The function of a protein depends critically on its ability to adopt a specific structure. Remarkably, a protein can fold efficiently to this native state from the many unfolded states on physiological time scales. Understanding how this process occurs is one of the great challenges in biology. Proteins can also form partially folded, collapsed states under both equilibrium and non-equilibrium conditions [1,2]. Such partially folded proteins resemble the intermediate states along the protein folding pathway, and thus play an important role in understanding the mechanisms of protein folding. Moreover these partially folded proteins have also been shown to participate in important cell functions.

A molten globule is a compact, partially folded protein that has native-like secondary structure and backbone folding topology, but lacks the extensive, specific side-chain packing interactions of the native structure [3]. Structural studies have shown that the side-chains in a molten globule can adopt a greater variety of conformations than in a native protein. The ability of the side chains to form the tight and specific interactions typical of a native protein, is the essential final step in the protein folding pathway. This packing process is considered to be energetically more difficult than forming the collapsed, disordered folding intermediates. However, little is known about the dynamics of this mostly folded state.

In order to understand the changes in protein dynamics that occur in the final stages of folding, we have used incoherent quasielastic neutron scattering (IQNS) to probe the differences in the dynamics between the native state and the almost completely folded, molten globule state of the protein, bovine α-lactalbumin in solution [4]. Because hydrogen scatters neutrons much more strongly than deuterium, the exchangeable protons were deuterated and D₂O was used as the solvent. The scattering from the protein is then dominated by the non-exchangeable protons in the side-chains. Figure 1 shows the measured scattering function, $S(Q, \omega)$, of bovine α-lactalbumin (BLA) and its molten globules (MBLA), as a function of the energy transfer $\hbar \omega$ at a momentum transfer $Q = 1.08$ Å⁻¹. The broader quasielastic peak of MBLA indicates that the side-chain protons within the molten globules move significantly faster than those in the native protein, which reflects the lack of the specific side chain interactions in MBLA compared to BLA.

In addition to the time scale, IQNS yields information on the geometry of the observed motion through the $Q$-dependence of $S(Q, \omega)$. We have analyzed the $Q$-dependence for BLA and MBLA...
using a model where some of the protons diffuse within a spherical cavity, while others are fixed on the ≈ 70 ps time scale of these measurements. This is intended to capture the physical picture of side chain motion within a constrained volume imposed by the backbone topology of the protein. Within this model, the scattering consists of two components, a δ-function and a Lorentzian, each broadened with the experimental resolution ≈ 60 µeV. Typical fits for individual spectra are shown in Fig. 1.

Figure 2 shows the half-width at half-maximum Γ of the Lorentzian component of the scattering as a function of Q². The initial linear region indicates that on longer length scales (small Q), the protons undergo spatially-restricted diffusive motions, while the crossover to a constant width at higher Q reflects the granularity of the motion at these shorter, atomic length scales. The elastic incoherent structure factor, which gives the time-averaged spatial distribution of the protons, is formed by dividing the intensity of the elastic (δ-function) component by the total integrated intensity measured at each Q. The EISF is shown as a function of Q in Fig. 3. The solid lines show fits to the EISF expected for diffusion within a sphere, showing that the length scale of the motion increases by about 25 % as the side chains become disordered. This is in contrast with the usual situation where slower motions tend to cover larger length scales. The fact that the EISF plateaus at a higher value for BLA than MBLA indicates that more of the side chain protons are immobilized in the protein’s native state.

The mean square amplitude <u²> of the high-frequency vibrational modes can be obtained from the Q-dependence of the total scattering intensity through the Debye-Waller factor. The values of <u²> extracted in this way are indistinguishable for BLA and MBLA, which suggests that chemical bond vibrational motions do not change significantly in the final stage of protein folding.

Overall, these results demonstrate that the side chains in molten globules are significantly more mobile than those in the native protein, and explore a larger length scale in a shorter time. This indicates that the specific side chain interactions responsible for the final step in protein folding both localize and slow the motions of the side chains.

REFERENCES