Supporting Information: Exploring Channelrhodopsin-2 and Gold Nanoclusters Interaction: a Route to Control the Protein Photocycle

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Table S1: Overview of the initial positions and types of interactions in the 26 MD simulations of ChR2 in interaction with the NC. For each initial position (see Figure S1) the number of simulations in which the NC interacts with the protein via C terminal (IvC), via N terminal (IvN), via membrane (IvM) and in which the NC does not interact with the protein (NoI) are reported.

	Tot	IvC	IvN	IvM	NoI
Start extracellular	9	1	2	0	6
Start intracellular	9	4	0	0	5
Start extracellular near protein	4	0	0	2	2
Start intracellular near protein	4	2	0	1	1
Additional simulations	5	0	3	2	0

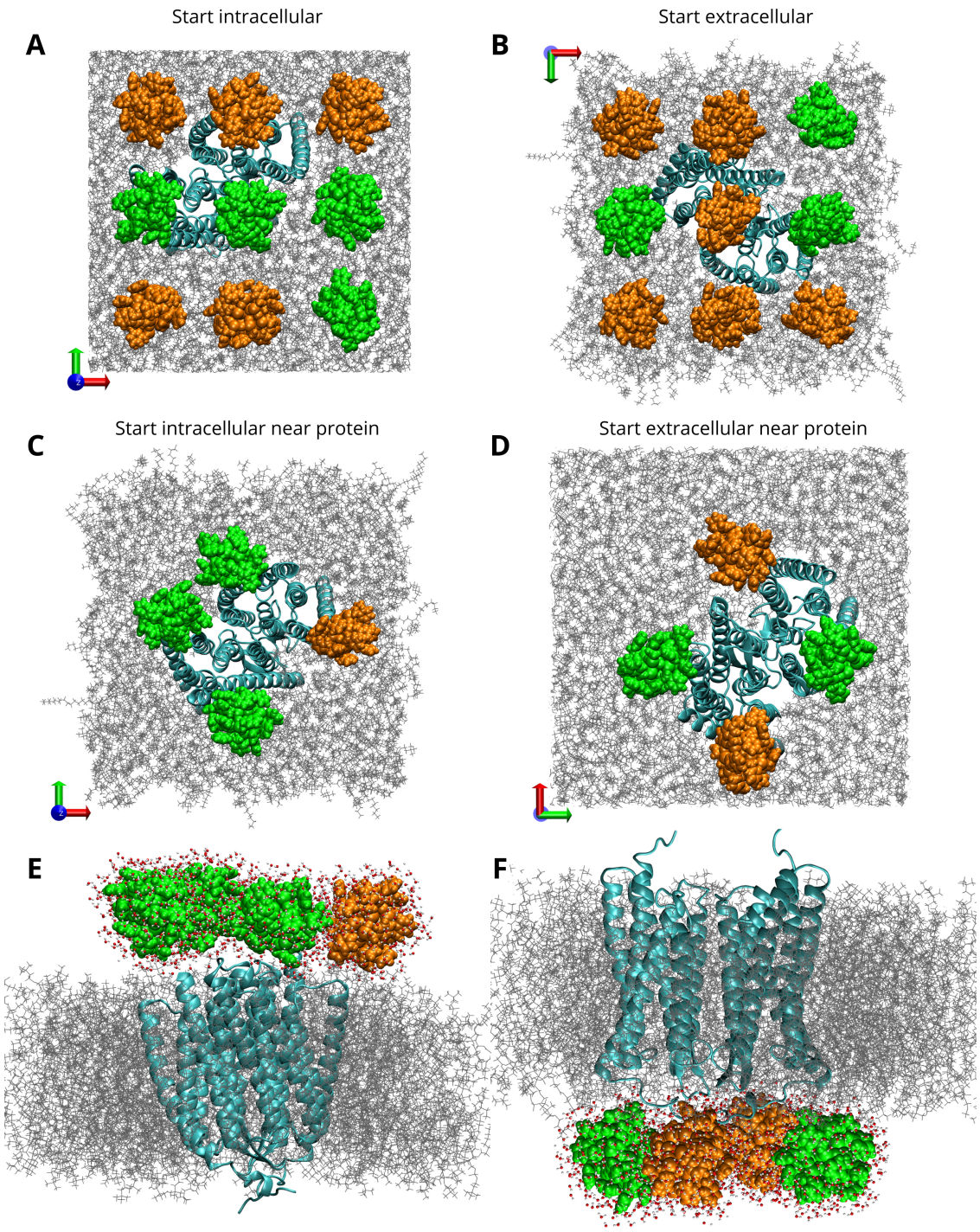

Figure S1: Initial position of the NC in the 26 MD simulations performed. The protein is represented in cyan, the lipid membrane in gray and solvent is not shown for clarity. The NCs that do/do not interact with ChR2 during the simulation are represented in orange and green, respectively. Panels A and B illustrate the initial structures in which the NCs are positioned at ≈3 nm from the membrane at the intracellular and extracellular regions, respectively. The grid is readily discernible. Panels C and D illustrate the initial structures in which the NCs are positioned near the protein, resulting in the formation of a thin layer of water (shown in red in panels E and F).

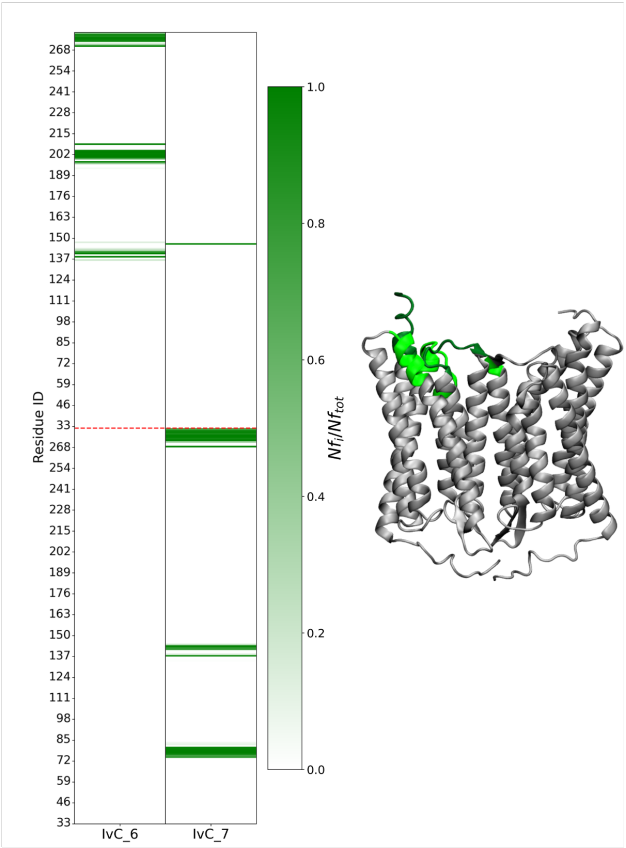

Figure S2: Contact maps between ChR2 and the NC for the two IvC group simulations not shown in Figure 2A of the main manuscript. The representation and color scale are the same as used in Figure 2.

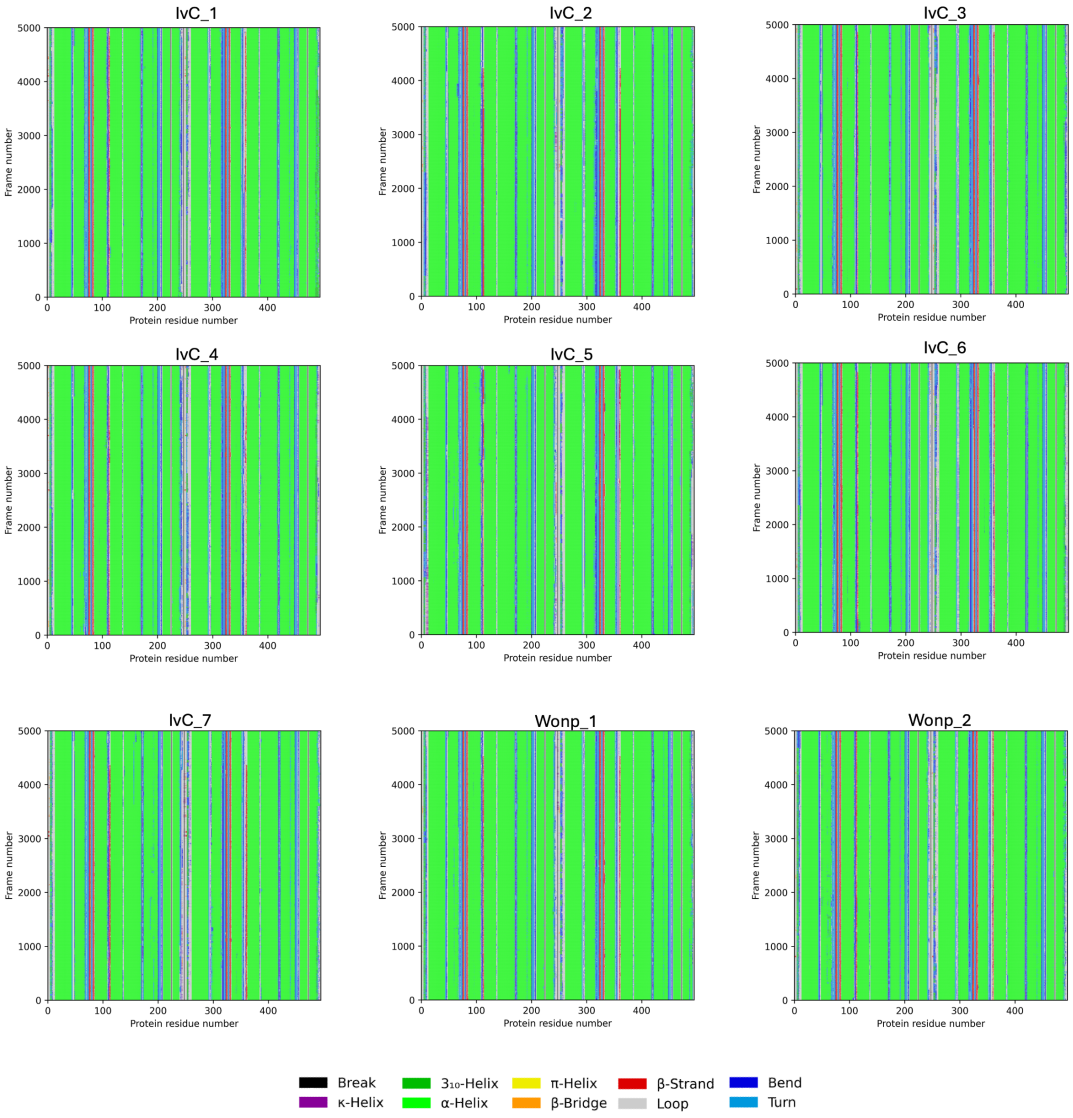

Figure S3: Secondary structure of ChR2 as provided by the DSSP analysis[S1] *along the IvC MD simulations compared to that along the MD simulation without the NC.*

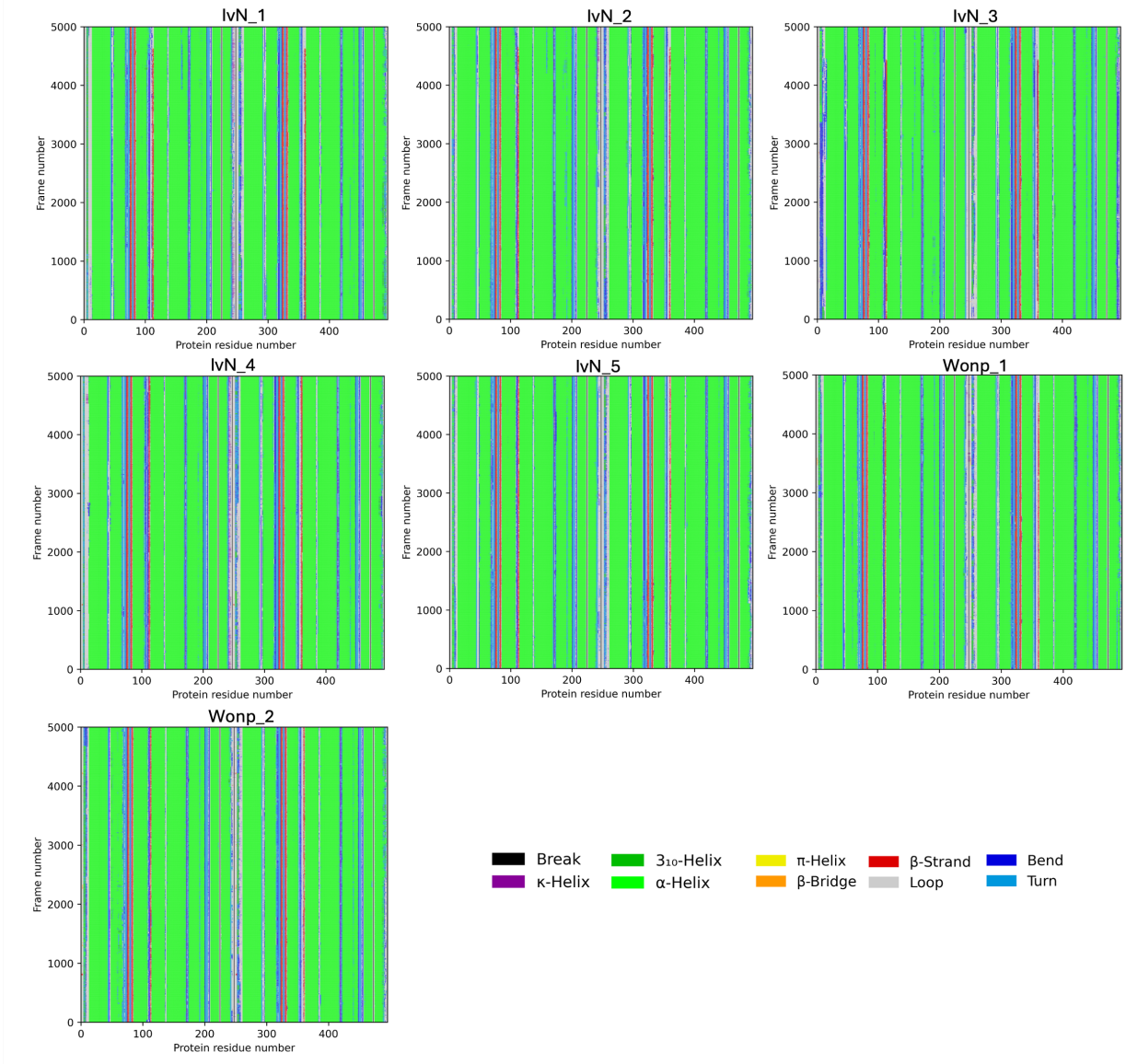

Figure S4: Secondary structure of ChR2 as provided by the DSSP analysis[S1] *along the IvN MD simulations compared to that along the MD simulation without the NC.*

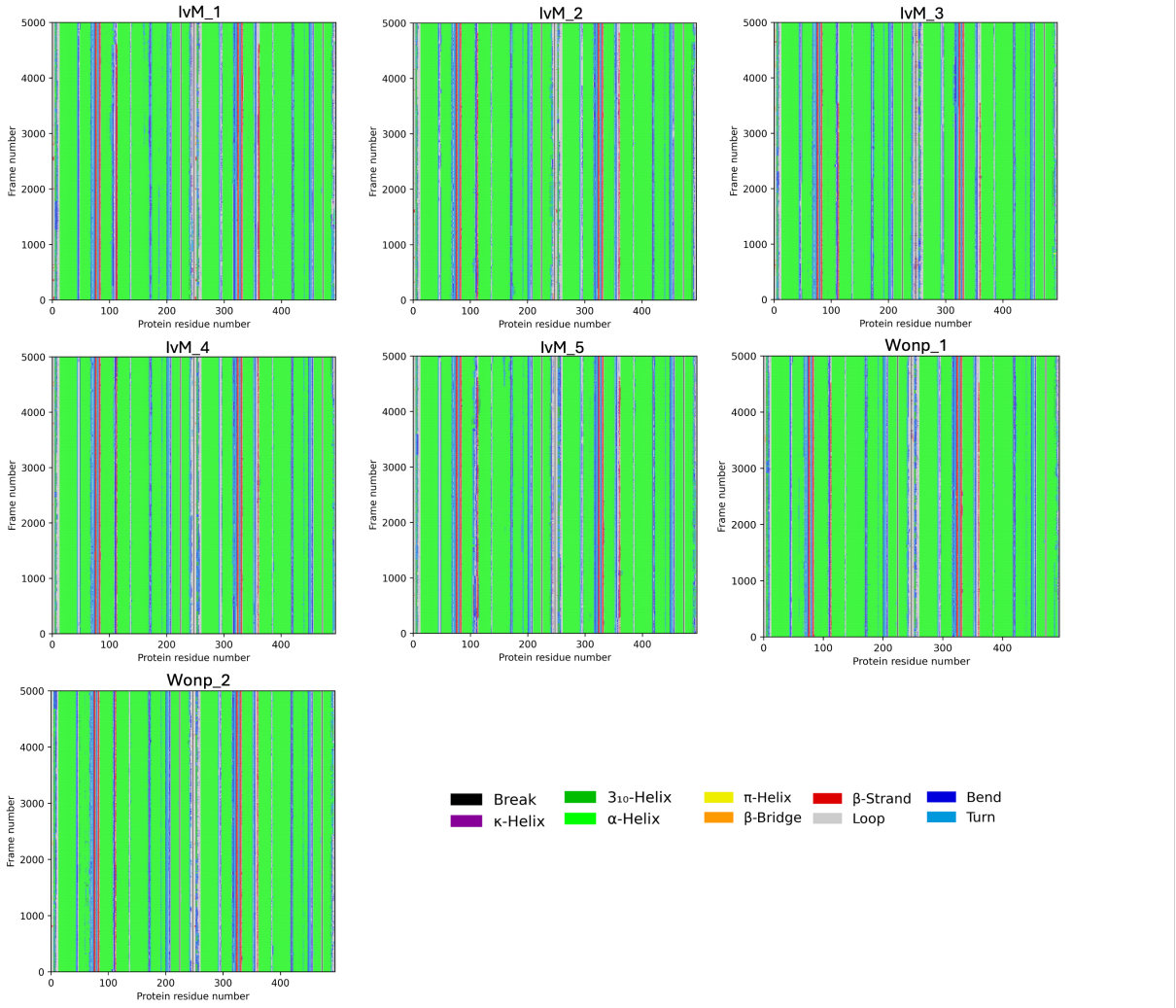

Figure S5: Secondary structure of ChR2 as provided by the DSSP analysis[S1] *along the IvM MD simulations compared to that along the MD simulation without the NC.*

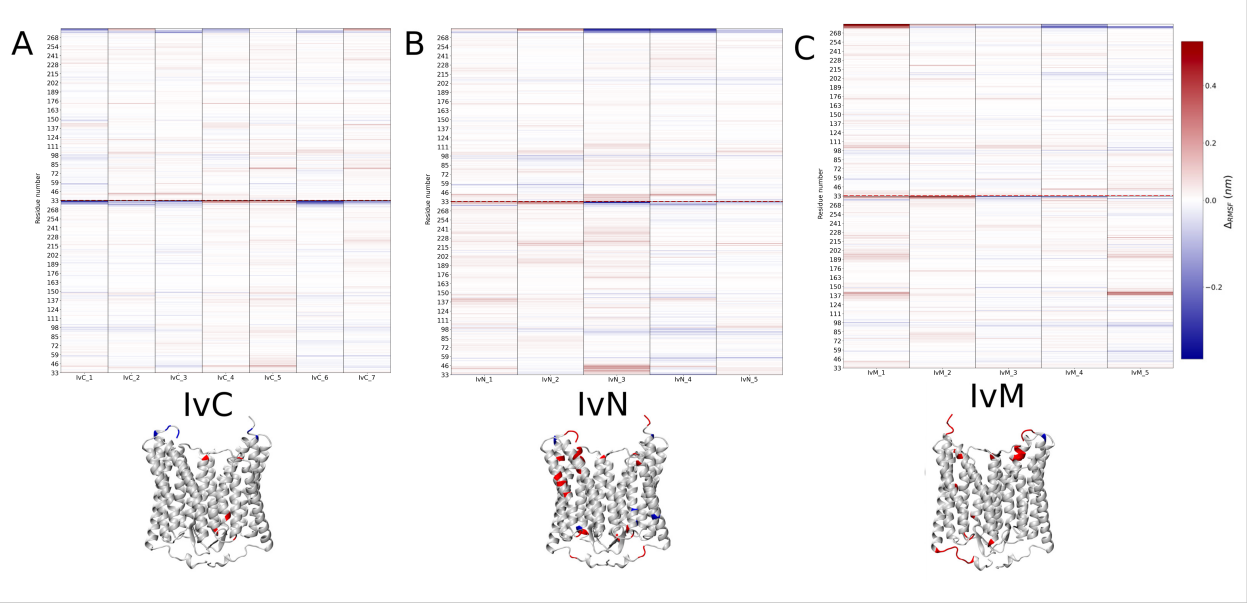

Figure S6: Color map reporting $\Delta_{RMSF} = (RMSF_{withNC} - RMSF_{withoutNC})$ *values for each residue in the IvC (A), IvN (B) and IvM (C). The color scale (from blue to red) indicates an increased (red) or reduced (blue) fluctuation of the i-th residue with respect to the average of the two MD simulations without NC. Below each panel a rapresentative snapshot of ChR2 is reported in which the protein regions showing the highest/lowest* Δ_{RMSF} *are highlighted in red/blue, respectively.*

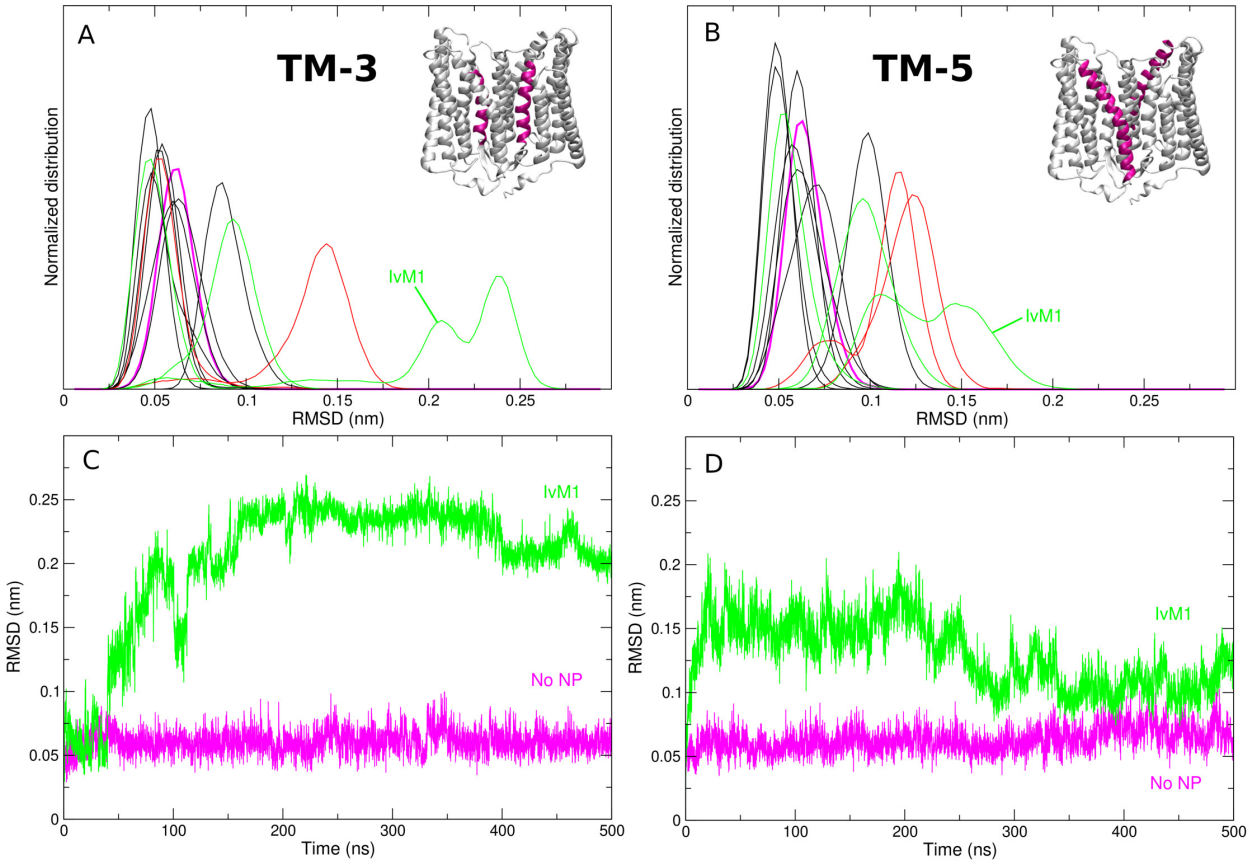

Figure S7: A and B: Normalized distribution of the RMSD of helices TM-3 (A) and TM-5 (B) along all simulations. The MD without the NC is reported in magenta, IvC, IvN and IvN simulations are reported in black, red and green, respectively. Insets: representative snapshot in which the considered helix is highlighted in magenta. C and D: Time evolution of the RMSD of helices TM-3 (C) and TM-5 (D) in the IvM1 simulation (green) and in the simulation without the NC (magenta).

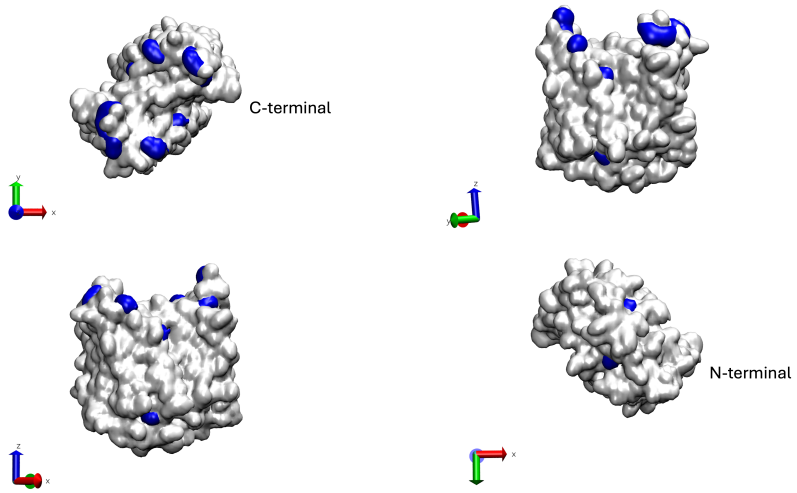

Figure S8: Spatial distribution of Histidine residues in ChR2, shown as a projection onto all sides of the protein. The protein is shown in surface representation (light grey), with Histidine (His) residues highlighted as blue spheres. The C-terminal and N-terminal regions are labeled, visually confirming the higher abundance of His residues in the C-terminal tail referenced in the main text.

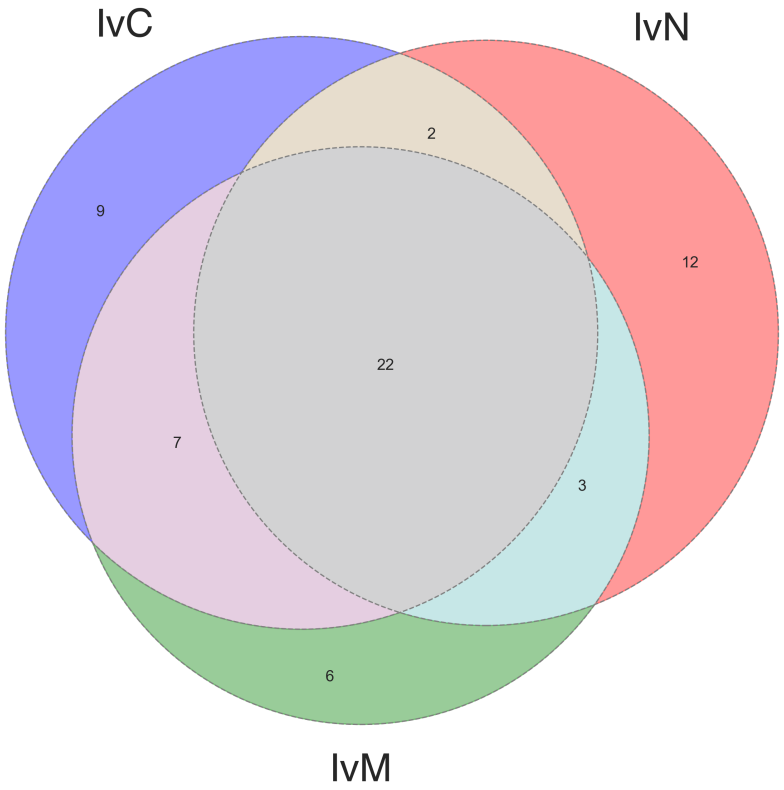

Figure S9: Venn diagram illustrating the overlap of hub residues among the IvC, IvN, and IvM groups. Hubs are defined as the minimal set of residues accounting for 50% of the total betweenness centrality in each network as described in the main text. The numbers indicate the cardinality of each unique and overlapping set. The central intersection highlights a core network of 22 residues common to all three systems.

References

(S1) Gorelov, S.; Titov, A.; Tolicheva, O.; Konevega, A.; Shvetsov, A. DSSP in GROMACS: Tool for Defining Secondary Structures of Proteins in Trajectories. *Journal of Chemical Information and Modeling* **2024**, *64*, 3593–3598, PMID: 38655711.