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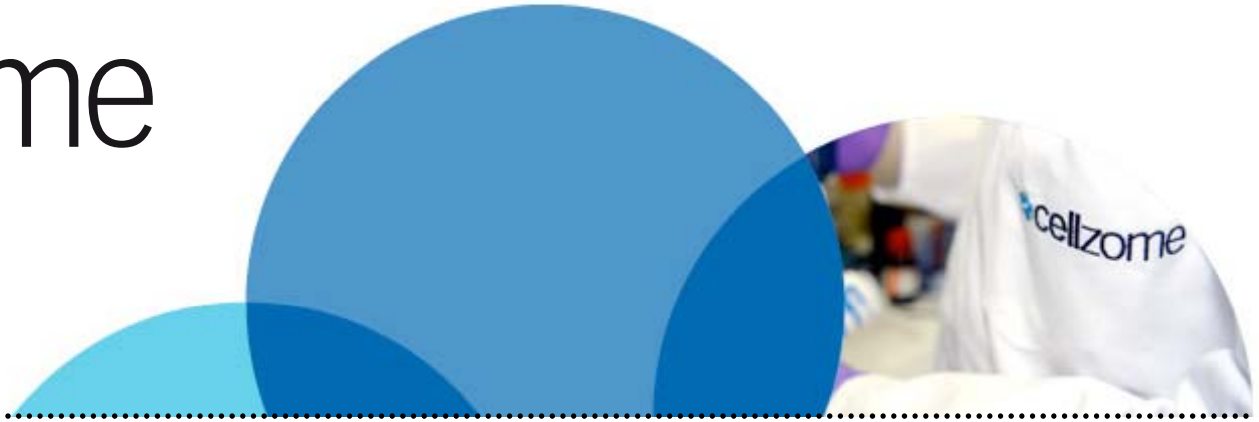

Chemoproteomic approach to epigenetic drug targets

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

2002 Nature

2004 Nature Cell Bio

2006 Nature

2007 Nature Biotech

2009 Nature

2011 Nature Biotech

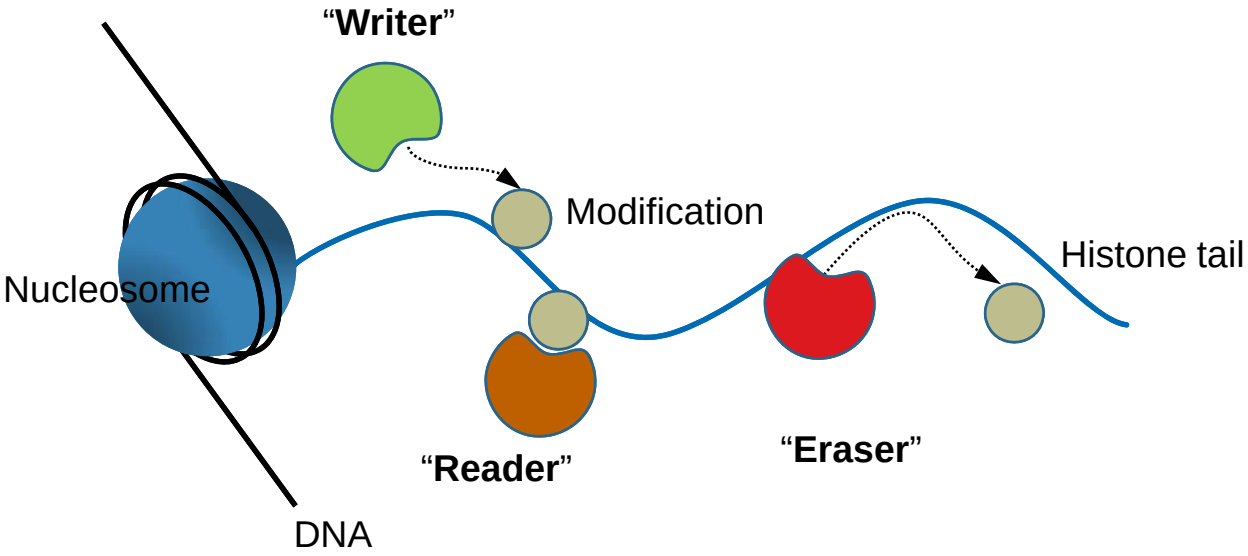
2011 Nature

2012 Nature Chem Bio, in press

Selective small molecule inhibitor discovered by chemoproteomic assay platform reveals regulation of Th17 cell differentiation by PI3Kγ

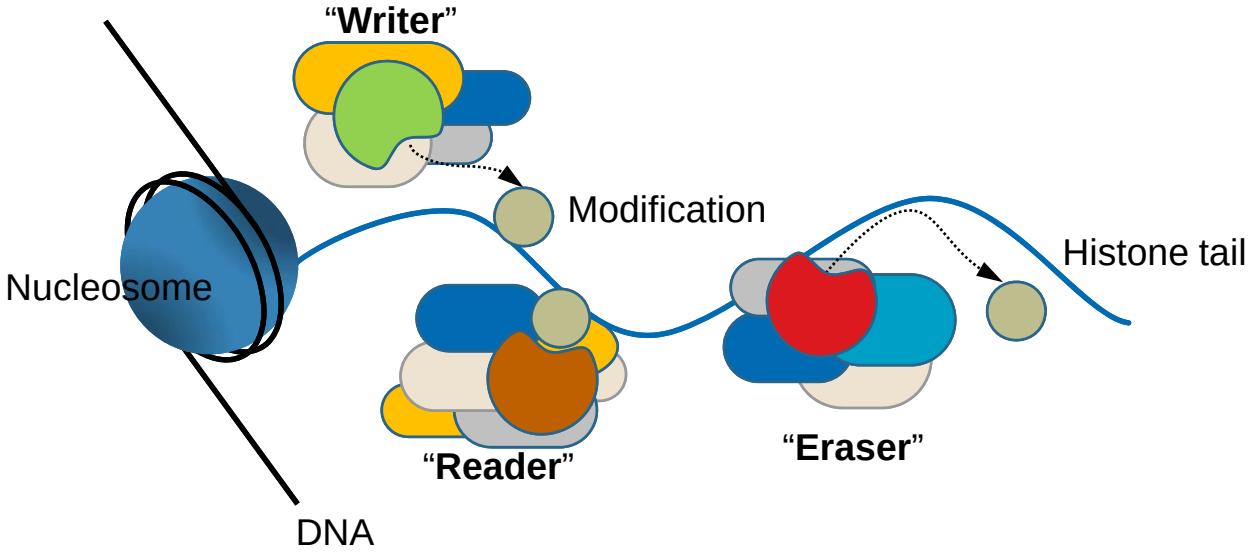
Giovanna Bergamini[1], Kathryn Bell[2], Satoko Shimamura[1], Thilo Werner[1], Andrew Cansfield[2], Katrin Müller[1], Jessica Perrin[1], Christina Rau[1], Katie Ellard[2], Carsten Hopf[3], Carola Doce[1], Daniel Leggate[2], Raffaella Mangano[2], Toby Mathieson[1], Alison O'Mahony[4], Ivan Plavec[4], Faiza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

Epigenetic Target classes



- **'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*

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Epigenetic Enzymes as Drug Targets

- 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
Histone Deacetylases (HDACs)	**11**
Sirtuins	7
Poly [ADP-ribose] polymerases	17
Bromodomain proteins	**40**
Chromodomain proteins	29
Tudor domain proteins	40

HDACs are catalytic subunits of megadalton protein complexes

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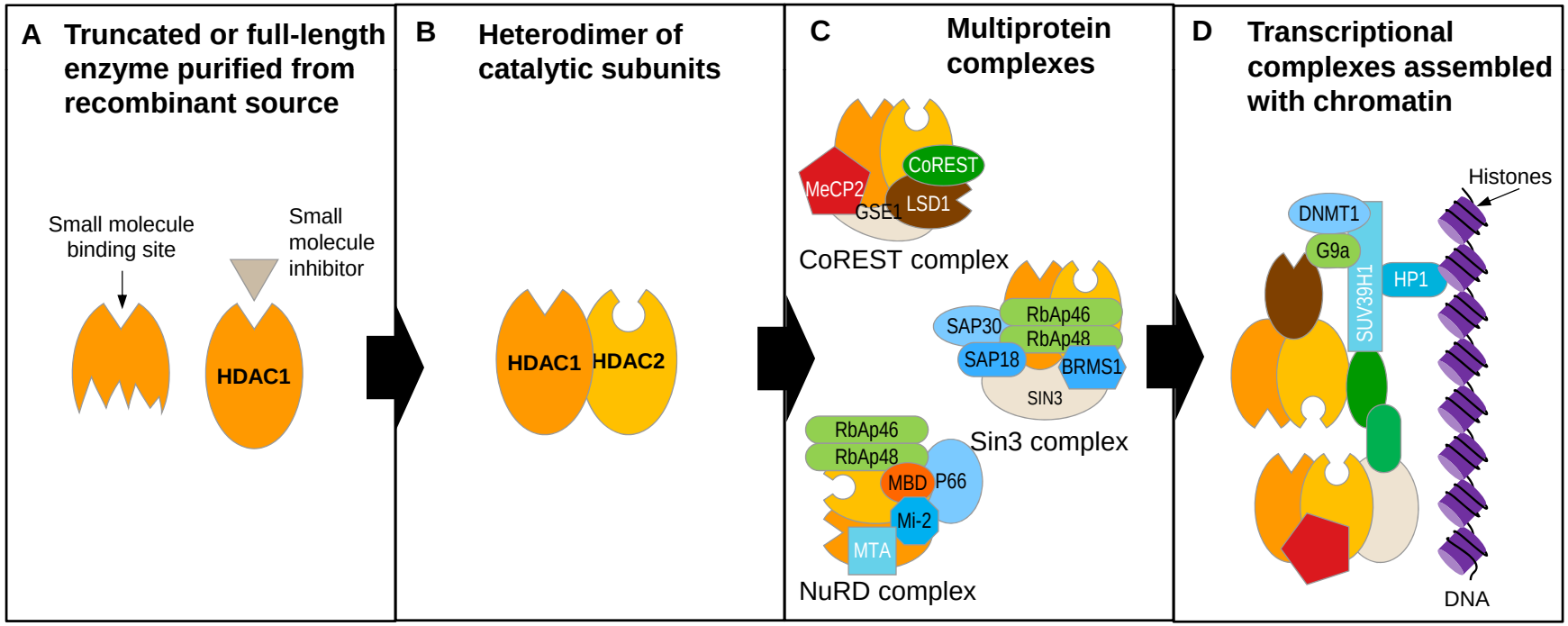
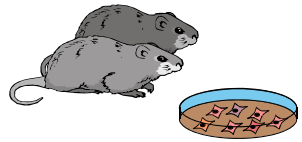
Can we use these protein complexes directly for Target Profiling and Drug Discovery?



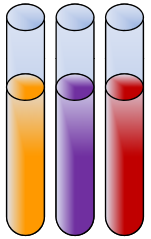
Basic chemoproteomics workflow

probe design and bead synthesis

tissue or cell preparation



optional sub-cellular fractionation



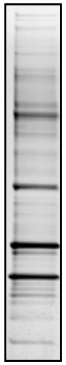
drug

matrix

affinity enrichment of bound proteins

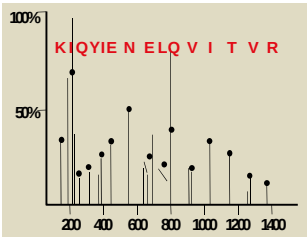
target identification and quantification

SDS-PAGE



protease digestion

LC-MS/MS (Orbitrap Velos)



Typical Experiment Design: Chemoproteomic competition assay

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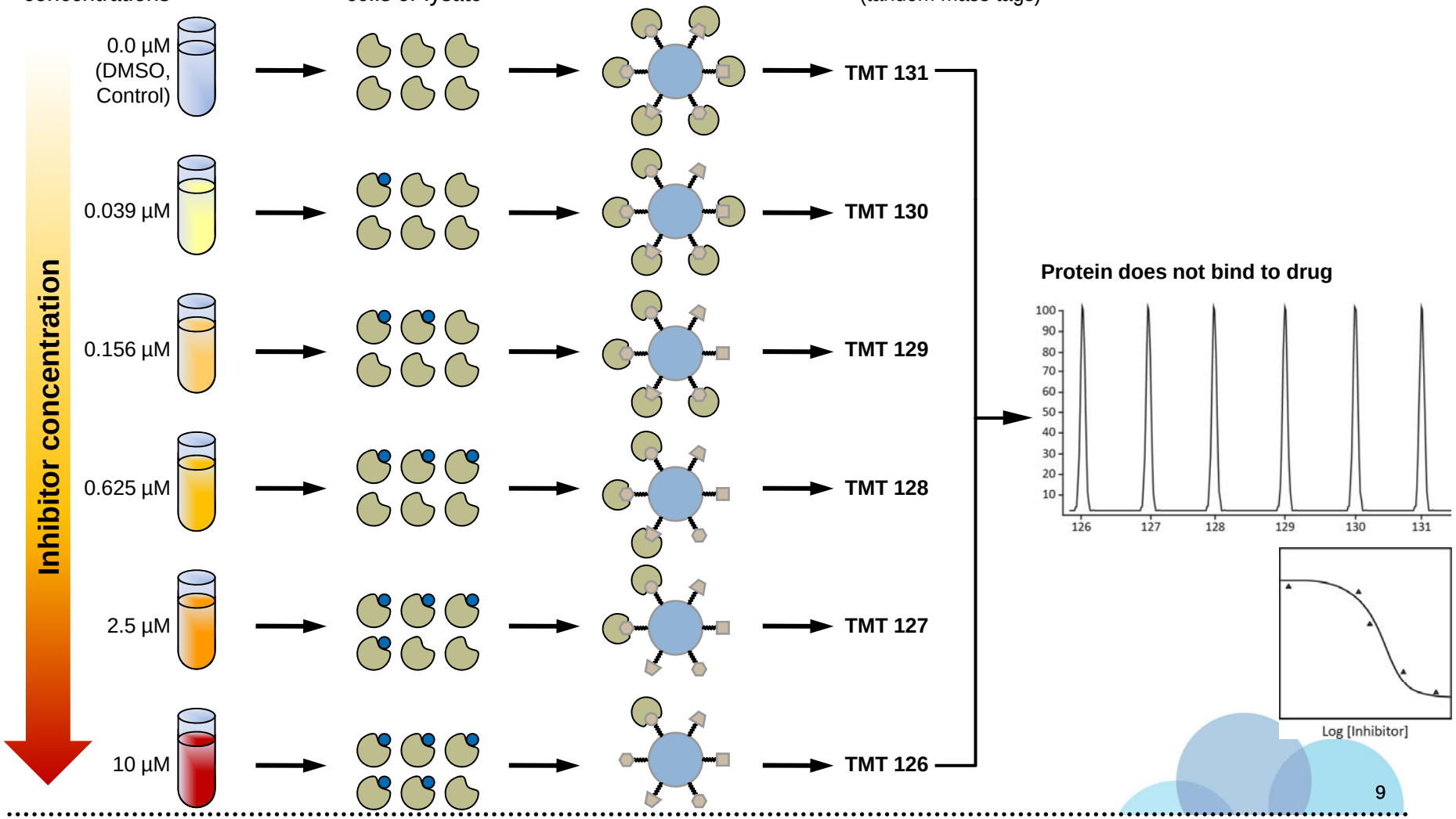
1. Add inhibitor to cells or cell lysate over a range of concentrations
2. Incubate - inhibitor binds to targets in cells or lysate
3. Add affinity matrix to lysates
4. Elute beads, digest, label with TMT6* (tandem mass tags)
5. Mix, run LC-MS/MS and quantify in MS/MS spectrum



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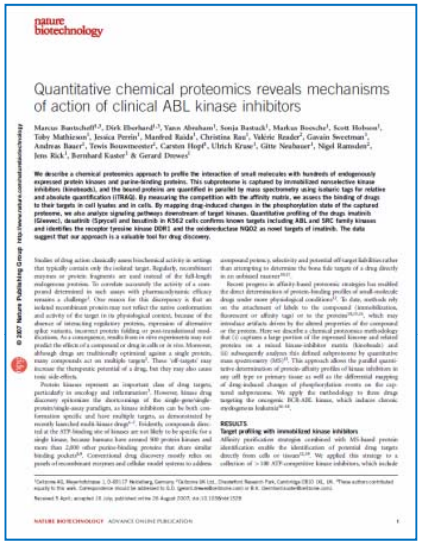
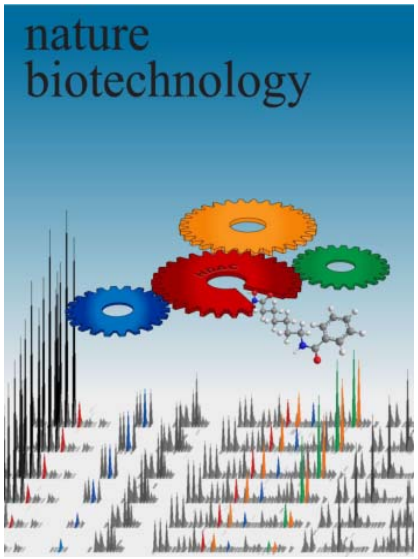
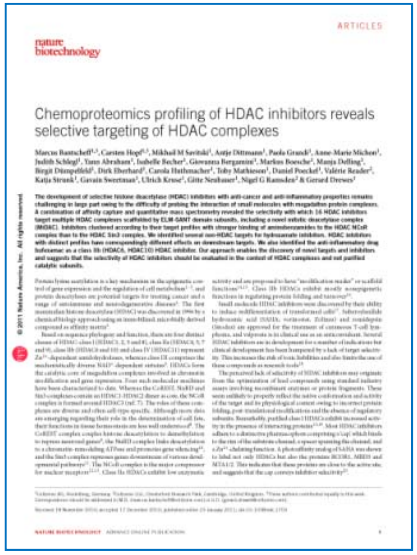
Target Classes

- Protein kinases (9/2007)



- HDACs (3/2011)





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

Chemoproteomic approach to epigenetic drug targets

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

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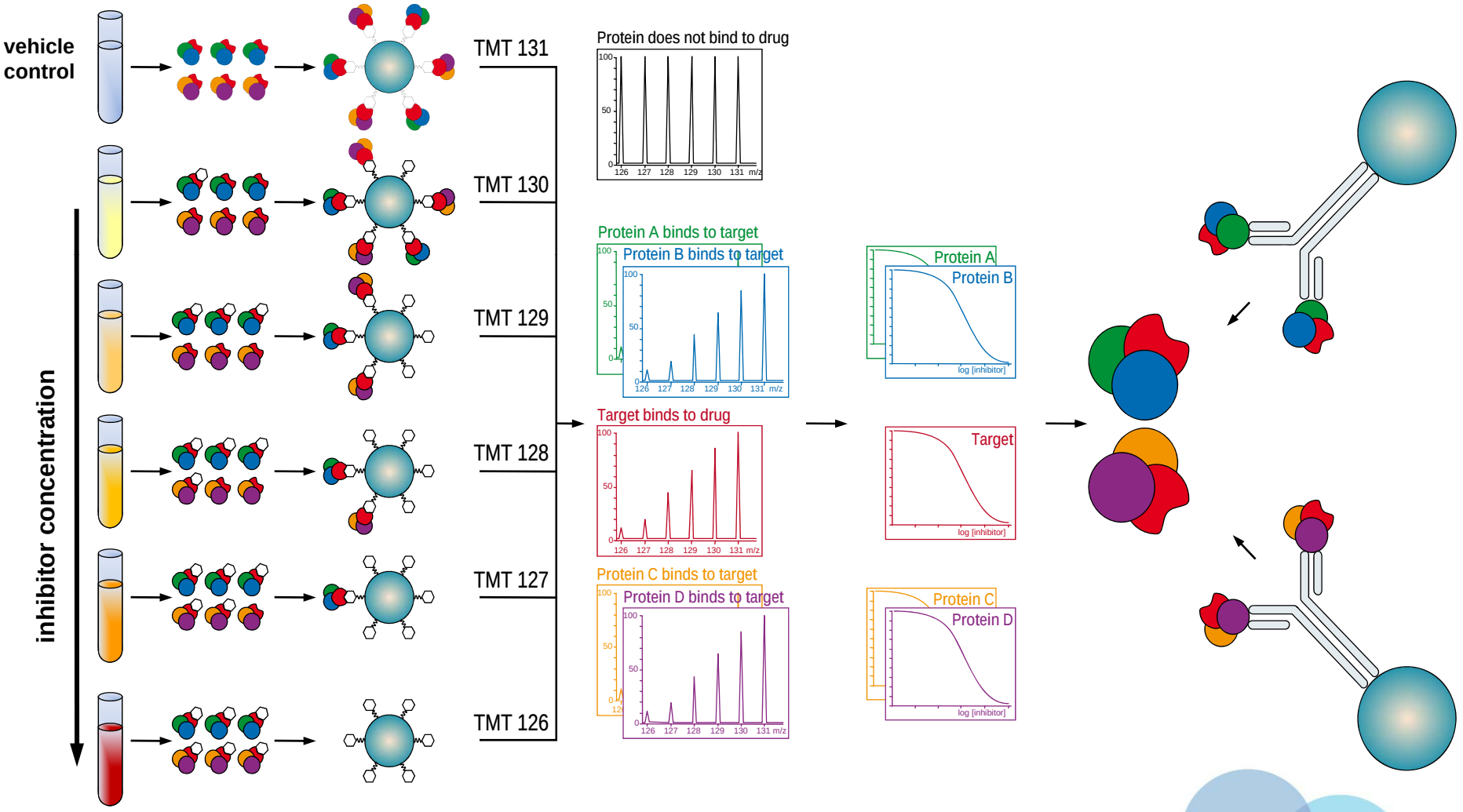
HDAC Target Profiling Strategy



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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		screening hit
Valproate	fatty acid	**marketed drug**
Apicidin	cyclic peptides	tool
Romidepsin		**marketed drug**

Examples of HDACi profiles in human leukemia cells

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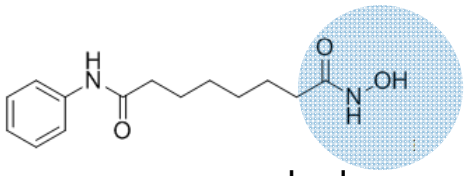
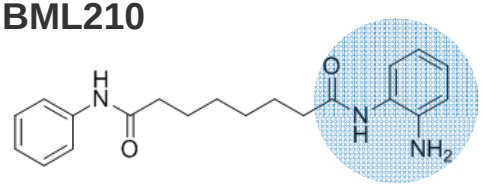
SAHA (vorinostat, Zolinza®)



hydroxamate

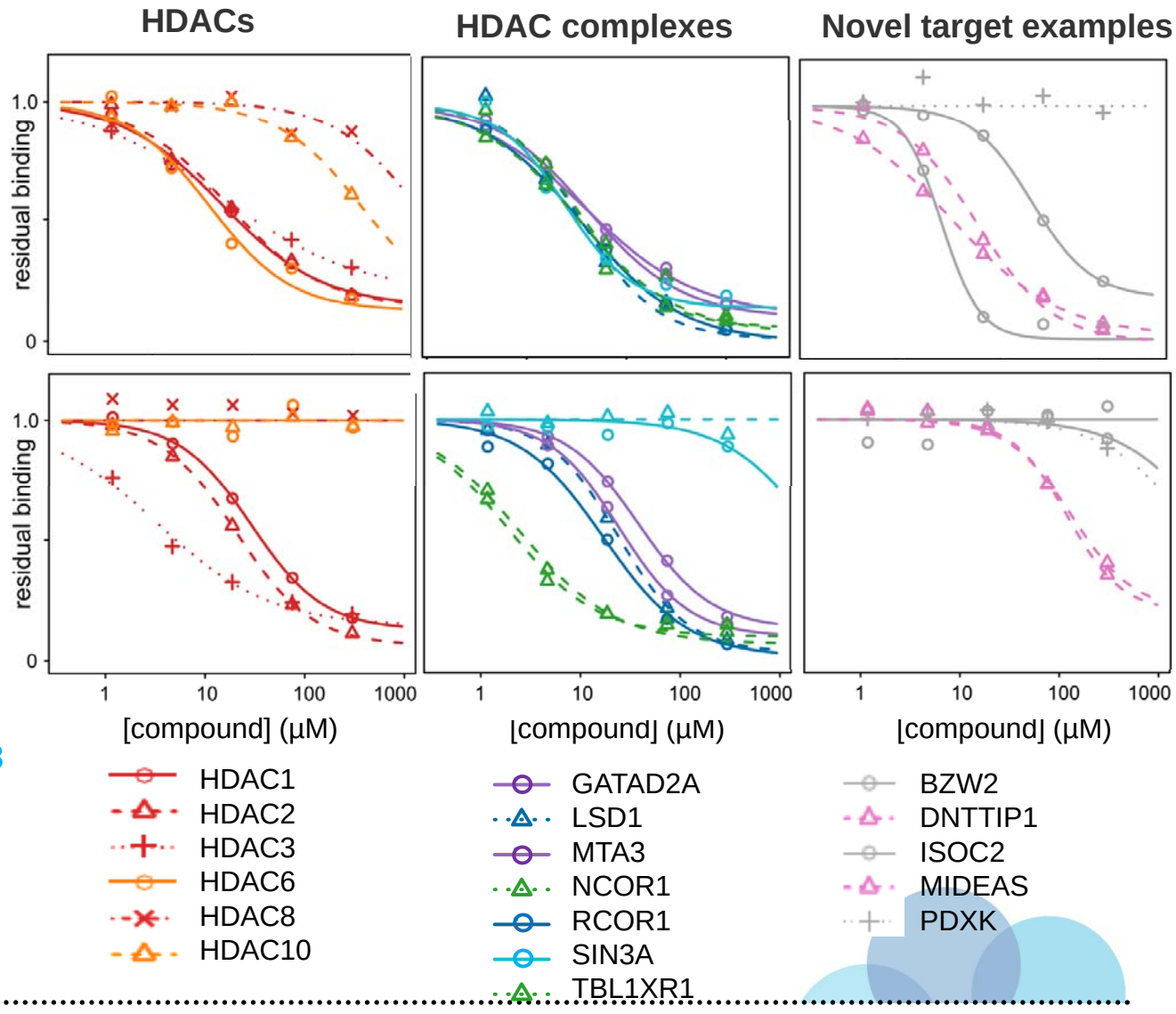
BML210



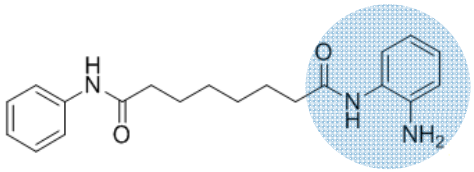
benzamide

Substitution of headgroup leads to:

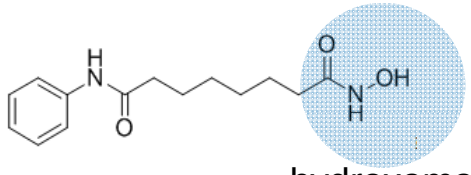
- HDAC3 / NCoR selectivity
- Impaired binding to the Sin3 complex
- different off-target profile



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



BML210 benzamide



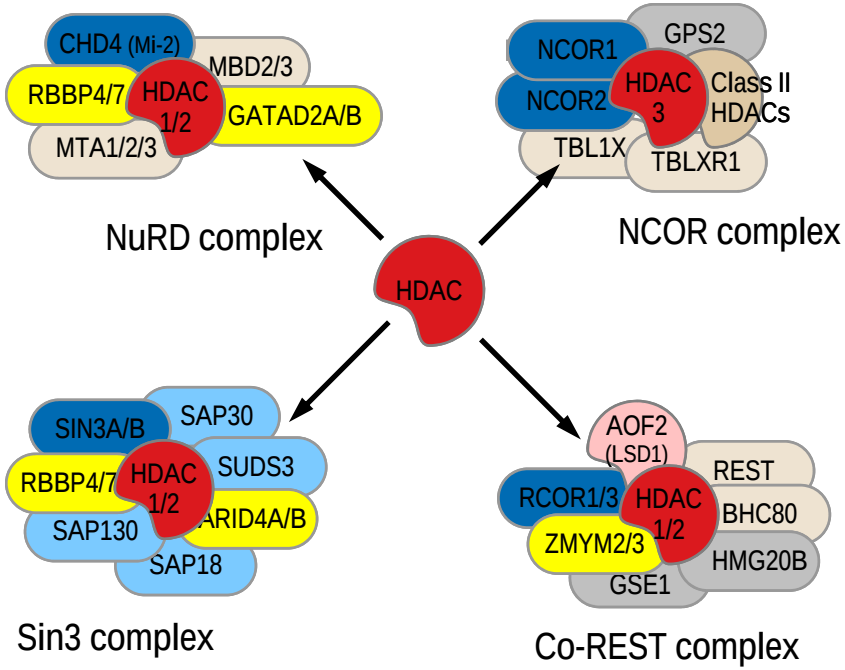
SAHA hydroxamate

BML210	40 µM

BML210	no inhib.

BML210	2 µM

BML210	20 µM



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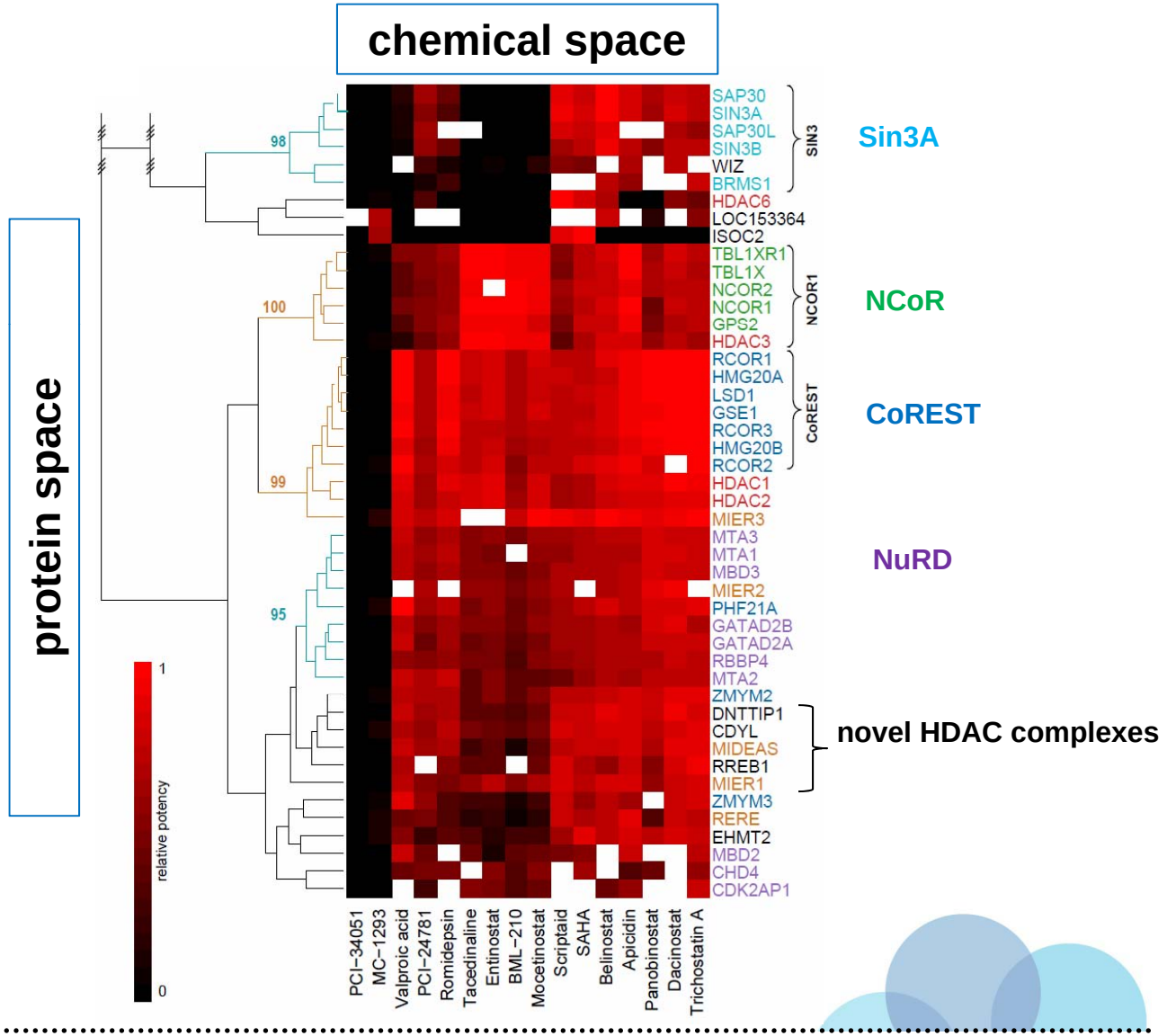
HDAC inhibitors "recognize" protein complexes

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



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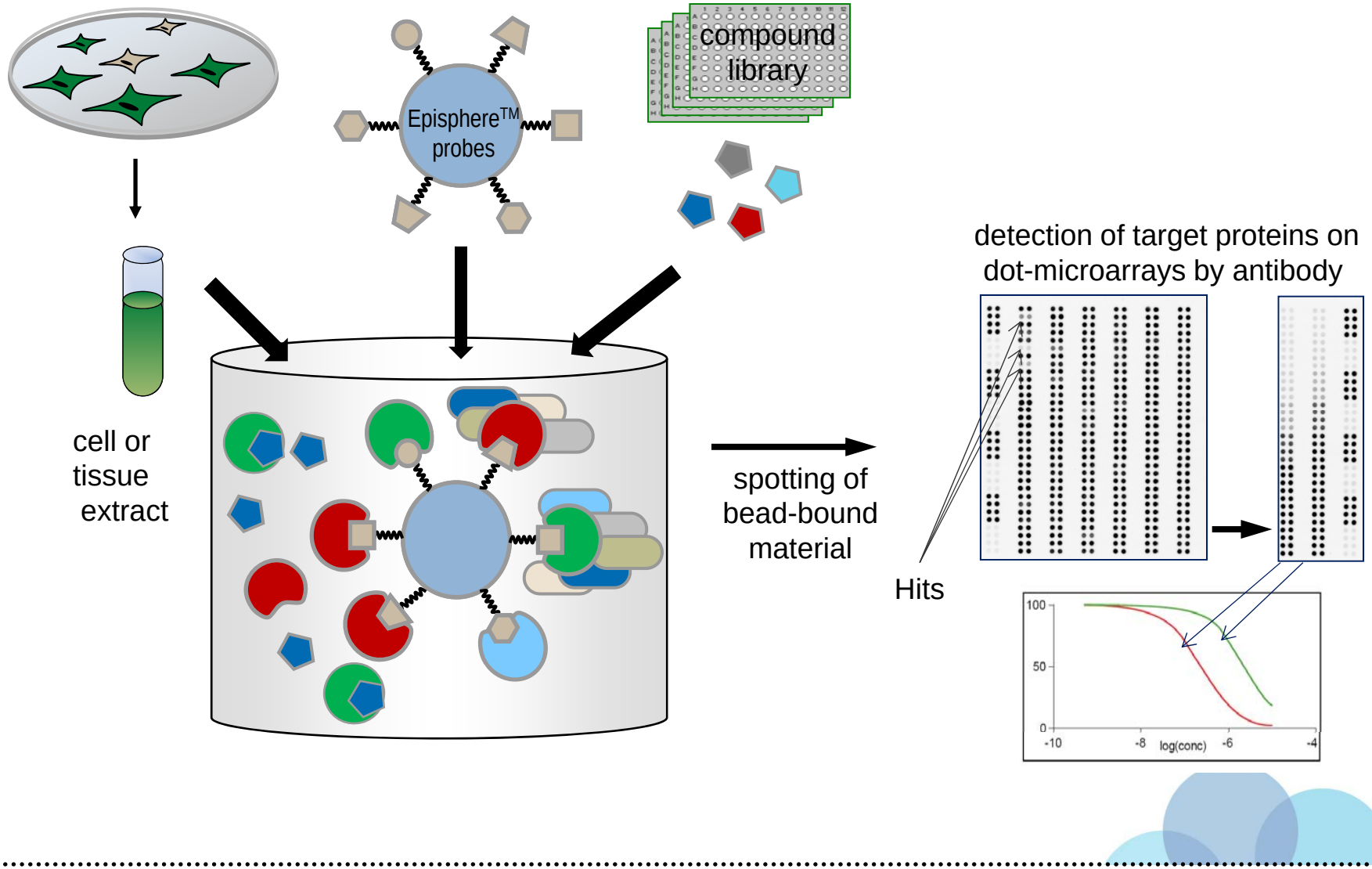
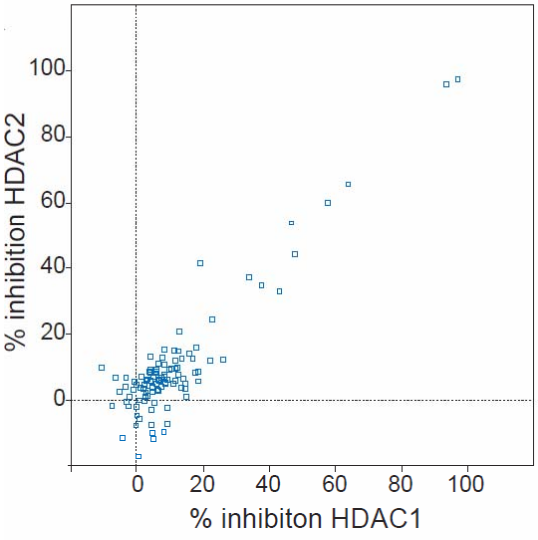
Screening with native protein complexes

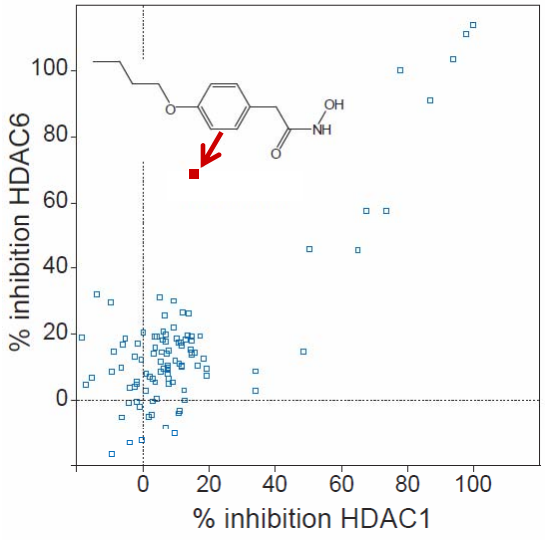


Chemoproteomic library profiling identifies selective HDAC inhibitors

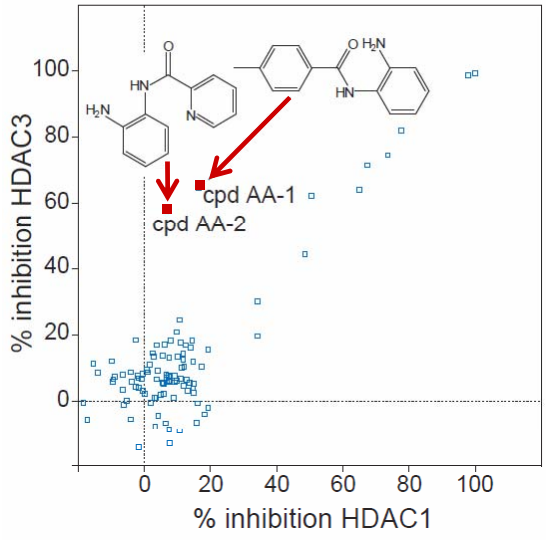
- **"Complexes" screened: HDAC1, HDAC2, HDAC3 and HDAC6**
- **Focused small molecule library, screening at 10µM**



HDAC2 vs HDAC1

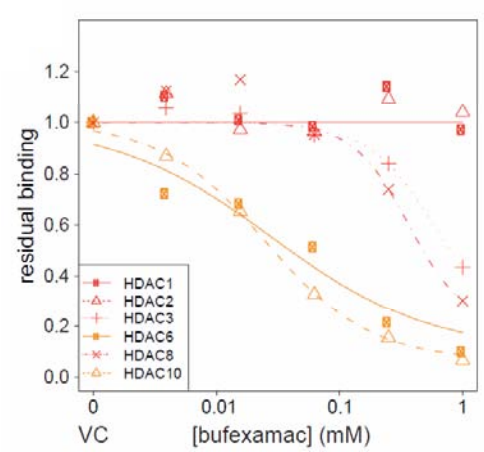


HDAC6 vs HDAC1

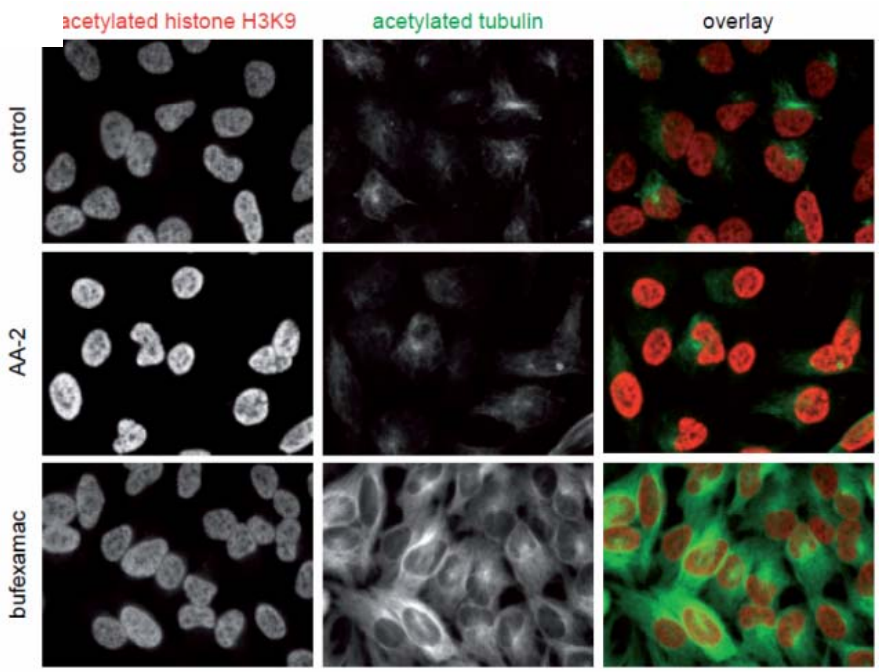


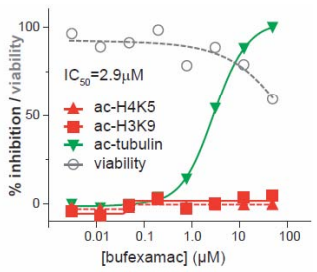
HDAC3 vs HDAC1

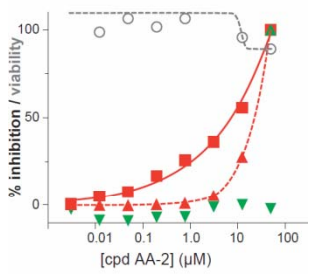
The NSAID bufexamac is a selective HDAC6/10 inhibitor



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.

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Summary

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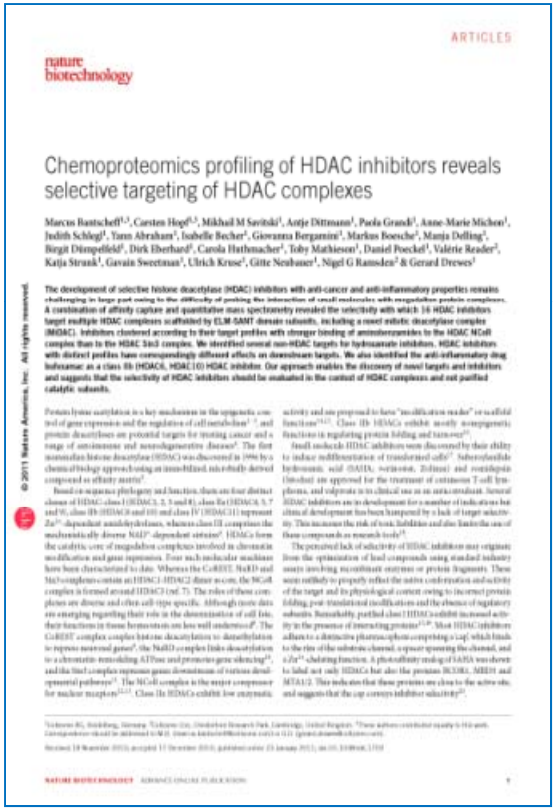
- Introduction
- Chemoproteomics target profiling of HDAC inhibitors
- Tripartite interaction proteomics: BET complexes

Epigenetic Target Classes amenable to chemoproteomic approaches

- HDACs (3/2011)



nature biotechnology

ARTICLES

Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes

Marcus Bantscheff[1,3], Carsten Hopf[1,3], Mikhail M Savitski[1], Antje Dittmann[1], Paola Grandi[1], Anne-Marie Michon[1], Judith Schlegl[1], Yann Abraham[1], Isabelle Becher[1], Giovanna Bergamini[1], Markus Boesche[1], Manja Delling[1], Birgit Dümpelfeld[1], Dirk Eberhard[1], Carola Huthmacher[1], Toby Mathieson[1], Daniel Poeckel[1], Valérie Reader[1], Katja Strunk[1], Gavain Sweetman[1], Ulrich Kruse[2], Gitte Neubauer[1], Nigel G Ramsden[2] & Gerard Drewes[1]

- Bromodomain proteins (10/2011)

EPINOVA DPU

+ T. Kouzarides

nature International weekly journal of science

LETTER

doi:10.1038/nature10509

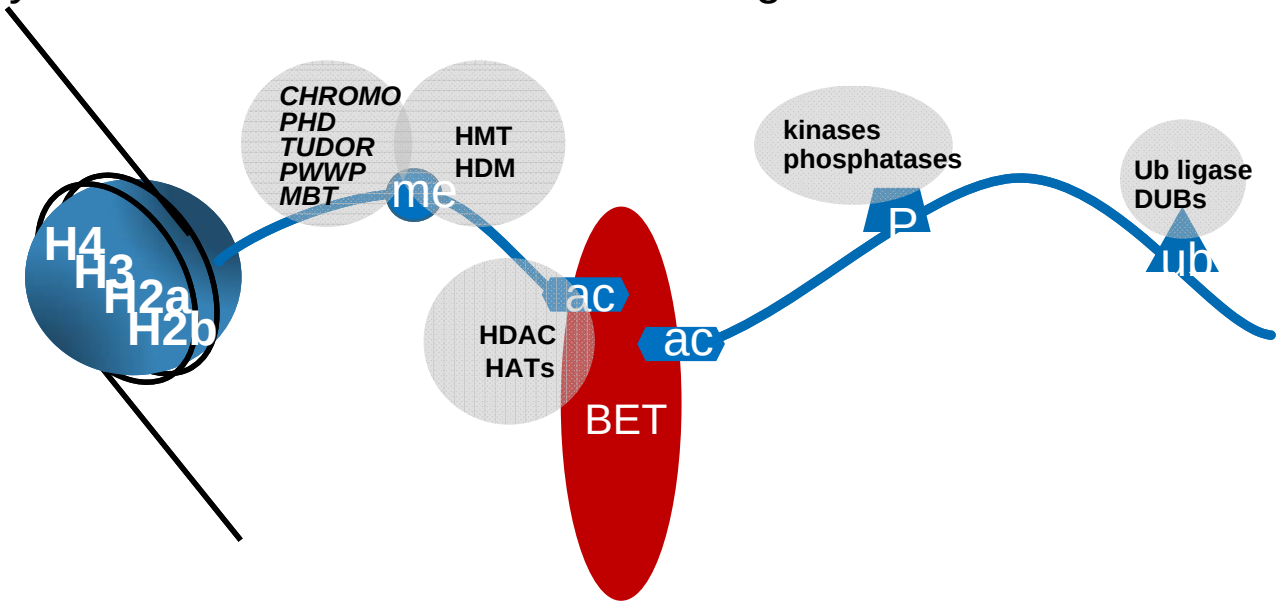
Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia

Mark A. Dawson[1,2*], Rab K. Prinjha[3*], Antje Dittmann[4*], George Giotopoulos[1], Marcus Bantscheff[4], Wai-In Chan[1], Samuel C. Robson[2], Chun-wa Chung[5], Carsten Hopf[4], Mikhail M. Savitski[4], Carola Huthmacher[4], Emma Gudgin[1], Dave Lugo[3], Soren Beinke[3], Trevor D. Chapman[3], Emma J. Roberts[3], Peter E. Soden[3], Kurt R. Auger[6], Olivier Mirguet[7], Konstanze Doehner[8], Ruud Delwel[9], Alan K. Burnett[10], Phillip Jeffrey[3], Gerard Drewes[4], Kevin Lee[3], Brian J. P. Huntly[1*] & Tony Kouzarides[2*]

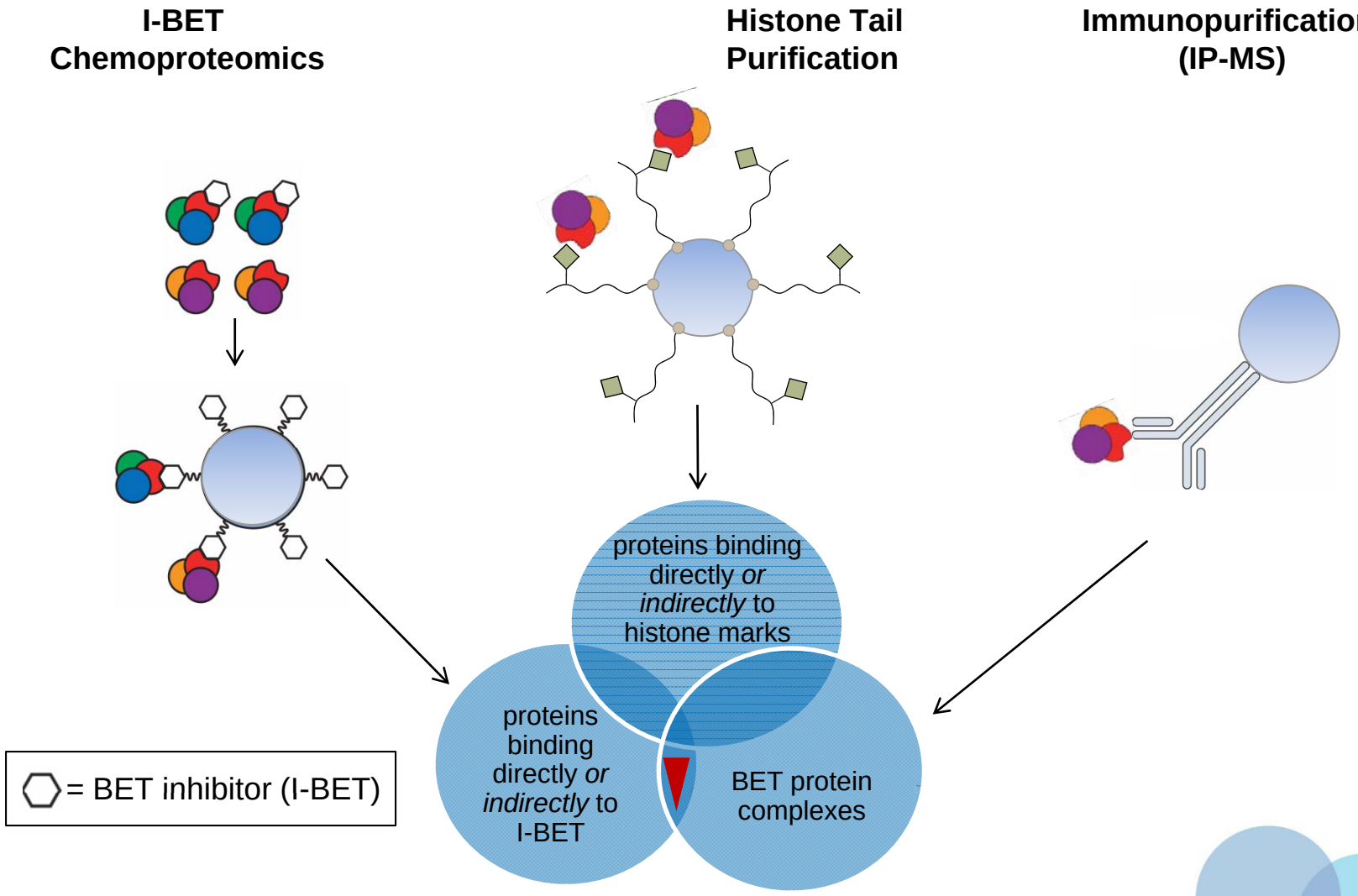
- PARPs
- K-Methyltransferases
- K-Demethylases

BET – bromodomain and extra-terminal domain proteins

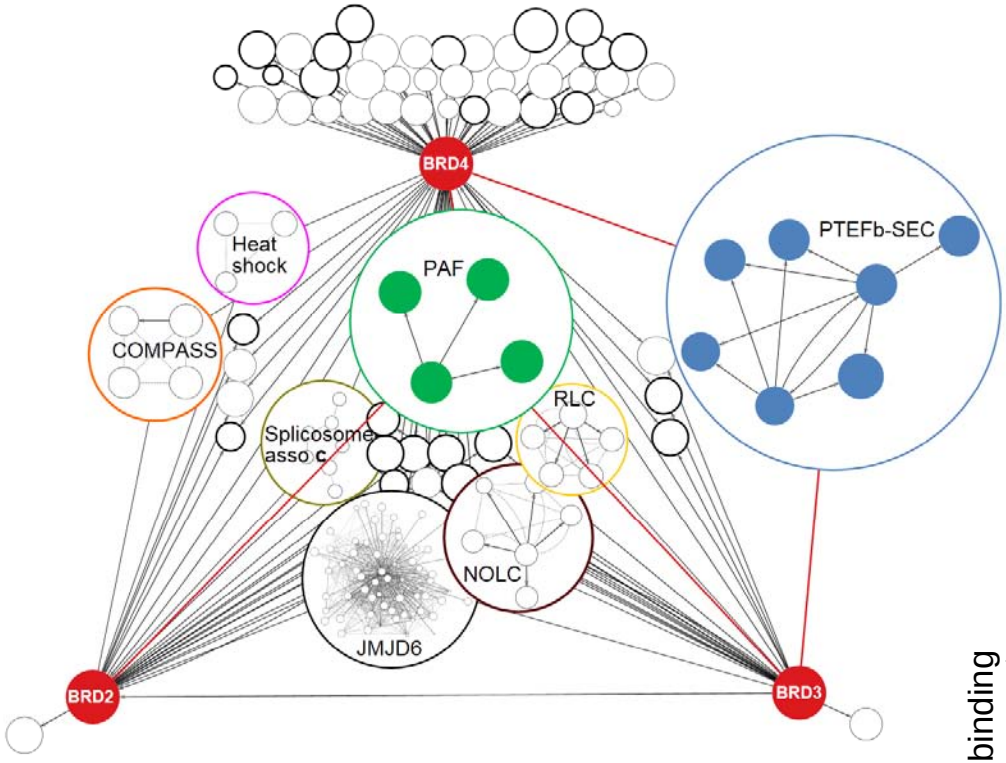
- Bromodomain: Acetyl-lysine recognizing domain – targets BETs and associated complexes to chromatin
- Facilitates transcriptional activation
- Highly conserved Bromodomains are target for small molecule inhibitors

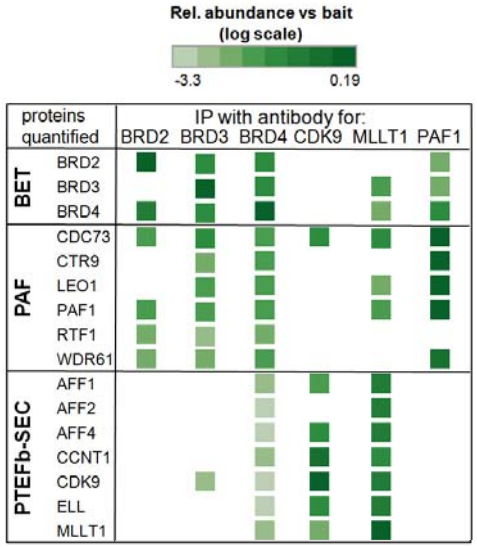


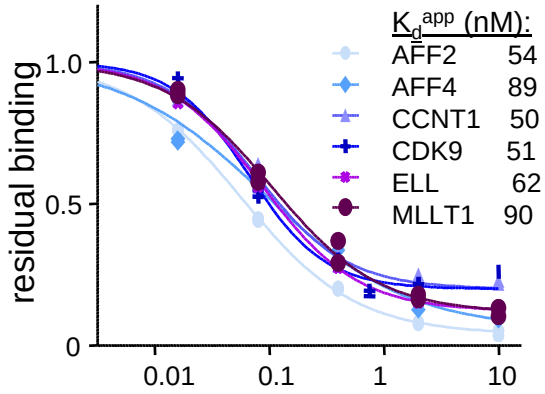
Tripartite interaction proteomics strategy

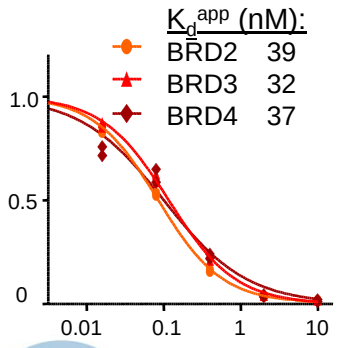


PTEFb-SEC and PAF are BRD3/4 associated complexes



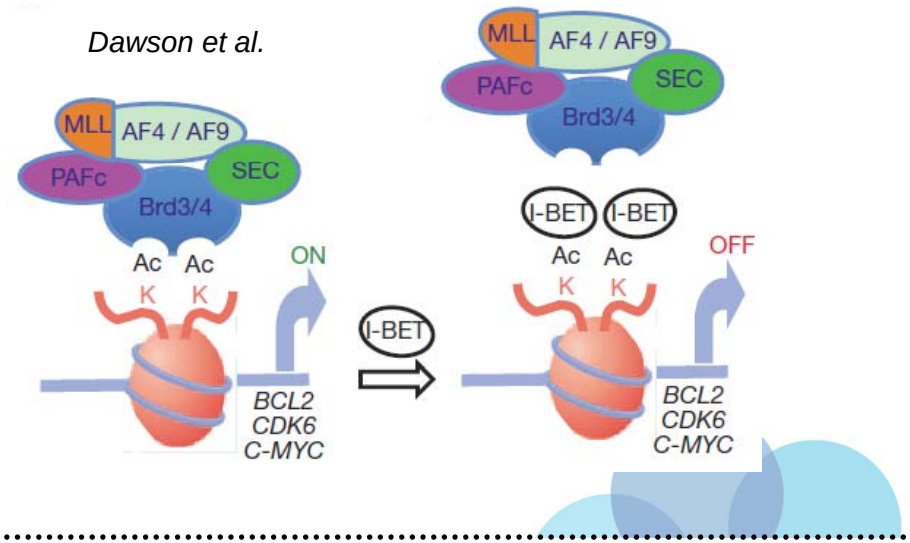






Summary

- 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 methyl-transferase 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



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The Cellzome Team

Special thanks:
Marcus Bantscheff
Mikhail Savitski

Antje Dittmann
Carsten Hopf

Gerard Drewes
Anne-Marie Michon
Paola Grandi