Advanced Vibrational Spectroscopies – from Developments in Physical Chemistry to Biomedical Applications

849. WE-Heraeus-Seminar

22 Feb - 26 Feb 2026
at the Physikzentrum Bad Honnef/Germany

The WE-Heraeus Foundation supports research and education in science, especially in physics.
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Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 849. WE-Heraeus-Seminar:

Vibrational spectroscopy has evolved from a core discipline of physical chemistry into a powerful tool in the life sciences, offering deep insights into molecular structures, dynamics, and interactions at an unprecedented level. Recent breakthroughs in ultrafast, surface-sensitive, and super-resolution techniques have propelled vibrational spectroscopy beyond traditional limitations. Molecular dynamics can be tracked at vibrational time-scales revealing transient structures and reaction pathways with intimate detail, detection limits are lowered towards few molecules using plasmonic platforms, and vibrational nanoscale imaging approaches now rival fluorescence super-resolution microscopy. As vibrational spectroscopies have been of great importance in fundamental to applied life sciences, the recent (nano)technological advancements hold great potential to further enhance such research. As such, fascinating phenomena that tune (bio)chemical reactivity via the structure and dynamics of the surrounding environment, for instance, electrostatic or (nano)confined conditions can be studied at greater detail. At the same time, the improvements in resolution and detection limits open new frontiers in biomedically relevant applications such as nanoscale bioanalytics and label-free disease diagnostics. This meeting will bring together leading experts and emerging researchers to explore these advancements, discuss current challenges, and highlight the exciting opportunities vibrational spectroscopy offers for unveiling the complex interplay of molecular structure and function in biological and medical contexts.

Scientific Organizers:

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Introduction

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Registration:

Martina Albert (WE Heraeus Foundation)
at the Physikzentrum, reception office
Sunday (17:00 h – 21:00 h) and Monday morning

Program

Program

Sunday, February 22, 2026

17:00 – 21:00	Registration	
18:00 – 21:00	*BUFFET SUPPER*	

Monday, February 23, 2026

08:00	*BREAKFAST*	
08:55 – 09:10	Scientific organizers	**Opening, Welcome and Intro**
09:10 – 10:20	Steven Boxer	**Vibrational Stark effect spectroscopy measures local electric fields in diverse environments**
10:20 – 10:50	*COFFEE BREAK*	
10:50 – 11:40	Thomas Elsaesser	**Electric interactions and vibrational excitations in liquids and proteins probed by ultrafast terahertz methods**
11:40 – 12:30	Peter Vöhringer	**The invisible made visible with invisible light: Photochemical dynamics of transition metal complexes**
12:30	*LUNCH*	

Program

Monday, February 23, 2026

14:00 – 14:50	Marius Horch	**2D-IR Spectroscopy of Bioorganometallic Targets**
14:50 – 15:40	Martina Havenith	**Terahertz calorimetry spotlights the role of water in biological processes**
15:40 – 16:00	Lorenz Mattes	**Temperature-jump dual-comb spectroscopy for time-resolved studies of peptide folding**
16:00 – 16:30	*COFFEE BREAK*	
16:30 – 17:20	Ioachim Pupeza	**Field-Resolved Spectroscopy for Biology and Medicine**
17:20 – 18:10	Thomas Ebbesen	**Manipulating Matter by Vibrational Strong Coupling to the Vacuum Electromagnetic Field**
18:30	*DINNER*	

Program

Tuesday, February 24, 2026

08:00	*BREAKFAST*	
08:55 – 09:10	Intro	
09:10 – 10:00	Hatice Altug	**Nanophotonic metasurfaces for surfaced enhanced spectroscopy, biosensing and microarrays**
10:00 – 10:20	Gergo Peter Szekeres	**Dissecting the local and macromolecular properties of glycosaminoglycans with infrared spectroscopy**
10:20 – 10:50	***Conference Photo*** *and COFFEE BREAK*	
10:50 – 11:40	Alexander Fellows	**Azimuthal-scanning and phase-resolved vibrational sum-frequency microscopy**
11:40 – 12:30	Patrycja Kielb	**Raman spectroscopy of UV-absorbing chromophores - from UV resonance Raman towards UV surface-enhanced esonance Raman spectroscopy**
12:30	*LUNCH*	

Program

Tuesday, February 24, 2026

14:00 – 14:50	Zsuzsanna Heiner	**Real-time and label-free monitoring of enzymatic cleavage at a model membrane interface**
14:50 – 15:40	Tilmann Kottke	**Time-resolved infrared spectroscopy on soluble receptors in living cells**
15:40 – 16:00	Izabella Brand	**Spectroelectrochemical characterization of rhodopsin membrane protien in recondsituted model membranes**
16 :00 – 16 :30	*COFFEE BREAK*	
16:30 – 18:30	**Poster session**	
18:30	*HERAEUS DINNER at the Physikzentrum* *(cold and warm buffet, with complimentary drinks)*	

Program

Wednesday, February 25, 2026

08:00	*BREAKFAST*	
08:55 – 09:10	Intro	
09:10 – 10:00	Mischa Bonn	**Molecular mechanics of aqueous interfaces from surface vibrational spectroscopy**
10:00 – 10:20	Karolina Augustyniak	**The fate of stem cell: FTIR and Raman imaging-based strategy to probe cell differentiation**
10:20 – 10:50	*COFFEE BREAK*	
10:50 – 11:40	Rainer Hillenbrand	**Scattering-type near-field optical nanospectroscopy**
11:40 – 12:30	Janina Kneipp	**Spectroscopy in the local fields of plasmonic structures for the characterization of biomolecules and cells**
12:30	*LUNCH*	
14:00 – 14:50	Francesco Simone Ruggeri	**Nano-Chemical Imaging & Spectroscopy of Protein Self-assembly in Neurodegeneration**
14:50 – 15:40	Caitlin Davis	**Optical photothermal infrared imaging using metabolic probes in biological systems**
15:40 – 16:10	*COFFEE BREAK*	

Program

Wednesday, February 25, 2026

16:10 – 17:00	Daniela Täuber	**Pioneering biomedical application of high-precision mid-IR photoinduced force microscopy (PiF-IR)**
17:00 – 17:50	Hervé Rigneault	**Super-resolved wide field multiphoton and coherent Raman imaging using speckle light**
17:50 – 18:10	Scientific organizers	**Poster Awards** **Concluding remarks**
18:30	*DINNER*	

Thursday, February 26, 2026

08:00	*BREAKFAST*

End of seminar and departure

Posters

Posters

Cornelius Bernitzky	**Ambient-Temperature Detection and Characterisation of a Metal Hydride by Nonlinear IR Spectroscopy: A Case Study on [NiFe] Hydrogenase**
Ruben Cruz	**Vibrational spectroscopy of fluorous systems**
Maria Gonzalez Viegas	**An Early-Nanosecond H-bonded Cysteine Intermediate in *Cr*LOV1 Revealed by Time-Resolved Infrared Spectroscopy**
Saskia Heermant	**Towards investigating Amyloid beta on a nanoscale in a liquod environment using nanoFTIR and s-SNOM**
Sarah Zulfa Khairunnisa	**Selective SERS Sensing of Environmental Pollutants via Hyperuniform Mesoporous Gold and Halogen-Bonding MOFs**
Max Alexander Klamke	**Coupled Catalysis Powers Enzymatic CO_2 Conversion**
Christian Langlouis	**Laser-excited temperature-jump IR spectroscopy to study the impact of lipid phase transitions on membrane proteins**
Nasim Mirzajani	**Mapping In-plane Orientational Order and Correlation Lengths in Molecular Films using Azimuthal-Scanning Vibrational Sum-Frequency Generation Microscopy**
Ferhat Mutlu	**Surface- Enhanced Infrared Absorption Spectroscopy on the Interaction of Cell Penetrating Peptides with Lipid Bilayers**
Manuel Oestringer	**Investigating lipid phase transitions and the insertion of the antimicrobial peptide Gramicidin S via time-resolved temperature-jump IR spectroscopy**
Ghada Omar	**Vibrational Spectroscopic Insights into Water Adsorption in MOF (UiO-66)**

Posters

Leonard Pfister	**NanoFTIR spectroscopy of liquids at interfaces**
Frank Hendrik Pilz	**Towards monitoring radical transfer in lytic polysaccharide monooxygenases using freeze-quench stopped flow adapted to cryo resonance Raman spectroscopy**
Aleksandra Pragnąca	**Spectral signature of hypoxia. Time-dependent effects on brain endothelium revealed by FTIR and Raman imaging**
Aoife Redlich	**Under Pressure: Photoswitchable Lipid Nanodiscs Reveal Protein Response to Lateral Forces in the Bilayer**
Ingo Rimke	**Advances in light sources for Coherent Raman and TPE microscopy**
Montserrat Roman Quintero	**Exploring the Orientation of a PAS-Domain Protein at Model Protein Interfaces with Distinct Secondary-Structure Content Across Nano- and Micro-Scales**
Ramona Schlesinger	**In situ observation of „native“ membrane protein folding by surface enhanced infrared absorption spectroscopy**
Aina-Sophie Schüen	**Development of UV SERRS to study molecular principles of DNA- and RNA-ligand interactions**
Alejandro Somoza	**Driving Force and Nonequilibrium Vibronic Dynamics: Simulating Electron Transfer on Noisy Quantum Computers**
Sven Stripp	**Coupled Catalysis Powers Enzymatic CO_2 Conversion**
Paul Stritt	**Protein-induced lipid dynamics and lipid-induced protein dynamics studied by QCL-based IR spectroscopy**

Abstracts of Lectures

(in alphabetical order)

Nanophotonic Metasurfaces for Surfaced Enhanced Spectroscopy, Biosensing and Microarrays

Hatice Altug
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Nanophotonics, which excels at confining light into nanoscale volumes and generating dramatically enhanced light-matter interaction is advancing next generation optical spectroscopy, biosensing and imaging systems. We leverage nanophotonics to introduce novel bioanalytical devices that can have impact on areas including life sciences, disease diagnostics, safety and point-of-care testing [1-2]. Our expertise covers integration of nanophotonics with microfluidics, surface chemistry, and data science toolkits. We engineer nanophotonic metasurfaces with operation wavelengths spanning from visible to Infrared spectrum to exploit powerful optical techniques such as label-free refractometric sensing, molecular and structural sensitive surface enhanced enhanced vibrational spectroscopy (infrared absorption – SEIRA, Raman scattering- SERS, Circular Dichroism - SEVCD), ultra-sensitive digital biosensing, and hyperspectral imaging [3-11]. We manufacture nanophotonic chips with scalable nanofabrication methods for high-throughput and low-cost production and integrate them with microfluidics for real-time measurements in aquaous medium [8, 10-12]. We utilize data science to extract spectrally, temporally and spatially resolved sensing and imaging signals for high device performance [9-11].

In this talk, I will present some of our recent progress on Mid-IR metasurfaces. I will introduce a new conpect using resonant gradient metasurfaces with high-Q qBIC modes to achieve ultra-broadband SEIRA biosensing and vibrational strong coupling [15]. I will show merging of such metasurfaces with broadband QCL sources to perform SEIRA measurements with faster speed [16]. Next, I will present a universal strategy for controlling the chiral response with resonant mid-IR metasurfaces via the interplay of meta-atom geometry and lattice arrangements [17]. Notably, this symmetry-controlled chiral response provides a new degree of freedom in optical signal processing and showcase its use for simultaneous mid-IR image encoding in circular dichroism and transmission. Next, I will present the first nanoplasmonic infrared microarray sensor for label-free and high-throughput drug screening based on structural protein biomarkers which we applied to compound used to suppress misfolding of neurodegenerative disease related proteins. Significantly, our microarray sensor successfully quantifies secondary structural changes in drug-treated protein samples, detecting both oligomers and fibrils, where conventional fluorescence-based assays fail [18].

Acknowledgements. We acknowledge funding from Swiss State Secretariat for Education, Research and lnnovation (SERI) under contract numbers 22.00081 and 22.00018, in connection to the Horizon Europe projects MIRAQLS (101070700) and TWISTEDNANO (101046424), respectively.

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[15] Richter et al. Advanced Materials, Vol. 36, 2314279 (2024)
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[17] Sinev et al. Nature Communications, (2025)
[18] Kavungal et al. Advanced Science (2025)

The fate of stem cell: FTIR and Raman imaging-based strategy to probe cell differentiation

K. Augustyniak[1], M. Lesniak[2], J. Bejar-Grimalt[3], D. Perez-Guaita[3], M. Unger[4], J. Z. Kubiak[2,5], R. Zdanowski[2], K. Malek[1]

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The application of stem cells in clinics, especially concerning their use in regenerative medicine, continues to grow. The better understanding of their secretory properties and cell signaling pathways together with their allo-/autogenic applications, often proves to be significant in the treatment of various diseases. Given the mentioned advancements, the need for customized methodical approaches to probe and describe stem cell-related processes is also increasing.

In this study, the label-free FTIR and Raman hyperspectral imaging, detecting the molecular composition, and combined with data mining, enables non-invasive monitoring of the stem cell osteogenesis [1]. Primary adipose tissue-derived mesenchymal stem cells (AD-MSCs) isolated from C57BL6 mice were cultured. Their differentiation into osteoblasts was followed within a few weeks by Raman, FTIR, and O-PTIR imaging guided by histological staining. The commercial MCT3-E1 cells were differentiated toward mature osteoblasts (t=21 days) which were considered as a positive control. A workflow of the data analysis was designed to identify spectral predictors of the osteogenesis phases and test machine-learning (ML) methods for the rapid control of the AD-MSC differentiation degree.

The employed methodology delivered critical findings that provide insights into the structure of mature osteoblasts monolayer and its chemism. The established spectral markers of osteoblasts composition rearrangement turned out to be crucial for the AD-MSC osteogenesis monitoring, particularly at the early stages. The selected ML methods proved the high sensitivity of the IRRS metrics to differentiation-based turnover. Through the tracking of raw AD-MSC chemical images, the prediction of osteoblasts-like spectral profile was possible, enabling the rapid probing of the course of osteogenesis within cultured cells. This study introduces a novel bioassay to identify molecular markers indicating metabolic and molecular transformations in the differentiating cells and to achieve rapid and machine learning-based monitoring of MSC growth that can be also relevant to other differentiation processes.

References

[1] K. Augustyniak et al., Towards rapid quality control of bone cell formation: Raman and FTIR imaging-based prediction of AD-MSC osteogenesis; under review.

Molecular Mechanics of Aqueous Interfaces from Surface Vibrational Spectroscopy

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Short abstract:

Understanding how water, ions, and surfaces interact across length scales is central to electrochemistry, nanofluidics, and catalysis. Using surface-selective vibrational spectroscopies, we demonstrate that water exhibits distinct behavior when confined at the nanoscale, with interfaces significantly influencing its structure, dynamics, and charge.

Long abstract:

Understanding how water, ions, and surfaces interact across length scales is central to electrochemistry, nanofluidics, and catalysis. In particular, the impact of hydrogen-bond network termination, interfacial charges, and their influence on the arrangement of counterions and water has been intensely debated. Across three studies, we have established a molecular-level picture of structure and dynamics at aqueous interfaces under confinement and electrostatic perturbation, using advanced nonlinear vibrational spectroscopy to probe both static properties and ultrafast dynamics.

We have demonstrated that, down to the angstrom-scale confinement, interfacial effects entirely determine the organization of confined water, disrupting bulk-like hydrogen bonding and producing asymmetric environments due to wall contact [Nature Commun. 16, 7288 (2025)]. These findings establish that nanofluidic behavior is governed not by the confined volume, but by its bounding interfaces. We extended this picture to show that even nominally neutral materials, such as hexagonal boron nitride, acquire a spontaneous surface charge at the aqueous interface [J. Am. Chem. Soc. 147, 30107 (2025)]. This intrinsic charging, observed in other solids as well, indicates that the formation of an electric double layer (EDL) is nearly universal at solid–liquid boundaries. Finally, we resolved the ultrafast dynamics of the aqueous EDL using femtosecond-resolved optical spectroscopy, showing that ionic rearrangements occur within tens of picoseconds — faster than diffusion-limited models predict [Science 388, 405 (2025)]. Together, these studies provide a molecularly consistent understanding of how interfacial polarization, confinement, and charge collectively define water's behavior in nanofluidic and electrochemical environments.

Vibrational Stark effect spectroscopy measures local electric fields in diverse environments

Steven G. Boxer, Stanford University, Stanford CA 94305

Non-covalent interactions in the condensed phase can be approximately described classically using the language of electric fields. We have enabled the molecular-scale measurement of electric fields using the framework of the vibrational Stark effect (VSE). Vibrational Stark spectroscopy was first measured in our lab in the condensed phase by applying an external electric field to simple aromatic nitriles in a frozen glass. This measurement gives the intrinsic Stark tuning rate which is the sensitivity of the vibrational frequency to an electric field. Stark tuning rates have been obtained for diverse diatomic oscillators including nitriles, carbonyls, alkynes, azides, C-D and C-F bonds and recently triatomic oscillators like carboxylate. Stark tuning rates can also be obtained by measuring vibrational frequency shifts in a wide range of solvent polarities (vibrational solvatochromism) and connecting frequencies to fields using MD simulations. Lastly, Stark tuning rates can be obtained by *ab initio* calculations using various levels of theory. These three methods each offer advantages and disadvantages, as well as different approximations. We look for convergence among these approaches or focus on the origin(s) of differences. Recent work uses nitrile frequency shifts and intensities as a benchmark for advanced polarizable force field simulations *[1]*. Once calibrated, these molecular-scale probes can be used to extract *local*, molecular scale information on electric fields in a variety of molecular systems.

Carbonyl groups (-C=O) are often involved in enzyme catalysis. Since our first demonstration that the fields projected on the carbonyl group at the active site can be very large and is directly correlated with the activation free energy for catalysis, we have expanded the scope to enzymes where the fields can be made larger than wild-type *[2]* and to two *[3]* and three *[4]* dimensions by incorporating multiple probes, mostly C-D bonds, in key locations and geometries around the active sites of several enzymes. Our goal is to understand the electrostatic contribution to catalysis, the role of electrostatic preorganization at the active site that may be responsible for the incredible catalytic proficiency of enzymes, and the coupling between conformational changes and electrostatics. Recent progress towards this goal will be described.

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Spectroelectrochemical characterization of rhodopsin membrane protien in recondsituted model membranes

J. Bozek[1], P. Bielski[2], K.W. Koch[2] and I. Brand[1]

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The membranes of the outer segment of a rod photoreceptor are rich in rhodopsin, a transmembrane protein. Rhodopsin is activated by light and initiates the primary signaling pathway in visual perception. The structure and function of rhodopsin are well known serving as an excellent model for our biomimetic studies.

Bicelles provide a perfect environment to introduce an active transmembrane protein into the bilayer core. A bicelle is a disc-shaped aggregate that contains a core lipid bilayer [DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine)] and a rim made of a detergent lipid [DHPC (1,2-dihexanoyl-sn-glycero-3-phosphocholine)]. Rhodopsin was introduced into the DMPC/DHPC bicelles. Dark adapted rhodopsin-filled bicelles were spread into a thioglucose modified Au(111) electrode surface to form a floating membrane. Electrochemical techniques provide information about the changes of the membrane conductivity and capacitance. The floating bilayers remained stable over a wide membrane potential window (-0.6 to 0.1 V), demonstrating their robustness for electrochemical studies.

Use of polarization modulation infrared reflection absorption spectroscopy (PM IRRAS) under electrochemical control allowed us to investigate changes in the packing and order of lipids and α-helices in rhodopsin as a function of the membrane potential. PM IRRAS studies revealed that the acyl chains in DMPC exist predominantly in a gel phase with an average tilt of 30° relative to the surface normal, suggesting a well-organized lipid arrangement. Furthermore, IR spectral analysis of rhodopsin within the bilayer confirmed that α-helices remain the dominant secondary structure element, indicating that the protein retains its native fold. The average orientation of the α-helices in rhodopsin depends on both the protein illumination state (dark adapted or photobleached) and the membrane potential. PM IRRAS made possible assignment of subtle changes in the protein arrangement in the membrane to its different activity-dependent states.

A floating membrane with embedded transmembrane protein enables studies of the membrane-based protein-protein interactions.

Optical photothermal infrared imaging using metabolic probes in biological systems

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Infrared spectroscopy is a powerful tool for identifying biomolecules. In biological systems, infrared spectra provide information on structure, reaction mechanisms, and conformational change of biomolecules. However, the promise of applying infrared imaging to biological systems has been hampered by low spatial resolution and the overwhelming water background arising from the aqueous nature of in cell and *in vivo* work. Recently, optical photothermal infrared microscopy (OPTIR) has overcome these barriers and achieved both spatially and spectrally resolved images of live cells and organisms. Here, we determine the most effective modes of collection on a commercial OPTIR microscope for work in biological samples. We examine three cell lines (Huh-7, differentiated 3T3-L1, and U2OS) and three organisms (*E. coli*, tardigrades, and zebrafish). Our results suggest that the information provided by multifrequency imaging is comparable to hyperspectral imaging while reducing imaging times twenty-fold. We also explore the utility of IR active probes for OPTIR using global and site-specific noncanonical azide containing amino acid probes of proteins. We find that photoreactive IR probes are not compatible with OPTIR. We demonstrate live imaging of cells in buffers with water. ^{13}C glucose metabolism monitored in live fat cells and *E. coli* highlights that the same probe may be used in different pathways. Further we demonstrate that some drugs (e.g. neratinib) have IR active moieties that can be imaged by OPTIR. Our findings illustrate the versatility of OPTIR, and together, provide a direction for future dynamic imaging of living cells and organisms.

References

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Manipulating Matter by Vibrational Strong Coupling to the Vacuum Electromagnetic Field

T. W. Ebbesen[1]

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Over the past decade, the possibility of manipulating material and chemical properties by using hybrid light-matter states has stimulated considerable interest [1,2]. Such hybrid light-matter states can be generated by strongly coupling the material to the spatially confined electromagnetic field of an optical resonator. Most importantly, this occurs even in the dark because the coupling involves the electromagnetic fluctuations of the resonator, the vacuum field. After introducing the fundamental concepts, examples of modified properties of strongly coupled systems, such as chemical reactivity, charge and energy transport, magnetism, will be given to illustrate the broad potential of light-matter states.

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Electric interactions and vibrational excitations in liquids and proteins probed by ultrafast terahertz methods

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Electric interactions have a significant impact on the properties of liquids, proteins, and molecular materials. The long-range character of Coulomb interactions and their ultrafast fluctuations in liquids and proteins at ambient temperature pose major challenges for a quantitative understanding. Novel methods of nonlinear terahertz (THz) spectroscopy [1] give experimental access to transient molecular electric fields, molecular dipole moments and interactions of electrons and ions with a polar environment. In this talk, recent results from THz Stark spectroscopy of molecular chromophores and two-dimensional THz spectroscopy of collective vibrational dynamics in polar liquids are presented.

Ultrafast THz Stark spectroscopy employs external THz electric fields of up to several megavolts/cm to modify electronic absorption spectra and derive transient excited-state dipole moments and polarizabilities of chromophores in a condensed-phase environment [2,3]. Working on a time scale shorter than molecular reorientation processes, this technique reveals the purely electronic response of a structurally 'frozen' molecular ensemble. Recent work gives insight in dipole moments of betaine-30 in polar liquids and of the retinal chromophore in bacteriorhodopsin and neorhodopsin, allowing for an in-depth analyis of excited-state electronic structure.

Electrons solvated in polar liquids such as water and alcohols display both single-particle and many-body excitations [4]. The latter are of a polaronic character and manifest in a distinct contribution to the THz dielectric response. Two-dimensional THz spectroscopy reveals long-lived coherent polaron oscillations, which arise upon impulsive excitation, are of a longitudinal character, and encompass an ensemble of several ten thousands of solvent molecules around the electron. Such polarons display a pronounced optical nonlinearity [5] and directly affect the optical dispersion of the liquid [6].

References

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Azimuthal-Scanning and Phase-Resolved Vibrational Sum-Frequency Microscopy

Ben John[1], Nasim Mirzajani[1], Tuhin Khan[1], Martin Wolf[1], Martin Thämer[1], and Alexander P. Fellows[1]

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Structural heterogeneity, manifesting as variations in density, composition, and packing, is an abundant feature in a wide range of molecular interfaces that play critical roles in important natural processes. In fact, in most cases, it is precisely this heterogeneity that profoundly impacts the behaviour of the system and controls its macroscopic functionality. Nevertheless, characterising the complex structural intricacies in such molecular assemblies is a formidable challenge.

Here, we introduce phase-resolved sum-frequency generation (SFG) microscopy as a method to resolve the heterogeneous structure in molecular films. By combining a resonant IR excitation with an off-resonant visible upconversion, we obtain wide-field images the resulting SFG signals. Owing to their second-order symmetry properties, the sign of the SFG response (which we ascertain through heterodyne-detection) is dependent on the absolute molecular orientation, thus giving access to the specific packing structure and being highly sensitive to orientational ordering. By performing full hyperspectral imaging in different polarisation combinations, we map the different components of the 3D structures. Furthermore, through an azimuthal-scanning scheme whereby SFG images are recorded as a function of sample rotation, we gain unprecedented access to the in-plane orientational distribution.

Here we demonstrate the capabilities of this microscope on model membrane systems consisting of phase-separated lipid monolayers. We firstly highlight the sub-monolayer sensitivity of the technique and, thereafter, utilise the full 5-dimensional imaging to characterise the heterogeneous structure across the film. Specifically, we map properties such as the density and composition, showing clear phase-separation and compositional enrichment, and we fully characterise the 3D orientational distribution of the molecules. From this, we decipher their orientational order, tilt angles, in-plane chiral packing motifs, and the conformational arrangements of their two tail-groups. This demonstration highlights the potential of SFG microscopy in characterising molecular structures through high-resolution, chemically specific, and label-free imaging of molecular organisation at complex interfaces.

Terahertz calorimetry spotlights the role of water in biological processes

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Terahertz (THz) calorimetry is a framework that allows for the deduction and quantification of changes in solvation entropy and enthalpy associated with biological processes in real-time (Figure 1). Fundamental biological processes are inherently non-equilibrium, and a small imbalance in free energy can trigger protein condensation or protein folding. Although biophysical techniques typically focus mainly on structural characterization, water is often ignored. 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 (ΔH) and entropic (ΔS) solvation contributions to the total Gibbs free energy lead to subtle energy differences of only a few kJ mol^{-1} that are capable of dictating biological functions. THz calorimetry spotlights these intermolecular coupled protein–water interactions. With experimental advances in THz technology, a new frequency window has opened, which is ideally suited to probe these low-frequency intermolecular interactions [1]. The future impact of these studies is based on the belief that the observed changes in solvation entropy and enthalpy are not secondary effects but dictate biological function.

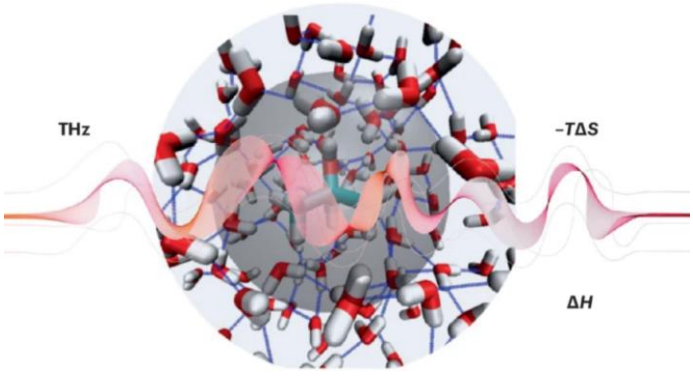

Figure 1: THz calorimetry spotlights the intermolecular coupled protein–water interactions and probes changes in solvation entropy and enthalpy associated with biological processes in real-time.

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Real-Time and Label-Free Monitoring of Enzymatic Cleavage at a Model Membrane Interface

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Biological membranes form highly dynamic reaction platforms for macromolecular interactions, yet resolving enzymatic processes at these interfaces remains challenging due to their structural and chemical complexity. Acid sphingomyelinase (aSMase) is a critical lysosomal enzyme responsible for sphingomyelin hydrolysis into ceramide and phosphorylcholine, and its dysfunction is central to Niemann–Pick disease pathology.[1] Conventional approaches to study lipid–enzyme interactions typically depend on fluorescent or chemical labels, which can disrupt membrane architecture and bias enzymatic activity. In this work, we introduce a label-free, real-time, and in situ strategy to monitor sphingomyelin hydrolysis at model membrane interfaces by combining Langmuir monolayers with vibrational sum-frequency generation (VSFG) spectroscopy. The Langmuir platform provides precise control over lipid packing and membrane composition, while VSFG selectively probes interfacial molecular vibrations,[2] enabling direct observation of PO_2^- stretching and amide I signals during enzymatic cleavage. Using this approach, we systematically examined how cholesterol content and environmental pH modulate aSMase activity, demonstrating that both membrane composition and acidity critically influence enzymatic hydrolysis.[3] By avoiding extrinsic probes, this method yields an unperturbed and physiologically relevant view of lipid–enzyme interactions. Overall, our label-free platform offers a robust framework for studying sphingolipid metabolism and provides valuable insight for therapeutic strategies targeting Niemann–Pick disease and related membrane-associated disorders.

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Acknowledgement

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2D-IR Spectroscopy of Bioorganometallic Targets

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Organometallic motifs play a key role in several metalloproteins. For instance, hydrogenases catalyze the reversible cleavage of dihydrogen, a clean fuel, by utilizing base-metal active sites containing CO and CN^- ligands. On the one hand, these ligands tune the electronic structure of the active sites; on the other hand, they give rise to structurally sensitive bond-stretching vibrations. Therefore, IR spectroscopy has long been used to study hydrogenases and other (bio)organometallic targets. To expand this approach, we have introduced experimental and computational 2D-IR spectroscopy to hydrogenase research.[1-7] Utilizing the multitude of vibrational transitions accessible by this approach, detailed insights into the vibrational and molecular structure of the enzymatic active sites were obtained. We found that diagonal and off-diagonal anharmonicities represent highly sensitive observables that can be calculated with exceptional accuracy.[3,4,6,7] Analyzing these quantities, the localization of CO and CN stretching modes could be determined for the first time.[1,3,4] In the localized limit, vibrational ladder climbing provides novel insights into the bond properties of the diatomic ligands that challenge the general understanding of organometallic bonding.[1,2] In the delocalized limit, information on active-site symmetry and its tuning by the protein matrix are obtained.[4,6] Due to strong coupling between bright and dark modes, dipole-forbidden transitions can also be probed.[5,7] This approach proved the presence of a bridging CO ligand in central catalytic states of high-activity hydrogenases, thereby resolving the debate about their catalytic mechanism.[5] In the same way, metal-hydride bond properties of another key intermediate could be probed.[7] All this information is encoded in the experimental 2D-IR data and understandable by anharmonic frequency calculations. This combination therefore represents a powerful and transferable approach for the elucidation of (bio)organometallic structure.

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Raman Spectroscopy of UV-absorbing chromophores - from UV resonance Raman towards UV surface-enhanced Resonance Raman Spectroscopy

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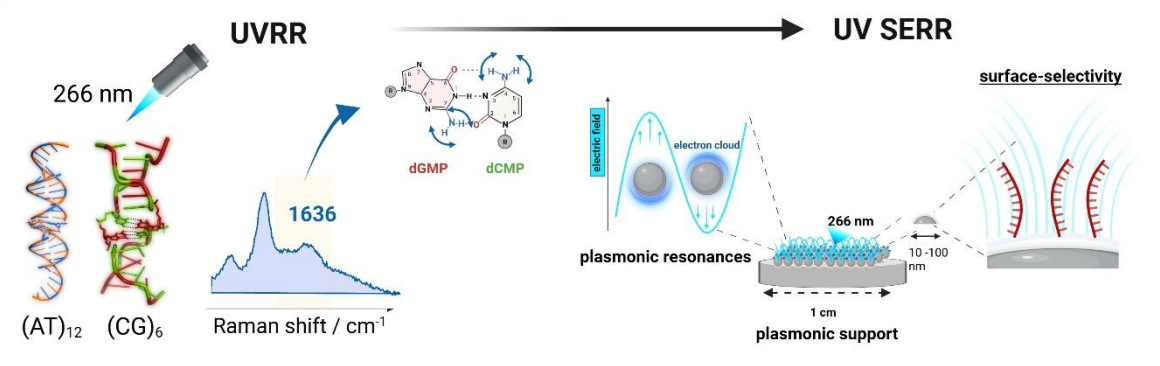
Raman spectroscopy has been essential for elucidating the molecular fingerprints of visible-light-absorbing biological chromophores by exploiting enhancement mechanisms. Resonance Raman (RR) spectroscopy provides label-free and chemically selective detection, while surface-enhanced Raman spectroscopy (SERS) lowers detection limits to femtomolar concentrations; their combination leverages the advantages of both approaches. However, many biologically important molecules absorb in the ultraviolet (UV), including nucleic acids and aromatic amino acids, yet their structures and structural changes remain comparatively underexplored due to the lack of UV-adapted spectroscopic technologies. We aim to close this gap by extending Raman spectroscopy into the UV regime, through the exploitation of UV electronic transitions of nucleic acids (NAs) in UV resonance Raman (UVRR) spectroscopy in solution, and by developing plasmonic supports for UV SERS. NAs provide a biologically central example, as their hybridization is a fundamental process in DNA biology and a key mechanism in aptamer-based therapeutics. In our recent work[1], using UVRR we accessed structural details of nucleic acid assembly and identified hydrogen-bonding-sensitive marker bands in individual nucleotides and G-C and A-T base pairs by following the conformational assembly during thermal hybridization. While UVRR provides ensemble-averaged information in solution, resolving NA-NA interactions in a conformation- and orientation-dependent manner requires the integration of UVRR and UV SERs. We are therefore currently working towards developing nanostructured UV-resonant plasmonic supports to enable studies of interfaced NA behavior with enhanced sensitivity and selectivity toward the conformational changes.



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Spectroscopy in the local fields of plasmonic structures for the characterization of biomolecules and cells

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Vibrational (Raman and infrared) spectroscopy has become indispensable for our understanding of the structure and interaction of organic molecules in materials and biomolecular samples.[1] Surface enhanced Raman scattering (SERS) is not restricted to the well-known one-photon excited spontaneous Raman process that gives information on molecular composition, structure, and interaction through vibrational probing with high sensitivity. High local fields, specifically those provided by localized surface plasmon resonances of metal nanostructures, can also support non-linear Raman scattering, as it depends on the involved fields to higher powers. Non-linear Raman processes provide a very sensitive access to the properties of metal nanomaterials and their interfaces with molecules and other materials.

The discussion will highlight the aspects of vibrational information that can be gained on molecule-molecule interactions, including those in complex environments. Apart from (field-enhanced) Raman scattering of known molecules, also their utilization in complex mixtures in living cells is within reach.[2] Possibilities to retrieve information from such data, and also their combination with nanoscale infrared experiments with biosamples [3] will be shown. As an example, SERS data from the membrane of eukaryotic cells [4] and nano-IR spectra obtained with an s-SNOM can be combined to learn more about the organization of the cell membrane directly in the living system.

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Time-Resolved Infrared Spectroscopy on Soluble Receptors in Living Cells

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Results from spectroscopic investigations on mechanisms of isolated proteins *in vitro* are challenged by the differences in conditions as compared to their native cellular environment. We developed in-cell infrared difference spectroscopy (ICIRD) as a non-invasive and label-free tool to study soluble receptors in living cells in attenuated total reflection and transmission [1]. Application to the photoreceptor light-, oxygen-, voltage (LOV) in living bacteria showed that there are deviations in the structural response to light as compared to isolated receptors, which however can be fully emulated by adding proteins as macromolecular crowders. Time-resolved spectroscopy on LOV-effector proteins verified an intact signaling mechanism with similar kinetics in living bacteria as *in vitro* [2]. We then expanded the application of ICIRD to include human cell lines as a highly complex system directly cultivated inside the spectrometer [3]. Here, cellular effects on the structure and response of LOV could not be rationalized by effects such as molecular crowding, dehydration, or temperature. Accordingly, we identified a strong impact of the eukaryotic cellular environment, which needs to be considered in physiology and optogenetic applications.

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Temperature-Jump Dual-Comb Spectroscopy for Time-Resolved Studies of Peptide Folding

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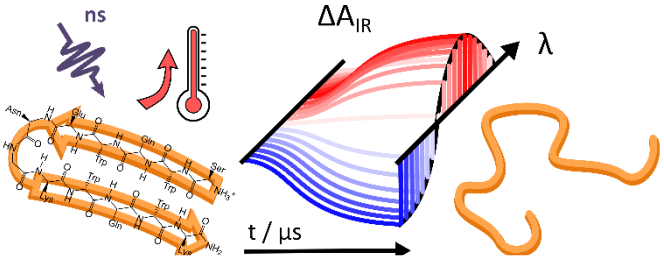

Temperature-jump IR spectroscopy reveals folding dynamics of a β-hairpin peptide. QCL-based dual-comb detection enables broadband spectral access and complements single-wavenumber methods with mechanistic details.

Time-resolved IR spectroscopy provides direct insight into protein conformational dynamics, yet capturing both fast kinetics and broad spectral information remains challenging. Single-wavenumber quantum cascade laser (SW-QCL) spectroscopy offers excellent signal-to-noise ratios and nanosecond time resolution. Combining dual-comb spectroscopy (DCS) with nanosecond laser-induced temperature-jump (T-jump) experiments establishes a versatile platform for monitoring peptide folding dynamics with high temporal resolution and simultaneous broadband spectral coverage. A nanosecond Ho:YAG laser pulse excites the overtone vibration of D_2O, inducing a rapid temperature jump of several Kelvins. DCS records amide I′ modes across almost 70 cm^{-1} spectral range with 1 µs time resolution, providing a broadband view of structural changes without the need for spectral scanning.

Using this approach, we investigate T-jump-induced dynamics of the β-hairpin model peptide Trpzip-Q_2. Protein and peptide folding are central to biological function, and misfolding can lead to disease-related aggregation, such as polyglutamine diseases, where expanded glutamine tracts promote oligomerization and neuronal toxicity. In the amide I′ region we can see the loss of ordered hairpin structure, the increase of disordered conformations, and - if present - the dynamics of oligomeric species. While SW-QCL measurements provide access to very fast dynamics at selected wavenumbers, DCS captures the simultaneous spectral evolution of all relevant structural markers, yielding a complementary picture of peptide conformational dynamics. [1]

We are extending this methodology by ^{13}C isotope labels in the side chains and backbone of the peptide. This allows residue-specific tracking of folding dynamics and a detailed mapping of mechanistic pathways. By combining broadband, microsecond-resolved DCS with rapid T-jump initiation, this platform opens new opportunities to investigate peptide and protein folding landscapes.

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Field-Resolved Spectroscopy for Biology and Medicine

Ioachim Pupeza

The outstanding control over the individual oscillations of the electromagnetic field of light afforded by modern femtosecond laser architectures, has enabled manifold breakthroughs in science and technology. This talk will review recent contributions of our research group to electric-field-resolved spectroscopy (FRS) in the infrared (IR) molecular fingerprint region [1]. Trains of millions of few-cycle IR pulses per second, with optical waveforms reproducible down to the (sub-)attosecond range [2] have now become available. Field-sensitive detection of the optical response of molecular samples to excitation with these waveforms has recently provided a combination of detection sensitivity, linear dynamic range and bandwidth unprecedented in molecular spectroscopy [3,4]. These results constitute key steps toward infrared (non-)linear spectroscopy of solids, liquids, and gases at the ultimate limits set by the nature of light. In particular, we will address novel biomedical applications of these spectroscopic techniques which are currently being developed in our group, including high-throughput FRS flow cytometry and breath analysis via FRS for the intensive-care unit.

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Super-resolved wide field multiphoton and coherent Raman imaging using speckle light

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In this presentation, I will discuss our recent advances in wide-field nonlinear imaging. We demonstrate that wide-field two-photon imaging over a field of view exceeding 200 µm is achievable using speckle illumination with short (150 fs), high-energy (µJ) laser pulses at a low repetition rate (100 kHz). Speckle illumination enables axial sectioning with a resolution of a few micrometers and improves spatial resolution to nearly twice below the diffraction limit through Random Illumination Microscopy (RIM). Additionally, I will show that RIM can be applied to Coherent Anti-Stokes Raman Scattering (CARS), provided that the nonlinear contrast is rendered incoherent [1].
I will then present our latest, unpublished results, in which we combined speckle illumination with temporal focusing. This approach enables two-photon and CARS imaging over fields of view greater than 500 µm, with axial sectioning at 10 frames per second.
These developments are aimed to perform chemical imaging over large field of views at video rates for biomedical applications [2].

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Nano-Chemical Imaging & Spectroscopy of Protein Self-assembly in Neurodegeneration

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A key challenge in biosciences is the comprehension of how the physical-chemical state and heterogeneity of biomolecules determine their role in cellular function and disease. Such as in the case of protein, whose self-assembly and formation of amyloid-like species is implicated in the onset of neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases [1]. While nanoscale imaging methods can characterise protein phase transitions, imaging alone is to the most part chemically blind hampering to retrieve their physical-chemical state. There is thus compelling need of innovative nano-analytical methods allowing single-molecule analysis of these biomolecular processes beyond simple imaging.

Here, we showcase how multimodal analysis by infrared nanospectroscopy (AFM-IR) can contribute to shedding light onto the molecular basis of protein self-assembly in neurodegeneration. We have first proved that AFM-IR can study protein self-assemblies to unravel their morphological, mechanical and chemical-structural properties in relationship to their function in life and malfunction in disease [2, 3]. We have then developed and pushed the sensitivity limits of AFM-IR to achieve single molecule and protein self-assembly level [4,5]. We can in turn apply this unpreceded sensitivity to answer critical questions in the field, such as unravelling the internal heterogenous structure of single protein condensates [3], characterise the secondary structure and post-translational state of single amyloid species [6]; as well as study the functional interaction of protein self-assemblies with other biomolecules in life [7], and with small molecules drugs against neurodegeneration [8].

Overall, our aim is to expand the capabilities of analytical nanoscience to shed light on the structure-activity relationship of biomolecules in life and disease.

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Dissecting the local and macromolecular properties of glycosaminoglycans with infrared spectroscopy

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Glycosaminoglycans (GAGs) are linear polysaccharides that play a crucial role in physiological processes due to their strong polyanionic character and varying sulfation pattern.[1] These molecules show significant structural diversity from chain length to sequence due to their non-template-driven biosynthesis and potential for epimerization along the chain. As a result, vibrational spectroscopy of GAGs generally focuses on sample purity assessment and sequencing, while their fundamental structural understanding has been mostly neglected. A deeper look into their vibrational signature, however, reveals unique features allowing us to elevate the structural understanding of GAGs to the levels established for proteins and nucleic acids.
In this work, the structural aspects of GAGs are probed using various IR spectroscopy techniques both in solution and in the gas phase.[2,3] Cryogenic gas-phase IR spectroscopy coupled with ion mobility separation allows for the isolation of specific conformers prior to excitation, which provides a detailed view of local structural features, including intra- and intermolecular interactions as well as the local symmetry. In parallel, condensed-phase methods offer more robustness against chain diversity and facilitate structural studies under physiologically relevant conditions. Here, ATR-FTIR results help assess the steric accessibility of sulfate groups, distinguishing between flexible and structurally defined configurations that govern interaction, whereas vibrational sum-frequency generation provides insight into the macromolecular conformation and chirality.

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Pioneering biomedical application of high-precision mid-IR photoinduced force microscopy (PiF-IR)

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Mid-infrared photoinduced force microscopy (PiF-IR) is a young imaging technique that combines powerful infrared (IR) illumination with mechanical detection via true non-contact atomic force microcopy (AFM). This enables the chemical characterization of surfaces with an unprecedented spatial (≈ 5 nm) and high spectral resolution, providing the sensitivity for local secondary protein structure evaluation [1]. We applied PiF-IR to map the local chemistry and structure of the antibiotic interaction on the surface of individual bacterial cells using the model system Bacillus subtilis and vancomycin [2] and to map the surface of retina pigment granules. AFM-IR methods [3] differ from conventional infrared (IR) spectroscopy by probing local and heterogeneous sample positions instead of achieving bulk IR spectra. This necessitates the consideration of additional parameters for the establishment of reliable quality control. Setup geometry as well as molecular alignment matter, resulting in anisotropic signal detection [2,4,5]. We used the unprecedented spatial resolution of PiF-IR and combined experiments with modeling [4,5] to study effects from molecular alignment [5] and hybrid field coupling [4] on nanostructured organic surfaces. The high precision of PiF-IR enabled us to evaluate chemical contaminations introduced during scanning by probe-sample contact [2]. Our home-written analysis software “hyPIRana” employs a chemometrics approach to retrieve local chemical information from hyperspectral PiF-IR data [1,2,5].

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The Invisible Made Visible with Invisible Light: Photochemical Dynamics of Transition Metal Complexes

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For decades, infrared (IR) spectroscopy has been a valuable tool to elucidate the static structure of molecules – in the gas phase, in liquid solution, and in solid matrices. When carried out with pulsed electric fields, it can provide direct access to the dynamic evolution of the structure of molecules, either at thermal equilibrium or in response to an external stimulus, e.g. a photonic excitation. Time-resolved IR-spectroscopy can be conducted in distinct modes of operation thereby giving access to time scales from tens of femtosecond to hundreds of seconds. [1]
Here, we will report on the utility of these techniques in studies of the photochemistry of transition metal (TM) complexes. Depending upon time, we will touch on either of the three specific topics:
(i) TM-carbon dioxide binding: using ligand-to-metal charge transfer excitation of TM-oxalates, CO_2 complexes can be prepared *in situ* and information about their molecular structure can be obtained from experimental time-resolved IR-data combined with electronic structure calculations. [2-4]
(ii) TM-nitride and nitrene formation: electronic excitation of late TM-azides yields non-isolable terminal nitrido and nitreno complexes through dinitrogen cleavage. Despite their fleeting nature, the chemical reactivity of these species can also be tested in intricate quenching studies. [5-8]
(iii) Titanium-based photo-redox catalysis: catalytic transformations relying on the earth-abundant metal, titanium, and light open the avenue to single-electron-transfer chemistry, i.e. chemistry with radicals! We will show how femtosecond spectroscopy can monitor the entry events into photo-redox catalytic cycles.

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Abstracts of Posters

(in alphabetical order)

Ambient-Temperature Detection and Characterisation of a Metal Hydride by Nonlinear IR Spectroscopy: A Case Study on [NiFe] Hydrogenase

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[NiFe] hydrogenases catalyse the conversion of dihydrogen (H_2) – a perfectly clean fuel – by utilizing a protein-embedded [NiFe] centre that carries CO and CN^- ligands. These can serve as structurally sensitive vibrational probes. Thus, infrared (IR) spectroscopy and harmonic vibrational analyses have long been used for studying hydrogenases. The active Ni_a-C state of the catalytic cycle is commonly accepted to harbour a bridging hydride ligand, based on low-temperature data from HYSCORE, ENDOR, and – more recently – NRV spectroscopy.[1,2] Direct detection at ambient temperature has not been reported so far, though, raising the question about the structure of the Ni_a-C intermediate under catalytic conditions. While IR spectroscopy could be used for probing the metal-hydride modes, these are overlapped by protein signals and potentially broadened beyond detection. Indirect detection by exploring the hydrogen/deuterium (H/D) isotope exchange sensitivity of the CO and CN stretch modes also failed in the sense that not even indirect proof through these very sensitive and unobscured diatomic ligands was observed. We have therefore expanded this strategy by introducing 2D-IR[3] and IR_{pump}-IR_{probe} spectroscopy and found that the hydride can be detected through pronounced H/D sensitivity of the vibrational lifetime of the CO stretch mode. Additionally, analysis of the CN stretch-mode anharmonicities in combination with generalized second-order vibrational perturbation theory (GVPT2) indicates that the geometry and charge distribution of the Ni_a-C state are constrained by the protein scaffold. This finding highlights the importance of structural confinement in metalloenzyme catalysis and provides new perspectives for designing synthetic H_2-converting catalysts. In total, these findings and strategies provide new perspectives for understanding structural, electronic, and dynamic properties of complex (bio)organometallic targets.

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Vibrational spectroscopy of fluorous systems

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Abstract:

Per- and polyfluoroalkyl substances (PFAS) have become common and persistent environmental contaminants. In particular, long-chain perfluorocarboxylic acids such as perfluorooctanoic acid (PFOA) are bioaccumulative and toxic to human health. This toxicity likely arises from the characteristic omniphobicity of perfluoroalkyl chains, which exhibit both hydrophobic and lipophobic behavior. In our research, we investigate the interactions between these molecules and their environment using FT-IR spectroscopy combined with density-functional theory (DFT) and molecular dynamics (MD) simulations. We first examine the use of C–F stretching vibrations to estimate local electric fields. We then explore how these molecules organize in self-assembled monolayers and how water arranges itself at the fluorous interface, using surface-enhanced infrared spectroscopy (SEIRAS). Our results show increased molecular ordering for perfluoroalkyl tails containing more than seven CF_2 groups, as well as the presence of a characteristic dangling-water orientation at hydrophobic fluorous surfaces.

An Early-Nanosecond H-bonded Cysteine Intermediate in *Cr*LOV1 Revealed by Time-Resolved Infrared Spectroscopy

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Abstract:
Light-oxygen-voltage (LOV) domains are blue-light photoreceptor domains found across bacteria, higher plants and green algae. Their small size and natural coupling to diverse effector domains, like sulfate transporters or serine/threonine kinases, has made them very suitable for optogenetic applications. Despite extensive study, the molecular mechanism behind formation of their signaling state, a flavin-cysteine thiol-adduct, remains unclear. To address this knowledge gap, we followed cysteine dynamics in the LOV1 domain of *Chlamydomonas reinhardtii* (C*r*LOV1) applying flash photolysis in the mid-infrared range with an external cavity quantum cascade laser. Time-resolved measurements probed the cysteine dynamics from 2 ns to 0.1 s. Early-ns spectra show that the reactive cysteine undergoes a H-bond change to a more hydrophilic environment. The decay of this H-bonded state (τ = 10 ns) is coupled to the rise of cysteine deprotonation (τ = 16 ns). In the μs regime, a minor second cysteine population also deprotonates (τ = 1.7 μs). Therefore, this study identifies a new intermediate in the *Cr*LOV1 photocycle: an H-bonded cysteine species that precedes deprotonation. Although analogous intermediates have been observed in cryo-trapping experiments of other LOV domains, this is the first direct time-resolved observation. Altogether, our findings provide the most complete temporal cysteine dynamics in a LOV domain to date. We propose the H-bonded cysteine species as a new key intermediate in the thiol-adduct formation and, therefore, in the photocycle of LOV domains.

Towards investigating Amyloid beta on a nanoscale in a liquod environment using nanoFTIR and s-SNOM

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Alzheimer's disease is tightly associated with amyloid beta (Aβ) peptides that misfold at membrane interfaces to form fibrils. This fibril formation leads to changes in the membrane and eventually to dysfunction or cell death. [1,2] Previously, we investigated the misfolding trajectory in the presence of different tethered lipid membrane systems using surface-enhanced infrared absorption (SEIRA) spectroscopy. [3] We monitored the changes in the secondary structures of Aβ during its interaction with the membranes and quantified the orientation and secondary structure by comparison of the experimental to computed spectra based on density functional theory (DFT). These methods showed us, that the lipid environment plays a critical role in the (mis-) folding process. Other groups demonstrated with AFM (Atomic Force Microscopy)-based methods for Aβ to form very inhomogeneous structures. However, most of the time, these measurements acquire dried molecules. We aim to determine whether similar nanoscale inhomogeneities of Aβ can be observed in a liquid and lipid environment using nano-FTIR (nanoscale Fourier-transform infrared spectroscopy) and s-SNOM (scattering-type scanning near-field optical microscopy) in combination with a SiNx membrane-covered flow cell. Towards this goal, we establish the foundation combining the “nano-aquarium” setup with planar lipid membranes to enable nanoscale-resolution spectra measurements and imaging of Aβ in various liquid environments.

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Selective SERS Sensing of Environmental Pollutants via Hyperuniform Mesoporous Gold and Halogen-Bonding MOFs

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Surface-enhanced Raman spectroscopy (SERS) is a powerful vibrational technique for trace molecular detection, but its application in complex environments is hindered by a lack of molecular selectivity and irreproducible plasmonic hotspots. We present a novel SERS substrate that overcomes these limitations by integrating a disordered hyperuniform mesoporous gold (mAu) film with a thin coating of a halogen-functionalized metal-organic framework (UiO-66-I). We quantitatively demonstrate that the hyperuniform mAu structure occupies an optimal regime between order and disorder, generating intense and spatially uniform electromagnetic hotspots, as confirmed by Weibull statistical analysis of simulated near-field enhancements. The UiO-66-I MOF coating serves a dual function: (1) its iodine-based linkers selectively recruit chlorinated aromatic hydrocarbons (CAHs) from complex aqueous matrices via specific halogen bonding (HaB), and (2) its high refractive index enhances light coupling and confines plasmonic modes near the sensor surface. This synergy of physical and chemical enhancement achieves a detection limit below 10^{-10} M for model CAHs like 1,4-dichlorobenzene, surpassing environmental standards by several orders of magnitude. The sensor demonstrates exceptional selectivity against common interferents (proteins, PAHs, inorganic ions) and excellent reusability over multiple cycles. This work establishes a general strategy combining tailored nano-architectonics with reticular chemistry to create highly selective and sensitive vibrational sensors for environmental monitoring and biomedical diagnostics.

Coupled Catalysis Powers Enzymatic CO_2 Conversion

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Hydrogenotrophic methanogens are microorganisms central to global carbon cycling and range among the largest biological sources of methane (CH_4), a potent greenhouse gas. At the heart of their energy metabolism lies the Hdr–Vhu–Fwd super-assembly, which couples H_2 oxidation with CO_2 reduction via flavin based electron bifurcation (FBEB).[1]

We solved the cryo-EM structure of this super-assembly from *Methanococcus maripaludis*, revealing an 8.000 kDa complex comprising 252 polypeptide chains and over 600 redox cofactors. This enormous architecture consists of two hexameric rings linked by a tetrameric core, forming a continuous, circular electron nanowire. In this unique arrangement, 12 polyferredoxin subunits (VhuB) connect the heterodisulfide reductase (Hdr) and Tungsten-containing formate dehydrogenase (Fwd) complexes, thereby coupling FBEB with CO_2 fixation and directly linking the last[2] and the first step of methanogenesis.

The [NiFe]-hydrogenase (VhuADGU) catalyses H_2 oxidation and serves as electron input module for FBEB. One electron is consumed in the “down-hill” reduction of heterodisulfide in the Hdr module while the “up-hill” electron reduces CO_2 to formate via the Fwd complex. Despite the large fingerprint of the Hdr–Vhu–Fwd super-assembly, we were able to follow the electronic coupling between subunits by means of *in situ* ATR FTIR spectroscopy.[3] The catalytic cofactor of Vhu comprises CO and CN^- ligands whose absorption frequency shifts characteristically with redox and protonation state.[4] Our data now indicate reversible redox changes in the presence of CO_2 and/or heterodisulfide, however, no re-oxidation in the absence of these substrates.

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Laser-excited temperature-jump IR spectroscopy to study the impact of lipid phase transitions on membrane proteins

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Membrane proteins function within dynamic lipid environments whose phase behavior strongly modulates protein structure and dynamics. Laser-excited temperature-jump (T-jump) infrared (IR) spectroscopy provides a powerful means to probe this interplay by inducing nanosecond-scale temperature perturbations and tracking relaxation processes from nanoseconds to milliseconds.[1] Our home-built single-wavenumber quantum cascade laser-based mid-IR spectrometer enables site-specific detection of lipid and protein vibrational modes across all relevant spectral regions with nanosecond time resolution.[2] Proteoliposomes, consisting of bacteriorhodopsin (BR) reconstituted into lipid vesicles, provide a system in which the conformational dynamics of the protein can be monitored, while a rapid T-jump drives the lipid phase transition.

Liposomes, lipid vesicles without reconstituted protein, undergo a highly cooperative gel-to-fluid transition, with relaxation times exhibiting pronounced *critical slowing down* near the main phase transition temperature. In contrast, proteoliposomes display markedly altered lipid relaxation dynamics, indicating that the presence of BR reduces lipid cooperativity and introduces increased kinetic complexity. Monitoring BR's secondary structure via the amide I' band further reveals thermally driven conformational changes that are coupled to lipid dynamics in both the gel phase and the phase transition region, with this coupling weakening for slower relaxation processes as well as in the fluid lamellar phase.

Overall, these results demonstrate that lipid phase transitions play a central role in modulating membrane protein conformational dynamics and highlight laser-excited T-jump IR spectroscopy as a precise tool for studying protein-lipid interactions.

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Mapping In-plane Orientational Order and Correlation Lengths in Molecular Films using Azimuthal-Scanning Vibrational Sum-Frequency Generation Microscopy

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Self-assembled molecular films are ubiquitous in nature, with highly ordered, anisotropic packing structures which govern their macroscopic properties and functional behavior. Local anisotropy in these systems is frequently accompanied with pronounced heterogeneity and long-range in-plane packing order extending from molecular to microscopic length scales. Experimental access to this structural complexity remains a formidable challenge. Recent advances have established phase-resolved sum-frequency generation (SFG) microscopy as a direct probe of thin-film structures, however, many properties have thus far remained unaddressed, including the width and shape of microscopic orientational distributions.

We present a method to overcome this limitation by implementing an azimuthal-scanning approach in SFG microscopy, enabling access to the full in-plane orientational distribution, far beyond average molecular orientation. Specifically, comparison of the complete set of rotational frequencies arising from the azimuthal dependence with simulated data illustrate how these frequencies respond differently to in-plane orientational disorder, deviations from perfect crystallinity, and more complex packing motifs such as bimodal arrangements.

We apply this framework to a model membrane consisting of a phase-separated mixed phospholipid monolayer, revealing that molecules within condensed domains exhibit micron-scale orientational correlations despite substantial in-plane orientational diversity, including a finite distribution width and markedly different orientations of the two lipid tail groups. Overall, this example highlights the potential of this method for future investigations into how packing structure governs functional behavior in lipid membranes. Beyond this specific system, the theoretical concepts introduced here are readily extendable to a wide range of systems, from molecular assemblies to phononic materials, and thus open new directions for structural elucidation at interfaces.

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Surface-Enhanced Infrared Absorption Spectroscopy on the Interaction of Cell Penetrating Peptides with Lipid Bilayers

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Abstract:

Cell-penetrating peptides (CPPs) are short chains of amino acids that can cross cell membranes effectively, allowing the transport of therapeutic molecules, such as drugs and genetic material, into cells. Due to their wide range of applications in fields such as drug delivery, gene therapy, and imaging, CPPs are important tools for improving biomedical treatments [1]. Despite their extensive use for delivering intracellular cargo, the exact mechanisms by which cell membranes interact with CPPs remain unclear. To shed light on this topic, we used surface-enhanced infrared absorption spectroscopy (SEIRAS) to study the interaction mechanism between CPPs consisting of decaarginine (R10) with alkyl or perfluorinated tags and a solid-supported lipid bilayer (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine, POPC). We observed discrepancies in the interaction between CPPs and the lipid bilayer when the tags were alkyl versus perfluoroalkyl [2]. To gain further insight into these interactions with consideration of the fluorous effect, we performed SEIRAS measurements at different peptide concentrations with various lengths of perfluorinated tags to evaluate their role in the system of interest [3]. This research is expected to contribute to a deeper understanding of the mechanisms underlying peptide-lipid interactions in the context of peptide-based intracellular delivery methods.

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Investigating lipid phase transitions and the insertion of the antimicrobial peptide Gramicidin S via time-resolved temperature-jump IR spectroscopy

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Antibiotic resistance is a growing global health threat, driving the need for novel antimicrobial strategies. Antimicrobial peptides (AMPs), which disrupt bacterial membranes primarily through lipid insertion, offer a promising platform for next-generation antibiotics. Gramicidin S (GS), a potent AMP, has been used clinically for over 80 years without the emergence of resistance, making it a valuable model system[1]. While most insights into GS insertion are derived from equilibrium measurements, time-resolved experimental data remain scarce.

Here, we use a laser-induced temperature jump to trigger a gel-to-fluid phase transition in biomimetic membranes, enabling insertion of GS. Using time-resolved quantum cascade laser infrared spectroscopy[2], we track lipid rearrangements, peptide insertion, and water dynamics from nanoseconds to milliseconds.

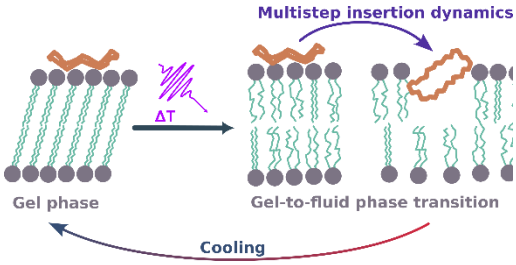

Figure: Schematic experimental design

Our results reveal that at the phase transition, the membrane exhibits slowed, highly stretched dynamics, suggesting strong intermolecular coupling and large domain fluctuations with poor packing. Altered and accelerated water dynamics at the phase transition indicate changes in membrane hydration. Transient GS insertion data show coupling to lipid dynamics and reveal a multistep insertion mechanism on a microsecond timescale that is highly lipid-phase dependent. Insertion depth, dynamics and insertion temperature depend on the membrane lipid composition and peptide concentration, indicating a cooperative, threshold-driven process governed by local peptide density.

Overall, our work demonstrates a complex, multistep insertion process that is tightly coupled to membrane dynamics, underscoring the importance of studying peptides and membranes as a coupled system.

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Vibrational Spectroscopic Insights into Water Adsorption in MOF (UiO-66)

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Abstract

Water adsorption plays a critical role in determining the physicochemical behavior and stability of metal–organic frameworks (MOFs), particularly under conditions relevant to aqueous and biologically inspired environments. In this study, vibrational spectroscopy is employed to investigate the adsorption and interaction of water molecules within the zirconium-based MOF UiO-66, a prototypical framework known for its exceptional chemical and hydrolytic stability. Infrared and Raman spectroscopic measurements provide molecular-level fingerprints of both the inorganic Zr–O clusters and the organic terephthalate linkers, enabling direct observation of framework–water interactions. Systematic changes in vibrational band positions, intensities, and line shapes reveal the formation of hydrogen-bonding networks and distinct adsorption environments within the pores. The results demonstrate how nano-confinement and local coordination sites influence the structure and dynamics of adsorbed water molecules, while preserving the structural integrity of the framework. These findings highlight the capability of vibrational spectroscopy as a label-free and non-destructive tool for probing host–guest interactions in MOFs and provide fundamental insights into water–framework coupling that are essential for understanding MOF performance in humid, aqueous, and biomedical-relevant conditions.

NanoFTIR spectroscopy of liquids at interfaces

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Liquids at interfaces and in nanocavities, as found in natural systems, experience a perturbed environment compared to bulk liquids. This leads to interesting new properties in various fields, for example, caused by the manipulation of the hydrogen bond networks in water. Experimental studies have indicated those properties but direct spectroscopic measurements to track these effects in minimal volumes of nanoconfined systems are far from straightforward. We used the method of nanoFTIR spectroscopy (nanoscale Fourier-transform infrared spectroscopy) to detect liquids in nanoconfinement, i.e. at interfaces or in nanochannels. NanoFTIR is a particularly suitable method towards this goal because it benefits from the use of a metallized atomic force microscopy tip as an antenna for infrared (IR) radiation, which enables recording IR spectra with spatial resolution of tens of nanometers. Nanochannels based on a silicon substrate in combination with hBN as a IR transparent top layer (in the wavenumber region between 1800 - 1600 cm^{-1}) showed to be suitable for the investigation of vibrational modes of liquids in nanocavities. Characterization with AFM (atomic force microscopy) and sSNOM (scattering-type scanning near-field optical microscopy) imaging of the system confirmed the successful construction of nanocavities fillable with liquids. Importantly, marker bands of dimethylformamide as model liquid at around 1660 cm^{-1} were successfully detected in channels with a depth from 100 nm down to 2-3 nm using nanoFTIR spectroscopy, the latter corresponding to 4-6 layers of molecules in the liquid.

Towards monitoring radical transfer in lytic polysaccharide monooxygenases using freeze-quench stopped flow adapted to cryo resonance Raman spectroscopy

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Lytic polysaccharide monooxygenases (LPMOs) are mono-copper enzymes that can cleave insoluble polysaccharides like cellulose, making them promising candidates for the industrial production of biofuels. H_2O_2 has been established as the preferred cosubstrate, yet excess H_2O_2 leads to rapid enzyme inactivation in the absence of substrate, indicating their vulnerability to oxidative damage. [1,2] In this context, naturally present Tyr/Trp redox chains in the protein matrix could protect the enzyme from damage by steering the oxidizing holes away from the LPMO's active site, as suggested in other metalloenzymes but not fully explored in LPMOs. [3]

In this work, we investigate the role and kinetics of oxidizing hole-transfer via Tyr/Trp pathways in *Serratia marcescens* AA10A LPMO under varying H_2O_2 and reductant concentrations using millisecond stopped-flow UV-Vis spectroscopy. By globally fitting time-dependent spectra to a mechanistic model, we assess how fast peroxidase-like turnover of reductant and slower intramolecular radical transfer ("hole-hopping") contribute to protecting LPMOs from oxidative damage. Our results show that reductant suppresses the formation of intramolecular Tyr/Trp-radicals. [4]

To unravel the chemical structure of the transient intermediates, we use rapid freeze-quench (RFQ) to stop the reaction at selected ms delay times and analyse them by cryo resonance Raman spectroscopy. This approach enables us to spectroscopically monitor trapped intermediates at varied time delays giving access to tracking the progress of the radical transfer, with a resolution to the radical environment or position within the protein matrix. Cryogenic conditions enhance intermediate stability and reduce photolytic damage, enabling longer acquisition times.

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Spectral signature of hypoxia. Time-dependent effects on brain endothelium revealed by FTIR and Raman imaging

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Brain endothelial cells, essential components of the blood–brain barrier, are particularly vulnerable to stress during neurodegenerative processes. Hypoxia, a typical aspect of these conditions, is frequently modelled *in vitro* to examine its cellular impact [1]. In this study, we investigated time-dependent impacts of hypoxia on human brain endothelial cells (HBEC-5i) via Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy (RS). Cells were grown in normal oxygen levels (37°C, 5% CO_2) and low oxygen conditions (1% O_2) for 3, 6, 9, 12, and 24 hours. Our analysis revealed that hypoxia leads to significant alterations in cellular structures. As a result of the adaptation mechanism, we observed significant alterations in lipid composition, including elongation of acyl chains, a transient decrease in fatty acid esters, and temporary changes in free fatty acids. Global changes in protein secondary structures were noted to occur under hypoxic conditions. Additionally, hypoxia caused a shift in DNA conformation from B-form to A-form. RNA structure was also impacted, as indicated by band shifts to lower wavenumbers, possibly reflecting changes in metabolic activity. These findings reveal brain endothelial cells' complex response to oxygen deficiency and highlight the value of FTIR and Raman spectroscopy in studying adaptations relevant to neurodegenerative research.

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Under Pressure: Photoswitchable Lipid Nanodiscs Reveal Protein Response to Lateral Forces in the Bilayer

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In this work we incorporate the photoswitchable lipid AzoPC into nanodiscs containing the protein bacteriorhodopsin (bR) and observe pronounced changes in the photocycle when the azobenzene moiety is switched between *trans* and *cis*.

AzoPC is known to modulate bilayer properties such as lateral pressure and thickness, which in turn alters the local membrane environment [1, 2]. It has previously been used to trigger mechanosensitive proteins, but here we apply it to perturb the bilayer and examine its effect on a non-mechanosensor [3]. Using time-resolved UV–Vis and infrared spectroscopy, we find that switching AzoPC from *trans* to *cis* allows reversible tuning of the bR photocycle rate and provides mechanistic insight into the lipidic environment through changes in activation energies.

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Advances in light sources for Coherent Raman and TPE microscopy

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We report on recent advances in commercial picosecond light sources designed for ultra-sensitive stimulated Raman scattering (SRS) imaging in the fingerprint region, as well as for Raman labeling applications. By reducing the repetition rate by a factor of two to 40 MHz and implementing additional optimizations, we achieved a signal-to-noise improvement of one order of magnitude compared to the previous light-source generation, see Fig. a. The shot-noise-limited performance enables SRS imaging of compounds at biologically relevant concentrations.

The system delivers several hundred milliwatts of output power with a spectral bandwidth of approximately 10 cm^{-1} and pulse durations of 2 ps for both Pump and Stokes beams, covering a vibrational range from 210 to 5450 cm^{-1}. This range encompasses all relevant vibrational bands while providing sufficient spectral resolution. Rapid tuning across the full wavelength range within about two seconds allows straightforward multispectral SRS imaging. The light source operates fully hands-free and combines Pump and Stokes beams both spatially and temporally, ensuring optimal overlap at the sample. We will present comparative measurements demonstrating the improvements in sensitivity and acquisition speed, as well as examples of fast multispectral SRS imaging of biological samples in the fingerprint region (Fig. b, c) and imaging of Raman labels.

To meet the differing requirements of chemical specificity in coherent Raman microscopy and short pulse durations for efficient excitation in two-photon excitation microscopy, we implemented a specialized OPO operating regime that produces a bandwidth of 30 cm^{-1} instead of 10 cm^{-1}. These pulses can be compressed to approximately 400–500 fs for two-photon excitation applications, as will be demonstrated. Switching between the two operating modes is straightforward.

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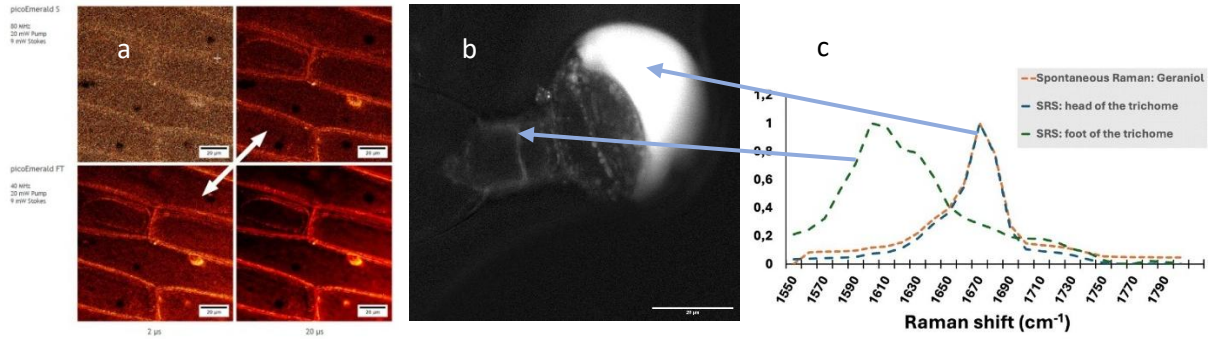

Fig. **a**: SRS images at 880cm^{-1} in epidermal onion cells. Upper row: images acquired with the 80MHz light source, picoEmerald S, lower row: 40MHz picoEmerald FT. Increasing the pixel dwell time from 2 µs to 20 µs significantly enhances image quality and feature visibility for both systems. Notably, at 2 µs dwell time, the picoEmerald FT achieves image quality comparable to the picoEmerald S at 20 µs., **b** and **c**: Spectral scan of Pelargonium secreting gland (trichome), pump 30mW, Stokes 28mW, 5µs pixel dwell time.

Exploring the Orientation of a PAS-Domain Protein at Model Protein Interfaces with Distinct Secondary-Structure Content Across Nano- and Micro-Scales

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Proteins leverage specialized domains to perceive signals and engage dynamically with other proteins. Among these, the PAS domain plays a crucial role in signal transduction, environmental sensing, and cellular regulation. The photoactive yellow protein (PYP), a blue-light receptor derived from bacteria, serves as a well-established model for the function of PAS domains and influences phototactic behavior. Although PAS domains frequently operate through dimerization, a direct binding partner for PYP has not yet been definitively identified. In this study, we investigate PYP's interaction with poly-L-lysine (PLL) at varying pH levels as an simplified model of protein-protein interaction. We applied a multi-modal approach—comprising circular dichroism (CD), atomic force microscopy (AFM), chiral vibrational sum-frequency generation (VSFG) spectroscopy, molecular dynamics simulations, VSFG spectral modeling, and nano-FTIR spectroscopy—to examine PYP's structural response at the PLL interface. CD provided comprehensive insights into the bulk structure, whereas VSFG offered surface-specific information regarding PYP's orientation and bonding via N-H and amide vibrations. AFM and nano-FTIR analyses uncovered nanoscale morphology and conformational details. PYP was observed to adopt a specific orientation on the PLL surface, predominantly driven by dipole-dipole interactions analogous to PAS domain dimer interfaces. Nano-FTIR data acquired at BESSY II revealed that the structure of PLL is pH- and temperature-dependent, thereby directly affecting PYP's interfacial behavior. These results diverge from bulk observations, underscoring the significance of local environmental factors in cell signaling. This research enhances our comprehension of PAS domain interactions and could facilitate the identification of physiological binding partners involved in PAS-mediated signaling pathways.

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In situ observation of „native“ membrane protein folding by surface enhanced infrared absorption spectroscopy

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The functional insertion, folding and assembly of proteins in biological membranes are essential for a variety of cellular activities, including cell signaling and the transport of molecules into and out of the cell. Here we present an innovative approach using surface-enhanced absorption spectroscopy (SEIRAS) to monitor translocon-unassisted membrane protein folding into artificial lipid bilayers (nanodiscs) during cell-free protein expression. In a first experiment, nanodiscs were attached to a Ni-NTA-modified gold surface via a His-tag while cell-free expression of the model protein bacteriorhodopsin (*Hs*BR) was processed in bulk solution. Time-resolved SEIRAS is sensitive only to events at the surface covered by the nanodiscs where folding takes place and provides structural folding information. After the gene transcription and translation processes had started, we observed the newly synthesized protein inserting into the nanodiscs and forming first secondary and then tertiary structures, indicating correct folding and integration of the chromophore all-*trans* retinal [1]. In a second round, we investigated the microbial rhodopsins sensory rhodopsin I (*Hs*SRI), sensory rhodopsin II (*Hs*SRII) and channel rhodopsin II (*Cr*ChR2) in comparison to *Hs*BR. These new microbial rhodopsins do not bind retinal properly, as indicated by the lack of visible absorption in in vitro cell expressions. SEIRAS experiments suggest that all the rhodopsins investigated lead to the production of polypeptides and show secondary structure formation. However, the condensation of helices in tertiary structure formation was not properly achieved, suggesting that the chromophore is not bound, which seems to be mandatory for the correct folding process [2]. Our method is suitable to analyze folding processes and to indicate steps for improvement in in vitro expression.

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Development of UV SERRS to study molecular principles of DNA- and RNA-ligand interactions

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The unique molecular behavior of Nucleic Acids (NA), i.e. the high efficiency and selectivity to recognize and hybridize complementary sequences, is a building stone of biology, and it is used in vast applications in therapeutic medicine. Hybridization, and subsequent folding of NA into a variety of structures are driven by non-covalent interactions like H-bonding, π-π-stacking and ionic complexation. To fully understand the molecular principles of NAs and explore their capabilities in medical and biotechnological applications, methods able to resolve the NA structure with a chemical resolution and during their functions are necessary.

In our recent work[1], we employed UV Resonance Raman (UVRR) spectroscopy to identify vibrational peaks of nucleotides sensitive to H-bonding. We then monitored the conformational assembly of oligonucleotide strands during thermal hybridization and identified marker bands for C-G and A-T base pairs formation, that are consistent with predictions from hybrid DFT-computational Raman spectra. As such, we demonstrated that UVRR is perfectly suited to provide a fingerprint of the base pairs and could be potentially used in mechanistic studies of NA-NA-interactions.

UV RR provides a unique fingerprint spectrum of each nucleobase. The label-free, non-destructive and non-invasive method can be further leveraged by additionally introducing surface enhancement, by bringing oligonucleotides in close proximity to a nanostructured surface that exhibits plasmonic resonance in the UV regime. Our current efforts are focused on testing various nanostructured supports that exhibit plasmonic resonance in the UV, which we aim to combine with UVRR in *UV surface enhanced resonance Raman spectroscopy* (UV SERRS). This will allow us to study monolayers of oligonucleotides and the mechanistic step under ligand binding.

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Driving Force and Nonequilibrium Vibronic Dynamics: Simulating Electron Transfer on Noisy Quantum Computers

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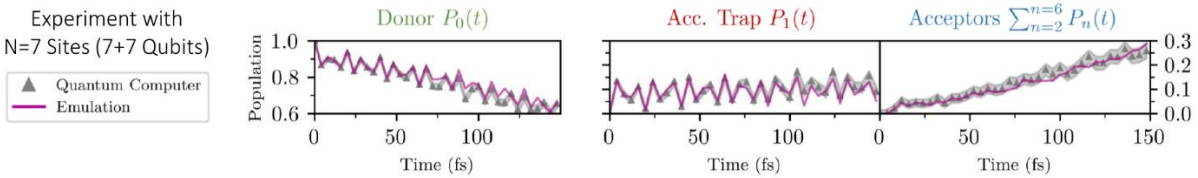
Electron-hole pairs in organic photovoltaics efficiently dissociate although their Coulomb-binding energy exceeds thermal energy at room temperature. The electronic-vibrational (vibronic) coupling of electronic states to structured vibrational environments is known to assist charge separation at specific driving forces. However, non-perturbative simulations of such large, spatially extended systems remain an unmet challenge in the presence of non-equilibrium, long-lived vibronic coherence. We present a toolbox for the digital quantum simulation of large open quantum systems with structured environments. It exploits the intrinsic damping of qubits to reproduce vibrational relaxation in combination with a model-specific error mitigation scheme. We validate our approach by studying a microscopic model of electron-transfer on IBM superconducting hardware and resolving electronic and vibronic transfer spectra of a one-dimensional donor-acceptor chain. Scalability is demonstrated by growing the chain length up to 10 electronic sites, an unprecedented size for chemical dynamics on a quantum computer to date. Microscopic models of vibronic electron transfer thus offer a portable, application-oriented benchmark for simulating long-lived entangled states on noisy quantum computers.



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Coupled Catalysis Powers Enzymatic CO_2 Conversion

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Abstract:
Hydrogenotrophic methanogens are microorganisms central to global carbon cycling and range among the largest biological sources of methane (CH_4), a potent greenhouse gas. At the heart of their energy metabolism lies the Hdr–Vhu–Fwd super-assembly, which couples H_2 oxidation with CO_2 reduction *via* flavin based electron bifurcation (FBEB).[1]
We solved the cryo-EM structure of this super-assembly from *Methanococcus maripaludis*, revealing an 8.000 kDa complex comprising 252 polypeptide chains and over 600 redox cofactors. This enormous architecture consists of two hexameric rings linked by a tetrameric core, forming a continuous, circular electron nanowire. In this unique arrangement, 12 polyferredoxin subunits (VhuB) connect the heterodisulfide reductase (Hdr) and Tungsten-containing formate dehydrogenase (Fwd) complexes, thereby coupling FBEB with CO_2 fixation and directly linking the last[2] and the first step of methanogenesis.
[NiFe]-hydrogenase catalyzes H_2 oxidation and serves as electron input modules for FBEB. One electron is consumed in the "down-hill" reduction of heterodisulfide in the Hdr module while the "up-hill" electron reduces CO_2 to formate *via* the Fwd complex.
Despite the large fingerprint of the Hdr–Vhu–Fwd super-assembly, we were able to follow the electronic coupling between subunits by means of *in situ* ATR FTIR spectroscopy.[3] The catalytic cofactor of [NiFe]-hydrogenase comprises CO and CN^- ligands whose absorption frequency shifts characteristically with redox and protonation state.[4] Our data now indicate reversible redox changes in the presence of CO_2 and/or heterodisulfide, however, no re-oxidation in the absence of these substrates.

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Protein-induced lipid dynamics and lipid-induced protein dynamics studied by QCL-based IR spectroscopy

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Membrane proteins do not act independently of their surrounding lipid environment. Previous studies on proteoliposomes using Fourier-transform infrared (FTIR) spectroscopy have shown that changes in membrane composition and order can alter protein kinetics of the proton pump bacteriorhodopsin (BR) [1]. However, these measurements provide only indirect information on how membrane dynamics are linked to protein activity.

To address this limitation, we employ time-resolved infrared spectroscopy based on single-wavenumber quantum cascade lasers (QCLs) [2] to study BR reconstituted into liposomes with fully deuterated alkyl chains (DSPC-d_{70}), which shift lipid vibrational modes into a separate spectral range, thereby allowing simultaneous observation of protein and lipid dynamics during the photocycle [3]. This approach enables site-specific tracking of protein protonation and conformational changes together with lipid responses from different regions of the bilayer, including alkyl chains, ester groups, and terminal methyl groups, with nanosecond time resolution over a time window spanning more than five decades.

Using a lipid system with a phase transition near room temperature (DMPC-d_{54}) allows separation of effects caused by temperature from those originating from changes in lipid phase. By jointly fitting protein and lipid transients, it is shown that lipid dynamics are strongly correlated with protein intermediates, while responding differently across the phase transition. This demonstrates that certain lipid motions are induced by protein activity and shaped by the physical state of the membrane.

To move beyond a purely responsive membrane, the next step is to actively control membrane dynamics in time. By introducing photoswitchable lipids (Azo-PC) into artificial membranes, light-driven changes of membrane structure independent of the protein photocycle can be established. This provides a platform to study how controlled membrane dynamics influence protein behavior.

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