MODELING OF LIGAND BINDING TO THE HIF-2α PROTEIN WITH ENHANCED SAMPLING METHODS

DEPARTMENT OF EARTH AND ENVIRONMENTAL SCIENCES
University of Milan-Bicocca

Lara CALLEA, Stefano MOTTA, Laura BONATI l.callea@campus.unimib.it

Department of Earth and Environmental Sciences, University of Milano – Bicocca, Milan, Italy

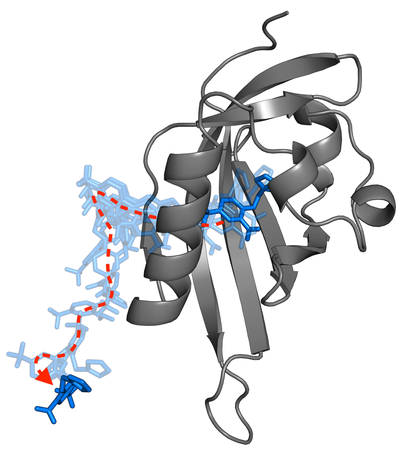
INTRODUCTION

Ligand-protein interactions play a key role in protein functions.
Ligand binding to biological targets can be studied using molecular dynamics (MD) simulations with enhanced sampling techniques to explore the long time-scales of such processes.
The aim of this study is to get an accurate mechanistic description of ligand binding to HIF-2α using computational methods able to simulate the dynamics of the process.
The study-case here investigated is the Hypoxia Inducible Factor-2α[1] (HIF-2α). The PAS-B domain of this protein contains a preformed cavity inaccessible to the solvent and able to bind artificial ligands. The ability of these ligands to act as potential anticancer drugs is attracting great interest. Several crystallographic structures of HIF-2α in complex with different ligands are available, and both thermodynamic and kinetic data of some ligands have been experimentally determined.

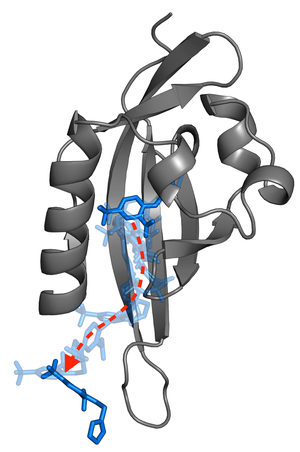
STEERED MD RESULTS

We performed 50 replicas of 25 ns for each path

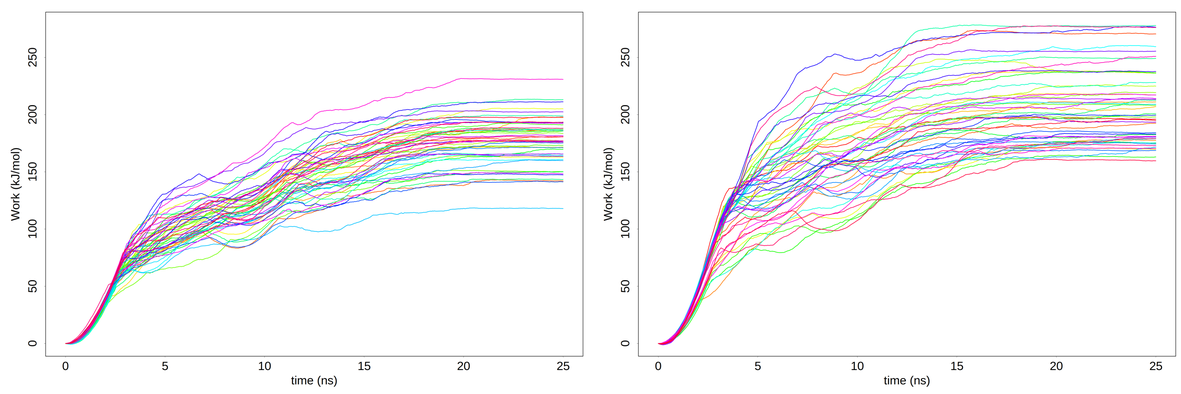
PATH 1: between Fα and Gβ



PATH 2: between Fα and Aβ/Bβ



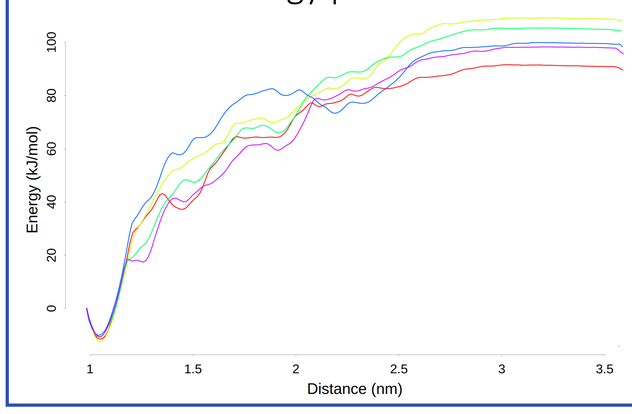
Plot of the work done on the system



The work profiles obtained for different replicas showed that the path 1 is preferred. Anyway, the simulations were not convergent.

UMBRELLA SAMPLING RESULTS

We selected 24 windows as starting points for 10 ns independent simulations. We analyzed the results using the WHAM method to recover the energy profile.



The starting path derived from steered MD had a high influence on the free energy calculation.

Therefore, in this case, the method did not provide a correct energetic characterization.

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METHODS

Among the enhanced sampling techniques, we tested and compared:

1. Steered MD[2] used to predict the possible paths.
   The harmonic bias potential moves along a coordinate (CV) pulling the system in this direction. In the case of ligand unbinding the distance between ligand and protein is generally used as CV.

2. Umbrella Sampling[3] used to the energetic characterization of the process. Multiple replicas are started, each with an harmonic bias centered in a different position of the CV. In this way each replica samples a different region, also called window.

3. Metadynamics[4,5] used both to predict the path and for the energetic characterization. The central idea is to bias the system along a set of CVs using a history dependent potential. To achieve this, a gaussian shape potential is added at regular time intervals to bias the system at the current position of the CVs.

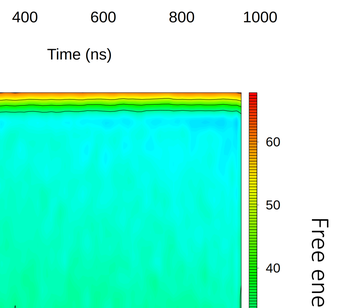
4. Path Collective Variables[6,7] used to optimize the paths.
   This method makes use of metadynamics to sample a path from the bound to the unbound states using a set of intermediate conformations. The CVs usually used are one to describe the progress along the path, S(X), and one to describe the distance from the path, Z(x).

METADYNAMICS + PATH CVs RESULTS

We selected 21 intermediate points from the path obtained from the steered MD simulation with the lowest unbinding work.

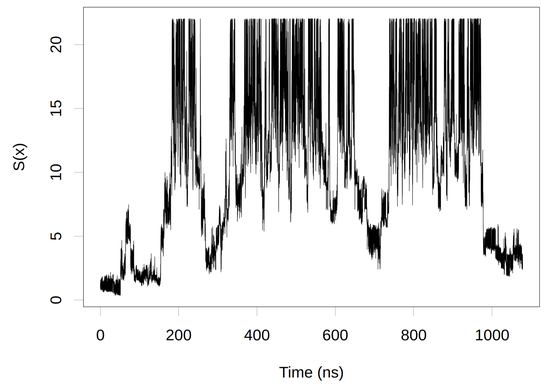
To refine the path, we calculated the distance matrix between pairs of intermediate points.

Regular distance matrix



Metadynamics simulation is still in progress and reached 1μs

BIAS FACTOR	10 K
HEIGHT	1 kJ/mol
WIDTH	0.10,0.04
FREQUENCY	10 ps



FUTURE DIRECTIONS

After the metadynamics simulations will be completed, we will select the best protocol on the basis of the results obtained for the study case and we will extend the study to other HIF-2a ligands for which the experimental information is available.