Title: An integrated protocol for relating Hydrogen-Deuterium exchange data to protein conformational ensembles

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Proteins do not maintain a single static 3D structure, but instead, exist in a dynamic equilibrium, constantly fluctuating between various conformations. While experimental structural determination – for example by X-ray crystallography – will capture one or a few stable structures, there is ample experimental and computational evidence on the existence of transient structures that likely play critical roles in protein function.

An important experimental method for characterizing protein structural dynamics is hydrogen-deuterium exchange, which was historically performed by NMR and now has been revived by mass-spectrometry methods that allow the study of much larger proteins.

For interpreting these data in terms of conformational ensembles, molecular dynamics (MD) simulations are extremely valuable, yet many significant conformational changes occur on timescales that are not routinely accessible, if at all, with conventional MD techniques.

In this study, we present a protocol based on well-tempered metadynamics (WTmetaD) to explore transient fluctuations of protein structure that are relevant for interpreting Hydrogen-Deuterium exchange data. The protocol was tested on the protein ubiquitin, which has been extensively characterized through both computational and experimental methods. We highlight important points of attention concerning the choice of collective variables for efficient exploration of the conformational landscape. We also discuss important parameters relevant to the calculation of Hydrogen-Deuterium exchange rates from computational trajectories. Our data show that the WTmetaD trajectories successfully sample the functional states of ubiquitin identified in independent NMR studies and that the overall conformational distribution aligns well with HDX data. The protocol is general and versatile, offering a robust framework for studying protein dynamics across various systems.