Disinhibition and inhibition of HCN2 channel function by ligand binding to the cyclic nucleotide binding domain

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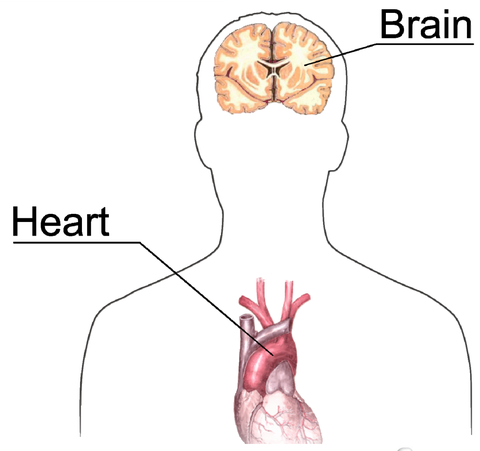
(Project-ID: HDD17)

Introduction

Hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels mediate electrical pacemaking activity in specialized cells of the heart and neurons of the brain [1]. The activity of HCN channels is controlled by two stimuli: (I) membrane hyperpolarization and (II) the binding of cyclic nucleotides (cNMP). However, the mechanism underlying the activation of HCN channels is presently only poorly understood.

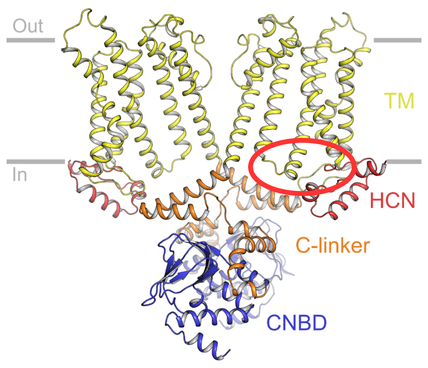


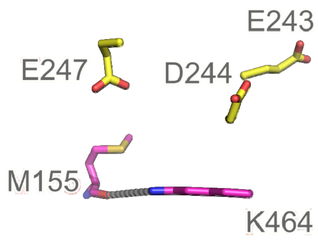
HCN channels are composed of four subunits. The transmembrane channel core of an HCN subunit consists of six α-helical segments and is linked to the intracellular cyclic nucleotide binding-domain (CNBD) via a C-linker region.

Here, we address the central question how changes in the conformational dynamics and energetics of the homotetrameric HCN2 upon cNMP binding relate to the ligand-dependent channel gating. Because cNMPs regulate a variety of targets, we further aimed to identify cNMP analogues selective for HCN2.

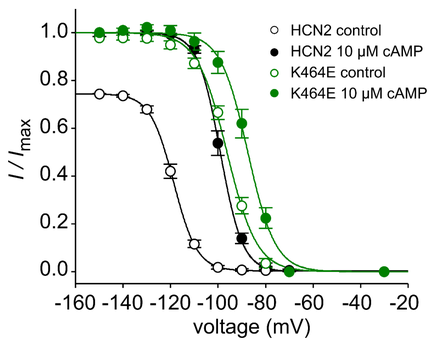
Full length HCN2

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.



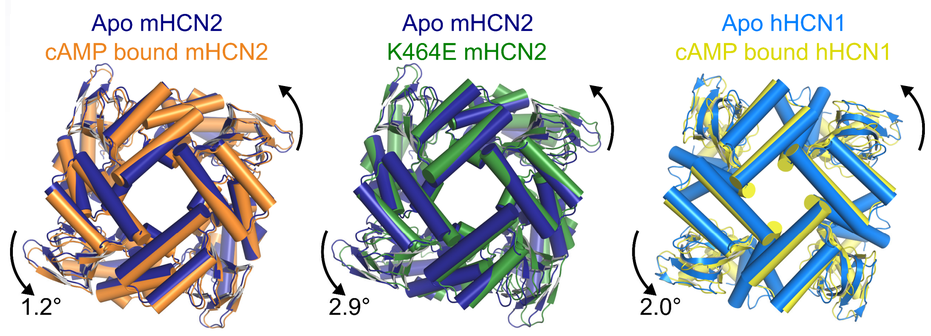


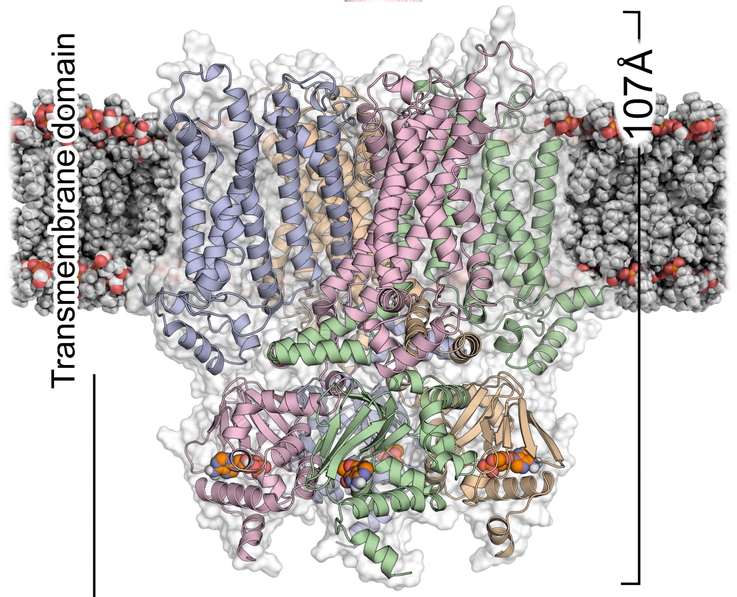
In vitro data revealed that K464 substitution changes the activation and the deactivation kinetics of HCN2.

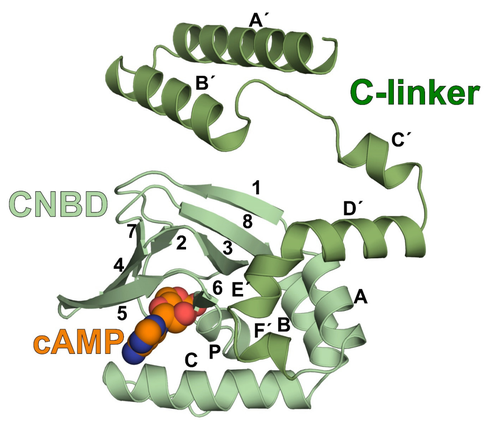


Data obtained for wild type at saturating [cAMP] and K464E without cAMP led us to speculate that apo K464E channels adopt a similar conformation as cAMP-bound wild type channels.

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.



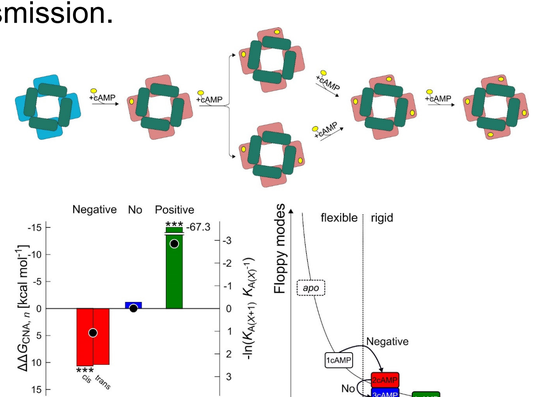


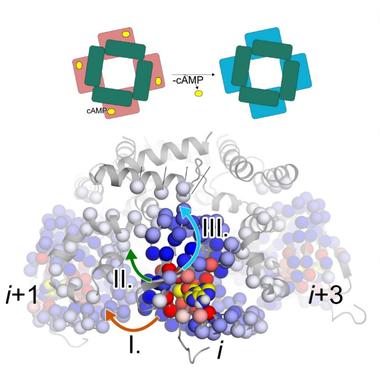


Allosteric transmission

We used a rigidity theory-based free energy perturbation approach for analyzing [2] the allosteric transmission through the isolated tetrameric CL-CNBD. After perturbing (removal of cAMP) the CL-CNBD, changes in statics allows for studying the intra-subunit cooperativity and the identification of key residues for signal transmission.

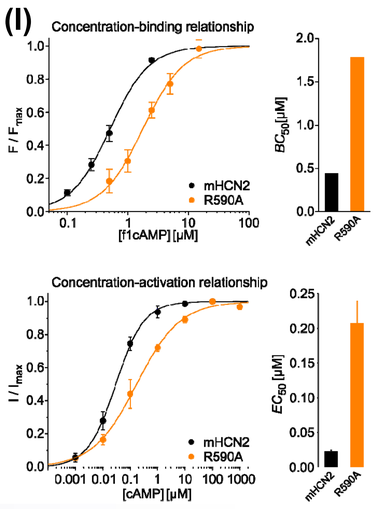
Binding of two, three, and four cAMP molecules to tetrameric CL-CNBD reveal a succession of negative, no, and positive for the cooperativity, in perfect qualitative agreement with experiments.





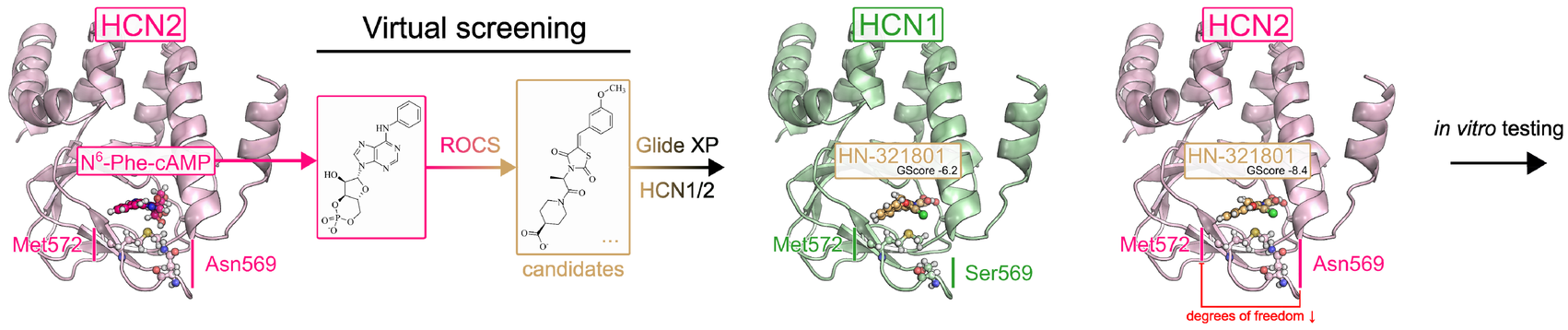
We observed three pathways along which changes in structural stability propagate, starting from the cAMP binding site: **(I)** a ring-like pattern to neighboring subunits, **(II)** a narrow path to the shoulder of the neighboring subunit, and **(III)** a broad path to the elbow of the same subunit.

Mutations of selected pathway residues modulate different cAMP binding responses in HCN2 channels through disjunct salt bridge interactions. Exemplary, mutation **(I)** involving the $R590_i$-$E617_{i+1}$ salt bridge impacts cAMP binding affinity, mediates cooperativity between cAMP binding sites, and, thereby, indirectly influences potency.



Determinants of selectivity

To identify a set of potential HCN2-selective cNMP analogues, we performed virtual screening experiments based on N^6-Phe-cAMP [3] using the ROCS methodology (OpenEye). 10,000 candidate molecules were docked into both HCN1 and HCN2 using the Glide XP approach (Schrödinger, LLC).



Compounds eligible for *in vitro* testing were selected based on the difference in docking score. A comparison of the HCN1 and HCN2 complexes indicates that a S569N substitution distant from the binding site imposes conformational constraints on the binding site residue M572. These constraints would lead to the formation of a conformationally stable hydrophobic patch that could be addressed by HCN2-selective compounds.

Summary

Our studies on the structural dynamics of the full-length model of HCN2 revealed that opposite subunits functionally interact to stabilize autoinhibition. Breaking these interactions either by cAMP binding or mutagenesis, allows the CL-CNBD to adopt a conformation in which autoinhibition is relieved and channel activation is promoted. As to the isolated CNBD, we identified three pathways for allosteric transmission upon cAMP binding deduced from rigidity analyses. Each pathway within the CL-CNBD modulates different cAMP binding responses in mHCN2 channels through disjunct salt bridge interactions. We further identified a set of potential HCN2-selective cAMP analogous.

References

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Acknowledgements

The authors gratefully acknowledge the computing time granted by the John von Neumann Institute for Computing (NIC) and for financial support by the research unit FOR 2518 “Funktionale Dynamik von Ionenkanälen und Transportern - DynIon”.