Differential Response to Ca^{2+} from Vertebrate and Invertebrate Calumenin is Governed by a Single Amino Acid residue

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List of Supplementary figures

FIGURE S1

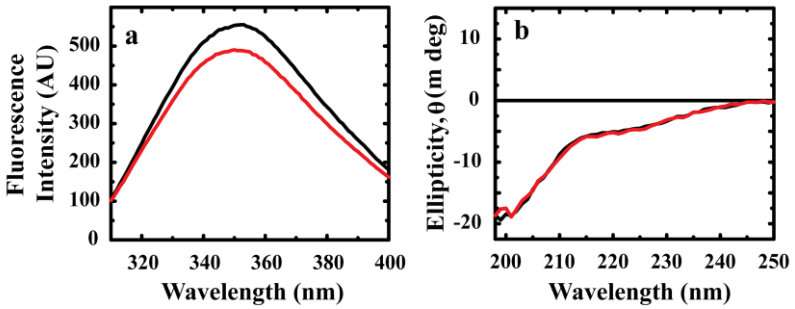

Figure S1. Binding of Mg^{2+} to HsCalu1 as monitored by Trp fluorescence and far-UV CD spectra. (a) Changes in tryptophan fluorescence emission spectra were monitored upon Mg^{2+}-binding by exciting HsCalu1 (0.5 µM) at $\lambda_{295\ nm}$ and emission spectra were recorded at $\lambda_{310\text{-}400\ nm}$. In the spectra, black and red lines denote apo and holo form (recorded in the presence of 5 mM $MgCl_2$) respectively. No conformational changes were observed upon Mg^{2+}-binding. (b) Far-UV CD spectra of purified HsCalu1 (4.5 µM) were recorded between 198-250 nm. Black line represents the apo form of the protein which is unstructured and unlike Ca^{2+}, Mg^{2+}-binding (5 mM $MgCl_2$) does not lead to any structural changes in HsCalu1which is shown as red line.

FIGURE S2

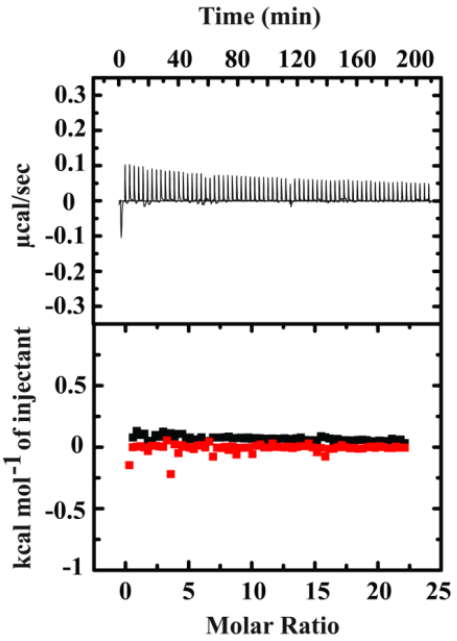

Figure S2. Binding of Mg^{2+} to HsCalu1 as determined by ITC. HsCalu1 (50 µM) and ligand (7 mM $MgCl_2$) were prepared in 50 mM Tris pH 8 containing 50 mM NaCl. Aliquots of $MgCl_2$ were titrated into HsCalu1. The upper panel of the thermogram shows the heat changes is due to the heat of dilution during each injection. The lower panel shows the heat evolved per injection (■) as a function of molar ratio. Whereas,(■) represents Mg^{2+} titration in buffer alone at 25˚C.

FIGURE S3

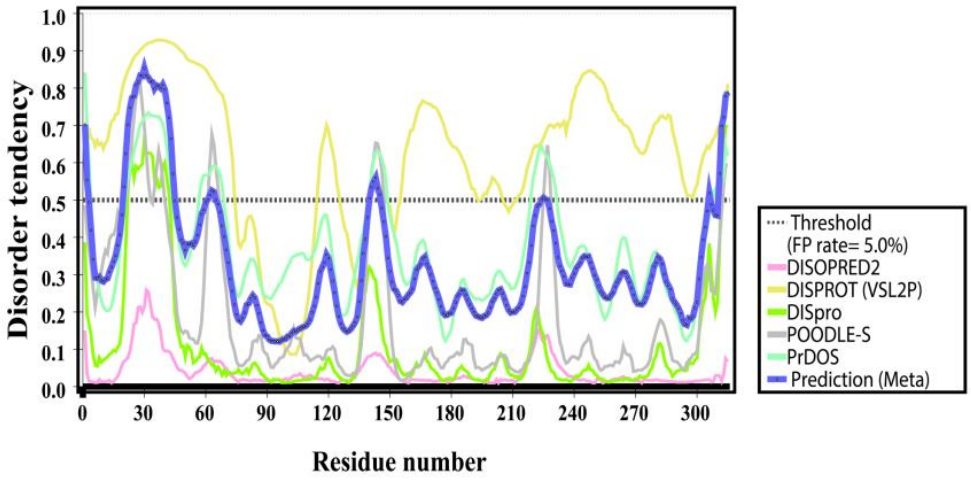

Figure S3. Prediction of structural disorder in HsCalu1 by MetaPrDOS. The primary amino acid sequence of HsCalu1 was analysed to predict unfolded sequences using MetaPrDOS. The plot shows the disorder tendency for each amino acid residue with highest predictive value of 0.5 for foldability. A default false positive rate of 5% was applied. Among different predictors used in the analysis, DISPROT suggested calumenin to be completely disordered whereas DISpro predicted as an ordered protein except for the N-terminal region. On the other hand, POODLE-S and PrDOS predicted three folded and an N-terminal disordered region. DISOPRED 2 predicted the protein as a folded protein not necessarily indicative of a disordered protein. The consensus drawn by Meta demonstrates that the N-terminal of the protein (EF1) as the disordered region.

FIGURE S4

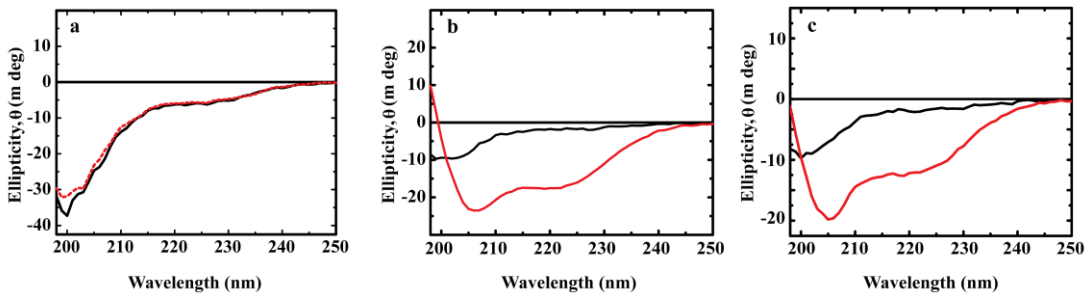

Figure S4. Far-UV CD spectra of EF1. Circular Dichroism spectra in the far-UV region ($\lambda_{198\text{-}250\ nm}$) were recorded in aqueous solution and also in the presence of organic solvents (90% methanol and 70% trifluoroethanol). The peptide was prepared in 50 mM Tris pH 8 containing 50 mM NaCl. (a) EF 1 peptide in buffer demonstrates a random-coil structure evident by a negative minima near 201-204 nm and binding to Ca^{2+} does not induce any secondary structure to the peptide shown as black and red solid line respectively. Far-UV CD spectra were also recorded in the presence of 90% methanol (red solid line; panel b) and also in 70% trifluoroethanol (red solid line; panel c) which shows α-helix formation evident by double negative minima near 208 and 222 nm. In both the panels (b and c), black solid line denotes the far-UV CD spectra of the peptide recorded in buffer.

FIGURE S5

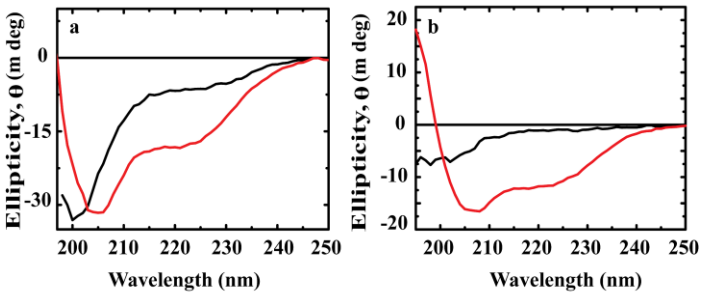

Figure S5. Effect of methanol on the secondary structure of EF12 and EF123. Far-UV CD spectra for fragments (EF12 and EF123) were recorded from $\lambda_{198\text{-}250\ nm}$ in a buffer containing 50 mM Tris pH 8, 50 mM NaCl and in the presence/absence of methanol (90 %). In both the spectra black and red solid lines represent peptide recorded in buffer and methanol respectively. Both the fragments EF12 (a) and EF123 (b) were unstructured when recorded in buffer and has the propensity to induce α-helix conformation when recorded in the presence of methanol shown by the double negative minima near 208 and 222 nm.

FIGURE S6

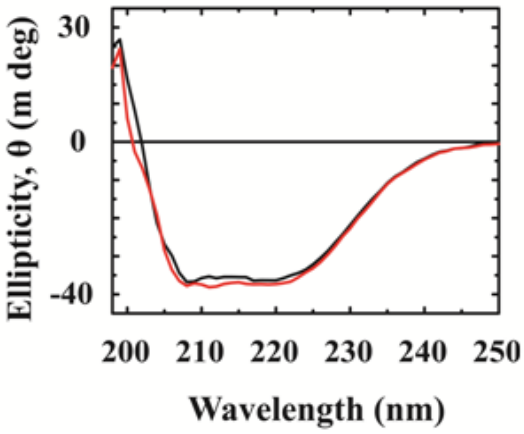

Figure S6. Far-UV CD spectra of L150G/G187L double mutant of HsCalu1. A double mutant was generated by substituting Gly at the sixth position in EF-hand 5 to Leu (G187L) in L150G mutant of HsCalu1 by site directed mutagenesis and the native conformation was determined by far-UV CD spectra. Far-UVCD spectra (198-250 nm) were recorded for the double mutant (L150G, G187L) in 50 mM Tris pH 8 containing 50 mM NaCl and in the presence/absence of 1 mM $CaCl_2$. Black and red solid lines represent apo and holo form (1 mM $CaCl_2$) respectively. The result shows that apo-L150G/G187L mutant is structured similar to L150G mutant of HsCalu1 implying the importance of Leu at the sixth position in EF-hand 4.

FIGURE S7

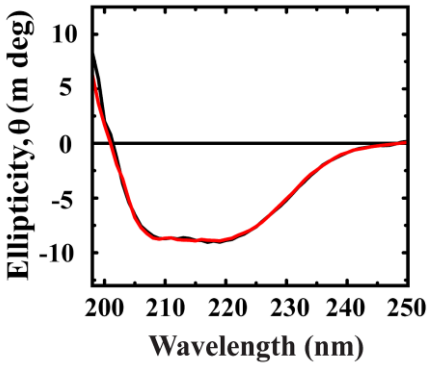

Figure S7. Far-UV CD spectra of G146L mutant of CeCalu. The single mutant was generated by substituting Gly at the sixth position in the corresponding EF-hand 4 to Leu (G146L) in CeCalu by site directed mutagenesis and confirmed by nucleotide sequencing. The native protein conformation of G146L of CeCalu was assessed by recording far-UV CD spectra (198-250 nm). The spectra were recorded in 50 mM Tris pH 8 containing 50 mM NaCl and in the presence/absence of 1 mM $CaCl_2$. In the spectra, black and red solid lines represent apo and holo forms (1 mM $CaCl_2$) of G146L mutant of CeCalu respectively. Apo-G146L mutant is natively structured and binding of Ca^{2+} does not induce any significant conformational changes in the protein.

FIGURE S8

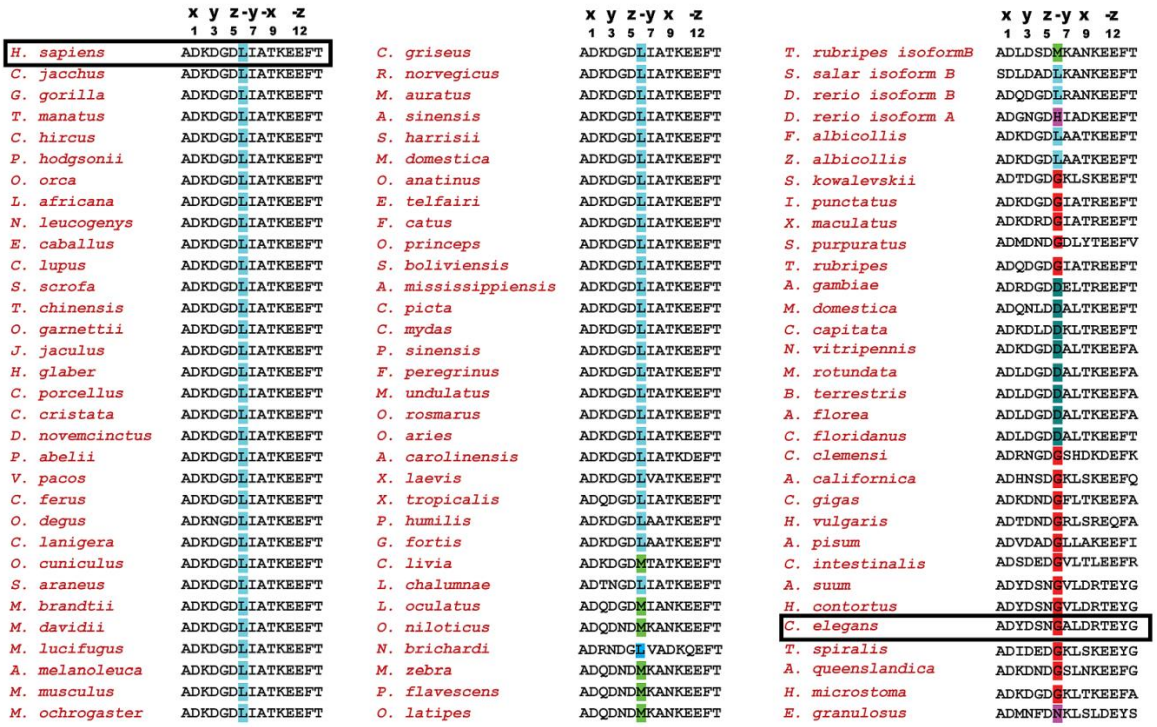

Figure S8. Multiple-sequence alignment of calumenin orthologs. Multiple sequence alignment was generated in MAFFT alignment tool employing blast search using human calumenin

(HsCalu1) as a query sequence. Redundant sequences (isoforms) were removed by CD-hit and only one representative sequence (the longest sequence) from each organism was collected and the multiple sequence alignment was calculated using MAFFT version 7.0 tool.[1] Only the alignment of corresponding EF-hand 4 loop sequences is shown here. The coordinating ligands for Ca^{2+}-binding is labeled at the top and the amino acid at the 6^{th} position is highlighted with different colors. Leu which is conserved in higher metazoans across chordates is highlighted in cyan blue. The amino acid Met is highlighted in green, His in purple, and Asp in blue. Gly which is conserved in lower metazoans, cnidarians and poriferans are highlighted in red. The Ca^{2+} coordinating residues present in EF-hand 4 loop for HsCalu1and CeCalu were shown in rectangular box.

References:

1. Katoh, K., Misawa, K., Kuma, K., and Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform, *Nucleic Acids Res. 30*, 3059-3066.