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Protein Complexes: Breaking Up is Hard to Do Well

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Abstract

Mass spectrometry of protein assemblies reveals size and stoichiometry. In this issue of Structure, Hall et al. (2013) demonstrate that gas-phase dissociations can recapitulate solution structure for complexes with few intersubunit salt bridges, high charge density, inflexible subunits, or small intersubunit interfaces.

Proteins interact with other proteins and molecules as they function in biology. Highly specific and dynamic associations of cognate binding partners and modular protein domains govern cell growth, differentiation, and intercellular communication. Yet, compared to characterizing these components of life individually, elucidating structures of their *assemblies* is more challenging, potentially hampering efforts to understand their function and modulation. Additionally, understanding the forces involved in macromolecular association and molecular recognition more thoroughly would benefit drug design efforts.

Although X-ray crystallography and NMR spectroscopy provide unrivaled high-resolution structural information, and new microscopy methods (e.g., cryoelectron microscopy) reveal impressive structural details of large protein assemblies, significantly advanced mass spectrometry (MS) has capabilities to offer structural biologists layers of insight. Mass measurements deliver information on stoichiometry of binding partners directly, even for multi-ligand hetero-complexes and molecular machines with masses well beyond 1 MDa (Heck, 2008). With electrospray ionization (ESI), MS can measure proteins and complexes from aqueous solution at near neutral pH, *i.e.*, “native” MS. Protein interactions are often sufficiently stable upon transitioning to the gas phase to support size/binding stoichiometry analyses. ESI's special gift for transforming solution-phase macromolecules into *gas-phase ionized* counterparts without disrupting covalent bonds *and* weak noncovalent interactions is key for applying MS to study protein complexes.

Throughout the history of mass spectrometry, the general concept of “breaking” molecules to derive molecular structural information has been a consistent theme. This approach has been applied to molecules small and large, e.g., sequencing peptides and identifying post-translational modifications. More recently, the strategy has been extended to protein complexes. It is hoped that fragmenting gas-phase protein complexes will yield structural

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insight into the topology of large protein assemblies. However, it is not entirely obvious that methods probing *dehydrated* proteins should yield structural information relevant to hydrated proteins, and most of the data gathered so far unsurprisingly suggests that structural fidelity and intermolecular forces are not identical for solution-phase and gas-phase complexes.

For example, a striking observation for the dissociation of gas-phase non-covalent complexes is that they tend to decompose unevenly upon collision-induced dissociation (CID), *e.g.*, streptavidin tetramers split into monomers and trimers, with half of the charge departing with the monomer (Schwartz et al., 1995). The accumulated multimer dissociation data has led to an appreciation that uneven decompositions are “typical”, argued to occur in the transition state from the unfolding of a single subunit that, in so doing, carries away the vast majority of charge and leaving behind a charged-stripped complex (Jurchen and Williams, 2003).

In the paper by Hall *et al.*, the authors attempt to find experimental conditions that can “direct dissociation through structurally informative pathways that mimic those followed in solution” for a series of protein complexes (Hall et al., 2013). The principles and the forces governing protein-protein interactions in solution are not fully understood. Electrostatic interactions such as ion pairs are important in protein folding, stability, flexibility, and function. Salt bridge networks can connect and stabilize protein subunits or join two secondary structures to form quaternary structures.

The Robinson group reports here that by increasing the ESI positive charge deposited on protein complexes, they can manipulate whether certain complexes decompose typically or atypically (Hall et al., 2013). The charge deposited on protein complexes was varied by adding different compounds to solutions dispersed by ESI. Typical methods to produce lesser-charged ions while preserving protein structure include proton transfer via gas-phase or solution-phase introduction of base. Higher charge state ions are created by spraying solutions containing other reagents, referred to as “supercharging agents” (Iavarone et al., 2001; Lomeli et al., 2010).

In contrast to unfolded monomers or small aggregates released in typical fragmentations, products of atypical dissociations are expected to be folded. Also, atypical dissociation products resemble those obtained by solution-phase disruption with weak denaturants. Thus, the authors argue that atypical dissociation comprises the preferred route for structural investigations and that for some protein complexes, especially for those with low subunit flexibility, fewer salt bridges, and smaller interfaces, solution-phase disassembly can be recapitulated in the gas phase if the complexes carry sufficient charge.

An alternative dissociation strategy, surface-induced dissociation (SID), activates ions in a single collision with a surface, in contrast to the multiple, sometimes glancing collisions that ions experience in CID (Zhou et al., 2012). Many complexes that dissociate typically by CID, decompose atypically by SID, attributed to the latter's fast deposition of energies exceeding the activation barrier for dissociation to atypical, but thermodynamically favored products. The supercharging approach of Hall *et al.* (Hall et al., 2013) provides an alternate means to access desirable, atypical products.

The collision cross section (CCS) comparisons for charge states generated with and without charge-manipulation have implications for mass spectrometry's current debate on how “supercharging” agents increase protein charge. Sterling *et al.* (Sterling et al., 2011) have argued that adding these agents to aqueous solutions increases protein charge by denaturation. An alternative view (Lomeli et al., 2010), that charge elevation can occur by interaction with very weakly basic (solution phase) reagents, is supported by measurements

here (Hall et al., 2013) showing that the high charge state ions produced by supercharging can, upon dissociation, deliver compact oligomers with collision cross-sections matching those calculated from atomic coordinates.

Such MS-based methods for characterizing proteins can augment existing knowledge for a given biochemical complex and can be integrated with data from a wide variety of methods to model and refine complex structures for biological assemblies (Alber et al., 2008).

Prior to the first x-ray crystal structure of a protein, Linus Pauling stated, “We may expect that as more precise information about the structure of these molecules is obtained in the future, a more penetrating understanding of biological reactions will develop, and that this understanding will lead to great progress in the fields of biology and medicine (Pauling, 1948).” Although much progress has occurred in the past 50+ years, Pauling's statement applies still. Structural methods based on MS, as the Robinson group have shown, will help advance our understanding of the functional role of proteins and biological macromolecules.

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