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Multiscale Time-Resolved Experiments to Follow Structural Dynamics of Chemical and Biochemical Systems

Chemical and biochemical dynamics span the entire timescale from femtoseconds to seconds and beyond. Molecular dynamics start with local processes, like bond breaking, solvation dynamics and photochemistry [1], or side chain rotation in the ultrafast regime from femtoseconds to nanoseconds. Those highly localized processes are linked to collective motions and chemical function like enzymatic catalysis [2, 3] or phase transitions, which are cover timescales from nano- to microsecond UV/VIS up to seconds and longer. Overall, chemical dynamics thus span more than 15 orders of magnitude in time.

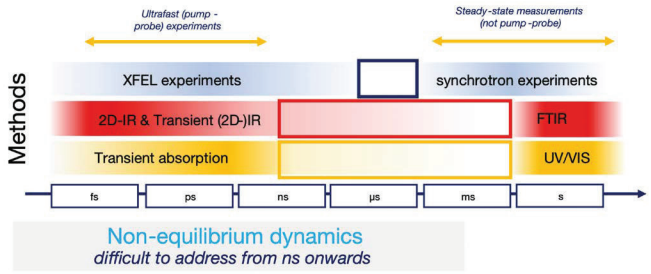

Fig. 1: Experimental strategies are required to overcome the gap in accessible timescales for pump-probe type experiments addressing non-equilibrium dynamics in chemistry and biochemistry to link the time regimes of local dynamics (fs-ns) with the timescales representing more collective motions and molecular function.

Experimental methods resolving real-time dynamics from femtoseconds onwards are mainly based on the pump-probe principle. Here a trigger, often an ultrafast laser pulse, is used to initiate non-equilibrium reaction dynamics followed by a probe pulse after a defined time delay. Variants of this experimental scheme are the basis for ultrafast optical spectroscopies in the UV/VIS and IR, which are routinely used to investigate light-induced dynamics e.g. in proteins, energy transport in new materials or reaction intermediates during photocatalysis [1], in many labs. Similarly, multidimensional nonlinear spectroscopies, like 2D-IR or 2D-ES are also based on the pump-probe principle, likewise ultrafast experiments utilizing new probe sources like X-Ray Free Electron lasers (ultrashort X-ray pulses) or electron pulses.

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In all these experiments the accessible timescales are regularly limited to studies from 10^{-14} s to 10^{-9} s, as time-delays are achieved by mechanical delay lines, which only allow delays up to few nanoseconds. Two synchronized but independent ultrafast laser sources allow to realize multiscale pump-probe experiments with continuous delays up to 1 ms (10^{-3} s) in case of 1 kHz laser systems or e.g. stroboscopic data collection schemes with 10^{-6} s timeresolution in case of >= 100 kHz probe sources. In case of all-optical experiments of table-top size, this enables full multiscale coverage to milliseconds. Several laboratories work on improved synchronization schemes for synchronizing two laser sources electronically with improved or negligible jitter.

While the use of two femtosecond laser sources allows for highest flexibility regarding possible experiments (because any wavemixing processes for required spectral ranges can be performed using high power regenerative amplifier laser systems with femtosecond pulses), also simpler solutions enabling specific multiscale experiments can be realized. In the lecture I presented recent work to realize pH-jump experiments up to milliseconds by the synchronization of a picosecond 1 kHz laser system (Nd:YAG) to a femtosecond Ti:Sa source [4]. While the Nd:YAG pump allows only limited pump wavelengths (SHG 532 nm, THG 355 nm), these are already sufficient to perform transient UV/VIS, transient IR and transient 2D-IR spectroscopy on molecular systems, which can be triggered at those respective wavelength.

One example for these types of experiments includes the study of protein structural dynamics upon pH jump: By exciting a photoacid sensitive at 355 nm a rapid change in pH within picoseconds can be realized, which is long-lived up to >100 microseconds and thus provides access to pH-sensitive, non-equilibrium dynamics over 7 orders of magnitude in time. This time-window is otherwise not continously accessible by other biophysical experimental tools, e.g. NMR spectroscopy or fluorescence spectroscopy. Processes, which can be studied by this approach include protein (un)folding and any peptide or protein structural changes, e.g. ligand binding sensitive to pH.

With the above-described setup for multiscale optical spectroscopy also experiments using photolabile protection groups (or often referred to as photocaged compounds) for triggering can be realized. Several photolabile protection groups have absorptions bands around 340-360 nm [5] and can be used to trigger

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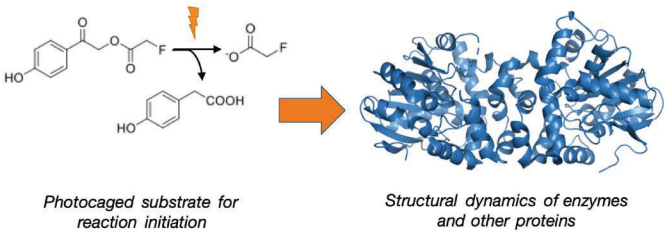

Fig. 2: Enzymatic catalysis can be studied over >10 orders of magnitude in time using a photocaged compound with substrate release time on the picosecond scale (here para-Hydroxyphenacyl) for triggering the dynamics [5], in this example of the enzyme Fluoroacetate dehalogenase [2, 3].

e.g. enzymatic catalysis in non-equilibrium time-resolved experiments.

In summary, multiscale optical spectroscopies using two laser sources and different triggering approaches, including direct laser excitation, laser-induced pH or temperature jumps and laser-induced ultrafast substrate release, are developing into a versatile tool box for addressing biochemical and chemical dynamics over 11 orders of magnitude in time reaching regularly delays from <100 fs to 1 ms.

However, specifically many biochemical processes possess relevant structural dynamics on the millisecond to seconds timescale. In a recent series of time-resolved serial X-Ray crystallography experiments at the synchrotron Petra III we demonstrated how this timewindow can be accessed in pump-probe type experiments of an irreversible enzymatic reaction [2, 3 see Fig. 2]. Building upon developments in sample delivery for time-resolved serial crystallography [6], namely the development of a fixed target able to provide 20.000-25.000 protein crystals on one silicon-based chip, we realized "hit-and-return" time-resolved serial crystallography [2]. In this approach, the immobilized sample on the chip is preilluminated with the pump laser and subsequently the sample is moved forward to pump the next sample. This is done for 1 or up to 512 sample positions before the chip is moved back to the initial starting position and the sample is probed. In this approach the nominal time-delay for any position equals the motion time between pump and probe, while the wall clock time is continuously used for preillumnation, thereby allowing pump-probe time-delays up to 30 s in an effective data collection scheme [3].

We applied this strategy to study the enzyme fluoroacetate dehalogenase [2] and were able to resolve room-temperature structures for 18 independent timepoints, covering 4 catalytic turnovers of the homodimeric enzyme. The time-resolved structural data provide insight to the catalytic mechanism and show a new mechanism for the regulation of the half-the-sites reactivity via a water-network of structured interfacial water molecules. This mechanism of water-mediated allostery is most likely a general mechanism for information transfer in proteins but couldn't be observed previously, as time-resolved room-temperature crystallography of irreversible processes, which can resolve water dynamics, wasn't available before.

Overall, multiscale experimental strategies will continue to provide new insights to many processes in chemistry and are important to link local dynamics with collective processes relevant for molecular function.

Literature

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Henrike Müller-Werkmeister (*1984) is a TT-professor for Physical Chemistry at University of Potsdam since fall 2017. A visit to DESY in 10th grade turned out as career-defining moment: In the exhibition „light of the future" she heard for the first time of the dream experiments to obtain molecular movies with then still-to-be-developed new light sources. This idea of filming the molecular nano-cosmos including proteins fascinated her and led her to first study Biochemistry, later in combination with Physics in Frankfurt. Her PhD work on developing ultrafast 2D-IR spectroscopy for studies of protein dynamics was awarded the Wilhelm-Ostwald-Nachwuchspreis by DBG, GDCh and Ostwald-Gesellschaft. Finally, during her postdoc as Marie-Curie Fellow in Toronto and Hamburg at Max-Planck-Institute for Structure and Dynamics of Matter, she was able to work on the dream experiments using XFELs to film proteins in action. Her independent group is targeting ultrafast structural dynamics in chemistry and biochemistry, mainly by 2D-IR spectroscopy and occasionally during X-ray experiments at large scale facilities.