Supporting Information for:

Cross Validation of the Structure of a Transiently Formed and Low Populated FF Domain Folding Intermediate Determined by Relaxation Dispersion NMR and CS-Rosetta

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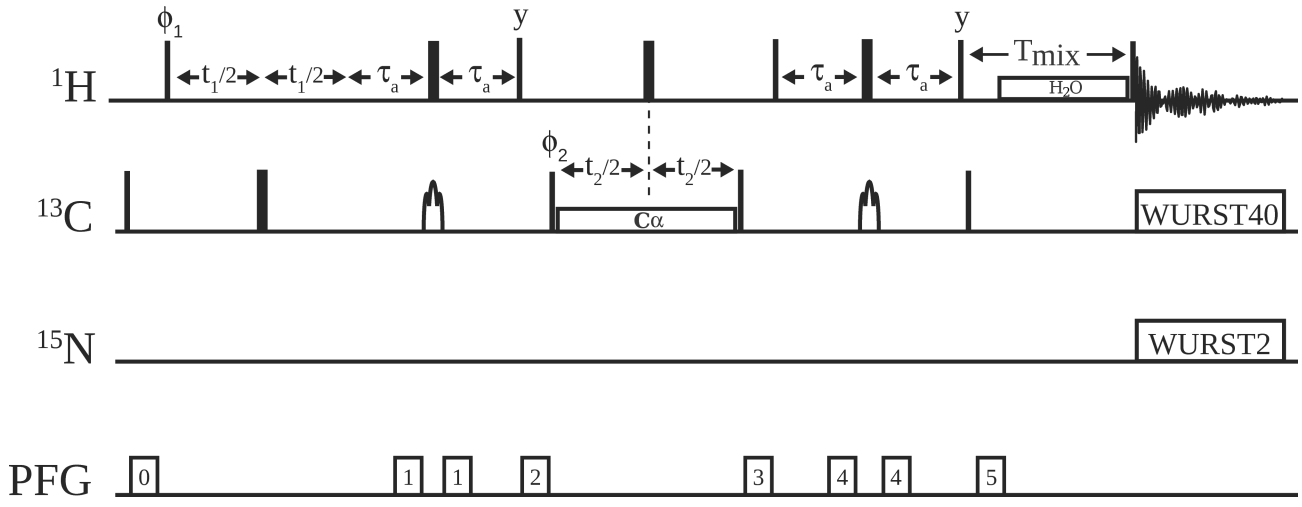

Figure S1. Pulse scheme for measurement of NOEs from Ala residues. Correlations of the form (ω^1H_{ALA},$\omega^{13}C_{ALA}$,ω^1H) are detected where ω^1H_{ALA} and $\omega^{13}C_{ALA}$ are Ala methyl chemical shifts and ω^1H is the chemical shift of a proximal proton. All narrow (wide) rectangular pulses are applied with 90° (180°) flip angles along the x axis, and unless indicated otherwise at the highest possible power levels. ^1H and ^{13}C radio frequency carriers are positioned at water and 19 ppm, respectively, until signal acquisition when the carbon carrier jumps to 75 ppm. During the mixing time a 30 Hz ^1H saturation field is applied to the water line. Shaped ^{13}C pulses (2 ms, Re-Burp profile[1], centered at 21.5 ppm, 800 MHz) are selective to excite primarily $^{13}C^{\beta}$ of Ala (along with other methyl spins). $^{13}C^{\alpha}$ decoupling during t_1 is achieved using constant adiabaticity WURST-8 decoupling[2], sweeping from 50 to 58 ppm. A second field is applied, swept from -12 to -20 ppm, to reduce Bloch-Siegert effects for the Ala methyl ^{13}C spins[3]. The net decoupling field strength is 0.59 kHz (rms 0.33 kHz). ^{13}C broad-band decoupling is achieved with a 2.6 kHz (max rf; 2.3 kHz rms) WURST-40 field centered at 75 ppm[2]. ^{15}N decoupling during acquisition was carried out using a WURST-2 field[2], with a bandwidth of 27 ppm,

centered at 116.5 ppm (0.75 kHz max rf; 0.46 kHz rms). The value of τ_a is set to 2 ms. The phase cycle is ϕ_1=x,-x; ϕ_2=2(x),2(-x); rec=x,2(-x),x. Quadrature in F_1 is achieved by States-TPPI[4] of ϕ_2. The gradient durations (ms) and strengths (G/cm, along Z) are: g0=(1,10), g1=(0.3,20), g2=(1,24), g3=(1.4,-40), g4=(0.5,-24), g5=(0.8,20).

Table S1. Structural statistics on the 10 lowest energy structures with no violations out of 100 calculated for I′ using XPLOR-NIH[5].

Restraints used for structure calculations

NOE distances	121
Dihedral angles	58

Average RMSD from idealized covalent geometry

Bond (Å)	0.0016±0.0002
Bond angles (°)	0.376±0.004
Improper (°)	0.323±0.068

Average RMSD from experimental distance restraints

NOEs (Å)	0.033±0.012

Average RMSD within the structural ensemble

Backbone atoms (Å)	0.79±0.23
Heavy atoms (Å)	1.22±0.25

Procheck[6] Ramachandran analysis

Residues in most favoured regions	85.0%
Residues in additional allowed regions	12.5%
Residues in generously allowed regions	2.5%
Residues in disallowed regions	0.0%

References

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(5) Schwieters, C. D.; Kuszewski, J. J.; Tjandra, N.; Clore, G. M. *J. Magn. Reson.* **2003**, *160*, 65-73.

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