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Identification of Calcium binding sites on calsequestrin 1 and its implications to polymerization

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Table S1: RMSD and Entropy calculations for CASQ1 in various systems at different [Ca^{2+}].

Simulation system (with number of Ca^{2+} ions)	**RMSD of backbone atoms** (in Å)			**Entropy** (in J/molK)
	CHAIN C	CHAIN B	DIMER	C-TER (residues 350-364)
120	2.8+/-0.5	2.7+/-0.7	3.3+/-0.7	1715
80	3.2+/-0.7	2.8+/-0.4	3.5+/-0.7	1705
40	3.0+/-0.8	3.9+/-0.8	4.9+/-1.2	1859
20	2.7+/-0.5	2.9+/-0.6	4.5+/-1.4	1896
0	3.5+/-0.6	4.0+/-0.9	5.7+/-1.4	1875

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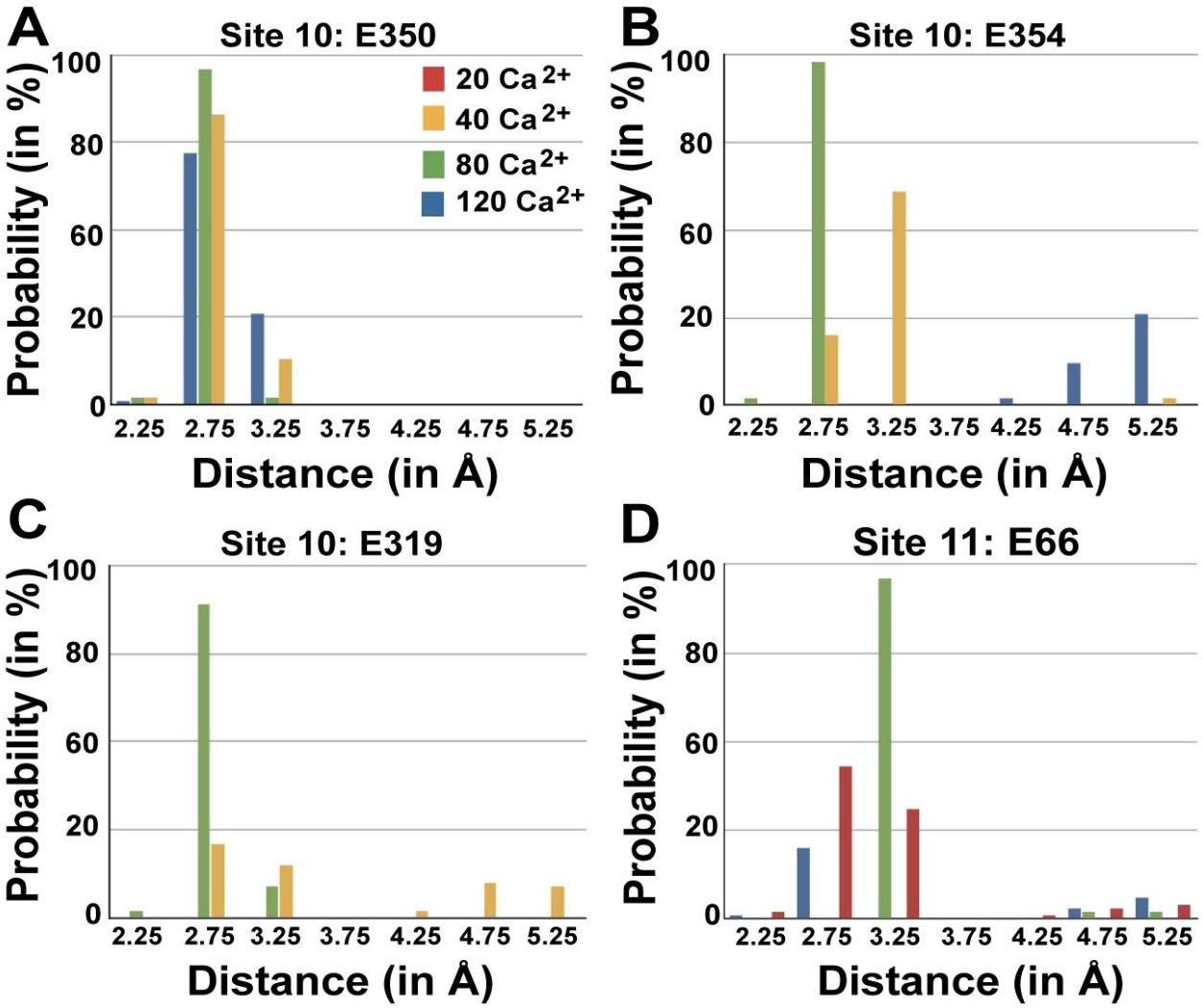
Supplemental Figure 1



Supplemental Figure 1: Probability of Ca^{2+}-binding at the [Ca^{2+}] dependent intermediate affinity sites on CASQ1. The site 10 (constituted by residues E319, E350 and E354) on CASQ1 is shown as an example of [Ca^{2+}] dependent intermediate affinity sites. **(A)** Residue E350 of site 10 show high probability of Ca^{2+}-binding at distance below 3.0Å when [Ca^{2+}] in the system is 40 and above. **(B)** Residue E354 in site 10 show high probability of Ca^{2+}-binding at distance around 3.0Å only when 40 or 80 Ca^{2+} ions are present in the system. But in the system with 120 Ca^{2+} ions the distance between the Ca^{2+} ions and the residue is high. **(C)** E319 binds Ca^{2+} ion with very high affinity and geometry when system [Ca^{2+}] is 80. E319 does not bind Ca^{2+} ions when [Ca^{2+}] in the system increased to 120. **(D)** Site 11 (formed by single acidic residue E66) on CASQ1 is a **[**Ca^{2+}] independent low affinity site. Many (~25 sites) Ca^{2+}-binding sites were found on CASQ1 surface that bind Ca^{2+} without any specific geometry at any [Ca^{2+}] randomly.

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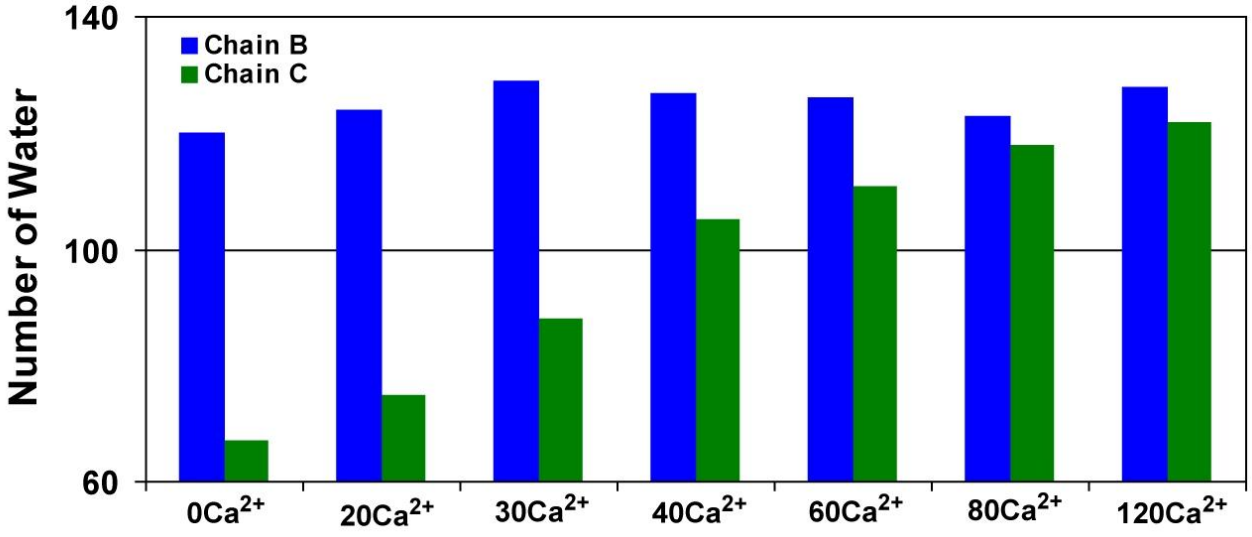
Supplemental Figure 2



Supplemental Figure 2: Presence of water molecules around the CAS region of each CASQ1 chain. CAS of chain B which is located at the inter-molecular back-to-back dimeric interface of CASQ1 does not show Ca^{2+}-dependent water binding. On the other hand the CAS of chain C bind more water with increasing Ca^{2+}-binding. It is important to point out that the back-to-back stacking which is stabilized by hydrophobic interactions is not dependent on water mediated stabilization unlike the free CAS.

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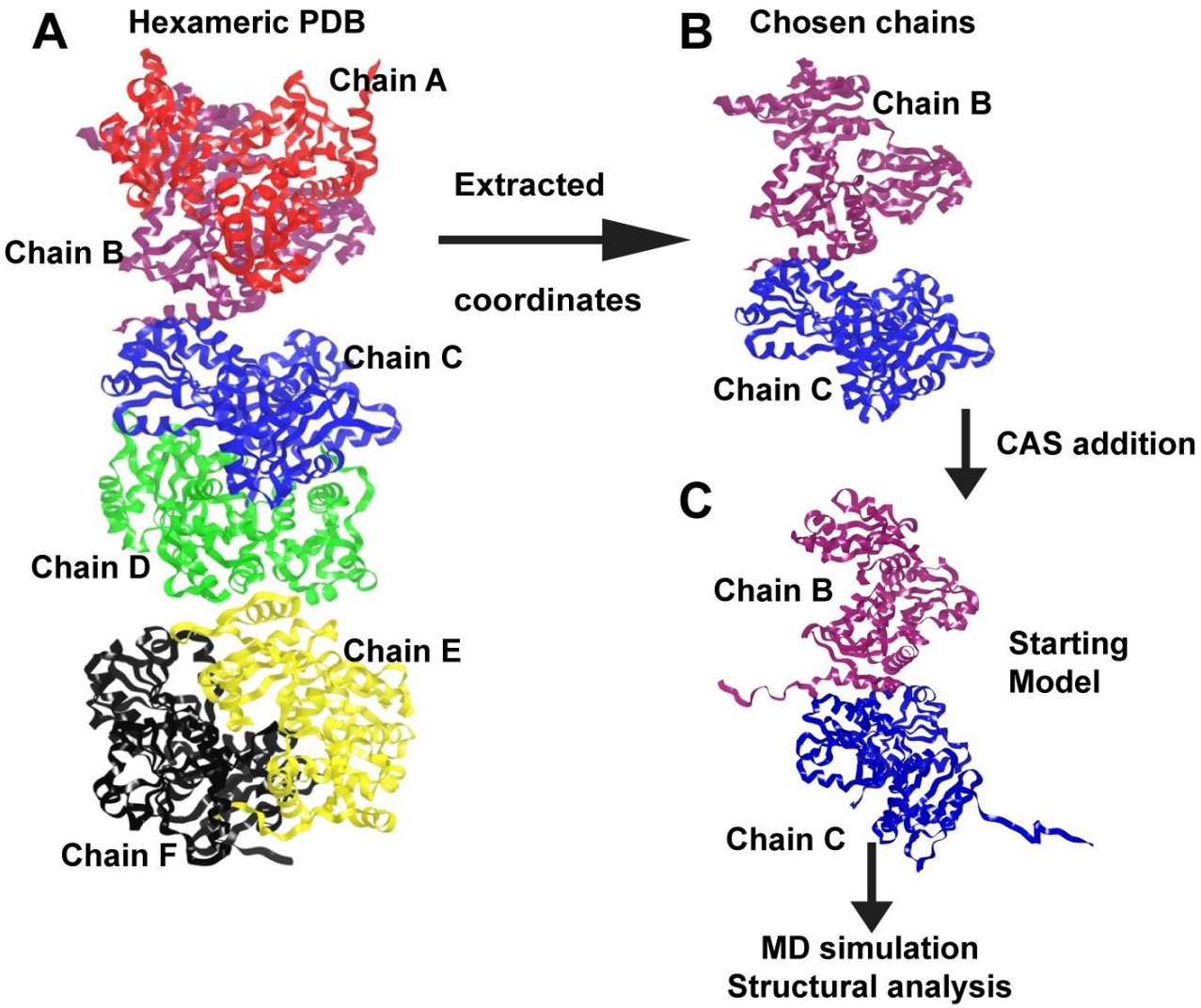
Supplemental Figure 3



Supplemental Figure 3: The flow chart of method employed in this paper. (A) The hexameric PDB id: 3UOM. **(B)** Borrowed the coordinates for the chain B and C that are in back-to-back interaction. **(C)** C-terminal missing residues are added and the incorrect residues are corrected.