Controlling The Folding and Misfolding of Potassium Channel Assembly

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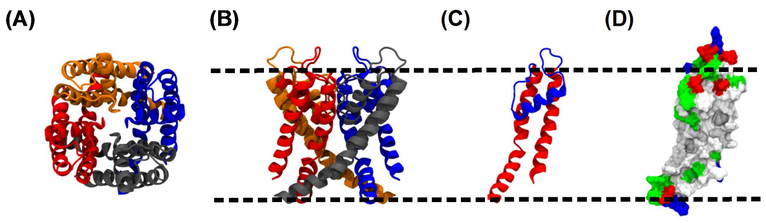
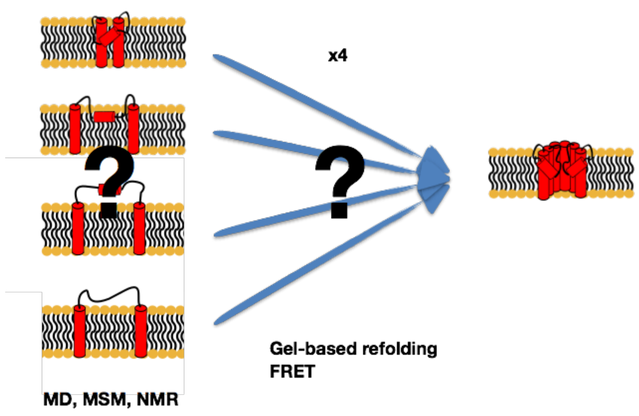
Abstract

We connect the dynamics of Kv1.2 and KcsA potassium channel pore domain monomers to the kinetics of tetramerization. In simulations, monomers adopt multiple conformations with the three helices folded. NMR studies also find the monomers to be dynamic and structurally heterogeneous. However, a KcsA construct with a disulfide bridge engineered between the two transmembrane helices has an NMR spectrum with well-dispersed peaks, suggesting that the monomer can be locked into a native-like conformation. During tetramerization, FRET results indicate that monomers rapidly oligomerize upon insertion into liposomes, likely forming a dense protein-rich phase. Folding within this protein-rich phase occurs along separate fast and slow routes, with tf ~ 40 and 1500 seconds, respectively. In contrast, constructs bearing the disulfide bond mainly fold via the faster pathway, suggesting that maintaining the TM helices in their native orientation reduces misfolding. Interestingly, folding is concentration independent in spite of the tetrameric nature of the channel, indicating that the rate-limiting step is unimolecular and occurs after monomer association in protein-rich phase. Finally, despite its name, the addition of KcsA's C-terminal "tetramerization" domain does not improve the kinetics of tetramerization.

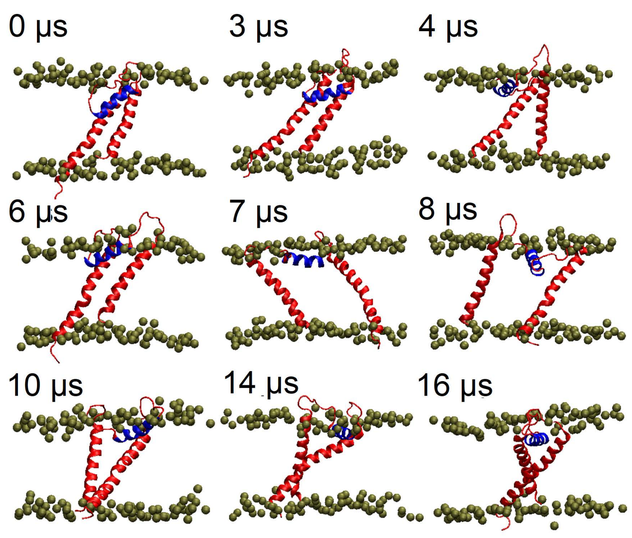


(A) Kv1.2 pore domain is viewed from top in cartoon with different color representing individual subunits. (B) Tetramer viewed from side, where black lines indicate hypothetical membrane boundaries. The pore is hidden from lipid bilayer by transmembrane helices. (C) Monomeric subunit of Kv1.2 pore domain is shown from its side. The pore loop structure is highlighted in blue, and the figure shows that the re-entrant structure buries unsatisfied hydrogen bond donors and acceptors into the membrane. (D) Kv1.2 pore domain monomer is shown in space-filling representation with residues highlighted by its residue type (Green= polar, Red = acidic, Blue = basic, and Grey = non-polar).

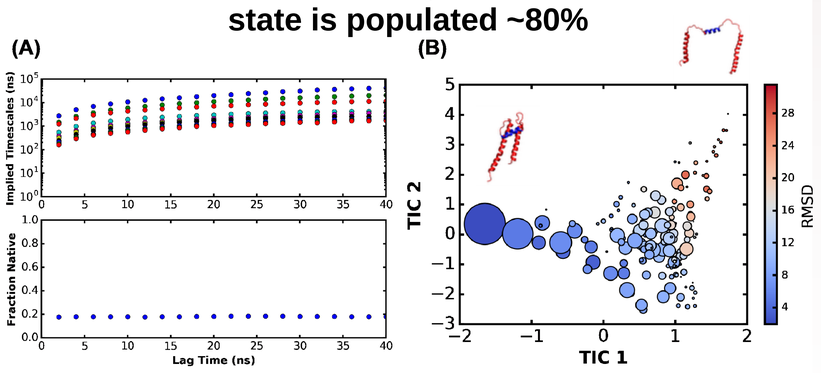
How do monomer dynamics affect tetramerization?



16 μs simulation of Kv1.2 monomer explores non-native states

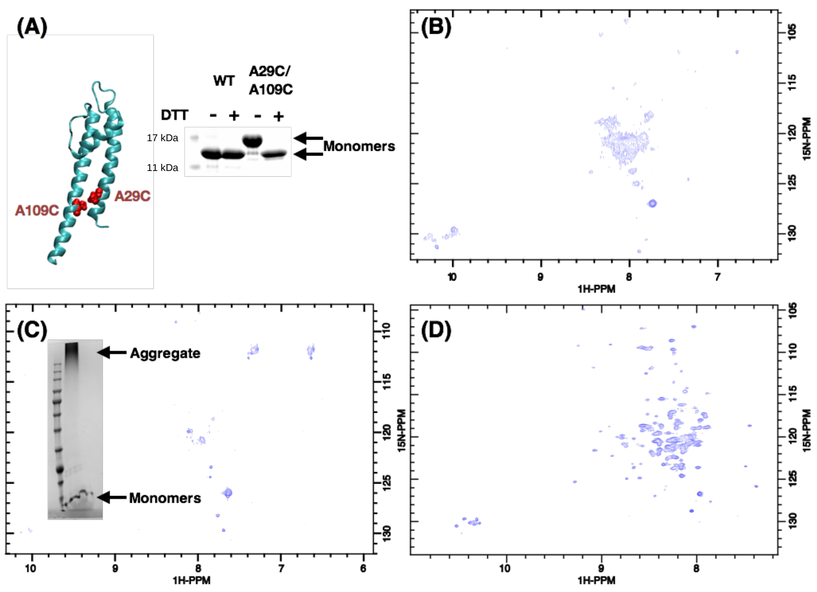


Markov State Model analysis shows non-native state is populated ~80%



MSM built on 400 μs of total simulation time. (A) Top: Implied timescale analysis of Markov State Model. Bottom: Lag-time analysis of fraction native indicates that ~20% of the population remains native-like**. (B)** Markov state model built with lag time of 20 ns is projected onto TIC1 and TIC2. The size of the circle is proportional to the population of each microstate and the color of the circle represents the RMSD of each microstate to the native structure (in the tetramer).

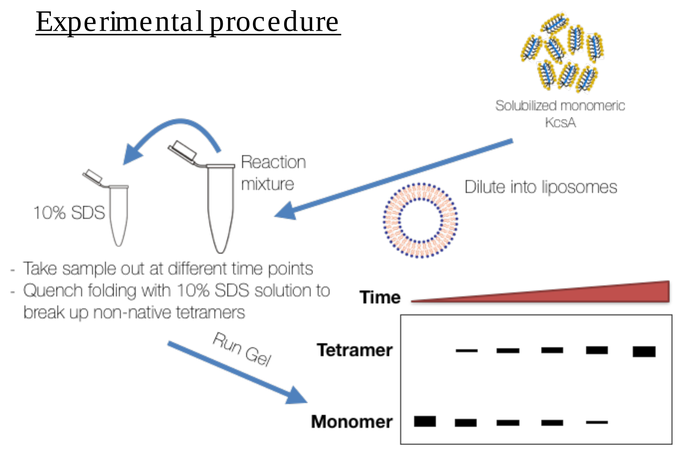
NMR shows disulfide bond "CC" mutant can be engineered to form a more "native-like" monomer



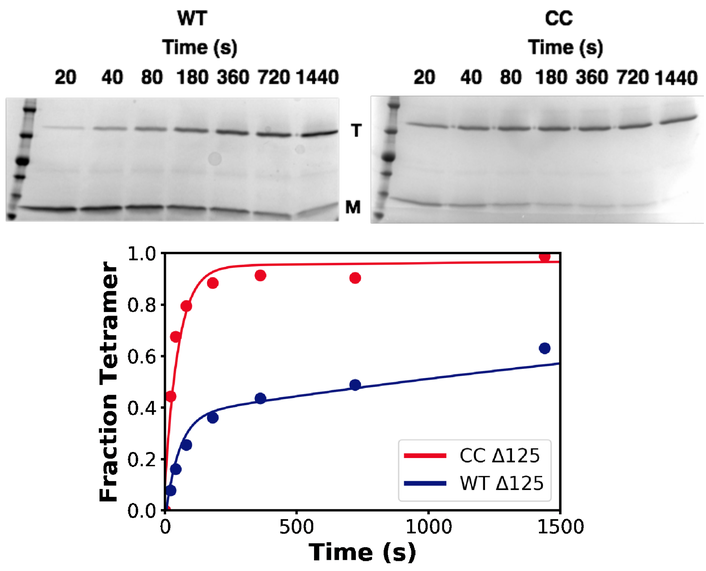
Disulfide bonding the two TM helices results in a less dynamic monomer. (A) The two Ala-to-Cys mutations made in KcsA is shown. A shift in the SDS-PAGE band in DTT confirms the presence of the disulfide bond. (B) NMR $_1$**H-**$_{15}$**N** HSQC spectrum of wild-type KcsA in MSP1D1 Δh5 nanodisc with DMPG lipids. (C) Wild-type KcsA solubilized in q=0.3 ([DMPC]:[DHPC]) bicelle has few peaks, and the SDS-PAGE gel shows the formation of soluble aggregates. (D) KcsA disulfide mutant is solubilized in the same bicelle mixture shows more well-dispersed peaks.

Native-like disulfide bond mutant has faster kinetics and higher yield of tetramer assembly

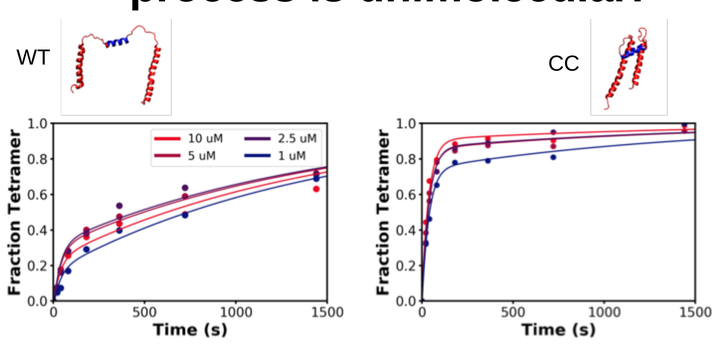
Experimental procedure



Gel results



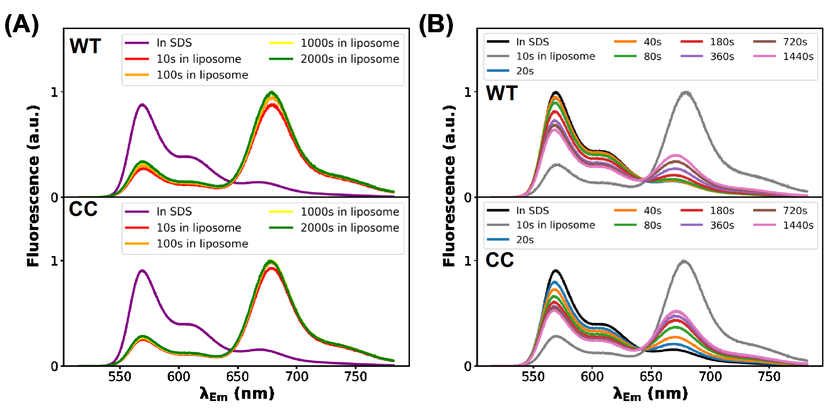
Rate-limiting step in tetramerization process is unimolecular!



FRET result shows rapid association before tetramerization

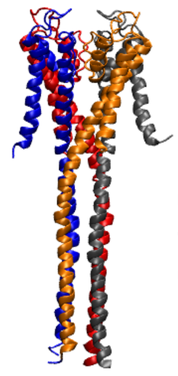
Cy3 (Donor)
- Emission ~ 580nm

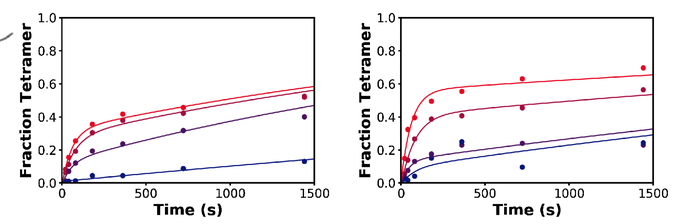
Cy5 (Acceptor)
- Emission ~ 680nm



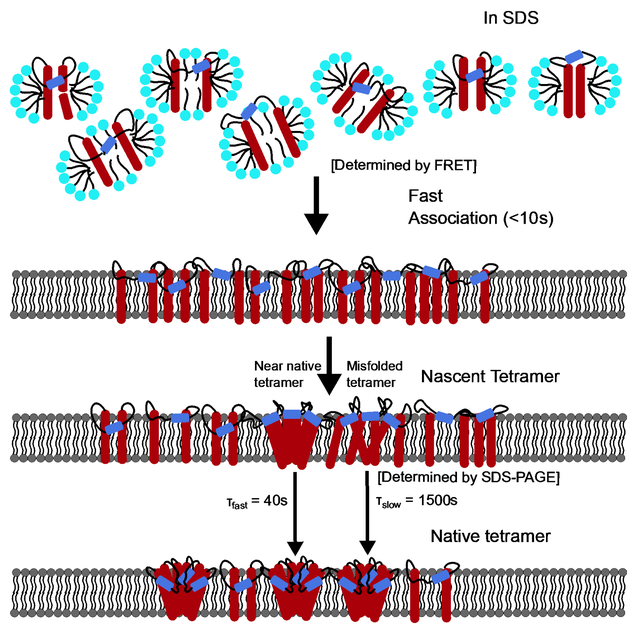
The initial FRET increase indicates that the monomers labeled with donor and acceptor dyes rapidly associate in the liposomes prior to tetramerization. The minimal subsequent change implies that the FRET level in the rapidly associated monomers is similar to the level of folded tetramers in liposomes. The observation that most of the change in FRET signal occurred before significant tetramer formation, along with an estimate of ~30 – 300 monomers per liposome at 1 – 10 mM monomer concentration, argues that most of the population forms a non-native oligomeric state upon insertion into liposomes with a FRET level comparable to that of native tetramers. The oligomers may become part of a protein-rich phase within the membrane.

Full-length (FL) KcsA tetramerization is concentration dependent and hinders tetramerization





Conclusions



Future Directions

- Identification of late, unimolecular rate-limiting step in tetramerization:
  - Pore helix insertion, twist of tetramer and/or channel opening?
- Lipid dependent folding studies
- Nature of the initial oligomeric association (phase separation?)
- Single-molecule microscopy
- Further investigation into C-terminal tetramerization domain

Acknowledgements

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