

# THz Calorimetry: A conceptional framework for a molecular understanding of solvation entropy and enthalpy

M. Havenith<sup>1</sup>

<sup>1</sup> Department of Physical Chemistry II, Ruhr University Bochum, Germany

E-mail: martina.havenith@rub.de

**Abstract** – We lay out the concept of terahertz (THz) calorimetry, a technique that allows to deduce and quantify changes in solvation entropy and enthalpy associated with biological processes in real-time. Fundamental biological processes are inherently non-equilibrium: A small imbalance in free energy can trigger protein condensation or folding. Strikingly, water being a generic solvent, the intermolecular protein-water interactions act as a strong competitor for intramolecular protein-protein interactions, leading to a delicate balance between functional structure formation and complete solvation. Characteristics for biological processes are large but competing enthalpic ( $\Delta H$ ) versus entropic ( $\Delta S$ ) solvation contributions to the total Gibbs free energy with subtle energy differences of only a few kJ/mol, which dictate the biological function. THz calorimetry is a technique that puts a spotlight on these intermolecular coupled protein-water interactions. THz calorimetry is based exclusively on spectroscopic observables of protein hydration. Therefore, unlike traditional calorimetry, measurements can be carried out even in inhomogeneous mixtures (protein condensates) and under non-equilibrium conditions. The future impact of these studies is based on the belief that the observed changes in solvation entropy and enthalpy are not mere epiphenomena but dictate biological function.

**Keywords** – THz spectroscopy, calorimetry, solvation

Exploring the unique role of water for life is one of the top future challenges in chemistry. Whether fast protein motion and solvent dynamics are correlated at the very heart of enzymatic reactions is still under heated debate. The underlying molecular mechanism of enthalpy-entropy (H/S) compensation in protein-ligand binding remains controversial. Systematic studies under steady state conditions revealed that differences in the structure and thermodynamic properties of the waters surrounding the bound ligands are an important contributor to the observed H/S compensation. These hydration free energies are dictated by a subtle balance of hydrophobic and hydrophilic interactions.

Calorimetry served as a powerful biophysical tool by measuring changes in thermodynamic state variables, but it is restricted to measurements in equilibrium and in macroscopic samples. THz calorimetry is a novel spectroscopic approach, which allows to deduce hydration free entropy and enthalpy based on spectroscopic observables (Fig. 1). Thereby we can probe time resolutions of up to picoseconds and probe inhomogeneous samples [1-5].

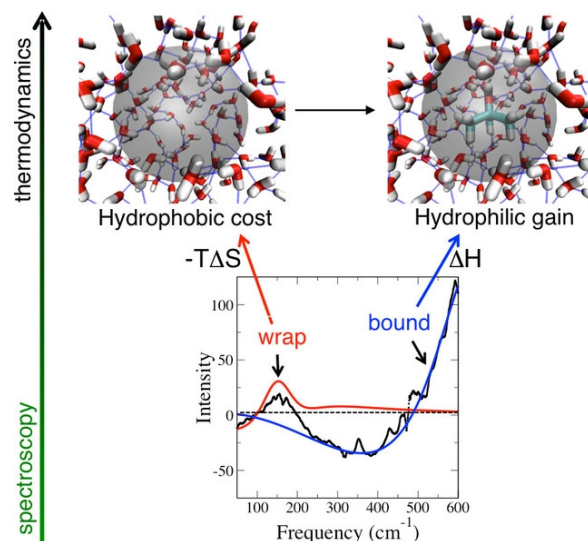


Fig. 1. THz calorimetry quantifies both terms from THz translational fingerprints of the hydration layer due to HB-stretching (red) and librational (blue). The hydration free energy is the sum of an entropic cost of cavity formation, involving formation of a HB-wrap over the whole hydration layer, and an enthalpic gain due to the attractive interactions with bound water molecules.

## ACKNOWLEDGMENTS

This work was financially supported by the Cluster of Excellence RESOLV (EXC 2033—390677874) funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and by the European Research Council (ERC) with the Advanced Grant 695437 (THz Calorimetry).

## REFERENCES

- [1] S. Pezzotti, B. König, S. Ramos, G. Schwaab, M. Havenith, *J. Phys. Chem. Lett.* **14**, 1556 (2023).
- [2] C. Hoberg, J.J. Talbot, J. Shee, T. Ockelmann, D. Das Mahanta, F. Novelli, M. Head-Gordon, M. Havenith, *Chem. Sci.* **14**, 4048 (2023).
- [3] F. Novelli, K. Chen, A. Buchmann, T. Ockelmann, C. Hoberg, T. Head-Gordon, M. Havenith, *PNAS* **120**, e2216480120 (2023).
- [4] S. Pezzotti, F. Sebastiani, E.P. van Dam, S. Ramos, V. Conti Nibali, G. Schwaab, M. Havenith, *Angew. Chem. Int. Ed.* **61**, e202203893 (2022).
- [5] J. Ahlers, E.M. Adams, V. Bader, S. Pezzotti, K.F. Winklhofer, J. Tatzelt, M. Havenith, *Biophys. J.* **120**, 1266 (2021)