

P5 – Measuring Adhesion Forces Between Influenza Virus and Living Cells

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Influenza Virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cells. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is estimated to be very low (10^{-13} M^{-1}). The attachment of influenza virus particles to living host cells was characterised on the level of single molecules using optical tweezers and atomic force spectroscopy. Unbinding events were analysed and revealed a multimodal rupture force distribution. This suggests sequential binding of multiple receptors. Treatment of the cells with neuraminidase (NA) which cleaves terminal sialic acid residues lead to a decrease of the binding probability by >50 %. This indicates a specific interaction between hemagglutinin and sialic acid during the force measurements.

Measuring Influenza Virus Adhesion to living cells

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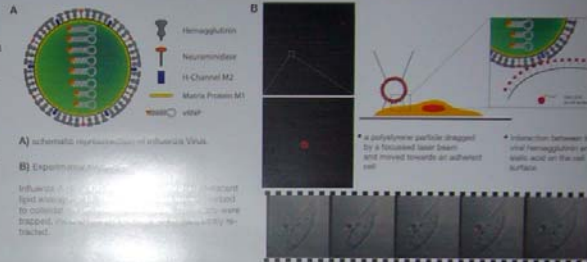


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Introduction and Aims

Influenza virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cells [1]. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is estimated to be very low (10^{-10} M^{-1}) [2]. The attachment of influenza virus particles to living host cells was characterized on the level of single molecules using optical tweezers and atomic force spectroscopy. Unbinding events were analyzed and revealed a multimodal rupture force distribution. This suggests sequential binding of multiple receptors. Treatment of the cells with neuraminidase (NA) which cleaves terminal sialic acid residues leads to a decrease of the binding probability by >50%. This indicates a specific interaction between hemagglutinin and sialic acid unravelled by force measurements.



Results

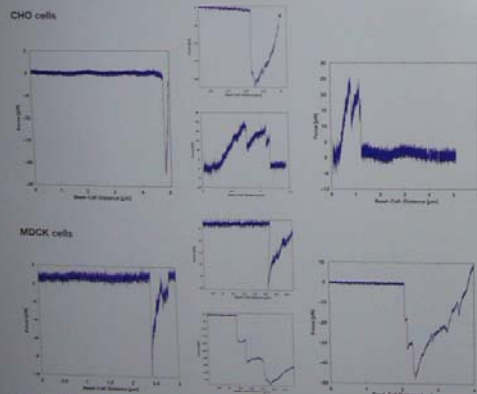
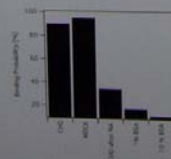


Figure 1: Force Traces of virus-cell interactions acquired by optical tweezers

Virus-coated beads were brought into cell-contact using optical tweezers and retracted without delay. Due to high sensitivity and low noise it was possible to record force traces with low-pN resolution. Measurements were carried out using epithelial cell lines CHO and MDCK. Both cell lines feature high levels of terminal sialic acid and are thus appropriate for influenza virus adhesion. In both cases single- and multiple rupture events were observed. We found binding probabilities of > 80% for CHO and MDCK. Even higher in the case of MDCK cells, which is due to higher SA surface density. NA treatment cleaves terminal sialic acid residues and lead to a reduction of the binding probability by > 50%. The binding probability of beads coated with BSA as negative control was below 10%.



Conclusion and Outlook

- good system to study the interaction of influenza virus and living cells on the single molecule level
- very high accuracy and low measurement noise (<1 pN)
- multimodal force distribution
- force peaks at 7, 15, 20 pN
- confirmation of the optical tweezers data by atomic force spectroscopy
- further analysis of AFM data on influenza adhesion
- dynamic force spectroscopy

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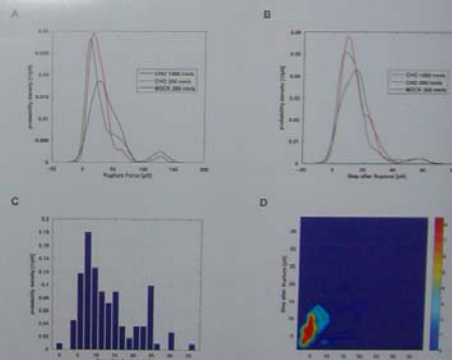


Figure 2: Distribution of Rupture Forces and Force Steps after Unbinding

The distribution of rupture forces and the corresponding rupture steps exhibit a multimodal shape. Within the force steps which represent the actual size of the bond a major peak at 7 pN and 2 minor peaks at 15 and 25 pN were observed B,C. The peaks are interpreted as the simultaneous unbinding of 1, 2 or 3 virus-cell bonds increasing retraction velocity from 200 to 1000 nm/s lead to a shift towards higher force values A,B. Plotting rupture force vs. force step reveals an accumulation of total rupture events at low force values D.

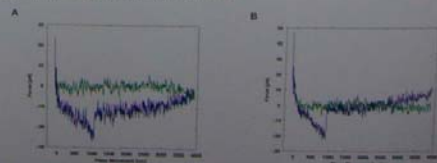


Figure 3: Confirmation of the Results via atomic force spectroscopy

Preliminary results from atomic force spectroscopy of influenza virus adhesion on living CHO cells. Influenza virions were tethered to the AFM tip as described [3]. Force traces revealed very similar unbinding forces. A and B show unbinding events at 10 pN and 20 pN which correspond to the rupture of one and two receptor-ligand bonds.

References

- [1] J. J. Skehel, D. C. Wiley, *Annu. Rev. Biochem.*, **2000**, *69*, 531 - 69
- [2] Mannen et al., *Angew. Chem. Int. Ed.*, **1980**, *37*, 2754 - 2794
- [3] Rankl et al., *PNAS*, **2008**, *105* (40), 17778 - 17783