

## Review

# Global aspects of viral glycosylation

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## Abstract

Enveloped viruses encompass some of the most common human pathogens causing infections of different severity, ranging from no or very few symptoms to lethal disease as seen with the viral hemorrhagic fevers. All enveloped viruses possess an envelope membrane derived from the host cell, modified with often heavily glycosylated virally encoded glycoproteins important for infectivity, viral particle formation and immune evasion. While N-linked glycosylation of viral envelope proteins is well characterized with respect to location, structure and site occupancy, information on mucin-type O-glycosylation of these proteins is less comprehensive. Studies on viral glycosylation are often limited to analysis of recombinant proteins that in most cases are produced in cell lines with a glycosylation capacity different from the capacity of the host cells. The glycosylation pattern of the produced recombinant glycoproteins might therefore be different from the pattern on native viral proteins. In this review, we provide a historical perspective on analysis of viral glycosylation, and summarize known roles of glycans in the biology of enveloped human viruses. In addition, we describe how to overcome the analytical limitations by using a global approach based on mass spectrometry to identify viral O-glycosylation in virus-infected cell lysates using the complex enveloped virus herpes simplex virus type 1 as a model. We underscore that glycans often pay important contributions to overall protein structure, function and immune recognition, and that glycans represent a crucial determinant for vaccine design. High throughput analysis of glycosylation on relevant glycoprotein formulations, as well as data compilation and sharing is therefore important to identify consensus glycosylation patterns for translational applications.

**Key words:** enveloped viruses, herpesvirus, mass spectrometry, mucin type O-glycosylation, viral glycans

## Introduction

Enveloped viruses are well adapted pathogens infecting a great variety of hosts from protozoans to mammals that hijack the host cell's molecular machinery for reproduction (International Committee on Taxonomy of Viruses 2012; Buchmann and Holmes 2015). They have evolved together with their hosts and developed various means to counteract the host's defense mechanisms (Christiansen et al. 2015; Beachboard and Horner 2016; Schuren et al. 2016). A characteristic feature of these viruses is a cell membrane-derived envelope modified with virally encoded proteins glycosylated by the host

glycosylation apparatus as they pass through the secretory pathway. While some viral glycoproteins are glycosylated at relatively few sites, others are densely glycosylated with N-linked and O-linked glycans. Prominent examples include the Ebola virus glycoprotein modified by a very high content of O-linked glycans, and the HIV-1 glycoprotein gp160 that is glycosylated by the addition of multiple N-linked glycans (Jeffers et al. 2002; Pabst et al. 2012; Yang W et al. 2014). Although specific functional roles have been assigned to distinct glycosites of multiple viruses (Goffard et al. 2005; Falkowska et al. 2007; Helle et al. 2010; Wang et al. 2013, 2017;

Lennemann et al. 2014; Bradel-Tretheway et al. 2015; Luo et al. 2015; Orlova et al. 2015; Suenaga et al. 2015; Stone et al. 2016; Wu et al. 2017), it is generally believed that the high levels of glycosylation serve as a protective shield from the host's immune system (Francica et al. 2010; Helle et al. 2011; Sommerstein et al. 2015; Behrens et al. 2016; Gram et al. 2016; Walls et al. 2016). The other major role of glycosylation occurs during virus entry where glycans on the host cell represent viral receptors interacting with carbohydrate binding proteins on the viral surface. A prominent example is the influenza viruses that attach to surface glycans residing on cellular glycoproteins (Air 2014). Hemagglutinins (HA) of human influenza viruses use  $\alpha$ 2,6-linked sialic acid moieties, while those of avian viruses bind preferentially to  $\alpha$ 2,3-linked sialic acids (Air 2014). Apart from influenza virus, several other nonenveloped human viruses, including adenoviruses, reoviruses and rotaviruses, use sialic acid-containing oligosaccharides as cell receptors (Stencel-Baerenwald et al. 2014). Sialic acid is not the only glycan that can be used by viruses for cell attachment. For example, flaviviruses, human respiratory syncytial virus and some of the herpesviruses use proteoglycans as a point of attachment to cells, whereas core fucosylation is suggested to play a role in HBV entry into hepatoma cells (Jolly and Sattentau 2013; Takamatsu et al. 2016). The important aspects of host glycans as receptors for viral infectivity will not be reviewed here as they are covered elsewhere (Jolly and Sattentau 2013; Stencel-Baerenwald et al. 2014; Kim et al. 2017). Instead, we will present the available information on glycosylation of human enveloped viruses. We will discuss general functions of glycosylation in various aspects of the viral life cycle and interaction with the immune system. In addition, we will discuss human herpesvirus glycosylation in more detail and include conclusions emerging from our recent global O-glycoproteomic analyses.

## N- and O-linked glycosylation of proteins

Glycosylation of viral envelope glycoproteins depends on the host glycosylation machinery. Viral glycoproteins, like cellular proteins, possess signal peptides directing them to the secretory pathway. Proteins targeted to the secretory pathway can be decorated with different kinds of post-translational modifications en route to the cell surface or another cellular compartment. Glycosylation is one of the most widespread and versatile modifications of proteins and is classified as N-, O- and C-linked, named after the acceptor amino acid atom to which the sugar moiety is attached. While there is only one type of N-linked glycosylation in terms of initiation, many types of O-glycosylation exist, classified based on the identity of initiating monosaccharides (Stanley 2011; Moremen et al. 2012). Most types of O-glycosylation are carried out in the secretory pathway, but cytosolic and nuclear proteins can also be glycosylated with N-acetylglucosamine (GlcNAc) by a cytosolic glycosyltransferase (Moremen et al. 2012; Yang and Qian 2017). Some types of O-glycosylation are specific to distinct classes of proteins or domains (Moremen et al. 2012; Haltom and Jafar-Nejad 2015; Larsen et al. 2017). In this review the term O-glycosylation will refer to GalNAc- or mucin-type O-glycosylation, which is one of the most widespread forms of protein O-glycosylation (Bennett et al. 2012). N-linked glycosylation of proteins is acquired cotranslationally as they fold in the endoplasmic reticulum (Rothman and Lodish 1977). The initial glycan moiety is comprised of nine mannose (Man), three glucose (Glc) and two GlcNAc residues that are transferred on proteins from a dolichol-phosphate-linked precursor to asparagine residues within NXS/T consensus sequons (X—all amino acids except P) (Robbins et al. 1977;

Bause 1983; Kornfeld and Kornfeld 1985). The initial glycan structure is trimmed to  $\text{Man}_8\text{GlcNAc}_2$  in the endoplasmic reticulum, with intermediates assisting in protein quality control (Hammond et al. 1994; Helenius and Aebi 2004). In the Golgi apparatus, the glycans can be further trimmed, elongated and branched by a differentially expressed set of glycosidases and glycosyltransferases to yield hybrid or complex-type N-glycans (Hunt et al. 1978; Rabouille et al. 1995; Schachter 2000). As proteins pass through the Golgi apparatus, they can also be modified with mucin-type O-glycans, initiated by a family of 20 GalNAc-transferases that add the initial GalNAc monosaccharide to Ser, Thr and possibly Tyr residues (Rottger et al. 1998; Halim et al. 2011; Bennett et al. 2012). The GalNAc-transferases exhibit somewhat overlapping, but also distinct substrate specificities and the expression of the isoenzymes is regulated in a tissue- and differentiation-specific manner (Sutherlin et al. 1997; Wandall et al. 1997, 2007; Mandel et al. 1999; Young et al. 2003; Tian and Ten Hagen 2006). Since there are no conserved protein sequence motifs for general or isoform-specific O-glycosylation, it is much more difficult to predict this modification (Gerken et al. 2011). However, prediction algorithms exist and are rapidly improving due to the vast increase in the number of identified O-glycosites using proteome-wide strategies. Nevertheless, experimental evidence is still required for reliable site identification (Steenfot et al. 2013). O-linked glycans can be further elongated by competing glycosyltransferases to form up to eight core structures, which adds to the heterogeneity of O-glycosylation and impedes its analysis (Brockhausen et al. 1990; Yeh et al. 1999; Dalziel et al. 2001; Schachter 2000). Although many types of protein glycosylation exist, N- and mucin-type O-linked glycosylation are the most studied in viral research. These types of glycosylation will also be the focus in the following sections.

## Methods for analysis of viral glycosylation

Historically, herpesviruses and togaviruses were some of the first viruses investigated for modification with carbohydrates, which led to important findings regarding general aspects of viral glycosylation. Early methods of analysis were based on monitoring the incorporation of radioactively labeled monosaccharides and amino acids into newly synthesized viral proteins during infection (Keller et al. 1970; Spear and Roizman 1970; Kim et al. 1976). It was thereby established that viral protein glycosylation took place at the cellular membranes and not the cytoplasmic compartment (Spear and Roizman 1970). Early glycoprofiling experiments utilized pronase digestion of labeled glycoproteins followed by gel filtration, which allowed separation of the short glycopeptides, bearing different glycan moieties (Hones and Roizman 1975; Schwarz et al. 1977). Moreover, differentially labeled sugars were used to provide insight into the putative composition of individual glycan structures (Sefton 1975). An important conclusion based on such experiments was that the extent of sugar incorporation into individual viral proteins differed depending on the infected cell type (Keller et al. 1970), establishing that viruses are dependent on the host glycosylation machinery. Use of glycosylation inhibitors, such as tunicamycin, 2-deoxy-D-glucose and glucosamine, provided additional insights into regulation of glycan synthesis, its impact on viral replication, and showed that glycosylation of viral proteins is critical for infectivity and cell-cell fusion (Knowles and Person 1976; Leavitt et al. 1977; Olofsson and Lycke 1980; Herrler and Compans 1983; Lambert and Pons 1983; Mann et al. 1983). Studies in cell lines deficient for specific glycosyltransferases or intracellular transport inhibitors provided additional means for investigating biological consequences of disrupted

glycan synthesis and maturation, respectively (Campadelli-Fiume et al. 1982; Serafini-Cessi, Dall'Olio, Scannavini, Campadelli-Fiume et al. 1983; Edwardson 1984).

Alongside numerous studies addressing viral N-glycan composition and function, it was discovered that viral envelope glycoproteins could also be modified with O-linked glycans (Olofsson et al. 1981; Shida and Dales 1981; Niemann et al. 1982; Gruber and Levine 1985; Montalvo et al. 1985; Gong et al. 1987; Lundstrom et al. 1991). As for human proteins, the Golgi apparatus was identified as the site of viral O-glycosylation (Johnson and Spear 1983; Locker et al. 1992). Historically it was presumed that few viruses were O-glycosylated and the function of this modification remained undetermined for some time (Feldmann et al. 1991; Bernstein et al. 1994). One of the first functions of viral O-glycosylation was discovered in vaccinia virus, where it has been demonstrated that the hemagglutinating activity of glycoprotein HA was entirely dependent on O-linked glycans (Shida and Dales 1981). A similar carbohydrate-dependent function was described for rubella virus, where treatment with a mix of glycosidases removing all glycans resulted in inhibition of hemagglutination (Ho-Terry and Cohen 1984).

Development of new biochemical methods facilitated the isolation and analysis of viral glycoproteins and glycans. Use of plant lectins or sera from immunized animals as well as vaccinated patients facilitated purification of viral envelope proteins and enabled analysis of glycans at the individual protein level (Eisenberg et al. 1979; Wenske et al. 1982; Friedrichs and Grose 1984; Respass et al. 1984; Montalvo et al. 1985). Introduction of chemical glycan release from the peptides or sequential enzymatic deglycosylation enabled more precise characterization of viral glycan size and composition compared to pronase digests (Burke and Keegstra 1979; Rasilo and Renkonen 1979), and led to determination of type and structure of N- and O-linked glycans for many viruses (Pesonen 1979; Pesonen, Kuismanen et al. 1982; Pesonen, Ronnholm et al. 1982; Niemann et al. 1984). The subsequent introduction of reverse phase HPLC further facilitated glycopeptide analysis, allowing separation of larger glycopeptides, generated by digestion of proteins with proteases of defined specificity, such as trypsin. Subsequent enzymatic glycan release enabled N-glycan analysis on isolated glycopeptides (Rosner and Robbins 1982; Cohen et al. 1983; Hsieh et al. 1983), and demonstrated site-specific glycan microheterogeneity in different hosts (Hsieh et al. 1983). Development and advancement of mass spectrometry-based applications had a large impact on the analysis of glycans, which was quickly adopted in the virology field. More recent advances in mass spectrometry-based glycoproteomics and glycoproteomics of enveloped viruses are discussed in the following sections.

### Glycoproteomics of viruses using mass spectrometry

Modern mass spectrometry-based methods of analysis enable robust characterization of N- or O-linked glycans in complex biological samples with unprecedented sensitivity and resolution. It has become routine practice in the characterization of recombinant therapeutics as well as vaccine candidates (Xie et al. 2011; Dubayle et al. 2015; Jacob et al. 2015). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is one of the most commonly used tools in modern day glycan analysis. Combining MALDI-MS with lectin microarray enables more reliable characterization of glycan structures, where lectin binding profiles can provide additional information on the glycosidic linkage (Mechref et al.

2003; Lei et al. 2015). Moreover, tandem MALDI-MS or tandem electrospray ionization MS allows fragmentation of selected precursor ions, enabling more detailed characterization of structures of a given composition (Mechref et al. 2003; Ritchie et al. 2010). The techniques were soon adapted for analysis of N-glycans on recombinant viral proteins and mature viral particles.

Applying mass spectrometry-based analysis to viral glycans released from mature viral particles illustrated the heterogeneity of N-glycan structures present on a single protein. For example, detailed studies on Dengue virions revealed enormous heterogeneity of N-glycan structures modifying two putative sites of Dengue virus glycoprotein, with 19 distinct structures identified (Lei et al. 2015). In addition, MS-based glycoproteomics demonstrated distinct glycosylation patterns in different host species for Chikungunya virus (Lancaster et al. 2016), confirming earlier observations on togaviruses (Hsieh et al. 1983).

MALDI-MS has also been used to compare glycan profiles of recombinantly expressed viral proteins and proteins isolated from native viruses. Comparison of N-glycan profiles on recombinant HIV-1 gp120 monomers and gp120/gp41 trimers, present on native viruses, revealed that while monomeric recombinant protein contained a high proportion of complex-type glycans, native trimers with preserved protein quaternary structure predominantly contained high-mannose type carbohydrates (Doores, Bonomelli et al. 2010; Bonomelli et al. 2011). The high-mannose type glycans consistently found in monomeric and trimeric formulations are part of the intrinsic mannose patch that contains densely spaced underprocessed glycans (Bonomelli et al. 2011). In contrast, analysis of dimeric viral glycoprotein of Hendra virus suggests that oligomerization does not always impede glycan processing (Bowden et al. 2010). Viral protein architecture, however, is not the only requirement for recapitulation of physiologically relevant glycosylation, as the repertoire of glycosyltransferases naturally also plays an important role. For example, N-glycans on gp120 on pseudovirions from the cell line HEK293T mainly carried  $\alpha$ 2,3-linked sialic acids, while N-glycans on gp120 from virions derived from peripheral blood mononuclear cells predominantly carried  $\alpha$ 2,6-linked sialic acids (Pritchard, Harvey et al. 2015). Such differences might have huge implications on immunogenicity of recombinant vaccine antigens, given that some broadly neutralizing antibodies to HIV-1 recognize glycan-containing epitopes (Doores and Burton 2010; Mouquet et al. 2012; McCoy and Burton 2017). Therefore, both antigen formulations and production cell lines must be considered very carefully.

Another concern when analyzing recombinant viral proteins as the source of information for viral glycosylation, is the occasional need for artificial formulations of recombinant viral proteins that often lack natural processing and oligomerization signals when not assembled and presented in the viral context (Bowden et al. 2008; Ritchie et al. 2010). For example, it has been shown that lack of proteolytic cleavage of the membrane-truncated soluble HIV-1 envelope glycoprotein (gp140) resulted in aberrant glycosylation that altered the conformation of the trimer (AlSalmi et al. 2015). Ebola virus glycoprotein represents another example of a highly elaborate protein complex assembly. It exists as three distinct species due to transcriptional stuttering—sGP, GP and ssGP—two of which are secreted (Lee and Saphire 2009). The membrane bound GP is proteolytically processed to GP1 and GP2, and forms a trimer at the cell surface (Lee and Saphire 2009). Nevertheless, formulations of GPs that differ from the natural viral context have been pursued for

glycoanalysis. *N*-glycan analysis was performed for soluble fragment of GP1 and sGP. The GP1 exhibited a mix of complex, hybrid and high-mannose structures, while sGP carried a higher proportion of processed structures (Ritchie et al. 2010). A more recent glycoprofiling of stabilized monomeric GP1,2 constructs from five different strains of Ebola virus confirmed the presence of heterogeneous and mostly complex-type *N*-glycans (Collar et al. 2016). This is in contrast to a highly variable *O*-glycan pattern in the different strains (Collar et al. 2016). A few other recombinant glycoproteins from Nipah virus and Machupo virus were expressed in their monomeric forms and reported to predominantly carry complex-type *N*-glycans (Bowden et al. 2008, 2009). In conclusion, it is difficult to predict glycosylation patterns of native oligomeric protein complexes based on studies of recombinant monomeric proteins. It is, however, sometimes the only solution because of difficulties in expression of correctly folded and authentic protein complexes.

MALDI-MS can also be applied for *O*-glycan analysis (Canis et al. 2010; Franc et al. 2013); however, it has not been widely used in the virology field and only a few examples of *O*-glycoprofiling of viral glycoproteins using MALDI-MS exist (Schmitt et al. 1999; Collar et al. 2016). It can probably be explained by lower interest in viral *O*-glycosylation as opposed to *N*-glycosylation, primarily due to poorly characterized functions of viral *O*-glycans and the presumption that most viral glycoproteins are not heavily *O*-glycosylated. In addition, more sophisticated methods allowing simultaneous determination of modified amino acids have rapidly taken over, which have in the recent years been widely used for analysis of viral *O*-glycosylation.

### Glycoproteomics of viruses

While glycoprofiling studies provide important information on distribution and composition of different glycan structures present on a given protein, it lacks the information on individual glycosite location, occupancy and glycan structure heterogeneity. The recent development of instruments equipped with ECD or ETD MS2 fragmentation has allowed for simultaneous determination of the peptide sequence and the position to which the carbohydrate moiety is attached (Leverly et al. 2015). When relevant reporter glycan oxonium ions are present and it is easy to predict the glycosylation position within peptides, HCD MS2 fragmentation can also be used (Wuhrer et al. 2007). Interfacing the mass spectrometer with capillary liquid chromatography enables separation of complex proteome-scale glycopeptide mixtures and allows identification of thousands of glycopeptides in a single run. At the single protein level, it is now possible to determine the exact position, structure heterogeneity, and site occupancy for each individual glycosite (Wuhrer et al. 2007). Both *N*- and *O*-glycosylation can be analyzed by tandem MS. Biosynthetic features of *N*-glycosylation enable relatively easy identification and quantification of deglycosylated sites by MS2 sequencing. Due to asparagine deamidation during enzymatic *N*-glycan removal, the site occupancy can be determined by calculating the intensity ratio of naked (Asn) and deglycosylated (Asp) peptides (Pabst et al. 2012; An et al. 2015). However, the more appropriate methodology includes carrying out the reaction using heavy-oxygen water ( $O^{18}$ -H<sub>2</sub>O), which discriminates deglycosylated sites from spontaneous Asn deamidation (Palmisano et al. 2012; Cao et al. 2017). It is also possible to evaluate the overall distribution of *N*-glycosite occupancy on intact glycoproteins by metabolically simplifying the glycans to homogeneous structures (Struwe et al. 2017). For *O*-linked glycans, complete *O*-glycan removal does not

result in chemical peptide modification and cannot be used for identification of glycosylated amino acid positions (Leverly et al. 2015). ETD and ECD MS2 techniques allow analysis of intact *O*-glycopeptides by fragmentation of the peptide backbone without the loss of *O*-glycan modification, however, it is of limited use for determining site occupancy (Wuhrer et al. 2007; Sihlbom et al. 2009; Zauner et al. 2012). Approximations are yet often made by comparing intensities of nonmodified and glycosylated peptides (Brautigam et al. 2013; Stansell et al. 2015). The relative quantitation of both *N*- and *O*-glycosylated peptides is not as accurate due to different ionization efficiencies of nonglycosylated peptides and peptides carrying complex glycans. Dependent on the complexity of the glycans, glycopeptides generally exhibit poorer ionization compared to peptides (Stavenhagen et al. 2013). The analysis of complex proteins therefore often requires enrichment with hydrophilic interaction chromatography or specific lectins, which is particularly relevant for proteome-wide applications (Bunkenborg et al. 2004; Zielinska et al. 2010; Khatri et al. 2014; Leverly et al. 2015). However, information regarding site occupancy needs to be sacrificed.

### *N*-glycoproteomics

Tandem mass spectrometry has been widely used for characterization of *N*-glycosylation sites on viral proteins, with respect to individual site occupancy status (macroheterogeneity) and site-specific structural diversity (microheterogeneity), as these are important features that can affect protein-protein interactions and immunogenicity. Comprehensive glycoproteomic analysis has, for example, mapped *N*-linked glycosylation of seasonal influenza A virus H3N2 HA, identifying more than 90 % site occupancy of all putative *N*-linked glycan sites (An et al. 2015). Moreover, the globular head glycosites, associated with host immune receptor interaction, were strictly high-mannose type (An et al. 2015). In a separate study on the highly pathogenic H5N1 influenza A virus, it was also shown that all potential *N*-glycosites were consistently occupied between several different strains (Blake et al. 2009), suggesting that *N*-glycosylation of HA is conserved between the different isolates of the same virus subtype, with a high occupancy of potential *N*-glycosites. In a similar way, it has been demonstrated that all predicted *N*-glycan sequons were utilized in Hepatitis C virus E2 and Murray Valley encephalitis virus NS1 (Blitvich et al. 2001; Iacob et al. 2008), where the majority of HCV E2 glycosites were modified with high-mannose type glycans (Iacob et al. 2008). On the densely glycosylated HIV-1 envelope glycoprotein several strategies have been employed to characterize the nature and location of the many glycans, with up to 27 mostly highly occupied *N*-glycosites identified, some of which were exclusively high-mannose type (Pabst et al. 2012; Go et al. 2013; Yang W et al. 2014). Tandem mass spectrometry has also been used for addressing differences in cell-type specific glycosylation and influence of protein conformation. Out of convenience, soluble HIV-1 gp120 or gp140 preparations are often analyzed. HIV-1 gp120 produced in CHO and 293 T cells had very similar occupancy, degree of fucosylation, sialylation, and glycan maturation, with a larger share of glycosites predominantly carrying hybrid and complex-type *N*-glycans (Go et al. 2013). The positions of some of the exclusively high-mannose *N*-glycans were located within the intrinsic mannose patch of gp120 (Go et al. 2013; Behrens et al. 2016). In contrast, a much higher proportion of the *N*-glycosites on gp140 expressed in CHO, recombinant trimers, or those on gp120 purified from native virions were modified with high-mannose type *N*-glycans (Pabst et al. 2012; Behrens et al. 2016;

Panico et al. 2016; Go et al. 2017). This again signifies the importance of analyzing the native protein conformation for generation of relevant glycosylation patterns. Glycosylation in different cell lines was also investigated for Hendra virus recombinant glycoprotein G, where all seven potential N-glycan sites were occupied in HeLa cells (Colgrave et al. 2012). In contrast, only four sites were N-glycosylated in HEK293, although the degree of glycan maturation was similar in both cell lines (Colgrave et al. 2012). To summarize the results obtained from various N-glycoproteomic and glycoproteomic studies, it seems that most of the putative N-glycosylation sequons are glycosylated with high occupancy on viral proteins. However, the site occupancy of viral protein N-glycosylation can vary in different producer cell lines. Moreover, high-mannose type N-glycans may constitute a substantial, if not the major, proportion of viral N-glycosites, particularly when native protein structure and oligomerization is taken into account. Thus, results obtained from analysis of recombinant monomeric proteins should be interpreted with caution also when considering the occupancy of individual N-glycosylation sites as well as complexity of glycan structures as discussed above.

### O-glycoproteomics

For decades very little information has been available regarding site-specific O-glycosylation of viral proteins. Some of the first described virus-derived site-specific O-glycans were on isoform M of HBV surface antigen purified from patient-derived viral particles (Schmitt et al. 1999). The O-glycosylation site was identified by combining MALDI analysis of exoglycosidase-treated glycopeptide and Edman sequencing of the underlying peptide. The position of the glycan attachment site was deduced by carboxypeptidase digestion and confirmed by collision-induced dissociation tandem mass spectrometry, representing some of the early glycoproteomic experiments of viral proteins (Schmitt et al. 1999). The recent advances in mass spectrometry-based proteomics have resulted in numerous studies addressing O-glycosylation of individual viral glycoproteins. O-glycoproteomic analyses have been performed for several recombinant or isolated viral glycoproteins, including HIV-1 gp120, influenza A virus HA1, HCV E2, HSV-1 gC and Hendra virus glycoprotein G (Colgrave et al. 2012; Brautigam et al. 2013; Go et al. 2013; Yang W et al. 2014; Norden et al. 2015; Stansell et al. 2015). Recombinantly expressed gp120 and HA1 were glycosylated at a single position each, which in both cases was modified with core 1 or core 2 elongated O-glycans (Stansell et al. 2015). Interestingly, site occupancy was much lower in recombinant gp140 undergoing proteolytic cleavage to gp120, assuming equivalent ionization efficiencies of nonmodified and glycosylated peptides. Recombinant gp140 possessed shorter less sialylated, predominantly core 1 O-glycans, again underlining the importance of native protein conformation for analogous studies (Stansell et al. 2015). In contrast, gp120 purified from T-cell derived virions was devoid of the single site found glycosylated in recombinantly expressed gp120 (Stansell et al. 2015). This, however, might be related to the viral strain used, as the single site was reported to be glycosylated in a separate study using virions from a different HIV-1 strain (Yang W et al. 2014). In addition, treatment of passaged or plasma-derived HIV-1 virions with antibodies against O-linked carbohydrate structures resulted in inhibition of cell entry, and virus neutralization (Hansen et al. 1990, 1991), suggesting that HIV-1 gp120 can indeed be O-glycosylated in vivo. Comparison of recombinant gp120 O-glycosylation in two different cell lines revealed predominant core 1 O-glycosylation in CHO cells, compared to core 1, core 2 and core 4 in 293 T cells

(Go et al. 2013). In a similar manner, Hendra virus glycoprotein G expressed in HeLa and HEK293 cells, differed considerably with different numbers of O-glycosites identified and carrying different core structures (Colgrave et al. 2012). Recombinant HCV E2 was O-glycosylated at six positions, with predominantly core 1 and core 2 O-glycan structures (Brautigam et al. 2013). More than 80 % occupancy was estimated for five of the six sites, whereas one site had very low occupancy. Moreover, a high level of structural heterogeneity was observed for the O-glycans localized at the individual sites, with up to 14 different structures identified (Brautigam et al. 2013). A recent study on O-linked glycosylation of HSV-1 mucin-like protein gC provided some insight into O-glycan synthesis, suggesting that the eleven O-glycosites were added in an orderly fashion, before elongation took place (Norden et al. 2015). These studies underscore the high heterogeneity of O-glycan structures, which are both cell type and protein specific, and the need for careful selection of candidates and expression cell lines for clinical applications. Moreover, comprehensive analysis of immune responses mounted by these different structures would be highly beneficial.

### Global O-glycoproteomics

While it is clear that single protein-targeted mass spectrometry approaches can provide comprehensive information on single site occupancy and structure heterogeneity, we lack robust methods of analysis for site-specific O-glycosylation in complex or proteome-wide samples. To solve this problem, we recently introduced a method for globally mapping O-glycosylation sites in glycoengineered cell lines lacking O-linked glycan elongation, which is based on *Vicia villosa* lectin (VVA) affinity enrichment of simple glycopeptides coupled to tandem mass spectrometry (Stentoft et al. 2011). The method is also applicable for analysis of wild type cells, predominantly expressing core 1 O-glycans, by using peanut agglutinin (PNA) enrichment of desialylated glycopeptides (Yang Z et al. 2014). We have applied these methods in the analysis of O-glycosylation of HSV-1 infected human fibroblasts by performing a sequential enrichment with PNA and VVA, thus reporting the first comprehensive viral O-glycoproteome (Bagdonaite et al. 2015). This approach provides several clear advantages: first of all, it allows simultaneous analysis of all viral glycoproteins expressed in an infected cell. Secondly, the strategy takes into account the endogenous glycosylation of a permissive cell, dictated by the repertoire of glycosyltransferases, as well as native conformations of proteins and the cytopathic effects of viral infection. Irrelevant cell lines are often chosen for recombinant expression of viral proteins. The glycosylation obtained in these cell lines does not always reflect the glycosylation pattern in a natural host.

Using herpesviruses as a model system, we have applied the same method for defining the O-glycoproteomes of other members of *Herpesviridae* family—HSV-2, VZV, HCMV and EBV (Bagdonaite et al. 2016; Iversen et al. 2016). The wide occurrence, associated complications and shortage of prophylactic measures make herpesviruses a relevant model system to analyze O-glycans and their importance in viral life cycle (Vazquez et al. 2001; Cohen et al. 2006; Adjei et al. 2008; Kramer et al. 2008; Oxman 2010; Shiley and Blumberg 2010; Lopo et al. 2011; Sauerbrei et al. 2011; Levine et al. 2012; van Rijckevorsel et al. 2012; Astuto et al. 2013; Conde-Glez et al. 2013; Fishman 2013; Gorfinkel et al. 2013; Odland et al. 2013; Pembrey et al. 2013; Rowe et al. 2013; Awasthi and Friedman 2014; Bradley et al. 2014; Fu et al. 2014; Sabugo et al. 2014; Sili et al. 2014; Chen et al. 2015; Cohen 2015; Korndewal et al. 2015;

Shaiegan et al. 2015). In addition, the large proteomes of herpesviruses highlight the benefits of global viral O-glycoproteomics. Human herpesviruses encode seven to 12 glycoproteins associated with the viral particle; however, many more viral proteins possess signal peptides and transit through the host secretory pathway. Some of them have previously been investigated for glycan modifications in focused studies. N-linked glycans have been identified on viral envelope glycoproteins from all 8 human herpesviruses (Wenske et al. 1982; Edson and Thorley-Lawson 1983; Friedrichs and Grose 1984; Serafini-Cessi et al. 1984, 1985, 1989; Montalvo et al. 1985; Montalvo and Grose 1986, 1987; Gong et al. 1987; Britt and Vugler 1989; Gong and Kieff 1990; Okuno et al. 1990, 1992; Foa-Tomasi et al. 1992; Nolan and Morgan 1995; Pfeiffer et al. 1995; Hata et al. 1996; Mukai et al. 1997; Chandran et al. 1998; Pertel et al. 1998; Huber and Compton 1999; Li et al. 1999; Zhu et al. 1999; Baghian et al. 2000; Skrincoy et al. 2000; Wu et al. 2000; Maresova et al. 2000; Theiler and Compton 2002; Koyano et al. 2003; Paulsen et al. 2005; Yamagishi et al. 2008; Gore and Hutt-Fletcher 2009; Luo et al. 2015), where individual glycoproteins have been demonstrated to exhibit variable extent and pattern of glycan chain maturation (Wenske et al. 1982; Edson and Thorley-Lawson 1983; Friedrichs and Grose 1984; Serafini-Cessi et al. 1984, 1985, 1989; Montalvo et al. 1985; Montalvo and Grose 1986, 1987; Britt and Vugler 1989; Gong and Kieff 1990; Okuno et al. 1990, 1992; Huber and Compton 1999; Maresova et al. 2000; Theiler and Compton 2002; Yamagishi et al. 2008). A relatively smaller proportion of envelope glycoproteins of herpesviruses have been investigated in terms of O-glycosylation, and in some of these envelope proteins O-glycans have been detected by biochemical assays (Serafini-Cessi, Dall'Olivo, Scannavini, Costanzo et al. 1983; Montalvo et al. 1985; Gong et al. 1987; Montalvo and Grose 1987; Serafini-Cessi et al. 1988, 1989; Britt and Vugler 1989; Kari et al. 1992; Yao et al. 1993; Nolan and Morgan 1995; Borza and Hutt-Fletcher 1998; Cardinali et al. 1998; Lake et al. 1998; Peng et al. 1998; Torrisi et al. 1999; Zhu et al. 1999; Wu et al. 2000; Theiler and Compton 2002; Xiao et al. 2007). Only a few of these proteins have merited more thorough investigation with most attention devoted to proteins containing mucin-like domains. HSV-1 attachment factor gC was the first envelope glycoprotein described to carry O-glycans, acquiring distinct structures in different cell types (Olofsson et al. 1981, 1983; Dall'Olivo et al. 1985; Lundstrom et al. 1987), and specific O-glycosites have recently been mapped to the mucin-like region (Bagdonaite et al. 2015; Norden et al. 2015). Furthermore, the HSV-2 and VZV orthologs were also found to be O-glycosylated (Zezulak and Spear 1983; Bagdonaite et al. 2016). Similarly, other mucin-like region-containing proteins such as HSV-1 gI, HSV-2 gG, EBV gp150 and gp350 have been shown to accommodate high density of O-glycosylation, and the types of O-glycan structures were identified for some of these proteins (Serafini-Cessi et al. 1985, 1989; Nolan and Morgan 1995; Borza and Hutt-Fletcher 1998; Norberg et al. 2007). The conserved viral fusion effector gB has been shown or predicted to be O-glycosylated in all herpesvirus subfamilies (Serafini-Cessi, Dall'Olivo, Scannavini, Costanzo et al. 1983; Gong et al. 1987; Montalvo and Grose 1987; Britt and Vugler 1989). The era of proteome-wide mass spectrometry-based applications allowed robust characterization of viral O-glycoproteomes (Bagdonaite et al. 2015, 2016; Iversen et al. 2016). The characterizations confirmed the identity of the majority of previously described O-glycoproteins of herpesviruses, and provided a tremendous expansion of site-specific O-glycosylation (Bagdonaite et al. 2015, 2016; Iversen et al. 2016). While GalNAc-type O-glycosylation is often associated with dense glycosylation in

mucin-like regions, it is also abundantly found in isolation or small clusters in human proteins (Stentoft et al. 2013), which is more difficult to predict. In agreement with this, we have demonstrated ample presence of isolated O-glycan sites on viral glycoproteins of HSV-1, HSV-2, VZV, HCMV and EBV by glycoproteomic approaches (Bagdonaite et al. 2015, 2016; Iversen et al. 2016). Location of O-glycosites identified via proteome-wide MS/MS approaches with respect to protein structural features suggests possible involvement in the protein-protein interactions (Bagdonaite et al. 2015, 2016), as exemplified in subsequent sections. Large scale glycoproteomic analyses of human herpesviruses of varying phylogeny (HSV-1, HSV-2, VZV, HCMV and EBV) have made it possible to compare the O-glycosite patterns in homologous proteins (Bagdonaite et al. 2015, 2016; Iversen et al. 2016). Comparison of O-glycosite conservation between alphaherpesviruses HSV-1 and HSV-2 suggests that sequence homology is an important determinant for O-glycosylation in closely related viruses (Figure 1A and F). Isolated homologous glycosites were mainly situated on highly homologous peptide stretches, whereas densely spaced glycosites in Pro/Ser/Thr-rich regions were glycosylated irrespective of low sequence identity, as expected (Bagdonaite et al. 2016). Several glycoproteins are homologous between all herpesviruses, including gB, gH, gL, gM and gN, of which gB, gH and gL comprise the conserved cell entry machinery (McGeoch et al. 2006). We identified a large number of O-glycosites on HSV-1 fusogenic effector gB, and predicted that a number of O-glycosites could be conserved in most, if not all, human herpesviruses (Figure 1A–C) (Bagdonaite et al. 2016). Based on multiple sequence alignments across investigated herpesvirus family members, enrichment of O-glycosylation was found in the extreme N-terminus of gB regardless of the underlying considerable sequence variation between different herpesviruses. This suggests that glycosylation patches are less dependent on the underlying sequence, and might serve a glycan specific function, such as protection of the N-terminal exposed region of gB from proteolytic degradation or immune recognition. In contrast, conserved single glycosites were predominantly found between HSV-1, HSV-2, and, to a smaller extent, VZV, and suggest that they mainly exert subfamily-specific functions. The conserved protein gH, which is another essential component of the fusion machinery, was found glycosylated in four out of five investigated viruses. Although no clear conserved pattern of glycosylation was observed, the O-glycosites were predominantly localized to the two exposed N-terminal domains involved in interaction with other viral proteins (Figure 1D and E) (Bagdonaite et al. 2016).

In summary, global O-glycoproteomics of viruses open up possibilities to rapidly “scan” the proteome of viruses for O-glycan modifications. Although the occupancy and the relevance of the individual glycan sites are still unknown, the information can be used to follow up by complimentary techniques at individual protein and glycosite level. It can be applied to any human virus of interest; given relevant propagation systems are available. The method, of course, has its limitations, such as a limited number of glycoforms that can be captured, as well as the availability of protein sequences in the databases, which is challenging when analyzing emerging or poorly annotated viruses, as well as clinical isolates. Another aim for the future is to make the results broadly available to the scientific community not only by means of publishing, but also by inclusion into public protein databases. Ideally, a virus database compiling structural data, sequence variability, available glycomic and glycoproteomic data as well as antigenic sites could be created to advance basic and applied research in virology. If sufficient experimental data is compiled, machine learning bioinformatic techniques could

be applied to predict glycosylation patterns of emerging viral strains within distinct virus species or even families.

### Roles of glycosylation in virus biology

Glycans on viral envelope glycoproteins play important roles in virus biology, with specific functions identified in various stages of viral infection (Table 1 and Figure 2). As previously mentioned, glycosylation is generally required for progeny formation and infectivity for many viruses (Knowles and Person 1976; Leavitt et al. 1977; Olofsson and Lycke 1980; Herrler and Compans 1983; Lambert and Pons 1983; Mann et al. 1983; Kuhn et al. 1988; Li et al. 2006; Reszka et al. 2010; Mathys and Balzarini 2015; Shrivastava-Ranjan et al. 2016; Mossenta et al. 2017). In some cases, mature N-glycans in particular are necessary for proper viral particle formation and release (Datema et al. 1984). As in human proteins, N-linked glycosylation is critical for proper folding of viral proteins (Land and Braakman 2001; Zai et al. 2013; Wu et al. 2017), and, in some cases, viral protein secretion (Hu et al. 1995; Lu et al. 1997; Yoshii et al. 2013; Mossenta et al. 2017). Drugs targeting glycoprotein maturation and exhibiting a favorable therapeutic ratio can therefore be used to combat viral infections, where no other therapeutics are available (Miller et al. 2012). Most importantly, N- and O-linked glycans shield immunodominant epitopes from immune recognition (Francica et al. 2010; Helle et al. 2011; Sommerstein et al. 2015; Behrens et al. 2016; Gram et al. 2016; Walls et al. 2016). In addition to general functions, specific types of glycans on viral glycoproteins often affect receptor binding and cell fusion (Lin et al. 2003; Lozach et al. 2003; Gramberg et al. 2005; Davis et al. 2006; Miller et al. 2008; Chen et al. 2014; Phoenix et al. 2016; Wang et al. 2016). Moreover, it is becoming increasingly clear that distinct glycans play separate roles in various stages of the viral cycle (Goffard et al. 2005; Falkowska et al. 2007; Helle et al. 2010; Wang et al. 2013, 2017; Lennemann et al. 2014; Bradel-Tretheway et al. 2015; Luo et al. 2015; Orlova et al. 2015; Wu et al. 2017). Some site-specific functions of viral glycosylation have been assigned for both N- and O-linked glycans mostly by site-directed mutagenesis studies. Identified roles of glycans within various stages of the viral cycle will be discussed in the following sections.

### Attachment and entry

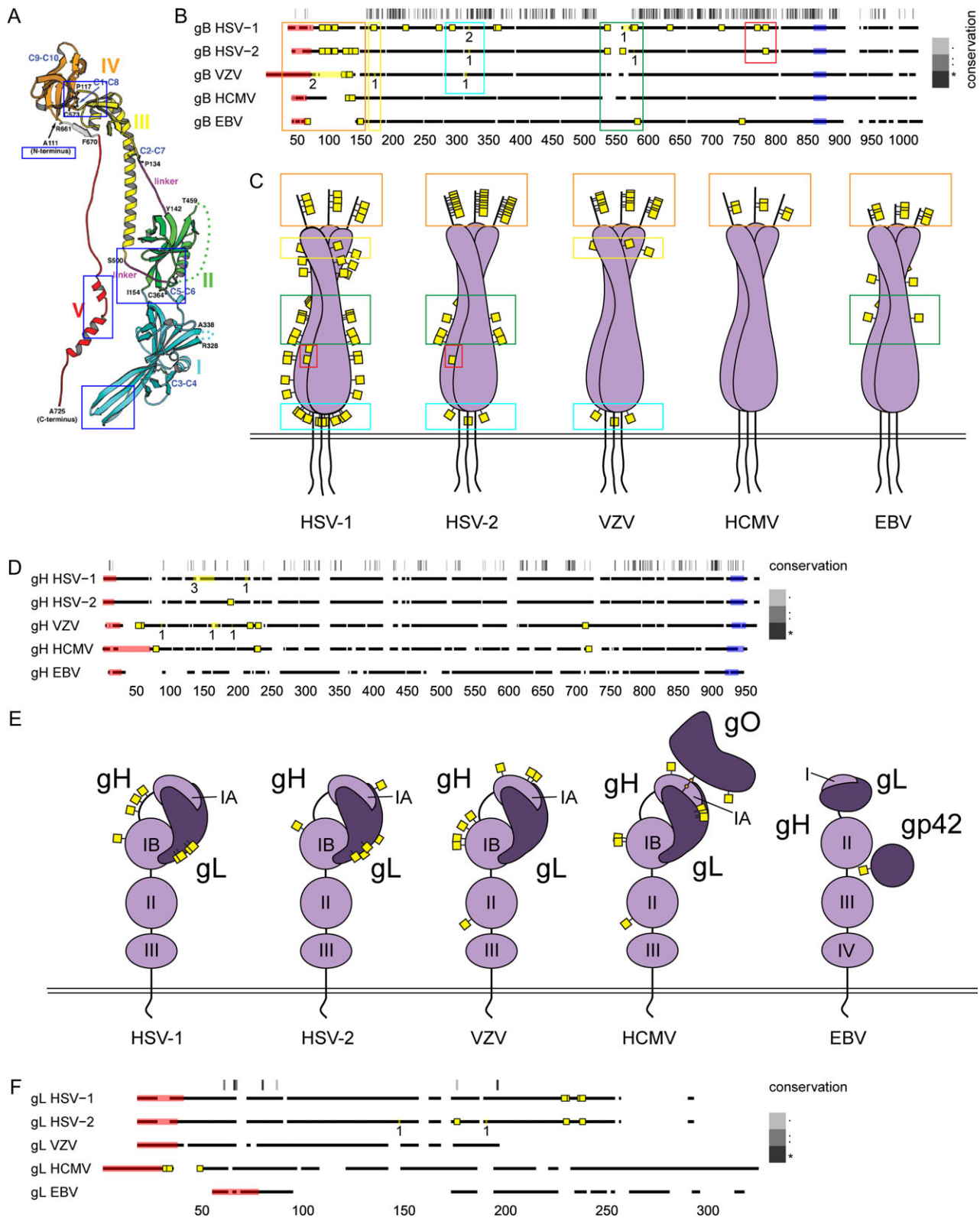
A number of viruses, such as HCV, Dengue virus, Ebola virus, SARS coronavirus, West Nile virus, Rift Valley fever virus and Japanese encephalitis virus use N-linked glycans as attachment and entry receptors by interacting with cellular lectins DC-SIGN, L-SIGN, LSECtin, ASGP-R or mannose receptor (Becker et al. 1995; Lin et al. 2003; Lozach et al. 2003; Gramberg et al. 2005; Davis et al. 2006; Miller et al. 2008; Chen et al. 2014; Phoenix et al. 2016; Wang et al. 2016). Mature N-glycans have been suggested to influence Lassa virus binding to cell entry receptor  $\alpha$ -dystroglycan (Shrivastava-Ranjan et al. 2016). In addition, specific N-glycans on HIV-1 have been shown to affect CD4 receptor binding on T cells or regulate coreceptor usage (Nakayama et al. 1998; Pollakis et al. 2001; Francois and Balzarini 2011; Lombardi et al. 2015). Systemic functional analysis of HIV-1 gp160 potential N-glycosylation sites has identified a number of additional glycosites affecting infectivity (Wang et al. 2013). In addition, a certain N-glycan on HIV-1 gp41 was shown to have a consistent effect in several viral strains (Mathys and Balzarini 2014). Specific sialylated N-glycans on VZV gB have been identified that are important for interaction with

myelin-associated glycoprotein and crucial for cell–cell fusion (Suenaga et al. 2015), and sialylation of virions in general was shown to affect entry of HSV-1 (Teuton and Brandt 2007). Similarly, specific N-linked glycans on HSV-2 gB influenced viral entry (Luo et al. 2015). An N-glycosylation site on IAV HA has also been shown to affect viral entry indirectly by modulating the avidity and specificity for sialosides (Wu et al. 2017). Finally, distinct putative N-glycosylation site mutations on HCV E1 and E2 resulted in diminished entry and altered CD81 binding (Goffard et al. 2005; Falkowska et al. 2007; Helle et al. 2010).

In contrast to the rather well documented roles of N-linked glycans, there are very few known examples, where specific O-glycans participate in virus–host interaction. Among these, two sialylated O-glycans on HSV-1 gB have been identified that determine binding to cellular receptor paired immunoglobulin-like type 2 receptor  $\alpha$  residing on immune cells (Wang et al. 2009; Arii et al. 2010; Bagdonaite et al. 2015). In addition, HSV-1 O-glycans are involved in a few other aspects of viral binding to the host cell, as deletion of the densely O-glycosylated region of attachment factor gC affects both the binding affinity to the cell surface, and the release of progeny virus via modulation of interactions with cell surface glycosaminoglycans (Altgarde et al. 2015). Another example includes the carbohydrate-dependent binding of filoviruses to the macrophage galactose lectin that is known to recognize GalNAc-O-glycans (Takada et al. 2004). In hepatitis C virus, the mutation of several putative O-glycosites has also been shown to decrease HCV E2 affinity for CD81, suggesting that O-glycans might be of a more general importance in mediating interaction with host cells (Falkowska et al. 2007). O-glycoproteomic analysis of Hendra virus glycoprotein G has recently sprouted the first, to the best of our knowledge, systematic functional analysis of known O-glycosylation sites, revealing a multitude of functions including attachment and entry to host cells (Colgrave et al. 2012; Stone et al. 2016). Importantly, most of the functions could also be identified in analogous O-glycosites of a closely related Nipah virus (Stone et al. 2016). Interestingly, only in Nipah virus a single O-glycosite significantly affected Ephrin B2 receptor binding (Stone et al. 2016). This is an important example of conserved O-glycan function between closely related viruses, and presents intriguing possibilities in the light of recently identified consistent O-glycosylation patterns of several herpesviruses. Besides confirming the HSV-1 O-glycosites involved in binding PILR $\alpha$  in vitro, a few other O-glycosites residing in protein regions expected to influence HSV-1 attachment via cell entry receptors nectin-1 and HVEM (Carfi et al. 2001; Krummenacher et al. 2005; Heldwein et al. 2006; Di Giovine et al. 2011; Gallagher et al. 2014), or VZV attachment to receptor insulin degrading enzyme (Berarducci et al. 2010) were identified (Bagdonaite et al. 2015, 2016). This is another example how MS-based approaches combined with structural knowledge can help narrow down a list of O-glycosite candidates for focused studies. In conclusion, glycans on viral entry proteins are widely used for modulation of receptor binding and entry, with both N-linked and O-linked glycans having the capacity to affect the interaction (Figure 2, top left panel). In some cases, though, it seems to be an effect of conformational stability, rather than direct interaction (Stone et al. 2016).

### Assembly and exit

Specific N-glycosites have been identified, that are important for glycoprotein secretion and viral particle formation in some viruses, including Zika virus, hepatitis viruses B and C (HBV and HCV),



**Fig. 1.** O-glycosylation of herpesvirus conserved fusion machinery. **(A)**, Crystal structure representation of HSV-1 gB monomer. From "Heldwein EE Lou H Bender FC Cohen GH Eisenberg RJ Harrison S 2006. Crystal structure of glycoprotein B from herpes simplex virus 1. *Science*, 313:217–220". Reprinted with permission from AAAS. Blue boxes mark the parts of the molecule where O-linked glycosylation sites are consistently found between at least two investigated herpesviruses. Modified with permission from the authors. **(B)**, **(D)** and **(F)**, Conservation of O-linked glycosylation sites on homologous envelope glycoproteins of human herpesviruses (from Bagdonaite et al., 2016). Reprinted with permission. © 2008 The American Society for Biochemistry and Molecular Biology. All rights reserved. Clustal Omega server was used to align amino acid sequences of gB **(B)**, gH **(D)** and gL **(F)** between HSV-1 (Bagdonaite et al. 2015), HSV-2 (Iversen et al. 2016),



HSV-2 and HIV-1 (Fournillier et al. 2001; Falkowska et al. 2007; Helle et al. 2010; Ito et al. 2010; Wang et al. 2013; Luo et al. 2015; Mossenta et al. 2017). A few N-glycosites on influenza A virus neuraminidase have been shown to affect protein activity and viral particle release (Wu et al. 2017). Also, it is well documented that N-linked glycosylation of alphaherpesvirus envelope proteins is required for trafficking to the cell surface and viral particle egress (Norrlid and Pedersen 1982; Montalvo et al. 1985; Luo et al. 2015; Suenaga et al. 2015). In particular, complex-type N-glycans are required for robust egress (Serafini-Cessi, Dall'Olio, Scannavini, Campadelli-Fiume et al. 1983). In varicella zoster virus the role of potential O-glycosylation sites has also been addressed by site-directed mutagenesis. Several glycosites were found to affect cell surface expression of the protein. However, such an approach is too laborious for analysis of all potential O-glycosylation sites, given the lack of conserved sequence motifs for O-glycosylation (Suenaga et al. 2015). As a more targeted approach, site-directed mutagenesis of some of the O-glycosites on Hendra virus glycoprotein G, identified via an O-glycoproteomic approach, affected the incorporation of glycoprotein F into pseudotyped virions. This suggests that the O-glycans might be required for protein–protein interaction (Stone et al. 2016). Similarly, in HIV gp120 O-glycosylation of a conserved threonine that is crucial for the association with gp41 has recently been shown to enhance infectivity and incorporation of gp120 into virions, though the virus maintains normal functionality in the absence of the O-glycan (Termini et al. 2017).

In conclusion, manipulation of glycans or glycosylation sites seems to affect both viruses that use direct budding and those using exocytosis. It is therefore likely that glycans affect many different aspects of viral particle formation (Figure 2, bottom right panel), possibly through an indirect consequence of disrupted trafficking of glycoproteins that are required for virus formation (Stone et al. 2016; Mossenta et al. 2017).

### Viral spread and innate immunity

Different viruses employ various spread mechanisms both systemically and within the site of infection, including cell–cell fusion, transmigration and neuroinvasion. Viruses often take advantage of components of the innate immune system to target the cell type of interest at distant locations. Glycans play an important role in these mechanisms (Figure 2, bottom left panel). In the deadly Hendra virus the globular head region of glycoprotein G is important for binding to the entry receptors Ephrin B2/B3. Mutation of N-glycosylation sites in the globular head of glycoprotein G increased cell–cell fusion, whereas removal of a glycosite in the stalk region of Hendra glycoprotein G abolished cell–cell fusion (Bradel-Tretheway et al. 2015). In case of O-glycans, however, removal of distinct

O-glycosites within a cluster in the stalk region resulted in both hypo- and hyperfusogenic phenotypes (Stone et al. 2016), and neither N- nor O-glycosite mutants exhibited impaired receptor binding (Bradel-Tretheway et al. 2015; Stone et al. 2016). This suggests that glycans influence other aspects of membrane fusion; possibly by affecting the thermodynamics of conformational changes. The impact of N-glycans in viral spread has also been demonstrated in *in vivo* models. Interestingly, the glycosylation status of West Nile virus glycoproteins has been associated with neuroinvasiveness, where abolishment of glycosites led to attenuation (Whiteman et al. 2010). Similarly, loss of a N-glycosite on Ross River virus glycoprotein E1 was associated with milder pathogenesis and increased virus clearance in a disease mouse model (Nelson et al. 2016). Finally, specific N-glycosylation patterns on both HA and NA on avian influenza A virus are required for efficient transmission in mammalian models (Park et al. 2016). A more direct role of glycans emerges from the viral exploitation of innate immune receptors as a helping hand during establishing the viral infection. Such innate immune receptors and other secreted glycan binding proteins present on infected host cells or antigen presenting cells are naturally the first line of defense against invading pathogens. For instance, secreted C-type lectins, ficolin-2 and mannose-binding lectin, inhibit HCV entry into hepatocytes (Brown et al. 2010; Zhao et al. 2014). However, these carbohydrate binding proteins can convey both protective and deleterious effects during viral infection, which are exploited by viruses to enter host cells. For example, secreted galectin-1 promotes entry of Nipah virus into endothelial cells by bridging viral and cellular glycans, when the glycan binding proteins are present during initial phases of infection. In contrast, galectin-1 inhibits viral spread, when added postinfection (Garner et al. 2015). Galectin-1 has also been shown to promote HIV-1 infection by crosslinking the viral particles and cell entry receptors, whereas influenza virus infection was inhibited (St-Pierre et al. 2011; Yang et al. 2011). As already mentioned, several viruses specifically use C-type lectin receptors for entry or transmission. Ebola virus utilizes an array of different C-type lectins, such as L-SIGN, DC-SIGN and LSECtin, for entry into various cell types (Lennemann et al. 2014). HIV-1 uses DC-SIGN on dendritic cells for migration to T cells (Hong et al. 2002). Although it is not entirely clear how the balance between antigen presentation and transmission is maintained, it has been proposed that homogeneous high-mannose type N-glycans enhance antigen presentation and virus degradation compared to intrinsically heterogeneous gp120 N-glycosylation (van Montfort et al. 2011). Interestingly, HIV-1 has also been shown to exploit sialic-acid binding lectin Siglec-1 for more efficient entry into macrophages, likewise promoting the existence of complex-type N-glycans on viral particles (Zou et al. 2011). In a similar context, we have demonstrated,

VZV (Bagdonaite et al. 2016), HCMV (Bagdonaite et al. 2016) and EBV (Bagdonaite et al. 2016). Protein backbones are depicted as broken black lines, where spaces represent gaps in the alignment. Individual alignments were drawn to scale (indicated below each graph). Sequence conservation is indicated above the aligned sequences for each set, and is represented by a greyscale barcode that maps to the clustal alignment score, as shown in the legend. In brief, for the clustal alignment score, an asterisk indicates positions with fully conserved residues, a colon indicates conservation of amino acids with strongly similar properties, whereas a period indicates conservation of amino acids with weakly similar properties. Predicted signal peptides and transmembrane regions are shaded in pink and blue, respectively. Unambiguous O-glycosylation sites are shown as yellow squares, whereas ambiguous sites are marked as yellow lines within the protein backbone, where the number below indicates the number of glycosites. An ambiguous O-glycosylation site from our previous publication (Bagdonaite et al. 2015, HSV-1 gB 109–123 (HexHexNAc)) was omitted from the graph, as we cannot exclude the possibility it could be part of an elongated structure on an adjacent site. Reference strain sequences were used for HSV-2, VZV and EBV due to incomplete or unavailable annotation of investigated strains. HSV-1—human herpes simplex virus type 1 (strain 17), HSV-2—human herpes simplex virus type 2 (strain HG52), VZV—varicella-zoster virus (strain Dumas), HCMV—human cytomegalovirus (strain Towne), EBV—Epstein-Barr virus (strain AG876). (C) and (E) Cartoon depiction of HSV-1 gB trimers (C) or gH-gL complexes and accessory proteins (E) of the five herpesviruses. O-glycosylation sites are shown as yellow squares. (B) and (C) Colored boxes mark association with herpesvirus gB domains as defined in (A).

**Table I.** Glycosylation of viral proteins of human enveloped viruses

Abbr.	Virus name	Family	Genus	Genome	N-glycosylated <sup>a</sup>	O-glycosylated <sup>a</sup>	Binding/entry/ haemagglutination/infectivity <sup>a</sup>	Spread <sup>a</sup>	Formation/release/ yield/protein transport <sup>a</sup>	Immune response <sup>a</sup>	Protein– protein interaction and other functions <sup>a</sup>
LASV	Lassa virus	<i>Arenaviridae</i>	<i>Arenavirus</i>	(–)ssRNA			Shrivastava-Ranjan et al. (2016)			Sommerstein et al. (2015)	
MACV	Machupo virus	<i>Arenaviridae</i>	<i>Arenavirus</i>	(–)ssRNA	Bowden et al. (2009);						
INKV	Inkoo virus	<i>Bunyaviridae</i>	<i>Orthobunyavirus</i>	(–)ssRNA	Pesonen, Ronnholm et al. (1982)						
RVFV	Rift Valley fever virus	<i>Bunyaviridae</i>	<i>Phlebovirus</i>	(–)ssRNA	Phoenix et al. (2016)		Phoenix et al. (2016)				
UUKV	Uukuniemi virus	<i>Bunyaviridae</i>	<i>Phlebovirus</i>	(–)ssRNA	Pesonen, Kuismanen et al. (1982)						
HCoV	Human coronavirus	<i>Coronaviridae</i>	<i>Alphacoronavirus</i>	(+)ssRNA						Walls et al. (2016)	
MHV	Mouse hepatitis virus <sup>b</sup>	<i>Coronaviridae</i>	<i>Betacoronavirus</i>	(+)ssRNA	Niemann et al. (1982)	Niemann et al. (1982); Niemann et al. (1984); Locker et al. (1992)			Niemann et al. (1982)		
SARS-CoV	SARS-related human coronavirus	<i>Coronaviridae</i>	<i>Betacoronavirus</i>	(+)ssRNA			Gramberg et al. (2005)				
EBOV	Ebola virus	<i>Filoviridae</i>	<i>Ebolavirus</i>	(–)ssRNA	Jeffers et al. (2002); Ritchie et al. (2010); Collar et al. (2016)	Jeffers et al. (2002); Ritchie et al. (2010); Collar et al. (2016)	Lin et al. (2003); Takada et al. (2004); Gramberg et al. (2005); Lennemann et al. (2014)		Wang et al. (2017)	Dowling et al. (2007); Francica et al. (2010); Noyori et al. (2013); Lennemann et al. (2014)	
MARV	Marburg virus	<i>Filoviridae</i>	<i>Marburgvirus</i>	(–)ssRNA	Feldmann et al. (1991)	Feldmann et al. (1991)	Becker et al. (1995); Gramberg et al. (2005)			Noyori et al. (2013)	
DENV	Dengue virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA	Miller et al. (2008); Dubayle et al. (2015); Lei et al. (2015)		Davis et al. (2006); Miller et al. (2008)			Rouvinski et al. (2015)	
JEV	Japanese encephalitis virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA	Zai et al. (2013)		Wang et al. (2016)		Zai et al. (2013)		
MVEV	Murray Valley encephalitis virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA	Blitvich et al. (2001)						
TBEV	Tick-borne encephalitis virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA	Yoshii et al. (2013)				Yoshii et al. (2013)		
WNV	West Nile virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA			Davis et al. (2006)	Whiteman et al. (2010)	Li et al. (2006)		
ZIKV	Zika virus	<i>Flaviviridae</i>	<i>Flavivirus</i>	(+)ssRNA			Mossenta et al. (2017)		Mossenta et al. (2017)		
HCV	Hepatitis C virus	<i>Flaviviridae</i>	<i>Hepacivirus</i>	(+)ssRNA	Iacob et al. (2008)	Brautigam et al. (2013)	Lozach et al. (2003); Goffard et al. (2005); Falkowska et al. (2007); Helle et al. (2010); Chen et al. (2014)		Fournillier et al. (2001); Goffard et al. (2005); Helle et al. (2010); Reszka et al. (2010); Orlova et al. (2015)	Fournillier et al. (2001); Falkowska et al. (2007); Liu et al. (2007); Brown et al. (2010); Helle et al. (2010); Anjum et al. (2013); Zhao et al. (2014); Li et al. (2016)	Meunier et al. (1999); Goffard et al. (2005); Orlova et al. (2015)
HBV	Hepatitis B virus	<i>Hepadnaviridae</i>	<i>Orthohepadnavirus</i>	dsDNA-RT	Schmitt et al. (1999)	Schmitt et al. (1999)			Lu et al. (1997); Ito et al. (2010); Julithe et al. (2014)	Julithe et al. (2014); Hyakumura et al. (2015)	

HCMV (HHV-5)	Human cytomegalovirus (human herpesvirus 5)	<i>Herpesviridae</i>	<i>Cytomegalovirus</i>	dsDNA	Kim et al. (1976) <sup>c</sup> ; Britt and Vugler (1989); Kaye et al. (1992); Al-Barazi and Colberg-Poley (1996); Jones et al. (1996); Margulies et al. (1996); Maidji et al. (1998); Huber and Compton (1999); Mullberg et al. (1999); Rehm et al. (2001); Gewurz et al. (2002); Theiler and Compton (2002); Griffin et al. (2005); Margulies and Gibson (2007); Lin et al. (2008); Stanton et al. (2010); Engel et al. (2011); Gabaev et al. (2014); Geyer et al. (2014); Cavaletto et al. (2015)	Britt and Vugler (1989); Kari et al. (1992); Theiler and Compton (2002); Gabaev et al. (2014); Geyer et al. (2014); Bagdonaite et al. (2016)				Kropff et al. (2012)
EBV (HHV-4)	Epstein-Barr virus (human herpesvirus 4)	<i>Herpesviridae</i>	<i>Lymphocryptovirus</i>	dsDNA	Edson and Thorley-Lawson (1983); Gong et al. (1987); Serafini-Cessi et al. (1989); Gong and Kieff (1990); Nolan and Morgan (1995); Paulsen et al. (2005); de Turenne-Tessier and Ooka (2007); Gore and Hutt-Fletcher (2009)	Gong et al. (1987) <sup>c</sup> ; Serafini-Cessi et al. (1989); Nolan and Morgan (1995); Borza and Hutt-Fletcher (1998); de Turenne-Tessier and Ooka (2007); Xiao et al. (2007); Bagdonaite et al. (2016)				D'Arrigo et al. (2013); Gram et al. (2016)
KSHV (HHV-8)	Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8)	<i>Herpesviridae</i>	<i>Rhadinovirus</i>	dsDNA	Chandran et al. (1998) <sup>c</sup> ; Pertel et al. (1998); Li et al. (1999); Zhu et al. (1999); Baghian et al. (2000); Wu et al. (2000); Chung et al. (2002); Koyano et al. (2003); Dela Cruz et al. (2004); Meads and Medveczky (2004); Dela Cruz et al. (2009); Wu et al. (2015)	Zhu et al. (1999); Wu et al. (2000)				Dela Cruz et al. (2004); Wu et al. (2015) <sup>d</sup>
HHV-6	Human herpesvirus 6	<i>Herpesviridae</i>	<i>Roseolovirus</i>	dsDNA	Okuno et al. (1990); Foa-Tomasi et al. (1992); Okuno et al. (1992); Pfeiffer et al. (1995) <sup>c</sup>	Cardinali et al. (1998); Torrisi et al. (1999)				
HHV-7	Human herpesvirus 7	<i>Herpesviridae</i>	<i>Roseolovirus</i>	dsDNA	Hata et al. (1996); Mukai et al. (1997) <sup>c</sup> ; Skrincoosky et al. (2000); Glosson et al. (2010)					Glosson et al. (2010) <sup>d</sup>
HSV-1 (HHV-1)	Herpes simplex virus type 1 (human herpesvirus 1)	<i>Herpesviridae</i>	<i>Simplexvirus</i>	dsDNA	Keller et al. (1970) <sup>c</sup> ; Spear and Roizman (1970) <sup>c</sup> ; Honess and Roizman (1975) <sup>c</sup> ; Eisenberg et al. (1979); Wenske et al. (1982); Cohen et al. (1983); Respass et al. (1984); Serafini-Cessi et al. (1984)	Keller et al. (1970) <sup>c</sup> ; Olofsson et al. (1981); Serafini-Cessi, Dall'Olio, Scannavini, Costanzo et al. (1983); Olofsson et al. (1983); Dall'Olio et al. (1985); Lundstrom et al. (1987); Serafini-Cessi et al. (1988);	Kuhn et al. (1988); Teuton and Brandt (2007); Wang et al. (2009); Altgarde et al. (2015)	Knowles and Person (1976); Campadelli-Fiume et al. (1982); Serafini-Cessi,	Olofsson and Lycke (1980); Norrild and Pedersen (1982); Serafini-Cessi, Dall'Olio, Scannavini,	Sodora et al. (1989)

Continued

Table I. Continued

Abbr.	Virus name	Family	Genus	Genome	N-glycosylated <sup>a</sup>	O-glycosylated <sup>a</sup>	Binding/entry/ haemagglutination/infectivity <sup>a</sup>	Spread <sup>a</sup>	Formation/release/ yield/protein transport <sup>a</sup>	Immune response <sup>a</sup>	Protein- protein interaction and other functions <sup>a</sup>
HSV-2 (HHV-2)	Herpes simplex virus type 2 (human herpesvirus 2)	<i>Herpesviridae</i>	<i>Simplexvirus</i>	dsDNA	Cohen et al. (1983); Zezulak and Spear (1983); Serafini-Cessi et al. (1985)	Peng et al. (1998); Norberg et al. (2007) <sup>c</sup> ; Bagdonaite et al. (2015); Norden et al. (2015)	Luo et al. (2015)	Luo et al. (2015)	Dall'Olio, Scannavini, Campadelli-Fiume et al. (1983)	Clo et al. (2012); Iversen et al. (2016)	Campadelli-Fiume et al. (1983); Altgarde et al. (2015)
VZV (HHV-3)	Varicella-zoster virus (human herpesvirus 3)	<i>Herpesviridae</i>	<i>Varicellovirus</i>	dsDNA	Friedrichs and Grose (1984); Montalvo et al. (1985); Montalvo and Grose (1986); Montalvo and Grose (1987); Yao et al. (1993); Maresova et al. (2000); Yamagishi et al. (2008)	Montalvo et al. (1985); Montalvo and Grose (1987); Yao et al. (1993); Bagdonaite et al. (2016)		Suenaga et al. (2015)	Montalvo et al. (1985)		
FLUAV	Influenza A virus	<i>Orthomyxoviridae</i>	<i>Influenzavirus A</i>	(-)ssRNA	Schwarz et al. (1977) <sup>c</sup> ; Edwardson (1984); Blake et al. (2009); An et al. (2015)	Stansell et al. (2015)	Park et al. (2016); Wu et al. (2017)		Edwardson (1984); Wu et al. (2017)	Skehel et al. (1984); Yang et al. (2011); Wu et al. (2017)	
HeV	Hendra virus	<i>Paramyxoviridae</i>	<i>Henipavirus</i>	(-)ssRNA	Bowden et al. (2010); Colgrave et al. (2012)	Colgrave et al. (2012)	Bradel-Tretheway et al. (2015); Stone et al. (2016)	Bradel-Tretheway et al. (2015); Stone et al. (2016)	Bradel-Tretheway et al. (2015); Stone et al. (2016)	Bradel-Tretheway et al. (2015)	Bradel-Tretheway et al. (2015); Stone et al. (2016)
NiV	Nipah virus	<i>Paramyxoviridae</i>	<i>Henipavirus</i>	(-)ssRNA	Bowden et al. (2008)		Stone et al. (2016)	Bradel-Tretheway et al. (2015); Stone et al. (2016)		Aguilar et al. (2006); Garner et al. (2015)	Stone et al. (2016)
MeV	Measles virus	<i>Paramyxoviridae</i>	<i>Morbillivirus</i>	(-)ssRNA	Hu et al. (1995)				Hu et al. (1995)		
HRSV	Human respiratory syncytial virus	<i>Paramyxoviridae</i>	<i>Pneumovirus</i>	(-)ssRNA	Lambert and Pons (1983); Gruber and Levine (1985)	Gruber and Levine (1985) <sup>c</sup>		Lambert and Pons (1983)	Lambert and Pons (1983)		
MuV	Mumps virus	<i>Paramyxoviridae</i>	<i>Rubulavirus</i>	(-)ssRNA	Herrler and Compans (1983)				Herrler and Compans (1983)		
VACV HIV-1	Vaccinia virus	<i>Poxviridae</i> <i>Retroviridae</i>	<i>Orthopoxvirus</i> <i>Lentivirus</i>	dsDNA ssRNA-RT	Shida and Dales (1981)	Shida and Dales (1981)	Shida and Dales (1981)				

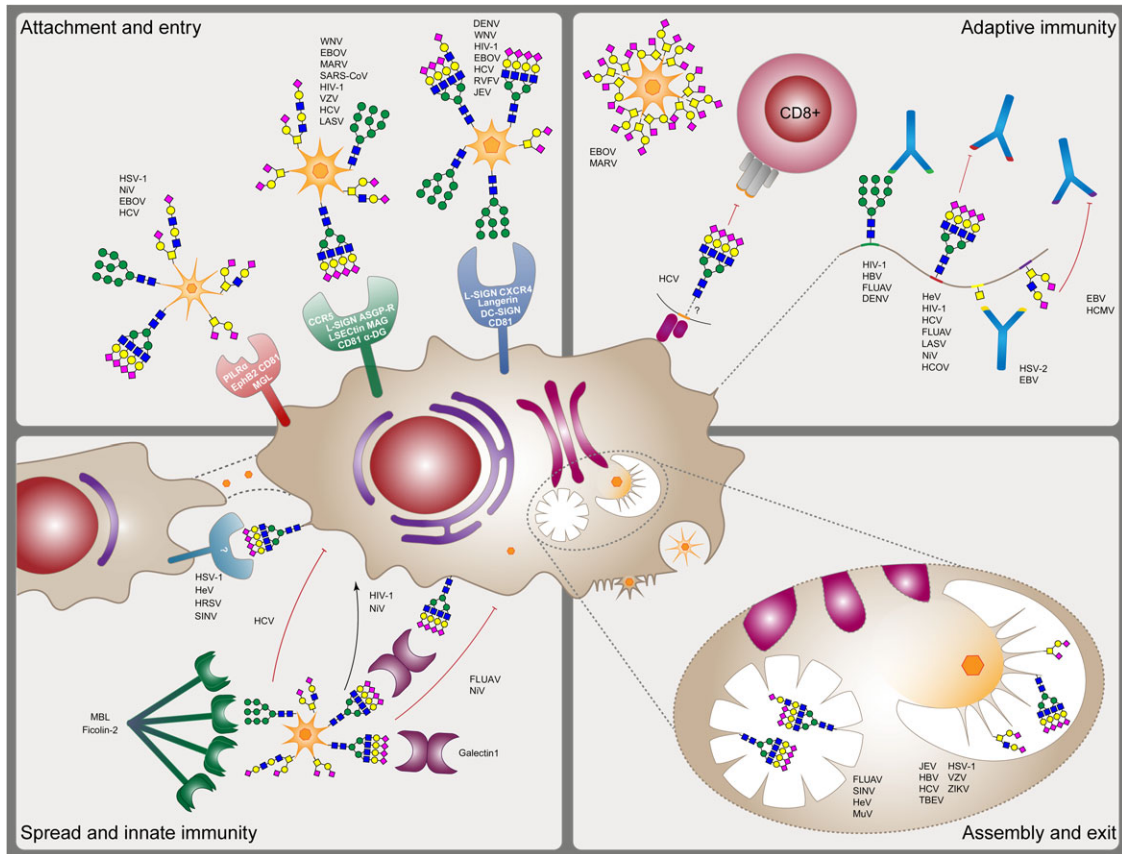
	Human immunodeficiency virus 1				Bernstein et al. (1994); Doores, Bonomelli et al. (2010); Bonomelli et al. (2011); Pabst et al. (2012); Go et al. (2013); Yang W et al. (2014); AlSalmi et al. (2015); Pritchard, Harvey et al. (2015); Behrens et al. (2016); Panico et al. (2016); Cao et al. (2017); Go et al. (2017); Struwe et al. (2017)	Bernstein et al. (1994); Go et al. (2013); Yang Z et al. (2014); Stansell et al. (2015)	Nakayama et al. (1998); Pollakis et al. (2001); Hong et al. (2002); Lin et al. (2003); Francois and Balzarini (2011); Zou et al. (2011); Wang et al. (2013); Mathys and Balzarini (2014); Lombardi et al. (2015); Mathys and Balzarini (2015); Termini et al. (2017)	Mathys and Balzarini (2014); Mathys and Balzarini (2015)	Mathys and Balzarini (2014); Mathys and Balzarini (2015)	Sanders et al. (2002); Wei et al. (2003); Wang et al. (2013); Doores and Burton (2010); St-Pierre et al. (2011); van Montfort et al. (2011); Pritchard, Spencer et al. (2015); Behrens et al. (2016); Lee et al. (2016)
CHIKV	Chikungunya virus	<i>Togaviridae</i>	<i>Alphavirus</i>	(+)ssRNA	Lancaster et al. (2016)					
RRV	Ross River virus	<i>Togaviridae</i>	<i>Alphavirus</i>	(+)ssRNA				Nelson et al. (2016)		
SFV	Semliki Forest virus	<i>Togaviridae</i>	<i>Alphavirus</i>	(+)ssRNA	Pesonen (1979); Rasilo and Renkonen (1979)					
SINV	Sindbis virus	<i>Togaviridae</i>	<i>Alphavirus</i>	(+)ssRNA	Sefton (1975) <sup>c</sup> ; Burke and Keegstra (1979); Hsieh et al. (1983)			Mann et al. (1983)	Leavitt et al. (1977); Datema et al. (1984)	
RUBV	Rubella virus	<i>Togaviridae</i>	<i>Rubivirus</i>	(+)ssRNA	Ho-Terry and Cohen (1984); Hobman et al. (1991); Lundstrom et al. (1991)	Ho-Terry and Cohen (1984) <sup>c</sup> ; Lundstrom et al. (1991)	Ho-Terry and Cohen (1984)			Hobman et al. (1991)

<sup>a</sup>Literature references are indicated as first author last name and year.

<sup>b</sup>Nonhuman infecting virus historically used as model for investigating betacoronaviruses.

<sup>c</sup>Indirect evidence.

<sup>d</sup>Other functions.



**Fig. 2.** Roles of glycosylation in the biology of enveloped viruses. The cartoon depicts functions of glycosylation described in the literature to affect various stages of the infectious cycle of enveloped viruses (Table I). The glycan chains presented on the generic viral particles are of illustrative manner and do not represent the glycoprofiles of individual viruses. Likewise, the glycan structures shown to interact with specific glycan binding molecules represent the most likely type of structure (high mannose or complex type) based on known carbohydrate binding specificities, unless specified otherwise in individual studies defining the interactions or glycoprofiling/glycoproteomic studies of the viruses in question. In the bottom right panel, the viruses are grouped according to the predominant mode of exit within the individual families. Virus abbreviations as in Table I. Monosaccharide symbols follow the SNFG (Symbol Nomenclature for Glycans) system (PMID 26,543,186, *Glycobiology* 25: 1323–1324, 2015).

that very early chemokine-mediated immune response to HSV-2 infection of the vaginal mucosa in mice relies on the presence of elongated O-linked glycans on the viral particles (Iversen et al. 2016). Although truncation of O-glycans might benefit the virus in escaping the early recognition by the host, elongated O-glycans are essential for viral replication (Bagdonaite et al. 2015; Iversen et al. 2016). Various carbohydrate structures from many bacteria and parasitic worms are known to elicit robust innate immune responses via toll-like and other receptors (Rabinovich and Toscano 2009; Prasanphanich et al. 2013). It is only conceivable that analogous carbohydrate-mediated mechanisms could exist for early detection of viral infections, but have not been described in detail.

### Adaptive immunity

During evolution, the immune system has developed redundant and fine-tuned mechanisms to identify and eliminate invading pathogens, including viruses. In turn, viruses continuously adapted to counteract or take advantage of the host's defense mechanisms, which is an ongoing battle. Glycans on viral glycoproteins have dual roles in virus–host interplay (Figure 2, top right panel). On one hand, glycans can be part of the immune determinants themselves, but on the other hand glycans can mask the antigenic protein epitopes from

recognition (Alexander and Elder 1984). Accumulating structural evidence suggests that glycans cover large surface areas of viral envelope proteins providing steric hindrance and physical shielding of vulnerable sites (Lee et al. 2016; Walls et al. 2016; Beniac and Booth 2017). Both N- and O-linked glycans can mask immunogenic B-cell epitopes, and, in some cases also determine or influence immunogenicity (Sodora et al. 1989; Hobman et al. 1991; Fournillier et al. 2001; Falkowska et al. 2007; Helle et al. 2010; Wang et al. 2013; Bradel-Tretheway et al. 2015). N-glycans covering structured domains and functional regions often confer protection from neutralization (Helle et al. 2010; Wang et al. 2013; Bradel-Tretheway et al. 2015; Wu et al. 2017). As an example, systemic functional analysis of N-glycosites in HCV revealed that protective N-linked glycans were mainly situated around the CD81 binding site on the HCV envelope glycoprotein E2 (Helle et al. 2010). In other cases, removal of specific N-linked glycans can alter the local antigenic landscape and broaden the immune response rather than simply uncovering underlying epitopes (Fournillier et al. 2001; Wang et al. 2013). In contrast, mutation of seemingly unrelated N-glycosites can reduce the sensitivity to neutralization at distant epitopes (Wang et al. 2013), highlighting the role of N-glycans on maintaining protein structure. In conclusion, mutation of potential N-glycosylation sites can result in unpredicted effects on protein

conformation and molecular dynamics, which differentially affect neutralization by antibodies, and are not explained solely by epitope recovery. Site-specific N-glycosylation has also been reported to affect cellular immunity to HCV E1, where deletion of specific glycans enhanced recognition by cytotoxic T cells (Liu et al. 2007).

Dense O-glycosylation of viral proteins has been widely described to provide “bulk” shielding from host immunity, and protect from antibody neutralization (Machiels et al. 2011; Kropff et al. 2012). Besides shielding select immunodominant epitopes (Machiels et al. 2011), highly O-glycosylated mucin-like domains, such as those found in Ebola and Marburg viruses, have been suggested to physically hinder the interaction between virus-infected cells and immune cells (Francica et al. 2010; Noyori et al. 2013). Conversely, the deletion of the Ebola virus mucin-like domain decreased the immune response in mice, although it did not have an effect on immunogenicity in vitro (Dowling et al. 2007). Finally, O-glycans not only shield, but in some cases can be presented as part of neo-epitopes when elongation of the glycans is prevented. As an example, several O-glycosylated immunodominant B-cell epitopes have been reported for HSV-2 and EBV, by probing O-glycopeptides with sera of infected patients, suggesting that the glycan moiety can be recognized by B-cells (Clo et al. 2012; D'Arrigo et al. 2013).

In the context of epitope shielding and immune recognition of viruses, it might be of importance that RNA viruses, such as influenza virus, HIV-1 and HCV, intrinsically have a very high mutation rate, thus minimizing the effect of adaptive immune response (Sanjuan et al. 2010; Anjum et al. 2013; Lauring et al. 2013; Lynch et al. 2015). Early on it was observed that the highest amino acid substitution rates occurred in antigenic regions of envelope glycoproteins, including such mutations that changed the number and position of glycosylation sites (Krystal et al. 1983; Skehel et al. 1984; Wei et al. 2003). Due to high density of glycans in such regions, some of the carbohydrate chains are not fully processed resulting in exposure of immature structures as seen in the mannose patch of HIV gp120 (Doores, Bonomelli et al. 2010). Paradoxically, such underprocessed structures are recognized by the immune system, giving rise to broad neutralizing antibodies (bnAbs), with the classical example being 2G12 (Sanders et al. 2002; Doores and Burton 2010). In addition, highly conserved glycosylation sites that are directly involved in binding to cell entry receptors or viral interaction partners often become targeted by bnAbs (Rouvinski et al. 2015). Interestingly, it has been demonstrated, that the recognition by such bnAbs is largely unaffected by deletion of individual N-glycans within the gp120 mannose patch, mimicking the consequences of intrinsic mutagenesis (Pritchard, Spencer et al. 2015). In some cases, however, mutations at distant glycosites may have subtle effects on epitope recognition (Behrens et al. 2016). Similarly, antigenicity of the HBV surface antigen isoform S did not suffer from introduction of additional N-glycosylation sites, and even resulted in stronger and more persistent immune responses (Hyakumura et al. 2015). In contrast, arenaviruses represent very well adapted pathogens where glycan shield density inversely correlates with potency of neutralizing antibodies (Sommerstein et al. 2015). However, in other instances, over-glycosylation of important functional regions of viral proteins result in better shielding from the immune system at a cost of binding affinity and virus production (Aguilar et al. 2006; Julithe et al. 2014; Lennemann et al. 2014; Lynch et al. 2015). As a compromise, variable glycosite occupancy can both fulfill the requirement for receptor binding via the nonglycosylated protein, and provide protection via the glycosylated counterpart (Julithe et al. 2014).

Being the components of viral particles exposed to the extracellular environment, viral envelope glycoproteins serve as major targets for vaccine development; however, protein sequence variability within and across strains, high mutation rate, as well as enormous heterogeneity of glycan structures makes it very difficult to identify immunogens evoking universal reactivity. Various expression systems are used for production of subunit vaccine candidates, such as bacteria, yeast, plant, insect and mammalian cell lines (Cox 2012; Kushnir et al. 2012; Redkiewicz et al. 2014). While some glycosylation types can be replicated in insect and mammalian cells, yeast cells will not carry out mucin type O-glycosylation and proteins produced in bacterial cells will also lack N-glycans. Even mammalian expression systems may lack the required glycosyltransferase repertoire to glycosylate relevant sites and build up relevant structures. Although there are many successful examples of vaccines produced in aforementioned systems, there is quite a number of infectious diseases that still lack vaccine coverage due to failure of vaccine candidates to induce adequate and lasting immune responses (Grimm and Ackerman 2013). Therefore, cell lines closer to the natural host cell type should be explored to more accurately reproduce the overall protein structure, modifications, and exposed antigenic sites for the primed immune system to be able to neutralize the naturally encountered antigen. Recently, efforts are being made to identify consensus glycosylation patterns, as well as production platforms leading to elicitation of desired immune responses and pathogen neutralization (Li et al. 2016; Go et al. 2017). In contrast to fast mutating RNA viruses, DNA viruses, including herpesviruses, have relatively stable genomes and rely on other means for counteracting the host's immune system (Sanjuan et al. 2010). It is therefore conceivable, that vaccine development should be less challenging for DNA viruses. However, most of herpesvirus-targeted subunit vaccines have failed so far. A recently developed HSV-2 vaccine lacking the main neutralizing antibody target is, however, showing great promise in mice. This vaccine evokes production of non-neutralizing antibodies to other envelope glycoproteins on infected cells, stimulating cellular NK cell immunity through engagement of Fcγ receptors (Petro et al. 2015). In conclusion, glycans on viral envelope glycoproteins have a tremendous impact on recognition by the host, and glycosylation heterogeneity makes it very difficult to identify universal vaccine candidates. While experimental evidence is key, development of accurate bioinformatic tools to predict glycosylation and likely mutation patterns would be of big value for vaccine research. This should become possible once a substantial number of viral strains are sequenced and analyzed for glycan modifications in native contexts.

### Roles of glycosylation on viral protein complex formation

While N-linked glycans situated on structural domains often shield functionally important protein regions from antibody neutralization, stem region N-glycans can affect glycoprotein stability and protein-protein interactions. For example, the mutations in the N-glycosite localized to the stem region of the Hendra virus glycoprotein G affected the conformation of the molecule, leading to increased oligomerization and interaction with its viral binding partner (Bradel-Tretheway et al. 2015). Moreover, the stalk mutation had similar effects in a closely related Nipah virus (Bradel-Tretheway et al. 2015). In Hepatitis C Virus, disruption of specific N-glycosites in the two highly N-glycosylated envelope glycoproteins E1 and E2 results in formation of unproductive E1–E2 heterodimers (Meunier

et al. 1999; Goffard et al. 2005; Orlova et al. 2015), whereas specific O-glycosites on Hendra virus protein G have been shown to affect association with protein F (Stone et al. 2016), suggesting both types of glycans can carry out similar functions. Similarly, O-glycosites have been found in regions of varicella zoster virus gE, important for interaction with partner gI (Bagdonaite et al. 2016), but the glycan specific functions are yet to be uncovered.

### Induction of host glycosylation machinery

In addition to hijacking host glycosylation machinery for modification of viral proteins, certain viruses induce changes in the expression levels of host glycosyltransferases. For example, it is documented that herpesviruses induce expression of host fucosyltransferases leading to expression of sLe<sup>x</sup> or Le<sup>y</sup> antigens (Nystrom et al. 2007; Norden et al. 2013, 2017). In addition, HSV-1 and HSV-2 infection lead to changes in gene expression related to glycosphingolipid synthesis (Miyaji et al. 2016). Also, in the case of HCV, it has been demonstrated that the total glycoprofile of HCV-infected hepatoma cells was shifted towards more fucosylated, sialylated, and in general complex N-glycans (Xiang et al. 2017). Another example includes HBV that has been shown to upregulate mannosidases, which supports increased glycan processing (Hu et al. 2016). In the case of influenza A virus, induction of the isoform, GalNAc-T3, has been detected in infected respiratory epithelial cells, possibly increasing O-glycosylation of airway mucins (Nakamura et al. 2015). The changes in expression levels of host carbohydrate active enzymes can either hint towards requirements for specific structures for functionality, or highlight the demand for increased glycosylation capacity to support viral glycoprotein glycosylation.

### Perspectives

It has long been known that glycosylation of viral envelope proteins is essential for infectivity and affects immune recognition. Surprisingly, few clinical applications have been developed using this knowledge, and glycosylation has rarely been considered in vaccine design. However, awareness is increasing and more and more studies are addressing the need of relevant glycosylation in vaccine formulations (Li et al. 2016; Go et al. 2017). The rapidly evolving analytical methods now allow precise characterization of the location and structure of both N- and O-linked glycosylation on viruses. Likewise, the advancement of glycobiology and gene editing techniques offers ample opportunities to investigate the functional roles of glycan structures and specific sites. Unreliable prediction of O-glycosylation have discouraged site-specific analysis of O-glycosylation in viruses, but now the structural information obtained by high throughput glycoproteomic approaches as well as bioinformatic analysis of glycosylation patterns can serve as a resource for defining targets for such studies. In addition, the knowledge base can be used for addressing the role of O-glycosylation for antigenicity and immunogenicity of subunit vaccine candidates. Currently it is difficult to obtain site occupancy information at a proteome-wide scale, but soon this will be possible with optimized targeted glycoproteomics strategies. Accumulating evidence suggest that O-glycosylation of viruses play important roles in various aspects of virus biology. The advent of readily available and robust genome engineering tools provides opportunities to investigate these findings in more detail and map the structural requirements for O-glycan structures on the virus and potential interaction partners on epithelial cells by using glycoengineered cell libraries (Radhakrishnan et al. 2014; Yang et al. 2015). The challenge is, however, to apply such knowledge for clinical purposes.

Since viral glycosylation is orchestrated by the capacity of the host cell, general glycosylation inhibitors are of limited use due to toxicity. It is therefore important to identify structural determinants of combinatorial self- and nonself-nature for safe targeting of viral glycans and boosting antiviral immunity.

### Conclusions

Enveloped viruses have long been known to hijack the host cell's glycosylation machinery for glycosylation of viral proteins. The development and advancement of mass spectrometry techniques have paid a tremendous contribution to the characterization of precise location and structure of glycans decorating the viral envelope proteins, and revealed substantial heterogeneity of glycan structures and site occupancy. Moreover, glycan density, as well as N- to O-glycan ratio, varies greatly among different classes of viruses, with some being highly glycosylated, and others having only a few glycan chains. Accumulating site-directed mutagenesis data suggests that individual glycosites play diverse important roles in virus biology. Moreover, glycans heavily contribute to the overall structure of the glycoprotein, epitope accessibility, and, in some cases, directly affect immune recognition. While there are some examples where carbohydrate moieties directly participate in interactions, in many cases disrupted biological functions are results of impaired protein structure, localization or function. Failure to develop universal vaccines for multiple viruses such as HIV-1, HCV and HSV suggest that comprehensive knowledge of structure and immunogenicity of viral envelope proteins in relevant biological systems is needed to achieve desired immune responses. While the location and function of N-linked glycans have been very well characterized for many viruses, discovery of O-glycosites has been neglected due to the nature of O-glycan synthesis and analytical difficulties. We have developed techniques to globally address site-specific O-glycosylation of enveloped viruses and revealed unprecedented magnitude of O-glycosylation in herpesviruses. We argue that the contribution of O-glycans needs to be addressed for vaccine development in order to have a complete picture of post-translational modifications affecting the protein function and immunogenicity. It is hoped that the data summarized in this review will ignite more interest in viral O-glycosylation, and give rise to focused studies leading to a better understanding of virus biology and improved means to prevent and combat the associated infections.

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### Conflict of interest statement

None declared.



## Abbreviations

GlcNAc, N-acetylglucosamine; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; PNA, peanut agglutinin; VVA, *Vicia villosa* lectin (VVA).

## References

- Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, Asare I. 2008. Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. *BMC Infect Dis.* 8:111.
- Aguilar HC, Matreyek KA, Filone CM, Hashimi ST, Levroney EL, Negrete OA, Bertolotti-Ciarlet A, Choi DY, McHardy I, Fulcher JA et al. 2006. N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. *J Virol.* 80:4878–4889.
- Air GM. 2014. Influenza virus-glycan interactions. *Curr Opin Virol.* 7: 128–133.
- Al-Barazi HO, Colberg-Poley AM. 1996. The human cytomegalovirus UL37 immediate-early regulatory protein is an integral membrane N-glycoprotein which traffics through the endoplasmic reticulum and Golgi apparatus. *J Virol.* 70:7198–7208.
- Alexander S, Elder JH. 1984. Carbohydrate dramatically influences immune reactivity of antisera to viral glycoprotein antigens. *Science (New York, NY).* 226:1328–1330.
- AlSalmi W, Mahalingam M, Ananthaswamy N, Hamlin C, Flores D, Gao G, Rao VB. 2015. A new approach to produce HIV-1 envelope trimers: Both cleavage and proper glycosylation are essential to generate authentic trimers. *J Biol Chem.* 290(32):19780–19795.
- Altgarde N, Eriksson C, Peerboom N, Phan-Xuan T, Moeller S, Schnabelrauch M, Svedhem S, Trybala E, Bergstrom T, Bally M. 2015. Mucin-like region of herpes simplex virus type 1 attachment protein gC modulates the virus-glycosaminoglycan interaction. *J Biol Chem.* 290(35):21473–21485.
- An Y, McCullers JA, Alymova I, Parsons LM, Cipollo JF. 2015. Glycosylation analysis of engineered H3N2 influenza A virus hemagglutinins with sequentially added historically relevant glycosylation sites. *J Proteome Res.* 14: 3957–3969.
- Anjum S, Wahid A, Afzal MS, Albecka A, Alsaleh K, Ahmad T, Baumert TF, Wychowski C, Qadri I, Penin F et al. 2013. Additional glycosylation within a specific hypervariable region of subtype 3a of hepatitis C virus protects against virus neutralization. *J Infect Dis.* 208:1888–1897.
- Arii J, Wang J, Morimoto T, Suenaga T, Akashi H, Arase H, Kawaguchi Y. 2010. A single-amino-acid substitution in herpes simplex virus 1 envelope glycoprotein B at a site required for binding to the paired immunoglobulin-like type 2 receptor alpha (PILRalpha) abrogates PILRalpha-dependent viral entry and reduces pathogenesis. *J Virol.* 84:10773–10783.
- Astuto M, Palermo CI, Costanzo CM, Ettore GC, Palmucci S, Franchina C, Russo R, Valastro P, Timpanaro V, Scalia G. 2013. Fatal pulmonary disease and encephalic complication in a man with HSV-1 Infection: A case report. *J Clin Virol.* 59(1):59–62.
- Awasthi S, Friedman HM. 2014. Status of prophylactic and therapeutic genital herpes vaccines. *Curr Opin Virol.* 6:6–12.
- Bagdonaite I, Norden R, Joshi HJ, Dabelsteen S, Nystrom K, Vakhrushev SY, Olofsson S, Wandall HH. 2015. A strategy for O-glycoproteomics of enveloped viruses—The O-glycoproteome of herpes simplex virus type 1. *PLoS Pathog.* 11:e1004784.
- Bagdonaite I, Norden R, Joshi HJ, King SL, Vakhrushev SY, Olofsson S, Wandall HH. 2016. Global mapping of O-glycosylation of varicella zoster virus, human cytomegalovirus, and Epstein-Barr virus. *J Biol Chem.* 291:12014–12028.
- Baghian A, Luftig M, Black JB, Meng YX, Pau CP, Voss T, Pellett PE, Kousoulas KG. 2000. Glycoprotein B of human herpesvirus 8 is a component of the virion in a cleaved form composed of amino- and carboxyl-terminal fragments. *Virology.* 269:18–25.
- Bause E. 1983. Structural requirements of N-glycosylation of proteins. Studies with proline peptides as conformational probes. *Biochem J.* 209: 331–336.
- Beachboard DC, Horner SM. 2016. Innate immune evasion strategies of DNA and RNA viruses. *Curr Opin Microbiol.* 32:113–119.
- Becker S, Spiess M, Klenk HD. 1995. The asialoglycoprotein receptor is a potential liver-specific receptor for Marburg virus. *J Gen Virol.* 76(Pt 2): 393–399.
- Behrens AJ, Vasiljevic S, Pritchard LK, Harvey DJ, Andev RS, Krumm SA, Struwe WB, Cupo A, Kumar A, Zitzmann N et al. 2016. Composition and antigenic effects of individual glycan sites of a trimeric HIV-1 envelope glycoprotein. *Cell Rep.* 14:2695–2706.
- Beniac DR, Booth TF. 2017. Structure of the Ebola virus glycoprotein spike within the virion envelope at 11 Å resolution. *Sci Rep.* 7:46374.
- Bennett EP, Mandel U, Clausen H, Gerken TA, Fritz TA, Tabak LA. 2012. Control of mucin-type O-glycosylation: A classification of the polypeptide GalNAc-transferase gene family. *Glycobiology.* 22:736–756.
- Berarducci B, Rajamani J, Zerboni L, Che X, Sommer M, Arvin AM. 2010. Functions of the unique N-terminal region of glycoprotein E in the pathogenesis of varicella-zoster virus infection. *Proc Natl Acad Sci USA.* 107: 282–287.
- Bernstein HB, Tucker SP, Hunter E, Schutzbach JS, Compans RW. 1994. Human immunodeficiency virus type 1 envelope glycoprotein is modified by O-linked oligosaccharides. *J Virol.* 68:463–468.
- Blake TA, Williams TL, Pirkle JL, Barr JR. 2009. Targeted N-linked glycosylation analysis of H5N1 influenza hemagglutinin by selective sample preparation and liquid chromatography/tandem mass spectrometry. *Anal Chem.* 81:3109–3118.
- Blitvich BJ, Scanlon D, Shiell BJ, Mackenzie JS, Pham K, Hall RA. 2001. Determination of the intramolecular disulfide bond arrangement and biochemical identification of the glycosylation sites of the nonstructural protein NS1 of Murray Valley encephalitis virus. *J Gen Virol.* 82:2251–2256.
- Bonomelli C, Doores KJ, Dunlop DC, Thaney V, Dwek RA, Burton DR, Crispin M, Scanlan CN. 2011. The glycan shield of HIV is predominantly oligomannose independently of production system or viral clade. *PLoS One.* 6:e23521.
- Borza CM, Hutt-Fletcher LM. 1998. Epstein-Barr virus recombinant lacking expression of glycoprotein gp150 infects B cells normally but is enhanced for infection of epithelial cells. *J Virol.* 72:7577–7582.
- Bowden TA, Crispin M, Graham SC, Harvey DJ, Grimes JM, Jones EY, Stuart DI. 2009. Unusual molecular architecture of the machupo virus attachment glycoprotein. *J Virol.* 83:8259–8265.
- Bowden TA, Crispin M, Harvey DJ, Aricescu AR, Grimes JM, Jones EY, Stuart DI. 2008. Crystal structure and carbohydrate analysis of Nipah virus attachment glycoprotein: A template for antiviral and vaccine design. *J Virol.* 82:11628–11636.
- Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI. 2010. Dimeric architecture of the Hendra virus attachment glycoprotein: Evidence for a conserved mode of assembly. *J Virol.* 84:6208–6217.
- Bradel-Tretheway BG, Liu Q, Stone JA, McNally S, Aguilar HC. 2015. Novel functions of Hendra virus G N-glycans and comparisons to Nipah virus. *J Virol.* 89:7235–7247.
- Bradley H, Markowitz LE, Gibson T, McQuillan GM. 2014. Seroprevalence of herpes simplex virus types 1 and 2—United States, 1999–2010. *J Infect Dis.* 209:325–333.
- Brautigam J, Scheidig AJ, Egge-Jacobsen W. 2013. Mass spectrometric analysis of hepatitis C viral envelope protein E2 reveals extended microheterogeneity of mucin-type O-linked glycosylation. *Glycobiology.* 23:453–474.
- Britt WJ, Vugler LG. 1989. Processing of the gp55-116 envelope glycoprotein complex (gB) of human cytomegalovirus. *J Virol.* 63:403–410.
- Brockhausen I, Moller G, Merz G, Adermann K, Paulsen H. 1990. Control of mucin synthesis: The peptide portion of synthetic O-glycopeptide substrates influences the activity of O-glycan core 1 UDPgalactose:N-acetyl-alpha-galactosaminyl-R beta 3-galactosyltransferase. *Biochemistry.* 29: 10206–10212.
- Brown KS, Keogh MJ, Owsianka AM, Adair R, Patel AH, Arnold JN, Ball JK, Sim RB, Tarr AW, Hickling TP. 2010. Specific interaction of hepatitis C virus glycoproteins with mannan binding lectin inhibits virus entry. *Protein Cell.* 1:664–674.

- Buchmann JP, Holmes EC. 2015. Cell walls and the convergent evolution of the viral envelope. *Microbiol Mol Biol Rev.* 79:403–418.
- Bunkenborg J, Pilch BJ, Podtelejnikov AV, Wisniewski JR. 2004. Screening for N-glycosylated proteins by liquid chromatography mass spectrometry. *Proteomics.* 4:454–465.
- Burke D, Keegstra K. 1979. Carbohydrate structure of Sindbis virus glycoprotein E2 from virus grown in hamster and chicken cells. *J Virol.* 29:546–554.
- Campadelli-Fiume G, Poletti L, Dall'Olio F, Serafini-Cessi F. 1982. Infectivity and glycoprotein processing of herpes simplex virus type 1 grown in a ricin-resistant cell line deficient in N-acetylglucosaminyl transferase I. *J Virol.* 43:1061–1071.
- Canis K, McKinnon TA, Nowak A, Panico M, Morris HR, Laffan M, Dell A. 2010. The plasma von Willebrand factor O-glycome comprises a surprising variety of structures including ABH antigens and disialosyl motifs. *J Thromb Haemost.* 8:137–145.
- Cao L, Diedrich JK, Kulp DW, Pauthner M, He L, Park SR, Sok D, Su CY, Delahunty CM, Menis S et al. 2017. Global site-specific N-glycosylation analysis of HIV envelope glycoprotein. *Nat Commun.* 8:14954.
- Cardinali G, Gentile M, Cirone M, Zompetta C, Frati L, Faggioni A, Torrisi MR. 1998. Viral glycoproteins accumulate in newly formed annulate lamellae following infection of lymphoid cells by human herpesvirus 6. *J Virol.* 72:9738–9746.
- Carfi A, Willis SH, Whitbeck JC, Krummenacher C, Cohen GH, Eisenberg RJ, Wiley DC. 2001. Herpes simplex virus glycoprotein D bound to the human receptor HveA. *Mol Cell.* 8:169–179.
- Cavaletto N, Lugini A, Gribaudo G. 2015. Inactivation of the human cytomegalovirus US20 gene hampers productive viral replication in endothelial cells. *J Virol.* 89(21):11092–11106.
- Chandran B, Bloomer C, Chan SR, Zhu L, Goldstein E, Horvat R. 1998. Human herpesvirus-8 ORF K8.1 gene encodes immunogenic glycoproteins generated by spliced transcripts. *Virology.* 249:140–149.
- Chen PC, Chuang PK, Chen CH, Chan YT, Chen JR, Lin SW, Ma C, Hsu TL, Wong CH. 2014. Role of N-linked glycans in the interactions of recombinant HCV envelope glycoproteins with cellular receptors. *ACS Chem Biol.* 9:1437–1443.
- Chen CY, Huang KY, Shen JH, Tsao KC, Huang YC. 2015. A large-scale seroprevalence of Epstein-Barr virus in Taiwan. *PLoS One.* 10:e0115836.
- Christiaansen A, Varga SM, Spencer JV. 2015. Viral manipulation of the host immune response. *Curr Opin Immunol.* 36:54–60.
- Chung YH, Means RE, Choi JK, Lee BS, Jung JU. 2002. Kaposi's sarcoma-associated herpesvirus OX2 glycoprotein activates myeloid-lineage cells to induce inflammatory cytokine production. *J Virol.* 76:4688–4698.
- Clo E, Kracun SK, Nudelman AS, Jensen KJ, Liljeqvist JA, Olofsson S, Bergstrom T, Blixt O. 2012. Characterization of the viral O-glycopeptide: A novel tool of relevance for vaccine design and serodiagnosis. *J Virol.* 86:6268–6278.
- Cohen JI. 2015. Epstein-barr virus vaccines. *Clin Transl Immunol.* 4:e32.
- Cohen DI, Davidovici BB, Smetana Z, Balicer RD, Klement E, Mendelson E, Green MS. 2006. Seroepidemiology of Varicella zoster in Israel prior to large-scale use of varicella vaccines. *Infection.* 34:208–213.
- Cohen GH, Long D, Matthews JT, May M, Eisenberg R. 1983. Glycopeptides of the type-common glycoprotein gD of herpes simplex virus types 1 and 2. *J Virol.* 46:679–689.
- Colgrave ML, Snelling HJ, Shiell BJ, Feng YR, Chan YP, Bossart KN, Xu K, Nikolov DB, Broder CC, Michalski WP. 2012. Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus. *Glycobiology.* 22:572–584.
- Collar AL, Clarke EC, Anaya E, Merrill D, Yarborough S, Anthony SM, Kuhn JH, Merle C, Theisen M, Bradfute SB. 2016. Comparison of N- and O-linked glycosylation patterns of ebolavirus glycoproteins. *Virology.* 502:39–47.
- Conde-Glez C, Lazcano-Ponce E, Rojas R, DeAntonio R, Romano-Mazzotti L, Cervantes Y, Ortega-Barria E. 2013. Seroprevalences of varicella-zoster virus, herpes simplex virus and cytomegalovirus in a cross-sectional study in Mexico. *Vaccine.* 31:5067–5074.
- Cox MM. 2012. Recombinant protein vaccines produced in insect cells. *Vaccine.* 30:1759–1766.
- Dall'Olio F, Malagolini N, Speziali V, Campadelli-Fiume G, Serafini-Cessi F. 1985. Sialylated oligosaccharides O-glycosidically linked to glycoprotein C from herpes simplex virus type 1. *J Virol.* 56:127–134.
- Dalziel M, Whitehouse C, McFarlane I, Brockhausen I, Gschmeissner S, Schwientek T, Clausen H, Burchell JM, Taylor-Papadimitriou J. 2001. The relative activities of the C2GnT1 and ST3Gal-I glycosyltransferases determine O-glycan structure and expression of a tumor-associated epitope on MUC1. *J Biol Chem.* 276:11007–11015.
- Datema R, Romero PA, Rott R, Schwarz RT. 1984. On the role of oligosaccharide trimming in the maturation of Sindbis and influenza virus. *Arch Virol.* 81:25–39.
- Davis CW, Mattei LM, Nguyen HY, Ansarah-Sobrinho C, Doms RW, Pierson TC. 2006. The location of asparagine-linked glycans on West Nile virions controls their interactions with CD209 (dendritic cell-specific ICAM-3 grabbing nonintegrin). *J Biol Chem.* 281:37183–37194.
- de Turenne-Tessier M, Ooka T. 2007. Post-translational modifications of Epstein Barr virus BARF1 oncogene-encoded polypeptide. *J Gen Virol.* 88:2656–2661.
- Dela Cruz CS, Lee Y, Viswanathan SR, El-Guindy AS, Gerlach J, Nikiforow S, Shedd D, Gradoville L, Miller G. 2004. N-linked glycosylation is required for optimal function of Kaposi's sarcoma herpesvirus-encoded, but not cellular, interleukin 6. *J Exp Med.* 199:503–514.
- Dela Cruz CS, Viswanathan SR, El-Guindy AS, Shedd D, Miller G. 2009. Complex N-linked glycans on Asn-89 of Kaposi sarcoma herpes virus-encoded interleukin-6 mediate optimal function by affecting cytokine protein conformation. *J Biol Chem.* 284:29269–29282.
- Di Giovine P, Settembre EC, Bhargava AK, Luftig MA, Lou H, Cohen GH, Eisenberg RJ, Krummenacher C, Carfi A. 2011. Structure of herpes simplex virus glycoprotein D bound to the human receptor nectin-1. *PLoS Pathog.* 7:29.
- Doores KJ, Bonomelli C, Harvey DJ, Vasiljevic S, Dwek RA, Burton DR, Crispin M, Scanlan CN. 2010. Envelope glycans of immunodeficiency viruses are almost entirely oligomannose antigens. *Proc Natl Acad Sci USA.* 107:13800–13805.
- Doores KJ, Burton DR. 2010. Variable loop glycan dependency of the broad and potent HIV-1-neutralizing antibodies PG9 and PG16. *J Virol.* 84:10510–10521.
- Dowling W, Thompson E, Badger C, Mellquist JL, Garrison AR, Smith JM, Paragas J, Hogan RJ, Schmaljohn C. 2007. Influences of glycosylation on antigenicity, immunogenicity, and protective efficacy of ebola virus GP DNA vaccines. *J Virol.* 81:1821–1837.
- Dubayle J, Vialle S, Schneider D, Pontvianne J, Mantel N, Adam O, Guy B, Talaga P. 2015. Site-specific characterization of envelope protein N-glycosylation on Sanofi Pasteur's tetravalent CYD dengue vaccine. *Vaccine.* 33:1360–1368.
- D'Arrigo I, Clo E, Bergstrom T, Olofsson S, Blixt O. 2013. Diverse IgG serum response to novel glycopeptide epitopes detected within immunodominant stretches of Epstein-Barr virus glycoprotein 350/220: Diagnostic potential of O-glycopeptide microarrays. *Glycoconj J.* 30:633–640.
- Edson CM, Thorley-Lawson DA. 1983. Synthesis and processing of the three major envelope glycoproteins of Epstein-Barr virus. *J Virol.* 46:547–556.
- Edwardson JM. 1984. Effects of monensin on the processing and intracellular transport of influenza virus haemagglutinin in infected MDCK cells. *J Cell Sci.* 65:209–221.
- Eisenberg RJ, Hydrean-Stern C, Cohen GH. 1979. Structural analysis of precursor and product forms of type-common envelope glycoprotein D (CP-1 antigen) of herpes simplex virus type 1. *J Virol.* 31:608–620.
- Engel P, Perez-Carmona N, Alba MM, Robertson K, Ghazal P, Angulo A. 2011. Human cytomegalovirus UL7, a homologue of the SLAM-family receptor CD229, impairs cytokine production. *Immunol Cell Biol.* 89:753–766.
- Falkowska E, Kajumo F, Garcia E, Reinus J, Dragic T. 2007. Hepatitis C virus envelope glycoprotein E2 glycans modulate entry, CD81 binding, and neutralization. *J Virol.* 81:8072–8079.

- Feldmann H, Will C, Schikore M, Slenczka W, Klenk HD. 1991. Glycosylation and oligomerization of the spike protein of Marburg virus. *Virology*. 182:353–356.
- Fishman JA. 2013. Overview: Cytomegalovirus and the herpesviruses in transplantation. *Am J Transplant*. 3:1–8.
- Foa-Tomas L, Guerrini S, Huang T, Campadelli-Fiume G. 1992. Characterization of human herpesvirus-6(U1102) and (GS) gp112 and identification of the Z29-specified homolog. *Virology*. 191:511–516.
- Fournillier A, Wychowski C, Boucreux D, Baumert TF, Meunier JC, Jacobs D, Muguet S, Depla E, Inchauspe G. 2001. Induction of hepatitis C virus E1 envelope protein-specific immune response can be enhanced by mutation of N-glycosylation sites. *J Virol*. 75:12088–12097.
- Franc V, Rehulka P, Raus M, Stulik J, Novak J, Renfrow MB, Sebela M. 2013. Elucidating heterogeneity of IgA1 hinge-region O-glycosylation by use of MALDI-TOF/TOF mass spectrometry: Role of cysteine alkylation during sample processing. *J Proteomics*. 92:299–312.
- Francica JR, Varela-Rohena A, Medvec A, Plesa G, Riley JL, Bates P. 2010. Steric shielding of surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein. *PLoS Pathog*. 6:e1001098.
- Francois KO, Balzarini J. 2011. The highly conserved glycan at asparagine 260 of HIV-1 gp120 is indispensable for viral entry. *J Biol Chem*. 286:42900–42910.
- Friedrichs WE, Grose C. 1984. Glycoprotein gp118 of varicella-zoster virus: Purification by serial affinity chromatography. *J Virol*. 49:992–996.
- Fu TM, An Z, Wang D. 2014. Progress on pursuit of human cytomegalovirus vaccines for prevention of congenital infection and disease. *Vaccine*. 32:2525–2533.
- Gabaei I, Elbasani E, Ameres S, Steinbruck L, Stanton R, Doring M, Lenac Rovis T, Kalinke U, Jonjic S, Moosmann A et al. 2014. Expression of the human cytomegalovirus UL11 glycoprotein in viral infection and evaluation of its effect on virus-specific CD8 T cells. *J Virol*. 88:14326–14339.
- Gallagher JR, Atanasiu D, Saw WT, Paradisgarten MJ, Whitbeck JC, Eisenberg RJ, Cohen GH. 2014. Functional fluorescent protein insertions in herpes simplex virus gB report on gB conformation before and after execution of membrane fusion. *PLoS Pathog*. 10:e1004373.
- Garner OB, Yun T, Pernet O, Aguilar HC, Park A, Bowden TA, Freiberg AN, Lee B, Baum LG. 2015. Timing of galectin-1 exposure differentially modulates Nipah virus entry and syncytium formation in endothelial cells. *J Virol*. 89:2520–2529.
- Gerken TA, Jamison O, Perrine CL, Collette JC, Moinova H, Ravi L, Markowitz SD, Shen W, Patel H, Tabak LA. 2011. Emerging paradigms for the initiation of mucin-type protein O-glycosylation by the polypeptide GalNAc transferase family of glycosyltransferases. *J Biol Chem*. 286:14493–14507.
- Gewurz BE, Ploegh HL, Tortorella D. 2002. US2, a human cytomegalovirus-encoded type I membrane protein, contains a non-cleavable amino-terminal signal peptide. *J Biol Chem*. 277:11306–11313.
- Geyer H, Hartung E, Mages HW, Weise C, Beluzic R, Vugrek O, Jonjic S, Kroccek RA, Voigt S. 2014. Cytomegalovirus expresses the chemokine homologue vXCL1 capable of attracting XCR1+ CD4- dendritic cells. *J Virol*. 88:292–302.
- Glosson NL, Gonyo P, May NA, Schneider CL, Ristow LC, Wang Q, Hudson AW. 2010. Insight into the mechanism of human herpesvirus 7 U21-mediated diversion of class I MHC molecules to lysosomes. *J Biol Chem*. 285:37016–37029.
- Go EP, Ding H, Zhang S, Ringe RP, Nicely N, Hua D, Steinbock RT, Golabek M, Alin J, Alam SM et al. 2017. A glycosylation benchmark profile for HIV-1 envelope glycoprotein production based on eleven Env trimers. *J Virol*. 91(9):e02428-16, pii.
- Go EP, Liao HX, Alam SM, Hua D, Haynes BF, Desaire H. 2013. Characterization of host-cell line specific glycosylation profiles of early transmitted/founder HIV-1 gp120 envelope proteins. *J Proteome Res*. 12:1223–1234.
- Goffard A, Callens N, Bartosch B, Wychowski C, Cosset FL, Montpellier C, Dubuisson J. 2005. Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. *J Virol*. 79:8400–8409.
- Gong M, Kieff E. 1990. Intracellular trafficking of two major Epstein-Barr virus glycoproteins, gp350/220 and gp110. *J Virol*. 64:1507–1516.
- Gong M, Ooka T, Matsuo T, Kieff E. 1987. Epstein-Barr virus glycoprotein homologous to herpes simplex virus gB. *J Virol*. 61:499–508.
- Gore M, Hutt-Fletcher LM. 2009. The BDLF2 protein of Epstein-Barr virus is a type II glycosylated envelope protein whose processing is dependent on coexpression with the BMRF2 protein. *Virology*. 383:162–167.
- Gorfinkel IS, Aoki F, McNeil S, Dionne M, Shafran SD, Zickler P, Halperin S, Langley J, Bellamy A, Schulte J et al. 2013. Seroprevalence of HSV-1 and HSV-2 antibodies in Canadian women screened for enrolment in a herpes simplex virus vaccine trial. *Int J STD AIDS*. 24:345–349.
- Gram AM, Oosenbrug T, Lindenberg MF, Bull C, Comvalius A, Dickson KJ, Wiegant J, Vrolijk H, Lebbink RJ, Wolterbeek R et al. 2016. The Epstein-Barr virus glycoprotein gp150 forms an immune-evasive glycan shield at the surface of infected cells. *PLoS Pathog*. 12:e1005550.
- Gramberg T, Hofmann H, Moller P, Lalor PF, Marzi A, Geier M, Krumbiegel M, Winkler T, Kirchhoff F, Adams DH et al. 2005. LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. *Virology*. 340:224–236.
- Griffin C, Wang EC, McSharry BP, Rickards C, Browne H, Wilkinson GW, Tomasec P. 2005. Characterization of a highly glycosylated form of the human cytomegalovirus HLA class I homologue gpUL18. *J Gen Virol*. 86:2999–3008.
- Grimm SK, Ackerman ME. 2013. Vaccine design: Emerging concepts and renewed optimism. *Curr Opin Biotechnol*. 24:1078–1088.
- Gruber C, Levine S. 1985. Respiratory syncytial virus polypeptides. IV. The oligosaccharides of the glycoproteins. *J Gen Virol*. 66(Pt 3):417–432.
- Halim A, Brinkmalm G, Ruetschi U, Westman-Brinkmalm A, Portelius E, Zetterberg H, Blennow K, Larsson G, Nilsson J. 2011. Site-specific characterization of threonine, serine, and tyrosine glycosylations of amyloid precursor protein/amyloid beta-peptides in human cerebrospinal fluid. *Proc Natl Acad Sci USA*. 108:11848–11853.
- Haltom AR, Jafar-Nejad H. 2015. The multiple roles of epidermal growth factor repeat O-glycans in animal development. *Glycobiology*. 25:1027–1042.
- Hammond C, Braakman I, Helenius A. 1994. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. *Proc Natl Acad Sci USA*. 91:913–917.
- Hansen JE, Clausen H, Nielsen C, Teglbjaerg LS, Hansen LL, Nielsen CM, Dabelsteen E, Mathiesen L, Hakomori SI, Nielsen JO. 1990. Inhibition of human immunodeficiency virus (HIV) infection in vitro by anticarbohydrate monoclonal antibodies: peripheral glycosylation of HIV envelope glycoprotein gp120 may be a target for virus neutralization. *J Virol*. 64:2833–2840.
- Hansen JE, Nielsen C, Arendrup M, Olofsson S, Mathiesen L, Nielsen JO, Clausen H. 1991. Broadly neutralizing antibodies targeted to mucin-type carbohydrate epitopes of human immunodeficiency virus. *J Virol*. 65:6461–6467.
- Hata A, Mukai T, Isegawa Y, Yamanishi K. 1996. Identification and analyses of glycoprotein B of human herpesvirus 7. *Virus Res*. 46:125–137.
- Heldwein EE, Lou H, Bender FC, Cohen GH, Eisenberg RJ, Harrison SC. 2006. Crystal structure of glycoprotein B from herpes simplex virus 1. *Science*. 313:217–220.
- Helenius A, Aebi M. 2004. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem*. 73:1019–1049.
- Helle F, Duverlie G, Dubuisson J. 2011. The hepatitis C virus glycan shield and evasion of the humoral immune response. *Viruses*. 3:1909–1932.
- Helle F, Vieyres G, Elkrief L, Popescu CI, Wychowski C, Descamps V, Castelain S, Roingeard P, Duverlie G, Dubuisson J. 2010. Role of N-linked glycans in the functions of hepatitis C virus envelope proteins incorporated into infectious virions. *J Virol*. 84:11905–11915.
- Herrler G, Compans RW. 1983. Posttranslational modification and intracellular transport of mumps virus glycoproteins. *J Virol*. 47:354–362.
- Ho-Terry L, Cohen A. 1984. The role of glycosylation on haemagglutination and immunological reactivity of rubella virus. *Arch Virol*. 79:139–146.
- Hobman TC, Qiu ZY, Chaye H, Gillam S. 1991. Analysis of rubella virus E1 glycosylation mutants expressed in COS cells. *Virology*. 181:768–772.

- Honess RW, Roizman B. 1975. Proteins specified by herpes simplex virus. XIII. Glycosylation of viral polypeptides. *J Virol.* 16:1308–1326.
- Hong PW, Flummerfelt KB, de Parseval A, Gurney K, Elder JH, Lee B. 2002. Human immunodeficiency virus envelope (gp120) binding to DC-SIGN and primary dendritic cells is carbohydrate dependent but does not involve 2G12 or cyanovirin binding sites: Implications for structural analyses of gp120-DC-SIGN binding. *J Virol.* 76:12855–12865.
- Hsieh P, Rosner MR, Robbins PW. 1983. Host-dependent variation of asparagine-linked oligosaccharides at individual glycosylation sites of Sindbis virus glycoproteins. *J Biol Chem.* 258:2548–2554.
- Hu A, Cathomen T, Cattaneo R, Norrby E. 1995. Influence of N-linked oligosaccharide chains on the processing, cell surface expression and function of the measles virus fusion protein. *J Gen Virol.* 76(Pt 3):705–710.
- Hu S, Jiang LB, Zou XJ, Yi W, Tian DY. 2016. Hepatitis B virus upregulates host expression of alpha-1,2-mannosidases via the PPARalpha pathway. *World J Gastroenterol.* 22:9534–9543.
- Huber MT, Compton T. 1999. Intracellular formation and processing of the heterotrimeric gH-gL-gO (gCIII) glycoprotein envelope complex of human cytomegalovirus. *J Virol.* 73:3886–3892.
- Hunt LA, Etchison JR, Summers DF. 1978. Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus. *Proc Natl Acad Sci USA.* 75:754–758.
- Hyakumura M, Walsh R, Thaysen-Andersen M, Kingston NJ, La M, Lu L, Lovrecz G, Packer NH, Locarnini S, Netter HJ. 2015. Modification of the asparagine-linked glycan density for the design of hepatitis B virus (HBV) virus-like particles with enhanced immunogenicity. *J Virol.* 89(22):11312–11322.
- Jacob RE, Perdivara I, Przybylski M, Tomer KB. 2008. Mass spectrometric characterization of glycosylation of hepatitis C virus E2 envelope glycoprotein reveals extended microheterogeneity of N-glycans. *J Am Soc Mass Spectrom.* 19:428–444.
- International Committee on Taxonomy of Viruses. 2012. *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses.* London: Academic Press.
- Ito K, Qin Y, Guarnieri M, Garcia T, Kwei K, Mizokami M, Zhang J, Li J, Wands JR, Tong S. 2010. Impairment of hepatitis B virus virion secretion by single-amino-acid substitutions in the small envelope protein and rescue by a novel glycosylation site. *J Virol.* 84:12850–12861.
- Iversen MB, Reinert LS, Thomsen MK, Bagdonaite I, Nandakumar R, Cheshenko N, Prabakaran T, Vakhrushev SY, Krzyzowska M, Kratholm SK et al. 2016. An innate antiviral pathway acting before interferons at epithelial surfaces. *Nat Immunol.* 17:150–158.
- Jacob L, Leib R, Ollila HM, Bonvalet M, Adams CM, Mignot E. 2015. Comparison of Pandemrix and Arepanrix, two pH1N1 A/S03-adjuvanted vaccines differentially associated with narcolepsy development. *Brain, Behav, Immun.* 47:44–57.
- Jeffers SA, Sanders DA, Sanchez A. 2002. Covalent modifications of the ebola virus glycoprotein. *J Virol.* 76:12463–12472.
- Johnson DC, Spear PG. 1983. O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the Golgi apparatus. *Cell.* 32:987–997.
- Jolly CL, Sattentau QJ. 2013. Attachment factors. *Adv Exp Med Biol.* 790:1–23.
- Jones TR, Wiertz EJ, Sun L, Fish KN, Nelson JA, Ploegh HL. 1996. Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proc Natl Acad Sci USA.* 93:11327–11333.
- Julithe R, Abou-Jaoude G, Sureau C. 2014. Modification of the hepatitis B virus envelope protein glycosylation pattern interferes with secretion of viral particles, infectivity, and susceptibility to neutralizing antibodies. *J Virol.* 88:9049–9059.
- Kari B, Radeke R, Gehr R. 1992. Processing of human cytomegalovirus envelope glycoproteins in and egress of cytomegalovirus from human astrocytoma cells. *J Gen Virol.* 73(Pt 2):253–260.
- Kaye J, Browne H, Stoffel M, Minson T. 1992. The UL16 gene of human cytomegalovirus encodes a glycoprotein that is dispensable for growth in vitro. *J Virol.* 66:6609–6615.
- Keller JM, Spear PG, Roizman B. 1970. Proteins specified by herpes simplex virus. 3. Viruses differing in their effects on the social behavior of infected cells specify different membrane glycoproteins. *Proc Natl Acad Sci USA.* 65:865–871.
- Khatri K, Staples GO, Leymarie N, Leon DR, Turiak L, Huang Y, Yip S, Hu H, Heckendorf CF, Zaia J. 2014. Confident assignment of site-specific glycosylation in complex glycoproteins in a single step. *J Proteome Res.* 13:4347–4355.
- Kim SY, Li B, Linhardt RJ. 2017. Pathogenesis and inhibition of flaviviruses from a carbohydrate perspective. *Pharmaceuticals (Basel).* 10(2):E44.
- Kim KS, Sapienza VJ, Carp RI, Moon HM. 1976. Analysis of structural polypeptides of purified human cytomegalovirus. *J Virol.* 20:604–611.
- Knowles RW, Person S. 1976. Effects of 2-deoxyglucose, glucosamine, and mannose on cell fusion and the glycoproteins of herpes simplex virus. *J Virol.* 18:644–651.
- Korndel MJ, Mollema L, Tchermiaeva I, van der Klis F, Kroes AC, Oudesluis-Murphy AM, Vossen AC, de Melker HE. 2015. Cytomegalovirus infection in the Netherlands: Seroprevalence, risk factors, and implications. *J Clin Virol.* 63:53–58.
- Kornfeld R, Kornfeld S. 1985. Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem.* 54:631–664.
- Koyano S, Mar EC, Stamey FR, Inoue N. 2003. Glycoproteins M and N of human herpesvirus 8 form a complex and inhibit cell fusion. *J Gen Virol.* 84:1485–1491.
- Kramer MA, Uitenbroek DG, Ujcic-Voortman JK, Pfrommer C, Spaargaren J, Coutinho RA, Dukers-Muijters NH. 2008. Ethnic differences in HSV1 and HSV2 seroprevalence in Amsterdam, the Netherlands. *Euro Surveill.* 13(24):18904, pii.
- Kropff B, Burkhardt C, Schott J, Nentwich J, Fisch T, Britt W, Mach M. 2012. Glycoprotein N of human cytomegalovirus protects the virus from neutralizing antibodies. *PLoS Pathog.* 8:e1002999.
- Krummenacher C, Supekar VM, Whitbeck JC, Lazear E, Connolly SA, Eisenberg RJ, Cohen GH, Wiley DC, Carfi A. 2005. Structure of unliganded HSV gD reveals a mechanism for receptor-mediated activation of virus entry. *EMBO J.* 24:4144–4153.
- Krystal M, Young JF, Palese P, Wilson IA, Skehel JJ, Wiley DC. 1983. Sequential mutations in hemagglutinins of influenza B virus isolates: Definition of antigenic domains. *Proc Natl Acad Sci USA.* 80:4527–4531.
- Kuhn JE, Eing BR, Brossmer R, Munk K, Braun RW. 1988. Removal of N-linked carbohydrates decreases the infectivity of herpes simplex virus type 1. *J Gen Virol.* 69(Pt 11):2847–2858.
- Kushnir N, Streatfield SJ, Yusibov V. 2012. Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. *Vaccine.* 31:58–83.
- Lake CM, Molesworth SJ, Hutt-Fletcher LM. 1998. The Epstein-Barr virus (EBV) gN homolog BLRF1 encodes a 15-kilodalton glycoprotein that cannot be authentically processed unless it is coexpressed with the EBV gM homolog BBRF3. *J Virol.* 72:5559–5564.
- Lambert DM, Pons MW. 1983. Respiratory syncytial virus glycoproteins. *Virology.* 130:204–214.
- Lancaster C, Pristatsky P, Hoang VM, Casimiro DR, Schwartz RM, Rustandi R, Ha S. 2016. Characterization of N-glycosylation profiles from mammalian and insect cell derived chikungunya VLP. *J Chromatogr B Analyt Technol Biomed Life Sci.* 1032:218–223.
- Land A, Braakman I. 2001. Folding of the human immunodeficiency virus type 1 envelope glycoprotein in the endoplasmic reticulum. *Biochimie.* 83:783–790.
- Larsen ISB, Narimatsu Y, Joshi HJ, Yang Z, Harrison OJ, Brasch J, Shapiro L, Honig B, Vakhrushev SY, Clausen H et al. 2017. Mammalian O-mannosylation of cadherins and plexins is independent of protein O-mannosyltransferase 1 and 2. *J Biol Chem.* 292(27):11586–11598.
- Lauring AS, Frydman J, Andino R. 2013. The role of mutational robustness in RNA virus evolution. *Nat Rev Microbiol.* 11:327–336.
- Leavitt R, Schlesinger S, Kornfeld S. 1977. Tunicamycin inhibits glycosylation and multiplication of Sindbis and vesicular stomatitis viruses. *J Virol.* 21:375–385.

- Lee JH, Ozorowski G, Ward AB. 2016. Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. *Science*. 351:1043–1048.
- Lee JE, Saphire EO. 2009. Ebola virus glycoprotein structure and mechanism of entry. *Future Virol*. 4:621–635.
- Lei Y, Yu H, Dong Y, Yang J, Ye W, Wang Y, Chen W, Jia Z, Xu Z, Li Z et al. 2015. Characterization of N-glycan structures on the surface of mature dengue 2 virus derived from insect cells. *PLoS One*. 10:e0132122.
- Lennemann NJ, Rhein BA, Ndungo E, Chandran K, Qiu X, Maury W. 2014. Comprehensive functional analysis of N-linked glycans on Ebola virus GP1. *mBio*. 5:e00862–00813.
- Leverly SB, Steentoft C, Halim A, Narimatsu Y, Clausen H, Vakhrushev SY. 2015. Advances in mass spectrometry driven O-glycoproteomics. *Biochim Biophys Acta*. 1850:33–42.
- Levine H, Balicer RD, Rozhavski V, Halperin T, Shreberk M, Davidovitch N, Huerta-Hartal M, Ankol OE. 2012. Seroepidemiology of Epstein-Barr virus and cytomegalovirus among Israeli male young adults. *Ann Epidemiol*. 22:783–788.
- Li J, Bhuvanankantham R, Howe J, Ng ML. 2006. The glycosylation site in the envelope protein of West Nile virus (Sarafend) plays an important role in replication and maturation processes. *J Gen Virol*. 87:613–622.
- Li M, MacKey J, Czajak SC, Desrosiers RC, Lackner AA, Jung JU. 1999. Identification and characterization of Kaposi's sarcoma-associated herpesvirus K8.1 virion glycoprotein. *J Virol*. 73:1341–1349.
- Li D, von Schaeuwen M, Wang X, Tao W, Zhang Y, Li L, Heller B, Hrebikova G, Deng Q, Ploss A et al. 2016. Altered glycosylation patterns increase immunogenicity of a subunit HCV vaccine inducing neutralizing antibodies which confer protection in mice. *J Virol*. 90(23):10486–10498.
- Lin YL, Chang PC, Wang Y, Li M. 2008. Identification of novel viral interleukin-10 isoforms of human cytomegalovirus AD169. *Virus Res*. 131:213–223.
- Lin G, Simmons G, Pohlmann S, Baribaud F, Ni H, Leslie GJ, Haggarty BS, Bates P, Weissman D, Hoxie JA et al. 2003. Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. *J Virol*. 77:1337–1346.
- Liu M, Chen H, Luo F, Li P, Pan Q, Xia B, Qi Z, Ho WZ, Zhang XL. 2007. Deletion of N-glycosylation sites of hepatitis C virus envelope protein E1 enhances specific cellular and humoral immune responses. *Vaccine*. 25:6572–6580.
- Locker JK, Griffiths G, Horzinek MC, Rottier PJ. 1992. O-glycosylation of the coronavirus M protein. Differential localization of sialyltransferases in N- and O-linked glycosylation. *J Biol Chem*. 267:14094–14101.
- Lombardi F, Nakamura KJ, Chen T, Sobrera ER, Tobin NH, Aldrovandi GM. 2015. A conserved glycan in the C2 domain of HIV-1 envelope acts as a molecular switch to control X4 utilization by clonal variants with identical V3 loops. *PLoS One*. 10:e0128116.
- Lopo S, Vinagre E, Palmilha P, Paixao MT, Nogueira P, Freitas MG. 2011. Seroprevalence to cytomegalovirus in the Portuguese population, 2002–2003. *Euro Surveill*. 16(25):19896, pii.
- Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, Staropoli I, Foug S, Amara A, Houles C, Fieschi F, Schwartz O, Virelizier JL et al. 2003. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J Biol Chem*. 278:20358–20366.
- Lu X, Mehta A, Dadmarz M, Dwek R, Blumberg BS, Block TM. 1997. Aberrant trafficking of hepatitis B virus glycoproteins in cells in which N-glycan processing is inhibited. *Proc Natl Acad Sci USA*. 94:2380–2385.
- Lundstrom M, Jeansson S, Olofsson S. 1987. Host cell-induced differences in the O-glycosylation of herpes simplex virus gC-1. II. Demonstration of cell-specific galactosyltransferase essential for formation of O-linked oligosaccharides. *Virology*. 161:395–402.
- Lundstrom ML, Mauracher CA, Tingle AJ. 1991. Characterization of carbohydrates linked to rubella virus glycoprotein E2. *J Gen Virol*. 72(Pt 4):843–850.
- Luo S, Hu K, He S, Wang P, Zhang M, Huang X, Du T, Zheng C, Liu Y, Hu Q. 2015. Contribution of N-linked glycans on HSV-2 gB to cell-cell fusion and viral entry. *Virology*. 483:72–82.
- Lynch RM, Wong P, Tran L, O'Dell S, Nason MC, Li Y, Wu X, Mascola JR. 2015. HIV-1 fitness cost associated with escape from the VRC01 class of CD4 binding site neutralizing antibodies. *J Virol*. 89:4201–4213.
- Machiels B, Lete C, Guillaume A, Mast J, Stevenson PG, Vanderplasschen A, Gillet L. 2011. Antibody evasion by a gammaherpesvirus O-glycan shield. *PLoS Pathog*. 7:e1002387.
- Maidji E, Tugizov S, Abenes G, Jones T, Pereira L. 1998. A novel human cytomegalovirus glycoprotein, gpUS9, which promotes cell-to-cell spread in polarized epithelial cells, colocalizes with the cytoskeletal proteins E-cadherin and F-actin. *J Virol*. 72:5717–5727.
- Mandel U, Hassan H, Therkildsen MH, Rygaard J, Jakobsen MH, Juhl BR, Dabelsteen E, Clausen H. 1999. Expression of polypeptide GalNAc-transferases in stratified epithelia and squamous cell carcinomas: Immunohistological evaluation using monoclonal antibodies to three members of the GalNAc-transferase family. *Glycobiology*. 9:43–52.
- Mann E, Edwards J, Brown DT. 1983. Polycaryocyte formation mediated by Sindbis virus glycoproteins. *J Virol*. 45:1083–1089.
- Maresova L, Kutinova L, Ludvikova V, Zak R, Mares M, Nemeckova S. 2000. Characterization of interaction of gH and gL glycoproteins of varicella-zoster virus: Their processing and trafficking. *J Gen Virol*. 81:1545–1552.
- Margulies BJ, Browne H, Gibson W. 1996. Identification of the human cytomegalovirus G protein-coupled receptor homologue encoded by UL33 in infected cells and enveloped virus particles. *Virology*. 225:111–125.
- Margulies BJ, Gibson W. 2007. The chemokine receptor homologue encoded by US27 of human cytomegalovirus is heavily glycosylated and is present in infected human foreskin fibroblasts and enveloped virus particles. *Virus Res*. 123:57–71.
- Mathys L, Balzarini J. 2014. The role of N-glycans of HIV-1 gp41 in virus infectivity and susceptibility to the suppressive effects of carbohydrate-binding agents. *Retrovirology*. 11:107.
- Mathys L, Balzarini J. 2015. Several N-glycans on the HIV envelope glycoprotein gp120 preferentially locate near disulphide bridges and are required for efficient infectivity and virus transmission. *PLoS One*. 10:e0130621.
- McCoy LE, Burton DR. 2017. Identification and specificity of broadly neutralizing antibodies against HIV. *Immunol Rev*. 275:11–20.
- McGeoch DJ, Rixon FJ, Davison AJ. 2006. Topics in herpesvirus genomics and evolution. *Virus Res*. 117:90–104.
- Meads MB, Medveczky PG. 2004. Kaposi's sarcoma-associated herpesvirus-encoded viral interleukin-6 is secreted and modified differently than human interleukin-6: Evidence for a unique autocrine signaling mechanism. *J Biol Chem*. 279:51793–51803.
- Mechref Y, Novotny MV, Krishnan C. 2003. Structural characterization of oligosaccharides using MALDI-TOF/TOF tandem mass spectrometry. *Anal Chem*. 75:4895–4903.
- Meunier JC, Fournillier A, Choukhi A, Cahour A, Cocquerel L, Dubuisson J, Wychowski C. 1999. Analysis of the glycosylation sites of hepatitis C virus (HCV) glycoprotein E1 and the influence of E1 glycans on the formation of the HCV glycoprotein complex. *J Gen Virol*. 80(Pt 4):887–896.
- Miller JL, de Wet BJ, Martinez-Pomares L, Radcliffe CM, Dwek RA, Rudd PM, Gordon S. 2008. The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog*. 4:e17.
- Miller JL, Lachica R, Sayce AC, Williams JP, Bapat M, Dwek R, Beatty PR, Harris E, Zitzmann N. 2012. Liposome-mediated delivery of iminosugars enhances efficacy against dengue virus in vivo. *Antimicrob Agents Chemother*. 56:6379–6386.
- Miyaji K, Furukawa JL, Suzuki Y, Yamamoto N, Shinohara Y, Yuki N. 2016. Altered gene expression of glycosyltransferases and sialyltransferases and total amount of glycosphingolipids following herpes simplex virus infection. *Carbohydr Res*. 434:37–43.
- Montalvo EA, Grose C. 1986. Neutralization epitope of varicella zoster virus on native viral glycoprotein gp118 (VZV glycoprotein gpIII). *Virology*. 149:230–241.
- Montalvo EA, Grose C. 1987. Assembly and processing of the disulfide-linked varicella-zoster virus glycoprotein gpII(140). *J Virol*. 61:2877–2884.

- Montalvo EA, Parmley RT, Grose C. 1985. Structural analysis of the varicella-zoster virus gp98-gp62 complex: Posttranslational addition of N-linked and O-linked oligosaccharide moieties. *J Virol.* 53:761–770.
- Moremen KW, Tiemeyer M, Nairn AV. 2012. Vertebrate protein glycosylation: Diversity, synthesis and function. *Nat Rev Mol Cell Biol.* 13: 448–462.
- Mossenta M, Marchese S, Poggianella M, Slon Campos JL, Burrone OR. 2017. Role of N-glycosylation on Zika virus E protein secretion, viral assembly and infectivity. *Biochem Biophys Res Commun.* 492(4):579–586.
- Mouquet H, Scharf L, Euler Z, Liu Y, Eden C, Scheid JF, Halper-Stromberg A, Gnanaprasam PN, Spencer DI, Seaman MS et al. 2012. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc Natl Acad Sci USA.* 109:E3268–E3277.
- Mukai T, Hata A, Isegawa Y, Yamanishi K. 1997. Characterization of glycoprotein H and L of human herpesvirus 7. *Microbiol Immunol.* 41:43–50.
- Mullberg J, Hsu ML, Rauch CT, Gerhart MJ, Kaykas A, Cosman D. 1999. The R27080 glycoprotein is abundantly secreted from human cytomegalovirus-infected fibroblasts. *J Gen Virol.* 80(Pt 2):437–440.
- Nakamura S, Horie M, Daidoji T, Honda T, Yasugi M, Kuno A, Komori T, Okuzaki D, Narimatsu H, Nakaya T et al. 2015. Influenza A virus-induced expression of a GalNAc transferase, GALNT3, via MicroRNAs is required for enhanced viral replication. *J Virol.* 90:1788–1801.
- Nakayama EE, Shioda T, Tatsumi M, Xin X, Yu D, Ohgimoto S, Kato A, Sakai Y, Ohnishi Y, Nagai Y. 1998. Importance of the N-glycan in the V3 loop of HIV-1 envelope protein for CXCR-4- but not CCR-5-dependent fusion. *FEBS Lett.* 426:367–372.
- Nelson MA, Herrero LJ, Jeffery JA, Hoehn M, Rudd PA, Supramaniam A, Kay BH, Ryan PA, Mahalingam S. 2016. Role of envelope N-linked glycosylation in Ross River virus virulence and transmission. *J Gen Virol.* 97 (5):1094–1106.
- Niemann H, Boschek B, Evans D, Rosing M, Tamura T, Klenk HD. 1982. Post-translational glycosylation of coronavirus glycoprotein E1: Inhibition by monensin. *EMBO J.* 1:1499–1504.
- Niemann H, Geyer R, Klenk HD, Linder D, Stirn S, Wirth M. 1984. The carbohydrates of mouse hepatitis virus (MHV) A59: Structures of the O-glycosidically linked oligosaccharides of glycoprotein E1. *EMBO J.* 3: 665–670.
- Nolan LA, Morgan AJ. 1995. The Epstein-Barr virus open reading frame BDLF3 codes for a 100–150 kDa glycoprotein. *J Gen Virol.* 76(Pt 6): 1381–1392.
- Norberg P, Olofsson S, Tarp MA, Clausen H, Bergstrom T, Liljeqvist JA. 2007. Glycoprotein I of herpes simplex virus type 1 contains a unique polymorphic tandem-repeated mucin region. *J Gen Virol.* 88:1683–1688.
- Norden R, Halim A, Nystrom K, Bennett EP, Mandel U, Olofsson S, Nilsson J, Larson G. 2015. O-linked glycosylation of the mucin domain of the herpes simplex virus type 1-specific glycoprotein gC-1 is temporally regulated in a seed-and-spread manner. *J Biol Chem.* 290:5078–5091.
- Norden R, Nystrom K, Adamiak B, Halim A, Nilsson J, Larson G, Trybala E, Olofsson S. 2013. Involvement of viral glycoprotein gC-1 in expression of the selectin ligand sialyl-Lewis X induced after infection with herpes simplex virus type 1. *APMIS.* 121:280–289.
- Norden R, Samuelsson E, Nystrom K. 2017. NFkappaB-mediated activation of the cellular FUT3, 5 and 6 gene cluster by herpes simplex virus type 1. *Glycobiology.* 27:999–1005.
- Norrild B, Pedersen B. 1982. Effect of tunicamycin on the synthesis of herpes simplex virus type 1 glycoproteins and their expression on the cell surface. *J Virol.* 43:395–402.
- Noyori O, Matsuno K, Kajihara M, Nakayama E, Igarashi M, Kuroda M, Isoda N, Yoshida R, Takada A. 2013. Differential potential for envelope glycoprotein-mediated steric shielding of host cell surface proteins among flaviviruses. *Virology.* 446:152–161.
- Nystrom K, Grahm A, Lindh M, Brytting M, Mandel U, Larson G, Olofsson S. 2007. Virus-induced transcriptional activation of host FUT genes associated with neo-expression of Ley in cytomegalovirus-infected and sialyl-Lex in varicella-zoster virus-infected diploid human cells. *Glycobiology.* 17:355–366.
- Odland ML, Strand KM, Nordbo SA, Forsmo S, Austgulen R, Iversen AC. 2013. Changing patterns of cytomegalovirus seroprevalence among pregnant women in Norway between 1995 and 2009 examined in the Norwegian Mother and Child Cohort Study and two cohorts from Sor-Trondelag County: A cross-sectional study. *BMJ Open.* 3:e003066.
- Okuno T, Sao H, Asada H, Shiraki K, Takahashi M, Yamanishi K. 1990. Analysis of a glycoprotein of human herpesvirus 6 (HHV-6) using monoclonal antibodies. *Virology.* 176:625–628.
- Okuno T, Shao H, Asada H, Shiraki K, Takahashi M, Yamanishi K. 1992. Analysis of human herpesvirus 6 glycoproteins recognized by monoclonal antibody OHV1. *J Gen Virol.* 73(Pt 2):443–447.
- Olofsson S, Blomberg J, Lycke E. 1981. O-glycosidic carbohydrate-peptide linkages of Herpes simplex virus glycoproteins. *Arch Virol.* 70:321–329.
- Olofsson S, Lycke E. 1980. Glucosamine metabolism of herpes simplex virus infected cells. Inhibition of glycosylation by tunicamycin and 2-deoxy-D-glucose. *Arch Virol.* 65:201–209.
- Olofsson S, Norrild B, Andersen AB, Pereira L, Jeansson S, Lycke E. 1983. Populations of herpes simplex virus glycoprotein gC with and without affinity for the N-acetyl-galactosamine specific lectin of Helix pomatia. *Arch Virol.* 76:25–38.
- Orlova OV, Drutsa VL, Spirin PV, Prasolov VS, Rubtsov PM, Kochetkov SN, Beljelarskaya SN. 2015. The role of HCV e2 protein glycosylation in functioning of virus envelope proteins in insect and mammalian cells. *Acta Naturae.* 7:87–97.
- Oxman MN. 2010. Zoster vaccine: Current status and future prospects. *Clin Infect Dis.* 51:197–213.
- Pabst M, Chang M, Stadlmann J, Altmann F. 2012. Glycan profiles of the 27 N-glycosylation sites of the HIV envelope protein CN54gp140. *Biol Chem.* 393:719–730.
- Palmisano G, Melo-Braga MN, Engholm-Keller K, Parker BL, Larsen MR. 2012. Chemical deamidation: A common pitfall in large-scale N-linked glycoproteomic mass spectrometry-based analyses. *J Proteome Res.* 11: 1949–1957.
- Panico M, Bouche L, Binet D, O'Connor MJ, Rahman D, Pang PC, Canis K, North SJ, Desrosiers RC, Chertova E et al. 2016. Mapping the complete glycoproteome of virion-derived HIV-1 gp120 provides insights into broadly neutralizing antibody binding. *Sci Rep.* 6:32956.
- Park S, Lee I, Kim JL, Bae JY, Yoo K, Kim J, Nam M, Park M, Yun SH, Cho WI et al. 2016. Effects of HA and NA glycosylation pattern changes on the transmission of avian influenza A(H7N9) virus in guinea pigs. *Biochem Biophys Res Commun.* 479(2):192–197.
- Paulsen SJ, Rosenkilde MM, Eugen-Olsen J, Kledal TN. 2005. Epstein-Barr virus-encoded BILF1 is a constitutively active G protein-coupled receptor. *J Virol.* 79:536–546.
- Pembrey L, Raynor P, Griffiths P, Chaytor S, Wright J, Hall AJ. 2013. Seroprevalence of cytomegalovirus, Epstein Barr virus and varicella zoster virus among pregnant women in Bradford: A cohort study. *PLoS One.* 8: e81881.
- Peng T, Ponce de Leon M, Novotny MJ, Jiang H, Lambris JD, Dubin G, Spear PG, Cohen GH, Eisenberg RJ. 1998. Structural and antigenic analysis of a truncated form of the herpes simplex virus glycoprotein gH-gL complex. *J Virol.* 72:6092–6103.
- Pertel PE, Spear PG, Longnecker R. 1998. Human herpesvirus-8 glycoprotein B interacts with Epstein-Barr virus (EBV) glycoprotein 110 but fails to complement the infectivity of EBV mutants. *Virology.* 251:402–413.
- Pesonen M. 1979. Sequence analysis of lactosamine type glycans of individual membrane proteins of Semliki Forest virus. *J Gen Virol.* 45:479–487.
- Pesonen M, Kuismanen E, Pettersson RF. 1982. Monosaccharide sequence of protein-bound glycans of Uukuniemi virus. *J Virol.* 41:390–400.
- Pesonen M, Ronnholm R, Kuismanen E, Pettersson RF. 1982. Characterization of the oligosaccharides of Inkoo virus envelope glycoproteins. *J Gen Virol.* 63:425–434.
- Petro C, Gonzalez PA, Cheshenko N, Jandl T, Khajoueinejad N, Benard A, Sengupta M, Herold BC, Jacobs WR. 2015. Herpes simplex type 2 virus deleted in glycoprotein D protects against vaginal, skin and neural disease. *eLife.* 4, doi:10.7554/eLife.06054.

- Pfeiffer B, Thomson B, Chandran B. 1995. Identification and characterization of a cDNA derived from multiple splicing that encodes envelope glycoprotein gp105 of human herpesvirus 6. *J Virol.* 69:3490–3500.
- Phoenix I, Nishiyama S, Lokugamage N, Hill TE, Huante MB, Slack OA, Carpio VH, Freiberg AN, Ikegami T. 2016. N-Glycans on the rift valley fever virus envelope glycoproteins Gn and Gc redundantly support viral infection via DC-SIGN. *Viruses.* 8(5):E149, pii.
- Pollakis G, Kang S, Kliphuis A, Chalaby MI, Goudsmit J, Paxton WA. 2001. N-linked glycosylation of the HIV type-1 gp120 envelope glycoprotein as a major determinant of CCR5 and CXCR4 coreceptor utilization. *J Biol Chem.* 276:13433–13441.
- Prasanphanich NS, Mickum ML, Heimburg-Molinaro J, Cummings RD. 2013. Glycoconjugates in host-helminth interactions. *Front Immunol.* 4: 240.
- Pritchard LK, Harvey DJ, Bonomelli C, Crispin M, Doores KJ. 2015. Cell- and protein-directed glycosylation of native cleaved HIV-1 envelope. *J Virol.* 89:8932–8944.
- Pritchard LK, Spencer DI, Royle L, Bonomelli C, Seabright GE, Behrens AJ, Kulp DW, Menis S, Krumm SA, Dunlop DC et al. 2015. Glycan clustering stabilizes the mannose patch of HIV-1 and preserves vulnerability to broadly neutralizing antibodies. *Nat Commun.* 6:7479.
- Rabinovich GA, Toscano MA. 2009. Turning 'sweet' on immunity: Galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol.* 9:338–352.
- Rabouille C, Hui N, Hunte F, Kieckbusch R, Berger EG, Warren G, Nilsson T. 1995. Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. *J Cell Sci.* 108(Pt 4):1617–1627.
- Radhakrishnan P, Dabelsteen S, Madsen FB, Francavilla C, Kopp KL, Steentoft C, Vakhrushev SY, Olsen JV, Hansen L, Bennett EP et al. 2014. Immature truncated O-glycophenotype of cancer directly induces oncogenic features. *Proc Natl Acad Sci USA.* 111(39):E4066–E4075.
- Rasilo ML, Renkonen O. 1979. The molecular size of glycans liberated by hydrazinolysis from Semliki Forest virus proteins. *Biochim Biophys Acta.* 582:307–321.
- Redkiewicz P, Sirko A, Kamel KA, Gora-Sochacka A. 2014. Plant expression systems for production of hemagglutinin as a vaccine against influenza virus. *Acta Biochim Pol.* 61:551–560.
- Rehm A, Stern P, Ploegh HL, Tortorella D. 2001. Signal peptide cleavage of a type I membrane protein, HCMV US11, is dependent on its membrane anchor. *EMBO J.* 20:1573–1582.
- Respass RA, Pancake BA, Edson CM, Schaffer PA. 1984. A rapid procedure for the enrichment of undenatured, antigenically active herpes simplex virus glycoproteins. *J Virol Methods.* 8:27–45.
- Reszka N, Krol E, Patel AH, Szewczyk B. 2010. Effect of tunicamycin on the biogenesis of hepatitis C virus glycoproteins. *Acta Biochim Pol.* 57: 541–546.
- Ritchie G, Harvey DJ, Stroehrer U, Feldmann F, Feldmann H, Wahl-Jensen V, Royle L, Dwek RA, Rudd PM. 2010. Identification of N-glycans from Ebola virus glycoproteins by matrix-assisted laser desorption/ionisation time-of-flight and negative ion electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 24:571–585.
- Robbins PW, Hubbard SC, Turco SJ, Wirth DF. 1977. Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. *Cell.* 12:893–900.
- Rosner MR, Robbins PW. 1982. Separation of glycopeptides by high performance liquid chromatography. *J Cell Biochem.* 18:37–47.
- Rothman JE, Lodish HF. 1977. Synchronised transmembrane insertion and glycosylation of a nascent membrane protein. *Nature.* 269:775–780.
- Rottger S, White J, Wandall HH, Olivo JC, Stark A, Bennett EP, Whitehouse C, Berger EG, Clausen H, Nilsson T. 1998. Localization of three human polypeptide GalNAc-transferases in HeLa cells suggests initiation of O-linked glycosylation throughout the Golgi apparatus. *J Cell Sci.* 111(Pt 1): 45–60.
- Rouvinski A, Guardado-Calvo P, Barba-Spaeth G, Duquerry S, Vaney MC, Kikutu CM, Navarro Sanchez ME, Dejnirattisai W, Wongwiwat W, Haouz A et al. 2015. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. *Nature.* 520:109–113.
- Rowe AM St, Leger AJ, Jeon S, Dhaliwal DK, Knickelbein JE, Hendricks RL. 2013. Herpes keratitis. *Prog Retin Eye Res.* 32:88–101.
- Sabugo F, Espinoza-Araya R, Meneses MF, Cuchacovich M. 2014. Acute herpes simplex virus 1 pneumonitis in a patient with systemic lupus erythematosus. *J Clin Rheumatol.* 20:42–44.
- Sanders RW, Venturi M, Schiffner L, Kalyanaraman R, Katinger H, Lloyd KO, Kwong PD, Moore JP. 2002. The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. *J Virol.* 76:7293–7305.
- Sanjuan R, Nebot MR, Chirico N, Mansky LM, Belshaw R. 2010. Viral mutation rates. *J Virol.* 84:9733–9748.
- Sauerbrei A, Schmitt S, Scheper T, Brandstadt A, Saschenbrecker S, Motz M, Soutschek E, Wutzler P. 2011. Seroprevalence of herpes simplex virus type 1 and type 2 in Thuringia, Germany, 1999 to 2006. *Euro Surveill.* 16(44):20005, pii.
- Schachter H. 2000. The joys of HexNAc. The synthesis and function of N- and O-glycan branches. *Glycoconj J.* 17:465–483.
- Schmitt S, Glebe D, Alving K, Tolle TK, Linder M, Geyer H, Linder D, Peter-Katalinic J, Gerlich WH, Geyer R. 1999. Analysis of the pre-S2 N- and O-linked glycans of the M surface protein from human hepatitis B virus. *J Biol Chem.* 274:11945–11957.
- Schuren AB, Costa AI, Wiertz EJ. 2016. Recent advances in viral evasion of the MHC Class I processing pathway. *Curr Opin Immunol.* 40:43–50.
- Schwarz RT, Schmidt MF, Anwer U, Klenk HD. 1977. Carbohydrates of influenza virus. I. Glycopeptides derived from viral glycoproteins after labeling with radioactive sugars. *J Virol.* 23:217–226.
- Sefton BM. 1975. Virus-dependent glycosylation. *J Virol.* 17:85–93.
- Serafini-Cessi F, Dall'Olio F, Malagolini N, Pereira L, Campadelli-Fiume G. 1988. Comparative study on O-linked oligosaccharides of glycoprotein D of herpes simplex virus types 1 and 2. *J Gen Virol.* 69(Pt 4):869–877.
- Serafini-Cessi F, Dall'Olio F, Pereira L, Campadelli-Fiume G. 1984. Processing of N-linked oligosaccharides from precursor- to mature-form herpes simplex virus type 1 glycoprotein gC. *J Virol.* 51:838–844.
- Serafini-Cessi F, Dall'Olio F, Scannavini M, Campadelli-Fiume G. 1983. Processing of herpes simplex virus-1 glycans in cells defective in glycosyl transferases of the Golgi system: Relationship to cell fusion and virion egress. *Virology.* 131:59–70.
- Serafini-Cessi F, Dall'Olio F, Scannavini M, Costanzo F, Campadelli-Fiume G. 1983. N-acetylgalactosaminyltransferase activity involved in O-glycosylation of herpes simplex virus type 1 glycoproteins. *J Virol.* 48: 325–329.
- Serafini-Cessi F, Malagolini N, Dall'Olio F, Pereira L, Campadelli-Fiume G. 1985. Oligosaccharide chains of herpes simplex virus type 2 glycoprotein gG.2. *Arch Biochem Biophys.* 240:866–876.
- Serafini-Cessi F, Malagolini N, Nanni M, Dall'Olio F, Campadelli-Fiume G, Tanner J, Kieff E. 1989. Characterization of N- and O-linked oligosaccharides of glycoprotein 350 from Epstein-Barr virus. *Virology.* 170: 1–10.
- Shaiegan M, Rasouli M, Zadsar M, Zolfaghari S. 2015. Meta-analysis of cytomegalovirus seroprevalence in volunteer blood donors and healthy subjects in Iran from 1992 to 2013. *Iran J Basic Med Sci.* 18:627–634.
- Shida H, Dales S. 1981. Biogenesis of vaccinia: Carbohydrate of the hemagglutinin molecules. *Virology.* 111:56–72.
- Shiley K, Blumberg E. 2010. Herpes viruses in transplant recipients: HSV, VZV, human herpes viruses, and EBV. *Infect Dis Clin North Am.* 24: 373–393.
- Shrivastava-Ranjan P, Bergeron E, Chakrabarti AK, Albarino CG, Flint M, Nichol ST, Spiropoulou CF. 2016. 25-Hydroxycholesterol inhibition of lassa virus infection through aberrant GP1 glycosylation. *mBio.* 7(6): e01808–e01816.
- Sihlbom C, van Dijk Hard I, Lidell ME, Noll T, Hansson GC, Backstrom M. 2009. Localization of O-glycans in MUC1 glycoproteins using electron-capture dissociation fragmentation mass spectrometry. *Glycobiology.* 19: 375–381.
- Sili U, Kaya A, Mert A, Group HSVES. 2014. Herpes simplex virus encephalitis: Clinical manifestations, diagnosis and outcome in 106 adult patients. *J Clin Virol.* 60:112–118.

- Skehel JJ, Stevens DJ, Daniels RS, Douglas AR, Knossow M, Wilson IA, Wiley DC. 1984. A carbohydrate side chain on hemagglutinins of Hong Kong influenza viruses inhibits recognition by a monoclonal antibody. *Proc Natl Acad Sci USA*. 81:1779–1783.
- Skrincosky D, Hocknell P, Whetter L, Secchiero P, Chandran B, Dewhurst S. 2000. Identification and analysis of a novel heparin-binding glycoprotein encoded by human herpesvirus 7. *J Virol*. 74:4530–4540.
- Sodora DL, Cohen GH, Eisenberg RJ. 1989. Influence of asparagine-linked oligosaccharides on antigenicity, processing, and cell surface expression of herpes simplex virus type 1 glycoprotein D. *J Virol*. 63:5184–5193.
- Sommerstein R, Flatz L, Remy MM, Malinge P, Magistrelli G, Fischer N, Sahin M, Bergthaler A, Igonet S, Ter Meulen J et al. 2015. Arenavirus glycan shield promotes neutralizing antibody evasion and protracted infection. *PLoS Pathog*. 11:e1005276.
- Spear PG, Roizman B. 1970. Proteins specified by herpes simplex virus, IV. Site of glycosylation and accumulation of viral membrane proteins. *Proc Natl Acad Sci USA*. 66:730–737.
- St-Pierre C, Many H, Ouellet M, Clark GF, Endo T, Tremblay MJ, Sato S. 2011. Host-soluble galectin-1 promotes HIV-1 replication through a direct interaction with glycans of viral gp120 and host CD4. *J Virol*. 85:11742–11751.
- Stanley P. 2011. Golgi glycosylation. *Cold Spring Harb Perspect Biol*. 3(4):a005199, pii.
- Stansell E, Panico M, Canis K, Pang PC, Bouche L, Binet D, O'Connor MJ, Chertova E, Bess J, Lifson JD et al. 2015. Gp120 on HIV-1 virions lacks O-linked carbohydrate. *PLoS One*. 10:e0124784.
- Stanton RJ, Baluchova K, Dargan DJ, Cunningham C, Sheehy O, Seirafian S, McSharry BP, Neale ML, Davies JA, Tomasec P et al. 2010. Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. *J Clin Invest*. 120:3191–3208.
- Stavenhagen K, Hinneburg H, Thaysen-Andersen M, Hartmann L, Varon Silva D, Fuchser J, Kaspar S, Rapp E, Seeberger PH, Kolarich D. 2013. Quantitative mapping of glycoprotein micro-heterogeneity and macro-heterogeneity: An evaluation of mass spectrometry signal strengths using synthetic peptides and glycopeptides. *J Mass Spectrom*. 48:627–639.
- Steenfot C, Vakhrushev SY, Joshi HJ, Kong Y, Vester-Christensen MB, Schjoldager KT, Lavrsen K, Dabelsteen S, Pedersen NB, Marcos-Silva L et al. 2013. Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. *EMBO J*. 32:1478–1488.
- Steenfot C, Vakhrushev SY, Vester-Christensen MB, Schjoldager KT, Kong Y, Bennett EP, Mandel U, Wandall H, Lavery SB, Clausen H. 2011. Mining the O-glycoproteome using zinc-finger nuclease-glycoengineered SimpleCell lines. *Nat Methods*. 8:977–982.
- Stencel-Baerenwald JE, Reiss K, Reiter DM, Stehle T, Dermody TS. 2014. The sweet spot: Defining virus-sialic acid interactions. *Nat Rev Microbiol*. 12:739–749.
- Stone JA, Nicola AV, Baum LG, Aguilar HC. 2016. Multiple novel functions of henipavirus O-glycans: The first O-glycan functions identified in the paramyxovirus family. *PLoS Pathog*. 12:e1005445.
- Struwe WB, Stuckmann A, Behrens AJ, Pagel K, Crispin M. 2017. Global N-glycan site occupancy of HIV-1 gp120 by metabolic engineering and high-resolution intact mass spectrometry. *ACS Chem Biol*. 12:357–361.
- Suenaga T, Matsumoto M, Arisawa F, Kohyama M, Hirayasu K, Mori Y, Arase H. 2015. Sialic acids on varicella-zoster virus glycoprotein B required for cell-cell fusion. *J Biol Chem*. 290(32):19833–19843.
- Sutherlin ME, Nishimori I, Caffrey T, Bennett EP, Hassan H, Mandel U, Mack D, Iwamura T, Clausen H, Hollingsworth MA. 1997. Expression of three UDP-N-acetyl-alpha-D-galactosamine:polypeptide GalNAc N-acetylgalactosaminyltransferases in adenocarcinoma cell lines. *Cancer Res*. 57:4744–4748.
- Takada A, Fujioka K, Tsuiji M, Morikawa A, Higashi N, Ebihara H, Kobasa D, Feldmann H, Irimura T, Kawaoka Y. 2004. Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J Virol*. 78:2943–2947.
- Takamatsu S, Shimomura M, Kamada Y, Maeda H, Sobajima T, Hikita H, Iijima M, Okamoto Y, Misaki R, Fujiyama K et al. 2016. Core-fucosylation plays a pivotal role in hepatitis B pseudo virus infection: A possible implication for HBV glyco-therapy. *Glycobiology*. 26(11):1180–1189.
- Termini JM, Church ES, Silver ZA, Haslam SM, Dell A, Desrosiers RC. 2017. Human immunodeficiency virus and simian immunodeficiency virus maintain high levels of infectivity in the complete absence of mucin-type O-glycosylation. *J Virol*. 91(19):e01228-17, pii.
- Teuton JR, Brandt CR. 2007. Sialic acid on herpes simplex virus type 1 envelope glycoproteins is required for efficient infection of cells. *J Virol*. 81:3731–3739.
- Theiler RN, Compton T. 2002. Distinct glycoprotein O complexes arise in a post-Golgi compartment of cytomegalovirus-infected cells. *J Virol*. 76:2890–2898.
- Tian E, Ten Hagen KG. 2006. Expression of the UDP-GalNAc: Polypeptide N-acetylgalactosaminyltransferase family is spatially and temporally regulated during Drosophila development. *Glycobiology*. 16:83–95.
- Torrisi MR, Gentile M, Cardinali G, Cirone M, Zompetta C, Lotti LV, Frati L, Faggioni A. 1999. Intracellular transport and maturation pathway of human herpesvirus 6. *Virology*. 257:460–471.
- van Montfort T, Eggink D, Boot M, Tuen M, Hioe CE, Berkhout B, Sanders RW. 2011. HIV-1 N-glycan composition governs a balance between dendritic cell-mediated viral transmission and antigen presentation. *J Immunol*. 187:4676–4685.
- van Rijckevorsel GG, Damen M, Sonder GJ, van der Loeff MF, van den Hoek A. 2012. Seroprevalence of varicella-zoster virus and predictors for seronegativity in the Amsterdam adult population. *BMC Infect Dis*. 12:140.
- Vazquez M, LaRussa PS, Gershon AA, Steinberg SP, Freudigman K, Shapiro ED. 2001. The effectiveness of the varicella vaccine in clinical practice. *N Engl J Med*. 344:955–960.
- Walls AC, Tortorici MA, Frenz B, Snijder J, Li W, Rey FA, DiMaio F, Bosch BJ, Veeler D. 2016. Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. *Nat Struct Mol Biol*. 23:899–905.
- Wandall HH, Hassan H, Mirgorodskaya E, Kristensen AK, Roepstorff P, Bennett EP, Nielsen PA, Hollingsworth MA, Burchell J, Taylor-Papadimitriou J et al. 1997. Substrate specificities of three members of the human UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase family, GalNAc-T1, -T2, and -T3. *J Biol Chem*. 272:23503–23514.
- Wandall HH, Irazoqui F, Tarp MA, Bennett EP, Mandel U, Takeuchi H, Kato K, Irimura T, Suryanarayanan G, Hollingsworth MA et al. 2007. The lectin domains of polypeptide GalNAc-transferases exhibit carbohydrate-binding specificity for GalNAc: Lectin binding to GalNAc-glycopeptide substrates is required for high density GalNAc-O-glycosylation. *Glycobiology*. 17:374–387.
- Wang J, Fan Q, Satoh T, Ariei J, Lanier LL, Spear PG, Kawaguchi Y, Arase H. 2009. Binding of herpes simplex virus glycoprotein B (gB) to paired immunoglobulin-like type 2 receptor alpha depends on specific sialylated O-linked glycans on gB. *J Virol*. 83:13042–13045.
- Wang P, Hu K, Luo S, Zhang M, Deng X, Li C, Jin W, Hu B, He S, Li M et al. 2016. DC-SIGN as an attachment factor mediates Japanese encephalitis virus infection of human dendritic cells via interaction with a single high-mannose residue of viral E glycoprotein. *Virology*. 488:108–119.
- Wang W, Nie J, Prochnow C, Truong C, Jia Z, Wang S, Chen XS, Wang Y. 2013. A systematic study of the N-glycosylation sites of HIV-1 envelope protein on infectivity and antibody-mediated neutralization. *Retrovirology*. 10:14.
- Wang B, Wang Y, Frabutt DA, Zhang X, Yao X, Hu D, Zhang Z, Liu C, Zheng S, Xiang SH et al. 2017. Mechanistic understanding of N-glycosylation in Ebola virus glycoprotein maturation and function. *J Biol Chem*. 292:5860–5870.
- Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS et al. 2003. Antibody neutralization and escape by HIV-1. *Nature*. 422:307–312.
- Wenske EA, Bratton MW, Courtney RJ. 1982. Endo-beta-N-acetylglucosaminidase H sensitivity of precursors to herpes simplex virus type 1 glycoproteins gB and gC. *J Virol*. 44:241–248.



- Whiteman MC, Li L, Wicker JA, Kinney RM, Huang C, Beasley DW, Chung KM, Diamond MS, Solomon T, Barrett AD. 2010. Development and characterization of non-glycosylated E and NS1 mutant viruses as a potential candidate vaccine for West Nile virus. *Vaccine*. 28:1075–1083.
- Wu CY, Lin CW, Tsai TI, Lee CD, Chuang HY, Chen JB, Tsai MH, Chen BR, Lo PW, Liu CP et al. 2017. Influenza A surface glycosylation and vaccine design. *Proc Natl Acad Sci USA*. 114:280–285.
- Wu H, Liu L, Xiao J, Chi M, Qu Y, Feng H. 2015. Glycosylation of KSHV encoded vGPCR functions in its signaling and tumorigenicity. *Viruses*. 7: 1627–1641.
- Wu L, Renne R, Ganem D, Forghani B. 2000. Human herpesvirus 8 glycoprotein K8.1: Expression, post-translational modification and localization analyzed by monoclonal antibody. *J Clin Virol*. 17:127–136.
- Wuhrer M, Catalina MI, Deelder AM, Hokke CH. 2007. Glycoproteomics based on tandem mass spectrometry of glycopeptides. *J Chromatogr B Analyt Technol Biomed Life Sci*. 849:115–128.
- Xiang T, Yang G, Liu X, Zhou Y, Fu Z, Lu F, Gu J, Taniguchi N, Tan Z, Chen X et al. 2017. Alteration of N-glycan expression profile and glycan pattern of glycoproteins in human hepatoma cells after HCV infection. *Biochim Biophys Acta*. 1861:1036–1045.
- Xiao J, Palefsky JM, Herrera R, Tugizov SM. 2007. Characterization of the Epstein-Barr virus glycoprotein BMRF-2. *Virology*. 359:382–396.
- Xie H, Doneanu C, Chen W, Rininger J, Mazzeo JR. 2011. Characterization of a recombinant influenza vaccine candidate using complementary LC-MS methods. *Curr Pharm Biotechnol*. 12:1568–1579.
- Yamagishi Y, Sadaoka T, Yoshii H, Somboonthum P, Imazawa T, Nagaike K, Ozono K, Yamanishi K, Mori Y. 2008. Varicella-zoster virus glycoprotein M homolog is glycosylated, is expressed on the viral envelope, and functions in virus cell-to-cell spread. *J Virol*. 82:795–804.
- Yang ML, Chen YH, Wang SW, Huang YJ, Leu CH, Yeh NC, Chu CY, Lin CC, Shieh GS, Chen YL et al. 2011. Galectin-1 binds to influenza virus and ameliorates influenza virus pathogenesis. *J Virol*. 85:10010–10020.
- Yang Z, Halim A, Narimatsu Y, Joshi HJ, Steentoft C, Schjoldager KT, Schulz MA, Sealover NR, Kayser KJ, Bennett EP et al. 2014. The GalNAc-type O-glycoproteome of CHO cells characterized by the SimpleCell strategy. *Mol Cell Proteomics*. 13(12):3224–3235.
- Yang X, Qian K. 2017. Protein O-GlcNAcylation: Emerging mechanisms and functions. *Nat Rev Mol Cell Biol*. 18(7):452–465.
- Yang W, Shah P, Toghi Eshghi S, Yang S, Sun S, Ao M, Rubin A, Jackson JB, Zhang H. 2014. Glycoform analysis of recombinant and human immunodeficiency virus envelope protein gp120 via higher energy collisional dissociation and spectral-aligning strategy. *Anal Chem*. 86:6959–6967.
- Yang Z, Wang S, Halim A, Schulz MA, Frodin M, Rahman SH, Vester-Christensen MB, Behrens C, Kristensen C, Vakhrushev SY et al. 2015. Engineered CHO cells for production of diverse, homogeneous glycoproteins. *Nat Biotechnol*. 33:842–844.
- Yao Z, Jackson W, Forghani B, Grose C. 1993. Varicella-zoster virus glycoprotein gpI/gpIV receptor: Expression, complex formation, and antigenicity within the vaccinia virus-T7 RNA polymerase transfection system. *J Virol*. 67:305–314.
- Yeh JC, Ong E, Fukuda M. 1999. Molecular cloning and expression of a novel beta-1, 6-N-acetylglucosaminyltransferase that forms core 2, core 4, and I branches. *J Biol Chem*. 274:3215–3221.
- Yoshii K, Yanagihara N, Ishizuka M, Sakai M, Kariwa H. 2013. N-linked glycan in tick-borne encephalitis virus envelope protein affects viral secretion in mammalian cells, but not in tick cells. *J Gen Virol*. 94:2249–2258.
- Young WW Jr., Holcomb DR, Ten Hagen KG, Tabak LA. 2003. Expression of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase isoforms in murine tissues determined by real-time PCR: A new view of a large family. *Glycobiology*. 13:549–557.
- Zai J, Mei L, Wang C, Cao S, Fu ZF, Chen H, Song Y. 2013. N-glycosylation of the premembrane protein of Japanese encephalitis virus is critical for folding of the envelope protein and assembly of virus-like particles. *Acta Virol*. 57:27–33.
- Zauner G, Kozak RP, Gardner RA, Fernandes DL, Deelder AM, Wuhrer M. 2012. Protein O-glycosylation analysis. *Biol Chem*. 393:687–708.
- Zezulak KM, Spear PG. 1983. Characterization of a herpes simplex virus type 2 75,000-molecular-weight glycoprotein antigenically related to herpes simplex virus type 1 glycoprotein C. *J Virol*. 47:553–562.
- Zhao Y, Ren Y, Zhang X, Zhao P, Tao W, Zhong J, Li Q, Zhang XL. 2014. Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape. *J Immunol*. 193:783–796.
- Zhu L, Puri V, Chandran B. 1999. Characterization of human herpesvirus-8 K8.1A/B glycoproteins by monoclonal antibodies. *Virology*. 262:237–249.
- Zielinska DF, Gnad F, Wisniewski JR, Mann M. 2010. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. *Cell*. 141:897–907.
- Zou Z, Chastain A, Moir S, Ford J, Trandem K, Martinelli E, Cicala C, Crocker P, Arthos J, Sun P. 2011. Siglecs facilitate HIV-1 infection of macrophages through adhesion with viral sialic acids. *PLoS One*. 6:e24559.