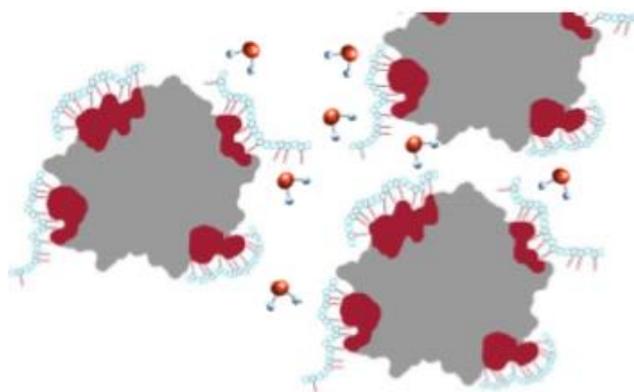


NVoy & Protein NMR

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INTRODUCTION

NMR spectroscopy is a powerful technique which enables the study of the structure, dynamics and function of proteins in solution. This technique requires maintaining high concentrations of protein over a period of several hours, and possibly at elevated temperatures, which is problematic for proteins prone to aggregation or with limited stability. Even a moderate tendency for aggregation can severely impede complex NMR projects, giving poor chemical shift dispersion and extreme line broadening of NMR resonances which can preclude the application of state-of-the-art 2D and 3D NMR techniques

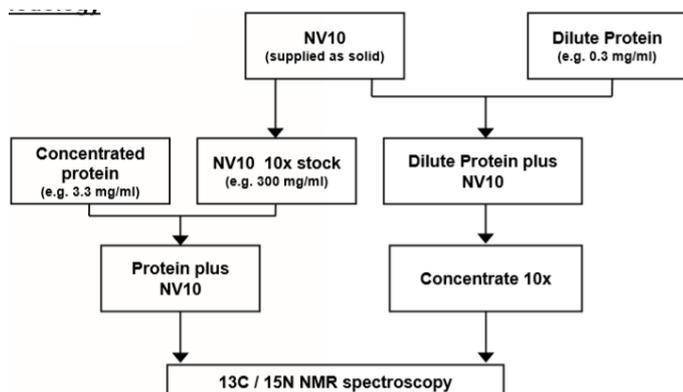


NVoy technology is a quantum leap in protein processing, production and analysis. It uses proprietary NV polymers to enhance protein solubility and stability through the formation of reversible multi-point complexes with proteins without altering their structure.

SUMMARY

NVoy technology can be used to protect, stabilise and improve the solubility of proteins by masking areas of surface exposed hydrophobicity. NVoy allows proteins to be concentrated and protected in solution, and is compatible with key NMR spectroscopy techniques using ^{13}C and ^{15}N labelled amino acids

METHODOLOGY



- NV10 can be prepared as a concentrated stock and added to a protein solution immediately prior to use.
- NV10 will co-concentrate with the protein of interest. As such the chosen protein:NV10 ratio is maintained during concentration and buffer exchange.

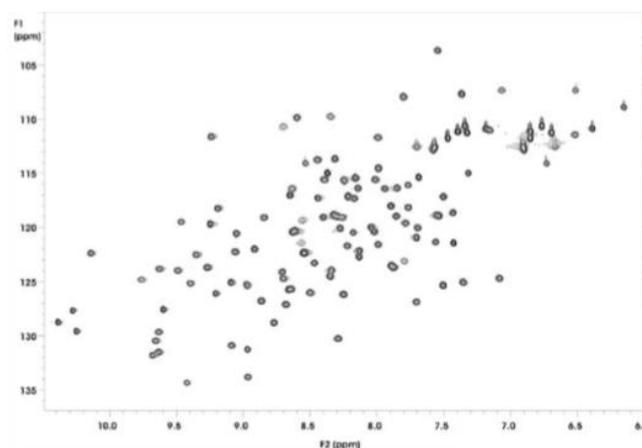
MATERIALS

Stabil-PAC Expedeon Product Code STP)

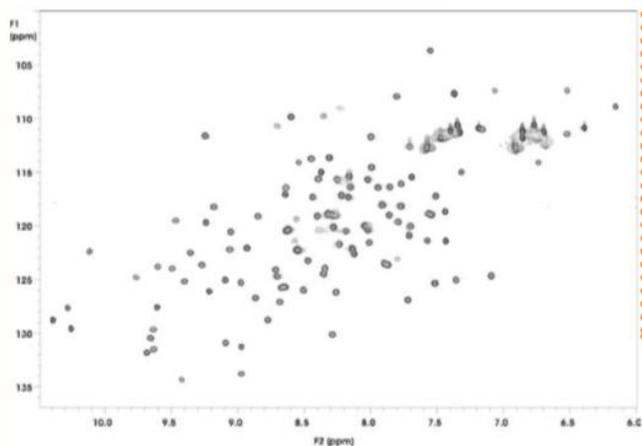
Varian Inova Spectrometer, 800 MHz

NMR spectroscopy Performed by: Dr A. Pastore & Dr L. Masino, NIMR, London UK.

CyaY is a small iron binding protein which is a bacterial ortholog of the human mitochondrial protein Frataxin. The loss of Frataxin is associated with the neurodegenerative disorder Friedreich's ataxia. Functional studies of this protein have utilised ^{15}N -HSQC NMR spectroscopy to clarify details of the iron-binding sites. ^{15}N -HSQC is a two-dimensional NMR technique which detects correlations between the nitrogen (^{15}N) atom of an NH_x group with the directly attached proton (^1H). Each amino acid residue, except proline, gives one signal that corresponds to the N-H of the amide group, with additional contributions from NH_x sidegroups depending on the conditions used. ^{15}N -HSQC NMR spectra of CyaY were recorded in the absence or presence of NV10.



^1H - ^{15}N -HSQC spectrum recorded at 27 °C on an 800 MHz Varian Inova Spectrometer in the absence of NV10. 3.4 mg/ml CyaY 20 mM Tris.HCl pH 7.0 50 mM NaCl



¹H-¹⁵N-HSQC spectrum recorded at 27 °C on an 800 MHz Varian Inova Spectrometer in the presence of NV10. 3.1 mg/ml CyaY 32.2 mg/ml NV10 20 mM Tris.HCl pH 7.0 50 mM NaCl.

CONCLUSION

Expedeon's NV10 polymer is invisible in ¹⁵N-HSQC NMR and shows no effect on the secondary structure of CyaY. NVoy technology enables the preparation of stable, concentrated protein solutions suitable for NMR applications using ¹⁵N and ¹³C labelled amino acids.

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