

Abstract No. brom227

Design and Testing of a Rapid Mixing Continuous Flow Cell for Time-Resolved FTIR Studies of Protein Folding in the Ultra Sub-Millisecond Time Domain

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Beamline(s): U2B

ABSTRACT: Our previous continuous flow cell is limited since the timescale of the initial hydrophobic collapse (60 –100 microseconds) is not accessible. Such limitation arises because it takes the solution 167 microseconds to exit the seal and leaking ensues at flow rates exceeding 1.5 ml/min. We have tested a new design in which the entire cell is constructed from two ZnSe windows. The TRS groove (45 x 200 microns) is cut along the length of one ZnSe substrate (25 x 12 x 2 mm³). The mixing groove (36 x 200 microns) is cut widthwise on the companion substrate. Once fastened together, syringe needles are inserted at both ends of the mixing groove and at the TRS groove exit. The two grooves form a “T” at one end of the rectangular window assembly and a sink with a 2.75 microliter retention volume forms at the contact point. Prior to carrying out a folding experiment, the minimum syringe pump flow rate needed for turbulent mixing at the contact point had to be established. Using an identical cell constructed from colorless CaF₂ windows, mixing of base with an aqueous solution of bromocresol purple (yellow in color) was monitored with visible microscopy. A homogeneous purple color change occurred at the contact point only at flow rates equal to or greater than 2.0 ml/min per syringe. The maximum flow rate tested was 2.8 ml/min (mixing dead time is 30 microseconds). The timescales accessible for protein refolding with this prototype “T” cell range between 0.042-1.7 ms.