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Crystal structure of the RNA-dependent RNA polymerase from influenza C virus

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Abstract

Negative-sense RNA viruses, such as influenza, encode large, multidomain RNA-dependent RNA polymerases that can both transcribe and replicate the viral RNA genome¹. In influenza virus, the polymerase (FluPol) is composed of three polypeptides: PB1, PB2 and PA/P3. PB1 houses the polymerase active site, whereas PB2 and PA/P3 contain, respectively, cap-binding and endonuclease domains required for transcription initiation by cap-snatching². Replication occurs through *de novo* initiation and involves a complementary RNA intermediate. Currently available structures of the influenza A and B virus polymerases include promoter RNA (the 5' and 3' termini of viral genome segments), showing FluPol in transcription pre-initiation states^{3,4}. Here we report the structure of apo-FluPol from an influenza C virus, solved by X-ray crystallography to 3.9 Å, revealing a new 'closed' conformation. The apo-FluPol forms a compact particle with PB1 at its

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Author Contributions N.H., F.T.V., D.I.S., J.M.G. and E.F. conceived and designed the study. N.H., K.E.O., I.S.M. and R.P.R. performed experiments. S.C. provided coordinates before publication and discussed the results. C.V. and G.B. aided with data analysis and provided valuable advice. N.H., F.T.V., K.E.O., R.P.R., D.I.S., J.M.G. and E.F. analysed data and wrote the paper.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Supplementary Information is available in the online version of the paper.

Author Information Atomic coordinates and structure factors for the reported crystal structure have been deposited in the Protein Data Bank under accession numbers 5D98 and 5D9A.

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Readers are welcome to comment on the online version of the paper.

centre, capped on one face by PB2 and clamped between the two globular domains of P3. Notably, this structure is radically different from those of promoter-bound FluPol_s^{3,4}. The endonuclease domain of P3 and the domains within the carboxy-terminal two-thirds of PB2 are completely rearranged. The cap-binding site is occluded by PB2, resulting in a conformation that is incompatible with transcription initiation. Thus, our structure captures FluPol in a closed, transcription pre-activation state. This reveals the conformation of newly made apo-FluPol in an infected cell, but may also apply to FluPol in the context of a non-transcribing ribonucleoprotein complex. Comparison of the apo-FluPol structure with those of promoter-bound FluPol_s allows us to propose a mechanism for FluPol activation. Our study demonstrates the remarkable flexibility of influenza virus RNA polymerase, and aids our understanding of the mechanisms controlling transcription and genome replication.

FluPol is a highly flexible protein complex; however, the conformational states it can adopt are uncharacterized. Understanding the nature of these conformational states is central to determining the regulatory mechanisms of this enzyme. To this end, we have determined the structure of FluPol from influenza C virus⁵ (FluPol_C), in the absence of promoter RNA. We expressed all three individual subunits of FluPol_C in insect cells by infection with a single baculovirus construct. FluPol_C purified from this system was active in both replication and transcription initiation (Extended Data Fig. 1). We crystallized apo-FluPol_C in two different crystal forms (Extended Data Table 1), and solved its structure at 3.9 Å (Extended Data Fig. 2) and 4.3 Å resolution.

Our model of FluPol_C (Fig. 1) comprises 711 of the 754 residues of PB1 (94.3%), 762 out of 774 for PB2 (98.4%) and 693 out of 709 for P3 (97.7%). FluPol_C forms a relatively compact structure (Fig. 1a, b). P3 folds into two domains connected by a long linker (Fig. 1c): an amino-terminal endonuclease domain (P3_{endo}) and a C-terminal domain (P3_C), which sandwiches PB1 at the heart of the molecule. PB1 has the canonical right-hand-like polymerase fold, possessing palm, fingers and thumb subdomains with additional N- and C-terminal extensions (PB1_{N-ext} and PB1_{C-ext}) that facilitate interactions with the other subunits (Fig. 1d). The thumb of PB1 is reinforced by P3_C. The priming loop of PB1, believed to facilitate *de novo* replication initiation⁴, is not visible in our structure and is probably disordered. PB2 stacks against one face of PB1, contacting both domains of P3. PB2 comprises 9 domains: the N-terminal PB1 interaction domain (PB2_{N-ter}), PB2_{N1}, PB2_{N2}, PB2_{lid} and PB2_{mid} domains, a cap-binding domain (PB2_{cap}), a linker domain (PB2_{cap-627 linker}), the 627 domain (PB2₆₂₇) and a C-terminal nuclear localization signal (NLS) (PB2_{NLS}) domain (Fig. 1e).

The fold of each FluPol_C domain is very similar to its counterpart in FluPol_A and FluPol_B, even though the sequence identity between these polymerases is only ~30% (Extended Data Table 2). The average root mean squared deviation (r.m.s.d.) values of Cα atoms between equivalent superposed domains of FluPol_C and FluPol_A, or of FluPol_C and FluPol_B are 1.6 Å or 1.5 Å, respectively, demonstrating that the FluPol fold is conserved across influenza A, B and C viruses. All key active site residues within FluPol_C are structurally conserved, and we confirmed, by mutation, that FluPol_C shares common mechanisms with FluPol_A (Extended Data Fig. 3a). The PB1 subunits of FluPol A, B and C belong to a structural grouping that

most closely resembles the polymerases of Reoviridae and Cystoviridae/Flaviridae (Extended Data Fig. 3b).

However, there are substantial differences between apo-FluPol_C and the activated structures for promoter-bound FluPol_A and FluPol_B. Most striking are the position of P3_{endo} and the arrangement of the C-terminal domains of PB2 (Fig. 2, Supplementary Video 1 and Extended Data Table 3). Thus, PB2₆₂₇, which in FluPol_A houses a crucial polymorphism (Glu627Lys) for the determination of viral host range and pathogenicity⁶, lies level with the endonuclease domain in the apo structure, (Fig. 2a), whereas in the activated structures it lies close to PB1_{palm} (Fig. 2b). The PB2_{mid} and PB2_{cap-627 linker} domains are rearranged *en bloc*, by a rotation of 140° and a translation of 30 Å, between the apo and activated conformations. PB2_{cap} also changes; in the apo structure, it is tucked in between the PB1_{palm} and PB2_{cap-627 linker}, while in the activated structures it does not extensively contact other domains. Finally, PB2_{NLS} packs between PB1_{C-ter} helix α23 and P3_{endo} helix α7 in apo-FluPol_C (Fig. 3a), but is near the base of the PB1_{palm} in the activated structures (Fig. 2b). The movement of PB2_{NLS} amounts to a rotation of 130° and a translation of 90 Å. The regions rearranged within PB2 match those that are disordered in the FluB2 structure⁴, lying immediately downstream of a conserved glycine (PB2 residue 255 in FluPol_C).

The buried area between PB2 and P3 (5,000 Å²) is more extensive in the apo than the activated, promoter-bound structures, reflecting new contacts between PB2 and P3_{endo} (Fig. 3a and Extended Data Fig. 4a, b). Additionally, the extreme C terminus of PB2 is visible in our apo structure (Extended Data Fig. 4b), forming a helix (α30) that packs against the back face of P3_{endo} (near P3 helices α2, α3 and α7). There are also many new contacts between PB2 and PB1 (Fig. 2 and Extended Data Fig. 4c).

One important consequence of the arrangement seen in apo-FluPol_C is that the cap-binding pocket is occluded (Fig. 3b). PB2_{cap} is folded in on the rest of the subunit, facing residues 520–535 from the PB2_{cap-627 linker} domain. This is consistent with the observation that promoter RNA is required for FluPol cap-binding and endonuclease activity^{7,8}. Thus, the structure we observe represents a closed, pre-activation state of FluPol and suggests that the viral RNA (vRNA) promoter causes the rearrangements necessary to form the activated structure.

Alternatively, the structure of FluPol_C could indicate a fundamental conformational difference between FluPols from different influenza viruses. To clarify this, we performed small angle X-ray scattering (SAXS) experiments with FluPol_C, as these allowed us to distinguish between closed and activated FluPol conformations (Extended Data Fig. 5a). The observed scattering profiles from FluPol_C were similar to a profile calculated from the FluPol_A crystal structure, indicating that FluPol_C can adopt the same activated conformation (Extended Data Fig. 5b). Thus, the change we see in the FluPol_C crystal is not an influenza virus type difference. However, promoter RNA was not required for the activated conformation to be detected, indicating that changes between apo and promoter-bound structures need not exclusively be caused by RNA binding. This suggests that the energy barrier between different FluPol conformations is low. Indeed, when placed into a phosphate-based buffer, FluPol_C adopted a currently uncharacterized conformation that was

even more open than that of the promoter-bound structures (Extended Data Fig. 5c). These results suggest that FluPol may be poised between several different conformations, with only subtle environmental changes needed for a particular conformation to be favoured.

In line with this assessment, differences around the promoter-binding site between the apo-FluPol_C and promoter-bound structures are small. Minor changes are evident around the pocket that binds the intra-base paired hook structure of the 5' strand of the vRNA promoter (Extended Data Fig. 6); however, sequence alignments suggest that these differences are influenza-virus-type-specific. More interesting are the differences around the binding site for the 3' strand of the vRNA promoter. In the apo structure, PB2 helix α 4, PB2_{N1} and the associated region of PB1_{thumb} lie ~5 Å further away from the polymerase core than in the promoter-bound structures (Fig. 3c). This change is transmitted to the neighbouring PB1_{C-ext}–PB2_{N-ter} interaction domain through PB1 helix α 22, resulting in a 20° rotation of this domain between the apo and vRNA promoter-bound conformations (Fig. 3d). Since the PB1_{C-ext}–PB2_{N-ter} domain lies next to PB2_{NLS} in apo-FluPol_C (Fig. 3a), this rotation could trigger the movement of PB2_{NLS} from its apo position, leading to the subsequent massive reorganization of FluPol after vRNA–promoter binding (Supplementary Video 2).

Notably, only one currently reported FluPol structure (FluB2) contains a fully ordered vRNA promoter (in the others, the 3' vRNA strand is either truncated or partially disordered)⁴. However, this does not display a stable activated conformation, as the C-terminal two-thirds of PB2 are not resolved. We suggest that this is because initial binding of the vRNA promoter, into a resting position away from the active site, generates a dynamic equilibrium between closed and activated conformations. The activated structure is only seen when the 3' end of the 3' vRNA promoter strand is either not present or disordered^{3,4}. Hence, the activated conformation might only be fully stabilized when this 3' end is released from its resting position to enter the polymerase active site. To test this hypothesis, we compared the ability of a full-length or truncated (lacking four nucleotides at the 3' end of the 3' strand) vRNA promoter to stimulate FluPol_C cap-dependent cleavage activity (Fig. 4). We reasoned that stabilization of an activated over a closed conformation would enhance capped-RNA cleavage, as the cap-binding pocket in PB2 becomes more accessible. In line with this, we observed a significant enhancement in capped RNA cleavage in the presence of the truncated promoter RNA (Fig. 4). The relative inefficiency of the full-length vRNA promoter to stimulate cleavage supports our assertion that initial promoter binding results in a closed/activated equilibrium. The mechanism behind this may involve the PB1 β -ribbon (177–212 in FluPol_A)³, which is disordered in the apo-FluPol_C structure, but adopts different conformations in the activated and FluB2 structures.

In summary, we have solved the structure of the RNA polymerase from an influenza C virus in the absence of RNA, uncovering a closed conformation accessible to FluPol. Our structure explains the observation that FluPol in the absence of promoter RNA is unable to perform cap-snatching^{7,8}, and we propose a mechanism for how vRNA promoter might bring about FluPol activation. However, the closed conformation captured here may have a wider functional relevance, because it could still be accessible to FluPol bound to a fully ordered vRNA promoter that does not enter the active site. Therefore, in the context of a non-transcribing viral ribonucleoprotein complex (RNP), containing FluPol, RNA and

nucleoprotein, FluPol may well adopt this closed conformation. In addition, dependent on stabilization of the PB1 priming loop, the closed conformation that we observe might still allow *de novo* initiation, as this is not dependent on cap-snatching. Thus, the conformation that we observe, in addition to being a transcription pre-activation state, could be relevant during genome replication initiation. This would allow the activity of FluPol within an RNP to be regulated by other viral factors and host proteins⁹⁻¹².

Our work underlines the tremendous flexibility of this protein complex. This flexibility offers an explanation for the differences between several low-resolution electron microscopy reconstructions of RNP-associated FluPol, as well as explaining why the promoter-bound structures do not fit well into these reconstructions¹³⁻¹⁶. Furthermore, since negative-sense RNA virus polymerases share a common organization, with a central polymerase core surrounded by various functional modular appendages^{1,17}, the conformational flexibility revealed here might be a theme among all of these polymerases and not just particular to FluPol¹⁸.

METHODS

Protein expression and purification

The three subunits of the influenza C/Johannesburg/1/1966 virus polymerase (PB1: AAF89738, PB2: AAF89739, P3: AAF89737) were co-expressed in Sf9 cells from codon-optimized genes (GeneArt) cloned into a single baculovirus using the MultiBac system¹⁹. Expression and purification of FluPol_C proceeded as previously described for FluPol_A¹², except that the gel filtration buffer used was 0.5 M NaCl, 25 mM HEPES-NaOH, pH 7.5 and 10% (v/v) glycerol. For crystallization and storage, protein purified in this buffer was supplemented with 0.5 mM TCEP, and 10 mM MgCl₂ or 10 mM CaCl₂.

Crystallization, data collection and structure determination

Crystals of FluPol_C, belonging to two different space groups, grew from sitting-drop vapour-diffusion experiments at 20 °C^{20,21}, set up using a protein:precipitant ratio of 2:1. In these experiments, 5 mg ml⁻¹ protein was mixed with either 70% (v/v) Morpheus G2 (Molecular Dimensions), supplemented with 0%–1% 1 M NaOH, to generate *P*₄₃₂₁² crystals; or with crystal-seeds and 0.2 M NaCl, 0.1 M Na-HEPES, pH 7.5 and 25% (w/v) PEG 4000, for *P*₂₁₂₁² crystals. For heavy atom derivatization, *P*₄₃₂₁² crystals were soaked in a solution of gold(i) potassium cyanide dissolved in mother liquor, for 2–3 h at 20 °C. Crystals were cryo-protected using 25% (v/v) glycerol in crystallization buffer, before flash-cooling in liquid nitrogen, and data collection on beamlines I03 and I04 at the Diamond Light Source, Didcot, UK. The beam size was matched to the crystal size and data were collected on a Pilatus 6M detector at a wavelength of 0.9763 Å (tetragonal native), 1.0350 Å (tetragonal derivative) and 0.9795 Å (orthorhombic native). Data collection statistics are shown in Extended Data Table 1. Data were processed using Xia2 (ref. 22) and HKL2000 (ref. 23). Initial phases were obtained by single isomorphous replacement with anomalous scattering (SIRAS), using data from native *P*₄₃₂₁² and gold-derivatized crystals. The *P*₄₃₂₁² data used at this stage was collected earlier (at a wavelength of 0.8634 Å) than that subsequently used in refinement. Heavy atoms were located with SHELX²⁴ and phases improved by two-fold

non-crystallographic averaging (the crystallographic asymmetric unit contained two heterotrimers) and solvent flattening (solvent content 76%) using Phenix.autosol²⁵. The tetragonal and the orthorhombic data were sharpened to 40 Å² and 36 Å², respectively. The $P2_12_12_1$ crystals were solved by molecular replacement (program Phaser²⁶), using the $P4_32_12$ structure as the search model. As expected the orthorhombic crystals possessed four heterotrimers in the crystallographic asymmetric unit, allowing phase improvement using non-crystallographic symmetry (NCS) averaging and solvent flattening using general averaging program (GAP) (D.I.S. and J.M.G., unpublished observations). The published fragments of FluPol_A (PDB accessions 4IUJ, 4AWH, 4CB4, 3A1G and 2VY7) were fitted by eye using Coot, which was used for all model building²⁷. Comparison with the complete FluPol_A and FluPol_B structures (4WSB and 4WSA, respectively), aided by the anomalous scattering from the sulphur atoms as markers, allowed us to build and refine complete models for FluPol_C. This provided a total of six independent views of the polymerase. Performing superpositions of these demonstrated that the molecule adopts a virtually identical conformation across all copies from both crystal forms (mean pairwise r.m.s.d. in C α was 0.94 Å between all pairs of molecules across both space groups). Refinement (Extended Data Table 1) used BUSTER²⁸ aided by NCS and initially phase restraints, and REFMAC²⁹ with secondary structure restraints using PROSMART³⁰.

SAXS experiments

SAXS measurements were performed on beamline B21 at Diamond Light Source, Didcot, UK. Samples were prepared onsite using a Shodex Kw-403 size exclusion column and Agilent HPLC. Approximately 40–60 µl of sample were collected for SAXS at 20 °C using a sample to detector distance of 3.9 m and X-ray wavelength of 1 Å. Samples were exposed for 300 s in 10 s acquisition blocks. Images were corrected for variations in beam current, normalized for exposure time and processed into 1D scattering curves using GDA and DAWN. Buffer subtractions and all other subsequent analysis were performed with the program ScÅtter (<http://www.bioisis.net/scatter>). Samples were checked for radiation damage by visual inspection of the Guinier region as a function of exposure time.

Data analysis

Figures and videos were prepared using PyMOL (<http://www.pymol.org>) and Chimera³¹. Structural comparisons used SHP³².

Polymerase activity assays

For the cap-dependent cleavage and transcription assays, FluPol_C (400 ng per reaction) was incubated for 2 h at 30 °C with or without (as indicated) NTPs (1 mM ATP, 0.5 mM each CTP/UTP/GTP), radiolabelled capped 20-nucleotide or 11-nucleotide RNAs, 0.6 µM each 5' and 3' vRNA promoter strands, in a reaction buffer containing 7.5 mM MgCl₂, 1.0 mM TCEP, 2 U µl⁻¹ RNasin (Promega), 20 mM HEPES-NaOH, pH 7.5, 100 mM NaCl and 5% (v/v) glycerol. For the *de novo* initiation and elongation assays, FluPol_C (400 or 800 ng per reaction, as indicated) was incubated for 2–3 h at 30 °C with 2.5 mM adenosine and 0.075 µM [α -³²P]GTP or 1 mM ATP, 0.5 mM each CTP/UTP, 0.1 mM GTP, 0.3 µM [α -³²P]GTP and (as indicated) 0.6 µM each 5' or 3' vRNA promoter strands, in the same reaction buffer as above. The reaction volume was 4 µl for all reactions. Products were denatured by boiling

(98 °C, 5 min) after the addition of formamide (4 µl) and separated on a denaturing 20% polyacrylamide gel, with the indicated size markers. Products were visualized by autoradiography. For all activity assays except the cleavage assays, the sequences of the promoter RNA oligonucleotides used were: 5'-AGCAGUAGCAAGGAG-3' (5' vRNA) and 5'-CUCCUGCUUCUGCU-3' (3' vRNA). The sequences of the RNAs used in the capped-RNA cleavage assays were 5'-AGCAGUAGCAAGGGG-3' (5'), 5'-UAUACCCCUGCUUC-3' (3' truncated) or 5'-UAUACCCCUGCUUCUGCU-3' (3' full length).

Capped and radiolabelled RNA was produced by incubating 5' diphosphate synthetic 20-nucleotide (5'-ppAAUCUAUAAUAGCAUUAUCC-3')^{3,4} or 11-nucleotide (5'-ppGAAUACUCAAG-3')^{33,34} RNA (Chemgenes), with [α -³²P]GTP, vaccinia virus capping enzyme (NEB) and 2'-O-methyltransferase (NEB), following the manufacturer's instructions. The resulting RNAs were gel purified before use in the above assays.

Extended Data

Extended Data Figure 1. Purification and characterization of FluPolC

a, Elution profile of FluPolC, after affinity purification over IgG-sepharose, from a size-exclusion chromatography column. Eluted protein was detected by measuring the absorbance at 280 nm. **b**, Fractions corresponding to the major peak eluting from the size-exclusion chromatography column were mixed and analysed by SDS-PAGE on a 15% polyacrylamide gel, alongside the indicated molecular mass markers. Protein was visualized

by Coomassie blue staining (PB1, 86.0 kDa; PB2, 87.2 kDa; P3, 81.9 kDa). **c**, Transcription and replication initiation assays. Lanes 2–6 test for transcription initiation. With the addition of vRNA promoter only (lanes 4 and 5), FluPol_C can cleave a capped and radiolabelled 20-nucleotide RNA, demonstrating promoter-dependent endonuclease activity. Lane 6 shows that with the addition of NTPs, this capped primer can be extended to produce a capped transcript, thus demonstrating transcription initiation activity. This result is confirmed by lanes 7–9, which test for extension of a capped and radiolabelled 11-nucleotide RNA primer. Extension only takes place when the polymerase is supplied with NTPs and promoter RNA (lane 9). Lanes 10–12 assay for replication initiation. Lane 12 shows that FluPol_C (400 ng per reaction) is able to synthesize ApG dinucleotide in a primer-independent manner. This demonstrates *de novo* replication initiation activity. Uncapped 20-nucleotide and 11-nucleotide primers are used as size markers in lane 1. The slow migration of the ApG dinucleotide compared to the markers is due to the lack of phosphate groups on the 5' end of this product³⁵. **d**, *De novo* initiation and elongation assay. FluPol_C (800 ng) was incubated for 3 h with NTPs, [α -³²P]GTP and 5' or 3' vRNA promoter strands, as indicated. In the presence of both promoter strands (lane 4), FluPol_C is able to produce a full-length copy of the template (14 nucleotides, corresponding to the major band), demonstrating *de novo* replication initiation and elongation activity. The minor slower and faster bands may correspond to non-templated extension and premature termination products, respectively.

Extended Data Figure 2. Data and model quality

Plots of CC^* , CC_{free} and CC_{work} against resolution, for the tetragonal crystal data set and model. The CC^* statistic assesses the signal present in the data, while CC_{free} and CC_{work} provide an estimate of the agreement between data and model. A CC^* value of 0.87 for the highest resolution shell indicates that these data contain useful information up to 3.9 Å. CC_{free} and CC_{work} are lower than CC^* , showing that the model does not overfit the data.

Extended Data Figure 3. Functional and structural relationships of FluPolC

a, Effect of amino acid mutations in FluPol_C on transcription and replication. Plasmids to express FluPol_C subunits and NP along with a plasmid expressing a negative-sense CAT reporter gene flanked by the terminal non-coding sequences of the influenza C virus NS gene segment³⁶ were transfected into 293T cells (ATCC). Total RNA was isolated using Trizol (Invitrogen) 30 h post transfection and viral RNAs were analysed by primer extension³⁷ using the following primers: 5'-CGCAAGGCGACAAGGTGCTGA-3' (for detection of vRNA, yielding a 127-nucleotide product) and 5'-ATGTTCTTTACGATGCGATTGGG-3' (for detection of mRNA and complementary RNA (cRNA), yielding 98–102-nucleotide and 89-nucleotide products, respectively). Primer extension products were analysed by 6% PAGE. Quantification of primer extension analysis using phosphorimaging is shown below. The mean and s.d. of three experiments are shown. Asterisks indicate a significant difference from wild type (WT), which was set to 100% (* $P < 0.05$; ** $P < 0.01$, based on a two-sample t -test). A double mutation of two aspartic acids Asp446/Asp447, that align with Asp445/Asp446 of influenza A virus PB1 found to be critical for activity³⁸, resulted in no detectable activity in the context of FluPol_C. Mutation of amino acid residues in the PB2 cap-binding and P3 endonuclease domains that align with critical amino acid residues in FluPol_A^{39–41} resulted in undetectable accumulation of viral mRNA although most of these mutants were still able to replicate. The exception was Phe416Ala in PB2 that inhibited both transcription and replication, suggesting that this mutation might not only affect cap-binding but overall PB2 folding, in agreement with previously observed inhibitory effects of mutations in the cap-binding domain on replication⁴². The requirement of these amino acid residues for mRNA synthesis is consistent with the hypothesis that FluPol_C generates capped RNA primers for transcription initiation in a manner similar to that of FluPol_A. **b**, Structure-based phylogenetic tree showing the relationship of PB1 from FluPol_C (C PB1) to other right-handed polymerases. Pairwise comparisons were performed using SHP³² and a phylogenetic tree constructed using PHYLIP. The branches are identified by the PDB accession code of the polymerase.

Extended Data Figure 4. New subunit interfaces in apo-FluPol_C

a, b, Two views of the interaction interface between PB2_{NLS} (green) and P3_{endo} (blue). Predicted polar contacts between the subunits are shown as dotted yellow lines. **c**, The interface between PB2_{cap} (green) and PB1_{palm} (orange). In all panels, residues at the interface were calculated using the 'Protein interfaces, surfaces and assemblies' service PISA at the European Bioinformatics Institute (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html)⁴³.

Extended Data Figure 5. SAXS analysis of FluPol_C

a. Calculated solution-state SAXS profiles for the crystal structures of vRNA-FluPol_A³ (activated conformation) and apo-FluPol_C (closed conformation). A distinguishing difference between these profiles is the dip at $q \sim 0.1 \text{ \AA}^{-1}$ in the FluPol_C curve. **b.** Solution-state SAXS profile of FluPol_C, without added promoter RNA, overlaid with the calculated curve for the vRNA-FluPol_A structure³. The good match between these curves suggests that in this particular buffer (0.5 M NaCl, 25 mM HEPES-NaOH, pH 7.5, 5% (v/v) glycerol), FluPol_C adopts the same globular conformation as the RNA bound state. **c.** Dimensionless

Kratky plot of apo-FluPol_C in the presence of 0.5 M NaCl, 25 mM HEPES-NaOH, pH 7.5 and 5% (v/v) glycerol (blue) or 100 mM KCl, 2% (w/v) sucrose and 100 mM sodium phosphate, pH 7.3, with (magenta) or without (grey) 200 mM proline. Cross-hairs denote the Guinier–Kratky point (1.732, 1.104), the peak position for an ideal, globular particle. As indicated by the upward shift of the peaks in the dimensionless Kratky plot, FluPol_C is less globular in the presence of phosphate than it is in the 0.5 M NaCl buffer. This effect can be lessened if proline is also present, potentially owing to increased molecular crowding.

Extended Data Figure 6. Differences at the 5' vRNA promoter binding site
Superposition of apo-FluPol_C (darker colours) and FluPol_A (lighter colours) structures, with sites of interest labelled.

Extended Data Table 1
Data collection, phasing and refinement statistics

	Tetragonal Native*	Tetragonal Derivative*	Orthorhombic Native*
Data collection			
Space group	$P4_32_12$	$P4_32_12$	$P2_12_12_1$
Cell dimensions			
a, b, c (Å)	185.66, 185.66, 598.22	184.22, 184.22, 598.75	107.28, 217.50, 597.75
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å) **	100.6 – 3.9 (4.0 – 3.9)	127.3 – 6.9 (7.1 – 6.9)	80.9 – 4.3 (4.4 – 4.3)
R_{sym} Or R_{merge}	0.196 (3.586)	0.157 (0.665)	0.204 (0.993)
$I/\sigma I$	13.2 (1.3)	16.5 (1.8)	5.5 (1.1)
Completeness (%)	98.8 (96.9)	99.1 (89.0)	99.0 (93.1)
Redundancy	21.2 (20.6)	14.5 (3.7)	3.3 (2.9)
$CC_{1/2}$ ***	(0.621)	(0.612)	(0.500)
Refinement			
Resolution (Å)	50.0 – 3.9		50.0 – 4.3
No. reflections	90,335		90,390
$R_{\text{work}}/R_{\text{free}}$	0.286/0.326		0.316/0.368
No. atoms			
Protein	34,720		69,371
Wilson B-factors (Å ²)			
Protein	205		190
R.m.s deviations			
Bond lengths (Å)	0.008		0.009
Bond angles (°)	1.12		1.24

* The native data sets were each collected from a single crystal, whereas the derivative data set was produced by merging data from two crystals.

** Highest resolution shell is shown in parentheses.

*** As described in ref. 44.

Extended Data Table 2
Sequence identities between subunits of FluPol from C/
Johannesburg/1/1966 and those from A/Little yellow-
shouldered bat/Guatemala/060/2010 or B/Memphis/
13/2003, calculated using EMBOSS Stretcher⁴⁵

FluPol Subunit	Sequence Identity with C (%)	
	A	B
PB1	38.4	40.8
PB2	23.3	25.2
P3 / PA	25.4	25.6

Extended Data Table 3
Domain position differences between apo-FluPolC and
promoter-bound FluPolA, calculated using SHP

Domain	Rotation (°)	Distance (Å)
PA _{Endo} / P3 _{Endo}	140	19
PB2 _{Mid} and PB2 _{cap-627} Linker	141	29
PB2 _{Cap}	122	30
PB2 ₆₂₇	163	79
PB2 _{NLS}	134	93

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structure of FluPol_C

a, b, Two views of the structure of the FluPol_C heterotrimer, coloured according to subunit (PB1, orange; PB2, green; P3, blue). The cap-binding pocket and endonuclease active site are shown as blue and red spheres, respectively. In **a**, the PB1 catalytic aspartates, residues 446 and 447, are also highlighted red. The position of PB2 residue 649 (equivalent to PB2 residue 627 in FluPol_A) is marked by a purple sphere. **c–e**, Structures of FluPol_C subunits P3 (**c**), PB1 (**d**) and PB2 (**e**), coloured and labelled by domain. **f**, Domain maps of each FluPol_C subunit.

Figure 2. Comparison of apo-FluPol_C with promoter-bound FluPol_A

a, Apo-FluPol_C, depicted in the same orientation and colouring as in Fig. 1b, but with the C-terminal domains of PB2 coloured as in Fig. 1e. Domains that do not change between apo and promoter-bound conformations are depicted as semi-transparent. **b**, Promoter-bound FluPol_A, shown as in **a**. **c**, **d**, The PB2 subunits of apo-FluPol_C (**c**) and promoter-bound FluPol_A (**d**), depicted as in **a** and **b**.

Figure 3. Critical changes between apo-FluPol_C and promoter-bound FluPol_A

a, Equivalent views of FluPol_C (left) and FluPol_A (right), showing domain arrangement differences. The inset shows the arrangement of P3_{endo} within the FluPol_C structure. **b**, Close-up of the FluPol_C (left) and FluPol_A (right) cap-binding domains. PB2 residues 520–535 in FluPol_C are coloured dark orange. The cap-binding pocket is shown with blue spheres. **c**, **d**, Two views of a superposition of apo-FluPol_C and FluPol_A, with FluPol_C coloured as in Fig. 1 and FluPol_A in lighter colours. 5' and 3' promoter RNAs in the FluPol_A structure are coloured pink and yellow, respectively. Arrows highlight differences between the two conformations.

Figure 4. Capped-RNA cleavage assays with FluPol_C

a, Representative autoradiograph of a capped-RNA cleavage assay. In each reaction, radiolabelled capped RNA was incubated at 30 °C for 2 h with FluPol_C and the indicated strands of the vRNA promoter. **b**, Quantification of cleavage, expressed as the percentage of cleaved to total RNA, from three replicates of this assay, performed with the same polymerase preparation. Mean cleavage percentage is plotted. Error bars show s.d. Asterisk

indicates a significant difference between cleavage with full or truncated vRNA promoter ($n = 3$, $P = 0.0003$, two-tailed t -test).