Supporting Information

Teilum et al. 10.1073/pnas.0907387106



Fig. S1. ¹⁵N CPMG relaxation dispersion curves recorded at 2 static magnetic fields (open symbols, 500 MHz; filled symbols, 600 MHz) and 3 temperatures (blue, 18 °C; green, 25 °C; red, 32 °C) on WT apo-SOD1. The effective transverse relaxation rate ($R_{2,eff}$) is plotted against the effective field strength (ν_{CPMG}) of the refocusing pulse train for datasets with significant dispersion (P < .0005). The solid lines represent a global fit with k_{ex} , p_F , and ΔH as global parameters. The optimized values for k_{ex} were (2.3 ± 0.2) × 10³ s⁻¹ at 32 °C, (2.2 ± 0.1) × 10³ s⁻¹ at 25 °C, and (2.4 ± 0.1) × 10³ s⁻¹ at 18 °C.



Fig. 52. Bootstrap analysis of the global fit routine. The robustness of the global fitting routine was assessed by global fits of 100 reduced datasets in which data for 20% of the residues included in the original global fit were randomly left out. The resulting distributions from the 100 fits for each variant show that the parameter distributions are centered around the optimized parameters obtained from the fits to the full datasets, and that no extreme outliers are observed.

<



Fig. S3. ¹⁵N CPMG relaxation dispersion curves for residues A60, G61, H63, and F64 in protein, where P62 is in the *cis* conformation. Symbols are as in Fig. S1.

DNAS



Fig. S4. Backbone amide ¹⁵N chemical shift differences in WT apo-SOD1 plotted versus residue number. The solid line and filled circles indicate $\Delta\omega_{FE}$ between the major and the minor states obtained from global fits of CPMG relaxation dispersions. The dashed line and open circles indicate the estimated change in chemical shift on unfolding, $\Delta\omega_{FU}$. In the regions that constitute loops IV and VII, $\Delta\omega_{FE}$ is larger than $\Delta\omega_{FU}$. Because $\Delta\omega_{FU}$ in these regions is small, the protein apparently becomes more structured in these loops in the minor state compared with the major state.

а

S A Z d



b



Fig. 55. ¹⁵N CPMG relaxation dispersions. (a) Reduced WT apo-SOD1 at 2 static magnetic fields (black circles, 500 MHz; blue triangles, 600 MHz) at 25 °C. The effective transverse relaxation rate ($R_{2,eff}$) is plotted against the effective field strength (ν_{CPMG}) of the refocusing pulse train for the 13 residues that show significant dispersions (P < 0.01). The "X" in the last panel indicates that the dataset could not be unambiguously assigned. The solid lines represent a global fit with optimized parameters, $k_{ex} = (3.4 \pm 0.3) \times 10^3 \text{ s}^{-1}$ and $p_E = 0.6\% \pm 0.1\%$. The exchange process is thus similar to that observed on disulfide oxidized apo-SOD1, although the exchange rate (k_{ex}) is faster. Due to the fast k_{ex} combined with the low p_E , only the residues with the largest chemical shift differences between the exchanging states can be observed in the CPMG relaxation dispersion experiments. Therefore, only 13 residues show significant exchange in reduced apo-SOD1. (b) Zn-loaded WT SOD at 2 static magnetic fields (black circles, 500 MHz; blue triangles, 600 MHz) at 25 °C. The effective field strength (ν_{CPMG}) of the refocusing pulse train. The solid lines are a global fit to the equation for slow exchange, $R_{2,eff} = R_2 + k_A(1 - \sin(\Delta\omega \tau_{CPMG})/\Delta\omega \tau_{CPMG})$, for the 18 residues that were found to participate in the slow process. The optimized rate constant for the flux away from the observed state is $k_A = 22.4 \pm 0.7 \text{ s}^{-1}$. In addition, 14 residues show evidence of exchange, but these could not be quantified accurately because of the very small dispersion magnitudes.

G10N_H	D11N_H 1.2 0.8 5 0.4 0 10 20 30 40 relaxing (4)(ms)	117N_H 1.2 0.8 0.0 0.10 20 30 40 relaxing delay (ms)	K23N_H 1.2 0.8 0.0 0.10 20 30 40 relaxing delay (ms)	E24N_H 1.2 0.8 	S25N_H 1.2 0.8 5 0.4 0.10 20 30 40 relaxing delay (ms)	L38N_H 1.2 0.8 0.4 0.0 0.10 20 30 40 relaxion delay (ms)	E40N_H 1.2 0.8 0.4 0.0 0.10_20_30_40 relaxing delay (ms)
H46N_H 6 4 2 0 0 0 0 0 0 0 0 0 0 0 0 0	E51N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	D52N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	N53N_H 1.2 0.8 0.4 0.00 10 20 30 40 relaxation delay (ms)	1.2 5.0.8 0.0 0.10 20 30 40 relaxation delay (ms)	A55N_H 1.2 0.8 0.0 0.10 20 30 40 relaxation delay (ms)	G56N_H	C57N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)
S59N_H 1.2 0.8 0.4 0.0 0.0 10 20 30 40 relaxation delay (ms)	A60N_H 1.2 0.8 0.4 0.0 10 20 30 40 relaxation delay (ms)	G61N_H 1.2 0.8 0.0 0.0 0.0 0.0 0.0 0.0 0.0	H63aN_H 1.2 0.8 0.4 0.0 10 20 30 40 relaxation delay (ms)	F64aN_H 1.2 0.8 0.4 0.0 0.0 0.0 0.0 0.0 0.0 0.0	L67N_H 1.2 0.8 0.4 0.0 1.2 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0	S68N_H 1.2 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	R69N_H 1.2 0.8 0.4 0.0 10 20 30 40 relaxation delay (ms)
K70N_H 1.2 0.8 0.4 0.0 0.0 0.0 30 40 relaxation delay (ms)	H71N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	G72N_H 1.2 0.8 5 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	G73N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	K75N_H 1.2 0.8 5 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	D76N_H 1.2 0.8 5 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	E77N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	E78N_H 1.2 0.8 0.0 0.0 0.0 0.0 0.0 0.0 0.0
R79N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	H80N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	V81N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	D92N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	S98N_H 1.2 0.8 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	E100N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	V103N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	G108N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)
D109N_H 1.2 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	A111N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	R115N_H 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	G127N_H 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	K128N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	G129N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	G130N_H 1.2 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	N131N_H 0.8 0.0 0.0 0.0 0.0 0.0 0.0 0.0
E132N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	E133N_H 1.2 0.0 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	S134N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	T135N_H 1.2 0.8 0.0 0.1 0.0 0.10 20 30 40 relaxation delay (ms)	K136N_H 1.2 0.8 0.0 0.1 0.0 0.10 20 30 40 relaxation delay (ms)	G138N_H 1.2 0.8 0.0 0.1 0.0 0.10 20 30 40 relaxation delay (ms)	G141N_H 1.2 0.8 0.0 0.0 0.10 20 30 40 relaxation delay (ms)	V148N_H 1.2 0.8 0.0 0.0 0.0 0.0 0.0 0.0 0.0
G150N_H 1.2 0.8 0.4 0.0 0.10 20 30 40 relaxation delay (ms)	1151N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)	Q153N_H 1.2 0.8 0.4 0.0 0 10 20 30 40 relaxation delay (ms)					

Fig. S6. Experimental data showing PRE decays. The relative cross-peak intensity (I_{ox}/I_{red}) is plotted against the relaxation delay. Only the 59 residues with data that fit an exponential decay significantly better (P < 0.01) than a constant value are shown. Because both I_{ox} and I_{red} decrease with relaxation delays, the data points at 30 and 40 ms generally are associated with large errors relative to the data points at shorter relaxation delays. Consequently, the data points at long relaxation delays weight less in the fit of the exponential decay.

VAS PNAS

G10N_H	D11N_H	Q15N_H	G16N_H	G37N_H	G41N_H	L42N_H	H43N_H
250 100 100 100 100 100 100 100 1	() <i>j j j j j j j j j j</i>	20 20 20 20 20 20 20 20 20 20 20 20 20 2	20 0 15 (*) 15 (*) 5 (*) 5	20 9) 15 5 20 0 400 800 v _{CPMG} (Hz)	- - - - - - - - - - - - - -	20 20 20 20 20 20 20 20 20 20 20 20 20 2	30 30 400 800 v _{CPMG} (H2)
H46N_H -(s) = 25 + 10 =	V47N_H (s) (s) (s) (s) (s) (s) (s) (s) (s) (s)	E49N_H © 20 \$10 \$10 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$	ESON_H	E51N_H () 255 () 255	N53N_H -(s) 500 0 400 800 v_CPMG (H2)	T54N_H	G56N_H s *********************************
C57N_H 	Т58N_H ^{••} 200 ^{••} 100 ^{••} 100 ^{••} 0 400 800 ^{••} v _{срид} (Hz)	G61N_H	H63 N_H	F64 N_H 	N65 N_H	L67N_H (16 17 18 18 19 19 19 19 10 10 10 10 10 10 10 10 10 10	S68N_H () 12 ()
K70N_H (16) (16) (17) (17) (18) (17)	G72N_H ⁻ 30 ⁺ 20 ⁺ 10 ⁺ 10 ⁺ 0 [−] 0 ⁺ 400 800 ⁺ v _{CPMG} (Hz)	G73N_H 	K75N_H (12) 12) 12) 12) 14) 14) 14) 14) 14) 14) 14) 14	E78N_H	R79N_H	H80N_H	V81N_H -(5) -(5) -(7)
G82N_H 	G85N_H () () () () () () () () () () () () () (N86N_H 20 15 15 15 0 400 800 v _{CPMG} (Hz)	V87N_H -() 250 -()	S102N_H 	V103N_H -(1104N_H 20 00000000000000000000000000000000000	S105N_H
A111N_H 	1112N_H (25 (112N_H (25) (112N_H (R115N_H 20 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	V118N_H () 25 () 100 () 100	E121N_H	A123N_H 30 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	L126N_H	G127N_H
K128N_H 	G129N_H 	G130N_H -(N131N_H	E133N_H	S134N_H 	T135N_H	T137N_H
G138N_H 12 8 4 0 0 0 0 0 0 0 0 0 0 0 0 0	N139N_H (A140N_H (250	G141N_H G14N_H G141N_H G14N	S142N_H () () () () () () () () () () () () () (R143N_H (-5) (-5	C146N_H -(-s) = = = = = = = = = = = = = = = = = = =	G147N_H

Fig. 57. Concentration dependence of the millisecond exchange process. ¹⁵N CPMG relaxation dispersion curves recorded at 600 MHz and 25 °C on 0.5 mM WT apo-SOD1. The solid lines represent a global fit of the experimental data. The optimized parameters for the exchange process are $p_E = 0.8\% \pm 0.1\%$ and $k_{ex} = (2.2 \pm 0.1) \times 10^3 \text{ s}^{-1}$.

SANG SANG



Fig. S8. ¹⁵N CPMG relaxation dispersion curves recorded on apo-SOD1 mutants A4V (*a*), G85R (*b*), and D90A (*c*) at 2 static magnetic fields (black circles, 500 MHz; blue triangles, 600 MHz) at 25 °C. The effective transverse relaxation rate ($R_{2,eff}$) is plotted against the effective field strength (ν_{CPMG}) of the refocusing pulse train. Solid lines represent a global fit.

S A Z d

b

DNAS

SANC



Fig. S8. Continued.

	-
•	
L	
	-

PNAS



Fig. S8. Continued.



Fig. S9. NMR chemical shift analysis of apo-SOD WT and mutants in 10 mM MES, 1 mM EDTA, and 10% D₂O (pH 6.3). (a) ¹H, ¹⁵N-HSQC spectra of WT and mutant SOD. (b) Chemical shift differences (¹H^N, ¹⁵N, and ¹³C^α) of the SOD1 mutants A4V, G85R, and D90A relative to the WT SOD1. For WT apo-SOD1, 142 out of the 148 nonproline ¹H^N and ¹⁵N chemical shifts were assigned. Double sets of peaks were identified and assigned for 7 residues (60, 61, 63, 64, 65, 72, and 73) that are followed by prolines at positions 62, 66, and 74. In addition, chemical shifts were assigned for ¹³C^α, ¹³C^β, and ¹³C' in 150, 124, and 148 residues, respectively. From the chemical shift of P62 C^β, the double set of peaks around this residue can be assigned to a minor (\approx 25%) *cis* conformation and a major (\approx 75%) *trans* conformation. C^β chemical shifts for the other proline residues were identified. In addition, ¹³C^α was assigned for 153 residues. For the A4V and G85R mutants, chemical shifts were assigned for 130/143/116/143 and 126/143/119/144 residues in the A4V and G85R mutants, respectively. No double sets of resonances were assigned for the set variants; see (a). (¹H^N, ¹⁵N)/¹³C^α/¹³C^β/¹³C' chemical shifts were assigned for the 50° these variants. All assignments have been deposited at the BioMagResBank; http://www.bmfb.wisc.edu (accession numbers: 15711, 15712, 15713, and 15714).

а

(mqq) _{15N}

Table S1. Data sizes and χ^2 results for global fits

PNAS PNAS

SOD variant	WT	A4V	G85R	D90A
Residues included	64	39	31	68
N _{datapoints}	6,144	1,404	1,116	2,312
Nparameters	453	119	95	206
χ^2	3,557	312.1	641.3	520.2
χ^2 red	0.62	0.24	0.63	0.25

The global fit for WT includes data acquired at 3 different temperatures. The global fits for the mutants include data acquired at a single temperature.

Table S2. Are k_{ex} and p_E different between mutants?

SANG SANG

Parameter tested	WT and A4V		WT and G85R		WT and D90A	
	k _{ex}	pE	k _{ex}	pE	k _{ex}	p _E
F-ratio	5.53	1.35	76.6	5.66	19.8	4.69
Р	0.019	0.24	2.6710 ⁻¹⁸	0.017	8.8210 ⁻⁶	0.031

Dispersion data for WT apo-SOD1 were fit pairwise with data for the mutants, taking either p_F or k_{ex} as a parameter shared between WT and mutant. These fits were compared with fits in which k_{ex} and p_E were optimized as individual parameters for each variant. The model with a shared k_{ex} (or p_F) and the model with individual k_{ex} (or p_F) for WT and mutant are nested models, with the former being a simpler version of the latter ($k_{ex,wt} = k_{ex,mut}$ or $p_{F,wt} = p_{F,mut}$). The *F* test was used to test the hypothesis that k_{ex} (or p_F) differs between WT and mutant against the null-hypothesis that k_{ex} (or p_F) is the same for the 2 variants.