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Supplementary Materials for

Tracking Ca^{2+} ATPase intermediates in real time by x-ray solution scattering

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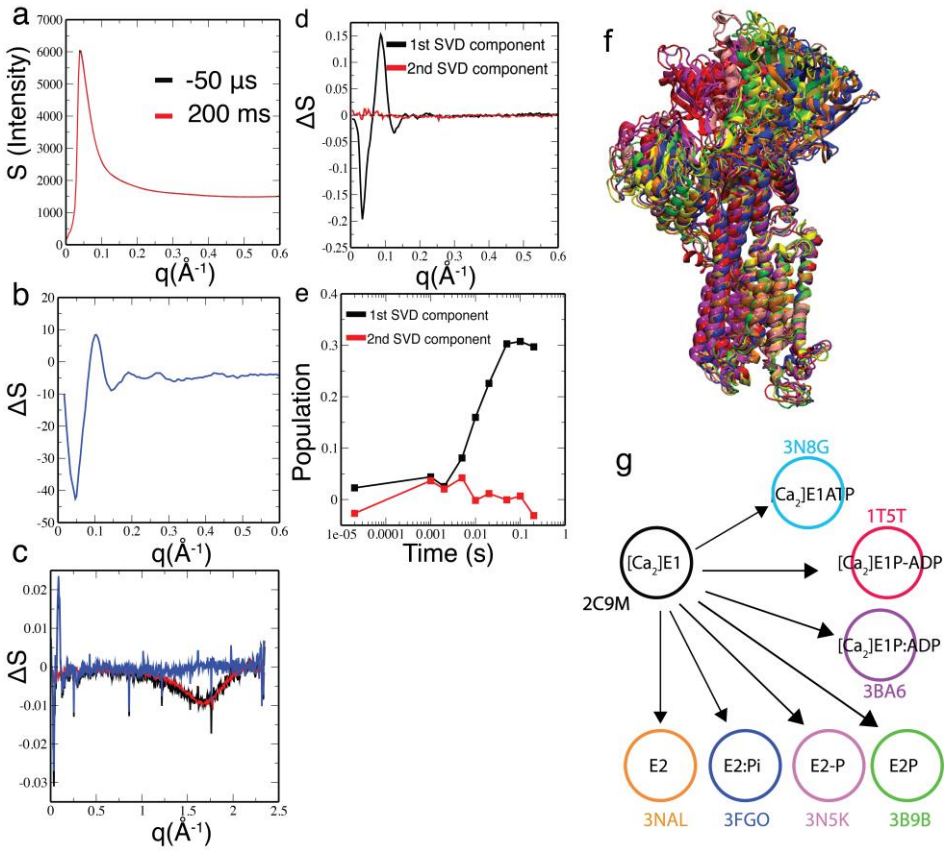

Fig. S1. Data analyses and targeted MD approach. a, X-ray scattering intensity profiles for 200 ms (i.e. after laser initiation) and -50 µs (i.e. before laser initiation). **b**, Difference X-ray scattering profile obtained from subtracting the -50 µs time point from the 200 ms time point. **c**, heating difference curve obtained from X-ray scattering of a dye solution (black) was scaled to the TR-XSS data (here represented by the 5 ms time point in red) by alignment in the 1.5 $Å^{-1}$ < q < 2.0 $Å^{-1}$ region where the effects from solvent expansion are observed. The resulting heating-free curves only show features associated with structural rearrangements in the protein and membrane in the sample (blue). SVD analysis of the SERCA TR-XSS dataset resulted in **d**, two major components and the corresponding **e**, relative amplitudes at each time point in the dataset. **f**, SERCA structures extracted from one targeted MD trajectory starting from the $[Ca_2]$E1 crystal structure to an intermediate state crystal structure

ranging from blue to red. **g**, schematic of the targeted MD simulation approach starting from [Ca_2]E1.

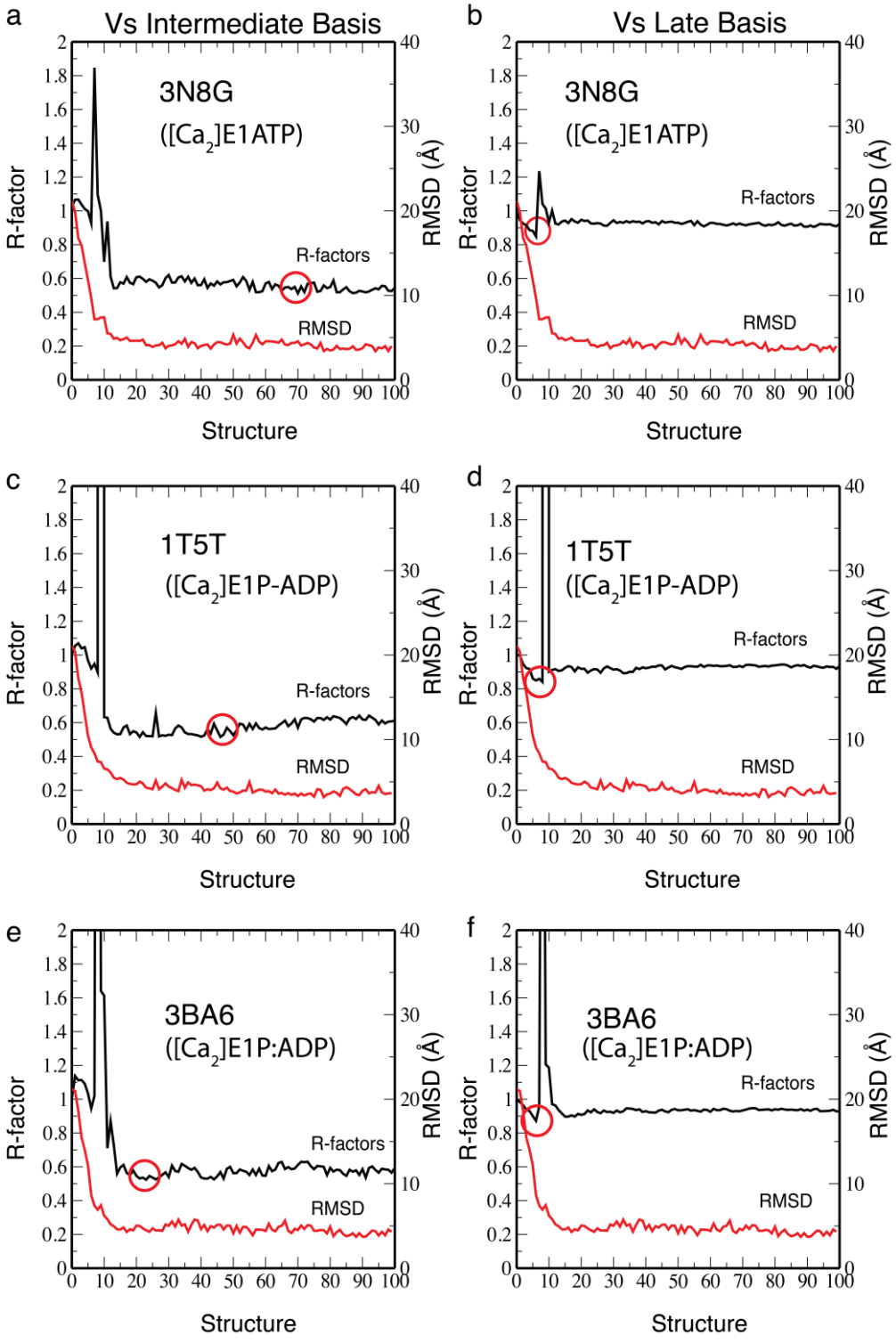

Fig. S2. Targeted MD of E1 states. Comparison of the RMSD and R values (vs Intermediate and Late basis spectra) from the TMD trajectories driving from 2C9M to E1 crystal structures 3N8G (**a**, **b**), 1T5T (**c**, **d**) and 3BA6 (**e**, **f**). The red circles correspond to the lowest obtained R-factors.

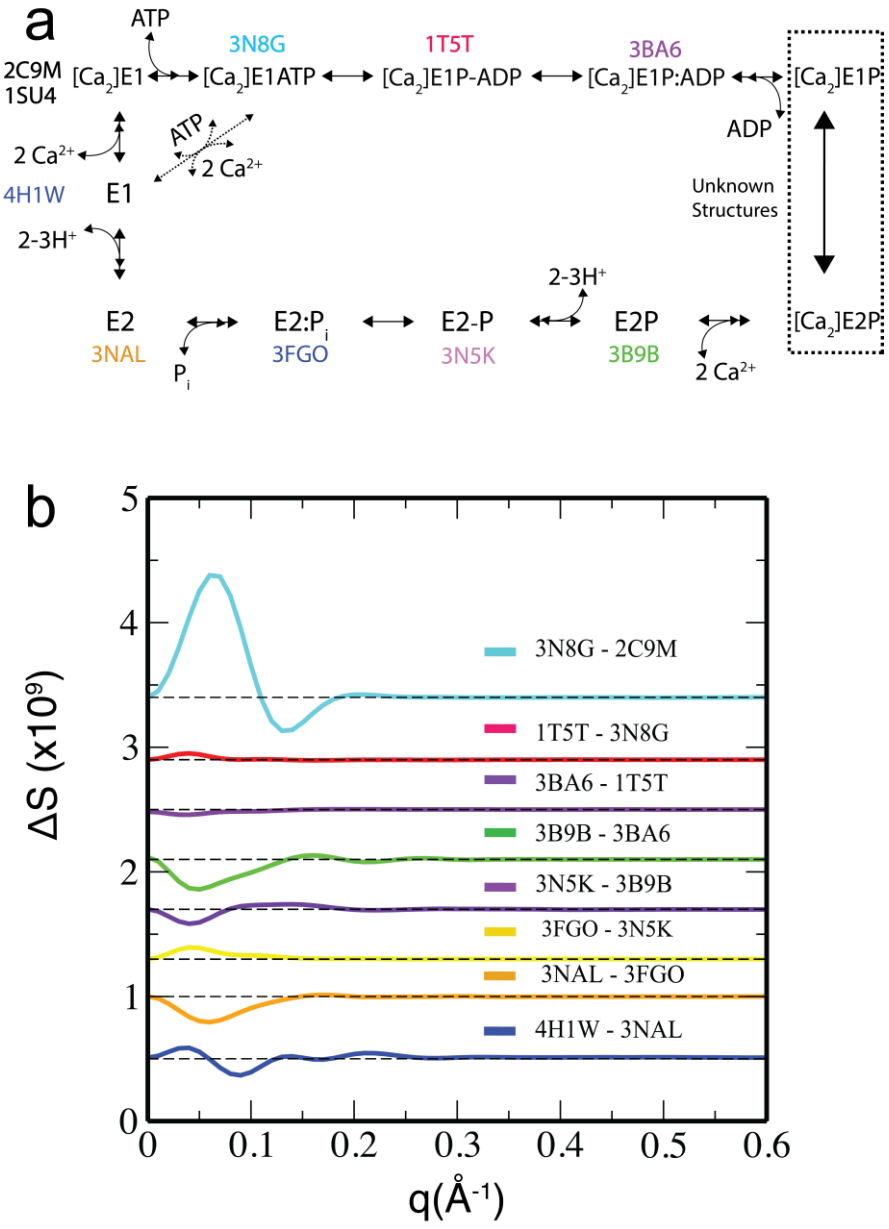

Fig. S3. Reaction scheme and sequential x-ray scattering differences. a, Reaction cycle schematic of principal SERCA states and representative crystal structures (PDB ID) used in the structural refinement procedure. **b**, Difference scattering profiles of the crystal structures for each of the transition states from its previous state in the reaction cycle.

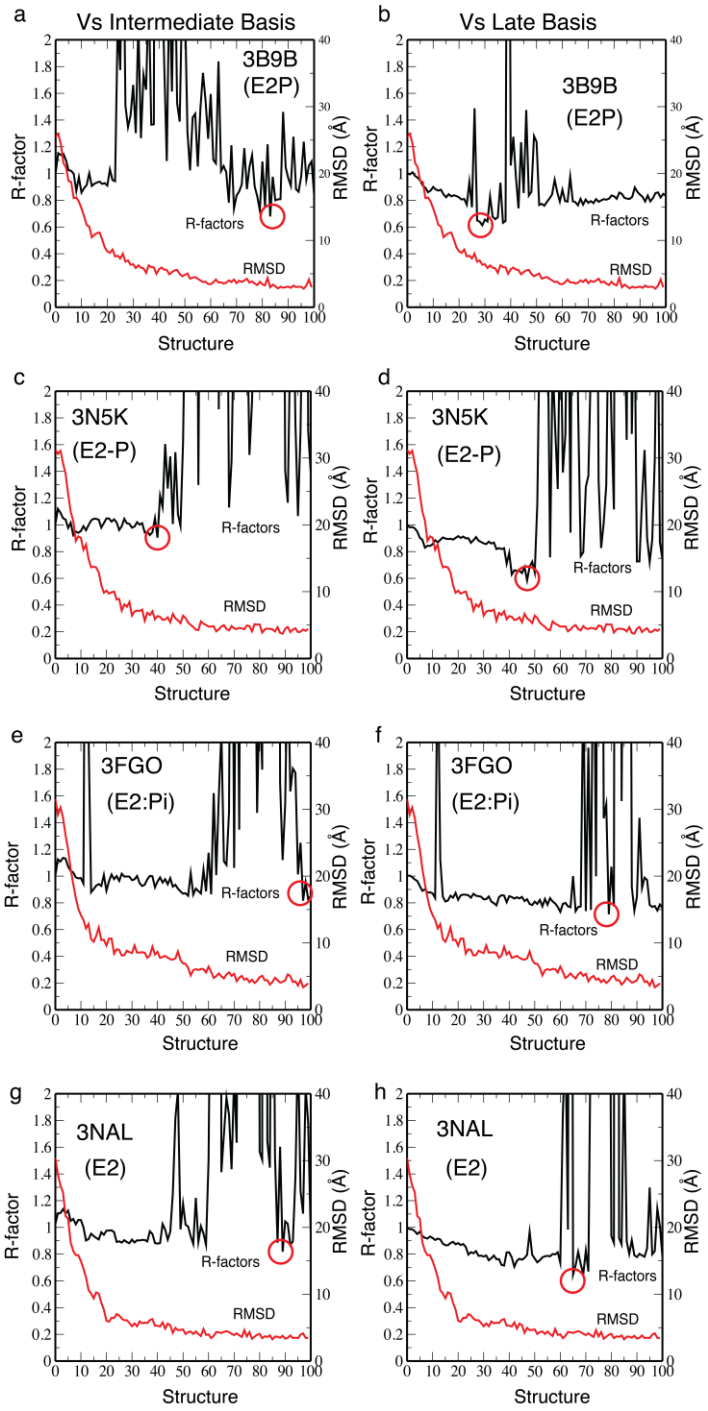

Fig. S4. Targeted MD of E2 states. Comparison of the RMSD and R values (vs Intermediate and Late basis spectra) from the TMD trajectories driving from 2C9M to E2 crystal structures 3B9B (**a**, **b**), 3N5K (**c**, **d**), 3FGO (**e**, **f**), 3NAL (**g**, **h**).

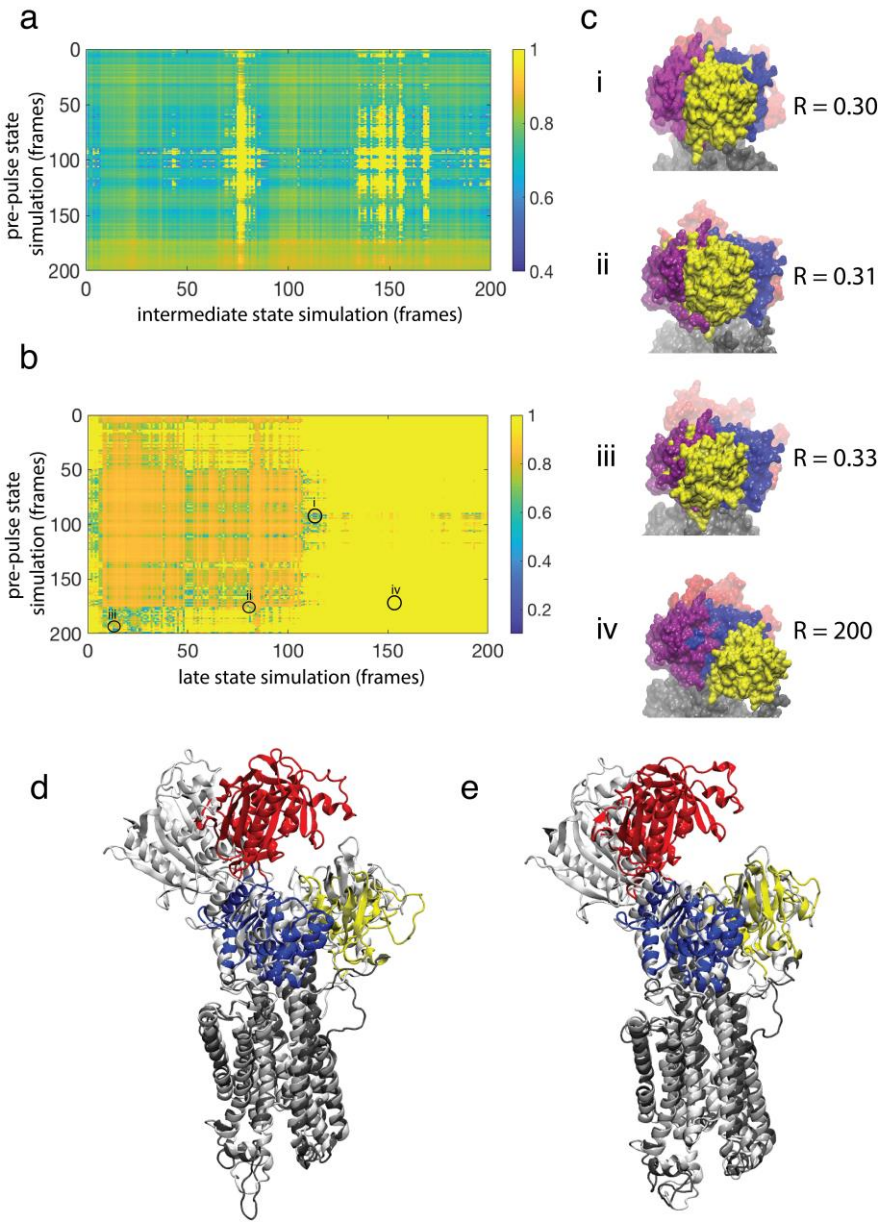

Fig. S5. R-factor analyses of the membrane simulations and pre-pulse TR-XSS models. R-factor matrices that correlate simulation trajectories to experimental **a** intermediate and **b**, late basis spectra**. c,** Structural differences in the A domain between the TR-XSS model (yellow), the [Ca_2]E1P:ADP (PDB ID: 3BA6) (blue), and E2P (PDB ID: 3B9B (magenta) states for different R-factors (i,ii,iii,iv). To enable contrasting of relevant (R-factors < 1) local minima, R-factors > 1 were given the same yellow color. TR-XSS models of the best fitting pre-pulse states for the **d**,

intermediate and **e**, late basis experimental spectra overlaid with the [Ca_2]E1 crystal structure (in white, PDB ID:2C9M)

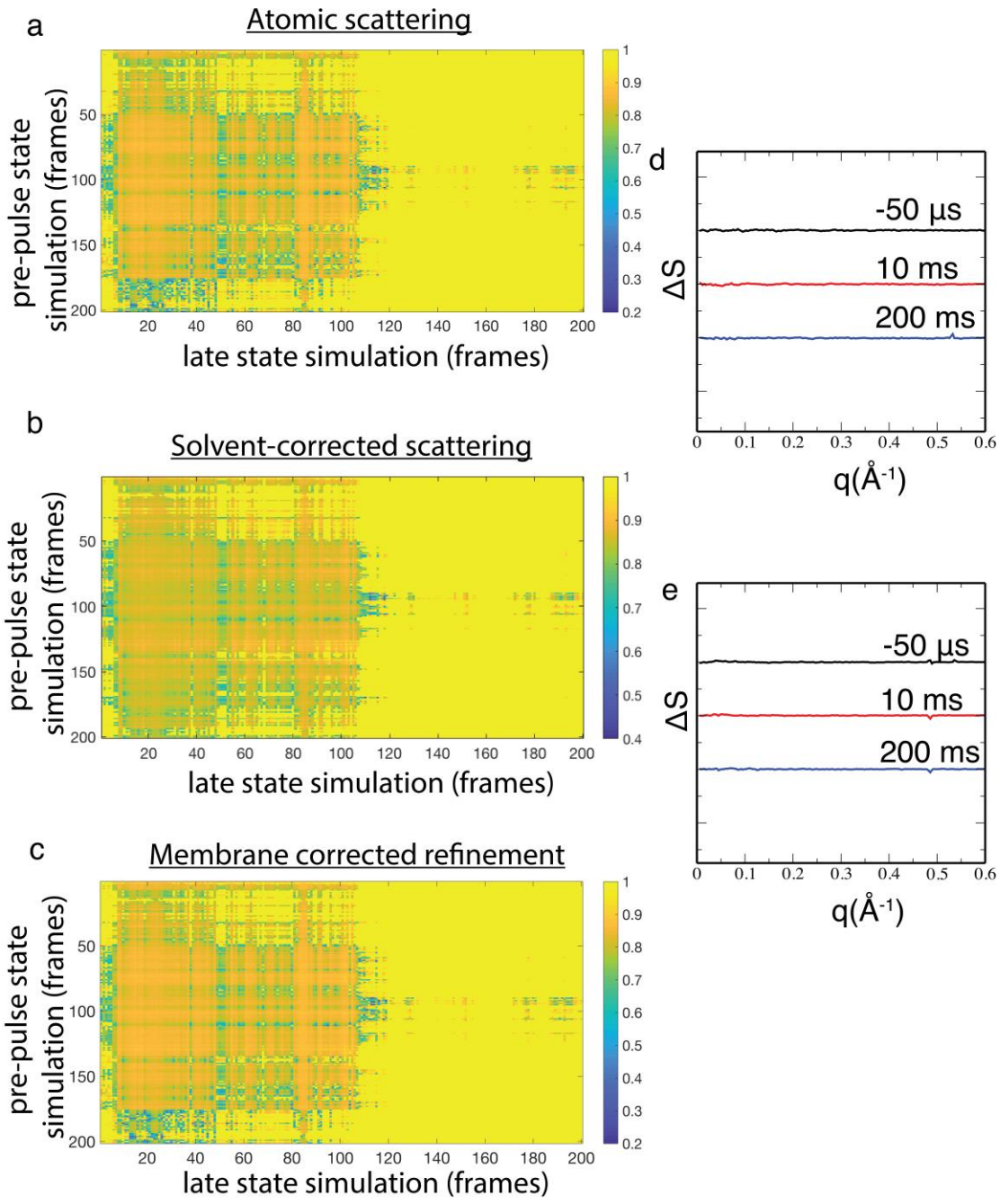

Fig. S6. Solvent and membrane scattering contributions and control TR-XSS experiments. R-factor matrices that correlate simulation trajectories to the late basis spectra compared between **a**, atomic scattering differences, **b**, solvent-corrected scattering, and **c**, membrane-corrected scattering refinement protocols. Control experiments at time delays -50 µs, 10 ms, and 200 ms showing no significant difference signal at 0 $Å^{-1}$ < q < 0.6 $Å^{-1}$ in **d**, buffer solution and **e**, caged ATP (10

mM) in buffer solution. The vertical scale is similar to that of the displayed experimental data in Fig. 2A.

Table S1. Generating difference scattering using linear combinations of calculated scattering from crystal structures of putative prepulse states. The R-factors were calculated from a single fit to the intermediate and late basis spectra. The calculated difference spectra were obtained by subtracting the following linear combinations of crystal structures from each intermediate state: Linear combination 1: $[Ca_2]$E1+ E2 + $[Ca_2]$E1ATP (PDB IDs 2C9M, 3NAL, 3N8G), Linear combination 2: $[Ca_2]$E1+ E2 (PDB IDs 2C9M, 3NAL), Linear combination 3: $[Ca_2]$E1+ $[Ca_2]$E1ATP (PDB IDs 2C9M, 3N8G), and Linear combination 4: E1 + E2 (PDB IDs 4H1W, 3NAL).

Transient enzymatic states	Crystal structure PDB ID	Linear Combination 1		Linear Combination 2	
		Intermediate state R-factor	Late state R-factor	Intermediate state R - factor	Late state R-factor
$[Ca_2]$E1ATP	3N8G	0.77	0.96	0.77	0.96
$[Ca_2]$E1P-ADP	1T5T	0.77	0.96	0.77	0.96
$[Ca_2]$E1P:ADP	3BA6	0.76	0.96	0.76	0.94
E2P	3B9B	0.68	0.91	0.70	0.93
E2-P	3N5K	0.72	0.83	0.73	0.92
E2:Pi	3FGO	0.78	0.92	0.77	0.94
E2	3NAL	0.92	0.88	0.64	0.89
Transient enzymatic states	Crystal structure PDB ID	Linear Combination 3		Linear Combination 4	
		Intermediate state R-factor	Late state R-factor	Intermediate state R - factor	Late state R-factor
$[Ca_2]$E1A	3N8G				

TP		0.74	0.94	0.77	0.96
[Ca_2]E1P-ADP	1T5T	0.74	0.95	0.77	0.96
[Ca_2]E1P:ADP	3BA6	0.73	0.94	0.76	0.94
E2P	3B9B	0.70	0.88	0.70	0.93
E2-P	3N5K	0.89	0.76	0.73	0.92
E2:Pi	3FGO	0.73	0.89	0.77	0.94
E2	3NAL	0.92	0.88	0.65	0.89

Table S2. Crystal structures (and PDB IDs) corresponding to principal transient enzymatic states in the SERCA reaction cycle considered in the structural refinement. The R-values were calculated from fitting the intermediate and late basis spectra to the difference spectra, which were obtained by subtracting calculated spectra from the [Ca_2]E1state (PDB ID: 2C9M) and each of the intermediate state crystal structures, simulated transition states- and states extracted from membrane simulations. For the membrane simulations, the mean and standard deviation from the 10 best scoring pairs are reported.

Transient enzymatic states	Crystal structure PDB ID	Crystal structure		Transition dynamics simulation R-factor		Membrane simulation R-factor (vs pre-pulse TR-XSS simulation)	
		Intermediate	Late	Intermediate	Late	Intermediate	Late
[Ca_2]E1ATP	3N8G	0.73	0.94	**0.51**	0.84	0.48 (0.50±0.02)	0.74 (0.75±0.01)
[Ca_2]E1P-ADP	1T5T	0.73	0.94	**0.51**	0.84		
[Ca_2]E1P:ADP	3BA6	0.73	0.95	**0.52**	0.87		
E2P	3B9B	0.65	0.90	0.67	**0.61**		
E2-P	3N5K	0.69	0.89	0.90	**0.58**	0.82 (0.83±0.01)	0.30 (0.30±0.01)
E2:Pi	3FGO	0.64	0.91	0.85	**0.71**		
E2	3NAL	0.64	0.88	0.81	**0.64**		

Table S3. Structural similarity (RMSD) between domains. M, A, P, and N in TR-XSS models of ten pairs pre-pulse, intermediate, and late state models which had the least R-values along with known crystal structures.

TR-XSS states	Reference State	RMSD M domain (Å)	RMSD A domain (Å)	RMSD P domain (Å)	RMSD N domain (Å)
Pre-pulse state (vs Intermediate basis spectrum)	$[Ca_2]$E1 (2C9M)	3.4 ± 0.1	12 ± 0.5	5.9 ± 0.6	27 ± 4
Pre-pulse state (vs Late basis spectrum)	$[Ca_2]$E1 (2C9M)	3.6 ± 0.1	10 ± 2.1	5.9 ± 1	29 ± 3
Intermediate	$[Ca_2]$E1ATP (3N8G)	5.7 ± 0.1	11 ± 0.7	5.8 ± 0.6	6.9 ± 3
Late	$[Ca_2]$E1P:ADP (3BA6)	6.4 ± 0.4	21 ± 3	7.3 ± 0.9	12± 2
Late	E2P (3B9B)	6.4 ± 0.2	17 ± 4	7.1 ± 1	23 ± 2
Late	E2~P (3N5K)	6.7 ± 0.5	20 ± 5	9.3 ± 1	21 ± 3

Table S4. List of abbreviations.

Abbreviation	**Expansion**
ESRF	European Synchrotron Radiation Facility
SR	Sarcoplasmic Reticulum
SERCA	Sarco/Endoplasmic Reticulum Ca^{2+} ATPase
TR-XSS	Time-Resolved X-ray solution scattering
SVD	Singular Value Decomposition
MD simulations	Molecular Dynamics simulations
ATP	Adenosine Tri-Phosphate
TM helices	Trans-Membrane helices
A domain	Actuator domain
N domain	Nucleotide-binding domain
P domain	Phosphorylation domain
RMSD	Root Mean Square Deviation
$[Ca_2]$E1	Calcium-bound, ATP-free state
$[Ca_2]$E1ATP	Calcium-occluded ATP-bound state
$[Ca_2]$E1P-ADP	Calcium-occluded, ADP-bound transition state of phosphorylation
$[Ca_2]$E1P:ADP	Calcium-occluded, ADP-bound phosphoryl aspartate state
E2P	Phosphoenzyme intermediate state following calcium release

E2-P	Transition state of dephosphorylation
E2:Pi	Phosphate product complex intermediate