Supplementary Material for "Design of self-assembling transmembrane helical bundles to elucidate principles required for membrane protein folding and ion transport"

Nathan H. Joh[1†]*, Gevorg Grigoryan[2], Yibing Wu[1], William F. DeGrado[1]*

[1] *Department of Pharmaceutical Chemistry, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA 94158, USA.*

[2] *Department of Computer Science and Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA.*

Supplementary Methods

Quantitation of Proteoliposomes

Rocker was reconstituted in large unilamellar vesicles (LUVs) consisting POPC, POPG and cholesterol mixed at a molar ratio of 4:1:2, with a peptide:lipid ratio (not counting cholesterol) of 1:1000 or 1.32:2000 by using the extrusion method (1). Briefly, organic solvent is removed from the mixture of lipid and peptide under N_2 then under at least 4 hours of lyophilization to form dry film. The film is re-suspended in the interior buffer to achieve the lipid concentration of 25 mM, and subjected to ten cycles of freeze-thaw-vortex. The resuspension is extruded 11 times across 100-nm sizing membrane. The buffer to the vesicle exterior is exchanged by washing the resulting LUVs using 0.5 ml Zeba 40k MWC size-exclusion spin column (Thermo Scientific) preconditioned in desired exterior buffer.

To quantify washed or dialyzed proteoliposomes, LC-MS is used in determining the ion count for POPC from an aliquot of proteoliposome, using isotope-labeled POPC as an internal standard (2). Proteoliposomes are then appropriately diluted in exterior buffer to achieve the final lipid concentration of 800 μM and kept at 18°C to be used for experiments on the same day.

Proton Flux Data Processing

In processing the stop-flow data for the proton-flux experiments, the measured F_{455}/F_{416} indicated the apparent interior $[H^+]$ deviating from the predetermined interior pH and approaching the predetermined exterior pH at the instance of mixing when $t = 0$, indicating a fraction of HPTS present on the vesicle exterior likely due to incomplete washing of the vesicles by spin size exclusion. So, if the apparent F_{455}/F_{416} ratio, r_{app}, is contributed by the fractions of HPTS inside (f_{in}) and outside (f_{out}) of the vesicles contributing F_{455}/F_{416} ratios that corresponds to the pH on the vesicle interior and exterior ($r_{in}^{\ t=0}$, r_{out}), respectively, thereby $r_{app} = f_{in} \times r_{in}^{t=0} + f_{out} \times r_{out}$. r_{out} and $r_{in}^{\ t=0}$ are both calculated using the predetermined interior and exterior $[H^+]$ via the formula, $[H^+] = 5.29(\frac{3.19}{F_{455}/F_{416}} - 1)$, obtained by non-linear curve fitting to describe the plots obtained for the HPTS/pH calibration from the HPTS-preloaded LUV standards (See ref. (1)), but in a form rearranged with the respect to the term, F_{455}/F_{416}. Finally, the corrected F_{455}/F_{416} subsequent to mixing, $r_{in}^{\ corr}$, is obtained by $r_{in}^{corr} = \frac{r_{app} - r_{out} + f_{in} \times r_{out}}{f_{in}}$, which is the rearranged form of the above equation that describes r_{app} after substituting f_{out} with 1 - f_{in}, where f_{in} is calculated by another rearranged form of the same equation, $f_{in} = \frac{r_{out} - r_{app}}{r_{out} - r_{in}^{t=0}}$. The interior proton concentration, $[H^+]$, was then determined from the F_{455}/F_{416} contributed by the corrected interior HPTS F_{455}/F_{416} ratio, $r_{in}^{\ corr}$, by using the function, $[H^+] = 5.29(\frac{3.19}{r_{in}^{corr}} - 1)$.

The time-courses for the concentration of intravesicular H^+, $[H^+]$, was fit to a double exponential function combined with a linear process (**Figure S2**), described as $[H^+] = N_0^a \exp(-k^a \times t) + N_0^b \exp(-k^b \times t) + mt + b$, where t is time; $N_0^{\ a}$

*Authors for correspondence: WFD (william.degrado@ucsf.edu), NHJ (nate.joh@amgen.com).
†Present address: Department of Attribute Sciences, Amgen Inc., Thousand Oaks, CA 91320, USA

and N_0^{b} are the initial quantity for 1st and 2nd process; and k^{a} and k^{b} are the rate constant for 1st and 2nd process; and m and b are the slope and $[H^+]$ at $t = 0$ for the linear component, respectively. The initial rate of transport, $V_i = \Delta[H^+]/\Delta t_{(t=0)}$, was calculated as the derivative at $t = 0$, $-N_0^{a}k^{a} - N_0^{b}k^{b} + m$. Ions accumulated per tetrameric bundle, or the equivalent in control vesicles, was calculated by assuming the surface area of 62.7 Å^2 occupied per a lipid molecule (not counting cholesterol) in 39.8-Å-thick bilayer(3) forming uniform vesicles with an outer diameter of 0.1 µm, as supported by dynamic light scattering and negative-stain electron microscopy (not shown).

Dependence of V_i on the exterior ion concentration, $[H^+_{out}]$, was analyzed by non-linear curve fitting of V_i in terms of ions accumulated per a tetrameric bundle equivalent to a function under one-protonation-site model below:

$$V_i([H^+_{Out}]) = \frac{r_1 \frac{[H^+_{Out}]}{10^{-pK_{a1}}}}{1 + \frac{[H^+_{Out}]}{10^{-pK_{a1}}}}$$

which is derived by the same approach described for the two-protonation-site model, below, by Balannik *et al* (4):

$$V_i([H^+_{Out}]) = \frac{r_1 \frac{[H^+_{Out}]}{10^{-pK_{a1}}} + r_2 \frac{[H^+_{Out}]^2}{10^{-pK_{a1}} \times 10^{-pK_{a2}}}}{1 + \frac{[H^+_{Out}]}{10^{-pK_{a1}}} + \frac{[H^+_{Out}]^2}{10^{-pK_{a1}} \times 10^{-pK_{a2}}}}$$

where pK_{a1} and pK_{a2} are the logarithmic acid dissociation constants; and r_1 and r_2, the maximal proton conductions; at the putative protonation sites 1 and 2, respectively.

Supplementary Figures

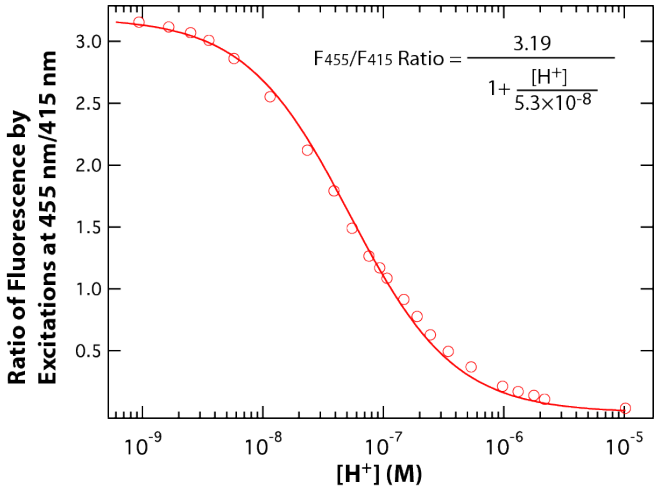

Figure S1. Standard Curve for HPTS Fluorescence as a function of LUV Interior pH. The ratio of areas under fluorescence emission spectra of LUV standards preloaded with HPTS upon excitation at 455 nm to those at 415 nm is plotted as a function of the internal proton concentration.

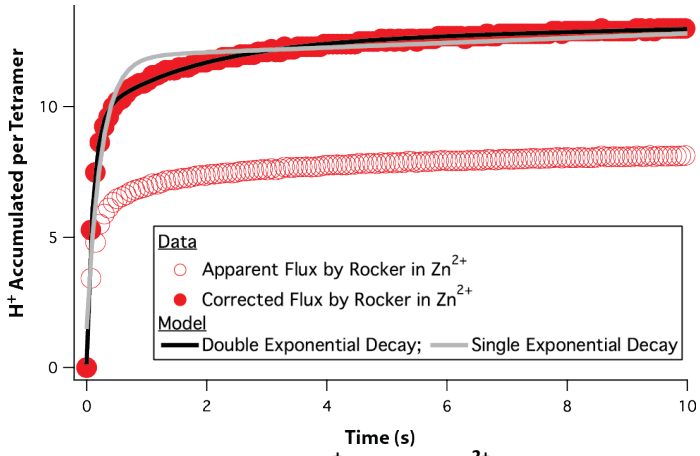

Figure S2. Time Course of H^+ Flux by Zn^{2+}-Bound Rocker in Vesicles with Initial Interior and Exterior pHs of 6.83 and 4.50. The corrected data are obtained by correcting the apparent data to account for incompletely washed HPTS on the vesicle exterior (see methods). The double exponential decay combined with line (black line) fits the flux data better than the single exponential decay combined with line (grey lines). The initial velocities at 0 sec are plotted as a function of the exterior pH to generate Figure 1 in the main text for this and other flux experiments.

Supplementary References

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