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Virion motility of sialoglycan-cleaving respiratory viruses

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Here, we review how respiratory viruses function as molecular motors by exploiting their glycan receptor-binding and -destroying glycoproteins. The resulting virion motility drives their mucus penetration and exploration of cell surfaces for locations permitting induction of entry. We discuss the role of virus morphology, kinetic parameters of receptor engagement and receptor heterogeneity, summarize physical models for virion motility and identify key gaps in our understanding of these dynamic processes.

The typical infection route of respiratory viruses, using human influenza A virus (IAV) as an example, is shown in Fig. 1. Virus transmission mostly proceeds via the airborne route through inhalation of virus-loaded respiratory droplets and aerosols (1) or by direct contact. In the lumen of the upper or lower parts of the airways, virus particles land within seconds on a mucus layer covering the epithelial cells (2). Mucus is an established restriction factor for IAV infection in vivo and in vitro[1,2]. Virions in the mucus will be expelled by ciliary beating and need to traverse this layer rapidly. Their hemagglutinin (HA) binds to sialic acid (Sia) decoy receptors displayed on mucins which may delay this step (3) but the remarkable efficiency of aerosol transmission (e.g. a very low IAV dose of 0.7–3.5 PFU causes seroconversion in 50% of subjects[3]) suggests efficient escape from decoy receptors. There is growing evidence that epithelial cells are rapidly reached by directional virus motility resulting for IAV from the interplay between Sia-receptor binding by HA and cleavage by neuraminidase (NA) (4) as will be discussed in more detail below.

While technical challenges have thus far prohibited determination of viral doses[4] inhaled under natural conditions few viral particles are expected to bind per cell at the initial stages of infection. As a consequence, virions must be highly efficient in finding sites that permit endocytic entry, e.g. by clathrin-mediated endocytosis or macropinocytosis[5]. Notably, IAV particles do not rely on constitutive endocytosis at the initial site of binding (5) nor actively induce endocytosis at that site. Instead, as shown by live particle tracking[6], binding leads to extensive cell surface roaming (6) followed in time by stalling of virus particles at as yet undefined "entry-competent" sites at which signaling (7) induces endocytosis (8) within a time-span of ~2 min[6].

Clearly, mucus penetration and cell surface roaming are crucial steps to keep optimized in view of escaping not only mucus expulsion but also (adaptive) immunity by minimizing extracellular presence. In this review we discuss the intricate machinery that is conceptually shared by members of at least four virus families in order to facilitate virus particle motility. Their sialoglycan receptors are (abundantly) present on the extracellular face of virtually every cell membrane and the soluble proteins in the mucus layer that covers the epithelial cells. As a leading principle, based on ample evidence, we propose that sialoglycan-binding virus particles become permanently, but dynamically, receptor-associated shortly after entering the respiratory tract. Motility, for penetrating the mucus layer and subsequently on the cell surface to find entry-competent sites, is largely dependent on the kinetic parameters of the interaction of these viruses with their sialoglycan receptors and the abundance and distribution of these receptors. These interactions critically involve receptor binding as well as receptor destruction by enzymatic cleavage. These two activities are present in virus envelope-embedded glycoproteins but are organized in a constellation that differs between members of the four virus families. Still, as we will discuss, evolution of the balance between receptor binding and cleavage is a unifying principle in regulating virus motility.

As a side note, the virus families covered in this review also contain members that can enter Sia-independently. Paramyxoviruses either use Sia or protein receptors[7,8]. Probably all coronaviruses rely on protein receptors, while some in addition require glycan receptors for attachment and/or opening of the spike protein to allow protein receptor binding via the RBD[9–12]. Also some orthomyxoviruses enter cells Sia-independently via MHC-II[13–17]. Why viruses within a virus family have evolved to enter via different types of receptors is not known. While the sheer abundance of glycan receptors may seem attractive, this abundance also comes at a cost as detailed in this review. Within this review we only focus on those viruses within these families that contain both sialoglycan-binding and -cleavage activities.

Multivalent binding and receptor cleavage as drivers of virus motility

The first challenge for a virus to enter a cell is binding to an attachment receptor. Whereas many virus species attach to a specific protein receptor with high affinity[18,19] (K_D ranging from 0.025 to 25 nM), influenza viruses and several coronavirus and paramyxovirus species

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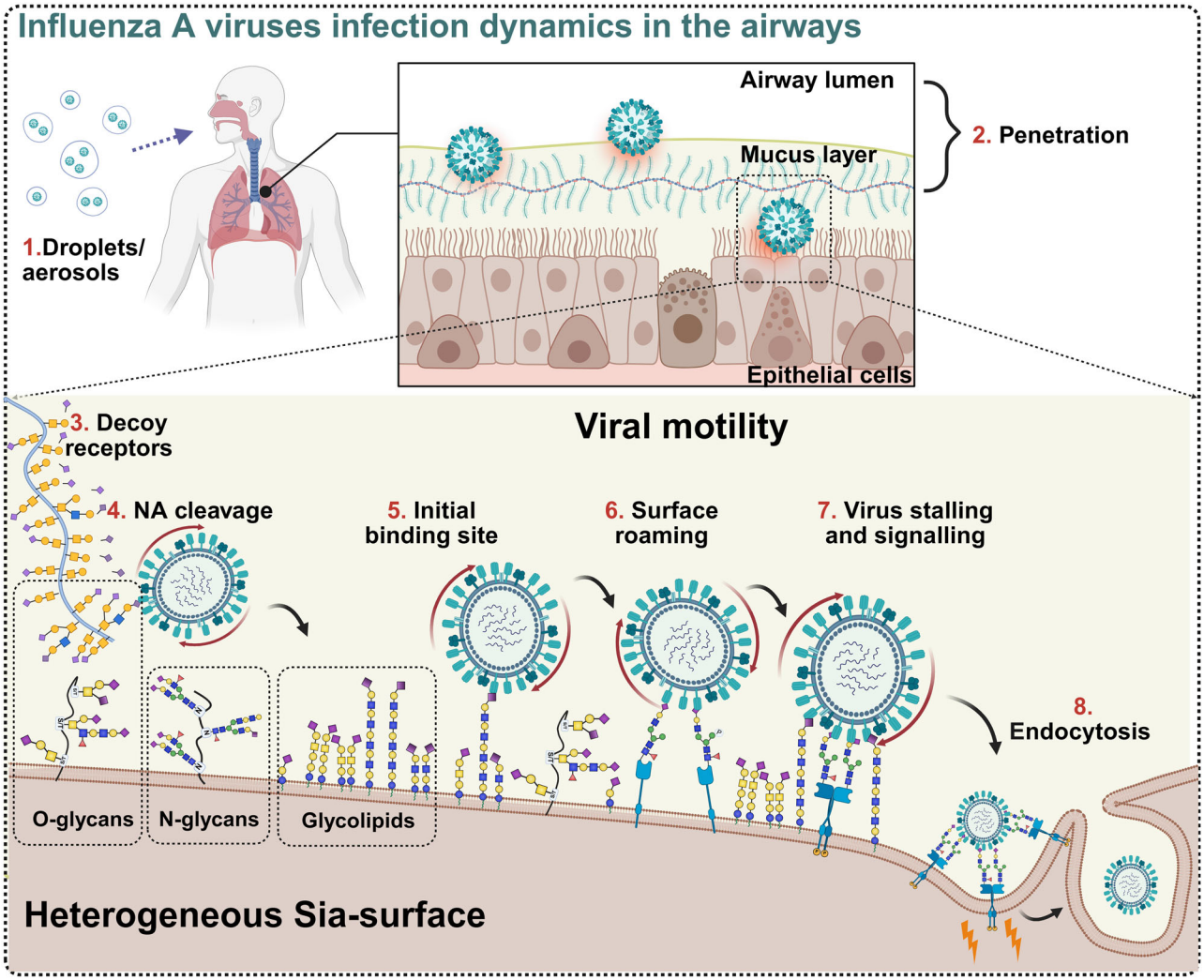

Fig. 1 | The route of IAV particles from inhalation of respiratory droplets/ aerosols until endocytic uptake. The numbered steps are described in the main text. Typical examples of glycan structures of the three major glycoconjugate groups are shown. Decoy receptors (3) are shown as an O-glycan-rich domain of soluble mucins but may also be present on the cell membrane, for instance on transmembrane mucins. Black arrows indicate virus motility. Clustering of glycosylated transmembrane proteins (7 and 8) may be crucial to signaling the induction of endocytic entry. Created in BioRender. Liu, M. (2025) https://BioRender.com/kxmkvsn.

bind to terminally located Sias of glycan chains attached to proteins and lipids. To compensate for the low affinity of individual viral protein-glycan interactions[20–25] (K_D ranging from 0.02 to 50 mM), viruses form multiple simultaneous receptor interactions (referred to as multivalent binding) for establishing high avidity binding required for cell infection[2,6–29]. Virus binding is not confined to unique sialoglycans displaying the highest affinity but can be supported simultaneously by different receptors with a range of affinities, resulting in a heteromultivalent binding mode[30]. In this way, a virus can efficiently exploit structurally diverse sialoglycan repertoires that, while varying between cell types and host species, are heterogeneously distributed on cell surfaces. Importantly, high avidity virus binding by multiple low affinity interactions determines its highly dynamic interaction with the cell surface and distinguishes it from binding to a high-affinity protein receptor. It can be imagined as an entity clinging with many "weak hands" to a ceiling full of handles, that can move around by exchanging "grips". This differs dramatically from the monovalent high-affinity interaction (tightly hanging to a "single grip") of viruses binding to a protein receptor that will direct the co-endocytosis of virions, either directly at the binding site or after surfing in continuous tight association with a motile protein receptor to an endocytic site. On the other hand, sialoglycan-binding viruses can bind any protein and/or lipid decorated with the matching glycan receptors but need to find proper sites at which endocytic vesicle formation can take place by moving over a landscape of sialoglycan receptors[31–33].

In addition to multivalent, low affinity binding to a sialoglycan-receptor surface, receptor-destroying activity has been shown to be essential for virus motility. Initially, receptor cleavage by viral envelope-embedded proteins was shown to prevent self-aggregation of virus particles and to promote shedding of newly formed virus particles by de-sialylation of viral envelopes and cell membranes[34,35]. Later, NA activity was shown to be important for the initiation of IAV infection in cells of differentiated mucus-secreting airway cell cultures[36] and recently directional motility on receptor-coated surfaces was shown to be critically dependent on receptor-destroying enzymes[37–40]. Thus, two virus components (receptor binding protein, RBP; receptor destroy enzyme, RDE) and a diverse receptor repertoire form the fundamental building blocks for a molecular motor (Fig. 2a). Before turning to mechanistic details of virus motility we briefly summarize the considerable diversity that is observed in the organization and structure of the participating viral proteins as well as in their preferred sialoglycan receptors.

Diversity of molecular motor components

Nearly all mammalian cells are decorated by the sialic acid (Sia) receptors on which we focus here. They are a family of nine-carbon backbone

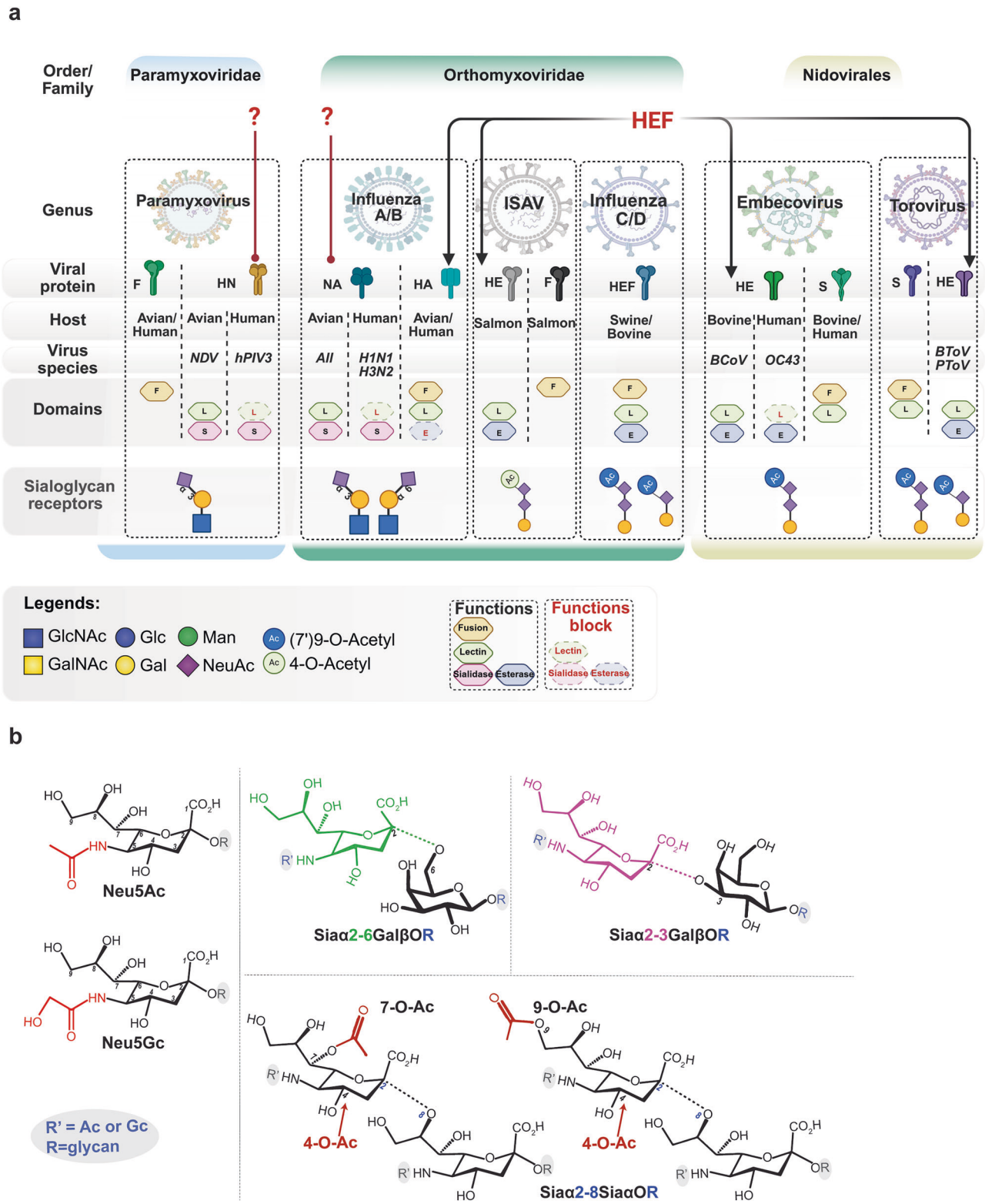

Fig. 2 | Ancestral relationships, functional domains and receptor specificity of viral envelope RBPs and RDEs involved in virus motility. a The distribution of the indicated lectin, enzyme and fusion functions over different proteins is schematically depicted for each of the major virus groups that are discussed. HA, HEs and HEF share a common ancestry with an unknown "HEF-like" precursor (red letter) while the origin of other proteins (e.g. NA and HN) cannot be resolved on basis of primary sequence conservation. HEF, HA and isavirus HE[116] form homotrimers, while corona- and torovirus HE are dimers[45]. Remnant domains that are still recognizable but have lost function are marked by dashed-line/red letter. Note that the esterase function has been lost in HA while keeping a weak lectin function at what is called a vestigial esterase site (VES). The preferred receptor usage is indicated[116,117], but exceptions can occur. For instance, some murine CoVs display specificity for 4-O-acetylated Sia[118]. Created in BioRender. Liu, M. (2025) https://BioRender.com/kxmkvsn. **b** Relevant examples of structural modifications of Sias are shown. The Neu5Gc modification may abolish HA binding but does not occur in humans. O-acetylation also occurs on N- and O-glycans.

monosaccharides that are mostly present as terminal residues of a wide diversity of cell surface-displayed glycans or glycans attached to secreted proteins[41]. Determining factors for specific binding to particular virus envelope glycoproteins are 1) specific enzymatic modifications of the Sia moiety[28], 2) the type of glycosidic linkage with the sub-terminal residue and 3) the structure of the underlaying glycan[42–45] (Figs. 1 and 2). Sialoglycans are the product of the consecutive activities of a large number of glycosyltransferases and other glycan-modifying enzymes that collectively synthesize a huge diversity of glycans constituting the cellular glycome[46,47]. The exact glycome composition is a major determinant of virus host species tropism and also determines the preferential site of infection within the respiratory or enteric tract. The three major glycan classes that can be distinguished are glycosphingolipids (GSLs), glycans linked to asparagine residues of proteins (N-glycans) and glycans linked to serine or threonine residues of proteins (O-glycans)(Fig. 1). A subset of O-glycans is densely spaced in, often repetitive, mucin domains of soluble and transmembrane mucins that form a decoy receptor layer protecting epithelial cells from infection[1,2].

Envelope proteins from viruses from the families of *Orthomyxoviridae* and *Paramyxoviridae* and the order of *Nidovirales* have been demonstrated or suggested to display receptor destruction-driven motility on sialoglycan-coated surfaces[20,37–40]. Limited, but significant, primary sequence conservation and structural similarities suggest a common ancestry of *Orthomyxoviridae* envelope proteins (HA, hemagglutinin-esterase fusion protein [HEF] and hemagglutinin-esterase protein [HE]) and HE of some Nidovirales (Fig. 2a). Evolutionary trajectories remain questionable but have resulted in a remarkably diverse modular arrangement of receptor-binding and -destructing moieties in a single or in two proteins. Whereas a single enzymatic site for receptor cleavage is present, multiple glycan binding sites (lectin sites) are present in some cases. An unknown virus probably donated the protein ancestral to the HEF of influenza C and D (ICV, IDV)[45]. It possesses a lectin domain for binding to (7,)9-O-acetylated Sias and an esterase domain for cleaving the O-acetyl groups. HEF, or it's precursor, is likely ancestral to HA of Influenza A and B viruses[48] and the HE of infectious salmon anemia virus (ISAV), an orthomyxovirus species infecting fish[49]. ISAV HE has lost the fusion function of HEF which is replaced by a separate, non-related, fusion protein (F)[50]. Remarkably, binding and cleavage of ISAV HE is specific for 4-O-acetylated Sias[50]. In case of IAV/IBV, HEF has evolved more drastically, into a protein boasting a lectin site that interacts with α2-3 or α2-6 linked Sias of the N-acetylneuraminic acid type (Fig. 2b) that are not O-acetylated[51]. For receptor destruction, it has therefore acquired a separate NA protein that cleaves α2-3 or α2-6 linked Sias. The HEF esterase site was shown to be maintained in HA as a vestigial esterase site (VES) that is inactive but, despite marginal primary sequence conservation, still able to bind Sia at very low affinity[52]. The class I fusion proteins HEF and HA are organized into homo-trimers and expose a fusion peptide upon a low pH-induced conformational change. Some Toroviruses (e.g. BToV, PToV) and coronaviruses of the Embeco subgenus (e.g. BCoV, OC43, HKU1, PHEV), both belonging to the Nidovirales, followed a different evolutionary track in acquiring a potential motility machine. Also in these cases, a HE ancestrally related to HEF has been acquired, most likely by independent horizontal gene transfers[45]. However, the fusion function was lost and replaced by acquiring a separate fusion protein (spike). Notably, this homo-trimeric spike protein harbors, in contrast to ISAV F protein, a lectin domain for binding (7,)9-O-acetylated Sias and has evolved into the major receptor-binding protein while the esterase activity remains located in the HE protein with its quaternary structure changed into a dimer. As yet another variation, several paramyxoviruses use a set-up in which a single hemagglutinin-neuraminidase (HN) protein of unknown origin displays an active site that provides both binding and cleavage function[44].

Adding to the complexity of molecular motors are the additional Sia-binding sites that can be present in all three virus groups. A subset of paramyxovirus HN[53] and influenza virus NA[54–56] proteins carry an additional lectin site named second sialic binding site (2SBS). Similarly, a subset of HE proteins (e.g. BCoV) have retained the lectin site from the enigmatic ancestor shared with HEF. The function of these secondary lectin sites was proposed to be mainly important for modulating receptor-destroying activity by directing glycans to the catalytic site[55,57]. Remarkably, loss of secondary lectin sites seems to be prominent for human strains. For all recorded IAV pandemics, the loss of a 2SBS occurred at, or shortly after, the start of a pandemic whereas a 2SBS is almost absolutely conserved in avian IAVs. Loss of a 2SBS has occasionally also been reported upon transfer of avian IAVs to other mammals[58]. Human coronavirus OC43, which likely derives from bovine coronavirus BCoV, was also associated with a loss of a functional lectin site in HE[57]. Finally, in contrast to avian paramyxovirus NDV, human paramyxovirus hPIV3 has lost its 2SBS[59] although it is not clear whether this is a human host-specific adaptation.

Motility of spherical virus particles on receptor surfaces

Evolution of the arrangement and functionality of lectin sites and hydrolytically active sites in viral envelope proteins (Fig. 2) likely affects virus motility. The first experimental evidence for motility on the cell surface or in the mucus layer[37–39] has been obtained for NA or HEF receptor-destruction-driven motility of, respectively, IAV and ICV particles. Theoretical models explaining directional motility of virus particles by an HA/NA "motor system" have been developed[60–63]. Motility of paramyxoviruses like Sendai virus (SeV) and Newcastle disease virus (NDV) was recently shown[40] but a role for receptor binding and destruction in motility of coronaviruses[20] has not yet been experimentally demonstrated. Different modes of (directional) motility are discussed below and depicted in a simplified way in Fig. 3.

Virus motility of IAV on the cell surface was first observed by Rust et al. who aimed to describe cell entry mechanisms exploited by IAVs by using live cell imaging[6]. Virus particles were shown to traverse the cell surface for minutes before stalling at a site at which entry only occurs after a lag phase of up to 2 min during which active signaling and recruitment of diverse endocytic machinery takes place. Sakai et al.[37] subsequently also used live cell imaging to demonstrate IAV motility on artificial receptor-coated surfaces and on the surface of human erythrocytes. Importantly, directional motility was dependent on NA activity, and NA inhibitors were shown to reduce virus entry, strongly suggesting the need for virus motility to reach sites of endocytosis. Two types of motility were observed. In absence of NA activity particles moved slowly, staying confined to a small area and displaying frequent change of direction (random motion) (Fig. 3a). In presence of NA activity, random motion was interrupted by fast, largely directional, gliding motility by crawling ("stepping") or rolling (Fig. 3b–d). Intermittent occurrence of the two types of motion allowed change of direction at the slow phase. Others used real-time kinetic analysis of IAV-receptor surface interactions by biolayer interferometry (BLI) revealing a crucial role for the balance between HA and NA in extensively clearing the receptor surface from sialoglycan receptors[38]. By the cooperative action of a small number of virus particles, imaginatively visualized as "lawn mowers" moving over the surface, receptor density becomes gradually decreased to levels at which virus particles dissociate. Recently, integrating confocal and atomic force microscopy, NA activity-dependent motility of IAV particles on the cell surface prior to cell entry[64] was confirmed and refined.

High avidity multivalent binding (K_D in the pM range[33,65]) results from monovalent HA-sialoglycan interactions with K_D values as low as 1–50 mM[66] (Table 1). These weak monovalent interactions have also been measured by atomic force microscopy[67] and were shown by single-particle tracking to be, relative to multivalent interaction, abundant[65,67]. Expectedly, the low affinity of a monovalent interaction results in a binding equilibrium that is far to the unbound state at the low virus concentrations encountered in vivo. Conversion to bivalent binding will be rare but the resulting enhancement of binding avidity will accelerate formation of additional interactions and lead to virtually irreversible multivalent binding. In contrast to single-particle tracking, BLI is an "ensemble method" that mainly records the accumulation of multivalently associated particles. Receptor destruction by NA activity is almost completely restricted to particles that are first bound to the receptor surface by HA-Sia interactions[38]. Variation of the K_D (equal to k_{off}/k_{on}) between IAV strains (1–50 mM) is determined by

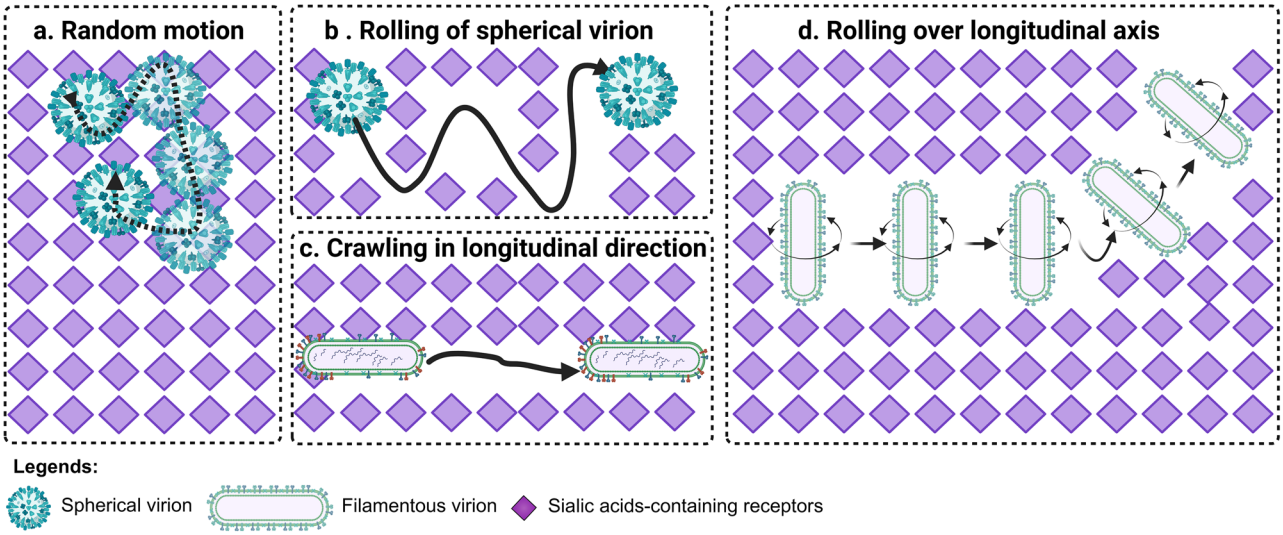

Fig. 3 | Schematic illustration of different motility modes. a Random IAV motion over short distances occurs in absence of NA activity. **b** In presence of receptor-destroying activity, spherical or short cylindrical IAV or ICV particles show fast directional motility alternating with periods of random motion that may result in a shift of direction. **c** Filamentous IAV particles display crawling motion. **d** ICV filament rolls over their longitudinal axis (indicated by the thin bended arrows) and display change of direction by flapping (indicated by the thick bended arrow). Created in BioRender. Liu, M. (2025) https://BioRender.com/kxmkvsn.

changes in their binding rate constant (k_{on}) as the k_{off} is highly conserved and results in a half-life of monovalent interactions of ~0.8 s[66]. This results in a dynamic binding mode where rapid exchange of Sias between HA and NA, results in progressive removal of Sia by NA activity that is essential for driving exploration of the cell surface in search for rare entry-competent sites. Motility on supported lipid bilayers was also microscopically observed for Sendai virus (SeV), the prototypical member of the respirovirus genus of the *Paramyxoviridae*[68]. Real-time kinetic analysis of receptor binding and clearance by paramyxoviruses suggested an IAV-like rolling mechanism for viruses that carry binding and cleavage functions of sialoglycan receptors in the single active site of the HN protein[40]. Up to date, reports on (cell surface) motility of coronaviruses have been lacking.

Motility of filamentous virus particles on receptor surfaces

The observations above for IAV were made on spherical or slightly cylindrical virus particles of ~100 nm diameter and 90–120 nm length (Table 1). However influenza viruses can display a filamentous morphology[69], especially when directly isolated from hosts, while mostly spherical respiroviruses like SeV are pleiomorphic in displaying a diversity of diameters[70]. Interestingly, a mostly filamentous ICV, carrying a HEF protein that combines a receptor binding site and a separate receptor cleavage site in a single protein, was also shown to display motility on receptor-coated surfaces[39]. Rolling occurred over the longitudinal side and was unidirectional for a while after which "flapping", by remaining attached to the receptor surface with the tip of the filament, can result in a change of direction (Fig. 3d). Similar to role of NA in IAV motility, the esterase activity of HEF was proposed to drive directional motility by clearing the virus trajectory from receptors and thereby preventing backward motion. In addition, the cylindrical shape of the particle will largely restrict sideward changes of direction that characterizes motility of spherical IAV particles. Filamentous ICV particles moved slower than spherical IAVs. Notably, this was not due to kinetic differences in binding and cleavage activities between ICV HEF and IAV HA and NA, nor by HEF combining both activities in one protein, while these are physically separated for HA and NA, but rather to differences in morphology: an ICV strain displaying spherical morphology showed fast directional motility, alternating with slow random motility, similar to spherical IAVs[39].

As receptor binding and cleavage activity of ICV are organized in a single HEF protein, their distribution along the filament is identical. In contrast, HA and NA of a filamentous IAV particles have been shown to be asymmetrically distributed along the filament with NA being concentrated at one end. Analysis of filamentous IAV motility on receptor-coated coverslips revealed trajectories in a direction lateral to its long axis[71] (Fig. 3c), in contrast to the orthogonal motility of filamentous ICV[39] (Fig. 3d). Remarkably, motility of IAV filaments occurred strictly in a polarized orientation, moving away from the NA-rich pole at the back end of the filament. This crawling motion was NA activity-dependent. In agreement, staining the coverslips after the experiment with a lectin, specific for desialylated glycans, revealed receptor-depletion along the trail followed by the virus filament.

A recent study on SeV further emphasized the significance of virus morphology on motility[72]. In this case, as for ICV HEF, receptor binding and cleavage are organized in a single HN protein. SeV particles are spherical but display a diameter range of 100–400 nm. Single particle tracking showed small SeV particles (~129 nm diameter) displaying binding efficiency highly dependent on receptor density (GD1a embedded in supported lipid bilayer). Similar effects were previously observed when increasing receptor density using BLI[38,73] or supported lipid bilayer assays[74] for IAVs and paramyxoviruses[40]. Larger SeV particles (~165 nm diameter), however, were less dependent on receptor density and moved at a lower speed[72]. Presumably, an increased number of interactions at the extended contact area of a larger particle increases binding efficiency while decreasing motility.

Motility of virus particles through mucus layers

Receptor-destroying activity is also an essential driver of directional mucus penetration by both filamentous and spherical virus particles[71,75,76]. The mucus layer that covers respiratory epithelia becomes gradually thinner from nasal cavity to bronchioles and is absent from alveoli. Soluble mucins like muc5AC and muc5B multimerize head-to-tail into filaments forming a mesh-like gel layer with a pore size of ~100–500 nm[1,2]. The extended filaments are densely covered with sialylated O-glycans, interspersed with N-glycans, and may form linear tracks to which viruses can bind and move along in dependence of receptor-destroying activity[2]. Despite the lack of detailed glycomic analyses, mucus is generally assumed to display abundant α2-3-linked Sias (2-3Sia)[77]. Therefore, mucus has been proposed to particularly block penetration by avian IAVs that preferably bind 2-3Sia[43]. Human IAVs prefer binding to α2-6-linked Sias (2-6Sia)[43] and may thus be less affected by the thick mucus layer of the upper respiratory tract. However, this has poorly been investigated in detail and raises questions. For

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Table 1 | Virus binding and motility factors

	Morphology	Dimensions	Envelope proteins[a]				Virus	Binding parameters[b]									Kinetic activity parameters[c]	
			Type	Height (nm)	Quatern. Struct.	Number[g]		K_D (uM)					k_{on} ($M^{-1}s^{-1}$)		k_{off} (s^{-1})		k_{cat} (s^{-1})	K_m (uM)
								3SLN	9-O-3SLN	6SLN	9-O-6SLN	9-O-8Sia	3SLN	6SLN	3SLN	6SLN		
CoV	spherical	80-120nm[103]	S	17-20[20]	trimer		HKU1[104]		75.7		101	5.8					No enzymatic activity	
							OC43[20]		49.7					14000		1		
			HE	5-7[48]	dimer		HKU1[105]											280[d]
							OC43[105]											1200[d]
Paramyxo	spherical	100-540nm[70,89]	HN	16	tetramer/ dimer		SeV[106]										16.5	929
							hPIV1[106]	79					6200		0.5		5.58	176-297
							hPIV2[107]	28					12000		0.34		49	440
							hPIV3[106]	4300					51		0.22		0.8-16	1.2-21x10^3
							NDV[106]										4.75	50.9
IAV	spherical or cylindrical	100 to 80x150[108]	HA	14	trimer	209-301[94]	H1N1										No enzymatic activity	
							H5N1[77]	2050		30000			274		0.67			
							H3N2[77]	3700-50400		2100-30600			19.6-258	28.8-382	0.76-1.34	0.63-1.16		
	filamentous	85 x >10000nm[69,109]	NA	13	tetramer	38-50[94]	H1N1[107,110]											153[e],7.5-31.5
							H5N1[111]											78.9-110.5
							H3N2[112,113]											4.1[f], 24-138
ICV	spherical	88-98nm[114,115]	HEF	15[115]	trimer		ICV											
	filamentous	diverse[114,115]																

[a]Protein size is presumably the same for different morphologies.

[b]Binding parameters for monovalent interactions.

[c]Kinetic parameters determined in soluble substrate cleavage assays using 2′-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) except when indicated otherwise.

[d]Determined by pNPA instead of MUNANA.

[e]Determined by STD-NMR instead of MUNANA.

[f]Determined by 5-N-acetyl-2-O-(3-methoxyphenyl)-alpha-D-neuraminic acid instead of MUNANA.

[g]Indicates trimer/tetramer number per virion.

instance, human paramyxoviruses specifically bind to α2-3 linked Sias but can efficiently infect the upper respiratory tract[78] indicating they can efficiently deal with a thick mucus layer that is rich in α2-3 linked Sias and which might suggest that avian IAVs should be capable of this too. Sialylated tracks provided by linear mucin filaments seem, more than the Sia landscape on the cell surface, ideally suited for guiding directional rolling motility of spherical particles. Still, the filaments form a network layer and high-magnification live-imaging only showed that filamentous IAVs display directional motility in a mucus layer without detailing the precise interaction with mucin filaments[71]. Overall it can be concluded that mucus penetration is directional and requires receptor-destroying activity but its mechanistic similarity to cell surface motility needs further exploration.

Theoretical models for virus motility

Physical models aiming to capture the observed virus motility phenomena have recently been developed. Sakai et al.[37] argued that the energy for the observed random motion of spherical IAV particles on a grid-like receptor surface originates from the impact of water molecules. Akin to the Brownian motion of small particles in solution, Brownian ratchet models[79,80] could apply to such virus motility. Directional motility requires an external energy source which in this case is provided by receptor-cleavage activity. Possibly, virus particles move, on average, away from receptor-depleted areas towards unvisited areas to "leave behind the burnt-bridge" (sometimes plastically described as a "lawnmower" going over a receptor surface). While burnt-bridge Brownian ratchet theories are frequently used to describe molecular motors, other physical models have recently been developed for influenza virus motility. Ziebert et al. have developed more elaborate models for IAV[61] (spherical or slightly cylindrical virions and carrying binding and cleavage sites in separate proteins) and ICV[60] (cylindrical or filamentous virions with binding and cleavage sites in the same protein). Differences in polarization of linkers (i.e. receptor-glycoprotein interactions) at the contact zone at the front and rear end of the particle give rise to a torque that drives rolling in the front-end direction. On basis of this model they proposed that rolling, orthogonal to the rotational axis of the particle, is inevitable in the presence of viral glycoproteins that bind and enzymatically cleave their ligands on the receptor surface. In their models, parameters were introduced to investigate the effects of variations in receptor density, binding strength, cleavage activity and virion morphology (cylindrical vs spherical). Directionality and reversal of motion appears to be affected by all these parameters.

For motility of filamentous IAVs through mucus layers, lateral to its long axis, a different model was proposed. Cases were investigated for either a polarized or uniform distribution of HA and NA[62]. For both cases, a minimal filament length was predicted to be required to switch from diffusional to directional motion. Polarized organization of NA at the rear-end resulted in more robust directionality than a uniform distribution. Also, too low or too high HA-receptor affinity both resulted in extreme loss of motility but, remarkably, locomotion was predicted to be largely insensitive to changes in NA activity. Finally, Ziebert et al. have proposed a model for mucus penetration by filamentous viruses[63] that involves an alternation between a symmetrical rod-like shape and curved shapes (described as "toro-elastic"). These morphological changes are predicted to be induced by short-range interactions between neighboring spikes. Rotation of such curved states will be invoked by receptor cleavage and lead to a propulsion of changes in filament shape that promotes virus motility. Collectively, these predictions impose another level of complexity on the evolutionary fine-tuning of the HA/NA balance that is supposed to be a crucial factor in virus fitness and a barrier for cross-species transmission[32].

Physical models are built on measured or predicted kinetic and physical parameters and can be experimentally challenged for further improvement. Yet, number and range of variables affecting in vivo virus motility is expectedly huge and often reluctant to quantification or experimental verification. For instance, mucus layers are difficult to reconstruct in vitro and virus morphology cannot easily be changed while remaining largely unexplored for its role in infection. A solely spherical IAV morphology has been suggested to be a cell culture adaptation but solid proof for this is lacking. Notably, spherical particles have been shown to efficiently penetrate a mucus layer in strict dependence on NA activity in apparent contrast to one of the model predictions mentioned above[62].

Towards improved models of virus binding dynamics and motility

We next discuss qualitative and quantitative data on viral and cellular factors affecting virus motility. Multivalent virus-binding avidity, usually determined in absence of receptor destroying activity, appears virtually irreversible with dissociation constants (K_D) as low as a few pM[65,81]. These avidities, however, often correspond poorly to infection efficiency[82–86]. Apparently, infection efficiency (also) depends on steps beyond initial binding, such as virus motility and cell surface roaming, or on the other hand virus stalling and subsequent induction of endocytosis (Fig. 1, steps 7–9). All these steps are determined by the concerted action of receptor binding and destruction, which coevolve to maintain a supposed optimal balance between them and their targeted receptor repertoire[66,81,87,88]. This balance is primarily determined by the kinetic binding and cleavage parameters of the individual proteins that also form the basis for the theoretical models. Unfortunately, only very little information on these parameters is available for a limited set of soluble glycans. This poses a challenge as the cell surface displays a diverse mixture of glycans of which many will contribute by an imperative heteromultivalent binding mode simply because glycans displaying higher and lower affinity can be located in close proximity. It seems unlikely that receptor binding and destruction can be optimally balanced for every individual receptor. Actually, the observed particle stalling on the cell surface may occur at places (microdomains) with a receptor composition (type and density) that is unbalanced and favors prolonged binding.

Table 1 presents a concise overview of, currently still limited, knowledge on virus parameters that may affect binding and motility of which some are discussed below. Recently, IAV HA-Sia monovalent receptor binding affinities were shown to fall within a relatively large 1–50 mM range despite their ability to support equally efficient infection levels[66]. In contrast, for coronavirus OC43 spike protein a much higher affinity (~50 μM) has been reported[20]. Remarkably, the dissociation rate constant (k_{off}) for OC43, HPIV1, HPIV2, HPIV3 and for a range of IAVs were all very similar, resulting in a half-life time of ~0.6–3 s. As a consequence, variation of the association rate is the main variable in determining affinity ($K_D = k_{off}/k_{on}$). Possibly, the large difference between OC43 and IAVs reflects differences in density of available receptors, with OC43 being dependent on binding to possibly less abundant O-acetylated Sias. Also, the number of interactions involved in multivalent binding may vary due to differences in density and motility of the receptor binding proteins in the viral envelope. Whereas HA and NA of IAV are tightly packed on the viral envelope, just as what is observed for paramyxoviruses[89], this appears less well established for coronaviruses, which, based on electron microscopy, seem to display more variable densities of spike proteins[90].

In many cases, modeling of binding dynamics still suffers from the current lack of precisely determined kinetic parameters. For example, kinetic parameters of secondary lectin sites of low affinity, for instance on NA of IAV (2SBS), HA of IAV (VES) and HE of BCoV (lectin site) should be considered in modeling of virus motility but have either not yet been determined or only to a limited extent (Table 1) without a systematic comparison of different strains. In addition, HEF of ICV/IDV and HN of NDV (2SBS) represent situations where a major lectin site and active site are present in the same protein. For HN, the active site also functions as the major site for attachment and a similar situation was observed for some IAV strains in which NA active site was mainly responsible for[91], or contributing to[38,92], binding.

Another important factor to consider is the accessibility and potential clustering of receptor binding and destroying enzymes. Whereas reported kinetic parameters are determined by the use of either soluble glycans or soluble proteins, natural multivalent binding takes place between receptor-coated surfaces and membrane-embedded viral proteins. This differs dramatically from a situation in which one of the components is accessible by diffusion in solution. When both are surface bound, density and a more

restricted orientation will determine accessibility of viral proteins to sialoglycan receptors. Importantly, accessibility may differ between receptor-binding and receptor-destroying proteins and have a large impact on their balance towards specific receptors. NA of IAV often appears to be organized into a few clusters[93,94] which has not been observed for coronavirus HE. HE of coronavirus has a ~2-fold lower height than its spike (Table 1) which will decrease accessibility, and thereby cleavage activity, for short glycans. HA and NA are commonly of similar height but deletions in the NA stalk region, making it shorter, seem to be an adaption of IAV strains originating from waterfowl to poultry. The resulting reduction in accessibility of NA to glycans might be a specific way of altering the HA/NA balance[73].

Finally, but of utmost importance, the lipid biolayers that form the viral envelope and cell membrane are highly fluid. Membrane-embedded proteins and lipids can display a high rate of lateral diffusion. Lipids can migrate at a rate as high as 1 μm per second, depending on the local lipid composition of the membrane, and transmembrane proteins can also diffuse at high rates but motility can be restricted by anchoring of cytoplasmic domains to the cytoskeleton or protein multimerization. However, depending on lipid composition, lipids are also found to be organized into lipid micro- and nano-domains where motility is restricted. Such domains, and their selective protein content have been shown to play an important role in IAV and SeV binding avidity, motility and signaling of endocytosis[72,95,96]. Diffusion of receptors will obviously affect their local density and thereby the kinetics of multivalent binding. It will, for instance, complicate the modeling of an "effective molarity" which was defined as the volume that an unbound ligand in a partly bound complex can probe[29,97]. One consequence of a fluid membrane for heteromultivalent binding has been shown in lectin binding studies. Lectins usually display two or more glycan binding sites in a single protein or a stable lectin multimer. Like recently shown for IAV, also lectins can bind to different receptors with different affinities. It was shown that initial multivalent binding occurs to receptors of higher affinity but in time these can be exchanged with receptors of lower affinity, resulting in heteromultivalent binding or eventually even to only weak receptors[98]. Clearly, virus motility will enable similar recruitment of weaker or stronger receptors that by themselves also diffuse in the membrane and which ultimately needs to be incorporated into models.

Concerning protein motility in the viral envelope, current knowledge mostly derives from molecular dynamics modeling for IAV. HA and NA did not seem to rotate a lot or move over extensive distances. Instead, clumps of neighboring proteins were shown to rapidly form and dissipate while the head domains of HA and NA displayed variable tilting up to angles of 60 degrees or 90 degrees respectively[99]. Tilting will likely affect transfer of bound receptors from on site to another and receptor scavenging, and thereby motility.

Concluding remarks and outlook

Since the initial discovery of sialogycans as receptors for many respiratory viruses and the presence of receptor-destroying activity in several of them, research on the dynamics and complexity of virus-receptor interactions is finally coming of age. It is now evident that receptor-destruction not merely serves to facilitate release of nascent virus particles but also plays a pivotal role in the motility of virions crucial to navigate the mucosal surfaces of the respiratory tract. As a consequence receptor binding and cleavage need to be balanced to the available sialoglycome of the cell surface as well as the mucus. Adaptations in this balance are required upon host species jumps and during antigenic drift. Yet, it is also becoming clear that the complexity of these interactions is huge, not only due to enormous species-specific variation in available receptors but also in viral moieties able to interact with these receptors. This complexity is increased further by multivalency effects both from the receptor and viral point of view. Perhaps not surprisingly, modeling these interactions has not yet yielded unifying concepts. Another topic that remains to be elucidated (and was not addressed in this review) is how viruses with sialoglycan-binding properties, but lacking receptor-destroying activity navigate the respiratory tract and prevent getting stuck. These include several adenoviruses, picornaviruses, polyomaviruses and reoviruses[100]. Evidence is evolving for such viruses that, in addition to interactions with Sia, specific protein receptors are essential to establish binding and cell entry[101,102]. Possibly, Sia moieties only make a minor contribution to the binding avidity of such viruses. In the absence of a specific protein receptor in the mucus layer, the binding avidity provided by Sias will be low enough to result in short-lived mucus-virus particle interaction in order to maintain motility. Clearly, it will be important to fully unravel virus-sialoglycan interactions to understand the rules of engagement for virion motility and the consequences thereof for host tropism, pathogenesis and virus evolution.

Data availability

No datasets were generated or analyzed during the current study.

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E.d.V. and C.A.M.d.H conceptualized and structured the manuscript. M.L. and E.d.V. wrote the main manuscript. M.L. prepared the figures. All authors have read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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