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Probing Interaction of a Cell Penetrating Peptide with Lipid at Air/Water Interface Using Heterodyne-Detected Vibrational Sum Frequency Generation Spectroscopy

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[Introduction]

Treatment of diseases often involves delivery of therapeutic molecules into cells. Thus, transportation of bio-molecules across cell membranes is an issue of utmost biological importance. The efficiency of such a process is governed by the interaction between the therapeutic molecule and the lipid bilayer of the cell membrane. Short-chain, basic amino acid-rich sequences, commonly known as Cell Penetrating Peptides (CPPs) are a class of molecules that facilitate this phenomenon.[1] However, a molecular level mechanism that describes this interfacial transport is currently lacking since such interfacial regions are often only a few nanometers in thickness and are difficult to investigate. Heterodyne-detected Vibrational Sum Frequency Generation (HD-VSFG) is a novel nonlinear spectroscopic tool which allows us to selectively monitor molecular process occurring at the interface.[2,3] In particular, the HD-VSFG experiment is capable of determining the net orientation of water molecules at various interfaces. The sign of the OH band as revealed in the imaginary $\chi^{(2)}$ spectrum of interfacial water provides a direct evidence about whether the water molecule is oriented with its H atoms pointing upwards or away from the interface.[2,3]

In this presentation, we report a HD-VSFG study that aims to unravel the interaction of a prototypical CPP molecule with a model lipid monolayer comprising of an anionic lipid.

[Experiment]

We chose deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (d-DPPG) as the model lipid for our study. It belongs to the biologically important class of membrane phospholipids and it is anionic in nature. Figure 1 shows the chemical structure of d-DPPG.

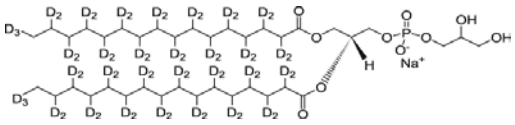


Figure 1. Chemical structure of deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (d-DPPG).

For CPP, we used an arginine oligomer (octa-arginine) in which the N-terminal was modified with a stearyl chain and its C-terminal amidified. Such modification has been reported to enhance the cellular uptake of cargo molecules quite significantly.[4] Thus stearyl octa-arginine (henceforth abbreviated as SR_8)

was used in our studies as a prototype to represent the behavior of CPPs. Figure 2 shows the chemical formula for the primary structure of SR_8.

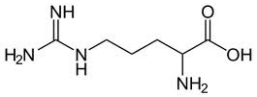
CH_3-$(CH_2)_{16}$-CONH-RRRRRRRR-$CONH_2$ where R= arginine = 

Figure 2. Chemical formula for Stearyl Octa-arginine

All measurements were carried out for lipid monolayer at the air/water interface with the subphase consisting of 10 mM Tris.HCl buffer solution (pH 7.5) at 23 ^oC. For our HD-VSFG experiments, the sample was excited simultaneously with a pair of pulses: i) a broadband infrared pulse (ω_2) containing frequencies in resonance with the vibrational transitions of interest and ii) a narrowband visible pulse (ω_1). The sum frequency generated from the sample was subsequently mixed with sum frequency light from a local oscillator to achieve heterodyne detection. The sum frequency, visible and infrared beams were s-, s- and p-polarized (ssp polarization) respectively, in the present work.

[Results and Discussion]

The interaction between the anionic negatively charged deuterated DPPG lipid interface and the arginine rich positively charged CPP is expected to be governed by electrostatic attraction. On addition of SR_8 to the d-DPPG monolayer, we observe a change in the sign of the OH band (above 3000 cm^{-1}) for interfacial water (Figure 3). This can be attributed to the modification of the lipid interface upon CPP adsorption. The net charge of the interface goes from negative to positive, causing a flip of the adjacent interfacial water molecules. The present study clarifies that water molecules are initially oriented with their H atoms up and re-orient themselves with their H atoms pointed downwards following adsorption of the positively charged CPP.

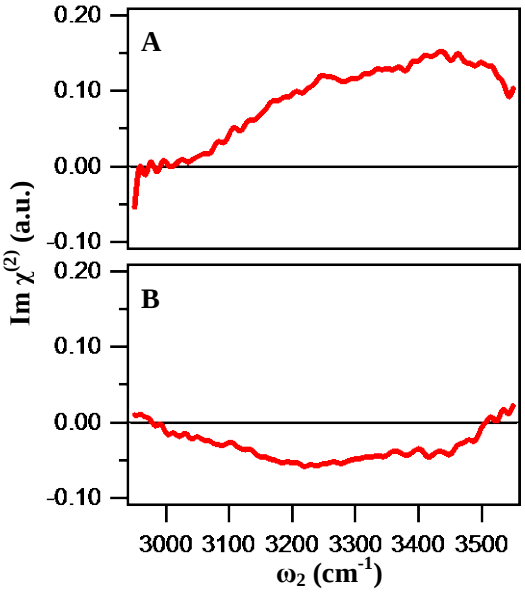


Figure 3. Im $\chi^{(2)}$ spectra of air/water interface with A) d- DPPG and B) after addition of 0.5 μM of SR_8 peptide.

[References]

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