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Exploring minor and transiently formed protein states using NMR spectroscopy.

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Proteins are inherently plastic molecules, whose function often critically depends on excursions between different molecular conformations. However, a rigorous understanding of the relation between a protein's structure, dynamics and function remains elusive. This is because many of the conformers on its energy landscape are only transiently formed and marginally populated (less than a few per cent of the total number of molecules), so that they cannot be individually characterized by most biophysical tools. Modern NMR spectroscopy provides some of the most powerful approaches for detecting and characterizing such minor conformers. In this talk, examples of these various approaches as well as some new experimental developments will be discussed.

1) Structure of a transient and low-populated state of a T4 lysozyme mutant (1)

In particular, a study will be presented of a lysozyme mutant from phage T4 that binds hydrophobic molecules and transiently populates an excited state to about 3% at 25˚C. Here we show that binding occurs only via the ground state, and present the atomic-level model of the 'invisible', excited state obtained using a combined strategy of relaxation-dispersion NMR and CS-Rosetta model building that rationalizes this observation. The model was tested using structure-based design calculations identifying point mutants predicted to stabilize the excited state relative to the ground state. In this way a pair of mutations were introduced, inverting the relative populations of the ground and excited states and altering function. These results suggest a mechanism for the evolution of a protein's function by changing the delicate balance between the states on its energy landscape.

2) Pushing the limits of relaxation-dispersion experiments: characterizing states with longer lifetimes (2)

Although states with millisecond lifetimes (0.5 - 5 ms) can be studied using relaxation-dispersion NMR methods, excited states with longer lifetimes could be studied only when they were populated to greater than ~10% and consequently visible in the NMR spectra. Borrowing from the MRI chemical exchange saturation transfer (CEST) approach we have developed a new experiment that can detect and characterize weakly populated (~1%) states with lifetimes between 5 and 50 milliseconds. The experiment and its application to the folding of FF A39G will be described.

References:

1. Solution structure of a minor and transiently formed state of a T4 lysozyme mutant. *Nature.* **477**, 111-4 (2011)
2. Studying "Invisible" Excited Protein States in Slow Exchange with a Major State Conformation. *J Am Chem Soc* **134**, 8148-61 (2012)

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