Energy analysis exercise 2021-22
Spike RBD-ACE2 Protein-protein interface analysis

Objective: To evaluate the relative contribution of interface residues to the interaction energy in a protein-protein complex. Case RBD-ACE2

Background: The growing amount of genomics data given by the abundance of large-scale sequencing project is releasing large amounts of protein sequence variants. Most proteins are known to work forming complexes either permanent or transient with other macromolecules. In most cases such complexes are key for the regulation of the activity of the involved proteins. There is an increasing interest in understanding the effect of sequence variants on the stability of such complexes. As an initial approach to that analysis, we pretend to evaluate the relative contribution of the amino acid residues forming the protein-protein interface in the interaction energy between the complex components.

As a case study we will concentrate in the complex between the Receptor Binding Domain (RBD) of SARS-Cov-2 Spike protein and its receptor the Angiotensin Converting Enzyme (ACE2). The formation of this complex is a key step in the viral infection. Most vaccines in the market try to block this binding. Analysis will be done on the structure **6m0j** from the PDB. This structure contains only the RBD domain from Spike.

Strategy: We will evaluate the contribution of individual amino acid residues through the following steps:

1. Determine amino acid residues that form the interface between the complex components (use 6m0j)
2. Determine the contribution to the stability of the complex by mimicking a traditional Ala-scanning experiment, i.e. replacing each residue in turn by Ala and evaluating the changes in the complex interaction energy.
3. Identify known SARS-Cov-2 Spike Variants and evaluate their effect on ACE2 Binding. Replace the appropriate residue with the variant and reevaluate the interaction energy.
4. (optional) Perform the same analysis using FoldX (http://foldxsuite.crg.eu/)

Methodology:

Preparation:

1. Obtain the required structure from the PDB.
2. Check at PDB which is the composition of a “Biological unit”. Remove all chains but those involved in the biological unit, if necessary
3. Remove all heteroatoms
4. Perform a quality checking on the structures, and add missing side-chains and hydrogen atoms and atom charges, using the biobb_structure_checking module

Step 1.

The interface between is defined by a list of residues on both chains that have at least one atom below a given distance.

1. Using pymol inspect visually the structure and choose a suitable distance in the way that all contact residues are included. Add 1-2 Å to that distance so the adjacent residues are also considered.
2. Prepare a python script (standalone or in a Jupyter notebook) to define the list of interface residues on each chain

Step 2

Interaction energy between chains correspond to the difference between the total energy of each chain in the bound state (the complex) and the unbound state (the two chains isolated in solution). To simplify the calculation, we will take the following approximations:

- We will assume that 3D structure does not change between bound and unbound states, hence bonded terms and non-bonded terms between atoms belonging to the same chain will not change and will not considered.
- We will consider solvation energies obtained from ASA values for all atom types (not only the hydrophobic ones).

With these assumptions prepare a python script or notebook to evaluate the Interaction energy among chains. Interaction energy between components of a A-B complex will come from the following:

$$\Delta G^{A-B} = \Delta G_{elect}{}^{A-B} + \Delta G_{vdw}{}^{A-B} + \Delta G_{Solv}{}^{A-B} - \Delta G_{Solv}{}^{A} - \Delta G_{Solv}{}^{B}$$

This can be done will all residues or only with the interface residues. Check that the interface from Step 1 represents most of the energy involved. If this is not the case modify the cut-off distance accordingly.

Optional: Derive and implement an algorithm to define the interface from the interaction energy instead of distance based.

Step 3

Determine the effect of replacing each interface residue by Ala in the overall ΔG^{A-B}, and make a plot of the results obtained, highlighting those residues that are more relevant for the stability of the interface. Discuss the results obtained in relation to the nature of the involved amino acids (hint, as Ala side-chain is part of all others, except Gly, there no need to do the replacement, just take into account the different atoms)

Step 4

Prepare images with pymol of the interface highlighting the relevant residues and interactions.

Step 5

Find the most relevant sequence variants for RBD (alfa, beta, and delta SARS-Cov-2 strains) and analyze the effect of the actual mutations on the energy. Build structure with the replaced side-chain, either using pymol or using biobb_structure_checking.

General hints

- You can prepare all functionality in a single script or (probably better), prepare a series of reusable scripts that run in a pipeline. Alternatively, you can prepare all steps in a Jupyter notebook
- For standalone scripts make sure that they have a proper command line in a way that can be re-used with different input PDBs or parameters.
- All output should be properly formatted and human readable.
- Prepare re-usable functions for calculating energy components

Parameters and energy calculations

- Parameters for electrostatic, var der Waals and solvation can be taken from https://github.com/jlgelpi/BioPhysics/tree/master/data (To be discussed in Practical Session) Python libraries to manage parameter files are also available at https://github.com/jlgelpi/BioPhysics
- Structure checking and management can be done with the biobb_structure_checking python module either from PiPy or BioConda distributions:
    - conda install -c bioconda biobb_structure_checking (preferred)
    - pip3 install biobb_structure_checking
- Surface areas will be calculated using NACCESS though Biopython (instructions at Biophysics github repo)
- Electrostatic energies will be calculated using the Mehler-Solmajer dielectric.

Equations

Vdw interaction:

$$Evdw_{ij} = 4\epsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right)$$

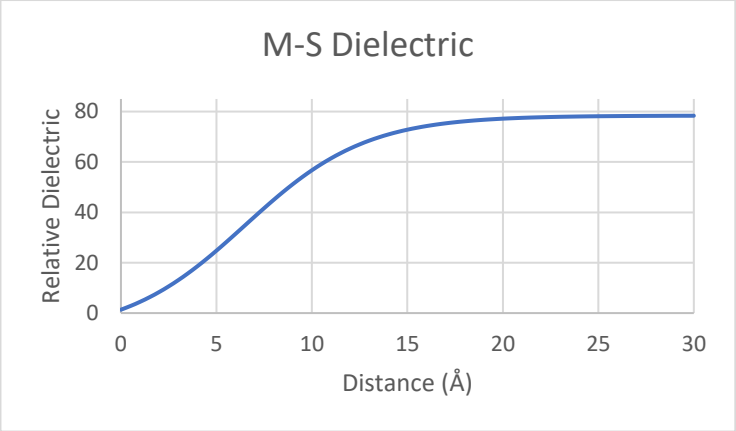
Note: Consider Combination Rules for vdw parameters

Electrostatic interaction:

$$E_{elec\ ij} = 332.16\ \frac{q_i q_j}{\varepsilon r_{ij}}$$

Mehler-Solmajer Dielectric

$$\varepsilon_r = \frac{86.9525}{1 - 7.7839\ e^{-0.3153\ r}} - 8.5525$$



Solvation:

$$\Delta G = \sum_{AtTypes} \sigma_i\ ASA_i$$

Note: We will use only this term for solvation, including also non-hydrophobic atoms

Software and Materials

Modelling, visualization: PyMol, Chimera

Scripting: Python (v3), BioPython modules. Other python modules for graphics and maths. Text Editor. Jupyter notebook optional

Other software: NACCESS (copy and instructions at the repository)

Structure setup: Biobb_structure_checking python library.

Software Version Control: Git

Evaluation

The exercise will be done in groups of **3 people** and will be evaluated from a written report (PDF, uploaded to ESCI Aula) including the analysis results, plots and the necessary figures. Code or notebook should be uploaded or made available in git repository.

The report should describe the operation performed, the results of the analysis and a critical discussion of the results