DNA-barcoded nucleosome library

Literature seminar #2

2020.5.25

M1 Tamiko Nozaki

Contents

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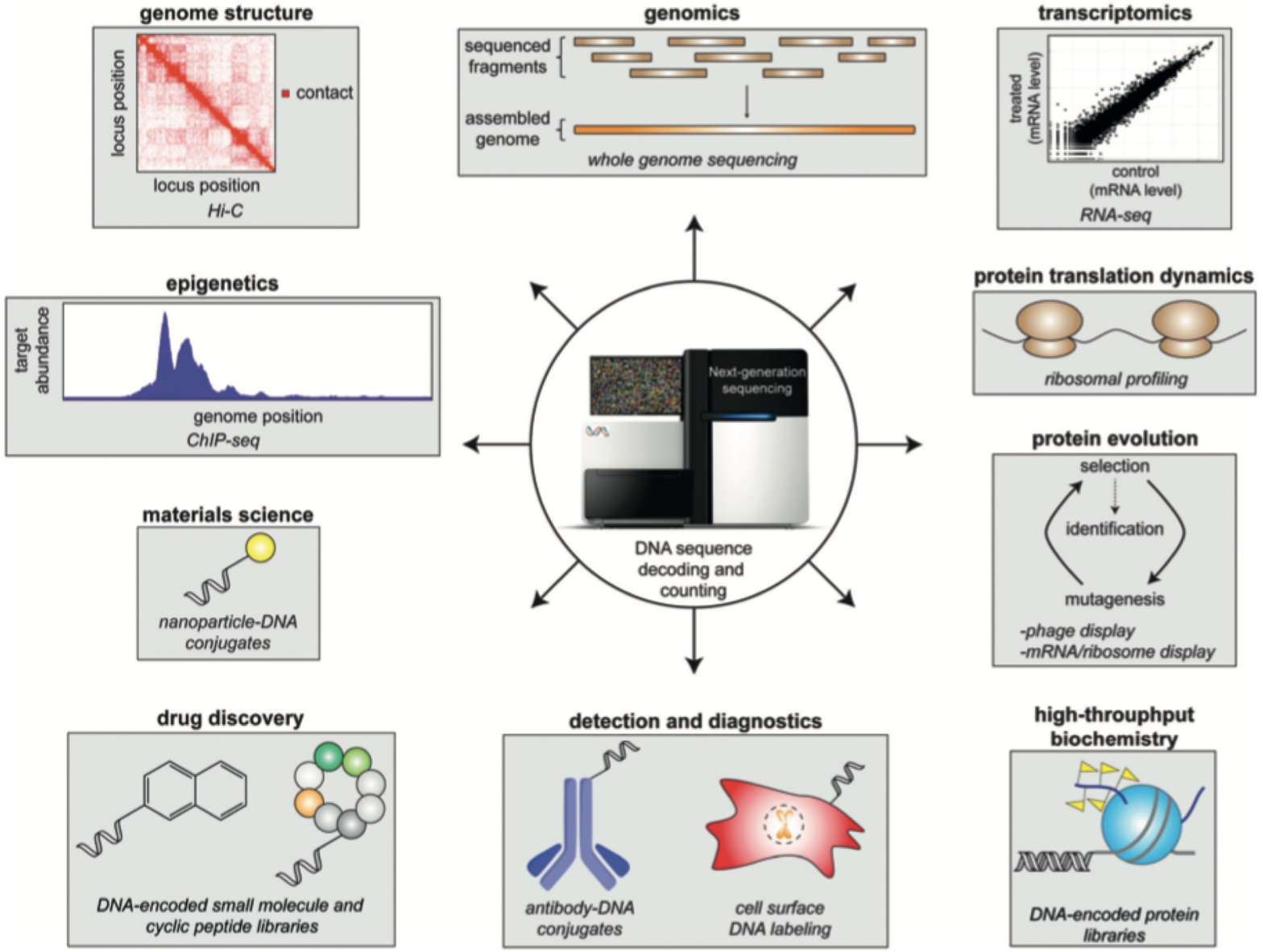
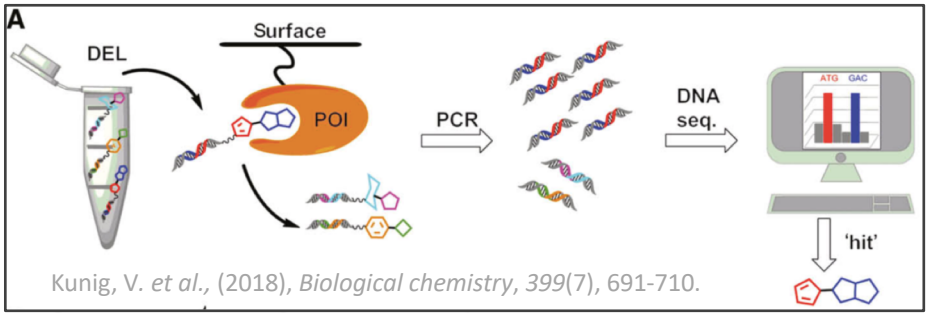
High-throughput sequencing-based methods and DNA-encoded molecules



Liszczak, G., & Muir, T. W. (2019). *ACIE*, *58*(13), 4144-4162.

Hits to therapeutic targets identified using DEC methods



Kunig, V. *et al.*, (2018), *Biological chemistry*, *399*(7), 691-710.

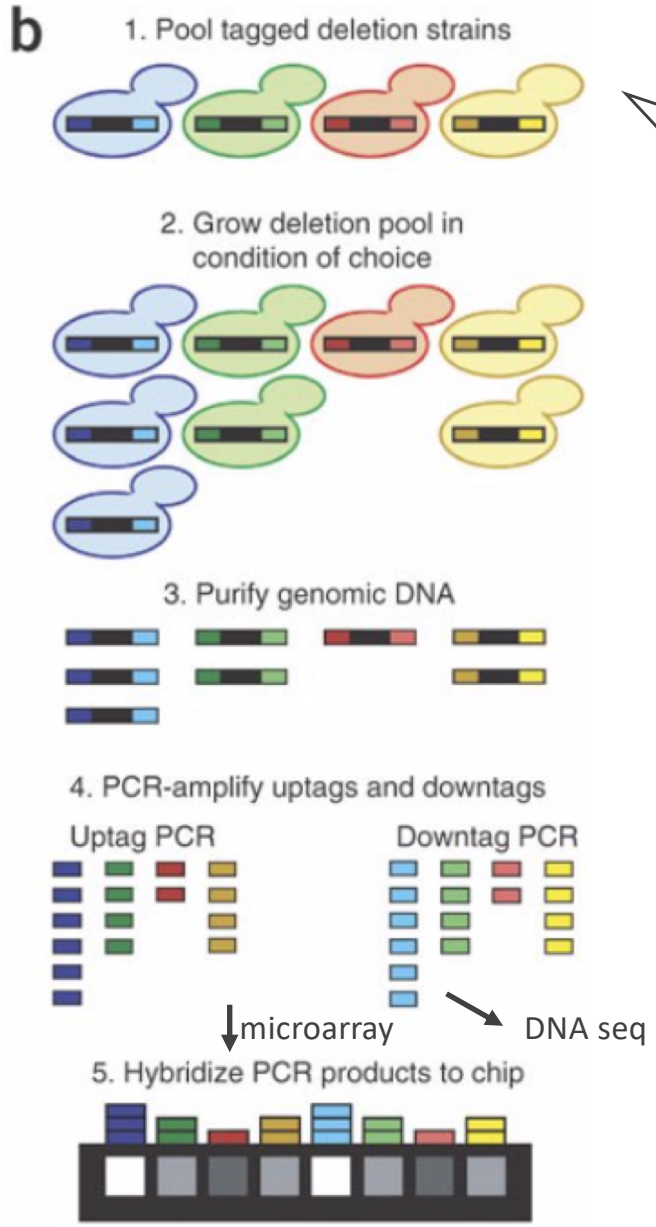
Target	Institution	Library structure and size	Exemplar compound	Activity	Status	Refs
Phosphoinositide 3-kinase-α (PI3Kα)	GlaxoSmith-Kline	3.5 million chemical compounds		10 nM IC_{50} in biochemical assays	Crystal structure available	97
X-chromosome-linked inhibitor of apoptosis protein (XIAP)	Ensemble	160,000 chemical compounds		140 nM IC_{50} in BIR2 biochemical assays, dimer more active	Active in mouse xenograft model	45
Hepatitis C virus NS4B protein	GlaxoSmith-Kline	Not disclosed		20 nM IC_{50} antiviral activity *in vitro*	Has antiviral activity but unattractive resistance profile	38

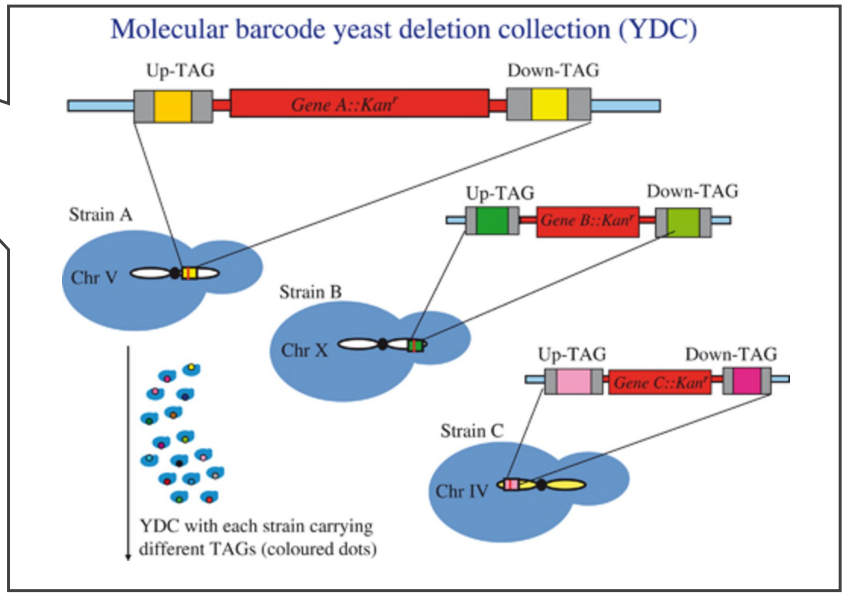
⋮

Reports of high-affinity hits for biological targets by usind DNA encoded library are now common.

Goodnow Jr, R. A. *et al.*, (2017). *Nature Reviews Drug Discovery*, *16*(2), 131.

Barcode technology in yeast



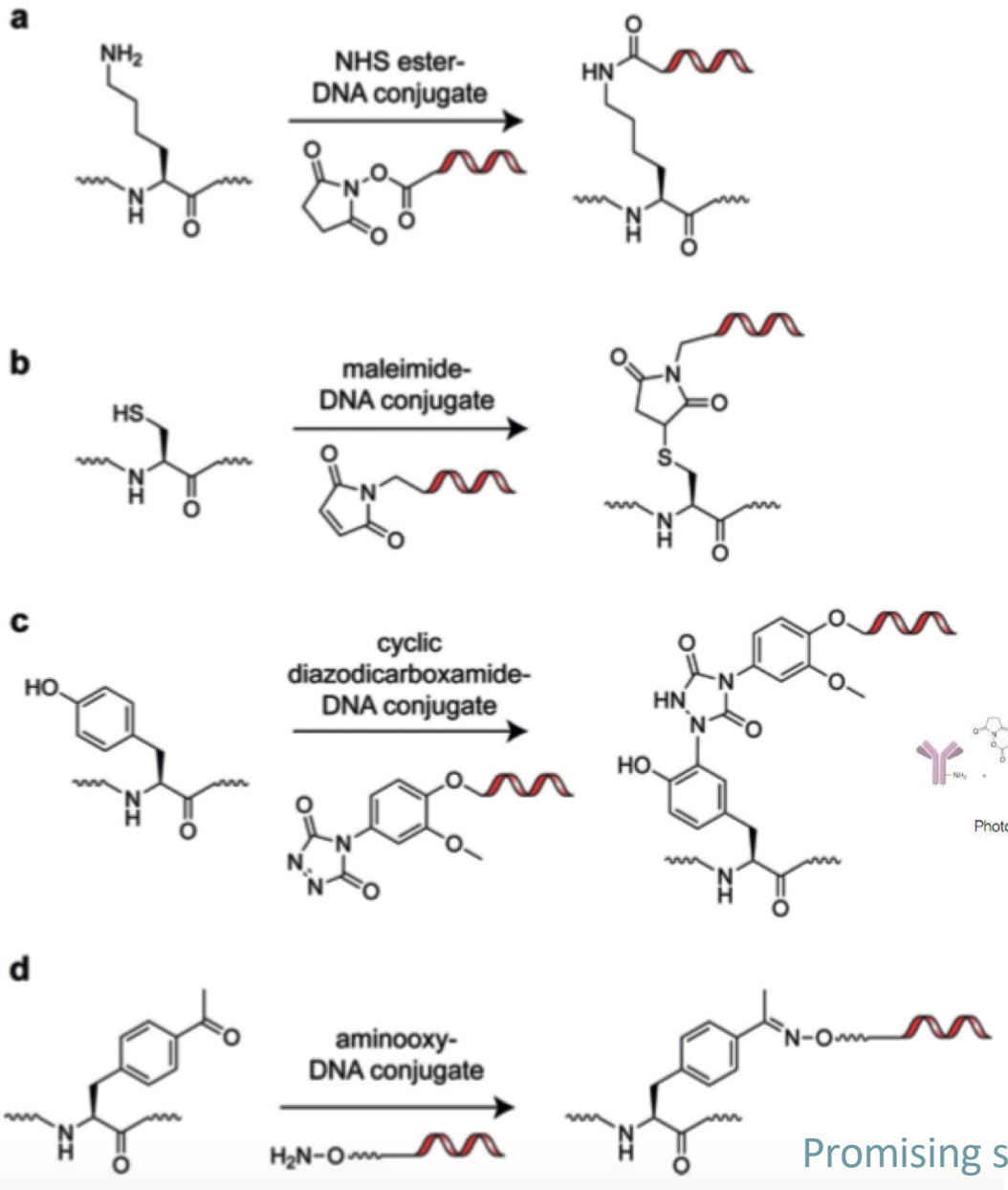


The simultaneous measurement by using barcode array provides a powerful system for identifying the genes required for growth in any condition of interest

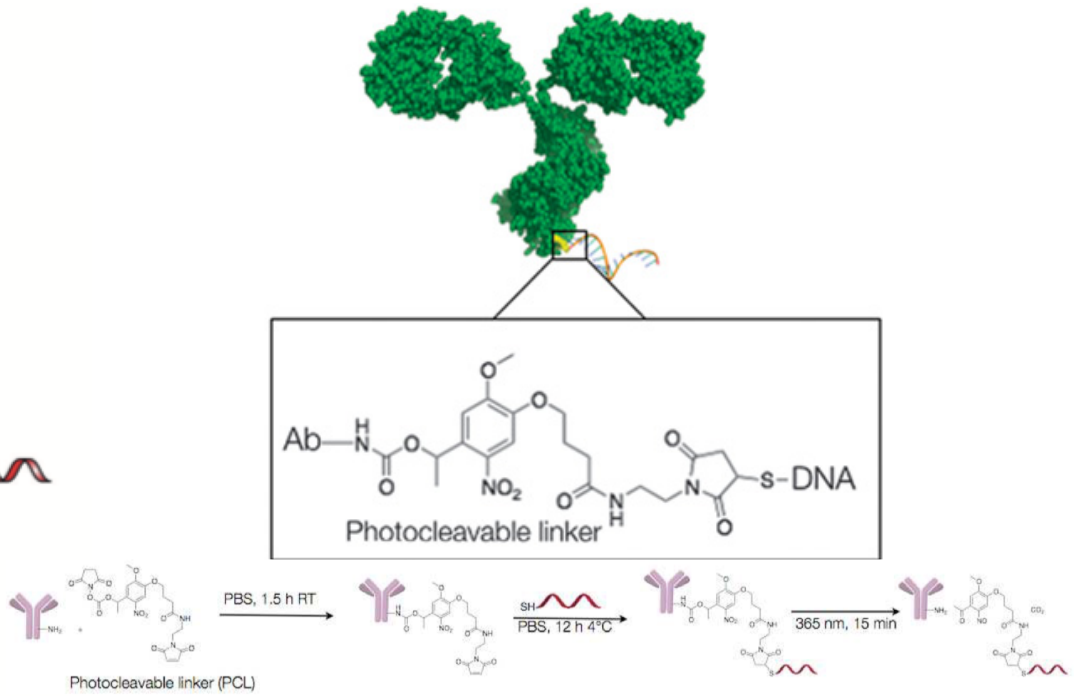
Pierce, S. E. *et al.*,(2006). *Nature methods*, *3*(8), 601-603.

Delneri, D. (2010). *FEMS yeast research*, *10*(8), 1083-1089.

Chemical Methods for Appending DNA to Natural and Unnatural Functional Groups



e.g. DNA-antibody conjugations

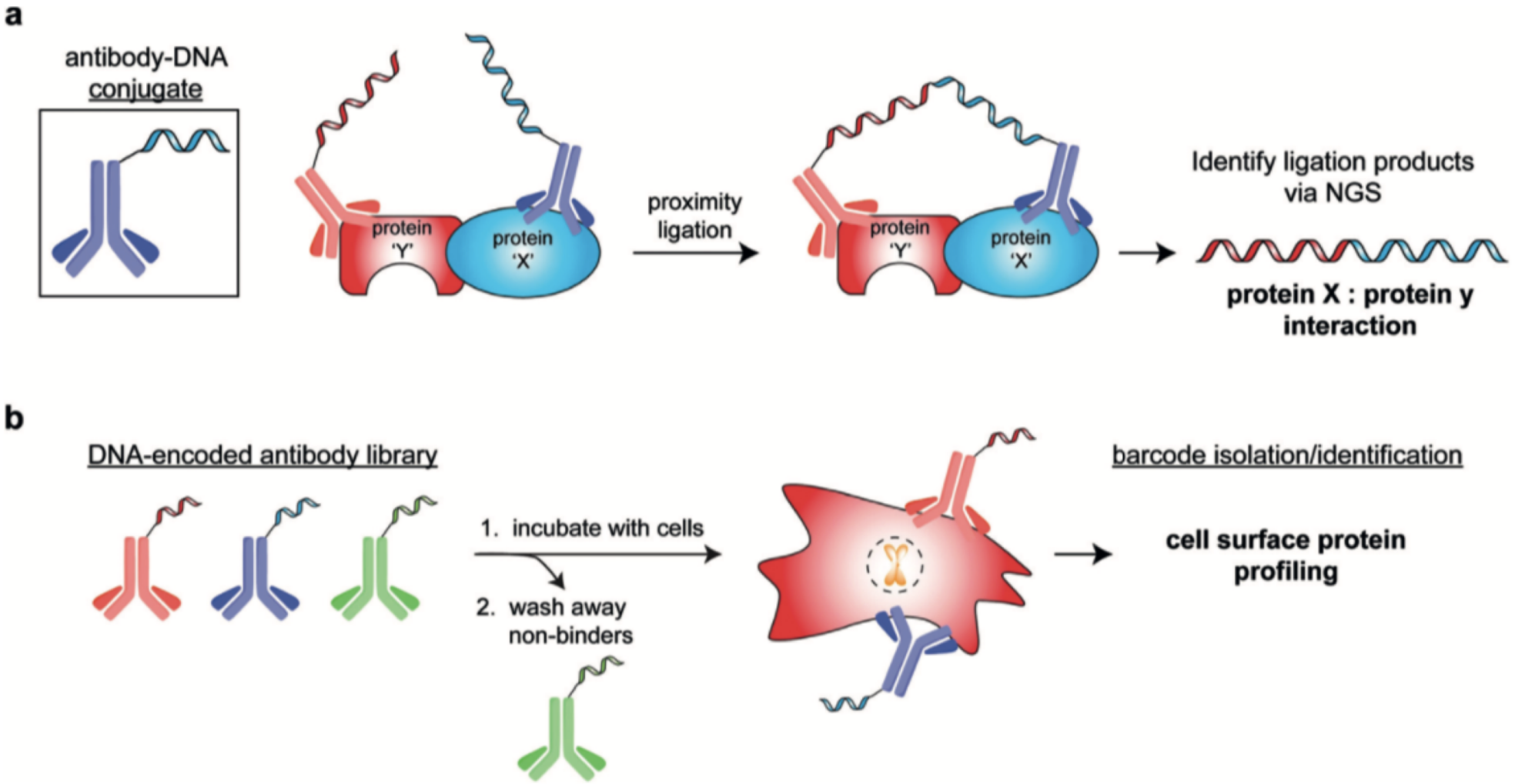


Ullal, A. V. *et al.* (2014). *Science translational medicine*, *6*(219), 219ra9-219ra9.

Promising strategy for targeting a single site in proteins

Liszczak, G., & Muir, T. W. (2019). *ACIE*, *58*(13), 4144-4162.

Antibody–DNA conjugate applications

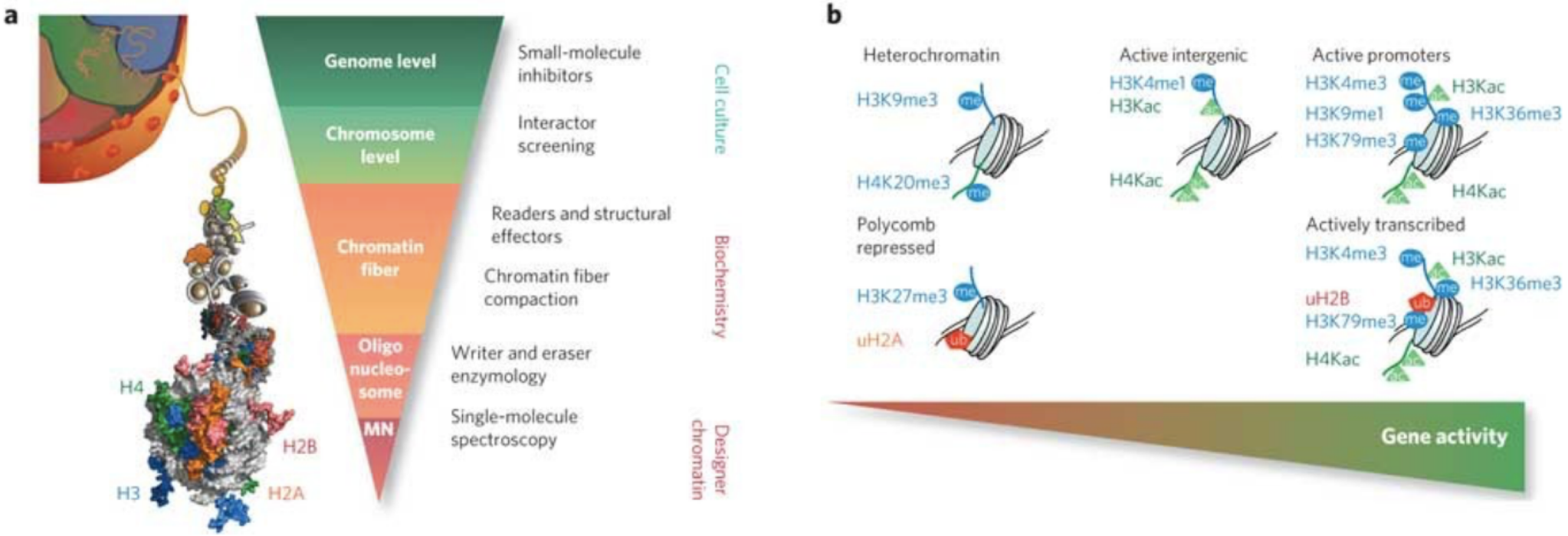


Antibodies represent one of the most common classes of proteins to which amino acid side chain-DNA conjugation methods have been applied.

Liszczak, G., & Muir, T. W. (2019). *ACIE*, *58*(13), 4144-4162.

Contents

Organization and analysis of chromatin

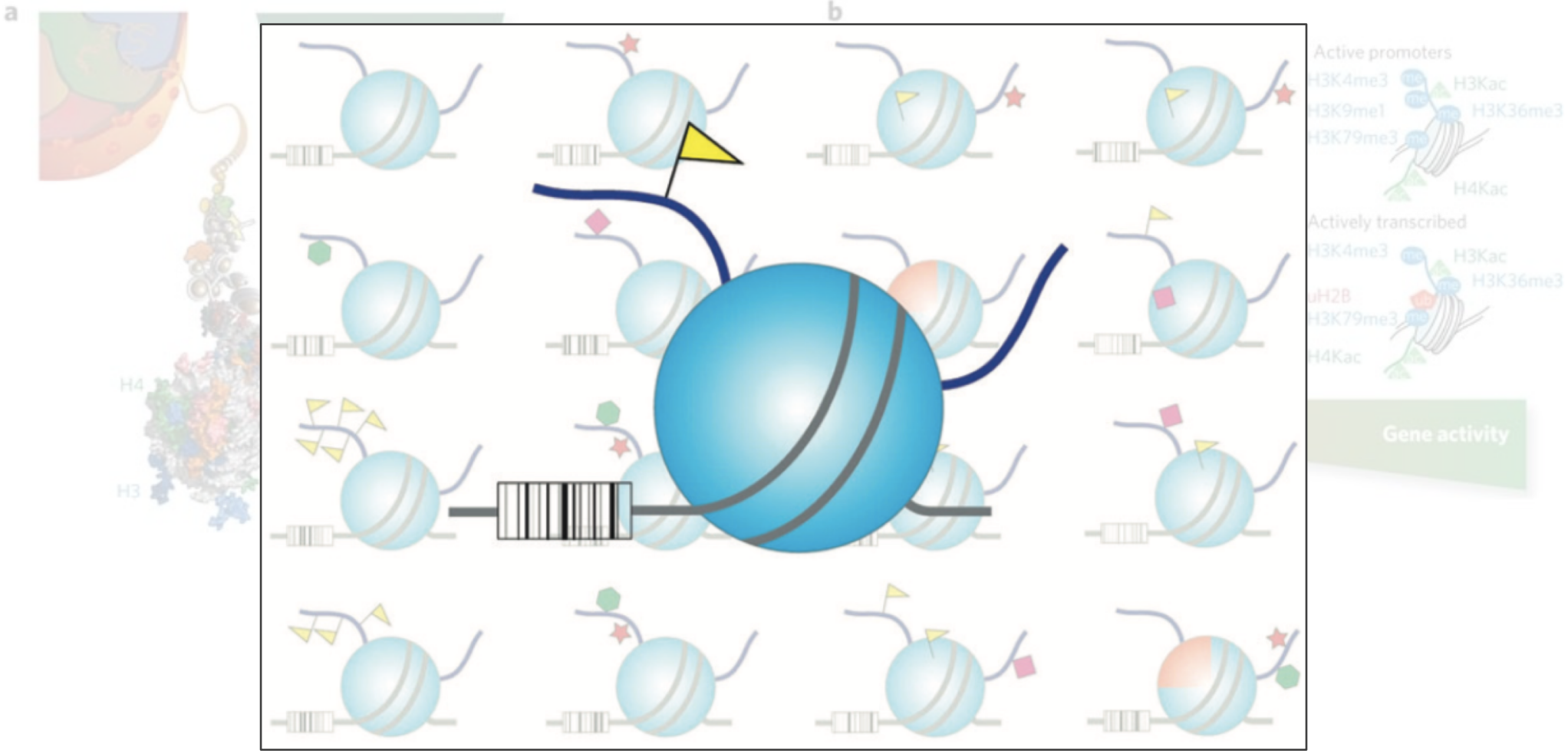


PTMs are dynamically inscribed into chromatin through covalent modifications of DNA and histones by 'writer' and 'eraser' enzymes; 'readers' further convert this chromatin landscape into defined transcriptional outputs.

Nucleosomes are indispensable substrates in biochemical studies that require the three-dimensional nucleosome architecture.

Fierz, B., & Muir, T. W. (2012). *Nature chemical biology*, *8*(5), 417.

DNA-barcoded nucleosome library



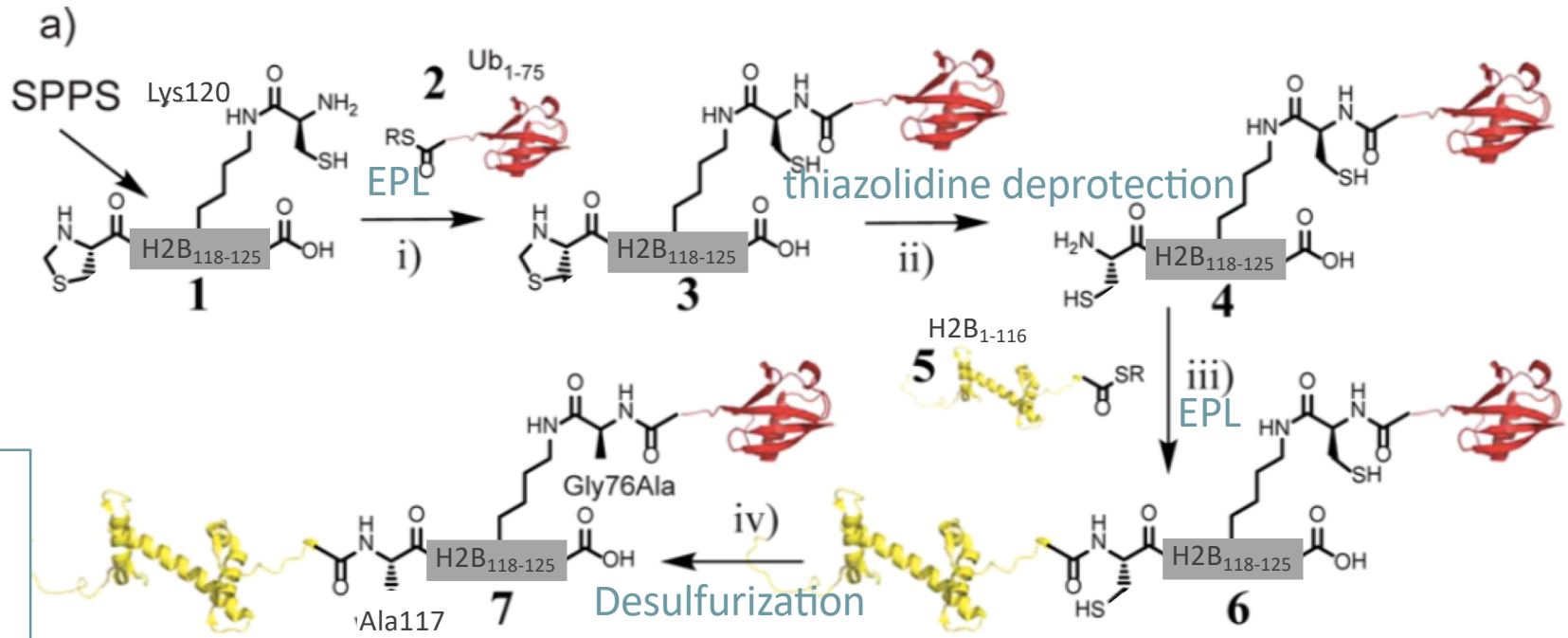
DNA-barcoded nucleosome library

= a versatile platform with high throughput and sensitivity

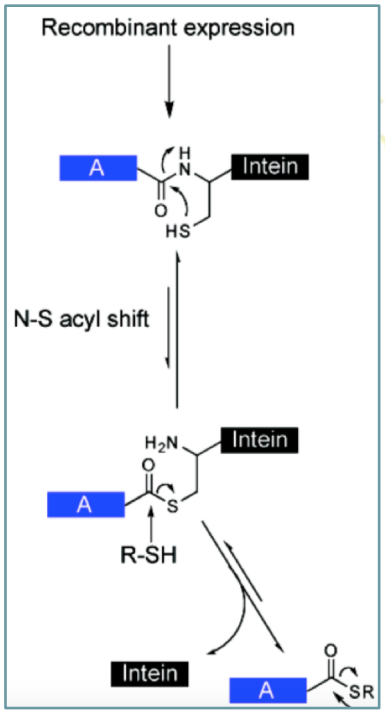
→ greatly accelerates biochemical investigations into chromatin recognition and signaling

Synthesis of modified mononucleosomes via a variety of protein chemistry approaches

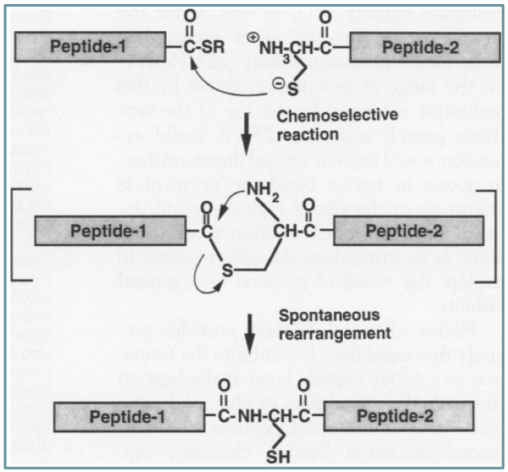
Mono-Ubiquitinated nucleosome



2 : Ub_{1-75}-thioester



McGinty, R. K.*et al,*(2008). *Nature*, *453*(7196), 812-816.



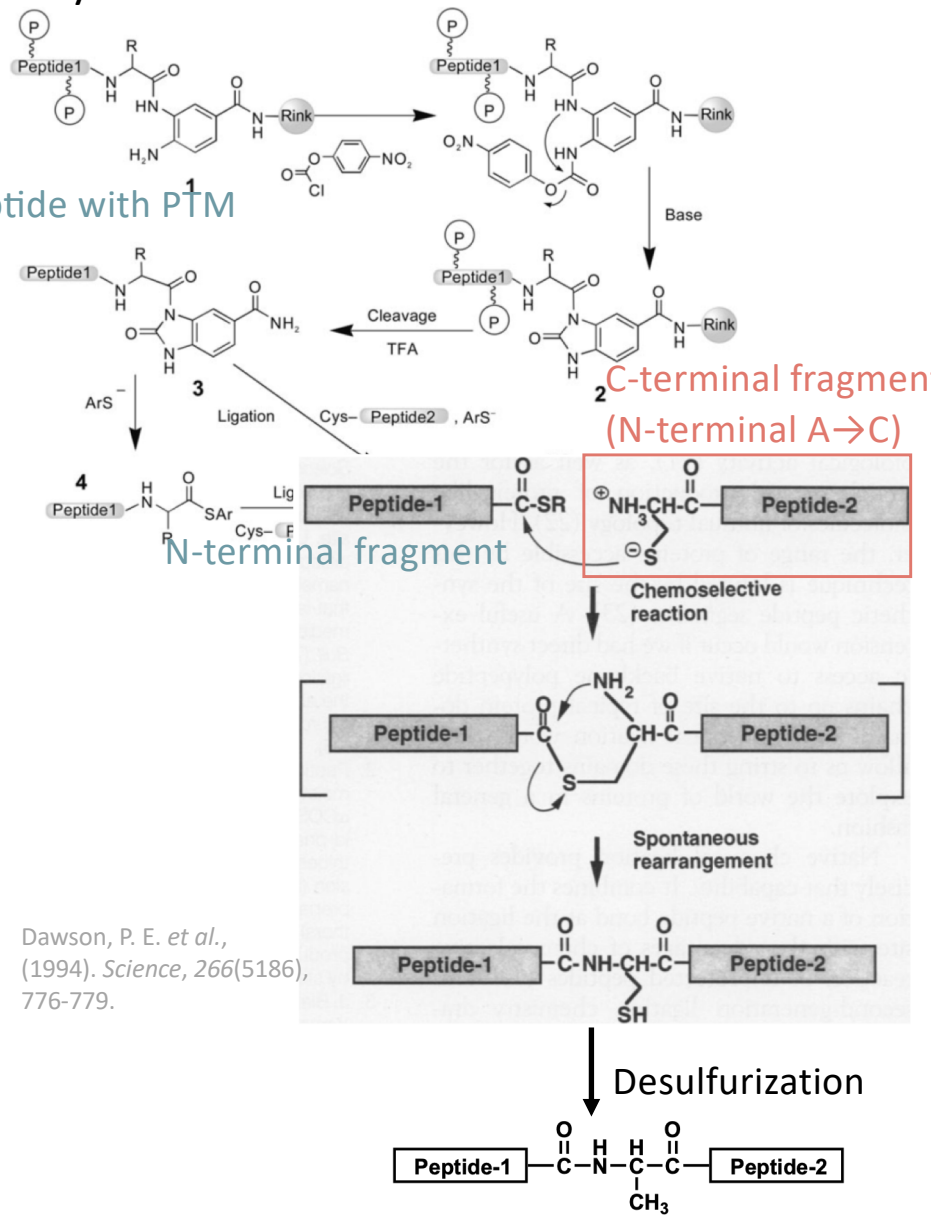
i), iii) EPL

Dawson, P. E. *et al.*, (1994). *Science*, *266*(5186), 776-779.

Fierz, B. *et al.*,(2012). *JACS.* **134**, 19548–19551 (2012). modified

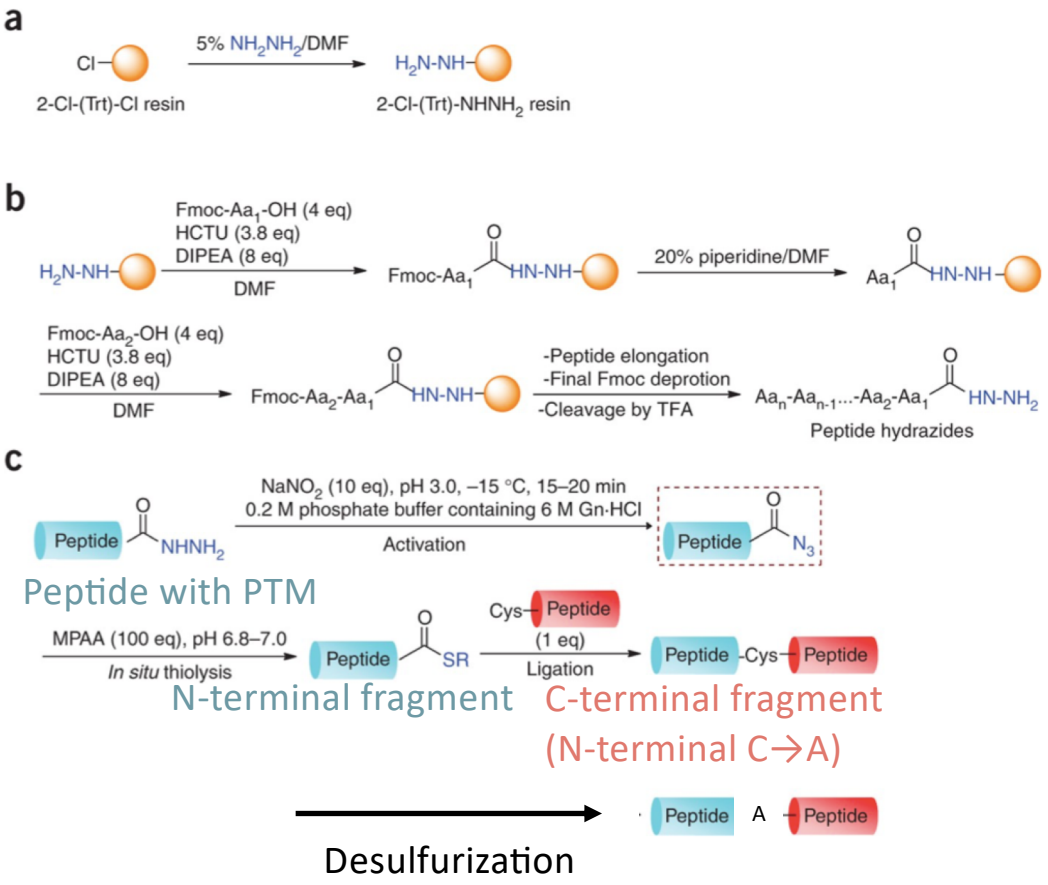
Synthesis of modified mononucleosomes via a variety of protein chemistry approaches

N-acylurea



Dawson, P. E. *et al.*, (1994). *Science*, *266*(5186), 776-779.

hydrazide

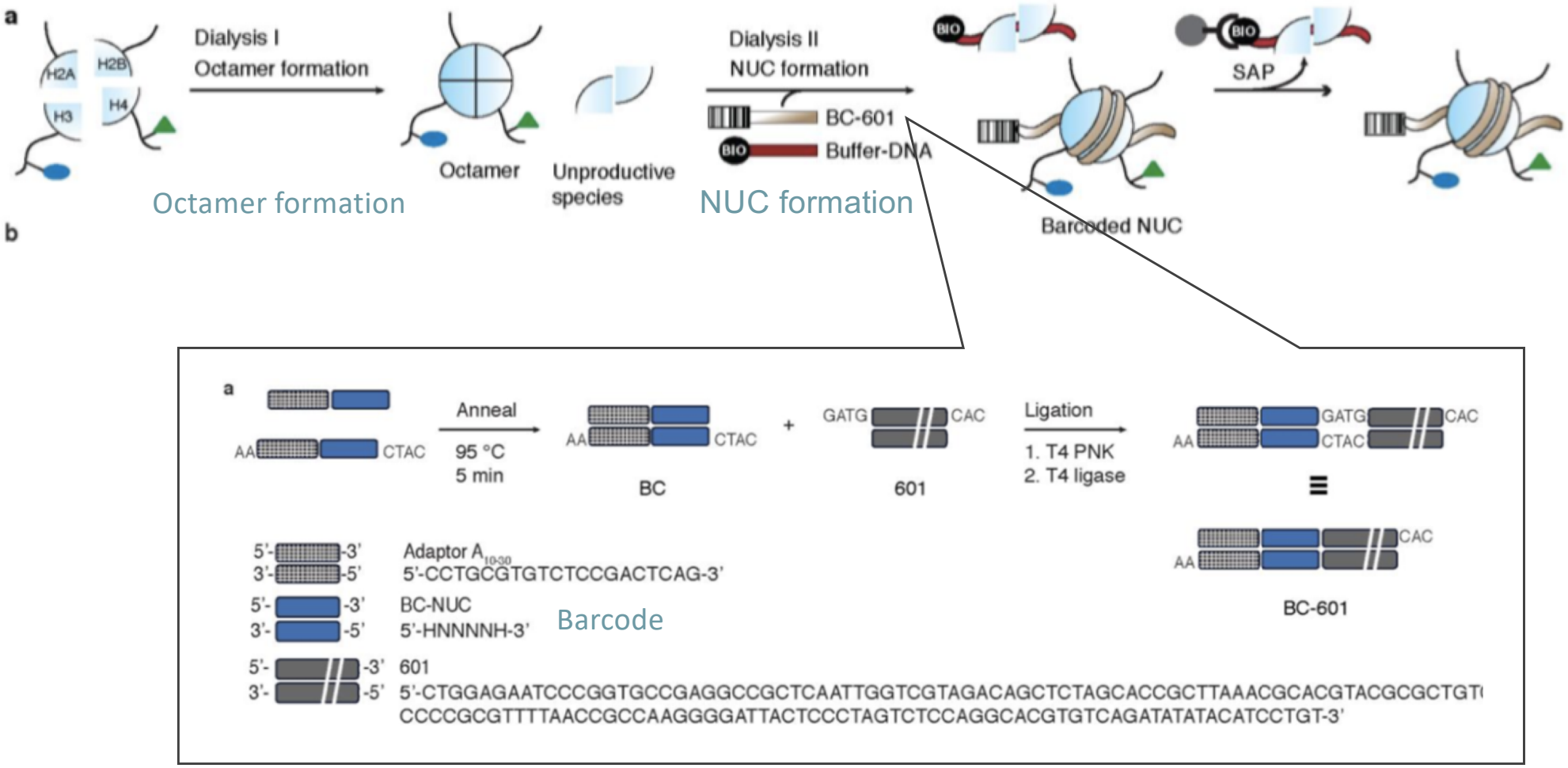


Zheng, J. S. *et al.*, (2013). *Nature protocols*, *8*(12), 2483.

Blanco-Canosa, J. B. *et al.*(2008),*ACIE*, *47*(36), 6851-6855.

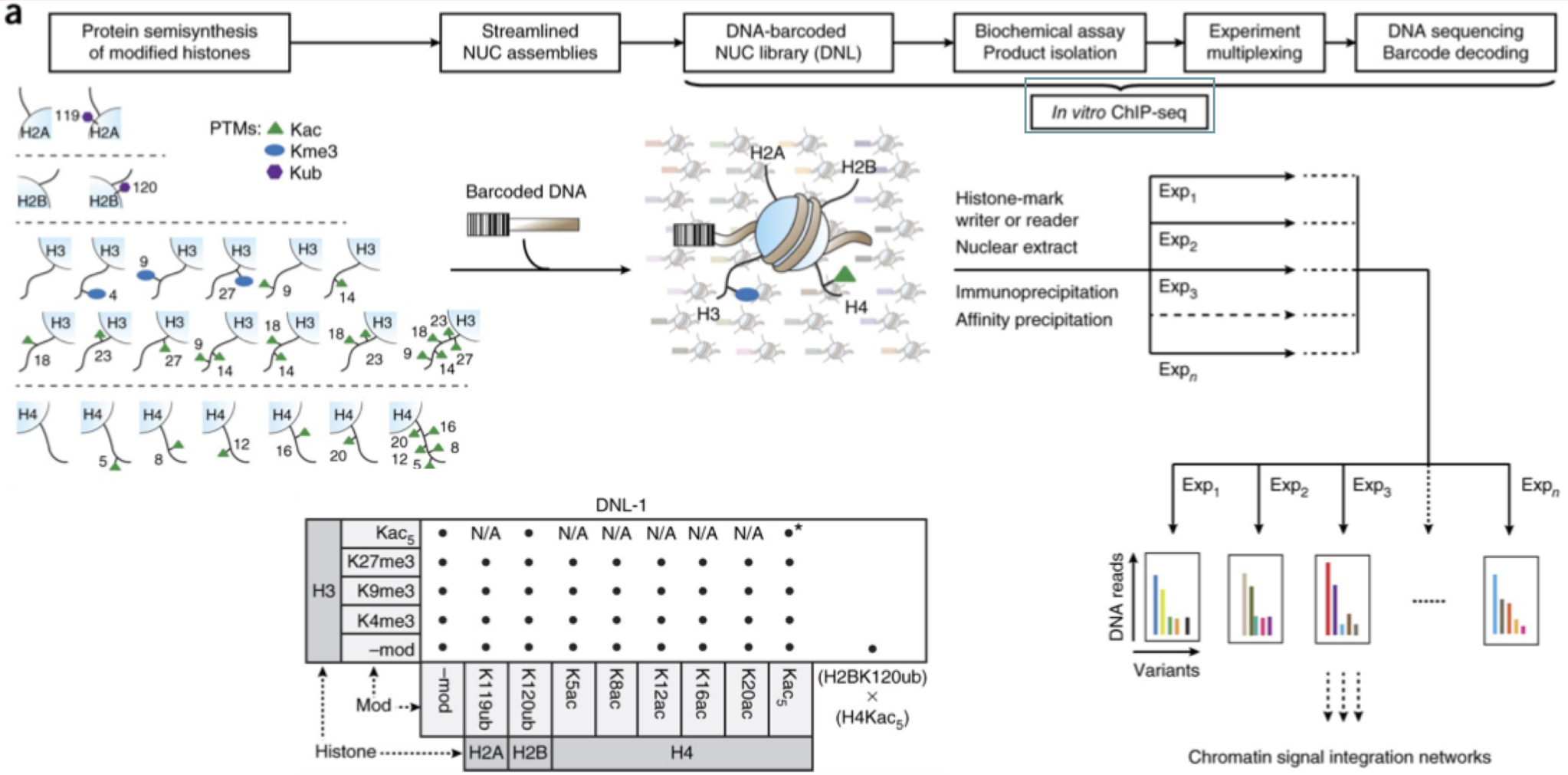
Fang, G. M., Li *et al.*, (2011). *ACIE*, *50*(33), 7645-7649.

Barcoded nucleosome assembly



Distinct combinations of histones were combined with 5’-barcoded versions of the strong nucleosome positioning sequence 601.

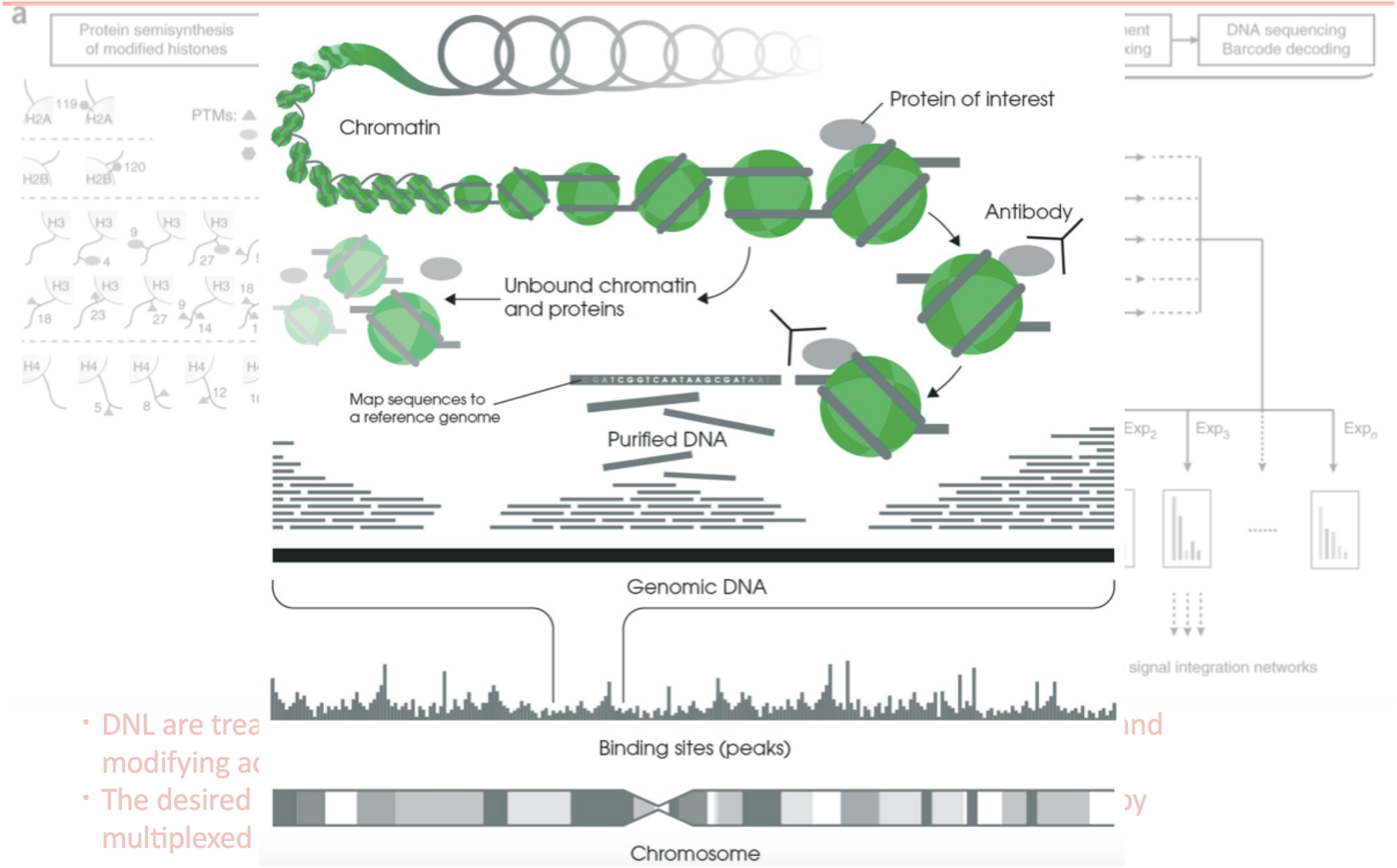
Preparation of a DNL and its use in *in vitro* ChIP-seq experiments



- DNL are treated with purified effectors or the combined chromatin recognizing and modifying activities of the nuclear proteome
- The desired products are isolated by chromatin immunoprecipitation, followed by multiplexed DNA-barcode sequencing

Nguyen, U. T *et al,*(2014). *Nature methods*, *11*(8), 834-840.

Preparation of a DNL and its use in *in vitro* ChIP-seq experiments



- DNL are trea … nd modifying ac
- The desired … by multiplexed

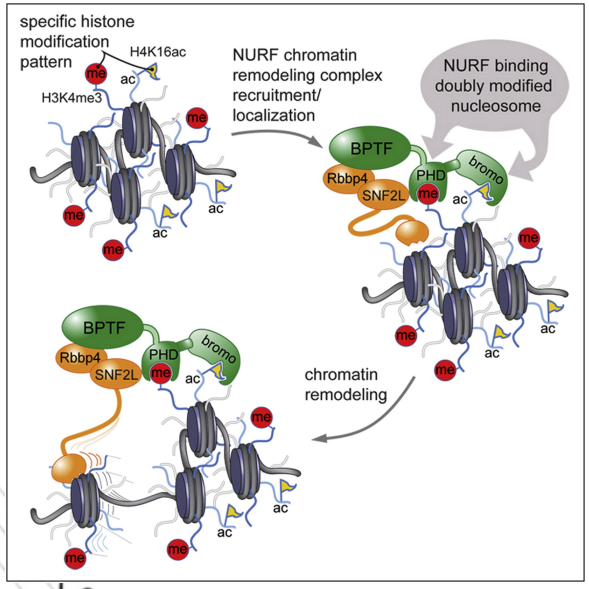
https://www.abcam.com/epigenetics/studying-epigenetics-using-chip

Profiling the substrate preference of histone-mark “readers”

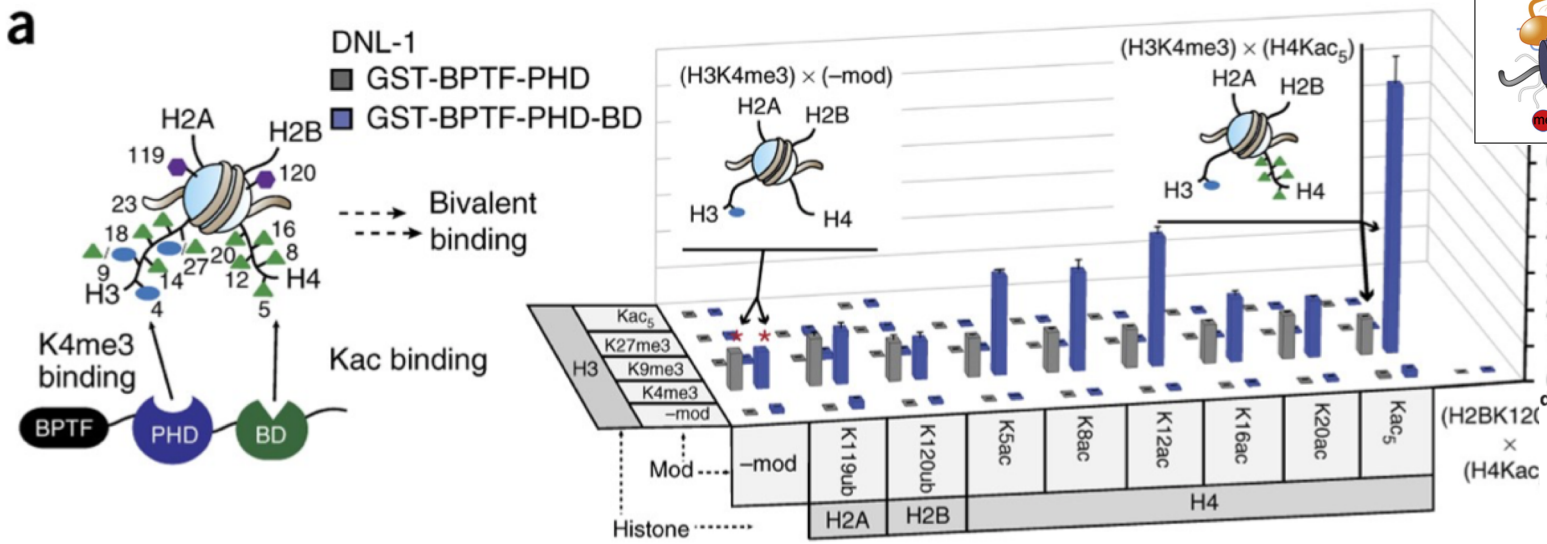
BPTF： the BD–PHD finger transcription factor

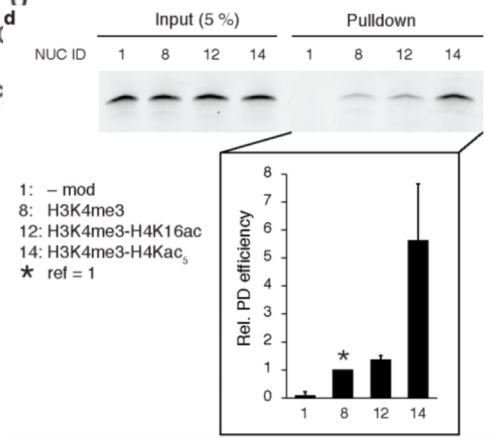
Previous peptide and nucleosome binding studies

- Nucleosomal binding increases with H3K4me3-H4K16ac
- Kac-binding pocket of BPTF's BD can accommodate only one K_{ac}



Ruthenburg, A. J., *et al.* (2011). *Cell*, *145*(5), 692-706.





- Only the BPTF-PHD-BD fusion associated bivalently to substrates carrying an H3K4me3 mark in conjunction with a monoacetylated lysine residue on H4
- A strong enhancement was found with $H4Kac_5$-containing nucleosomes

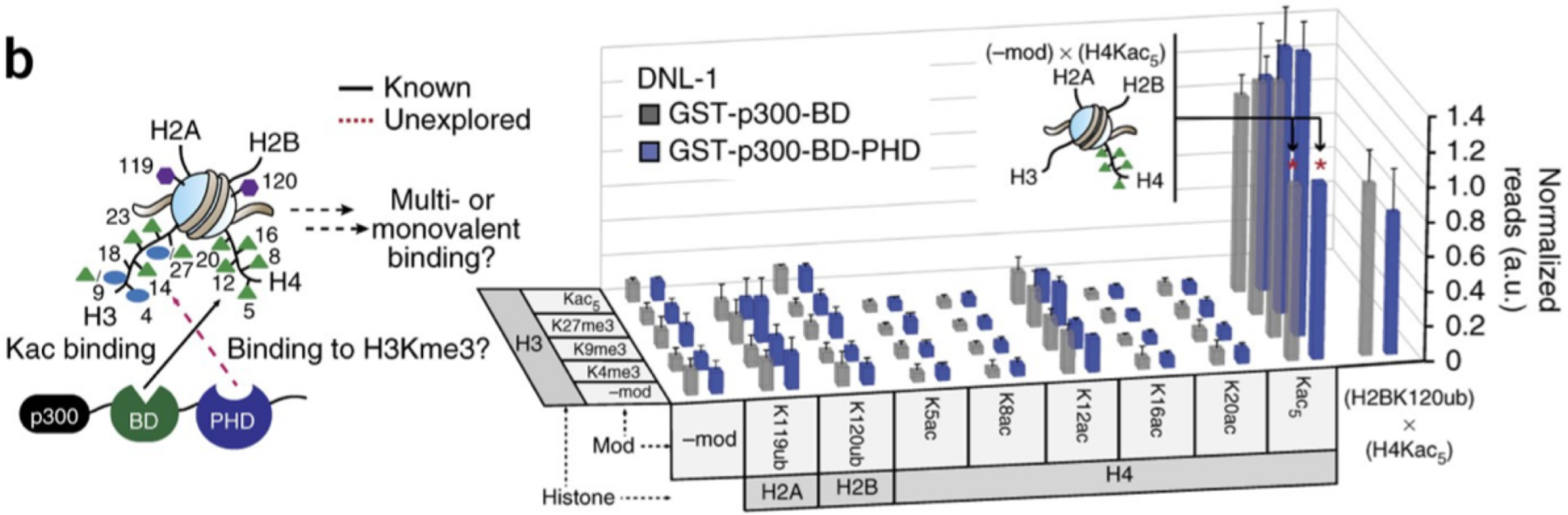
Nguyen, U. T *et al,*(2014). *Nature methods*, *11*(8), 834-840.

Profiling the substrate preference of histone-mark “readers”

P300：transcriptional coactivator, acetyltransferase

Previous peptide study

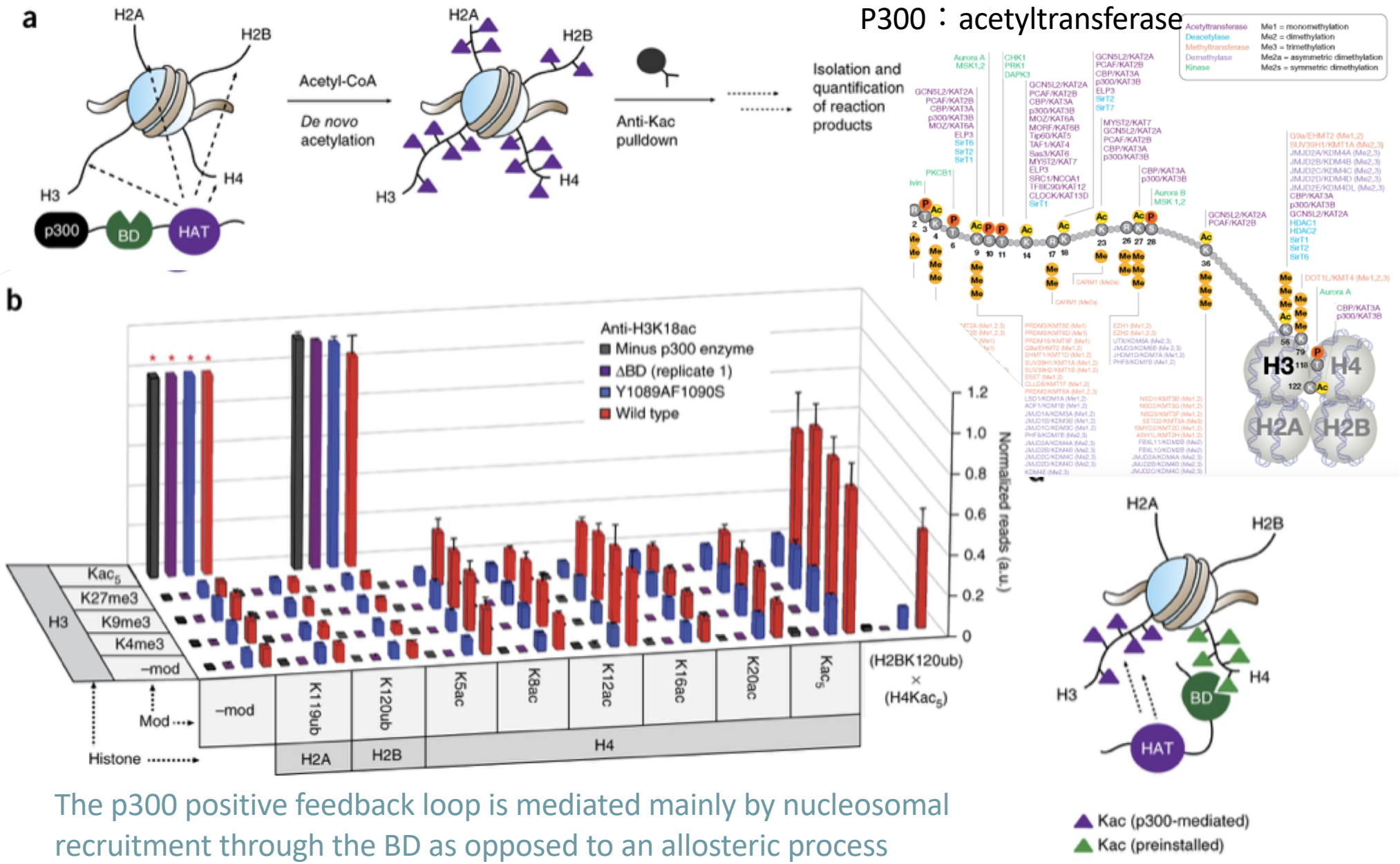
- Peptides that interacted with BD-PHD contained at least one acetylated residue, and peptides that displayed the strongest affinity contained combinations of multiple acetylated histone H4 tail.



A BD-mediated robust association to substrates with hyperacetylated H4 tails was observed.

Profiling the regulation of histone-mark “writers”

P300：acetyltransferase



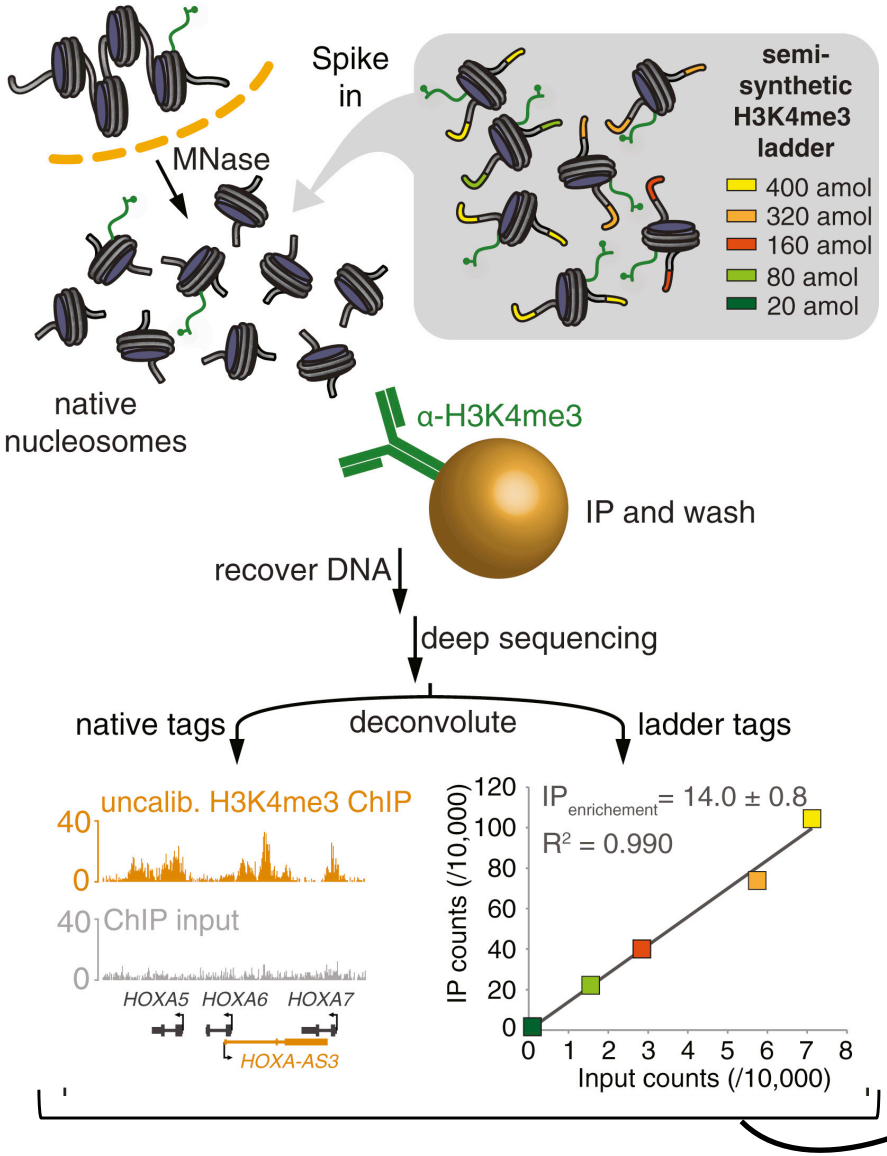
The p300 positive feedback loop is mediated mainly by nucleosomal recruitment through the BD as opposed to an allosteric process

Nguyen, U. T *et al,*(2014). *Nature methods*, *11*(8), 834-840.

https://www.cellsignal.com/contents/science-cst-pathways-epigenetics/epigenetic-writers-and-erasers-of-histone-h3/pathways-epi-h3

DNA-barcoded mononucleosomes as internal standards to calibrate ChIP-seq data

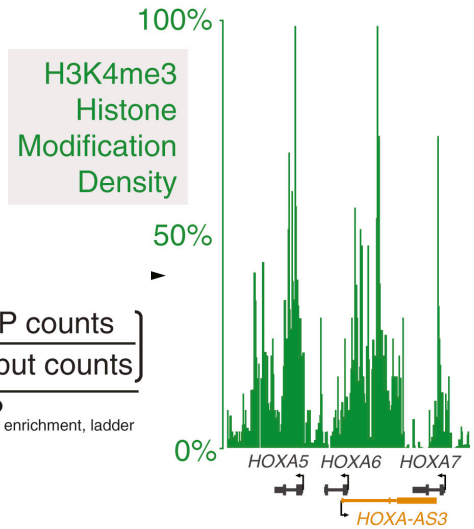
Internal Standard Calibrated ChIP (ICeChIP) → unbiased trans-experimental comparisons



"barcode" that encodes each member's concentration

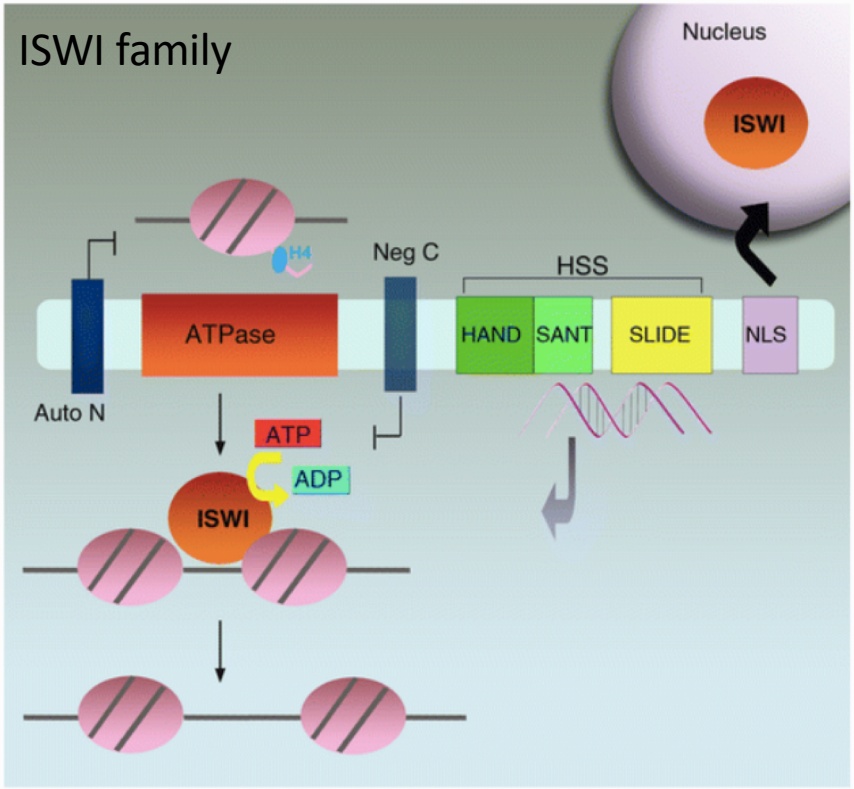
The greatest source of experimental error of ChIP is the frequently poor affinity, specificity, and reproducibility of the antibodies employed to capture desired epitopes.

$$HMD(\%)_{/bp} = 100\% * \left[\frac{\text{IP counts}}{\text{input counts}} \right] / \text{IP}_{\text{enrichment, ladder}}$$



Contents

Chromatin remodelers



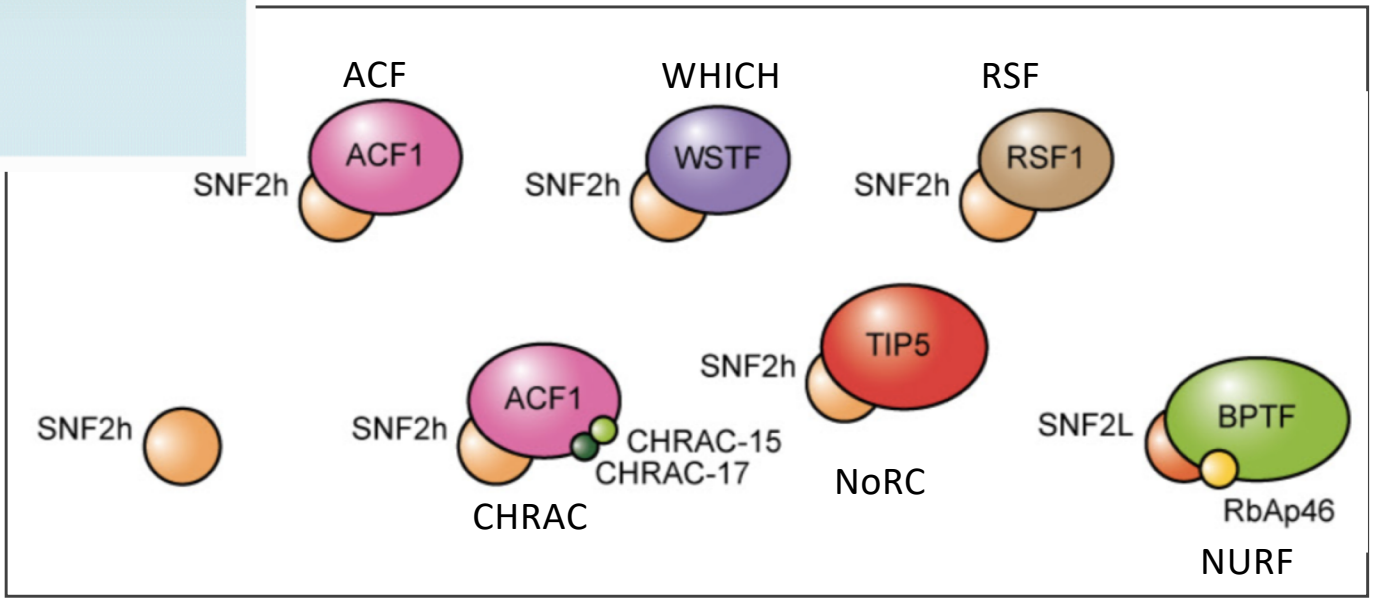
Toto, M. *et al,* (2014). *Chromosoma*, *123*(1-2), 91-102.

・The ISWI chromatin remodelling ATPase constitutes an important subfamily within the SNF2 superfamily of ATPase.

・A basic patch at the base of the H4 tail is a major determinant of ISWI recognition.

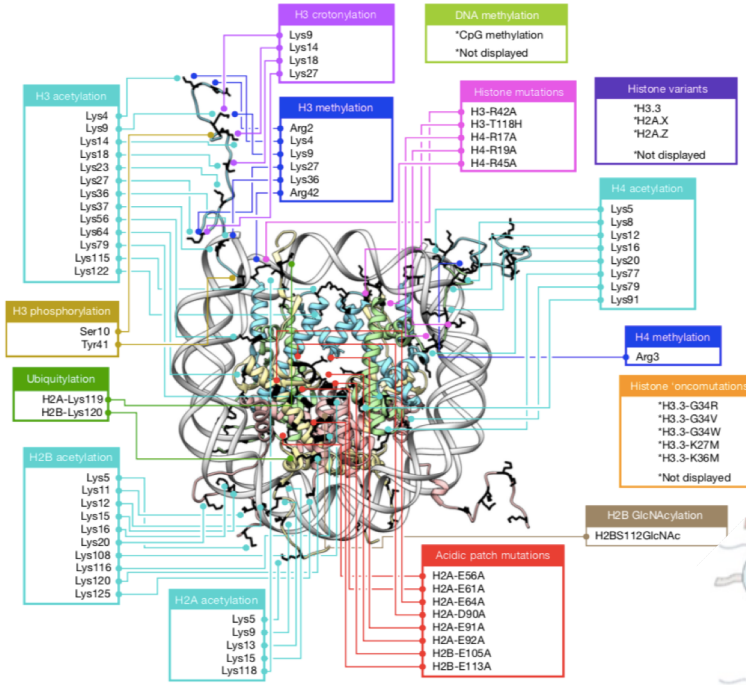
・ISWI remodelers are stimulated by histone variant H2A.Z.

ISWI remodellers



Dann, G. P. *et al,* (2017). *Nature*, *548*(7669), 607-611.

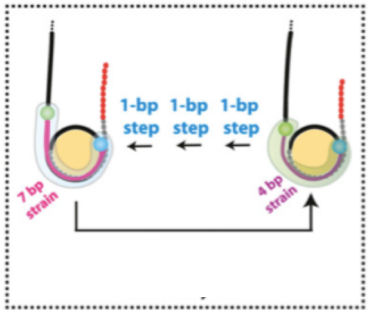
Nucleosome remodelling assay for ISWI family chromatin remodellers using MN library

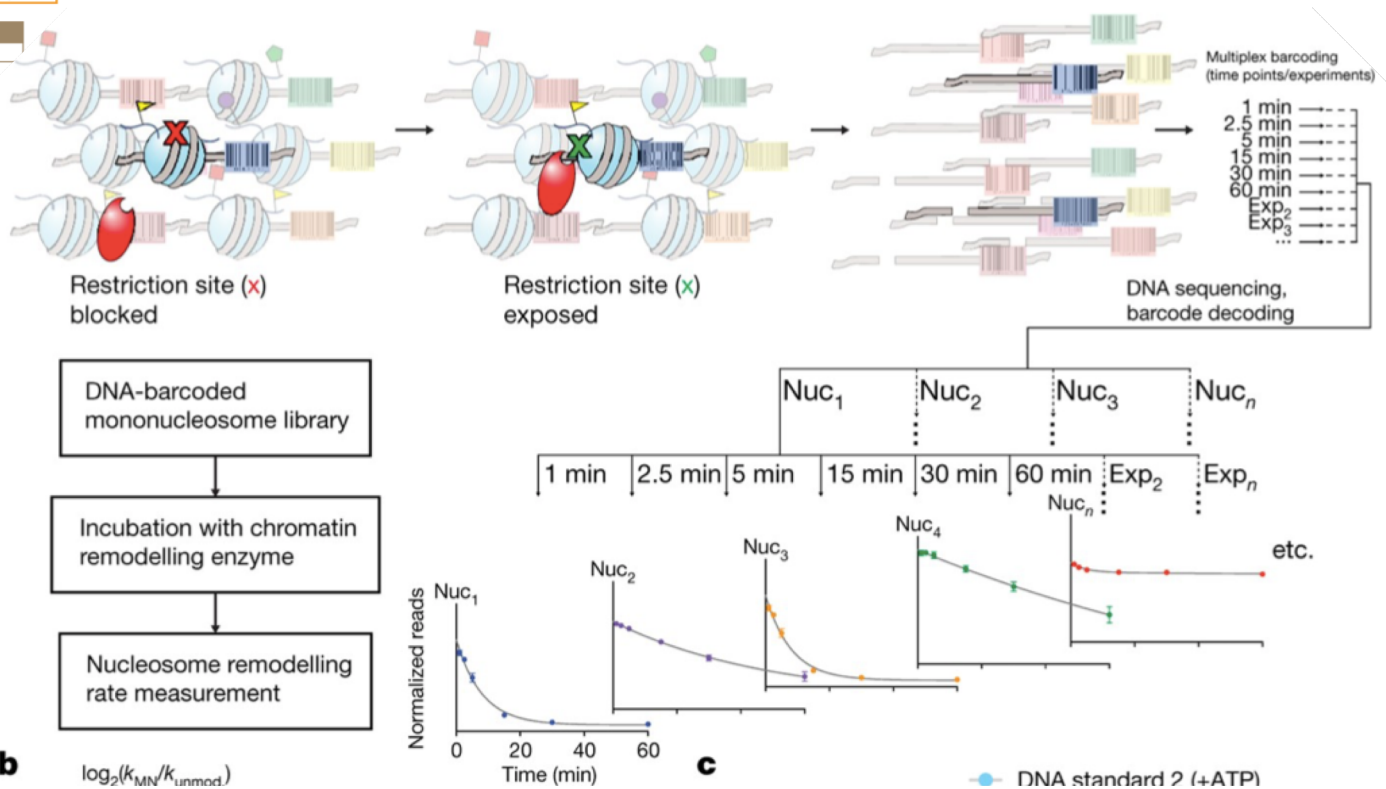


MN library

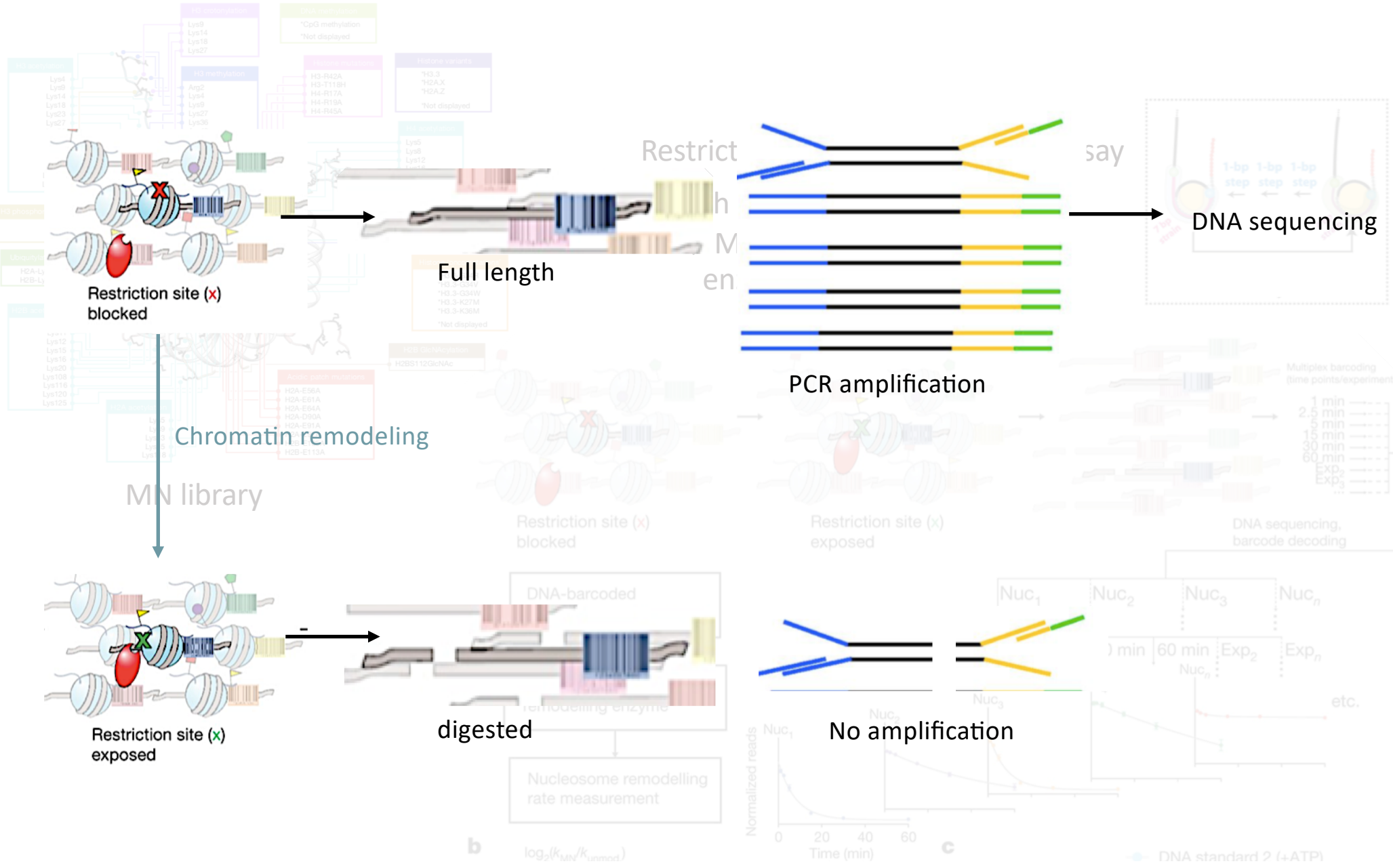
Restriction enzyme accessibility assay

High remodeling activity
→ More sensitive to restriction enzyme at early time point



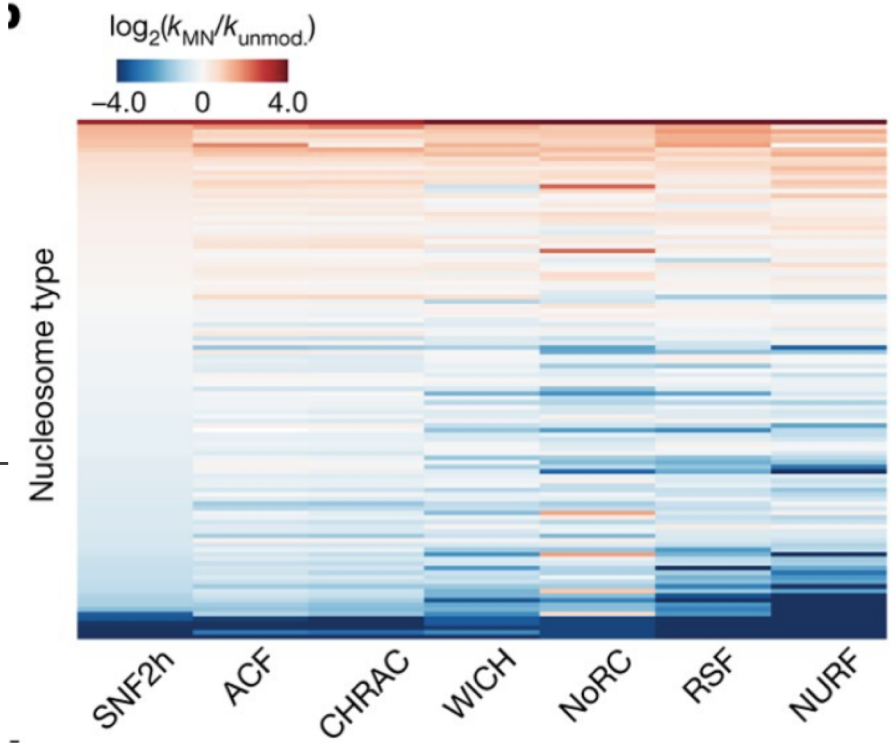


Nucleosome remodelling assay for ISWI family chromatin remodellers using MN library

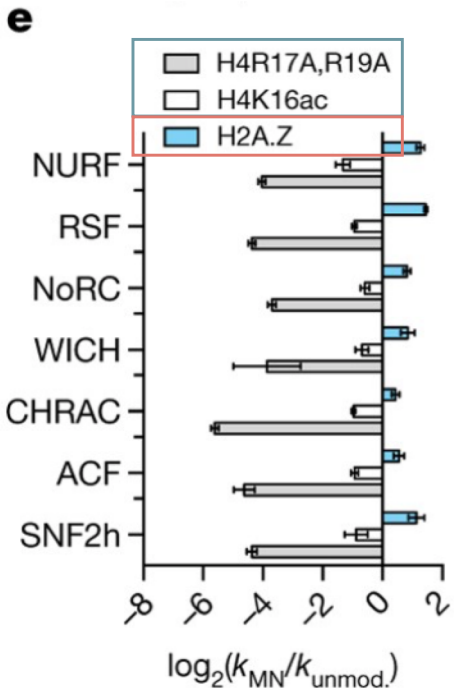


Evaluation of ISWI remodeling assay against the nucleosome library

Heat map displaying ISWI remodeling data against the nucleosome library.



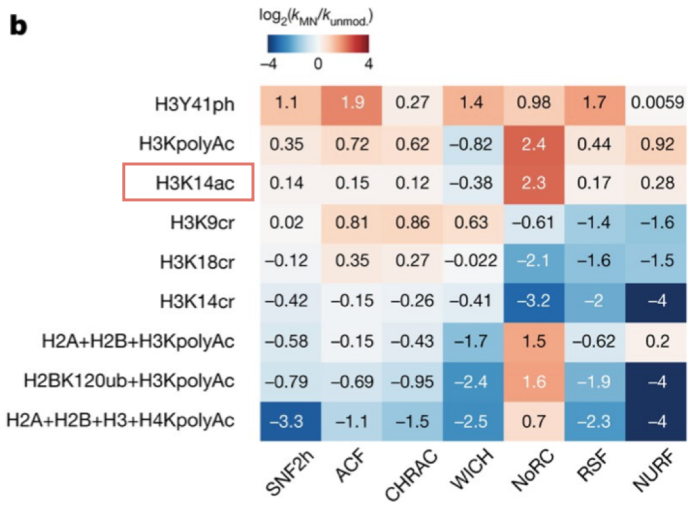
A DNA standard present in the library that is unoccupied by a histone octamer was always faster than nucleosome remodeling.



- Nucleosomes containing modifications or mutations in the basic patch of the H4 tail were poor substrates of ISWI remodelers.
- Nucleosomes containing the histone variant H2A.Z led to enhanced remodeling activity.

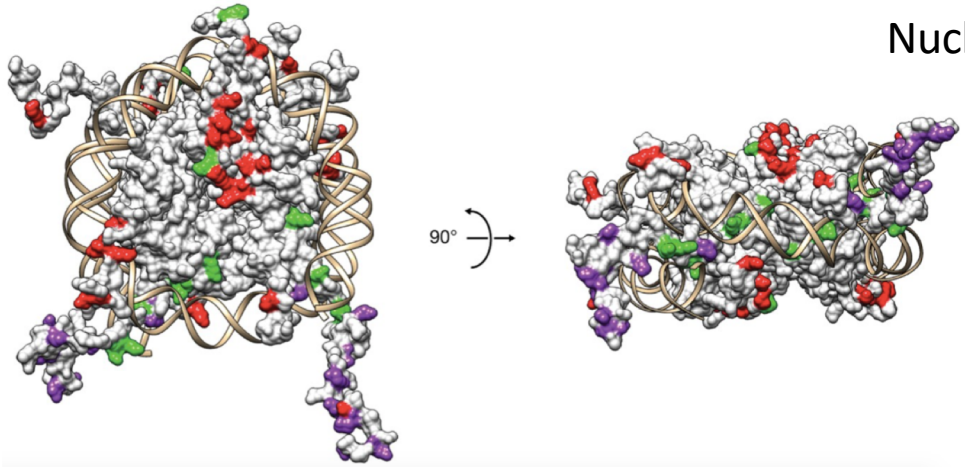
→MN library results are consistent with previous work.

Specialization of ISWI remodelers for diverse nucleosome modifications.



- Subsets of the nucleosome library drive differences in remodeler activity
- A specific nucleosome type had a broad range of effects across different remodelers

Single-site modifications mapped onto the nucleosome



Nucleosome remodeling activity across all ISWI remodelers

consistently positive

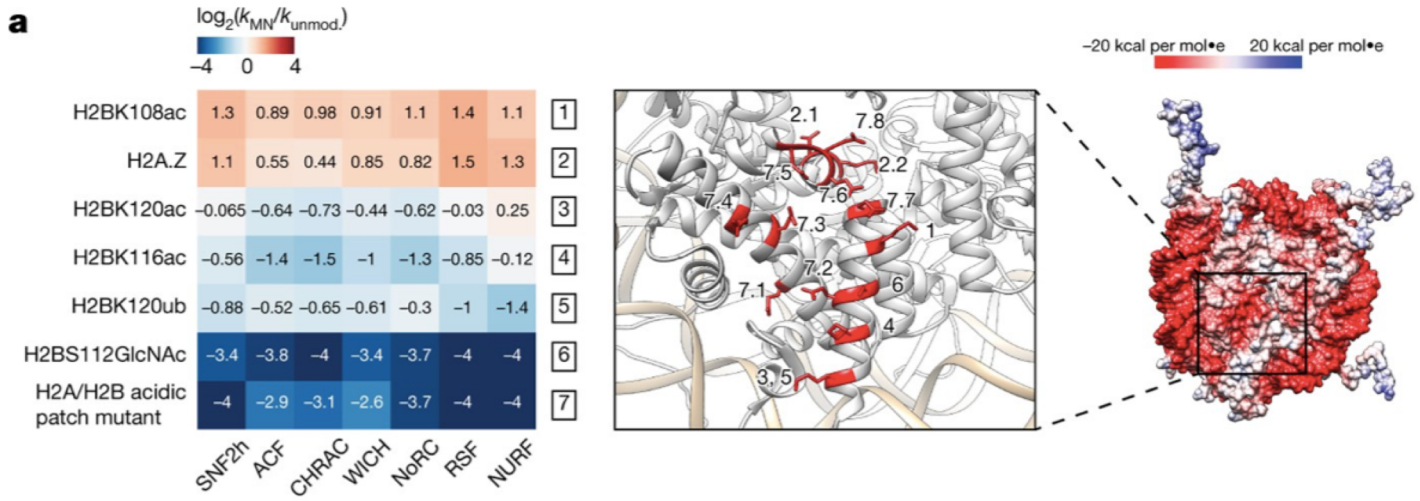
consistently negative

：more accessible regions of the nucleosome

variable effects

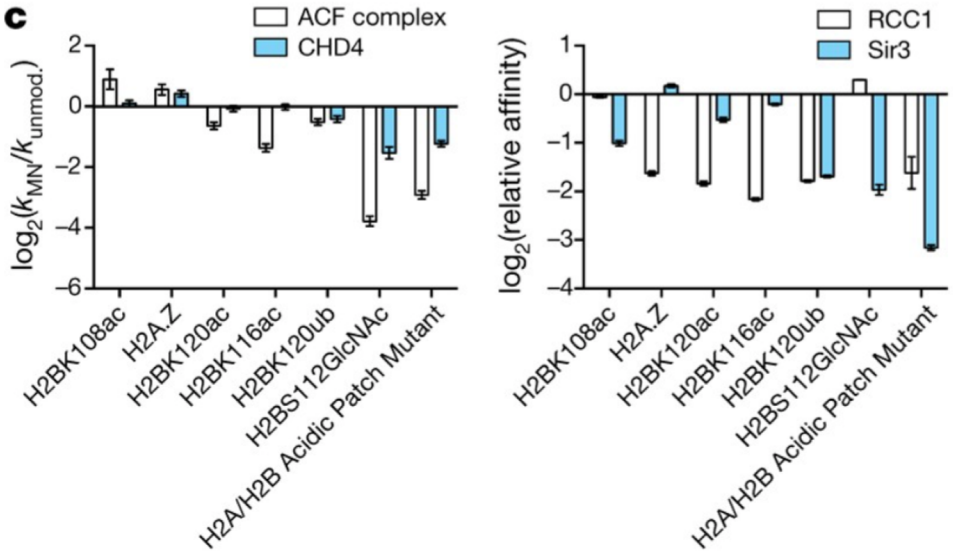
： residing under the DNA

The nucleosome acidic patch is crucial for remodeling and regulatable by histone PTMs



(7.1, H2AE56A; 7.2, H2BE113A; 7.3, H2AE61A; 7.4, H2AE64A; 7.5, H2AD90A; 7.6, H2AE92A; 7.7, H2BE105A; 7.8, H2AE91A; 2.1, N94D (H2A→H2A.Z); 2.2, K95S (H2A→H2A.Z)

- Acidic patch disrupted nucleosome was inefficiently remodeled by all ISWI family remodelers examined.
- PTMs near the acidic patch showed both stimulation and inhibition of nucleosome sliding activity.
  → mechanism mediated by the ATPase subunit

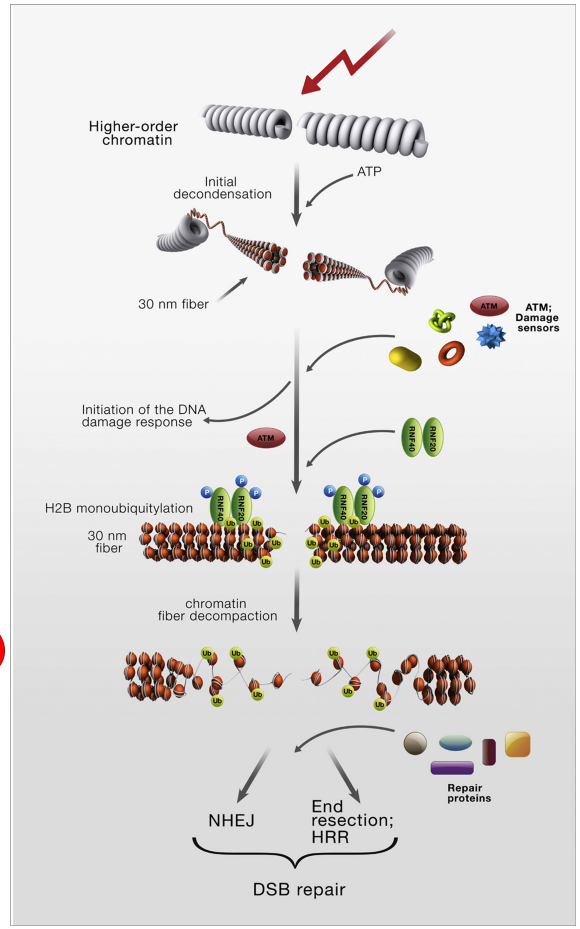


The acidic patch is subject to dynamic regulation by nucleosome modifications

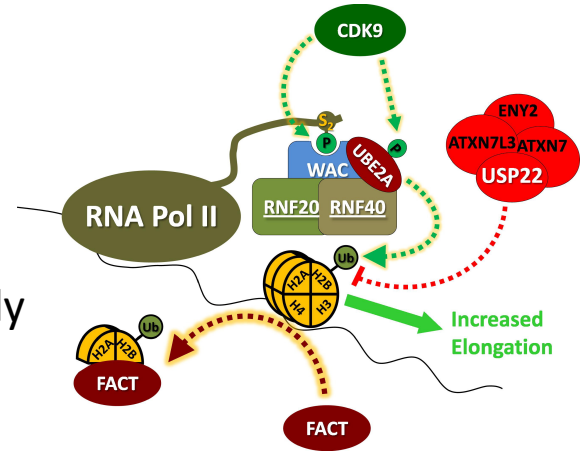
Contents

H2BK120 Ub

- **H2BK120 Ub function**
  - Active transcriptional elongation
  - Binding of the histone chaperone complex, FACT
  - Decompaction of higher-order structure of chromatin
  - DNA damage response (DDR) to double-strand breaks



- **Machinery for installing H2BK120**
  - H2BK120ub is localized primarily to actively transcribed genes
  - E2 ligase UBE2A/B and the hetero-dimeric RING-type E3 ligase RNF20/40
  - Efficient ubiquitylation of H2B is coupled to ongoing transcription



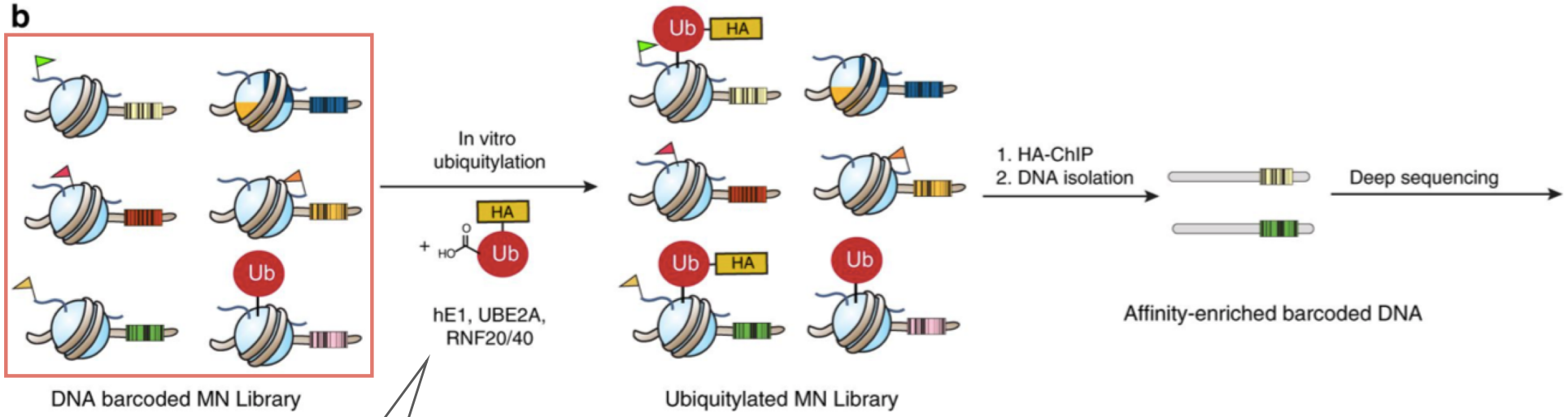
Johnsen, S. A. (2012). *FEBS letters*, *586*(11), 1592-1601. Moyal, L *et al.* (2011). *Molecular cell*, *41*(5), 529-542.

Wojcik, F. *et al.*,(2018). *Nature communications*, *9*(1), 1-11.

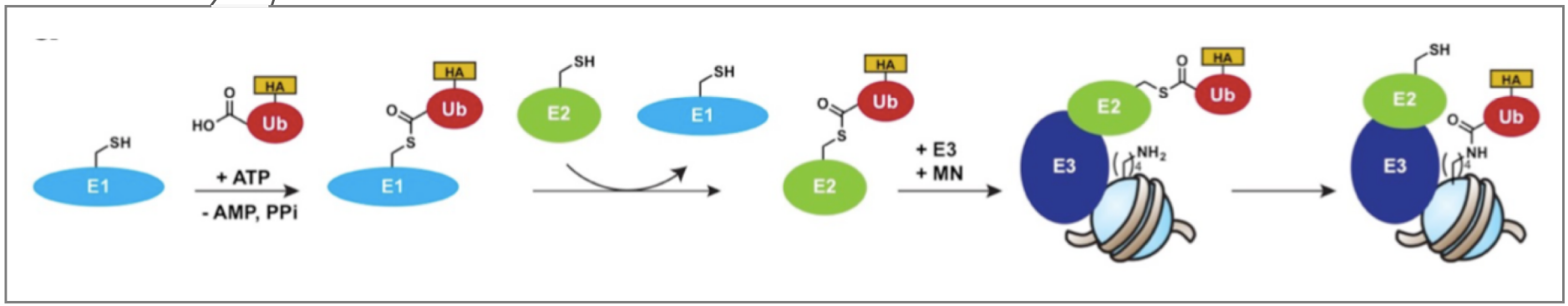
Library-based screen for the effects of PTMs on de novo H2B ubiquitylation

Do pre-existing chromatin modifications or within gene coding regions influence H2BK120ub?

in vitro screen of ubiquitylation using MN Library



In vitro ubiquitylation with RING-type enzyme and HA-tagged ubiquitin

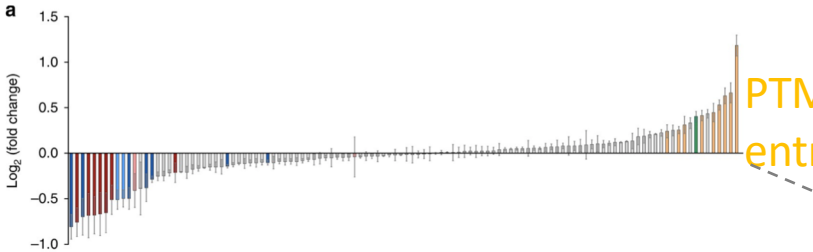


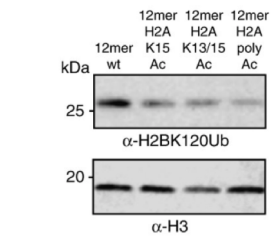
MN library based screening corresponds to several hundred enzymatic measurements

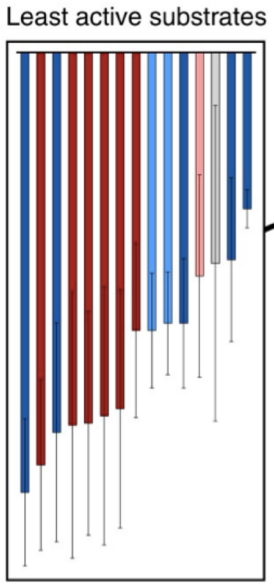
H2BK120ub deposition is sensitive to some nucleosomal modifications

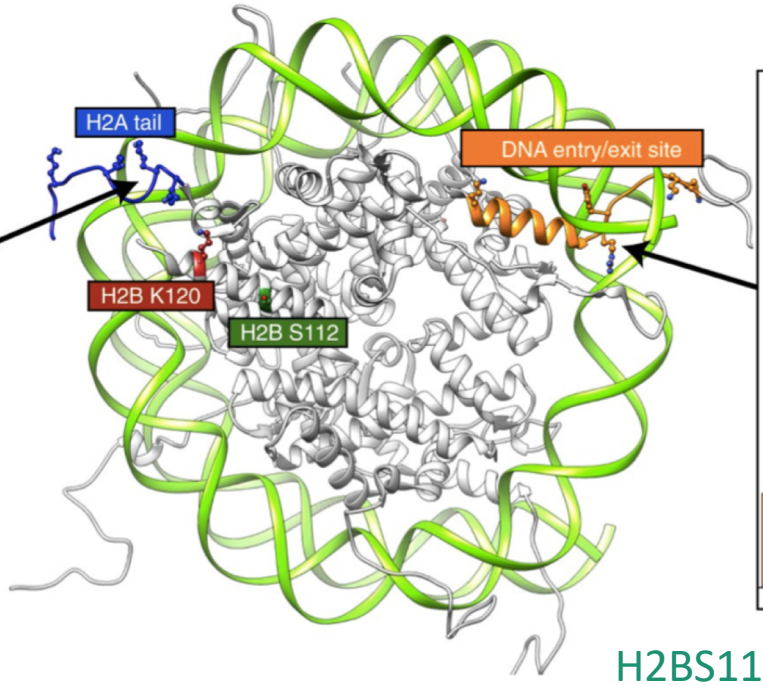
H2A.Z. and Kac on the N-terminal tail of H2A inhibit H2BK120ub.

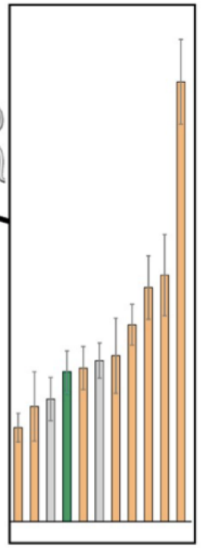
PTMs proximal to the DNA entry/exit site stimulate H2BK120ub

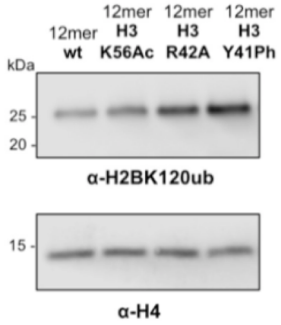


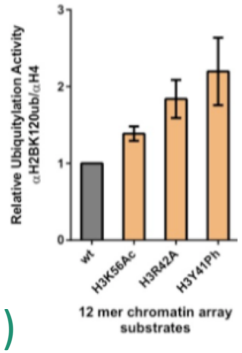


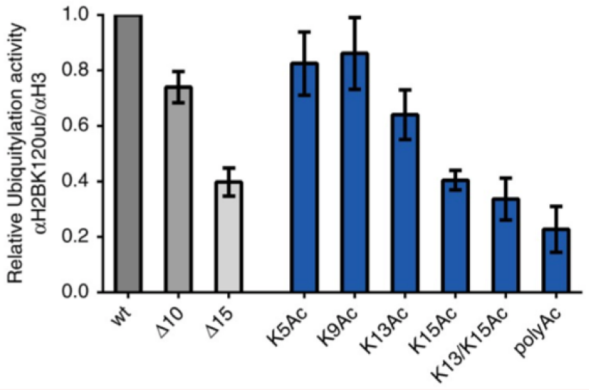












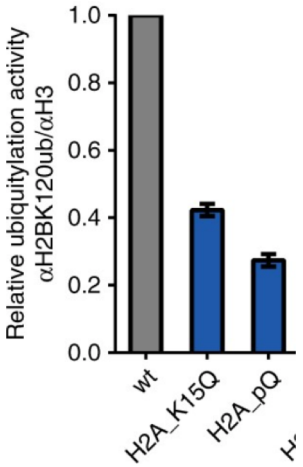
H2BS112GlcNAc (reported)

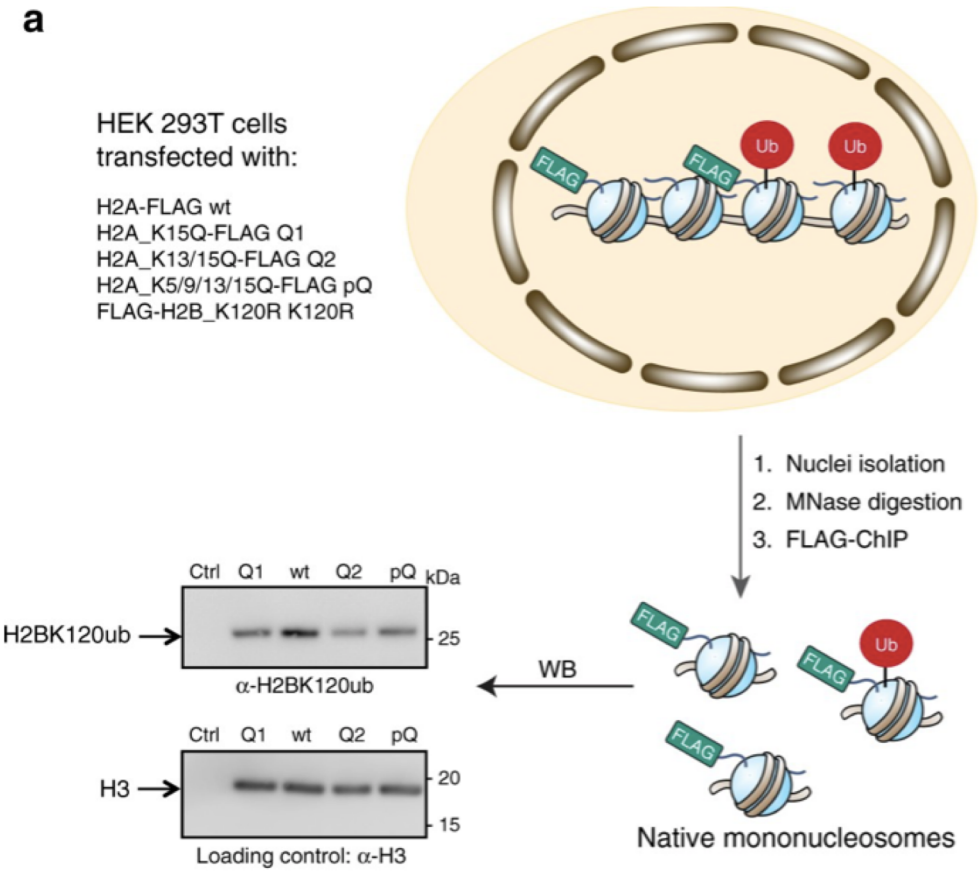
・UBE2A:RNF20/40 apparatus is extremely sensitive to changes in the H2A tail

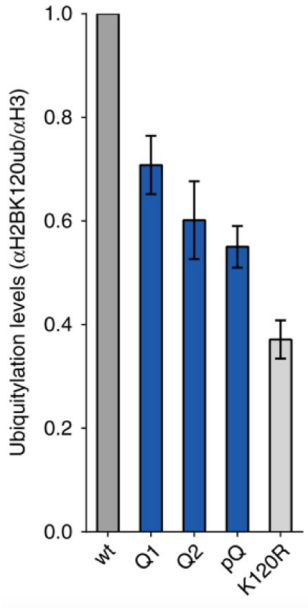
・The presence of positive charges on both K13 and K15 is especially important.

Modifications to the H2A tail affect H2BK120ub in cells

In vitro





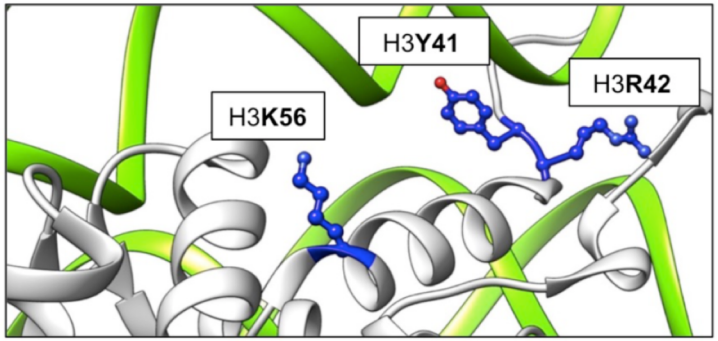


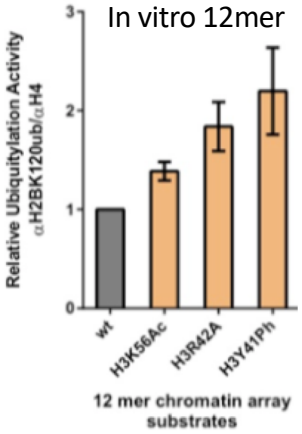
Replacement of H2AK15 either alone or in tandem with K13 led to reduction of H2BK120ub compared with a WT H2A control.

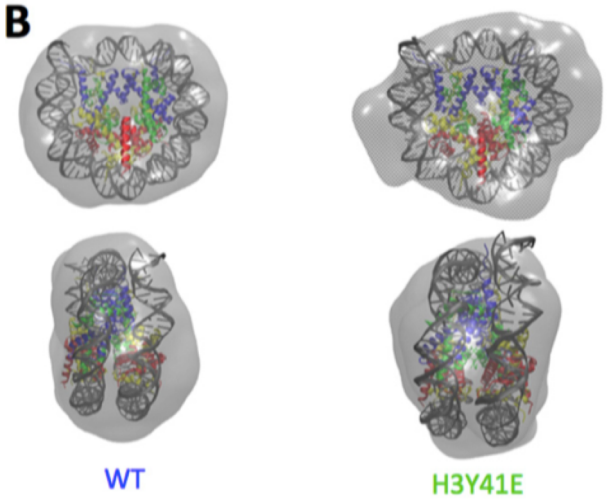
・The majority of the isolated cellular MNs will be asymmetric, containing both WT (i.e., endogenous) and mutant copies of H2A.

✓ acetylation of the H2A N terminus negative regulates H2B ubiquitylation.

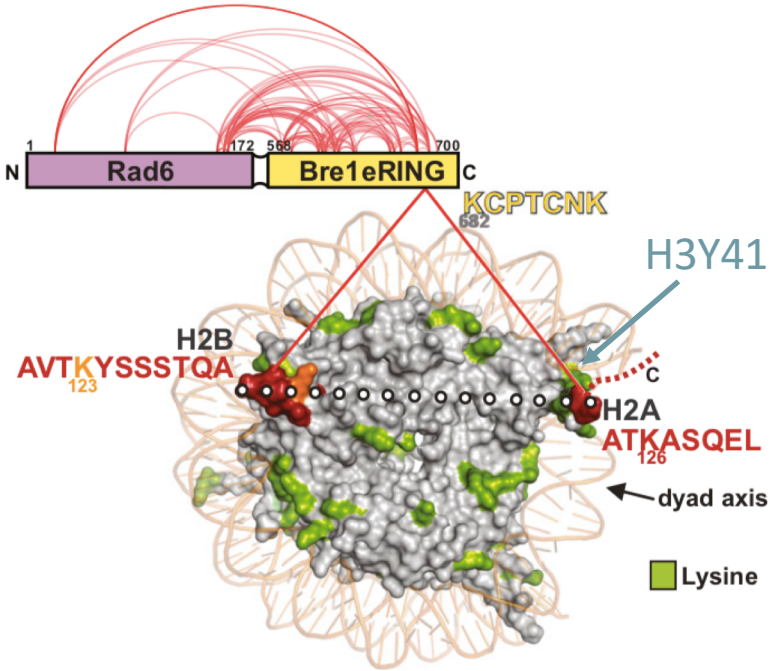
Crosstalk between H2BK120ub and modifications in the DNA entry/exit site







Brehove, M. *et al.*, (2015). , *Journal of Biological Chemistry*, *290*(37), 22612-22621.



Gallego, L. D. et al., *Proc. Natl Acad. Sci. USA* **113**, 10553–10558 (2016).

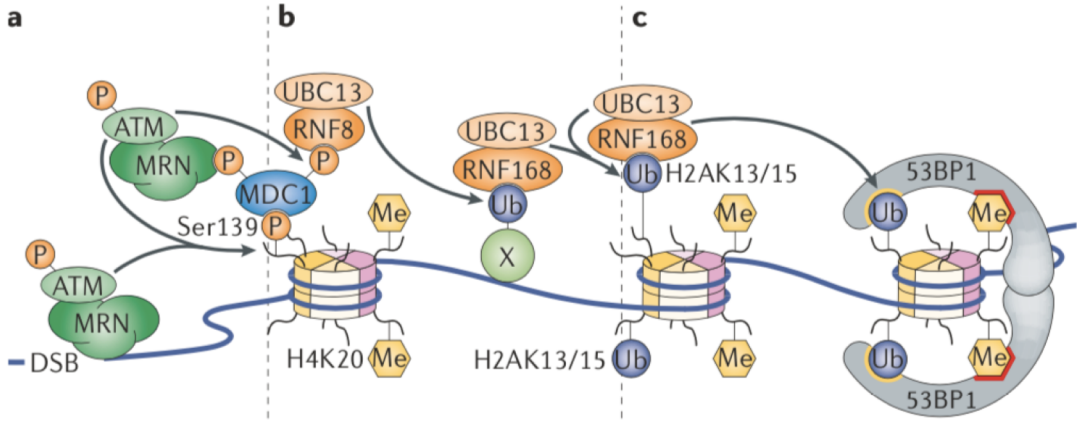
H3Y41：disrupt DNA-histone contacts leading to increased breathing of DNA on the nucleosome

- H3Y41 is proximal to the DNA entry/exit.
- DNA entry/exit site on the nucleosome
  ：a region previously implicated in the binding of E3 ligase Bre1 (the yeast version of the RNF20/40)

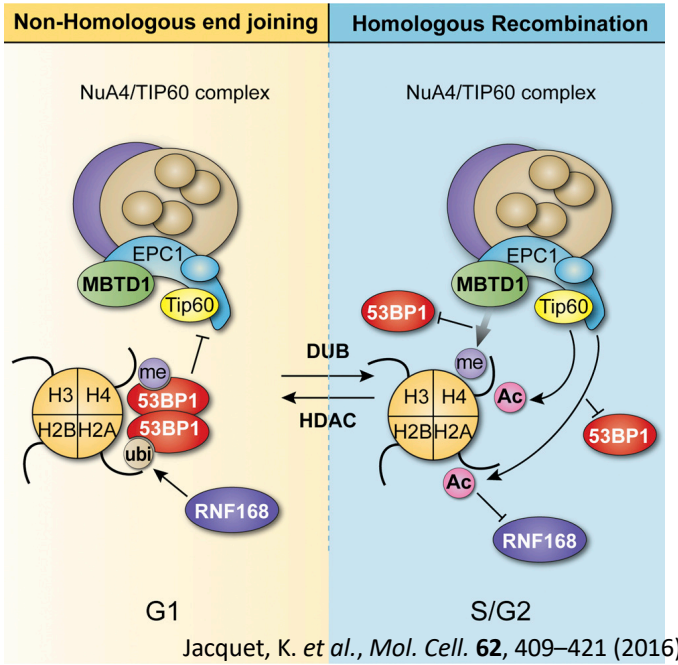
Modifications that modulate the local structure of the DNA entry/exit site, such as H3Y41ph, might stimulate ubiquitylation activity through an enzyme-binding mechanism.

Wojcik, F. *et al.*,(2018). *Nature communications*, *9*(1), 1-11.

Model for the crosstalk between H2BK120ub and the H2A/H2A.Z N-terminal tail



Panier, S., & Boulton, S. J. (2014)., *Nature reviews Molecular cell biology*, *15*(1), 7-18.



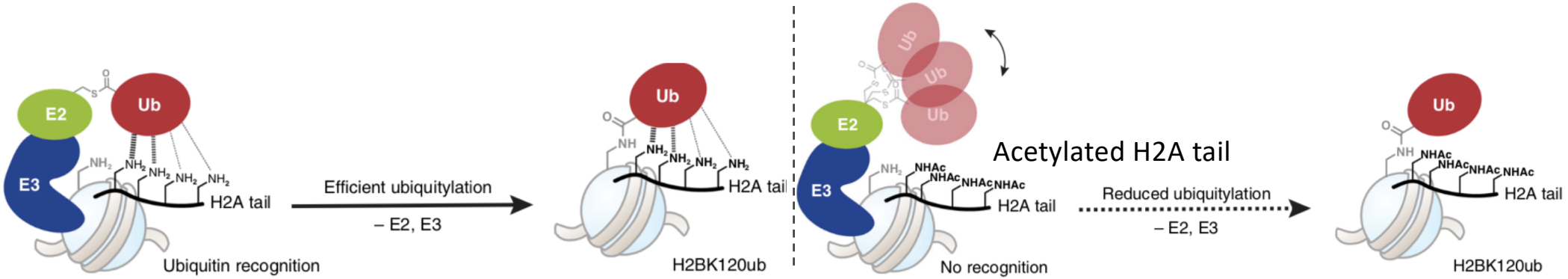
Jacquet, K. *et al.*, *Mol. Cell.* **62**, 409–421 (2016).

H2AK15Ac ⊣ H2AK15Ub by the RNF168 →DDR pathway

⊥

H2BK120Ub by RNF20/40 →DDR pathway

The crosstalk may help control the timing of H2B K120Ub at DDR (DNA damage response).



H2A tail would position ubiquitin correctly so that the transfer from RNF20/40-UBE2A-Ub to H2BK120 is ensured.

Wojcik, F. *et al.*,(2018). *Nature communications*, *9*(1), 1-11.

Contents

Summary

- ✓ DNA barcoded nucleosome library allows high throughput and sensitive PTM analysis.
- ✓ DNL enables to investigate PTM-based recruitment and modulation of histone-mark readers and writers, and shows how PTM signals, alone or synergistically, result in composite systems-level signal outputs through the combined action of the nuclear proteome.
- ✓ A systematic analysis of the effects of nucleosome modifications on chromatin remodeling activity, generated a dataset that exists as a resource to drive the design of future biochemical and cell-based studies.
- ✓ Crosstalk between PTM was revealed by using DNA barcoded mononucleosome library.