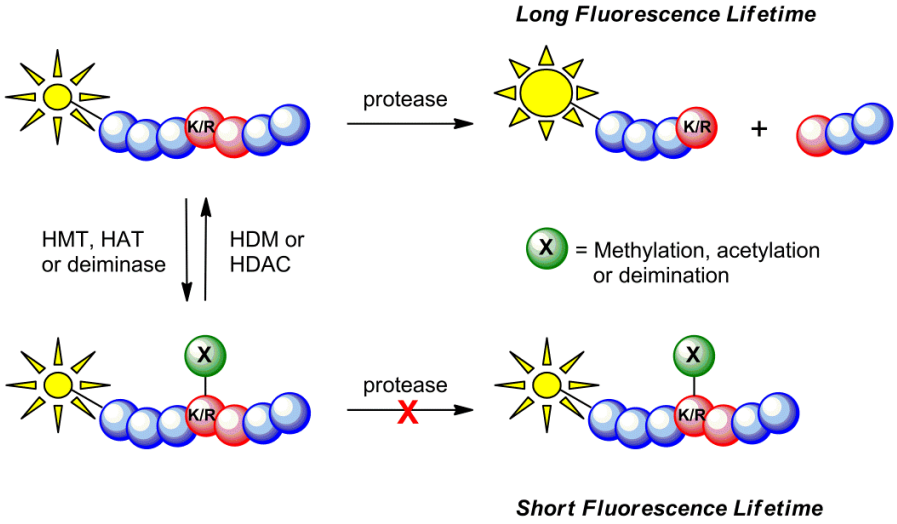
FLEXYTE® Epigenetic Assays

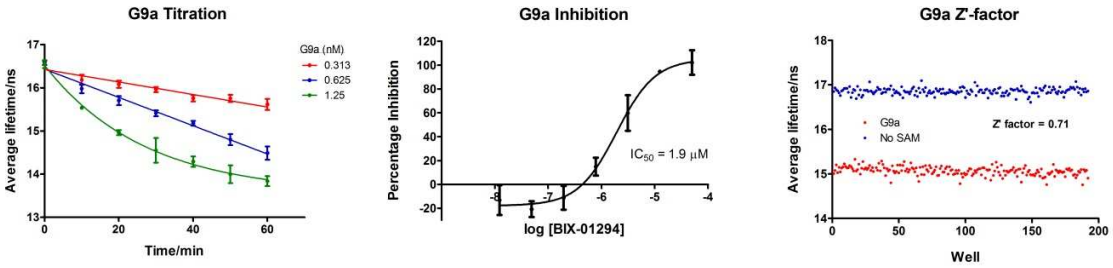
Robust assay technologies for epi-enzymes and epi-readers

Enzymes that modify lysine and arginine residues on histone tails and other proteins, together with the enzymes that recognise these marks, play an important role in controlling gene expression and protein transcription. Such epi-enzymes and epi-readers have received considerable attention in the field of drug discovery due to their role in oncology and inflammatory disease areas. Consequently, there is a requirement for robust assay technologies suitable for screening these targets.

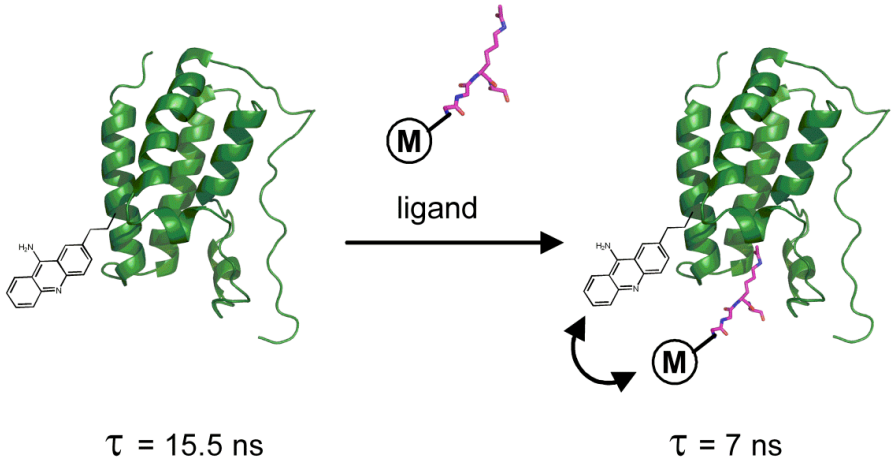
To meet this need, we have developed:

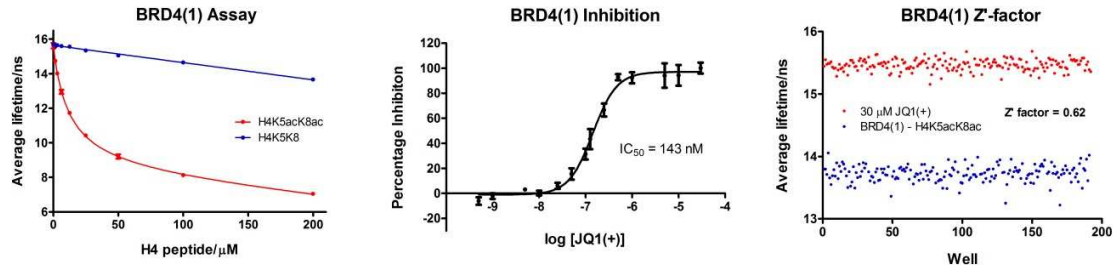
- FLEXYTE® fluorescence lifetime (FLT) epi-enzyme assays for a wide spectrum of epigenetic enzymes including protein methyltransferases, protein demethylases, histone acetyltransferases, histone deacetylases and protein arginine deiminases.
- These assays are based on histone peptide substrates site-specifically labelled with our long lifetime fluorophore, 9-aminoacridine (9AA), and a modulator residue that reduces the FLT of the substrate. The 9AA and modulator moieties are positioned on either side of the lysine or arginine residue that is modified by the epi-enzyme. Depending on the nature of the modification, the peptide either becomes resistant or susceptible to protease-induced cleavage leading to a change in FLT as the 9AA and modulator moieties become separated. Hence, action of the epi-enzyme is reported through changes in FLT.





- FLEXYTE® fluorescence lifetime (FLT) epi-reader assays targeted towards proteins that recognise acetylated or methylated lysine residues in histones including bromodomain, BRD4(1) and malignant brain tumour domain, L3MBTL1. Such interactions are an area of increasing focus in oncology and inflammatory diseases.
- For these assays, the protein of interest is expressed recombinantly and site-specifically labelled with our long lifetime fluorophore, 9-aminoacridine (9AA). The ligand incorporates a known small molecule modulator, M, that reduces the FLT of 9AA when the ligand is bound to the protein. Displacement of the ligand, for example by inhibitors of the protein-ligand interaction, abolishes this quenching effect leading to an increase in FLT. Hence, protein-ligand interactions can be monitored directly through changes in FLT.





FLEXYTE® FLT epigenetic assays are homogeneous, antibody free, and easily miniaturised. Through the use of simple reagent chemistries, fluorophores with long lifetimes, and advancements in instrumentation (ameon®, TTP LabTech) these assays offer a distinct advantage over competing technologies and have the ability to mitigate the most common forms of compound-related assay interference.