Supporting information

Supporting figures:

Fig. S1 Allosteric processes of nCaM recorded by fraction of discrepant native contacts Q. Native contact map of *Apo* (PDB ID 1CFD) and *Holo* (PDB ID 1J7O) structures of nCaM. Contacts only in *Apo* structure show in solid red triangles, only in *Holo* structure show in solid green circles; common in both *Apo* and *Holo* structures present in solid black squares. (B) and (C) The variation of fraction of discrepant native contacts Q versus simulation time in Traj1 and Traj2 respectively, where the green line is for *Holo* and the red line is for *Apo*, with native contacts corresponding to the same color points in (A). The vertical lines correspond to the time Ca^{2+}-binding completely, same as those in Fig 1C and 1D.

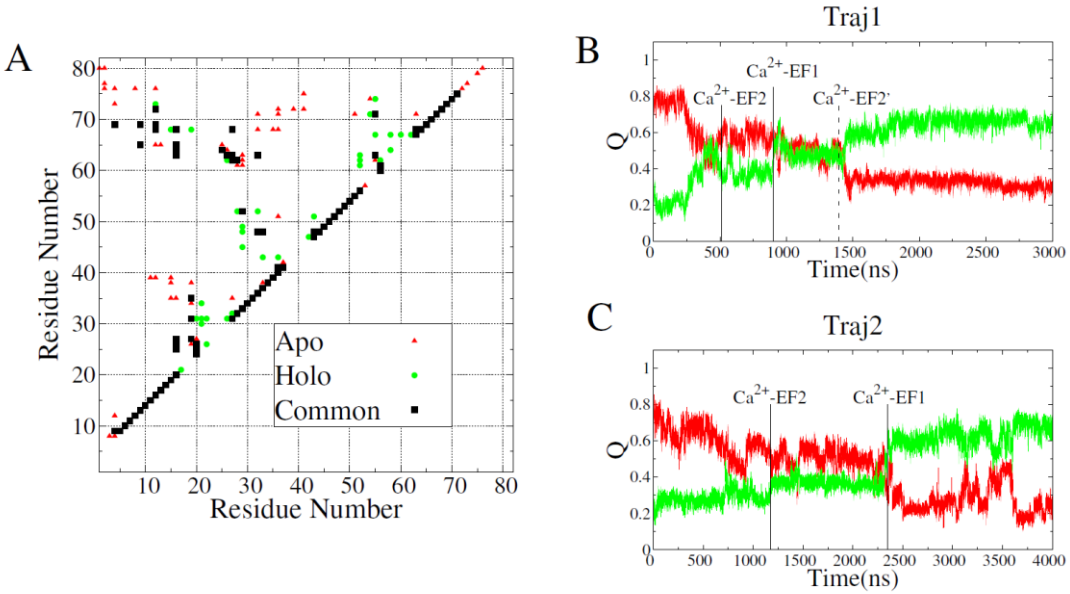


Fig. S2 Ca^{2+} binding process. (A) and (B) Ca^{2+} binding process in Traj1 and Traj2. For each column, the first and the second panels present the variation of distance between Ca^{2+} ion and six oxygen atoms supposed to be ligated in each EF-loop. Lines in different color indicate the oxygen atoms from different amino acids. The third and the fourth panels display the recorded torsion φ (in red) and ψ (in green) of Ile27 and Ile63. The bottom panels denote the RMSD records of EFβ-scaffold defined by Grabarek,[1, 2] taking *Holo* structure as reference.

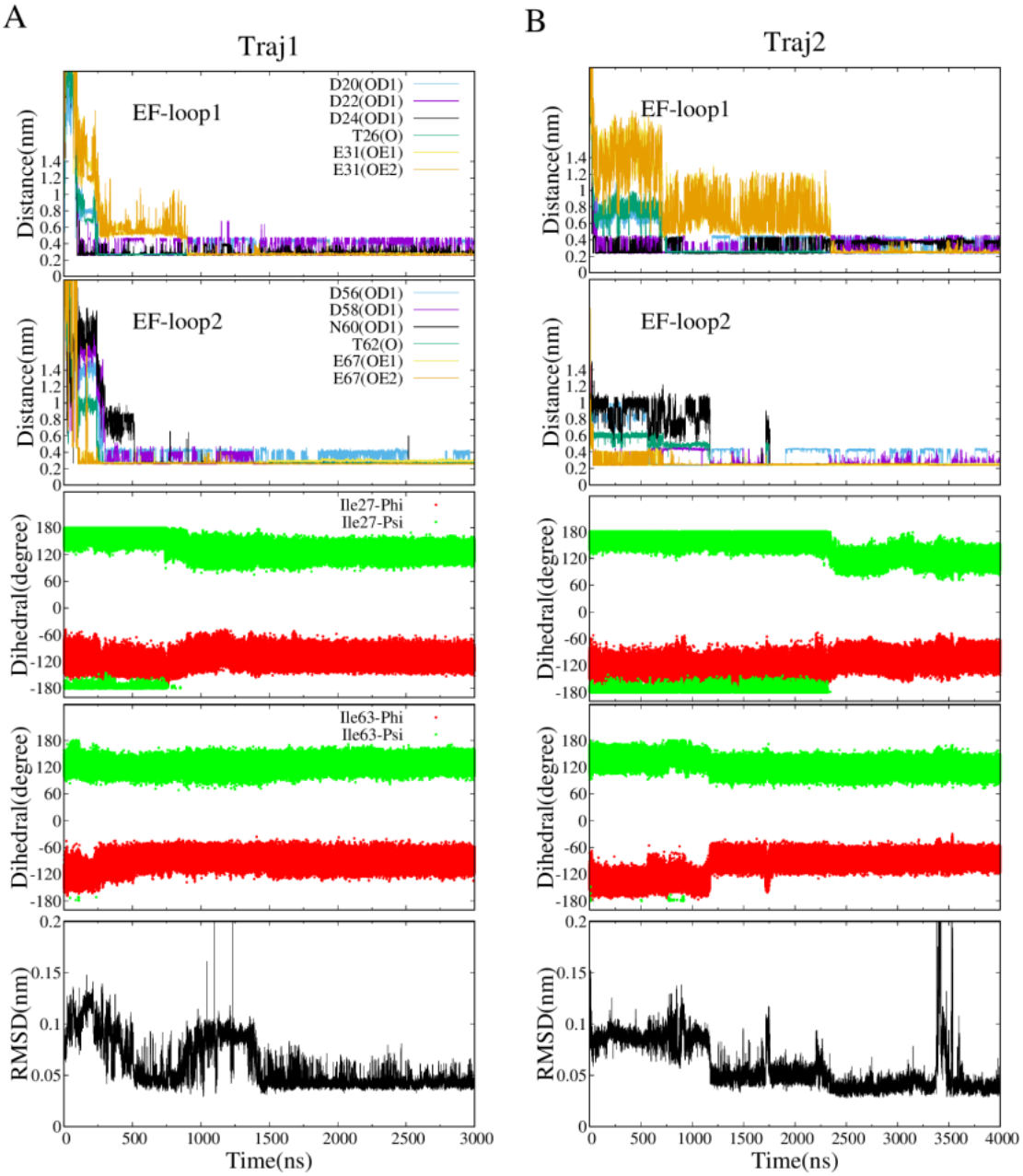


Fig. S3 RMSF of each residue in equilibrium simulations. The red line presents the RMSF values from 1.01 μs simulation for *Apo* structure; the green line for *Holo* structure with Ca^{2+} parameters from Amber99sb-ildn; the blue line for *Holo* structure with modified Ca^{2+} parameters.

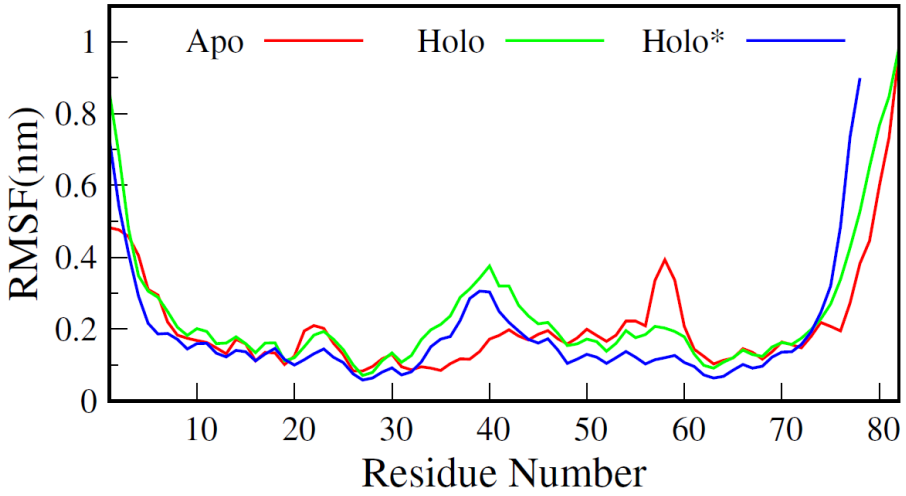


Fig. S4 Binding of other Ca^{2+} ions. (A) One example of Ca^{2+} ion dynamically binds to positively charged residue E6 and D50 indicated by the measurement of minimal distance between them. (B) The exceptional Ca^{2+} ion which binds to D58 and D64 at around 1395 ns and then keep staying the position until end in Traj1.

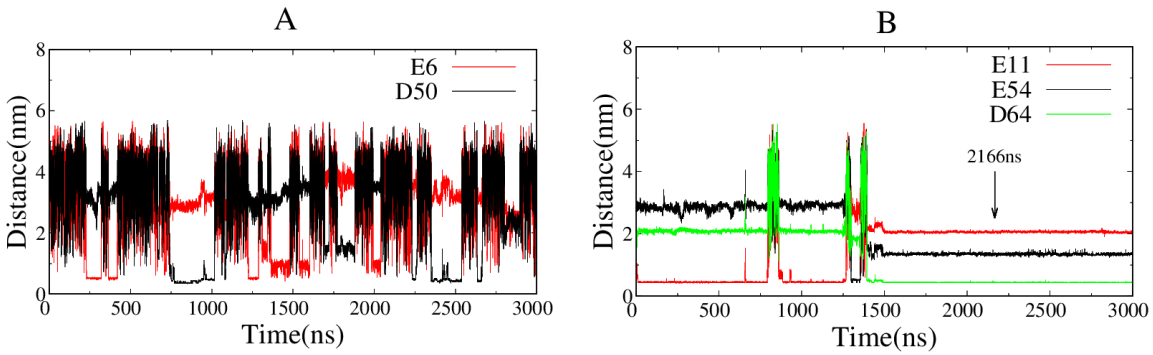


Fig. S5 Conformation changes of EF-hand motifs in specific simulation time regions of Traj1. (A) and (B) Conformational changes of EF-hand motifs in time region 945ns-965ns and 1360ns-1500ns in Traj1. Same as Fig 4, for each column, the top three panels present for EF-hand 1 and the fourth to the sixth panels present for EF-hand 2; the bottom panel presents RMSD of EFβ-bridge region. The angles (black line for φ and magenta line for θ) and outer-end-point distance between two helixes in EF-loop are shown in the second and the fifth, as well as the third and the six panels, respectively, with the corresponding value in *Holo* structure shown as horizontal line in same color. The red and blue lines in the panels of RMSD records indicate the reference structure comes from experimental Apo and Holo conformation respectively. The vertical line in (B) denotes the moment when exceptional Ca^{2+} ion binds with D58 and D64.

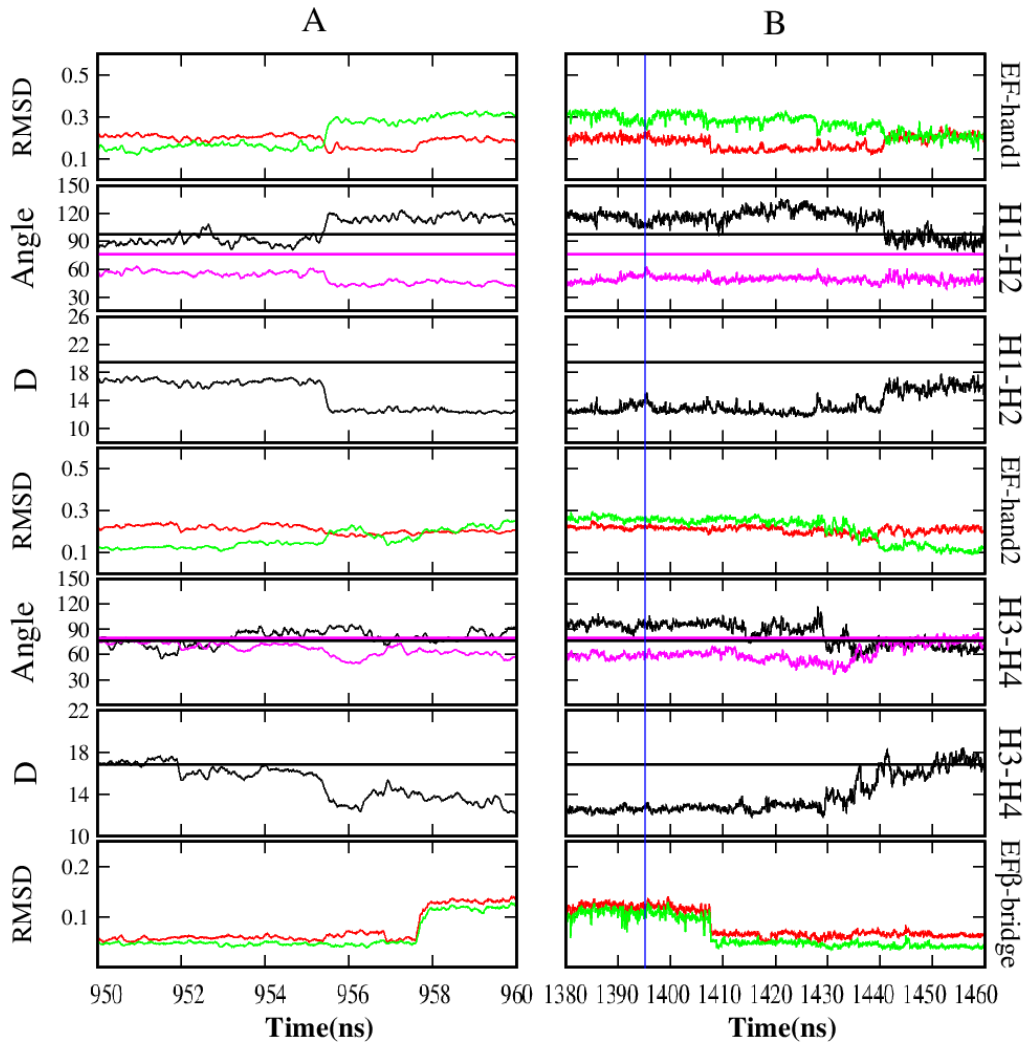
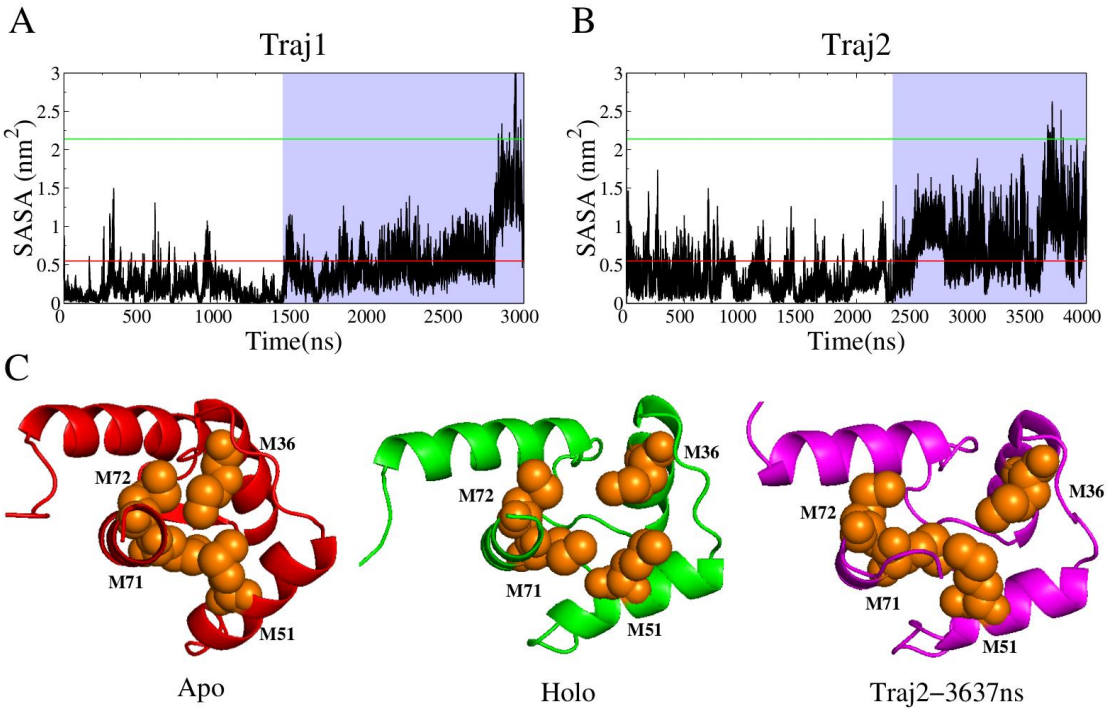


Fig. S6 Hydrophobic core explosion analysis for nCaM. The SASA summation of four methionines (M36/M51/M71/M72) versus simulation time in Traj1(A) and Traj2(B). The red and green lines indicate the calculated SASA values for Apo and *Holo* structures determined by experiments, respectively. The shadowed indicate the time regions of transformed *Holo* conformations. (C) Cartoon structures of *Apo* (PDB code 1CFD) and *Holo* (PDB code 1J7O) nCaM and the snapshot (3637 ns with SASA 2.19 nm^2) in Traj2. The side chains of four residues M36, M51, M71 and M72 are presented in brown sphere.



Supporting tables:

Table S1. Ca^{2+} and other ions parameters. Here, ε is the depth of the potential well and σ is the finite distance at which the inter-particle potential is zero for ions in Lennard-Jones (LJ) 12-6 potential. CN indicates the coordination number of hydration oxygen. IOD indicates the ion−oxygen distance in the first solvation shell. The Modified Ca^{2+} and Pb^{2+} as well as Ba^{2+} parameters come from table 8 of (Li et al., 2013)[3] where they were used with TIP3P water model. Ca^{2+} parameter in Amber99sb-ildn force field comes from (Aqvist, 1990)[4] Parameters of "Ca^{2+} (experiment)" come from (Jalilehvand et al., 2001).[5]

Ion parameter	σ(Å)	ε(kcal/mol	charge(e)	CN	IOD(Å)
Ca^{2+} (Experiment)	-	-	-	8	2.46
Ca^{2+}(AMBER99SB-ILDN)	3.0524	0.459789	2	8.9	2.7
Ca^{2+} (Modified)	2.9382	0.105929	2	8	2.49
Pb^{2+}	3.1092	0.170180	2	8.7	2.62
Ba^{2+}	3.5974	0.406646	2	9.7	2.94

Table S2. MD simulations in this work and the residue order of Ca^{2+} binding in each simulation. The bold indicates the allosteric transition is detected in the simulation (No.3 corresponds to Traj1 and No.10 corresponds to Traj2). The italic indicates the EF-loop binds with two Ca^{2+} ions, one in its right position, the other ligated residue D58 and D64 same as Fig 3A.

Ca^{2+} paramete	**No**	**Simulation Time**	**Ca^{2+} binding order**
AMBER99 SB-ILDN	1	1010ns	Ca^{2+}-free (*Apo*)
	2	1010ns	Ca^{2+}-loaded **(***Holo*)
	3	**3000ns**	**EF-loop1 D24(96ns)→D22(104ns)→T26(247ns)→D20(248ns)→E31(899.5ns)** **EF-loop2 E67(250ns)→T62(242ns)→D56(288ns)→D58(299ns)→N60(513ns)**
	4	4495ns	EF-loop1 D24(320ns) →T26(463ns) →D20(468ns) *EF-loop2 D58(11ns) →D56(56ns) →T62(123ns) →E67(588ns) →N60(2723ns)*
	5	4760ns	EF-loop1 E31(100ns) EF-loop2 D58(177ns) →E67(228ns)
	6	5000ns	EF-loop1 D24(10ns) →D22(24ns) →T26(69ns) →D20(99ns) EF-loop2 E67(21ns) →D56(34ns) →D58(59ns) →T62(307ns) →N60(4892ns)
	7	5000ns	EF-loop1 D24(23ns) →D22(244ns) →E31(3689ns) EF-loop2 E67(34ns) →D56(582ns) →D58(650ns)
	8	5000ns	EF-loop1 D24(490ns) →D22(500ns) →T26(790ns) EF-loop2 E67(200ns) →D58(417ns) → T62(655ns) →D56(1022ns)
Modified	9	1010ns	Ca^{2+}-loaded **(***Holo*)
	10	**4000ns**	**EF-loop1 D24(32ns)→D22(71ns)→T26(735ns) →D20(759ns)→E31(2348ns)** **EF-loop2 D58(31ns)→D56(562ns)→E67(715ns)→T62(1171ns)→N60(1173ns)**
	11	5000ns	EF-loop1 D24(6ns) →D22(335ns) →T26(385ns) →D20(386ns) EF-loop2 D58(27ns) →E67(28ns) →D56(4458ns)
	12	5000ns	EF-loop1 D24(74ns) →D22(90ns) →D20(181ns) →T26(185ns) EF-loop2 D56(27ns) →58D(1142ns) →E67(3778ns)
	13	4165ns	EF-loop1 D24(24ns) →D22(30ns) →D20(78ns) →T26(82ns) →E31(2246ns) EF-loop2 D58(126ns) →T62(361ns) →D56(849ns) → E67(3926.7ns)
	14	4450ns	EF-loop1 D22(159ns) →D24(183ns) →D20(509ns) →T26(511ns) EF-loop2 D58(56ns) →D56(142ns)
	15	4450ns	**EF-loop1 D24(2ns) →D22(36ns) →T26(183ns) →D20(633ns) →E31(2192ns)** EF-loop2 D58(42ns) →D56(43ns)

Table S3. The moments of Ca^{2+} binding completely and the time regions of conformational transition in Traj1 and Traj2.

	Ca^{2+} binding completely		**Conformational transition**		
	Ca^{2+}-EF-loop1	**Ca^{2+}-EF-loop2**	**EF-hand1**	**EF-hand2**	**nCaM**
Traj1(1)	899.5	513.1	934.2-934.4	906.5-934.4	906.5-934.4
Traj1(2)	-	-	1427.7-1441.1	1433.5-1441.1	1427.7-1441.1
Traj2	2347.9	1173.4	2393.3-2393.9	2315.4-2320.4	2315.4-2393.9

The time unit is nanosecond.

Table S4. The values of angle θ, φ and outer-end-point distance *D* between two helixes in each EF-hand from experimentally determined structures (*Apo* from PDB 1CFD and *Holo* from PDB 1J7O) according to VGM definition. [6]

	Structure	θ (degrees)	φ (degrees)	*D* (Å)
EF-hand1	*Apo*	44.92	117.01	12.18
	Holo	76.00	97.45	19.46
EF-hand2	*Apo*	50.84	102.01	13.13
	Holo	79.75	76.27	16.86

Table S5. Artificial Ca^{2+} binding simulation with different strategies. The italic indicates the EF-loop binds with two Ca^{2+} ions, one in its right position, the other ligated residue D58 and D64 as shown in Fig 3A. The bold indicates the allosteric transition is detected in the simulation. The brackets means the residue is ligated by Ca^{2+} during the simulation but disassociated later.

Ca^{2+} parameters	Strategy	No	Simulation time	Ligated residues
AMBER99SB-ILDN	A	1	1000ns	EF-loop1 E31 *EF-loop2 D56,D58,N60,T62,E67*
		2	1000ns	EF-loop1 D22,D24 EF-loop2 D56,D58,N60,T62
		3	**1000ns**	**EF-loop1 D20,D22,D24(d),T26,E31** **EF-loop2 D56,D58,N60(d),T62,E67**
		4	1000ns	EF-loop1 E31 EF-loop2 D56,D58,N60,T62,E67
		5	1000ns	EF-loop1 EF-loop2 D56,D58,T62,E67
	B	6	724ns	EF-loop1 D20,D22,D24,T26,E31 EF-loop2 D56,D58,T62,E67
	C	**7**	**500ns**	**EF-loop1 D20,D22,D24,T26,E31** **EF-loop2 D56,D58,N60,T62,E67**
Modified	A	8	1000ns	EF-loop1 D22,D24,T26 EF-loop2 D56,D58,T62,E67
		9	1000ns	EF-loop1 D20,D22,D24,T26 EF-loop2 D56,D58,E67
		10	1000ns	EF-loop1 D22,E31 EF-loop2 E67
		11	1000ns	EF-loop1 D20,D22 EF-loop2 D56,D58,E67
		12	1800ns	EF-loop1 D20,D22,D24,T26 EF-loop2 D56,D58,N60,T62,E67
	B	**13**	**340ns**	**EF-loop1 D20,D22,D24,T26,E31** **EF-loop2 D56,D58,N60,T62,E67**
	C	14	893ns	EF-loop1 D20,D22,D24,T26,E31 EF-loop2 D56,D58,N60,T62,E67

Strategy A – Ca^{2+} ions were placed near EF-loops in starting structure.

Strategy B – Based on strategy A, the distance restraint between Ca^{2+} ion and the six oxygens supposed to bind with in each EF-loop was executed in pre-equilibration simulation.

Strategy C– Based on strategy A, the distance restraint between Ca^{2+} ion and the six oxygens supposed to bind with in each EF-loop was executed in whole simulation.

References

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2. Z. Grabarek, *J. Mol. Biol,* 2006, **359** , 509-525.
3. P.F. Li, B.P. Roberts, D.K. Chakravorty, K.M. Merz, *J Chem Theory Comput*, 2013, **9** , 2733-2748.
4. J. Aqvist, *J. Phys. Chem,* 1990, **94**, 8021-8024.
5. F. Jalilehvand, D. Spangberg, P. Lindqvist-Reis, K. Hermansson, I. Persson, M. Sandstrom, *J Am Chem Soc,* 2001, **123** , 431-441.
6. K.L. Yap, J.B. Ames, M.B. Swindells, M. Ikura, *Proteins,* 1999, **37**, 499-507.