Supporting Information for:

Identification of Amino Acid Epimerization and Isomerization in Crystallin Proteins by Tandem LC-MS

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Content:

Table S1. Source ions for R and S values

R Value for CID

Peptide	Fragment 1	Fragment 2	R value
RGYALG	a_5^+	-17	1.13
MEHFRW	b_3^+	-17	1.20
LVFFAEDVGSNK	b_{10}^+	b_5^+	1.88
RPPGFSPFR	b_6^+	-17	1.55
PHCKRM	b_5^+	y_4^+	1.27
RYLPT	b_2^+	-17	1.35

R value for RDD

Peptide	Fragment 1	Fragment 2	R value
LDLAGR	-56L	-43L	1.22
IQTGLDATHAER	-72E	-18	1.70
DAEFR	y_4^+	-59E	1.18
HGPLGPL	-56L	-43L	1.21
NGPLQAGQPGER	$y_{11}\bullet^{2+}$	-18	2.01
PSKYEPFV	$b_5\bullet^+$	$y_7\bullet^+$	2..31

S value for CID

Peptide	Fragment 1	Fragment 2	R value
TVLDSGISEVR (L-Asp)	b_2^+	y_6^+	1.63
TVLDSGISEVR (D-isoAsp)	y_4^+	y_8^+	1.13
TVLDSGISEVR (D-Asp)	y_4^+	y_5^+	1.58
TVLDSGISEVR (isoAsp)	y_6^+	-18	1.62
TVLDSGISEVR (L-Asp)	b_2^+	y_9^{2+}	1.72
TVLDSGISEVR (isoAsp)	y_8^+	-18	1.22

S value for RDD

Peptide	Fragment 1	Fragment 2	R value
TVLDSGISEVR (isoAsp)	$y_9\bullet^+$	$y_6\bullet^+$	2.38
TVLDSGISEVR (L-Asp)	b_5^+	$y_3\bullet^+$	2.02
TVLDSGISEVR (D-isoAsp)	b_5^+	$y_9\bullet^+$	2.22
TVLDSGISEVR (D-Asp)	$y_9\bullet^+$	$y_9\bullet^{2+}$	3.21
TVLDSGISEVR (iso-Asp)	$b_3\bullet^+$	y_2^+	2.07
TVLDSGISEVR (L-Asp)	b_6^+	$b_4\bullet^+$	2.70

Table S2. Other identified proteins predicted to be crystallins from the *ovis aries* database (UniProt 2014 06, 26,849 entries)

UniprotKB Acession Number	Gene Name	Sequence Coverage %	Unique Peptides Detected	Log (E)
W5P9A5	CRYBA1	92.6%	12	-506.4
W5NUB1	CRYBA4	53.9%	9	-383.0
W5QCG5	CRYBB2	71.6%	9	-334.9
W5QC77	CRYGB	58.9%	6	-304.3
W5NTX9	CRYBB1	49.2%	9	-260.1
W5QH67	CRYGS	66.3%	7	-217.3
W5PSF8	CRYZ	50%	9	-197.9
W5QC70	CRYGC	24.1%	3	-145.3
W5QCA3	CRYGA	37.4%	4	-119.9
W5QET5	CRYBA2	6.6%	1	-16.4

Figure S1 (a) LC chromatogram for peptide TVLDSGISEVR in sheep eye crystallin digestion mixture.

(b) CID of [TVLDSGISEVR + 2H]$^{2+}$ at 24.76min, 26.59min, 28.53min separately. The red stars represent fragments from a co-eluting peptide HFSPEDLTVK.

The peptide eluting at 24.76 minute has the S_{CID} value of 30, 1.3, 6.7, 2.1 comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides;

The peptide eluting at 26.59 min has the S_{CID} value of 2.7, 13, 3.5, 3.9 comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides

The peptide eluting at 28.53 min has the S_{CID} value of 4.8, 10, 2.1, 5.2 comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides

The spectra at 24.76min and 28.53min are identified as L-isoAsp and D-Asp containing peptide.

The peptide eluting at 26.59 minutes does not match any of the isomers, indicating that potentially the serine residue is also epimerized or that the remaining two Asp isomers are co-eluting.

(c) CID spectra of four synthetic peptides L-Asp, D-Asp, L-isoAsp, and D-isoAsp TVLDSGISEVR.

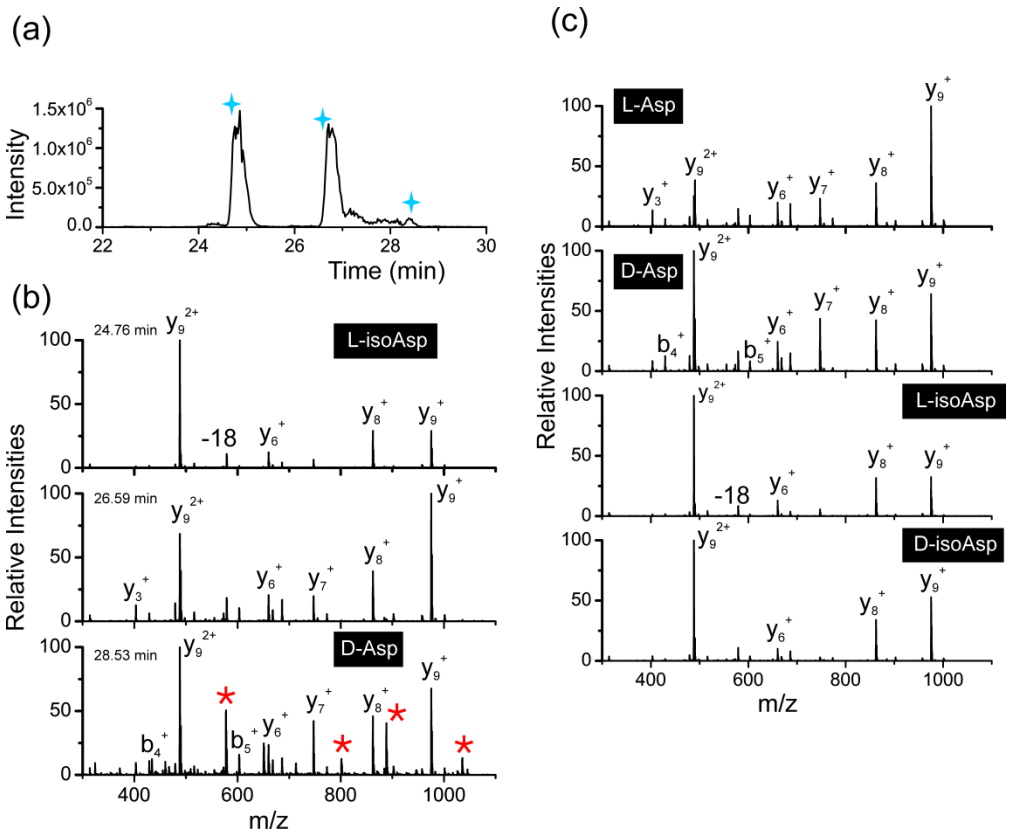

Figure S2. (a) LC chromatogram for peptide ^{I}TVLDSGISEVR in sheep eye crystallin digestion mixture after modification.

(b) RDD spectra of [^{I}TVLDSGISEVR + 2H]$^{2+}$ at 64.68min, 67.57min, 70.44min separately. The red stars represent fragments from a co-eluting peptide.

The peptide eluting at 64.68 minute has the S_{RDD} value of 40, 2.6, 59, 20, comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides.

The peptide eluting at 67.57 min has the S value of 62, 16, 79, 2.4 comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides

The peptide eluting at 70.44 min has the S_{RDD} value of 3.1, 25, 6.7, 43, comparing to L-Asp, L-isoAsp, D-Asp, D-isoAsp containing synthetic peptides.

The three spectra are identified as L-isoAsp, D-isoAsp, and L- Asp containing peptide (S_{RDD}<3.2). Since RDD provides much higher values than CID, the identification is clear in RDD.

(c) RDD spectra of four synthetic peptides L-Asp, D- Asp, L-isoAsp, and D-isoAsp ^{I}TVLDSGISEVR.

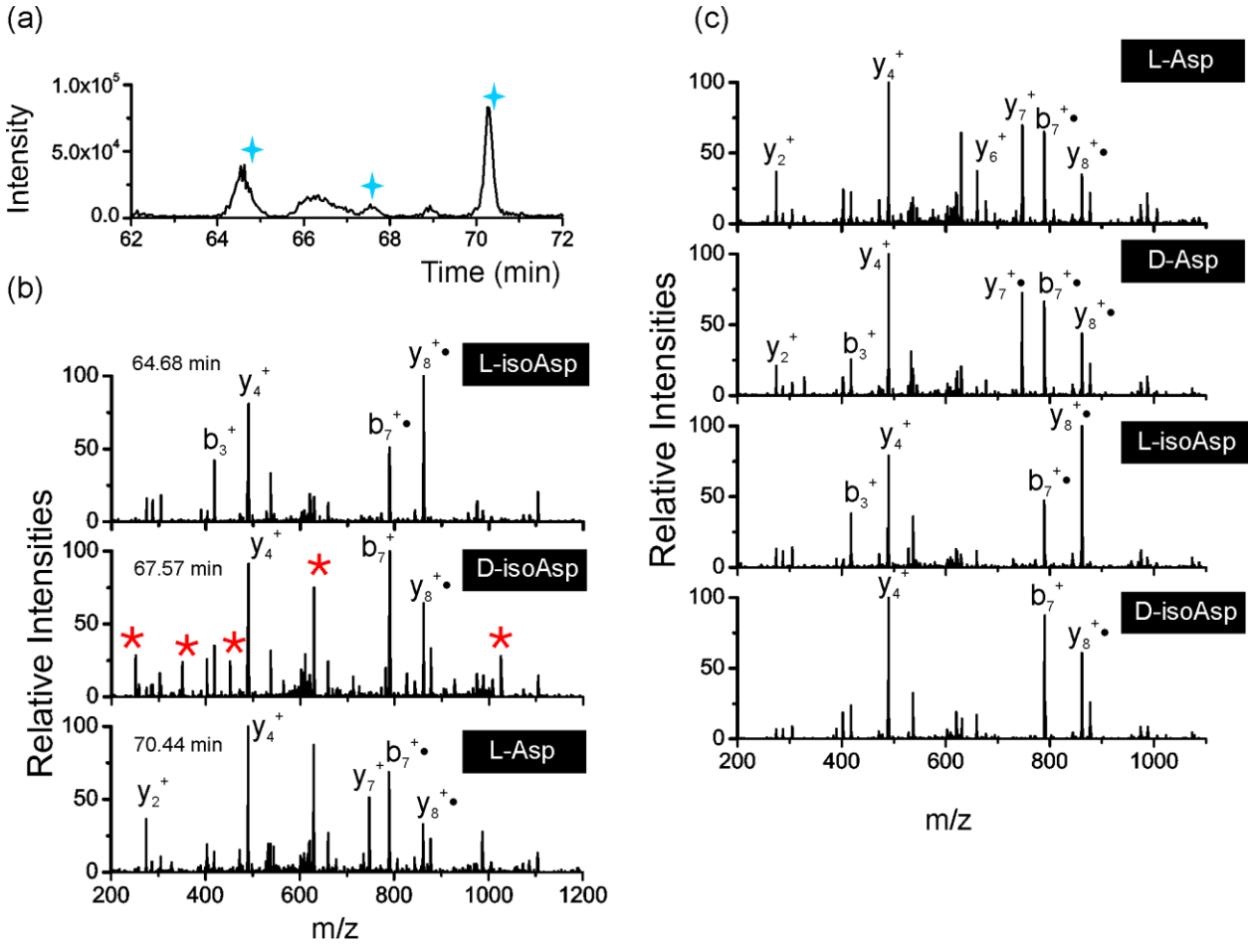

Figure S3. (a) LC chromatogram for peptide HFSPEDLTVK in sheep eye crystallin digestion mixture. (b) CID of $[HFSPEDLTVK + 2H]^{2+}$ at 32.44min and 35.39min, separately. The R_{isomer} value is 1.5 (y_9^+ & -18). (c) CID of $[HFSPEDLTVK + H]^+$ at 32.64min and 35.53min, separately. The R_{isomer} value is 7.3 (b_8^+ & y_5^+). (d) LC chromatogram for peptide ^{I}HFSPEDLTVK in sheep eye crystallin digestion mixture after modification. (e) RDD of $[HFSPEDLTV^{I}K + 2H]^{2+}$ at 61.97min and 66.25min, separately. The R_{isomer} value is 25.3 (y_6^+& y_4^+).

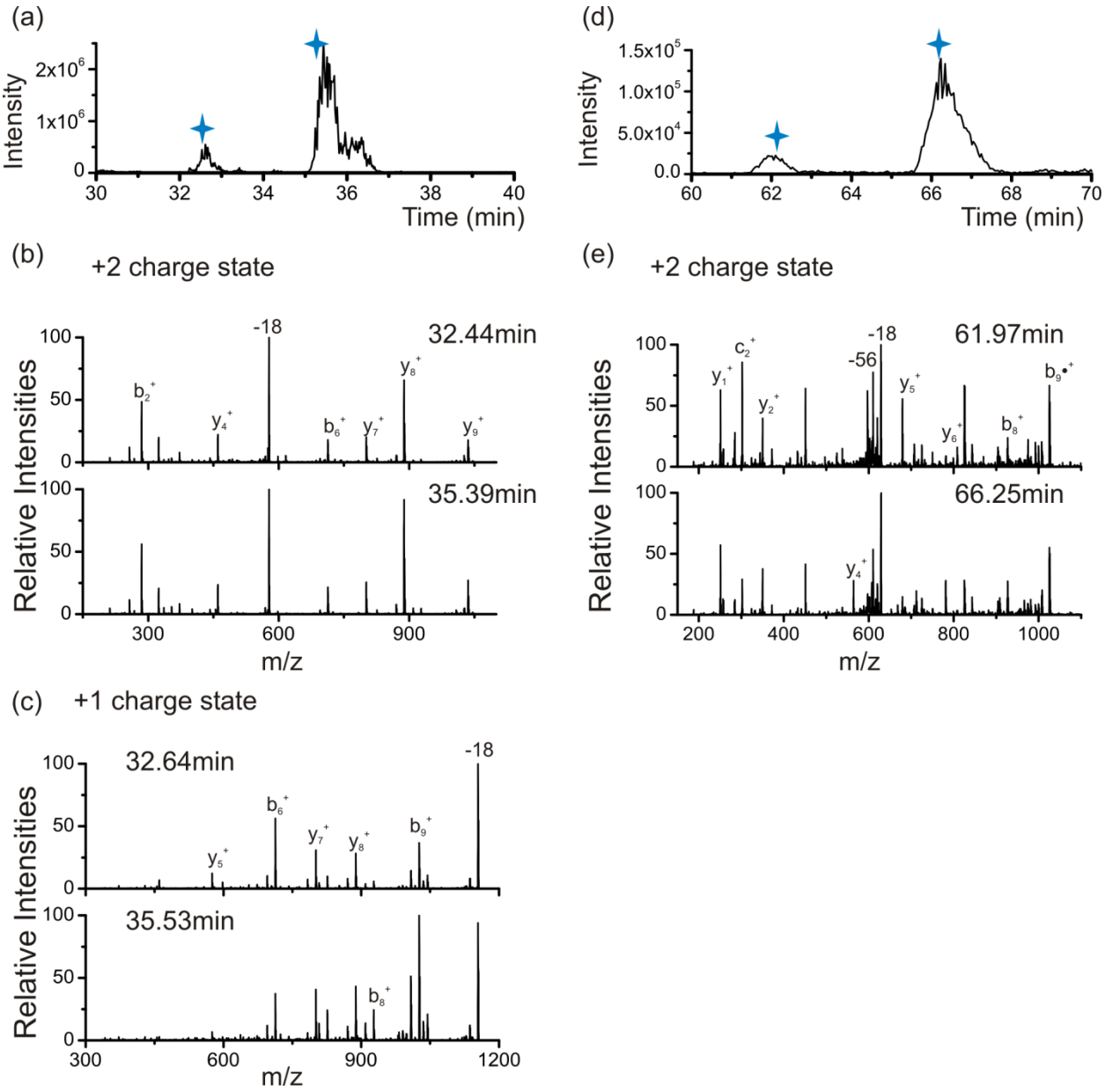

Figure S4. (a) LC chromatogram for peptide Ac-AEQHSAPEQAAAGK in sheep eye crystallin digestion mixture (b) CID spectra of [Ac-AEQHSAPEQAAAGK + 2H]$^{2+}$ at 22.18min and 22.48min, separately. The two spectra are identified as L-serine and D-serine containing peptide by comparing with (c). (c) CID spectra of two synthetic peptides L-serine and D-serine Ac-AEQHSAPEQAAAGK.

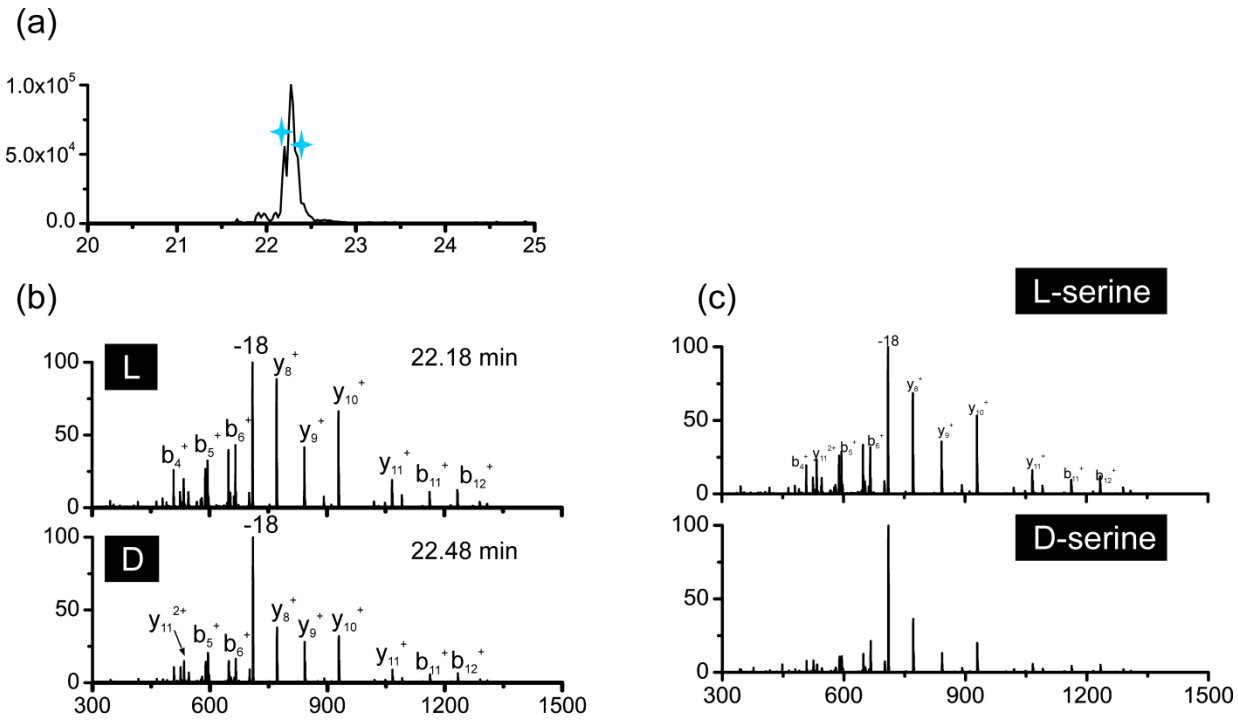

Figure S5. (a) LC chromatogram for peptide LFDQFFGEGLFEYDLLPFLSSTISPYYR in sheep eye crystallin digestion mixture. (b) CID of [LFDQFFGEGLFEYDLLPFLSSTISPYYR + 3H]$^{3+}$ at 84.02min, 84.89min, and 86.06min, separately. The R_{isomer} value is 5.3 for 84.02min and 84.49min (y_4^+ & y_{13}^{2+}), 2.3 for 84.02min and 86.06min (b_{15}^+ & b_{16}^+), 2.1 for 84.89min and 86.06min (y_5^+& b_{12}^+).

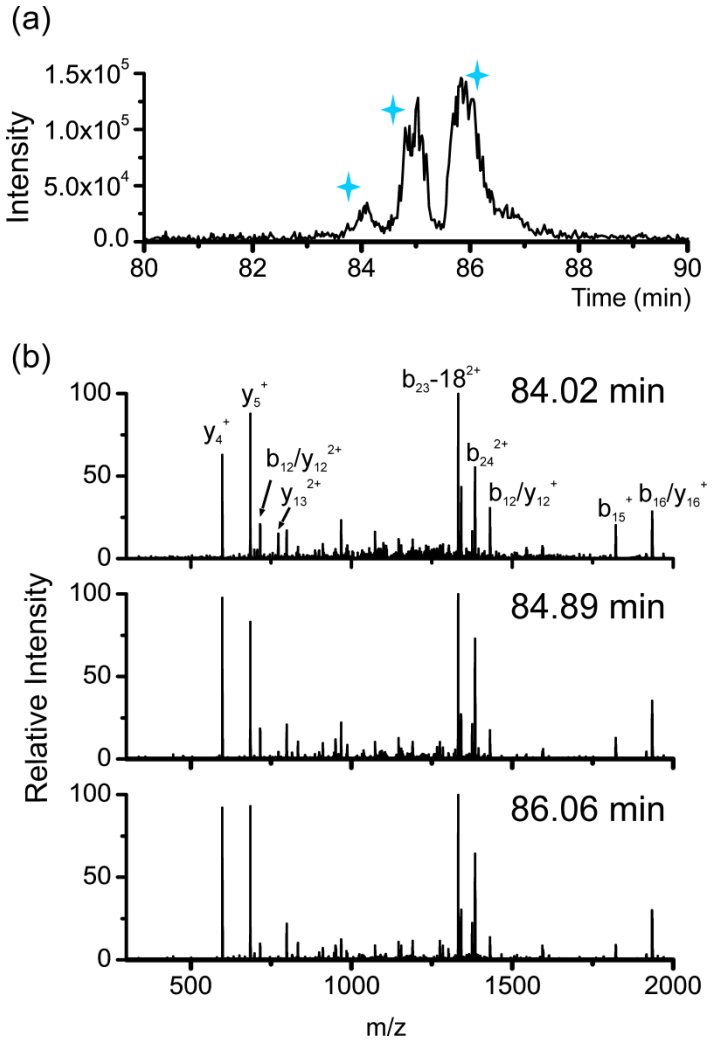

Figure S6. (a) LC chromatogram for peptide QDEHGFISR (N-terminal glutamine cyclized) in sheep eye crystallin digestion mixture. (b) CID of $[QDEHGFISR + 2H]^{2+}$ at 27.98min and 30.68min, separately. The R_{isomer} value is 2.8 (b_7^+& $y6^+$).