

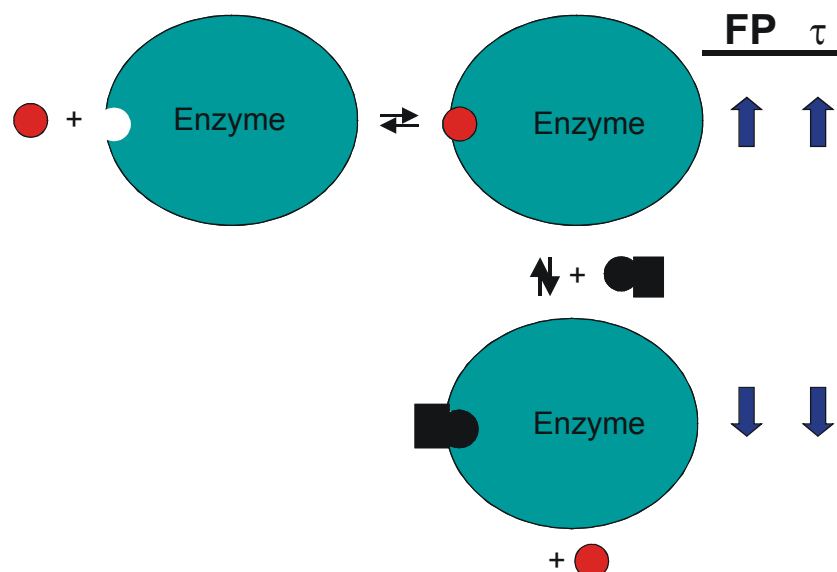
Application Note 12

Histone deacetylase Activity Assay Using FLT and Nano-FP read-out

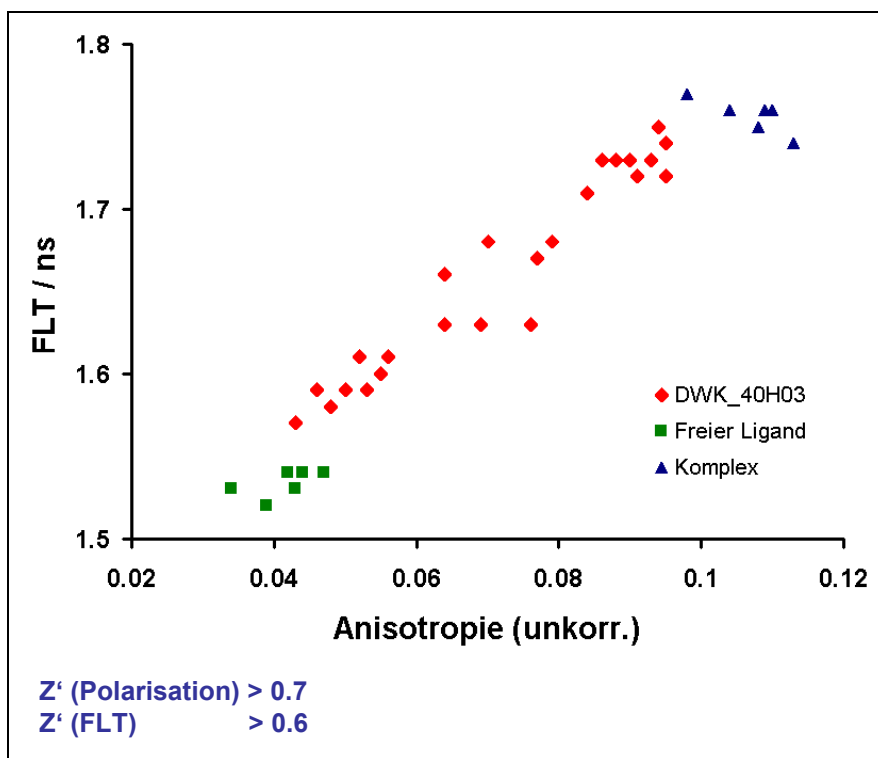
date: 22/04/2008

Assay description:

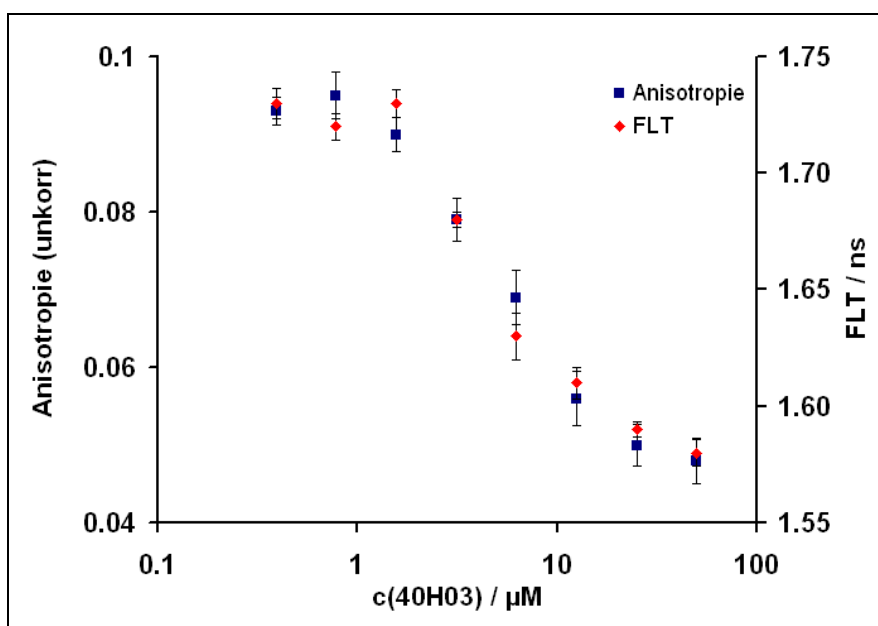
- the assay is intended to monitor the enzymatic activity of zinc dependent Histone deacetylases in order to identify potential inhibitors of HDAC's
- the assay was developed by Prof Meyer-Almes, Hochschule Darmstadt – University of Applied Sciences
- the generic assay method uses the simultaneous read-out of fluorescence lifetime (FLT) and nanosecond time-resolved fluorescence polarization (Nano-FP)
- the assay principle is based on the displacement of a fluorescent tracer molecule from the active site of the enzyme
- upon binding of the tracer molecule to the enzyme both, the fluorescence lifetime and the fluorescence polarization is increased
- the dual read-out improves data statistics significantly and reduces the number of false positive hits



Correlation of FLT and Nano-TRF:



Dose-response curve of an inhibitor:



Instrument settings for Fluorescence lifetime measurements:

filter settings:

filter cube:	excitation:	640/20
	beam splitter:	660 DCLP
	emission:	680/30
laser frequency:	30 Hz	
laser energy:	10 %	
gain Channel 1:	reduced gain	
measurement window for FLT:	50 ns	
integration window for Nano-FP	4 – 20 ns	

Summary:

- **library screening for unknown enzyme inhibitors is under way**
- **only one assay component has to be fluorescently labelled**
- **the dual assay read-out reduces the number of false positives, thus improving data statistics**
- **work is under way to demonstrate the transfer of the generic assay principle to other enzymatic assays**

Acknowledgement:

We wish to thank Prof. Meyer-Almes, Hochschule Darmstadt – University of Applied Sciences, for his valuable contributions to improvements of fluorescence lifetime assay technology.