

Sarcolipin is a conformation-dependent regulator of the sarcoplasmic reticulum calcium pump SERCA

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Abstract

Muscle relaxation is enabled by the sarco-endoplasmic reticulum Ca^{2+} -ATPase (SERCA), which removes calcium from the cytosol and returns it to the lumen of the sarcoplasmic reticulum. During transport, SERCA adopts a variety of conformational states which differ in their structure and affinity for substrates. The transition between these states is mediated by calcium and ATP binding and the formation of an aspartyl-phosphate intermediate, which enables the transport of calcium across the membrane. SERCA function is highly regulated because of the importance of calcium in processes such as muscle contraction-relaxation. A family of tissue-specific transmembrane regulatory subunits interact with SERCA, exemplified by sarcolipin (SLN) in skeletal muscle and phospholamban (PLN) in cardiac muscle. SLN and PLN are known to alter the apparent calcium affinity and maximal activity of SERC. In the present study, we investigated SLN inhibition of SERCA under conditions that varied the substrate-dependent conformational state of SERCA. Measuring both calcium-dependent ATP hydrolysis and charge translocation, we found that SLN inhibition was dependent on the initial state of SERCA. Under substrate conditions that poised SERCA in the calcium-free E2 state, SLN was more inhibitory and impacted both the maximal activity and apparent calcium affinity of SERCA. In contrast, SLN inhibition was reduced under pre-incubation conditions that favored the calcium-bound E1 state of SERCA. We conclude that SLN is capable of distinct modes of interaction with SERCA depending on the conformational state, and that the mode of interaction exhibits conformational memory in that the initial state persists during steady-state turnover of SERCA.

Introduction

The sarco-endoplasmic reticulum calcium pump (SERCA) maintains the low resting-state calcium levels in the cytosol that are essential for many physiological signaling processes. A well-characterized example is the relaxation phase of the contraction-relaxation cycle in muscle. Following calcium release and muscle contraction, SERCA uses the energy of ATP to transport calcium ions into the sarcoplasmic reticulum (SR) lumen to achieve muscle relaxation. The calcium transport mechanism of SERCA has been extensively studied (e.g. (Sorensen, Moller & Nissen, 2004; Toyoshima & Mizutani, 2004; Toyoshima et al., 2000; Toyoshima & Nomura, 2002)). SERCA progresses through a series of intermediates that alternate between high-affinity (E1) and low-affinity (E2) states for calcium. Calcium transport requires the binding of ATP ($\text{E2} \cdot \text{ATP}$) and the cooperative binding of two calcium ions ($\text{E1} \cdot \text{ATP} \cdot \text{Ca}$) to form a catalytically competent conformation of SERCA. The energy of ATP is captured in the form of a phospho-enzyme intermediate ($\text{E1} \sim \text{P} \cdot \text{Ca}$) and released through a conformational change that facilitates calcium translocation ($\text{E2} \cdot \text{P}$). Following calcium release into the SR lumen, the phosphate is hydrolyzed and released, returning SERCA to a low-affinity state (E2). From this point, SERCA is poised to begin the transport cycle anew. It is important to note that the presence or absence of substrates can be used to poise SERCA in different conformational states (e.g. E2, $\text{E2} \cdot \text{ATP}$, $\text{E1} \cdot \text{Ca}$) and that the crystal structures of these conformational states have been determined.

SERCA activity is regulated by several tissue-specific transmembrane peptides called the regulins, which include phospholamban (PLN) and dwarf open reading frame (DWORF) in cardiac muscle and sarcolipin (SLN) and myoregulin (MLN) in skeletal muscle (Anderson et al., 2015; Anderson et al., 2016; Nelson et al., 2016). SLN is a 31-residue, α -helical integral membrane protein, consisting of a short cytoplasmic domain that contains a regulatory phosphorylation site, a transmembrane domain, and a highly conserved luminal tail. Like PLN, SLN has been reported to reduce the apparent calcium affinity of SERCA (Gorski et al., 2013; Odermatt et al., 1998) by interacting with SERCA in an inhibitory groove formed by transmembrane helices M2, M6, and M9. Crystal structures revealed binding of SLN to the inhibitory groove of SERCA, which stabilized a calcium-free E1-like intermediate of SERCA that appears poised between the calcium-free E2 and calcium-bound E1 states (Toyoshima et al., 2013; Winther et al., 2013). The transmembrane portion of SLN mediates this interaction; however, SLN regulation is strongly dependent on the unique and highly conserved luminal Arg²⁷-Ser-Tyr-Gln-Tyr³¹ domain of SLN. While SLN was thought to be functionally homologous to PLN, the mechanism by which SLN regulates SERCA is distinct (Gorski et al., 2013).

In addition to SLN's role in regulating calcium homeostasis in skeletal muscle, it has been suggested that SLN also plays a role in thermogenesis and energy metabolism (Bal et al., 2012). SLN was reported to uncouple SERCA ATPase activity from calcium transport by promoting the backflow of calcium into the cytosol during ATP hydrolysis. A mechanism for SLN-mediated uncoupling through the interaction of SLN's N-terminus with SERCA has been put forward (Autry, Thomas & Espinoza-Fonseca, 2016). Given that skeletal muscle accounts for approximately 40% of adult body mass, the energy lost from SERCA uncoupling would make a significant contribution to heat generation and thermogenesis. This unique physiological role of SLN suggests a unique SERCA regulatory mechanism. The crystal structures of SERCA in the presence of SLN revealed an unexpected conformation of SERCA – an E1-like, calcium-free state that appears poised between the E2 and E1 states (Toyoshima et al., 2013; Winther et al., 2013). These structures are very similar to the crystal structure of SERCA-PLN complex (Akin et al., 2013). While these structures provide a plausible mechanism for SERCA inhibition, they yield little insight into the functional differences between PLN and SLN and the potential uncoupling behavior of SLN.

In our previous work, we reported that the conformational state of SERCA influenced the interaction with PLN, suggesting that multiple conformational states of the SERCA-PLN complex were possible. Moreover, once a particular complex was engaged, the functional effect persisted through multiple SERCA turnover events of the catalytic cycle, and we termed this process “conformational memory” (Smeazzetto et al., 2017). Herein, we performed a similar study of SERCA regulation by SLN. Through the use of particular substrates, SERCA was poised in the calcium-free E2 state (pre-incubation in the absence of substrates), the calcium-bound E1 state (pre-incubation with calcium), ATP-bound E2 state (pre-incubation with ATP), and a physiological resting state (pre-incubation with ATP & resting calcium). Proteoliposomes containing SERCA in the absence and presence of SLN were pre-incubated under these different substrate conditions. Following pre-incubation, calcium-dependent ATPase activity and charge translocation were measured. SLN decreased the maximal activity of SERCA under all substrate conditions tested. In contrast, SLN altered the apparent calcium affinity of SERCA only under one

condition, pre-incubation in the absence of substrates. There was no evidence of SERCA uncoupling in the SERCA-SLN proteoliposomes. Nonetheless, the results indicated two possible SERCA-SLN regulatory complexes that depend on the conformation of SERCA. Once formed, these conformations are stable through steady-state turnover of SERCA. While both SERCA-PLN (Smeazzetto et al., 2017) and SERCA-SLN complexes exhibited conformational memory, the results support the concept that SLN is a distinct regulatory subunit of SERCA.

Results

The co-reconstituted proteoliposomes used in this study approximate the SR membrane in protein density, orientation, and lipid composition (Ceholski, Trieber & Young, 2012; Gorski et al., 2013; Trieber, Afara & Young, 2009; Trieber et al., 2005; Young et al., 1997). The proteoliposomes are unilamellar, SERCA molecules are oriented in the membrane with the cytoplasmic domains accessible to substrates on the outer surface (Young et al., 1997), and the lipid-to-protein ratio is ~ 120 to 1 (Trieber et al., 2005). These proteoliposome characteristics mimic native skeletal muscle SR membranes, and they allow precise measurement of steady-state calcium-dependent ATPase activity (e.g. (Ceholski et al., 2012; Gorski et al., 2013; Trieber et al., 2009)) and pre-steady state calcium transport (Smeazzetto et al., 2017).

SERCA ATPase activity and calcium transport

The proteoliposomes containing SERCA in the absence and presence of SLN were pre-incubated under four different substrate conditions: (i) pre-incubation in the absence of substrates (substrate jump condition), (ii) pre-incubation with calcium (ATP jump condition), (iii) pre-incubation with ATP (calcium jump condition), and (iv) pre-incubation under physiological conditions (80 nM calcium, 4 mM ATP; physiological calcium jump condition). Following pre-incubation under these substrate conditions, we measured calcium-dependent ATP hydrolysis and calcium transport by SERCA (Fig. 1B, C). ATP hydrolysis was measured as a function of calcium concentration (0.1–10 μ M free calcium). The data were fit to the Hill equation and the kinetic parameters maximal activity ($V_{\max}$) and apparent calcium affinity (K_{Ca}) were determined from the resultant curve fits (Fig. 2). The kinetic parameters obtained under various starting conditions are summarized in Table 1.

Charge translocation by SERCA was also measured as a function of calcium concentration using a surface electrogenic even reader (SEER; SURFE²R N1, Nanion Technologies GmbH). Proteoliposomes containing SERCA in the absence and presence of SLN were adsorbed to a solid supported membrane (SSM) on the surface of a gold electrode as previously described (Smeazzetto et al., 2017; Tadini-Buoninsegni, 2020) (Fig. 1C). The current transients observed following pre-incubation and initiation of the reaction were analyzed for peak current (nA) and total charge translocation (nC). The data were plotted as peak current (Fig. 3) and total charge translocation (Fig. 4) versus calcium concentration. These data were then fit to the Hill equation and the kinetic parameters maximal activity ($V_{\max}$) and apparent calcium affinity (K_{Ca}) were determined from the resultant curve fits (Table 1). Peak current

measurements resulted in statistically significant differences in the maximal activity ($V_{\max}$) of SERCA, and charge translocation measurements resulted in statistically significant differences in the apparent calcium affinity (K_{Ca}) of SERCA. Overall, the trends observed with ATPase activity measurements (Fig. 2) were consistent with the charge translocation measurements (Figs. 3 & 4).

Table 1

Kinetic parameters for SERCA and SERCA-SLN proteoliposomes derived from steady-state ATPase activity measurements and pre-steady state charge transfer measurements.

Starting conditions	Kinetic parameters		
	K_{Ca} (μM)	V_{max} ($\mu moles/min/mg$) (nA) (nC)	n_H
Substrate jump			
Pre-incubate in the absence of substrates; start reaction with 4 mM ATP and 0.1 to 10 μM Ca^{2+}			
SERCA ^{ATPase-activity}	0.42 ± 0.02	4.1 ± 0.1	1.5 ± 0.1
SERCA-SLN ^{ATPase-activity}	0.97 ± 0.04	2.8 ± 0.1	1.6 ± 0.1
SERCA ^{Peak-current}	1.00 ± 0.10	3.9 ± 0.3	1.5 ± 0.1
SERCA-SLN ^{Peak-current}	1.30 ± 0.10	2.1 ± 0.3	1.3 ± 0.1
SERCA ^{Charge-translocation}	0.53 ± 0.04	0.80 ± 0.05	1.6 ± 0.1
SERCA-SLN ^{Charge-translocation}	0.94 ± 0.09	0.70 ± 0.09	1.5 ± 0.1
ATP jump			
Pre-incubate with 0.1 to 10 μM Ca^{2+} ; start reaction with 4 mM ATP			
SERCA ^{ATPase-activity}	0.27 ± 0.02	3.0 ± 0.1	1.1 ± 0.1
SERCA-SLN ^{ATPase-activity}	0.45 ± 0.07	2.5 ± 0.1	1.0 ± 0.1
SERCA ^{Peak-current}	0.83 ± 0.03	4.8 ± 0.5	2.0 ± 0.1
SERCA-SLN ^{Peak-current}	1.04 ± 0.06	3.7 ± 0.7	1.6 ± 0.1
SERCA ^{Charge-translocation}	0.48 ± 0.02	0.90 ± 0.10	1.7 ± 0.1
SERCA-SLN ^{Charge-translocation}	0.61 ± 0.03	0.70 ± 0.10	1.6 ± 0.1
Calcium jump			
Pre-incubate with 4 mM ATP; start reaction with 0.1 to 10 μM Ca^{2+}			
SERCA ^{ATPase-activity}	0.35 ± 0.02	3.9 ± 0.1	1.5 ± 0.1

Starting conditions	Kinetic parameters		
	K_{Ca} (μM)	V_{max} ($\mu moles/min/mg$) (nA) (nC)	n_H
Substrate jump			
Pre-incubate in the absence of substrates; start reaction with 4 mM ATP and 0.1 to 10 μM Ca^{2+}			
SERCA-SLN ^{ATPase-activity}	0.37 $\pm$ 0.03	2.3 $\pm$ 0.1	1.6 $\pm$ 0.1
SERCA ^{Peak-current}	1.18 $\pm$ 0.05	3.8 $\pm$ 0.3	1.3 $\pm$ 0.1
SERCA-SLN ^{Peak-current}	1.40 $\pm$ 0.10	1.6 $\pm$ 0.3	1.1 $\pm$ 0.1
SERCA ^{Charge-translocation}	0.65 $\pm$ 0.02	0.84 $\pm$ 0.06	1.5 $\pm$ 0.1
SERCA-SLN ^{Charge-translocation}	0.98 $\pm$ 0.08	0.46 $\pm$ 0.06	1.3 $\pm$ 0.1
Physiological calcium jump			
Pre-incubate with 4 mM ATP and 80 nM Ca^{2+} ; start reaction with remaining Ca^{2+}			
SERCA ^{ATPase-activity}	0.37 $\pm$ 0.01	3.5 $\pm$ 0.1	1.5 $\pm$ 0.1
SERCA-SLN ^{ATPase-activity}	0.46 $\pm$ 0.03	3.0 $\pm$ 0.1	2.0 $\pm$ 0.2
SERCA ^{Peak-current}	0.86 $\pm$ 0.03	3.5 $\pm$ 0.3	1.5 $\pm$ 0.1
SERCA-SLN ^{Peak-current}	0.91 $\pm$ 0.06	2.9 $\pm$ 0.4	1.6 $\pm$ 0.1
SERCA ^{Charge-translocation}	0.47 $\pm$ 0.03	0.61 $\pm$ 0.06	1.7 $\pm$ 0.1
SERCA-SLN ^{Charge-translocation}	0.59 $\pm$ 0.03	0.55 $\pm$ 0.07	1.7 $\pm$ 0.1

Values shaded in grey indicated SERCA and SERCA-SLN comparisons that were statistically significant ($p > 0.01$).

Substrate jump condition

Proteoliposomes containing SERCA in the absence and presence of SLN were pre-incubated in the absence of substrates. This pre-incubation condition favors the E2 state of SERCA, which is a low-affinity state for calcium binding. The substrate jump condition involved the simultaneous addition of calcium and ATP to initiate ATPase activity (Fig. 2 & Table 1). As previously observed (Gorski et al., 2013), SLN

altered both the maximal activity ($V_{\max}$) and apparent calcium affinity (K_{Ca}) of SERCA. Proteoliposomes containing SERCA alone yielded a $V_{\max}$ of $4.1 \pm 0.1 \mu\text{mol}/\text{min}/\text{mg}$, while proteoliposomes containing SERCA and SLN yielded a $V_{\max}$ of $2.8 \pm 0.1 \mu\text{mol}/\text{min}/\text{mg}$. Similarly, proteoliposomes containing SERCA alone yielded a K_{Ca} value of $0.42 \pm 0.02 \mu\text{M}$ calcium, compared to $0.97 \pm 0.04 \mu\text{M}$ calcium for proteoliposomes containing SERCA and SLN. The increase in K_{Ca} indicates a decrease in the apparent calcium affinity of SERCA in the presence of SLN, which is a characteristic feature of SERCA inhibition. Thus, SLN decreased both the maximal activity and apparent calcium affinity of SERCA under these conditions.

The substrate jump condition was next used to activate calcium transport by SERCA-containing proteoliposomes adsorbed to an SSM. Peak currents (nA) and total charge translocation (nC) were measured for current traces over a range of calcium concentrations. Proteoliposomes containing SERCA alone yielded a $V_{\max}$ of $3.9 \pm 0.3 \text{ nA}$, while proteoliposomes containing SERCA and SLN yielded a $V_{\max}$ of $2.1 \pm 0.3 \text{ nA}$ (Fig. 3). Similarly, the K_{Ca} values were found to be 0.53 ± 0.04 and $0.94 \pm 0.09 \mu\text{M}$ calcium for SERCA in the absence and presence of SLN, respectively (Fig. 4). Thus, the $V_{\max}$ and K_{Ca} values determined under these conditions using SEER technology were comparable to the values obtained from the calcium-dependent ATPase activity measurements (Table 1).

ATP jump condition

Proteoliposomes containing SERCA in the absence and presence of SLN were next pre-incubated with calcium concentrations ranging from 0.1 to 10 μM before initiating SERCA ATPase activity (ATP jump; Fig. 2 & Table 1). This pre-incubation condition favors the E1 state of SERCA, which is a high-affinity state for calcium binding. Compared to the substrate jump condition, the overall impact of SLN on SERCA activity was significantly reduced. The maximal activity of SERCA was lower with a modest depression in the presence of SLN ($V_{\max}$ values of $3.0 \pm 0.1 \mu\text{mol}/\text{min}/\text{mg}$ for SERCA alone compared to $2.5 \pm 0.1 \mu\text{mol}/\text{min}/\text{mg}$ for SERCA-SLN). In addition, the effect of SLN on the apparent calcium affinity (K_{Ca}) of SERCA was more modest, and the observed K_{Ca} values were shifted to higher affinity ($0.27 \pm 0.02 \mu\text{M}$ calcium for SERCA alone compared to $0.45 \pm 0.07 \mu\text{M}$ calcium for SERCA-SLN). Consequently, pre-incubation of SERCA proteoliposomes with calcium resulted in a higher-affinity, slower pump, which manifested as an increase in the apparent calcium affinity of SERCA (decreased value for K_{Ca}) and a decrease in the maximal activity of SERCA. SLN inhibition of SERCA was still evident, but considerably diminished by pre-incubation with calcium.

The ATP jump condition was used to activate calcium transport by SERCA-containing proteoliposomes adsorbed to an SSM using SEER technology. The measured charge translocation compared well with the ATPase activity measurements. Proteoliposomes containing SERCA alone yielded a $V_{\max}$ of $4.8 \pm 0.5 \text{ nA}$, while proteoliposomes containing SERCA and SLN yielded a $V_{\max}$ of $3.7 \pm 0.7 \text{ nA}$ (Fig. 3). While there was an apparent reduction in $V_{\max}$, these values were not statistically different. K_{Ca} was found to be $0.48 \pm$

0.02 and 0.61 ± 0.03 μM calcium for SERCA in the absence and presence of SLN, respectively (Fig. 4). Thus, inhibition of SERCA calcium transport by SLN was also diminished following pre-incubation of SERCA in the presence of calcium (Table 1).

Calcium jump condition

The proteoliposomes containing SERCA in the absence and presence of SLN were pre-incubated with 4 mM ATP (absence of calcium), and SERCA ATPase activity was initiated by the addition of calcium (calcium jump condition; **Fig. 2** & Table 1). This pre-incubation condition favors the ATP-bound, calcium-free E2 state of SERCA. The V_{max} values were determined to be 3.9 ± 0.1 $\mu\text{mol}/\text{min}/\text{mg}$ for SERCA alone compared to 2.3 ± 0.1 $\mu\text{mol}/\text{min}/\text{mg}$ for SERCA in the presence of SLN. The V_{max} values observed for pre-incubation with ATP were comparable to the substrate jump condition. In contrast, the effect of SLN on the apparent calcium affinity of SERCA was not observed with ATP pre-incubation (K_{Ca} of 0.35 ± 0.02 μM calcium for SERCA alone compared to 0.37 ± 0.03 μM calcium for SERCA-SLN). These measurements indicated that ATP pre-incubation preserved the effect of SLN on the V_{max} of SERCA. However, there was no change in the apparent calcium affinity of SERCA, indicating that this aspect of SLN inhibition was lost in the E2·ATP state of SERCA.

The calcium jump condition was next used to activate calcium transport by SERCA-containing proteoliposomes. SERCA alone yielded a V_{max} of 3.8 ± 0.3 nA, while proteoliposomes containing SERCA and SLN yielded a V_{max} of 1.6 ± 0.3 nA (Fig. 3). This was the largest decrease in SERCA V_{max} observed in the presence of SLN. K_{Ca} was found to be 0.65 ± 0.02 and 0.98 ± 0.08 μM calcium for SERCA in the absence and presence of SLN, respectively (Fig. 4). Thus, for the ATPase activity and charge translocation measurements, the primary effect of SLN on SERCA under ATP pre-incubation conditions was depression of SERCA's V_{max} (Table 1).

Physiological calcium jump

The different pre-incubation conditions described above poised SERCA in well-known conformational states – E2, E1, and E2·ATP, respectively. However, these pre-incubation conditions differ from the physiological resting state (mM ATP and nM calcium). To mimic the physiological resting state, SERCA and SERCA-SLN proteoliposomes were pre-incubated with 4 mM ATP and 80 nM calcium, near the resting level of cytosolic calcium that myocytes experience. The ATPase activity of SERCA and SERCA-SLN proteoliposomes under these conditions was determined (physiological calcium jump condition; **Fig. 2** & Table 1). The observed V_{max} depression remained between SERCA and SERCA-SLN proteoliposomes, though it was reduced (V_{max} values of 3.5 ± 0.1 and 3.0 ± 0.1 $\mu\text{mol}/\text{min}/\text{mg}$, respectively). The K_{Ca} shift observed between SERCA and SERCA-SLN proteoliposomes was also reduced (K_{Ca} values of 0.37 ± 0.01 and 0.46 ± 0.03 μM calcium, respectively). This small change in the apparent calcium affinity of SERCA was akin to the weak SLN inhibition observed for the calcium pre-

incubation (ATP jump) and ATP pre-incubation (calcium jump) conditions. These trends in the presence of SLN – $V_{\max}$ depression and weak SERCA inhibition – agreed with previous observations for SERCA in the presence of PLN (Smeazzetto et al., 2017).

The physiological calcium jump condition was next used to activate calcium transport by SERCA-containing proteoliposomes. Similar to the ATPase activity measurements for this pre-incubation condition, the effects of SLN on the $V_{\max}$ (3.5 ± 0.3 and 2.9 ± 0.4 nA respectively; **Fig. 3**) and K_{Ca} (0.47 ± 0.03 and 0.59 ± 0.03 μ M calcium, respectively; **Fig. 4**) of SERCA were reduced. Thus, the primary effect of SLN on SERCA under physiological pre-incubation conditions was depression of SERCA's $V_{\max}$ (Table 1). These data are consistent with the ATPase activity of SERCA in the absence and presence of SLN (Fig. 2 & Table 1).

Summary of Results

SLN consistently decreased the maximal activity ($V_{\max}$) of SERCA under all conditions tested, using both ATPase activity and charge translocation as measures of SERCA function. This effect was more pronounced under calcium-free pre-incubation conditions that promoted the E2 state of SERCA (Figs. 2 & 3; substrate jump & calcium jump conditions) and diminished under calcium pre-incubation conditions that promoted the E1 state of SERCA (Figs. 2 & 4; ATP jump & physiological calcium jump conditions). SLN altered the apparent calcium affinity of SERCA (K_{Ca}) under one condition (Table 1; substrate jump condition), though there were minor changes in K_{Ca} under most other conditions.

Comparative charge translocation by SERCA, SERCA-SLN, and SERCA-PLN

SLN has been reported to uncouple SERCA calcium transport from ATP hydrolysis as a means of heat production in non-shivering thermogenesis (Bal et al., 2012; Mall et al., 2006; Sahoo et al., 2015). This is considered a unique property of SLN regulation of SERCA in skeletal muscle, and not a property of PLN regulation of SERCA in cardiac muscle. To investigate the possible uncoupling of calcium transport from ATP hydrolysis, we compared the ATP hydrolysis rates ($V_{\max}$ in Table 2) with the total charge translocation for SERCA in the absence and presence of SLN (**Figure S1**). The underlying assumption was that uncoupling should manifest as a selective reduction in charge movement for SERCA in the presence of SLN. We also compared the total charge translocation by SERCA in the presence of SLN to SERCA in the presence of PLN (Fig. 5). No differences were observed in the kinetic parameters derived from total charge translocation (Table 2), suggesting that calcium transport is similar in SERCA-SLN and SERCA-PLN proteoliposomes. In addition, under most pre-incubation conditions, the maximal activity of SERCA in ATPase activity measurements ($V_{\max}$; Table 2) was higher for SERCA-PLN compared to SERCA-SLN. This suggests that coupling of ATP-hydrolysis to charge-translocation was higher for SERCA-PLN and lower for SERCA-SLN. Since we would expect a signature feature of SERCA uncoupling

to involve higher ATP hydrolysis and lower calcium transport, this is the opposite trend expected for uncoupling of SERCA by SLN.

While there was no evidence of SERCA uncoupling in the SERCA-SLN proteoliposomes, there were advantages and disadvantages in using this membrane reconstitution system. The SERCA-SLN proteoliposomes allowed detailed functional measurements and mechanistic insights in a highly purified system, though they lacked the complexity of the cell and additional factors that may modulate cellular calcium homeostasis. The combination of calcium-dependent ATPase activity and charge translocation measurements allowed comparison of both ATP hydrolysis and calcium transport by SERCA. However, the charge translocation measurements were not quantitative in terms of the number of transported calcium ions or the number of SERCA molecules contributing to the signal. To overcome this, we compared the relative charge translocation by SERCA, SERCA-SLN, and SERCA-PLN proteoliposomes.

Table 2

Kinetic parameters for SERCA-SLN and SERCA-PLN proteoliposomes derived from steady-state ATPase activity measurements and pre-steady state charge transfer measurements (total charge translocation).

Starting conditions	Kinetic parameters		
	K_{Ca}	V_{max}	n_H
	(μM)	(nC)	
Substrate jump			
Pre-incubate in the absence of substrates; start reaction with 4 mM ATP and 0.1 to 10 μM Ca^{2+}			
SERCA-SLN ^{ATPase-activity}	0.97 ± 0.04	2.8 ± 0.1	1.6 ± 0.1
SERCA-PLN ^{ATPase-activity}	0.89 ± 0.04	5.9 ± 0.1	2.0 ± 0.1
SERCA-SLN ^{Charge-translocation}	0.94 ± 0.09	0.70 ± 0.09	1.5 ± 0.1
SERCA-PLN ^{Charge-translocation}	1.02 ± 0.06	0.95 ± 0.02	1.3 ± 0.1
ATP jump			
Pre-incubate with 0.1 to 10 μM Ca^{2+} ; start reaction with 4 mM ATP			
SERCA-SLN ^{ATPase-activity}	0.45 ± 0.07	2.5 ± 0.1	1.0 ± 0.1
SERCA-PLN ^{ATPase-activity}	0.82 ± 0.01	3.9 ± 0.1	1.4 ± 0.1
SERCA-SLN ^{Charge-translocation}	0.61 ± 0.03	0.70 ± 0.10	1.6 ± 0.1
SERCA-PLN ^{Charge-translocation}	0.49 ± 0.03	0.62 ± 0.01	1.8 ± 0.2
Calcium jump			
Pre-incubate with 4 mM ATP; start reaction with 0.1 to 10 μM Ca^{2+}			
SERCA-SLN ^{ATPase-activity}	0.37 ± 0.03	2.3 ± 0.1	1.6 ± 0.1
SERCA-PLN ^{ATPase-activity}	0.84 ± 0.03	5.1 ± 0.1	1.3 ± 0.2
SERCA-SLN ^{Charge-translocation}	0.98 ± 0.08	0.46 ± 0.06	1.3 ± 0.1
SERCA-PLN ^{Charge-translocation}	0.96 ± 0.05	0.54 ± 0.01	1.7 ± 0.1
Physiological calcium jump			
Pre-incubate with 4 mM ATP and 80 nM Ca^{2+} ; start reaction with remaining Ca^{2+}			
SERCA-SLN ^{ATPase-activity}	0.46 ± 0.03	3.0 ± 0.1	2.0 ± 0.2

Starting conditions	Kinetic parameters		
	K_{Ca} (μM)	V_{max} (nC)	n_H
Substrate jump			
Pre-incubate in the absence of substrates; start reaction with 4 mM ATP and 0.1 to 10 μM Ca^{2+}			
SERCA-PLN ^{ATPase-activity}	0.65 ± 0.03	2.8 ± 0.1	3.0 ± 0.4
SERCA-SLN ^{Charge-translocation}	0.59 ± 0.03	0.55 ± 0.07	1.7 ± 0.1
SERCA-PLN ^{Charge-translocation}	0.66 ± 0.03	0.59 ± 0.01	2.1 ± 0.2

DISCUSSION

The pre-incubation conditions described above poised SERCA in two main conformational states: calcium-free E2 and calcium-bound E1. The calcium-free E2 state was promoted by the substrate jump condition (pre-incubation in the absence of substrates) and the calcium jump condition (pre-incubation in the presence of ATP). The calcium-bound E1 state was promoted by the ATP jump condition (pre-incubation in the presence of calcium) and the physiological calcium jump condition (pre-incubation in resting-state conditions). The E2 substrate jump condition is our typical method for initiating functional measurements of SERCA in the presence of SLN (Gorski et al., 2013). Under this condition, SERCA and SLN are pre-incubated in the absence of calcium and ATP, and the simultaneous addition of substrates initiated SERCA ATPase activity and calcium transport. As a result, the inhibition of SERCA by SLN manifested as a decrease in both the apparent calcium affinity and maximal activity of SERCA (Table 1). The decrease in the apparent calcium affinity of SERCA in the presence of SLN is consistent with previous observations by others (Odermatt et al., 1998); however, this effect of SLN was only observed for the substrate jump condition. The primary effect of SLN under all conditions was a depression of SERCA's maximal activity. This effect was more pronounced for the E2 states of SERCA and diminished for the E1 states of SERCA. Thus, the interaction of SLN with SERCA exhibited a clear preference for the calcium-free E2 states of SERCA.

The physiological jump condition pre-incubated SERCA under resting-state physiological conditions followed by a calcium jump to elicit SERCA ATPase activity and charge translocation. Under this pre-incubation condition, SERCA slowly progresses through the calcium transport cycle. The turnover number was approximately 11 per minute ($0.1 \mu moles/min/mg$ at $25^\circ C$), while SERCA's maximum turnover rate in proteoliposomes was ~ 450 per minute for SERCA alone ($4.1 \mu moles/min/mg$ at $25^\circ C$). The slow turnover of SERCA through the calcium transport cycle presumably allowed SLN to interact with any preferred conformational state. That said, SERCA may preferentially occupy states that precede the rate-limiting steps in the reaction cycle, particularly the $E1 \sim P$ to $E2P$ transition. Thus, the $E1 \sim P$

conformation of SERCA was a likely candidate for SLN interaction. Consistent with this notion, SERCA regulation by SLN was most similar to the calcium pre-incubation condition (ATP jump; Tables 1). We categorized this as a condition that promoted the calcium-bound E1 state of SERCA, which could explain the diminished effect of SLN on SERCA activity (Fig. 2, 3, & 4).

SERCA apparent calcium affinity and maximal activity

Under most conditions tested, only a slight shift in the apparent calcium affinity of SERCA was observed in the presence of SLN (Table 1). Surprisingly, the presence of substrates – calcium, ATP, or both – resulted in a weak effect of SLN on the apparent calcium affinity of SERCA. The exception was pre-incubation of SERCA in the absence of substrates, which resulted in a more inhibitory interaction of SLN and a significant shift in the apparent calcium affinity of SERCA (substrate jump condition; Table 1). These results suggested that the physical interaction between SERCA and SLN was sensitive to the conformational state of SERCA and that at least two distinct SERCA-SLN complexes exist. One complex caused a decrease in the maximal activity of SERCA. This was most pronounced for the complex of SLN with the calcium-free E2 states of SERCA, but clearly present under all SERCA conformations tested. A second complex resulted in a decrease in both the apparent calcium affinity and maximal activity of SERCA. These results were unique for the complex of SLN with the substrate-free conformation of SERCA (E2). Surprisingly, this was not observed for the complex of SLN with the calcium-free, ATP-bound conformation (E2·ATP) of SERCA. In a previous study of the SERCA-PLN complex (Smeazzetto et al., 2017), we reached a similar conclusion that the calcium-free E2·ATP state of SERCA had distinct structural and functional properties from the substrate-free E2 state of SERCA. This was a surprising finding given that the structures of SERCA in the E2 and E2·ATP states are similar (e.g. compare PDB 1IWO with 2C88 or 3FG0, respectively).

SERCA-SLN complexes

The results described above point toward two distinct conformational states for the SERCA-SLN complex. One conformation resulted in weaker inhibition of SERCA with minor changes in SERCA kinetics (pre-incubation with calcium in the ATP jump & physiological jump conditions). A second conformation resulted in potent inhibition of SERCA with large changes in SERCA kinetics (pre-incubation without calcium in the substrate jump & calcium jump conditions). We suggest that the weaker inhibitory state corresponds to the known crystal structure of the SERCA-SLN complex (Toyoshima et al., 2013; Winther et al., 2013), and the potent inhibitory state corresponds to the previous model of the SERCA-PLN complex (Seidel et al., 2008; Toyoshima et al., 2003).

One conformation must correspond to the crystal structure of the SERCA-SLN complex (Toyoshima et al., 2013; Winther et al., 2013), where SERCA is found in a calcium-free E1-like state (Fig. 6A,B). This calcium-free E1-like state is closer to the calcium-bound E1 state of SERCA than it is to the calcium-free E2 state. Nonetheless, SLN interacts with M2, M6, and M9 of SERCA to form an inhibitory complex. The nature of the complex suggests that SLN slows transmembrane domain movements that allow SERCA to progress through the calcium transport cycle. However, as SERCA progresses through the transport

cycle, SLN must alternately populate two interaction sites – one site that is inhibitory and a second site that is non-inhibitory and releases SERCA for progression through the transport cycle. In the transition from the calcium-free E1-like state to the calcium-bound E1 state of SERCA, the upward movement of transmembrane segment M2 is a major structural change. Side-chain interactions mediate the SLN complex with M2, and only a small change in the position of SLN is required to disengage. Since this complex is poised closer to the calcium-bound state of SERCA, it appears consistent with the weaker inhibitory effect of SLN under some SERCA pre-incubation conditions (ATP jump and physiological jump conditions, which pre-incubated SERCA in the presence of calcium).

We suggest that the second conformation corresponds to the calcium-free E2 state of SERCA, as predicted by the original molecular models for the SERCA-PLN complex (Gustavsson et al., 2013; Seidel et al., 2008; Toyoshima et al., 2003) (Fig. 6C,D). The two conformations, the E1-like state and the E2 state, differ in how SLN is oriented in the inhibitory groove of SERCA formed by transmembrane segments M2, M6, and M9 (Fig. 6E,F & inset). The binding groove is shallow (partially closed) in the E1-like state of SERCA and deeper (fully open) in the E2 state. This allows SLN to penetrate further into the inhibitory groove in E2, which results in a 5–7 Å lateral translation of SLN compared to the E1-like complex. The position of SLN in the E2 complex partially occupies the position M2 will take in the transition to the calcium-bound E1 state of SERCA. While SLN needs to relocate to allow the movement of M2, the repositioning of SLN required in the E2 complex is larger compared to the E1-like complex. Since this complex is poised further from the calcium-bound state of SERCA and SLN partially occupies the position M2 will take, it appears consistent with the potent inhibitory effect of SLN under some SERCA pre-incubation conditions. These include the substrate jump and calcium jump conditions, which pre-incubated SERCA in the absence of calcium and promoted the calcium-free E2 state. Thus, these conditions seemed consistent with an E2 SERCA-SLN complex that has a potent inhibitory effect on SERCA.

Conformational memory

The concept of conformational memory has been explored in protein conformational dynamics, inherently disordered proteins, amyloids, and chemical reactions (Csermely et al., 2020; Maeda, Taketsugu & Morokuma, 2012; Makarov, 2021; Schorner et al., 2015; Tian, Meng & Zhang, 2020). Several proteins exhibit conformational memory by retaining information about past conformations or interactions that influence future activity, e.g. the retention of a ligand bound state after ligand dissociation (Csermely et al., 2020; Tripathi, Uversky & Giuliani, 2025). SERCA is a molecular machine that undergoes a wide range of conformational states during the transport cycle, all of which require dynamics and flexibility in domain motions. The transitions between these different conformational states of SERCA have been assumed to include spontaneous conformational fluctuations in a stochastic process. However, the availability of substrates can influence the energy landscape for conformational fluctuations and shift from a stochastic reaction coordinate to one with reduced degrees of freedom between SERCA intermediates (conformational memory). The same argument can be made for the interaction of SLN with SERCA. The reaction coordinate between SERCA inhibition by SLN and SERCA's

progression through the calcium transport cycle is not stochastic. We suggest that SLN cycles between inhibitory and non-inhibitory interactions with SERCA, and that these interactions are linked by conformational memory – past SERCA-SLN interactions influence future transport cycles (Fig. 7).

It is important to remember that SLN remains associated with SERCA during the catalytic cycle (Sahoo et al., 2013). Therefore, the two SERCA-SLN complexes described above must alternately populate inhibitory interactions and secondary interactions that are non-inhibitory and release SERCA for progression through the transport cycle. This is most notable for the E2 SERCA-SLN complex that results in potent inhibition of SERCA. In the transition from the calcium-free E2 state to the calcium-bound E1 state, SLN must relocate away from the position transmembrane segment M2 will occupy. This requires a large change in how SLN interacts with SERCA. However, in the transition from the calcium-free E1-like state to the calcium-bound E1 state, side chain interactions between SLN and M2 are broken. This requires a small change in how SLN interacts with SERCA. Conformational memory refers to the exclusive nature of these SLN transitions during steady-state turnover of SERCA. In each case, SLN can reversibly transition between the initial inhibitory interaction (E2 or E1-like) and the associated secondary (non-inhibitory) interaction. These interactions are linked and SLN cannot transition to a different inhibitory interaction (from E2 to E1-like or E1-like to E2). Conformational memory suggests that the secondary, non-inhibitory interaction encodes information (memory) that allows SLN to return to the original inhibitory interaction with each new transport cycle (Fig. 7).

The presence of conformational memory can be observed in the measurement of pre-steady-state charge translocation versus steady-state ATPase activity by SERCA (Fig. 1). In the charge translocation measurements, SERCA molecules undergo a single turnover event that loads the proteoliposomes with calcium and generates a current response (Tadini-Buoninsegni et al., 2006). Poising SERCA in different conformational states prior to initiating charge translocation influenced the kinetic parameters for SERCA alone, as well as SERCA in the presence of SLN. This was not surprising for the pre-steady-state measurements. In the ATPase activity measurements, SERCA molecules undergo continuous turnover under conditions where all variables and substrate concentrations remain constant. Poising SERCA in different conformational states prior to initiating ATPase activity influenced the kinetic parameters for both SERCA alone and SERCA in the presence of SLN, even under steady-state continuous turnover. This suggests that the initial inhibitory conformations of SERCA encode information on how the SERCA-SLN complex can progress through the calcium transport cycle, and this conformational memory is retained in multiple turnover events.

MATERIALS & METHODS

All reagents used were of the highest purity available. Reagents used for proteoliposome reconstitution include egg yolk phosphatidylcholine (PC), egg yolk phosphatidic acid (PA) (Avanti Polar Lipids, Alabaster AL), and octaethylene glycol monododecyl ether ($C_{12}E_8$) (Barnet Products, Englewood Cliff, NJ). Reagents used for couple-enzyme ATPase measurements include ATP, NADH, phosphoenolpyruvate, pyruvate kinase, and lactate dehydrogenase (Sigma-Aldrich, Oakville, ON).

Protein purification

Recombinant human SLN was expressed and purified as previously described (Douglas et al., 2005; Gorski et al., 2013). SERCA1a was purified from rabbit skeletal muscle SR as described (Eletr & Inesi, 1972; Stokes & Green, 1990) with the following modifications. 100 mg of rabbit SR was suspended in extraction buffer (10 mg/mL $C_{12}E_8$, 8 mM $CaCl_2$, 50 mM MOPS pH 7.0, 5 mM DTT, 20% glycerol) and stirred for 15 minutes at 4°C. Solubilized SR was centrifuged at $100,000 \times g$ for 20 minutes and loaded onto a packed column containing Reactive Green Resin (Sigma-Aldrich, Oakville, ON) equilibrated with extraction buffer. The column was washed with two column volumes of wash buffer (1% $C_{12}E_8$, 1 mM $CaCl_2$, 20 mM MOPS pH 7.0, 1 mM DTT, 20% glycerol) and eluted with elution buffer (10 mg/mL $C_{12}E_8$, 8 mM $CaCl_2$, 50 mM MOPS pH 7.0, 5 mM DTT, 20% glycerol, 10 mM ADP, 50 mM NaCl). Elution fractions were evaluated for SERCA purity by SDS-PAGE and calcium-dependent ATPase activity.

Co-reconstitution of SERCA and SLN

Co-reconstitution of SLN and SERCA was performed as previously described (Glaves et al., 2020; Gorski et al., 2013) with the following modifications. 75 μg of lyophilized SLN was suspended in 100 μL trifluoroethanol: 2-propanol (5:1) and mixed with 360 μg egg yolk PC and 40 μg egg yolk PA. The mixture was then dried under $N_2(g)$ while vortexing to form a thin film of lipid and peptide, and placed under vacuum overnight. The lipid-peptide thin films were rehydrated in buffer (20 mM imidazole, pH 7.0, 100 mM KCl, and 0.02% NaN_3) at 37°C for 10 min, cooled to room temperature, and detergent solubilized by the addition of $C_{12}E_8$ (0.2% final concentration) with vigorous vortexing. Detergent-solubilized SERCA1a was added (300 μg), and the reconstitution was stirred gently at room temperature. Detergent was slowly removed by the addition of Bio-Beads SM-2 resin (Bio-Rad Laboratories, Hercules, CA) over a 4-h time course (final ratio of 25 biobeads: 1 detergent w/w). After detergent removal, the reconstitution was centrifuged over a 20–50% sucrose step gradient for 1 h at $100,000 \times g$. The resultant layer of reconstituted proteoliposomes was removed, flash frozen in liquid nitrogen and stored at -80°C. The final molar ratios were 120 lipids: 4.5 SLN: 1 SERCA and this SERCA-SLN ratio was confirmed by quantitative SDS-PAGE (Young, Jones & Stokes, 2001).

Calcium-dependent ATPase activity measurements

The calcium-dependent ATPase activity of SERCA1a in co-reconstituted proteoliposomes was determined by a coupled-enzyme assay over a range of calcium concentrations (0.1–10 μM). This assay has recently been adapted to a microplate reader and 96-well format (Armanious et al., 2024; Fisher et al., 2021). Proteoliposomes containing SERCA1a alone (negative control) and SERCA1a with SLN (experimental) were evaluated (~ 10 –20 nM SERCA1a at 30°C). Reactions were initiated under different conditions that poised SERCA1a in distinct conformational states: (i) pre-incubation in the absence of substrates (substrate jump condition; 0.1–10 μM calcium, 4 mM ATP; assay initiated by the simultaneous addition of calcium and ATP); (ii) pre-incubation in the presence of calcium (ATP jump condition; 4 mM ATP; assay initiated by the addition of ATP); (iii) pre-incubation in the presence of ATP

(calcium jump condition; 0.1–10 μM calcium; assay initiated by the addition of calcium); and (iv) physiological pre-incubation condition (physiological jump condition; 80 nM calcium, 4 mM ATP; assay initiated by the addition of calcium to achieve 0.1–10 μM calcium). Kinetic parameters V_{max} (maximal activity), K_{Ca} (apparent calcium affinity), and n_{H} (cooperativity) were determined by nonlinear least-squares fitting of the activity data to the Hill equation (Sigma Plot software, SPSS, Chicago, IL). Errors were calculated as the standard error of the mean for a minimum of four independent reconstitutions. Comparison of the kinetic parameters was carried out using one-way analysis of variance (between subjects), followed by the Holm-Sidak test for pairwise comparisons.

Charge translocation measurements

Charge translocation measurements were performed with a SURFE²R N1 surface electrogenic event reader (Nanon Technologies, Munich, Germany). The temperature was maintained at $\sim 23^{\circ}\text{C}$. Charge movement was measured by adsorbing proteoliposomes containing SERCA in the absence and presence of SLN onto a solid supported membrane (Fig. 1; alkane thiol/phospholipid bilayer anchored to the surface of a gold electrode) (Smeazzetto et al., 2017; Tadini Buoninsegni et al., 2004). Following adsorption of the proteoliposomes to the SSM, charge translocation by SERCA was initiated by a concentration jump of a suitable substrate. The substrate pre-incubation and jump conditions (i-iv) are described above. These conditions induced charge translocation (calcium transport) across the proteoliposome membrane, and a current transient was recorded due to capacitive coupling between the proteoliposome and SSM (**Figure S1**). The peak amplitude and numerically integrated current transient (total charge translocation) are related to the net charge movement in the proteoliposomes, which depends upon the electrogenic calcium transport by SERCA. The SSM technique detects pre-steady-state current transients within the first catalytic cycle of SERCA, and it is not sensitive to stationary currents following the first cycle (Tadini-Buoninsegni et al., 2006; Tadini-Buoninsegni et al., 2008). The peak amplitude (nA) and total charge translocation (nC) were plotted versus calcium concentration. Kinetic parameters V_{max} (maximal activity), K_{Ca} (apparent calcium affinity), and n_{H} (cooperativity) were determined by nonlinear least-squares fitting of the activity data to the Hill equation (Sigma Plot software, SPSS, Chicago, IL). Errors were calculated as the standard error of the mean for a minimum of four independent reconstitutions. Comparison of the kinetic parameters was carried out using one-way analysis of variance (between subjects), followed by the Holm-Sidak test for pairwise comparisons.

Declarations

Data and Materials Availability

All experimental data are available in the main text and supplemental data.

COMPTING InterestS

The authors declare that there are no competing interests associated with this manuscript.

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Author Contribution

Conceptualization - JOP, MJL, HSY; Methodology - JOP, MJL, PL, HSY; Investigation - JOP, HSY; Visualization - JOP, HSY; Supervision – HSY; Writing – HSY; Writing, review & editing - JOP, HSY.

Data Availability

All experimental data are available in the main text and supplemental data.

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Figures

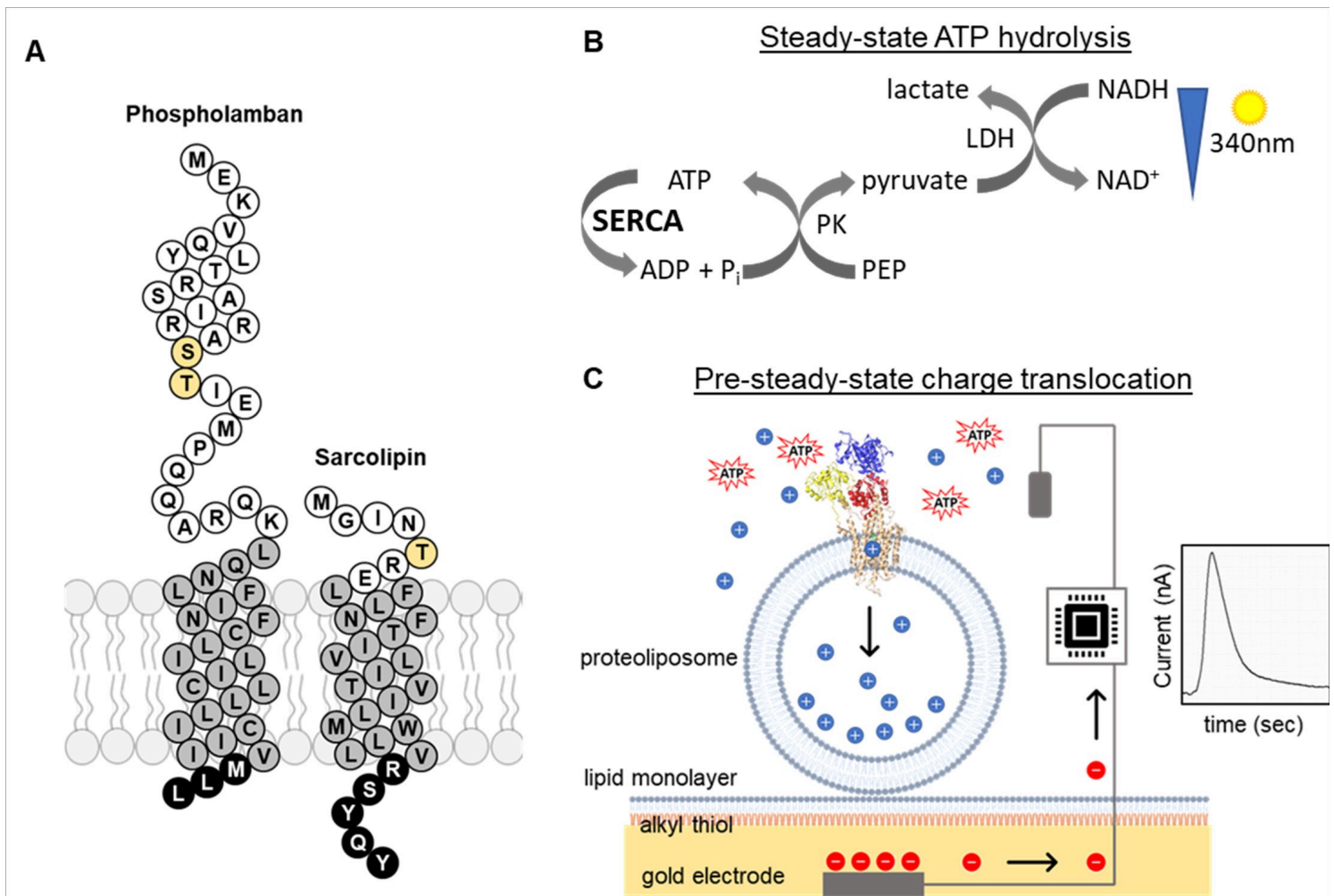


Figure 1: Topology models for PLN and SLN and the *in vitro* assays used to measure SERCA activity. **(A)** The cytoplasmic (white), transmembrane (grey), and luminal domains (black) of PLN and SLN are indicated. **(B)** The coupled enzyme assay used to measure the calcium dependent ATPase activity of SERCA. Through the enzymatic activity of pyruvate kinase (PK) and lactate dehydrogenase (LDH), calcium-dependent ATP hydrolysis by SERCA is linked to NADH oxidation. The hydrolysis of ATP is stoichiometrically linked to the disappearance of NADH, which can be measured as the absorbance at 340 nm. ATP is simultaneously regenerated to maintain steady-state conditions. **(C)** Charge translocation measured by capacitive coupling on solid supported membranes (SSM) using a surface electrogenic event reader (SEER). SERCA-containing proteoliposomes are adsorbed to the SSM (alkyl thiol and lipid monolayer) and calcium transport into the proteoliposomes induces a current transient that can be measured with SEER technology.

Figure 1

See image above for figure legend.

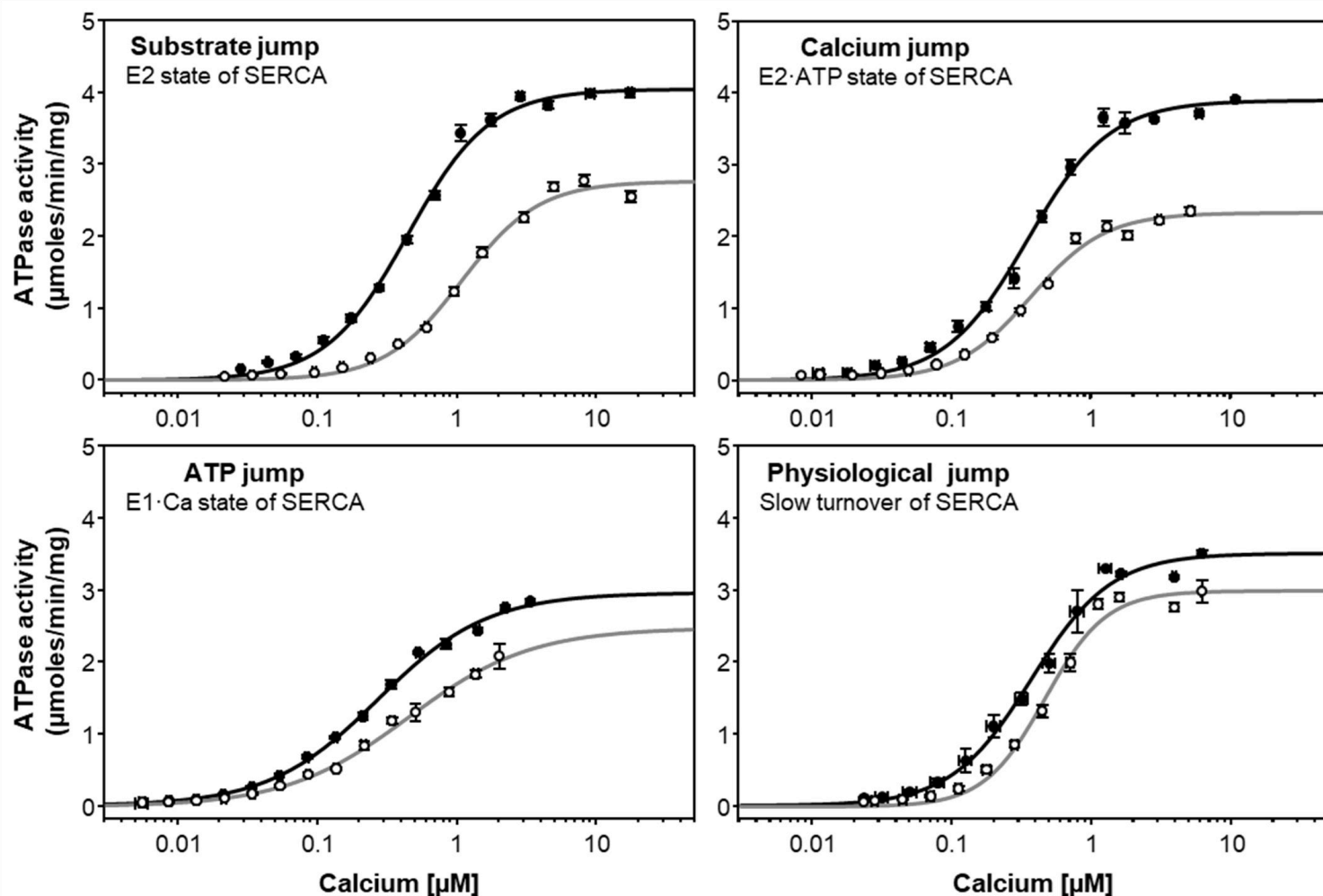


Figure 2: Calcium-dependent ATPase activity data for SERCA in the absence and presence of SLN. ATPase activity as a function of calcium concentration is shown for SERCA alone (*solid black line*) and SERCA in the presence of wild-type SLN (*solid grey line*). **Substrate jump** – SERCA was pre-incubated in the absence of substrates and ATP and calcium were added to initiate SERCA activity. **Calcium jump** – SERCA was pre-incubated in the presence of ATP and calcium was added to initiate SERCA activity. **ATP jump** – SERCA was pre-incubated in the presence of calcium and ATP was added to initiate SERCA activity. **Physiological jump** – SERCA was pre-incubated in the presence of ATP and calcium (80 nM) and additional calcium was added to initiate SERCA activity. Each data point is the mean $\pm$ SEM ($n \geq 4$). The V_{max} , K_{Ca} , and Hill coefficients (n_H) are given in Table 1.

Figure 2

See image above for figure legend.

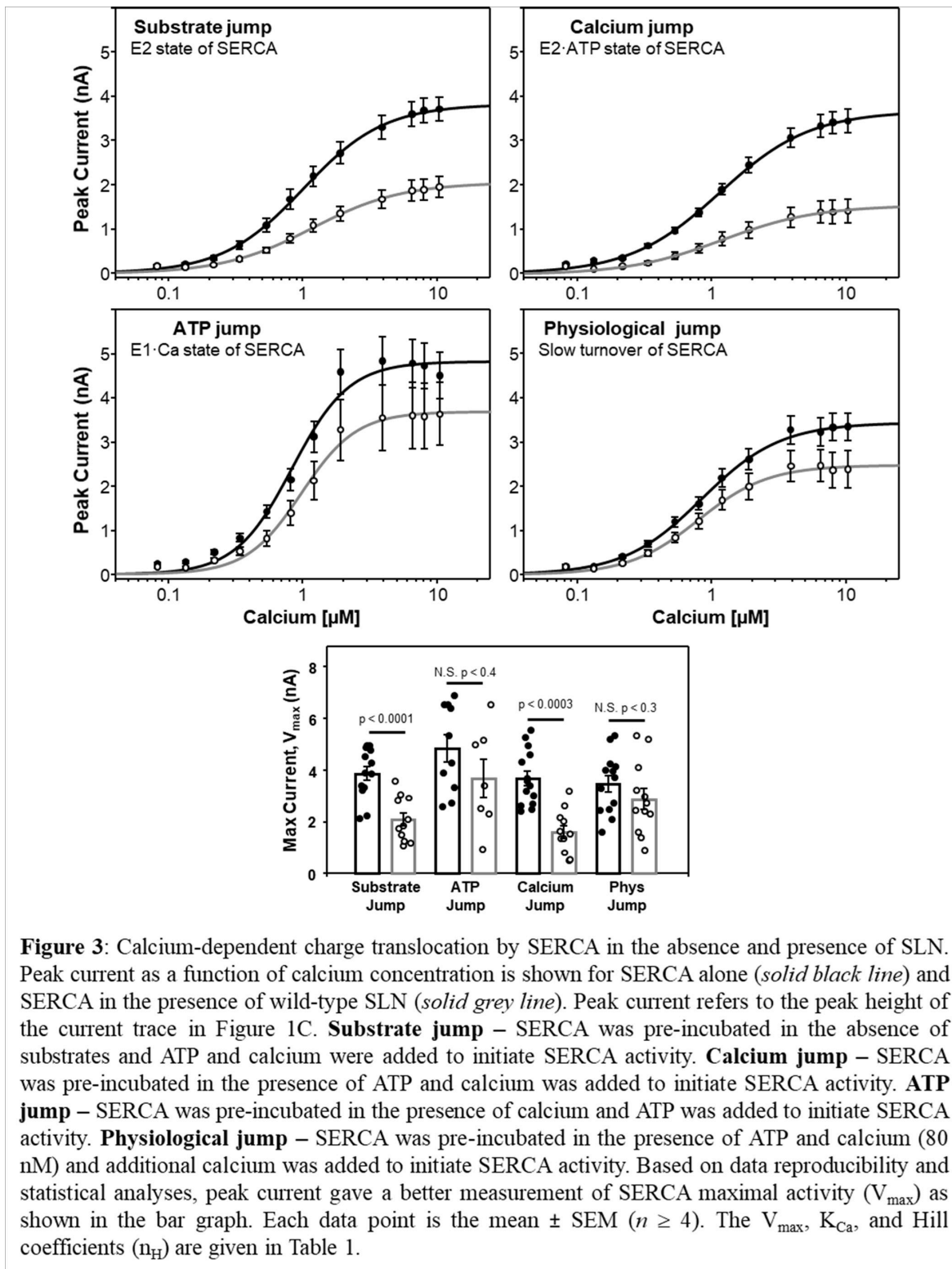


Figure 3: Calcium-dependent charge translocation by SERCA in the absence and presence of SLN. Peak current as a function of calcium concentration is shown for SERCA alone (*solid black line*) and SERCA in the presence of wild-type SLN (*solid grey line*). Peak current refers to the peak height of the current trace in Figure 1C. **Substrate jump** – SERCA was pre-incubated in the absence of substrates and ATP and calcium were added to initiate SERCA activity. **Calcium jump** – SERCA was pre-incubated in the presence of ATP and calcium was added to initiate SERCA activity. **ATP jump** – SERCA was pre-incubated in the presence of calcium and ATP was added to initiate SERCA activity. **Physiological jump** – SERCA was pre-incubated in the presence of ATP and calcium (80 nM) and additional calcium was added to initiate SERCA activity. Based on data reproducibility and statistical analyses, peak current gave a better measurement of SERCA maximal activity (V_{max}) as shown in the bar graph. Each data point is the mean $\pm$ SEM ($n \geq 4$). The V_{max} , K_{Ca} , and Hill coefficients (n_{H}) are given in Table 1.

Figure 3

See image above for figure legend.

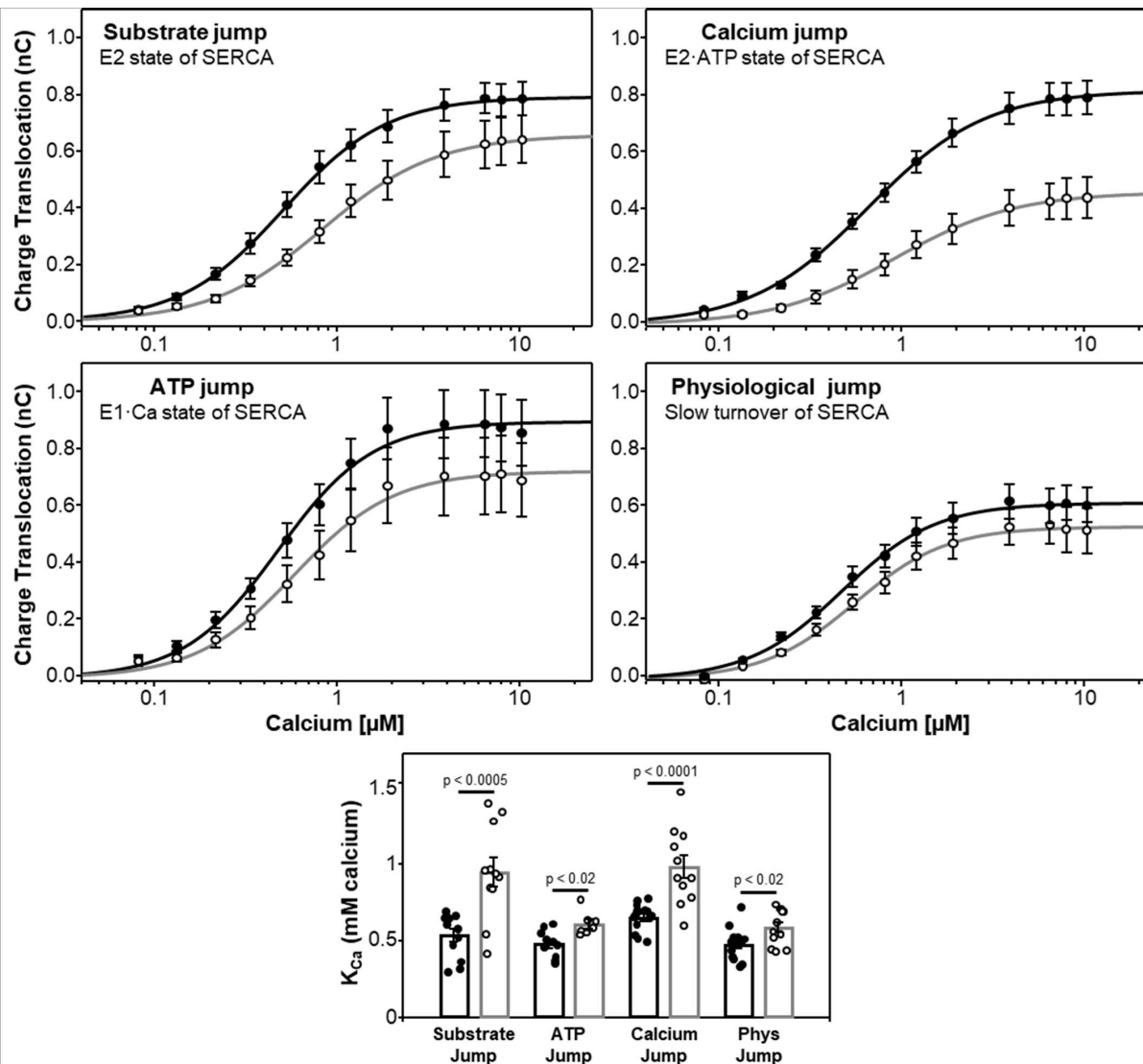


Figure 4: Calcium-dependent charge translocation by SERCA in the absence and presence of SLN. Total charge translocation as a function of calcium concentration is shown for SERCA alone (*solid black line*) and SERCA in the presence of wild-type SLN (*solid grey line*). Total charge translocation refers to the peak integration of the current trace in Figure 1C. **Substrate jump** – SERCA was pre-incubated in the absence of substrates and ATP and calcium were added to initiate SERCA activity. **Calcium jump** – SERCA was pre-incubated in the presence of ATP and calcium was added to initiate SERCA activity. **ATP jump** – SERCA was pre-incubated in the presence of calcium and ATP was added to initiate SERCA activity. **Physiological jump** – SERCA was pre-incubated in the presence of ATP and calcium (80 nM) and additional calcium was added to initiate SERCA activity. Based on data reproducibility and statistical analyses, total charge translocation gave a better measurement of SERCA apparent calcium affinity (K_{Ca}) as shown in the bar graph. Each data point is the mean $\pm$ SEM ($n \geq 4$). The V_{max} , K_{Ca} , and Hill coefficients (n_{H}) are given in Table 1.

Figure 4

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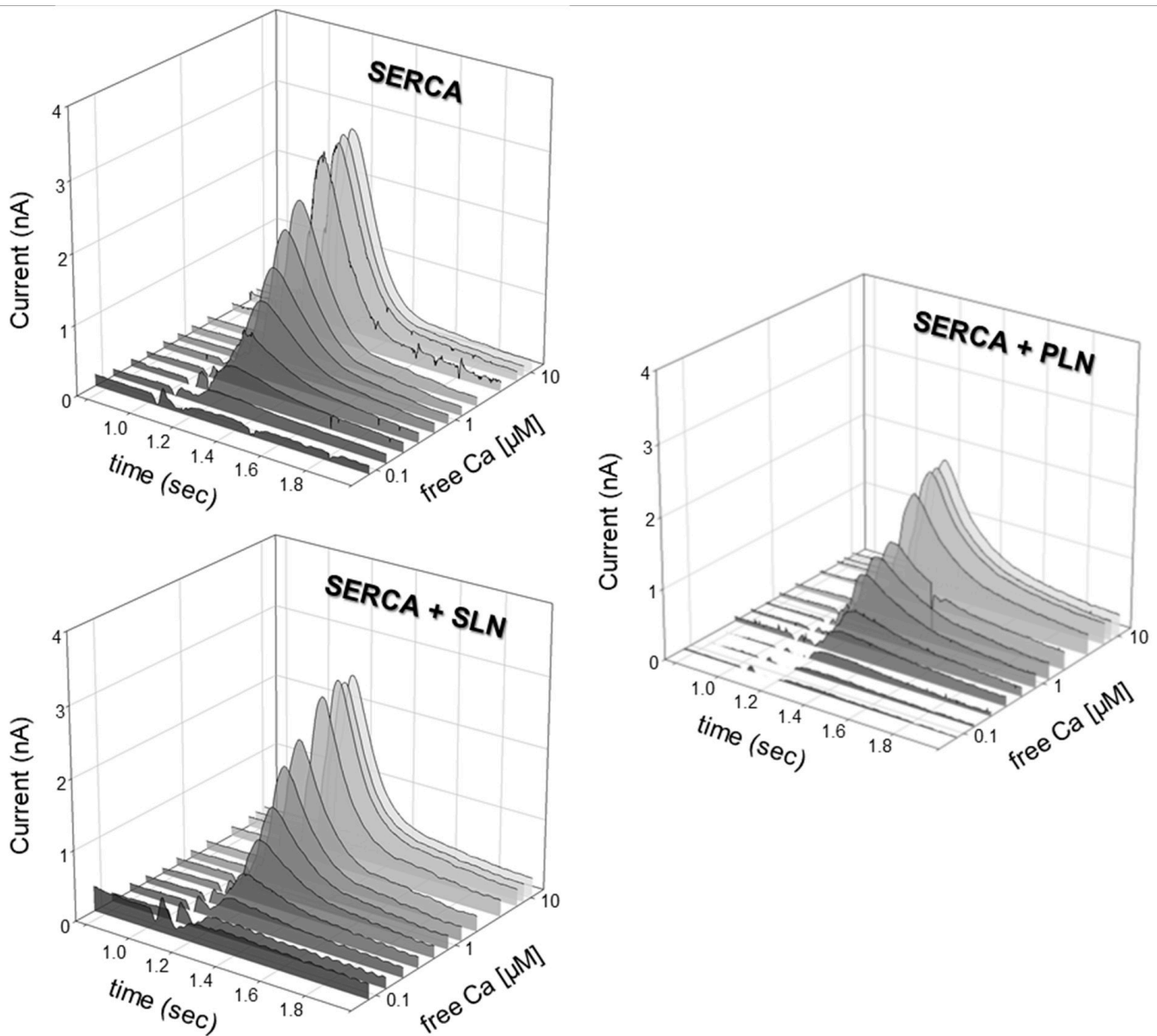


Figure 5: Current traces for SERCA alone, SERCA in the presence of SLN, and SERCA in the presence of PLN. SERCA was pre-incubated under physiological conditions in the presence of 4 mM ATP and 60 nM calcium. Various calcium concentrations (0.1 – 10 μM total free calcium) were added to initiate charge translocation using SEER technology. The resultant current traces (nA versus time) were measured for each calcium concentration. Notice the similarity in overall charge translocation by SERCA alone and SERCA in the presence of SLN. Under these conditions, charge translocation by SERCA in the presence of PLN is significantly reduced.

Figure 5

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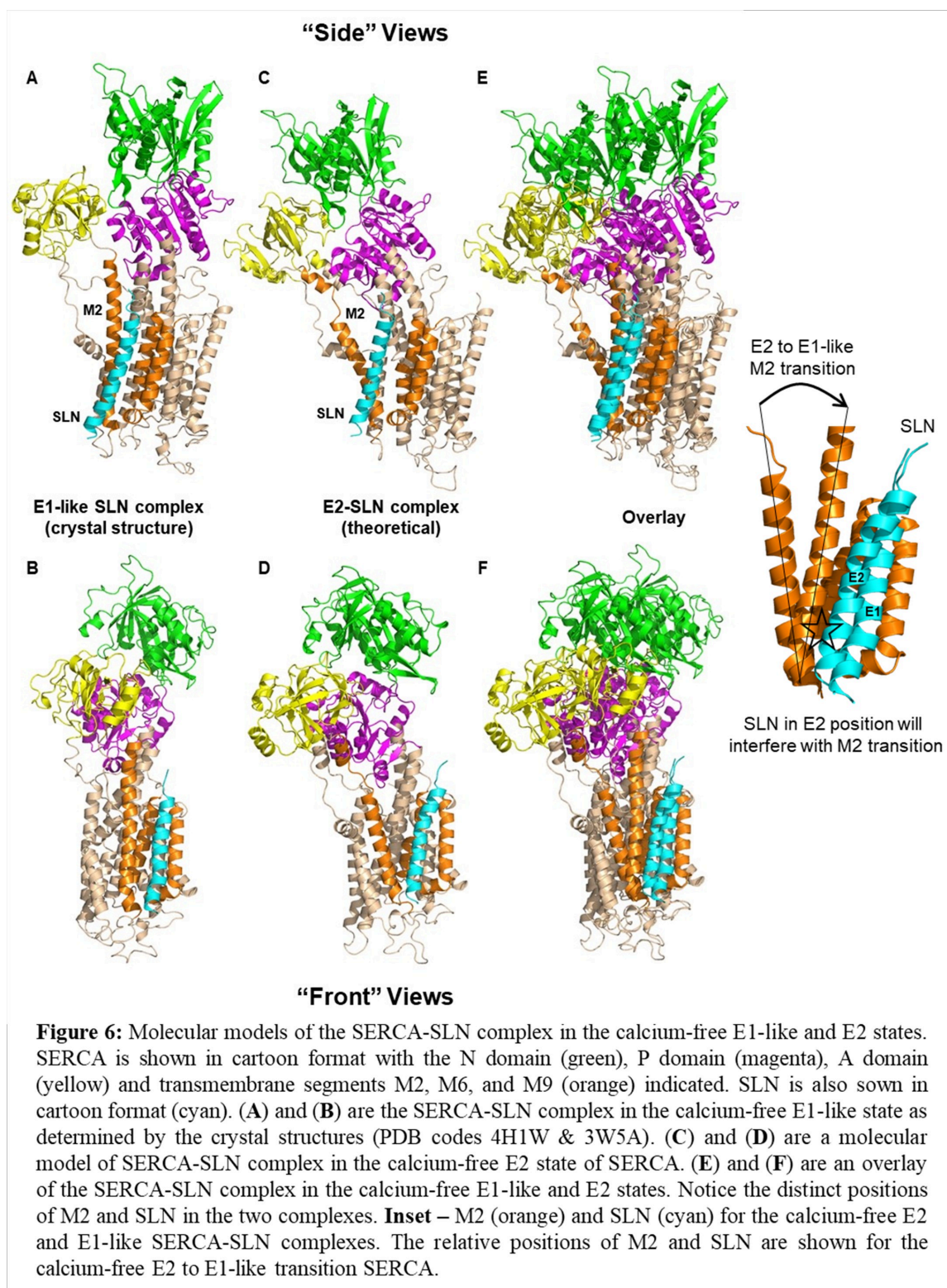


Figure 6

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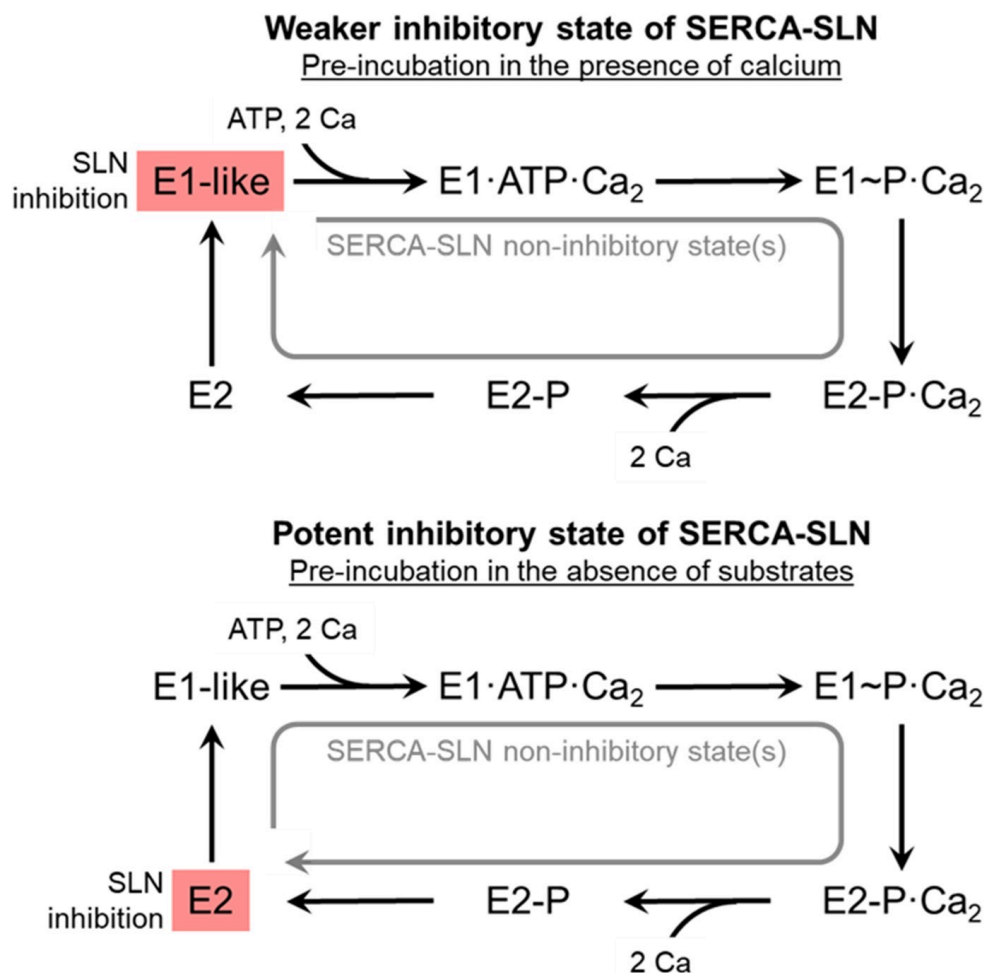


Figure 7: Simplified reaction scheme for SERCA indicating the two potential inhibitory conformations and conformational memory. The first reaction scheme (**upper panel**) is based on the E1-like conformation of SERCA and the known crystal structures. SLN establishes an inhibitory interaction with the E1-like state of SERCA (highlighted in red) and progression through the calcium transport cycle involves non-inhibitory interactions that return to the E1-like SERCA-SLN inhibitory complex (grey arrow). The second reaction scheme (**lower panel**) is based on the E2 conformation of SERCA and the previous molecular models. SLN establishes an inhibitory interaction with the E2 state of SERCA (highlighted in red) and progression through the calcium transport cycle involves non-inhibitory interactions that return to the E2 SERCA-SLN inhibitory complex (grey arrow). The initial inhibitory state (E1-like or E2) and the non-inhibitory states are linked by conformational memory.

Figure 7

See image above for figure legend.

Supplementary Files

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- [floatimage8.png](#)