Coupling and uncoupling of allosteric signals underlying ligand binding and gating in HCN2 channels

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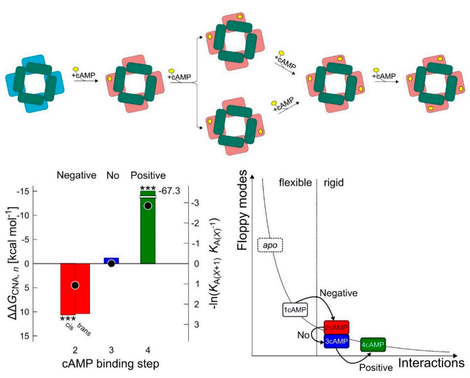
Introduction

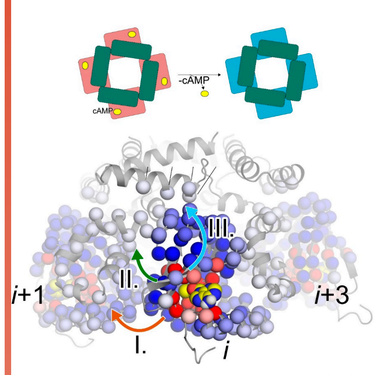
Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels generate the inward I_f/I_h current that acts as a pacemaker in cardiac and neuronal cells. The gating of these channels is modulated through the cyclic nucleotide-binding domain (CNBD) and the C-linker (CL) in the C-terminal channel region. Over the past years, we have studied how cyclic nucleotide monophosphate (cNMP) binding drives changes in the conformational dynamics and energetics of the tetrameric CL-CNBD in HCN2 channels to determine how these changes affect the ligand-dependent channel gating.

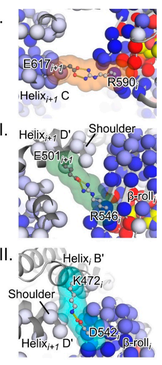
Allosteric transmission

We used a rigidity theory-based free energy perturbation approach[1] for analyzing the allosteric transmission through the isolated tetrameric CL-CNBD. After perturbing (removal of cAMP) the CL-CNBD, changes in statics allow for studying the intra-subunit cooperativity and the identification of key residues for signal transmission.

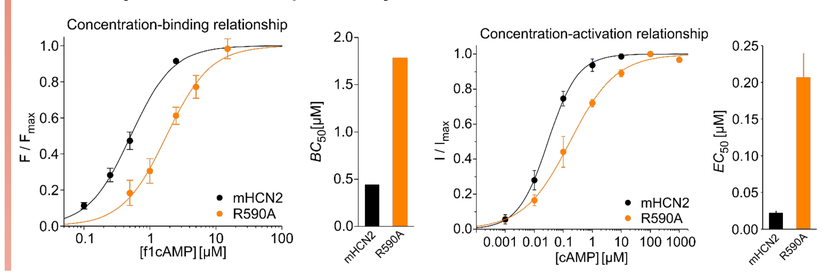
Binding of two, three, and four cAMP molecules to tetrameric CL-CNBD reveal a succession of negative, no, and positive for the cooperativity, in perfect qualitative agreement with experiments.

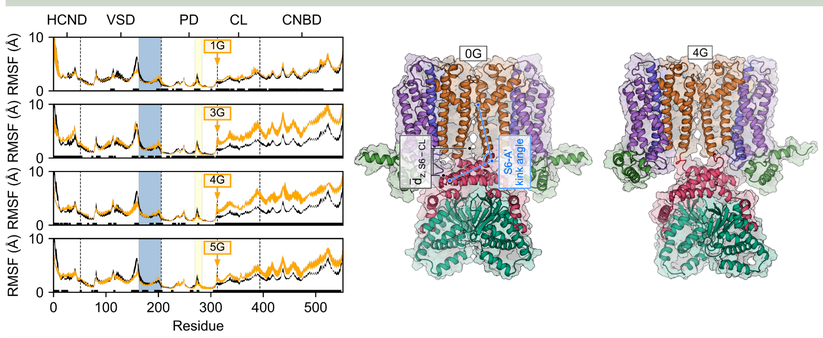


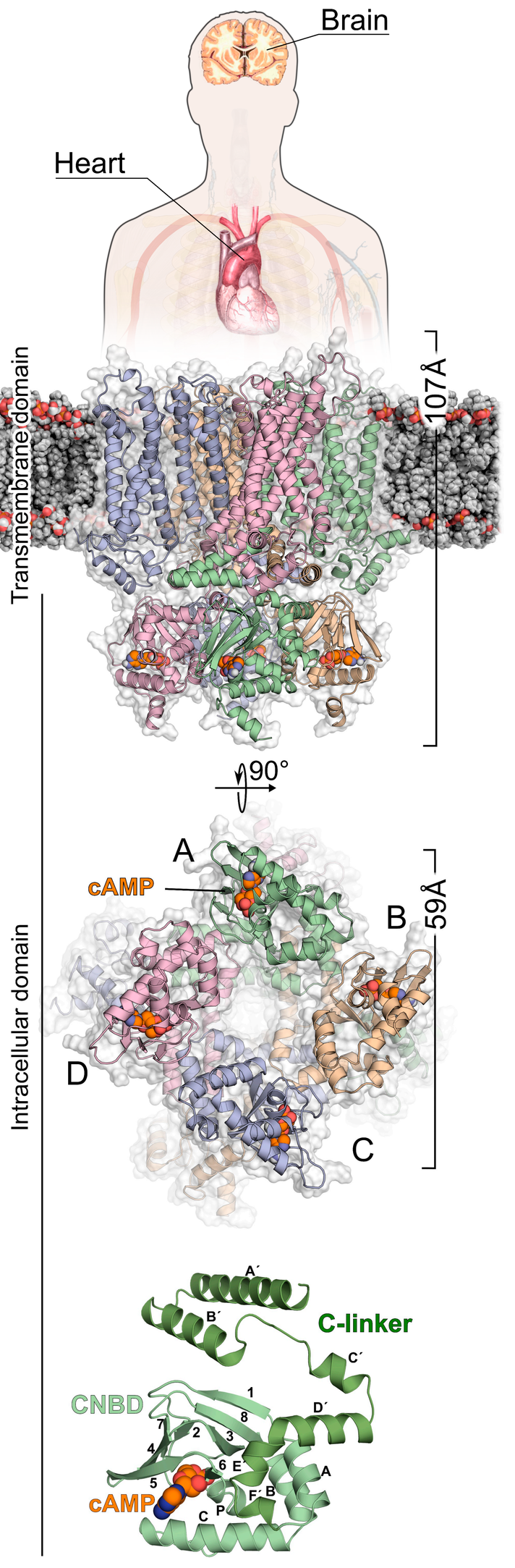




We observed three pathways along which changes in structural stability propagate, starting from the cAMP binding site: **(I)** a ring-like pattern to neighboring subunits, **(II)** a narrow path to the shoulder of the neighboring subunit, and **(III)** a broad path to the elbow of the same subunit. Mutations of selected pathway residues modulate different cAMP binding responses in HCN2 channels through disjunct salt bridge interactions. Exemplary, mutation **R590A** involving the $R590_i$-$E617_{i+1}$ salt bridge impacts cAMP binding affinity, mediates cooperativity between cAMP binding sites, and, thereby, indirectly influences potency.[2]

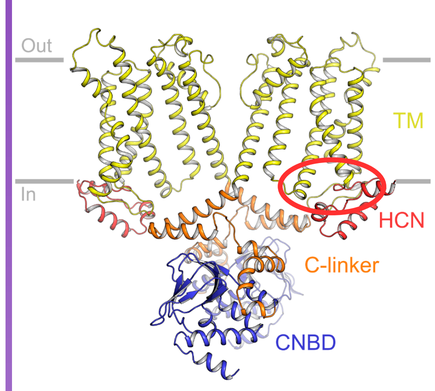


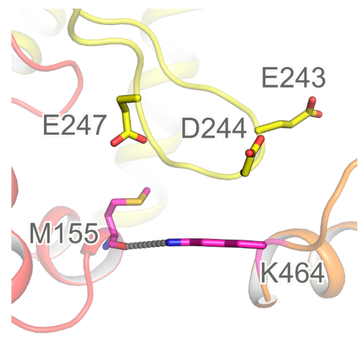




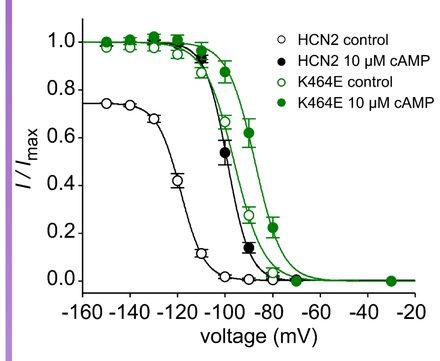
Closed-state stabilization

Most functional studies focused on interactions between neighboring subunits. However, little is known regarding the interactions between opposite subunits. We show that K464 of the C-linker is essential for stabilizing the closed state of the HCN2 channel.



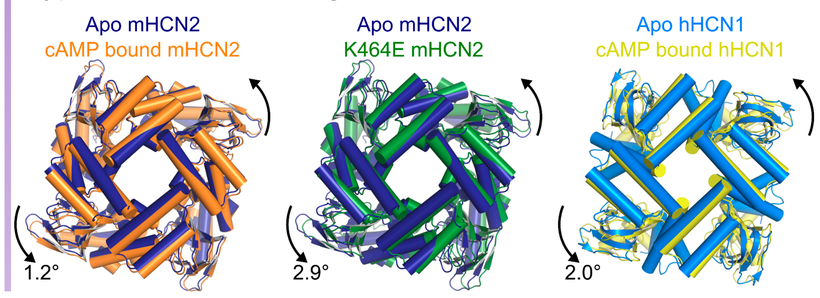


In vitro data revealed that K464 substitution changes the activation and the deactivation kinetics of HCN2.



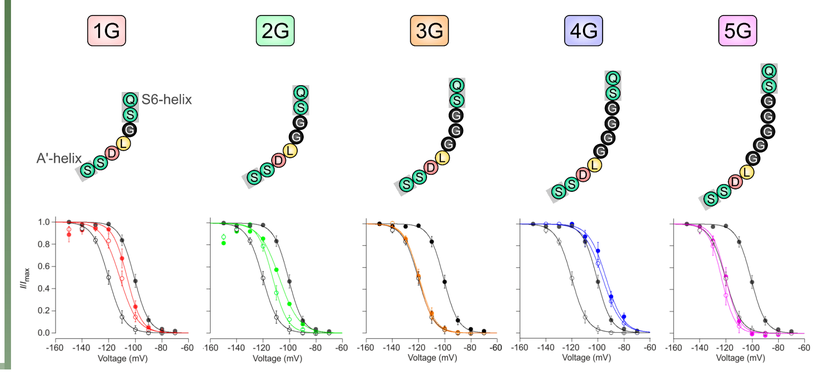
Data obtained for wild type at saturating [cAMP] and K464E without cAMP led us to speculate that *apo* K464E channels adopt a similar conformation as cAMP-bound wild type channels.

MD simulations of the K464E channel revealed a rotation of the CL-CNBD relative to the *apo* wild type channel pore, which agrees in terms of direction and magnitude with structural changes induced by cAMP binding to wild type HCN2 or homologous HCN channels.[3]

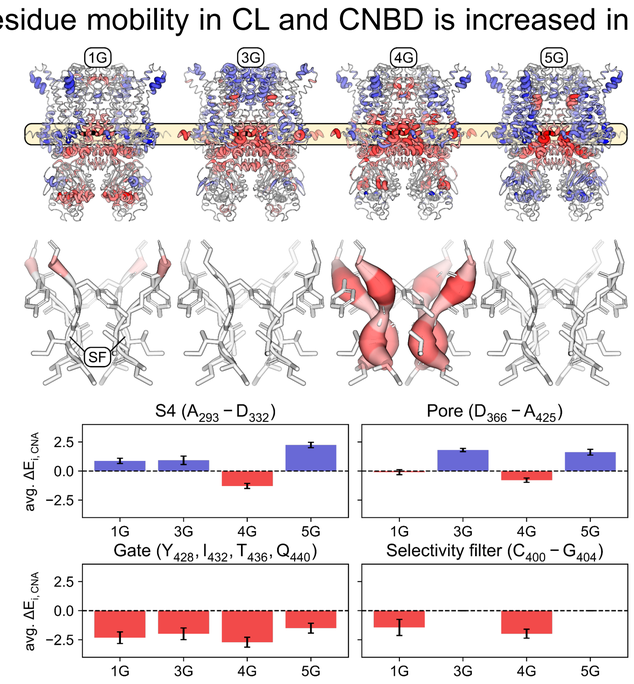


Periphery-pore communication

To study the communication between CL-CNBD and pore region, we structurally uncoupled the C-linker from the transmembrane region by inserting 1-5 glycines between S6 gate and A'-helix.



We demonstrated, that a single glycine is sufficient to abolish the cAMP effect on activation. Concentration-activation relationships were shifted to depolarized voltages in all constructs except 3G and 5G – the strongest effect was found in 4G. Similarly, the activation kinetics were accelerated in all constructs, again with the strongest effect in 4G. MD simulations and Constraint Network Analysis revealed that the average residue mobility in CL and CNBD is increased in all constructs and that the junction between the S6 and A′-helix is turned into a flexible hinge, thus destabilizing the gate in all constructs. 3G and 4G exhibit a stronger downward displacement of the CL-CNBD than wild-type HCN2 and the other constructs, resulting in an increased kink angle between S6 and A′-helix, which in turn loosens contacts between the S4-helix and the CL.[4]



Summary

Our studies on the structural dynamics of the full-length model of HCN2 revealed **I)** three pathways for allosteric transmission upon cAMP binding, **II)** that opposite subunits functionally interact to stabilize autoinhibition, and **III)** detailed insights into the functional coupling between CL-CNBD and the channel pore.

References

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