Structural determination of the C2-V5 complex representing an intra-molecular interaction from PKCα

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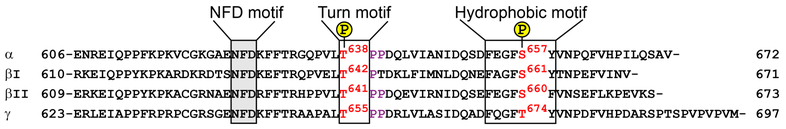
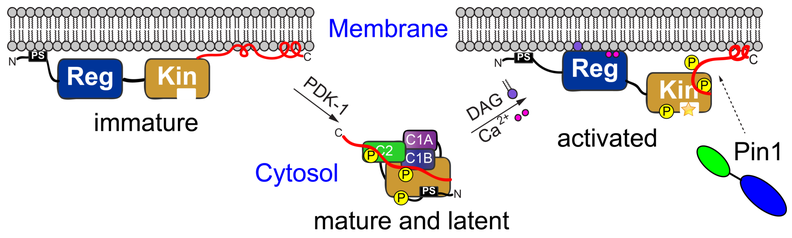
Introduction

V5 domains are the extreme C-terminal 60 to 80 amino acid segments of Protein Kinase C (PKC) isozymes, which are the least conserved sequences among PKCs[1].



Above: The alignment of V5 domains from conventional PKC isoforms. Phosphorylated residues are in red.

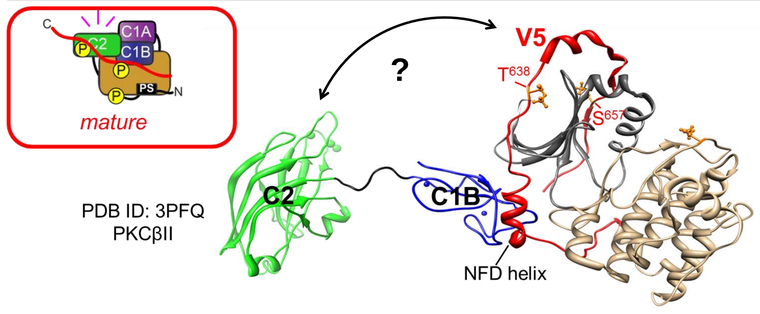
We have previously demonstrated that V5 domain is intrinsically disordered and can serve as a membrane anchor, using solution NMR methods and other biochemical techniques[5]. Functionally, V5 domains are proposed to play roles in (1) the maturation of newly-synthesized PKCs[1]; (2) intra-molecular interactions to maintain the latent form of PKCs until activation[2,3]; and (3) inter-molecular interactions with multiple regulating proteins, including peptidyl-prolyl isomerase Pin1[4].



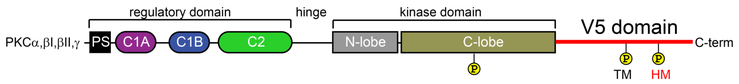
Above: A model of conventional PKC maturation and activation. PDK-1 is the upstream kinase responsible for processing the immature PKC. Calcium ions and diacylglycerol (DAG) are second messengers required for PKC activation. Pin1 was proposed to down-regulate conventional PKC (cPKC) through interactions with V5 domain[4].

The objective of this work is to investigate the protein-protein interactions involving V5 domain. Our data demonstrate the dole of the V5 domain in intra-molecular interaction with the C2 regulatory domain. We use a combination of data-driven molecular dynamics methods to determine the complex structure of C2-V5.

V5 and C2 are not adjacent in the primary structure of PKC

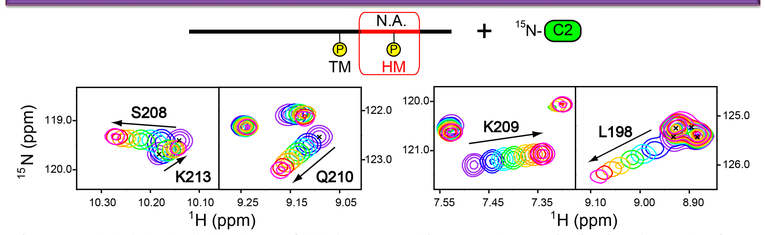


Above: The auto-inhibitory interaction between C2 domain and V5 domain does not exist in the crystal structure of PKCβII.

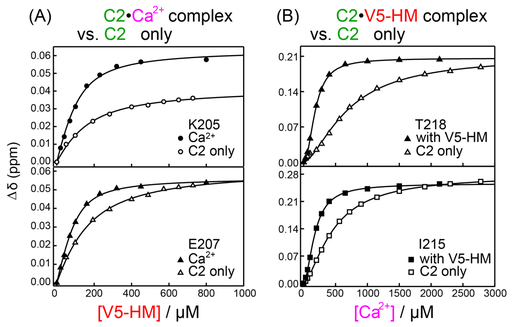


Above: The domain structure of PKCα. Calcium binding C2 domain is far away from the C-terminal V5 domain in the primary structure.

V5 interacts with the C2 domain and increases its affinity for Ca^{2+}



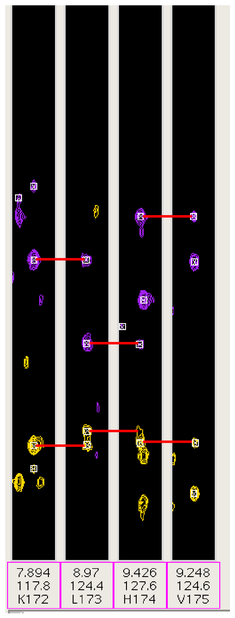
Above: NMR-detected titration of C2 domain with V5-HM reveals the binding interface.



Left: V5-HM may sensitize C2 domain to Ca^{2+} ions through the auto-inhibitory interaction. (A) Ca^{2+} increases C2 affinity for V5-HM. (B) V5-HM increases C2 affinity for Ca^{2+} .

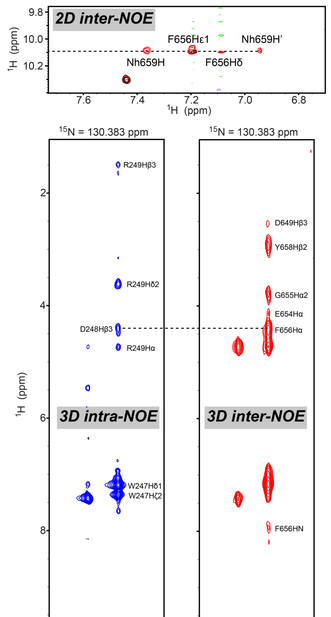
NMR data provide atomic details of the protein-peptide complex

To calculate the C2-V5 protein-peptide complex structure by NMR, the first step is to acquire atomic level information with a series of two- and three-dimensional NMR experiments. Two types of information are required: (1) chemical shift values assigned for each atom available in both protein and peptide components; (2) distance information between atom pairs, including intra- and inter-molecular pairs.



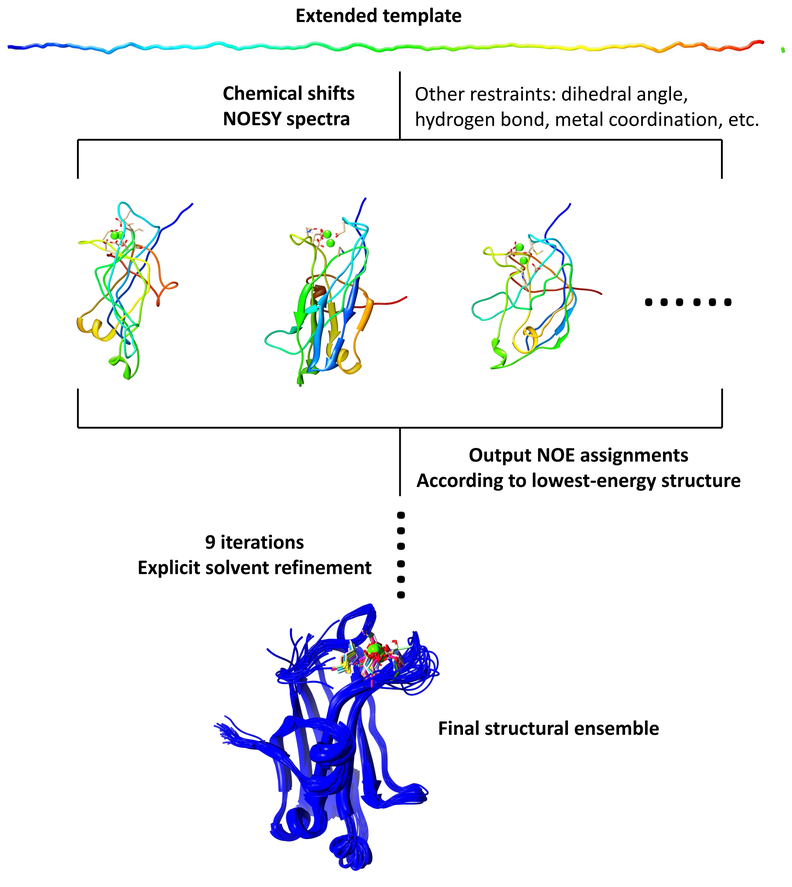
Above: triple resonance spectra showing chemical shift assignments, connecting the protein residues.

Below: NOESY spectra showing NOE peaks containing both intra- and inter-molecular distance information



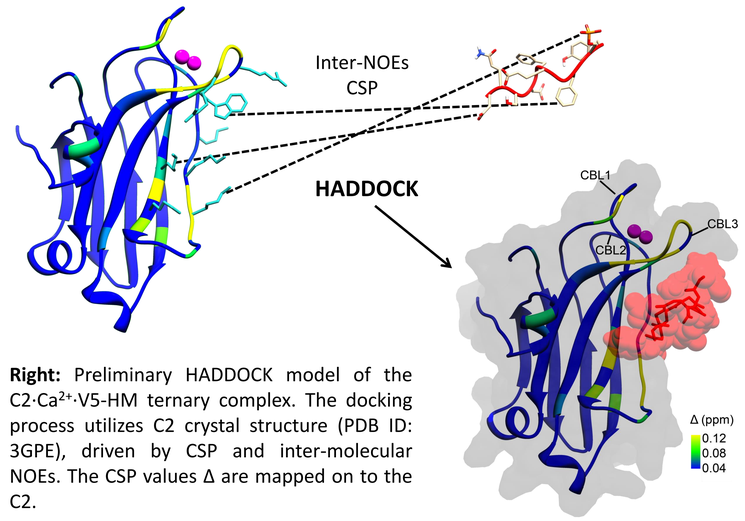
Determine the structure of C2 domain in the complex by ARIA

ARIA (**A**mbiguous **R**estraints for **I**terative **A**ssignment) is a software for automated NOE assignment and NMR structure calculation[6]. The basic input information for ARIA requires chemical shift values unambiguously assigned to each available atom, and the NOE spectral data. Since there are more than 8,000 NOE peaks in the NOESY spectra, it is very difficult and time-consuming to manually assign atomic identities with accuracy. Due to this reason, we use ARIA program package to assist the NOE assignments in a iterative manner.



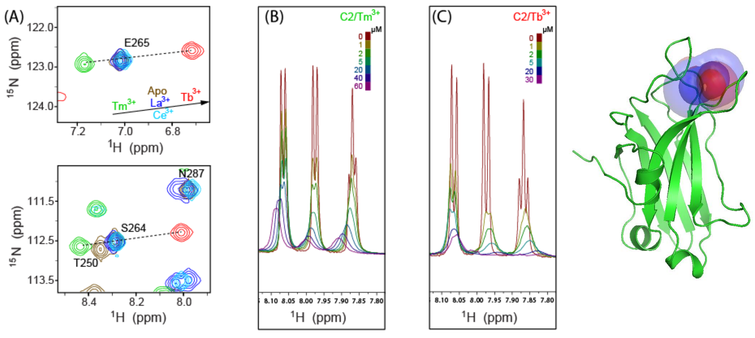
Towards the structure determination of the ternary C2·Ca^{2+}·V5-HM complex with HADDOCK

To determine the ternary C2·Ca^{2+}·V5-HM complex structure, we use HADDOCK package (*H*igh *A*mbiguity *D*riven biomolecular *DOCK*ing) based on biochemical and/or biophysical information[7].



Right: Preliminary HADDOCK model of the C2·Ca^{2+}·V5-HM ternary complex. The docking process utilizes C2 crystal structure (PDB ID: 3GPE), driven by CSP and inter-molecular NOEs. The CSP values Δ are mapped on to the C2.

Additional restraints: pseudocontact shifts (PCS)



Above: Potential use of pseudo contact shifts (**PCS**) to refine the complex structure with the new PCS-HADDOCK[8]. (A) ^{15}N-^{1}H HSQC overlay showing [U-^{15}N] C2 bound to equimolar amount of La^{3+}, Tm^{3+}, Ce^{3+} and Tb^{3+}. Doping experiments of V5-HM peptide with pre-formed (B) C2/Tm^{3+} complex; (C) C2/Tb^{3+} complex. (D) The isosurface of Tm^{3+} anisotropic magnetic susceptibility tensors, determined from backbone amide proton and nitrogen PCS values and PDBID 3TWY with the first metal coordination site, using the Numbat software[9].

Conclusions and future directions

We have shown by solution NMR techniques and other methods that V5 is an Intrinsically Disordered Protein domain and potentially serves as a membrane anchor for immature PKC to partition in the cellular membrane. V5 is involved in intra-molecular interactions with C2. These interactions may stabilize the latent form of mature PKC and enhance C2 affinity to Ca^{2+}. In order to determine the solution NMR structure of the ternary C2·Ca^{2+}·V5-HM complex, we use ARIA to determine the structure of C2 domain in the complex form. Preliminary docking of the final complex was also performed by HADDOCK, leading to a final high-quality structural ensemble.

Future directions include:

1) Include PCS restraints into complex structural calculation.
2) Optimise the docking protocol for the final ternary complex structure.
3) Guided by the complex structure, investigate the auto-inhibitory role of the C2-V5 interaction in full-length PKC.

References

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