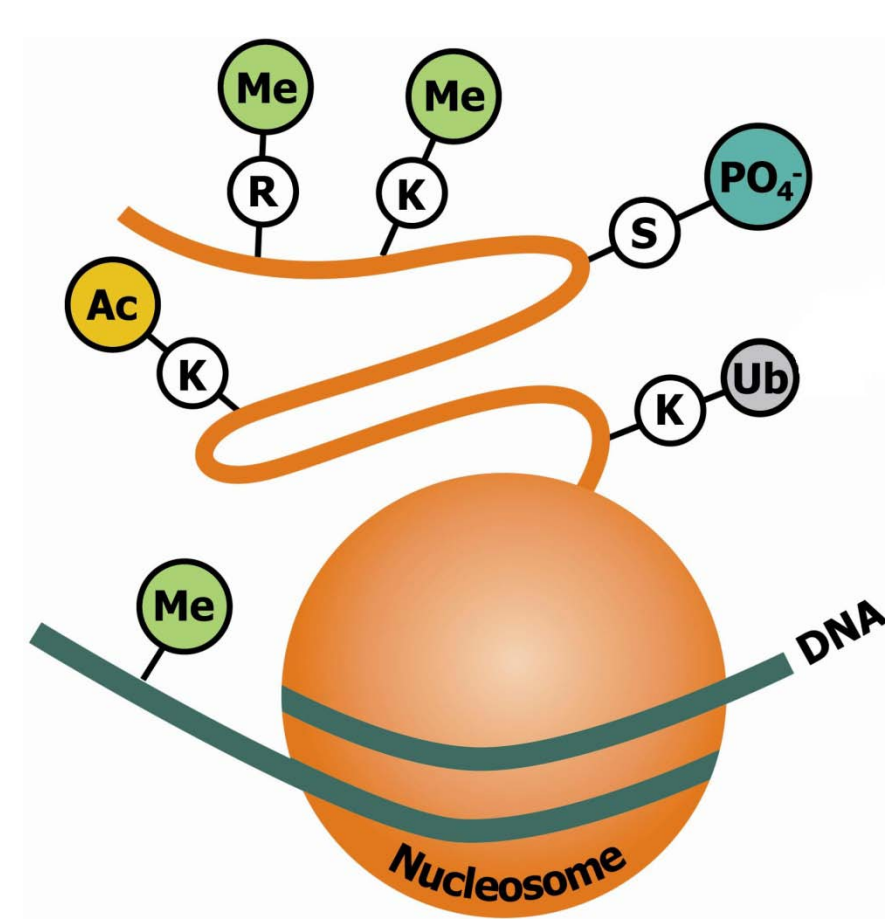


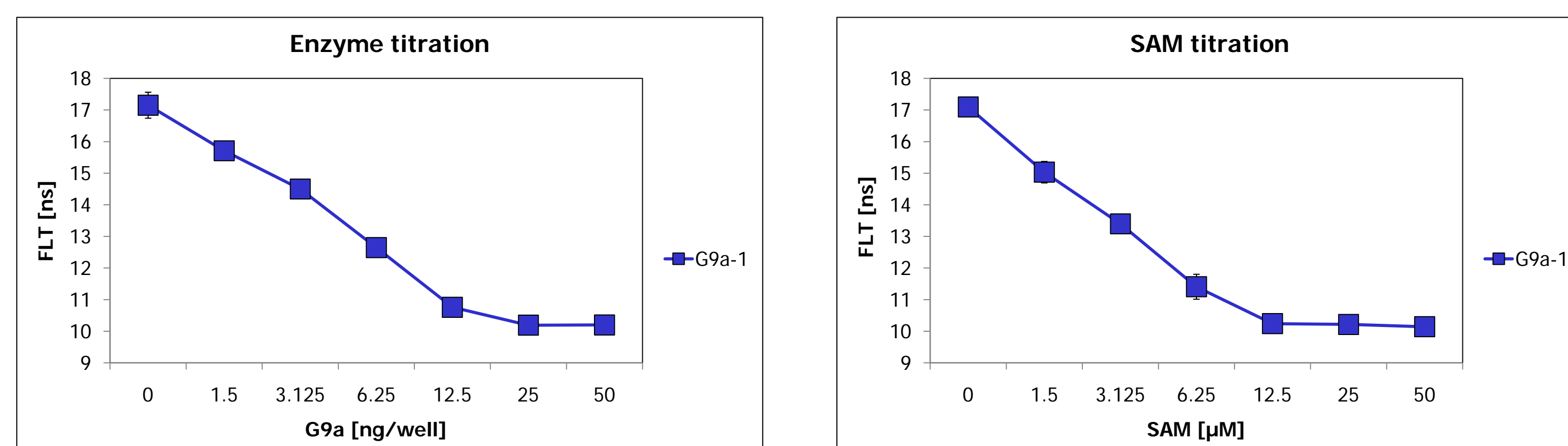
Abstract

For many novel targets the chemical ligand space and structural information is largely unknown. Hit finding campaigns are therefore dependent on large chemical diversity. In the specific case of histone methyltransferases (HMTs) we have been able to apply an efficient process of intelligent selection of screening subsets for primary screening, rather than screening the full diverse deck. The information from this primary screening is fed back into the selection of subsequent screening sets (hit expansion). For the validation of hit series several orthogonal assay technologies and selectivity assays are used. In a G9a case-study employing a mini screen of 2,112 selected compounds, successful assay development and hit finding applying this compound selection strategy is demonstrated. FLEXYTE® FLT and LC3000 MSA assays were established for validation of primary hits. The importance of applying orthogonal assay technologies in early stages of the drug discovery process is shown.

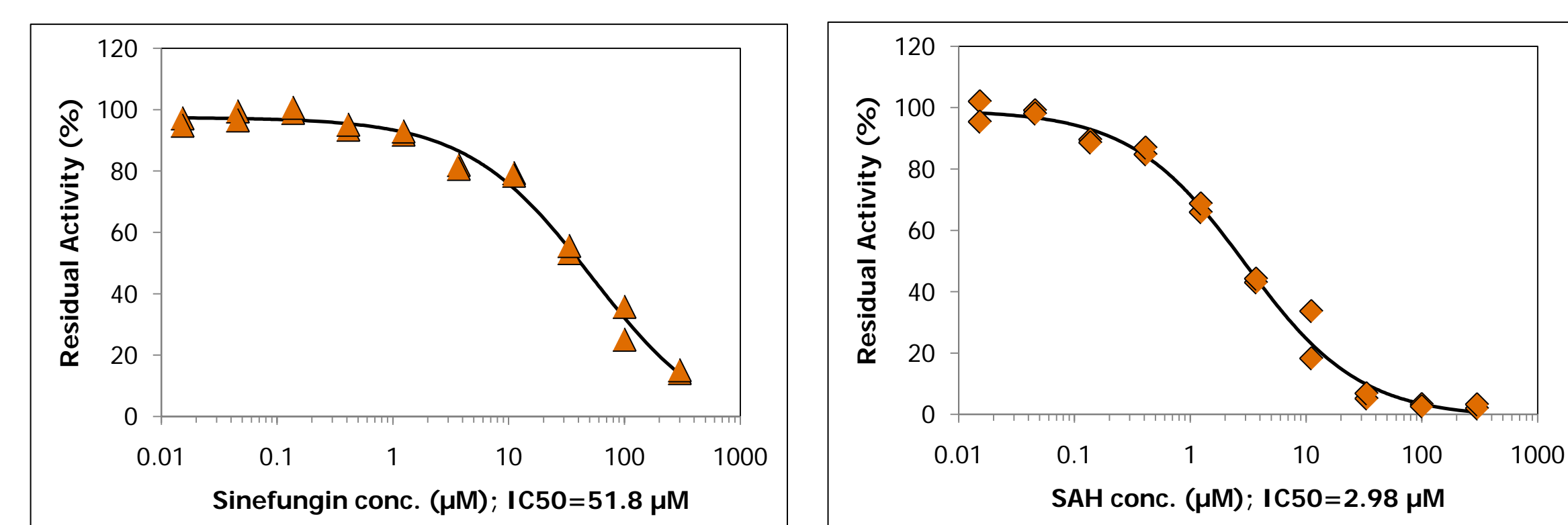


Schematic representation of selected enzymatic protein modifications in epigenetics. Covalent epigenetic modifications of DNA and chromatin proteins include histone phosphorylation, methylation and demethylation, acetylation and deacetylation, ubiquitination etc.

Almac FLEXYTE G9a HMT assay transfer



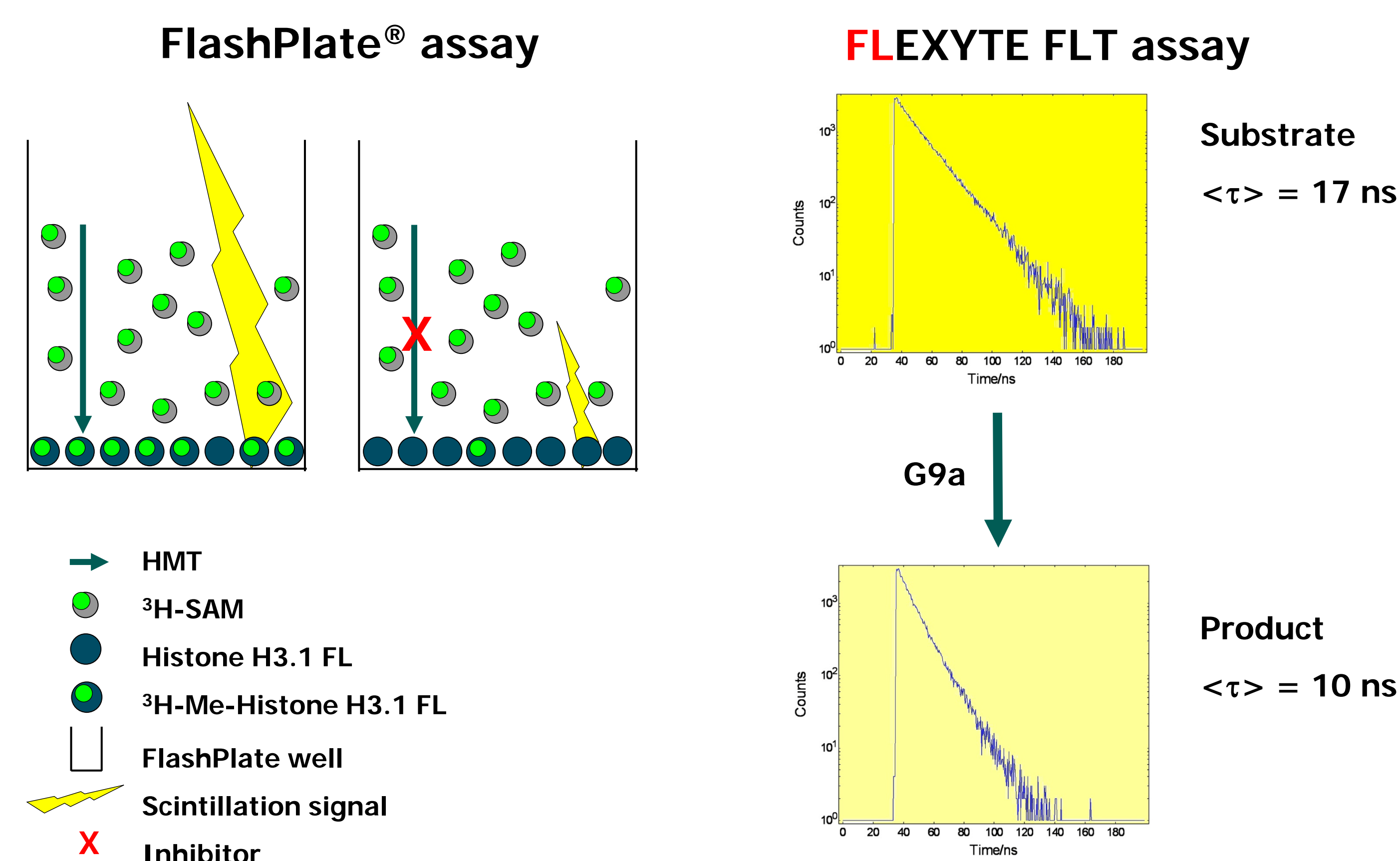
Assay transfer of FLEXYTE G9a HMT assay. G9a (BPS Bioscience) was titrated in the presence of 1 µM G9a-1 peptide substrate and 10 µM SAM (left). The reaction was measured after 90 min reaction time using the NanoTaurus™ FLT reader (Edinburgh instruments). SAM was titrated in the presence of 15 ng/well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

Methods

- Isotopic assays using FlashPlates (standard and Image FlashPlates) or SPA beads (blue shifted and red shifted) are routinely used at BioFocus for addressing HMTs.
- A broad spectrum of selectivity and orthogonal assays can be applied for compound validation. LC3000 MSA (Caliper) and a new FLEXYTE Fluorescence Lifetime (Almac) assay for G9a were used at BioFocus.



Principles of isotopic FlashPlate and Fluorescence lifetime assays (Almac)

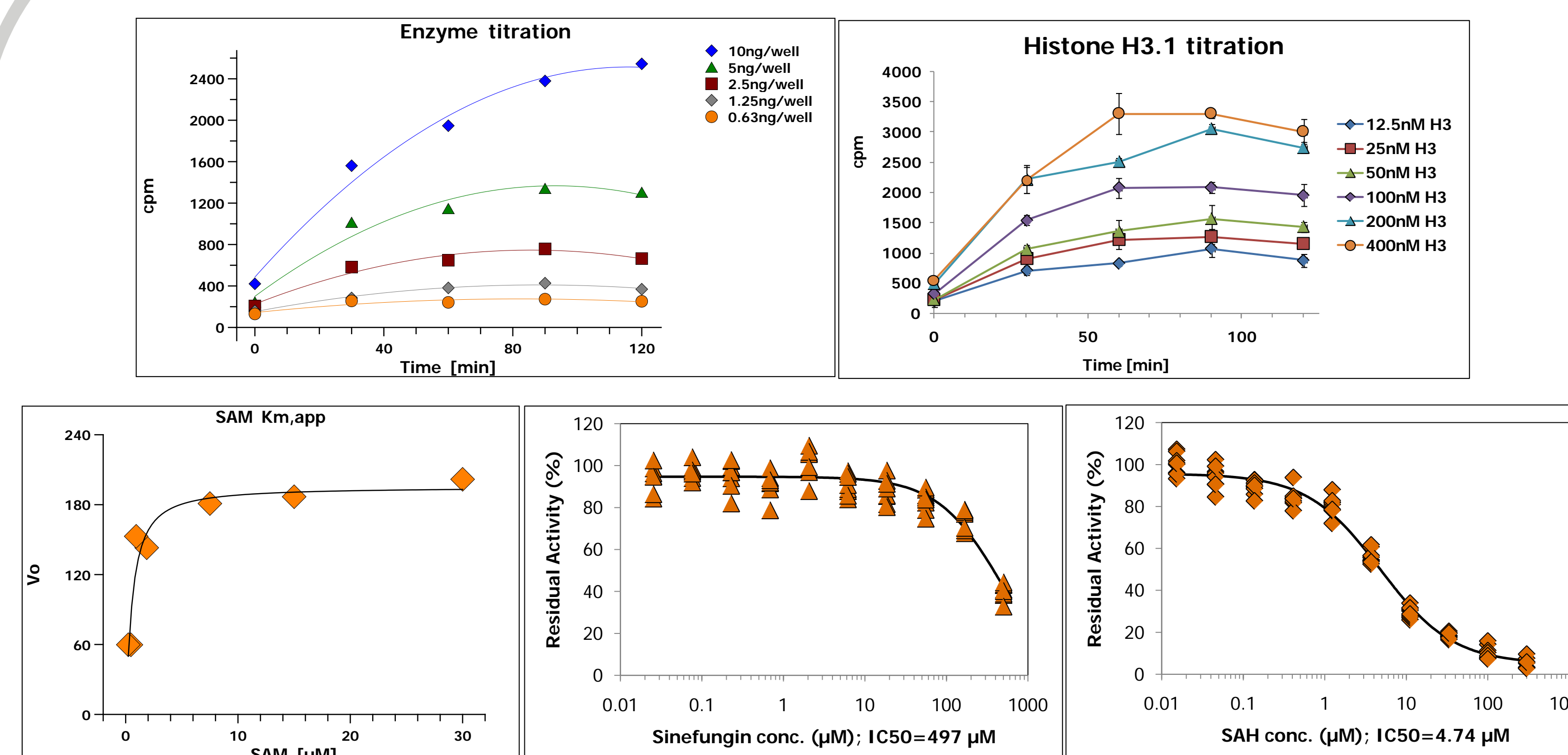
Compound selection approach

- 2,112 compounds were selected using complementary virtual screening (VS) approaches. The entire BioFocus compound collection comprising ~870,000 compounds was used.
- Structure-based virtual screening (SBVS)
 - G9a X-ray crystal structure (PDB 3nni) was used
 - Docking (Program Glide) in SAM binding site + post processing
- Ligand-based virtual screening (LBVS)
 - Chemoinformatics-based hit expansion methods:
 - Similarity searches using different fingerprint sets
 - Bioisosteric transformation and enumerations
 - Ring generalization and ring assembly modification
 - Pharmacophore tree (DiscNgin)
 - SAH and two reference compounds (BIX-01294 and UNC0224) ¹ were used as query structures for the LBVS approaches.

| Source | Frequency |
|--------------------------|-----------|
| HitExpansion OR SVLTK | 7 |
| HitExpansion | 727 |
| DiscNgin | 504 |
| HitExpansion OR DiscNgin | 2 |
| SVLTK | 616 |
| DOCK | 255 |
| DiscNgin OR SVLTK | 1 |

Compound selection strategy for G9a MiniScreen. 872 compounds were selected using SBVS, 736 using combined hit expansion methods, and 506 with DiscNgin's pharmacophore trees. The overlap between the methods was remarkably small. SVLTK: BioFocus post-processing tool kit.

G9a HMT FlashPlate assay



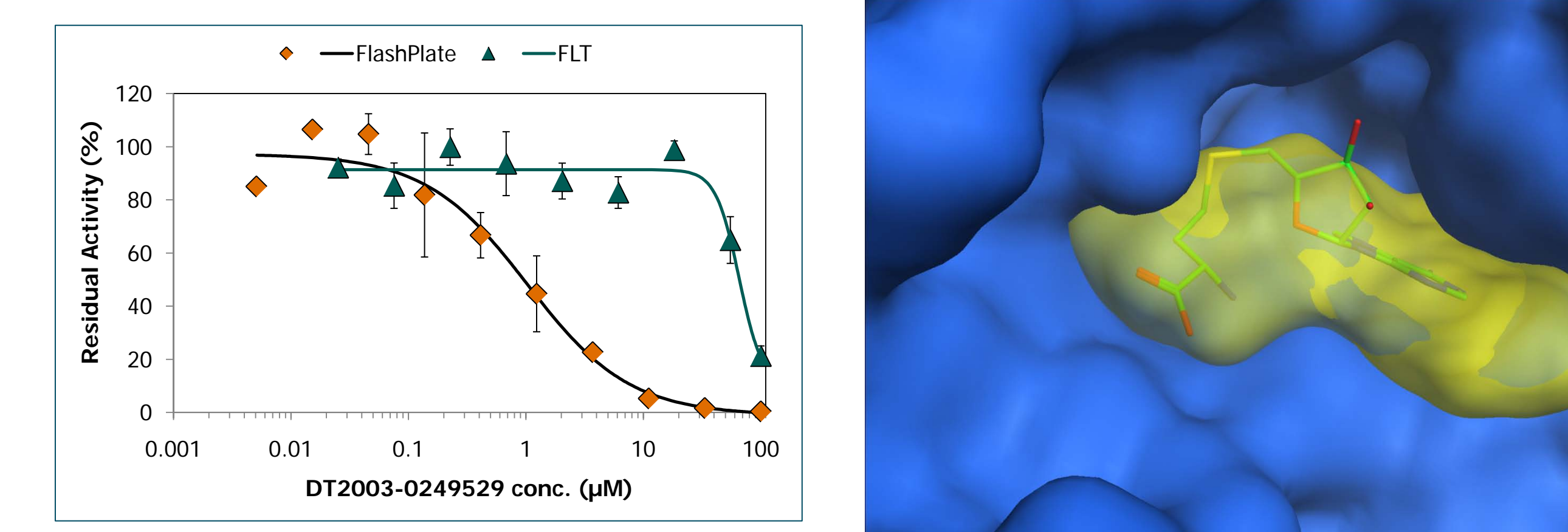
Assay development of isotopic G9a FlashPlate assay. Final assay conditions were 5 ng G9a (BPS BioScience) per well (1.3 nM), 3 µM SAM, 50 nM full length Histone H3.1 (BPS Bioscience) and a reaction time of 90 min.

Hit rates of Mini screen against G9a

| | > 30% Inhi. (hit rate) | > 40% Inhi. (hit rate) | > 50% Inhi. (hit rate) | > 60% Inhi. (hit rate) |
|--------------|------------------------|------------------------|------------------------|------------------------|
| HTS | 233 (11%) | 149 (7.1%) | 105 (5.0%) | 82 (3.9%) |
| Confirmation | 95 (41%) | 58 (39%) | 32 (30%) | 15 (18.3%) |

2,112 compounds at 10 µM were screened against G9a in the FlashPlate assay. Increased primary hit rates and confirmation rates within expectations were observed.

Hit validation using FlashPlate and Almac FLEXYTE G9a HMT assay



- 32 compounds were selected for IC50 determination based on inhibitory activity and chemical structure.
- 24 of 32 compounds in dose response confirmed in FlashPlate assay with IC50s ranging from 0.6 to 40 µM.
- Only three compounds confirmed in FLEXYTE G9a HMT assay. Orthogonal assays can aid triaging of hits.
- Docking of confirmed compound DT2003-0249529 (transparent volume) to G9a SAM binding pocket (stick model for SAM) is shown.

BioFocus offers

- Assay technologies for a broad spectrum of epigenetics targets
- Computational chemistry tools for selection of screening decks
- Significantly increased hit rates through customized compound selection strategies
- Track record for rapid epigenetic target hit discovery

➔ Fast-track to novel chemical entities inhibiting epigenetics targets

References

1. Wigle et al. (2010), *Chemistry and Biology*, 17, 695-704