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Surface Induced Dissociation: Dissecting Noncovalent Protein Complexes in the Gas phase

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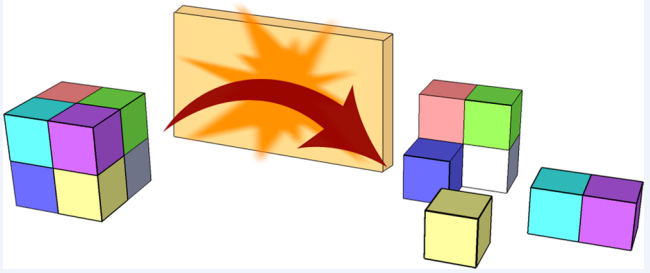
CONSPECTUS: The quaternary structures of proteins are both important and of interest to chemists, because many proteins exist as complexes in vivo, and probing these structures allows us to better understand their biological functions. Conventional structural biology methods such as X-ray crystallography and nuclear magnetic resonance provide high-resolution information on the structures of protein complexes and are the gold standards in the field. However, other emerging biophysical methods that only provide low-resolution data (e.g. stoichiometry and subunit connectivity) on the structures of the protein complexes are also becoming more important to scientists. Mass spectrometry is one of these approaches that provide lower than atomic structural resolution, but the approach is higher throughput and provides not only better mass information than other techniques but also stoichiometry and topology.



Fragile noncovalent interactions within the protein complexes can be preserved in the gas phase of MS under gentle ionization and transfer conditions. Scientists can measure the masses of the complexes with high confidence to reveal the stoichiometry and composition of the proteins. What makes mass spectrometry an even more powerful method is that researchers can further isolate the protein complexes and activate them in the gas phase to release subunits for more structural information. The caveat is that, upon gas-phase activation, the released subunits need to faithfully reflect the native topology so that useful information on the proteins can be extracted from mass spectrometry experiments. Unfortunately, many proteins tend to favor unfolding upon collision with neutral gas (the most common activation method in mass spectrometers). Therefore, this typically results in limited insights on the quaternary structure of the precursor without further manipulation of other experimental factors.

Scientists have observed, however, that valuable structural information can be obtained when the gas-phase proteins are activated by collision with a surface. Subcomplexes released after surface collision are consistent with the native quaternary structure of several protein systems studied, even for a large chaperone protein, GroEL, that approaches megadalton mass. The unique and meaningful data generated from surface induced dissociation (SID) have been attributed to the fast and energetic activation process upon collision with a massive target, the surface.

In this Account, we summarize our SID studies of protein complexes, with emphasis on the more recent work on the combination of ion mobility (IM) with SID. IM has gained popularity over the years not only as a gas-phase separation technique but also as a technique with the ability to measure the size and shape of the proteins in the gas phase. Incorporation of IM before SID allows different conformations of a protein to be separated and examined individually by SID for structural details. When IM is after SID, the cross sections of the SID products can be measured, providing insight on the dissociation pathways, which may mimic disassembly pathways. Furthermore, the separation by IM greatly reduces the peak overlapping (same m/z) and coalescence (merging) of SID products, improving the resolving power of the method. While there are still many unanswered questions on the fundamental properties of gas-phase proteins and a need for further research, our work has shown that SID can be a complementary gas-phase tool providing useful information for studying quaternary structures of noncovalent protein complexes.

STRUCTURAL BIOLOGY OF PROTEIN COMPLEXES IN VACUUM

Mass spectrometry (MS) has become one of the many structural biology tools for proteins because of the discovery of electrospray ionization (ESI),[1,2] which allows these large and fragile biomolecules to be ionized and transferred into the gas phase intact. The observation that noncovalent interactions in protein complexes can be preserved in gas phase analysis under carefully tuned instrumental conditions[3−5] has led to a new area of research, which is now often referred to as "native mass spectrometry",[6] where the proteins are electrosprayed from aqueous buffers that are mass spectrometer compatible but also mimic physiological conditions (usually ammonium acetate as a volatile buffer with minimal nonvolatile content[5]). With additions of detergents to the buffer and proper tuning conditions, some membrane protein complexes can also be transferred and detected in the gas phase by mass

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spectrometry.[7] The molecular weight of the species can be measured with higher resolution and better accuracy than some other low-resolution biophysical techniques, allowing the stoichiometry of noncovalent protein complexes to be determined with high confidence.[5,8] Additionally, the resolving power of mass spectrometry offers great advantages for analysis of samples that are heterogeneous or dynamic.[6,8,9] Even small mass differences from ligand binding[10,11] or post-translational modifications[12] can be detected on the scale of the intact protein complexes and are otherwise difficult to characterize by conventional methods but provide rather important insight on biological functions of proteins.

Recently, the combination of ion mobility (IM) with mass spectrometry has provided additional insights on structures of protein complexes in the gas phase. The principle of IM is the differential migration of ions based on their size, shape, and charge under the driving force of an electric field while being retarded by bath gas. Collisional cross section (CCS) of the ions can also be deduced from IM experiments. The consistency between experimentally measured gas-phase CCSs and CCSs calculated from crystal structures for a collection of protein complexes suggested that many protein complexes preserve the overall shape of their solution structure when sprayed from buffers under physiological pH.[8,13] IM studies have implied that, within the time scale of the experiments, the proteins retain memory of their solution structures and many protein complexes preserve native-like conformations for a range of charge states examined,[13−16] although there could be aberrant behavior for complexes with certain structural features. The CCS measurements from IM can be used as constraints to sieve out the most plausible candidate structures in computational modeling for determination of protein conformation.[17] The methodology, albeit at low resolution, has been validated with a number of well-characterized protein complexes,[18] and it can be quite powerful when combined with other techniques such as electron microscopy to determine the structures of unknown proteins.[19]

DISSECTING GAS-PHASE PROTEIN IONS BY TANDEM MASS SPECTROMETRY

Perhaps the most intriguing capability of mass spectrometry is the isolation of one species at a certain mass-to-charge ratio (m/z) and dissociation to obtain more structural information, rather than looking at an ensemble of proteins in solution. This is often referred to as "tandem mass spectrometry" or MS/MS. Following the same principle as MS/MS of smaller molecules, large noncovalent protein complexes can be disrupted by activation in the gas phase,[20,21] releasing substructures that reveal the connectivity of subunits in the original precursor complex. These experiments are largely performed on ESI/quadrupole/time-of-flight mass spectrometers[5] because of the capability of detecting high m/z on these instruments (recent advances in instrumentation also allow detection of large protein complexes on high-resolution mass spectrometers such as Orbitrap[12] and Fourier transform ion cyclotron resonance mass analyzers[22]). The most commonly used activation method in the instrument platforms used for this type of analysis, collision induced dissociation (CID), typically results in the ejection of highly charged monomers and complementary ($n-1$)-mers for many protein complexes.[20,23] The asymmetric charge state distribution in CID (i.e., the monomer takes a disproportionally large amount of charge given the small mass compared with the full complex) has been attributed, based on some experimental and theoretical studies, to the unfolding of the monomer subunits.[24−26] CID can be extremely helpful in cleaning-up nonspecific adducts of salt and buffer molecules on the protein complexes in order to improve the accuracy of molecular weight measurement and confirming stoichiometry determination based on the stripped complex with the loss of monomers.[5,20] It is also interesting to note that the CID unfolding profile (i.e., CCS change as a function of increasing activation energy) of a ligand-bound protein monitored by IM can be unique to the identity of ligands and used as a "fingerprint" for protein−ligand screening.[27] However, the ubiquitous dissociation pattern of monomer ejection has limited the structural information that can be obtained from MS/MS experiments for many protein complexes. Despite some intriguing success of using CID in combination with other methods for structural elucidation of large protein machineries, releasing subcomplexes for establishing contact maps of the subunits is primarily achieved using solution disruption techniques.[8,28,29]

INITIAL OBSERVATION OF SYMMETRIC DISSOCIATION BY SURFACE COLLISION

The formation of predominantly highly charged monomer products in typical multistep collisional activation (CID) of protein complexes inspired us to attempt alternative activation with a much more massive target, a surface. The first SID experiment of nonspecific noncovalent cytochrome C dimer (+11) on a modified quadrupole/time-of-flight (QTOF) mass spectrometer in our group (QTOF2, Waters Corporation, Manchester, United Kingdom)[30] showed striking differences from CID (Figure 1).[31] In contrast to the highly asymmetric

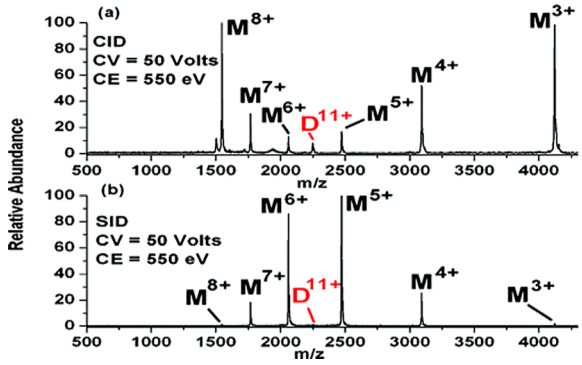

Figure 1. MS/MS of cytochrome C dimer (+11). (a) CID of cytochrome C dimer results in monomers with a highly asymmetric charge state distribution where the most intense pair is the +8 and +3 monomers. (b) SID of the cytochrome C dimer produces monomers with a symmetric charge state distribution with the most intense monomers at +6 and +5, which are about half the charge of the precursor. Reprinted with permission from ref 31. Copyright 2006 American Chemical Society.

charge state distribution of the monomer products normally seen for CID (Figure 1a), the SID spectrum (Figure 1b) features monomer products with a symmetric charge state distribution that is centered around half the charge of the precursor. Similar behaviors (charge distributed evenly among subunits of homooligomeric complexes) have been observed for several other protein complexes in their native oligomeric stoichiometries, including transthyretin (TTR) tetramer, hemoglobin tetramer, C-reactive protein (CRP) pentamer,

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and serum amyloid P (SAP) pentamer. SID of these tetramer and pentamer proteins results in mostly monomers that carry 1/4 or 1/5 of the precursor charge, respectively.[23]

The symmetric charge distribution of the product ions in SID (i.e., charge states of the products are proportional to mass) led to our hypothesis that the subunits are released from the precursor as folded products; otherwise they should have carried more charge due to the larger surface area of the extended, unfolded conformations as seen in the CID monomer products. We have attributed the drastic differences between CID and SID spectra to the differences in the activation process (Figure 2).[23] Due to the large size of protein

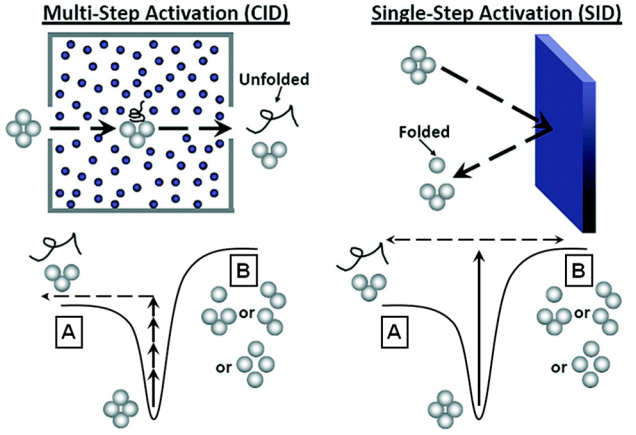

Figure 2. Schematic representations of CID and SID of noncovalent protein complexes with corresponding simplified potential energy diagrams shown at the bottom (reaction coordinate on *x* axis, potential energy on *y* axis. The scale of energy is arbitrary because the actual energies of the processes are unknown). In CID, the protein traversing a pressurized collision cell undergoes multiple steps of collisional activation that could lead to structural rearrangement/unfolding and result in ejection of an unfolded subunit (pathway A). The rapid, single-step, and energetic activation process in SID can favor the faster direct dissociation (pathway B) of the complex into folded subunits that carry charges proportional to their mass without large scale structural rearrangements. Adapted from ref 23. Copyright 2009 American Chemical Society.

complexes compared with the gaseous atomic/molecular targets in CID, the internal energy deposition into the protein upon each collision is not very large and typically requires multiple collision events to reach the dissociation threshold. The surface in the SID experiment has a much greater effective mass as a collision target, which results in much more efficient energy transfer upon collision because of the larger relative mass.[32] The higher efficiency of internal energy deposition of SID allows dissociation pathways with high barriers to be accessed within the practically restricted acceleration voltages, which are usually at a maximum of a couple of hundred volts in most QTOF platforms. In some cases, it is even possible to dissociate very stable, CID-resistant proteins by SID.[33] Furthermore, in contrast to CID, which is likely to be a multistep activation, SID is proposed to be approximately a single-step activation (activation time estimated to be on the order of a few hundred microseconds for CID, but only picoseconds for SID based on small molecule and peptide studies; however, the time for the dissociation event after activation can be significantly longer, especially for large proteins).[23,34]

Data for the dissociation of a heterocomplex, toyocamycin nitrile hydratase, illustrates well the potential of SID for quaternary structure determination of protein complexes.[35] This complex does not have a known high-resolution structure due to difficulty in crystallization. Molecular weight measurement of the intact complex in the mass spectrum reveals that the protein is a hexamer consisting two copies of each α, β, and γ subunit. While CID only results in ejection of individual α and β subunits, SID shows preferential cleavage of the hexamer into $\alpha\beta\gamma$ trimers, strongly suggesting that the hexamer is a dimer of $\alpha\beta\gamma$ trimers. This example clearly implies that SID can be used to obtain critical information on quaternary structure for protein complexes that are difficult to study with conventional high-resolution biophysical methods.

IMPORTANCE OF PROTEIN CONFORMATION AND THE INCORPORATION OF ION MOBILITY FOR MORE STRUCTURAL INFORMATION

In addition to the discovery of the unique dissociation behavior of protein complexes in SID, it was observed that SID spectra are sensitive to the conformation of the precursor on our simple QTOF instrument based on the result of in-source activated hemoglobin tetramer.[23] Elevated sampling cone voltage (CID in the source) caused unfolding of the tetramer as indicated by an increase in the CCS of the tetramer when monitored by IM on a separate IM-MS instrument. While an additional stage of CID in the collision cell for the unfolded tetramer (after the first stage of CID in the source) does not reveal any significant differences, the SID spectra acquired on our instrument clearly featured more monomer products with high charges for the precursor unfolded in the source. The high charge of monomer products is representative of unfolded conformations of the subunit.[24,36,37] Thus, this change in hemoglobin tetramer conformation in the ion source at elevated cone voltage was faithfully reported by SID because the unfolding of precursors presumably results from partially unfolded monomers that manifest themselves as highly charged monomer products in the SID spectra. Similar behavior for in-source activated CRP has also been observed where SID reveals the differences in precursor conformations but CID does not.[38,39] It is critical that native-like conformations are sampled in tandem MS experiments in the gas phase so that the dissociation information can correlate reliably to the quaternary structures of the native protein complexes. In this regard, the fact that SID is sensitive to precursor conformation makes this method suitable for providing insights on gas-phase conformations of protein complexes. Therefore, combining SID with IM, which brings in additional information regarding the gas-phase conformations of the protein ions, became an obvious route to understanding how much "native structure" is preserved in the protein ions and in their dissociation products after SID.

Consequently, we incorporated our SID design into an ion mobility–mass spectrometer (Synapt G2, Waters Corporation, Manchester, United Kingdom),[40] in order to have additional structural information from IM. The instrument is essentially a QTOF but equipped with multiple traveling wave ion guides as ion transfer, CID cell, and mobility separation devices.[41] The design of the custom SID device[40] was adapted from the previous design in the QTOF2[30] but with smaller dimensions. The SID device can be installed either after the IM cell or before the IM cell for different experiments. With the configuration shown in Figure 3a (IM-SID), SID can be

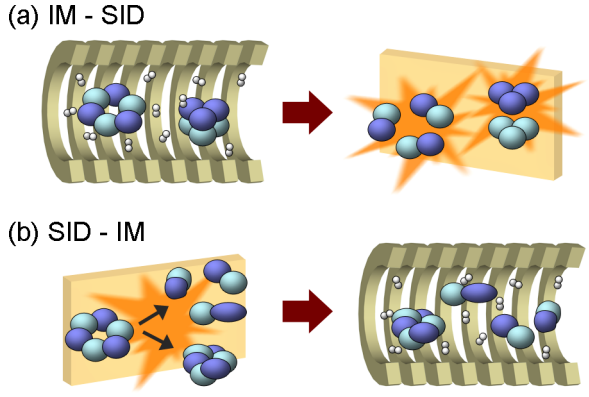

Figure 3. Cartoon illustrations of the instrument configurations for (a) IM-SID and (b) SID-IM. IM-SID is used for SID on conformation-selected precursors, while SID-IM can provide mobility separation or CCS measurements on product ions or deformed precursors after activation.

performed on ions that may be overlapping and indistinguishable in m/z but separated by drift time in the IM cell. Alternatively, the SID can also be placed in front of the IM cell so that the product ions can be separated and analyzed based on the drift time measurement (Figure 3b, SID-IM).

IM-SID: SORTING OUT STRUCTURAL DETAILS OF PROTEINS WITH DIFFERENT CONFORMATIONS

IM offers an additional dimension of separation that greatly improves the resolving power for mixtures based on their charge, size, and shape. It has been shown that even molecules with similar m/z but different chemical compositions can be well separated into individual mobility space in IM-MS experiments.[42] For studies of protein complexes, IM allows species at low abundances to be identified with higher sensitivity because overlapping signal and chemical noise can be removed by the separation in drift time.[43−45] Because SID is sensitive to precursor conformation as discussed earlier, the IM-SID configuration becomes beneficial to separate out conformers of protein complexes and explore their differences in conformation by SID.

Figure 4 illustrates the separation and CID/SID of the conformers of SAP decamers.[40] It was observed in the IM-MS spectrum that SAP decamers have a bimodal distribution of drift time, indicating the existence of multiple conformations. Because the compact conformers exit the IM cell earlier than the less compact conformers, the CID/SID spectrum for each conformer could be individually evaluated using the IM-SID platform by extracting the CID/SID spectra at the drift time corresponding to each conformer. The CID spectra of both conformers show the typical asymmetric dissociation pattern of monomer ejection with high charges (Figure 4a,b). Both conformations undergo unfolding pathways, so the CID spectra do not report differences between the conformers (Figure 4e). SID can clearly discriminate the two conformers with the extra peaks in the high m/z region of the spectrum for the compact conformer (Figure 4c,d). Interestingly, the additional products for the compact conformer are mostly subcomplexes with even numbers of subunits (dimer, tetramer, and hexamer, around m/z 6000−10000). Thus, we hypothesized that the pentamer-to-pentamer interface in the compact SAP decamers is stronger than the case of the less compact decamers, resulting in even-number subcomplexes upon SID with pairs of subunits from

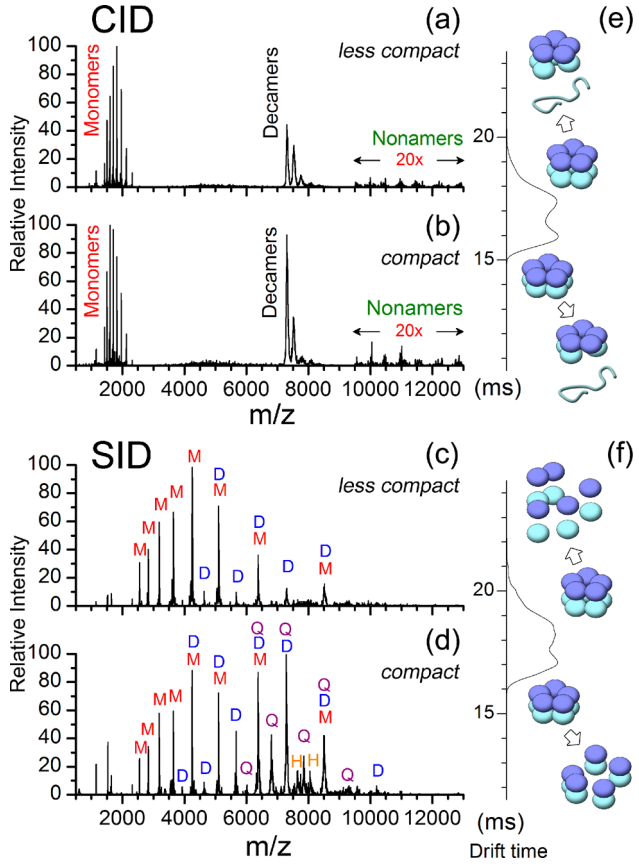

Figure 4. CID of the (a) less compact and (b) compact SAP decamer. SID of the (c) less compact and (d) compact SAP decamer. Major peaks are labeled in the spectra (M, monomer; D, dimer; Q, tetramer; H, hexamer). (e, f) Corresponding drift time distribution showing separation of CID/SID product packets originated from less compact and compact SAP decamers. Insets show the schematics for plausible dissociation pathways. Reprinted with permission from ref 40. Copyright 2012 American Chemical Society.

each pentamer ring (Figure 4f). It is a plausible explanation because SAP is a glycoprotein and the covalently attached glycans (most likely their orientation) could have affected the intersubunit interactions in the decamer structure. The data illustrate the application of the IM-SID platform as a gas-phase analytical tool for reporting conformational differences of protein complexes.

SID-IM: MONITORING CONFORMATIONAL TRANSITIONS AFTER ACTIVATION

Another critical aspect of the drift time measurement is that the collisional cross section (CCS) of the ions can be deduced.[15] The unique information provided by the SID-IM configuration is that the CCS of the ions released from the surface can be monitored.[36,46] Figure 5 illustrates the experiment where the CCS of the remaining precursor after SID/CID is monitored for C-reactive protein (CRP). The typical monomer ejection pathway is again prevalent in CID, while SID shows multiple types of products from monomers up to tetramers. Although the remaining precursor still consists of about half of the total signal in the CID spectrum, the conformation of the precursor has extended greatly as manifested by the shift in CCS. In contrast, extensive dissociation into various low-charge

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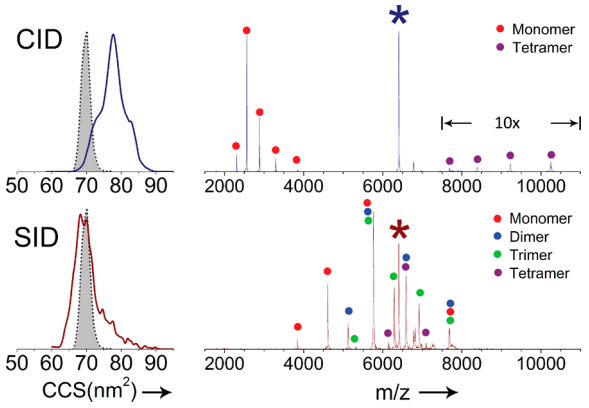

Figure 5. CCS profiles of the remaining CRP pentamer precursor (+18) in CID at 100 V (top left, blue curve) and SID at 40 V (bottom left, red curve), with the gray-shaded peaks representing the measured CCS distributions of the pentamer precursor without activation. The protein was sprayed in a mixture of 80 mM ammonium acetate and 20 mM triethyl ammonium acetate. The corresponding tandem mass spectra of CRP pentamer in CID (top) and SID (bottom) are shown on the right. Product ions are labeled with colored circles and precursor ions with asterisks. Reprinted with permission from ref 36. Copyright 2012 John Wiley and Sons.

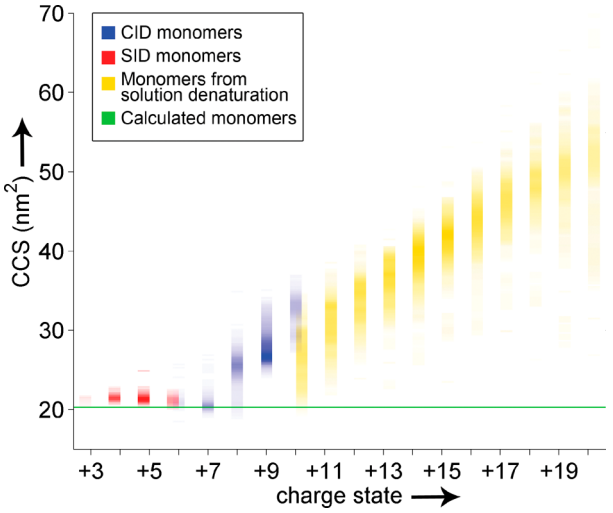

Figure 6. CCS profile of CRP monomer products at different charge states for CID (blue) and SID (red) from native CRP pentamer precursor, as well as solution denatured CRP monomers (yellow). The green line corresponds to the calculated CCS for the CRP monomer in the crystal structure (trajectory method in MOBCAL[48]). Color depth of the spots is proportional to the square root of the relative abundance of the species. Monomer fragments in SID are compact and in reasonable agreement with monomer CCS calculated from the crystal structure. Reprinted with permission from ref 36. Copyright 2012 John Wiley and Sons.

products is observed in SID without significant shift in CCS of the remaining precursor.

Likewise, the CCS of the product ions from SID can also be monitored using this instrument setup and compared with the CCS of CID products. We noticed for this and other examples that the charge state is strongly correlated with the CCS of CID/SID products, largely independent of collision energy and activation method.[47] The fact that monomer products of CRP by SID are low-charged (higher m/z) correlates well with the smaller CCSs of these SID products (red spots in Figure 6) compared with the CCSs of the CID products (blue spots) and solution-denatured monomers (yellow spots). These CCS data on both the remaining precursor and the products further support our previous hypothesis[23] on the differences between CID and SID. The symmetric charge distribution of the SID products leads to lower charge states of the products than CID, consistent with the IM results that the low charged products are indeed compact and folded. The minimal CCS change for the remaining precursor of CRP provides additional evidence for our previous hypothesis[23] that less unfolding is involved in SID than CID.

The charge state of the precursor was reduced by doping in triethylammonium acetate in the spraying buffer in this study because the remaining precursor of CRP could barely be detected even at low SID collision energies at higher precursor charge states. The charge states of the proteins detected in the mass spectra can be readily controlled by solution additives (charge reducing[37,49] or supercharging[50]), and it has been shown that the charge state of proteins is an important factor that affects the dissociation, unfolding, and conformation in the gas phase.[37,46,51−53] Supercharged proteins tend to dissociate/unfold more readily but may dissociate with some symmetric charge partitioning in CID.[51,53] Proteins with fewer charges show a suppressed level of dissociation/unfolding but may collapse into more compact structures upon activation.[37,46] Compact monomer products and even covalent peptide backbone fragmentation can be observed in CID of charge reduced proteins.[28,37] It is demonstrated through both experimental and theoretical studies that charge density and charge migration have strong influence on the gas-phase unfolding process.[25,53−55] We also examined how SID of CRP and concanavalin A (ConA) are affected by charge.[46] In general, SID seems to benefit from charge reduction because more subunit contacts can be preserved in the products (i.e., more multimer products with charge reduction) while unfolding is also suppressed with fewer charges on the precursor.

Examination of the CCSs of multimer products for CRP and ConA has revealed that the higher oligomeric SID products may be more collapsed than the conformations clipped from the full native complex, a result that makes sense intuitively as the remaining subunits try to decrease exposed surface area/charges to lower the free energy of the system.[46] We have observed a few protein-specific cases where the CCSs of some multimer products change as a function of collision energy. Ideally, if the experimental conditions can be tuned so that the native intersubunit contacts of the subcomplexes can be preserved, the CCSs of these product ions will then serve as direct experimental constraints for computer modeling of the protein structure, following the same principle as the solution disruption techniques reported in the literature[5,53] but possibly with higher throughput. At this stage, more fundamental research is required to understand the conformational changes that occur for these multimer products in SID before any modeling work of unknown structures can be applied. Efforts in improving accuracy and throughput for measurements of experimental CCS[56] and CCS calculation algorithms[57] are also critical in pushing this method forward for analysis of real-world protein complexes.

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SID-IM: SIMPLIFYING SPECTRAL INTERPRETATION WITH MOBILITY SEPARATION

IM can also be simply utilized as a separation technique in the SID-IM configuration (Figure 3b). In this case, the product ions from SID are separated in drift time. This can actually be extremely helpful for interpreting SID spectra with rich dissociations containing many possible overlapping species in m/z. For example, for a homocomplex such as TTR tetramer, the dimer products with even numbers of charge will have the same m/z as monomer products with half the charge and are difficult to discriminate without theoretical deconvolution.[23] When the protein has more subunits or the protein is a heterocomplex, the interpretation of the SID spectra becomes increasingly difficult. The CID and SID of an undecameric protein, *trp* RNA-binding attenuation protein (TRAP) from *Bacillus stearothermophilus* are shown in Figure 7a,b, respectively. While CID again shows the typical pattern of monomer ejection, SID shows a variety of products in the high m/z region, some of which are unresolved (higher than m/z 4000). The addition of the drift time dimension in Figure 7c obviously better separated the m/z-overlapped products in the drift time axis. Each type of oligomeric product appears to reside on a single "trendline" in the plot because they have similar compact CCS and their drift times only scale with charge. The separation in the drift time axis obviously increased the resolving power as shown by the successful identification of the species around m/z of 6000−8000, which are otherwise unresolved due to overlapping and coalescence in the m/z axis (e.g., +5 tetramer and +10 octamer overlap at the same m/z of 6650; while +9 octamer and +10 nonamer are only expected to differ by about 90 around the m/z of 7400 thus although they do not directly overlap, the broadness of the peak makes them coalesce in the m/z axis. Neither of the cases can be resolved by m/z alone, although the latter may be resolved by mass analyzers with higher resolution). The experiment is exemplary of a simple but quite practical application of SID-IM for structural studies of complicated protein systems.

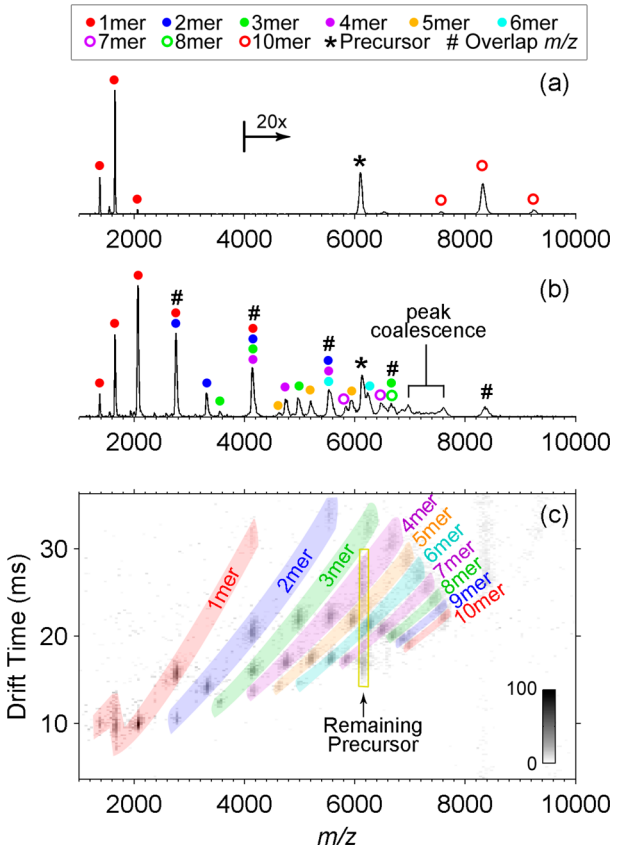

Figure 7. (a) CID spectrum and (b) SID spectrum of +15 TRAP undecamer, each at an acceleration voltage of 100 V. While CID of the protein show the typical monomer ejection pathway, SID results in the dissociation of the undecamer to many different products, some of which cannot be resolved by m/z alone in the high m/z region. (c) IM-MS plot of the SID spectrum of +15 TRAP undecamer. The color depth of the spots in the plot indicates the relative abundance of the species in square root scale (color bar inset bottom right). The compactness of the spots implies that most of the SID products have narrow distributions of drift times in the y axis, thus suggesting they are mostly in folded states, except the remaining precursor, which has a widely stretched distribution of drift time indicative of a distribution of extended structures. The major products from monomers to decamers are outlined and labeled.

PROBING INTERFACES OF PROTEIN COMPLEXES BY SID

The proportional charge distribution and formation of oligomers of every possible size (from monomer up to $(n-1)$-mer where n is the oligomeric state of the precursor) in the SID spectra observed for CRP, TRAP, and several other homocomplexes[23,36,46] are consistent with their known structures when considering the symmetries of these proteins. They possess identical interfaces between subunits thus there is unbiased cleavage over the whole complex into products with all possible numbers of subunits. The chemical nature of the binding interface (solvent accessible surface area, number of salt bridges, hydrogen bonding, etc.) can be correlated to the dissociation behavior of the protein complexes upon activation in the gas phase.[53,58] Therefore, it would be ideal if SID specifically disrupts interfaces to release subcomplexes reflecting the native structure of the precursor.

We have also looked at homocomplexes that possess multiple types of interfaces,[33,59] as well as heterocomplexes mentioned earlier[35] and DNA/RNA−protein complexes including nucleosome and RNase P (work in progress), which show structurally meaningful products in SID. For example, SID of a large protein complex, GroEL, showed the charge reduced tetradecamer precursor (charge state +50) dissociated into heptamers that are consistent with the native topology of the complex (two stacked heptamer rings). Similar observations for several other proteins, including the dissociation into trimeric subcomplexes of a CID-resistant hexamer, glutamate dehydrogenase,[33] have led to the conclusion that it is possible to dissect protein complexes with SID to reveal substructures and subunit connectivity because it tends to favorably disrupt the weakest interactions in the protein complex. It is noteworthy that the SID spectrum for GroEL at the charge state of +71 without the addition of charge reducing reagent was overwhelmed by asymmetric dissociations, although some heptamer products at very low abundance were still detected. The differences in SID for different charge states emphasizes the need for manipulating the charge state of proteins to optimize the conditions to suppress uncontrolled unfolding and extract useful structural information from native-like gas-phase protein complexes.

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CONCLUSION AND OUTLOOK

The application of the gas-phase MS/MS tool, SID, for quaternary structure studies of protein complexes has been demonstrated. SID reports differences in protein conformations and tends to selectively disrupt weak interfaces to release substructures representing native topology. When SID is incorporated after IM, the mobility separation allows conformational isomers to be individually examined. When SID is placed before IM, the conformations of product ions can be studied to better understand the dissociation pathways and fundamental properties of gas-phase protein ions. The encouraging results suggest that it is possible to achieve dissociation of protein complexes with minimal unfolding by SID, revealing structural information that is difficult to obtain by direct CID where unfolding is prevalent. The compactness and proportional charge distribution of SID products for several protein complexes have been attributed to the single-step and high-energy activation process upon surface collision, in contrast to the multistep, lower-energy activation process in CID under typical operation conditions. Additionally, the IM after SID also greatly reduces spectral interpretation complexity and increases the resolving power by separating overlapping or coalescing species in m/z.

While the current research has been primarily focused on well-characterized, soluble proteins for initial development of SID as a technique, ongoing and future research will expand the application of SID to more complicated additional biologically relevant systems such as membrane proteins and protein−DNA/RNA complexes to fully explore the capability of SID in structural biology. Improving the design of SID for even better sensitivity is still a continuing goal to make this technique more applicable to systems that might be demanding even for intact MS measurement due to large size or complexity. It is also of great interest to incorporate SID into other instrument platforms. In particular, high resolution mass analyzers could provide high resolution spectra of the SID products that may bear critical structural information (e.g., noncovalently bound small ligands, or a variety of heterogeneous post-translational modifications) that cannot be resolved on the current QTOF platforms and that is difficult to analyze by other analytical methods.

Several challenges remain in the application of MS/MS as a tool for structural biology of protein complexes. The fragile gas-phase protein complexes can rearrange or unfold into non-native structures even before MS/MS, as induced by many factors including excess activation in the ion source and charge state.[47,52,60] SID is useful for the continuation of these studies, because SID is sensitive to precursor conformation and can be used as an additional probe of the conformation of the protein ions. It is also important to systematically characterize the correlation of gas-phase dissociation patterns with the structures of known protein complexes to correctly interpret an MS/MS spectrum for an uncharacterized protein. Efforts to establish the relationship between protein interface and CID behavior by Hall et al.[53] are very promising. SID shows great potential in this respect considering the unique structural information it can provide for the interfaces between subunits as discussed in the previous section. We anticipate that mass spectrometry methods for studying protein quaternary structure will continue to overcome these challenges and fully emerge as a high-throughput technique for structural biology, with SID as a valuable member of the toolbox, providing complementary structural information to the existing methods.

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Notes

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Vicki Wysocki is Professor and Ohio Eminent Scholar at The Ohio State University. She received her Ph.D. in Chemistry at Purdue University in 1987. After postdoctoral work at Purdue and Naval Research Laboratory (NRC fellow), she joined Virginia Commonwealth University as an Assistant Professor in 1990. She was promoted to Associate Professor in 1994. Vicki moved to University of Arizona in 1996 and was promoted to Professor in 2000. She joined The Ohio State University in 2012. Her research interests include surface induced dissociation of noncovalent protein complexes, peptide fragmentation mechanisms, and development of disease diagnoses by proteomics methods.

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