Supporting Information

Vallurupalli *et al.* 10.1073/pnas.0804221105

Structure Calculation Protocol

Experimental Restraints. In the discussion that follows, the crystal structure (1jo8) of the apo form of the Abp1p SH3 domain (1) was used as the representative ground state ("visible") structure after (*i*) addition of the missing N-terminal residue, Ala 1, and (*ii*) addition of hydrogen atoms [using the hbuild feature of X-PLOR (2)]. This was followed by energy minimization with all heavy atoms fixed.

Structures of the invisible state corresponding to the Ark1p peptide bound form of the Abp1p SH3 domain have been calculated from ^{15}N, ^{1}HN, $^{13}C^{\alpha}$, and ^{13}CO chemical shifts, along with anisotropic restraints including ^{1}HN-^{15}N, $^{1}H^{\alpha}$-$^{13}C^{\alpha}$, ^{1}HN-^{13}CO RDCs, and ^{13}CO RCSAs. RDC and RCSA values were recorded on several samples, as described in *Materials and Methods*, with amounts of alignment media tailored to the size of the interaction measured. To employ all of the anisotropic restraints in a single structure calculation, the RDC and RCSA values must be scaled appropriately (3). The scaling factors were obtained by calculating the components of the alignment frame of the ground state structure individually for each type of anisotropic restraint (for example, ^{1}HN-^{15}N RDCs) by fitting RDCs measured for the (visible) state *P* of the

$$P + L \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} PL$$

exchanging system to the x-ray structure of the *P* state (apo form of the Abp1p SH3 domain). The D_a values (4) so obtained were subsequently scaled to a common set of bond lengths (^{1}HN-^{15}N) and gyromagnetic ratios (^{1}HN,^{15}N). Different amounts of alignment media were used to record (*i*) ^{1}HN-^{15}N RDCs, (*ii*) $^{1}H^{\alpha}$-$^{13}C^{\alpha}$ RDCs, and (*iii*) ^{1}HN-^{13}CO RDCs and ^{13}CO RCSAs. D_a values of −5.5, −3.4, and −15.3 Hz were obtained for *i*, *ii*, and *iii*, respectively, and $^{1}H^{\alpha}$-$^{13}C^{\alpha}$ and ^{1}HN-^{13}CO RDCs were scaled by 5.5/3.4 and 5.5/15.3. Before scaling the ^{13}CO RCSAs by 5.5/15.3, we first offset values to correct for differences in peak positions between aligned and unaligned samples caused by splitting of the lock signal upon alignment. The offset was determined by minimizing the difference between experimental and calculated RCSA values of the ground state structure; this was done by fitting RCSAs of the apo protein (*P*) to those predicted on the basis of the x-ray structure of the unliganded form of the SH3 domain.

A flowchart summarizing the protocol used for structure determination is provided in Fig. S3, with additional details given in what follows. As described in *Results and Discussion*, regions for which $\Delta\varpi_{\mathrm{RMS}} < 0.05$ were assumed to have the same conformation in both the ground (apo) and "excited" (ligand-bound) states. This was enforced by fixing the coordinates of all of the atoms in these regions (gray in Fig. 2*C*) during the torsion angle molecular dynamics (TAMD) protocol, as implemented in Xplor-NIH (5). Covalent geometry was maintained throughout all calculations by using the BOND, ANGLE, and IMPROPER (dihedral angle) energy terms. The RAMA torsion angle database term (6), which biases dihedral angles toward those expected from a database of high-resolution structures, was also used. Typically in NMR structure calculations, only a repulsive nonbonded interaction term is used, with the "attractive component" provided through experimentally derived distance restraints and a radius of gyration potential (7). To this point, it is not possible to measure such restraints for an "invisible" state, and we have therefore used the full Lennard-Jones potential to describe nonbonded interactions. Experimental RDC and RCSA restraints were imposed by using a flat bottom harmonic potential with the width determined by experimental errors (5), with (ϕ, ψ) restraints enforced using the CDIH potential term of X-PLOR (Table S3). We have chosen not to use the alignment tensor values, D_a and *R*, estimated from the histogram of Fig. S1 as fixed input in structure calculations because we cannot be sure that there is a near-isotropic sampling of interaction vector orientations. Rather, D_a and *R* were allowed to float during the structure calculation protocol (5). It is clear, however, from inspection of Fig. S1 that $D_a < 0$ (see below), and this was enforced by imposing a torsion angle restraint on the four "atoms" that describe the orientation tensor.

Generating Structures with Random Conformations for Variable Regions 1–3 of Fig. 2*C*. TAMD is performed on the ground state structure (5) for 200 ps at 5,000 K, followed by a "production" run (250 ps of TAMD, 5,000 K), in which structures are stored every 25 ps. The 10 structures generated by this method have a wide range of conformations for the variable regions (Fig. 3*A*). Only BOND, ANGLE, IMPROPER, and NONBONDED (Lennard-Jones) potential energy terms were used at this stage.

Initial Generation of (ϕ, ψ) Torsion Angle Restraints from Chemical Shift Data. In total, 57 ^{15}N, 56 ^{1}HN, 50 $^{13}C^{\alpha}$, and 49 ^{13}CO chemical shifts of the invisible state were obtained through analysis of relaxation dispersion profiles. (ϕ, ψ) torsion angle restraints were obtained for close to 60% (22/37) of the residues that compose variable regions 1–3 by using the program TALOS (8). Values of (ϕ, ψ) were assigned only if the prediction was deemed "good" by the program. The restraints were imposed with a width of max($\pm 2\sigma$, $\pm 30°$), where σ is the standard deviation of the TALOS best matches.

Conformation of Each Variable Region. To improve the rate of convergence of the structure calculation protocol, we individually optimized the conformations of each of regions 1–3 using TALOS-derived (ϕ, ψ) dihedral angle restraints, as well as RDC and RCSA restraints via a simulated annealing TAMD protocol. In this procedure all but one of the variable regions are deleted from each of the 10 high-temperature structures, calculated as described above. Each of the structures so obtained is subjected to 100 ps of TAMD at 5,000 K, with weak dihedral (20 kcal·mol^{-1}·rad^{-2}), RDC (0.002 kcal·mol^{-1}·Hz^{-2}), and RCSA (5×10^{-5} kcal·mol^{-1}·ppb^{-2}) restraints. The ANGLE, IMPROPER, and RAMA energy terms (but not the BOND and NONBONDED terms) were scaled at this high-temperature TAMD stage by factors of 0.4, 0.1, and 0.002. The temperature of the system was gradually reduced to 25 K over 798 ps whereas the force constants of the dihedral, RDC and RCSA terms were increased to 400 kcal·mol^{-1}·rad^{-2}, 0.4 kcal·mol^{-1}·Hz^{-2}, and 0.01 kcal·mol^{-1}·ppb^{-2}, respectively; the scaling factors for the ANGLE, IMPROPER and RAMA terms were also increased to 1. The structure at the end of the run was stored for further analysis, and the process was repeated 48 times for each starting structure, with different starting random velocities, to generate a total of 480 structures. The 10 lowest energy structures were selected. If a TALOS dihedral restraint was consistently violated, the restraint was removed and the whole processes repeated again. (This occurred for only one residue, Phe-31.)

Generation of new (ϕ, ψ) Torsion Angle Restraints. The 10 lowest energy structures obtained as described above for each of regions 1–3 were used to generate a new set of (ϕ, ψ) dihedral restraints for residues within the variable regions (color in Fig. 2*C*). The (ϕ, ψ) restraints were calculated as the mean of the dihedral angles obtained from the generated structures, imposed with a width of max($\pm 2\sigma$, $\pm 30°$), where σ is the standard deviation of (ϕ, ψ) in the 10 lowest energy structures; restraints were not imposed if 2σ was greater than 50°. Note that this process generates restraints for residues even if there were originally none (based on TALOS calculations from chemical shifts).

Determination of the Invisible, Ark1p-Bound Abp1p SH3 Domain Structure. A TAMD protocol was used to generate structures of the excited state starting from the 10 initial structures obtained from MD runs at 5,000 K that produce random conformations for regions 1–3 (Fig. 3*A*, see above). The newly derived and more comprehensive set of (ϕ, ψ) restraints were used, along with the complete set of RDC and RCSA restraints, to produce structures. The TAMD protocol used was very similar to that used to optimize individual variable regions, except that the starting dihedral angle restraint force constant was 5 kcal·mol^{-1}·rad^{-2}; 96 structures were generated from each starting structure to produce a total of 960 structures.

All rmsd values quoted in the text were obtained by superimposing the "fixed" regions (gray in Fig. 2*C*) and subsequently calculating pairwise rmsd values by including only the variable regions (1–3) in the computations.

1. Fazi B, *et al.* (2002) Unusual binding properties of the SH3 domain of the yeast actin-binding protein Abp1: Structural and functional analysis. *J Biol Chem* 277:5290–5298.
2. Brünger AT (1992) X-PLOR (Yale University, New Haven, CT), Version 3.1.
3. Clore GM, Gronenborn AM, Bax A (1998) A robust method for determining the magnitude of the fully asymmetric alignment tensor of oriented macromolecules in the absence of structural information. *J Magn Reson* 113:216–221.
4. Tjandra N, Bax A (1997) Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium. *Science* 278:1111–1114.
5. Schwieters CD, Kuszewski JJ, Clore GM (2006) Using Xplor-NIH for NMR molecular structure determination. *Prog Nucl Magn Reson Spectrosc* 48:47–62.
6. Kuszewski J, Clore GM (2000) Sources of and solutions to problems in the refinement of protein NMR structures against torsion angle potentials of mean force. *J Magn Reson* 146:249–254.
7. Kuszewski JJ, Gronenborn AM, Clore GM (1999) Improving the packing and accuracy of NMR structures with a pseudopotential for the radius of gyration. *J Am Chem Soc* 121:2337–2388.
8. Cornilescu G, Delaglio F, Bax A (1999) Protein backbone angle restraints from searching a database for chemical shift and sequence homolgy. *J Biomol NMR* 13:289–302.

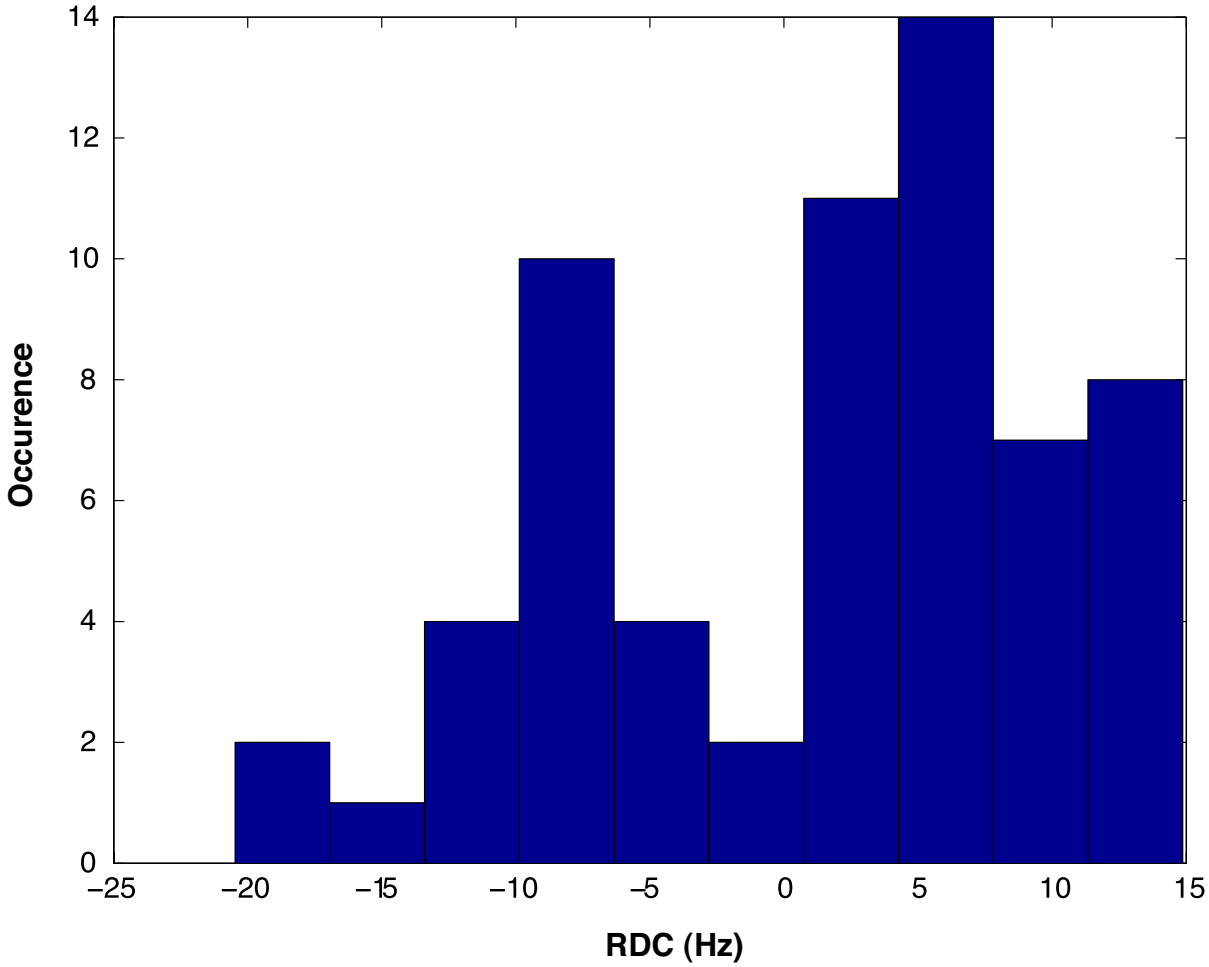

Fig. S1. Distribution of CPMG-derived RDCs for the invisible state. The histogram consists of 24 ^{1}HN-^{15}N, 2 $^{1}H^{\varepsilon 1}$-$^{15}N^{\varepsilon 1}$, 21 $^{1}H^{\alpha}$-$^{13}C^{\alpha}$, and 16 ^{1}HN-^{13}CO RDC values. The $^{1}H^{\alpha}$-$^{13}C^{\alpha}$ and ^{1}HN-^{13}CO RDCs have been scaled to the ^{1}HN-^{15}N values to account for differences in gyromagnetic ratios and distances and different concentrations of alignment media (Pf1 phage) in the samples used for measurements (as described in *Materials and Methods*). It is clear that the right-hand side corresponds to the well sampled edge of the histogram, $D_{YY} = -D_a(1 + 1.5R)$; therefore, $D_a < 0$.

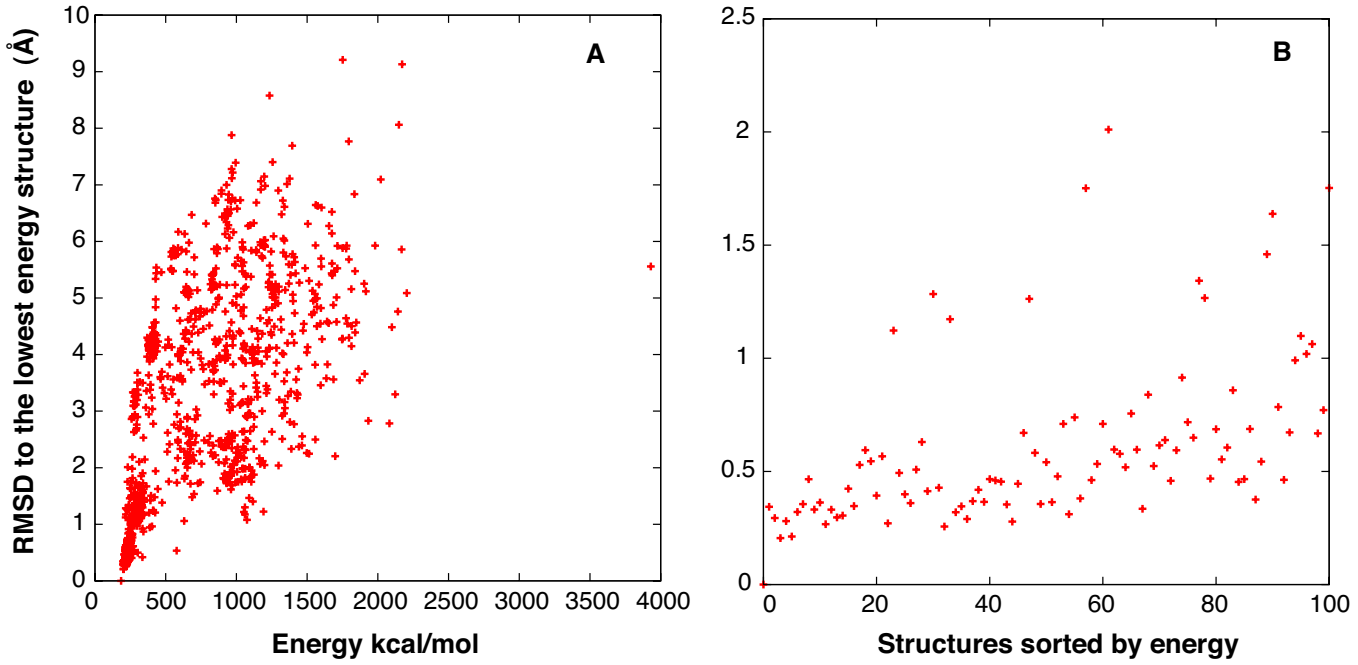

Fig. S2. Convergence of the TAMD structure determination protocol. (*A*) Plot of the RMSD of each of the 960 determined structures to the lowest energy structure. (*B*) Plot of the RMSD of the 100 lowest energy structures to the lowest energy structure. A good correlation between energy and RMSD to the lowest energy structure is obtained, and the best 20 structures have an RMSD <0.5 Å to the lowest energy structure. Here, the structures are superimposed on the fixed regions (gray in Fig. 2*C*), and rmsd is calculated for the N, C^{α}, and CO atoms in the variable regions.

Protocol for Invisible State Structure Determination Using CPMG Derived Chemical Shifts, RDCs and RCSAs.

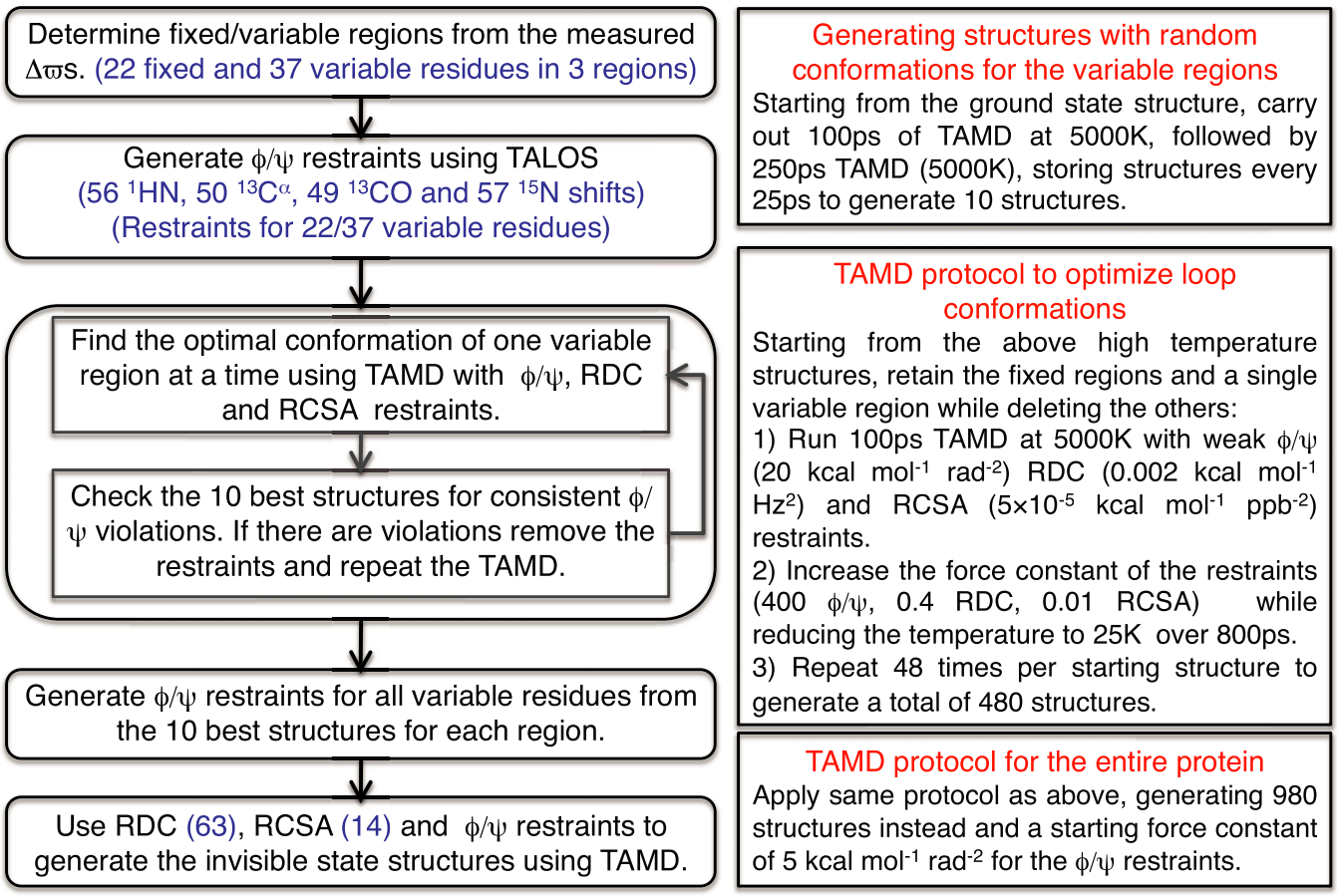

Fig. S3. Flow chart of the structure-determination protocol.

Table S1. Nitrogen chemical shifts (ppm) of the SH3 domain component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments

	ϖ_{bound}, ppm		$\Delta\varpi = \varpi_{free} - \varpi_{bound}$, ppm	
Residue	CPMG	Direct	CPMG	Direct
A1	126.83*	126.90		
P2				
W3	116.18†	116.29	**0.121 ± 0.044**	**−0.113**
A4	120.85†	120.71	**0.152 ± 0.035**	**0.142**
T5	113.32 ± 0.01	113.34	−0.393 ± 0.015	−0.407
A6	127.52 ± 0.03	127.49	−0.155 ± 0.034	−0.123
E7	125.35*	125.31		
Y8	113.09 ± 0.01	113.08	0.850 ± 0.012	0.858
D9	118.66 ± 0.02	118.65	−0.351 ± 0.017	−0.338
Y10	121.77 ± 0.01	121.75	−0.595 ± 0.012	−0.574
D11	125.75 ± 0.02	125.77	0.365 ± 0.017	0.345
A12	123.70 ± 0.02	123.70	0.278 ± 0.020	0.275
A13	125.74 ± 0.02	125.73	0.305 ± 0.019	0.316
E14	113.06 ± 0.01	113.06	1.075 ± 0.013	1.074
D15	117.87 ± 0.02	117.75	1.964 ± 0.023	2.080
N16	113.58 ± 0.01	113.58	0.671 ± 0.012	0.676
E17	117.77 ± 0.01	117.75	1.039 ± 0.013	1.062
L18	122.35 ± 0.01	122.35	0.517 ± 0.013	0.510
T19	114.34 ± 0.02	114.34	0.366 ± 0.016	0.365
F20	120.59†	120.51	**0.114 ± 0.046**	**0.083**
V21	116.79†	116.87	**0.122 ± 0.043**	**−0.080**
E22	120.53*	120.49		
N23	117.26†	117.09	**0.162 ± 0.033**	**0.164**
D24	122.59†	122.68	**0.108 ± 0.049**	**−0.093**
K25	120.27 ± 0.03	120.24	−0.158 ± 0.034	−0.131
I26	130.67 ± 0.03	130.69	0.159 ± 0.034	0.141
I27	118.98*	118.98		
N28	115.82†	115.64	**0.177 ± 0.030**	**0.178**
I29	117.13†	116.98	**0.156 ± 0.034**	**0.154**
E30	124.76 ± 0.01	124.78	0.452 ± 0.014	0.433
F31	128.36 ± 0.02	128.31	−2.008 ± 0.024	−1.956
V32	117.38 ± 0.06	117.12	7.152 ± 0.063	7.418
D33	120.43 ± 0.02	120.46	−1.781 ± 0.020	−1.806
D34	118.56 ± 0.04	118.48	−3.790 ± 0.046	−3.703
D35	116.47 ± 0.01	116.48	0.943 ± 0.012	0.937
W36	122.49 ± 0.01	122.52	0.544 ± 0.013	0.513
W37	119.94 ± 0.02	119.92	1.683 ± 0.019	1.702
L38	121.00 ± 0.03	121.01	0.204 ± 0.027	0.200
G39	109.39 ± 0.01	109.46	−0.572 ± 0.013	−0.644
E40	119.76*	119.77		
L41	125.87 ± 0.04	125.89	0.133 ± 0.041	0.113
E42	129.28*	129.20		
K43	113.61*	113.60		
D44	114.24*	114.28		
G45	109.80*	109.81		
S46	118.28*	118.26		
K47	119.48*	119.55		
G48	108.57 ± 0.03	108.57	0.161 ± 0.034	0.165
L49	119.62 ± 0.01	119.51	0.917 ± 0.012	1.021
F50	115.17 ± 0.04	115.18	0.142 ± 0.037	0.129
P51				
S52	120.57 ± 0.01	120.58	1.242 ± 0.015	1.230
N53	116.14 ± 0.01	116.10	−0.869 ± 0.012	−0.830
Y54	119.16*	119.13		
V55	109.26 ± 0.02	109.25	−0.252 ± 0.022	−0.243
S56	113.84†	113.92	**0.099 ± 0.054**	**−0.081**
L57	129.01*	129.10		
G58	111.54†	111.66	**0.142 ± 0.038**	**−0.122**
N59	123.28*	123.29		

Shifts are compared with those obtained directly when the complex is the major (visible) state in solution. Residues in bold indicate that no sign information about $\Delta\omega$ could be obtained.

*A two-site chemical exchange model did not generate statistically significant improvements in fits over a model of no exchange (constant $R_2(\nu_{CPMG})$) at the 98% confidence level. Values of the excited state chemical shifts were assumed the same as the ground state.

†The sign of $\Delta\varpi$ could not be obtained accurately from changes in peak positions among the following four experiments that are used to quantify the sign [Skrynnikov NR, Dahlquist FW, Kay LE (2002) Reconstructing NMR spectra of ''invisible'' excited protein states using HSQC and HMQC experiments. *J Am Chem Soc* 124:12352–12360]: (HSQC at 11.7T, HSQC at 18.8T, HMQC at 11.7T and HMQC at 18.8T) i.e., $\max(|\varpi_{HSQC,500} - \varpi_{HMQC,500}|, |\varpi_{HSQC,800} - \varpi_{HMQC,800}|, |\varpi_{HSQC,500} - \varpi_{HSQC,800}|) < 3.0$ ppb. However, the absolute value of $\Delta\varpi$ is so small that the chemical shift of the bound state can be assumed to be identical to that of the free state.

Table S2. Amide proton chemical shifts (ppm) of the SH3 domain component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments

	ω_{bound} (ppm)		$\Delta\omega = \omega_{free} - \omega_{bound}$ (ppm)	
Residue	CPMG	Direct	CPMG	Direct
A1	8.336*	8.343		
P2				
W3	7.824*	7.823		
A4	10.086*	10.084		
T5	8.306 ± 0.004	8.302	−0.046 ± 0.004	−0.042
A6	8.942 ± 0.006	8.946	−0.033 ± 0.006	−0.037
E7	9.410*	9.400		
Y8	7.449 ± 0.004	7.447	−0.049 ± 0.004	−0.047
D9	8.598 ± 0.004	8.590	−0.155 ± 0.004	−0.147
Y10	8.220 ± 0.005	8.223	0.041 ± 0.005	0.038
D11	7.090 ± 0.003	7.090	0.106 ± 0.003	0.106
A12	7.709 ± 0.003	7.701	−0.095 ± 0.003	−0.087
A13	8.854 ± 0.005	8.857	0.227 ± 0.005	0.224
E14	7.085 ± 0.004	7.086	−0.048 ± 0.004	−0.049
D15	8.671 ± 0.008	8.667	0.319 ± 0.008	0.323
N16	7.324 ± 0.013	7.351	0.606 ± 0.013	0.579
E17	7.561*	7.555		
L18	8.756 ± 0.003	8.758	0.099 ± 0.003	0.097
T19	7.387 ± 0.004	7.385	−0.048 ± 0.004	−0.046
F20	9.013*	9.023		
V21	9.253*	9.279		
E22	8.786†	8.798	**0.019 ± 0.009**	**0.012**
N23	8.905*	8.900		
D24	8.645*	8.648		
K25	8.172 ± 0.005	8.170	−0.039 ± 0.005	−0.037
I26	9.321*	9.322		
I27	9.827*	9.820		
N28	9.059*	9.065		
I29	7.898*	7.899		
E30	9.236 ± 0.008	9.243	−0.024 ± 0.008	−0.031
F31	8.722 ± 0.006	8.711	−0.239 ± 0.006	−0.228
V32	7.730 ± 0.004	7.717	−0.064 ± 0.004	−0.051
D33	8.315 ± 0.009	8.342	0.354 ± 0.009	0.327
D34	8.169 ± 0.003	8.167	0.090 ± 0.003	0.092
D35	8.720 ± 0.003	8.717	0.072 ± 0.003	0.075
W36	7.827 ± 0.008	7.848	0.348 ± 0.008	0.327
W37	7.876 ± 0.012	7.907	0.463 ± 0.012	0.432
L38	8.358 ± 0.009	8.399	0.360 ± 0.009	0.319
G39	8.833 ± 0.003	8.841	−0.107 ± 0.003	−0.115
E40	8.587*	8.580		
L41	9.521*	9.500		
E42	8.190*	8.188		
K43	9.240*	9.246		
D44	6.822*	6.820		
G45	8.411*	8.398		
S46	8.686*	8.696		
K47	8.374*	8.371		
G48	8.751 ± 0.005	8.746	−0.037 ± 0.005	−0.032
L49	9.106 ± 0.008	9.099	−0.320 ± 0.008	−0.313
F50	9.234 ± 0.004	9.230	0.056 ± 0.004	0.060
P51				
S52	7.870 ± 0.005	7.865	−0.231 ± 0.005	−0.226
N53		7.967		
Y54	7.792 ± 0.004	7.794	0.061 ± 0.004	0.059
V55	7.419 ± 0.008	7.413	−0.022 ± 0.008	−0.016
S56	8.604†	8.615	**0.014 ± 0.012**	**0.011**
L57	9.158†	9.187	**0.026 ± 0.007**	**0.029**
G58	8.487*	8.495		
N59	7.478*	7.462		

Shifts are compared with those obtained directly when the complex is the major (visible) state in solution. Signs are measured relative to the ^{15}N shift in a series of double quantum and zero quantum experiments [Korzhnev DM, Neudecker P, Mittermaier A, Orekhov VY, Kay LE (2005) Multiple-site exchange in proteins studied with a suite of six NMR relaxation dispersion experiments: An application to the folding of a Fyn SH3 domain mutant. *J Am Chem Soc* 127:15602–15611].

*A two-site chemical exchange model did not generate statistically significant improvements in fits over a model of no exchange [constant $R_2(\nu_{CPMG})$] at the 98% confidence level. Chemical shifts of the ground and excited states were assumed to be the same.

†The sign of $\Delta\varpi$ could not be obtained accurately; however, the absolute value of $\Delta\varpi$ is so small that the chemical shift of the bound state can be assumed to be identical to that of the free state.

Table S3. Alpha-carbon ($^{13}C^{\alpha}$) chemical shifts (ppm) of the SH3 component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments

	ϖ_{bound}, ppm		$\Delta\varpi = \varpi_{free} - \varpi_{bound}$, ppm	
Residue	CPMG	Direct	CPMG	Direct
A1	50.58 ± 0.02	50.69	*0.08 ± 0.02*	*−0.04*
P2	63.59†	63.59	**0.14 ± 0.01**	**0.00**
W3	54.22†	54.19	**0.11 ± 0.01**	**0.03**
A4	50.89†	50.80	**0.11 ± 0.01**	**0.09**
T5	60.77 ± 0.01	60.74	−0.14 ± 0.01	−0.12
A6	53.18†	53.14	**0.07 ± 0.01**	**0.04**
E7	56.36 ± 0.01	56.42	0.18 ± 0.01	0.11
Y8	54.89 ± 0.01	54.84	−0.14 ± 0.01	−0.09
D9	54.41 ± 0.02	54.58	0.19 ± 0.02	0.02
Y10	58.85 ± 0.01	58.85	0.15 ± 0.01	0.15
D11	52.24†	52.18	**0.13 ± 0.01**	**0.06**
A12	53.54 ± 0.01	53.52	−0.11 ± 0.01	−0.09
A13	52.10†	52.00	**0.13 ± 0.01**	**0.10**
E14	54.35 ± 0.01	54.38	−0.70 ± 0.01	−0.73
D15	56.82 ± 0.01	56.80	−0.22 ± 0.01	−0.21
N16		51.50	**0.83 ± 0.01**	**0.82**
E17	54.89 ± 0.01	54.95	0.32 ± 0.01	0.26
L18				
T19	61.73*	61.74		
F20	55.53 ± 0.01	55.51	0.10 ± 0.01	0.11
V21	59.08 ± 0.02	59.18	0.10 ± 0.02	0.00
E22	58.51†	58.53	**0.10 ± 0.01**	**0.02**
N23	56.16 ± 0.01	56.28	*0.11 ± 0.01*	*−0.01*
D24	56.05 ± 0.01	56.15	*0.09 ± 0.01*	*−0.01*
K25	55.98†	55.97	**0.07 ± 0.02**	**0.02**
I26				
I27				
N28	53.87†	53.84	**0.09 ± 0.01**	**0.02**
I29				
E30	54.48 ± 0.01	54.42	−0.28 ± 0.01	−0.22
F31	54.74 ± 0.02	54.65	−0.13 ± 0.02	−0.05
V32	64.30 ± 0.10	64.08	1.96 ± 0.10	2.18
D33	53.36 ± 0.01	53.43	0.14 ± 0.01	0.08
D34	57.80 ± 0.01	57.78	−0.56 ± 0.01	−0.54
D35	55.12 ± 0.01	55.31	*0.13 ± 0.01*	*−0.06*
W36	56.95 ± 0.01	56.92	−0.39 ± 0.01	−0.36
W37	51.56 ± 0.01	51.59	0.40 ± 0.01	0.38
L38				
G39	45.86	45.88		
E40	52.62†	52.64	**0.15 ± 0.01**	**−0.02**
L41				
E42	59.83 ± 0.01	59.67	*−0.14 ± 0.01*	*0.03*
K43	58.60†	58.57	**0.10 ± 0.01**	**0.02**
D44	53.56†	53.40	**0.09 ± 0.02**	**0.15**
G45	46.16	46.16		
S46	59.80†	59.82	**0.09 ± 0.01**	**−0.02**
K47	54.96 ± 0.01	55.01	0.10 ± 0.01	0.04
G48	45.61	45.55		
L49				
F50	55.13 ± 0.01	55.11	0.31 ± 0.01	0.32
P51	60.74 ± 0.01	60.68	0.29 ± 0.01	0.35
S52	60.47 ± 0.01	60.45	−0.43 ± 0.01	−0.41
N53	53.22 ± 0.01	53.23	0.50 ± 0.01	0.48
Y54	59.10 ± 0.01	59.09	−0.12 ± 0.01	−0.11
V55	58.51 ± 0.02	58.43	−0.16 ± 0.02	−0.09
S56	56.23†	56.28	**0.10 ± 0.01**	**−0.05**
L57				
G58	43.56	43.56		
N59	54.62*	54.62		

Shifts are compared with those obtained directly when the complex is the major (visible) state in solution. Chemical shifts for Leu and Ile are not available (blank rows) due to the labeling scheme employed, which leads to the presence of $^{13}C^{\alpha}$-$^{13}C^{\beta}$ scalar couplings [Lundstrom P, *et al.* (2007) Fractional 13C enrichment of isolated carbons using [1–13C]- or [2–13C]-glucose facilitates the accurate measurement of dynamics at backbone Calpha and side-chain methyl positions in proteins. *J Biomol NMR* 38:199–212]. Although such couplings are also present in the case of Val, they can be "suppressed" during the CPMG element by using selective pulses.

*A two-site chemical exchange model did not generate statistically significant improvements in fits over a model of no exchange (constant $R_2(\nu_{CPMG})$) at the 98% confidence level. Chemical shifts of the ground and excited state were assumed to be identical.

†The sign of $\Delta\varpi$ could not be obtained accurately, ($\max(|\varpi_{HSQC,500} - \varpi_{HMQC,500}|, |\varpi_{HSQC,800} - \varpi_{HMQC,800}|, |\varpi_{HSQC,500} - \varpi_{HSQC,800}|) < 3.0$ ppb; residues marked in bold); however, the absolute value of $\Delta\varpi$ is so small that the chemical shift of the bound state can be assumed to be identical to that of the free state. Residues for which the determined sign does not agree with expectations based on measurements recorded directly on the bound state are marked in bold italics.

Table S4. Carbonyl (^{13}CO) chemical shifts (ppm) of the SH3 domain component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments and compared with those obtained directly when the complex is the major (visible) state in solution

	ϖ_{bound}		$\Delta\varpi = \varpi_{free} - \varpi_{bound}$	
Residue	CPMG	Direct	CPMG	Direct
A1				
P2	174.02*	173.96		
W3	174.29*	174.29		
A4	175.11*	175.09		
T5	174.88*	174.82		
A6	178.43 ± 0.02	178.44	0.101 ± 0.019	0.090
E7	174.07 ± 0.02	174.01	0.132 ± 0.019	0.196
Y8	173.19 ± 0.01	173.19	0.255 ± 0.010	0.258
D9	175.57 ± 0.01	175.58	0.190 ± 0.012	0.175
Y10	172.65 ± 0.03	172.65	0.056 ± 0.034	0.056
D11	173.25*	173.26		
A12	178.46 ± 0.04	178.44	−0.048 ± 0.039	−0.028
A13	177.43 ± 0.01	177.44	−0.320 ± 0.010	−0.324
E14	177.41 ± 0.02	177.40	−0.108 ± 0.023	−0.099
D15	175.63 ± 0.01	175.64	0.424 ± 0.010	0.414
N16	175.51*	175.52		
E17	176.57*	176.61		
L18				
T19	174.45*	174.38		
F20	174.89*	174.90		
V21	174.97*	174.97		
E22	176.64*	176.69		
N23	175.12*	175.13		
D24	175.50*	175.47		
K25	175.86 ± 0.03	175.86	−0.059 ± 0.032	−0.056
I26	174.90*	174.90		
I27	174.92*	174.91		
N28	174.95*	174.93		
I29	176.11 ± 0.02	176.11	0.086 ± 0.023	0.086
E30		176.50	**0.865 ± 0.024**	**0.905**
F31	176.67 ± 0.01	176.67	−0.464 ± 0.011	−0.464
V32	174.34 ± 0.04	174.34	1.756 ± 0.040	1.757
D33	173.69 ± 0.02	173.66	0.838 ± 0.021	0.871
D34	177.49 ± 0.01	177.50	0.231 ± 0.011	0.220
D35		174.99	**0.778 ± 0.019**	**0.751**
W36	172.55 ± 0.01	172.54	0.411 ± 0.010	0.421
W37	172.54 ± 0.01	172.54	−0.382 ± 0.010	−0.374
L38				
G39	177.66†	177.67	**0.057 ± 0.033**	**−0.011**
E40	175.66†	175.61	**0.081 ± 0.024**	**0.056**
L41				
E42	178.54†	178.53	**0.053 ± 0.035**	**0.008**
K43	176.76*	176.75		
D44	177.22*	177.22		
G45	174.47*	174.47		
S46	172.62 ± 0.08	172.61	−0.024 ± 0.077	−0.017
K47	176.87*	176.91		
G48	171.50 ± 0.02	171.50	−0.115 ± 0.017	−0.112
L49				
F50				
P51	178.44 ± 0.01	178.47	0.324 ± 0.011	0.294
S52	174.52 ± 0.01	174.50	0.607 ± 0.013	0.619
N53	175.20 ± 0.01	175.18	0.481 ± 0.011	0.493
Y54	175.49 ± 0.01	175.50	−0.282 ± 0.010	−0.292
V55	173.78*	173.81		
S56	175.46*	175.44		
L57				
G58	172.43†	172.43	**0.035 ± 0.054**	**0.003**
N59				

^{13}CO shift values for Leu and His could not be obtained (blank rows) due to the labeling scheme employed [Hansen DF, Vallurupalli P, Lundstrom P, Neudecker P, Kay LE (2008) Probing chemical shifts of invisible states of proteins with relaxation dispersion nmr spectroscopy: How well can we do? *J Am Chem Soc* 2667–2675]. Shifts for residues preceding Pro are not available (blank rows) because of the HNCO type of experiment used [Vallurupalli P, Hansen DF, Kay LE (2008) Probing structure in invisible protein states with anisotropic NMR chemical shifts. *J Am Chem Soc* 130:2734–2735.].

*A two-site chemical exchange model did not generate statistically significant improvements in fits over a model of no exchange (constant $R_2(\nu_{CPMG})$) at the 98% confidence level. Chemical shifts in the ground and excited states were assumed to be identical.

†The sign of $\Delta\varpi$ could not be obtained accurately; however, the absolute value of $\Delta\varpi$ is so small that the chemical shift of the bound state can be assumed to be identical to that of the free state.

Table S5. Residual dipolar couplings of the SH3 domain component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments and compared with those obtained directly [IPAP (1, 2)] when the complex is the major (visible) state in solution

	$^1D(^{15}N\text{-}^1HN)$, Hz		$^1D(^{13}C - ^1H^{\alpha})$, Hz		$^2D(^{13}CO - ^1HN)$, Hz	
Residue	CPMG	Direct	CPMG	Direct	CPMG	Direct
A1		5.8 ± 0.1		−13.0 ± 0.1		
P2				−10.5 ± 0.2		3.5 ± 0.5
W3				−13.1 ± 0.1		−8.7 ± 0.3
A4		14.4 ± 0.1		−17.0 ± 0.1		4.1 ± 1.7
T5	10.5 ± 1.5	11.8 ± 0.6	−9.8 ± 4.3	−9.5 ± 0.2		−10.0 ± 0.5
A6		2.9 ± 0.6		−11.0 ± 0.1	−6.2 ± 1.9	−5.3 ± 0.3
E7		6.7 ± 0.7		−5.3 ± 0.4	−2.3 ± 3.8	2.7 ± 0.5
Y8	−10.8 ± 1.5	−12.7 ± 0.1	10.2 ± 4.7	7.1 ± 0.2	8.0 ± 1.8	7.0 ± 0.3
D9	−17.0 ± 1.7	−16.1 ± 0.5			8.8 ± 2.6	8.6 ± 0.8
Y10	−8.1 ± 1.5	−9.7 ± 0.6	14.2 ± 3.5	20.9 ± 0.3		4.0 ± 0.3
D11	−7.9 ± 1.6	−9.5 ± 0.1		7.0 ± 0.2		7.4 ± 0.3
A12	−0.3 ± 2.1	−1.5 ± 0.1	−15.8 ± 4.9	−19.3 ± 0.1	−11.3 ± 2.4	−9.1 ± 0.4
A13	9.7 ± 1.8	11.6 ± 0.5		−2.4 ± 0.1	8.0 ± 2.0	5.3 ± 0.3
E14	4.5 ± 1.5	3.8 ± 0.1	−4.8 ± 5.4	−10.7 ± 0.2		−3.0 ± 1.1
D15	12.1 ± 2.7	3.2 ± 0.2	−4.8 ± 2.8	−5.8 ± 0.1	−13.1 ± 3.4	−7.5 ± 1.0
N16	−4.9 ± 1.5	−6.7 ± 0.6		7.0 ± 0.2		6.2 ± 0.4
E17	7.0 ± 1.5	5.9 ± 0.2	−15.0 ± 4.2	−12.4 ± 0.5		
L18	13.7 ± 1.5	14.8 ± 0.1		14.8 ± 0.5		
T19	−10.3 ± 1.5	−12.5 ± 0.1		8.4 ± 0.3		9.1 ± 0.5
F20		−15.6 ± 0.1	−11.2 ± 4.3	−6.8 ± 0.1	−1.7 ± 2.6	−1.2 ± 0.3
V21		−18.3 ± 0.1		14.4 ± 0.3		14.1 ± 0.3
E22		−13.3 ± 0.2		−14.6 ± 0.2	−11.1 ± 3.7	−7.9 ± 1.1
N23		10.8 ± 0.6		−20.7 ± 0.1		−3.3 ± 0.9
D24		−11.6 ± 0.1		−4.5 ± 0.2	5.3 ± 3.1	3.0 ± 1.0
K25		9.5 ± 0.6		−10.9 ± 0.3		−10.5 ± 0.4
I26	5.7 ± 4.0	10.2 ± 0.2		−19.1 ± 0.5		0.4 ± 0.5
I27		16.5 ± 0.6		−17.4 ± 0.5		0.4 ± 0.5
N28		6.3 ± 1.1		−1.1 ± 0.2		−1.6 ± 1.0
I29		8.6 ± 0.1				−2.6 ± 0.6
E30	3.0 ± 1.5	2.2 ± 0.6	−5.4 ± 2.9	−4.9 ± 0.3		
F31	−8.2 ± 2.3	−9.1 ± 0.6		0.0 ± 0.0	−6.7 ± 2.5	−10.3 ± 0.3
V32				18.1 ± 0.3		6.8 ± 0.3
D33	2.1 ± 1.7	6.1 ± 0.6	0.1 ± 4.1	−2.4 ± 0.2		−5.2 ± 1.1
D34	6.7 ± 3.8	4.4 ± 0.6	11.2 ± 3.7	4.6 ± 0.5	−9.1 ± 2.6	−5.6 ± 1.1
D35	5.5 ± 1.5	5.1 ± 0.1		−4.1 ± 0.2		9.8 ± 1.3
W36	4.3 ± 1.6	3.6 ± 0.6	5.0 ± 2.6	2.1 ± 0.3	−3.6 ± 3.4	−4.3 ± 0.3
W37	−5.0 ± 2.7	−3.1 ± 0.7	−5.2 ± 2.8	1.1 ± 0.2	14.9 ± 1.7	10.6 ± 0.3
L38		5.9 ± 0.6				
G39		5.1 ± 0.1				0.3 ± 0.3
E40		3.9 ± 2.3		−11.4 ± 0.4		−8.7 ± 0.4
L41		12.3 ± 0.5				
E42		2.0 ± 0.1				12.4 ± 0.3
K43		−6.8 ± 0.6		1.0 ± 0.1		−6.2 ± 1.3
D44		6.0 ± 0.2		−8.1 ± 0.2		
G45		8.7 ± 0.1				12.8 ± 0.3
S46		−1.2 ± 0.1		−1.0 ± 0.1	−2.9 ± 2.7	−2.1 ± 0.3
K47		12.7 ± 0.3		−12.1 ± 0.2	−9.6 ± 2.3	−9.3 ± 0.9
G48		10.9 ± 0.7			−0.9 ± 2.0	−1.4 ± 0.3
L49	8.9 ± 1.5	14.1 ± 1.1				
F50		4.5 ± 0.6	−3.5 ± 3.0	−1.2 ± 0.3		
P51				−3.6 ± 0.6		−6.7 ± 1.1
S52	5.2 ± 1.5	5.4 ± 0.6		3.9 ± 0.2	6.7 ± 3.1	1.9 ± 0.5
N53	6.0 ± 1.5	6.9 ± 0.1	−15.3 ± 3.4	−13.1 ± 0.2	6.0 ± 2.1	3.3 ± 0.4
Y54		−4.3 ± 0.1	25.4 ± 4.4	24.4 ± 0.3	−5.5 ± 1.7	−3.1 ± 0.3
V55		8.8 ± 0.6		−8.6 ± 0.4	−4.5 ± 2.2	−3.5 ± 0.3
S56		10.5 ± 0.2		−11.9 ± 0.1		−8.8 ± 0.3
L57		2.5 ± 0.1		17.3 ± 0.5		
G58		5.2 ± 0.1		9.6 ± 76.6		−0.5 ± 0.3
N59		1.2 ± 0.5		0.8 ± 0.1		
$W36^{SC}$	−11.6 ± 1.4	−14.9 ± 0.3				
$W37^{SC}$	7.2 ± 1.5	10.7 ± 0.3				

All residual dipolar couplings are measured in Pf1 phage alignment media. Blanks indicate that data cannot be obtained due to the labeling scheme or the nature of the experiment performed.

1. Yang D, Nagayama K (1996) A sensitivity-enhanced method for measuring heternuclear long-range coupling constants from the displacement of signals in two 1D subspectra. *J Magn Reson Ser A* 118:117–121.
2. Ottiger M, Delaglio F, Bax A (1998) Measurement of J and dipolar couplings from simplified two-dimensional NMR spectra. *J Magn Reson* 131:373–378.

Table S6. ^{13}CO RCSA values of the SH3 domain component of the Abp1p SH3–Ark1p peptide complex derived from CPMG relaxation dispersion experiments and compared with values obtained directly when the complex is the major (visible) state in solution

	RCSA(^{13}CO), ppb	
Residue	CPMG	Direct
A1		
P2		−17 ± 3
W3		−120 ± 2
A4		−81 ± 2
T5		35 ± 8
A6	−153 ± 22	−111 ± 2
E7	−66 ± 24	−40 ± 9
Y8	35 ± 15	39 ± 1
D9	−82 ± 16	−60 ± 9
Y10		71 ± 0
D11		−118 ± 4
A12		108 ± 1
A13	−212 ± 16	−158 ± 2
E14		−88 ± 6
D15	67 ± 15	73 ± 4
N16		−70 ± 6
E17		
L18		
T19		−27 ± 8
F20		−142 ± 3
V21		−48 ± 1
E22		44 ± 9
N23		−106 ± 1
D24		−92 ± 1
K25		74 ± 4
I26		−128 ± 6
I27		−70 ± 3
N28		21 ± 4
I29		44 ± 2
E30		
F31	150 ± 19	129 ± 2
V32	−119 ± 56	−114 ± 1
D33		2 ± 1
D34	−5 ± 15	2 ± 9
D35		25 ± 9
W36	−154 ± 17	−87 ± 1
W37	−62 ± 14	−54 ± 0
L38		
G39		−34 ± 3
E40		32 ± 2
L41		
E42		−17 ± 1
K43		102 ± 2
D44		
G45		−106 ± 0
S46		145 ± 0
K47		95 ± 5
G48		68 ± 1
L49		
F50		
P51		149 ± 7
S52	−269 ± 24	−186 ± 6
N53	79 ± 16	58 ± 5
Y54	0 ± 14	3 ± 2
V55		144 ± 3
S56		−107 ± 1
L57		
G58		14 ± 1
N59		
W36^{SC}		
W37^{SC}		

Blanks indicate that data cannot be obtained due to the labeling scheme employed and/or the nature of the experiment.

Table S7. Residual dipolar couplings of the SH3 domain component of the Abp1p SH3 – Ark1p peptide complex derived from CPMG relaxation dispersion experiments and compared with dipolar couplings obtained directly (IPAP) when the complex is the major (visible) state in solution

	$^1D(^{15}N - {}^1HN)$, Hz	
Residue	CPMG	Direct
A1		−2.2 ± 0.1
P2		
W3		1.2 ± 0.1
A4		−1.7 ± 0.1
T5	−13.9 ± 1.8	−4.6 ± 0.1
A6	−10.1 ± 3.4	−4.4 ± 0.1
E7		2.0 ± 0.1
Y8	17.5 ± 1.1	6.5 ± 0.1
D9	15.5 ± 1.4	6.3 ± 0.1
Y10	−8.3 ± 1.1	−4.1 ± 0.1
D11	−5.4 ± 2.0	−1.9 ± 0.1
A12	−10.0 ± 1.6	−4.3 ± 0.1
A13	−6.7 ± 1.8	−1.0 ± 0.1
E14	0.8 ± 1.0	0.9 ± 0.1
D15	4.3 ± 1.7	2.6 ± 0.1
N16	−4.4 ± 1.1	−3.0 ± 0.1
E17	−1.9 ± 1.0	0.3 ± 0.1
L18	−13.0 ± 1.2	−4.6 ± 0.1
T19	−2.3 ± 1.9	−1.8 ± 0.1
F20		−2.7 ± 0.1
V21		−1.0 ± 0.1
E22		0.7 ± 0.1
N23		−5.5 ± 0.1
D24		0.1 ± 0.1
K25		−5.5 ± 0.1
I26		
I27		−0.2 ± 0.1
N28		1.8 ± 0.1
I29		4.2 ± 0.1
E30	−1.6 ± 1.3	−0.3 ± 0.1
F31	1.5 ± 1.8	1.5 ± 0.1
V32		−2.5 ± 0.1
D33	1.0 ± 1.2	2.0 ± 0.1
D34	−5.8 ± 2.5	1.1 ± 0.1
D35	12.0 ± 1.0	6.5 ± 0.1
W36	2.8 ± 1.4	2.5 ± 0.1
W37	6.1 ± 1.3	2.9 ± 0.1
L38		−0.8 ± 0.1
G39		
E40		−1.2 ± 0.1
L41		3.7 ± 0.1
E42		−0.9 ± 0.1
K43		−1.4 ± 0.1
D44		−5.4 ± 0.1
G45		2.7 ± 0.1
S46		5.8 ± 0.1
K47		2.0 ± 0.1
G48		
L49	5.3 ± 1.0	2.3 ± 0.1
F50	−3.1 ± 4.0	−0.9 ± 0.1
P51		
S52	4.2 ± 1.2	2.6 ± 0.1
N53	4.5 ± 1.2	2.7 ± 0.1
Y54		8.4 ± 0.1
V55		−3.3 ± 0.1
S56		−5.7 ± 0.1
L57		−4.7 ± 0.1
G58		4.0 ± 0.1
N59		1.7 ± 0.1
$W36^{SC}$	−2.5 ± 1.2	−0.9 ± 0.1
$W37^{SC}$	−13.5 ± 1.7	−4.8 ± 0.1

All residual dipolar couplings were measured in PEG/hexanol alignment media.

Table S8. (ϕ,ψ) dihedral angle restraint list derived using TALOS written as an input file

```
! Made on 1-Apr-08
! 1. A 4 Phi -130.03 +/- 60.06 (-190.09 to -69.97)

!assign (resid   3 and name  C) (resid   4 and name  N)
!       (resid   4 and name CA) (resid   4 and name  C) 1.0  -130.03    60.06 2

! 2. T 5 Phi -117.83 +/- 48.00 (-165.83 to -69.83)

assign (resid   4 and name  C) (resid   5 and name  N)
       (resid   5 and name CA) (resid   5 and name  C) 1.0  -117.83    48.00 2

! 3. A 6 Phi -67.10 +/- 31.62 (-98.72 to -35.48)

assign (resid   5 and name  C) (resid   6 and name  N)
       (resid   6 and name CA) (resid   6 and name  C) 1.0   -67.10    31.62 2

! 4. Y 8 Phi -130.81 +/- 42.24 (-173.05 to -88.57)

assign (resid   7 and name  C) (resid   8 and name  N)
       (resid   8 and name CA) (resid   8 and name  C) 1.0  -130.81    42.24 2

! 5. D 11 Phi -135.67 +/- 30.00 (-165.67 to -105.67)

assign (resid  10 and name  C) (resid  11 and name  N)
       (resid  11 and name CA) (resid  11 and name  C) 1.0  -135.67    30.00 2

! 6. A 12 Phi -81.95 +/- 51.74 (-133.69 to -30.21)

assign (resid  11 and name  C) (resid  12 and name  N)
       (resid  12 and name CA) (resid  12 and name  C) 1.0   -81.95    51.74 2

! 7. N 16 Phi -93.66 +/- 39.46 (-133.12 to -54.20)

assign (resid  15 and name  C) (resid  16 and name  N)
       (resid  16 and name CA) (resid  16 and name  C) 1.0   -93.66    39.46 2

! 8. E 17 Phi -102.23 +/- 60.22 (-162.45 to -42.01)

assign (resid  16 and name  C) (resid  17 and name  N)
       (resid  17 and name CA) (resid  17 and name  C) 1.0  -102.23    60.22 2

! 9. T 19 Phi -121.67 +/- 41.32 (-162.99 to -80.35)

assign (resid  18 and name  C) (resid  19 and name  N)
       (resid  19 and name CA) (resid  19 and name  C) 1.0  -121.67    41.32 2

! 10. F 20 Phi -131.09 +/- 30.00 (-161.09 to -101.09)

!assign (resid  19 and name  C) (resid  20 and name  N)
!       (resid  20 and name CA) (resid  20 and name  C) 1.0  -131.09    30.00 2

! 11. V 21 Phi -130.04 +/- 39.86 (-169.90 to -90.18)

!assign (resid  20 and name  C) (resid  21 and name  N)
!       (resid  21 and name CA) (resid  21 and name  C) 1.0  -130.04    39.86 2

! 12. N 23 Phi -68.39 +/- 30.00 (-98.39 to -38.39)

!assign (resid  22 and name  C) (resid  23 and name  N)
!       (resid  23 and name CA) (resid  23 and name  C) 1.0   -68.39    30.00 2

! 13. K 25 Phi -118.75 +/- 50.18 (-168.93 to -68.57)

!assign (resid  24 and name  C) (resid  25 and name  N)
!       (resid  25 and name CA) (resid  25 and name  C) 1.0  -118.75    50.18 2

! 14. I 27 Phi -134.26 +/- 35.12 (-169.38 to -99.14)

!assign (resid  26 and name  C) (resid  27 and name  N)
!       (resid  27 and name CA) (resid  27 and name  C) 1.0  -134.26    35.12 2

! 15. I 29 Phi -105.58 +/- 53.22 (-158.80 to -52.36)

assign (resid  28 and name  C) (resid  29 and name  N)
       (resid  29 and name CA) (resid  29 and name  C) 1.0  -105.58    53.22 2

! 16. E 30 Phi -126.79 +/- 43.02 (-169.81 to -83.77)

assign (resid  29 and name  C) (resid  30 and name  N)
       (resid  30 and name CA) (resid  30 and name  C) 1.0  -126.79    43.02 2
```

Table S8. Continued.

```
! 17. F 31 Phi -96.67 +/- 30.00 (-126.67 to -66.67)

assign (resid  30 and name  C) (resid  31 and name  N)
       (resid  31 and name CA) (resid  31 and name  C) 1.0   -96.67    30.00 2

! 18. V 32 Phi -75.83 +/- 34.68 (-110.51 to -41.15)

assign (resid  31 and name  C) (resid  32 and name  N)
       (resid  32 and name CA) (resid  32 and name  C) 1.0   -75.83    34.68 2

! 19. D 34 Phi -62.47 +/- 30.00 (-92.47 to -32.47)

assign (resid  33 and name  C) (resid  34 and name  N)
       (resid  34 and name CA) (resid  34 and name  C) 1.0   -62.47    30.00 2

! 20. D 35 Phi -78.92 +/- 30.00 (-108.92 to -48.92)

assign (resid  34 and name  C) (resid  35 and name  N)
       (resid  35 and name CA) (resid  35 and name  C) 1.0   -78.92    30.00 2

! 21. W 36 Phi -112.20 +/- 46.22 (-158.42 to -65.98)

assign (resid  35 and name  C) (resid  36 and name  N)
       (resid  36 and name CA) (resid  36 and name  C) 1.0  -112.20    46.22 2

! 22. W 37 Phi -145.51 +/- 30.00 (-175.51 to -115.51)

assign (resid  36 and name  C) (resid  37 and name  N)
       (resid  37 and name CA) (resid  37 and name  C) 1.0  -145.51    30.00 2

! 23. L 38 Phi -118.11 +/- 69.94 (-188.05 to -48.17)

assign (resid  37 and name  C) (resid  38 and name  N)
       (resid  38 and name CA) (resid  38 and name  C) 1.0  -118.11    69.94 2

! 24. E 40 Phi -118.46 +/- 37.88 (-156.34 to -80.58)

assign (resid  39 and name  C) (resid  40 and name  N)
       (resid  40 and name CA) (resid  40 and name  C) 1.0  -118.46    37.88 2

! 25. L 41 Phi -83.58 +/- 30.00 (-113.58 to -53.58)

!assign (resid  40 and name  C) (resid  41 and name  N)
!       (resid  41 and name CA) (resid  41 and name  C) 1.0   -83.58    30.00 2

! 26. E 42 Phi -58.03 +/- 30.00 (-88.03 to -28.03)

!assign (resid  41 and name  C) (resid  42 and name  N)
!       (resid  42 and name CA) (resid  42 and name  C) 1.0   -58.03    30.00 2

! 27. K 43 Phi -68.82 +/- 30.00 (-98.82 to -38.82)

!assign (resid  42 and name  C) (resid  43 and name  N)
!       (resid  43 and name CA) (resid  43 and name  C) 1.0   -68.82    30.00 2

! 28. D 44 Phi -103.94 +/- 46.72 (-150.66 to -57.22)

!assign (resid  43 and name  C) (resid  44 and name  N)
!       (resid  44 and name CA) (resid  44 and name  C) 1.0  -103.94    46.72 2

! 29. G 45 Phi 86.87 +/- 30.00 (56.87 to 116.87)

!assign (resid  44 and name  C) (resid  45 and name  N)
!       (resid  45 and name CA) (resid  45 and name  C) 1.0    86.87    30.00 2

! 30. L 49 Phi -123.88 +/- 39.38 (-163.26 to -84.50)

assign (resid  48 and name  C) (resid  49 and name  N)
       (resid  49 and name CA) (resid  49 and name  C) 1.0  -123.88    39.38 2

! 31. P 51 Phi -75.76 +/- 42.62 (-118.38 to -33.14)

assign (resid  50 and name  C) (resid  51 and name  N)
       (resid  51 and name CA) (resid  51 and name  C) 1.0   -75.76    42.62 2

! 32. S 52 Phi -68.49 +/- 30.00 (-98.49 to -38.49)

assign (resid  51 and name  C) (resid  52 and name  N)
       (resid  52 and name CA) (resid  52 and name  C) 1.0   -68.49    30.00 2

! 33. S 56 Phi -105.94 +/- 41.94 (-147.88 to -64.00)
```

Table S8. Continued.

```
assign (resid  55 and name  C) (resid  56 and name  N)
       (resid  56 and name CA) (resid  56 and name  C) 1.0  -105.94    41.94 2

! 34. L 57 Phi -90.82 +/- 54.94 (-145.76 to -35.88)

!assign (resid  56 and name  C) (resid  57 and name  N)
!       (resid  57 and name CA) (resid  57 and name  C) 1.0   -90.82    54.94 2


! 35. A 4 Psi 150.15 +/- 30.00 (120.15 to 180.15)

!assign (resid   4 and name  N) (resid   4 and name CA)
!       (resid   4 and name  C) (resid   5 and name  N) 1.0   150.15    30.00 2

! 36. T 5 Psi 137.27 +/- 34.46 (102.81 to 171.73)

assign (resid   5 and name  N) (resid   5 and name CA)
       (resid   5 and name  C) (resid   6 and name  N) 1.0   137.27    34.46 2

! 37. A 6 Psi 125.78 +/- 30.00 (95.78 to 155.78)

assign (resid   6 and name  N) (resid   6 and name CA)
       (resid   6 and name  C) (resid   7 and name  N) 1.0   125.78    30.00 2

! 38. Y 8 Psi 156.16 +/- 30.00 (126.16 to 186.16)

assign (resid   8 and name  N) (resid   8 and name CA)
       (resid   8 and name  C) (resid   9 and name  N) 1.0   156.16    30.00 2

! 39. D 11 Psi 135.65 +/- 50.50 (85.15 to 186.15)

assign (resid  11 and name  N) (resid  11 and name CA)
       (resid  11 and name  C) (resid  12 and name  N) 1.0   135.65    50.50 2

! 40. A 12 Psi 132.53 +/- 30.00 (102.53 to 162.53)

assign (resid  12 and name  N) (resid  12 and name CA)
       (resid  12 and name  C) (resid  13 and name  N) 1.0   132.53    30.00 2

! 41. N 16 Psi -14.30 +/- 44.94 (-59.24 to 30.64)

assign (resid  16 and name  N) (resid  16 and name CA)
       (resid  16 and name  C) (resid  17 and name  N) 1.0   -14.30    44.94 2

! 42. E 17 Psi 147.36 +/- 37.56 (109.80 to 184.92)

assign (resid  17 and name  N) (resid  17 and name CA)
       (resid  17 and name  C) (resid  18 and name  N) 1.0   147.36    37.56 2

! 43. T 19 Psi 140.56 +/- 40.66 (99.90 to 181.22)

assign (resid  19 and name  N) (resid  19 and name CA)
       (resid  19 and name  C) (resid  20 and name  N) 1.0   140.56    40.66 2

! 44. F 20 Psi 156.05 +/- 30.00 (126.05 to 186.05)

!assign (resid  20 and name  N) (resid  20 and name CA)
!       (resid  20 and name  C) (resid  21 and name  N) 1.0   156.05    30.00 2

! 45. V 21 Psi 160.92 +/- 30.00 (130.92 to 190.92)

!assign (resid  21 and name  N) (resid  21 and name CA)
!       (resid  21 and name  C) (resid  22 and name  N) 1.0   160.92    30.00 2

! 46. N 23 Psi -29.64 +/- 39.08 (-68.72 to 9.44)

!assign (resid  23 and name  N) (resid  23 and name CA)
!       (resid  23 and name  C) (resid  24 and name  N) 1.0   -29.64    39.08 2

! 47. K 25 Psi 122.43 +/- 30.00 (92.43 to 152.43)

!assign (resid  25 and name  N) (resid  25 and name CA)
!       (resid  25 and name  C) (resid  26 and name  N) 1.0   122.43    30.00 2

! 48. I 27 Psi 163.68 +/- 34.02 (129.66 to 197.70)

!assign (resid  27 and name  N) (resid  27 and name CA)
!       (resid  27 and name  C) (resid  28 and name  N) 1.0   163.68    34.02 2

! 49. I 29 Psi 142.42 +/- 30.00 (112.42 to 172.42)
```

Table S8. Continued.

```
assign (resid  29 and name  N) (resid  29 and name CA)
       (resid  29 and name  C) (resid  30 and name  N) 1.0   142.42    30.00 2

! 50. E 30 Psi 134.52 +/- 30.00 (104.52 to 164.52)

assign (resid  30 and name  N) (resid  30 and name CA)
       (resid  30 and name  C) (resid  31 and name  N) 1.0   134.52    30.00 2

! 51. F 31 Psi 152.00 +/- 50.76 (101.24 to 202.76)

assign (resid  31 and name  N) (resid  31 and name CA)
       (resid  31 and name  C) (resid  32 and name  N) 1.0   152.00    50.76 2

! 52. V 32 Psi -15.21 +/- 50.08 (-65.29 to 34.87)

assign (resid  32 and name  N) (resid  32 and name CA)
       (resid  32 and name  C) (resid  33 and name  N) 1.0   -15.21    50.08 2

! 53. D 34 Psi -42.99 +/- 30.00 (-72.99 to -12.99)

assign (resid  34 and name  N) (resid  34 and name CA)
       (resid  34 and name  C) (resid  35 and name  N) 1.0   -42.99    30.00 2

! 54. D 35 Psi -10.97 +/- 30.00 (-40.97 to 19.03)

assign (resid  35 and name  N) (resid  35 and name CA)
       (resid  35 and name  C) (resid  36 and name  N) 1.0   -10.97    30.00 2

! 55. W 36 Psi 135.84 +/- 60.06 (75.78 to 195.90)

assign (resid  36 and name  N) (resid  36 and name CA)
       (resid  36 and name  C) (resid  37 and name  N) 1.0   135.84    60.06 2

! 56. W 37 Psi 162.21 +/- 30.00 (132.21 to 192.21)

assign (resid  37 and name  N) (resid  37 and name CA)
       (resid  37 and name  C) (resid  38 and name  N) 1.0   162.21    30.00 2

! 57. L 38 Psi 153.85 +/- 30.00 (123.85 to 183.85)

assign (resid  38 and name  N) (resid  38 and name CA)
       (resid  38 and name  C) (resid  39 and name  N) 1.0   153.85    30.00 2

! 58. E 40 Psi 140.85 +/- 35.72 (105.13 to 176.57)

assign (resid  40 and name  N) (resid  40 and name CA)
       (resid  40 and name  C) (resid  41 and name  N) 1.0   140.85    35.72 2

! 59. L 41 Psi 117.23 +/- 30.00 (87.23 to 147.23)

!assign (resid  41 and name  N) (resid  41 and name CA)
!       (resid  41 and name  C) (resid  42 and name  N) 1.0   117.23    30.00 2

! 60. E 42 Psi -37.44 +/- 30.00 (-67.44 to -7.44)

!assign (resid  42 and name  N) (resid  42 and name CA)
!       (resid  42 and name  C) (resid  43 and name  N) 1.0   -37.44    30.00 2

! 61. K 43 Psi -25.36 +/- 38.82 (-64.18 to 13.46)

!assign (resid  43 and name  N) (resid  43 and name CA)
!       (resid  43 and name  C) (resid  44 and name  N) 1.0   -25.36    38.82 2

! 62. D 44 Psi -3.51 +/- 30.00 (-33.51 to 26.49)

!assign (resid  44 and name  N) (resid  44 and name CA)
!       (resid  44 and name  C) (resid  45 and name  N) 1.0    -3.51    30.00 2

! 63. G 45 Psi -5.29 +/- 31.68 (-36.97 to 26.39)

!assign (resid  45 and name  N) (resid  45 and name CA)
!       (resid  45 and name  C) (resid  46 and name  N) 1.0    -5.29    31.68 2

! 64. L 49 Psi 145.22 +/- 30.00 (115.22 to 175.22)

assign (resid  49 and name  N) (resid  49 and name CA)
       (resid  49 and name  C) (resid  50 and name  N) 1.0   145.22    30.00 2

! 65. P 51 Psi 127.81 +/- 48.14 (79.67 to 175.95)
```

Table S8. Continued.

```
assign (resid  51 and name  N) (resid  51 and name CA)
       (resid  51 and name  C) (resid  52 and name  N) 1.0   127.81    48.14 2

! 66. S 52 Psi -21.12 +/- 30.00 (-51.12 to 8.88)

assign (resid  52 and name  N) (resid  52 and name CA)
       (resid  52 and name  C) (resid  53 and name  N) 1.0   -21.12    30.00 2

! 67. S 56 Psi 126.84 +/- 30.00 (96.84 to 156.84)

assign (resid  56 and name  N) (resid  56 and name CA)
       (resid  56 and name  C) (resid  57 and name  N) 1.0   126.84    30.00 2

! 68. L 57 Psi 133.59 +/- 30.00 (103.59 to 163.59)

!assign (resid  57 and name  N) (resid  57 and name CA)
!       (resid  57 and name  C) (resid  58 and name  N) 1.0   133.59    30.00 2
```