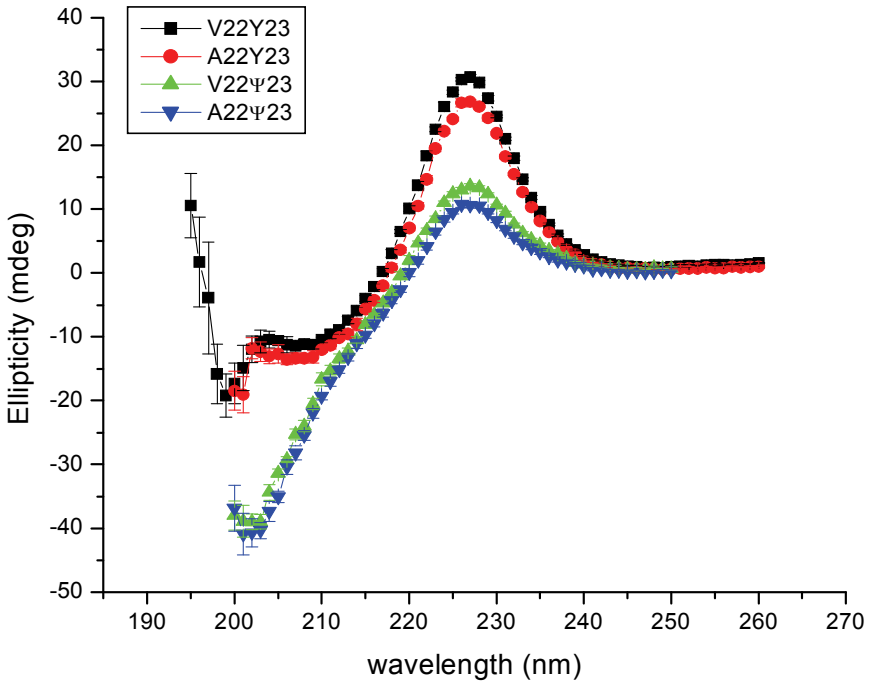
Supplementary Information for "Localized Thermodynamic Coupling between Hydrogen Bonding and Microenvironment Polarity Substantially Stabilizes Proteins"

Jianmin Gao, Daryl A. Bosco, Evan T. Powers*, and Jeffery W. Kelly*

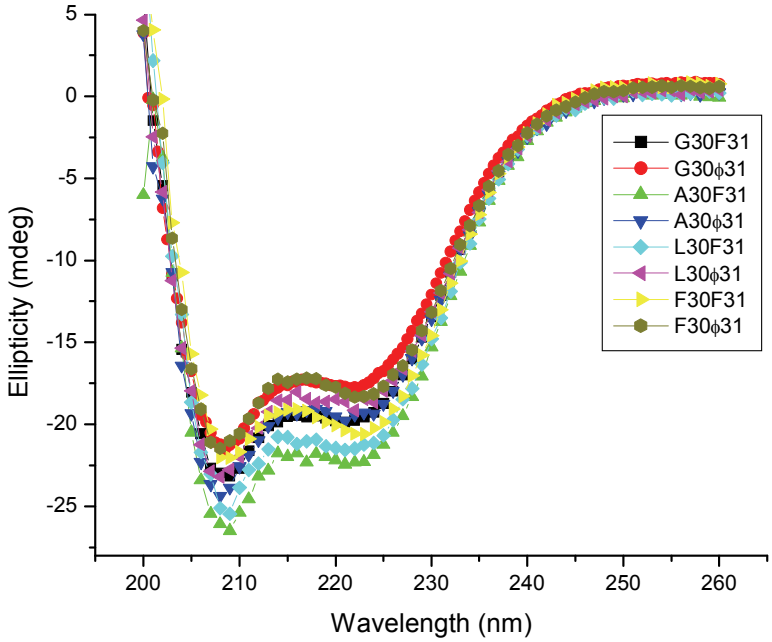
Departments of Chemistry and Molecular and Experimental Medicine and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC265, La Jolla, CA 92037, USA

*To whom correspondence should be addressed. E-mail: jkelly@scripps.edu, epowers@scripps.edu

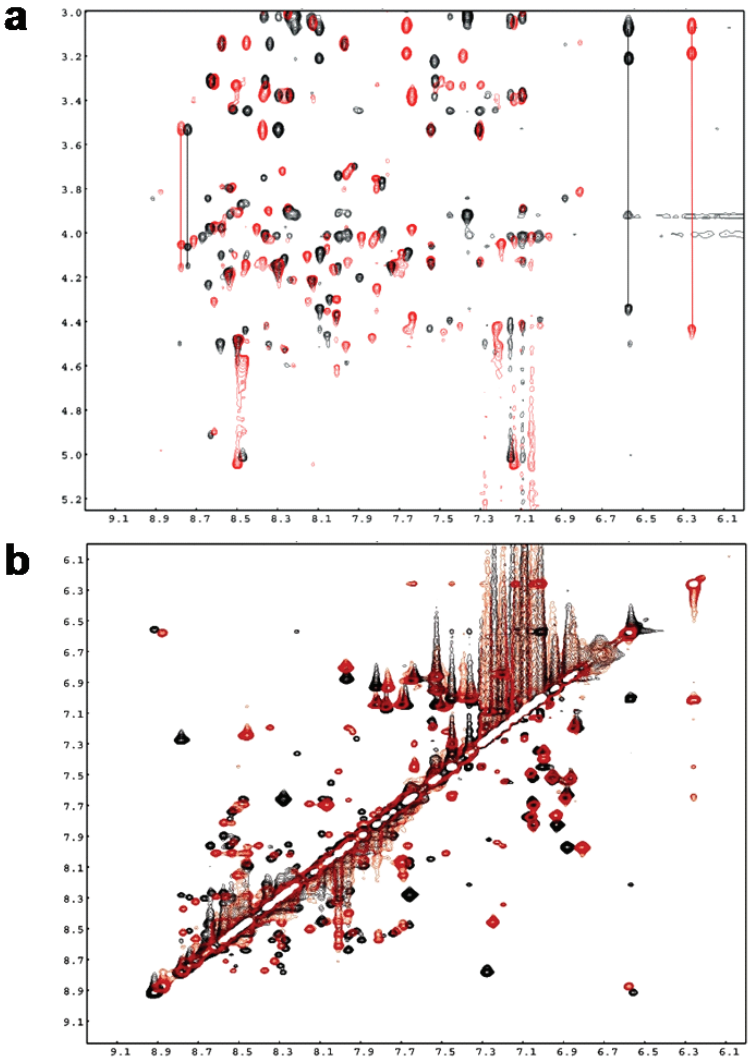
Supplementary Figure 1. Circular dichroism spectra of loop-1–modified Pin WW domain variants. The characteristic maxima were observed for all variants, indicating that they adopt a native WW domain fold.



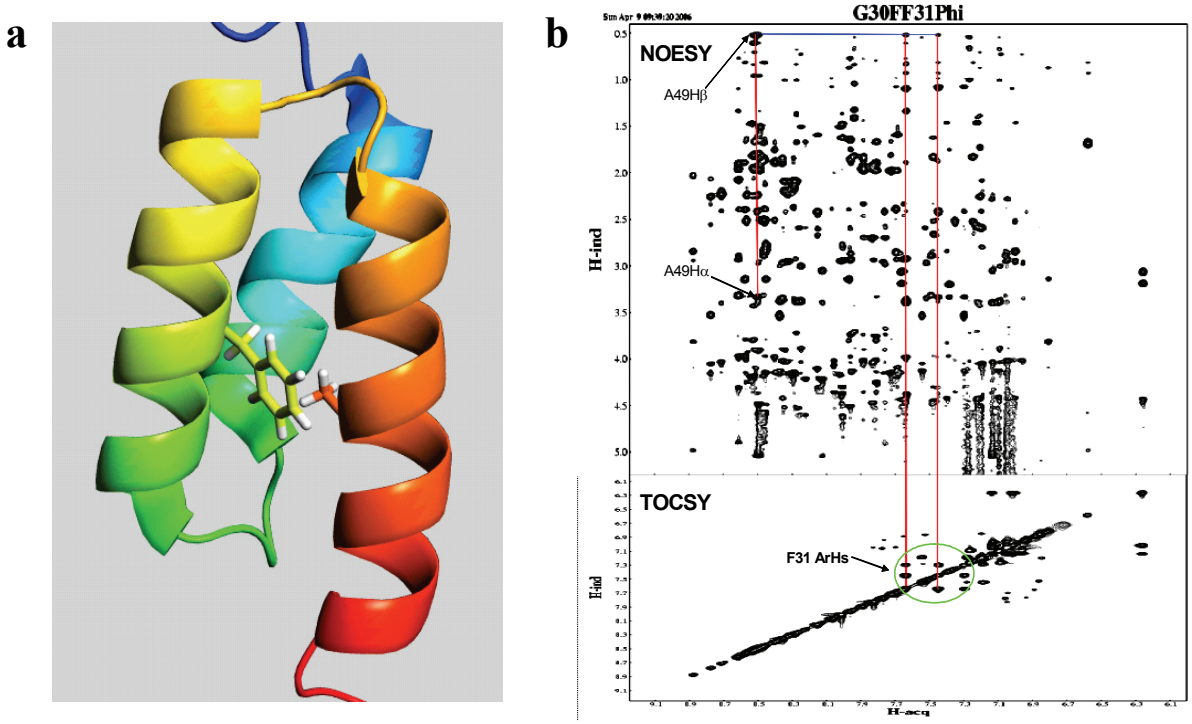
Supplementary Figure 2. Circular dichroism spectra of protein A* variants. Far-ultraviolet circular dichroism spectra exhibit ellipticity minima at 208 nm and 222 nm, characteristic of α-helical structures, indicating that all protein A* variants adopt the native fold.



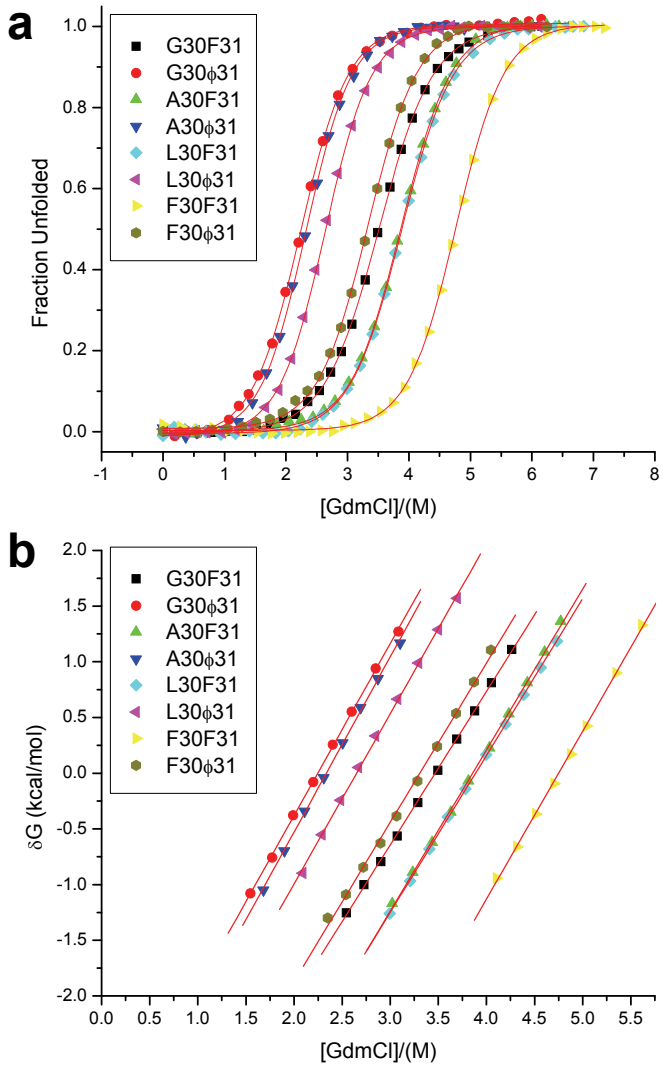
Supplementary Figure 3. ^{1}H-^{1}H NOESY spectra of protein A* variants G30F (black) and G30F/F31ϕ (red). (**a**) NH-Hα region. (**b**) NH-NH region. The fact that G30F and G30F/F31ϕ exhibit analogous patterns in the fingerprint regions of their 2D NOESY spectra suggests that the ester mutant G30F/F31ϕ adopts the same structure as the all amide variant G30F. The axes in both (**a**) and (**b**) are ^{1}H chemical shifts in p.p.m.



Supplementary Figure 4. ^{1}H-^{1}H NOESY data for protein A* variant G30F/F31ϕ. (**a**) Native protein A structure exhibiting the signature contact between Phe31 and Ala49 side chains. (**b**) Combining NOESY and TOCSY allows NOESY cross peak assignment. Cross peaks between aromatic protons of Phe31 and β-protons of Ala49 indicates that this interaction, which is characteristic of the native state, is present in the G30F/F31ϕ variant of protein A*. The axes in (**b**) are ^{1}H chemical shifts in p.p.m.



Supplementary Figure 5. Denaturation analysis of protein A* variants. (**a**) Guanidine hydrochloride denaturation curves of protein A* variants showing two-state cooperative transitions. (**b**) Thermodynamic data were extracted from fitting the denaturation curves according to a two-state model and are summarized in Table S1.



Supplementary Table 1. Summary of thermodynamic data for the protein variants used in this study. C_m is the denaturant concentration at which the protein in question is 50% unfolded and the m value is the slope of a plot of ΔG_f vs. the concentration of denaturant.

Protein	variants	C_m (M)	*m* values (kcal mol^{-1} M^{-1})	$-\Delta G_f$ (kcal mol^{-1}
Protein A*	Wild-type (A1)	3.51±0.01	1.38±0.01	4.78±0.03
	F31ϕ (E1)	2.24±0.01	1.54±0.02	3.47±0.03
	G30A (A2-A)	3.87±0.01	1.44±0.02	5.58±0.05
	G30A/F31ϕ (E2-A)	2.32±0.01	1.57±0.02	3.67±0.04
	G30L (A2-L)	3.88±0.01	1.41±0.01	5.47±0.02
	G30L/F31ϕ (E2-L)	2.44±0.01	1.53±0.02	3.77±0.06
	G30F (A2-F)	4.76±0.01	1.49±0.02	7.15±0.06
	G30F/F31ϕ (E2-F)	3.33±0.01	1.43±0.03	4.73±0.07
Loop-1–modified Pin WW domain	V22A (A1)	4.64±0.02	0.99±0.01	4.58±0.01
	V22A/Y23ψ (E1)	1.47±0.02	1.04±0.02	1.52±0.02
	Wild-type (A2)	5.85±0.02	0.99±0.02	5.65±0.11
	Y23ψ (E2)	1.52±0.03	0.92±0.03	1.42±0.05
Pin WW domain	Wild-type (A1)			3.32±0.1†
	W11ω (E1)			2.20±0.05†
	G30DF (A2)	3.34±0.02	1.04±0.02	3.47±0.04
	G30DF/W11ω (E2)	2.11±0.02	1.12±0.01	2.34±0.04

† From Deechongkit et al. *J. Am. Chem. Soc.* **126**, 16762-16771 (2004).

Supplementary Table 2. Summary of perturbation energies and backbone solvent accessible surface areas from amide-to-ester mutants in the literature. Only mutants in which the perturbed amide donates, but does not accept, a hydrogen bond are included (type 3 mutants according to the nomenclature of Powers *et al.*[1]).

Protein	Mutation	Backbone exposed surface area (Å^2)[a]	Fractional backbone exposure	$\Delta G_{f,wt}-\Delta G_{f,mut}$
Pin WW domain[2,3] (pdb ID: 2KCF)	W11ω	22.4	0.53 (exposed)	–1.1
	E12ε	5.0	0.11 (buried)	–1.5
	R14ρ	5.4	0.12 (buried)	–3.9
	S16σ	16.6	0.40 (exposed)	–1.1
	S19σ	32.5	0.77 (exposed)	–0.6
	Y23ψ	0.4	0.01 (buried)	–2.2
	N30ν	17.7	0.39 (exposed)	–1.8
	A31α	13.1	0.28 (exposed)	–0.8
	Q33θ	4.9	0.11 (buried)	–3.1
T4 lysozyme (pdb ID: 2LZM)[4]	(I)L50λ	28.4	0.68 (exposed)	–0.7
Eglin C (pdb ID: 1EGL)[5]	G70γ	20.7	0.24 (exposed)	–0.4
P22 Arc repressor (pdb ID: 1MYK)[6]	L8λ	5.8	0.14 (buried)	–1.2
V9F mutant of villin headpiece (pdb ID: 1YRF)[7]	F10ϕ	10.2	0.24 (exposed)	–1.5
Protein L (pdb ID: 1HZ6)[8]	F106ϕ	1.8	0.04 (buried)	–0.55
Average, buried sites	n/a	n/a	≤ 0.2	–2.1 ± 0.5
Average, exposed sites	n/a	n/a	> 0.2	–1.0 ± 0.2

[a]Exposed surface areas were calculated using the program GETAREA[9].

Supplementary References

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