

**OF1 Conformational evidence for the effects of protein stabilization on charge-state distributions using ion-mobility mass spectrometry**

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Travelling wave IMMS separated unfolded and folded conformations of the DnaB-N protein

Charge state distributions of proteins in ESI mass spectra vary depending on the degree of unfolding. Previously, ESI mass spectrometry was used to compare charge state distributions of the N-terminal domain of DnaB from *Escherichia coli* (DnaB-N) with those of the same protein which had been stabilized via *in vivo* cyclization (amide bond formation between the N- and C-termini) [1]. The use of a stabilized cyclized protein enabled the effects of the ionization process on charge state distribution to be observed without altering solution conditions.

Ion-mobility mass spectrometry (IMMS) may be used to supply information regarding protein unfolding, as the cross-sectional areas of unfolded and folded proteins are quite distinct.

In the current work, a travelling wave IMMS (Synapt HDMS™) was used to investigate the conformations of the linear and cyclized proteins. The ion-mobility drift times (related to the cross-sectional area) of the two proteins were compared at the same  $m/z$ . The linear protein showed there were two populations with distinct drift times, consistent with the presence of both folded and unfolded forms. Only one population (one drift time) was measured for the cyclized protein, indicating only the folded form was present.

[1] S.J. Watt, M.M. Sheil, J.L. Beck, P. Prosselkov, G. Otting, N.E. Dixon. *J. Am. Soc. Mass Spectrom.* 2007, 18: 1605-1611.