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Supporting Information

Superoxide Dismutase 1 Folding Stability as a Target for Molecular Tweezers in SOD1-Related Amyotrophic Lateral Sclerosis

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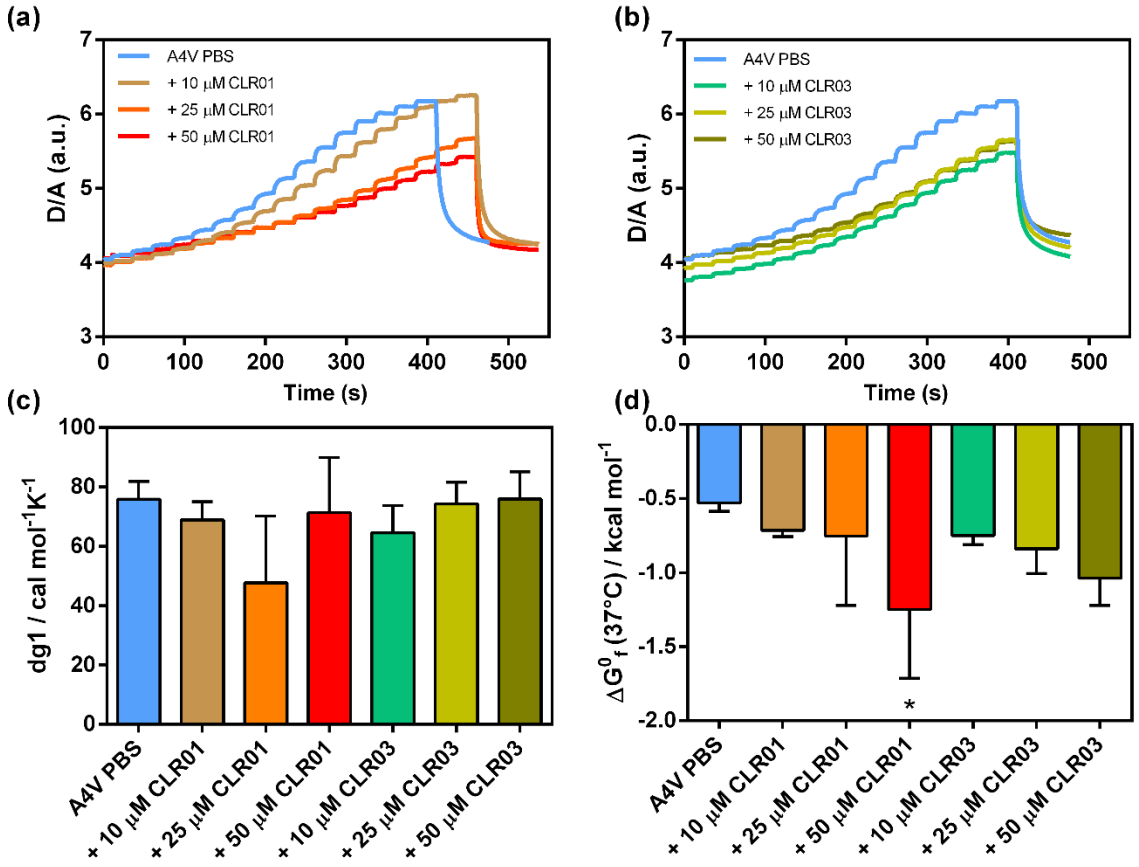

Figure S1: Exemplary D/A plots of FReI measurements of $SOD1_{bar}$ A4V in the presence of different concentrations of (a) CLR01 and (b) CLR03. Evaluation of respective (c) cooperativity parameters (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. Data is shown as mean and s.d.. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*p < 0.05, **p < 0.01, ****p < 0.0001). n = 5.

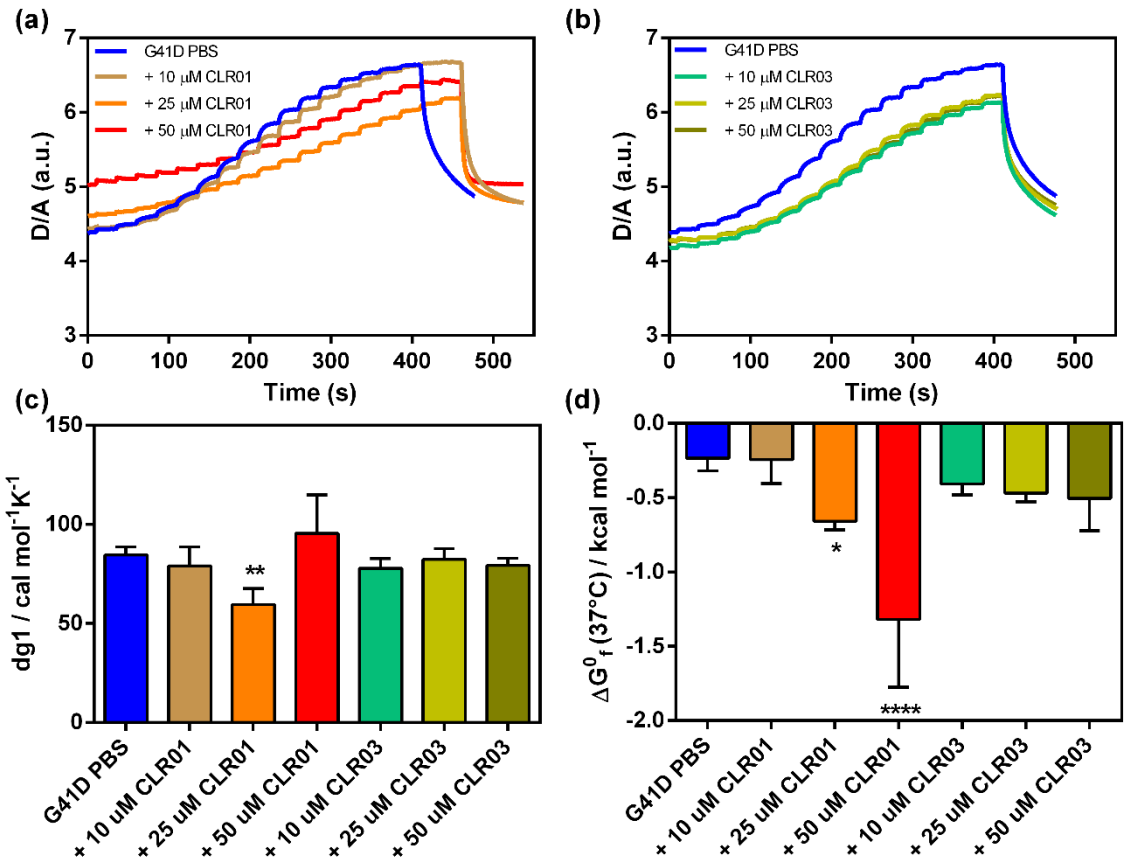

Figure S2: Exemplary D/A plot of FReI measurement of $SOD1_{bar}$G41D in the presence of different concentration of (a) CLR01 and (b) CLR03. Evaluation of respective (c) cooperativity parameters (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. Data is shown as mean and s.d.. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). n = 5.

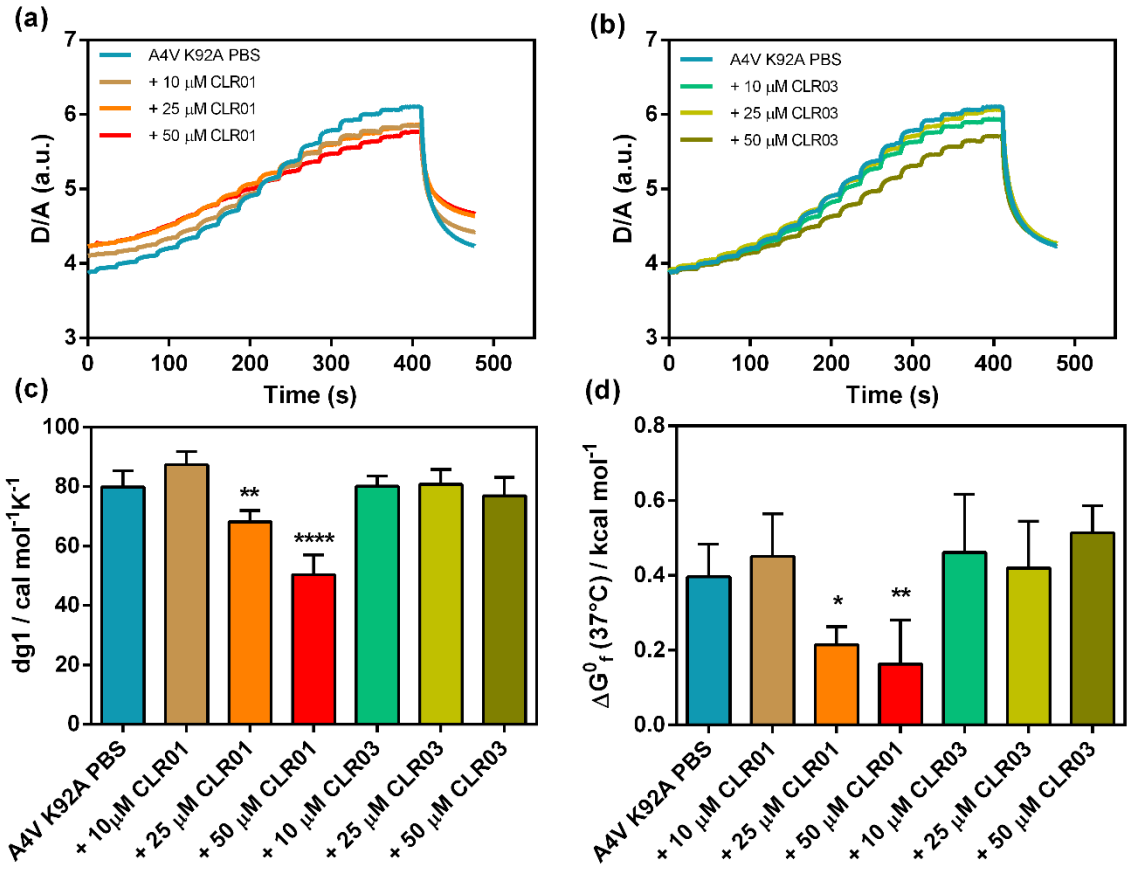

Figure S3: Exemplary D/A plot of FReI measurement of $SOD1_{bar}$A4V K92A in presence of different concentration of (a) CLR01 and (b) CLR03. (c) cooperativity parameter (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. The mean value and its corresponding standard deviation are shown. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). n = 5.

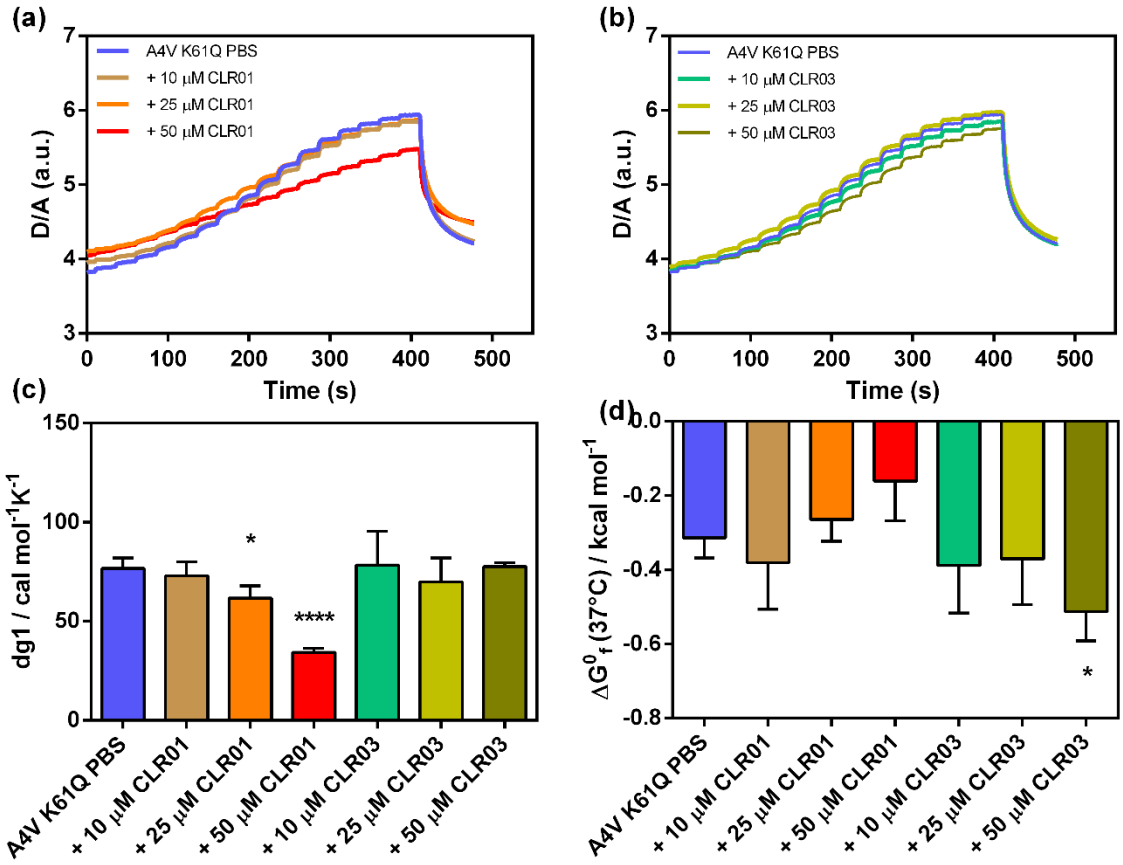

Figure S4: Exemplary D/A plot of FReI measurement of $SOD1_{bar}$A4V K61Q in presence of different concentration of (a) CLR01 and (b) CLR03. (c) cooperativity parameter (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. The mean value and its corresponding standard deviation are shown. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). n = 5.

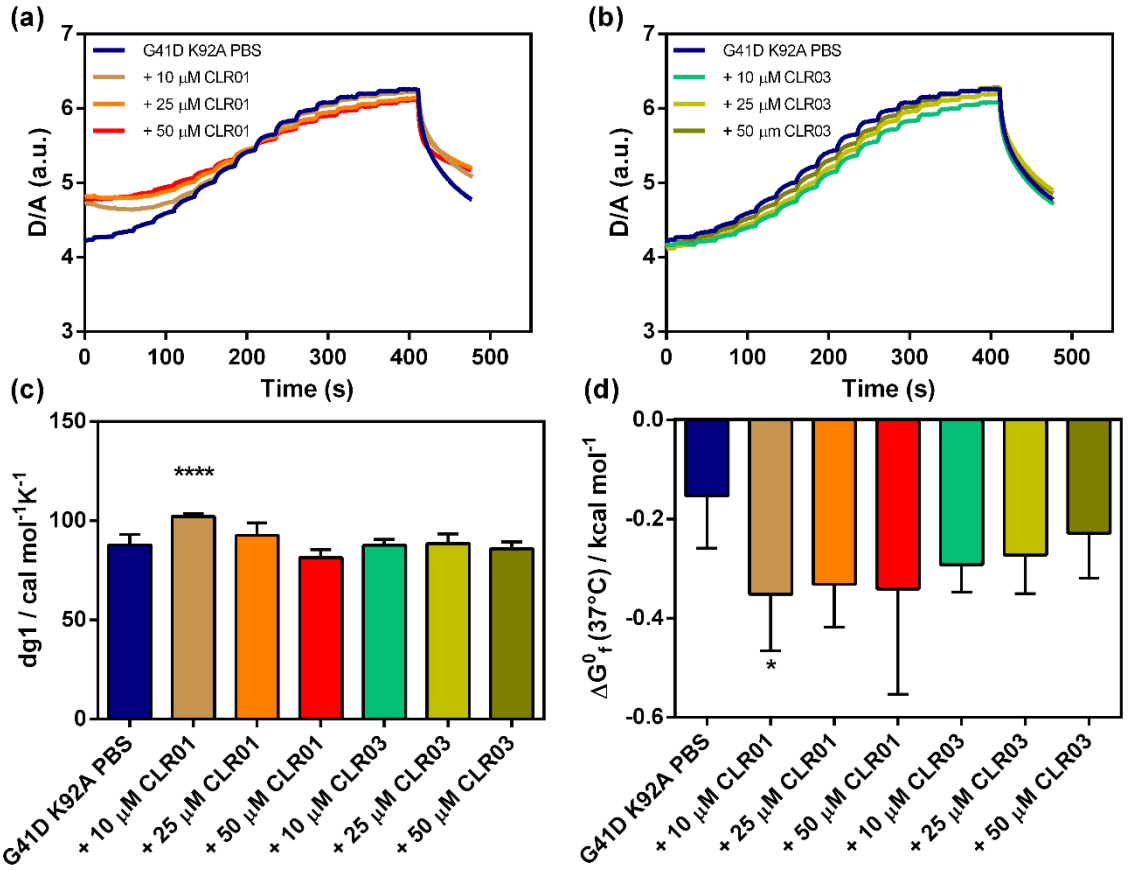

Figure S5: Exemplary D/A plot of FReI measurement of $SOD1_{bar}$G41D K92A in presence of different concentration of (a) CLR01 and (b) CLR03. (c) cooperativity parameter (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. The mean value and its corresponding standard deviation are shown. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). n = 5.

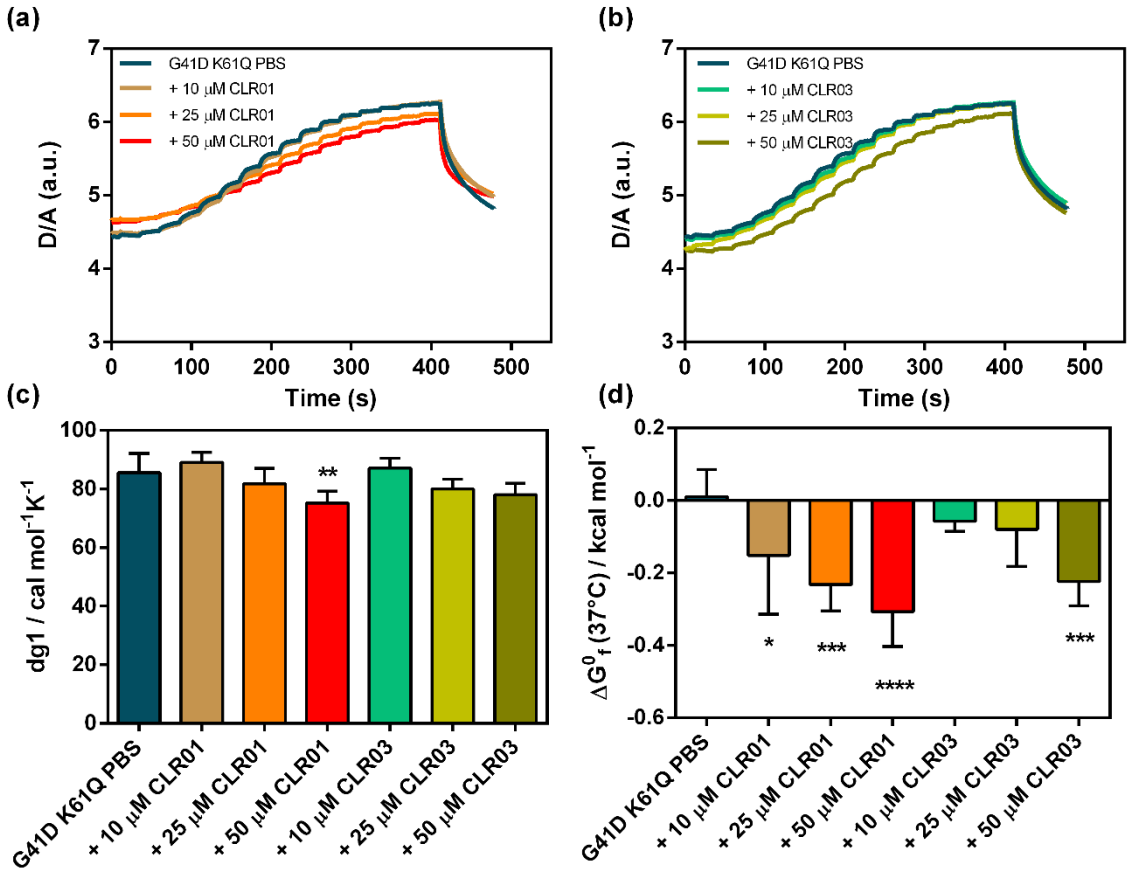

Figure S6: Exemplary D/A plot of FReI measurement of $SOD1_{bar}$G41D K61Q in presence of different concentration of (a) CLR01 and (b) CLR03. (c) cooperativity parameter (dg1) and (d) standard free energy change of folding (ΔG^0_f) at 37 °C in presence of CLR01/03. The mean value and its corresponding standard deviation are shown. Using one-way ANOVA with post-hoc analysis, significant statistical differences with respect to the PBS measurement were determined, which are indicated by asterisks (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). n = 5.

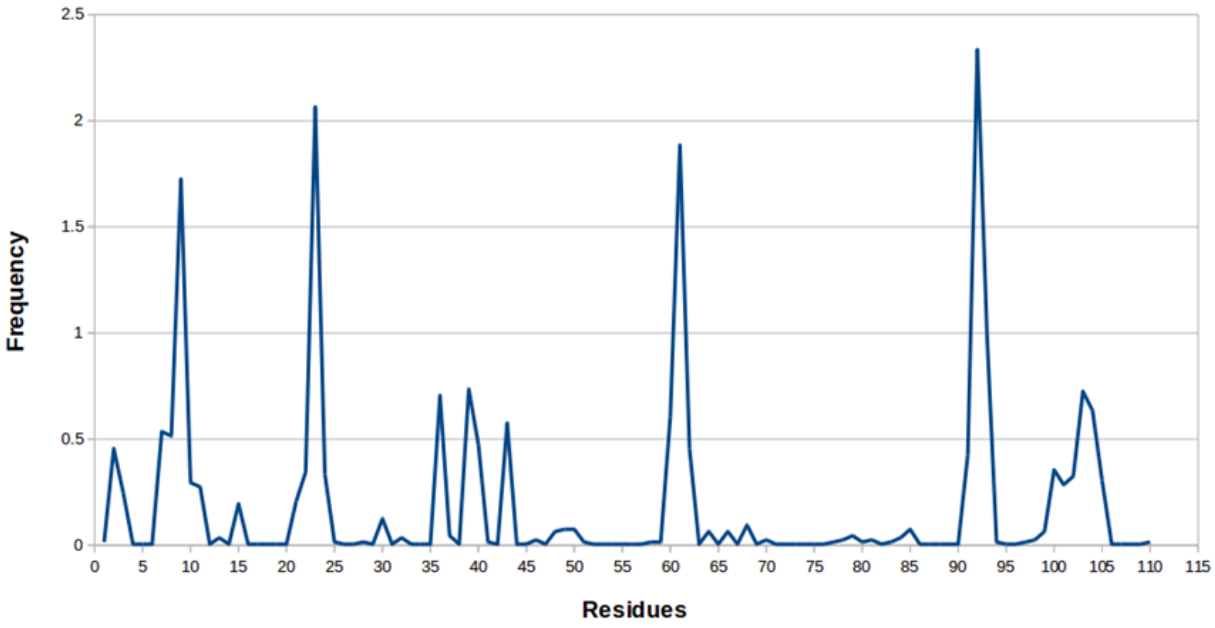

Figure S7: Prevalence of tweezers in the vicinity of each residue of SOD1. The vicinity was defined as 4 Å distance between the phosphorous atoms of the tweezers and any heavy atom of the amino acid. The frequency values correspond to the average number of CLR01´s phosphorous atoms within the vicinity of every residue along the simulations. NOTE: frequency values larger than two indicate that a second molecule of CLR01 is close (< 4 Å) to the corresponding residue during part of the simulation. The four peaks with frequencies above 1.5 correspond to K9, K23, K61 and K92.

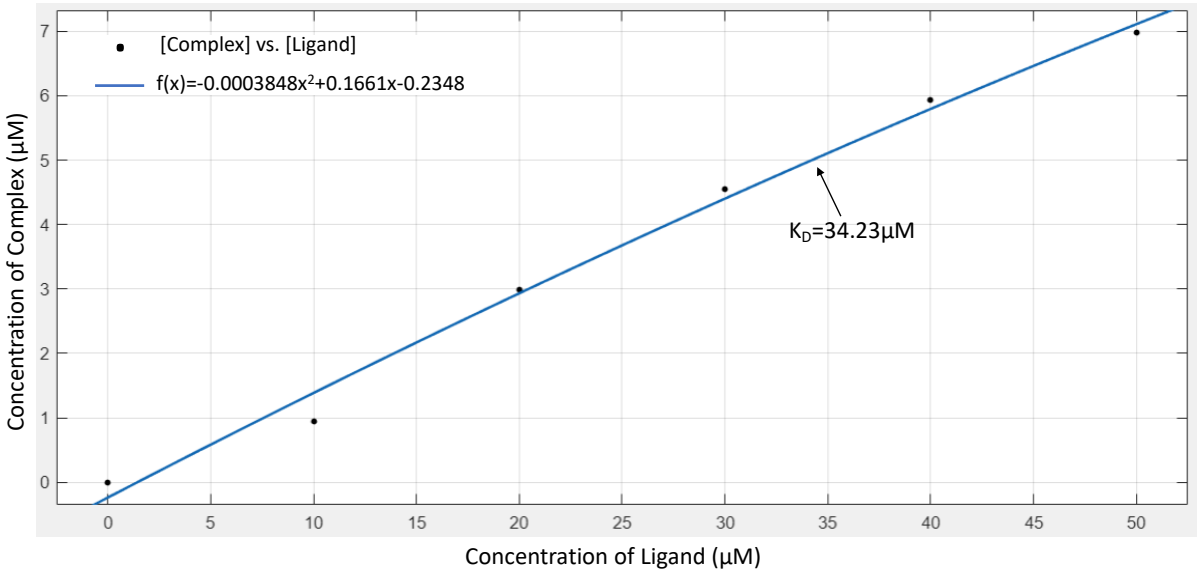

Figure S8: The curve utilized to calculate a K_D value for CLR01 binding to $SOD1_{bar}$. The concentration of $SOD1_{bar}$ bound to CLR01 was plotted against the concentration of total ligand. A quadratic function was fitted to the data points and the K_D was calculated to be the concentration of ligand at which 50% of $SOD1_{bar}$ is bound (5µM).

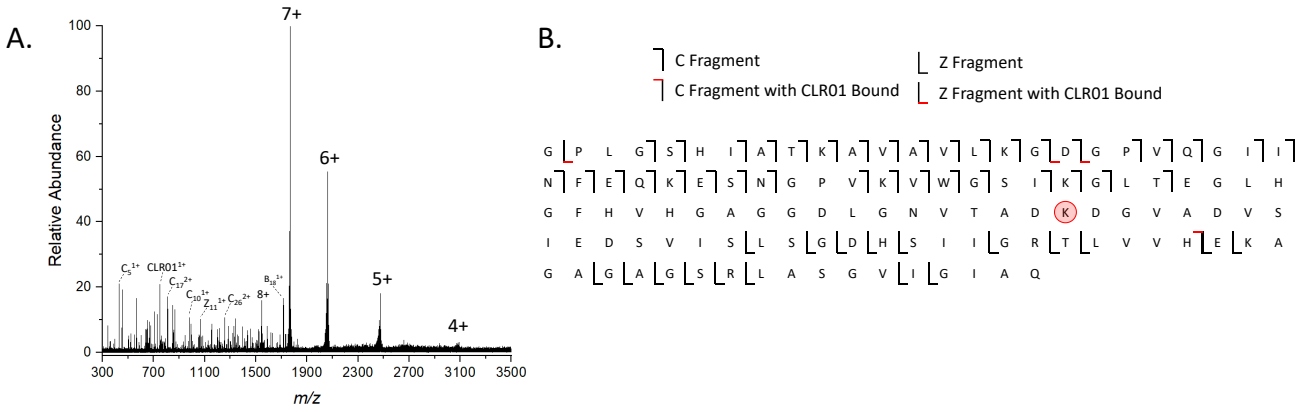

Figure S9: (A) An ECD fragmentation spectrum of the $SOD1_{bar}$/CLR01 complex, and (B) a representation of the peaks present in the TD mass spectrum. Analysis of the MS fragmentation revealed that CLR01 binds $SOD1_{bar}$ on K61 (as highlighted by red circle)

Table S1: Thermodynamic parameters as obtained from FReI data analysis: Melting temperature (T_m), cooperativity parameter ($dg1$) and modified standard free energy change of folding (ΔG^0_f) at 37 ℃ for $SOD1_{bar}$A4V, $SOD1_{bar}$G41D and the double mutants in PBS and in presence of CLR01/03.

	T_m (K)	$dg1$±s.d. (kcal mol^{-1}K^{-1})	ΔG^0_f ±s.d. (at 310 K) (kcal mol^{-1})
		$SOD1_{bar}$A4V	
PBS	316.98±0.53	0.076±0.005	-0.53±0.06
CLR01 10μM	320.38±0.57	0.069±0.007	-0.71±0.04
CLR01 25 μM	325.23±3.33	0.048±0.022	-0.75±0.47
CLR01 50 μM	327.08±2.47	0.072±0.019	-1.25±0.47
CLR03 10 μM	321.75±1.52	0.065±0.010	-0.75±0.06
CLR03 25 μM	321.25±1.49	0.074±0.007	-0.84±0.16
CLR03 50 μM	323.63±1.52	0.076±0.010	-1.04±0.18
		$SOD1_{bar}$G41D	
PBS	312.81±1.13	0.084±0.005	-0.23±0.08
CLR01 10μM	313.25±2.27	0.079±0.010	-0.24±0.16
CLR01 25 μM	321.28±2.19	0.060±0.007	-0.66±0.06
CLR01 50 μM	323.58±1.88	0.096±0.019	-1.32±0.46
CLR03 10 μM	315.25±0.98	0.079±0.005	-0.41±0.07
CLR03 25 μM	315.73±0.88	0.081±0.005	-0.47±0.06
CLR03 50 μM	316.45±3.09	0.079±0.005	-0.50±0.22
		$SOD1_{bar}$A4V K92A	
PBS	312.81±1.13	0.079±0.005	0.40±0.09
CLR01 10μM	313.25±2.27	0.088±0.005	0.45±0.11
CLR01 25 μM	321.28±2.19	0.069±0.005	0.22±0.05
CLR01 50 μM	323.58±1.88	0.050±0.007	0.16±0.12
CLR03 10 μM	315.25±0.98	0.081±0.002	0.46±0.16
CLR03 25 μM	315.73±0.88	0.081±0.005	0.42±0.13
CLR03 50 μM	316.45±3.09	0.076±0.007	0.51±0.07
		$SOD1_{bar}$A4V K61Q	
PBS	314.11±0.76	0.076±0.005	-0.32±0.05
CLR01 10μM	315.17±1.45	0.074±0.007	-0.38±0.12
CLR01 25 μM	314.31±0.84	0.062±0.007	-0.27±0.06
CLR01 50 μM	314.85±3.50	0.033±0.002	-0.16±0.11
CLR03 10 μM	315.39±2.61	0.079±0.017	-0.39±0.13
CLR03 25 μM	315.33±1.49	0.069±0.012	-0.37±0.12
CLR03 50 μM	316.63±1.12	0.076±0.002	-0.51±0.08
		$SOD1_{bar}$G41D K92A	
PBS	311.79±1.29	0.88±0.005	-0.15±0.11
CLR01 10μM	313.45±1.13	0.103±0.002	-0.35±0.11

CLR01 25 µM	313.55±0.71	0.093±0.007	-0.33±0.09
CLR01 50 µM	314.11±2.33	0.081±0.005	-0.34±0.21
CLR03 10 µM	313.33±0.64	0.088±0.002	-0.29±0.05
CLR03 25 µM	313.13±1.05	0.088±0.005	-0.27±0.08
CLR03 50 µM	312.69±1.14	0.086±0.002	-0.23±0.09
		$SOD1_{bar}$G41D K61Q	
PBS	309.88±0.89	0.086±0.007	0.01±0.07
CLR01 10µM	311.73±1.90	0.088±0.002	-0.15±0.16
CLR01 25 µM	312.89±1.07	0.081±0.005	-0.23±0.07
CLR01 50 µM	314.05±1.13	0.074±0.005	-0.31±0.10
CLR03 10 µM	310.67±0.33	0.086±0.002	-0.06±0.03
CLR03 25 µM	311.03±1.27	0.079±0.002	-0.08±0.10
CLR03 50 µM	312.85±0.81	0.079±0.005	-0.22±0.07

Table S2. Convergence analysis of the binding free energy calculation with the complex CLR01 – K23. The table summarizes the estimated at every checkpoint of the method, the associated error, the importance sampling ratio (ISR) and the convergence status of the checkpoint. The last row of the table contains the weighted average among the converged checkpoints, the resulting work of the harmonic shape restraints applied to the protein in its bound and unbound states, and the final unbiased estimation recovered by

subtracting the work from the average of the CL-FEP checkpoints. The convergence analysis was performed with an oversampling of 2. All values are in kcal/mol.

TITLE:	ΔG	ERROR	ISR	CONVERGENCE
POINT_0:	-3.20	0.82	13.25	WARNING3
POINT_1:	-2.19	0.49	14.93	OK
POINT_2:	-1.81	0.56	19.50	OK
POINT_3:	-2.15	0.63	26.82	WARNING3
POINT_4:	-2.66	0.76	18.84	WARNING3
POINT_5:	-3.18	0.76	43.59	WARNING3
POINT_6:	-1.84	0.69	25.84	WARNING3
POINT_7:	0.91	0.65	24.75	WARNING3
POINT_8:	-3.24	0.43	25.33	OK
POINT_9:	-3.27	0.59	24.18	OK
POINT_10:	-2.67	0.59	39.35	WARNING3
POINT_11:	-2.64	0.49	21.34	OK
POINT_12:	-2.17	0.55	29.51	OK
POINT_13:	-2.65	0.51	32.42	OK
POINT_14:	-2.59	0.53	38.03	OK
AVERAGE*	-2.60	0.53		
WORK OF RESTRAINTS**	1,56	-		
TOTAL	-4.16	0.53		

*ISR-weighted average among converged checkpoints

**Work computed as the difference between the averages of the harmonic restraints in the bound and unbound protein. This value should be subtracted from the results of evaluating CL-FEP to recover an unbiased estimate

Table S3. Convergence analysis of the binding free energy calculation with the complex CLR01 – K61. The table summarizes the estimated at every checkpoint of the method, the associated error, the importance sampling ratio (ISR) and the convergence status of the checkpoint. The last row of the table contains the weighted average among the converged checkpoints, the resulting work of the harmonic shape restraints applied to the protein in its bound and unbound states, and the final unbiased estimation recovered by subtracting the work from the average of the CL-FEP checkpoints. The convergence analysis was performed with an oversampling of 2. All values are in kcal/mol.

TITLE:	ΔG	ERROR	ISR	CONVERGENCE
POINT_0:	-5.09	0.84	11.24	WARNING3
POINT_1:	1.80	0.74	24.82	WARNING3
POINT_2:	-1.87	0.61	29.20	WARNING3
POINT_3:	-1.10	0.66	22.05	WARNING3
POINT_4:	0.14	0.66	21.86	WARNING3
POINT_5:	-0.88	0.57	33.76	OK
POINT_6:	2.02	0.60	34.84	WARNING3
POINT_7:	-0.36	0.69	31.57	WARNING3
POINT_8:	-0.07	0.74	37.65	WARNING3
POINT_9:	0.30	0.60	25.96	WARNING3
POINT_10:	-0.96	0.68	87.07	WARNING3
POINT_11:	-1.30	0.46	37.97	OK
POINT_12:	-1.14	0.57	30.45	OK
POINT_13:	-0.86	0.50	35.49	OK
POINT_14:	-0.55	0.52	26.18	OK
AVERAGE*	-0.97	0.52		
WORK OF RESTRAINTS**	0.99	-		
TOTAL	-1.96	0.52		

*ISR-weighted average among converged checkpoints

**Work computed as the difference between the averages of the harmonic restraints in the bound and unbound protein. This value should be subtracted from the results of evaluating CL-FEP to recover an unbiased estimate

Table S4. Convergence analysis of the binding free energy calculation with the complex CLR01 – K92. The table summarizes the estimated at every checkpoint of the method, the associated error, the importance sampling ratio (ISR) and the convergence status of the checkpoint. The last row of the table contains the weighted average among the converged checkpoints, the resulting work of the harmonic shape restraints applied to the protein in its bound and unbound states, and the final unbiased estimation recovered by subtracting the work from the average of the CL-FEP checkpoints. The convergence analysis was performed with an oversampling of 2. All values are in kcal/mol.

TITLE:	ΔG	ERROR	ISR	CONVERGENCE
POINT_0:	-2.52	0.92	25.12	WARNING3
POINT_1:	-1.91	0.67	21.12	WARNING3
POINT_2:	-0.70	0.74	23.01	WARNING3
POINT_3:	-4.07	0.59	18.12	OK
POINT_4:	-5.08	0.68	25.78	WARNING3
POINT_5:	-3.90	0.72	26.57	WARNING3
POINT_6:	-3.48	0.78	26.92	WARNING3
POINT_7:	-5.61	0.45	32.04	OK
POINT_8:	-3.24	0.81	29.19	WARNING3
POINT_9:	-3.43	0.68	32.15	WARNING3
POINT_10:	-3.25	0.51	33.23	OK
POINT_11:	-4.08	0.44	43.25	OK
POINT_12:	-3.58	0.55	23.00	OK
POINT_13:	-3.67	0.54	45.30	OK
POINT_14:	-3.34	0.49	44.33	OK
AVERAGE*	-3.91	0.74		
WORK OF RESTRAINTS**	-0.1	-		
TOTAL	-3.81	0.74		

*ISR-weighted average among converged checkpoints

**Work computed as the difference between the averages of the harmonic restraints in the bound and unbound protein. This value should be subtracted from the results of evaluating CL-FEP to recover an unbiased estimate