Characterization of intracellular water investigated with terahertz spectroscopy

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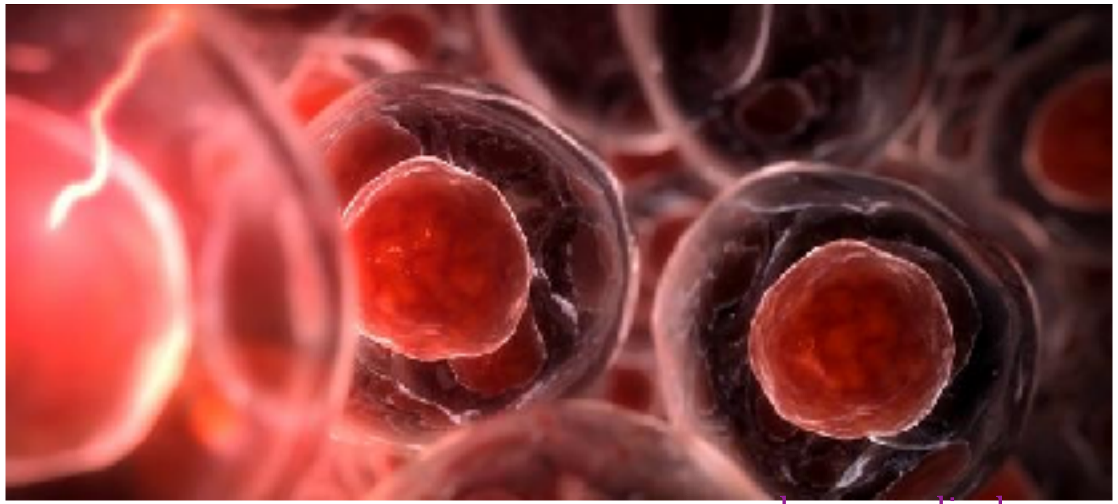

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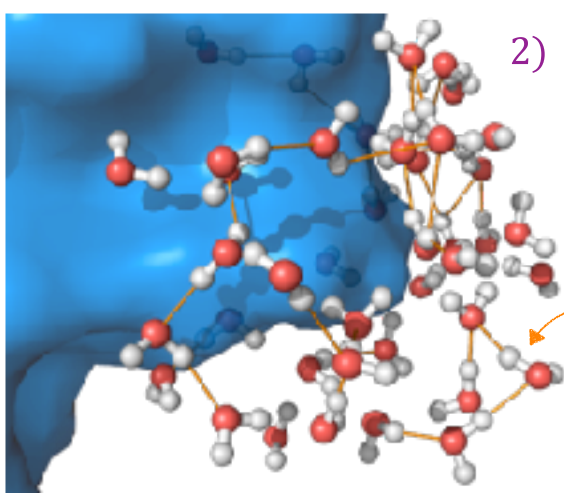
Water as a "matrix of life"

Water is essential for the function of biomolecules

→ Water behaviors in malignant tissues are different from those in heathy ones[1)]

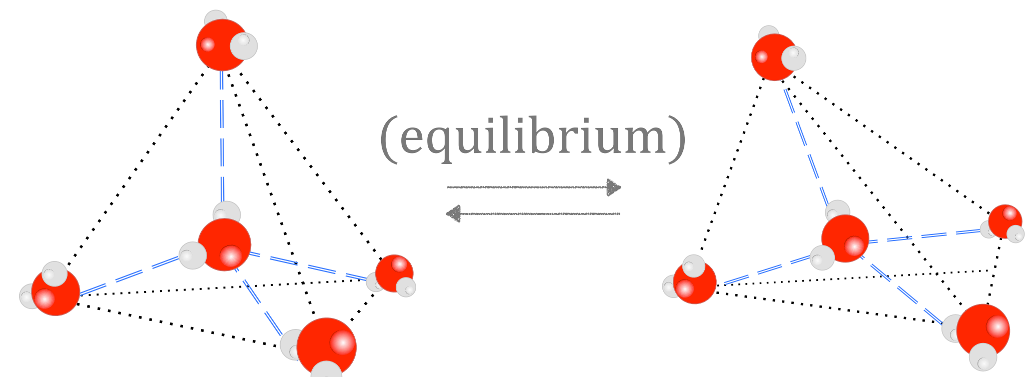
< Hydration to biomolecules >



Dynamically retarded water (= stable)

Hydration water
→ activates biological functions[2)]

< Reaction field >



Low-density liquid (LDL: ~0.91g/cm^3)

High-density liquid (HDL: ~1.18g/cm^3)

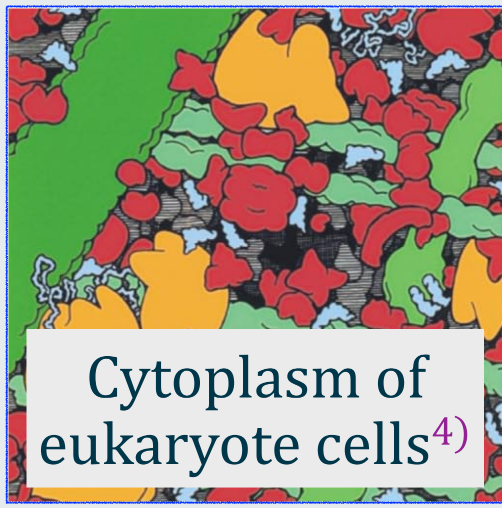
Hydrogen-bond (HB) network
→ affects reactivities and metabolisms[3)]

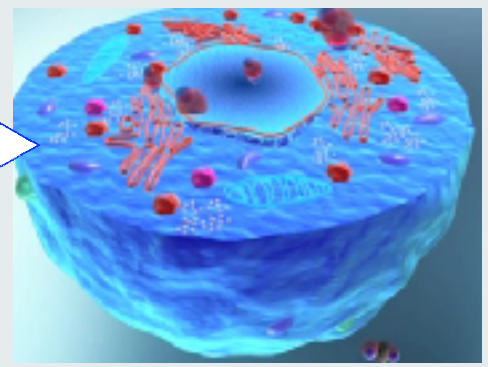
"Hydration state" and "HB structure" may reflect "personality" of the cell

1) R. Damadian, Science, 171, 1151, (1971).
2) J. T. King & K. J. Kubarych, JACS 134, 18705, (2012).
3) P. M. Wiggins, Cell. Biol. Int. 20, 429, (1996).

Mysterious cellular water

Characteristics of intracellular water are mostly unexplored





< Difficulty in probing cellular water >

① Diverse and heterogeneous cell components

② Discriminating intra- and extracellular water

→ i.e. H/D substitution or freezing

☞ Water in "intact" cells: completely veiled

☞ Terahertz (THz) spectroscopy to reveal intracellular water

(1) 1THz ↔ 1ps in time

☞ Fluctuation of the water HBs

= HB dynamics directly observed

(2) 1THz ↔ 300μm in wavelength

☞ Scattering by cell components

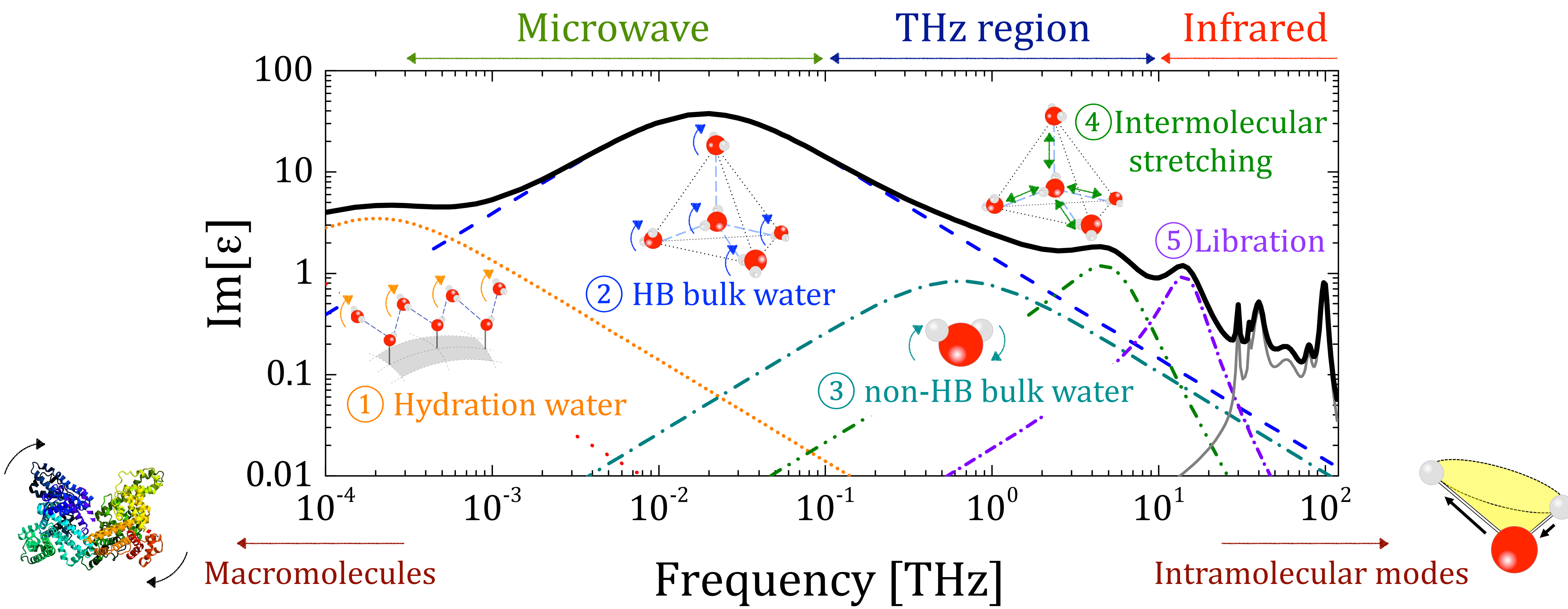
= negligibly small

4) D. S. Goodsell, The Machinery of Life: Springer, (1992).

Motivation

THz spectroscopy

→ Imaginary part Im[ε] of protein aqueous solution[5,6)]



→ Contribution of macromolecules (< 300mg/ml) = negligibly small[7)]

☞ Reduction in the bulk water relaxation = (1) hydration state

☞ non-HB water & intermolecular modes = (2) HB structure

5) C. Cametti et al., J. Phys. Chem. B 115, 7144, (2011). 6) H. Yada et al., Chem. Phys. Lett. 464, 166, (2008). 7) K. Shiraga et al., Biophys. J. (in press).

Exploration of cellular water

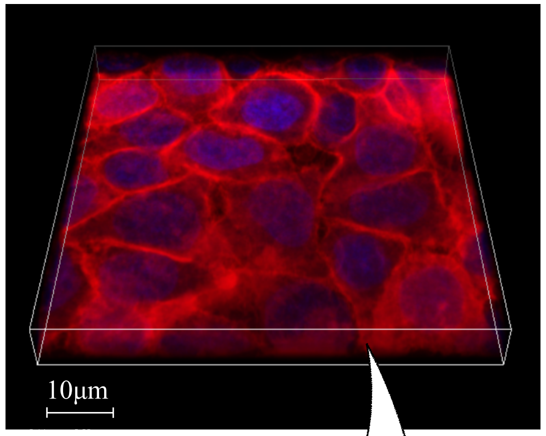
< Objective >

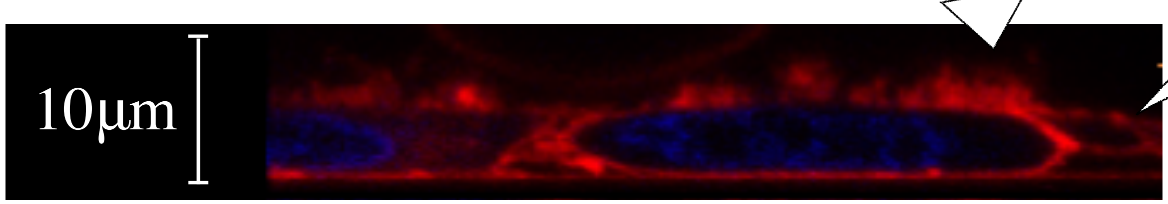
Evaluation of the "hydration state" and "HB structure" in intact cells based on the dielectric responses in the THz region

Sample cell: HeLa (human cervical cancer)

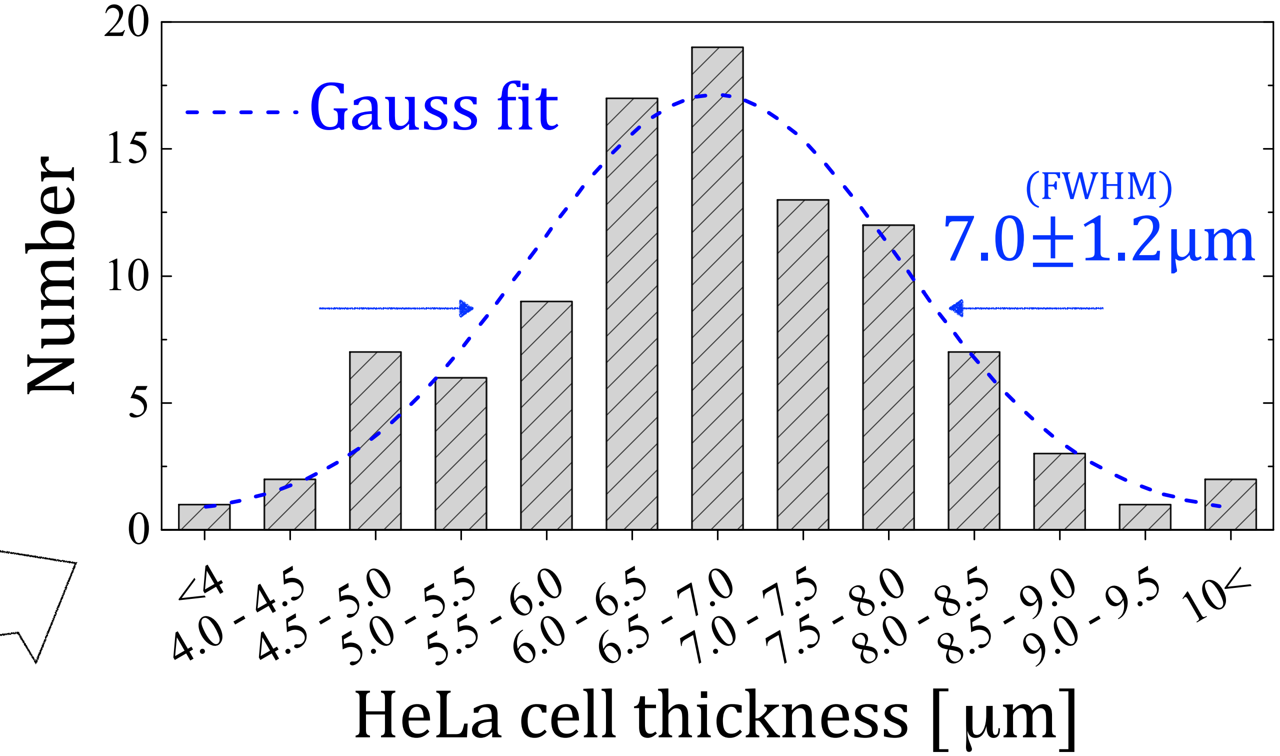
Optical image

Fluorescent 3D image

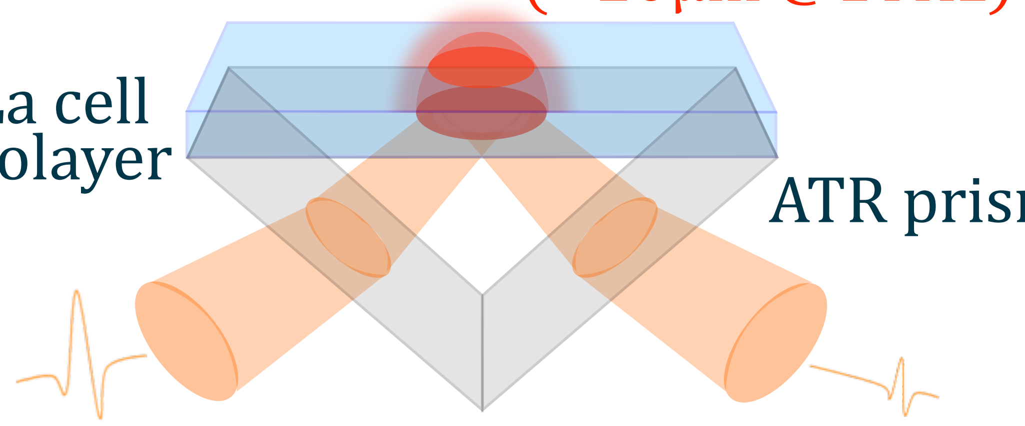




< Histogram: HeLa cell thickness >



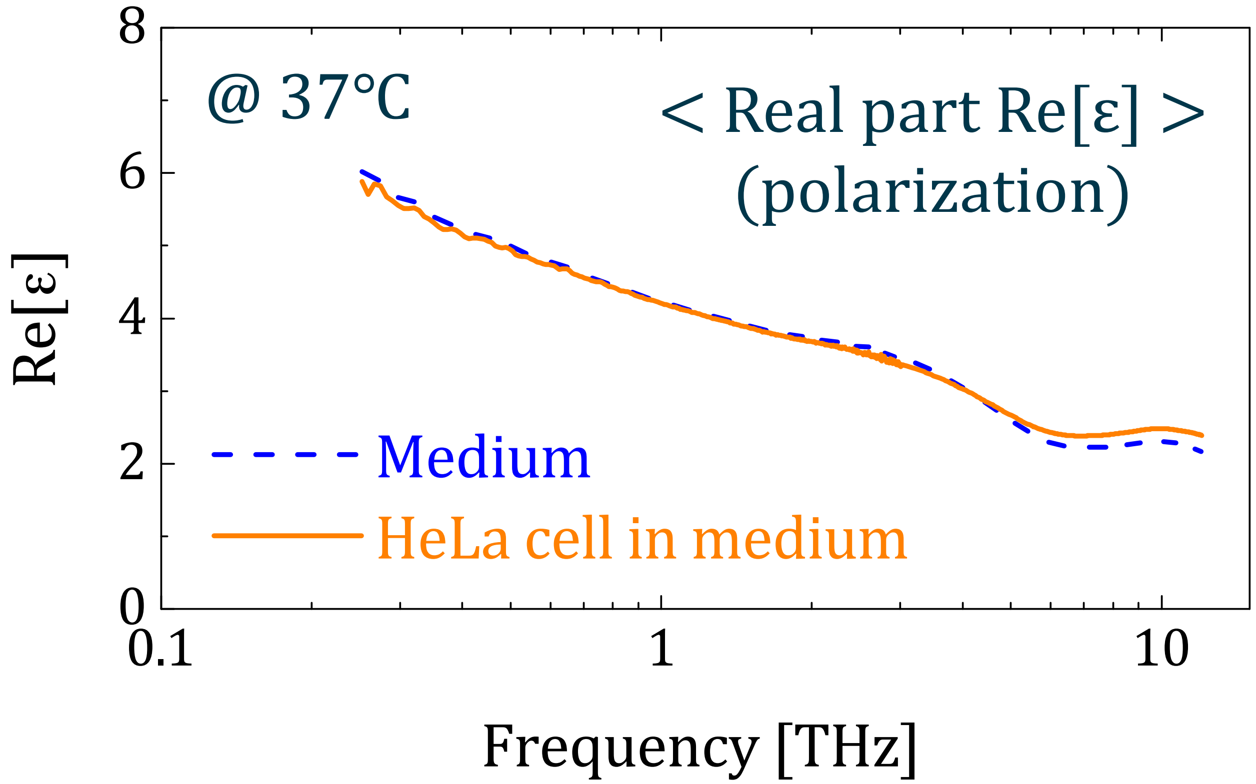
THz-ATR spectroscopy

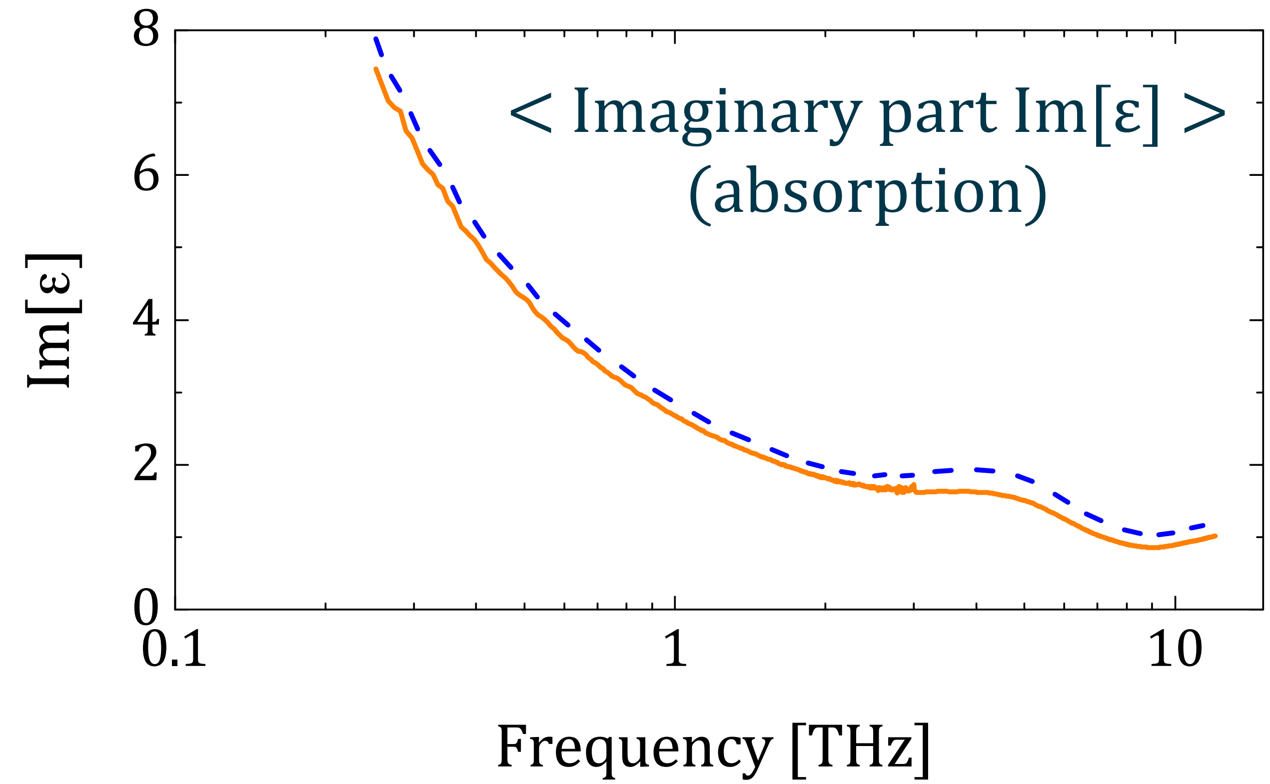


☞ HeLa cells cultured on the ATR prism

① THz time-domain ATR spectroscopy
→ 0.25–3 THz
→ Directly determine ε̃(ω)

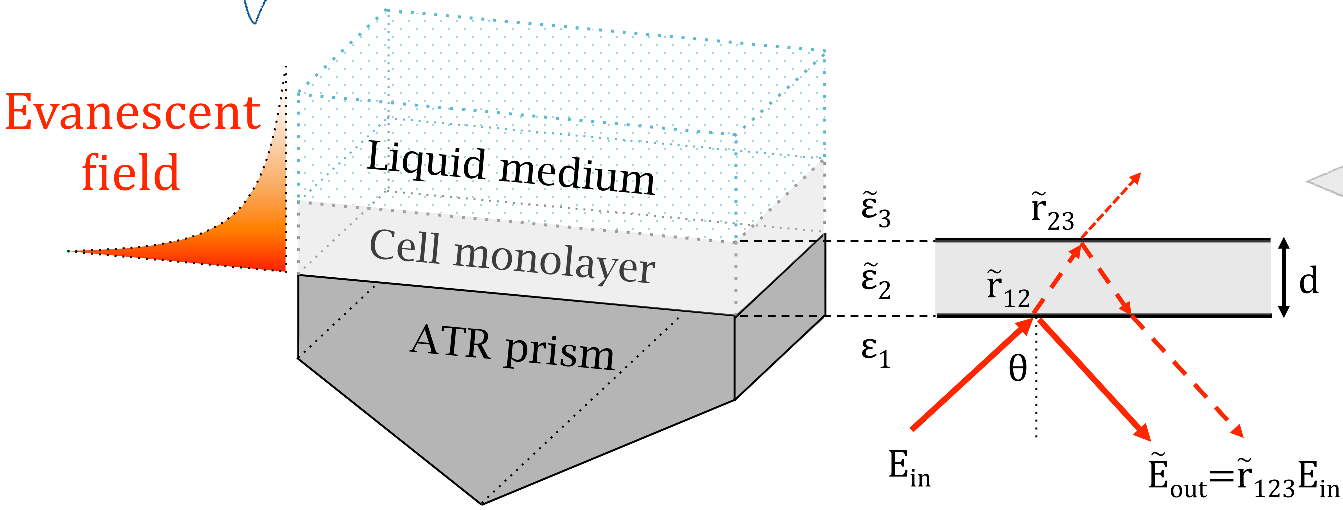
② Fourier transform ATR spectroscopy
→ 3–12 THz
→ KK transform to determine ε̃(ω)





Experimental

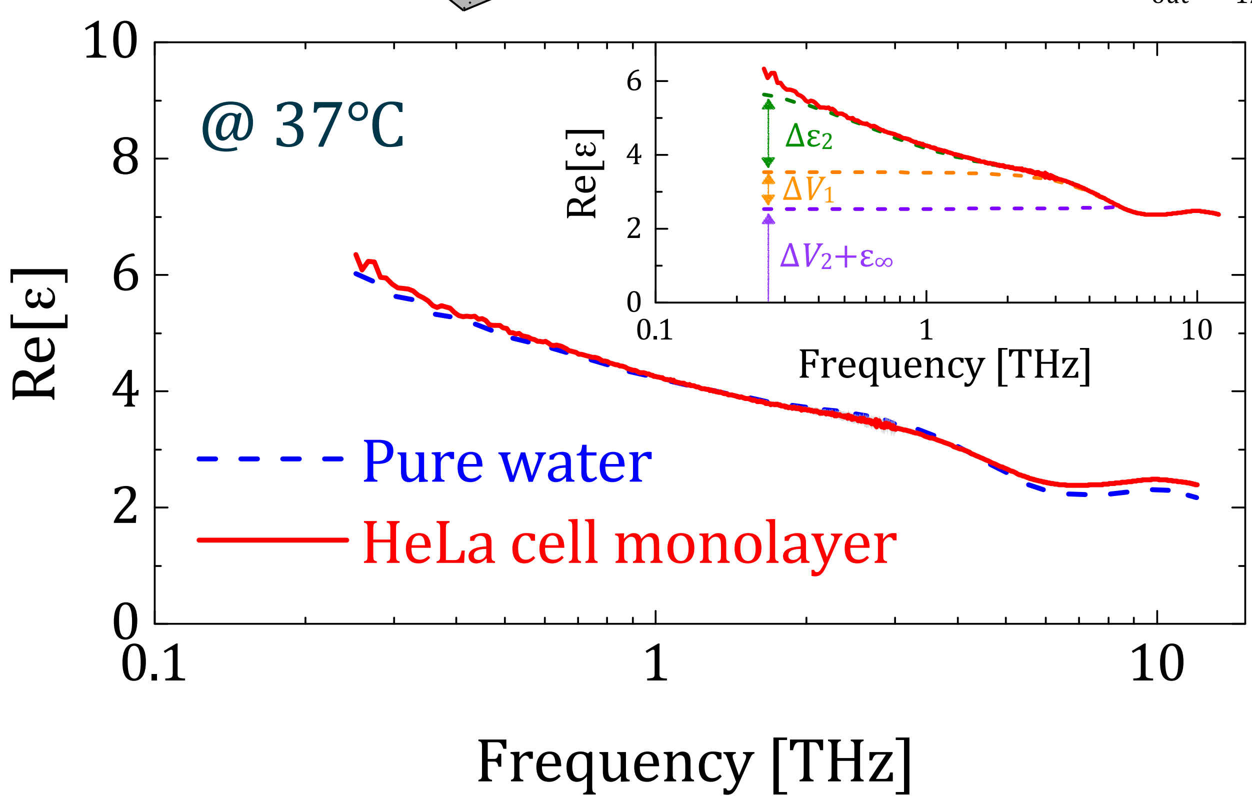
Two-interface model

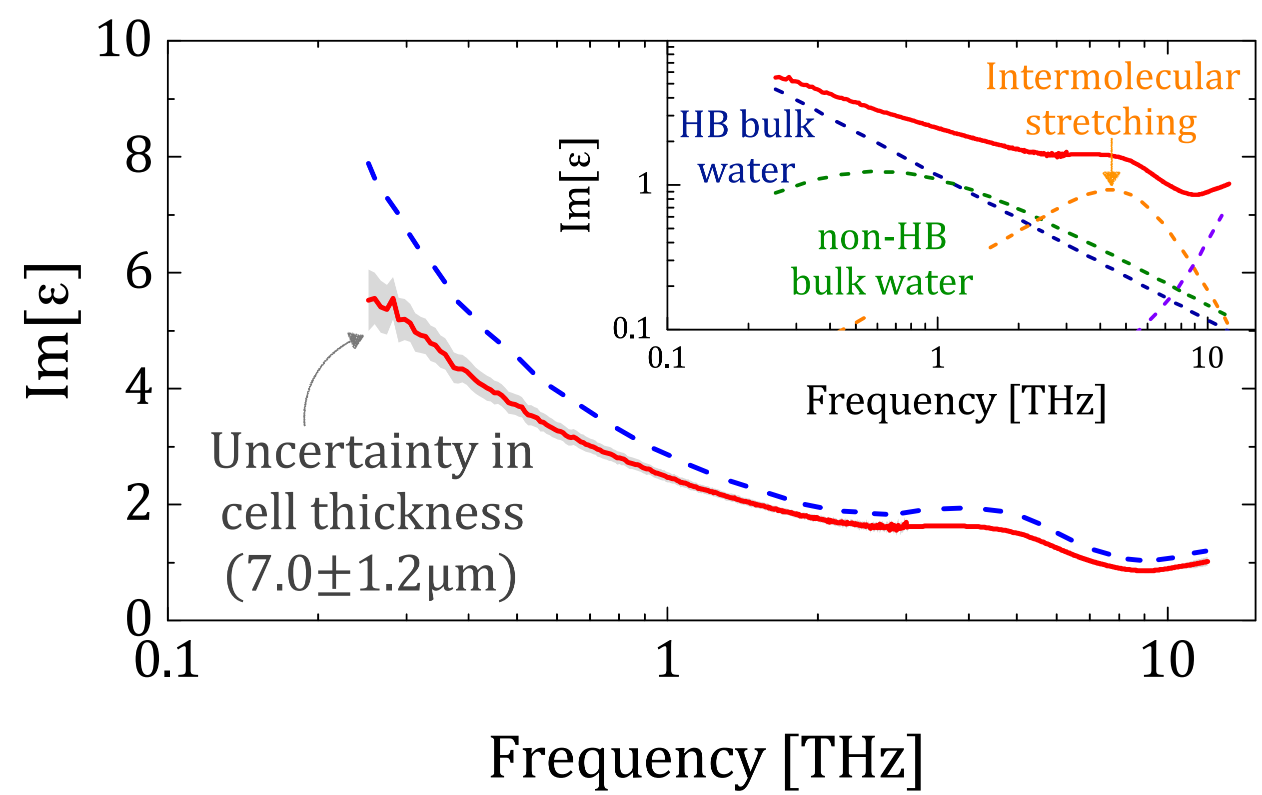


< Fresnel's equation >

$$\tilde{r}_{123}(\omega) = \frac{\tilde{r}_{12}(\omega) + \tilde{r}_{23}(\omega)\exp\left[i\,4\pi d\sqrt{\varepsilon_1(\omega)\sin^2\theta - \tilde{\varepsilon}_2(\omega)}/\lambda\right]}{1 + \tilde{r}_{12}(\omega)\tilde{r}_{23}(\omega)\exp\left[i\,4\pi d\sqrt{\varepsilon_1(\omega)\sin^2\theta - \tilde{\varepsilon}_2(\omega)}/\lambda\right]}$$

→ ε_2 is derived if ε_1, ε_3, θ and d are given[8)]





$$\tilde{\varepsilon}(\omega) = \frac{\Delta\varepsilon_1}{1+i\omega\tau_1} + \frac{\Delta\varepsilon_2}{1+i\omega\tau_2} + \frac{\Delta V_1\omega_1^{\,2}}{\omega_1^{\,2}-\omega^2+i\omega\gamma_1} + \frac{\Delta V_2\omega_2^{\,2}}{\omega_2^{\,2}-\omega^2+i\omega\gamma_2} + \varepsilon_\infty$$ [7)]

HB bulk water | non-HB bulk water | Intermolecular stretching | Libration

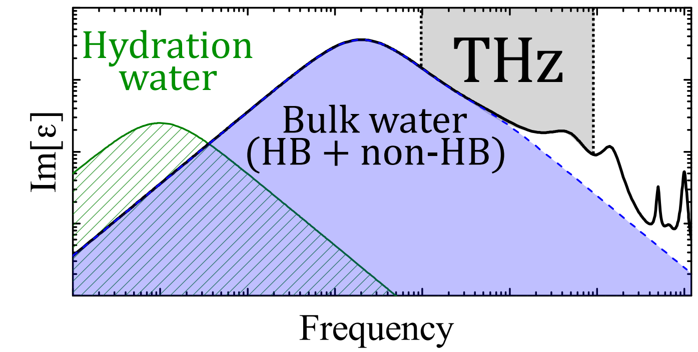
- $\Delta\varepsilon_i$: Relaxation strength
- τ_i : Relaxation time
- ΔV_i : Vibration strength
- ω_i : Resonant frequency
- γ_i : Damping constant

8) K. Shiraga et al., J. Infrared Milli. THz Waves. 35, 493, (2014).

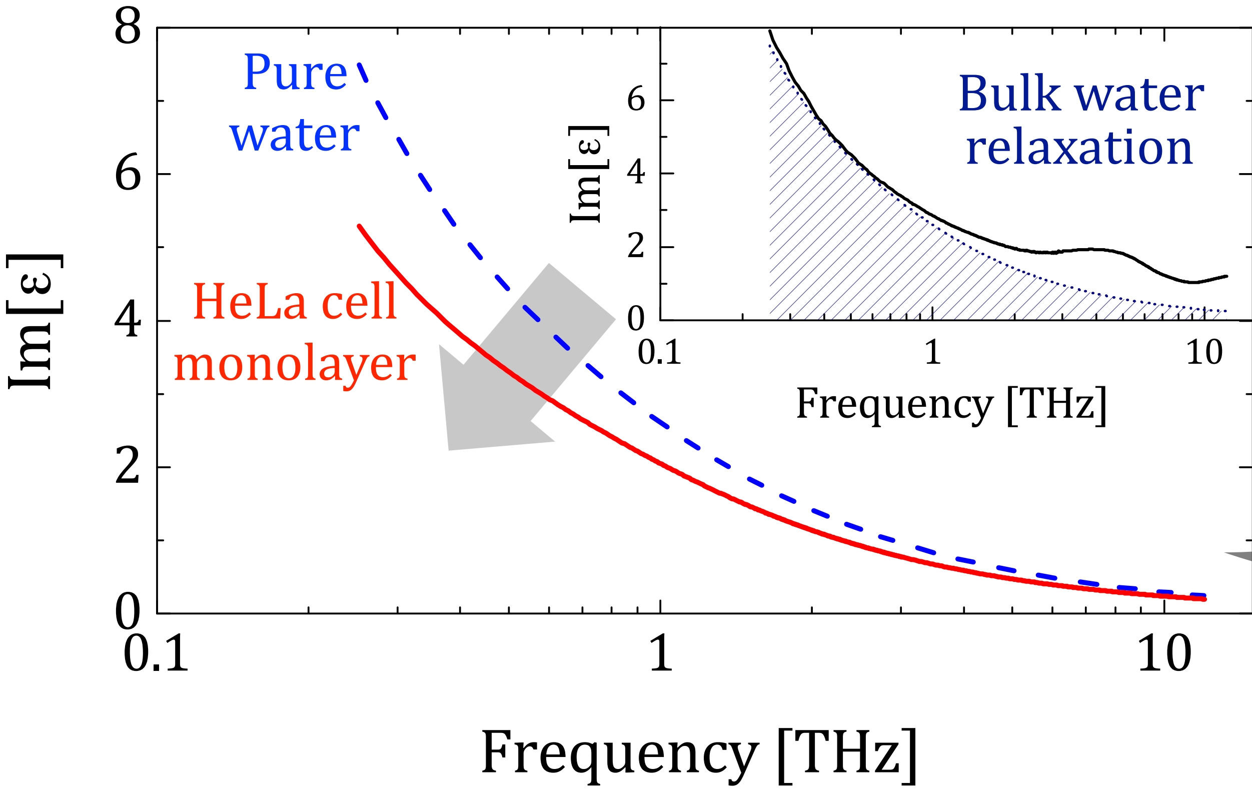
(1) Hydration state

Decrease in the bulk water relaxation

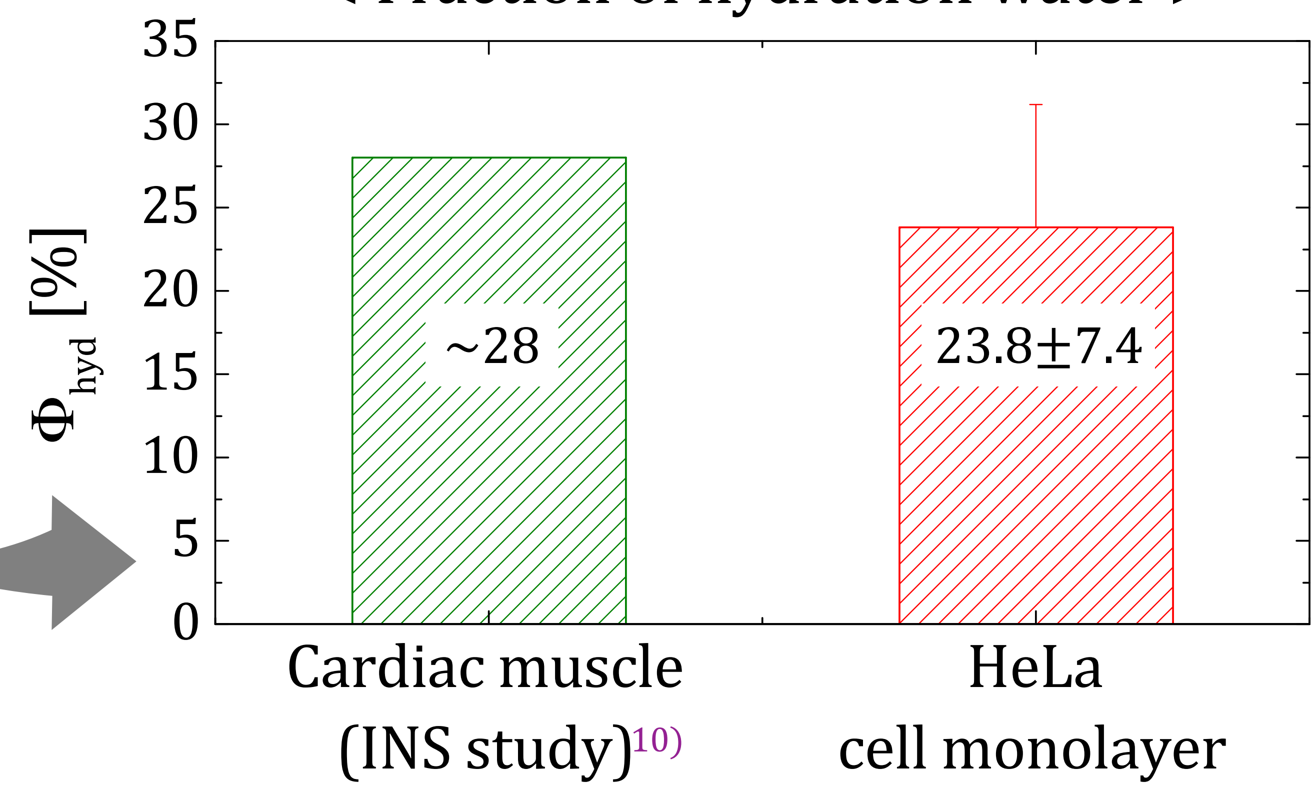
☞ "Bulk water"→"hydration water" transition



< Bulk water relaxation >



< Fraction of hydration water >



$$\tilde{\varepsilon}(\omega)-1=\underbrace{\frac{4\pi(N_w-n_hN_s)\tilde{g}(\omega)}{1-\tilde{\alpha}_{vib.}(\omega)\tilde{h}(\omega)/r_w{}^3}\left\{\tilde{\alpha}_{vib.}(\omega)+\frac{\tilde{\alpha}_1(\omega)+\tilde{\alpha}_2(\omega)}{1-\alpha_w h_w/r_w{}^3}\right\}}_{\text{Bulk}}$$

$$+\underbrace{\frac{4\pi n_h N_s\tilde{g}(\omega)}{1-\tilde{\alpha}_{vib.}(\omega)\tilde{h}(\omega)/r_h{}^3}\tilde{\alpha}_{vib.}(\omega)}_{\text{Hydration}}$$

$$+\underbrace{\frac{4\pi N_s\tilde{g}(\omega)}{1-\alpha_s\tilde{h}(\omega)/r_s{}^3}\alpha_s}_{\text{Solute}}$$

Onsager's equation[9]

n_h: hydration number

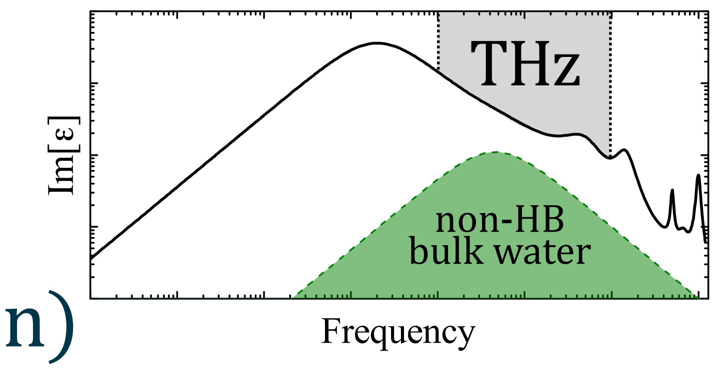
→ Similar with "frozen" muscle tissue[10] (inelastic neutron scattering measurement)

☞ Hydration state in the intact cells

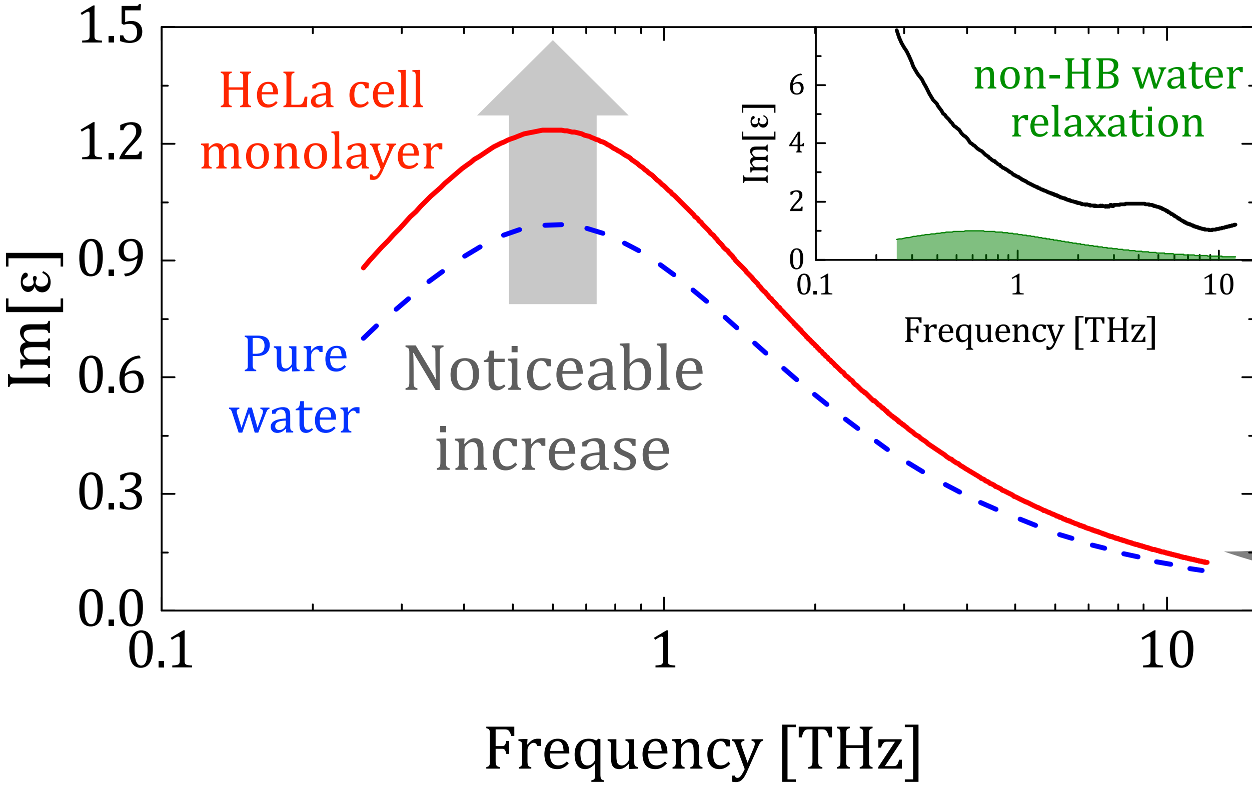
9) K. Shiraga et al., Appl. Phys. Lett. 106, 253701, (2015).
10) R. C. Ford et al., JACS 126, 4682, (2004).

(2) HB structure

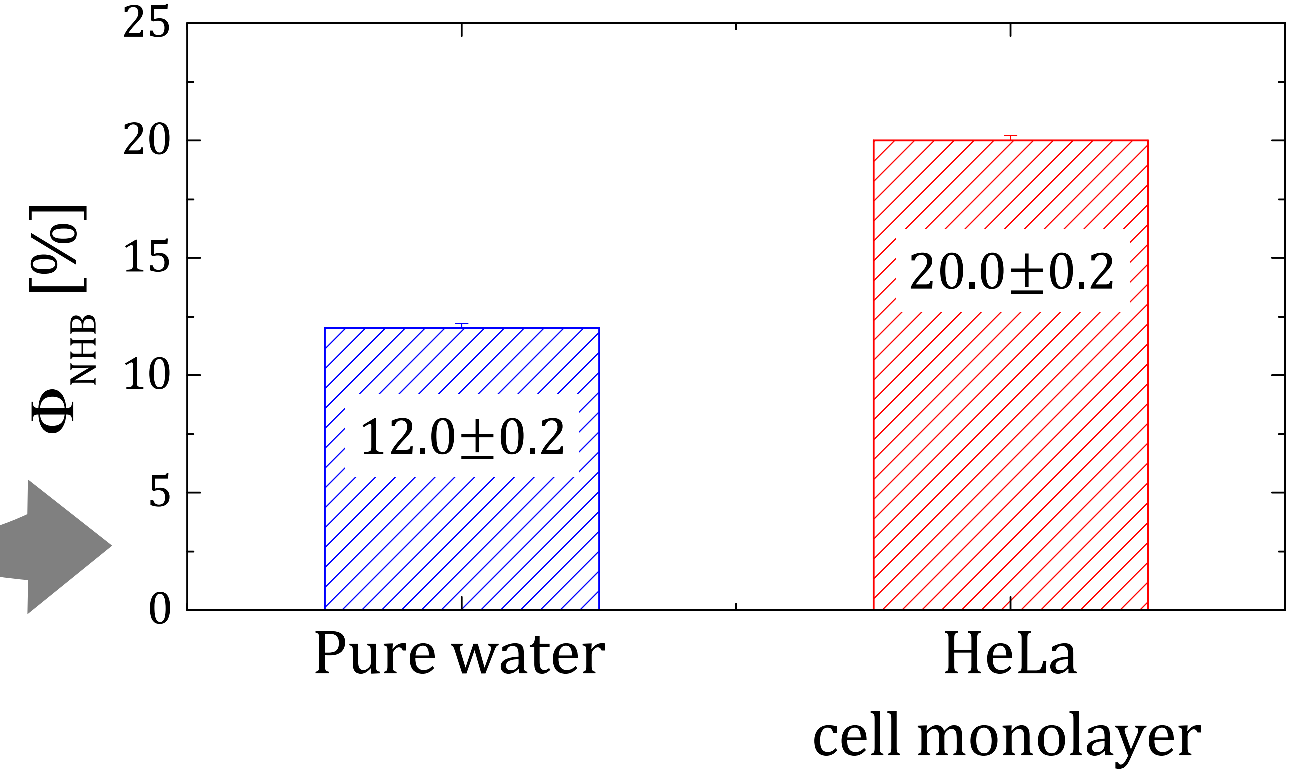
Individual relaxation of non-HB bulk water
☞ freed from the HB network (i.e. HB fragmentation)



< non-HB bulk water relaxation >



< Fraction of non-HB bulk water >



→ Fraction of non-HB water (Φ_{NHB});[11)]

$$\Phi_{NHB} = \frac{\text{(non-HB water amount)}}{\text{(Bulk water amount)}} = \frac{\dfrac{\Delta\varepsilon_2}{g_{NHB}}}{\dfrac{\Delta\varepsilon_1}{g_{HB}} + \dfrac{\Delta\varepsilon_2}{g_{NHB}}}$$

$g_{HB(NHB)}$: Kirkwood correlation factor of HB (NHB) water

→ HeLa intracellular water;
greater amount of non-HB water

☞ *"destructured"* HB network

11) K. Shiraga et al., Biophys. J. (in press).

HeLa intracellular water

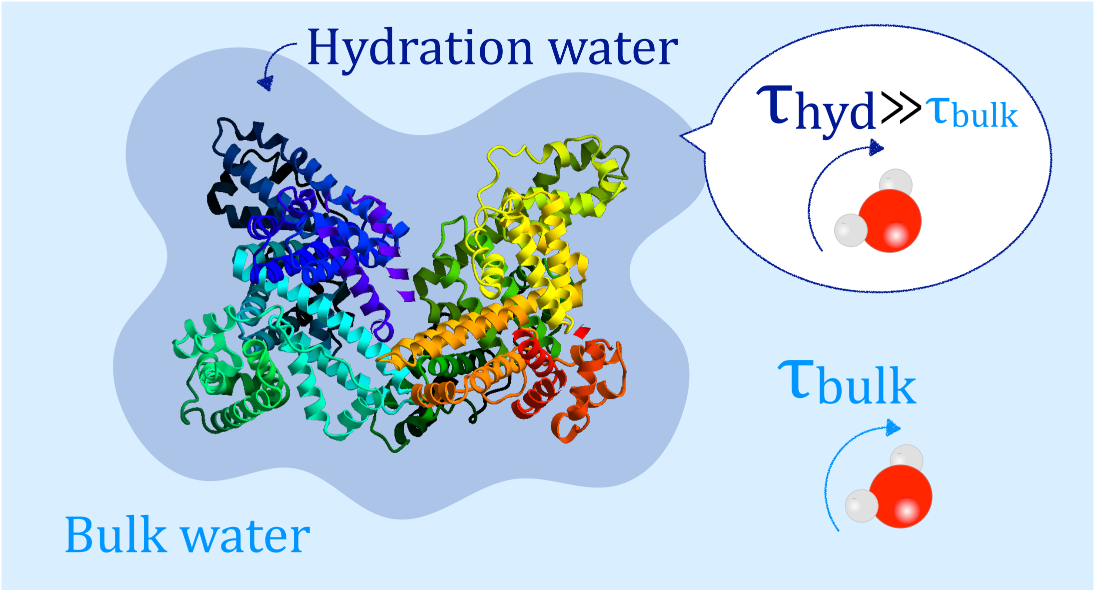
Two different characteristics of water were found in HeLa cells:

(1) Hydration state

☞ Hydration water amounts to ~24%;

(= reorientationally retarded water)

→ Dynamically "stabilized"



☞ The rest ~75% behaves as bulk water

(2) HB structure

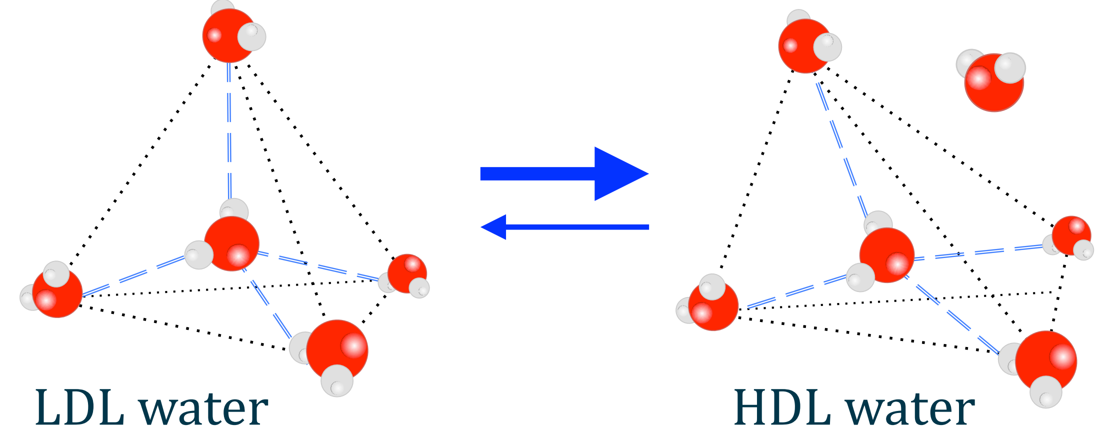
☞ Increase in the non-HB bulk water

☞ More heterogeneous HB structure

(result not shown in this presentation)

→ Structurally "disordered"

< HB structure of intracellular water >



☞ The fraction of HDL water increases

Conclusion

Dielectric responses in the THz region (0.25−12 THz);

→ "Hydration state" and "HB *destructuring*" in intact cells

☞ ~24% of intracellular water is classified as hydration water

☞ Population of HDL-like water is increased in the cell interior

THz spectroscopy: new tool to access intracellular water

Relationship between intracellular water and cell activity is unexplored
→ Reveal "biological importance" of intracellular water

Acknowledgement

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