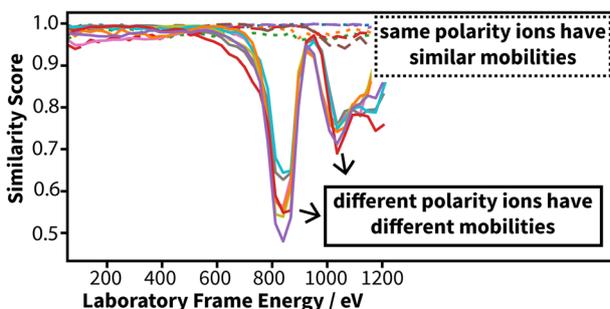


# Collision-Induced Unfolding Is Sensitive to the Polarity of Proteins and Protein Complexes

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**Abstract.** Collision-induced unfolding (CIU) uses ion mobility to probe the structures of ions of proteins and noncovalent complexes as a function of the extent of gas-phase activation prior to analysis. CIU can be sensitive to domain structures, isoform identities, and binding partners, which makes it appealing for many applications. Almost all previous applications of CIU have probed cations. Here, we evaluate the application of CIU to anions and compare the results for

anions with those for cations. Towards that end, we developed a “similarity score” that we used to quantify the differences between the results of different CIU experiments and evaluate the significance of those differences relative to the variance of the underlying measurements. Many of the differences between anions and cations that were identified can be attributed to the lower absolute charge states of anions. For example, the extents of the increase in collision cross section over the full range of energies depended strongly on absolute charge state. However, over intermediate energies, there are significant difference between anions and cations with the same absolute charge state. Therefore, CIU is sensitive to the polarity of protein ions. Based on these results, we propose that the utility of CIU to differentiate similar proteins or noncovalent complexes may also depend on polarity. More generally, these results indicate that the relationship between the structures and dynamics of native-like cations and anions deserve further attention and that future studies may benefit from integrating results from ions of both polarities.

**Keywords:** Ion mobility, Collision-induced unfolding, Protein structure

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## Introduction

In native mass spectrometry (MS), native-like ions are generated using electrospray ionization of proteins, nucleic acids, lipids, and/or other biological molecules in aqueous solutions. Native-like ions can retain noncovalent interactions that were present in solution, and therefore, native MS can probe the oligomeric state [1], topology [2], and stability [3] of biomolecular complexes in solution. This information can be particularly valuable when the biomolecular complex is too

large or small, heterogeneous, or dynamic for other structural tools such as nuclear magnetic resonance, X-ray crystallography, and cryo-electron microscopy. For example, native MS of hepatitis B virus capsid proteins provides evidence for complete capsids containing 180 and 240 subunits [4], consistent with high-resolution characterization, as well as the stoichiometry of antigen-binding domain binding [5] and mechanisms of the assembly process [6, 7], which would have been challenging to investigate using conventional techniques.

Most native MS experiments have probed native-like cations, although some properties of native-like anions have been investigated [8–11]. Typically, native-like anions have lower absolute charge states than the corresponding cations generated from the same solutions [9, 10]. This systematic difference has been attributed to different extents of charge-carrier emission during the final stages of desolvation [10], with lower emission

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energies and therefore greater numbers of competitive emission events [12] for anions. An atomic-level understanding of the structural differences between cations and anions remains elusive. One obvious difference is that native-like anions,  $[M-nH^+]^{n-}$ , are proton deficient, whereas native-like cations,  $[M+nH^+]^{n+}$ , are proton rich. Even though these difference will affect hydrogen bonding, salt bridges, and other noncovalent interactions within the ion [13, 14], the collision cross section ( $\Omega$ ) values determined using ion mobility (IM) MS of native-like ions of medium- to large-mass proteins and protein complexes appear to depend weakly on charge state [10, 15] and polarity [10]. This suggests that the structural differences between native-like anions and cations may be modest, highly localized, and/or beyond the resolution of current IM measurements.

Collisional activation of native-like ions can result in the loss of nonspecific adducts, changes in conformation, and eventually dissociation [16]. Monitoring changes in conformation (collision-induced unfolding, CIU [17]) and extents of dissociation (collision-induced dissociation, CID) as a function of energy can provide information that is complementary to the associated IM and MS characterization of minimally activated ions. For example, CIU can be sensitive to charge state [18–20], domain structure [21], isoform identity [22], and ligand binding [23–26]. Consequently, there is great interest in using CIU “fingerprints” to differentiate similar proteins [22, 24, 27, 28], particularly in cases where the associated native MS and native IM-MS data are not sensitive to those difference. For example, native-like cations of IgG subclasses with different disulfide bonding have similar IM arrival-time distributions but exhibit unique CIU features upon the activation [22]. Almost all applications of energy-dependent native MS have probed native-like cations. Based on the similarity of the results from CIU of 21+ and 21– ions of serum amyloid P (SAP) pentamers, Hall and coworkers reported that “the conformation and the gas-phase behavior during collisional activation are the same for SAP pentamer, regardless of whether it has an excess of positive or negative charges” [20]. For complexes between cholera toxin B subunit homopentamers and five GM1 pentasaccharide ligands, CID of cations results predominantly in the loss of a protein subunit (without a ligand), whereas CID of anions results predominantly in the loss of a ligand (without a protein) [29]. These differences suggest that the CID products are a consequence of polarity-dependent structural changes that occur in the gas phase prior to dissociation [29].

It is challenging to assess the general utility of CIU to differentiate similar proteins or complexes because negative results, i.e., cases where CIU is not sensitive to those differences, are usually not reported. Here, in order to broaden the potential utility of CIU, we demonstrate the application of CIU to anions and compare the results for anions with those for cations. Towards that end, we developed a “similarity score” that we use to quantify the differences between the results of different CIU experiments and evaluate the significance of those differences relative to the variance of the underlying measurements.

## Methods and Materials

### *Sample Preparation*

Avidin from egg white (A2667, Invitrogen, Carlsbad, CA) and  $\beta$ -lactoglobulin A from bovine milk (L7880, Sigma-Aldrich Co., St. Louis, MO) were each buffer exchanged into aqueous 200 mM ammonium acetate at pH 7.0 using centrifugal concentrators (10 kDa MWCO, Spin-X UF, Corning, Inc.) and a centrifuge operated at 4 °C. For charge-reduction experiments, ions were generated from an aqueous solution containing 50 mM trimethylamine and 200 mM ammonium acetate.

### *Ion Mobility Mass Spectrometry and Collision-Induced Unfolding*

Samples with a protein concentration of 10 micromolar were each loaded into glass capillaries with inner diameters of 0.78 mm that were pulled to approximately 1 to 3  $\mu$ m on one end using a micropipette puller (P-97, Sutter Instruments, Novato, CA). Electrical contact with the solution was achieved by inserting a platinum wire electrode into the wide end of the capillary [30]. To minimize analyte carryover between samples, the electrode was washed with aqueous 25% HCl (v/v) and rinsed with ultrapure (18.2 M $\Omega$ ) water. Data were acquired using a Waters Synapt G2 HDMS hybrid mass spectrometer (Waters Co., Wilmslow, UK) [31] in which the traveling-wave IM cell was replaced with a radiofrequency (RF) confining drift cell [32] that contained 1.7 Torr He. To preserve the noncovalent interactions, the following instrument parameters were used: capillary voltage,  $\sim$  1.0 kV; sampling cone, 20 V; extraction cone, 5 V; source temperature,  $\sim$  30 °C; and trap collision energy, 4 V. To determine the mobilities of minimally activated ions, those ions were introduced into the RF-confining drift cell that was operated using at least 9 drift voltages ranging from 104 to 354 V [32].  $\Omega$  values of minimally activated ions and the mobility-independent transport times of ions ( $t_0$ ) were determined from the slopes of plots of drift time versus reciprocal drift voltage as described previously [32]. For CIU experiments, ions were accelerated prior to entering the trap cell using a range of trap injection voltages from 4 to 61 V with the RF confining drift cell operated at a drift voltage of 104 V.

### *Data Analysis*

Mass spectra were analyzed using MassLynx v4.1 (Waters, Co., Milford, MA). Arrival-time distributions were extracted and analyzed using in-house software [33]. The intensities of each arrival-time distribution were normalized so that their sum equals one. To aid in the comparison of IM data, arrival-time distributions were converted to apparent  $\Omega$  distributions and then interpolated along a common  $\Omega$  axis. The axes used for avidin and  $\beta$ -lactoglobulin contained 1000 and 500 points, respectively.

## Results

### *Effects of Polarity on the Mass Spectra and Ion Mobility of Native-like Ions*

Figure 1a shows a representative native mass spectrum of avidin that was measured while operating the instrument in positive ion mode and exhibits peaks that are assigned to the 17+, 16+, 15+, and 14+ tetramers of avidin. This spectrum and these assignments are consistent with many examples in the literatures [10, 34]. Figure 1b shows the analogous spectrum obtained in negative ion mode, which exhibits peaks that are assigned to the 14-, 13-, and 12- tetramers of avidin. The difference in the absolute charge states observed for the two polarities is similar to that reported previously [10]. However, we are not aware of any other reports of native-like anions of avidin in the literature that could be used for comparison. The relative lack of analogous spectra of anions can partially be attributed to the propensity of corona discharge at the tip of electrospray emitters in negative ion mode [35]. However, corona discharge was rare in the present experiments, which may be attributable to the low onset voltage for electrospray in this implementation [30].

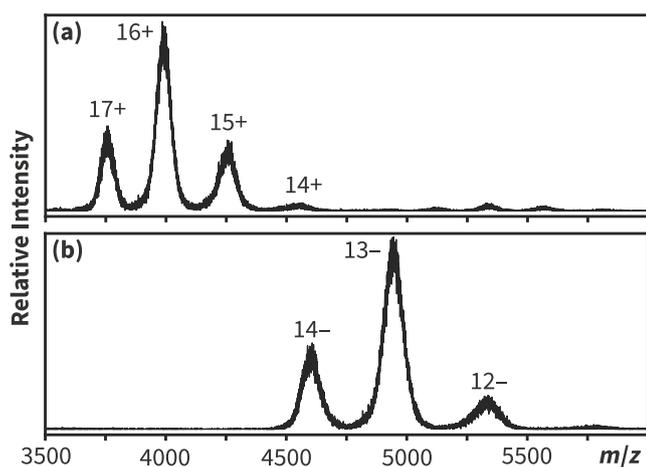
The native-like cations and anions of avidin were also analyzed using IM-MS, which revealed that the median  $\tilde{\Omega}$  values of these ions are all similar to each other (Table S1, determined from cumulative distribution function of each apparent  $\tilde{\Omega}$  distribution as shown in Figure S1). These results corroborate previous results, suggesting that gas-phase structure can depend weakly on charge and polarity under native-like conditions [10, 20]. However, similar  $\tilde{\Omega}$  values for native-like cations and anions does not preclude possible differences between anions and cations that are not resolved in these IM measurements. To investigate the potential

differences between the cations and anions, we subjected avidin ions to CIU.

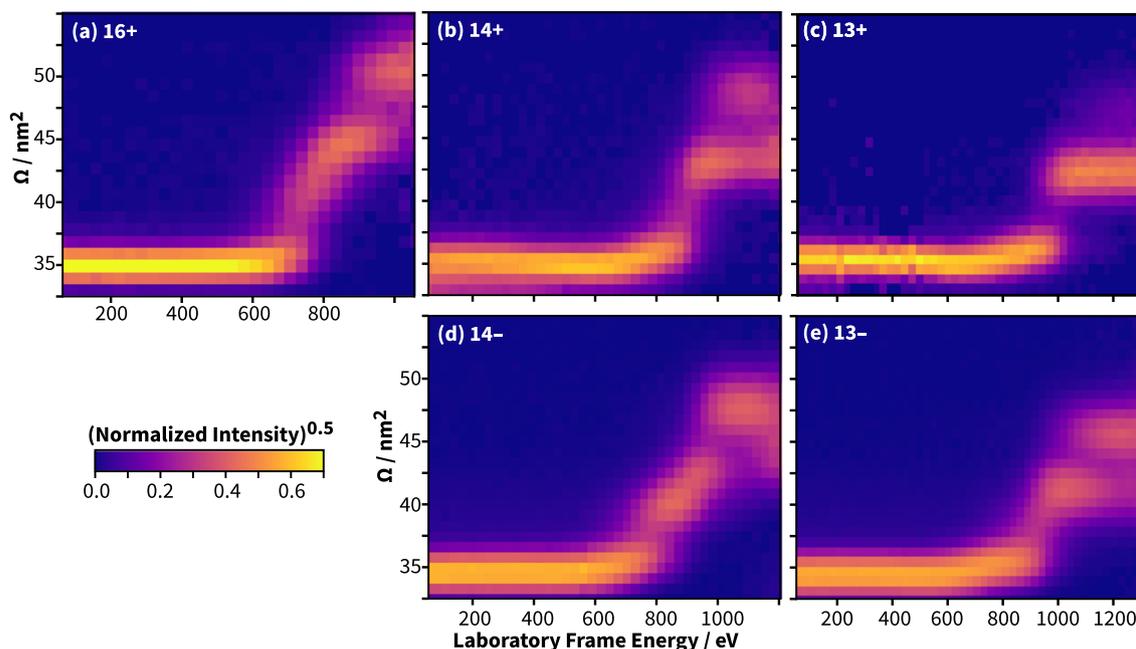
### *Effects of Polarity on the Collision-Induced Unfolding of Native-like Ions*

Selected charge states of native-like cations and anions of avidin were subjected to CIU by varying their laboratory-frame kinetic energy as they enter the trap cell that is positioned immediately prior to the IM cell. Laboratory-frame energy is the product of the voltage used to inject ions in the trap cell and the absolute value of charge state of the ion [36]. The resulting products were then separated by IM, and the resulting apparent  $\tilde{\Omega}$  distributions are shown in Figure 2. The initial distributions are similar for all charge states, consistent with the  $\tilde{\Omega}$  values discussed above. With increasing energy, features appear that are attributed to partially unfolded and mostly unfolded structures, consistent with a previous study of native-like cations of avidin [34]. Although features for folded, partially unfolded, and mostly unfolded avidin ions are observed in all cases, the appearance energy and  $\tilde{\Omega}$  of these features appear to depend on charge state and polarity to varying extents. Differences are most apparent for the partially unfolded ions. Aspects of these differences will be quantified and discussed in more detail below.

Even though the  $\tilde{\Omega}$  distributions at low energies are similar for charge states characterized in both polarities, the distributions formed at the highest energies appear to exhibit decreasing magnitudes with decreasing absolute charge state (Figure 2). Figure 3 shows the  $\tilde{\Omega}$  values at the highest (Figure S2) and lowest (Figure S1) energies for each species. The  $\tilde{\Omega}$  values at high energy increase with absolute charge states, which is consistent with visual inspection of the CIU data (Figure 2). The  $\tilde{\Omega}$  value at high energy for the 14- ions is slightly greater than that for the 14+ ions. Because these are the only ions with the same absolute charge state that are observed in both polarities, it is challenging to draw more general conclusions about the effects of polarity on the maximum expansion of protein ions by CIU. In order to further investigate the effects of polarity separately from charge state, trimethylamine was added to an avidin sample, which was then analyzed in positive ion mode. In these experiments, trimethylamine acts as a charge-reducing agent [37] and enables the formation of 13+ avidin ions. Interestingly,  $\tilde{\Omega}$  value at high energy for the 13- ions is less than that for the 13+ ions. However, the binding of trimethylamine is evidenced by the mass spectra shown in Figure S3; the centroids of the peaks in the originating from the sample with trimethylamine correspond to masses that are ~556 Da heavier (equivalent to nearly 10 molecules of trimethylamine) than those originating from the sample without trimethylamine. Based on these results alone, we are unable to conclude whether the differences are a consequence of polarity or the presence of trimethylamine in the sample used to generate the cations. Therefore, this topic will require further investigation in the future.

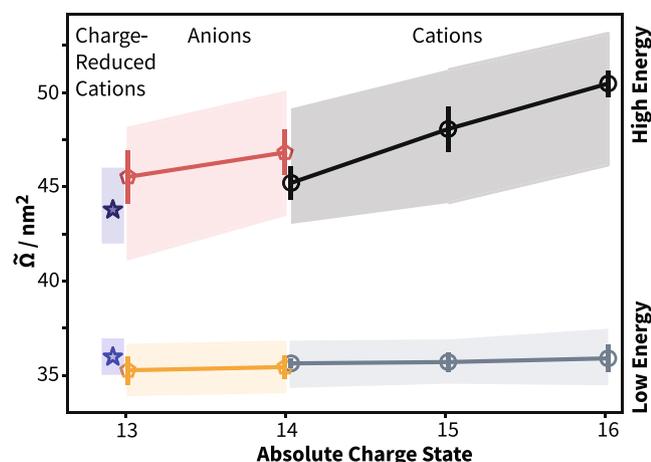


**Figure 1.** Native mass spectra of avidin in positive (a) and negative (b) polarity obtained from an aqueous solution of 200 mM ammonium acetate at pH 7.0. The predominant peaks are assigned to tetramers, with charge states as labeled. The weak signals at higher  $m/z$  are assigned to dimers of the avidin tetramer, which is attributed to nonspecific aggregation during electrospray ionization [30]



**Figure 2.** Results from collision-induced unfolding (CIU) of selected charge states of the avidin tetramer. Ions were generated from an aqueous solution containing 200 mM ammonium acetate, except the 13+ ions were generated from a solution that also contained 50 mM trimethylamine. The heat maps display the apparent  $\Omega$  distributions as a function of laboratory frame energy, based on data acquired using 2 V increments of the trap injection voltage. Laboratory frame energy was increased until the precursor was depleted due to dissociation

Although the differences between the  $\tilde{\Omega}$  values at high energies 14+ and 14- are subtle, the CIU results for these ions appear to exhibit more significant differences over intermediate energies (Figure 2 b and d). For comparison, the apparent  $\Omega$

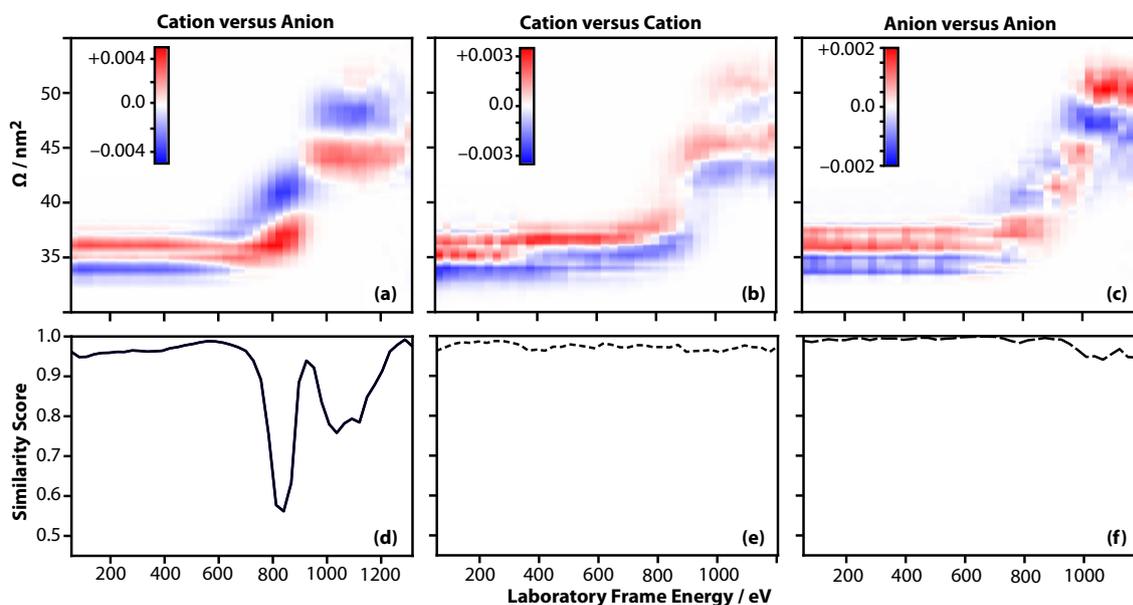


**Figure 3.** The median  $\tilde{\Omega}$  value at low (20 V) and high (16+, 64 V; 15+, 68 V; 14+, 86 V; 13+, 118 V; 14-, 86 V; 13-, 100 V) trap injection voltage. Each marker represents the average of the  $\tilde{\Omega}$  values determined for three independent technical replicates. The vertical bars span the 95% confidence interval of the replicates. The upper and low bound of the shaded region represent the  $\Omega$  values at 90% and 10% of the cumulative distribution function respectively, as shown in Figs. S1 and S2

distributions for these ions at selected energies are shown in Figure S4. To highlight the differences between the CIU results for these ions of opposing polarity, the difference between those results is plotted in Figure 4a. For comparison, Figure 4 b and c show the difference between the  $\Omega$  distribution of two independent replicates of 14+ and 14- ions, respectively. Although the magnitudes of the differences between replicate analyses of ions of the same polarity (Figure 4 b and c) are less than the differences between analyses of ions of different polarity (Figure 4a), it was challenging to interpret these differences using these plots alone. For example, the differences at low energies appear as red and blue streaks, but it appears that those differences are the consequence of very slight differences in underlying distributions (e.g., Figure S4a) and that the distributions may have been undersampled in the original experiments. However, both the difference plot (Figure 4a) and the individual  $\Omega$  distributions (Figure S4) indicate that the greatest differences occur over intermediate energies, in the region associated with partially unfolded structures.

In order to quantitatively compare the apparent  $\Omega$  distributions underlying these CIU results, we developed a similarity score that depends on the dot products of the pair of distributions and the dot products of duplicate distributions. The similarity score of the distributions for ions  $\alpha$  and  $\beta$  is defined as:

$$\text{Similarity Score} = \frac{1}{2} \left( \frac{I_{\alpha} \cdot I_{\beta}}{I_{\alpha} \cdot I_{\alpha}} + \frac{I_{\alpha} \cdot I_{\beta}}{I_{\beta} \cdot I_{\beta}} \right) \quad (1)$$



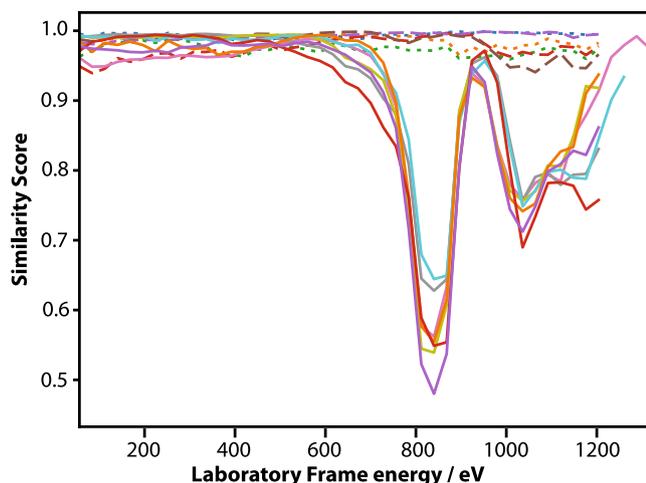
**Figure 4.** (a) The differences between the CIU intensities for 14+ and 14– avidin from native-like conditions. Reds indicate greater relative intensities for the cations (positive values), whereas blues indicate greater relative intensities for the anions (negative values). The differences between the CIU intensities based on two independent technical replicates of 14+ and 14– avidin are shown in (b) and (c), respectively. (d) The similarity scores (Eq. (1)) for the apparent  $\Omega$  distributions of 14+ and 14– avidin used for (a). The similarity scores for the apparent  $\Omega$  distributions used for (b) and (c) are plotted in (e) and (f), respectively

where  $I_\alpha$  and  $I_\beta$  are the intensities of the distribution for  $\alpha$  and  $\beta$ , respectively. Possible scores range between zero and one. A similarity score of one indicates that the two distributions are identical. Conversely, differences in the two distributions will result in similarity scores that are less than one. Figure 4d shows the similarity scores as a function of laboratory frame energy determined from CIU analysis of 14+ and 14– avidin ions, i.e., where  $\alpha$  and  $\beta$  have different polarities. This trace is near one for energies  $< 600$  eV and near 950 eV but decreases to significantly lower values near 840 and 1050 eV. Figures S4a to S4e show representative apparent  $\Omega$  distributions of 14+ and 14– avidin ions at 56, 840, 924, 1036, and 1288 eV. Visual inspection of the distributions at each energy provides context for interpreting the scores determined using Eq. (1) and corroborates the preceding evaluation of similarity/difference based only on similarity scores. The results demonstrate the utility of this score for quantifying the similarity between two distributions and identifying the laboratory frame energies that yield the greatest differences for samples from different classes.

Figure 4e and f plot the similarity score traces determined for the same data used for the analysis in Figure 4 b and c, respectively. These traces illustrate typical similarity scores for comparisons between members of the same class. Relative to the traces determined when  $\alpha$  and  $\beta$  have the opposite polarity, these traces are largely independent of energy. To provide context for this data and illustrate sources of variance, Figures S5 and S6 show apparent  $\Omega$  distributions and similarity scores at selected

energies for two pairs of technical replicates. Figure S5 shows that the results for a pair of technical replicates selected for the anions have similarity scores that range from 0.97 to 0.99. At intermediate energies, it appears that a slightly greater fraction of the ions in one replicate have unfolded than in the other, but the differences between the two replicates are very subtle. Figure S6 shows that the results for a pair of technical replicates selected for the cations have similarity scores that range from 0.96 to 0.99. Those slightly lower values appear to be a consequence of a systematic  $\sim 1$  nm<sup>2</sup> difference in  $\Omega$  between the two replicates, which were acquired 4 months apart. Systematic differences could be reduced by acquiring data for different samples on the same day or by standardizing the mobility data [33].

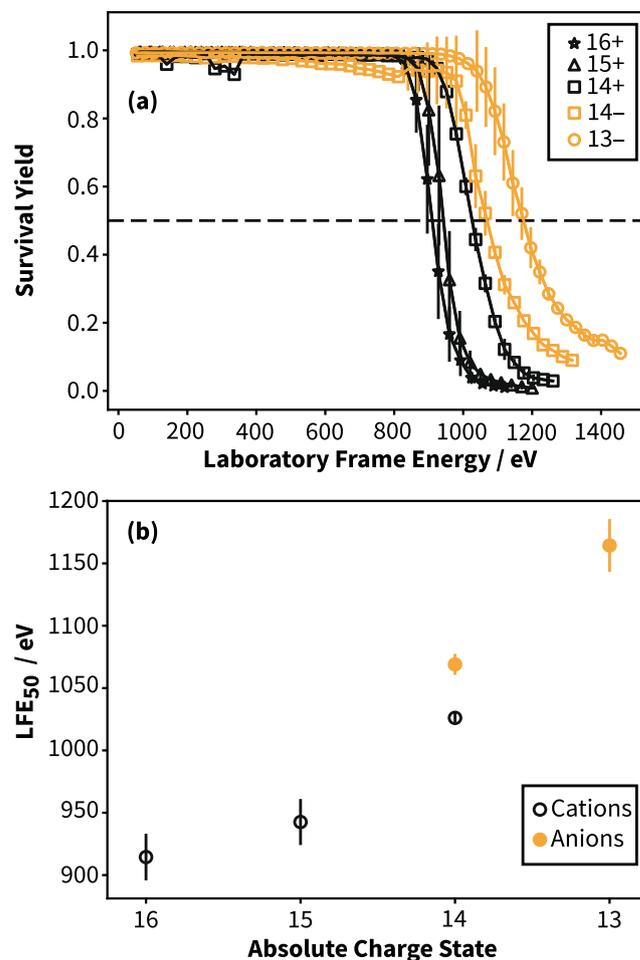
Figure 5 shows similarity score traces that were determined for many independent CIU analyses of 14+ and 14– ions. These results clearly show that the differences between the CIU results for ions of different polarities are significantly greater than the differences between technical replicates for ions of the same polarity. That is, even though the  $\bar{\Omega}$  values at the lowest and the highest energies depend subtly on polarity, the  $\Omega$  values of the ions generated at intermediate energies depend strongly on polarity. An alternative method for analyzing these results is CIU Suite 2 [38]. For example, Figure S7 shows the comparison plot and RMSD values generated using that method, default settings, and the same experimental data used for Figure 4. The RMSD value for ions of different polarity (25%, Figure S7a) is larger than those for ions of the same polarity



**Figure 5.** Similarity scores of results for 14+ and 14- avidin ions (solid lines) determined from unique pairs of three replicate CIU analyses of 14+ and 14- avidin. Similarity scores for unique pairs of replicate CIU analyses of 14+ (dotted lines) and 14- (dashed lines) avidin

(10%, Figures S7b and S7c). By comparison, the similarity scores in Figure 5 (1) exhibit far larger differences between ions of different polarity and ions of the same polarity and (2) directly indicate the energies that are most useful for differentiating between species of interest.

In order to evaluate whether other proteins also exhibit polarity-dependent differences, CIU was used to characterize monomers of  $\beta$ -lactoglobulin, which has a mass of 18.4 kDa. Figure S8 shows the native mass spectra of the 7+ and 7- ions, and that features for unadducted protein ions (i.e., no nonspecific adducts) are resolved in both polarities. Unadducted forms of these ions were each quadrupole selected (Figure S8) and subjected to CIU. Unadducted ions are preferred for these experiments because previous studies have shown that that ions subjected to CIU can be stabilized through interactions with nonspecific adducts [34, 39, 40]; unfortunately, this was not possible in the preceding example due to presence of multiple glycoforms of avidin [41] and other challenges associated with native MS of higher mass proteins, including decreased apparent resolving power associated with broader isotope distributions [42], increased binding of nonspecific adducts [43], and increased scattering with background gas during time-of-flight mass analysis. Figure S9a shows the apparent  $\Omega$  distributions of 7+ and 7- ions as a function of laboratory frame energy, which reveals very different CIU fingerprints. For example, at 518 eV the 7- anions exhibit a bimodal  $\Omega$  distribution that is significantly broader than that for the 7+ cations (Figure S4f). The similarity scores are plotted in Figure S9b and indicate that the differences between the results for the anions and cations increase with increasing laboratory frame energy. These results for  $\beta$ -lactoglobulin complement those for avidin and provide further support that the CIU of native-like ions can depend on polarity.



**Figure 6.** Results from collision-induced dissociation (CID) of avidin ions from native-like conditions. Representative mass spectra are shown in Figure S10. (a) Survival yield of the precursor ions as function of laboratory frame energy. The bars span the 95% confidence interval of three independent technical replicates. Note that replicates were not collected for laboratory frame energies that are far greater or lower than the LFE<sub>50</sub>. (b) LFE<sub>50</sub> for each charge state is shown in both polarities. The bars span the 95% confidence interval based on three independent technical replicates

### Effects of Polarity on CID Efficiency

The highest energies used for CIU of the avidin tetramer also resulted in CID, yielding monomers and trimers of avidin (Figure S10). Figure 6 a shows the survival yield of quadrupole-selected tetramers ions:

$$\text{Survival Yield} = \frac{\sum I(\text{tetramers})}{\sum I(\text{monomers}) + \sum I(\text{trimers}) + \sum I(\text{tetramers})} \quad (2)$$

The survival yield of the tetramer decreases with increasing laboratory frame energy as a consequence of the formation of monomers and trimers (Figure S10). Activation of the precursor ions also results in the appearance of charge-reduced and higher-charge ions (Figures S11 and S12); such charge-transfer

products have been reported previously [44]. In general, anions exhibit greater propensity for charge stripping than the corresponding cations (Figure S13). Note that survival yields were calculated using all ions observed for each oligomeric state, including any charge-transfer products.

To help compare the stability of each precursor during CID, we determined “LFE<sub>50</sub>” values, which are defined as the laboratory frame energy that results in a survival yield of 50%. These values for all charge states and both polarities are plotted in Figure 6b. Note that in some cases, charge-stripped tetramers may have the same *m/z* as some trimers. The likelihood of this occurring is greatest for the anions, which exhibit the greatest extent of charge stripping. In order to estimate the possible contributions from such interferences, we calculated LFE<sub>50</sub> values for the 13<sup>-</sup> and 14<sup>-</sup> ions assuming that the ambiguous ions are entirely trimers (the lower limit) or charge-stripped tetramers (the upper limit). The lower and upper limits of the LFE<sub>50</sub> for 14<sup>-</sup> avidin are 1077 and 1089 eV, respectively. Those limits for 13<sup>-</sup> avidin are 1141 and 1191 eV, respectively. Since these ranges are small, we did not evaluate this possibility further and use the upper limits in the remaining discussion.

Overall, the CID efficiency for the native-like anions is less than that of the native-like cations, which to some extent is a consequence of the lower absolute charge states for the former. Figure 6b shows that the LFE<sub>50</sub> of 14<sup>-</sup> avidin is higher than that of the 14<sup>+</sup> avidin by 43 eV. 14<sup>+</sup> and 14<sup>-</sup> avidin possess the same charge state but may differ in the presence of mobile protons or charge carriers. For context, numerous studies have concluded that CID of multiprotein complexes involves charge migration to one of the protein subunits, which leads to unfolding and the subsequent ejection of that subunit [20, 45–49]. Therefore, it is possible that less-efficient charge migration in anions, which are proton deficient, may also contribute to their lower CID efficiency. There may be additional consequences of the number of protons in these ions and the concomitant differences in the number of hydrogen bonds and salt bridges [13] present in their structures.

## Conclusions

Native-like anions and cations of the avidin tetramer were probed using IM-MS, CIU, and CID. Visual inspection of the CIU data for avidin (Figure 2) indicates that there are differences between the results for anions and cations. To analyze the significance of those differences, we developed a “similarity score” (Eq. (1)) that we used to quantify the differences between results for ions of difference polarities relative to the differences between results for technical replicates of the same polarity (Figure 5). Many of the differences between anions and cations that were identified can be attributed to the lower absolute charge states of anions (Figure 1). For example, the extents of increase in  $\bar{Q}$  values over the full range of energies depended strongly on absolute charge state (Figure 3). However, over intermediate energies, there are significant

differences between anions and cations with the same absolute charge state (Figures 4, 5, and S4). Differences between the CIU of anions and cations of  $\beta$ -lactoglobulin were also observed and characterized (Figure S9). These results all indicate that CIU is sensitive to the polarity of protein ions. The CID efficiency of avidin ions also appears to depend on polarity (Figure 6). Characterizing the differences between these properties for additional multiprotein complexes may enable more general insights into the role of charge mobility on dissociation of multiprotein complexes.

Very few native IM-MS experiments have probed anions. Based on results for a selected set of proteins and protein complexes, we reported previously that native-like cations and anions (with minimal activation) can have very similar  $\Omega$  values and suggested that there was “no inherent benefit to selecting a specific polarity for capturing a more native-like structure” [10]. Because the present results indicate that the CIU of equally charged ions is sensitive to polarity, we propose that the utility of CIU to differentiate similar proteins or noncovalent complexes may depend on polarity. Therefore, we recommend that analyte polarity should be considered during the development of native IM-MS methods for specific applications. More generally, these results indicate that the relationship between the structures and dynamics of native-like cations and anions deserve further attention and that future studies may benefit from integrating results from ions of both polarities.

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