Capturing protein structure ensembles of *E. coli* phosphofructokinase-2 using hydrogen-deuterium exchange mass spectrometry.

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Protein folding and binding have been usually studied through spectroscopic, fluorometric and/or calorimetric techniques that address global structural changes. Notwithstanding, capturing local structural changes experienced by a given protein due to chemical, temperature or mutational perturbations, or due to protein binding, is still conceived as a challenging task.

It is well known that amide backbone hydrogens are naturally exchanged with the surrounding water molecules in the solvent. Hence, D₂O can be used as a non-specific label in solution, enabling amide protons to be exchanged by deuterium, thus assessing the solvent accessibility of a protein by an increase in mass. Hydrogen-deuterium exchange, followed by protein fragmentation through proteolysis and analysis of the resulting peptides by mass spectrometry, allows sampling of structural changes of local regions of a given protein over time.

Using the dimeric enzyme phosphofructokinase-2 (Pfk-2) from *Escherichia coli* as a model, we used hydrogen-deuterium exchange mass spectrometry (HXMS) to determine the local structural changes occurring during its highly cooperative cold denaturation process. While the native-state of Pfk-2 described by HXMS fully reconstructs the dimer structure solved by X-ray crystallography, the cold-denatured state builds up upon solvent penetration throughout the structure and concurrent dissociation into monomers, with a kinetic unfolding rate of ~1×10⁻⁴ s⁻¹. Further high-resolution characterization of the partially unfolded monomeric state of a single point mutant of Pfk-2 and of an homologous dimeric enzyme allows reconstruction of the first structural changes after dissociation of the native dimer and illustrates the mechanism by which Pfk-2 and other enzymes from the ribokinase superfamily unfold. FONDECYT 1110137, 1130510 & 11140601.