Supporting Information for Analytical Chemistry

Supporting Information

An Interlaboratory Evaluation of Drift Tube Ion Mobility - Mass Spectrometry Collision Cross Section Measurements

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Abstract

Collision cross section (CCS) measurements resulting from ion mobility – mass spectrometry (IM-MS) experiments provide a promising orthogonal dimension of structural information in MS based analytical separations. As with any molecular identifier, interlaboratory standardization must precede broad range integration into analytical workflows. In this study we present a reference drift tube ion mobility mass spectrometer (DTIM-MS) where improvements on the measurement accuracy of experimental parameters influencing IM separations provide standardized drift tube, nitrogen CCS values ($^{DT}CCS_{N2}$) for over 120 unique ion species with the lowest measurement uncertainty to date. The reproducibility of these $^{DT}CCS_{N2}$ values are evaluated across three additional laboratories on a commercially-available DTIM-MS instrument. The traditional stepped field CCS method performs with a relative standard deviation (RSD) of 0.29% for all ion species across the three additional laboratories. The calibrated single field CCS method, which is compatible with a wide range of chromatographic inlet systems, performs with an average, absolute bias of 0.54% to the standardized stepped field $^{DT}CCS_{N2}$ values on the reference system. The low RSD and biases observed in this interlaboratory study illustrate the potential of DTIM-MS for providing a molecular identifier for a broad range of discovery based analyses.

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Interlaboratory Study Stepped field Agilent Reference Method DB.xlsx

This excel data file contains *m/z*, CCS, and other descriptors of the biological standards analyzed in this study in a database format.

Table S1. Chemical standards information for lipids and metabolites.

Compound	Sigma-Aldrich p/n	Agilent p/n
Lipids		
C12:0	61609-5G	
C15:0	91446-5G	
C16:1	76169-1G	
C16:0	76119-5G	
C17:0	H3500-1G	
C18:3	L2376-500MG	
C18:2	L1376-500MG	
C18:1	O1008-1G	
C18:0	85679-500MG	
C20:4	A3611-100MG	
C20:3	E4504-10MG	
C20:2	E3127-25MG	
C20:1	44878-100MG	
C20:0	A3631-500MG	
C24:0	L6641-100MG	
Metabolites		
Creatinine	C4255-10G	
L-proline		AA Standard 5061-3330
L-leucine		AA Standard 5061-3330
L-isoleucine		AA Standard 5061-3330
L-aspartic acid		AA Standard 5061-3330
Homocysteine	44925-25MG	
L-glutamic acid		AA Standard 5061-3330
L-lysine		AA Standard 5061-3330
L-methionine		AA Standard 5061-3330
L-histidine		AA Standard 5061-3330
L-phenylalanine		AA Standard 5061-3330
Uric acid	U0881-10G	
L-arginine		AA Standard 5061-3330
L-tyrosine		AA Standard 5061-3330
Glucose	G8270-100G	
L-cystine		AA Standard 5061-3330
Pyridoxal 5’-phosphate	P9255-1G	
Cortisol	H4001-1G	
Levomefolic acid	M0132-5MG	
Peptides		
Angiotensin I	A9650-1MG	
Angiotensin II	A9525-1MG	
Bradykinin	B3259-1MG	
Renin	R8129-1MG	
Substance P	S6883-1MG	
Neurotensin	N6383-1MG	
Melittin	M2272-1MG	

Tune Mix Ions		
Betaine		G1969-85000
Trifluoroaceitc acid ammonium salt		G1969-85000
Hexamethoxyphosphazine		G1969-85000
Hexakis(1H, 1H, 3H-tetrafluoropropoxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 5H-octafluoropentoxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 7H-dodecafluoroheptoxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 9H-perfluorononyloxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 4H-hexafluorobutyloxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 6H-decafluorohexyloxy) phosphazine		G1969-85000
Hexakis(1H, 1H, 8H-tetradecafluorooctyloxy) phosphazine		G1969-85000
Tris(trifluoromethyl)-1,3,5-triazine		G1969-85000
Tris(heptafluoropropyl)-1,3,5-triazine		G1969-85000

Table S2. Chemical Standards Information for peptides and proteins

Compound	**Sigma-Aldrich p/n**	**Agilent p/n**
Proteins		
Ubiquitin	U6253-5MG	
Cytochrome c	C2506-50MG	
Digest Peptides from Cytochrome c		
GITWK		
IFVQK		
YIPGTK		
MIFAGIK		
EDLIAYLK		
TGPNLHGLFGR		
TGQAPGFTYTDANK		
EETLMEYLENPK		

Table S3. Drift Tube Settings for Stepped Field Experiments

Time Sequence	Time (min)	Drift Tube Entrance (V)	Drift Tube Exit (V)	Rear Funnel Entrance (V)	Rear Funnel Exit (V)
1	0.0 – 0.5	1074	224	217.5	45
2	0.5 – 1.0	1174	224	217.5	45
3	1.0 – 1.5	1274	224	217.5	45
4	1.5 – 2.0	1374	224	217.5	45
5	2.0 – 2.5	1474	224	217.5	45
6	2.5 – 3.0	1574	224	217.5	45
7	3.0 – 3.5	1674	224	217.5	45

Table S4. Drift Tube Settings for Stepped Field Experiments – Fragile Ion Tune

Time Sequence	Time (min)	Drift Tube Entrance (V)	Drift Tube Exit (V)	Rear Funnel Entrance (V)	Rear Funnel Exit (V)
1	0.0 – 0.5	1067	217	210.5	38
2	0.5 – 1.0	1167	217	210.5	38
3	1.0 – 1.5	1267	217	210.5	38
4	1.5 – 2.0	1367	217	210.5	38
5	2.0 – 2.5	1467	217	210.5	38
6	2.5 – 3.0	1567	217	210.5	38
7	3.0 – 3.5	1667	217	210.5	38

Table S5. Drift Tube Settings for Single Field Experiments

Time Sequence	Time (min)	Drift Tube Entrance (V)	Drift Tube Exit (V)	Rear Funnel Entrance (V)	Rear Funnel Exit (V)
1	0.0 – 0.5	1574	224	217.5	45

Single Field Experiment

The tune mix CCS values obtained in this study on the Reference System are used for the single field experiments as they represent the lowest measurement uncertainty to date due to thorough error analysis of the experimental parameters that influence CCS measurements. In a single field experiment, the calibrant tune mix ions are first run in a single field experiment and their drift times are measured and aligned with the stepped field CCS values mentioned above. The slope (ß) and intercept (t_{fix}) (mentioned in the manuscript for Eqn (3)) are determined from the regression of these measured drift times and standard CCS values. The sample is then run at the exact same single field settings as the tune ions and according to Eqn 3 the drift times of the analytes present are converted into CCS values.

Table S6. Correcting CCS Data for Instrument Improvements on Agilent Reference System

	This is a 0.5% factor	*This is a 0.2% factor*	*This is 0.2% factor*	
Original CCS	**Update Measured Length**	**Apply Voltage Slope**	**Apply Temperature Correction**	**New Agilent Value**
	$= (78.12/78.302)^2$			
	0.9954	1.00284 (+) 1.0013 (-)	1.0019	
622 Example				
203.20	202.26	202.83	203.22	203.22
1633 Example				
320.60	319.11	319.53	320.13	320.13

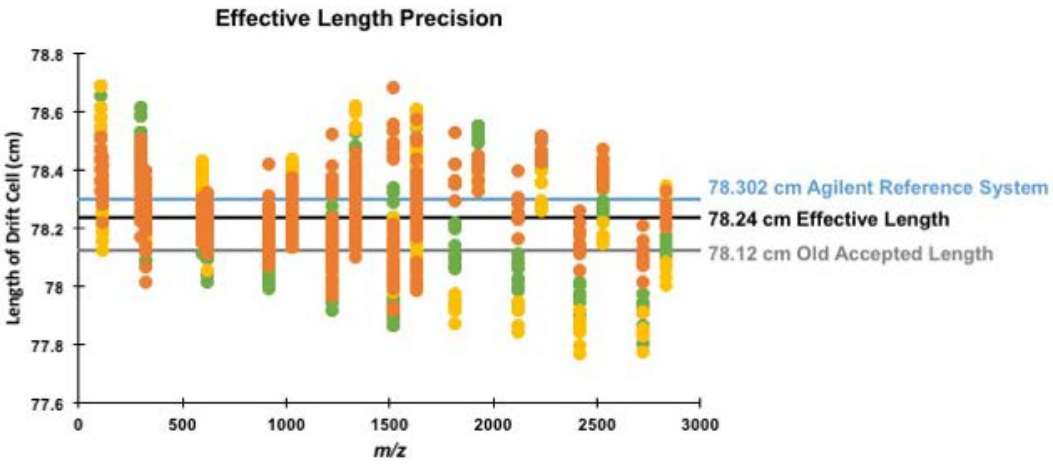


Figure S1. To account for the instrument updates on the Agilent Reference System as well as end effects due to front and rear funnels on both ends of the drift cell an effective length was determined for the other three labs in this study (BOKU, green; PNNL, orange; and Vanderbilt, yellow). An effective length was determined for each tune mix CCS measurement obtained on each of the three instruments, on three different days, and at four different tune settings. This resulted in 1,413 effective lengths which average to 78.24 ± 0.16 cm. This precision across the three different labs, different tune settings, and over the mass range gives us confidence in using 78.24 cm as the new drift tube length. The metrology measured drift cell length for the gridded Agilent Reference System is also noted as 78.302 cm and the previously accepted production length of 78.12 cm is shown for reference.

Table S7. Converting CCS Values with Effective Length

	This is the 3 day, 4 tune settings Agilent CCS Reference Value		*This is the consensus Effective Length of 78.24 cm*	
Original CCS (Å^2)	**Compare to Agilent Reference System Value**	**Determine Effective Length (cm)**	**Use Effective Length to Obtain New CCS**	**New CCS (Å^2)**
622 Example				
203.30	$\sqrt{}$(203.30/202.96) = 1.000849	1.000849 * 78.12 = 78.19	203.3 * (78.12/78.24)^2	202.69
1633 Example				
321.30	$\sqrt{}$(321.30/319.03) = 1.003552	1.003552 * 78.12 = 78.40	321.30 * (78.12/78.24)^2	320.35

Table S8. Average CCS Values from BOKU, PNNL and VU for Tune Mix Ions

Tune Mix Ions – Positive Mode				
m/z	**CCS (Å^2)**	**%RSD *(interlab.)***	**n**	**Bias**
118	121.51 ± 0.21	0.17%	81	0.17%
322	153.67 ± 0.20	0.13%	108	-0.04%
622	202.67 ± 0.24	0.12%	108	-0.14%
922	243.05 ± 0.34	0.14%	108	-0.24%
1222	281.25 ± 0.55	0.20%	108	-0.34%
1522	315.79 ± 0.81	0.26%	99	-0.37%
1822	350.43 ± 1.76	0.50%	27	-0.23%
2122	381.40 ± 1.54	0.40%	27	-0.43%
2422	410.28 ± 1.49	0.36%	27	-0.65%
2722	437.94 ± 1.39	0.32%	27	-0.74%
Tune Mix Ions – Negative Mode				
m/z	**CCS (Å^2)**	**%RSD *(interlab.)***	**n**	**Bias**
113	108.93 ± 0.25	0.23%	45	0.65%
302	140.66 ± 0.32	0.23%	108	0.44%
602	180.94 ± 0.31	0.17%	108	0.09%
1034	255.59 ± 0.53	0.21%	108	0.10%
1334	285.29 ± 0.78	0.27%	108	0.19%
1634	319.53 ± 1.34	0.42%	108	0.16%
1934	354.32 ± 0.64	0.18%	27	0.50%
2234	382.39 ± 0.79	0.21%	27	0.43%
2534	413.67 ± 1.03	0.25%	27	0.16%
2834	431.98 ± 1.12	0.26%	27	-0.15%

Table S9. Positive Mode Tune Mix Values (Å^2)

Lab	Collision Cross Section Data				
	250 Fragile	250 Stable	1700	3200	Summary
	118 Tune Mix Ion				
Agilent	121.41 ± 0.22	121.37 ± 0.14	121.13 ± 0.22		121.30 ± 0.20 (0.17%)
BOKU	121.57 ± 0.17	121.56 ± 0.17	121.69 ± 0.17		121.51 ± 0.21 (0.17%)
PNNL	121.69 ± 0.12	121.53 ± 0.14	121.60 ± 0.09		
VU	121.27 ± 0.25	121.36 ± 0.12	121.33 ± 0.09		
	322 Tune Mix Ion				
Agilent	153.79 ± 0.22	153.71 ± 0.13	153.52 ± 0.21	153.89 ± 0.22	153.73 ± 0.23 (0.15%)
BOKU	153.80 ± 0.19	153.76 ± 0.16	153.66 ± 0.13	153.43 ± 0.19	153.67 ± 0.20 (0.13%)
PNNL	154.02 ± 0.21	153.80 ± 0.14	153.71 ± 0.17	153.43 ± 0.36	
VU	153.52 ± 0.13	153.72 ± 0.08	153.57 ± 0.13	153.60 ± 0.22	
	622 Tune Mix Ions				
Agilent	203.06 ± 0.18	203.66 ± 0.22	202.77 ± 0.25	202.93 ± 0.35	202.96 ± 0.27 (0.14%)
BOKU	202.61 ± 0.22	202.56 ± 0.23	202.53 ± 0.23	202.27 ± 0.26	202.67 ± 0.24 (0.12%)
PNNL	202.94 ± 0.24	202.87 ± 0.19	202.69 ± 0.14	202.68 ± 0.33	
VU	202.52 ± 0.20	202.87 ± 0.13	202.83 ± 0.14	202.70 ± 0.16	
	922 Tune Mix Ions				
Agilent	243.71 ± 0.19	243.70 ± 0.20	243.48 ± 0.28	243.68 ± 0.45	243.64 ± 0.30 (0.12%)
BOKU	242.67 ± 0.38	242.66 ± 0.29	242.69 ± 0.33	243.06 ± 0.31	243.05 ± 0.34 (0.14%)
PNNL	243.09 ± 0.37	243.21 ± 0.21	243.29 ± 0.19	243.89 ± 0.39	
VU	242.81 ± 0.15	243.14 ± 0.20	243.06 ± 0.12	243.01 ± 0.17	
	1221 Tune Mix Ions				
Agilent	282.29 ± 0.20	282.14 ± 0.32	281.87 ± 0.29	282.49 ± 0.71	282.20 ± 0.47 (0.17%)
BOKU	280.45 ± 0.31	280.46 ± 0.32	280.47 ± 0.40	281.86 ± 0.38	281.25 ± 0.55 (0.20%)
PNNL	280.88 ± 0.41	281.34 ± 0.24	281.88 ± 0.20	283.16 ± 0.47	
VU	280.95 ± 0.44	281.14 ± 0.34	281.18 ± 0.15	281.21 ± 0.23	
	1521 Tune Mix Ions				
Agilent	316.96 ± 0.21	316.75 ± 0.37	316.53 ± 0.32	317.63 ± 0.73	316.96 ± 0.60 (0.19%)
BOKU	314.31 ± 0.31	314.51 ± 0.40	314.58 ± 0.51	317.01 ± 0.49	315.79 ± 0.81 (0.26%)
PNNL	315.09 ± 0.52	315.55 ± 0.26	316.47 ± 0.20	319.17 ± 0.61	
VU		315.94 ± 0.63	315.42 ± 0.24	315.62 ± 0.17	
	1821 Tune Mix Ions				
Agilent				351.25 ± 0.62	351.25 ± 0.62 (0.18%)
BOKU				350.27 ± 0.56	350.43 ± 1.76 (0.50%)
PNNL				352.53 ± 0.60	
VU				348.48 ± 0.24	

	2121 Tune Mix Ion				
Agilent				383.03 ± 0.64	383.03 ± 0.64 (0.17%)
BOKU				381.03 ± 0.54	381.40 ± 1.54 (0.40%)
PNNL				383.30 ± 0.63	
VU				379.86 ± 0.35	
	2421 Tune Mix Ion				
Agilent				412.96 ± 0.58	412.96 ± 0.58 (0.14%)
BOKU				409.90 ± 0.55	410.28 ± 1.49 (0.36%)
PNNL				412.10 ± 0.66	
VU				408.84 ± 0.47	
	2721 Tune Mix Ion				
Agilent				441.21 ± 0.59	441.21 ± 0.59 (0.13%)
BOKU				437.29 ± 0.64	437.94 ± 1.39 (0.32%)
PNNL				439.69 ± 0.64	
VU				436.84 ± 0.46	

Table S10. Negative Tune Mix CCS Values (Å^2)

Lab	Collision Cross Section Data				
	250 Fragile	250 Stable	1700	3200	Summary
	112 Tune Mix Ion				
Agilent	108.23 ± 0.20				108.23 ± 0.20 (0.19%)
BOKU	109.15 ± 0.19				108.93 ± 0.25 (0.23%)
PNNL	108.80 ± 0.16	108.80 ± 0.19			
VU	108.92 ± 0.33	108.98 ± 0.22			
	301 Tune Mix Ion				
Agilent	140.37 ± 0.18	140.15 ± 0.07	139.93 ± 0.15	139.70 ± 0.18	140.04 ± 0.29 (0.21%)
BOKU	141.09 ± 0.18	140.95 ± 0.29	140.86 ± 0.13	140.57 ± 0.14	140.66 ± 0.32 (0.23%)
PNNL	140.77 ± 0.09	140.77 ± 0.12	140.55 ± 0.10	140.10 ± 0.16	
VU	140.74 ± 0.10	140.74 ± 0.05	140.61 ± 0.15	140.12 ± 0.07	
	601 Tune Mix Ions				
Agilent	180.83 ± 0.19	180.75 ± 0.11	180.60 ± 0.13	180.89 ± 0.29	180.77 ± 0.21 (0.12%)
BOKU	181.01 ± 0.27	180.96 ± 0.40	180.82 ± 0.25	180.73 ± 0.22	180.94 ± 0.31 (0.17%)
PNNL	180.79 ± 0.19	180.80 ± 0.10	180.55 ± 0.14	180.75 ± 0.14	
VU	181.23 ± 0.25	181.20 ± 0.14	181.20 ± 0.30	181.22 ± 0.07	
	1033 Tune Mix Ions				
Agilent	255.52 ± 0.25	255.59 ± 0.14	255.16 ± 0.21	255.09 ± 0.35	255.34 ± 0.32 (0.13%)
BOKU	255.58 ± 0.28	255.99 ± 0.34	255.83 ± 0.23	254.81 ± 0.16	255.59 ± 0.53 (0.21%)
PNNL	255.32 ± 0.20	256.00 ± 0.14	255.42 ± 0.23	254.94 ± 0.25	
VU	256.02 ± 0.28	256.06 ± 0.09	256.15 ± 0.47	254.99 ± 0.28	
	1333 Tune Mix Ions				
Agilent	284.49 ± 0.27	282.75 ± 0.18	285.03 ± 0.22	284.76 ± 0.31	284.76 ± 0.31 (0.11%)
BOKU	284.42 ± 0.24	285.59 ± 0.69	285.99 ± 0.46	285.17 ± 0.16	285.29 ± 0.78 (0.27%)
PNNL	284.16 ± 0.24	285.45 ± 0.19	285.85 ± 0.50	284.94 ± 0.27	
VU	284.93 ± 0.48	285.01 ± 0.21	286.46 ± 1.15	285.49 ± 0.16	
	1633 Tune Mix Ions				
Agilent	318.26 ± 0.34	319.55 ± 0.15	319.83 ± 0.26	319.48 ± 0.28	319.03 ± 0.70 (0.22%)
BOKU	317.73 ± 0.34	319.39 ± 1.22	320.30 ± 0.79	320.52 ± 0.17	319.53 ± 1.34 (0.42%)
PNNL	317.40 ± 0.26	318.90 ± 0.29	320.90 ± 0.70	319.79 ± 0.32	
VU	318.88 ± 1.00	318.87 ± 0.39	320.95 ± 1.28	320.76 ± 0.38	
	1933 Tune Mix Ions				
Agilent				352.55 ± 0.27	352.55 ± 0.27 (0.08%)
BOKU				355.10 ± 0.21	354.32 ± 0.64 (0.18%)
PNNL				354.10 ± 0.37	
VU				353.76 ± 0.23	

	2233 Tune Mix Ion				
Agilent				380.74 ± 0.31	380.74 ± 0.31 (0.08%)
BOKU				382.81 ± 0.26	382.39 ± 0.79 (0.21%)
PNNL				382.97 ± 0.30	
VU				381.40 ± 0.47	
	2533 Tune Mix Ion				
Agilent				412.99 ± 0.31	412.99 ± 0.31 (0.07%)
BOKU				413.30 ± 0.35	413.67 ± 1.03 (0.25%)
PNNL				414.67 ± 0.47	
VU				413.03 ± 1.16	
	2833 Tune Mix Ion				
Agilent				432.62 ± 0.35	432.62 ± 0.35 (0.08%)
BOKU				431.51 ± 0.44	431.98 ± 1.12 (0.26%)
PNNL				432.83 ± 0.46	
VU				431.60 ± 1.56	

Table S11. Stepped Field CCS Values for Biological Standards (Å^2)

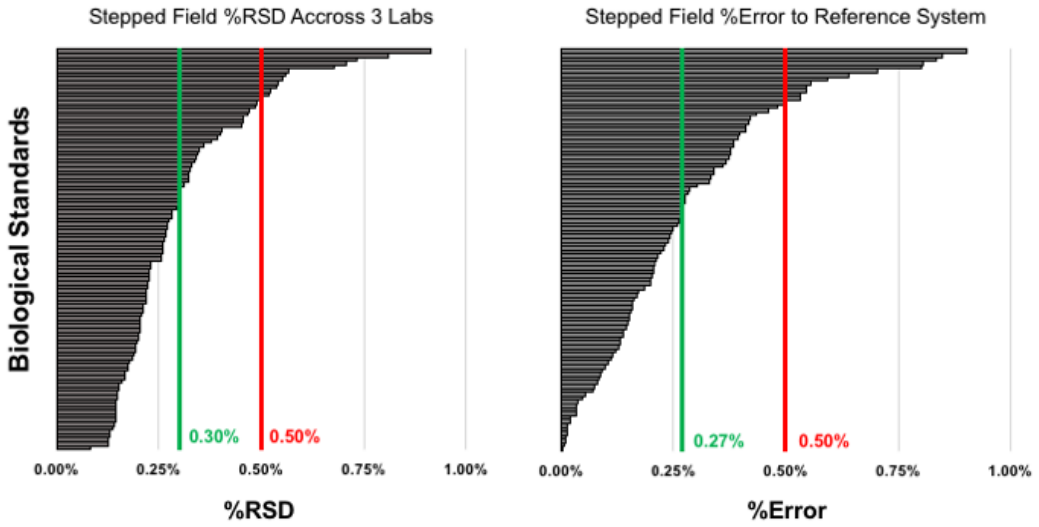
Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent	% Error
Lipids									
C12:0	199.1704	$[M-H]^-$	155.89 ± 0.15	154.96 ± 0.15	155.53 ± 0.27	155.46 ± 0.44	0.28 %	154.82 ± 0.10	0.41%
C15:0	241.2173	$[M-H]^-$	166.16 ± 0.12	165.59 ± 0.17	166.05 ± 0.41	165.93 ± 0.35	0.21 %	165.34 ± 0.06	0.36%
C16:1	253.2173	$[M-H]^-$	168.45 ± 0.06	167.86 ± 0.12	168.57 ± 0.15	168.29 ± 0.34	0.20 %	167.95 ± 0.06	0.20%
C16:0	255.233	$[M-H]^-$	169.78 ± 0.00	169.17 ± 0.06	169.57 ± 0.25	169.51 ± 0.30	0.18 %	169.12 ± 0.06	0.23%
C17:0	269.2486	$[M-H]^-$	174.13 ± 0.21	173.49 ± 0.12	174.06 ± 0.41	173.90 ± 0.38	0.22 %	173.41 ± 0.10	0.28%
C18:3	277.2173	$[M-H]^-$	175.06 ± 0.00	174.53 ± 0.10	174.63 ± 0.17	174.74 ± 0.26	0.15 %	174.88 ± 0.06	0.08%
C18:2	279.233	$[M-H]^-$	176.19 ± 0.15	175.37 ± 0.15	176.14 ± 0.46	175.90 ± 0.47	0.27 %	175.69 ± 0.06	0.12%
C18:1	281.2486	$[M-H]^-$	176.62 ± 0.06	176.07 ± 0.06	176.74 ± 0.10	176.48 ± 0.32	0.18 %	176.46 ± 0.15	0.01%
C18:0	283.2643	$[M-H]^-$	178.22 ± 0.06	177.95 ± 0.27	177.62 ± 0.12	177.93 ± 0.30	0.17 %	177.66 ± 0.06	0.15%
C20:4	303.233	$[M-H]^-$	183.30 ± 0.15	182.21 ± 0.06	182.91 ± 0.51	182.81 ± 0.55	0.30 %	183.66 ± 0.17	0.46%
C20:3	305.2486	$[M-H]^-$	183.20 ± 0.06	182.64 ± 0.21	182.21 ± 0.45	182.68 ± 0.50	0.27 %	183.02 ± 0.06	0.18%
C20:2	307.2643	$[M-H]^-$	183.44 ± 0.10	182.38 ± 0.17	183.01 ± 0.12	182.94 ± 0.48	0.26 %	183.32 ± 0.12	0.21%
C20:1	309.2799	$[M-H]^-$	185.23 ± 0.17	183.95 ± 0.21	184.79 ± 0.52	184.66 ± 0.64	0.34 %	184.29 ± 0.06	0.20%
C20:0	311.2956	$[M-H]^-$	186.56 ± 0.06	186.36 ± 0.23	186.20 ± 0.36	186.37 ± 0.27	0.14 %	186.17 ± 0.10	0.11%
C24:0	367.3582	$[M-H]^-$	202.15 ± 0.06	200.57 ± 0.10	201.04 ± 0.35	201.25 ± 0.72	0.36 %	201.11 ± 0.06	0.07%
Metabolites									
Creatinine	112.0516	$[M-H]^-$	121.43 ± 0.17	120.65 ± 0.20	121.25 ± 0.00	121.11 ± 0.38	0.31 %	120.70 ± 0.15	0.34%
Creatinine	114.0662	$[M+H]^+$	124.35 ± 0.06	123.93 ± 0.21	123.83 ± 0.06	124.04 ± 0.26	0.21 %	123.87 ± 0.00	0.14%
L-proline	116.0706	$[M+H]^+$	126.38 ± 0.27	126.48 ± 0.27	125.87 ± 0.36	126.24 ± 0.38	0.30 %	126.22 ± 0.21	0.02%
L-leucine	130.0874	$[M-H]^-$	132.72 ± 0.21	133.89 ± 0.14	134.31 ± 0.13	133.64 ± 0.72	0.54 %	132.51 ± 0.01	0.85%
L-isoleucine	130.0874	$[M-H]^-$	131.58 ± 0.19	132.62 ± 0.10	132.84 ± 0.18	132.34 ± 0.60	0.45 %	131.29 ± 0.06	0.81%
L-aspartic acid	132.0302	$[M-H]^-$	120.95 ± 0.00	120.71 ± 0.50	120.51 ± 0.15	120.72 ± 0.32	0.27 %	120.39 ± 0.40	0.28%
L-leucine	132.1019	$[M+H]^+$	135.37 ± 0.11	136.54 ± 0.16	137.03 ± 0.41	136.31 ± 0.77	0.57 %	135.56 ± 0.07	0.56%
L-isoleucine	132.1019	$[M+H]^+$	133.71 ± 0.09	134.80 ± 0.19	135.06 ± 0.38	134.52 ± 0.66	0.49 %	133.81 ± 0.04	0.53%

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent	% Error
Homocysteine	136.0427	$[M+H]^+$	131.40 ± 0.20	130.53 ± 0.25	130.73 ± 0.15	130.89 ± 0.43	0.33 %	130.78 ± 0.06	0.08%
Creatinine	136.0481	$[M+Na]^+$	133.49 ± 0.17	133.41 ± 0.17	133.11 ± 0.35	133.34 ± 0.27	0.21 %	132.99 ± 0.35	0.26%
L-glutamic acid	146.0459	$[M-H]^-$	126.01 ±0.06	125.64 ± 0.12	125.87 ± 0.10	125.84 ± 0.18	0.14 %	125.65 ± 0.15	0.15%
L-lysine	147.1128	$[M+H]^+$	132.01 ± 0.20	131.80 ± 0.35	131.40 ± 0.10	131.74 ± 0.34	0.26 %	131.62 ± 0.53	0.09%
L-methionine	150.0583	$[M+H]^+$	133.88 ± 0.38	133.65 ± 0.78	133.61 ± 0.10	133.71 ± 0.46	0.34 %	134.08 ± 0.40	0.27%
L-histidine	154.0622	$[M-H]^-$	130.50 ± 0.00	130.13 ± 0.06	130.33 ± 0.12	130.32 ± 0.17	0.13 %	130.01 ± 0.10	0.24%
L-histidine	156.0768	$[M+H]^+$	132.71 ± 0.10	132.41 ± 0.10	132.31 ± 0.10	132.47 ± 0.20	0.15 %	132.74 ± 0.12	0.20%
L-phenyl-alanine	164.0717	$[M-H]^-$	141.59 ± 0.06	140.95 ± 0.27	141.49 ± 0.41	141.34 ± 0.38	0.27 %	141.29 ± 0.20	0.04%
L-phenyl-alanine	166.0863	$[M+H]^+$	141.59 ± 0.32	141.02 ± 0.21	141.26 ± 0.10	141.29 ± 0.32	0.23 %	141.28 ± 0.06	0.01%
Uric acid	167.0211	$[M-H]^-$	127.71 ± 0.10	127.08 ± 0.00	127.41 ± 0.06	127.40 ± 0.28	0.22 %	126.93 ± 0.06	0.37%
L-arginine	173.1044	$[M-H]^-$	138.47 ± 0.15	138.24 ± 0.27	138.41 ± 0.21	138.37 ± 0.21	0.15 %	138.03 ± 0.06	0.25%
L-arginine	175.119	$[M+H]^+$	136.87 ± 0.15	136.63 ± 0.00	136.43 ± 0.00	136.64 ± 0.20	0.15 %	136.84 ± 0.06	0.15%
L-histidine	178.0587	$[M+Na]^+$	135.52 ± 0.00	134.82 ±0.20	135.19 ± 0.68	135.18 ± 0.47	0.35 %	135.48 ± 0.50	0.22%
L-tyrosine	180.0666	$[M-H]^-$	146.11 ± 0.15	145.75 ± 0.12	146.11 ± 0.35	145.99 ± 0.27	0.19 %	145.59 ± 0.35	0.28%
L-tyrosine	182.0812	$[M+H]^+$	146.68 ± 0.20	146.42 ± 0.31	146.55 ± 0.42	146.55 ± 0.30	0.21 %	146.45 ± 0.21	0.07%
Glucose	203.0526	$[M+Na]^+$	147.68 ± 0.06	147.72 ± 0.15	147.32 ± 0.12	147.57 ± 0.22	0.15 %	147.35 ± 0.29	0.15%
L-cystine	239.0166	$[M-H]^-$	144.64 ± 0.15	144.47 ± 0.10	144.47 ± 0.27	144.53 ± 0.18	0.13 %	144.39 ± 0.10	0.10%
L-cystine	241.0311	$[M+H]^+$	149.97 ± 0.12	149.57 ± 0.06	149.73 ± 0.06	149.76 ± 0.19	0.13 %	150.08 ± 0.06	0.21%
Pyridoxal Phosphate	246.0173	$[M-H]^-$	150.97 ± 0.12	150.54 ± 0.15	150.94 ± 0.15	150.82 ± 0.24	0.16 %	150.80 ± 0.10	0.01%
Pyridoxal Phosphate	248.0319	$[M+H]^+$	151.73 ± 0.17	151.38 ± 0.25	151.81 ± 0.00	151.64 ± 0.25	0.17 %	151.94 ± 0.10	0.20%
L-cystine	263.0131	$[M+Na]^+$	152.05 ± 0.15	151.34 ± 0.06	151.81 ± 0.17	151.73 ± 0.33	0.22 %	151.81 ± 0.10	0.05%
Pyridoxal Phosphate	270.0138	$[M+Na]^+$	162.04 ± 0.06	162.03 ± 0.21	162.03 ± 0.15	162.03 ± 0.13	0.08 %	161.40 ± 0.20	0.39%
Cortisol	363.2166	$[M+H]^+$	189.68 ± 0.12	188.88 ± 0.15	189.45 ± 0.06	189.34 ± 0.37	0.20 %	189.27 ± 0.10	0.03%
Cortisol	385.1985	$[M+Na]^+$	213.49 ± 0.15	213.91 ± 0.15	214.78 ± 0.12	214.06 ± 0.58	0.27 %	213.72 ± 0.00	0.16%
Levome-folic acid	458.1798	$[M-H]^-$	200.75 ± 0.15	199.57 ± 0.00	200.37 ± 0.10	200.23 ± 0.53	0.27 %	200.57 ± 0.12	0.17%

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent	% Error
Levome-folic acid	460.1939	$[M+H]^{+}$	197.92 ± 0.23	196.85 ± 0.27	197.12 ± 0.06	197.30 ± 0.52	0.26 %	197.52 ± 0.27	0.11%
Peptides									
GITWK	302.6763	$[M+2H]^{2+}$	275.31 ± 1.65	276.85 ± 0.65	272.22 ± 0.38	274.79 ± 2.23	0.81 %	273.43 ± 0.21	0.50%
IFVQK	317.6998	$[M+2H]^{2+}$	285.07 ± 0.04	289.88 ± 1.92	284.96 ± 0.36	286.64 ± 2.62	0.91 %	285.50 ± 0.25	0.40%
Angiotensin I	324.93	$[M+4H]^{4+}$	546.49 ± 0.32	548.67 ± 0.38	552.75 ± 0.41	549.30 ± 2.77	0.50 %	549.24 ± 0.06	0.01%
YIPGTK	339.6947	$[M+2H]^{2+}$	291.90 ± 0.16	293.54 ± 0.38	291.56 ± 0.05	292.34 ± 0.94	0.32 %	292.28 ± 0.38	0.02%
Angiotensin II	349.52	$[M+3H]^{3+}$	433.38 ± 0.12	435.63 ± 0.56	437.91 ± 0.06	435.64 ± 1.98	0.45 %	436.24 ± 0.21	0.14%
Bradykinin	354.19	$[M+3H]^{3+}$	445.98 ± 0.46	448.16 ± 0.41	447.29 ± 0.10	447.15 ± 1.00	0.22 %	447.61 ± 0.12	0.10%
MIFAGIK	390.2278	$[M+2H]^{2+}$	305.00 ± 0.13	307.12 ± 1.00	304.74 ± 0.06	305.62 ± 1.24	0.40 %	305.66 ± 0.06	0.01%
Angiotensin I	432.90	$[M+3H]^{3+}$	474.60 ± 0.15	476.05 ± 0.36	475.71 ± 0.21	475.45 ± 0.69	0.15 %	474.71 ± 0.15	0.16%
Renin	440.49	$[M+4H]^{4+}$	634.53 ± 0.38	636.34 ± 0.99	637.34 ± 0.35	636.07 ± 1.36	0.21 %	634.60 ± 0.35	0.23%
Substance P	449.92	$[M+3H]^{3+}$	493.97 ± 0.52	497.36 ± 1.13	497.83 ± 0.55	496.39 ± 1.94	0.39 %	495.74 ± 1.29	0.13%
EDLIAYLK	482.7711	$[M+2H]^{2+}$	340.62 ± 0.88	345.04 ± 0.27	340.02 ± 0.10	341.89 ± 2.42	0.71 %	340.98 ± 0.06	0.27%
Angiotensin II	523.78	$[M+2H]^{2+}$	354.86 ± 0.06	354.43 ± 0.65	355.97 ± 0.31	355.09 ± 0.78	0.22 %	353.79 ± 0.17	0.37%
Bradykinin	530.79	$[M+2H]^{2+}$	343.20 ± 0.12	343.47 ± 0.65	344.24 ± 0.36	343.64 ± 0.60	0.17 %	343.33 ± 0.10	0.09%
Neurotensin	558.31	$[M+3H]^{3+}$	526.11 ± 0.00	525.54 ± 1.01	527.92 ± 0.46	526.53 ± 1.21	0.23 %	525.02 ± 0.23	0.29%
Melittin	569.96	$[M+5H]^{5+}$	809.60 ± 0.25	811.40 ± 0.61	812.91 ± 0.98	811.30 ± 1.55	0.19 %	808.61 ± 0.60	0.33%
Melittin	569.96	$[M+5H]^{5+}$	850.35 ± 0.46	853.16 ± 1.93	852.56 ± 0.52	852.02 ± 1.64	0.19 %	844.40 ± 0.25	0.90%
TGPNLH-GLFGR	584.8147	$[M+2H]^{2+}$	367.46 ± 0.53	372.56 ± 0.32	367.77 ± 0.17	369.26 ± 2.50	0.68 %	369.09 ± 0.10	0.05%
Renin	586.98	$[M+3H]^{3+}$	520.99 ± 0.20	519.58 ± 0.82	521.76 ± 0.45	520.77 ± 1.07	0.21 %	518.81 ± 0.36	0.38%
GITWK	604.3453	$[M+H]^{+}$	237.30 ± 0.55	238.31 ± 0.15	236.80 ± 0.06	237.47 ± 0.72	0.31 %	236.33 ± 0.12	0.48%
IFVQK	634.3922	$[M+H]^{+}$	250.00 ± 0.41	250.71 ± 0.23	249.06 ± 0.12	249.93 ± 0.75	0.30 %	248.88 ± 0.15	0.42%
Angiotensin I	648.85	$[M+2H]^{2+}$	387.37 ± 0.20	386.13 ± 1.12	388.41 ± 0.15	387.30 ± 1.14	0.30 %	387.30 ± 0.20	0.00%
Substance P	674.37	$[M+2H]^{2+}$	399.77 ± 0.06	398.87 ± 0.46	400.88 ± 0.41	399.84 ± 0.93	0.23 %	399.88 ± 0.20	0.01%
YIPGTK	678.3821	$[M+H]^{+}$	255.94 ± 0.73	256.30 ± 0.15	254.93 ± 0.12	255.72 ± 0.72	0.28 %	254.75 ± 0.15	0.38%

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent	% Error
Melittin	712.20	$[M+4H]^{4+}$	760.16 ± 0.20	757.08 ± 0.06	760.03 ± 0.51	759.09 ± 1.53	0.20 %	756.79 ± 0.53	0.30%
TGQAPGF TYTDANK	735.8466	$[M+2H]^{2+}$	399.13 ± 0.44	402.95 ± 0.27	398.10 ± 0.06	400.06 ± 2.23	0.56 %	399.01 ± 0.21	0.26%
EETLME-YLENPK	748.3529	$[M+2H]^{2+}$	413.21 ± 0.36	418.20 ± 0.32	414.32 ± 0.10	415.24 ± 2.28	0.55 %	414.23 ± 0.21	0.24%
MIFAGIK	779.4484	$[M+H]^{+}$	276.71 ± 0.45	277.01 ± 0.21	275.74 ± 0.06	276.49 ± 0.63	0.23 %	275.34 ± 0.06	0.42%
Neurotensin	836.96	$[M+2H]^{2+}$	435.96 ± 0.21	434.89 ± 0.50	434.89 ± 0.32	435.25 ± 0.62	0.14 %	434.32 ± 0.21	0.21%
Renin	879.97	$[M+2H]^{2+}$	461.37 ± 0.17	461.10 ± 0.55	462.27 ± 0.40	461.58 ± 0.64	0.14 %	460.39 ± 0.41	0.26%
Melittin	949.26	$[M+3H]^{3+}$	724.17 ± 0.17	720.95 ± 0.36	723.47 ± 0.36	722.86 ± 1.49	0.21 %	721.07 ± 0.53	0.25%
EDLIAYL K	964.5349	$[M+H]^{+}$	310.63 ± 2.41	308.68 ± 0.06	308.15 ± 0.17	309.15 ± 1.66	0.54 %	307.47 ± 0.12	0.55%
Angiotensin II	1046.54	$[M+H]^{+}$	313.48 ± 0.10	314.20 ± 0.46	313.95 ± 0.23	313.87 ± 0.41	0.13 %	314.38 ± 0.15	0.16%
Bradykinin	1060.57	$[M+H]^{+}$	314.35 ± 0.38	312.97 ± 0.76	314.88 ± 0.70	314.07 ± 1.02	0.32 %	315.25 ± 0.30	0.38%
TGPNLH-GLFGR	1168.622 1	$[M+H]^{+}$	331.20 ± 0.32	331.37 ± 0.60	331.94 ± 0.12	331.51 ± 0.48	0.15 %	331.08 ± 0.06	0.13%
Angiotensin I	1296.69	$[M+H]^{+}$	356.01 ± 0.13	355.90 ± 5.19	356.50 ± 0.11	356.14 ± 2.61	0.73 %	357.31 ± 0.27	0.33%
Substance P	1347.74	$[M+H]^{+}$	361.00 ± 0.23	359.66 ± 0.23	360.96 ± 0.31	360.54 ± 0.70	0.19 %	362.51 ± 0.21	0.54%
Melittin	1423.38	$[M+2H]^{2+}$	612.01 ± 0.21	610.23 ± 0.91	614.12 ± 0.91	612.12 ± 1.81	0.30 %	613.37 ± 0.12	0.20%
TGQAPGF TYTDANK	1470.687 3	$[M+H]^{+}$	365.59 ± 3.27	366.96 ± 0.46	365.69 ± 0.25	366.08 ± 1.78	0.49 %	365.46 ± 0.36	0.17%
EETLME-YLENPK	1495.698 5	$[M+H]^{+}$	383.65 ± 1.14	387.44 ± 0.45	386.20 ± 0.45	385.76 ± 1.80	0.47 %	386.80 ± 0.27	0.27%
Proteins									
Ubiquitin	612.414	$[M+14H]^{14+}$	2715.62 ± 2.44	2734.60 ± 10.02	2720.21 ± 5.17	2723.48 ± 10.34	0.38%	2727.44 ± 4.95	0.15%
Cytochrome c	618.9162	$[M+20H]^{20+}$	3826.99 ± 4.87	3852.06 ± 1.51	3818.88 ± 5.23	3832.65 ± 15.42	0.40%	3816.11 ± 0.79	0.43%
Cytochrome c	651.438	$[M+19H]^{19+}$	3757.49 ± 3.48	3767.68 ± 3.48	3746.63 ± 4.74	3757.27 ± 9.73	0.26%	3741.89 ± 0.82	0.41%
Ubiquitin	659.446	$[M+13H]^{13+}$	2578.85 ± 1.63	2591.90 ± 2.23	2588.57 ± 5.55	2586.44 ± 6.64	0.26%	2577.71 ± 0.64	0.34%
Cytochrome c	687.5725	$[M+18H]^{18+}$	3668.95 ± 0.67	3641.74 ± 1.66	3651.62 ± 0.66	3654.10 ± 11.97	0.33%	3566.34 ± 1.57	0.03%
Ubiquitin	714.316	$[M+12H]^{12+}$	2427.98 ± 2.96	2433.11 ± 5.36	2410.61 ± 8.20	2423.90 ±11.42	0.47%	2424.69 ± 0.88	0.03%
Cytochrome c	727.9594	$[M+17H]^{17+}$	3560.97 ± 2.41	3519.89 ± 3.84	3547.40 ± 3.42	3542.75 ± 18.35	0.52%	3538.14 ± 0.28	0.13%
Cytochrome c	773.3916	$[M+16H]^{16+}$	3435.20 ± 4.33	3436.11 ± 33.57	3423.98 ± 2.56	3431.76 ± 17.95	0.52%	3403.30 ± 2.10	0.84%

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent	% Error
Ubiquitin	779.161	$[M+11H]^{11+}$	2360.15 ± 0.67	2357.44 ± 3.40	2367.36 ± 4.52	2361.65 ± 5.27	0.22%	2349.14 ± 0.78	0.53%
Ubiquitin	856.978	$[M+10H]^{10+}$	2201.57 ± 0.86	2205.63 ± 1.30	2215.95 ± 6.15	2207.71 ± 7.16	0.32%	2192.31 ± 0.61	0.70%
Ubiquitin	952.081	$[M+9H]^{9+}$	2073.39 ± 1.04	2066.92 ± 2.63	2066.28 ± 5.75	2068.86 ± 4.68	0.23%	2052.43 ± 0.65	0.80%
Ubiquitin	1070.964	$[M+8H]^{8+}$	1962.83 ± 0.85	1964.24 ± 3.32	1963.20 ± 4.37	1963.42 ± 2.85	0.15%	1950.96 ± 0.24	0.64%
Ubiquitin	1223.8	$[M+7H]^{7+}$	1783.44 ± 0.80	1784.88 ± 2.89	1771.76 ± 9.85	1780.02 ± 8.08	0.45%	1773.24 ± 1.27	0.38%
Ubiquitin	1223.8	$[M+7H]^{7+}$	1880.69 ± 1.54	1885.82 ± 2.89	1894.20 ± 3.66	1886.90 ± 6.40	0.34%	1875.77 ± 1.03	0.59%



Precision Across 3 Labs		Percent Error to Agilent Reference System	
Biological Standard	**%RSD**	**Biological Standard**	**%Error**
Angiotensin I $[M+4H]^{4+}$	0.50%	GITWK $[M+2H]^{2+}$	0.50%
Cytochrome c $[M+17H]^{17+}$	0.52%	L-isoleucine $[M+H]^{+}$	0.53%
Cytochrome c $[M+16H]^{16+}$	0.52%	Ubiquitin $[M+11H]11^{+}$	0.53%
EDLIAYLK $[M+H]^{+}$	0.54%	Substance P $[M+H]^{+}$	0.54%
L-leucine $[M-H]^{-}$	0.54%	EDLIAYLK $[M+H]^{+}$	0.55%
EETLMEYLENPK $[M+2H]^{2+}$	0.55%	L-leucine $[M+H]^{+}$	0.56%
TGQAPGFTYTDANK $[M+2H]^{2+}$	0.56%	Ubiquitin $[M+7H]^{7+}$	0.59%
L-leucine $[M+H]^{+}$	0.57%	Ubiquitin $[M+8H]^{8+}$	0.64%
TGPNLHGLFGR $[M+2H]^{2+}$	0.68%	Ubiquitin $[M+10H]^{10+}$	0.70%
EDLIAYLK $[M+2H]^{2+}$	0.71%	Ubiquitin $[M+9H]^{9+}$	0.80%
Angiotensin I $[M+H]^{+}$	0.73%	L-leucine $[M-H]^{-}$	0.81%
GITWK $[M+2H]^{2+}$	0.81%	Cytochrome c $[M+16H]^{16+}$	0.84%
IFVQK $[M+2H]^{2+}$	0.91%	L-leucine $[M-H]^{-}$	0.95%
		Melittin $[M+5H]^{5+}$	0.90%

Figure S2. Bar graphs showing the %RSD across the three labs and the %error of the average of the three labs to the Agilent Reference System. The average %RSD and %error is shown in green and an arbitrary threshold of 0.50% is shown in red. The biological standards that fall above the 0.50% threshold are listed in the table with their corresponding %RSD or %error. Reasons why these fall above the 0.50% threshold are discussed in the manuscript.

Table S12. Single Field CCS Values for Biological Standards (Å^2)

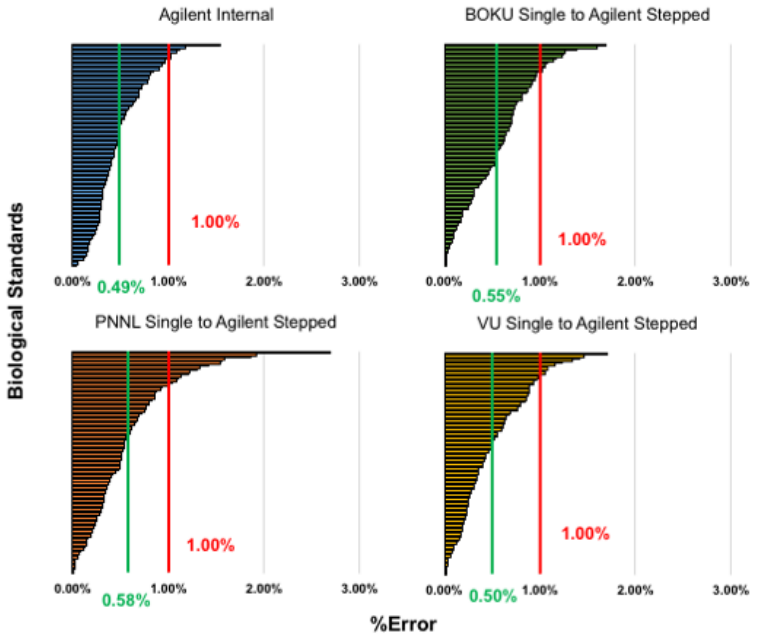
Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent
C12:0	199.1704	$[M-H]^-$	154.39 ± 0.02	155.60 ± 0.07	154.85 ± 0.06	154.95 ± 0.61	0.40 %	154.28 ± 0.02
C15:0	241.2173	$[M-H]^-$	165.11 ± 0.01	166.13 ± 0.06	165.68 ± 0.11	165.64 ± 0.51	0.31 %	164.86 ± 0.02
C16:1	253.2173	$[M-H]^-$	167.64 ± 0.02	168.51 ± 0.07	167.79 ± 0.08	167.98 ± 0.47	0.28 %	167.74 ± 0.05
C16:0	255.233	$[M-H]^-$	169.11 ± 0.01	170.05 ± 0.03	169.53 ± 0.03	169.56 ± 0.47	0.28 %	168.86 ± 0.08
C17:0	269.2486	$[M-H]^-$	173.34 ± 0.02	173.99 ± 0.24	173.52 ± 0.04	173.62 ± 0.34	0.19 %	172.89 ± 0.17
C18:3	277.2173	$[M-H]^-$	174.54 ± 0.02	174.92 ± 0.13	174.39 ± 0.03	174.62 ± 0.28	0.16 %	174.28 ± 0.15
C18:2	279.233	$[M-H]^-$	175.52 ± 0.01	175.96 ± 0.18	175.09 ± 0.06	175.52 ± 0.44	0.25 %	175.10 ± 0.01
C18:1	281.2486	$[M-H]^-$	175.92 ± 0.01	176.68 ± 0.13	175.95 ± 0.06	176.18 ± 0.43	0.24 %	175.98 ± 0.02
C18:0	283.2643	$[M-H]^-$	177.47 ± 0.01	178.16 ± 0.13	177.50 ± 0.03	177.71 ± 0.39	0.22 %	177.00 ± 0.09
C20:4	303.233	$[M-H]^-$	182.32 ± 0.01	184.12 ± 0.08	182.20 ± 0.20	182.88 ± 1.08	0.59 %	182.31 ± 0.23
C20:3	305.2486	$[M-H]^-$	182.48 ± 0.01	183.03 ± 0.03	182.28 ± 0.06	182.60 ± 0.39	0.21 %	182.18 ± 0.04
C20:2	307.2643	$[M-H]^-$	182.44 ± 0.03	183.22 ± 0.67	182.40 ± 0.05	182.69 ± 0.46	0.25 %	182.61 ± 0.00
C20:1	309.2799	$[M-H]^-$	184.60 ± 0.01	184.83 ± 0.05	183.86 ± 0.09	184.43 ± 0.51	0.27 %	183.98 ± 0.01
C20:0	311.2956	$[M-H]^-$	186.01 ± 0.03	186.63 ± 0.10	185.68 ± 0.16	186.11 ± 0.48	0.26 %	185.45 ± 0.06
C24:0	367.3582	$[M-H]^-$	201.21 ± 0.02	201.33 ± 0.16	200.50 ± 0.01	201.01 ± 0.45	0.22 %	200.66 ± 0.05
Creatinine	112.0516	$[M-H]^-$	119.02 ± 0.62	119.58 ± 0.09	119.08 ± 0.05	119.23 ± 0.31	0.26 %	118.84 ± 0.08
Creatinine	114.0662	$[M+H]^+$	122.81 ± 0.01	123.69 ± 0.06	122.92 ± 0.13	123.14 ± 0.48	0.39 %	122.98 ± 0.03
L-proline	116.0706	$[M+H]^+$	125.08 ± 0.04	126.25 ± 0.10	125.09 ± 0.17	125.48 ± 0.67	0.54 %	125.38 ± 0.09
L-leucine	130.0874	$[M-H]^-$	131.00 ± 0.04	131.70 ± 0.06	132.56 ± 0.02	131.75 ± 0.78	0.59 %	131.15 ± 0.01
L-isoleucine	130.0874	$[M-H]^-$	129.62 ± 0.03	130.28 ± 0.03	130.95 ± 0.02	130.28 ± 0.66	0.51 %	129.83 ± 0.01
L-aspartic acid	132.0302	$[M-H]^-$	118.97 ± 0.07	119.43 ± 0.15	119.35 ± 0.06	119.25 ± 0.25	0.21 %	119.16 ± 0.04
L-leucine	132.1019	$[M+H]^+$	134.59 ± 0.07	134.84 ± 0.06	135.59 ± 0.03	135.01 ± 0.52	0.39 %	134.58 ± 0.03
L-isoleucine	132.1019	$[M+H]^+$	132.82 ± 0.02	133.08 ± 0.08	133.83 ± 0.01	133.24 ± 0.53	0.40 %	132.88 ± 0.04

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent
Homocysteine	136.0427	$[M+H]^+$	130.00 ± 0.08	130.85 ± 0.12	130.73 ± 0.05	130.53 ± 0.46	0.35 %	129.59 ± 0.63
Creatinine	136.0481	$[M+Na]^+$	132.45 ± 0.04	134.05 ± 0.05	133.05 ± 0.06	133.18 ± 0.81	0.61 %	132.61 ± 0.36
L-glutamic acid	146.0459	$[M-H]^-$	124.29 ± 0.02	124.96 ± 0.19	124.55 ± 0.11	124.60 ± 0.34	0.27 %	124.48 ± 0.01
L-lysine	147.1128	$[M+H]^+$	130.90 ± 0.19	130.95 ± 0.11	130.45 ± 0.62	130.77 ± 0.27	0.21 %	131.22 ± 0.14
L-methionine	150.0583	$[M+H]^+$	133.00 ± 0.13	132.91 ± 0.25	132.89 ± 0.11	132.93 ± 0.06	0.04 %	133.03 ± 0.48
L-histidine	154.0622	$[M-H]^-$	128.69 ± 0.03	129.51 ± 0.04	128.87 ± 0.02	129.02 ± 0.43	0.33 %	128.83 ± 0.01
L-histidine	156.0768	$[M+H]^+$	131.80 ± 0.02	132.78 ± 0.06	131.83 ± 0.01	132.14 ± 0.56	0.42 %	131.94 ± 0.03
L-phenyl-alanine	164.0717	$[M-H]^-$	139.93 ± 0.21	140.09 ± 0.57	139.91 ± 0.05	139.98 ± 0.10	0.07 %	139.94 ± 0.04
L-phenyl-alanine	166.0863	$[M+H]^+$	140.39 ± 0.05	141.27 ± 0.02	140.21 ± 0.03	140.62 ± 0.57	0.41 %	140.30 ± 0.13
Uric acid	167.0211	$[M-H]^-$	125.59 ± 0.02	126.53 ± 0.09	125.66 ± 0.06	125.93 ± 0.52	0.41 %	125.55 ± 0.07
L-arginine	173.1044	$[M-H]^-$	137.04 ± 0.02	137.77 ± 0.14	137.16 ± 0.03	137.32 ± 0.39	0.29 %	137.08 ± 0.02
L-arginine	175.119	$[M+H]^+$	136.32 ± 0.01	137.37 ± 0.01	136.36 ± 0.03	136.68 ± 0.59	0.43 %	136.45 ± 0.01
L-histidine	178.0587	$[M+Na]^+$	134.62 ± 0.10	135.60 ± 0.14	134.35 ± 0.25	134.86 ± 0.66	0.49 %	134.40 ± 0.44
L-tyrosine	180.0666	$[M-H]^-$	144.40 ± 0.06	145.12 ± 0.51	144.44 ±0.09	144.65 ± 0.41	0.28 %	144.42 ± 0.08
L-tyrosine	182.0812	$[M+H]^+$	145.72 ± 0.06	146.66 ± 0.20	145.51 ± 0.08	145.96 ± 0.61	0.42 %	145.58 ± 0.13
Glucose	203.0526	$[M+Na]^+$	146.88 ± 0.00	147.67 ± 0.06	146.73 ± 0.01	147.09 ± 0.50	0.34 %	146.94 ± 0.08
L-cystine	239.0166	$[M-H]^-$	143.45 ± 0.03	144.19 ± 0.07	143.63 ± 0.06	143.76 ± 0.38	0.27 %	143.58 ± 0.02
L-cystine	241.0311	$[M+H]^+$	149.43 ± 0.02	150.52 ± 0.01	149.48 ± 0.01	149.81 ± 0.62	0.41 %	149.48 ± 0.03
Pyridoxal Phosphate	246.0173	$[M-H]^-$	149.49 ± 0.03	150.44 ± 0.03	149.52 ± 0.02	149.82 ± 0.54	0.36 %	149.36 ± 0.04
Pyridoxal Phosphate	248.0319	$[M+H]^+$	151.25 ± 0.01	152.42 ± 0.03	151.44 ± 0.02	151.70 ± 0.63	0.41 %	151.37 ± 0.03
L-cystine	263.0131	$[M+Na]^+$	151.33 ± 0.04	152.30 ± 0.01	151.35 ± 0.03	151.66 ± 0.56	0.37 %	151.26 ± 0.13
Pyridoxal Phosphate	270.0138	$[M+Na]^+$	161.50 ± 0.07	162.64 ± 0.03	161.56 ± 0.02	161.90 ± 0.64	0.40 %	161.47 ± 0.21
Cortisol	363.2166	$[M+H]^+$	189.08 ± 0.04	190.30 ± 0.03	188.92 ± 0.19	189.44 ± 0.76	0.40 %	188.34 ± 0.01
Cortisol	385.1985	$[M+Na]^+$	213.62 ± 0.08	215.03 ± 0.06	213.22 ± 0.08	213.96 ± 0.95	0.44 %	212.80 ± 0.08
Levome-folic acid	458.1798	$[M-H]^-$	199.48 ± 0.00	200.15 ± 0.06	199.35 ± 0.12	199.66 ± 0.43	0.22 %	199.00 ± 0.01

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent
Levome-folic acid	460.1939	$[M+H]^{+}$	197.72 ± 0.02	198.23 ± 0.59	197.39 ± 0.02	197.78 ± 0.42	0.21 %	197.17 ± 0.04
GITWK	302.6763	$[M+2H]^{2+}$	273.46 ± 0.09	274.44 ± 0.33	273.64 ± 0.14	273.85 ± 0.52	0.19 %	274.51 ± 1.02
IFVQK	317.6998	$[M+2H]^{2+}$	285.90 ± 0.04	287.44 ± 0.41	286.09 ± 0.02	286.47 ± 0.85	0.30 %	286.78 ± 0.08
Angiotensin I	324.93	$[M+4H]^{4+}$	548.31 ± 0.03	555.47 ± 0.15	551.96 ± 0.15	551.91 ± 3.58	0.65 %	550.99 ± 0.07
YIPGTK	339.6947	$[M+2H]^{2+}$	292.80 ± 0.04	293.03 ± 0.11	292.96 ± 0.04	292.92 ± 0.13	0.04 %	293.76 ± 0.13
Angiotensin II	349.52	$[M+3H]^{3+}$	435.39 ± 0.06	441.02 ± 0.13	437.32 ± 0.31	437.91 ± 2.86	0.65 %	437.30 ± 0.13
Bradykinin	354.19	$[M+3H]^{3+}$	447.50 ± 0.29	453.08 ± 0.41	448.27 ± 0.14	449.62 ± 3.02	0.67 %	449.08 ± 0.39
MIFAGIK	390.2278	$[M+2H]^{2+}$	306.50 ± 0.04	307.30 ± 0.55	306.34 ± 0.03	306.70 ± 0.52	0.17 %	305.56 ± 0.42
Angiotensin I	432.90	$[M+3H]^{3+}$	477.40 ± 0.06	481.98 ± 0.10	477.30 ± 0.06	478.89 ± 2.68	0.56 %	477.05 ± 0.04
Renin	440.49	$[M+4H]^{4+}$	638.02 ± 0.41	644.69 ± 0.16	638.11 ± 0.10	640.27 ± 3.82	0.60 %	637.65 ± 0.24
Substance P	449.92	$[M+3H]^{3+}$	496.17 ± 0.11	502.78 ± 0.34	496.57 ± 0.26	498.51 ± 3.71	0.74 %	496.51 ± 0.38
EDLIAYLK	482.7711	$[M+2H]^{2+}$	341.82 ± 0.09	343.80 ± 0.17	342.43 ± 0.01	342.68 ± 1.01	0.30 %	342.46 ± 0.07
Angiotensin II	523.78	$[M+2H]^{2+}$	357.15 ± 0.03	358.51 ± 0.07	355.33 ± 0.05	357.00 ± 1.60	0.45 %	355.10 ± 0.03
Bradykinin	530.79	$[M+2H]^{2+}$	345.64 ± 0.03	347.81 ± 0.05	345.07 ± 0.06	346.17 ± 1.44	0.42 %	344.99 ± 0.04
Neurotensin	558.31	$[M+3H]^{3+}$	530.42 ± 0.07	533.22 ± 0.08	528.55 ± 0.10	530.73 ± 2.35	0.44 %	528.29 ± 0.03
Melittin	569.96	$[M+5H]^{5+}$	815.60 ± 0.12	824.14 ± 0.41	816.16 ± 0.05	818.63 ± 4.78	0.58 %	815.39 ± 0.10
Melittin	569.96	$[M+5H]^{5+}$	858.72 ± 0.15	867.13 ± 0.56	852.47 ± 2.60	859.44 ± 7.36	0.86 %	854.38 ± 0.15
TGPNLH-GLFGR	584.8147	$[M+2H]^{2+}$	369.20 ± 0.03	370.94 ± 0.11	370.02 ± 0.02	370.04 ± 0.89	0.24 %	369.62 ± 0.10
Renin	586.98	$[M+3H]^{3+}$	527.10 ± 0.04	528.44 ± 0.11	524.43 ± 0.13	526.66 ± 2.04	0.39 %	524.12 ± 0.07
GITWK	604.3453	$[M+H]^{+}$	238.30 ± 0.03	236.28 ± 0.23	237.19 ± 0.01	237.24 ± 0.99	0.42 %	235.86 ± 0.01
IFVQK	634.3922	$[M+H]^{+}$	250.70 ± 0.11	248.40 ± 0.22	249.19 ± 0.01	249.52 ± 1.17	0.47 %	248.08 ± 0.02
Angiotensin I	648.85	$[M+2H]^{2+}$	389.79 ± 0.02	390.60 ± 0.10	388.99 ± 0.04	389.79 ± 0.81	0.21 %	388.41 ± 0.10
Substance P	674.37	$[M+2H]^{2+}$	400.99 ± 0.06	402.87 ± 0.11	400.58 ± 0.04	401.48 ± 1.22	0.30 %	400.10 ± 0.05
YIPGTK	678.3821	$[M+H]^{+}$	256.30 ± 0.00	254.21 ± 0.15	255.33 ± 0.02	255.29 ± 1.06	0.41 %	254.03 ± 0.02

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent
Melittin	712.20	$[M+4H]^{4+}$	764.81 ± 0.06	765.89 ± 0.23	761.71 ± 0.07	764.14 ± 2.17	0.28 %	760.82 ± 0.13
TGQAPGF TYTDANK	735.8466	$[M+2H]^{2+}$	400.90 ± 0.06	401.40 ± 0.13	400.85 ± 0.04	401.03 ± 0.32	0.08 %	399.97 ± 0.13
EETLME-YLENPK	748.3529	$[M+2H]^{2+}$	415.70 ± 0.03	415.98 ± 0.41	416.73 ± 0.07	416.15 ± 0.52	0.13 %	414.73 ± 0.09
MIFAGIK	779.4484	$[M+H]^{+}$	277.10 ± 0.03	274.37 ± 0.18	276.03 ± 0.01	275.81 ± 1.35	0.49 %	274.49 ± 0.02
Neurotensin	836.96	$[M+2H]^{2+}$	437.38 ± 0.04	438.85 ± 0.07	435.13 ± 0.03	437.12 ± 1.87	0.43 %	435.43 ± 0.07
Renin	879.97	$[M+2H]^{2+}$	462.46 ± 0.08	465.36 ± 0.10	461.25 ± 0.06	463.02 ± 2.12	0.46 %	461.12 ± 0.04
Melittin	949.26	$[M+3H]^{3+}$	725.02 ± 0.04	728.30 ± 0.07	721.95 ± 0.06	725.09 ± 3.18	0.44 %	722.45 ± 0.02
EDLIAYL K	964.5349	$[M+H]^{+}$	308.42 ± 0.55	305.96 ± 0.18	308.52 ± 0.02	307.63 ± 1.45	0.47 %	306.57 ± 0.03
Angiotensin II	1046.54	$[M+H]^{+}$	313.21 ± 0.11	315.67 ± 0.56	313.33 ± 0.03	314.07 ± 1.39	0.44 %	313.66 ± 0.03
Bradykinin	1060.57	$[M+H]^{+}$	313.53 ± 0.05	316.25 ± 0.70	313.63 ± 0.12	314.47 ± 1.54	0.49 %	314.00 ± 0.12
TGPNLH-GLFGR	1168.6221	$[M+H]^{+}$	331.53 ± 0.26	329.44 ± 0.61	332.09 ± 0.03	331.02 ± 1.39	0.42 %	330.07 ± 0.02
Angiotensin I	1296.69	$[M+H]^{+}$	354.77 ± 0.12	358.49 ± 0.48	355.11 ± 0.10	356.12 ± 2.06	0.58 %	355.62 ± 0.41
Substance P	1347.74	$[M+H]^{+}$	360.09 ± 0.07	363.37 ± 0.30	360.49 ± 0.07	361.32 ± 1.79	0.50 %	361.44 ± 0.04
Melittin	1423.38	$[M+2H]^{2+}$	613.42 ± 0.13	618.59 ± 0.37	613.64 ± 0.08	615.22 ± 2.92	0.48 %	614.26 ± 0.03
TGQAPGF TYTDANK	1470.6873	$[M+H]^{+}$	365.37 ± 0.60	363.50 ± 0.47	366.08 ± 0.26	364.98 ± 1.33	0.37 %	364.37 ± 0.18
EETLME-YLENPK	1495.6985	$[M+H]^{+}$	385.83 ± 1.33	384.62 ± 0.64	386.77 ± 0.11	385.74 ± 1.08	0.28 %	384.84 ± 1.32
Ubiquitin	612.414	$[M+14H]^{14+}$	2728.47 ± 1.57	2725.64 ± 6.73	2731.76 ± 0.59	2728.62 ± 3.06	0.11 %	2728.87 ± 1.74
Cytochrome c	618.9162	$[M+20H]^{20+}$	3842.93 ± 0.84	3851.74 ± 0.00	3829.88 ± 0.00	3841.52 ± 11.00	0.29 %	3832.36 ± 0.00
Cytochrome c	651.438	$[M+19H]^{19+}$	3768.76 ± 1.60	3760.52 ± 4.36	3759.95 ± 0.79	3763.08 ± 4.93	0.13 %	3757.99 ± 0.00
Ubiquitin	659.446	$[M+13H]^{13+}$	2592.02 ± 0.00	2595.43 ± 1.43	2604.75 ± 0.54	2597.40 ± 6.59	0.25 %	2594.38 ± 0.54
Cytochrome c	687.5725	$[M+18H]^{18+}$	3680.42 ± 0.76	3656.05 ± 15.24	3670.27 ± 1.99	3668.91 ± 12.24	0.33 %	3670.41 ± 0.75
Ubiquitin	714.316	$[M+12H]^{12+}$	2443.35 ± 0.51	2441.14 ± 1.32	2460.31 ± 0.00	2448.27 ± 10.49	0.43 %	2444.26 ± 0.00
Cytochrome c	727.9594	$[M+17H]^{17+}$	3572.41 ± 1.24	3520.95 ± 3.90	3560.36 ± 0.71	3551.24 ± 26.91	0.76 %	3554.73 ± 0.70
Cytochrome c	773.3916	$[M+16H]^{16+}$	3445.64 ± 1.35	3391.96 ± 2.38	3439.01 ± 0.67	3425.54 ± 29.27	0.85 %	3420.25 ± 2.39

Compound	m/z	Ion	BOKU	PNNL	VU	Average	% RSD	Agilent
Ubiquitin	779.161	$[M+11H]^{11+}$	2362.82 ± 0.47	2359.54 ± 0.00	2378.26 ± 0.46	2366.87 ± 10.00	0.42 %	2362.34 ± 0.00
Ubiquitin	856.978	$[M+10H]^{10+}$	2212.27 ± 0.73	2203.54 ± 0.72	2223.39 ± 0.00	2213.07 ± 9.95	0.45 %	2204.84 ± 0.41
Ubiquitin	952.081	$[M+9H]^{9+}$	2077.68 ± 0.38	2067.26 ± 0.38	2076.24 ± 0.38	2073.73 ± 5.64	0.27 %	2063.50 ± 0.00
Ubiquitin	1070.964	$[M+8H]^{8+}$	1969.37 ± 0.34	1963.92 ± 0.00	1972.07 ± 0.33	1968.45 ± 4.15	0.21 %	1960.53 ± 0.33
Ubiquitin	1223.8	$[M+7H]^{7+}$	1789.38 ± 0.30	1784.66 ± 0.51	1789.80 ± 0.59	1787.95 ± 2.85	0.16 %	1785.41 ± 0.29
Ubiquitin	1223.8	$[M+7H]^{7+}$	1885.66 ± 0.51	1886.96 ± 0.29	1843.71 ± 0.29	1872.11 ± 24.60	1.31 %	1884.36 ± 0.29



Agilent		BOKU	
Biological Standard	**%RSD**	**Biological Standard**	**%Error**
Renin $[M+3H]^{3+}$	1.02%	Uric acid	1.00%
L-aspartic acid	1.03%	Ubiquitin $[M+13H]^{13+}$	1.05%
L-leucine	1.03%	Cytochrome c $[M+16H]^{16+}$	1.05%
Uric acid	1.08%	Ubiquitin $[M+8H]^{8+}$	1.08%
L-isoleucine	1.11%	Renin $[M+3H]^{3+}$	1.08%
Melittin $[M+5H]^{5+}$	1.18%	Ubiquitin $[M+9H]^{9+}$	1.16%
Creatinine	1.54%	Ubiquitin $[M+11H]^{11+}$	1.24%
PNNL		Creatinine	1.34%
Melittin $[M+3H]^{3+}$	1.00%	Ubiquitin $[M+10H]^{10+}$	1.42%
Neurotensin $[M+2H]^{2+}$	1.04%	Ubiquitin $[M+12H]^{12+}$	1.47%
Renin $[M+2H]^{2+}$	1.08%	Ubiquitin $[M+7H]^{7+}$	1.71%
Angiotensin II $[M+2H]^{2+}$	1.10%	**Vanderbilt**	
Angiotensin I $[M+2H]^{2+}$	1.13%	L-histidine	1.02%
Melittin $[M+4H]^{4+}$	1.20%	Neurotensin $[M+3H]^{3+}$	1.03%
Bradykinin $[M+3H]^{3+}$	1.22%	Uric acid	1.05%
Bradykinin $[M+2H]^{2+}$	1.30%	Melittin $[M+4H]^{4+}$	1.06%
Angiotensin II $[M+2H]^{2+}$	1.33%	L-glutamic acid	1.08%
Substance P $[M+3H]^{3+}$	1.42%	L-leucine	1.14%
Angiotensin I $[M+3H]^{3+}$	1.53%	L-aspartic acid	1.18%
Neurotensin $[M+3H]^{3+}$	1.56%	Ubiquitin $[M+9H]^{9+}$	1.23%
Renin $[M+4H]^{4+}$	1.59%	Cytochrome c $[M+16H]^{16+}$	1.24%
Renin $[M+3H]^{3+}$	1.86%	L-isoleucine	1.27%
Melittin $[M+5H]^{5+}$	1.92%	Creatinine	1.39%
Melittin $[M+5H]^{5+}$	2.69%	Renin $[M+3H]^{3+}$	1.60%
		Melittin $[M+5H]^{5+}$	1.70%

Figure S3. Absolute error of single field CCS values compared to stepped field CCS values obtained on the Reference System. The biological samples listed in the table are those that fall above 1% error. The reasons for the larger deviations are discussed in the manuscript.

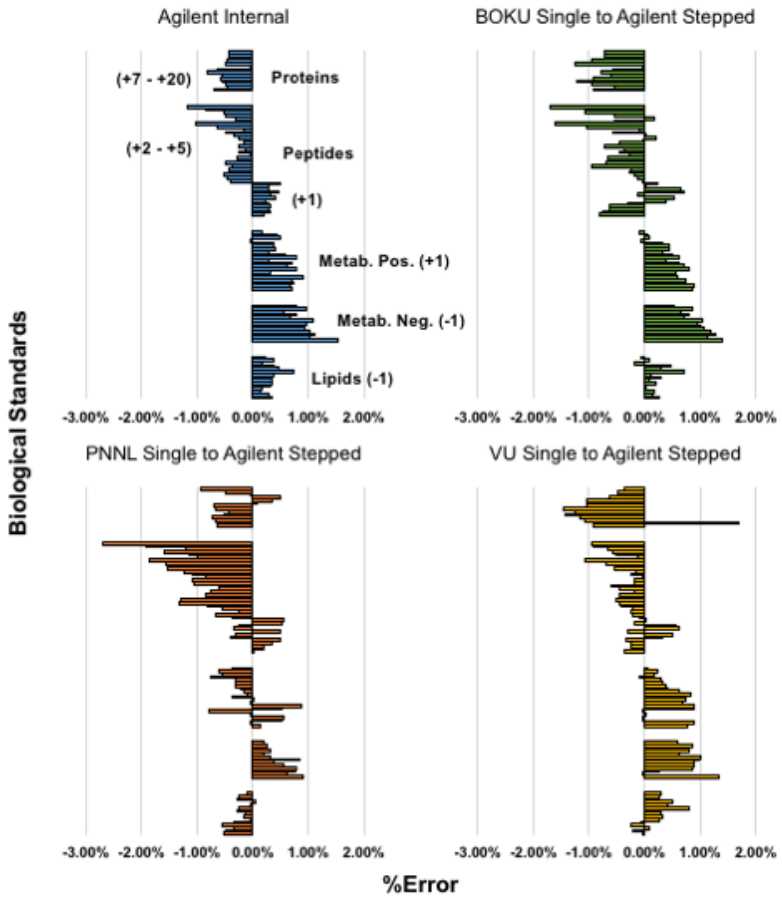


Figure S4. The bar graphs from Figure 4B comparing single field CCS values from each lab to stepped field CCS values on the Reference System are shown separately for clarity. The molecular classes are listed on the "Agilent Internal" plot with the other three plots following the same order.

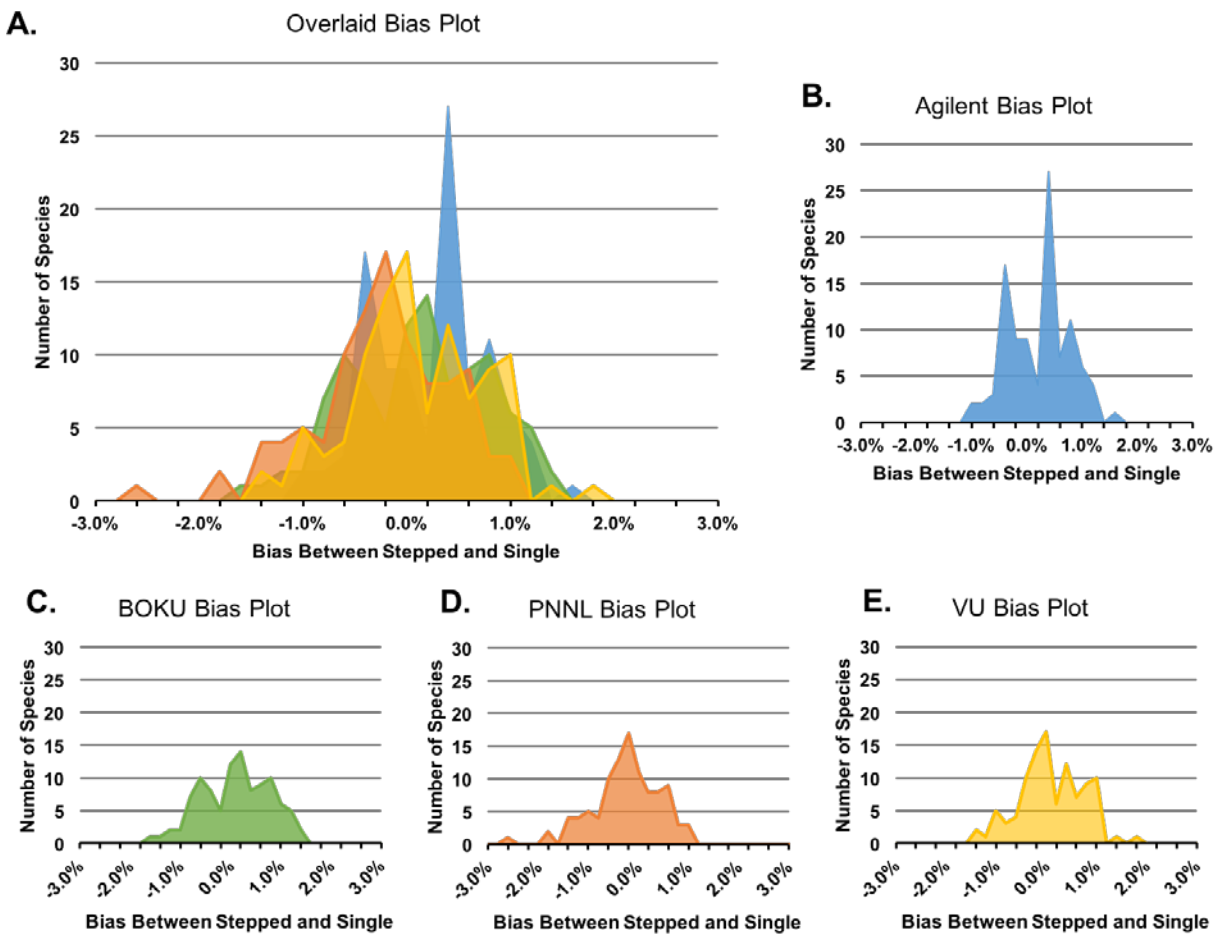


Figure S5. Bias plots for single field data from each lab compared to stepped field data acquired on the Reference System. Results for all 4 labs are overlaid in A. Results for each lab are shown on individual plots for clarity for B. Agilent, C. BOKU, D. PNNL, and E. Vanderbilt. These plots further emphasize that a majority of the single field CCS measurements are within 1% across the four labs.

Theoretical Simulation Details

Theoretical CCS ranges were determined for the molecules examined in this study. This involved the classic two step approach of sampling conformational space of each molecule followed by a theoretical CCS calculation for each conformation. Initial structures were either optimized in Gaussian09[1] or built in MOE[2] or XLEAP[3] software. Gaussian was used for molecules under 800 Da to obtain an electrostatic description of the molecule. Peptides larger than 800 Da were built in XLEAP with both existing amino acid residues and Gaussian optimized residues for those not included in XLEAP (i.e. –COOH capped C-termini, N-termini with neutralized side chains) to better mimic the gas-phase charged species. Distance geometry, a computationally-inexpensive method, was utilized allowing a large number of conformers (1-20,000 depending on molecule size and flexibility) to be generated in a short amount of CPU time (usually hours when parallelized).[4] Distance geometry is mathematical approach to sampling conformational space as it treats all the atoms in the molecule as points in space and uses linear algebra to convert a set of distance between all pairwise points to three dimensional coordinates of a molecule. The mathematical basis of this approach allows it to randomly sample conformational space very quickly. This gives us confidence that we are sampling all conformational space of the molecules in this study in a very time efficient manner. The resulting conformations from distance geometry were submitted to an energy minimization in Amber to create low energy conformations.

All conformations were then submitted to MOBCAL[5] to calculate a projection approximation (PA) theoretical CCS value. PA in MOBCAL calculates theoretical CCS values very quickly, but these theoretical CCS values can only be aligned with helium data. Based on the PA, helium CCS values and RMSD clustering a subset of conformers is selected for submission to the projected superposition approximation (PSA)[6-9]webserver. PSA also determines theoretical CCS values very quickly and has a set of preliminary nitrogen parameters so theoretical nitrogen CCS values can be determined. Since the current format of PSA is through their webserver submitting 20,000 conformations is not possible so selecting a subset of these conformations is desirable. Based on the MOBCAL PA CCS calculation the 10 highest and 10 lowest PA, helium CCS conformers are selected along with 20 low energy conformations that span the enter PA, helium CCS range. An additional 20 conformers are selected based on RMSD clustering, meaning the 20 most structurally unique conformations are also selected for submission to PSA. This set of 60 conformers is reasonable for submission to PSA and should be sufficient to give us a theoretical CCS range in nitrogen space for the molecules in this study.

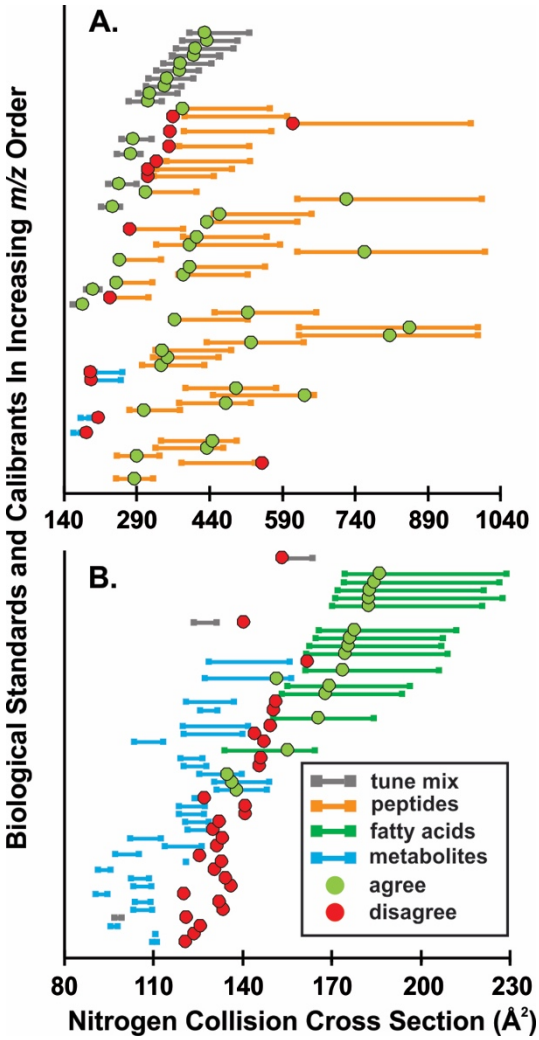


Figure S6. Comparison of experimental stepped field CCS values to CCS ranges based on computational conformational space sampling are shown in A. for larger molecules and in B. for smaller molecules. Computational results are represented with horizontal bars colored according to molecular class. Experimental values are overlaid as either green circles showing agreement or red circles showing disagreement. Ions are listed in increasing *m/z* order to avoid overlap of isomers and dense *m/z* regions specifically at low masses. Agreement between experiment and theory varies based on chemical class and molecule size. Good agreement is observed for tune mix ions above 600 m/z as shown in **Figure S6A**, but not for smaller tune mix ions (118-322 m/z) in **Figure S6B**. Peptide ions which range from 300-1500 m/z and +1 to +5 charge states in **Figure S6A** show good agreement between experiment and theory. Fatty acids which range from 200-368 m/z in **Figure S6B** also show excellent agreement between theoretical ranges and experimental CCS measurements. In contrast, the theoretical methods used here generated CCS ranges which did not encompass experimental CCS values for the majority of metabolites ions in **Figure S6B**, all of which are below 500 m/z. It is noted here that the PSA nitrogen parameters used are preliminary and were not developed for small molecule CCS prediction.[6-9] For small molecules, the contribution of the cross section of the nitrogen buffer gas becomes significant, and experimental CCS_{N2} values obtained at low CCS ranges should converge on the CCS of the

nitrogen gas polarization limit (ca. 93 $Å^2$),[10] which is evident in the experimental values in **Figure S6B**. While other calculations exist for computing theoretical CCS values such as the trajectory method which better simulates the effect of nitrogen as a buffer gas, this method is much more computationally expensive than PSA. This is a concern when attempting to calculate theoretical CCS values for a large number of conformations that span the conformational space of even small molecules like many of the metabolites investigated in this work. Although more work could be done in attempt to provide more accurate theoretical CCS values the work in its current state serves two main purposes. First, to show how current theoretical methods would benefit from future parameterization which relies on accurate experimental CCS values presented in this manuscript. Second, this work provides visual insight into the conformational landscape of the molecules in this study and the theoretical CCS values that currently represent them. Conformations representing these ranges are included in Figure S6-S19.

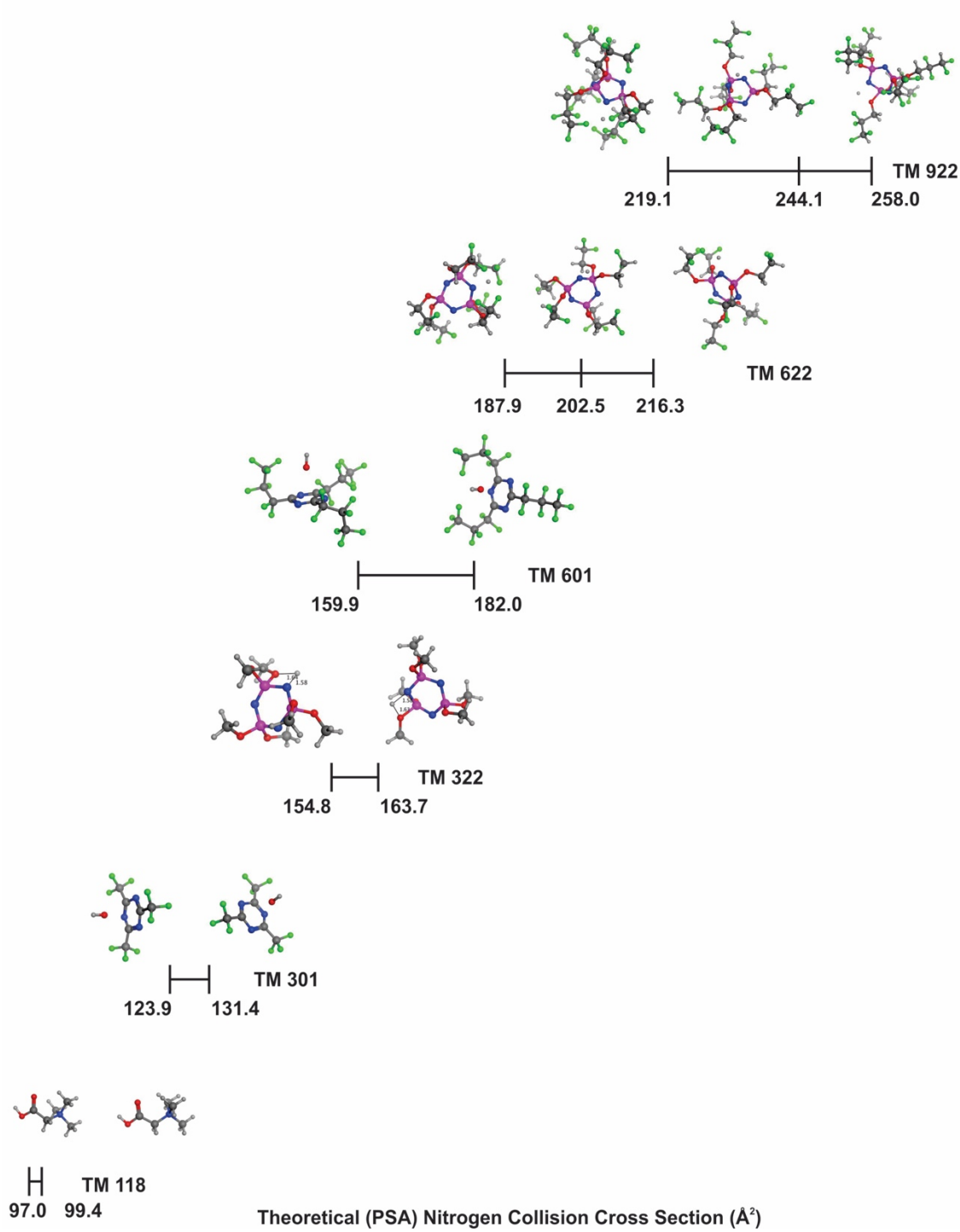


Figure S7. Sample conformations from computational sampling for the tune mix ions. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, phosphorus with pink, and fluorine with green.

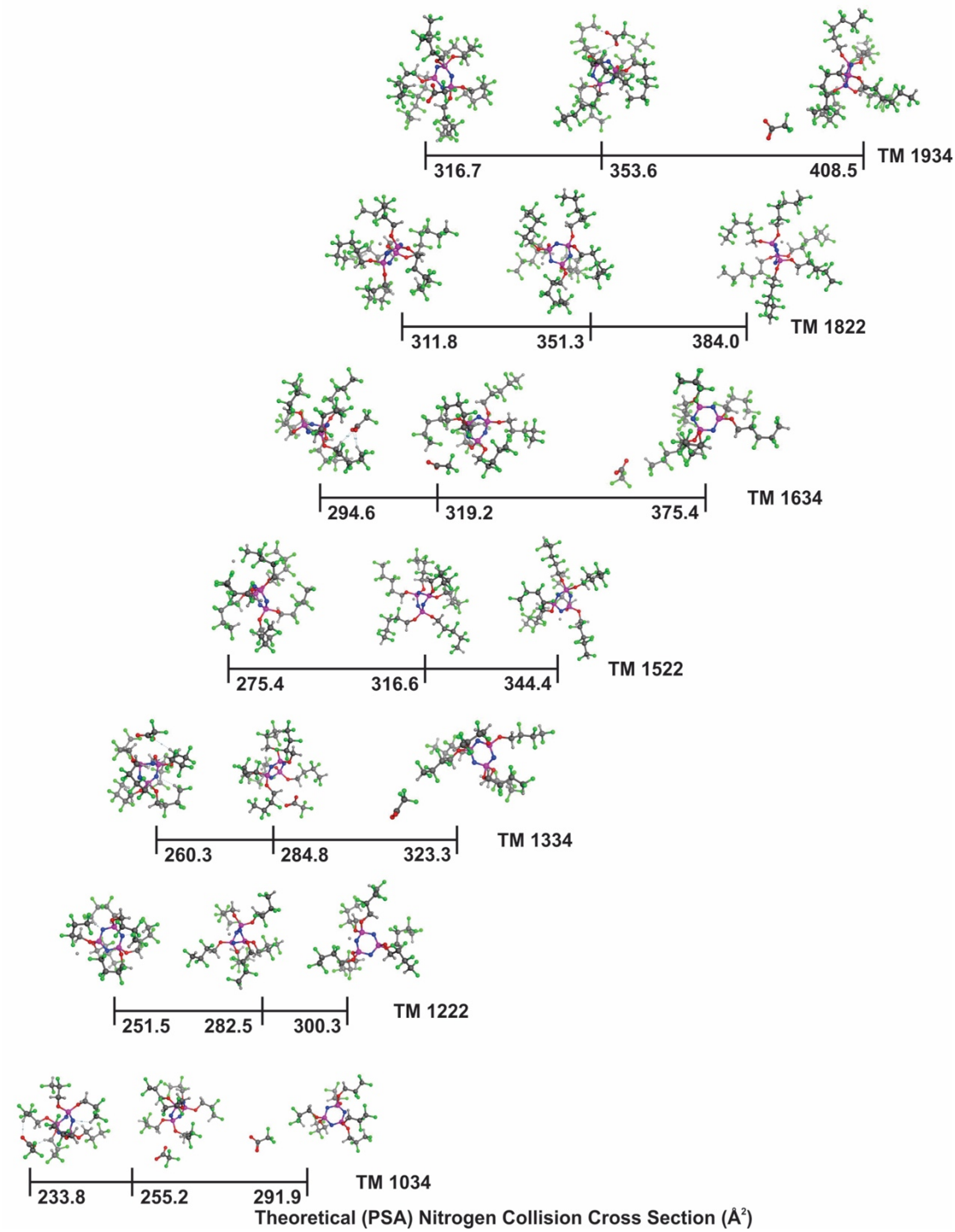


Figure S8. Sample conformations from computational sampling for the tune mix ions. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, phosphorus with pink, and fluorine with green.

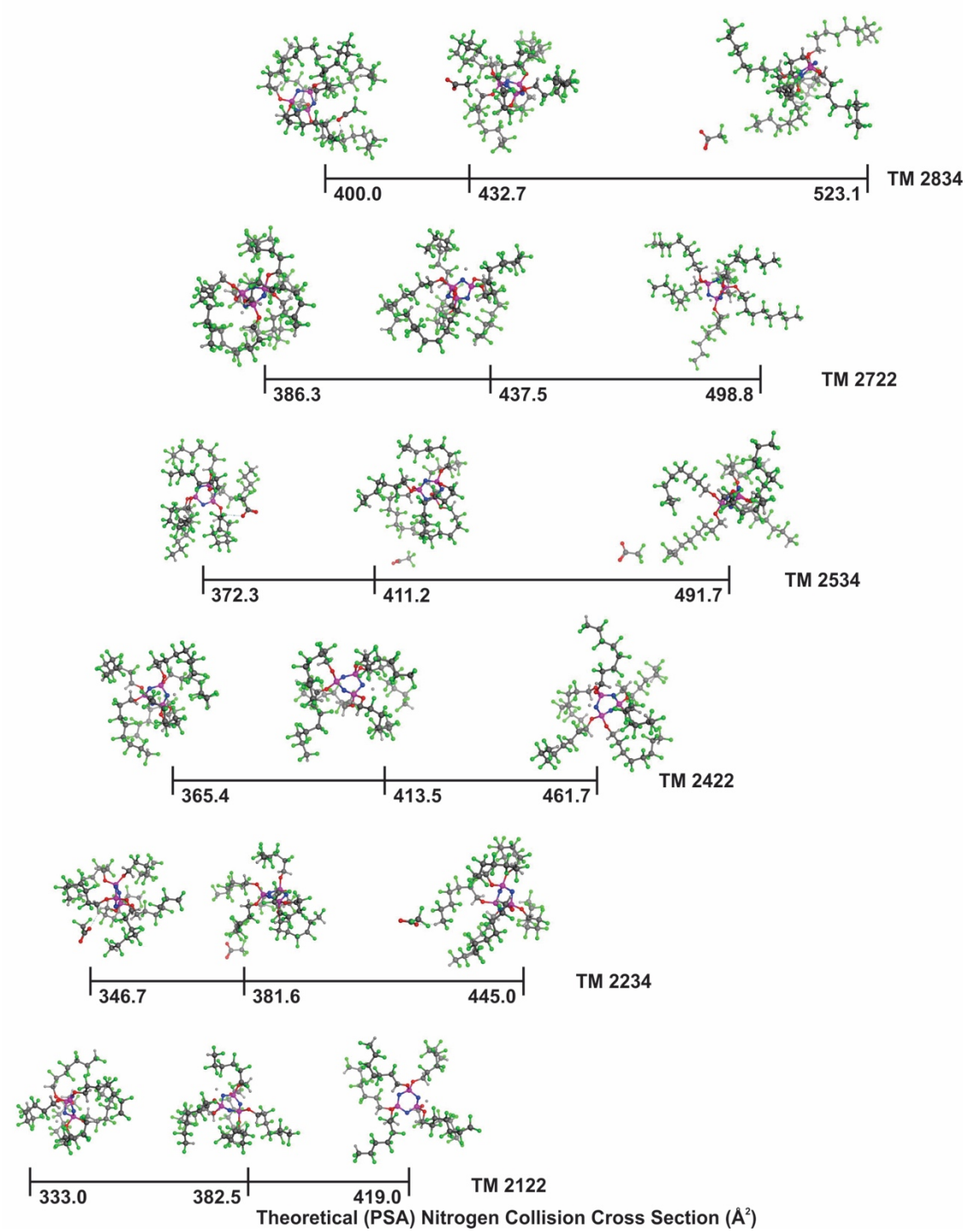


Figure S9. Sample conformations from computational sampling for the tune mix ions. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, phosphorus with pink, and fluorine with green.

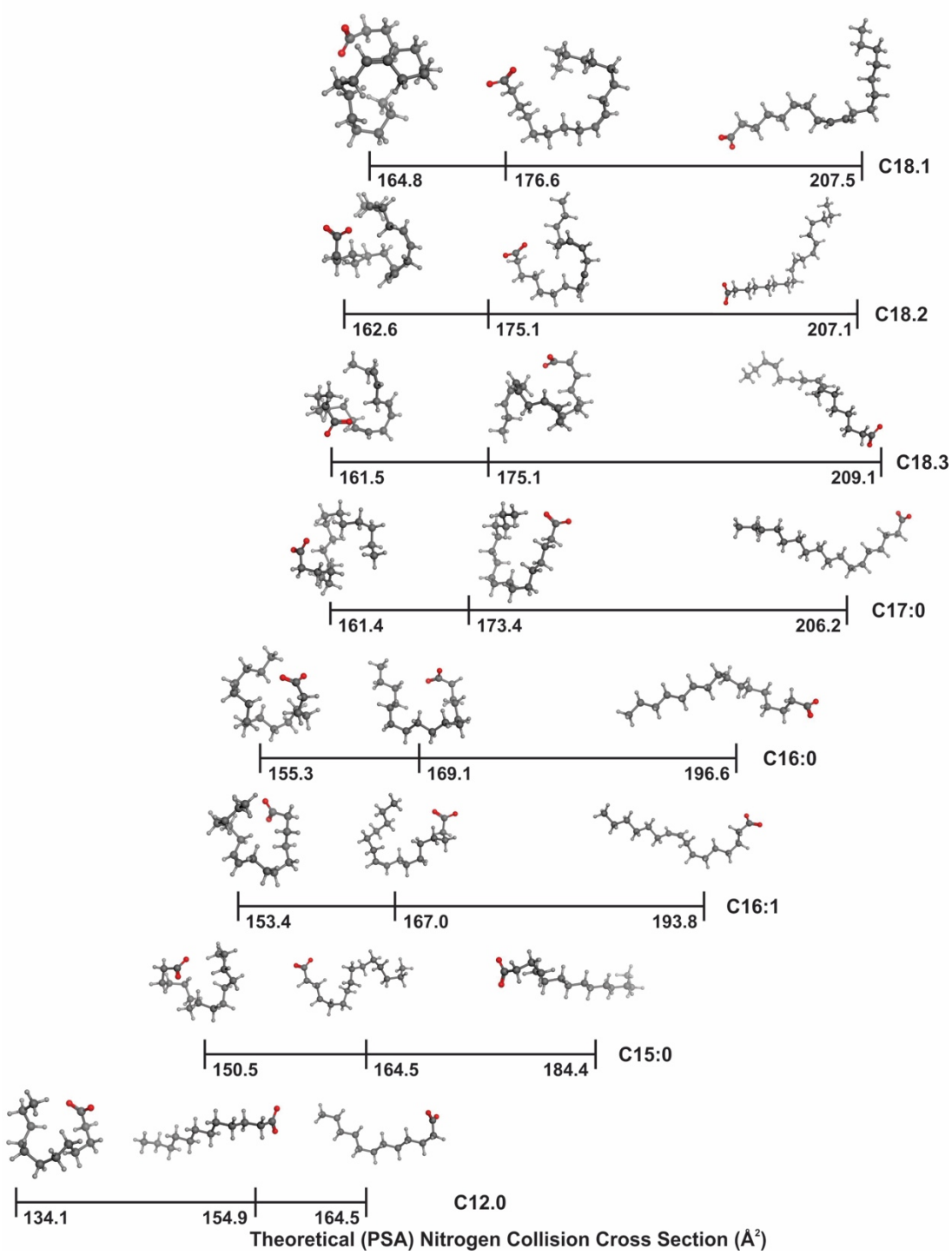


Figure S10. Sample conformations from computational sampling for the fatty acids. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. Carbon is represented with dark grey, hydrogen with light grey, and oxygen with red.

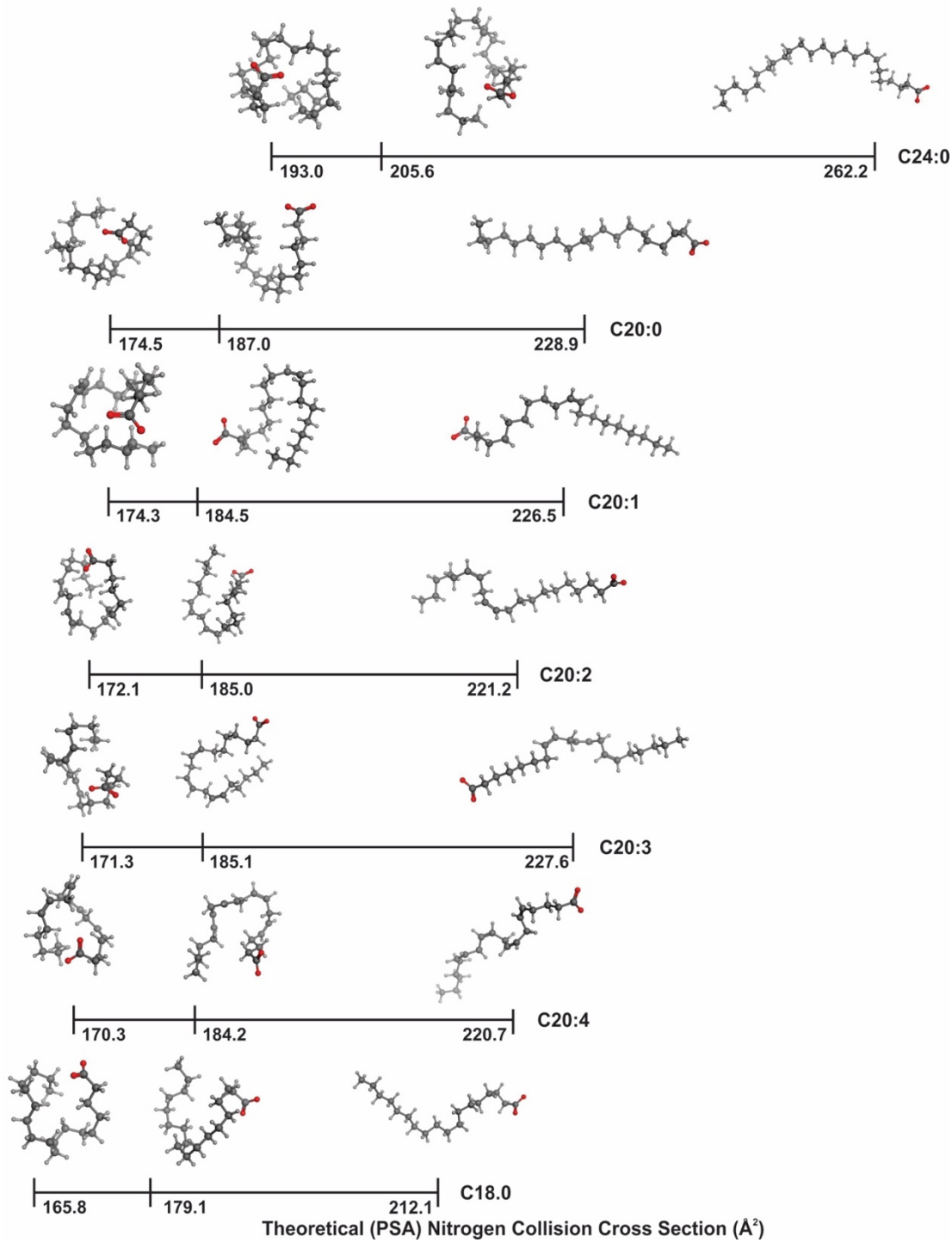


Figure S11. Sample conformations from computational sampling for the fatty acids. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. Carbon is represented with dark grey, hydrogen with light grey, and oxygen with red.

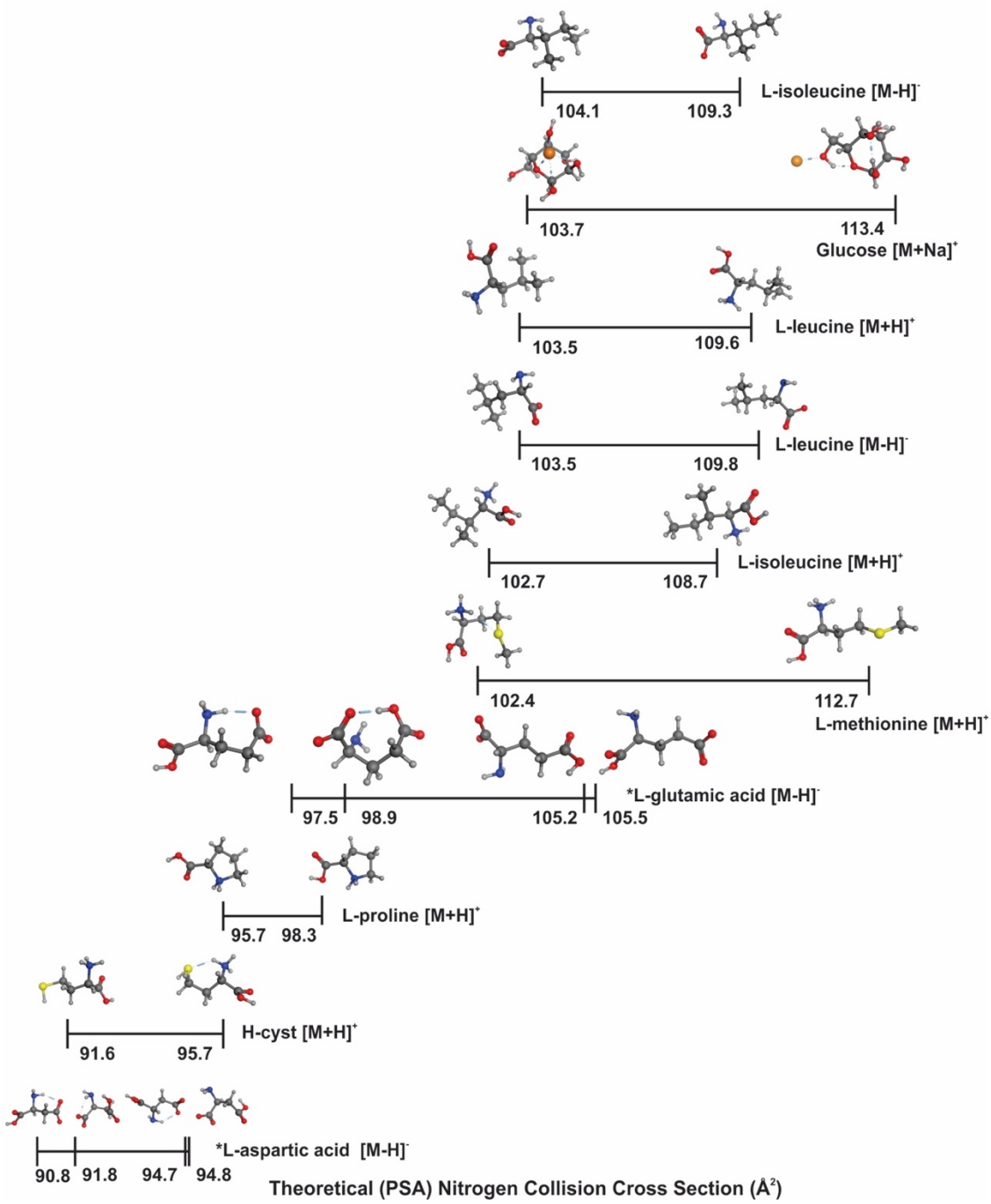


Figure S12. Sample conformations from computational sampling for the metabolites. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. An * indicates metabolites that had more than one possible protonation sites with conformations shown for the additional protonation site. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, sulfur with yellow, nitrogen with blue, and sodium with orange.

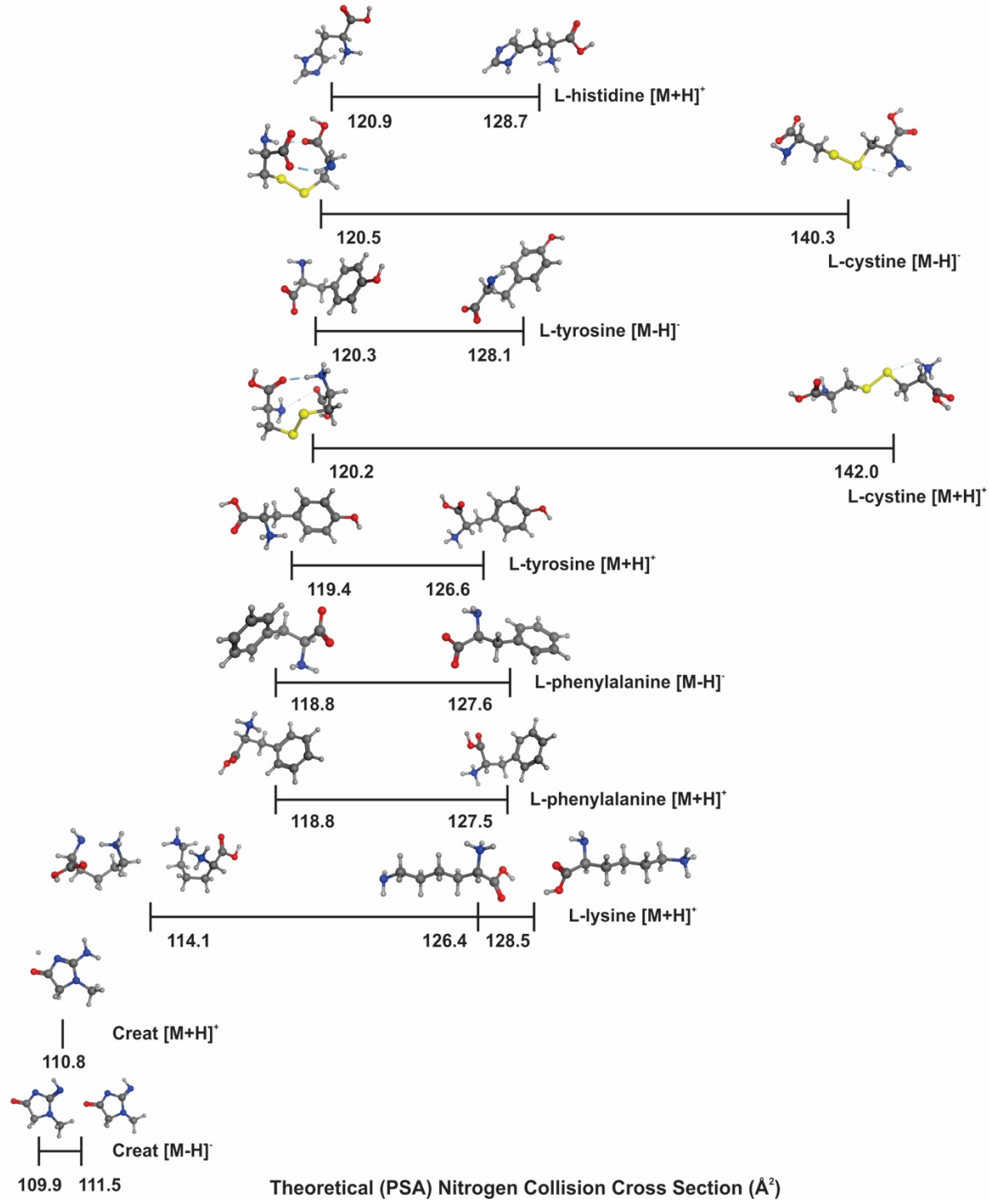


Figure S13. Sample conformations from computational sampling for the metabolites. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, sulfur with yellow, and nitrogen with blue.

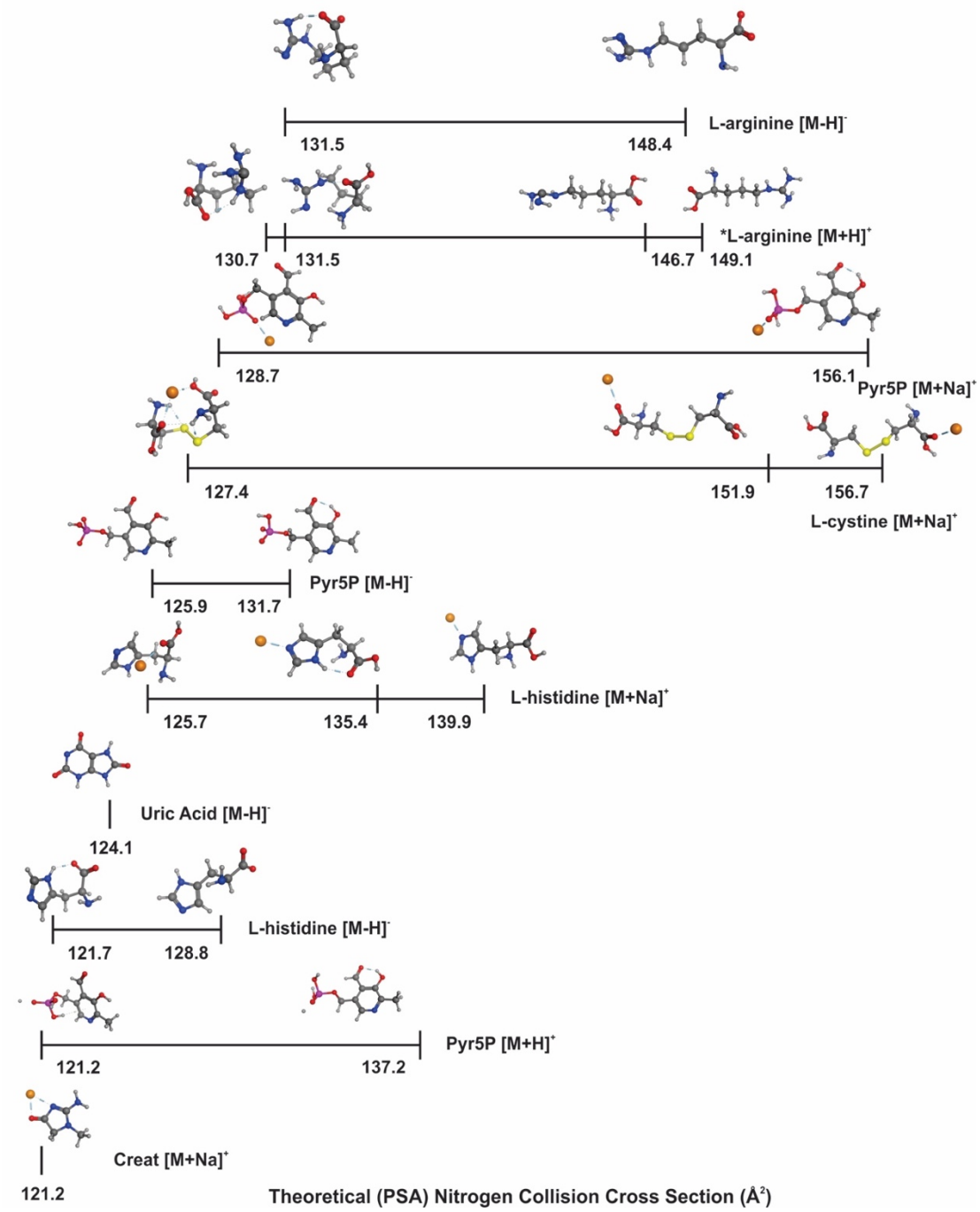


Figure S14. Sample conformations from computational sampling for the metabolites. A representative conformation is shown for the lowest and highest theoretical CCS values as well as for one that closely agrees with the experimental CCS value. If no conformation is indicated within the range, the experimental CCS value fell outside the range. An * indicates metabolites that had more than one possible protonation sites with conformations shown for the additional protonation site. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, sulfur with yellow, nitrogen with blue, and sodium with orange.

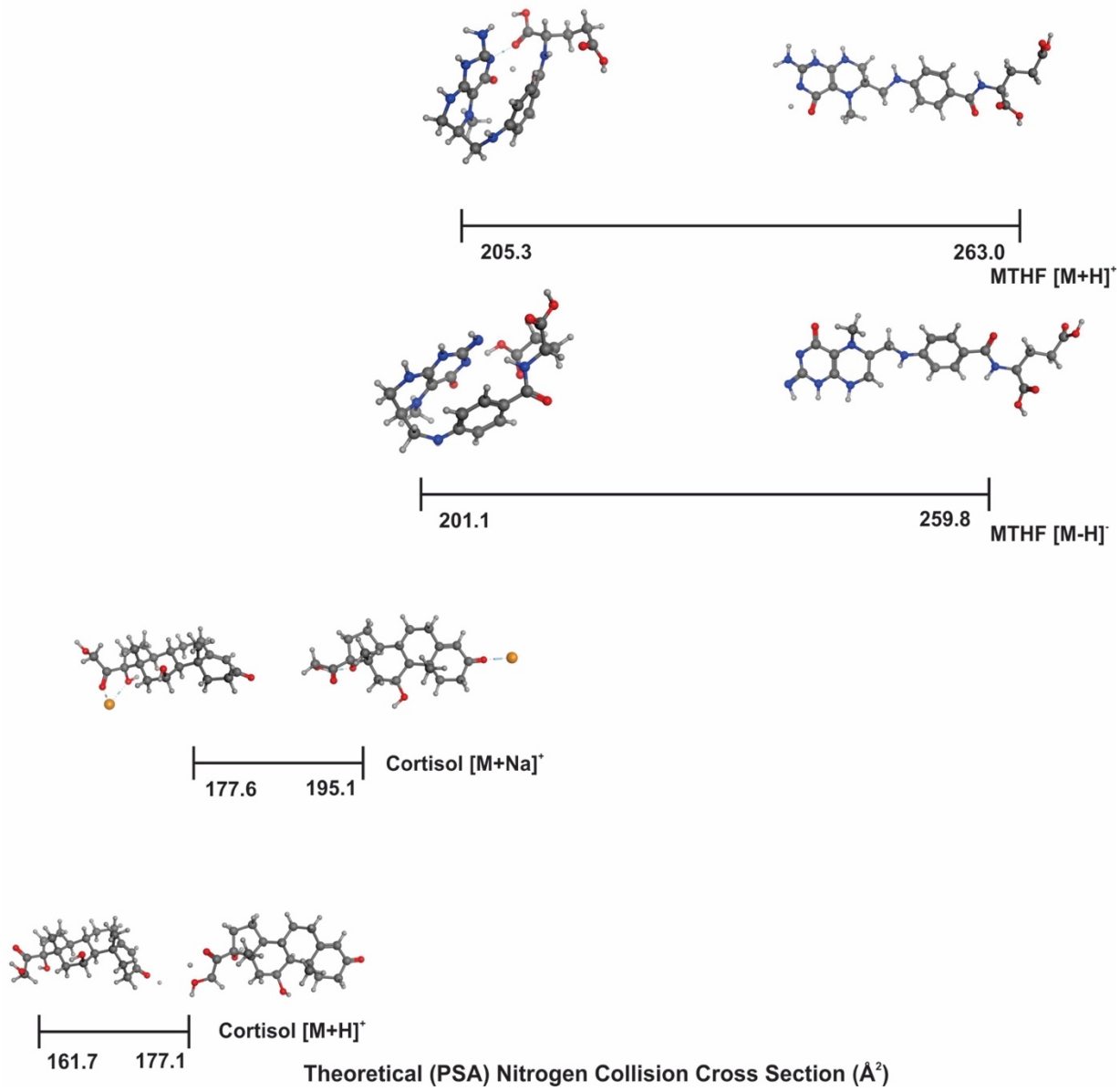


Figure S15. Sample conformations from computational sampling for the metabolites. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, and sodium with orange.

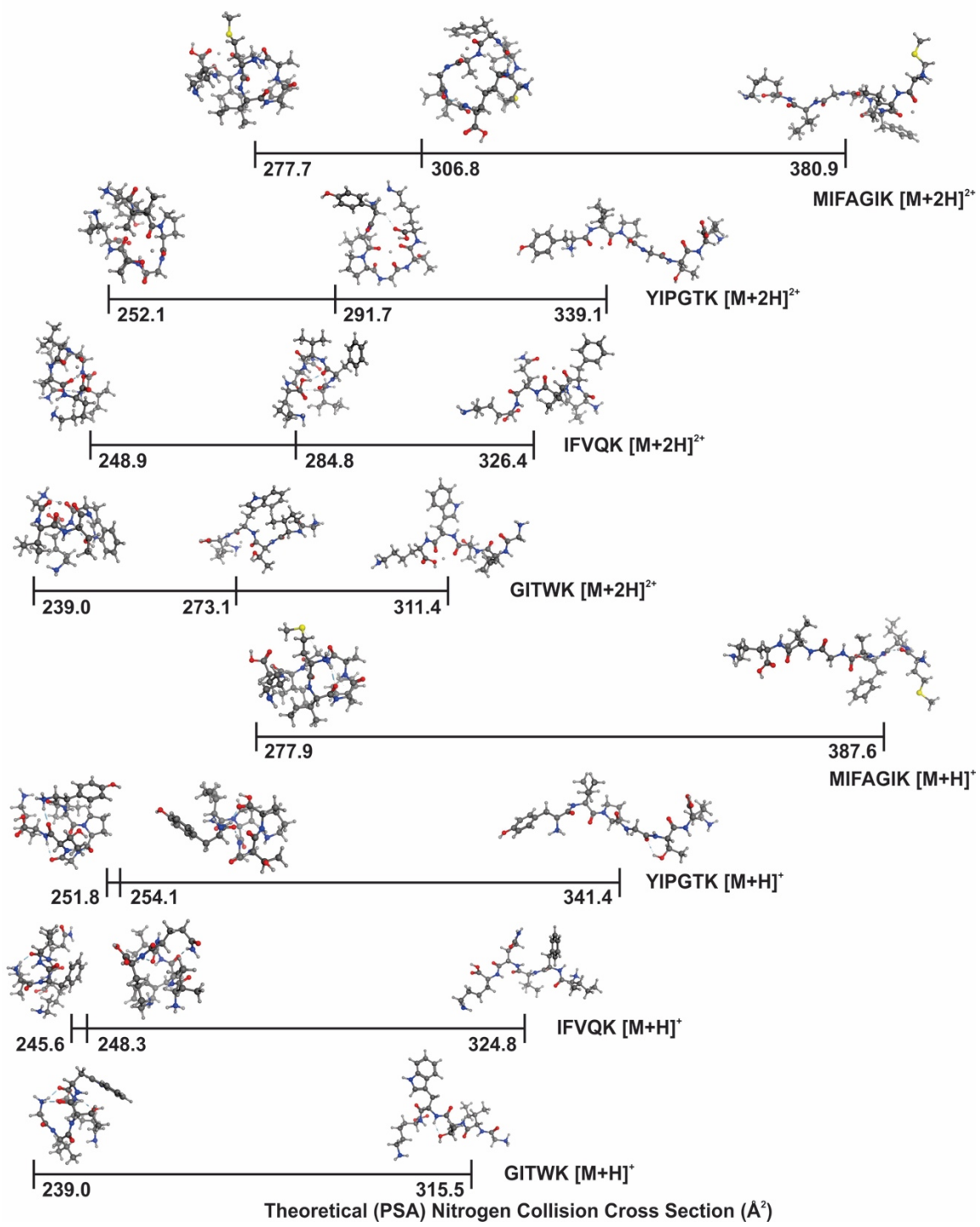


Figure S16. Sample conformations from computational sampling for the peptides. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with bluem and sulfur with yellow.

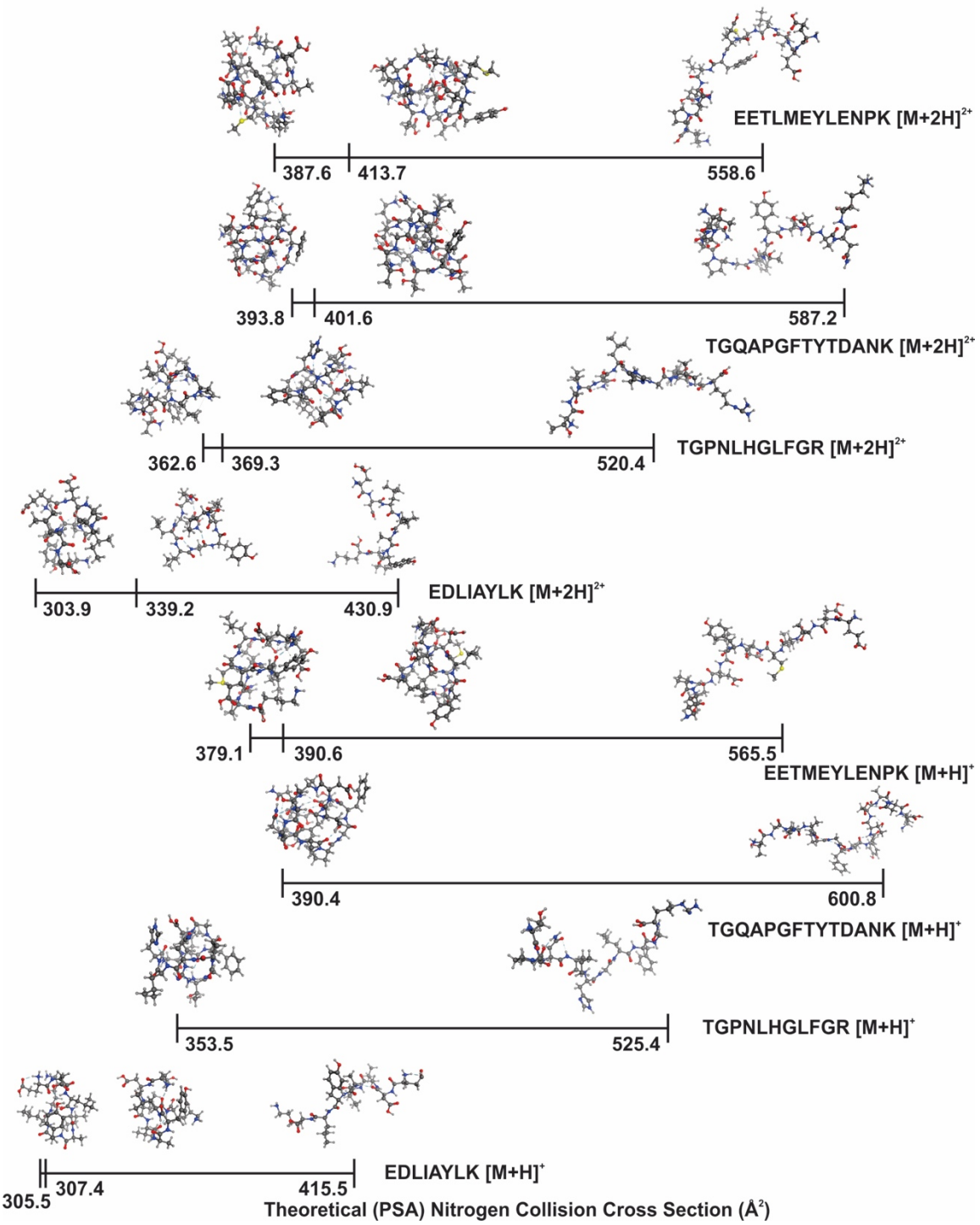


Figure S17. Sample conformations from computational sampling for the peptides. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, and sulfur with yellow.

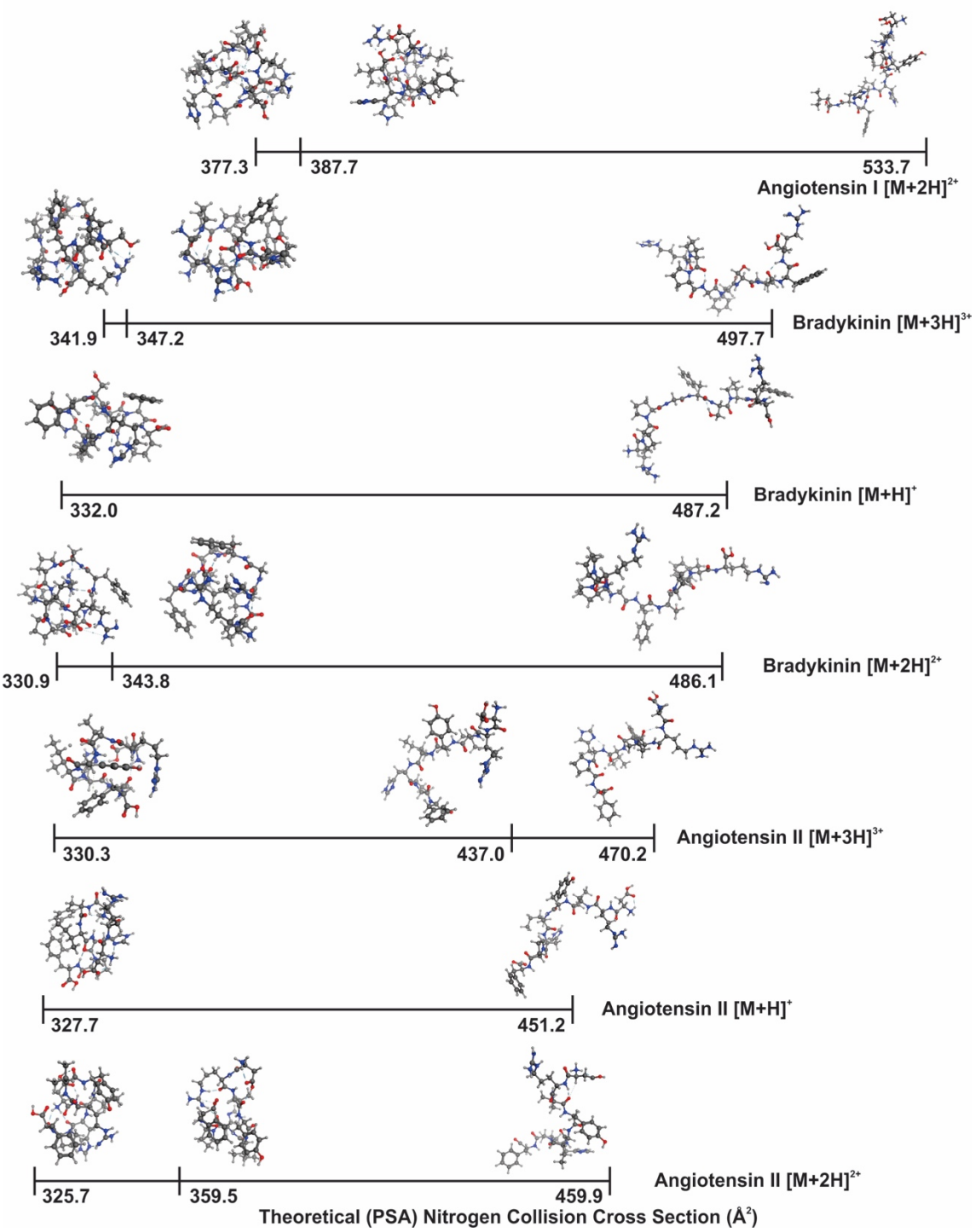


Figure S18. Sample conformations from computational sampling for the peptides. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, and sulfur with yellow.

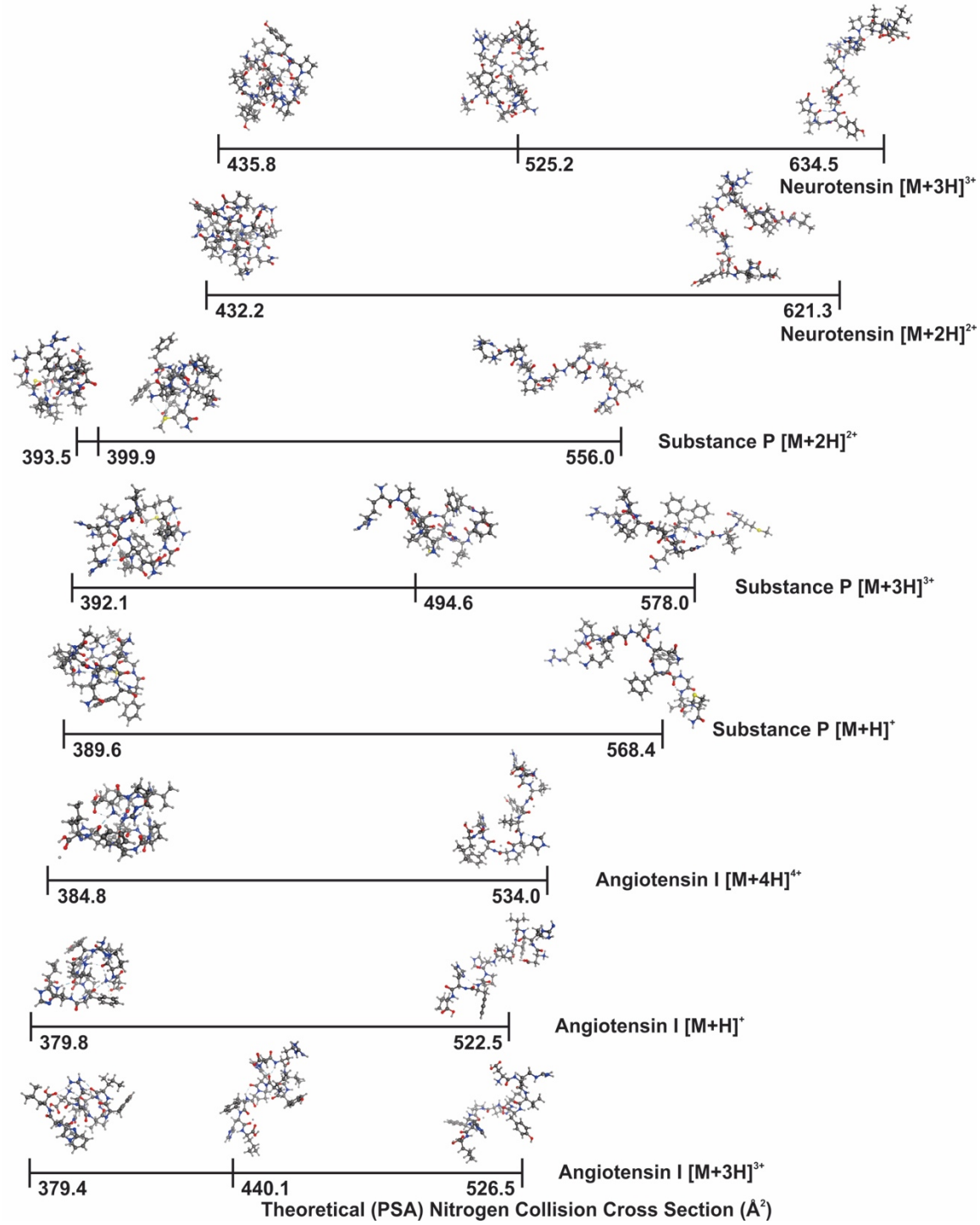


Figure S19. Sample conformations from computational sampling for the peptides. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, and sulfur with yellow.

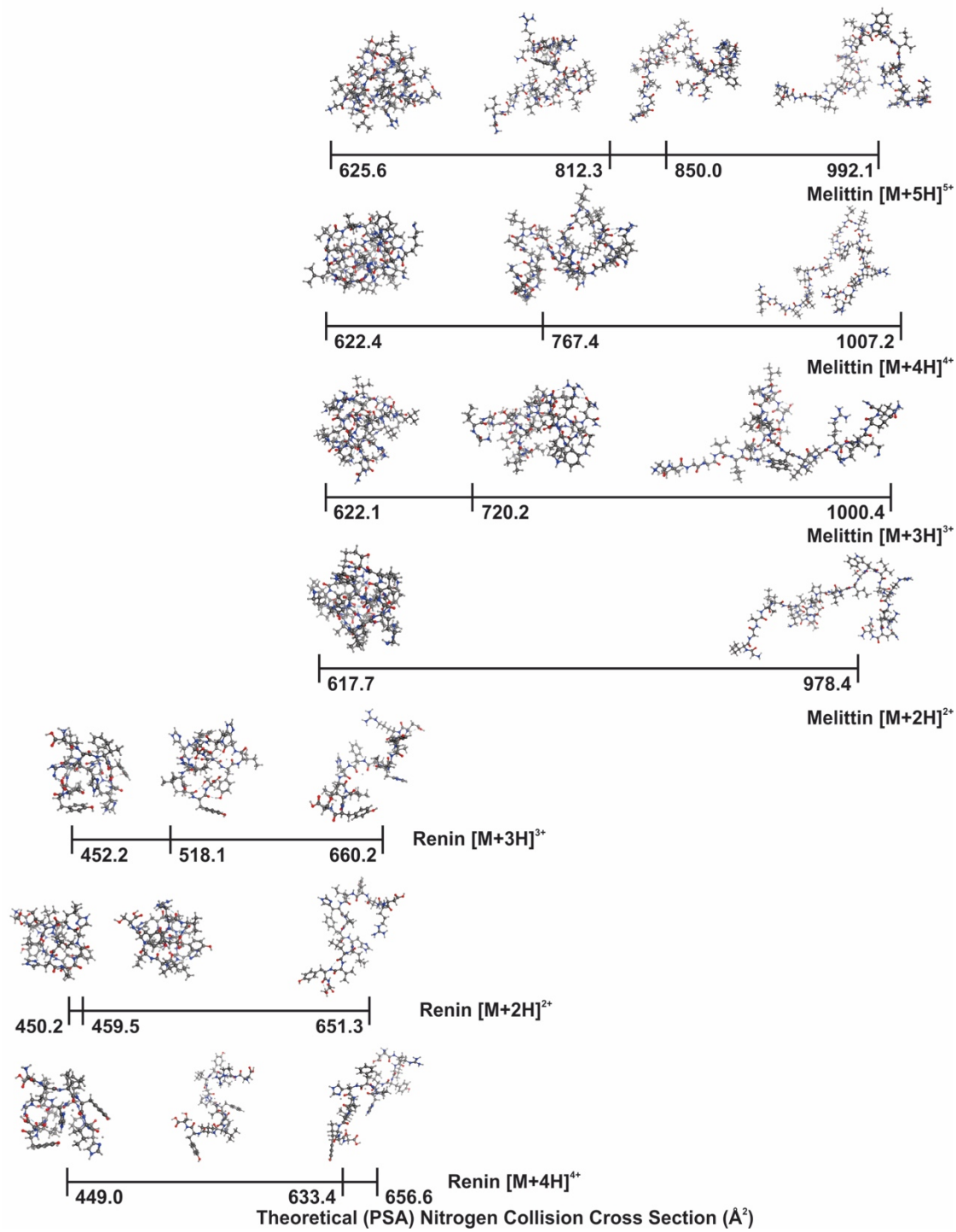


Figure S20. Sample conformations from computational sampling for the peptides. A representative conformation is shown for the lowest and highest theoretical CCS values. If no conformation is indicated within the range, the experimental CCS value fell outside the range. Carbon is represented with dark grey, hydrogen with light grey, oxygen with red, nitrogen with blue, and sulfur with yellow.

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