Supporting Information

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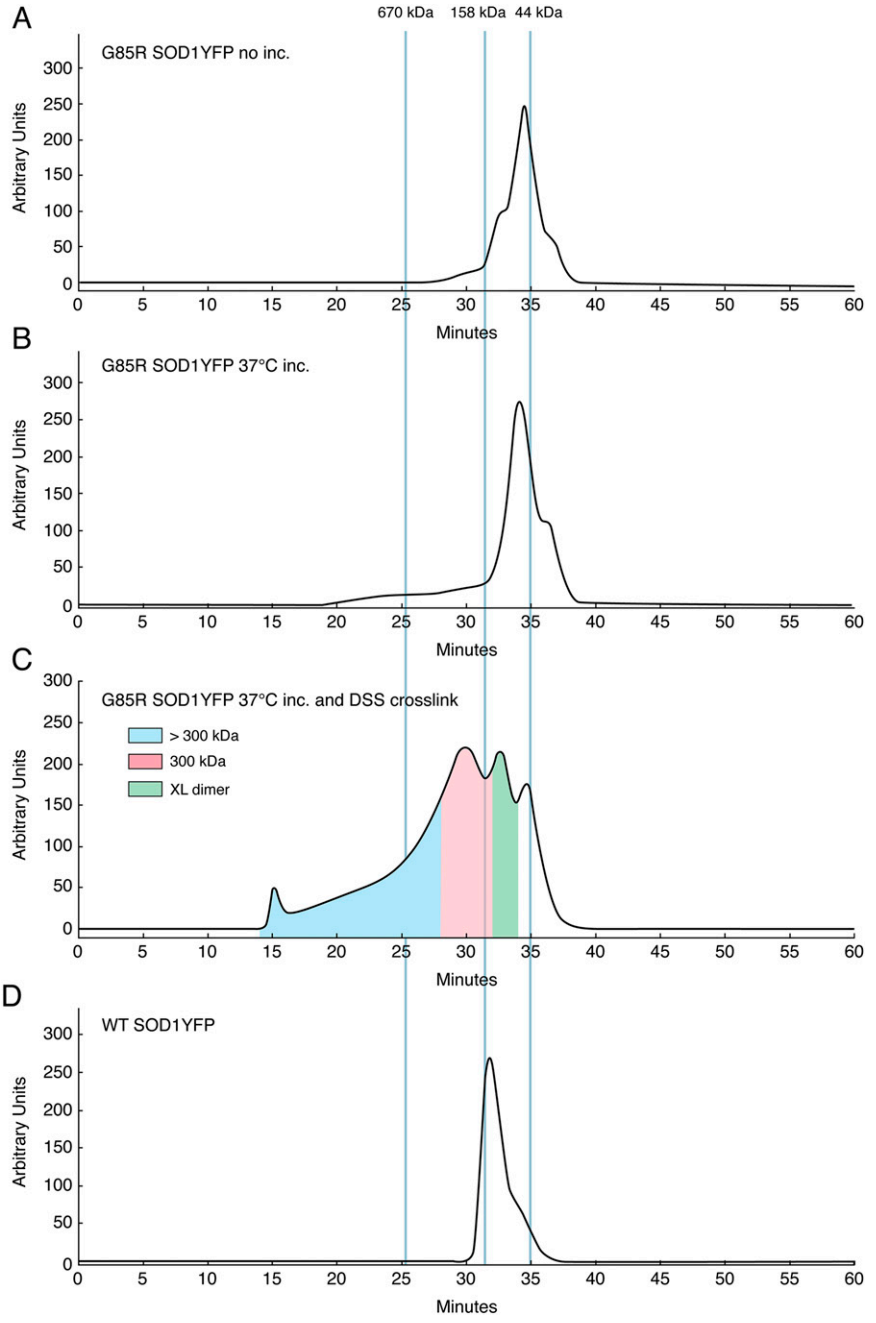

Fig. S1. Gel filtration profiles (Superose 6), monitored by yellow fluorescent protein (YFP) fluorescence, of purified G85R superoxide dismutase 1 (SOD1)YFP, (*A*) without incubation; (*B*) incubated at 37 °C for 24 h; (*C*) incubated at 37 °C for 24 h and then disuccinimidyl suberate (DSS) crosslinked; and of WT SOD1YFP, (*D*) without incubation. The profile of WT SOD1YFP incubated at 37 °C for 24 h did not differ from that shown in *D*. The elution positions of protein size markers are indicated by the vertical lines: thyroglobulin (670 kDa), γ-globulin (158 kDa), and ovalbumin (44 kDa). The size of the latter marker corresponds closely to the size of SOD1YFP monomer (43.5 kDa). Late-eluting species in the top two traces correspond to degradation products, which are essentially free YFP, as indicated by SDS/PAGE and immunodetection on Western blots with anti-YFP and anti-SOD1 antibodies. Note that 37 °C incubation (without cross-linking) reproducibly shifts the G85R SOD1YFP peak toward earlier elution and also produces a broad "left shoulder" of early-eluting material that begins at fraction 20.

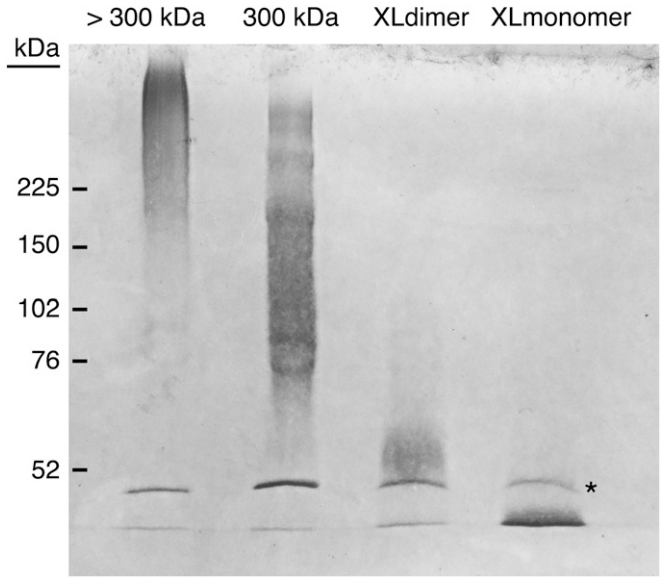

Fig. S2. Analysis of DSS-crosslinked (XL) G85R superoxide dismutase 1 (SOD1)YFP gel filtration fractions by SDS/PAGE and Coomassie staining. Indicated gel filtration fractions were applied without boiling to a 6% polyacrylamide SDS gel. Size markers are indicated at the left. Note that migration positions of the XL material are relatively broad and, in general, the material appears to migrate more rapidly than corresponding marker proteins, presumably related to hydrodynamic shape. For example, the XL monomer migrates both behind and at the dye front in this gel. XL dimer appears to migrate more slowly than the 52-kDa marker. The sharp Coomassie-stained species marked by an asterisk is not fluorescent when assessed by PhosphorImager analysis, compared with the smeared species, and is thus not an SOD1-YFP species.

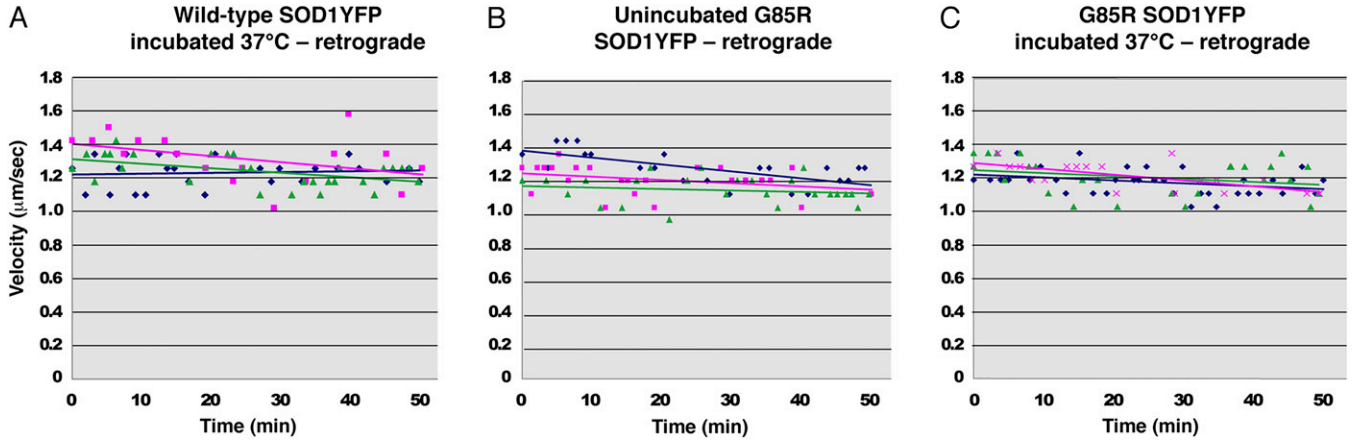

Fig. S3. Retrograde transport is not significantly affected by addition of WT superoxide dismutase 1 (SOD1)YFP or G85R SOD1YFP. Incubations were carried out as described in Fig. 1, and retrograde transport was measured by video-enhanced contrast DIC microscopy. These measurements were acquired simultaneously with those of anterograde transport shown in Fig. 1. (*A*) WT SOD1YFP, incubated 24 h at 37 °C before addition; (*B*) G85R SOD1YFP, not incubated before addition; and (*C*) G85R SOD1YFP incubated 24 h at 37 °C before addition. Three separate experiments for each condition are shown; lines are linear least-squares fits to the correspondingly colored data points.

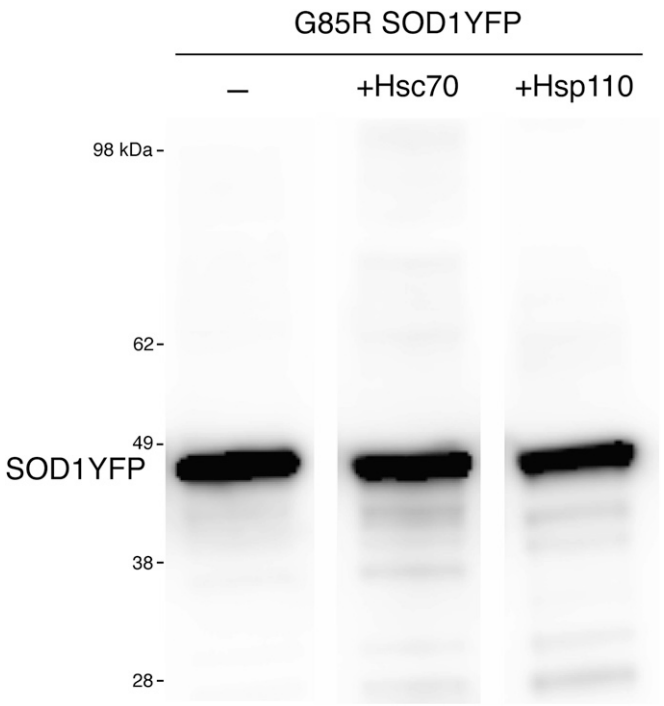

Fig. S4. Chaperone additions do not affect the levels of intact G85R superoxide dismutase 1 (SOD1)YFP during incubation with the squid axoplasm. Western blot analysis was carried out on identical aliquots of squid axoplasm incubated with the additives indicated, probed with anti-YFP antiserum. Chaperone additions do not lead to appreciable degradation of the input G85R SOD1YFP.

Table S1. Affinity capture of axoplasm proteins by WT SOD1YFP and G85R SOD1YFP

	Spectral counts[†]	
Protein*	WT-captured	G85R-captured
Hsc(p)70-71[‡]	19	97
Hsc(p)70-72[‡]	21	109
DnaJA1	16	57
DnaJA 2	14	30
TCP1zeta	2	12
TCP1delta	0	12
TCP1gamma	0	5
Hsp90	0	10
β-actin	6	42
α-tubulin (4 isoforms)[§]	38	202
β-tubulin (4 isoforms)[§]	54	190
Tropomyosin	3	10
α-spectrin	15	101
β-spectrin	6	62
Neurofilament, light chain	28	203
Hu-li-tai shao (adducin)	3	49
Dystonin	3	60
Discs large protein	2	12
Ankyrin2	3	17
Stathmin	10	27
Doublecortin domain containing 2	17	42
Sperm surface protein Sp17 (obscurin)	0	42
Formin-binding protein	0	12
Microtubule-associated futsch	2	15
Synapse-associated protein 1	13	37
Complexin	7	15
Synapsin	13	77
Syntaxin-binding protein 5	0	7
Endophilin B	18	42
Cytoplasmic Cu/Zn SOD	9	32
Copper chaperone for SOD	0	25
Kinesin heavy chain	10	40
Dynein heavy chain	12	42
Calpain 3	2	20
Calpain 9	0	12

Note that some of these interactions may be indirect (e.g., through binding of SOD1 to organelles or the cytoskeleton). For example, direct interaction between kinesins and SOD1 has not been detected (Morfini and Brady, unpublished results).

*Proteins were identified after MS by homology searching with BLAST+ (National Center for Biotechnology Information) (*Methods*). The "best hits" for the most prominent species that were different between the WT and mutant SOD1YFP affinity-captured samples are shown.

[†]Spectral counts for the WT capture are the raw counts from the Sequest analysis of the MS data. For the G85R capture, the raw counts have been normalized by a factor of 2.5 to account for the lower recovery of SOD1YFP in that sample.

[‡]Two distinct Hsc(p)70 isoforms are present, but it was not possible to assign one or the other as Hsp70 rather than Hsc70.

[§]Multiple isoforms of α- and β-tubulin are present as indicated, but it was not possible to assign them unequivocally.

Table S2. Proteins affinity-captured from axoplasm incubated with G85R SOD1YFP and not from axoplasm incubated with both G85R and Hsp110

Protein*	G85R-captured spectral counts†
Intermediate filament protein	10
Myosin heavy chain B	10
Neurofilament protein	7
Sperm surface protein 17 (obscurin)	42
Formin-binding protein 1	12
Ankyrin 1	5
SH3 domain-containing adapter protein	18
Coronin-2B	7
LIM domain-containing protein	10
Spartin	5
Syntaxin-binding protein 5	7
Synapse-associated protein	13
Clathrin assembly protein AP180	5
Endophilin-B1	42
Ca^{2+}-dependent secretion activator	18
Reticulon-4	10
DnaJC7	5
Copper chaperone for SOD1	25
ELAV2-like	13

In the affinity capture experiment from axoplasm with both G85R SOD1YFP and added Hsp110, human Hsp110 (93 spectral counts reflecting 51% sequence coverage) was recovered along with G85R SOD1YFP (1273 spectral counts, 82.5% coverage).

*Proteins were identified after MS as in Table S1. The significant best hits that were present in axoplasm incubated with G85R SOD1YFP and not present when incubation was with both G85R and Hsp110 are listed.

†Raw spectral counts for G85R have been normalized as in Table S1 to reflect the different recovery of SOD1YFP in the two experiments.