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# The SERCA residue Glu340 mediates interdomain communication that guides Ca<sup>2+</sup> transport

Maxwell M. G. Geurts<sup>a,1</sup>, Johannes D. Clausen<sup>b,c,1</sup>, Bertrand Arnou<sup>b,d,1</sup>, Cedric Montigny<sup>d</sup>, Guillaume Lenoir<sup>d</sup>, Robin A. Corey<sup>a</sup>, Christine Jaxel<sup>d</sup>, Jesper V. Møller<sup>b</sup>, Poul Nissen<sup>c,e</sup>, Jens Peter Andersen<sup>b</sup>, Marc le Maire<sup>d</sup>, and Maike Bublitz<sup>a,2</sup>

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The sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA) is a P-type ATPase that transports Ca<sup>2+</sup> from the cytosol into the sarco(endo)plasmic reticulum (SR/ER) lumen, driven by ATP. This primary transport activity depends on tight coupling between movements of the transmembrane helices forming the two Ca<sup>2+</sup>-binding sites and the cytosolic headpiece mediating ATP hydrolysis. We have addressed the molecular basis for this intramolecular communication by analyzing the structure and functional properties of the SERCA mutant E340A. The mutated Glu340 residue is strictly conserved among the P-type ATPase family of membrane transporters and is located at a seemingly strategic position at the interface between the phosphorylation domain and the cytosolic ends of 5 of SERCA's 10 transmembrane helices. The mutant displays a marked slowing of the Ca<sup>2+</sup>-binding kinetics, and its crystal structure in the presence of Ca<sup>2+</sup> and ATP analog reveals a rotated headpiece, altered connectivity between the cytosolic domains, and an altered hydrogen bonding pattern around residue 340. Supported by molecular dynamics simulations, we conclude that the E340A mutation causes a stabilization of the Ca<sup>2+</sup> sites in a more occluded state, hence displaying slowed dynamics. This finding underpins a crucial role of Glu340 in interdomain communication between the headpiece and the Ca<sup>2+</sup>-binding transmembrane region.

Ca<sup>2+</sup> binding | P-type ATPase | SERCA | tryptophan fluorescence | molecular dynamics simulations

The Ca<sup>2+</sup>-ATPase of sarco(endo)plasmic reticulum (SERCA) is an ion-translocating ATPase belonging to the P-type family of membrane transporters. It pumps cytosolic Ca<sup>2+</sup> ions across the sarco- or endoplasmic reticulum (SR/ER) membrane at the expense of ATP, a vital function in all living cells, particularly in the context of muscle contraction, Ca<sup>2+</sup> signaling, and cell survival (1).

The molecular Ca<sup>2+</sup> transport mechanism of SERCA is based on a cyclic transition through different conformations of the 110-kDa membrane protein, allowing alternating access to the Ca<sup>2+</sup>-binding sites from the cytosol and the SR/ER lumen. Binding and hydrolysis of ATP is mediated by SERCA's cytosolic "headpiece" which consists of three roughly globular domains, the nucleotide-binding (N), phosphorylation (P), and actuator (A) domains, while the Ca<sup>2+</sup>-binding sites are located in the transmembrane (TM) part of the protein, which consists of 10  $\alpha$ -helices (M1 through M10) (Fig. 1A). ATP binding and the subsequent transient phosphorylation of a conserved aspartate residue are coupled to a sequence of binding of two Ca<sup>2+</sup> ions from the cytosol, followed by occlusion (a state where the ions are shielded from access to either side of the membrane), and luminal release of these ions. This is associated with conformational transitions between so-called *E1*, *E1P*, *E2P*, and *E2* forms (Fig. 1B, "P" representing phosphorylation and brackets representing ion occlusion) (1, 2).

A number of SERCA1a crystal structures have shed light on the nature of the conformational changes associated with Ca<sup>2+</sup> transport (reviewed in refs. 3–5). There are two Ca<sup>2+</sup>-binding sites within the TM domain of SERCA, denoted sites I and II based on a proven sequential order of Ca<sup>2+</sup> binding (6). The Ca<sup>2+</sup> coordination in site I is mediated by residues in helices M5, M6, and M8, and in site II by residues in M4 and M6. Ca<sup>2+</sup> binding from the cytosol to SERCA involves the characteristically kinked M1 helix (Fig. 1A): a large vectorial movement of M1 toward the luminal side of the membrane mediates Ca<sup>2+</sup> access from the cytosolic side, whereas Ca<sup>2+</sup> occlusion after binding depends on an M1 movement in the opposite direction, allowing the formation of a hydrophobic cluster around the kink that shields the Ca<sup>2+</sup> sites. This goes along with a smaller shift of M3 relative to M5 (7, 8). The transition from the *E1P* to the *E2P* state is associated with a large rotation of the A domain, which causes a distortion of the coordination geometry at the high-affinity Ca<sup>2+</sup>-binding sites and a concomitant loss of Ca<sup>2+</sup> affinity, along with ADP release (9) and the formation of a luminal exit pathway for Ca<sup>2+</sup> release (10).

## Significance

We present a crystal structure, functional data, and molecular dynamics (MD) simulations of the sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA) mutant E340A. The mutation slows Ca<sup>2+</sup>-binding kinetics, and the structural differences between wild type and E340A indicate that the mutation disrupts a central interdomain "communication hub" governing Ca<sup>2+</sup> binding/dissociation. MD simulations reveal altered dynamics in regions mediating Ca<sup>2+</sup> occlusion, a critical step in SERCA's alternating access mechanism. The mutation stabilizes a more occluded state of the Ca<sup>2+</sup> sites. The strict conservation of Glu340 among P-type ATPases is the result of its critical role in interdomain communication between the cytosolic headpiece and the transmembrane domain, ensuring a delicate balance between dynamics of ion binding, occlusion, and release—key steps in the transport process.

Author contributions: J.V.M., P.N., J.P.A., M.I.M., and M.B. designed research; M.M.G.G., J.D.C., B.A., C.M., G.L., R.A.C., C.J., and M.B. performed research; M.M.G.G., C.M., G.L., R.A.C., and M.B. analyzed data; M.B. wrote the paper; and C.M., G.L., P.N., J.P.A., and M.I.M. contributed to the final editing of the paper.

The authors declare no competing interest.

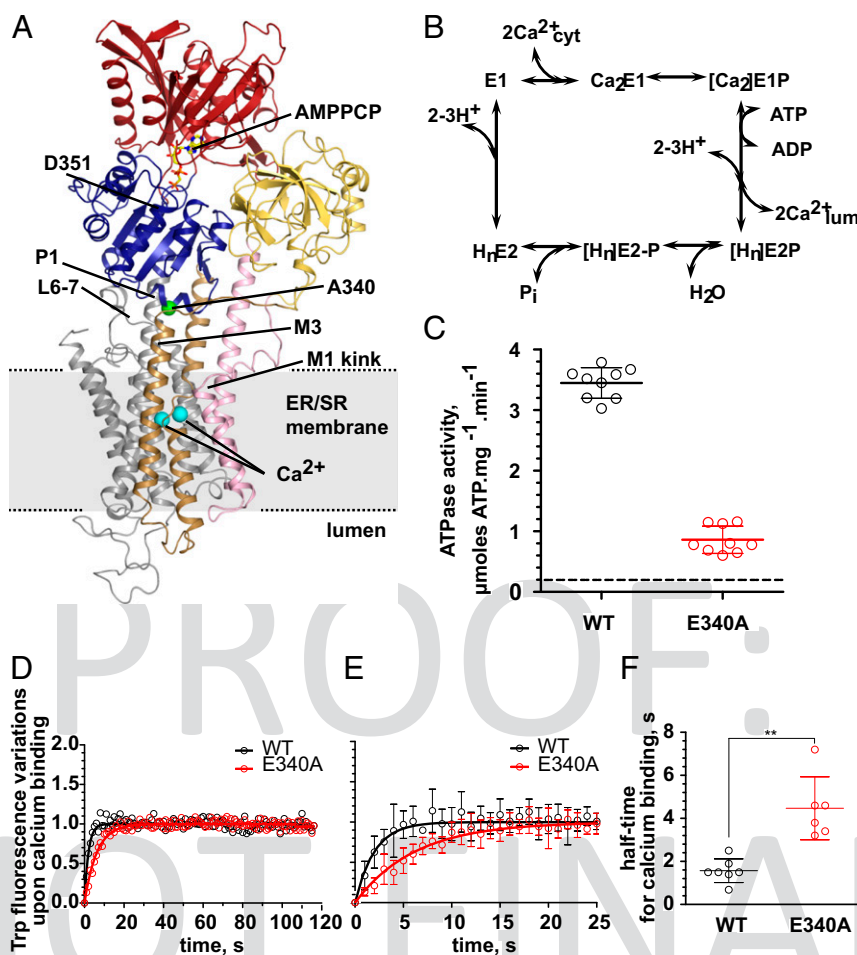
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**Fig. 1.** SERCA reaction scheme, structure, and activity measurements. (A) Crystal structure of SERCA E340A shown as cartoon. Nucleotide-binding (N) domain is red, actuator (A) domain yellow, phosphorylation (P) domain blue, M1-2 pink, M3-4 brown, M5-10 gray. AMPPCP is shown as ball-and-stick, Ca<sup>2+</sup> ions as cyan spheres. Residue 340 is indicated by a green sphere. (B) Schematic reaction cycle of SERCA. (C) Steady-state ATPase activity of purified WT SERCA (black) or E340A mutant (red) in an enzyme-coupled assay. WT:  $3.45 \pm 0.26$  ( $n = 9$ ); E340A:  $0.86 \pm 0.22$  ( $n = 9$ ); background (dashes):  $0.18 \pm 0.06$  ( $n = 18$ ). Three independent experiments were done from two different batches of purified protein. (D) Time course of Ca<sup>2+</sup>-binding transition determined by tryptophan fluorescence. Each time point represents the average of several measurements ( $n = 7$  for WT and  $n = 6$  for E340A from two independent experiments). (E) Magnified view of the early time points in D. Error bars correspond to the SD from the mean ( $n = 7$  for WT and  $n = 6$  for E340A). (F) Half-time for Ca<sup>2+</sup> binding for WT SERCA and E340A.  $t_{1/2}$  were determined on each individual experiment.  $t_{1/2}$  are  $1.6 \pm 0.6$  s for WT and  $4.5 \pm 1.5$  s for E340A with  $P = 0.0023$  (one-tailed paired  $t$  test).

Both the P and the A domains of the cytosolic headpiece are in contact with the 10-transmembrane helical domain, albeit by different structural elements: The A domain is connected via three loops (A-M1, M2-A, and A-M3) that are thought to relay the movements of the A domain to the M1 to M3 helices. The P domain, however, is structurally very tightly integrated into the cytosolic ends of M4 and M5. M4 is linked to the so-called P1 helix (Pro337-Cys344), a short  $\alpha$ -helix that runs roughly parallel to the membrane surface, at the membrane-facing side of the P domain. In such a location, P1 may be a key element of inter-domain communication: it connects directly to a  $\beta$ -strand in the P domain ending with the phosphorylated aspartate residue, and it makes contact with the cytosolic end of M3 and to the loop between M6 and M7 (L6-7), which has been shown to play an important role in SERCA catalysis (11–17) (Fig. 1A).

To understand the Ca<sup>2+</sup> transport mechanism it is mandatory to obtain information on the structural relations of residues critical in mediating the communication between the membranous Ca<sup>2+</sup>-binding sites and the cytosolic phosphorylation site. Glu340 is a centrally positioned residue in the P1 helix at the interface between the phosphorylation domain and the cytosolic

ends of M3 through M7 (Fig. 1A). It is almost universally conserved throughout the large P-type ATPase superfamily. With the exception of polyamine transporting pumps and some bacterial representatives that mostly have a glutamine or asparagine residue at this position, all animal P-type ATPases known to pump ions or lipids strictly possess glutamate (SI Appendix, Table S1) (18).

Mutation of Glu340 seems to affect the partial reactions involving cytoplasmic Ca<sup>2+</sup> interactions (17), thus raising the question of whether this residue plays an important role in linking the P and the TM domains. Structural information supporting such a role has, however, been lacking.

In general, structural information on SERCA mutants is relatively scarce with only four published crystal structures to date (19–21). The main reason for this shortage is that purification and crystallization of recombinantly produced SERCA is challenging, with low protein yields and poor stability in the absence of native lipids compared to the native enzyme purified from skeletal muscle.

In this study, we have determined the crystal structure of yeast-expressed rabbit SERCA1a mutant E340A at 3.2-Å

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249 resolution in the  $\text{Ca}_2\text{E1}$  form with bound ATP analog  
250 (AMPPCP), and we examined its functional properties, including  
251 ATPase activity and  $\text{Ca}^{2+}$ -binding kinetics. This is notably a  
252 determination of the structure of a SERCA mutant being cata-  
253 lytically active, i.e., capable of completing a full catalytic cycle,  
254 albeit with altered kinetics. Furthermore, molecular dynamics  
255 (MD) simulations of both wild-type (WT) and E340A structures  
256 embedded in a lipid bilayer supported our conclusions derived  
257 from structural and functional studies. Our data link the struc-  
258 ture to Glu340's functional importance and provide insight into  
259 the interdomain communication that guides  $\text{Ca}^{2+}$  transport  
260 by SERCA.

## 261 Results

262 **SERCA E340A Has a Lower ATPase Activity and Slower  $\text{Ca}^{2+}$ -Binding**  
263 **Kinetics than WT.** The effect of the E340A mutation on yeast-  
264 expressed SERCA function was addressed by using an enzyme-  
265 coupled assay to estimate the specific activity of the E340A  
266 mutant in detergent. We observed that the ATPase activity of the  
267 E340A mutant is about 25% of the WT (0.86 vs. 3.45  $\mu\text{moles}$   
268  $\text{ATP}/\text{mg}/\text{min}$ ,  $n = 9$ ) (Fig. 1C and *SI Appendix*, Fig. S1), i.e., a  
269 marked slowing of the enzyme cycle similar to, although not as  
270 extensive, as that reported in prior studies carried out in mi-  
271 crosomal membranes (11 to 13%) (14, 17). The presence of a  
272 large excess of  $\text{C}_{12}\text{E}_8$  in our preparation, a mild detergent that  
273 has been described to accelerate specifically the  $\text{E2} \rightarrow \text{Ca}_2\text{E1}$   
274 transition, provides a likely explanation for the less drastic effect  
275 of the E340A mutation observed here (22–24).

276 In order to assess whether this overall deceleration is due to a  
277 reduced rate of  $\text{Ca}^{2+}$  binding to SERCA E340A, we measured  
278 the time course of the  $\text{Ca}^{2+}$ -binding transition, by recording  
279 changes in intrinsic tryptophan fluorescence upon addition of  
280  $\text{Ca}^{2+}$  to the E2 state of the protein. The E2 to  $\text{Ca}_2\text{E1}$  transition  
281 of E340A is significantly slower than that of the WT, with an  
282 increase of  $t_{1/2}$  of about threefold (Fig. 1D–F). This suggests that  
283 the effect of the E340A mutation on the overall turnover of  
284 SERCA may be caused by a delay in the conformational change  
285 associated with  $\text{Ca}^{2+}$  binding, i.e., the  $\text{H}_n\text{E2} \rightarrow \text{E1} \rightarrow \text{Ca}_2\text{E1}$   
286 transition (see further discussion below).

287 **The Mutation E340A Causes Global Changes in SERCA's Domain**  
288 **Arrangement.** Our crystal structure of SERCA E340A at 3.2-Å  
289 resolution (Fig. 1A and *SI Appendix*, Table S2) allows for a de-  
290 tailed structural comparison with the WT structure. When su-  
291 perposed, the WT and E340A structures deviate by a substantial  
292 rmsd of 2.3 Å over all main chain atoms. Nevertheless, when  
293 superposed on the C-terminal transmembrane helix bundle M5  
294 to M10 (residues 750 to 994, main chain rmsd = 0.49 Å), the  
295 E340A mutant and WT have an almost identical arrangement of  
296 the remaining transmembrane helices M1 to M4 (main chain  
297 rmsd = 0.56 Å), with a small 1.6-Å bend at the cytosolic end of  
298 M3 in E340A (Fig. 2A), and a slightly more kinked helix M1  
299 ( $105^\circ$  in E340A vs.  $109^\circ$  in WT). In line with this, the  $\text{Ca}^{2+}$ -  
300 binding sites look identical in WT and E340A.

301 In contrast to the almost identical arrangement in the TM  
302 domain, there is a pronounced shift (approximately 10 Å) in the  
303 position of the cytosolic headpiece, which is rotated further  
304 “downward” toward the membrane surface in E340A (Fig. 2A  
305 and B and *Movie S1*). At the membrane-facing side of the P  
306 domain, the distance between Pro337 at the distal end of the P1  
307 helix and Pro312 in M4 (the nearest residue to Pro337 at the  
308 approximate level of the membrane surface) decreases from 15.6  
309 Å in WT to 12.3 Å in E340A. The pivot points of this headpiece  
310 movement are at the cytosolic ends of M2, M4, and M5. Whereas  
311 M2 and M4 stay very rigid throughout their entire lengths, M5  
312 undergoes a slight bend in the region between its N-terminal end  
313 (at Phe740), which is embedded in the P domain, up till residue  
314 Gly750, which sits at the “rear side” of P1, exactly opposite

315 residue 340 (Fig. 2B). On the “front side” of P1, the N-terminal  
316 end of M3 moves in the opposite direction of the shifting P1  
317 helix, bending slightly inwards with its first two N-terminal  
318 windings (Fig. 2A, *Inset* and *Movie S1*).

319 While the domain bodies themselves do not change between  
320 WT and E340A (main chain rmsd of 0.37 Å, 0.37 Å, and 0.32 Å  
321 for the single P, N, and A domains, respectively), their concerted  
322 movement relative to the rest of the structure in E340A is  
323 reflected by an overall main chain rmsd for the entire headpiece  
324 of 1.16 Å. The P and N domains appear to move together as one  
325 rigid body (main chain rmsd = 0.47 Å for the P/N body), whereas  
326 the A domain is displaced slightly further, with the distance  
327 between the N and the A domain increasing by approximately  
328 3 Å in the final position.

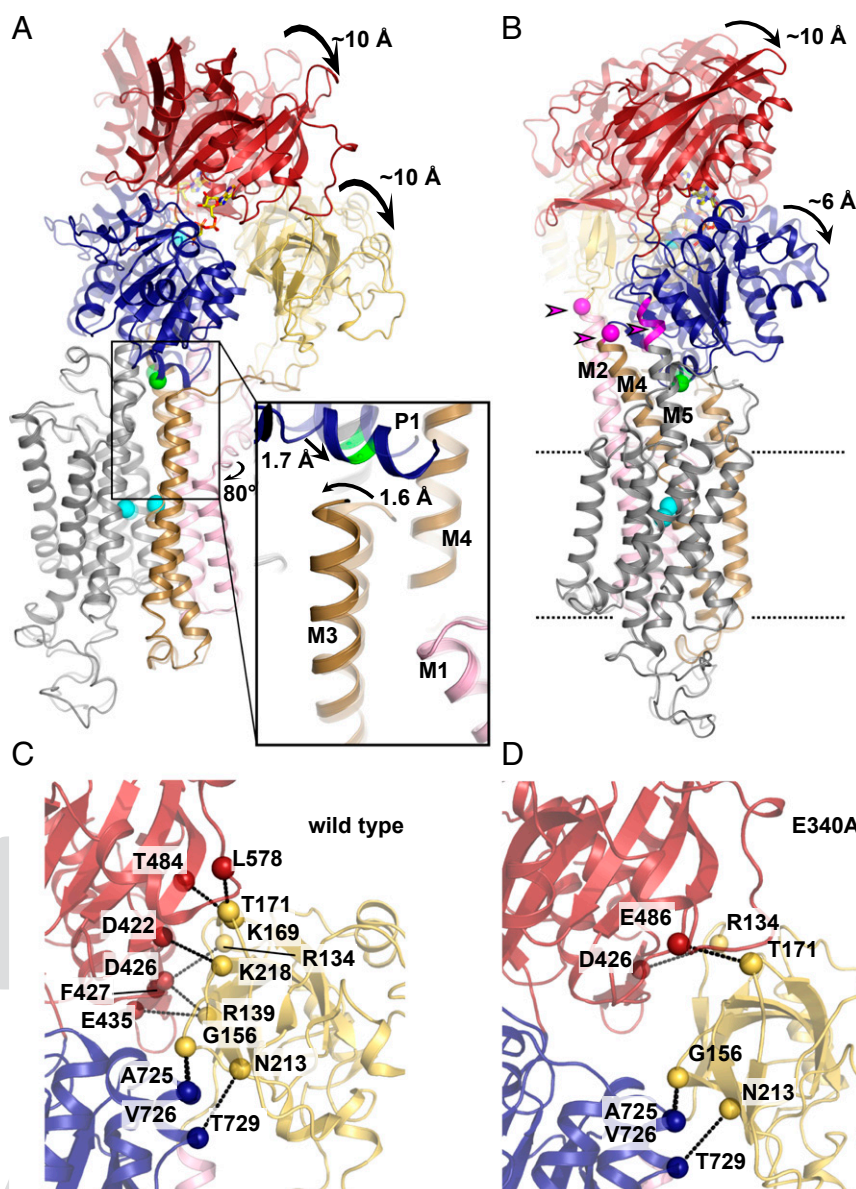
329 Intriguingly, the downward rotation of the cytosolic headpiece  
330 in E340A is an extension of exactly the same trajectory as the  
331 movement of WT SERCA when it shifts from empty MgE1 to  
332 the  $\text{Ca}^{2+}$ -bound E1 form ( $\text{Ca}_2\text{E1}$ ): when  $\text{Ca}^{2+}$  binds to WT  
333 SERCA, the entire P1 to M3 network moves upwards relative to  
334 L6-7 and M5, by about 4.5 Å, while the overall headpiece rotates  
335 down toward the membrane surface (8). In E340A, this head-  
336 piece downward rotation continues further by about 10 Å (as  
337 visualized in a superposed morph between the MgE1 [Protein  
338 Data Bank {PDB} 4H1W] and the  $\text{Ca}_2\text{E1}$  WT [PDB 3N8G], and  
339 E340A [PDB 6RB2] structures; see *Movie S2*). This extension of  
340 the WT  $\text{Ca}^{2+}$ -binding trajectory is reflected in a larger main  
341 chain rmsd between the empty WT structure and the  $\text{Ca}^{2+}$ -  
342 bound states in E340A (5.6 Å) than in WT SERCA (4.7 Å).

343 The observation of an “overshooting”  $\text{Ca}^{2+}$ -binding move-  
344 ment in E340A led us to ask whether this mutation might lead to  
345 a stabilization of a  $\text{Ca}^{2+}$  occluded state, the state that follows  
346  $\text{Ca}^{2+}$  (and nucleotide) binding in the catalytic cycle.  $\text{Ca}^{2+}$  oc-  
347 clusion is normally stabilized by the initiation of phosphorylation  
348 by ATP and not by  $\text{Ca}^{2+}$  binding alone (7). We hence looked at  
349 further structural deviations of SERCA E340A from WT,  
350 starting at the contacts between the three domains of the cyto-  
351 solic headpiece (N, P, and A domains).

352 **Connectivity between the N and A Domains Is Looser in E340A.** While  
353 the P/N body moves as one rigid body as described above, the  
354 distance between the N and A domains increases in E340A. The  
355 main chain rmsd of 1.4 Å for the N/A body is the largest rms  
356 deviation measured for any element of the two structures, indi-  
357 cating that this is the largest structural change induced by the  
358 mutation. This change leads to the loss of six of eight polar  
359 contacts between the N and the A domains (Arg134 to Phe427,  
360 Arg139 to Asp426 and Glu435, Lys169 to Thr484, Thr171 to  
361 Leu578, and Lys218 to Asp422), leaving only two contacts intact  
362 between these two domains (Arg134 to Asp426 and Thr171 to  
363 Glu486), whereas the A domain's contacts to the P domain re-  
364 main intact (Gly156 to Ala725 and Val726, Asn213 to Thr729)  
365 (Fig. 2C and D). This loss of N-A contacts is also evident from  
366 our MD simulations (see below and Fig. 4C).

367 **Local Changes at the Mutated Residue 340.** In the  $\text{Ca}_2\text{E1}$  form of  
368 WT SERCA (PDB 3N8G), the Glu340 side chain is in hydrogen  
369 bonding distance to the side chain OH group of Thr247 and the  
370 main chain nitrogen of Leu249 at the N-terminal end of M3, with  
371 additional electrostatic attraction by the partial positive charge  
372 of the helix N terminus. A third interaction of Glu340 is a water-  
373 mediated contact to Arg822 in L6-7 (Fig. 3A).

374 Interestingly, the local conformation around the Glu  $\rightarrow$  Ala  
375 mutation (within a radius of  $\sim 10$  Å) is very similar to WT, with  
376 measurable shifts of under 2 Å (Figs. 2A and 3A and B). While  
377 the local similarity may seem surprising at first glance, looking at  
378 the larger global structural changes described above, it becomes  
379 clear that residue A340 is located very close to the pivot point for



**Fig. 2.** Comparison of SERCA WT (PDB 3N8G) and E340A crystal structures. Coloring is as in Fig. 1. (A) Domain rearrangements in E340A. Superposition of E340A with WT (transparent). (Inset) Local changes near residue 340. (B) Side view of A, with the pivot regions at the cytosolic ends of M2, M4, and M5 highlighted in magenta and by arrowheads. (C and D) Polar contacts between the A domain (yellow) and the N (red) and P (blue) domains in WT SERCA (B) and E340A (C).

the described headpiece rotation and therefore does not move much itself.

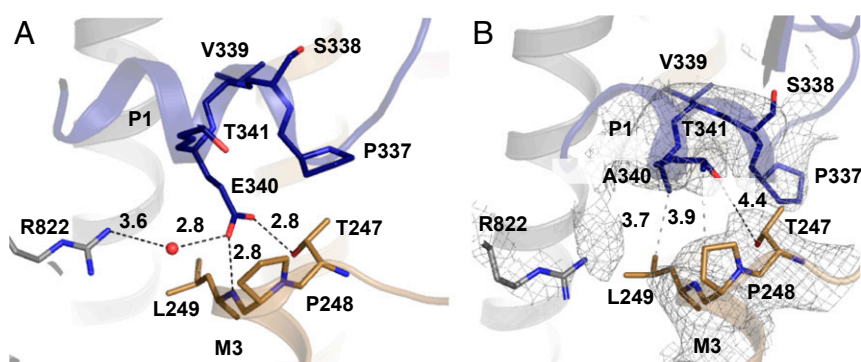
The overall distance between P1 and M3 is slightly smaller than in WT, and the nature of the contact between these two structural elements is very different in E340A: there are no apparent polar interactions between P1 and M3, except for a distant (4.4 Å) contact between Thr341 in P1 and Thr247 in M3 (Fig. 3B). Instead, the electrostatic attraction to the partially positive M3 N terminus is replaced with hydrophobic interactions (Ala340 to Leu249, Pro337 to the methyl group of Thr247, and Thr341 to Pro248). In fact, this interaction appears to be rather tight, as shown by our MD simulations (see below and Fig. 4B).

Removal of the Glu340 side chain abolishes the contact to Arg822, and the entire L6-7 loop (Phe809 to Ser830) is displaced laterally by ~3 Å, increasing the distance between Arg822 and

the P1 helix. In order to test the hypothesis that the loss of the Arg822-Glu340 contact in E340A contributes to the functional impairment of this mutation, and to validate the structural and functional differences between WT and E340A described above, we conducted MD simulations.

#### Molecular Dynamics Simulations Support a More Occluded State of E340A.

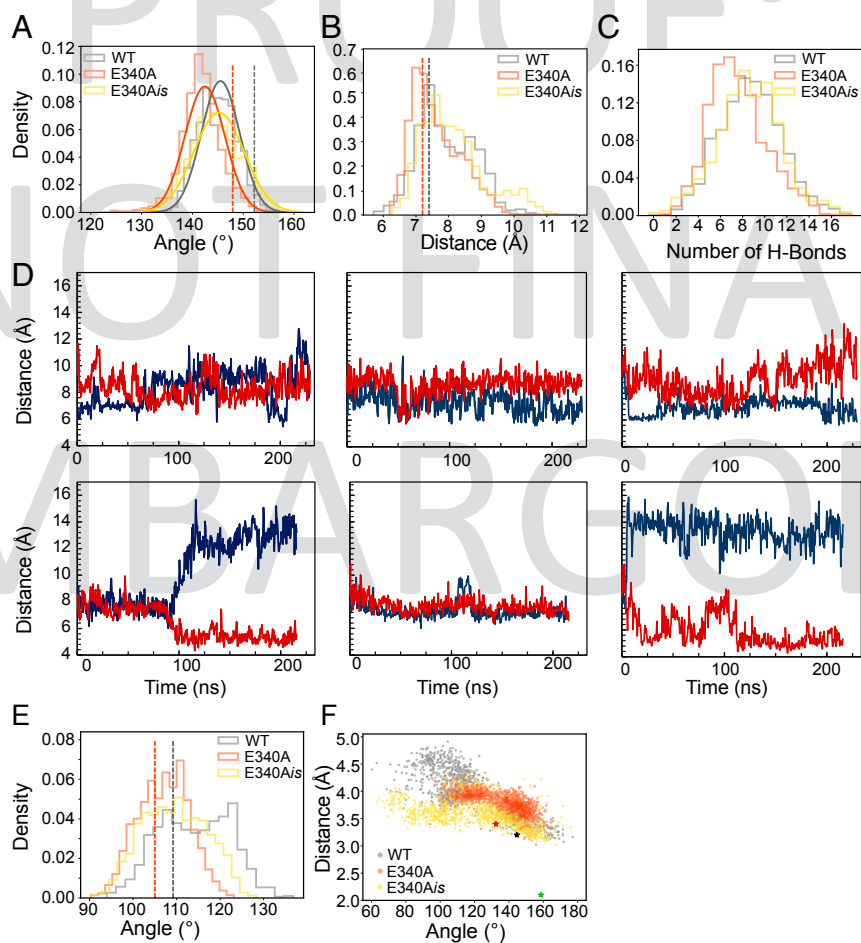
In order to assess whether the differences we found between the crystal structures of WT SERCA and E340A might reflect stable conformations occurring in the native membrane environment, and to find further explanations for the observed functional impairment of the mutant, we carried out  $3 \times 200$  ns of all-atom MD simulations of each structure reconstituted in a POPC lipid bilayer. As an internal control, we generated an  $\alpha$ -14 in silico mutant E340A<sub>is</sub> by truncation of the Glu340 side chain



**Fig. 3.** Local structural changes near the E340A mutation. (A) Local hydrogen bond network (black dashes) around residue 340 in WT SERCA. (B) Local hydrogen bond network (black dashes) and hydrophobic contacts (gray dashes) around residue 340 in E340A. Gray mesh:  $2mF_o-DF_c$  electron density map contoured at  $1.0 \sigma$ .

from the WT  $Ca_2E1$ -AMPPCP structure and subjected it to the same simulations.

To quantify the conformational downward shift of the cytosolic headpiece relative to the membrane, we determined the angle between three points; Leu13 in the A domain and Thr86



**Fig. 4.** Molecular dynamics simulations of SERCA WT (PDB 3N8G), E340A and modeled mutant E340A<sub>is</sub>. WT is shown in gray, E340A in orange, and E340A<sub>is</sub> in yellow. Histograms give density as an arbitrary unit on the y axis. Dotted lines and asterisks refer to reference values in respective crystal structures. (A) Angle histogram between the soluble headpiece and the transmembrane domain, measured between Leu13 in the A domain and Thr86 and Leu98 in M2. (B) Distance histogram between C $\alpha$  atoms of Leu249 at the tip of M3 and residue 340. (C) Histogram of hydrogen bonds between the A-domain and the N/P-domain body. (D) Distance traces between Arg822-N $\epsilon$  and Glu/Ala340-C $\alpha$  (blue) and between Leu249-C $\alpha$  and Pro824-C $\alpha$  (red). *Left to Right*: Simulation runs 1 to 3. *Top traces*, WT; *Bottom traces* E340A. (E) Histogram of kink angles in M1, measured between C $\alpha$  atoms of Trp50, Arg63, and Val74. (F) Geometry at the catalytic site. Angles between the O- and P $\gamma$ -atoms of the terminal phosphoanhydride bond of ATP and the closest carboxyl oxygen (O $\delta$ ) of Asp351 versus the distance between O $\delta$  and P $\gamma$ . Green asterisk: 1T5T, ADP-AlFx complex.

621 and Leu98 in M2 (*SI Appendix, Fig. S2A*). When comparing the  
622 two crystal structures, the angle in E340A is 5° more acute (147°  
623 than in WT (152°). During the course of the simulations, both  
624 structures relax toward more acute angles, but the difference  
625 between them stays about the same, with E340A having a median  
626 angle of 142° and WT of 146° (Fig. 4A). This confirms that the  
627 difference in headpiece angle is a true feature of E340A that  
628 persists in a dynamic membrane environment.

629 Coherent with the above finding, the P1 helix remains closer to  
630 the tip of M3 in E340A, as shown by the distance between the C $\alpha$   
631 atoms of residue 340 and Leu249 at the tip of M3 (Fig. 4B and *SI*  
632 *Appendix, Fig. S2B*). The WT structure exhibits a bimodal  
633 distribution of distances with peaks at 7.5 Å and 8.8 Å, whereas  
634 E340A has a prominent peak at a shorter distance than WT (6.9  
635 Å).

636 The loss of connectivity between the A domain and the N/P  
637 body described above is also apparent from the MD simulations.  
638 The number of H bonds between these elements remains re-  
639 duced in E340A throughout the simulation (Fig. 4C).

640 In the three analyses described above, the in silico mutant  
641 E340A<sub>is</sub> gives similar distributions to WT, likely because the  
642 rather large domain movements are too slow to be seen after the  
643 short simulation time of 200 ns. When looking at more local  
644 dynamics, however, E340A<sub>is</sub> does behave more similarly to  
645 E340A, as seen in the analyses below.

646 The crystal structure suggests that Glu340 can engage in up to  
647 three hydrogen bonds: with Thr247, the backbone of Leu249,  
648 and a water-mediated contact to Arg822 (Fig. 3A). The link to  
649 Arg822 in L6-7 is completely lost in E340A (Fig. 3B). Despite  
650 the loss of this ionic contact, there is only a slight displacement  
651 of L6-7, which is held in place by contacts to L8-9 and other regions  
652 of the P domain. Accordingly, we could not detect an increased  
653 motility of L6-7 in our MD simulations (*SI Appendix, Fig. S3A*).  
654 Nevertheless, our simulation data show that—in the absence of  
655 the Glu340 side chain—Arg822 can swing out of the site between  
656 P1 and M3 (*SI Appendix, Fig. S3B*). This is measurable as a  
657 sudden increase in the distance between Arg822 N $\epsilon$  and Ala340  
658 C $\beta$  from about 7 Å to 13 Å in E340A and to more than 14 Å in  
659 E340A<sub>is</sub> (Fig. 4D and *SI Appendix, Fig. S3C*). In WT SERCA,  
660 Arg822 does not swing out spontaneously, and the distance stays  
661 at 7 to 10 Å (Fig. 4D). Importantly, once moved to the “out”  
662 position, Arg822 does not return to the “in” position over the  
663 course of any of our simulations of E340A and E340A<sub>is</sub> (Fig. 4D  
664 and *SI Appendix, Fig. S3C*). The outward swing of Arg822 does,  
665 however, not lead to a loss of contact between P1, M3, and L6-7,  
666 as might be expected at first glance. On the contrary, concomi-  
667 tant with the outward swing of Arg822 from the wedge between  
668 P1 and M3, the space is taken up by Leu249 at the tip of M3,  
669 which closes in on the gap and approaches Pro824 in the L6-7  
670 loop (Fig. 4B and *SI Appendix, Fig. S3D*), reducing the distance  
671 between the two C $\alpha$  atoms from 10 Å (E340A<sub>is</sub>) or 7.5 Å  
672 (E340A) to 5 Å (Fig. 4D and *SI Appendix, Fig. S3C*). This ap-  
673 proach of M3 toward L6-7 is mutually exclusive with the in po-  
674 sition of Arg822, as seen by a conspicuous correlation of the  
675 distance plots between Arg822 and Ala340 and between Leu249  
676 and Pro824. This correlation is also evident in the in silico mu-  
677 tant E340A<sub>is</sub> but absent from the WT (Fig. 4D and *SI Appendix,*  
678 *Fig. S3C*), suggesting that the changes in the contact network  
679 around P1 play a central role in mediating the observed struc-  
680 tural changes in E340A.

681 Comparing the dynamics of the transmembrane segments  
682 throughout the simulations, we found a remarkable difference in  
683 the kinked region of M1 between WT and both E340A and  
684 E340A<sub>is</sub>: M1 is rigidly kinked in E340A, while in WT the kink  
685 region displays more dynamics and can straighten to some extent  
686 (Fig. 4E and *SI Appendix, Fig. S4*). This finding is particularly  
687 interesting in light of prior studies showing an involvement of M1  
688 with Ca<sup>2+</sup> binding and occlusion (7, 8).

689 In order to further probe our simulation data with respect to  
690 Ca<sup>2+</sup> occlusion in E340A, we analyzed the nucleotide-binding  
691 site. Ca<sup>2+</sup> occlusion is coupled to phosphorylation of Asp351,  
692 and a more occluded conformation of SERCA would be  
693 expected to be more prone to being phosphorylated. We hence  
694 compared the distances between the ATP  $\gamma$ -phosphate and the  
695 respective closer of the two carboxyl oxygen atoms of Asp351,  
696 along with the angles between the carboxyl oxygen and the O-P  
697 bond of the terminal phosphoanhydride in ATP (*SI Appendix,*  
698 *Fig. S5*). For comparison, the crystal structure in which the fully  
699 occluded transition state of phosphorylation has been trapped  
700 with ADP-AIF<sub>x</sub> (7) the distance and angle between the Asp351  
701 O $\delta$  and the aluminum atom are 2.1 Å and 159°, respectively. As  
702 seen in Fig. 4F, the WT Ca<sub>2</sub>E1-AMPPCP structure displays a  
703 large spread of angles (between 60° and 180°) and distances  
704 (between 3 and 5 Å), many of which are not compatible with an  
705 in-line associative hydrolysis mechanism. In E340A, the distances  
706 take up a much narrower spread mostly between 3.5 and 4 Å, and  
707 the angles are more restricted toward the catalytically competent  
708 obtuse region (between 110° and 170°). E340A<sub>is</sub> displays an in-  
709 termediate distribution. Our data are therefore consistent with a  
710 more occluded state in both the ion-binding and the nucleotide-  
711 binding regions of E340A.

## 712 Discussion

713 We have addressed the molecular basis for intramolecular  
714 communication in SERCA by analyzing the structure and func-  
715 tional properties of the SERCA mutant E340A. The Glu340  
716 residue is strongly conserved among P-type ATPase membrane  
717 transporters and is located at a central position of the ATPase, at  
718 the interface between the phosphorylation domain and trans-  
719 membrane segment, where the Ca<sup>2+</sup>-binding sites are located.

720 The structure of the mutant differs from the equivalent wild-  
721 type structure in: 1) a lowered cytosolic headpiece relative to the  
722 membrane, 2) altered connectivity between the nucleotide-  
723 binding and the actuator domains, and 3) a shift in the Ca<sup>2+</sup>-  
724 gating transmembrane helix M3. MD confirms the structural  
725 changes observed and moreover reveals altered dynamics in re-  
726 gions involved in Ca<sup>2+</sup> occlusion, in particular a rigidly kinked  
727 M1. These structural findings point to a stabilization of a more  
728 occluded conformation and are discussed below in relation to the  
729 functional properties of the E340A mutant.

730 The downward movement of the cytosolic headpiece toward  
731 the membrane in E340A is an extension of exactly the same  
732 trajectory as the movement of WT SERCA when it shifts from  
733 empty MgE1 to the Ca<sup>2+</sup>-bound E1 form (Ca<sub>2</sub>E1). Hence, the  
734 loss of Glu340 allows an overshoot of this closure movement  
735 after ion binding, which is pivoting around P1. At the same time,  
736 within the headpiece, the A domain gets displaced and loses  
737 more than half of its contacts with the N domain. The exact  
738 functional implication of this effect is not entirely clear. Crystal  
739 structures, X-ray solution scattering, and our MD simulations  
740 show that the A domain undergoes the largest and most diverse  
741 movements during the catalytic cycle (9, 10, 20, 25), and through  
742 its direct linkage to TM helices M1 to M3 it directly affects the  
743 geometry of the Ca<sup>2+</sup>-binding sites. On the other hand, its po-  
744 sition relative to the N and P domains dictates whether the site of  
745 ATP hydrolysis can adopt a catalytically competent conforma-  
746 tion or not. Hence, any alteration of the dynamics of the A do-  
747 main will coactively alter SERCA catalysis.

748 The striking similarity of the Ca<sup>2+</sup>-binding sites and their  
749 immediate surroundings in the WT and E340A crystal structures  
750 indicates that the differences in the Ca<sup>2+</sup> binding and dissocia-  
751 tion behavior are not caused by a perturbation of the geometry at  
752 the Ca<sup>2+</sup> sites in the TM domain but rather by altered kinetics of  
753 the actual binding process. One of the few structural differences  
754 in the transmembrane region is a small inward shift of the tip of  
755 M3 in E340A. Interestingly, a mutation of Leu249 at the tip of



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M3 to alanine leads to increased rates of both Ca<sup>2+</sup> binding and dissociation (26). Furthermore, in the SERCA Ca<sup>2+</sup>-binding site mutant E309Q, which is defective in both Ca<sup>2+</sup> binding and occlusion, the tip of M3 is outward shifted (20), highlighting the immediate influence of small changes to the spacing between P1 and M3 for the ion-binding dynamics in SERCA.

The loss of the electrostatic interaction between Glu340 and Arg822 in L6-7 appears to have only a small structural effect at first glance. However, prior studies have implicated an important role of L6-7 in SERCA catalysis (16, 17, 26). For example, the D813A/D818A mutation leads to a dramatic loss of Ca<sup>2+</sup> affinity (16). There are also conspicuous similarities between the behavior of the mutant E340A and some L6-7 mutants, including R822A: a slowing of the Ca<sup>2+</sup>-binding transition from E2 and of Ca<sup>2+</sup> dissociation (17). The loop L6-7 has moreover been suggested to be part of an ion access pathway in both SERCA and the Na<sup>+</sup>/K<sup>+</sup>-ATPase (12, 13, 15, 27), and/or to contribute to the coordination of events between the cytosolic and transmembrane domains (14, 16, 17). A very recent study on SERCA2b has found that small, local conformational changes in the area around the L6-7-P1 interaction—in this case induced by long-range effects from changes to the luminal region of the M domain—are accompanied by changes to the entire headpiece arrangement (28). In light of our finding that the E340A mutation allows for an irreversible swinging out of Arg822, which is correlated to a concomitant inward movement of M3, the reverse conclusion must be that the interaction network between Arg822, Glu340, and Leu249 is critical for maintaining a proper architecture of the Ca<sup>2+</sup> entry and exit pathways.

Finally the “rigidification” of the M1 kink which we see in our MD simulations is also in line with a shift of E340A toward a slightly more occluded state than WT. The kink at Leu60 in M1 permits the conserved residue Phe57 to engage in a hydrophobic cluster that shields off the Ca<sup>2+</sup>-binding sites as a prerequisite for ion occlusion. Notably, a straight M1 helix has been seen in nucleotide-free inward-open E1 structures (6, 20), and is associated with an unoccluded Ca<sub>2</sub>E1 state of SERCA. Most notably, when probing the reported interrelation of ion occlusion and phosphorylation (7), we find the geometry of the phosphorylation site to be more narrowly clustered around catalytically competent values.

**How Does the Structural Stabilization of the Occluded State Manifest Itself in Functional Terms?** Our tryptophan fluorescence data obtained with the detergent solubilized yeast enzyme used for crystallization show that the E340A mutation caused a pronounced slowing of the reaction sequence H<sub>n</sub>E2 → E1 → Ca<sub>2</sub>E1 consisting of proton release, Ca<sup>2+</sup> binding, and the associated conformational changes. This effect explains the reduced ATPase activity of the E340A mutant relative to WT, and the slowing agrees with the change in the dynamics of Ca<sup>2+</sup> gating suggested from the structural changes in relation to the Ca<sup>2+</sup>-binding sites. It should be noted that yeast-expressed SERCA has been demonstrated to be as active and stable as SERCA extracted from the SR, both in membranes and in detergent, when measured under the appropriate experimental conditions, making the comparison straightforward and reliable (29–31).

The E340A mutant has previously been analyzed functionally following expression in COS cells (17), showing effects qualitatively similar to the present results. In accordance with our finding of a more occluded E340A structure, the previous functional analysis in COS cell microsomes (17) showed that the E340A mutation not only slows down Ca<sup>2+</sup> binding, but also the reverse reaction Ca<sub>2</sub>E1 → E1 (Ca<sup>2+</sup> dissociation from the high-affinity sites toward the cytosol). The mutation was moreover found not to affect SERCA’s apparent vanadate affinity (which is typically affected by E2 to E1 shifts), suggesting that the slowing of H<sub>n</sub>E2 → E1 → Ca<sub>2</sub>E1 is due to inhibition of the latter

part of this reaction sequence (i.e., actual Ca<sup>2+</sup> binding). The mutation also had no significant effect on the remaining partial reaction steps of the cycle including the phosphorylation (Ca<sub>2</sub>E1 → Ca<sub>2</sub>E1P), the rate-limiting Ca<sub>2</sub>E1P → E2P transition, and the dephosphorylation E2P → E2 (17). Hence, the kinetic constraints observed for the E340A mutant are consistent with the hypothesis that perturbation of the hydrogen-bonding network around Glu340 causes a slowing, in both directions, of the structural changes associated with the binding of the second Ca<sup>2+</sup> ion to the CaE1 state and the subsequent occlusion step.

In conclusion, our data suggest that the E340A mutation stabilizes a conformational state closer to occlusion and phosphorylation than the WT structure. This slows down Ca<sup>2+</sup> entry and release but favors phosphorylation by ATP. Hence, the role of Glu340 in WT SERCA is to maintain the necessary structural flexibility for rapid Ca<sup>2+</sup> exchange at the binding sites and to relay this flexibility to the site of phosphorylation.

This key function provides an explanation for the evolutionary acquisition and strict conservation of this glutamate residue throughout the entire P-type ATPase family, irrespective of their substrate specificity.

## Materials and Methods

**Chemicals.** Octaethylene glycol monododecyl ether (C<sub>12</sub>E<sub>8</sub>) was purchased from Nikkol Chemical (BL-85Y), and n-dodecyl β-D-maltopyranoside (DDM) was from Anatrace (D310). Streptavidin sepharose high-performance resin was provided by GE Healthcare (17-5113-01). Thapsigargin (TG stock solution was prepared at 1 mg/mL in DMSO, i.e., about 1.5 mM) was from VWR International (586005). All other chemical products were purchased from Sigma. Sequence for SERCA1a heterologous expression was from rabbit.

**Cloning, Expression, and Purification.** SERCA1a E340A cDNA (SERCA1a[E340A]) was recovered from pMT2 vector initially used for expression in COS cells (17) and cloned into the yeast expression plasmid pYeDP60, with a C-terminal biotin acceptor domain (32), resulting in pYeDP60\_SERCA1a[E340A]-Bad. The construct was checked by sequencing (Eurofins MWG). The *Saccharomyces cerevisiae* yeast strain W303.1b/Gal4 (α, leu2, his3, trp1::TRP1-GAL10-GAL4, ura3, ade2-1, canr, cir) was the same as previously described (32). Transformation was performed according to the lithium acetate/single-stranded carrier DNA/PEG method (33).

Expression and purification of the E340A mutant SERCA1a was done as previously described for the WT enzyme (32). Briefly, after overexpression in the yeast *S. cerevisiae*, light membranes were prepared and solubilized by DDM for subsequent purification by streptavidin affinity chromatography (30, 32, 34). Purified WT SERCA1a was recovered in a buffer containing 50 mM Mops-Tris pH 7, 100 mM KCl, 5 mM MgCl<sub>2</sub>, 2.1 mM CaCl<sub>2</sub>, 40% glycerol (vol/vol), and 0.5 mg/mL DDM, together with some thrombin remaining from the elution procedure. The protein concentration in the purified fraction was typically in the 0.05- to 0.15-mg/mL range, depending on the batch. Further purification for crystallization trials was carried out as described previously for the SERCA E309Q mutant (20), involving exchange of DDM with C<sub>12</sub>E<sub>8</sub> and relipidation of the purified SERCA with DOPC.

**ATPase Activity Measurements.** Steady-state ATPase activity measurements were performed using an enzyme-coupled assay by measuring the rate of NADH oxidation (followed by absorbance at 340 nm) in the presence of 0.02 mg/mL lactate dehydrogenase, 0.04 mg/mL pyruvate kinase, 1 mM phosphoenolpyruvate, 0.3 to 0.4 mM NADH, 5 mM MgATP (31) and in the additional presence of 1 mg/mL C<sub>12</sub>E<sub>8</sub> and 50 μM free Ca<sup>2+</sup> to limit time-dependent inactivation of the SERCA1a (22), at 30 °C and pH 7.5.

**Tryptophan Fluorescence.** Intrinsic fluorescence was measured with a Fluorolog spectrofluorimeter (Horiba), in a temperature-regulated and continuously stirred 2-mL quartz cuvette. Excitation and emission wavelengths were set at 295 and 320 nm, with bandwidth of 2 and 5 nm, respectively. Integration time for recording of the signal was 2 s. SERCA1a intrinsic fluorescence changes were measured with purified WT or purified E340A mutant suspended at a protein concentration of about 10 μg/mL in a buffer containing 50 mM Mes-Tris pH 6.5, 5 mM MgCl<sub>2</sub>, 20% glycerol (vol/vol) and 2 mg/mL DDM, at 20 °C. Initial Ca<sup>2+</sup> concentration was adjusted to 105 μM on top of the contaminating Ca<sup>2+</sup> (3 to 5 μM) already present in the buffer. This was followed by the addition of 5 mM EGTA (EG), reducing [Ca<sup>2+</sup>]<sub>free</sub> to

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about 100 nM. An extra addition of 12.5 mM CaCl<sub>2</sub> (Ca) allowed to reach a final [Ca<sup>2+</sup>]<sub>free</sub> of about 7.6 mM to recover the initial level of fluorescence. Data were fitted with the GraphPad Prism program with an exponential two-phase association law on the first 60 s of the raw data following Ca<sup>2+</sup> addition.

**Crystallization.** Purified SERCA E340A at a concentration of 8 to 10 mg/mL was supplemented with Ca<sup>2+</sup>, Mg<sup>2+</sup>, and AMPPCP to 10, 3, and 1 mM, respectively, and crystallization trials were carried out using the vapor diffusion technique. The crystallization drops were prepared as 1- $\mu$ L sitting drops, with each drop containing a 1:1 mixture of the concentrated, DOPC-relipidated, and C<sub>12</sub>E<sub>8</sub>-solubilized Ca<sup>2+</sup>-ATPase solution and the reservoir solution. Diffraction-quality crystals were obtained with reservoir solutions containing 200 mM lithium acetate, 18 to 22% PEG6K, 6 to 9% glycerol, and 3% tert-butanol. The E340A crystals varied markedly in appearance from crystals obtained with WT under similar conditions. Hence, the E340A crystals displayed a rectangular stick morphology, whereas WT crystals were diamond shaped (compare figure S2 in ref. 20).

**Data Collection, Processing, and Refinement.** Crystals were flash frozen and tested at the microfocus beam line ID23-2 at ESRF. The best dataset diffracted to 3.2- $\text{Å}$  resolution. In accordance with the variant crystal forms, also the space group and unit cell parameters were different from WT SERCA. Hence, WT crystallizes in space group C2 with unit cell parameters (163  $\text{Å}$ , 76  $\text{Å}$ , and 151  $\text{Å}$ ; 90°, 109°, and 90°), and E340A in space group P2<sub>1</sub>2<sub>1</sub>2 with unit cell parameters (126  $\text{Å}$ , 232  $\text{Å}$ , and 50  $\text{Å}$ ; 90°, 90°, and 90°), which are hitherto unknown parameters for SERCA. Phasing was done by molecular replacement with the Ca<sub>2</sub>E1-AMPPCP form (1T55). Initial rigid-body refinement with each domain as a single rigid body was followed by all-atom and TLS refinement in PHENIX (35).

Molecular graphics figures were prepared with PyMOL (36) and morphs with Morphinator (37).

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**Molecular Dynamics Simulations.** Simulations were built using the coordinates from PDB 3N8G (WT) and PDB 6RB2 (E340A). The protein atoms were described using the CHARMM36 force field (38), and built into a lipid bilayer composed of POPC molecules and solvated with TIP3P water and Na<sup>+</sup> and Cl<sup>-</sup> to 150 mM in a 120 × 120 × 180  $\text{Å}$  box. Systems were built using CHARMM-GUI (39, 40). Generation of the in silico mutant E340A<sub>is</sub> was done with CHARMM-GUI.

The systems were energy minimized using the steepest descent method, then equilibrated with positional restraints on heavy atoms for 100 ps in the NPT ensemble at 310 K with the V-rescale thermostat and semiisotropic Parrinello-Rahman pressure coupling (41, 42). Production simulations were run in triplicate without positional restraints, with 2-fs time steps for a minimum of 200 ns.

All simulations were run using GROMACS 2019 (43). Simulations were analyzed using GROMACS tools and images were made using VMD (44). Graphs were plotted using Matplotlib (45).

**Data Availability.** X-ray crystallography data have been deposited in the Protein Data Bank under accession no. 6RB2.

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