Regulation of Bestrophins by Ca^{2+}: a Theoretical and Experimental Study

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Text S1

Test of the accuracy of the force field used in the MD simulation.

Structures and energetic of Ca^{2+}-binding proteins are difficult to reproduce with current classical force fields [1,2]. Factors, which limit the accuracy, include the lack of polarizability and charge transfer effects [3]. Here we tested the accuracy of three commonly used force fields: the Aqvist [4], the Bradbrook [5] and the AMBER [6,7] force fields. We performed 20-ns MD calculations of TSP-1 $T3_6$ for which the X-ray structure is available [8] and compared X-ray and MD structures.

TSP-1 $T3_6$ is a 39 amino acids long loop resembling EF hands motif in the spacing of acidic side chains [9,10]. It binds five Ca^{2+} ions by eleven Asp and two Asn residues (See Figure). Here we considered a model in which all five ($Ca_{1\text{-}5}$) calcium ions were present. The TSP-1 $T3_6$ domain was inserted in a box of edges 46, 51 and 49 Å filled with ~3700 water molecules and 2 Cl^- counterions. Computational details for the relaxation and unrestrained MD simulations were the same as reported in the main text of the paper.

In the three simulations, the final structure turned out to be rather similar to the X-ray structure: the RMSD values calculated onto the N-terminal and C-terminal halves are indeed equal to 3.2 Å and 2.4 Å, respectively (see table below) and $Ca_{1\text{-}4}$ remained bound. However, the total backbone RMSD from the X-ray structure was relatively large because of conformational changes of Ca_5–binding groups (D880 and D887) upon Ca_5 solvation after few ns (~2 ns for Aqvist- and Bradbrook-based MD, ~1.3 ns for the AMBER-based MD). The loss of the Ca^{2+} ion might be caused by several factors, including the limitations of the accuracy of the force field describing the Ca^{2+} ions [2] and/or the absence of electrostatic packing forces in the simulation with respect to the crystal, along with a difference in temperature (the simulation was run at 300 K, the crystal structure was solved at 100 K). Force-matching methods, which were already applied by us for other metal-containing systems [11], might be used to further address this issue, which is however beyond the

scope of the present paper and the severe computational resources that would be needed prevent the application of these methods to the present problem. In all of the other calculations presented in this work we have indeed used the AMBER force field.

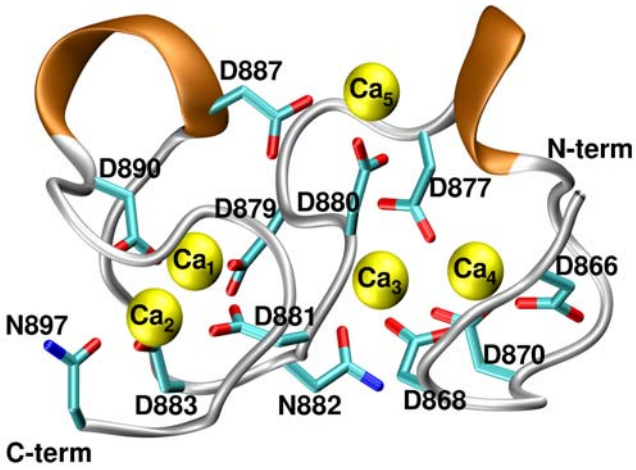

Figure. Crystal structure of TSP-1 $T3_6$ domain. Five Ca^{2+} ions are bonded to the Asp/Asn residues. For clarity, Ca^{2+} ions are indicated as $Ca_{1\text{-}5}$ according to their binding positions. The same labels are used throughout the text in the model of the hBest1 Asp-rich domain.

Because of its limitations, we used molecular modeling to identify possible Ca^{2+}-binding conformations of the Asp-rich domain and alanine mutations, which could affect Ca^{2+} binding. No attempts were made to predict the structure of disease-linked mutants [12] and/or mutants for which electrophysiological experiments have been previously carried out [13] .
N-term corresponds to residues from A865 to H878, whereas C-term corresponds to residues from D879 to N897.

Table. TSP-1 $T3_6$ backbone RMSD values relative to the X-ray structure after 20-ns MD simulations based on three different force fields.

	Aqvist [4]	Bradbrook [5]	AMBER [6,7]
Total RMSD (Å)	3.5	5.2	5.5
RMSD of N-term (Å)	3.5	3.8	3.2
RMSD of C-term (Å)	2.6	4.3	2.4

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