

Table 2 Kinetic parameters of src SH3 folding<sup>1</sup>

	Mutant	ln(k <sub>f</sub> )	ln(k <sub>u</sub> )	m <sub>f</sub>	m <sub>u</sub>	ΔΔG <sub>u</sub>	Φ <sub>f</sub>
β1	WT	3.55 ± 0.03	1.07 ± 0.04	1.02 ± 0.019	0.54 ± 0.013	NA	NA
	T9A	3.67 ± 0.03	2.35 ± 0.04	1.01 ± 0.037	0.46 ± 0.017	-0.64 ± 0.08	-0.11 ± 0.04
	F10A	3.41 ± 0.03	2.39 ± 0.03	1.06 ± 0.029	0.51 ± 0.012	-0.84 ± 0.07	0.10 ± 0.03
	F10I	3.67 ± 0.04	4.06 ± 0.03	1.08 ± 0.11	0.44 ± 0.091	-1.65 ± 0.17	-0.05 ± 0.02
	V11A	3.45 ± 0.04	3.79 ± 0.03	0.85 ± 0.057	0.55 ± 0.033	-1.64 ± 0.12	0.03 ± 0.02
	A12G	3.46 ± 0.03	2.73 ± 0.05	1.08 ± 0.049	0.47 ± 0.026	-1.00 ± 0.09	0.05 ± 0.02
	L13A	3.62 ± 0.02	3.87 ± 0.06	1.20 ± 0.098	0.36 ± 0.02	-1.49 ± 0.13	-0.03 ± 0.01
	Y14A	3.59 ± 0.03	1.68 ± 0.03	0.96 ± 0.042	0.50 ± 0.011	-0.31 ± 0.06	-0.08 ± 0.09
	D15A	3.70 ± 0.03	2.02 ± 0.08	0.98 ± 0.051	0.41 ± 0.03	-0.43 ± 0.13	-0.22 ± 0.10
	Y16A	3.40 ± 0.07	4.94 ± 0.06	↘	0.39 ± 0.018	-2.27 ± 0.26	0.03 ± 0.03
RT loop	Y16F	3.54 ± 0.04	1.37 ± 0.02	0.97 ± 0.036	0.65 ± 0.093	-0.18 ± 0.10	↘
	S18A	3.80 ± 0.04	0.47 ± 0.06	0.96 ± 0.028	0.55 ± 0.051	0.52 ± 0.10	0.28 ± 0.06
	R19A	3.64 ± 0.04	1.22 ± 0.07	1.08 ± 0.034	0.61 ± 0.017	-0.07 ± 0.08	↘
	T20A	3.69 ± 0.03	1.10 ± 0.04	1.01 ± 0.033	0.52 ± 0.017	0.06 ± 0.07	↘
	T22A	3.60 ± 0.03	1.14 ± 0.03	1.00 ± 0.03	0.52 ± 0.016	-0.01 ± 0.07	↘
	D23A	3.43 ± 0.05	1.91 ± 0.07	1.06 ± 0.083	0.51 ± 0.043	-0.56 ± 0.13	0.13 ± 0.07
	L24A	2.76 ± 0.03	3.44 ± 0.03	1.45 ± 0.06	0.45 ± 0.0089	-1.79 ± 0.09	0.26 ± 0.01
	S25A	3.49 ± 0.04	2.40 ± 0.03	0.95 ± 0.041	0.58 ± 0.04	-0.82 ± 0.08	0.03 ± 0.04
	F26A	2.17 ± 0.03	3.21 ± 0.04	↘	0.40 ± 0.0096	-1.97 ± 0.10	0.40 ± 0.01
	K27A	3.60 ± 0.04	1.79 ± 0.05	1.01 ± 0.051	0.65 ± 0.045	-0.44 ± 0.11	-0.06 ± 0.09
diverging turn	K28A	3.73 ± 0.03	1.45 ± 0.04	0.88 ± 0.017	0.49 ± 0.022	-0.09 ± 0.07	↘
	G29A	2.30 ± 0.04	2.74 ± 0.03	1.54 ± 0.088	0.43 ± 0.017	-1.66 ± 0.12	0.44 ± 0.02
	E30A	1.50 ± 0.03	2.34 ± 0.03	1.09 ± 0.054	0.65 ± 0.027	-1.94 ± 0.13	0.62 ± 0.02
	R31A	3.43 ± 0.03	1.45 ± 0.10	1.00 ± 0.06	0.57 ± 0.035	-0.32 ± 0.12	0.23 ± 0.08
	L32A	1.40 ± 0.08	2.61 ± 0.11	↘	↘	-2.26 ± 0.37	0.55 ± 0.05
n-src loop	L32V	3.10 ± 0.04	2.68 ± 0.03	1.03 ± 0.05	0.56 ± 0.031	-1.21 ± 0.11	0.22 ± 0.02
	Q33A	3.17 ± 0.04	1.00 ± 0.04	1.11 ± 0.033	0.55 ± 0.027	-0.21 ± 0.09	↘
	I34A	1.40 ± 0.04	-0.63 ± 0.03	1.39 ± 0.027	0.51 ± 0.03	-0.32 ± 0.12	↘
	I34V	3.09 ± 0.05	0.75 ± 0.09	1.16 ± 0.093	0.50 ± 0.019	-0.09 ± 0.12	↘
	V35A	2.6 ± 0.06	1.33 ± 0.05	1.27 ± 0.046	0.59 ± 0.041	-0.77 ± 0.12	0.77 ± 0.05
	N36A	3.64 ± 0.05	1.50 ± 0.05	1.12 ± 0.035	0.47 ± 0.017	-0.20 ± 0.09	↘
	N37A	3.91 ± 0.03	1.30 ± 0.04	0.94 ± 0.032	0.59 ± 0.023	0.07 ± 0.06	↘
	G40A	2.99 ± 0.05	1.02 ± 0.06	0.92 ± 0.037	0.55 ± 0.036	-0.28 ± 0.08	↘
	W42A	3.03 ± 0.05	2.83 ± 0.02	1.62 ± 0.048	0.45 ± 0.0084	-1.29 ± 0.10	0.25 ± 0.03
	W43A	3.24 ± 0.03	2.97 ± 0.06	1.16 ± 0.069	0.35 ± 0.025	-1.20 ± 0.11	0.15 ± 0.02
β3	W43I	4.45 ± 0.05	3.30 ± 0.03	1.10 ± 0.081	0.62 ± 0.073	-0.77 ± 0.13	↘
	L44A	2.10 ± 0.05	2.52 ± 0.05	1.88 ± 0.065	0.41 ± 0.0095	-1.64 ± 0.15	0.54 ± 0.03
	A45G	1.71 ± 0.07	0.86 ± 0.06	1.70 ± 0.044	0.39 ± 0.011	-0.92 ± 0.15	1.20 ± 0.08
	H46A	3.47 ± 0.03	2.00 ± 0.02	0.99 ± 0.029	0.58 ± 0.016	-0.62 ± 0.06	0.08 ± 0.04
	S47A	1.23 ± 0.04	1.29 ± 0.03	1.50 ± 0.035	0.44 ± 0.0074	-1.46 ± 0.09	0.95 ± 0.03
	L48A	2.82 ± 0.04	1.38 ± 0.03	1.20 ± 0.059	0.51 ± 0.019	-0.61 ± 0.08	0.72 ± 0.04
	S49A	2.98 ± 0.06	0.07 ± 0.06	1.20 ± 0.061	0.61 ± 0.026	0.18 ± 0.11	↘
distal hairpin	T50A	0.99 ± 0.05	1.59 ± 0.02	1.84 ± 0.054	0.47 ± 0.012	-1.79 ± 0.10	0.86 ± 0.02
	G51A	1.39 ± 0.04	1.02 ± 0.06	1.51 ± 0.11	0.41 ± 0.019	-1.21 ± 0.14	1.06 ± 0.06
	Q52A	3.29 ± 0.03	1.41 ± 0.08	1.03 ± 0.059	0.49 ± 0.021	-0.35 ± 0.12	0.45 ± 0.09
	T53A	2.33 ± 0.06	1.65 ± 0.04	1.38 ± 0.048	0.56 ± 0.021	-1.11 ± 0.11	0.68 ± 0.03
	G54A	3.79 ± 0.02	4.59 ± 0.05	1.60 ± 0.17	0.36 ± 0.02	-1.81 ± 0.12	-0.08 ± 0.01
	Y55A	2.10 ± 0.04	2.34 ± 0.04	1.64 ± 0.063	0.39 ± 0.0062	-1.52 ± 0.10	0.56 ± 0.02
	I56A	1.34 ± 0.03	2.02 ± 0.02	1.64 ± 0.067	0.46 ± 0.016	-1.84 ± 0.10	0.71 ± 0.02
β4	P57A	2.98 ± 0.04	2.89 ± 0.04	1.36 ± 0.098	0.45 ± 0.014	-1.36 ± 0.11	0.24 ± 0.02
	S58A	4.06 ± 0.03	1.14 ± 0.05	0.99 ± 0.034	0.58 ± 0.023	0.24 ± 0.08	↘
	N59A	3.55 ± 0.03	1.28 ± 0.04	0.87 ± 0.041	0.58 ± 0.016	-0.14 ± 0.07	↘
	Y60A	3.48 ± 0.05	0.65 ± 0.04	1.06 ± 0.034	0.47 ± 0.034	0.23 ± 0.09	↘
3 <sub>10</sub> helix	V61A	3.67 ± 0.04	3.29 ± 0.02	1.15 ± 0.044	0.44 ± 0.013	-1.18 ± 0.09	-0.06 ± 0.03
	A62G	3.59 ± 0.04	1.97 ± 0.03	1.12 ± 0.045	0.55 ± 0.019	-0.53 ± 0.08	-0.02 ± 0.07
	P63A	3.64 ± 0.04	1.44 ± 0.08	1.04 ± 0.062	0.48 ± 0.031	-0.14 ± 0.11	↘
	S64A	3.66 ± 0.03	0.40 ± 0.04	0.95 ± 0.026	0.59 ± 0.018	0.44 ± 0.06	0.14 ± 0.05
β5	D65A	3.69 ± 0.03	0.98 ± 0.05	0.92 ± 0.032	0.57 ± 0.02	0.13 ± 0.07	↘

<sup>1</sup>All experiments were done at pH 6 and 295 K. Kinetic measurements were done by stopped-flow fluorescence. Rate of folding (k<sub>f</sub>) is reported at 0.3 M Gnd; rate of unfolding (k<sub>u</sub>) is reported at 4 M Gnd to avoid extrapolation. ΔΔG<sub>u</sub>, Φ<sub>f</sub> and standard errors were calculated as described in the Methods section.

<sup>2</sup>Parameters could not be reliably measured.

<sup>3</sup>Mutation has no (or very small) effect on stability, that is, ΔΔG<sub>u</sub> ≤ 0.20 kcal mol<sup>-1</sup>.

<sup>4</sup>Mutation either increases or decreases both k<sub>f</sub> and k<sub>u</sub>.