Supporting Information

Filamentous phage studied by magic-angle spinning NMR: resonance assignment and secondary structure of the coat protein in Pf1.

Amir Goldbourt[§], *Benjamin J. Gross*[§], *Loren A. Day*[‡], *Ann E. McDermott**[§]

[§]Department of Chemistry, Columbia University, New York, NY 10027, and

[‡]Public Health Research Institute, 225 Warren Street, Newark, NJ 07103

*Corresponding author: aem5@columbia.edu

Figure S1

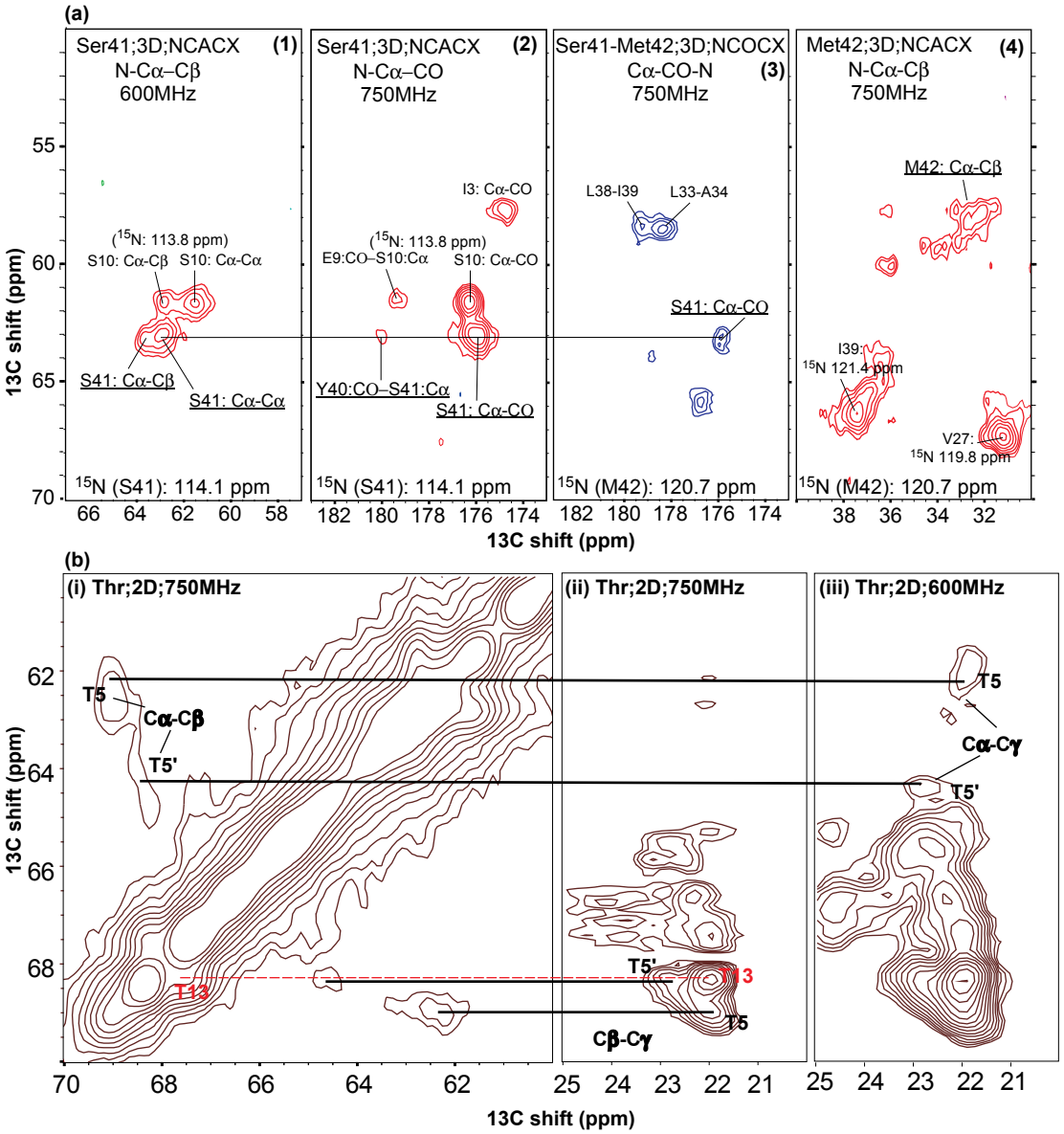

Figure S1: Magic-angle spinning solid state NMR spectra of intact Pf1 filamentous phage. Data were collected at temperatures above 10° C to ensure that the virus is in the high temperature form.

(a) Multiple-conformations of Met42 are demonstrated using strips showing the ^{13}C-^{13}C planes extracted from NCACX and NCOCX 3D experiments. The strips connect residue Ser41 to Met42. Sequential contacts are indicated by the solid line: N_{S41}-$C\alpha_{S41}$-$C\beta_{S41}$ (1), N_{S41}-$C\alpha_{S41}$-CO_{S41} (2) and N_{M42}-CO_{S41}-$C\alpha_{S41}$ (3) all share a common Cα (subscripts denote the residue corresponding to the assigned atom). Strips (2) and (3) share both CO and Cα shifts of S41. Strip (4) shows the Met42 dispesive Cα-Cβ peak at the ^{15}N frequency similar to strip (3). The relevant cross-peaks for Ser41 and Met42 are underlined. Additional contacts in the 3D that are within 1ppm of the M42 ^{15}N frequency are shown, and their ^{15}N shifts at the peak maxima are indicated. Sequential contacts in the 3D were establish also via N_i-$C\alpha_i$-CO_{i-1} transfers, as shown in two cases: S41-Y40 and S10-E9.

(b) Relevant regions from 2D homonuclear ^{13}C-^{13}C correlation spectra show at least two conformations for Thr5: T5 and T5'. Cross-peaks are shown for Cα/Cβ (i), Cβ/Cγ (ii) and Cα/Cγ (iii). The red dotted line indicates the Cα/Cβ and Cβ/Cγ cross-peaks belonging to Thr13.

Table S1: Chemical shift assignments of the magic-angle spinning solid state NMR spectra of the Pf1 filamentous phage coat protein. Chemical shift values were generated by Sparky (1) and are given in ppm. Standard deviations (calculated by Sparky) are given in parenthesis for atoms that were assigned in more than 4 cross-peaks. Unassigned atoms are indicated by a double dash ('--') and empty entries indicate that the sidechain of the particular residue does not have the atom in that column. All spectra were referenced externally to Adamantane CH_2 line at 40.48 ppm (^{13}C) and NH_4Cl at 39.27 ppm (^{15}N). Linewidths (w, in Hz) and signal-to-noise (sn) (gray color) are given for many of the peaks, and are based on 1-bond transfer cross-peaks in the 750 MHz, 10ms mixing ^{13}C-^{13}C homonuclear spectra. Mostly the peak with the highest signal-to-noise was chosen to analyze the cross-peak linewidth using the Sparky integrator. In the bottom of the table, residues with at low cross-peaks intensities (< 30% of the average peak intensity value), broad peaks and undetected peaks, are indicated.

	Cα	w(hz)/sn	Cβ	w(Hz)/sn	CO	w(Hz)/sn	Cγ1/Cγ2	w(Hz)/sn	Cδ1/Cδ2	w(Hz)/sn	Cε	Cζ	N	Nε/Nζ	Nη
Gly1	44.3(.08)	87/104			171.2(.21)	133/14							--		
Val2	65.3(.08)	130/14	32.9(.06)	108/15	175.4(.17)		22.6(.12)/22.0(.04)	100/9;116/11					124.9(.34)		
Ile3	57.7(.10)	137/12	40.5(.13)	235/6	174.9(.12)	111/20	27.2/17.7(.05)	?/?;100/6	14.6				113.7(.15)		
Asp4	54.6(.16)	186/9	41.1(.07)	98/8	177.2(.07)	118/16	--						124.2(.20)		
Thr5(1)	62.3(.17)		69.0(.10)	127/43	177.0(.11)		22.0(.05)						116.1(.32)		
Thr5(2)	63.4(.06)		68.8		177.2		22.3						--		
Thr5(3)	64.4(.06)		68.4(.02)	132/14	--		22.8	*160/14*					--		
Ser6	62.5(.06)		--		176.8								122.4(.04)		
Ala7	54.4(.07)	112/77	18.8(.06)	109/67	180.6(.09)	120/26							124.8(.14)		
Val8	65.8(.06)	150/23	31.5(.07)	119/30	176.7(.05)		23.1(.04)/22.5(.05)						117.9(.12)		
Glu9	60.1(.06)	97/22	29.3(.07)	115/21	179.3(.08)		36.0(.05)	93/12	182.9(.13)	168/10			120.3(.17)		
Ser10	61.6(.06)	115/21	62.8(.05)	*155/41*	176.4(.08)	97/36							113.8(.17)		
Ala11	55.1(.08)	115/71	19.6(.04)	74/66	180.4(.12)	102/19							123.4(.16)		
Ile12	64.7(.11)	152/23	36.4(.09)	134/24	178.2(.08)		29.8(.09)/18.0		13.2(.09)				117.9(.18)		
Thr13	67.4(.07)	107/31	68.4(.06)	72/33	177.5(.12)		21.9(.04)	76/68					118.8(.13)		
Asp14	57.7(.10)		40.9(.09)	168/12	179.6(.18)	177/21	177.3(.02)						124.1(.19)		
Gly15	47.6(.12)				175.8(.07)								109.2(.25)		
Gln16	59.3(.09)	96/31	28.6(.05)	85/35	179.1(.08)		34.7(.08)	105/20	177.9(.08)	105/36			121.8(.16)	107.9	
Gly17	47.6(.05)				176.9								107.6(.17)		
Asp18	58.0(.07)		40.9(.07)		178.9(.14)		177.6						124.4(.09)		
Met19	60.6(.06)	97/25	35.3(.05)	116/25	178.3(.06)		32.9(.05)	88/28			18.0		118.7(.16)		
Lys20	60.7(.04)	96/42	32.8(.05)	88/25	180.0(.06)		26.6(.04)	92/26	30.1(.05)		42.3(.06)		118.6(.06)	33.6	
Ala21	55.0(.07)	97/88	17.9(.05)	61/94	179.5(.12)	70/25							123.6(.11)		
Ile22	65.5(.12)		37.9(.06)		178.0(.10)		30.0(.05)/17.8(.05)		14.6(.05)				117.4(.09)		
Gly23	47.3(.11)				175.7(.07)								106.8(.20)		
Gly24	47.4(.05)				176.9								108.3(.15)		
Tyr25	58.9(.12)	132/12	37.6(.07)	116/16	179.6(.12)		129.3(.09)		132.2(.07)		118.8(.08)	157.8(.06)	122.5(.13)		
Ile26	64.3(.13)		36.5(.09)		179.3(.10)		28.6(.07)/17.9(.09)		11.0(.10)				119.6(.16)		
Val27	67.3(.13)	124/34	31.3(.08)	134/40	177.4(.11)		22.2(.3)/--						119.7(.16)		
Gly28	47.4(.12)				177.0								106.1(.09)		
Ala29	54.3(.13)	*140/14*	19.4(.3)	>200/15	179.2(.08)								122.4(.15)		
Leu30	57.9(.07)	110/22	43.4(.05)	109/21	179.9(.08)		27.6(.14)		24.7/--				119.5(.06)		
Val31	65.2(.05)		--		175.8(.10)		--						117.7		
Ile32	65.4(.07)	93/21	36.9(.06)	112/20	177.2(.11)		29.1(.05)/17.1(.06)	80/34;60/40	14.1(.08)				122.1(.16)		
Leu33	58.5(.07)		42.7(.10)		178.4(.10)		27.6(.07)		24.8(.06)/--				119.4(.12)		
Ala34	54.6(.06)		18.3(.05)		180.6(.08)								121.0(.14)		
Val35	66.7(.07)	109/33	31.5(.08)	112/37	177.6(.16)		24.0(.10)/22.3(.03)						117.4(.11)		
Ala36	56.0(.07)	113/54	17.6(.09)	122/47	177.8(.07)								122.1(.13)		
Gly37	47.4(.07)				177.3								99.8(.11)		
Leu38	58.3(.09)		41.2(.11)		179.1		26.9(.06)		23.8(.05)/25.7(.11)				124.1(.14)		
Ile39	66.4(.08)	93/26	37.4(.07)	82/28	178.6(.09)		30.7(.10)/18.0(.09)		14.5(.13)		--		121.5(.14)		
Tyr40	57.9(.07)	125/11	36.2(.09)	127/13	180.1(.11)		129.9(.17)		--		--	--	119.1(.17)		
Ser41	63.0(.10)		63.6		176.1(.13)								114.1(.15)		
Met42(1)	57.9		32.1		--		--				--		120.7		
Met42(2)	58.4		32.7		--		--				--		120.8		
Leu43	57.3(.07)	111/24	42.9(.06)	99/26	178.6(.09)		28.2(.07)		24.8(.06)/--				117.4(.10)		
Arg44	58.8(.11)	*198/11*	31.3(.08)	107/11	177.2		29.3(.07)		43.7(.06)			159.9(.06)	118.4(.20)	85.2	72.6
Lys45	57.0(.13)	172/6	33.8(.12)	>200/6	175.9(.12)		25.6(.08)		29.3(.06)		43.7		116.6(.13)	--	
Ala46	55.4(.06)	*205/9*	19.6(.06)	*167/7*	181.8(.05)	133/9							127.4(.16)		

^{13}C average RMSD is 0.08(.05) ^{15}N average RMSD is 0.14(.06)

Residues with Low peak intensities (<30% of average):

Residue	atoms involved in weak/broad cross-peaks (undetected in parenthesis)
Ile3	Cβ,Cγ1,Cγ2,Cδ
Thr5	Cα,Cβ
Ile12	Cγ1,Cδ
Tyr25	Cδ,Cε,Cζ
Ala29	Cβ (Broad peak but strong)
Tyr40	Cγ,Cz,(Cδ,Cε)
Met42	Cα,Cβ
Arg44	**Cα,Cβ** (Broad peak but strong)
Lys45	Cα,Cβ,Cγ,Cδ,Cε

(1) T. D. Goddard and D. G. Kneller, SPARKY 3,• University of California, San Francisco

Table S2: Comparison of Chemical shift predictions using SHIFTX (1) for the three monomers of the Pf1 coat protein subunits in the asymmetric repeating unit of model 1QL2 (2).
The three monomers 1-3 are compared pair-wise, and the table contains only those amino acids, for which the shift deviations are larger than |0.4| ppm for at least one of the atoms: N, CO, Cα, and Cβ. The values of the chemical shift differences are indicated with a bold font in the table. Δ_{ij} designates chemical shift difference of a particular atom between monomers *i* and *j*. The backbone RMSD for the three pairs are all ~0.1 Å. The all-atom RMSD is larger (1.2-1.5 Å), however shifts for the sidechain are not predicted by the program and are expected to be large as well. For 26 residues (64 atoms), at least one atom is expected to have a significant shift in the NMR spectra. Such differences are not observed in our spectra.

	Δ_{12}				Δ_{23}				Δ_{13}			
	N	Cα	Cβ	CO	N	Cα	Cβ	CO	N	Cα	Cβ	CO
I3	**-0.4**	0.0	0.2	-0.1	-0.2	0.1	0.1	0.0	0.2	0.1	-0.1	0.1
T5	**0.6**	-0.1	-0.3	-0.2	-0.2	0.0	-0.2	-0.1	**-0.8**	0.0	0.1	0.1
S6	**0.4**	0.0	0.0	0.1	0.3	0.0	0.0	0.1	-0.1	0.0	0.0	-0.1
E9	**0.6**	0.0	0.0	0.0	**0.9**	0.0	0.0	-0.1	0.3	0.0	0.0	-0.1
I12	0.0	**-0.4**	0.0	0.0	**0.4**	-0.3	0.0	0.0	**0.4**	0.1	0.0	0.0
T13	**-0.8**	**0.5**	**-1.1**	-0.1	0.3	-0.2	**-1.6**	0.1	**1.1**	**-0.7**	**-0.5**	0.2
D14	**-2.0**	-0.3	-0.1	0.0	**-1.9**	-0.2	0.1	0.0	0.1	0.1	0.2	0.1
M19	**1.0**	-0.3	-0.1	0.0	**0.9**	-0.3	-0.1	0.1	-0.1	0.1	0.0	0.0
K20	**0.5**	0.2	0.3	-0.1	**0.6**	0.1	0.0	-0.1	0.0	-0.1	-0.3	0.0
G23	0.3	0.0	0.0	0.0	**-0.6**	0.0	0.0	0.0	**-0.9**	0.0	0.0	0.0
G24	**0.7**	0.1	0.0	0.0	**0.9**	0.1	0.0	0.2	0.2	0.0	0.0	0.2
Y25	0.1	-0.2	0.1	**-0.4**	0.3	**0.4**	0.0	0.0	0.2	**0.7**	-0.1	**0.5**
I26	0.1	0.1	0.1	**0.5**	-0.3	0.0	-0.1	0.3	**-0.4**	0.0	-0.2	-0.2
V27	-0.2	**-0.4**	0.0	0.0	0.0	0.0	0.0	0.0	0.2	**0.5**	0.0	0.0
G28	**0.5**	-0.1	0.0	0.1	0.1	0.0	0.0	0.1	**-0.4**	0.1	0.0	0.0
A29	**-0.5**	0.1	0.0	0.1	**-0.5**	0.2	0.0	0.1	-0.1	0.2	0.0	0.1
L33	0.0	0.2	**0.4**	0.0	0.2	-0.1	0.0	-0.1	0.1	-0.2	**-0.4**	-0.1
A34	-0.1	-0.1	0.1	-0.1	**0.5**	-0.1	-0.1	0.2	**0.6**	0.0	-0.2	0.2
A36	**-0.9**	0.1	0.1	0.0	**-0.4**	0.1	0.1	-0.1	**0.5**	-0.1	-0.1	-0.1
G37	**-0.4**	-0.2	0.0	-0.3	-0.1	0.1	0.0	-0.2	0.3	0.3	0.0	0.1
L38	**-0.6**	**-0.5**	0.1	**-0.4**	**0.5**	-0.2	0.0	**-0.4**	**1.1**	0.3	-0.1	0.1
I39	-0.2	**-0.6**	-0.1	0.0	**-0.5**	**-0.7**	**1.1**	0.0	-0.3	-0.1	**1.2**	0.0
Y40	**0.9**	-0.2	-0.2	0.2	**0.4**	-0.1	0.1	-0.2	**-0.4**	0.1	**0.4**	**-0.4**
S41	-0.2	0.3	0.0	-0.1	0.0	-0.1	0.0	0.0	0.3	**-0.4**	0.0	0.1
L43	**-0.5**	-0.3	0.0	-0.2	-0.2	0.0	0.0	-0.1	0.3	0.3	0.0	0.1
R44	**-2.5**	-0.2	-0.1	0.1	**-2.9**	0.0	0.0	0.0	**-0.4**	0.2	0.1	-0.1

1. Neal, S., Nip, A. M., Zhang, H. & Wishart, D. S. (2003) *J. Biomol. NMR* **26,** 215-240.
2. Welsh, L. C., Symmons, M. F. & Marvin, D. A. (2000) *Acta Cryst. D* **56,** 137-150.

Table S3a: Secondary structure probabilities derived from the program PSSI Version 2.0 (1) for the Pf1 filamentous phage coat protein. (i) Results obtain for the assignments given in Table 1. (ii) Results obtained after re-referencing the spectra according to (2) with the following shifts: C' -0.6ppm; Cα -0.7ppm; Cβ -0.36ppm; N -0.25ppm. Regions with low helix probability (<60%) are in italics. **Abbreviations:** SS: Secondary structure; F-SS: Filtered secondary structure; H: α-Helix; C: random coil; B: β-strand.

(i)	β-strand	Coil	α-Helix	SS	F-SS
G1	*0.45*	*0.55*	*0.00*	*C*	*C*
V2	*0.06*	*0.62*	*0.32*	*C*	*C*
I3	*0.23*	*0.77*	*0.00*	*C*	*C*
D4	*0.13*	*0.87*	*0.00*	*C*	*C*
T5	*0.17*	*0.50*	*0.33*	*C*	*C*
S6	0.00	0.25	0.75	H	H
A7	0.00	0.02	0.98	H	H
V8	0.00	0.02	0.98	H	H
E9	0.00	0.00	1.00	H	H
S10	0.00	0.03	0.97	H	H
A11	0.00	0.02	0.98	H	H
I12	0.00	0.00	1.00	H	H
T13	0.00	0.00	1.00	H	H
D14	0.00	0.00	1.00	H	H
G15	0.01	0.30	0.69	H	H
Q16	0.00	0.00	1.00	H	H
G17	0.00	0.05	0.95	H	H
D18	0.00	0.01	0.99	H	H
M19	0.00	0.01	0.99	H	H
K20	0.00	0.00	1.00	H	H
A21	0.00	0.02	0.98	H	H
I22	0.00	0.00	1.00	H	H
G23	0.00	0.20	0.80	H	H
G24	0.01	0.15	0.84	H	H
Y25	0.00	0.05	0.95	H	H
I26	0.00	0.00	1.00	H	H
V27	0.00	0.00	1.00	H	H
G28	0.00	0.04	0.95	H	H
A29	0.00	0.13	0.87	H	H
L30	0.00	0.02	0.98	H	H
V31	0.00	0.10	0.90	H	H
I32	0.00	0.01	0.99	H	H
L33	0.00	0.01	0.99	H	H
A34	0.00	0.01	0.99	H	H
V35	0.00	0.00	1.00	H	H
A36	0.00	0.02	0.98	H	H
G37	*0.04*	*0.43*	*0.53*	*H*	*H*
L38	0.00	0.01	0.99	H	H
I39	0.00	0.00	1.00	H	H
Y40	0.00	0.13	0.87	H	H
S41	0.00	0.04	0.96	H	H
M42	0.00	0.10	0.90	H	H
L43	0.00	0.04	0.96	H	H
R44	0.00	0.14	0.86	H	H
K45	*0.15*	*0.81*	*0.03*	*C*	*H*
A46	0.00	0.17	0.83	H	C

(ii)	β-strand	Coil	α-Helix	SS	F-SS
G1	*0.72*	*0.28*	*0.00*	*B*	*C*
V2	*0.21*	*0.72*	*0.07*	*C*	*C*
I3	*0.17*	*0.83*	*0.00*	*C*	*C*
D4	*0.13*	*0.87*	*0.00*	*C*	*C*
T5	*0.31*	*0.59*	*0.10*	*C*	*C*
S6	0.01	0.42	0.57	H	H
A7	0.00	0.08	0.92	H	H
V8	0.00	0.09	0.91	H	H
E9	0.00	0.00	1.00	H	H
S10	0.00	0.10	0.90	H	H
A11	0.00	0.05	0.95	H	H
I12	0.00	0.02	0.98	H	H
T13	0.00	0.00	1.00	H	H
D14	0.00	0.02	0.98	H	H
G15	*0.03*	*0.65*	*0.33*	*C*	*H*
Q16	0.00	0.01	0.99	H	H
G17	0.01	0.20	0.80	H	H
D18	0.00	0.03	0.97	H	H
M19	0.00	0.03	0.97	H	H
K20	0.00	0.00	1.00	H	H
A21	0.00	0.07	0.93	H	H
I22	0.00	0.01	0.99	H	H
G23	*0.01*	*0.56*	*0.44*	*C*	*C*
G24	*0.02*	*0.49*	*0.49*	*C*	*C*
Y25	0.02	0.16	0.82	H	H
I26	0.00	0.01	0.99	H	H
V27	0.00	0.00	1.00	H	H
G28	0.01	0.19	0.80	H	H
A29	*0.00*	*0.46*	*0.53*	*H*	*H*
L30	0.00	0.03	0.97	H	H
V31	*0.04*	*0.49*	*0.47*	*C*	*H*
I32	0.00	0.03	0.97	H	H
L33	0.00	0.02	0.98	H	H
A34	0.00	0.02	0.98	H	H
V35	0.00	0.01	0.99	H	H
A36	0.00	0.04	0.96	H	H
G37	*0.06*	*0.79*	*0.15*	*C*	*H*
L38	0.00	0.01	0.99	H	H
I39	0.00	0.00	1.00	H	H
Y40	*0.01*	*0.45*	*0.54*	*H*	*H*
S41	0.00	0.09	0.91	H	H
M42	0.00	0.17	0.83	H	H
L43	0.00	0.16	0.83	H	H
R44	0.01	0.36	0.63	H	H
K45	*0.32*	*0.67*	*0.01*	*C*	*H*
A46	0.00	0.23	0.77	H	C

Table S3b: Torsion angle prediction results are shown for those amino acids that vary following a re-referencing protocol. Results are shown from TALOS(3) and PREDITOR(4) programs: (i) Specific torsion angles that change their value in more than 10 degrees when re-referencing the shifts in Table S1. Re-referencing and torsion angle prediction for PREDITOR were calculated with the java online interface. The following correction values were obtained for the shifts: C': -0.66ppm; Cα: -0.56ppm; Cβ: -0.40ppm; N: -0.45ppm. These values were also used for correcting the input shifts for TALOS. (ii) The ten lowest score results for residue Gly24 (in the triplet G23-G24-Y25) from running TALOS with re-referenced chemical shifts. In italics – the database entries used to determine ϕ and ψ values for G24 in (i).

(i)	TALOS					PREDITOR			
	data		Re-ref			data		Re-ref	
Res.	ϕ	ψ	ϕ	ψ	Res.	ϕ	ψ	ϕ	ψ
V2	-102	132	-69	-33	A29	-65	44	-71	19
T5	-93	157	-113	138	K45	-94	30	-97	-1
G24	-66	-41	48	39					
K45	-105	8	-94	-11					

(ii)	ϕ	ψ	score	R1	R2	R3	Source
1	-73	-39	79.88	G68	G69	Y70	Mbp
2	-65	-42	81.16	N110	G111	Y112	pin1
3	*35*	*34*	*81.63*	*D131*	*G132*	*D133*	*calmodulin_complexed*
4	*40*	*55*	*81.97*	*D131*	*G132*	*D133*	*calmodulin*
5	76	-161	84.70	G32	G33	K34	L25
6	-70	-41	84.73	G98	E99	W100	Hpt
7	*68*	*20*	*84.73*	*H648*	*G649*	*I650*	*malate*
8	*60*	*36*	*84.94*	*D22*	*G23*	*D24*	*calmodulin*
9	60	-148	85.10	N12	G13	L14	Hpr
10	*38*	*49*	*85.11*	*D95*	*G96*	*N97*	*calmodulin*

1. Wang, Y. J. & Jardetzky, O. (2002) *Prot. Sci.* **11,** 852-861.
2. Wang, Y. J. & Wishart, D. S. (2005) *J. Biomol. NMR* **31,** 143-148.
3. Cornilescu, G., Delaglio, F. & Bax, A. (1999) *J. Biomol. NMR* **13,** 289-302.
4. Neal, S., Berjanskii, M., Zhang, H. & Wishart, D. S. (2006) *Magn. Reson. Chem.* **44,** S156-S167; http://wishart.biology.ualberta.ca/shiftor/cgi-bin/preditor_current.py

Table S4: Experimental parameters.
Experimental parameters are shown for the 2D and 3D spectra recorded on several samples of the Pf1 filamentous phage. For the processing parameters, only the main scheme used for assignment is mentioned. Additional proccessing protocols have been used for verification. Relevant abbreviations are given below each part of the Table. All spectra were acquired using the TPPI (Time Proportional Phase Increment) scheme[1] in order to obtain phase sensitive detection in the indirect dimensions.

3D experiments				
Experiment:	3D NCACX	3D NCOCX	3D NCACX	3D NCOCX
sample number	3b	3b	3a	3a
^{1}H freq. [MHz]	750	750	600	600
spectrometer	Bruker,NYSBC	Bruker,NYSBC	Varian	Varian
spinning freq. (ν_r), [kHz]	14.5	14.5	13.5	13.5
temperature (VT) [^{o}K]	~290[b]	~290[b]	283[a]	283[a]
acq. points(t1/t2/t3)	96/98/2048	104/50/2048	110/100/2048	110/80/2048
acq. time(t1/t2/t3) [ms]	6.6/6.5/25.6	7.2/6.9/25.6	16.3/14.8/30.7	16.3/11.9/30.7
carrier freq. (ppm)	120.6/56.2/103.2	120.3/178.7/102.6	90.0/56.5/135.4	90.0/178/.0135.4
H_{90}/C_{90} [μs]	3.7/5.0	3.7/5.1	3.9/5.6	3.9/5.7
CP power(ν_H/ν_N) [kHz]	~50/~35	~50/~35	~60/~35	~60/~35
CP contact time [ms]	2.0	2.0	1.0	1.0
CP shape	lru,H,20%	lru,H,20%	tru,N,11%	tru,N,11%
DCP power (ν_C/ν_N) [kHz]	2.5ν_r/1.5ν_r	1.5ν_r/2.5ν_r	2.5ν_r/1.5ν_r	2.5ν_r/1.5ν_r
DCP contact time [ms]	4.0	4.0	3.5	4.0
DCP shape	tru,C,20%	lru,C,10%	lrd,C,28%	lrd,C,30%
DARR[2] mixing time (ms)[c]	10.0	10.0	15.0	10.0
^{1}H decoupling [kHz]	~85	~85	~75	~75
TPPM (pw/phase)	6.0μ/15°	6.0μ/15°	6.9μ/15°	6.9μ/15°
relx delay/scans	2.9s/16	2.9s/16	3.0s/16	3.0s/16
SW(F1/F2/F3) [kHz]	7.25/7.5/80.0	7.25/3.625/80.0	3.375/3.375/66.7	3.375/3.375/66.7

proc. parameters F1/F2				
Total # of points	256/256/4096	256/128/4096	256/256/4096	256/256/4096
apodization	EM(40)/CB/CB	EM(40)/CB/CB	EM(40)/CB/CB	EM(40)/SP/SP
linear prediction	-/f98/-	-/f50/-	-/-/-	-/-/-

Abbreviations: freq.: Frequency; acq.: acquisition; $H(C)_{90}$: Proton(Carbon) 90 degree pulse length; CP: cross-polarization; $\nu_H/\nu_C/\nu_N$: radio frequency power of the specified channel; DCP: Band selective CP (see manuscript materials and methods section); TPPM - Two-pulse phase-modulation decoupling scheme; pw/phase: Pulse width and phase in TPPM decoupling; relx: relaxation; SW: Spectral width; CP/DCP shape: [t/l]r[u/d],[C/H/N], X%: [tangent/linear]-shape ramp [up/down] on [13C/1H/15N] channel with X% amplitude variations; t1(F1)/t2(F2)/t3(F3) - time(frequency) domain of 15N, CO/CA, and acquisition dimensions, respectively; EM(40): apodization of the FID with 40 Hz exponential broadening; CB: apodization with a cosine-bell function; f98 - Forward linear prediction of 98 points; NYSBC: spectrometer located at the New York Structural Biology Center.

Homonuclear ^{13}C-^{13}C DARR 2D				
acq. parameters				
sample number	2	3a	3b	1
^{1}H freq. [MHz]	750	600	750	400
spectrometer	Bruker,NYSBC	Bruker,NYSBC	Varian	Varian
spinning freq. (ν_r), [kHz]	15.0	11.5	14.5	9.0
temperature (VT) [^{o}K]	~296[b]	283[a]	~291[b]	288[a]
acq. points(t1/t2)	1676/2048	1000/4096	1200/2048	768/1024
acq. time(t1/t2) [ms]	12.6/25.6	10.0/41.0	9.0/25.6	10.7/28.4
carrier freq. (ppm)	111.0	135.4	102.7	93.5
H_{90}/C_{90} [μs]	3.6/5.0	4.0/6.0	3.7/5.0	2.9/3.4
CP power (ν_H/ν_C) [kHz]	~65/~50	~55/~40	~50/~35	
CP contact time [ms]	1.25	1.0	1.25	1.0
CP shape	lru,H,20%	tru,C,15%	lru,H,20%	lrd,C,20%
DARR mixing time (ms)	6.0	6.0	10.0	1.0
^{1}H decoupling [kHz]	~85	~75	~85	~75
TPPM (pw/phase)	5.3μ/15°	6.9μ/15°	6.0μ/15°	5.8μ/17°
relx delay/scans	3s/16	3s/16	3s/16	4s/24
SW(F1/F2) [kHz]	70.4/80.0	50.0/100.0	66.7/80.0	36.0/36.0

proc. parameters F1/F2				
Total # of points	2048/4096	2048/4096	2048/4096	1024/4096
apodization	1:GM(80;65)/CB 2:EM(10)/CB	1:GM(80;65)/CB 2:GM(50;65)/CB	1:GM(50;50)/CB 2:EM(40)/EM(40)	EM(40)/CB

Additional abbreviations: DARR: Dipolar Assisted Rotational Resonance(2);
GM(x;y): Lorentz (-x[Hz]) to Gauss (+y[Hz]) transformation, t1(F1)/t2(F2) - indirect dimension time(Frequency)/acquisition dimension time(Frequency)

Temperature: RF heating was not considered and can increase sample temperature by ~10 deg. For the Varian spectrometer, heating due to the air friction during spinning was also not considered. Thus, all experiments were performed in temperatures well above the transition point of Pf1 and the virus is considered to be in the high temperature form at all times.

[a] VT gas temperature for Varian spectrometers.

[b] Temperature calibrated for spinning.

[C] DARR condition(1) at n=1: $\nu_r=\nu_c$

Samples:
U-[^{13}C,^{15}N] Pf1 Virion, ~30 μL of PEG precipitated and fully hydrated:
#1: ~10mg; #2: ~10mg; #3a: ~7 mg, 30% Ethylene Glycol; #3b: ~7 mg, 30% Ethylene Glycol.

References:
(1) R.R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, Oxford, 1987.
(2) Takegoshi, K., Nakamura, S. & Terao, T. (2001) Chem. Phys. Lett. 344, 631-637.