Time-resolved IR studies of a variety of IR probes for studying local electrostatics and solvent structures in myoglobin, reverse micelles, and peptides

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Introduction and Experimental Techniques

Over the years, ultrafast IR pump-probe and 2D-IR methods have been widely used to study ultrafast dynamics of biomolecules and their model systems. Although the technique itself has been found to be extremely powerful, a lot of potential applications of 2D-IR have yet to be discovered. Very often it is the case that the success of an experiment relies wholly upon the choice of a suitable IR probe that can provide critical and site-specific information on the local electrostatic environment and solvent (protein residue) structure. Although many vibrational probes have been already studied, which include nitriles (CN), thio- (SCN) and selenocyanates (SeCN), or the very popular azides (N_3), several new applications of those IR probes are presented.
Moreover, we have recently put a lot of effort in developing new IR probes based on isocyano(NC)-derivatized amino acids including β-isocyanoalanine and p-isocyanophenylalanine, which have been chosen as appropriate models for both aliphatic and aromatic amino acid residues in proteins.

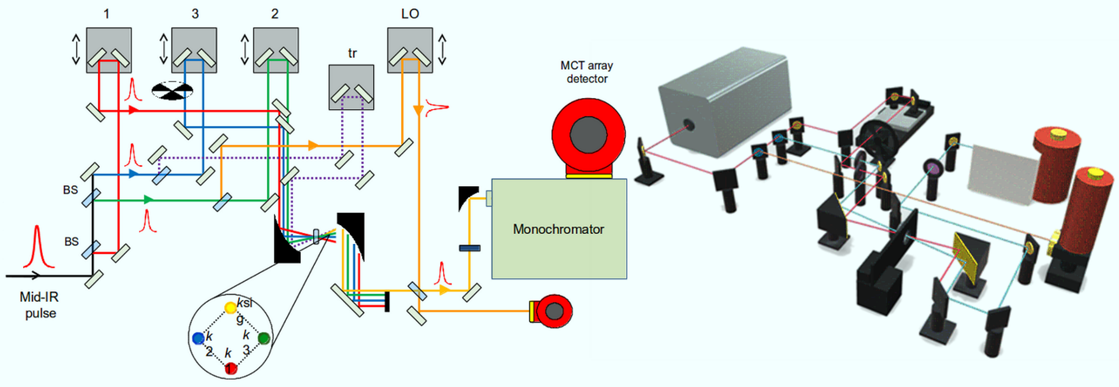


Figure 1: Scheme of the photon echo setup used for the 2D-IR measurements (left) along with simple 3D model of the laser system used in our IR pump-probe studies (right).

Water dynamics in Reverse Micelles

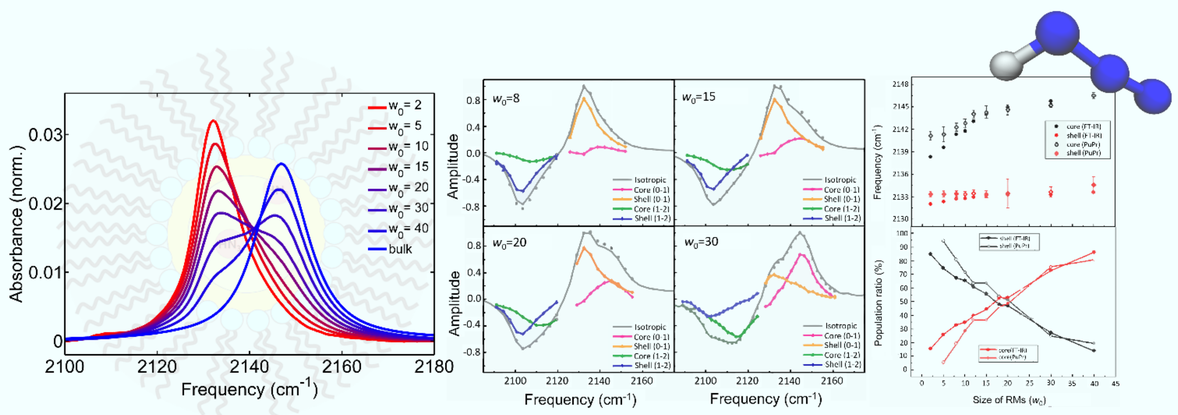


Figure 2: We have investigated the vibrational spectroscopy of HN_3 confined in an AOT RM. The hydrazoic acid has been found to possess most of important characteristics of good IR probes for studying water structures in nanoconfined environment. The spectral components commonly referred to as core and shell are well-separated and easily identified in the IR absorption spectra. The core part of the RM has been found to be affected by spatial confinement, resulting in changes in vibrational frequencies and spectral line shape, whereas the water structure in the shell region is not. The fs IR pump-probe spectroscopy revealed that the vibrational relaxation process of HN_3 molecules in the shell part occurs on much slower time scale, indicating weak H-bonding interaction with H_2O molecules, whereas the vibrational lifetime of the HN_3 molecules in the core region shows a weak dependence on RM size.

Pump-Probe spectroscopy of MbNCS and MbNCSe complexes

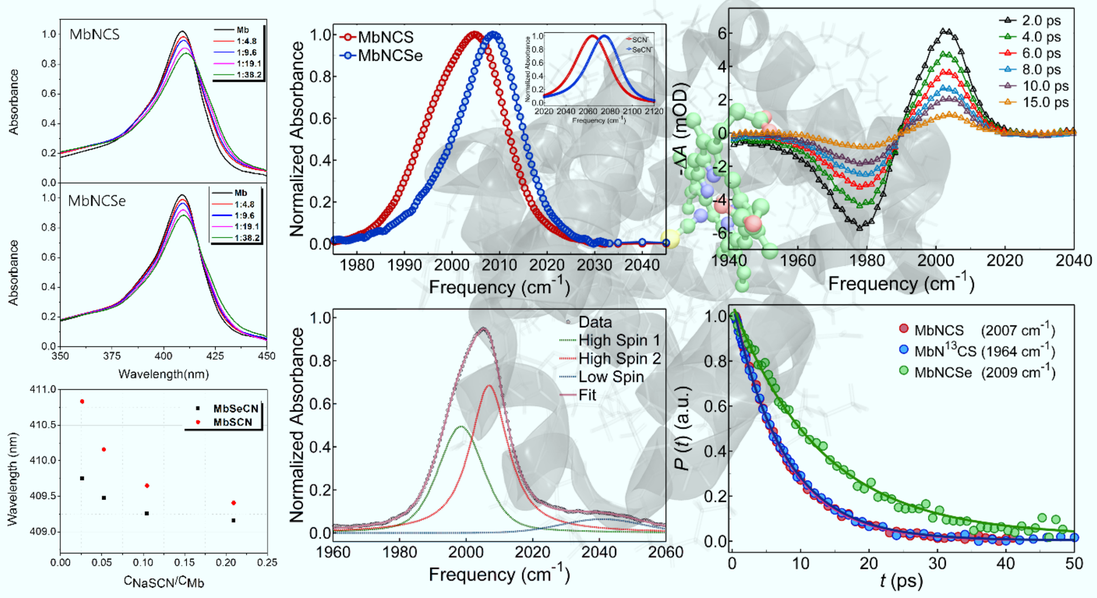


Figure 3: We have investigated the structure and vibrational dynamics of SCN and SeCN-bound myoglobin proteins. The changes in the electronic spectra suggest that, in both cases, mostly high spin complexes are formed. The FTIR spectra of MbNCS shows a doublet that may originate from different structural conformations as well as a shoulder peak coming from different binding mode (different spin state). The vibrational lifetimes of MbNCS and MbNCSe were determined to be 7.2 $\pm$ 0.12 ps and 13.6 $\pm$ 0.37 ps, respectively. SCN^- and $SeCN^-$ ions appear to be promising probes to study open-shell complexes of hemoproteins due to their long vibrational lifetime and the fact that their electronic spin state and binding mode show high globin dependency

2D-IR Spectroscopy of MbNCS (MbNCSe)

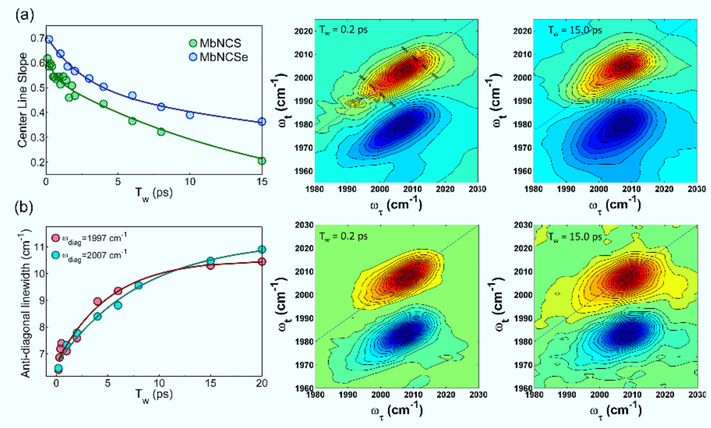


Figure 4: To provide more insights into structural origins of the doublet feature observed in the IR spectrum of MbNCS and to quantify the ultrafast structural fluctuations within the distal cavity of ion-bound myoglobin, we have measured a series of 2D-IR photon echo spectra and analyzed the temporal evolution of their lineshapes. No chemical exchange process has been observed in the 2D-IR spectra of MbNCS, indicating that the transition between the corresponding two conformation states occurs on much slower time scales compared to the vibrational lifetime of the probe. Spectral diffusion process has been quantified with FFCFs obtained through center line slope analyses.

Development of new IR probes based on isocyanide-derivatized amino acids

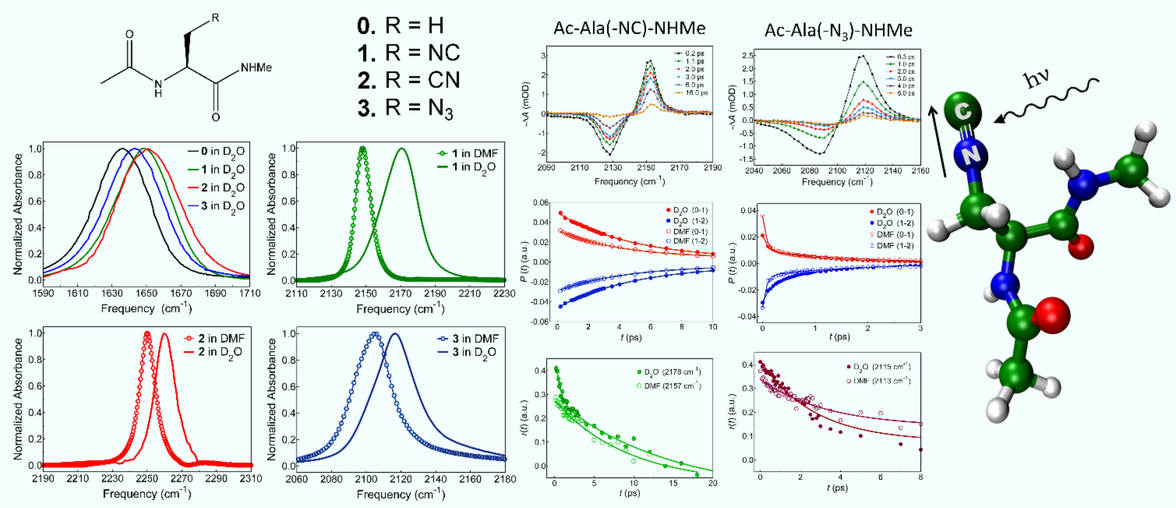


Figure 5: An infrared probe based on isonitrile (NC)-derivatized alanine was synthesized and the vibrational properties of its NC stretching mode were investigated using FTIR and femtosecond IR pump-probe spectroscopy. It was found that the NC stretching mode is very sensitive to the hydrogen-bonding ability of solvent molecules. Moreover, its transition dipole strength is larger than that of nitrile (CN) in nitrile-derivatized IR probe. The vibrational lifetime of the NC stretching mode was found to be 5.5 $\pm$ 0.2 ps in both D_2O and DMF solvents, which is several times longer than that of the azido (N_3) stretching mode in azido-derivatized IR probe.

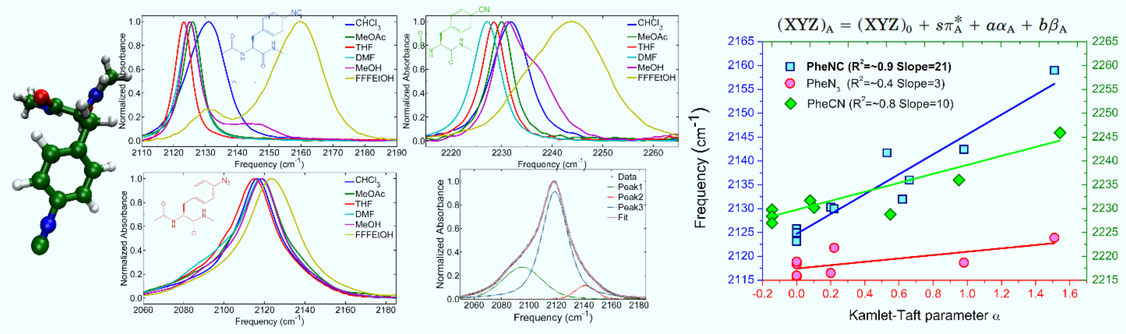
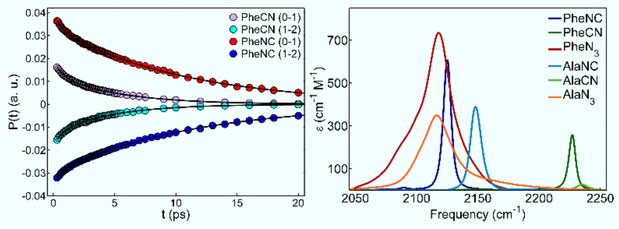


Figure 6: The previous study on alanine amino acids was extended to aromatic systems by synthesizing (NC)-derivatized phenylalanine (PheNC). Interestingly, centre frequencies and linewidths (not shown) of both aliphatic and aromatic isocyanides show high correlation with H-bond donating abilities of solvents, described by Kamlet-Taft parameter α. It appears that isocyanides are twice more sensitive to H-bonding interactions as compared to nitriles. Also, the azide compounds show low degree of correlation with α, and are highly contaminated with Fermi resonance contributions. The origin of enhanced sensitivity of isocyanides are currently studied by using coarse-grained model of solvatochromism.



Compound	ε [M^{-1} cm^{-1}]	$\varepsilon_{arom}/\varepsilon_{aliph}$	D [10^{-2} D^2]	D_{arom}/D_{aliph}
AlaNC	390	1.6	2.21	1.2
PheNC	610		2.71	
AlaCN	27	9.6	0.15	6.6
PheCN	259		0.99	
$AlaN_3$	333	2.2	4.8	2.7
$PheN_3$	736		12.9	

Figure 7: Changes in band intensities, when going from aliphatic to aromatic systems, were studied for all alanine and phenylalanine derivatives considered in our work. It is seen that the largest intensity enhancement is observed for the CN group, whereas our new NC probe shows similar dipole strength regardless of whether the functional group is attached to an aliphatic or aromatic carbon. Interestingly, the vibrational lifetime of PheNC was found to be 11.0 ps, which is more than twice longer than that of PheCN (4.7 ps). We believe that all these properties combined will allow isocyanides to completely replace the nitrile probes in the nearest future.

References

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