# Towards a Molecular Movie: Real Time Observation of Hydrogen Bond Breaking by Transient 2D-IR Spectroscopy in a Cyclic Peptide

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## MOTIVATION

Our view of structural properties and hydrogen bonds in biomolecules is mainly based on the static architecture, probed by X-ray and neutron crystal structure analysis and nuclear magnetic resonance (NMR). Changes in these architectures on ultrafast timescales are, however, very difficult or impossible to observe by these techniques. Thus our motivation is the generation of a "Molecular Movie", which allows to make protein folding visible. Cyclo(Boc-Cys-Pro-Aib-Cys-OMe)*(1)* is a cyclic disulfide-bridged tetrapeptide that adopts a -turn structure due to an intramolecular hydrogen bond. To study protein- or peptide folding, a triggering event (that perturbs the peptide from a "starting" conformation) is required to initiate the folding or unfolding process. In time-resolved spectroscopy, in general, the triggering event is a short laser pulse. The disulfide bridge of the peptide is well-suited, since the disulfide bridge is a weak covalent bond (bond dissociation energy, 64.5 kcal $mol^{-1}$) and thus provides a predetermined breaking point. Hence a disulfide bridge can easily be cleaved by UV light*(2)*.

## IR BAND ASSIGNMENT

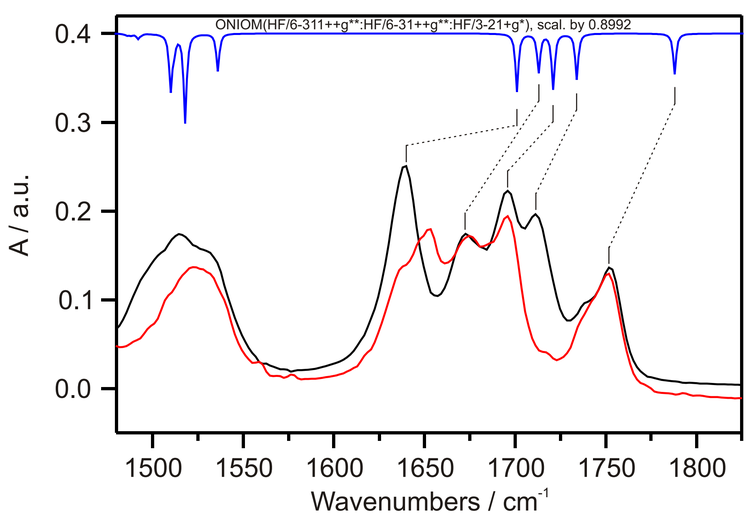
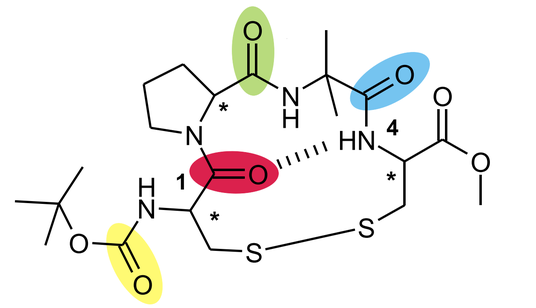



**FIGURE 1. Blue:** Calculated vibrational spectrum of the peptide; **Black:** FTIR spectrum of the peptide; **Red:** FTIR spectrum taken after chem. modification (Boc deprotection) of the peptide (spectral resolution 2 $cm^{-1}$, spacer size 0.05 mm, [*D3*]-$CH_3CN$).



**SCHEME 1.** Cyclo(Boc-Cys-Pro-Aib-Cys-OMe); Carbonyl groups are colored to facilitate the band assignment.

## UV-IR EXPERIMENTS

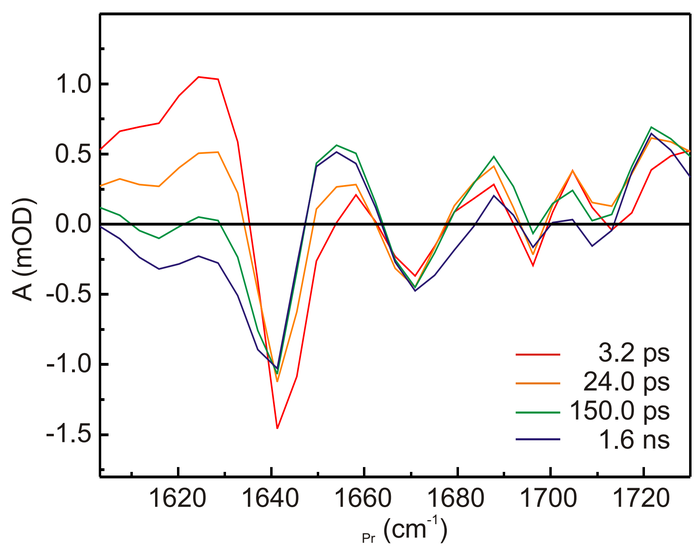



**FIGURE 2.** Difference infrared absorption spectra at different time delays after photolysis of the peptide with a $_{exc.}$=266 nm. Bands appearing on irradiation are pointing upward; bands disappearing are pointing downward.

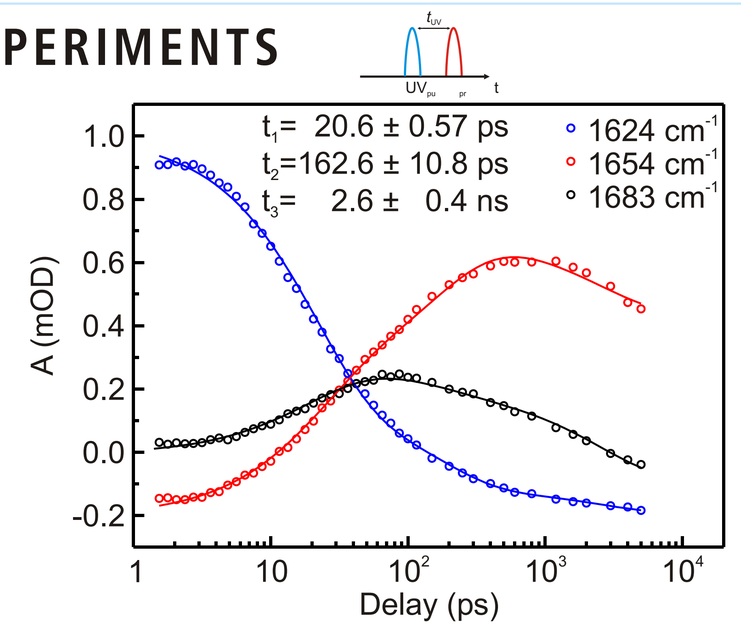



**FIGURE 3.** Dynamics of selected spectral positions. The solid lines correspond to a simultaneous fit of the time traces by a function containing three exponentials.

We determined the dynamics of the peptide upon ultrafast cleavage of the disulfide bridge using time-resolved mid-IR spectroscopy (T1D). The pulsed photolysis resulted in an instantaneous red shift of the amide I bands. Within the first 200 ps the initial red shifted signal has transformed into a blue shifted spectrum. This behavior can be observed independent of concentration ranging from 14 mM to 200 mM (Fig. 2). Only at high concentrations the transient spectrum starts to alter again after approximately 1 ns. We then observe concentration dependent kinetics covering the time range up to 10 µs. The transient spectra can be fit by three global time constants which allow us to make a tentative assignment of the dynamics:

$t_1$ = ~20 ps: *Cooling*
$t_2$ = ~165 ps: *Conformational Change ???*
$t_3$ = ~2.5 ns: *Diffusion controlled reaction of the liberated thiyl radicals*

## 2D EXPERIMENTS

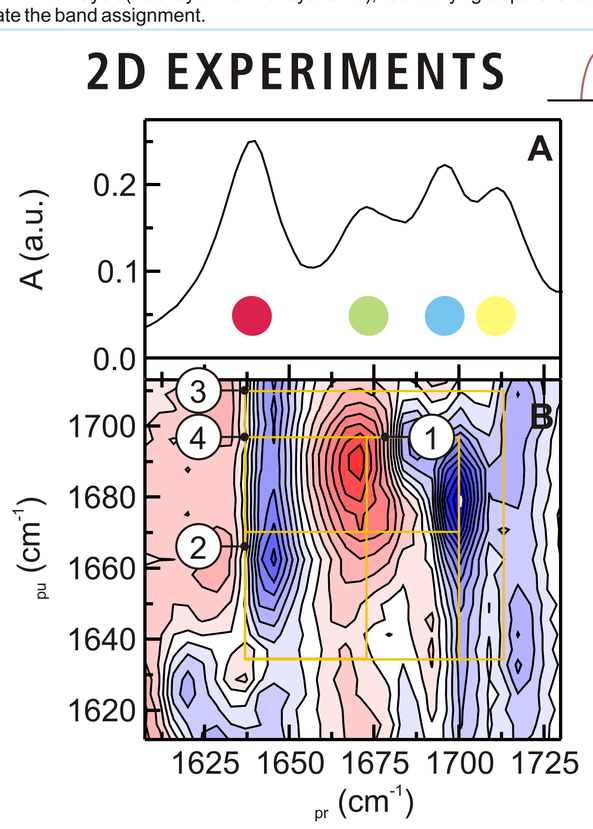



**FIGURE 4. A:** FTIR absorption spectrum of the amide I region of the peptide (spectral resolution 2 $cm^{-1}$, [*D3*]-$CH_3CN$). Band assignments correspond to the colored regions of the chemical structure in Scheme 1. **B:** Weighted difference between perpendicular and parallel polarization 2D-IR spectra. Blue signals: bleach; Red signals: excited state absorption. Crosspeaks due to coupling of neighboring amide groups are highlighted by black indicators and numbers.

We observe four pairs of crosspeaks. Three of them are caused by coupling between neighboring amide groups: Pro-Aib①, Cys(1)-Pro② and Cys(1)-Boc③. In addition we detect a crosspeak caused by a non-covalent interaction, namely the intramolecular hydrogen bond. This crosspeak is caused by the interaction between Cys(1)-Aib④. Due to the large spatial distance we do not observe crosspeaks for Boc-Aib and Boc-Pro.

## TRANSIENT 2D EXPERIMENTS *(3)*

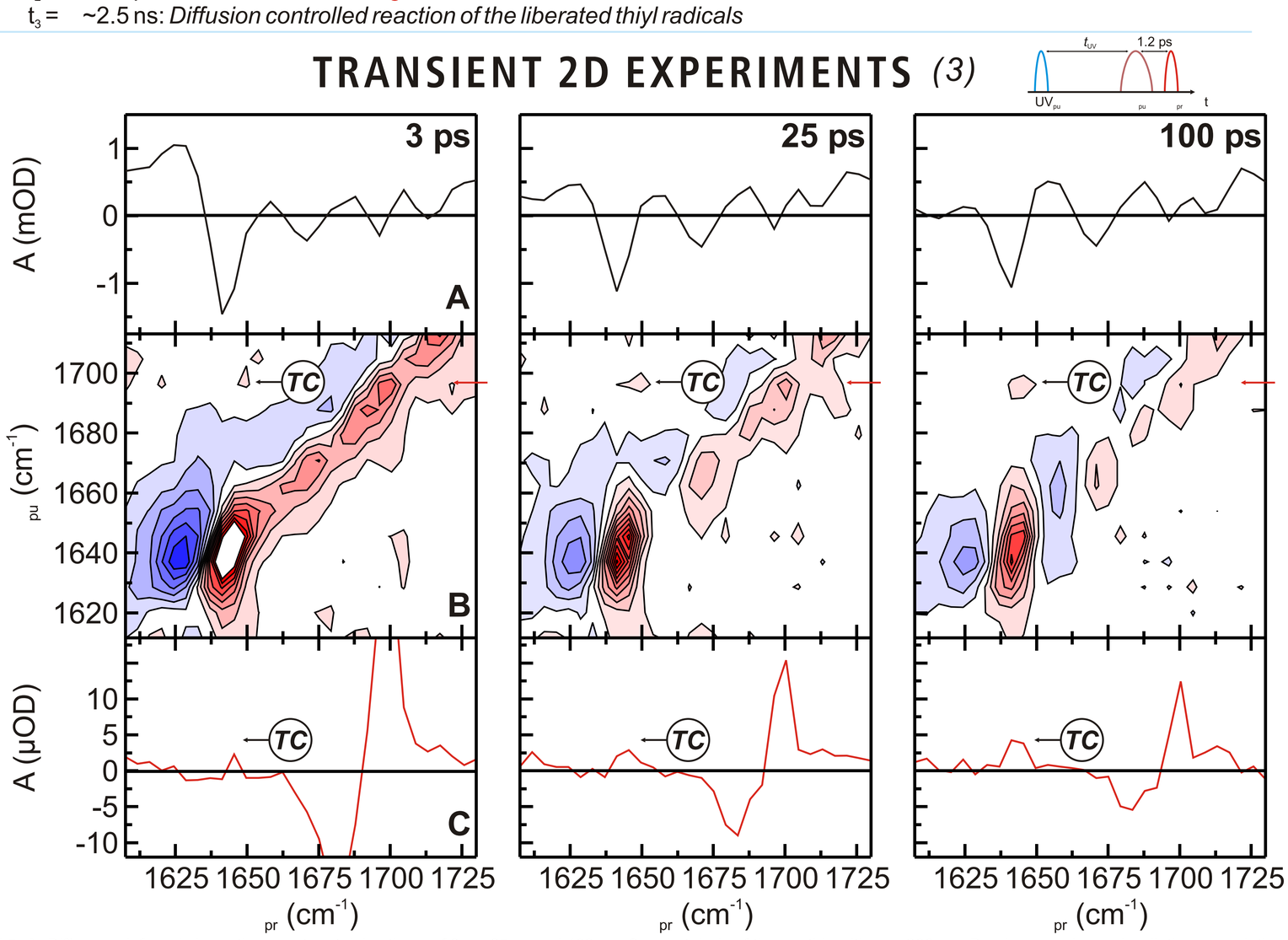
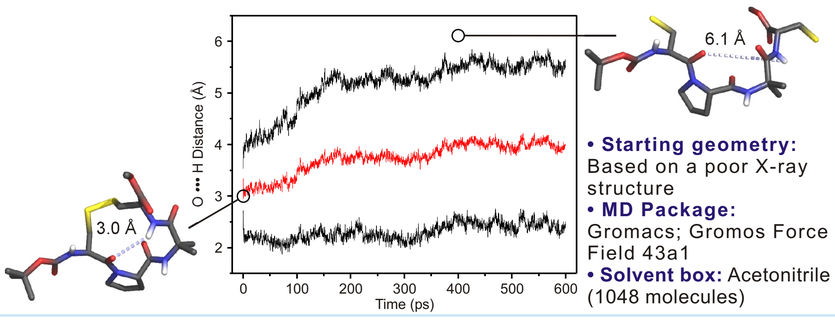



**FIGURE 5. A:** Difference infrared absorption spectra at delay times of 3 ps, 25 ps and 100 ps after photolysis of the peptide with $_{exc.}$=266 nm. Bands appearing on irradiation are pointing upward; bands disappearing are pointing downward. **B:** T2D-IR spectra at UV pump-2D-IR probe delay times of 3 ps, 25 ps and 100 ps. The red arrow indicates the position of the vertical cut shown in Fig. 5C. In the T2D spectra, negative signals are depicted in blue and positive signals, in red. The labeled arrow highlights the transient crosspeak. **C:** Vertical cut through the T2D-IR spectra at the position highlighted by the red arrow. The labeled arrow highlights the transient crosspeak.

## MD SIMULATIONS




- **Starting geometry:** Based on a poor X-ray structure
- **MD Package:** Gromacs; Gromos Force Field 43a1
- **Solvent box:** Acetonitrile (1048 molecules)

## CONCLUSION

- Transient 2D infrared spectroscopy reveals weakening of a crosspeak caused by an intramolecular hydrogen bond. Real time observation of intramolecular hydrogen bond breaking in a small cyclic peptide upon ultrafast cleavage of the disulfide bridge.
- The dynamics of the cyclic tetrapeptide can be described by a "three-step mechanism", giving rise to three global time constants:
  - Cleavage of the disulfide bridge and dissipation of excess energy to the solvent (about 20 ps).
  - Disappearance of the stabilizing intramolecular hydrogen bond and concomitant transformation of the starting -turn motif into a random structure implicated by continuous bleaching of the corresponding transient crosspeak, taking place on a time scale of a few hundred picoseconds, which is in perfect agreement with our MD-simulations.
  - Quenching of the liberated thiyl radicals by diffusion controlled reactions.

**References:**

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(2) (a) Volk, M.; Kholodenko, Y.; Lu, H. S. M.; Gooding, E. A.; DeGrado, W. F.; Hochstrasser, R. M. *J. Phys. Chem. B* **1997**, *101*, 8607. (b) Lu, H. S. M.; Volk, M.; Kholodenko, Y.; Gooding, E.; Hochstrasser, R. M.; DeGrado, W. F. *J. Am. Chem. Soc.* **1997**, *119*, 7173.

(3) Bredenbeck, J.; Helbing, J.; Behrendt, R.; Renner, C.; Moroder, L.; Wachtveitl, J.; Hamm, P. *J. Phys. Chem. B* **2003**, *107*,8654.

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