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Structural basis of the partially open central gate in the human CNGA1/CNGB1 channel explained by additional density for calmodulin in cryo-EM map

Diane C.A. Barret [a], Gebhard F.X. Schertler [a,b], U. Benjamin Kaupp [c,d], Jacopo Marino [a,*]

[a] Laboratory of Biomolecular Research, Paul Scherrer Institut, Switzerland
[b] Department of Biology, ETH-Zurich, Switzerland
[c] Center for Advanced European Studies and Research (CAESAR), Bonn, Germany
[d] Life and Medical Sciences Institute LIMES, University of Bonn, Germany

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SUMMARY

The recently reported structure of the human CNGA1/CNGB1 CNG channel in the open state (Xue et al., 2021a) shows that one CNGA1 and one CNGB1 subunit do not open the central hydrophobic gate completely upon cGMP binding. This is different from what has been reported for CNGA homomeric channels (Xue et al., 2021b; Zheng et al., 2020).

In seeking to understand how this difference is due to the presence of the CNGB1 subunit, we find that the deposited density map (Xue et al., 2021a) (EMDB 24465) contains an additional density not reported in the images of the original publication. This additional density fits well the structure of calmodulin (CaM), and it unambiguously connects the newly identified D-helix of CNGB1 to one of the CNGA1 helices ($A1_R$) participating in the coiled-coil region. Interestingly, the CNGA1 subunit that engages in the interaction with this additional density is the one that, together with CNGB1, does not open completely the central gate. The sequence of the D-helix of CNGB1 contains a known CaM-binding site of exquisitely high affinity – named CaM2 (Weitz et al., 1998) –, and thus the presence of CaM in that region is not surprising.

The mechanism through which CaM reduces currents across the membrane by acting on the native channel (Bauer, 1996; Hsu and Molday, 1993; Weitz et al., 1998) remains unclear. We suggest that the presence of CaM may explain the partially open central gate reported by Xue et al. (2021a). The structure of the open and closed states of the CNGA1/CNGB1 channel may be different with and without CaM present.

1. Introduction

Cyclic nucleotide-gated (CNG) channels in photoreceptors and olfactory neurons form a family of ion channels, which serve as targets for cGMP- and cAMP-signaling pathways that are activated by light and odorants, respectively (Kaupp and Seifert, 2002; Matulef and Zagotta, 2003).

CNG channels, composed of CNGA and CNGB subunits, belong to the superfamily of voltage-gated ion channels (VGICs) that share a common topology: the channels comprise six transmembrane segments, a cytoplasmic C-linker composed of helices A′ to F′, and a cyclic nucleotide-binding domain (CNBD) composed of helices A to C and a beta-roll. Upon gating, the CNBD undergoes conformational changes that are transmitted via the C-linker to the four S6 transmembrane segments, which move to open a central hydrophobic gate formed by residues F389 and V393 in the human CNGA1 subunit, and F872 and I876 in the human CNGB1 subunit (Xue et al., 2021b).

CNG channels are cation-selective ion channels, but a large fraction of the current is carried by Ca^{2+} ions (Frings et al., 1995; Nakatani and Yau, 1988; Perry and McNaughton, 1991). Ca^{2+} partially blocks the channel (Yiannis and Yau, 1996); the Ca^{2+} flux through the CNG channel is pivotal for controlling the activity of several enzymes, in particular the guanylyl cyclase, via various Ca^{2+}-binding proteins including calmodulin (CaM) (Polans et al., 1996; Yau and Hardie, 2009).

The activity of the rod CNG channel is tuned by CaM, which decreases the apparent affinity for cGMP binding; CaM binds with high affinity to the CNGB1 but not to the CNGA1 subunit, thereby decreasing Ca^{2+} influx (Chen et al., 1994; Hsu and Molday, 1993; Weitz et al.,

* Corresponding author.
E-mail address: jacopo.marino@psi.ch (J. Marino).

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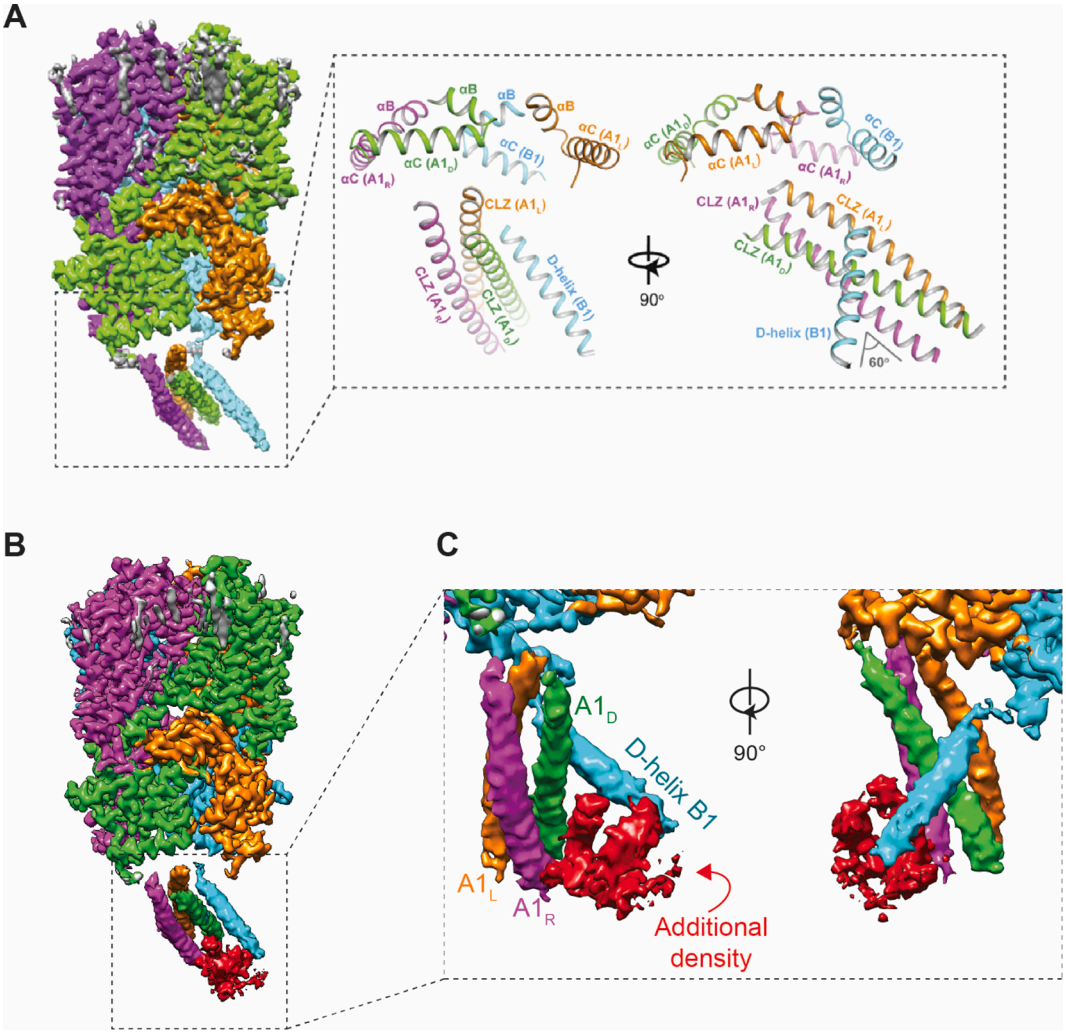

Fig. 1. (A) Panel A is taken from the original publication of Xue et al. (2021a). Light blue, density corresponding to CNGB1; orange, density corresponding to $A1_L$ (CNGA1); magenta, density corresponding to $A1_R$; and green, density corresponding to $A1_D$. (B) Overview of the density map EMDB 24465, with colored density according to Xue et al. (2021a). The density map was opened in Chimera (Pettersen et al., 2004) and a Gaussian filter (width 0.842) was applied, after which the map was colored according to 4.5 Å radius from the relative atomic coordinates (PDB: 7RHL). Figures reported in this manuscript are based on this map. In red is shown the additional density that connects the CNGB1 D-helix to CNGA1 $A1_R$. (C) Close up of the panel C in the coiled-coil region.

1998). This modulation might be required to tune the sensitivity of photoreceptor cells and olfactory neurons (Bradley et al., 2004, 2001; Trudeau and Zagotta, 2003).

Two CaM-binding sites are located at the N- and C-terminus of the CNGB1 subunit, referred to as CaM1 and CaM2, respectively (Weitz et al., 1998). CaM1 is strictly required for modulation of the CNGA1/CNGB1 heteromeric channels, whereas CaM2 is not (Grunwald et al., 1998; Weitz et al., 1998). CaM2 is localized on a segment adjacent to the cGMP-binding domain and has a higher affinity for CaM than CaM1 (Weitz et al., 1998); the role of CaM2 site remains to be fully understood (Weitz et al., 1998). The structural basis for the control by CaM of the gating mechanism itself remains ill-defined. CaM features a two-lobe structural architecture with the N-terminal domain (N-lobe) and the C-terminal domain (C-lobe), and each lobe harbors two Ca^{2+} binding sites (Babu et al., 1988). The affinity of CaM for the native rod CNG channel is high, with maximal effects on currents observed at approximately <5 nM CaM (Bauer, 1996; Grunwald et al., 1998; Hsu and Molday, 1993; Weitz et al., 1998). Binding of CaM to peptides that correspond to CaM1 and CaM2 sequences or modulation of the rod CNG channel requires very low concentrations of Ca^{2+} (40–50 nM) (Nakatani et al., 1995; Sagoo and Lagnado, 1996; Weitz et al., 1998). CaM modulates many ion channels by a variety of mechanisms, including CaM that is not bound to Ca^{2+} (Adams et al., 2014; Sachyani et al., 2014), and CaM is permanently associated with the olfactory CNG channel in a Ca^{2+}-independent fashion (Bradley et al., 2004).

2. Results and discussion

We analyzed the density map deposited by Xue et al. (2021a) corresponding to CNGA1/CNGB1 in the *apo* state (EMDB 24465). Only on this density map the authors performed an additional step of focused 3D classification with a mask around the CLZ (C-terminal leucine zipper)

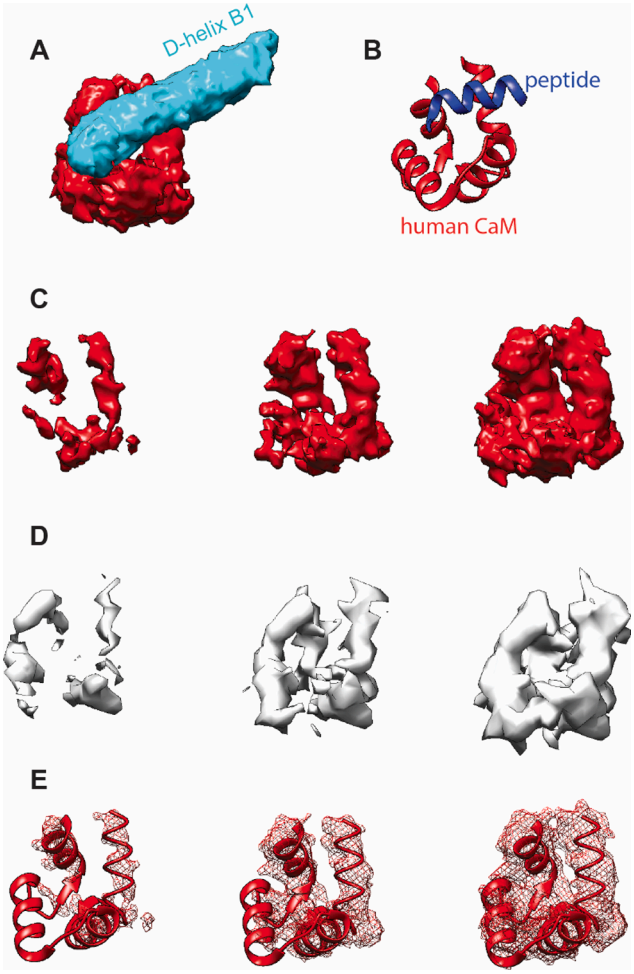

Fig. 2. (A) Isolated density attributed to CaM (red) contacting the D-helix of CNGB1 (light blue). (B) One of the lobes of human CaM (PDB: 7BF2) bound to a peptide. (C) Isolated density attributed to CaM shown at different contour levels (from left to right: 0.0167, 0.0139, and 0.0114), compared with corresponding (D) density calculated (molmap in Chimera) from the crystal structure of human CaM (PDB: 7B2F) shown at different contour levels (from left to right: 0.634, 0.532, and 0.415). In (E) the isolated cryo-density is show as mesh with the docked model (red) of CaM.

coiled-coil region (CNGA1 residues 623–679); this step allows to visualize the density corresponding to the three C-terminal CNGA1 helices that form the coiled-coil. This region is not clearly visible in the other density maps corresponding to the cGMP-bound (EMBD 24461 and EMBD 24462) and cGMP-*l*-*cis*-diltiazem-bound states (EMBD 24463).

In the provided map (EMDB 24465), each of the three C-terminal helices of the CNGA1 subunits form a coiled-coil structure, consistent with data indicating that three CNGA1 subunits are connected at their C-terminus by a leucine-zipper domain (Shuart et al., 2011). Near the CNGA1 coiled-coil, the authors identify a helix named D, which they assign as continuation of the C-helix at the C-terminus of CNGB1 (Xue et al., 2021a). As reported in the original figure (Fig. 1A), this D-helix is tilted nearly 60° with respect to the axis formed by the coiled-coil, and contacts the C-terminal region of one of the three CNGA1 subunits referred to as $A1_D$.

Notably, we find that the density map contains an additional density that is set apart from noise, and that connects unambiguously the CNGB1 D-helix to the C-terminal $A1_R$-helix (Fig. 1B–C). This density is neither visible nor discussed in the main figures, however it is visible in Fig. S3 (Xue et al., 2021a).

This additional density, and its orientation relative to the D-helix are surprisingly reminiscent of the binding mode of human CaM to peptides (Fig. 2A), with a nearly identical orientation between the D-helix and the peptide bound to CaM (Fig. 2B). Rigid-body docking of a molecular model of human CaM (PDB: 7BF2) revealed an excellent fit (Fig. 2C–E), with a cross-correlation of 0.94 between model and density. This suggests that part of CaM (the N-lobe) is not visible in the density map, either because it is excluded from the mask used to perform 3D classification or because it is flexible.

The amino-acid sequence assigned by Xue et al. (2021a) within the density of the D-helix contains part of the CaM2-binding site; however, as the authors note, the density map in that region does not allow to model side chains. By analyzing the map, we suggest that it is questionable whether the D-helix is indeed connected to the C-helix (Fig. 3A). In fact, the D-helix of CNGB1 may actually be part of the N-terminal sequence containing the CaM1 site. To explain these possibilities, we discuss two structural models (Fig. 3B, C). The hypothesis that the D-helix may correspond to the N-terminus of CNGB1 is supported by biochemical experiments that identify strong interaction between an N-terminal sequence of CNGB1 containing the CaM1 site and the C-terminal region forming the coiled-coil of CNGA1 (Trudeau and Zagotta, 2002a, 2002b). Further investigations are required to fully assign this additional D-helix to the C-terminus or N-terminus of CNGB1.

Of note, our results do not question the quality of the structural data presented by Xue et al. (2021a). The additional density in the map that we suggest being CaM is located in a peripheral and flexible region, which might have led Xue et al. to be cautious about its interpretation. Despite the inherent limitations of the quality of the map in that region, we suggest that the presence of CaM should be further investigated and characterized. This characterization would benefit from making available all density maps where local refinement has been performed, as done for the map of the *apo* state (EMDB 24465). The presence of CaM in the sample may be explained by the relatively high abundance of endogenous CaM in HEK cells, which can modulate heterologously expressed ion channels (Biswas et al., 2008; Sun and MacKinnon, 2017). Although CaM cannot be recognized near 16 kDa in the SDS-PAGE of Fig. S1 (Xue et al., 2021a), this might be explained by the low concentration of sample loaded, and by the notorious difficulty in staining small proteins. However, mass spectrometry should be able to identify CaM unequivocally.

The presence of CaM in these structures has profound implications for the conclusions made by Xue et al., because the CNGA1/CNGB1 channel structure might be significantly different in presence or absence of CaM.

We suggest that binding of CaM to CNGB1 and $A1_R$ might explain the partially open central gate. In this model, in absence of CaM, the coiled-coil region lies perpendicular to the membrane axis. If present, CaM binds to the CaM site (either contributed by the N-terminus or C-terminus of CNGB1), and CaM brings this CNGB1 sequence near the coiled-coil region. This causes the coiled-coil region to tilt (Xue et al., 2021a), which may cause a lower degree of conformational freedom within the CNBD of CNGB1 and one CNGA1 subunit ($A1_R$), and thus result in only a modest or no rotation and dilation of their S6 segments, as reported for the structures of the two observed open conformations (Xue et al., 2021a).

Further analysis will be required to fully characterize the structural basis for CaM modulation of the native CNGA1/CNGB1 channel.

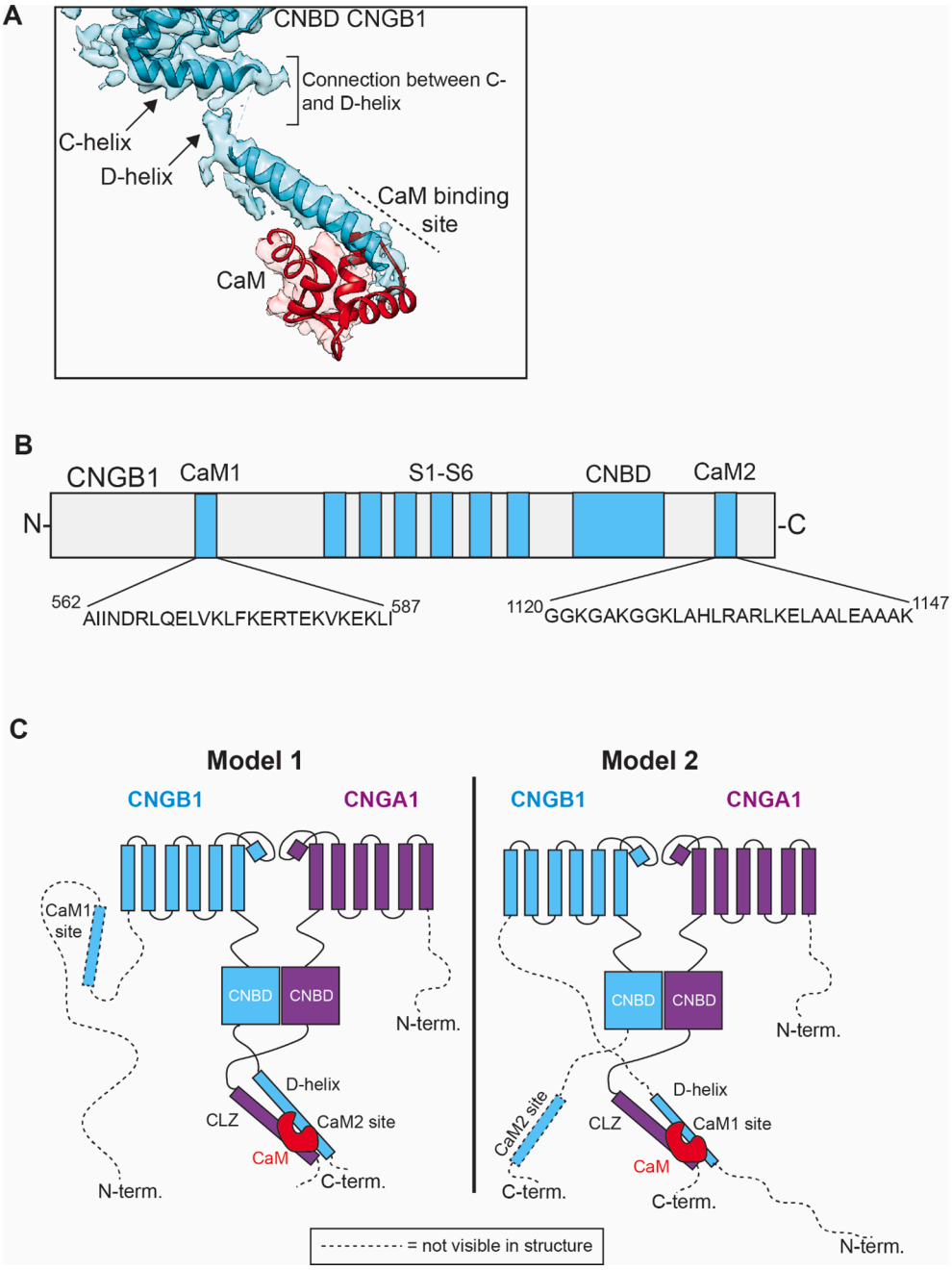

Fig. 3. (A) Details of the density map (EMBD: 24465) corresponding to the CNGB1 subunit in the region of the CNBD, including the C-helix and the additional helix (referred to as D-helix in the text). The position of the sequence corresponding to the CaM2 site is indicated according to the current assignment of the amino acid sequence provided by Xue et al. (2021a). (B) Location and sequences of the CaM-binding sites in the human CNGB1 subunit (UniProt: Q14028). (C) Proposed models for interpreting the identity of the CNGB1 D-helix visualized in the density map. Model 1 is consistent with the interpretation of Xue et al. (2021a), with the additional CNGB1 helix being assigned to a D-helix as continuation of the C-helix of the CNBD. In Model 2, instead, the additional CNGB1 helix is proposed being part of the N-terminal sequence which contains the CaM1-binding site. As shown in Model 2, the sequence of CNGB1 C-terminal to the C-helix is not visible in the density map.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adams, P., Ben-Johny, M., Dick, I., Inoue, T., Yue, D., 2014. Apocalmodulin itself promotes ion channel opening and Ca^{2+} regulation. Cell 159 (3), 608–622. https://doi.org/10.1016/j.cell.2014.09.047.

Babu, Y.S., Bugg, C.E., Cook, W.J., 1988. Structure of calmodulin refined at 2.2 Å resolution. J. Mol. Biol. 204 (1), 191–204. https://doi.org/10.1016/0022-2836(88)90608-0.

Bauer, P.J., 1996. Cyclic GMP-gated channels of bovine rod photoreceptors: affinity, density and stoichiometry of Ca^{2+}-calmodulin binding sites. J. Physiol. 494, 675–685.

Biswas, S., Deschênes, I., DiSilvestre, D., Tian, Y., Halperin, V.L., Tomaselli, G.F., 2008. Calmodulin regulation of NaV1.4 current: Role of binding to the carboxyl terminus. J. Gen. Physiol. 131, 197–209. https://doi.org/10.1085/jgp.200709863.

Bradley, J., Bönigk, W., Yau, K.-W., Frings, S., 2004. Calmodulin permanently associates with rat olfactory CNG channels under native conditions. Nat. Neurosci. 7 (7), 705–710. https://doi.org/10.1038/nn1266.

Bradley, J., Reuter, D., Frings, S., 2001. Facilitation of calmodulin-mediated odor adaptation by cAMP-gated channel subunits. Science (80-.) 294 (5549), 2176–2178.

Chen, T.Y., Illing, M., Molday, L.L., Hsu, Y.T., Yau, K.W., Molday, R.S., 1994. Subunit 2 (or β) of retinal rod cGMP-gated cation channel is a component of the 240-kDa

channel-associated protein and mediates Ca2+-calmodulin modulation. Proc. Natl. Acad. Sci. U. S. A. 91, 11757–11761. https://doi.org/10.1073/pnas.91.24.11757.
Frings, S., Seifert, R., Godde, M., Kaupp, U.B., 1995. Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. Neuron 15 (1), 169–179. https://doi.org/10.1016/0896-6273(95)90074-8.
Grunwald, M.E., Yu, W., Yu, H., Yau, K., 1998. Identification of a Domain on the b-Subunit of the Rod cGMP-gated Cation Channel That Mediates Inhibition by Calcium-Calmodulin. J. Biol. Chem. 273, 9148–9157.
Hsu, Y.-T., Molday, R.S., 1993. Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. Nature 361 (6407), 76–79. https://doi.org/10.1038/361076a0.
Kaupp, U.B., Seifert, R., 2002. Cyclic Nucleotide-Gated Ion Channels. Physiol. Rev. 82 (3), 769–824. https://doi.org/10.1152/physrev.00008.2002.
Matulef, K., Zagotta, W.N., 2003. Cyclic Nucleotide-Gated Ion Channels. Annu. Rev. Cell Dev. Biol. 19 (1), 23–44. https://doi.org/10.1146/annurev.cellbio.19.110701.154854.
Nakatani, K., Koutalos, Y., Yau, K.W., 1995. Ca^{2+} modulation of the cGMP-gated channel of bullfrog retinal rod photoreceptors. J. Physiol. 484, 69–76. https://doi.org/10.1113/jphysiol.1995.sp020648.
Nakatani, K., Yau, K.W., 1988. Calcium and magnesium fluxes across the plasma membrane of the toad rod outer segment. J. Physiol. 395, 695–729. https://doi.org/10.1113/jphysiol.1988.sp016942.
Perry, R.J., McNaughton, P.A., 1991. Response properties of cones from the retina of the tiger salamander. J. Physiol. 433, 561–587. https://doi.org/10.1113/jphysiol.1991.sp018444.
Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25 (13), 1605–1612. https://doi.org/10.1002/jcc.20084.
Polans, A., Baehr, W., Palczewski, K., 1996. Turned on by Ca^{2+}! The physiology and pathology of Ca2+-binding proteins in the retina. Trends Neurosci. 19 (12), 547–554. https://doi.org/10.1016/S0166-2236(96)10059-X.
Sachyani, D., Dvir, M., Strulovich, R., Tria, G., Tobelaim, W., Peretz, A., Pongs, O., Svergun, D., Attali, B., Hirsch, J., 2014. Structural Basis of a Kv7.1 Potassium Channel Gating Module: Studies of the Intracellular C-Terminal Domain in Complex with Calmodulin. Structure 22 (11), 1582–1594. https://doi.org/10.1016/j.str.2014.07.016.
Sagoo, M.S., Lagnado, L., 1996. The action of cytoplasmic calcium on the cGMP-activated channel in salamander rod photoreceptors. J. Physiol. 497, 309–319. https://doi.org/10.1113/jphysiol.1996.sp021770.
Shuart, N.G., Haitin, Y., Camp, S.S., Black, K.D., Zagotta, W.N., 2011. Molecular mechanism for 3:1 subunit stoichiometry of rod cyclic nucleotide-gated ion channels. Nat. Commun. 2 (1) https://doi.org/10.1038/ncomms1466.
Sun, J., MacKinnon, R., 2017. Cryo-EM structure of a KCNQ1/CaM complex reveals insights into congenital long QT syndrome, 169 (6), 1042–1050.e9. https://doi.org/10.1016/j.cell.2017.05.019.
Trudeau, M.C., Zagotta, W.N., 2003. Calcium/calmodulin modulation of olfactory and rod cyclic nucleotide-gated ion channels. J. Biol. Chem. 278 (21), 18705–18708. https://doi.org/10.1074/jbc.R300001200.
Trudeau, M.C., Zagotta, W.N., 2002a. Mechanism of calcium/calmodulin inhibition of rod cyclic nucleotide-gated channels. Proc. Natl. Acad. Sci. U. S. A. 99 (12), 8424–8429. https://doi.org/10.1073/pnas.122015999.
Trudeau, M.C., Zagotta, W.N., 2002b. An Intersubunit Interaction Regulates Trafficking of Rod Cyclic Nucleotide-Gated Channels and Is Disrupted in an Inherited Form of Blindness. Neuron 34 (2), 197–207.
Weitz, D., Zoche, M., Müller, F., Beyermann, M., Körschen, H.G., Kaupp, U.B., Koch, K. W., 1998. Calmodulin controls the rod photoreceptor CNG channel through an unconventional binding site in the N-terminus of the β-subunit. EMBO J. 17, 2273–2284. https://doi.org/10.1093/emboj/17.8.2273.
Xue, J., Han, Y., Zeng, W., Jiang, Y., 2021a. Structural mechanisms of assembly, permeation, gating, and pharmacology of native human rod CNG channel. Neuron 110, 1–10. https://doi.org/10.1016/j.neuron.2021.10.006.
Xue, J., Han, Y., Zeng, W., Wang, Y., Jiang, Y., 2021b. Structural mechanisms of gating and selectivity of human rod CNGA1 channel. Neuron 109 (8), 1302–1313.e4. https://doi.org/10.1016/j.neuron.2021.02.007.
Yau, K.-W., Hardie, R.C., 2009. Phototransduction Motifs and Variations. Cell 139 (2), 246–264. https://doi.org/10.1016/j.cell.2009.09.029.
Yiannis, K., Yau, K.-W., 1996. Regulation of sensitivity in verebrate photoreceptors by calcium. Trends Neurosci. 19, 73–81.
Zheng, X., Fu, Z., Su, D., Zhang, Y., Li, M., Pan, Y., Li, H., Li, S., Grassucci, R.A., Ren, Z., Hu, Z., Li, X., Zhou, M., Li, G., Frank, J., Yang, J., 2020. Mechanism of ligand activation of a eukaryotic cyclic nucleotide−gated channel. Nat. Struct. Mol. Biol. 27 (7), 625–634. https://doi.org/10.1038/s41594-020-0433-5.