

The **FLEXYTE**TM Assay Platform: Exploiting Fluorescence Lifetime for Drug Screening Applications



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Introduction

The **FLEXYTE**TM assay platform is a screening technology that utilises fluorescence lifetime (FLT) as the reporting modality. The application of this intrinsic fluorescence property to biochemical and cell based assays is attractive as the inherent properties of this method enable more robust assays to be configured and background interference from fluorescent compound libraries to be minimised, leading to fewer false positives in drug screening applications.¹

Key to the success of the **FLEXYTE**TM assay platform technology has been the development and application of 9-aminoacridine (9AA) as a long lifetime fluorescent reporter ($\tau = 17$ ns).² By exploiting the fluorescence properties of this dye, 9AA labelled substrates have been developed, which produce significant changes in fluorescence lifetime upon modification by a variety of target enzymes.

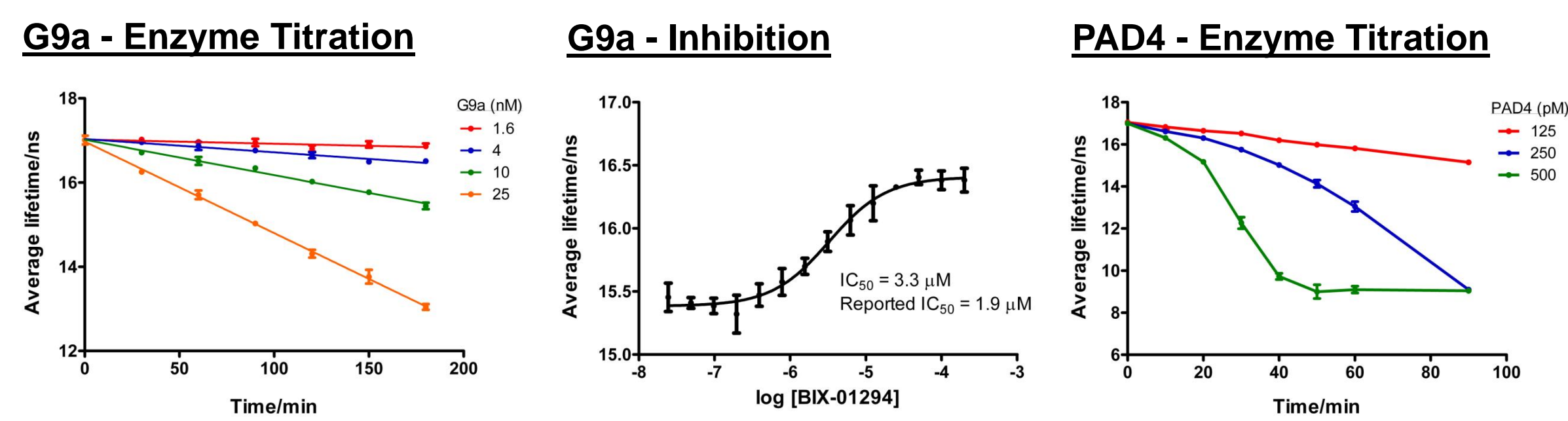
We previously launched **FLEXYTE**TM Ser / Thr protein kinase and **FLEXYTE**TM protease platforms which offer a homogeneous, antibody free, non-radioactive, and cost effective solution for screening these target classes. We now report,

- The extension of our established Ser / Thr kinase platform technology to Tyr kinases.
- The development of FLT based assays for monitoring Tyr kinase activity in real-time.
- The development of **FLEXYTE**TM assays for the epigenetic target classes of histone methyltransferases and deiminases.

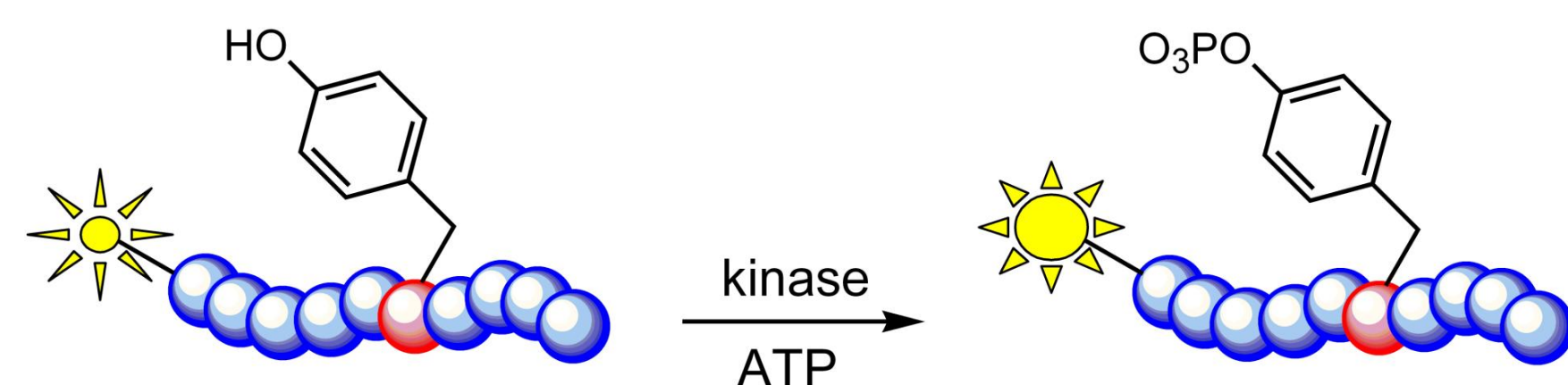
These homogeneous and antibody free assays enable the benefits of FLT technology to be extended to these important areas.

Epigenetic Targets

- FLT assays have now been developed for the protein methyltransferase and deiminase target class
- The **FLEXYTE**TM technology provides an homogeneous antibody free platform to satisfy the current needs for assaying these enzyme classes

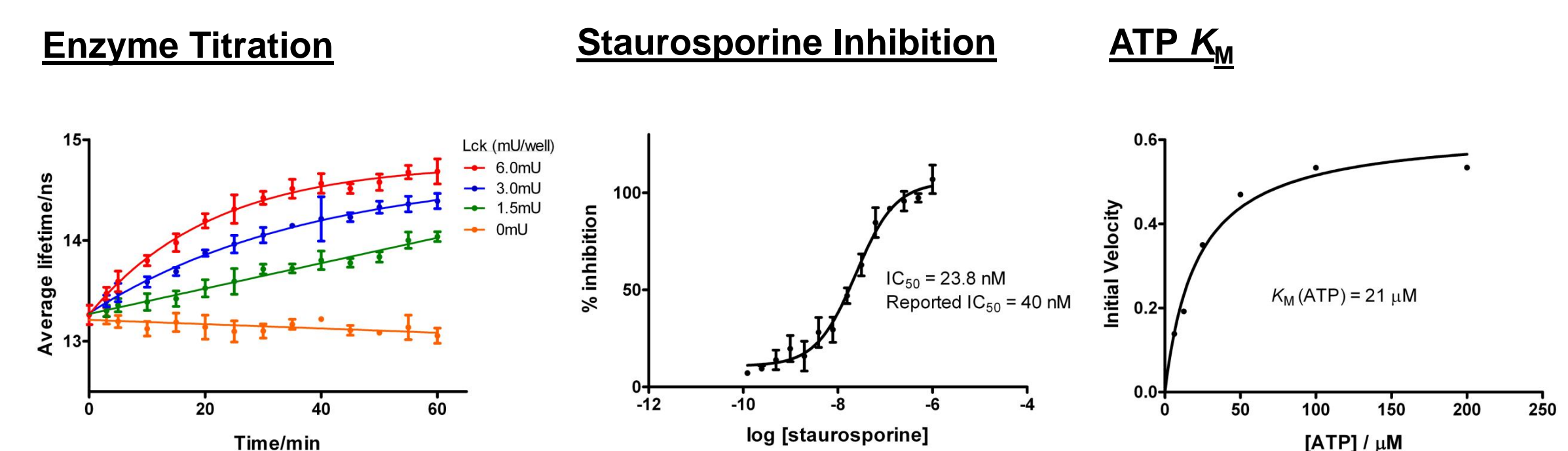


Real-Time Tyrosine Kinase Assay

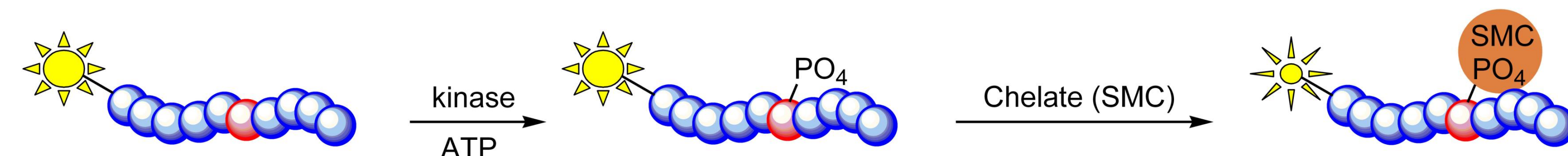


The average fluorescence lifetime of 9AA is reduced by the presence of a tyrosine residue in the peptide sequence

Phosphorylation by the kinase alleviates this effect leading to an increase in the average fluorescence lifetime of 9AA



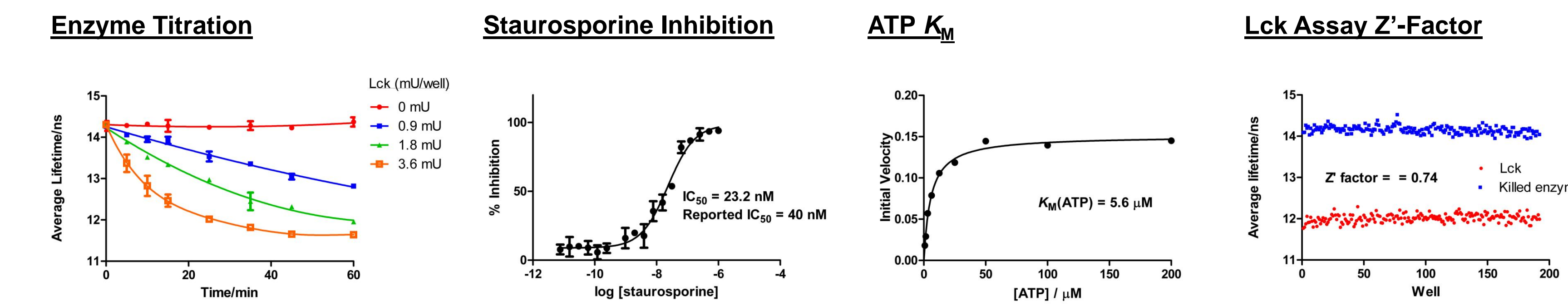
Universal Ser / Thr / Tyr Protein Kinase Assay



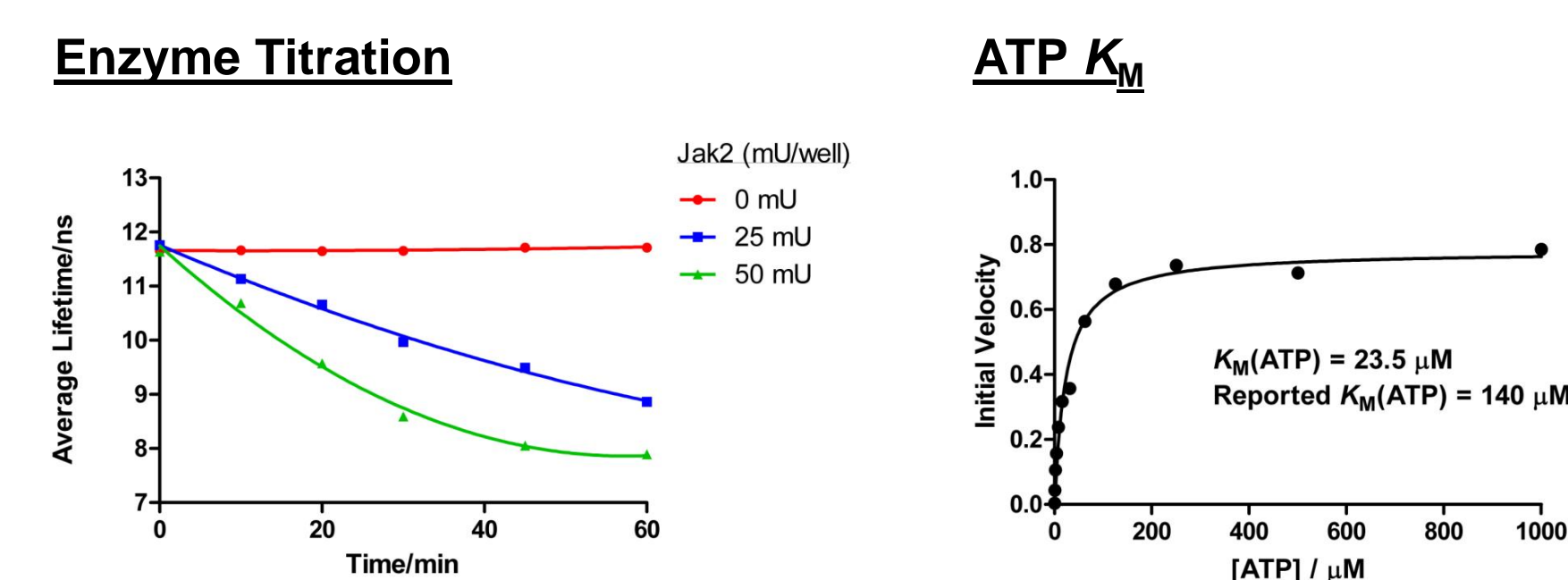
Dye labelled peptide substrate with long fluorescence lifetime
 Kinase phosphorylates substrate on Ser, Thr or Tyr
 SMC complexes the phosphate group and effects a reduction in fluorescence lifetime of up to 5 ns

- Universal approach is validated for a panel of Ser / Thr and Tyr kinases.
- Generic substrates (KS 1-3) configured for assaying a broad panel of Ser / Thr protein kinases (>100).
- Tailored substrates developed to meet the requirements of other Ser / Thr and Tyr kinases.
- **FLEXYTE**TM protein kinase assays give excellent Z'-factors (typically >0.8).
- Compatible with a wide range of ATP and substrate concentrations.
- Assays are easily miniaturised to low volumes. Application to HTS demonstrated.

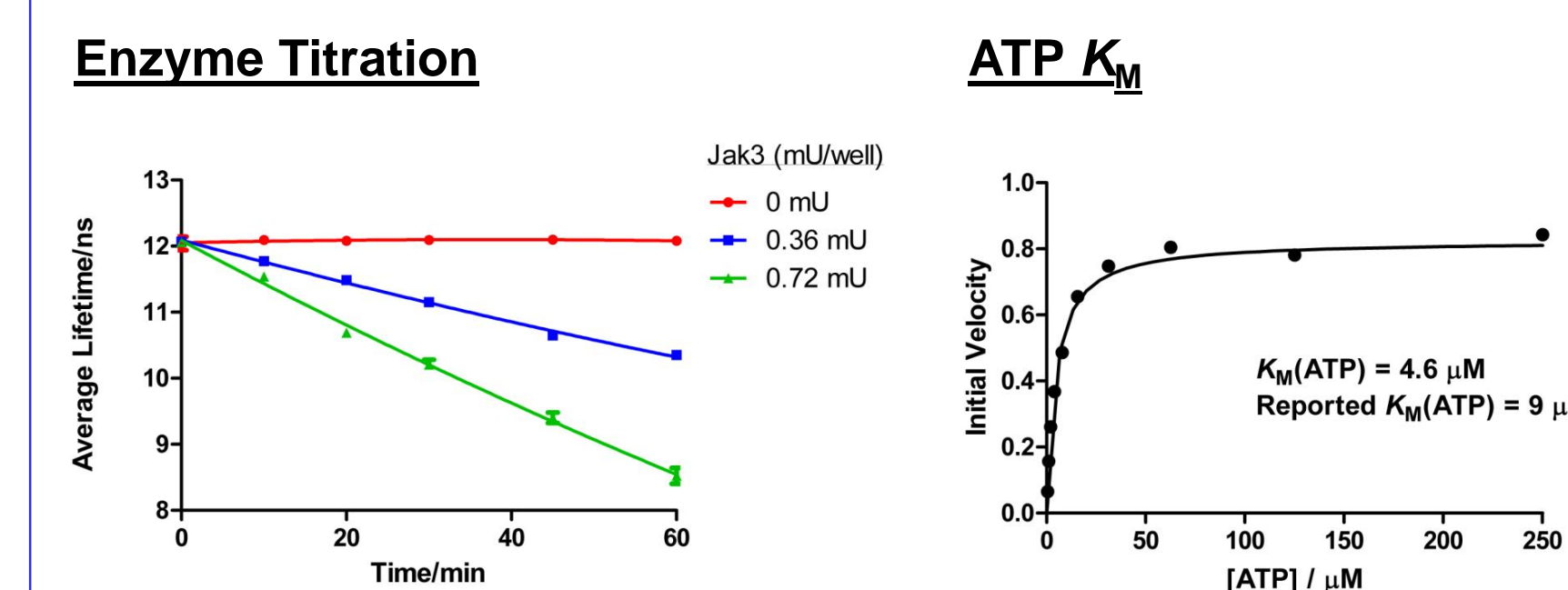
Lck Tyr Kinase Assay



Jak2 Tyr Kinase Assay

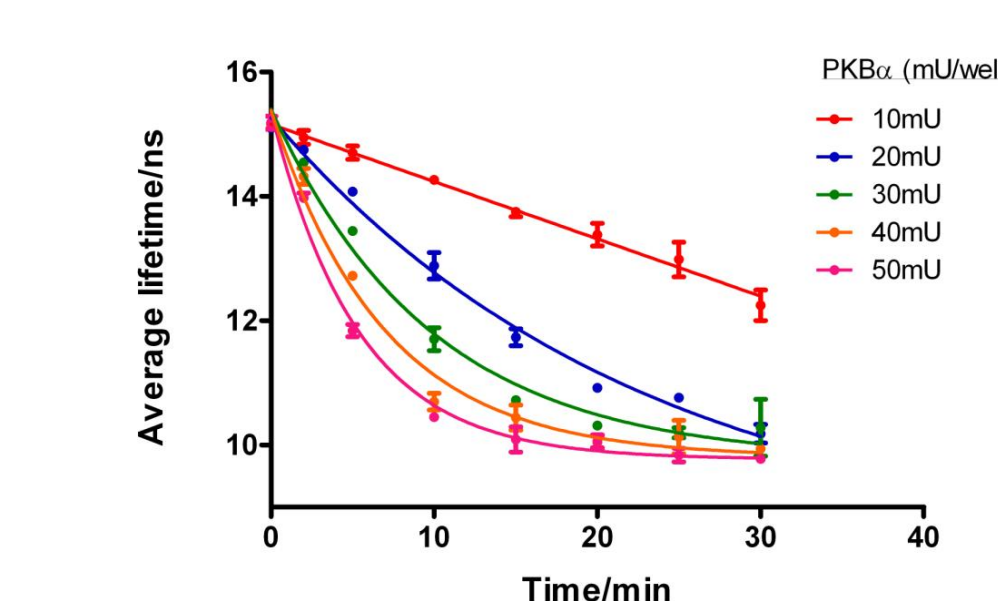


Jak3 Tyr Kinase Assay

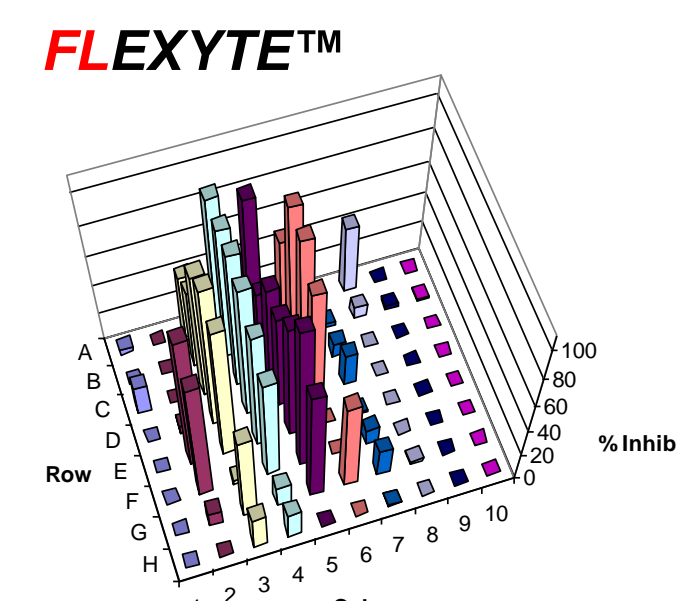


Generic Ser / Thr Kinase Assays

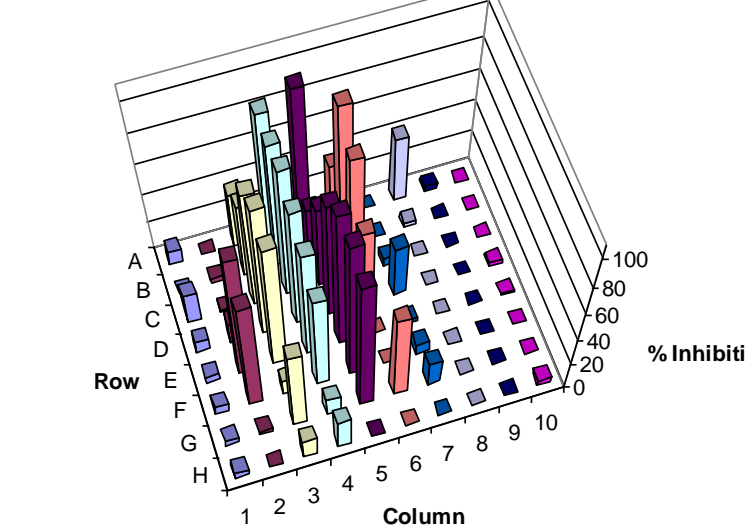
PKB α Assay - Enzyme Titration



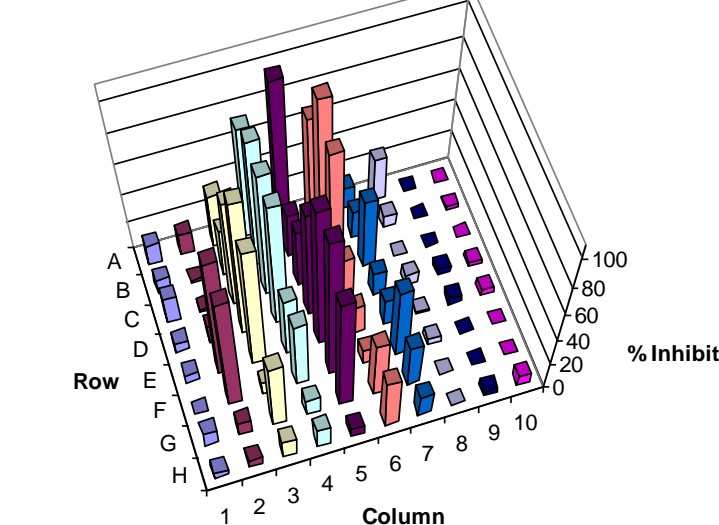
PKB α Assay - Inhibitor Screen



Radiometric

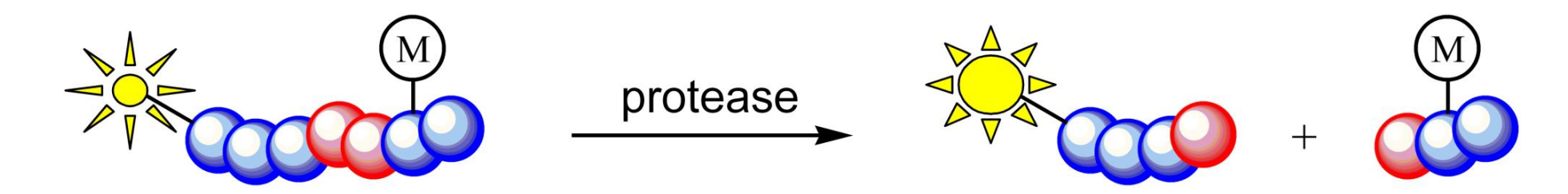


TR-FRET



FLEXYTETM PKB α assay shows excellent comparison with gold-standard radiometric assay and TR-FRET for hit finding (Pearson correlations: 0.98 and 0.93 respectively)

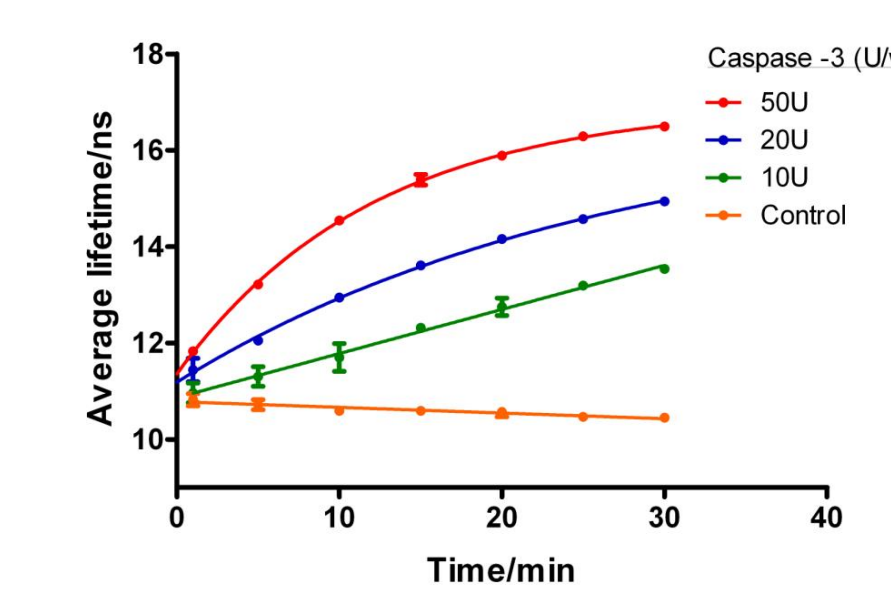
Real-Time Protease Assay



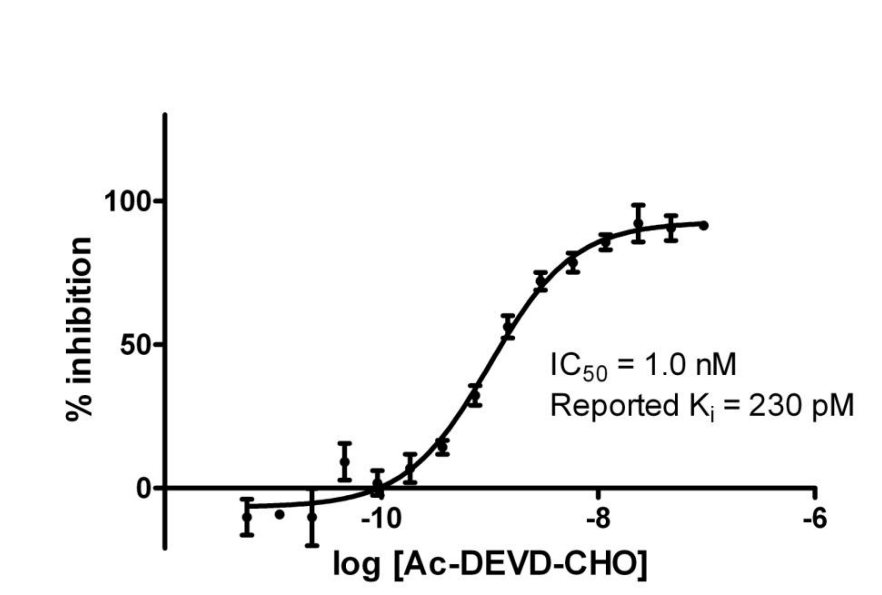
Dye labelled peptide substrate with reduced fluorescence lifetime due to presence of modulator in sequence
 Protease cleaves substrate releasing dye from modulator resulting in an increase in fluorescence lifetime of up to 6 ns

- **FLEXYTE**TM protease assays are homogeneous real-time assays that allow for kinetic measurements to be performed
- Excellent Z'-factors (>0.8) demonstrate that **FLEXYTE**TM protease assays are suitable for HTS and profiling applications
- Generic approach enables **FLEXYTE**TM assays to be configured for a broad range of proteases

Caspase 3 - Enzyme Titration



Caspase 3 - Inhibitor Assay



Conclusion

FLEXYTETM Protein Kinase Assays

- A universal **FLEXYTE**TM protein kinase assay has now been developed for Ser / Thr and Tyr kinases based on fluorescence lifetime as the reporting modality
- This platform technology provides an antibody free, non-radioactive, and cost effective solution for protein kinase screening
- Generic substrates enable **FLEXYTE**TM assays to be configured for a broad panel of Ser / Thr protein kinases in a rapid fashion (covering > 100 different kinases)
- The technology has been shown to be extendable to phosphatase assays using bespoke 9AA-labelled peptide substrates

FLEXYTETM Protease Assays

- **FLEXYTE**TM assays have been developed for proteases, with enzyme activity reported directly by an increase in fluorescence lifetime
- This universal platform provides homogeneous, reliable, real-time monitoring of protease activity for a broad range of targets

FLEXYTETM Epigenetic Targets

- New **FLEXYTE**TM assays are being developed for Epigenetic targets to meet the growing requirements for robust assays in this area
- Protein methyltransferases and deiminases have been exemplified using the **FLEXYTE**TM technology
- Assays are in development for other epigenetic target classes

[1] D. M. Gakamsky, R. B. Dennis and S. D. Smith, Use of fluorescence lifetime technology to provide efficient protection from false hits in screening applications, *Anal. Biochem.*, 2010, **409**, 89-97.

[2] B. A. Maltman, C. J. Dunsmore, S. C. M. Couturier, A. E. Tirnaveanu, Z. Delbederi, R. A. S. McMordie, G. Naredo, R. Ramage and G. Cotton, 9-Aminoacridine peptide derivatives as versatile reporter systems for use in fluorescence lifetime assays, *Chem. Commun.*, 2010, **46**, 6929-6931.

[3] M. J. Paterson, C. J. Dunsmore, R. Hurteaux, B. A. Maltman, G. J. Cotton and A. Gray, A fluorescence-lifetime based assay for serine and threonine kinases that is suitable for high-throughput screening, *Anal. Biochem.*, 2010, **402**, 54-64.