

# General Audience Report

**Project title**

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

**HPC System(s) and corresponding centre(s)**

JUWELS Booster, NIC

**Project ID**

HCN2COOP

**Principal investigator**

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and

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**Project contributor(s)**

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In this project, Prof. Dr. Holger Gohlke, Dr. Michele Bonus, and Dr. Christopher Pfleger investigated hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels, which are homotetrameric voltage-gated cation channels. The rhythmicity of channel opening arises from channel activation during hyperpolarization of the plasma membrane, which occurs during the repolarization phase of regular action potentials. Binding of cyclic nucleotides such as cAMP or cGMP to HCN channels modulates the channel's response to hyperpolarization by increasing the activation rate, shifting the voltage of half-maximal activation to less hyperpolarized potentials, and increasing the maximal current. However, the mechanisms by which HCN channels are activated and modulated by cyclic nucleotides are not well understood, which complicates the development of drugs that specifically target these channels. Such compounds hold great promise for treating heart disease, pain, inflammatory conditions, and epileptic disorders.

During the project, the team achieved several key objectives, which they present in two publications. The large size of the systems, exceeding 350,000 atoms, in conjunction with the outstandingly long simulation time of in total more than 240 microseconds, required the efficient use of Graphics Processing Units (GPUs) on the JUWELS Booster system. First, by combining electrophysiological techniques with molecular dynamics (MD) simulations and our in-house software for rigidity analysis ("Constraint Network Analysis", CNA), they dissected the mechanisms by which a *structural* uncoupling of the cyclic nucleotide-binding domain (CNBD) from the transmembrane domain of HCN2 channels leads to *functional* uncoupling: We characterized the structural effects evoked by glycine insertions of different lengths in the elbow region of the C-linker (CL) (**Figure 1**), and provided a granular explanation of the electrophysiological phenotypes of five HCN2 constructs. Collectively, these results provided deeper insights into the intricate activation mechanisms of HCN2 channels (Yüksel et al., 2022, DOI: 10.3389/fphys.2022.895324).

Second, the team contributed to constructing a complete thermodynamic profile of the interaction between different subunits in heterotetrameric cyclic nucleotide-gated (CNG) channels, which are close homologs of HCN2 channels. By combining structural and mathematical modeling with electrophysiological techniques, they unraveled microscopic cooperativity in heterotetrameric CNG channels in unprecedented detail and derived a complex heterotetrameric allosteric (CHA) model of channel activation that is applicable to other multisubunit membrane receptors and ion channels (Schirmeyer et al., 2021, DOI: 10.1073/pnas.2100469118).

These results – obtained through a combination of structural modeling and MD simulations performed by us and electrophysiological experiments and mathematical modeling performed in the working group of Prof. Dr. Klaus Benndorf, Jena – have contributed significantly to the understanding of the coupling mechanisms of allosteric signals underlying the ligand binding and gating in HCN and CNG channels.

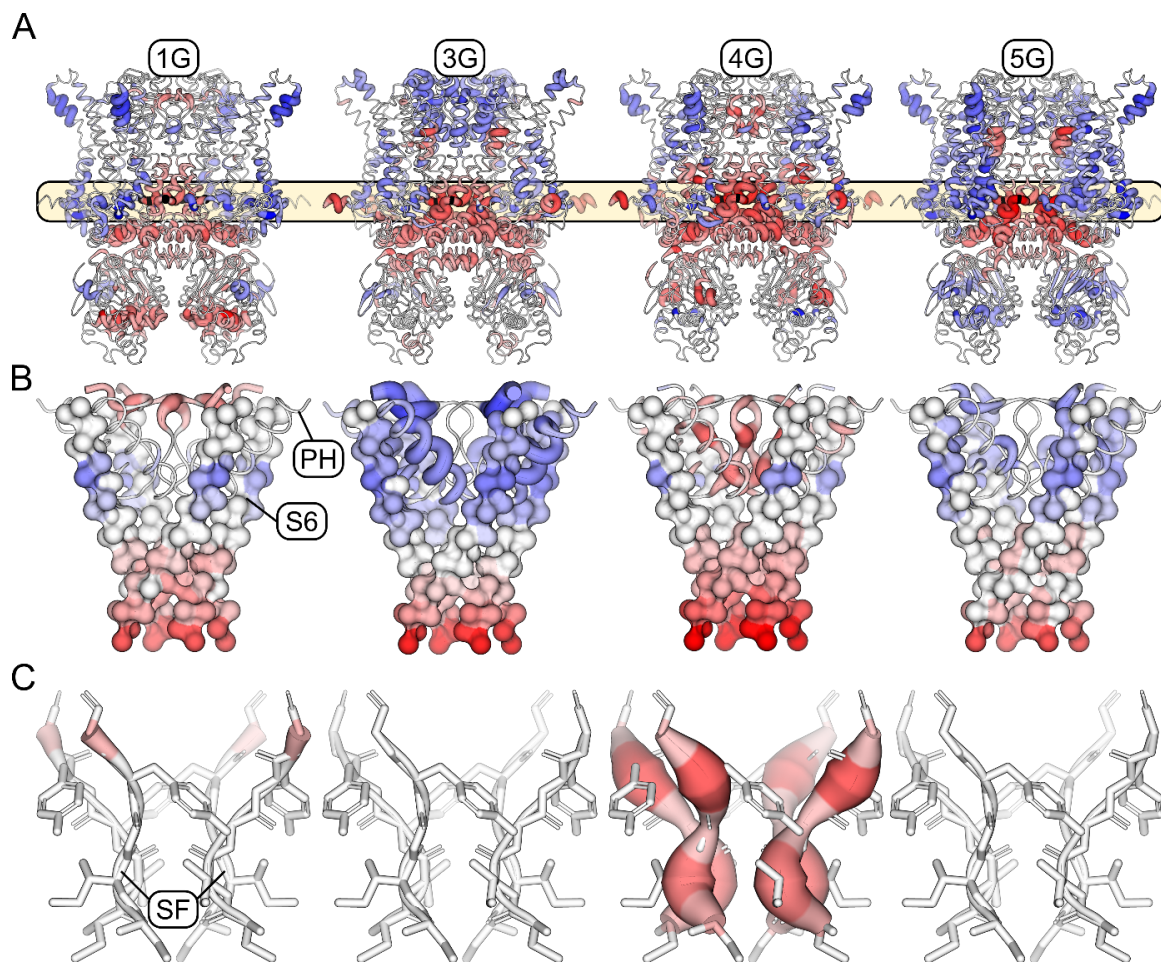
## Teaser

Prof. Dr. Holger Gohlke and his team have made fundamental discoveries about hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels. By investigating the activation and modulation mechanisms of these channels through a combination of molecular dynamics simulations and electrophysiological techniques and mathematical modeling (with Prof. Dr. Klaus Benndorf, Jena), they uncovered crucial insights. Their findings shed light on the structural effects caused by glycine insertions in the C-linker region and elucidated the thermodynamic profile of heterotetrameric cyclic nucleotide-gated (CNG) channels. This research not only enhances our understanding of channel activation but might also pave the way for the development of targeted drugs for heart disease, pain, inflammation, and epilepsy.

In the granting period, the following publications were published:

Yüksel, S., Bonus, M., Schwabe, T., Pflieger, C., Zimmer, T., Enke, U., Saß, I., Gohlke, H., Benndorf, K., Kusch, J. Uncoupling of voltage- and ligand-induced activation in HCN2 channels by glycine inserts. *Front. Physiol.* 2022, 13, 895324. DOI: 10.3389/fphys.2022.895324

Schirmeyer, J., Hummert, S., Eick, T., Schulz, E., Schwabe, T., Ehrlich, G., Kukaj, T., Wiegand, M., Sattler, C., Schmauder, R., Zimmer, T., Kosmalla, N., Bonus, M., Gohlke, H., Benndorf, K. Thermodynamic profile of mutual subunit control in a heteromeric receptor. *Proc. Natl. Acad. Sci.* 2021, 118, e2100469118. DOI: 10.1073/pnas.2100469118



**Figure 1: Stabilized and destabilized regions of the glycine-linker constructs according to rigidity analyses.** **A:** Mapping of the differences  $\Delta E_{i,CNA} = E_{i,CNA(0G)} - E_{i,CNA(nG)}$  – averaged across simulations and subunits – onto the structure of the wildtype channel (**0G**). Regions that are significantly stabilized in the respective construct compared to **0G** (positive  $\Delta E_{i,CNA}$  values) are shown in shades of blue. Regions that are significantly destabilized compared to **0G** (negative  $\Delta E_{i,CNA}$  values) are shown in shades of red. Shading is defined by a divergent color map with  $\Delta E_{i,CNA} \leq -5.5$  kcal mol<sup>-1</sup>: red,  $\Delta E_{i,CNA} = 0$  kcal mol<sup>-1</sup>: white, and  $\Delta E_{i,CNA} \geq 5.5$  kcal mol<sup>-1</sup>: blue. The thickness of the cartoon representation is scaled using the absolute value  $|\Delta E_{i,CNA}|$ . Residues for which the differences were not significant ( $p \geq 0.05$ ) were assigned  $\Delta E_{i,CNA} = 0$  kcal mol<sup>-1</sup>. **B:** Similar representation as in panel A with a focus on S6, the pore helix, and the selectivity filter. S6 is depicted in a surface representation of its C-alpha trace. **C:** Same representation as in panel A with a focus on the selectivity filter. Figure taken from ref. DOI 10.3389/fphys.2022.895324.