

# Structural Genomics Consortium and Drug Discovery

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SGC Toronto



SGC Oxford



SGC Stockholm

# Outline

- Epigenetic code and protein mediators
- Hypothesis – epigenetic proteins are likely to be superior targets for Drug Discovery
- Epigenetic probe consortium – partners and objectives
- Progress
  - bromodomains, recognition sites, initial probe and selectivity
  - overall
- Planned short term deliverables

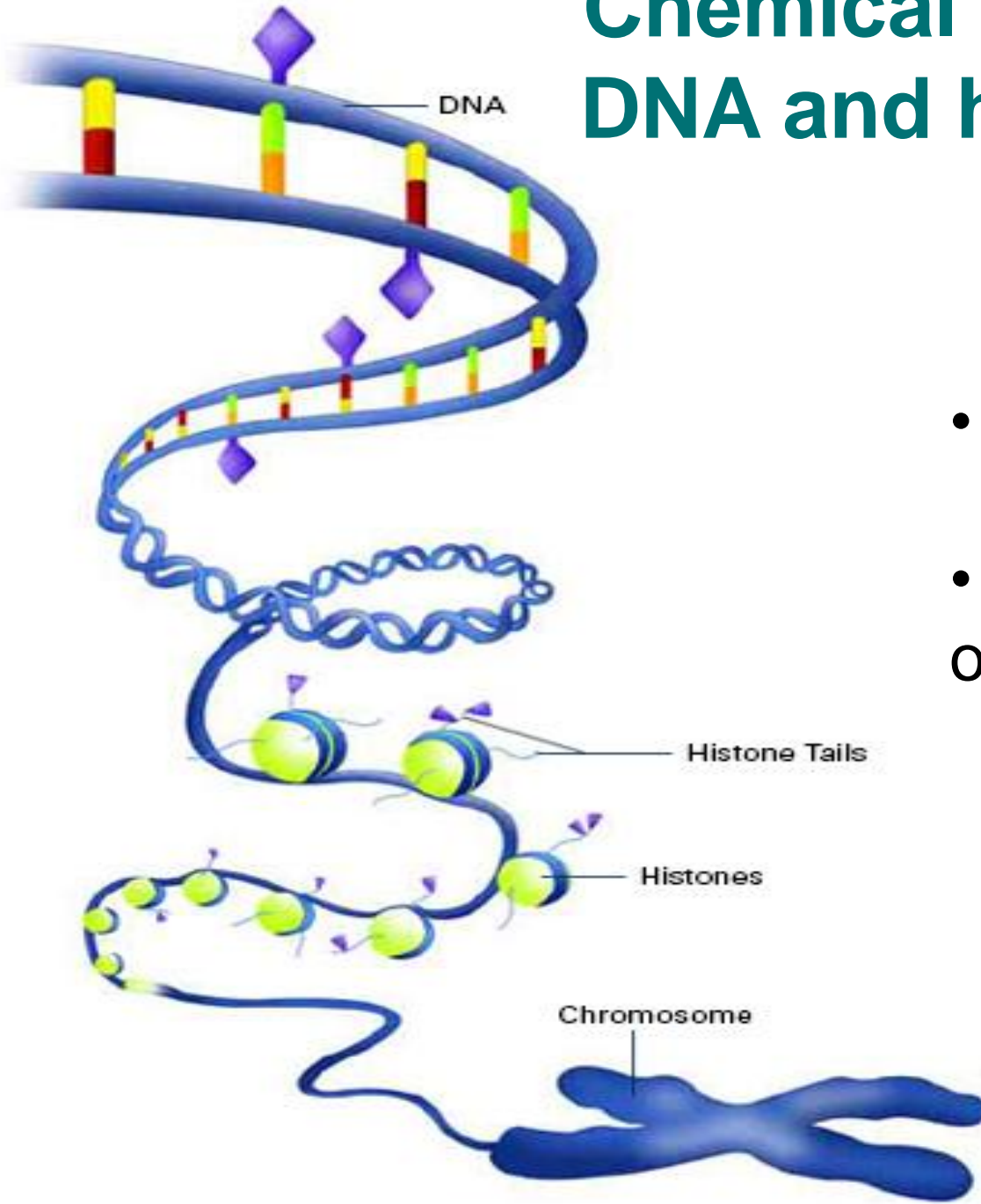
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# Epigenetic Code

- Genetic code is same in all cells in an organism
- Epigenetic code is cell and tissue specific
- Gene expression modulated by chromatin folding, **chemical modifications** and recruitment of transcriptional machinery
- Epigenetic modifications are inherited, but may be responsive to environmental influence eg injury, stress, exposure to toxins, diet....

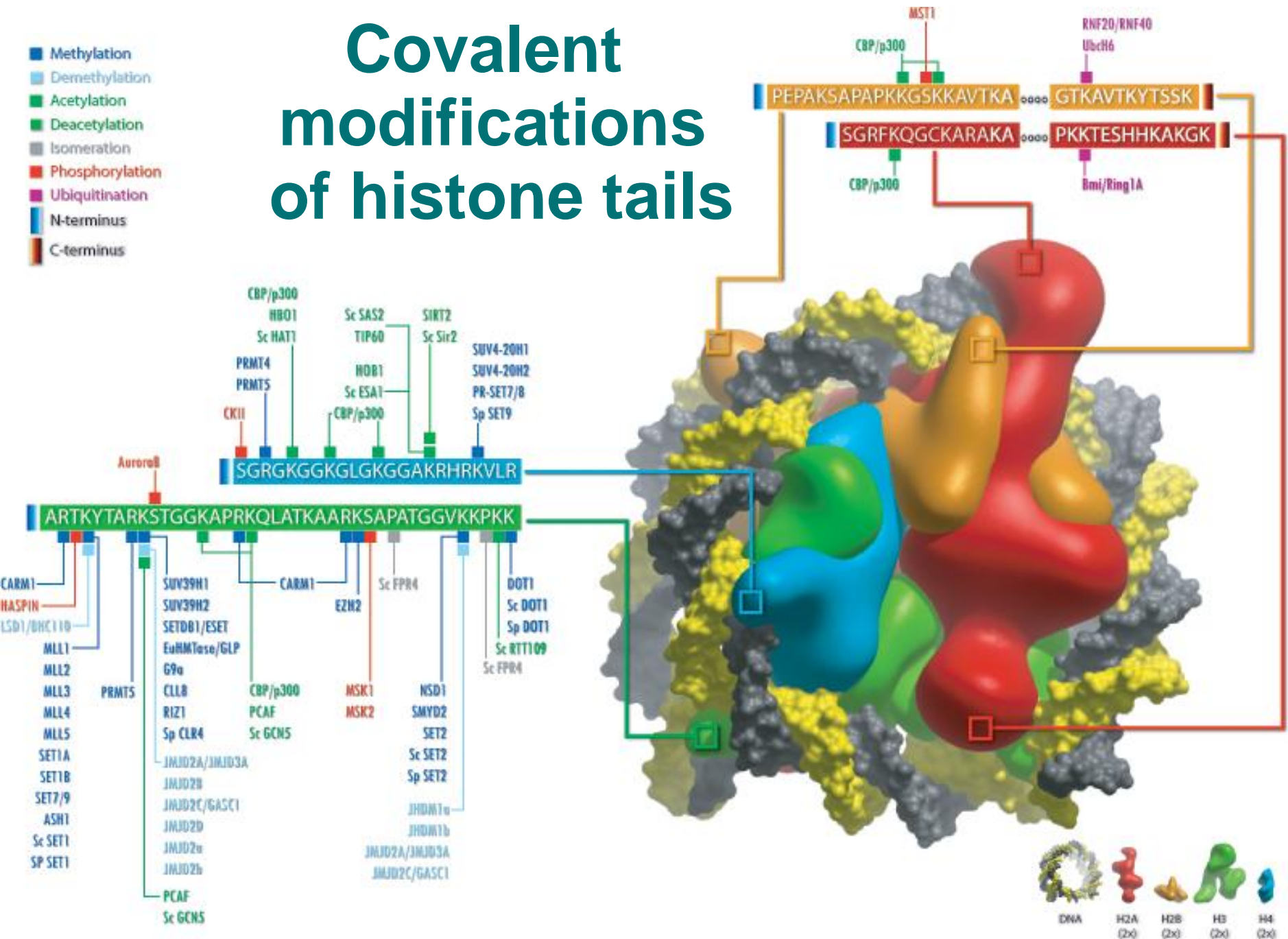
# Chemical modifications of DNA and histone proteins



- DNA Methylation
- Covalent modifications of histone tails

# Covalent modifications of histone tails

- Methylation
- Demethylation
- Acetylation
- Deacetylation
- Isomeration
- Phosphorylation
- Ubiquitination
- N-terminus
- C-terminus



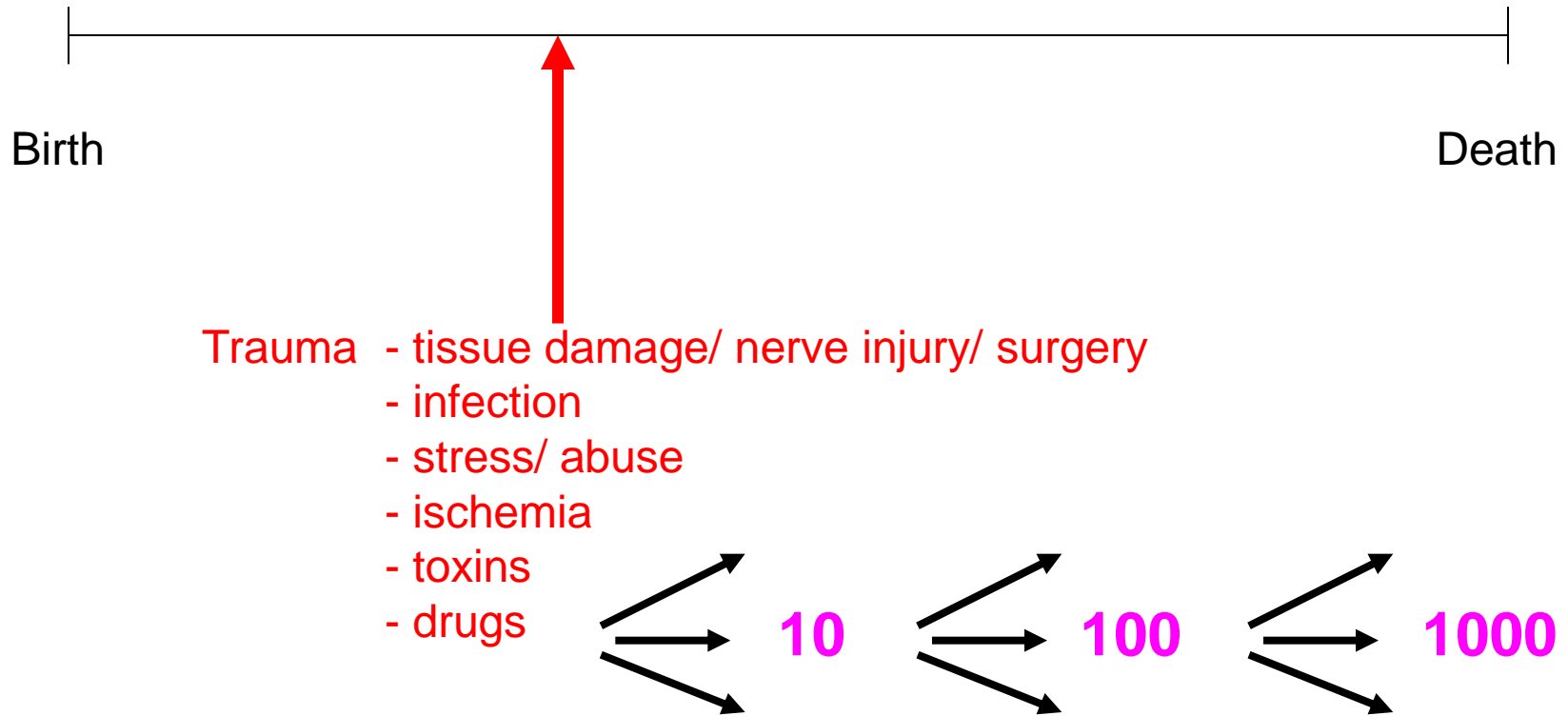
# Modifications may be activating or repressing

	H3K4	H3K9	H3K27	H4K20
Me	Activation	Activation	Activation	Activation
Me3	Activation	Repression	Repression	
Ac	Activation	Activation		

# Outline

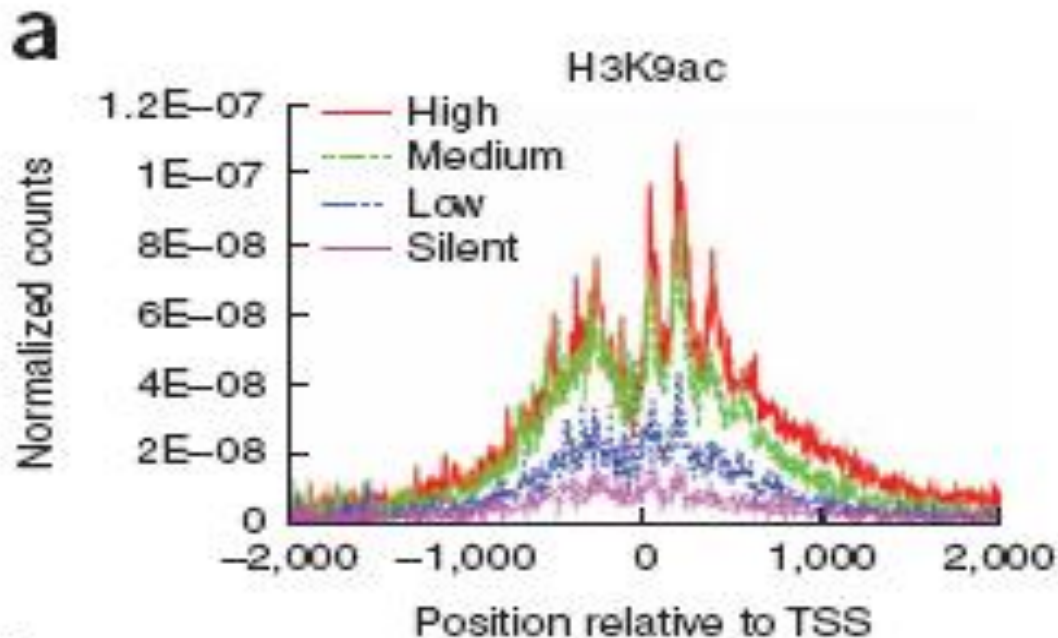
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# Modulating a late stage mediator is unlikely to be effective



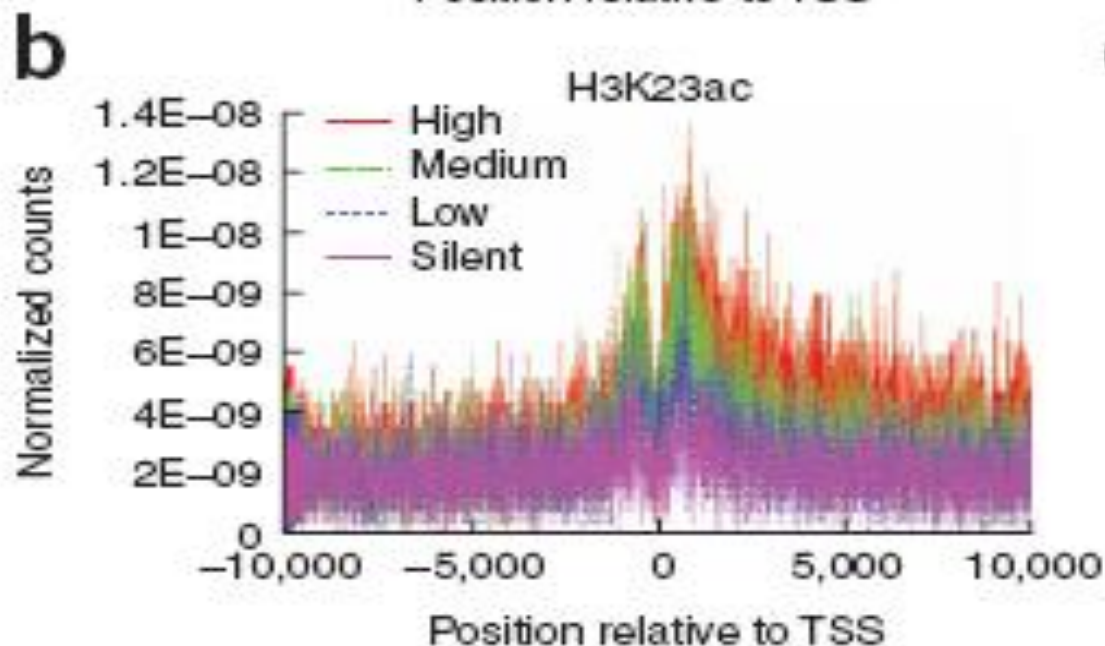
Nos of genes/ proteins, up/down regulated

# A modification can effect multiple genes



CD4+ T cells

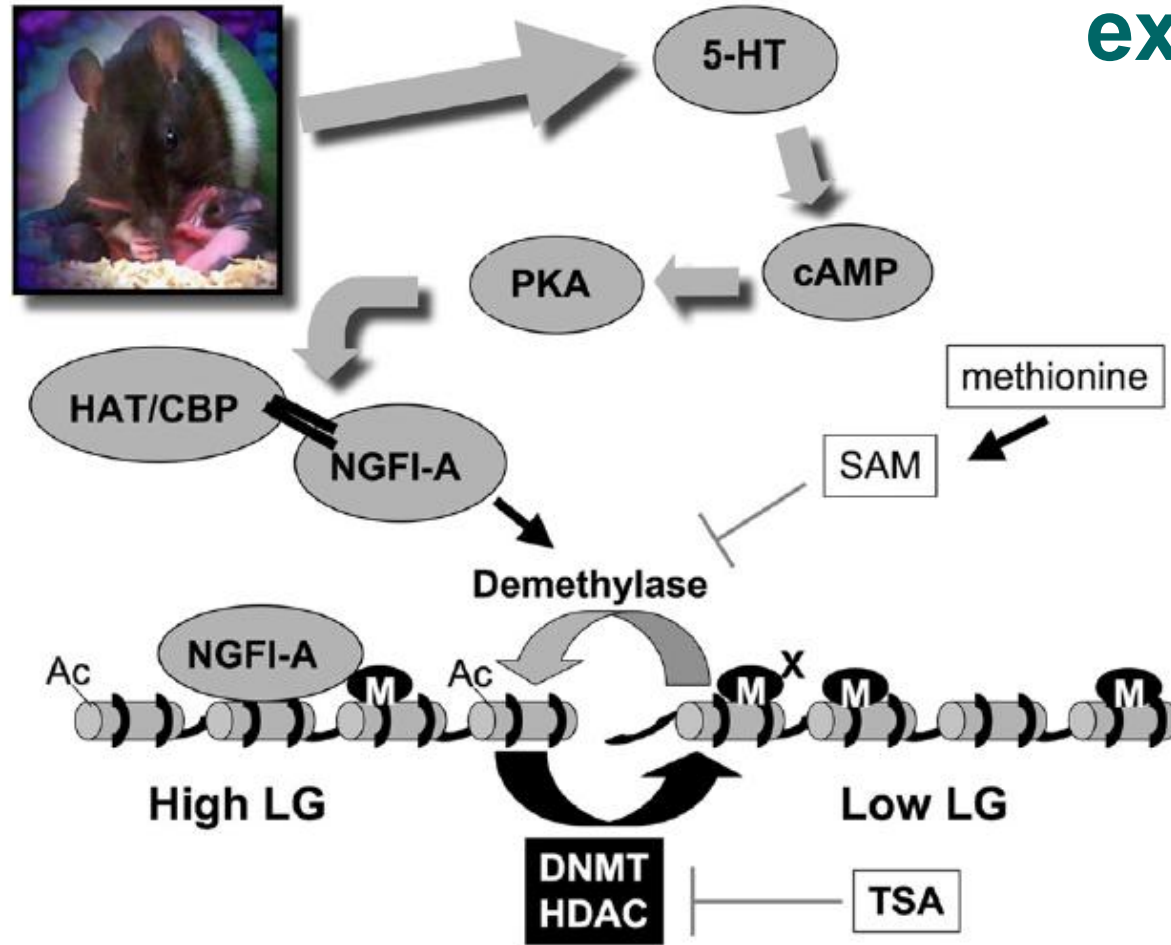
17 modifications in  
3286 promoters



More modifications  
associated with further  
increased expression

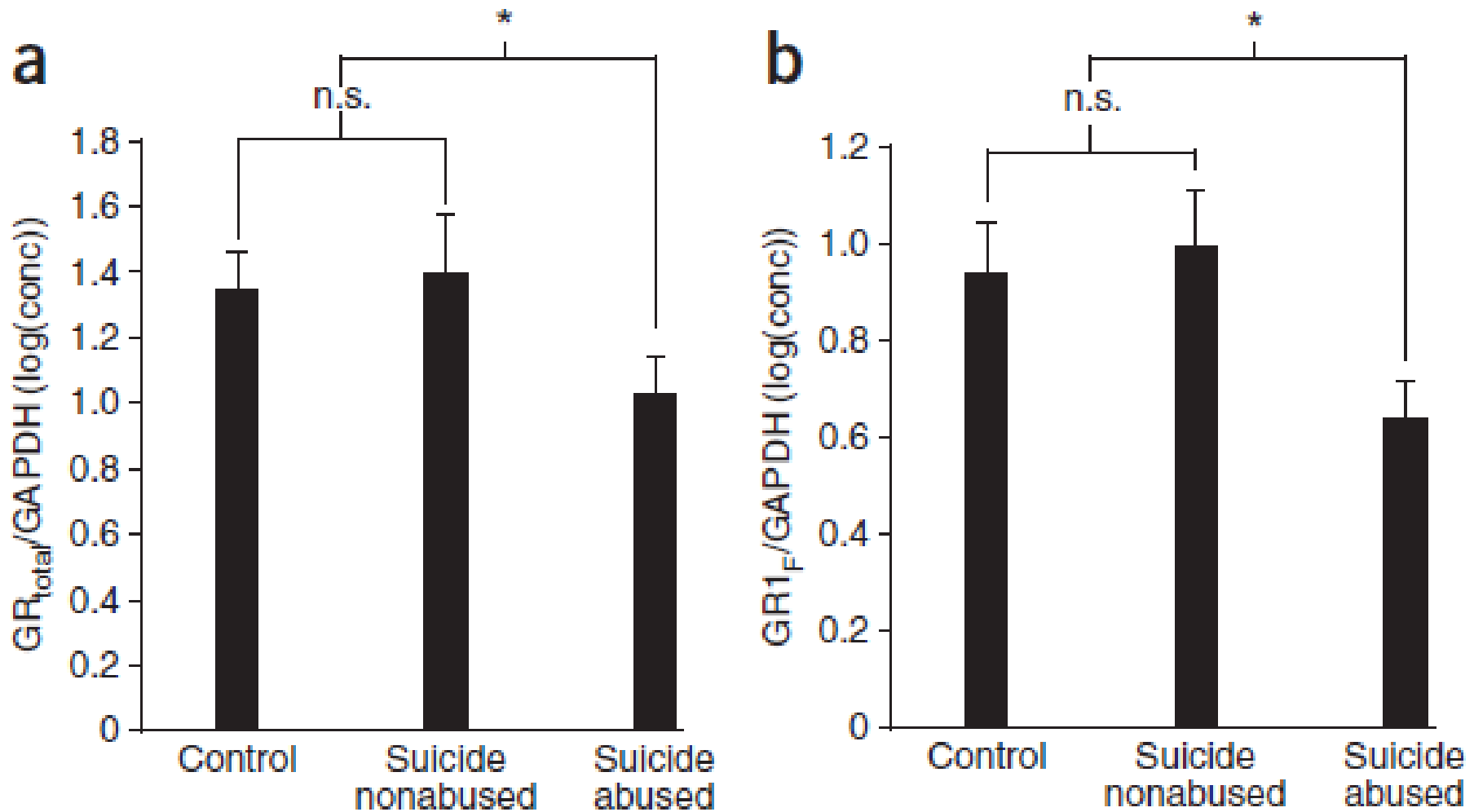
TSS = Transcription start site

# Maternal care alters glucocorticoid receptor expression



- Maternal care: activates 5HT7, increases cAMP, TF NGFI-A and histone acetylation, decreases DNA demethylation and increases GR expression
- Methionine promotes methylation and Low LG phenotype
- HDAC inhibits increase acetylation and High LG phenotype

# Childhood abuse decreases glucocorticoid receptor

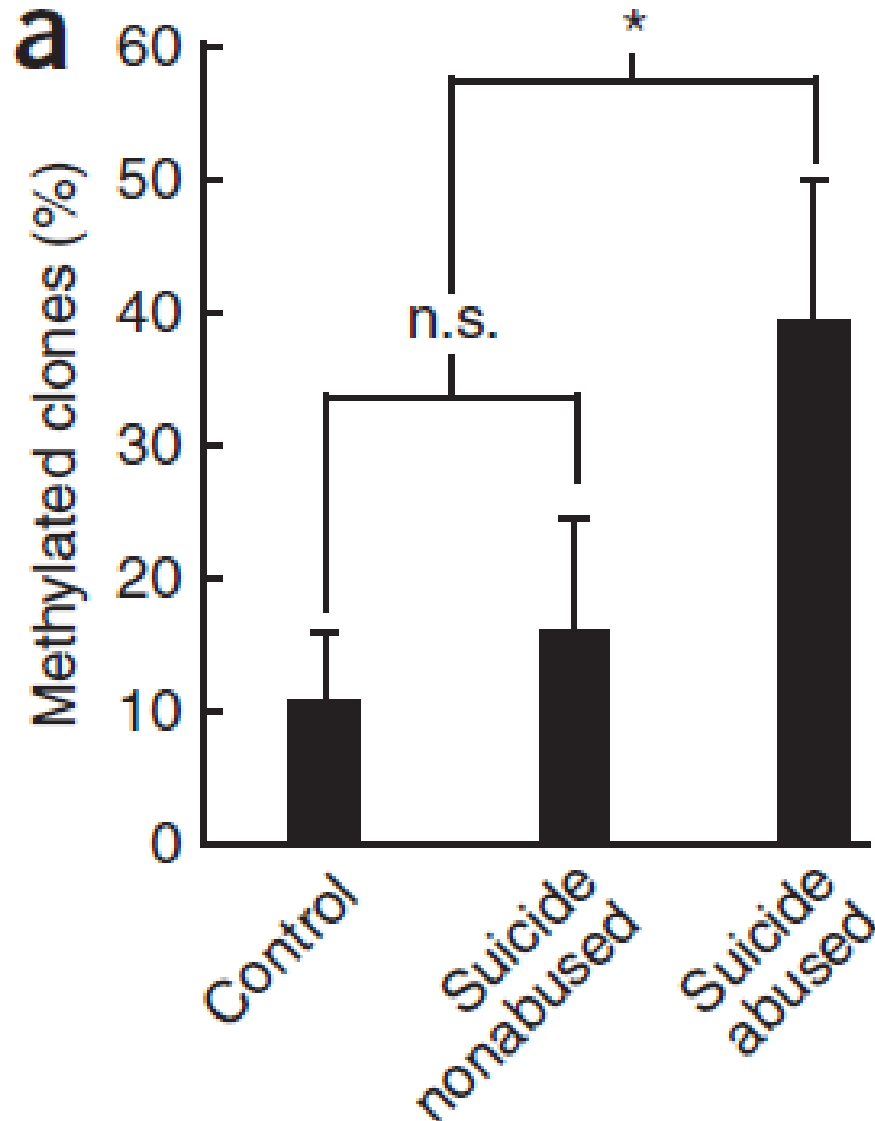


Post mortem hippocampus

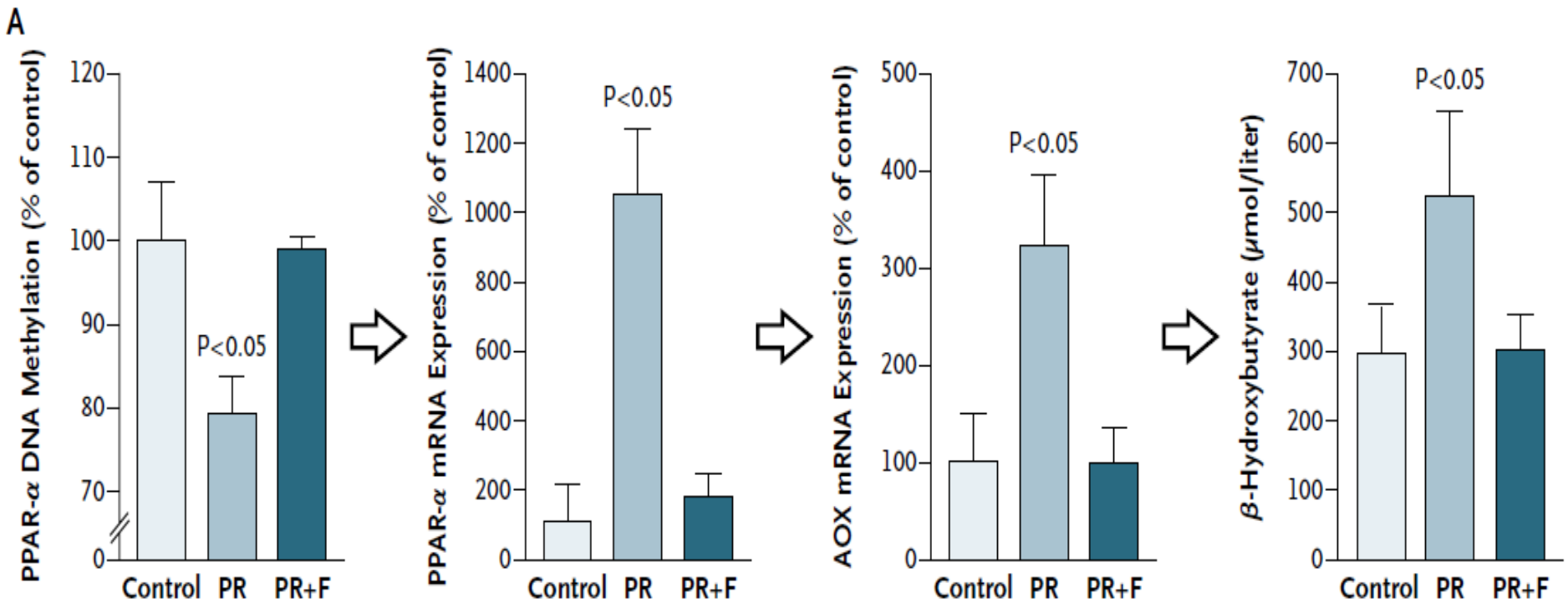
Decreased GR, leads to increased stress response

Also decreased NGFI-A

# Childhood abuse increases methylation of glucocorticoid receptor



# Prenatally undernourished rats show changes in methylation and gene expression

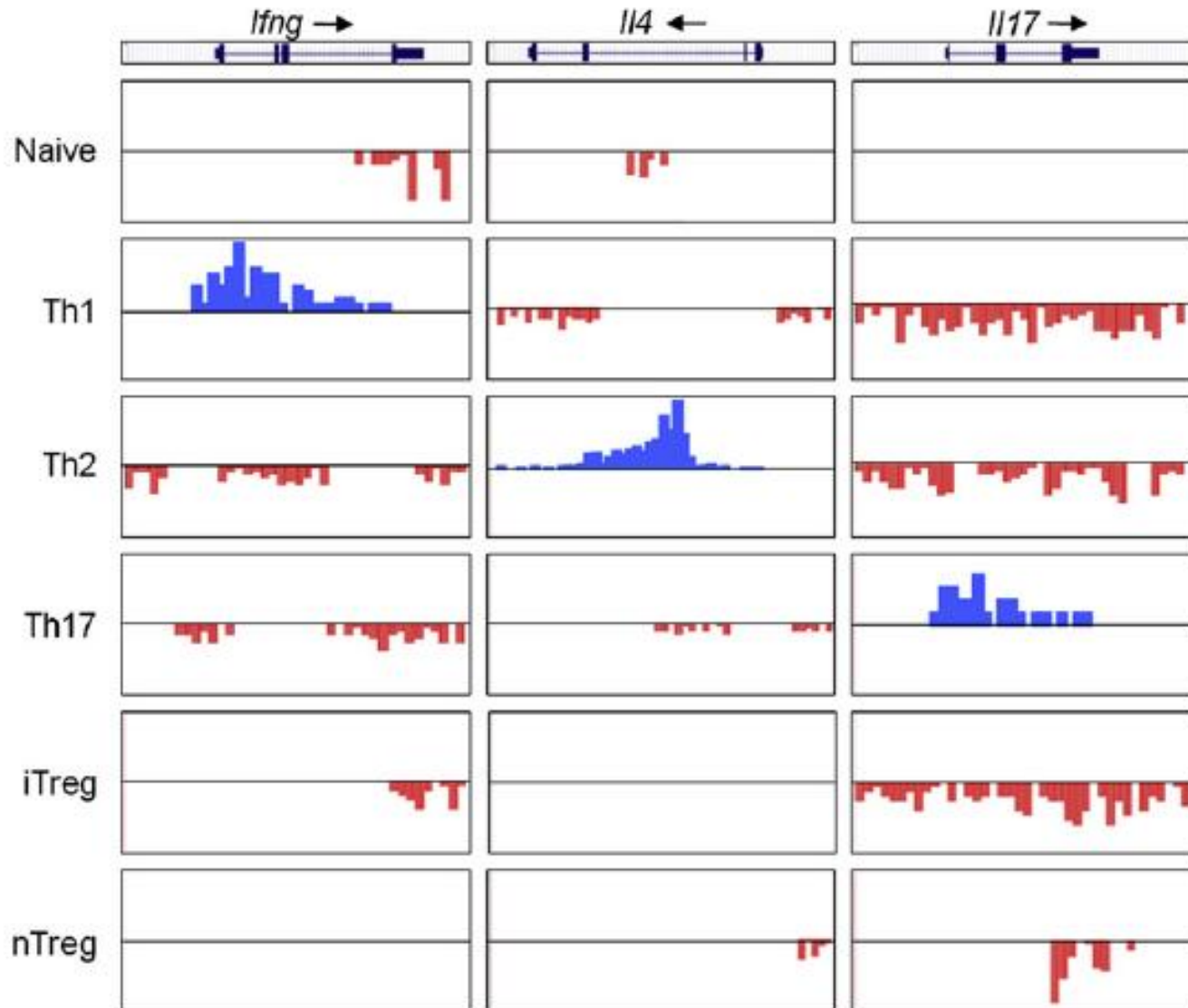


Protein restriction in pregnant mother leads to following in offspring

- Reduced promoter methylation of PPAR $\alpha$
- Increased PPAR $\alpha$  mRNA
- Increased acyl CoA oxidase mRNA
- Increased circulating ketone  $\beta$  hydroxybutyrate

Effects reversed by folate in maternal diet

# T cell differentiation is associated with modifications of signature cytokines



- Genome wide maps - ChIP Seq

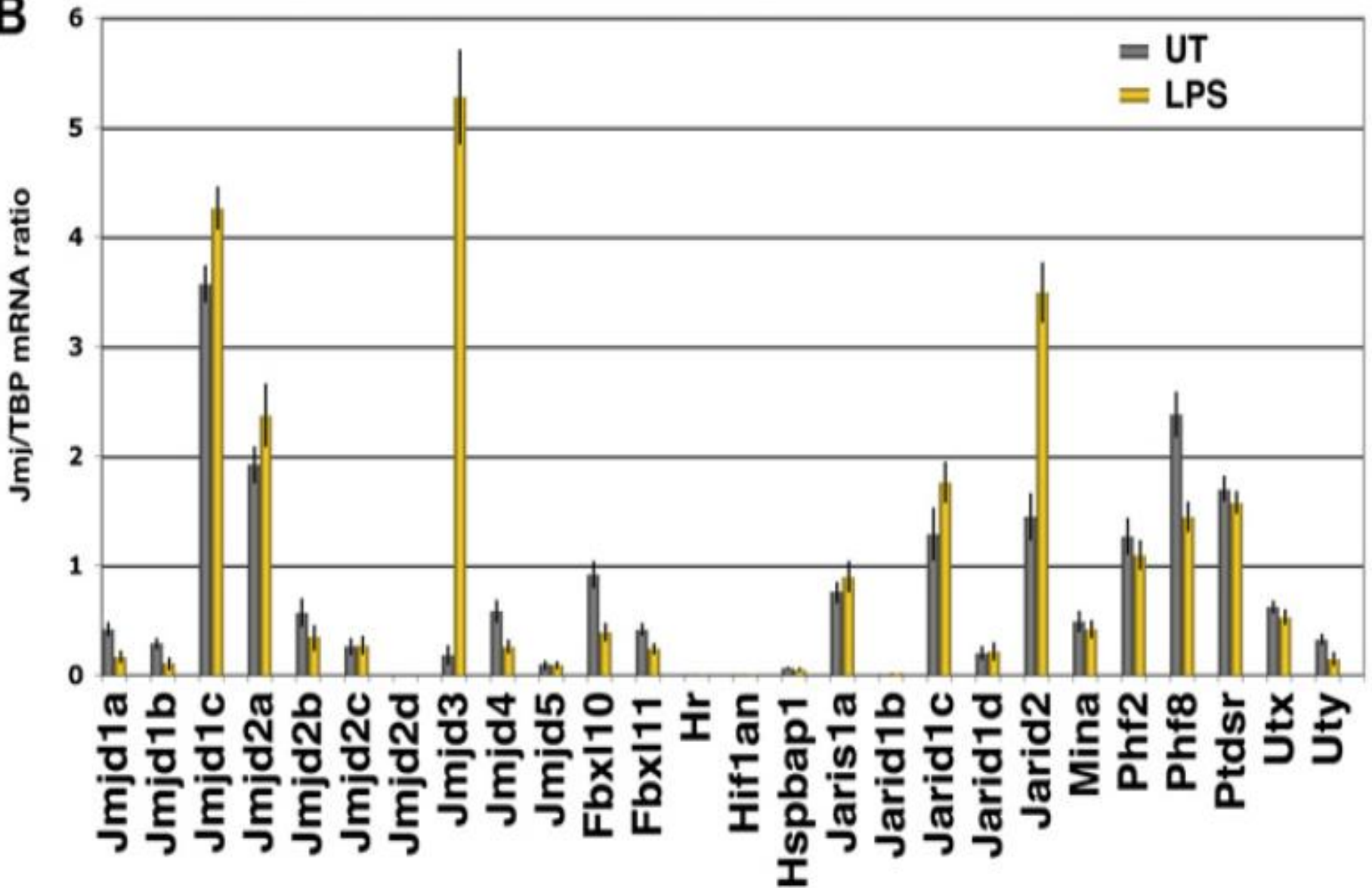
- Signature cytokines as expected

■ H3K4me3 - activating

■ H3K27me3 - repressing

# Jmjd3 is increased in activated macrophages

**B**



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# Aim: 40+ probes in 4 years

## Oxford project (25 probes)

- Lysine demethylases
- Bromo domains
- Tudor domains

## Toronto project (12 probes)

- Histone methyl transferases
- Histone acetyl transferases
- Methyl lysine binding domains

## Stockholm (5 probes)

- Arginine methyl-transferases
- Poly (ADP ribose) polymerases
- Plant homeodomain methyl lysine binding domains

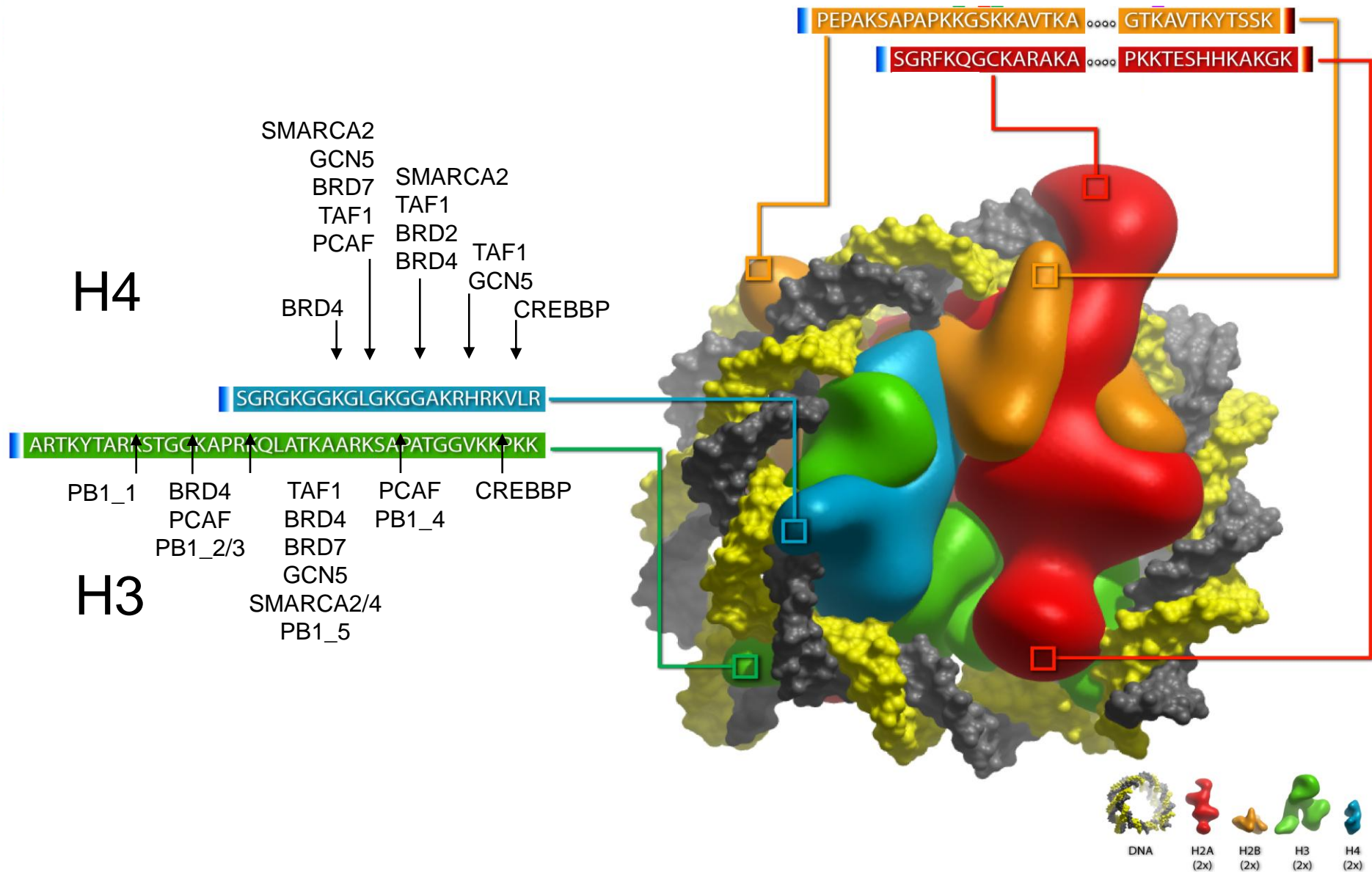
# Probe criteria

- $K_d$  or  $K_i \leq 100\text{nM}$  in purified protein assay
- Selectivity  $\geq 30$ -fold vs proteins in other subfamilies
- Demonstration of direct interaction with target protein in cells at  $\leq 1\mu\text{M}$

# Outline

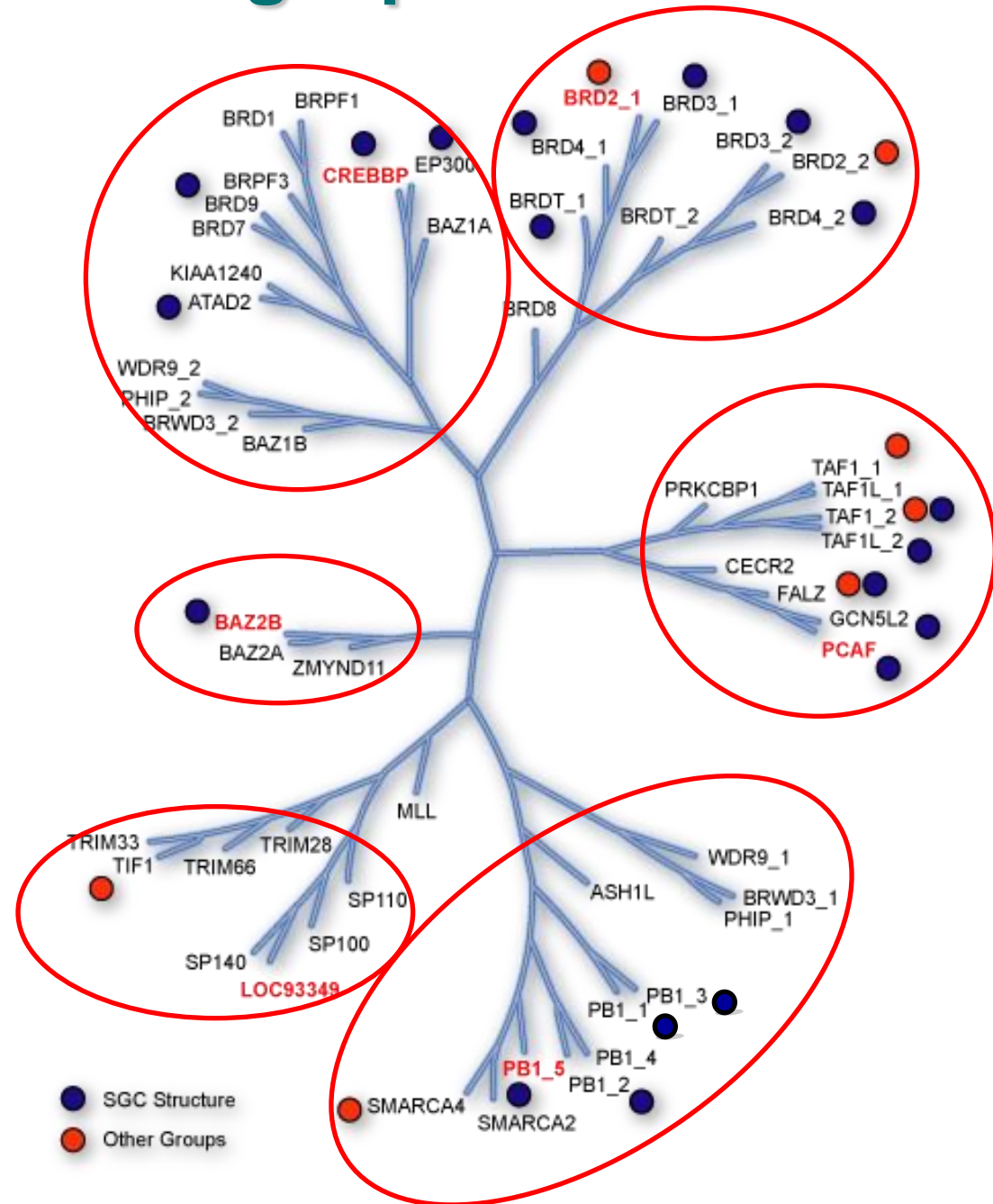
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- Hypothesis – epigenetic proteins are likely to be superior targets for Drug Discovery
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  - **overall**
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# Bromodomain recognition of KAc on histone tails

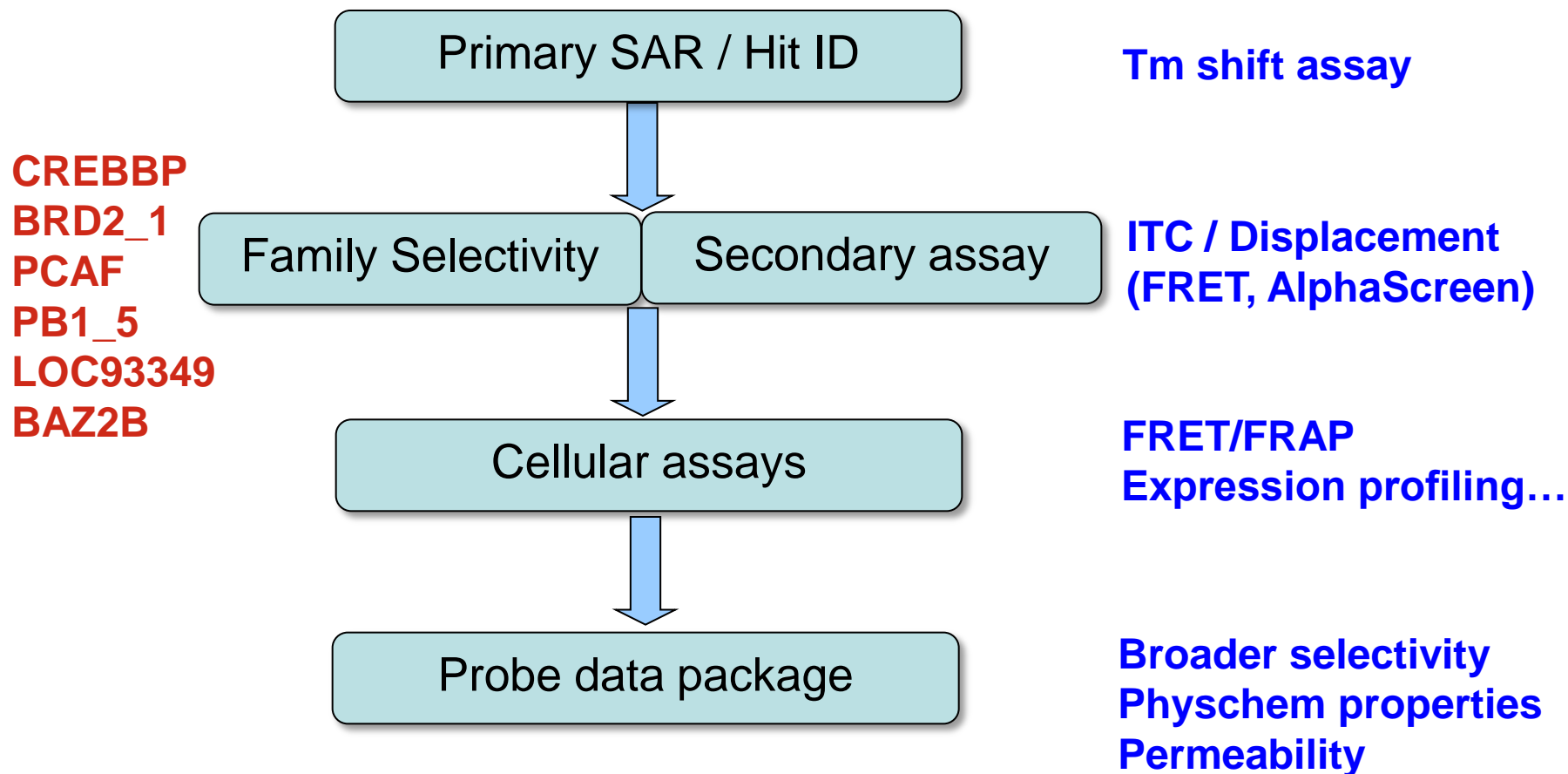


# Bromodomains: initial target probes and selectivity panel

- a representative from each subfamily
- expression, purification and crystallization (incl complexes)
- 20 structures solved, further 13 pending

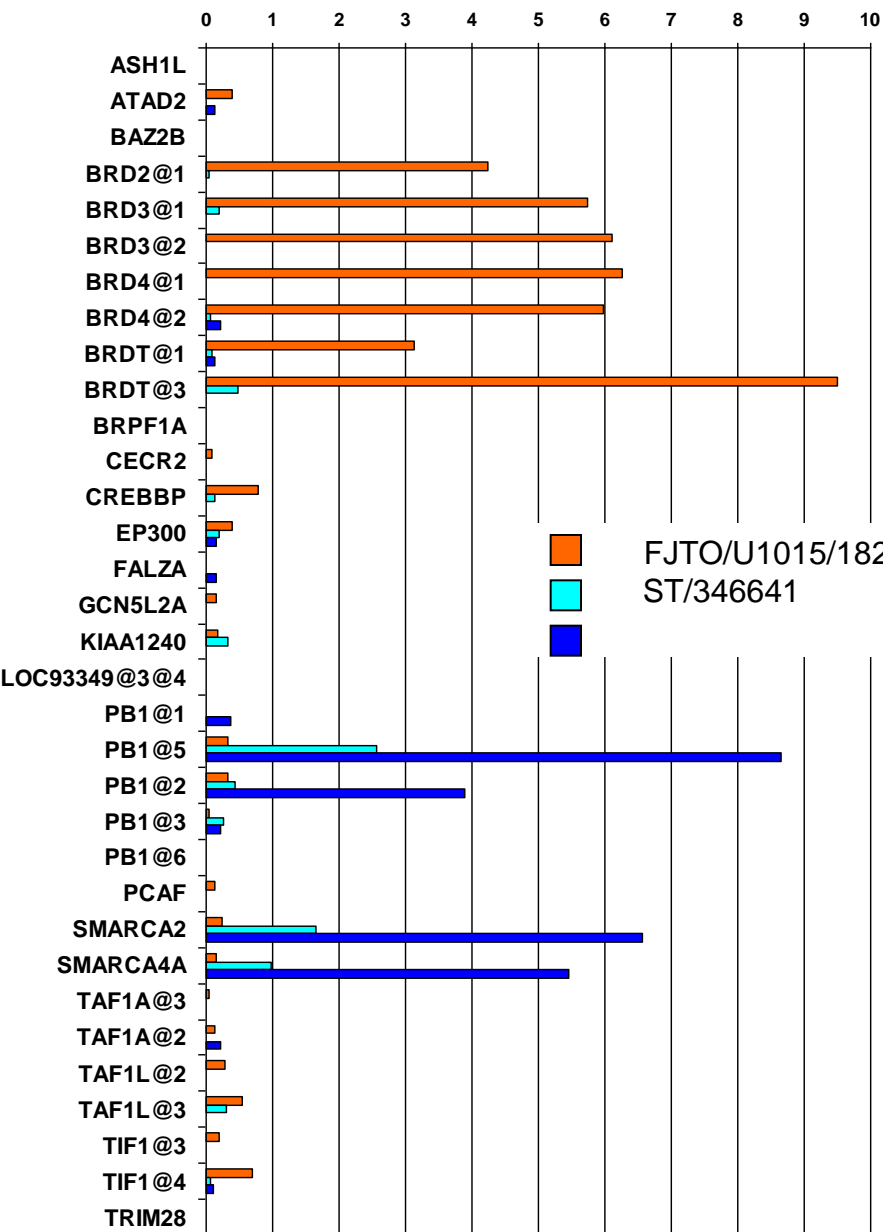


# Bromodomain screening cascade

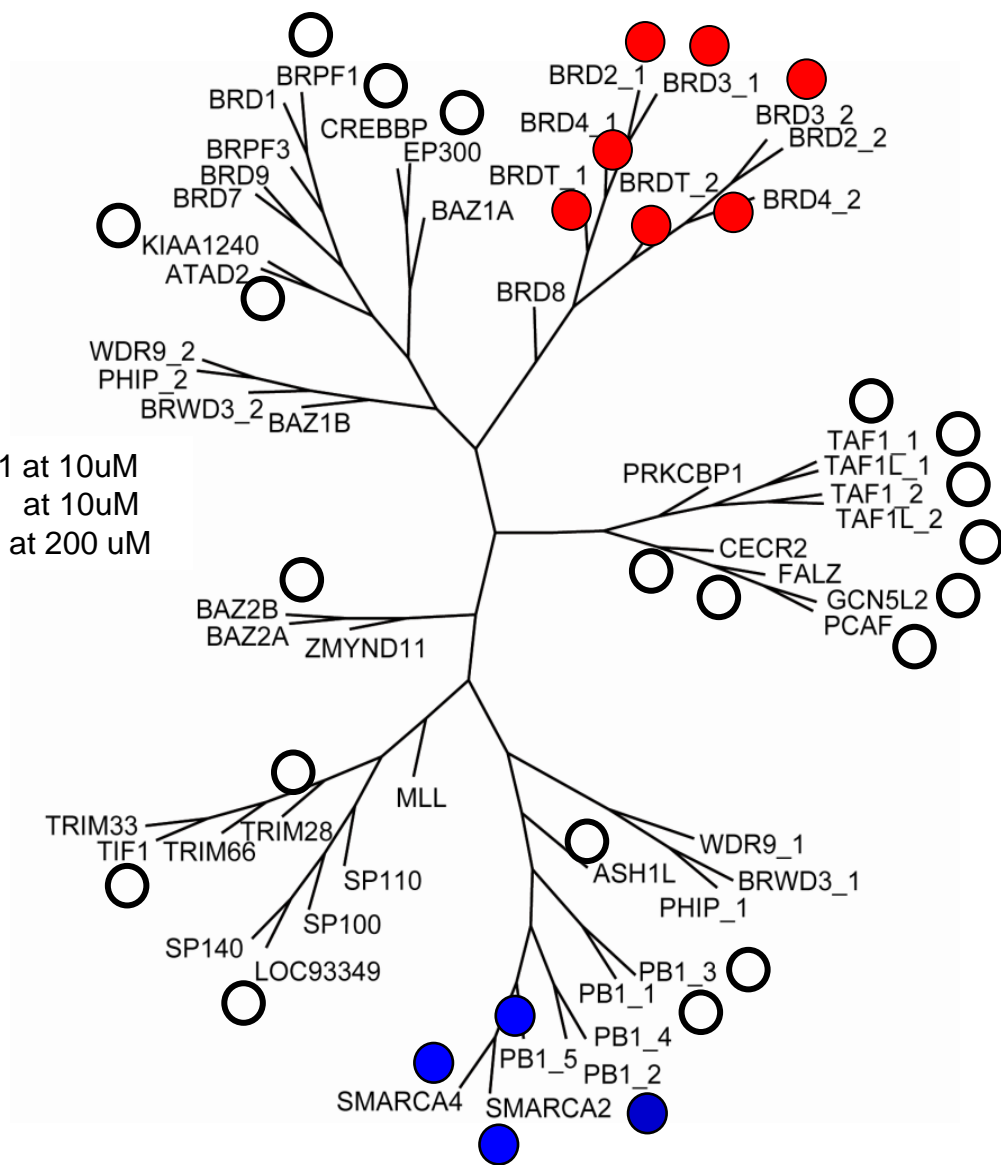


# Subfamily-specific hits

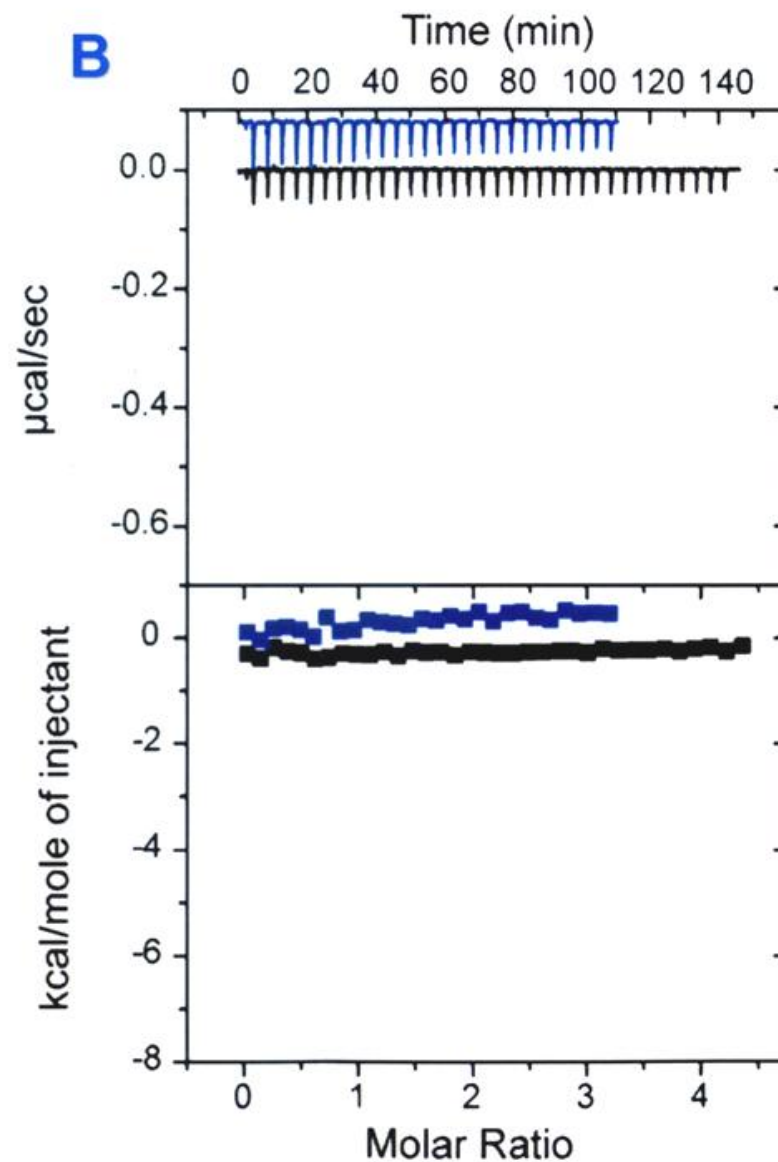
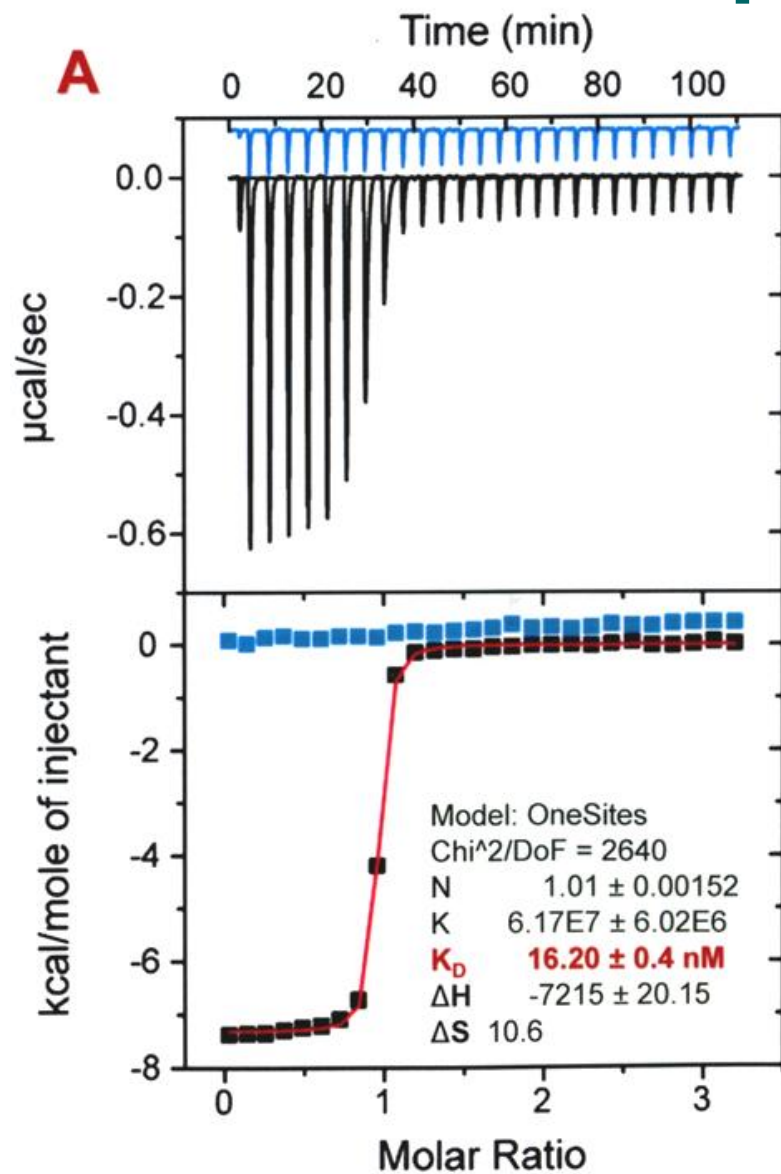
T<sub>m</sub> shift (C)



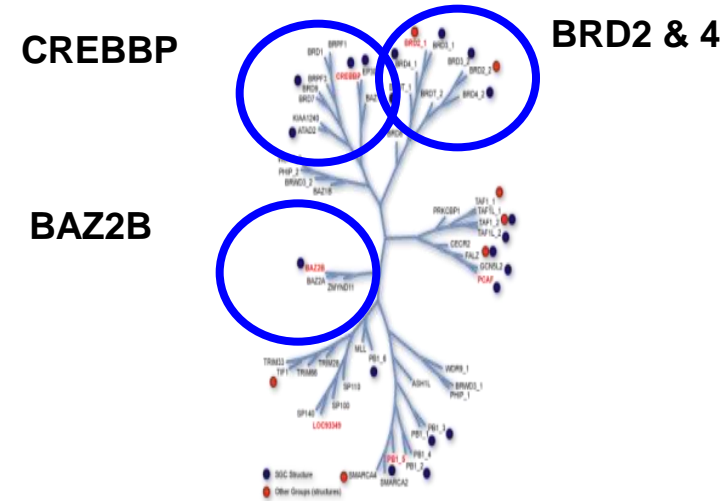
Panel of 32 bromodomains



# BRD4 pre probe shows enantiomeric specificity



# Pre-probe selectively displaces H4KAc<sub>4</sub> from BRD2 and BRD4



Pre-probe	Bromodomain	T <sub>m</sub> shift / °C	K <sub>d</sub> (ITC) / nM	IC <sub>50</sub> (Alphascreen) / nM
BDGBJ86	BRD2_1	4.2	70	200
	BRD4_1	6.0	16	200
	CREBBP	0.8	16200	7500
	BAZ2B	0	nd	202000

# Status of BRD4 pre-probe

- Tm shift  $>3^{\circ}\text{C}$  vs subfamily (BRD2, BRD3, BRD4 and BRDT)
- Tm shift  $<1^{\circ}\text{C}$  vs other sub families (25 bromodomains)
- ITC Kd = 16 nM vs BRD4
- Physicochemical characterization (solubility, permeability)
- Co-crystal structure with BRD4
- Broader cross-screening
- Cellular activity:
  - Direct interruption of BRD4-histone interaction
  - Phenotypic readouts

# Cellular assays for BRD4

<b>Observation</b>	<b>PI</b>	<b>Assay</b>	<b>Reference</b>
BRD4-Histone interaction	Keiko Ozato, NIH	Cellular FRET / GFP FRAP assays	FEBS Lett. (2008) 582 1501–1507
BRD4 regulates Aurora B expression	Ashok Venkitaraman Cambridge	H3S10-P immunofluorescence	Mol. Cell Biol. (2009) doi:10.1128
BRDT KO: ES cell – increase in H1t	Debra Wolgemuth, Columbia	(RT)-PCR levels of histone H1t	Development (2007) 134, 3507-3515

# Bromodomain status



SGC

Family	Protein	1 Assay	2 Assay	Structure	1 hits	Validated hits	Chemistry	Cellular assay
I	BRD4	Alpha	ITC	Y	Y	Y	Complete	Complete
II	CREBBP	Alpha	ITC	Y	Y	Y	ongoing	
III	BAZ2B	Alpha	ITC	Y	Y	Y	ongoing	
IV	FALZ	Alpha	ITC	Y	Y	ongoing		
V	PB1	Tm shift	ITC	Y		fragment		
VI	LOC93443	Tm shift						

- BRD4: probe  $K_d = 16\text{nM}$  by ITC, planned deposition in April
- CREBBP, BAZ2B, FALZ: assays in place, hit ID / optimization in progress
- PB1: fragment hit identified & confirmed by crystallography, but no binding assay
- BAZ2B HTS completed, FALZ (or BRD2) scheduled

# 56 structures to date



Protein family		Substrate	Total Genes	Total * domains	Structures (SGC)
Lysine demethylase		K(Me) <sub>n</sub>	21	21	4 (2)
Methyl-transferase***		K&R	80	80	27 (6)
R O Y A L	Tudor domain	RMe <sub>2</sub>	26	45	14 (2)
	Tandem Tudor	K(Me) <sub>2/3</sub>			
	Chromo-domain**	K(Me) <sub>3</sub>	32	41	16 (3)
	MBT repeat domain	K(Me) <sub>3</sub>	11	31	25 (9)
Acetyl-transferase***		K	45	45	25 (14)
Bromodomain		K(Ac)	42	56	26 (20)

\* in human genome

\*\* includes double chromodomain & chromo barrel domains

\*\*\* only those related to epigenetics.

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# Planned deliverables in 2010

- 6 probes
- completed 10+ HTSs
- established parallel KO effort
- establish antibody generation capability

# Summary

- Structures and well characterised chemical probes and antibodies are critical for dissecting role of these targets in normal and disease states
- Offer potential for considerable efficacy in chronic and complex diseases
- Our aim is not to secure any IP, merely to work with the best to facilitate Drug Discovery
- Already achieved considerable structural coverage and are identifying sub family specific probes



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# SGC

## SGC

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[www.thesgc.org](http://www.thesgc.org)

# Demethylase status



SGC

Family	Protein	1 Assay	2 Assay	Structure	1 hits	Validated hits	Chemistry	Cellular assay
I	JMJD2E	Alpha	MS	Y	Y	Y	ongoing	ongoing
II	FBXL11	Alpha	MS	Y	Y	Y	ongoing	
III	JMJD3	Alpha	MS		Y	Y	ongoing	
IV	JMJD1							
V	JARID							

- JMJD2E: lead series active in cells, optimization in progress
- FBXL11, JMJD3: assays in place, hit ID / optimization in progress
- JMJD1, JARID: active construct screens in progress
- JMJD2E HTS completed, FBXL11 in work-up
- PHF8 (FBXL11 family) structure solved
- JMJD2A & C Alpha assays established

↑  
Chemistry/Screening

Potent, Selective  
And Cell Active

Potent and  
Selective

Potent  
 $XC_{50} < 100$  nM

Weak  
 $XC_{50} < 20$   $\mu$ M

None

● G9a

● GLP

● L3MBTL1 ● HAT1  
● L3MBTL3  
● MBTD1 ● GCN5L2

● SETD7

● CBX7 ● HDAC6  
● SETD8 ● MYST3  
● PRMT3

DOT1L  
PRDM2  
EP300

Active Protein

In-Vitro Assay

Cell-Based Assay

→  
Assay Development