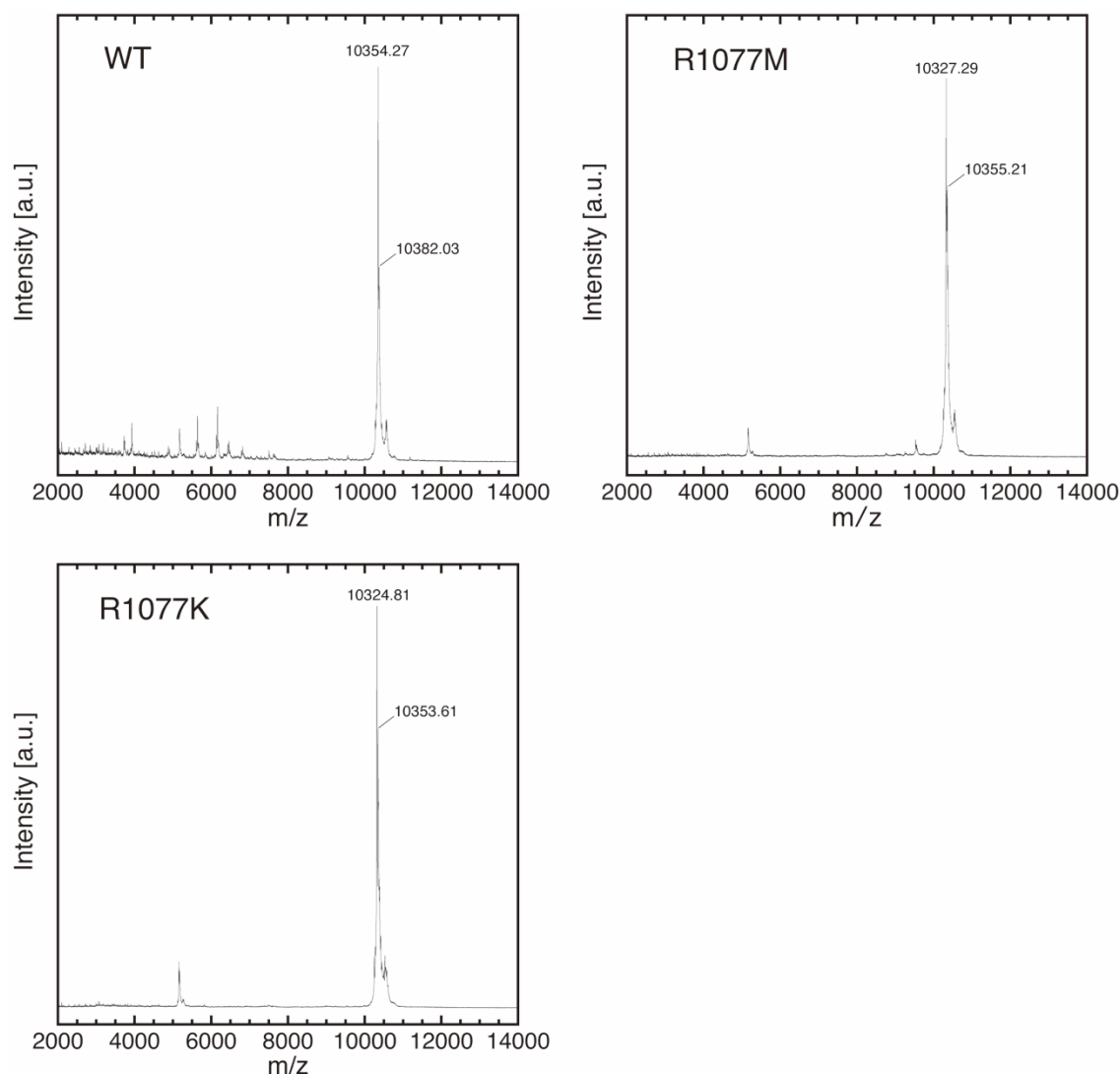*Eriko Aoki, Daisuke Sato, Kazuo Fujiwara, and Masamichi Ikeguchi*

Figure S1. MALDI-TOF-MS spectra of mHiaTD (WT), R1077M, and R1077K. Mass spectra were acquired on an Autoflex II TOF/TOF spectrometer (Bruker). The expected masses of mHiaTD, R1077M, and R1077K are 10353.53, 10328.54, and 10325.51, respectively. For all proteins, an additional peak with a molecular mass that was greater than the main peak value by 28 (probably formylation) was observed.
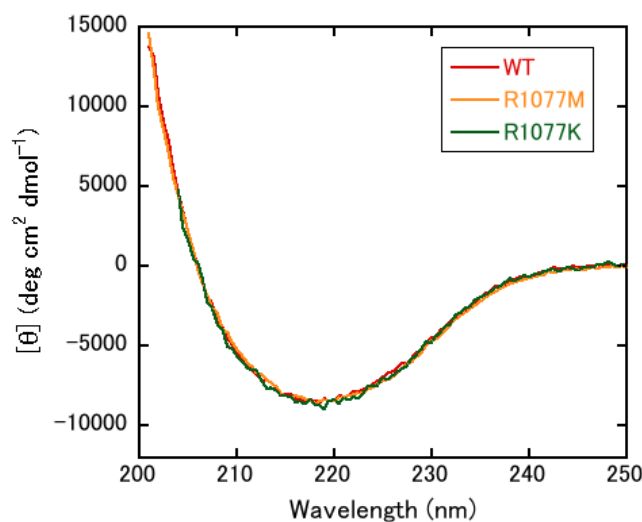
Figure S2. CD spectra of purified mHiaTD (WT), R1077M, and R1077K in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 0.6% (v/v) C_8E_4.
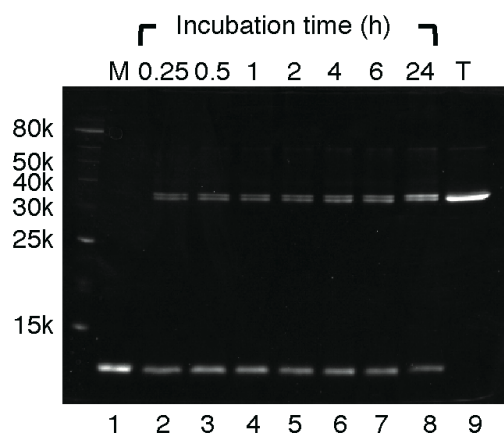

Figure S3. Reassembly of R1077M in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 0.6% (v/v) C_8E_4. (A) SDS–PAGE of R1077M reassembled over the incubation time indicated. Lane M corresponds to the dissociated R1077M (in 8 M urea), and lane T corresponds to the undissociated trimeric R1077M purified from the membrane fraction of *E. coli*. The protein concentration was 113 μg/mL.
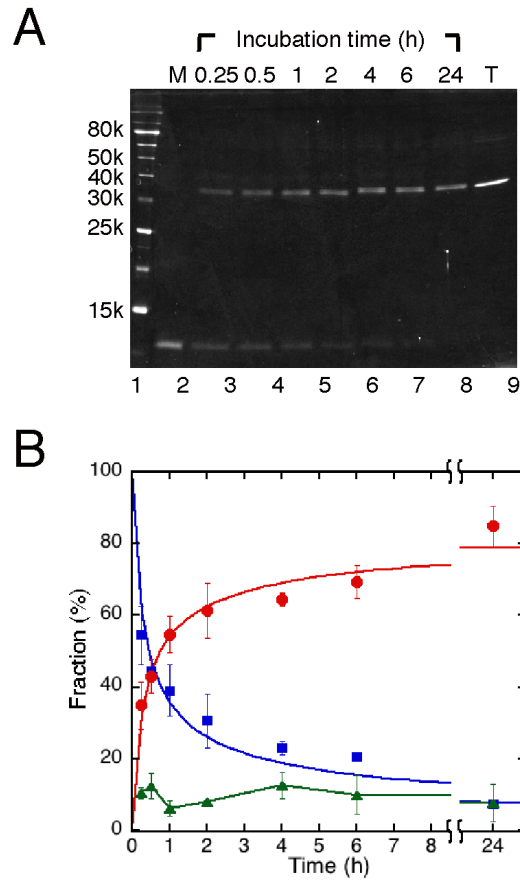
Figure S4. Reassembly kinetics of R1077K at the concentration of 23 μg/mL in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 0.6% (v/v) C_8E_4. (A) SDS–PAGE of R1077K reassembled over the incubation time indicated. Lane M corresponds to the dissociated R1077K (in 8 M urea), and lane T corresponds to the undissociated trimeric R1077K purified from the membrane fraction of *E. coli*. (B) Time course of the monomer (blue), trimer (red), and high-molecular-weight aggregate (green) fractions. The error bars represent the standard error of two independent experiments. The fitting curves were drawn as described in the text and using the parameters shown in Table 1.

Table S1. Distances of possible hydrogen bonding atomic pairs

Donor Chain/Residue	Atom	Acceptor Chain/Residue	Atom	Distance
A/R1077	Nη1	A/L1037	O	2.9
A/R1077	Nη1	Water/87	O	2.7
A/R1077	Nη2	Water/191	O	3.0
A/R1077	Nη2	C/L1037	O	3.1
C/R1077	Nη1	C/L1037	O	2.9
C/R1077	Nη1	Water/146	O	2.7
C/R1077	Nη2	Water/191	O	2.8
C/R1077	Nη2	D/L1037	O	3.2
D/R1077	Nη1	Water/191	O	3.2
D/R1077	Nη1	D/L1037	O	3.0
D/R1077	Nη1	Water/101	O	2.6
D/R1077	Nη2	Water/191	O	2.8
D/R1077	Nη2	A/L1037	O	3.1
Water/101	O	D/S1035	O	2.9
Water/101	O	A/S1035	O	2.7
Water/101	O	D/A1034	O	3.0
Water/146	O	C/A1034	O	2.9
Water/146	O	D/S1035	O	2.7
Water/146	O	C/S1035	O	3.1
Water/87	O	A/S1035	O	2.9
Water/87	O	C/S1035	O	2.8
Water/87	O	A/A1034	O	3.1

Distances were calculated by PyMol based on the coordinates of PDB entry 2GR8.