



Discover novel chemotypes for Epigenetics targets

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Outline

- Historical view
- Overview of epigenetics targets
- Target classes
 - therapeutic relevance
 - known inhibitors
- Strategies for hit-finding for novel chemotypes
- Assay technologies

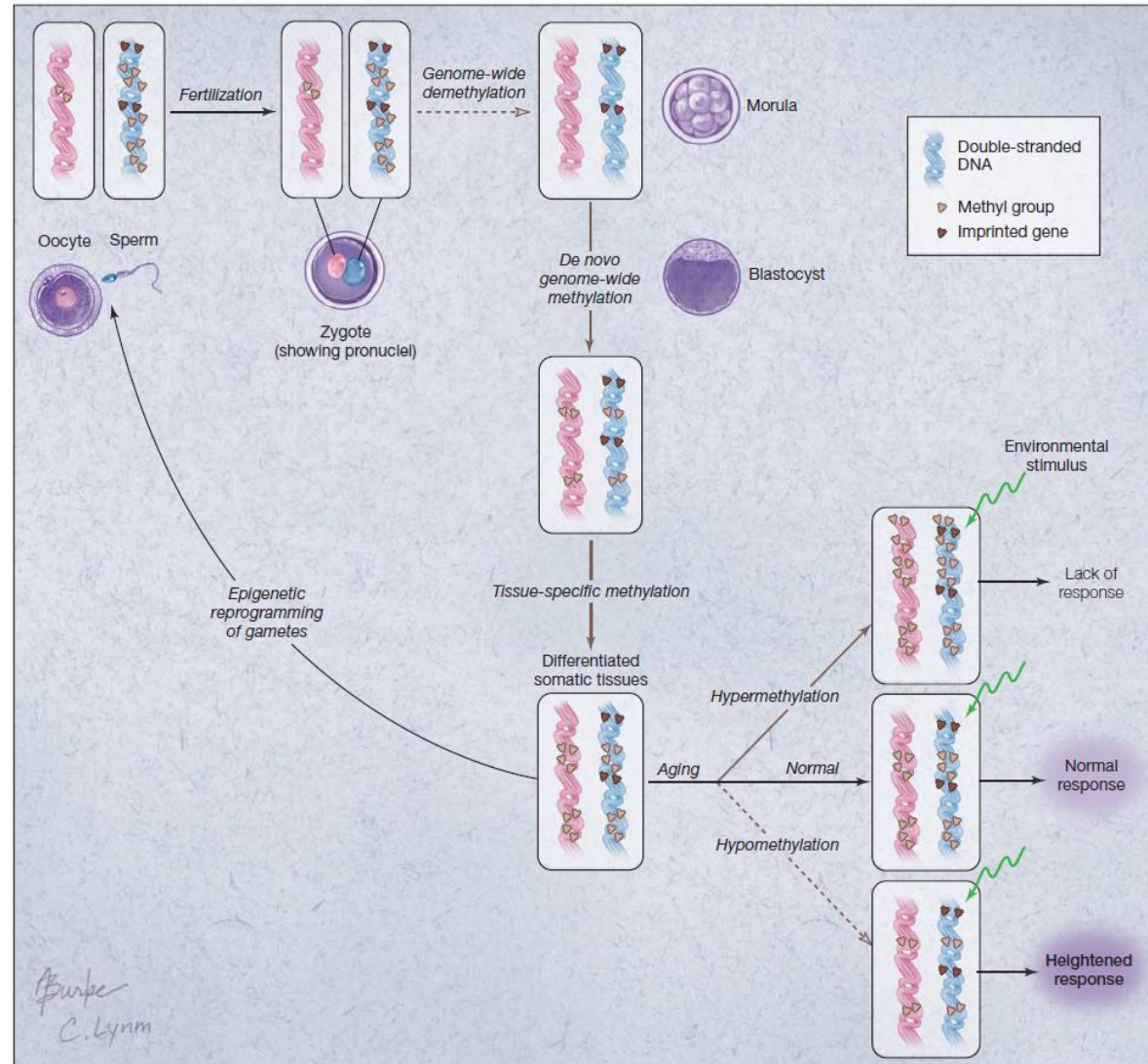


Epigenetics

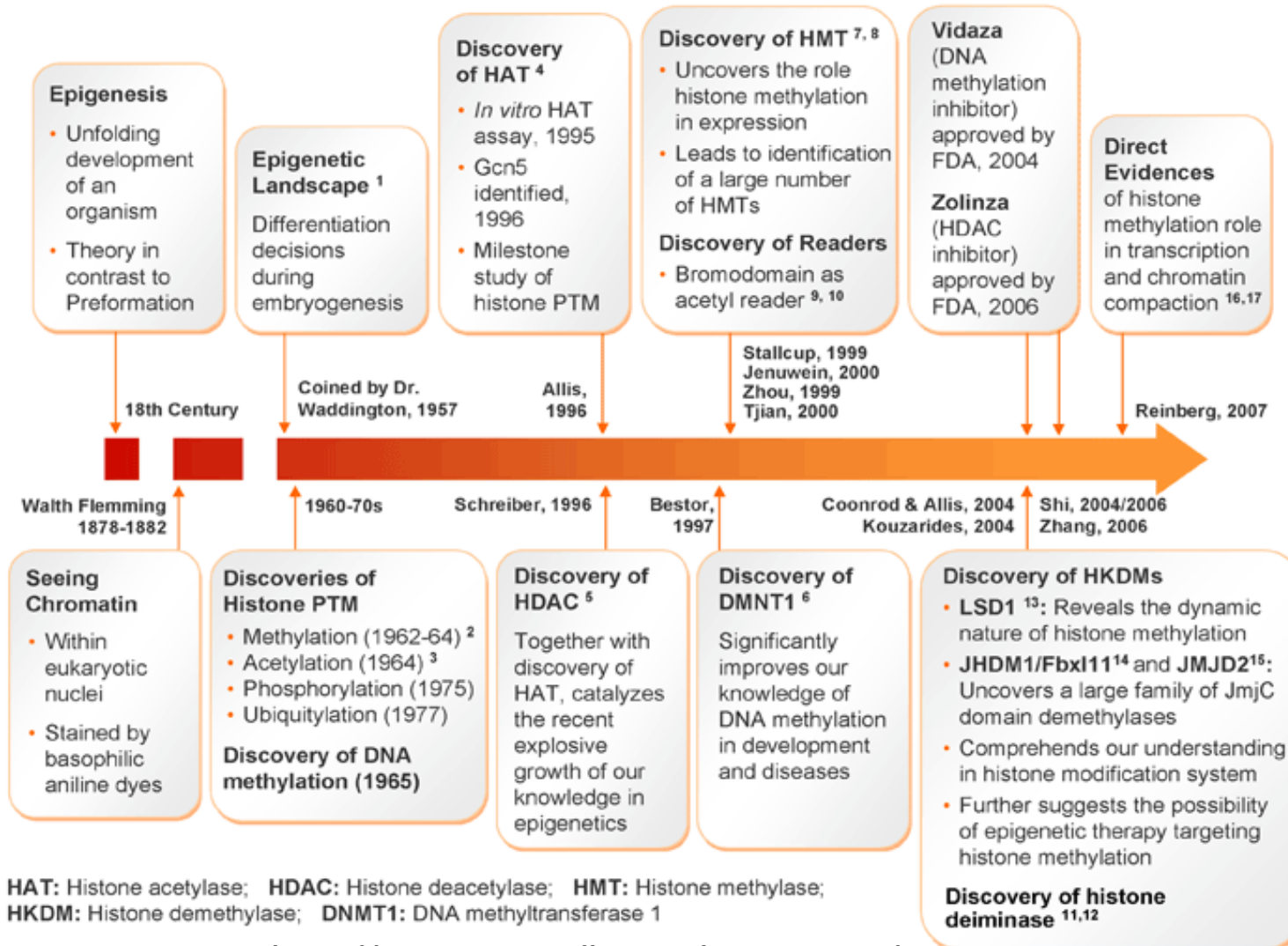
- The epigenetics state of an organism has a life cycle - the DNA sequence does not
- Currently, the main therapeutic areas are oncology and metabolic disorders
 - also inflammation, neurodegeneration, cardiovascular
- Despite being a “new, hot” area of research
 - epigenetics research has been ongoing for some time
 - three drugs affecting epigenetics targets are on the market, current market estimate is \$560M
 - another 30 drugs in development

Epigenetics – the life cycle of the epigenome

- Cell type specific regulations
- Disease states
- Developmental biology
- Age related modifications
- Environmental Changes



Historical view



<http://www.constellationpharma.com/>



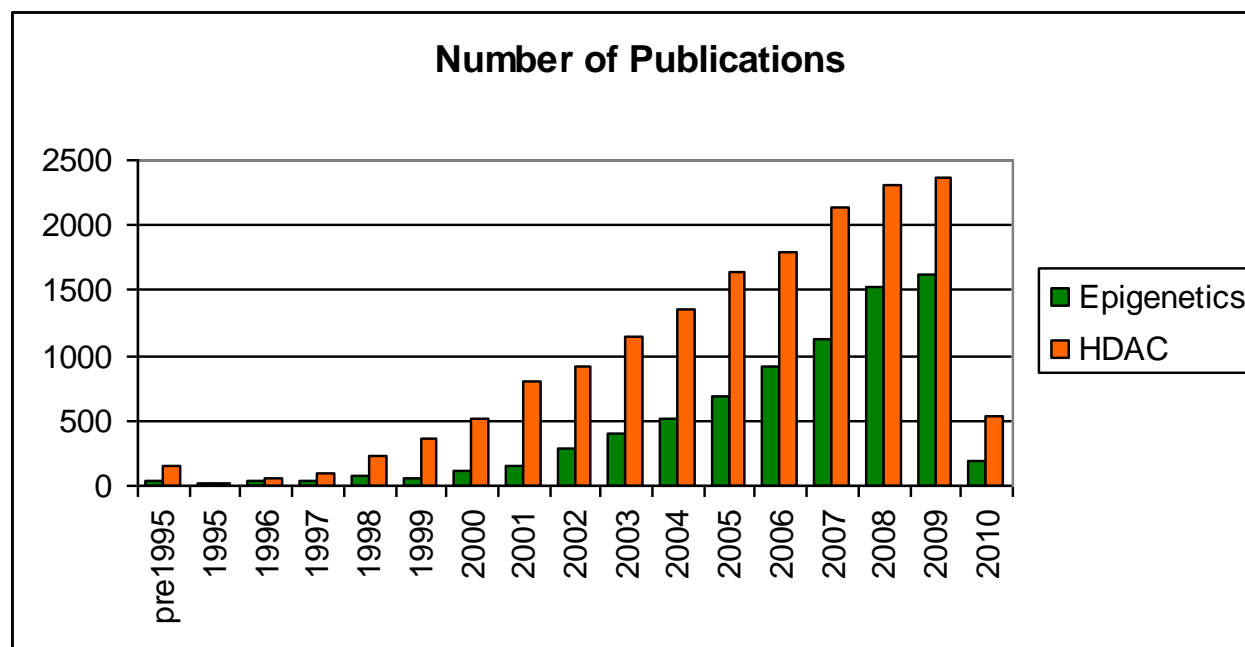
Epigenetics:

Methylome/Acetylome to Medicine

- Array-based approaches to identify targets and validated functionality of inhibitors
 - differences of methylation/acetylation between tissue diseases, esp in cancer
 - ligand-mediated PCR and methylated DNA immunoprecipitation
- Drug discovery and development
 - hope for agents that modify the epigenome globally
 - highly specific modulators of epigenetic target enzymes and pathways (e.g IGF-II signalling through IGF1R inhibition)

Current interest

- Epigenetics is a particularly “hot” area of current research with many large pharma companies involved together with several smaller biotechs dedicated to the area
 - CellCentric, Epizyme, Constellation, Structural Genomics Consortium...



Source: SciFinder®

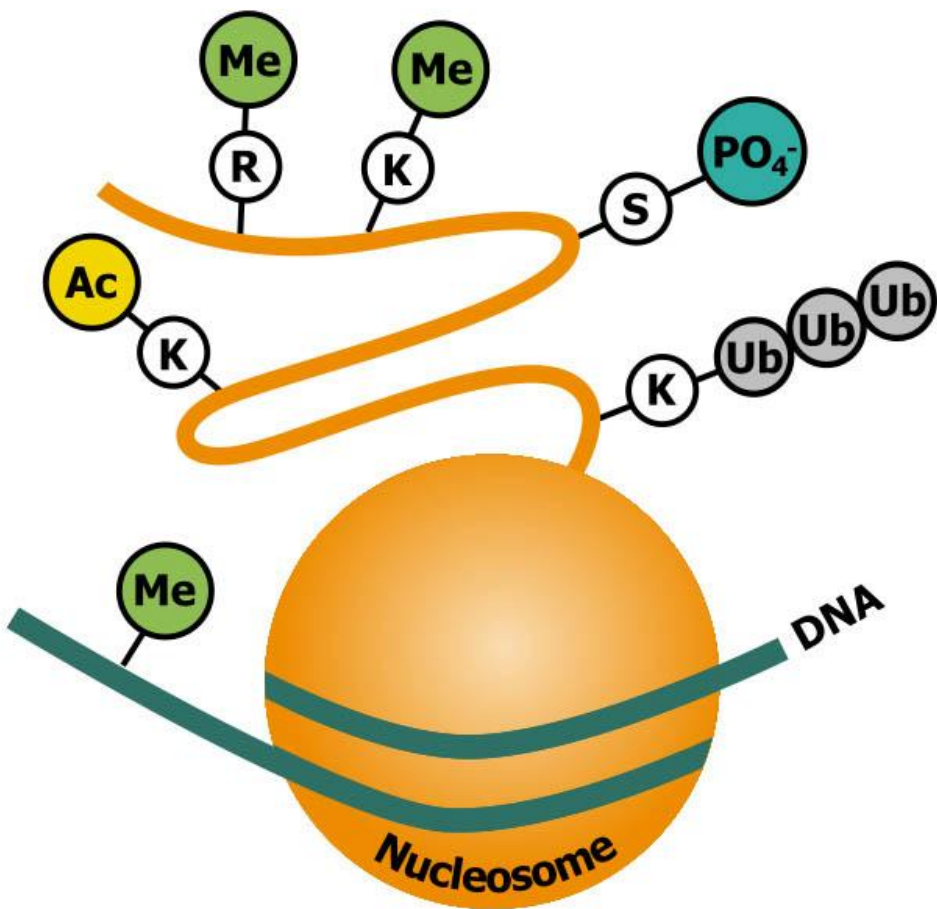
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Epigenetics – sub-classification

- Enzymes involved in epigenetics generally catalyse the transfer of small chemical groups (acetyl, methyl, phosphate)
- The area is sub-divided into the type of transfer that takes place
 - acetyltransferases/deacetylases
 - methyl transferases/demethylases
 - kinases/phosphatases
 - ubiquitin/SUMO Ligases

Epigenetics Targets



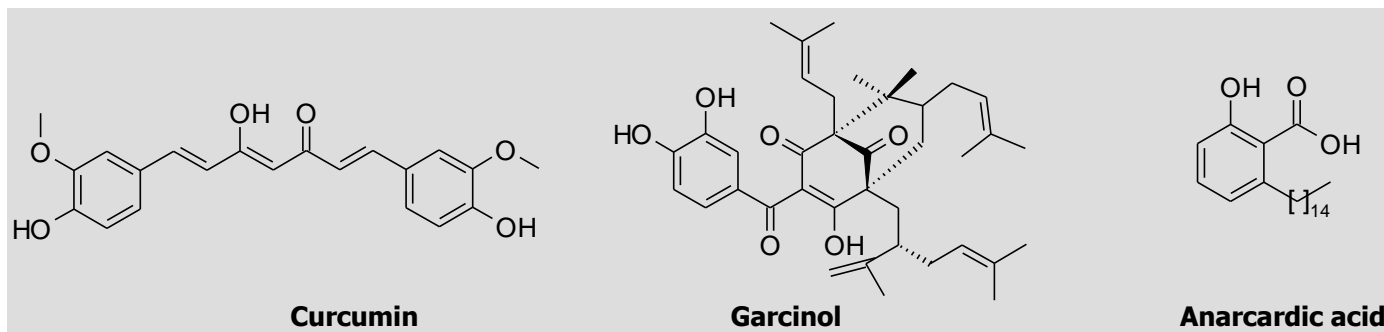


Acetylation/deacetylation

- Histone acetyl transferases (HAT) – widely unexplored family of enzymes
- Histone deacetylases (HDAC) – a small sub-family comprising ~18 enzymes, among the most widely studied sub-family in epigenetics with several clinical inhibitors known
 - provide/remove recruitment signals for non-histone proteins involved in transcriptional activation and silencing
 - change chromatin structure and physical interactions between histone and DNA

Histone acetyl transferases (HAT): emerging drug targets

- Target family involved in cancer, asthma, COPD, viral infections (HIV), learning, memory
- Main families (often part of larger multiprotein complexes):
 - GNAT, P300/CBP, MYST, Rtt109
- Known inhibitors:
 - curcumin, garcinol, bisubstrate inhibitor PCAF and p300, anacardic acid





Acetylome analysis

- Novel acetylation sites discovered
- Actively transcribed chromatin is hyperacetylated

Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions

Chunaram Choudhary,^{1,2} Chanchal Kumar,¹ Florian Gnad,¹ Michael L. Nielsen,^{1,2} Michael Rehman,³ Tobias C. Walther,³ Jesper V. Olsen,^{1,2} Matthias Mann^{1,2*}

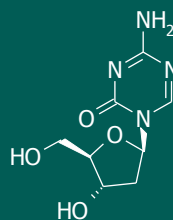
Lysine acetylation is a reversible posttranslational modification of proteins and plays a key role in regulating gene expression. Technological limitations have so far prevented a global analysis of lysine acetylation's cellular roles. We used high-resolution mass spectrometry to identify 3600 lysine acetylation sites on 1750 proteins and quantified acetylation changes in response to the deacetylase inhibitors suberoylanilide hydroxamic acid and MS-275. Lysine acetylation preferentially targets large macromolecular complexes involved in diverse cellular processes, such as chromatin remodeling, cell cycle, splicing, nuclear transport, and actin nucleation. Acetylation impaired phosphorylation-dependent interactions of 14-3-3 and regulated the yeast cyclin-dependent kinase Cdc28. Our data demonstrate that the regulatory scope of lysine acetylation is broad and comparable with that of other major posttranslational modifications.

From: Choudhary C et al, Science. 2009 Aug 14;325(5942):834-40

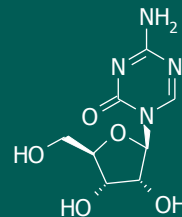
Methylation/demethylation

- Histone methyl transferases (HMT) add a methyl group to the side chain of an Arginine (R) or Lysine (K) residue
 - ~70 family members known
- Histone demethylases (HDM) responsible for removal of a methyl group
 - ~30 family members known
- DNA methyltransferases (NMT): two marketed drugs

Launched/NDA



decitabine

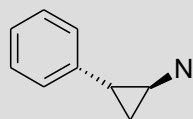


5-Azacytidine

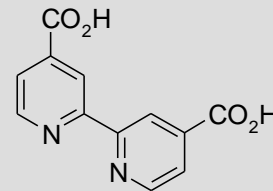
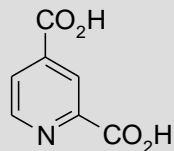
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Histone Demethylases

- LSD1 discovered in 2004 by Shi et al
 - methylation is reversible!
 - lead to the revision of functional roles of HMTs
- Lysine residue modification: mono, di and trimethylation
- Arginine residue modification: mono, dimethylation, symmetric and asymmetric



Trancypromine
(an MAO inhibitor)
LSD1 inhibitor



JMJ inhibitors



Histone Demethylases - Families

- LSD – two functions
 - repressor of neuronal genes in non-neuronal cells (part of CoREST)
 - bound to Androgen Receptor: change in substrate specificity
- Jmjc family:
 - 27 members, can remove all 3 methyls
- PADI4
 - demethylation leads to citrulline (not a true demethylase)
- Diseases:
 - cancer, development, metabolic diseases
 - inhibitors are known for PADI4 and LSD1

▪ Lit. Natoli G et al, Current Opinion Drug Discovery and Development 2009, 12 (5) 607-613



Histone Demethylases

Enzyme family	Enzyme	Substrate specificity
PADI	PADI4	H3R2, H3R8, H3R17, H3R26 and H4R3
LSD	LSD1	H3K4me2 and H3K4me1
	LSD2	ND
JMJC	JHDM1	H3K36me2 and H3K36me1
	JHDM2	H3K9me2 and H3K9me1
	JHDM3/JMJD2	H3K9me3, H3K9me2, K36me3 and K36me2
	JARID	H3K4me3 and H3K4me2
	PHF8/PHF2	ND
	UTX/UTY	ND
	JmjC only	Asn hydroxylation and ND

JhDM, JmjC-domain-containing histone demethylase; JMJC, PADI, peptidylarginine deiminase; Jumonji-C; ND, not determined

From: Klose RJ and Zhan Y, Nature Reviews, Molecular cell biology, Vol8, 2007, 307pp

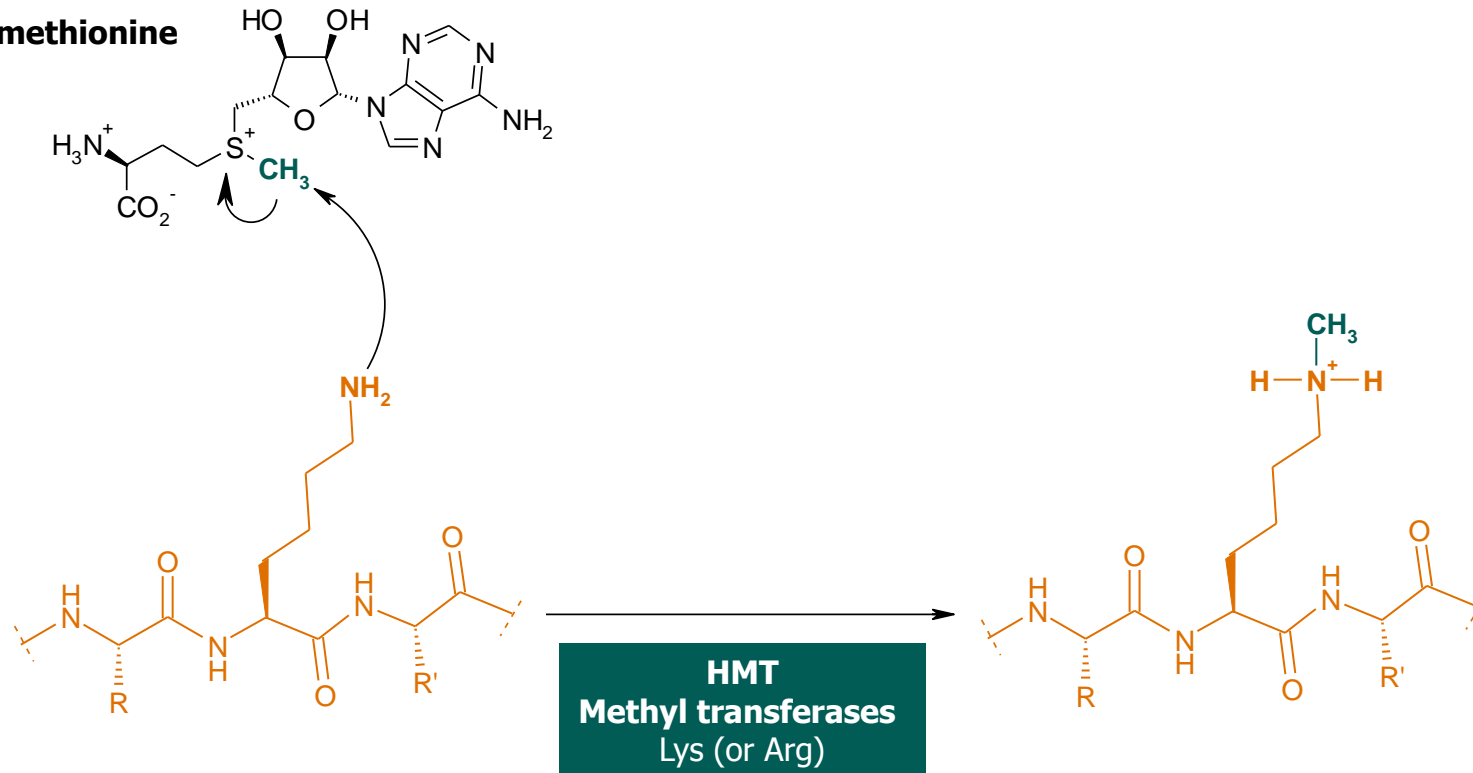


Histone Methyltransferases (HMT)

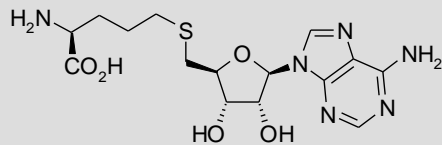
- Important role in regulation of gene-expression patterns
- No change in charge, but basicity, hydrophobicity
 - leads to changed affinity to other proteins
 - no effect on DNA-protein interaction
- Families:
 - SET domain lysine MT (mono, di, trimethylation)
 - non-SET domain lysine MT (mono, di, trimethylation)
 - arginine MT (mono, dimethylation, symmetric, non-symmetric)
- Other target proteins known

Methylation

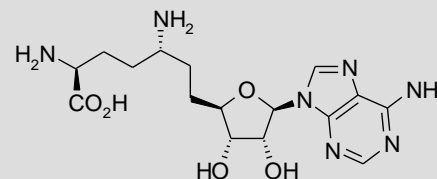
S-adenosyl methionine



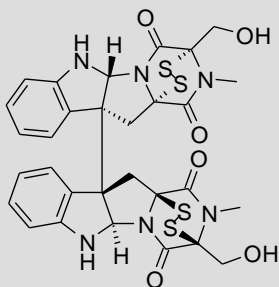
Methyl transferase inhibitors – non-clinical



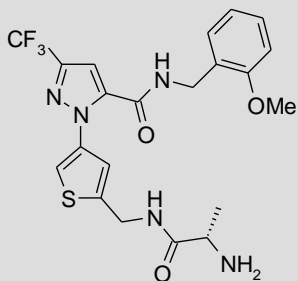
SAH - S-adenosyl homocysteine



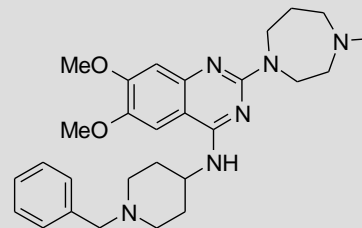
Sinefungin



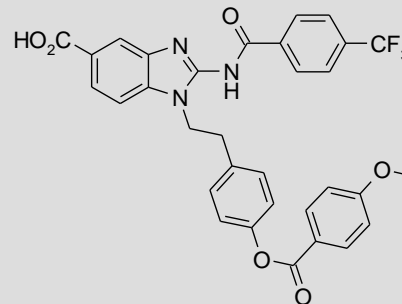
Chaetocin



BMCL, 2008, 18, 4338



BIX-01294



BIX-01338

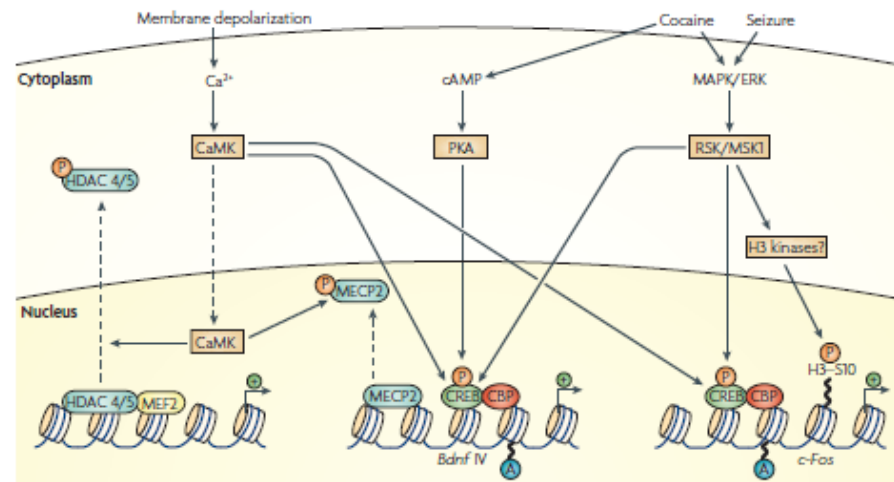


Methyl transferases in cancer

PMT	Methylation substrate	Cancer
SUV39H1	H3K9	Colon
EHMT2 (KMT1C)	H3K9	Lung, prostate, hepatocellular carcinoma
MLL (KMT2A)	H3K4	Leukaemia
NSD1	H3K36	Acute myeloid leukaemia (AML)
WHSC1 (NSD2)	H3K36 / H4K20	Myeloma
WHSC1L (NSD3)	H3K4	Lung and breast. Childhood AML
DOT1L (KMT4)	H3K79	MLL-rearranged leukaemias
SMYD3	H3K4	Breast, liver, colon, gastric
EZH2 (KMT6)	H3K27	Breast, liver, colon, gastric, prostate, bladder
SETD7 (KMT7)	H3K4	Breast
PRDM14	<i>Unknown</i>	Breast
CARM1 (PRMT4)	H3R17, KMT6, NCOA3	Breast, prostate
PRMT5	H3R8, p53, SMD1&3, SUPT5H	Lymphoma
KMTD1 (Eu-HMTase1)	ND	Gland tumors
SMYD3 (KMT4)	ND	Suppression of p53 dependent transcription
SET8/PR-SET7	ND	Suppression of p53 dependent transcription

Phosphorylation

- Kinases, e.g. AurB, GSK3Beta, JAK2
- Intracellular signalling cascades for regulation of chromatin remodelling; direct and indirectly regulated via kinases



From: Tsankova et al, Nature Reviews Neuroscience, Vol8, 2007, 355ff



Other enzyme classes

- Ubiquitin ligases: addition of ubiquitin to lysine residues
- Deubiquitinases
- SUMO ligases (covalent addition of SUMO = ubiquitin related modifier proteins)

Hit finding strategies

target discovery

compound collections

natural products

assay development

Accelerating Drug Discovery

hit finding hit to lead
lead optimization



Hit finding – chemical space

- “Epigenetics targets present several challenges for the development of small-molecules inhibitors.....many of these targets are in enzyme classes that are poorly covered by existing therapeutics.”

Best, J.D. and Carey, N. Drug Discovery Today, 2010,

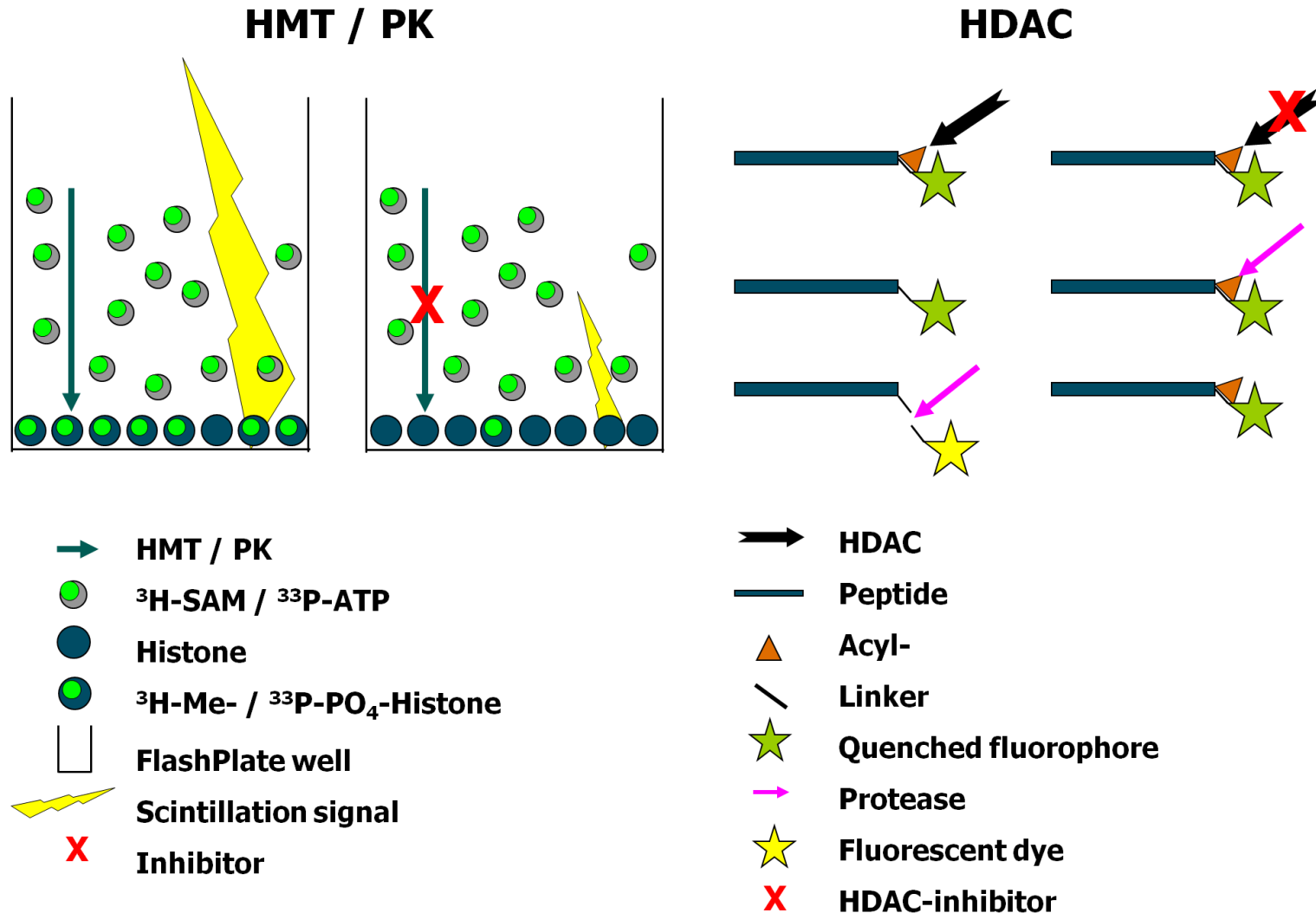
- Therefore compound collections that are heavily based around compounds synthesized or selected for previous projects are unlikely to provide useful starting points
- Is accessing the largest diversity across chemical space the best way forward?



Epigenetics targets and assay formats

- Assay format HMT: detection of ^3H -Me incorporated in histones from ^3H -SAM on Flashplates
- Assay format HDAC: fluorescence intensity from self-quenched, deacetylated substrate after protease cleavage
- Caliper LabChip platform assays are currently established for histone acetyltransferase, deacetylase, demethylase and methyltransferase
- Binding assays and kinetic interaction studies on SPR platform

Assay principles HMT and HDAC





Screening collection

- BioFocus has the largest screening deck of any Contract Research Organisation
 - >900,000 compounds
- Covering the greatest amount of chemical space ensures a better chance of identifying hit compounds
- Coupled with BioFocus' computational and chemoinformatics expertise in compound selection access the largest chemical space whilst screening smaller numbers of compounds
- The collection is constantly maintained with new compounds added annually, and degraded compounds removed



Screening libraries

- Access to 4 key compound collections

Diverse Compound Library

- ~830,000 compounds
- >60,000 scaffolds represented
- Selected for diversity & purity

SoftFocus® Libraries

- >60,000 compounds
- Chemogenomically designed compounds
- GPCR's, kinases, ion channels, PP

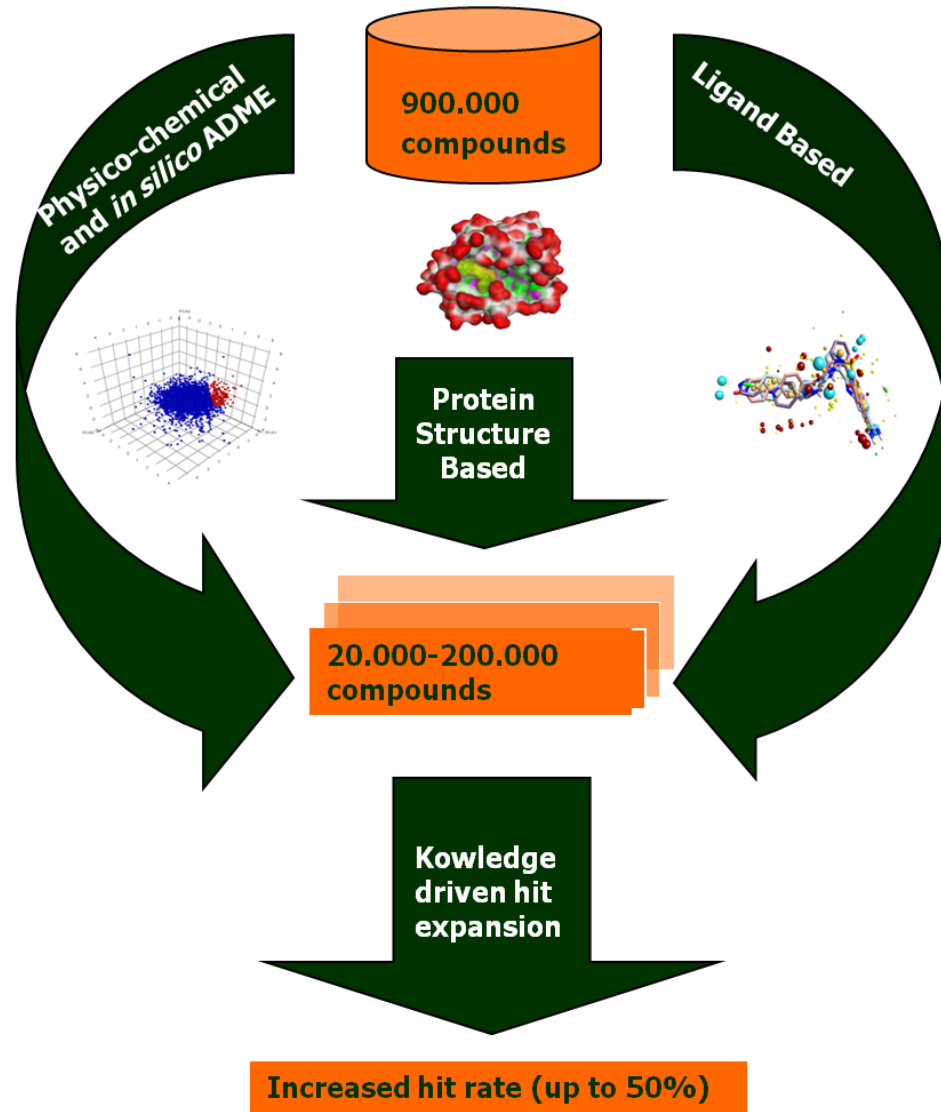
Pre-purified natural products

- >140,000 subfractions
- Derived from actinomycetes & fungi

Virtual screening database

- >4 million compounds
- Virtual screening & docking
- 2D and 3D pharmacophore & similarity searching

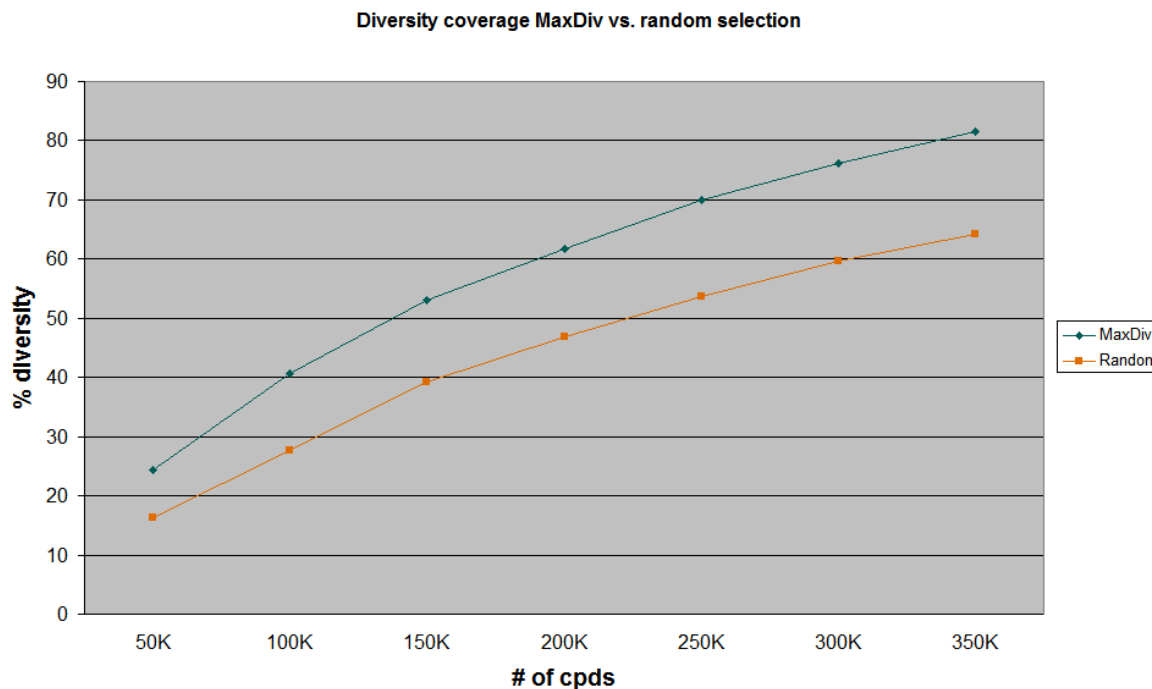
Intelligent Compound Selection



Selection Strategy

Diversity coverage

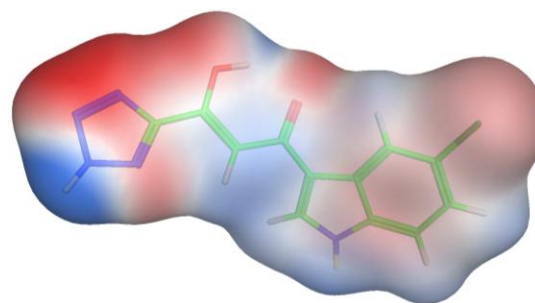
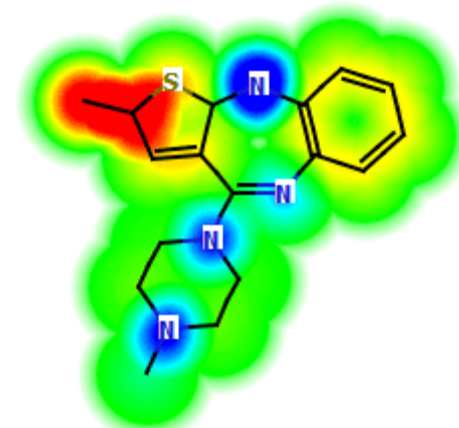
- With the MaximumDiverse approach, optimal balance between diversity coverage and bias can be found
 - by selecting 350'000 compounds, 82% of the overall diversity would be covered



Compound Selection Strategy

Property-based

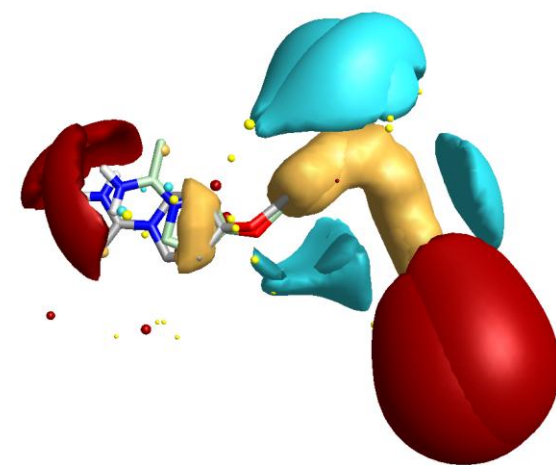
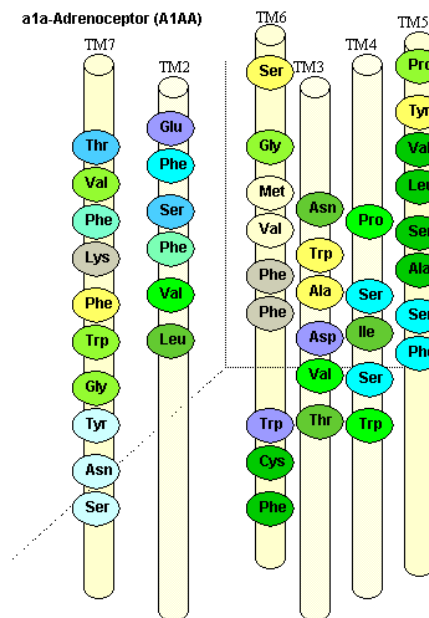
- Optimize or balance physico-chemical property profile for intended therapeutic area
- Chemoinformatics
 - chemical and property space analysis, e.g. with PCA
- Calculation of *in silico* ADME parameters with Stardrop
 - standard lead- and drug-likeness criteria
 - blood-brain barrier penetration (BBB)
 - PSA, logD, logS
 - CYP P450 affinity models
 - HIA and P-gp binary categories



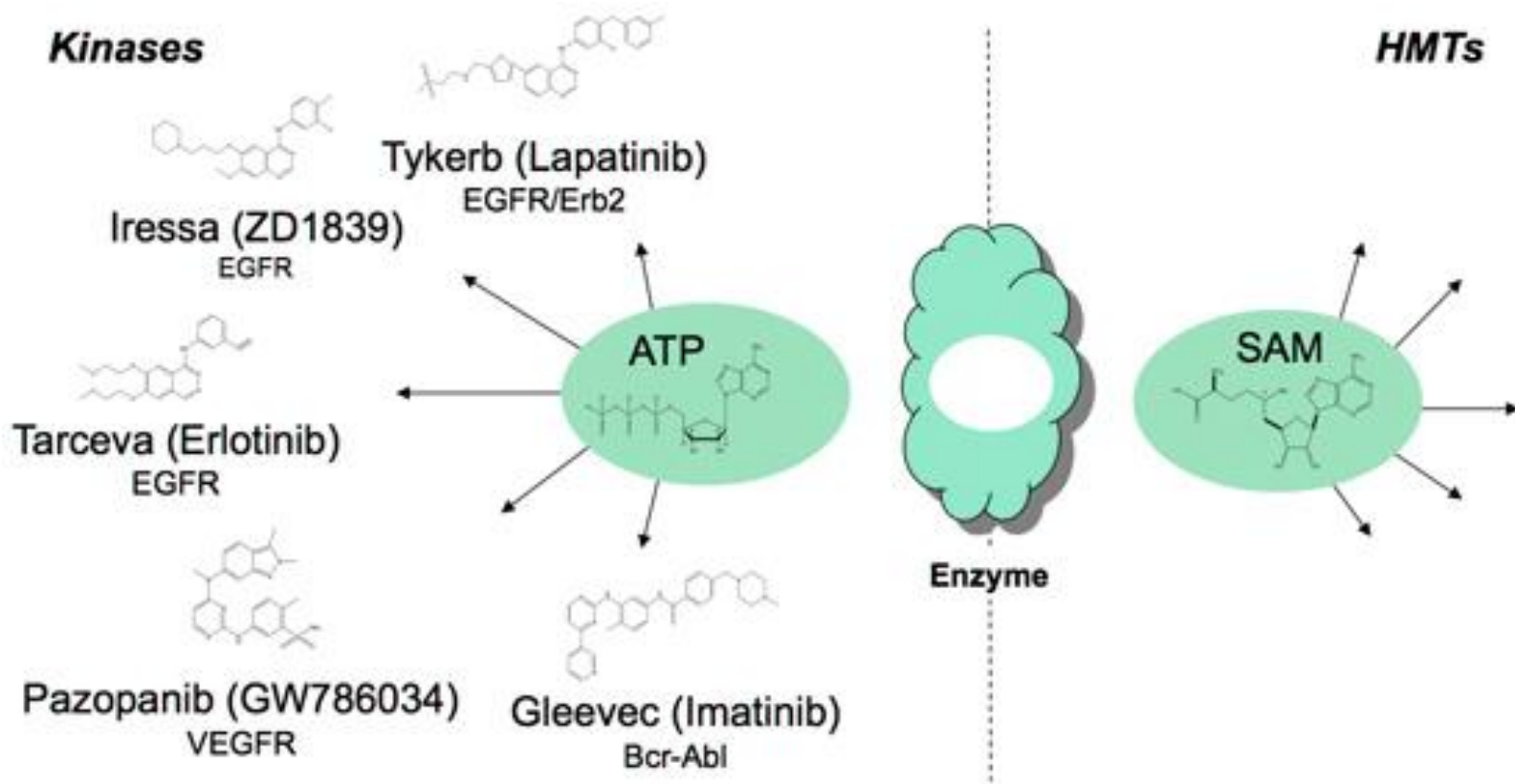
Compound Selection strategy

Ligand-based

- Compilation of information from various sources
- Generation of bioactive conformation hypothesis
 - Cresset field-based approaches
- 3D-Pharmacophore models and searches
- QSAR methods
 - qualitative and quantitative 3D-(Q)SAR methods
 - descriptor-based activity models (e.g. MLR-GA)
- Chemoinformatics-based active-likeness scoring

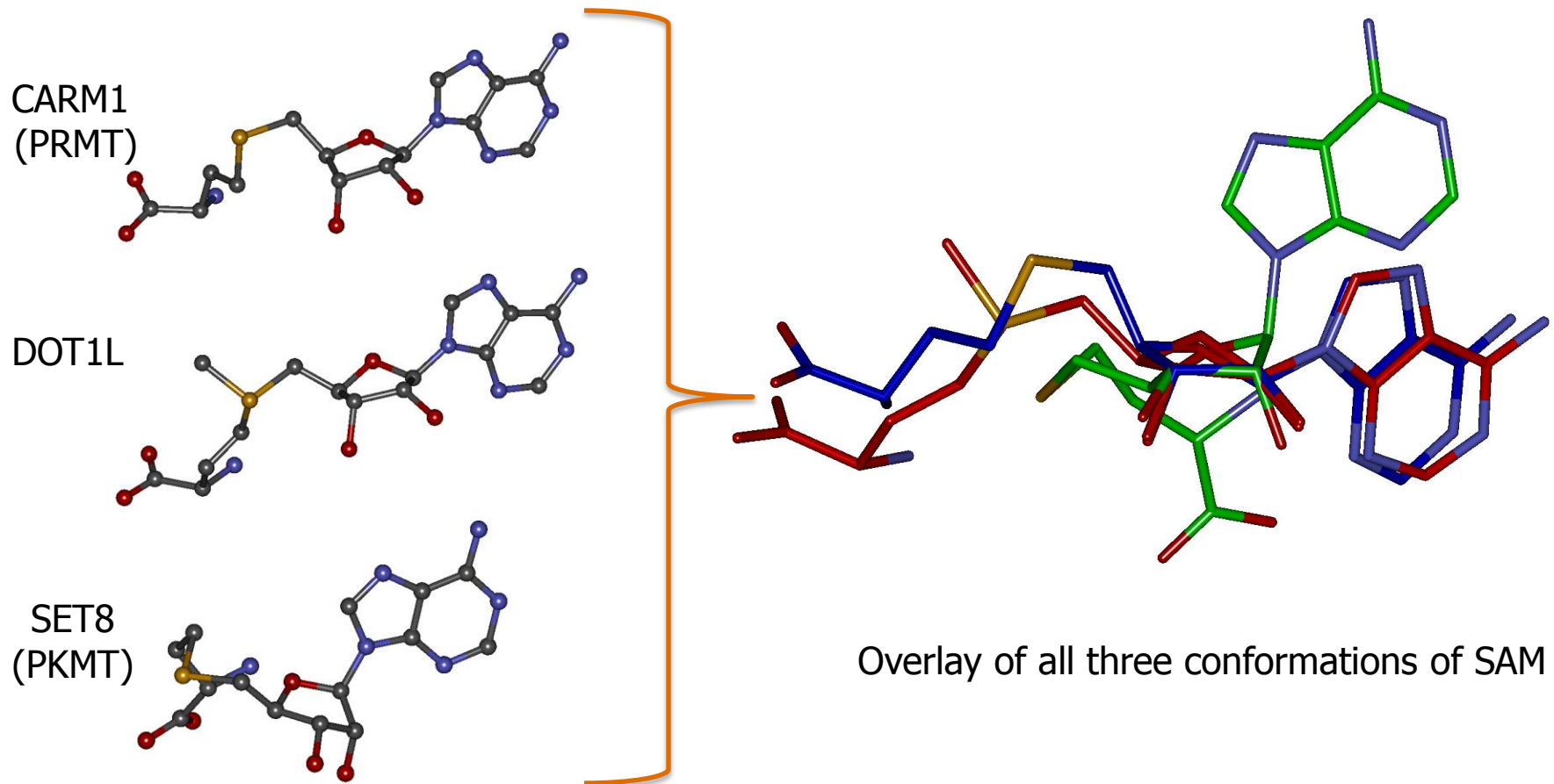


SAM binding as common feature for HMTs



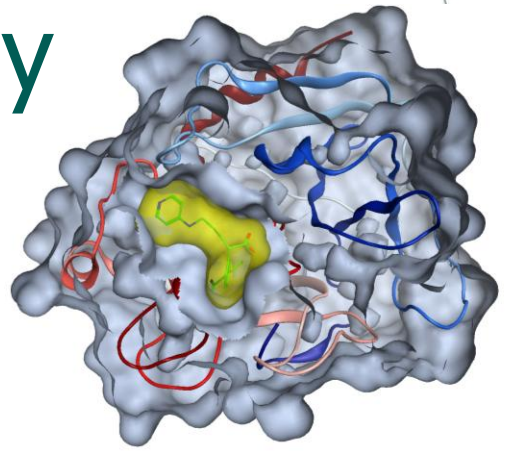
From: Copeland RA et al, Nat Rev Drug Discov. 2009 Sep;8(9):724-32. Review.

SAM binding as common feature for HMTs – conformational change

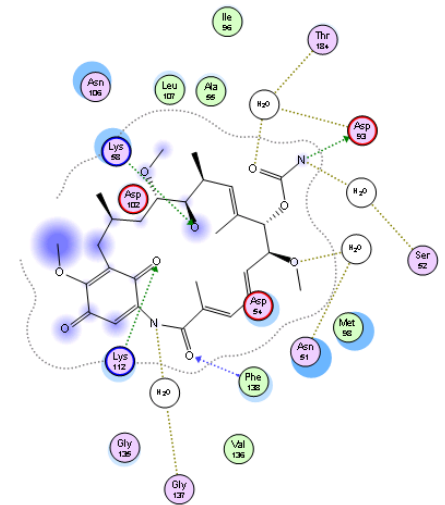


Compound Selection Strategy

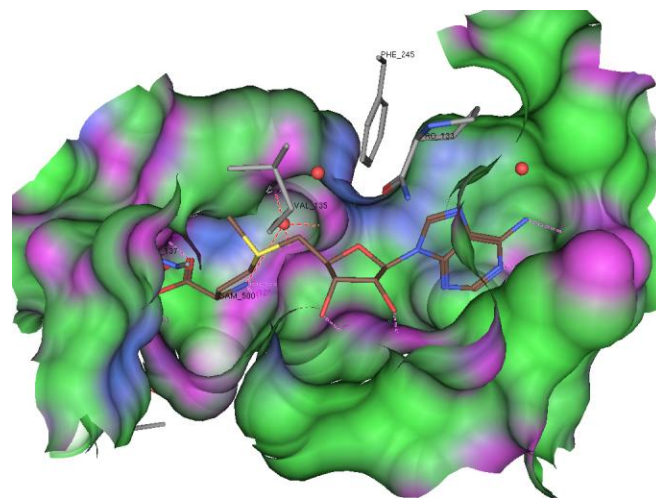
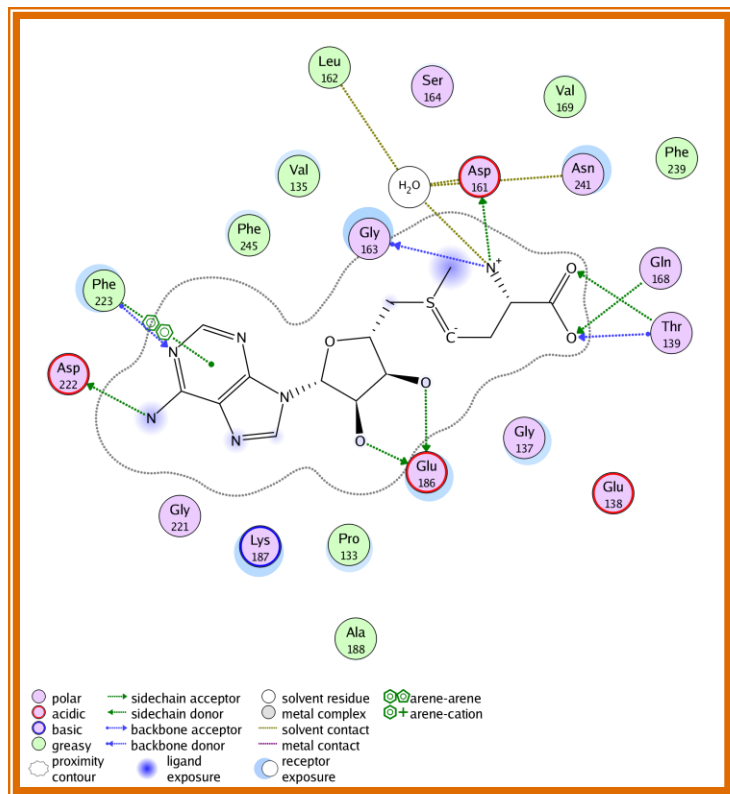
3D structure-based



- Homology model generation, if applicable
- Protein preparation
 - detailed analysis and comparison of available structures, including
 - crystal packing effects
 - protein flexibility
 - hydration pattern
 - select the best model structure(s) and ensure confidence in the binding site(s)
 - validation and optimization of docking protocol
- High-throughput docking on cluster
- Knowledge-based post-filtering using proprietary toolkit



HMT crystal structures

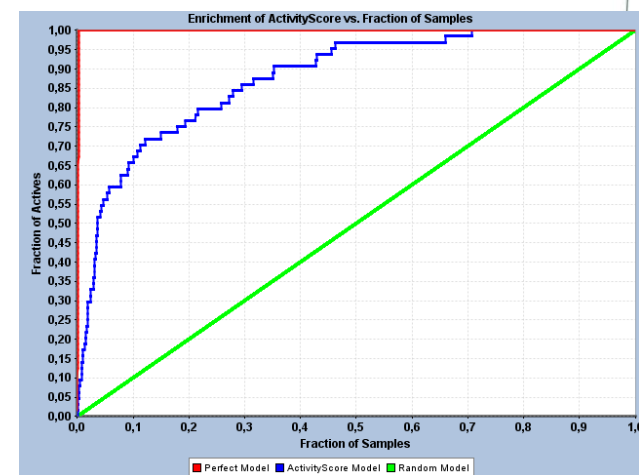
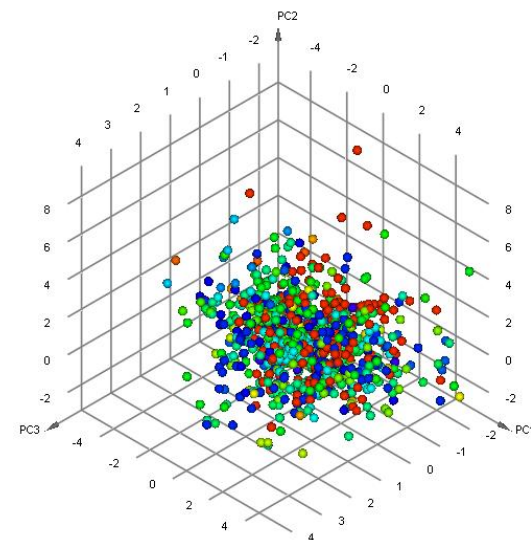


- Crystallisable targets
- Enclosed binding site (between SAH binding domain and barrel like domain)
- SAM binding but several different folds
- Diversity of interaction points

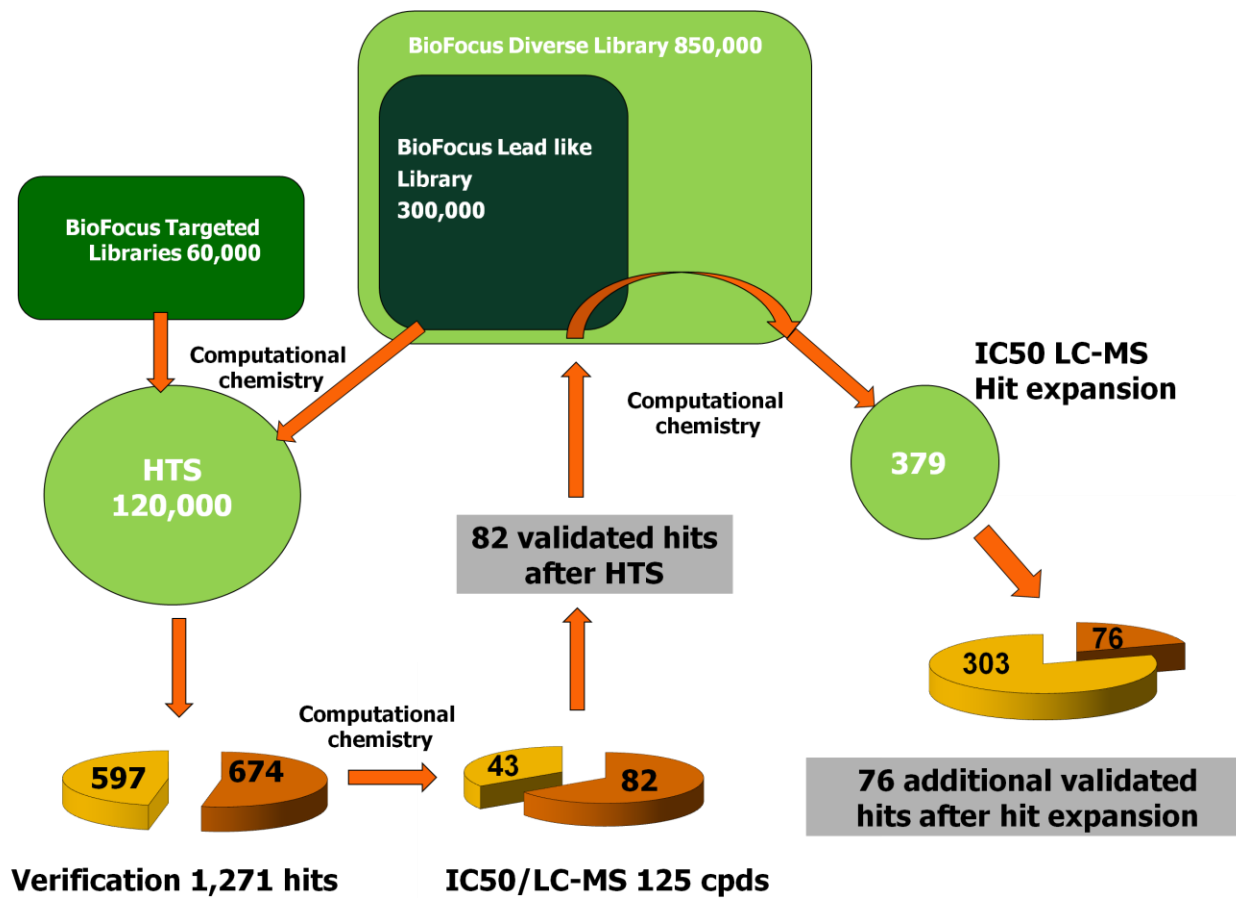
Hit Expansion

Purpose and Concepts

- Consolidate and expand knowledge about active regions in chemical space
 - find derivatives of active scaffolds and establish or broaden SAR information
 - rescue of active scaffolds from low-activity data
 - scaffold hopping
 - strengthen knowledge base for direct use in H2L campaign
- Ability to distill knowledge from HTS, and direct selection towards potent hits



Case study: epigenetics targets





Cellular follow up assays in Epigenetics

MOA analyses of compounds

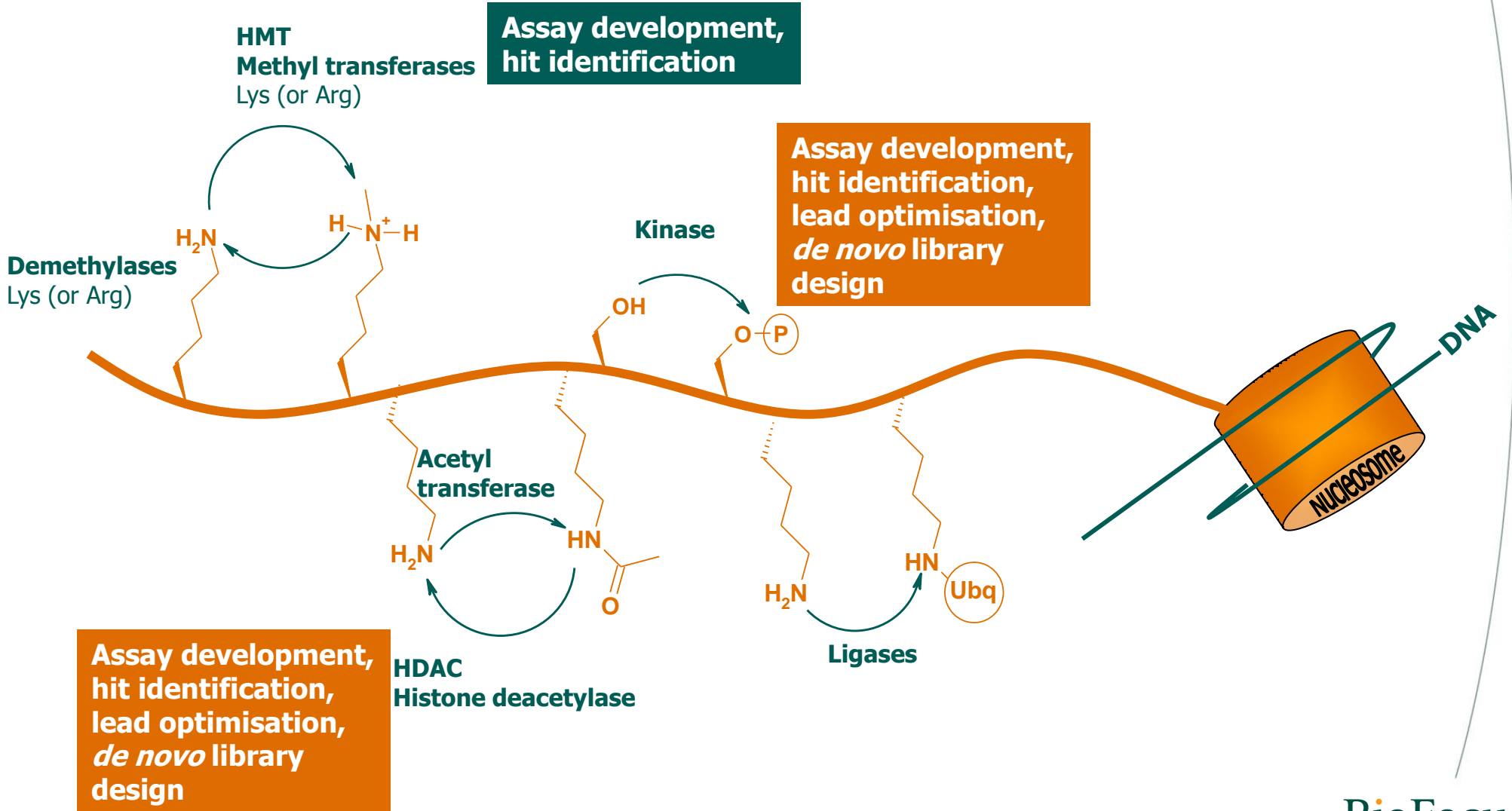
- Histone hypomethylation assay (Spannhoff et al, J Med Chem 50, 2319-2325)
 - HepG2 cells
 - H-Me specific mAbs, 2nd Ab Eu-labelled, TR-FI read-out
- Cellular HAT/HDAC ELISA (Aherne et al, Methods 26, 245-253)
 - HCT116 or HT29 colon carcinoma cells
 - 1st Ab anti H3-Ac, 2nd Ab Eu-labelled, TR-FI read-out
- Reporter gene functional assays
- Apoptosis assays
- ChIP assays to assess chromatin modifications of known downstream targets
- ChIP-chip and ChIP-seq for genome wide identification of transcriptional targets



Hit finding experience epigenetics targets

- Several assay development and hit finding projects with a variety of different epigenetics targets successfully completed
- Hit finding and consecutive hit expansion actives from entirely novel chemotypes identified
- Profiling of hits against other family members
- Cellular confirmation and mode of action studies with primary screening hits

Epigenetics Targets





Summary

- Intelligent selection of Screening decks with chemoinformatics tools
- Significantly increased hit rates through hit expansion
- Track record for rapid epigenetics target hit discovery

 Fast-track to novel chemical entities inhibiting epigenetics targets



Useful References/Weblinks

- Histone sequence database: <http://genome.nhgri.nih.gov/histones/>
- Human epigenome consortium: <http://www.epigenome.org/>
- Epigenome network of excellence: <http://epigenome.eu/>
- Natoli G. et al, Current Opinion Drug Discovery and Development 2009, 12 (5) 607-613
- Best J.D. and Carey N. Drug Discov Today. 2010 Jan;15(1-2):65-70. Epub 2009 Nov 6.
- Klose RJ and Zhan Y, Nature Reviews, Mol cell biol, Vol8, 2007, 307pp
- Copeland RA et al, Nat Rev Drug Discov. 2009 Sep;8(9):724-32. Review.
- Spannhoff et al, J Med Chem 50, 2319-2325
- Feinberg AP, JAMA, 2008 Mar 19; 299 (11), 1345-50

 Thank you!

target discovery
compound collections
natural products
assay development
Accelerating Drug Discovery
hit finding *hit to lead*
lead optimization

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