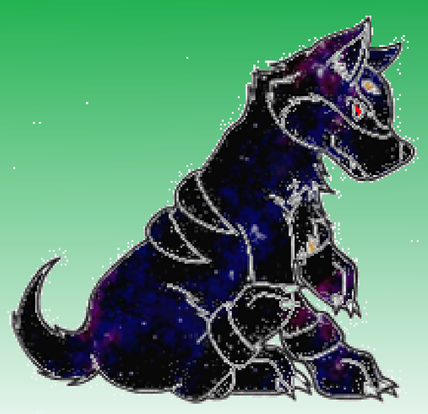

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Modeling a closed connexin hemichannel

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Introduction

Connexin are proteins that form two types of channels: gap-junction channels (GJC) and hemichannels (HC). While the first are important to cell-to-cell communication; the latter can provide a dangerous communication between cytoplasm and the external millieu. HCs have a wide pore with low selectivity, that allows the passive diffusion of solutes, from ions through small peptides, including ATP molecules. Open HCs can lead to apoptosis in several cases. HC are closed in physiological conditions, which are mediated by factors like external calcium concentration, membrane electrical potential, pH, among others. Sadly, the structure of a closed HC have remained elusive for the research community. Most experimentally obtained HC structure are in an open conformation. There are published evidence regarding N-terminal helix (NTH) and parahelix (PH) and their role on different types of gating.

Acknowledgements

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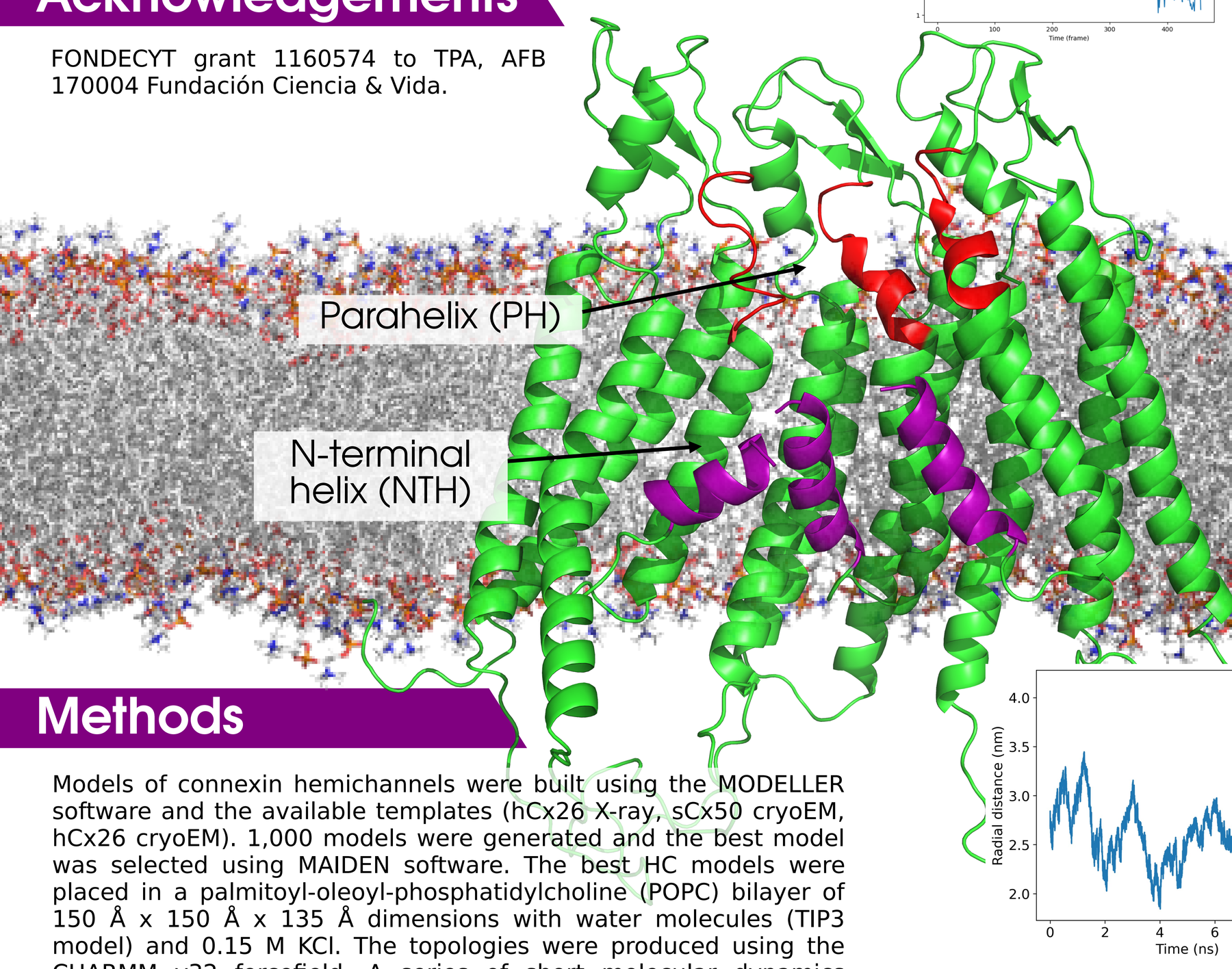


Methods

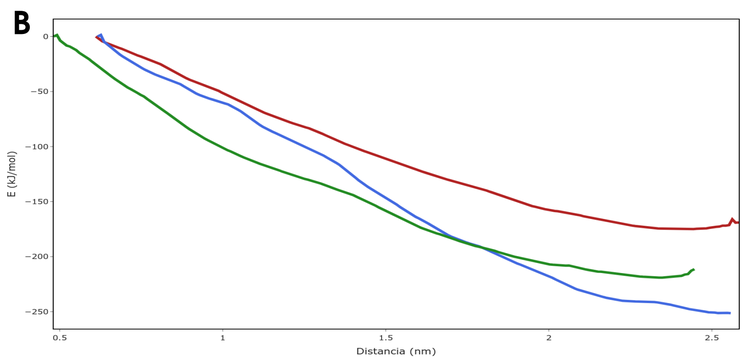
Models of connexin hemichannels were built using the MODELLER software and the available templates (hCx26 X-ray, sCx50 cryoEM, hCx26 cryoEM). 1,000 models were generated and the best model was selected using MAIDEN software. The best HC models were placed in a palmitoyl-oleoyl-phosphatidylcholine (POPC) bilayer of 150 Å x 150 Å x 135 Å dimensions with water molecules (TIP3 model) and 0.15 M KCl. The topologies were produced using the CHARMM v32 forcefield. A series of short molecular dynamics simulations were run at NVT ensemble starting with a position restraint in all non-hydrogen atoms, after which these restraints were gradually removed. In the final simulations the temperature was held at 310ºK using a Nose-Hoover thermostat and pressure was held at 1 atm using a Parrinello-Rhaman barostat in a NPT ensemble. Finally, steered simulations and umbrella sampling were run with GROMACS 2021 and metadynamics were run using GROMACS 2021 patched with PLUMED 2.7. Analyses of simulations including distance measurements were performed using GROMACS packages and the MDAnalysis module of Python.

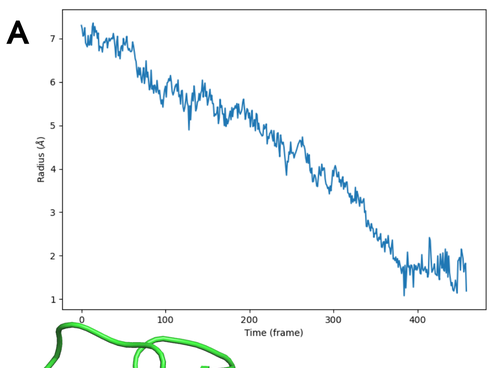
References

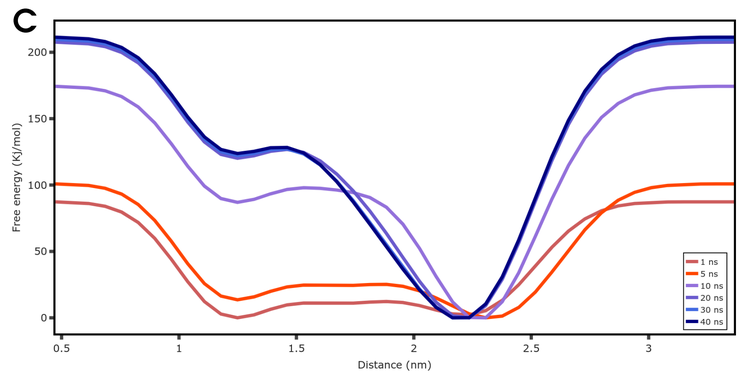
• Bennett, BC et al. Nature communications.
• Maeda et al., 2009. Nature 458: 597-602
• Flores et al., 2020. Nat commun 11: 4331
• Brotherton et al., 2022. Structure 30: 697-706
• Postic et al., 2015. Biochimie 115: 155-161

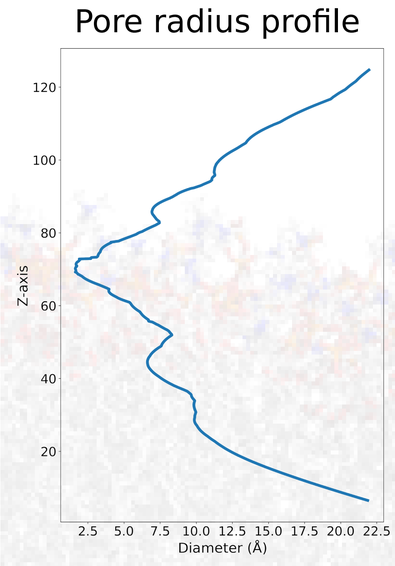
Results

Figure 1. Closing Cx26 HC at the PH region. (A). Radius calculated from the distance between opposing E42 residues, during an steered MD simulation. (B) Free energy calculated from umbrella sampling. (C) Free energy calculated from metadynamics.









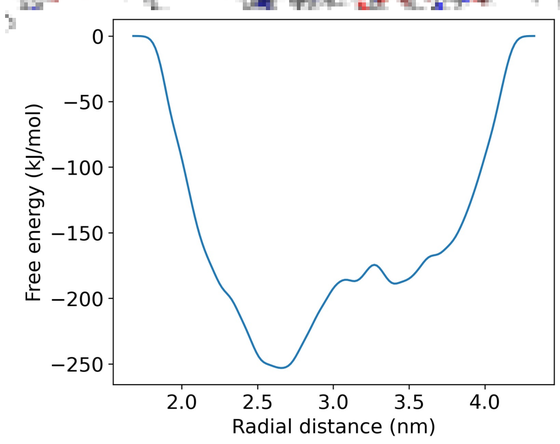


Figure 2. Closing Cx26 HC at the NTH region. (A). Radius calculated from the distance between center of mass of opposing NTH during a metadynamic. (B) Free energy calculated from metadynamics.

Conclusions

We were able to forcefully close Cx26 HC using two non-equilibrium simulation techniques: steered simulation and metadynamics. To calculate free energy we rely on metadynamics itself and umbrella sampling. The present results indicate that Cx26 in its open conformation is very stable. On the contrary, the closed conformation, in the PH or in the NTH region, are unstable. A meta-stable intermediate was observed while closing the channel around PH region, which should be studied. Other reaction coordinates are currently being explored to close the HC in the region of NTH.