Water at biological membranes: structure, dynamics and biomolecular sensing

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Water at biological membranes: structure, dynamics and biomolecular sensing

Outline

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Structure

Sensing

Structure??

Dynamics

Structure from dynamics

Conclusions

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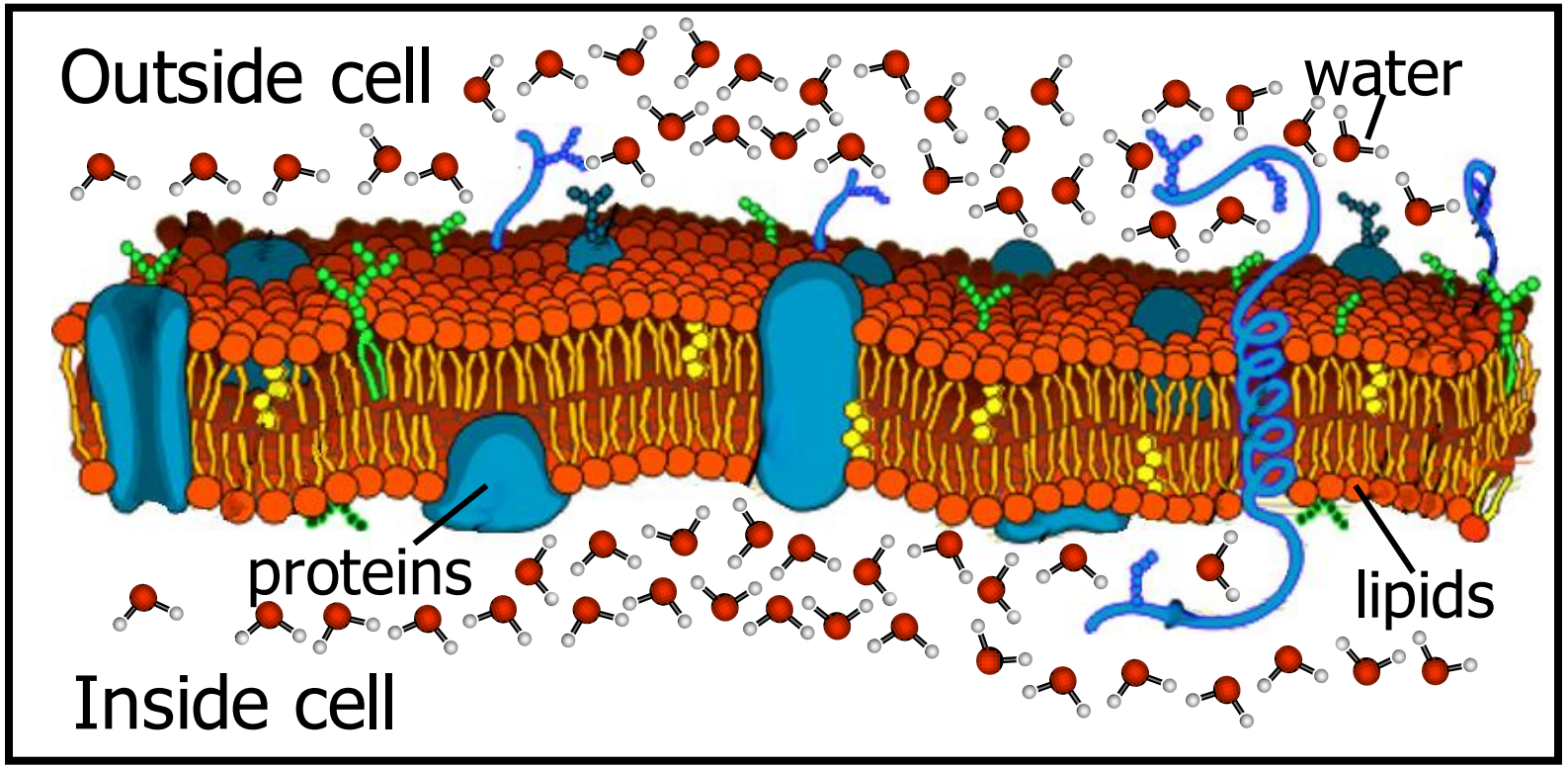
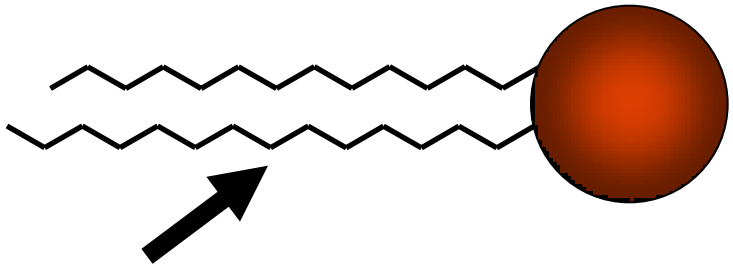
Biological membranes



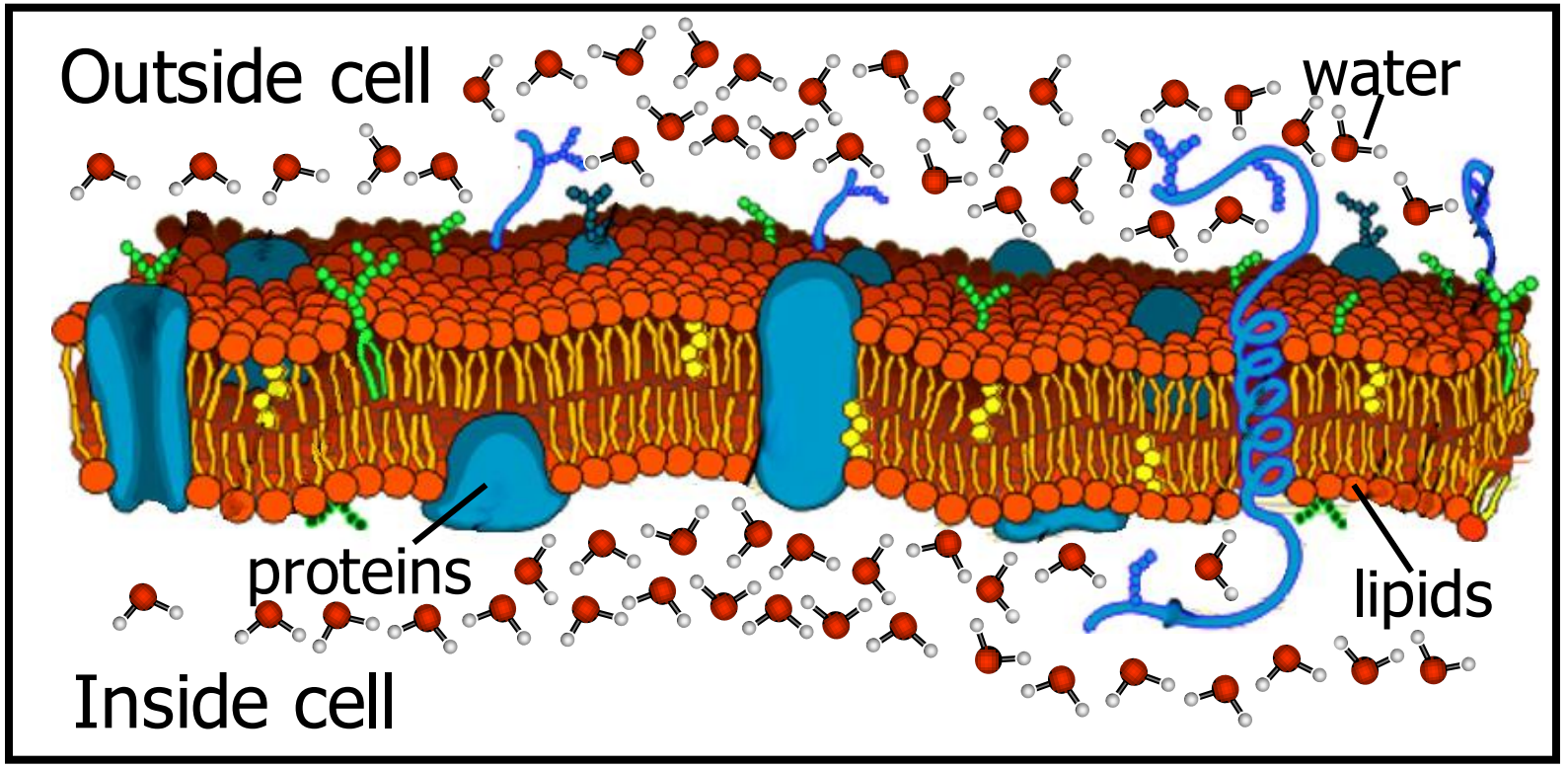


Phospholipids: building block of cell membrane

hydrophobic alkyl chains

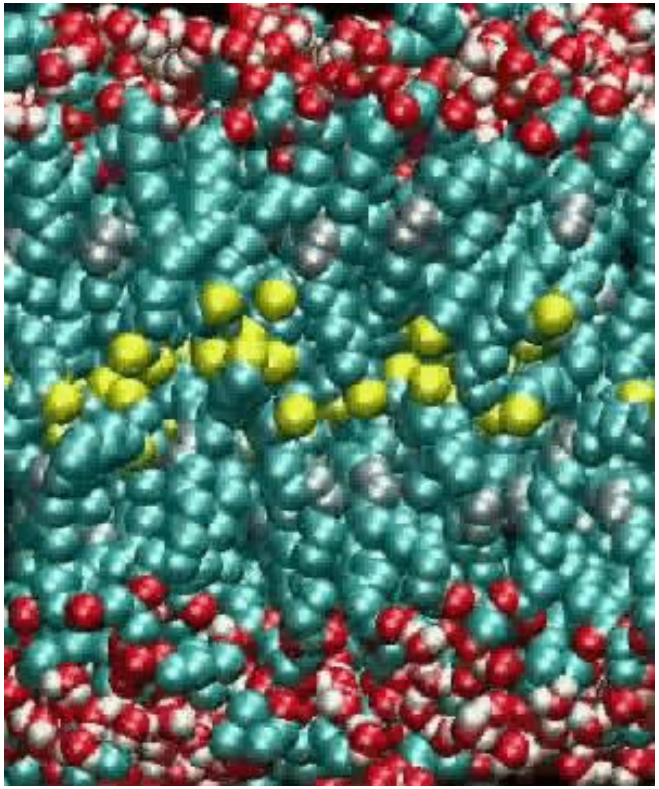
hydrophilic "polar" head group

Biological membranes



- Important biomolecular interactions at cell membrane
- Functionality = interplay between lipids/proteins/water

Membrane-bound water



Most information on membrane-bound water has been obtained through MD simulations:

- residence time: exchange with bulk on ps-ns timescales
- preferential orientation
- heterogeneity??

MD simulation (~100 ps) Schulten group UIUC

Membrane-bound water

- The one-molecule thick layer of water (~3 Å) at the membrane
- Many biological reactions happen at membrane surfaces: structure and dynamics of membrane-bound water important for these processes

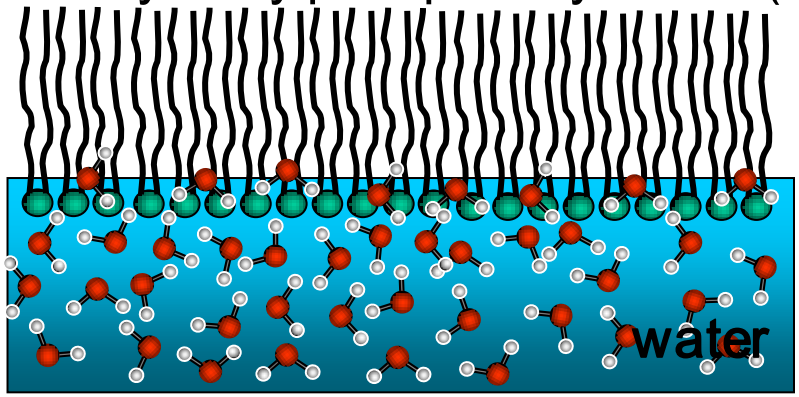
the challenge

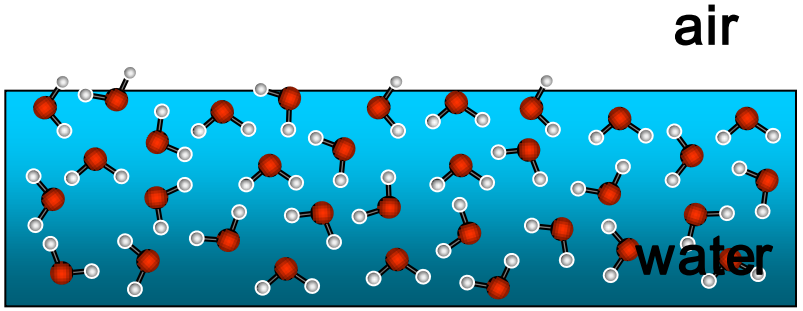
Direct probing of membrane-bound water

Model system: Lipid monolayer on water

- Self-assembled monolayers of lipids: good membrane model
- Compare lipid-water interface with air-water interface: distinguish effects due to termination of bulk from lipid-specific effects

Dimyristoylphosphatidylserine (DMPS)

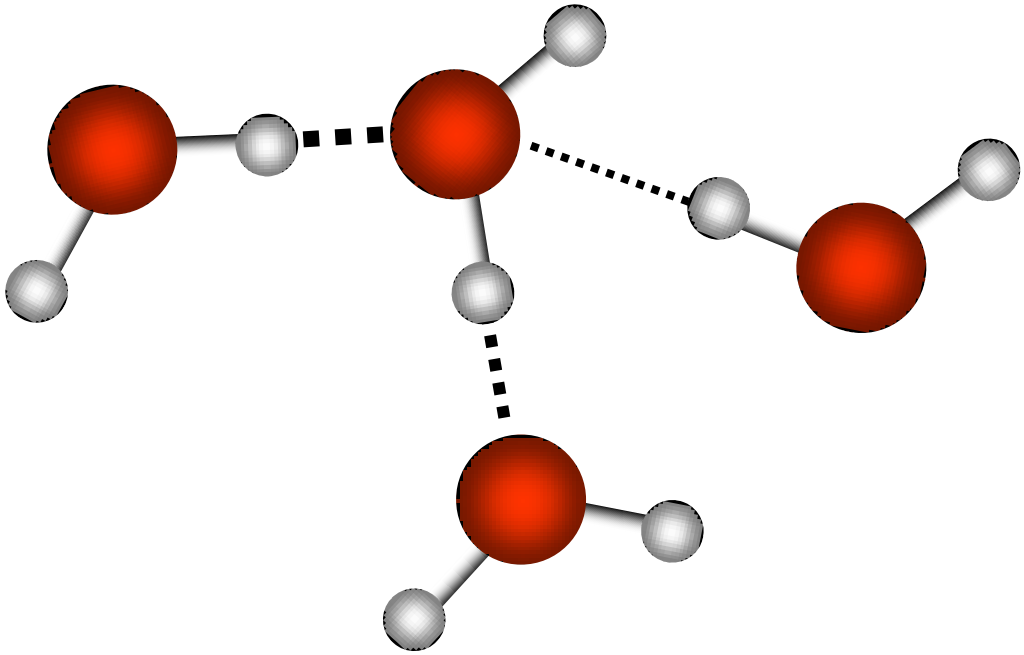


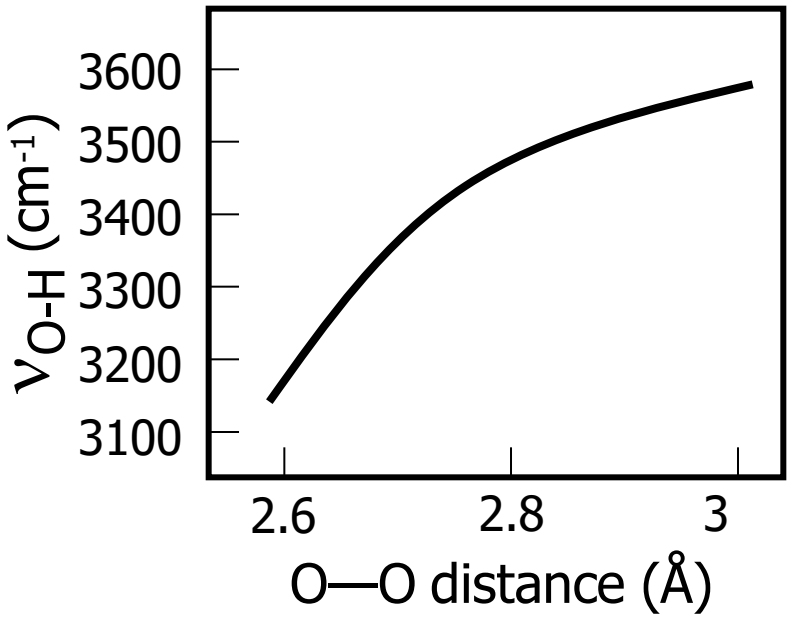


How to investigate structure of 1 ML water?

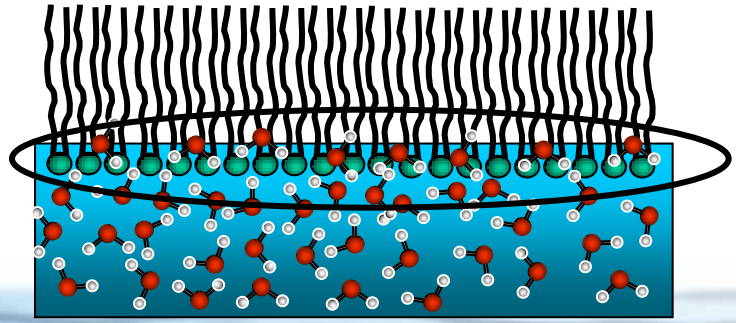
How to investigate water structure?

Water displays strong variation in H-bond strengths, which affect O—H stretch vibration





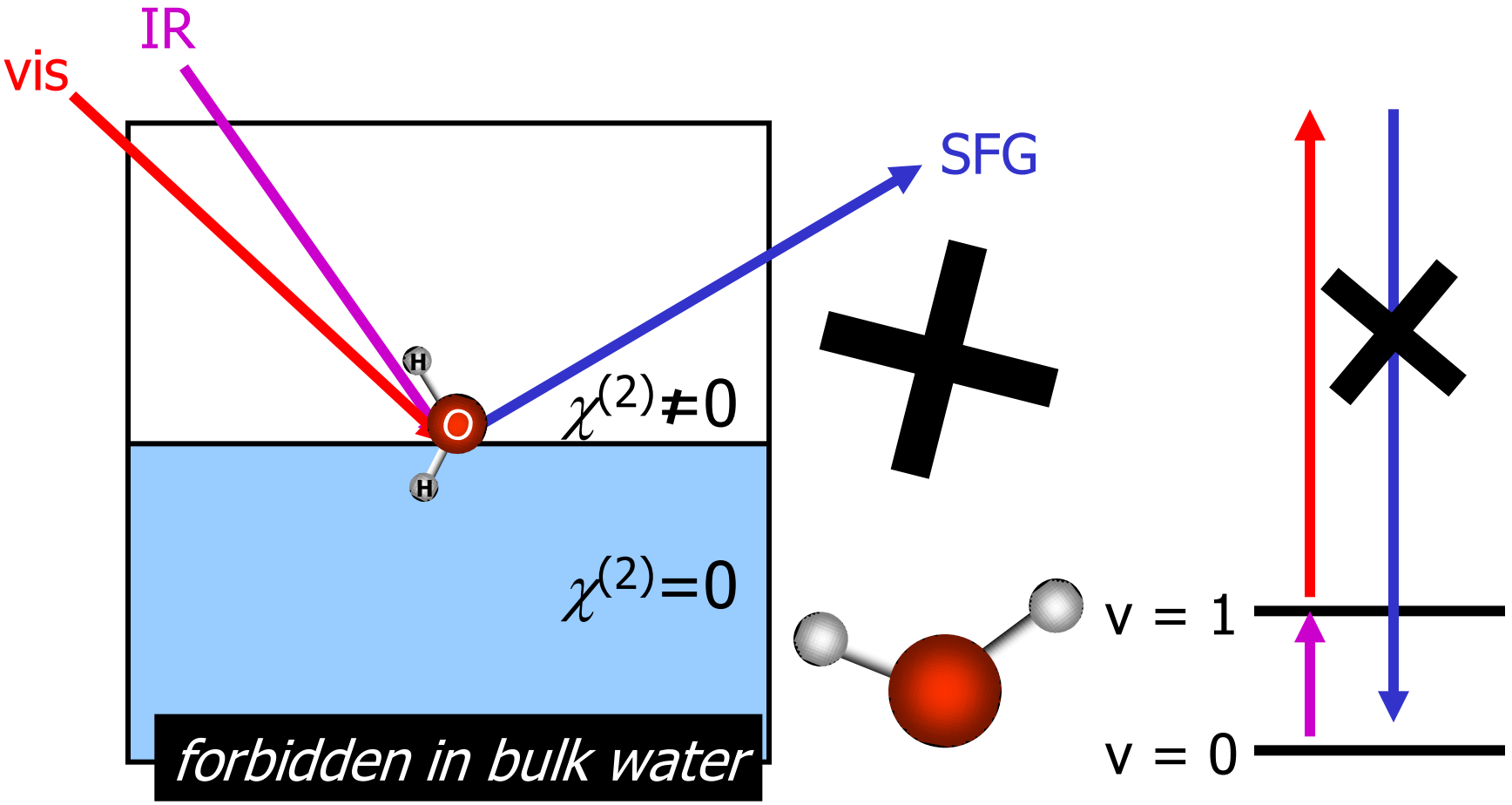
O—H stretch vibration is a marker of local water environment



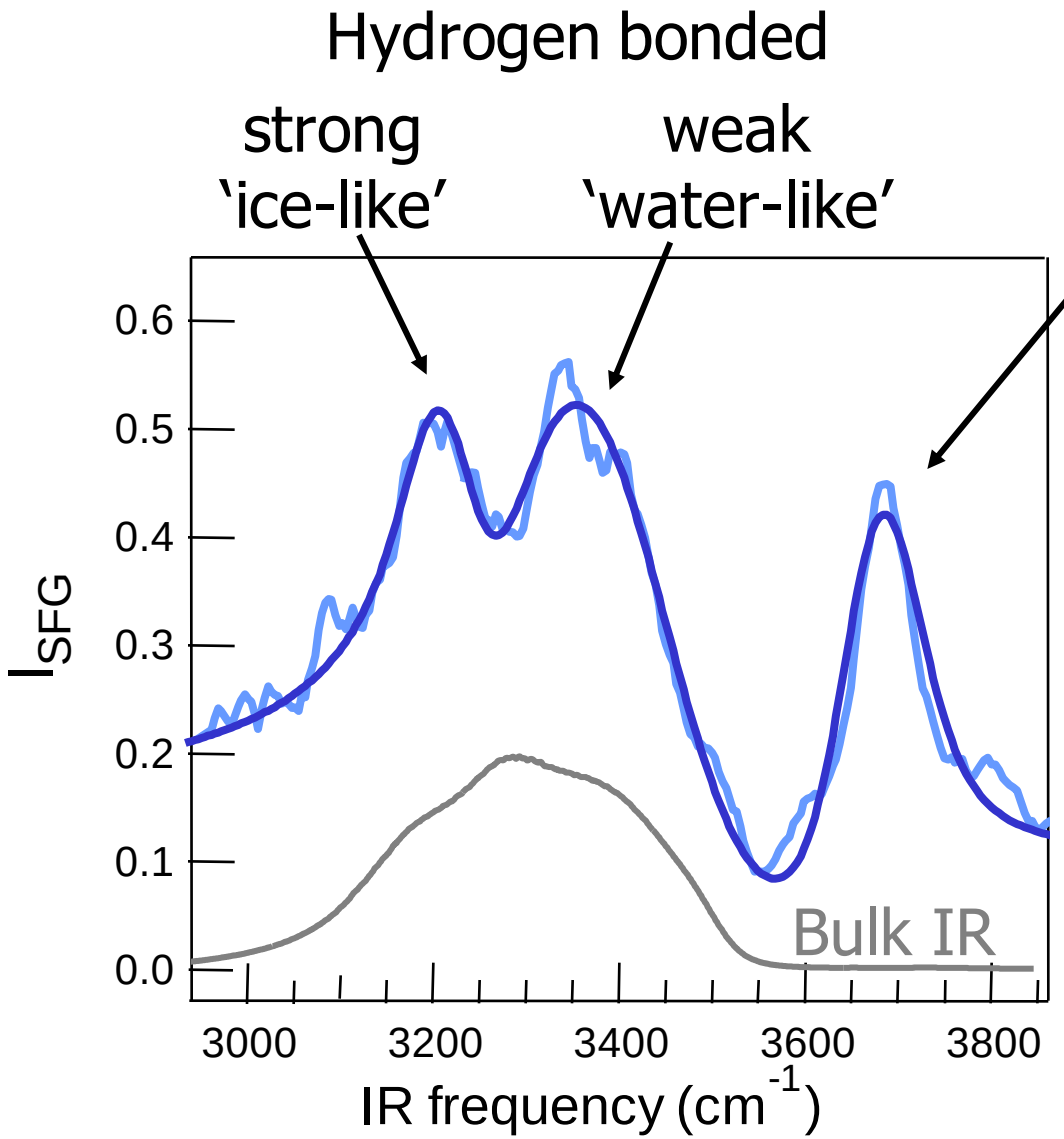
How to detect ONE MOLECULAR layer of water?

Sum Frequency Generation (SFG)

provides vibrational spectrum of surface monolayer



SFG spectrum of water/air interface vs bulk IR spectrum



OH-stretch vibrations of H_2O

Water at biological membranes: structure, dynamics and biomolecular sensing

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Structure from dynamics

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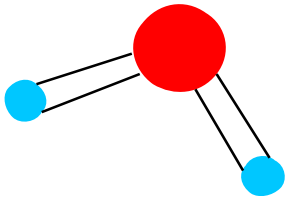
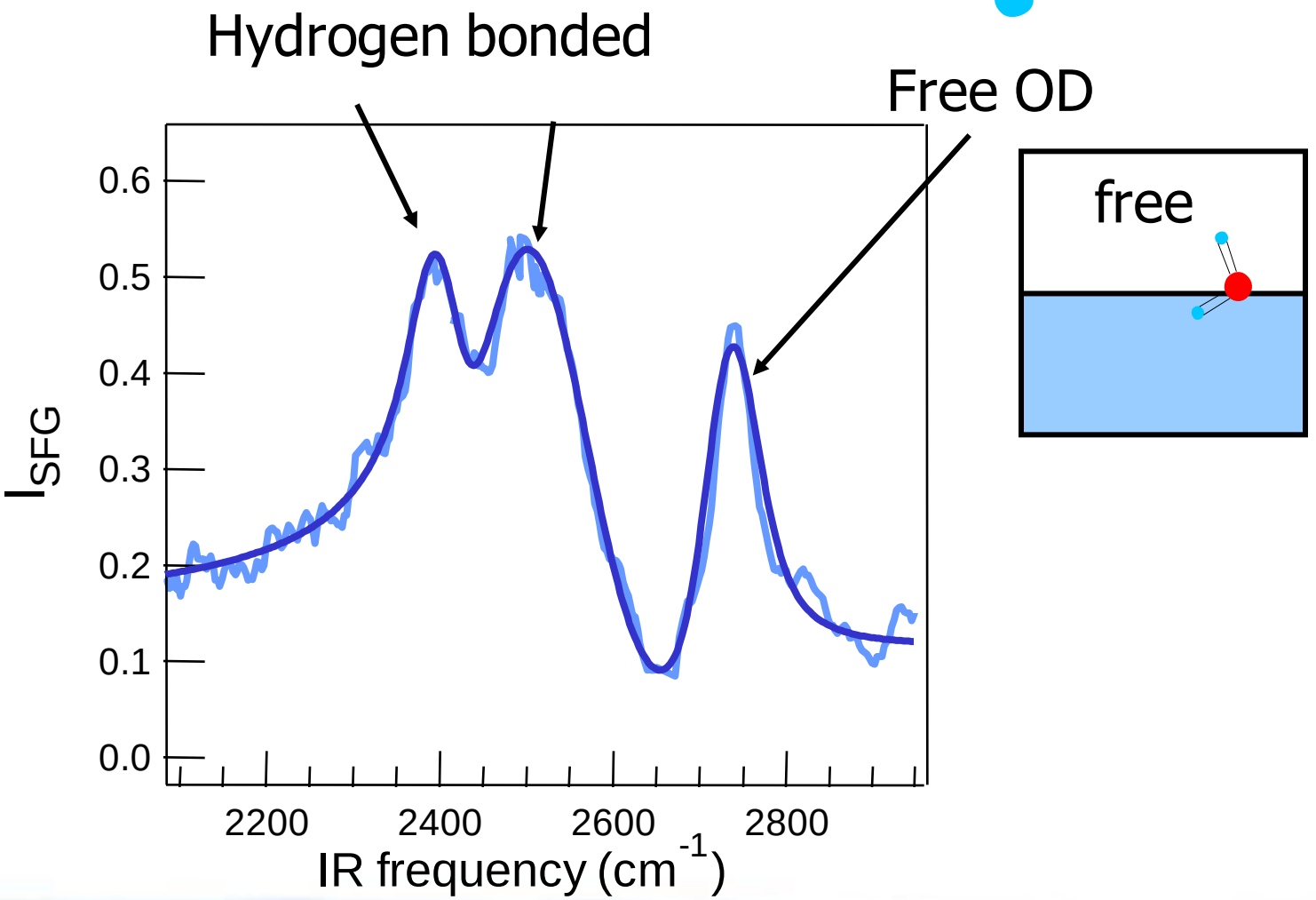
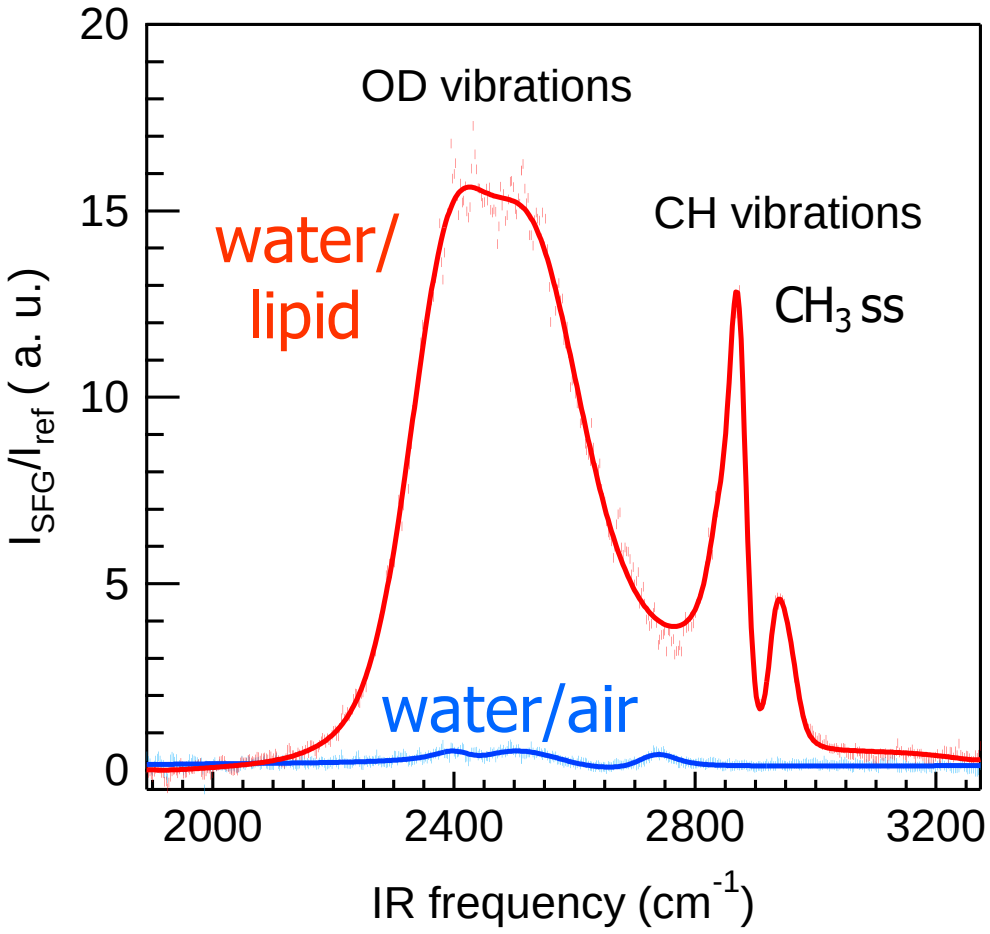
SFG spectrum of the Air-D_2O Interface

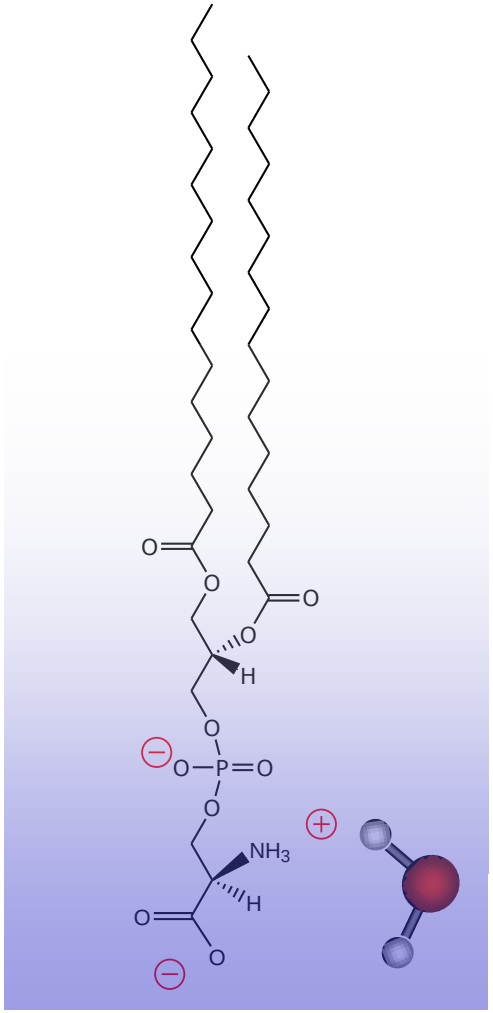
OH-stretch vibrations of D_2O





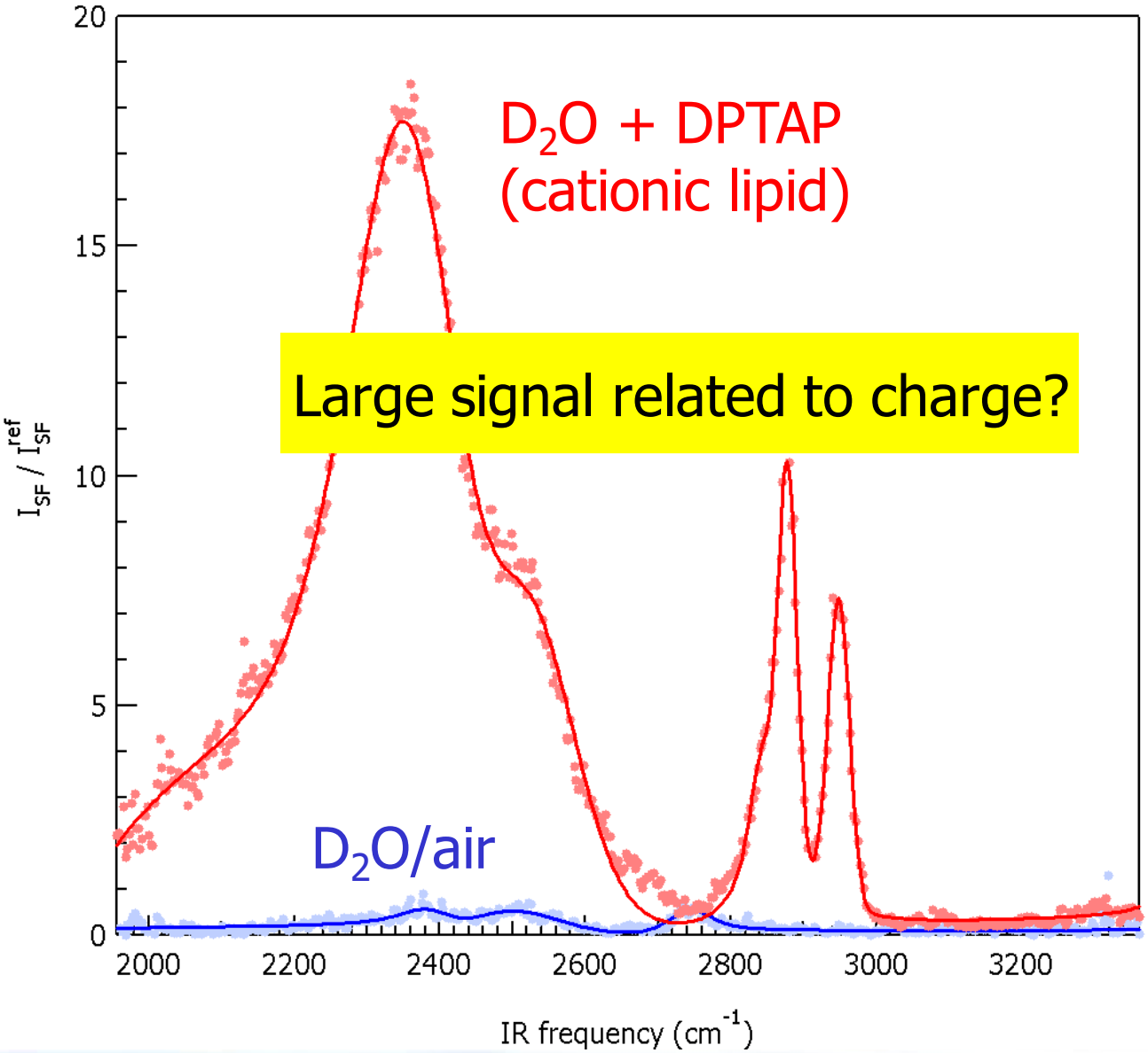
SFG spectrum of D_2O-lipid interface

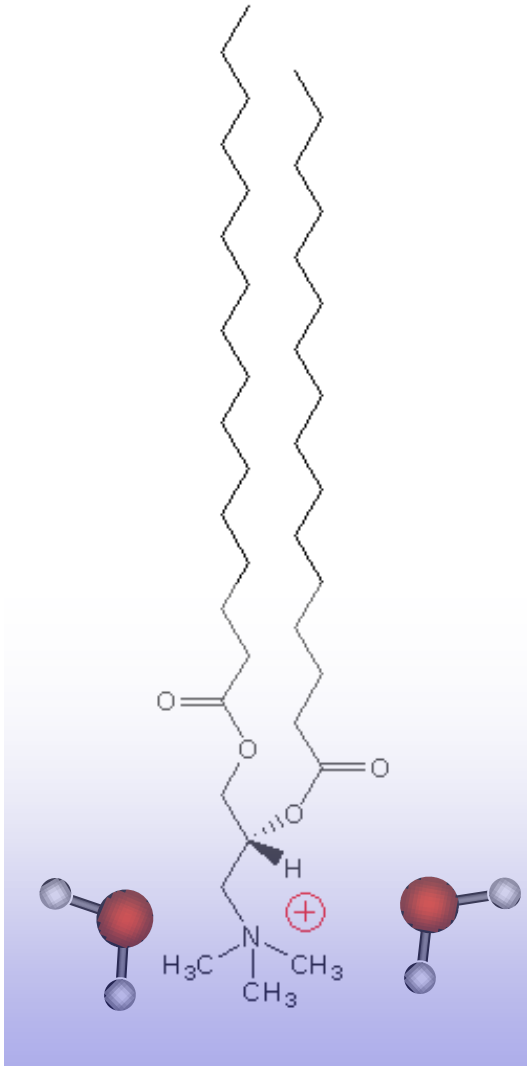




Why is the signal so much larger at the water-lipid interface?

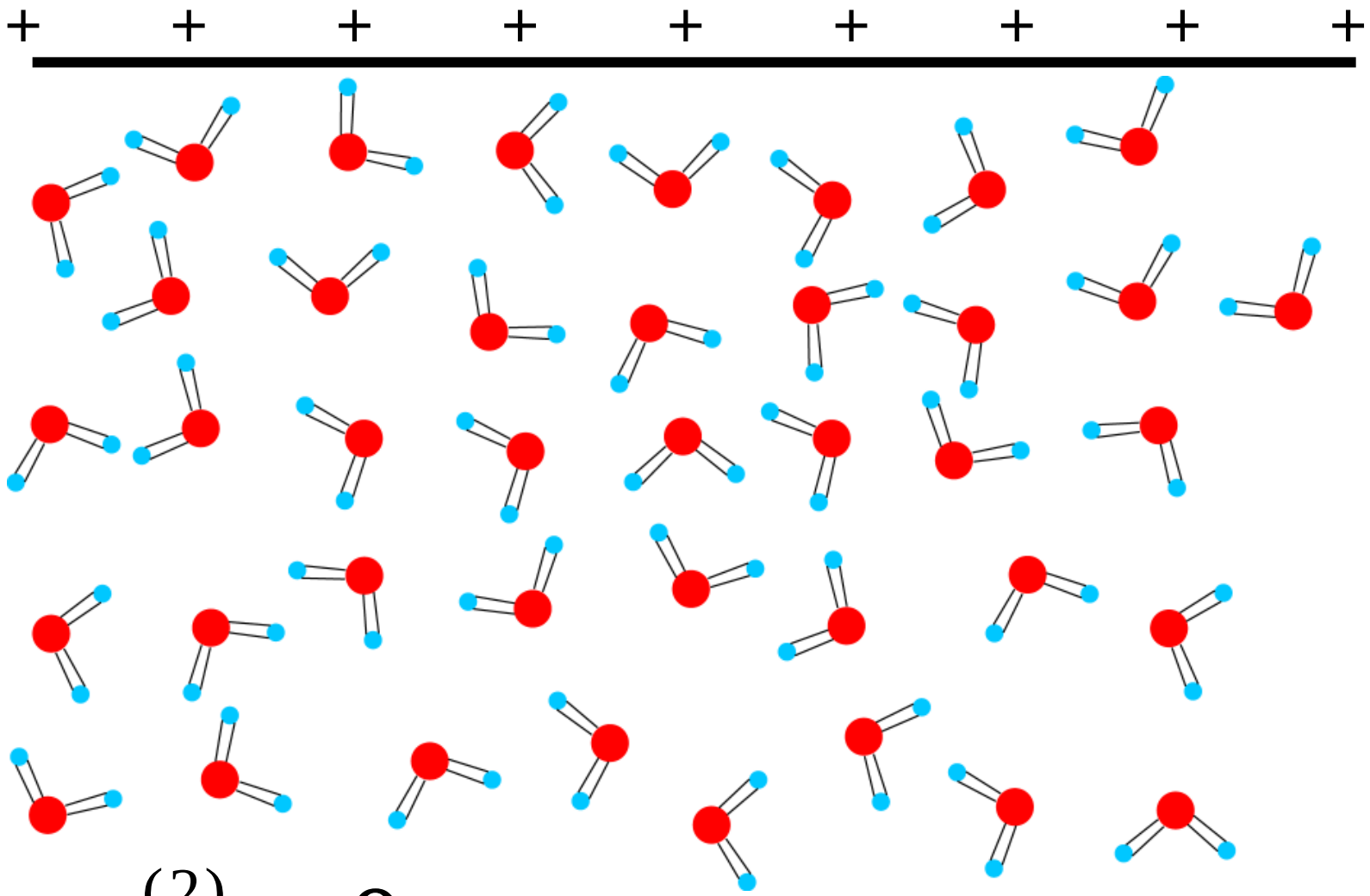
Large signal also observed for cationic lipid monolayer





dipalmitoyl trimethyl ammonium propane

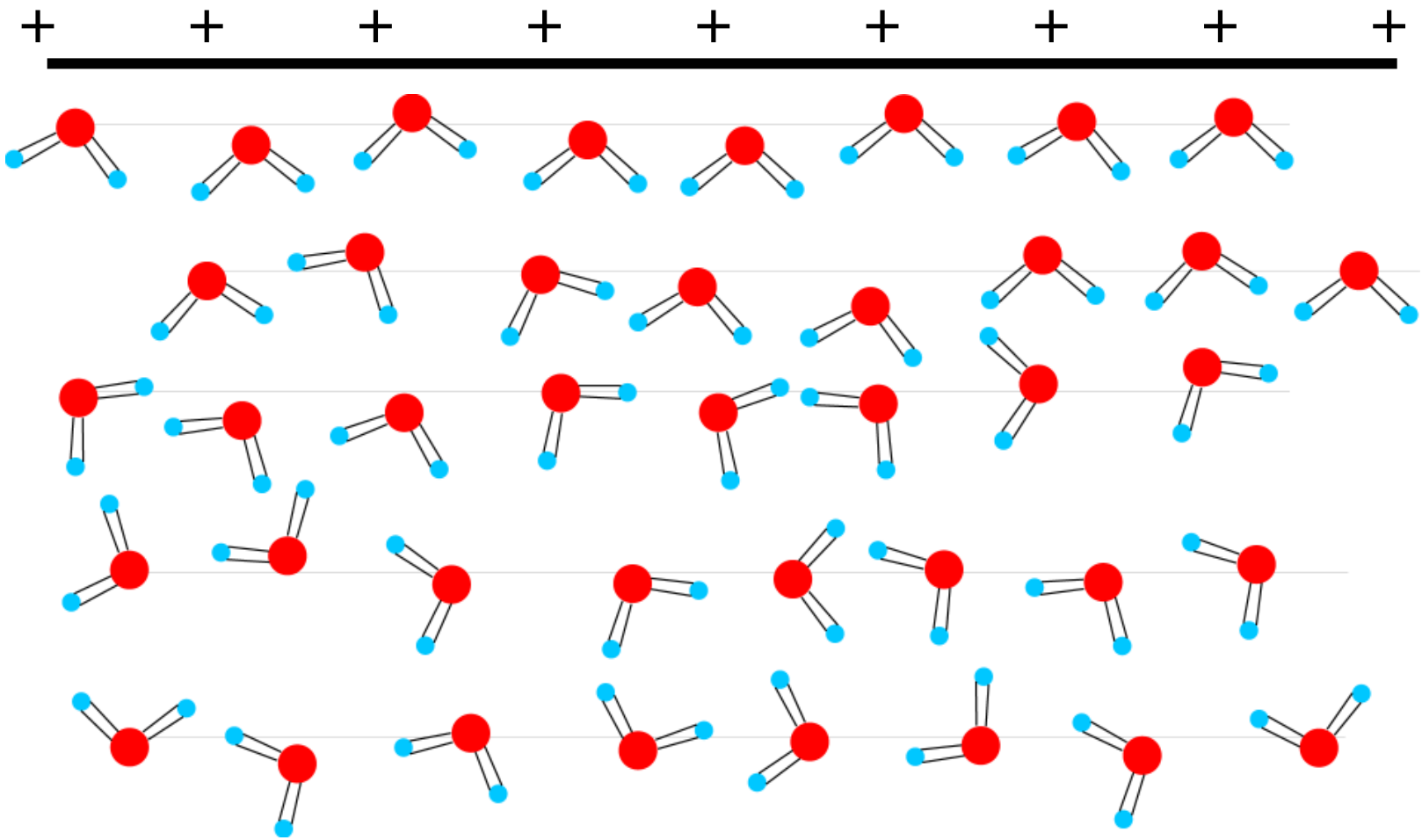
Water Alignment



$$\chi^{(2)} = 0$$

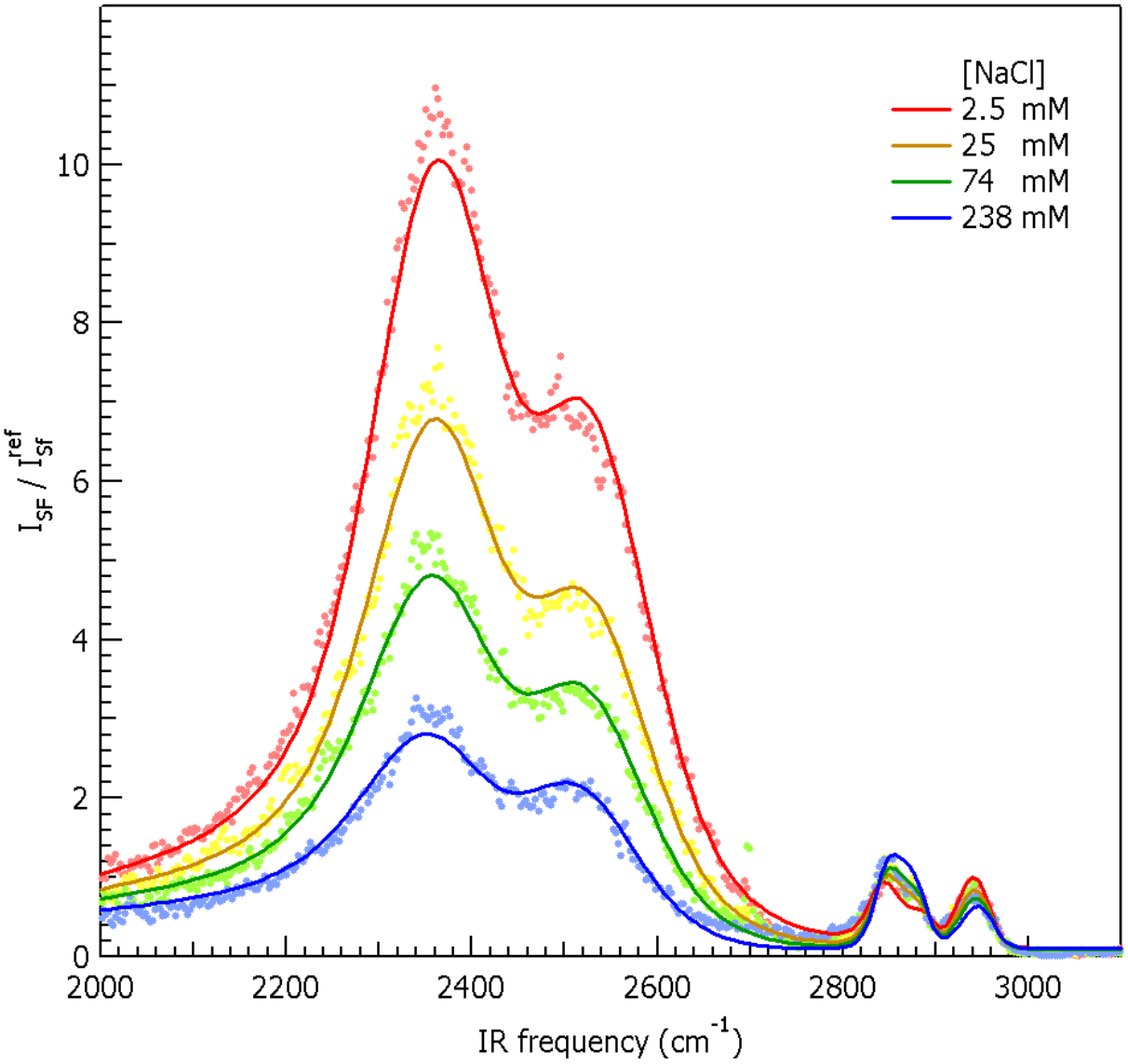
In centro-symmetric environment (bulk water)

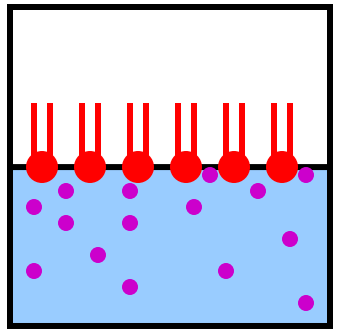
Water Alignment

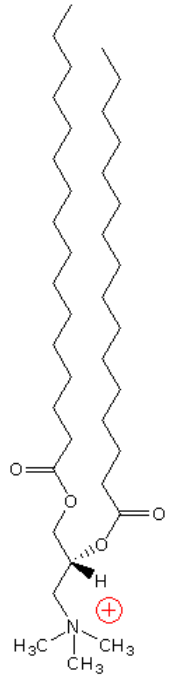


$\chi^{(2)} \neq 0$ in headgroup region due to water alignment → large SFG signal!

Adding NaCl Lowers Water Signal



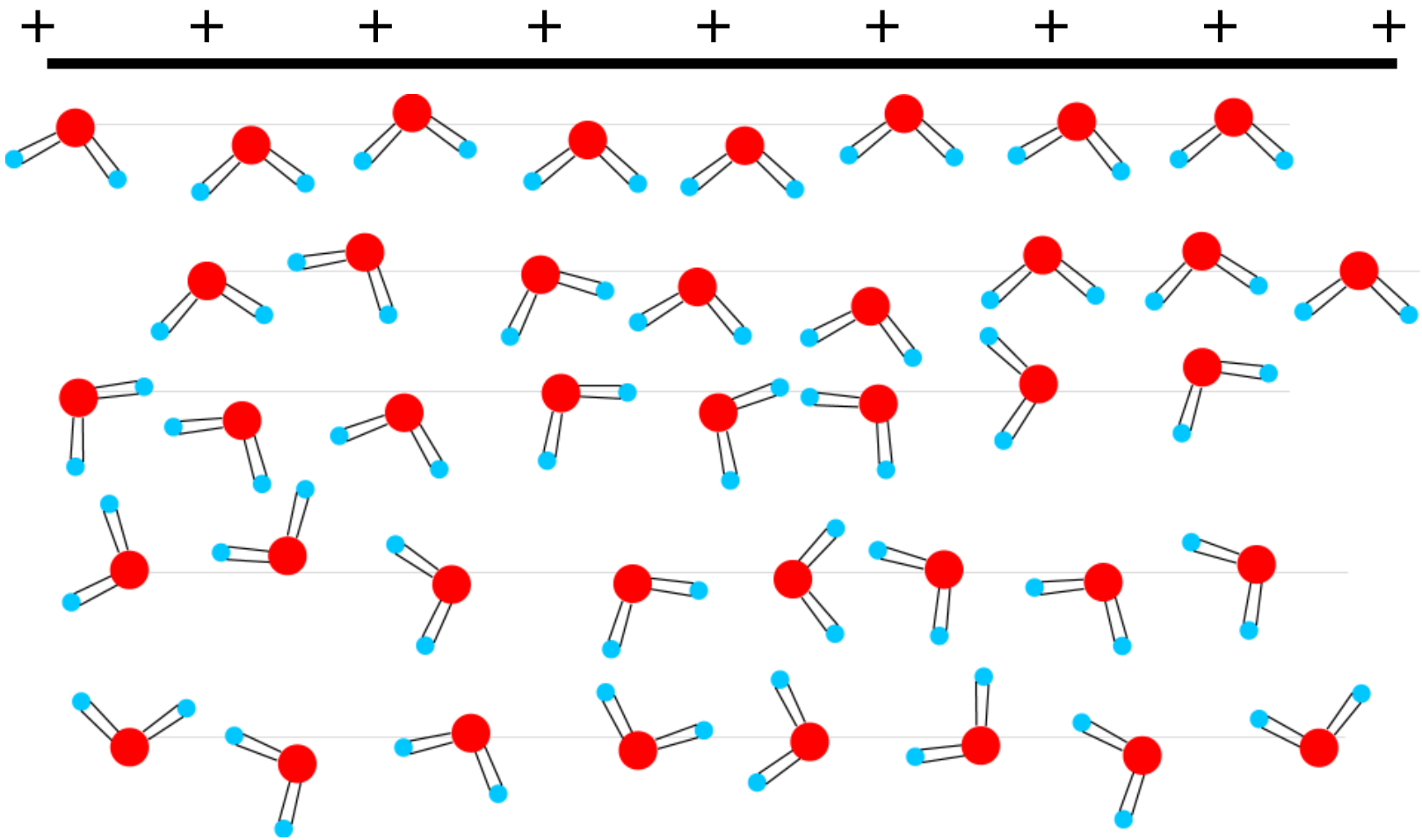




+

NaCl

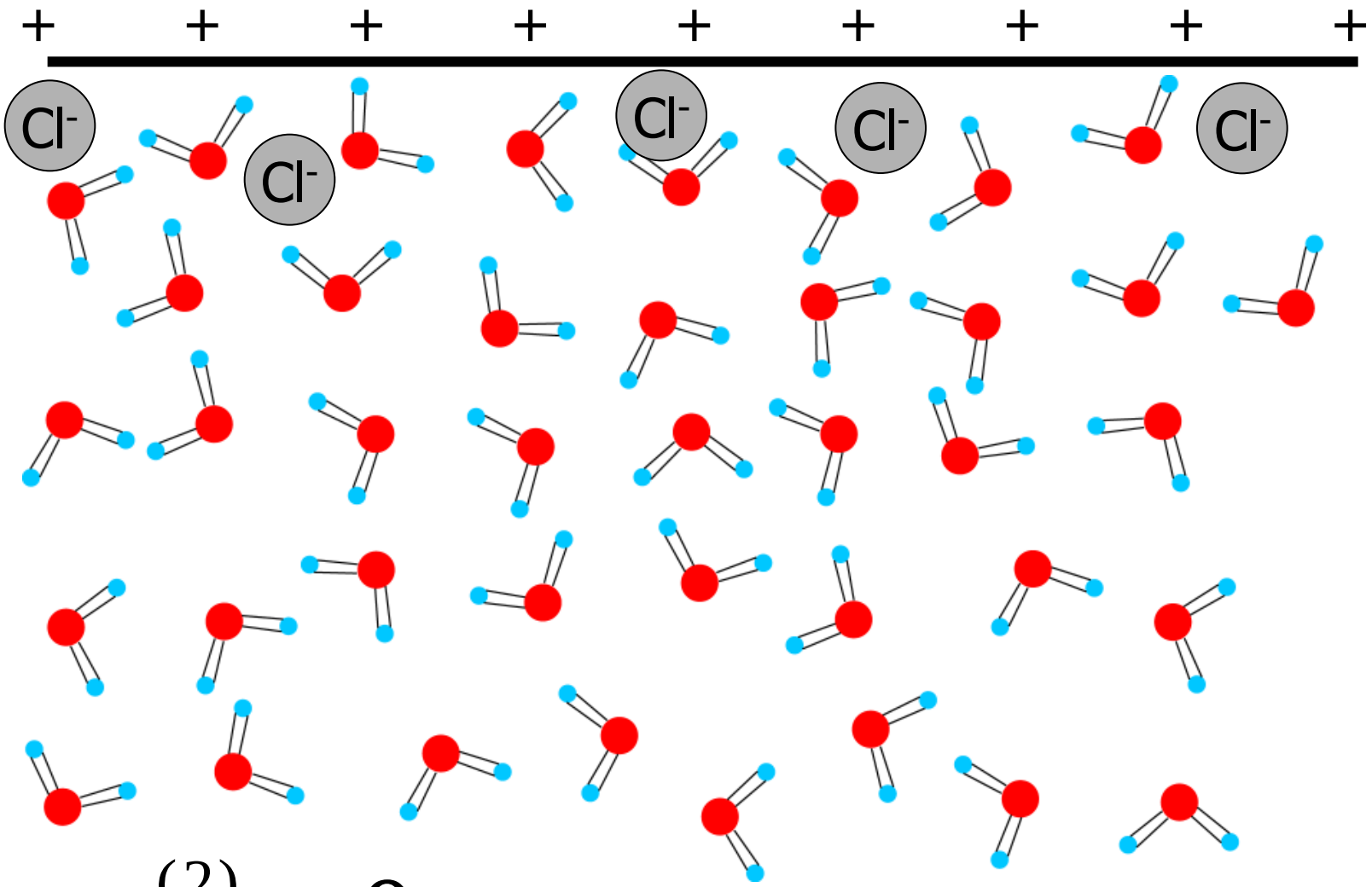
Water Alignment



$$\chi^{(2)} \neq 0$$

in headgroup region due to water orientation → large SFG signal!

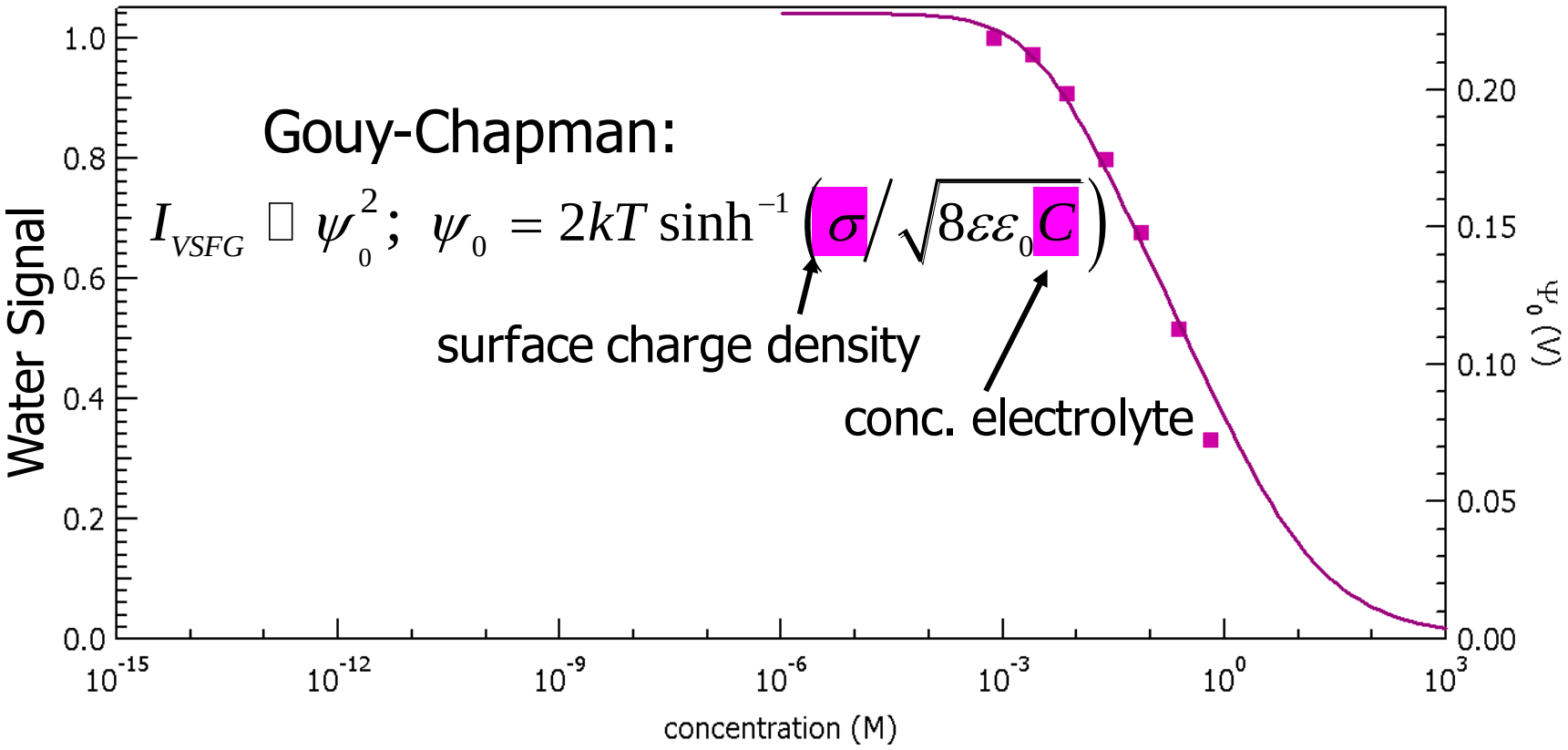
Water Alignment



$$\chi^{(2)} = 0$$

Lipid field is shielded by Cl^- ions: disorder is restored

Water Signal Depends on Surface Potential



We can use the water signal for DNA detection:

DNA is a poly-electrolyte

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We can use the water signal for DNA detection

- Important for life sciences, forensic and medical diagnostics
- Most common approach: *labeling* with (fluorescent) marker → optical detection with high sensitivities, but requires extensive (bio-)chemical treatment.

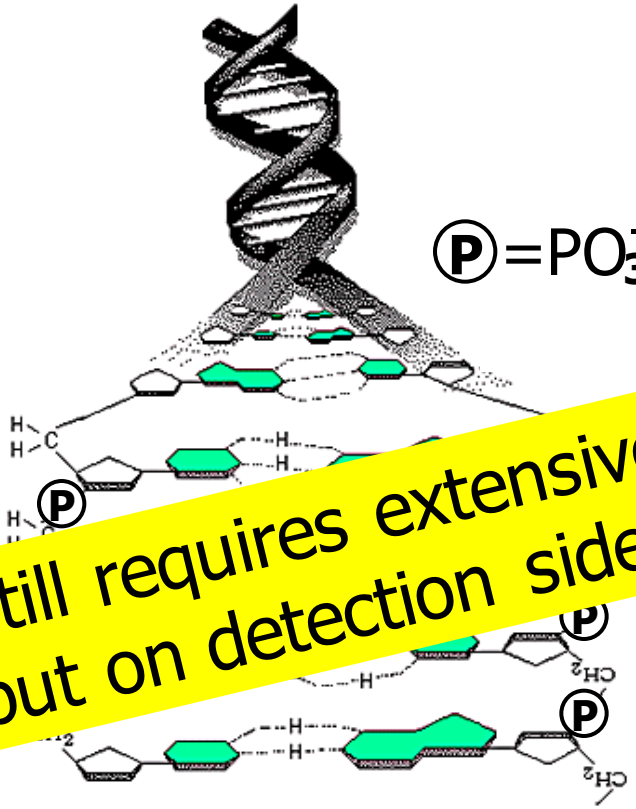
→ much interest in label-free DNA detection schemes

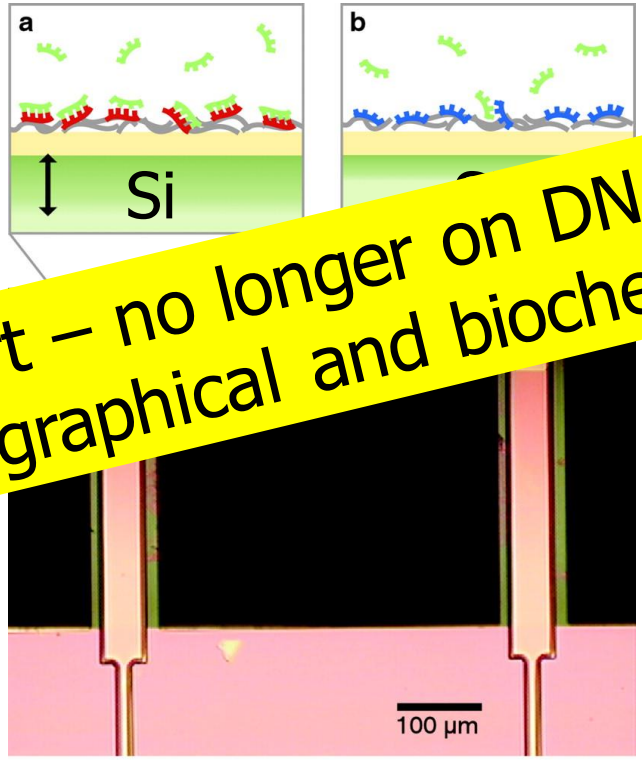
Label-free DNA detection schemes

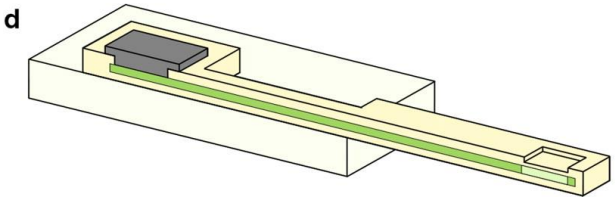
publication	approach	sensitivity	detection
Fritz, 2002 PNAS **99,** 14142	lithography	1 nM	electronic
Pouthas, 2004 APL **84,** 1594	nano-lithography	10 µM	electronic
Hahm, 2004 Nano Lett. **4,** 51	nanowires	10 fM	electronic
Star, 2006 PNAS **103,** 1594	carbon nanotubes	1 nM	electronic

General principle of label-free schemes

Field-effect transistor-type geometries that rely on changes in surface fields due to adsorption of ***anionic*** DNA







Still requires extensive effort – no longer on DNA side, but on detection side (lithographical and biochemical)

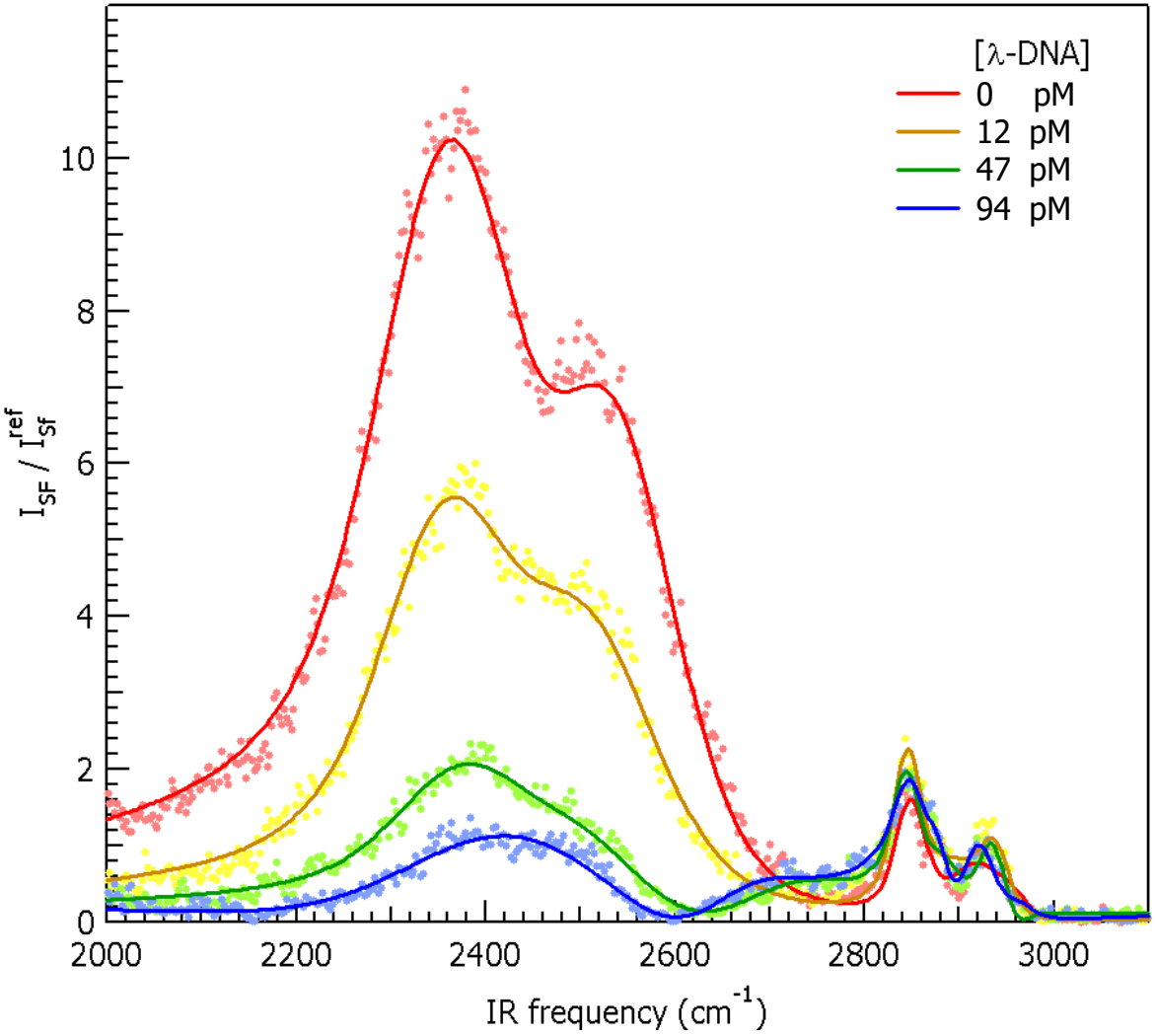
Fritz, PNAS **99,** 14142 (2002).

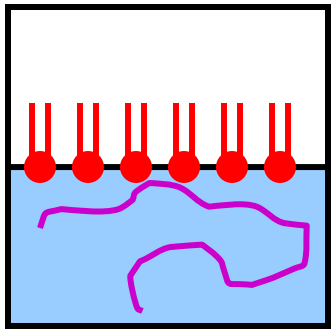
Our scheme

- Also employ poly-anionic character of DNA
- Detect optically – not electrically – using *water* as the reporter for the presence of DNA

Adding DNA

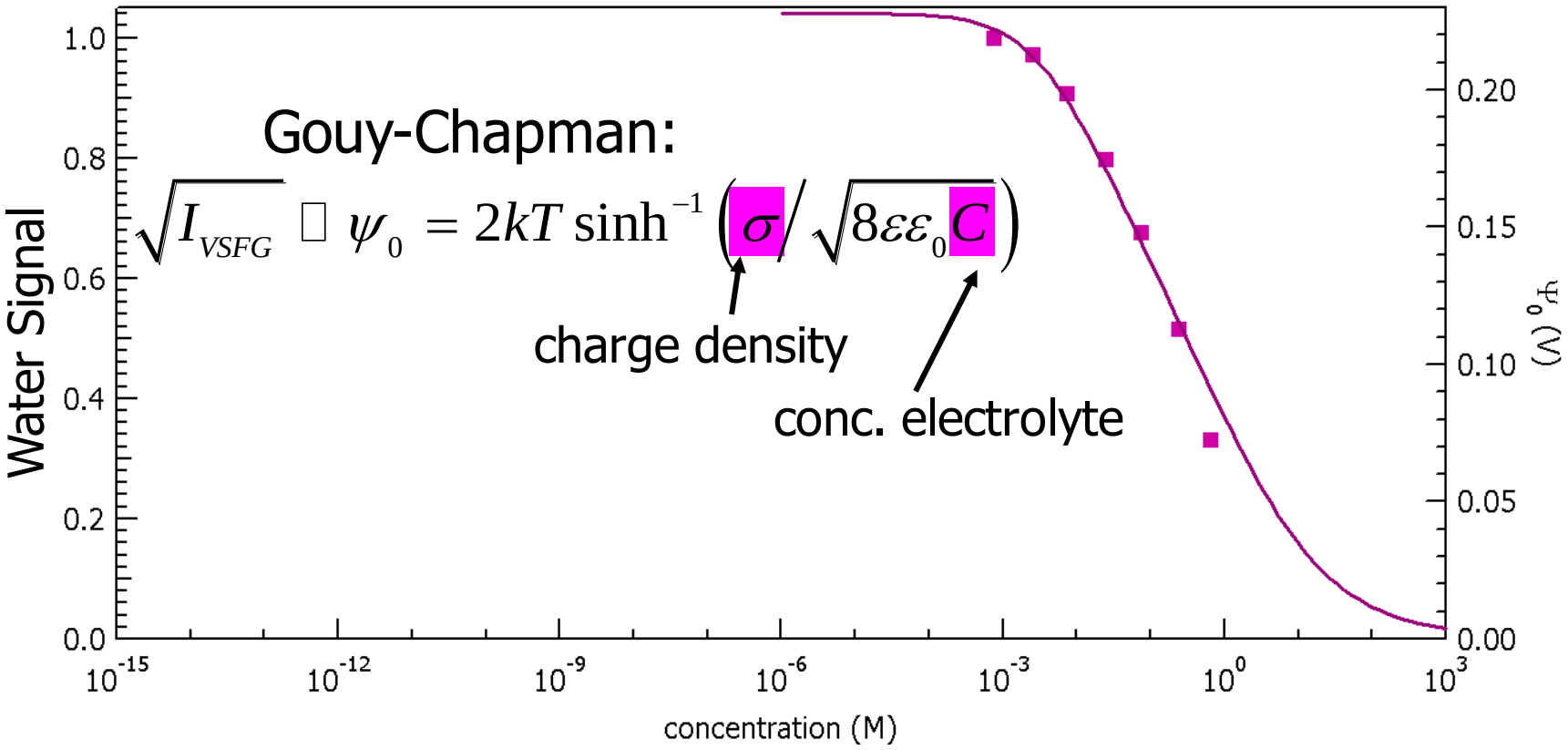
a polyanion





λ-DNA:
48502 bp

Water Signal Depends on Surface Potential

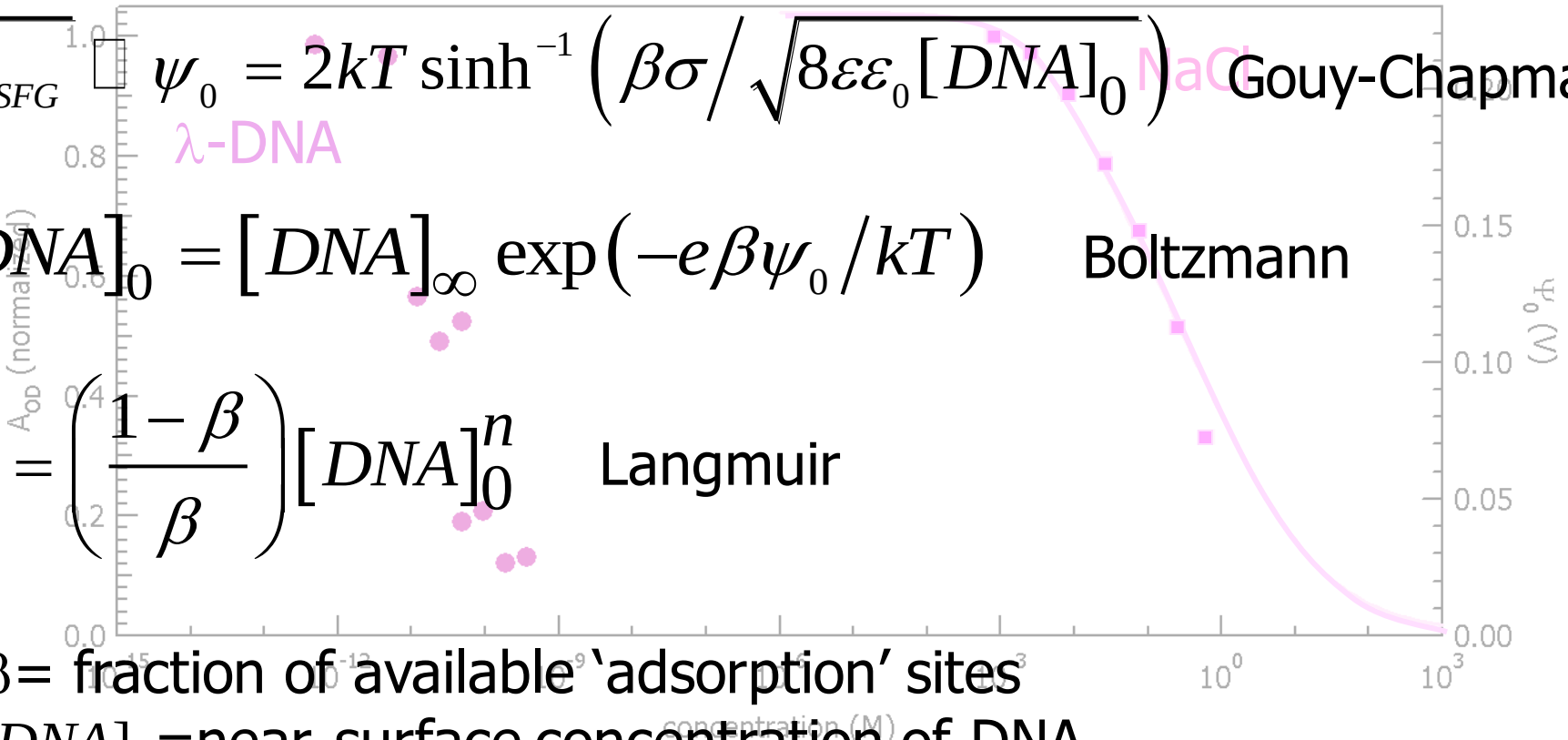


DNA Concentration Dependence: picomolar sensitivity!

$$\sqrt{I_{SFG}} \propto \psi_0 = 2kT \sinh^{-1}\left(\beta\sigma \Big/ \sqrt{8\varepsilon\varepsilon_0 [DNA]_0}\right)$$ Gouy-Chapman

$$[DNA]_0 = [DNA]_\infty \exp\left(-e\beta\psi_0 / kT\right)$$ Boltzmann

$$K = \left(\frac{1-\beta}{\beta}\right)[DNA]_0^n$$ Langmuir



β= fraction of available 'adsorption' sites

$[DNA]_0$=near-surface concentration of DNA

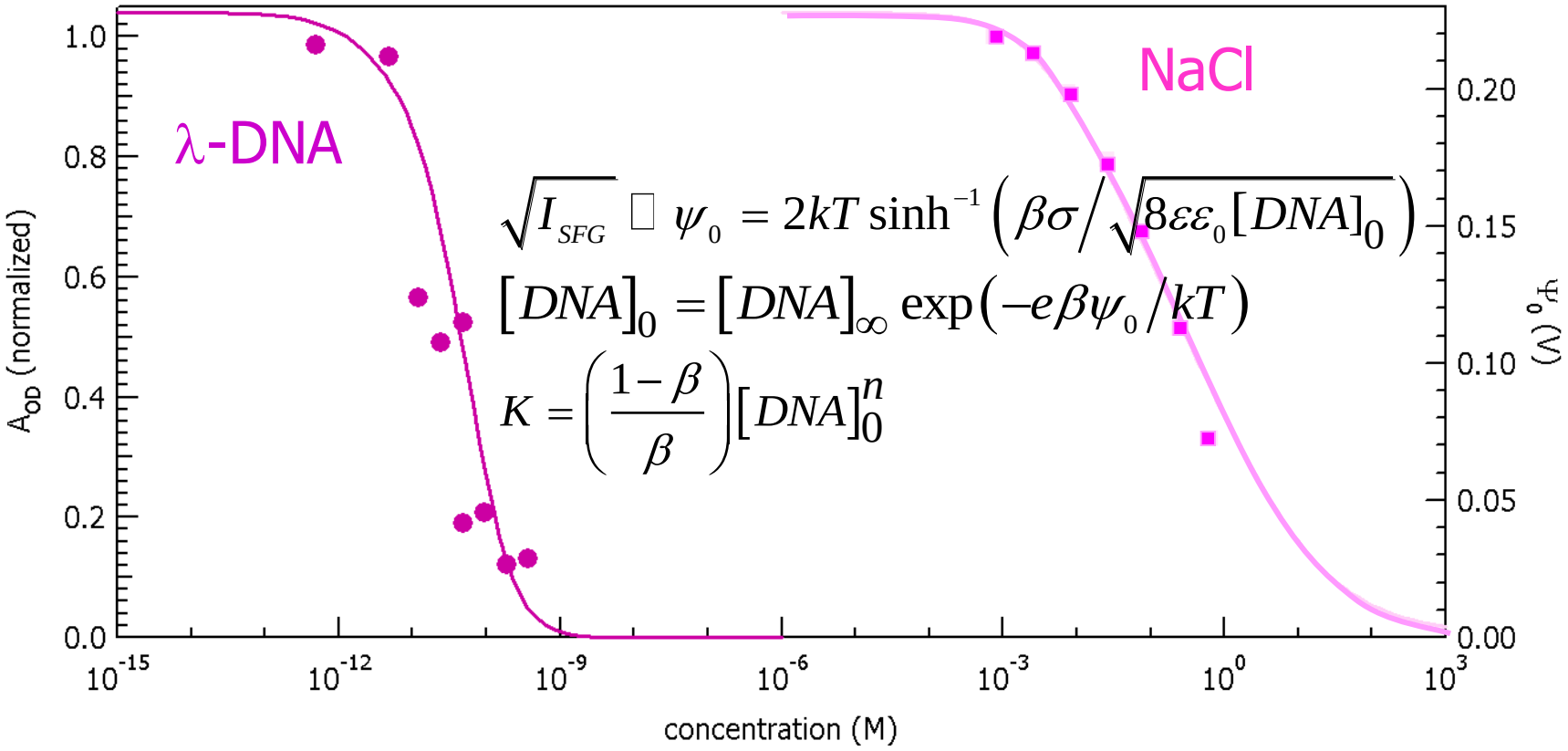
$[DNA]_\infty$=bulk concentration of DNA

K=association constant

n= cooperativity constant

Following Miranda, CPL 1998

DNA Concentration Dependence: picomolar sensitivity!

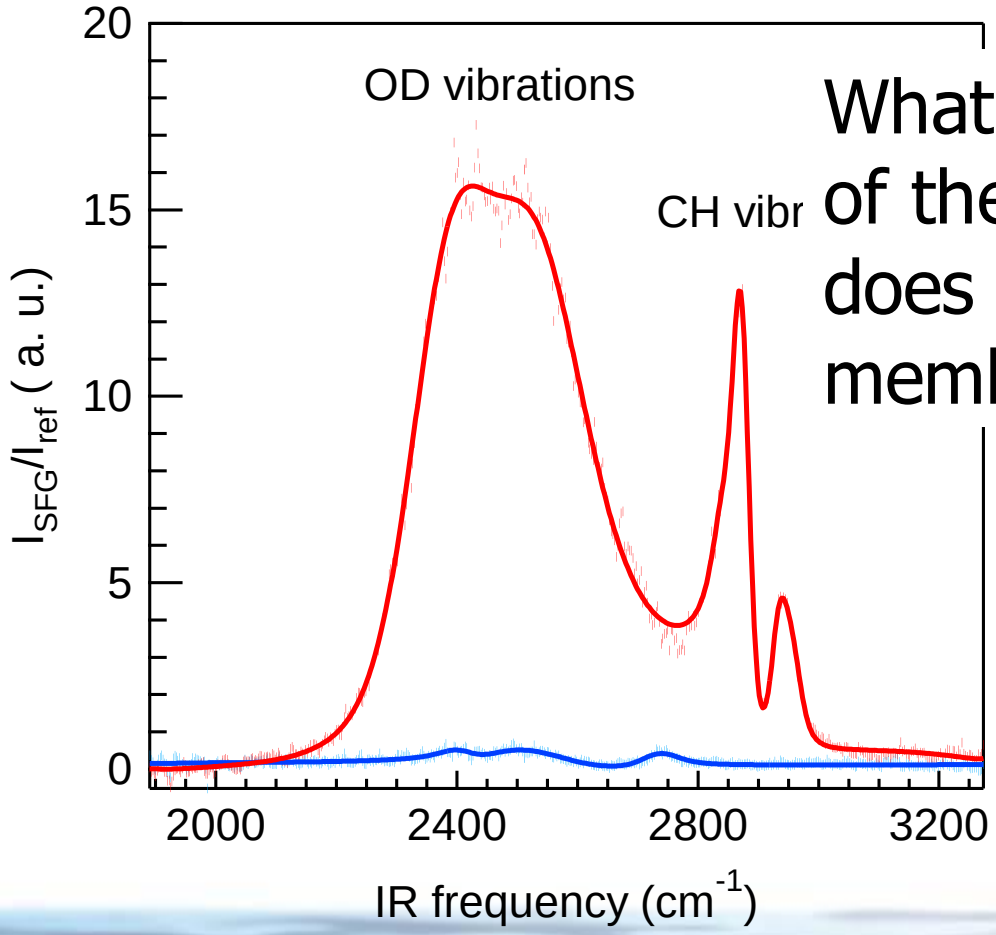


$K = 2.6 \times 10^4 \mathrm{M}^{-1}\mathrm{l}^{-1}$ per base

Cooperativity n=3 → Highly sensitive DNA detection, readily made specific

Conclusion (intermediate…)

- Sensitive, labelfree DNA detection by optical detection of water vibrations (also useful for toxins).



What determines the shape of the SFG spectra; what does it tell us about the membrane-bound water?

water-lipid

water-air

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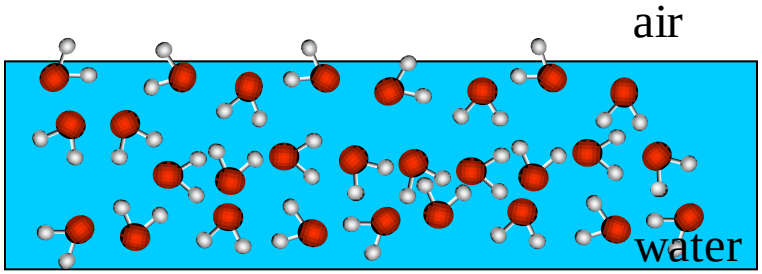
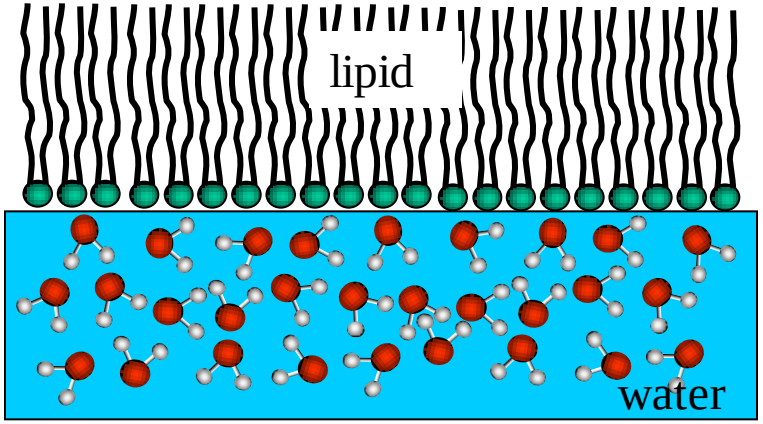
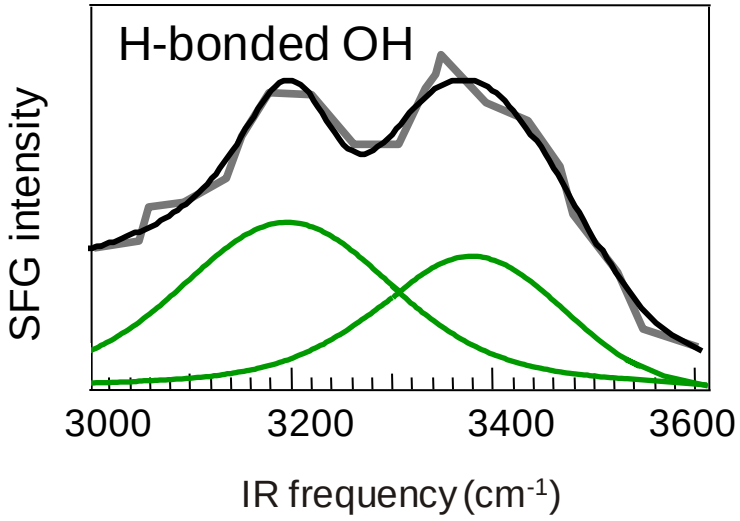
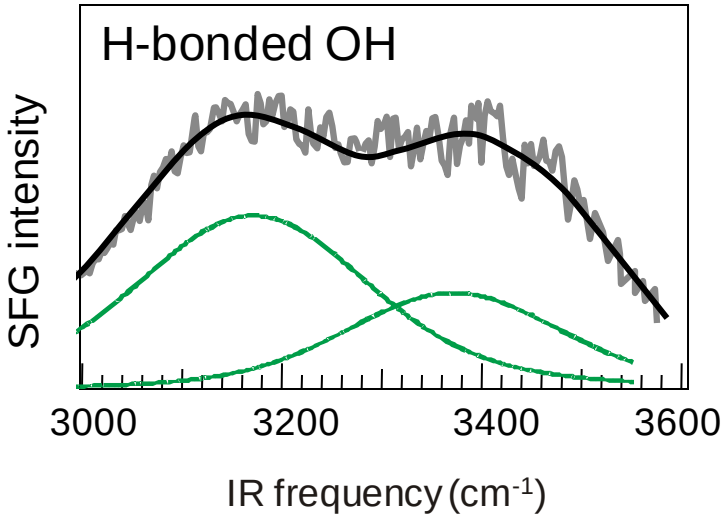
Comparison of air-water and lipid-water interfaces



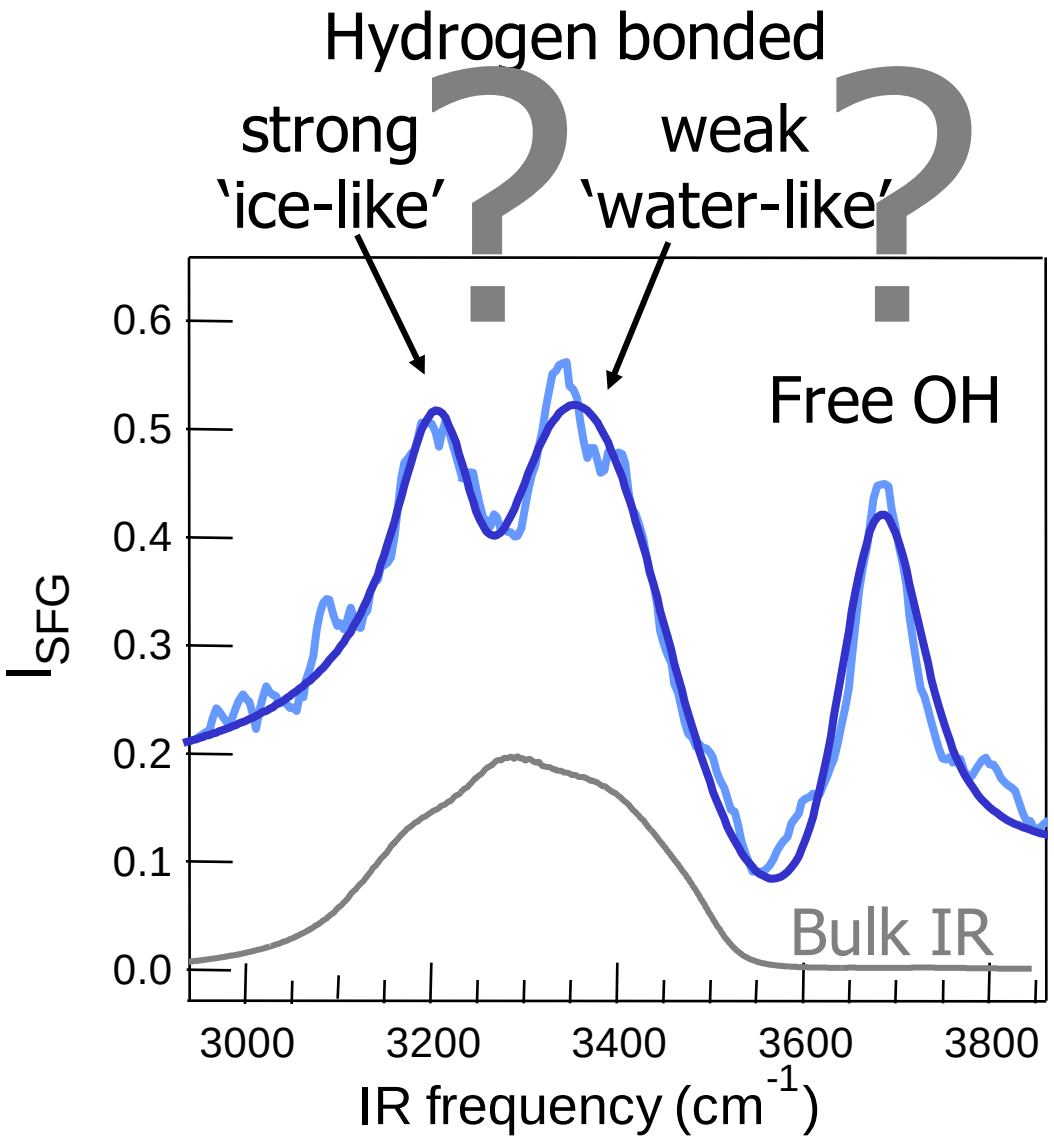


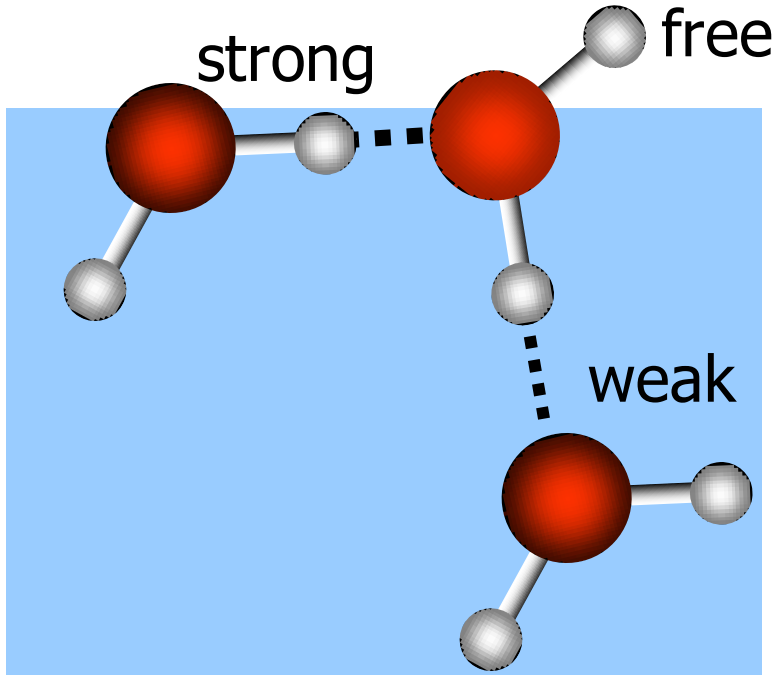




Similar spectra: same interfacial water?

SFG spectrum of water/air interface vs bulk IR spectrum

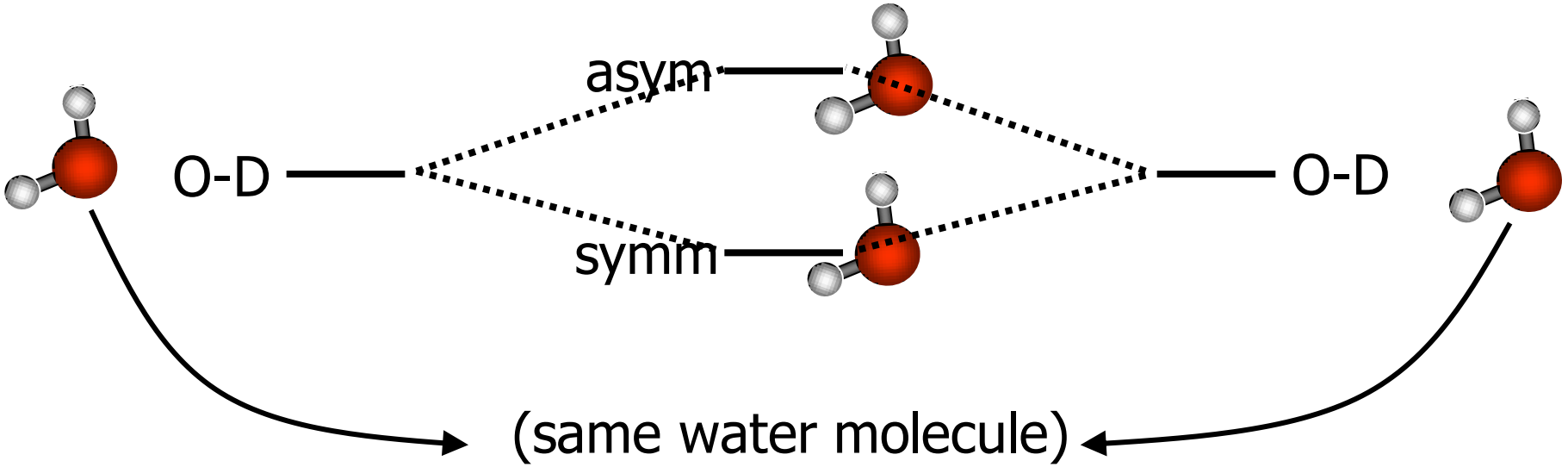




Interpretation in terms of quasi-static sub-structures[1] at surface doubtful, given recent experimental results for bulk water[2]

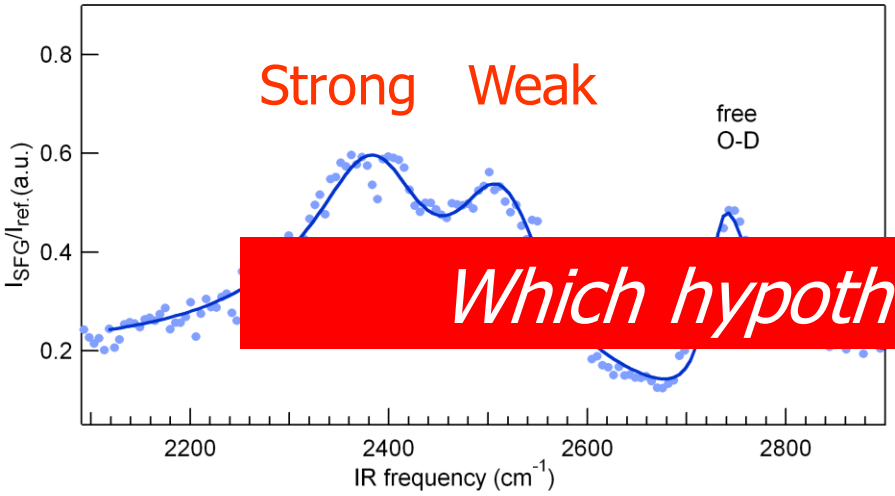
[1] Shen et al., PRL 1993; [2]Woutersen, Nature, 1999; Cowan, Nature 2005.

Remember the H_2 molecule?

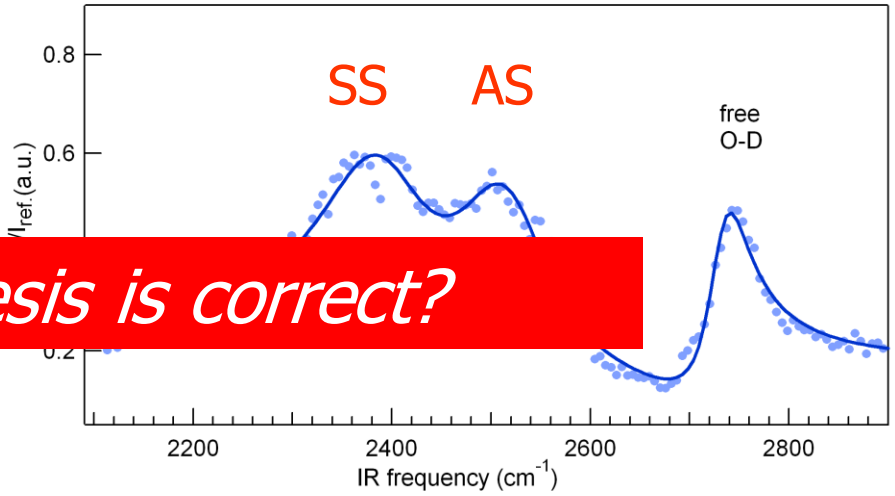


Hypotheses

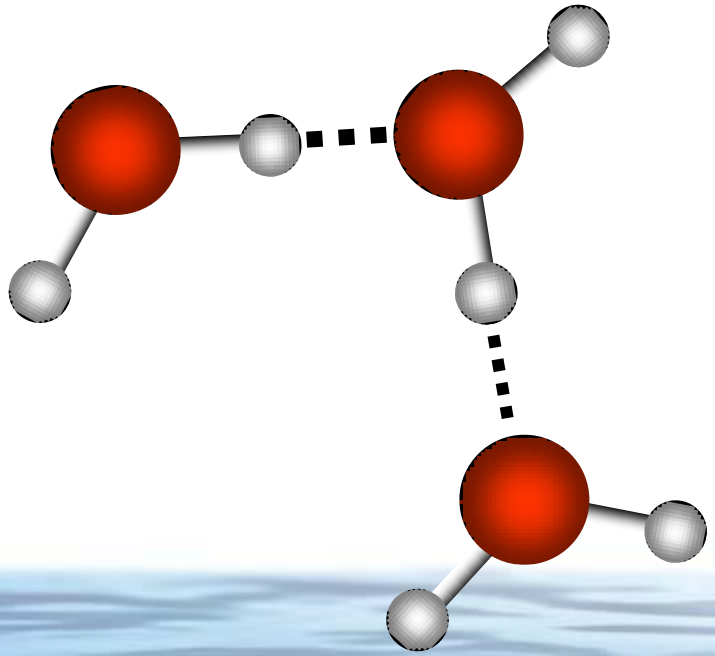
'Ice/Liquid-like' hypothesis

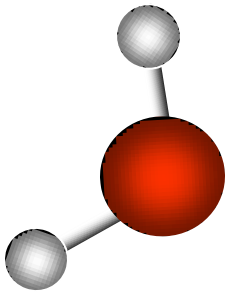


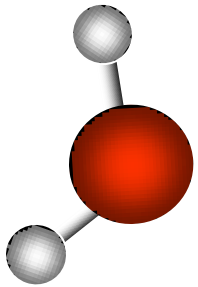
O-H coupling hypothesis



Which hypothesis is correct?



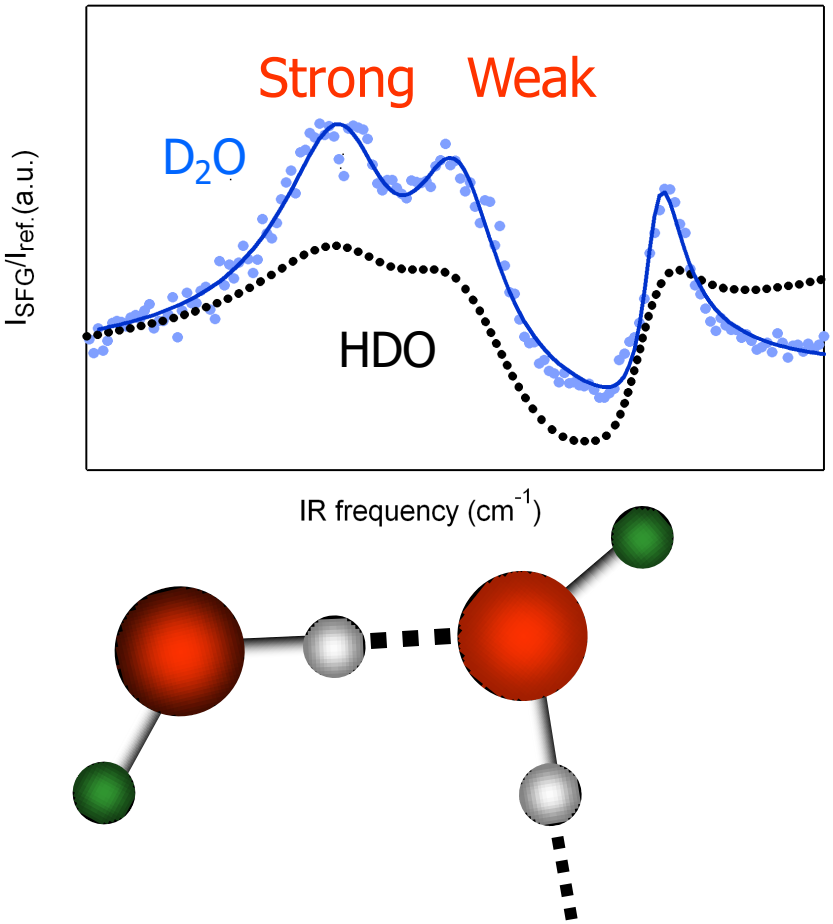




Testing the hypothesis

using isotopic dilution experiments

'Ice/Liquid-like' hypothesis

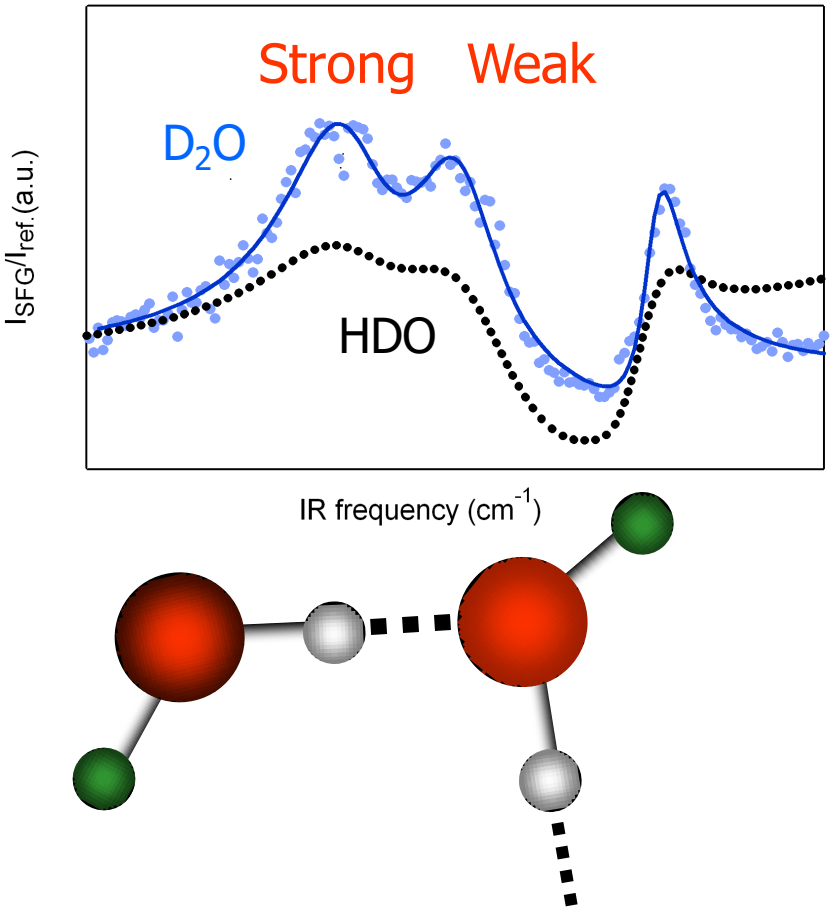


the amplitude of the two-peaks should change over the whole spectrum

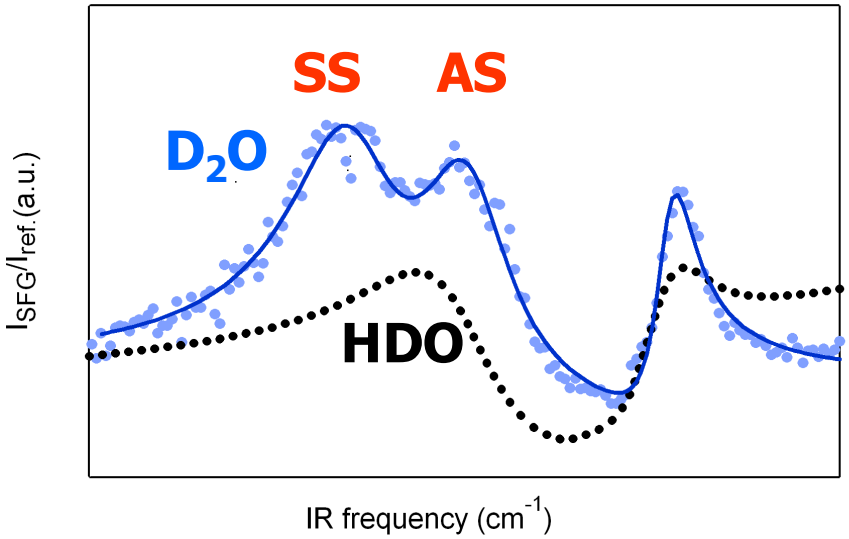
Testing the hypothesis

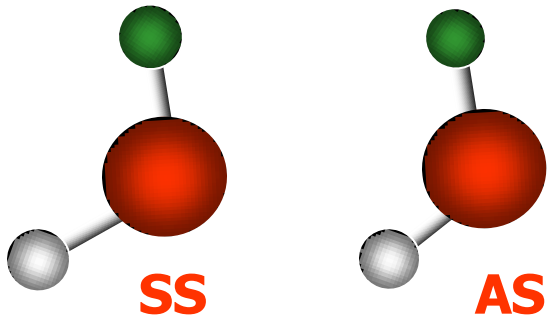
using isotopic dilution experiments

'Ice/Liquid-like' hypothesis



O-H coupling hypothesis

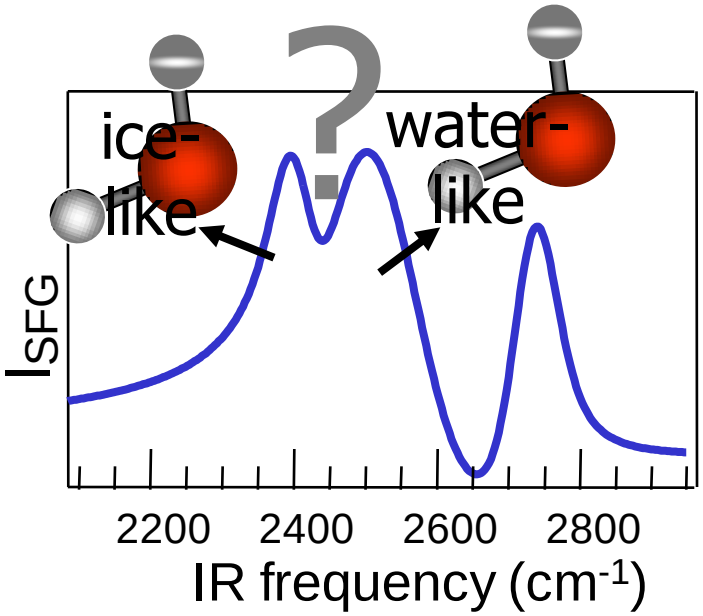




SFG spectrum should change from double-peak to a single-peak structure

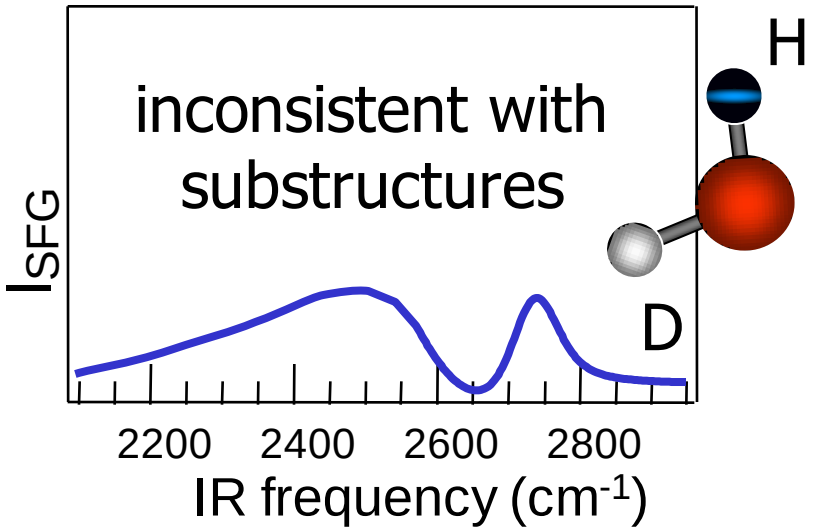
H-bonded doublet is due to substructures? → Isotopic dilution

pure D_2O

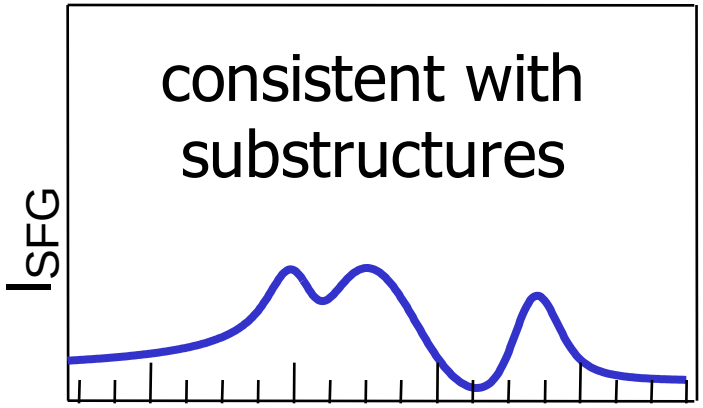


H_2O:D_2O 2:1

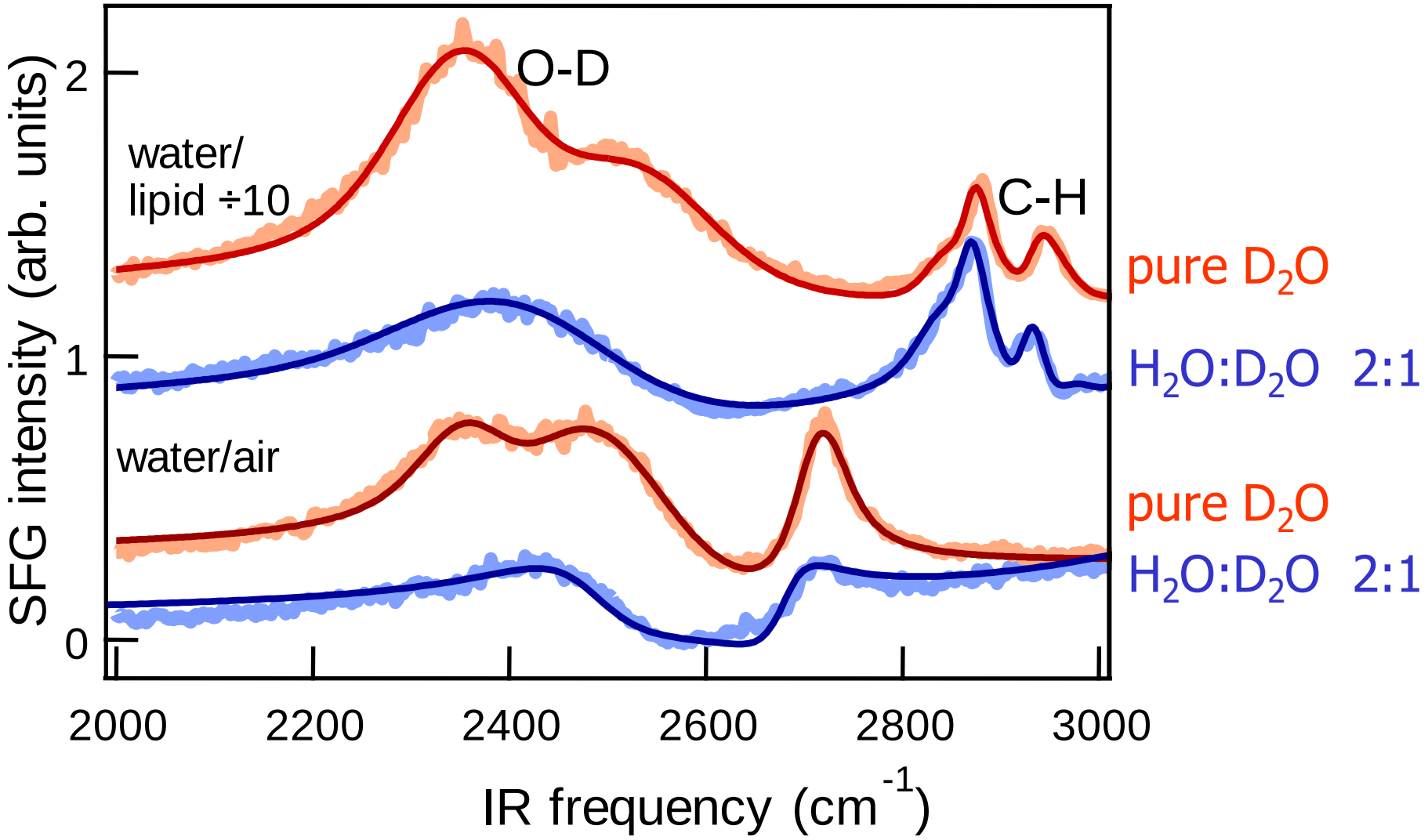
H_2O:HDO:D_2O 4:4:1



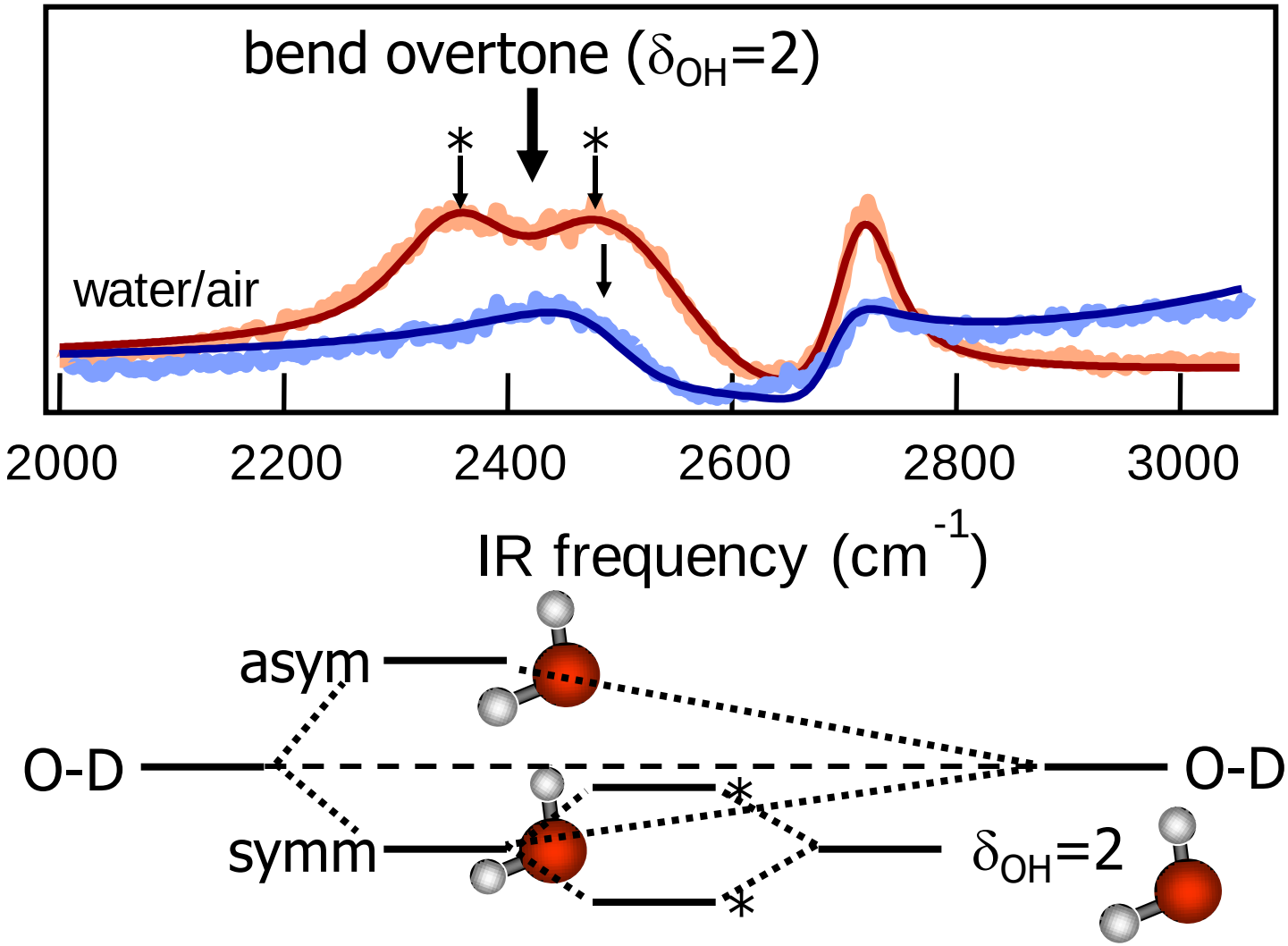
Splitting of peak by distinct substructures or intramolecular coupling (symm. & asym. stretches)??



H-bonded doublet is NOT due to substructures



H-bonded doublet is due to Fermi resonance



D_2O → HOD switches off coupling

Water at biological membranes: structure, dynamics and biomolecular sensing

Outline

Introduction

Structure

Sensing

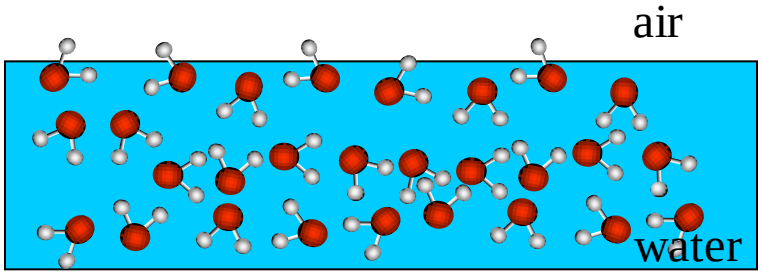
Less structure

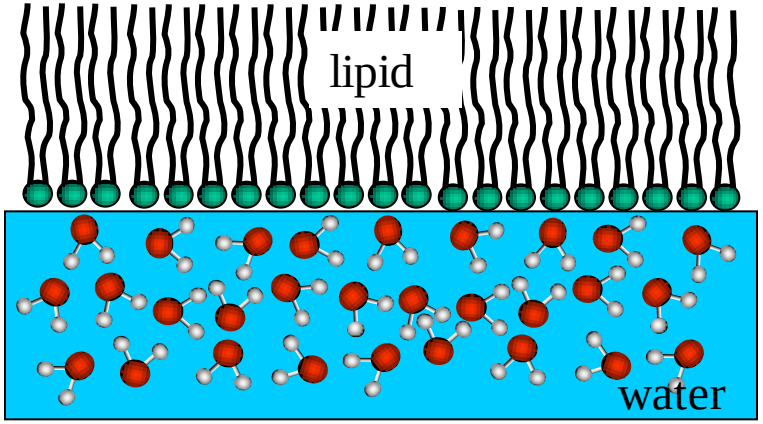
Dynamics

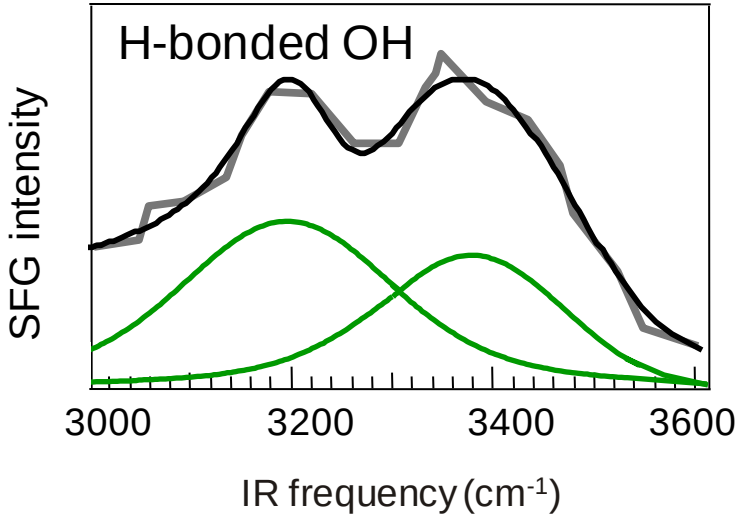
Structure from dynamics

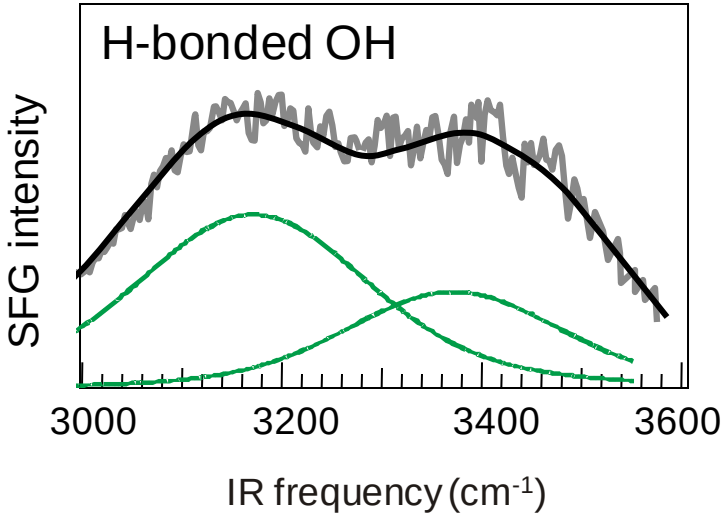
Conclusions

Double-peaked structure is due to Fermi resonance…









…and contains little info on structure.

Broad and featureless spectra hide water dynamics.

Water at biological membranes: structure, dynamics and biomolecular sensing

Outline

Introduction

A bit of structure

Sensing

Structure??

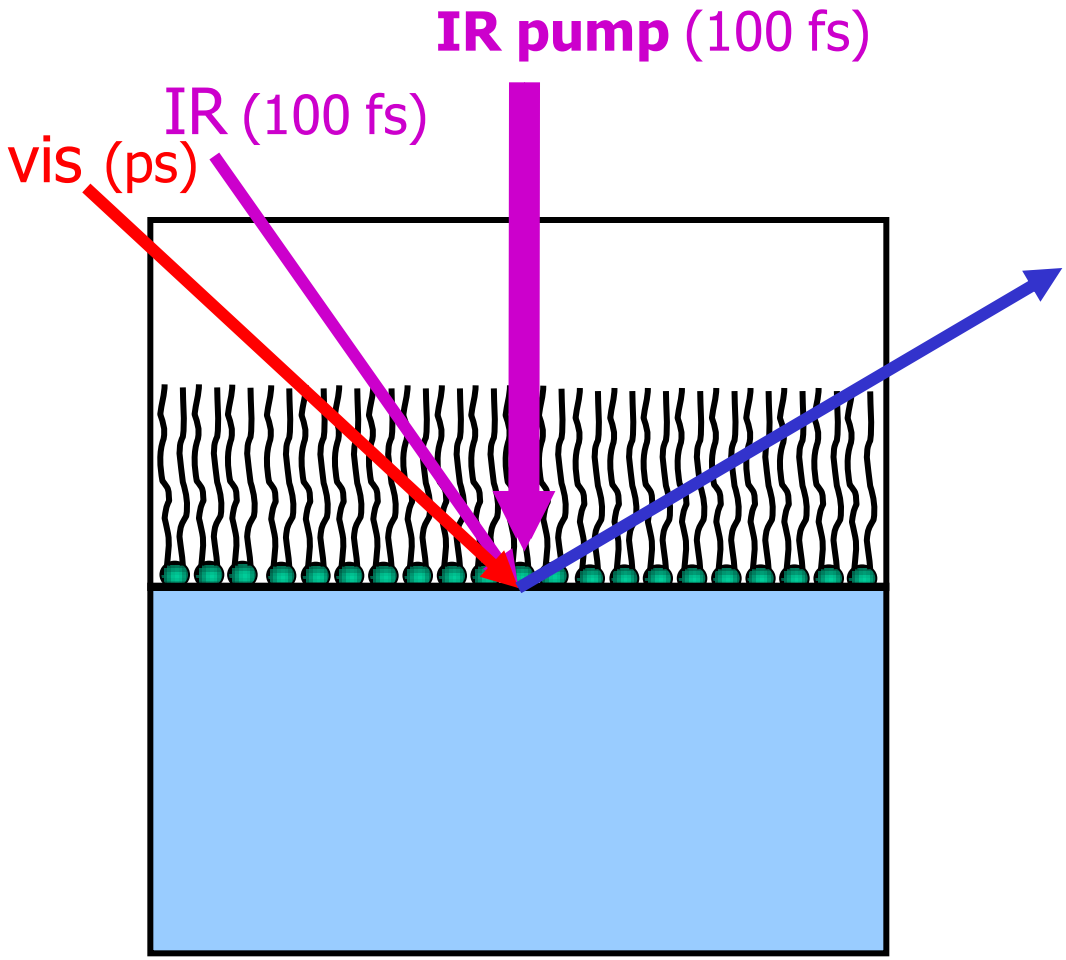
Dynamics

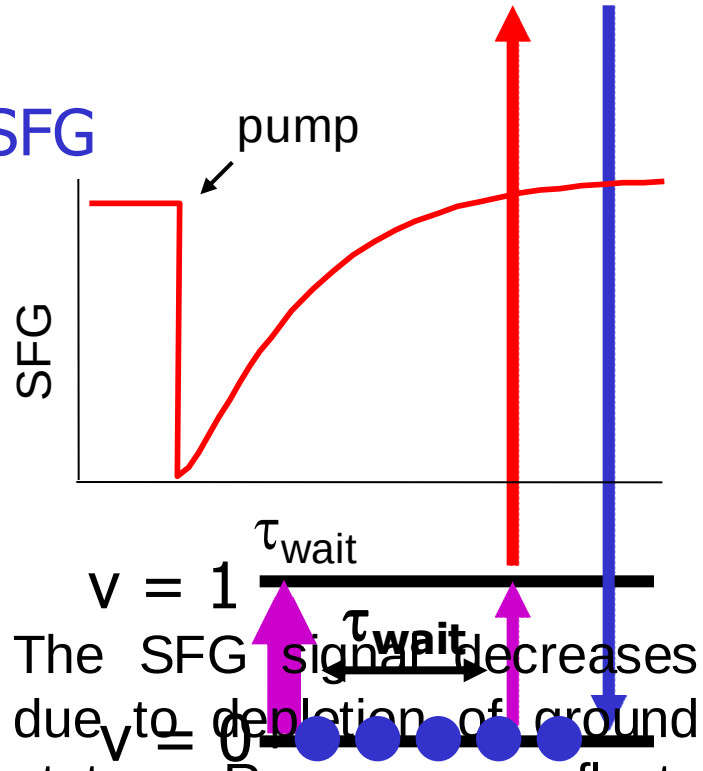
Structure from dynamics

Conclusions

Fs interfacial vibrational dynamics: From $\chi^{(2)}$ to $\chi^{(4)}$

Surface-specific femtosecond time-resolved spectroscopy*

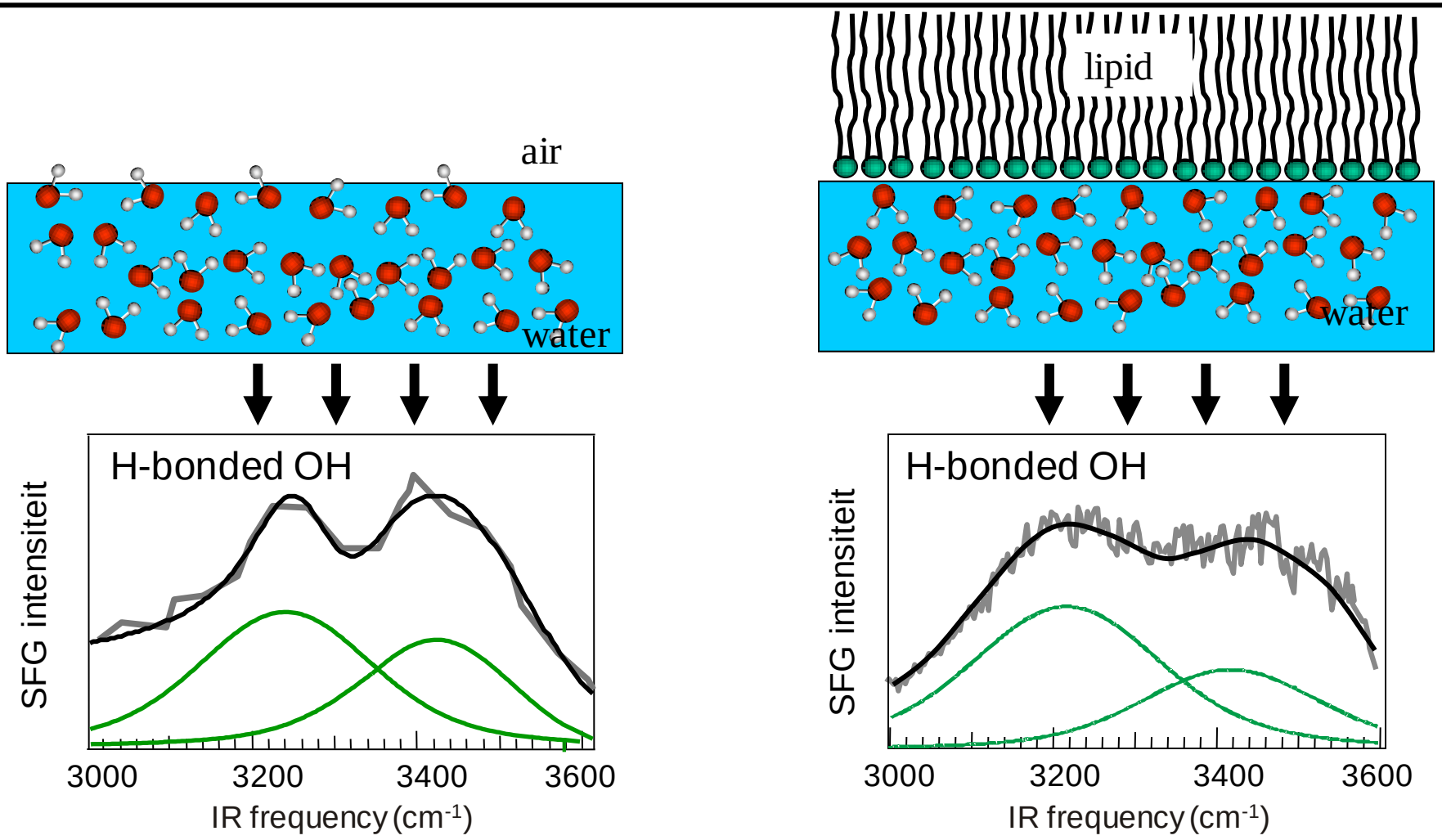




The SFG signal decreases due to depletion of ground state. Recovery reflects vibrational relaxation.

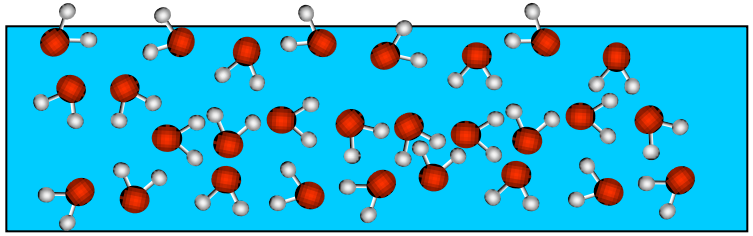
*Backus… Bonn, Science 2005 ; McGuire, Science 2006; Smits… Bonn PRL 2007

Comparison air-water and lipid-water interfaces



Record changes in SFG signal at specific frequencies as a function of time after vibrational excitation

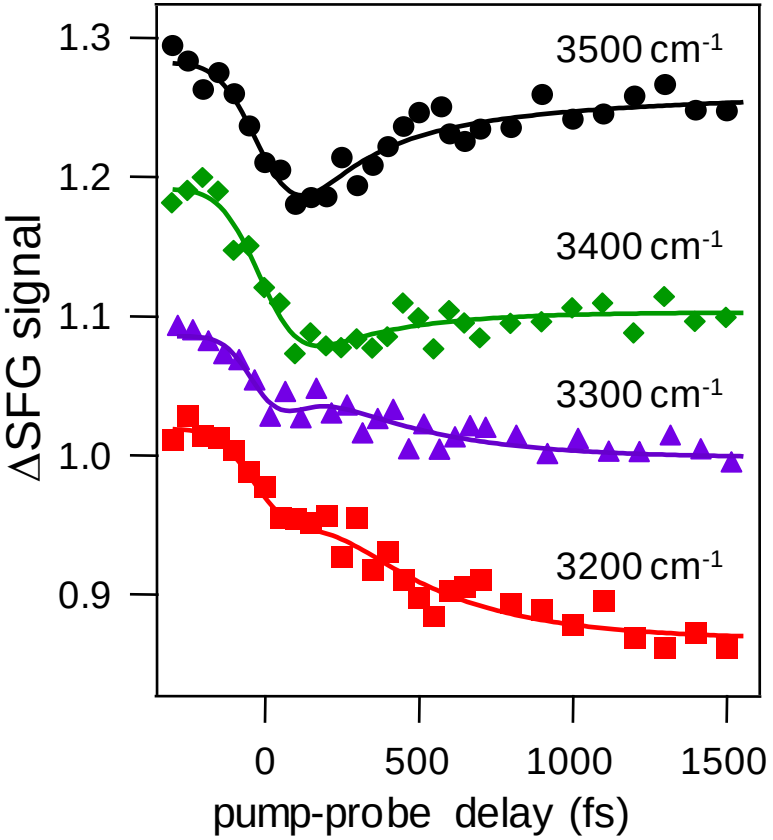
Dynamics at the water-air interface



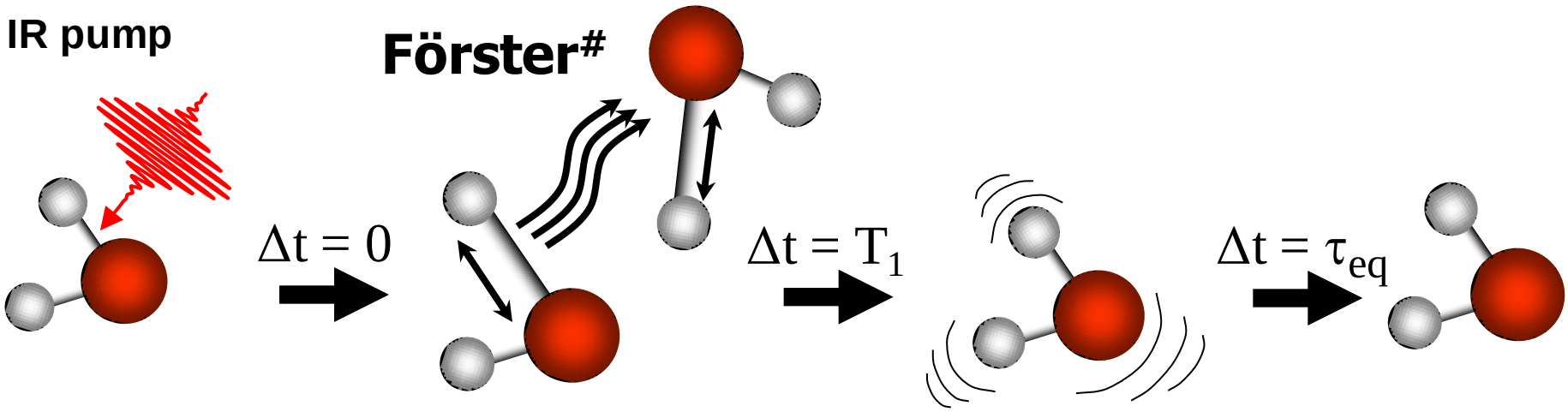
Dynamics at water/air interface:

- Distinct spectral response
- Two time constants
- No pump-polarization dependence

just like bulk!



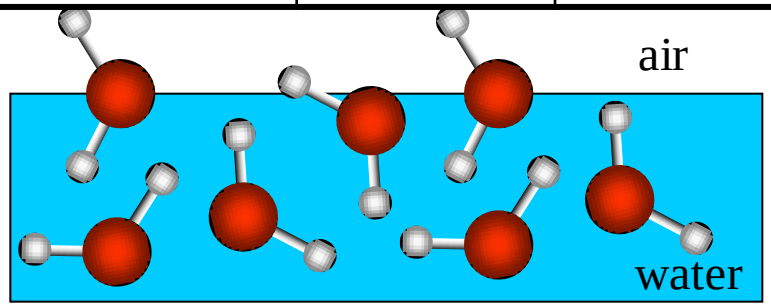
Ultrafast energy transfer between surface and bulk water

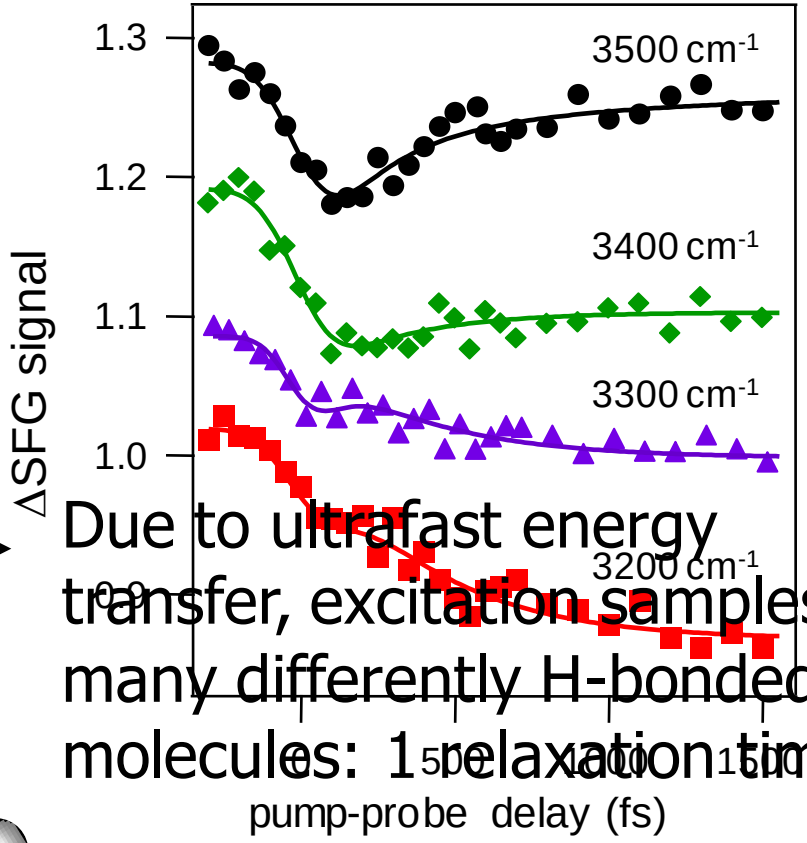


#Woutersen, *Nature* 1999;*Cowan, *Nature* 2005; @Lock, *J. Chem. Phys* 2002

Ultrafast energy transfer between surface and bulk water

υ (cm^{-1})	T_1 (fs)	τ_{eq} (fs)
3500 3400 3300 3200	190*	400@

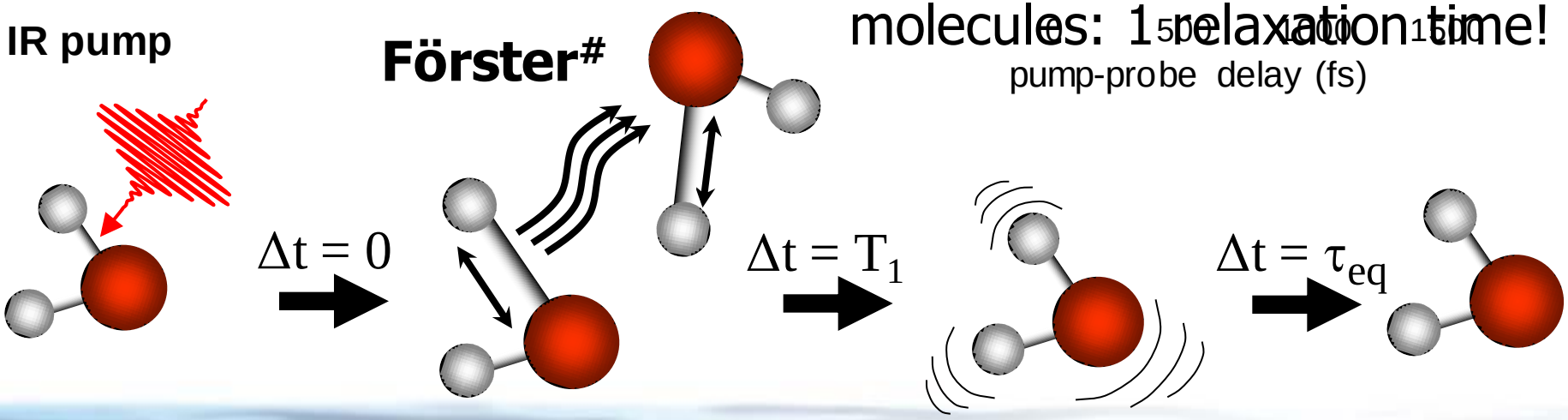




Due to ultrafast energy transfer, excitation samples many differently H-bonded molecules: 1 relaxation time!

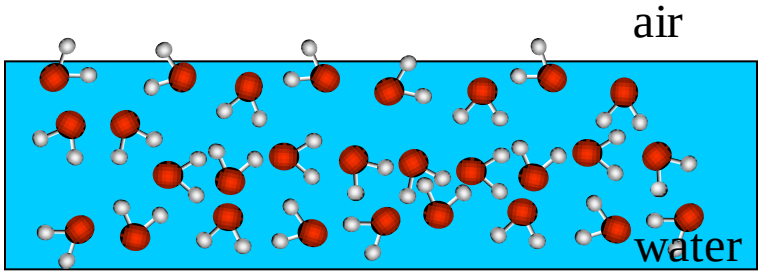
IR pump

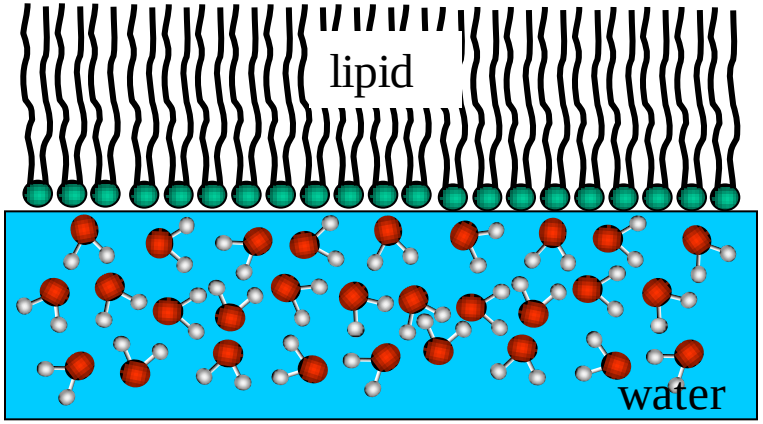
Förster#

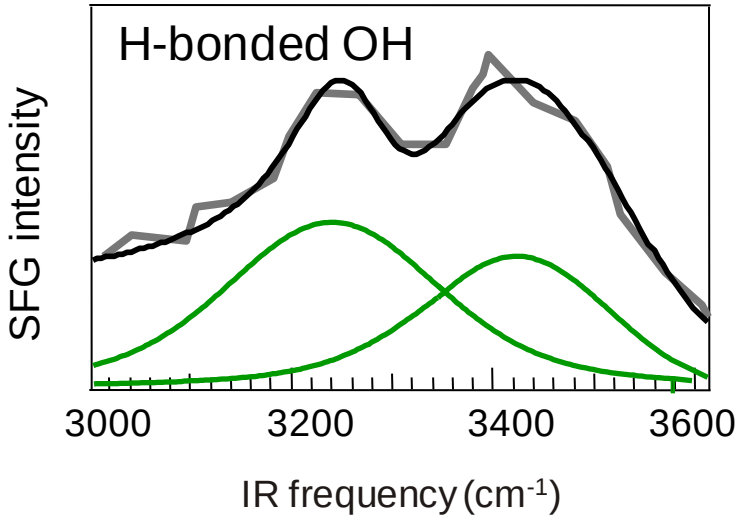


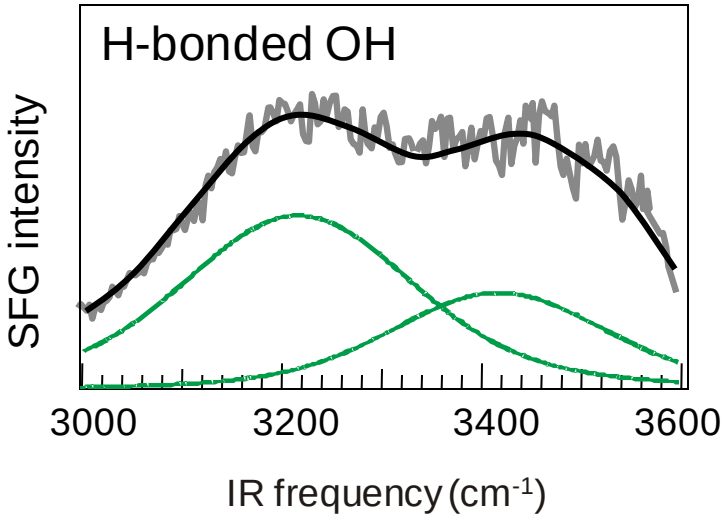
#Woutersen, *Nature* 1999;*Cowan, *Nature* 2005; @Lock, *J. Chem. Phys* 2002

Comparison air-water and lipid-water interfaces



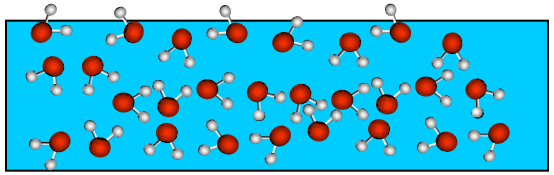


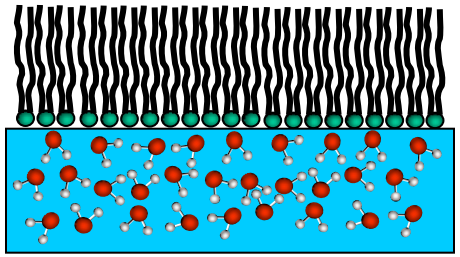


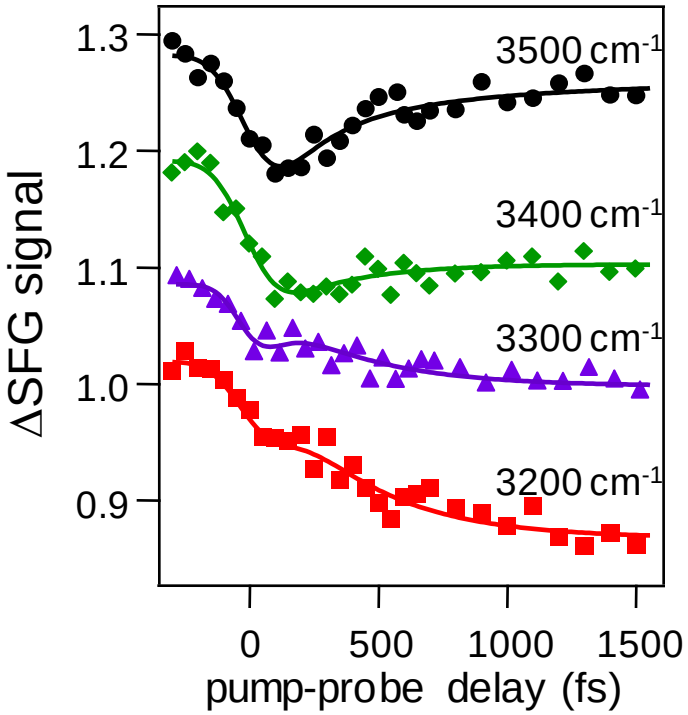


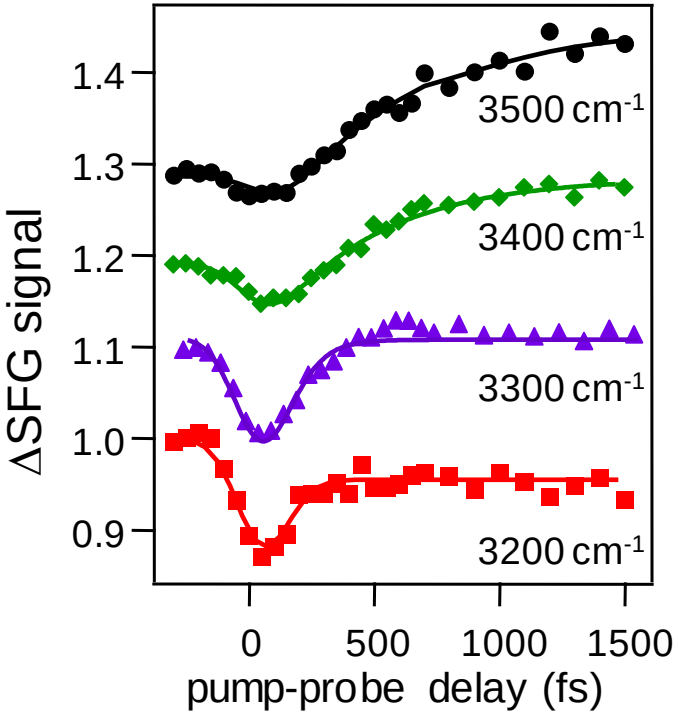
Similar spectra: same interfacial water?

Different dynamics for the two interfaces





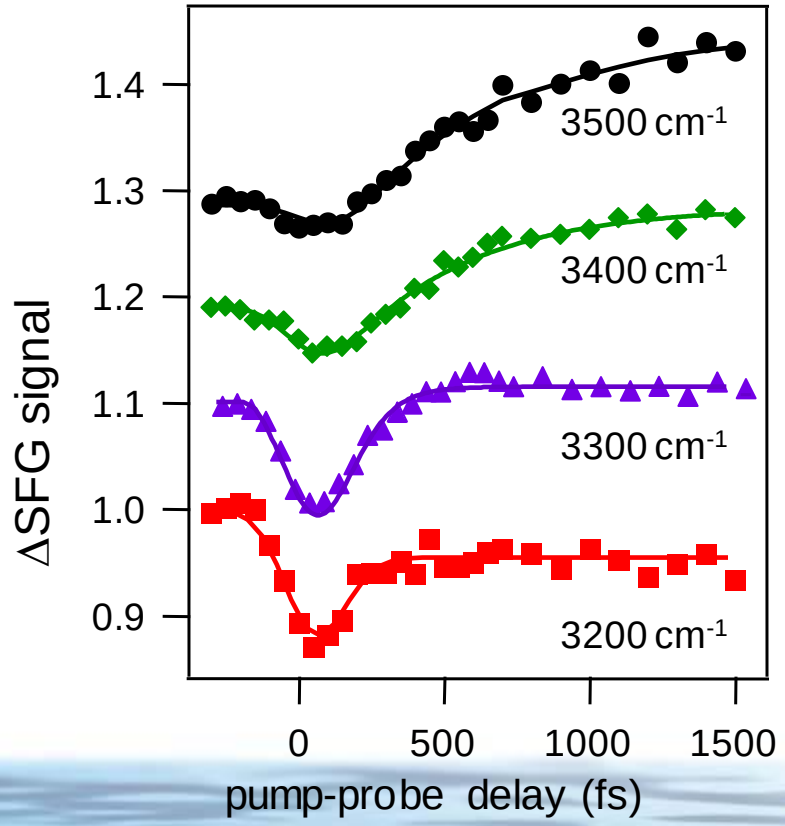




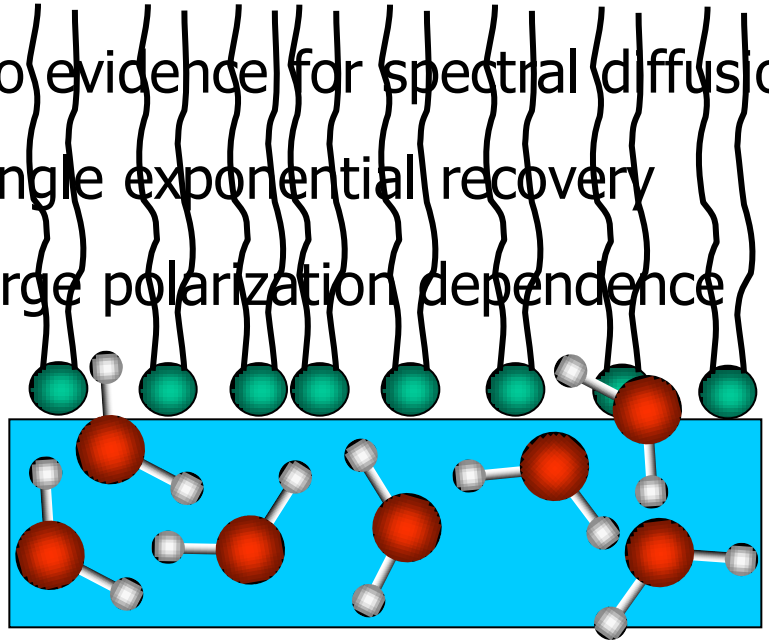
Dynamics at water/air interface reflect *bulk* behaviour;
Not so for water/lipid interface

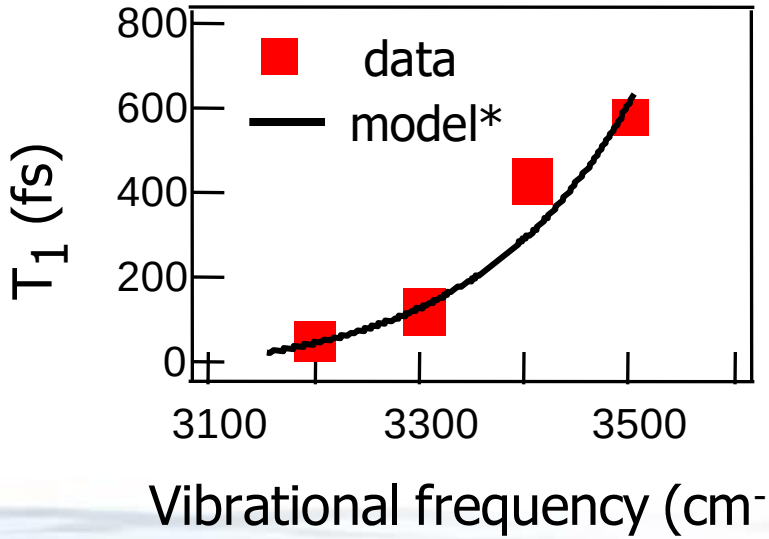
Membrane-bound water is energetically isolated from the bulk

υ (cm^{-1})	T_1 (fs)
3500	570
3400	430
3300	130
3200	<100



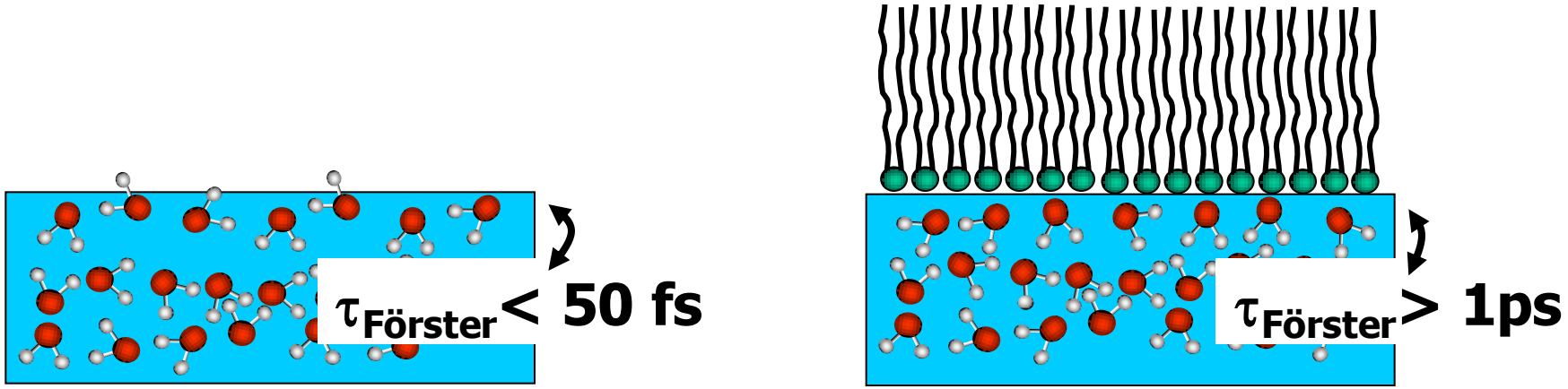
- No evidence for spectral diffusion
- single exponential recovery
- large polarization dependence





*Staib, A.; Hynes, J. T. *Chem.Phys.Lett.* 1997

Membrane-bound water is energetically isolated from the bulk



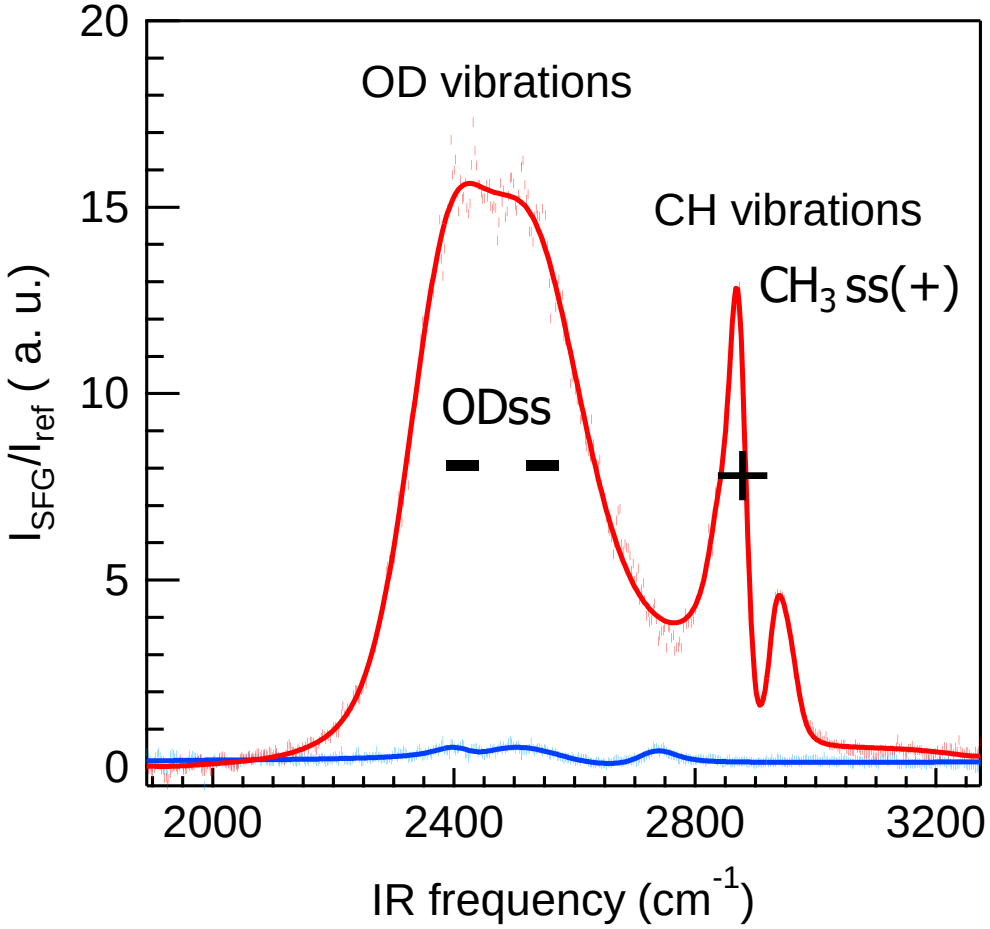
How? Why?

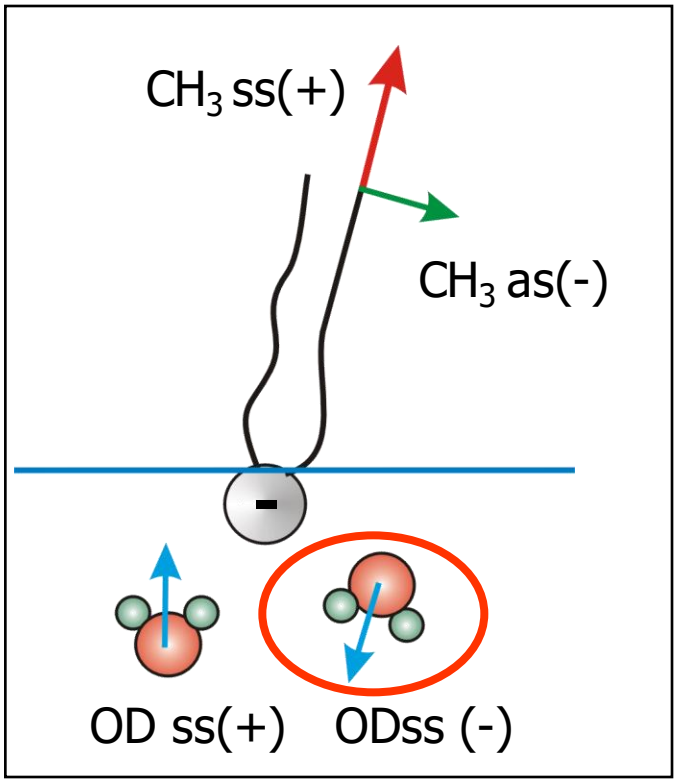
Answer lies in steady-state SFG spectra
of membrane-bound water

Water bound to an anionic lipid monolayer

$$I_{SFG} \propto \left|\chi^{(2)}\right|^2 ; \chi^{(2)} = \sum_n \chi_n^{(2)}$$

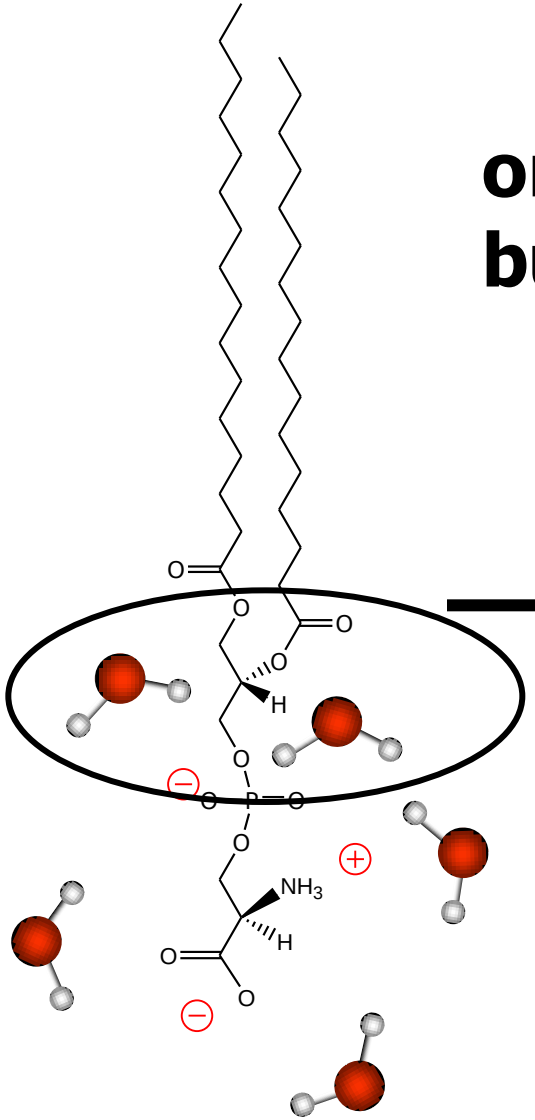
Sign of $\chi^{(2)}$ → molecular orientation





Water oriented with oxygen towards negative lipid??

We are looking at headgroup water



oriented the right way,
but at other side of charge

This is the water we are observing with SFG.
Spatially isolated from bulk → slow Forster energy transfer

Water at biological membranes: structure, dynamics and biomolecular sensing

Outline

Introduction

A bit of structure

Sensing

Less structure

Dynamics

Structure from dynamics

Water at biological membranes: structure, dynamics and biomolecular sensing

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Structure from dynamics

Conclusions

Conclusions

- Water surface structure is simpler than has been thought (PRL **100**, 173901 (2008))
- At many interfaces, surface water exchanges vibrational energy rapidly with the underlying bulk (PRL **98**, 098302 (2007))
- In contrast, membrane-bound water does not show fast energy exchange – it does not just terminate the bulk and constitutes an intrinsic part of membrane (JACS **129**, 9608 (2007))

~~Membrane = lipids~~

Membrane = lipids + water

- Sensitive, labelfree DNA detection by optical detection of water vibrations (JACS **129**, 8420 (2007))