Supporting Information

Jensen et al. 10.1073/pnas.1103270108

SI Methods

Recombinant Protein Production and Purification. Cloning, expression, and purification of the isotopically labeled isolated measles virus N_{TAIL} domain and the C-terminal domain of P (sometimes known as XD) were described previously (1, 2). Experiments were carried out in 50 mM phosphate buffer at pH 7 with 50 mM NaCl.

Cloning, Expression, and Purification of Intact Measles Virus Nucleocapsids. The cloning procedure of the measles virus nucleoprotein gene (strain Edmonston B) into the expression vector pET22b (+) was described previously (3). The vector was transformed into *Escherichia coli* Rosetta™ (λDE3)/pRARE strain (Novagen) for expression of the recombinant protein. Unlabeled protein was obtained in Luria-Bertani medium, while the uniformly isotopically labeled ^{15}N and $^{15}N/^{13}C$ protein samples were produced in M9 minimal medium supplemented with 1.0 g/L of $^{15}NH_4Cl$, 2.0 g/L of ^{13}C glucose and Minimum Essential Medium (MEM) vitamins (Gibco). The cells were grown at 37 °C until the optical density (OD) at 600 nm reached 0.6 and the protein expression was then induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 14–16 h at 30 °C. Cells were harvested by centrifugation and then suspended in lysis buffer (10 mL/L of bacteria culture) containing 20 mM Tris-HCl, 150 mM NaCl at pH 7.5 (buffer A), supplemented with 1 mM $MgSO_4$, complete™ protease inhibitor cocktail tablets (Roche), DNAse I (Sigma), and lysozyme (Fluka) and incubated for 30 min on ice. Cells were completely disrupted by sonication on ice and the debris was removed by centrifugation for 20 min at 16,000 × *g*, 4 °C. Typically, 5–8 mL of the supernatant was layered onto a continuous gradient of 23–26 mL of CsCl (20–40% w/w in buffer A). The gradient was centrifuged at 25,000 rpm for 15 h at 12 °C (SW28 Beckman rotor using UltraClear™ tubes of 38.5 mL), and the visible nucleocapsid band was collected by puncturing the tube. The sample was dialyzed into buffer A and layered onto a glycerol cushion 15% (v/v in buffer A) and then centrifuged as described for the CsCl gradient. The capsid on the bottom was resuspended in 50 mM sodium phosphate buffer pH 7.0 with 50 mM NaCl and dialyzed in the same buffer overnight. Sample was centrifuged at 16,000 × *g*, 1 min at 4 °C and the quality of the capsid preparation in the supernatant was checked by SDS-PAGE and electron microscopy (negative staining) as previously described (3). Protein concentrations were measured by absorbance spectroscopy using BioRad Bradford's method based-protein assay. The yield of ^{15}N- and $^{15}N/^{13}C$-labeled measles virus nucleoprotein was about 78 mg/L. The protein solution was frozen in liquid nitrogen and stored at −80 °C at final concentration ranges of 0.2 to 0.4 mM. Trypsin-digested nucleocapsids were obtained as described previously and comprised residues 14–405 (4).

Capsid EM Negative Staining (Sample Quality Control). Noncleaved and cleaved capsids were resuspended and dialyzed in the same buffer used for the NMR studies (e.g., 50 mM sodium phosphate buffer pH 7.0 50 mM NaCl). Samples were centrifuged at 16,000 × *g*, 1 min, 4 °C and the quality of the capsid preparation in the supernatant was checked by SDS-PAGE and electron microscopy. Briefly, the capsids were diluted to a concentration of about 0.1 mg/mL and were adsorbed onto the clean face of a carbon film on mica, negatively stained with 2% (w/v) uranyl acetate and observed under low-dose conditions with a JEOL 1200 EX II microscope at 100 kV and a nominal magnification of 40,000X.

1. Longhi S, et al. (2003) The C-terminal domain of the measles virus nucleoprotein is intrinsically disordered and folds upon binding to the C-terminal moiety of the phosphoprotein. *J Biol Chem* 278:18638–18648.
2. Gely S, et al. (2010) Solution structure of the C-terminal X domain of the measles virus phosphoprotein and interaction with the intrinsically disordered C-terminal domain of the nucleoprotein. *J Mol Recognit* 23:435–447.
3. Desfosses A, Goret G, Farias Estrozi L, Ruigrok RWH, Gutsche I (2011) Nucleoprotein-RNA orientation in the measles virus nucleocapsid by three-dimensional electron microscopy. *J Virol* 85:1391–1395.
4. Schoehn G, et al. (2004) The 12 A structure of trypsin-treated measles virus N-RNA. *J Mol Biol* 339:301–312.

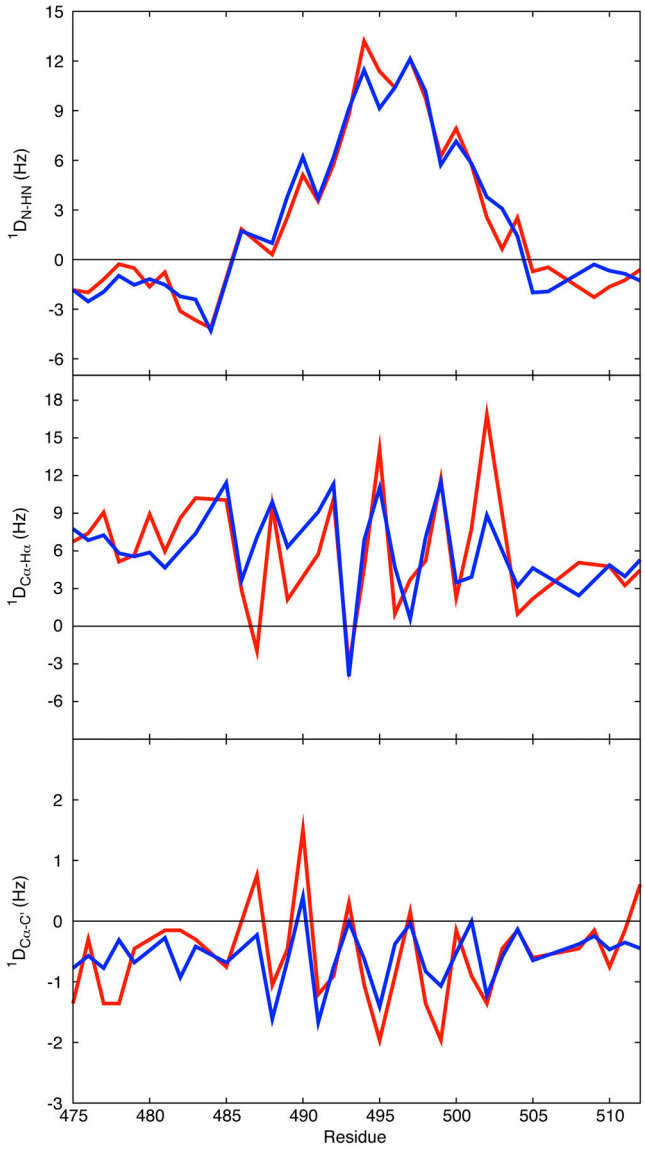

Fig. S1. Comparison of different types of experimental (red) and back-calculated (blue) RDCs in the molecular recognition element of N_{TAIL}. The back-calculated RDCs were obtained as a population-weighted average corresponding to the conformational equilibrium depicted in Fig. 1 (main text).

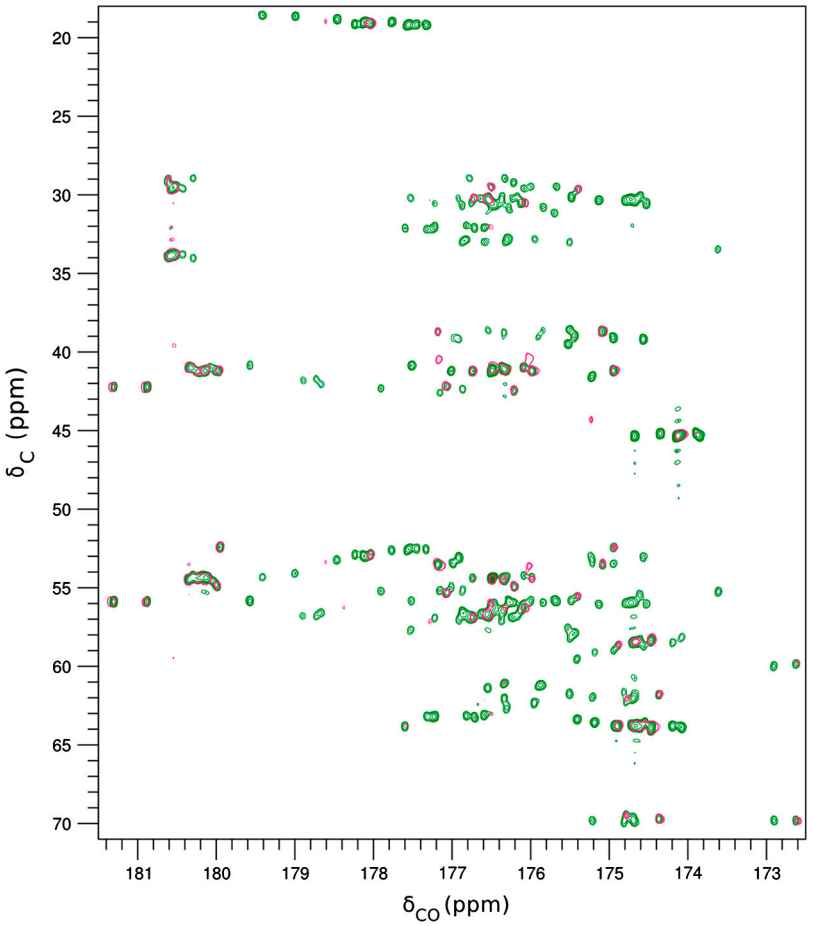

Fig. S2. ^{13}C detected correlation spectra from free N_{TAIL} (green) and full-length capsid (red) recorded at a ^{1}H frequency of 700 MHz and 25 °C. The spectrum of the free N_{TAIL} was acquired using the CBCACO pulse sequence (1, 2) with 1,024 and 192 complex points and sweep widths of 10.5 and 12.7 kHz in the direct and indirect dimensions, respectively. The spectrum of the intact capsid was acquired using the HCBCACO pulse sequence (3) with 1,024 and 192 complex points and sweep widths of 10.5 and 12.7 kHz in the direct and indirect dimensions, respectively.

1 Duma L, Hediger S, Lesage A, Emsley L (2003) Spin-state selection in solid-state NMR. *J Magn Reson* 164:187–195.
2 Bermel W, et al. (2006) Protonless NMR experiments for sequence-specific assignment of backbone nuclei in unfolded proteins. *J Am Chem Soc* 128:3918–3919.
3 Bermel W, et al. (2009) H-start for exclusively heteronuclear NMR spectroscopy: the case of intrinsically disordered proteins. *J Magn Reson* 198:275–281.

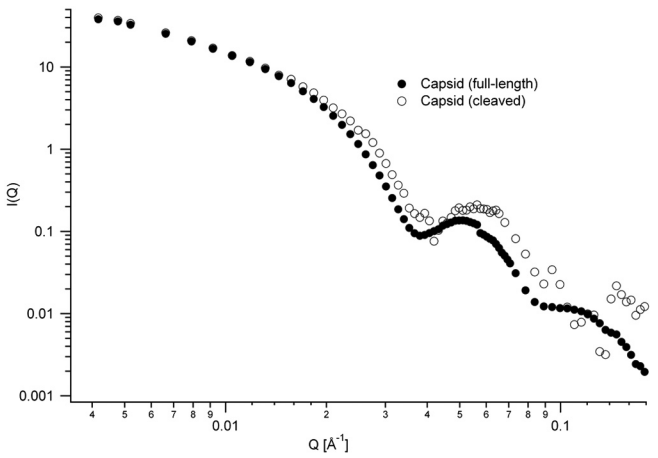

Fig. S3. Small angle neutron scattering data $I(Q)$ of the intact capsid and the cleaved, trypsin-digested form (no N_{TAIL}) in a double-logarithmic representation. The data of the cleaved form are noisier as a consequence of the lower concentration.

Table S1. Data reproduction from ensembles with different combinations of helical conformers

Number of helical conformers	χ^2*	Number of optimized parameters†	Helical conformers‡	Population (%)§	Significance¶
1	433	4	485–502	34	
2	231	7	486–498	22	$P < 0.0001$
			492–502	37	
3	186	10	485–502	19	$P < 0.0001$
			492–497	32	
			494–499	23	
4	163	13	485–502	13	$P = 0.0041$
			489–502	10	
			492–497	30	
			494–499	22	
5	154	16	485–502	13	$P = 0.1043$
			489–496	12	
			492–497	19	
			492–502	12	
			494–499	21	

*The target function for the χ^2 included all 114 experimental data points (three types of RDCs and Cα chemical shifts).
†One helix implies the optimization of three parameters: starting amino acid, final amino acid, and the population. In addition, a scaling factor is optimized to take into account the absolute level of alignment for the RDCs.
‡Range of the invoked helices.
§The population of the invoked helices. The remaining conformers are completely unfolded.
¶Significance of the improvement of this model as compared to the simpler model calculated using a standard *F*-test.

Table S2. The six best ASTEROIDS solutions assuming that N_{TAIL} samples four specific, helical conformers in conformational equilibrium with a completely unfolded form

Solution	χ^2	Helical conformers	Population (%)
1	163	485–502	13
		489–502	10
		492–497	30
		494–499	22
2	167	485–502	16
		489–499	8
		492–497	30
		494–499	20
3	168	485–502	12
		489–497	17
		492–501	19
		494–499	19
4	169	485–502	11
		489–497	18
		492–502	19
		494–499	21
5	170	485–502	14
		491–495	23
		492–501	17
		494–499	24
6	173	485–502	17
		492–497	23
		492–499	13
		494–499	19