SPECTROSCOPY

# Ultrafast spectroscopy: timing is everything



**Two groups present applications of two-dimensional optical spectroscopic methods for investigating dynamic biological processes on pico- to femtosecond timescales.**

Perhaps it is time to replace that tired cliché "faster than a speeding bullet" with something like, "faster than two-dimensional optical spectroscopy"?

By using a new breed of two-dimensional optical techniques, researchers can reap the benefits of both high structural resolution and fast temporal resolution. These technologies are the subject of two recent reports investigating the structural and temporal properties of dynamic peptide structures (Kolano *et al.*, 2006; Zhuang *et al.*, 2006).

Although X-ray crystallography is the most widely used tool for investigating the structure of proteins and of other biomolecules, it generates a static snapshot. Nuclear magnetic resonance (NMR) spectroscopy can be used to observe dynamic motions, but NMR is a relatively 'slow' technique and cannot resolve dynamic information below the millisecond regime. Whereas this is suitable for investigations of many biological processes, it cannot answer questions, for example, about the early stages of protein folding or unfolding. Optical spectroscopies offer much higher time resolution but provide only low-resolution structural information. Therefore, the structural investigation of ultrafast biological processes has been mainly the domain of computational researchers. But some biophysicists are venturing into new territory with two-dimensional optical methods.

Peter Hamm of the Institute of Physical Chemistry at the University of Zurich has been using transient two-dimensional infrared (2D-IR) spectroscopy to study molecular dynamics on the picosecond timescale. Infrared spectroscopy is widely used by chemists for its ability to signal the presence of functional groups in a molecule owing to their characteristic vibrational motions. "In [transient] 2D-IR spectroscopy," explains Hamm, "two IR pulses are interacting with two different parts of a molecule, and in this way we learn something about their relative distance and orientation."

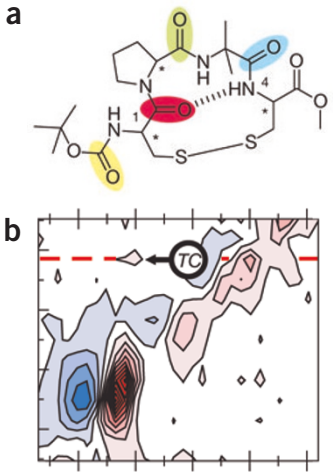


**Figure 1** | The investigation of hydrogen bond dynamics with transient 2D-IR. (**a**) Structure of the model peptide. (**b**) Transient 2D-IR spectra. The arrow labeled 'TC' indicates the crosspeak representing the changing hydrogen-bond strength. Reprinted from *Nature*.

Hamm and his colleagues recently showed that transient 2D-IR can be applied to investigate hydrogen-bond dynamics in a model peptide with β-turn architecture (Kolano *et al.*, 2006).

The six-residue peptide contained two cysteine residues, forming a disulfide bridge and constraining the β-turn (**Fig. 1a**). The structure also contained an intramolecular hydrogen bond. As the disulfide bridge can be cleaved by UV light, this served as a convenient phototrigger for 'breaking' the β-turn structure. Hamm and colleagues were able to monitor the weakening of the hydrogen bond and the opening of the β-turn with transient 2D-IR spectroscopy (**Fig. 1b**). The β-turn opening took about 160 picoseconds, which was close to the 240 picoseconds they had predicted using molecular dynamics simulations.

Shaul Mukamel of the University of California, Irvine, has also been interested in applying ultrafast spectroscopic methods for monitoring protein dynamics. He and his colleagues have now proposed a new class of two-dimensional chirality-induced techniques with optimized spectral resolution (Zhuang *et al.*, 2006).

Circular dichroism spectroscopy is used to characterize peptide or protein secondary structure, as chiral α-helical and β-sheet structures give strong, characteristic spectra. However, explains Mukamel, "The identification of spectrally resolved features associated with various secondary structures is not always possible for large proteins, and [its] application is limited by low resolution." To adapt chirality-induced techniques for the investigation of more complex molecules, Mukamel and colleagues optimized methods for generating two-dimensional correlation plots, in which the crosspeaks change their sign depending on their chirality. "Such signals are particularly sensitive to structural details and can greatly improve the spectral resolution of two-dimensional techniques," he says.

Mukamel and colleagues used their two-dimensional chirality-induced method in a simulation study to investigate fast peptide folding events on a femtosecond timescale. But this technique should not be limited to model systems, as Mukamel comments, "Studies of energy and electron transfer pathways in biological complexes will greatly benefit from using chirality-specific techniques to refine the spectroscopic information."

These sorts of tools, coupling ultrafast temporal resolution with high spatial resolution will undoubtedly become increasingly important for biophysical studies. "Molecular processes, on a microscopic, atomistic level, are intrinsically superfast," says Hamm. "We tend to forget this, because very often the outcome of a chemical or biological process is slow. But that does not mean to say that they are intrinsically slow. In many cases, the outcome is slow just because two partners need to find each other, but once they find each other, the actual process is super-fast."

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RESEARCH PAPERS

Kolano, C. *et al.* Watching hydrogen-bond dynamics in a β-turn by transient two-dimensional infrared spectroscopy. *Nature* **444**, 469–472 (2006).

Zhuang, W. *et al.* Two-dimensional vibrational optical probes for peptide fast folding investigation. *Proc. Natl. Acad. Sci. USA* **103**, 18934–18938 (2006).