

## **SUPPORTING INFORMATION**

### **The L-isoaspartate modification within protein fragments in the aging lens can promote protein aggregation**

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#### **Materials included:**

Tables S1-S9

Figures S1-S2

<u>Sequence</u>	<u>Positions in Master Proteins</u>	<u>Theo. MH+</u> <u>[Da]</u>	<u>Abundances</u> <u>44 year old</u>	<u>Abundances</u> <u>76 year old</u>	<u>Ion Score</u>
LFRTVLDSGISEVR	P02489 [52-65]	1591.88023	2.2	197.8	70
VLDSGISEVR	P02489 [56-65]	1074.57896	12.9	187.1	65
SADGMLTFCGPKIQ	P02489 [134-147]	1524.71851	6	194	62
RTVLDSGISEVR	P02489 [54-65]	1331.72775	68.3	131.7	61
SLFRTVLDSGISEVR	P02489 [51-65]	1678.91226		200	61
SADGMLTFCGPKI	P02489 [134-146]	1396.65993		200	61
SADGMLTFCGPKIQ	P02489 [134-147]	1540.71343	21.3	178.7	61
LDVKHFSPEDLTVK	P02489 [75-88]	1627.869	0.8	199.2	58
RTVLDSGISEVRSD	P02489 [54-67]	1533.78672	191	9	57
TVLDSGISEVRSD	P02489 [55-67]	1377.68561	200		55
VQDDFVEIH	P02489 [89-97]	1101.52111	22.9	177.1	51
LDVKHFSPEDLTVKVQD	P02489 [75-91]	1970.02293	1.6	198.4	51
STISPYRQSLF	P02489 [42-53]	1461.73726	0.9	199.1	49
DDFVEIH	P02489 [91-97]	874.39412	85.5	114.5	47
KVQDDFVEIH	P02489 [88-97]	1229.61608	183.7	16.3	45
DVKHFSPEDLTVKVQD	P02489 [76-91]	1856.93887	28.1	171.9	42
VQDDFVEIHG	P02489 [89-98]	1158.54258		200	42
IFLDVK	P02489 [73-78]	734.4447	181.3	18.7	37
SCSLADGMLTFCGPKIQ	P02489 [130-147]	1971.89728	6.2	193.8	37
VKVQDDFVEIH	P02489 [87-97]	1328.68449	173.1	26.9	37
RLFDQFFGE	P02489 [21-29]	1158.55783	42.2	157.8	37
TISPYRQSLF	P02489 [43-53]	1374.70523	10.3	189.7	36
RLFDQF	P02489 [21-26]	825.42536	75.9	124.1	35
LFRTVLDSGI	P02489 [52-61]	1120.63609		200	34
LDVKHFSPEDLTVKVQ	P02489 [75-90]	1854.99599	0.6	199.4	34
SADGMLTFCGPKIQT	P02489 [134-148]	1625.76619		200	34
LSSTISPYRQ	P02489 [40-50]	1314.66884	200		34
LFRTVLDSGISEVRSD	P02489 [52-67]	1793.9392	24.8	175.2	33
HFSPEDLTVKVQ	P02489 [79-90]	1399.72161		200	33
LFRTVLD	P02489 [52-58]	863.49853	87.7	112.3	33
SLFRTVLDSGIS	P02489 [51-62]	1294.70014	51.8	148.2	33
SSTISPYRQSLF	P02489 [41-53]	1548.76928	10.8	189.2	32
TVKVQDDFVEIH	P02489 [86-97]	1429.73217	200		32
DFVEIH	P02489 [92-97]	759.36718	41.4	158.6	29
LDVKHFSPEDLTVKV	P02489 [75-89]	1726.93741		200	29
LDVKHF	P02489 [75-80]	758.41955	5.6	194.4	28
RLFDQFF	P02489 [21-27]	972.49378	80.7	119.3	27
SLFRTVLDSGISEVRSD	P02489 [51-67]	1880.97123	27.9	172.1	26

**TABLE S1:**  $\alpha$ A crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

Sequence	Positions in Master Theo. MH+ Abundances				
	Proteins	[Da]	44 year old	76 year old	Ion Score
VLGDVIEVH	P02511 [93-101]	980.54112	57.2	142.8	73
GEHLESDFPT	P02511 [29-40]	1357.66342	9.8	190.2	61
RIPADVPLTIT	P02511 [123-134]	1310.73144	2.2	197.8	49
RIPADVPLTITS	P02511 [123-135]	1397.76347	2.7	197.3	46
GDVIEVH	P02511 [95-101]	768.38864		200	43
HFSPEELKVKVLGD	P02511 [83-96]	1597.85843		200	41
EELKVKVL	P02511 [87-94]	957.59791	8.9	191.1	39
RAPSWFDGLSEMR	P02511 [56-69]	1652.78495		200	38
VLGDVIEVHGK	P02511 [93-103]	1165.65755		200	37
REEKPAVTAAPKK	P02511 [163-175]	1424.82199		200	37
RIPADV	P02511 [123-129]	785.41519	1.8	198.2	37
HLLESDFPT	P02511 [31-40]	1171.59937	5.6	194.4	36
RLFDQFFGE	P02511 [22-30]	1158.55783	42.2	157.8	36
SSLSSDGVLTVNGPRKQ	P02511 [135-151]	1744.9188	69.4	130.6	34
RLFDQF	P02511 [22-27]	825.42536	75.9	124.1	34
REEKPAVTAAPK	P02511 [163-174]	1296.72703		200	34
YLRPPSFL	P02511 [48-55]	992.55638	17.1	182.9	34
HFSPEELKVKVL	P02511 [83-94]	1425.81003	0.6	199.4	33
SSLSSDGVLTVNGPRKQ	P02511 [136-151]	1657.88677	73.3	126.7	33
LSSDGVLTVNGPRK	P02511 [137-150]	1442.79617		200	31
FHRKYRIPADVPLTIT	P02511 [118-134]	2042.11817		200	31
HFSPEELKVK	P02511 [83-92]	1213.65755		200	31
PEELKVKVL	P02511 [86-94]	1054.65067	6.2	193.8	30
RIPADVPLTITSS	P02511 [123-136]	1484.7955		200	30
SPEELKVKVL	P02511 [85-94]	1141.6827	9.8	190.2	30
VSGPERTIPITREEKPAVTAAPKK	P02511 [152-175]	2575.45661	74.6	125.4	29
RFSVNLDVKHF	P02511 [74-84]	1361.73245	21.8	178.2	28
LDVKHFSPEELKVK	P02511 [79-92]	1668.93193		200	28
RIPADVPL	P02511 [123-131]	995.55202	20.2	179.8	27
SSLSSDGVLTVNGPRKQV	P02511 [135-152]	1843.98722		200	27
LDVKHF	P02511 [79-84]	758.41955	5.6	194.4	27
KYRIPADVPLTIT	P02511 [121-134]	1601.88973		200	26
RIPADVPLTITSSLS	P02511 [123-138]	1684.91159		200	26
RLFDQFF	P02511 [22-28]	972.49378	80.7	119.3	26
VKHFSPEELKVKVL	P02511 [81-94]	1652.9734		200	26

**TABLE S2:**  $\alpha$ B crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

<u>Sequence</u>	<u>Positions in Master Proteins</u>	<u>Theo. MH+ [Da]</u>	<u>Abundances 44 year old</u>	<u>Abundances 76 year old</u>	<u>Ion Score</u>
GPWKITIYD	P05813 [29-37]	1092.57242	200		47
GWFNNEVGSMKIQ	P05813 [152-164]	1509.71547		200	28
TIFEKENFIGRQ	P05813 [127-138]	1481.7747	16	184	27
SKMTIF	P05813 [124-129]	726.38547	1.4	198.6	27
WEISDDYPSLQAM	P05813 [139-151]	1554.67809	0.4	199.6	26

**TABLE S3:**  $\beta$ A3 crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

<u>Sequence</u>	<u>Proteins</u>	<u>Theo. MH+</u> <u>[Da]</u>	<u>Abundances</u>		<u>Ion Score</u>
			<u>44 year old</u>	<u>76 year old</u>	
TIFEQENFLGKK	P53673 [108-119]	1453.76856		200	55
IFEQENFLGKK	P53673 [109-119]	1352.72088	1.4	198.6	45
GELSDDYPSLQAM	P53673 [120-132]	1425.62024	1.8	198.2	45
SRLTIFEQENFLGKK	P53673 [105-119]	1809.98576		200	42
DSRLTIFEQENFLGKK	P53673 [104-119]	1925.0127		200	42
SGAWVCSQFPGYRGF	P53673 [146-160]	1718.77439	30.8	169.2	36
ERLTSFRPA	P53673 [89-97]	1076.58472		200	32
FQVQSIR	P53673 [186-192]	877.48903	24.1	175.9	32
GELSDDYPSLQ	P53673 [120-130]	1223.54264	6.3	193.7	31
SRLTIF	P53673 [105-110]	736.4352	8.8	191.2	30
SGAWVCSQFPGYRG	P53673 [146-159]	1571.70597	115.8	84.2	29
GWEGNEVGSFHVH	P53673 [133-145]	1454.64475	200		29
FLGKKGELSDDYPSLQAM	P53673 [115-132]	1998.9841		200	27
KGELSDDYPSLQAM	P53673 [119-132]	1553.7152		200	27

**TABLE S4:**  $\beta$ A4 crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

Sequence	Positions in Master Proteins	Theo. MH+ [Da]	Abundances 44 year old	Abundances 76 year old	Ion Score
HLEGSFPVLA	P53674 [238-247]	1069.56767		200	62
HLEGSFPVLATEPPK	P53674 [238-252]	1621.85843	21	179	59
EGSFPVLATEPPK	P53674 [240-252]	1371.71546	2.3	197.7	55
SFPVLATEPPK	P53674 [242-252]	1185.6514	54.3	145.7	54
TLAPTTVPITSAK	P53674 [38-50]	1299.75184	22.2	177.8	50
RDKQWHLEGSFPVLATEPPK	P53674 [233-252]	2335.21934		200	48
RLRDKQWHLEGSFPVLATEPPK	P53674 [231-252]	2604.40452		200	45
VVFELENFQGR	P53674 [62-72]	1337.68483		200	44
GSFPVLATEPPK	P53674 [241-252]	1242.67286	39.7	160.3	39
WHLEGSFPVLATEPPK	P53674 [237-252]	1807.93775		200	39
MFILEKG	P53674 [113-119]	837.45389	18.7	181.3	38
NFRGEMFILEKG	P53674 [108-119]	1440.7304		200	30
TTLAPTTVPITSA	P53674 [37-49]	1272.70456	12.7	187.3	28
LEGSFPVLATEPPK	P53674 [239-252]	1484.79952	38.9	161.1	28
WHLEGSFPVLA	P53674 [237-247]	1255.64698		200	27
RLMSFRPIKM	P53674 [135-144]	1278.71733		200	26

**TABLE S5:**  $\beta$ B1 crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

<u>Sequence</u>	<u>Positions in Master Proteins</u>	<u>Theo. MH+ [Da]</u>	<u>Abundances</u>		<u>Ion Score</u>
			<u>44 year old</u>	<u>76 year old</u>	
KMEIIDDVPSFHAH	P43320 [121-135]	1753.8214		200	51
KMEIIDDVPSFHA	P43320 [121-134]	1616.76248		200	48
KMEIIDDVPSFHAHG	P43320 [121-136]	1810.84286		200	41
MEIIDDVPSFHAH	P43320 [122-135]	1625.72643		200	35
SVRVQSGTWVGYQYPGYRGL	P43320 [143-162]	2273.14618		200	34
KMEIIDDVPSFH	P43320 [121-133]	1545.72537		200	33
SGTWVGYQYPGYRGL	P43320 [148-162]	1703.81763		200	33
KMEIIDDVPSFHAHGYQEKVSSRVQ	P43320 [121-147]	3114.53131	3.1	196.9	31
PKIIF	P43320 [17-22]	730.48617	8.3	191.7	29
SHELNGPCPNLKETGVEKAGSVLVQ	P43320 [31-55]	2663.34574		200	26
EIIDDVPSFHAH	P43320 [123-135]	1494.68595	5.4	194.6	26

**TABLE S6:**  $\beta$ B2 crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

<u>Sequence</u>	<u>Positions in Master Proteins</u>	<u>Theo. MH+ [Da]</u>	<u>Abundances</u>		<u>Ion Score</u>
			<u>44 year old</u>	<u>76 year old</u>	
GELAGPEDALARQVE	Q12934 [79-93]	1554.77583	66.3	133.7	77
FIETPIPLFTQSH	Q12934 [324-336]	1529.79986		200	66
RVELQAQTTTLEQAIK	Q12934 [239-254]	1829.0127	5.8	194.2	61
FIETPIPLFTQ	Q12934 [324-334]	1305.70892	1.1	198.9	53
AQRVELQAQTTTLEQAIK	Q12934 [237-254]	2028.10839			51
QQIIHTTTPPASIVTS	Q12934 [191-205]	1592.86425	2.6	197.4	50
SLAALQGLGERVA	Q12934 [40-52]	1284.72703	12.3	187.7	48
GELAGPEDALARQV	Q12934 [79-92]	1425.73323		200	48
QQIIHTTTPPASIVT	Q12934 [191-204]	1505.83222	6.1	193.9	47
TTTLEQAIKSA	Q12934 [246-256]	1162.63139	6.5	193.5	45
LDAFQRLGEL	Q12934 [72-81]	1161.62625		200	45
GELAGPEDALAR	Q12934 [79-90]	1198.60624		200	42
ISILQQIIH	Q12934 [187-195]	1064.64626	12	188	42
FIETPIPLFT	Q12934 [324-333]	1177.65034	4.5	195.5	42
SLAALQGLGERVAAH	Q12934 [40-54]	1492.82305		200	40
KTVEVESIEKIS	Q12934 [620-632]	1460.82065	2.4	197.6	39
QQIIHTTTPPASIVTSGM	Q12934 [191-207]	1780.9262		200	38
IETPIPLFTQ	Q12934 [325-334]	1158.6405	0.4	199.6	37
RLQLEAQ	Q12934 [157-163]	857.48394	2.2	197.8	36
FIETPIPLFTQSHG	Q12934 [324-337]	1586.82132		200	35
NKEADEALLHNL	Q12934 [145-156]	1366.69612	6.4	193.6	34
LTEREVAAL	Q12934 [213-221]	1001.56259		200	34
RQLDAFQRL	Q12934 [70-78]	1146.63782	12.8	187.2	33
GMREEKLLTEREVAAL	Q12934 [206-221]	1844.98986		200	33
IETPIPLFTQSH	Q12934 [325-336]	1382.73144		200	33
LDAFQRL	Q12934 [72-78]	862.47813	13.3	186.7	33
AFQRLGELAGPE	Q12934 [74-85]	1287.66918	177.5	22.5	32
IETPIPLF	Q12934 [325-332]	929.53425		200	32
MREEKLLTEREVAAL	Q12934 [207-221]	1787.96839		200	31
VIVETMIGKTK	Q12934 [644-654]	1218.71262	200		31
FIETPIPL	Q12934 [324-331]	929.53425		200	31
FIETPIPLF	Q12934 [324-332]	1076.60266		200	30
QLDAFQRL	Q12934 [71-78]	990.53671		200	30
RIIEIEGNRLT	Q12934 [311-321]	1313.75357	9.2	190.8	28
AALQGLGERVAAHVQ	Q12934 [42-56]	1519.83395	39.6	160.4	27
REEKLLTEREVAAL	Q12934 [208-221]	1656.92791	6.1	193.9	27
LEEGREVLSHL	Q12934 [225-235]	1281.67974		200	27
RVELQAQTTTLEQAIS	Q12934 [239-255]	1916.04473		200	27
SLAALQGLGERVAAHVQ	Q12934 [40-56]	1719.95004		200	26

**TABLE S7:** Filensin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

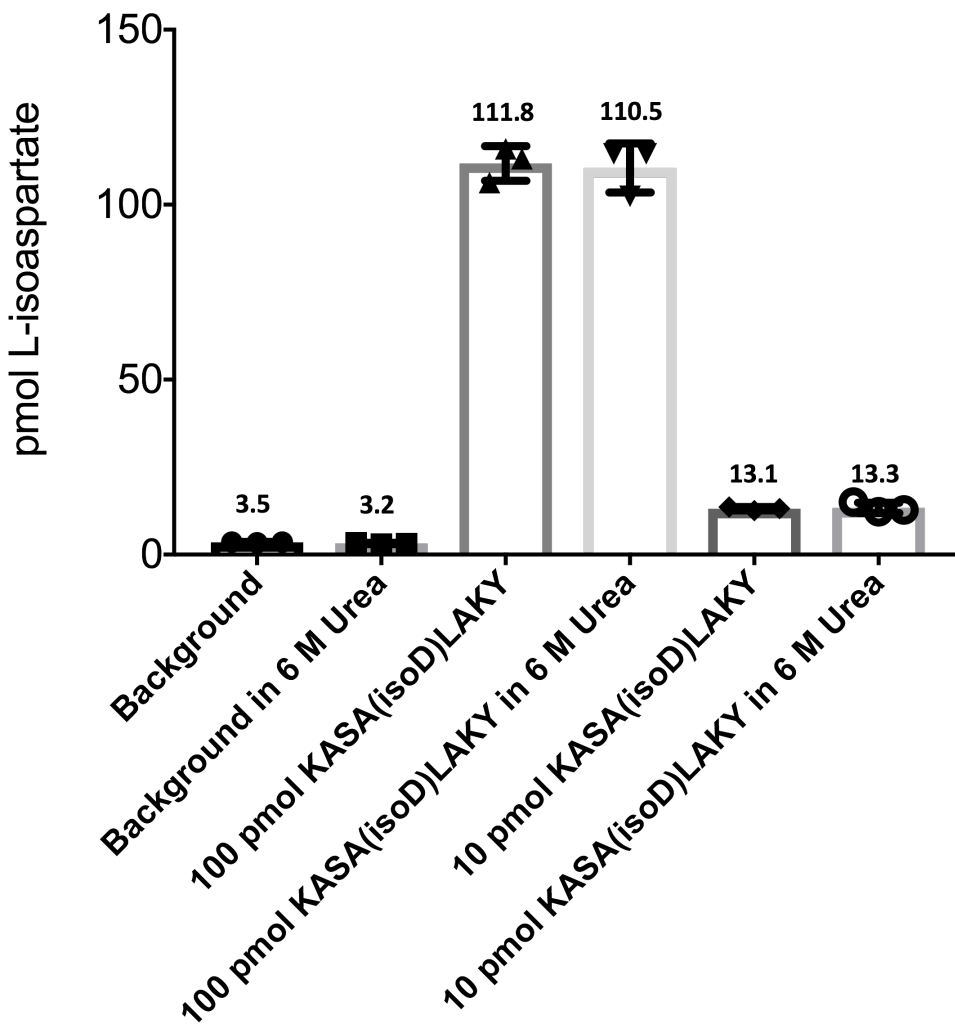


<u>Sequence</u>	<u>Positions in Master Proteins</u>	<u>Theo. MH+ [Da]</u>	<u>Abundances</u>		<u>Ion Score</u>
			<u>44 year old</u>	<u>76 year old</u>	
YILPQGEYPEYQRWM	P22914 [60-74]	1972.9262		200	45
IFEKGDMSGQ	P22914 [98-107]	1127.53677		200	43
GEYPEYQRWM	P22914 [65-74]	1358.5834	13.2	186.8	41

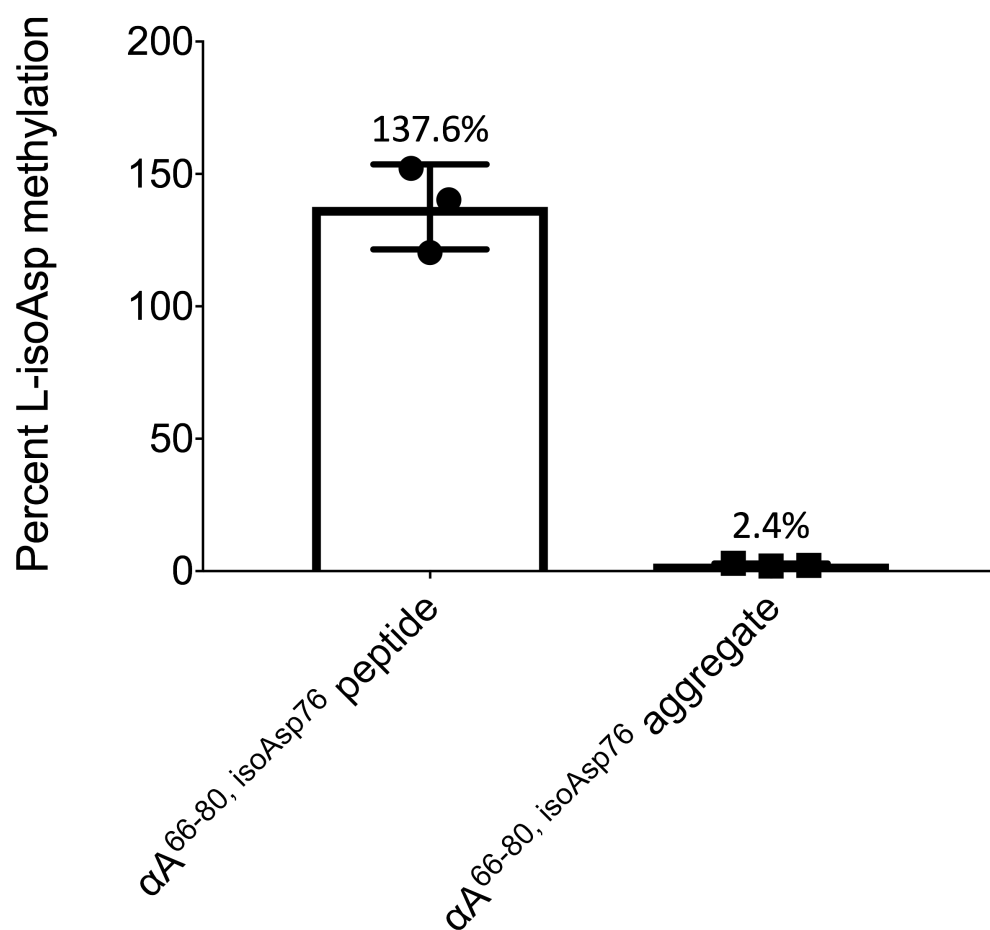
**TABLE S8:**  $\gamma$ S crystallin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

Sequence	Positions in Master Proteins	Theo. MH+ [Da]	Abundances		Ion Score
			44 year old	76 year old	
QQVGEAVLENARL	Q13515 [162-174]	1426.76487		200	60
WASSCQQVGEAVLENARL	Q13515 [157-174]	2017.976		200	57
KVIDEANLTKM	Q13515 [211-221]	1261.68205	6.3	193.7	56
TGLDDILETIRIQ	Q13515 [266-278]	1486.81115		200	56
QQVGEAVLENARLM	Q13515 [162-175]	1557.80535	4	196	55
RASWASSCQQVGEAVLENARL	Q13515 [154-174]	2332.14625		200	53
SSCQQVGEAVLENARLM	Q13515 [159-175]	1891.90006	2.1	197.9	51
RASWASSCQQVGEAVLENARLM	Q13515 [154-175]	2463.18674		200	50
KVIDEANLTKMDLESQIE	Q13515 [211-228]	2076.05291		200	48
SWASSCQQVGEAVLENARL	Q13515 [156-174]	2105.00803		200	47
WASSCQQVGEAVLENARLM	Q13515 [157-175]	2149.01648	0.2	199.8	45
QVGEAVLENARLM	Q13515 [163-175]	1429.74677		200	44
SCQQVGEAVLENARLM	Q13515 [160-175]	1804.86803	1.2	198.8	44
QVGEAVLENARL	Q13515 [163-174]	1298.70629	23.7	176.3	41
SVFLQGLRSSGL	Q13515 [82-93]	1263.70556	7	193	40
SLYKVIDEANLTKM	Q13515 [208-221]	1624.86147	12.4	187.6	39
YHALLD	Q13515 [405-410]	731.37227		200	30
GLRSSGLATVPAPGLER	Q13515 [87-103]	1680.93914	20.9	179.1	30
SWASSCQQVGEAVLENARLM	Q13515 [156-175]	2236.04851		200	30
HALLDREE	Q13515 [406-413]	982.49523	2.9	197.1	29
SCQQVGEAVLENARL	Q13515 [160-174]	1673.82754		200	29
YHALLDREE	Q13515 [405-413]	1145.55856	4.4	195.6	29
SVFLQGLRSSGLATVPAPGLERD	Q13515 [82-104]	2370.27758		200	29
HMSQTQEEKLAAAL	Q13515 [304-317]	1556.77372		200	28
SVFLQGLR	Q13515 [82-89]	919.53598	9	191	28
AAAAEINSLY	Q13515 [202-210]	1067.48915		200	28
ALLDREESG	Q13515 [407-415]	989.48981		200	27
DILETIRIQ	Q13515 [270-278]	1100.631	15.2	184.8	27
GAVVGRL	Q13515 [364-370]	671.41989		200	26
SVFLQGLRSSGLA	Q13515 [82-94]	1334.74268	2.4	197.6	26
AVLENARLM	Q13515 [167-175]	1016.55573	11	189	26
SVFLQGLRSS	Q13515 [82-91]	1093.60003	31.3	168.7	26
SVFLQGLRSSGLATVPAPGLER	Q13515 [82-103]	2255.25064		200	26

**TABLE S9:** Phakinin peptides. Lens peptides were isolated from the gel filtration HMW fraction and identified as described in “Experimental Procedures.” All peptides with an ion score of 26 or higher are displayed with their corresponding theoretical mass and relative abundances in a 44-year-old and 76-year-old whole lens sample on a scale of 0-200.

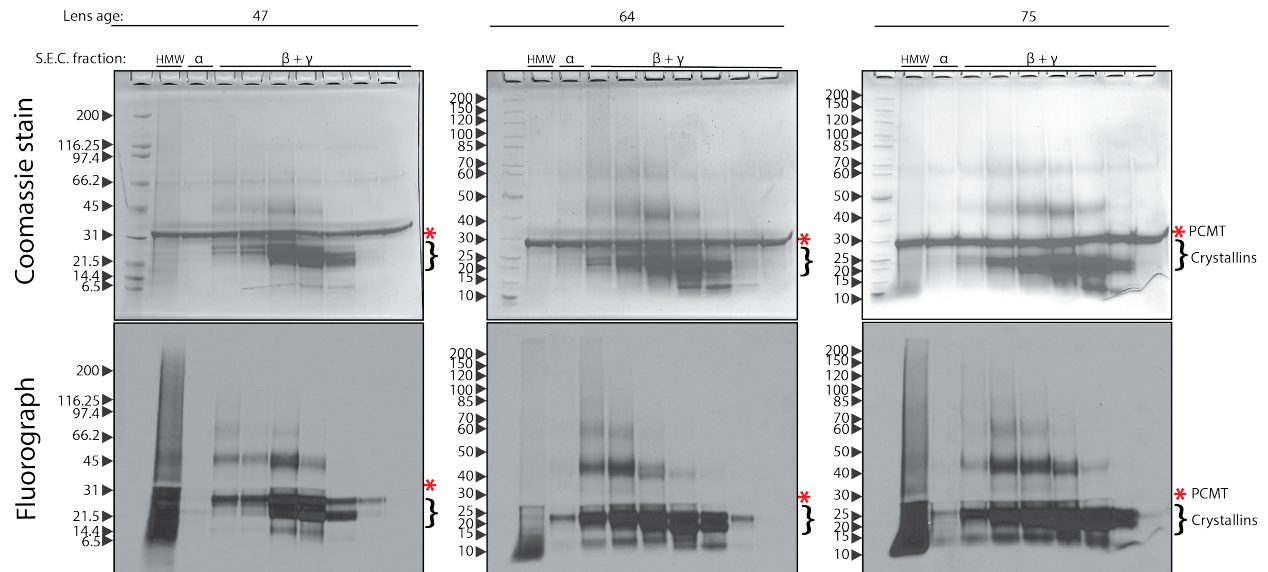


**Fig. S1: Exogenous PCMT1 labeling of L-isoAsp is not affected by urea.** KASA(isoD)LAKY peptide was resuspended in 50 mM Tris-HCl, pH 7.9, 150 mM NaCl, and 6 M urea to match buffer conditions of re-solubilized water-insoluble lens extracts (“in 6 M urea” indicated on the x-axis), or in 50 mM Tris-HCl, pH 7.9, 150 mM NaCl buffer lacking urea to match buffer conditions of water-soluble lens extracts. These buffers alone were used in the “Background” reactions. Buffer alone, 100 pmol of KASA(isoD)LAKY, and 10 pmol of KASA(isoD)LAKY were analyzed for L-isoAsp content by PCMT1 methylation as described in the Experimental Procedures section. Reactions were performed in triplicate, and the average value is displayed above each column. Error bars represent the standard deviation.

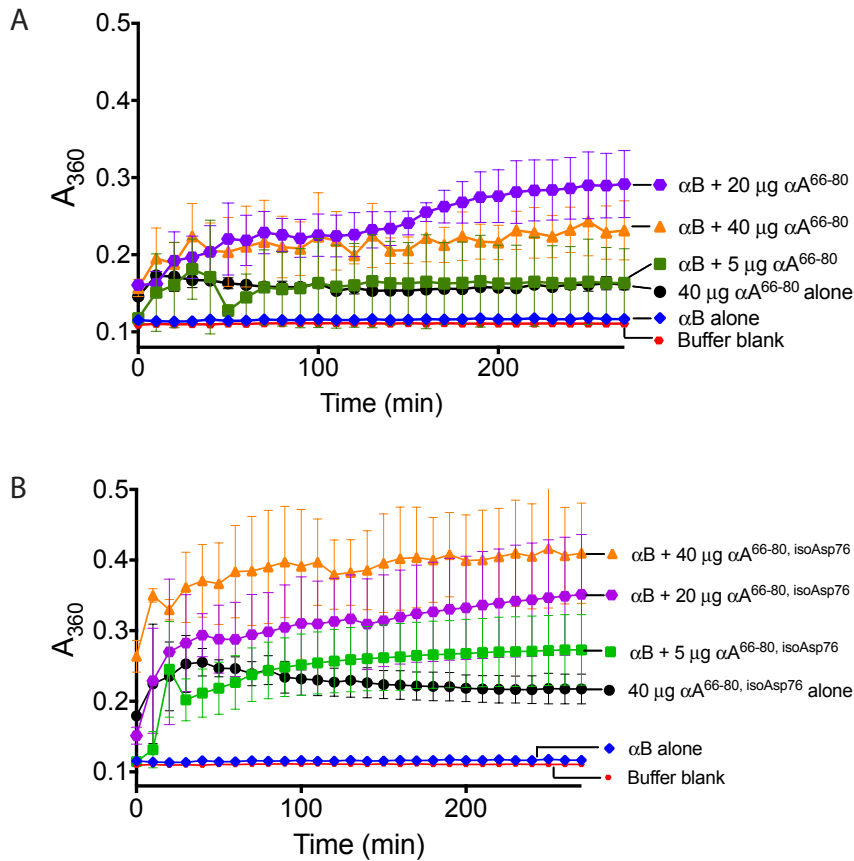


**Fig. S2: Exogenous PCMT1 cannot methylate aggregated  $\alpha A^{66-80, isoAsp76}$  peptide.** Aggregates of  $\alpha A^{66-80, isoAsp76}$  were formed at 1 mg/mL in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl (TBS). Soluble peptide solutions of  $\alpha A^{66-80, isoAsp76}$  were prepared by dissolving  $\alpha A^{66-80, isoAsp76}$  in TBS with 10% DMSO and filtering immediately prior to PCMT1 methylation assays, carried out as described in the Experimental Procedures section. Values of “Percent L-isoAsp methylation” were calculated by dividing the pmol of L-isoAsp methylated by the pmol of L-isoAsp present in the reaction (70 pmol soluble peptide analyzed, 1000 pmol aggregate analyzed). Error bars represent the standard deviation.

Size exclusion chromatography-separated lens nuclear extracts



**Fig. S3: Analysis of L-isoAsp damage in the native size-exclusion fractions of human nuclear lens extracts by SDS-PAGE reveals the HMW gel filtration fraction contains significant amounts of LMW L-isoaspartyl-containing species.** Fractions from size exclusion chromatography as in Fig. 4 were trichloroacetic acid-precipitated, resuspended in 100 mM Tris-HCl, pH 7.9, 150 mM NaCl, 0.1% SDS, 6 M Urea and L-isoAsp sites within the fractions were radiolabeled by PCMT1 and [<sup>3</sup>H] AdoMet as described in the Fig. 3 legend. Labeled fractions were then separated by SDS-PAGE on a 4-12% gradient gel. The Coomassie-stained gel is shown in the upper panels; the fluorograph (one week exposure) is shown in the lower panels. In the experiment with the 47-year-old extract, molecular weight markers shown in Fig. 3 were used; in the 64- and 75-year-old-samples the molecular weight standards include synthetic polypeptides of the designated molecular weights (Thermo-Scientific, PageRuler Unstained Protein Ladder, catalog # 26614). The bands corresponding to PCMT1 in the Coomassie-stained gel are indicated by asterisks. The migration positions of intact crystallins are indicated by brackets on the right hand side of the gels. Labels above lanes represent the corresponding peaks from the size exclusion run (see Fig. 4). The age of lens sample in years is designated above the corresponding gel.



**Fig. S4: The  $\alpha\text{A}^{66-80, \text{isoAsp76}}$  peptide aggregates purified  $\alpha\text{B}$  crystallin more than the native  $\alpha\text{A}^{66-80}$  peptide.**  $\alpha\text{B}$  crystallin ( $40\ \mu\text{g}$ ) was incubated with designated amounts of the  $\alpha\text{A}^{66-80}$  (top panel) or  $\alpha\text{A}^{66-80, \text{isoAsp76}}$  (bottom panel) peptide in  $50\ \text{mM}$  sodium phosphate,  $\text{pH}\ 7.0$ ,  $100\ \text{mM}$   $\text{NaCl}$ , and  $10\ \text{mM}$  phenanthroline as described in the “Experimental Procedures” section. Aggregation was monitored by light scattering at  $A_{360}$ . Symbols represent the mean and error bars represent the standard deviation of three technical replicates.