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Parvovirus Glycan Interactions

Lin-Ya Huang[#], **Sujata Halder**[#], and **Mavis Agbandje-McKenna**[*]
Department of Biochemistry and Molecular Biology, Center for Structural Biology, McKnight Brain Institute, College of Medicine, University of Florida, Gainesville, FL, 32610, USA

Abstract

Members of the *Parvoviridae* utilize glycan receptors for cellular attachment and subsequent interactions determine transduction efficiency or pathogenic outcome. This review focuses on the identity of the glycan receptors utilized, their capsid binding footprints, and a discussion of the overlap of these sites with tropism, transduction, and pathogenicity determinants. Despite high sequence diversity between the different genera, most parvoviruses bind to negatively charged glycans, such as sialic acid and heparan sulfate, abundant on cell surface membranes. The capsid structure of these viruses exhibit high structural homology enabling common regions to be utilized for glycan binding and at the same time the sequence diversity at the common footprints allows for binding of different glycans or differential binding of the same glycan.

Introduction

Viruses are durable nanomachines evolved to utilize an assortment of strategies to manipulate a host cell's replication machinery for successful infection. The key initial step in this process is the attachment to cell surface receptors. This is followed by internalization into the cytoplasm and delivery of the viral genome to the appropriate replication compartment; the cytoplasm for most RNA packaging viruses and the nucleus for those that package DNA. Initial binding is often mediated by ‘attachment factors’ that concentrate the virus on the cell surface and prime it to interact with secondary receptors or co-receptors for internalization.

Glycans and glycoconjugates, displayed on cell surface, serve in communication as well as primary receptors for many viruses. The variability of glycan structures expressed in different species and in different tissues within the same species creates diversity in viral tissue tropism [1]. Mostly, the glycoepitopes consist of negatively charged terminal sialic acid (SIA) or sulfated oligosaccharide motifs of glycosaminoglycans (e.g. heparan sulfate (HS)) and thus mediate electrostatic interactions with the viral capsid. The virus capsid

[*]Corresponding author, mckenna@ufl.edu, telephone: 1 352 392 5694; Mailing address, University of Florida, Dept. of Biochemistry and Molecular Biology, 1600 SW Archer Road, Gainesville, FL, 32610-0245.
[#]Equal contribution

receptor binding motif can be projections or depressions conformed on the assembled capsid surface of non-enveloped viruses, or glycoproteins decorating the lipid membrane of enveloped viruses.

The *Parvoviridae*, a family of ssDNA viruses, have evolved to 'hijack' the interaction functionality of glycans for gaining cellular entry during infection. Receptor-mediated attachment and entry are essential first steps in their infection [2-4]. Following an introduction to the family, this review will discuss current knowledge on (I) glycan receptors utilized for cellular entry and (II) mapped glycan receptor binding footprints with mention of overlaps with transduction efficiency (for non-pathogenic members being exploited as gene delivery vectors), and pathogenesis (for autonomous members) determinants.

The Parvoviridae

The *Parvoviridae*, small (~260 Å diameter) non-enveloped T=1 icosahedral viruses, package linear ssDNA genomes of 4 to 6 kb [5]. The family is divided into two subfamilies based on host range: the *Parvovirinae* infect vertebrates and the *Densovirinae* infect insects and arthropods [6]. Due to limited information on the *Densovirinae* with respect to receptor utilization, this review will focus on the *Parvovirinae*. The *Parvovirinae* is further subdivided into five genera: *Amdovirus*, *Bocavirus*, *Dependovirus*, *Erythrovirus*, and *Parvovirus*, with type members *Aleutian mink Disease Virus* (AMDV), *Bovine Parvovirus* (BPV), *Adeno-associated virus serotype 2* (AAV2), *Human Parvovirus B19* (B19), *Minute Virus of Mice* (MVM), respectively, based on genomic architecture and protein sequence-based phylogenetic analyses [6]. Their capsid open reading frame (*cap*) encodes two or three (depending on virus) overlapping structural viral proteins (VP) which assemble the T=1 capsid [7]. The dependovirues rely on co-infection with a complex helper virus (such as *Adenovirus*, *Herpes Simplex Virus* or *Papillomavirus*) for productive infection [8-13] and are non-pathogenic. The other genera replicate independently of helper virus function ('autonomously replicating') and contain nonpathogenic and pathogenic members [14-17].

The capsid structures for several parvoviruses, including the type member for each *Parvovirinae* genera (Fig. 1), have been determined by X-ray crystallography and/or cryo-electron microscopy and image reconstruction (cryo-reconstruction) (reviewed in [18,19] and unpublished data). Despite low sequence similarity (e.g. 14% to 36% between genera), the ordered VP region (VP2 or VP3 depending on virus) is highly conserved with a superposable core eight-stranded β-barrel and αA helix (Fig. 1A). The tops of the loops between these conserved regions are varied in sequence and structure (even within each genus) and defined as variable regions (VRs) I-IX or VR1-8 for dependo and autonomous parvoviruses, respectively [20,21]. The capsid surface is characterized by depressions at the 2-fold axes (dimple) and surrounding a cylindrical channel at the 5-fold axes (canyon), and protrusions at or surrounding the 3-fold axes (Fig. 1B-F). A wall is located between the depressions at the 2-fold axes and surrounding the 5-fold channel, the "2/5-fold wall" (Fig. 1B-F) [18,19]. The VRs cluster at the 5-fold axes, the 3-fold protrusions, and depression at the 2-fold axes to create local variations of the characteristic capsid surface features exhibited by members of each genus. Mutagenesis, biochemical, and structural studies

demonstrate that residues in these VRs play important roles in viral life infection, including viral-receptor binding (reviewed in [14,19,22]).

Glycan receptor utilization by the dependoviruses and capsid recognition sites

Currently, over one hundred AAV genomic isolates have been reported [23-33], with thirteen (AAV1-13) serotypes described for the human and non-human primate sequences. Due to their ability to package and delivery foreign genes to different tissue types and the lack of associated disease, AAVs are being developed and used as gene delivery vectors, and serve as the first approved gene therapy treatment [34-40]. AAVs share ∼60 to 99% identity and display differential cell and tissue tropisms as well as transduction efficiency *in vitro* and *in vivo* [23,41]. For several of these viruses, cell infectivity and transduction is reported to be dictated by the ability of their capsids to recognize cell surface glycans (Table 1) as primary attachment receptors. AAV1 binds both α2-3 and α2-6 N-linked SIA [42-44]; AAV2, AAV3, and AAV13 bind heparan sulfate proteoglycan (HSPG) [27,45-48]; AAV4 and AAV5 bind α2-3 O-linked and α2-3 N-linked sialic acids, respectively, ([42,49-52] and our unpublished data); AAV6, a tissue culture adapted recombinant between AAV1 and AAV2, binds both HS and α2-3 and α2-6 N-linked SIA [44,52-54], with SIA being the determinant of transduction; AAV9 binds terminal N-linked galactose (GAL) [55,56]. The nature of the glycosylation (if any) required for cellular recognition by AAV7, AAV8, and AAV10-12 are not known. Thus, the human and non-human primate AAVs fall into 3 groups with respect to their glycan receptor usage: HSPG for AAV2, AAV3, AAV6, and AAV13; SIA for AAV1, AAV4, AAV5, and AAV6; GAL for AAV9. Bovine AAV (bAAV) utilizes terminal gangliosides, more commonly observed in glycolipids, and chitotriose, a trimer of β1,4-linked *N*-acetyl-D-glucosamine, for cellular infection [57,58].

Cell binding, transduction assays, mutagenesis, and structural approaches have been utilized to identify the capsid amino acid residues involved in receptor recognition for most of the AAVs for which glycan receptors are known (Table 1; Fig. 2A-C), with the interaction between AAV2 and HSPG the best characterized. Mutagenesis identified the AAV2 HS binding footprint as five basic residues: R484, R487, K532, R585, and R588 (Fig. 2A-C), with R585 and R588 (VP1 numbering) the most critical [45,46]. Cryo-reconstruction of the AAV2-HS complex confirmed this binding site, as adjacent residues, at the inner wall of the protrusions surrounding the 3-fold axis on AAV2 capsid (Fig. 2A-C) [59,60]. These residues are located on VR-V, VR-VI, and VR-VIII. Structural and biochemical approaches identified the HS binding site on AAV3B, a minor variant of AAV3, as R594 (in VR-VIII) located also on the inner wall of the 3-fold protrusion but not overlapping with the AAV2 footprint (Fig. 2A-C) [61,62]. For AAV6, two separate mutagenesis studies identified two non-adjacent residues as being important for HS recognition; K459 (VR-IV) located close to the top of the 3-fold protrusion and K531 (VR-VI) located at the base of 3-fold protrusion facing the 2-fold axis (Fig. 2A-C) [53,63,64]. Random capsid VP mutagenesis and *in vivo* assays suggest that the SIA binding properties of AAV4 are controlled by residues K492, K503, M523, G581, Q583, and N585. These residues are located at the top of the 3-fold protrusion: K492 and K503 within VR-V, and G581, Q583, and N585 within VR-VIII. [65].

Mutagenesis studies for AAV5 suggest the involvement of A581 (VR-VIII) in SIA binding and transduction [66,67]. This residue is also located on the inner wall of the 3-fold protrusion (Fig. 2A-C). By mutagenesis and computational molecular docking, the GAL binding residues on AAV9 were identified as D271 (VR-I), N272 (VR-I), Y446, N470, and W503 (VR-V) that form a pocket at the base of the 3-fold protrusion facing the 5-fold axis (Fig. 2A-C) [56]. Residues Y466, N470, and W503, are the 3/26 reported to directly or indirectly affect AAV9 GAL binding by AAV Barcode-Seq [68]. An analogous pocket on AAV1 has been identified as its SIA binding site by structural mapping (unpublished data). Residue K528 in AAV13, which is structurally equivalent to K531 in AAV6, dictates its HS interaction [27]. Thus regardless of the glycan recognized, the 3-fold protrusion serves as the binding region on several AAV capsids (Fig. 2A-C). These sites, mostly located in VRs, overlap residues reported to affect the transduction properties of the AAVs (Table 1) (reviewed in [22]).

Glycans receptor utilization by the autonomous parvoviruses and capsid recognition sites

Pathogenic members of the autonomous parvoviruses are associated with serious diseases in the young of the species infected and immunocompromised adults, while nonpathogenic members establish asymptomatic but persistent infections (reviewed in [17]). The glycans involved in cellular recognition are known for most of the type members of the autonomous *Parvovirinae*, except amdovirus, and for genus parvovirus this information is available for several members. BPV (bocavirus) binds to glycophorin A via O-linked α2,3-linked sialic acid [69-71]. B19 (erythrovirus), a human pathogen, binds to the neutral glycosphingolipid erythrocyte P antigen (globoside/Gb4) on erythroid progenitor cells [72,73], and another structurally and biosynthetically unrelated glycolipid called paragloboside (neolactotetraglycosylceramide or nLc4) [74]. However, Kaufmann and coworkers [75] reported that recombinant B19 capsids do not bind to globoside Gb4 *in vitro*.

For several members of the parvovirus genus, terminal SIA serves as a glycan receptor (Table 1), although for *Canine Parvovirus* (CPV) and *Feline Panleukopenia Virus* (FPV), SIA recognition is not essential for infection, but rather utilized during hemagglutination (HA) of erythrocytes [76]. CPV and FPV recognize both α2-3 and α2-6 linked N-glycolyl neuraminic acid, a non-human SIA. Host cellular infection by CPV and FPV requires recognition of their respective host transferrin receptor type-1 with differential N-glycosylation dictating viral host specificity ([77-80]. For other members, for example *H-1 Parvovirus* (H-1PV), LuIII, MVM, and *Porcine Parvovirus* (PPV), SIA binding is essential for infection and the recognition is to N-acetyl neuraminic acid. MVM utilizes terminal SIA on an unknown glycoprotein for cellular recognition [4] and binds specifically to α2-3 N-linked sialylated glycans including the $s(Le^x)_3$ motif [81,82]. This Lewis X motif is over expressed in cancer cells and likely dictates MVM's oncotropism [83]. MVM exists in several highly homologous strains, including the prototype strain (MVMp) and the immunosuppressive strain (MVMi) which share 97% sequence identity. These viruses are reciprocally restricted for growth in each other's permissive cell type, although both viruses are able to infect the SV40 transformed human fibroblast cell line NB324K [84]. MVMp is

non-pathogenic and results in an asymptomatic infection [85], while MVMi causes a lethal infection and is neurotropic [86,87]. Additional recognition of α2-8 linked multi-SIA glycans, over-expressed in neuronal cells, by MVMi likely mediates its neurotropism [81,88]. Similar to MVM, H-1PV, and LuIII also recognize α2-3 linked SIA glycans, especially the sLe^x motif ([89] and unpublished data). Significantly, these rodent parvoviruses (MVM, H-1PV, and LuIII) exhibit tumor specific cytotoxic activity *in vitro* and oncosuppressive activity *in vivo* and are under development as viral vectors for anticancer gene therapy (reviewed in [90-92]). PPV binds to SIAs on cell surface glycoproteins yet to be identified [93].

Similar to the dependovirues, a multitude of approaches have been utilized to characterize the glycan recognition site for a number of the autonomous parvoviruses. Cryo-reconstruction localized the B19 globoside footprint to the depression between the protrusions surrounding the 3-fold axes of the capsid [94], similar to the HS and SIA sites for the AAVs. The globoside footprint includes residues Q399, Q400, Y401, T402, D403, Q404, and E406 (VP2 numbering, Table 1, Fig. 2D-F). For CPV, mutations of residues R377K, E396K, R397G/E on the wall of the depression surrounding the 2-fold axis of the capsid reduced or abolished HA, hence, SIA recognition [95-97]. Residues N93, G299, A300, and N323 control CPV/FPV host range and pathogenicity determination as well as transferrin receptor attachment [77,98-100]. These residues are located at the top and shoulder of the 3-fold protrusions [101,102] (Fig. 3A-C). Mutagenesis, cell binding assays, and structural studies indicate that the H-1PV SIA footprint contains I368 and H374 located at the 2-fold depression ([89] and unpublished data). For MVM, mutagenesis, and structural studies have also identified the depression at the 2-fold axis as the SIA binding site [103]. The binding pocket is shallow and surrounded by charged and hydrophobic residues that include K241, M243, I362, K368, Y396, W398, D399, T401, F403, D553, Y558, and I578. Significantly, the residues determining *in vitro* tropism (T317 and G321), conferring fibrotropism on MVMi (D399,S 460, D553, Y558), *in vivo* pathogenicity (V325, I362, K368), and those associated with the development of leukopenia (G321, A551, V575) are localized in the vicinity of this SIA binding pocket [104-109] (Fig. 3D-F). In addition, tissue tropism and pathogenicity determinants for ADV, CPV, and PPV overlap with this region [14]. There is no information on residues controlling BPV, LuIII, or PPV capsid – SIA interactions. The available information identifies the 2-fold (CPV and MVM) and 3-fold (B19) depressions as important for glycan receptor engagement for the autonomous viruses.

Commonality in utilization and binding region are features of *Parvovirinae* glycan interactions, although recognition determinants differ

A role for glycan recognition in dictating successful cellular infection and as a determinant of tissue tropism is well established for the *Parvovirinae*. Evidence points to a role in dictating transduction efficiency and host pathogenicity for dependo and autonomous parvoviruses, respectively, due to overlap of the capsid residues involved. However, this possibility requires further investigation given the ubiquity of SIA and HS which are the most commonly recognized glycans. Thus parvovirus tissue (especially for the AAVs) and host specificity likely requires post cell entry interactions, including the recognition of

specific internalization receptors/co-receptors which are often glycosylated [110,111]. Regardless of the outcome of infection, pathogenic or non-pathogenic, commonality in utilization and binding region is a feature of *Parvovirinae* glycan interactions, despite the sequence diversity that exists between genus members (14-36%) and within each genera (e.g. 50-66% for parvovirus genus).

The known interaction sites involve residues at the 3-fold depression (e.g. AAVs and B19); pocket at base of 3-fold protrusion (AAV1 and AAV9), or 2-fold depression (e.g. CPV and MVM) (Figs. 2 and 3). Significantly, both the 2- and 3-fold depressions are utilized to bind SIA, e.g. in MVM and AAV4/AAV5, respectively, despite low sequence and structure similarity at these capsid regions. In addition, analogous capsid regions/pockets are used to bind different glycans, e.g. SIA, globoside, and HS at the 3-fold depression, and SIA and GAL at the base of the 3-fold protrusions. Furthermore, while 3/5 AAV2 HS binding footprint residues are conserved in most of the AAV serotypes, the critical R585 and R588 are not. For AAV3B and AAV6/AAV13, their HS binding residues, R594 and K531/K528, respectively, are not conserved in other HS binding AAVs. For SIA binding, 4/6 SIA binding residues in AAV4 (K492, K503, G581, and N585) are not conserved in AAV1 and AAV5, the serotypes that also bind SIA and while AAV5's A581 position is structurally conserved in other AAVs, including AAV2, the adjacent residues and structure are not. With respect to the common pocket for SIA and GAL binding by AAV1 and AAV9, respectively, N470, the most critical residue for the AAV9 phenotype, is G470 in AAV1. In B19, the globoside binding pocket residues are not conserved in other genus type members. For MVM, the SIA binding footprint residues are conserved in H-1PV consistent with a common pocket, but not for SIA binding dependovirus members, likely leading to their use of the 3-fold region for this interaction. Importantly, these glycan recognition sites overlap with transduction and pathogenicity determinants. These observations suggest a parvovirus capsid evolution to establish host recognition niches rather than conservation of glycan binding pockets. Similar binding site localization points to structural motifs evolved to serve an essential functional role and begin to establish a receptor binding pattern that could inform efforts to use these capsids for targeted therapies or develop viral infection inhibitors.

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Highlights

- Glycan interactions are an essential first step in successful host infection by the *Parvovirinae* subfamily of the *Parvoviridae*.
- The binding sites for glycans overlap with determinants of transduction efficiency and pathogenicity for non-pathogenic and pathogenic members, respectively.
- The *Parvovirinae* exhibit commonality in the glycans recognized across genera and they utilize common capsid regions for binding to disparate glycans.
- Identifies capsid regions that can inform engineering efforts for tissue targeted gene delivery therapies or development of viral infection inhibitors.

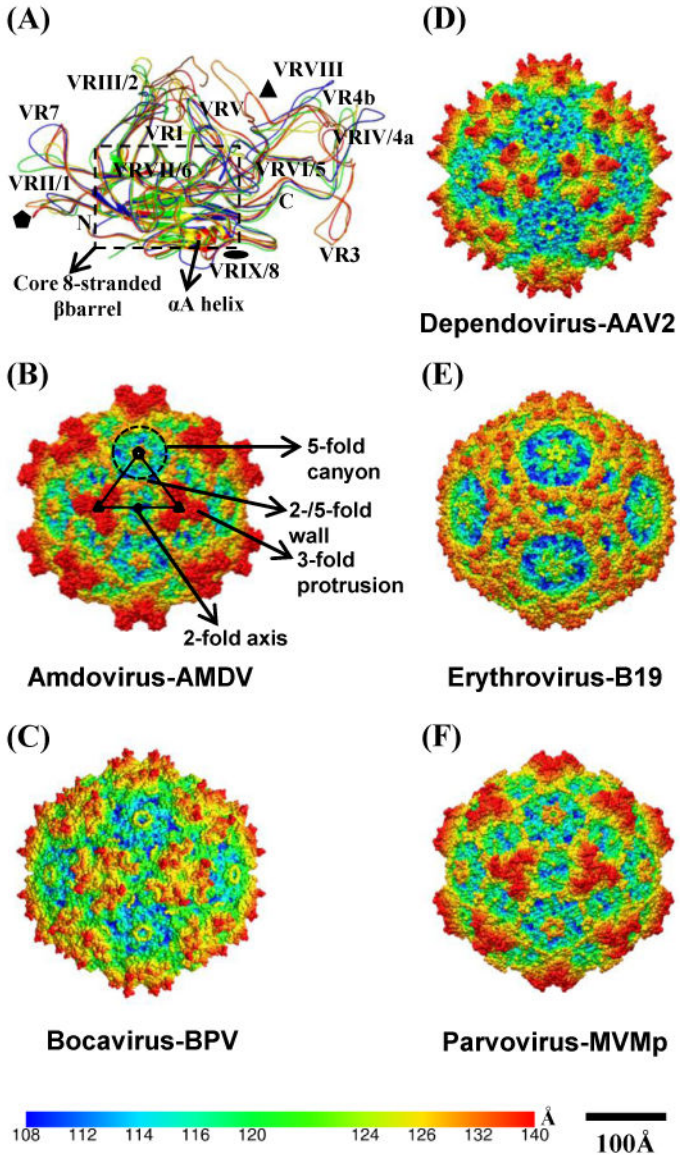

Fig.1.

Parvovirinae capsid structure. (A) Structure superposition of the structurally ordered VP region for type members of the *Parvovirinae* subfamily: amdovirus - ADV (orange); bocavirus – BPV (yellow); dependovirus - AAV2 (blue); erythrovirus - B19 (green); and parvovirus – MVM (red). The N-terminus (N), C-terminus and variable regions (VRI-IX, VR1-8) are labeled, the conserved core eight-stranded β-barrel and αA are delineated by a dashed box. (B-F) Depth cued (from capsid center to surface: blue-green-yellow-red) capsid surface representation of the viruses shown in (A). A viral asymmetric unit (AU, black triangle), bounded by a 5-fold axis (filled pentagon) and two 3-fold axes (filled triangles) separated by 2-fold axis (filled oval), is shown on the AMDV capsid image in (B). The topological features of the parvovirus capsid, such as 3-fold protrusion, 2-fold depression, 5-fold canyon and 2/5-fold wall are labeled on the AMDV image. A horizontal color bar for radial distance (Å) from the center of the capsid and a horizontal scale bar (100Å) for

diameter measurements are provided. Panel (A) was generated using the PyMol program [112] and (B-F) were generated using the UCSF-Chimera program [113].

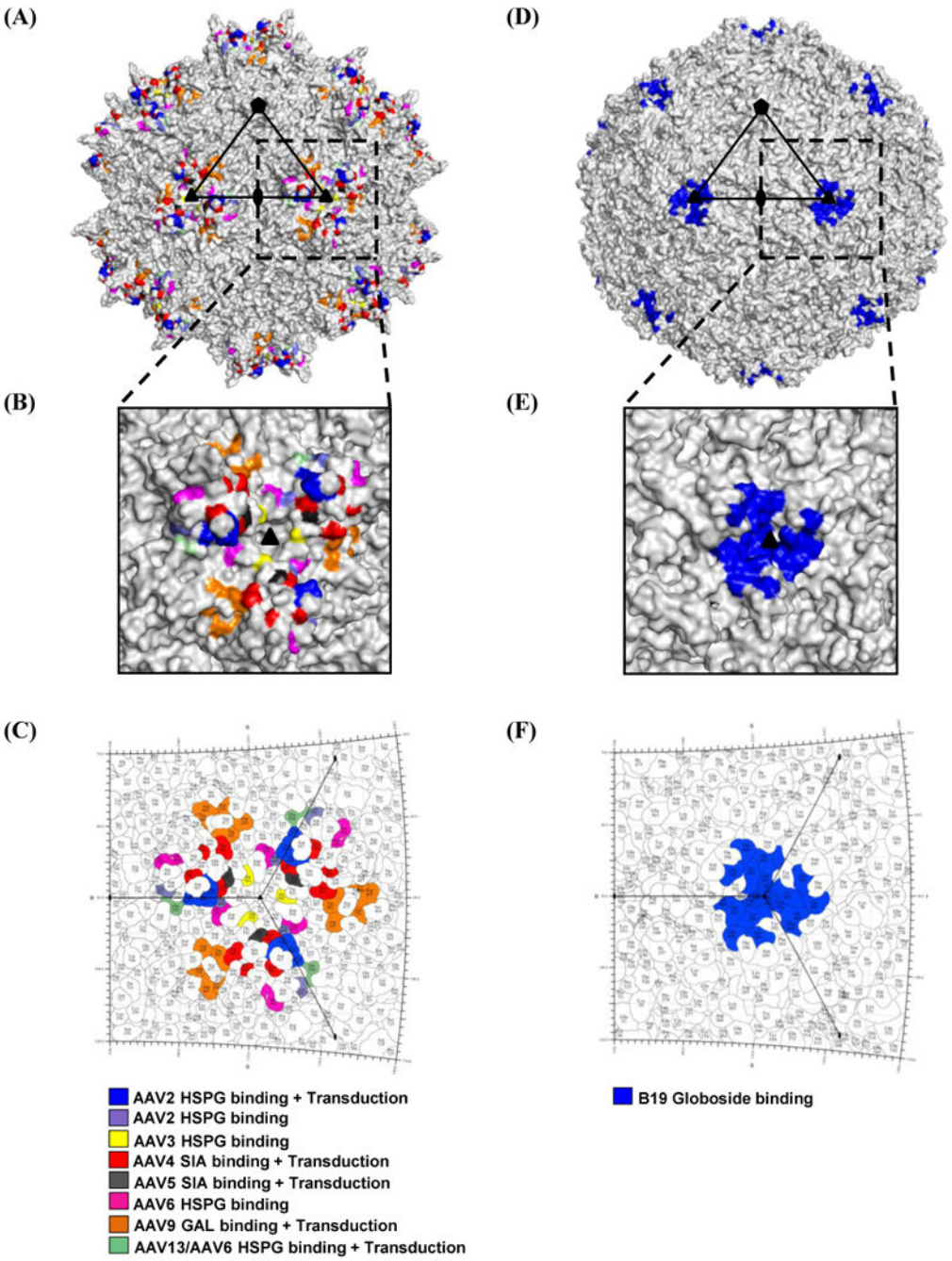

Fig.2.

Capsid surface images for AAV2 and B19. (A) Capsid surface image of AAV2 on which the residues involved in glycan receptor binding for AAV2, AAV3B, AAV4, AAV5, AAV6, AAV9, and AAV13 are highlighted along with residues determining transduction phenotypes. The view is approximately down the icosahedral 2-fold axis. (B) A zoom of the receptor binding footprints in the section delineated by a dashed box in (A). (C) Stereographic Roadmap projection [114] of the glycan footprint shown in (A) viewed down the threefold axis. Residues are labeled by type (three letter code) and number (VP1

numbering). The amino acid residues that are exposed on the capsid exterior are visible in this image. The boundary for each residue is shown in black, and a color key is provided. The AU is depicted as in Fig. 1B. (D, E, and F) Capsid surface image of B19, zoom of the dashed box in (D) and Roadmap projection, respectively, of the globoside footprint at the 3-fold axis. The image labels are as in (A-C). Panels (A, B, D, and E) were generated using the UCSF-Chimera program [113] and (C and F) were generated using the RIVEM program [114].

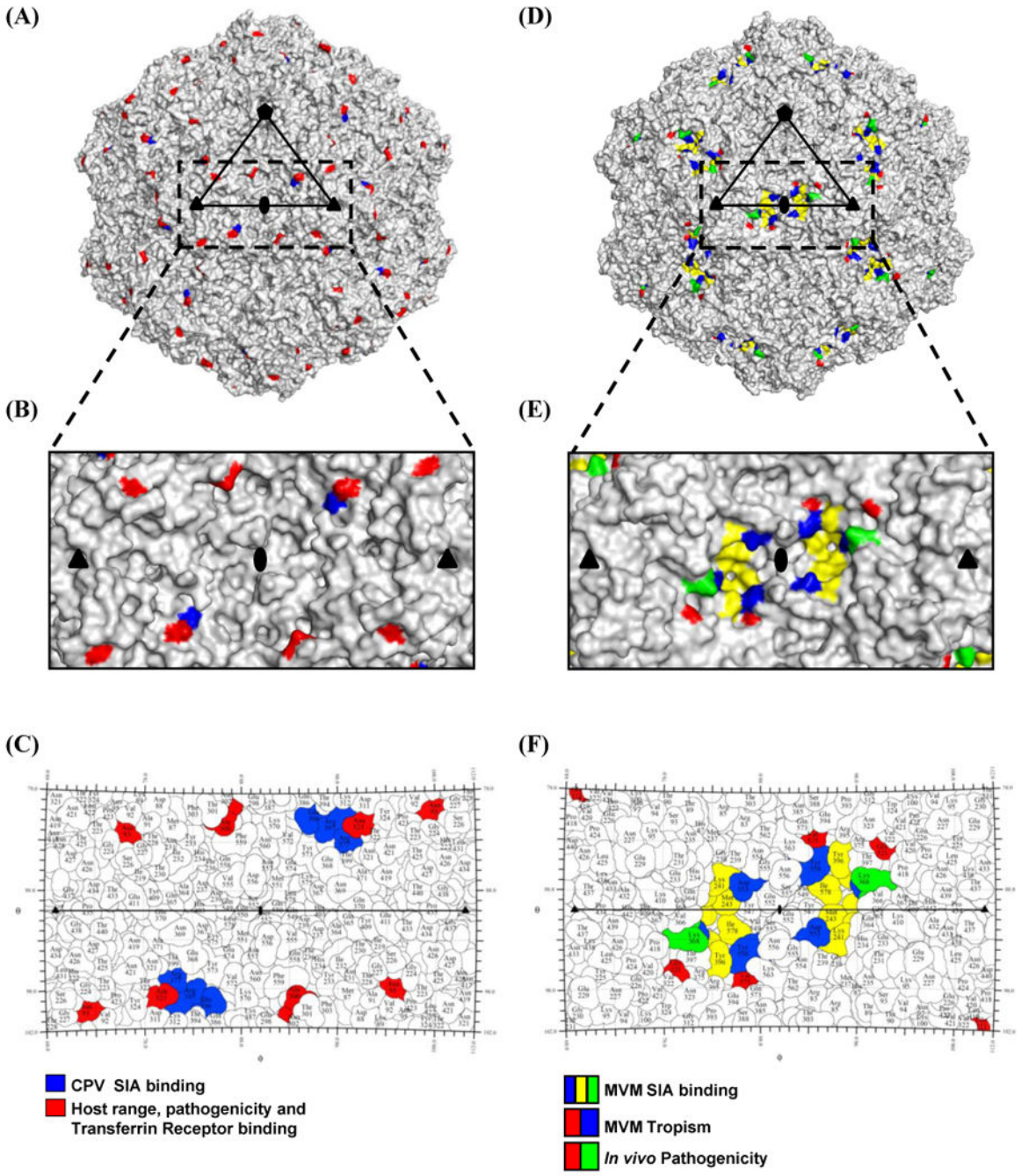

Fig. 3.

Capsid surface images for CPV and MVM. (A-C) Capsid surface image of CPV, zoom of the dashed box in (A), and Roadmap projection, respectively, of the residues involved in SIA binding and host tropism/pathogenicity/transferrin receptor binding. (D-F) Capsid surface image of MVM, zoom of the dashed box in (D), and Roadmap projection, respectively, of the residues involved in SIA binding, tissue tropism, and pathogenicity. The

image labels are as in Fig. 2. Panels (A, B, D, and E) were generated using the UCSF-Chimera program [113] and (C and F) were generated using the RIVEM program [114].

Table 1

Parvoviruses, their hosts and glycan receptors

Virus	Host	Glycan Receptors	Phenotype	Residues	References
AAV1	Humans	α2-3 and α2-6 N-linked sialic acid (SIA)	SIA binding	unpublished	[42-44], unpublished data
AAV2	Humans	Heparan sulfate proteoglycan (HSPG)	HSPG binding/Transduction	R484, **R487**, K532, **R585**, **R588**	[45,46,48]
AAV3b	Humans	HSPG	HSPG binding	**R594 (A593)**	[47,61,62]
AAV4	NHP*	α2-3 O-linked SIA	SIA binding/Transduction	**K492 (S498), K503 (T503), M523 (M523), G581 (N582), Q583 (Q584), N585 (G586)**	[49,51,65]
AAV5	Humans	α2-3 and α2-6 N-linked SIA	SIA binding/Transduction	**A581 (A591)**	[42,49-52,66,67]
AAV6	Humans	α2-3 and α2-6 N-linked SIA, HSPG	HSPG binding/Transduction	K459 (S458), K493 (S492), **K531 (E530)**, R576 (Q575)	[44,52-54,63,64]
AAV9	Humans	Galactose (GAL)	GAL binding/Transduction	**D271 (D269), N272 (N270), Y446 (Y444), N470 (D469), W503 (W502)**	[55,56]
AAV13	NHP	HSPG	HSPG binding/Transduction	**K528 (E530)**	[27]
Bovine AAV	Bovine	Gangliosides, Chitotriose	Receptor Binding	N/D	[57,58]
BPV	Bovine	α2,3 O-linked SIA (Glycophorin A)	SIA Binding	N/D	[69-71]
B19	Humans	Erythrocyte P antigen (Globoside)	Globoside binding	Q399, Q400, Y401, T402, D403, Q404, E406	[72-74,94]
MVM	Rodents	α2-3 and α2-8 N-linked SIA	SIA binding/Host tropism/pathogenicity	K241, M243, *I362*, *K368*, Y396, W398, D399, T401, F403, D553, Y558, T578	[4,81,82,103-109]
H-1PV	Rodents	α2-3 N-linked SIA	SIA binding	I368, H374	[89],
CPV and FPV	Canine,	α2-3 and α2-6 N-linked Neu5Gc	SIA binding	R377, E396, R397	[95-97]
			Host range/pathogenicity/Transferrin receptor binding	N93, G299, A300, N323	[77,98-100]
PPV	Swine	α2-3 and α2-6 N and O- linked SIA	SIA Binding	N/D	[93]

*NHP=Non-Human Primate;

N/D: not defined; Residues in parentheses are AAV2 VP1 numbering; residues in **bold** are overlapping glycan binding and transduction determinants; residues in *italics* are overlapping glycan binding and pathogenicity determinants; residues underlined are overlapping glycan binding and tropism determinants.