DESCRIPTION OF CALCULATION PROCEDURE

1. The structure listed in PDB is extended with H atoms using PyMol (Python 3.5 module) (www.pymol.org (Aug 2019))
2. Energy minimization is performed to eliminate steric clashes introduced by the previous step (http://www.mdtutorials.com/gmx/lysozyme/ (Aug 2019))
    a. Gromacs version 5.0.7 is used to perform energy minimization, with *pdb2gmx* used to convert the structure and obtain a topology. This command builds topologies for molecules consisting of distinct structural units such as amino acid residues.
    b. The OPLS-AA/L all-atom force field (2001 amino acid dihedrals) is applied.
    c. The protein is placed in a cubic bounding box whose dimensions exceed that of the protein by at least 1.0 nm along each edge.
    d. Water is added to simulate the solvent.
    e. *grompp* and *minim.mdp* are invoked to perform energy minimization.
3. Additional files are generated to facilitate computation of energy levels
    a. *index.ndx* – contains the names and numbers of chains, atoms, residues etc.
    b. *topol.tpr* – contains the startup structure, its molecular topology and all parameters describing the simulation.
4. The *g_mmbps* package is employed. This package was developed in the framework of Open Source Drug Discovery Continuum (OSDD), which promotes collaboration on design and discovery of drugs targeting some diseases such as malaria, tuberculosis, leishmaniasis etc. *g_mmbps* relies on two popular open source software toolkits – Gromacs and APBS – and has a similar UI to other Gromacs tools (Kumari *et al* 2014; Baker *et al* 2001)
5. Gromacs 5.0.7 and APBS 1.3 (1.5 for 2N0A) are used to calculate potential energy in vacuum – generates a file listing van der Waals and electrostatic interaction with the neighbors (omitting the close neighbor residues : i±1).