Published in final edited form as:
Acc Chem Res. 2011 March 15; 44(3): 171–179. doi:10.1021/ar100110d.

Relating Form and Function of EF-hand Calcium Binding Proteins

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Conspectus

The EF-hand is one of the most common structural motifs in animal genomes and EF-hand Ca^{2+} binding proteins (EFCaBPs) are widely distributed throughout the cell. However, researchers remain confounded by a lack of understanding of how their sequences provide them with their specific functions or what molecular mechanisms enable them to distinguish among their diverse cellular targets. Such knowledge could define the roles of EFCaBPs in health and disease and ultimately enable control or *de novo* design of Ca^{2+} dependent activities for medical and biotechnology applications. In this Account, we describe our research using combined structural and biochemical approaches.

The first structural goal was to define conformational changes induced by binding Ca^{2+} and our group and others established that solution NMR spectroscopy is well suited to this task. We showed the structural differences and pinpointed residues critical to the differences in Ca^{2+} response of calbindin D_{9k} and calmodulin (CaM), homologous EFCaBPs from different functional classes, using direct structure determination and site directed mutagenesis/protein engineering. Structure combined with biochemistry provided the foundation to identify the fundamental mechanism of cooperativity in the binding of Ca^{2+} ions, which provides the ability of EFCaBPs to detect the relatively small changes in concentration that constitute Ca^{2+} signals. Using calbindin D_{9k} as a model system, we characterized the structure and fast time scale dynamics of each of the four ion binding states in a typical EF-hand domain and showed direct evidence that site-site communication lowers the free energy cost of reorganization for binding the second ion.

Our work has also extended models of how EFCaBPs interact with their cellular targets. By determining the unique dimeric architecture of S100 proteins, we described the implications for how these proteins transduce signals and went on to characterize interactions with peptide fragments of important cellular targets. Studies of the CaM homolog centrin revealed novel characteristics of it binding of Ca^{2+} and its interaction with its cellular target Kar1. These results provided clear examples of how subtle differences in sequence fine-tune EFCaBPs to interact with their specific targets.

The structural approach stands at a critical cross road, shifting in emphasis between descriptive structural biochemistry, and integrated biology and medicine. Our dual molecular switch model for Ca^{2+} regulation of gating functions of voltage-gated sodium channels in which both CaM and an intrinsic EF-hand domain serve as coupled Ca^{2+} sensors is presented. A second example involves novel EFCaBP extracellular function, i.e. the role of S100A8/S100A9 heterodimer in the innate immune response to bacterial pathogens. A mechanism for the antimicrobial activity of S100A8/S100A9 was discovered. Interactions of S100A8/S100A9 and S100B with the cell surface receptor for advanced glycation end products are described. Biochemical and structural studies are now uncovering the mechanisms by which EFCaBPs work and are helping to define

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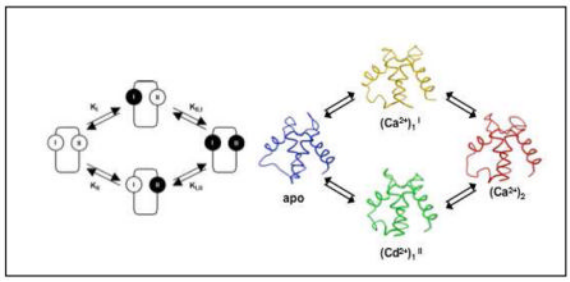
their biological activities, while simultaneously expanding knowledge of the roles of these proteins in normal cellular physiology and the pathology of disease.



Introduction

The calcium ion (Ca^{2+}) is a common currency widely used for regulation of signaling pathways in cells. EF-hand Ca^{2+} binding proteins (EFCaBPs) are central players in all aspects of Ca^{2+} signaling events, with diverse roles ranging from controlling the opening and closing of Ca^{2+} channels to modulating the intensity and duration of Ca^{2+} signals to transducing these signals into biochemical and biomechanical responses.[1] The importance of EFCaBPs is evident from their direct association with diseases ranging from Alzheimer's, cardiac arrhythmia syndromes, complications of diabetes, chronic inflammatory disorders and cancer. [2–5]

The EF-hand is one of the most common structural motifs in animal genomes; over 1000 have been identified from their unique sequence signatures.[6] However, there is a lack of understanding of how their sequences provide them with their specific functions or what molecular mechanisms enable them to distinguish among their diverse cellular targets. The transduction of a Ca^{2+} signal can be viewed as a two-step process involving an initial activation of the EFCaBP by the ionic signal, followed by binding to and modulation of a target. This formulates the relationship between sequence and function in terms of two key questions: (i) How does the sequence specify the response to Ca^{2+} binding? (ii) How do different EF-hand proteins interact with and modulate their targets? Answers to these two questions will enhance our understanding of the roles of EFCaBPs in health and disease and ultimately enable control or *de novo* design of Ca^{2+} dependent activities for medical and biotechnology applications.

Eighteen years ago I helped write Sture Forsén's *Account* entitled, "The Molecular Anatomy of a Calcium-Binding Protein", about the EFCaBP calbindin D_{9k}.[7] In the years since then the study of EFCaBPs has greatly evolved. One of the first goals of structural research on EFCaBPs was to define the conformational changes induced by binding Ca^{2+}. The first three-dimensional structure of an EFCaBP was determined for parvalbumin by X-ray crystallography in the early 70's,[8] and over the subsequent 15 years, crystal structures of a number of other EFCaBPs were determined.[9] However, due to the inability to obtain crystals of multiple binding states of these proteins, the structural response to Ca^{2+} binding could not be determined directly for any of them. In 1987, I set out to use solution NMR spectroscopy as a tool to directly define the Ca^{2+}-induced changes in EFCaBPs because this approach would be readily amenable to studies of different states of the protein. Although it took more than five years, this strategy was ultimately validated by determining the structure of calbindin D_{9k} in the presence and absence of Ca^{2+}.[10,11] The approach was also utilized for studying calmodulin. troponin and other EF-hand proteins [e.g. Refs. [12–15]].

The database of 3D structures built up over the years has revealed many nuances of EFCaBPs and their responses to the binding of Ca^{2+} ions.[16,17] In addition, considerable progress was made in understanding fundamental mechanisms used by EF-hand proteins to generate key functional attributes such as cooperativity in binding ions.[18,19] Structural studies of EF-hand proteins are now shifting in emphasis towards developing a broader understanding of how sequence specifies function and how targets are activated. The results are uncovering the mechanisms by which EFCaBPs work, while simultaneously expanding knowledge of their roles in normal cellular physiology and malfunctions in disease. This *Account* provides a selection of examples of our contributions, not a comprehensive review, and spans the range from fundamental studies of EFCaBP structure to multi-disciplinary structure-function analyses addressing specific issues of human health.

EFCaBP structure and cooperativity in the binding of Ca^{2+}

Although the EF-hand is a common helix-loop-helix motif, the basic EF-hand structural unit is a pair of motifs that together form a structurally stable four-helix bundle domain (Fig. 1). EF-hand proteins are comprised of one or more of these domains. There are two primary functional classes of EFCaPBs: Ca^{2+} sensors such as calmodulin (CaM), which transduce Ca^{2+} signals; and Ca^{2+} signal modulators such as calbindin D_{9k} (ClB), which modulate the shape and/or duration of Ca^{2+} signals and help maintain Ca^{2+} homeostasis. These differences in function correlate with differences in the conformational changes induced by Ca^{2+} binding.[20] For example, despite 25% sequence identity and very similar structures in the apo state, there is a striking difference in the conformational change triggered by Ca^{2+} binding to ClB and CaM.[12–14,20] Activation by Ca^{2+} binding causes each of the EF-hand domains of CaM to undergo a significant opening of their structure resulting in exposure of a hydrophobic patch that is critical for interaction with downstream targets (Fig. 2). ClB, on the other hand, remains in a ‘closed’ conformation upon Ca^{2+} binding that is more similar to its apo state.[21] It is now well accepted that subtle differences in sequence fine tune EF-hand domains to generate the specific Ca^{2+}-induced conformational response that distinguish signal modulator from signal transducer EFCaBPs.[22–24]

The pairing of EF-hand Ca^{2+} binding motifs so that each domain binds two Ca^{2+} ions is a key property of EFCaBPs. Cooperativity is particularly important for the Ca^{2+} sensor proteins because it provides their ability to function as on-off switches by responding to the relatively subtle Ca^{2+} signals of ~100-fold change in Ca^{2+} concentration. Working with the small single domain protein calbindin D_{9k} as a model system, my laboratory carried out a series of detailed analyses of the molecular basis for cooperative binding of Ca^{2+} ions by EFCaBPs.

To deconvolute the cooperative Ca^{2+} binding, each of the four ion binding states (no ions, site I filled only, site II filled only, both sites filled) must be fully characterized. The half-saturated states pose a significant challenge and can be studied through the use of metal ion substitution or site directed mutagenesis. A model for the half-saturated state with Ca^{2+} bound only in site I was obtained by mutating the Ca^{2+} chelating side chain of Asn56 in site II to Ala.[25] The complementary half-saturated state with Ca^{2+} bound only in site II could be studied by using Cd^{2+} in place of Ca^{2+} because Cd^{2+} has a more stringent requirement for its coordination geometry that is not met in the non-canonical “S100-specific” site I of calbindin D_{9k} (*vide infra*).[26]

Comparative analyses of the differences in structures and fast time scale (ps-ns) dynamics of each of the four states of the protein provided unique insights into the mechanism of cooperativity.[18,19] In particular, we demonstrated that binding of the first ion (in either site) causes the protein to shift far closer to the fully Ca^{2+}-loaded state than the apo state. A

particularly exciting discovery was that the binding of a single ion in site I caused significant shift in the structure and the motional dynamics in site II. Thus, the reorganization associated with the first binding step lowers the free energy cost of reorganization for the second binding step. These findings revealed the importance of long-range effects and site-site communication in the cooperative binding of Ca^{2+} ions. They also provided clear evidence that the EF-hand domain functions as a globally cooperative structural unit.[21] We have proposed that the tight structural integration of the domain reflected in efficient site-site communication provides fine tuning of responses to Ca^{2+} binding, which is integral to the diversity in functionality needed to generate, control and transduce Ca^{2+} signals.[19]

The tuning of conformational response to Ca^{2+} binding by EF-hand proteins

In order to determine how sequence dictates EFCaBP function, we have investigated what factors control the differences in the Ca^{2+}-induced conformational changes of EF-hand proteins differentiating Ca^{2+} sensors and signal modulators. We hypothesized that reorganization of packing in and around the hydrophobic core drives the bulk of the conformational response to Ca^{2+} binding.[21] To test this proposal, we undertook the rational design of 'calbindomodulin', i.e. calbindin D_{9k} re-engineered to adopt an open conformation in the Ca^{2+}-loaded state and function like the N-terminal Ca^{2+} sensor domain of calmodulin (CaM-N).

It was first thought that ClB does not open upon binding Ca^{2+} because the conformation of the non-canonical N-terminal EF-hand of ClB already occupies a conformation ready to bind Ca^{2+}. However, homology models showed that there would be exposed hydrophobic residues and packing conflicts if ClB were to occupy the open conformation.[21,22] The initial calbindomodulin design was developed on the basis of sequence alignments of EFCaBPs, an homology model of ClB in the open conformation, and comparisons of all available three-dimensional structures of EFCaBPs. Results of these analyses were analyzed in the context of the biophysical properties of the side chains to formulate hypotheses about their importance for regulating Ca^{2+}-induced conformational change. Fifteen mutations were chosen for the first calbindomodulin design (CBM-1) with the goal of improving solvation properties, minimizing steric conflicts in the open conformation, and reorganizing the closed conformation of the apo protein.[22]

The gene for CBM-1 was synthesized and the protein purified. A 1.44 Å X-ray crystal structure (PDB 1QX2) showed that the protein core is reorganized in a manner consistent with transition from a closed to an open conformation upon Ca^{2+} binding.[22] However, although a deep hydrophobic pocket similar to that of CaM was created in CBM-1 (Fig. 3), access to this binding site was occluded by the linker, which did not flip out of the way as in CaM, but rather, packed onto the hydrophobic patch as a result of favorable hydrophobic interactions. Thus, while CBM-1 was a largely successful design, additional engineering is required to convert the ClB signal modulator into a functional Ca^{2+} sensor.

Structural and functional diversity in EFCaBPs- moving beyond the CaM paradigm

Although our knowledge of intrinsic structural features that distinguish different EF-hand proteins is quite advanced, there is a dearth of information about the molecular mechanisms relating structure to specificity of EFCaBP function. How can so many homologous proteins work in parallel and selectively participate in signal transduction pathways? Initially, models of how EF-hand proteins interact with their targets were largely derived from studies of CaM, which contains four EF-hands organized into two EF-hand domains connected by a flexible tether. Figure 5A shows the paradigm 'wrap around' mode of CaM binding to

target. Since the initial models, numerous examples of diversity in target interactions with CaM and other EFCaBPs have been identified.[27]

Centrin

Centrin (also known as caltractin) shares approximately 50% sequence homology with CaM, and like CaM contains two structurally independent EF-hand domains. Unlike CaM, the Ca^{2+} affinities of the two domains are rather different, and the C-terminal domain (Cen-C) does not appear to function as a normal Ca^{2+} sensor.[28] Our high-resolution structure of the complex of Cen-C with a fragment from the target protein Kar1 provided a clear example of how subtle differences in the sequence of Cen and CaM fine-tune these homologous EFCaBPs to interact selectively with specific targets (Fig. 4).[29] This study also revealed that sub-stoichiometric amounts of Ca^{2+} are sufficient to activate Cen-C binding to Kar1, which implies that centrin and Kar1 will be constitutively bound at the basal level of Ca^{2+} in the cell. Remarkably, there are no targets known that interact specifically with the N-terminal domain, even though it has all of the properties of a Ca^{2+} sensor.[30,31] However, studies of the interaction between centrin and the target protein Sfi1 showed that both domains are engaged in binding in an extended mode.[32]

S100 proteins

The S100 sub-family is among the most distinctive of EFCaBPs and are found exclusively in vertebrates. They are believed to have evolved to enable activation of specific biochemical pathways in parallel to the activity of classical Ca^{2+} sensors such as the ubiquitous CaM. The importance of S100 proteins is underscored by their deregulated expression in neurodegenerative and inflammatory disorders, cardiomyopathies and cancer.[2] In fact, S100 proteins serve as diagnostic markers in the clinic[33] and their potential as therapeutic targets, for example in cancer, is under active investigation.[34]

S100 proteins are readily distinguished from other Ca^{2+} binding EF-hand proteins because they have a S100-specific 14-residue loop in their N-terminal EF-hands, which deviates substantially from the highly conserved 12 residue canonical Ca^{2+} binding loop.[9] S100 proteins contain two EF-hands organized into a single EF-hand domain that forms integrated homo and heterodimers with a distinctive dimeric architecture (Fig. 5) first seen in our structure of calcyclin (S100A6).[35] [ClB is an ancestral S100 protein that is significantly shorter in length and remains monomeric.] The very start difference in the organization of the two EF-hand domains of S100 proteins and CaM led us to propose that the molecular basis of Ca^{2+} signal transduction by S100 proteins must be distinct from that of the CaM-like EFCaBPs.[36] The details of the Ca^{2+}-induced changes in S100 proteins are now well characterized by high resolution structures.[37] These structures show that the extent of Ca^{2+}-induced changes is more modest than in the classical CaM-like Ca^{2+} sensors because their unique N-terminal EF-hands change very little when they bind Ca^{2+}.[36] Thus, although both S100 proteins and CaM have two target binding sites, the fundamental difference in their responses to binding Ca^{2+} and their structural organization enforces differences in their modes of interaction with targets (Fig. 5).[27,37]

Structures of a number of complexes of S100 proteins with peptide fragments of target proteins have been determined.[37] These structures reveal that although the target binding sites are in similar locations on the S100 proteins, the peptide fragments of the target do not all have the same structure and do not all bind to the S100 protein in the same way.[27] This observation speaks to the specificity of S100-target interactions and demonstrates the importance of subtle structural details in the binding surface of the S100 protein and target protein.[27,38] Recently, our laboratory reported the structure of a complex of S100A6 (calcyclin), which revealed a mode of interaction with target that had not been seen

previously.[39] This observation demonstrates that important discoveries continue to be made about the how S100 proteins recognize and engage their cellular targets. Moreover, despite the wealth of structural information, there still remains much to be learned about how S100 proteins activate signaling events in normal cells and participate in disease.

An EFCaBP with extracellular function-calprotectin

S100 proteins are also unique among EFCaBPs because they can be exported outside cells.[40] Once outside the cell, they function by activating cell surface receptors and also via their ability to bind trace metals such as Zn^{2+} and Mn^{2+} at sites that are distinct from the Ca^{2+} binding sites. However, virtually no information is available relating the activities of S100 proteins in the extracellular environment to their biochemical basis for function. We have been investigating extracellular functions of the S100A8/S100A9 heterodimer termed calprotectin (CP), which is abundantly expressed in certain white blood cells. CP is linked directly to inflammation and the innate immune response and is found at high levels in patients suffering from cystic fibrosis, rheumatoid arthritis, chronic bronchitis, AIDS, diabetes and cancer.[4] While specific functions have been reported for the isolated subunits, the two proteins are almost always expressed together and they exhibit a strong preference for formation of the heterodimer.[41,42]

Convincing evidence has been obtained that CP exerts potent antimicrobial activity in abscesses infected with the critical pathogen *Staphylococcus aureus* through its chelation of Zn^{2+} and Mn^{2+}.[43] These findings support a mechanism in which CP lowers the free concentration of essential trace metals such that they fall below the levels required for growth and survival of bacterial pathogens.[43,44] The importance of CP binding Zn^{2+} extends beyond sequestration of ions as evidence has accumulated suggesting that Zn^{2+} also modulates the interaction of CP with receptors on the surface of cells.[45]

Among the cell surface receptors, the best characterized is RAGE (Receptor for Advanced Glycation End products). AGEs are a heterogeneous mixture of proteins and lipids modified by glucose metabolites, which accumulate to unusually high levels in diabetics. It turns out that their receptor RAGE can be activated by a variety of ligands, including S100 proteins. The relative abundance of S100 proteins and RAGE in tissues correlates with specific diseases. For example, high plasma levels of CP are found in inflamed tissues[5] and CP has been shown to stimulate RAGE signaling in human prostate cancer.[46] Hence, RAGE is under active investigation as a therapeutic target.[47,48]

In order to understand how CP functions, our laboratory is studying the structure of RAGE and the molecular basis its activation by S100 protein (in collaboration with Guenter Fritz, Unversity of Freiburg, Germany). Our studies have shown that two of the three extracellular Ig domains form an integrated structural unit (VC1) independent of the third Ig domain.[49] Biochemical studies revealed that the VC1 unit contains the primary ligand binding sites for AGEs and S100 proteins.[49–51] Our recent NMR studies combined with the high resolution x-ray crystal structure of VC1 provided a structural model of the S100B-VC1 complex (Fig. 6).[52] These studies serve as an initial basis for the design of inhibitory mutants for functional analysis and the development of chemical probes to explore the therapeutic potential of RAGE-directed chemotherapy.

A complex calcium dependent regulatory apparatus in human cardiac Na^+ channels

Voltage-gated sodium (Na_V) channels are responsible for generating the influx of Na+ ions through excitable membranes in nerve, heart, and skeletal muscle. In humans, even

relatively mild perturbation of Na_V function can result in cardiac arrhythmias and other disorders[3,53], and Na_V channels are also important for the management of pain by local anesthetics.[54]

Several years ago it was discovered that changes in intracellular calcium ($[Ca^{2+}]_i$) affect the function of Na_V channels and that CaM was involved.[55,56] Figure 7A shows a schematic diagram of the $Na_V1.5$, emphasizing the regions involved in Ca^{2+} effects. CaM is recruited to channels by binding to a C-terminal 'IQ' motif, a characteristic sequence associated with the localization of CaM to cellular regions where it is required. Our group demonstrated that $Na_V1.5$ has a second means by which it responds to $[Ca^{2+}]_i$, one which involves *direct* binding of Ca^{2+} ions to an EF-hand domain that is just upstream from the IQ motif.[57] Remarkably, we observed high affinity binding of Ca^{2+} to the EF-hand domain only in a construct containing the IQ motif.[58] In addition, we showed the affinity and mode of interaction of CaM with the IQ motif was altered by the level of Ca^{2+}. These studies suggested that the modulation of channel function by CaM and the EF-hand domain is coupled through the IQ motif, which led us to a proposal that the IQ motif serves as a molecular switch.[58]

Although knowledge of the molecular basis for sensing Ca^{2+} was valuable, there remained a critical gap to understanding how this altered channel function. We speculated that either CaM or the EF-hand domain must interact with the ion channel pore or the lid that covers the pore, which is termed the D3D4 linker. To investigate if CaM binding is directly involved, we submitted the $Na_V1.5$ sequence to the Calmodulin Target Database server, which searches for calmodulin binding domains (CDBs).[59] A number of CBDs were returned, including one site located precisely within the D3D4 linker. Isothermal Titration Calorimetry (ITC) was used to show that CaM binds the full D3D4 linker strongly in the presence of Ca^{2+} but ~20-fold weaker in absence of Ca^{2+}. Since CDBs are known to contain contributions from hydrophobic interactions, three hydrophobic residues (Phe1520-Ile1521-Phe1522) were substituted with alanines and ITC revealed these mutations caused a 20-fold drop in binding affinity relative to the native sequence (Fig. 7B,C).[60] These FIF-AAA mutations were then engineered into the full-length $Na_V1.5$ and the corresponding decrease in channel function confirmed the functional significance of the interactions.

In order to elucidate the detailed mechanism of the EF-hand domain, its structure was determined by NMR (Fig. 8) and combined with NMR chemical shift perturbations induced by binding of an IQ peptide, to construct a model of the complex. The model shows the IQ motif binds between Helices I and IV (Fig. 8) and predicts residues F1855 and L1786 are crucial for the interaction.[61] Functional analysis on channels with the corresponding F1855A and L1786A mutations showed that the interaction between the IQ motif and EF-hand domain is required for proper Ca^{2+} sensing. The clinical implications of calcium-dependent regulation of $Na_V1.5$ are highlighted by findings that mutations within CTD-EF, the IQ motif and the D3D4 linker are arrhythmogenic, placing patients at risk for sudden cardiac death.[62,63] Ongoing studies will allow us to translate our structural and biochemical information to clinical outcomes, and to evaluate the potential for development of anti-arrhythmia therapies targeted to the $Na_V1.5$ Ca^{2+} sensing apparatus.

Concluding Remarks

The ~500 structures of EFCaBPs and their complexes with peptide fragments of cellular targets and small molecule ligands provide a thorough description of how EF-hand proteins respond to calcium. However, the predictive power of this knowledge remains limited, and the impact of this information is relatively modest because the structural effects on the downstream targets are largely unknown. The grand challenge ahead is determining the

changes in target molecules induced by interaction with EFCaBPs. Structural and biochemical studies of EFCaBPs have begun to bridge the gap from basic descriptive structural biochemistry to integrated biology and medicine, suggesting that promising, exciting applications in therapeutics and biotechnology lie close at hand.

Acknowledgments

I am deeply indebted to Professor Sture Forsén of the Department of Physical Biochemistry at Lund University in Sweden for his many years of invaluable mentorship, collaboration, and friendship, particularly as I was launching this research program as the core of my first faculty position. I also thank Professor Joachim Krebs, then in the Department of Biochemistry at the ETH-Zürich, for teaching me the ins and outs of the biochemistry of EFCaBPs during my postdoctoral studies. I acknowledge colleagues in the EFCaBP research community, in particular fellow protein NMR spectroscopists Mitsu Ikura, Brian Sykes, Gary Shaw, David Weber, and Jim Ames, who have set a high standard of excellence while maintaining a stimulating environment for friendly competition that has propelled the field forward. I also acknowledge the many outstanding contributions from past members of my group, without whom this research would not have been possible. I thank Drs. Jonathan Sheehan and Steven Damo for critical reading of the manuscript and assistance in preparing the Conspectus figure. EFCaBP research in my laboratory was supported by grants from the National Institute of General Medical Science and the American Cancer Society.

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Biography

Walter J. Chazin was born in Lackawanna, NY in 1954. He received chemistry degrees from McGill University (BSc ‘75) and Concordia University (Ph.D ‘83) in Montréal, then studied with Kurt Wüthrich at the E.T.H. in Switzerland (1983–1985) and Peter Wright at the Scripps Research Institute (1986). After 12 years on the faculty at Scripps, he moved to Vanderbilt University in 1999 where he is Chancellor’s Professor of Biochemistry and Chemistry, Ingram Professor of Cancer Research, and Director of the Center for Structural Biology and the Molecular Biophysics Training Program. His current research interests are in the integrated application of structural approaches to understand (i) the functioning of DNA replication, damage response and repair machines, (ii) the mechanisms of action of protein ubiquitination machines, and (iii) the molecular basis for the biological activities of EF-hand calcium binding proteins.

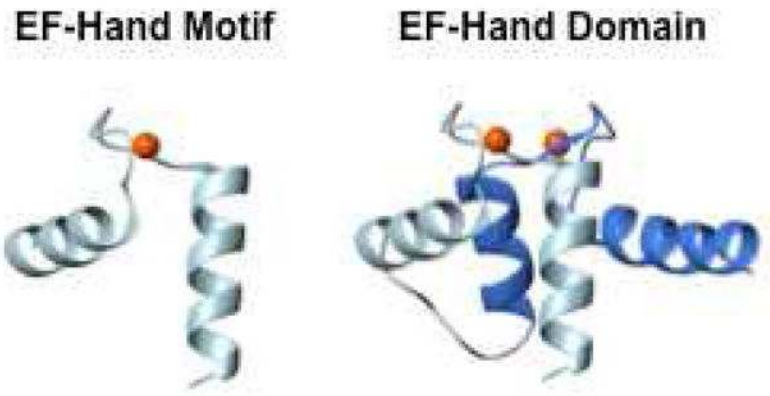

Figure 1.
Ribbon diagram of EF-hand motif and domain.

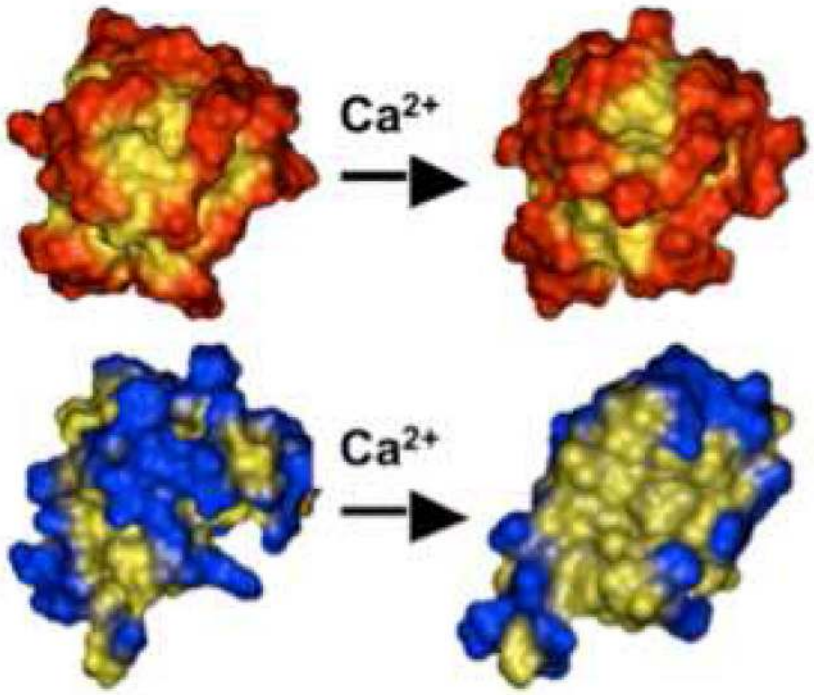

Figure 2.
Differences in Ca^{2+}-induced conformational changes in ClB (red) versus CaM-N (blue). Hydrophobic accessible surface is colored yellow.

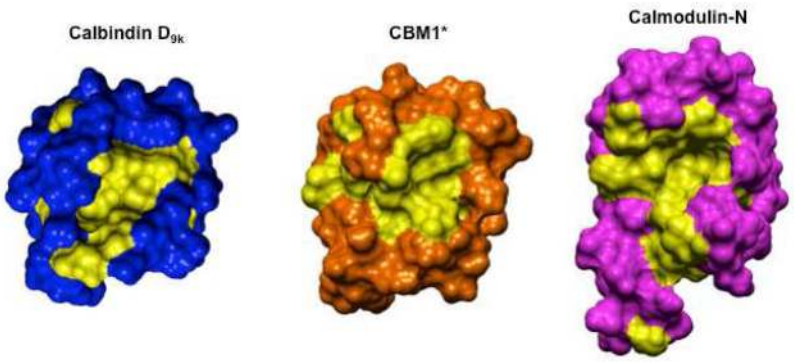

Figure 3.
The conformation for CBM-1 that is more similar to CaM than ClB. Comparison of the hydrophobic accessible surfaces (yellow) of Ca^{2+}-loaded ClB, CBM-1 and CaM. [Adapted from Ref. [22]]

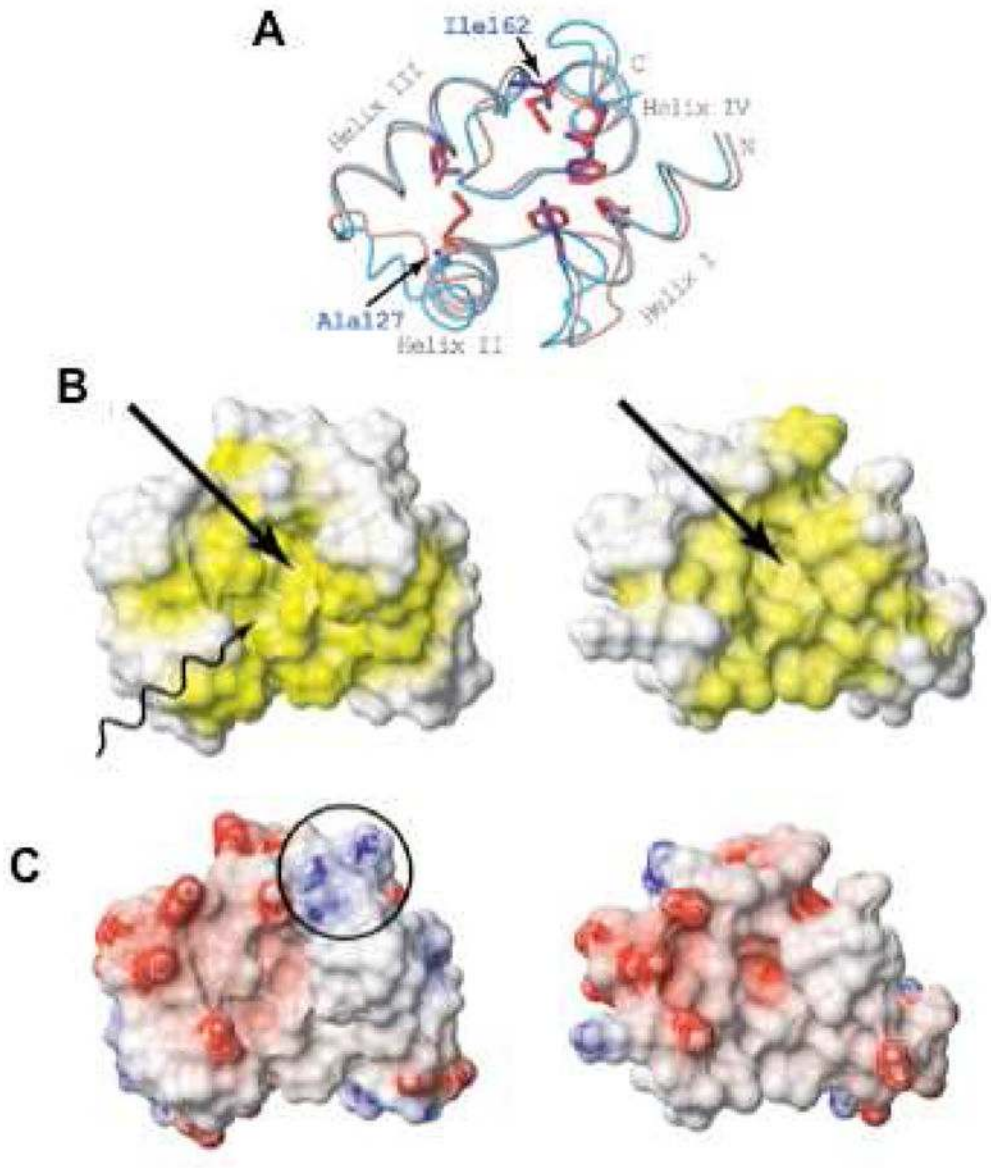

Figure 4.
Target binding selectivity of EF-hand proteins. A. Overlay of the structures of Cen-C (blue) and CaM-N (red) from their complexes with peptide fragments of Kar1p (1OQP) and smooth muscle myosin light chain kinase (smMLCK,1CDL), respectively. Selected side chains in the hydrophobic core are included. B. Comparison of the target binding surfaces of the two complexes with arrows highlighting key differences. C. Comparison of the electrostatic field (blue +, red −) and shape of a key area on the respective target binding surfaces. [Taken from Ref. [29]]

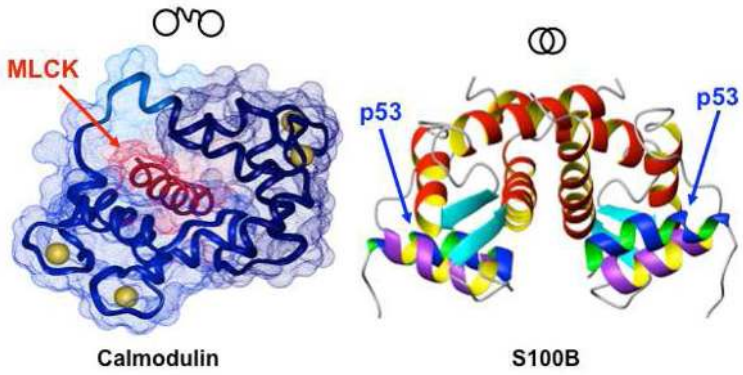

Figure 5.
Fundamental differences in target binding by CaM and S100B. Comparison of the complexes of CaM with a peptide fragment of smMLCK (1CDL) and S100B with a peptide fragment of p53 (1DT7). The positions of the respective target binding sites are highlighted with arrows.

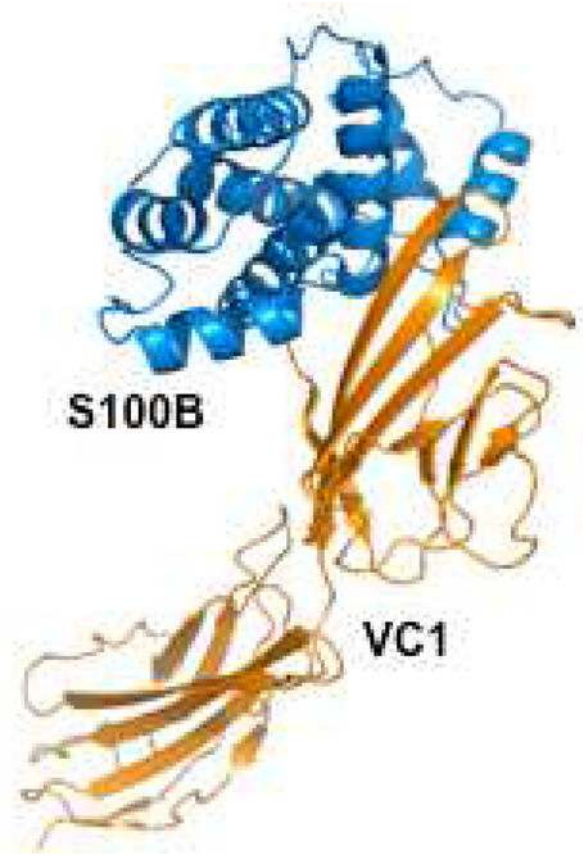

Figure 6.
Ribbon diagram of the NMR-based structural model of the complex of S100B (blue) with the VC1 activation domain of RAGE (gold). [Adapted from Ref. [52]]

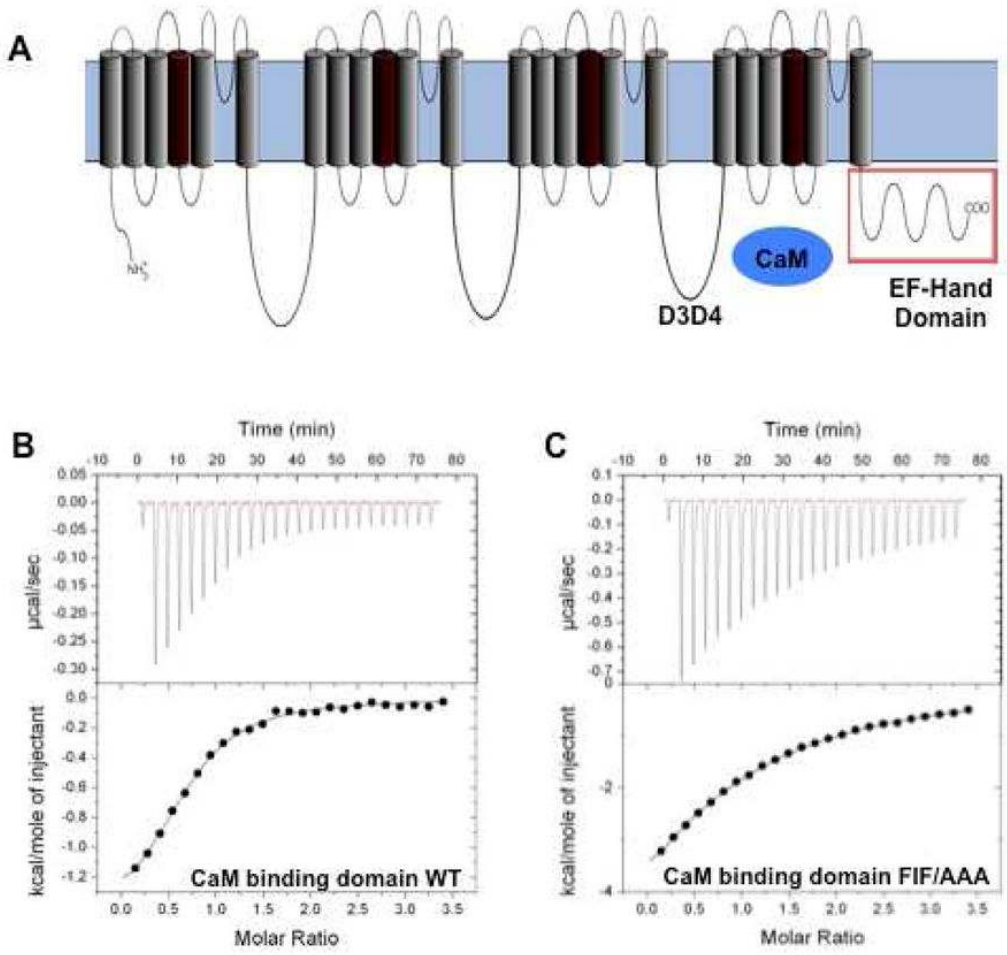

Figure 7.

Interaction of CaM with the D3D4 linker of $Na_V1.5$. (A) Schematic diagram of $Na_V1.5$ highlighting the key elements involved in the Ca^{2+} sensing apparatus. (B,C) Binding isotherms determined by isothermal titration calorimetry demonstrating (B) the Ca^{2+}-dependent interaction of CaM with the D3D4 linker and (C) loss of affinity when three key hydrophobic residues in the CaM binding region are mutated to Ala. [Panels B and C taken from Ref. [60]]

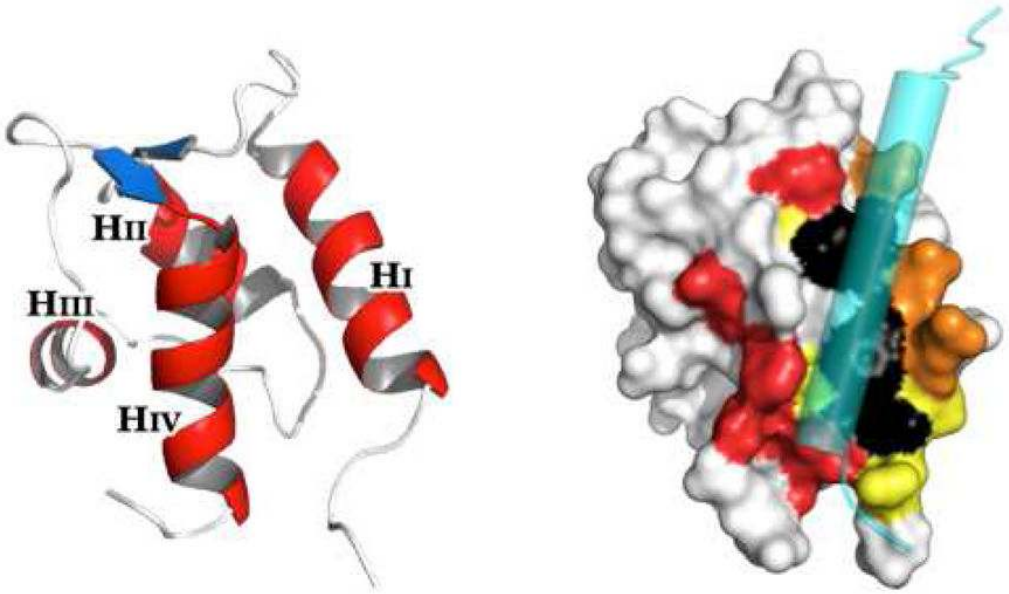

Figure 8.
Structure of the $Na_V1.5$ EF-hand domain and surface representation with a cylindrical cartoon of the IQ motif peptide docked on the basis of NMR binding data. The residues with chemical shift perturbations induced by the IQ motif peptide are colored yellow, orange and red for progressively larger perturbations and black for adjacent residues whose NMR resonances could not be monitored. [Taken from Ref. [61]]