# Two-dimensional infrared spectroscopy of a site-specifically labeled photoswitchable allosteric protein

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We have measured 2D-IR difference spectra for several different mutants of the PDZ2 domain protein using azidohomoalanine as a label. The 2D-IR difference spectra upon inducing the conformational change of a site-specifically labeled protein, as well as difference spectra for temperature depending unfolding of the protein are reported.

It has been shown that azidohomoalanine is a good vibrational label that can be inserted site-specifically into proteins, can serve as a reporter for the changes in local environment and it is possible to measure azidohomoalanine labelled proteins down to a sub-millimolar concentration, with good signal to noise ratio [1, 2]. In order to investigate the signal propagation upon a conformational change of protein, we have incorporated azidohomoalanine as a site-specific label into the photoswitchable PDZ2 domain, previously used as a model system for investigating allosteric proteins [3].

Our results show that azidohomoalanine is suitable as a label for reporting large changes, e.g., moving the label from a hydrophobic environment to a solvent exposed surrounding, as it happens upon thermal unfolding. One of the mutant PDZ2 domain has shown a significant change in azidohomoalanine absorption upon conformational switching. When the label is placed at other positions in the protein, the changes in surrounding are too small to observe a difference in absorption. Therefore, we have been investigating additional labels, with larger oscillator strength, which may allow us to gain sensitivity to very small variations in surrounding.

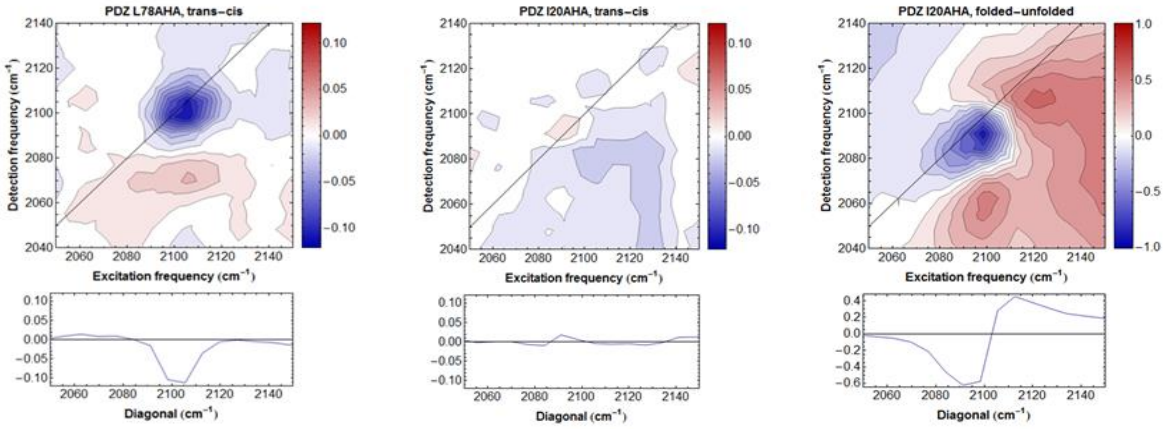


Fig. 1: 2D-IR difference spectra of some of the Aha-labeled PDZ2 domain mutants. Different mutants of the PDZ2 domain were measured. All samples were measured in buffered at protein concentrations between 0.6 to 1.3 mM and at a temperature of $10^0$C. Below each 2D-IR spectrum a cut along the diagonal is shown.

[1]Bloem, Robbert, et al. "Ligand binding studied by 2D IR spectroscopy using the azidohomoalanine label." The Journal of Physical Chemistry B 116.46 (2012): 13705-13712.
[2]Koziol, Klemens L., et al. "Fast infrared spectroscopy of protein dynamics: advancing sensitivity and selectivity." Current opinion in structural biology 34 (2015): 1-6.
[3]Buchli, Brigitte, et al. "Kinetic response of a photoperturbed allosteric protein." Proceedings of the National Academy of Sciences 110.29 (2013): 11725-11730.