

Supplementary Table 1. Crystal data collection statistics.

X-ray wavelength (Å)	0.9792	
Space group	P2 ₁ 2 ₁ 2 ₁	
Unit cell dimensions	a = 31.6 Å, b = 43.6 Å, c = 65.6 Å, α = β = γ = 90°	
Resolution* (Å)	26.2 – 1.25	(1.27 - 1.25)
No. of unique reflections	23934	(749)
Completeness	92.5%	(59.3%)
R-merge	0.053	(0.48)
CC1/2 (Å ²)	-	(0.82)
I/σ	10.9	(2.04)
Redundancy	6.1	(3.1)
Wilson plot B-factor (Å ²)	10.9	
Molecules per asymmetric unit	1	
No. of protein residues	92	

* Numbers in parenthesis are shown for the highest resolution shell.

Supplementary Table 2. Structure refinement statistics.

Resolution range (Å)	26.2 - 1.25	(1.28 – 1.25)
Reflections	23883	(1228)
σ cutoff	None	
R-value (all) (%)	12.3	
R-value (R-work) (%)	12.2	(20.7)
Free R-value (%)	14.9	(24.6)
Rms deviations from ideal geometry		
bond length (Å)	0.011	
angle (degrees)	1.53	
chiral (Å)	0.096	
No. of atoms		
protein	771	
water	148	
Mean B-factor (Å²)		
all atoms	17.3	
protein atoms	14.3	
protein main chain	11.9	
protein side chain	16.3	
water	32.7	
Molprobit Ramachandran plot statistics		
Residues in favored regions (%)	100.0	
Residues in allowed regions (%)	100.0	
Residues in disallowed region (%)	0.0	

Supplementary Figure Legend

Fig. 1. Malate dehydrogenase (MDH) refolding time course. MDH was heat denatured and diluted into chaperone mixtures including the indicated J-protein and Hsp70, as well as Hsp104 and Sse1. Aliquots were removed at the indicated times and enzymatic activity determined. Activity of non-denatured enzyme was taken as 100%. Hsp70: Ssa1 (Hsp70 WT) or Ssa1 lacking the EEVD (Hsp70 Δ EEVD) (a) Sis1, Xdj1, and Ydj1 with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 1c. (b) Sis1 and Sis1 with Xdj1 J-domain (J_{Xdj1}Sis1) or Ydj1 J-domain (J_{Ydj1}Sis1) with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 2b. (c) Sis1 WT and chimeras having substituted segments 14-20 (A), 38-44 (B) or 52-61 (C) of Ydj1 with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 3c. (d) Sis1 WT and variants with indicated substitutions with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 4b. (e) Sis1 WT, E50A and R73A with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 5c. (f) Sis1 WT and variants having indicated lysines substituted by asparagine with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 6b. (g) Sis1 WT and Sis1 variants having lysine substitutions with or without additional substitution at E50 or R73, with Hsp70 WT or Hsp70 Δ EEVD. Activity at 90 min, Fig. 6c.

Supplementary Fig. 1.

