Henrike Müller-Werkmeister

Structural dynamics of carbohydrates and protein-carbohydrate interactions studied by 2D-IR spectroscopy

Carbohydrates constitute one of the four main classes of biomacromolecules, alongside proteins, DNA and lipids. While they possess a myriad of important biological functions, i.e. in molecular recognition or cell metabolism, they are difficult to study by molecular biophysical chemistry tools due to their high structural diversity at low chemical complexity.

In protein biophysics detailed information can be gained by studying site-specific vibrational reporter groups i.e. in FTIR or 2D-IR spectroscopy, the later giving access to dynamics on the femtosecond and picosecond timescale (see figure 1 and caption for details). We have now demonstrated that similar vibrational reporter groups, like thiocyanate (-SCN) or azide (-N_3) can be employed to 2D-IR studies of carbohydrates [1, 2].

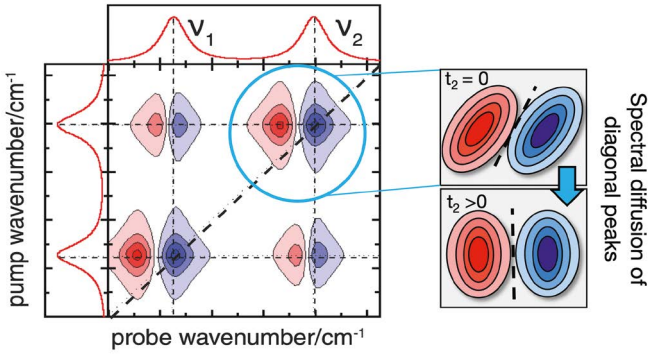

Fig. 1: 2D-IR spectra are obtained by a three-pulse sequence of two femtosecond pump pulses followed by a probe pulse after t_2 time. The intrinsic time resolution is limited by the vibrational lifetime of the studied oscillator. Spectra contain diagonal peaks (labeled in the blue circle), comprised of an ESA (red) and SE/GSB (blue) component as well as diagonal peaks indicative of energy transfer or coupling between oscillators. Diagonal peaks give access to homogeneous (anti-diagonal) and inhomogeneous line width (diagonal) at early waiting times. The time evolution of diagonal peaks is often analyzed by its "Center Line Slope" (CLS) and indicative of spectral diffusion within the studied ensemble.

β-glucose can be synthesized with an -SCN label at C_2 position and studied in H_2O (see figure 2), an important prerequisite for biophysical applications. The vibrational dynamics, i.e. the lifetime and linewidth accessible in 2D-IR spectroscopy resemble the properties of the -SCN oscillator in methyl-thiocyanate. However, the spectral diffusion dynamics are already altered for the vibrational reporter group when added to a monosaccharide like glucose. Here data indicate the presence of two subensembles which don't interchange on the accessible timescale of ~15 ps, indicative of dynamics intrinsic to the carbohydrate.

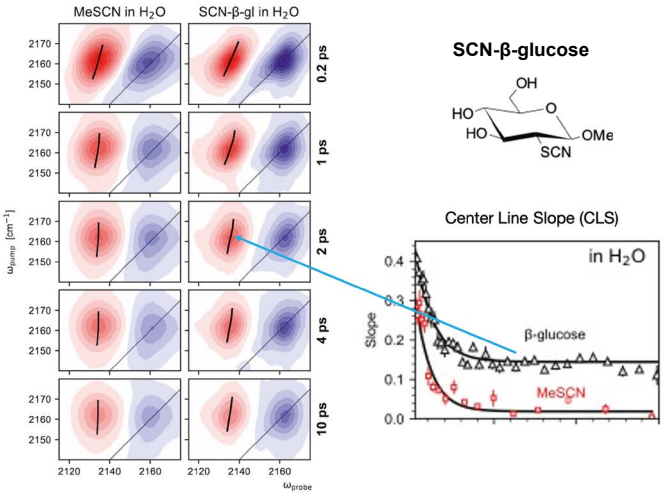

Fig. 2: 2D-IR spectra (diagonal peak) of SCN-labeled glucose in comparison to the SCN oscillator in methyl-thiocyanate in H_2O. The SCN group has a long vibrational lifetime and allows observation of dynamic processes in a time window up to >15 ps. The CLS analysis (bottom right) reveals the presence of two subensembles for SCN-glucose, which don't undergo exchange on the accessible timescale, indicated by the offset (in contrast to the MeSCN data, data published in [1]).

While SCN is an optimal vibrational reporter group based on its lifetime, its extinction coefficient is low and suboptimal for studies requiring low mM concentrations, such as protein-carbohydrate interaction studies. We hence have further tested several azide-labelled carbohydrates (available commercially due to applications of click chemistry/for microscopy) and found that by combining high IR pump intensities in 2D-IR experiments with concentrations ~5-10 mM, signals are accessible in H_2O for azide-labelled carbohydrates. Similarly, as for SCN-β-glucose data reveal intrinsic dynamics of the carbohydrates studied [2]. The built library of carbohydrates for IR spectroscopy allows now to target protein-carbohydrate interactions and research question in glycobiology in general. A first example our group is studying from different perspectives is the first reaction step in glycolysis: Here the enzyme hexokinase catalyzes the transformation of glucose to glucose-1-phosphate accompanied by

Prof. Dr. Henrike Müller-Werkmeister
Current address: Universität Potsdam
Institut für Chemie, Physikalische Chemie
Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm/D
henrike.mueller-werkmeister@uni-potsdam.de
From 01.09.2026: TU Graz, Institute of Physical and Theoretical Chemistry
Stremayrgasse 9, 8010 Graz/A

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the conversion of ATP to ADP (see figure 3.). We have studied the binding of different labeled glucose-derivates (with -N_3 as vibrational reporter) to map the active site of hexokinase. 2D-IR is here a great match as hexokinase has a highly flexible binding pocket, difficult to monitor in i.e. X-Ray crystallography with high resolution. However, mapping the local inhomogeneities and solvent accessibility with a vibrational reporter group in 2D-IR and comparison to MD simulations confirms the high flexibility of the binding pocket and gives insight into the reaction mechanism of this enzyme (data not shown, publication in preparation, [3]).

Another strength of 2D-IR, in comparison to FTIR, beside the intrinsic time-resolution and increased information due to the multidimensional spectra, is its higher sensitivity. Signals in 2D-IR scale with the transition dipole moment of a studied oscillator to the 4th power, while in FTIR this relation is quadratic. This means, signals which appear weak in FTIR and i.e. are obscured by solvent background, are stronger in 2D-IR. We have used this benefit to monitor the kinetics (rather than ultrafast dynamics) of the hexokinase reaction over ~2h using the signatures of ATP/ADP [4]. While in FTIR spectra specifically the signature of the phosphate stretch vibrations in ADP is weaker, this signal can be analyzed in depth in 2D-IR spectra. Extracting pump-slice amplitude spectra (PSA) from the 2D-IR spectra allows us to plot and analyze the kinetics as pseudo-first order reaction. The approach to study phosphate signature of ATP/ADP during enzymatic catalysis by 2D-IR is not limited to this specific reaction but can be broadly applied to many biophysical questions. We have abstained from studying the Glucose-1-phosphate signature as the ATP/ADP is a more general approach. Kinetic studies using 2D-IR become feasible due to higher laser intensities in the mid-IR improving signal-to-noise while reducing data collection times. With high rep rate experiments coming along this kind of studies can become more broadly applicable also beyond biophysical studies.

In summary this contribution showcased the application of 2D-IR spectroscopy to biophysical research, with a focus on carbohydrate dynamics and enzymatic catalysis.

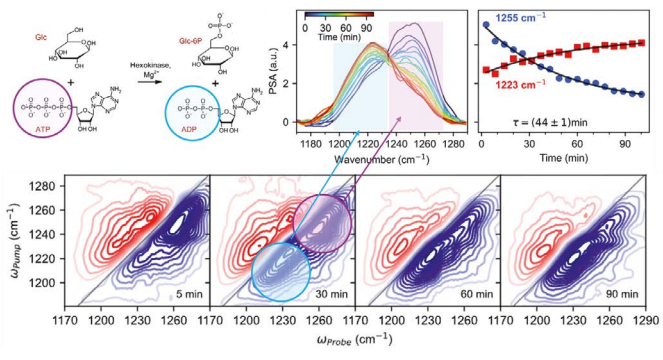

Fig. 3: Kinetics of the enzymatic reaction of hexokinase monitored by 2D-IR on the phosphate stretch vibrations of ATP/ADP: top left reaction, bottom: 2D-IR spectra for a waiting time of 200fs at different times after start of the reaction, top middle: extracted pump slice amplitude spectra, top right: resulting kinetic traces (from [4]).

Literature

[1] P. Gasse et al. *J. Chem. Phys.* **158**, 145101, 2023

[2] P. Gasse, et al. *J. Chem. Phys.* **161** 195101, 2024.

[3] P. Gasse, F. Sobral, T. Stensitzki, A. Nunes-Alves, H.M. Müller-Werkmeister, unpublished results, in preparation

[4] T. Stensitzki et al., in revision, 10.26434/chemrxiv-2025-j5szt

Prof Dr. Henrike Müller-Werkmeister

Henrike Müller-Werkmeister (*1984) has been a TT-professor for Physical Chemistry at Uni Potsdam since 2017 and is starting as full professor at TU Graz in fall 2026. While originally a biochemist by training (Diploma studies in Frankfurt/Main), she early on developed an interest in complex physical/physicochemical experiments. After her PhD work on developing 2D-IR for studies of protein dynamics (honored by the Wilhelm-Ostwald-Nachwuchspreis by DBG, GDCh and Ostwald-Gesellschaft), she spent her Marie-Curie funded postdoc focusing on time-resolved X-Ray and electron diffraction at U Toronto and MPSD Hamburg. The Müller-Werkmeister lab is using 2D-IR spectroscopy for research in Biophysics, but also materials or on fundamental energy transfer processes.