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Unique insights to intrinsically disordered proteins provided by ion mobility mass spectrometry

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Entire functional proteins as well as large regions of proteins lack structural elements which are resolvable via crystallography or NMR. These intrinsically disordered proteins (IDPs) or regions (IDRs) are often involved in cell regulation processes, for example in signalling hubs and as a result aberrant behaviour can cause or be representative of disease. As a consequence there is a pressing need to develop alternative structural methods for IDPs and the interactions that they may form with other proteins and/or with potential inhibitors of binding. One such method is ion mobility mass spectrometry (IM–MS) coupled with careful application of electrospray ionisation, which shows great promise as a technique that does not 'care' if a protein is structured or not. We highlight recent work which has employed IM–MS to study conformational heterogeneity in disordered proteins, and discuss the opportunities, as well as the challenges of this approach.

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Introduction

Intrinsically disordered proteins defined as possessing function from a disordered structure can perhaps be considered as sitting on one end of a structure to disorder continuum [1–3]. It is convenient to define five different classifications of protein structure from fully structured to unstructured (Figure 1).

The most unusual case is that shown by proteins which have a narrow spread in the net number of charged groups accompanied by a large spread of collision cross section values as shown here for the example of the linked folded domain protein. We hypothesise that this behaviour would occur from fuzzy complexes.

With increased recognition of the existence and importance of IDPs [17] there are reports which predict between 30% and 70% of all eukaryotic proteins are either IDPs or contain intrinsically disordered regions (IDRs) [18–20]. Recent experimental work by Picotti *et al.* supports the lower end of this spread [21], regardless, for IDPs the central dogma that relates one structure to a given function no longer holds and it is more appropriate to consider IDPs as structural ensembles with promiscuous functions. IDPs have been shown to fold upon binding [22,23] and perhaps more generally to form fuzzy complexes with a myriad of sampled interactions and hence active conformational states [24,25]. Since IDPs are implicated in many diseases, including neurodegenerative diseases and cancer, it is important to gain better understanding of their structure(s) function(s) relationships. The lack of resolvable structure reduces the effectiveness of crystallography for this class of protein, and IDPs are more commonly examined with NMR, SAXS, and other spectroscopies [26••]. Ion mobility spectrometry with mass spectrometry has also been applied to the study of IDPs and shows some promise with its capability to resolve structure and stoichiometry, and also because it reports the net number of charges on the molecule under examination.

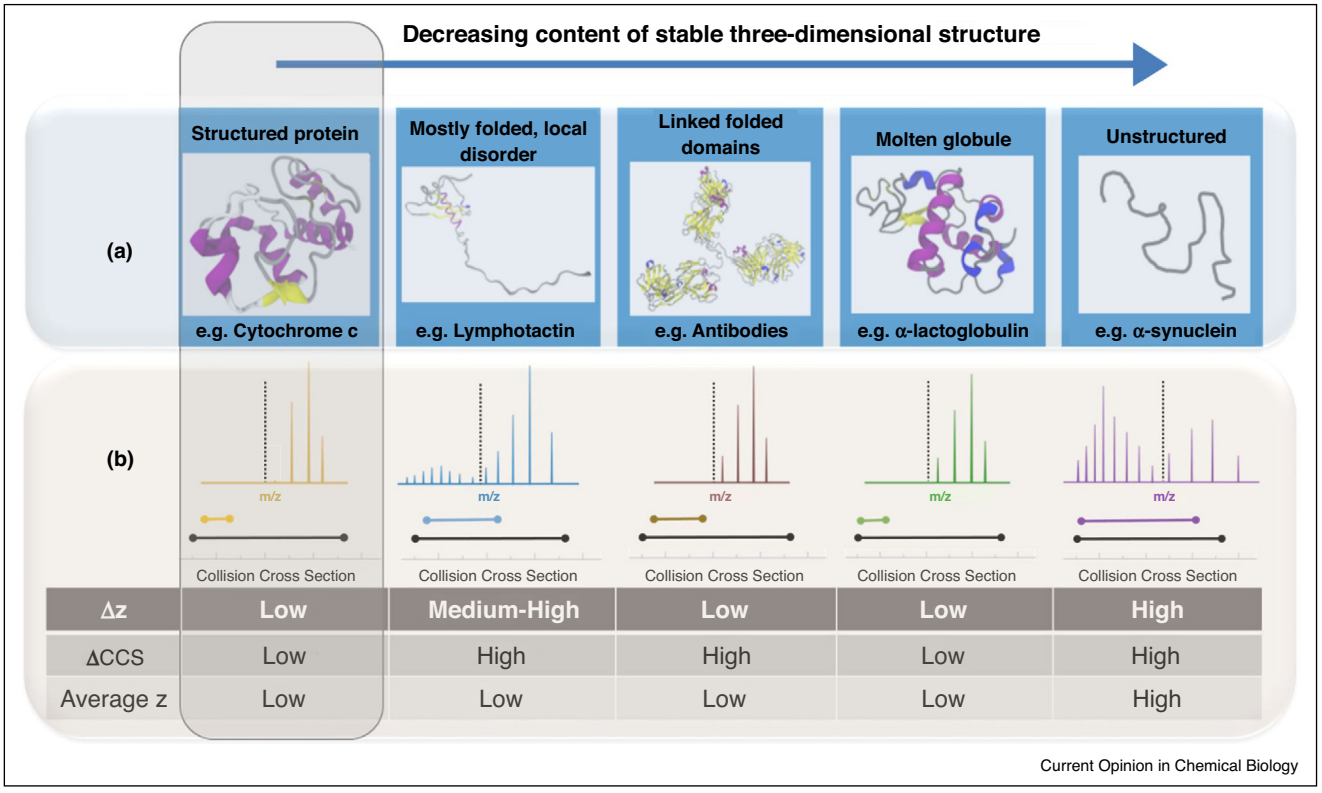
Ion mobility mass spectrometry (IM–MS)

An IM–MS instrument carries out five basic operations: sample introduction, compound ionisation, ion mobility separation, mass separation, and ion detection. There are a variety of methods employed to form gas-phase ions for IM–MS analysis [27], but for proteins the preeminent method is electrospray ionisation (ESI) [28] and in order to best preserve non covalent interactions from solution the related technique of nano-ESI [29–31] is most commonly used. Four methods are commonly available for of ion mobility separation: aspiration ion mobility spectrometry (AIMS) [32], differential-mobility spectrometry (DMS) also known as field-asymmetric waveform ion mobility spectrometry (FAIMS) [33], drift-tube ion mobility spectrometry (DTIMS) [27] and travelling-wave ion mobility spectrometry (TWIMS) [34]. For the purpose of this review we will only consider the latter two since they have had the largest application for IDPs [26••,35•–39••].

IM–MS to study amyloid forming IDPs

Over the past 15 years a number of studies have used IM–MS to examine the early stages of the aggregation of peptides which go on to form amyloid fibrils [40–43].

Figure 1



Progression of protein structure from stable three dimensional structure to unstructured and the corresponding ion mobility mass spectrometry observables. **(a)** We have adapted a representation first shown by Dyson and Wright [4] to highlight the continuum of protein structure from ordered to disordered. In our version we illustrate this with proteins that have been examined with mass spectrometry [5–9]. From left to right the exemplar proteins are the electron transport protein cytochrome *c* (PDB: 3CYT), readily able to form a crystal structure; the metamorphic protein Lymphotactin (PDB: 1J8I) for which NMR provides some information regarding the location and structure of the disordered N and C termini present in both the monomeric and dimeric forms [10]; a monoclonal human antibody — immunoglobulin G (PDB: 1IGT) where structured regions rich in β-sheet are connected by flexible disordered linking regions [8,11]; α-lactoglobulin (PDB: 1HMK) which is a molten globule consisting of a compact form with mobile side chains. This state was first reported for cytochrome *c* [12] highlighting the progression of structure that can be adopted by proteins; and finally α-synuclein a highly disordered amyloid forming protein associated with the progression of Parkinson's Disease [13,14]. **(b)** Schematic to show the read out from mass spectrometry following electrospray ionisation under gentle conditions that act to preserve solution structure(s). The charge state distributions for each class of protein are shown along with the corresponding spread in collision cross section values (coloured lines) and the predicted possible range of collision cross section values (black lines) based on a toy model described previously which predicts the most compact and most extended CCS values for a given linear protein sequence [15]. The black dotted line on the schematic mass spectra shows the upper value for the net charge (*z*) on the protein that would correspond to a globular/folded form according to de la Mora [16], it can be seen for structured proteins and molten globules the mass spectrometry data falls below this upper limit, whereas for partially or completely disordered proteins much of the signal intensity is above this line indicative of extended forms.

Bowers [42,44–46], and Ashcroft [43,47,48], amongst the most prolific authors. Whilst amyloid forming peptides and proteins are often also categorised as IDPs the focus of these excellent studies has principally been to understand the assembly mechanisms and to develop MS methods that can help to screen for inhibitors of assembly. Several groups have focused more on the use of mass spectrometry to study the intrinsic structure of IDPs for example, Grandori *et al.* [49] who used experiment and theory to explain the charge state distributions (CSD) obtained following ESI–MS of disordered proteins. They conclude whilst proton transfer does occur during electrospray and/or drift, that structural rearrangements associated with this do not have a substantial effect on structure. From this is it suggested that the CSD does indeed provide a convenient 'read out' of the solution phase form as indicated in Figure 1b.

α-Synuclein has been extensively studied with mass spectrometry as well as other techniques due to its significance in the progression of Parkinson's Disease as well as its use as an exemplary IDP for biophysical studies [44,50,51]. In 2015 Phillips *et al.* [9] used hybrid MS approaches including IM–MS to reveal the conformational flexibility of α-synuclein [9]. These experiments revealed the presence of multiple conformational

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