

Funnel-Metadynamics 2.0

Graphic user interface and implementation in Plumed

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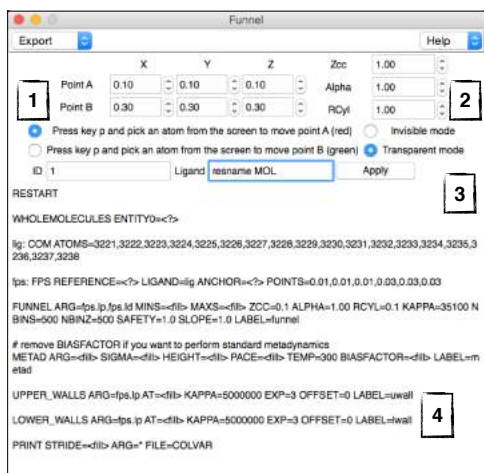
Background

Funnel Metadynamics (FM) is a computational method proven to be successful in obtaining an accurate description of the free energy surface (FES) in ligand/protein^{1,2,3} and ligand/DNA binding interaction⁴. Using this method, one can predict experimental data, such as crystallographic ligand binding modes and experimental binding free energies^{1,2}.

In detail, using FM a funnel-shape restraint potential is applied to the target molecule (see Figure below). This potential is a combination of a cone restraint, that includes the ligand binding site, and a cylindrical part that is directed towards the solvent. Using the funnel potential during the simulation, the system does not feel any repulsive bias when the ligand explores regions inside the funnel area, while as the ligand reaches the edge of the funnel, a repulsive bias is applied to the system. In so doing, in the bulk water the repulsive potential reduces the space to explore to a cylindrical region, thus favouring the observation of multiple binding/unbinding events that lead to a faster convergence of the results and a quantitatively well-characterised FES.

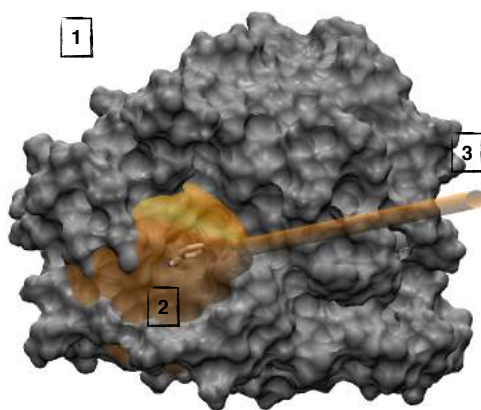
Here we present a graphical interface for the setup and analysis of FM calculations together with a new implementation of FM in PLUMED (v2.3b)⁵. At variance with the previous version, the target molecule (i.e. protein or DNA) is free to rotate and translate in the cartesian space, simulating motion that might be relevant degrees of freedom of the system.

Pre-processing



1. Define the axis of the funnel potential;
2. Set up the parameters to customise the funnel shape for the target binding site;
3. Transparent and invisible modes for the funnel;
4. Writing the FM input might be tedious, we do it for you!

FM Simulation



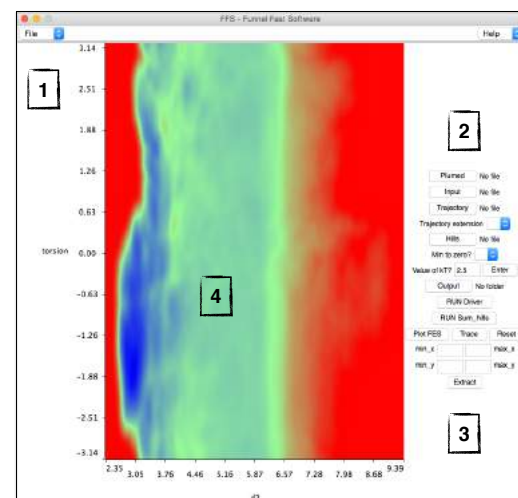
1. The target molecule is fully free, thus its roto-translational entropy is taken into consideration;
2. The funnel potential is set at the beginning of the simulation, then the protein is aligned to frame 0 when computing HILLS and COLVAR;
3. An upper wall is set where the ligand is fully solvated and does not interact with any target's atom (higher than short range interaction cutoff).

Objectives

The setup of FM calculations might be not user friendly and this fact could discourage people to use such technique. Therefore, we have developed a graphical user interface (GUI) that allows setting in an interactive way the parameters necessary to run FM simulations and analysing visually the FM results in a straightforward fashion. These modules have been implemented in the widely used molecular visualisation program "Visual Molecular Dynamics" (VMD)⁶.

This advance, together with the implementation of FM in the new version of PLUMED (v2.3b), is expected to spread its use in binding free-energy studies with potential impact in drug design both for academia and pharmaceutical companies.

Post-processing



1. Plot your free energy surface directly in VMD!
2. Commands to call post-processing plumed tools from VMD;
3. Extract from the trajectory uploaded in VMD the frames of interest in the FES choosing the interval in the collective variables space;
4. Right click on mouse to extract the frames directly from the FES!

Results

The first FM implementation was tested with the system benzamide/trypsin, therefore we show the results obtained with the same system and the new FM code in PLUMED 2. From previous studies¹, we know that there are 3 main states for this system: two minima in the bound state (A and B) and an unbound solvated state (C).

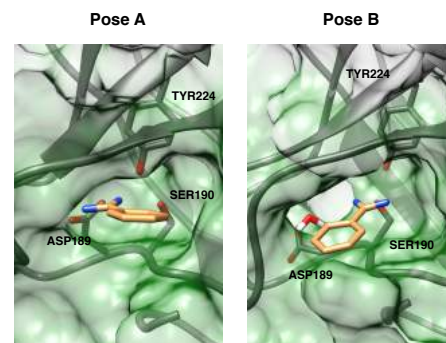
- Pose A is the crystallographic structure and is known to be the most stable, with a direct interaction of the amine of benzamide with a carboxylic group of the aspartic acid in position 189 of trypsin.

- Pose B is similar to pose A, though it is slightly rotated and the amine of benzamide interacts with serine 190 of trypsin, while the empty space left in front of ASP189 is filled by a molecule of water. Considering the previous studies^{1,7}, the difference in energy between this state and pose A is expected 2-5 kcal/mol¹.

- Pose C represents the ligand in the unbound state and fully solvated. It is at a distance where no interaction with the trypsin is possible and its free-energy difference with the lowest energy bound state, ΔG_b^0 , is reported -8.5 ± 0.7 kcal/mol¹. This value is obtained using the formula $\Delta G_b^0 = -\frac{1}{\beta} \ln(K_b)$, where K_b is the binding constant calculated as $K_b = C^0 \pi R_{cyl}^2 \int_{site} e^{-\beta(W(z)-W_{ref})} dz$. C^0 is the standard concentration

of 1 M and is equal to $1/1.660 \text{ \AA}^{-3}$, πR_{cyl}^2 is the surface of the cylinder used as restraint potential in the unbound state, whereas $W(z)$ and W_{ref} are the free-energy values in the bound and unbound states, respectively, β is constant and equal to $1/k_B T$, where k_B is the Boltzmann constant and T the temperature of the system.

Here below, we compare the FES computed by the new version of FM (left) with those reported by Limongelli *et al.* in the original paper (right)¹. We note that the two FESs show common features such as the presence of two energy minima and the lowest energy state A, which corresponds to the X-ray pose. However, the left simulation is still ongoing and has not converged yet after 340 ns. As a matter of fact, the alternative binding mode, pose B, has been only partially explored and the solvated state region (C) is not isoenergetic, symptom of a not converged FES.



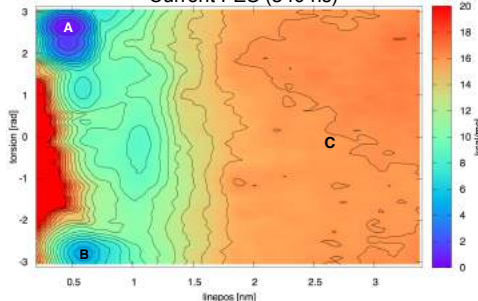
Acknowledgements

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Current FES (340 ns)



Reference FES (500 ns)¹

