

Support Information

“Aggregation of α -crystallins in kynurenic acid-sensitized UVA photolysis under anaerobic conditions”

by

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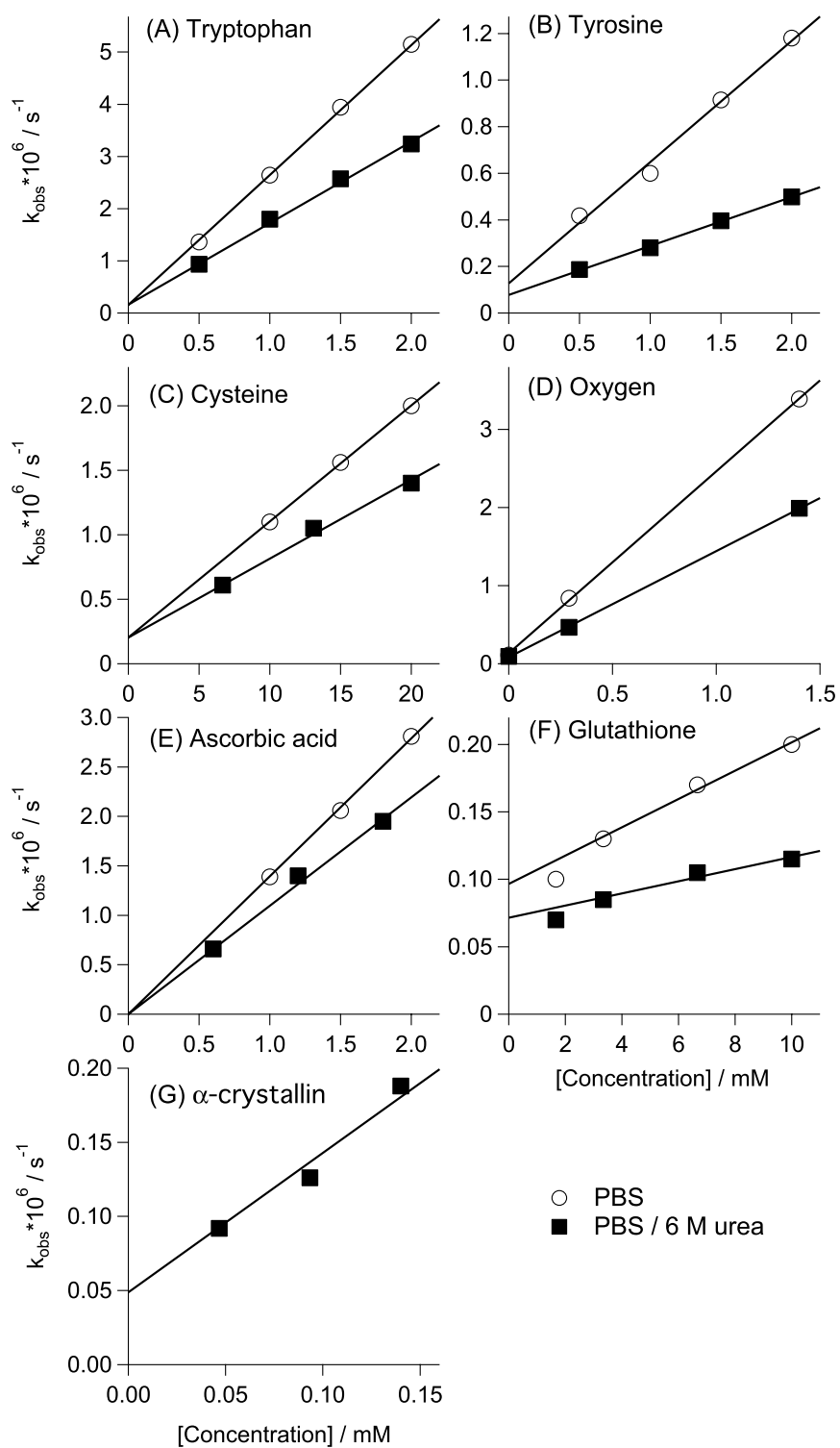


Figure S1. Concentration dependencies of the observed rate constant (k_{obs}) of the triplet KNA quenching in reactions with (A) *L*-tryptophan, (B) *L*-tyrosine, (C) *L*-cysteine, (D) oxygen, (E) ascorbic acid, (F) glutathione and (G) α -crystallin in PBS (open circles) and PBS / 6 M urea solution (filled squares).

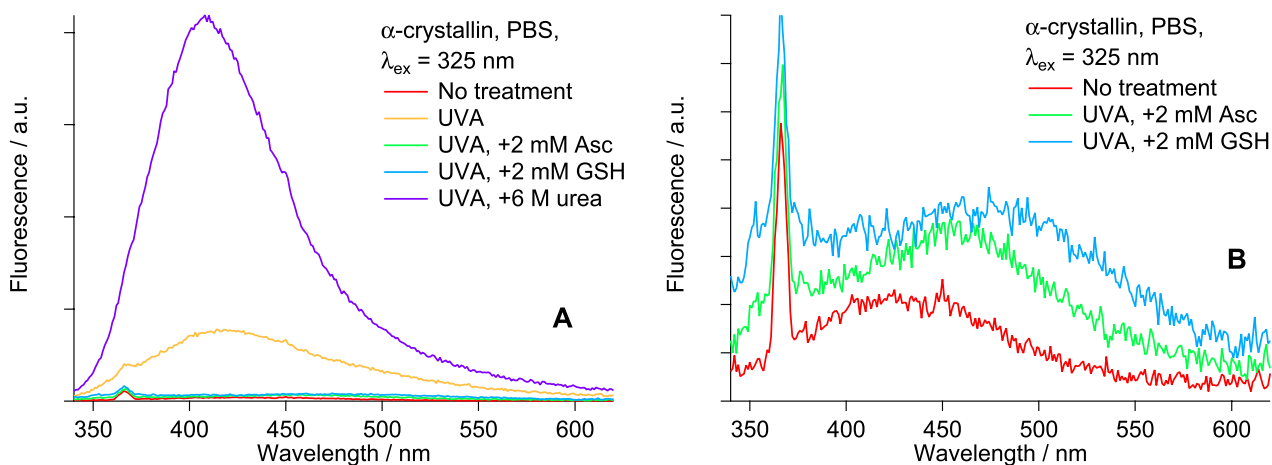


Figure S2. (A) Fluorescence emission spectra of purified α -crystallin after 20 min of KNA-sensitized UVA photolysis in the absence and presence of antioxidants or 6 M urea recorded with the excitation at 325 nm. (B) The same spectra on smaller scale. Peaks at 366 nm on emission spectra are signals from Raman scattering.

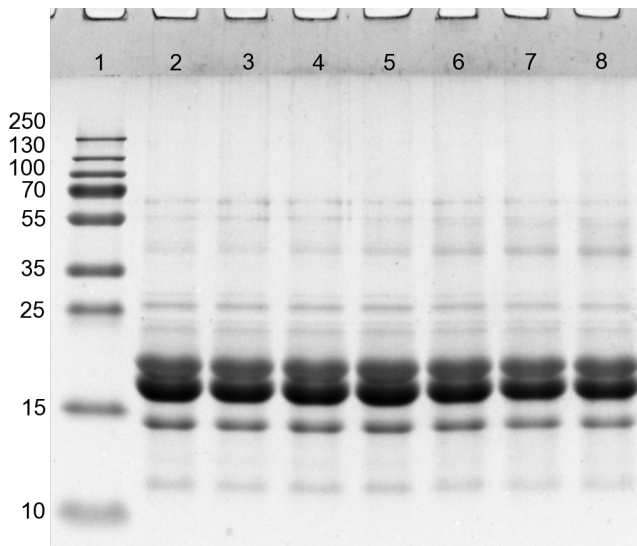


Figure S3. 15% SDS-PAGE of α -crystallin after UVA photolysis in PBS solution. Lane 1 – molecular mass standards (kDa); lane 2 – α -crystallin without any treatment; lane 3 – after 20 min of argon bubbling; lanes 4-8 –after 1, 2, 5, 10, and 20 min of UVA photolysis.

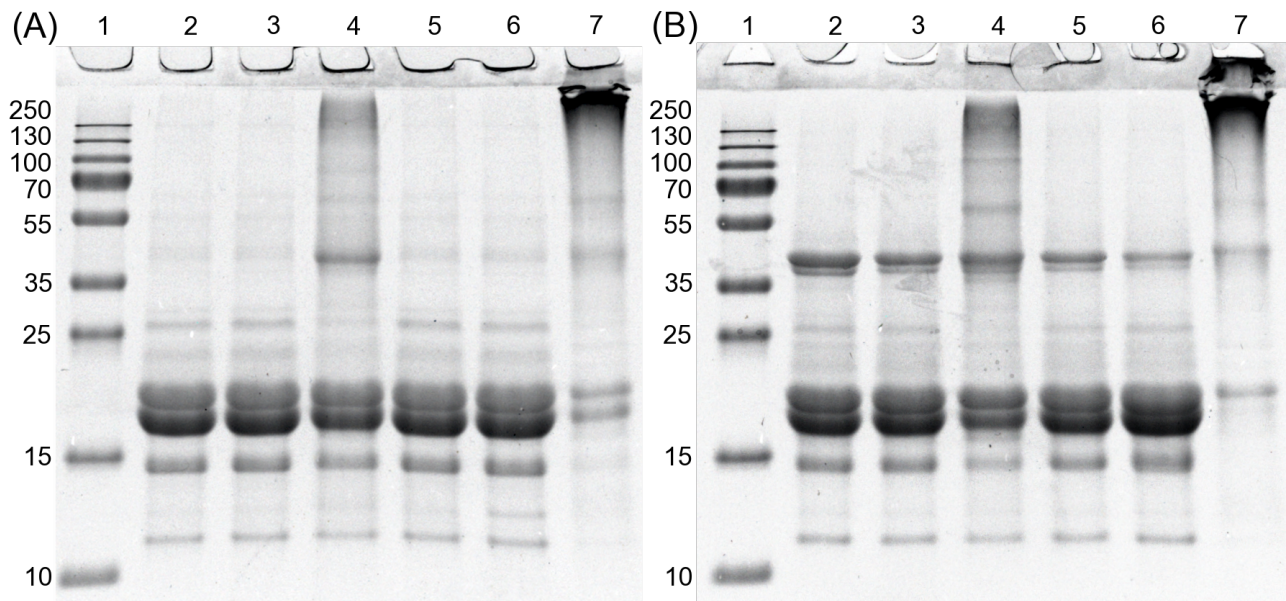


Figure S4. 15% SDS-PAGE of α -crystallin after KNA-sensitized UVA photolysis. The protein samples treated (A) with β -mercaptoethanol and (B) without β -mercaptoethanol before loading onto the gel. For both gels: lane 1 – molecular mass standards (kDa); lane 2 – α -crystallin without any treatment; lane 3 – after 20 min of argon bubbling; lane 4 – after 20 min of photolysis in the absence of antioxidants; lane 5 – after 20 min of photolysis in the presence of 2 mM Asc; lane 6 – after 20 min of photolysis in the presence of 2 mM GSH; lane 7 – after 20 min of UVA photolysis in 6 M urea.

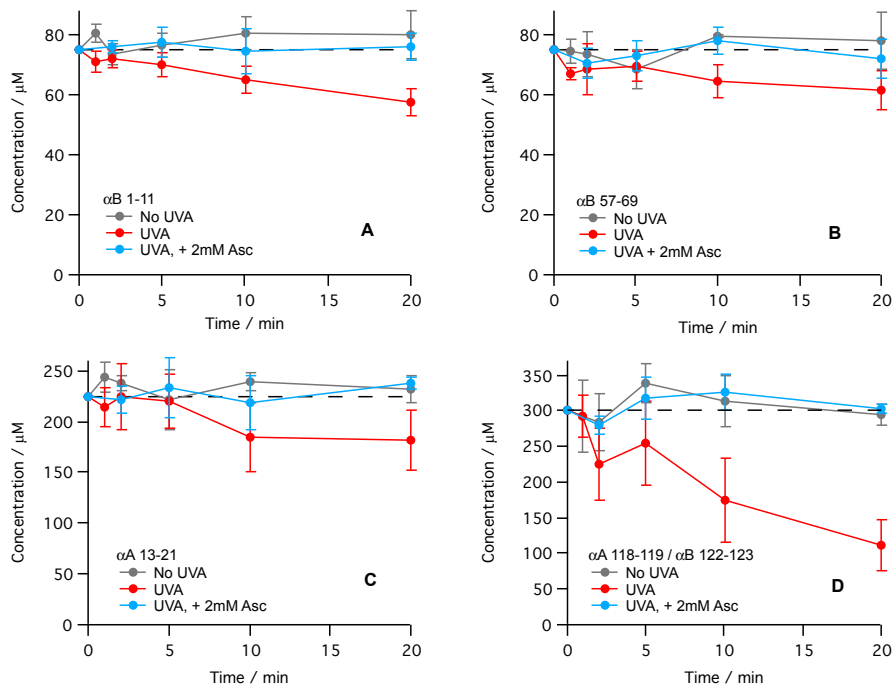


Figure S5. Time dependences of concentrations of α -crystallin peptides: (A) residues 1-11 of α B-crystallin (Trp9), (B) residues 57-69 of α B-crystallin (Trp60); (C) residues 13-21 of α A-crystallin (Tyr18); (D) residues 118-119 of α A-crystallin (Tyr118) and residues 122-123 of α B-crystallin (Tyr122). Grey circles – control non-irradiated sample; red circles – KNA-sensitized UVA photolysis; blue circles – the same photolysis in the presence of 2 mM Asc. Each point is the average value \pm standard deviation of three independent experiments.

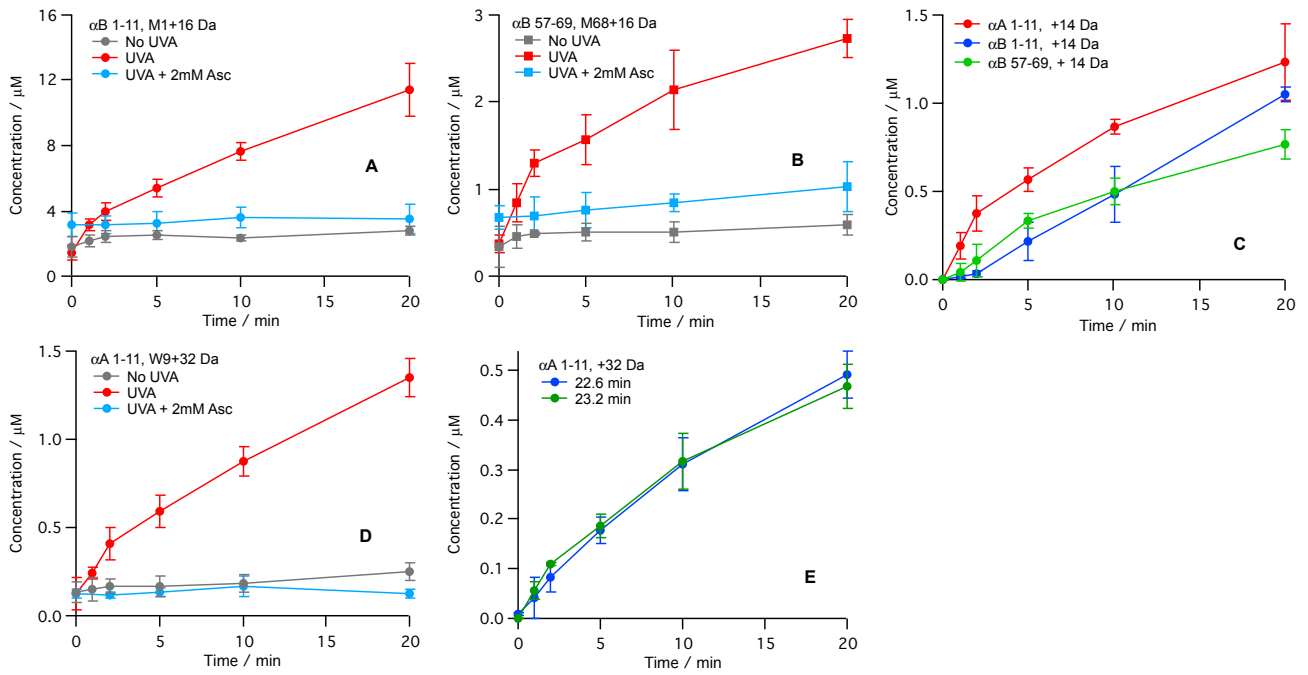


Figure S6. Time dependences of concentrations of α -crystallin peptides: (A) residues 1-11 of αB -crystallin with oxidized Met1; (B) residues 57-69 of αB -crystallin with oxidized Met68; (D) residues 1-11 of αA -crystallin with doubly oxidized Trp9. Grey circles – control non-irradiated sample; red circles – KNA-sensitized UVA photolysis; blue circles – the same photolysis in the presence of 2 mM Asc. (C) Time dependences of concentrations of 1-11 residues of αA -crystallin peptide, 1-11 and 57-69 residues of αB -crystallin peptides (red, blue and green circles, respectively) with modification +13.979 Da. (E) Time dependences of concentrations of 1-11 residues of αA -crystallin peptide with modification +31.988 Da eluted at 22.6 and 23.2 min (blue and green circles, respectively). Each point is the average value \pm standard deviation of three independent experiments.

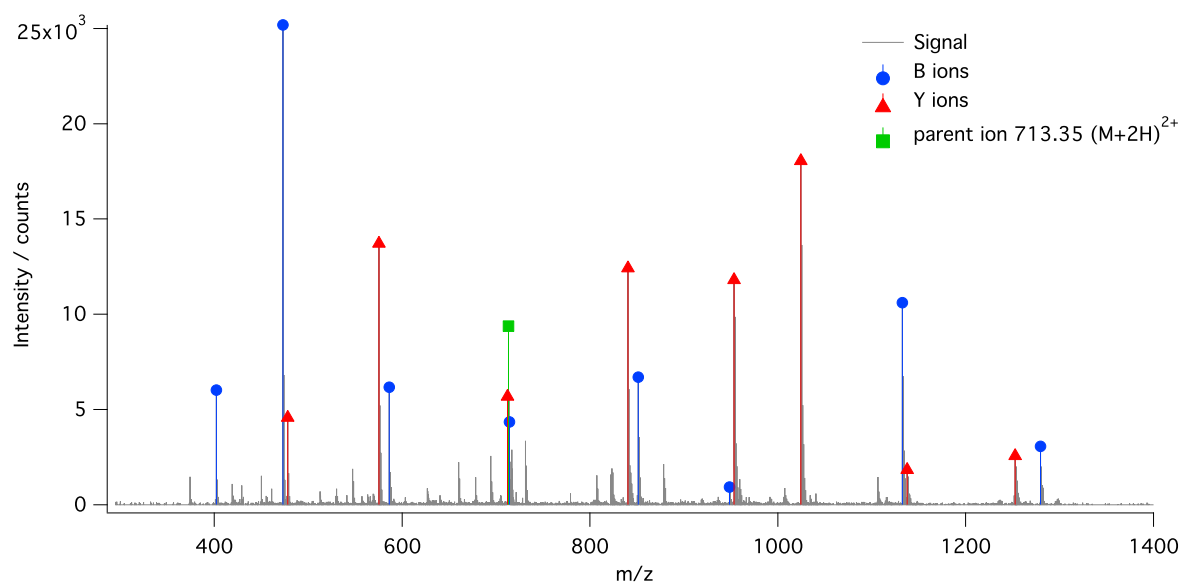
MS/MS data

All theoretical m/z and pI values for peptides under MS/MS analysis were calculated with Fragment Ion Calculator:

<http://db.systemsbiology.net:8080/proteomicsToolkit/FragIonServlet.html>

α A crystallin, W9-2.017 Da
Sequence: Ac-MDIAIQHPWFK, pI: 6.49531

Retention time 27.3 min
Fragmentation of 713.35 (M+2H)²⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M	1		174.05896	1425.69904		11
D	2		289.08590	1252.64796	(1252.6313)	10
I	3	(402.1717)	402.16996	1137.62102	(1137.6105)	9
		(374.1721 -CO, a ₃)				
A	4	(473.2069)	473.20708	1024.53695	(1024.5305)	8
I	5	(586.2899)	586.29114	953.49984	(953.4972)	7
Q	6	(714.3522)	714.34972	840.41578	(840.4141)	6
H	7	(851.4026)	851.40863	712.35720	(712.3529)	5
P	8	(948.4545)	948.46139	575.29829	(575.2957)	4
W-2	9	(1132.5251)	1132.52510	478.24552	(478.2423)	3
F	10	(1279.5948)	1279.59352	294.18181		2
K	11		1407.68848	147.11340		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1424.69122	1425.64149
(M+H) ⁺	1425.69904	1426.64943
(M+2H) ²⁺	713.35346	713.82871
(M+3H) ³⁺	475.90493	476.22180
(M+4H) ⁴⁺	357.18066	357.41835

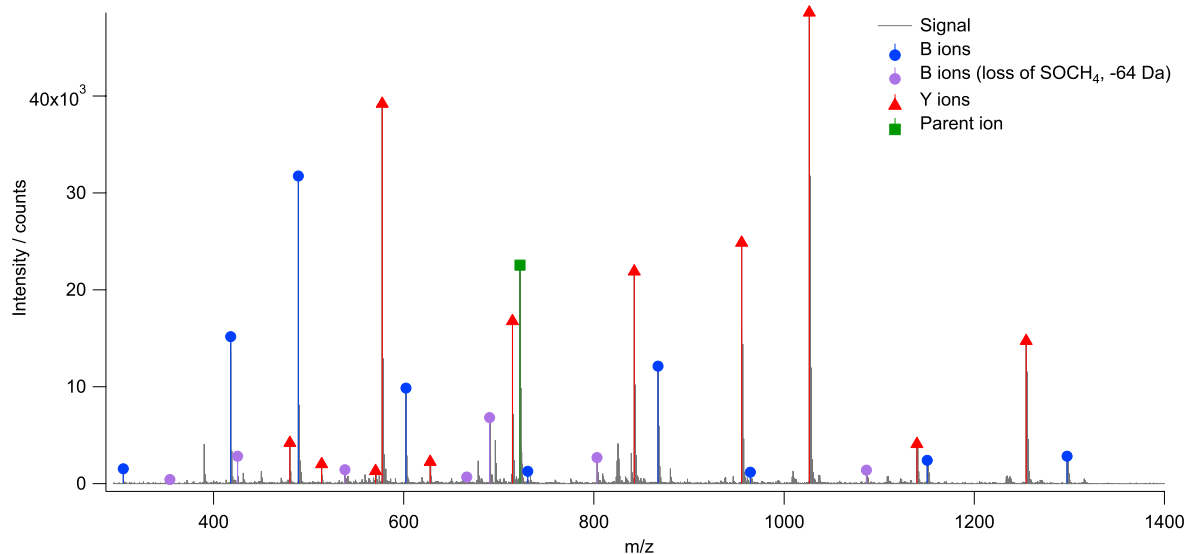
Modifications:

* To residue W added the value -2.0156

* N-terminus modification: 42.0106

α A crystallin, M1+15.995
 Sequence: Ac-MDIAIQHPWFK, pI: 6.49531

Retention time 21.6 min
Fragmentation of 722.35 (M+2H)²⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M+16	1		190.05386	1443.70954		11
D	2	(305.0808)	305.08080	1254.66356	(1254.6573) (627.8278 M+2H ⁺)	10
I	3	(418.1641) (390.1680 -CO / a ₃ ion) (354.1630 -SOCH ₄)	418.16486	1139.63662	(1139.6344) (570.3174 M+2H ⁺)	9
A	4	(489.2001) (425.2043 -SOCH ₄)	489.20198	1026.55255	(1026.5496) (513.7803 M+2H ⁺)	8
I	5	(602.2871) (538.2901 -SOCH ₄)	602.28604	955.51544	(955.5113)	7
Q	6	(730.3441) (666.3310 -SOCH ₄)	730.34462	842.43138	(842.4297)	6
H	7	(867.3979) (803.4007 -SOCH ₄)	867.40353	714.37280	(714.3768)	5
P	8	(964.4513)	964.45629	577.31389	(577.3100)	4
W	9	(1150.5298) (1086.5310 -SOCH ₄)	1150.53560	480.26112	(480.2571)	3
F	10	(1297.6064)	1297.60402	294.18181		2
K	11		1425.69898	147.11340		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1442.70172	1443.65199
(M+H) ⁺	1443.70954	1444.65993
(M+2H) ²⁺	722.35871	722.83395
(M+3H) ³⁺	481.90843	482.22530
(M+4H) ⁴⁺	361.68329	361.92097

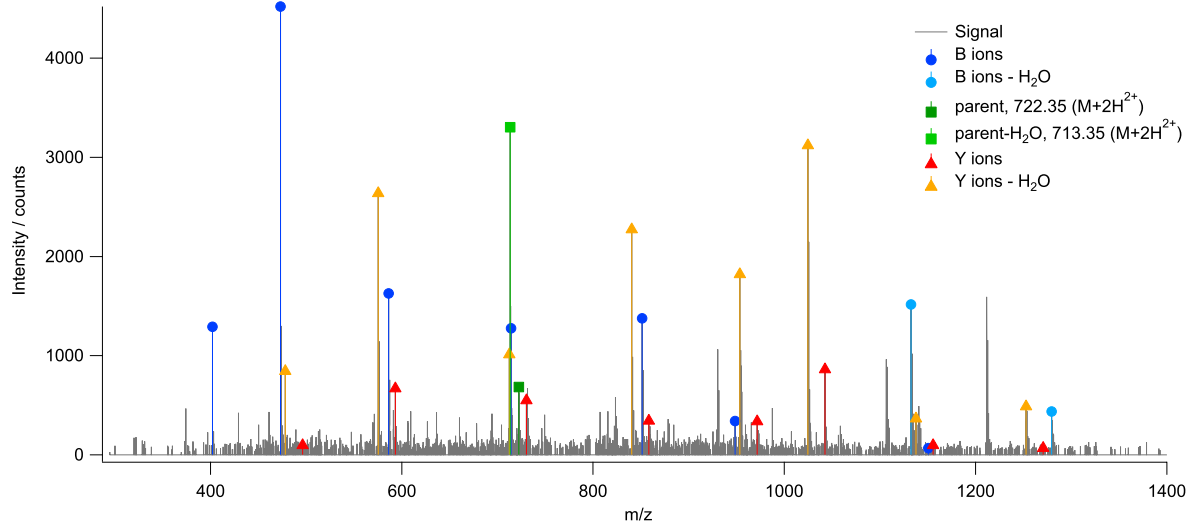
Modifications:

* To residue M added the value 15.9949

* N-terminus modification: 42.0106

α A crystallin, W9+15.995
 Sequence: Ac-MDIAIQHPWFK, pI: 6.49531

Retention time 23.1 min
Fragmentation of 722.35 (M+2H)²⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M	1		174.05896	1443.70954		11
D	2		289.08590	1270.65846	(1270.6570)	10
					(1252.6548 -H ₂ O)	
I	3	(402.1675)	402.16996	1155.63152	(1155.6046)	9
					(1137.6066 -H ₂ O)	
A	4	(473.2060)	473.20708	1042.54745	(1042.5411)	8
					(1024.5313 -H ₂ O)	
I	5	(586.2855)	586.29114	971.51034	(971.5105)	7
					(953.4930 -H ₂ O)	
Q	6	(714.3478)	714.34972	858.42628	(858.4221)	6
					(840.4142 -H ₂ O)	
H	7	(851.4028)	851.40863	730.36770	(730.3606)	5
					(712.3567 -H ₂ O)	
P	8	(948.4550)	948.46139	593.30879	(593.3040)	4
					(575.2950 -H ₂ O)	
W+16	9	(1150.5259)	1150.53560	496.25602	(496.2509)	3
			(1132.5160 -H ₂ O)		(478.2413 -H ₂ O)	
F	10		1297.60402	294.18181		2
			(1279.5856 -H ₂ O)			
K	11		1425.69898	147.11340		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1442.70172	1443.65199
(M+H) ⁺	1443.70954	1444.65993
(M+2H) ²⁺	722.35871	722.83395
(M+3H) ³⁺	481.90843	482.22530
(M+4H) ⁴⁺	361.68329	361.92097

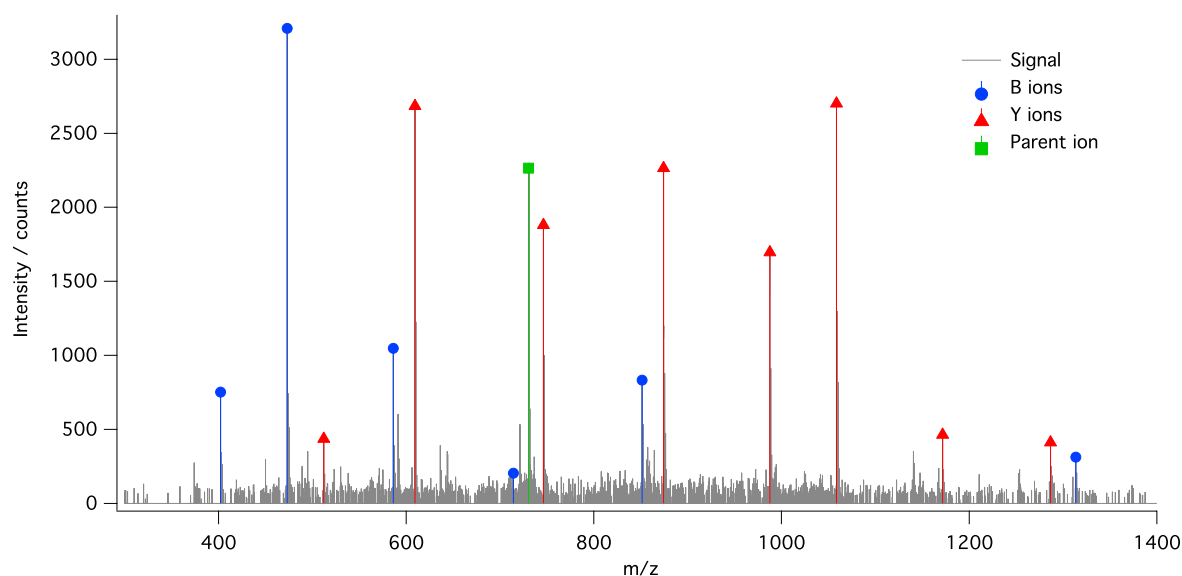
Modifications:

* To residue W added the value 15.9949

* N-terminus modification: 42.0106

α A crystallin, W+31.9898
 Sequence: Ac-MDIAIQHPWFK, pI: 6.49531

Retention time 25.6 min
Fragmentation of 730.35 (M+2H)²⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M	1		174.05896	1459.70444		11
D	2		289.08590	1286.65336	(1286.6462)	10
I	3	(402.1614)	402.16996	1171.62642	(1171.6321)	9
A	4	(473.2060)	473.20708	1058.54235	(1058.5355)	8
I	5	(586.2929)	586.29114	987.50524	(987.5021)	7
Q	6	(714.3437)	714.34972	874.42118	(874.4149)	6
H	7	(851.4028)	851.40863	746.36260	(746.3622)	5
P	8		948.46139	609.30369	(609.3014)	4
W+32	9		1166.53050	512.25092	(512.2437)	3
F	10	(1313.5934)	1313.59892	294.18181	2	
K	11		1441.69388	147.11340	1	

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1458.69662	1459.64689
(M+H) ⁺	1459.70444	1460.65483
(M+2H) ²⁺	730.35616	730.83141
(M+3H) ³⁺	487.24006	487.55693
(M+4H) ⁴⁺	365.68201	365.91970

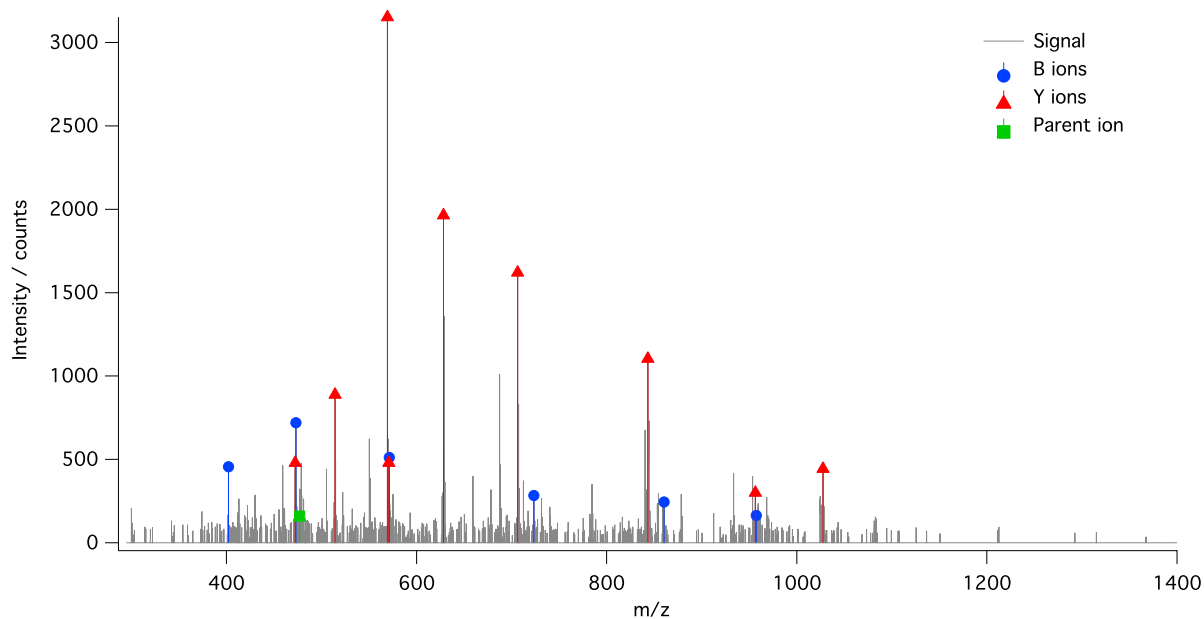
Modifications:

* To residue W added the value 31.9898

* N-terminus modification: 42.0106

α B crystallin 1-11, W9-2.017 Da
 Sequence: Ac-MDIAIHHPWIR, pI: 6.69056

Retention time 25.2 min
Fragmentation of 476.91 (M+3H)³⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	#	(+1)
Ac-M	1		174.05896	1428.72118		11	
D	2		289.08590	1255.67009		10	
I	3	(402.1645)	402.16996	1140.64315	(628.3348 M+2H ⁺)	9	
A	4	(473.2060)	473.20708	1027.55909	(570.8260 M+2H ⁺) (1027.5483) (514.2803 M+2H ⁺)	8	
I	5		586.29114	956.52197	(956.5174)	7	
H	6	(723.3714)	723.35005	843.43791	(843.4409)	6	
H	7	(860.3963)	860.40896	706.37900	(706.3755)	5	
P	8	(957.5092)	957.46173	569.32009	(569.3171)	4	
W-2	9		1141.52544	472.26732	(472.2655)	3	
I	10	(571.3209 M+2H ⁺)	1254.60950	288.20361		2	
R	11		1410.71061	175.11955		1	

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1427.71335	1428.64813
(M+H) ⁺	1428.72118	1429.65607
(M+2H) ²⁺	714.86452	715.33202
(M+3H) ³⁺	476.91231	477.22401
(M+4H) ⁴⁺	357.93620	358.17001

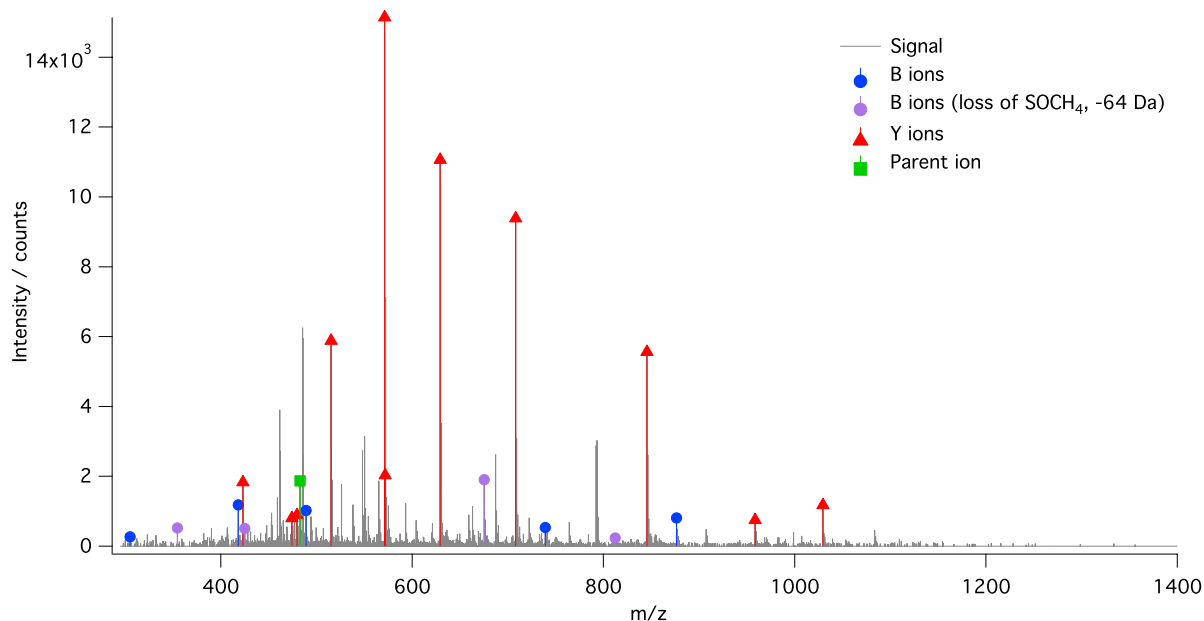
Modifications:

* To residue W added the value -2.0156

* N-terminus modification: 42.0106

α B crystallin 1-11, M1+15.995
 Sequence: Ac-MDIAIHPWIR, pI: 6.69056

Retention time 21.6 min
Fragmentation of 482.91 (M+3H)³⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M+16	1		190.05386	1446.73168		11
D	2	(305.0778)	305.08080	1257.68569		10
I	3	(418.1643)	418.16486	1142.65875	(629.3443 M+2H ⁺)	9
A	4	(489.2005)	489.20198	1029.57469	(571.8285 M+2H ⁺) (1029.5679)	8
I	5		602.28604	958.53757	(515.291 M+2H ⁺) (958.5324)	7
H	6	(739.3403)	739.34495	845.45351	(479.7736 M+2H ⁺) (845.4446)	6
H	7	(876.4097)	876.40386	708.39460	(423.2298 M+2H ⁺) (708.3927)	5
P	8		973.45663	571.33569	(571.3334)	4
W	9		1159.53594	474.28292	(474.2858)	3
I	10		1272.62000	288.20361		2
R	11		1428.72111	175.11955		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1445.72385	1446.65863
(M+H) ⁺	1446.73168	1447.66656
(M+2H) ²⁺	723.86977	724.33727
(M+3H) ³⁺	482.91581	483.22751
(M+4H) ⁴⁺	362.43882	362.67263

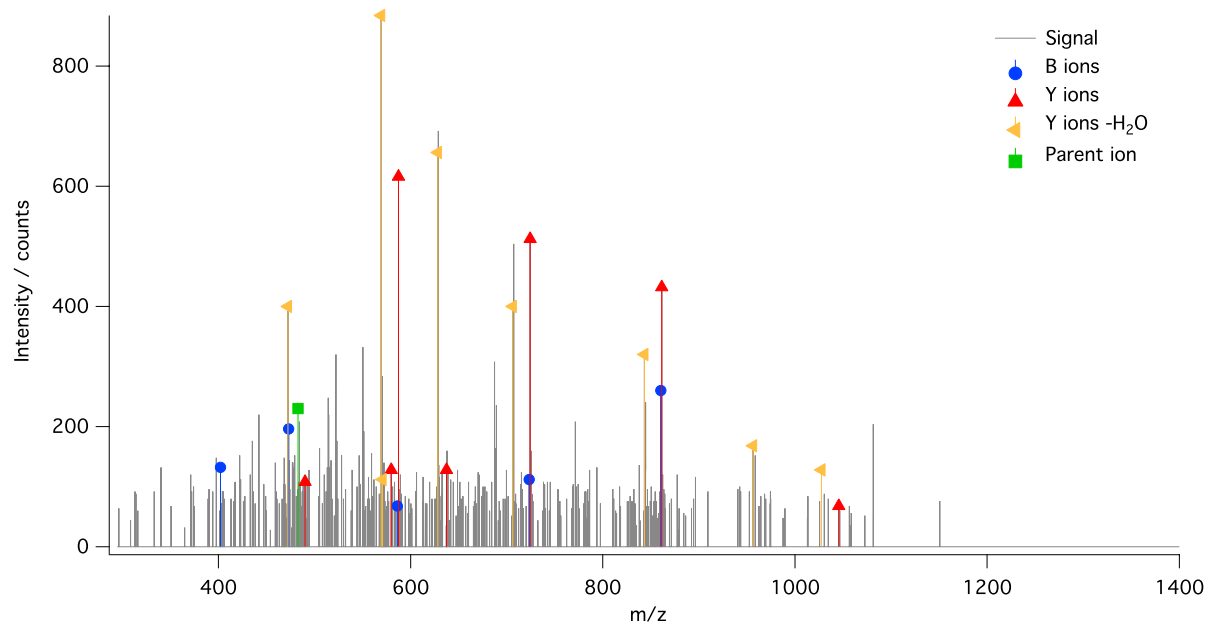
Modifications:

* To residue M added the value 15.9949

* N-terminus modification: 42.0106

α B crystallin 1-11, W9+15.995
 Sequence: Ac-MDIAIHPWIR, pI: 6.69056

Retention time 23.7 min
Fragmentation of 482.91 (M+3H)³⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
Ac-M	1		174.05896	1446.73168		11
D	2		289.08590	1273.68059		10
					(637.3393 M+2H ⁺)	
					(628.3404 M+2H ⁺ -H ₂ O)	
I	3	(402.1687)	402.16996	1158.65365		9
					(579.8311 M+2H ⁺)	
					(570.8276 M+2H ⁺ -H ₂ O)	
A	4	(473.2074)	473.20708	1045.56959	(1045.5574)	8
					(1027.5707 -H ₂ O)	
I	5	(586.2946)	586.29114	974.53247		7
					(956.5200 -H ₂ O)	
H	6	(723.3610)	723.35005	861.44841	(861.4473)	6
					(843.4432 -H ₂ O)	
H	7	(860.3986)	860.40896	724.38950	(724.3886)	5
					(706.3773 -H ₂ O)	
P	8		957.46173	587.33059	(587.3313)	4
					(569.3178 -H ₂ O)	
W+16	9		1159.53594	490.27782	(490.2767)	3
					(472.2735 -H ₂ O)	
I	10		1272.62000	288.20361		2
R	11		1428.72111	175.11955		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1445.72385	1446.65863
(M+H) ⁺	1446.73168	1447.66656
(M+2H) ²⁺	723.86977	724.33727
(M+3H) ³⁺	482.91581	483.22751
(M+4H) ⁴⁺	362.43882	362.67263

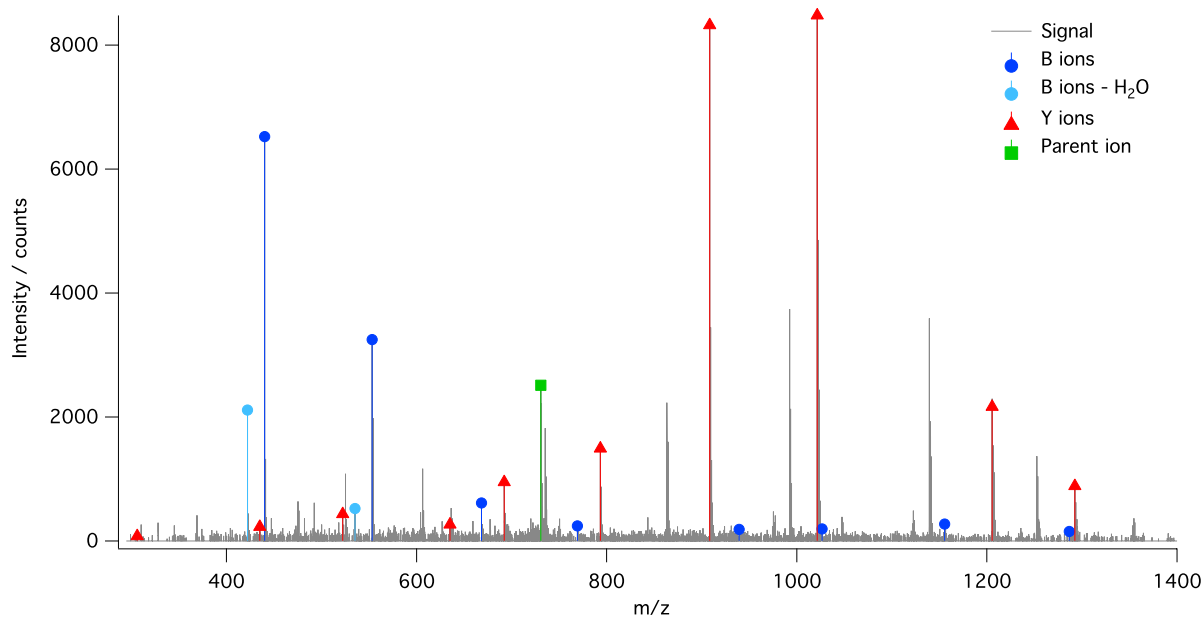
Modifications:

* To residue W added the value 15.9949

* N-terminus modification: 42.0106

α B crystallin 57-69, W60 - 2.017 Da
 Sequence: APSWIDTGLSEMR, pI: 4.37040

Retention time 25.3 min
Fragmentation of 730.85 (M+2H)²⁺



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
A	1		72.04498	1460.68448		13
P	2		169.09775	1389.64737		12
S	3		256.12978	1292.59460	(1292.5841)	11
W-2	4	(440.1932)	440.19349	1205.56258	(1205.5620)	10
		(422.1812 -H ₂ O)				
I	5	(553.2791)	553.27755	1021.49886	(1021.4937)	9
		(535.2575 -H ₂ O)				
D	6	(668.3018)	668.30449	908.41480	(908.4143)	8
T	7	(769.3506)	769.35217	793.38786	(793.3880)	7
G	8		826.37364	692.34018	(692.3432)	6
L	9	(939.4434)	939.45770	635.31872	(635.3056)	5
S	10	(1026.5054)	1026.48973	522.23465	(522.2314)	4
E	11	(1155.5258)	1155.53232	435.20262	(435.2028)	3
M	12	(1286.5687)	1286.57281	306.16003	(306.1520)	2
R	13		1442.67392	175.11955		1

Mass/Charge Table

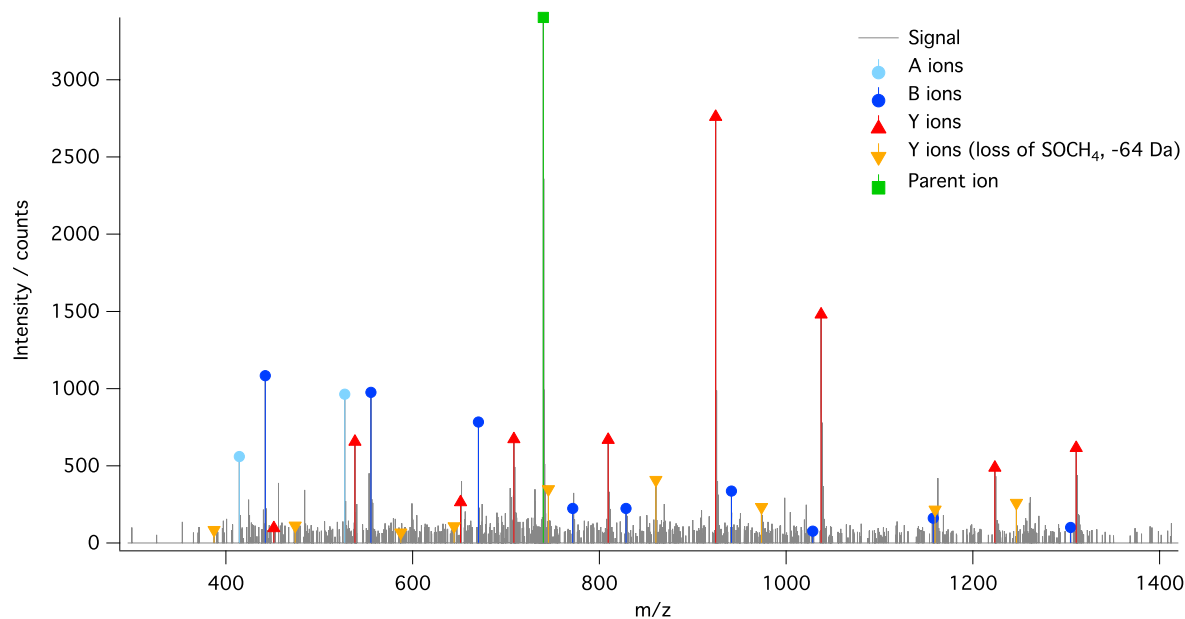
	Mass	
	Mono	Avg
(M)	1459.67666	1460.62481
(M+H) ⁺	1460.68448	1461.63275
(M+2H) ²⁺	730.84618	731.32036
(M+3H) ³⁺	487.56674	487.88290
(M+4H) ⁴⁺	365.92702	366.16418

Modifications:

* To residue W added the value -2.0156

α B crystallin 57-69, M68 + 15.995 Da
 Sequence: APSWIDTGLSEMR, pI: 4.37040

Retention time 21.8 min
Fragmentation of ion 739.85 (M+2H)²



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
A	1		72.04498	1478.69498		13
P	2		169.09775	1407.65787	(1407.6582)	12
					(704.3305 M+2H ⁺)	
S	3		256.12978	1310.60510	(1310.6022)	11
					(1246.5961 -SOCH ₄)	
W	4	(442.2083)	442.20909	1223.57308	(1223.5662)	10
		(414.2117 -CO, a ₄)			(1159.5698 -SOCH ₄)	
		(424.1969 -H ₂ O)				
I	5	(555.2918)	555.29315	1037.49376	(1037.4894)	9
		(527.2962 -CO, a ₅)			(973.4951 -SOCH ₄)	
D	6	(670.3146)	670.32009	924.40970	(924.4023)	8
					(860.4095 -SOCH ₄)	
T	7	(771.3650)	771.36777	809.38276	(809.3815)	7
					(745.3779 -SOCH ₄)	
G	8	(828.3839)	828.38924	708.33508	(708.3339)	6
					(644.3394 -SOCH ₄)	
L	9	(941.4709)	941.47330	651.31362	(651.3086)	5
					(587.3171 -SOCH ₄)	
S	10	(1028.5016)	1028.50533	538.22955	(538.2260)	4
					(474.2352 -SOCH ₄)	
E	11	(1157.5492)	1157.54792	451.19752	(451.1953)	3
					(387.2030 -SOCH ₄)	
M+16	12	(1304.5782)	1304.58331	322.15493	(322.1555)	2
R	13		1460.68442	175.11955		1

Mass/Charge Table

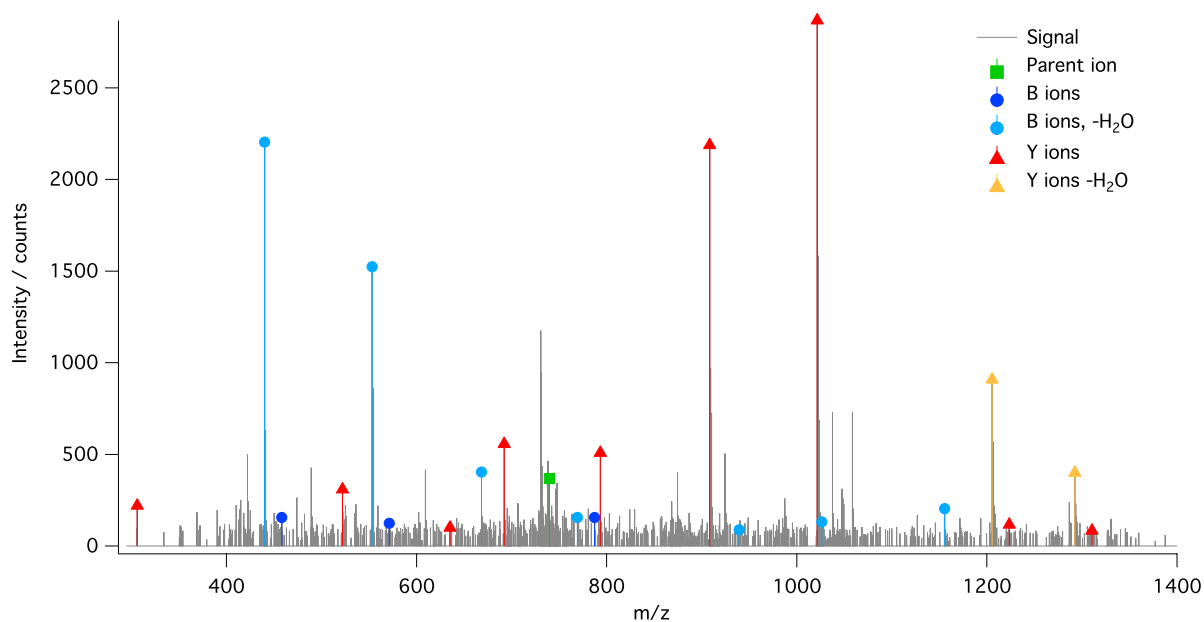
	Mass	
	Mono	Avg
(M)	1477.68716	1478.63531
(M+H) ⁺	1478.69498	1479.64324
(M+2H) ²⁺	739.85143	740.32561
(M+3H) ³⁺	493.57024	493.88640
(M+4H) ⁴⁺	370.42965	370.66680

Modifications:

* To residue M added the value 15.9949

α B crystallin 57-69, W60 + 15.995 Da
 Sequence: APSWIDTGLSEMR, pI: 4.37040

Retention time 22.7 min
Fragmentation of ion 739.85 (M+2H)²



Fragment Ion Table, monoisotopic masses

Seq	#	(Exp)	B	Y	(Exp)	# (+1)
A	1		72.04498	1478.69498		13
P	2		169.09775	1407.65787		12
S	3		256.12978	1310.60510	(1310.6132)	11
					(1292.5939 -H ₂ O)	
W+16	4	(458.2345)	458.20399	1223.57308	(1223.5500)	10
		(440.1880 -H ₂ O)			(1205.5504 -H ₂ O)	
I	5	(571.2629)	571.28805	1021.49886	(1021.4983)	9
		(553.2730 -H ₂ O)				
D	6		686.31499	908.41480	(908.4097)	8
		(668.2985 -H ₂ O)				
T	7	(787.3810)	787.36267	793.38786	(793.3840)	7
		(769.3511 -H ₂ O)				
G	8		844.38414	692.34018	(692.3399)	6
L	9		957.46820	635.31872	(635.3142)	5
		(939.4528 -H ₂ O)				
S	10		1044.50023	522.23465	(522.2255)	4
		(1026.4903 -H ₂ O)				
E	11		1173.54282	435.20262		3
		(1155.5302 -H ₂ O)				
M	12		1304.58331	306.16003	(306.1561)	2
R	13		1460.68442	175.11955		1

Mass/Charge Table

	Mass	
	Mono	Avg
(M)	1477.68716	1478.63531
(M+H) ⁺	1478.69498	1479.64324
(M+2H) ²⁺	739.85143	740.32561
(M+3H) ³⁺	493.57024	493.88640
(M+4H) ⁴⁺	370.42965	370.66680

Modifications: * To residue W added the value 15.9949