Computational Study of a Metamorphic Protein

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Abstract

Metamorphic proteins, also known as fold-switchers, are capable of interconverting their structure between two or more stable native states to perform different functions in response to changes in the environment. The origin and purpose of fold-switchers in protein evolution have been the subject of an exciting literature debate[1,2,3]. The selective access of different folds and functions suggests potential applications in drug discovery and molecular target treatment. A small molecule that interferes with the fold switch could directly control the biological process, offering a new avenue for function modulators. Known fold-switchers are present in both eukaryotic and prokaryotic cells and are involved in essential biological processes such as chemotaxis, cell cycle regulation, ion transport, and regulation of transcription and translation[4,5,6].
Here we show a computational study of the metamorphic Collagen Binding Domain (CBD) from bacterial Collagenase. This enzyme degrades collagen tissue in cases of gangrene. The metamorphic properties of CBD are induced by changes in calcium concentration, as demonstrated by X-Ray, NMR, and SAXS. We simulated both metamorphic states of the CBD and identified atomistic details of the pseudo-transitions in metamorphic regions of the domain.

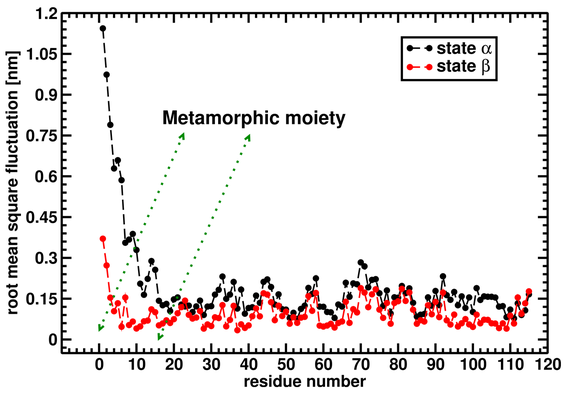
References:
[1] Davidson, A. R. *PNAS* 105, 2759–2760 (2008)
[2] Tian, P. *et al. PLOS Computational Biology* 16, e1008285 (2020)
[3] Sikosek, T. *et al. PLOS Computational Biology* 8, e1002659 (2012)
[4] Lei, Y. *et al. Microbes and Infection* 14, 262–267 (2012)
[5] Luo, X. *et al. Nature Structural & Molecular Biology 2004 11:4* 11, 338–345 (2004)
[6] Porter, L. L. *Protein Science* 30, 1723–1729 (2021)

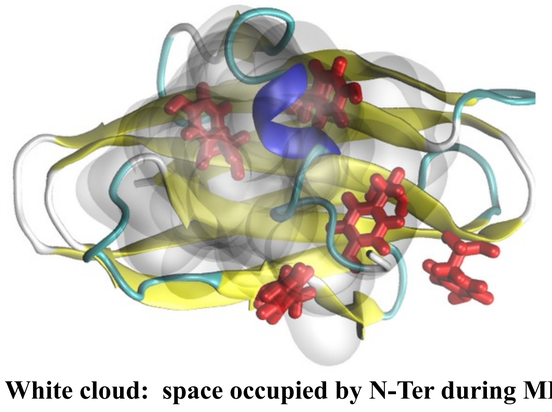
Collagen Binding Domain

The CBD domain is a component of the enzyme collagenase, which enables the bacteria responsible for gangrene to disseminate within tissues. The CBD domain is responsible for binding to collagen fibres, while the remainder of the enzyme is involved in the hydrolysis of collagen fibres. The stability of these interactions is enabled by the presence of solvent exposed TYRs. The affinity of CBD for collagen is regulated by the concentration of Ca^{2+} ions. The high concentration of Ca^{2+} ions in the extracellular matrix causes the N-terminal of CBD to change conformation, **from state α to state β**, thereby freeing the TYRs responsible for binding to collagen fibres from steric hindrance.

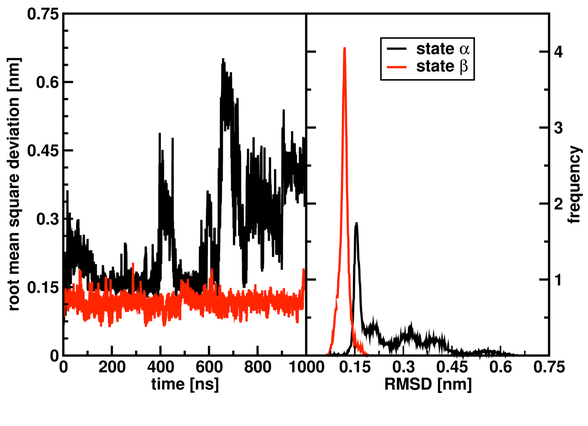
Molecular Dynamics of CBD

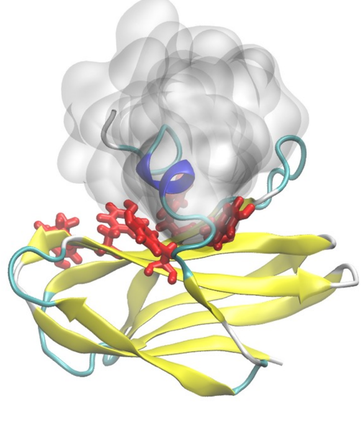
The results of the molecular dynamics simulations demonstrate that when the N-termini residues are in the alpha-helix conformation, they are capable of concealing the TYR-mediated bond with the collagen fibres. The N-termini exhibit fluctuations upon the TYR residues, thereby disrupting any potential interaction between CBD with other bio-molecule.





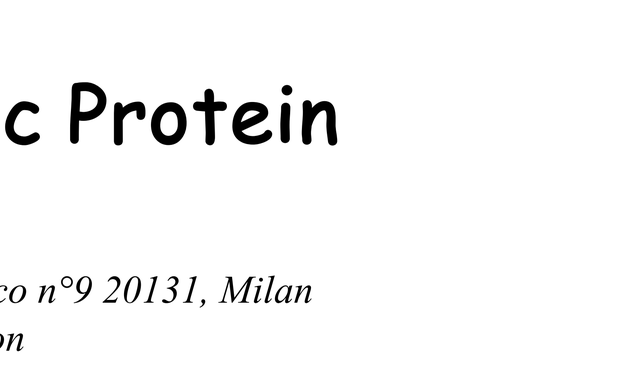
White cloud: space occupied by N-Ter during MD

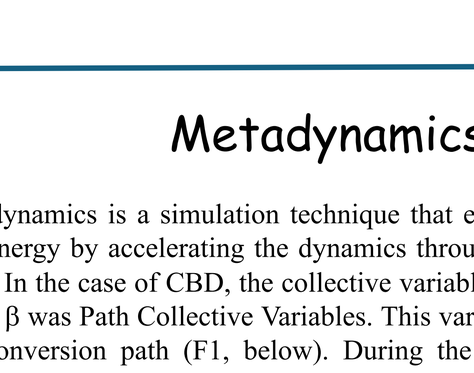


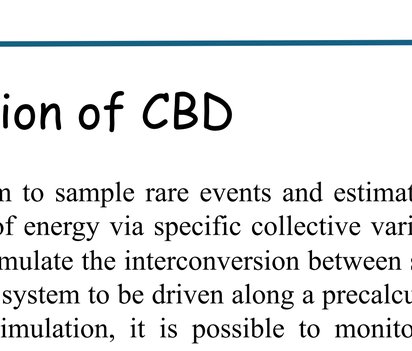


Metadynamics Simulation of CBD

Metadynamics is a simulation technique that enables the system to sample rare events and estimate the free energy by accelerating the dynamics through the addition of energy via specific collective variables (CV). In the case of CBD, the collective variable employed to simulate the interconversion between states α and β was Path Collective Variables. This variable enables the system to be driven along a precalculated interconversion path (F1, below). During the course of the simulation, it is possible to monitor the progress of the 'reaction' along the path with the component ***s*** and the distance from the path with the CV component ***z*** (F2, below). The reweighted free energy surface is presented in F2 below. The simulation enabled the transition from the initial state (**state α**) to a final state (**state β**) with a deviation from the crystal structure of just 0.138 nm (F3 and F4 below).

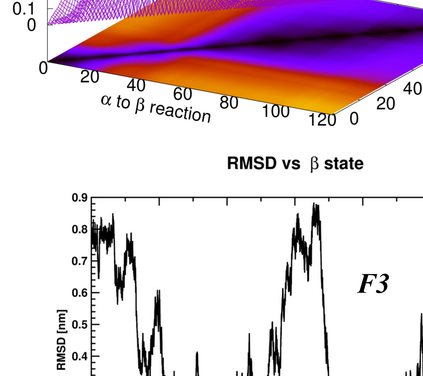


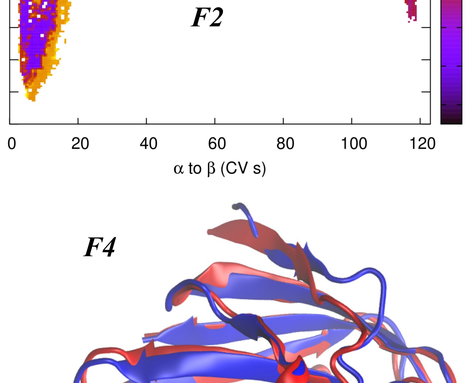


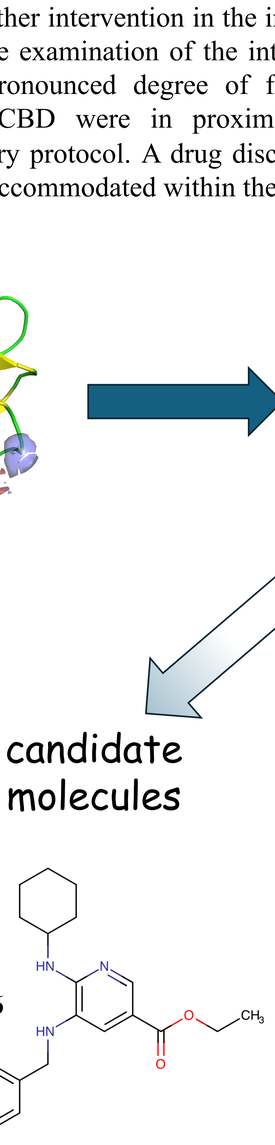


Drug Discovery

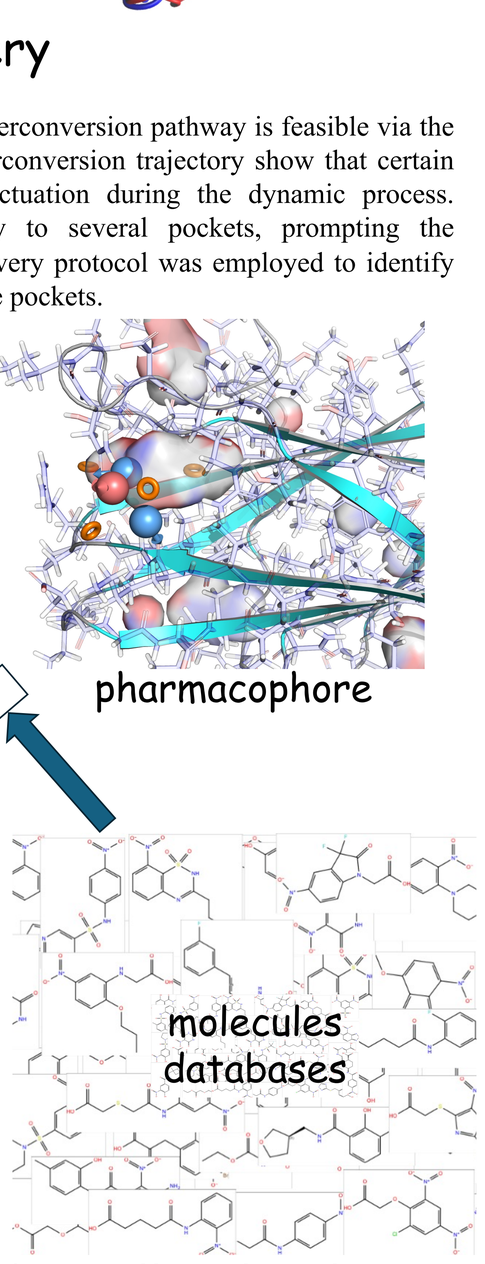
The question thus arises as to whether intervention in the interconversion pathway is feasible via the utilisation of small molecules. The examination of the interconversion trajectory show that certain moieties of CBD exhibited a pronounced degree of fluctuation during the dynamic process. Additionally, these regions of CBD were in proximity to several pockets, prompting the implementation of a drug discovery protocol. A drug discovery protocol was employed to identify potential molecules that could be accommodated within these pockets.





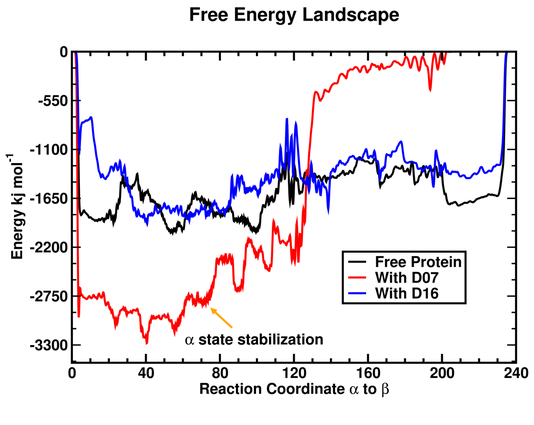


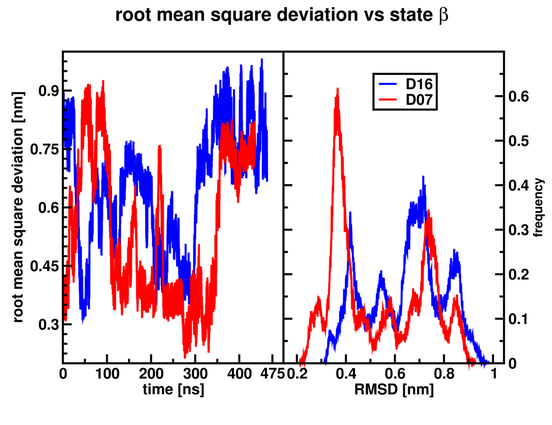
candidate molecules



Metadynamics of CBD with small molecule

The structural configuration of the complexes formed between CBD and the identified small molecule, as determined through the drug discovery process, was obtained by docking simulation. It is noteworthy that a single molecule is capable of stabilising the α-state of CBD, thereby rendering it more challenging to transition to the β-state.





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