Evaluating fluorescence lifetime technology for identification and characterisation of DUB inhibitors

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MISSION Therapeutics
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2nd - 3rd September 2015

MISSION Therapeutics

- Company started in July 2011
- Major funding of £26m
- Financed to develop first-in-class inhibitors of deubiquitylating enzymes (DUBs)
- Core biology in DNA damage response (DDR) and oncology
- Focused on DUB drug discovery platform

Moneta,
Babraham Research Campus,
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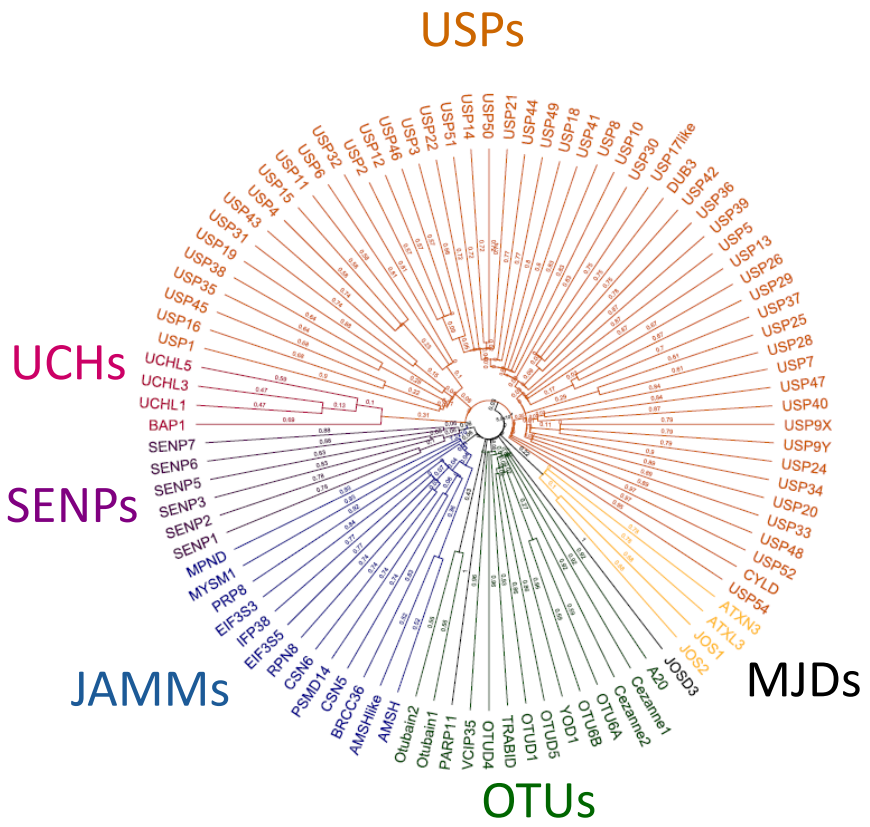
The DUB family of enzymes

Attractive targets for drug discovery

- DUBs control every aspect of ubiquitin biology
- Intracellular peptidases: endo-peptidases
- Approx. 100 DUBs in human cells
- Majority are cysteine peptidases
- Cysteine protease catalytic site:
  - Common catalytic mechanism and similar overall architectures but high degree of divergence; allowing development of drug selectivity
  - Several known cysteine enzyme classes such as cathepsins and caspases where small molecule inhibitors have been developed



DUBs remain an under-exploited target class for drug discovery

Benefits of fluorescence lifetime

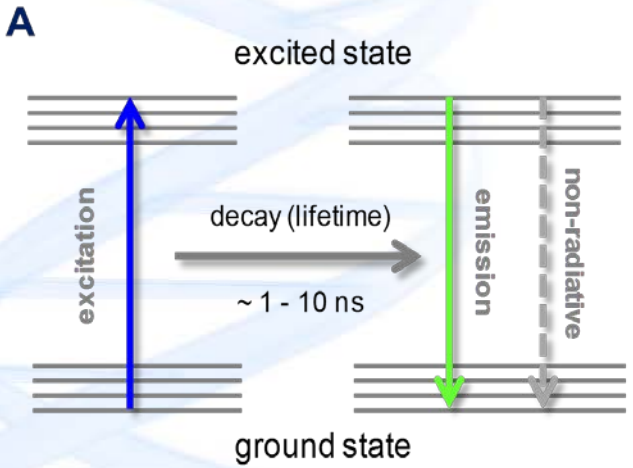
TECHNOLOGY / LIMITATION	FI	FP	TRF	TR-FRET	FRET	FLT	FCS+ plus	Alpha Screen	SPA	Lead Seeker	ECL	Bio LUMI
Inner filter effect	●			●	●		●		●	●	●	●
Quenching	●	●		●	●	●	●	●			●	●
Autofluroscence	●	●			●		●					
Bk. phosphorescence			●	●						●	●	●
Light scattering	●						●		●			●
Photo bleaching	●						●					
Volume/meniscus	●	●	●					●	●	●		●
Light sensitivity								●				
Molecular size		●					●					
Tracer conc.	●		●				●	●	●	●	●	●
Temp. stability	●						●	●				●
Robustness score	8	4	3	3	3	1	8	5	4	4	4	7

Comley J, *Drug Discovery World*, 2003, 91-98

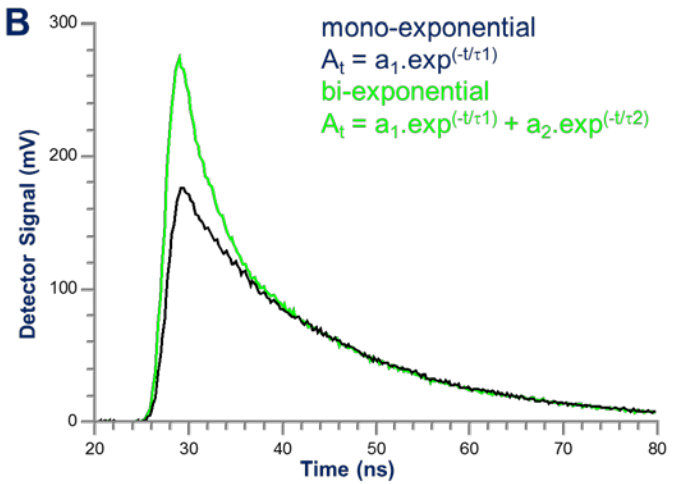
'FLT is in principle the most robust assay format'

What is fluorescence lifetime?

- Fluorescence lifetime: average time a fluorophore stays in the excited state before emitting a photon and returning to ground state



The relationship between excitation and emission to lifetime for a fluorophore



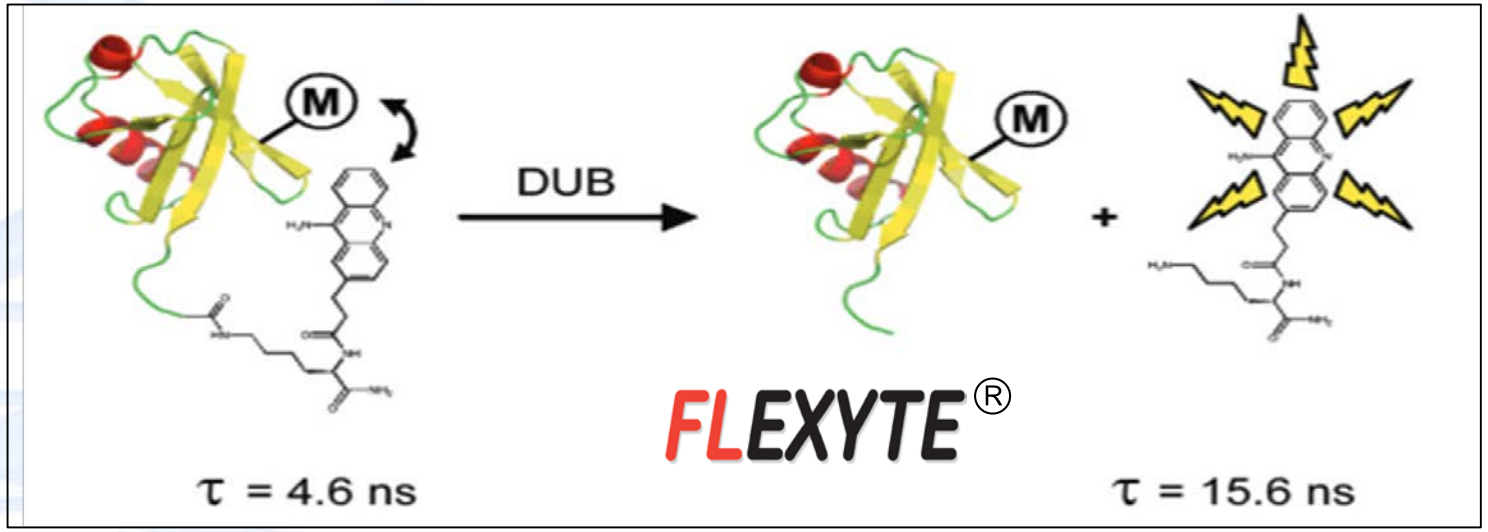
Typical fluorescence decay curves following a short excitation pulse. For single fluorophores, fitting to a mono-exponential equation yields the FLT value (τ)

Ameon reader from TTP Labtech

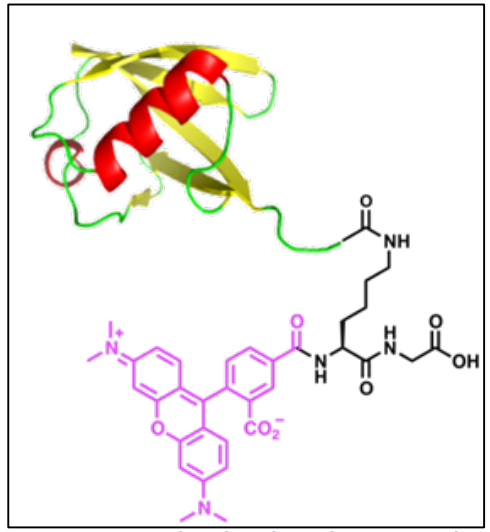
G. Cotton, W. Bowen, R Grundy *et al*. Fluorescence Lifetime Assays, Drug Discovery World, Fall 2013

FLEXYTE® DUB assay principle

- The FLT FLEXYTE® DUB assay technology utilises a full-length isopeptide-linked ubiquitin substrate labelled with Almac's 9-aminoacridine (9AA) long lifetime fluorophore and incorporating a FLT modulator which reduces the FLT of 9AA in the substrate. Cleavage of scissile isopeptide bond by the DUB is reported through an increase in FLT



FLEXYTE® DUB assay principle



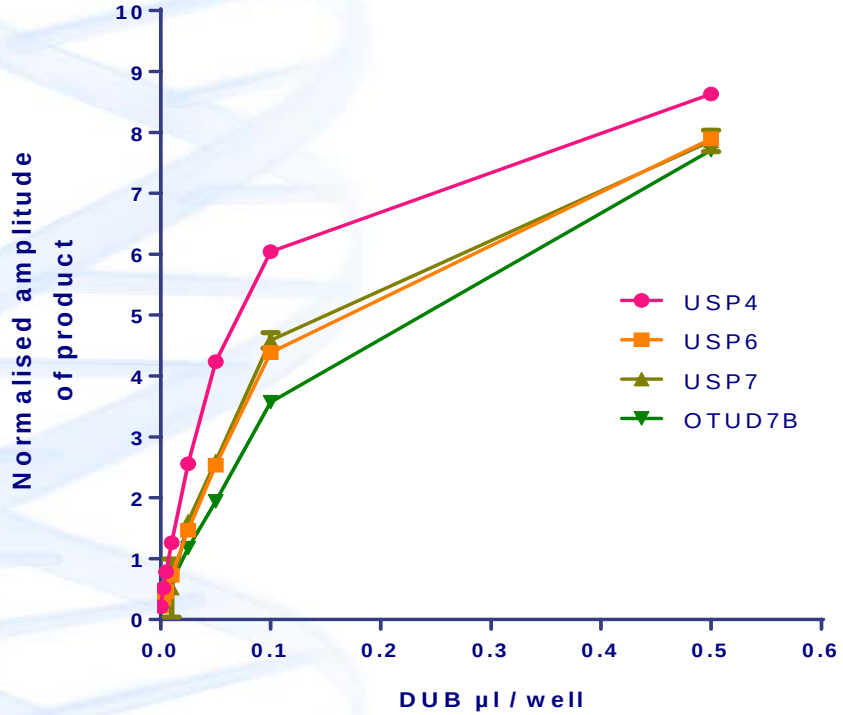
Isopeptide linked FP substrate

- The FLEXYTE® assay technology was compared to *MISSION's* standard fluorescence polarisation (FP) screening assays

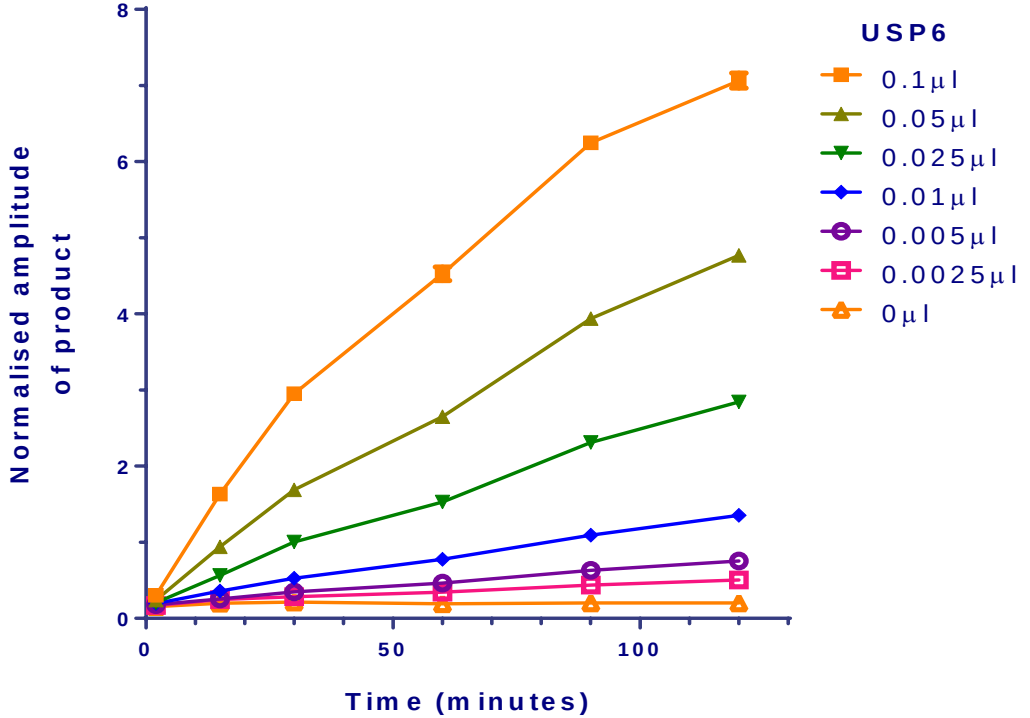
G. Cotton, W. Bowen, R Grundy *et al*. Fluorescence Lifetime Assays, Drug Discovery World, Fall 2013

Developing FLT assays for DUBs

- USP4, USP6, USP7 and OTUD7B were profiled in the FLT assay



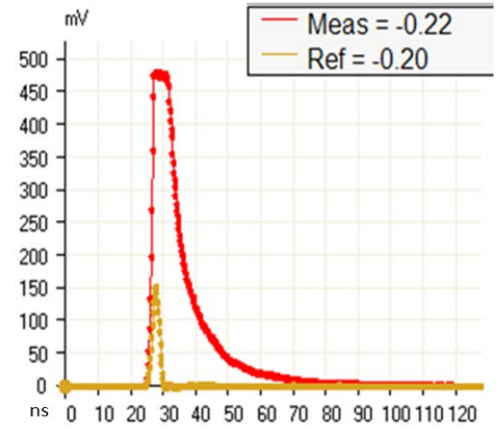
Comparison of DUB activities in FLT assays



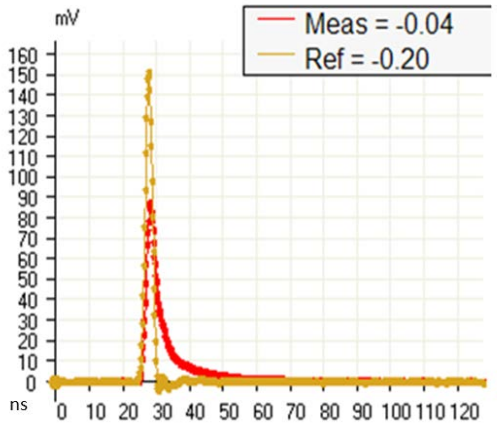
USP6 activity over time, measured by change in FLT

Detection of false positives

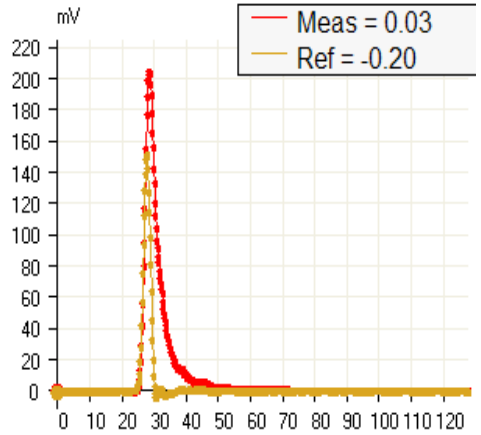
- Compounds that were quenchers, insoluble or fluorescent were easily identifiable in FLT assay



Profile of an insoluble compound



Profile of a quencher



Profile of a hit compound

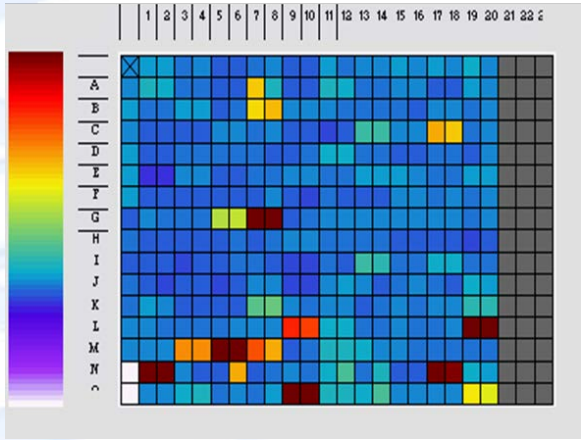
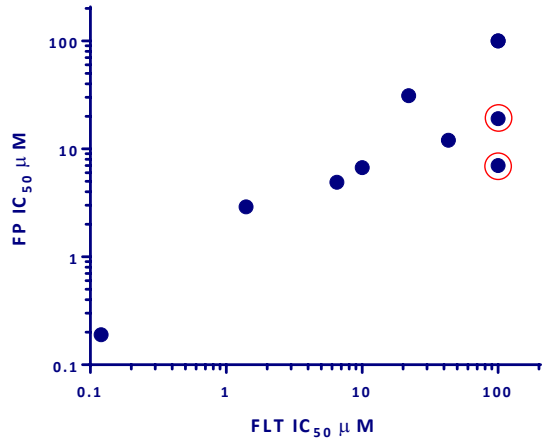


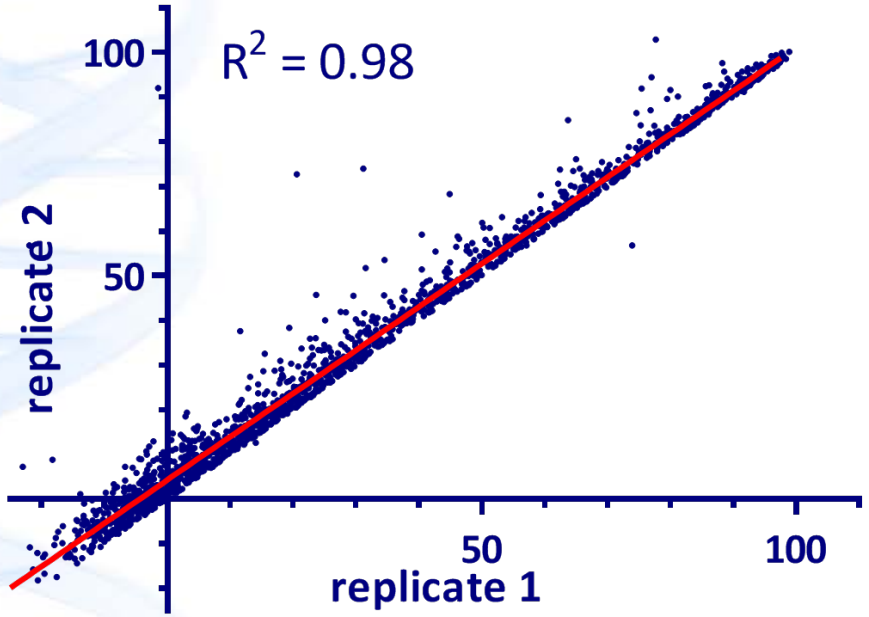
Plate map showing insoluble compounds in a SPS



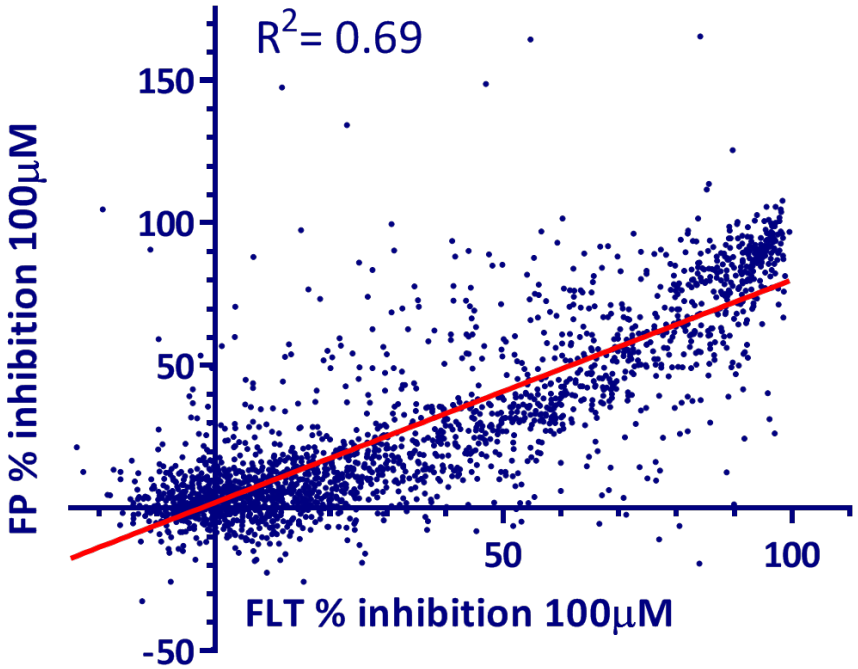
Correlation of 10 compounds profiled in USP6 assay

FLT single point library screen

- 2400 compounds were tested for % inhibition at 100µM in the USP7 FLT assay
  - 139 compounds were identified as insoluble; 4 were quenchers



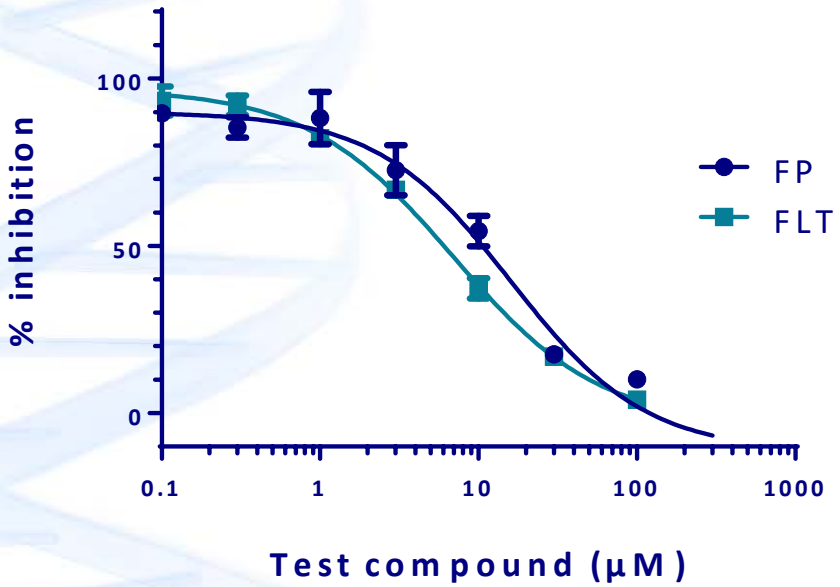
Correlation between technical replicates for FLT SPS



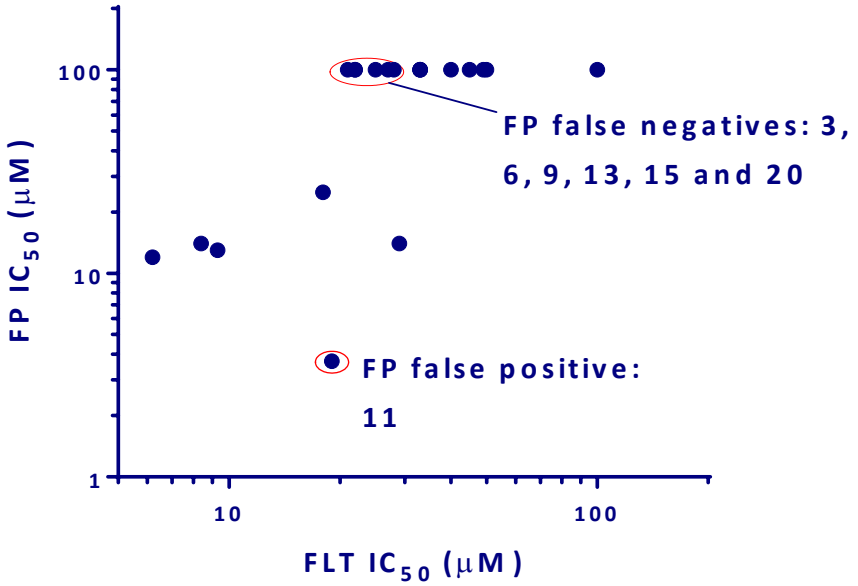
Correlation of USP7 FLT and FP SPS assays

Comparison of FLT and FP IC_{50}

- 20 compounds from the USP7 SPS FLT assay were retested to measure IC_{50} and compared to FP result



IC_{50} curves for a test compound in USP7 FLT and FP assays



Correlation of USP7 FLT and FP IC_{50} assays for 20 compounds

Conclusions

- FLT reader technology can be used with FLEXYTE® substrates to create DUB activity assays for high throughput screening
- The assay technology allows for detection of fluorescence, quenching and solubility related false positives in screening sets
- In a library screen FLT detected a similar number of hits when compared to a FP assay, but also flagged some false positives and false negatives
- FLT is an alternative technology to traditional fluorescence based assays with the advantage of up front removal of a number of common types of false positives and could help streamline drug discovery cascades

Acknowledgements

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