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Refolding of Denaturant-Induced Unfolded State of Cytochrome C and a TIM Barrel Protein Studied by Submillisecond Continuous-Flow SAXS. E. Kondrashkina¹, O. Bilsel², C. Kayatekin², T. Irving¹, C.R. Matthews², ¹BioCAT APS, Dept. of Biological, Chemical, and Physical Sciences, Illinois Inst. of Technology, Argonne, IL 60439, ²Dept. of Biochemistry and Molecular Pharmacology, Univ. of Massachusetts Medical School, Worcester, MA 01605.

Protein folding dynamics is of great interest as being closely related to protein functions and the origin of many diseases. However, many proteins collapse within the first hundred of microseconds that makes time resolution of SAXS experiments an important issue. A microfluidic continuous-flow mixer was interfaced to the BioCAT undulator beamline possessing beam focusing capabilities. It made possible to achieve time resolution of about 0.1 millisecond using final protein concentrations as low as 1 mg/ml. Refolding of guanidine-induced denatured state of cytochrome c studied by this technique in submillisecond and millisecond range demonstrated at least two stages in progressive increase of compactness of the protein molecules indicated by the decrease in radius of gyration from 24.5 to 15.5 Å. The SAXS data from the α -subunit of tryptophan synthase demonstrated that collapse of urea-denatured state of the protein occurred within the first 150 microseconds of dilution experiment. The measured radius of gyration of 33 Å was significantly smaller than that for the denatured state (43 Å).

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