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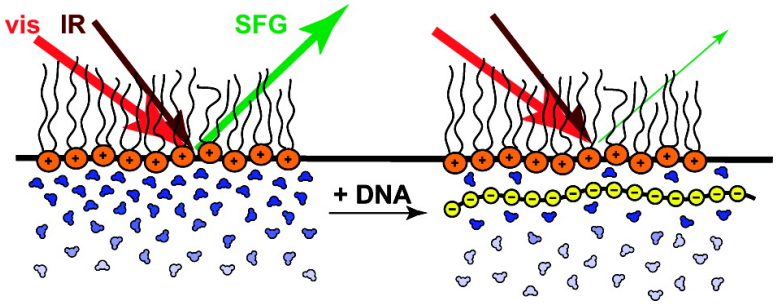

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Sensitive Probing of DNA Binding to a Cationic Lipid Monolayer

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Cationic lipids are widely used as in vitro DNA transfection vectors and show promise as non-viral carriers for in vivo gene therapy.[2] In solution, DNA and cationic lipids form complexes[3] that can enter the cell through endocytosis. One of the major obstacles in gene transfection, however, is the release of the DNA from the complex, in order for transcription to occur. Understanding and ultimately controlling the electrostatic interactions between these lipids and DNA is therefore essential to optimize these genetic carriers. Here we show how vibrational sum-frequency generation (VSFG) can be used to study the binding of DNA to a monolayer of cationic lipids and reveal the role that water plays in this interaction.

Water at the interface of charged lipids is more ordered than bulk water through the alignment of the water dipoles by the surface electric field,[4] as depicted schematically in Figure 1. The interaction of DNA at the lipid interface will result in a screening of the positive lipid charges and will therefore affect the interfacial water structure. We demonstrate here that the interfacial water structure, revealed using surface-specific vibrational techniques, contains detailed information on the lipid−DNA interaction.

VSFG has been shown to be an ideal tool to investigate the structure of interfacial water.[5,6] VSFG can probe the O−H stretch vibration of water, which is very sensitive to the local water environment. In VSFG, an infrared (IR) and visible beam are combined at an interface to generate a signal with a frequency which is the sum of the infrared and visible frequencies.[5] When the IR frequency is resonant with a molecular vibration, the sum-frequency signal is strongly enhanced. As such, VSFG measures the intrinsic molecular properties of the sample and no labeling is required. Moreover, VSFG is surface specific by an inherent suppression of the isotropic bulk signal, as its selection rules require a non-centrosymmetric environment.

The lipid-aligned water molecules represent such a non-centrosymmetric environment. Due to the specific, absolute orientation of the interfacial water molecules, the symmetry is broken not just at the water−lipid interface but for the entire region that is under the influence of the static electric field imposed by the charged lipids. As a result, the water VSFG signal is strongly enhanced at the water-charged lipid interface.[7]

This effect is clearly demonstrated in Figure 2a: A monolayer of 1,2-dipalmitoyl-3-trimethylammonium propane (DPTAP), a cationic lipid, was spread on a D_2O interface and VSFG spectra were collected (see Supporting Information). The intensity of the OD stretch resonances (2000−2700 cm^{-1}) increases by more than an order of magnitude. The much narrower resonances around 2900 cm^{-1} are due to the lipid CH stretch modes.

The electric field of charged surfactants can be screened by the addition of ions to the water subphase. Indeed, the addition of NaCl to the subphase at millimolar concentrations (at a constant lipid area of 60 $Å^2$ per molecule) reduces the water signal by lifting the preferential alignment of water molecules (Figure 2a).

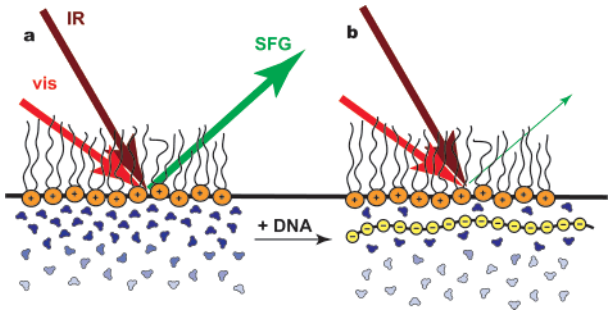

Figure 1. (a) The presence of a cationic lipid monolayer at the air−water interface aligns the first few water layers. This breaks the symmetry, giving rise to a large vibrational sum-frequency generation (VSFG) signal. (b) Due to the strong binding of DNA to the cationic lipids, the electric charges are screened and the orientational order of water is lost, leading to a sharp decrease of the water signal at DNA concentrations as low as 12 pM.

The addition of λ-phage DNA (48 502 basepairs) to the subphase results in a reduction of the water signals in the same manner, but already *at concentrations as low as 12 pM DNA* (Figure 2b). Around 100 pM, the signals reach the levels of a bare water interface, which shows that already at these low concentrations the electric field is fully screened by the polyanionic DNA. This implies that tens of percent of the DNA present in the 4 mm deep trough is localized at the interface.

Given the strong binding of the DNA to the DPTAP monolayer, one might expect that water is essentially "squeezed out" between the lipid and the DNA. This is confirmed by simple thermodynamic considerations, which predict the water layer between the lipid and the DNA to be ∼1 Å thin (see Supporting Information). Indeed, when we examine the SFG spectrum in the presence of 93 pM DNA (corresponding to a DNA surface coverage of ∼90%) in Figure 2b, significant changes in the water spectrum are observed.

When NaCl is added to the subphase, only the amplitude of the water signal varies with concentration, but in the presence of DNA, an additional broad band around 2700 cm^{-1} appears that increases in intensity with increasing DNA concentration. This high frequency is indicative of non- or very weakly hydrogen-bonded OD groups. A similar feature was recently found in surfactant monolayers containing sugar headgroups.[8] By analogy, we assign this OD resonance to water that is disconnected from the bulk hydrogen bond network. Such a situation may be expected in the strongly confined water layer between the lipid monolayer and the DNA strands, as indicated in Figure 1. The spectral changes occurring upon interfacial adsorption of DNA are witness to pronounced restructuring of interfacial water.

The effect of the ions and DNA on the water organization is quantified by considering the VSFG intensity associated with the lipid-bound water obtained from the fits to the data[9] and comparing these to a simple electrostatic binding model (see Supporting Information). The results of this procedure are shown in Figure 3. The total normalized VSFG water signal E is found to be

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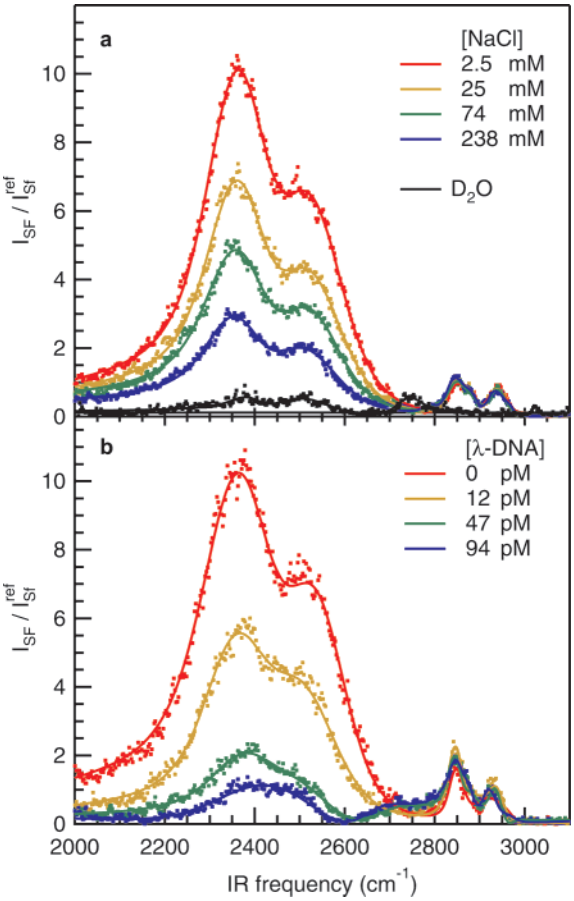

Figure 2. VSFG spectra of water and lipid (DPTAP) at the lipid/D_2O interface. (a) Selected spectra and global fit results for different concentrations of NaCl in the subphase. The spectrum of a bare air/D_2O interface is shown for comparison. (b) Selected spectra and fits for different concentrations of λ-DNA in the subphase.

proportional to the surface potential Ψ_0, which, for the NaCl solution, can be described by Gouy−Chapmann theory, using only the known surface charge and ion concentrations. This confirms the purely electrostatic nature of the interaction between the 1:1 electrolyte and the cationic lipid.

For the water signal as a function of DNA concentration, it is important to realize the cooperativity in the adsorption process: if a negatively charged nucleotide interacts at the surface, others are necessarily close by, increasing the probability of their interaction. This is evident from the steeper slope of the DNA interaction curve in Figure 3. Such cooperative effects can be described by a modified Langmuir adsorption isotherm with an association constant K and a cooperativity (or Hill's) constant n.

A good description of the data was found for $\log K = 10 \pm 1$ (expressed in concentration of DNA strands) and cooperativity constant $2 < n < 7$. This association constant, when expressed per available binding site, that is, per DNA nucleotide, corresponds to 10^4−10^6 M^{-1} L in good agreement with thermodynamic studies of bulk association.[10]

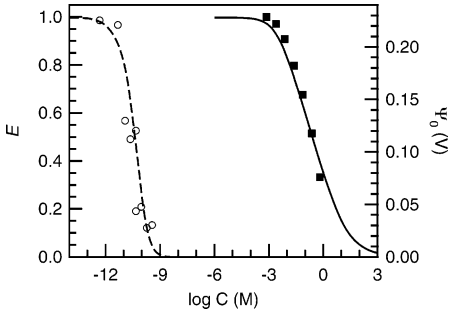

Figure 3. The normalized total water OD stretch signal E obtained from the fits to the VSFG data as a function of NaCl (closed squares) and λ-DNA concentration (open circles). Theoretical surface potentials for a surface charge $\sigma = 0.27$ Cm^{-2} (see Supporting Information) are plotted against the right axis. The Gouy−Chapmann model (continuous line) has no adjustable parameters. The binding model (dashed line) was plotted for log $K = 10$ and $n = 3$ (see Supporting Information for details).

In summary, the vibrational response of interfacial water has been used to obtain novel information on the interaction of cationic lipids and DNA. The DNA−lipid complexation induces a dramatic restructuring of surface water. We find that less than one monolayer of water remains between the DNA and the lipid, and that the water reorients. The approach presented here allows for the quantification of the DNA−lipid association constant, which is important for lipid-based transfection agents.

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Supporting Information Available: Experimental procedures, thermodynamic analysis, and electrostatic binding model. This material is available free of charge via the Internet at http://pubs.acs.org.

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