Open access • Journal Article • DOI:10.1146/ANNUREV.PHYSCHEM.59.032607.093757

Two-Dimensional Infrared Spectroscopy of Photoswitchable Peptides

Peter Hamm, Jan Helbing, Jens Bredenbeck

Institutions: University of Zurich

Published on: 04 Apr 2008 - Annual Review of Physical Chemistry (Annual Reviews)

Topics: Two-dimensional infrared spectroscopy, Spectroscopy and Transverse relaxation-optimized spectroscopy

Year: 2008

Two-dimensional infrared spectroscopy of photoswitchable peptides

Hamm, P; Helbing , J; Bredenbeck, J

Two-dimensional infrared spectroscopy of photoswitchable peptides

Abstract

We present a detailed discussion of the complimentary fields of the application of two-dimensional infrared (2D-IR) spectroscopy in comparison with two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy. Transient 2D-IR (T2D-IR) spectroscopy of nonequilibrium ensembles is probably one of the most promising strengths of 2D-IR spectroscopy, as the possibilities of 2D-NMR spectroscopy are limited in this regime. T2D-IR spectroscopy uniquely combines ultrafast time resolution with microscopic structural resolution. In this article we summarize our recent efforts to investigate the ultrafast structural dynamics of small peptides, such as the unfolding of peptide secondary structure motifs. The work requires two ingredients: 2D-IR spectroscopy and the possibility of triggering a structural transition of a peptide on an ultrafast timescale using embedded or intrinsic photoswitches. Several photoswitches have been tested, and we discuss our progress in merging these two pathways of research.

Two-Dimensional Infrared Spectroscopy of Photoswitchable Peptides

Peter Hamm, Jan Helbing, and Jens Bredenbeck

Physikalisch–Chemisches Institut, Universität Zürich, 8057 Zürich, Switzerland; email: phamm@pci.unizh.ch

Annu. Rev. Phys. Chem. 2008. 59:291–317

First published online as a Review in Advance on November 7, 2007

The *Annual Review of Physical Chemistry* is online at http://physchem.annualreviews.org

This article's doi: 10.1146/annurev.physchem.59.032607.093757

0066-426X/08/0505-0291$20.00

Key Words

2D-IR spectroscopy, ultrafast time-resolved spectroscopy, multidimensional spectroscopy, peptide dynamics, peptide folding

Abstract

We present a detailed discussion of the complimentary fields of the application of two-dimensional infrared (2D-IR) spectroscopy in comparison with two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy. Transient 2D-IR (T2D-IR) spectroscopy of nonequilibrium ensembles is probably one of the most promising strengths of 2D-IR spectroscopy, as the possibilities of 2D-NMR spectroscopy are limited in this regime. T2D-IR spectroscopy uniquely combines ultrafast time resolution with microscopic structural resolution. In this article we summarize our recent efforts to investigate the ultrafast structural dynamics of small peptides, such as the unfolding of peptide secondary structure motifs. The work requires two ingredients: 2D-IR spectroscopy and the possibility of triggering a structural transition of a peptide on an ultrafast timescale using embedded or intrinsic photoswitches. Several photoswitches have been tested, and we discuss our progress in merging these two pathways of research.

1. INTRODUCTION

Two-dimensional infrared (2D-IR) spectroscopy: vibrational spectroscopy that elucidates connectivity between vibrational states by expanding the spectrum into two dimensions

NMR: nuclear magnetic resonance

MD: molecular dynamics

The importance of the structural dynamics of biomolecules for their function is widely appreciated. Two-dimensional infrared (2D-IR) spectroscopy is a novel spectroscopic tool that uniquely combines ultrafast time resolution with appreciable structural resolution. The transferability of the concepts of multidimensional nuclear magnetic resonance (NMR) spectroscopy (1) to IR spectroscopy has been postulated already in the earliest publications on 2D NMR (2); however, the first 2D-IR spectrum was measured only relatively recently (3). In analogy to 2D-NMR spectroscopy, in 2D-IR spectroscopy the connectivity of various vibrational states can be related to local contacts of the corresponding molecular groups, which is the basic principle of structural determination in both cases. Equivalents of all fundamental NMR experiments [COSY (4, 5), NOESY (6), and EXSY (7–9)] have been demonstrated in the IR range in the meantime (10).

By nature of the close analogy, it might seem that 2D-IR spectroscopy's biggest competitor will always be 2D-NMR spectroscopy, which would put the bar high! Both 2D-NMR and 2D-IR spectroscopy are capable of resolving certain functional groups in a molecule, work (mostly) in the solution phase, and aim to elucidate the structure as well as dynamics of solution-phase systems. Over the course of the past 20–30 years, NMR spectroscopy has proven to be so much more versatile that it has largely superseded conventional (1D) IR spectroscopy for standard analytic purposes for essentially two reasons: (*a*) NMR spectra are more structured and allow one to resolve individual resonances from much larger molecules than IR spectroscopy. (*b*) The interpretation and assignment of NMR spectra work on the basis of simple empirical rules, whereas the assignment of IR spectra is currently challenging to even the most powerful quantum-chemistry codes for only a midsize molecule with, for example, 30 atoms.

When we began to develop 2D-IR spectroscopy as a tool to determine molecular structure, we concentrated on a small peptide, trialanine (6, 11–13). Much to our surprise, the conformation of that molecule, or rather the distribution of conformations, was not known at the time although it has important model character: The backbone of trialanine contains only one central (ϕ, ψ)-pair of dihedral angles and hence reflects the intrinsic structural propensity of the peptide bond itself without the additional complication of intermolecular hydrogen bonds in larger peptides that form secondary or tertiary structure motifs. In a series of papers (6, 11–13), we determined the preferred conformation and the relative populations of conformations, as well as the flexibility of trialanine. We found that trialanine populates predominantly (approximately 80%) the so-called poly-prolin II structure (P_{II}). Other structural elements such as α-helical and β-sheet motifs seemed to contribute only little. This result was unexpected because the P_{II} structure is not common in larger proteins, and standard molecular dynamics (MD) force fields predicted different structural distributions (14). Our results (among others) led to suggestions for modifications of the AMBER force field (15).

Recently, our structural prediction of trialanine has essentially been confirmed in a combined NMR and MD simulation study, going just a bit beyond the most elementary methods of the rich toolbox of NMR spectroscopy (16). This is good and

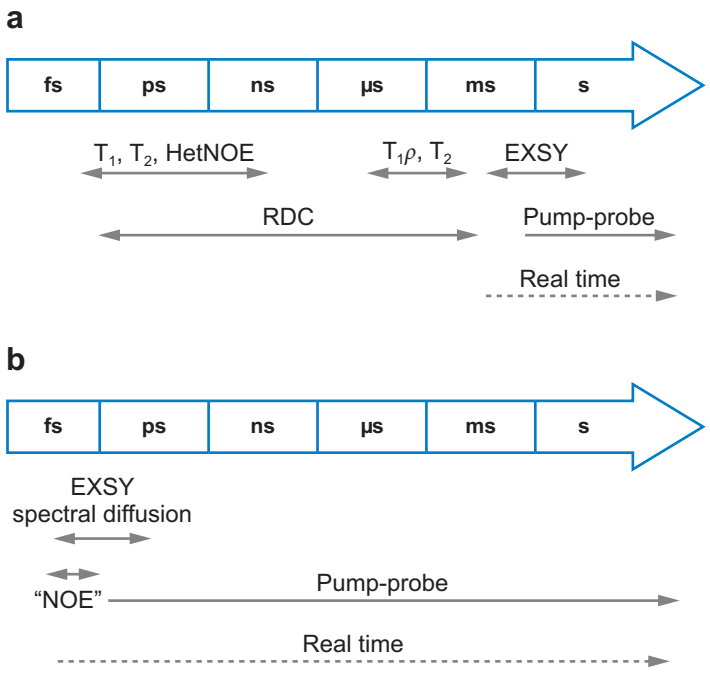

Figure 1

Time arrow from femtoseconds to seconds, together with time regimes that can be covered by various techniques of (*a*) two-dimensional nuclear magnetic resonance and (*b*) two-dimensional infrared spectroscopy. Panel *a* is adapted from Reference 17. EXSY, exchange spectroscopy; HetNOE, heteronuclear Overhauser effect; NOE, nuclear Overhauser effect; RDC, residual dipolar coupling.

bad news: On the one hand, it validates the use of 2D-IR spectroscopy in determining the unknown structure of small peptides, even when the experimenter cannot put any biased expectation into the structural analysis. On the other hand, it suggests that if 2D-NMR spectroscopy tries hard enough, it almost always will beat 2D-IR spectroscopy in terms of pure structural-resolution capabilities.

Many have argued that the large potential of 2D-IR spectroscopy, as compared with 2D-NMR spectroscopy, is its many orders of magnitude higher intrinsic time resolution. However, one must carefully specify what exactly is meant by time resolution. **Figure 1*a*** shows the time regimes that can be covered by numerous variants of NMR spectroscopy, which do in fact include the ultrafast picosecond and even subpicosecond range (17). For example, in the mid-1970s, Fung & McGaughy (18) deduced the orientational diffusion time of bulk water using NMR spectroscopy, revealing a value of 2.3 ps for D_2O. Only approximately 20 years later (19), IR spectroscopy came into the position to repeat the measurement, revealing that the process is more complex. The generally accepted view now is that the anisotropy decay is biphasic with a sub-100-fs librational component prior to a 2.5–3-ps diffusive component (20, 21). Hence, although NMR spectroscopy is essentially right for the diffusive part, it completely misses the fast component and cannot differentiate the contributions in a heterogeneous process—a subtle but important difference.

The ultrafast time regime is accessed by the intrinsically very slow NMR spectroscopy indirectly through relaxation methods (T_1, T_2, HetNOE, $T_1\rho$, and residual dipolar couplings; see **Figure 1*a***). For example, in the case of the orientational diffusion of water discussed above, a simple theory exists that relates the T_1 relaxation time

EXSY: exchange spectroscopy

of the proton spin transition, which is what is actually measured in the experiment and is quite slow (0.45 s), to the fast orientational correlation time τ_c of the molecule (18). Both quantities are inversely proportional; hence the faster the molecular structural process is, the slower the spin relaxation. The important point is that this theory relies on a model assumption—free orientational diffusion—which is not extremely well justified in the case of water (22). NMR relaxation techniques cannot validate the model assumption made to interpret the data and, hence, tend to oversimplify complex systems such as liquid water. For a truly diffusive process (e.g., the reorientation of a larger, close to spherical molecule in solution), the results from NMR spectroscopy are usually correct.

The important difference between IR and NMR spectroscopy is the time range that can be accessed in real time. That is, the experimenter modifies a time variable somewhere in the measuring instrument—e.g., the timing of one part of an NMR pulse sequence with respect to the rest, or the sledge of an optical delay line—and that time is related directly to the time a particular structural process takes in a one-to-one manner (e.g., the time for interconversion of two molecular species). (For the purpose of this review, we distinguish between structural and energy relaxation processes, the latter of which can be investigated as well by 2D-IR spectroscopy.) In the context of this definition, this is exchange spectroscopy (EXSY) and pump-probe spectroscopy (**Figure 1**).

In an EXSY experiment, an initial part of a pulse sequence (preparation phase) is used to label a subensemble of molecules in a certain conformation by exciting a spectrally distinct spin or vibrational state. The subensemble is then given time to change its conformation during a waiting period, after which the conformation is finally read out. By varying the waiting period, one observes the conformational transition in real time. EXSY has an upper limit of the accessible time window, which is dictated by the excitation lifetime of the spin or vibrational state used as a label. In the case of 2D-IR spectroscopy, the time window ranges, for example, from approximately 100 fs for the frequency fluctuations of the OH vibration in water (i.e., spectral diffusion) (23–26) to typically a few tens of picoseconds for hydrogen bond on and off times in solute-solvent systems (7–9).

In a pump-probe experiment, a molecular system is perturbed in some way (e.g., by triggering a photoreaction or by a temperature jump) and thereby brought into a nonequilibrium state. As a result, all molecules in the sample volume undergo a conformational transition in a concerted way, and 2D-NMR or 2D-IR spectroscopy is then used to elucidate a snapshot structure of that ensemble on the fly. The lower time limit of pump-probe spectroscopy is dictated by the spectroscopic method (approximately 1 ps for 2D-IR spectroscopy and tens of milliseconds for 2D-NMR spectroscopy), whereas no intrinsic limitation is imposed toward longer timescales.

From all the methods indicated in **Figure 1**, pump-probe spectroscopy is the only one that allows access to the nonequilibrium regime. Comparing **Figures 1*a*,*b***, one sees clearly that the biggest potential of IR spectroscopy, as compared with NMR spectroscopy, lies in the almost unlimited time window accessible to such nonequilibrium experiments. This is why we made the strategic decision to explore and develop this capability of 2D-IR spectroscopy: measuring snapshot structures of fast evolving

molecular systems by means of transient 2D-IR (T2D-IR) spectroscopy. This is where the dynamical aspect of NMR spectroscopy almost completely breaks down.

T2D-IR spectroscopy is a versatile technique that can be applied to all sorts of problems in chemistry and biophysics. For example, we have studied in detail the polarization dependence of the 2D-IR spectra of a metal-to-ligand charge transfer complex in its electronically excited state (27), established a technique that allows one to correlate vibrations in the electronic ground state to those in a photoproduct state (i.e., labeling vibrations by light) (28), applied that technique to elucidate the connectivity of binding sites after the photodissociation of CO in myoglobin (29), and studied solvation phenomena beyond the linear response regime (30). Recently, T2D-IR experiments have also been reported by Tokmakoff and coworkers (31, 32), as well as Kubarych and coworkers (33). In this review we focus on the folding dynamics of photoswitchable peptides (34–36). Our approach requires two ingredients that we currently explore as parallel research paths in our laboratory: (*a*) 2D-IR and T2D-IR spectroscopy (Section 2.1) and (*b*) concepts of the ultrafast photoswitching of peptides (Section 2.2). In Section 3, we report on our current progress in merging these two pathways of research.

Transient two-dimensional infrared (T2D-IR) spectroscopy: two-dimensional infrared spectroscopy of transient species

Photoswitchable peptides: peptides whose conformation can be switched through light by means of a photo-isomerizable or photocleavable moiety embedded in or attached to the peptide

2. METHODOLOGY

2.1. Two-Dimensional Infrared and Transient Two-Dimensional Infrared Spectroscopy

Although the first 2D-IR experiment was a pulsed-frequency domain experiment (3), a technique that has been adapted and refined by various groups (4, 6, 7, 11–13, 37–41), time domain–pulsed Fourier transform techniques have been devised as well (8, 9, 23–26, 42–57). Variants of 2D vibrational spectroscopy are 2D-Raman spectroscopy (58–60) and DOVE spectroscopy (i.e., doubly vibrationally enhanced four-wave mixing, a hybrid frequency–time domain spectroscopy) (61, 62). 2D spectroscopy has also been applied to electronic transitions (63–68), addressed extensively by theoretical groups (10, 69–82), and reviewed from various perspectives (5, 83–91).

The information content of the time domain and frequency domain implementations of 2D-IR spectroscopy is largely identical (55, 92): The pulsed-frequency domain experiment preselects the pump frequency with the help of a tunable Fabry-Perot filter, whereas the time domain–Fourier transform techniques achieve the pump process by two phase-locked, spectrally broad pulses that interfere in the sample. In the latter case, the spectral information is regained by Fourier transforming the data after the measurement. With respect to the probe process, both techniques are identical. The time domain–Fourier transform technique has conceptual advantages (highest possible spectral resolution, higher sensitivity, more control over pulse parameters of all three field interactions), which, however, come at the expense of a more difficult experiment (interferometic stability, accurate determination of the absolute phase) (92). Zanni and coworkers (54, 55) have recently proposed a hybrid method based on an acousto-optical modulator that seems to combine the advantages of both approaches.

It is more intuitive to discuss the outcome of a 2D-IR experiment in a frequency domain context. First let us consider two vibrators that are uncoupled, in which case a characteristic double-peak structure is obtained for each vibrator on the diagonal of the 2D-IR spectrum (**Figure 2*a***). Whenever the pump frequency is resonant with one

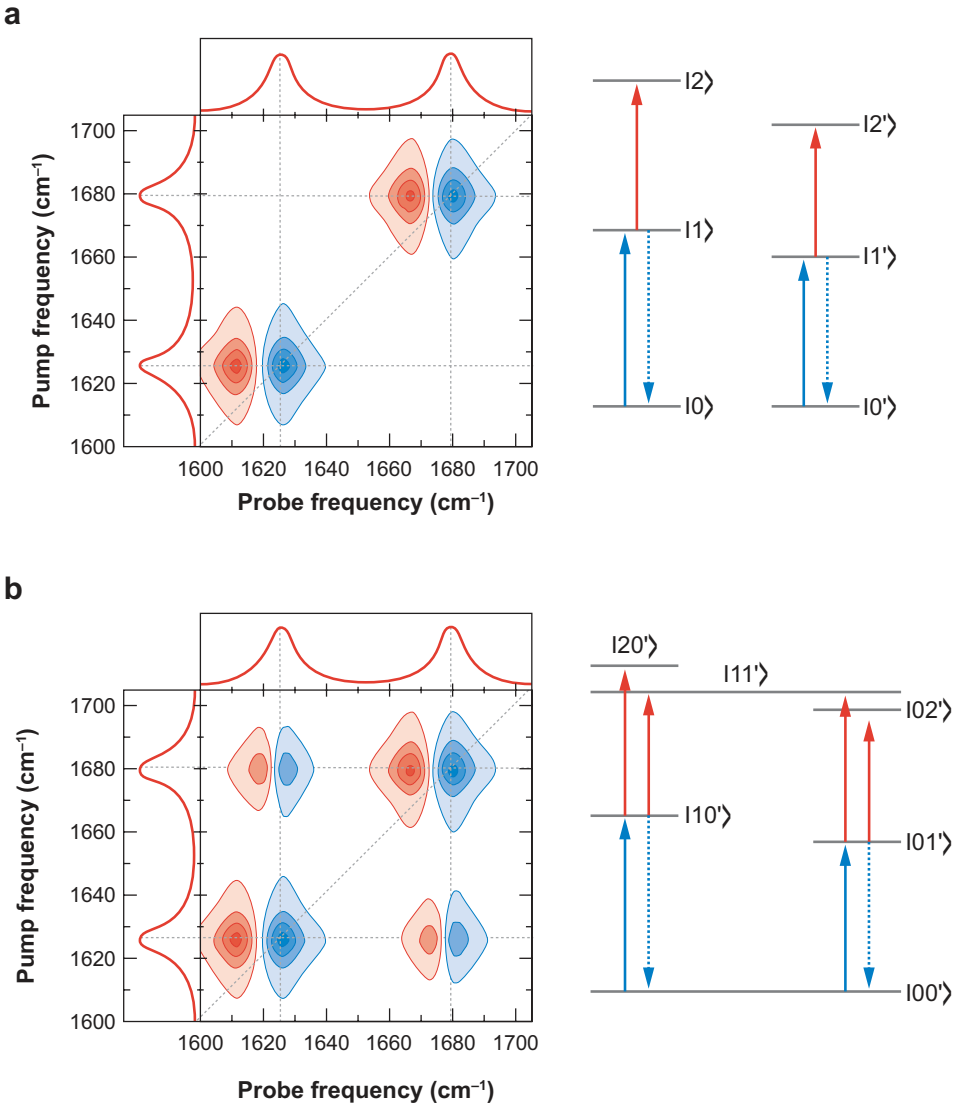

Figure 2

Two-dimensional infrared spectrum of two vibrators that are (*a*) not coupled and (*b*) coupled. Negative, bleach and stimulated emission is presented in blue, and positive, excited-state absorption is presented in red.

of the vibrators, it is promoted into the first excited state ($|1\rangle$ or $|1'\rangle$), and the probe beam then probes the fundamental 0–1 transition (bleach and stimulated emission), as well as the first 1–2 overtone transition (excited-state absorption). Because chemical bonds are always weakly anharmonic with slightly different frequencies of the 0–1 and the 1–2 transitions, the two peaks separate along the probe frequency axis. Both peaks have opposite signs, and we usually color code them blue (negative, bleach and stimulated emission) and red (positive, excited-state absorption).

When the coupling between the two vibrators is switched on (**Figure 2*b***) by bringing them into close spatial proximity, additional cross-peaks appear in the off-diagonal region of the 2D-IR spectrum. This is because the $|00'\rangle \rightarrow |10'\rangle$ transition, for example—exciting the first vibrator while the second is in its ground state—is different in frequency from the $|01'\rangle \rightarrow |11'\rangle$ transition, in which the second vibrator is already in its first excited state. This so-called intermode anharmonicity is a direct manifestation of the coupling between the two vibrators (86). The coupling can be of static nature (in the sense of a level scheme as shown in **Figure 2*b***) or of dynamic nature by the energy flow between the two vibrators (6). In any case, the intensity of the cross-peak carries information on the distance, whereas the anisotropy of the cross-peak reveals the relative orientation of the corresponding molecular groups. Both are important structural indicators that can be used to determine the unknown structure of small peptides from 2D-IR spectroscopy (4, 11, 12, 43, 49).

For reasons discussed in Section 1, we concentrate on an extension of 2D-IR spectroscopy, T2D-IR spectroscopy (27–30, 34–36). For that purpose, we introduce an additional optical or ultraviolet (UV) pulse preceding the 2D-IR pulse sequence, which triggers a photochemical reaction, or a conformational transition, of the molecular system under study. The evolving nonequilibrium ensemble is then probed by the 2D-IR part of the experiment, and we may call the outcome a UV-pump 2D-IR probe experiment.

Because the UV pump pulse in general does not convert 100% of the reactant species into the photoproduct species, the 2D-IR spectrum in the presence of the UV-switch pulse always contains contributions from both. To eliminate contributions of molecules that have not absorbed any UV photons, one records two sets of 2D-IR spectra simultaneously—one with the UV-switch pulse on and one with the UV-switch pulse off—and subtracts one from the other (34). Hence, just as it is common practice in conventional pump-probe spectroscopy, our T2D-IR spectra are in fact T2D-IR difference spectra.

To facilitate the interpretation of T2D-IR spectroscopy, we introduce the schematic response of an idealized molecule with one single homogeneously broadened oscillator, the frequency of which shifts on triggering the photoreaction. In analogy to the data presented in **Figure 2**, the vibrator gives rise to a 2D-IR spectrum with two peaks in each of the initial and product states: a negative (blue) bleach and stimulated emission signal at the vibrator's original frequency and a positive (red) excited-state absorption signal, which is red-shifted owing to the intrinsic anharmonicity of the oscillator. The T2D-IR spectrum, however, is the difference of the 2D-IR spectra of the initial and the transient species (**Figure 3**). In a transient 1D difference spectrum, negative contributions arise from the depleted initial population, whereas

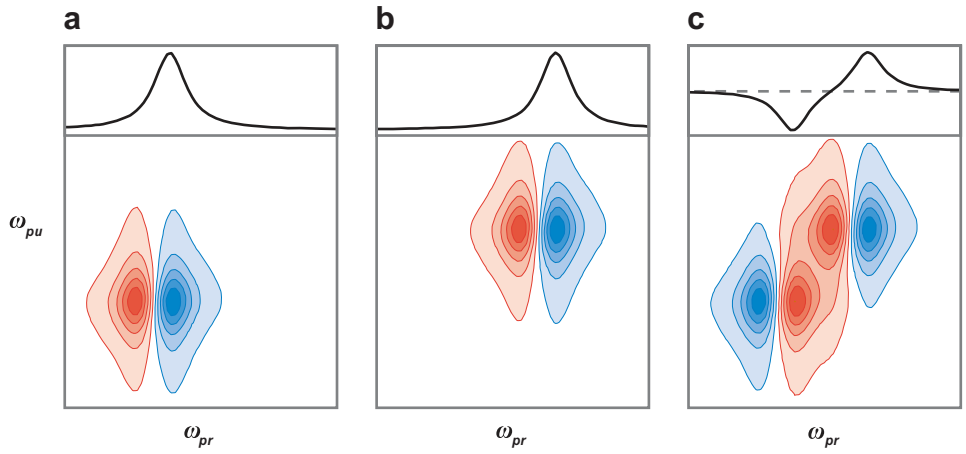

Figure 3

Schematic illustration of transient one-dimensional (1D) (*top panels*) and two-dimensional infrared (2D-IR) (*bottom panels*) spectroscopy. The absorption bands shift from the steady state (*a to b*), each yielding a characteristic 2D-IR spectrum. Panel *c* shows the difference 1D and 2D-IR spectra. Negative response is depicted in blue and positive response in red. Figure adapted from Reference 34.

positive contributions stem from the transient photoproduct (**Figure 3*c***, top panel). Accordingly, the signs of the various 2D-IR peaks of the initial and the product state are interchanged also in the T2D-IR difference spectrum (**Figure 3*c***, bottom panel).

If the frequency shift on the photoreaction, as well as the anharmonicities of reactant and product states, is large compared to the line width of a band, four peaks are expected in total along the diagonal for each individual resonance. Principally speaking, the same is true for each cross-peak. However, we demonstrate below that this is not the case in general, and the various contributions overlap and tend to strongly cancel each other owing to their opposite signs. In particular for transient cross-peaks, in which we anticipate a change in coupling and hence a change in intermode anharmonicity, only the two peaks related to the conformation with the stronger coupling tend to survive this cancellation effect (35). However, T2D-IR lineshapes have not been thoroughly studied from the theoretical side.

2.2. Photoswitchable Peptides

The second important ingredient for studying nonequilibrium structural dynamics is the possibility of initiating a conformational transition of a peptide or a protein on an ultrafast picosecond timescale. The best established approach to achieve this is a laser-induced temperature jump to initiate, for example, the folding or unfolding of a peptide (93–101). Temperature jumps, in principle, can be very fast and are only limited by the thermal equilibration time of the bulk solvent that is in the range of a few tens of picoseconds, depending on the concentration of the molecule used to absorb light and to deposit energy into the solvent (102). Nevertheless, most

temperature-jump studies have been limited to the 10-ns regime for technical reasons: Q-switched Raman-shifted Nd-YAG lasers were used to generate the intense IR pulse with an energy of typically 1 mJ needed to heat the solvent water directly by exciting an overtone of either its OH or OD stretch vibration.

However, the intrinsic time resolution of 2D-IR spectroscopy is significantly faster (1 ps) than the pulse duration of suitable Q-switched lasers (10 ns). We felt that by limiting the experiment's overall time resolution to 10 ns, we would potentially miss important advantages of 2D-IR spectroscopy (although this time resolution might be sufficient for most of the peptide dynamics problems studied in temperature-jump experiments).

Temperature jumps introduce another problem in combination with a 2D-IR experiment as a probe process: A significant amount of energy is deposited in a relatively small volume, which causes a sudden jump of pressure in a small sample volume. Researchers have devised strategies to reduce artifacts from the resulting shock waves and the resulting index of refraction gradients for conventional pump-probe experiments (103), but 2D-IR spectroscopy, consisting of a sequence of IR pulses, is more critical in this regard. That the pulsed-frequency domain experiment is not a phase-sensitive technique has proven to be a crucial advantage. Only recently, Tokmakoff and coworkers (31, 32) figured out how to cope with the problem of phase distortions using the time domain–Fourier transform technique.

We have chosen another approach to initiate conformational transitions in small peptides. By incorporating a photoswitch into a peptide that undergoes a photochemical reaction after electronic excitation, one may alter the conformation of the peptide in a controlled way. The switching time is ultrafast in most cases (≈1 ps). Furthermore, because the photoswitch is directly incorporated into the molecular system, its action is more local than that of a temperature jump. As a consequence, the overall energy pumped into the sample volume is much smaller (at least a factor of 10); hence problems with shock waves are considerably reduced (albeit still present in some cases). We distinguish between photoswitches, which are reversible, and phototriggers, which are one-time switches. Various photoswitches have been proposed in the literature, of which we tested a few in combination with T2D-IR spectroscopy: azobenzene (**Figure 4*a*,*b***) as well as disulfide bridges and thiopeptides as intrinsic photoswitches (**Figure 4*c*,*d***). The use of photoswitches for various applications is reviewed in References 104–106.

3. TRANSIENT TWO-DIMENSIONAL INFRARED SPECTROSCOPY OF PHOTOSWITCHABLE PEPTIDES

3.1. Azobenzene

The *cis-trans* isomerization of azobenzene is widely used for photoswitching (106). It is characterized by a high quantum yield (50%), high photostability, ultrafast switching speed (approximately 1 ps), and the fact that *cis* and *trans* isomers have absorption bands that are well separated, therefore allowing a selective switching in both directions (107). Various groups (108–121) have directly incorporated azobenzene

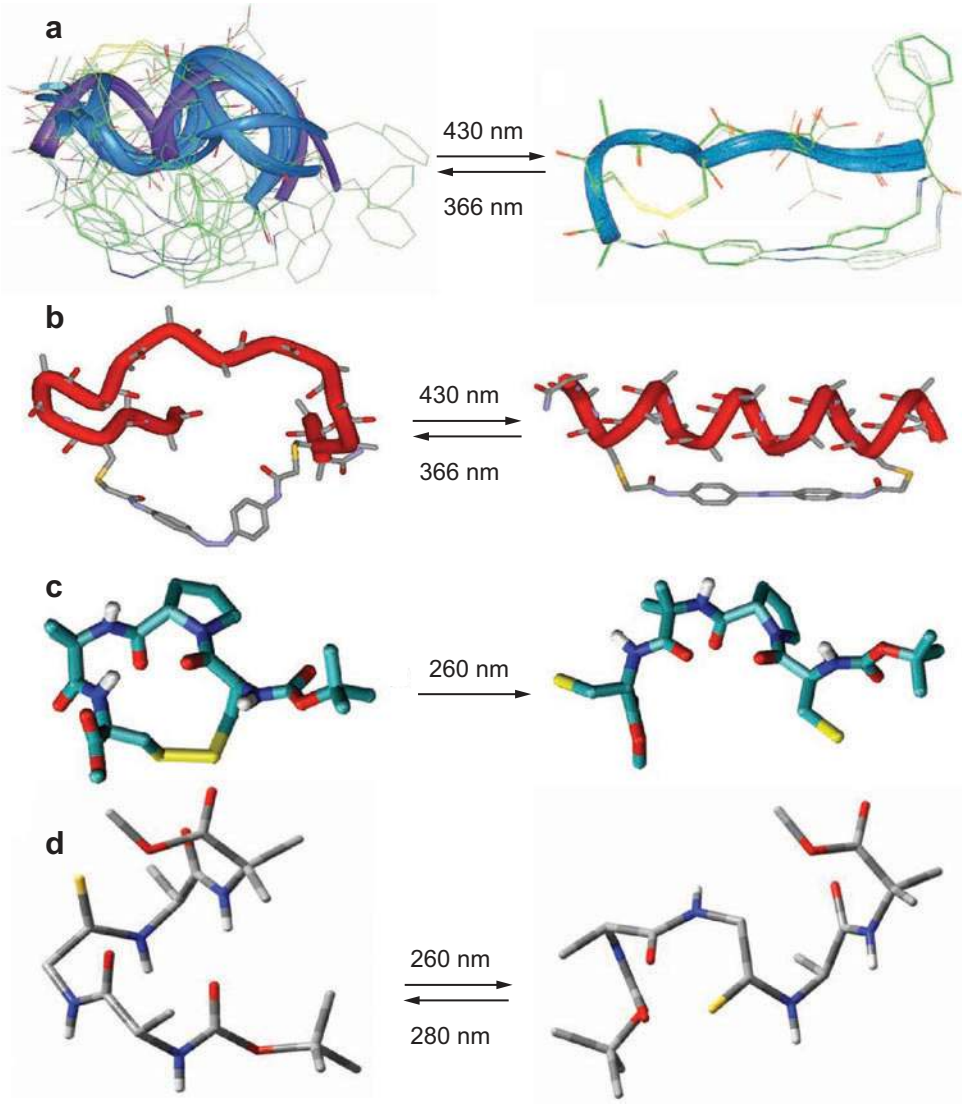

Figure 4

Photoswitchable peptides and intrinsic photoswitches studied in our lab: azobenzene (*a*) embedded into a peptide backbone (*b*) or cross-linking amino acid side chains, and (*c*) a photocleavable disulfide bridge and (*d*) thiopeptides.

into the backbone of peptides, switching the peptide conformation by photo-exciting the azo-moiety. In a first step, we concentrated on a small cyclized peptide designed by Moroder and coworkers (112): The peptide resembles the active site of thioredoxin reductase (**Figure 4*a***) and was cyclized by an azobenzene-based ω-amino acid. Researchers have applied UV spectroscopy, reporting the response of the

chromophore (122–124), as well as IR spectroscopy, which addresses directly the dynamics of the peptide backbone (125).

Isomerization of the photoswitch couples directly to a conformational transition of the peptide backbone. In fact, we find that the peptide backbone can be stretched on an ultrafast 20-ps timescale upon the *cis-trans* isomerization of an azo-moiety (125). After this initial driven phase, the molecule keeps on equilibrating on a slower nanosecond timescale (diffusive dynamics), but the major part of the conformational transition clearly occurs within the first 20 ps: The transient pump-probe signals have built up already 20 ps after the UV pump pulse (**Figure 5*a***) and change only marginally between 20 ps and 1.7 ns (**Figure 5*a*,*c*,*e***). The 20-ps timescale might seem surprisingly fast for a peptide's conformational transition. However, because a major fraction of the energy of the excitation photon (290 kJ mol^{-1}) is used to drive the conformational transition, we conclude that the peptide backbone is stretched with an artificially high force. Nonequilibrium MD simulations of the photoinduced molecular process are in remarkable qualitative agreement with experimental results, revealing multiple timescales for the initial cooling phase as well as for the fast driven and slower diffusive conformational response of the peptide backbone (126, 127). Quantitatively speaking, however, the timescale of the driven phase, particularly, is predicted to slow by approximately one order of magnitude from the MD simulation (122, 126, 127), for reasons that currently are not clear.

Amide I band: vibrational band mainly located on the carbonyl groups of the peptide backbone, which exhibits characteristic spectral features sensitive to secondary structure motifs of peptides and proteins

Nevertheless, such cyclized azo-peptides appear to be ideal model systems to test the capability of 2D-IR spectroscopy in combining ultrafast time resolution with direct structural resolution. This is why we used this system for our first demonstration of T2D-IR spectroscopy (34). Despite the fact that hardly any time dependence is observed in the pump-probe spectra between 20 ps and 1.7 ns (**Figure 5*a*,*c*,*e***), we find large differences in the T2D-IR spectra (**Figure 5*b*,*d*,*f***), illustrating the gain of information in the latter as compared to the former. In particular, we find only a small signal from the photoswitched peptides in the T2D-IR spectrum after 20 ps (expected at position 2 in **Figure 5*b***) although it gives rise to a band in the 1D spectrum (**Figure 5*a***, position 1). Only the T2D-IR signal of the initial *cis* state is observed. After 200 ps, however, the band of the *trans*-ensemble has grown in (**Figure 5*d***, position 3), and within 1.7 ns we observe a tilt of this band toward the diagonal of the 2D spectrum (**Figure 5*f***, position 4). Interestingly, the T2D-IR spectra can be modeled almost quantitatively by varying the homogeneous line width γ_t of the amide I band of the photoproduct by 10%–15% between 20 ps and 1.7 ns (**Figure 5*b*,*j*,*l***). In particular, the low intensity of the transient product band at 20 ps and its rise at later times are perfectly matched. T2D-IR spectroscopy is sensitive to relatively small changes in the lineshape function owing to strong cancellation effects in the double-difference spectrum.

The homogeneous line width γ_t in 2D-IR experiments is a measure of fast (<1 ps) fluctuations of the peptide backbone. The timescale relevant for homogeneous broadening (<1 ps) is too fast for the system to cross any significant barrier on the order of k_BT. However, there may be regions on the potential energy surface far from equilibrium that are more shallow, on which the molecule can move freely and hence quickly. The change of homogeneous width during the diffusion-controlled phase

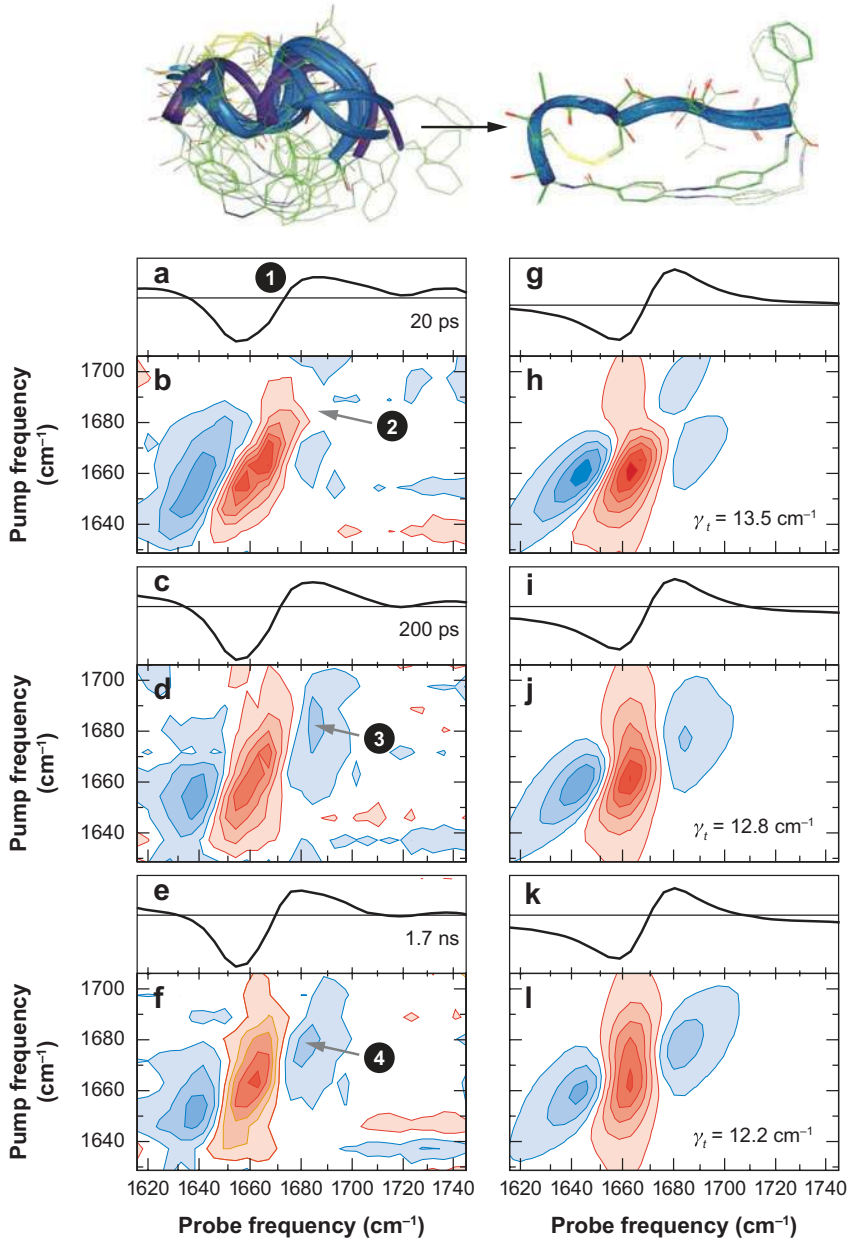

Figure 5

Time-resolved pump-probe (*top of each panel*) and transient two-dimensional infrared spectra (*bottom of each panel*) at $T = 20$ ps (*top panels*), 200 ps (*middle panels*), and 1.7 ns (*bottom panels*). The left column shows experimental results, whereas the right column shows simulations. Figure adapted from Reference 34.

revealed by T2D-IR may reflect the width of the conformation space accessible on a fast timescale, which becomes more confined as the backbone approaches its global energy minimum.

In ongoing work, we concentrate on an alternative approach designed by Woolley and coworkers (110, 113–115), who used an azobenzene moiety to cross-link two amino acid side chains of a polypeptide (**Figure 4*b***). Depending on its end-to-end distance, the photoswitch either increases or decreases the helix propensity of the peptide upon *cis-trans* isomerization (110, 113–115). However, in contrast to the previous example, the photoswitch couples much less directly to the conformational transition of the peptide backbone. In particular, the photoswitch does not force the peptide into an α-helical structure upon *cis-trans* isomerization; it just opens the folded state as a new region of conformational space. Helix folding occurs in a diffusive manner and hence on orders of magnitude slower timescales of 100 ns to 1 μs than in the example discussed above (128–130). Helix folding is thermally activated, and the folding rate varies strongly with temperature. Furthermore, at lower temperatures, the folding kinetics deviates strongly from mono-exponential, providing clear evidence that it is not a trivial two-state process. T2D-IR experiments are currently underway.

3.2. Intrinsic Photoswitches

The disulfide bridge between two cysteines, which occurs in natural peptides and proteins, may be used as a predetermined breaking point that photodissociates upon electronic excitation at ≈260 nm and hence as a phototrigger. Hochstrasser and coworkers (131, 132) first reported this concept in their pioneering work. A 20-residue peptide was designed to fold into an α-helix after photocleavage, while no distinct secondary structure was possible in the initial bridged state. Unfortunately, geminate recombination of the liberated thiyl radicals occurred before helix formation could be detected. We have recently used this approach for a much smaller peptide (**Figure 4*c***) (133) that forms a β-turn structure in the disulfide-bridged configuration. Upon photocleavage of the disulfide bridge, the hydrogen bond in the β-turn is destabilized and the ring structure opens. In contrast to the molecule studied by Hochstrasser and coworkers (131, 132), this molecule is much more strained owing to the shortness of the backbone and stays open on long timescales. In fact, only approximately half the radicals are quenched after 1 ms either intra- or intermolecularily (134).

The peptide features a completely resolved and easy to assign IR spectrum of the five C=O groups without having to invoke isotope labeling. This fortunate situation made it the ideal test case for T2D-IR spectroscopy and allowed us to directly extract microscopic structural information on an ultrafast timescale for the conformational transition of the molecule (35). **Figure 6*b*** shows an equilibrium 2D-IR spectrum of the molecule that is rather congested because essentially all C=O groups are coupled to each other. **Figure 6*d***, in contrast, shows a T2D-IR spectrum 100 ps after photocleavage of the disulfide bridge. The diagonal is dominated by signals similar to those in **Figure 5**. More important with regard to the structural resolution, only one dominant peak remains in the off-diagonal region, related to the coupling

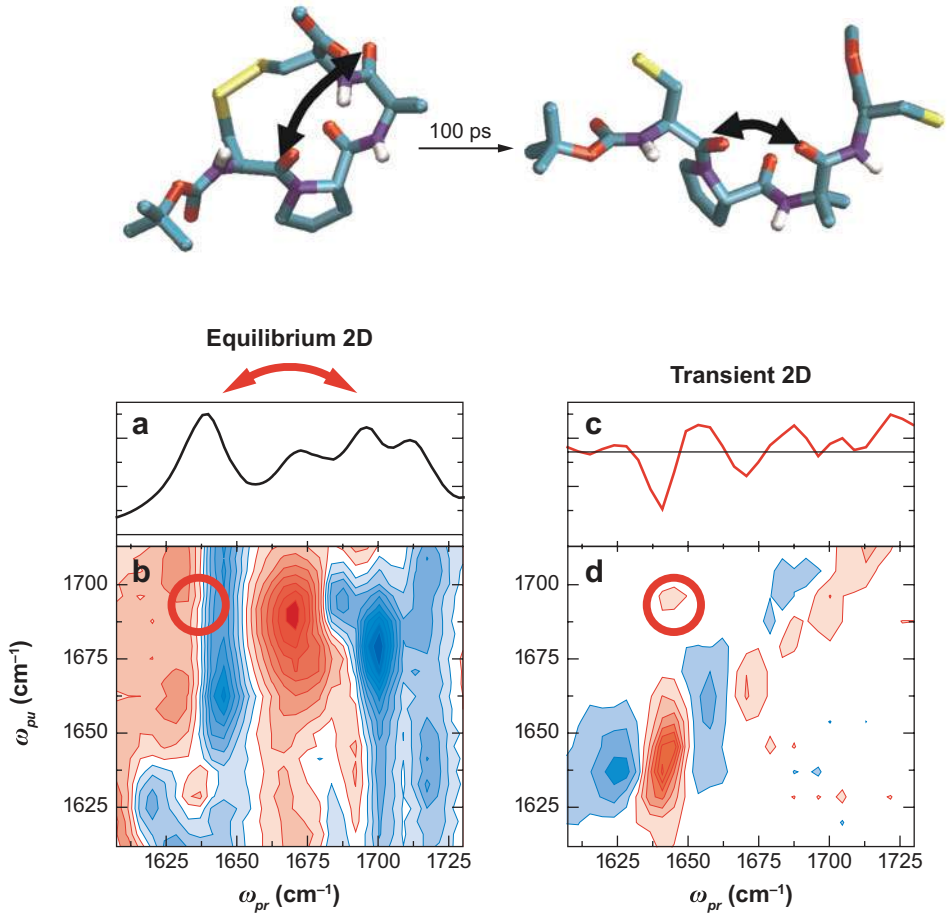

Figure 6

(*Left column*) Equilibrium and (*right column*) transient nonequilibrium spectra 100 ps after photocleavage of cyclo-(Boc-Cys-Pro-Aib-Cys-OMe). The top panels (*a*,*c*) show one-dimensional infrared (1D-IR) spectra, and the bottom panels (*b*,*d*) show the corresponding two-dimensional infrared (2D-IR) spectra. The equilibrium 2D-IR spectrum (*b*) is the weighted difference between parallel and perpendicular polarization measurement (to enhance the cross-peak contribution), whereas the transient 2D-IR spectrum (*d*) is with all three laser beams polarized parallel. The cross-peak (*circled in red*) in either spectrum relates to the coupling across the hydrogen bond in the β-turn structure. The example structures were obtained from an accompanying molecular dynamics simulation. Figure adapted from Reference 35.

between Cys(1)-Aib(3) across the intramolecular hydrogen bond. The corresponding cross-peak is weak in the equilibrium case (**Figure 6*b***) and is hidden underneath the wings of the much stronger cross-peaks of Boc-Cys(1) and Cys(1)-Pro(2), both related to nearest neighbors in the sequence. In contrast, Cys(1) and Aib(3) are far in sequence, but close in space in the folded structure. Therefore, upon opening of

the ring structure, changes in coupling are expected only for Cys(1) and Aib(3) in the T2D-IR spectrum, which is a difference 2D-IR spectrum between the photoproduct state and reactant state. A time series showed that the transient cross-peak rises on a 100-ps timescale, in good agreement with accompanying results from MD simulations (35). The transient cross-peak constitutes a direct and easy to interpret structural indicator.

Another potential intrinsic photoswitch is the –CO–NH– peptide unit itself, which is thermodynamically more stable in the *trans* state, but which can be switched to the *cis* conformation by electronic $\pi\pi^*$ excitation (135), forcing the peptide to change its secondary structure (136, 137). However, the required excitation wavelength lies deep in the UV (190 nm), which is hard to reach technically and can destroy the molecule. Excitation at 190 nm is also nonselective and can randomly switch any peptide unit in a polypeptide. One can avoid both problems by replacing one peptide unit with a thiopeptide (i.e., –CSNH–) unit (**Figure 4*d***) (138–142). This red-shifts the wavelength of the $\pi\pi^*$ transition of the *trans* isomer to ≈260 nm (143, 144), which is easily accessible by frequency tripling a Ti:S laser. Furthermore, incorporating a thioamide linkage results in only minor changes of a peptide's secondary structure (145, 146). A combination of theoretical and time-resolved experimental studies on the photoswitch itself (*N*-methylthioacetamide) has shown that the isomerization quantum yield is high (30%–40% in the *trans-cis* and 60–70% in the *cis-trans* direction) although the molecule remains trapped in a relatively long-lived excited state for 200–300 ps, in which it is almost free to rotate about the thioamide bond (147, 148). Nevertheless, thiopeptides are particularly useful as model systems for T2D-IR spectroscopy because the isomerization of the thioamide bond changes the relative orientation of the adjacent peptide units, which are sufficiently close to each other for amide I mode coupling. In analogy to trialanine in 2D-IR spectroscopy, the smallest test molecule for T2D-IR is thus a tetrathiopeptide, with two amide I oscillators separated by the thio-photoswitch. The first thiopeptide we have investigated using T2D-IR spectroscopy [Boc-Ala-Gly(=S)-Ala-Aib-OMe] (36, 149) in addition contains two protecting groups (Boc and Aib/OMe, providing two more C=O oscillators), which stabilize the *trans* molecules in a looped conformation via an intramolecular hydrogen bond. In the T2D-IR spectra, we observed resolution enhancement along the diagonal as well as a transient cross-peak due to the breaking of that hydrogen bond (36).

4. DISCUSSION

Comparing the results of Reference 34 (**Figure 5**) with those of Reference 35 (**Figure 6**), one may suggest concrete design criteria to render T2D-IR spectroscopy an expressive method to time resolve transient structures. In the case of the azobenzene-switched cyclic octapeptide of Reference 34, the first example investigated above, the amide I′ band was not spectrally resolved; hence, we could not interpret the T2D-IR spectra in terms of structural motifs. The structural information did not go any deeper than that of the corresponding 1D spectroscopy; nevertheless, additional dynamic information could be extracted. In contrast, in the

case of the disulfide-bridged β-turn (35), the somewhat smaller peptide has a fully resolved amide I′ spectrum. As a result, we can identify specific local contacts whose time dependence we can interpret in terms of the breaking of certain loop structures.

In the examples in References 35 and 36, bands are resolved because the different C=O groups are sitting in different chemical environments. For larger peptides, this is not the case in general. However, spectral resolution can always be regained when employing isotope labeling (12, 46, 49, 149). The synthesis of site-selectively isotope-labeled peptides is not particularly demanding. For example, in our studies of the folding of a photoswitchable α-helix (**Figure 4*b***), we employed double $^{13}C^{16}O$ and $^{13}C^{18}O$ isotope labeling to single out two specific sites from the unstructured main amide I′ band of unlabeled groups. $^{13}C^{16}O$ labeling causes a frequency downshift of the amide I′ band of approximately 30 cm^{-1}, whereas $^{13}C^{18}O$ shifts it by 50 cm^{-1}. In that way, one can identify the local contact between a specific pair of amino acids [e.g., the $i \rightarrow i+4$ configuration of a α-helical loop (49)] and potentially measure its time dependence. These experiments promise to give unprecedented structural insights into which helix loop is forming at what time and to what extent during the folding process.

An important, but not thoroughly explored issue is the lineshapes of T2D-IR spectra. If the frequency shifts induced by the photoreaction are larger than the line width of the band, we expect a total of four spectrally resolved bands for each diagonal and cross-peak in the T2D-IR spectrum (**Figure 3**). For example, this is the case for the metal-to-ligand charge transfer of a metal-carbonyl compound we studied extensively ($[Re(CO)_3(dmbpy)Cl]$, dmbpy = 4,4′-dimethyl-2,2′bipyridine) (27). However, with regard to photoswitchable peptides, the transient frequency shifts induced by a conformational transition are typically very small—much smaller than the line width of the amide I band. In this case, the two contributions (**Figure 3*a*,*b***) overlap and tend to cancel each other. If the lineshape functions of reactant and product species are identical (which is not the case in general), then we expect the T2D-IR to be symmetric (**Figure 3*c***). Owing to the strong cancellation between reactant and product contributions, however, small changes in the lineshape functions of either contribution may cause strong effects for the resulting difference T2D-IR spectrum (see **Figure 5*b***).

The theoretical modeling of these cancellation effects is quite demanding and has not been fully achieved. First, it requires very high accuracy to obtain still reasonable results after subtracting the two almost identical contributions from the reactant and product state; second, almost all lineshape calculations are based on an equilibrium assumption [i.e., in the sense that the frequency fluctuation correlation function, which is at the basis of any lineshape analysis, is assumed to depend on the time difference only and not on absolute time: $\langle\omega(t_1)\omega(t_2)\rangle = \langle\omega(0)\omega(t_2 - t_1)\rangle$] (70). However, it is not clear after which time one can think about an evolving structural ensemble as being in a quasi-equilibrium. We have shown that this assumption is definitely not valid on a 10-ps timescale (30), but we do not know whether it might become reasonable on a 100-ps to 1-ns timescale (34, 35). This problem calls for significant effort from the theoretical side [preliminary results suggest that the small change in homogeneous linewidth used in our empirical fits (**Figure 5*b*,*j*,*l***) can indeed be reproduced from a nonequilibrium all-atom MD simulation] (150).

5. CONCLUSION

NMR is a high-resolution spectroscopy, capable of spectrally isolating thousands of individual resonances. However, the narrow line width implies that direct time resolution of NMR spectroscopy is intrinsically slow. The time resolution is ultimately limited by the dephasing (T_2) time of the spectroscopic transition used as molecular probe (typically 1 ms for spin transitions), rather than by the measurement instrument. In contrary, typical dephasing times of vibrational transitions in the solution phase are in the range of 1 ps; hence IR bands are broad, and the number of resolved bands in a given spectral window is restricted. Nevertheless, the fast dephasing time is responsible for the high direct time resolution of 2D-IR spectroscopy. Spectral congestion can be circumvented to a significant extent by isotope labeling. In that sense, 2D-IR and 2D-NMR spectroscopy should not be viewed as competing methods; rather 2D-IR spectroscopy should be further developed as a complementary method. In the above sections we focus on transient nonequilibrium 2D-IR spectroscopy as one of the most promising strengths of 2D-IR spectroscopy (it certainly is not the only one). Learning to use the structural-resolution capabilities of 2D-IR spectroscopy, also in equilibrium, is of course an inevitable prerequisite before extending it to the nonequilibrium regime, and work in this direction is ongoing and, of course, still required.

Because the typical length and time scales of T2D-IR spectroscopy coincide exactly with what is accessible to MD simulations, the latter can directly be tested against experiment. MD simulations currently are the only approach to understand and visualize the complicated structure and dynamics—and hence the function—of biomolecules on an atomic level. Yet the MD approach clearly is based on, in part crude, approximations, whereas experimental techniques that can provide pictures of similar clarity are scarce. 2D-IR spectroscopy combines ultrafast time resolution with microscopic structural resolution in an unprecedented manner; hence it has the potential to become as expressive as all-atom MD simulations. The big challenge of 2D-IR spectroscopy is to extend the method to larger molecular systems (i.e., proteins rather than peptides), circumventing spectral congestion with intelligent strategies of isotope labeling, the introduction of new vibrational probes, or well-designed methods of difference spectroscopy. Nevertheless, as summarized above, significant steps toward a molecular movie of ultrafast structural dynamics extracted directly from experimental data have already been made.

SUMMARY POINTS

1. One of the biggest strengths of 2D-IR spectroscopy is the investigation of nonequilibrium structural processes.
2. T2D-IR spectroscopy uniquely combines ultrafast time resolution with microscopic structural resolution.
3. 2D-IR and 2D-NMR spectroscopy should be viewed as complementary rather than competing methods.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are thankful to numerous contributions from our collaborators, in particular Christoph Kolano, Mariusz Kozinski, Luis Moroder, Christian Renner, Wolfram Sander, Gerhard Stock, Josef Wachtveitl, Sander Woutersen, and Wolfgang Zinth. Our work has been financially supported by the University of Zurich, the Swiss Science Foundation (SNF), and the German Science Foundation (DFG).

LITERATURE CITED

1. Ernst RR, Bodenhausen G, Wokaun A. 1987. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*. Oxford: Clarendon
2. Aue WP, Bartholdi E, Ernst RR. 1976. Two-dimensional spectroscopy: application to nuclear magnetic resonance. *J. Chem. Phys.* 64:2229–46
3. **Hamm P, Lim M, Hochstrasser RM. 1998. Structure of the amide I band of peptides measured by femtosecond nonlinear-infrared spectroscopy. *J. Phys. Chem. B* 102:6123–38**
4. Hamm P, Lim M, DeGrado WF, Hochstrasser RM. 1999. The two-dimensional IR nonlinear spectroscopy of a cyclic penta-peptide in relation to its three-dimensional structure. *Proc. Natl. Acad. Sci. USA* 96:2036–41
5. Ge NH, Hochstrasser RM. 2002. Femtosecond two-dimensional infrared spectroscopy: IR-COSY and THIRSTY. *PhysChemComm* 5:17–26
6. Woutersen S, Mu Y, Stock G, Hamm P. 2001. Subpicosecond conformational dynamics of small peptides probed by two-dimensional vibrational spectroscopy. *Proc. Natl. Acad. Sci. USA* 98:11254–58
7. Woutersen S, Hamm P. 2001. Hydrogen-bond lifetime measured by time-resolved 2D-IR spectroscopy: *N*-methylacetamide in methanol. *Chem. Phys.* 266:137–47
8. Zheng J, Kwak K, Asbury J, Chen X, Piletic IR, Fayer MD. 2005. Ultrafast dynamics of solute-solvent complexation observed at thermal equilibrium in real time. *Science* 309:1338–43
9. Kim YS, Hochstrasser RM. 2005. Chemical exchange 2D IR of hydrogen-bond making and breaking. *Proc. Natl. Acad. Sci. USA* 102:11185–90
10. Scheurer C, Mukamel S. 2001. Design strategies of pulse sequences in multidimensional optical spectroscopy. *J. Chem. Phys.* 115:4989–5004
11. **Woutersen S, Hamm P. 2000. Structure determination of trialanine in water using polarization sensitive two-dimensional vibrational spectroscopy. *J. Phys. Chem. B* 104:11316–20**
12. Woutersen S, Hamm P. 2001. Isotope-edited two-dimensional vibrational spectroscopy of trialanine in aqueous solution. *J. Chem. Phys.* 114:2727–37

3. First 2D-IR spectrum measured.

11. First demonstration that 2D-IR can be used to determine the unknown structure of a small peptide.

13. Woutersen S, Pfister R, Hamm P, Mu Y, Kosov DS, Stock G. 2002. Peptide conformational heterogeneity revealed from nonlinear vibrational spectroscopy and molecular dynamics simulations. *J. Chem. Phys.* 117:6833–40
14. Mu YG, Kosov DS, Stock G. 2003. Conformational dynamics of trialanine in water. 2. Comparison of AMBER, CHARMM, GROMOS, and OPLS force fields to NMR and infrared experiments. *J. Phys. Chem. B* 107:5064–73
15. Gnanakaran S, Garcia AE. 2003. Validation of an all-atom protein force field: from dipeptides to larger peptides. *J. Phys. Chem B* 107:12555–57
16. Graf J, Nguyen PH, Stock G, Schwalbe H. 2007. Structure and dynamics of the homologous series of alanine peptides: a joint molecular dynamics/NMR study. *J. Am. Chem. Soc.* 129:1179–89
17. Lakomek NA, Carlomagno T, Becker S, Griesinger C, Meiler J. 2006. A thorough dynamic interpretation of residual dipolar couplings in ubiquitin. *J. Biomol. NMR* 34:101–15
18. Fung BM, McGaughy TW. 1976. Molecular motions in liquid. I. Rotation of water and small alcohols studied by deuteron relaxation. *J. Chem. Phys.* 65:2970–76
19. Woutersen S, Emmerichs U, Bakker HJ. 1997. Femtosecond mid-infrared pump-probe spectroscopy of liquid water: evidence for a two-component structure. *Science* 278:658–60
20. Fecko CJ, Loparo J, Roberts ST, Tokmakoff A. 2005. Local hydrogen bonding dynamics and collective reorganization in water: ultrafast infrared spectroscopy of HOD/D_2O. *J. Chem. Phys.* 122:054506
21. Rezus YLA, Bakker HJ. 2005. On the orientational relaxation of HDO in liquid water. *J. Chem. Phys.* 123:114502
22. Laage D, Hynes JT. 2006. A molecular jump mechanism of water reorientation. *Science* 311:832–35
23. Asbury JB, Steinel T, Stromberg C, Gaffney KJ, Piletic IR, et al. 2003. Hydrogen bond dynamics probed with ultrafast infrared heterodyne-detected multidimensional vibrational stimulated echoes. *Phys. Rev. Lett.* 91:237402
24. Yeremenko S, Pschenichnikov MS, Wiersma DA. 2003. Hydrogen-bond dynamics in water explored by heterodyne-detected photon echo. *Chem. Phys. Lett.* 369:107–13
25. Cowan ML, Bruner BD, Huse N, Dwyer JR, Chugh B, et al. 2005. Ultrafast memory loss and energy redistribution in the hydrogen bond network of liquid H_2O. *Nature* 434:199–202
26. Eaves JD, Loparo JJ, Fecko CJ, Roberts ST, Tokmakoff A, Geissler PL. 2005. Hydrogen bonds in liquid water are broken only fleetingly. *Proc. Natl. Acad. Sci. USA* 102:13019–22
27. Bredenbeck J, Helbing J, Hamm P. 2004. Transient two-dimensional IR spectroscopy: exploring the polarization dependence. *J. Chem. Phys.* 121:5943–57
28. Bredenbeck J, Helbing J, Hamm P. 2004. Labeling vibrations by light: Ultrafast transient 2D-IR spectroscopy tracks vibrational modes during a photoreaction. *J. Am. Chem. Soc.* 126:990–91

29. Bredenbeck J, Helbing J, Nienhaus K, Nienhaus GU, Hamm P. 2007. Protein ligand migration mapped by nonequilibrium 2D-IR exchange spectroscopy. *Proc. Natl. Acad. Sci. USA* 104:14243–48
30. Bredenbeck J, Helbing J, Hamm P. 2005. Solvation beyond the linear response regime. *Phys. Rev. Lett.* 95:083201
31. Chung HS, Ganim Z, Jones KC, Tokmakoff A. 2007. Transient 2D IR spectroscopy of ubiquitin unfolding dynamics. *Proc. Natl. Acad. Sci. USA* 104:14237–42
32. Chung HS, Khalil M, Smith AW, Tokmakoff A. 2007. Transient two-dimensional IR spectrometer for probing nanosecond temperature-jump kinetics. *Rev. Sci. Instrum.* 78:063101
33. McCanne R, Nee MJ, Kubarych KJ. 2007. *Ultrafast nonequilibrium chemical exchange: phototriggered Fourier transform multidimensional IR spectroscopy*. Presented at Am. Chem. Soc. Natl. Meet., 233rd, Chicago
34. Bredenbeck J, Helbing J, Renner C, Behrendt R, Moroder L, et al. 2003. Transient 2D-IR spectroscopy: snapshots of the nonequilibrium ensemble during the picosecond conformational transition of a small peptide. *J. Phys. Chem. B* 107:8654–60
35. Kolano C, Helbing J, Kozinski M, Sander W, Hamm P. 2006. Watching hydrogen bond dynamics in a β-turn by transient two-dimensional infrared spectroscopy. *Nature* 444:469–72
36. Cervetto V, Pfister R, Hamm P, Helbing J. 2007. Transient 2D-IR spectroscopy of thiopeptide isomerization and hydrogen bond breaking. *J. Phys. Chem. B.* To be submitted
37. Rubtsov IV, Wang J, Hochstrasser RM. 2003. Dual frequency 2D-IR of peptide amide-A and amide-I modes. *J. Chem. Phys.* 118:7733–36
38. Larsen OFA, Bodis P, Buma WJ, Hannam JS, Leigh DA, Woutersen S. 2005. Probing the structure of a rotaxane with two-dimensional infrared spectroscopy. *Proc. Natl. Acad. Sci. USA* 102:13378–82
39. Volkov VV, Chelli R, Righini R. 2006. Domain formation in lipid bilayers probed by two-dimensional infrared spectroscopy. *J. Phys. Chem. B* 110:1499–501
40. Barbour LW, Hegadorn M, Asbury JB. 2006. Microscopic inhomogeneity and ultrafast orientational motion in an organic photovoltaic bulk heterojunction thin film studied with 2D IR vibrational spectroscopy. *J. Phys. Chem. B* 110:24281–86
41. DeCamp MF, DeFlores LP, Jones KC, Tokmakoff A. 2007. Single-shot two-dimensional infrared spectroscopy. *Opt. Express* 15:233–41
42. Asplund MC, Zanni MT, Hochstrasser RM. 2000. Two-dimensional infrared spectroscopy of peptides by phase-controlled femtosecond vibrational photon echoes. *Proc. Natl. Acad. Sci. USA* 97:8219–24
43. Zanni MT, Gnanakaran S, Stenger J, Hochstrasser RM. 2001. Heterodyned two-dimensional infrared spectroscopy of solvent-dependent conformations of acetylproline-NH_2. *J. Phys. Chem. B* 105:6520–35

34. First T2D-IR spectrum measured.

35. First demonstration that T2D-IR combines microscopic structure information with picosecond time resolution for nonequilibrium processes.

44. Khalil M, Demirdöven N, Tokmakoff A. 2003. Obtaining absorptive line shapes in two-dimensional infrared vibrational correlation spectra. *Phys. Rev. Lett.* 90:047401
45. Krummel AT, Mukherjee P, Zanni MT. 2003. Inter and intrastrand vibrational coupling in DNA studied with heterodyned 2D-IR spectroscopy. *J. Phys. Chem. B* 107:9165–69
46. Mukherjee P, Kass I, Arkin IT, Zanni MT. 2006. Picosecond dynamics of a membrane protein revealed by 2D IR. *Proc. Natl. Acad. Sci. USA* 103:3528–33
47. Demirdöven N, Cheatum CM, Chung HS, Khalil M, Knoester J, Tokmakoff A. 2004. Two-dimensional infrared spectroscopy of antiparallel β-sheet secondary structure. *J. Am. Chem. Soc.* 126:7981–90
48. Cowan ML, Ogilvie JP, Miller RJD. 2004. Two-dimensional spectroscopy using diffractive optics based phase-locked echoes. *Chem. Phys. Lett.* 386:184–89
49. Fang C, Hochstrasser RM. 2005. Two-dimensional infrared spectra of the $^{13}C{=}^{18}O$ isotopomers of alanine residues in an α-helix. *J. Phys. Chem. B* 109:18652–63
50. Kurochkin DV, Naraharisetty SRG, Rubtsov IV. 2005. Dual-frequency 2D IR on interaction of weak and strong IR modes. *J. Phys. Chem. A* 109:10799–802
51. Volkov V, Schanz R, Hamm P. 2005. Active phase stabilization in Fourier-transform two-dimensional infrared spectroscopy. *Opt. Lett.* 30:2010–12
52. Maekawa H, Toniolo C, Moretto A, Broxterman QB, Ge NH. 2006. Different spectral signatures of octapeptide 3_{10}- and α-helices revealed by two-dimensional infrared spectroscopy. *J. Phys. Chem. B* 110:5834–37
53. Ding F, Mukherjee P, Zanni MT. 2006. Passively correcting phase drift in two-dimensional infrared spectroscopy. *Opt. Lett.* 31:2918–20
54. Shim SH, Strasfeld DB, Fulmer EC, Zanni MT. 2006. Femtosecond pulse shaping directly in the mid-IR using acousto-optic modulation. *Opt. Lett.* 31:838–40
55. Shim SH, Strasfeld DB, Ling YL, Zanni MT. 2007. Using pulse shaping to automate 2D-IR spectroscopy for more precise characterization of structure and dynamics. *Proc. Natl. Acad. Sci. USA* 104:14197–202
56. Kozinski M, Garrett-Roe S, Hamm P. 2007. Vibrational spectral diffusion of CN^- in water. *Chem. Phys.* In press
57. Nee MJ, McCanne R, Kubarych KJ, Joffre M. 2007. Two-dimensional infrared spectroscopy detected by chirped pulse upconversion. *Opt. Lett.* 32:713–15
58. Golonzka O, Demirdöven N, Khalili M, Tokmakoff A. 2000. Separation of cascaded and direct fifth-order Raman signals using phase-sensitive intrinsic heterodyne detection. *J. Chem. Phys.* 113:9893–96
59. Milne CJ, Li YL, Jansen TLC, Huang L, Miller RJD. 2006. Fifth-order Raman spectroscopy of liquid benzene: experiment and theory. *J. Phys. Chem. B* 110:19867–76
60. Kaufman LJ, Heo J, Ziegler LD, Fleming GR. 2002. Heterodyne-detected fifth-order nonresonant Raman scattering from room temperature CS_2. *Phys. Rev. Lett.* 88:207402
61. Zhao W, Wright JC. 2000. Doubly vibrationally enhanced four wave mixing: the optical analog to 2D-NMR. *Phys. Rev. Lett.* 84:1411–14

62. Donaldson PM, Guo R, Fournier F, Gardner EM, Barter LM, et al. 2007. Direct identification and decongestion of Fermi resonances by control of pulse time-ordering in 2D-IR spectroscopy. *J. Chem. Phys.* 127:114513
63. Gallagher SM, Albrecht AW, Hybl JD, Landin BL, Rajaram B, Jonas DM. 1998. Heterodyne detection of the complete electric field of femtosecond four-wave mixing signals. *J. Opt. Soc. Am. B* 15:2338–45
64. Tian PF, Keusters D, Suzaki Y, Warren WS. 2003. Femtosecond phase-coherent two-dimensional spectroscopy. *Science* 300:1553–55
65. Brixner T, Stenger J, Vaswani HM, Cho M, Blankenship RE, Fleming GR. 2005. Two-dimensional spectroscopy of electronic couplings in photosynthesis. *Nature* 434:625–28
66. Li X, Zhang T, Borca CN, Cundiff ST. 2006. Many-body interactions in semiconductors probed by optical two-dimensional Fourier transform spectroscopy. *Phys. Rev. Lett.* 96:057406
67. Engel GS, Calhoun TR, Read EL, Ahn TK, Mancal T, et al. 2007. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. *Nature* 446:782–86
68. Szocs V, Palszegi T, Lukes V, Sperling J, Milota F, et al. 2006. Two-dimensional electronic spectra of symmetric dimers: intermolecular coupling and conformational states. *J. Chem. Phys.* 124:124511
69. Tanimura Y, Mukamel S. 1993. Two-dimensional femtosecond vibrational spectroscopy of liquids. *J. Chem. Phys.* 99:9496–511
70. Mukamel S. 1995. *Principles of Nonlinear Optical Spectroscopy*. Oxford, UK: Oxford Univ. Press
71. Hamm P, Woutersen S. 2002. Coupling of the amide I modes of the glycine di-peptide. *Bull. Chem. Soc. Jpn.* 75:985–88
72. Kwac K, Cho M. 2003. Molecular dynamics simulation study of *N*-methylacetamide in water. II. Two-dimensional infrared pump-probe spectra. *J. Chem. Phys.* 119:2256–63
73. Kwac K, Lee H, Cho M. 2004. Non-Gaussian statistics of amide I mode frequency fluctuation of *N*-methylacetamide in methanol solution: linear and nonlinear vibrational spectra. *J. Chem. Phys.* 120:1477–90
74. Schmidt JR, Corcelli SA, Skinner JL. 2004. Ultrafast vibrational spectroscopy of water and aqueous *N*-methylacetamide: comparison of different electronic structure/molecular dynamics approaches. *J. Chem. Phys.* 121:8887–96
75. Dijkstra AG, Knoester J. 2005. Collective oscillations and the linear and two-dimensional infrared spectra of inhomogeneous β-sheets. *J. Phys. Chem. B* 109:9787–98
76. Gorbunov RD, Kosov DS, Stock G. 2005. Ab initio-based exciton model of amide I vibrations in peptides: definition, conformational dependence, and transferability. *J. Chem. Phys.* 122:224904
77. Zhuang W, Abramavicius D, Hayashi T, Mukamel S. 2006. Simulation protocols for coherent femtosecond vibrational spectra of peptides. *J. Phys. Chem. B* 110:3362–74

78. Jansen TL, Knoester J. 2006. Nonadiabatic effects in the two-dimensional infrared spectra of peptides: application to alanine dipeptide. *J. Phys. Chem. B* 110:22910–16
79. Bounouar M, Scheurer C. 2006. Reducing the vibrational coupling network in *N*-methylacetamide as a model for ab initio infrared spectra computations of peptides. *Chem. Phys.* 323:87–101
80. Torii H. 2006. Effects of intermolecular vibrational coupling and liquid dynamics on the polarized Raman and two-dimensional infrared spectral profiles of liquid *N*,*N*-dimethylformamide analyzed with a time-domain computational method. *J. Phys. Chem. A* 110:4822–32
81. Gorbunov RD, Nguyen PH, Kobus M, Stock G. 2007. Quantum-classical description of the amide I vibrational spectrum of trialanine. *J. Chem. Phys.* 126:054509
82. Kjellberg P, Brüggemann B, Pullerits T. 2007. Two-dimensional electronic spectroscopy of an excitonically coupled dimer. *Phys. Rev. B* 74:024303
83. Mukamel S. 2000. Multidimensional femtosecond correlation spectroscopies of electronic and vibrational excitations. *Annu. Rev. Phys. Chem.* 51:691–729
84. Hamm P, Hochstrasser RM. 2001. Structure and dynamics of proteins and peptides: femtosecond two-dimensional infrared spectroscopy. In *Ultrafast Infrared and Raman Spectroscopy*, ed. MD Fayer, pp. 273–347. New York: Marcel Dekker
85. Zanni MT, Hochstrasser RM. 2001. Two-dimensional infrared spectroscopy: a promising new method for the time resolution of structures. *Curr. Opin. Struct. Biol.* 11:516–22
86. Woutersen S, Hamm P. 2002. Nonlinear 2D vibrational spectroscopy of peptides. *J. Phys. Condens. Matter* 14:R1035–62
87. Jonas DM. 2003. Two-dimensional femtosecond spectroscopy. *Annu. Rev. Phys. Chem.* 54:425–63
88. Cho M. 2006. Coherent two-dimensional optical spectroscopy. *Bull. Korean Chem. Soc.* 27:1940–60
89. Zheng J, Kwak K, Fayer MD. 2007. Ultrafast 2D IR vibrational echo spectroscopy. *Acc. Chem. Res.* 40:75–83
90. Bredenbeck J, Helbing J, Kolano C, Hamm P. 2007. Ultrafast 2D-IR spectroscopy of transient species. *Chemphyschem* 8:1747–56
91. Scheurer C, Steinel T. 2007. 2D infrared chemical exchange spectroscopy of ultrafast isomerizations. *Chemphyschem* 8:503–5
92. Cervetto V, Helbing J, Bredenbeck J, Hamm P. 2004. Double-resonance versus pulsed Fourier transform 2D-IR spectroscopy: an experimental and theoretical comparison. *J. Chem. Phys.* 121:5935–42
93. Williams S, Causgrove TP, Gilmanshin R, Fang KS, Callender RH, et al. 1996. Fast events in protein folding: helix melting and formation in a small peptide. *Biochemistry* 35:691–97
94. Thompson PA, Eaton WA, Hofrichter J. 1997. Laser temperature jump study of the helix-coil kinetics of an alanine peptide interpreted with a 'kinetic zipper' model. *Biochemistry* 36:9200–10

95. Lednev IK, Karnoup AS, Sparrow MC, Asher SA. 1999. α-Helix peptide folding and unfolding activation barriers: a nanosecond UV resonance Raman study. *J. Am. Chem. Soc.* 121:8074–86
96. Sabelko J, Ervin J, Gruebele M. 1999. Observations of strange kinetics in protein folding. *Proc. Natl. Acad. Sci. USA* 96:6031–36
97. Thompson PA, Muñoz V, Jas GS, Henry ER, Eaton WA, Hofrichter J. 2000. The helix-coil kinetics of a heteropeptide. *J. Phys. Chem. B* 104:378–89
98. Huang CY, Klemke JW, Getahun Z, DeGrado WF, Gai F. 2001. Temperature-dependent helix-coil transition of an alanine based peptide. *J. Am. Chem. Soc.* 123:9235–38
99. Werner JH, Dyer RB, Fesinmeyer RM, Andersen NH. 2002. Dynamics of the primary processes of protein folding: helix nucleation. *J. Phys. Chem. B* 106:487–94
100. Huang CY, Getahun Z, Zhu Y, Klemke JW, DeGrado WF, Gai F. 2002. Helix formation via conformation diffusion search. *Proc. Natl. Acad. Sci. USA* 99:2788–93
101. Petty SA, Volk M. 2004. Fast folding dynamics of an α-helical peptide with bulky side chains. *Phys. Chem. Chem. Phys.* 6:1022–30
102. Phillips CM, Mizutani Y, Hochstrasser RM. 1995. Ultrafast thermally induced unfolding of RNase A. *Proc. Natl. Acad. Sci. USA* 92:7292–96
103. Wray WO, Aida T, Dyer RB. 2002. Photoacoustic cavitation and heat transfer effects in the laser-induced temperature jump in water. *Appl. Phys. B* 74:57–66
104. Mayer G, Heckel A. 2006. Biologically active molecules with a 'light switch'. *Angew. Chem. Int. Ed. Engl.* 45:4900–21
105. Pieroni O, Fissi A, Angelini N, Lenci F. 2001. Photoresponsive polypeptides. *Acc. Chem. Res.* 34:9–17
106. Feringa BL, ed. 2001. *Molecular Switches*. Weinheim, Ger.: Wiley
107. Nägele T, Hoche R, Zinth W, Wachtveitl J. 1997. Femtosecond photoisomerization of *cis*-azobenzene. *Chem. Phys. Lett.* 272:489–95
108. Ulysse L, Cubillos J, Chmielewski J. 1995. Photoregulation of cyclic peptide conformation. *J. Am. Chem. Soc.* 117:8466–67
109. Behrendt R, Renner C, Schenk M, Wang FQ, Wachtveitl J, et al. 1999. Photomodulation of the conformation of cyclic peptides with azobenzene moieties in the peptide backbone. *Angew. Chem. Int. Ed. Engl.* 38:2771–74
110. Kumita JR, Smart OS, Woolley GA. 2000. Photo-control of helix content in a short peptide. *Proc. Natl. Acd. Sci. USA* 97:3803–8
111. Renner C, Behrendt R, Spörlein S, Wachtveitl J, Moroder L. 2000. Photomodulation of conformational states. I. Mono- and bicyclic peptides with (4-amino)phenylazobenzoic acid as backbone constituent. *Biopolymers* 54:489–500
112. Renner C, Cramer J, Behrendt R, Moroder L. 2000. Photomodulation of conformational states. II. Mono- and bicyclic peptides with (4-aminomethyl)-phenylazobenzoic acid as backbone constituent. *Biopolymers* 54:501–14
113. Flint DG, Kumita JR, Smart OS, Woolley GA. 2002. Using an azobenzene cross-linker to either increase or decrease peptide helix content upon *trans*-to-*cis* photoisomerization. *Chem. Biol.* 9:391–97

114. Woolley GA. 2005. Photocontrolling peptide α helices. *Acc. Chem. Res.* 38:486–93
115. Borisenko V, Woolley GA. 2005. Reversibility of conformational switching in light-sensitive peptides. *J. Photochem. Photobiol. A Chem.* 173:21–28
116. Aemissegger A, Krautler V, van Gunsteren WF, Hilvert D. 2005. A photoinducible β-hairpin. *J. Am. Chem. Soc.* 127:2929–36
117. Renner C, Moroder L. 2006. Azobenzene as conformational switch in model peptides. *Chembiochem* 7:869–78
118. Jurt S, Aemissegger A, Güntert P, Zerbe O, Hilvert D. 2006. A photoswitchable miniprotein based on the sequence of avian pancreatic polypeptide. *Angew. Chem. Int. Ed. Engl.* 45:6297–300
119. Dong SL, Loweneck M, Schrader TE, Schreier WJ, Zinth W, et al. 2006. A photocontrolled β-hairpin peptide. *Chemistry* 12:1114–20
120. Rehm S, Lenz MO, Mensch S, Schwalbe H, Wachtveitl J. 2006. Ultrafast spectroscopy of a photoswitchable 30-amino acid de novo synthesized peptide. *Chem. Phys.* 323:28–35
121. Kusebauch U, Cadamuro SA, Musiol HJ, Lenz MO, Wachtveitl J, et al. 2006. Photocontrolled folding and unfolding of a collagen triple helix. *Angew. Chem. Int. Ed. Engl.* 45:7015–18
122. Spörlein S, Carstens H, Satzger H, Renner C, Behrendt R, et al. 2002. Ultrafast spectroscopy reveals subnanosecond peptide conformational dynamics and validates molecular dynamics simulation. *Proc. Natl. Acad. Sci. USA* 99:7998–8002
123. Satzger H, Root C, Renner C, Behrendt R, Moroder L, et al. 2004. Picosecond dynamics in water-soluble azobenzene-peptides. *Chem. Phys. Lett.* 396:191–97
124. Löweneck M, Milbradt AG, Root C, Satzger H, Zinth W, et al. 2006. A conformational two-state peptide model system containing an ultrafast but soft light switch. *Biophys. J.* 90:2099–108
125. Bredenbeck J, Helbing J, Sieg A, Schrader T, Zinth W, et al. 2003. Picosecond conformational transition and equilibration of a cyclic peptide. *Proc. Natl. Acad. Sci. USA* 100:6452–57
126. Nguyen PH, Stock G. 2006. Nonequilibrium molecular dynamics simulations of a photoswitchable peptide. *Chem. Phys.* 323:36–44
127. Nguyen PH, Gorbunov RD, Stock G. 2006. Photoinduced conformational dynamics of a photoswitchable peptide: a nonequilibrium molecular dynamics simulation study. *Biophys. J.* 91:1224–34
128. Bredenbeck J, Helbing J, Kumita JR, Woolley GA, Hamm P. 2005. α-Helix formation in a photoswitchtide tracked from picoseconds to microseconds by time resolved IR spectroscopy. *Proc. Natl. Acad. Sci. USA* 102:2379–84
129. Hamm P, Helbing J, Bredenbeck J. 2006. Stretched versus compressed exponential kinetics in α-helix folding. *Chem. Phys.* 323:54–65
130. Ihalainen JA, Bredenbeck J, Pfister R, Helbing J, Chi L, et al. 2007. Folding and unfolding of a photoswitchable peptide from picoseconds to microseconds. *Proc. Natl. Acad. Sci. USA* 104:12749–54
131. Volk M, Kholodenko Y, Lu HSM, Gooding EA, DeGrado WF, Hochstrasser RM. 1997. Peptide conformational dynamics and vibrational Stark effects following photoinitiated disulfide cleavage. *J. Phys. Chem. B* 101:8607–16

132. Lu HSM, Volk M, Kholodenko Y, Gooding E, Hochstrasser RM, DeGrado WF. 1997. Aminothiotyrosine disulfide, an optical trigger for initiation of protein folding. *J. Am. Chem. Soc.* 119:7173–80
133. Kolano C, Gomann K, Sander W. 2004. Small cyclic disulfide peptides: synthesis in preparative amounts and characterization by means of NMR and FT-IR spectroscopy. *Eur. J. Org. Chem.* 20:4167–76
134. Kolano C, Helbing J, Bucher G, Sander W, Hamm P. 2007. Intramolecular disulfide bridges as a phototrigger to monitor the dynamics of small cyclic peptides. *J. Chem. Phys. B* 111:11297–302
135. Li P, Chen XG, Shulin E, Asher SA. 1997. UV resonance Raman ground and excited state studies of amide and peptide isomerization dynamics. *J. Am. Chem. Soc.* 119:1116–20
136. Sifferlen T, Rueping M, Gademann K, Jaun B, Seebach D. 1999. β-Thiopeptides: synthesis, NMR solution structure, CD spectra, and photochemistry. *Helv. Chim. Acta* 82:2067–93
137. Frank R, Jakob M, Thunecke F, Fischer G, Schutkowski M. 2000. Thioxylation as one-atom-substitution generates a photoswitchable element within the peptide backbone. *Angew. Chem. Int. Ed. Engl.* 39:1120–22
138. Jensen OE, Lawesson SO, Bardi R, Piazzesi AM, Toniolo C. 1985. Studies on amino acids and peptides. 8. Synthesis and crystal structure of two monothiated analogs of Boc-Gly-S-Ala-Aib-OMe. *Tetrahedron* 41:5595–606
139. Lehmann J, Linden A, Heimgartner H. 1999. Site-selective incorporation of thioamide-linkages into a growing peptide. *Tetrahedron* 55:5359–76
140. Deleted in proof
141. Zhao J, Wildemann D, Jakob M, Vargas C, Schiene-Fischer C. 2003. Direct photomodulation of peptide backbone conformations. *Chem. Commun.* 21:2810–11
142. Satzger H, Root C, Gilch P, Zinth W, Wildemann D, Fischer G. 2005. Photoswitchable elements within a peptide backbone-ultrafast spectroscopy of thioxylated amides. *J. Phys. Chem. B* 109:4770–75
143. Harada I, Tasumi M. 1980. Formation of transient *cis N*-methylthioacetamide under ultraviolet-laser irradiation. *Chem. Phys. Lett.* 70:279–82
144. Kato C, Hamaguchi H, Tasumi M. 1985. Transient resonance Raman study on the *trans-cis* photoisomerization of *N*-methylthioacetamide. *J. Phys. Chem.* 99:407–10
145. Miwa JH, Patel AK, Vivatrat N, Popek SM, Meyer AM. 2001. Compatibility of the thioamide functional group with β-sheet secondary structure: incorporation of a thioamide linkage into a β-hairpin peptide. *Org. Lett.* 3:3373–75
146. Miwa JH, Pallivathucal L, Gowda S, Lee KE. 2002. Conformational stability of helical peptides containing a thioamide linkage. *Org. Lett.* 4:4655–57
147. Helbing J, Bregy H, Bredenbeck J, Pfister R, Hamm P, et al. 2004. A fast photoswitch for minimally perturbed peptides: investigation of the *trans-cis* photoisomerization of *N*-methylthioacetamide. *J. Am. Chem. Soc.* 126:8823–34
148. Cervetto V, Bregy H, Hamm P, Helbing J. 2006. Time-resolved IR spectroscopy of *N*-methylthioacetamide: *trans* → *cis* isomerization upon n-π^* and π-π^* excitation and *cis* → *trans* photoreaction. *J. Phys. Chem. A* 110:11473–78

149. Cervetto V, Pfister R, Kolano C, Bregy H, Heimgartner H, Helbing J. 2007. Coexistence of cyclic H-bonded and open tetrapeptide conformation. *Chem. Eur. J.* 13:9004–11
150. Stock G. 2007. Private communication.

Annual Review of
Physical Chemistry

Volume 59, 2008

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Physical Chemistry* articles may be found at http://physchem.annualreviews.org/errata.shtml