

**Light induced conformational isomerisation of helical photoswitchable S-peptide and perturbation of S-protein/S-peptide complex**

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Transient infrared spectroscopy is a promising technique for time-resolved investigation of proteins' conformational changes induced by e.g. peptide binding. Model system that we are currently interested in is ribonuclease S (RNase S), a non-covalent complex composed of S-protein and S-peptide. RNase S is produced by subtilisin cleavage of ribonuclease A at specific position and consists of tightly associated S-peptide (residues 1-20) and S-protein (residues 21-124) which possess full enzymatic activity. Dissociated S-peptide has predominantly random coil conformation in solution, whereas alpha helical conformation is favored in the bound state to folded S-protein<sup>1</sup>. Our main aim is to gain further insights in mechanism of S-peptide – S-protein binding by using different modified forms of S-peptide. By covalently modifying S-peptide, we managed to introduce a bridging photoswitchable water soluble azobenzene molecule which allows us to perturb the structure in a controlled manner. This perturbation should be large enough to lead to dissociation of the S-peptide from the complex, which would allow us to monitor time-resolved changes of S-peptide and S-protein conformation upon ligand unbinding. Furthermore, incorporation of site-specific and sensitive infrared labels, either in S-peptide or S-protein, would provide even more specific and detailed information on conformational changes that occur<sup>2</sup>.

[1] Annett Bachmann, et al. *Proceedings of the National Academy of Sciences*, **2011**, 108, 3952-3957.

[2] Robert Bloem, et al. *Journal of Physical Chemistry B*, **2012**, 116, 13705-13712.