Supporting Information

Determination of Leu Side-Chain Conformations in Excited Protein States by NMR Relaxation Dispersion

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Protein sample preparation:

A ^{2}H-, Ile-[$\delta1^{13}CH_3$], Leu,Val-[$^{13}CH_3$,$^{13}CH_3$]-labeled sample of the G48M Fyn SH3 domain was expressed and purified as described previously[1]. The protein concentration was ≈1 mM and the sample contained 50 mM sodium phosphate, 1 mM EDTA, 1mM NaN_3, 90%/10% H_2O/D_2O, pH 7.0. A sample of the A39V/N53P/V55L mutant of the Fyn SH3 domain was prepared by protein over-expression in M9 minimal media with 3g/L [1-^{13}C]-glucose as the sole carbon source, as described in detail previously[2,3]. The sample was 1.0 mM in protein, 0.2 mM EDTA, 0.05% NaN_3, 50 mM sodium phosphate, 90%/10% H_2O/D_2O, pH 7.0.

Resonance assignments of A39V/N53P/V55L Fyn SH3:

Sequence-specific resonance assignments of methyl [^{13}C,^{1}H] correlations of the A39V/N53P/V55L Fyn SH3 domain were obtained from C(CO)NH[4] and [^{1}H,^{15}N] NOESY-HSQC[5] spectra of suitably isotope labeled samples and from comparison with spectra of WT Fyn and G48M Fyn SH3 domains that were assigned using standard triple resonance based approaches[1,6].

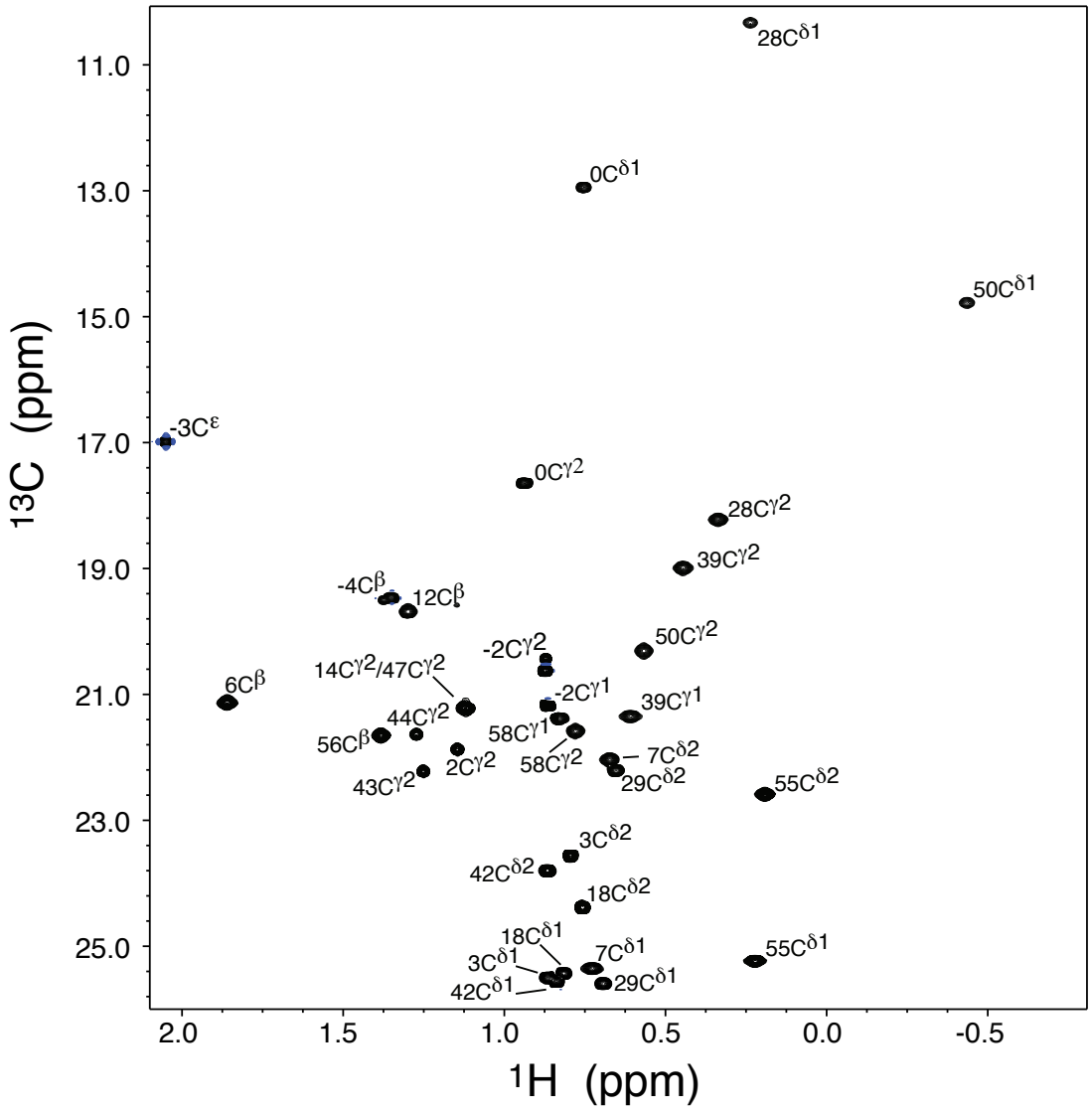

Figure S1. Methyl ^{13}C, ^{1}H correlation map of the A39V/N53P/V55L Fyn SH3 domain, 800 MHz, 20ºC.

NMR spectroscopy and data analysis:

Single quantum ^{13}C relaxation dispersion profiles[7] were recorded at 23 ºC (20 ºC) and at magnetic fields strengths of 11.4 and 18.8 T for the G48M (A39V/N53P/V55L) Fyn SH3 domain. Relaxation dispersion profiles, $R_{2,\mathrm{eff}}(\nu_{\mathrm{CPMG}})$, were generated from peak intensities, $I_1(\nu_{\mathrm{CPMG}})$, in a series of 2D ^{1}H-^{13}C correlation maps measured as a function of CPMG frequency, $\nu_{\mathrm{CPMG}} = 1/(4\tau_{\mathrm{CP}})$, where $2\tau_{\mathrm{CP}}$ is the interval between consecutive 180º-refocusing pulses of the CPMG sequence. Effective relaxation rates were calculated as $R_{2,\mathrm{eff}}(\nu_{\mathrm{CPMG}}) = \ln(I_0/I_1(\nu_{\mathrm{CPMG}}))/T_{\mathrm{relax}}$, where I_0 is the peak intensity in a reference spectrum recorded without the relaxation delay T_{relax} [8]. Dispersion profiles were recorded with T_{relax} = 50 ms (40 ms) and 26 (14) values of ν_{CPMG} ranging from 25 to 1000 Hz (50-950 Hz), along with 2 repeats for error analysis for the G48M (A39V/N53P/V55L) mutant of the Fyn SH3 domain.

All data sets were processed and analyzed with the NMRPipe program[9] and signal intensities were quantified using the program FuDA (http://pound.med.utoronto.ca/software.html). Relaxation dispersion data were analyzed using a two-state exchange model, $A \underset{k_{\mathrm{BA}}}{\overset{k_{\mathrm{AB}}}{\rightleftharpoons}} B$ and the best-fit model parameters were extracted as described previously[10] using the program CATIA which is available from http://pound.med.utoronto.ca/software.html. Briefly, the parameters are obtained by minimization of the target function,

$$\chi^2(\xi) = \sum \frac{\left(R_{2,eff}^{clc}(\xi) - R_{2,eff}^{exp}\right)^2}{\left(\Delta R_{2,eff}^{exp}\right)^2} \qquad \text{(S1)}$$

where $R_{2,eff}^{exp}$ and $\Delta R_{2,eff}^{exp}$ are experimental effective relaxation rates and their uncertainties, respectively, $R_{2,eff}^{clc}(\xi)$ are calculated relaxation rates obtained by numerical

integration of the Bloch-McConnell equation[11], $\zeta = \{x_1,\ldots,x_{npar}\}$ denotes the set of adjustable model parameters (including $\Delta\varpi$) and the summation in eq S1 is over the number of experimental data points.

Table S1. Leu $C^{\delta1}/C^{\delta2}$ chemical shifts of the folded and unfolded states of the G48M Fyn SH3 domain along with p_{trans}.

Residue	Folded (ground) state[a]				Unfolded (excited) state[b]			
	$\delta(C^{\delta1})$ (ppm)	$\delta(C^{\delta2})$ (ppm)	$\Delta\delta(^{13}C^{\delta})$	p_{trans}	$\delta(C^{\delta1})$ (ppm)	$\delta(C^{\delta2})$ (ppm)	$\Delta\delta(^{13}C^{\delta})$	p_{trans}
L3	25.02	24.02	1.00	0.60	24.57±0.01	23.35±0.01	1.22	0.62
L7	25.19	21.88	3.31	0.83	24.71±0.01	23.40±0.01	1.31	0.63
L18	25.29	24.70	0.58	0.56	24.90±0.01	23.03±0.01	1.88	0.69
L29	25.56	22.18	3.38	0.84	24.74±0.01	23.29±0.01	1.45	0.65
L42	25.09	23.49	1.59	0.66	24.84±0.02	23.05±0.01	1.79	0.68

[a]Measured directly from spectra; stereospecific assignments were obtained via the method of Neri[12].

[b]Measured via CPMG relaxation dispersion. Uncertainties are obtained with the covariance matrix method[13].

Table S2. Leu $C^{\delta1}/C^{\delta2}$ chemical shifts of the folded state and the folding intermediate of the A39V/N53P/V55L Fyn SH3 domain along with p_{trans}.

Residue	Folded (ground) state[a]				Folding intermediate (excited) state[b]			
	$\delta(C^{\delta1})$ (ppm)	$\delta(C^{\delta2})$ (ppm)	$\Delta\delta(^{13}C^{\delta})$	p_{trans}	$\delta(C^{\delta1})$ (ppm)	$\delta(C^{\delta2})$ (ppm)	$\Delta\delta(^{13}C^{\delta})$	p_{trans}
L3	25.50	23.80	1.70	0.67	25.50±0.03	24.21±0.01	1.29	0.63
L7	25.35	22.03	3.32	0.83	25.45±0.04	22.67±0.01	2.78	0.78
L18	25.43	24.38	1.05	0.61	25.43±0.02	23.74±0.01	1.69	0.67
L29	25.59	22.21	3.38	0.84	25.59±0.02	22.21±0.08	3.38	0.84
L42	25.56	23.56	2.00	0.70	25.56±0.07	23.20±0.01	2.36	0.74
L55	25.23	22.58	2.65	0.77	25.03±0.02	23.65±0.02	1.38	0.64

[a]Measured directly from spectra; stereospecific assignments were obtained via the method of Neri[12].

[b]Measured via CPMG relaxation dispersion. Uncertainties are obtained with the covariance matrix method[13].

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