

## WIDE-ANGLE X-RAY SCATTERING CAN REVEAL HIERARCHICAL MAP OF UNFOLDING-REFOLDING TRANSITION OF PROTEIN

The X-ray solution scattering technique has a great advantage for studying nanoscale structures of biological materials and those complexes *in situ*. High-brilliant X-ray from a third generation synchrotron radiation (SR) source has been opening a new horizon not only in protein crystallography but also in protein structure analyses in solutions. How proteins fold into their native structures is still one of the main problems in structural biology. Numerous studies on protein folding have become possible because of advances in both experimental methods and theoretical approaches. The experimental resolutions in space and time have been also improving continuously. A time-resolved small-angle X-ray scattering (SAXS) study at a third-generation SR source demonstrates that the collapse process of a protein is now observable in time and spatial resolutions of  $> 1$  ms and  $> 30$  Å, respectively [1]. However, there exists some intrinsic difficulties in folding-kinetics studies for obtaining information on the intramolecular structures of proteins in solutions. Alternatively, the experimental demand for time resolution still competes with that for spatial resolution.

On the other hand, under equilibrium conditions, high-spatial-resolution scattering data of proteins in solutions can be measured using X-ray from a third generation SR source. We have shown that the experimental wide-angle X-ray scattering (WAXS) curves of several typical globular proteins in different structural classes (all  $\alpha$ , all  $\beta$ ,  $\alpha+\beta$  and  $\alpha/\beta$ ) well reflect not only overall structures (molecular size and shape) but also intramolecular structures such as interdomain correlation, intradomain structures, and secondary structures [2]. Figure 1 shows the experimental and theoretical scattering curves of hen-egg-white lysozyme (HEWL) and horse skeletal muscle myoglobin with the schematic image of the three-dimensional HEWL structure. As shown in Fig. 1, the experimental SR-WAXS curves of the proteins are comparable to the theoretical curves in the full  $q$ -range calculated from the atomic coordinates of the proteins. The scattering curves in the regions of A, B, C and D mostly correspond to the different hierarchical structural levels of proteins, namely, the quaternary and tertiary structures, the interdomain correlation and intradomain structures, and the secondary structures including closely packed side chains, respectively.

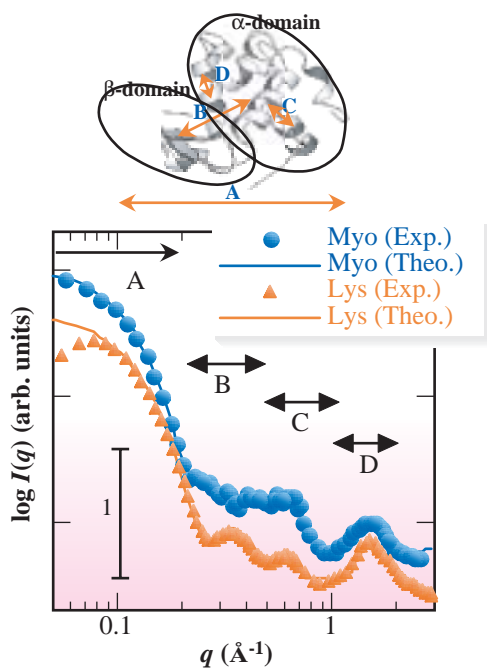


Fig. 1. Experimental and theoretical wide-angle X-ray scattering curves of hen-egg-white lysozyme (HEWL) and horse skeletal muscle myoglobin with schematic image of three-dimensional HEWL structure. The regions of A, B, C and D mostly correspond to the different hierarchical structural levels.

Based on the above results, we have studied the hierarchical features of the thermal unfolding-refolding structural transitions of HEWL in the temperature range from 13 to 84 °C at pH 2.2, 3.1, 3.6, and 4.5 [3] at beamline **BL40B2**. We have successfully obtained high-statistic WAXS data on the reversible unfolding-refolding process in the spatial range from  $\sim 2$  to  $\sim 125$  Å, which covers all hierarchical structures of a small globular protein from the tertiary structure to the secondary structure (Fig. 2). We have found that the pH dependence of the thermal structural transition of HEWL is well characterized by the different hierarchical levels and the transition cooperatively among them. In this study we have presented a new hierarchical map depiction of unfolding-refolding transitions, which we call the structural hierarchy (SH) map. According to a method established previously [4], that is, scattering data at wide range of  $q$  values allowed us to determine molar fraction  $\alpha$  ( $\alpha \leq 1$ ) of the native-like protein structure defined by data at each  $q$  range, thus producing a map of the amount of the native-like protein structure as a function of  $q$  range, namely, as a function of the hierarchical level or resolution. This map can show the detailed feature of

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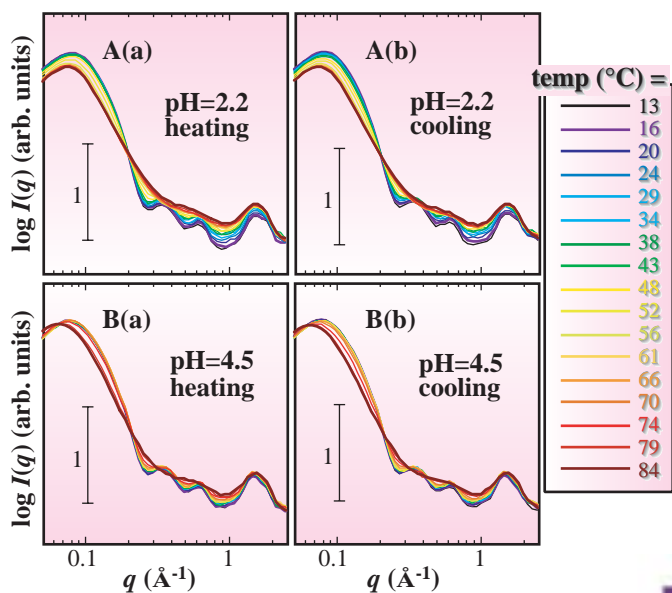


Fig. 2. Temperature dependence of WAXS curve  $I(q)$  of HEWL (pH 2.2, A; pH 4.5, B). (a) heating (unfolding); (b) cooling (refolding).

the unfolding-refolding transition of a protein depending on various structural hierarchical levels. Figure 3 shows the SH maps during heating (unfolding) and cooling (refolding) at pHs 2.2 and 4.5, where we display the  $\alpha$  values in rainbow colors and contour lines. In Fig. 3, the contour lines of  $\alpha = 0.5$  in the SH maps at different  $q$  values correspond to the transition midpoints for different hierarchical levels. The transition-midpoint temperatures  $T_m$  of the  $\alpha$  values below  $q = 0.2 \text{ \AA}^{-1}$  (tertiary structure) agree well with those determined by calorimetric measurements. Except for structural insensitive regions around  $q = 1 \text{ \AA}^{-1}$  and above  $q = 1.8 \text{ \AA}^{-1}$ , the density and slope of the contour lines depict the thermal stability and transition feature for each hierarchical level, which would relate to the transition cooperativeness among different hierarchical levels. At pH 4.5, the contour lines for all hierarchical levels are densely packed and parallel around the same transition midpoint temperature, suggesting that the collapse and

regeneration of the native-like protein structure proceed concurrently or cooperatively for all hierarchical levels in a two-state transition. The SH map at pH 2.2 shows that the transition midpoints for each hierarchical levels greatly differ from each other, suggesting that the transition proceeds in a multistate manner. SH maps would be related to a hierarchical fine structure of protein-folding energetics such as a “folding funnel” or an “energy landscape.”

Thus, SR-WAXS data of proteins obtained using X-ray from a third generation source are sufficiently powerful to clarify detailed transition features for all the hierarchical levels under equilibrium conditions.

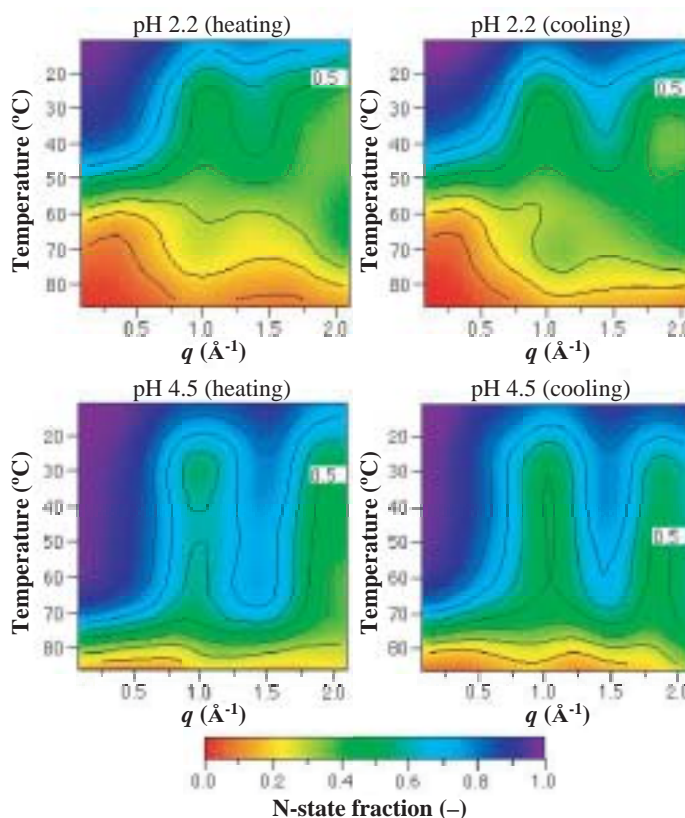


Fig. 3. Hierarchical maps of unfolding-refolding transitions of HEWL given by native molar fraction  $\alpha$  at different  $q$  values. The  $\alpha$  values are displayed in rainbow colors and contour lines.  $\alpha = 0.5$  corresponds to the transition midpoint for each hierarchical level.

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## References

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