Supplementary Material for MS 2013BIOPHYSJ302227:

"NMR determines transient structure and dynamics in the disordered C-terminal domain of WASp interacting protein", Haba, Gross et al.

Expression and purification of WIP^C

A coding region for residues 407-503 of WIP was cloned into a pET28 plasmid (Novagen) between its XhoI and BamHI restriction sites. The resulting plasmid was used to transform BL21 competent cells and to overexpress the protein GSSHHHHHH-WIP(407-503)–LEHHHHHH (WIP^C) in M9 minimal medium containing $^{15}NH_4Cl$ (1 g/L), $^{13}C_6$-D-glucose (2.5 g/L) (1). Cultures were grown to OD_{280} ~ 0.6-0.8 and induced with 1 mM isopropyl-thio-D-galactose (IPTG), followed by expression at 27 °C for 14-18 h, yielding uniformly ^{15}N,^{13}C-labeled WIP^C. Cells were lysed and the supernatant loaded on a Ni^{++}-affinity column. WIP^C eluted at 300 mM imidazole at sufficient purity for purposes of NMR data acquisition. For analytical purposes, size exclusion columns were typically run in 20 mM phosphate buffer (pH 7), 150 mM NaCl and 10 mM βME on a Superdex 75 column. The buffer was exchanged and the sample concentrated to 1.0-1.5 mM in a Vivaspin centrifugation tube with a molecular cutoff of 10 kDa. β-mercaptoethanol (βME, 10 mM) or dithiothreitol (DTT, 1-5 mM) were maintained in the sample to ensure residue C^{446} remains reduced throughout the experiments. The protein was assayed on SDS-PAGE prior to NMR measurements, exhibiting a single (>95%) band at 12-13 kDa. Typical yields were 4-6 mg of purified WIP^C per liter of M9 culture.

Acquisition of NMR data

Backbone assignment. Backbone assignment utilized a ^{13}C'-detected strategy (CON spectrum as readout), based on the 3D-experiments CANCO, CBCACON, CBCANCO, and C-(CC-TOCSY)-CON (2,3), and the 5D-experimnents CACONCACO and NCOCANCO (4,5). 5D-NMR experiments used non-uniform sampling and T_1-relaxation optimized excitation to acquire the high-dimensionality experiment in reasonable time. Experiments for purposes of resonance assignment were performed at 298 K. 2D-CON-based experiments were typically acquired in interleaved in-phase-anti-phase (IPAP) manner with 200-256 complex points, an acquisition time of 35-45 ms in the ^{15}N dimension, and 1024 complex points and an acquisition time of 145 ms in the observed ^{13}C dimension. 3D-experiments were acquired with 32–40 complex points and 7–7.5 ms acquisition time for Cα-based experiments, and 80-120 complex points and 4-6 ms acquisition time for $^{13}C^{\alpha/\beta}$ evolution. ^{15}N evolution was achieved during a 33 ms constant-time period concomitantly with refocusing of the N-C' coupling, and 1024 complex points and an acquisition time of 145 ms were maintained for the observed ^{13}C dimension.

The (H)CACONCACO experiment was measured with the spectral widths set to 6010 (aq) × 4000 ($^{13}C^{\alpha}$) × 2000 (^{15}N) × 1600 ($^{13}C'$) × 4000 ($^{13}C^{\alpha}$) Hz. The (H)NCOCANCO experiment was measured with the spectral widths set to 6010 (aq) × 2000 (^{15}N) × 4000 ($^{13}C^{\alpha}$) × 1600 ($^{13}C'$) × 2000 (^{15}N) Hz. For both experiments, 1024 complex points were collected in the acquisition dimension and 1800 hypercomplex points were randomly distributed using the Poisson disc algorithm (6) over the four indirectly detected dimensions with the maximum evolution periods adjusted to 27, 30 and 50 ms for the $^{13}C\alpha$, $^{13}C'$, and ^{15}N dimensions, respectively. The interscan delay was set to 0.2 s. Figure S1 details the methodology by which the 5D-NMR data were used to perform backbone assignment.

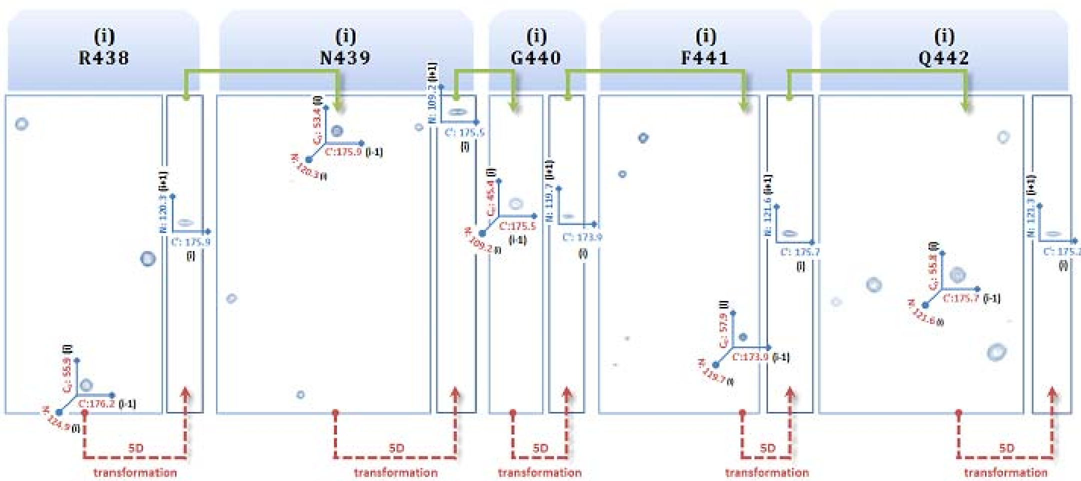

Figure S1. 5D-NMR-based assignment of WIP^{C} resonance frequencies. Assignment of the sequence R^{438}NGFQ^{442} using the 5D (H)NCOCαNCO experiment. Each light blue panel represents a ($^{13}C\alpha^{i}$, $^{13}C'^{i-1}$) plane with given $^{15}N^{i}$ frequency value from the auxiliary 3D experiment, and each dark blue framed spectra represents a ($^{15}N^{i+1}$, 13C'^{i}) 'hyper-plane' at a given (^{15}N, $^{13}C\alpha$, $^{13}C'$) frequency set. For assignment, the cross-peak correlating the frequencies of $^{13}C'^{437}$/$^{13}C\alpha^{438}$ with $^{15}N^{438}$ is selected from a 3D spectrum. A 'hyper-plane' corresponding to these coordinates is then extracted from the 5D spectrum, affording the two additional frequencies of $^{13}C'^{438}$ and $^{15}N^{439}$. This process is then repeated for the newly assigned $^{13}C'^{438}$/$^{13}C\alpha^{439}$ and $^{15}N^{439}$, successively assigning all residues in the N → C direction.

The ubiquitous presence of proline residues required a modification of the original 5D-NMR sequence, due to the unique spin topology of proline ^{15}N nuclei which are coupled to three aliphatic ^{13}C nuclei instead of the usual two. The 50 ms constant-time evolution was originally designed to direct magnetization transfer via the stronger intra-residual interaction and suppress the unwanted inter-residual connectivity, utilizing the difference between the $^{1}J[^{15}N_{i},{}^{13}C^{\alpha}_{i}]$ and $^{2}J[^{15}N_{i},{}^{13}C^{\alpha}_{i-1}]$ couplings (5). However, a side effect of this transfer scheme is a discrimination against the desired intra-residual connectivity in prolines. Therefore, a second version of the experiment was acquired with the ^{15}N evolution period was shortened to 33 ms. The intra- and inter-residual correlations could still be correctly identified on the basis of their intensities.

The $^{1}J(NC^{\alpha})$-selective HCBCANCO experiment (7) was measured with the spectral widths set to 6010 (aq) × 2000 (^{15}N) × 10000 ($C^{\alpha/\beta}$) × 3125 ($H^{\alpha/\beta}$) Hz, and maximum acquisition times of 8, 7, and 40 ms for $^{1}H\alpha/\beta$, $^{13}C\alpha/\beta$, and ^{15}N dimensions, respectively. The experiment was measured with 1024 complex points in the directly detected dimension and 2000 randomly distributed hypercomplex points in the indirectly detected dimensions with 8 scans per increment and the recovery delay set to 0.75 s.

Relaxation experiments. Relaxation delays of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 s were used for measuring ^{15}N longitudinal relaxation (R_1), and spin-lock pulse durations of 4, 24, 44, 64, 94, and 124 ms were applied for measuring the ^{15}N rotating-frame transverse relaxation ($R_{1\rho}$) using a 2.1 kHz spin-lock pulse. In both cases delay durations were randomized and the reference spectrum was repeated after acquisition of all spectra to exclude sample degradation effects. To account for offset effects, R_2 was determined using the relation $R_2 = (R_{1\rho} - R_1\sin^2\theta)/\cos^2\theta$, where $\tan\theta$ is the ratio between the ^{15}N offset and the 2.1 kHz pulse strength. ^{15}N-{^{1}H}-NOEs were estimated by comparison of two HSQC-like spectra with excitation on steady-state ^{15}N magnetization, with and without saturation of the H^N nuclei. Saturation was effected by a series of 150° pulses for the duration of the recycling delay (4 s).

Supporting References

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