

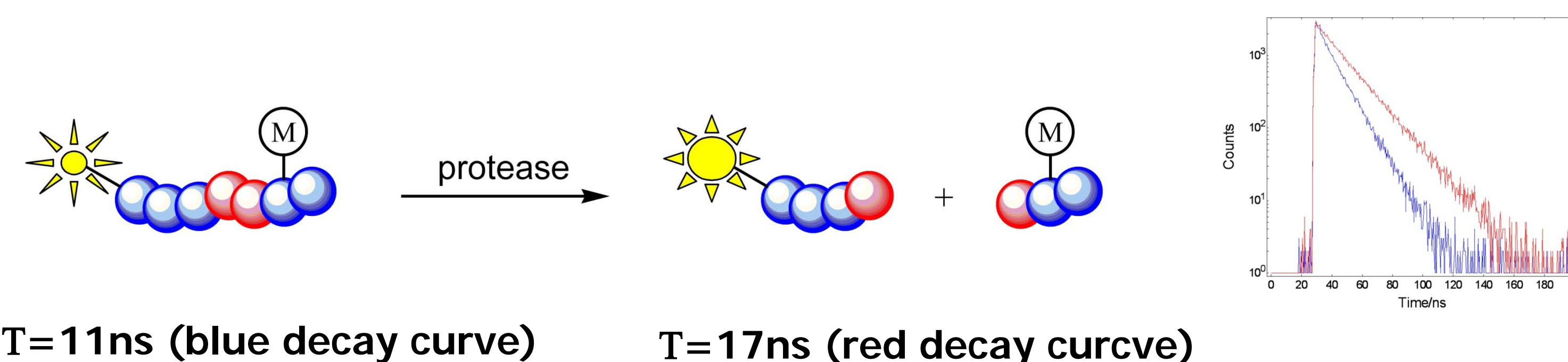
## Abstract

Fluorescence lifetime (FLT) has recently gained new attraction as a screening technology in drug discovery through the introduction of the high throughput screening (HTS) compatible NanoTaurus™ FLT plate reader (Edinburgh Instruments) and significant improvements in substrate and assay design using the new fluorophore 9AA as a long lifetime fluorophore reporter (Almac FLEXYTE® assays). By its nature, FLT has many benefits over other screening technologies; the most important of these are markedly reduced assay interferences. Here we describe three novel FLT assays used at BioFocus. Assay conditions were optimized and activities of reference compounds were determined and compared to results obtained in orthogonal assay technologies (Fluorescence intensity for metalloprotease and Caliper LC3000 mobility shift assay (MSA) for p38alpha).

Fragment-based drug discovery (FBDD) is considered an increasingly important area of drug discovery combining structural biology, binding studies and functional assays which assess the activity of fragments against molecular targets. A subset (42 compounds) of the BioFocus fragment library was tested in the FLT and Caliper LC3000 MSA assays against p38α. A good correlation of inhibitory activities was observed when both functional assays were compared, which adds the FLT format as a valuable option for FBDD assays.

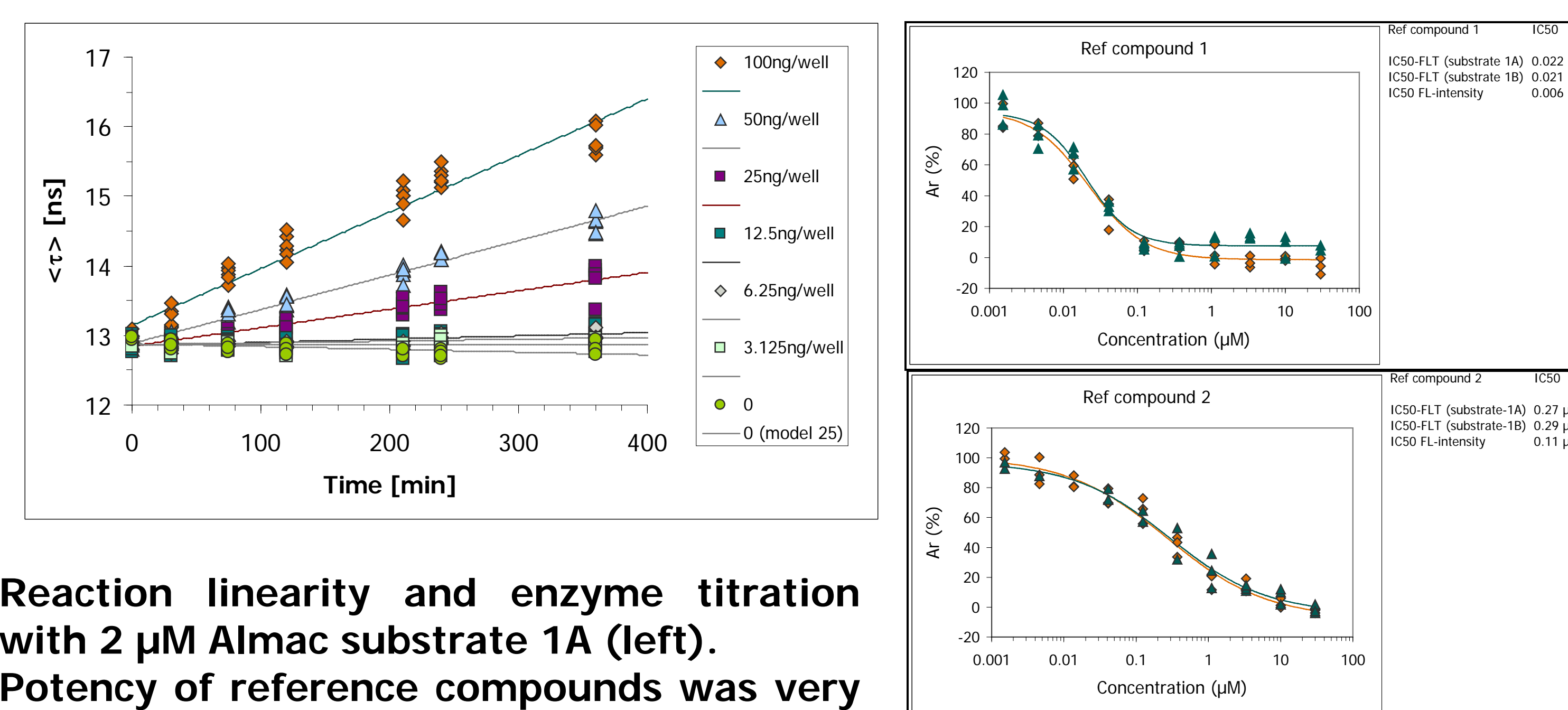
Together with the excellent precision obtained using the combination of the NanoTaurus FLT reader and the Almac FLEXYTE assay technology these results render FLT a highly attractive assay technology for fragment-based drug discovery.

## Almac FLEXYTE protease assay technology

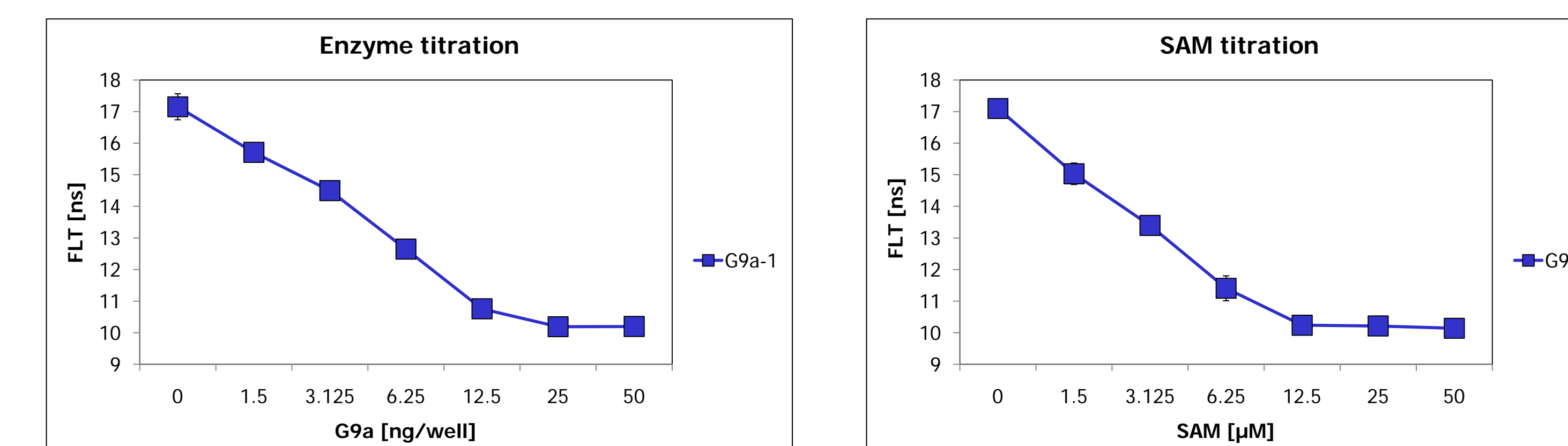


FLT assay principle for protease assays: FLT reducing modulator M is cleaved off which results in full FLT of the fluorophore 9-AA (courtesy: Almac)

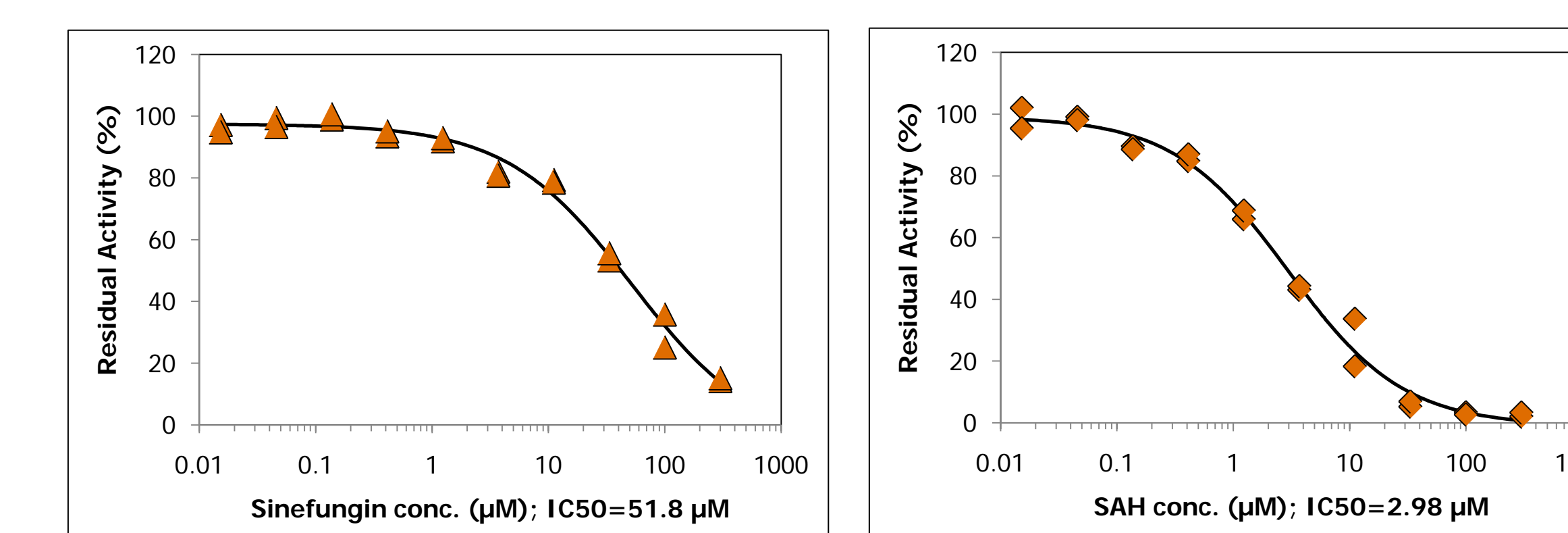
## Metalloprotease FLT assay development



## Almac FLEXYTE G9a HMT assay transfer

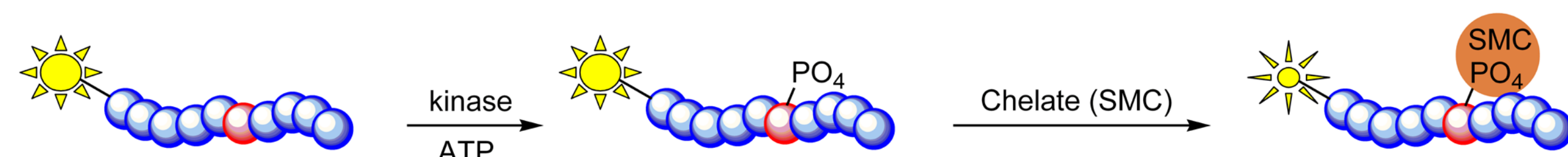


Assay transfer of FLEXYTE G9a HMT assay. G9a (BPS Bioscience) was titrated in the presence of 1 μM G9a-1 peptide substrate and 10 μM SAM (left). The reaction was measured after 90 min reaction time using the NanoTaurus FLT reader (Edinburgh instruments). SAM was titrated in the presence of 15 ng/well G9a and 1 μM G9a-1 peptide (right).

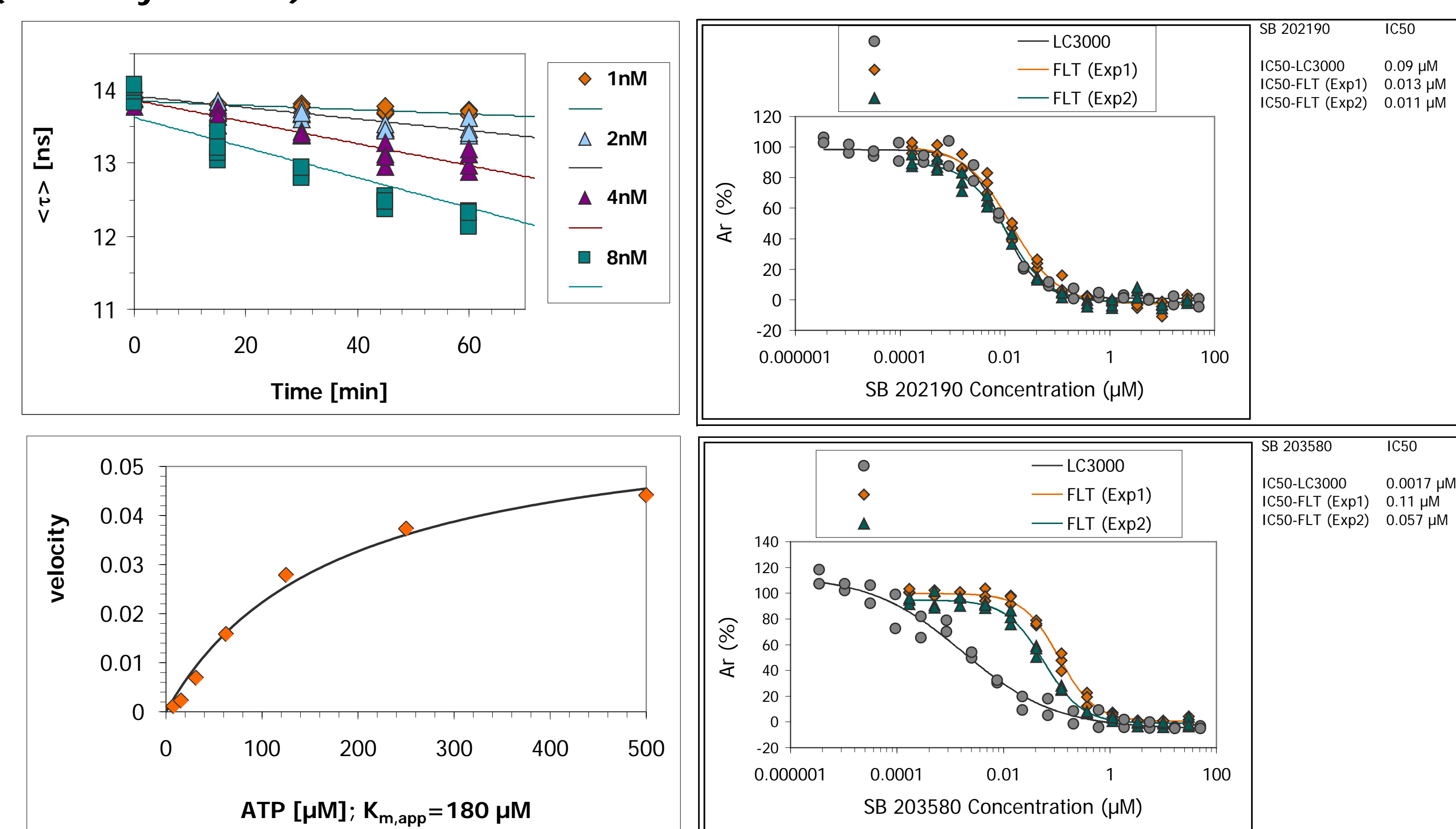


Assay transfer of FLEXYTE G9a HMT assay. IC50 determination for reference compounds Sinefungin and SAH. IC50 values of 52 μM for Sinefungin and 3 μM for SAH were determined. Final assay conditions were: 15 ng G9a per well (8 nM), 5 μM SAM, 1 μM substrate (Almac), 25 μl reaction volume, reaction time of 90 min.

## p38α FLEXYTE kinase assay development

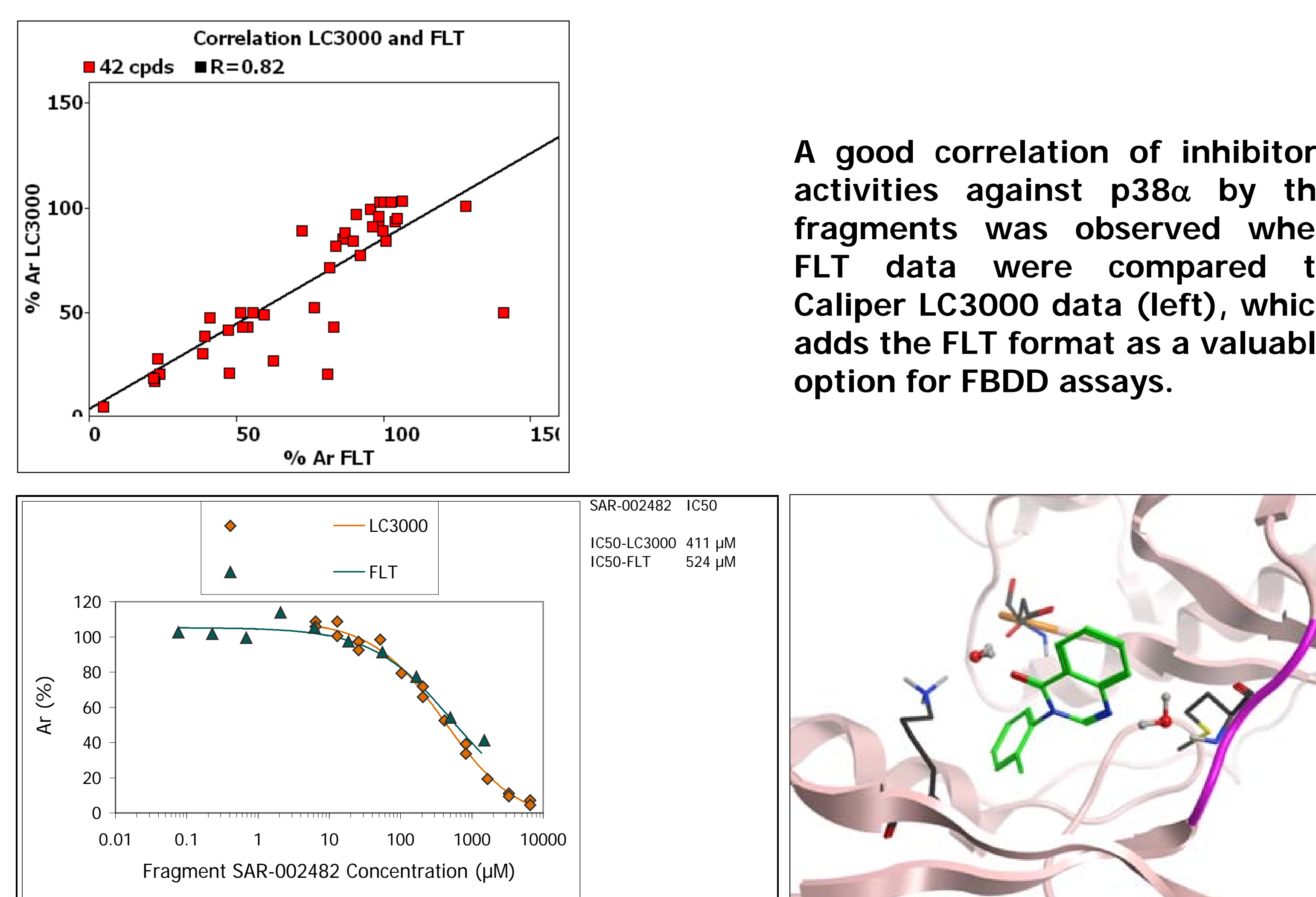


FLT assay principle for kinase assays: Small molecule chelate (SMC) complexes phosphate group and effects a reduction in fluorescence lifetime of up to 5 ns (courtesy: Almac)



For p38α kinase assay development the reaction linearity (upper left), optimal enzyme concentration, ATP Km (lower left), and potency of ATP-competitive inhibitors SB202190 and SB203580 (right) were assessed using a FLEXYTE peptide substrate developed for p38α (Almac).

## Testing of a subset of the BioFocus fragment library against p38α



Functional data in LC3000 and FLT (left) were supported by SPR binding studies and X-ray crystallography as shown for fragment-based SAR-002482 (right).

## Summary

- BioFocus has extended its panel of assay technology offerings by FLT which can be applied to a large spectrum of targets in high throughput screening mode.
- Due to reduced assay interferences in FLT the number of false negatives in screening campaigns can be reduced.
- FLT format is a valuable option for fragment-based drug discovery programs (FBDD).
- The excellent precision and data quality obtained using the combination of the NanoTaurus FLT reader and the Almac FLEXYTE assay technology renders FLT a highly attractive assay technology for drug discovery in particular target classes.
- Outlook: Fragment-based screening of epigenetics targets using FLT becomes now possible

Acknowledgements: For this study the NanoTaurus FLT reader was kindly provided and installed by Edinburgh Instruments. The Almac FLEXYTE assay technology was used throughout this study and reagents and technical support was provided by Almac. Special thanks to Geoff Irvine (Edinburgh Instruments) and Colin Dunsmore (Almac). Thanks to Céline Klein and Virginie Voegtlin for expert technical assistance.