Utrecht University

Novel aspects of the dynamics of influenza A virus-receptor interactions revealed by biolayer interferometry

Hongbo Guo[1], Floor van der Vegt[1], Keanu Leenknegt[1], James Paulson[2], Frank van Kuppeveld[1], Cornelis de Haan[1], Erik de Vries[1]

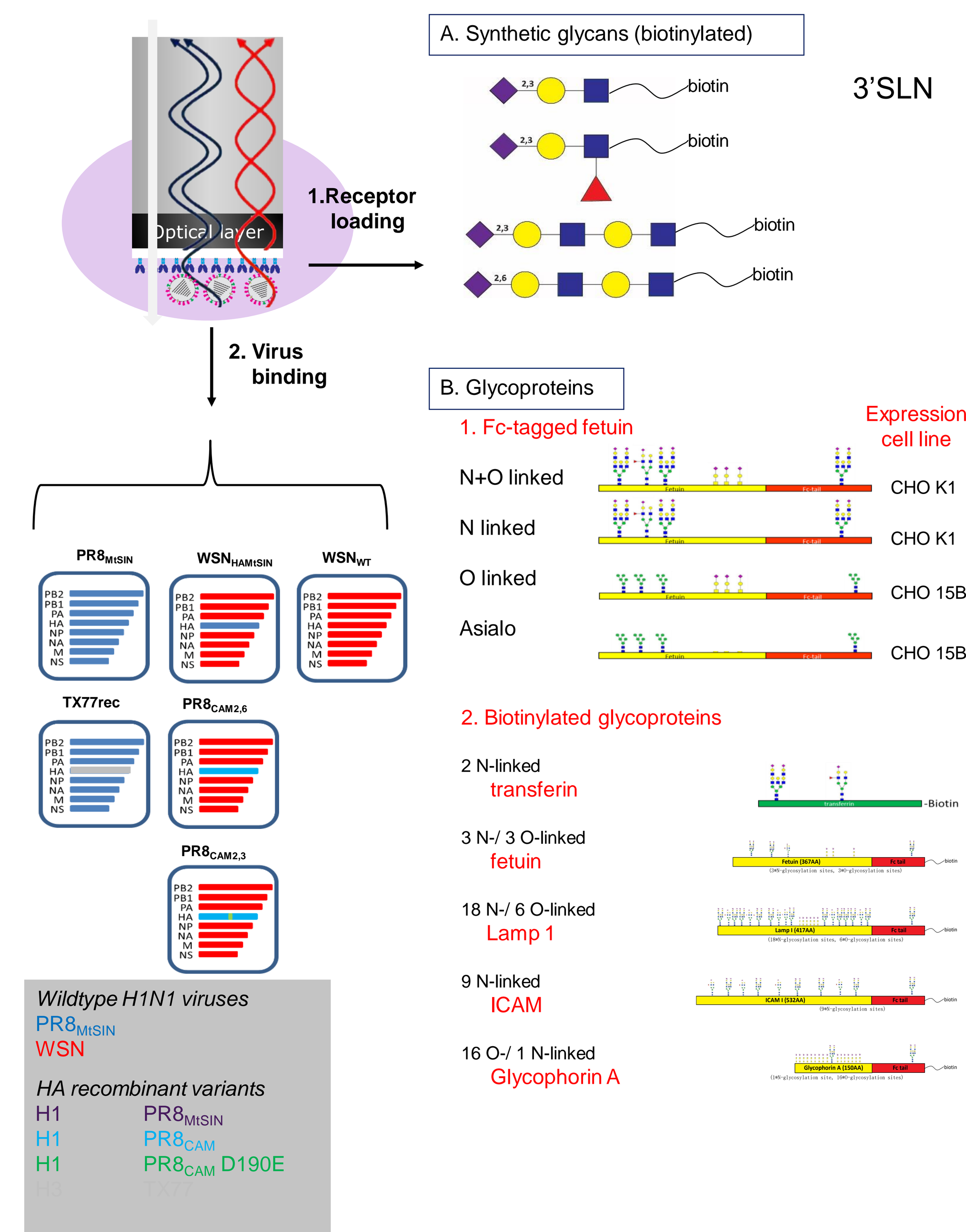
1 Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
2 Departments of Cell and Molecular Biology, Chemical Physiology, and Immunology and Microbial Science, The Scripps Research Institute, La Jolla, USA

INTRODUCTION

Host and cell tropism of Influenza viruses is determined by the specificity of the hemagglutinin (HA) attachment protein, which binds sialic acid receptors, and the neuraminidase (NA) receptor-destroying enzyme, which is required for virion release from cells and decoy receptors like mucus. The HA/NA balance is crucial for virus fitness. Virus binding to sialic acid-coated surfaces is polyvalent in nature and cannot be described by equilibrium kinetics models, thereby hampering the use of endpoint binding assays. Biolayer interferometry (BLI) monitoring of virus particle binding to sialoside-coated sensors opens novel ways to determine the HA/NA balance in real time, including the effects of natural competitors like mucus and antibodies.

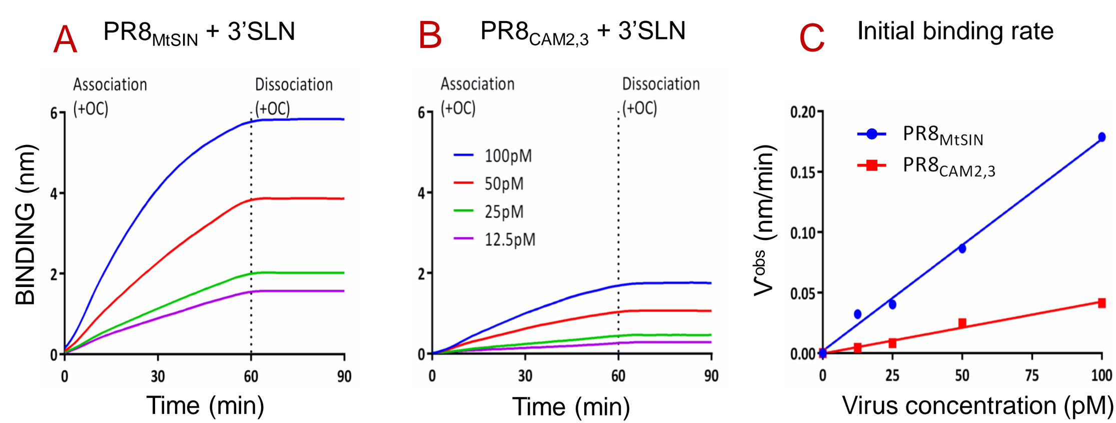
METHODS

Biotinylated synthetic glycans (A) or specifically engineered recombinant glycoproteins (B) carrying different numbers of N- or O-linked glycans were loaded (step 1) to BLI biosensors and subsequently (step 2) used for a range of multistep HA- or HA/NA-dependent realtime association or release assays.



RESULTS

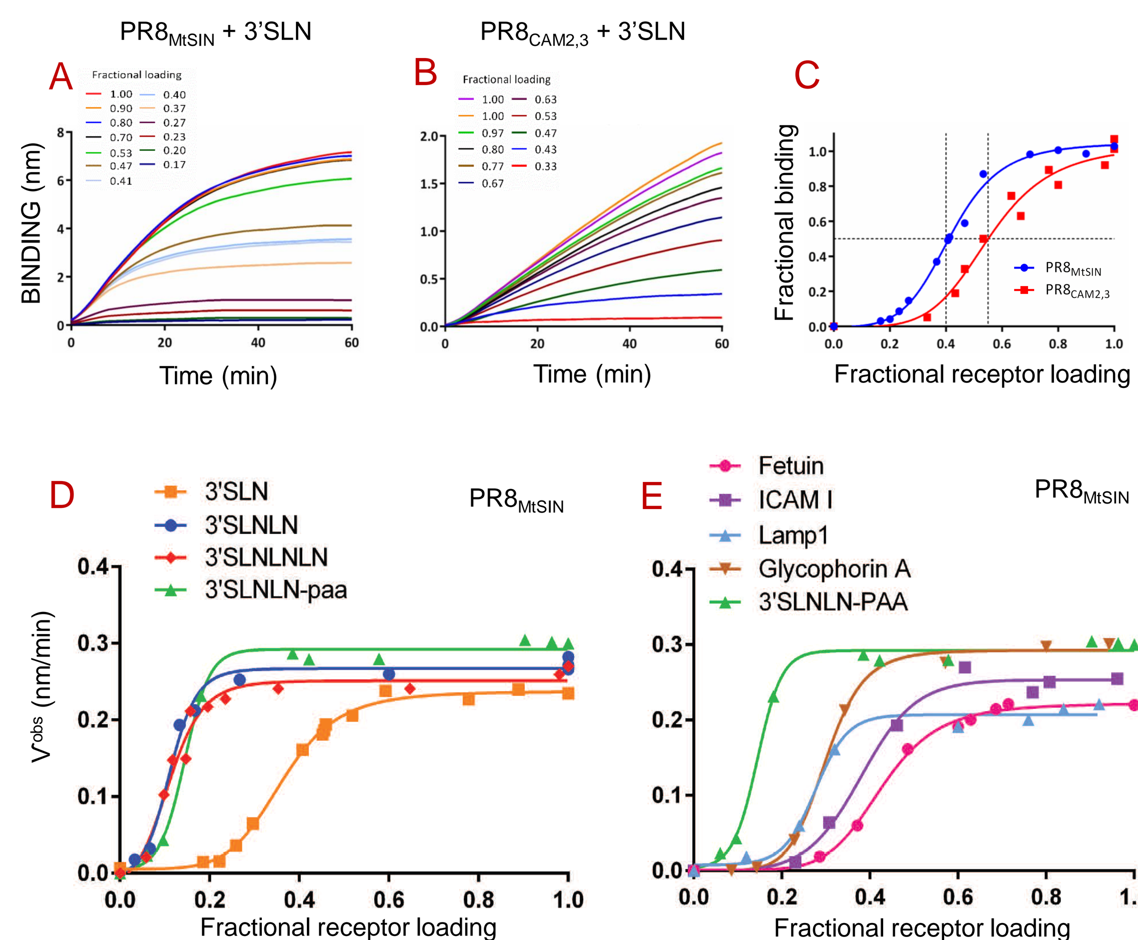
Figure 1. Initial binding rate shows a linear correlation with virus concentration: $v^{obs} = k_{on}.[virus]$ $(k_{off} \sim 0)$



Streptavidin sensors were maximally loaded with receptor (3'SLN) and bound with a concentration series of virus ($PR8_{SIN}$ (A) or $PR8_{CAM2,3}$ (B)) in presence of oseltamivir carboxylate (OC) to inhibit NA activity. After 60 min sensors were moved to PBS + OC for dissociation. Even after 6 hours (not shown) no dissociation was observed. In (C) the initial binding rate is plotted against virus concentration.

CONCLUSION: Virus binding is virtually irreversible in absence of NA activity prohibiting determination of K_D. Determination of the observed initial binding rate is a quantifiable parameter ($v^{obs} = k_{on}.[virus]$).

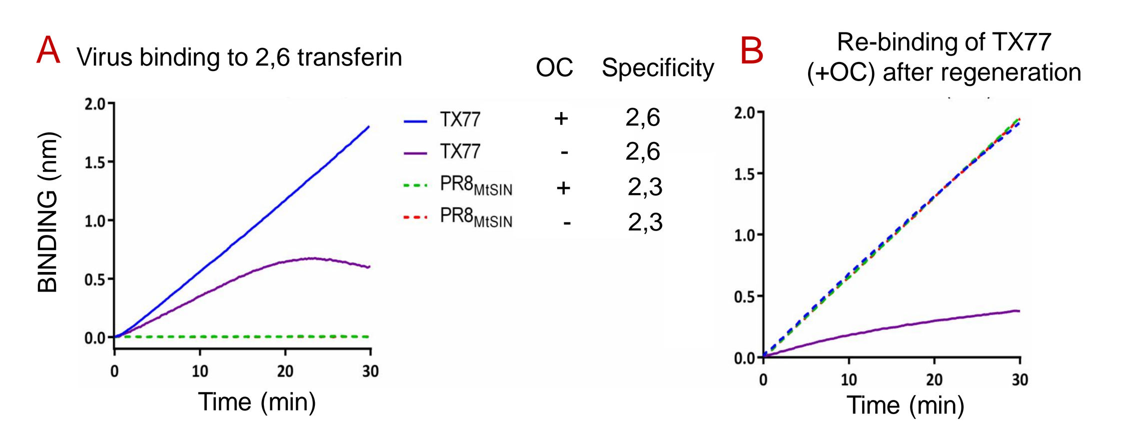
Figure 2. The effect of receptor density on virus binding



Streptavidin sensors were loaded with receptor (3'SLN) concentration range resulting in receptor loading densities as indicated. 10pM of virus (A,B) was bound for 1 hour in presence of OC. Binding levels obtained after 1 hour were plotted as fraction of maximal binding against fractional receptor loading (C). Clearly such a plot will look different when using different virus binding times. Therefore, in D and E fractional receptor loading for a range of synthetic glycans or glycoproteins was plotted against initial binding rate which is independent of binding time.

CONCLUSION: Receptor density allows for a quantitative comparison of binding strength of virus to different receptors. A weaker binder requires higher receptor density for reaching maximum binding rate.

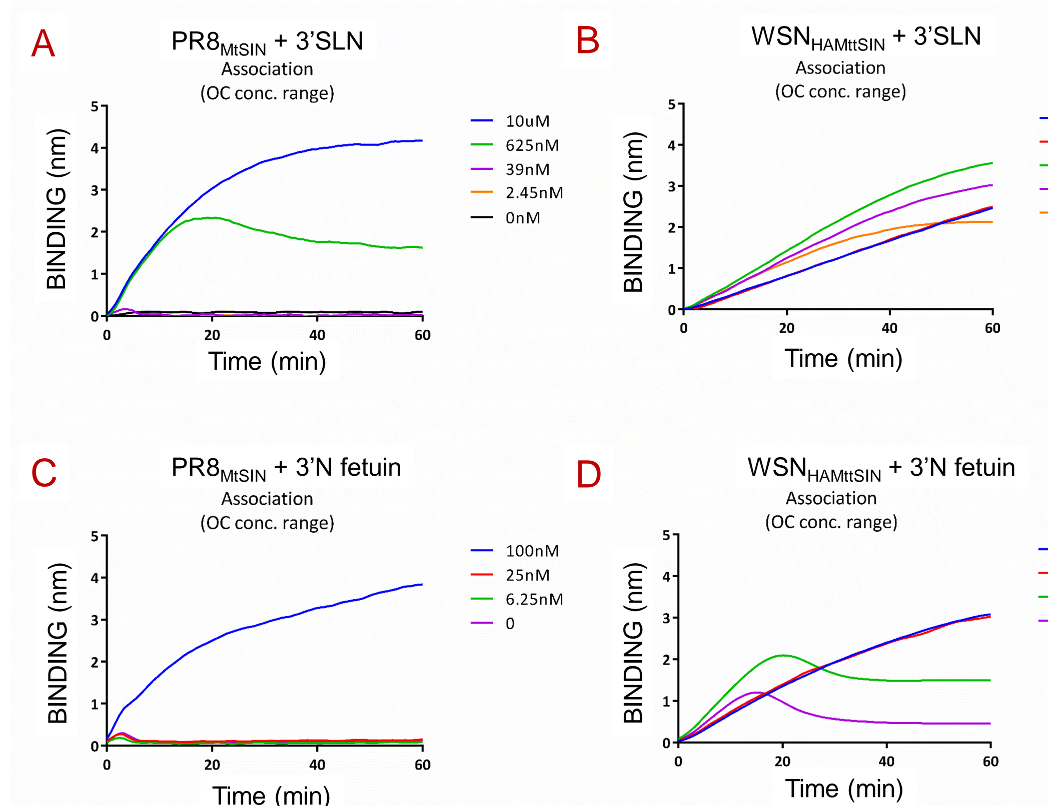
Figure 3. NA activity requires HA-dependent virus binding to the receptor-coated surface



Streptavidin sensors were maximally loaded with receptor (2,6 transferin (A)) and bound with 100 pM TX77 virus (2,6 specific binder) or $PR8_{SIN}$ (2,3 specific binder) in presence or absence of oseltamivir carboxylate (OC) as indicated. Both viruses have the same NA. Clearly, in absence of OC the neuraminidase activity of TX77 results in decreased binding in time whereas the 2,3 specific $PR8_{SIN}$ does not bind. After 30 min the sensors were cleared of virus by regeneration at pH2 leaving the receptors at the sensor surface. All sensors were re-bound with TX77 virus to determine whether sialic acids were removed in the previous step.

CONCLUSION: When neuraminidase is not associated with the receptor surface via HA it cannot act efficiently.

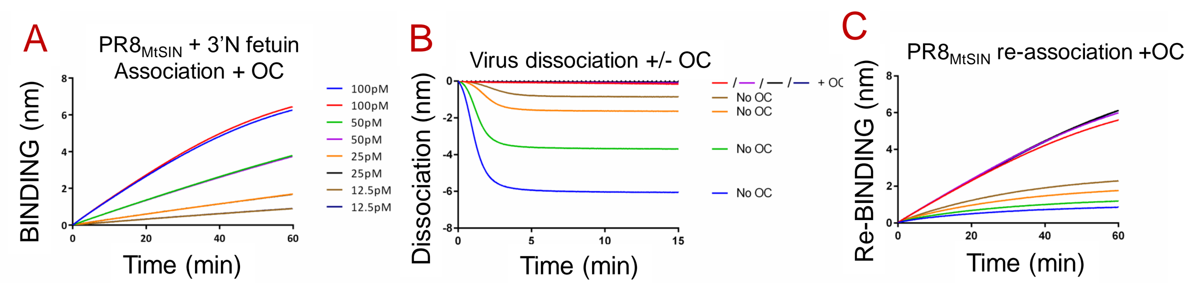
Figure 4. NA can contribute to virus-binding



Streptavidin sensors were maximally loaded with receptor (3'SLN (A,B) or 3'N fetuin (C,D)) and bound with 100 pM virus ($PR8_{SIN}$ (A,C) or $WSN_{HAMtSIN}$ (B,D)) in presence of a concentration range of oseltamivir carboxylate (OC). Clearly, in absence of OC the neuraminidase activity results in decreased binding in time. The NA of $PR8_{SIN}$ is more active than the NA of $WSN_{HAMtSIN}$. Remarkably, the initial binding rate of $WSN_{HAMtSIN}$ is reduced at increased OC concentrations, indicating that blocking the NA active site prevents a contribution to virus binding.

CONCLUSION: A neuraminidase with relatively low activity can contribute to the initial binding rate and can be measured by BLI.

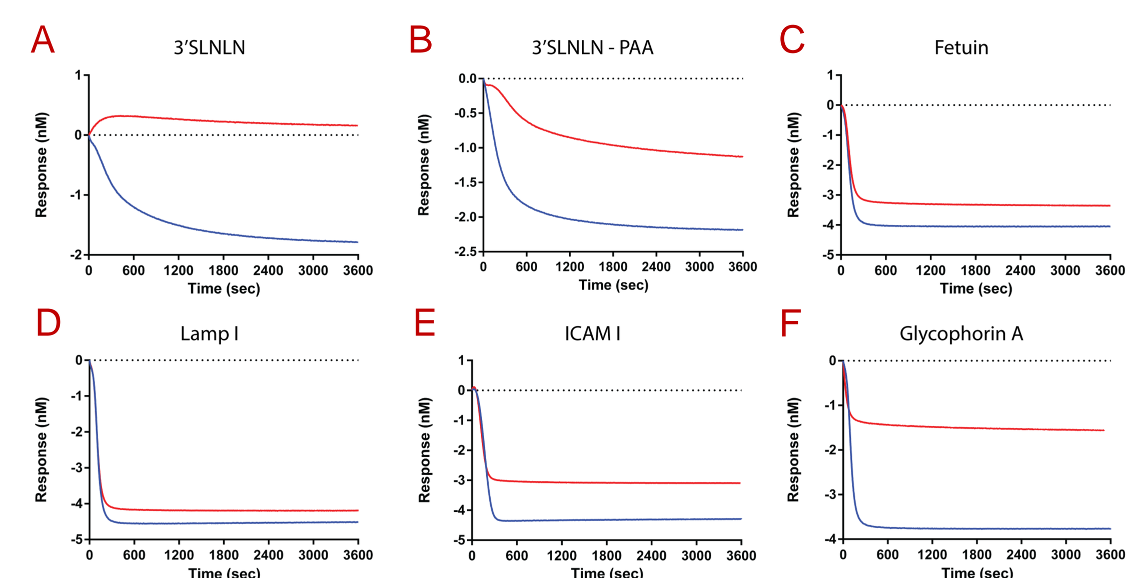
Figure 5. Virus particles are moving over the receptor surface before NA activity-driven dissociation occurs



Streptavidin sensors were maximally loaded with the 3'N fetuin receptor and bound in duplo with 4 concentrations of $PR8_{SIN}$ virus in presence of OC (A). For each duplo, one sensor was further incubated for 1 hour in PBS+OC and one in PBS in absence of OC allowing efficient NA-driven virus self-elution (B). Subsequently all virus was stripped from the sensors by regeneration at pH2 (leaving the 3'N fetuin intact). In panel (C) re-binding with 100 pM $PR8_{SIN}$ was used to asses the effect of neuraminidase activity in the previous step. Remarkably, even a low virus loading level in the initial binding step (~10% saturation) results in highly reduced binding in the second binding (C). Thus, a small amount of bound virus is able to clear the complete surface of sialic acids in step (B). Extensive control experiments have shown that the amounts of virus released from the surface in (B) are to low to give efficient re-binding.

CONCLUSION: Virus particles move over a receptor-coated surface and are only released when the whole surface is efficiently cleared from sialic acid. BLI can be used to quantify this process. Movement probably depends on the low affinity of a single HA-SIA interaction allowing a highly dynamic binding mode in which individual HA-SIA interactions are continously formed and broken. The presence of multiple interactions allows the virus to remain firmly bound to the surface.

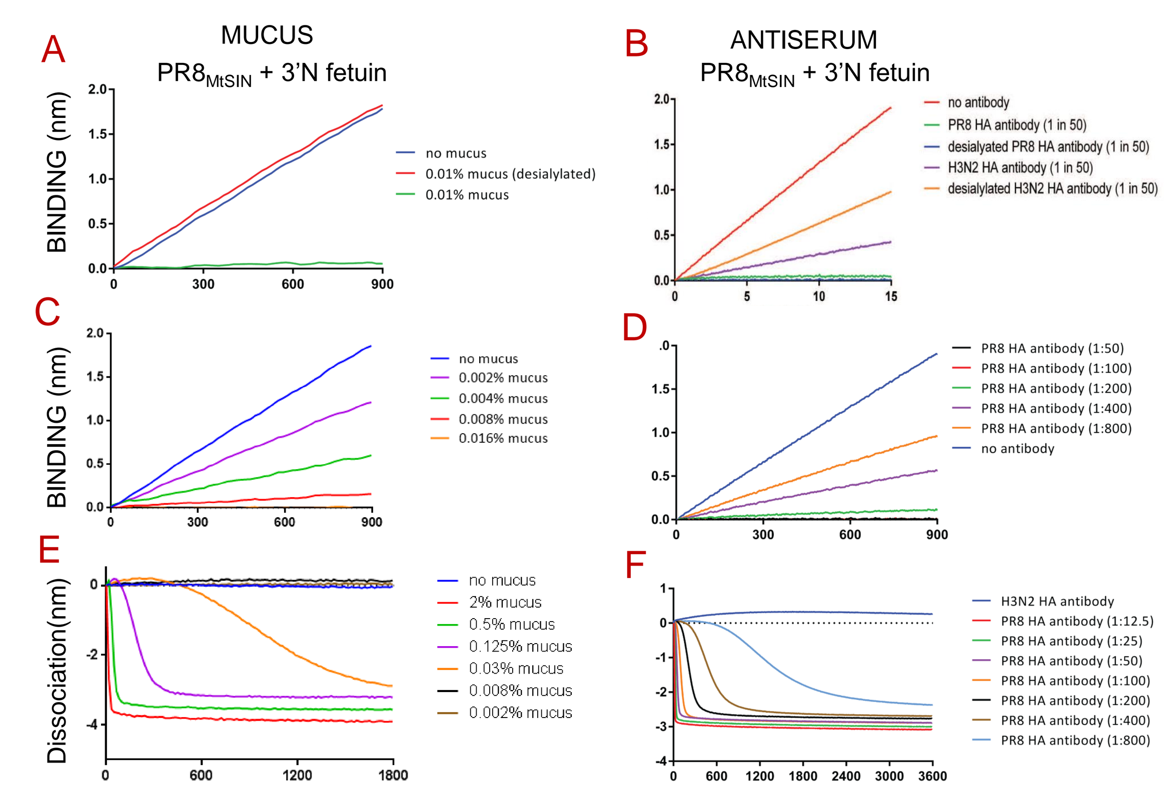
Figure 6. Virus binding matures upon prolonged binding



Streptavidin sensors were maximally loaded with the indicated receptors and bound in duplo with 100 pM $PR8_{SIN}$ virus for 15 min in presence of OC. For each duplo, one sensor was further incubated for 1 hour in PBS+OC (prolonged association) and subsequently moved to PBS without OC to determine NA-driven virus elution (red lines) whereas the other sensor was directly moved to PBS without OC (blue lines). It appears that prolonged association of virus reduces NA-driven virus elution.

CONCLUSION: Virus association with a receptor-coated surface maturates in time. Whereas viruses initially move over the surface (Fig. 5) due to the highly dynamic mode of individual HA-SIA interactions, allowing the interference of NA and resulting in virus elution, such movement is arrested in time. Possibly multiple interactions with the monomers of a single HA-trimer increase in time and affect movement of virus.

Figure 7. Mucus and antisera drive virus release from receptor-coated surfaces



Streptavidin sensors were maximally loaded with receptor (3'N fetuin) and bound with 100 pM $PR8_{SIN}$ virus that was pre-incubated with potential human mucus or antiserum inhibitors as indicated in the panels (A-D). In panels E and F virus was first bound to the sensors and subsequently incubated in presence of mucus or antiserum. All steps were preformed in presence of oseltamivir. Clearly, pre-incubation with mucus reduces virus binding in a sialic acid dependent way demonstrating the decoy receptor function of mucus (A,C). Pre-incubation with antiserum is, as expected, antigen specific in reducing virus binding (B,D). Remarkably, pre-bound virus, which in absence of oseltamivir does not dissociate (Fig. 1), can be rapidly eluted by addition of mucus or specific antisera (E,F). The highly dynamic binding mode of virus to a receptor surface (e.g. Fig. 5) most likely enables mucins or antibodies to slip in between HA and receptor (note that the Kd of individual HA-SIA interactions is in the sub-milimolar range (0.1 – 3 mM).

CONCLUSION: BLI provides a direct and quantitative mode for determining interference of decoy receptors or antisera with virus binding. Virus elution by such molecules might be of physiological importance.