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Impact of charge state on 193 nm ultraviolet photodissociation of protein complexes

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Supporting Information

Table S1. Range of charge states obtained from each of the solutions for each of the protein complexes. Charge states with insufficient abundances for MS/MS analysis are not included. The full range of charge states observed are shown in Figures 1 and S1-S4.

Protein	MW / oligomeric state	Charge state distribution ¹		
Complex		Supercharging	Standard	Charge-reducing
Cu/Zn Superoxide dismutase	31 kDa/ homodimer		10+ - 11+	8+ - 9+
Streptavidin	52 kDa / homotetramer	15+	13+ - 14+	10+ - 11+
Transthyretin	56 kDa/ homotetramer	16+ - 17+	13+ - 15+	11+ - 12+
Hemoglobin	64 kDa/ heterotetramer		15+ - 17+	11+ - 13+
C-reactive protein	115 kDa/ homopentamer	26+ - 27+	23+ - 25+	17+ - 19+

¹ The solution conditions are as follows: 20 mM m-NBA and 80 mM ammonium acetate for supercharging conditions; 100 mM ammonium acetate for standard native conditions, and 20 mM TEAA and 80 mM ammonium acetate for charge-reducing conditions.

Table S2. Structural features of each protein complex as observed from the associated PDB structures. Average interfacial area and number of hydrogen bonds (HB) and salt bridges (SB) for each subunit were obtained from PDBePISA. Values for Hb were averaged for α and β subunits.

Complay	Subunits	PDB	Disulfide Bonds	Interfacial contacts		
Complex				Area (Ų)	HB	SB
SOD	2	1CB4	1	661	5	0
SA	4	1SWB	0	2139	25	3
TTR	4	4TLT	0	1620	31	1
Hb	4	1BBB	0	1578	16	2
CRP	5	1B09	1	1275	14	16



Figure S1. ESI-MS of streptavidin in a) 20 mM m-NBA, 80 mM ammonium acetate solution for supercharging, b) 100 mM ammonium acetate (standard native MS solution), and c) 20 mM triethylammonium acetate, 80 mM ammonium acetate for charge-reducing native solutions.



Figure S2. ESI-MS of transthyretin in a) 20 mM m-NBA, 80 mM ammonium acetate solution for supercharging, b) 100 mM ammonium acetate (standard native MS solution), and c) 20 mM triethylammonium acetate, 80 mM ammonium acetate for charge-reducing native solutions.



Figure S3. ESI-MS of hemoglobin in a) 20 mM m-NBA, 80 mM ammonium acetate solution for supercharging, b) 100 mM ammonium acetate (standard native MS solution), and c) 20 mM triethylammonium acetate, 80 mM ammonium acetate for charge-reducing native solutions. Asterisks denote apo-homodimers containing two β -subunits.



Figure S4. ESI-MS of C-reactive protein in a) 20 mM m-NBA, 80 mM ammonium acetate solution for supercharging, b) 100 mM ammonium acetate (standard native MS solution), and c) 20 mM triethylammonium acetate, 80 mM ammonium acetate for charge-reducing native solutions.



Figure S5. MS/MS spectra of 11+ (top) and 8+ SOD (bottom) using a) a lab frame collision energy of 1.2 keV for HCD, b) 1 mJ UVPD (1 pulse), and c) 3 mJ UVPD (1 pulse).



Figure S6. MS/MS spectra of transthyretin in 17+ (top) and 11+ (bottom) charge states using a) a lab frame collision energy of 1 keV for HCD, b) 1 mJ UVPD (1 pulse), and c) 3 mJ UVPD (1 pulse). The precursor ion is labelled with a star.



Figure S7. MS/MS spectra of hemoglobin in 17+ (top) and 12+ (bottom) charge states using a) a lab frame collision energy of 1 keV for HCD, b) 1 mJ UVPD (1 pulse), and c) 3 mJ UVPD (1 pulse). The precursor ion is labelled with a star. No dimeric products were observed.



Figure S8. MS/MS spectra of C-reactive protein in 27+ (top) and 17+ (bottom) charge states using a) a lab frame collision energy of 1 keV for HCD, b) 1 mJ UVPD (1 pulse), and c) 3 mJ UVPD (1 pulse). The precursor ion is labelled with a star.



Figure S9. Fractional abundance of product ions normalized to the total ion current of each HCD or UVPD spectrum from selected precursors, as illustrated for a) 10+ to 15+ streptavidin, b) 11+ to 17+ transthyretin, c) 12+ to 17+ hemoglobin, and e) 17+ to 19+ and 23+ to 27+ CRP.



Figure S10. Truncated m/z range of UVPD mass spectra of 13+ streptavidin from standard native conditions activated with a single laser pulse with energy from 0.5 to 3.0 mJ. A shift to lower monomer charge state (higher m/z) is observed with increasing pulse energy.



Figure S11. Location and relative abundance of sequence ions generated from 3 mJ UVPD of all of the charge states generated from supercharging, standard native, and charge-reducing solutions for a) superoxide dismutase, b) streptavidin, c) transthyretin, d) α -hemoglobin, e) β -hemoglobin, and f) C-reactive protein. The abundances of fragment ions that originate from cleavages at each backbone position are mapped from the N-terminus to the C-terminus across the entire protein sequence. The gradient scale shows the natural log of the fractional abundances of fragment ions with yellow representing the greatest abundance and black representing the lowest/no abundance.



Figure S12. Fragment maps obtained using ProSight Lite for each charge state of SOD from 3 mJ UVPD (1 pulse). The red box on the N-terminus reflects the additional mass accounting for an acetylation modification. The gray boxes on Cys55 and Cys144 reflect the loss of one hydrogen each to account for the disulfide bond. Backbone cleavage markers are shown as colored flags: a,x: green; b,y: blue; c,z: red. On the right is the crystal structure of SOD (pdb 1cb4) colored to reflect the average normalized abundances of the backbone cleavages that yield the sequence ions on each fragment map.



Figure S13. Fragment maps obtained using ProSight Lite for each charge state of SA from 3 mJ UVPD (1 pulse). Backbone cleavage markers are shown as colored flags: a,x: green; b,y: blue; c,z: red. On the right is the crystal structure of SA (pdb 1swe) colored to reflect the average normalized abundances of the backbone cleavages that yield the sequence ions on each fragment map.

-	TD	
L	IR	

11+	G[P[T[G T G E]S]K]C P]L MÌVÌKÌVÌL[D[A]V[R G S[P]A 25 26 I N VÌA V[H V F R K A A[D[D[T W E[P F A]S]G]K T S 50 51 E]S]G E L]HÌGÌLÌT[T]EÌEÌFÌVÌEÌGÌI [Y[K[V[EÌ] ID[T 75 76 [kls Y]W[K[ALL[G[I][S[P[F]H]E]HIALE[V]V]F[T[A]N[D]S 100 101 [G[P]R[R[Y[T[I]A]ALL]L[S[P[Y]S]Y[S]T]T[A[V V T]N P 125 126 K]E C	1
12+	GLPLTLG TLGLE SLKJCLPJL M VLKJVJL DJAJVLRLG S PLA 25 26 I NJVLA V H V FLR KLALA D D TLW ELPJF AJS G K T S 50 51 EJSLGJEJLJHLGLITJTETE ELFLVLELGLILVLKLLELILDT 75 76 [KLSLYW]KLALLLGLILS[PLFLHLELHLALELVLVLFLTALNLD[S 100 101 [G[PLRLVLTLILALLLS[PLYLS]VLS]TLTALVJV T NJP 125 126 K]E C	1
13+	$ \begin{bmatrix} G[P[T G T G E S K C P]L]M V K]V L D A V[R G S P A 25 \\ 26 I N[V]A[V H[V F R K A]A D D]T]W]E P F[A[S[G K T S 50 \\ 51]E S[G E]L]H G]L T T E[E]E F]V E[G I Y K[V E[I [D[T 75 \\ 76 K]S[Y W K[A L[G[I [S[P]F]H]E[H[A]E[V V]F[T[A]N[D[S 100 \\ 101[G P R[R]Y[T[I [A[A]L[L]S[P[Y]S[Y]S[T[T[A V V T N P 125 \\ 126 K E]]]]]]]]]]]]]]]]]] $	
14+	GLPLTLG T GLE S KICIPILIM VIKIVL DIALVLRLGLSLPLA 25 26 I NUV A VLHIVLFLRIK AIA D DLT WLEIPIFLALSIGIKLTLS 50 51 LE SLGLEILIH GLLITITELELFLVLELGLILVKIVLELLDT 75 76 [KLSIYWLKLALLGLILSIPIFLHLELHLALELVVLFLTAINDIS 100 101 LGIPLRLRIVILLALLLSIPIYLSIVLSITLTALV V T N P 125 126 K]E C	
15+	GLPLTLG TLG E S KICLPILIM VIKIVL DA V R G S P A 25 26 I N V A VLH VLF R K AA DDLT W ELP F A SLGLK TLS 50 51 LELS GLELLHIGLTTTE ELEIFIVE GILVLKLVLELILDT 75 76 KLSIY WLKALLGLILSLPLFLELHALELALALELLSLPLYLSLTLALV V T N P 125 101 LGLPLRLKLVLLSLPLYLSLVLSLTLALV V T N P 125	



 26[I Ν[V Α[V]Η V F[R]K[A Α[D[D[T]W]E[P[F[A S[G K T S 50

 51 E S G E L H]G]L]T[T[E]E]E[F]V]E G I]Y]K[V[E[I]D]T 75

 76[K[S Y[W K[A]L[G]L]S[P[F]H]E]H]A[E[V[V[F[T]A[N[D]S 100

 101[G]P[R]R]Y[T[I]A]ALL[L[S[P[Y[S[Y[S[T]T]A]V V T N]P 125

 126]K]E C



Figure S14. Fragment maps obtained using ProSight Lite for each charge state of TTR from 3 mJ UVPD (1 pulse). Backbone cleavage markers are shown as colored flags: a,x: green; b,y: blue; c,z: red. On the right is the crystal structure of TTR (pdb 2rox) colored to reflect the average normalized abundances of the backbone cleavages that yield the sequence ions on each fragment map.

α-Hb

VLL S P ALD K T N V KJALAJWJGJKLVJGJALHJAJGLE YJG 25 2°JAJEJALLEJRJMJFJLJSJFLPJTJTJKJTJYJFLP HJFDLLJSJH 50 51JG S A Q V KJGJHJGLK K VJAJD ALL TLNLALVJALHJVDDD 75 76 MLP NLA LJSLA L SLDL HJAJH K L R V DLP V NLFLKLL 100 101LLSLHJC L L V T LLALALHLLLPLALEJFLTLPLALVJHLALSLL 125 126(DLKLFJLLALSLV S T V L T S K Y R C

β-**Hb**

N HL T P ELE K SLA VLTALIWGKVVNVDEVGG 25 20]EIALLG R LILLVVVVVPWTQRFFFESFG DLLS T 50 51]P D AVVM G NPK VKA H GKK V LGA FS DLG L 75 76 ALH L D NLL KLG TLFLA TL S ELL H CLDLK L H VLDLP 100 101 LEINLFLRLLLGINVLLVCLV L A HIHFLGIKLEFTPP 125 126 VLQLALVQLKUV AGV A N A L A H KY H C

V H L T P ELE K S A VITALIWIGIKIVINIVDEIVLGG 25 26]EJALIGIR LIL VIVIYP W TIQIRIFLFIEISIFIG DILIST 50 51]PID A VIM G NIP KIVIK AH G K K V LLG AFIS D GL 75 76 A HLL DIN L KIGIT FLA T LLS EILLH C D KLLHIVLDIP 100 101 E N FLRILL GINIVLLVLCIV LIA HLH FLGIKLELFITIPIP 125 126[VLQLALALYLQLKIV V A G VIA NIALL A H K Y H ○

V H L T P ELELK SLAV T A LÌWÌGÌK VÌNÌVÌD EÌVÌGÌG 25 26 ELALLIGIRLLILÌVÌVÌYÌP W TÌQÌRÌFÌFÌEÌSÌFÌG DÌL 5 T 50 51 P DÌAÌVÌMÌGÌNÌP K VÌKÌAÌHÌG KİKLVÌLÌGÌAÌFÌS D GL 75 76ÌALHLLDÌN L K G TLFLALTLL S ELL H CLD K LLHLVÌDLP 100 101 LEINLFLRLLL GÌNLVLLVLCLVLLALHLHLFLGÌKLEIFLTLPLP 125 126ÌVLQLALALY QLK V V A G V A N AÌLÌALH K Y H ○

 V [H L T[P E[E K S A]V T A]L]W]G]K]V]N]V]D E[V]G]G 25

 26]E]A]L]G]R]L]L]V]V]Y]P W]T]Q]R]F F]E]S]F]G]D L]S]T 50

 51]P]D]A V M G N[P K]V]K[A]H G K]K V L G A]F]S D]G[L 75

 76 A[H]L[D N]L K G[T F]A T[L]S[E L H C[D]K]L[H V[D[P 100

 101 E[N[F[R[L L]G]N V[L]V]C[V]L[A[H H]F[G]K[E[F[T[P[P 125

 126[V]Q[A[A[Y]Q[K]V[V A G V A N A L A H K Y H C

16+
I U H L T P E E K S A VÌT AÌLÌWÌGÌKÌVÌNÌVÌDÌEÌVÌGÌG 25
26]EÌALLGÌRÌLÌLÌV VÌYÌP WÌT QÌRÌFÌFÌEÌSÌFÌGÌD L SÌT 50
51ÌPÌDÌA V MÌGÌNÌP K V KÌA H GIKIK VÌL G AÌFÌS D GÌL 75
76[A H L DÌNLL K GIT FÌA TLL S ELL H CÌD KLLIHIVIDIP 100
101[EIN FIRILL G N VLLIVIC VLL AIHIHIFIGÌKIEIFITIPIP 125
126[VIQ ALAIVIQÌKIVIV AÌGIV A N AÌL A H K Y H C

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        V[H L T P E E K S A V]T]A L]W]G]K]V]N]V]D E]V]G]G 25

        2°]E]A]L G]R L L V V]Y]P[W]T]Q]R]F]F]E S F]G]D]L S]T 50

        51]P D A V M G N]P[K V K]A H G K K V L G A F]S D G]L 75

        **

        76 A H L[D N L K G T F A T[L S[E L H]C D K L[H V[D[P 100

        101 E[N]F]R L[L G[N V L[V]C[V L]A H[H]F[G[K]E[F[T]P[P 125

        126 [V]Q[A[A]Y[Q K V[V A G V A N]A L A H K Y H ○
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Figure S15. Fragment maps obtained using ProSight Lite for each charge state of Hb from 3 mJ UVPD (1 pulse) for the alpha subunit (left) and beta subunit (right). Backbone cleavage markers are shown as colored flags: a,x: green; b,y: blue; c,z: red. On the bottom is the crystal structure of Hb (pdb 1qzx) colored to reflect the average normalized abundances of the backbone cleavages that yield the sequence ions on each fragment map.

	N Q T D M S R K A FÌVÌFÌPÌKÌEÌSÌDÌTÌSÌYÌVÌSÌLÌKÌA P 25		N Q T D M S R K A]F]V]F]P]K]E]S]D]T]S]Y]V]S]L]K <mark>]</mark> A]P ²⁵
	26 LITKPILKAFT V C LHFYTLELSSTRGYS 50		20]L]T]K[P]L]K]A <mark>[</mark> F]T V]C L H F Y[T]E]L]S S]T]R G Y S 50
	⁵¹ I [F S [Y] A] T K R Q D N E I I I F W S K D I G Y S F 75		51 I]F]S]Y A T]K]R Q D]N]E I]L]I FWSKD I]GYSF 75
	76] T V] G [G S E I] L F E V P E V] T V A P V H] I C T S W 100		76 T V G G SLE IL F EV P E VLT V A PV H I C TS W 100
17+	101 E SALS G I V E F W VLD G K P RLV R KLSL KK G Y 125	24+	101 ETS A S GLI V E FWVLDLGLK P RVRK SLLK K GLY 125
	126 T V G ALE A SLILI LLG QLE QDLSLFLGLGLNLFLELGLSQ 150		126 TIV G A E A SII I L G Q E Q DISIFIGIGINIFIEIGISIQ 150
	151 [S [L [V] G [D [I [G [N [V [N [M W[D [F [V [L [S [P [D [E [I] N [T [I] Y 175		151 LSLLVGDLIGNVVNMVDLFVLLSLPD ELINLTIY 175
	176[L[G[G[P[F[S[P[Ν[V[L[Ν[Ψ[R[Α[L[Κ[Υ[Ε[V[Q[G[Ε[V F Τ 200		176 ΙΙΙGΙGΙΡΙFΙSΙΡΙΝΙVΙΙΙΝΊΝΙΡΙΑΙΙΙΙΚΙΥΙΕΙVΙQΙGΙΕΙV F T 200
	201 KPQLWPC		201 K P Q L W P C
	N Q T D M S R K A F V]F P]K]E]S]D]T]S]Y]V]S]L]K]A[P 25		N QLT DLM S R K A F V]F]P]K]E]S]D]T]S]Y]V]S]L]K]A]P 25
	26]L]T]K P]L]K]A]F T V C L H F Y]T]E]L S S T]R G Y S 50		²ĕ]L]T]K]P]L]K]AĽF]T]VŪC LHFYTE]LSS]TRGYS ⁵♡
	51 I F S Y]A]T K R[Q]D[N]E]IL I F W S K D]I]G Y S F 75	25+	51 I F S]Y]A]T]K]R Q]D N]E]I L I F W S K D I G Y]S F 75
	76 T V G G]S ELILL F E V P E V T VLA P V H I C T S W 100		76 T V G G S E I L F E VÌPÌE V T V AÌPÌV HÌ I 🕻 T S[W 100
18+	101 ELSLASGIVEFWVDGKPRVRKSLKKGY 125		101 E]S ALS G ILV E FW VLDLG KLPRVK SLLK K G Y 125
10	126 T V G ALE ALSLI ILLG Q E Q D SLEGGNEELGLS Q 150		126 T V G A ELASII I L G Q ELQ DES FLOLOUNEFLE G SLQ 150
	151 LSLLVGLUIGNVNMWDLFVLLSLPDELINLTILY 175		151 [S[L[V[G[D]] G[N[V[N]M W]D] F[V[L[S[P[D[E]] [N T I] Y 175
	176 LLGGPFSPNVLLNWRALLKYEVQGE V F T 200		176LLGGGPFSSPNVLLNWRALLKYEVQGE VFT 200
	201 K P Q L W P C		201 K P Q L W P C
	N Q T D M S R K A F V]F]P]K]E]S]D]T]S]Y]V]S]L]K]A]P 25		N Q T DĮM S R K A F VĮFĮPĮKĮEĮSĮDĮTĮSĮYĮVĮSĮLĮKĮAĮP 25
	26]L]T[K P]L]K]A]F]T V CILHFY]T E]LS S]T]R]G]Y S 50		²° <mark>]</mark> L]T]KÌPÌLÌKÌAÌF[T[V[<mark>C</mark> [L H[F[Y T E L]S SÌT]R GÌYÌS ⁵°
	51]I]F]S Y]A T K R Q]D N E]IL I F]W S[K DLI G Y S F 75	26+	51 I F S Y A T K R Q D N E I L I F W S K D I G Y S F 75
	76 T V G G S E I L F E V P E V T V ALP V H I C T S W 100		⁷⁶ T V G G SLE I]L FLE V PLEÌV TÌV AÌPÌV HÌI CT S W 100
19+	101 E SALS G I V ELF W V D GLKLPLRLV R KLS LK K GLY 125		101]ELS]ALSLGLI]VLE FLWLV DGKPLRLVRKLSLKKGY 125
10	126 TIVG ALE A SII I LLG QE QLDLSLFLGLG NLFLELGLSLQ 150		126 T V G A E A S I I L G Q E Q D SLFLG GUNLFLEGLS Q 150
	151 LSLL VEGLIE GIN VINIM WDEFVLLSEPDELINLTLIY 175		151 [S[L[V[G[D[I[G[Ν[V[Ν[Μ[Μ[Ν[D[F[V[L[S]P]D]E]I[Ν]Τ]I]Υ 175
	176LL[G[G[PLFLS[P[NLVLL[N[WLRLALL[KLYLELVLQ[GLE V F T 200		176 ΙΙ [G[G]Ρ]F[S]Ρ[Ν[V[Ι[Ν[Ψ[R[A]Ι[Κ[Υ[Ε[V[Q]G[Ε V F T 200
	201 K P Q L W P C		201 K P Q L W P C
23+	N Q T D M S R K A)FÌVÌF PÌKÌEÌSÌDÌTÌSÌYÌVÌSÌLÌKÌA P 25		N QLTLDUM S R K A F VLFLPLK]E]S]DLT]SLY]V]SLLK]A]P 25
	26]L]T]K]P]L]K]A]F]T V C L]H F Y T]E L S S T R G Y S 50	27+	26 L]T]K]PLL]K]A]FLT V[C L HLFLY T ELL]S S T R G Y S 50
	51]IFSYATKRQDNEILIFWSKDIGYSF 75		51 I F S Y A T K R Q D N E I L I [F]W S K D I G Y S F 75
	76 T V G G S E] I L FLE V] P E V TLVA P V H I C T S W 100		76 T) V G G S E I L) F E V P E V T V A P V) H I C T S W 100
	101 ELSIALS G I V ELF W V DLG K P R VIRIKLSLL K K G Y 125		101 ELS ALSLG I V E F WLV DLGLKLPRVRK S L K K G Y 125
	126 T V G A E ALS ILI L G Q E Q DISIFIGION FLEIGIS Q 150		126 T V G A E A S I I L G Q E Q DLSLF G GLNLFLELGSQ 150
	151 SLLVGDIGNVNMVDFVLLSPDEIINTIY 175		151 [S]L[V[G[D]] [G[N]V[N[M[W[D][F[V]L[S[P[D][F]]]N]T]]]Y 175
	176LLGGGPLFLSLPINLVLLNWRALLKLYLELVQG E V F T 200		¹⁷⁶ [L]G]G]P[F[S[P[Ν[V]L[Ν]W[R[Α[L[Κ[Υ[Ε]V[Q[G]E V]F T 200
	201 K P Q L W P C		201 K P Q LWP C



Figure S16. Fragment maps obtained using ProSight Lite for each charge state of CRP from 3 mJ UVPD (1 pulse). The gray boxes on Cys36 and Cys97 reflect the loss of one hydrogen each to account for the disulfide bond. Backbone cleavage markers are shown as colored flags: a,x: green; b,y: blue; c,z: red. On the bottom is the crystal structure of CRP (pdb 1b09) colored to reflect the average normalized abundances of the backbone cleavages that yield the sequence ions on each fragment map.



Figure S17. Weighted average charge of ejected monomers using varying lab frame collision energies for HCD of the precursors of a) 10+ to 15+ streptavidin, b) 11+ to 17+ transthyretin, c,d) 12+ to 17+ hemoglobin, and e) 17+ to 19+ and 23+ to 27+ CRP. Ejected monomers of Hb are shown separately as c) α -subunits and d) β -subunits. Error bars indicate the standard deviation of replicate data. Values in parentheses next to each precursor charge indicate the theoretical charge state of each monomer following charge-symmetric partitioning.



Figure S18. Energy variable fragmentation graphs showing the abundance of low-charged monomers (3+, 4+), high-charged monomers (5+, 6+), and sequence ions from the a) 11+, b) 13+, and c) 15+ precursors of SA using varying laser pulse energies for UVPD.



Figure S19. Energy variable fragmentation graphs showing the abundance of low-charged monomers (3+, 4+), high-charged monomers (5+, 7+), and sequence ions from the a) 11+, b) 14+, and c) 17+ precursors of TTR using varying laser pulse energies for UVPD.



Figure S20. Energy variable fragmentation graphs showing the abundance of low-charged monomers (4+), high-charged monomers (6+, 8+), and sequence ions from the a) 12+, b) 15+, and c) 17+ precursors of α -Hb using varying laser pulse energies for UVPD.



Figure S21. Energy variable fragmentation graphs showing the abundance of low-charged monomers (4+), high-charged monomers (6+, 8+), and sequence ions from the a) 12+, b) 15+, and c) 17+ precursors of β -Hb using varying laser pulse energies for UVPD.



Figure S22. Energy variable fragmentation graphs showing the abundance of low-charged monomers (5+), high-charged monomers (8+, 9+), and sequence ions from the a) 17+, b) 23+, and c) 27+ precursors of CRP using varying laser pulse energies for UVPD.



Figure S23. Energy variable fragmentation graphs showing the summed abundance of monomers and trimers from charge reduced (a, b), standard (c, d), and supercharged streptavidin (e) upon UVPD.



Figure S24. Energy variable fragmentation graphs showing the summed abundance of monomers and trimers from charge reduced (a, b), standard (c, d, e), and supercharged transthyretin (f, g) upon UVPD.



Figure S25. Energy variable fragmentation graphs showing the summed abundance of monomers, trimers, and tetramers from charge reduced (a, b, c), standard (d, e, f), and supercharged C-reactive protein (g, h) upon UVPD. Charge states of tetramers and trimers that overlap with other subcomplexes were not included (e.g. 12+ trimer, 16+ tetramer).