A structure-based rationale for sialic acid independent host-cell entry of Sosuga virus

Alice J. Stelfox and Thomas A. Bowden

The bat-borne paramyxovirus, Sosuga virus (SosV), is one of many paramyxoviruses recently identified and classified within the newly established genus Pararubulavirus, family Paramyxoviridae. The envelope surface of SosV presents a receptor-binding protein (RBP), SosV-RBP, which facilitates host-cell attachment and entry. Unlike closely related hemagglutinin neuraminidase RBPs from other genera of the Paramyxoviridae, SosV-RBP and other pararubulavirus RBPs lack many of the stringently conserved residues required for sialic acid recognition and hydrolysis. We determined the crystal structure of the globular head region of SosV-RBP, revealing that while the glycoprotein presents a classical paramyxoviral six-bladed β-propeller fold and structurally classifies in close proximity to paramyxoviral RBPs with hemagglutinin-neuraminidase (HN) functionality, it presents a receptor-binding face incongruent with sialic acid recognition. Hemadsorption and neuraminidase activity analysis confirms the limited capacity of SosV-RBP to interact with sialic acid in vitro and indicates that SosV-RBP undergoes a nonclassical route of host-cell entry. The close overall structural conservation of SosV-RBP with other classical HN RBPs supports a model by which pararubulaviruses only recently diverged from sialic acid binding functionality.

Significance

Bat populations constitute a reservoir for numerous viruses with human and animal spillover potential. Sosuga virus (SosV), from the genus Pararubulavirus, family Paramyxoviridae, is a prominent example as it has been implicated to be responsible for severe disease in an infected patient. Through investigation of the virion envelope-displayed SosV host-cell receptor binding protein, we provide a molecular-level rationale for how SosV undergoes a sialic acid-independent host-cell entry pathway, which contrasts the glycan reliance of related orthorubulaviruses, including mumps virus. By analogy to glycan-independent host-cell attachment of pathogenic henipaviruses, these data support a model whereby the evolutionary departure of SosV and other pararubulaviruses from a sialic acid-specific ancestral paramyxovirus may contribute to the extensive known host range of these emerging pathogens.

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Olu, stabilizes Arg (35–38). Site-directed mutagenesis of the individual residues within this conserved site has been shown to result in ablation of enzymatic activity (38, 39). Paramyxoviruses also express an “Asn–Arg–Lys–Ser–Cys–Ser” hexapeptide motif, a stretch of amino acids conserved among most paramyxoviral HN RBPs. Mutagenesis studies of the HN RBP from Newcastle disease virus (NDV) revealed that the first four residues of this motif (Asn–Arg–Lys–Ser) are essential for neuraminidase activity (40). Furthermore, NDV-RBP has been shown to present a second sialic acid binding site, which is located at the homodimeric interface and implicated in maintaining avidity during the fusion process (41, 42).

Pararubulaviruses are closely related to orthorubulaviruses such as mumps virus (MuV), which encode RBPs with HN functionality. However, despite this close genetic relationship, a recent study revealed that pararubulavirus likely utilize a sialic acid independent mode of entry (7), a finding rationalized by the lack of the conserved amino acid sequence required for binding and hydrolyzing sialic acid. Here, through analysis of the RBP from SosV, an emerging member of the Pararubulavirus genus associated with human infection, we provide an integrated structural and functional rationale for how pararubulaviruses undergo sialic acid-independent host-cell entry and egress. These data demonstrate the pathobiological distinctiveness of pararubulaviruses and highlight the diverse host-cell entry pathways available to paramyxoviruses more generally.

Results

SosV-RBP Lacks Hemadsorption and Neuraminidase Activity. The RBPs of SosV and other pararubulaviruses exhibit the highest level of sequence conservation with the RBPs of orthorubulaviruses (e.g., MuV-RBP) (10), a group of viruses with HN activity (43).Interestingly, while the RBP of SosV and other pararubulaviruses retain all seven residues of the sialidase catalytic site, which are conserved among the sialidase protein family more widely (35–38), the glycoproteins retain only the two C-terminal amino acids (Cys–Ser) of the hexapeptide motif known to be necessary for paramyxovirus RBP HN functionality (Fig. 1A). The absence of these crucial residues has also been observed in other recently classified pararubulaviruses, including Menangle virus (MenV), Tieviot virus (TeVv), and Tioman virus (TioV), with experimental data confirming that sialic acid is not integral to infection of permissive cells (7, 44–46).

We performed hemadsorption (47) and neuraminidase activity (48) assays to assess whether the absence of the hexapeptide motif found in HN RBPs impairs the ability of SosV-RBP to bind and hydrolyze sialic acid. In line with previous studies, which demonstrate that disruption of this key motif in NDV-RBP compromises neuraminidase activity (40), a human embryonic kidney (HEK) 293T cells presenting full-length SosV-RBP exhibited no detectable neuraminidase and minimal hemadsorption functionalities (Fig. 1B). The absence of these crucial residues has also been observed in other recently classified pararubulaviruses, including Menangle virus (MenV), Tieviot virus (TeVv), and Tioman virus (TioV), with experimental data confirming that sialic acid is not integral to infection of permissive cells (7, 44–46).

The Structure of SosV-RBP Is Most Closely Related to Paramyxoviral HN Glycoproteins. We sought to assess whether the functional independence of SosV-RBP from paramyxoviral RBPs with known HN functionality was reflected at a structural level. A soluble construct of SosV-RBP was engineered to incorporate the full hexapeptide motif (termed eSosV). The seven conserved sialidase residues (35, 37) and hexapeptide motif (40) are labeled according to residue and blade location (35) and annotated above the alignments. (B) SosV-RBP neuraminidase (48) and hemadsorption (47) activity normalized to cell surface expression and a NDV-RBP control. (C) Free sialic acid concentration detected following incubation of NDV-RBP, SosV-RBP, “NRKS” mutant eSosV-RBP, and mock-transfected cell supernatant with fetuin (49). For y = n = 6, error bars represent the SD.

Two near-identical molecules of SosV-RBP were observed in the asymmetric unit (root-mean-square deviation [RMSD] of 0.5 Å over 366 aligned Ca atoms). Residues ranging from 158 to 582 correspond to the canonical six-bladed β-propeller of the paramyxoviral attachment glycoprotein (25), with each blade (b1 to b6) composed of four antiparallel β-strands (Fig. 2A). SosV-RBP was crystallized and the structure was determined to 2.50-Å resolution using the structure of MuV-RBP (PDB ID code 5B2C) (43) as a molecular replacement search model (SI Appendix, Table S1).

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and Asn455) (Fig. 2A), suggestive that these sites may be occupied on the native virion.

Overlay analysis reveals that SosV-RBP shares the greatest level of structural conservation with orthoavula-, orthorubula-, and respirovirus HN RBPs (1.7–2.1 Å RMSD upon superposition of equivalent Ca atoms), when compared with protein-binding morbilliviral H RBPs (3.1 Å RMSD upon overlay with measles virus RBP; MV-RBP) and henipaviral G RBPs (2.2–2.3 Å RMSD) (Fig. 2B). The relatively close structural correspondence of the SosV-RBP β-propeller scaffold with other paramyxoviral RBPs with HN functionality is also reflected upon structure-based phylogenetic analysis (Fig. 2C) (59, 60). Indeed, in line with our previous investigations, which demonstrate that paramyxoviral RBPs structurally classify according to receptor usage (23, 61, 62), the relatively close proximity of SosV-RBP to other RBPs with HN functionality, with respect to henipaviral and morbilliviral RBPs, may reflect that SosV-RBP only recently diverged from sialic acid-binding functionality.

**Unique Dimeric Assembly Supports Sialic Acid-Independent Functionality.**

Two molecules of SosV-RBP were observed in the asymmetric unit of the crystal and form a putative homodimer through the interaction of the first (β1) and sixth (β6) blades of the β-propeller (Fig. 3). Although we cannot preclude the possibility of preferential crystallization, we note that the formation of such higher-order oligomers has precedent in other dimeric and tetrameric paramyxoviral RBP structures, including NDV-RBP (19, 36), PIV3-RBP (63), PIV5-RBP (18, 20, 64), HeV-RBP (22), and MuV-RBP (43). In addition, the formation of this putative homodimer does not occlude N-linked glycosylation, as expected and consistent with previous analysis of paramyxovirus RBPs (22). The interaction between SosV-RBP protomers occludes...
This dramatic difference in loop conformation results in a difference in the toroidal axis of the acid-binding paramyxoviral RBPs, but peels outwards away from the typical liganded and unliganded HN RBP (Fig. 4). (A) The sialic acid active site of structurally characterized RBPs, including MuV-RBP (43), PIV5-RBP (20), PIV3-RBP (63), and NDV-RBP (36), localizes to a cavity at the top center of the β-propeller fold (Fig. 4A). Structural and functional analyses have comprehensively detailed the conserved RBP–glycan interactions facilitated by the seven conserved sialidase residues and hexapeptide motifs, which are essential for HN activity (20, 35–38, 43, 63). In addition to our hemadsorption and sialidase activity analysis (Fig. 1B and C), we collected crystallographic data on SosV-RBP crystals soaked with (3-sialyllactose, 30 mM) and co-crystallized in the presence of sialyllactose (3- and 6-sialyllactose, at a 5 times greater molar concentration than protein). However, consistent with our hemadsorption and neuraminidase activity assays (Fig. 1B and C), we could find no evidence for glycan binding at the sialic acid cavity (38), nor at the region equivalent to the secondary sialic acid binding site on NDV-RBP (41).

Examination of our unliganded SosV-RBP structure provides a molecular rationale for the absence of this interaction. Indeed, while the glycoprotein presents some features that are conserved with paramyxoviral RBPs with known HN functionality, including a conserved cation binding site (SI Appendix, Fig. S2) (36, 63, 64, 68) and residues that would contribute to recognition of the glycerol moiety of sialic acid (Fig. 4A and B), the overall configuration of the putative active site is incompatible with known modes of sialic acid recognition. First, we observe that SosV-RBP hexapeptide residues Leu243SosV-RBP and His245SosV-RBP impede into the region where the O₄ of sialic acid is positioned in other paramyxovirus RBP–sialic acid complex structures (Fig. 4A and B and SI Appendix, Fig. S3). Second, analysis of the electrostatics in the SosV-RBP cavity reveals dramatically different surface charge properties when compared to paramyxoviral RBPs with HN functionality, where the SosV-RBP presents an extended acidic patch, which is unlikely to be favorable for binding of sialylated glycoconjugates (Fig. 4C). Third, the β5L23 loop in SosV-RBP, which encodes Arg514 (R₅) of the triarginyl motif and does not fold inward, as observed in other sialic acid-binding paramyxoviral RBPs, but peels outwards away from the toroidal axis of the β-propeller toward a small external cavity. This dramatic difference in loop conformation results in a ∼14-Å distance between the equivalent Co atom of Arg5 in SosV-RBP and the typical liganded and unliganded HN RBP (Fig. 4A and SI Appendix, Fig. S4). We note that the equivalent region in the HN RBPs is structurally conserved and remains unchanged upon ligand recognition (20, 38, 43, 63). Such a large local structural difference in the β-propeller fold may be, in part, attributed to the presence of a disulphide bond in SosV-RBP between residues Cys522 and Cys527 of β5S3 and β5S4 (SI Appendix, Fig. S4), respectively, which is not present in other paramyxoviral RBPs with HN functionality, and may contribute to an open conformation of the β5L23 loop (Fig. 4A). Combined, the dramatic overall structural and physicochemical differences observed between SosV-RBP and paramyxoviral RBPs with HN functionality is consistent with our hemadsorption and neuraminidase activity analysis (Fig. 1B and C), and provides a structural rationale for why the replacement of the hexapeptide motif alone does not result in SosV-RBP obtaining the capability to hydrolyze sialic acid (Fig. 1C).

Discussion
The considerable toll exacted by bat-borne viruses, such as pathogenic henipaviruses, coronaviruses, and filoviruses, upon human...
health and animal husbandry has provoked worldwide initiatives focused on exploring virus diversity in wildlife and identifying determinants of emergence (69). While a number of sociological, epidemiological, and economic parameters are essential in defining the spillover potential of these emerging and reemerging viruses (70), the ability of virus-displayed glycoproteins to productively attach to and interact with a human host-cell constitutes a fundamental barrier for zoonosis (71, 72). Here, we provide insights into such molecular-level restrictions at the stage of host-cell entry and egress for bat-borne SosV, a recently identified pararubulavirus associated with severe febrile disease.

In line with studies on related pararubulaviruses (7, 44–46), our hemadsorption and sialidase analysis reveals that the absence of the hexapeptide motif in SosV-RBP, which is well conserved in paramyxovirus RBPs with known sialic acid functionality (e.g., MuV-RBP, PIV3-RBP, NDV-RBP, and PIV5-RBP), results in limited hemadsorption and no sialidase activity (Fig. 1). Interestingly, we note that a vaccine strain of MuV has been reported to bind α2,8 sialic acid (73–75). However, this somewhat broadened receptor tropism did not involve a departure from α2,3 and α2,6 specificity, indicative that if SosV-RBP also interacted with this neurotrophic-associated glycan, we would have likely observed hemadsorption and neuraminidase activity in our functional assays (Fig. 1). Similarly, while there was no evidence for sialic acid binding in our crystal soaking and cocrystallization experiments, given that trace hemadsorption activity was observed with respect to an NDV-RBP control (Fig. 1B), we cannot discount the possibility of low-affinity interactions with sialic acid. Indeed, such interactions have been shown to augment MERS-CoV infection (76).

Although we were unable to produce a full-length eSosV-RBP construct that includes the four hexapeptide residues missing from SosV-RBP in sufficient yield to assess the effect of these residues on hemadsorption, solubly produced eSosV-RBP exhibited no measurable neuraminidase activity (Fig. 1 and SI Appendix, Fig. S1). We rationalize why introduction of the hexapeptide motif is not sufficient to confer hydrolysis activity by showing that SosV-RBP is structurally and physicochemically incompatably with the established mode of paramyxovirus HN-glycan recognition (Fig. 4). Specifically, we find that the region corresponding to the sialic acid binding site on SosV-RBP presents less favorable surface charge properties (Fig. 4C), is partially occluded by N-linked glycosylation presented by Asn265, and is sterically disrupted by local structural elements (Fig. 4 A and B), including the protruding residues, Leu243 and His245 (SI Appendix, Fig. S3).

Assuming that an ancestral precursor to SoSv utilized sialic acid as a receptor, it seems plausible that the observed structural differences at the sialic acid recognition site may have arisen following the acquisition of binding motifs to a unique receptor (e.g., either protein or glycan specific). Alternatively, given the structural plasticity of the β-propeller (25), it is possible that another site on SosV-RBP may be utilized for receptor recognition, and structural diversification at the original sialic acid binding site may have occurred due to the absence of functional constraints to maintain efficient sialic acid recognition capacity. Furthermore, we note that the mode of SosV-RBP homodimerization deviates from that of the conserved ~60° association angle observed in sialic acid-specific MuV-RBP, hPIV5-RBP, PIV3-RBP, and NDV-RBP structures, a feature in common with protein-binding HeV-RV and MV-RBP glycoproteins, and supportive of the hypothesis that the acquisition of new receptor-binding modularity may require alteration to the higher-order attachment glycoprotein assembly (18, 22).

Interestingly and consistent with genetic analysis (8), structure overlay of available paramyxoviral attachment glycoprotein structures reveals that the overall six-bladed β-propeller fold of SosV-RBP more closely matches sialic acid-binding RBPs than hemiparoviral or morbilliviral RBPs (Fig. 2B). Combined with the observation that SosV-RBP does not appear to share structural features required for ephrin or SLAMF1/nectin-4 recognition (Fig. 2B), we suggest that SosV may have more recently diverged from a common sialic acid-specific ancestral paramyxovirus than known protein-specific henipavirus and morbilliviruses (Fig. 2C). Moreover, this analysis demonstrates the smallest known level of structural reorganization to the β-propeller scaffold required for sialic acid-independent paramyxovirus host-cell attachment.

The burden of newly emerging viruses in humans may be underestimated. For example, a recent study in Uganda reported that 62% of cases of severe febrile illness were misdiagnosed as malaria due to resource limitations in clinics (77). While the potential biomedical and economic impact of pararubulavirus emergence remains to be fully established, several pararubulaviruses, in addition to SoSv, have shown the ability to cross the species barrier and cause disease. For example, MenV infects both pigs, fruit bats, and humans (45, 78), and laboratory models of the bat-borne Achimota viruses (15) have been found to cause respiratory disease in ferrets.

Our integrated structural and functional investigation, combined with studies supporting the sialic acid independence of Menangle virus (MenV), Teviot virus (TevPV), and Tioman virus (TioV) (7), indicate that pararubulavirus RBPs are functionally distinct from characterized paramyxoviral HN, HN, and G RBPs, and undergo a novel host cell entry pathway. While the receptor(s) utilized by SosV remains unknown, this work broadens our appreciation of the diverse host receptors utilized by paramyxoviruses. Future efforts to characterize the receptor(s) utilized by this group of emerging pathogens will be essential for understanding cellular, tissue, and species tropism characteristics, as well as rationalizing the spillover potential of these emerging viruses.

Materials and Methods

Protein Production. SosV-RBP (GenBank accession no. NC_025343.1) cDNA was synthesized by GeneArt LTD. SosV-RBP (residues 125–582) was cloned into the pURD vector alongside a 3C protease cleavable N-terminal SUMO tag and hexahistidine tag (79) and used to generate a stable HEK293T cell line (80). When required for crystallization, upscaling of stably expressing cells was performed in the presence of kifunensine (81). All proteins were purified using immobilized metal-affinity chromatography (IMAC) and subsequently N-linked glycans were cleaved at the di-N-acetylgalactosamine core using endoglycosidase F1 (EndoF1) (10 μg/mg protein, 12 h, 21 °C). Size-exclusion chromatography (SEC) was used to purify the cleaved products (SEC) in 150 mM NaCl, 10 mM Tris pH 8.0, 0.1 M sodium cacodylate pH 6.5, 20% PEG 8000. Crystals were immobilized in 150 mM sodium cacodylate pH 6.5, 20% PEG 8000. Crystals were immersed in 20% glycerol prior to cryo-cooling by plunging into liquid nitrogen.

Data collection was performed at wavelength 0.9795 Å at beamline I04, Diamond Light Source (DLS), United Kingdom. Images were integrated and scaled using the XIA2 pipeline (83). SosV-RBP was solved with MuV-HN (PDB ID code 5B2C) as the search model (43), using Phaser within the PHENIX suite (84, 85). Noncrystallographic symmetry restraints were employed throughout, and Translation–liberation–screw parameters were employed for later rounds of refinement. Structures were validated with Molprobity (86). Crystallographic data processing and refinement statistics are presented in SI Appendix, Table S1. The atomic coordinates and structure factors of SosV-RBP were deposited in the Protein Data Bank (PDB), PDB code 6SG8 (89).

Cocrystallography and Structure Determination. SosV-RBP crystals were grown using nanoliter-scale sitting-drop vapor diffusion at room temperature, using 100 nL of protein and 100 nL of reservoir (82). Crystals grew after 25 d in a precipitant containing 0.2 M magnesium acetate tetrahydrate, 0.1 M sodium cacodylate pH 6.5, 20% PEG 8000. Crystals were immersed in 20% glycerol prior to cryo-cooling by plunging into liquid nitrogen.

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Hemadsorption Assay. The hemadsorption method to determine sialic acid binding was adapted from that developed by Morrison and McGinnies (47). Briefly, full-length SosV-RBP (residues 1–582) and NDV-RBP (residues 1–577,
PBS pH 7.4 following 18 h incubation at 37 °C, 5% CO₂, counted and seeded HEK293T cells (91). Lipofectamine-2000 transfected cells were washed utilized to measure expression of full-length SosV-RBP and NDV-RBP on Cell Surface Expression. ELISA (enzyme-linked immunosorbent assay) was at 365 nm for excitation and 450 nm for emission using a CLARIOStar plate reader. Twenty-four hours after transfection, HEK 293T monolayers were washed with PBS, pH 7.4, counted and seeded in a 96-well black nontransparent plate at a density of 25,000 cells per mL. Cells were blocked by pipetting in 1,500 rpm for 5 min, and supernatant was replaced with 0.1 M sodium acetate, pH 6.0 containing 1 mM MU-Neu5Ac. The plate was incubated for 1 h prior to addition of horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Vector Laboratories, product no. PI-1000), for 1 h at 37 °C. Cells were subsequently reacted with thiobarituric acid to form a pink product, which was fluorometrically measured (λex = 555/nm = 585 nm) using a CLARIOStar plate reader (BMG Labtech).

Neuraminidase Assay. Neuraminidase activity was determined through hydrolysis of the substrate 2′-4-(methylumbelliferyl)-β-N-acetylneuraminic acid (MU-Neu5Ac; Sigma-Aldrich, product no. M8639), as described previously (38, 48). The full-length SosV-RBP and NDV-RBP constructs presented above were transfected with Lipofectamine 2000 into HEK 293T cells. Twenty-four hours after transfection, HEK 293T monolayers were washed with PBS, pH 7.4, counted and seeded in a 96-well black nontransparent plate at a density of 25,000 cells per mL. Cells were pelleted by spinning at 1,500 rpm for 5 min, and supernatant was replaced with 0.1 M sodium acetate, pH 6.0 containing 1 mM MU-Neu5Ac. The plate was incubated for 1 h at 37 °C prior to addition of 0.25 M glycine buffer, pH 10.7 to stop the reaction. The amount of free 4-methylumbelliferone was fluorometrically determined at 365 nm for excitation and 450 nm for emission using a CLARIOStar plate reader (BMG Labtech).

Cell Surface Expression. ELISA (enzyme-linked immunosorbent assay) was utilized to measure expression of full-length SosV-RBP and NDV-RBP on HEK 293T cells (91). Best-transfected cells were washed with PBS pH 7.4 following 18 h incubation at 37 °C, 5% CO₂, counted and seeded into an ELISA plate at a density of 25,000 cells per mL. Cells were bound overnight at 4 °C prior to fixation for 15 min in 4% paraformaldehyde. Following thorough washing, cells were blocked in PBS-5% milk for 1 h and subsequently reacted with rabbit anti-6xhis-tag antibody (Abcam, product no. ab9108) for 1 h at room temperature. Cells were repeatedly washed prior to addition of horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Qiagen, product no. 34660) diluted 1:200 in PBS pH 7.4 (Gibco). Plates were washed and stained with HEK293T cell supernatants containing soluble NDV-RBP, WT SosV-RBP, eSosV-RBP, and the mock-transfected control. Neuraminidase activity was assayed by measuring levels of free sialic acid (FSA) following incubation with 50 μM fetuin (Sigma Aldrich, product no. F3004) for 18 h at 37 °C in PBS, pH 7.4 (Gibco). Plates were stained with 10 μM mono-sodium hippurate buffer, pH 10.7 to stop the reaction.

Production of the Hexapetide Motif eSosV-RBP Mutant. The splice-by-overlap extension PCR method was utilized to generate a soluble construct of SosV-RBP (eSosV-RBP, residues 125–582) bearing the following hexapeptide motif site-directed substitutions: R242N, L243R, K244Y, and H245S (Fig. 1A). eSosV-RBP was cloned into a pLHsec vector containing an N-terminal SUMO tag and hexahistidine tag (79).

Warren Method for Determining Free Sialic Acid. Alongside a mock-transfected negative control, HEK 293T cell monolayers were transiently transfected using Lipofectamine 2000 with eSosV-RBP, WT SosV-RBP, and NDV-RBP (residues 47–570) similarly cloned into a pLHsec vector encoding an N-terminal SUMO tag and hexahistidine tag. An ELISA plate was incubated overnight at 4 °C with monoclonal anti-histidine-derived monomeric and dimeric anti-SUMO antibody (Qiagen, product no. 34660) diluted 1:200 in PBS, pH 7.4 (Gibco). Plates were washed and stained with HEK293T cell supernatants containing soluble NDV-RBP, WT SosV-RBP, eSosV-RBP, and the mock-transfected control. Neuraminidase activity was assayed by measuring levels of free sialic acid (FSA) following incubation with 50 μM fetuin (Sigma Aldrich, product no. F3004) for 18 h at 37 °C in PBS, pH 7.4 (Gibco). Using a sialic acid assay kit (Sigma Aldrich, product no. MAK314) based on the Warren method for assaying sialic acid (49), FSA was oxidized to formylpyruvic acid and subsequently reacted with thiobarbituric acid to form a pink product, which was fluorometrically measured (λex = 555/nm = 585 nm) using a CLARIOStar plate reader (BMG Labtech).

Structural Phylogenetic Analysis. Structural phylogenetic analysis was performed with the Structural Homology Program (SHP) (59, 60) using paramyxoviral RBP monomers. The resulting evolutionary distance matrix was used to construct an unrooted phylogenetic tree with the PHYLOGeny Inference Package (PHYLIP) (92).

Dimer Angle Analysis. Analysis of relative angles monomers within the paramyxoviral dimers was performed with UCSF Chimera (93). To calculate the angle between the monomers of a dimer, planes representing the top faces of the monomers were constructed based upon conserved stretches of paramyxoviral RBP sequence using the “Define plane functionality.”

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