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Supporting Material

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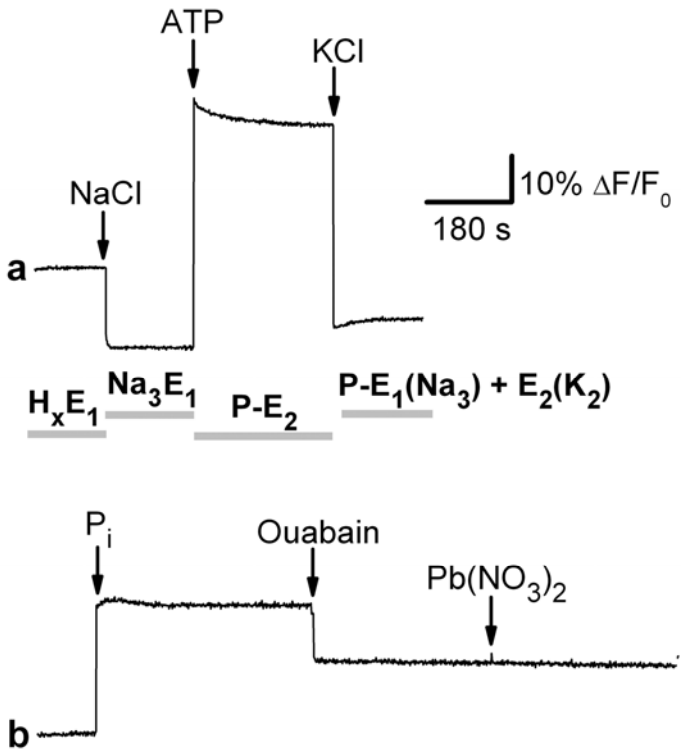

Figure S1 (a) Steady-state fluorescence pattern representative of a standard experiment performed in standard buffer. The reagents are added in correspondence of the arrows. The final concentrations are 50 mM NaCl, 0.5 mM ATP and 20 mM KCl. Each steady-state fluorescence level is determined by the conformation (or the conformations) indicated on the bottom of the trace. Besides $E_2(K_2)$, the final level is determined by the $P\text{-}E_1(Na_3)$ or the Na_3E_1 conformation (see text). (b) Control experiment carried out in standard buffer by adding 10 µM Pb^{2+} ions after inhibition of the pump by 100 µM ouabain. Prior to inhibition, the protein was confined in the $P\text{-}E_2$ conformation by performing backdoor phosphorylation with 0.5 mM P_i (from TRIS-P_i, pH 7.0).

Ion	*Initial conformation*	*$K_{0.5}$ (mM) or pK*		*n*	
		- Pb	*+ Pb*	*- Pb*	*+ Pb*
Na^+	H_xE_1	10.7 ± 0.9	9.9 ± 0.9	1.06 ± 0.08	1.09 ± 0.08
	P-E_2 [(a)]	39 ± 6	35 ± 2	1.3 ± 0.2	2.0 ± 0.2
K^+	H_xE_1	n.d.[(b)]	n.d.	n.d.	n.d.
	P-E_2 [(a)]	0.46 ± 0.02	0.44 ± 0.02	1.13 ± 0.05	1.14 ± 0.06

[(a)] obtained by backdoor phoshorylation.
[(b)] n.d. : not determined

Table S1 Values of $K_{0.5}$ (or pK) and *n* obtained from Na^+ and K^+-titrations in the E_1 and P-E_2 conformations in the absence and presence of 10 µM free Pb^{2+}.

APPENDIX

The simulation was carried out following the approach reported in Peinelt and Apell, 2002 (44). In particular, the reaction scheme reported in Fig. 6 (upper panel) can be represented mathematically as follows (protons are omitted for simplicity):

$$[E_1] = K_1[E_2] \quad \text{(A1)}$$

$$[P][E_2] = K_2[P-E_2] \quad \text{(A2)}$$

$$[Pb][P-E_2] = K_3[P-E_2^{Pb}] \quad \text{(A3)}$$

$$[Pb][E_2] = K_4[E_2^{Pb}] \quad \text{(A4)}$$

$$[Pb][E_1] = K_5[E_1^{Pb}] \quad \text{(A5)}$$

$$[E_1^{Pb}] = K_6[E_2^{Pb}] \quad \text{(A6)}$$

$$[P][E_2^{Pb}] = K_7[P-E_2^{Pb}] \quad \text{(A7)}$$

$$[E_1]+[E_2]+[P-E_2]+[E_1^{Pb}]+[E_2^{Pb}]+[P-E_2^{Pb}] = 1 \quad \text{(A8)}$$

The equilibrium constants K_6 and K_7 are not independent parameters, since relative to cycling sections of the entire scheme ($K_6 = K_1K_4/K_5$; $K_7 = K_2K_3/K_4$). The first five independent equations (A1 to A5), together with the normalization condition (A8), constitute a 6x6 equation system whose solution is

$$[E_1] = x_1/D \quad \text{(A9)}$$

$$[E_2] = x_2/D \quad \text{(A10)}$$

$$[P-E_2] = x_3/D \quad \text{(A11)}$$

$$[E_1^{Pb}] = x_4/D \quad \text{(A12)}$$

$$[E_2^{Pb}] = x_5/D \quad \text{(A13)}$$

$$[P-E_2^{Pb}] = x_6/D \quad \text{(A14)}$$

where

$$x_1 = K_1 K_2 K_3 K_4 K_5 \quad \text{(A15)}$$

$$x_2 = K_2 K_3 K_4 K_5 \quad \text{(A16)}$$

$$x_3 = [\mathrm{P}] K_3 K_4 K_5 \quad \text{(A17)}$$

$$x_4 = [\mathrm{Pb}] K_1 K_2 K_3 K_4 \quad \text{(A18)}$$

$$x_5 = [\mathrm{Pb}] K_2 K_3 K_5 \quad \text{(A19)}$$

$$x_6 = [\mathrm{P}][\mathrm{Pb}] K_4 K_5 \quad \text{(A20)}$$

$$D = x_1 + x_2 + x_3 + x_4 + x_5 + x_6 \quad \text{(A21)}$$

The total steady-state fluorescence intensity is determined by the linear combination of the specific fluorescence level of each intermediate, f_i, weighted by the concentration of the intermediate at equilibrium (31):

$$F_{total} = f_1[\mathrm{E}_1] + f_2[\mathrm{E}_2] + f_3[\mathrm{P}-\mathrm{E}_2] + f_4[\mathrm{E}_1^{\mathrm{Pb}}] + f_5[\mathrm{E}_2^{\mathrm{Pb}}] + f_6[\mathrm{P}-\mathrm{E}_2^{\mathrm{Pb}}] \quad \text{(A22)}$$

The values of K_i and f_i used to obtain the simulation curves of Fig. 4 (solid lines), are reported in Table 2.

ADDITIONAL REFERENCE

44. Peinelt C., and H.-J. Apell. 2002. Kinetics of the Ca^{2+}, H^+, and Mg^{2+} interaction with the ion-binding sites of the SR Ca-ATPase. Biophys J 82:170-181.