

Dynamics of peptide structures by multiple pulse 2D IR spectroscopy

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Abstract. The 2D IR COSY spectra of peptides is shown to yield novel information about structure and dynamics not available from linear IR methods. Three pulse correlation spectroscopy with heterodyning and passive phase control is carried out with rephasing and nonrephasing sequences yielding absolute value, real and purely absorptive 2D spectra for a variety of polarization conditions. The diagonal peaks are removable by polarization choices and improvements in resolution are obtained, especially for more complex systems such as α -helices. IR COSY studies are reported of amide-I, amide-A (N-H and N-D) and the coupling and angular relations between these modes are obtained through novel two color methods.

1. Introduction

An ideal approach to visualizing chemical and biological processes would involve snapshots of atomic positions of participating molecules throughout the course of chemical reactions or conformational changes. Although unlikely to be obtained from a single experimental technique, a combination of methods may well achieve this goal. New experiments have demonstrated the promise of using 2D IR methods to simplify IR spectroscopy of complex systems and thus obtain structural features with sufficient time resolution to resolve processes during chemical reactions and conformational dynamics. The 2D IR spectroscopy (1) naturally accesses the time scales that are hard to reach by 2D NMR and X-ray diffraction methods in solutions. Exciting biological applications include structural dynamics of nucleotides, peptides (2-4), and perhaps small proteins. Other applications in photochemistry, polymer dynamics, and liquids (see paper herein by Ge and Hochstrasser) are certainly possible and promising approaches.

2. The heterodyned 2D IR experiments

In IR COSY and THIRSTY (three pulse IR stimulated echo spectroscopy) experiments, three passively phase locked IR pulses with wavevectors k_1 , k_2 and k_3 arrive at the sample after time intervals τ and T generating an electric field pulse $E(t; \tau, T)$ in the direction $-k_1 + k_2 + k_3$ which is heterodyne detected by the local oscillator pulse k_{LO} . The echo field is a sum of Bohr frequencies corresponding to

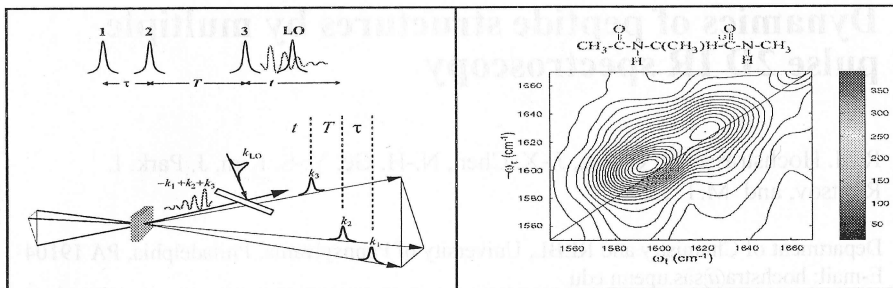


Fig. 1. 2D IR time domain interferometry. Fig. 2. 2D IR spectrum of alanine dipeptide

various Liouville pathways for the excitation and transfer between vibrational populations and coherences. Important structural constraints are also obtainable. For transition charge potentials the distance constraints are $\langle 1/r^3 \rangle$ from anharmonicity and $\langle 1/r^6 \rangle$ from energy transfer. The cross peak polarizations yield averages $\langle P_1 \rangle$ or $\langle P_2 \rangle$ of angles between transition dipoles, which are not model dependent, and their distribution functions. A typical 2D IR peptide spectrum is shown in Fig. 2 for the alanine dipeptide, which shows the ^{12}C and ^{13}C elongated diagonal shapes from the inhomogeneous structure distributions, the cross peaks from coupling the two anharmonic amide units that are energetically separated by isotopic substitution (also being done with $^{18}\text{O} = ^{13}\text{C}$) and the structural differences between the ends of the molecule in agreement with MD simulations (5).

3. Structures and dynamics by 2D IR

Novel 2D IR phenomena have been demonstrated. **Polarization conditions.** Polarization conditions were found that eliminate diagonal peaks from the 2D IR spectra. For the polarization condition $(\pi/4, -\pi/4, \pi/2, 0)$, the signal is $\langle \mu_1 \cdot \mu_3 \mu_2 \cdot \mu_4 - \mu_1 \cdot \mu_4 \mu_2 \cdot \mu_3 \rangle$ (6,7) which vanishes for a single dipole involvement (the symbols refer to the dipole directions at four times). An example of a diketone is given in Figure 3. Removing the intense diagonal peaks allows a clear observation of the phase of the cross peaks in the real 2D spectrum and represents a significant advance in simplifying complex spectra. The systematic use of polarization conditions considerably clarifies the 2D spectra of alpha helices because only the transitions between symmetric and antisymmetric excitonic levels of the α -helix need be exposed. The energy distributions and relaxation dynamics of delocalized states of the helix could be tested, free from interference by the diagonal peaks, based on the coupling between the amide oscillators. An example of the 20 unit helix Ac-AAAAkAaaaKAAAkAAAk-NH₂, where the lower case a's (alanines) contain $^{13}\text{C}=\text{O}$ groups down shifted by ca. 30 cm^{-1} , is shown in Fig. 3. The a-A coupling is clearly seen in these spectra (arrow), as is the broad distribution of two particle excitations of the α -helix.

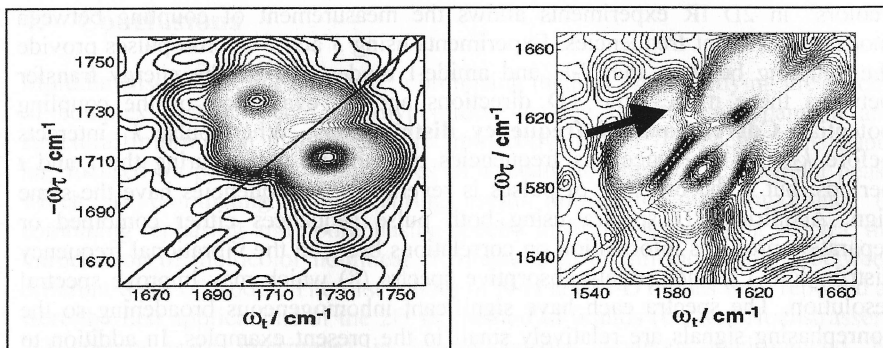


Fig. 3. 2D IR spectra of 1,3-cyclohexanedione (left) and the 20 unit helix (right)

The heterodyned photon echoes of amide-A modes. Heterodyned echoes of N-H modes of peptides have been obtained. These modes undergo very rapid (sub-picosecond) relaxation dependent on whether or not the N-H bond is hydrogen bonded and the frequency correlation functions signal the motions of nearby solvent molecules.

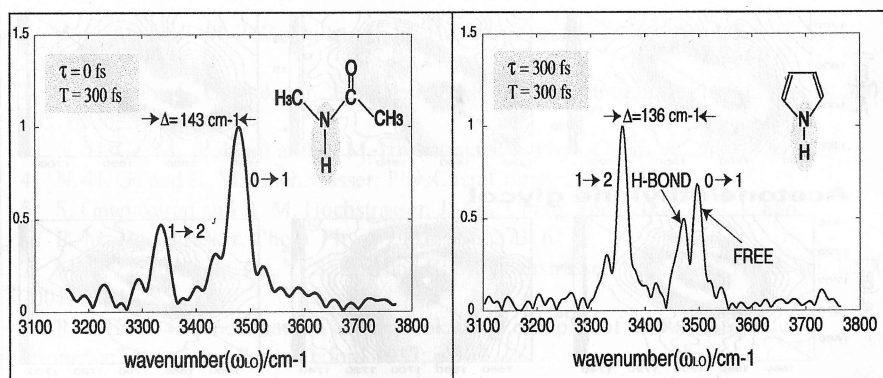


Fig. 4. Heterodyned photon echoes from N-methylacetamide (left) and pyrrole (right)

Examples of heterodyned N-H echoes are shown in Fig. 4 for some values of T and τ . For N-H the anharmonicity is ca. 140 cm^{-1} so self-localization of N-H excitation occurs in multiple unit peptides and the coupling scheme is quite different from that used for amide-I. **Two color experiments.** The coupling matrix, needed to interpret two color heterodyned echo signals for molecules with multiple N-H and amide-I modes incorporates the N-H/amide-I off-diagonal anharmonicity. We therefore used nonlinear IR methods to obtain these benchmarks for a number of peptide models. Typically the N-H/amide-I coupling is found to be 2 or 3 cm^{-1} (see paper by Rubtsov et al.). The use of two infrared

“colors” in 2D IR experiments allows the measurement of coupling between modes of different frequencies. Experiments using 3 and 6 micron pulses provide the coupling between the N-H and amide-I modes and on the energy transfer between these modes, the TD directions and an evaluation of the coupling potential. **Correlations of frequency distributions.** When pulse k_1 interacts before k_2 , the coherence has frequencies of opposite signs during the t and t' periods, but if the ordering of pulses is reversed, the frequencies have the same sign. Acquiring 2D spectra using both pulse sequences either combined or separately provides information on correlations between the vibrational frequency distributions (3) and purely absorptive spectra (8) which may improve spectral resolution. The spectra each have significant inhomogeneous broadening so the nonrephasing signals are relatively small in the present examples. In addition to time ordering, the polarization of each laser pulse can be individually varied in all quadrants allowing linear combinations of Liouville paths to be observed and hence the exclusion or emphasis of particular pieces of the 2D IR spectra. Fig. 5. shows some recent results (the corresponding real parts of the 2D IR spectra were also presented).

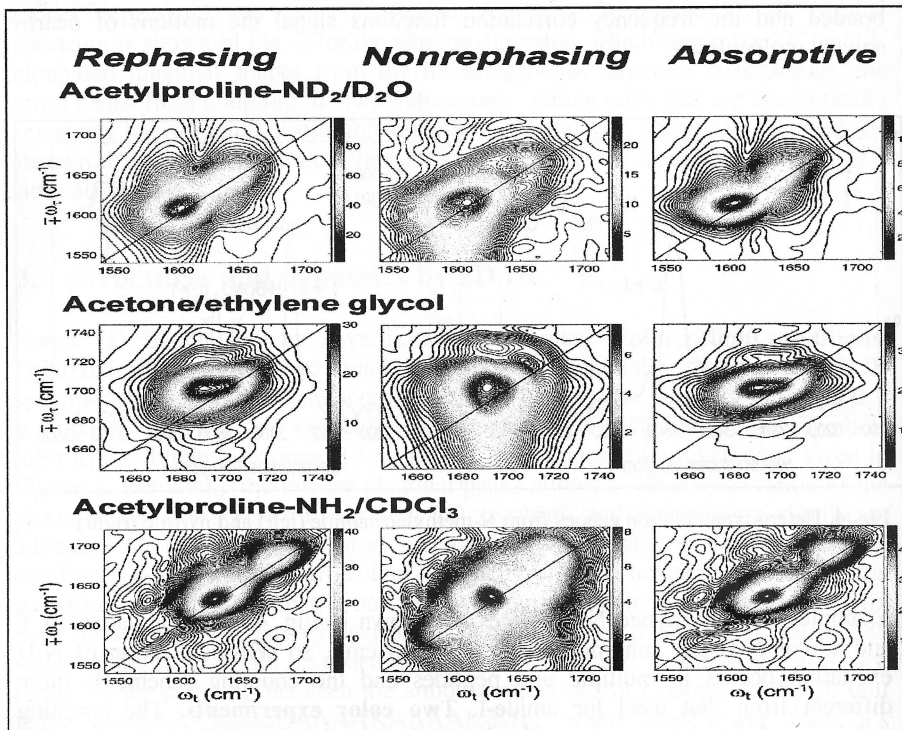


Fig. 5. Examples of absolute 2D IR spectra having oscillatory parts from the two times: left, FT($\cos \cos + \sin \sin$); center, FT($-\cos \cos - \sin \sin$); and right, FT(left +right).

4. Conclusions

Multidimensional IR appears to be a promising method for simplifying the spectra of complex systems such as polypeptides, leading to sharper dynamical and structural information than obtainable from conventional IR spectra. This paper has focused on polarization properties as a means to remove the diagonal peaks which are dominating and obscuring useful information, the importance of combining rephasing and nonrephasing spectra in further simplifying spectra, the introduction of multicolor nonlinear IR methods to obtain the coupling between different types of modes. The first echoes of N-H and N-D modes were reported as were the first applications of the 2D IR method to liquids (Ge and Hochstrasser). 2D spectra were interpreted in inhomogeneous, aqueous environments by comparing to molecular dynamics simulations (5).

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