Table II. **Biochemical properties of mutant cofilins**

Parameter	Cofilin	Cofilin-M2	Cofilin-M3
Stability: ΔG (kcal mol^{-1})	9.10	7.91	10.32
Mg-ATP-actin monomer affinity, K_d (μM)	0.08	0.11	0.17
ADP-actin filament apparent affinity, K_a (μM^{-1})	0.15	0.012	0.08
ADP-actin filament affinity, K_d (μM)	0.33	1.7	0.89
ADP-actin filament binding cooperativity, ω	19.9	48.7	14.2
ADP-actin filament association rate constant, k_+ (μM^{-1} s^{-1})	12.5	1.4	3.8
ADP-actin filament dissociation rate constant, k_- (s^{-1})	200	4600	140
Ratio of k_-/k_+ (μM)	16	3300	37
Optimal severing concentration (nM)	10	500	100
Maximum serving rate (events per 1,000 subunits s^{-1})	0.032	0.009	0.012

Stability was determined from the dependence of the intrinsic fluorescence on urea concentration (Fig. S2 B). Affinity for ATP-actin monomers was measured from the effect on nucleotide exchange (Fig. 1 C and S2 C). Association equilibrium constants K_a and cooperativity ω were calculated from best fits to equilibrium binding data of Fig. 1 D. Apparent dissociation equilibrium constants K_d were calculated as $K_d = 1/(\omega * K_a)$. Association k_+ and dissociation k_- rate constants for cofilin binding pyrenyl-actin filaments were calculated from kinetic curves of Figs. 1, E–E′, and S2 D. Severing rates were measured by TIRF microscopy (Figs. 1 F and S2 E).