Monolayer detection

with sum-frequency generation spectroscopy

E. H. G. Backus, T. Weidner, Max Planck Institute for Polymer Research, Mainz, Germany
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Application Note

Introduction

Interfaces play important roles in various fields like atmospheric chemistry, catalysis, electrochemistry, and biochemistry. Studying an interface is a real challenge as the interfacial molecules are surrounded by many more bulk molecules. The vibrational sum frequency generation (VSFG) spectroscopic technique has been shown to be a useful technique to study specifically interfacial molecules. In VSFG, an infrared and a visible laser pulse are combined and subsequently a signal at the sum frequency of the two is generated. If the infrared is in resonance with a molecular vibration, the signal is strongly enhanced, making the technique chemical sensitive. As this second order technique will not generate a signal in centrosymmetric media, only a signal will be obtained from the interface where the symmetry has been broken. This technique is thus extremely well suited to study interfacial layers of centrosymmetric media like water and ice, but also to study monolayers of molecules deposited on water or to study interfaces between solids and liquids. Here, we demonstrate how this technique can obtain information about the interfacial structure and hydration of a protein in contact with water. The interaction of peptides with interfaces is of fundamental interest for biomaterials design, biosensors and food science.

Experimental setup

In our VSFG setup part of the output of a 10 mJ amplified laser system (Spectra-Physics) centered at 800 nm with a pulse duration around 40 fs is frequency converted in an optical parametric amplifier with a nonlinear difference frequency mixing stage (TOPAS, Lightconversion) to generate light between 3 and 6 µm. Another part of the 800 nm light is frequency narrowed by a homebuilt pulseshaper. The focused visible and infrared beams are temporally and spatially overlapped at the interface. The reflected VSG light is dispersed in a Shamrock 303i (SR-303i-A-SIL) spectrograph and detected by a newton EMCCD-detector DU970P-BV, both made by Andor Technology. The high sensitivity, low dark current, and low read noise of this detector make it possible to detect the very weak signals of the monolayer of molecules at the interface with good signal to noise. A picture of our VSFG setup is depicted in Figure 1.

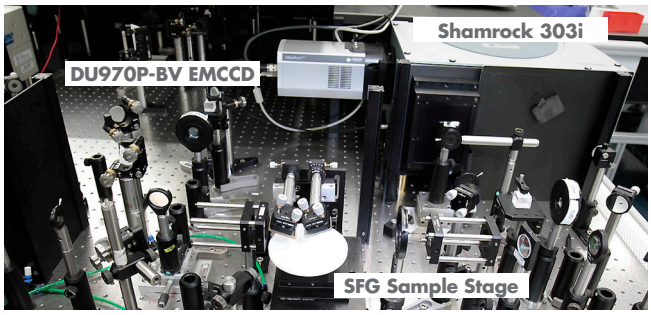

Fig. 1 Picture of the vibrational sum frequency generation setup

Results

Figure 2a shows an SFG spectrum of the model amphiphilic peptide LKa14 self-assembled into a monolayer film at the air-water interface. The peptides consist of 14 amino acids and carry hydrophobic leucines and hydrophilic lysines arranged in a sequence which induces a stable a-helical secondary structure. The spectra show strong, narrow features below 3000 cm^{-1}, which can be assigned to the C–H stretching modes of the leucine isopropyl groups.[1] Since in SFG only ordered species generate an appreciable net signal, we can conclude the leucines become ordered upon peptide adsorption, most likely due to hydrophobic interactions with the air water interface. Broad peaks above 3000 cm^{-1} are assigned to water molecules in close contact with the peptide film. In general such water modes contain important information about details of interfacial protein hydration. In the specific case of LKa14 peptides, we can conclude that while the leucine sites are oriented towards the hydrophobic air interface, the lysines, which are located on the opposite side of the peptide helix, are facing the water phase and interact with the surrounding waters. Fig. 2b illustrates the current picture of how LKa14 is structured at the air water interface and other hydrophobic surfaces.

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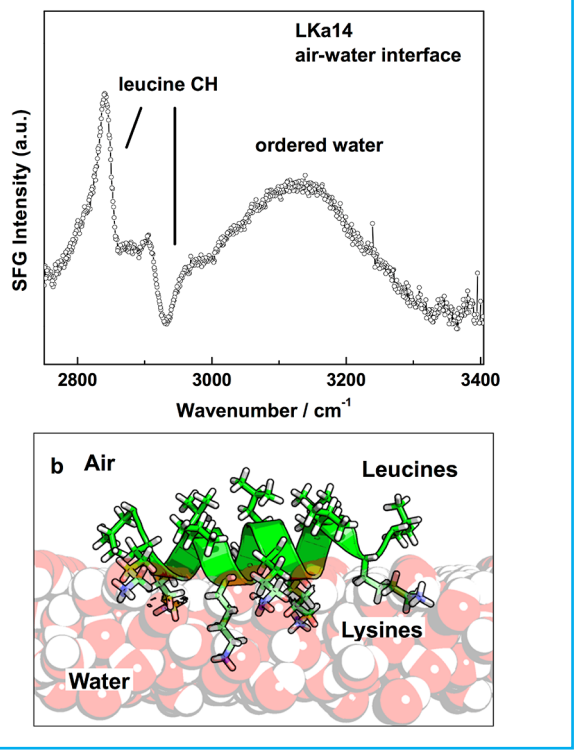

Fig. 2 (a) SFG spectrum of a lysine-leucine model peptide at the air water interface. (b) Model of the peptide adsorption geometry. Leucines order at the interface while lysine binds surrounding water molecules.

References

[1] Weidner, T.; Samuel, N. T.; McCrea, K.; Gamble, L. J.; Ward, R. S.; Castner, D. G., Assembly and Structure of α-Helical Peptide Films on Hydrophobic Fluorocarbon Surfaces. Biointerphases 2010, 5, 9-16.

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