Architecture and dynamics of proteins and aqueous solvation complexes
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3 Experimental section

In the following, the three experimental setups that have been used in collecting the data for this thesis are presented.

3.1 One-color infrared pump-probe setup

The experiments described in Chapters 4 and 5 were performed with a femtosecond mid-infrared setup based on a commercial regenerative Titan:sapphire amplifier system (Spectra Physics Hurricane), delivering 100 fs-pulses centered around 800 nm and a pulse energy of 1 mJ at a repetition rate of 1 kHz. About 700 µJ of the pulses are used to pump a commercial optical parametric amplifier (OPA, Spectra Physics) based on β-barium borate (BBO). The OPA is seeded by a white-light continuum generated by focusing a small fraction of the 800 nm-beam into a sapphire plate. The white-light seed and a fraction of the 800 nm-pump beam are then overlapped in a BBO-crystal, leading to the generation of signal and idler beams in the wavelength ranges of $\lambda_S = 1250$-1330 nm (signal) and $\lambda_I = 2000$-2200 nm (idler). The signal beam is filtered out by a dichroic mirror and the idler is used to seed a second amplification process in the same BBO-crystal driven by ~ 570 µJ of the 800 nm-pump beam, yielding final pulse energies of 40 µJ (signal) and 20 µJ (idler). The desired wavelengths are selected by angle tuning of the BBO-crystal. The resulting idler beam is frequency doubled in a second BBO-crystal to a wavelength of 1000-1100 nm and is subsequently used as a seed for parametric amplification pumped by the remaining part (~ 300 µJ) of the 800 nm-beam in either a lithium niobate (LiNbO$_3$) or a potassium titanyl phosphate (KTiPO$_4$) crystal, leading to the generation of mid-infrared pulses with a center wavelength of $\lambda_{IR} = 3$-4 µm and a pulse length of ~ 150 fs. Pulse energies are typically in the range of 5-9 µJ. Residual signal and (doubled) idler beams are filtered out by a germanium-based long-wave pass (LWP) filter with a cut-off wavelength of 2.5 µm.

The mid-infrared pulses are used in a pump-probe experiment as schematically depicted in Figure 3.1. Reflections from the front and the back side of a calcium fluoride (CaF$_2$) wedge are used as probe and reference beams, respectively. The transmitted part (~ 90%) is used as the pump beam. Its plane of polarization is rotated by a λ/2-plate to an angle of 45° relative to the plane of polarization of the probe beam. A chopper running at half the repetition of the laser system is placed in the beam path of the pump to block every other shot, so that long term drifts are minimized and only pump-induced absorption changes are monitored. The probe beam is delayed in time by sending it over a motorized delay stage. All beams are focussed by a gold-coated off-axis parabolic mirror.
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3.1

**Figure 3.1.** Schematic outline of the infrared pump-probe experiment that was used for the experiments in Chapters 4 and 5 of this thesis. The mid-infrared pulses are delivered from an optical parametric amplifier/difference frequency generation stage as described in the text and split into three parts. The pump-beam resonantly excites the OH-stretch (OD-stretch) vibration of water molecules, and a weak, time-delayed probing pulse measures the pump-induced absorption changes. The probe and the reference pulse are frequency-dispersed by a spectrograph and imaged on an MCT-array detector of 2x32 pixels.

with a focal length of $f = 100$ mm into the sample and recollimated by an identical mirror. The sample itself is usually a liquid layer of 1-100 μm thickness, sandwiched between two CaF$_2$ windows. The polarization components of the probe beam parallel and perpendicular to the pump beam are selected by a wire grid polarizer on CaF$_2$, placed behind the sample, that can be rotated over 90°. The probe and reference beams are sent into an imaging spectrograph (Lot Oriel) and imaged on a 2x32 pixel array of a mercury cadmium telluride (MCT)-based infrared detector (Infrared Associates), placed in the exit plane of the spectrograph. The signal is amplified by a gated boxcar-integrator and digitized by a 16-bit A/D-converter card (Measurement Computing). The transient absorption change is calculated as

$$
\Delta \alpha = - \log_{10} \frac{I_{pr} / I_{o, pr}}{I_{ref} / I_{o, ref}}
$$

(3.1)

where $I_{pr}$ and $I_{ref}$ denote the recorded intensity of probe and reference beams in the presence of the pump, and $I_{o, pr}$ and $I_{o, ref}$ denote the probe and reference intensity in the absence of the pump. Dividing the recorded probe intensity $I_{pr}$ by the intensity of the reference beam $I_{ref}$ eliminates pulse-to-pulse fluctuations and increases the signal-to-noise ratio of the experiment. If we let $\Delta \alpha_{\parallel}$ denote the absorption change monitored with the polarization component of the probe beam that is parallel to the plane of polarization of the pump beam and $\Delta \alpha_{\perp}$ the absorption change with the perpendicularly polarized component of the probe beam, we can calculate the isotropic absorption change $\Delta \alpha_{iso}$ as
The isotropic signal is unaffected by orientational effects and reflects vibrational relaxation and spectral diffusion. In addition, we also construct the anisotropy parameter $R(\omega,t)$ given by Eq. (2.74), which reflects the orientation of the transition dipole moment of the probed vibration.

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$$\Delta \alpha_{\text{iso}}(\omega, t) = \frac{1}{3}(\Delta \alpha_{\parallel}(\omega, t) + 2\Delta \alpha_{\perp}(\omega, t))$$

(3.2)

The isotropic signal is unaffected by orientational effects and reflects vibrational relaxation and spectral diffusion. In addition, we also construct the anisotropy parameter $R(\omega,t)$ given by Eq. (2.74), which reflects the orientation of the transition dipole moment of the probed vibration.

3.2 Two-dimensional infrared setup

The experimental setup for two-dimensional infrared (2D-IR) spectroscopic experiments in Chapters 6 and 7 is shown in Figure 3.2. The experiments were performed with a frequency-domain implementation of two-dimensional spectroscopy (spectral hole burning) that makes use of spectrally narrow excitation pulses. In this experiment, mid-infrared pulses with a center wavelength of $\sim 6 \mu m$ were generated in a commercial high-energy optical parametric amplifier (HE-Topas, Light Conversion) pumped by 35 fs 800 nm pulses with a pulse energy of 4.5 mJ delivered by a commercial regenerative Titanium:sapphire amplifier (Legend Elite Duo, Coherent) at a repetition rate of 1 kHz. The first parametric amplification step in a BBO-crystal is seeded by a white-light continuum, generated by focussing a small fraction of 800 nm-light into a sapphire-plate. Signal and idler beams with wavelength of $\lambda_S \approx 1.4 \mu m$ and $\lambda_I \approx 1.8 \mu m$, respectively, are then further amplified to final pulse energies of 1.125 mJ (signal) and 0.875 mJ (idler) in two subsequent BBO-crystals. Mid-infrared pulses with a pulse energy of $\sim 25 \mu J$ and a bandwidth of 350 cm$^{-1}$ are generated via a type I difference-frequency generation (DFG) process by overlapping the signal and idler beams in a 0.6 mm thick silver thiogallate crystal (AgGaS$_2$, cut-angle $\theta = 39^\circ$). After the generation of the mid-infrared pulses, the residual signal and idler beams are filtered out by a germanium-based long-wave pass filter with a cut-off wavelength of 4 $\mu m$. The use of a longer cut-off wavelength (compared to the one-color infrared pump-probe setup) is required to filter out infrared light at 3 $\mu m$, resulting from a second harmonic generation process of the mid-infrared pulses at 6 $\mu m$ that is close to being phase-matched in the AgGaS$_2$ crystal.

The mid-infrared pulses are split in pump, probe and reference pulses as described in Section 3.1. The pump light is directed into an air-spaced, tunable Fabry-Perot etalon, consisting of two parallel, highly reflecting mirrors (reflectivity R = 90%). The spacing between the mirrors and thus the optical path length difference, that determines which frequency components interfere constructively and destructively, can be adjusted to generate spectrally narrow excitation pulses from the broad-band mid-infrared pulses. The narrow-band excitation pulses have a Lorentzian-shape profile with a full-width at half maximum of 12-15 cm$^{-1}$, which corresponds in the time-domain to a single-sided exponential with a characteristic decay time $\tau$ in the range of 500-600 fs, as inferred from two-photon absorption in an indium-arsenide wafer. Pump, probe
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Figure 3.2. A transmission through the sample, a small fraction of the narrow-band pump pulse is directed into the spectrograph and dispersed on a 32-pixel MCT-array. The measured pump-pulse spectrum is then used as the input signal for the feedback-loop that controls the piezo-driven mirror mount. To compensate for the drift of the piezo-actuators on a time-scale of minutes, a reoptimization of the mirror positions after every delay scan is required (typically ∼5 mins) is required.

and reference beams are focussed by a gold-coated off-axis parabolic mirror with a focal length of $f = 75$ mm into the sample and recollimated by an identical mirror. The measurement of the pump-induced absorption changes $\Delta \alpha_{\parallel}, \Delta \alpha_{\perp}$ is performed in essentially the same way as was described in Section 3.1. A two-dimensional data set is assembled by scanning the spectrally narrow pump pulse over the desired frequency range, which requires adjusting the spacing between the mirrors of the etalon. In practice, this is achieved by placing one of the two mirrors on a piezo-driven mount and monitoring the pulse spectrum by directing a weak reflection of the pump light from a CaF$_2$-window into the spectrograph and imaging it on the MCT detector. The signal from the MCT detector is used in an automated feedback routine to drive the piezo-actuators, thereby allowing the tuning of the spectrally narrow excitation pulse to the desired center frequency.

3.3 Sum-frequency generation spectroscopy setup

The surface sum-frequency generation experiment is based on a commercial Titanium:sapphire regenerative amplifier (Coherent Legend Elite) that delivers 35 fs pulses centered at 800 nm with a pulse energy of 3.5 mJ at a repetition rate of 1 kHz. Approximately two thirds of the output is used to pump a home-built optical parametric amplifier (OPA) based on $\beta$-barium borate, generating sig-
nal and idler pulses around 1270 nm and 2150 nm, respectively. Mid-infrared pulses are generated by mixing the signal and idler pulses in a type I difference frequency mixing process (DFG) in a 0.6 mm thick silver thiogallate crystal (AgGaS$_2$). The resulting femtosecond mid-infrared pulses (IR) are centered around 3200 cm$^{-1}$, have a bandwidth of $\sim$ 350 cm$^{-1}$ and a pulse energy of 8 - 10 $\mu$J. About 450 $\mu$J of the 800 nm-output is sent through an air-spaced etalon to generate pulses with a narrow spectral bandwidth of $\sim$ 15 cm$^{-1}$ centered around 800 nm.

The SFG technique probes the elements of the second-order susceptibility tensor $\chi^{(2)}$. The polarizations of the IR, visible (= 800 nm) and the detected sum-frequency light determine which elements are probed. The polarizations are denoted with s or p, depending on whether the light is polarized perpendicular (s-polarized) or parallel (p-polarized) to the plane of incidence of the beams. A polarization configuration psp implies that the detected SFG-light is p-polarized, the visible (VIS) is s-polarized, and the mid-infrared beam is p-polarized. The polarizations of the VIS and the IR beams are set by means of $\lambda/2$-plates in combination with a wire-grid polarizer (IR) or a Glan-Laser-prism (VIS). The VIS and the IR beams are focused on the sample surface by lenses with focal lengths of $f = 200$ mm and $f = 100$ mm, respectively. The beams are sent to the sample with an angle of incidence of $\sim 50^\circ$ and $\sim 55^\circ$, respectively, and are spatially and temporally overlapped at the sample to generate light at the sum-frequency (SFG). For intensity (non-phase-resolved) SFG-measurements, the generated SFG-light is sent into a spectrograph, after filtering out the residual 800 nm light, and dispersed on a thermoelectrically-cooled EMCCD-camera (Andor Technologies). A polarizing beam-splitter cube in the detection path is used to select the polarization of the generated SFG-light. The SFG-spectra are normalized to a non-resonant reference SFG-signal from a z-cut quartz crystal. The signal is integrated for typically 10 minutes in both direct and phase-resolved SFG-experiments.

Phase-resolved SFG spectra are obtained by interfering the SFG light generated by the sample with the SFG light generated from a local oscillator. The lay-out is schematically depicted in Figure 3.3. The IR- and the VIS-beam are first focused on a bare gold mirror to generate a local oscillator (LO) before they are refocused by a spherical mirror together with the LO-beam on the sample. The LO-beam from gold is time-delayed ($\sim 2$ ps) by transmitting it through a fused silica plate. The SFG-signals from gold and from the sample propagate collinearly through the detection path and generate a spectral interferogram on the CCD-camera after being dispersed by a spectrograph. In Figure 3.4A, a spectral interferogram from a phase-resolved SFG-experiment recorded under ssp-polarization is shown. The fringe pattern originates from the interference between the time-delayed LO-SFG-signal ($E_{LO}$) and the SFG-signal generated from an aqueous sample ($E_{sample}$). The recorded intensity of the heterodyne-detected SFG (HD-SFG) signal is given by:

$$I_{HD-SFG}(\omega) \propto |E_{sample}(\omega)|^2 + |E_{LO}(\omega)|^2 + E^*_{sample}(\omega)E_{LO}(\omega)e^{i\omega\Delta t} + E_{sample}(\omega)E^*_{LO}(\omega)e^{-i\omega\Delta t}$$ (3.3)
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Figure 3.3. Top: schematic of the experimental geometry of the sum-frequency generation spectroscopy setup used for intensity (non-phase resolved) measurements. Mid-infrared pulses (IR) are generated by a home-built optical parametric amplifier and spectrally narrow upconversion pulses (VIS, $\Delta \nu \approx 15 \text{ cm}^{-1}$) are generated by transmission of a fraction of the 800 nm laser fundamental through an air-spaced etalon. The desired polarization of the generated SFG-light is selected with a polarizing beam splitter (PBS) and subsequently frequency-dispersed in a spectrograph and imaged on a CCD-camera. Bottom: Experimental arrangement used for phase-resolved measurements. A non-resonant SFG-field (local oscillator, LO) is generated from the surface of a bare gold mirror and delayed in time by transmission through a silica plate. Subsequently, all three beams (IR, VIS, LO) are recombined on the sample surface, where a second SFG-generation process takes place. The interference between the SFG-field generated at the sample and the time-delayed local oscillator leads to a fringe pattern in the detected interferogram.

The cross-term $E_{\text{sample}} E_{\text{LO}}^* e^{-i\omega \Delta t}$ of this equation contains the electric field of the SFG-signal originating from the sample and therefore offers access to the complex non-linear susceptibility. As was shown in Section 2.2, the SFG-field is related to the non-linear susceptibility as $E_{\text{SFG}} \propto \chi^{(2)} E_{\text{VIS}} E_{\text{IR}}$. To retrieve the complex-valued $\chi^{(2)}$ of the sample, an inverse Fourier transformation of the recorded interferogram $FT^{-1}[I_{HD-SFG}(\omega)]$ is performed. The imaginary part of the complex time-domain signal obtained in this way is shown in Figure 3.4B. The desired cross-term indicated in Figure 3.4B can now be isolated by setting the signal at $t \leq 0.8 \text{ ps}$ to zero and subsequently Fourier-transforming the
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Figure 3.4. (A) Spectral interferogram recorded from an aqueous solution of an antifreeze protein under ssp-polarization setting. (B) Imaginary part of the time-domain signal obtained by applying an inverse Fourier transformation to the data in panel (A). The data for delay times \( t \leq 0.8 \) ps (indicated by the dashed line) are set to zero. (C) Imaginary part of the complex SFG-spectrum from the sample obtained by Fourier transformation of the filtered signal in panel (B).

filtered time-domain signal back to the frequency domain. In practice, the signal for \( t \leq 0.8 \) ps is filled with random numbers from a gaussian distribution with zero mean and a width comparable to the experimental noise level to avoid artifacts in the Fourier transformation arising from discontinuities in the signal. The imaginary part of the cross-term \( E_{\text{sample}}(\omega)E_{\text{LO}}^*(\omega)e^{-i\omega\Delta t} \) obtained after filtering is shown in Figure 3.4C. The non-linear susceptibility \( \chi_{\text{sample}}(\omega) \) is then obtained by dividing the signal shown in Figure 3.4C by a reference signal, which is obtained by replacing the sample with a z-cut quartz crystal with a known \( \chi^{(2)} \). After applying the same filtering process as outlined above, \( \chi^{(2)}_{\text{sample}}(\omega) \) is obtained as

\[
\chi^{(2)}_{\text{sample}}(\omega) = \chi^{(2)}_{\text{ref}} \frac{E_{\text{sample}}(\omega)E_{\text{LO}}^*(\omega)e^{-i\omega\Delta t}}{E_{\text{ref}}(\omega)E_{\text{LO}}^*(\omega)e^{-i\omega\Delta t}}
\]

(3.4)

where \( \chi^{(2)}_{\text{ref}} \) and \( E_{\text{ref}} \) denote the nonlinear susceptibility of the (reference) quartz-crystal and the SFG-field generated from the crystal, respectively.