SGC

# Epigenetics: A pioneer area for drug discovery across multiple therapeutic areas

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Sept 30th 2010

SGC Toronto

SGC Oxford

SGC Stockholm

# Structural Genomics Consortium

Public-private consortium funding pre-competitive protein-based science

- GSK, Merck, Novartis
- Canadian, Swedish granting agencies
- Wellcome Trust, UK

Goals: 3D protein structures for biological and drug discovery (~160/year)

Three sites: Toronto, Oxford, Stockholm

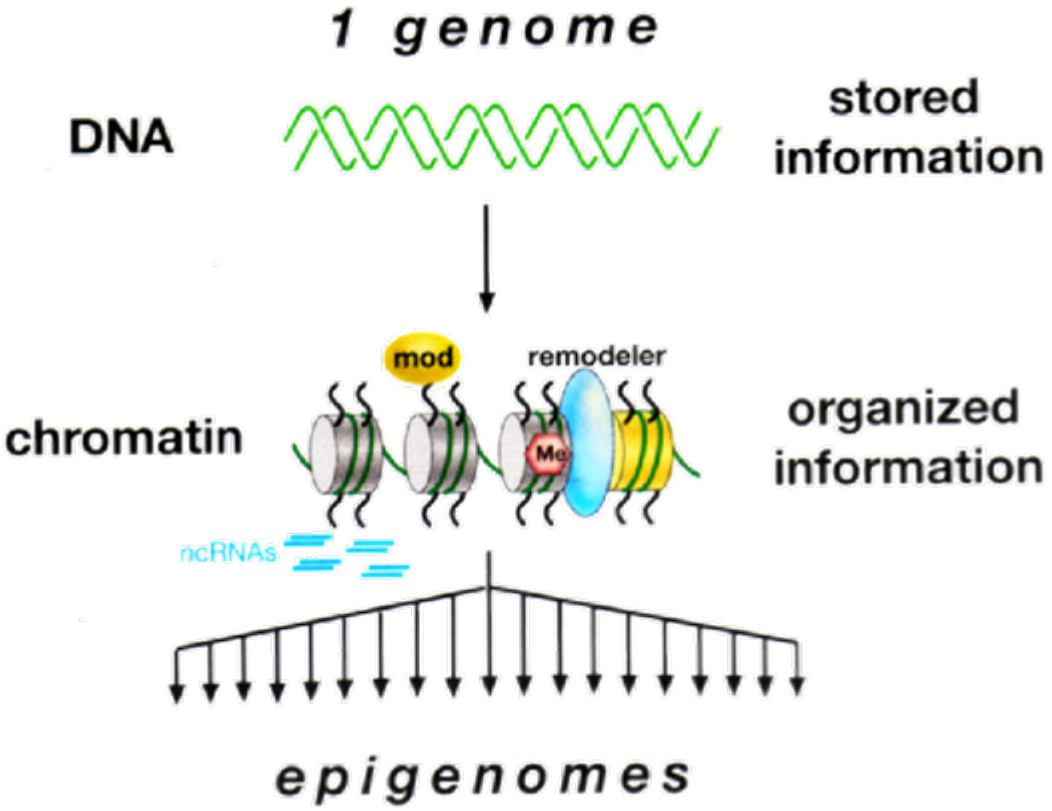
All data and reagents are made publicly available, without restriction on use

- 3D structures
- Protein expression clones
- Protocols for expression and crystallization
- Other protein-based protocols and methodologies
- www.TheSGC.org

***New initiatives in developing chemical probes and protein capture reagents for biological discovery and potential new drug targets***

# Epigenetics

**Heritable changes in phenotype caused by mechanisms other than changes in the underlying DNA sequence**

# Epigenetics: Pioneer Target Area

***Biologically attractive, "pioneer target area"***

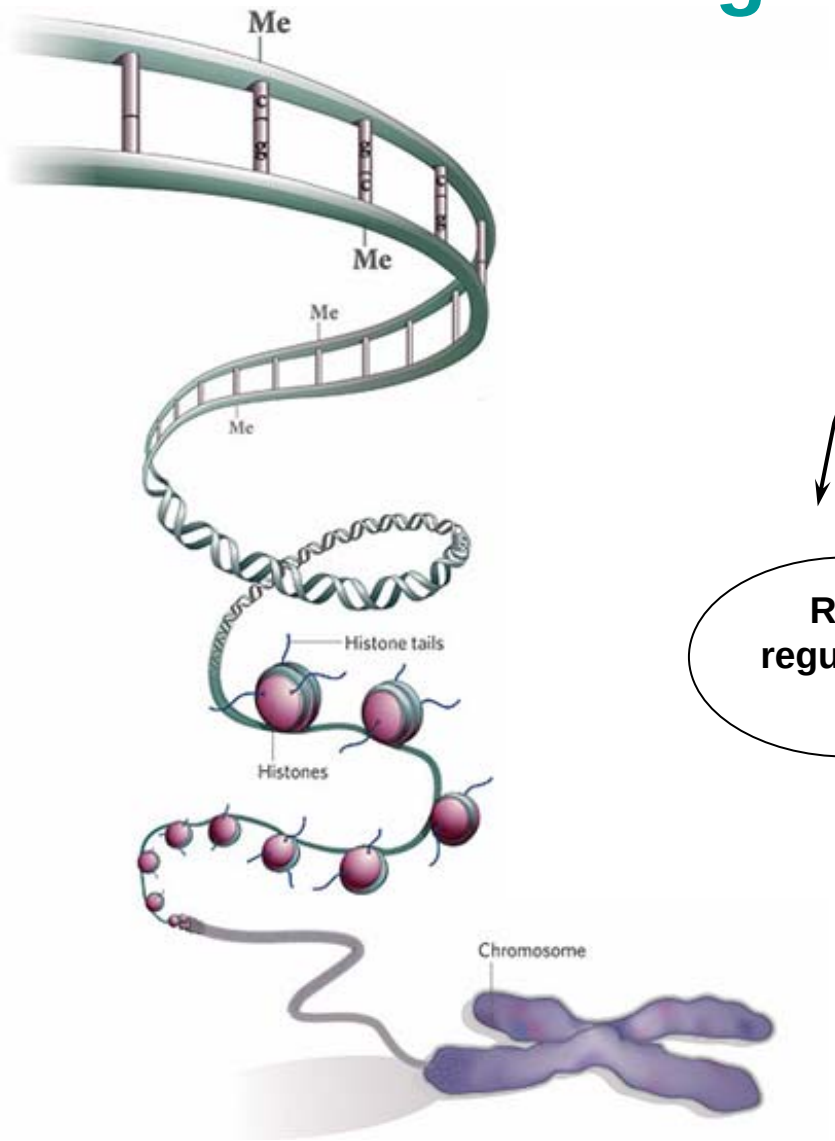
- Play a key role in development, differentiation and stem cell biology
- Underlie many chronic diseases: cancer, inflammation, psychiatric disorders
- Directly impact transcriptional programs, DNA repair & metabolism
- Intense area of research for which there is a receptive community to test chemical probes and protein capture reagents

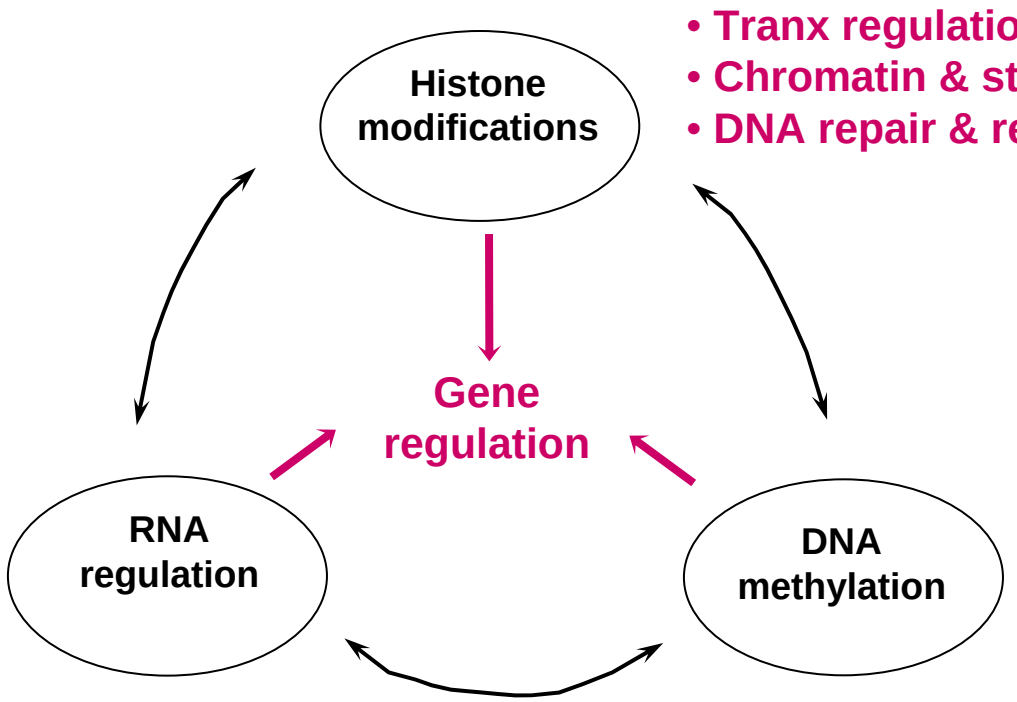
***Epigenetic targets appear to be Druggable***

- SAHA (HDAC inhibitor) approved for cutaneous T-cell lymphoma
- Inhibitors of DNA MTases shown to reactivate silenced genes
- nM inhibitors of Bromo domains have been developed and can affect transcriptional programs.

***Opportunity for discovery of new biology and new drug targets using chemical biology approaches***

# Three basic mechanisms of epigenetic regulation

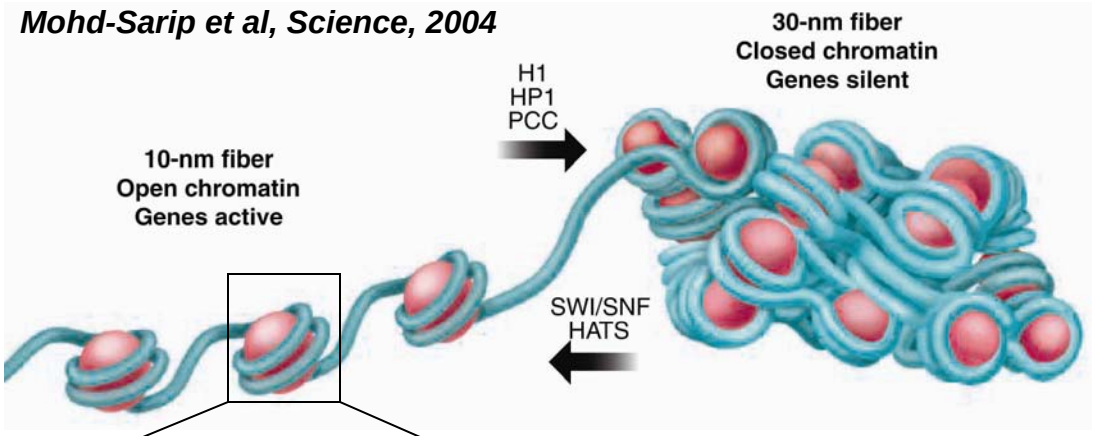



- Tranx regulation
- Chromatin & struct
- DNA repair & rep

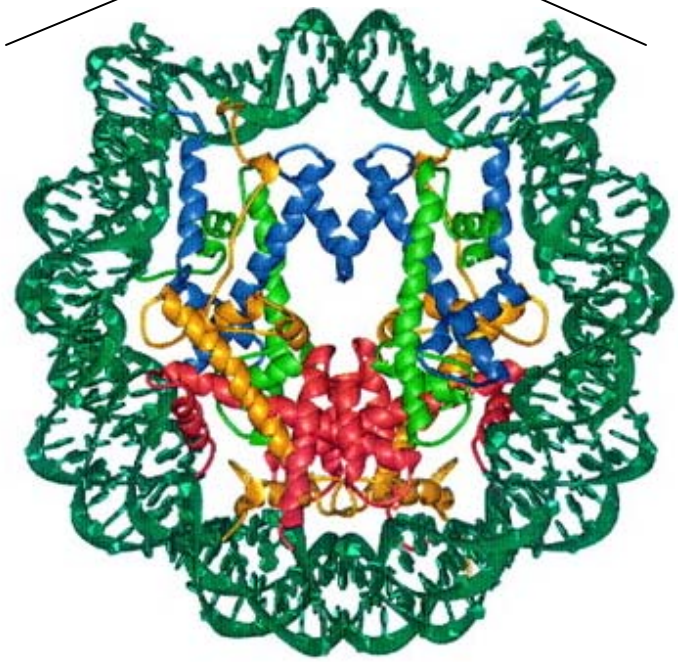
Gene silencing

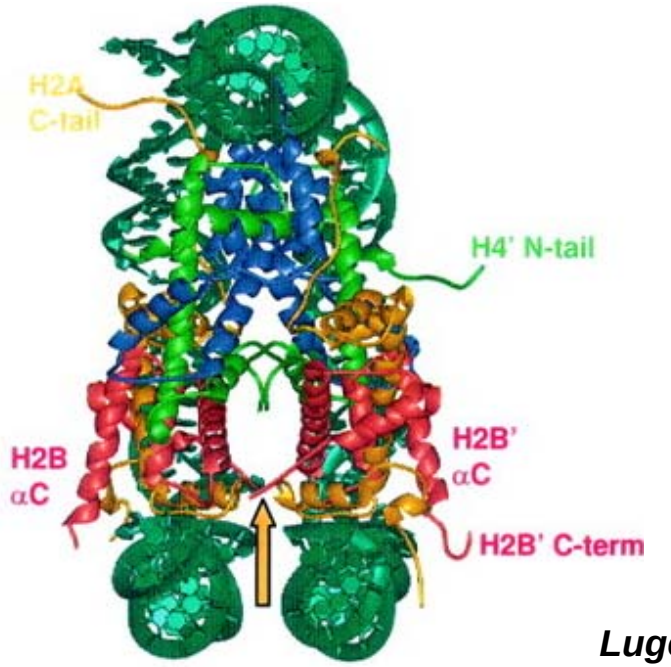
# Gene expression is regulated by chromatin structure and its covalent modifications




*Strahl & Allis, 2000*

**The "Histone Code" hypothesis: Covalent modifications of histone tails encode heritable regulatory information**



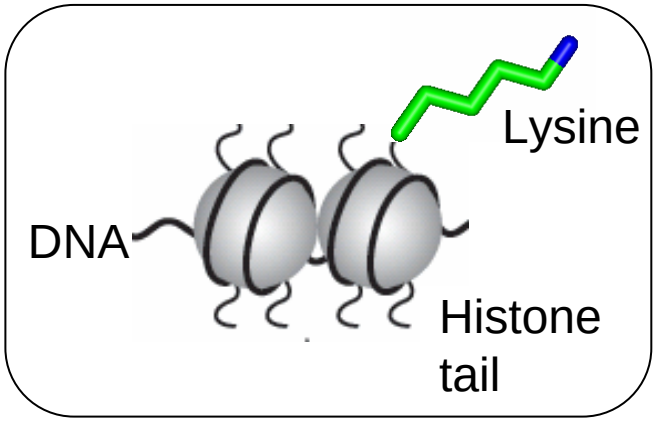


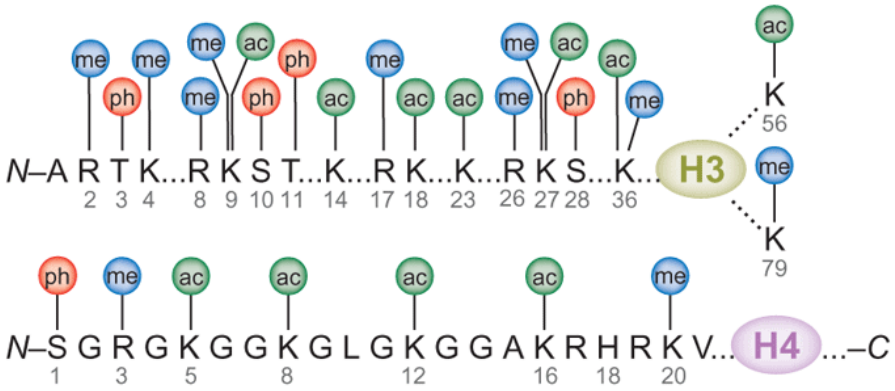

**Histones**

- H2A
- H2B
- H3
- H4

*Luger et al., Nature, 1997*

# Acetyl- and methyl- lysines are an important component of the histone code



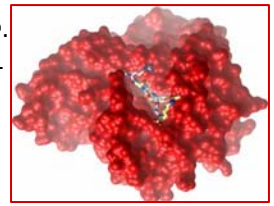

| Lysine Modification | Write | Read | Erase |
|---|---|---|---|
| Acetyl | HAT | BROMO | HDAC |
| Methyl | HMT | Royal Family PHD | KDM |

Oxford

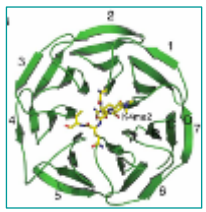
Toronto

# Readers, Writers and Erasers of Histone Marks: Key Focus of SGC Structural Effort

Ng et al, ***Nature***. 2007, 448:87-91



Shuetz et al, ***EMBO J*** 2006, 25:4245-52



JMJD2

PRDM2

SUV39H2

EHMT1

EHMT2


CBX2

CBX3

CBX4

CBX7

WDR5

SETMAR

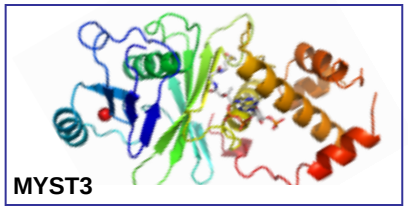
**Histone Acetyltransferases**: not so specific

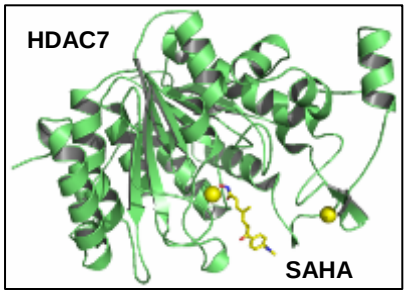
**HDACs**: Sirt5 and HDAC7 - substrates unknown

**Histone Methyltransferases**: site specific

**Readers**: mixed specificity

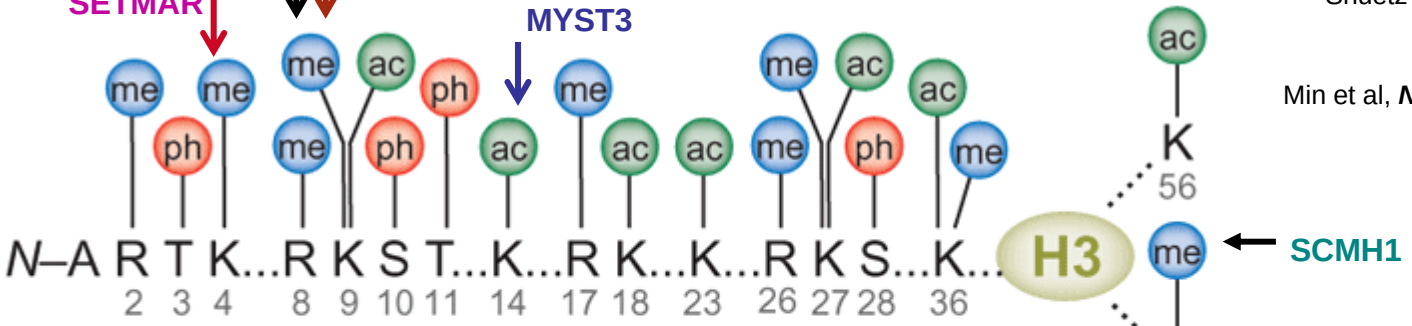
**Demethylase**: specific



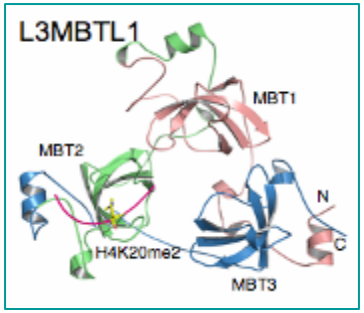




Shuetz et al, ***J. Biol Chem.*** 2007, 283:11355

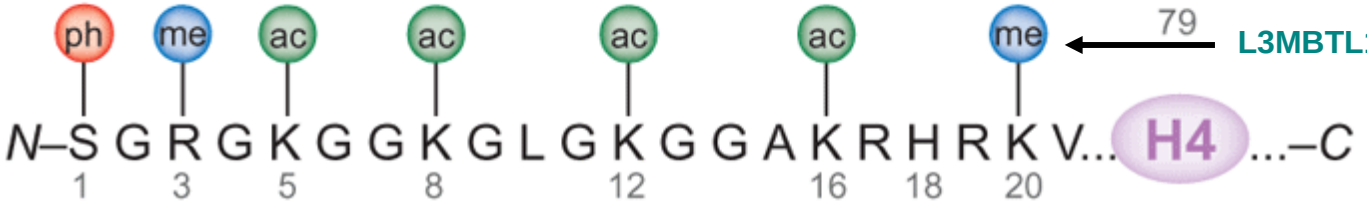
MYST3




Min et al, ***Nature Struct Mol. Biol*** 2007,14:1229




BRDT BRD3 BRD4



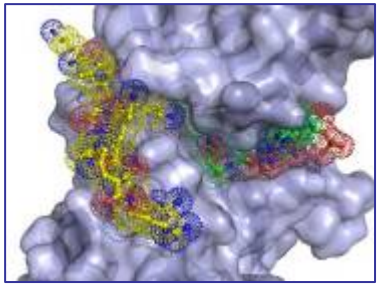

L3MBTL1&2

TIP60 HAT1

TIP60

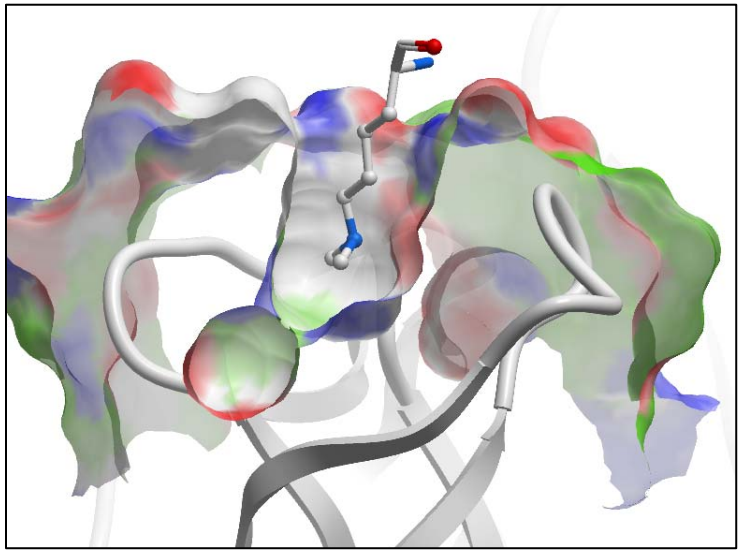
TIP60 HAT1

TIP60 MYST1

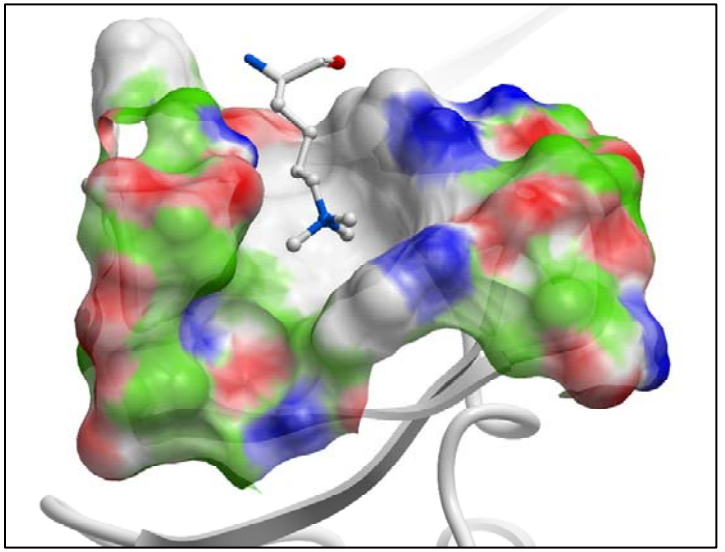


HAT1 with H4K12Ac and CoA

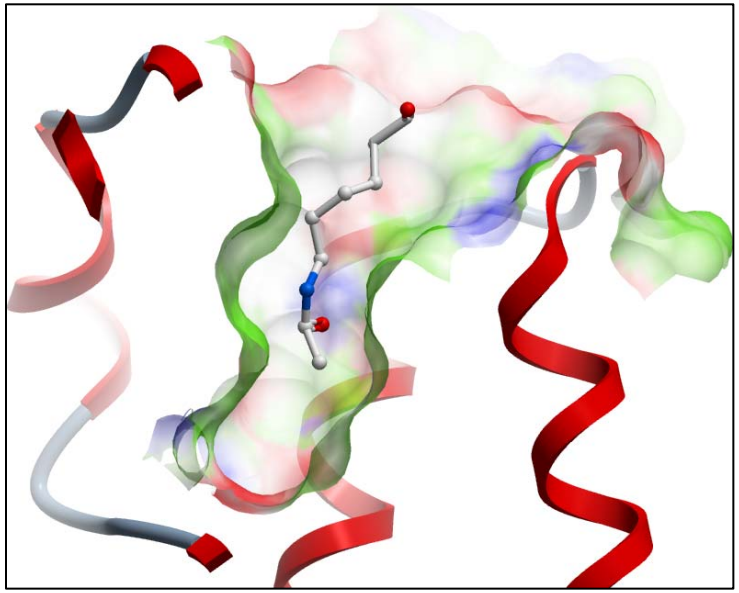
# Can we exploit the variability in Lysine binding sites?



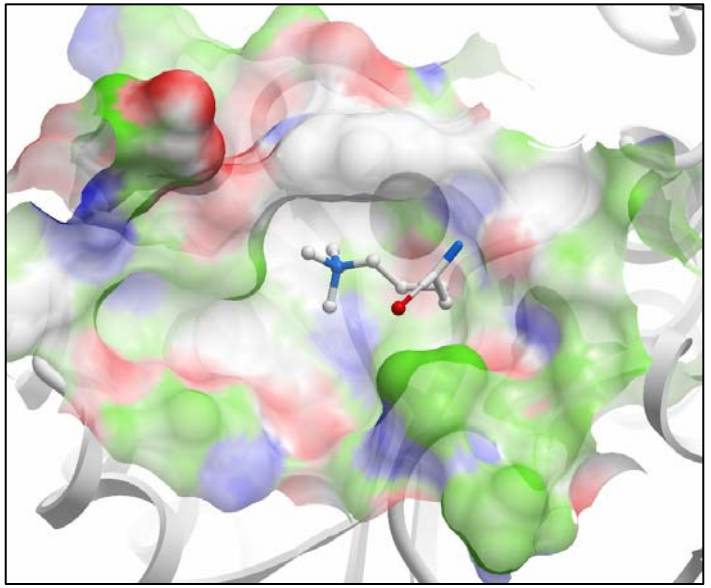
MBT Domain (mono & di-methyl)



Tudor Domain (di- & tri-methyl)



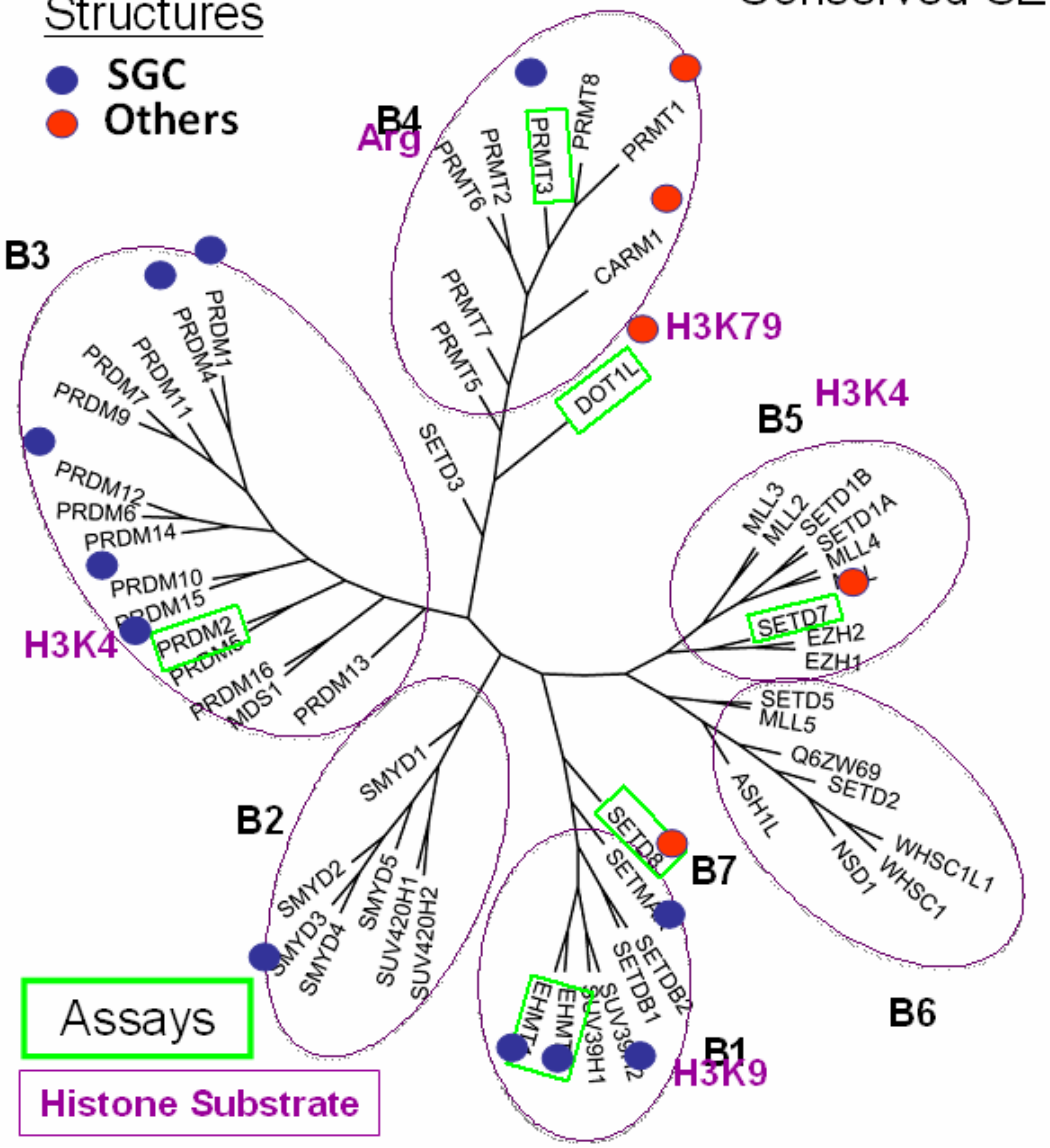
Bromo Domain (acetyl)



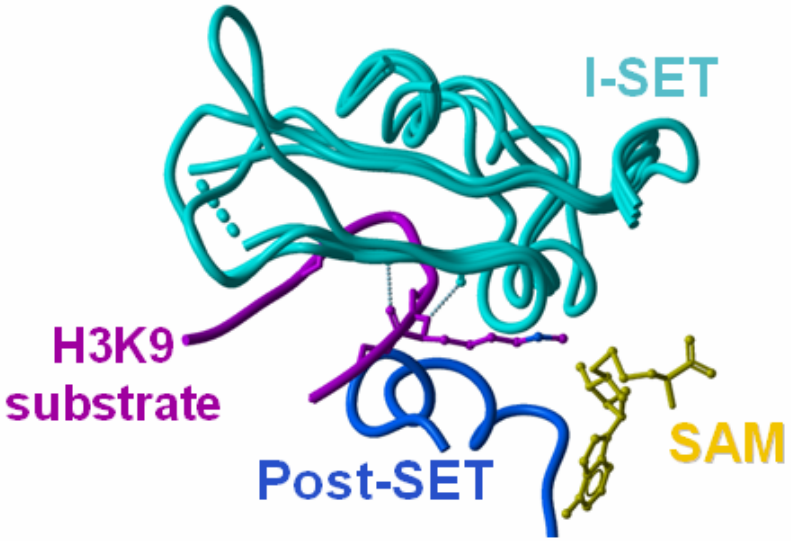
Chromo Domain (tri-methyl)

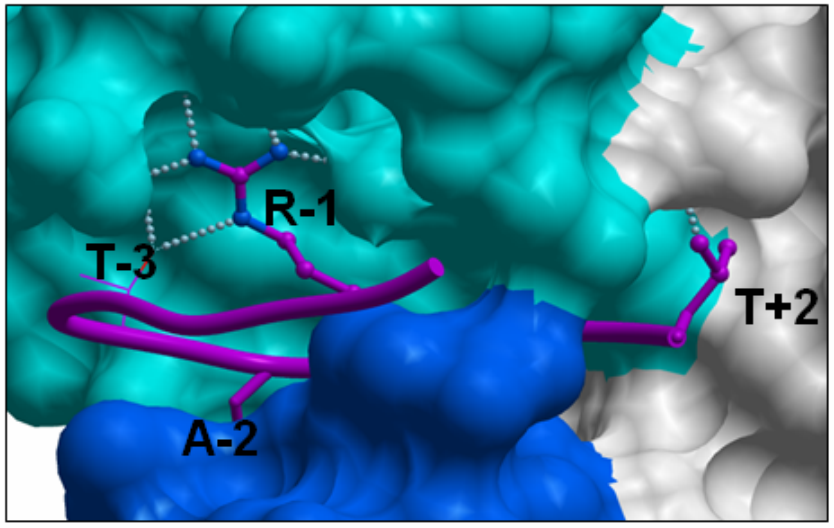
# Histone Methyltransferase Family Approach: Opportunities for selectivity

Structures
- SGC
- Others




Conserved SET domain has unique substrate pockets



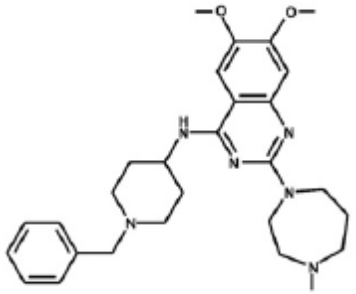



# Case Study: G9a methyltransferase

Molecular Cell
Technique

## Reversal of H3K9me2 by a Small-Molecule Inhibitor for the G9a Histone Methyltransferase

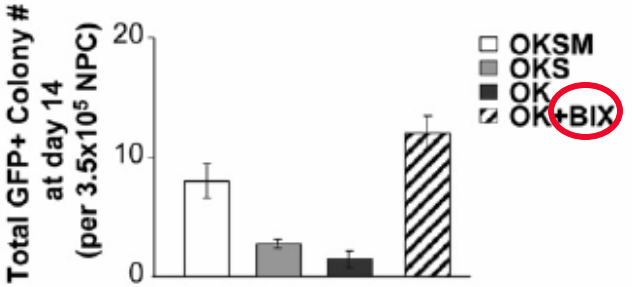
Stefan Kubicek,[1] Roderick J. O'Sullivan,[1] E. Michael August,[2] Eugene R. Hickey,[2] Qiang Zhang,[2] Miguel L. Teodoro,[2] Stephen Rea,[1,3] Karl Mechtler,[1] Jennifer A. Kowalski,[2] Carol Ann Homon,[2] Terence A. Kelly,[2] and Thomas Jenuwein[1,*]



BIX-01294

Cell Stem Cell
Correspondence

## A Combined Chemical and Genetic Approach for the Generation of Induced Pluripotent Stem Cells




Yan Shi,[1] Jeong Tae Do,[2] Caroline Desponts,[1] Heung Sik Hahm,[1] Hans R. Schöler,[2] and Sheng Ding[1,*]

# But, also implicated in Addiction, Cognition/Behavior, Viral Response and Cancer

SCIENCE VOL 327 8 JANUARY 2010

## Essential Role of the Histone Methyltransferase G9a in Cocaine-Induced Plasticity

Ian Maze,[1] Herbert E. Covington III,[1] David M. Dietz,[1] Quincey LaPlant,[1,2] William Renthal,[2] Scott J. Russo,[1] Max Mechanic,[2] Ezekiell Mouzon,[1] Rachael L. Neve,[3] Stephen J. Haggarty,[4,5] Yanhua Ren,[1] Srihari C. Sampath,[6] Yasmin L. Hurd,[1] Paul Greengard,[7] Alexander Tarakhovsky,[6] Anne Schaefer,[7] Eric J. Nestler[1]*

678 Neuron 64, 678–691, December 10, 2009

## Control of Cognition and Adaptive Behavior by the GLP/G9a Epigenetic Suppressor Complex

Anne Schaefer,[1,4] Srihari C. Sampath,[2,4,6] Adam Intrator,[1] Alice Min,[1] Tracy S. Gertler,[3] D. James Surmeier,[3] Alexander Tarakhovsky,[2,5,*] and Paul Greengard[1,5,*]

***J Biol Chem. 2010 Mar 24.***
**Involvement of histone H3 Lysine 9 (H3K9) methyl transferase G9a in the maintenance of HIV-1 latency and its reactivation by BIX01294. Imai K, Togami H, Okamoto T.**

## G9a and Glp Methylate Lysine 373 in the Tumor Suppressor p53*[S]

Received for publication, September 8, 2009, and in revised form, January 5, 2010 Published, JBC Papers in Press, January 29, 2010, DOI 10.1074/jl

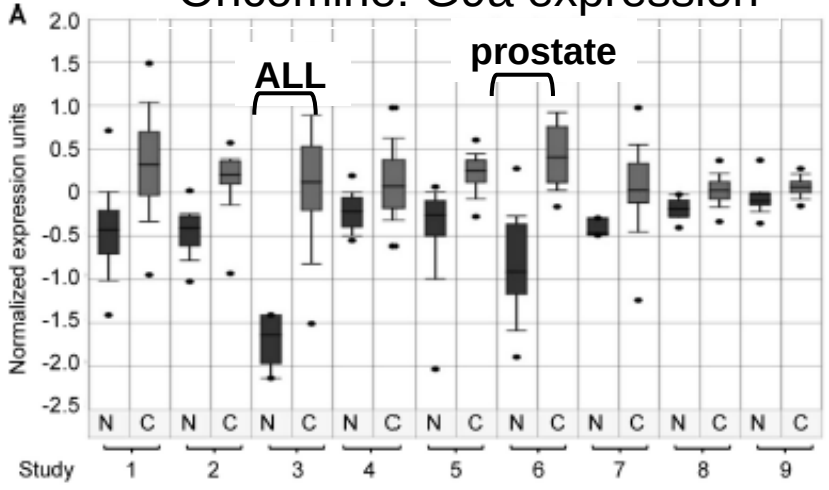
Jing Huang[‡§1], Jean Dorsey[§], Sergei Chuikov[¶], Xinyue Zhang[‡], Thomas Jenuwein[‖**], Danny Reinbe and Shelley L. Berger[§§§]

Mol Cancer Res 2009;7(6). June 2009

## Distinct Roles for Histone Methyltransferases G9a and GLP in Cancer Germ-Line Antigen Gene Regulation in Human Cancer Cells and Murine Embryonic Stem Cells

Petra A. Link,[1] Omkaram Gangisetty,[1] Smitha R. James,[1] Anna Woloszynska-Read,[1] Makoto Tachibana,[2] Yoichi Shinkai,[2] and Adam R. Karpf[1]

Oncomine: G9a expression




Leukemia (2010) 24, 81–88

## EVI-1 interacts with histone methyltransferases SUV39H1 and G9a for transcriptional repression and bone marrow immortalization

S Goyama[1,2], E Nitta[1], T Yoshino[1], S Kako[1], N Watanabe-Okochi[1], M Shimabe[1], Y Imai[1], K Takahashi[2] and M Kurokawa[1]

# Chemical biology for target discovery/validation

**Goal:** Develop *well characterized* small molecules to be used to link pharmacological inhibition of an individual target (or small group of targets) with cellular biology/pathways/phenotype

- **Potent:** IC50 < 100 nM *in vitro*
- **Selective:** 30x over related proteins
- **Cell permeable:** IC50 < 1 uM in cell
- **Low/No *cellular* toxicity**

- **Make available to research community**

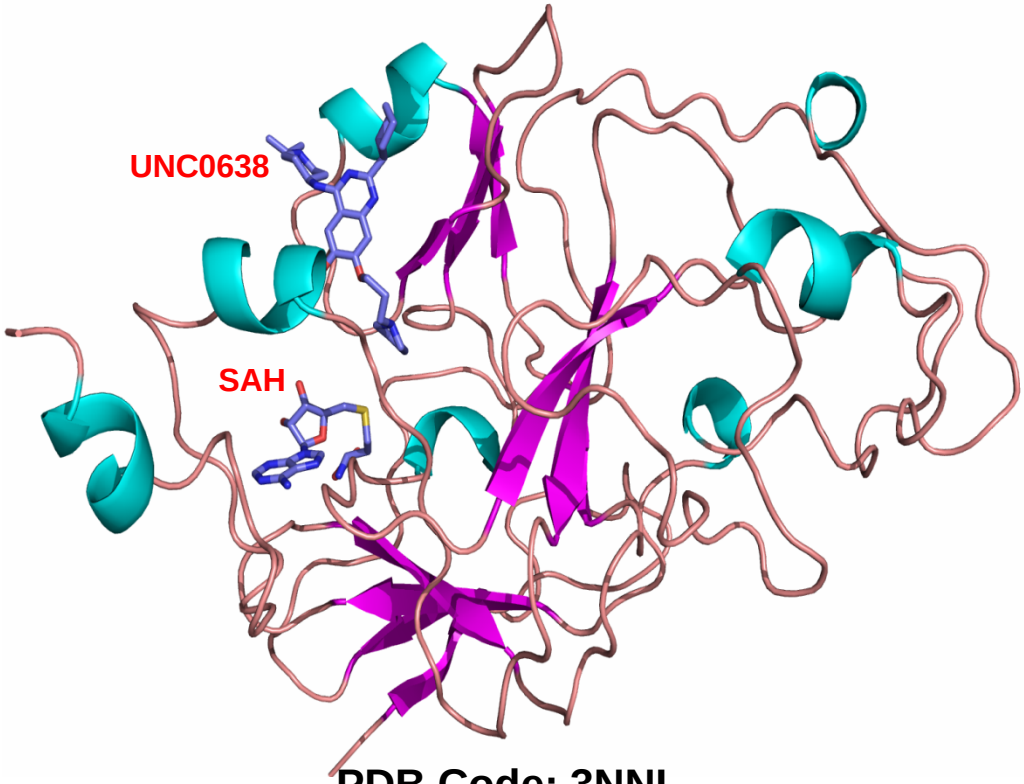
# Unique Consortium: Epigenetics PPP

**<u>Objective</u>: identify 40 probes and make compounds & data publicly available (no restriction on use) over 4 years**

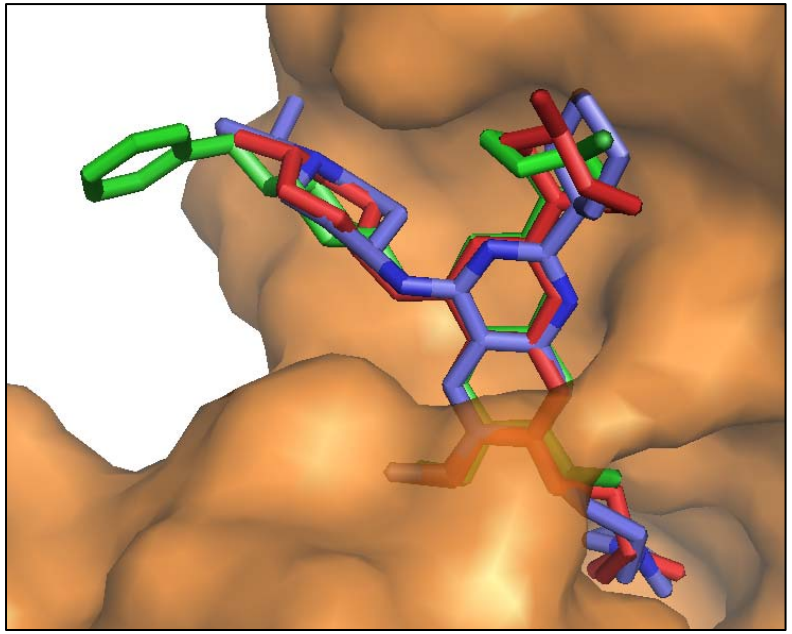
**<u>Participants</u>:** **<u>Funder</u>**

- **SGC – Toronto (**HMTs, Royal Family, HATs**)** **Ontario $4.6M**
- **SGC – Oxford (**KDMs, Bromo domains**)** **Wellcome Trust $8M**
- **SGC – Stockholm (**PARPs**)** **Swedish Sci. F. $ 3M**
- **GSK, Pfizer, Novartis Chemistry (**8 med chemist FTEs each**)**
  - commit to release "public probe" other compounds not disclosed
- **NIH Chemical Genomics Center (**20 HTS slots – data public**)**
- **Ontario Inst. Cancer Research (**2 FTE med chemists**)**
- **Frye Lab, University N. Carolina (**4 FTE med chem/assay dev**)**

# Structure based design of potent and selective G9a antagonist




**PDB Code: 3NNI**



**UNC0638 (PDB 3NNI)**

**UNC0224 (PDB 3K5K)**

**BIX-01294 (PDB 3FPD)**

- UNC0638 occupies histone binding groove and does not interact with SAM binding pocket. Same binding mode as BIX01294 & UNC0224

# Associated Data Published on SGC Website

etting Started | Latest Headlines

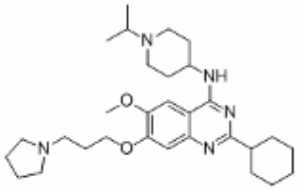
Probes for G9a/G... | Structural Genomics Consortium Datab...

www.TheSGC.org/chemical_probes/UNC0638

## UNC0638: Selective chemical probe for G9a/GLP methyltransferases

UNC0638 Data Sheet | How to obtain this probe | UNC0638 released on June 1, 2010

### UNC0638



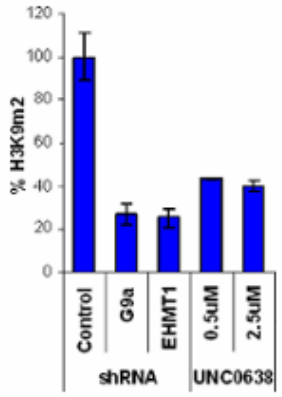
Click on the 'Properties' tab above for more details

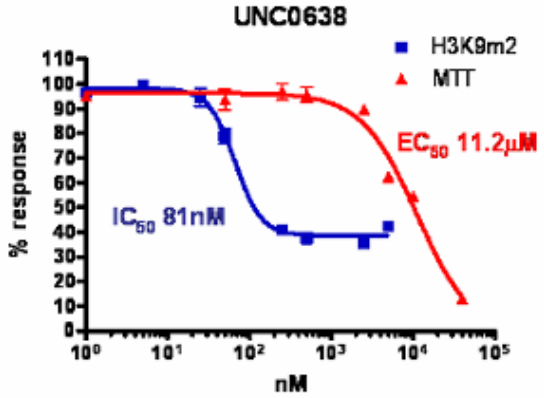
### Biology of the G9a/GLP methyltransferases

G9a (EHMT2) and GLP (EHMT1) catalyze the mono and dimethylation of lysine 9 of histone 3 (H3K9) and other non-histone substrates such as p53 and WIZ.

### Cellular Activity

Significant reduction in H3K9 dimethylation at 100nM in MDA-MB231 cells as measured by fluorescence immunostaining without significant cellular toxicity.




Click on the 'Cell-based Assay Data' tab above for more details

### Selectivity Within Target Family

| Protein | $IC_{50}$/nM (Activity) | Tm shift °C [1] |
|---|---|---|
| G9a (EHMT2) | <15 | 4 |
| GLP (EHMT1) | 19 ± 1 | 8 |
| SETD7 | >10,000 | nt |
| SETD8 | >10,000 | nd |
| PRMT3 | >10,000 | nd |
| SUV39H2 | >10,000 | nt |
| DOT1L | nt | nd |
| PRDM1 | nt | nd |
| PRDM10 | nt | nd |
| PRDM12 | nt | nd |
| SMYD3 | nt | nd |
| JMJD2E | 4660 (AlphaScreen) | nt |
| HTATIP | nt | nd |

(nt=not tested, nd=not detected, [1] singlicate determination @ 100 µM)

### Selectivity Beyond Target Family

**>30% Inhib @ 1 µM**

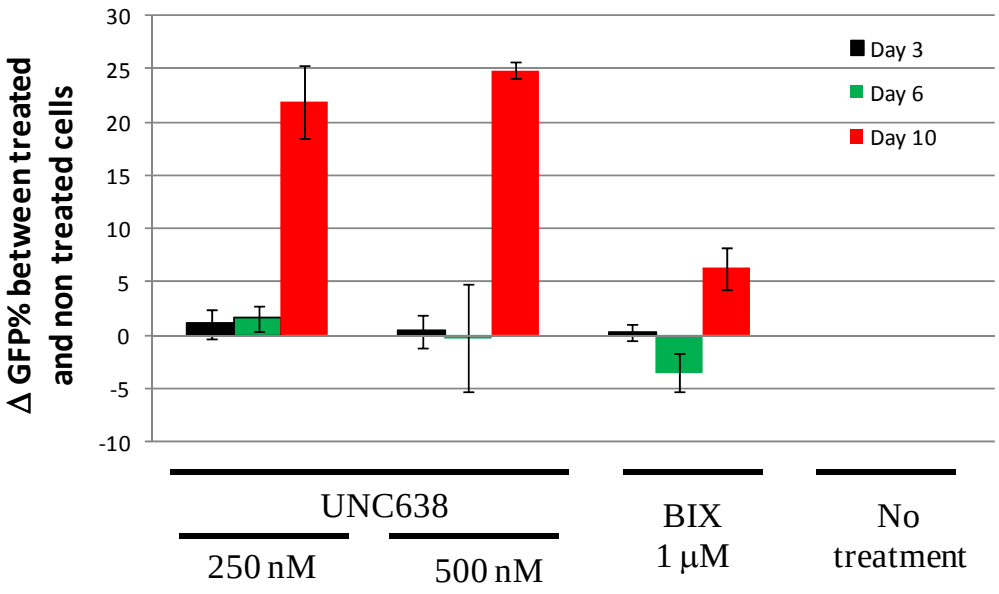
# Functional consequences of G9a/GLP inhibition?

## Well tolerated in variety of cancer cell lines

| Cell Lines | | $IC_{50}$ (nM) | |
|---|---|---|---|
| | | H3K9me2 | MTT |
| Breast carcinoma | MDA231 | 81 ± 9 | 11,000 ± 710 |
| | MCF7 | 70 ± 12 | 7,600 |
| Prostate carcinoma | PC3 | 59 | 14,000 |
| | 22RV1 | 48 | 4,500 |
| Colon carcinoma | HCT116 wt | 210 | 11,000 |
| | HCT116 p53-/- | 240 | 11,000 |
| Human fibroblast | IMR90 | 120 | 2,300 |

*Dalia Barsyte*

## Re-activates expression of retroviral GFP reporter protein



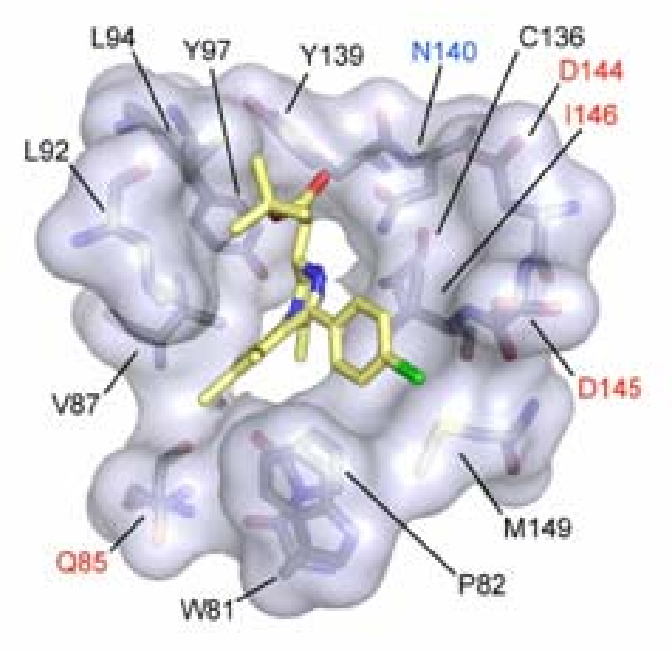

*James Ellis, HSC*

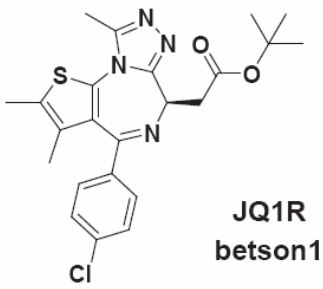
ARTICLE

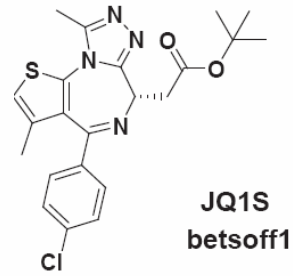
doi:10.1038/nature09504

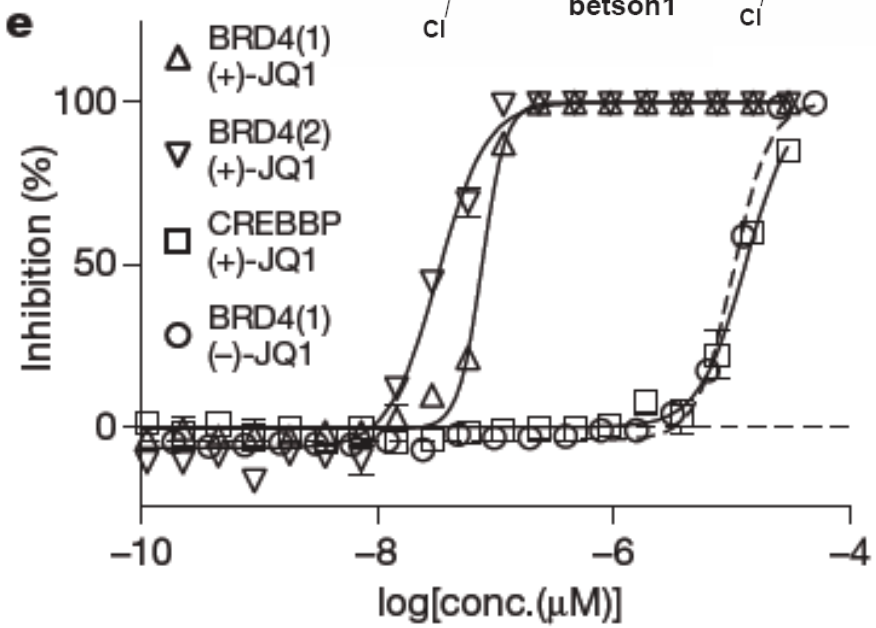
# Selective inhibition of BET bromodomains

Panagis Filippakopoulos[1]*, Jun Qi[2]*, Sarah Picaud[1]*, Yao Shen[3], William B. Smith[2], Oleg Fedorov[1], Elizabeth M. Morse[2], Tracey Keates[1], Tyler T. Hickman[4], Ildiko Felletar[1], Martin Philpott[1], Shonagh Munro[5], Michael R. McKeown[2,6], Yuchuan Wang[7], Amanda L. Christie[8], Nathan West[2], Michael J. Cameron[4], Brian Schwartz[4], Tom D. Heightman[1], Nicholas La Thangue[5], Christopher A. French[4], Olaf Wiest[3], Andrew L. Kung[8,9], Stefan Knapp[1,5] & James E. Bradner[2,6]

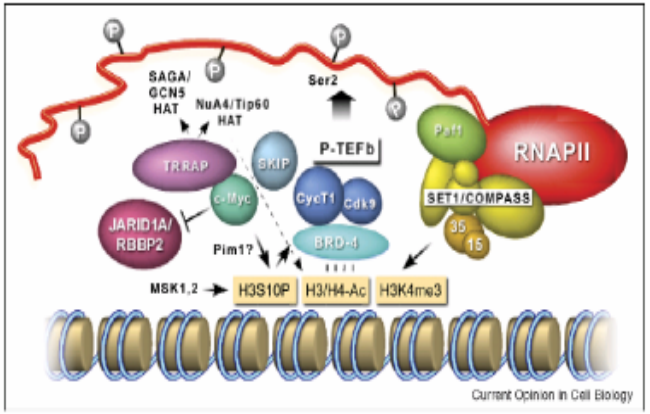


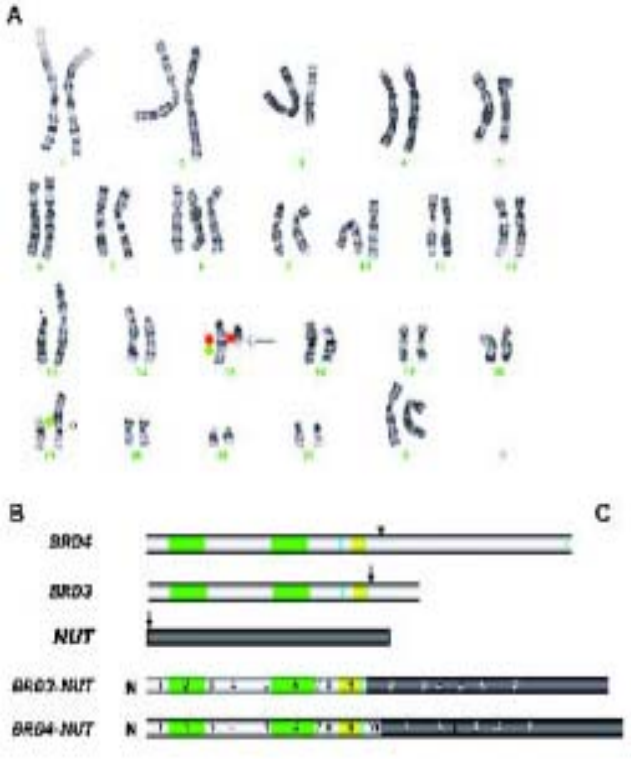



# BET family of BRD proteins




**Regulation of P-Tefb(cdk9) mediated transcription**

- KO of BRD4/2 results in G1 arrest and apoptosis and suppresses many genes required for growth
- P-TEFb and BRD functions with the oncogene c-Myc
- c-Myc interacts with the H3Kme3 specific demethylase JARID1A

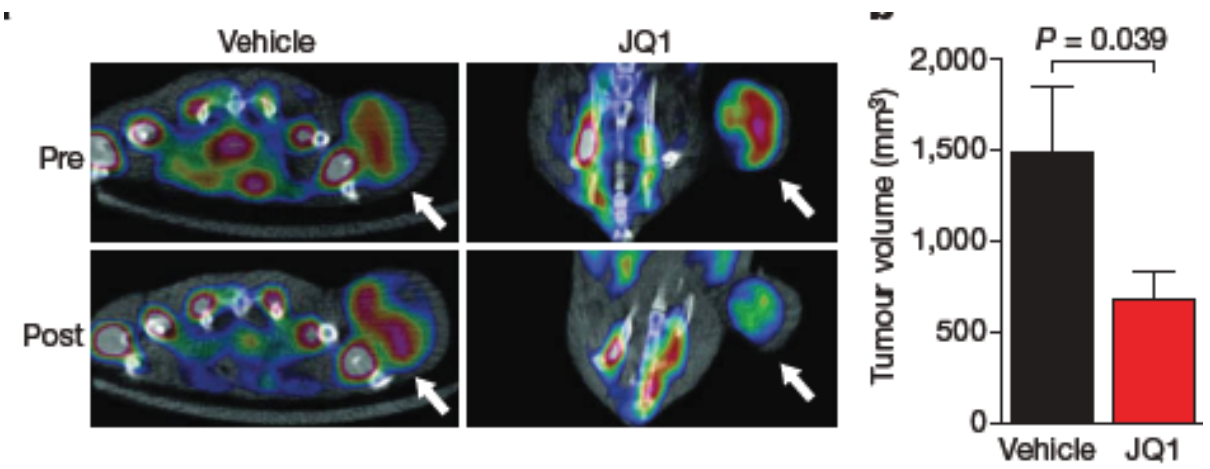



**BET in Disease: NMC**

- *NUT midline carcinoma (NMC)* is a rare, highly lethal cancer that occurs in children and adults.
- NMCs uniformly present in the midline, most commonly in the head, neck, or mediastinum, as poorly differentiated carcinomas
- Rearrangement of the Nuclear protein in testis (*NUT*) that creates a *BRD4-NUT* fusion gene
- Variant rearrangements, some involving the *BRD3* gene
- NMC is diagnosed by fluorescence *in situ* hybridization. However, most cases of NMC currently go unrecognized.

# BET Probe: effective against NMC xenograft

*Jay Bradner, Dana Farber*




## Cellular and *in vivo* studies

- Induces terminal differentiation of BRD4-NUT cell lines derived from MLC patients
- Anti-proliferative effects on cell lines that carry BRD-NUT fusion at 100nM
- Induces apoptosis in BRD-NUT cell lines
- Dissolves nuclear foci typically observed in BRD-NUT cell lines and biopsies
- Reduces tumour growth in xenograft models (50mg/kg IP, enantiomer)
- Displaces BRD4 from E-selectin and TNFα promoter in ChIP assays

Collaboration with J. Bradner Lab, Harvard Medical School

# Summary

- Open access research tools
  - Proteins, structural info, production protocols
  - Chemical Probes
  - Protein capture reagents

- Proactive engagement of community for
  - biological discovery
  - target validation
  - ultimately new therapeutics

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www.TheSGC.org


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