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Mass spectrometry beyond the native state

Shane A Chandler and Justin LP Benesch

Native mass spectrometry allows the study of proteins by probing in vacuum the interactions they form in solution. It is a uniquely useful approach for structural biology and biophysics due to the high resolution of separation it affords, allowing the concomitant interrogation of multiple protein components with high mass accuracy. At its most basic, native mass spectrometry reports the mass of intact proteins and the assemblies they form in solution. However, the opportunities for more detailed characterisation are extensive, enabled by the exquisite control of ion motion that is possible in vacuum. Here we describe recent developments in mass spectrometry approaches to the structural interrogation of proteins both in, and beyond, their native state.

Address
Department of Chemistry, Physical & Theoretical Chemistry Laboratory, University of Oxford, Oxford OX1 3QZ, UK

Corresponding author: Benesch, Justin LP (justin.benesch@chem.ox.ac.uk)

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Introduction

Native mass spectrometry (MS) is the study of proteins and other biomolecules in the vacuum of a mass spectrometer, having initially maintained their noncovalent interactions upon transferral from solution [1,2]. A variety of experimental data and simulations have shown that the native state of proteins and their complexes can persist on the millisecond timescale of MS experiments [3,4]. This provides ample opportunity for interrogation of not just native structure, but also the free-energy landscape accessible to the protein [5,6]. This can be viewed in terms of a canonical protein (un)folding funnel, where protein ions can populate states differing in their atomic coordinates and energy (Figure 1). While the native states of proteins are relatively accessible to structural biologists and biophysicists, probing states populated only to low levels at equilibrium, or fleetingly during assembly and folding, remains extremely challenging [7]. Here we discuss how and why MS-based experiments can contribute to this endeavour.

We first consider how the protein folding funnel can be explored within the mass spectrometer, discussing the role played by protein charge state in populating different minima. We evidence how these represent starting points for exploring higher levels of the protein folding funnel by activating ions within the mass spectrometer. In the second part of this review, we describe emerging gas-phase labelling, spectroscopy, and imaging methods that are being integrated with native MS. These approaches promise to provide unprecedented detail regarding the structure of proteins in vacuum, including during excursions from the native state.

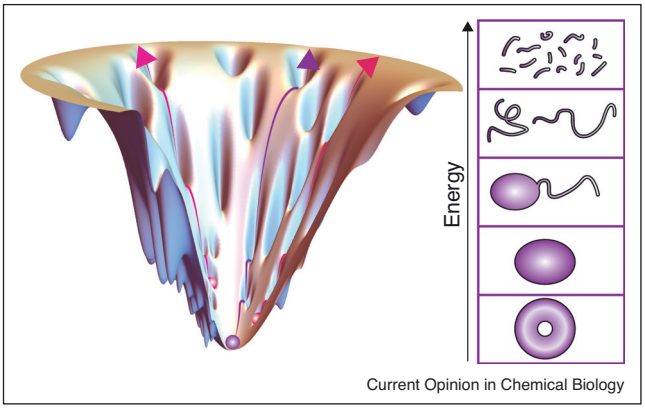
Coulombic exploration of the free energy landscape

Protein ions generated by (nano)electrospray ionisation each populate a distribution of charge states. Oftentimes the gross structure, evidenced by collision cross-section (CCS) values obtained from ion mobility (IM) measurements, appears to be unaffected by this charge state variation [8]. However, in cases where the range of charge states populated is broad relative to the average, or when the protein has an intrinsic tendency towards disorder, significant variations in CCS are observed [9,10]. This demonstrates how, as in solution [11], different charge states of a protein in vacuum correspond to different free-energy minima in the free energy landscape (Figure 1a).

To investigate how global protein structure depends on charge state, a number of recent studies have performed IM-MS on protein ions that are selected within the mass spectrometer. Charge manipulation has been performed via ion-ion collisions with a reaction partner of opposite polarity, most commonly performed by abstraction of a proton [12,13], or transfer of an electron [14,15]. A number of studies have shown that reduction of charge on native conformers of ubiquitin and cytochrome C results in a step-wise compaction [13–16,17•,18]. In other cases, increases in CCS upon charge reduction are observed [14,19]. This demonstrates the impact of charge on the initial position on the folding funnel, and that other local minima can be occupied by charge modulation. These minima therefore represent alternative starting points for exploration of the conformational landscape.

In order to navigate the protein folding funnel, the ions can be activated within the mass spectrometer (Figure 1),

Figure 1



Proteins exist on a rugged free-energy landscape in vacuum [5] that can be framed in terms of a protein folding funnel with the native state at the global minimum [7]. Different charge states of the protein can be considered as other minima on this surface, and thereby represent alternative starting points for exploration of the funnel. This can be achieved by gas-phase activation of the protein ions [20], enabling access to different unfolded states. At high levels of activation, dissociation of non-covalently bound units and even fragmentation of the polypeptide backbone can occur, providing information as to protein composition and identity.

typically by energetic collisions with a bath gas. By varying the amount of energy supplied, the types of bonds broken and level of structural information obtained can be controlled. At a low level of gas-phase activation, proteins first undergo structural rearrangements and unfolding due to breakage of intramolecular non-covalent bonds [20]. IM-MS measurements have shown that this occurs via a number of discrete transitions in CCS (Figure 2a), diagnostic of the trajectory taken by the protein as it escapes the folding funnel. The structural evolution of a protein depends strongly on its charge state [15,17•,21], and tandem IM-MS experiments reveal complex conformational inter-dependencies [22], consistent with the view of different minima in the folding funnel being populated and leading to alternative pathways out (Figure 1).

As a consequence, the transitions observed and the energies at which they occur represent a characteristic signature for the protein charge state being investigated. This can be exploited as a fingerprint for comparison, enabling, for instance, distinction between different antibody structures and interactions [23–26]. Unfolding has also been actively pursued as a measure of ligand-stabilisation of protein structure [27], revealing measures consistent with solution data [28], as well as reflecting interactions that are strengthened in vacuum [29•]. The number of unfolding transitions appears to reflect the number of independent structural domains seen in solution [30]. However, assignment of the transitions observed to individual regions of the protein is difficult. One way to address this is to use specific small-molecule probes, and correlate their release upon activation with the transitions in CCS (Figure 2b) [31••]. Potentially a more general strategy will come from performing electron-mediated fragmentation of the protein during its unfolding pathway [32].

Increasing activation further results in the dissociation of non-covalently attached protein subunits or other ligands. In general, dissociation of multimeric assemblies appears to proceed via the expulsion of single, highly charged subunits [20]. The identity of the dissociation products can yield compositional information on the complex, while the asymmetric partitioning of charge between products can be exploited to mine spectra not resolvable by MS alone [33–35]. However, fast activation, in particular by collision with a surface, can lead to information regarding the assembly and architecture of the complex [36,37]. This can be rationalised by the activation allowing access to the higher reaches of the protein energy landscape without transitioning through lower-energy intermediates via polypeptide unfolding [38]. Remarkably, this information appears to be retained on the timescale of seconds [39].

At the highest energies accessible in the mass spectrometer, covalent fragmentation of the polypeptide backbone can be induced (Figure 2a) [20]. This means that 'top-down' proteomics is possible from the starting point of a native protein assembly [40]. Exploiting this capability enables the attribution of properties of individual protein assemblies to differences in their primary sequence [41]. A particularly attractive means for reducing native proteins into peptide fragments comes from irradiation of a protein complex using UV photons [42]. This has been shown to operate on a fast timescale, providing comprehensive sequence coverage [43,44•], while retaining non-covalent interactions [43] and promoting a more symmetric dissociation pathway [45]. Interestingly, regions of enhanced fragmentation correlate reasonably well with solvent exposure in solution, suggesting that aspects of the native fold are retained (Figure 2c) [46••]. Similar correlations have also been found when using electron-mediated fragmentation [32,47].

Structural interrogation of native and activated states

Aside from mass and charge, the information most frequently obtained from native MS experiments is CCS, through the implementation of an orthogonal IM dimension [48]. While the CCS contains significant information on the global shape of proteins [49,50], and can be used to aid structural modelling [51,52], complementary approaches are needed to probe structure on a local level. An attractive strategy is to perform labelling