The cost of long catalytic loops in folding and stability of the ALS-associated protein SOD1

FAN YANG [1], HUABING WANG [1], DEREK T. LOGAN [2], XIN MU [1], JENS DANIELSSON [1] AND MIKAEL OLIVEBERG [1] *

Fitting of chevron plots including curved unfolding limb. From the chevron data of apoSOD1^{66+32} it is apparent that protein shifts between and early and late transition-state (‡'→‡'') at high urea concentration, yielding a downward kink of the unfolding limb, $\log k_u^{H_2O} + m_u[\text{urea}]$ in manuscript Eq. 5 was expanded to [1]

$$\log k_{u,\,obs} = \log k_u^{H_2O} + m_{u'}[\text{urea}] - \log(1 + K_{part}10^{m''[\text{urea}]}), \qquad \text{(Eq. S1)}$$

where $K_{part} = [‡'] / [‡'']$ is the equilibrium constant between the two transition-states ‡' and ‡'', respectively, and

$$\log K_{part} = (\log k_u - \log k_{u''}), \qquad \text{(Eq. S2)}$$

where $\log k_u = \log k_u^{H_2O} + m_u[\text{urea}]$ (c.f. Eq. 3) and $\log k_{u''} = \log k_{u''}^{H_2O} + m_{u''}[\text{urea}]$ are the rate constants for unfolding over ‡' and ‡'', respectively, and $m'' = m_u - m_{u''}$. Notably, $\log k_u$ is here equivalent to $\log k_u$ in manuscript Eq. 5, i.e. unfolding over the dominant ‡', captured by a v-shaped fit. The parameters for apoSOD1^{66+32} yielded by Eq. S2 are given in manuscript Table 1.

Fitting of folding rates of low stability proteins. For ϕ-value mutations where low unfolding midpoints prevents accurate fitting of the chevron refolding limb, i.e. L117A and V119A of apoSOD^{33+16}, k_f was estimated from k_u and the unfolding amplitudes by the following relationship: $K_{D\text{-}N} = k_u/k_f = [D]/[N]$, where [D] and [N] were derived from the sigmoidal plots of the unfolding amplitudes vs. [urea] in the unfolding transition region [2].

SI Tables

Table S1. Statistics of crystallographic data, processing and refinement. Values in parentheses are for the highest resolution shell, and R_{free} was calculated with 5.1 % of the reflections excluded in refinement.

Data processing	
Space Group	*H32*
Cell dimensions	
a, b, c (Å)	83.21, 83.21, 133.48
α, β, γ (º)	90.0, 90.0, 120.0
Resolution (Å)	63.41−1.79 (1.83−1.79)
Unique reflections	16779 (810)
Completeness (%)	98.7 (80.6)
Multiplicity	9.6 (6.6)
I/σI	20.1 (2.4)
R_{merge}	0.081 (0.746)
Refinement	
Resolution (Å)	63.41−1.79 (1.84−1.79)
R_{work}	0.177 (0.272)
R_{free}	0.203 (0.262)
Protein atoms	837
Water atoms	61
Glycerol atoms	6
Sulfate ion	5
R.m.s. deviation	
Bond lengths (Å)	0.025
Angles (º)	2.411
Mean *B* value (Å^2)	31.4
Ramachandran plot	
Most favoured (%)	96.5
Allowed (%)	3.5

Disallowed (%)	0.0

Table S2. Parameters ($c^{Eq.}$ and $c^{kin.}$) from linear fits of data Figure S3, plot shown in Figure 7B.

	$c^{Eq.}$	$c^{kin.}$
I18V	1.41±0.02	1.02±0.15
I35V	1.14±0.05	0.96±0.02
F45A	1.34±0.19	1.88±0.051
V47A	1.07±0.05	1.24±0.10
L117A	1.08±0.20	0.89±0.18
V119A	1.15±0.22	0.70±0.24

Table S3. Parameters ($c^{Eq.*}$, $c^{kin.*}$ and α-value) from linear fits of data Figure S4.

	$c^{Eq.*}$	$c^{kin.*}$	α
pwt	0.061±0.003	0.021±0.002	0.34±0.02
I18V	0.070±0.007	0.019±0.004	0.28±0.03
I35V	0.056±0.004	0.018±0.001	0.32±0.01
F45A	0.068±0.002	0.036±0.001	0.53±0.03
V47A	0.053±0.004	0.023±0.003	0.44±0.03
L117A	0.056±0.003	0.017±0.002	0.31±0.02
V119A	0.059±0.004	0.014±0.004	0.24±0.05

SI Figures and Legends

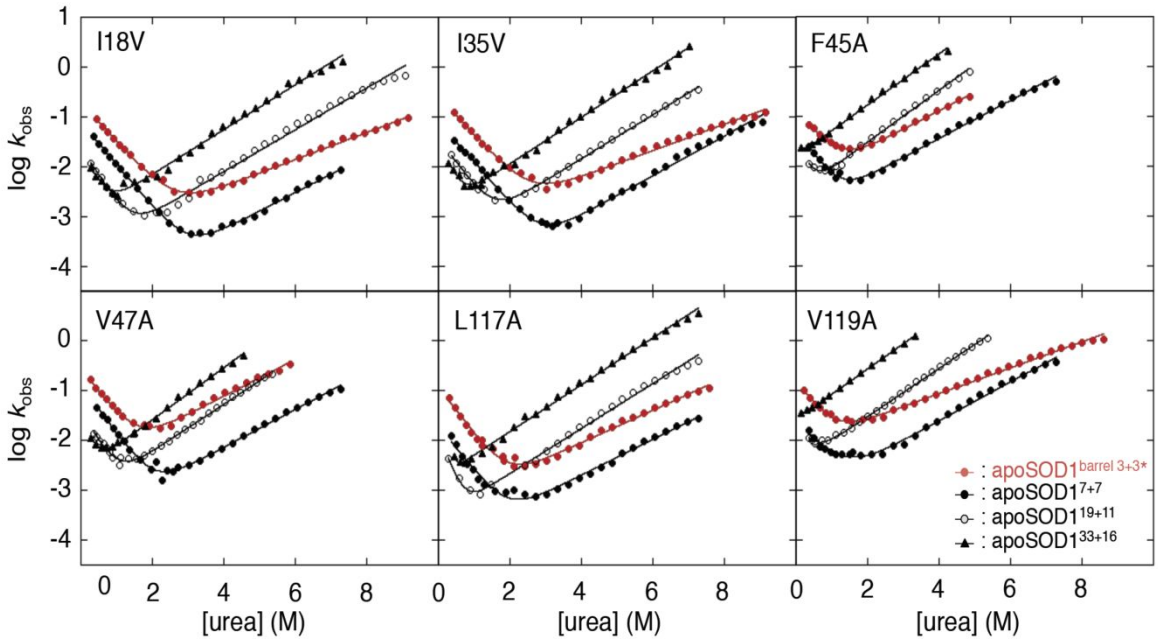

Figure S1. The folding and unfolding kinetics of the apoSOD1 ϕ-value mutations, where k_{obs} is in units of s^{-1}. The parameters obtained by fitting of manuscript Eq. 5 are shown in manuscript Table 4.

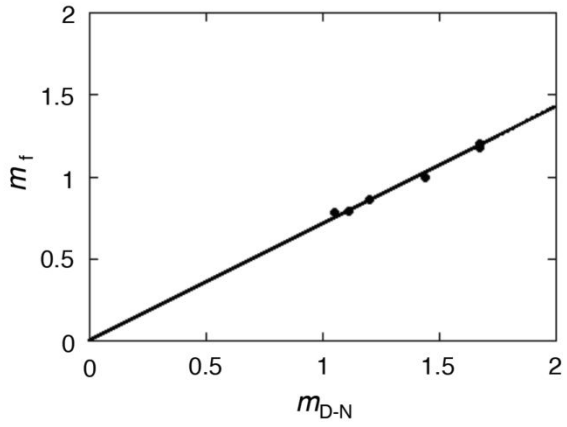

Figure S2. The plot of m_f vs. $m_{D\text{-}N}$ shows a linear relationship with $\beta^{\ddagger} = m_f / m_{D\text{-}N} = 0.71$, and suggests no transition-state movement following loop extension. Values are from Table 1.

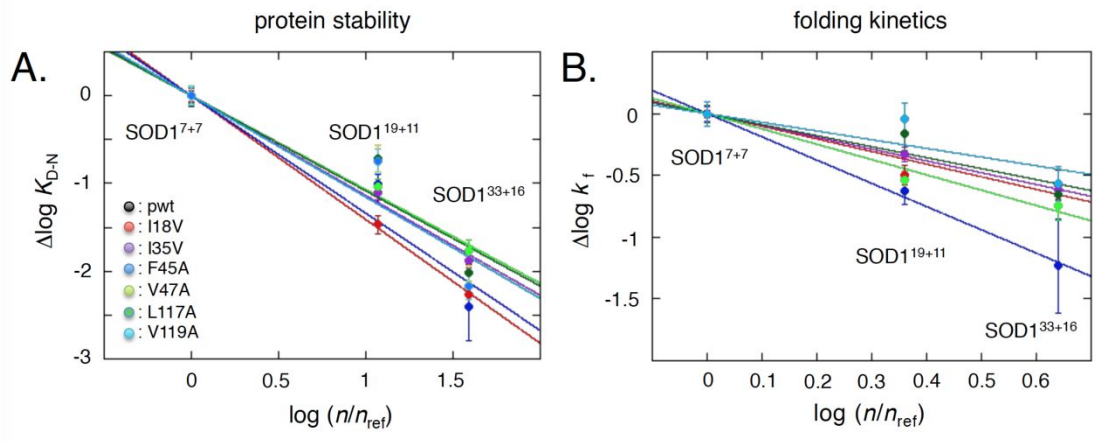

Figure S3. Effects on the apoSOD1 ϕ–value mutations on stability ($\log K_{D-N}$, Eq. 2) and refolding kinetics ($\log k_f$, Eq. 3) upon loop-length alteration ($\log n/n_{ref}$, Eq. 4). The slopes $c^{Eq.}$ and $c^{kin.}$ are shown in Figure 7B and Table S3. **A**. Effects on protein stability. **B**. Effects on refolding kinetics.

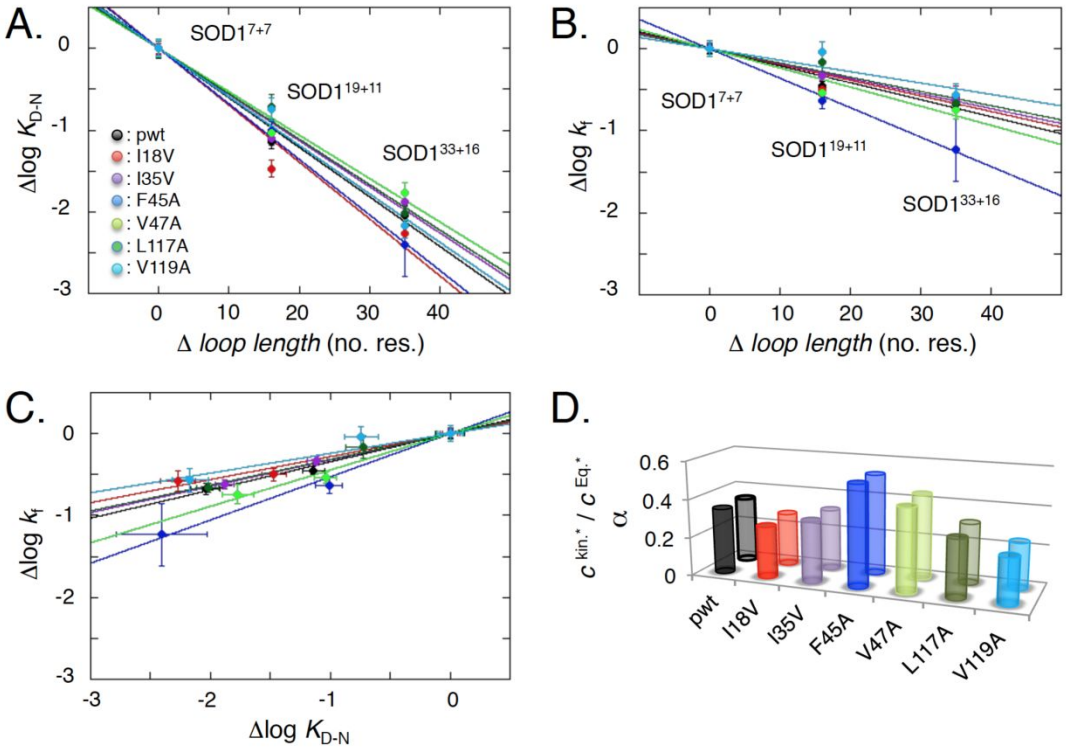

Figure S4. Mutational effects on the apoSOD1 loop-length titration, and derivation of Leffler α. Loop lengths are plotted in linear scale to allow with results in previous studies. Colour coding as in Panel D. **A**. Δlog $K_{D\text{-}N}$ vs. Δ*loop length*, i.e. number of residues, where the slopes denote $c^{kin.*}$. **B**. Δlog k_f vs. Δ*loop length*, where the slopes denote $c^{Eq.*}$. **C**. Δlog k_f vs. Δlog $K_{D\text{-}N}$ plots, where the slopes represent the Leffler α values [3]. **D**. Comparison of the values of $c^{kin.*}$ / $c^{Eq.*}$ derived from panels A and B (front bars), and the Leffler α values derived from panel D (rear bars). Values are shown in Table S3.

SI References

1. Wang, H.; Lang, L.; Logan, D. T.; Danielsson, J.; Oliveberg, M., Tricking a Protein To Swap Strands. *Journal of the American Chemical Society* **2016**, *138* (48), 15571-15579.
2. Nordlund, A.; Oliveberg, M., Folding of Cu/Zn superoxide dismutase suggests structural hotspots for gain of neurotoxic function in ALS: parallels to precursors in

amyloid disease. *Proceedings of the National Academy of Sciences of the United States of America* **2006,** *103* (27), 10218-23.
3. Sanchez, I. E., Protein folding transition states probed by loop extension. *Protein science : a publication of the Protein Society* **2008,** *17* (1), 183-6.