

Appendix 1. Crystallin species, sequences, and modifications from Coomassie-stained 2D-DIGE gel of a 69-year-old human lens identified by ESI-QTRAP LC-MS/MS.

Spot No.	α -Crystallin	β - and γ -Crystallin	CP49	Filensin
1		γ D: Residue #2-10 GKITLYEDR γ D: #4-10 ITLYEDR γ D: #154-163 YQDWGATNAR	Present only in Spots 22 and 28	Present only in Spot 35
2	α A: Residue #55-65 TVLDGISEVR α A: #79-88 HFSPEDLTVK α B: #1-11 <u>M</u> DIAIHHP <u>W</u> IR [Acet(N-term);Oxi(MHW)] α B: #12-22 RPFPPFHSPSR α B: #57-69 APSW <u>F</u> DTGLSE <u>M</u> R [Oxi(MHW)] α B: #57-72 APSWFDTGLSEMRLEK α B: #73-82 DRFSVNLDVK α B: #83-90 HFSPEELK α B: #83-92 <u>H</u> FSPEELK <u>V</u> K [Eth(N-term)] α B: #93-103 VLGDVIEVHGK α B: #108-116 <u>Q</u> DEHGFIS <u>R</u> [Eth(N-term)] α B: #124-149 IPADV <u>D</u> PLTITSSLS <u>D</u> GVLT <u>V</u> <u>N</u> GPR [Deam(NQ)]			
3	α A: #55-65 TVLDGISEVR α B: #12-22 RPFPPFHSPSR α B: #57-69 APSWFDGLSEMR α B: #57-72 APSWFDTGLSEMRLEK	β A3: #33-44 ITIYDQENFQGK β A3: #33-45 ITIYDQENFQGKR β A3: #96-109 WDAWSGSNAYHIER β A3: #126-137 <u>M</u> TIFEKEN <u>F</u> IGR [Oxi(M)]		

	α B: #73-82 DRFSVNLDVK	β A3: #197-211 EWGSHAQTSQIQSIR
	α B: #83-90 HFSPEELK	β B3: #26-37 VILYELENFQGK
	α B: #83-92 HFSPEELKVK	β B3: #26-38 VILYELENFQGKR
	α B: #93-103 VLGDVIEVHGK	
4	α B: #1-11 <u>M</u> DIAIHHPWIR [Acet(N-term);Oxi(M)]	β A3: #33-45 ITIYDQENFQGKR
	α B: #12-22 RRFFPFHSPSR	β A3: #96-109 WDAWSGSNAYHIER
	α B: #57-69 APSW <u>F</u> DTGLSE <u>M</u> R [Oxi(MHW)]	β A3: #126-137 <u>M</u> TIFEKENFIGR [Oxi(M)]
	α B: #57-72 APSWFDTGLSEMRLEK	β A3: #197-211 EWGSHAQTSQIQSIR
	α B: #73-82 DRFSVNLDVK	β B1: #151-160 ISLFEGANFK
	α B: #83-90 HFSPEELK	γ D: #2-10 GKITLYEDR
	α B: #83-92 HFSPEELK <u>V</u> [Cam(K)]	γ D: #154-163 YQDWGATNAR
	α B: #91-103 VKVLGDVIEVHGK	
	α B: #93-107 VLGDVIEVHGKHEER	
	α B: #108-116 <u>Q</u> DEHGFISR [Eth(N-term)]	
	α B: #124-149 IPADVDPLTITSSLSSDGVLT <u>V</u> GPR [Deam(NQ)]	
	α B: #164-174 EEKPAVTAAPK	
5	α B: #1-11 MDIAIHHPWIR	β A3: #33-45 ITIYDQENFQGKR
	α B: #12-22 RPFFPFHSPSR	β A3: #126-137 <u>M</u> TIFEKENFIGR [Oxi(M)]
	α B: #57-69 APSW <u>F</u> DTGLSE <u>M</u> R [Oxi(M)]	γ D: #2-10 GKITLYEDR
	α B: #57-72 APSWFDTGLSEMRLEK	γ D: #60-77 RGDYADHQQWMGLSDSVR
	α B: #73-82 DRFSVNLDVK	γ D: #61-77 GDYADHQQWMGLSDSVR
	α B: #83-92 HFSPEELKVK	γ D: #143-152 QYLLMPGDYR

6	<p> αB: #93-103: VLGDVIEVHGK αB: #108-116: QDEHGFISR αA: #55-65 TVLDSGISEVR αB: #12-22 RPFPPFHSPSR </p> <p> αB: #57-69 APSWFDTGLSEMR [Oxi(M)] αB: #57-72 APSWFDTGLSEMRLEK αB: #83-90 HFSPEELK αB: #83-92 HFSPEELKVK [Meth(H,N-term);Eth(N-term)] αB: #91-103 VKVLGDVIEVHGK αB: #93-103 VLGDVIEVHGK [Eth(N-term)] αB: #93-107 VLGDVIEVHGKHEER αB: #158-174 TIPITREEKPAVTAAPK </p>	<p> γD: #143-153 QYLLMPGDYRR γD: #154-163 YQDWGATNAR βA3: #33-44 ITIYDQENFQ GK βA3: #96-109 WDAWSGSNAYHIER βA3: #126-137 MTIFEKENFIGR [Oxi(M);Sulph(M)] βA3: #197-211 EWGSHAQTSQIQSIR γD: #78-89 SCRLIPHSGSHR γD: #143-152 QYLLMPGDYR </p> <p> γD: #143-153 QYLLMPGDYRR γD: #154-163 YQDWGATNAR γS: #8-14 ITFYEDK [Eth(K)] γS: #73-79 WMGLNDR </p>
7	<p> αA: #13-21 TLGPFYPSR αA: #146-157 IQTGLDATHAER </p> <p> αB: #1-11 MDIAIHPWIR [Acet(N-term);Cam(N-term);Oxi(M)] αB: #12-22 RPFPPFHSPSR </p> <p> αB: #57-69 APSWFDTGLSEMR </p> <p> αB: #57-72 APSWFDTGLSEMRLEK αB: #83-90 HFSPEELK αB: #83-92 HFSPEELKVK αB: #150-157 KQVSGPER </p>	<p> βA3: #33-44 ITIYDQENFQ GK βA3: #33-45 ITIYDQENFQ GK [Cam(K)] βA3: #91-95 GEYPR </p> <p> βA3: #96-109 WDAWSGSNAYHIER [Eth(N-term);Oxi(HW)] βA3: #126-137 MTIFEKENFIGR [Cam(K);Deam(NQ);Oxi(M)] βA3: #197-211 EWGSHAQTSQIQSIR βB2: #109-120 IILYENPNFTGK γB: #4-10 ITFYEDR γB: #60-77 RGEYDPDYQQWMGLSDSIR </p>

	α B: #164-174 <u>E</u> EKPAVTAAPK [Cam(N-term)]	γ B: #155-164 FLDWGAPNAK
		γ S: #8-14 ITFYEDK
		γ S: #73-79 WMGLNDR
		γ S: #85-95 AVHLPSSGGQYK
8	α B: #73-82 DRFSVNLDVK	β A3: #33-44 ITIYDQENFQ GK
	α B: #83-92 HFSPEELKVK	β A3: #33-45 ITIYDQENFQ GK R
	α B: #93-107	β A3: #197-211
	VLGDVIEVHGKHEER	EWGSHAQTSQIQSIR
8B	α B: #73-82 DRFSVNLDVK	β A3: #33-44 ITIYDQENFQ GK
	α B: #93-107	β A3: #96-109
	VLGDVIEVHGKHEER	WDAWSGSNAYHIER
		β A3: #126-137 <u>M</u> TIFEKENFIGR [Oxi(M)]
9	α B: #150-157 KQVSGPER	β A3: #33-44 ITIYDQENFQ GK
	α B: #158-163 TIPITR	β A3: #33-45 ITIYDQENFQ GK R
	α B: #164-174 EEKPAVTAAPK	β A3: #96-109
		WDAWSGSNAYHIER
		β A3: #126-131 MTIFEK
		β A3: #126-137 MTIFEKENFIGR
		β A4: #14-25 <u>M</u> VVWDEDGFQGR [Oxi(M)]
		β A4: #107-118 LTIFEQENFLGK
		β A4: #107-119 LTIFEQENFLGKK
10	α A: #1-11 <u>M</u> DVTIQHPWFK [Acet(N-term);Oxi(M)]	β A3: #33-44 ITIYDQENFQ GK
	α A: #13-21 TLGPFYPSR	β A3: #96-109
		WDAWSGSNAYHIER
		[Deam(NQ)]
	α A: #55-65 TVLDSGISEVR	β A3: #126-137 <u>M</u> TIFEKENFIGR [Oxi(M)]
	α A: #79-88 HFSPEDLTVK	β A4: #14-25 MVVWDEDGFQGR
	α A: #89-99 VQDDFVEIHGK	β A4: #107-118 LTIFEQENFLGK
	α A: #146-157 <u>I</u> QTGLDATHAER [Meth(N-term,Q,R); Eth(N-term)]	
	α B: #1-11 <u>M</u> DIAIHHPWIR	

	[Acet(N-term);Oxi(M)]	
	α B: #83-92 HFSPEELKVK	
11	α A: #1-11 <u>M</u> DVTIQHPWFK	β A3: #33-44 ITIYDQENFQGK
	[Acet(N-term);Oxi(M)]	
	α A: #13-21 TLGPFYPSR	β A3: #33-45 ITIYDQENFQGKR
	α A: #55-65 TVLD SGISEVR	β A3: #126-137 MTIFEKENFIGR
	α A: #79-88 <u>H</u> FSPEDLTVK	β A4: #107-118 LTIFEQENFLGK
	[Meth(H,N-term);Eth(N-term)]	
	α A: #89-99 VQDDFVEIHGK	
	α A: #146-157 IQTGLDATH <u>A</u> ER	
	[Meth(H)]	
11B	α A: #1-11 <u>M</u> DVTIQHP <u>W</u> FK	β A4: #14-25 <u>M</u> VVWDEDGFQGR
	[Acet(N-term);Oxi(MHW)]	[Oxi(M)]
	α A: #12-21 RTLGPFYPSR	β A4: #107-118 LTIFEQENFLGK
	α A: #13-21 <u>I</u> LGPFYPSR [Eth(N-term)]	
	α A: #50-65	
	QSLFRTVLD SGISEVR	
	α A: #55-65 TVLD SGISEVR	
	α A: #79-88 HFSPEDLTVK	
	α A: #89-99 <u>V</u> QDDFVEIHGK	
	[Eth(N-term);Meth(N-term,Q)]	
	α A: #146-157 IQTGLDATH <u>A</u> ER	
	[Meth(H)]	
	α A: #158-173	
	AIPVSREEKPTSAPSS	
	α B: #1-11 MDIAIHHPWIR	
	α B: #93-103 VLGDVIEVHGK	
12	α A: #13-21 TLGPFYPSR	γ S: #8-19 ITFYEDKNFQGR
	α A: #55-65 TVLD SGISEVR	
	α A: #146-157 IQTGLDATHAER	
12B	α B: #83-90 HFSPEELK	β A3: #33-44 ITIYDQENFQGK
		β B1: #203-214 GYQYLLEPGDFR
		β B2: #91-101 TDSLSSLRPIK
		β B2: #109-120 IILYENPNFTGK
		β B2: #173-188

13 αA: #55-65 TVLDSGISEVR
 DSSDFGAPHPQVQSVR
 γC: #4-10 ITFYEDR
 γS: #8-14 ITFYEDK [Eth(K)]
 γS: #8-19 ITFYEDKNFQGR
 γS: #73-79 WMGLNDR
 γS: #85-95 AVHLPSGGQYK
 γS: #159-174
 KPIDWGAASPAVQSFR
 [Deam(NQ);Fkyn(W);Oxi(HW)]
 βA4: #14-25 MVVWDEDGFQGR
 [Oxi(M)]
 βA4: #107-118 LTIFEQENFLGK
 βB1: #61-72 LVVFELENFQGR
 βB2: #82-89 WDSWTSSR
 βB2: #109-120 IILYENPNFTGK
 βB2: #161-168 GLQYLLEK
 βB2: #173-188
 DSSDFGAPHPQVQSVR
 γS: #8-19 ITFYEDKNFQGR
 γS: #85-95 AVHLPSGGQYK
 [Meth(H)]
 γS: #149-155 QYLLDKK
 γS: #156-174
 EYRKPIDWGAASPAVQSFR
 [Oxi(HW)]
 γS: #159-174
 KPIDWGAASPAVQSFR
 [Meth(K,N-
 term);Oxi(HW);Fkyn(W);Phos(ST
 Y)]
 14 βA3: #33-44 ITIYDQENFQ GK
 βA3: #33-45 ITIYDQENFQ GK
 βB1: #61-72 LVVFELENFQGR
 γS: #8-19 ITFYEDKNFQGR
 γS: #73-79 WMGLNDR
 γS: #85-95 AVHLPSGGQYK

14B γ S: #159-174
 KPIDWGAASPAVQSFR
 [Oxi(HW)]
 β B2: #69-76 KGEQFVFEK
 β B2: #91-101 TDSLSSLRPIK
 β B2: #109-120 IILYENPNFTGK
 β B2: #109-121 IILYENPNFTGKK
 β B2: #121-140
 KMEIIDDVPSFHAHGYQEK
 β B2: #122-140
 MEIIDDVPSFHAHGYQEK
 [Oxi(M)]
 β B2: #146-160
 VQSGTWVGYQYPGYR
 β B2: #169-188
 GDYKDSSDFGAPHPQVQSVR
 15 β A3: #33-44 ITIYDQENFQGK
 β A4: #107-118 LTIFEQENFLGK
 [Deam(NQ)]
 β B1: #61-72 LVVFELENFQGR
 β B2: #69-76 GEQFVFEK
 β B2: #109-120 IILYENPNFTGK
 β B2: #161-168 GLQYLLEK
 β B2: #169-188
 GDYKDSSDFGAPHPQVQSVR
 γ S: #8-19 ITFYEDKNFQGR
 γ S: #85-95 AVHLPSSGGQYK
 γ S: #159-174
 KPIDWGAASPAVQSFR
 [Deam(NQ);Oxi(HW)]
 15B β A3: #33-44: ITIYDQENFQGK
 β B2: #109-120 IILYENPNFTGK
 β B2: #109-120 IILYENPNFTGK
 β B2: #161-172 GLQYLLEKGDYK
 β B2: #169-188
 GDYKDSSDFGAPHPQVQSVR

		<p> βB2: #173-188 DSSDFGAPHPQVQSVR γS: #8-19 ITFYEDKNFQGR γS: #85-95 AVHLPSGGQYK γS: #159-174 KPIDWGAASPAVQSFR [Fkyn(W)] </p>
16	α A: #55-65 TVLDSGISEVR	<p> βB1: #51-60 AAELPPGNYR βB1: #61-72 LVVFELENFQGR βB1: #61-73 LVVFELENFQGR βB1: #74-86 AEFSGECSNLADR βB1: #124-132 WNTWSSSYR βB1: #151-160 ISLFEGANFK βB1: #188-202 VSSGTWVGYPGYR βB1: #203-214 GYQYLLEPGDFR βB1: #234-252 DKQWHLEGSFPVLATEPPK βB1: #236-252 QWHLEGSFPVLATEPPK βB2: #91-101 TDSLSSLRPIK βB2: #109-120 IILYENPNFTGK βB2: #109-121 IILYENPNFTGKK βB2: #146-160 VQSGTWVGYPGYR βB2: #161-168 GLQYLLEK βB2: #169-188 GDYKDSSDFGAPHPQVQSVR βB2: #173-188 DSSDFGAPHPQVQSVR </p>
17		<p> βB1: #51-60 AAELPPGNYR βB1: #61-72 LVVFELENFQGR βB1: #61-73 LVVFELENFQGR βB1: #74-86 AEFSGECSNLADR βB1: #111-118 GEMFILEK </p>

βB1: #124-132 WNTWSSSYR
βB1: #188-202
VSSGTWVG^WYQYPGYR
βB1: #236-252
QWHLEGSFPVLATEPPK
βB2: #2-18
ASDHQTQAGKPQSLNPK
[Acet(N-term)]
βB2: #69-81 GEQFVFEKGEYPR
[Deam(NQ)]
βB2: #77-89
GEYPRWDSWTSSR
βB2: #82-89 WDSWTSSR
[Fkyn(W); Oxi(HW)]
βB2: #90-101 RTDSLSSLRPIK
βB2: #91-108
TDSLSSLRPIKVDSQEHK
[Deam(NQ)]
βB2: #109-120 IILYENPNNFTGK
[2Deam(NQ)]
βB2: #109-121 IILYENPNFTGKK
[Eth(N-term)]
βB2: #121-140
KMEIIDDVPSFHAHGYQEK
[Oxi(M)]
βB2: #122-140
MEIIDDVPSFHAHGYQEK
[Deam(NQ);Oxi(M)]
βB2: #146-160
VQSGTWVG^WYQYPGYR
[Fkyn(W);Oxi(HW)]
βB2: #161-168 GLQYLLEK
βB2: #161-172 GLQYLLEKGDYK
[Deam(NQ)]
βB2: #169-188
GDYKDSSDFGAPHPQVQSVR

β B2: #173-188
DSSDFGAPHPQVQSVR
 β B1: #51-60 AAELPPGNYR
 β B1: #61-72 LVVFELENFQGR
 β B1: #151-160 ISLFEGANFK
 β B1: #188-202
VSSGTWVG \underline{Y} QYPGYR
 β B1: #234-252
DKQWHLEGSFPVLATEPPK
[Deam(NQ)]
 β B2: #2-18
ASDHQTQAGKPQSLNPK
 β B2: #82-89 WDSWTSSR
 β B2: #90-101 RTDSLSSLRPIK
 β B2: #91-101 TDSLSSLRPIK
 β B2: #91-108
TDSLSSLRPIKVDSQEHK
 β B2: #109-120 IILYENPNFTGK
[Eth(N-term)]
 β B2: #109-121 IILYENPNFTGKK
[Deam(NQ)]
 β B2: #121-140
KMEIIDDVPSFHAHG \underline{Y} QEK
[Deam(NQ);Eth(N-term);Oxi(M)]
 β B2: #122-140
MEIIDDVPSFHAHG \underline{Y} QEK
[Deam(NQ); Oxi(M,2HW)]
 β B2: #146-160
VQSGTWVG \underline{Y} QYPGYR
[Fkyn(W);Oxi(HW)]
 β B2: #161-168 GLQYLLEK
 β B2: #161-172 GLQYLLEKGDYK
 β B2: #169-188
GDYKDSSDFGAPHPQVQSVR
 β B2: #173-188
DSSDFGAPHPQVQSVR

		[Deam(NQ)]
		βB2: #173-189
19	αA: #55-65 TVLDSGISEVR	DSSDFGAPHPQVQSVRR
		βB1: #61-72 LVVFELENFQGR
		βB1: #203-214 GYQYLLEPGDFR
		βB2: #82-89 WDSWTSSR
		βB2: #90-101 RTDSLSSLRPIK
		βB2: #91-101 TDSLSSLRPIK
		βB2: #109-120 IILYENPN <u>N</u> FTGK
		[Deam(NQ)]
		βB2: #109-121 IILYENPNFTGKK
		βB2: #146-160
		VQSGT <u>W</u> VGYQYPGYR
		[Oxi(HW)]
		βB2: #161-168 GLQYLLEK
		βB2: #169-188
		GDYKDSSDFGAPHPQVQSVR
		βB2: #173-188
		DSSDFGAPHPQVQSVR
19B	αA: #1-11 <u>M</u> DVTIQHP <u>W</u> FK	βA3: #33-44 ITIYDQENFQGK
	[Acet(N-term);Oxi(MHW)]	
	αA: #1-12 MDVTIQHPWFKR	βA4: #107-118 LTIFEQENFLGK
	αA: #13-21 TLGPFYPSR	βB2: #109-120 IILYENPNFTGK
	αA: #55-65 TVLDSGISEVR	
	αA: #79-88 <u>H</u> FSPEDLTVK	
	[Eth(N-term);Meth(H,N-term)]	
	αA: #89-99 <u>V</u> QDDFVEIHGK	
	[Meth(N-term,Q)]	
	αA: #146-157 <u>I</u> QTGLDATH <u>A</u> ER <u>R</u>	
	[Eth(N-term);Meth(H,N-term,Q,R);Oxi(HW)]	
	αA: #158-173	
	AIPVSREEKPTSAPSS	
	αB: #1-11 MDIAIHHPWIR	
	αB: #12-22 RPFFPFHSPSR	
20	αB: #57-72	βA3: #33-44 ITIYDQENFQGK

APSWFDTGLSEMRLEK [Oxi(M)]

α B: #83-90 HFSPEELK

α B: #124-149

IPADVDPLTITSSLSDGVLTVN

GPR [Deam(NQ)]

β B1: #61-72 LVVFELENFQGR

[Deam(NQ)]

β B2: #82-89 WDSWTSSR

β B2: #109-120 IILYENPNFTGK

β B2: #161-168 GLQYLLEK

β B2: #169-188

GDYKDSSDFGAPHPQVQSVR

β B2: #173-188

DSSDFGAPHPQVQSVR

γ S: #8-14 ITFYEDK

γ S: #8-19 ITFYEDKNFQGR

[Eth(N-term)]

γ S: #73-79 WMGLNDR

γ S: #147-154 GRQYLLDK

γ S: #159-174

KPIDWGAASPAVQSFR

[Deam(NQ);Eth(N-term);Meth(K,N-term);Oxi(HW)]

β A3: #33-44 ITIYDQENFQGK

β B1: #51-60 AAELPPGNYS

β B1: #61-72 LVVFELENFQGR

β B1: #73-86

RAEFSGECNLADR

β B1: #188-202

VSSGTWVGYQYPGYR

β B1: #203-214 GYQYLLEPGDFR

β B2: #69-81 GEQVFEKGEYPR

β B2: #90-101 RTDSLSSLRPIK

β B2: #91-101 TDSLSSLRPIK

β B2: #91-108

TDSLSSLRPIKVDSQEHK

β B2: #109-120 IILYENPNFTGK

21

α A: #13-21 TLGPFYPSR

α A: #55-65 TVLDSGISEVR

α B: #83-90 HFSPEELK

α B: #124-149

IPADVDPLTITSSLSDGVLTVN

GPR [Deam(NQ)]

		[Eth(N-term)] βB2: #109-121 IILYENPNFTGKK βB2: #122-140 MEIIDDVPSFHAHGYQEK βB2: #146-160 VQSGT <u>W</u> VGYQYPGYR [Oxi(HW)] βB2: #161-168 <u>GL</u> QYLLEK [Eth(N-term)] βB2: #173-188 DSSDFGAPHPQVQSVR		
22	αA: #55-65 TVLDSGISEVR		CP49: #201-211 AAEEEINSLY K	
	αA: #79-88 HFSPEDLTVK αA: #89-99 VQDDFVEIHGK			
28			CP49: #77-89 ALGISSVFLQ GLR CP49: #174-191 <u>L</u> MLQTETIQA GADDFKER [Oxi(M)]	
35	αA: #55-65 TVLDSGISEVR			Filensin: #78-90 LGELAGPEDAL AR

Spot numbers refer to spots in Figure 4. Underlined amino acids mark sites of modification, and modifications are shown in brackets.
Abbreviations Used: Acet – Acetylation; Cam – Carbamylation; Deam – Deamidation; Eth – Ethylation; Fkyn – Formylkynurenin = Double oxidation of Trp; Kyn – Kynurenin = Triple oxidation of Trp; Meth – Methylation; Oxi – Oxidation = Single oxidation; Phos – Phosphorylation; Sulph - Sulphone