Chapter 2 - Supplementary information

1 Rosetta calculations - Double mutants

Supporting information of the calculations of the binding energy of several variants of the $K_V7.2$ channel, these calculations were performed with the package called flexddG [1] of the rosetta software (https://www.rosettacommons.org/), more info also in the tutorial of the package [2].

We have all calculations separated in folders named after the mutation under study, since all calculations have the mutation W344R, suffix MUT is added: **Q341A-MUT Q341D-MUT Q341F-MUT Q341H-MUT Q341K-MUT Q341M-MUT Q341C-MUT Q341E-MUT Q341G-MUT Q341I-MUT Q341L-MUT Q341N-MUT**.

All folders containing the outputs have the same architecture, which will be described now:

inputs/6FEG/: Inputs needed by rosetta

→ '6FEG.pdb': input structure for the protocol, from [3].

→ 'chains_to_move.txt': chain moved.

→ 'mutation.resfile': definition of the analyzed mutation.

rosetta_scripts/: Code of the protocol with its options.

→ 'flexddG.wt.xml': main code for flexddG protocol.

→ 'flexddG.options': parser options needed for rosetta command.

output/6FEG/01/: output of the first simulation, up to output/6FEG/50/, output data bases or structures are not provided due to storage limitation.

→ 'rosetta.out': main output file, the scores obtained for the structures are appended to this file.

analysis_output/: output in spreadsheet format.

→ '-results.csv': $\Delta\Delta G$ and score terms of the backrub steps.

→ '-struct_scores_results.csv': same as results.csv but with different order

./: parent folder of the simulation with extra files.

→ 'run.py': python script that performs the whole simulation, its output is the final $\Delta\Delta G$

→ 'extract_structures.py': reads output databases and extract structures in pdb format

→ 'analyze_flex_ddG.py': reads the output and writes the previously commented spreadsheet output files

For each set of calculations, we have followed the same workflow, this is, we introduce small random rotations in the angles formed by the atoms of the backbone around the mutation site, defining the mobile shell as those amino acids within 8Å of the mutation, and these modifications are accepted or rejected following the Metropolis criterion at a temperature of 1.2kT for improving the sampling; trajectories of 50,000 backrub steps were collected, saving the coordinates each 5000 steps, these structures obtained are the inputs for two modification in parallel, on the one hand they are fed to the packing protocol to optimize the side chains and a posterior minimization,

and on the other hand the mutation of interest is performed and the side chain of the mutation is optimized and minimized, obtaining two optimized Monte Carlo trajectories, the wild type and the mutated one. All steps of these two trajectories are energetically evaluated with the Rosetta energy function (REF 2015) [4], by computing the score of the two chains separated and bound, leading to $\Delta G = G_{complex} - G_{unbound}$ and subsequently compared to the mutated one, giving the final result of $\Delta\Delta G = \Delta G_{Mutation} - \Delta G_{WT}$, that it is average over all trajectory. Full trajectories are not provided as the resulting folder will be too big.

References

[1] Kyle A. Barlow et al. "Flex ddG: Rosetta Ensemble-Based Estimation of Changes in Protein-Protein Binding Affinity upon Mutation". eng. In: *The journal of physical chemistry. B* 122.21 (May 2018). PMC5980710[pmcid], pp. 5389–5399. ISSN: 1520-5207. DOI: 10.1021/acs.jpcb.7b11367.

[2] https://github.com/Kortemme-Lab/flex_ddG_tutorial. Accessed: September of 2022.

[3] Ganeko Bernardo-Seisdedos et al. "Structural basis and energy landscape for the Ca^{2+} gating and calmodulation of the Kv7.2 K^+ channel". In: *Proceedings of the National Academy of Sciences* 115.10 (2018), pp. 2395–2400. DOI: 10.1073/pnas.1800235115.

[4] Rebecca F. Alford et al. "The Rosetta All-Atom Energy Function for Macromolecular Modeling and Design". eng. In: *Journal of chemical theory and computation* 13.6 (June 2017). PMC5717763[pmcid], pp. 3031–3048. ISSN: 1549-9626. DOI: 10.1021/acs.jctc.7b00125.