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Engineering AAV receptor footprints for gene therapy

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Abstract

Adeno-associated viruses (AAV) are currently at the forefront of human gene therapy clinical trials as recombinant vectors. Significant progress has been made in elucidating the structure, biology and tropisms of different naturally occurring AAV isolates in the past decade. In particular, a spectrum of AAV capsid interactions with host receptors have been identified and characterized. These studies have enabled a better understanding of key determinants of AAV cell recognition and entry in different hosts. This knowledge is now being applied toward engineering new, lab-derived AAV capsids with favorable transduction profiles. The current review conveys a structural perspective of capsid–glycan interactions and provides a roadmap for generating synthetic strains by engineering AAV receptor footprints.

Introduction

The last decade has witnessed the transition of Adeno-associated viruses (AAV) from gene transfer vectors to clinically viable, therapeutic candidates. Discovered as a contaminant in Adenovirus preparations during the mid 1960s [1], at first AAV failed to garner much clinical interest due to its complete lack of pathogenicity [2]. AAV is helper-dependent, meaning its replication relies on the presence of factors from a helper virus, such as adenovirus or herpes simplex virus [3,4]. As a member of the Dependovirus genus of *Parvoviridae*, AAV has a $T = 1$ icosahedral capsid, ~25 nm in diameter. The 4.7 kb single-stranded DNA genome contains two open reading frames: *Rep* and *Cap* [5]. *Rep* encodes four alternatively spliced proteins for viral genome packaging and replication, while *Cap*, encodes the capsid structural proteins VP1, VP2, and VP3 along with the Assembly

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Conflict of interest

Aravind Asokan is a co-founder at Stridebio LLC and an inventor on patents owned by UNC-CH.

Activating Protein (AAP) [5–7]. VP1, VP2, and VP3 share a common beta barrel domain, but differ in their N-terminal extension [5–7].

The AAV genome is flanked by inverted terminal repeats (ITRs), which are crucial for packaging, replication, and integration into the host genome. The ITRs represent the only essential cis-acting element of the AAV genome, enabling the incorporation of different transgenes between the ITRs, which can then be packaged into AAV capsids to generate recombinant AAV vectors (rAAV) [8]. Recombinant AAVs can be generated via transfection of plasmids containing the ITR-flanked transgene, an AAV capsid gene, and necessary helper genes, followed by harvest of transfected cells and purification of virus [5–7]. The application of rAAVs in gene therapy applications has rapidly mushroomed over the past decade. During this time, significant improvements to rAAV technology have been made with the goal of improving gene transfer efficiency, altering tropism and/or reducing antigenicity [9,10]. Structural studies of AAV capsids combined with rational mutagenesis as well as combinatorial protein engineering strategies combined with directed evolution have yielded several lab-derived AAV strains [9,10]. Here, we specifically review AAV capsid engineering strategies focused on exploiting knowledge of AAV capsid structure and interactions with host cell surface glycan receptors. Potential future studies focused on re-engineering other AAV–receptor footprints are also discussed.

AAV and host receptors

Several distinct AAV serotypes and variants have been discovered and isolated from different animal species including humans over the past decade [9,11–14]. It is now well known that different AAV isolates can utilize a variety of cell surface glycans for cell surface binding, including heparan sulfate (HS), N-linked sialic acids (SIA), O-linked sialic acids or mucins (MUC), or galactose (GAL) [5,15–19]. Some naturally occurring AAV serotypes recognize two different glycans, although typically only one of the carbohydrates serves as a cognate receptor. For instance, AAV6, can bind both HS and $\alpha 2,3/\alpha 2,6$ SAs [20]. Other AAV serotypes such as AAV12 have yet to be conclusively linked with a particular host receptor [12,21]. This spectrum of glycan receptors is thought to mediate the diverse tropisms displayed by distinct serotypes and has consequently provided the basis for selecting optimal AAV vectors for specific therapeutic applications [5,22,23,24].

Subsequent to glycan binding, cellular uptake of AAV serotypes has been shown to involve specific co-receptors on the cell surface. For instance, the fibroblast growth factor receptor (FGFR) and/or hepatocyte growth factor receptor (HGFR)/C-Met have been implicated in AAV2 and AAV3 cell entry [25,26]. Further, platelet-derived growth factor (PDGFR) and epidermal growth factor receptor (EGFR) have been implicated in the cellular uptake of AAV5 and AAV6, respectively [27,28]. In addition, an essential role for integrins has been demonstrated for AAV2 and AAV9 [29–32]. More recently, a genetic screen identified a previously uncharacterized transmembrane protein, KIAA0319L (denoted as AAVR) as being essential for endocytosis and golgi trafficking of multiple AAV isolates [33]. Notably, the latter study questions the extent of direct or essential involvement of co-receptors such as FGFR and C-met in AAV infection. However, while this study has demonstrated the importance of AAVR in transduction, more thorough comprehension of the

interaction between AAV and AAVR is required. Soluble AAVR has been shown to inhibit AAV infection, and an uptake-deficient AAVR with a C-terminal deletion including an endocytic sorting motif has proven incapable of rescuing infection in an AAVR knockout [33^{**}]. Although these data suggest a cell-surface role for AAVR, the exact nature of the place of AAVR in the AAV uptake pathway remains to be determined. In depth study of AAVR should elucidate whether this new receptor facilitates attachment at the cell surface level, uptake following binding, endosomal escape, trafficking, later steps in the AAV life cycle, or some combination of these roles. Given that many studies have established the crucial role of glycans as AAV cell surface attachment factors and biodistribution determinants, better comprehension of AAVR function could advance our understanding of the mechanisms of AAV infection. In the case of a confirmed cell surface role for AAVR, interrogation of the structural aspects of the AAVR interaction with AAV could catalyze our ability to manipulate this interaction at the capsid level, as exemplified by studies with AAV glycan receptors discussed below.

Structural determinants of AAV glycan interactions

The VP3 capsid protein subunit of AAV consists of 8 antiparallel beta strands forming a barrel. The beta strands are interconnected by variable loops, which are localized on the capsid surface. The VP1, VP2 and VP3 monomers assemble with twofold, threefold, and fivefold axes of symmetry to create the 60-subunit AAV capsid [5,7,34^{**},35]. The twofold axis of symmetry is characterized by depressions, where the variable loops form the barrier between these depressions [5]. The variable loops also form shoulders atop the protrusions that make up the threefold axis of symmetry, as well as the surface topology associated with the fivefold pore, as shown in Figure 1 [5]. Overall, the interdigitating variable loops form specific surface topologies at the different symmetry axes that are unique to each AAV isolate. In addition to dictating capsid–glycan interactions, the variable loops play a crucial role in determining antigenicity and potentially other secondary interactions that involve cell entry or postentry trafficking events.

The HS recognizing motif on the AAV serotype 2 capsid was the first identified glycan receptor footprint in the AAV family. Following mutational analysis of a series of basic residues (R484, R487, R585, R588, and K532) located within surface spikes on the threefold axis spikes, studies demonstrated that these residues are essential for HS binding [36–38]. These results were further confirmed by cryo-EM reconstruction of AAV2-HS complexes [39]. Three other serotypes/isolates, namely, AAV3B, AAV6 and AAV strain VR-942/AAV13, have been shown to bind HS, although only AAV3B and AAV13 appear to require HS for transduction [12,36,37,40–44]. The binding affinities of AAV3B and AAV6 are known to be weaker than that of AAV2 to HS [16,40–42,45,46]. Similar structural and biochemical approaches identified the key HS binding residue on AAV3B as R594 located on the inner wall of the threefold protrusions and for AAV6, K459 and K531 located on or near the threefold protrusions. However, the positions of these residues do not overlap with the HS footprint on AAV2 [16,20,40,42–45]. Lastly, residue K528 in AAV13 or VR-942, which is structurally equivalent to K531 in AAV6 has been implicated in HS recognition [41].

The MUC binding residues on the AAV4 capsid were identified following a random library-based approach as K492, K503, M523, G581, Q583, and N585. These residues are located at the top of the threefold protrusion [47]. Recent studies utilizing X-ray crystallographic and mutational analysis demonstrated that AAV5 has two potential SIA binding sites [48,49**]. Although one site is located in the depression centered within the threefold spikes, the other site is located under the HI loop at the fivefold symmetry axis. Although both sites appear essential for transduction, only the former threefold site appears to be directly involved in SIA recognition. The amino acid residues identified within this primary SIA footprint on the AAV5 capsid by structural analysis include M569, A570, T571, G583, T584, Y585, N586, and L587 [49**]. Mutational analysis further demonstrated that M569, Y585 and L587 were the most crucial in identifying SIA glycans on the cell surface. Lastly, using site-directed mutagenesis and computational molecular docking studies, residues within the GAL binding footprint on AAV9 were identified as D271, N272, Y446, N470, and W503 [50,51]. These studies are corroborated by other studies evaluating structure–function correlates of AAV9-GAL interactions [23,52,53,54**]. These residues cluster together to form a pocket at the base of the threefold protrusions. An analogous glycan footprint on AAV1 and AAV6 has recently been identified comprised of residues forming a SIA binding pocket (unpublished data). Structural diagrams of different AAV serotypes and their cognate glycan receptor footprints are shown in Figure 2.

Re-engineering AAV capsid glycan interactions

A thorough structural understanding of AAV capsid glycan interactions has enabled rational manipulation of glycan footprints on the AAV capsid surface. This reengineering approach has yielded novel, synthetic AAV strains with potential applications in therapeutic gene transfer. Specifically, structure-inspired design has been utilized to abrogate capsid binding to glycan receptors, alter binding affinity, and more recently engineer orthogonal glycan receptor interactions. Structural diagrams of different lab-derived, synthetic AAV strains and their engineered footprints are shown in Figure 3.

Altering endogenous AAV glycan interactions

The earliest example demonstrating the ability to alter AAV glycan interactions was demonstrated by mutating the basic HS binding footprint on AAV2 capsids. Mutations R585E and R588E yielded AAV2 mutants defective in HS binding, while conservative R585K and R588K conversions had minimal effect [36,37]. Additional single point mutations on R484, R487, or K532 decreased HS binding affinity, while a R484E/R585E HS binding mutant demonstrated markedly decreased transduction in the liver, while continuing to transduce heart with efficiency similar to parental AAV2 [37]. These early studies paved the way for strategies to re-engineer AAV capsid glycan footprints with the goal of developing new vectors displaying favorable tissue tropism and biodistribution profiles.

One such example was focused on substituting amino acid residues derived from different AAV serotypes/isolates onto the 585-RGNRQA-590 motif on the AAV2 capsid. This hexapeptide motif contains the key AAV2 HS binding residues R585 and R588. Consistent

with earlier mutagenesis studies, several chimeras displayed loss of liver transduction similar to the R484E/R585E HS binding AAV2 mutants. However, one specific AAV2 chimera containing amino acid residues 585-QQNTAP-590 derived from AAV8, identified as AAV2i8, demonstrated reduced liver tropism and was redirected to cardiac and skeletal muscle tissue, which were transduced with high efficiency [55]. This reagent is undergoing further development as a recombinant vector for therapeutic gene transfer in cardiac and musculoskeletal indications [56]. Similar studies involving random mutagenesis of the AAV9 capsid yielded two liver-detargeted mutants reminiscent of the AAV2i8 phenotype [52,53]. The two AAV9 mutants, denoted AAV9.45 and AAV9.61 with N498V or W503R mutations retained cardiac and musculoskeletal transduction similar to the parent, but displayed markedly reduced hepatic sequestration and transduction. The latter phenotype was later corroborated by mechanistic studies, which demonstrated that AAV9.45 and AAV9.61 displayed reduced affinity for GA [52,53].

Another instance of re-engineering AAV–glycan interactions is exemplified by studies centered on the antigenically distinct monkey isolate, AAV4. Random mutagenesis of key amino acid residues K492, K503, M523, G581, Q583, and N585 yielded novel AAV4 mutants with decreased binding affinity to O-linked α 2,3 sialic acid or mucin (MUC) [47]. Although defective upon systemic injection, one particular mutant AAV4.18, containing K492E, K503E and N585S substitutions displayed a unique transduction profile in the brain. Specifically, increased spread of AAV4.18 throughout the brain parenchyma and selective transduction of migrating progenitors was observed [57]. This acquired tropism and increased CNS spread were attributed to a switch in receptor specificity from MUC to α 2,8-linked polysialic acid (PSA), an established marker of neurogenesis [57]. Based on similar rationale, re-engineering of residues within the AAV5 SIA binding footprint is likely to yield novel AAV5 mutants with altered glycan binding affinity and potentially, altered tropism. Together, the latter studies suggest that re-engineering existing receptor footprints on AAV capsids could yield mutants with altered affinity for the cognate glycans. In general, decreasing glycan binding affinity appears to be more feasible than increasing affinity, although examples of such modifications have been reported earlier for AAV–HS interactions [40,45]. In general, the phenotypes associated with re-engineered AAV capsids that display decreased affinity to glycans can be sub-divided into predominantly three categories: (i) transduction-deficient; (ii) liver-detargeted and/or (iii) displaying altered tropism for other tissues such as heart or the brain.

Engineering orthogonal AAV glycan interactions

The primate-origin strains, AAV1 and AAV6 are closely related and differ only by six amino acids in the capsid protein VP subunits. Although both strains utilize SIA as a cell surface attachment factor, AAV6 also binds HS by virtue of a basic cluster of residues. As indicated earlier, a lysine residue at position 531 is key toward mediating this interaction [45]. Although a K531E mutation in the AAV6 capsid ablated heparin binding, the inverse E531K mutation in AAV1 imparted HS binding ability. Moreover, incorporation of an E533K mutation to the AAV8 capsid conferred HS binding to AAV8 [45]. These studies corroborate the notion that orthogonal glycan receptor footprints can be engineered onto AAV capsid templates, with the goal of achieving expanded tropism or improved transduction.

One example in this regard pertains to engraftment of the AAV9 GAL footprint onto other capsid templates. For instance, key residues involved directly in GAL recognition or flanking the footprint were grafted onto the AAV2 capsid to yield a novel, dual glycan capsid, identified as AAV2g9 [54]. This new chimeric strain utilizes both HS and GAL as cell surface receptors and displays robust transduction in different tissues. Recent studies indicate that AAV2g9 can afford robust and widespread CNS transduction with minimal systemic leakage (unpublished data). Further, the GAL footprint was also found to enhance the transduction efficiency of the liver-detargeted AAV2i8, a first generation AAV chimeric described earlier. Lastly, the GAL footprint has since been incorporated into different AAV strains including AAV1, 3B, 6 and 8 supporting the plasticity of different AAV capsid structures.

More recently, structural studies identified two SIA binding sites on the AAV5 capsid, both of which are essential for transduction [49]. However, only one of the sites appears to foster SIA dependent transduction. The availability of two orthogonal sites presents a unique opportunity to re-engineer either of these footprints to alter tropism. Receptor footprint engraftment has also facilitated interrogation of glycan usage in transduction of the retina via intravitreal injection [58*]. Intravitreal injection of gain and loss of HS mutants demonstrated that HS is essential for AAV2 intravitreal administration, yet does not act as the sole determinant of transduction via this route [58*]. Together, these studies support the notion that orthogonal glycan footprints recognizing GAL, SIA, HS or other carbohydrates can be engineered onto different AAV templates to potentially yield new and improved vectors for gene therapy applications.

In summary, knowledge of the structural biology of AAV capsids and AAV capsid–glycan receptor interactions has provided a path toward developing new and chimeric AAV strains. Protein engineering strategies that are either rational/site-directed and/or combinatorial in nature can be exploited to re-engineer the different receptor footprints associated with various AAV isolates. Overall, host factors essential for AAV infection continue to be identified [33**] and our mechanistic understanding of how AAV–receptor interactions dictate cell-specific, tissue-specific and species-specific tropism continues to evolve. Exploiting the knowledge provided by such advances could help develop engineering strategies that can afford greater control over AAV tropism, appropriate matchmaking of AAV technology with specific indications and the development of personalized approaches to human gene therapy.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Atchison RW, Casto BC, Hammon M: Adenovirus-associated defective virus particles. *Science* 1965, 49:754–756.
2. Carter BJ: Adeno-associated virus and the development of adeno-associated virus vectors: a historical perspective. *Mol Ther* 2004, 10:981–989. [PubMed: 15564130]
3. Alazard-Dany N, Nicolas A, Ploquin A, Strasser R, Greco A, Epstein AL, Fraefel C, Salvetti A: Definition of herpes simplex virus type 1 helper activities for adeno-associated virus early replication events. *PLoS Pathog* 2009, 5:e1000340. [PubMed: 19282980]
4. Geoffroy MC, Salvetti A: Helper functions required for wild type and recombinant adeno-associated virus growth. *Curr Gene Ther* 2005, 5:265–271. [PubMed: 15975004]
5. Agbandje-McKenna M, Kleinschmidt J: AAV capsid structure and cell interactions In *Adeno-associated Virus: Methods and Protocols*. Edited by Snyder R, Moullier P. Humana Press; 2011:47–92
6. Wu Z, Asokan A, Samulski RJ: Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol Ther* 2006, 14:316–327. [PubMed: 16824801]
7. Van Vliet K, Blouin V, Brument N, Agbandje-McKenna M, Snyder R: The role of the adeno-associated virus capsid in gene transfer In *Methods in Molecular Biology*, Vol. 437: Drug Delivery Systems. Edited by Jain K Humana Press; 2008:51–90. [PubMed: 18369962]
8. Salganik M, Hirsch ML, Samulski RJ: Adeno-associated virus as a mammalian DNA vector. *Microbiol Spectr* 2015, 3.
9. Grimm D, Zolotukhin S: E Pluribus Unum: 50 years of research, millions of viruses, and one goal – tailored acceleration of AAV evolution. *Mol Ther* 2015, 23:1819–1831. [PubMed: 26388463]
This paper provides an excellent review of combinatorial approaches to AAV capsid engineering. The approaches outlined in this review do not rely on pre-existing structural information, but instead utilize directed evolution.
10. Hastie E, Samulski RJ: Adeno-associated virus at 50: a golden anniversary of discovery, research, and gene therapy success – a personal perspective. *Hum Gene Ther* 2015, 26:257–265. [PubMed: 25807962]
11. Mori S, Wang L, Takeuchi T, Kanda T: Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein. *Virology* 2004, 330:375–383. [PubMed: 15567432]
12. Schmidt M, Voutetakis A, Afione S, Zheng C, Mandikian D, Chiorini JA: Adeno-associated virus type 12 (AAV12): a novel AAV serotype with sialic acid-independent and heparan sulfate proteoglycan-independent transduction activity. *J Virol* 2008, 82:1399–1406. [PubMed: 18045941]
13. Bossis I, Chiorini JA: Cloning of an avian adeno-associated virus (AAAV) and generation of recombinant AAAV particles. *J Virol* 2003, 77:6799–6810. [PubMed: 12768000]
14. Bello A, Tran K, Chand A, Doria M, Allocca M, Hildinger M, Beniac D, Kranendonk C, Auricchio A, Kobinger GP: Isolation and evaluation of novel adeno-associated virus sequences from porcine tissues. *Gene Ther* 2009, 16:1320–1328 30. [PubMed: 19626054]
15. Ströh LJ, Stehle T: Glycan engagement by viruses: receptor switches and specificity. *Annu Rev Virol* 2014, 1:285–306. [PubMed: 26958723]
16. Wu Z, Miller E, Agbandje-mckenna M, Samulski RJ: α 2,3 and α 2,6 N-linked sialic acids facilitate efficient binding and transduction by adeno-associated virus types 1 and 6. *J Virol* 2006, 80:9093–9103. [PubMed: 16940521]
17. Shen S, Bryant KD, Brown SM, Randell SH, Asokan A: Terminal N-linked galactose is the primary receptor for adeno-associated virus 9. *J Biol Chem* 2011, 286:13532–13540. [PubMed: 21330365]
18. Kaludov N, Brown KE, Walters RW, Zabner J, Chiorini JA: Adeno-associated virus serotype 4 (AAV4) and AAV5 both require sialic acid binding for hemagglutination and efficient transduction but differ in sialic acid linkage specificity. *J Virol* 2001, 75:6884–6893. [PubMed: 11435568]
19. Walters RW, Min S, Yi P, Keshavjee S, Brown KE, Welsh MJ, Chiorini JA, Zabner J: Binding of adeno-associated virus type 5 to 2,3-linked sialic acid is required for gene transfer. *J Biol Chem* 2001, 276:20610–20616. [PubMed: 11262413]

20. Ng R, Govindasamy L, Gurda BL, McKenna R, Kozyreva OG, Samulski RJ, Parent KN, Baker TS, Agbandje-Mckenna M: Structural characterization of the dual glycan binding adeno-associated virus serotype 6. *J Virol* 2010, 84:12945–12957. [PubMed: 20861247]
21. Quinn K, Quirion MR, Lo C, Mispilon JA, Epstein SL, Chiorini JA: Intranasal administration of adeno-associated virus type 12 (AAV12) leads to transduction of the nasal epithelia and can initiate transgene-specific immune response. *Mol Ther* 2009, 19:1990–1998.
22. Kotterman MA, Schaffer DV: Engineering adeno-associated viruses for clinical gene therapy. *Nat Rev Genet* 2014, 15:445–451. [PubMed: 24840552]
- 23•. Adachi K, Enoki T, Kawano Y, Veraz M, Nakai H: Drawing a high-resolution functional map of adeno-associated virus capsid by massively parallel sequencing. *Nat Commun* 2014, 5:1–14. A new method termed AAV Barcode-Seq is utilized to interrogate libraries of mutant AAVs to determine amino acids key in structure and function. This paper demonstrates a comprehensive approach outlining methods to establish structure–function correlates of AAV capsids at the single amino acid level.
24. Mays LE, Wilson JM: The complex and evolving story of T cell activation to AAV vector-encoded transgene products. *Mol Ther* 2009, 19:16–27.
25. Ling C, Lu Y, Kalsi JK, Jayandharan GR, Li B, Ma W, Cheng B, Gee SW, McGoogan KE, Govindasamy L et al.: Human hepatocyte growth factor receptor is a cellular coreceptor for adeno-associated virus serotype 3. *Hum Gene Ther* 2010, 21:1741–1747. [PubMed: 20545554]
26. Kashiwakura Y, Tamayose K, Iwabuchi K, Hirai Y, Shimada T, Matsumoto K, Nakamura T, Watanabe M, Oshimi K, Daida H: Hepatocyte growth factor receptor is a coreceptor for adeno-associated virus type 2 infection. *J Virol* 2005, 79:609–614. [PubMed: 15596854]
27. Weller ML, Amornphimoltham P, Schmidt M, Wilson PA, Gutkind JS, Chiorini J: A. Epidermal growth factor receptor is a co-receptor for adeno-associated virus serotype 6. *Nat Med* 2010, 16:662–664. [PubMed: 20473307]
28. Di Pasquale G, Davidson BL, Stein CS, Martins I, Scudiero D, Monks A, Chiorini JA: Identification of PDGFR as a receptor for AAV-5 transduction. *Nat Med* 2003, 9:1306–1312. [PubMed: 14502277]
29. Summerford C, Bartlett JS, Samulski RJ: AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. *Nat Med* 1999, 5:78–82. [PubMed: 9883843]
30. Asokan A, Hamra JB, Govindasamy L, Agbandje-McKenna M, Samulski RJ: Adeno-associated virus type 2 contains an integrin alpha5beta1 binding domain essential for viral cell entry. *J Virol* 2006, 80:8961–8969. [PubMed: 16940508]
31. Kaminsky PM, Keiser NW, Yan Z, Lei-Butters DC, Engelhardt JF: Directing integrin-linked endocytosis of recombinant AAV enhances productive FAK-dependent transduction. *Mol Ther* 2012, 20:972–983. [PubMed: 22233580]
32. Shen S, Berry GE, Castellanos Rivera RM, Cheung RY, Troupes AN, Brown SM, Kafri T, Asokan A: Functional analysis of the putative integrin recognition motif on adeno-associated virus 9. *J Biol Chem* 2015, 290:1496–1504. [PubMed: 25404742]
- 33•. Pillay S, Meyer NL, Puschnik AS, Davulcu O, Diep J, Ishikawa Y, Jae LT, Wosen JE, Nagamine CM, Chapman MS, Carette JE: An essential receptor for adeno-associated virus infection. *Nature* 2016, 530:108–112. [PubMed: 26814968] This recent study reports the discovery of a universal receptor essential for AAV host cell entry. Resolution of the interplay between glycan receptors and AAVR in mediating AAV infection is likely to expand our understanding of the biology of AAV vectors.
- 34•. Huang L, Halder S, Agbandje-mckenna M: Parvovirus glycan interactions. *Curr Opin Virol* 2014, 7:108–118. [PubMed: 25047752] This paper provides a comprehensive review on parvovirus glycan interactions, outlining interactions between parvoviruses and their host glycan receptors at the structural level. AAV serotype-specific distinctions in glycan usage are also delineated.
35. Diprimio N, Asokan A, Govindasamy L, Agbandje-mckenna M, Samulski RJ: Surface loop dynamics in adeno-associated virus capsid assembly. *J Virol* 2008, 82:5178–5189. [PubMed: 18367523]

36. Opie SR, Warrington KH, Agbandje-McKenna M, Zolotukhin S, Muzyczka N: Identification of amino acid residues in the capsid proteins of adeno-associated virus type 2 that contribute to heparan sulfate proteoglycan binding. *J Virol* 2003, 77:6995–7006. [PubMed: 12768018]
37. Kern A, Schmidt K, Leder C, Müller OJ, Wobus CE, Bettinger K, Von der Lieth CW, King JA, Kleinschmidt JA: Identification of a heparin-binding motif on adeno-associated virus type 2 capsids. *J Virol* 2003, 77:11072–11081. [PubMed: 14512555]
38. Xie Q, Bu W, Bhatia S, Hare J, Somasundaram T, Azzi A, Chapman MS: The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc Natl Acad Sci* 2002, 99:10405–10410. [PubMed: 12136130]
39. Levy HC, Bowman VD, Govindasamy L, McKenna R, Nash K, Warrington K, Chen W, Muzyczka N, Yan X, Baker TS, Agbandje-McKenna M: Heparin binding induces conformational changes in Adeno-associated virus serotype 2. *J Struct Biol* 2009, 165:146–156. [PubMed: 19121398]
40. Lerch TF, Chapman MS: Identification of the heparin binding site on adeno-associated virus serotype 3b (AAV-3B). *Virology* 2012, 423:6–13. [PubMed: 22169623]
41. Schmidt M, Govindasamy L, Afione S, Kaludov N, Agbandje-mckenna M, Chiorini JA: Molecular characterization of the heparin-dependent transduction domain on the capsid of a novel adeno-associated virus isolate, AAV (VR-942). *J Virol* 2008, 82:8911–8916. [PubMed: 18524816]
42. Xie Q, Lerch TF, Meyer NL, Chapman MS: Structure function analysis of receptor-binding in adeno-associated virus serotype 6 (AAV-6). *J Virol* 2011, 420:10–19.
43. Donnell JO, Taylor KA, Chapman MS: Adeno-associated virus-2 and its primary cellular receptor-Cryo-EM structure of a heparin complex. *Virology* 2009, 385:434–443. [PubMed: 19144372]
44. Zhang F, Aguilera J, Beaudet JM, Xie Q, Lerch TF, Davulcu O, Colon W, Chapman MSRL: Characterization of interactions between heparin/glycosaminoglycan and adeno-associated virus. *Biochemistry* 2013, 52:6275–6285. [PubMed: 23952613]
45. Wu Z, Asokan A, Grieger JC, Govindasamy L, Agbandje-mckenna M, Samulski RJ: Single amino acid changes can influence titer, heparin binding, and tissue tropism in different adeno-associated virus serotypes. *J Virol* 2006, 80:11393–11397. [PubMed: 16943302]
46. Lerch TF, Xie Q, Chapman MS: The structure of adeno-associated virus serotype 3B (AAV-3B): insights into receptor binding and immune evasion. *Virology* 2010, 403:26–36. [PubMed: 20444480]
47. Shen S, Troupes AN, Pulicherla N, Aravind A: Multiple roles for sialylated glycans in determining the cardiopulmonary tropism of adeno-associated virus 4. *J Virol* 2013, 87:13206–13213. [PubMed: 24067974]
48. Govindasamy L, Dimattia MA, Gurda BL, Halder S, McKenna R, Chiorini JA: Structural insights into adeno-associated virus serotype 5. *J Virol* 2013, 87:11187–11199. [PubMed: 23926356]
- 49•. Afione S, Dimattia MA, Halder S, Pasquale GDi, Agbandje-mckenna M, Chiorini JA: Identification and mutagenesis of the adeno-associated virus 5 sialic acid binding region. *J Virol* 2015, 89:1660–1672. [PubMed: 25410855] Combined structural and computational analysis lead to elucidation of glycan binding footprints on the AAV5 capsid, followed by validation of their function in transduction.
50. Dimattia MA, Nam H, Vliet KVan, Mitchell M, Bennett A, Gurda BL, McKenna R, Olson NH, Sinkovits RS, Potter M et al.: Structural insight into the unique properties of adeno-associated virus serotype 9. *J Virol* 2012, 86:6947–6958. [PubMed: 22496238]
51. Bell CL, Gurda BL, Vliet KVan, Agbandje-mckenna M, Wilson JM: Identification of the galactose binding domain of the adeno-associated virus serotype 9 capsid. *J Virol* 2012, 86:7326–7333. [PubMed: 22514350]
52. Pulicherla N, Shen S, Yadav S, Debbink K, Govindasamy L, Agbandje-mckenna M, Asokan A: Engineering liver-detargeted AAV9 vectors for cardiac and musculoskeletal gene transfer. *Mol Ther* 2009, 19:1070–1078.
53. Shen S, Bryant KD, Sun J, Brown SM, Troupes A, Pulicherla N, Asokan A: Glycan binding avidity determines the systemic fate of adeno-associated virus type 9. *J Virol* 2012, 86:10408–10417. [PubMed: 22787229]
- 54•. Shen S, Horowitz ED, Troupes AN, Brown SM, Pulicherla N, Samulski RJ, Agbandje-McKenna M, Asokan A: Engraftment of a galactose receptor footprint onto adeno-associated viral capsids

- improves transduction efficiency. *J Biol Chem* 2013, 288:28814–28823. [PubMed: 23940044]
- Novel engineering strategy demonstrates the modularity of glycan footprints on AAV capsids and the ability to generate novel AAV strains with orthogonal receptor usage and function.
55. Asokan A, Conway JC, Phillips JL, Li C, Hegge J, Sinnott R, Yadav S, Diprimio N, Nam H, Agbandje-mckenna M et al.: Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. *Nat Biotechnol* 2009, 28:79–82. [PubMed: 20037580]
56. Rotundo IL, Lancioni A, Savarese M, Orsi LD, Iacomino M, Nigro G, Piluso G, Auricchio A, Nigro V: Use of a lower dosage liver-detargeted AAV vector to prevent hamster muscular dystrophy. *Hum Gene Ther* 2013, 430:424–430.
57. Murlidharan G, Corriher T, Ghashghaei HT, Asokan A: Unique glycan signatures regulate adeno-associated virus tropism in the developing brain. *J Virol* 2015, 89:3976–3987. [PubMed: 25631075]
58. Boye SL, Bennett A, Scalabrino ML, McCullough KT, Vliet KVan, Choudhury S, Ruan Q, Peterson J, Agbandje-mckenna M, Boye SE: Impact of heparan sulfate binding on transduction of retina by recombinant adeno-associated virus vectors. *J Virol* 2016, 90:4215–4231. [PubMed: 26865709] This recent study uses various heparan sulfate mutants of AAV to question the role of HS binding in retinal transduction via intravitreal injection. These experiments demonstrate the utility of understanding glycan usage in determining the biology of distinct transduction routes.
59. Xiao C, Rossmann MG: Interpretation of electron density with stereographic roadmap projections. *J Struct Biol* 2007, 158:182–187. [PubMed: 17116403]

Figure 1. Structural analysis of adeno-associated virus serotype 2 (AAV2) residues targeted for modification. **(a)** Cartoon representation of the AAV2 VP3 subunit monomer obtained using SWISS-MODEL, with regions subject to receptor footprint modifications highlighted in blue. **(b)** Surface rendering of an AAV2 capsid model, with 60 VP3 subunits generated using $T=1$ icosahedral symmetry coordinates with VIPERdb. Regions subject to receptor footprint modifications are shown in blue. **(c)** Cartoon of AAV2 VP3 subunit trimer created on VIPERdb, with modified regions highlighted in blue. **(d)** Side view of AAV2 capsid trimer, with surface modified regions highlighted in blue.

Figure 2.

Stereographic Roadmap projections [59] of glycan receptor footprints as viewed down the threefold symmetry axis (as shown in the cartoon inset) on different naturally occurring AAV serotype capsids. Only surface exposed amino acids are shown, with each residue boundary depicted in black. The green regions depict the spikes on the threefold surface, while the blue regions represent the surrounding depressions. Residues colored in red show the different glycan footprints for (a) AAV2-HS; (b) AAV4-MUC; (c) AAV5-SIA; and (d) AAV9-GAL.

Figure 3.

Stereographic Roadmap projections [59] of engineered glycan receptor footprints as viewed down the threefold symmetry axis (as shown in the cartoon inset) on different lab-derived, synthetic AAV strains. Only surface exposed amino acids are shown, with each residue boundary depicted in black. The green regions depict the spikes on the threefold surface, while the blue regions represent the surrounding depressions. **(a)** Residues colored in orange depict engineered residues on AAV2i8; **(b)** yellow residues depict the HS footprint and pink residues depict the GAL footprint engineered on the dual glycan binding AAV2g9 strain; **(c)** pink residues depict the GAL footprint engineered onto AAV2i8 to generate AAV2i8g9; and **(d)** pink residues depict GAL footprint engineered on AAV8g9.