Fast Events in Protein Folding following Ultrarapid Mixing
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GICA BAKAJIN, LLNL — A continuous flow microfluidic mixer fabricated out of
fused silica was used to study microsecond time scales of protein folding by monitor-
ing natural tryptophan fluorescence. This mixer uses hydrodynamic focusing and
diffusion to lower the concentration of the initial denaturant, inducing the protein
to fold. The mixing time can be as fast as 8 µs and allows access to times that
are inaccessible in conventional mixers. Using a confocal microscope we observe the
UV fluorescence spectrum from naturally occurring tryptophans in 3 well-studied
proteins, cytochrome c, apomyoglobin and lysozyme, as a function of time after
rapid mixing. Single value decomposition of the time dependent spectra reveal two
separate processes: 1) a spectral shift which occurs within the mixing time and 2) a
fluorescence decay occurring between 100 and 300 microseconds. We attribute the
first process to hydrophobic collapse and the second process the formation of the first
tertiary contacts. While the slower rate obviously depends on the details of the fold-
ing trajectory of each protein, we note that all three measured rates anti-correlate
well with the fraction of secondary structure formed. This work demonstrates that
hydrophobic collapse is much faster than had been estimated with slower mixing
methods and is in good agreement with measured rates of intramolecular diffusion
in unstructured peptides.