The sweet spot: defining virus–sialic acid interactions

Jennifer E. Stencel-Baerenwald[1,2], Kerstin Reiss[3,4]*, Dirk M. Reiter[3,5], Thilo Stehle[3,6] and Terence S. Dermody[1,2,6]*

Abstract | Viral infections are initiated by attachment of the virus to host cell surface receptors, including sialic acid-containing glycans. It is now possible to rapidly identify specific glycan receptors using glycan array screening, to define atomic-level structures of virus–glycan complexes and to alter the glycan-binding site to determine the function of glycan engagement in viral disease. This Review highlights general principles of virus–glycan interactions and provides specific examples of sialic acid binding by viruses with stalk-like attachment proteins, including influenza virus, reovirus, adenovirus and rotavirus. Understanding virus–glycan interactions is essential to combating viral infections and designing improved viral vectors for therapeutic applications.

[1]*Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine.*
[2]*Elizabeth B. Lamb Center for Pediatric Research, Vanderbilt University School of Medicine.*
[3]*Interfaculty Institute of Biochemistry, University of Tübingen, 72076 Tübingen, Germany.*
[4]*Present address: Institute of Complex Systems (ICS-6), Forschungszentrum Jülich, 52425 Jülich, Germany.*
[5]*Present address: Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK.*
[6]*Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.*
**These authors contributed equally to this work.*
Correspondence to T.S., T.S.D.
e-mails: Thilo.stehle@uni-tuebingen.de; Terry.dermody@Vanderbilt.edu
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Viral attachment to receptors that are expressed on host cells initiates infection and therefore, viral receptors are determinants of host range and govern host cell susceptibility. Various cell surface carbohydrates, including sialylated glycans[1–6], glycosaminoglycans[7–10] and human blood group antigens (HBGAs)[11,12], function as host cell receptors for viral attachment and entry.

Although viruses have been known for some time to use cell surface carbohydrates to bind to host cells, recent advances in glycan array (also known as glycan microarray) screening technology have accelerated the identification of glycan receptors. Together with new structural information about how viruses bind to glycans, the interactions between viruses and glycans can now be analysed in unprecedented detail.

In this Review, we highlight the molecular and structural determinants of virus–sialylated glycan interactions and the influence of glycan binding on viral tropism, with an emphasis on well-studied examples, including influenza virus, reovirus, adenovirus and rotavirus (TABLE 1). Although this group of sialic acid-binding viruses is not exhaustive[4,13,14], all four have stalk-like attachment proteins, which enables more direct comparisons of virus–glycan interactions to be made. Specifically, we examine how glycan array studies and structure determination, coupled with *in vivo* experiments to establish the function of sialic acid binding in pathogenesis, have provided insights into the remarkable complexity of virus–sialic acid relationships. In addition, we discuss how information that has been gained from studies of these viruses has yielded general principles of virus–glycan interactions that may aid in the design of antiviral drugs and viral vectors.

Virus–sialic acid interactions

Sialic acids are derivatives of neuraminic acid, which is a nine-carbon monosaccharide that is ubiquitously expressed in higher vertebrates[15]. The C5 carbon is frequently modified with an *N*-acetyl group to form *N*-acetylneuraminic acid (Neu5Ac), which can be further hydroxylated to form *N*-glycolylneuraminic acid (Neu5Gc)[15] (FIG. 1a). Additional modifications of neuraminic acid involve acetylation, methylation and sulphation of its hydroxyl groups. Sialic acids are often α-linked from the C2 carbon to carbohydrate chains on glycoproteins and glycolipids (FIG. 1b). In the host, sialic acids function in cell–cell adhesion, in cell signalling (especially within the immune system) and in development[16,17]. In addition, they are known to be key components of receptors for many viruses and bacterial toxins[18–21]. Virus interactions with sialylated glycans are usually of low affinity and are strengthened by the multivalency of the virus[1].

Studying virus–sialic acid interactions

To gain a comprehensive understanding of virus–sialylated glycan interactions, it is crucial to identify the precise glycan receptor, define the molecular and structural basis of the interaction and establish the contribution of binding to sialylated glycan receptors in disease.

Table 1 | **Sialic acid-binding viruses***

Virus (family)	Morphology	Genome	Sialic acid-binding protein	Functional role	Sialylated glycans bound
Influenza virus (*Orthomyxoviridae*)	Spherical or filamentous; enveloped	ssRNA	Haemagglutinin	Attachment, membrane fusion	α2,3- and α2,6-linked sialylated glycans[5,20,27,52–55,59–60]
			Neuraminidase	Receptor destruction, release of progeny	
Reovirus (*Reoviridae*)	Icosahedral; non-eneveloped	dsRNA	σ1	Attachment	T1 serotype: GM2 (REF. 19) T3 serotype: α2,3-, α2,6- and α2,8-linked sialylated glycans[66]
Adenovirus (*Adenoviridae*)	Icosahedral; non-eneveloped	dsDNA	Fibre	Attachment	Ad37: GD1a[21]
Rotavirus (*Reoviridae*)	Icosahedral; non-eneveloped	dsRNA	VP4	Attachment, membrane penetration	RRV: GM3 (REF. 99) Wa: GM1 (REF. 100) HAL1166: A-type HBGA[11]

*Listed are the viruses that are discussed in this Review (see REFS 4,13,14 for other examples). Ad37, adenovirus 37; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; HBGA, human blood group antigen; RRV, rhesus rotavirus; ssRNA, single-stranded RNA.

Identification of glycan receptors. The interaction between a virus and sialylated glycan is often first investigated using cell-based infectivity assays in which attachment is blocked by sialic acid-binding lectins or enzymatic removal of sialic acids by neuraminidases. However, neuraminidase does not efficiently remove sialic acid from branched gangliosides such as GM1 (REF. 22). In addition, the specificity of neuraminidase is limited to the type of sialic acid linkage. Conversely, glycan arrays can be used to discern finer differences in virus–glycan binding preferences by enabling rapid, high-throughput screening of several glycans as potential virus receptors[23–26]. This technology has been used to identify glycan ligands of adenovirus[21], influenza virus[27,28], polyomavirus[14], reovirus[19] and rotavirus[11,29], among other viruses.

Glycan array screening is analogous to the more familiar microarrays that are used to study gene expression. Glycans are immobilized on an array and then incubated with whole virus or the viral attachment protein to identify specific glycan receptors for viruses[19,21,30] and compare glycan-binding preferences of different virus strains[28,31]. Binding is usually quantified using fluorescence-based detection systems. Different glycan arrays vary in glycan composition[32] and the mode of glycan immobilization; for example, covalent binding of amine-terminating glycans to *N*-hydroxysuccinimide (NHS)-activated glass slides[23] or glycan linkage to lipids that are printed on nitrocellulose-coated glass slides (known as neoglycolipid (NGL)-based arrays[25,33,34]). Although arrays from the different platforms vary in the composition of the glycans on the array, as well as the glycan-coupling method, both types of arrays have been useful in identifying glycan receptors for viruses. Different glycan array platforms have previously been compared in-depth[32,35,36].

Structural studies of virus–glycan interactions. Structural studies of virus–glycan interactions enable the identification of regions of the viral attachment protein and glycan that contribute to binding and facilitate the engineering of mutant viruses that can be used to investigate the physiological consequences of glycan engagement[37–40]. Although X-ray crystallography is not a new technique, advances in nearly every step of the crystallographic process have accelerated structural determination[41]. Protein purification techniques have also improved, and the use of robots in crystal screening reduces the amount of protein required[42]. Complementary methods, such as nuclear magnetic resonance (NMR) spectroscopy, are well suited for mapping protein–glycan interactions in solution[13,19,43]. Taken together, glycan array, crystallography and functional studies provide a more complete understanding of virus–sialylated glycan engagement.

Influenza virus

Influenza virus is a segmented, single-stranded RNA (ssRNA) virus in the *Orthomyxoviridae* family that infects mammals and birds; infections with influenza virus are common in humans. The trimeric viral haemagglutinin protein binds to sialic acid, commonly Neu5Ac, to adhere to host cells. Influenza viruses engage α2,3-linked and α2,6-linked sialic acid attached to a penultimate galactose of the glycan receptor. Avian influenza viruses primarily bind to α2,3-linked sialic acid, whereas human influenza viruses preferentially bind to α2,6-linked sialic acid[5,20]. The virus-encoded neuraminidase protein catalyses removal of Neu5Ac from the cell surface and viral glycoproteins to release newly formed virions.

Binding of influenza virus haemagglutinin to sialic acid. Influenza virus haemagglutinin is anchored in the viral envelope and projects away from the viral surface. The haemaglutinin trimer is composed of the globular HA1 domain, which engages sialic acid, and the stalk-like HA2 domain, which facilitates membrane fusion (FIG. 2a). The carbohydrate-binding site is conserved in all influenza subtypes and is located in a shallow groove in the HA1 domain[44]. The orientation of Neu5Ac and its interactions with HA1 are also mostly conserved among influenza virus strains[44–47]. In influenza virus haemagglutinin–sialic acid interactions, the Neu5Ac

Glycans
A nonspecific term for a polysaccharide or polymeric carbohydrate.

α-linked
A term used to describe a Neu5Ac that is incorporated into a polysaccharide via a glycosidic bond in which the alpha anomer, or C1 carbon, of Neu5Ac is in the axial position on the opposite side of the plane of the C6 carbon.

Lectins
Proteins, usually of plant origin, that bind to carbohydrates on the surface of animal cells; they also agglutinate red blood cells.

Neuraminidases
Enzymes, usually of microbial origin, that catalyse the removal of terminal sialic acids on the surface of cells or microorganisms.

Gangliosides
A type of glycolipid, commonly composed of a ceramide tail and a glycan portion that contains at least one sialic acid moiety.

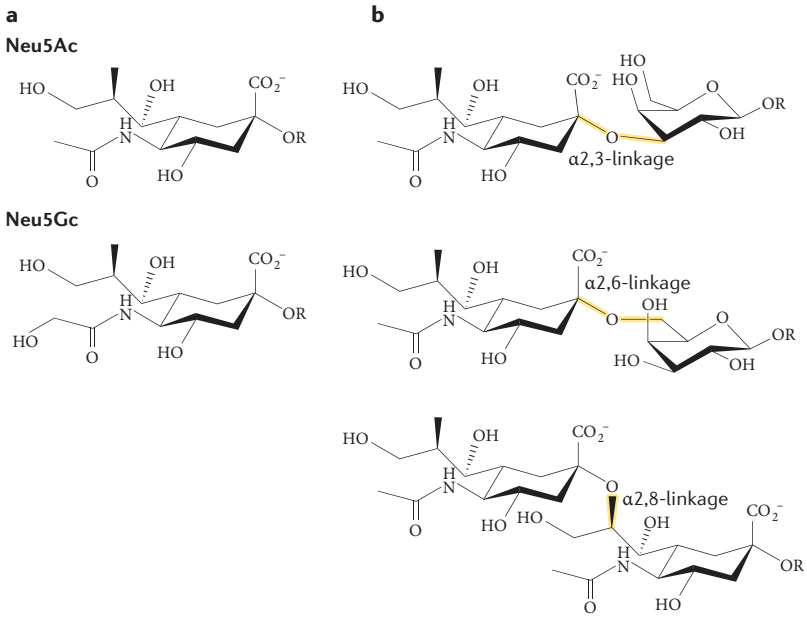

Figure 1 | **Sialic acid types and glycosidic linkage.** **a** | Sialic acids are nine-carbon monosaccharide derivatives of neuraminic acid. The two most common sialic acids are *N*-acetyl neuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). The C5 carbon in Neu5Ac is modified with an *N*-acetyl group, which can be further hydroxylated to form Neu5Gc. The hydroxyl groups at C4, C7, C8 and C9 are subject to various modifications (not shown). Common constituents include *O*-acetyl, *O*-sulphate, *O*-lactyl, *O*-methyl and *O*-phosphate groups. **b** | Sialic acids are attached to carbohydrate chains on glycoproteins and glycolipids via different glycosidic linkages. The most common linkage types are α2,3-linkage to a galactose residue, α2,6-linkage to a galactose moiety or to an *N*-acetylgalactosamine moiety, and α2,8-linkage to another sialic acid moiety on a glycan.

carboxylate inserts deeply into the carbohydrate-binding site of HA1 (FIG. 2b,c), where it forms two hydrogen bonds with adjacent residues, and the glycerol and *N*-acetyl chains form hydrogen bonds with additional residues in viral haemagglutinin. Moreover, the methyl group of the *N*-acetyl chain is inserted into a hydrophobic pocket in the virus-binding site (FIG. 2d), which is a common feature that is observed in virus–glycan interactions. Rotation around the glycosidic bond enables the galactose molecule to adopt either a *cis* or a *trans* position with respect to the *N*-acetyl group of Neu5Ac to accommodate different haemagglutinin molecules[48]. Avian influenza virus haemagglutinin molecules are commonly bound in a *trans* conformation, whereas human receptors are commonly found in a *cis* conformation. Structural analysis of avian H5 and H7 strains showed that a point mutation that changes the conformation from *trans* to *cis* leads to an increase in affinity for α2,6-linked sialic acid[49,50].

Determinants of influenza virus binding specificity. Although all influenza strains are thought to bind to sialic acids, the context of these monosaccharides in the recognized glycan structures varies. Glycans that contain α2,3-linked sialic acid have restricted conformational freedom and form a cone-like glycan structure. Conversely, glycans that contain α2,6-linked sialic acid have greater conformational flexibility[51], and such glycans form umbrella-like shapes (FIG. 2e). The linkage between sialic acid and galactose in the receptor molecules thus determines the affinity of HA1 for a given oligosaccharide by defining the topology of the glycan.

The 1918, 1957 and 1968 pandemic influenza viruses were not of human origin but acquired receptor-binding specificity for glycans that contain α2,6-linked sialic acid[27,52–55]. Glycan arrays have helped to define the binding preferences of pandemic strains and are thus aiding in understanding mechanisms of the host jump. A conserved region of haemagglutinin of the 1918 pandemic H1N1 strain A/South Carolina/1/1918, which differs from the consensus amino acid sequence of the avian virus by E190D and G225D mutations, preferentially binds to α2,6-linked sialyl-oligosaccharides. Conversely, pandemic strain A/New York/1/1918, which differs from the avian influenza virus consensus sequence by an E190D substitution, binds to both α2,3-linked and α2,6-linked sialyl-oligosaccharides. The presence of a glycine at position 225 in either the avian or human strain enables binding to α2,3-linked sialic acids, whereas an aspartic acid at that position does not enable binding to this receptor[28,56]. In addition, mutation of residue 190 in A/New York/1/1918 to the avian consensus sequence results in exclusive binding to α2,3-linked sialyl-oligosaccharides like the avian counterpart[28]. Thus, these two residues are determinants of influenza virus receptor-binding specificity. Interestingly, the presence of a glycine at position 225 in some H1N1 isolates from the 2009 pandemic is also associated with increased binding to α2,3-linked sialic acids[57]. Glycan arrays showed that the binding preferences of the 2009 H1N1 pandemic influenza virus more closely resemble the binding preferences of the swine influenza virus isolates rather than those of seasonal strains[27,58].

Despite this work, it is clear that the classification of influenza virus strains that are specific for either α2,3- or α2,6-linked sialic acids is too simplistic; for example, glycan array screening of seasonal H3N2 influenza virus strains did not identify a single moiety that all of the 45 strains tested bound[59], and the preference of different strains for certain ligands changed with time. A study that investigated the binding specificity of human H3N2 influenza viruses that were isolated from 1968 to 2012 showed that early isolates preferentially bind to short and branched sialylated glycans, whereas more recent strains bind with high avidity to sialic acids that are attached to long polylactosamine chains[59].

Although human influenza viruses bind to glycans that contain α2,6-linked sialic acid, the linkage type alone is not sufficient to explain strain-specific binding preferences. The affinity and avidity of haemagglutinin for α2,3- and α2,6-linked sialic acid, and not just the capacity to engage either or both ligands, influence influenza virus transmission[48,60]. Sialic acid modifications, including fucosylation and sulphation, also influence binding: influenza viruses that primarily bind to glycans that contain α2,3-linked sialic acids interact with greater avidity with glycans that contain a sulphate or sialic acid on position six of the penultimate *N*-acetylglucosamine

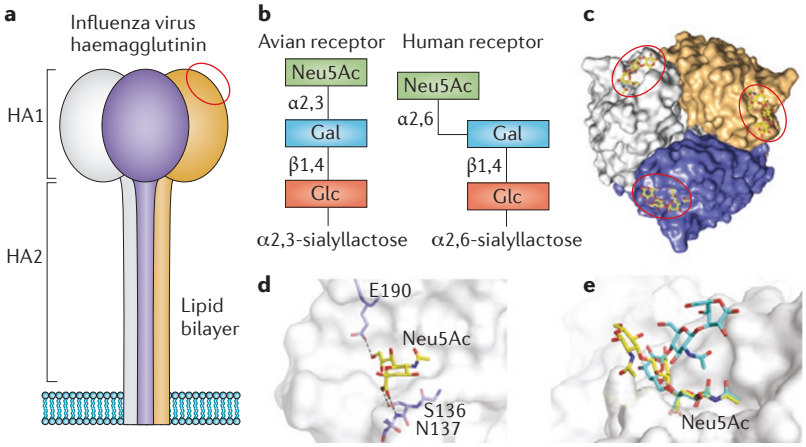

Figure 2 | **Influenza virus binding to differentially linked sialic acids. a** | Schematic of the trimeric influenza virus haemagglutinin, with the monomers depicted in purple, orange and grey. Haemagglutinin is a transmembrane protein that is composed of the globular HA1 domain and the stalk-like HA2 domain. Each HA1 domain in the trimer binds to sialic acid (commonly *N*-acetyl neuraminic acid (Neu5Ac)), and the binding site is indicated in one monomer with a red circle. **b** | Avian influenza viruses preferentially bind to host cell receptors that contain α2,3-linked sialic acid, and human-adapted viruses bind to receptors that contain α2,6-linked sialic acid moieties. Schematics of an example of an avian influenza virus receptor (α2,3-sialyllactose) and a human influenza virus receptor (α2,6-siallylactose) are shown. Glucose (Glc), galactose (Gal) and Neu5Ac are depicted as blocks. **c** | Surface representation of trimeric haemagglutinin (monomers are shown in purple, orange and grey) in complex with Neu5Ac (in yellow as a stick representation) (Protein Data Bank (PDB) accession 1HGG). Red circles indicate the glycan-binding site. **d** | Close-up view of the glycan-binding site of haemagglutinin. Selected crucial contacts between the haemagglutinin residues Ser136, Asn137 and Glu190 (purple) and Neu5Ac (yellow) are highlighted (grey dashes). The glycan receptors are shown in stick representation, with oxygen atoms in red and nitrogen atoms in blue. **e** | Superposition of an avian influenza virus haemagglutinin in complex with α2,3-sialyllactosamine (yellow) (PDB accession 2WR2) and the human receptor α2,6-sialyllactosamine (cyan) (PDB accession 2WR7). The avian receptor generally has a linear conformation, whereas the human receptor is more flexible and has an umbrella-like topology.

(GlcNAc) than with glycans that are fucosylated at this site[28,60]. These studies suggest that the attachment of influenza virus to sialic acid is determined by the linkage of the sialic acid as well as other factors, such as the length, branching and sialic acid modifications of the glycan.

Glycan binding influences viral transmission. As described above, the substitution of a few amino acid residues in haemagglutinin can alter the receptor specificity of influenza virus. In addition, mutation of a few residues in haemagglutinin and other viral proteins also influences influenza virus transmission. Ferrets are a useful animal model to study influenza virus infection as they mimic the tropism and pathogenesis observed in humans[61]. Mutations in haemagglutinin from the H5 subtype influence the spread of influenza virus between ferrets[50,62,63], as these mutations lead to a shift in the binding specificity from α2,3- to α2,6-linked sialic acid. This alteration in binding enables the virus to adhere to nasal turbinates, which are known to express α2,6-linked sialic acid[63]. In addition to mutations in the glycan-binding site that result in a shift in haemagglutinin-binding preference, mutations in viral proteins that regulate transcription and replication also contribute to the transmission phenotype[63]. Thus, although a shift in the binding specificity of haemagglutinin influences the transmission of influenza virus, it is not the only determinant. The role of glycans in influenza virus pathogenesis has recently been reviewed in depth[64].

Serotypes
A subclassification of a virus species that shares antigens and for which antibodies are cross-reactive.

Reovirus

Reoviruses are non-enveloped viruses that belong to the *Reoviridae* family. They contain ten segments of double-stranded RNA (dsRNA), which are encapsidated within two concentric protein shells. Nearly all mammals function as hosts for reovirus, but disease is restricted to the very young. Reovirus infections are common in humans and most are exposed by adulthood[65]. Attachment of reovirus to host cells is mediated by the outer-capsid protein σ1, which is a trimeric fibre that protrudes from the surface of the virion. The σ1 attachment protein has three structurally distinct domains — the head, the body and the tail (FIG. 3a) — and binds to both carbohydrate and protein receptors. Reovirus serotypes differentially bind to sialic acid[19,66] in an initial adhesive step[1] before serotype-independent engagement of junctional adhesion molecule A (JAM-A)[67–69], which is expressed at tight junctions that link polarized cells as well as on some leukocytes[70–73].

Reovirus–glycan interactions. Haemagglutination studies suggested that the T1 and T3 serotypes differentially bind glycans[2,74]. T1 reovirus agglutinates erythrocytes of human and non-human primates, whereas T3 reovirus agglutinates erythrocytes of various mammalian species. T3 reovirus, but not T1 reovirus, binds to glycophorin, which is a sialylated glycoprotein that is expressed on erythrocytes[75–77]. Glycan array screening provided new information on the specificity of different reovirus serotypes for distinct glycans. T1 reovirus σ1 specifically engages the GM2 glycan[19] (FIG. 3b,c), whereas T3 reovirus σ1 binds to a range of sialylated glycans[66] (FIG. 3d,e).

The structural basis for reovirus–glycan interactions. The σ1 proteins from T1 and T3 reovirus have been crystallized in complex with sialylated glycans[19,66] (FIG. 3b–e). Interestingly, the glycan-binding sites of T1 and T3 reovirus are located in different domains of σ1. The carboxy terminal head domain of T1 σ1 binds to the GM2 glycan[19], whereas the body domain is the glycan-binding region of T3 σ1 (REF. 66). The T1 and T3 σ1 head domains also bind to JAM-A[67], but the binding sites for GM2 and JAM-A in the T1 σ1 head domain are distinct, which suggests that T1 σ1 can interact with both receptors independently. The terminal Neu5Ac and *N*-acetylgalactosamine (GalNAc) moieties of the branched GM2 glycan contact the T1 σ1 head domain (FIG. 3c). The carboxyl group of Neu5Ac forms a hydrogen bond with the side chain of Gln371 in the attachment protein, whereas the Neu5Ac *N*-acetyl nitrogen and the glycerol chain form hydrogen bonds with residues in the σ1 backbone[19]. The finding that most of the interactions between T1 σ1 and Neu5Ac occur via

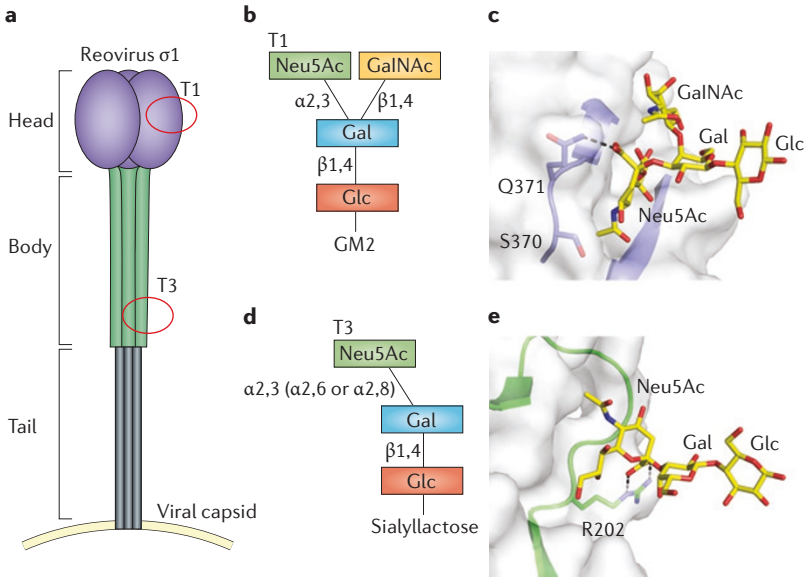

Figure 3 | **T1 and T3 reovirus σ1 proteins differentially bind to sialylated glycans.** **a** | Schematic showing the reovirus attachment protein σ1, which is a trimeric fibre that is composed of three structurally distinct domains: the head (purple), body (green) and tail (grey). The glycan-binding sites of serotypes 1 (T1) and 3 (T3) are located in different domains of σ1 (indicated with red circles). **b** | T1 reovirus binds to the GM2 glycan, which is composed of glucose (Glc) and galactose (Gal), with an α2,3-linked *N*-acetyl neuraminic acid (Neu5Ac), and β1,4-linked *N*-acetylgalactosamine (GalNAc). **c** | Close-up view of the T1 reovirus–GM2 interaction (Protein Data Bank (PDB) accession 4GU3). The protein surface is depicted in white, and the glycan-binding site is shown as a ribbon tracing in blue. Ser370 and Gln371 are crucial residues that are involved in GM2 binding and are shown in stick representation. The glycan receptor is shown in stick representation (yellow), with oxygen (red) and nitrogen atoms (blue). **d** | T3 reovirus binds to α2,3-, α2,6-, and α2,8-linked sialylated glycans. **e** | Close-up view of T3 reovirus σ1 in complex with α2,3-linked sialyllactose (PDB accession 3S6X). The protein surface is shown with the glycan-binding site depicted as a ribbon tracing in green. Arg202, which is required for the virus–sialic acid interaction, is shown in stick representation. Contacts between viral residues and sialic acid are depicted as grey dashes.

backbone elements and not via amino acid side chains is rare in virus–glycan interactions and was confirmed by structure-guided mutagenesis studies[19]. Of note, the methyl group of the Neu5Ac *N*-acetyl chain inserts into a hydrophobic pocket, which is similar to the interaction that is observed for influenza virus haemagglutinin. The GalNAc moiety of the GM2 glycan is located in a surface-exposed shallow pocket of σ1 and provides contact via van der Waals interactions, which increase the specificity of T1 σ1 for GM2.

Although the precise glycan ligands for T3 reovirus σ1 are not known, T3 σ1 can bind to α2,3-, α2,6-, and α2,8-linked Neu5Ac (FIG. 3d) using a loop that connects β-spirals 2 and 3 in the body domain. Neu5Ac is anchored in the σ1 binding site by a bidentate salt bridge that is formed between the Neu5Ac carboxylate and Arg202 of T3 σ1 (FIG. 3e). This salt bridge is required for the interaction, as replacement of Arg202 with alanine or tryptophan abolishes the sialic acid-binding capacity of T3 reovirus[66]. Additional hydrogen bonds between the hydroxyl, acetyl and glycerol groups of Neu5Ac and the backbone carbonyl groups of T3 σ1 strengthen the interaction. Similarly to both influenza virus haemagglutinin and T1 reovirus σ1, the Neu5Ac *N*-acetyl methyl group inserts into a partially hydrophobic pocket of T3 reovirus σ1 (REF. 66).

Glycan binding and reovirus tropism. Binding of the reovirus attachment protein to sialic acid is crucial for viral tropism and spread. Alteration of one or two residues in σ1 is sufficient to disrupt this interaction[3,19,66]. Binding of T3 reovirus to sialic acid promotes dissemination from the mouse intestines to sites of secondary replication, including the brain, heart and liver, and leads to infection of the bile duct epithelium, which results in biliary obstruction[78]. Moreover, sialic acid-binding T3 reoviruses replicate to higher titres in the mouse spinal cord and brain and are substantially more virulent than strains that do not bind to sialic acid[79]. Concordantly, sialic acid-binding T3 reoviruses infect primary cultures of cortical neurons more efficiently than strains that do not bind to sialic acid[79], and infectivity of sialic acid-binding strains is reduced following neuraminidase treatment[79,80]. It is not established whether the sialic acid-binding capacity of T1 reovirus influences its pathogenesis, but preliminary findings suggest that this might be the case (J.E.S.-B. and T.S.D., unpublished observations).

Reovirus displays serotype-dependent pathology in the central nervous system (CNS) of newborn mice. The viral gene that encodes the σ1 attachment protein determines these serotype-dependent differences in neural tropism[81–84], probably via the differential engagement of σ1 with cell surface receptors. Therefore, given that T1 and T3 reoviruses have distinct glycan-binding preferences, it is possible that differential glycan expression correlates with the serotype-dependent differences in the CNS tropism of reovirus. However, this model remains speculative and requires a comprehensive evaluation of the glycan expression profiles *in vivo*.

Adenovirus

Adenoviruses are non-enveloped double-stranded DNA (dsDNA) viruses in the *Adenoviridae* family that infect humans, other mammals and birds. Some adenovirus strains cause conjunctivitis or upper respiratory illness in humans, whereas others only rarely produce symptoms in immunocompetent individuals. Like reovirus, adenovirus serotypes differ in sialic acid binding; for example, although most adenoviruses use protein receptors[85], the species D adenovirus 37 (Ad37) agglutinates human erythrocytes[86] in a neuraminidase-sensitive manner[87,88], which indicates that this adenovirus binds to sialic acid.

Structural basis of adenovirus–glycan binding. Similarly to reovirus σ1, adenovirus binds to host cells using a filamentous trimeric fibre that extends from the viral capsid at the twelve icosahedral vertices (FIG. 4a). The C-terminal region of the fibre folds into a globular structure (known as the knob), which binds to protein or carbohydrate receptors in a species-specific manner[85]. Interactions with sialic acid are strengthened by the presence of multiple attachment molecules per virion.

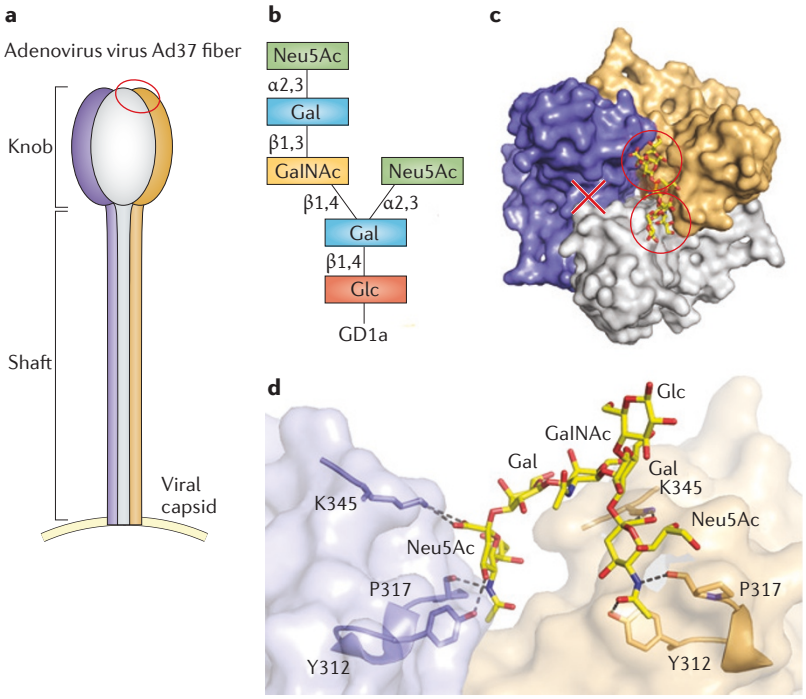

Figure 4 | **Interaction between adenovirus 37 and glycan.** **a** | Schematic representation of the trimeric adenovirus 37 (Ad37) fibre, which is the viral attachment protein that binds to the GD1a glycan. The monomers are depicted in purple, orange and grey. The glycan-binding site is located in the knob domain of two monomers (indicated by a red circle). **b** | Schematic showing the GD1a glycan, which is the Ad37 glycan receptor on host cells. GD1a is composed of glucose (Glc), galactose (Gal), a terminal α2,3-linked sialic acid and *N*-acetylgalactosamine (GalNAc), depicted as blocks. **c** | Surface representation of a top view of the Ad37 fibre knob in complex with the GD1a glycan (Protein Data Bank (PDB) accession 3N0I). The monomers are coloured as in part **a**, and the GD1a glycan is shown in stick representation (in yellow, with oxygen atoms in red and nitrogen atoms in blue). The two *N*-acetyl neuraminic acid (Neu5Ac)-binding sites that are occupied by the GD1a glycan are marked with red circles, and the third potential binding site (X) remains unoccupied. **d** | Surface representation of two knob monomers bound to the GD1a glycan, which is shown in stick representation (oxygen and nitrogen atoms as in part **c**). The interaction between the Ad37 fibre knob and sialic acid is mediated by several interactions (depicted as grey dashes), including a salt bridge between Lys345 and the Neu5Ac carboxylate, and hydrogen bonds between residues Tyr312 and Pro317 of the Ad37 knob and the *N*-acetyl chain of Neu5Ac of GD1a.

Glycan array screening assays showed that Ad37 binds specifically to the GD1a glycan, which is a branched hexasaccharide with two arms that terminate in α2,3-linked Neu5Ac[21] (FIG. 4b). An initial structural analysis of the trimeric Ad37 fibre knob identified three equivalent binding sites for Neu5Ac[89]. However, another crystal structure of the knob–GD1a complex shows a stoichiometry 'mismatch', in which two knob monomers engage the two terminal Neu5Ac groups of GD1a in an identical manner and the third sialic acid-binding site on the knob remains unoccupied[21] (FIG. 4c). Bivalent binding of GD1a increases the affinity of the interaction compared with the interaction of the fibre knob with monovalent sialyllactose alone[21]. The interaction between the Ad37 fibre knob and sialic acid involves a salt bridge between Lys345 and the Neu5Ac carboxylate. Hydrogen bonds between the Ad37 knob and additional Neu5Ac functional groups strengthen the interaction (FIG. 4d). A central salt bridge that anchors the Neu5Ac carboxylate group to the viral protein is required for binding[89], similarly to binding of T3 reovirus σ1 to glycan[66].

Glycan binding specificity and cell tropism. Sialic acid binding also influences the susceptibility of cells to infection by certain adenovirus types. Soluble GD1a diminishes attachment to human corneal epithelial cells of species D adenovirus serotypes Ad8, Ad19a, Ad19p and Ad37, but not species C adenovirus serotype Ad5 (REF. 21), which suggests that different types have specific binding preferences for cellular receptors. Furthermore, this finding suggests that compounds that mimic GD1a might function as antiviral agents[90]. Ad8, Ad19a and Ad37 cause epidemic keratoconjunctivitis, whereas Ad19p does not[21]; however, the binding of all strains is GD1a-dependent, and therefore factors other than GD1a might contribute to serotype-dependent tropism[21].

Rotavirus

Rotaviruses are non-enveloped dsRNA viruses that belong to the *Reoviridae* family. These viruses are a leading cause of childhood diarrhoea worldwide. Rotavirus attachment is dependent on glycans and is mediated by the trimeric outer-capsid protein VP4 (REF. 91). Rotavirus infectivity is increased following proteolytic cleavage of the VP4 trimer into amino-terminal VP8* and C-terminal VP5* subunits. The VP8* subunit mediates attachment of the virus by binding to cell surface glycans[92] (FIG. 5a), whereas the VP5* subunit facilitates membrane penetration[93].

Glycans that are bound by rotavirus. Many animal rotavirus strains, including rhesus rotavirus (RRV), bind to terminal sialic acid-containing receptors[94–99], such as GM3 (REF. 99). Some human rotaviruses, including strain Wa, bind to sialylated receptors in which the sialic acid is attached to one branch of biantennary glycans, such as ganglioside GM1 (REF. 100), but other human rotavirus strains, such as HAL1166, do not. The combination of glycan array screening and crystallographic analysis of VP8* from the human strain HAL1166 (P[14] VP4 genotype) showed that this virus specifically binds to A-type HBGAs[11] (FIG. 5b). HBGAs are oligosaccharides that are expressed on erythrocytes and epithelial cells and are also present in mucosal secretions. In addition, human P[11] rotavirus strains, which cause diarrhoea in neonates, bind to HBGA precursors[12,29].

Structural basis of glycan-binding specificity. Remarkably, rotaviruses bind to sialylated and non-sialylated glycans using the same site in VP8* (REF. 11). The crystal structure of RRV VP8* (P[3] VP4 genotype) in complex with sialic acid showed that VP8* assumes a galectin-like fold[94]. Galectins are glycan-binding proteins that usually bind ligands at a conserved binding site at the top of the galectin molecule. However, this site is blocked in VP8*, and the virus instead engages sialic acid via a different interface on the side of the spike-shaped VP8* protein[94] (FIG. 5c).

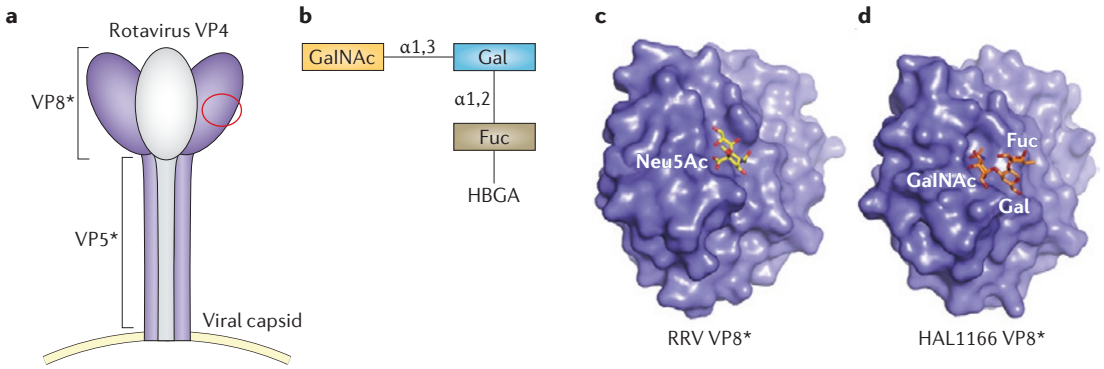

Figure 5 | **The VP8* domain of rotavirus VP4 differentially engages glycans.** **a** | The schematic depicts the rotavirus outer-capsid protein VP4, which is composed of VP5* and VP8*. The protein is a trimer, but only two of the three monomers are visible in some structures, and hence the third monomer is depicted in grey. The VP8* subunit binds to glycans (the binding site is indicated by the red circle), whereas the VP5* subunit facilitates membrane penetration. **b** | The glycan ligand for the human HAL1166 rotavirus, human blood group antigen (HBGA), comprises *N*-acetylgalactosamine (GalNAc), galactose (Gal) and fucose (Fuc). **c** | The crystal structure of rhesus rotavirus (RRV) VP8* in complex with *N*-acetyl neuraminic acid (Neu5Ac) (Protein Data Bank (PDB) accession 1KQR). The protein surface is shown in purple, and Neu5Ac is depicted in stick representation (yellow carbon with red oxygen atoms and blue nitrogen atoms). **d** | The crystal structure of human rotavirus strain HAL1166 VP8* (purple) in complex with HBGA (with orange carbons) (PDB accession 4DRV). The glycan receptors are shown as orange sticks with red oxygen atoms and blue nitrogen atoms. The HAL1166 VP8* binds to a completely different glycan at the same position at which RRV VP8* engages Neu5Ac.

The crystal structure of rotavirus strain HAL1166 VP8* in complex with A-type HBGA (FIG. 5d) shows subtle modifications in the binding site of VP8* that render it incapable of binding to sialic acid and instead enable binding to A-type HBGA. The change in specificity is due to the insertion of a single amino acid, Asn187, in the binding pocket, which reorients a neighbouring tyrosine, Tyr188, such that its side chain blocks binding to sialic acid via steric hindrance. At the same time, the reoriented tyrosine can form hydrophobic contacts with HBGA[11]. As the remaining residues in the binding site are mostly conserved among sialic acid-binding and non-sialic acid-binding rotaviruses, it is clear that a single amino acid substitution in the receptor-binding pocket has a substantial effect on glycan specificity. By contrast, minor amino acid changes in influenza virus lead to altered specificity for similar types of glycans, such as sialylated oligosaccharides with different linkages. Structurally, glycans such as GM1 and HBGAs have little in common and it is therefore remarkable that rotaviruses can switch between entirely different classes of glycans via such small changes in the VP8* receptor-binding pocket.

Glycan binding and cell tropism. Rotavirus pathogenesis varies between neonates and older children. Whereas a broad group of rotavirus strains cause disease in older children[29,101], neonatal infection is commonly asymptomatic, and only a few select strains, including the P[11] VP4 serotype, preferentially infect and cause diarrhoea[29] in neonates[101]. Glycan array screening showed that the P[11] serotype binds to glycan precursors of HBGAs[12,29]. These blood group precursor glycans are more commonly expressed in neonates[12] compared with older children or adults, which may explain the age restriction of rotavirus disease. Of note, the P[4] and P[8] genotypes that target older children bind to HBGAs but not to the precursors[29,102,103]. In addition, the VP8* of HAL1166, which engages A-type HBGA, agglutinates only type A erythrocytes. This suggests that human polymorphisms influence susceptibility to rotavirus infection, and individuals with blood group A may be at increased risk for infection with G8 P[11] rotavirus[11].

RNA interference-mediated knockdown of genes that are involved in the synthesis of gangliosides decreases the capacity of human, porcine, bovine and simian rotaviruses to infect cells *in vitro*[104]. Moreover, ovine erythrocytes, which are naturally covered with sialic acid, interfere with rotavirus replication in mice by blocking Neu5Ac-binding sites on the virus and preventing attachment to other cells. Concordantly, neuraminidase treatment of these erythrocytes negates the therapeutic effect[105]. Taken together, these studies demonstrate a relationship between glycan-binding capacity and rotavirus pathogenesis.

Inhibition of virus–sialic acid interactions

Sialic acid-binding viruses include important human pathogens, such as adenovirus, influenza virus and rotavirus, as well as viruses with therapeutic applications, such as adenovirus and reovirus, which are being tested as gene-delivery vectors and oncolytic agents[106,107]. Therefore, manipulating the interactions of these viruses with sialic acid may improve therapeutic design and efficacy. Influenza virus attachment and release necessitate interactions with sialic acid and are important antiviral targets. Structure-based therapeutic design led to the development of oseltamivir and zanamivir, which are sialic acid derivatives that inhibit influenza virus neuraminidase and block the release of progeny virions[108,109]. Structural analysis shows that the sialic acid-binding pocket in group 1 neuraminidase proteins (N1,N4, N5

and N8) is larger than that observed in group 2 neuraminidase proteins (N2, N3, N6, N7 and N9), and group 2 neuraminidase proteins were used for the design of oseltamivir and zanamivir[110]. Therefore, it will probably be possible to generate group-specific neuraminidase inhibitors that fit more tightly in the active site[110]. A new class of neuraminidase inhibitor forms a stable covalent intermediate of neuraminidase, inhibits neuraminidase activity for extended intervals and has been shown to be effective in prophylaxis and therapy for influenza virus infection in mice[111].

The influenza virus haemagglutinin is also an attractive drug target. However, it binds to sialylated glycans with low affinity, and it has been difficult to generate monovalent sialic acid derivatives that compete with native glycans[112]. Unfortunately, polyvalent sialic acid derivatives that target haemagglutitin are difficult to deliver into host cells and have considerable toxicity[113]. An interesting alternative approach to block haemagglutinin involves liposomes coated with lactoseries tetrasaccharide c (LSTc), which is an α2,6-linked sialic acid-bearing pentasaccharide[114]. This approach provides a framework to design a multivalent, but safe, delivery vehicle[114].

Whereas most influenza antiviral therapeutic agents target the virus, DAS181 targets the host receptors. This drug is a fusion protein comprising an epithelial anchoring domain and a sialidase, which removes α2,3- and α2,6-linked sialic acid from respiratory epithelial cells[115,116]. DAS181 is effective against influenza A and B strains *in vitro*[115] and protects mice from lethal challenge with H1N1 (REF. 115) and H5N1 (REF. 116) isolates. Phase II clinical trials showed that DAS181 reduced viral shedding in humans[117]. Thus, both virus and host determinants of sialic acid binding provide antiviral targets.

The crystal structure of Ad37 in complex with the GD1a glycan led to the development of trivalent sialic acid-based compounds that interact with all three binding pockets of the Ad37 fibre knob, thus engaging the knob with high avidity. Such compounds could be delivered topically, which bypasses potential problems of systemic drug delivery and could thus be useful for the treatment of epidemic keratoconjunctivitis. As it is unlikely that the Ad37–GD1a interaction is unique, multivalent sialic acid-based inhibitors form a template for the design of antiviral drugs in cases in which there are multiple sialic acid-binding sites in close proximity on multimeric viral attachment proteins.

Knowledge that has been gained from studies of virus–glycan interactions may be particularly useful to retarget viruses either for use as gene delivery vehicles or oncolytic agents. Reoviruses are naturally cytotoxic and preferentially infect transformed cells[118–121]. Targeting of transformed cells, coupled with the relative avirulence of these viruses in humans following the first few weeks of life, makes reovirus a suitable candidate for oncolytic therapy. Phase I and Phase II clinical trials have shown that the reovirus strain T3 Dearing (Reolysin; Oncolytics Biotech) is safe and non-toxic even at high doses[122–124]. T3 Dearing is now being tested in Phase III clinical trials for the treatment of head and neck cancer[125].

The sialylation pattern in transformed cells is altered compared with that in untransformed cells[126]. Sialic acid abundance is increased in transformed cells owing to overexpression of sialyltransferases[127]. Understanding reovirus–glycan interactions could improve tumour targeting. In this regard, a T3 Dearing virus that lacks the σ1 head domain is less toxic in the host but retains its oncolytic potential[128]. This truncated T3 reovirus cannot bind to JAM-A, which indicates that the virus must adhere to cells using only sialic acids or using a receptor that has not been identified. It is possible that the altered glycan profile of cancer cells enables all three sialic acid-binding sites of the T3 σ1 trimer to be occupied, which increases the avidity of the binding interaction. Structural studies of reovirus σ1–sialic acid interactions[19,66], coupled with structure-guided mutagenesis[39,40], can also facilitate the generation of strains that have increased affinity for sialic acids and that may have increased tumour specificity and oncolytic potential.

Future directions

Structure–function studies of sialic acid-binding viruses with stalk-like attachment proteins show that these viruses primarily engage the sialic acid moiety using a small number of contacts. Additional residues confer specificity for a given linkage or glycan type. The location of the glycan-binding site is often conserved among attachment proteins of different strains of the same virus — for example, as seen for influenza virus and rotavirus. However, some viruses, such as reovirus, have evolved distinct glycan-binding regions in their attachment proteins, depending on viral serotype. A common feature of virus–glycan binding is the insertion of the methyl group of the *N*-acetyl chain of Neu5Ac into a hydrophobic pocket of the viral attachment protein. However, it is remarkable that even viruses with similarly structured, stalk-like attachment proteins, such as reovirus σ1 and adenovirus fibre, engage similar Neu5Ac-based glycans in an entirely different way. Even more remarkable is that the same protein from different serotypes of reovirus uses different binding sites for the same Neu5Ac.

The capacity to bind to sialyloligosaccharides contributes to host range, as exemplified by influenza virus, and influences tropism, as exemplified by adenovirus, influenza virus and reovirus. However, a comprehensive understanding of the role of glycan binding in viral tropism has been hindered by the lack of information about the specific glycans that are present on tissues that are targeted by viruses. Studies using plant lectins and immunohistochemistry suggest that the generalized binding preference of human influenza virus strains for α2,6-linked sialic acid and of avian strains for α2,3-linked sialic acid[20,28] reflects the pattern of sialic acid expression of the target host[129,130]. However, these expression studies are limited in specificity to the sialic acid linkage type, which is insufficient to explain differences in glycan binding. A remaining challenge is to increase our understanding of glycan expression profiles *in vivo*. This knowledge gap currently presents the largest obstacle to attaining a comprehensive understanding of virus–glycan interactions and their functions in disease. Mass spectrometry[131],

microarray technology[132] and shotgun glycomics are being used to define the glycome and tissue-specific glycan expression profiles. In shotgun glycomics, glycolipids and glycoproteins are extracted from organs, tissues or cells, and labelled. The identity and composition of these glycans are determined by high-throughput liquid chromatography (HPLC). This approach[133,134], coupled with glycan array screening, could provide a framework for studying organ- and cell type-specific glycan use by viruses, as shown for swine influenza virus[134].

The characterization of glycan expression on the cell surface is required to synergize glycan array technology with pathogenesis studies. Unfortunately, none of the glycan array platforms fully represent the glycans that are found on the lung and bronchial epithelium[131], which can lead to discrepancies between glycan array screening data and functional studies. For example, glycan array screening indicates that certain influenza virus strains bind similarly to specific glycans, whereas such strains differ in their capacity to bind to lung tissue explants. Thus, the physiologically relevant receptors are not known.

Shotgun glycomics could be complemented to incorporate glycans onto arrays in their relative biological abundance. Virus–sialic acid interactions are usually of low affinity. Physiologically relevant glycan receptors are presumably expressed on the surface in moderate to high abundance to facilitate efficient attachment. Further studies investigating the avidity of haemagglutinin for glycans, as well as the tissue distribution of these carbohydrates, will improve our understanding of glycan receptors for influenza and other viruses. Future work in this field will determine how the intricate glycan-binding preferences that are displayed by viruses function in disease and provide new ideas for altering glycan use to improve therapeutic applications.

1. Barton, E. S., Connolly, J. L., Forrest, J. C., Chappell, J. D. & Dermody, T. S. Utilization of sialic acid as a coreceptor enhances reovirus attachment by multistep adhesion strengthening. *J. Biol. Chem.* **276**, 2200–2211 (2001).
2. Chappell, J. D., Duong, J. L., Wright, B. W. & Dermody, T. S. Identification of carbohydrate-binding domains in the attachment proteins of type 1 and type 3 reoviruses. *J. Virol.* **74**, 8472–8479 (2000).
3. Chappell, J. D., Gunn, V. L., Wetzel, J. D., Baer, G. S. & Dermody, T. S. Mutations in type 3 reovirus that determine binding to sialic acid are contained in the fibrous tail domain of viral attachment protein s1. *J. Virol.* **71**, 1834–1841 (1997).
4. Tsai, B. *et al.* Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J.* **22**, 4346–4355 (2003).
5. Rogers, G. N. *et al.* Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* **304**, 76–78 (1983).
6. Neu, U., Bauer, J. & Stehle, T. Viruses and sialic acids: rules of engagement. *Curr. Opin. Struct. Biol.* **21**, 610–618 (2011).
7. Silva, L. A. *et al.* A single-amino-acid polymorphism in Chikungunya virus E2 glycoprotein influences glycosaminoglycan utilization. *J. Virol.* **88**, 2385–2397 (2014).
8. Gardner, C. L. *et al.* Natural variation in the heparan sulfate binding domain of the eastern equine encephalitis virus E2 glycoprotein alters interactions with cell surfaces and virulence in mice. *J. Virol.* **87**, 8582–8590 (2013).
9. Gardner, C. L., Ebel, G. D., Ryman, K. D. & Klimstra, W. B. Heparan sulfate binding by natural eastern equine encephalitis viruses promotes neurovirulence. *Proc. Natl Acad. Sci. USA* **108**, 16026–16031 (2011).
10. Tiwari, V., Maus, E., Sigar, I. M., Ramsey, K. H. & Shukla, D. Role of heparan sulfate in sexually transmitted infections. *Glycobiology* **22**, 1402–1412 (2012).
11. Hu, L. *et al.* Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature* **485**, 256–259 (2012).
This study identifies HBGA as a receptor for human rotavirus strain HAL1166 and defines the glycan-binding site using X-ray crystallography. Interestingly, only modest changes in the glycan-binding site greatly alter glycan-binding specificity among rotavirus strains.
12. Liu, Y. *et al.* Poly-LacNAc as an age-specific ligand for rotavirus P[11] in neonates and infants. *PLoS ONE* **8**, e78113 (2013).
13. Neu, U. *et al.* Structures of Merkel cell polyomavirus VP1 complexes define a sialic acid binding site required for infection. *PLoS Pathog.* **8**, e1002738 (2012).
14. Neu, U. *et al.* Structure–function analysis of the human JC polyomavirus establishes the LSTc pentasaccharide as a functional receptor motif. *Cell Host Microbe* **8**, 309–319 (2010).
15. Varki, A. Multiple changes in sialic acid biology during human evolution. *Glycoconjugate J.* **26**, 231–245 (2009).
This review provides insights into the host–pathogen 'arms race' throughout the course of human evolution.
16. Varki, A. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. *Nature* **446**, 1023–1029 (2007).
17. Schwarzkopf, M. *et al.* Sialylation is essential for early development in mice. *Proc. Natl Acad. Sci. USA* **99**, 5267–5270 (2002).
18. Svennerholm, L. Interaction of cholera toxin and ganglioside G(M1). *Adv. Exp. Med. Biol.* **71**, 191–204 (1976).
19. Reiss, K. *et al.* The GM2 glycan serves as a functional co-receptor for serotype 1 reovirus. *PLoS Pathog.* **8**, e1003078 (2012).
20. Rogers, G. N., Pritchett, T. J., Lane, J. L. & Paulson, J. C. Differential sensitivity of human, avian, and equine influenza A viruses to a glycoprotein inhibitor of infection: selection of receptor specific variants. *Virology* **131**, 394–408 (1983).
21. Nilsson, E. C. *et al.* The GD1a glycan is a cellular receptor for adenoviruses causing epidemic keratoconjunctivitis. *Nature Med.* **17**, 105–109 (2011).
This study identifies GD1a as a cellular receptor for Ad37 and defines the glycan-binding site using NMR spectroscopy and X-ray crystallography.
22. Miller-Podraza, H., Bradley, R. M. & Fishman, P. H. Biosynthesis and localization of gangliosides in cultured cells. *Biochemistry* **21**, 3260–3265 (1982).
23. Blixt, O. *et al.* Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc. Natl Acad. Sci. USA* **101**, 17033–17038 (2004).
24. Feizi, T., Fazio, F., Chai, W. & Wong, C. H. Carbohydrate microarrays — a new set of technologies at the frontiers of glycomics. *Curr. Opin. Struct. Biol.* **13**, 637–645 (2003).
25. Liu, Y. *et al.* Neoglycolipid-based oligosaccharide microarray system: preparation of NGLs and their noncovalent immobilization on nitrocellulose-coated glass slides for microarray analyses. *Methods Mol. Biol.* **808**, 117–136 (2012).
26. Liu, Y., Palma, A. S. & Feizi, T. Carbohydrate microarrays: key developments in glycobiology. *Biol. Chem.* **390**, 647–656 (2009).
This review provides a comprehensive description of glycan array technology.
27. Childs, R. A. *et al.* Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. *Nature Biotech.* **27**, 797–799 (2009).
This study uses glycan array technology to determine the glycan-binding specificity of pandemic H1N1 in comparison to the 2009 seasonal strain. It is an example of how glycan arrays can be used to identify specific glycan receptors for emerging virus strains.
28. Stevens, J. *et al.* Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. *J. Mol. Biol.* **355**, 1143–1155 (2006).
This study is an important example of how glycan array technology can be used to determine differences in receptor specificity between influenza virus strains.
29. Ramani, S. *et al.* The VP8* domain of neonatal rotavirus strain G10P[11] binds to type II precursor glycans. *J. Virol.* **87**, 7255–7264 (2013).
This study defines the glycan receptor specificity of neonatal rotavirus infections.
30. Neu, U., Woellner, K., Gauglitz, G. & Stehle, T. Structural basis of GM1 ganglioside recognition by simian virus 40. *Proc. Natl Acad. Sci. USA* **105**, 5219–5224 (2008).
31. Xu, R. *et al.* Preferential recognition of avian-like receptors in human influenza A H7N9 viruses. *Science* **342**, 1230–1235 (2013).
32. Gulati, S., Lasanajak, Y., Smith, D. F., Cummings, R. D. & Air, G. M. Glycan array analysis of influenza H1N1 binding and release. *Cancer Biomark.* **14**, 43–53 (2014).
33. Fukui, S., Feizi, T., Galustian, C., Lawson, A. M. & Chai, W. Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate–protein interactions. *Nature Biotech.* **20**, 1011–1017 (2002).
34. Palma, A. S., Feizi, T., Childs, R. A., Chai, W. & Liu, Y. The neoglycolipid (NGL)-based oligosaccharide microarray system poised to decipher the *meta*-glycome. *Curr. Opin. Chem. Biol.* **18**, 1–8 (2014).
35. Wang, L. *et al.* Cross-platform comparison of glycan microarray formats. *Glycobiology* **24**, 507–517 (2014).
36. Padler-Karavani, V. *et al.* Cross-comparison of protein recognition of sialic acid diversity on two novel sialoglycan microarrays. *J. Biol. Chem.* **287**, 22593–22608 (2012).
37. Engelhardt, O. G. Many ways to make an influenza virus — review of influenza virus reverse genetics methods. *Influenza Other Respir. Viruses* **7**, 249–256 (2013).
38. Fodor, E. *et al.* Rescue of influenza A virus from recombinant DNA. *J. Virol.* **73**, 9679–9682 (1999).
39. Boehme, K. W., Ikizler, M., Kobayashi, T. & Dermody, T. S. Reverse genetics for mammalian reovirus. *Methods* **55**, 109–113 (2011).
This study describes methods for mammalian reovirus reverse genetics in detail.
40. Kobayashi, T., Ooms, L. S., Ikizler, M., Chappell, J. D. & Dermody, T. S. An improved reverse genetics system for mammalian orthoreoviruses. *Virology* **2**, 194–200 (2010).
41. Adams, P. D. *et al.* Advances, interactions, and future developments in the CNS, Phenix, and Rosetta structural biology software systems. *Annu. Rev. Biophys.* **42**, 265–287 (2013).

42. Wlodawer, A., Minor, W., Dauter, Z. & Jaskolski, M. Protein crystallography for aspiring crystallographers or how to avoid pitfalls and traps in macromolecular structure determination. *FEBS J.* **280**, 5705–5736 (2013).
43. Rademacher, C., Krishna, N. R., Palcic, M., Parra, F. & Peters, T. NMR experiments reveal the molecular basis of receptor recognition by a calicivirus. *J. Am. Chem. Soc.* **130**, 3669–3675 (2008).
44. Weis, W. *et al.* Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. *Nature* **333**, 426–431 (1988).
45. Nobusawa, E. *et al.* Comparison of complete amino acid sequences and receptor-binding properties among 13 serotypes of hemagglutinins of influenza A viruses. *Virology* **182**, 475–485 (1991).
46. Watowich, S. J., Skehel, J. J. & Wiley, D. C. Crystal structures of influenza virus hemagglutinin in complex with high-affinity receptor analogs. *Structure* **2**, 719–731 (1994).
47. Sauter, N. K. *et al.* Binding of influenza virus hemagglutinin to analogs of its cell-surface receptor, sialic acid: analysis by proton nuclear magnetic resonance spectroscopy and X-ray crystallography. *Biochemistry* **31**, 9609–9621 (1992).
48. Xiong, X. *et al.* Receptor binding by an H7N9 influenza virus from humans. *Nature* **499**, 496–499 (2013).
49. Xiong, X. *et al.* Receptor binding by a ferret-transmissible H5 avian influenza virus. *Nature* **497**, 392–396 (2013).
50. Zhang, Y. *et al.* H5N1 hybrid viruses bearing 2009/H1N1 virus genes transmit in guinea pigs by respiratory droplet. *Science* **340**, 1459–1463 (2013).
51. Chandrasekaran, A. *et al.* Glycan topology determines human adaptation of avian H5N1 virus hemagglutinin. *Nature Biotech.* **26**, 107–113 (2008).
52. Matrosovich, M. *et al.* Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J. Virol.* **74**, 8502–8512 (2000).
53. Connor, R. J., Kawaoka, Y., Webster, R. G. & Paulson, J. C. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* **205**, 17–23 (1994).
54. Imai, M. & Kawaoka, Y. The role of receptor binding specificity in interspecies transmission of influenza viruses. *Curr. Opin. Virol.* **2**, 160–167 (2012).
55. Stevens, J., Blixt, O., Paulson, J. C. & Wilson, I. A. Glycan microarray technologies: tools to survey host specificity of influenza viruses. *Nature Rev. Microbiol.* **4**, 857–864 (2006).
56. Glaser, L. *et al.* A single amino acid substitution in 1918 influenza virus hemagglutinin changes receptor binding specificity. *J. Virol.* **79**, 11533–11536 (2005).
57. Liu, Y. *et al.* Altered receptor specificity and cell tropism of D222G hemagglutinin mutants isolated from fatal cases of pandemic A(H1N1) 2009 influenza virus. *J. Virol.* **84**, 12069–12074 (2010).
58. Bradley, K. C. *et al.* Comparison of the receptor binding properties of contemporary swine isolates and early human pandemic H1N1 isolates (Novel 2009 H1N1). *Virology* **413**, 169–182 (2011).
59. Gulati, S. *et al.* Human H3N2 influenza viruses isolated from 1968 to 2012 show varying preference for receptor substructures with no apparent consequences for disease or spread. *PLoS ONE* **8**, e66325 (2013).
This study reveals the remarkable diversity in influenza virus haemagglutinin binding preferences using glycan array screening.
60. Crusat, M. *et al.* Changes in the hemagglutinin of H5N1 viruses during human infection — influence on receptor binding. *Virology* **447**, 326–337 (2013).
61. Belser, J. A., Katz, J. M. & Tumpey, T. M. The ferret as a model organism to study influenza A virus infection. *Dis. Models Mechanisms* **4**, 575–579 (2011).
62. Imai, M. *et al.* Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* **486**, 420–428 (2012).
63. Linster, M. *et al.* Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus. *Cell* **157**, 329–339 (2014).
This study identifies the precise genetic changes that are required for influenza virus transmissibility.
64. de Graaf, M. & Fouchier, R. A. Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *EMBO J.* **33**, 823–841 (2014).
This study reviews the function of receptor binding in influenza virus pathogenesis in detail.
65. Tai, J. H. *et al.* Prevalence of reovirus-specific antibodies in young children in Nashville, Tennessee. *J. Infect. Dis.* **191**, 1221–1224 (2005).
66. Reiter, D. M. *et al.* Crystal structure of reovirus attachment protein σ1 in complex with sialylated oligosaccharides. *PLoS Pathog.* **7**, e1002166 (2011).
67. Kirchner, E., Guglielmi, K. M., Strauss, H. M., Dermody, T. S. & Stehle, T. Structure of reovirus s1 in complex with its receptor junctional adhesion molecule-A. *PLoS Pathog.* **4**, e1000235 (2008).
68. Barton, E. S. *et al.* Junction adhesion molecule is a receptor for reovirus. *Cell* **104**, 441–451 (2001).
69. Antar, A. A. R. *et al.* Junctional adhesion molecule-A is required for hematogenous dissemination of reovirus. *Cell Host Microbe* **5**, 59–71 (2009).
70. Bazzoni, G. *et al.* Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. *J. Biol. Chem.* **275**, 20520–20526 (2000).
71. Ebnet, K., Schulz, C. U., Meyer Zu Brickwedde, M. K., Pendl, G. G. & Vestweber, D. Junctional adhesion molecule interacts with the PDZ domain-containing proteins AF-6 and ZO-1. *J. Biol. Chem.* **275**, 27979–27988 (2000).
72. Maschio, A. D. *et al.* Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhabited by an antibody to junctional adhesion molecule (JAM). *J. Exp. Med.* **190**, 1351–1356 (1999).
73. Lechner, F. *et al.* Antibodies to the junctional adhesion molecule cause disruption of endothelial cells and do not prevent leukocyte influx into the meninges after viral or bacterial infection. *J. Infect. Dis.* **182**, 978–982 (2000).
74. Lerner, A. M., Cherry, J. D. & Finland, M. Haemagglutination with reoviruses. *Virology* **19**, 58–65 (1963).
75. Fukuda, M., Lauffenburger, M., Sasaki, H., Rogers, M. E. & Dell, A. Structures of novel sialylated *O*-linked oligosaccharides isolated from human erythrocyte glycophorins. *J. Biol. Chem.* **262**, 11952–11957 (1987).
76. Pahlsson, P., Blackall, D. P., Ugorski, M., Czerwinski, M. & Spitalnik, S. L. Biochemical characterization of the *O*-glycans on recombinant glycophorin A expressed in Chinese hamster ovary cells. *Glycoconjugate J.* **11**, 43–50 (1994).
77. Eggers, H. J., Gomatos, P. J. & Tamm, I. Agglutination of bovine erythrocytes: a general characteristic of reovirus type 3. *Proc. Soc. Exp. Biol. Med.* **110**, 879–881 (1962).
78. Barton, E. S. *et al.* Utilization of sialic acid as a coreceptor is required for reovirus-induced biliary disease. *J. Clin. Invest.* **111**, 1823–1833 (2003).
This study demonstrates a linkage between sialic acid binding and alterations in reovirus tropism.
79. Frierson, J. M. *et al.* Utilization of sialylated glycans as coreceptors enhances the neurovirulence of serotype 3 reovirus. *J. Virol.* **86**, 13164–13173 (2012).
This work shows that sialic acid-binding capacity increases reovirus-induced neurovirulence in mice.
80. Konopka-Anstadt, J. L. *et al.* The Nogo receptor NgR1 mediates infection by mammalian reovirus. *Cell Host Microbe* **15**, 681–691 (2014).
81. Weiner, H. L., Drayna, D., Averill, D. R. Jr & Fields, B. N. Molecular basis of reovirus virulence: role of the S1 gene. *Proc. Natl Acad. Sci. USA* **74**, 5744–5748 (1977).
82. Weiner, H. L., Greene, M. I. & Fields, B. N. Delayed hypersensitivity in mice infected with reovirus. I. Identification of host and viral gene products responsible for the immune response. *J. Immunol.* **125**, 278–282 (1980).
83. Morrison, L. A., Sidman, R. L. & Fields, B. N. Direct spread of reovirus from the intestinal lumen to the central nervous system through vagal autonomic nerve fibers. *Proc. Natl Acad. Sci. USA* **88**, 3852–3856 (1991).
84. Tyler, K. L., McPhee, D. A. & Fields, B. N. Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. *Science* **233**, 770–774 (1986).
85. Cupelli, K. & Stehle, T. Viral attachment strategies: the many faces of adenoviruses. *Curr. Opin. Virol.* **1**, 84–91 (2011).
86. Kemp, M. C., Hierholzer, J. C., Cabradilla, C. P. & Obijeski, J. F. The changing etiology of epidemic keratoconjunctivitis: antigenic and restriction enzyme analyses of adenovirus types 19 and 37 isolated over a 10-year period. *J. Infect. Dis.* **148**, 24–33 (1983).
87. Arnberg, N., Edlund, K., Kidd, A. H. & Wadell, G. Adenovirus type 37 uses sialic acid as a cellular receptor. *J. Virol.* **74**, 42–48 (2000).
88. Wadell, G. Hemagglutination with adenovirus serotypes belonging to Rosen's subgroups II and 3. *Proc. Soc. Exp. Biol. Med.* **132**, 413–421 (1969).
89. Burmeister, W. P., Guilligay, D., Cusack, S., Wadell, G. & Arnberg, N. Crystal structure of species D adenovirus fiber knobs and their sialic acid binding sites. *J. Virol.* **78**, 7727–7736 (2004).
90. Spjut, S. *et al.* A potent trivalent sialic acid inhibitor of adenovirus type 37 infection of human corneal cells. *Angew. Chem. Int. Ed. Engl.* **50**, 6519–6521 (2011).
This paper reports the development of a multivalent inhibitor of viral infection that was inspired by the crystal structure of the Ad37 fibre knob in complex with its carbohydrate receptor; the inhibitor is more potent than monovalent sialic acid.
91. Yeager, M., Dryden, K. A., Olson, N. H., Greenberg, H. B. & Baker, T. S. Three-dimensional structure of rhesus rotavirus by cryoelectron microscopy and image reconstruction. *J. Cell Biol.* **110**, 2133–2144 (1990).
92. Fiore, L., Greenberg, H. B. & Mackow, E. R. The VP8 fragment of VP4 is the rhesus rotavirus hemagglutinin. *Virology* **181**, 553–563 (1991).
93. Denisova, E. *et al.* Rotavirus capsid protein VP5* permeabilizes membranes. *J. Virol.* **73**, 3147–3153 (1999).
94. Dormitzer, P. R., Sun, Z. Y., Wagner, G. & Harrison, S. C. The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. *EMBO J.* **21**, 885–897 (2002).
This study identifies the sialic acid-binding site of rhesus rotavirus and defines residues that are required for this glycan interaction.
95. Kraschnefski, M. J. *et al.* Effects on sialic acid recognition of amino acid mutations in the carbohydrate-binding cleft of the rotavirus spike protein. *Glycobiology* **19**, 194–200 (2009).
96. Blanchard, H., Yu, X., Coulson, B. S. & von Itzstein, M. Insight into host cell carbohydrate-recognition by human and porcine rotavirus from crystal structures of the virion spike associated carbohydrate-binding domain (VP8*). *J. Mol. Biol.* **367**, 1215–1226 (2007).
97. Yu, X. *et al.* Structural basis of rotavirus strain preference toward *N*-acetyl- or *N*-glycolylneuraminic acid-containing receptors. *J. Virol.* **86**, 13456–13466 (2012).
98. Monnier, N. *et al.* High-resolution molecular and antigen structure of the VP8* core of a sialic acid-independent human rotavirus strain. *J. Virol.* **80**, 1513–1523 (2006).
99. Haselhorst, T. *et al.* Recognition of the GM3 ganglioside glycan by Rhesus rotavirus particles. *Angew. Chem. Int. Ed. Engl.* **50**, 1055–1058 (2011).
100. Haselhorst, T. *et al.* Sialic acid dependence in rotavirus host cell invasion. *Nature Chem. Biol.* **5**, 91–93 (2009).
101. Sowmyanarayanan, T. V. *et al.* Severity of rotavirus gastroenteritis in Indian children requiring hospitalization. *Vaccine* **30** (Suppl. 1), 167–172 (2012).
102. Huang, P. *et al.* Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *J. Virol.* **86**, 4833–4843 (2012).
103. Liu, Y. *et al.* Rotavirus VP8*: phylogeny, host range, and interaction with histo-blood group antigens. *J. Virol.* **86**, 9899–9910 (2012).
104. Martinez, M. A., Lopez, S., Arias, C. F. & Isa, P. Gangliosides have a functional role during rotavirus cell entry. *J. Virol.* **87**, 1115–1122 (2013).
105. Yolken, R. H., Willoughby, R., Wee, S. B., Miskuff, R. & Vonderfecht, S. Sialic acid glycoproteins inhibit *in vitro* and *in vivo* replication of rotaviruses. *J. Clin. Invest.* **79**, 148–154 (1987).
106. Kaufmann, J. K. & Nettelbeck, D. M. Virus chimeras for gene therapy, vaccination, and oncolysis: adenoviruses and beyond. *Trends Mol. Med.* **18**, 365–376 (2012).
107. Russell, S. J., Peng, K. W. & Bell, J. C. Oncolytic virotherapy. *Nature Biotech.* **30**, 658–670 (2012).
108. von Itzstein, M. *et al.* Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **363**, 418–423 (1993).
109. Kim, C. U. *et al.* Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J. Am. Chem. Soc.* **119**, 681–690 (1997).

110. Russell, R. J. *et al.* The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design. *Nature* **443**, 45–49 (2006).
111. Kim, J. H. *et al.* Mechanism-based covalent neuraminidase inhibitors with broad-spectrum influenza antiviral activity. *Science* **340**, 71–75 (2013).
112. Takemoto, D. K., Skehel, J. J. & Wiley, D. C. A surface plasmon resonance assay for the binding of influenza virus hemagglutinin to its sialic acid receptor. *Virology* **217**, 452–458 (1996).
113. Matrosovich, M. & Klenk, H. D. Natural and synthetic sialic acid-containing inhibitors of influenza virus receptor binding. *Rev. Med. Virol.* **13**, 85–97 (2003).
114. Hendricks, G. L. *et al.* Sialylneolacto-*N*-tetraose c (LSTc)-bearing liposomal decoys capture influenza A virus. *J. Biol. Chem.* **288**, 8061–8073 (2013).
This study shows that liposomes that are coated with LSTc bind to influenza virus and function as antiviral agents.
115. Malakhov, M. P. *et al.* Sialidase fusion protein as a novel broad-spectrum inhibitor of influenza virus infection. *Antimicrob. Agents Chemother.* **50**, 1470–1479 (2006).
116. Belser, J. A. *et al.* DAS181, a novel sialidase fusion protein, protects mice from lethal avian influenza H5N1 virus infection. *J. Infect. Dis.* **196**, 1493–1499 (2007).
117. Wathen, M. W., Barro, M. & Bright, R. A. Antivirals in seasonal and pandemic influenza — future perspectives. *Influenza Other Respir. Viruses* **7** (Suppl. 1), 76–80 (2013).
118. Hashiro, G., Loh, P. C. & Yau, J. T. The preferential cytotoxicity of reovirus for certain transformed cell lines. *Arch. Virol.* **54**, 307–315 (1977).
119. Norman, K. L., Hirasawa, K., Yang, A. D., Shields, M. A. & Lee, P. W. Reovirus oncolysis: the Ras/RalGEF/p38 pathway dictates host cell permissiveness to reovirus infection. *Proc. Natl Acad. Sci. USA* **101**, 11099–11104 (2004).
120. Strong, J. E., Coffey, M. C., Tang, D., Sabinin, P. & Lee, P. W. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. *EMBO J.* **17**, 3351–3362 (1998).
121. Coffey, M. C., Strong, J. E., Forsyth, P. A. & Lee, P. W. Reovirus therapy of tumors with activated Ras pathway. *Science* **282**, 1332–1334 (1998).
122. Gollamudi, R. *et al.* Intravenous administration of Reolysin, a live replication competent RNA virus is safe in patients with advanced solid tumors. *Invest. New Drugs* **28**, 641–649 (2009).
123. Vidal, L. *et al.* A phase I study of intravenous oncolytic reovirus type 3 Dearing in patients with advanced cancer. *Clin. Cancer. Res.* **14**, 7127–7137 (2008).
124. Sahin, E., Egger, M., McMasters, K. & Zhou, H. Development of oncolytic reovirus for cancer therapy. *J. Cancer Ther.* **4**, 1100–1115 (2013).
125. Kyula, J. N., Roulstone, V., Karapanagiotou, E. M., Melcher, A. A. & Harrington, K. J. Oncolytic reovirus type 3 (Dearing) as a novel therapy in head and neck cancer. *Expert Opin. Biol. Ther.* **12**, 1669–1678 (2012).
126. Raval, G. *et al.* TNF-α induction of GM2 expression on renal cell carcinomas promotes T cell dysfunction. *J. Immunol.* **178**, 6642–6652 (2007).
127. Harduin-Lepers, A. *et al.* Sialyltransferases functions in cancers. *Frontiers Biosci.* **4**, 499–515 (2012).
128. Kim, M. *et al.* Attenuated reovirus displays oncolysis with reduced host toxicity. *Br. J. Cancer* **104**, 290–299 (2011).
129. Shinya, K. *et al.* Avian flu: influenza virus receptors in the human airway. *Nature* **440**, 435–436 (2006).
130. Baum, L. G. & Paulson, J. C. Sialyloligosaccharides of the respiratory epithelium in the selection of human influenza virus receptor specificity. *Acta Histochem. Suppl.* **40**, 35–38 (1990).
131. Walther, T. *et al.* Glycomic analysis of human respiratory tract tissues and correlation with influenza virus infection. *PLoS Pathog.* **9**, e1003223 (2013).
The authors use glycomics approaches to improve understanding of the glycome on human lung tissue and compare these findings with results from traditional glycan array experiments.
132. Comelli, E. M. *et al.* A focused microarray approach to functional glycomics: transcriptional regulation of the glycome. *Glycobiology* **16**, 117–131 (2006).
133. Song, X. *et al.* Shotgun glycomics: a microarray strategy for functional glycomics. *Nature Meth.* **8**, 85–90 (2011).
134. Byrd-Leotis, L. *et al.* Shotgun glycomics of pig lung identifies natural endogenous receptors for influenza viruses. *Proc. Natl Acad. Sci. USA* **111**, E2241–E2250 (2014).
This study demonstrates how shotgun glycomics can be used to identify influenza virus receptors.

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Competing interests statement
The authors declare no competing interests.

DATABASES
Protein Data Bank (PDB): http://www.rcsb.org/pdb/home/home.do
1HGG | 2WR2 | 2WR7 | 4GU3 | 3S6X | 3N0I | 1KQR | 4DRV

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