

Figure S1. Multiple sequence alignment. The Deep MSA tool was used to identify conserved residues starting with **A** tunicate $\beta\gamma$ -crystallin **B** human γ S-crystallin **C** human γ D-crystallin and **D** zebrafish γ M7-crystallin. In all cases, the most common conserved residues are serine and glycine, along with the tryptophans commonly found in the hydrophobic core of each Greek key domain, the tyrosines associated with the tyrosine corners, and charged residues on the surface. Insets show the structure of each protein with strongly conserved residues highlighted. Cysteine and methionine are generally not among the more strongly conserved residues.

A0A9J7L863_BRAFL --MATNSISI YDEEGLRGTK HTFTDTVADV P--AGQNSCF SIKVTSGEWL VYSYNDFEGT SIRLPTGIYP DPGHIRSNHV NPANTTWNDM VRSLRPVRQD
 F6Q2R9_CIOIN ----MGKIIL FEDVEFGGKK LELETSVSDI NVHGFNDIVS SIIVESGTWF VFDEEGFSGP SYKLTPGKYP NPGSWGNG-- ----DDE LSSVKQ--
 S4REP0_PETMA TLGCAPIIF FEERDFKGRR FEYSQDCNM A--SNLSRON SIRVEGGAWV IYERPDFKGN MYIMEPGEYP EYQRWNGY-- ----NDH IGSRSIRGV
 A0A8C4N311_EPTBU ----KIQVVL YEEEFECGRS QE--MCVTI A--IYLF-- ----VCSWV IYEEPGFQGR QLLLEEGERP RPSAWNGY-- ----SGR LGSIRPICCV

A0A9J7L863_BRAFL AVTLYDKDD- ---FCGAEQV VTCD--PDV EMDNCCSVVI RGGWWSLYPY GSYSGGYPPS NTEKTMGPNG YRVQLVAGGE TITIGSIKKV ECPYDPFFLT
 F6Q2R9_CIOIN ----MGKIIL FEDVEFGGKK LELETSVSDI NVHGFNDIVS SIIVESGTWF VFDEEGFSGP SYKLTPGKYP NPGSWGNG-- ----DDE LSSVKQ--
 S4REP0_PETMA SIPTCGQCTG GGEIEGAERK EDCPHLHDF NRMEVRSVRV HDGAWVAYEE PHYRGR-- --QYLLEKGD YRKSEYAGM SPTMQSVRCI RRF-----
 A0A8C4N311_EPTBU SSTFHN----- ----HLLS STRVVPHEYL EGRWVAYEE ANFCGE-- --QYLLEKGD YRSHLDWGGG DPSIGSVRPV FLVRLPMTMK

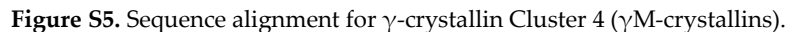
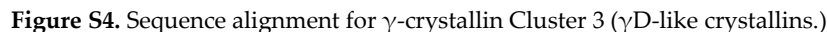
A0A9J7L863_BRAFL PFFPGTTVGP FHHVLPL
 F6Q2R9_CIOIN -----
 S4REP0_PETMA -----
 A0A8C4N311_EPTBU LHF-----

Figure S2. Sequence alignment for γ -crystallin Cluster 1 ($\beta\gamma$ -crystallins).

A0A8V0Z9D9_CHICK -----LSR AVTFYEDKNF LGRRYECDD CDPFHTYLSR CNSIRVEGGT WVAYERPNYS GNMVVLRRGE YPDYHHWMLG NDRLGSKAV HIPSGAQGHI
 A0A151NH35_ALLMI MTIDFALVGF QVTFYEDKNF QGRRYECDD CDPFHTYLSR CNSIRVQGA WVAYERPNFS GNTYVLTGPE YPDYQHWMLG NDRLSSCKAI QIPSGGQSI
 Q5XTP1_DANRE -----MG KIVFFEDKNF QGRRYECDD CDPFHTYLSR CNSIRVESGA WVAYERPNYT GNRVLLTRGE YPEYLGWMLG NDRLSSCKII RFTSGMQYKV
 H2ZWR5_LATCH ----VSLFFQ QITFYEDKNF QGRRYECDD CDPFHTYLSR CNSIRVENGA WVAYERPNYG GYMYVLNRGE YPEYQHWMLG NDRLSSCKMI HFSSGSQYKI
 E9QE97_DANRE ----MGRVST KIIFEDKNF QGRRYECDD CDPFHTYLSR CNSIRVESGA WVAYERPNFI GYQYVLTGPE YPDYQHWMLG NDRLSSCKMI HFVSGSEHKI
 A0A803TS30_ANOCA ---MGSSPDK TITFYEGKNF QGRRYECDD CLDFHTYLSR CNSIRVEGGA WVAYERPNFA GNMVVLTHGD YPEYQHWMLG NDRLSSCKYI QLSGGQYKI
 CRYGS_MACFL ----MSKSVK KITFYEDKNF QGRRYECDD CDPFHTYLSR CNSIRVTGGA WVAYERPNFS GNMVILTGDD YPDYHHWMLG NDRLSSCKVI HSSGGQYKI
 F6SLN9_ORNAN ----MSKTGA KITFYEDKNF QGRRYECDD SADFHTYLSR CNSIRVDGGA WVAYERPNFA GAMVVLTRGE YPDYQHWMLG SDRLGSKAI HSSGGQYKI
 CRYGS_MOUSE ----MSKTGG KISFYEDKNF QGRRYECDD CADFRSYLSR CNSIRVEGGT WAVAYERPNFS GHMYILPQGE YPEYQHWMLG NDRLGSKRAV HSSGGQAKI
 CRYGS_BOVIN ----MSKAGT KITFYEDKNF QGRRYECDD CADFRSYLSR CNSIRVEGGT WAVAYERPNFA GYMYILPQGE YPEYQHWMLG NDRLSSCKRAV HSSGGQYKI
 CRYGS_HUMAN ----MSKTGT KITFYEDKNF QGRRYECDD CDPFHTYLSR CNSIRVEGGT WAVAYERPNFA GYMYILPQGE YPEYQHWMLG NDRLSSCKRAV HLPSSGGQYKI

A0A8V0Z9D9_CHICK QVFEKGFSG QMFATEDCP SILEECHFRE VHACRVLEGI WVFYEHFNIR GRQYLLPKGE YRQPVWGA VTPAVQSFRRS AE
 A0A151NH35_ALLMI QIFEKGFSG QMFATEDCP SVMDEFHMR IHACKVLGGV WVFYEHFNIR GRQYLLEKGE YRKPVEWGA VTPAVQSFRCI VE
 Q5XTP1_DANRE QLYDKPDFTG QAIESIEDCP SVQERFLRE VNSCKVLDGY WIFYEHPNIR GHQYFLKGN YRKPVDWGA CPSVQSFRRF TE
 H2ZWR5_LATCH QVFDKGNFAG QMFATEDCP SVMERFRIR IHACKVLEGV WVFYEHFNIR GRQYLLEKGE YRKPVDWGA CPTVQSFRRR ME
 E9QE97_DANRE QLYDKGFAG QVYETDDCP SVVERFRTRE VHSCKVLDGI WIFYEHPNIR GRQYLLKGE YRKPVDWGA CPTVQSFRRR TE
 A0A803TS30_ANOCA QFWEKGFSG QMYETDDCP SVMQFHLRE IHSCKVLDG VVFYEQPNY GRQYFLKGE YRKPVEWGA SPVVQSFRRV AE
 CRYGS_MACFL QIFEKGFSG QMYETDDCP SVMQFHLRE IHSCKVLDG VVFYEQPNY GRQYFLKGE YRKPVDWGA CAVQSFRRR ME
 F6SLN9_ORNAN QIFEKGFSG QMFATEDCP SVLEQFHLRE IHSCKVLEGV WVFYEQPNY GRQYLLDKE YRKPVDWAA GPTVQSFRRV VE
 CRYGS_MOUSE QVFEKGFNG QMYETDDCP SIMEQFHLRE IHSCKVLEGT WIFYELPNIR GRQYLLDKE YRKPVDWGA SPAIQSFRRR VE
 CRYGS_BOVIN QIFEKGFNG QMYETDDCP SIMEQFHLRE VHSCKVLEGA WIFYELPNIR GRQYLLDKE YRKPVDWGA SPAVQSFRRR VE
 CRYGS_HUMAN QIFEKGFSG QMYETDDCP SIMEQFHLRE IHSCKVLEGV WIFYELPNIR GRQYLLDKE YRKPVDWGA SPAVQSFRRR VE

Figure S3. Sequence alignment for γ -crystallin Cluster 2 (γ S-like crystallins).



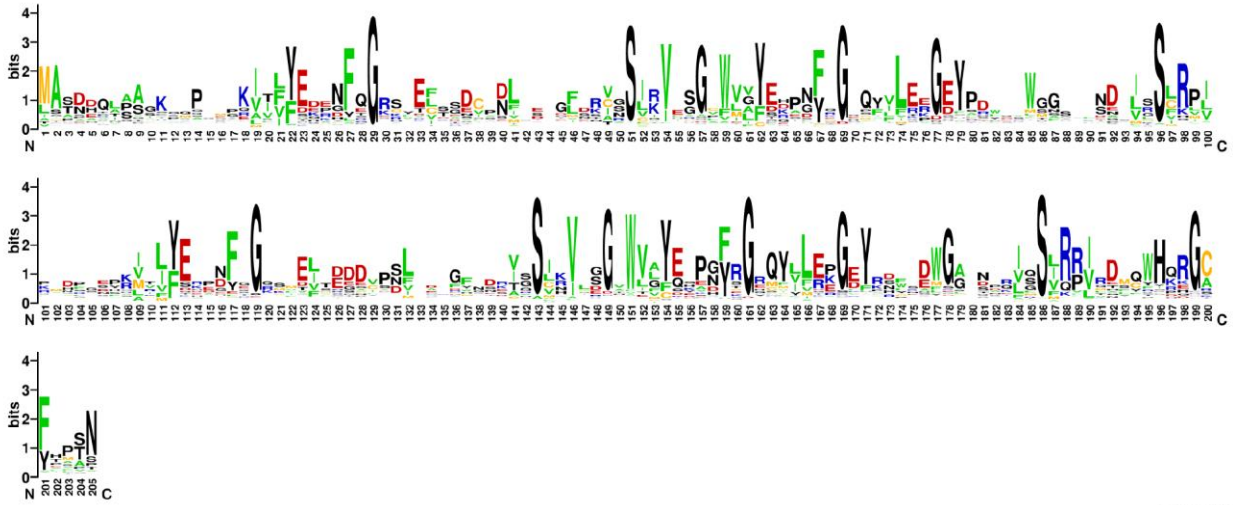


Figure S6. Multiple sequence alignment. The Deep MSA tool was used to identify conserved residues starting with human β 2-crystallin. As for the γ -crystallins, the most common conserved residues are mostly serine, glycine, and aromatic residues, and not cysteine or methionine.

Table S1: Cysteine and methionine content of β -crystallins in representative vertebrates.

organism	protein	UniProt ID	method	length	Cys (no./%)	Met (no./ %)
<i>Mus musculus</i>	β A1-crystallin	CRBA1_MOUSE	x	198	8/4.0	4/2.0
<i>Ornithorhynchus anatinus</i>	β A1-crystallin	F7BTY4_ORNAN	x	204	10/4.9	5/2.5
<i>Danio rerio</i>	β A1-crystallin	Q567D9_DANRE	x	199	11/5.5	8/4.0
<i>Danio rerio</i>	β A1-crystallin	Q6DH14_DANRE	x	196	7/3.6	9/4.6
<i>Danio rerio</i>	β A1b-crystallin	Q6DGY4_DANRE	x	198	12/6.0	12/6.0
<i>Danio rerio</i>	β A1c-crystallin	B5M4A7_DANRE	x	206	10/4.9	8/3.9
<i>Latimeria chalumnae</i>	β A1-crystallin	H3AXI2_LATCH	x	199	10/5.0	12/6.0
<i>Latimeria chalumnae</i>	β A1-crystallin-like	H2ZY80_LATCH	x	213	9/4.2	13/6.1
<i>Anolis carolinensis</i>	β A1-crystallin	H9GJE8_ANOCA	x	215	4/1.9	7/3.3
<i>Gallus gallus</i>	β A2-crystallin	CRBA2_CHICK	x	196	3/1.5	4/2.0
<i>Alligator mississippiensis</i>	β A2-crystallin	A0A151NRA2_A LLMI	x	208	4/1.9	3/1.4
<i>Latimeria chalumnae</i>	β A2-crystallin	H3B2W8_LATCH	x	213	7/3.3	6/2.8
<i>Danio rerio</i>	β A2-crystallin	Q6DGY2_DANRE	x	197	8/4.1	7/3.6
<i>Danio rerio</i>	β A2-crystallin	Q6IQU2_DANRE	x	197	8/4.1	9/4.6
<i>Macropus fuliginosis</i>	β A2-crystallin	CRBA2_MACFL	x	197	4/2.0	2/1.0
<i>Ornithorhynchus anatinus</i>	β A2-crystallin	A0A6I8NZG8_O RNAN	x	241	4/1.6	4/1.6
<i>Bos taurus</i>	β A2-crystallin	CRBA2_BOVIN	x	197	4/2.0	1/0.5
<i>Mus musculus</i>	β A2-crystallin	CRBA2_MOUSE	x	197	7/3.6	1/0.5
<i>Homo sapiens</i>	β A2-crystallin	CRBA2_HUMAN	x	197	6/3.0	1/0.5

<i>Gallus gallus</i>	βA3-crystallin	CRBA1_CHICK	x	215	7/3.3	7/3.3
<i>Alligator mississippiensis</i>	βA3-crystallin	A0A151N992_A LLMI	x	215	6/2.8	6/2.8
<i>Homo sapiens</i>	βA3-crystallin	CRBA1_HUMAN	x	215	5/2.3	6/2.8
<i>Bos taurus</i>	βA3-crystallin	CRBA1_BOVIN	x	215	8/3.7	6/2.8
<i>Alligator mississippiensis</i>	βA4-crystallin	A0A151MUM2_A LLMI	x	260	14/5.4	3/1.2
<i>Gallus gallus</i>	βA4-crystallin	CRBA4_CHICK	x	196	7/3.6	1/0.5
<i>Homo sapiens</i>	βA4-crystallin	CRBA4_HUMAN	x	196	5/2.5	2/1.0
<i>Mus musculus</i>	βA4-crystallin	CRBA4_MOUSE	x	196	4/2.0	2/1.0
<i>Latimeria chalumnae</i>	βA4-crystallin	H3ASM1_LATCH	x	205	9/4.4	7/3.4
<i>Danio rerio</i>	βA4-crystallin	Q52JI7_DANRE	x	196	11/5.6	5/2.6
<i>Danio rerio</i>	βB1-crystallin	A7E2K5_DANRE	x	221	9/4.1	15/6.8
<i>Danio rerio</i>	βB1-crystallin	E7F8M1_DANRE	x	219	9/4.1	16/7.3
<i>Danio rerio</i>	βB1-crystallin-like	A7E2I4_DANRE	x	208	8/3.8	12/5.8
<i>Danio rerio</i>	βB1-crystallin	Q6DGZ8_DANRE	x	232	9/3.9	12/5.2
<i>Latimeria chalumnae</i>	βB1-crystallin	H3A1B8_LATCH	x	219	10/4.6	14/6.4
<i>Latimeria chalumnae</i>	βB1-crystallin	H3AWX7_LATCH	x	211	10/4.7	10/4.7
<i>Latimeria chalumnae</i>	βB1-crystallin	H3AS10_LATCH	x	254	11/4.3	9/3.5
<i>Gallus gallus</i>	βB1-crystallin	CRBB1_CHICK	x	238	5/2.1	8/3.4
<i>Alligator mississippiensis</i>	βB1-crystallin	A0A151MUJ3_A LLMI	x	238	3/1.3	6/2.5
<i>Bos taurus</i>	βB1-crystallin	CRBB1_BOVIN	x	253	4/1.6	5/2.0
<i>Mus musculus</i>	βB1-crystallin	CRBB_MOUSE	x	250	4/1.6	5/2.0

<i>Homo sapiens</i>	β B1-crystallin	CRBB1_HUMAN	x	252	1/0.4	4/1.5
<i>Latimeria chalumnae</i>	β B2-crystallin	H3AY34_LATCH	x	205	4/2.0	1/0.5
<i>Gallus gallus</i>	β B2-crystallin	BRBB2_CHICK	x	219	3/1.4	2/0.9
<i>Alligator mississippiensis</i>	β B2-crystallin	A0A151NSD4_A LLMI	x	204	3/1.5	1/0.5
<i>Macropus fuliginosis</i>	β B2-crystallin	B2ZF66_MACFL	x	205	1/0.5	2/1.0
<i>Ornithorhyncus anatinus</i>	β B2-crystallin	F7A5Y5_ORNAN	x	187	3/1.6	1/0.5
<i>Danio rerio</i>	β B2-crystallin	Q52JI4_DANRE	x	208	6/2.9	1/0.5
<i>Homo sapiens</i>	β B2-crystallin	CRBB2_HUMAN	x	205	1/0.5	2/1.0
<i>Bos taurus</i>	β B2-crystallin	CRBB2_BOVIN	x	205	2/1.0	2/1.0
<i>Mus musculus</i>	β B2-crystallin	CRBB2_MOUSE	x	205	2/1.0	3/1.5
<i>Danio rerio</i>	β B3-crystallin	Q52JI3_DANRE	x	259	4/1.5	3/1.2
<i>Latimeria chalumnae</i>	β B3-crystallin	H3AXG5_LATCH	x	211	2/0.9	5/2.4
<i>Gallus gallus</i>	β B3-crystallin	CRBB3_CHICK	x	211	1/0.5	4/1.9
<i>Ornithorhyncus anatinus</i>	β B3-crystallin	F6Z4S4_ORNAN	x	211	3/1.4	1/0.5
<i>Homo sapiens</i>	β B3-crystallin	CRBB3_HUMAN	x	211	2/0.9	1/0.5
<i>Mus musculus</i>	β B3-crystallin	CRBB3_MOUSE	x	211	3/1.4	1/0.5
<i>Bos taurus</i>	β B3-crystallin	CRBB3_BOVIN	x	211	2/0.9	2/0.9

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A0A9J7L863_BRAFL  ----- --MATNSISI YDEEGLRGTK HTFTDTVADV PA--GQNSCF SIKVTSGEWL
A0A8C4N311_EPTBU ----- --KIQVVL YEEEFGRS QEMVTLA-- ----- IYLFVSSWV
F6Q2R9_CIOIN ----- --MGKILL FEDVEFGGKK LELETSVSDL NVHGFNDIVS SIIVESGTFW
S4REP0_PETMA ----- TLGAPQIIF FEERDFGKRR FEYSQDCNM AS--NLSRCN SIRVEGGAWV
A0A6I8NZG8_ORNAN MRLLRGREGS VVAGEHEDES EMDKGSIVSG TQAQEQREQE HIRSMSSISP QDSGRARLTL WEEEFQGRR CELIGDCPSI RDRSGFRVR SVKVESGAWV
CRBA2_MACFL ----- --VAGEHEDES ----- --MSCTL ----- QGSSPARLTL WEEENFQGRR CELMSDCSSI RELSGFRVR SVKVESGAWV
CRBA2_BOVIN ----- --MSSAPA QGPAPASLTL WDEEDFQGRR CRLISDCANI GERGGLRVR SVKVENGAWV
CRBA2_MOUSE ----- --MSSAPA PGSAPVCLTL WDEEDFQGRR CRLISDCANV CERGALRRVR SVKVENGAWV
CRBA2_HUMAN ----- --MSSAPA PGAPASLTL WDEEDFQGRR CRLISDCANV CERGGLPRVR SVKVENGAWV
H3B2W8_LATCH ----- --WPAFLNIS ----- --EVEKR SDFKMTTQQI ETMGQWKITV WEEENFQGRR CEFMMECPNI MER-GFHKIR SVKVECGPWV
CRBA2_CHICK ----- ----- ----- --MTSEAM DTLGQYKITV WEEENFQGRR CEFLECPNI MER-GFRKIR SIKVENGPWV
A0A151NRA2_ALLMI ----- --MRG ----- --LRVCS KGIAMTTQAM DTLGQYKITV WEEENFQGRR CEFLECPNI LER-GFRKIR SIKVENGPWV
Q6DGY2_DANRE ----- ----- ----- --MNPQEQ EQRGKFRMTV FEEEHFQGRS CEFSECPNI LER-DFRKIR SIKVENGPWV
Q6IQU2_DANRE ----- ----- ----- --MNQREQ EQQ-QMRITV WEEENFQGRR CEFLECPNI LER-DFQKIR SIKVNDGPWV

A0A9J7L863_BRAFL VVSYNDEFT SIRLPTGIYP DPGHIRSNHV NPANTTWNDM VRSIRPVVRQ- ---DAVTLY DKDDFCGAE- ---QVVTDD PDVE---M DNCCSVVIRG
A0A8C4N311_EPTBU IYEEPGFQGR QLLLEEGEFP RPSAWGGYSG R----- LGSIRPICCV SSTFHNHL----- LSST RVVPEHYLEG
F6Q2R9_CIOIN VFDDEGFSGP SYKLTGKYP NPGSWGND E-----LSS-----VRQQ-----
S4REP0_PETMA IYERPDFGN MYIMEPEYYP EYQRWGYND H-----IGSRSIRGV S-----IP TQG TGGGE EGAERKEDC PHLH-DRFNM REVRSVRVHD
A0A6I8NZG8_ORNAN GFEPDFQGG QFILEKGDYP CWLAWSGSSG Y-----HTDQ LLSFRLLICA NHSDSRVTLF EGENFQGG-- --KFEVNDDY PSLPAMGWT SVVGSIMVGS
CRBA2_MACFL GFEPDFQGG QFILEKGDYP RSTAWSGSSG Y-----RTDQ LLSFRPILCA NHSDSRVTLF EGENFQGG-- --KFELSDDY PSLPAMGWAS KDVGSLKVT
CRBA2_BOVIN AFEYPDFQGG QFILEKGDYP RWSAWSGSAG H-----HSDQ LLSFRPVLCA NHSDSRVTLF EGENFQGG-- --KFEVNDDY PSLPAMGWAS KDVGSLKVT
CRBA2_MOUSE AFEYPDFQGG QFILEKGDYP CWSAWSGSSG H-----HSNQ LLSFRPVLCA NHSDSRVTLF EGENFQGG-- --KFELSDDY PSLPAMGWT KDVGSLKVT
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H3B2W8_LATCH GFEPDFQGG QFILEKGDYP RWEAWSGNSG Y-----RTEH LLSFRPVKCA NHSDSKVTLF EGENFQGR-- --KFELDDY PSLPAMGWGN KEVASIKVNS
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A0A151NRA2_ALLMI GFEPDFQGG QFILEKGDYP RWEAWSGSSG Y-----RTEH LLSFRPVKCA NHSDSKVTLF EGENFQGR-- --KFELSDDY PSLPAMGWGT KEVASIKVNS
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Figure S7. Sequence alignment for β -crystallin Cluster 1 ($\beta\gamma$ and β A2-crystallins).

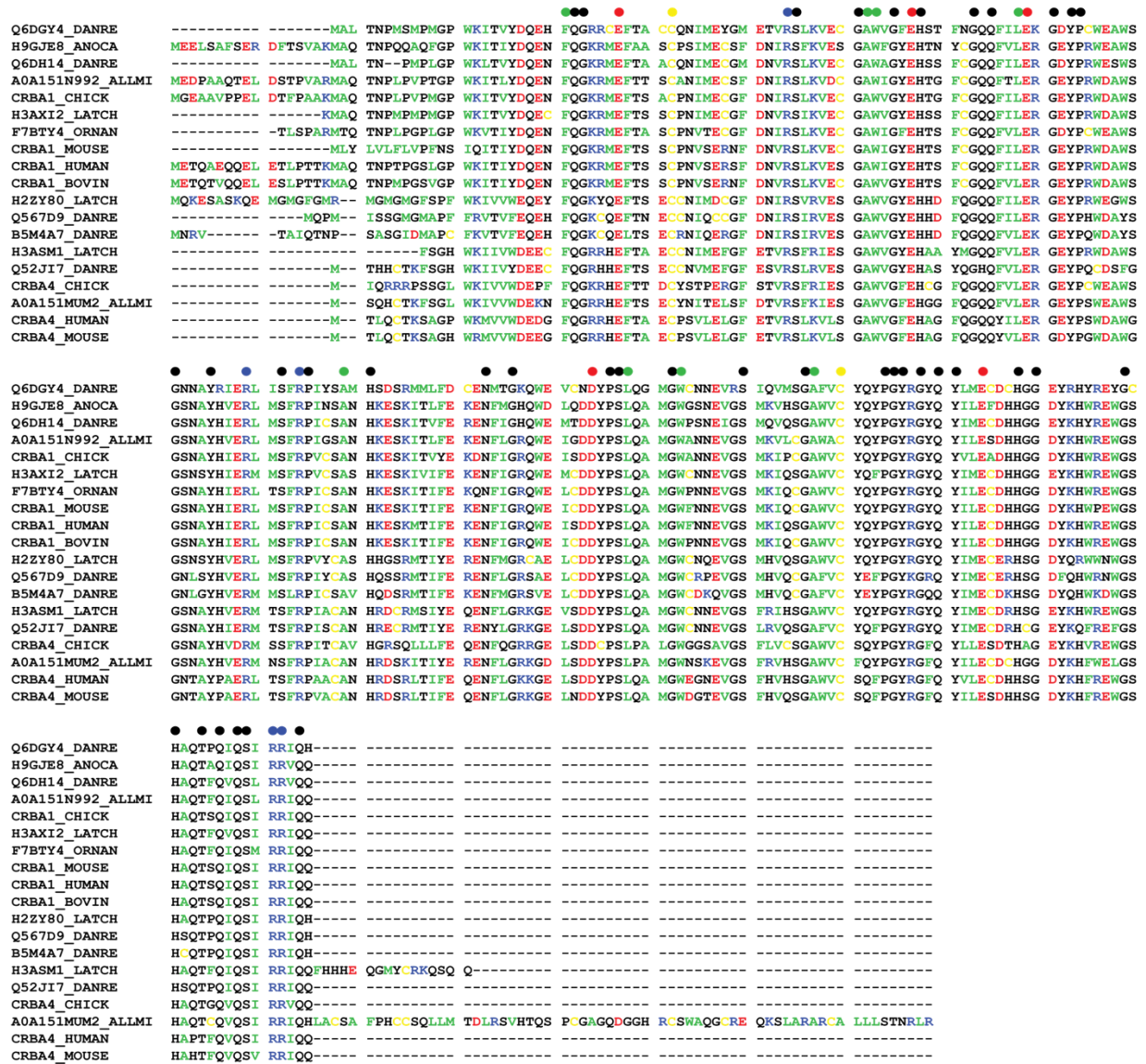


Figure S8. Sequence alignment for β -crystallin Cluster 2 (β A4-crystallins).

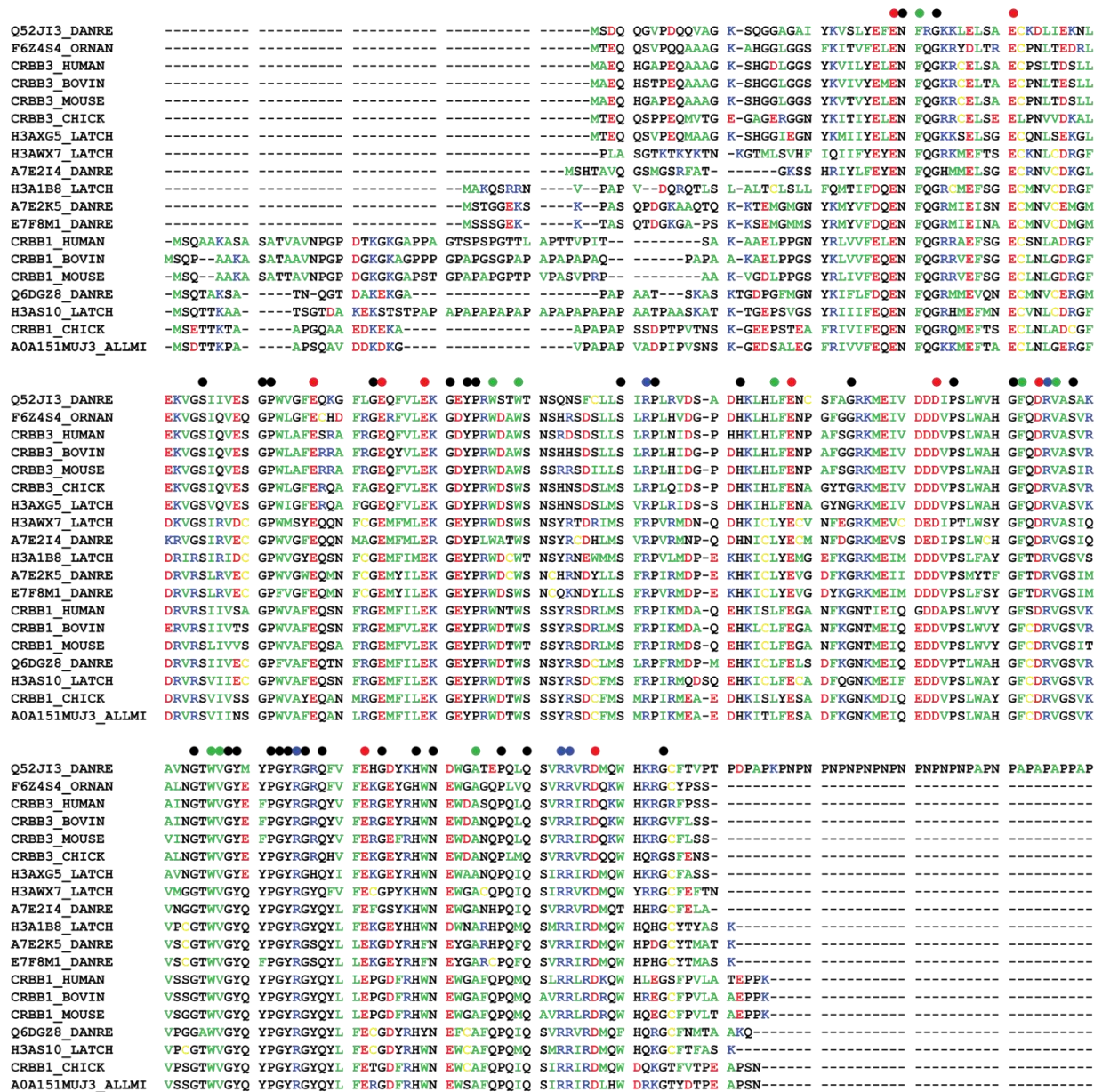


Figure S9. Sequence alignment for β -crystallin Cluster 3 (β B1- and β B3-crystallins). Q52JI3_DANRE has an additional 7 amino acids (SATAASS) that were truncated to limit the figure to one page.

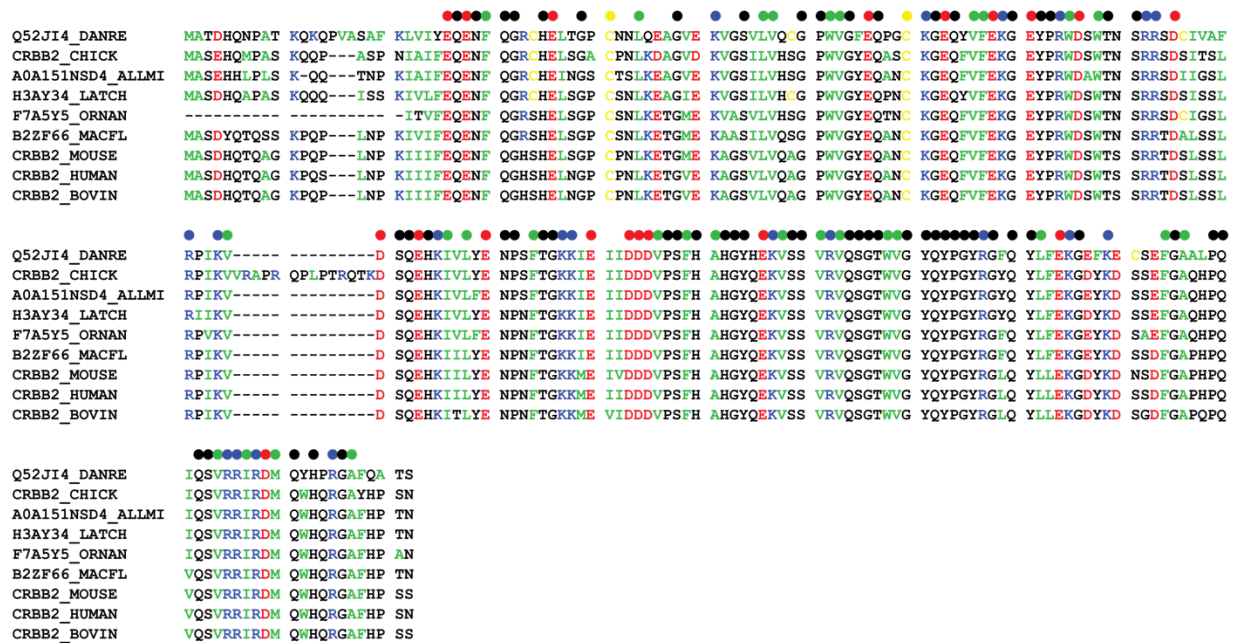


Figure S10. Sequence alignment for β -crystallin Cluster 4 (β 2-crystallins).