Highly retarded thermal *cis*-to-*trans* relaxation of an azobenzene-based photoswitch enables photocontrol of the enzyme activity of a histone deacetylase-like amidohydrolase from *Bordetella/Alcaligenes* strain FB188 by a single light pulse

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Introduction

The photocontrol of enzyme activity offers novel and promising opportunities for biotechnological applications. Due to nowadays available light sources light-triggered sequential bioprocessing systems as well as diagnostic and lab assays[1] based on lab-on-a-chip technology could be temporally controlled by light in a highly spatial resolution. The photoisomerization of azobenzene derivatives in their *cis*- and *trans*-conformational states by ultraviolet (UV) and visible (VIS) light (Fig. 1) can be used as an approach to reversibly control the function of proteins.[1-3]

The aim of this work was to investigate and to characterize a photoswitchable biocatalyst which could be applied to control deacetylation reactions of biomolecules or chemical compounds in vitro in a spatial and temporal defined manner. For this approach a bacterial histone deacetylase-like amidohydrolase (HDAH) from *Bordetella/Alcaligenes* strain FB188[4] was used which catalyzes the deacetylation of ε-acetylated lysine side chains of histone and non-histone proteins. The enzyme was sensitized to light by a monofunctional azobenzene derivative (4-phenylazomaleinanil; 4-PAM**)** which was covalently attached to defined cysteines adjacent to the active site of several HDAH variants. The scheme of reversible photocontrol of the HDAH activity by *cis/trans* isomerization of the attached azobenzene moiety is shown in Fig. 2.

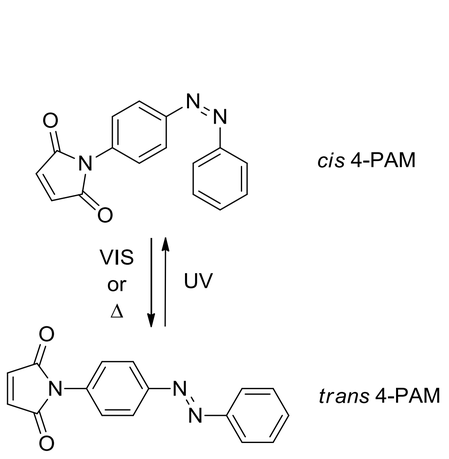

Fig. 1. Photoisomerization and thermal relaxation of 4-PAM. UV light irradiation leads to the *cis*- and VIS light irradiation as well as heat to the *trans*-state.

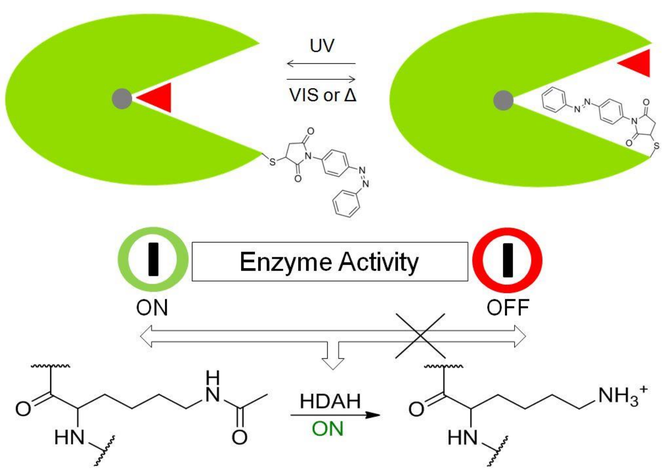

Fig. 2. Reversible photocontrol of the HDAH activity. The *cis*-state of 4-PAM activates (ON) and the *trans*-state (OFF) inactivates HDAH.

Results

Six different HDAH variants with a single surface accessible cysteine residue were generated and covalently modified by 4-PAM (Fig. 3). In particular, two 4-PAM/HDAH conjugates were shown to be controlled by light (M30C, Fig. 4 A and S20C, Fig. 4 B) whose enzyme activities were significantly changed upon UV (*cis*-state) or VIS (*trans*-state) light irradiation, compared to HDAH wild typ (wt) (Fig. 4 C). The reversible decrease and increase of the 4-PAM/M30C conjugate activity by means of multiple alternated VIS- and UV light irradiation steps could also be demonstrated (Fig. 5)

UV/Vis spectra of the thermal *cis*-to-*trans* relaxation of unbound 4-PAM and covalently attached to HDAH-wt, S20C and M30C (Fig. 6 A-D) and its extended kinetic (Fig. 7 A-D, up to 70 h) was investigated. The thermal *cis*-to-*trans* relaxation kinetic of unbound 4-PAM could be fitted by a simple one exponential function (Fig. 7 A) whereas 4-PAM/HDAH conjugates (Fig. 7, HDAH-wt (B), S20C (C) and M30C (D)) showed a two exponential function. Especially the relaxation constants (k_{slow}) of S20C and M30C were highly retarded (~ 50-fold). This strong deceleration of the thermal *cis*-to-*trans* relaxation seems to be caused by stabilization of the *cis*-state due to noncovalent interactions with adjacent amino acids on the protein surface.

Furthermore, molecular dynamic (MD) simulations were performed to investigate the photoswitch mechanism as well as to identify potential amino acids which are involved in the stabilization of the *cis*-state in both HDAH variants (M30C, Fig. 8 A1 and S20C, Fig 8, B1).

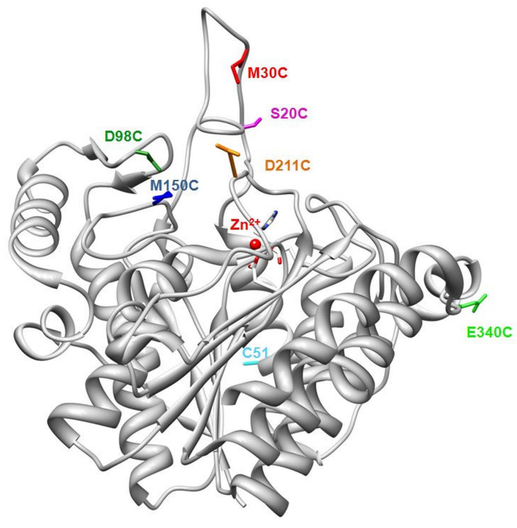

Fig. 3. Crystal structure of HDAH (PDB ID 2VCG). Colored positions indicate each amino acid which was mutated to cysteine by site-directed-mutagenesis.

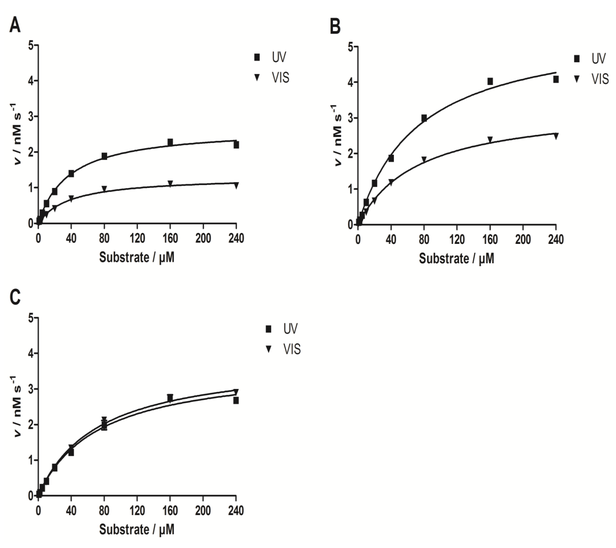

Fig. 4. Michaelis-Menten kinetics of 4-PAM/HDAH conjugates M30C (A), S20C (B) and wt (C) upon UV- and VIS light irradiation. Curves were fitted to Michaelis-Menten equation and the catalytic velocity (*v*) described as turnover of substrate per second (nM s^{-1}). Values are mean ± SEM and n = 3.

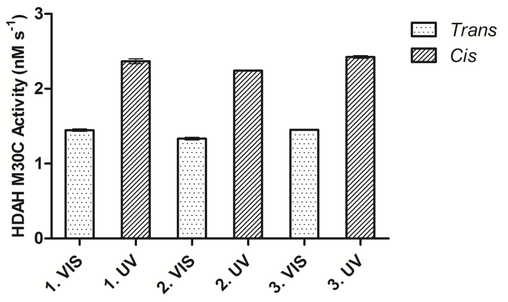

Fig. 5. Reversible photocontrol of the enzymatic activity of the 4-PAM/M30C conjugate upon VIS- and UV light irradiation. The sample was alternately irradiated with VIS- and UV light and the enzyme activity expressed as (nM s^{-1}). Values are mean ± SEM and n = 3.

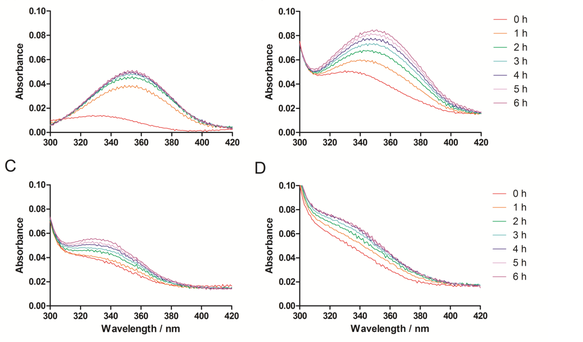

Fig. 6. UV-VIS spectra of the thermal 4-PAM isomerization from its *cis*- to its *trans*-conformational state in its unbound (A) and conjugated state on HDAH-wt (B), S20C (C) and M30C (D). Samples were irradiated by UV light and UV/VIS spectra recorded in a time interval of 1 h.

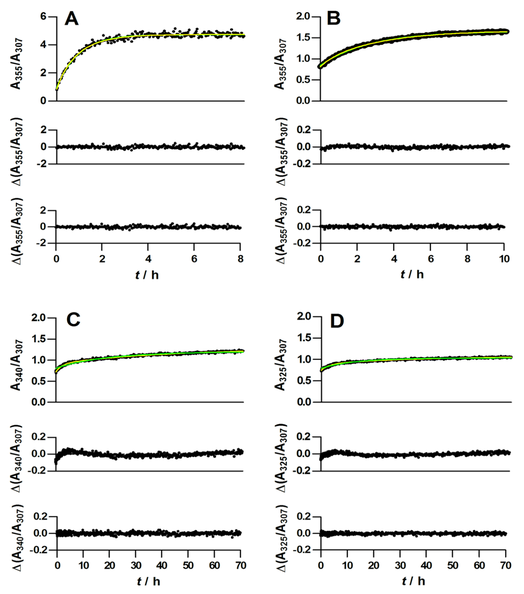

Fig. 7. Thermal *cis*-to-*trans* relaxation kinetics of 4-PAM in its unbound (A) and conjugated state on HDAH-wt (B), S20C (C) and M30C (D). Samples were irradiated by UV light and thermal *cis*-to-*trans* relaxation of 4-PAM recorded up to 70 h. Green solid lines represent fit curves to a one- and yellow fit curves to a two-exponential model (A-D). Corresponding residuals for the one- and two-exponential function were listed consecutively beneath.

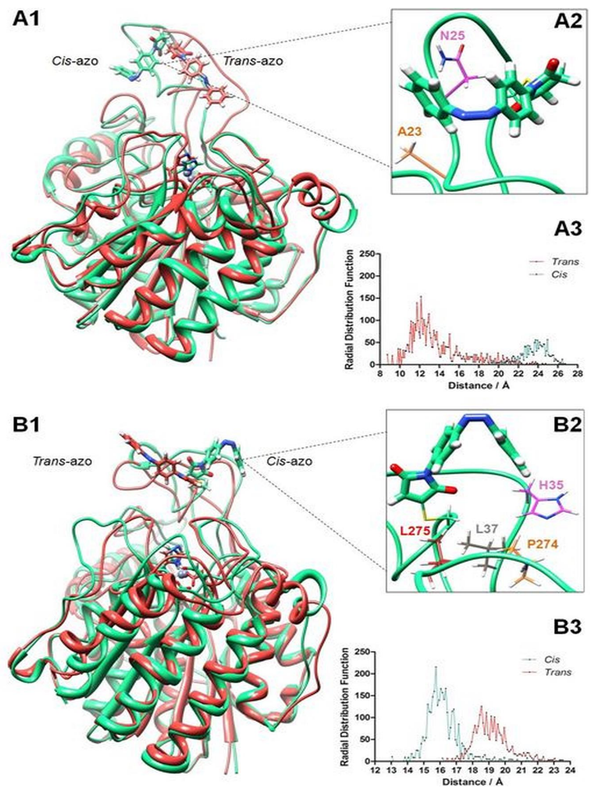

Fig. 8. Snapshots of MD-simulations of 4-PAM/HDAH M30C (A1) and S20C (B1) conjugates in *cis*- (green ribbon, *Cis*-azo) and *trans*-state (red ribbon, *Trans*-azo) after 19 ns. A2 (4-PAM/M30C) and B2 (4-PAM/S20C) depict interactions between the attached azobenzene moiety in its *cis*-state and adjacent amino acids. A3 (4-PAM/M30C) and B3 (4-PAM/S20C) show the distribution of mean distances between the aromatic *para*-carbon atom of the phenyl group of the azobenzene moiety and the zinc ion (Zn^{2+}; dark gray sphere in the middle marks the active site).

Conclusion

The enzyme activity of HDAH can be reversibly controlled multiple times by 4-PAM up to 40 %. A complete inactivation (OFF) and activation (ON) of the HDAH activity could not be shown. The thermal *cis*-to-*trans* relaxation of 4-PAM covalently attached to HDAH variants S20C and M30C was highly retarded and allows single light pulse switching and therefore maintaining the thermodynamically less stable *cis*-state several hours. The stabilization of the attached *cis*-azobenzene moiety by adjacent amino acids will be investigated further by site-directed mutagenesis and NMR experiments.

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