Supplementary Materials

X-ray structure of NS1 from a highly pathogenic H5N1 influenza virus

Zachary A. Bornholdt[1] and B. V. Venkataram Prasad[1,2*]

[1]Department of Molecular Virology and Microbiology

[2]Department of Biochemistry and Molecular Biology

Baylor College of Medicine

One Baylor Plaza

Houston TX 77030

Supplementary Figure Legends

Figure S1. Possible alternative connections between RBD and ED in the full length H5N1 NS1 crystal structure. Due to higher than average temperature factors in the C-terminal end (67-74) of the RBD it is possible for those residues to bend and create an alternative connectivity (shown by red dashed lines) instead of the connectivity proposed in Fig. 1 (shown by blue spheres). However, the appearance of the electron density, although relatively weak, in both 2Fo-Fc and Fo-Fc maps after removing these residues supports the connectivity as shown in **Fig. 1** (shown here by blue spheres). Either of these connections lead to the same consequences in terms of tubular formation, which is consistent with the cryo-EM results (**Fig. 3**), and the biological functions of NS1 as discussed in the main text.

Figure S2. A multiple sequence alignment (performed using CLUSTAL W[1]) of the A/Vietnam/1203/2004 H5N1 NS1, A/Puerto Rico/8/34 H1N1 NS1 and A/Udorn/72 H3N2 NS1 discussed in the manuscript. The RBD, linker domain and ED regions in the sequences are shaded **blue, green** and **red** respectively.

Figure S3. Similar to Fig. 2a, but showing in addition RBD monomer of the A/Udorn/72 NS1[2] (colored in **ruby**, pdb id: 1AIL) superimposed to each H5N1 RBD with residue R38 and K41 displayed as sticks (colored in **blue)** to demonstrate how they may project out from the wild type H5N1 RBD. The residues critical for dsRNA (in both the H5N1 and H3N2 RBD) and CPSF binding are colored in blue and red respectively.

Figure S4. Similar to Fig. 3b but showing in addition the RBD monomer of the A/Udorn/72 NS1[2] (colored in **ruby**, pdb id: 1AIL) superimposed to each H5N1 RBD with residue R38 and K41 displayed as sticks (colored in **blue**) to demonstrate how they

may project out from the wild type H5N1 RBD into the central tunnel for dsRNA binding. Around the outside of the tube the residues implicated in CPSF binding are colored in **red**.

Figure S5. Size exclusion chromatography was performed on H5N1 NS1 during the last step of purification using a High Load™ 16/60 Superdex™. The size exclusion peak for H5N1 NS1 corresponds to a dimer of approximately 50 kDa. The inset displays an SDS-PAGE analysis of the size exclusion fractions through the H5N1 NS1 peak; which displays a single band at 25 kDa, the H5N1 NS1 monomer.

Fig. S1

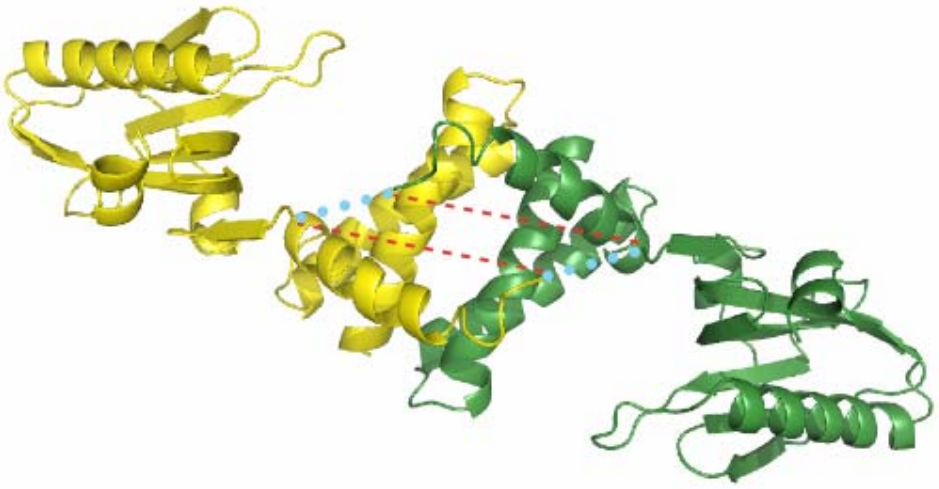
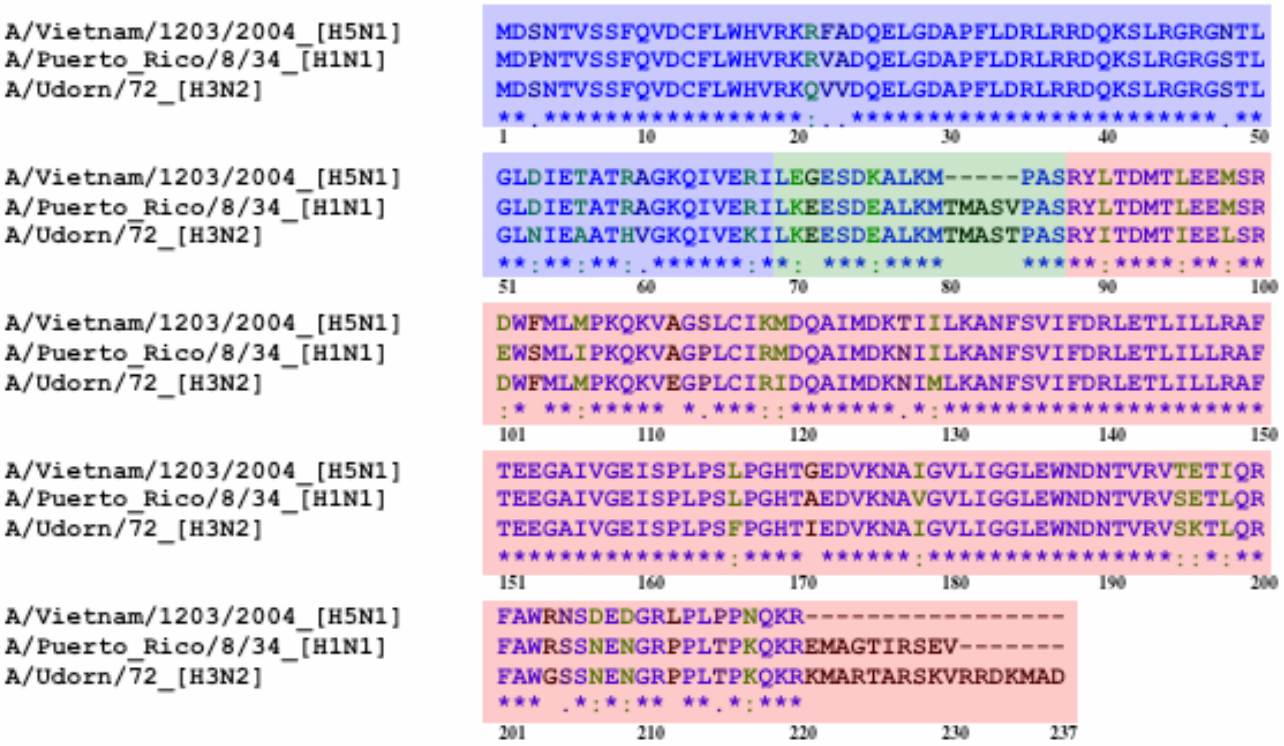


Fig. S2



Consensus key

* - single, fully conserved residue
: - conservation of strong groups
. - conservation of weak groups
  - no consensus

dsRNA Binding Domain:

Linker Domain:

Effector Domain:

Fig. S3

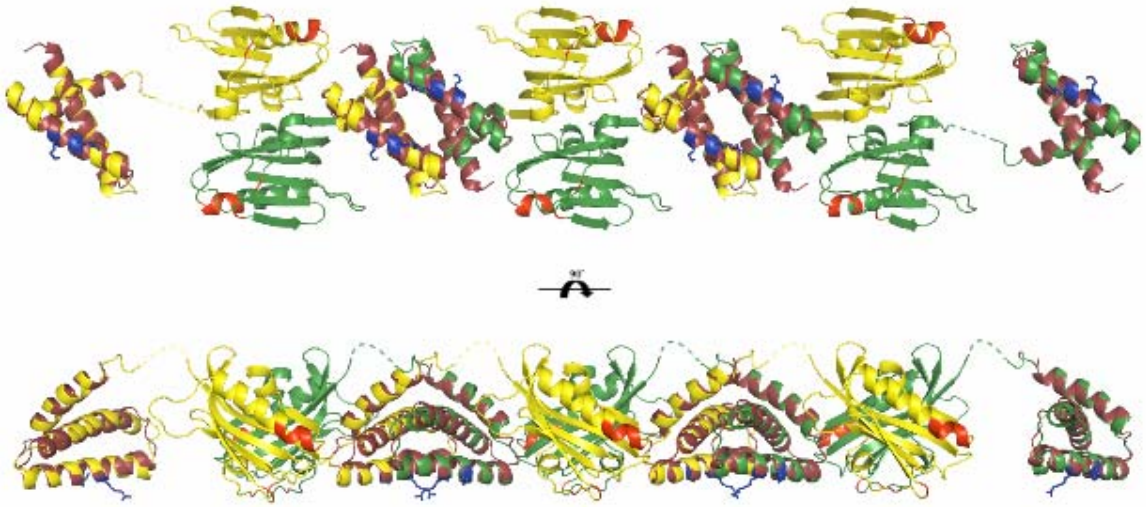


Fig. S4

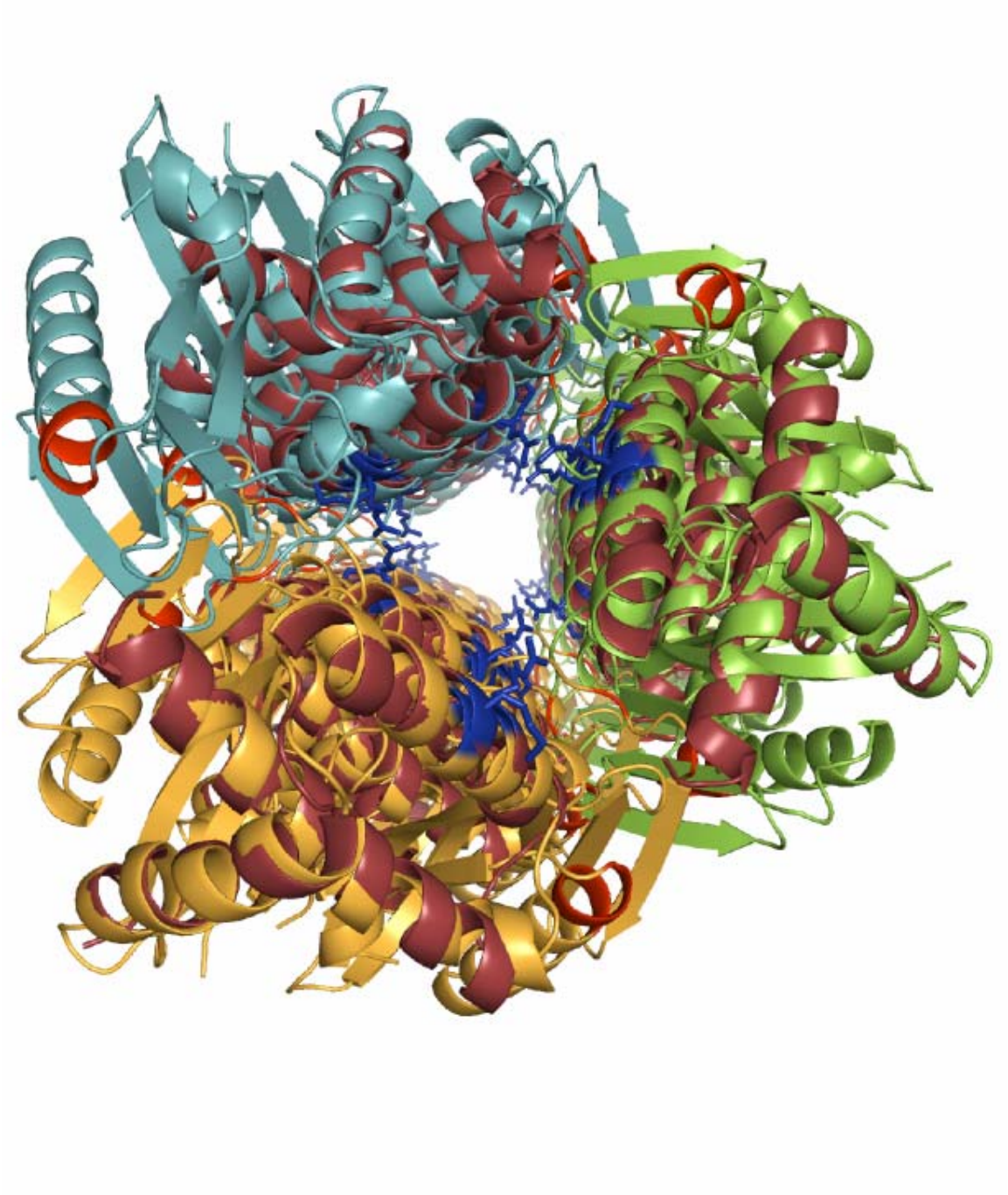


Fig. S5

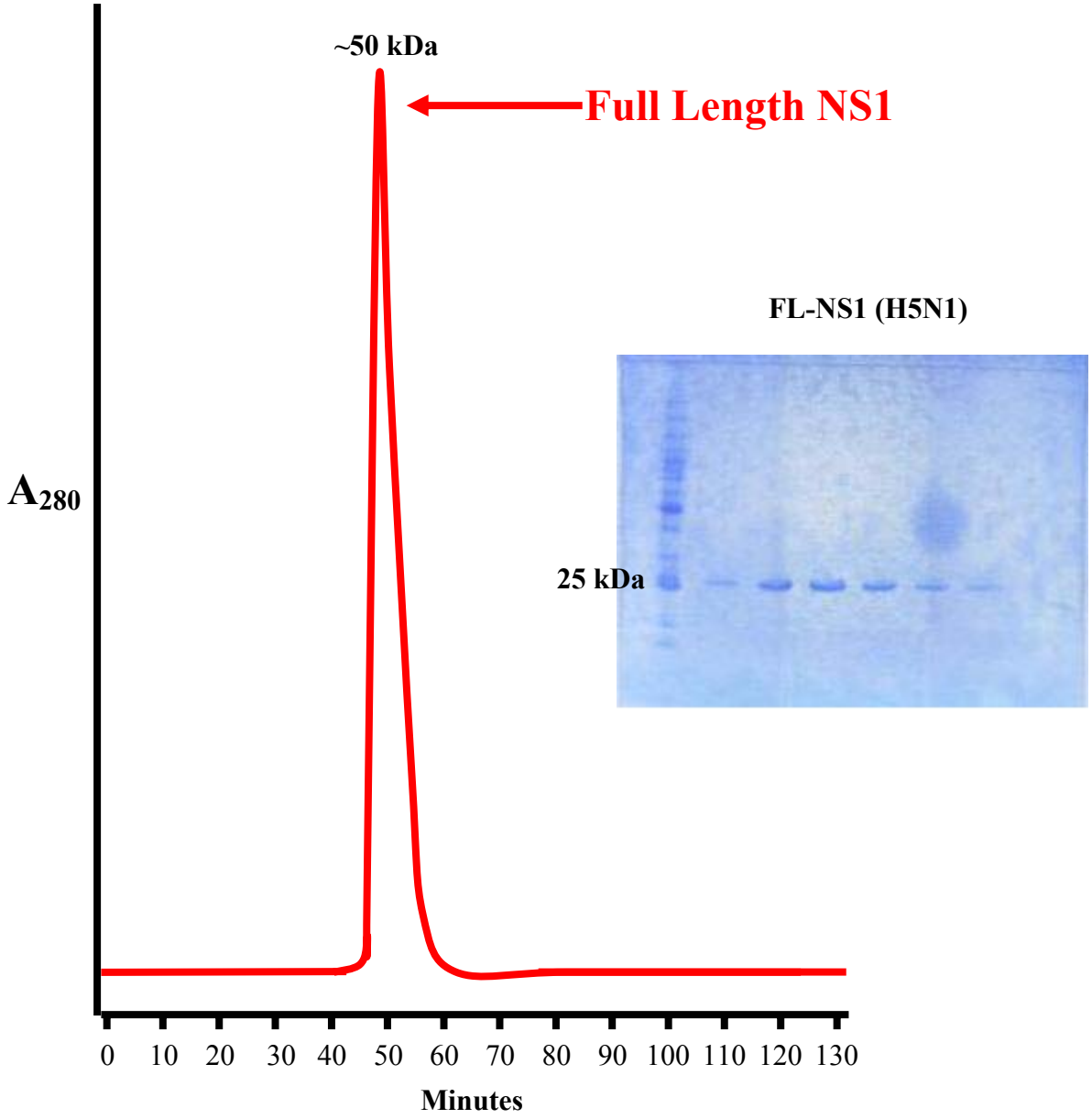

~50 kDa
Full Length NS1
FL-NS1 (H5N1)
25 kDa
A280
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Minutes

Table S1

Data collection and refinement statistics

Data Collection	
Wavelength	1.0332 Å
Space group	P 6_5 2 2
Cell dimensions	
a, b, c (Å)	106.12, 106.12, 69.63
α, β, γ (º)	90, 90, 120
Resolution (Å)	50 - 2.7 (2.8-2.7)
R_{sym} or $R_{merge\ (\%)}$	10.0 (40.5)
Completeness (%)	100.0 (100.0)
Redundancy	13.4 (14.1)
Refinement	
Resolution (Å)	30 - 2.7
No. reflections	6,718
$R_{work/}$ R_{free}	0.27 / 0.29
Average B-factors (Å^2)	52.4
R.m.s deviations	
Bond lengths (Å)	0.016
Bond angles (º)	1.9

* Values in parentheses are for the highest resolution shell.

Supplementary Figure References

[1] Thompson, J. D., Higgins, D. G., and Gibson, T. J., CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673-4680 (1994).

[2] Liu, J. et al., Crystal structure of the unique RNA-binding domain of the influenza virus NS1 protein. *Nat Struct Biol* **4**, 896-899 (1997).