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# **Chemoproteomic approach to epigenetic drug targets**

Gerard Joberty

**Introduction to epigenetic Drug Discovery  
Chesterford Research Park, March 28th, 2012**

# Chemoproteomic approach to epigenetic drug targets

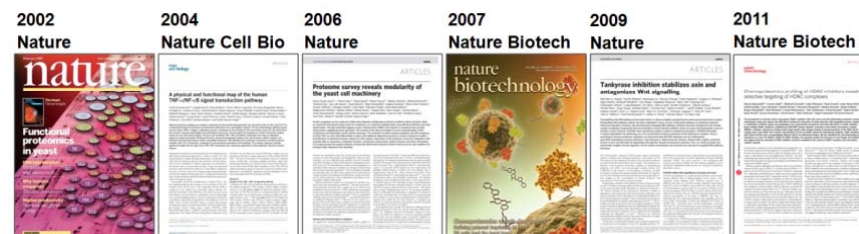
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- Introduction
- Chemoproteomics target profiling of HDAC inhibitors
- Tripartite interaction proteomics: BET complexes



# Who we are ..

- Biotech company spun off from the EMBL in Heidelberg
- 100 people in Heidelberg (Germany) and in Cambridge (UK)
- Use of Chemoproteomics Platform to discover small molecule drugs for novel targets (kinases, epigenetic enzymes and reader proteins)
- Therapeutic focus on chronic inflammation and oncology
- Collaborations with Pharma and Academia



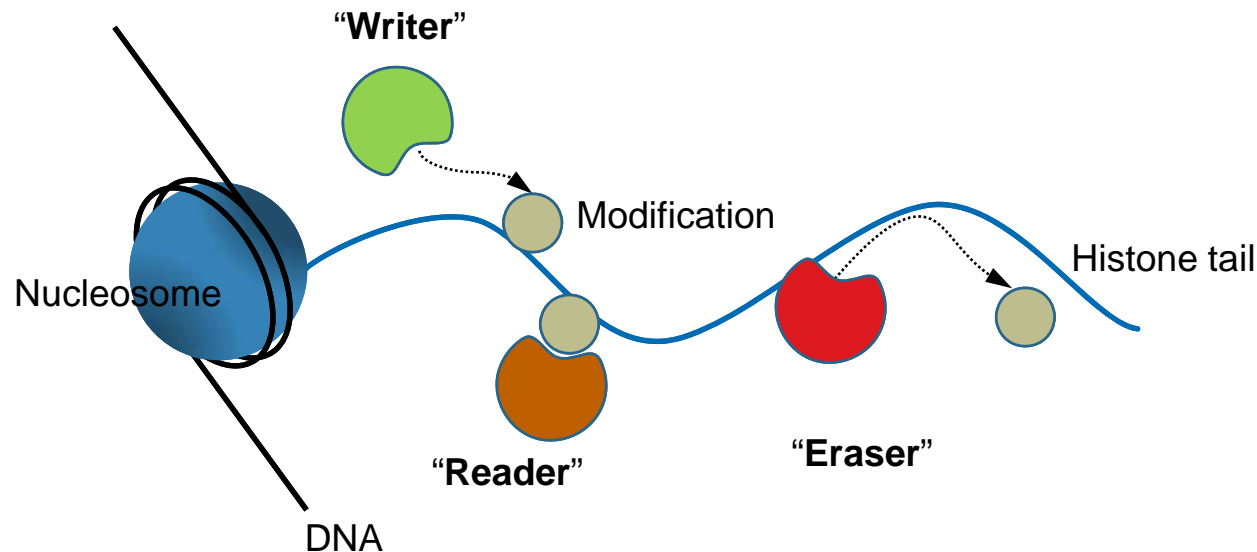
## 2012 Nature Chem Bio, in press

**Selective small molecule inhibitor discovered by chemoproteomic assay platform reveals regulation of Th17 cell differentiation by PI3K $\gamma$**

Giovanna Bergamini<sup>1</sup>, Kathryn Bell<sup>2</sup>, Satoko Shimamura<sup>1</sup>, Thilo Werner<sup>1</sup>, Andrew Cansfield<sup>2</sup>, Katrin Müller<sup>1</sup>, Jessica Perrin<sup>1</sup>, Christina Rau<sup>1</sup>, Katie Ellard<sup>2</sup>, Carsten Hopf<sup>2</sup>, Carola Doce<sup>1</sup>, Daniel Leggate<sup>2</sup>, Raffaella Mangano<sup>2</sup>, Toby Mathieson<sup>1</sup>, Alison O'Mahony<sup>4</sup>, Ivan Plavec<sup>4</sup>, Faiza Rharbaoui<sup>1</sup>, Friedrich Reinhard<sup>1</sup>, Mikhail M. Savitski<sup>1</sup>, Nigel Ramsden<sup>2</sup>, Emilio Hirsch<sup>3</sup>, Gerard Drewes<sup>1</sup>, Oliver Rausch<sup>2</sup>, Marcus Bantscheff<sup>1\*</sup> and Gitte Neubauer<sup>1\*</sup>

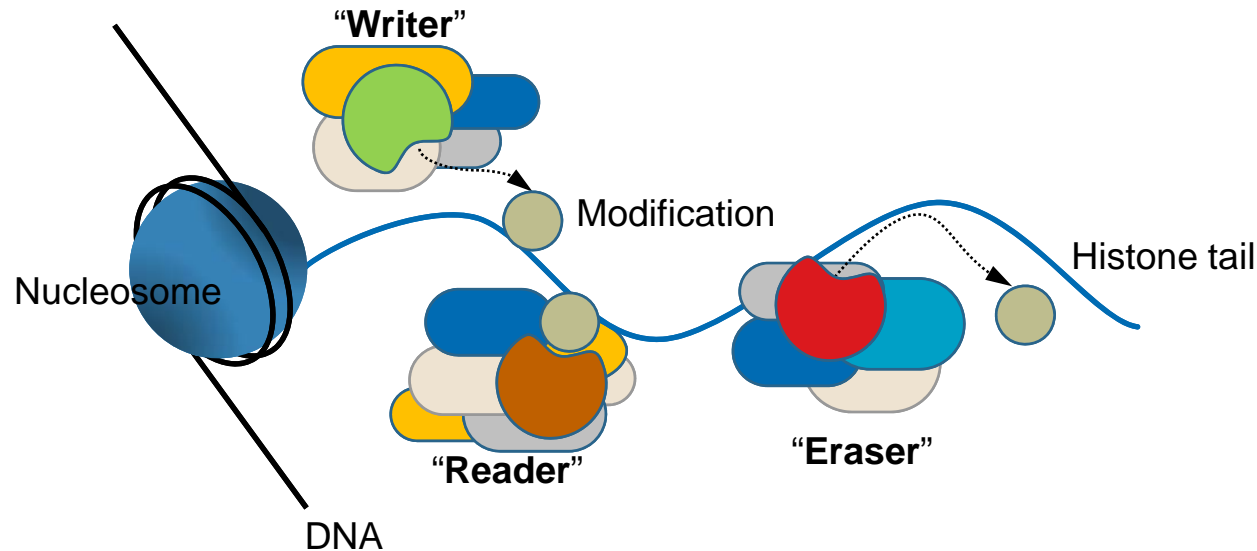
# Epigenetic Target classes

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- **'Writers'** – enzymes that add modifications to histones: Methyltransferases, Acetyltransferases...
- **'Erasers'** – enzymes that take modifications off histones: Demethylases, Deacetylases...
- **'Readers'** – proteins that recognise the modifications: Bromodomains (acetylated lysines), Chromodomains, PHD domains (methylated lysines)

# Epigenetic Targets Operate in Large Protein Complexes



- Epigenetic targets are part of large multi-protein complexes
- Complex components regulate activity, location and specificity of enzymes
- *Action of drugs is determined by interaction with entire protein complex*

# Epigenetic Enzymes as Drug Targets

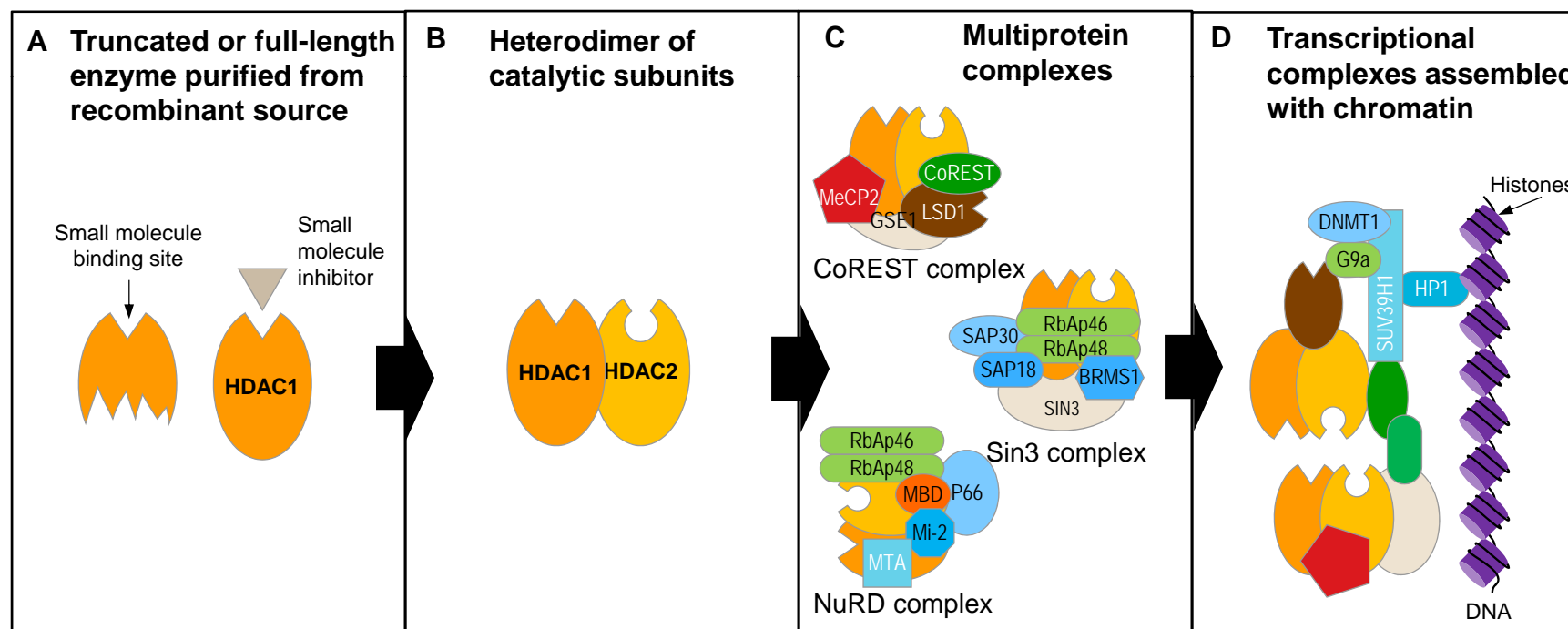
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- Opportunity for selective inhibitors with well understood MoA
- First small molecule inhibitors have been approved for cancer:
  - First demonstration of beneficial effect targeting epigenetic enzymes
  - Non-selective HDAC inhibitors (Zolinza®, Istodax®)
- But: lack of suitable assays hampers lead optimization
  - Often unreliable data with recombinant proteins
  - Selectivity cannot be measured reliably

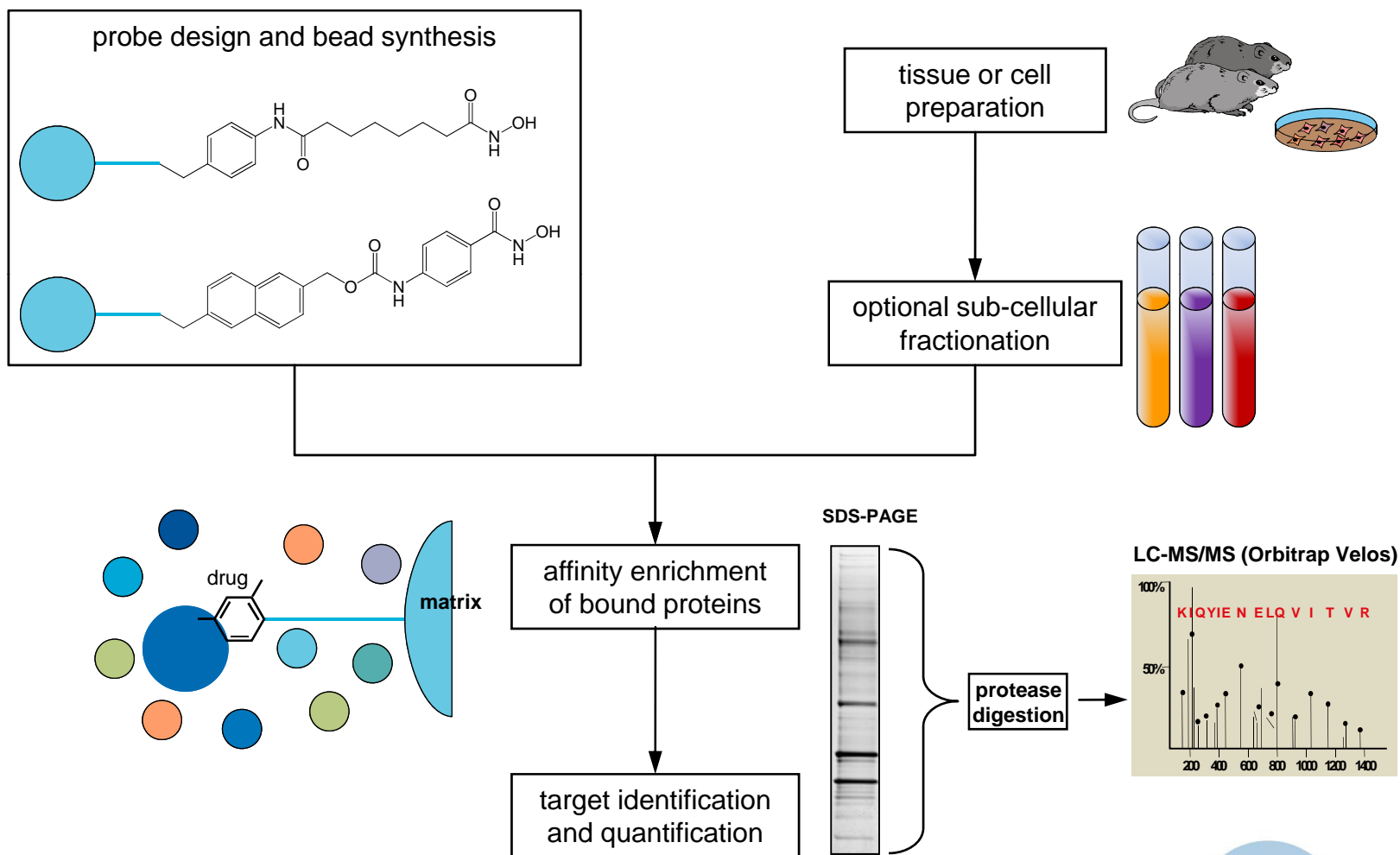
Target class	members
Histone K/R Methyltransferases	64
Histone Demethylases	33
Histone Acetyltransferases	21
<b>Histone Deacetylases (HDACs)</b>	<b>11</b>
Sirtuins	7
Poly [ADP-ribose] polymerases	17
<b>Bromodomain proteins</b>	<b>40</b>
Chromodomain proteins	29
Tudor domain proteins	40

# HDACs are catalytic subunits of megadalton protein complexes

Can we use these protein complexes directly for Target Profiling and Drug Discovery?



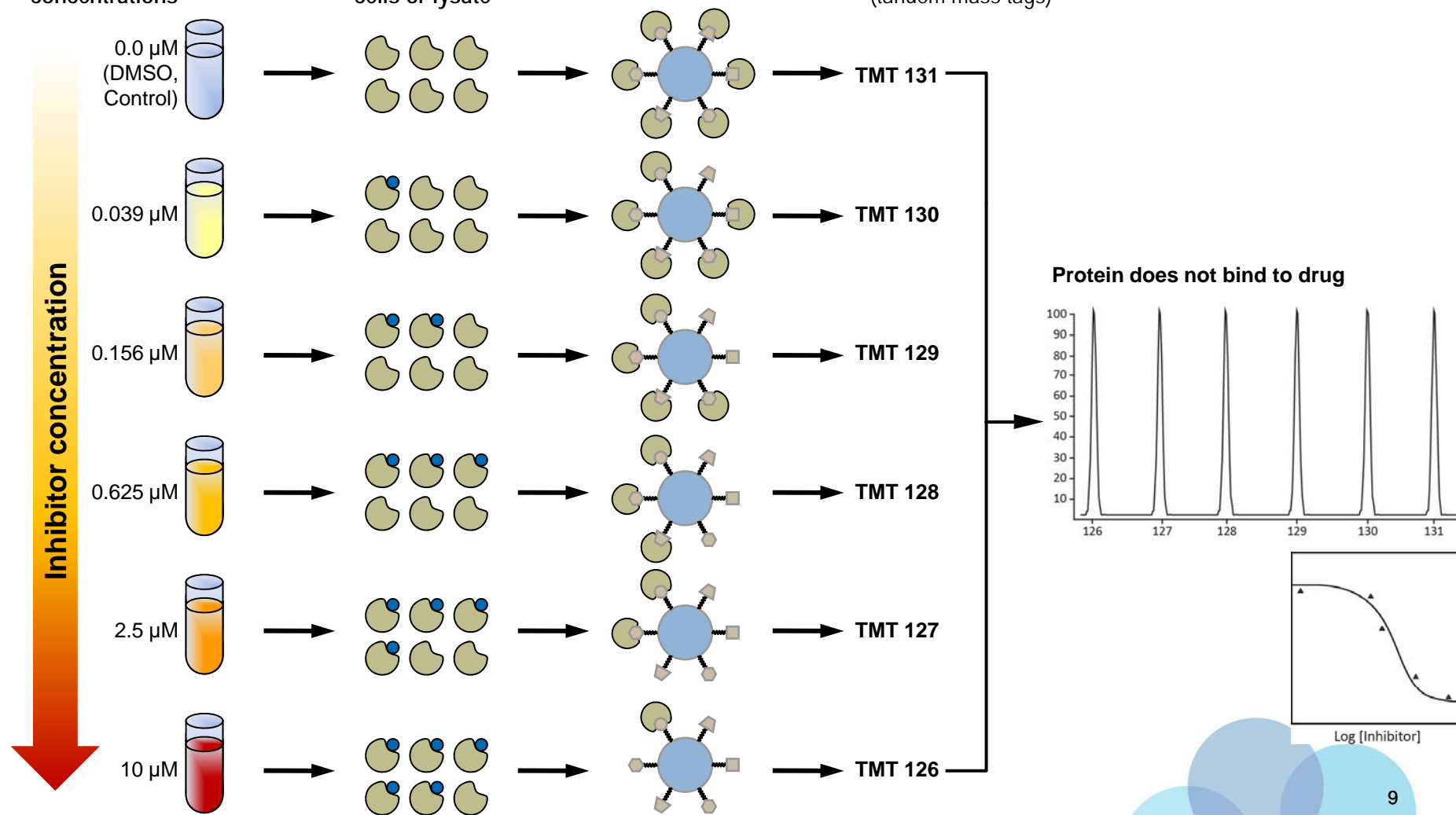
# Basic chemoproteomics workflow





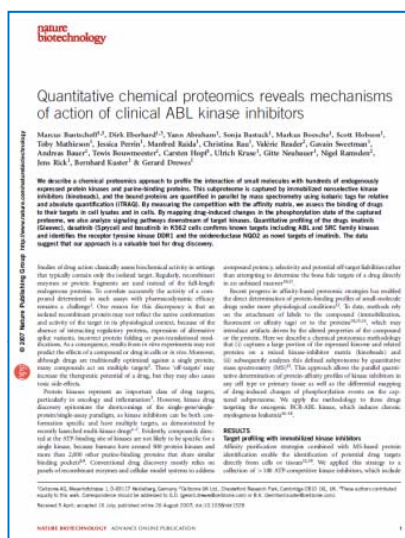
# Typical Experiment Design: Chemoproteomic competition assay

1. Add **inhibitor** to cells or cell lysate over a range of concentrations
2. Incubate - **inhibitor** binds to targets in cells or lysate
3. Add affinity matrix to lysates
4. Elute beads, digest, label with TMT6\* (tandem mass tags)
5. Mix, run LC-MS/MS and quantify in MS/MS spectrum

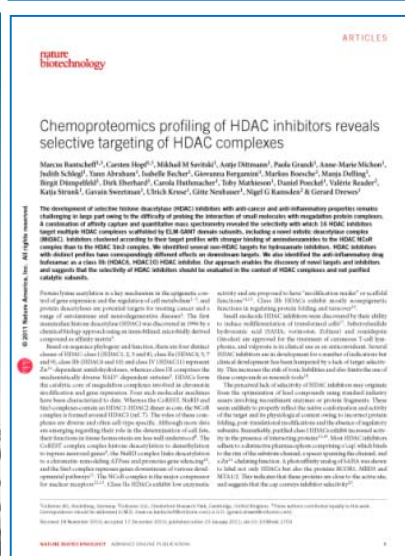
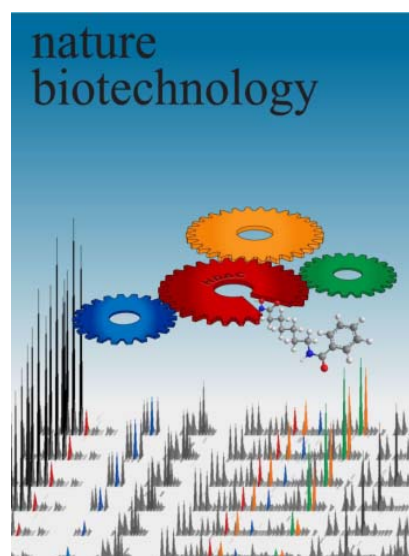


# Target Classes

- Protein kinases  
(9/2007)



- HDACs  
(3/2011)



- PARPs
- K-Methyltransferases
- K-Demethylases
- Bromodomains

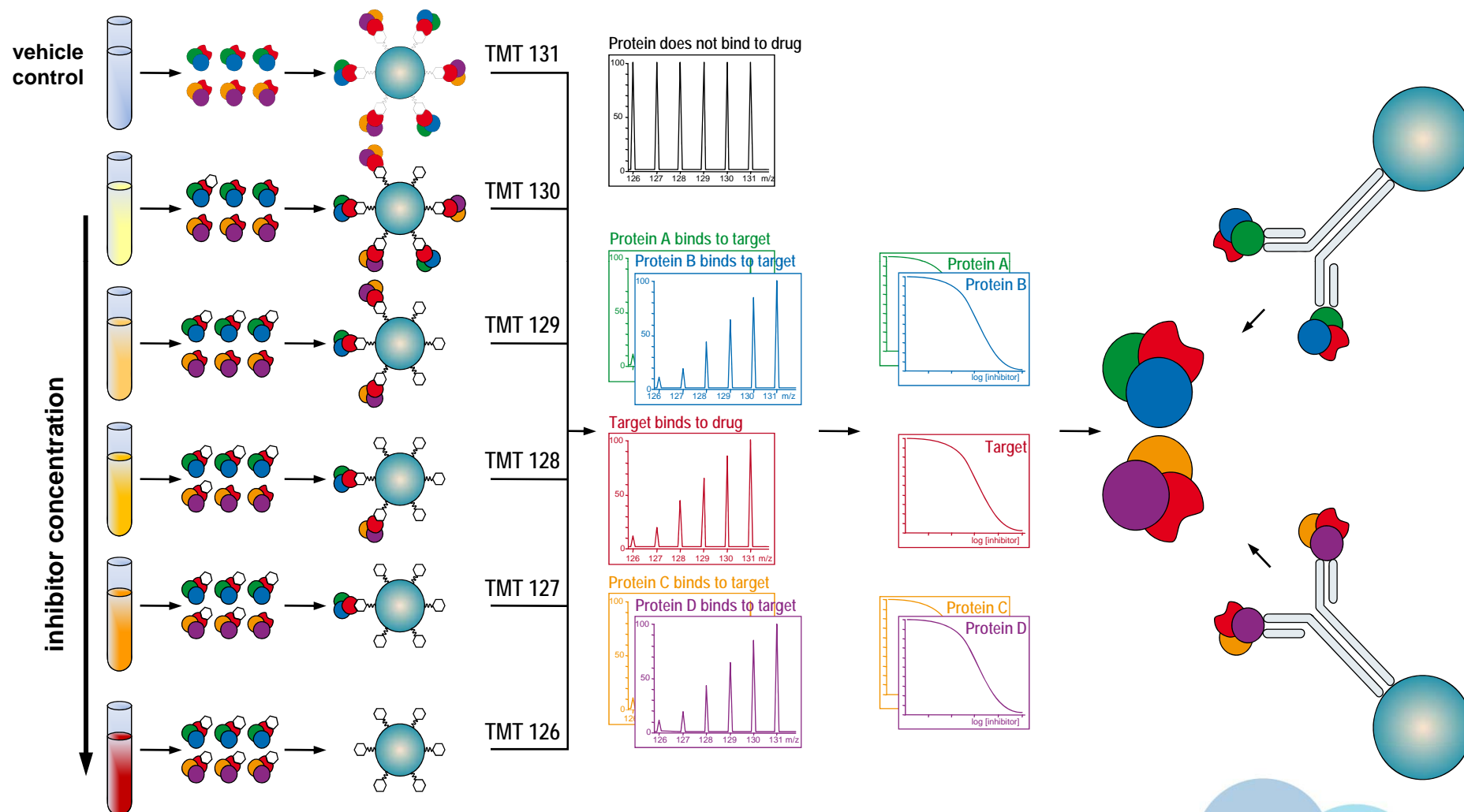
# Chemoproteomic approach to epigenetic drug targets

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# HDAC Target Profiling Strategy

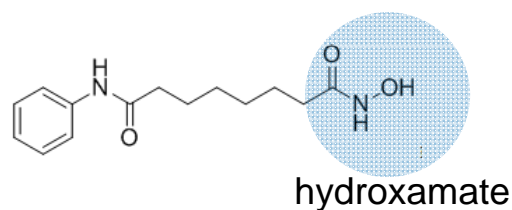


# Panel of 20 HDAC inhibitors used in this study

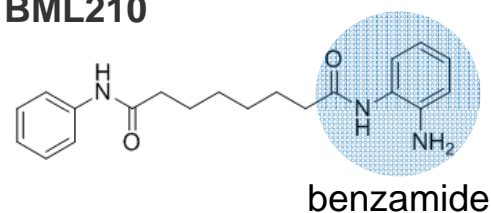
compound	class	status
SAHA	hydroxamates	marketed drug
TSA		tool
Belinostat (PXD-101)		Phase II
Dacinostat (LAQ-824)		Phase I
Scriptaid		tool
Panobinostat (LBH-589)		Phase II
PCI-24781		Phase I/II
PCI-34051		preclinical
MC 1293		tool
Bufexamac		screening hit / marketed drug
Entinostat (MS-275)	aminobenzamides	Phase II
CI-994 (Tacedinaline)		Phase II
Mocetinostat (MGCD-0103)		Phase II
BML-210		tool
AA-1		screening hit
AA-2	fatty acid	screening hit
Valproate		marketed drug
Apicidin	cyclic peptides	tool
Romidepsin		marketed drug

# Examples of HDACi profiles in human leukemia cells

## SAHA (vorinostat, Zolinza®)

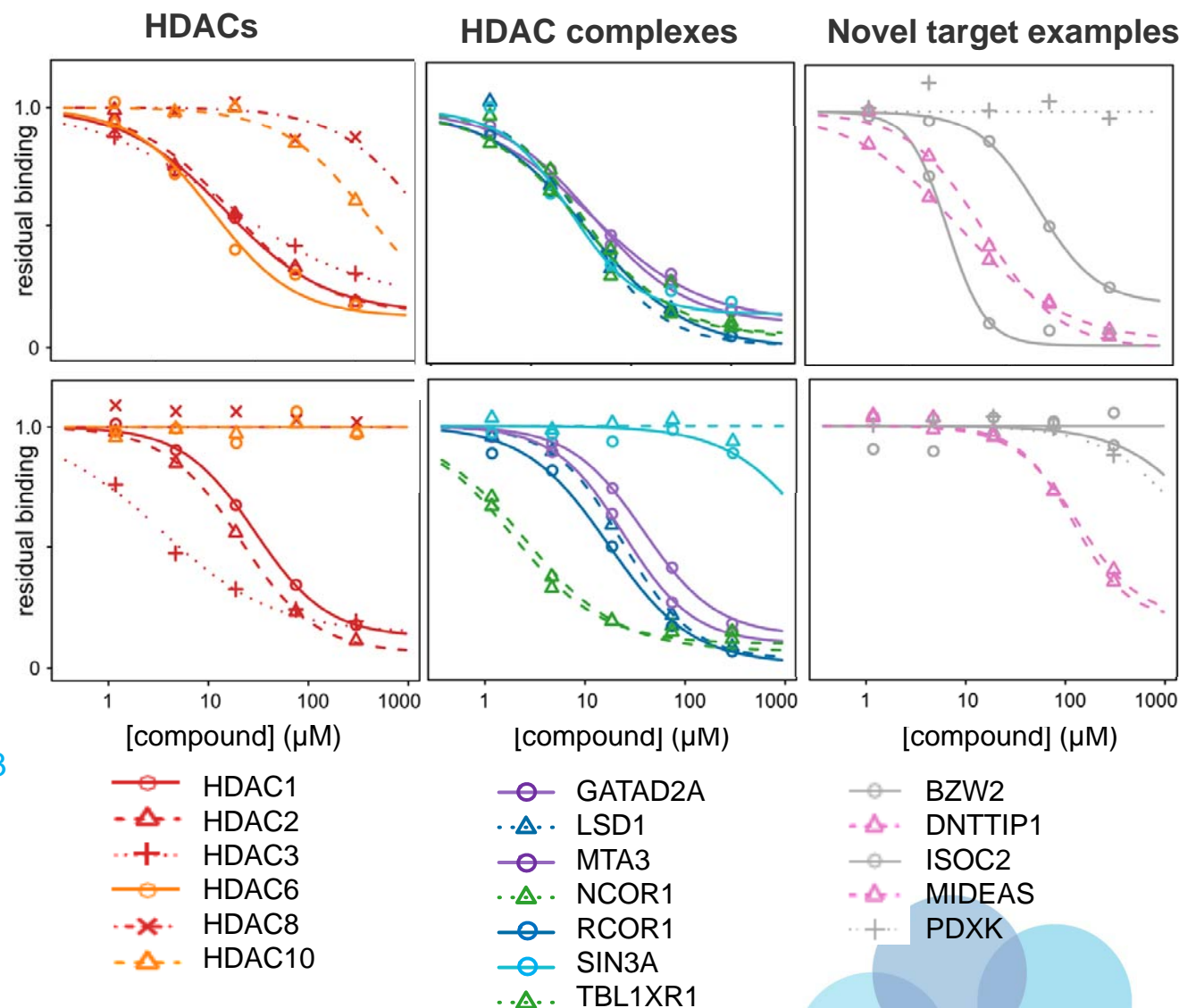


## BML210

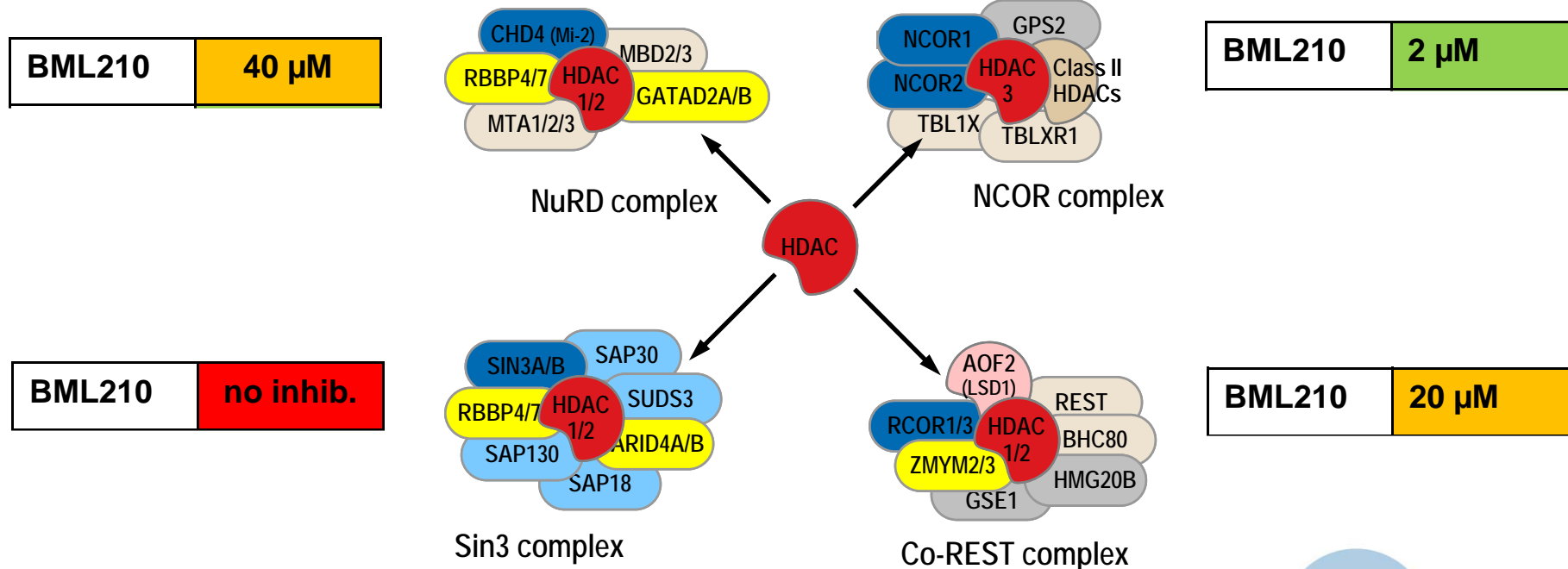
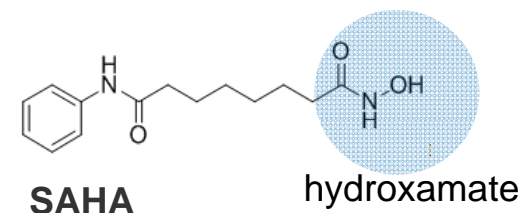
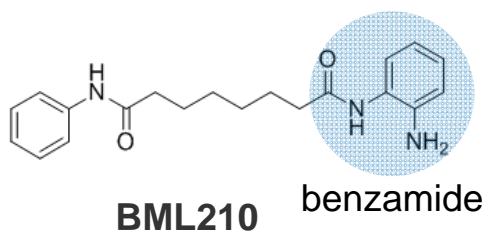


Substitution of headgroup leads to:

- HDAC3 / NCoR selectivity
- Impaired binding to the Sin3 complex
- different off-target profile



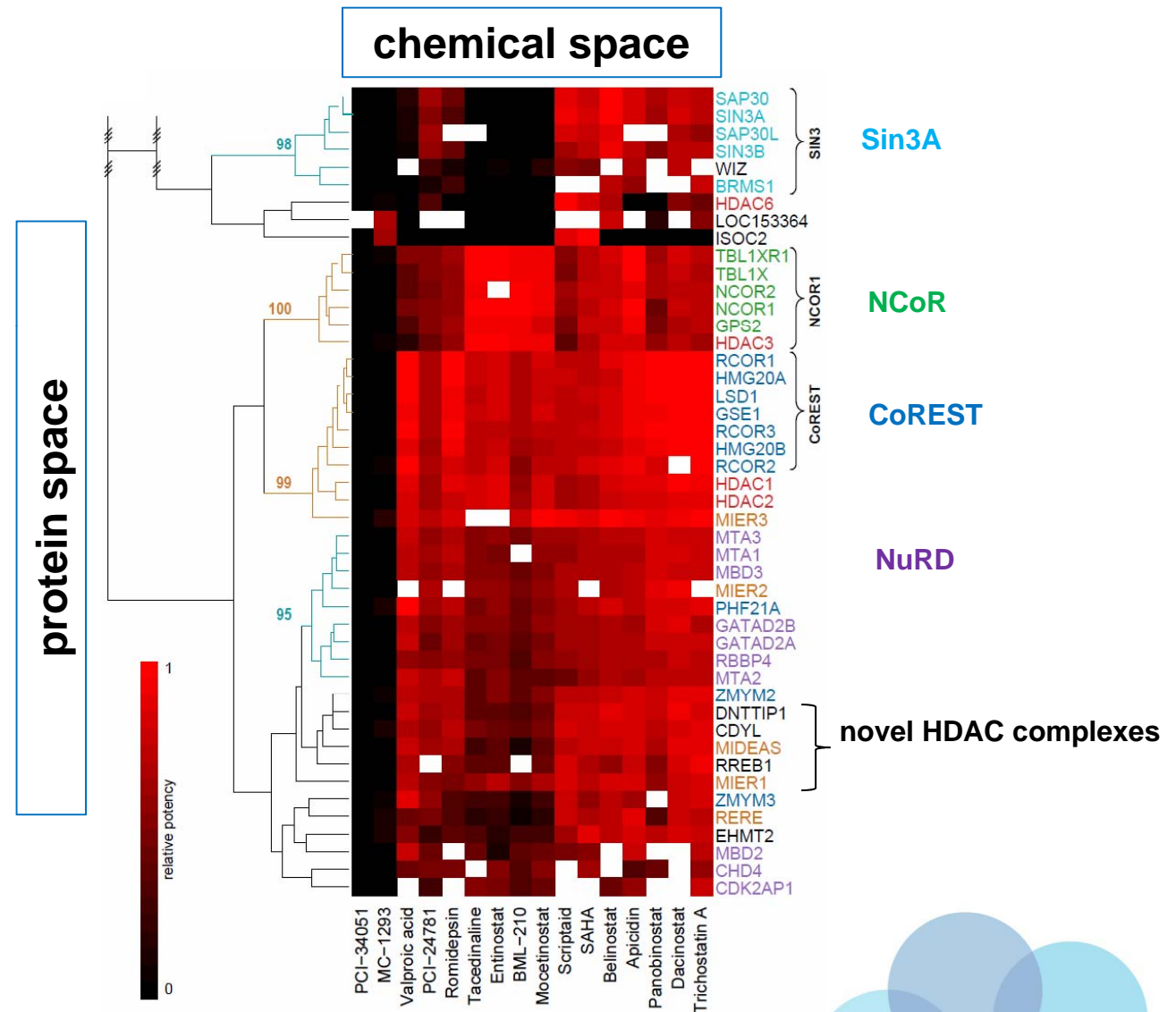
# HDAC inhibitors: benzamides display target and **complex** selectivity





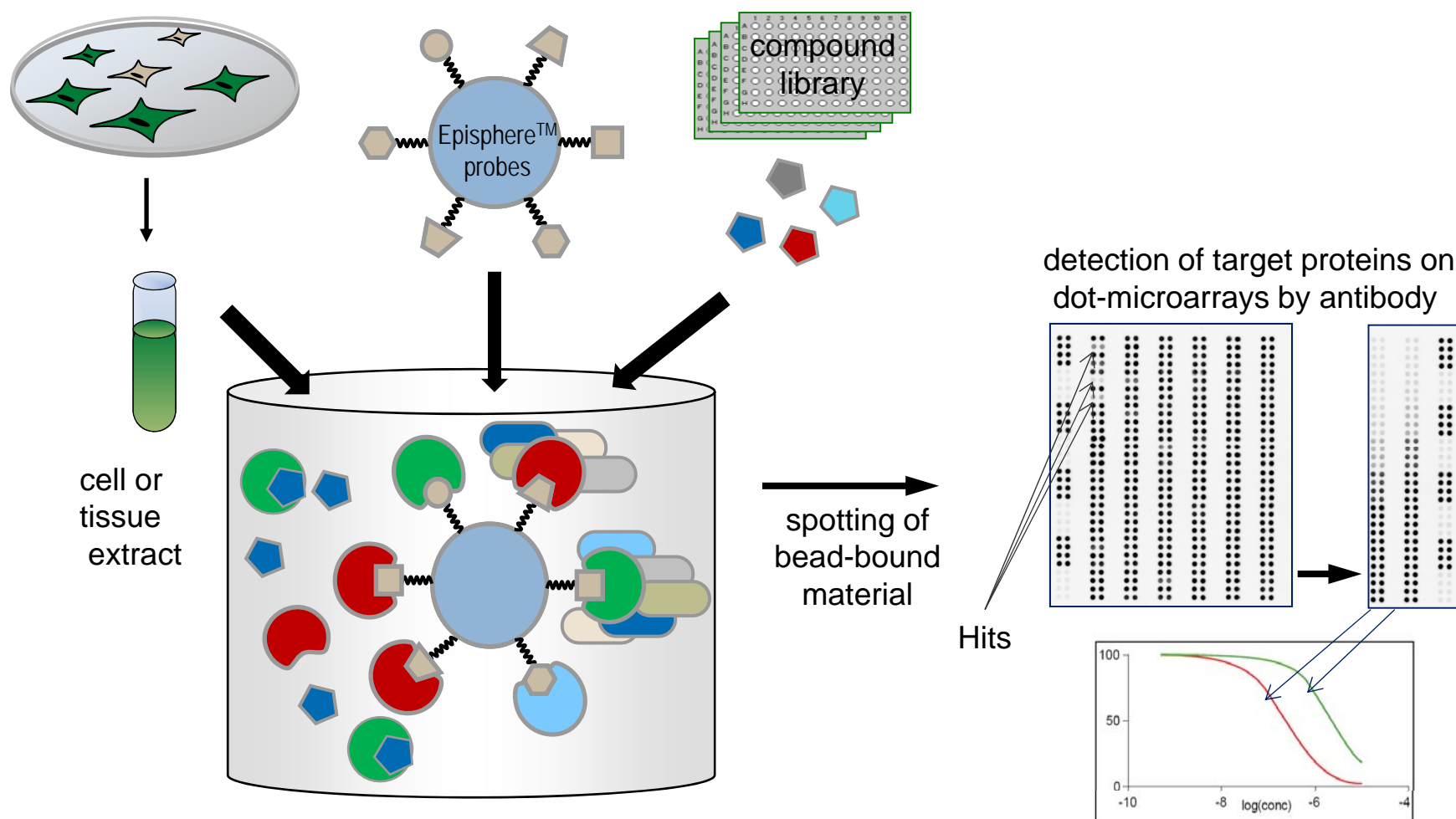
# HDAC inhibitors "recognize" protein complexes

Clustering of all affected proteins versus the 16 inhibitors delineates complexes!



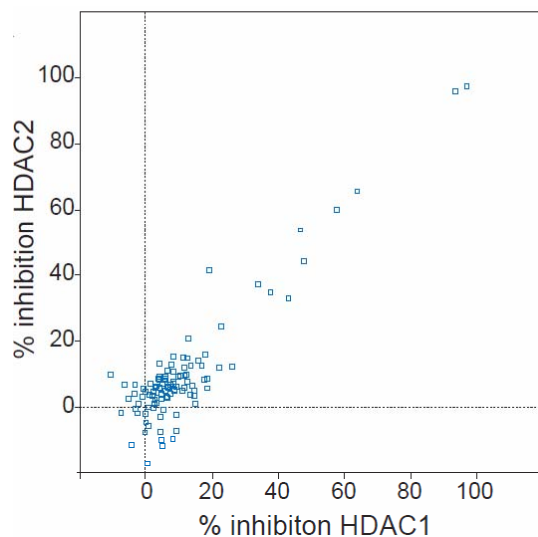


# Screening with native protein complexes

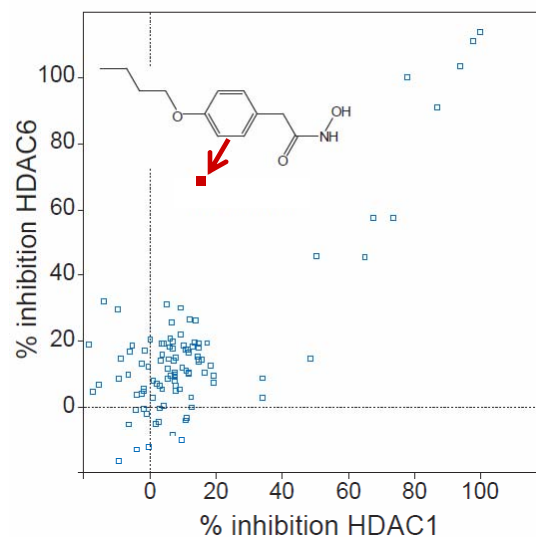


# Chemoproteomic library profiling identifies selective HDAC inhibitors

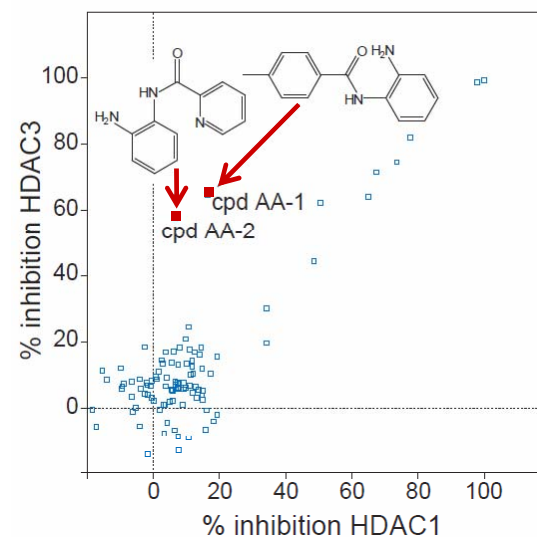
- "Complexes" screened: HDAC1, HDAC2, HDAC3 and HDAC6
- Focused small molecule library, screening at 10 $\mu$ M



**HDAC2 vs HDAC1**



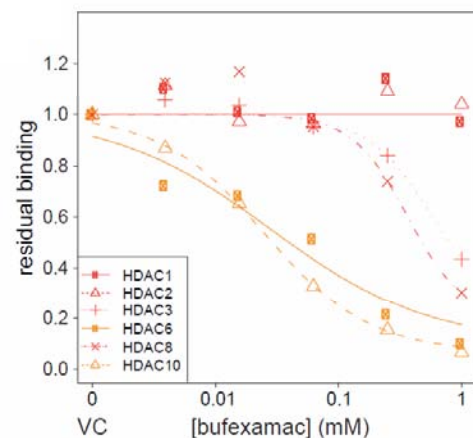
**HDAC6 vs HDAC1**



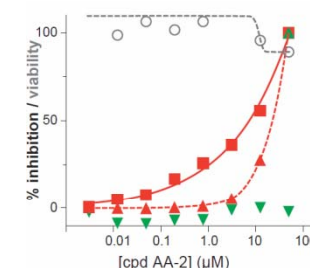
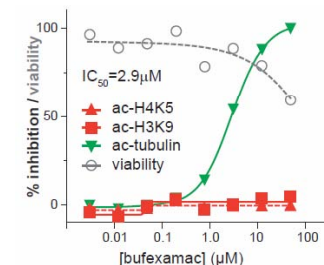
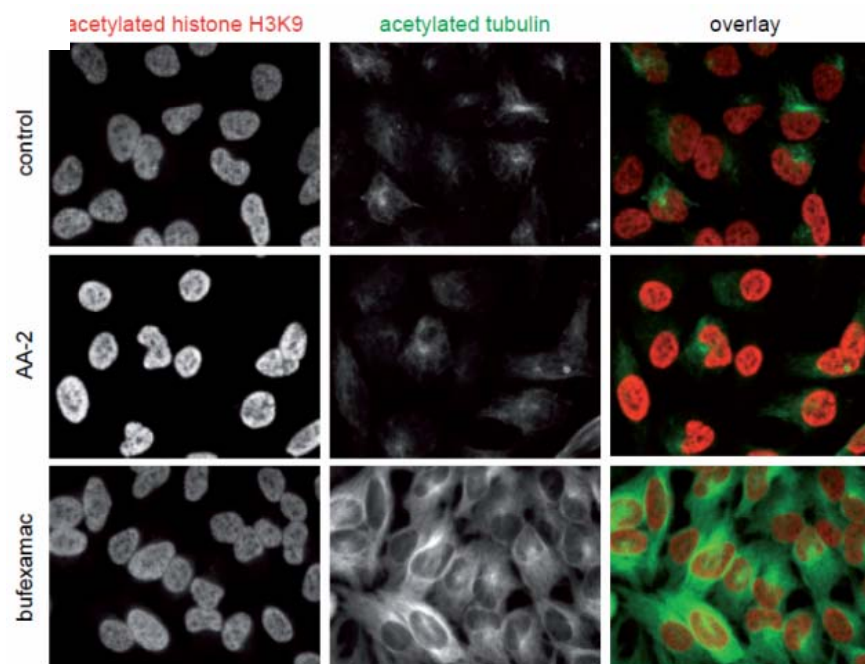
**HDAC3 vs HDAC1**



# The NSAID bufexamac is a selective HDAC6/10 inhibitor



Chemoproteomic selectivity profile of bufexamac in K562 cells



- treatment of HeLa cells with bufexamac elicits hyperacetylation of tubulin
- treatment with the HDAC3 compounds leads to hyperacetylation of histones.



## Summary

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- HDAC inhibitors "recognize" protein complexes differentially
- Some HDAC1/2 inhibitors (benzamides, Valproate) display clear complex selectivity
- Benzamides show preferential inhibition of the HDAC3/NCoR complex vs. HDAC1/2 complexes
- We identified novel HDAC1/2 complexes including a complex upregulated in mitosis
- NSAID Bufexamac is a selective HDAC6/10 inhibitor

# Chemoproteomic approach to epigenetic drug targets

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# Epigenetic Target Classes amenable to chemoproteomic approaches

## • HDACs (3/2011)

**Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes**

Marcus Bantscheff<sup>1,2</sup>, Carsten Hopf<sup>1,2</sup>, Mikhail M Savitski<sup>1</sup>, Antje Dittmann<sup>1</sup>, Paola Grandi<sup>1</sup>, Anne-Marie Michon<sup>1</sup>, Judith Schlegl<sup>1</sup>, Yann Abraham<sup>1</sup>, Isabelle Recher<sup>1</sup>, Giovanna Bergamini<sup>1</sup>, Markus Boesche<sup>1</sup>, Manja Delling<sup>1</sup>, Birgit Dimpfle<sup>1,3</sup>, Dirk Eberhard<sup>1</sup>, Carola Huthmacher<sup>1</sup>, Toby Mathiesen<sup>1</sup>, Daniel Poock<sup>1</sup>, Valérie Reader<sup>1</sup>, Karja Strunk<sup>1</sup>, Gavin Sweetman<sup>1</sup>, Ulrich Kruse<sup>1</sup>, Gite Neubauer<sup>1</sup>, Nigel G Raaijmakers<sup>1</sup> & Gerard Drewes<sup>1</sup>

**ARTICLES**

The development of selective histone deacetylase (HDAC) inhibitors with anti-cancer and anti-inflammatory properties remains challenging in large part owing to the difficulty of probing the interactions of small molecules with regulatory protein complexes. A combination of affinity capture and quantitative mass spectrometry revealed the selectivity with which 18 HDAC inhibitors target multiple HDAC complexes enriched by ELN-SMRT domain subunits, including a novel histone deacetylase complex (HDAC1). Inhibitors clustered according to their target profiles with stronger binding of antineoplastic to the HDAC HDAC1 complex than to the HDAC HDAC2 complex. We identified several non-HDAC targets for hydroxamate inhibitors. HDAC inhibitors with distinct profiles have correspondingly different effects on downstream targets. We also identified the anti-inflammatory drug ibuprofen as a class II (HDAC1, HDAC10) HDAC inhibitor. Our approach enables the discovery of novel targets and inhibitors and suggests that the selectivity of HDAC inhibitors should be evaluated in the context of HDAC complexes and not purified catalytic subunits.

Protein lysine acetylation is a key mechanism in the epigenetic control of gene expression and the regulation of cell metabolism<sup>1,2</sup>, and protein deacetylases are potential targets for treating cancer and a range of autoimmune and neurodegenerative diseases<sup>3</sup>. The first mammalian histone deacetylase (HDAC) was discovered in 1996 by a chemical biology approach using an imino-linked substrate-derived compound as effector agent<sup>4</sup>.

Based on sequence phylogeny and function, there are four distinct classes of HDAC: class I (HDAC1, 2, 3 and 8), class II (HDAC4, 5, 7 and 9), class III (HDAC6 and 10) and class IV (HDAC11) represent Zn<sup>2+</sup>-dependent metalloproteases, whereas class II comprises the mechanistically diverse NAD<sup>+</sup>-dependent sirtuins<sup>5</sup>. HDACs form the catalytic core of regulatory complexes involved in chromatin modification and gene expression. Four such molecular machines have been characterized to date. Whereas the CoREST, NuRD and MLL complexes contain an HDAC1/HDAC2 dimer core, the NuRD complex is formed around HDAC1 and 2. The roles of these complexes are diverse and often cell-type specific. Although these data are emerging regarding their role in the chromatinization of all cells, their functions in these mechanisms are less well understood<sup>6,7</sup>. The CoREST complex complex histone deacetylation to deacetylation to repress neuronal genes<sup>8</sup>, the NuRD complex histone deacetylation to chromatin remodeling of T-cell and prostate gene silencing<sup>9</sup>, and the MLL complex represses gene transcription of various developmental pathways<sup>10</sup>. The HDAC1 complex is the major component for nuclear receptors<sup>11,12</sup>. Class IIa HDACs exhibit low enzymatic activity and are proposed to have "modulation reader" or scaffolding functions<sup>13,14</sup>. Class IIb HDACs exhibit acetylase-independent functions in regulating protein folding and turnover<sup>15</sup>.

Small molecule HDAC inhibitors were discovered by their ability to induce differentiation of transformed cells<sup>16</sup>. Subsequently hydroxamate, zinc chelators, sirtuin, and sirtuin-like inhibitors (classified as approved for the treatment of cutaneous T-cell lymphoma, and valproate is in clinical use as an anticonvulsant, general HDAC inhibitors are in development for a number of indications but clinical development has been hampered by a lack of target selectivity. This increases the risk of toxic inhibition and also limits the use of these compounds as research tools<sup>17</sup>.

The potential lack of selectivity of HDAC inhibitors may originate from the optimization of lead compounds using standard industry assays involving recombinant enzymes or protein fragments. These assays routinely do not properly reflect the native conformation and activity of the target and its physiological context owing to incorrect protein folding, poor translational modifications and the absence of regulatory subunits. Remarkably, purified class II HDACs exhibited increased activity in the presence of interacting proteins<sup>18,19</sup>. Small HDAC inhibitors induce a distinctive pharmacophore comprising a cap which binds to the zinc of the catalytic domain, a spacer spanning the channel, and a Zn<sup>2+</sup>-binding function. A phylogenetic analysis of HDACs was done to label not only HDACs but also the proteins HDAC1, HDAC2 and HDAC3. This indicates that these proteins are close to the active site, and suggests that the cap covers inhibitor selectivity<sup>20</sup>.

**NATURE BIOTECHNOLOGY** ADVANCE ONLINE PUBLICATION

## • Bromodomain proteins (10/2011)

**EPINOVA DPU**

+ T. Kouzarides

- PARPs
- K-Methyltransferases
- K-Demethylases

**nature** International weekly journal of science

**LETTER**

doi:10.1038/nature10599

**Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia**

Mark A. Dawson<sup>1,2\*</sup>, Rab K. Prinjha<sup>1,2\*</sup>, Antje Dittmann<sup>1,2\*</sup>, George Girotopoulos<sup>1</sup>, Marcus Bantscheff<sup>1</sup>, Wai-In Chan<sup>1</sup>, Samuel C. Robson<sup>1</sup>, Chun-wa Chung<sup>1</sup>, Carsten Hopf<sup>1</sup>, Mikhail M. Savitski<sup>1</sup>, Carola Huthmacher<sup>1</sup>, Emma Gudgin<sup>1</sup>, Dave Lugo<sup>1</sup>, Soren Beisike<sup>1</sup>, Trevor D. Chapman<sup>1</sup>, Emma J. Roberts<sup>1</sup>, Peter E. Soden<sup>1</sup>, Kurt R. Auger<sup>1</sup>, Olivier Milguet<sup>1</sup>, Konstanze Doehner<sup>1</sup>, Ruedi Dettweil<sup>1</sup>, Alan K. Burnett<sup>1,2</sup>, Phillip Jeffrey<sup>1</sup>, Gerard Drewes<sup>1</sup>, Kevin Lee<sup>1</sup>, Brian J. P. Huntly<sup>1,2</sup> & Tony Kouzarides<sup>1,2</sup>

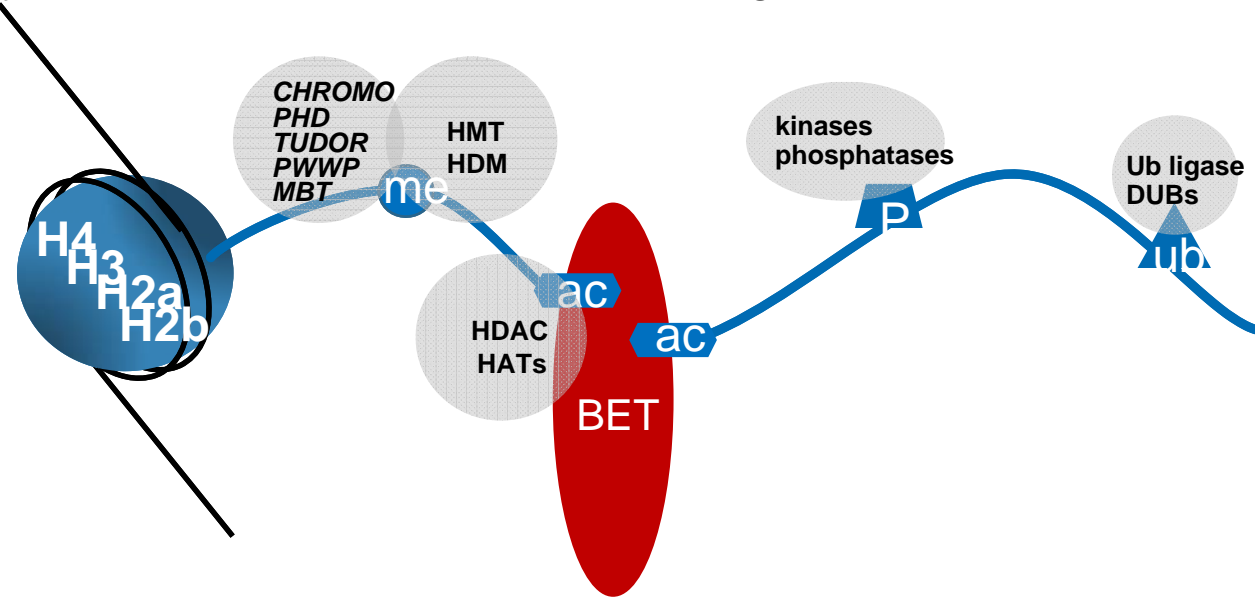
Recurrent chromosomal translocations involving the mixed lineage leukaemia (MLL) gene initiate aggressive forms of leukaemia, which are often refractory to conventional therapies<sup>1</sup>. Many MLL-fusion partners are members of the super elongation complex (SEC), a critical regulator of transcriptional elongation, suggesting that aberrant control of this process has an important role in leukaemia induction<sup>2,3</sup>. Here we use a global proteomic strategy to demonstrate that MLL fusions, as part of SEC<sup>2,3</sup> and the polymerase-associated factor complex (PAF)<sup>4,5</sup>, are associated with the BET family of acetyl-lysine recognizing, chromatin 'adaptor' proteins. These data provided the basis for therapeutic intervention in MLL-fusion leukaemia, via the displacement of the BET family of proteins from chromatin. We show that a novel small molecule inhibitor of the BET family, GSK1210151A (I-BET151), has profound efficacy against human and murine MLL-fusion leukaemic cell lines, through the induction of early cell cycle arrest and apoptosis. I-BET151 treatment in two human leukaemia cell lines with different MLL fusions alters the expression of a common set of genes whose function may account for these phenotypic changes. The mode of action of I-BET151 is, at least in part, due to the inhibition of transcription at key genes (*BCD2*, *C-MYC* and *CDK6*) through the displacement of BRD3/4, PAFc and SEC components from chromatin. *In vivo* studies indicate that I-BET151 has significant therapeutic value, providing survival benefit in two distinct mouse models of murine MLL-AFP9 and human MLL-AFL leukaemia. Finally, the efficacy of I-BET151 against human leukaemia stem cells is demonstrated, providing further evidence of its potent therapeutic potential. These findings establish the displacement of BET proteins from chromatin as a promising epigenetic therapy for these aggressive leukaemias.

Dysregulation of chromatin modifiers is a recurrent and sentinel event in oncogenesis<sup>6</sup>. Therapeutic strategies that selectively alter the recruitment and/or catalytic activity of these enzymes at chromatin therefore hold great promise as targeted therapies<sup>7</sup>. In this regard the bromodomain and extra-terminal (BET) family of proteins (BRD2, BRD3, BRD4 and BRD9) provide an ideal 'druggable' target, because they share a common highly conserved tandem bromodomain at their

of I-BET151 (ref. 9) were incubated with HL60 nuclear extracts and bound proteins were analysed by quantitative mass spectrometry (Supplementary Table 1). This approach identified the BET isoforms and a large number of co-purifying proteins (Supplementary Tables 1 and 2), indicating that the BET isoforms reside in many distinct protein complexes. In the second approach, immunoprecipitation analyses with selective antibodies against BRD2/3/4 were performed (Supplementary Fig. 1 and Supplementary Tables 3 and 4). This was complemented with additional immunoprecipitations using selected antibodies against complex members ('baits') selected from the subset of proteins that were identified in the first approach (Fig. 1b right panel, Supplementary Fig. 2 and Supplementary Table 3). In the third approach, bead-immobilized histone H4(1-21):K5acK8acK12ac acetylated peptides were used to purify protein complexes. These data were combined to highlight a list of complexes identified in all three methods (Fig. 1b left panel, Supplementary Fig. 3 and Supplementary Table 1). Finally, specificity of the I-BET151 and histone tail matrix was further assessed by competition experiments (Fig. 1c, Supplementary Figs 4, 5 and Supplementary Table 2). Together these data indicate the direct determination of the targets of the inhibitor, and the proteins associated with the target, with subunits of protein complexes exhibiting closely matching half-maximum inhibitory concentration (IC<sub>50</sub>) values<sup>9</sup>. Taken together these stringent and complementary approaches provide a high confidence global data set encompassing all known<sup>11-15</sup> and several novel BET protein complexes (Fig. 1b and Supplementary Fig. 3). Among the novel complexes, we observed a prominent enrichment and dose-dependent inhibition of several components of the PAFc<sup>4,5</sup> and SEC<sup>2,3</sup> (Fig. 1b, c), which were confirmed by reciprocal immunoprecipitations in HL60 cells (Fig. 1b). Moreover, reciprocal immunoprecipitations in two MLL-fusion leukaemia cell lines (MV411 and RS411) confirmed the relationship of SEC with BRD4 in different cellular contexts (Fig. 1d). Together these data indicate that BRD2 and BRD4 associate with the PAFc and SEC, and may function to recruit these complexes to chromatin. Given that these complexes are crucial for malignant transformation by MLL fusions<sup>2,3</sup> we used the hypothesis that displacement of BET proteins from chromatin may have a therapeutic role in these leukaemias.

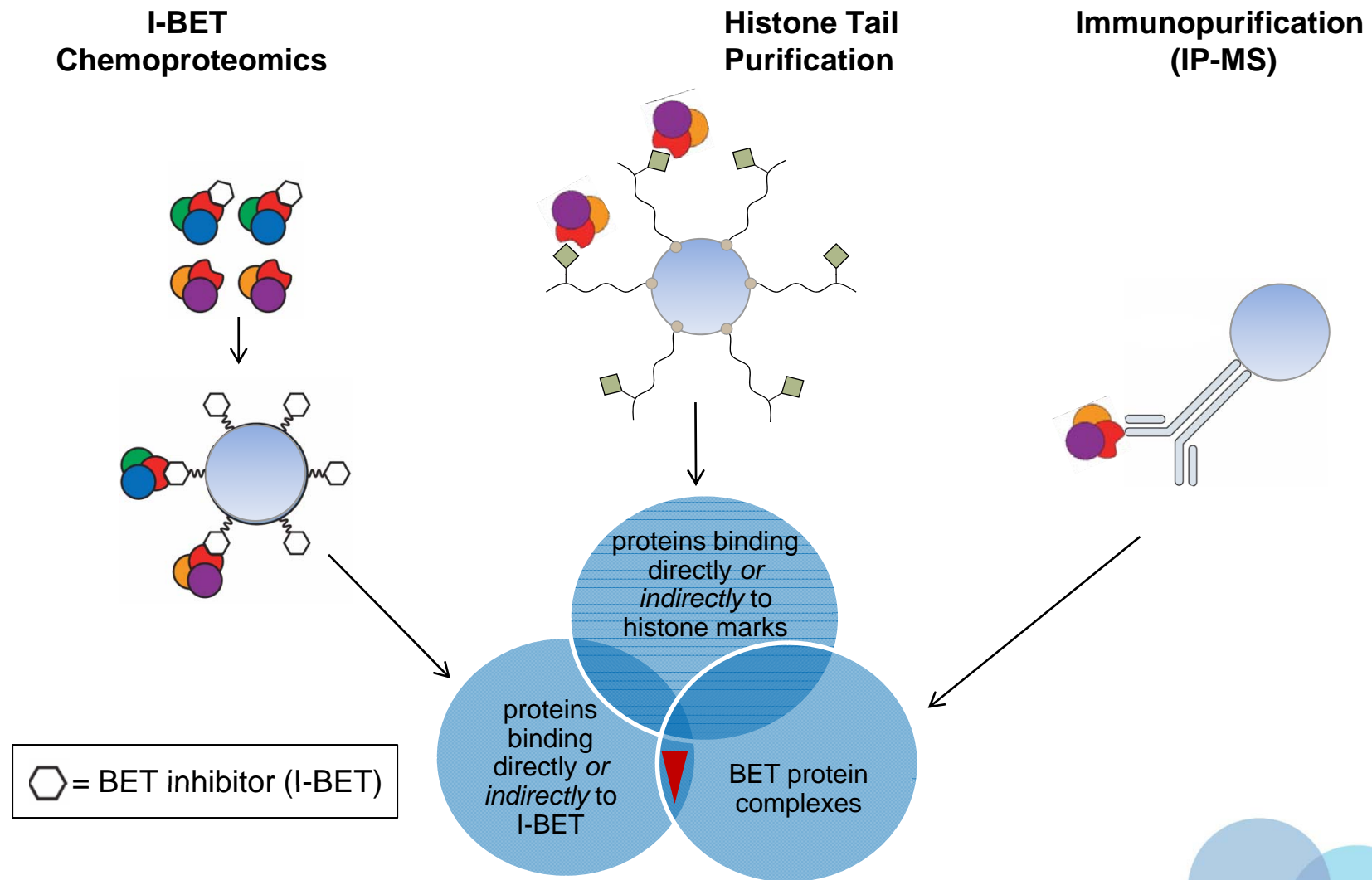
# BET – bromodomain and extra-terminal domain proteins

- Bromodomain: Acetyl-lysine recognizing domain – targets BETs and associated complexes to chromatin
- Facilitates transcriptional activation
- Highly conserved Bromodomains are target for small molecule inhibitors



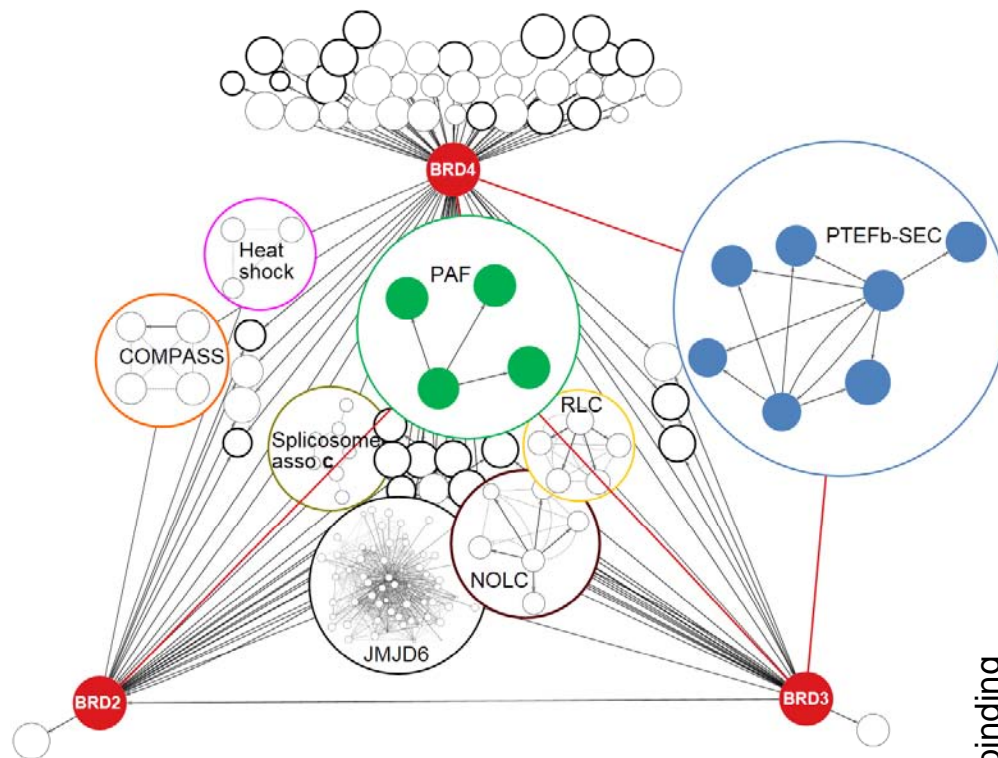


# Tripartite interaction proteomics strategy





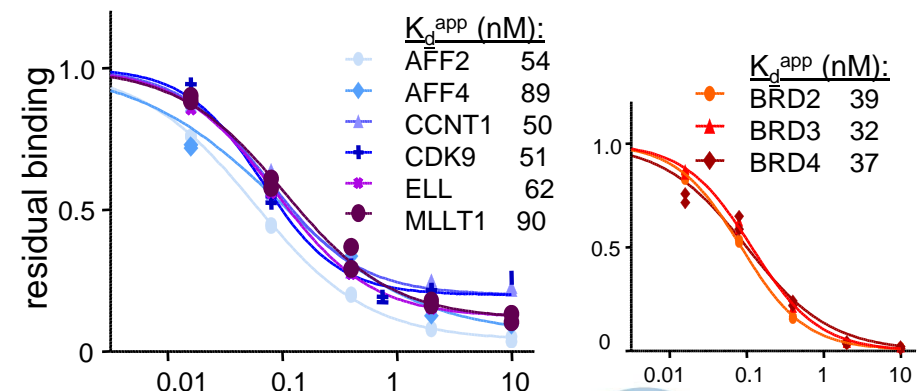
# PTEFb-SEC and PAF are BRD3/4 associated complexes



Rel. abundance vs bait (log scale)

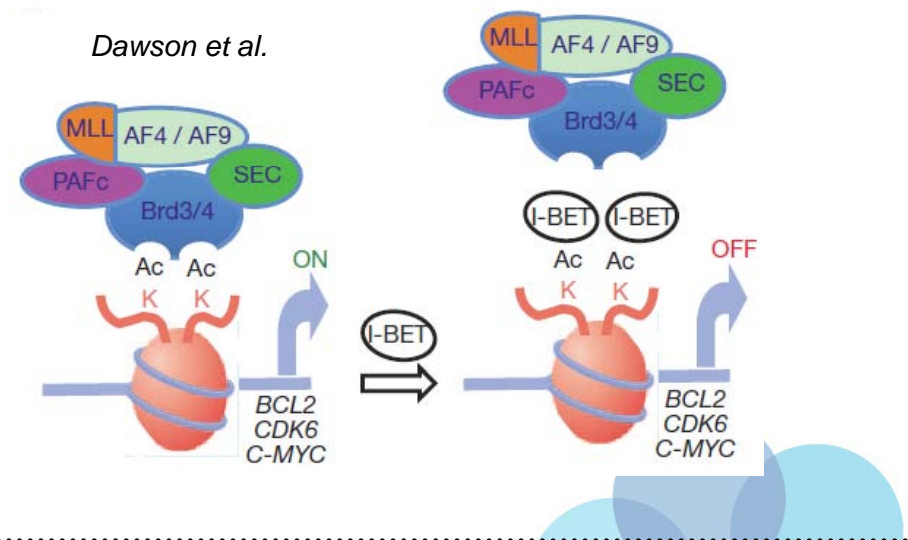
-3.3 0.19

proteins quantified	BRD2	BRD3	BRD4	CDK9	MLLT1	PAF1
<b>BET</b>						
BRD2	■	■	■			■
BRD3	■	■	■			■
BRD4	■	■	■			■
<b>PAF</b>						
CDC73	■	■	■	■	■	■
CTR9	■	■	■			■
LEO1	■	■	■			■
PAF1	■	■	■			■
RTF1	■	■	■			■
WDR61	■	■	■			■
<b>PTEFb-SEC</b>						
AFF1			■	■	■	■
AFF2			■	■	■	■
AFF4			■	■	■	■
CCNT1			■	■	■	■
CDK9		■	■	■	■	■
ELL			■	■	■	■
MLLT1			■	■	■	■



## Summary

- We used three orthogonal proteomic approaches to identify BET associated complexes
- Epigenetic complexes PAF and PTEFb-SEC are associated with BRD3 and BRD4
- PTEFb-SEC subunits (AFF4, AFF9...) are often found fused with MLL methyltransferase in mixed lineage leukemia, resulting to a deregulation of gene expression and aggressive leukemia
- BET inhibitors offers a therapeutic solution by preventing the recruitment of the chimera complex



# The Cellzome Team

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Special thanks:  
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Carsten Hopf

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Anne-Marie Michon  
Paola Grandi

