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Commentary

Does stromal interaction molecule-1 have five senses?

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ABSTRACT

A single calcium (Ca^{2+}) binding site within the canonical EF-hand loop was thought to govern the stromal interaction molecule-1 (STIM1) structural changes that lead to activation of Orai1 Ca^{2+} channels. Recent work by Gudlur et al., published in *Nat Commun* [9(1):4536], suggests that the STIM1 endoplasmic reticulum (ER) luminal domain has ~5 additional Ca^{2+} binding sites, which underlie a surprising new proposal for Ca^{2+} sensing.

Store operated calcium entry (SOCE) is the cellular process whereby endoplasmic reticulum (ER) luminal calcium (Ca^{2+}) depletion causes Ca^{2+} channels on the plasma membrane (PM) to open, elevating cytosolic Ca^{2+} and effecting myriad signaling pathways. Stromal interaction molecule-1 (STIM1) functions as the ER Ca^{2+} sensor, and Orai1 makes up the PM Ca^{2+} channel; together, these proteins principally mediate SOCE in most cell types [1,2]. Within its ER lumen-oriented region, STIM1 contains canonical and non-canonical EF-hand motifs that interact and form a hydrophobic pocket when Ca^{2+} is coordinated in the canonical EF-hand loop, analogous to hundreds of other EF-hand domains [3,4]. This EF-hand pocket closely associates with the adjacent sterile α-motif (SAM) domain of STIM1, forming a compact globular structure in the presence of Ca^{2+} [5] (Fig. 1A). Together, the EF-hand and SAM domains of STIM1 are termed 'EFSAM'. The cytosol-oriented region of STIM1 contains a series of conserved coiled-coils. In SOCE, ER luminal Ca^{2+} depletion causes structural changes in STIM1 EFSAM, which propagate to the cytosolic coiled-coils, ultimately promoting direct coupling to Orai1 and opening of the Ca^{2+} channels [1,2].

In isolation, Ca^{2+}-loaded EFSAM exists as a monomer, and high-resolution structures have been elucidated in this stable state [5,6]. In contrast, Ca^{2+}-depleted EFSAM is destabilized, partially unfolds, dimerizes and oligomerizes, biophysical changes previously suggested to trigger the cytosolic structural rearrangements that enable the activation of Orai1 channels [7,8]. Biochemical analyses of the isolated EFSAM and canonical EF-hand motif suggest a single Ca^{2+} binding site with a dissociation constant (K_d) of ~200–500 μM [7–9]. Given that the STIM1 coiled-coils are dimeric in the quiescent state [1], Gudlur et al. [10], cleverly designed a soluble EFSAM construct fused to the *Thermus thermophilus* GroP-like gene *E* (GrpE) protein to study the Ca^{2+} sensing properties of EFSAM when constricted in dimeric GrpE space.

Gudlur et al., found that Ca^{2+} depletion increased the EFSAM-EFSAM FRET within artificial EFSAM-GrpE dimers. However, the midpoint of the transition to lower FRET was found to occur at ~1–10 μM of Ca^{2+}, much lesser than the estimated Ca^{2+} binding affinity of the canonical EF-hand [7–9] and the midpoint for STIM1 activation previously characterized in cells [11,12]. Remarkably, isothermal titration calorimetry (ITC) and D4 cameleon fluorescence Ca^{2+} sensor competition experiments suggested ~5-6 Ca^{2+} binding sites exist per EFSAM monomer. Interestingly, disruption of Ca^{2+} coordination within the canonical EF-hand loop via the D76A mutation abrogated all Ca^{2+} binding sites.

To study the structural determinants of the multiple apparent Ca^{2+} binding sites, three clusters of negative charge-neutralizing EFSAM mutations were designed and incorporated into full-length STIM1. A 4-residue mutation cluster at the N-terminal region and a 6-residue mutation cluster on the SAM domain did not affect the ability of full-length STIM1 to form ER Ca^{2+} depletion-dependent puncta. On the other hand, an 11-residue mutation cluster introduced in the EF-hand domain (i.e. D77N/D82N/E86Q/D89N/E90Q/E94Q/D100N/E111Q/D112N/E118Q/D119N, where underlined residues are located in the canonical EF-hand loop) inhibited the ability of STIM1 to form puncta. Subsequent ITC experiments using EFSAM-GrpE containing these EF-hand domain mutations showed only a single Ca^{2+} binding site. However, when Gudlur et al., mutated only a subset of these residues (i.e. E94Q/D100N/E111Q/D112 N/E118Q/D119N), a fraction of full-length STIM1 molecules were found to constitutively form puncta, and the remaining fraction showed activation after partial ER Ca^{2+} store depletion. They attributed this phenotype to weaker Ca^{2+} binding affinity based on ITC data acquired on EFSAM-GrpE containing this subset of EF-hand domain mutations still suggesting ~5-6 binding sites.

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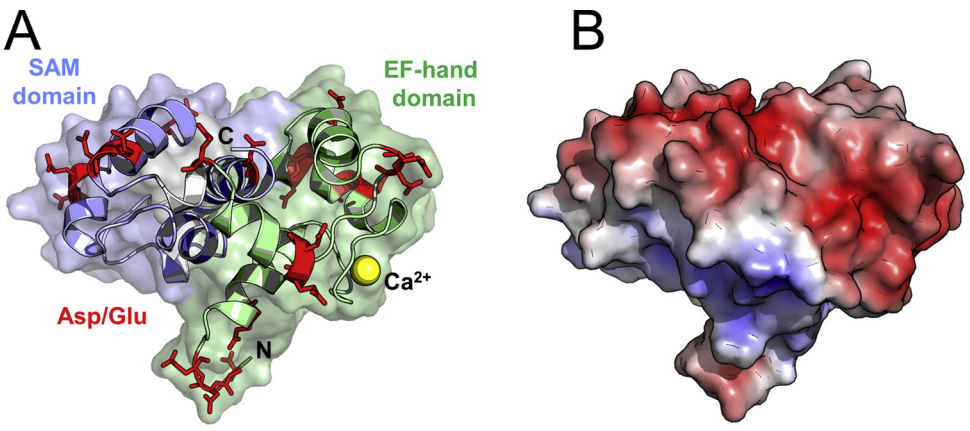

Fig. 1. Human STIM1 EFSAM solution structure. (A) Backbone cartoon representation of Ca^{2+} loaded STIM1 EFSAM. The EF-hand domain is shaded green, and the SAM domain is shaded blue. The Ca^{2+} coordinated in the canonical EF-hand loop is shown as a yellow sphere. All Asp and Glu residues, excluding the canonical EF-hand loop, are shown as red sticks. (B) Electrostatic surface potential of Ca^{2+} loaded STIM1 EFSAM. The surface potential is shown as a gradient between +5 (blue) and −5 (red) kT/e, calculated at pH 7.4 and 37 °C. The images in (*A*) and (*B*) were rendered using the 2K60.pdb coordinate file.

Interestingly, far-UV circular dichroism (CD) spectra of EFSAM-GrpE showed only a minor Ca^{2+}-induced (i.e. 1 or 10 mM $CaCl_2$) increase in apparent α-helicity, in part due to the ~50% unchanging contribution by GrpE, while spectra of EFSAM-GrpE containing the full set of EF-hand domain or D76A mutations were insensitive to Ca^{2+}. However, minor Ca^{2+}-induced changes in secondary structure were also supported by hydrogen-deuterium amide exchange experiments performed in the absence and presence of 30 μM Ca^{2+}, revealing highly protected backbone amides. After engineering Cys residues into buried EFSAM positions, Gudlur et al., also used thiol-specific biotinylation of full length STIM1 embedded in isolated ER membranes followed by pull-down experiments to show similar Cys accessibilities in the presence or absence of Ca^{2+}. Nevertheless, these EFSAM-GrpE and full-length STIM1 data must be interpreted with caution since the biochemical assay buffers contained 5% glycerol, a known EFSAM structure stabilizing agent [8], and thiol-mediated biotinylation is influenced by a number of factors besides folding, complicating interpretations on conformation in intact cells.

Collectively, the work by Gudlur et al., advocates that *i*) EFSAM binds ~5-6 Ca^{2+} ions, dependent on Ca^{2+} coordination by the canonical EF-hand loop; *ii*) the binding of at least one site occurs with a midpoint of dissociation of ~1–10 μM; *iii*) large scale unfolding of the EFSAM domain is not required to induce an active STIM1 conformation; *iv*) most of the Ca^{2+}-depletion-dependent STIM1-STIM1 FRET observed in cells can be accounted for by EFSAM-EFSAM dimerization. These data are integrated into two models of Ca^{2+} sensing by the authors. In the first model, they suggest that, despite the ~1–10 μM Ca^{2+} sensitivity detected using EFSAM-GrpE, the midpoint of Ca^{2+} dissociation at all sites is ~200 μM. In this scenario, the Ca^{2+} binding to the EFSAM surface stabilizes the EF-hand loop binding, and Ca^{2+} dissociation from the surface sites is required to activate STIM1. In the second model, they suggest that Ca^{2+} binds to the EF-hand loop with ~1–10 μM affinity, but additional Ca^{2+} binding at ~5–6 peripheral sites are required to stabilize the full-length molecule in an inactive conformation, and it is the binding and unbinding at the peripheral sites that controls the conformational switch.

Neither scenario involves large scale EFSAM unfolding, a supposition reinforced by past studies showing isolated EFSAM retains structure in the Ca^{2+}-depleted state [8] and highly structured EFSAM chimeras can activate STIM1 [6]. From an EF-hand perspective, both scenarios integrate ~5 Ca^{2+} binding sites peripheral to canonical Ca^{2+} coordination in the EF-hand loop as the crucial sensing event. There are at least 865 members in the EF-hand superfamily, and to the best of our knowledge, there is no analogous EF-hand-mediated Ca^{2+} sensing mechanism reported to date that similarly links peripheral Ca^{2+} binding [3,4]. Thus, several prudent questions arise regarding the new Ca^{2+} sensing mechanism proposed. Could GrpE-mediated constitutive dimerization obscure conformational changes that occur in EFSAM? This question is particularly imperative given that GrpE normally participates in preventing aggregation of denatured proteins [13]. Further, when structurally coupled, two independent EF-hand pairs such as the N-lobe or C-lobe of calmodulin mutually enhance the Ca^{2+} binding affinity within each lobe [3]. How many Ca^{2+} binding sites could be quantitatively derived if cooperativity induced by EFSAM dimerization is considered? How could mutations designed to disrupt peripheral Ca^{2+} binding in the same EFSAM region cause both loss-of-function and gain-of-function phenotypes in STIM1? What is the physiological relevance of the ~10 μM Ca^{2+} sensitivity? Structurally, the EF-hand domain creates a highly negative surface electrostatic potential (Fig. 1B). Does the negatively charged surface of the EF-hand domain directly bind Ca^{2+} and at what precise sites? Are the negatively charged residues allosterically coupled to more distant binding sites? Ultimately, the high-resolution structural elucidation of Ca^{2+} depleted EFSAM, direct atomic level Ca^{2+} binding experiments, and molecular dynamics simulations will begin to tease out the answers to these questions. New surprising discoveries always generate new important questions.

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