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Related Links

Products
Products at a glance
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Applications
Applications Overview
Automated Circular Dichroism Protein stability
Pharmacokinetics
Protein Folding
Protein Structure
Biochemical Kinetics
Chemical Kinetics

Techniques
Techniques Overview
Circular Dichroism
Dynamic Multi-mode Spectroscopy
Stopped-Flow
Laser flash
Global Analysis

References
Product References

Spectroscopy Article
"Structure and Thermodynamics of a Monoclonal Antibody Biotherapeutic in Different Formulations"

Protein Folding

Studies using Stopped-flow Fluorescence Kinetic Spectroscopy with Steady-state and Stopped-flow Circular Dichroism Spectroscopy.

Protein folding is the ultimate process where the information contained in genes is transformed into the final functional unit, an active folded protein from the unfolded primary amino acid sequence.

Misfolded proteins almost always fail to function correctly. Also excess misfolded protein can accumulate and interfere with the functioning of the cell. Consequently misfolded proteins are a feature of a large number of diseases, including Alzheimer's, Creutzfeldt-Jakob disease (CJD), cystic fibrosis, and many cancers. Understanding the difference in the folding process can bring insight into the causes of these diseases at the molecular level.

In many protein production environments, from small scale research applications to large industrial scale bio-pharmaceutical production, it is important to ensure that an expressed and purified protein is correctly folded. Comparison of circular dichroism (CD) spectra, using the Chirascan CD spectrophotometer, a particularly powerful tool for this application.

Understanding the mechanism of protein folding is a very active research areas due to its fundamental importance to biology. The techniques of stopped-flow spectroscopy, particularly using CD and fluorescence, are used widely for this application. Below are two application notes demonstrating CD stopped-flow as a method for investigating protein folding mechanisms and kinetics.

At the bottom there are 5 references utilising APL fluorescence and CD stopped-flow systems to study protein folding. There is also one reference utilising an APL laser flash system to study ultrafast protein folding events.

Multiple Wavelength CD Kinetics — Refolding of the Protein Lysozyme

In protein research applications it is of great interest to study protein refolding as this gives information on the protein characteristics. For example, Hen egg lysozyme refolding occurs through well defined steps, and is fairly typical of a protein refolding reaction.

This application note demonstrates the performance of the Chirascan CD spectrometer equipped with the SF.3 stopped flow accessory for very fast stopped-flow circular dichroism kinetics. In particular, the quality and consistency of stopped-flow CD data sets recorded at single and multiple wavelengths on the Chirascan is demonstrated. Pro-K global analysis software is introduced as a tool for analysing multiple wavelength CD data sets.

Download the full Application Note here.

Steady-state and Kinetic CD — Refolding of Cytochrome-C

Horse heart cytochrome-C is an example of a more complex situation where refolding occurs via several intermediates including a heme misligated side pathway. The refolding process and its individual rates depend upon a number of factors including pH and refolding agent concentration.

Far-UV circular dichroism spectra and very fast stopped-flow CD kinetics are highly demanding spectroscopic techniques. In this application note where an APL CD stopped-flow instrument is

used to study horse heart cytochrome-C by acquiring steady-state and kinetic CD data in the far UV. The simultaneous acquisition of CD and fluorescence kinetic data is also demonstrated giving valuable information on the protein.

Download the full Application Note here.

Relevant References

Listed below are 5 selected reference for studies of protein folding using Applied Photophysics instruments. A complete searchable database with all references can be accessed by logging into the APL members area.

Authors	Title	Year	Keywords	Journal/Proceedings
Naomi Courtemanche and Doug Barrick	Folding thermodynamics and kinetics of the leucine-rich repeat domain of the virulence factor Internalin B [Abstract] [URL]	2008	repeat protein, leucine-rich repeat, protein folding, kinetics	PROTEIN SCI, 2008, Vol 17, pp 43-53
Xie, J.-B.; Zhou, J.-M.	Trigger Factor Assisted Folding of Green Fluorescent Protein [Abstract] [URL]	2008	Protein Folding, FRET	BIOCHEMISTRY-USA, 2008, Vol 47, Issue 1, pp 348-357
Beat Fierz, Helmut Satzger, Christopher Root, Peter Gilch, Wolfgang Zinth, and Thomas Kiefhaber	Loop formation in unfolded polypeptide chains on the picoseconds to microseconds time scale [Abstract] [URL]	2007	conformational substates , femtoseconds spectroscopy , peptide dynamics, protein folding, triplet□triplet energy transfer	PROC NAT ACAD SCI USA, 2007, Vol 104, Iss 7, pp 2163-2168
Sarah Batey and Jane Clarke	Apparent cooperativity in the folding of multidomain proteins depends on the relative rates of folding of the constituent domains [Abstract] [URL]	2006	alpha-helix, protein folding, spectrin, m value, equilibrium denaturation	PROC NAT ACAD SCI USA, 2006, Vol 103, Iss 48, pp 18113-18118
Anna Nordlund and Mikael Oliveberg	Folding of Cu/Zn superoxide dismutase suggests structural hotspots for gain of neurotoxic function in ALS: Parallels to precursors in amyloid disease [Abstract] [URL]	2006	neurodegenerative disease, protein folding, transition state	PROC NAT ACAD SCI USA, 2006, Vol 103, Iss 27, pp 10218-10223

Back to top

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