

Figure S1: Depiction of the final 20-structure family for  $\gamma$ S-WT (transparent grey, colored by heteroatom) and the energy-minimized average NMR structure (opaque green colored by heteroatom), including sidechains. Related to Figure 1 and Table 1.



Figure S2: Depiction of the final 20-structure family for  $\gamma$ S-G18V (transparent grey, colored by heteroatom) and the energy-minimized average NMR structure (opaque blue colored by heteroatom), including sidechains. Related to Figure 1 and Table 1.



Figure S3: Comparison of two structural refinements without NOE restraints to residues 16-20 and without angular RDC restraints to residue 18 performed with (top) and without (bottom) the angular backbone restraint provided from the HNHA experiment for V18. Note that this single restraint is sufficient to keep V18 in place in all of the top scoring structures. Even if the J-coupling restraint is omitted, only five of 50 calculated structures land with V18 in an "allowed" dihedral angle conformation. Related to Figure 1 and Table 1.



Figure S4: Particle size of  $\gamma$ S-WT and  $\gamma$ S-G18V at 2.0 mM concentration in 10 mM acetate buffer pH 4.5 measured by dynamic light scattering on a Zetasizer Nano ZS (Malvern Instruments, Ltd.). Under these conditions  $\gamma$ S-WT and  $\gamma$ S-G18V are fully monomeric, with particle diameters of 39.7 and 40.9 Å respectively. Related to Figure 1 and Table 1.



Figure S5: DLS data of  $\alpha$ B,  $\gamma$ S-WT,  $\gamma$ S-G18V and mixtures thereof in 10 mM phosphate buffer pH 6.9 where (A) is the distribution of particle size by number and (B) is the distribution of particle size by intensity. The concentrations of the  $\gamma$ S-WT,  $\gamma$ S-G18V, and the  $\alpha$ B samples are at 1.5 mM while the mixtures with  $\alpha B$  consist of a 2:1 molar ratio of  $\alpha B:\gamma S$ . Both sets of data show that  $\gamma S$ -G18V forms large aggregates at pH 6.9 and that the  $\alpha B/\gamma S$ -G18V mixture forms larger aggregates than that of either the  $\alpha$ B-only and  $\alpha$ B/ $\gamma$ S-WT samples. Related to Figure 2.

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15	Res	D (Hz)	Res	D (Hz)	Res	D (Hz)	Res
16							
17	2	1.7	41	17.5	83	16.1	127
19	3	3.9	42	-22.7	84	17.9	129
10	4	-14.4	43	24.6	85	20.2	130
19	5	-9.2	44	22.9	86	1.1	134
20	6	12.2	45	14.5	87	7.6	$137\epsilon$
21	7	12.9	46	7.0	88	-28.0	138
22	8	20.9	47	21.4	90	14.3	139
23	9	17.6	$47\epsilon$	-27.1	91	-1.4	140
24	10	13.2	48	17.6	92	-9.9	141
21	11	-9.5	49	6.3	93	12.0	142
25	12	-21.1	50	-12.2	94	-26.3	144
26	13	-10.3	51	-30.1	96	18.4	145
27	14	9.4	52	-35.8	97	0.3	146
28	15	-35.6	54	-23.2	99	-27.9	147
29	16	-28.3	55	-11.9	100	-19.2	148
30	17	-11.9	56	-42.8	101	11.2	150
21	18	-27.8	57	-27.6	102	16.3	151
22	19	20.9	58	-9.6	104	-27.6	152
32	20	19.7	61	0.8	105	-17.3	156
33	21	1.1	62	20.1	106	-20.3	158
34	22	-7.9	04	-10.6	107	20.3	159
35	23	21.3	03	0.2	108	-32.2	101
36	24	2.0	67	10.4	109	-10.9	102
37	25	3.0	69	-27.0	110	-12.5	103
20	20	2.7	70	-27.8	111	11.8	164
20	28	-22.7	70	-10.0	112	-20.3	165
39	20	85	71	-33.0	113	10.5	165
40	30	-14.1	72	-2.3	115	-6.9	167
41	31	-30.8	73e	25.7	117	5.7	169
42	32	-6.0	74	-3.9	118	-20.6	170
43	33	-42.5	75	-11.2	119	-36.7	171
44	34	-18.7	76	14.2	120	-42.1	172
11	35	-24.3	77	21.0	121	-26.7	173
45	36	-1.3	78	15.8	122	-29.3	174
46	37	-11.9	79	-15.4	123	-2.1	175
47	38	-10.4	80	-40.1	124	-6.1	176
48	39	23.2	81	-45.2	125	-21.0	177
49	40	23.9	82	16.4	126	-34.9	178
50							
51							
51							
54							
53							
54							

Table S1:  $\gamma$ S-WT RDCs. Related to Figure 1 and Table 1.

D (Hz)

-27.9

21.2

17.5

14.0

24.1 -22.7

2.7 -26.0

-22.7

-22.7 -34.0

-41.4

-40.2

-29.8

4.4 -1.1

3.6

-22.7

-22.7 -6.5

-23.4

-20.6

-35.4

-0.3

-4.1

-17.4 -14.0

3.5

5.1

-34.9 23.5

13.1

14.8 25.3

-5.9 3.2

9.6

-2.4

20.9

63 64

Res	D (Hz)	Res D (Hz)	Res D (Hz)	Res D (Hz)
2	2.6	43 24.6	85 22.3	127 -33.8
3	3.4	44 24.4	86 1.4	128 -35.1
4	-7.7	45 8.4	87 7.2	129 21.1
5	-16.3	46 9.1	88 -22.2	130 14.4
6	15.1	47 14.9	90 25.0	134 1.2
7	18.4	$47\epsilon$ 17.9	91 -4.6	$137\epsilon$ 14.3
8	24.1	48 21.0	92 -13.2	138 20.4
9	-13.4	49 1.5	93 13.5	139 -2.1
10	8.6	50 -21.7	94 11.2	140 -35.1
11	-23.1	51 -55.3	96 17.4	142 -20.3
12	-33.8	52 -34.7	97 -4.9	144 -45.4
13	10.6	54 -31.5	99 -29.4	145 -38.3
14	6.3	56 -49.1	100 -23.4	146 -46.4
16	-26.5	57 -34.5	101 13.0	147 -35.4
17	-14.1	58 1.6	102 16.5	148 -1.3
18	-25.1	61 2.8	104 -31.9	150 3.5
19	-25.5	62 35.8	105 -20.4	151 5.4
21	-45.7	64 -11.6	106 -25.0	159 -24.7
22	16.6	65 4.7	107 23.0	161 -25.8
23	22.1	66 17.5	108 -38.6	162 -41.4
24	14.1	67 2.8	109 -14.8	163 -2.3
25	-1.4	69 -20.3	110 -17.3	$163\epsilon$ 23.4
26	-3.2	70 -19.9	111 10.7	164 -6.5
27	7.4	71 -41.4	112 6.6	165 -21.8
29	-30.3	72 -39.8	113 -19.4	166 -18.8
30	-33.8	73 -5.0	114 19.0	167 2.9
31	-30.1	$73\epsilon$ 28.4	115 -10.2	169 5.5
32	-5.1	74 10.6	117 3.3	170 -39.9
33	-44.0	75 -12.7	118 -27.4	171 -45.8
34	-17.9	77 24.0	119 -39.9	172 12.3
35	-39.3	78 14.3	120 22.1	173 16.7
36	-16.7	79 -19.5	121 -41.1	174 9.2
37	-36.7	80 -29.2	122 -33.3	175 -10.7
38	-35.4	81 -55.9	123 -14.9	176 5.0
39	26.4	82 18.9	124 -6.5	177 12.8
40	10.4	83 18.1	125 -25.1	178 -5.1
41	25.2	84 16.9	126 -43.5	

Table S2:  $\gamma$ S-G18V RDCs. Related to Figure 1 and Table 1.

## **Supplemental Experimental Procedures**

**Structure Calculations** The structures for  $\gamma$ S-WT and  $\gamma$ S-G18V were refined using the full NOE restraint set, residual dipolar couplings, TALOS+ generated dihedral restraints, H-N-HA derived 3J couplings, and hydrogen-bonding data from the H-D exchange experiments. 100 structures were calculated from an extended conformation using high-temperature torsion angle dynamics, a simulated annealing, followed by a cartesian space energy minimization. The average of the 10 lowest-energy structures was calculated and used as a starting structure for a final 200-structure calculation using a simulated annealing and Cartesian space energy minimization. The 20 lowest-energy structures were used as the final structure family. (Supplementary Figures S1 and S2, Table 1).

Structure families are shown for  $\gamma$ S-WT and  $\gamma$ S-G18V in Supplementary Figures S1 and S2. In the  $\gamma$ S-G18V structure, V18 does not swing out into the solvent, but instead remains buried within the surrounding loop. The first 17 residues of the structure are largely unaffected by the substitution, while the dihedral angles shift from  $\phi = 73.7$  °,  $\psi = -163.0$  ° for G18 to  $\phi = 129.8$  °,  $\psi = -134.3$  ° for V18. Consistent with the change in amide chemical shifts observed in  $\gamma$ S-G18V, the R19 backbone dihedral angles are substantially changed from their wild-type configurations. The presence of V18 shifts the R19 dihedral angles from  $\phi = -58.6^{\circ}$ ,  $\psi = -177.2^{\circ}$  to  $\phi = -110.5$ °,  $\psi = 17.9$ °. The 180° change in the  $\psi$  angle of R19 disrupts the R20–C23  $\beta$ -sheet and the disruption propagates down the backbone. The exact structural cause of the  $\psi$ -inversion may be from steric clashes between the V18 sidechain and R19 carbonyl or other effects early in the folding of  $\gamma$ S-crystallin from the presence of the highly hydrophobic V18. Additional structural calculations indicate that the V18 side chain remains buried even upon exclusion of all distance restraints to V18 (Supplementary Figure S3 (top)), and the adoption of a favorable backbone dihedral angle configuration would require the elimination of restraints from several surrounding residues, producing an extensive structural disruption to the surrounding loop region that is not supported by the NMR data (Supplementary Figure S3 (bottom)).

**Monomeric**  $\gamma$ **S-WT and**  $\gamma$ **S-G18V under NMR conditions** Samples of natural abundance wild-type  $\gamma$ S-crystallin ( $\gamma$ S-WT), and the variant  $\gamma$ S-G18V were prepared in the NMR sample buffers in concentrations comparable to those used in structural NMR experiments. Light scattering measurements performed on these samples (Supplementary Figure S4) indicated that both and  $\gamma$ S-G18V were monomeric under these buffer conditions.  $\gamma$ S-WT,  $\gamma$ S-G18V,  $\alpha$ B-crystallin and mixtures thereof were also prepared in 10 mM phosphate, pH 6.9 in concentrations comparable to those used in NMR binding experiments. Light scattering measurements were then performed on these samples (Supplementary Figure S5). See main text for discussion of data.

## Supplementary Information: Preferential and specific binding of human $\alpha$ B-crystallin to cataract-related variant of $\gamma$ S-crystallin

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## **Supplemental Data**



Figure 1: Depiction of the final 20-structure family for  $\gamma$ S-WT (transparent grey, colored by heteroatom) and the energy-minimized average NMR structure (opaque green colored by heteroatom), including sidechains. Related to Figure 1 and Table 1.



Figure 2: Depiction of the final 20-structure family for  $\gamma$ S-G18V (transparent grey, colored by heteroatom) and the energy-minimized average NMR structure (opaque blue colored by heteroatom), including sidechains. Related to Figure 1 and Table 1.



Figure 3: Comparison of two structural refinements without NOE restraints to residues 16-20 and without angular RDC restraints to residue 18 performed with (top) and without (bottom) the angular backbone restraint provided from the HNHA experiment for V18. Note that this single restraint is sufficient to keep V18 in place in all of the top scoring structures. Even if the J-coupling restraint is omitted, only five of 50 calculated structures land with V18 in an "allowed" dihedral angle conformation. Related to Figure 1 and Table 1.



Figure 4: Particle size of  $\gamma$ S-WT and  $\gamma$ S-G18V at 2.0 mM concentration in 10 mM acetate buffer pH 4.5 measured by dynamic light scattering on a Zetasizer Nano ZS (Malvern Instruments, Ltd.). Under these conditions  $\gamma$ S-WT and  $\gamma$ S-G18V are fully monomeric, with particle diameters of 39.7 and 40.9 Å respectively. Related to Figure 1 and Table 1.



Figure 5: DLS data of  $\alpha$ B,  $\gamma$ S-WT,  $\gamma$ S-G18V and mixtures thereof in 10 mM phosphate buffer pH 6.9 where (A) is the distribution of particle size by number and (B) is the distribution of particle size by intensity. The concentrations of the  $\gamma$ S-WT,  $\gamma$ S-G18V, and the  $\alpha$ B samples are at 1.5 mM while the mixtures with  $\alpha B$  consist of a 2:1 molar ratio of  $\alpha B:\gamma S$ . Both sets of data show that  $\gamma S$ -G18V forms large aggregates at pH 6.9 and that the  $\alpha B/\gamma S$ -G18V mixture forms larger aggregates than that of either the  $\alpha$ B-only and  $\alpha$ B/ $\gamma$ S-WT samples. Related to Figure 2.

ResD (Hz)ResD (Hz)21.74117.533.942-22.74-14.44324.65-9.24422.9612.24514.5712.9467.0820.94721.4917.6 $47\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036			Table	1: $\gamma$ S-W
2 $1.7$ $41$ $17.5$ 3 $3.9$ $42$ $-22.7$ 4 $-14.4$ $43$ $24.6$ 5 $-9.2$ $44$ $22.9$ 6 $12.2$ $45$ $14.5$ 7 $12.9$ $46$ $7.0$ 8 $20.9$ $47$ $21.4$ 9 $17.6$ $47\epsilon$ $-27.1$ 10 $13.2$ $48$ $17.6$ $11$ $-9.5$ $49$ $6.3$ $12$ $-21.1$ $50$ $-12.2$ $13$ $-10.3$ $51$ $-30.1$ $14$ $9.4$ $52$ $-35.8$ $15$ $-35.6$ $54$ $-23.2$ $16$ $-28.3$ $55$ $-11.9$ $17$ $-11.9$ $56$ $-42.8$ $18$ $-27.8$ $57$ $-27.6$ $20$ $19.7$ $61$ $0.8$ $21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $62$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $27.3$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ <	Res	D (Hz)	R	es D (Hz)
33.942-22.74-14.44324.65-9.24422.9612.24514.5712.9467.0820.94721.4917.647 $\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.357-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.	2	1.7	4	1 17.5
4-14.44324.65-9.24422.9612.24514.5712.9467.0820.94721.4917.647 $\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.139 <td< td=""><td>3</td><td>3.9</td><td>42</td><td>2 -22.7</td></td<>	3	3.9	42	2 -22.7
5-9.24422.9612.24514.5712.9467.0820.94721.4917.647 $\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.575-13.231-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	4	-14.4	4.	3 24.6
612.24514.5712.9467.0820.94721.4917.647 $\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.438-10.480-40.13923.281-45.24023.98216.4	5	-9.2	44	4 22.9
712.9467.0820.94721.4917.647 $\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	6	12.2	4:	5 14.5
8   20.9   47   21.4     9   17.6   47 $\epsilon$ -27.1     10   13.2   48   17.6     11   -9.5   49   6.3     12   -21.1   50   -12.2     13   -10.3   51   -30.1     14   9.4   52   -35.8     15   -35.6   54   -23.2     16   -28.3   55   -11.9     17   -11.9   56   -42.8     18   -27.8   57   -27.6     19   20.9   58   -9.6     20   19.7   61   0.8     21   1.1   62   20.1     22   -7.9   64   -10.6     23   21.3   65   62     24   15.6   66   16.4     25   3.0   67   -27.0     26   2.7   69   -27.8     27   -22.7   70   -16.6     28   -31.9   71   -27.4     29 <td>7</td> <td>12.9</td> <td>40</td> <td>5 7.0</td>	7	12.9	40	5 7.0
917.6 $47\epsilon$ -27.11013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	8	20.9	4'	7 21.4
1013.24817.611-9.5496.312-21.150-12.213-10.351-30.1149.452-35.815-35.654-23.216-28.355-11.917-11.956-42.818-27.857-27.61920.958-9.62019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.873 $\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	9	17.6	4'	$7\epsilon$ -27.1
11-9.549 $6.3$ 12-21.1 $50$ -12.213-10.3 $51$ -30.1149.4 $52$ -35.815-35.6 $54$ -23.216-28.3 $55$ -11.917-11.9 $56$ -42.818-27.8 $57$ -27.61920.9 $58$ -9.62019.7610.8211.1 $62$ 20.122-7.9 $64$ -10.62321.3 $65$ $62$ 2415.6 $66$ 16.4253.0 $67$ -27.0262.7 $69$ -27.827-22.770-16.628-31.9 $71$ -27.429 $8.5$ $72$ -33.030-14.1 $73$ -2.331-30.8 $73\epsilon$ 25.732-6.0 $74$ -3.933-42.5 $75$ -11.234-18.7 $76$ 14.235-24.3 $77$ 21.036-1.3 $78$ 15.837-11.9 $79$ -15.438-10.4 $80$ -40.13923.2 $81$ -45.24023.9 $82$ 16.4	10	13.2	43	8 17.6
$12$ $-21.1$ $50$ $-12.2$ $13$ $-10.3$ $51$ $-30.1$ $14$ $9.4$ $52$ $-35.8$ $15$ $-35.6$ $54$ $-23.2$ $16$ $-28.3$ $55$ $-11.9$ $17$ $-11.9$ $56$ $-42.8$ $18$ $-27.8$ $57$ $-27.6$ $19$ $20.9$ $58$ $-9.6$ $20$ $19.7$ $61$ $0.8$ $21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	11	-9.5	49	9 6.3
13 $-10.3$ $51$ $-30.1$ 14 $9.4$ $52$ $-35.8$ 15 $-35.6$ $54$ $-23.2$ 16 $-28.3$ $55$ $-11.9$ 17 $-11.9$ $56$ $-42.8$ 18 $-27.8$ $57$ $-27.6$ 19 $20.9$ $58$ $-9.6$ 20 $19.7$ $61$ $0.8$ 21 $1.1$ $62$ $20.1$ 22 $-7.9$ $64$ $-10.6$ 23 $21.3$ $65$ $6.2$ 24 $15.6$ $66$ $16.4$ 25 $3.0$ $67$ $-27.0$ 26 $2.7$ $69$ $-27.8$ 27 $-22.7$ $70$ $-16.6$ 28 $-31.9$ $71$ $-27.4$ 29 $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	12	-21.1	50	-12.2
149.452 $-35.8$ 15 $-35.6$ 54 $-23.2$ 16 $-28.3$ 55 $-11.9$ 17 $-11.9$ 56 $-42.8$ 18 $-27.8$ 57 $-27.6$ 1920.958 $-9.6$ 2019.7610.8211.16220.122 $-7.9$ 64 $-10.6$ 2321.3656.22415.66616.4253.067 $-27.0$ 262.769 $-27.8$ 27 $-22.7$ 70 $-16.6$ 28 $-31.9$ $71$ $-27.4$ 29 $8.5$ $72$ $-33.0$ 30 $-14.1$ $73$ $-2.3$ 31 $-30.8$ $73\epsilon$ $25.7$ 32 $-6.0$ $74$ $-3.9$ 33 $-42.5$ $75$ $-11.2$ 34 $-18.7$ $76$ $14.2$ 35 $-24.3$ $77$ $21.0$ 36 $-1.3$ $78$ $15.8$ 37 $-11.9$ $79$ $-15.4$ 38 $-10.4$ $80$ $40$ $23.9$ $82$ $16.4$	13	-10.3	5	1 -30.1
15 $-35.6$ $54$ $-23.2$ 16 $-28.3$ $55$ $-11.9$ 17 $-11.9$ $56$ $-42.8$ 18 $-27.8$ $57$ $-27.6$ 19 $20.9$ $58$ $-9.6$ 20 $19.7$ $61$ $0.8$ 21 $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	14	9.4	52	2 -35.8
$16$ $-28.3$ $55$ $-11.9$ $17$ $-11.9$ $56$ $-42.8$ $18$ $-27.8$ $57$ $-27.6$ $19$ $20.9$ $58$ $-9.6$ $20$ $19.7$ $61$ $0.8$ $21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	15	-35.6	54	4 -23.2
$17$ $-11.9$ $56$ $-42.8$ $18$ $-27.8$ $57$ $-27.6$ $19$ $20.9$ $58$ $-9.6$ $20$ $19.7$ $61$ $0.8$ $21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	16	-28.3	5:	5 -11.9
$18$ $-27.8$ $57$ $-27.6$ $19$ $20.9$ $58$ $-9.6$ $20$ $19.7$ $61$ $0.8$ $21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $71.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	17	-11.9	50	-42.8
1920.9 $58$ $-9.6$ 2019.7610.8211.16220.122 $-7.9$ 64 $-10.6$ 2321.3656.22415.66616.4253.067 $-27.0$ 262.769 $-27.8$ 27 $-22.7$ 70 $-16.6$ 28 $-31.9$ 71 $-27.4$ 298.572 $-33.0$ 30 $-14.1$ $73$ $-2.3$ 31 $-30.8$ $73\epsilon$ 25.732 $-6.0$ $74$ $-3.9$ 33 $-42.5$ 75 $-11.2$ 34 $-18.7$ $76$ $14.2$ 35 $-24.3$ $77$ $21.0$ 36 $-1.3$ $78$ $15.8$ 37 $-11.9$ $79$ $-15.4$ 38 $-10.4$ $80$ $-40.1$ 39 $23.2$ $81$ $-45.2$ 40 $23.9$ $82$ $16.4$	18	-27.8	5	7 -27.6
2019.7610.8211.16220.122-7.964-10.62321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.8 $73\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	19	20.9	58	8 -9.6
$21$ $1.1$ $62$ $20.1$ $22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	20	19.7	6	1 0.8
$22$ $-7.9$ $64$ $-10.6$ $23$ $21.3$ $65$ $6.2$ $24$ $15.6$ $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73 < -2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	21	1.1	62	2 20.1
2321.3656.22415.66616.4253.067-27.0262.769-27.827-22.770-16.628-31.971-27.4298.572-33.030-14.173-2.331-30.8 $73\epsilon$ 25.732-6.074-3.933-42.575-11.234-18.77614.235-24.37721.036-1.37815.837-11.979-15.438-10.480-40.13923.281-45.24023.98216.4	22	-7.9	64	4 -10.6
$24$ 15.6 $66$ $16.4$ $25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	23	21.3	63	5 6.2
$25$ $3.0$ $67$ $-27.0$ $26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	24	15.6	60	5 16.4
$26$ $2.7$ $69$ $-27.8$ $27$ $-22.7$ $70$ $-16.6$ $28$ $-31.9$ $71$ $-27.4$ $29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	25	3.0	6	-27.0
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	26	2.7	6	9 -27.8
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	27	-22.7	70	J -16.6
$29$ $8.5$ $72$ $-33.0$ $30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	28	-31.9	7	1 -2/.4
$30$ $-14.1$ $73$ $-2.3$ $31$ $-30.8$ $73\epsilon$ $25.7$ $32$ $-6.0$ $74$ $-3.9$ $33$ $-42.5$ $75$ $-11.2$ $34$ $-18.7$ $76$ $14.2$ $35$ $-24.3$ $77$ $21.0$ $36$ $-1.3$ $78$ $15.8$ $37$ $-11.9$ $79$ $-15.4$ $38$ $-10.4$ $80$ $-40.1$ $39$ $23.2$ $81$ $-45.2$ $40$ $23.9$ $82$ $16.4$	29	8.5 14 1	7.	2 -33.0 2 33
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21	-14.1	1.	-2.3 
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21	-30.8	1.	25.7
33   -42.3   73   -11.2     34   -18.7   76   14.2     35   -24.3   77   21.0     36   -1.3   78   15.8     37   -11.9   79   -15.4     38   -10.4   80   -40.1     39   23.2   81   -45.2     40   23.9   82   16.4	32 22	-0.0	74	+ -3.9 5 11 0
34   -10.7   70   14.2     35   -24.3   77   21.0     36   -1.3   78   15.8     37   -11.9   79   -15.4     38   -10.4   80   -40.1     39   23.2   81   -45.2     40   23.9   82   16.4	23 24	-42.3	7.	5 -11.2 5 14.2
35   -24.3   77   21.0     36   -1.3   78   15.8     37   -11.9   79   -15.4     38   -10.4   80   -40.1     39   23.2   81   -45.2     40   23.9   82   16.4	34	-10.7	70	7 210
30   -11.5   78   13.8     37   -11.9   79   -15.4     38   -10.4   80   -40.1     39   23.2   81   -45.2     40   23.9   82   16.4	33 36	-24.5	7	/ 21.0 8 15 9
37   -11.9   79   -13.4     38   -10.4   80   -40.1     39   23.2   81   -45.2     40   23.9   82   16.4	27	-1.5	70	5 13.8 G 15.4
30 -10.4 60 -40.1   39 23.2 81 -45.2   40 23.9 82 16.4	31 20	-11.9	/	y -13.4
40 23.9 82 16.4	20 20	-10.4	0	J -40.1
то 23.7 02 10.4	39 40	23.2	ð. 04	1 -43.2 7 164
	40	23.9	δ.	2 10.4

 $\gamma \text{S-WT}$  RDCs. Related to Figure 1 and Table 1.

Res

83

84

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86

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99

100

101

102

104

105

106

107

108

109

110

111

112

113

114

115

117

118

119

120

121

122

123

124

125

126

D (Hz)

16.1

17.9

20.2

1.1

7.6

-28.0

14.3

-1.4

-9.9

12.0

-26.3

18.4

0.3

-27.9

-19.2

11.2

16.3

-27.6

-17.3

-20.3

20.3

-32.2

-10.9

-12.5

-11.8

-20.3

10.5

-6.9

5.7

-20.6

-36.7

-42.1

-26.7

-29.3

-2.1

-6.1

-21.0

-34.9

8.1

Res

127

129

130

134

 $137\epsilon$ 

138

139

140

141

142

144

145

146

147

148

150

151

152

156

158

159

161

162

163

 $163\epsilon$ 

164

165

166

167

169

170

171

172

173

174

175

176

177

178

D (Hz) -27.9

21.2

17.5

14.0

24.1

-22.7

-26.0

-22.7

-22.7

-34.0

-41.4

-40.2

-29.8

4.4

-1.1

3.6

-22.7

-22.7

-6.5

-23.4 -20.6

-35.4

-0.3

20.9

-4.1

-17.4

-14.0

3.5

5.1

-34.9

23.5

13.1

14.8

25.3

-5.9

3.2

9.6

-2.4

2.7

63 64 65

Res	D (Hz)	Res D (Hz)	Res D (Hz)	Res	D (Hz)
2	2.6	43 24.6	85 22.3	127	-33.8
3	3.4	44 24.4	86 1.4	128	-35.1
4	-7.7	45 8.4	87 7.2	129	21.1
5	-16.3	46 9.1	88 -22.2	130	14.4
6	15.1	47 14.9	90 25.0	134	1.2
7	18.4	$47\epsilon$ 17.9	91 -4.6	$137\epsilon$	14.3
8	24.1	48 21.0	92 -13.2	138	20.4
9	-13.4	49 1.5	93 13.5	139	-2.1
10	8.6	50 -21.7	94 11.2	140	-35.1
11	-23.1	51 -55.3	96 17.4	142	-20.3
12	-33.8	52 -34.7	97 -4.9	144	-45.4
13	10.6	54 -31.5	99 -29.4	145	-38.3
14	6.3	56 -49.1	100 -23.4	146	-46.4
16	-26.5	57 -34.5	101 13.0	147	-35.4
17	-14.1	58 1.6	102 16.5	148	-1.3
18	-25.1	61 2.8	104 -31.9	150	3.5
19	-25.5	62 35.8	105 -20.4	151	5.4
21	-45.7	64 -11.6	106 -25.0	159	-24.7
22	16.6	65 4.7	107 23.0	161	-25.8
23	22.1	66 17.5	108 -38.6	162	-41.4
24	14.1	67 2.8	109 -14.8	163	-2.3
25	-1.4	69 -20.3	110 -17.3	$163\epsilon$	23.4
26	-3.2	70 -19.9	111 10.7	164	-6.5
27	7.4	71 -41.4	112 6.6	165	-21.8
29	-30.3	72 -39.8	113 -19.4	166	-18.8
30	-33.8	73 -5.0	114 19.0	167	2.9
31	-30.1	$73\epsilon$ 28.4	115 -10.2	169	5.5
32	-5.1	74 10.6	117 3.3	170	-39.9
33	-44.0	75 -12.7	118 -27.4	171	-45.8
34	-17.9	77 24.0	119 -39.9	172	12.3
35	-39.3	78 14.3	120 22.1	173	16.7
36	-16.7	79 -19.5	121 -41.1	174	9.2
37	-36.7	80 -29.2	122 -33.3	175	-10.7
38	-35.4	81 -55.9	123 -14.9	176	5.0
39	26.4	82 18.9	124 -6.5	177	12.8
40	10.4	83 18.1	125 -25.1	178	-5.1
41	25.2	84 16.9	126 -43.5		

Table 2:  $\gamma$ S-G18V RDCs. Related to Figure 1 and Table 1.

## **Supplemental Experimental Procedures**

**Structure Calculations** The structures for  $\gamma$ S-WT and  $\gamma$ S-G18V were refined using the full NOE restraint set, residual dipolar couplings, TALOS+ generated dihedral restraints, H-N-HA derived 3J couplings, and hydrogen-bonding data from the H-D exchange experiments. 100 structures were calculated from an extended conformation using high-temperature torsion angle dynamics, a simulated annealing, followed by a cartesian space energy minimization. The average of the 10 lowest-energy structures was calculated and used as a starting structure for a final 200-structure calculation using a simulated annealing and Cartesian space energy minimization. The 20 lowest-energy structures were used as the final structure family. (Supplementary Figures S1 and S2, Table 1).

Structure families are shown for  $\gamma$ S-WT and  $\gamma$ S-G18V in Supplementary Figures S1 and S2. In the  $\gamma$ S-G18V structure, V18 does not swing out into the solvent, but instead remains buried within the surrounding loop. The first 17 residues of the structure are largely unaffected by the substitution, while the dihedral angles shift from  $\phi = 73.7$  °,  $\psi = -163.0$  ° for G18 to  $\phi = 129.8$  °,  $\psi = -134.3$  ° for V18. Consistent with the change in amide chemical shifts observed in  $\gamma$ S-G18V, the R19 backbone dihedral angles are substantially changed from their wild-type configurations. The presence of V18 shifts the R19 dihedral angles from  $\phi = -58.6^{\circ}$ ,  $\psi = -177.2^{\circ}$  to  $\phi = -110.5$ °,  $\psi = 17.9$ °. The 180° change in the  $\psi$  angle of R19 disrupts the R20–C23  $\beta$ -sheet and the disruption propagates down the backbone. The exact structural cause of the  $\psi$ -inversion may be from steric clashes between the V18 sidechain and R19 carbonyl or other effects early in the folding of  $\gamma$ S-crystallin from the presence of the highly hydrophobic V18. Additional structural calculations indicate that the V18 side chain remains buried even upon exclusion of all distance restraints to V18 (Supplementary Figure S3 (top)), and the adoption of a favorable backbone dihedral angle configuration would require the elimination of restraints from several surrounding residues, producing an extensive structural disruption to the surrounding loop region that is not supported by the NMR data (Supplementary Figure S3 (bottom)).

**Monomeric**  $\gamma$ **S-WT and**  $\gamma$ **S-G18V under NMR conditions** Samples of natural abundance wild-type  $\gamma$ S-crystallin ( $\gamma$ S-WT), and the variant  $\gamma$ S-G18V were prepared in the NMR sample buffers in concentrations comparable to those used in NMR experiments. Light scattering measurements performed on these samples (Supplementary Figure S4) indicated that both and  $\gamma$ S-G18V were monomeric under these buffer conditions.