Protein-Protein Interactions in Calcium Transport Regulation Probed by Saturation Transfer Electron Paramagnetic Resonance

>>SUPPORTING MATERIAL<<

Zachary M. James, Jesse E. McCaffrey, Kurt D. Torgersen, Christine B. Karim, and David D. Thomas

University of Minnesota, Minneapolis, MN 55455

Sample	pK_{Ca}
SERCA Only	6.48 ± 0.02
PLB	5.96 ± 0.01
pPLB	6.26 ± 0.02
36-TOAC-PLB	5.97 ± 0.01
36-TOAC-pPLB	6.27 ± 0.02

Table S1. pK_{Ca} values from functional experiments shown in Fig. 3.

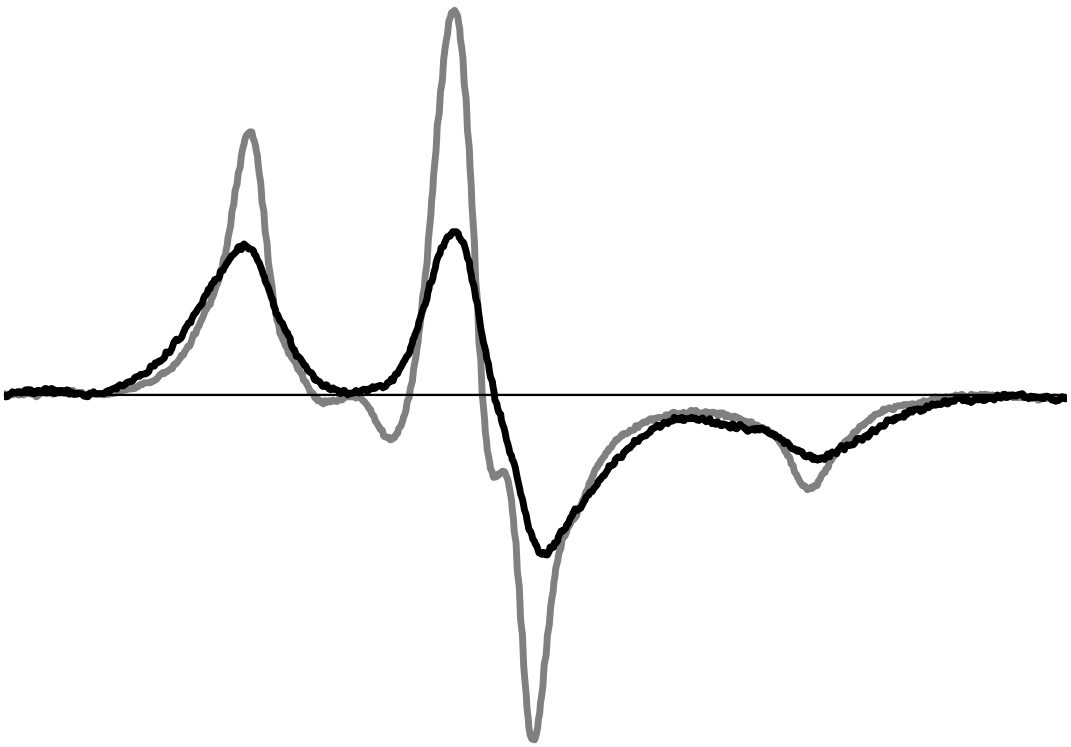

Fig. S1. Conventional EPR spectra of 36-TOAC PLB at 1000 L/P (gray, same conditions as in Fig. 4) and 20 L/P (black), showing greatly enhanced spin-spin interactions due to aggregation at 20 L/P.

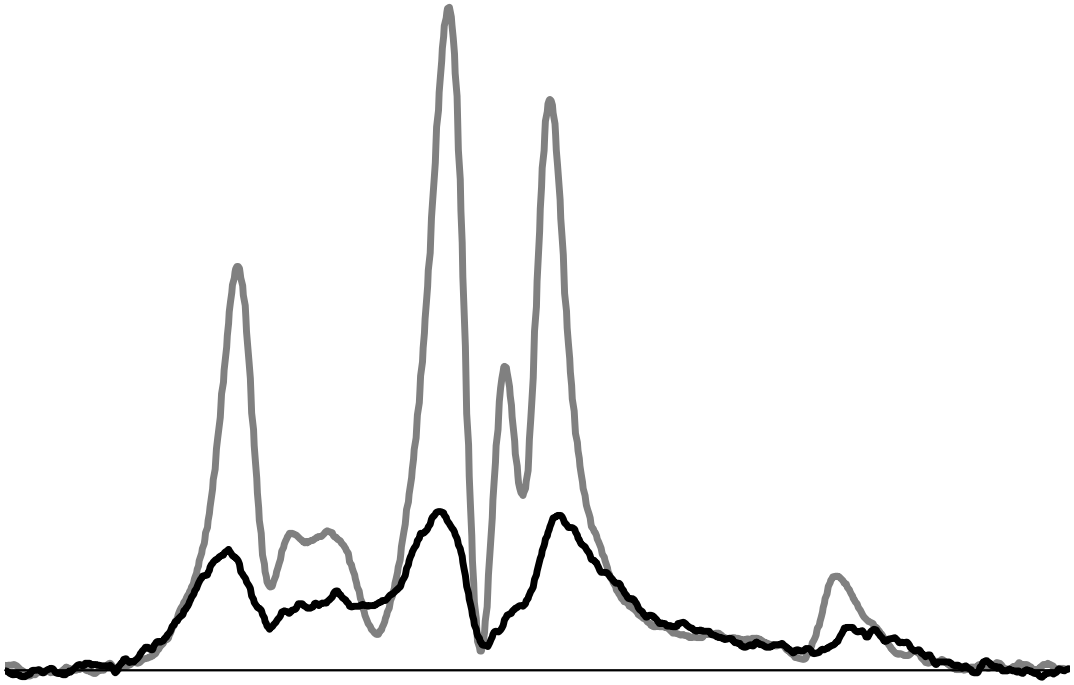

Fig. S2. STEPR spectra of 36-TOAC PLB at 1000 L/P (gray, same conditions as in Fig. 4) and 20 L/P (black). The black spectrum is strongly attenuated by spin-spin interactions (1), which are evident in the conventional EPR spectra of Fig. S1. Thus STEPR is quite sensitive to self-aggregation of this protein, which does not occur above 600 L/P.

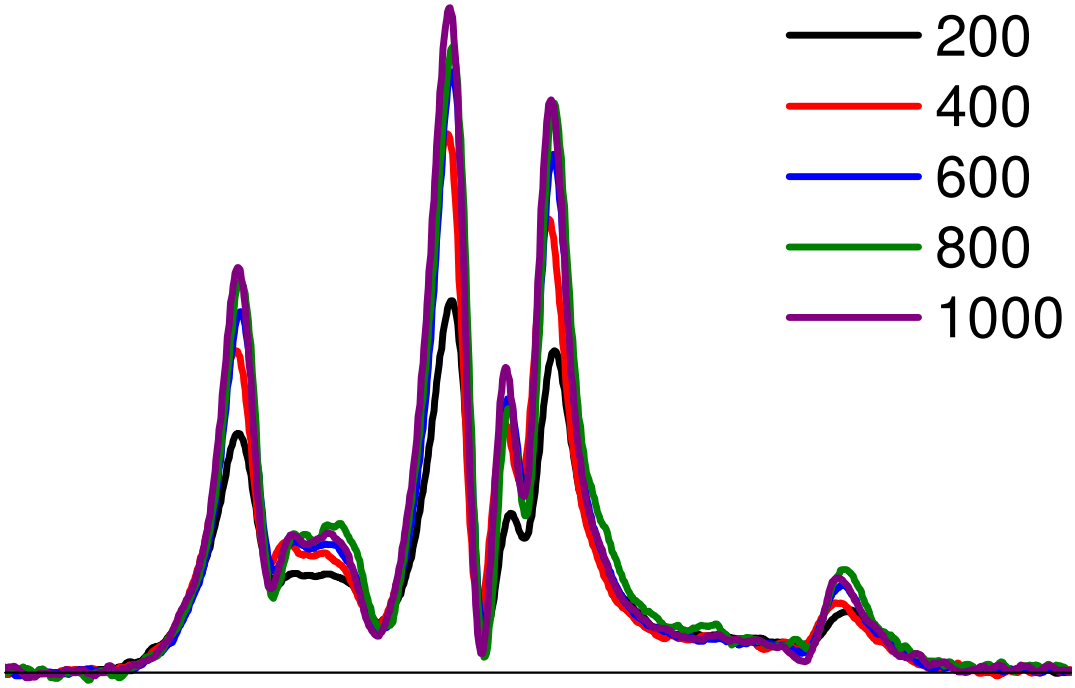

Fig. S3. STEPR spectra of 36-TOAC-PLB as a function of L/P (see figure legend), corresponding to the same samples as in Fig. 4. The increase in spectral intensity with increasing L/P (leveling off at high L/P) is consistent with decreasing spin-spin interactions (1), as documented in Fig. 4. STEPR lineshapes are essentially invariant above 600 L/P, supporting the conclusion that AFA-PLB is monomeric.

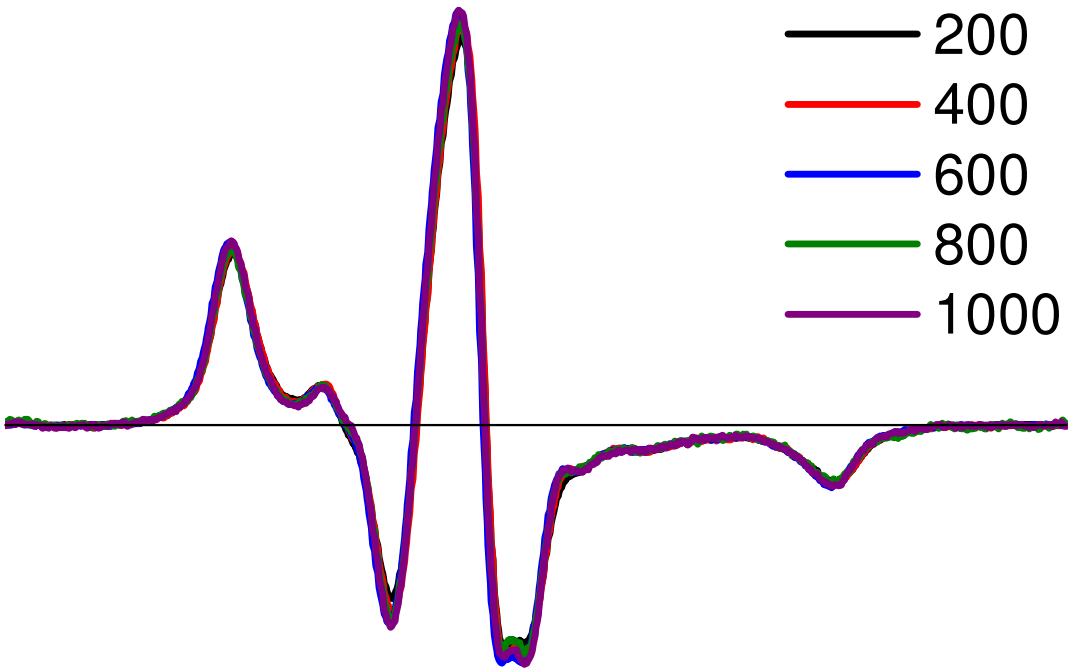

Fig. S4. Conventional EPR spectra of MSL-SERCA as a function of L/P, corresponding to the data shown in Fig. 5. The invariant spectra show that there are no significant changes in nanosecond rotational motion or spin-spin interactions due to variation of L/P, so all changes in STEPR spectra (Fig. 5) are due to μs rotational diffusion of SERCA. Average $2T_{\parallel}' = 68.2$ G.

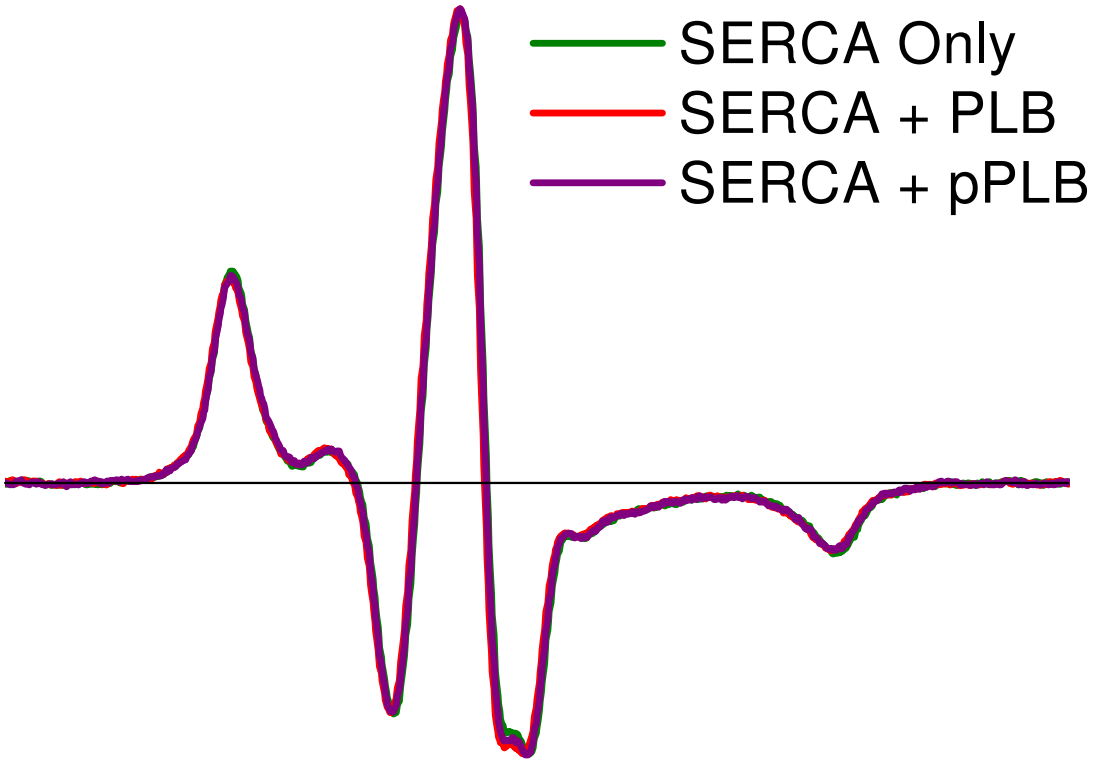

Fig. S5. Conventional EPR of MSL-SERCA reconstituted with unlabeled PLB, corresponding to the data shown in Fig. 7. The invariant spectra show that there are no significant changes in nanosecond rotational motion or spin-spin interactions due to PLB binding or phosphorylation, so STEPR spectra (Fig. 7) report microsecond rotational diffusion of SERCA.

Supporting References

1. Horvath, L. I., L. Dux, H. O. Hankovszky, K. Hideg, and D. Marsh. 1990. Saturation transfer electron spin resonance of Ca2(+)-ATPase covalently spin-labeled with beta-substituted vinyl ketone- and maleimide-nitroxide derivatives. Effects of segmental motion and labeling levels. Biophys J 58:231-241.