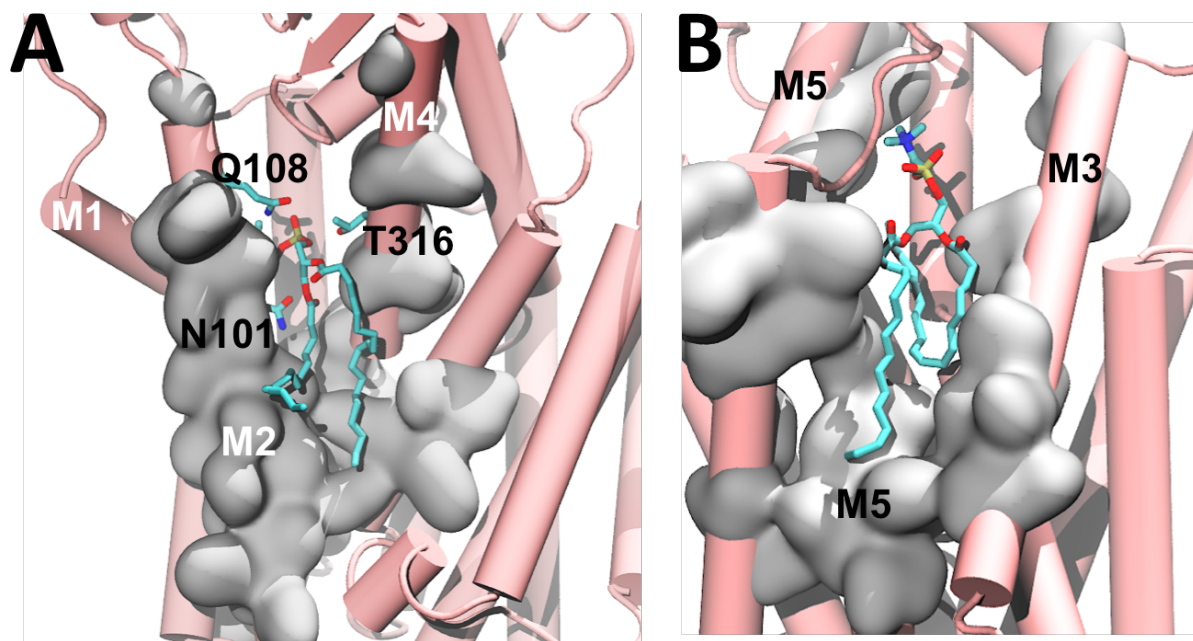


## Supplementary Information

### Probing the effects of nonannular lipid binding on the stability of the calcium pump SERCA

L. Michel Espinoza-Fonseca

Center for Arrhythmia Research, Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI 48109, USA



**Figure S1. Binding of nonannular lipids to SERCA during the early stages of the E2 to E1 transition of the pump.** (A) Upon deprotonation of transport site residues Glu309 and Glu771 and in the absence of an initially bound nonannular lipid, the pump undergoes structural changes in the transmembrane and cytosolic domains that initiate the transition toward the competent E1 state<sup>1,2</sup>. In a previous study by our group<sup>2</sup>, we found that during these structural change, a single lipid molecule binds to the nonannular site at  $t=0.2 \mu\text{s}$  and remains bound to the pump for the remainder of the simulation time ( $t=3.6 \mu\text{s}$ ). Remarkably, the nonannular lipid interacts with residues that participate in nonannular lipid binding to this site, i.e., Asn101, Gln108 and Thr316. (B) In the same trajectory of the early stages of the E2-to-E1 transition, metal-proton exchange at the transport sites induce a physical separation of helices M3 and M5, thus exposing several hydrophobic residues at the M3-M5 interface, which is occupied by a nonannular lipid. In both cases, these nonannular lipids appear to protect SERCA by ‘sealing’ transient hydrophobic patches in the transmembrane domain that result from the large structural changes in the pump during the E2-to-E1 transition. Based on these observations, we propose nonannular lipid binding is not necessary for the stability of the E2 state, but we speculate that it becomes functionally significant during the E2-to-E1 transition of the pump.

## References

- 1 Inesi, G., Lewis, D., Toyoshima, C., Hirata, A. & de Meis, L. Conformational fluctuations of the Ca<sup>2+</sup>-ATPase in the native membrane environment. Effects of pH, temperature, catalytic substrates, and thapsigargin. *J Biol Chem* **283**, 1189-1196, doi:M707189200 [pii] 10.1074/jbc.M707189200 (2008).
- 2 Fernandez-de Gortari, E. & Espinoza-Fonseca, L. M. Preexisting domain motions underlie protonation-dependent structural transitions of the P-type Ca(2+)-ATPase. *Phys Chem Chem Phys* **19**, 10153-10162, doi:10.1039/c7cp00243b (2017).