

## Rapid Kinetic and Spectroscopy instruments

### $\mu$ SFM-20 - $\mu$ volume operation (updated August 13, 2009)

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This series of test was designed to demonstrate the operation of the  $\mu$ SFM-20 when using low total volume of protein samples.

Instrument used were : **SFM-20** stopped-flow, spectrometer was a **J-810 Jasco** CD spectrometer equipped with the SFM-Jasco adaptor.

Reaction used was the refolding of lysozyme by dilution in NaAc buffer.

Refolding was observed by recording the CD signal at 230 nm and at the same time the fluorescence emission at wavelength > 295 nm.

0.7 mg of lysozyme were dissolved in **233  $\mu$ L** of 6 M GuHCl giving a concentration of 3 mg/mL of denatured enzyme.

This volume of 233  $\mu$ L of solution were transferred in a 5 mL syringe and installed in the stopped-flow. 33  $\mu$ L were pushed to prime the system, resulting in a usable volume of 200  $\mu$ L.

The stopped flow was set to deliver 15  $\mu$ L of the enzyme solution per shot against 135  $\mu$ L of the NaAc buffer contained in the second syringe. Observation cuvette was a **FC-20** and temperature of the experiment was **5 °C**

After 2 shots necessary for setting the PM tubes high voltages (for CD and fluorescence), 11 shots were available for real experiments.

With this amount a series of 8 shots could be executed. Kinetics of CD and fluorescence changes were recorded allowing highly sufficient statistics for evaluation of the refolding process.

Results are shows in the figures on the next page. This shows the average of the 8 shots.

The possibility to obtain variable mixing ratio by a simple programming of the instrument (i.e. without changing the syringes) is one of the major advantages of the Bio-Logic stopped-flow instruments. The microprocessor control of the stepping motors giving 6400 steps per revolution of the motor gives a smooth and quasi continuous movement of the syringe over a very large range of flow rate.

