Dansyl-Ligands for FRET-based Histone Deacetylase Assays

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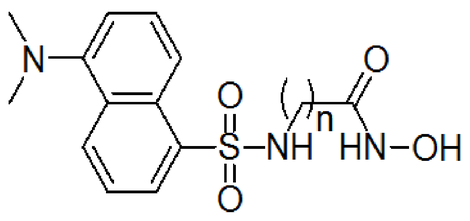
Introduction

Histone deacetylases (HDACs) and their homologues have emerged as proven protein targets predominantly for cancer. In addition, there is growing evidence that HDACs homologues are also involved in such different therapeutic areas like neurodegeneration and cardiac as well as parasitic and viral diseases. Kinetic parameters of compound binding to a pharmaceutical target protein are indispensable information for the optimization of lead structure.

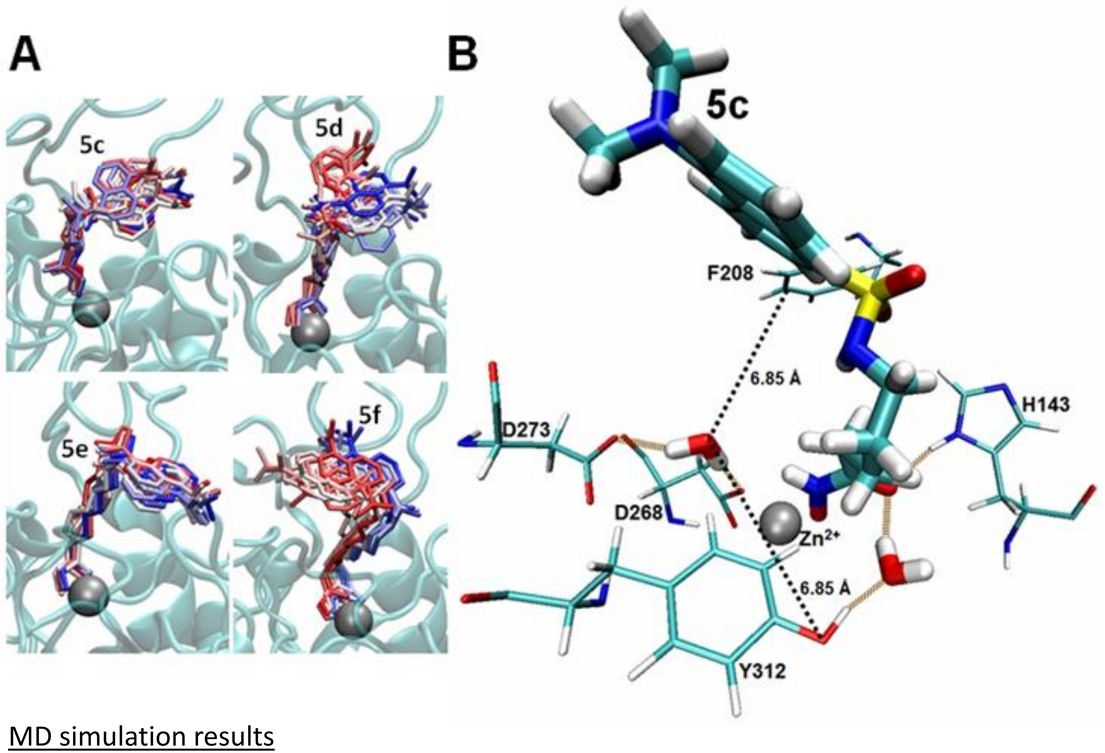
In this study a kinetic competitive binding assay for HDAH, a bacterial HDAC-homolog, has been developed to determine on- and off-rates of HDAC inhibitors.

Results



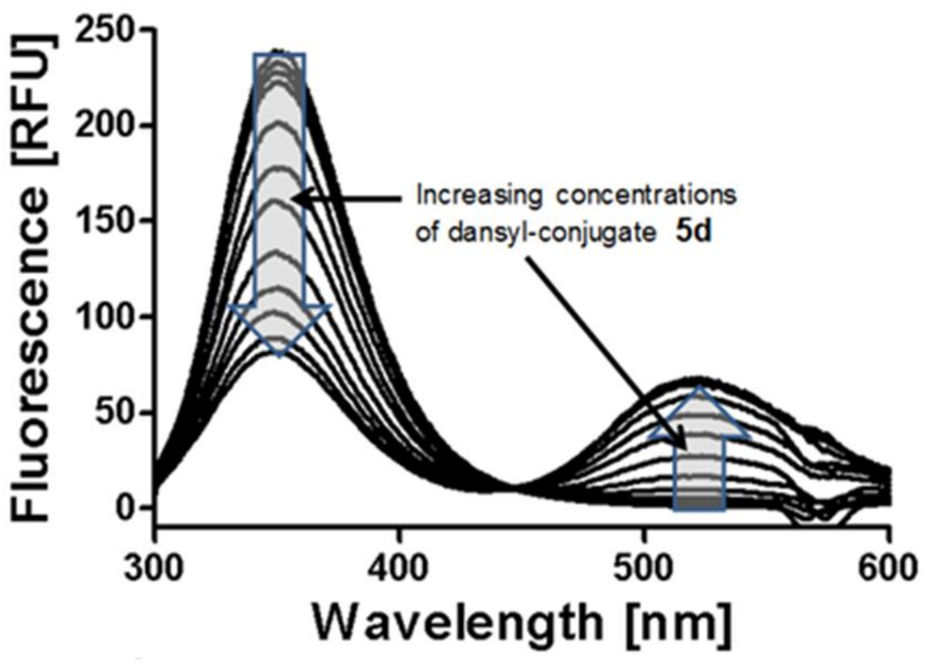
5a, n = 2
5b, n = 3
5c, n = 4
5d, n = 5
5e, n = 6
5f, n = 7

Synthesized Probes for HDAH enzyme.



MD simulation results

A) snapshots of dansyl-ligands **5a-f** in the active site of HDAH. B) Structural water molecules within the binding pocket of the complex between **5c** and HDAH. Yellow lines between water molecules and protein or ligand represent hydrogen bonds.

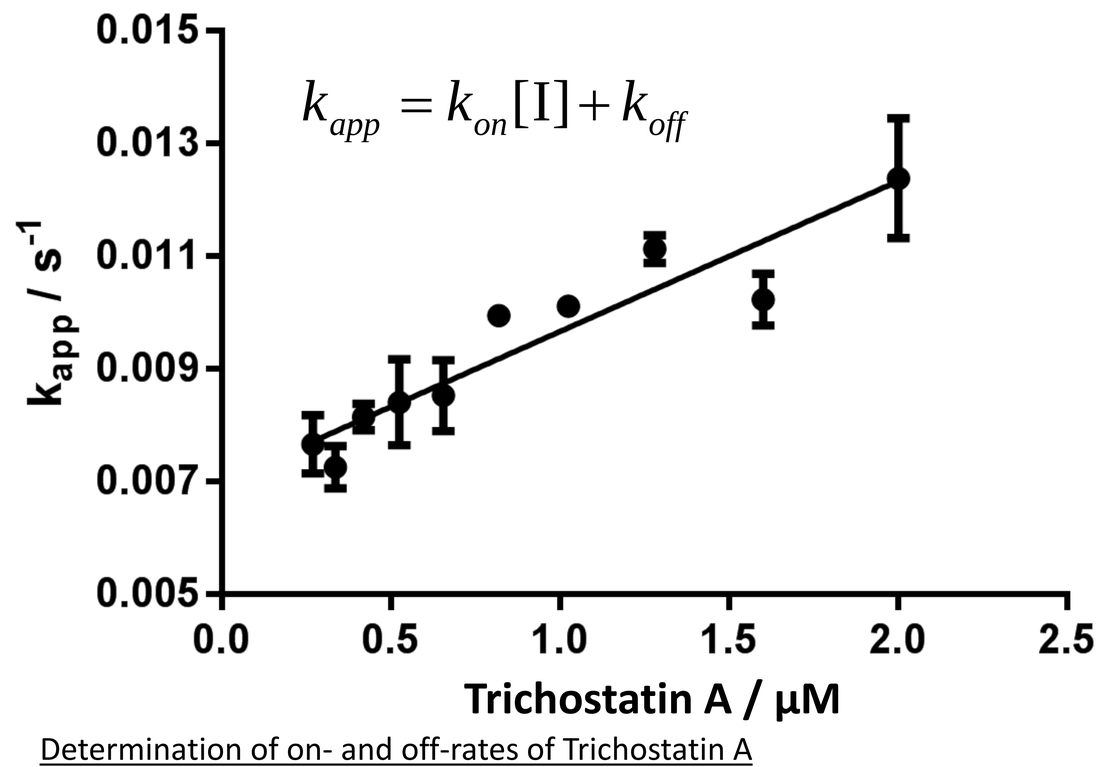


FRET-Signal upon addition of Dansyl-probes to HDAH

100 nM HDAH was titrated with increasing concentrations of **5d** ranging from 12.5 nM to 12.8 µM (11 concentrations) at 25^{0}C.

Diplacement kinetics of Trichostatin A

0.25-2 µM Trichostatin A were added to a preformed and equilibrated mixture of 50 nM HDAH and 5 µM **5c**. The data were fit using a one-exponental decay function yielding k_{app}. The off-rate of **5c** was previously determined to be much faster than the binding reaction betweenTrichostatin A and HDAH.



Determination of on- and off-rates of Trichostatin A

The on-rate of the binding of Trichostatin A corresponds to the slope and was calculated to be (2.7 ± 0.3) x 10^3 $M^{-1}s^{-1}$ and the off-rate corresponds to the y-axis intercept yielding (7.0 ± 0.3) x 10^{-3} s^{-1}. Assuming a simple binding equilibrium between Trichostatin A and HDAH, the binding constant can be calculated as ratio of the rate constants to be 2.6 µM.

Conclusions

We have synthesized dansyl-modified hydroxamic acid ligands with different linker length and measured the resulting fluorescence resonance energy transfer signal from intrinsic tryptophans to the dansyl-moiety of ligands bound to HDAC similar amidohydrolyse (HDAH) from *Alcaligenes*, a close homologue to human HDAC6, as model protein. The dansyl-ligands were displaceable by the common HDAC inhibitor SAHA demonstrating the potential for a homogeneous binding assay to screen larger collections of compounds. The developed assay principle also allowed the measurement of the association and dissociation kinetics of dansyl-ligand binding to HDAH in the sub-second timeframe. The kinetic parameters are invaluable supplementary decision criteria for the optimization of drug candidates.