Photocontrolled AzoChignolin β-Hairpin Peptidomimetics

Tom Podewin[1], Stefan M. Hofmann[2], Michael S. Rampp[2], Elisa Weber[2], Ilvana Turkanovic[2], Wolfgang Zinth[2] and Anja Hoffmann-Röder[1]

[1]Department of Organic Chemistry, Faculty for Chemistry and Pharmacy, Ludwig-Maximilians-University Munich
[2]Department for BioMolecular Optics, Fakulty for Physics, Ludwig-Maximilians-University Munich

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

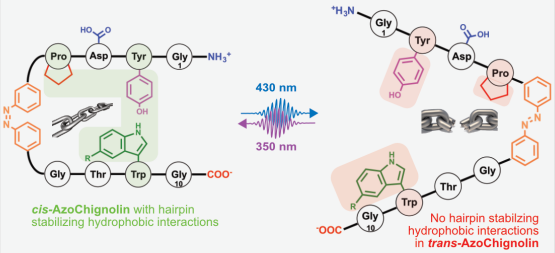
CUP Campus Chemie und Pharmazie

β-structures belong to the most frequent secondary structure elements in proteins and can serve as nucleation sites for protein folding. In this context the understanding of the folding dynamics of small β-structures on various timescales is of major importance. Here we present a newly synthesized light switchable β-hairpin model peptide based on the recently introduced **Chignolin**[1] and use CD-, NMR- and time-resolved IR spectroscopy to obtain information on structural dynamics.

For photo-control of the structure the central turn region of **Chignolin** (Glu[5], Thr[6]) was replaced by the ultrafast photoswitch AMPP.[2,3] This light-triggered peptide, called **AzoChignolin**[4], can be reversibly switched from a folded β-hairpin structure to an unfolded disordered structure monitored by previously mentioned spectroscopies[4]. With azobenzene in *cis*-isomeric state the peptide shows folded structures, contrary to unfolded peptide structures for *trans*-azobenzene.

AzoChignolin[4] - A photoswitchable β-hairpin peptide

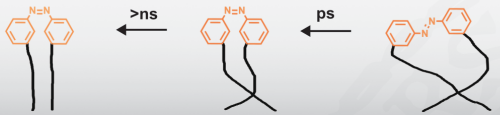
AzoChignolin can be reversibly switched from folded structures with *cis*-AMPP to disordered/unfolded structures with *trans*-AMPP.



***cis*-AzoChignolin** with hairpin stabilizing hydrophobic interactions

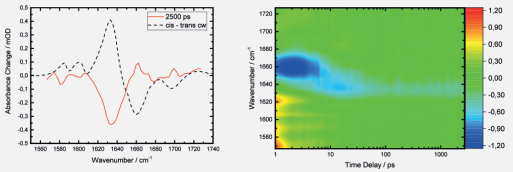
No hairpin stabilzing hydrophobic interactions in ***trans*-AzoChignolin**

AzoChignolin folding and unfolding takes place on a nanosecond timescale, while *cis/trans* isomerisation of AMPP occurs ultrafast within picoseconds.



Folding of AzoChginolin

Time-resolved infrared spectroscopy of the amid I‘ band of the *trans* ensemble to obtain information on structural dynamics during folding reaction.

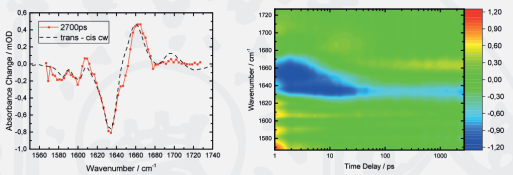


The IR difference spectrum after 2500 ps does not resemble the continuous wave (cw) spectrum. However, it resembles the unfolding spectrum after 2500 ps, showing the unfolding of "folded" *trans* structures within the first nanoseconds.

→ **The real folding dynamics must occur on the ns to µs timescale**

Unfolding of AzoChginolin

Time-resolved infrared spectroscopy of the photostationary cis ensemble to observe unfolding reaction.

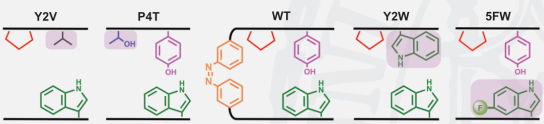


The IR difference spectrum after 2500 ps resembles the cw spectrum, with some minor differences. This indicates interstrand unfolding reactions start within the first few nanoseconds.

→ **Interstrand unfolding involves rapid processes. However it is not finished within 2.7 ns, with dynamics occuring on the ns to µs timescale**

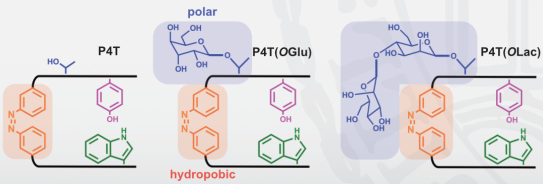
Novel AzoChignolin derivatives - Modifing the hairpins

Increasing or decreasing hydrophobic interactions allows for aimed alternations in folding and unfolding behavior and thus modifications were introduced to **AzoChignolin**.



increasing hydropobic interactions

A drawback of **AzoChignolin** peptides: low solubility in aq. solvents.
The solution: introducing polar sugar residues to **P4T-AzoChignolin**.



increasing solubility in aq. media

Outlook

Further enhancment of the **solubility in aq. media** by introduction of polar groups to **AzoChignolin** peptides.

Time-resolved infrared spectroscopy up to the **100 µs** range to observe the entire **folding** and **unfolding** reactions.

Time resolved measurements are *en route* to observe dynamics in **AzoChignolin** compounds with **modified hydrophobic interactions.**

Investigations on modified **AzoChignolin** samples show that hydrophobic interactions are of major importance for stabilizing the folded structure.

[1] Honda S., Yamasaki K., Sawada Y., Morii H., *Structure* (2004) **12** p. 1507-1518. [2] Aemissegger A., Kräutler, V., van Gunsteren W.F., Hilvert D., *J. Am. Chem. Soc.* (2005) **127**, 2929. [3] Dong S.-L., Löweneck M., Schrader T.E., Schreier W.J., Zinth W., Moroder L., Renner C., *Chem Eur. J.* (2006) **12**, 1114. [4] Podewin T., Rampp M. S., Turkanovic I., Karaghiosoff K. L., Hoffmann-Röder A., Zinth W., *ChemComm* (2015) **19** p. 4001-4004.