

## Self-association of a Folding Intermediate of Acetylcholinesterase: the Effect of Glycerol

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Beamline(s): X12B

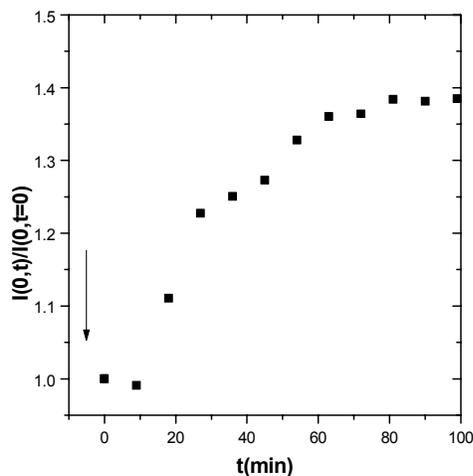
**Introduction:** Partially folded forms of proteins are encountered during protein synthesis; as a hallmark of “prion diseases”; and during the bulk purification of bioengineered proteins. In the latter two cases, folding intermediates exhibit a marked tendency to self-associate. We are using the well-characterized molten globule form of acetylcholinesterase (1) as a model system for studying the kinetics and thermodynamics of the self-association of protein folding intermediates.

**Methods and Materials:** Dilute (15-2 mg/ml) aqueous solutions of acetylcholinesterase were prepared as described previously (1). The molten globule form was generated by addition of 1.2-1.5M Gdn-HCl. X-ray scattering data acquisition was performed with MAR imaging plates or with a multiwire linear position sensitive detector. Exposure time for each frame was 200-300 seconds. Data analysis programs were written by M. Capel and O. Glatter.

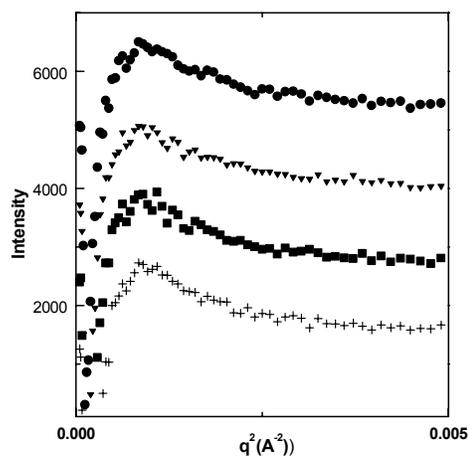
**Preliminary Results:** Progressive increase in the intensity of low angle X-ray scattering of acetylcholinesterase (2mg/ml) in aqueous solution containing 1.5M Gdn-HCl is indicative of self-association. It is found to be a strongly non-linear function of time (**Figure 1**). 10wt% glycerol (which does not affect the partial unfolding) stabilizes the monomer form of the molten globule: no change in the X-ray scattering curve is observed during approximately one hour after addition of denaturant. (**Figure 2**).

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References: (1) D. Kreimer, R. Szosenfogel, D. Goldfarb, I. Silman, L. Weiner, “Two-state transition between molten globule and unfolded states of acetylcholinesterase as monitored by electron paramagnetic resonance spectroscopy”, *Proc. Natl. Acad. Sci.* **91**,12145-12149 (1994).



**Figure 1.** Increase of  $q=0$  scattered intensity (calculated using the Indirect Transformation Procedure) as a function of time after addition of Gdn-HCl (arrow) monitors self-association of acetylcholinesterase molten globule molecules.



**Figure 2.** X-ray scattering curves from acetylcholinesterase molten globule molecules in the presence of 10wt% glycerol. Approximate times after addition of denaturant (from top to bottom): 5min; 17min; 42min; 60min