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Bouncing off the walls: excited protein complexes tell their story

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"Native" mass spectrometry has become a valuable tool for structural biology. Modified instruments which are capable of surface-induced dissociation (Quintyn et al., 2015) allow dissection of protein complexes in a way which is reminiscent of their native topology and architecture.

It seems ironic that when using mass spectrometry for the analysis of "native" protein complexes, we take great care to preserve non-covalent interactions intact during their transfer from solution – just so that we can then apply novel, sophisticated methods in order to fragment them again in the gas phase. Advances such as the surface-induced dissociation (SID) method described by Quintyn et al. on page XXX however are not as contradictory as they first sound, given that the controlled disassembly of macromolecular complexes gives us an opportunity to understand how the specific parts work together. The ability to gently release proteins from the native-like environment of a buffered, aqueous solution is owed to the use of nano-electrospray ionization, in combination with careful tuning of the interface and mass analyzer of the instrument (in particular acceleration voltages and gas pressures). With non-denaturing conditions for m/z analysis thus established, this approach has shown to be capable of keeping particles together as large and complex as intact viruses, ribosomes or ATPase (1), allowing to measure their mass and (in combination with ion mobility) also their overall size and shape.

Why then would we want to disassemble these carefully preserved complexes again in the gas phase? Many particularly challenging questions regarding biomolecular structure and function target dynamic aspects such as conformational transitions or assembly pathways of subunits. This can quickly lead to a situation where different protein conformations and/or assembly states co-exist in the sample. Such ensembles can be particularly difficult to analyze with methods which either average across populations or favor one type of species, e.g. a particularly ordered or symmetrical one. Mass spectrometry does not suffer from such limitations, and smart dissociation techniques can greatly facilitate the interpretation of subunit composition and stoichiometry, as well as delivering information on the relative stability of noncovalent interactions.

Native MS is now increasingly applied in structural biology – from ligand binding studies to assembly pathways of dynamic and heterogeneous complexes, and from the characterization of structural disorder to integral membrane proteins in a detergent or lipid environment. Its ability to simultaneously detect dynamic, heterogeneous protein ensembles makes it a powerful addition to traditional structural techniques such as X-ray crystallography, NMR spectroscopy and electron microscopy. Additional advantages of MS include the relatively low sample consumption and wide range of masses and sizes that can be studied. As such, native mass spectrometry is often used to study large non-covalent protein complexes. The observed mass of the complex alone might be enough to solve the subunit stoichiometry, but provides no further structural insight. To obtain information about the subunit topology, complexes can be destabilized in solution by chaotropes such as organic solvents, thereby producing a range of subcomplexes which can be used to generate a connectivity map (2). Combining such information with low-resolution data obtained from e.g. ion mobility MS, electron microscopy or SAXS in an integrated structural approach, with the help of molecular modeling, often succeeds in constructing a model of the complex (3).

Collision-Induced Dissociation (CID) is the fragmentation "workhorse" in mass spectrometers and used very widely for MS/MS sequencing of tryptic peptides. In native MS, it can be employed for top-down sequencing, subunit dissociation of protein complexes as well as following protein unfolding in the gas-phase (**figure 1**) (4). In CID, ions accelerated by an electric field collide with inert gas. A large protein complex with 5 nm cross section will for example undergo more than 10^4 collisions in a typical collision cell (3 - 5 x 10^{-2} mbar, 10 cm length) in a quadrupole-time of flight type instrument, thus building up internal energy in an ergodic (quasi-thermal) process. The asymmetric dissociation of an exposed subunit which takes the least activation to unfold is ultimately charge-driven (5), and as such the structural information that CID provides is limited. The low number of charges remaining on the residual complex often also prevents further gas-phase experiments.

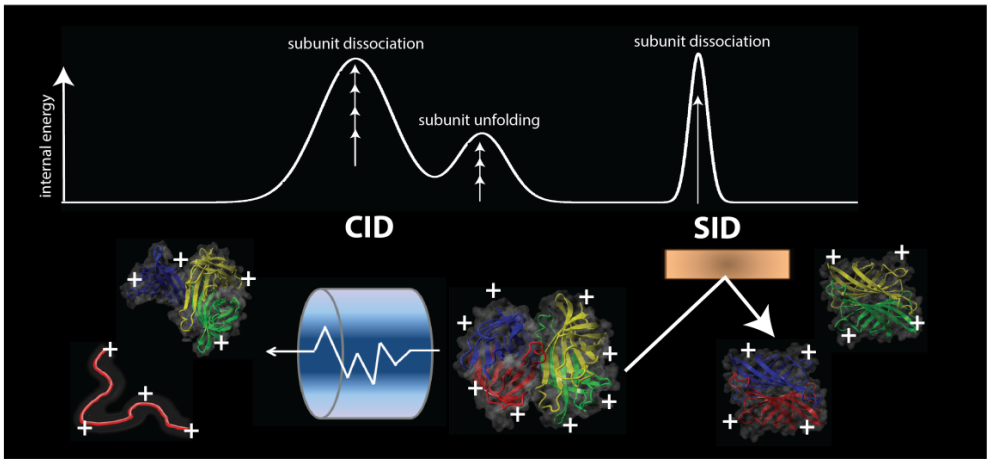

Figure 1. *Comparison of Collision Induced Dissociation (CID) and Surface Induced Dissociation (SID) of tetrameric avidin. CID is a multi-step fragmentation approach where the internal energy of the ions is increased by collisions with an inert gas (often argon). As this "heating" of the ions occurs rather slowly, the energy can dissipate. Dissociation proceeds via unfolding of typically the smallest subunit which is exposed in the complex, which in turn leads to asymmetric charge partitioning. Once there are no interactions left between the unfolded monomer and the remaining compact complex, the subunit dissociates, taking roughly half of the total charge with it, according to its share of the total exposed surface. Due to the nature of this process, the structural information that is obtained from CID experiments is rather limited. In comparison, SID is a near instantaneous process. In SID the ions are collided with a surface target (which has in theory an unlimited mass), where the energy transfer is limited to a single event. This results in nearly symmetrical dissociation, both in terms of charge and topology. Quintyn et al. show that the dissociation of complexes is related to the area of their interacting surfaces, thus resembling their assembly pathways.*

The need for structurally more informative fragmentation data of protein complexes has been largely addressed by the application of Surface-Induced Dissociation (SID). The approach has come a long way, initially being used to study the fragmentation of small molecules and peptides (6), but recently demonstrating to efficiently fragment the 800 kDa GroEL 14-mer (7). These developments have largely been made possible by efforts of Vicki Wysocki and coworkers. Although the processes which occur during the short interaction between the charged protein complex and the surface are not fully understood yet, the strength of SID lies in the fact that the energy transfer is very rapid (figure 1), seemingly not giving the ions enough time to change their structure substantially. As a consequence virtually no unfolding or charge redistribution are observed, resulting in dissociation products that are reflective of the topology of the complex studied (8). In the work by Quintyn et al. on page XXX, the authors study the dissociation pathways of three tetrameric protein complexes with D2 symmetry, with and without additional ligands bound. They show that SID, unlike CID, proceeds via cleavage of the smallest dimer-dimer interface. The method can also shed light on the structural effect of ligands, depending on the site and their mode of binding. In combination with ion mobility analysis, this work presents the most convincing evidence yet for the power of the direct MS/MS approach taken by the authors, for the investigation of complex stability and architecture by SID.

Mass spectrometry approaches are rapidly evolving to provide structural information on a proteome-wide scale. This was recently demonstrated in an impressive study using limited digestion, which could detect altered digestion patterns in a large number of yeast proteins, due to changes in their conformational or assembly state (9). At the intact protein level, the relation between the exact proteoform (encompassing sequence variations, post-translational modifications and other forms of editing) and the propensity of a protein to form alternative complexes remains a challenge which would lend itself to study by SID (top-down) or, on a large scale, the limited digestion approach (middle-down). Top-down experiments using other alternative fragmentation techniques, such as UV photodissociation (UV-PD) or electron capture or transfer dissociation (ECD or ETD), are also rapidly gaining interest for the dissociation of large native complexes, given that they can also deliver information on the exposed protein surface (10). Taken together, the different types of mass spectrometry data combined with ion mobility and SID dissociation under similar conditions provide a powerful tool to investigate the stability and assembly pathway of dynamic protein complexes.

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