

## Construction of a Rapid-Mix Flow Cell for Sub-Millisecond Protein Folding Studies (1)

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Infrared spectroscopy provides an excellent means for examining the secondary structure of proteins. The deconvolution of the Amide I band ( $1600 - 1700 \text{ cm}^{-1}$ ), which is primarily a C=O stretching mode of the peptide linkage that forms the protein backbone, provides details of the percent of the protein structure that is  $\alpha$ -helical,  $\beta$ -sheet,  $\beta$ -turn, or extended coil. Using sensitive infrared spectroscopic techniques and powerful deconvolution methods such as Fourier self deconvolution and second derivative analysis, infrared spectroscopy can study the detailed changes in secondary structure with unfolding. It is beneficial to study protein folding in a time-resolved fashion, however the time limit of a conventional stopped flow mixers is on the order of several milliseconds and requires relatively large solution volumes. However, the use of bright light sources, such as lasers (for Raman or fluorescence spectra) and synchrotron infrared light (for FTIR spectra), significantly reduce the large areas (and hence volumes) that are necessary. Volume elements on the order of  $10 \mu\text{m} \times 10 \mu\text{m} \times 10 \mu\text{m}$  ( $\sim 1 \text{ pl}$ ) are sufficient to obtain high quality spectra.