Application Note - Chirascan Stopped-Flow Accessory (SF.3)

Multiple Wavelength CD Kinetics- Refolding of the Protein Lysozyme

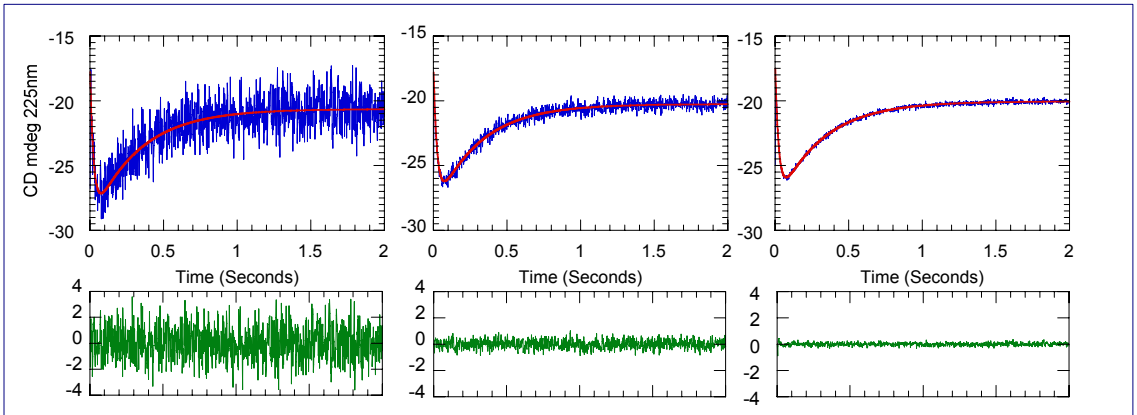
This application note demonstrates the performance of the Stopped-flow accessory (SF.3) with the Chirascan for measurement of transient CD kinetics. The method used closely follows the method published in the Applied Photophysics Pi-star application note 3 allowing a direct comparison of the performance of the two instruments.

Lysozyme refolding

Hen egg white lysozyme refolding occurs through well defined steps[1], and is fairly typical of a protein refolding reaction. This reaction was used to demonstrate the performance of the SF.3 with the Chirascan in a typical rapid kinetic protein folding reaction.

Single wavelength CD Kinetics

Using the SF.3 with the Chirascan, unfolded Lysozyme (2.2mg/mL) in 6M guanidine hydrochloride in a 250µL drive syringe was refolded by dilution with 10mM pH7 phosphate buffer (10mM) from a 2.5mL drive syringe (1:10 ratio) to final concentrations of 200µg/mL lysozyme and 0.545M guanidine hydrochloride. The total volume required per shot was 250µL utilising 50µg of protein per acquisition. The effect of averaging multiple traces to reduce random noise and increase fit confidence is shown in Figure 1. The SF.3 with Chirascan was able to produce data that could be fitted from a single acquisition. The high performance of the Chirascan minimises the amount of sample required for CD-kinetic experiments.



One acquisition

Analysis of two exponetial fit.
a_1. exp(-k_1.x) + a_2.exp(-k_2.x)+c

Parameter	Value	Error
c mdeg	-20.64	0.07
a_1 mdeg	12.72	0.97
k_1 s^{-1}	**43**	**5.593**
a_2 mdeg	-8.67	0.3807
k_2 s^{-1}	**3.133**	**0.1877**

Average of 16 acquisition

Analysis of two exponetial fit.
a_1. exp(-k_1.x) + a_2.exp(-k_2.x)+c

Parameter	Value	Error
c mdeg	-20.26	0.01708
a_1 mdeg	11.62	0.2411
k_1 s^{-1}	**41.1**	**1.502**
a_2 mdeg	-8.29	0.1056
k_2 s^{-1}	**3.306**	**0.05565**

Average of 128 acquisition

Analysis of two exponetial fit.
a_1. exp(-k_1.x) + a_2.exp(-k_2.x)+c

Parameter	Value	Error
c mdeg	-20.05	0.006288
a_1 mdeg	11.52	0.08841
k_1 s^{-1}	**41.84**	**0.56**
a_2 mdeg	-8.10	0.03688
k_2 s^{-1}	**3.231**	**0.01989**

Figure 1 CD kinetic traces and 2 exponential curve fits at 225nm during the refolding of lysozyme, showing good correlation of the fitted rate constants for 1, 16 and 128 averaged traces.

Multiple Wavelength CD Kinetics

Global analysis of multple wavelength kinetic data sets yields more robust kinetic parameters than may be achieved from single wavelength data, as the proposed kinetic model must satisfy the experimental data across the whole spectral range. The refolding of lysozyme was investigated using the SpectraKinetic acquisition mode of the SF.3 on the Chirascan, ie an automated series of stopped-flow drives across a user specified wavelength range. Kinetic traces (average of 16 repeat acquisitions) were collected at 2nm intervals between 211 and 247nm. Two traces at 217 and 227nm are shown in the top left of Figure 2.

The complete SpectraKinetic data set was analysed using the Pro-K global analysis software. The data fitted well to a two-step model, A→B→C, with the resultant rate constants of k_1=43.4s^{-1} and k_2=3.42s^{-1}. The data and results are summarised in Figure 2 below.

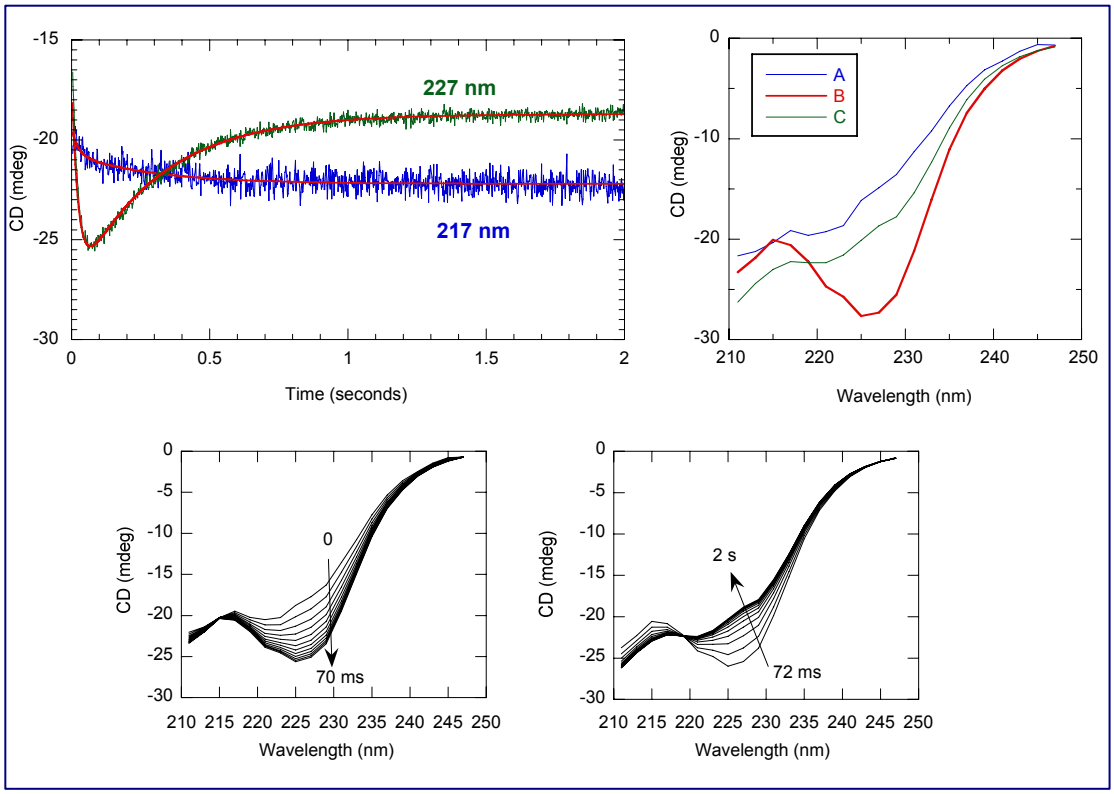


Figure 2 Multi-wavelength global analysis of the refolding of Lysozyme. Top left: 2 selected wavelengths showing the fit to the model A→B→C in red. Top right: The calculated spectra of the three species modelled in the global analysis. Bottom: the 2 spectral changes observed during the reaction.

References

[1] Hooke *et al.*, Biochemistry, 1994, **33**, 5876.

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