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Molecular Restructuring of Water and Lipids upon the Interaction of DNA with Lipid Monolayers

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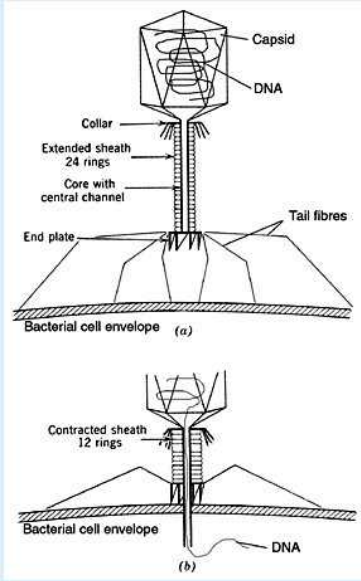
Abstract: Understanding the molecular mechanism of DNA/lipid interaction is critical in optimizing the use of lipid cofactors in gene therapy. Here, we address this question by employing label-free vibrational sum frequency (VSF) spectroscopy to study the interaction of DNA with lipid monolayers of the cationic lipids DPTAP(1,2-dipalmitoyl-3-trimethylammonium-propane) and diC14-amidine as well as the zwitterionic lipid DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) in the presence and absence of calcium. Our approach has the advantage both of allowing us to explicitly probe intermolecular interactions and of providing insight into the structure of water and lipids around DNA at the lipid interface. We find, by examination of the OD stretch of interfacial D_2O, that water structure differs markedly between systems containing DNA adsorbed to cationic and those that contain DNA adsorbed to zwitterionic lipid monolayers (in the presence or absence of Ca^{2+}). The spectral response of interfacial water in the cationic system is consistent with a highly structured, undercoordinated, structural 'type' of water. Further, by investigation of CH stretch modes of the diC14-amidine lipid tails, we demonstrate that the adsorption of DNA to this lipid leads to increased ordering of lipid tails.

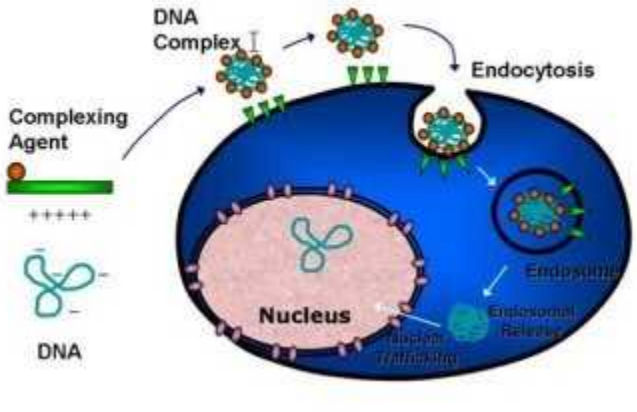
Motivation



<viral infection>

⊙ Species-specific.

⊙ Hard to control DNA contents.



<Non-viral transfection>

⊙ Efficiency of transfection
depends on complexing agent.

⊙ Choosing DNA is available.

➡ **Lipid cofactor is important**
in gene therapy and non-viral transfection.

Previous result

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Sensitive Probing of DNA Binding to a Cationic Lipid Monolayer

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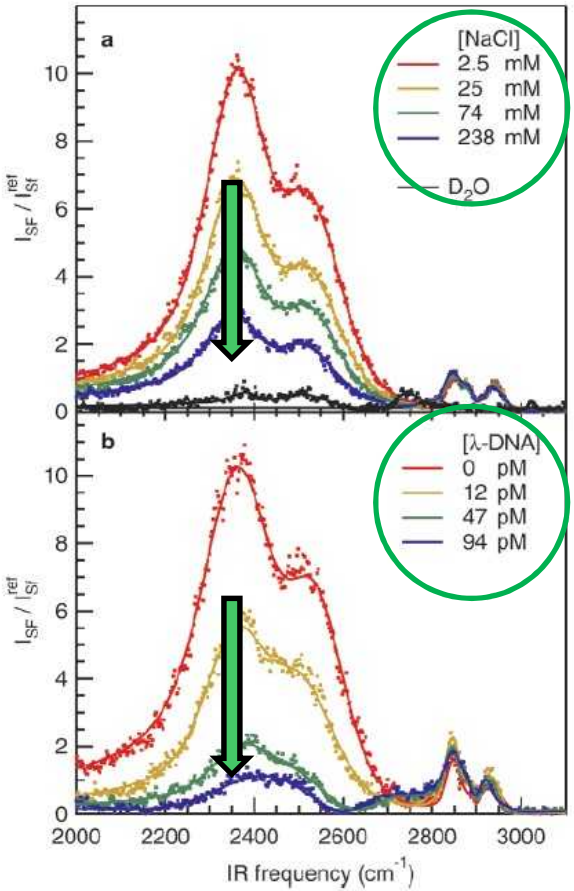
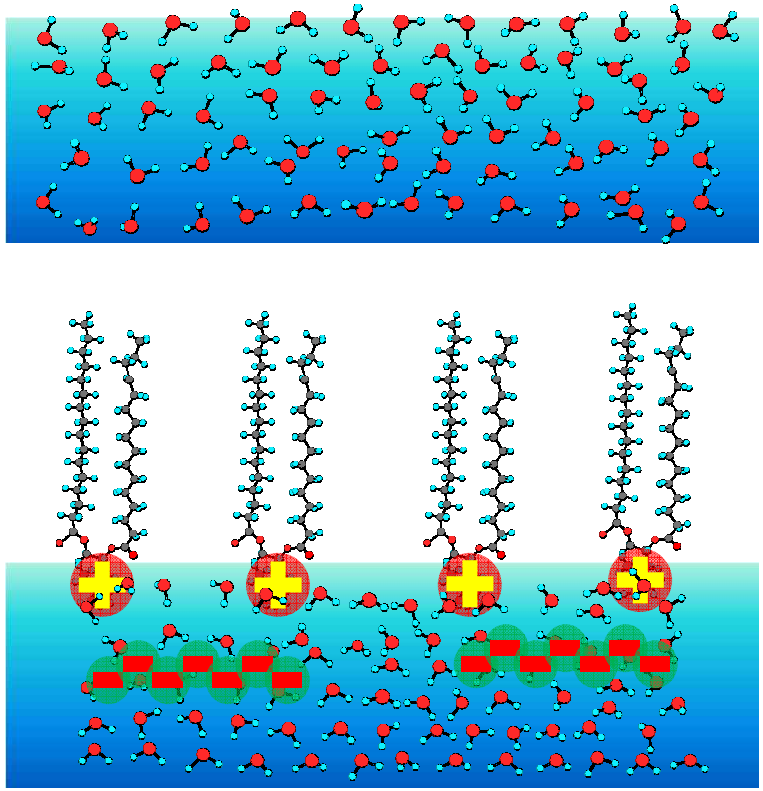
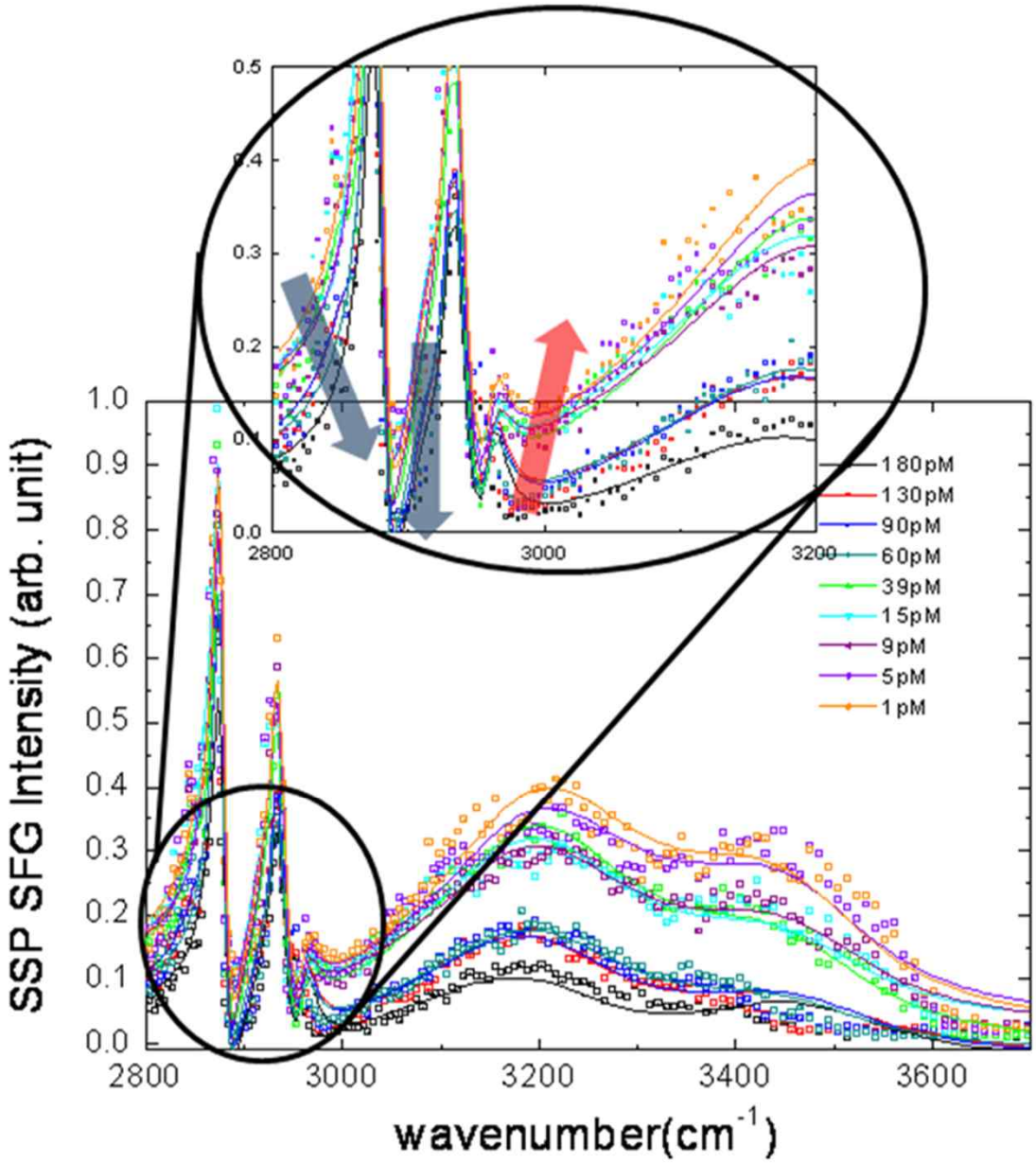




In H_2O case…..



Increasing concentration of λ-phage DNA

Decreasing of OH strength

Samples

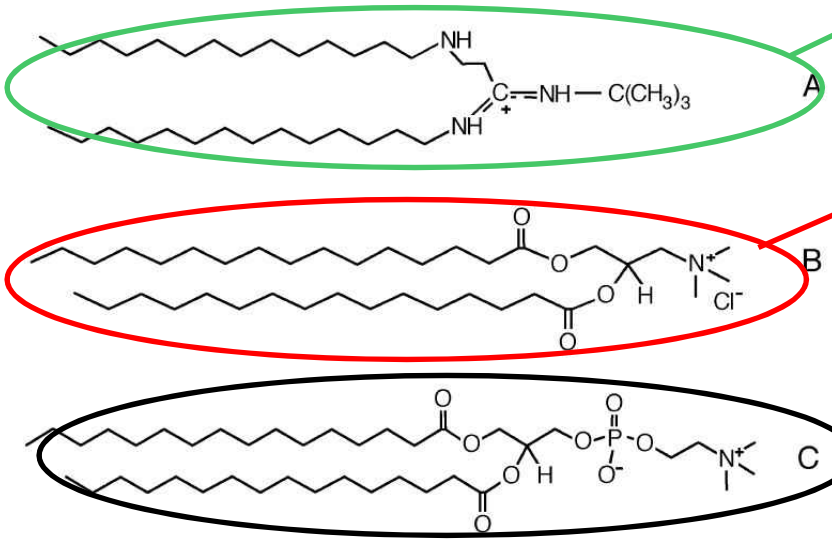


Figure 1. Molecular structure of (A) diC14-amidine; (B) DPTAP; and (C) DPPC.

Intensively studied for non-viral transfection

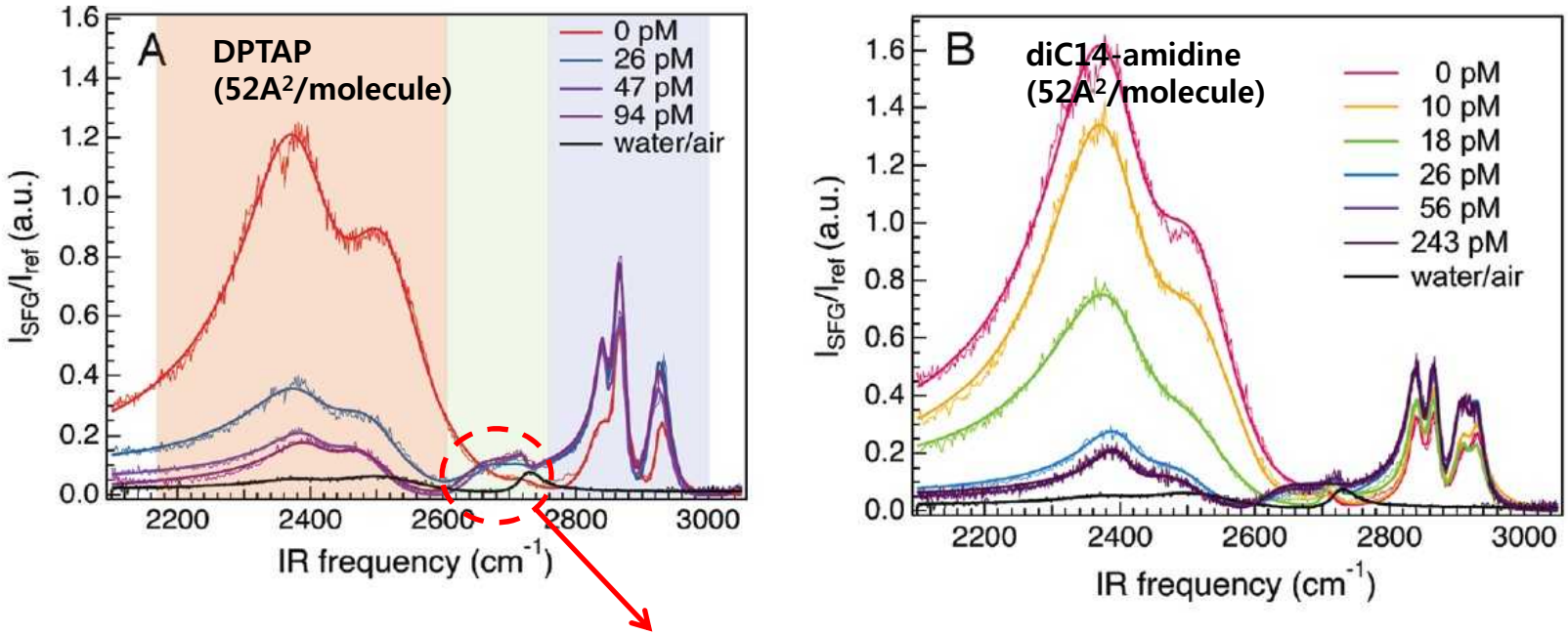
Toxic to mammalian cell

With (DNA+ $CaCl_2$)

Non toxic (usually found in living organism)

SFG spectra on cationic lipid monolayer

* SSP polarization combination / 35° for Vis (800nm) & 40° for IR (3300nm~5000nm)

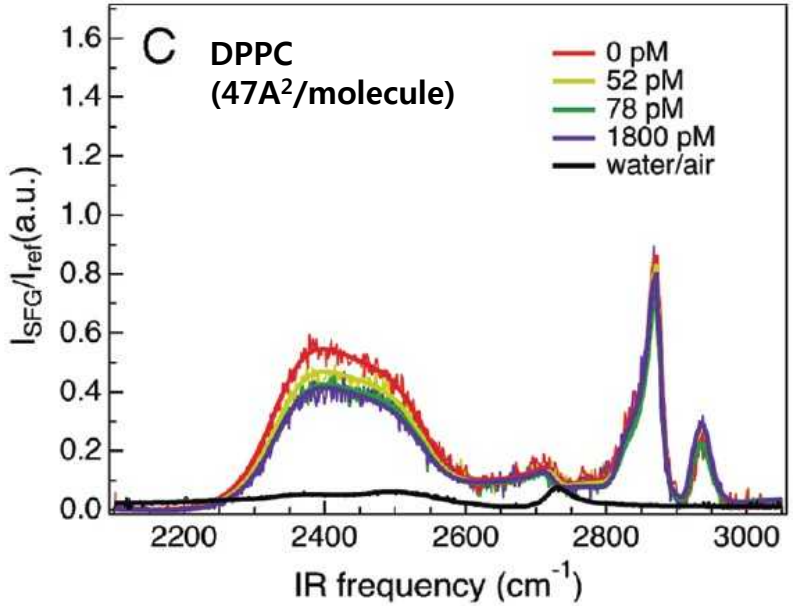


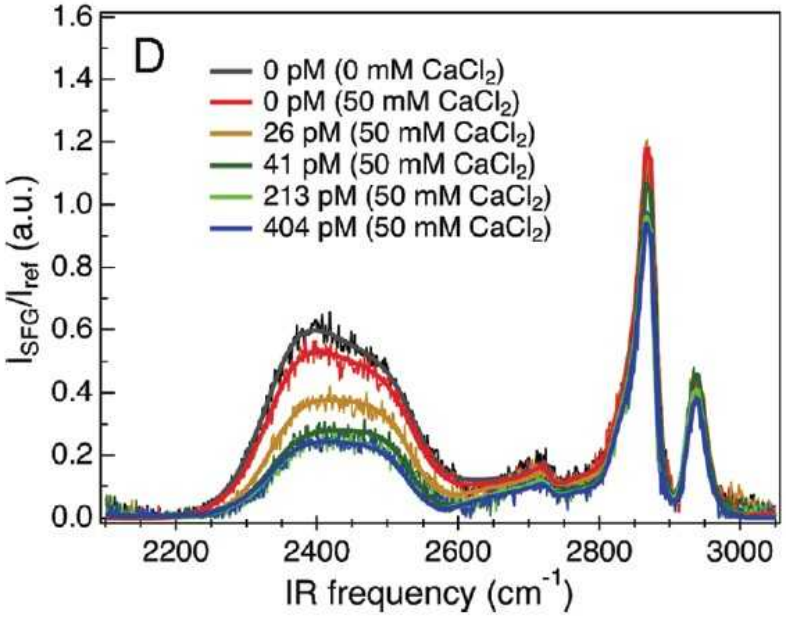
Contribution from weakly hydrogen bonded D_2O
due to DNA adsorption (hydrophobic pocket)

➡ ~100mM of λ-DNA is enough to reduce surface electric field.

➡ Interfacial water structure is changed.

SFG spectra on zwitterionic lipid monolayer





In the case of Zwitterionic lipid, DNA doesn't affect too much.

Ca^{2+} change the headgroups more cationic.

OD stretch intensities

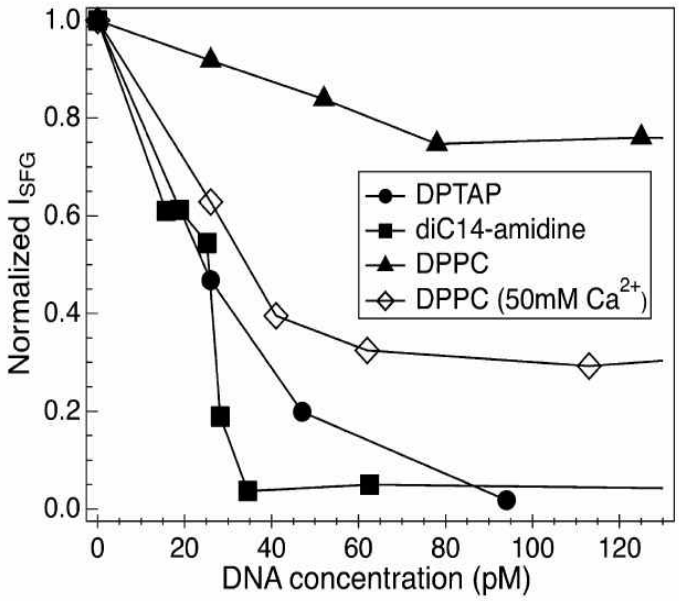


Figure 3. Fitted hydrogen bonded OD stretch intensities (i.e., $(A_n/\Gamma_n)^2$) from data in Figure 2. The hydrogen bonded OD stretch peak is highlighted in the transparent red rectangle in Figure 2A. These results clearly show that, while OD intensity underneath all lipid monolayers decreases with increasing DNA concentration, the change underneath the charged lipids is much larger.

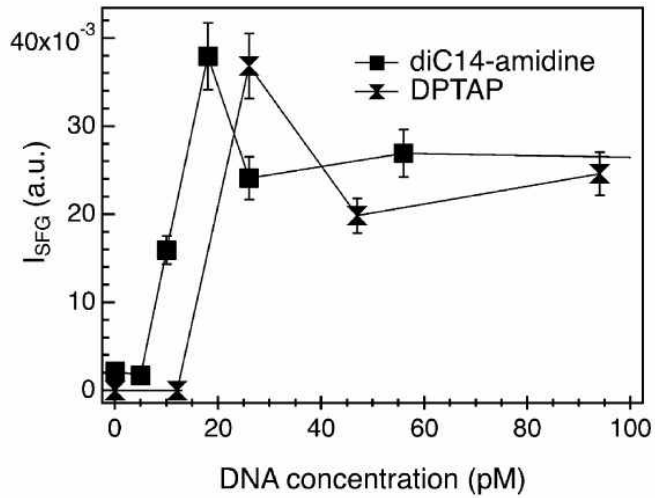


Figure 4. Fitted spectral intensities for 'weak' OD stretch peak for the spectra shown in Figure 2, panels A and B. These results clearly indicate that the intensity of the 'weak' OD stretch peak plateaus above ≈30 pM bulk concentration of DNA.

As DNA concentration increase, hydrogen bonded OD contribution is decreased.

'weakly' bonded OD contribution increase with DNA concentration.

Isotopic dilution

➡ 6.25% of D_2O / 37.5% of HDO / 56.25% of H_2O

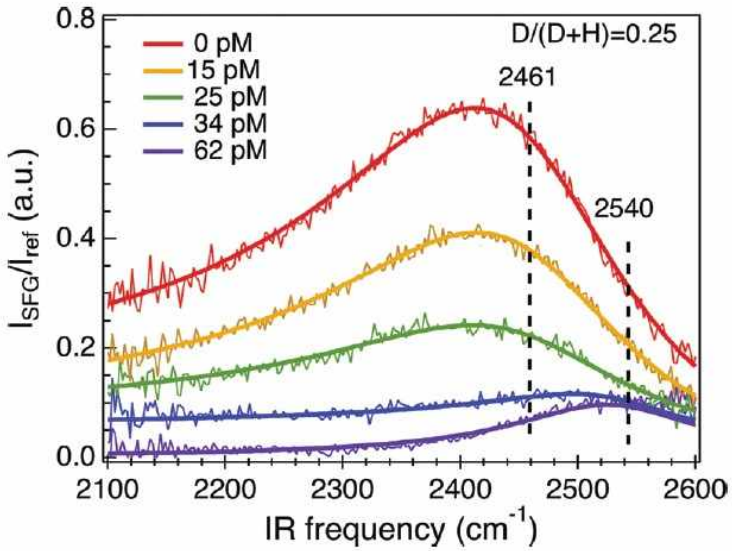
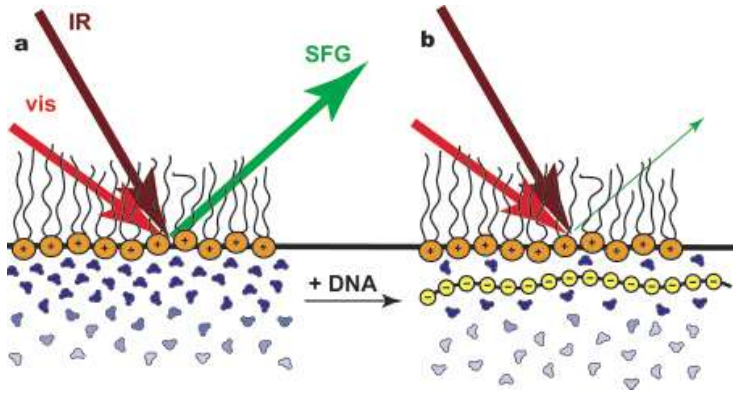


Figure 5. Spectra of the OD stretch frequency window of HOD in H_2O given a phosphate buffered subphase containing increasing concentration of DNA beneath a diC14-amidine monolayer. OD stretch spectra of HOD have only a single peak structure and clearly show that the resonance shifts toward higher frequencies as it decreases in amplitude with increasing DNA concentration.

➡ Resonance frequency of hydrogen bonded OH is also changed in high DNA concentration.



Structural change of monolayer with DNA

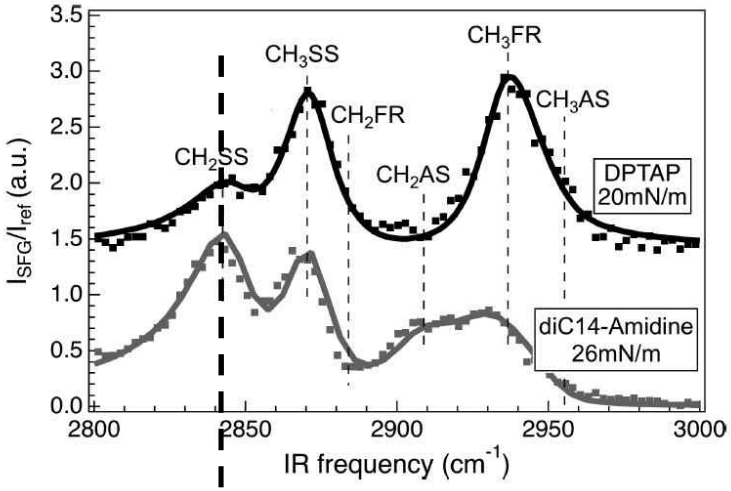
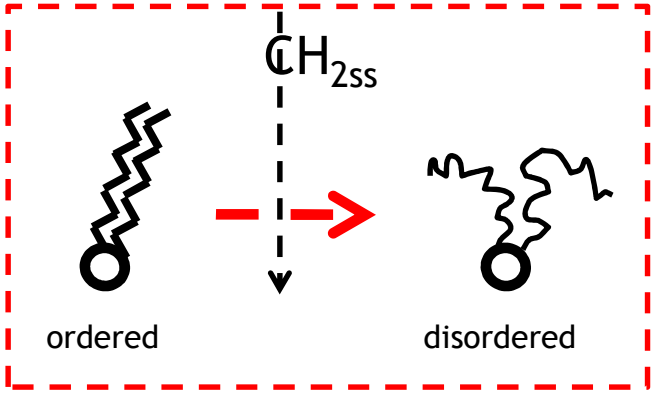


Figure 6. SFG spectra (dots) of diC14-amdine at 26 mN/m (gray) and DPTAP (black) at 20 mN/m. The vertical lines indicate the positions of methylene symmetric stretch (CH_2SS), methyl symmetric stretch (CH_3SS), methylene asymmetric stretch-Fermi resonance (CH_2FR), methylene asymmetric stretch (CH_2AS), methyl symmetric stretch-Fermi resonance (CH_3FR) and methyl asymmetric stretch (CH_3AS). The DPTAP spectrum is offset for clarity. The solid curves are fits to the data using a Lorentzian model.



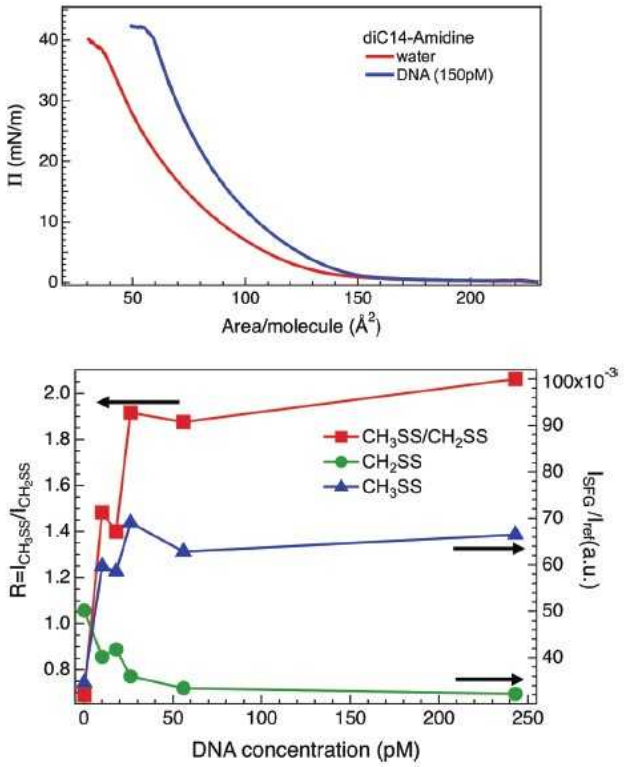


Figure 7. (Upper panel) $\pi-$Area isotherms for a diC14-amidine monolayer above a phosphate buffered subphase in the absence (red line) and in the presence (blue line, 150 pm) of DNA. For a given lipid density, the addition of DNA to the subphase results in an increase in surface pressure, or equivalently, a condensation of lipid molecules. (Lower panel) VSF intensity of CH_2SS and CH_3SS resonances (triangle and circle, right axis) and their ratio R (squares, left axis) of diC14-amidine monolayer on DNA solution with various concentrations. The solid lines are guides to the eye.