

# Shining light on Nature's raincoat



KONRAD MEISTER is postdoc in the group of Ultrafast Spectroscopy, AMOLF.

## → References

K. Meister, *et al.*, Observation of pH-induced Protein Reorientation at the Water Surface, *The Journal of Physical Chemistry Letters* 8, 1772-1776 (2017).

K. Meister, A. Paananen, H. J. Bakker, Identification of the response of N-H vibrations in vibrational sum-frequency generation spectroscopy of aqueous protein films, *Physical Chemistry Chemical Physics* 19, 10804-10807 (2017).

K. Meister, *et al.*, Self-Assembly of Hydrophobin classes at the Air-Water Interface, *The Journal of Physical Chemistry Letters* 7, 4067-4071 (2016).

## ↘ Figure

(A) Schematic structure of a hydrophobin protein.  
 (B) Principle of an SFG experiment. In this technique we combine invisible infrared pulses with visible pulses that are fired at the sample. A new light beam is generated at the sum frequency of the input infrared and visible beams, but only if the frequencies of the infrared light match the frequencies of the molecular vibrations of ordered interfacial molecules. This aspect makes the technique extremely surface-specific and has allowed us to study hydrophobins directly at the relevant water-air interface.  
 (C) Typical SFG spectrum of a hydrophobin film at the interface. The detected signals originate from structural elements of the proteins and help us to determine their molecular structure and orientation at the interface.

→ Nature provides us with a variety of fascinating examples from which we can learn and take inspiration on how to make new functional designer materials. One such example is hydrophobins, a group of surface-active proteins produced by filamentous fungi and known for their unique functions related to the interaction of interfaces. In nature, hydrophobins are secreted to reduce the surface tension of the aqueous environment, which otherwise can be a barrier to the growth of hyphae into the air and subsequent spore production. Hydrophobins are also retained in fungal structures, where they assemble into stable and very elastic films that provide a natural water-repellent coating (a natural rain coat) on the outer surface of the fungi. Hydrophobins have already led to industrial applications including foams, dispersions and functional coatings. Their clear geometric separation of hydrophilic (water-loving) and hydrophobic (water-fearing) regions further inspired chemists to make molecular Janus particles. The molecular origin of the hydrophobins' remarkable interfacial properties remains unclear; however, a detailed molecular-structure understanding of these proteins adsorbed to their relevant place of action, the water-air interface, holds the key for understanding their diverse bi-

ological functions and will further enhance their biotechnological applicability. The reason for the current gaps in our knowledge is not the shortage of relevant questions but rather the lack of techniques that can probe liquid surfaces with high selectivity.

In order to unravel the secrets of hydrophobin films we used an advanced nonlinear technique called vibrational sum-frequency generation (SFG) spectroscopy. The technique relies on the interaction of the proteins with light. Chemical groups absorb part of the light waves, which generates a unique signature at various frequencies that can be used to identify specific structural elements and groups that are present within the protein. Using SFG spectroscopy we could determine the detailed molecular structure of hydrophobin directly at the water-air interface. We found that upon hydrophobin adsorption to the water surface, the three-dimensional solution structure remained intact and assembled into an extremely stable and highly ordered film.

Interestingly, we further noticed that, when we changed the acidity of the water on which the hydrophobins are floating, the individual hydrophobin molecules changed their orientation at the interface. This was revealed by changes

in the vibrational signature and confirmed by spectral calculations performed by our collaborators at the University of Amsterdam.

An interesting question was whether the observed reorientations on the molecular level also have an effect on the macroscopic film properties such as the stability and elasticity. Together with collaborators in Finland we observed that the acidity-induced reorientation correlated with a large decrease in the elasticity of the film. We explain this observation with the change of the charge state of particular amino acids, which we could directly follow using the vibrational signal. When negatively charged, the residues have strong interactions with their neighbours, which are not possible once neutralised. This results in an altered orientation and less stable and elastic films.

A certain orientation on the molecular level is apparently crucial to give hydrophobin films their extraordinary macroscopic film properties.

The finding that these properties can be affected by changing an external factor, in this case the acidity of the solution, opens the door to fine-tune the properties of hydrophobin films with desired properties.  $\Omega$

