CONFORMATIONAL DYNAMICS IN A NICOTINIC RECEPTOR HOMOLOGUE PROBED BY SIMULATIONS

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Open / closed conformation

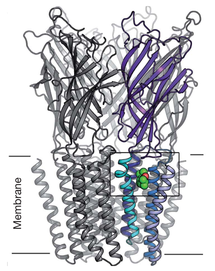

Fig. 1: Side view of GLIC in cartoon representation (anesthetics shown as spheres).

- Bacterial homologues of eukaryotic pentameric ligand-gated ion channels (LGICs, Fig.1 & 2) [1,3]
- Structural and functional models of signal transduction in the nervous system.
- Two crystal structures in distinct conformations.
- Gloeobacter violaceus (GLIC) [1] is gated by protons
- Crystallized at acidic pH [4]
- **Open pore**
- MD simulations: protein is stable on a 20 ns timescale [1] (Fig. 2)
- M2 helices that form the wall of the pore fluctuate around an open (Fig. 2 & 4) **water-filled pore** (Fig. 6a).

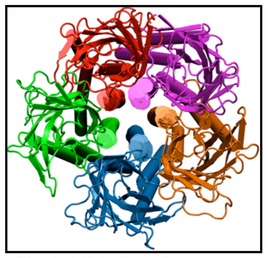

Fig. 2: M2 helices fluctuations during MD simulation.

Fig. 3: Our one microsecond long simulation lead to the closure of 2 subunits by a sequential «domino mecanism»

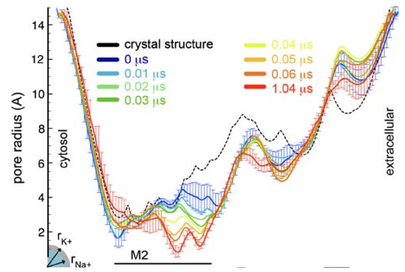

Fig. 4: Pore radius as a fonction of the pore distance.

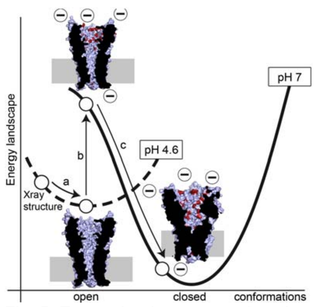

Fig. 5: Energy landscape as a function of the conformations (that are relative to pH).

- GLIC undergoes large motion at neutral pH
- 1 µs MD simulation of the channel's pH stimulated gating mechanism [2]
- Open channel equilibrated instantly set to neutral pH (Fig. 5)
- Simulations show:
  - **channel closure** rapidly takes place at the level of the hydrophobic furrow
  - **quaternary twist** increases progressively
  - **two-step domino**-like mechanism suggested by observed transitions (Fig. 3)
- channel dehydratation (Fig. 6b)
- cation reservoirs at specific places (Fig. 6c)

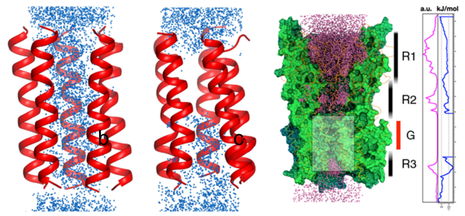

Fig 6: Pore hydratation at pH=4.6 and pH=7.0.

General anesthetics binding

Construct	IC50 (mol.L^{-1})
WT	2.4x10^{-5}
V242M	3.2x10^{-6}
T255A	1.9x10^{-6}

Tab. 1: Propofol activity on wild type and GLIC mutants.

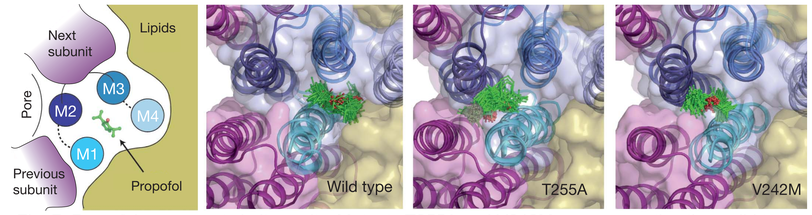

Fig. 7: Propofol movements in its cavity. Mutants T255A and V242M are more active than wild type, which seems correlated with the contacts to the M2 helices.

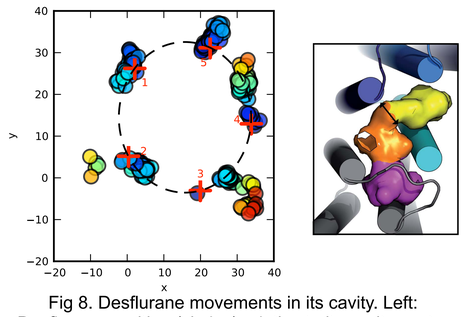

Fig 8. Desflurane movements in its cavity. Left: Desflurane position (circles) relative to its cavity center (red crosses). Coloring is a function of the ligand-cavity distance. Right: tunnel (orange) that links two adjacent subunits (yellow and purple).

- We crystallized G.A. bound to GLIC (Fig.1) [5]
- MD simulations:
  - contacts between G.A. and M2 helices correlated with activity (Tab. 1 & Fig. 7)
  - desflurane can enter a connection tunnel between 2 adjacent subunits (Fig. 8)
- Related work by Klein *et al.* [6].:
  - MD simulations
  - isoflurane
  - multiple binding sites (transmembrane, extracellular, pore)
- Related work by Tang *et al.* [7]:
  - theoretical & experimental methods
  - halotane & thiopental
  - multiple binding sites including pore
- Related work by Murail *et al.* (private communication): ethanol binding to GlyRa receptor bears some similarities

To the best of our knowledge, our study is the only one supported by a crystal structure.

Conclusion - Perspectives

Many questions concerning GLIC still remain unsolved.

- Ion selectivity
  - ion permeation
  - protonation state of all key residues.
  - classical pKa calculations yield contradictory results and original approaches will be necessary.
- Gating mechanism
  - observe the full transition
  - twist movement central in the process
  - which interactions initiate this movement?
  - how do forces propagate across the structure ?
- Anesthesia.
  - crystal structure of general anesthetics bound to GLIC provides important insight
  - how do these small molecules operate?
  - how is the channel inactivated?
  - why are some molecules more efficient than others?

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[2] Nury et al. One-microsecond molecular dynamics simulation of channel gating in a nicotinic receptor homologue. PNAS (2010) vol. 107, 6275
[3] Hilf et Dutzler. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature (2008) vol. 452, 375
[4] Bocquet et al. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. Nature (2007) vol. 445, 116
[5] Nury et al. X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. Nature (2011) vol. 469, 428
[6] Brannigan et al. Multiple binding sites for the general anesthetic isoflurane identified in the nicotinic acetylcholine receptor transmembrane domain. PNAS (2010) vol. 107, 14122
[7] Chen et al. Anesthetic binding in a pentameric ligand-gated ion channel: GLIC. Biophys J (2010) vol. 99, 1801

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