Allosteric Modulation of the Sarcoplasmic Reticulum Ca^{2+} ATPase by Thapsigargin via Decoupling of Functional Motions

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Supplementary Methods

Model setup of SERCA

This study included models based on three different states (PDB accession codes in parenthesis): [Ca_2]E1-AlF_4^--ADP (5XA8), (Ca_2)E1 (5XA7), E2-TG (5XAB) and the [Ca_2]E1P-ADP 4G insertion mutant of SERCA that we built using the MODELLER interface of UCSF Chimera (based on 5XA8).[1-3] We modelled TG-bound structures for the [Ca_2]E1P-ADP-TG (5XA8), (Ca_2)E1-TG (5XA7) and the [Ca_2]E1P-ADP-TG 4G insertion mutant of SERCA by aligning the structure of the TG-bound E2 state (5XAB) to the original model of each of the aforementioned states based on TG's binding pocket, followed by transferring the TG molecule and stepwise minimization of the resulting structures — no major clashes were found between the TG molecule and the different states of SERCA. We also performed simulations for Ca^{+2}-free E1-TG after removing the Ca^{+2} from (Ca_2)E1-TG and a TG-free E2 state after removing TG from the E2-TG model.

The protonation states for the ionizable residues were as follows (i) E908 was protonated for our E1 and E1P models and (ii) residues E309, E771 and E908 were protonated for the E2 models. Protonation states were assigned on the basis of previous studies[4-6] and also verified using PROPKA3[7]. A double negatively-charged phosphorylation was modelled at D351 in the phosphorylated E1P states using previously derived parameters[6] instead of the AlF_4 found in the crystal structures .The necessary cofactors for each state and co-crystalized water and ions were kept in the models.

General setup of MD simulations

SERCA models were inserted into a 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) membrane bilayer according to the orientation in the OPM database.[8] Parameters for the simulations were generated using the CHARMM-GUI web interface,[9-11] using the CHARMM36m force field[12] with CGenFF [13] for TG with partial charges generated from AM1-BCC calculations.[14] The appropriate number of potassium and chloride ions was added to the systems to a concentration of ~150mM. Particle-mesh Ewald was used to treat electrostatic interactions using a cut-off distance of 10 Å.[15] The resulting system was geometry-optimized and then equilibrated for 2 ns in the NVT and NPT ensembles respectively using restraints on the heavy atoms that were tapered down during the equilibration followed by a production run in the NPT ensemble at T=300 K using Nose-Hoover thermostat[16] and constant pressure using the Parrinello-Rahman barostat.[17] All hydrogen bonds were constrained using the LINCS algorithm[18] and time steps was set to 2fs. All simulations used the TIP3P water model and a box of 130×130×160 $Å^3$. System orientation and distance between protein and the box edge is illustrated in Figure 1. Simulations were performed using either GROMACS 5.0.4 or Gromacs2016.3 [19] and Table S1 provides an overview of

#	State	PDB ID	Construct	TG	Time (μs)	#Rep
1	[Ca_2]E1P-ADP	5XA8	WT	Yes	1.8μs	1
2	[Ca_2]E1P-ADP	5XA8	4G mutant	Yes	1.9μs	1
3	[Ca_2]E1P-ADP	5XA8	WT	No	1.5μs	1
4	[Ca_2]E1P-ADP	5XA8	4G mutant	No	1.5μs	1
5	(Ca_2)E1	5XA7	WT	No	1μs	1
6	(Ca_2)E1	5XA7	WT	Yes	1μsx3	3
7	E2	5XAB	WT	No	0.9μs	1
8	E1	5XA7	WT	Yes	0.99μs	1

Table S1. Overview of simulations performed and discussed.

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Supplementary figures

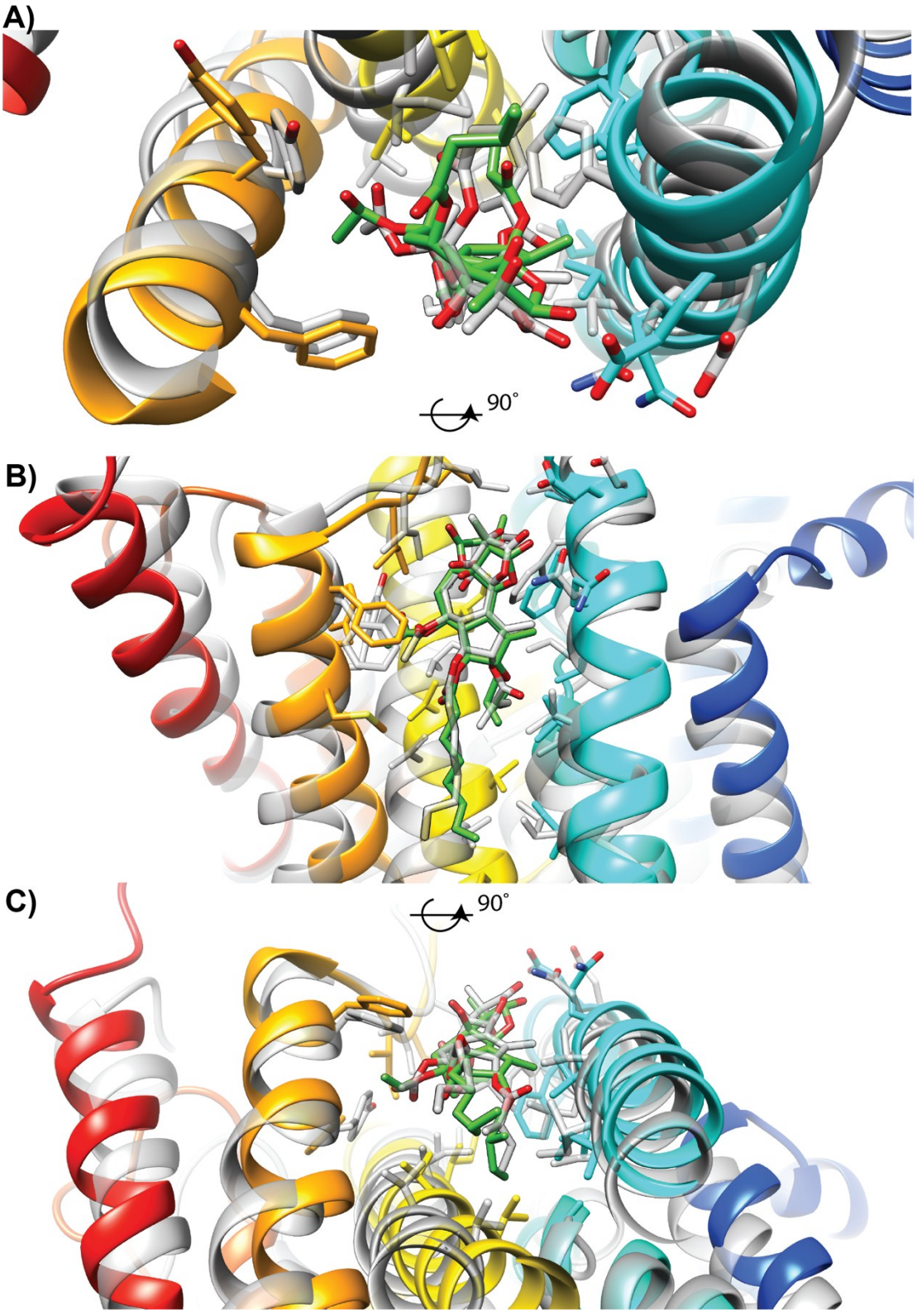

Figure S1. Comparison of the E2-TG binding pocket and the TG binding pocket in the [Ca_2]E1P-ADP structure after minimization from three different perspectives**.**

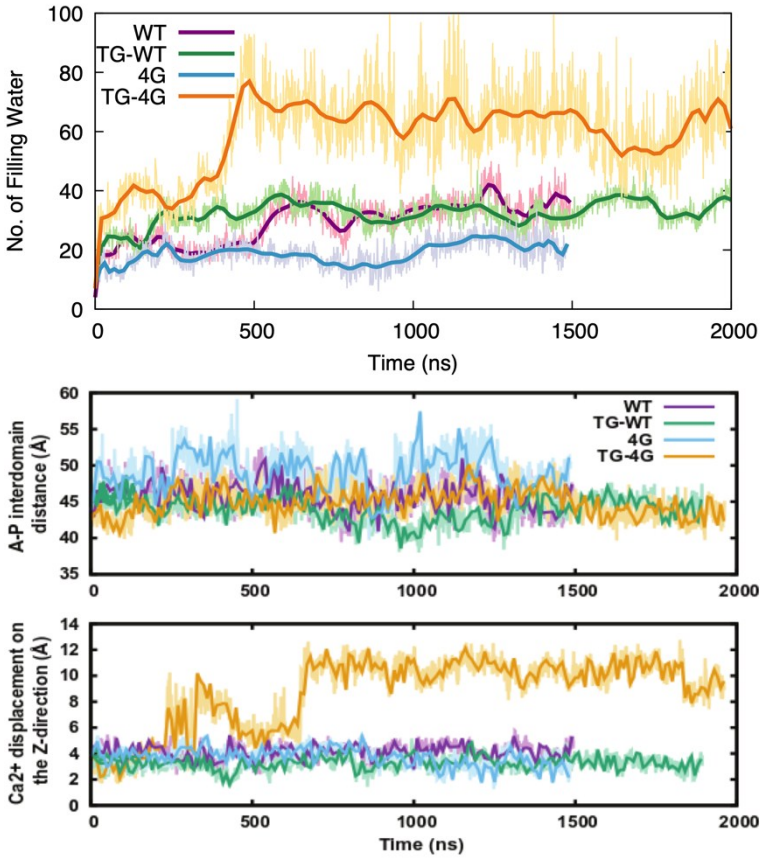

Figure S2. Comparison between the behaviour of the phosphorylated [Ca_2]E1P-ADP simulations. **Upper panel:** Number of water within the transmembrane bundle. **Middle panel**: Inter-domain distances between cytoplasmic A-P domains. **Lower panel**: Z-component of distance between the Ca^{2+} ion and the Cγ atom in E308.

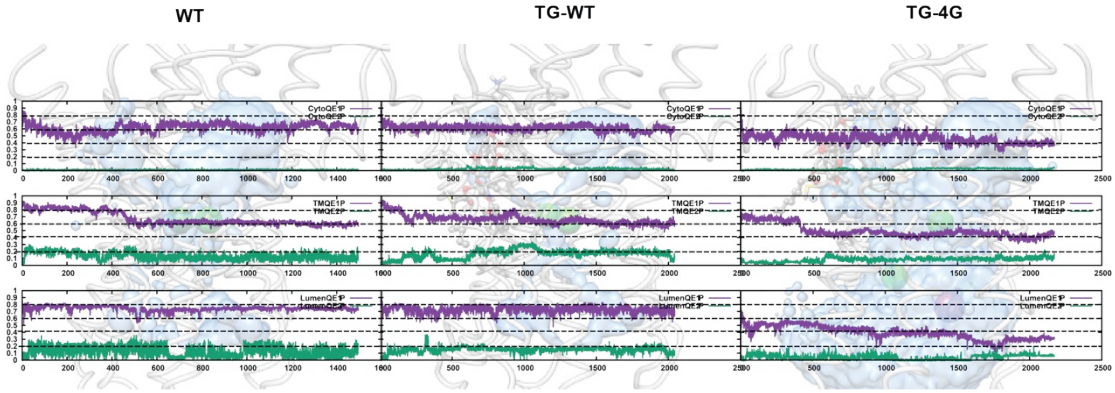

Figure S 3. Time evolution for the fraction of E1P/E2P specific native contacts formed during the WT, TG-WT and TG-4G simulations. A state-specific contact is defined as a contact that is present in one of the two states and is broken in the other state by a ratio 1:1.4 as described in references [20-22].

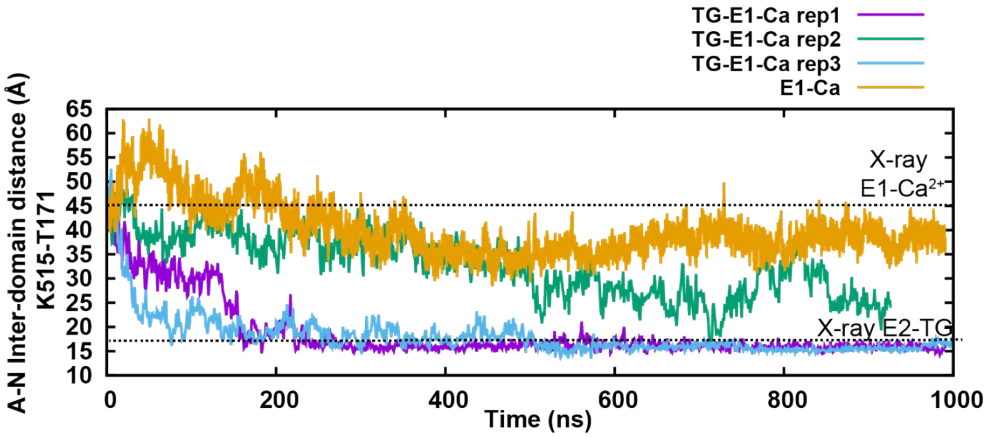

Figure S4. Time evolution of the A-N inter-domain distance during the (Ca_2)E1-TG and the (Ca_2)E1 simulations.

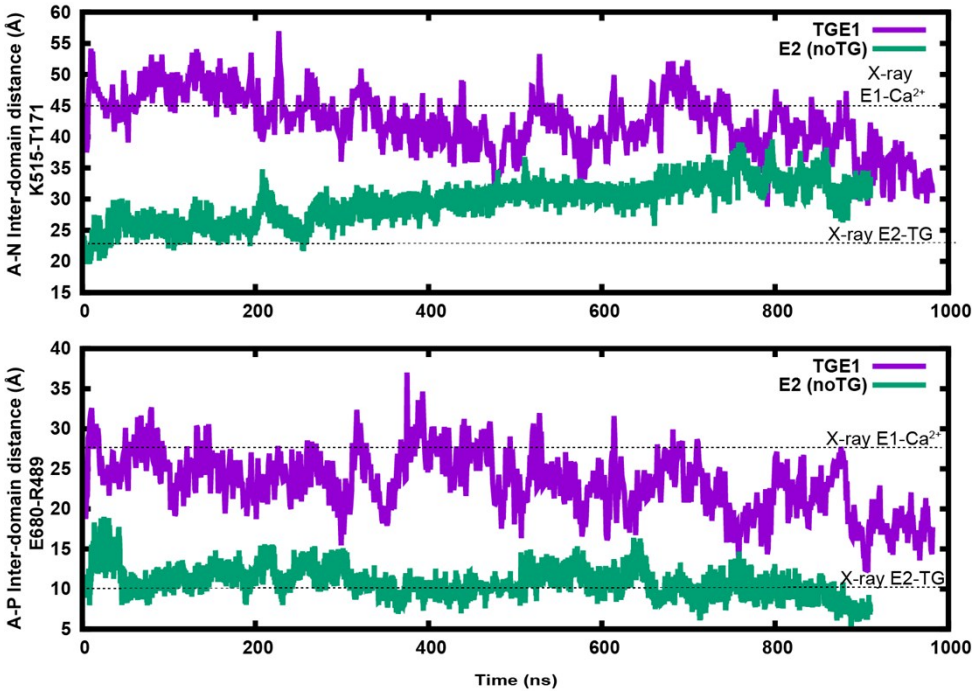

Figure S5. Upper panel: A-N inter-domain distance. **Lower panel**: P-N inter-domain distance evolution during the TG-bound E1 and the TG-free E2 simulations.

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