

Table 1. Kinetic parameters of folding from stopped-flow experiments*

Mutant	$k_f(0.5\text{ M})/\text{s}^{-1}$	m_f/M^{-1}	$k_u(5.0\text{ M})/\text{s}^{-1}$	m_u/M^{-1}	$\Phi_{\text{TS(UF)}}$
wt	50.4 ± 3.6	1.78 ± 0.05	0.39 ± 0.02	0.87 ± 0.04	
L3A	50.2 ± 2.7	1.99 ± 0.05	2.85 ± 0.07	0.86 ± 0.02	0.002 ± 0.045
E5V	83.3 ± 4.7	1.97 ± 0.04	0.17 ± 0.01	0.98 ± 0.05	0.377 ± 0.076

*Kinetic parameters were extracted from the chevron plots of **L3A** and **E5V** Fyn SH3 (in the wt background) shown in Fig. 7 assuming a two-state folding model $\text{U} \leftrightarrow \text{F}$, with $\Phi_{\text{TS(UF)}}$ calculated from $k_{\text{UF}} = k_f(0.5\text{ M})$ and $k_{\text{FU}} = k_u(5.0\text{ M})$ according to Eqs. 1 and 2 as described in the section “Methodology of Φ -Value Analysis.” Errors reported here are the fitting errors when using Eq. S1, Fig. 7, propagated to obtain error estimates for $\Phi_{\text{TS(UF)}}$. Unfortunately, Φ values could not be measured by stopped-flow fluorescence for **L3A/A39V/N53P/V55L** and **E5V/A39V/N53P/V55L** Fyn SH3 themselves because the dead time of our stopped-flow instrument precluded collection of a sufficient number of data points along the folding arm of the chevron plot for these very fast-folding and unstable mutants (data not shown).