Peptides in motion

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English Summary

The control of biological processes has always been a challenge for chemists and biochemists. The ability to interfere with a specific biological function helps scientists to achieve a better understanding of the process itself and, if the process is correlated with pathology, it might be possible to gain new insights in the development of diseases and eventually to discover new treatments. Towards this goal, the control of biological processes might be achieved using light as a stimulus. Light of the appropriate wavelength is not invasive and usually bioorthogonal: it does not interfere with almost any biological function. The use of light usually doesn't lead to contamination, except when there is photo-degradation or photo-oxidation. Finally, it can be delivered with high precision to achieve control of function in time and space.

Photoswitches are molecules that change structure/geometry upon irradiation with light. The most prominent biological photoswitch is the retinal in our eyes and the most widely used photoswitches used for the control of peptides and proteins are azobenzenes, stilbenes and diarylethenes. In the first chapter, the structure of these photoswitchable molecules and the strategies to incorporate them into peptides are described. Illustrative examples of photoswitchable peptides are highlighted, with special focus on peptidic domains, including photosensitive β-hairpins and zinc-fingers.

The first part of the thesis (chapter 2, 3, 4) describes the development of methodological approaches to incorporate photoswitches into biomacro molecules. The second part of the thesis describes two novel examples of photoresponsive peptides.

In the second chapter, a photoswitchable molecule, azobenzene, is used as a linker to immobilize the enzyme lipase from *Candida rugosa* on a quartz surface (Figure 1a). The bifunctional linker allows deactivation of the immobilized enzyme by irradiation with visible light (Figure 1b). Despite the irreversibility of the process, this system constitutes the first example of covalent immobilization of an enzyme with a photoswitchable linker. The design of the linker with two groups, possessing orthogonal reactivity, enables a modular approach to be taken to immobilize enzymes on surfaces. The presented results enable new approaches for biosensor construction, especially in analytical applications, where the duration of enzymatic reaction is crucial and it has to be terminated in a manner that is mild and orthogonal to other elements of the system.

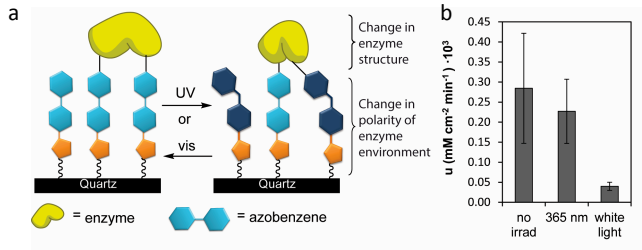

Figure 1: a) Design of immobilized lipase from *Candida rugosa* on quartz surface using a photoswitchable linker. b) Specific activity of the immobilized enzyme after irradiation at λ=365 nm and after irradiation with white light.

In the third chapter, a new family of azobenzene photoswitches was developed. These azobenzenes can be introduced to azide-bearing targets using Staudinger-Bertozzi ligation in aqueous, as well as organic media and without any additional reagents or catalysts (Figure 2). The molecular photoswitches, formed upon this ligation, show high fatigue resistance for the reversible switching process in water, photostationary states with up to 95% of the *cis* isomer, and thermal stabilities of the *cis* isomer ranging from milliseconds to days. In this chapter, also possible applications of the azobenzene Staudinger tags are explored.

Model studies have been conducted on the use of these compounds for the introduction of azobenzene photoswitches on quartz surfaces. To confirm that these compounds can be used for the modification of biomolecules, its application for site-selective incorporation of the photochromic residue into the structure of azide-modified zinc finger protein is presented. Attempts were made also to use one of this photoswitch directly in solid phase peptide synthesis, but the reaction is prevented by steric hindrance probably due to the proximity of the azide functionality to the resin.

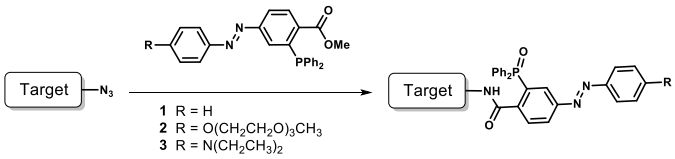

Figure 2: Staudinger-Bertozzi ligation of molecular photoswitches to an azide-bearing target.

Approaches to control the function of peptides with light would benefit from expanding the number of switch architectures that can be used. The field of photoresponsive peptides is limited so far to switches that exhibit a geometrical change between only two stereoisomers and that show thermal instability of the

photoisomer. The research described in the fourth chapter aims to find a synthetic strategy to insert, for the first time, an overcrowded-alkene switch into the backbone of peptides (Figure 3). Towards this goal, strategies for solid phase peptide synthesis are described, focusing on the choices of resins and protecting groups.

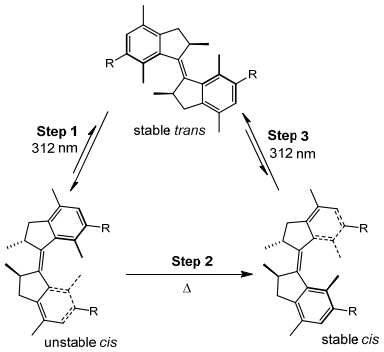

Figure 3: Overcrowded alkene switch.

In the fifth chapter, we used the developed methodology to insert an overcrowded alkene photoswitch to control a model β-hairpin peptide (Figure 4). This photoresponsive unit undergoes a large conformational change and has two thermally stable isomers. The geometrical change which has major influence on the secondary structure and the aggregation of the peptide, permitting the phototriggered formation of amyloid-like fibrils. The different behaviour of the isomers in aggregation was studied (Figure 4).

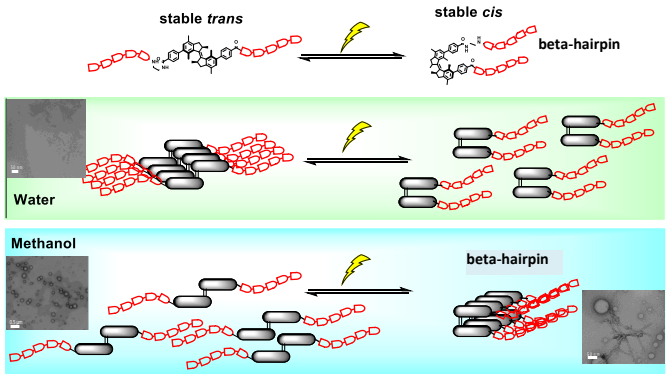

Figure 4: Model β-hairpin peptide functionalized with an overcrowded alkene and representation of different aggregation behaviour in methanol and water.

The final chapter, Chapter 6, describes the insertion of an azobenzene unit in a zinc-finger domain (Figure 5). The photochemical isomerization studies of this system showed that the azobenzene has a photostationary state of at least 45% of the *cis*

isomer and shows reversible photoisomerization. Furthermore, the half-life of the *cis* isomer depends on the presence of zinc ions. Both *trans* and *cis* isomers bind zinc ion and form the secondary structure of zinc finger, therefore, both *trans* and *cis* forms can bind to DNA. Interestingly, *cis*-AMPB-Sp1-f3 is a stronger zinc-binder, but a weaker DNA-binder than *trans*-AMPB-Sp1-f3. This might be explained by a better stabilization of the α-helix in the *trans*-isomer. This alternative approach to obtain control of the secondary structure of the zinc finger and, therefore, of the binding to DNA of the zinc finger domain, is generally applicable to control zinc finger domains as it does not interfere with specific α-helix-DNA interactions.

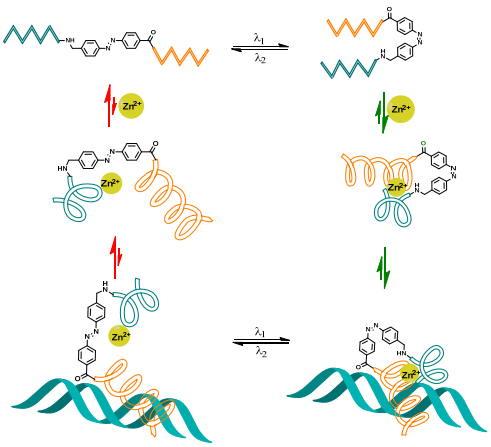

Figure 5: Photoswitchable zinc finger in which the azobenzene interferes with the secondary structure and therefore with the DNA-binding.

The results presented in this thesis aimed at establishing new methods for modification of peptides, enzymes and proteins with photoswitches. In particular, the overcrowded alkene switch was inserted for the first time in peptides. Ultimately, this work may provide tools for modification of photosensible surfaces with biomolecules and for constructing new photoresponsive biosystems.