Chemical Engineering Graduate Seminar

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3:30 – 4:30 PM | February 3, 2004 | CPE 2.218

"Unboiling the Egg: Protein Disaggregation and Refolding under High Hydrostatic Pressures"

Protein aggregates are a serious problem facing the biotechnology industry. Aggregation in therapeutic protein products can result in extreme side effects (death!), and during the production of recombinant proteins aggregation causes dramatically reduced process yields and heavy environmental costs. I will discuss a newly developed high-pressure protein folding process that offers high process yields and low solvent requirements.

Hydrostatic pressures between 1 and 3 kbar cause dissociation of multimeric proteins. At higher pressures, typically 5-10 kbar, monomeric proteins will unfold. There is thus a pressure "window" between about 2 and 5 kbar wherein the native state of monomeric proteins is thermodynamically favored, but multimeric proteins will dissociate into their subunits. We have used high pressures to refold proteins from aggregates and to fold proteins from inclusion bodies. By applying pressures in the "window", we dissolve the aggregates in a fashion similar to the dissociation of multimeric proteins, and concomitantly refold the protein because the native state is still favored. High yields of folded protein are obtained, and folding yields are independent of protein concentration at concentrations to 10g/L. For protein aggregates containing disulfide-crosslinked aggregates, application of high hydrostatic pressures in the presence of disulfide shuffling agents produced higher yields of active protein than conventional, chaotrope-based refolding processes.