APPLICATION NOTE

NVOY & Circular Dichroism

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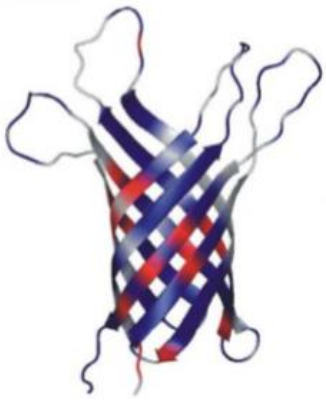
INTRODUCTION

Circular dichroism spectroscopy is an analytical technique used to estimate the secondary and tertiary structure of proteins. This technique can be used to confirm whether structure has been retained during protein processing, but is frequently adversely affected by additives such as solubility enhancers and detergents.



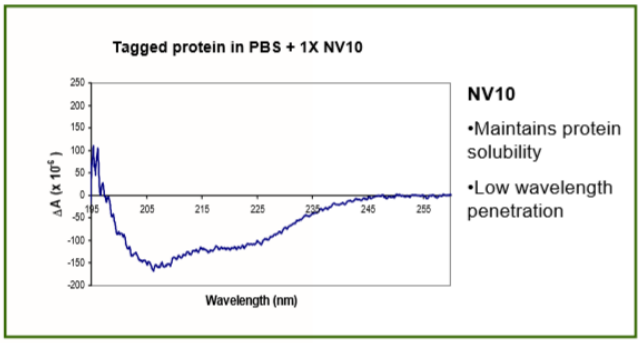
NVoy technology is a quantum leap in protein processing, production and analysis. It uses proprietary NV polymers to enhance protein solubility and stability through the formation of reversible multi-point complexes with proteins without altering their structure.

SUMMARY

NVoy technology can be used to protect, stabilize and improve the solubility of proteins by masking areas of surface exposed hydrophobicity and is directly compatible with far UV circular dichroism spectroscopy.

EXAMPLE 1

Membrane proteins are traditionally solubilised and stabilised in solution by detergents. Expedeon's NVoy technology provides an alternative strategy, and has been demonstrated to maintain the solubility of a tagged membrane protein during buffer exchange into a detergent- and denaturant-free working buffer. Far-UV circular dichroism analysis demonstrates that the membrane protein is both soluble and has retained structure during buffer exchange.

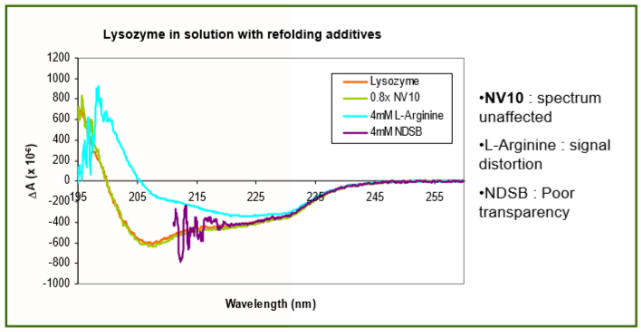


Example 1: Far UV-CD spectrum of a tagged membrane protein demonstrating retained secondary structure.

EXAMPLE 2

Solubilising additives which suppress aggregation either distort the far-UV CD signal, by contributing a signal of their own, (L-arginine), or have poor UV transparency preventing accurate signal detection (NDSB). A far-UV CD spectrum of lysozyme was collected, either in buffer alone, buffer with 0.8 x NV10, buffer containing 4 mM L-arginine or buffer containing 4 mM NDSB.

While the spectrum of lysozyme with NV10 is indistinguishable from that of lysozyme in buffer alone, the spectrum containing L-arginine has a distorted signal below 230 nm, while that containing NDSB cannot be collected below 215 nm. In each of the latter cases the resulting spectra prevent secondary structure information from being collected.



Example 2: Far UV-CD spectra of lysozyme in buffers containing common refolding additives.

Expedeon's NVoy technology is highly compatible with circular dichroism analysis, and enables the study of secondary structure in proteins with solubility problems such as membrane proteins and proteins expressed as inclusion bodies.

MATERIALS

Stabil-P.A.C. incorporating NVoy technology (Expedeon, STP)
Hen egg white lysozyme (Sigma)
L-Arginine (Fluka)
NSDB 256 (Fluka)
PBS (GIBCO)

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedeon.com
For further information see our website: www.expedeon.com