STUDY OF THE INTERACTION BETWEEN GENERAL ANESTHETICS AND A BACTERIAL HOMOLOGUE TO THE HUMAN NICOTINIC RECEPTOR.

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Overview

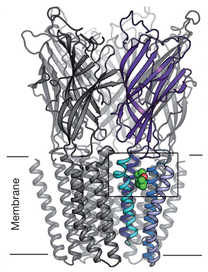
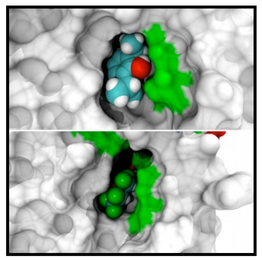


Fig. 1 (left): Side view of GLIC in cartoon representation (anesthetics shown as spheres).

Fig. 2 (right): Propofol (top) and desflurane (bottom) bound to GLIC (charged residues in green, hydrophobic in white).



- Bacterial homologues of eukaryotic pentameric ligand-gated ion channels (LGICs, Fig.1) [1,3]
- Structural and functional models of signal transduction in the nervous system.
- Gloeobacter violaceus (GLIC) [1] is gated by protons
- Crystallized at acidic pH [4] with an open pore
- 2 structures of GLIC with general anesthetics (GA) bound to it: desflurane & propofol [5]

Methods: Extensive Sampling Close To The Crystal Structure

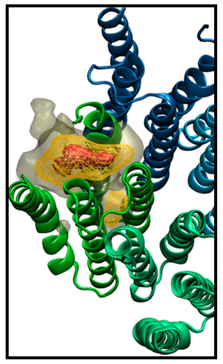


Fig. 6: Sampling of the cavity by desflurane.

- Setup
  - All atom MD simulations: anesthetic + GLIC + membrane
  - Either 1 or 5 GAs bound to GLIC
  - 125 different GA configurations determined by clustering
  - Protonation state same as in [1]

Production
- NAMD + CHARMM27
- 8 ns to sample crystal structure
- 310K, 1bar

Table 1: Amount of time computed for each system

	WT	T255A
Desflurane x 5	25 x 8 ns = 200 ns	25 x 8 ns = 200 ns
Propofol x 5	25 x 8 ns = 200 ns (ongoing)	3 x 25 x 8 ns = 600 ns
Desflurane x 1	30 x 8 ns = 240 ns (ongoing)	-

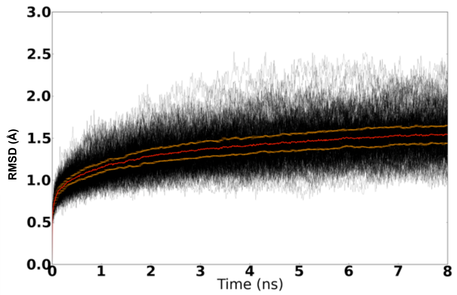


Fig. 5: RMSD per subunit for all simulations (average appears in red, standard deviation in orange).

2 binding sites? 3 sites? more?

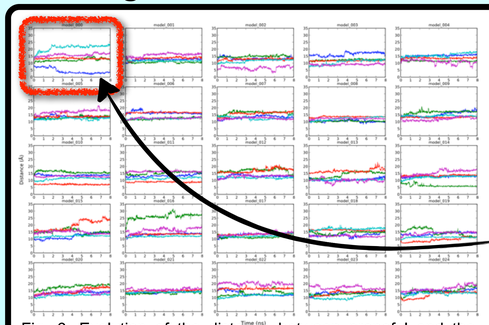


Fig. 6: Evolution of the distance between propofol and the center of the inter-subunit site.

- Fig. 6 shows sampling of propofol for all subunits for 25 (out of 75) simulations
- Intra-subunit site shown in crystallography (Fig.2 & 7)[5]
- Small desflurane enters an inter-subunit site
- Bigger propofol enters this site too!
- E. Lindahl *et al.* see this site too! (poster B351)
- Several other binding sites suggested[6,7]

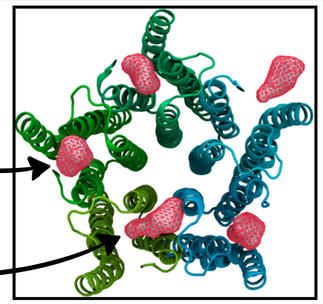


Fig. 7: Propofol density averaged over an 8 ns MD. Highlighting both intra and inter-subunit sites.

[1] Bocquet et al. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature (2008) vol. 457, 111
[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
[8] Delalande et al. Multi-resolution approach for interactively locating functionally linked ion binding sites by steering small molecules into electrostatic potential maps using a haptic device. PacSympBiocomput (2010) pp. 205-15

A more detailed contact map

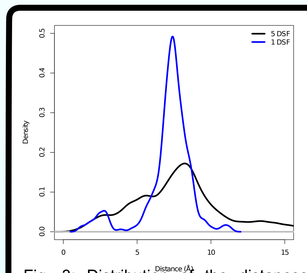


Fig. 3: Distribution of the distances between the desflurane and the center of the cavity.

- Hypothesis: «an allosteric effect could prevent GA molecules to go deep inside a cavity once a neighbor cavity is filled»
- Fig. 3 does not show such a behavior, a cooperative effect could even exist (ongoing work).

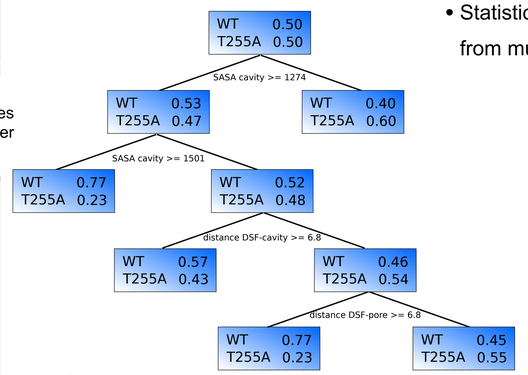
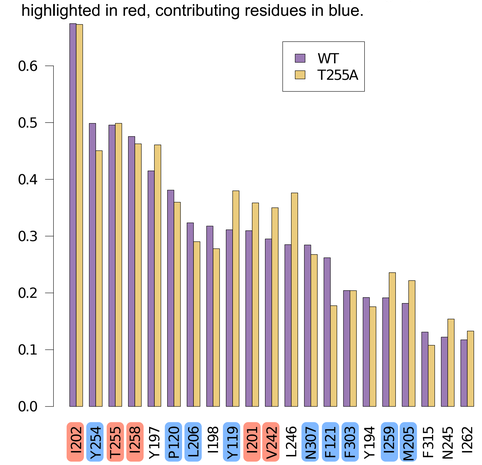


Fig. 4: Decision tree that aims to split WT and mutant channels.

- Statistical clustering methods can't discriminate wildt-type from mutant channels using MD-based descriptors.

Fig. 5: Normalized number of contacts between the protein and the desflurane. Comparison between WT and T255A mutant. Key residues suggested by crystallography are highlighted in red, contributing residues in blue.



- Y197 and I198 could play a key role
- 2 «key» residues (red on Fig. 5) might not be as important as anticipated
- GAs «pushing» M2 seems unlikely as we don't see any difference between the contacts of the WT and mutant channels (Fig. 5 & 8)

Conclusion - Perspectives

- More statistics is needed to conclude on the potential effect of anesthetic concentration
- More statistics is needed to study the GLIC-propofol interaction (with WT MD simulations
- Long simulations could provided additional informations on anesthetic induced conformational changes
- Interactive simulations using virtual reality could provide a additional insight in the paths from the solvent to the cavity.

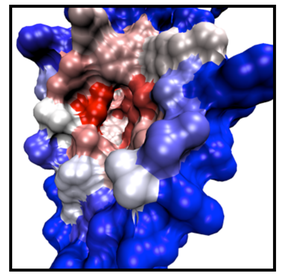


Fig 8: Cavity colored with respect to the number of contacts with desflurane

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