13g Measurements of Protein Folding and Unfolding Kinetic Pathways Using a Microfluidic Approach

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The folding and unfolding transition kinetics of proteins were studied using a microfluidic chip platform. The chip exploits the advantages of microfluidics in order to identify the optimal pathways for protein unfolding and refolding using only nanogram amounts of reagents and protein. An assay was developed to monitor protein folding and unfolding events through accurate on-chip dilutions using urea and guanidine hydrochloride and a molecular probe, 8-Anilino-1-naphthalenesulfonic acid, which interacts strongly with unfolded proteins via exposed hydrophobic regions. The unfolding mechanism of protein molecules is described using two or more sequential pathways such as a short time scale “rapid expansion” event followed by a long time scale “slow opening” of molecules. The on-chip protein refolding transition showed a two step process in response to decreased concentration of chemical denaturant. Free energy refolding curves suggests an initial rapid collapse of protein molecules to an intermediate structure followed by a slow refolding. Refolding curves were analyzed using standard methods and compared to accepted off-chip assay results for similar protein systems. A binding interaction study with drug molecules was also investigated using partially folded states. Refolding in the presence of drug showed destabilization of the molecule and subsequent unfolding. Results will be presented for three kinds of protein molecules.