

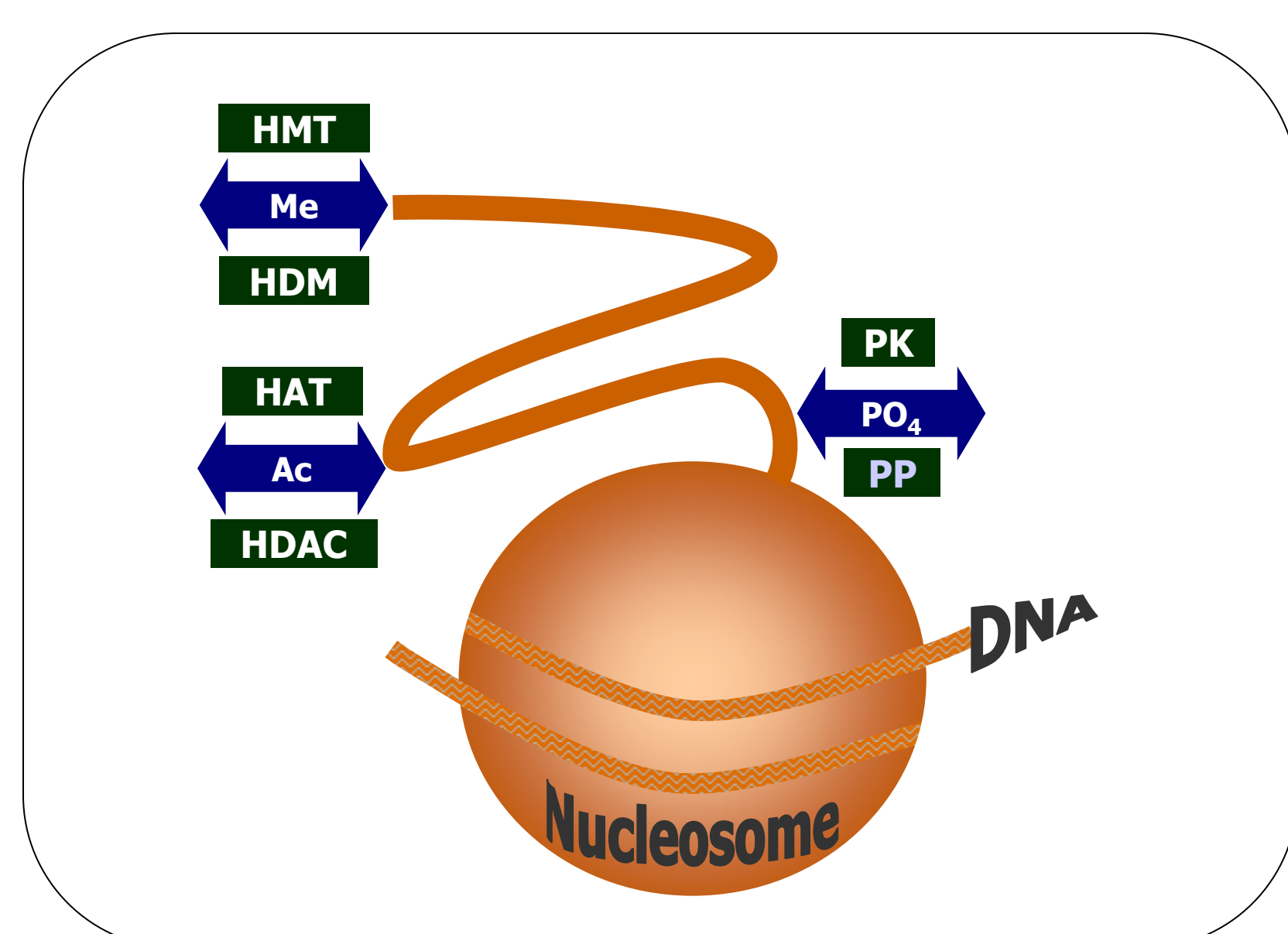
Abstract

For novel targets the chemical ligand space and structural information is largely unknown. Hit-finding campaigns are therefore typically performed with very large compound libraries in order to identify good starting points for lead optimization.

Rather than screening our full compound collection, we have been able to address a broad range of novel epigenetics targets with intelligent selection of library subsets for primary screening. The information from the primary screening is fed back into the initial models and through a knowledge driven hit-expansion the hit rates are significantly increased.

Overview of epigenetics target classes

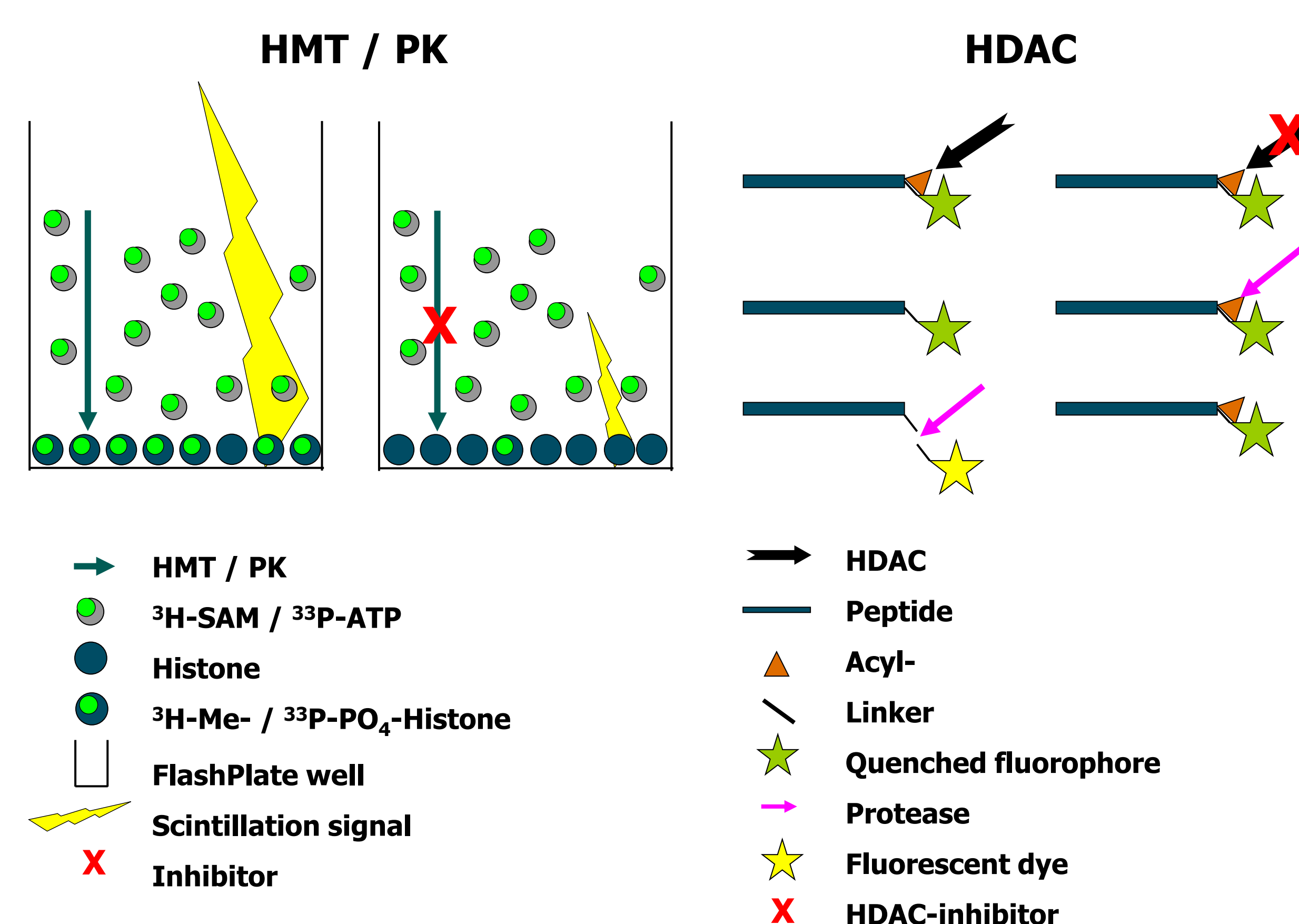
- covalent epigenetic modifications of DNA and chromatin proteins include histone phosphorylation, methylation, acetylation, ribosyl transfer, ubiquitinylation, proteolytic cleavage etc.
- BioFocus has gained much experience in early drug discovery programs targeting epigenetic modulators as e.g. histone kinases (PK), methyltransferases (HMT), and deacetylases (HDAC)



Schematic representation of selected enzymatic protein modifications in epigenetics

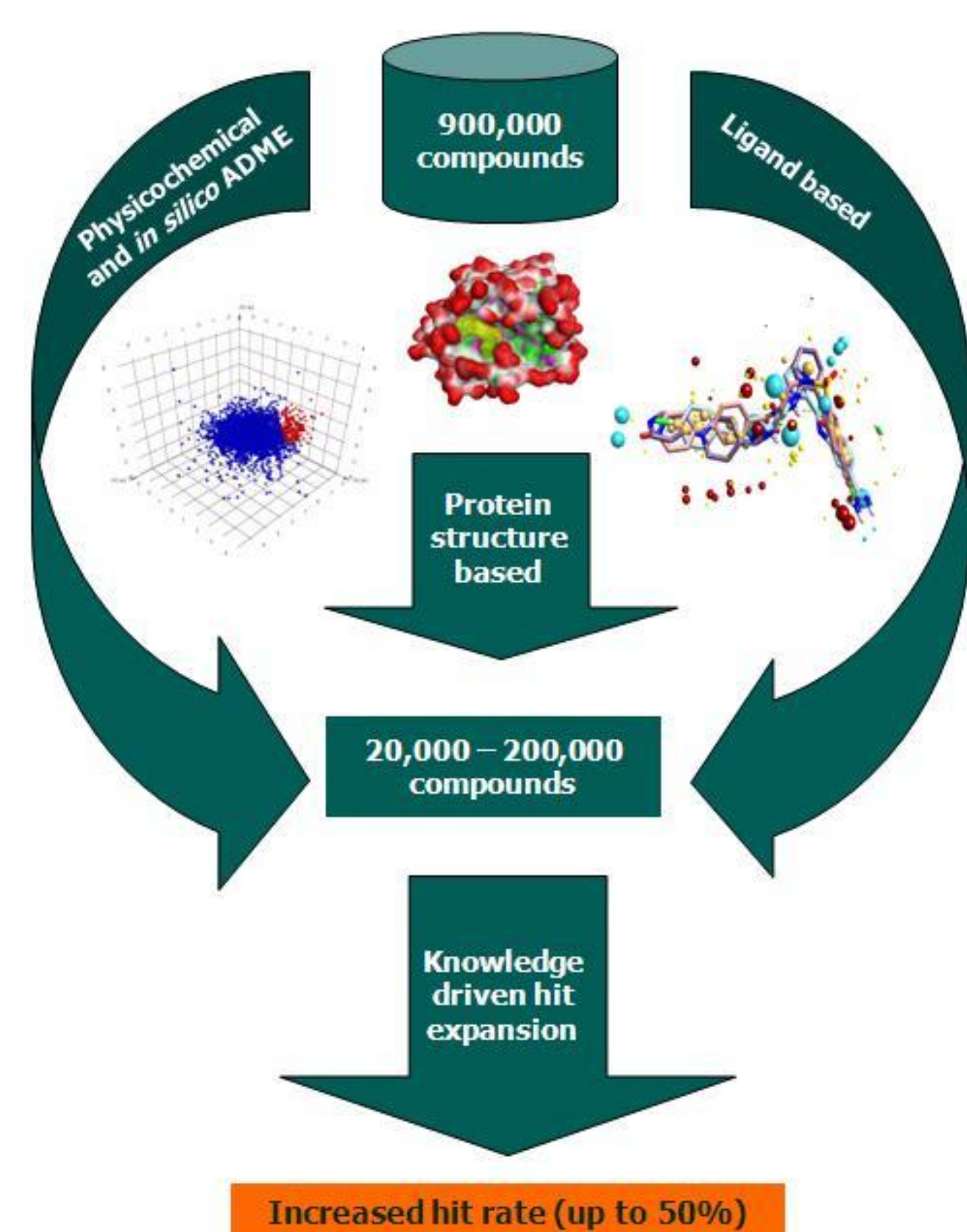
Examples shown are Histone phosphorylation (PO_4 , kinase PK and phosphatase PP assays), methylation (Me, methyltransferase HMT and demethylase DM assays), and acetylation (Ac, acetyltransferase HAT and deacetylase HDAC assays). Other modifications such as ribosyl transfer, ubiquitinylation, proteolytic cleavage, etc., are not depicted.

HTS assay formats



- standard primary HTS assay routinely used at BioFocus for addressing HMTs are isotopic assays using FlashPlate assays (standard and Image FlashPlates) or SPA beads for read-out (blue shifted and red shifted)
- broad panel of assay technologies in place for kinase drug discovery (FlashPlate, harvester filter plates, ATP consumption and ADP production, fluorescence polarization, TR-FRET, and MSA on Caliper LC3000)
- two step fluorescence intensity assay for HDAC was established using a substrate modified by a quenched fluorophore
- a broad spectrum of selectivity and orthogonal assays can be applied for compound validation

Intelligent screening approach



Computational chemistry workflows are individually tailored and optimized for selecting the most suited compounds from our 900,000 compounds collection

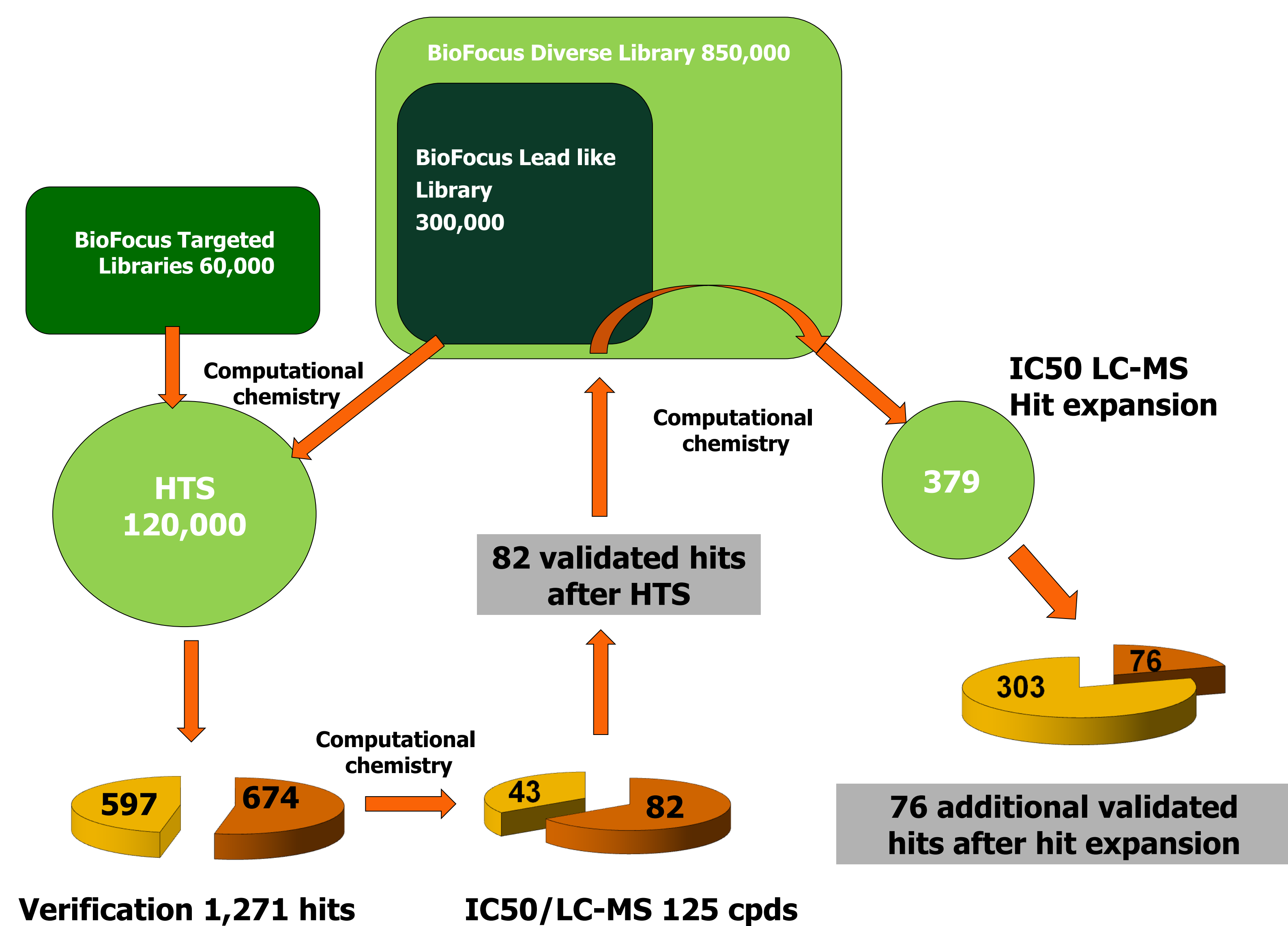
HTS compound selection:

- filtering towards the physicochemical property profile for the intended therapeutic area with *in silico* ADME descriptors
- using available knowledge of known active compounds, such as compilation of SAR information of various sources.
- exploiting target 3D structure information
- proprietary computational chemistry tools

Hit expansion: re-mining of primary HTS data

- expand and consolidate the knowledge about active regions in chemical space
- generation and broadening of SAR information of active scaffolds
- scaffold hopping
- individual hits or scaffolds of known active compounds can be used to mine the non tested part of the compound library for promising structures

Case study: Histone modifying Kinase



BioFocus has

- assay technologies for a broad spectrum of epigenetics targets available
- chemoinformatics tools for intelligent selection of screening decks
- significantly increased hit rates through hit expansion
- track record for rapid epigenetic target hit discovery

Fast-track to novel chemical entities inhibiting epigenetics targets