Supplementary information for

Structure of a Highly Active Cephalopod S-crystallin Mutant: New Molecular Evidence for Evolution from an Active Enzyme into Lens-Refractive Protein

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References

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Extended Data Table 1

Summary of crystallographic information for octopus S-crystallin Q108F mutant

Data Collection	
Space group	$P6_422$
Cell dimensions	
a, *b*, *c* (Å)	114.3, 114.3, 63.9
α, β, γ (°)	90, 90, 120
Resolution[a] (Å)	30-2.35 (2.43-2.35)
R_{merge}[b] (%)	16.4 (53.2)
$I / \sigma I$	13.3 (4.0)
Completeness (%)	99.9 (100.0)
Redundancy	7.1 (7.2)
Refinement	
Number of reflections	10,177 (1,251)
R factor[c] (%)	19.5
Free *R* factor[d] (%)	25.0
Number of atoms	1,782
Protein	1,713
Ligand/ion	20/5
Water	44
B-factors	
Protein	24.7
Ligand/ion	23.5/28.7
Water	26.6
rmsd	
Bond length (Å)	0.009
Bond angles (°)	1.3
Ramachandran analysis[e] (%)	
Favored	91.4
Allowed	8.6
Disallowed	0

[a] The numbers in parentheses are for the highest-resolution shell.

[b] $R_{merge} = \sum_h \sum_i \left| I_{hi} - \langle I_h \rangle \right| / \sum_h \sum_i I_{hi}$, where I_{hi} is the integrated intensity of a given reflection and $\langle I_h \rangle$ is the mean intensity of multiple corresponding symmetry-related reflections.

[c] $R = \sum_h \left| F_h^o - F_h^c \right| / \sum_h F_h^o$, where F_h^o and F_h^c are the observed and calculated

structure factors, respectively.

[d] Free *R* is *R* calculated using a random 5% of data excluded from the refinement.

Extended Data Table 2

Primer used for making the S-crystallin mutants

Primer	Sequence (5' to 3')
R43K-F	CAGAATGGGACAGCATGAAAAACAAGATGCCATGTCA
R43F-R	TGACATGGCATCTTGTTTTTCATGCTGTCCCATTCTG
H49N-F	ATGAGAAACAAGATGCCATGTAACATGATGCCAATGTTGG
H49N-R	CCAACATTGGCATCATGTTACATGGCATCTTGTTTCTCAT
Q64A-F	AACAGAACCCAAATTCCCGCGAGTATGGCTATGGCCAG
Q64A-R	TGGCCATAGCCATACTCGCGGGAATTTGGGTTCTGTTG
L100F-F	CAGACTGCTTCTATGACATCTTTGACGATTACATGAGAA
L100F-R	TTCTCATGTAATCGTCAAAGATGTCATAGAAGCAGTCTG
D101A-F	TGCTTCTATGACATCTTGGCTGATTACATGAGAATGTAC
D101A-R	GTACATTCTCATGTAATCAGCCAAGATGTCATAGAAGCA
D101N-F	GCTTCTATGACATCTTGAACGATTACATGAGAATG
D101N-R	CATTCTCATGTAATCGTTCAAGATGTCATAGAAGC
M104V-F	GACATGTTGGACGATTACGTGAGAATGTACCAGGATG
M104V-R	CATCCTGGTACATTCTCACGTAATCGTCCAAGATGTC
Q108F-F	GACGATTACATGAGAATGTACTTCGATGGTAACTGCAGAATGATG
Q108F-R	CATCATTCTGCAGTTACCATCGAAGTACATTCTCATGTAATCGTC
C112G-F	ATGTACCAGGATGGTAACGGCAGAATGATGTTCCAGCGA
C112G-R	TCGCTGGAACATCATTCTGCCGTTACCATCCTGGTACAT
L100F/D101N-F	GACTGCTTCTATGACATCTTCAACGATTACATGAGAATGTAC
L100F/D101N-R	GTACATTCTCATGTAATCGTTGAAGATGTCATAGAAGCAGTC
M104V/Q108F-F	GACATCTTGGACGATTACGTGAGAATGTACTTCGATGGT
M104V/Q108F-R	ACCATCGAAGTACATTCTCACGTAATCGTCCAAGATGTC
L100F/D101N/M104V-F	GACTGCTTCTATGACATCTTCAACGATTACGTGAGAATGTAC
L100F/D101N/M104V-R	GTACATTCTCACGTAATCGTTGAAGATGTCATAGAAGCAGTC
Δloop(112-122)-F	ATGTACCAGGATGGTAACAGCAGCTCCTCTGAGAAG
Δloop(112-122)-R	CTTCTCAGAGGAGCTGCTGTTACCATCCTGGTACAT
Δloop(112-122)/Q108F-F	GATTACATGAGAATGTACTTCGATGGTAACAGCAGCTCC
Δloop(112-122)/Q108F-R	GGAGCTGCTGTTACCATCGAAGTACATTCTCATGTAATC

Extended Data Table 3

Steady-state kinetic parameters of S-crystallin mutants

Protein[a]	[Protein] (μM)	$K_{m,GSH}$ (mM)[c]	$K_{m,CDNB}$ (mM)[c]	k_{cat} (s^{-1})[c]
R43K	0.3	0.23 ± 0.07	1.3 ± 0.1	0.40 ± 0.01
H49N	0.5	0.13 ± 0.02	5.1 ± 1.8	0.60 ± 0.14
Q64A	5.4	6.3 ± 0.5	unsaturated	0.03 ± 0.003
L100F	0.07	2.5 ± 0.3	2.8 ± 0.4	3.7 ± 0.3
D101A	0.4	2.6 ± 0.5	2.2 ± 0.6	1.2 ± 0.2
D101N	0.5	0.4 ± 0.04	2.5 ± 0.6	0.41 ± 0.05
M104V	0.09	1.1 ± 0.1	0.65 ± 0.07	2.3 ± 0.1
Q108F	0.02	8.2 ± 0.8	unsaturated	10.6 ± 0.6
C112G	1.7	0.02 ± 0.002	unsaturated	0.08 ± 0.002
L100F/Q108F	0.02	3.7 ± 0.5	1.4 ± 0.2	10.8 ± 0.5
D101A/Q108F	0.03	4.1 ± 0.5	5.3 ± 1.2	24.3 ± 3.5
D101N/Q108F	0.01	5.1 ± 0.5	0.67 ± 0.11	13.4 ± 0.6
M104V/Q108F	0.02	10.0 ± 1.6	4.1 ± 0.7	11.6 ± 1.1
L100F/M104V/Q108F	0.02	3.7 ± 0.4	2.4 ± 0.3	8.5 ± 0.5
Δloop[b]	0.6	2.2 ± 0.2	3.3 ± 0.6	0.38 ± 0.04
Δloop/L100F	0.07	5.7 ± 0.9	4.5 ± 1.0	2.0 ± 0.3
Δloop/D101N	1.1	5.3 ± 0.6	3.9 ± 0.7	0.4 ± 0.04
Δloop/M104V	0.7	6.1 ± 1.3	2.3 ± 0.5	0.32 ± 0.02
Δloop/Q108F	0.05	6.2 ± 0.9	1.9 ± 0.5	5.2 ± 0.4

[a] For GSH titration, the concentration of CDNB was 3.5 mM. For CDNB titration, the concentration of GSH was 10 mM.

[b] The loop between α4 and α5 helices (residue 112-122) was deleted and is designated as Δloop.

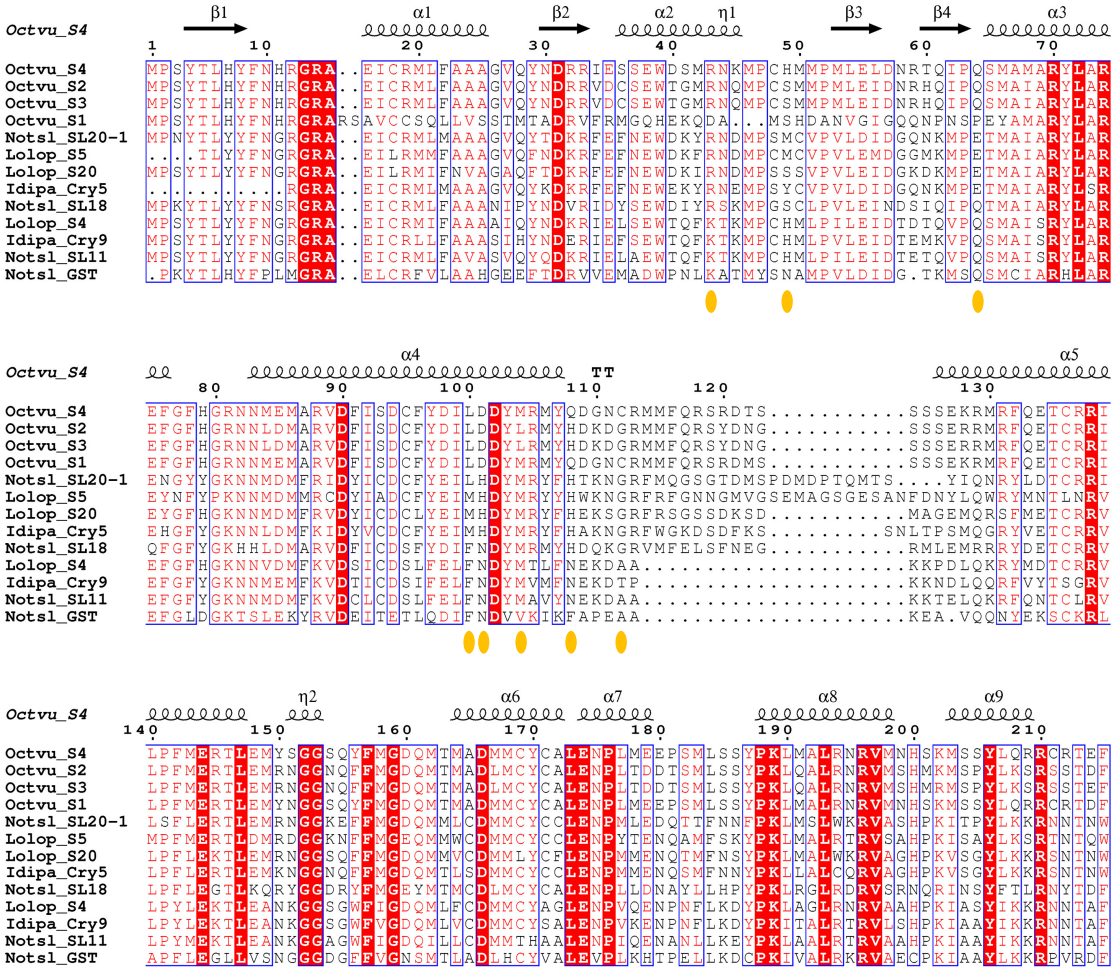
[c] Data were fitted to the Michaelis-Menten equation and the R_{sqr} values were 0.980 to 0.999, respectively. All the assays were repeated at least twice to ensure reproducibility.

Extended Data Table 4

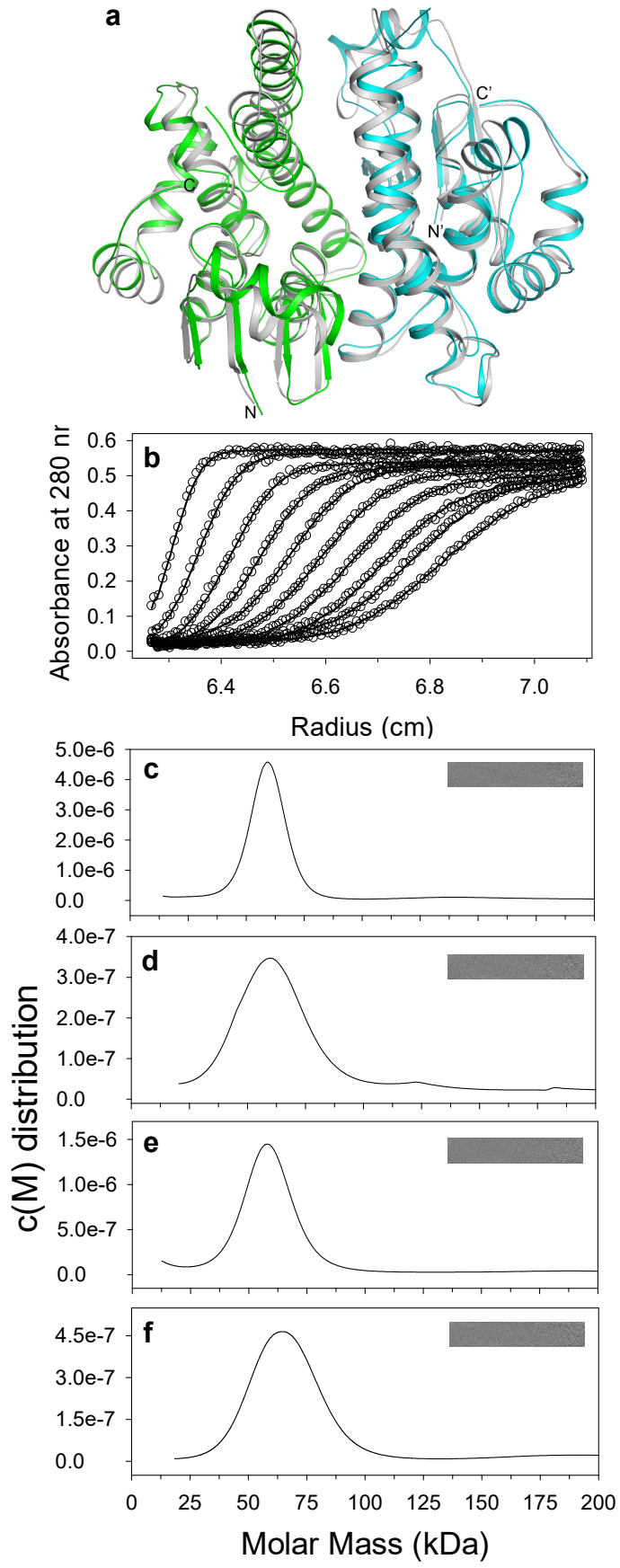
Thermal stability of S-crystallin mutants with or without 1 mM GSH

Protein[a]	T_m (ºC)	T_m in GSH (ºC)	ΔT_m1 (mutant-WT)	ΔT_m2 (in GSH-apoform)
L100F	42.8 ± 1.7	48.1 ± 2.6	-5.4	5.3
D101N	45.5 ± 1.1	48.8 ± 1.4	-2.7	3.3
M104V	45.1 ± 1.2	47.4 ± 1.9	-3.1	2.3
Q108F	49.1 ± 0.7	48.8 ± 1.4	0.9	-0.3
C112G	50.7 ± 0.7	58.2 ± 2.2	2.5	7.5
L100F/Q108F	43.7 ± 0.9	43.6 ± 1.3	-4.5	-0.1
D101N/Q108F	45.2 ± 1.3	46.3 ± 1.4	-3.0	1.1
M104V/Q108F	44.1 ± 1.7	44.8 ± 1.7	-4.1	0.7
L100F/M104V/Q108F	41.1 ± 0.6	43.3 ± 1.8	-7.1	2.1
Δloop	46.9 ± 0.9	46.5 ± 1.4	-1.3	-0.4
Δloop/L100F	45.5 ± 1.3	45.2 ± 1.5	-2.7	-0.3
Δloop/D101N	43.1 ± 1.2	45.4 ± 1.5	-5.1	2.3
Δloop/M104V	45.9 ± 0.8	46.7 ± 1.9	-2.3	0.8
Δloop/Q108F	43.5 ± 0.9	43.1 ± 1.8	-4.7	-0.4

[a] The protein concentration was at 7.2 μM. The ellipticity at 222 nm was monitored at varying temperature ranging from 25 to 85ºC. The results were fitted to the two-state unfolding model to calculate T_m of S-crystallin.

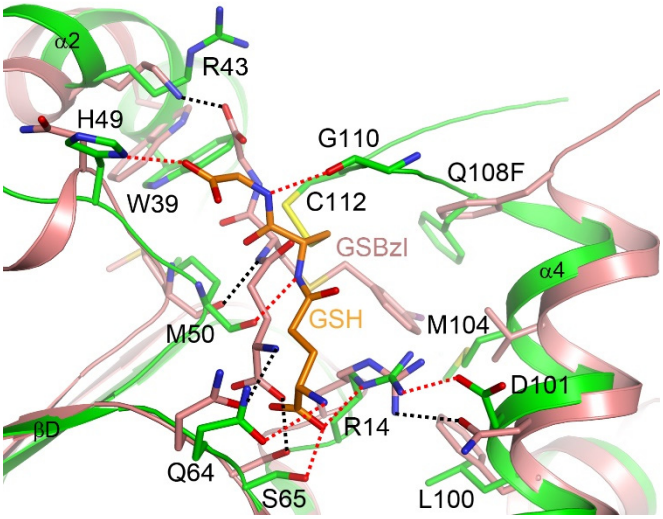


Extended Data Fig. 1. Sequence alignment of S-crystallins and GST-σ from cephalopods. Modified from an output from ESPript[1]. For comparison, Octvu_S4 shares 52-82% amino acid sequence identity with Octvu_S2 (accession number: P27014), S3 (Q25626), S1 (P27013), Notsl_SL20-1 (P18425), Lolop_S5 (Q25359), Lolop_S20 (Q25371), Idipa_Cry5 (A0A0H5ANU6), Lolop_S4 (Q25357), Notsl_SL18 (P27016; residues 1-120 and 214-308 were used), Idipa_Cry9 (A0A0H5ARE1), Notsl_SL11 (P18426) and 38% amino acid sequence identity with Notsl_GST (1GSQ_A), respectively. Orange ovals indicate some important residues in the active site that have been mutated in the present studies.

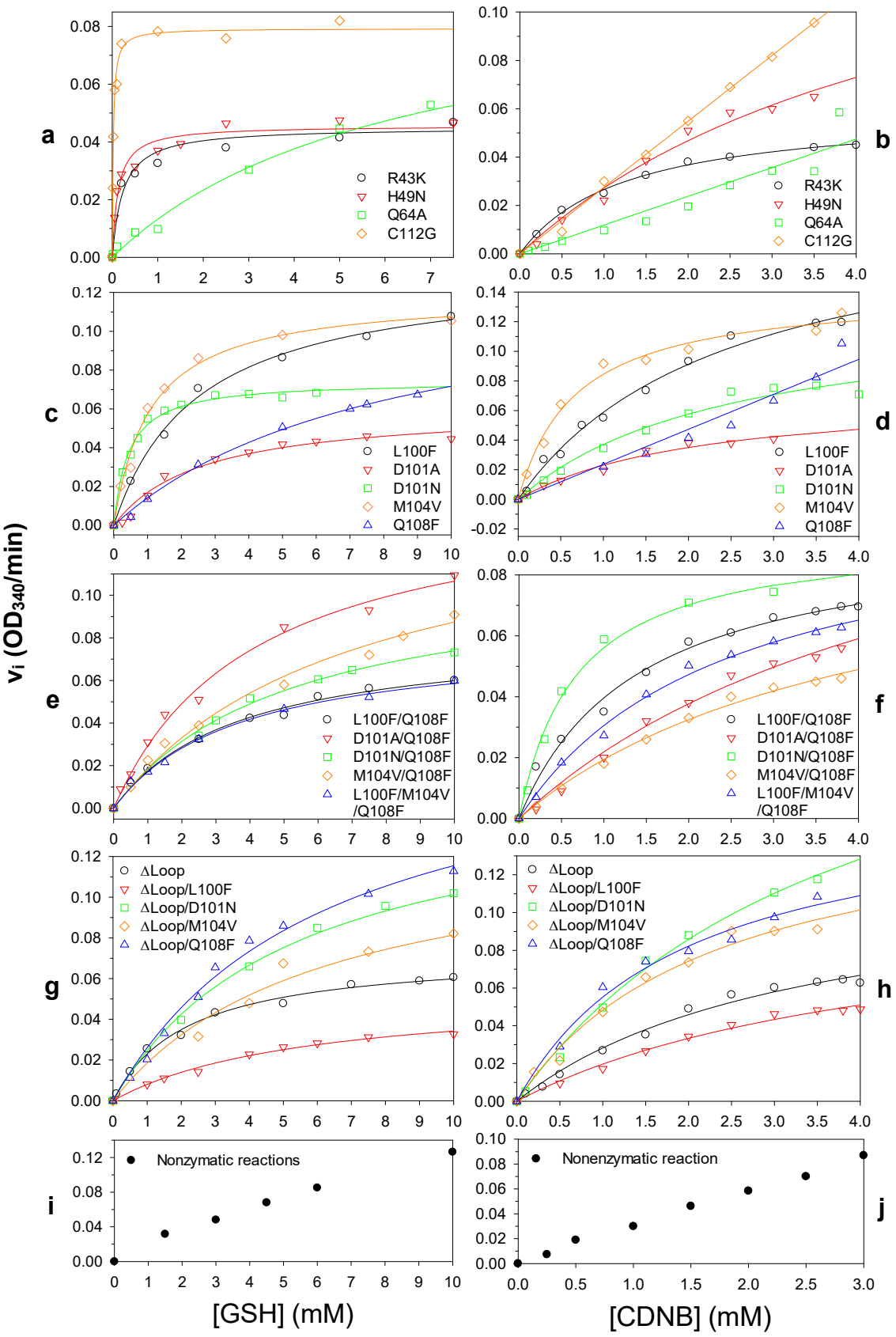


Extended Data Fig. 2. The octopus S-crystallin forms a stable dimer. (a) Overlay of

the dimeric S-crystallin and GST-σ. The dimers show similar orientation. (b) Traces of absorbance at 280 nm of the S-crystallin in 100 mM phosphate buffer (pH 6.5) during the sedimentation-velocity experiment[2-4] by analytical ultracentrifugation. The protein concentration was 1 mg/ml. For clarity, only every two scan is shown. The circles represent experimental data and the lines are the results after fitting to the Lamm equation using SEDFIT[5]. (c-f) The continuous c(M) distribution of S-crystallin and its mutants, L100F/D101N, ΔLoop/L100F/D101N and L100F/D101N/M104V/Q108F, all show a major species located at ~60 kDa. It indicates that it is a dimer as the molar mass of monomeric S-crystallin is 28 kDa. The residual bitmaps of the raw data and the best-fit result are shown as the gray bar. The protein concentration for the distribution analysis was 0.025 mg/ml (0.9 μM).

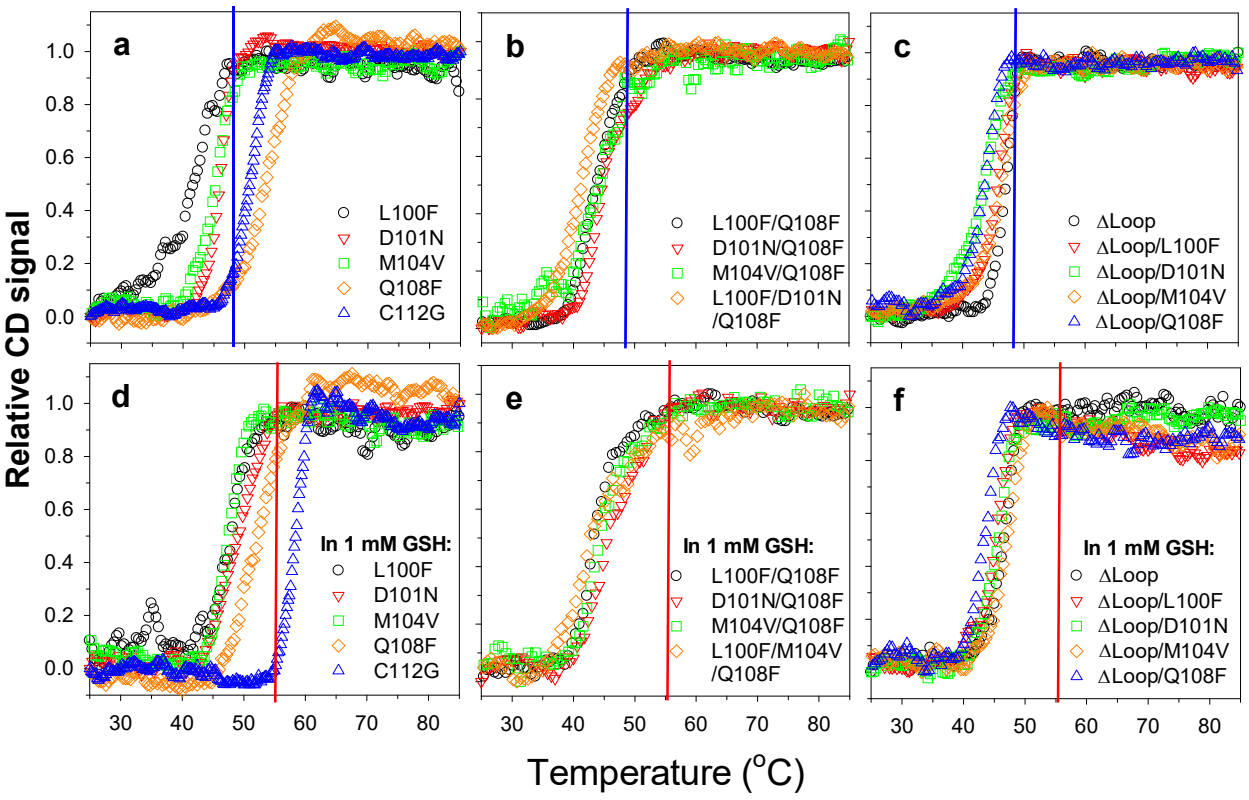


Extended Data Fig. 3. Overlay of the active sites of octopus S-crystallin Q108F mutant in complex with GSH (colored by green) and GST-σ in complex with S-(3-iodobenzyl) glutathione (GSBzI) (colored by salmon)[6]. The iodobenzyl ring of GSBzI is located in the hydrophobic pocket consisted by the residues Phe98, Val102 and Phe106 of GST-σ, while the equivalent residues in S-crystallin are Leu100, Met104 and Gln108, respectively.

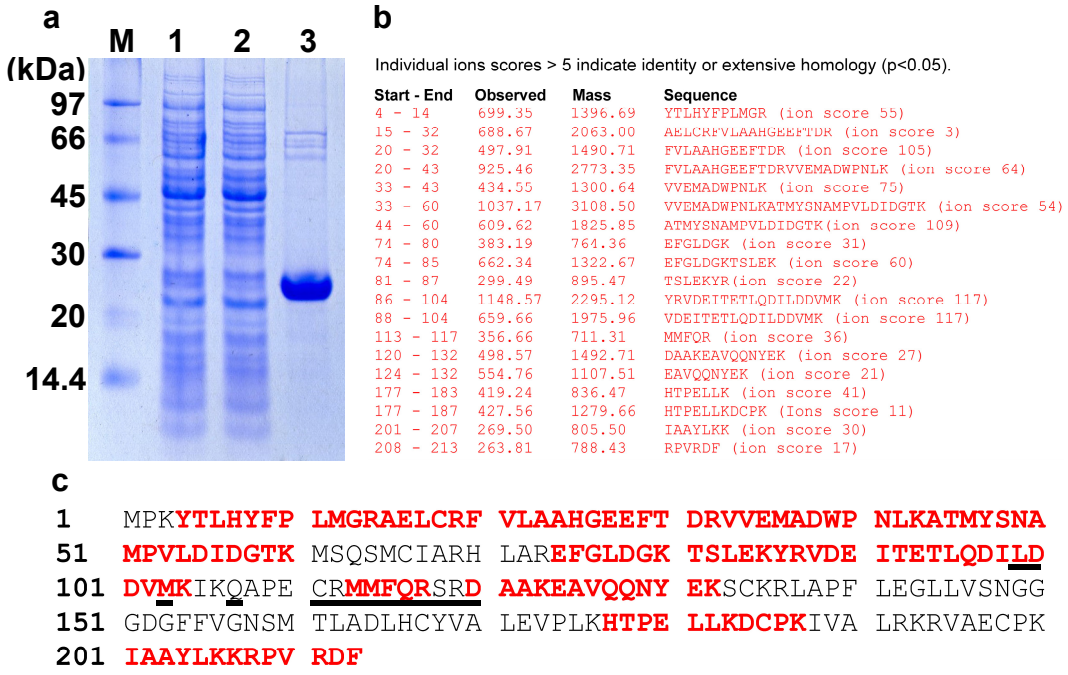


Extended Data Fig. 4. The GST activity of various S-crystallin mutants. Plots of the initial velocity as a function of the concentration of the two substrates GSH (a, c, e, g) and CDNB (b, d, f, h) for S-crystallin mutants and nonenzymatic reactions (i, j) were

shown, respectively. The solid lines are the best-fit by the Michaelis-Menten equation. The kinetic parameters such as K_m and k_{cat} from the best fit were shown in Table 1 and Extended data Table 3.



Extended Data Fig. 5. Thermal stability of S-crystallin mutants without (a-c) and with (d-f) 1 mM GSH by circular dichroism spectroscopy. Plot of the relative CD signal from the ellipticity at 222 nm as a function of the temperature for the S-crystallin mutants, respectively. The protein concentration was at 7.2 μM. The results were fitted to calculate the T_m, which are shown in Table 2. For comparison, the blue and red lines showed the T_m of wild-type S-crystallin without and with 1 mM GSH, respectively.



Extended Data Fig. 6. Expression and purification of recombinant "S-crystallin"-like GST. (a) Protein identification by SDS-PAGE. M: molecular marker. Lane 1-3: cytoplasmic fraction, flow through, elution from the nickel affinity column. (b-c) Protein sequence identification by mass spectrometry. The protein was digested by trypsin and then analyzed by LC-MS/MS spectrometry. There are 19 matched peptides observed (b) and 61% sequence coverage are shown in bold red (c). Underlines show the point mutations and loop insertion.