APPLICATION NOTE

NVOY & Mass spectrometry

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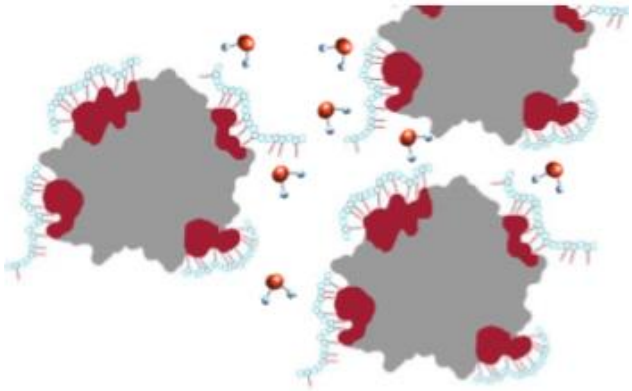
INTRODUCTION

Mass spectrometry is a frequently used analytical technique to accurately determine protein mass and structure. However, this technique requires relatively high concentrations of protein (>5 µM), which is problematic in particular for larger proteins and proteins prone to aggregation. Moreover, since mass spec does not readily tolerate commonly used protein stabilisers and solubilisers, e.g. detergents, glycerol and arginine, it is often difficult to obtain a suitably concentrated protein solution.

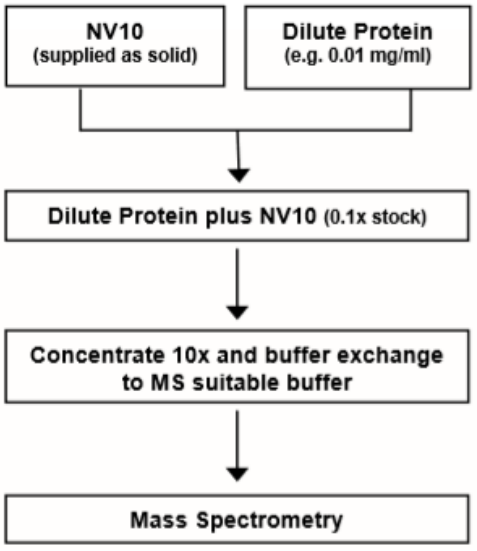


NVoy technology is a quantum leap in protein processing, production and analysis. It uses proprietary NV polymers to enhance protein solubility and stability through the formation of reversible multi-point complexes with proteins without altering their structure.

SUMMARY

NVoy technology can be used to protect, stabilise and improve the solubility of proteins by masking areas of surface exposed hydrophobicity thus preventing aggregation through hydrophobic interaction, allowing aggregation prone proteins to be concentrated to a level suitable for mass spectrometry.

Methodology



NV10 will co-concentrate with your protein of interest. As such the chosen proteinNV10 ratio is maintained during concentration and buffer exchange

Tolerance Chart

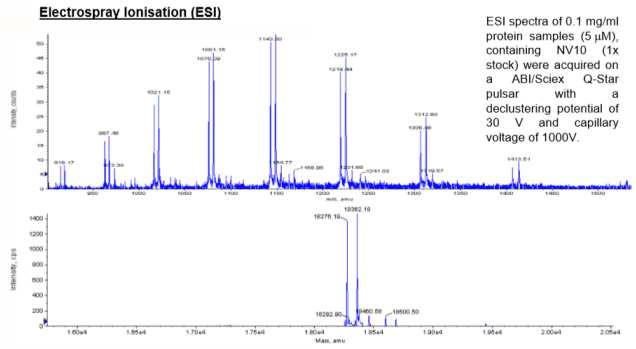
Surfactant	MALDI (wt.%)	ESI (wt.%)	Reference
Glycerol	1.2	n.a.	1
n-Hexyl-glucoside	n.a.	0.1	2
n-Octyl sucrose	n.a.	0.1	2
n-Dodecyl-glucoside	n.a.	0.1	2
PEG2000	0.1	n.a.	1
CHAPS	0.01	<0.1	1 & 2
Triton-X-100	0.1	<0.1	1 & 2
Zwittergent,3-16	0.1	n.a.	1
Tween20	0.1	<0.1	1 & 2
SDS	0.01	0.01	1 & 2
CTAB	n.a.	<0.1	2
NV10	**0.25**	**0.25**	**Unpublished Data**

1. Henzel, W. J. and Stults, J. T. Current Protocols in Protein Science (1996) 16.2.1-16.2.11. Edited by Coligan, J. E. 2. Ogorzalek, R. R. et al. Methods in Molecular Biology (1996) 61, 141-160. Edited by Chapman J. R.

MATERIALS

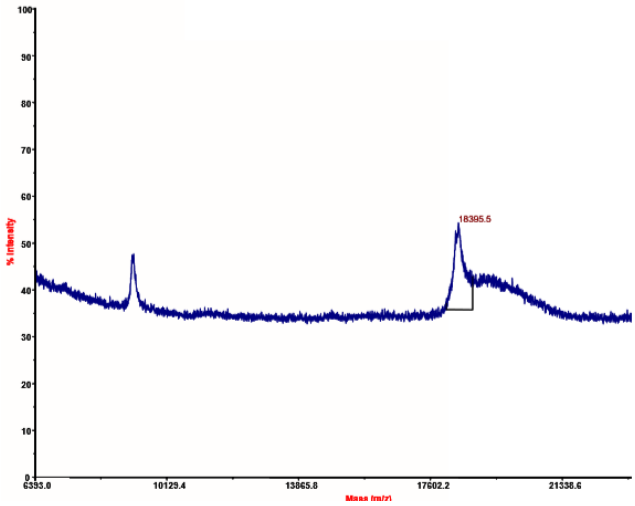
Stabil-P.A.C. (Expedeon, STP)
Spec (ESI) β-Lactoglobulin A/B (Sigma, L-0130
ABI/Sciex Q-Star Pulsar Mass (MALDI)
ABI 4700 Proteomics Analyser

RESULTS



The protein ionised well in the presence of NV10 (1x stock). Deconvolution of the spectrum gives two protein species corresponding to the masses of β-Lactoglobulin A and β-Lactoglobulin B.

Matrix Assisted Laser Desorption Ionisation (MALDI)



Maldi MS spectra of 0.1 mg/ml protein samples (5 µM), containing NV10 (1x stock) were acquired on an ABI 4700 Proteomics Analyser with TOF/TOF optics. The mass range m/z 6000 - 23000 was analysed.

The protein sample was run in an α-cyano-4-hydroxycinnamic acid matrix (10 mg/ml in 50% MeOH:water).

The aqueous protein sample was mixed in a 1 to 1 ratio with the matrix solution.

The protein ionised well in the presence of NV10 (1x stock) and a well-defined protein peak was observed at 18.4 kDa, which corresponds to the mass of β-Lactoglobulin.
Expedeon's NVoy technology enables the preparation of concentrated protein solutions suitable for direct application in mass spectrometers using either electrospray ionisation (ESI) or matrix assisted desorption ionisation (MALDI)

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