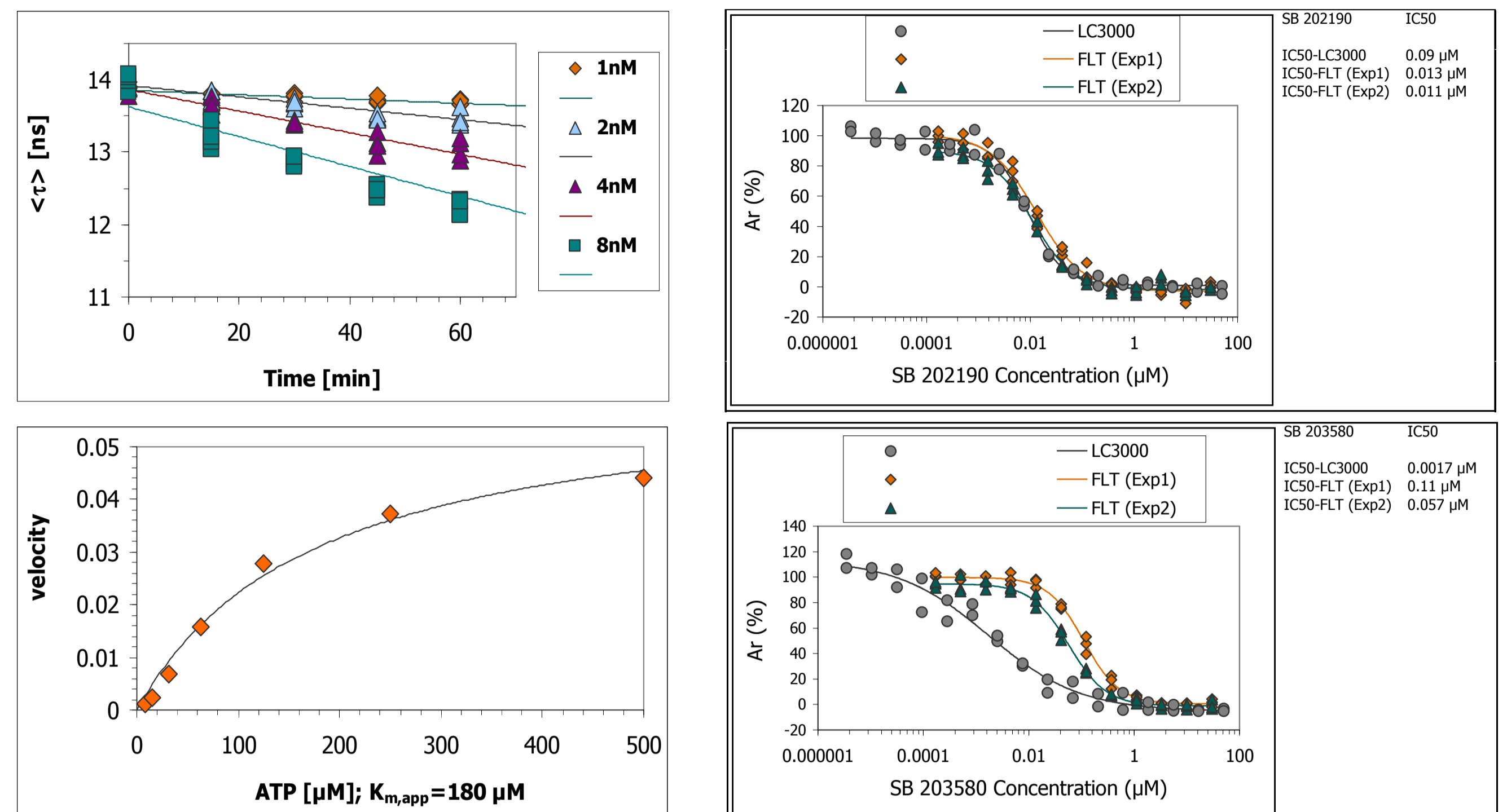


## Abstract

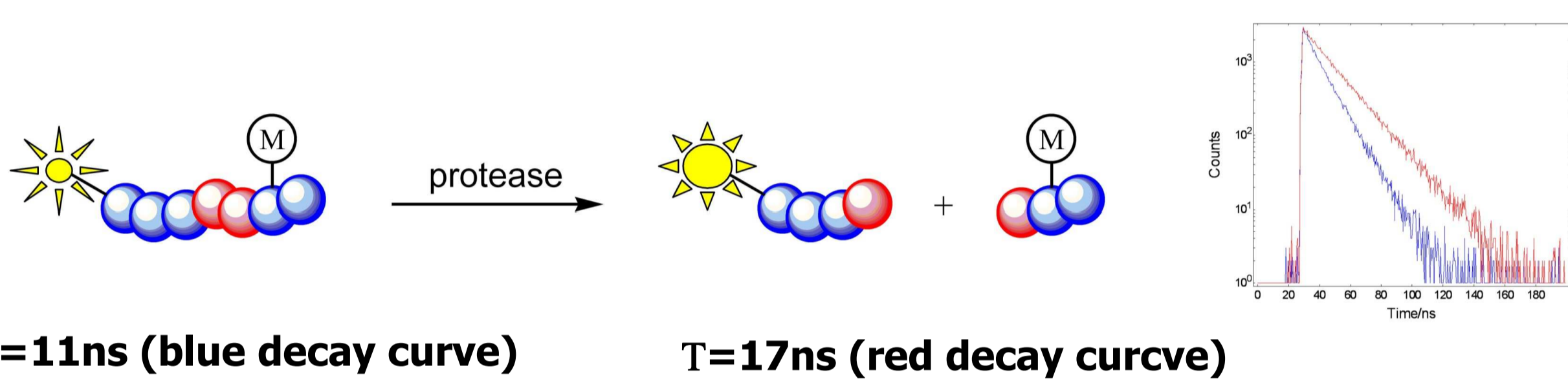
Fluorescence lifetime (FLT) has recently gained new attraction as a screening technology in drug discovery through the introduction of the high throughput screening (HTS) compatible NanoTaurus FLT plate reader (Edinburgh Instruments) and significant improvements in substrate and assay design using the new fluorophore 9-aminoacridine (9-AA) as a long lifetime fluorophore reporter (Almac FLEXYTE™). By its nature, FLT has many benefits over other screening technologies, the most important of these are markedly reduced assay interferences. In this case study at BioFocus FLT assays were developed for a metalloprotease and for p38 $\alpha$  kinase. Assay conditions for both FLT assays were optimized and validated by potency determination of reference compounds. Compound activities from the BioFocus diverse compound and fragment libraries were determined and compared to orthogonal assay technologies (fluorescence intensity (FI) for metalloprotease and Caliper LC3000 mobility shift assay (MSA) for p38 $\alpha$ ). Together with the excellent precision obtained using the combination of the NanoTaurus FLT reader and the Almac FLEXYTE™ assay technology the obtained results render FLT a highly attractive assay technology for drug discovery in particular target classes.

## p38 $\alpha$ FLT assay development



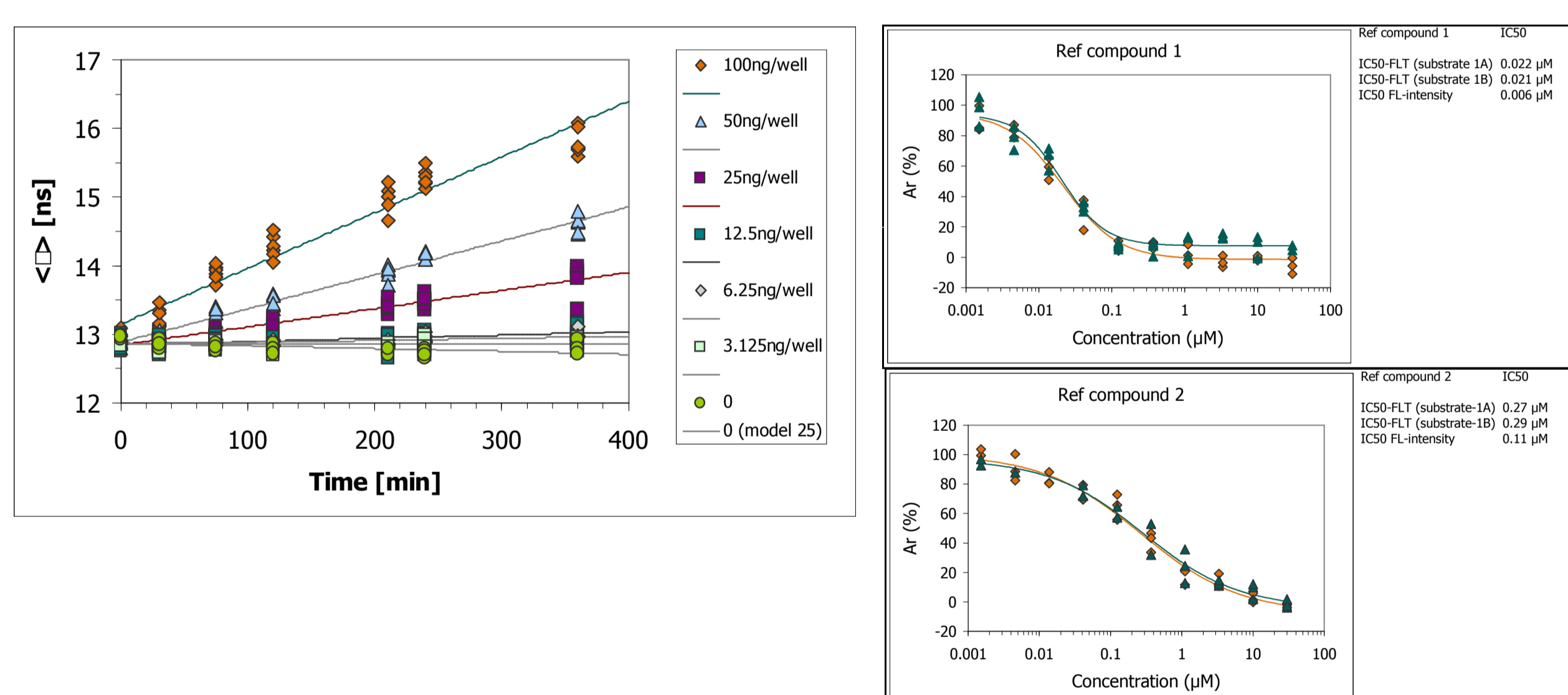
For p38 $\alpha$  kinase assay development the reaction linearity, optimal enzyme concentration, ATP  $K_m$ , and potency of ATP-competitive inhibitors SB202190 and SB203580 were assessed using a FLEXYTE™ peptide substrate developed for p38 $\alpha$  (Almac).

## Almac FLEXYTE™ 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)

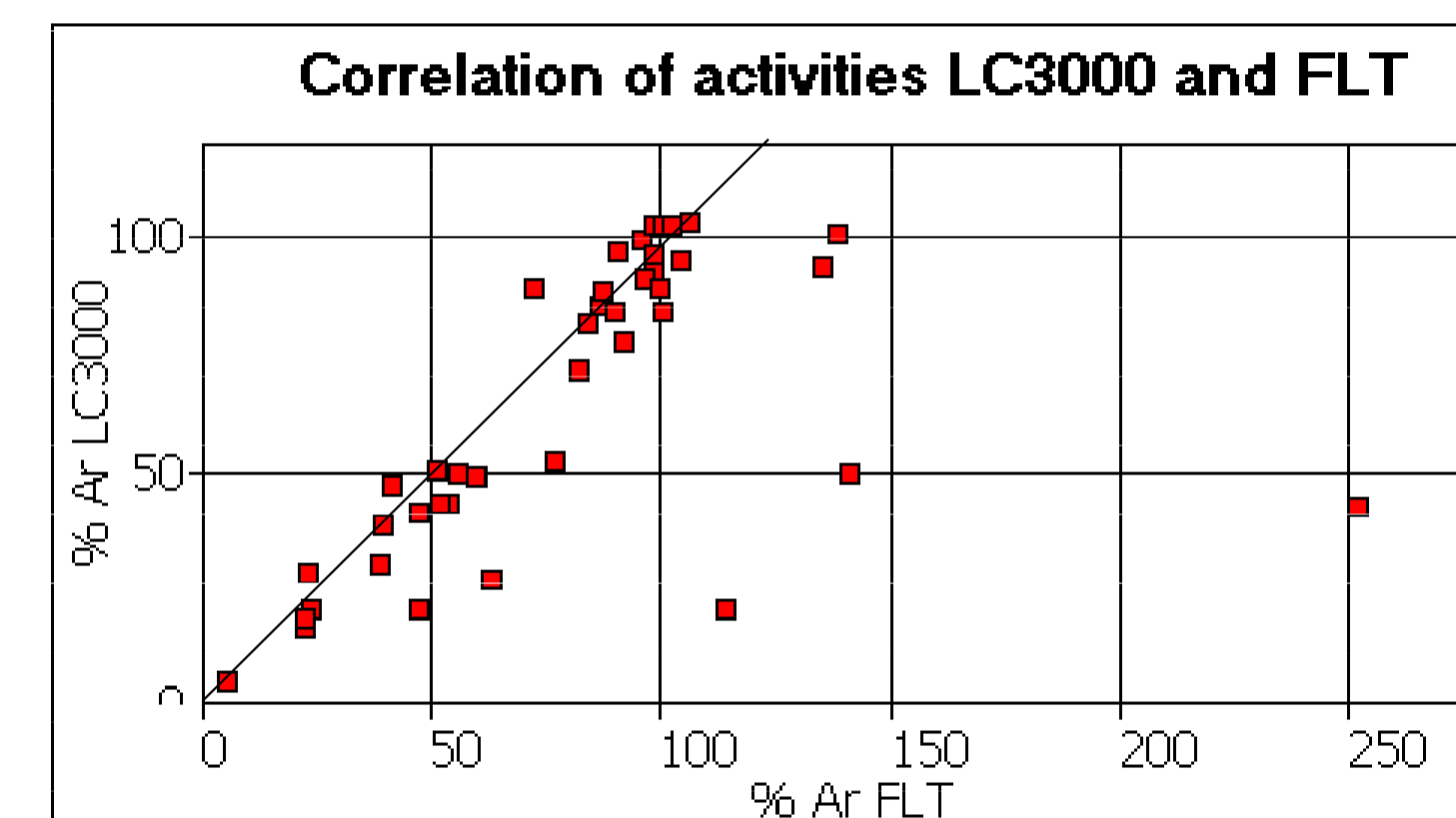
## Metalloprotease FLT assay development



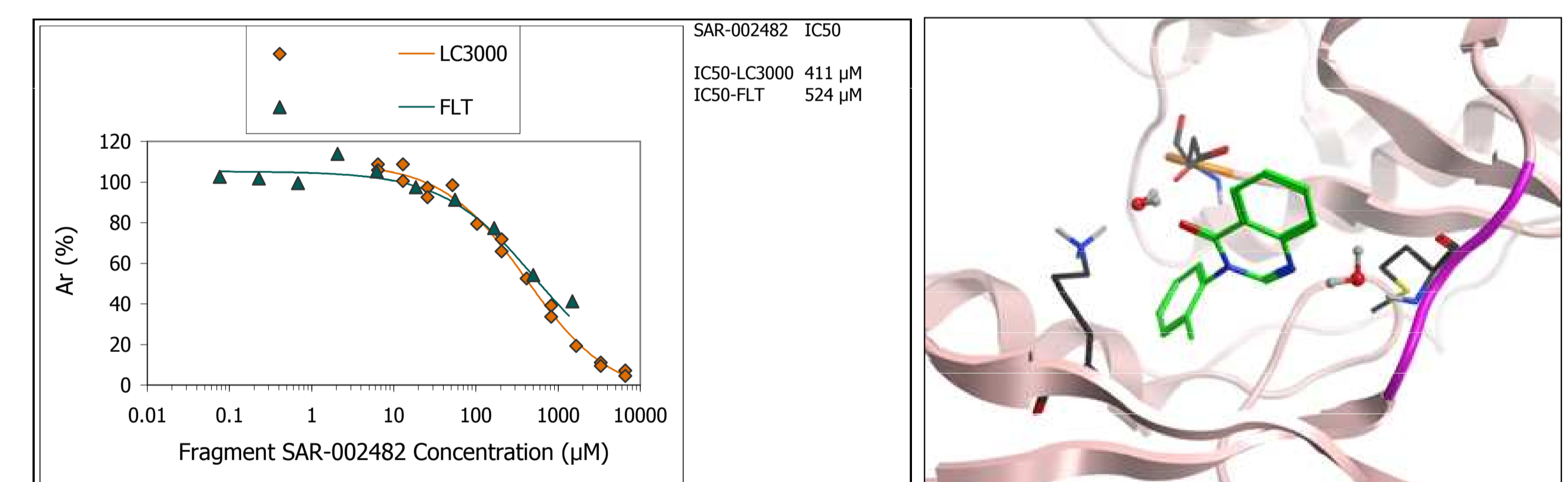
Reaction linearity and enzyme titration with 2  $\mu M$  f.c. Almac substrate 1A (left) Potency of reference compounds was very comparable in FLT and FI assays (right).

## Testing of a subset of the BioFocus fragment library against p38 $\alpha$

Fragment-based drug discovery is considered an increasingly important area of drug discovery combining structural biology, binding studies and functional assays.

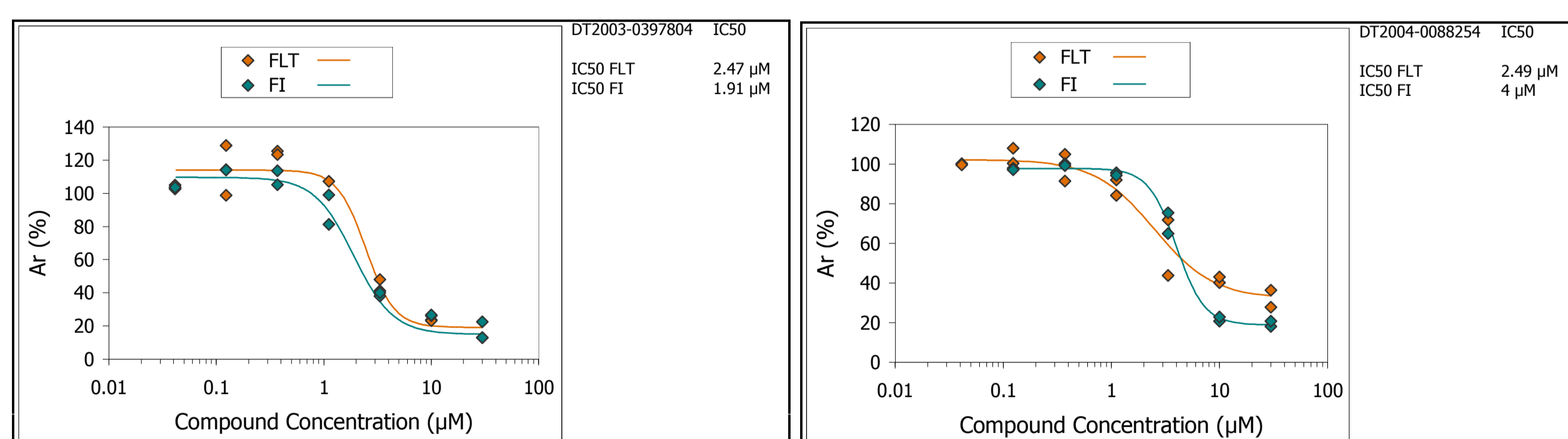


A subset (42 compounds) of the BioFocus fragment library was tested against the kinase p38 $\alpha$  in the FLT and Caliper LC3000 MSA assays. A good correlation of inhibitory activities against p38 $\alpha$  by the fragments was observed when both functional assays were compared (left), which adds the FLT format as a valuable option for FBDD assays.

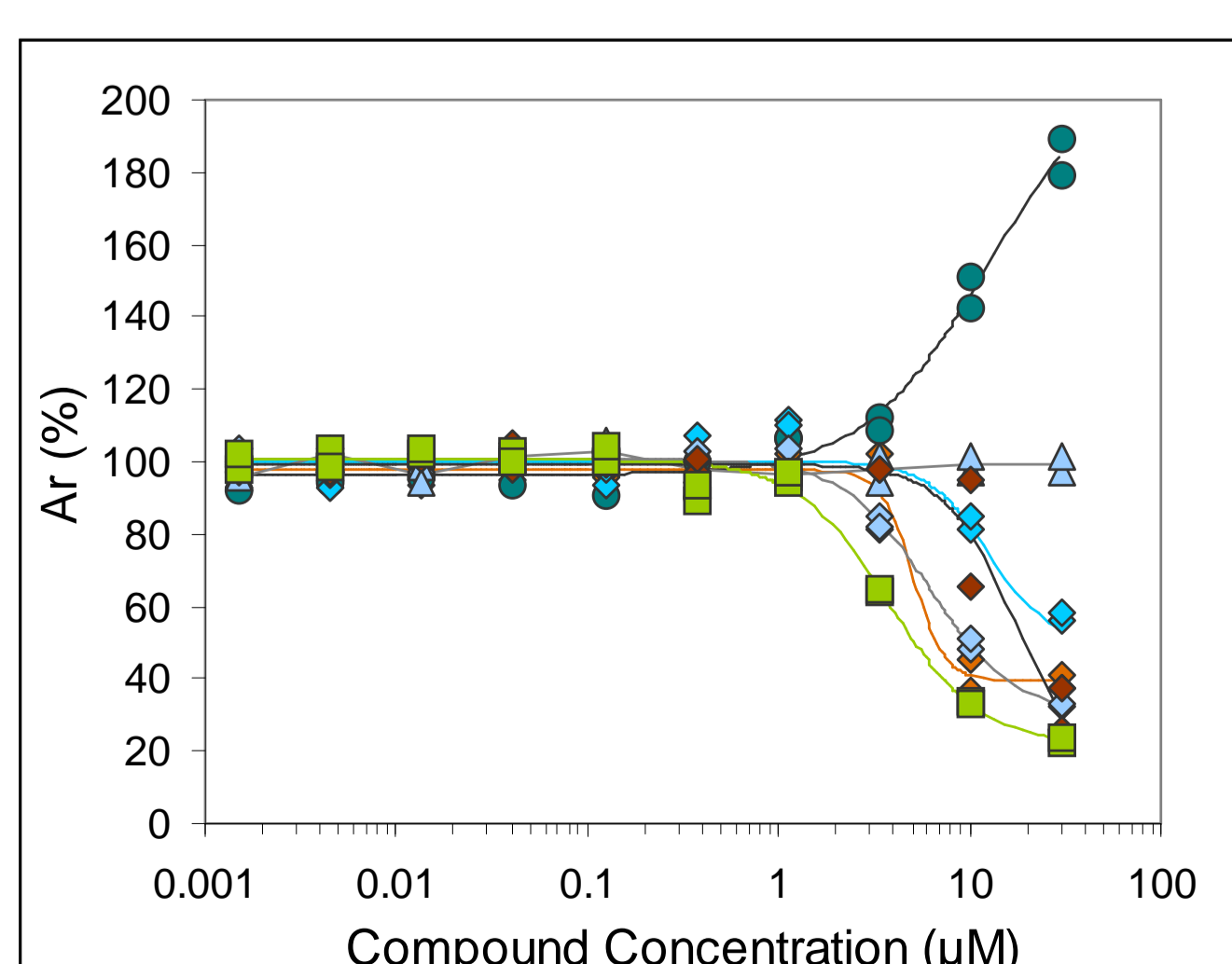


Functional data in LC3000 and FLT (left) were supported by SPR binding studies and x-ray crystallography as shown for fragment-based SAR-002482 (right).

## Testing of compound subsets of the BioFocus diverse collection in the protease FLT assay



Validated active compounds identified in the FI assay were tested in the FLT assay. Comparable potencies and efficacies in both assay formats were found.



Using the FLT assay a number of additional inhibitory compounds from the BioFocus diverse compound collection were identified which were not pursued further in the FI based assay due to assay interference caused by compound fluorescence.

5 of 7 compounds in the FLT assay (left) showed dose dependent inhibition of the protease, one was inactive and one still interfered with the read out.

## 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.

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.