

Rapid Kinetic and Spectroscopy instruments

SFM specialized adaptation for Jasco CD spectrometer J-600, J-700 & J-800 series - test experiments (Updated August 13, 2009)

Refolding of Acyl-CoA Binding Protein (ACBP). (Courtesy of Dr. Kragelund, Copenhagen DK)

Instrument :

J-810 + SFM-20 + additional detection channel for simultaneous recording of intrinsic fluorescence.

Description of the test :

Syringe 1 : 3 mg/mL of ACBP in 6 M GuHCl, 20 mM NaAc pH 5.3.

Syringe 2 : 20 mM NaAc pH 5.3

For each shot 15 μ L of ACBP were diluted against 135 μ L of NaAc (1/10 dilution)

Data capture was always triggered from the start of the flow to observe cuvette washing, actual flow stop is indicated in the figures.

All experiments were performed at 5°C.

NOTE : These experiments were executed starting from a total volume of 400 μ L of protein solution. This amount was used to prime stopped-flow instrument, wash the cuvette, to set the CD instrument and finally to execute the actual shots used for collecting the data shown below.

Results

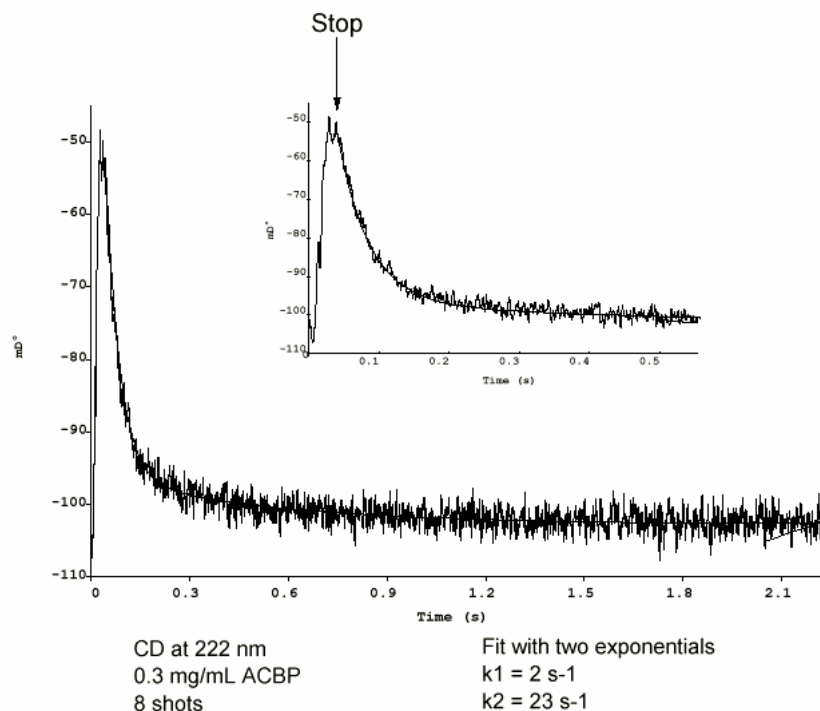
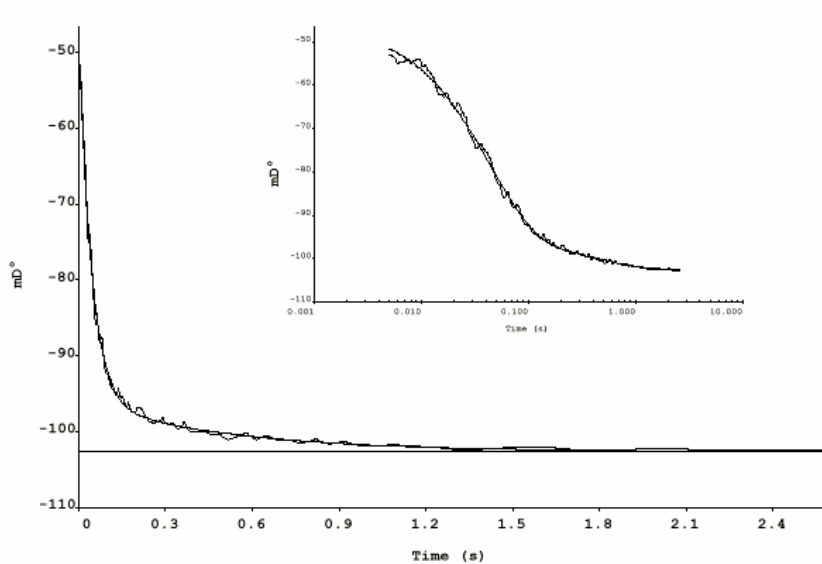


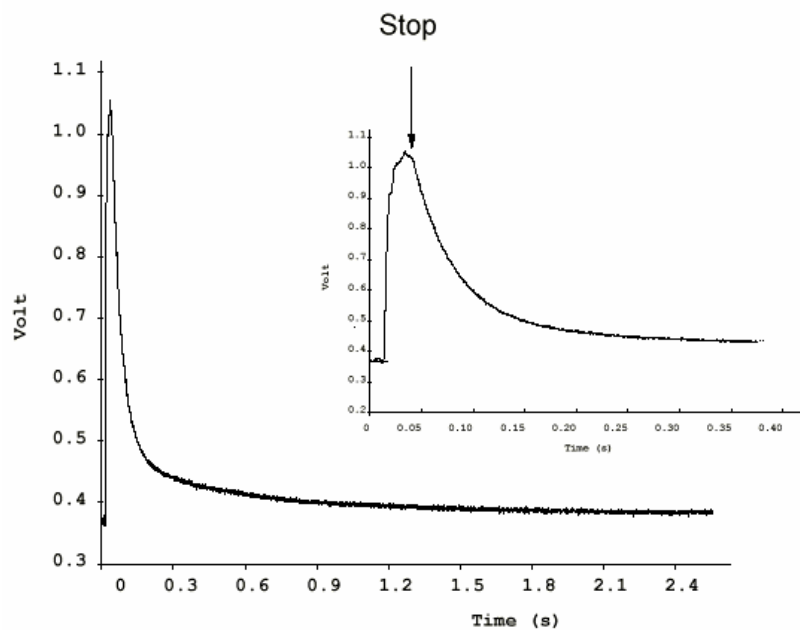
Figure 1 : CD recordings at 222 nm. Cuvette was FC-20 (light path 2 mm). Final concentration of protein was 0.3 mg/mL A fit with two exponentials is shown superimposed on the data.



CD at 222 nm
0.3 mg/mL ACBP
8 shots

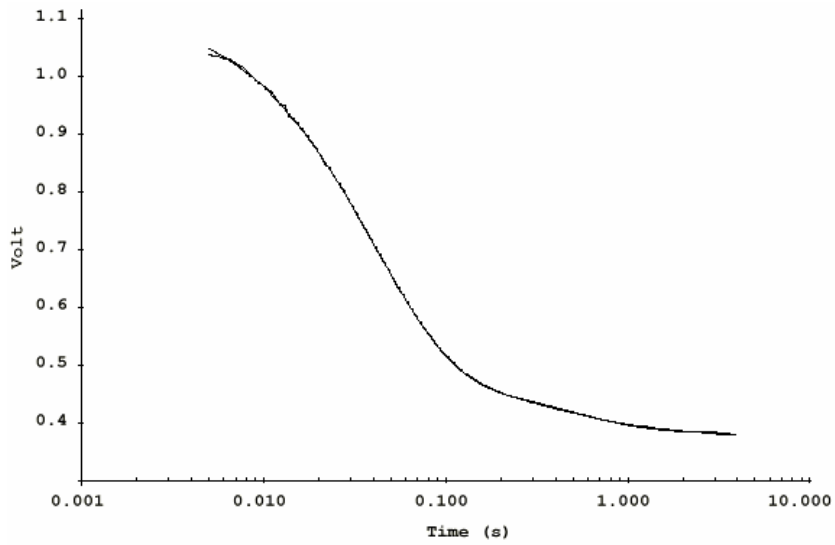
Fit with two exponentials
Same parameters as figure 1

Figure 2 : same as figure 1. Data were transformed to logarithmic sampling and logarithmic filtering.



Fluorescence Exc : 222 nm, Em. > 295
0.3 mg/mL ACBP
8 shots

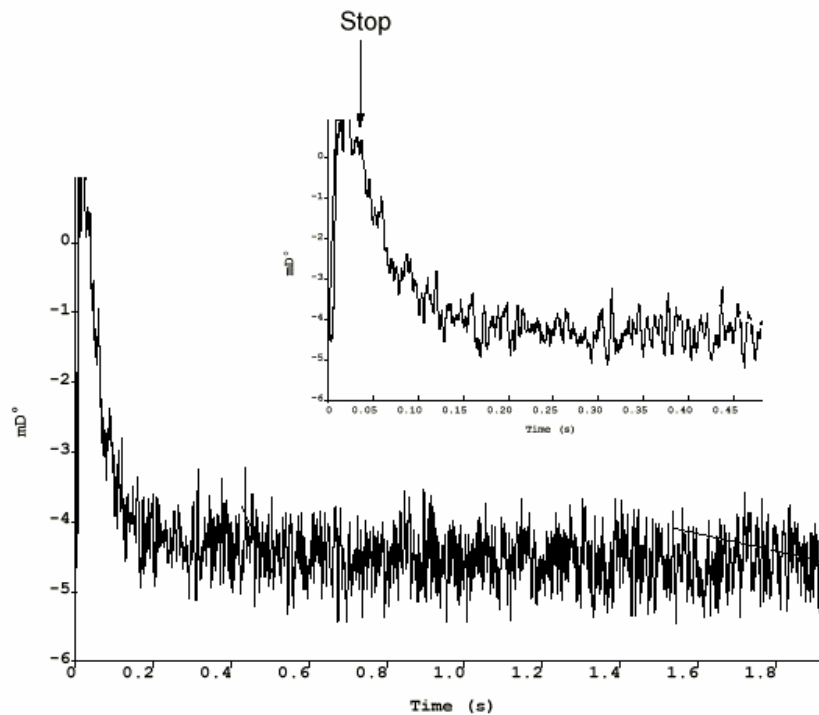
Figure 3 : fluorescence recordings taken at the same time as the CD



Fluorescence Exc : 222 nm, Em. > 295
 0.3 mg/mL ACBP
 8 shots

Fit with two exponentials
 $k_1 = 2 \text{ s}^{-1}$
 $k_2 = 23 \text{ s}^{-1}$

Figure 4 : same as figure 3. Data were transformed to logarithmic sampling and logarithmic filtering. A fit with two exponentials is shown superimposed on the data.



CD at 286 nm
 0.3 mg/mL ACBP
 10 shots

Figure 5 : CD recordings at 286 nm. Cuvette was TC-100/10 (light path 10 mm)