Applications of DBD dyes

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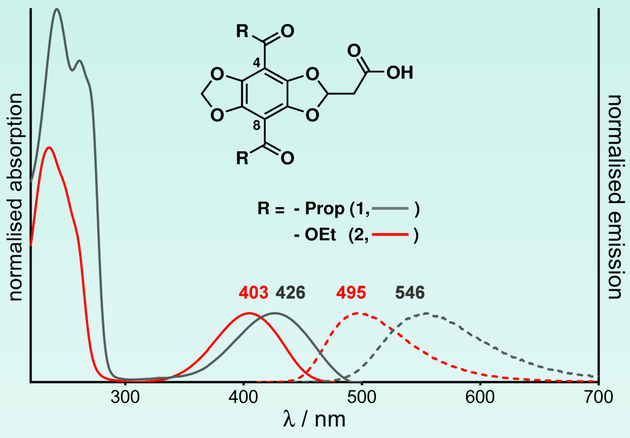
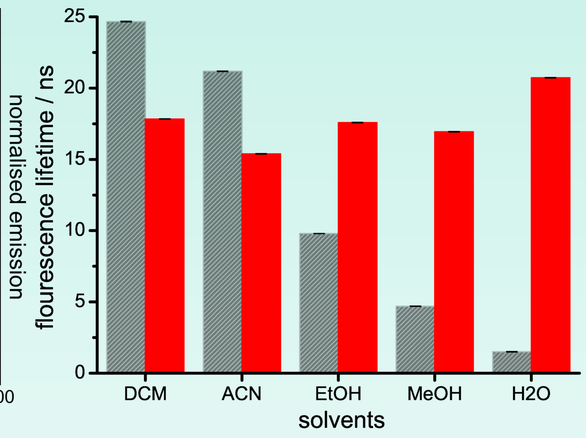
Introduction

Fluorescence dyes based on [1,3]dioxolo[4,5-*f*][1,3]benzodioxole (DBD) with electron withdrawing groups in 4 and 8 position show remarkable *large* STOKES *shifts* combined with *long fluorescence lifetimes* in appropriate solvents.[1, 2] In particular DBD dyes of the first generation called acyl-DBD dyes (**1**) are *sensitive to the polarity* of their microenvironment.[3] Subsequently the motivation was to synthesize specialized DBD derivates for bioanalytical applications.The outcome of this are various applications which we want to introduce now.



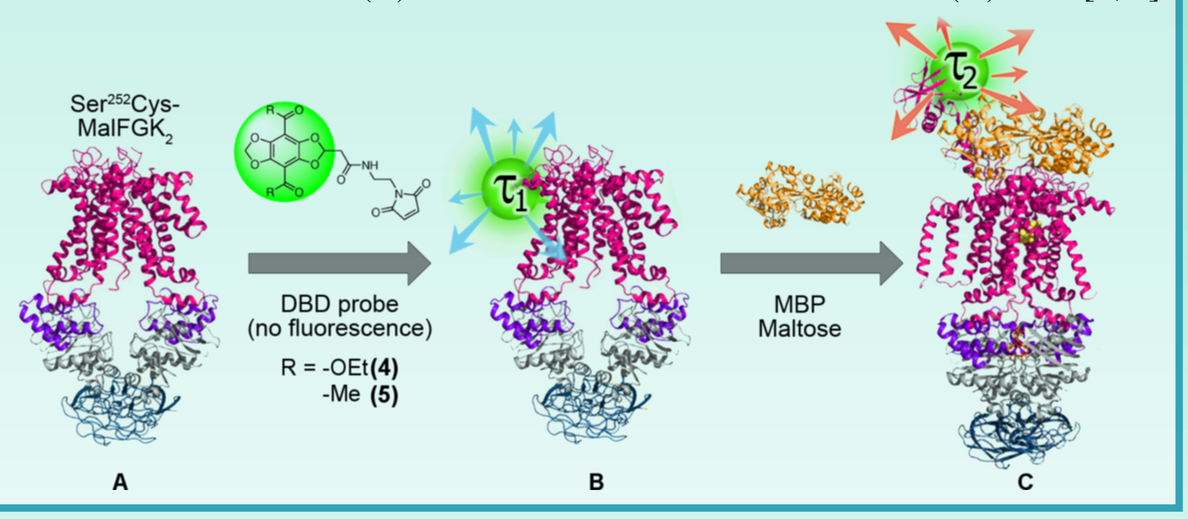
UV-Vis-absorption and fluorescence emission spectra of **1** and **2** in ACN.



Measured fluorescence lifetime of **1** and **2** in different solvents.

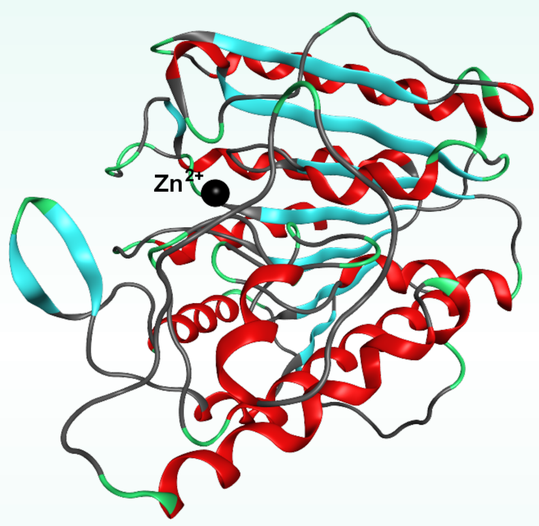
Conformational Study

Similar to other fluorophores, DBD dyes are quenched by maleimides so that the DBD dyes **4** and **5** show no fluorescence. Binding to a pre-introduced thiol group of a protein results in an increased fluorescence intensity and decay time (τ_1). Because the decay time of DBD dyes (especially of acyl-DBD dyes) depends extremely on the polarity of the microenvironment a conformational change of a protein caused by a cofactor can be measured by τ_2. Exemplarily this could be used to study the maltose ATP-binding cassette (ABC) transporter during a transition from a cofactor-less (**B**) to an intermediate ATP/ADP-bound (**C**) state.[4, 5]



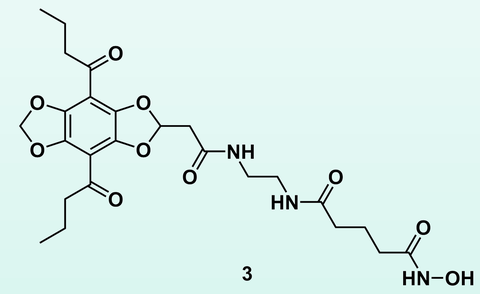
FLT-based binding assay for APAHs

Acetylpolyamine amidohydrolases (APAHs) of *Pseudomonas aeruginosa* containing the deacetylase binding domain with a Zn^{2+} ion are members of the histone deacetylase family (HDACs) which plays an important role in pharmacology. For this reason the development of an efficient fluorescence lifetime (FLT)-based binding assay is exemplarily described on the basis of APAHs.



Due to their extraordinary photophysical properties DBD dyes fulfil the conditions (large STOKES shifts, long FLTs) for a robust FLT-based assay for APAHs. For this purpose a novel ligand probe **3** was successfully synthesized.

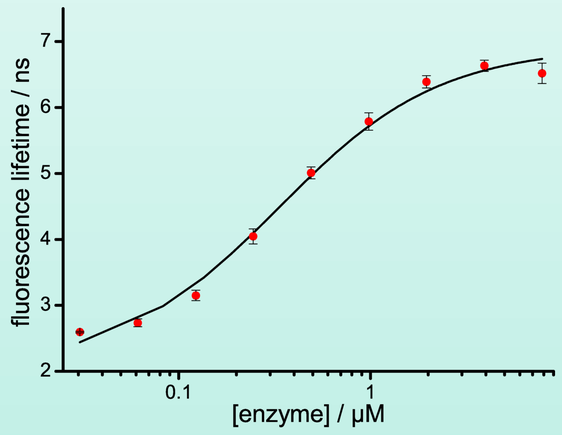
To determine the binding constants for inhibitors first the binding of three APAHs (PA0321, PA1409 and PA3774) to the DBD ligand **3** were analyzed by titration. Afterwards the binding constants can be calculated from the measured FLT. The results are summarized in the following table. Analogously the binding constants of inhibitors were obtained from displacement titrations. To validate this, reference experiments with enzyme activity assays were executed. The results are published by MEYER-ALMES *et al.*.[6]



3

	PA0321	PA1409	PA3774
ΔFLT/ns	4.9± 0.1	7.19 ± 0.06	5.29 ± 0.08
$K_d/\mu M$	0.3 ± 0.3	1.31 ± 0.05	0.19 ± 0.01

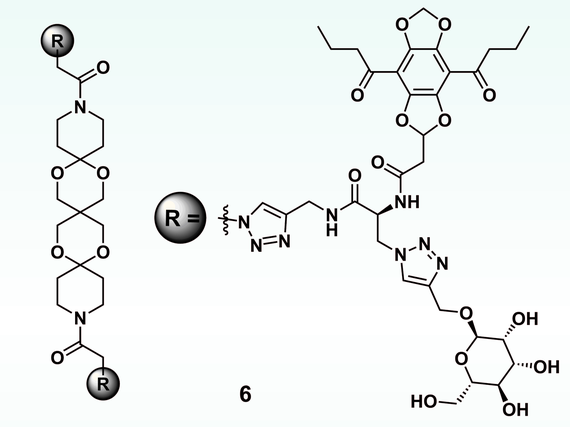
Changes in FLT and binding constants K_d of **3** to the respective APAH.



Titration curve of 50 mM **3** with the enzyme PA0321.

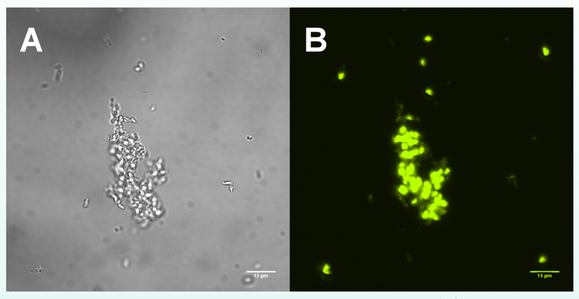
Biosensor for mannose-receptors

Another interesting application of DBD dyes was developed by the conjugation of the acyl DBD **1** with a bivalent molecular rod and mannose to form a biosensor **6** with appropriate binding to mannose-receptors. To test the system *E. coli* bacteria were incubated with different concentrations of **6**. Afterwards the solutions were analyzed by fluorescence microscopy.



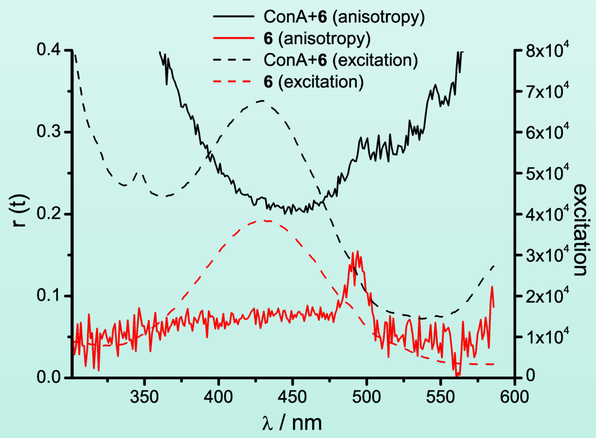
6

The figure shows a successful binding of **6** to the bacteria via the mannose group. This is validated by the dependency of the fluorescence emission of **6** on the polarity of the microenvironment. An outstanding fact is the agglutination of the bacteria which was observed for the bivalent biosensor **6**.

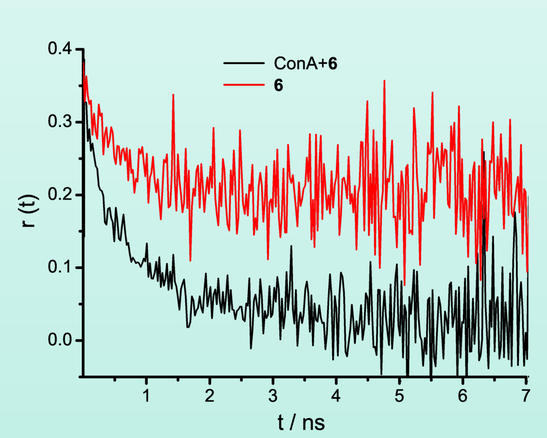


Microscopy of *E. coli* bacteria with 10^{-13} M **6** (**A**: bright-field , **B**: fluorescence intensity).

This effect can be explained by the simultaneous binding of two bacteria to one molecule of **6** and was additonally analyzed by fluorescence anisotropy. It can be observed that the steady state anisotropy of **6** increases after the addition of the protein ConA caused by the expected binding between them while the correlated rotational lifetime remains constant.



Steady state fluorescence anisotropy of **6** with and without protein ConA.



Time dependent fluorescence anisotropy of **6** with and without protein ConA.

References

[1] *Patent-No.* EP2399913A1, P. Wessig, K. Möllnitz, R. Wawrzinek, **2011**.

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Conclusion

To summarize the compounds **1** and **2** of the DBD dye class with extraordinary photophysical properties could be modified to the derivates **3-6** to afford various bioanalytical applications. While the hydroxamic acid **3** functions as a ligand probe for a new type of a binding assay for enzymes of the HDAC family, the maleimides **4** and **5** could be used to analyze the conformational change of the ABC-transporter during a transition from the cofactor-less to an intermediate ADP/ATP-bound state.[4, 5, 6] Furthermore a bivalent biosensor **6** could be synthesized succesfully. On the one hand low concentrated solutions of **6** stains selectively *E. coli* bacteria. On the other hand the bivalent structure causes an agglutination of bacteria.