MOLECULAR DYNAMICS AND BINDING MECHANISMS OF VOLATILE ANESTHETICS TARGETING HUMAN TUBULIN

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Introduction

Anesthesia, despite being the cornerstone of modern surgery, is to this date a biological puzzle. While scientific efforts still have not managed to frame its pharmacology in an exhaustive theoretical framework, microtubules inside neurons are thought to be essential for memory formation and consciousness [1]. The potential ability of volatile anesthetics to alter or dampen the vibrational properties of microtubules justifies the spatiotemporal characterization of the interaction between such molecules and the tubulin dimer through the use of computational molecular modelling.

Methods

Human tubulin isotypes βVI, βIIa and βIVa were modeled as αI-β dimers through homology modelling from the PDB 3J6F [2] template, and simulated in GROMACS 2019.1 for 100ns using the AMBER ff99SB-ILDN force field [3], with explicit water and ions at 0.1M, at 300 K and 1 bar, in three replicas. Simulations were performed both without and with Ethylene (ET), Desflurane (DF), Halothane (HT) or Methoxyflurane (MF) in the solvent at 10 mM [4]. Secondary Structure analysis on MD simulations was performed using DSSP, while interaction hotspots were determined as a per-residue contact probability over each frame, which is reported for each sector of the tubulin surface in a spherical coordinate system. Main binding clefts were obtained from the heatmaps and the interaction of the anesthetics therein has been further analyzed using the MM/PBSA method.

Results

The analysis of MD trajectories predicts distinct patterns of interaction of each anesthetic, with a significant fraction of interaction sites located both on the luminal surface ($\varphi<0$), and on the α subunit ($\theta<0$) of the microtubule for all anesthetics except ET, as visible in Fig. 1 for isotype βIVa, the most expressed isotype in the brain [5].

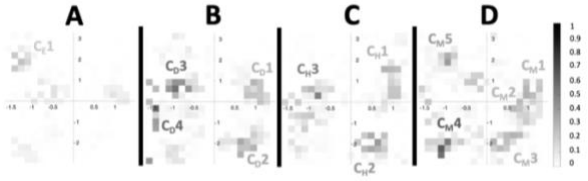

Figure 1: Anesthetic contact heatmaps, i.e. (θ,φ) probability plots, for each anesthetic (A: ET, B:DF.,C:HT,D:MF) on isotype βIVa. Main binding clefts are highlighted. Colorbar is 0 to 1 probability.

No significant interactions occurred with ET, which is the weakest among the four compounds in terms of clinical potency. This analysis highlights the mechanical compatibility of the contact between the three anesthetics DF, HT and MT, and tubulin in specific areas of the surface, hence suggesting the existence of preferential transient binding pockets on the tubulin dimer. These clefts are highlighted in Fig. 1, and a binding energy estimate for each ligand in each of those clefts is provided in Fig. 2.

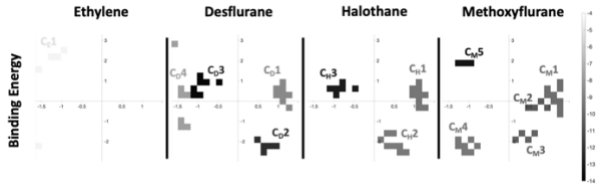

Figure 2: Per-area MM/PBSA binding energy estimate for isotype βIVa. Left to right: ET; DF; HT; MF. Color scale is -4 kcal/mol to -14 kcal/mol.

Binding energy estimates of DF, HT and MT were comparable, between -7 and -14 kcal/mol, while ET confirmed much weaker interactions, below 4 kcal/mol. No significant secondary structure alterations were determined in the presence of anesthetics in the binding clefts, as expected by the transient nature of binding events.

Discussion

The results suggest the lack of a unique and stable binding site for volatile anesthetics on tubulin, but highlight the existence of specific hotspots where anesthetics interact for a relevant fraction of time. These are areas where the contact is mechanically most favorable. The binding events do not follow a *lock-and-key* paradigm, but rather occur frequently inside different, mechanically favorable clefts. This might have consequences on the assembly of protofilaments, on locally induced instantaneous dipoles and on higher-scale vibrational characteristics whose involvement in conscious brain activity and information transmission is currently debated and requires further studies.

References

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