Experimental report

12/09/2024

Proposal: **8-02-1036** **Council:** 4/2024

Title: Neutron reflectometry studies of ultrathin water layers on virus surface models

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DOPC- and DGS -based SLBs, 3. The same with influenza HA proteins

1. Near-monolayers of densely packed Tobacco Mosaic Viruses, 2. DOPC- and DGS -based SLBs, 3. The same with influenza HA proteins

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	01/06/2024	05/06/2024

Abstract:

The transmission of airborne viruses, such as influenza, relies on drying of the virus particles. It is unknown to which extent the particles dry, and at which humidity they can ¿survive¿. It is however surprising that low air humidity (<40%) appears to be advantageous. The use of spectroscopy and probe microscopy has provided hints on stable ultrathin water layers, but no quantitative results. Hence, the thickness of water layers on viruses requires neutron reflectivity studies. The results will allow to improve our simple strategies against transmission (social distancing, air flow) and to devise new ones. The proposed experiments rely on carefully selected models that focus on a very resilient (but harmless) plant virus, and on lipid and protein layers, which emulate the deadliest virus, influenza.

Background

The transmission of viruses, usually from organism to organism, is a phase during which virus particles (virions) can be seen as nanoparticles. Understanding and possibly influencing this pathway can be, in addition to vaccination, a powerful strategy against pandemic spreading, such as the 1918 influenza pandemic. Airborne viruses, such as influenza and CoV, can reside for hours as aerosols, which is the decisive factor in the typical influenza waves in winter (on the northern hemisphere), which claim yearly hundreds of thousands of fatalities. The current threat in late 2024 is based on the H5N1 variant, which is fatal for various animals ("bird flu", spreading in cattle), but luckily not for humans. It is unknown, in which form water is bound to virions, and how the virions proceed from total hydration (in and at a living cell) to the dry state of an aerosol. Bilayers form the surface of enveloped virions such as CoV and influenza. While such layers, supported on Si wafers (Supported Lipid Bilayers, SLBs) are very well established also at ILL, we have introduced a bilayer/HA model to ILL, where the hemagglutinin (HA) proteins, the "spikes" on the viral surface, are bound to functionalized lipids via NTA-Ni(II) route. Our model for a non-enveloped virion, which is practically not influenced by its environment, is Tobacco Mosaic Virus (TMV), a harmless plant virus, which we used for the first time at ILL.

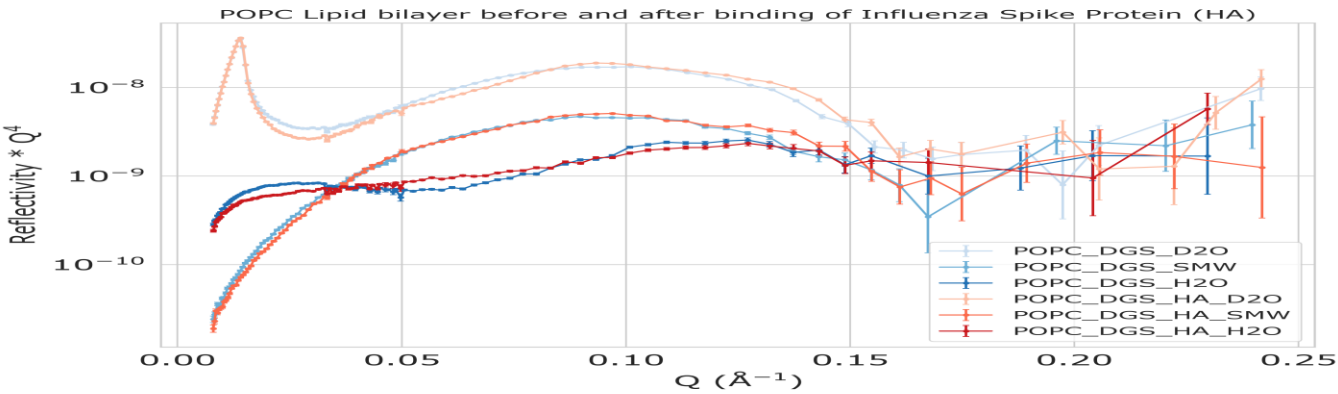
Lipid bilayers in contact with liquid water

In our experiments at ILL, we had first performed solid/liquid experiment of our SLBs (based on the standard lipid POPC and a small fraction of the modified lipid DGS), in absence of HA. The preparation turned out to be straightforward, based on excellent facilities (chemistry and soft matter surface laboratories) and high-quality Si wafer surfaces. For the experimental conditions, we first worked with the liquid cell with:

Liquid water

Liquid deuterium oxide

Both mixed, matching the neutron refractive index of Si (SMW)



The mixed lipid layer (POCP and DGS, with/out the "spike" protein HA) under three conditions: In deuterium oxide, in water, and in Si index-matching water (SMW). The reflectivity is multiplied by Q^4 to enhance the features appearing at typical lipid thickness (around 4 nm). The small, but notable and reproducible differences in presence of HA should be due to an average increase of the layer height, and on inhomogeneities. Note that HA is a large trimeric protein, the spike dimensions are about 12 nm height and 8 nm diameter.

Lipid bilayers in contact with humid air

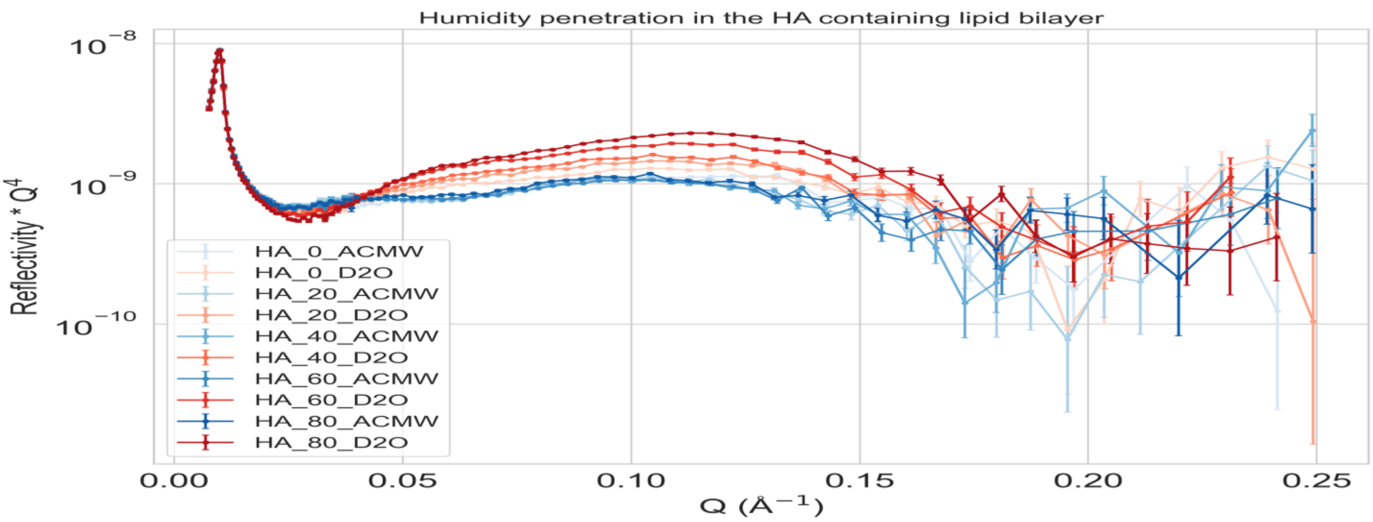
The wafers were then carefully moved from the liquid cell, kept submerged in a water-filled container to maintain hydration, placed in a custom-made teflon tray, and slowly dried in the environmental chamber, filled with humidified air. For the humidity, we explored a large part of the parameter space:

Deuterium oxide vapour

Mixed water/deuterium oxide vapour, matching the neutron refractive index of air (ACMW)

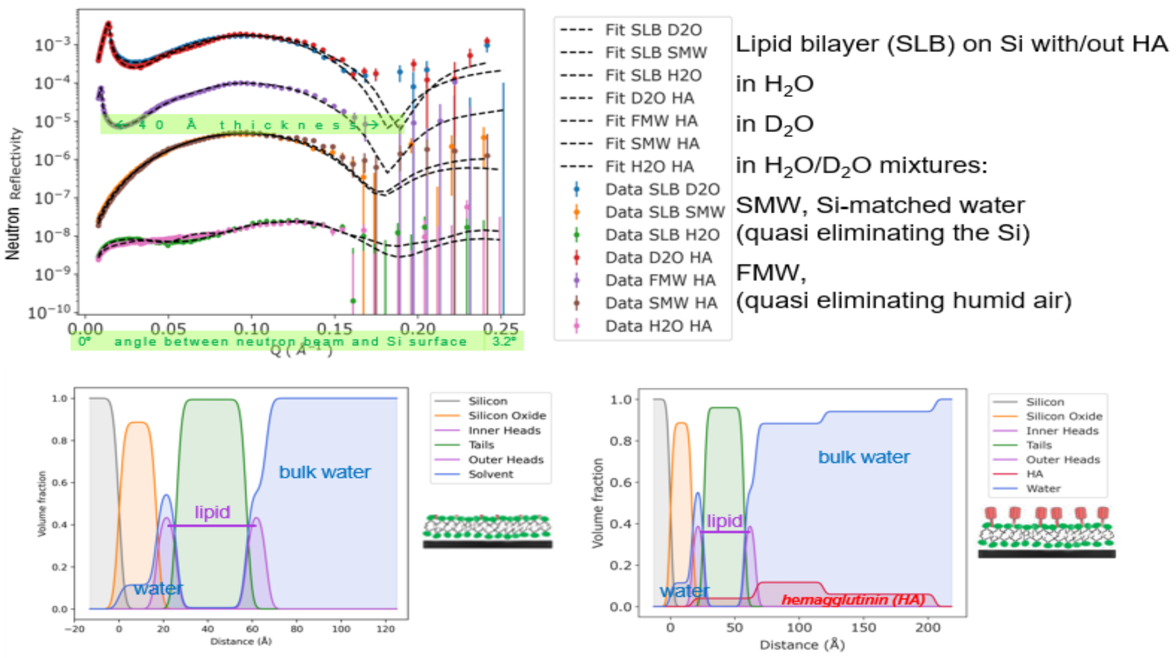
The layers turned out to be very sensitive to dehydration. In some cases, the preliminary results are poorly compatible with relevant data obtained by AFM and fluorescence methods, but at low humidity (<30%) both agree, and widespread destruction was found.

The experiments were repeated for the SLB/HA mixed layer (the HA is bound with Ni(II) to the modified lipid DGS; the large majority of the layer is still POPC). The exact concentration of the HA can be deduced from the assembly conditions, but it was not independently verified by nanoscale imaging (e.g. by AFM). It was verified that HA has an enormous influence on stability, especially at low humidity. The expected degradation at very high humidity, known from the behaviour of the influenza virion, could not be verified due to restrictions in time, and due to the difficult and slow attainment of high humidity, a general problem in experiments.



Experimental series with the same lipid bilayer (with HA) in air humidities from 0% to 80%. When the humidity is produced from deuterium oxide (reddish traces), water adsorption is found, which correlates with humidity. For ACMW, air contrast matching, the blueish traces show little variation, possibly based on penetration of water into the SLB.

Prof. Bittner presented our preliminary results in a seminar at nanogune, and on a poster at a high-level international conference (Gordon Research Conference "Biosurfaces", June 2024 in Il Ciocco):



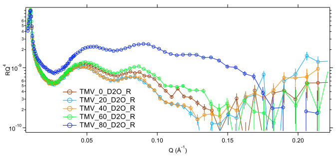
Volume fraction profiles, based on data fitting: ***Water penetration, hemagglutinin hydration***

Experiments with Tobacco Mosaic Virus (TMV)

The adsorption of TMV from aqueous suspensions was not detected. No experiment gave a hint at the presence of an organic layer, although the thickness of adsorbed TMV would be very well suited, and the hydrophilic nature of the wafer and the virion is well known. We then proceeded to a dry TMV monolayer, built from a 0.1 mg/ml virion suspension (these suspensions are free of salts). The layer, of correct height, was verified, which is, to our knowledge, a first in neutron science. We continued to investigate the same sample under various air humidities, in the parameter space:

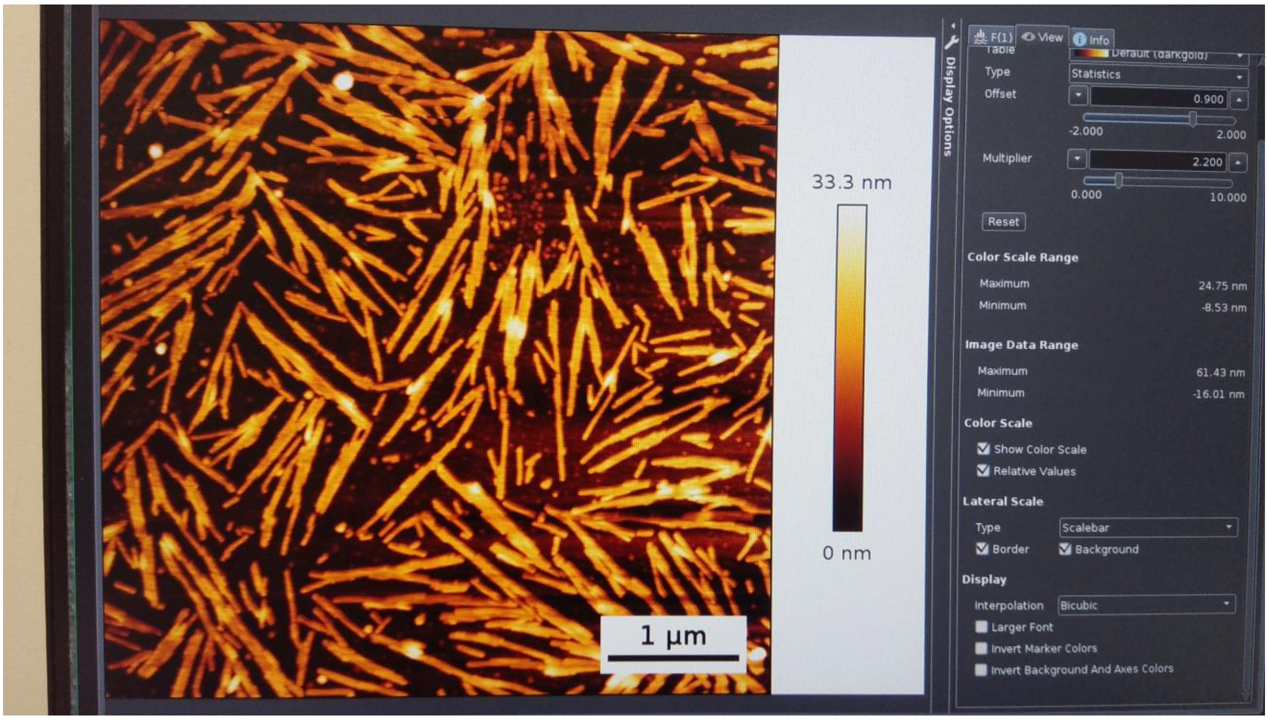
Deuterium oxide vapour

Mixed water/deuterium oxide vapour, matching the neutron refractive index of air (ACMW)



Water adsorption on Tobacco Mosaic Virus, in deuterium oxide vapour (0% to 80% humidity)

Preliminary evaluation showed well-established oscillations in the reflectivity curves; the height of ca 15 nm organic layer is very well suited for or studies. A water layer above 1 nm thickness only forms at 80% air humidity, which is in rough agreement with our AFM studies. However, there are also smaller changes at 60%, which we need to evaluate in detail. They are not evident in AFM data. Finally, the sample was checked at nanogune by AFM and showed a very well assembled submonolayer of TMV. Here, we demonstrated for the first time the extremely low beam damage of neutrons on adsorbed virions (TMV is completely resilient to water, but neither to electrons nor photons).



AFM scan (noncontact mode) of a large area of the Si wafer (dark brown), covered by well-adsorbed viuses (light brown). The scan was recorded after many hours of exposure to the neutron beam.