Exploring HIF-2α Unbinding Pathways via Path CVs and Metadynamics

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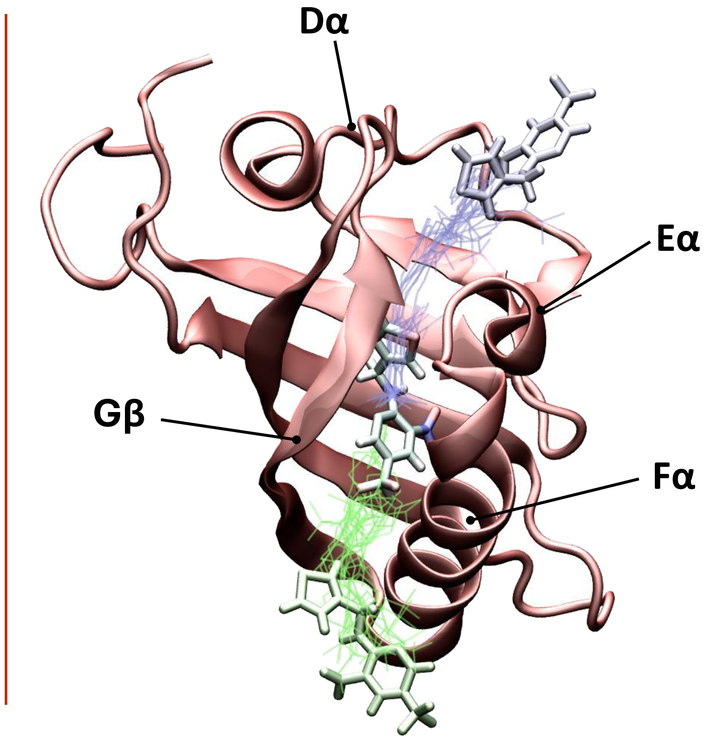
Targeting HIF-2α

- The Hypoxia-Inducible Factor 2α (HIF-2α) is a transcription factor involved in the regulation of numerous genes in response to changes in oxygen concentrations. In low oxygen conditions, the PAS-B domain of HIF-2α binds the aryl hydrocarbon receptor nuclear translocator (ARNT), forming the functionally active heterodimer. [1]
- Since HIF-2α pathways are often misregulated in several cancers, disrupting the HIF-2α-ARNT interaction is a promising therapeutic strategy. Notably, the HIF-2α-PAS-B domain features a druggable 290 Å^3 buried cavity. [2]
- Currently, Belzutifan is the only available HIF-2α inhibitor. It has been approved to be used in patients with von Hippel-Lindau disease who require therapy for associated renal cell carcinoma (RCC), central nervous system hemangioblastomas, or pancreatic neuroendocrine tumors (pNET). [3]
- Computational prediction of binding free energies and kinetic rates is key to the rational design of HIF-2α inhibitors.
- Previous Weighted Ensemble (WE) simulations have identified two possible dissociation pathways: [1]
  - Pathway 1, involving ligand exit between **Fα helix** and **Gβ strand** (contribution 63%);
  - Pathway 2, involving ligand exit through **Eα** and **Dα helices** (contribution 37%).
- **Aim of the work:** using the WE-generated trajectories to compute free energy profiles and association/dissociation barriers for a reference compound (THS-017) along Pathway 1 and 2 employing Path-Based Metadynamics.



Path Collective Variables [4]

$$\begin{cases} S(\mathbf{x}) = \dfrac{\sum_{i=1}^{N} i\, e^{-\lambda(\mathbf{x}-\mathbf{x}_i)^2}}{\sum_{i=1}^{N} e^{-\lambda(\mathbf{x}-\mathbf{x}_i)^2}} \\ Z(\mathbf{x}) = -\dfrac{1}{\lambda}\ln\left[\sum_{i=1}^{N} e^{-\lambda(\mathbf{x}-\mathbf{x}_i)^2}\right] \end{cases}$$

$$\lambda = -\frac{\ln 0.1}{\langle(\mathbf{x}-\mathbf{x}_i)^2\rangle}$$

Well-Tempered Metadynamics [5,6]

$$V(\mathbf{s},t) = \sum_{\kappa\tau<t} W(\kappa\tau)\exp\left(-\sum_{i=1}^{d}\frac{\left(\mathbf{s}_i - \mathbf{s}_i\left(q(\kappa\tau)\right)\right)}{2\sigma_i^2}\right)$$

$$W(\kappa\tau) = W_0\exp\left(-\frac{V\left(\mathbf{s}\left(q(\kappa\tau)\right),\kappa\tau\right)}{k_B\Delta T}\right)$$

$$V(\mathbf{s},t\to\infty) = -\frac{\Delta T}{T+\Delta T}F(\mathbf{s}) + C$$

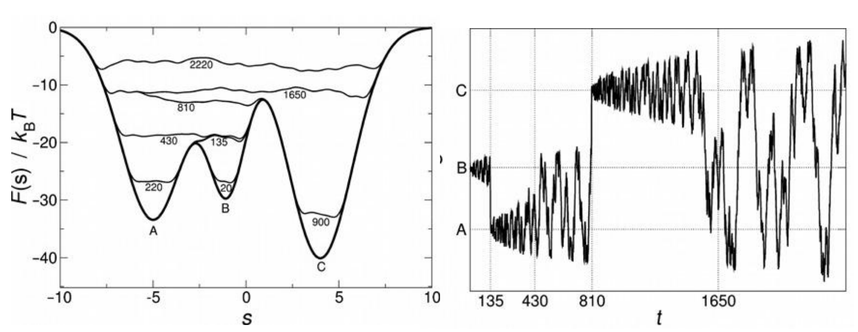
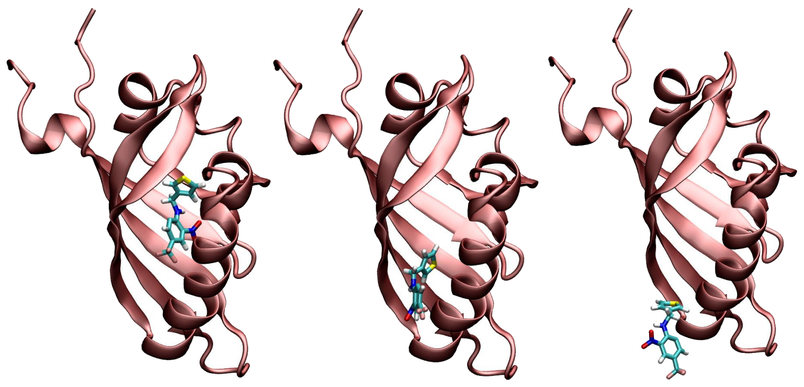
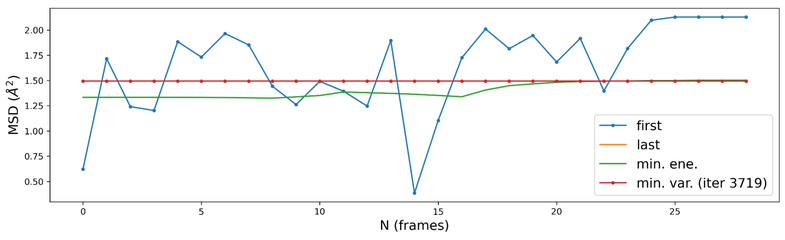


Figure adapted from [7]

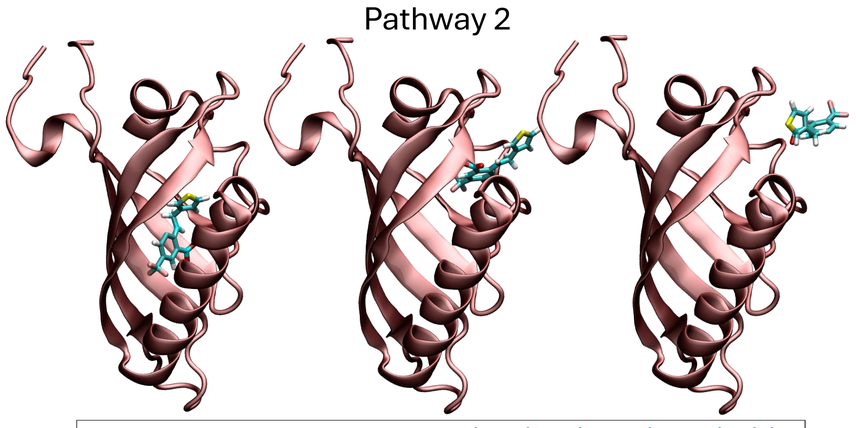
PCVs Parametrization

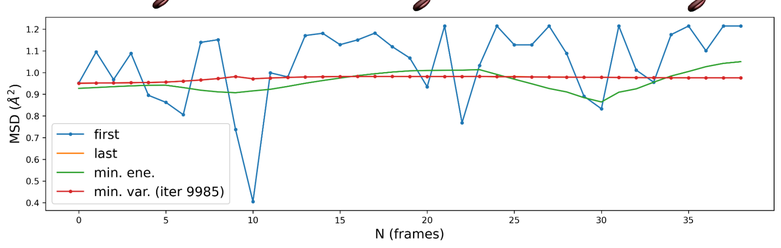
Pathway 1





Pathway 2

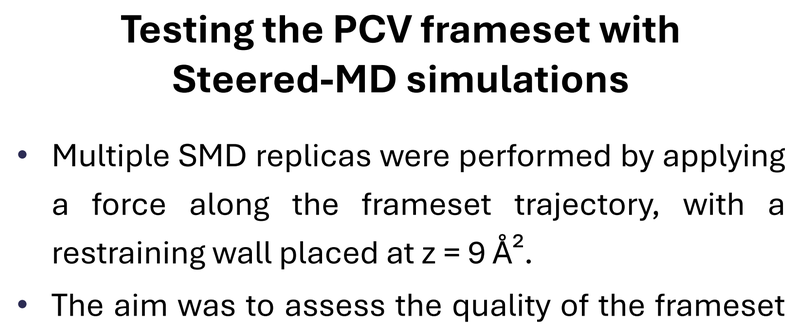




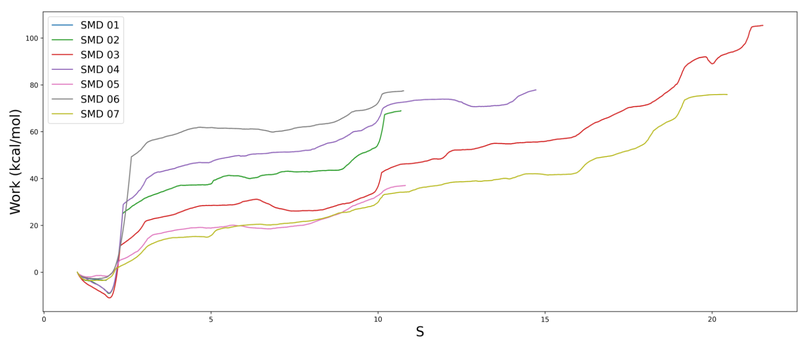
Testing the PCV frameset with Steered-MD simulations

- Multiple SMD replicas were performed by applying a force along the frameset trajectory, with a restraining wall placed at z = 9 Å^2.
- The aim was to assess the quality of the frameset by evaluating how the space described by the CVs was sampled and identifying potential regions along the trajectory where clashes with protein segments might occur.

Pathway 1



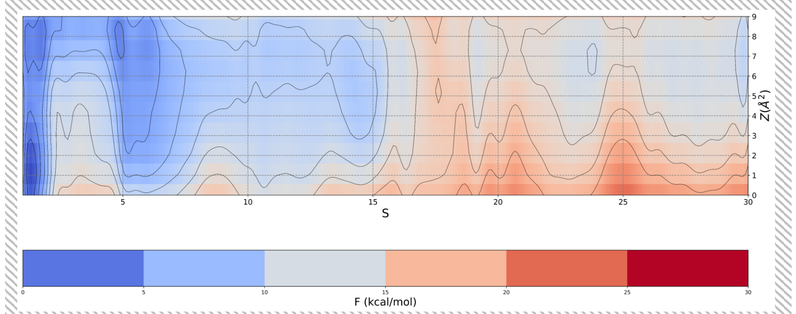
Pathway 2



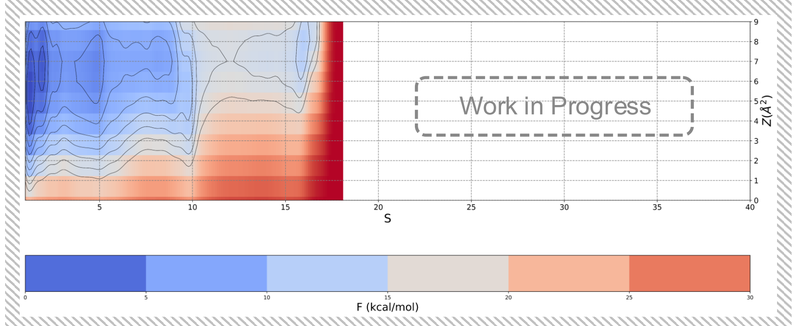
Preliminary Free Energy Profiles

- Preliminary results of WT-Metad simulations.

Pathway 1



Pathway 2



Simulation Parameters

- Force Field: AMBER19SB
- Bias deposition pace: every 500 steps
- Initial bias height: 1 kJ/mol
- Sigma: 0.3 (s); 0.03 (z)
- Bias factor: 8

Learn more about the methodology

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