Real-time multidimensional NMR: a complementary off-equilibrium tool for structural biology

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Today, an impressive amount of high-resolution structural information is available for ground-state conformations of proteins, nucleic acids, and their complexes. Much less is known, however, on the conformational properties of sparsely populated high-energy conformations (excited states), encounter and transition states that are important for a detailed understanding of the function and, in some cases, disease-related misfunction of these molecules. Real-time multidimensional NMR spectroscopy provides a powerful and unique tool for investigating, at atomic resolution, conformational transitions that involve energy barriers of more than a few kcal/mol (figure 1). For real-time NMR applications, the conformational transition is induced by a rapid change in the sample conditions, followed by continuous NMR data acquisition to monitor changes in NMR observables over time. Real-time NMR has benefited substantially from fast multidimensional NMR data acquisition methods, e.g. the SOFAST and BEST techniques developed in our laboratory [1,2].

I will present some recent advancement we have made in this field: i) an improved fast mixing device has been designed that allows the removal of the injector from the NMR detection volume after mixing, thus allowing the use of lower samples volumes while ensuring high magnetic field homogeneity [3]; ii) a sensitivity-optimized CPMG-based relaxation-dispersion experiment provides a powerful new tool for site-resolved investigation of conformational exchange dynamics occurring in timely unstable or transiently populated molecular states [4]; and iii) a Hadamard-encoded ^{15}N-edited imino ^{1}H SOFAST pulse sequence allows site-resolved monitoring of RNA refolding events [5].

We will demonstrate how these new real-time NMR tools proved useful for further characterizing the major folding intermediate of the amyloidogenic protein β2 microglobulin, that is believed to be at the onset of the aggregation process, and for investigating at single-nucleotide resolution the RNA-melting activity of CspA, the major cold shock protein of *E. coli*.

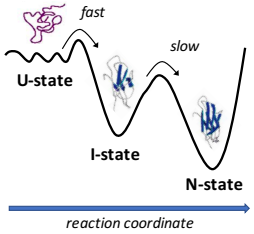

Figure 1: One-dimensional energy landscape for protein folding, involving an ensemble of unstructured states (U), a folding intermediate (I), and the native state (N).

References:

(1) Schanda & Brutscher, Very fast two-dimensional NMR spectroscopy for real-time investigation of dynamic events in proteins on the time scale of seconds., *J. Am. Chem. Soc.* 127 (2005) 8014–8015.
(2) Schanda et al., Speeding up three-dimensional protein NMR experiments to a few minutes., *J. Am. Chem. Soc.* 128 (2006) 9042–9043.
(3) Franco et al., Optimized fast mixing device for real-time NMR applications, *J. Magn. Reson.* (2017) 281, 125-129.
(4) Franco et al., Probing conformational exchange dynamics in a short-lived protein folding intermediate by real-time relaxation-dispersion NMR, *J. Am. Chem. Soc.* 139 (2017) 1065–1068.
(5) Rennella et al, RNA binding and chaperone activity of the E. coli cold-shock protein CspA, *Nucleic Acids Res.* (2017) 1–14.