Supplemental Data

The Membrane Topography of Diphtheria Toxin T Domain Linked to A Chain Reveals a Transient Transmembrane Hairpin and Potential Translocation Mechanisms

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Supplemental Experimental Procedures:

AT fluorescence: For samples in aqueous solution, 4μg/ml of Cysless AT protein was incubated either in neutral pH buffer (10mM Tris-HCl, 150mM NaCl, pH 8.1) or low pH buffer (167mM acetate, 67mM Tris, 150mM NaCl, pH 4.3). For lipid-containing samples, 4μg/ml of Cysless AT protein was incubated with either 7:3 (mol:mol) DOPC/DOPG or DMoPC/DOPG SUV containing 0.2mM lipid in low pH buffer. The sample volume is 800μl. After 20 min incubation at room temperature, fluorescence emission spectra were measured, using an excitation wavelength of 280nm.

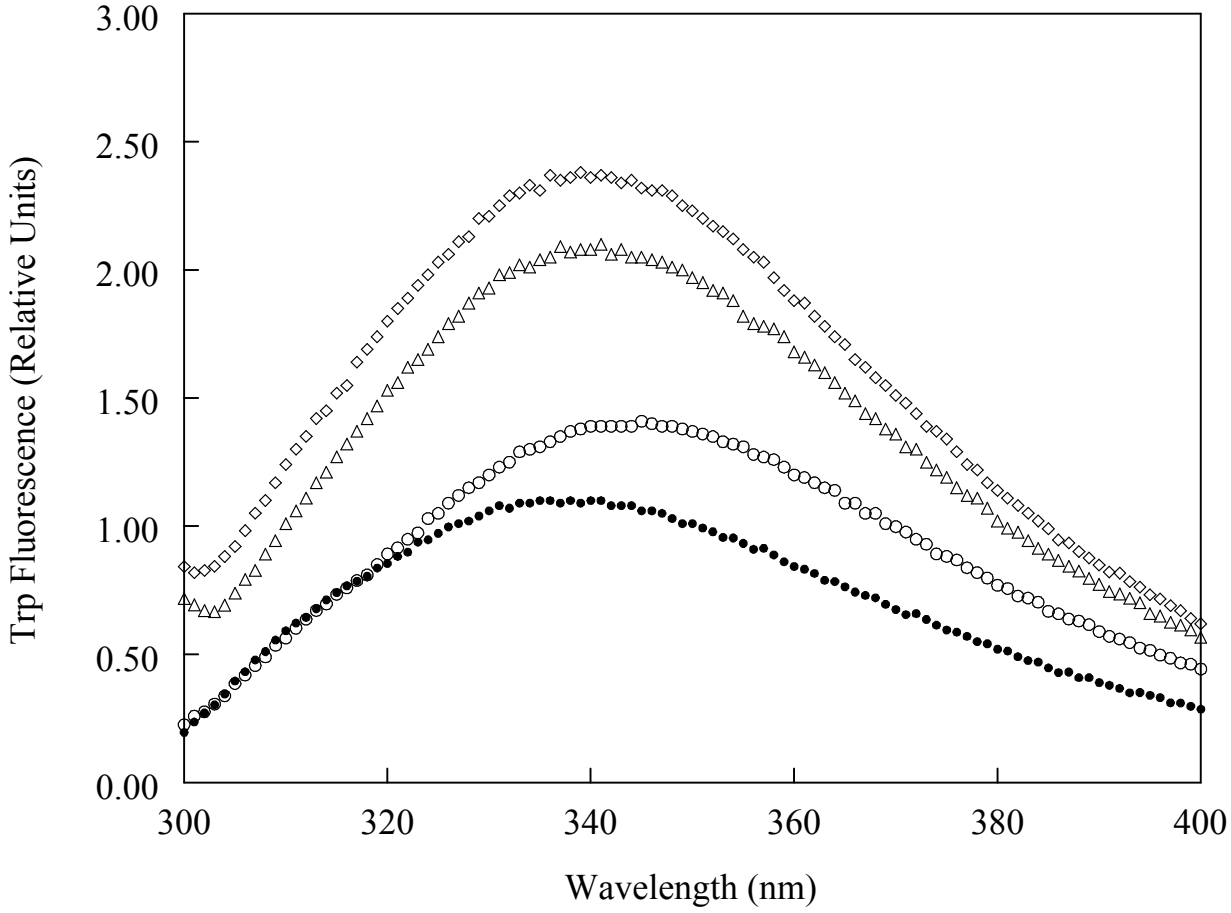
Fluorescence vs. lipid concentration 4μg/ml of Cysless AT protein was incubated with different amounts of SUV in 800μl of low pH buffer. After 20 min incubation at room temperature, tryptophan fluorescence was measured as described above. The ratio of emission intensities at 330nm and 350nm, F_{330}/F_{350}, was then calculated.

Fluorescence vs. pH: 4μg/ml of Cysless AT protein was incubated without and with DOPC/DOPG SUV or DMoPC/DOPG SUV (200 μM lipid) for 20min at room temperature in different pH buffers. Buffers from pH 8.1 to pH 5.7 contained 10 mM Tris, 150mM NaCl and pH was adjusted by addition of different amounts of acetate acid into the pH 8.1 buffer. Buffers from pH 5.66 to pH 4.0 contained 167mM acetate, 67mM Tris, 150mM NaCl, and pH was adjusted by addition of different amounts of 2M NaOH or HCl to the pH 4.3 buffer. Protein fluorescence was measured as described above. The ratio of emission intensities at 330nm and 350nm, F_{330}/F_{350}, was then calculated.

Pore formation vs. pH: 1μg/ml Cysless AT protein was incubated with LUV (0.1mM lipid) containing entrapped BODIPY-SA (66ng/ml in the total volume) for 20min in 800μl of the same buffers used for measuring fluorescence vs. pH. A background sample contained LUV (with entrapped BODIPY-SA) without AT protein in pH 4.3 buffer. BODIPY fluorescence (excitation at 488nm and emission at 516 nm) was measured every 30s. At the 150 s time point 4μl of 4μM biocytin dissolved in water was added (final concentration of 20nM biocytin).

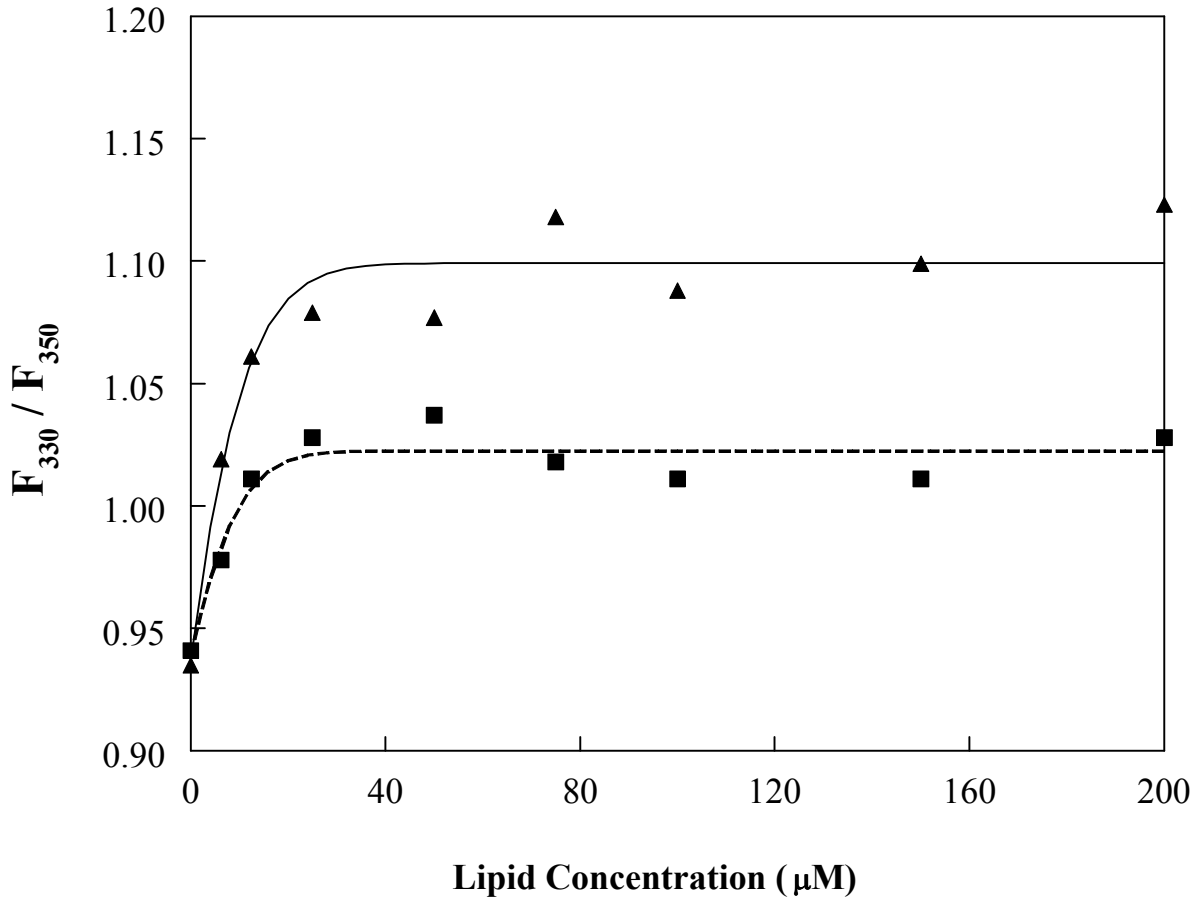
Arg C digestion experiments: Bimane-labeled AT proteins at a concentration of 200-300 μg/ml were incubated at room temperature for 1h with Arg C (0.75μg/ml) in 90 mM Tris-Cl, 8.5 mMCaCl2, 5 mM DTT and 0.5 mM EDTA, pH 7.6. Digestion was confirmed by SDS-PAGE (8-25% Phastsystem gradient gels), staining with Coomassie blue. The digested proteins were incorporated into SUV at low pH, and bimane fluorescence measured as described.

Supplemental Figure S1:



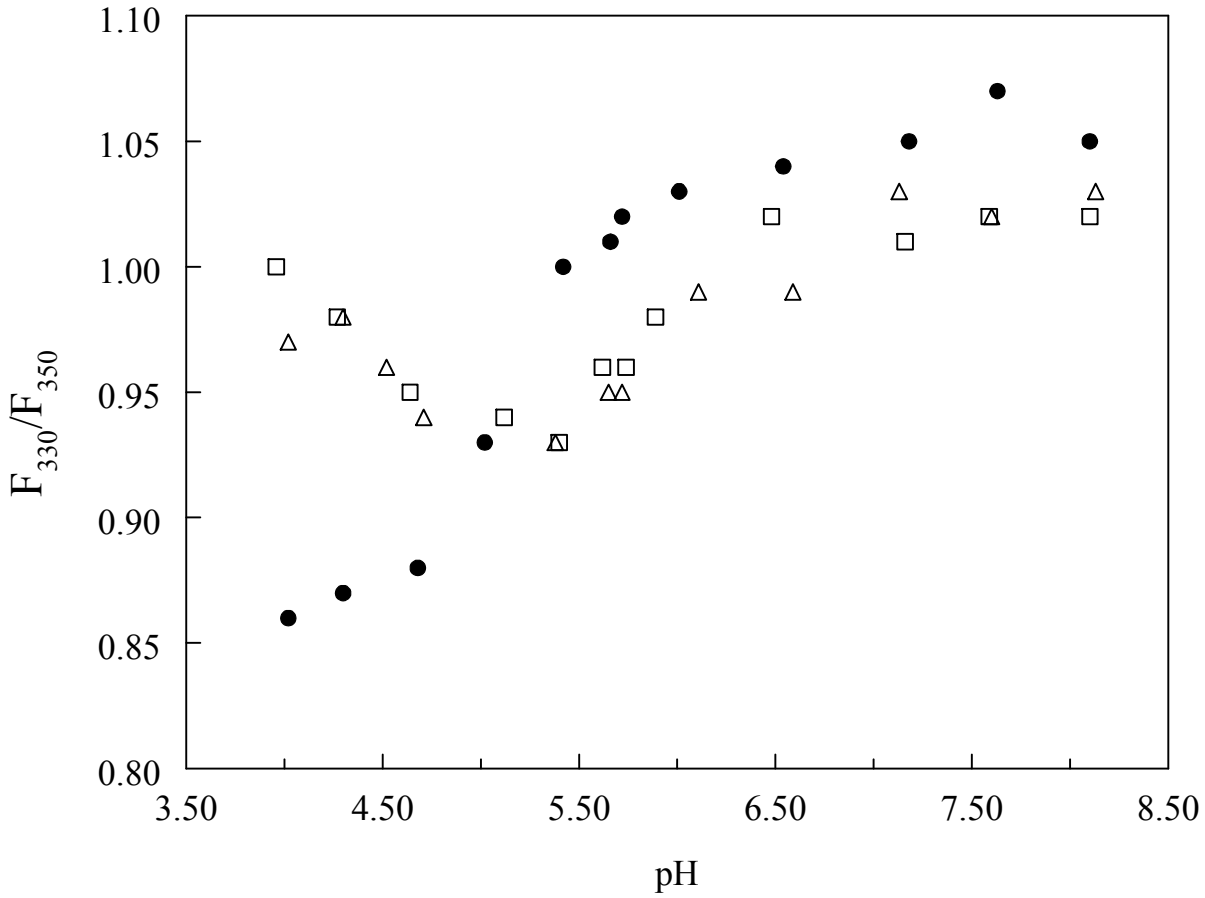
Cysless AT protein fluorescence in (from lowest to highest intensity) aqueous solution at pH 8.0 (filled circles), in aqueous solution at pH 4.3 (open circles), inserted into DMoPC/DOPG SUV at pH 4.3 (triangles), and inserted into DOPC/DOPG SUV at pH 4.3 (diamonds). Protein concentration was 4 μg/ml and lipid concentration was 0.2 mM.

Supplemental Figure S2:



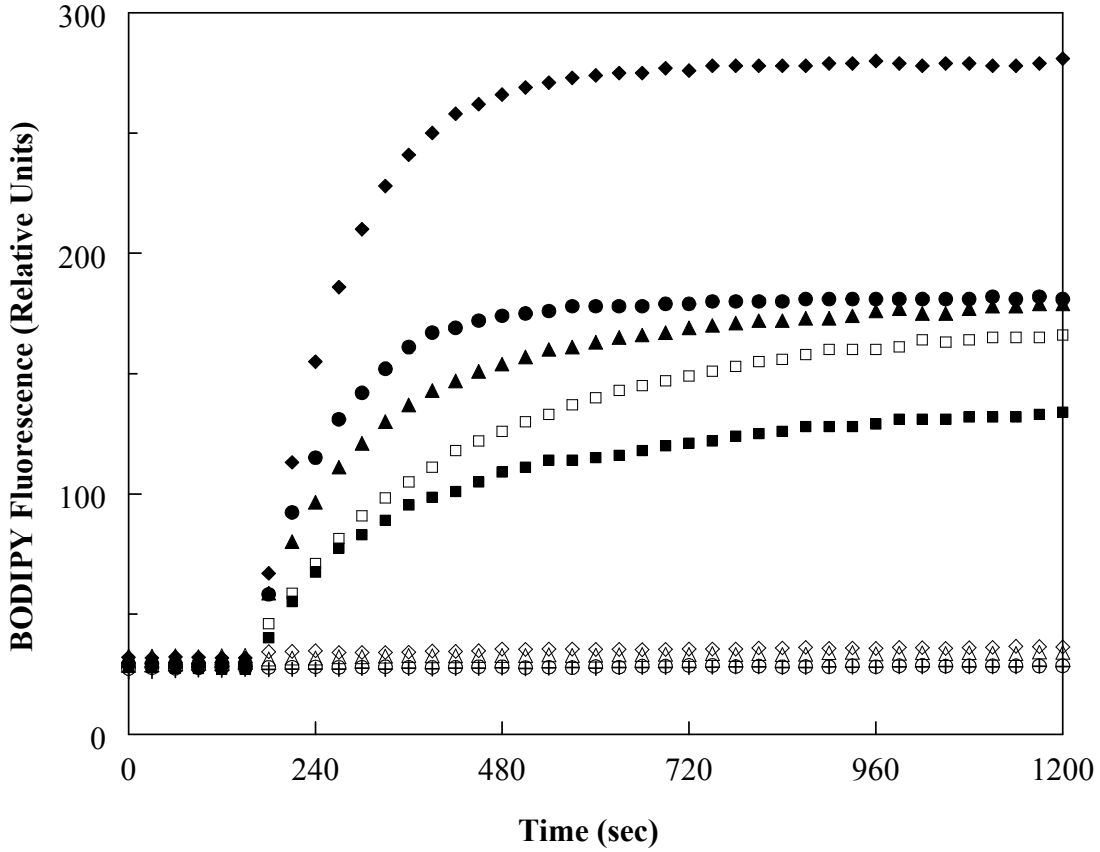
Tryptophan fluorescence of Cysless AT protein incubated with different concentrations of DOPC/DOPG SUV (triangles) or DMoPC/DOPG SUV (squares) at pH 4.3. Protein concentration 4 μg/ml

Supplemental Figure S3:



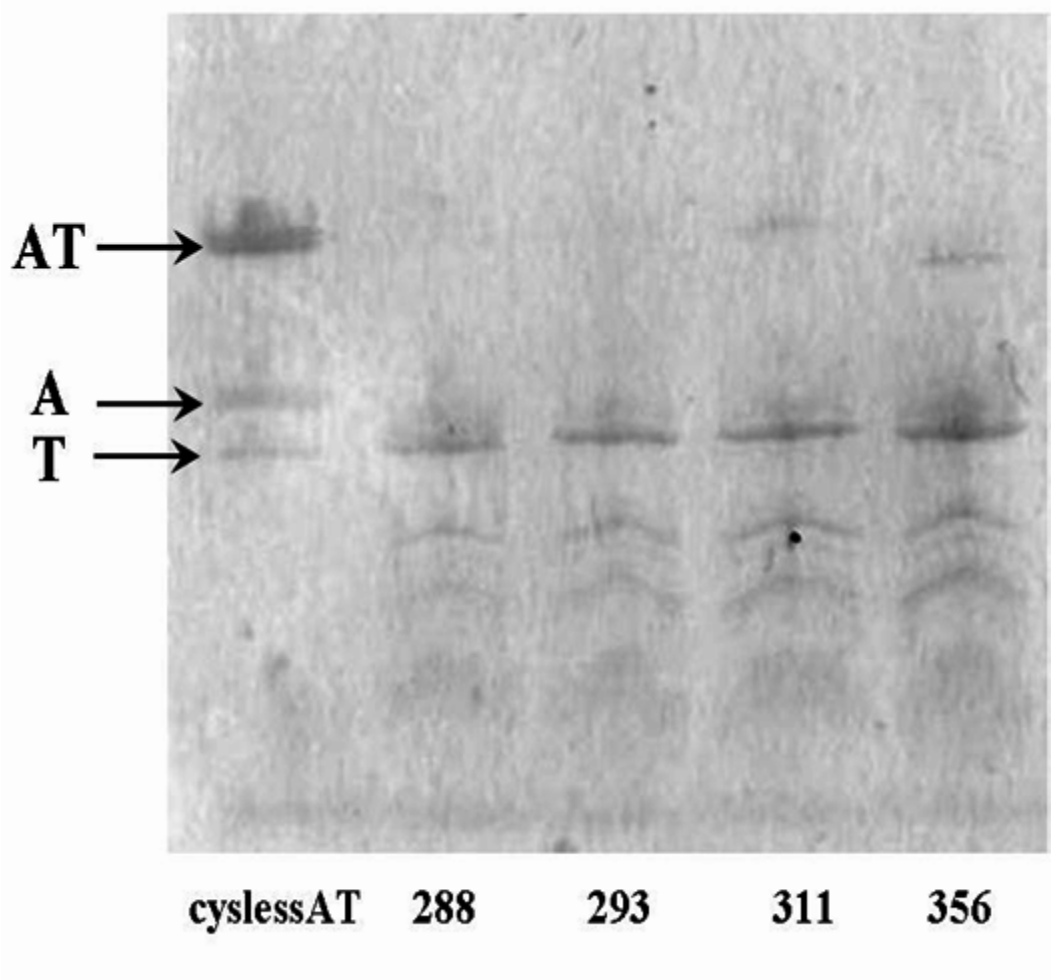
Cysless AT protein fluorescence in aqueous solution (circles), within DOPC/DOPG SUV (triangles) and DMoPC/DOPG SUV (squares) in different pH buffers. Protein concentration was 4 μg/ml and lipid concentration was 0.2 mM.

Supplemental Figure S4:



Time dependence of BODIPY-SA fluorescence due to pore formation by Cysless AT protein inserted into DOPC/DOPG LUV at various pH. Samples contained LUV (100 μM lipid) with 1 μg/ml Cysless AT protein and 66 ng/ml BODIPY-SA that was vesicle-entrapped. At 150 s after the sample was placed in the fluorimeter, biocytin was added to a concentration of 20 nM. Sample pH was: (open triangles) pH 8.1, (open circles) pH 7.2, (open diamonds) pH 6.2 (fix figure), (filled circle) pH 5.7, (filled triangles) pH 5.66, (filled diamond) pH 5.3, (squares) pH 4.7, (filled squares) pH 4.3, (+) background with no protein at pH 4.3.

Supplemental Figure S5:



SDS gel profile of Arg C digested Cysless AT protein. From left to right, lane 1 Cysless AT protein with wild type linkage between A chain and T domain. Notice small amount of digestion into separate A chain and T domain. This was avoided in other experiments by use of AT protein with nick site residues mutated to Gly. Lane 2-5 Bimane-labeled AT protein with Gly linkage and Cys at residue number shown. A small amount of products due to incomplete digestion are present.