Figure S1

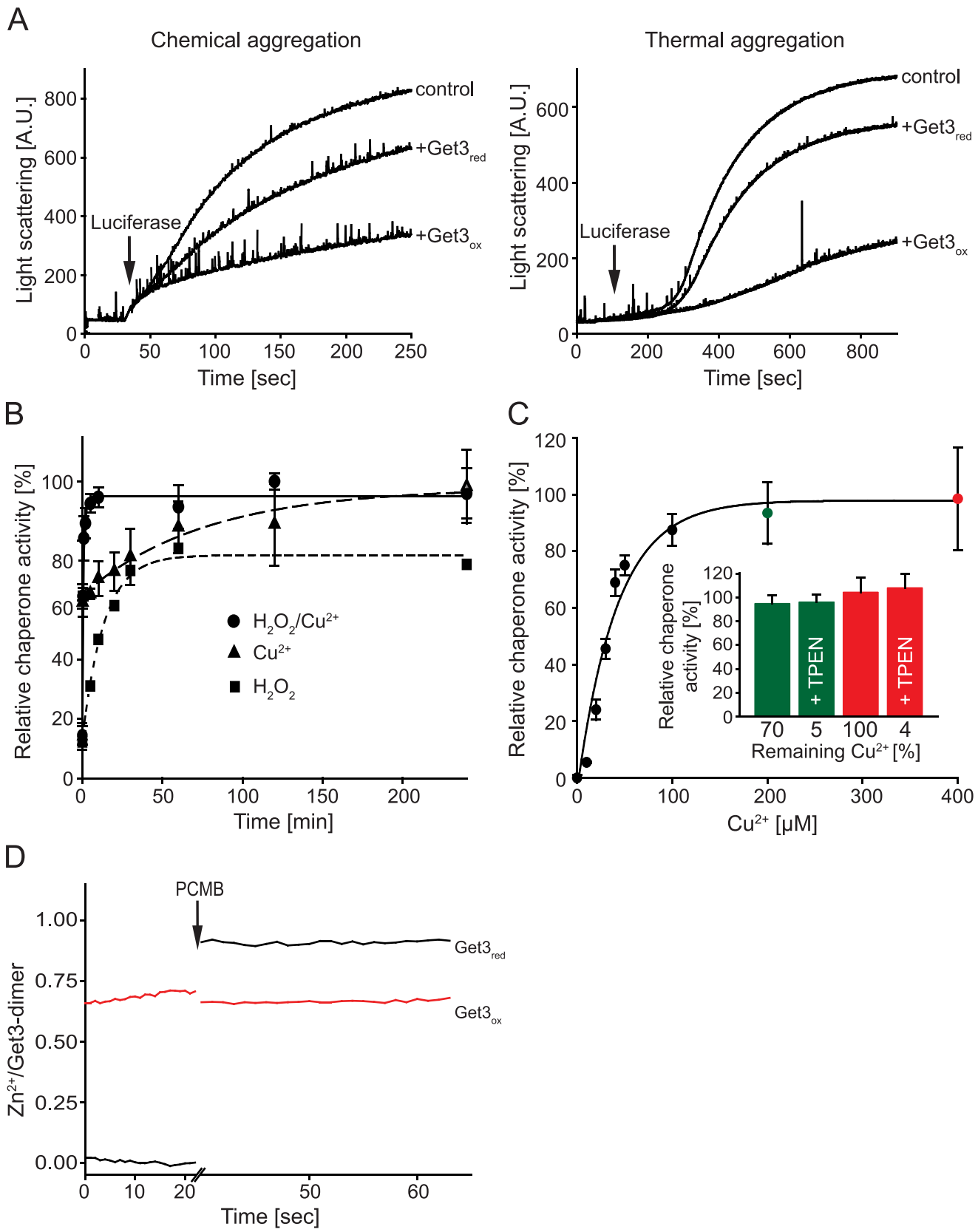
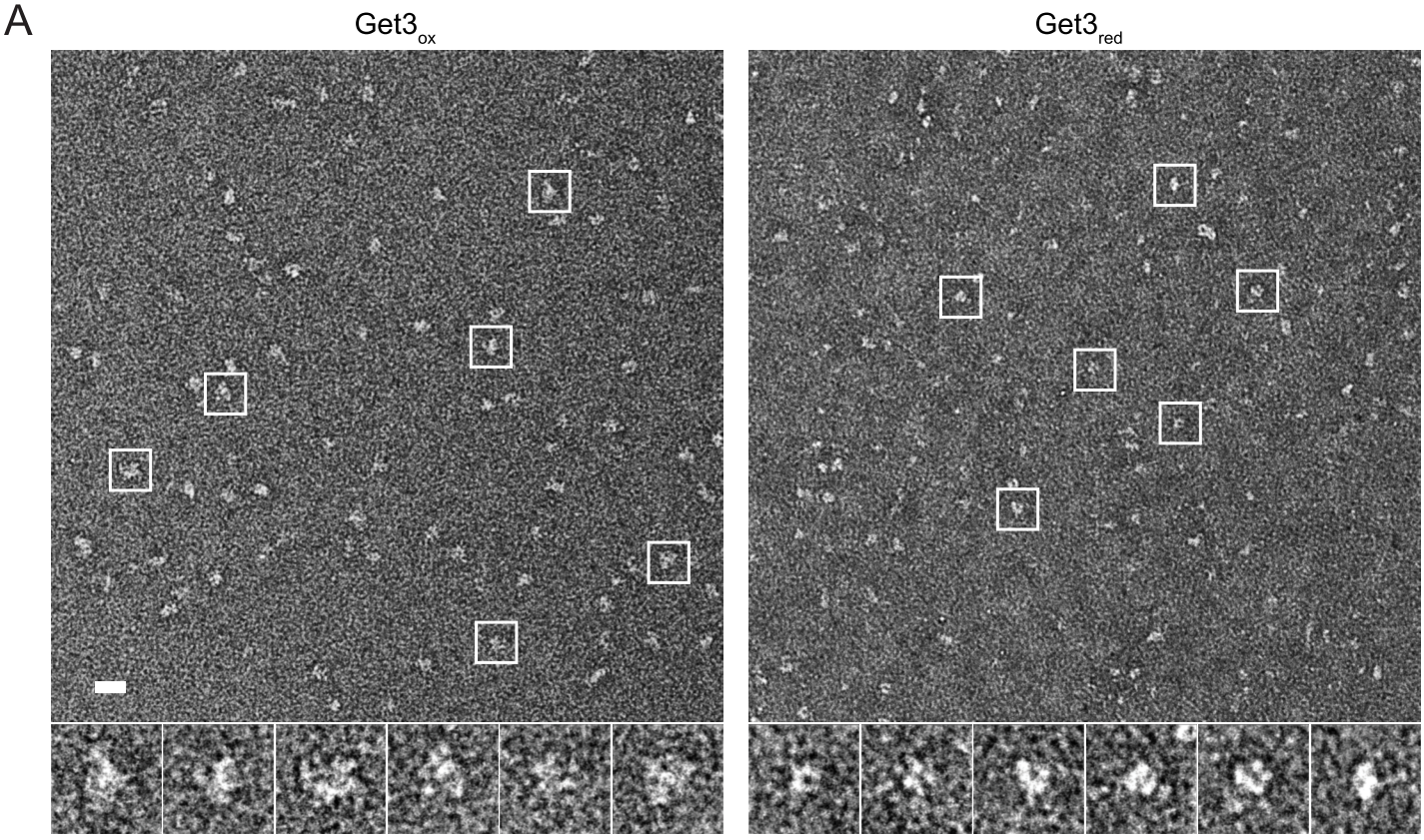
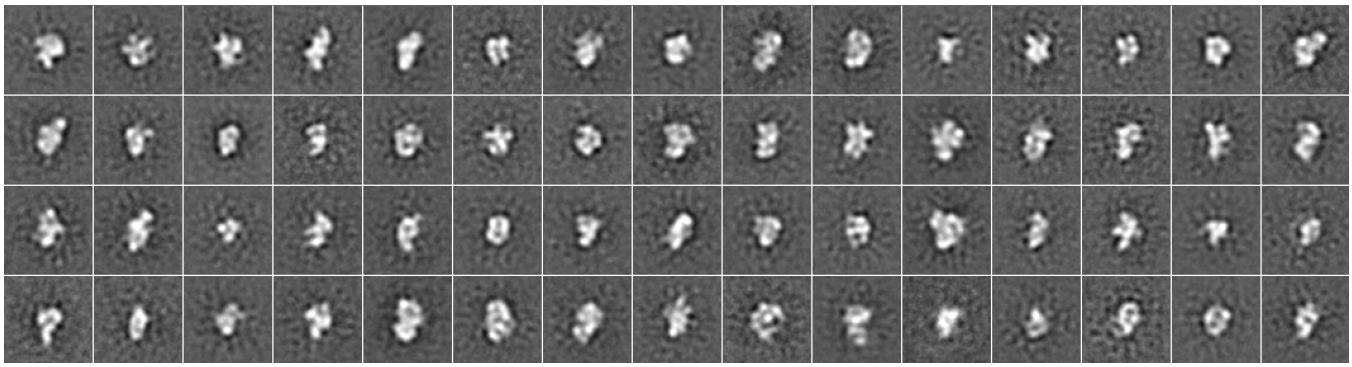

A
Chemical aggregation
Thermal aggregation
Light scattering [A.U.]
Time [sec]
control
+Get3red
+Get3ox
Luciferase
B
Relative chaperone activity [%]
Time [min]
H2O2/Cu2+
Cu2+
H2O2
C
Relative chaperone activity [%]
Cu2+ [μM]
+ TPEN
Remaining Cu2+ [%]
D
PCMB
Zn2+/Get3-dimer
Get3red
Get3ox
Time [sec]

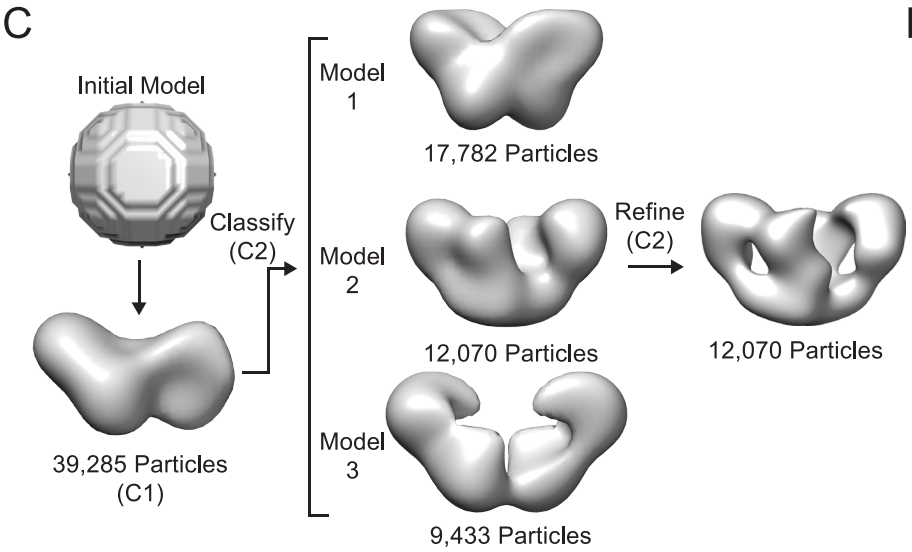
Figure S1 (associated with Figure 1). Get3 functions as chaperone when oxidized. **A.** Effect of $Get3_{red}$ or $Get3_{ox}$ on the light scattering of chemically denatured luciferase (Get3:luciferase ratio 8:1) or thermally unfolding luciferase at 43°C (Get3:luciferase ratio 1:1). Light scattering of luciferase in the absence of added chaperones is shown (control). **B.** The chaperone function of 0.3 µM $Get3_{red}$ before and at defined time points after incubation in either 2 mM H_2O_2/50 µM Cu^{2+} (circles), 50 µm Cu^{2+} (triangles) or 2 mM H_2O_2 (squares) at 37°C was determined by analyzing the influence of Get3 on the aggregation of 0.075 µM chemically denatured citrate synthase (CS). The light scattering signal of CS in the absence of added chaperones was set to 0% chaperone activity, whereas the light scattering signal in the presence of fully oxidized Get3 was set to 100%. **C.** The chaperone function of 0.3 µM $Get3_{red}$ before and 4 min after addition of various concentrations of copper at 37°C was analyzed as described. Insert: $Get3_{red}$ that was incubated in either 200 µm $CuCl_2$ (green bars) or 400 µM $CuCl_2$ (red bars) for 4 min was either incubated with a 100-fold excess of the copper chelator TPEN for 10 min or left untreated. The samples were then loaded onto gelfiltration columns equilibrated with metal-free buffer and tested for their remaining copper content by ICP analysis. The same samples were tested for their chaperone activity as described above. At least 3-6 replicates were performed and the standard error is shown. **D.** Oxidation of Get3 causes zinc release. Reduced and oxidized Get3 (2 µM) were incubated in the presence of 100 µM 4-(2-pyridylazo)resorcinol (PAR), which forms bright red complexes with zinc. PAR is unable to compete with high affinity sites, like cysteine-coordinating zinc centers for zinc binding. Addition of parachloromercuri-benzoic acid (PAR/PCMB) leads to thiol-mercaptide bond formation which releases

cysteine-coordinated metal that now interacts with PAR. Signal from surface-attached metal was subtracted from the baseline.

Figure S2


A
Get3ox
Get3red


B


C
Initial Model
Classify
(C2)
39,285 Particles
(C1)
Model
1
17,782 Particles
Model
2
12,070 Particles
Refine
(C2)
12,070 Particles
Model
3
9,433 Particles

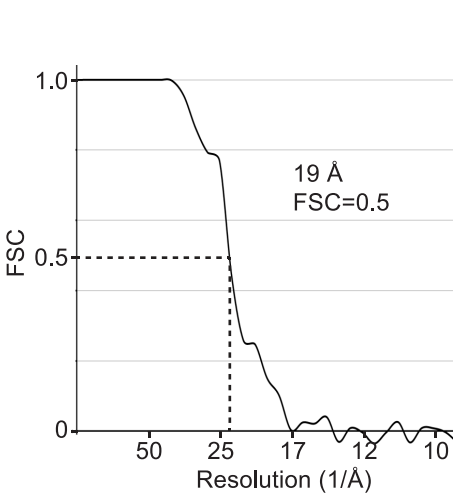

D
1.0
0.5
0
FSC
19 Å
FSC=0.5
50
25
17
12
10
Resolution (1/Å)

Figure S2 (associated with Figure 3). Negative-stain EM and 3D reconstruction.

A. Representative micrograph images and selected particles of $Get3_{ox}$ (left panel) and $Get3_{red}$ (right panel) are shown. Scale bar equals 200 Å and the box size is 260 Å. **B.** Representative reference-free class averages of $Get3_{ox}$, generated using SPIDER (Frank et al., 1996). **C.** 3D reconstruction method and **D.** gold-standard Fourier Shell Correlation (FSC) curve of the final refinement showing a 19Å resolution at an FSC=0.5 criterion, performed using RELION (Scheres, 2012).

Figure S3

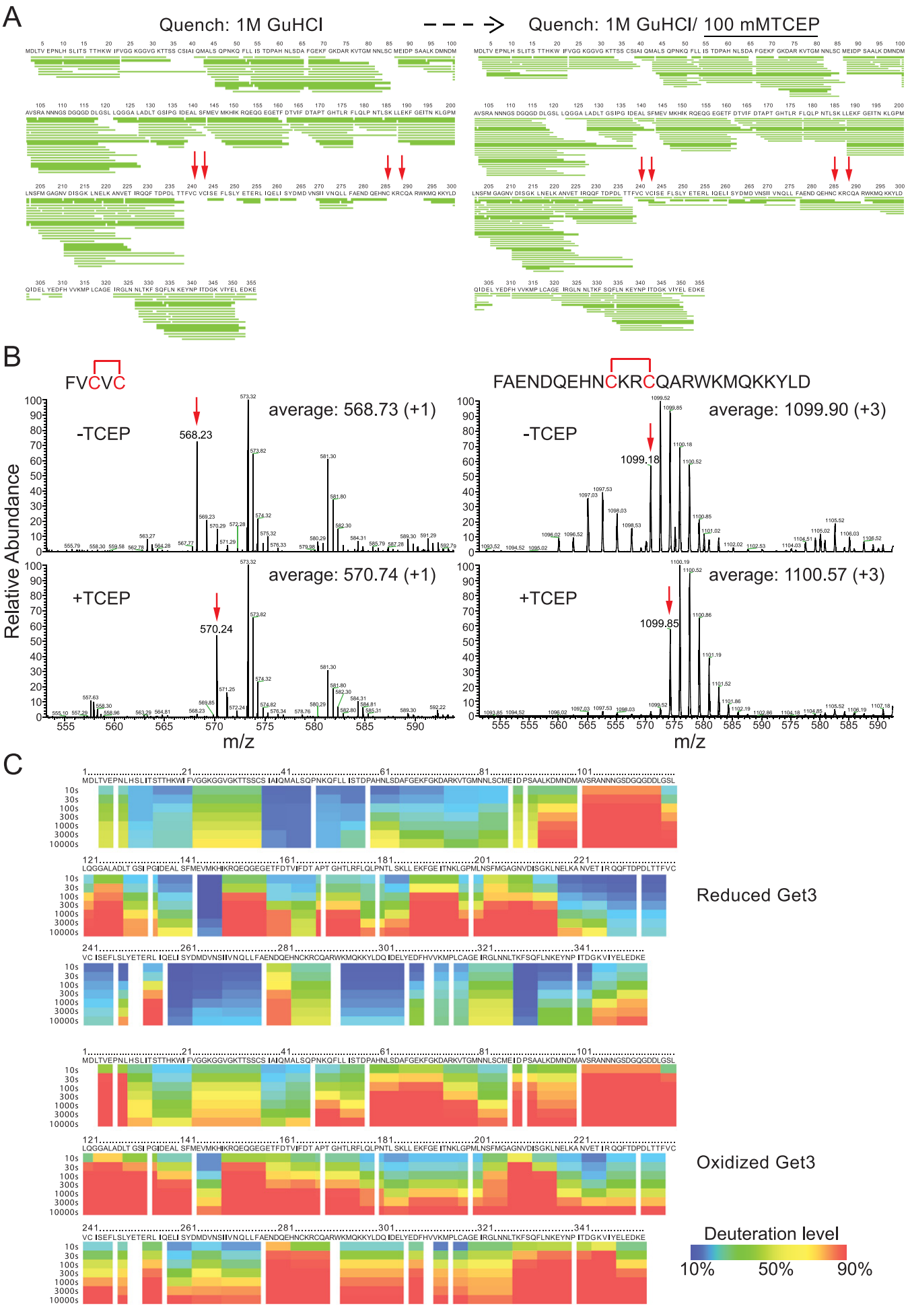
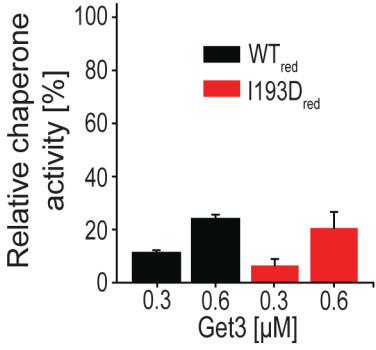

A
Quench: 1M GuHCl
Quench: 1M GuHCl/ 100 mMTCEP
B
FVCVC
FAENDQEHNCKRCQARWKMQKKYLD
-TCEP
+TCEP
average: 568.73 (+1)
average: 570.74 (+1)
average: 1099.90 (+3)
average: 1100.57 (+3)
568.23
570.24
1099.18
1099.85
Relative Abundance
m/z
C
Reduced Get3
Oxidized Get3
Deuteration level
10%
50%
90%

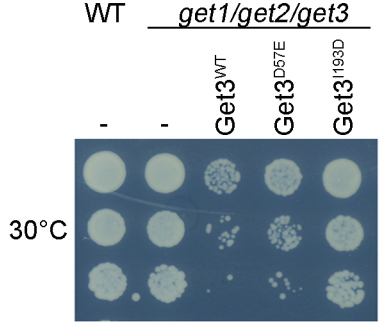
Figure S3 (associated with Figure 4). Oxidative stress mediated disulfide bond formation and conformational rearrangements of Get3. A. Pepsin digest of $Get3_{ox}$ in the absence of reducing agents revealed significant differences in the patterns of proteolytic cleavage surrounding the two pairs of conserved cysteines (red arrows). However, quenching in the presence of 100 mM TCEP, a thiol reductant, reconstituted the cleavage pattern of $Get3_{red}$. **B.** MS spectra for Get3 peptides containing the CXC- and CXXC-motif with and without TCEP reveal disulfide bond formation within the peptide chain. The calculated mass of the first monoisotopic peaks (red arrow) in both peptides shows a mass increase of ~ 2 Da upon addition of the reducing agent TCEP, consistent with the reduction of one disulfide bond. **C.** Deuterium incorporation into peptides of $Get3_{red}$ and $Get3_{ox}$ after quenching and pepsin digest in the presence of 100 mM TCEP. The deuteration level was calculated as described in the experimental procedures.

Figure S4

A



B



C

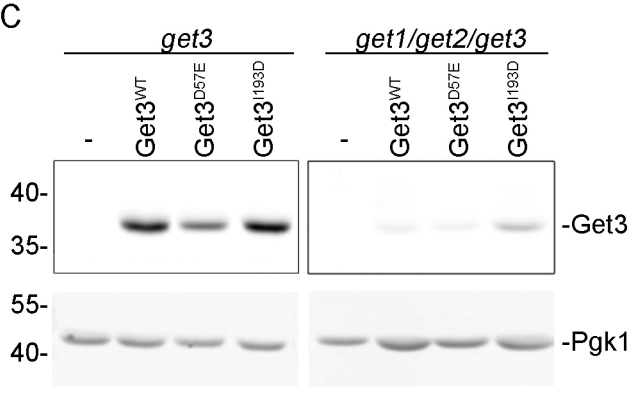


Figure S4 (associated with Figure 5 and 6). TA-binding function and chaperone function can be separated *in vivo*. A. Both wild-type Get3 and the tail-anchored binding deficient mutant Get3I193D were prepared in their reduced form and tested for their influence on the aggregation behavior of chemically denatured CS (see figure legend 1 for details). **B.** Serial dilutions of *get1/get2/get3* cells, transformed with constructs containing the coding sequence of Get3 variants, were spotted and grown at 30°C. **C.** *get3* and *get1/get2/get3* cells were transformed with constructs containing the coding sequence of wt or mutant Get3 variants. Protein lysates were analyzed by immunoblot using a Get3 specific serum. Pgk1 was used as loading control.

Supplemental Table S1 (associated with Figure 4)

Supplemental Table S2 (associated with Figure 5 and 6)

***E. coli* and *S. cerevisiae* strains used in this study**

Strain	Genotype	Background	Ref.*
E. coli Bl21 DE3	F- *ompT gal dcm lon hsdSB(rB- mB-) λ(DE3 [lacI lacUV5- 7 gene 1 ind1 sam7 nin5]*		Novagen
BY4741	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	S288C	1
get3	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydl100c*::KanR	BY4741	2
get1/*get2*/*get3*	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 get1*::KanR, *get2*::NatR, *get3*::PhleoR	BY4741	3

Plasmids used in this study

Name	Description	Marker	Ref.*
pJ488	pQE80-10His-ZZ-Get3	Amp	4
pAA1349	pQE80-10His-ZZ-Get3$_{D57E}$	Amp	5
pAD1474	pet280 Get3$_{I193D}$	Kan	6
pG307	p416Met25-Get3^{WT}	Ura	5
pG309	p416Met25-Get3^{D57E}	Ura	5
ppG310	p416Met25-GFP-Get3^{WT}	Ura	5
pG308	p416Met25-GFP-Get3^{D57E}	Ura	5
pAA1307	p415Met25-mCherry-Sed5	Leu	5
pX1157	p413Met25-GFP-Sed5	His	5
pAF1558	p416Met25-Get3^{I193D}	Ura	this study
pAF1559	p416Met25-GFP-Get3^{I193D}	Ura	this study

*1(Brachmann et al., 1998); 2(Jonikas et al., 2009); 3(Schuldiner et al., 2008); 4(Metz et al., 2006); 5(Powis et al., 2013); 6(Mateja et al., 2009)

Supplemental Material and Methods

Plasmids construction - For p416Met25-Get3^{I193D}, the coding sequence of Get3^{I193D} was amplified by PCR from pLAC33-Get3^{I193D} (Mateja et al., 2009) using the primers 5'-TATGATACTAGTATGGATTTAACCGTGGAA-3' and 5'-ATCATACTCGAGCTATTCCTTATCTTCTAA-3' containing SpeI and XhoI restriction sites, respectively. For p416Met25-GFP-Get3^{I193D}, a SpeI/BamHI fragment was originated from p416Met25-Get3^{I193D} and ligated to p416Met25-GFP-Get3^{WT} previously digested with the same restriction endonucleases.

Get3 Purification - Get3 wild-type and the D57E mutant were expressed in *E. coli* Rosetta2(DE3)/pLysS (Novagen) from a pQE80 derivative as a fusion of two Z domains (IgG-binding domain of protein A) to Get3 (Metz et al., 2006) containing a tobacco etch virus (TEV) protease cleavage site between an N-terminal 6×His tag in front of the Z domain and the polylinker. Get3^{I193D} was expressed from a pet280 vector (kind gift of Robert Keenan) containing a TEV protease cleavage site between the N-terminal 6×His tag and a polylinker. After cells reached an A_{600} of ~0.6, protein expression was induced with 0.4 mM IPTG and cells were cultivated for another 4 h at 30 °C. The cells were pelleted and resuspended in extraction buffer (50 mM Tris, 50 mM NaCl, 2 mM MgAc, 1 mM imidazole, optional 2 mM DTT, pH 7.5), supplemented with one tablet of protease inhibitor (Roche) and 1 mM PMSF (Sigma Aldrich). Cells were lysed using a commercial French press (3 x 1300 psi). The cleared lysate was purified by a nickel-NTA column (QIAGEN). For further purification an anion-exchange chromatography column (Q-sepharose HP, GE Healthcare) was used. The purified protein was simultaneously cleaved with 6×His-tagged TEV protease and dialyzed for 20 h at 4°C against cleavage

buffer (50 mM Tris, 50 mM NaCl, pH 7.5) containing 0.5 mM DTT and 0.5 mM EDTA. Uncleaved Get3 and the 6×His-tagged TEV protease was removed by subtractive Ni-NTA purification. The efficiency of cleavage was quantified by SDS-PAGE and was >90%. Get3 concentrations were determined spectroscopically using a Jasco spectrophotometer V-550.

Chaperone activity assays - 12 µM CS was denatured in 40 mM HEPES, 6 M guanidine hydrochloride (Gdn-HCl), pH 7.5 overnight at RT. To initiate protein aggregation, chemically denatured CS was diluted to a final concentration of 0.075 µM into 40 mM HEPES, pH 7.5 at 30°C under continuous stirring. The maximum in light scattering signal was reached after 4 min of CS incubation and was set to 0% chaperone activity. To determine the effect of Get3 on CS aggregation, Get3 was diluted into the buffer and light scattering was monitored after addition of chemically denatured CS using a Hitachi F4500 fluorescence spectrophotometer equipped with a temperature-controlled cuvette holder and stirrer. Excitation and emission wavelengths were set to 360 nm. To monitor the activation and inactivation kinetics of Get3, the protein was incubated with the respective oxidants or reductants as described. At defined time points, aliquots were taken and Get3 was diluted to a final concentration of 0.3 µM into assay buffer. Chaperone activity measurements were monitored as described.

EM Sample imaging and analysis - Samples were imaged under low dose conditions using a G2 Spirit TEM (FEI) operated at 120 keV. Micrographs were taken at 52,000x magnification with 2.16 Å per pixel using a 4k x 4k CCD camera (Gatan). Single particles were selected using E2boxer in EMAN2 (Tang et al., 2007) and totaled 39,285 for $Get3_{ox}$ and 7,731 for $Get3_{red}$. Reference free 2D classification and analysis of Get3

was performed using SPIDER (Radermacher et al., 1987) and generated 400 classes for Get3 $_{ox}$ and 100 classes for Get3 $_{red}$. 3D refinement was performed using RELION (Scheres, 2012) by first running '3D classification' on the entire data set, binned two-fold, using a sphere as an initial model and no imposed symmetry (Figure S2C). The resulting model was used for an additional 3D classification involving 20 rounds with 3 classes and two-fold symmetry imposed, using the entire un-binned dataset. The best model, based on similarity to the reference-free 2D averages (Figure 3D), was subjected to additional refinement, using '3D Refine' with two-fold symmetry and converged after 9-rounds, calculated to be 19 Å by the 'gold-standard' Fourier shell correlation procedure (Figure S2D) (Scheres, 2012). Additional symmetries were tested, however only the two-fold remained consistent with the asymmetric model and the 2D averages (data not shown).

Hydrogen/Deuterium exchange experiments combined with mass spectrometry - The H/D exchange experiments were initiated by diluting 45 µl of reduced $Get3_{red}$ (~36 µM) or $Get3_{ox}$ (~32 µM) with 135 µl of D_2O buffer (8.3 mM Tris, 50 mM NaCl, in D_2O, pD_{READ} 7.2) at 0°C. At 10, 30, 100, 300, 1000, 3000, and 10000 sec, 24 µl of the reaction were removed and quenched by adding 36 µl of optimized quench buffer (1.0 M Gdn-HCl, 100 mM TCP, 0.8 % formic acid, 16.6 % v/v glycerol) at 0°C. The quenched samples were incubated on ice for 5 min, and then frozen at -80°C. In addition, non-deuterated control samples (incubated in non-deuterated D_2O buffer) and equilibrium-deuterated back exchange control samples (incubated in D_2O buffer containing 0.5% formic acid overnight at 25°C) were prepared.

Analysis of plasmid-induced Get3 expression levels –1 ml of mid-log phase yeast

culture was pelleted by low speed centrifugation, resuspended in 0.1 M NaOH and incubated for ten minutes at room temperature. Cells were recovered by centrifugation and dissolved in 1X SDS loading buffer. 10 µl of lysate per lane were separated by SDS-PAGE and analyzed by immunoblot using a mouse monoclonal antibody against Pgk1 (Molecular Probes) or a guinea pig serum against Get3 (Metz et al., 2006).

Supplemental References

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