With regard to the aspects of biomolecular hydration dynamics we previously discussed (fig. 10.1) our findings breed individual branches to a network of challenging biophysical tasks for equilibrium and real-time terahertz (THz) applications as well as a call for powerful computational tools for detailed simulations of dynamical processes in the THz region.

Based on our KITA study of Ub* folding [121] we may speculate that measuring folding kinetics in the THz region of our p-Ge laser spectrometer will be challenging as the 2 to 3 THz region provides additional insight to long-range influences on hydration dynamics induced by protein surface charge [85] and structural destabilization [20]. Due to the more powerful p-Ge laser THz source, it may be possible to resolve a slow kinetic phase of Ub* folding between 0.05 and 2 s. During the 0.05 and 2 s time span dynamics of merely a dozen of water molecules is involved in hydrophobic core finalization. To date full scale folding scenarios for two-state folding proteins are achievable which simulate the folding free energy landscape of the protein in detail [165]. However, computational approaches will be required to support the interpretation of complex hydration dynamics during folding which occurs as...
coupled vibrational motions of hydration water with proteins [166]. Thus dipole-dipole autocorrelation functions of ubiquitin and water dynamics are required for simulation of the denatured and folded state aqueous ubiquitin solutions by molecular dynamics simulations in the KITA range from 0.1 to 1 THz and in the p-Ge laser range from 2 to 3 THz respectively [31]. Predictions for the more complex scenario of water network motions during enzyme catalysis are even more sophisticated since multi-body problems with cross-interacting components are involved which are given by a bunch of substrate molecules, an enzyme with specific polarity changes at the active site, and the multitude of solvent molecules with specific rotational and translational dynamics and hydrogen bond lifetimes depending on their location in the reaction volume, at the active site, in the dynamical hydration shell of enzyme or substrate, or in the bulk. Thus only \textit{ab initio} molecular dynamics simulations [26] promise a suitable solution for simulating a precise THz absorption spectrum including the collective modes of all discussed fractions of the kinetic ensemble. Dynamic properties and their spatial extension of the biochemically active species embedded in the aqueous solvent with its fluctuations in polarization [167] are determined by these collective THz modes. However the computation time required for full-scale \textit{ab initio} simulations of such a kinetic ensemble exceeds by far the range of feasibility.

Our systematic hydration study of monovalent salts (fig. 10.2) showed that small cations and anions interact differently with the dynamically coordinated water network [168]. In the THz region cations oscillate in the water network with an oscillation frequency depending on the ion mass whereas the heavier anions did not exhibit such a mass-dependence, an observation which turns out to be \textit{vice versa} in Raman spectroscopy at
Figure 10.3: Terahertz temperature-jump experiment (THz-T-jump). By rapid temperature increase shock denaturation of a model protein (here: AFP III from pdb file 1HG7) is induced. The temperature change is just sufficient (typically a few Kelvin) to pass the denaturation barrier. Subsequently the protein will refold while the solution temperature slowly drops back to the initial equilibrium conditions. While the protein is refolding, the THz electric field is read out successively in order to follow the water network dynamics during folding in real-time.

In vitro folding mechanisms astonishingly resemble co-translational in vivo folding at the ribosome [171] though quite different time spans occur with a generation rate of 2 to 4 amino acids per second of the evolving polypeptide chain at a eukaryotic ribosome [172] compared to in vitro conditions where a complete folding transition to the native state is performed during nanoseconds up to seconds. Therefore it will be interesting to compare in vitro folding to upcoming in vivo techniques like the heat-shock induced denaturation experiment which initiates in vivo refolding [173] and is monitored by fluorescence-dependent fast relaxation imaging (FReI). An improved technical tool for real-time in vitro studies of folding is our THz-T-jump setup (fig. 10.3) which imitates in vitro T-jump techniques by fluorescence spectroscopy [174] and allows for real-time studies of water network reorganization dynamics during a folding reaction with sub-microsecond time resolution. A complementary technique to study denaturation by application of high-pressure (p-jump) has been developed recently [175-176] which like T-jump is a method based on thermodynamically-induced denaturation and refolding proteins. Additionally mechanical denaturation by nano-manipulation of folded proteins by improved atomic force microscopy.
has been developed as an alternate option to gain insight to folding mechanisms [177]. Our KITA and the proposed THz-T-jump technique enrich the concert of methods to study folding with specific focus on hydration water dynamics and long-range water network dynamics, a feature which is “invisible” to complementary techniques available today.

KITA studies of enzyme-substrate interactions with their solvent during molecular recognition and catalytic turnover promise a novel entrance to solvent dynamics-based drug design rather than reducing the design of antibodies and enzymes to protein structure and conformational protein dynamics, or as Martin Chaplin announced in his review on the biological relevance of water [178] “liquid water is not a ‘bit player’ in the theatre of life, it’s the headline act.”