# The interaction analysis of linker histone and nucleosome for developing new nucleosome ligand

2023/08/04

Literature Seminar2

M1 Takeuchi

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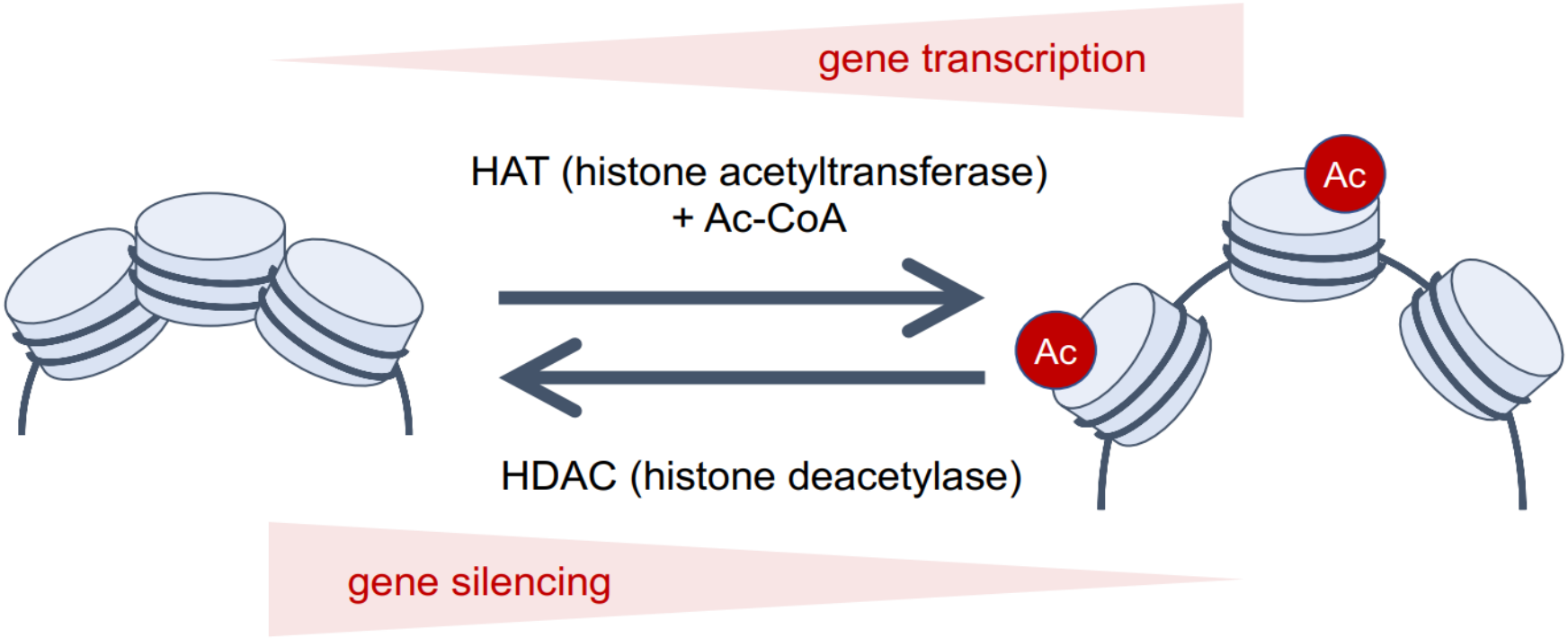
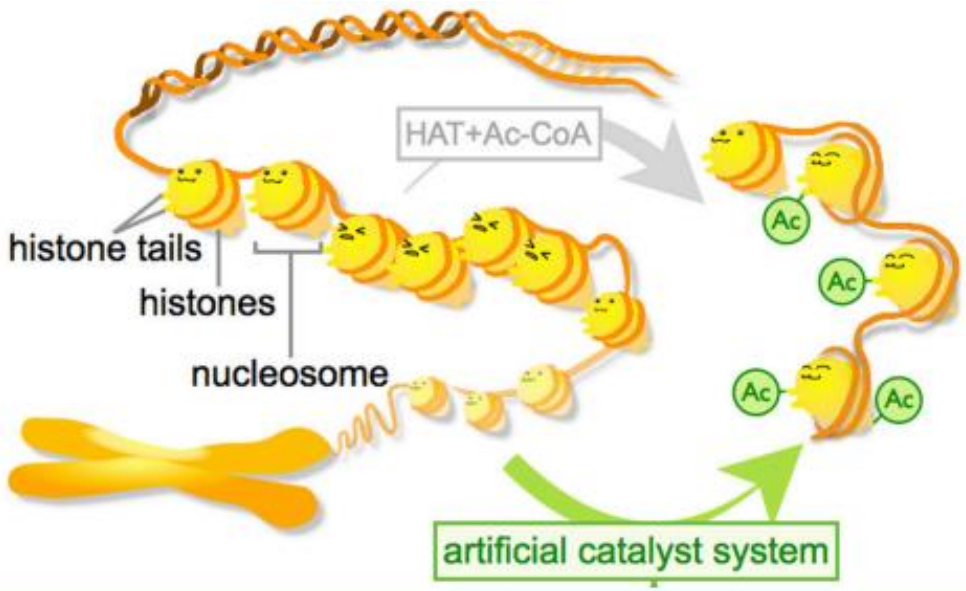
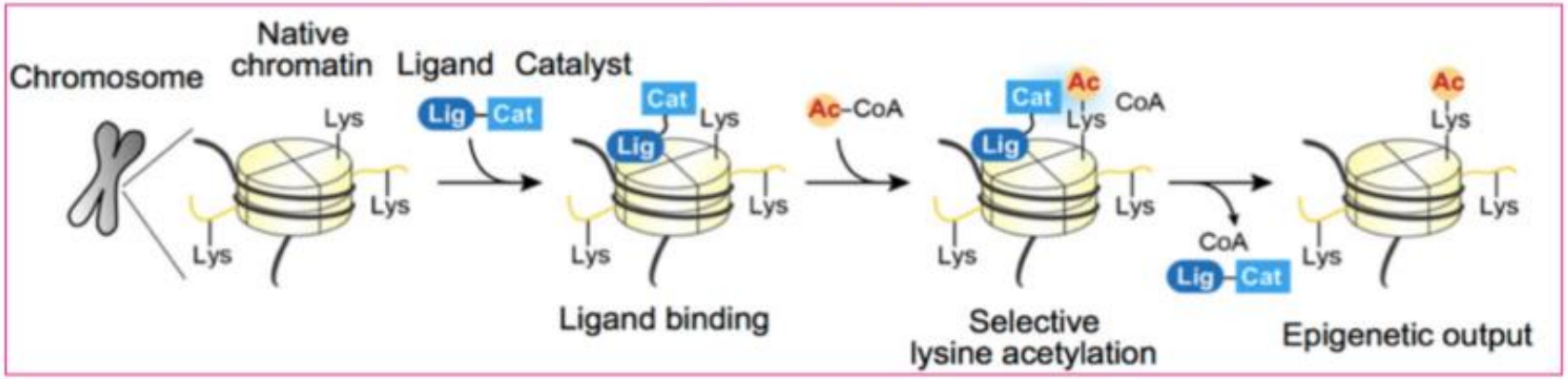
# Histone post-translational modification (PTMs)




- PTMs have a significant impact on the structure and function of proteins.
- Histone PTMs can change the structure of chromatin.
- Dysregulation of histone acetylation can result in the development of diseases.
  → **Regulation of histone acetylation levels can lead to the development of novel therapeutic strategies.**

# Chromatin acetylation by an artificial catalyst system

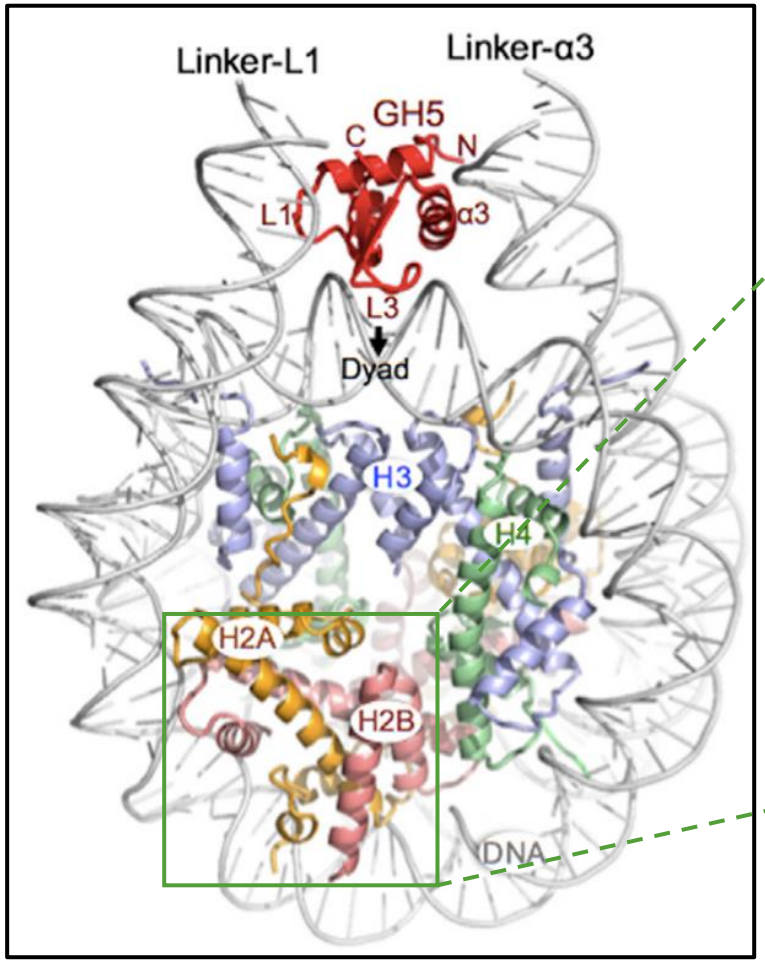


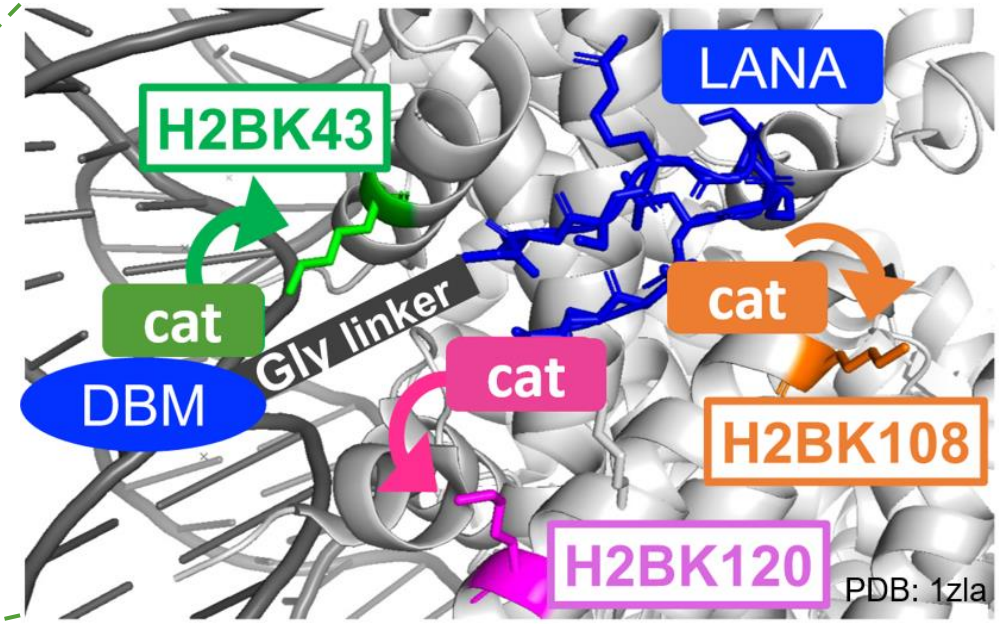




- Chemical catalysts enables various PTMs in various positions of proteins and histones.

Ishiguro, T. et al., *Chem,* **2017**, *2*(6), 840-859. Amamoto, Y. et al., *JACS*, **2017**, *139*(22), 7568-7576.

# Existing strategy: limited acetylation position (near acidic patch)



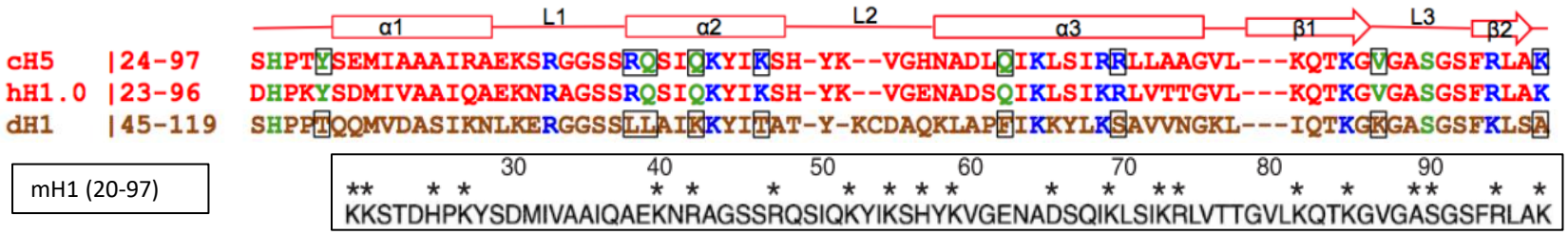

**The use of LANA as the ligand**

**→ H2BK120, H2BK108, H2BK43**

**→ Limited acetylation position (only near histone acidic patch)**

# New nucleosome ligand candidate: linker histone H1

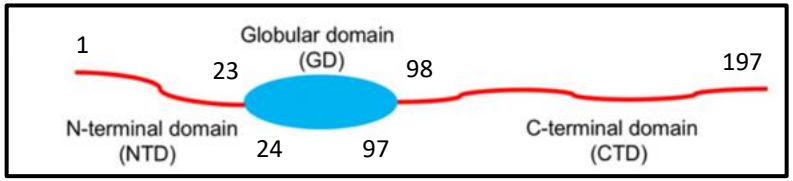
## Sequence alignment of the linker histone H1 globular domains



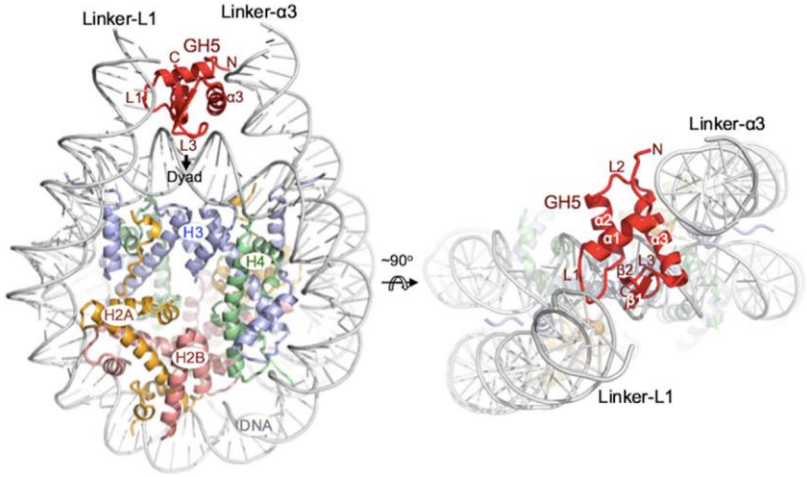

(cH5: chicken linker histone H1 (H5), hH1.0: human, dH1: *Drosophila*, mH1 : mouse)

## Structural feature of linker histone

- NTD contributes little to nucleosome binding
- GD can bind to the nucleosome.
- CTD interacts with linker DNA and is important for higher-affinity binding of linker histones to the nucleosome.

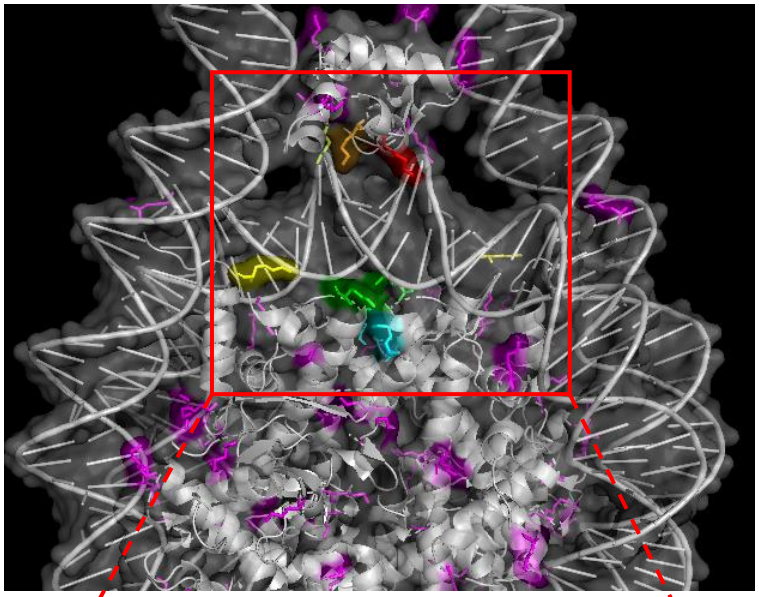


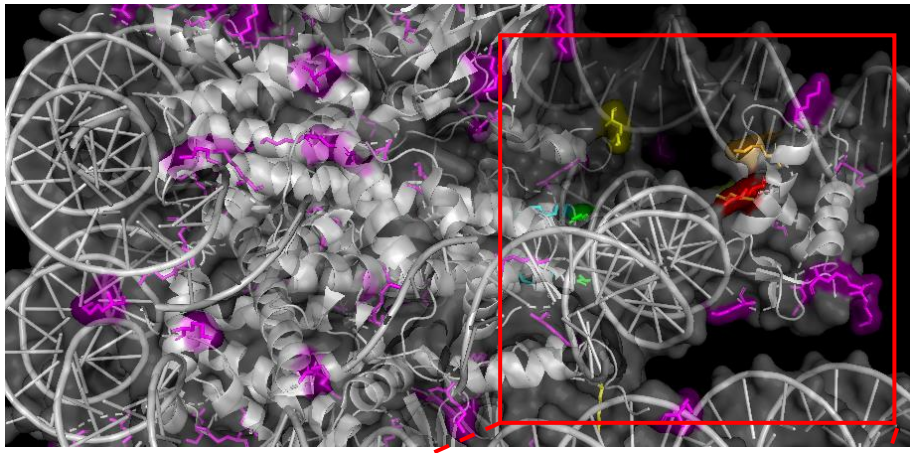

## Co-crystal Structure of the GH5 (GD of H5) and the nucleosome with linker-DNA (3.5 Å )

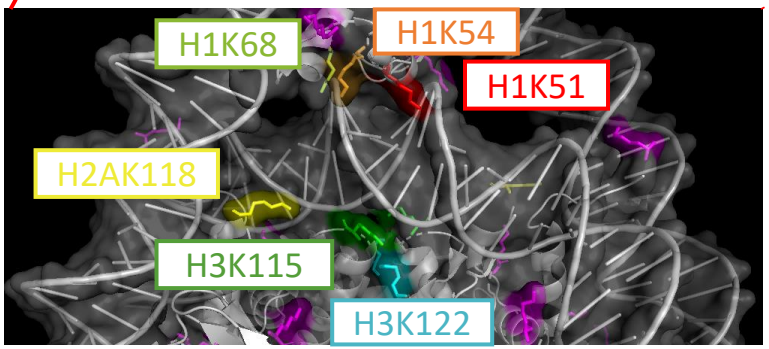



Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

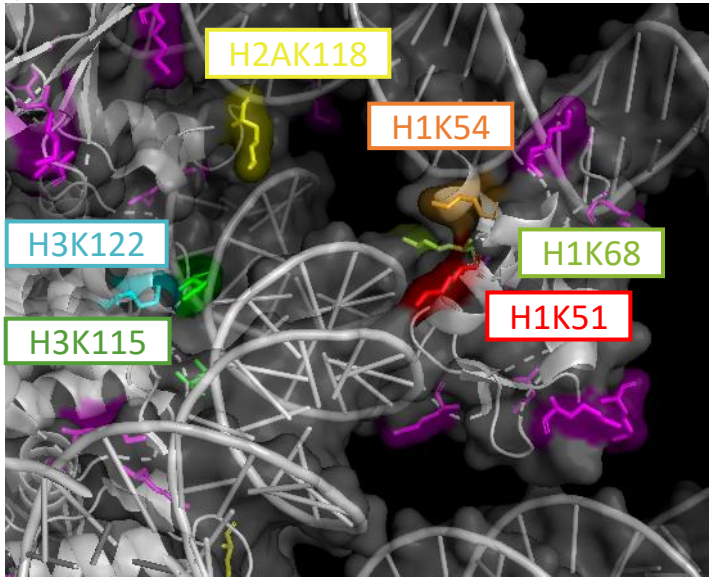
# Lysine residues near the dyad: H2AK118, H3K115, H3K122








90° 90°




(hH1.0 and chromatosome)
(PDB: 7k5x)

# PTMs of H2AK118, H2AK119, H3K115, H3K122

**H2AK118:** acetylation, methylation, ubiquitination, crotonylation, 2-hydroxyisobutyrylation, β-hydroxybutyrylation

**H2AK119:** ubiquitination, crotonylation, β-hydroxybutyrylation

**H3K115:** acetylation, butyrylation

**H3K122:** acetylation, methylation, succinylation, crotonylation, malonylation, butyrylation, 2-hydroxyisobutyrylation, β-hydroxybutyrylation

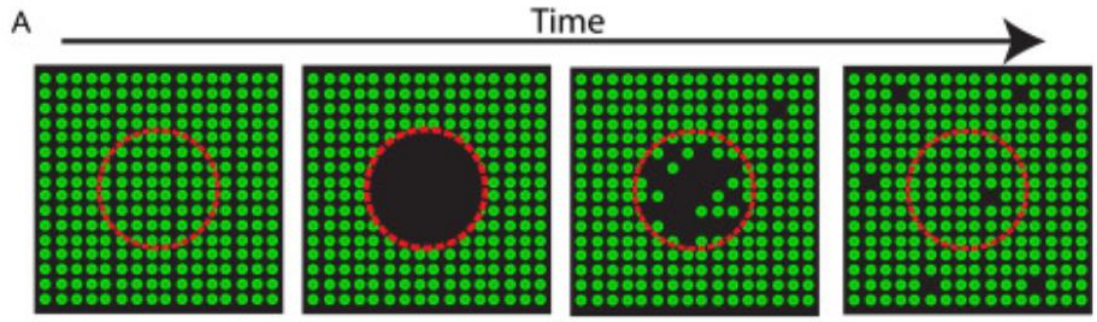
Liu, J. *et. al., Journal of Cellular and Molecular Medicine,* **2021,** *25*(15), 7101-7109

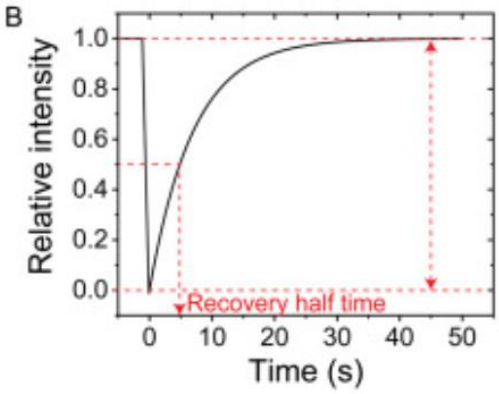
# Contents

# FRAP (Fluorescence Recovery After Photobleaching)

mouse

## The concept of FRAP experiment




Shorter recovery half time (t50) → weaker binding affinity

## Sequence alignment of the mGH1 (GD of mouse H1) (20-97)

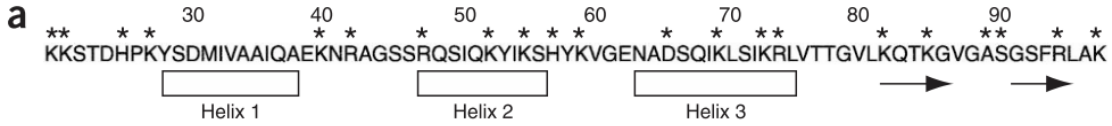
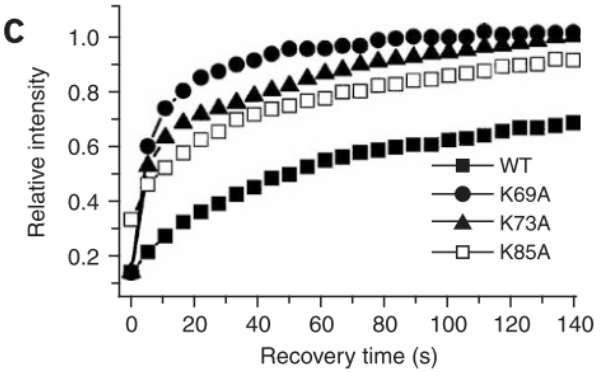



**Figure 1** FRAP analysis of mutant and wild-type (WT) H1-GFP. (**a**) Sequence of the globular domain of $H1^0$. Residues are numbered from the initiator methionine to allow direct comparison to the H5 sequence. Asterisks indicate amino acids mutated in this study. Boxes and arrows below the sequence indicate α-helices and β-sheets, respectively, as determined from the crystal structure of H5 (ref. 21).

(**c**,**d**) Quantitative FRAP analysis of stable transfectants expressing H1-GFP constructs containing single mutations in putative binding residues.

## FRAP (WT: mH1-GFP) (mouse BALB/c 3T3 cells)

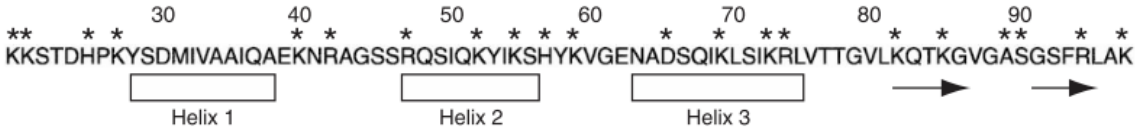



Brown, D. T. *et. al., Nature Structural and Molecular Biology,* **2006,** *13*(3), 250-255

# Definition of two distinct binding sites of mouse GH1

mouse

## Sequence alignment of the mGH1 (GD of mouse H1) (20-97)




## FRAP (WT: mH1-GFP) (mouse BALB/c 3T3 cells)

**Table 1 Quantitative FRAP analysis of mutant H1 constructs**

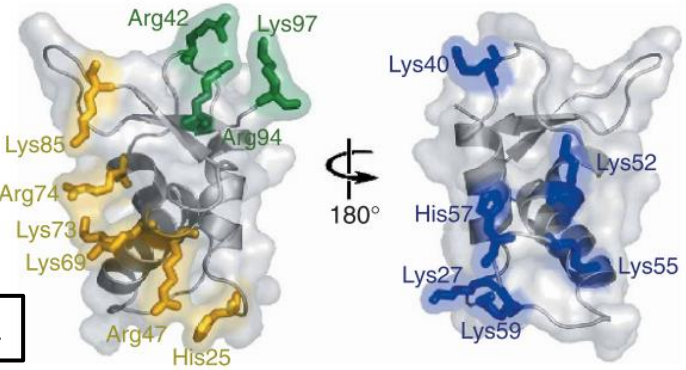
| Genotype | $t_{50}$ (sec)[a] | $t_{80}$ (sec)[a] | Genotype | $t_{50}$ (sec) | $t_{80}$ (sec) |
|---|---|---|---|---|---|
| Wild type | 52 ± 3.2 | 270 ± 7.3 | D65K[b] | 8 ± 0.3 | 46 ± 2.4 |
| Site 1 | 1 ± 0.3 | 6 ± 1.1 | K55D D65K[b] | 47 ± 3.6 | 189 ± 12.5 |
| Site 2 | 1 ± 0.4 | 8 ± 1.4 | H57A | 34 ± 2.8 | 169 ± 11.6 |
| S12 | <1 | 4.2 ± 0.8 | H57E | 28 ± 2.6 | 170 ± 10.8 |
| K20A | 37 ± 3.1 | 160 ± 5.2 | K59A | 37 ± 4.5 | 142 ± 8.2 |
| K21A | 38 ± 3.4 | 159 ± 6.5 | K59D | 37 ± 3.4 | 139 ± 9.6 |
| H25G | 12 ± 0.8 | 53 ± 2.3 | E62H | 76 ± 5.7 | 280 ± 10.2 |
| H25E | 10 ± 1.2 | 47 ± 4.1 | K69A | 4 ± 0.3 | 22 ± 1.6 |
| H25K | 32 ± 4.3 | 190 ± 8.6 | K69R | 23 ± 3.2 | 119 ± 7.5 |
| K27T | 53 ± 4.2 | 208 ± 12.1 | K73A | 8 ± 0.4 | 40 ± 2.8 |
| K40A | 39 ± 2.8 | 152 ± 9.5 | K73E | 4 ± 0.3 | 20 ± 3.1 |
| K40E | 34 ± 2.5 | 132 ± 9.1 | K73R | 49 ± 3.9 | 201 ± 11.9 |
| R42A | 16 ± 1.5 | 65 ± 3.2 | R74A | 14 ± 1.8 | 50 ± 3.1 |
| R42E | 10 ± 1.1 | 35 ± 2.2 | K82V | 63 ± 4.6 | 208 ± 12.7 |
| R42K | 45 ± 3.3 | 181 ± 9.2 | Q83D | 4 ± 0.3 | 17 ± 2.3 |
| R47A | 5 ± 0.2 | 28 ± 1.2 | K85A | 10 ± 1.1 | 72 ± 5.7 |
| R47E | 2 ± 0.3 | 20 ± 2.1 | K85E | 4 ± 0.3 | 18 ± 1.9 |
| R47K | 22 ± 2.2 | 121 ± 8.7 | K85R | 49 ± 4.2 | 197 ± 8.6 |
| R47L | 8 ± 0.5 | 41 ± 3.1 | A89D | 3 ± 0.2 | 19 ± 3.1 |
| K52A | 28 ± 1.6 | 120 ± 7.3 | S90D | 3 ± 0.2 | 21 ± 2.7 |
| K55A[b] | 12 ± 1.2 | 52 ± 3.8 | R94A | 17 ± 1.4 | 56 ± 2.6 |
| K55E[b] | 3 ± 0.2 | 20 ± 1.7 | R97A | 20 ± 1.3 | 84 ± 3.8 |
| K55D[b] | 5 ± 0.2 | 24 ± 1.3 | | | |

[a]Values for $t_{50}$ and $t_{80}$ were determined as previously described[24]. [b]See **Supplementary Figure 1**.

(exception: K55)

## Map of the interaction surface of mGH1

Site 2 (flexible domain, linker DNA)




Site 1 (inflexible domain, dyad DNA)

**Figure 4** Map of the interaction surface of $H1^0$ based on data from **Table 1**. Yellow, binding residues in site 1; green, site 2; blue, nonbinding residues.

Nine positively charged residues mutants:

t50 < 20 s and t80 < 90 s

Brown, D. T. *et. al., Nature Structural and Molecular Biology,* **2006,** *13*(3), 250-255

# Definition of two distinct binding sites of *drosophila* GH1

*drosophila*

## Sequence alignment of the dGH1 (GD of *drosophila* H1)


## ITC (Isothermal Titration Calorimetry)

A

| H1 | $K_D$(μM) |
|---|---|
| $H1_{1-256}$ (WT) | 0.29 ± 0.02 |
| $H1_{1-256}$ | 0.28 ± 0.02 |
| $H1_{37-211}$ | 0.21 ± 0.02 |
| H46A | 0.38 ± 0.07 |
| K58A | 0.83 ± 0.08 |
| K61A | 0.46 ± 0.05 |
| R63A | 0.37 ± 0.05 |
| K72A | 0.36 ± 0.03 |
| K73A | 0.21 ± 0.03 |
| K80A | 0.37 ± 0.04 |
| K85A | 0.30 ± 0.03 |
| K91A | 0.7 ± 0.2 |
| K92A | 0.20 ± 0.02 |
| K95A | 0.83 ± 0.09 |
| K102A | 0.56 ± 0.08 |
| K107A | 0.55 ± 0.05 |
| K109 | 0.39 ± 0.04 |
| K116A | 0.58 ± 0.09 |

Except the WT $H1_{1-256}$, all others include quadruple mutations. Additional single mutations are based on $H1_{37-211}$.

D_gH1_mut $^{45}$SHPPTQQMIDAAIKNLKERGGSSLLAIKKYITATYKVDAQKLAPFIKKYLKSLVVNGKLIQTKGKGASGSFKLS$^{118}$

M_gH1$^{0}$ $^{24}$DHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYKVGENADSQ-IKLSIKRLVTTGVLKQTKGVGASGSFRLA$^{76}$

## Map of the interaction surface of dGH1

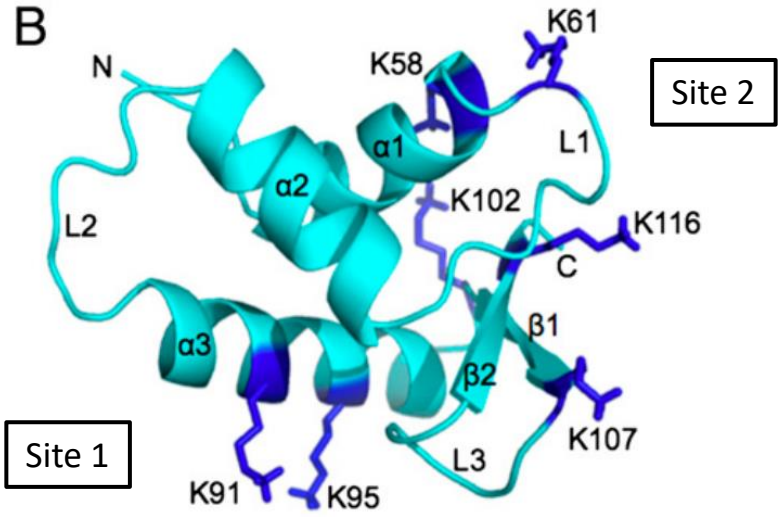



**Fig. 4.** Effects of mutations in gH1 on the binding affinity of $H1_{37–211}$ to the nucleosome. (*A*) Effects of mutation of surface residues in gH1 on the binding affinity between $H1_{37–211}$ and the nucleosome. (*B*) Structural illustration of the distribution of the gH1 residues whose Ala mutations lead to a large decrease in binding affinity.

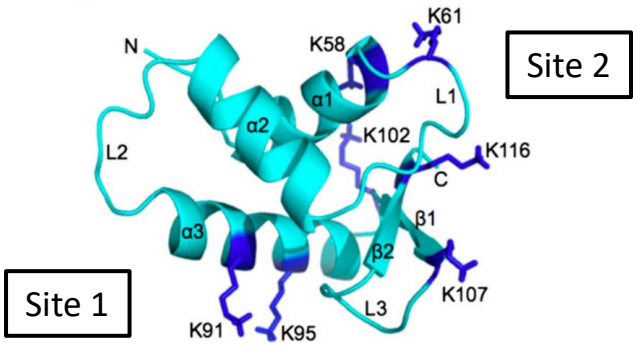
Zhou, B. R. *et. al., PNAS,* **2013,** *110*(48), 19390-19395

# Proposed binding mode model (Off-dyad binding mode)

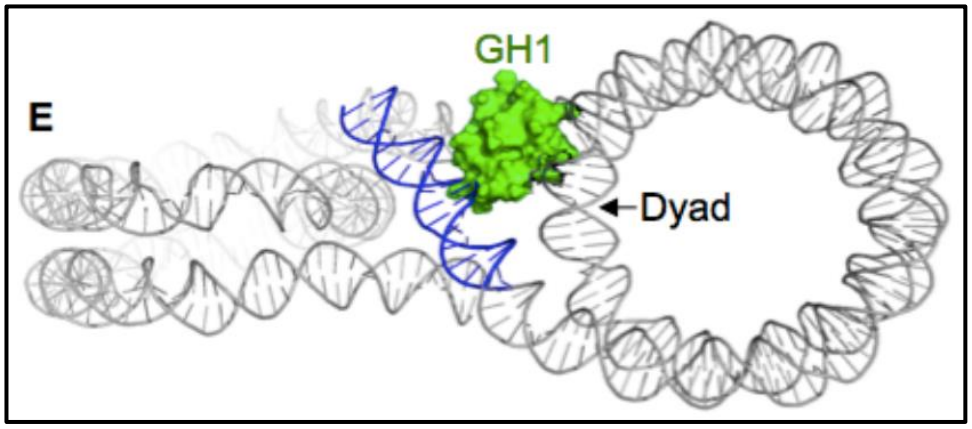
*drosophila*

## Sequence alignment of the GH1 (GD of *drosophila* H1)


## Map of the interaction surface of dGH1




## Off-dyad binding mode (model)




(using HADDOCK program)

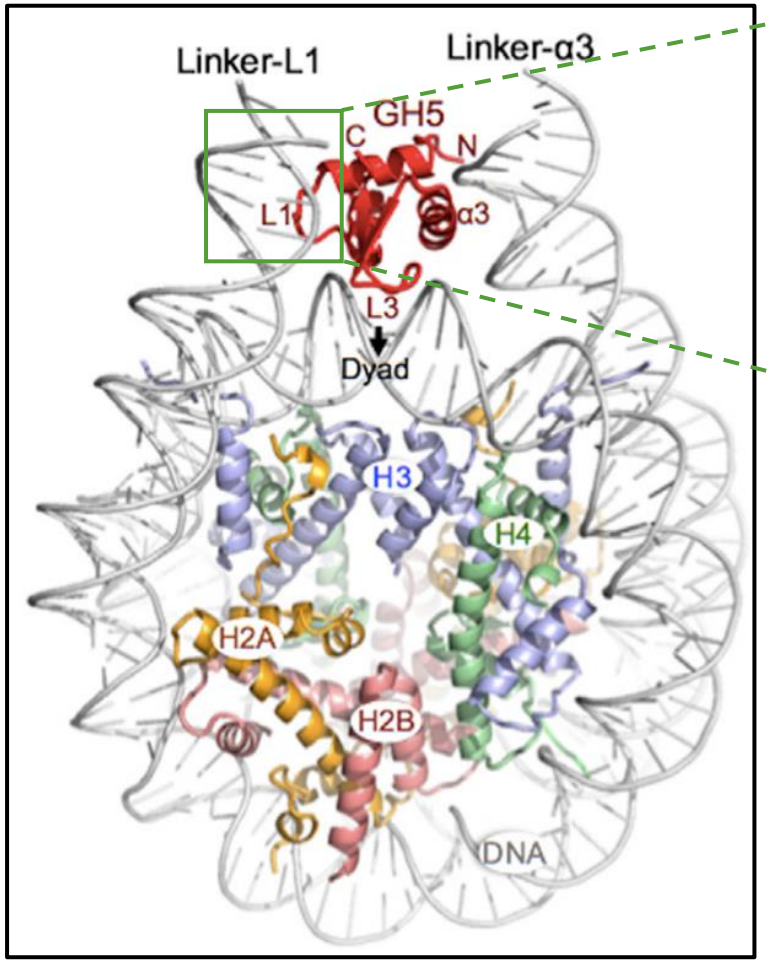
- **K91 and K95 were forced to interact with the nucleosomal DNA near the dyad. (①)**
- **K58, K102, K107, and K116 were forced to interact with the nearby linker DNA. (②)**
- **①, ② → docking calculation**
- **It is possible that GH1 may interact with both DNA linkers, one strongly and the other weakly.**

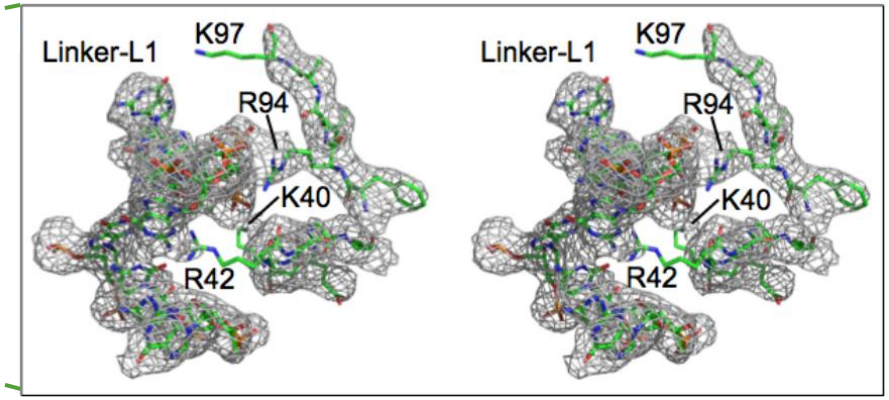
Zhou, B. R. *et. al., PNAS,* **2013,** *110*(48), 19390-19395

# Contents

# Detailed interactions between GH5 and linker-L1

chicken




(3.5 Å resolution)

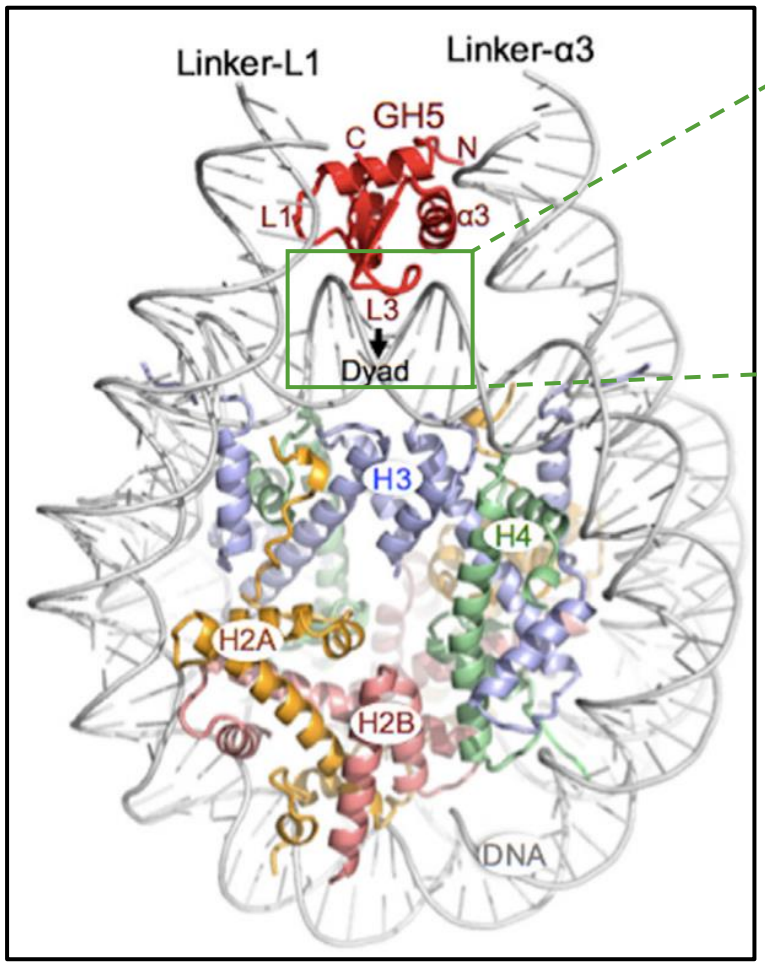
- **Crystal structure is consistent with on-dyad binding mode.**
- **R94 has stable interaction with the linker-L1.**
- **K40, R42, and K97 have substantial local dynamic motions.**

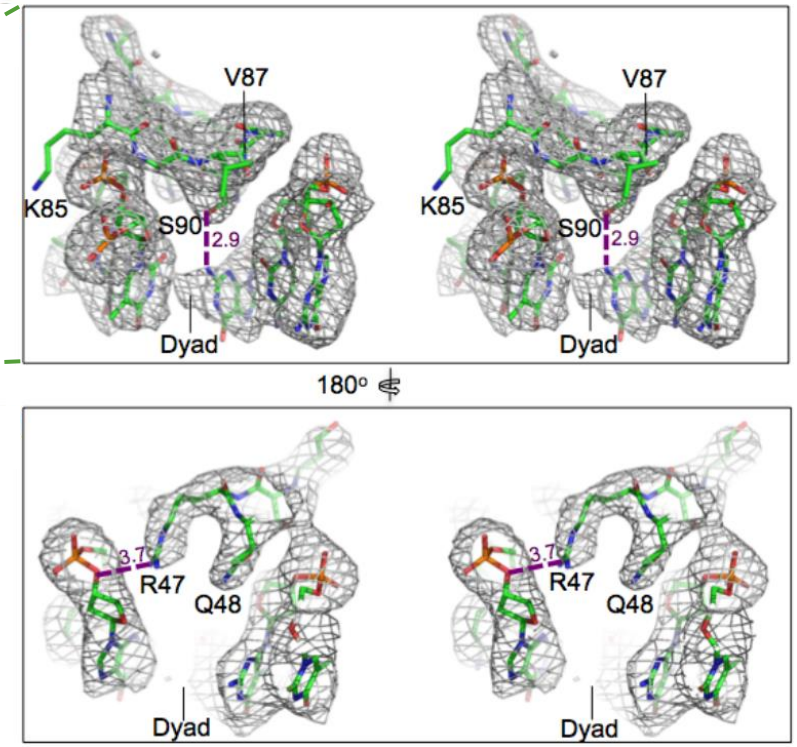
GH5: GD of chicken linker histone H1 (H5)

Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

# Detailed interactions between GH5 and the DNA at the dyad

chicken



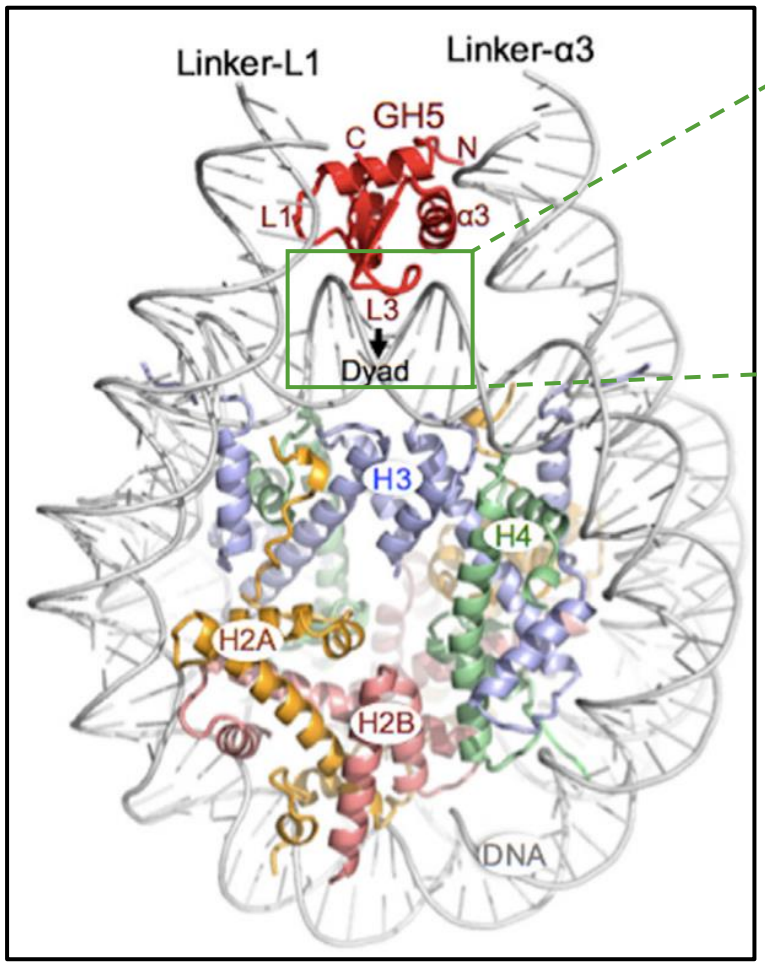



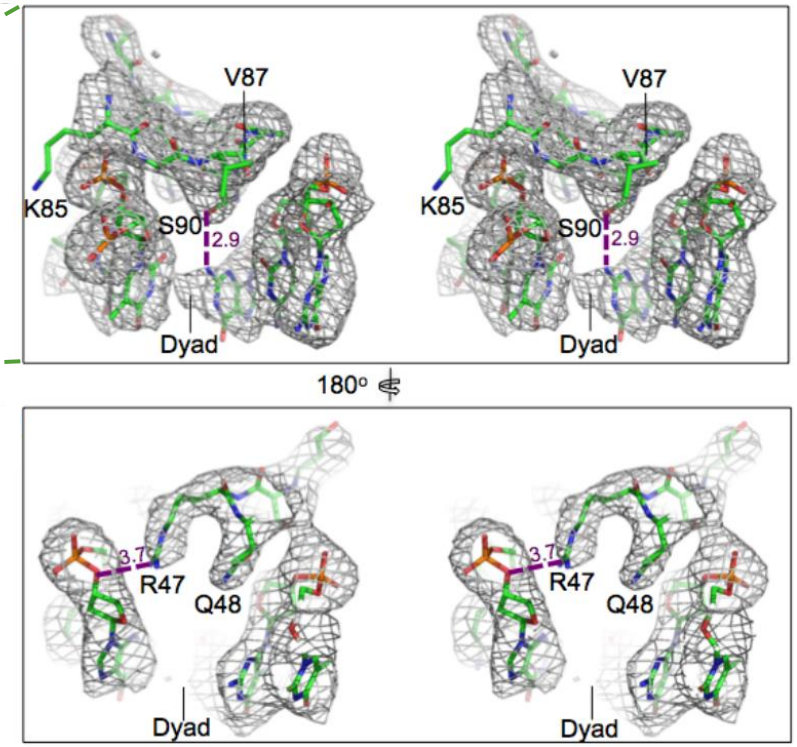

- **R47 has stable interaction with the dyad.**
- **K85 has substantial local dynamic motions.**

Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

# Detailed interactions between GH5 and the DNA at the dyad

chicken



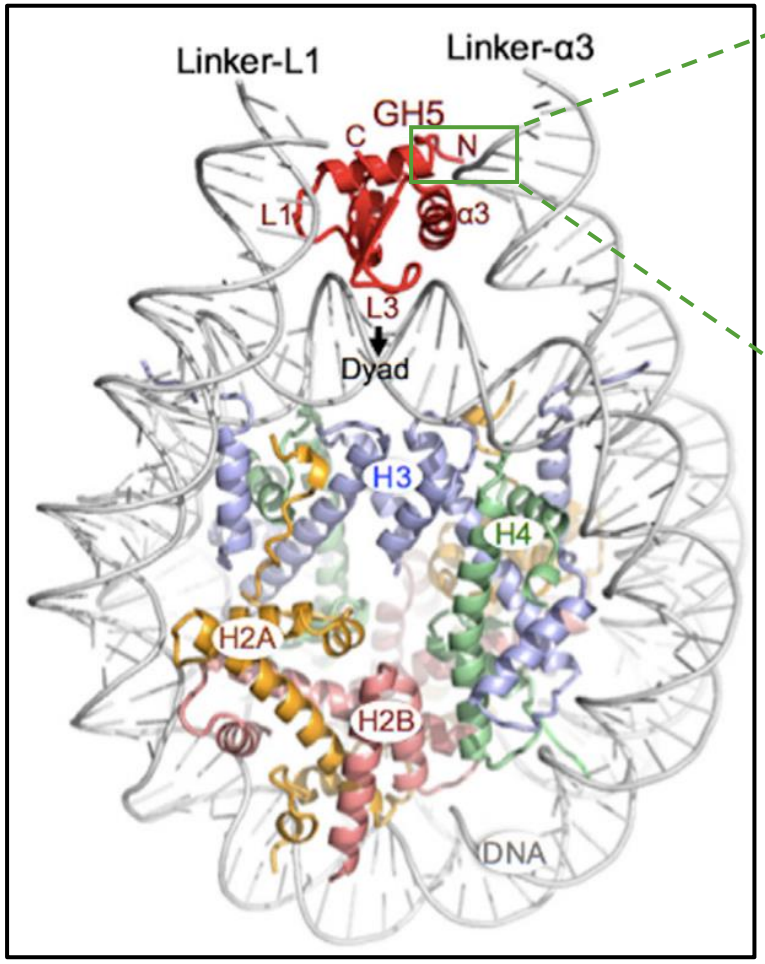



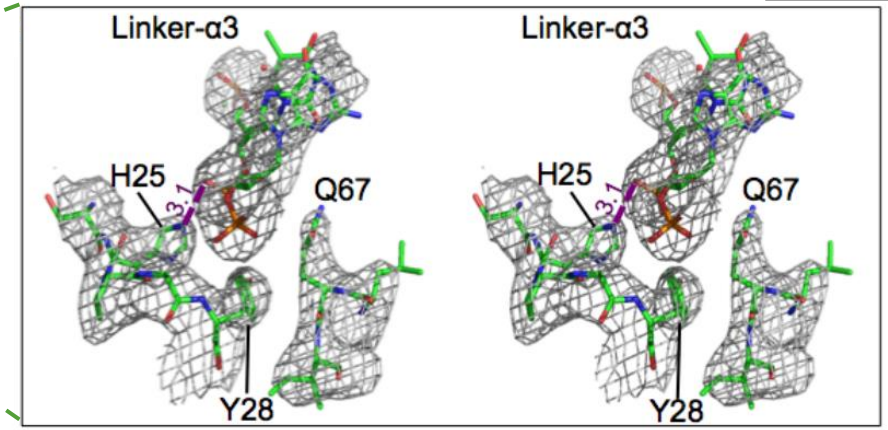

- **The charge-neutral residues Q48, V87, and S90 insert into the minor groove of the DNA at the dyad**

Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

# Detailed interactions between GH5 and linker-α3

chicken



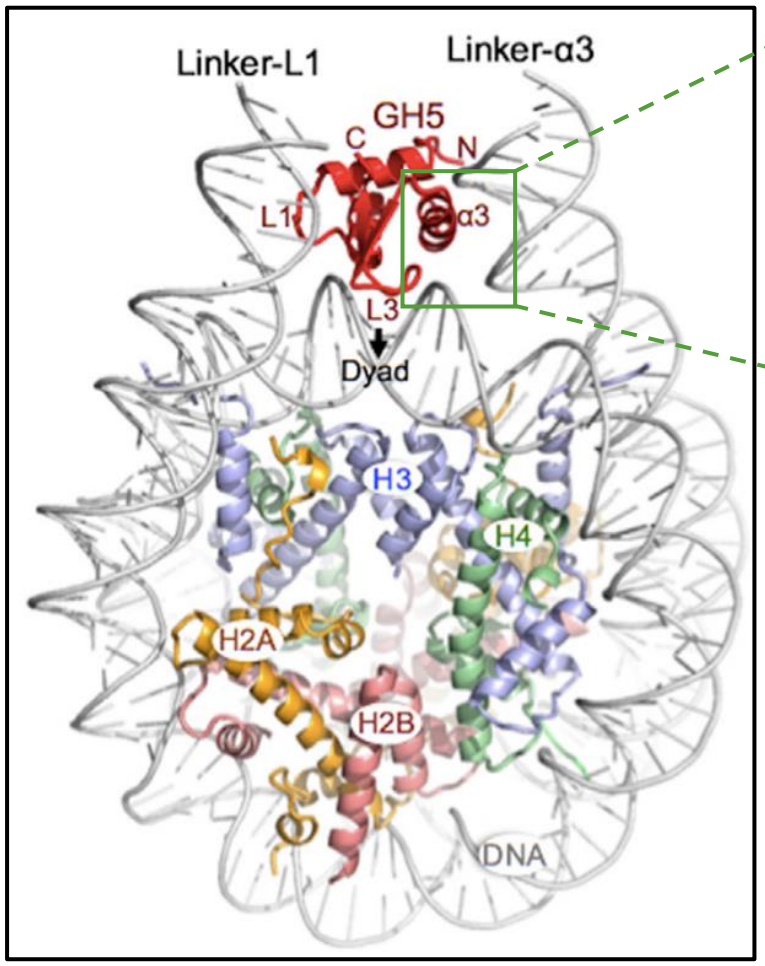



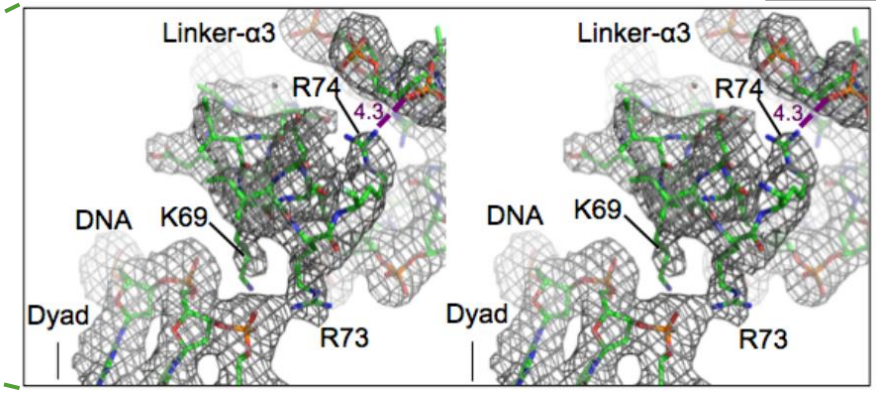

- **H25, Y28, Q67 are in close contact with the linker-α3**

Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

# Detailed interactions between GH5 and DNA near the dyad and linker-α3

chicken

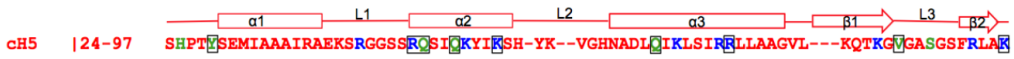


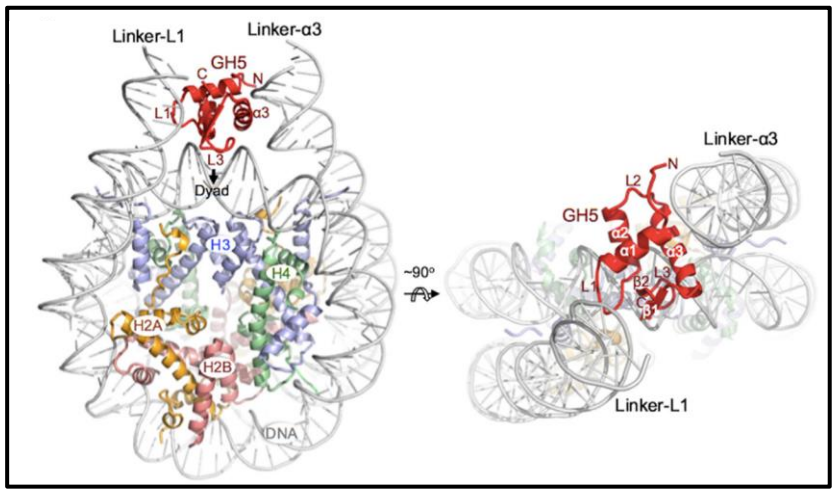




- **R73, R74 have stable interaction with the dyad and linker-α3.**
- **K69 has substantial local dynamic motions.**

Zhou, B. R. *et. al., Molecular Cell,* **2015,** *59*(4), 628-638

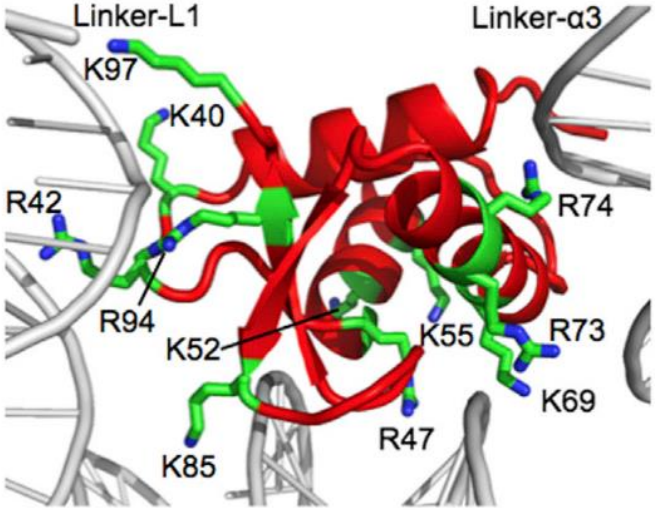
# Short summary of Detailed interactions between GH5 and nucleosome



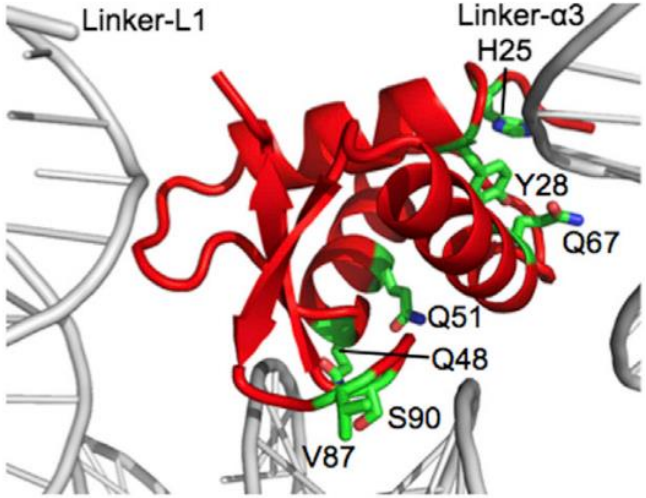




## Positively charged GD residues

chicken




## Non-charged GD residues




**Interactions between GH5 residues and nucleosome**

**Linker-L1**: K40(L1), R42(L1), R94(β2), K97(β2-CTD)

**Dyad**: R47(α2), Q48(α2), K85(L3), V87(L3), S90(L3)

**Linker-α3**: H25(NTD-α1), Y28(α1), Q67(α3)

**Dyad and linker-α3**: K69(α3), R73(α3), R74(α3)

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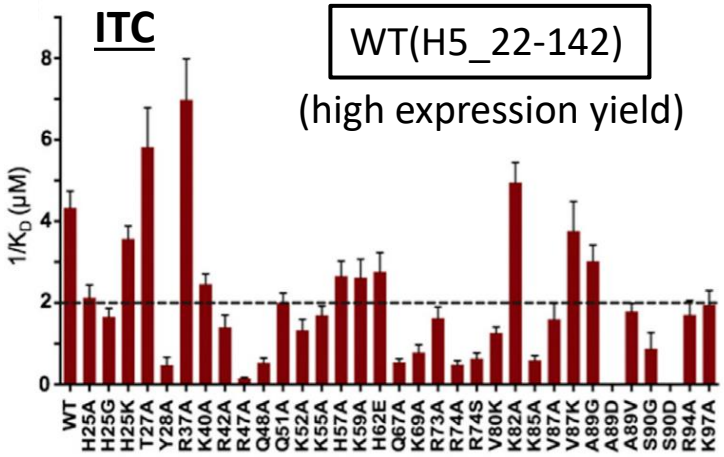
# Effects of mutations in the GD on the binding affinity of H5 to the nucleosome

**ITC**

WT(H5_22-142)

(high expression yield)

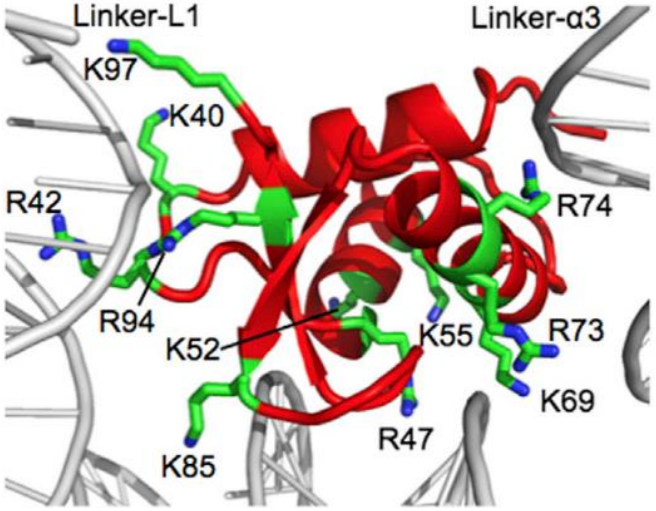



| Construct | $K_D$ (μM) | Construct | $K_D$ (μM) |
|---|---|---|---|
| H5 (22_102)[a] | 0.35 ± 0.05 | H62E | 0.36 ± 0.06 |
| H5 (22_142)[a] | 0.23 ± 0.02 | Q67A | 1.8 ± 0.3 |
| H25A | 0.47 ± 0.07 | K69A | 1.3 ± 0.3 |
| H25G | 0.60 ± 0.07 | R73A | 0.6 ± 0.1 |
| H25K | 0.28 ± 0.02 | R74A | 2.0 ± 0.4 |
| T27A | 0.17 ± 0.03 | R74S | 1.6 ± 0.3 |
| Y28A | 2 ± 1 | V80K | 0.8 ± 0.1 |
| R37A | 0.14 ± 0.02 | K82A | 0.20 ± 0.02 |
| K40A | 0.41 ± 0.04 | K85A | 1.7 ± 0.3 |
| R42A | 0.7 ± 0.2 | V87A | 0.6 ± 0.2 |
| R47A | 7 ± 1 | V87K | 0.27 ± 0.05 |
| R47A/Q48L/S49A | 4 ± 1 | A89G | 0.33 ± 0.04 |
| Q48A | 2 ± 0.4 | A89D | ND |
| Q51A | 0.50 ± 0.06 | A89V | 0.56 ± 0.06 |
| K52A | 0.8 ± 0.2 | S90G | 1.1 ± 0.5 |
| K55A | 0.59 ± 0.07 | S90D | ND |
| H57A | 0.37 ± 0.05 | R94A | 0.6 ± 0.1 |
| K59A | 0.38 ± 0.07 | K97A | 0.5 ± 0.1 |

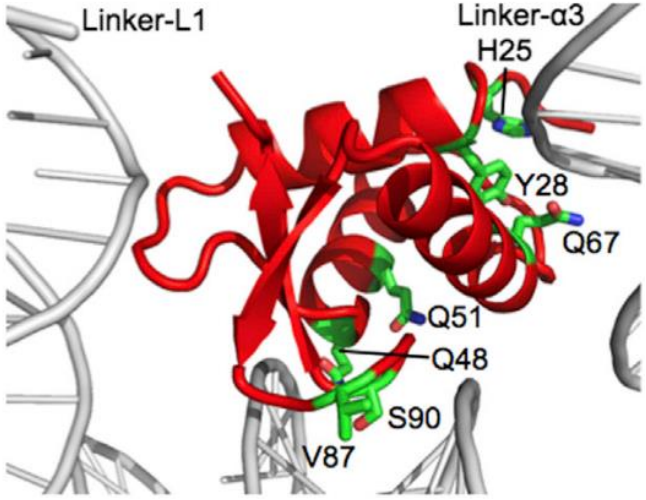
[a]For $H5_{22–102}$, n = 0.96 ± 0.02 and ΔH = 3.20 ± 0.06 kcal/mol. For $H5_{22–142}$, n = 0.71 ± 0.01 and ΔH = 5.22 ± 0.07 kcal/mol. n is the stoichiometry and ΔH is the binding enthalpy. All mutations are based on $H5_{22–142}$. The nucleosome used in the experiment includes 167 bp DNA.

chicken

**Positively charged GD residues**




**Non-charged GD residues**

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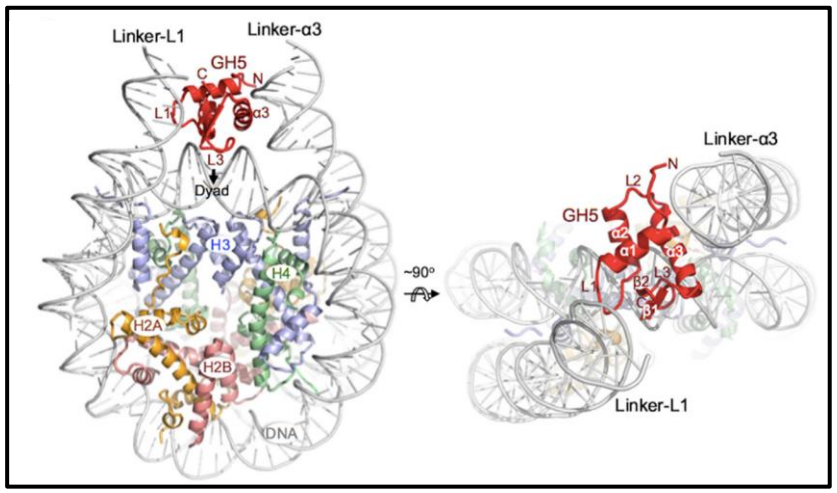
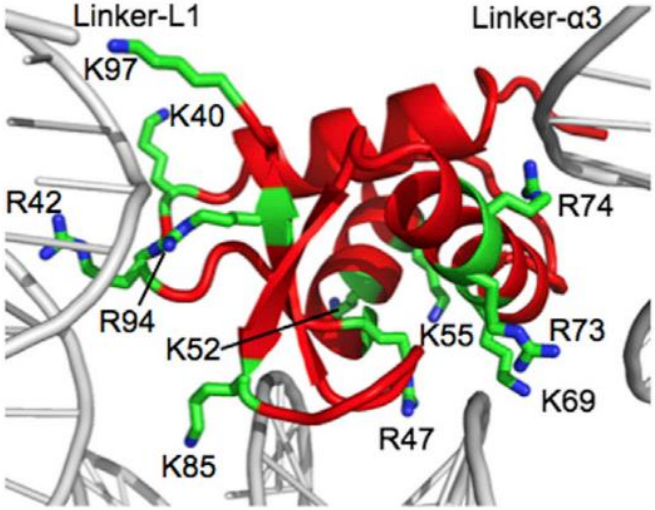
# Short summary of Detailed interactions between GH5 and nucleosome

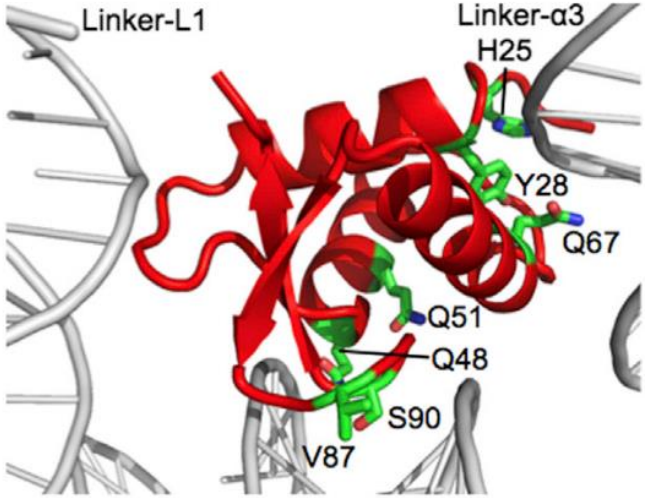




## Positively charged GD residues

chicken




## Non-charged GD residues




## Interactions between GH5 residues and nucleosome

Linker-L1: K40(L1), R42(L1), R94(β2), K97(β2-CTD)

Dyad: **R47(α2), Q48(α2), K85(L3),** V87(L3), **S90(L3)**

Linker-α3: H25(NTD-α1), **Y28(α1), Q67(α3)**

Dyad and linker-α3: **K69(α3),** R73(α3), **R74(α3)**

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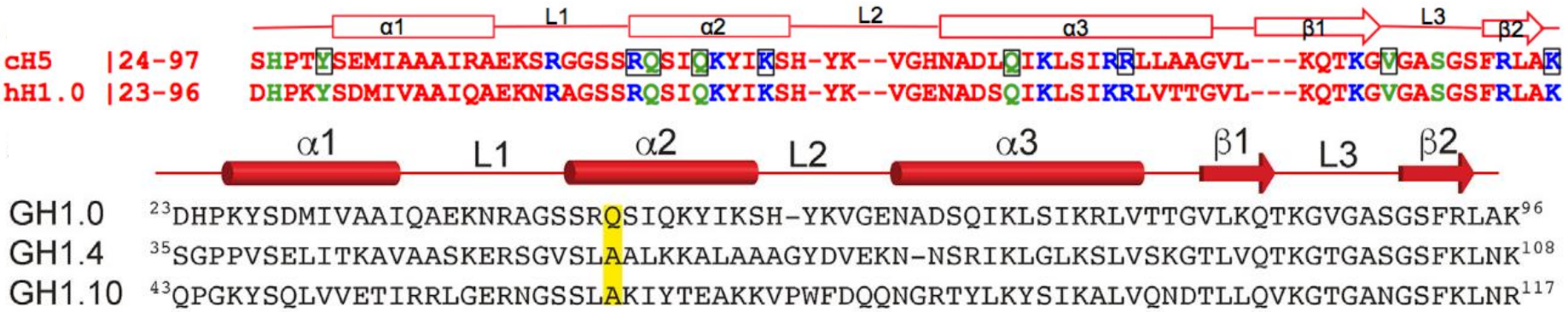
# Contents

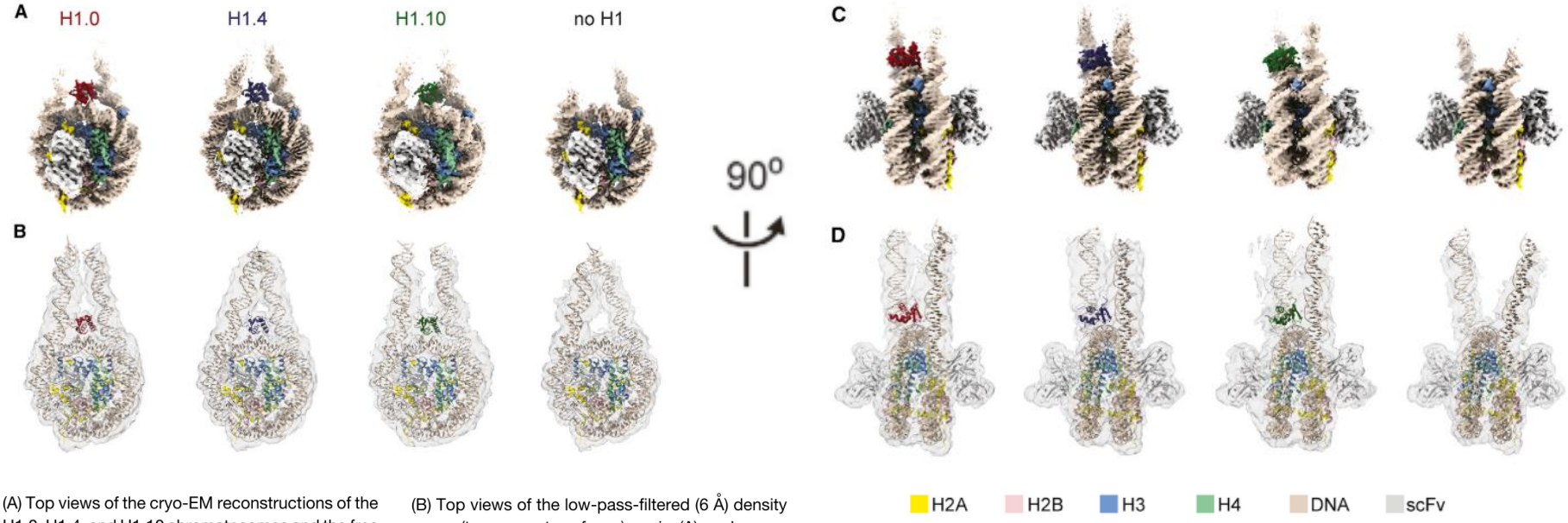
# Cryo-EM structures are consistent with on-dyad binding mode.

human

## Sequence alignment of the linker histone globular domains




## (A, C) Cryo-EM (2.8 Å to 3.1 Å), (B, D) low-pass-filtered (6 Å) density map




(A) Top views of the cryo-EM reconstructions of the H1.0, H1.4, and H1.10 chromatosomes and the free nucleosome.

(B) Top views of the low-pass-filtered (6 Å) density maps (transparent surfaces) as in (A) and corresponding atomic structural models.

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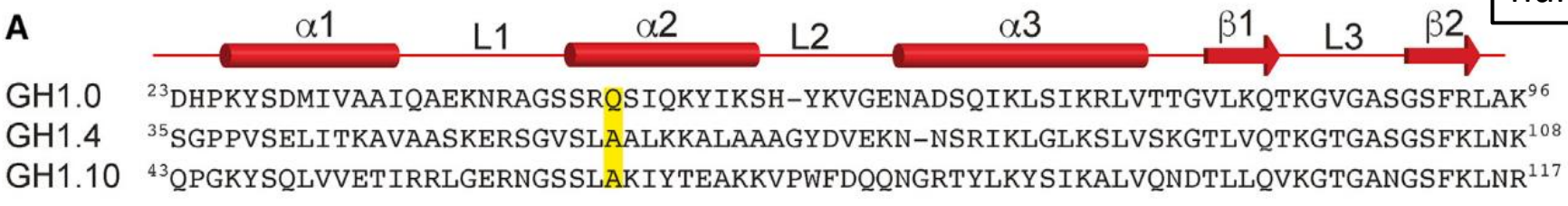
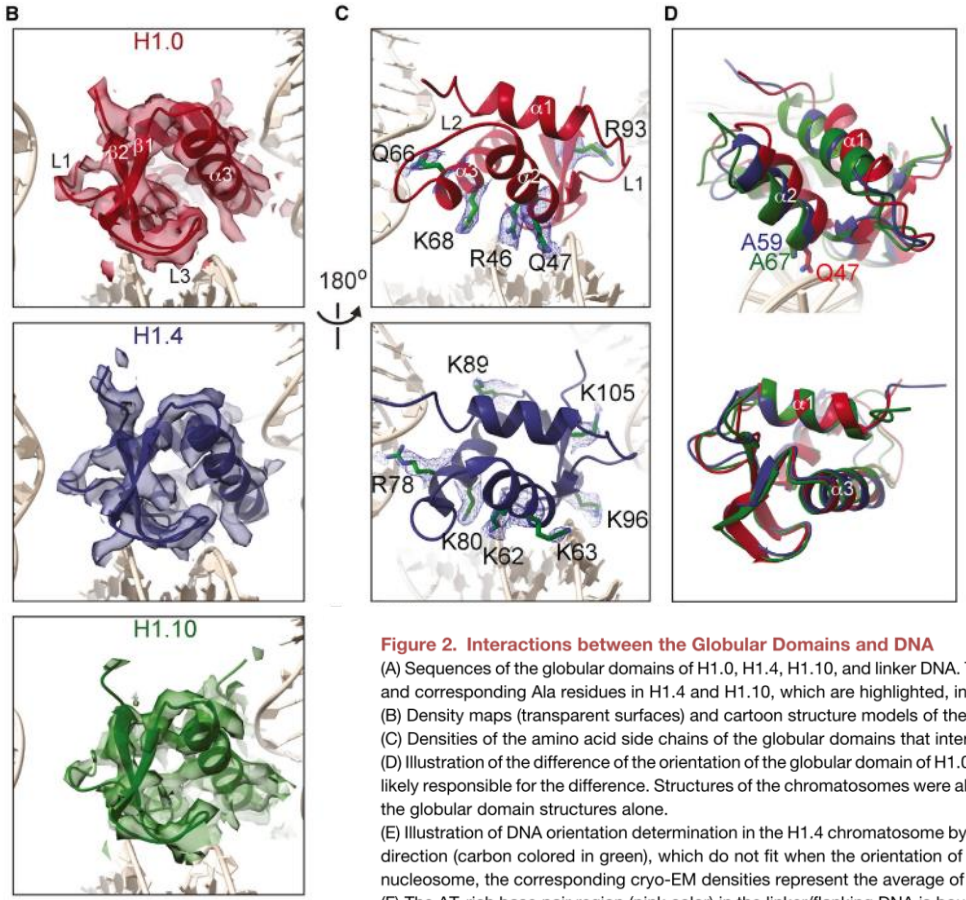
# Interactions between DNA and the GDs of isoforms

human




Even though the structures of the H1 GDs were similar, interactions between H1 GDs and nucleosome are different (→ next page)

**Figure 2. Interactions between the Globular Domains and DNA**

(A) Sequences of the globular domains of H1.0, H1.4, H1.10, and linker DNA. The diagram on the top shows the secondary structures. The residue Gln47 in H1.0 and corresponding Ala residues in H1.4 and H1.10, which are highlighted, interact with dyad DNA.

(B) Density maps (transparent surfaces) and cartoon structure models of the globular domains.

(C) Densities of the amino acid side chains of the globular domains that interact with the nucleosomal and linker DNAs.

(D) Illustration of the difference of the orientation of the globular domain of H1.0 relative to those of H1.4 and H1.10 in the chromatosomes and the residues that are likely responsible for the difference. Structures of the chromatosomes were aligned on core histones in the top panel. The bottom panel showed the alignment of the globular domain structures alone.

(E) Illustration of DNA orientation determination in the H1.4 chromatosome by the fitting of cryo-EM densities with DC-95:DG-103 and DC-99:DG-99 pairs in one direction (carbon colored in green), which do not fit when the orientation of the DNA is reversed (carbon colored in gold) (left). In contrast, in the case of free nucleosome, the corresponding cryo-EM densities represent the average of the two positions from opposite DNA orientations (right).

(F) The AT-rich base pair region (pink color) in the linker/flanking DNA is bound by the α3 helix through residues Arg78 of H1.4.

See also Figures S1 and S2.

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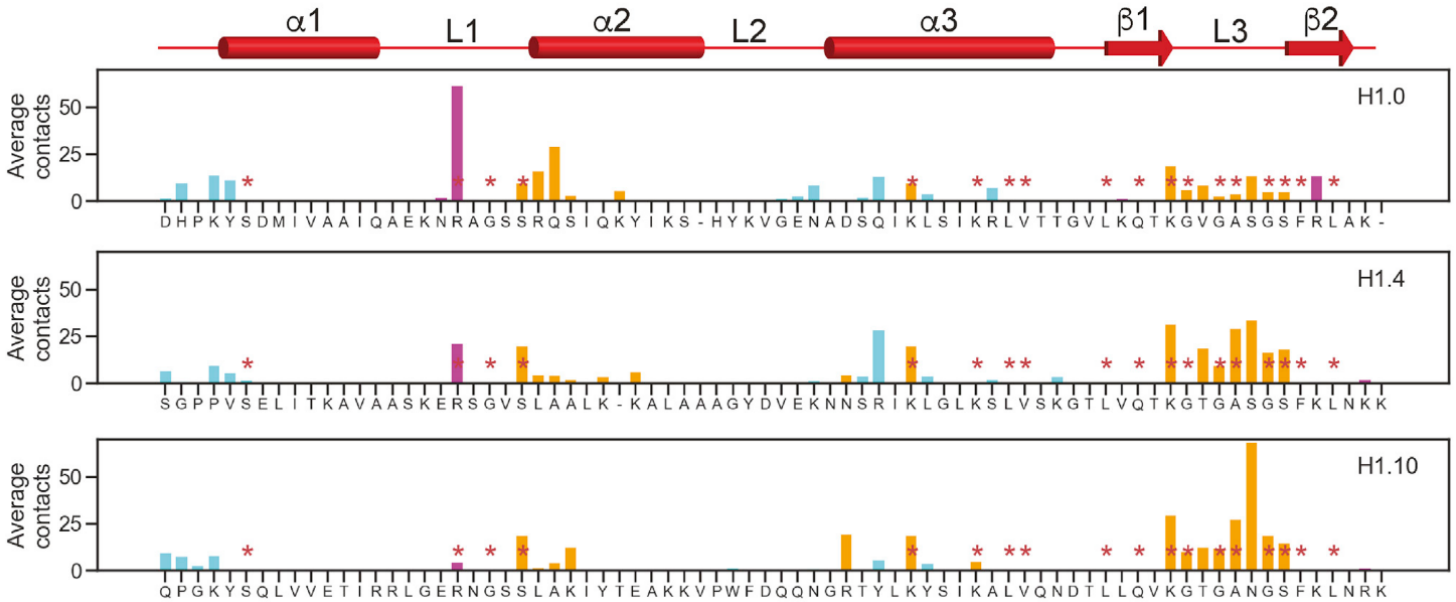
# Variant GDs redistribute interactions with the nucleosomal and linker DNA.

human

**The number of contacts between the GD and DNA averaged over MD trajectories**




the distance between a heavy atom in the globular domain is within 5 Å of a heavy atom from DNA.

- the majority of contacts occurred between the residues from the NT of the α2 helix and the CT of the L3 loop in the GH1 and the DNA near the dyad.
- H1.10 interacts mostly with the nucleosomal DNA near the dyad region, whereas H1.0 and H1.4 globular domains have more pronounced interactions with the linker strands.

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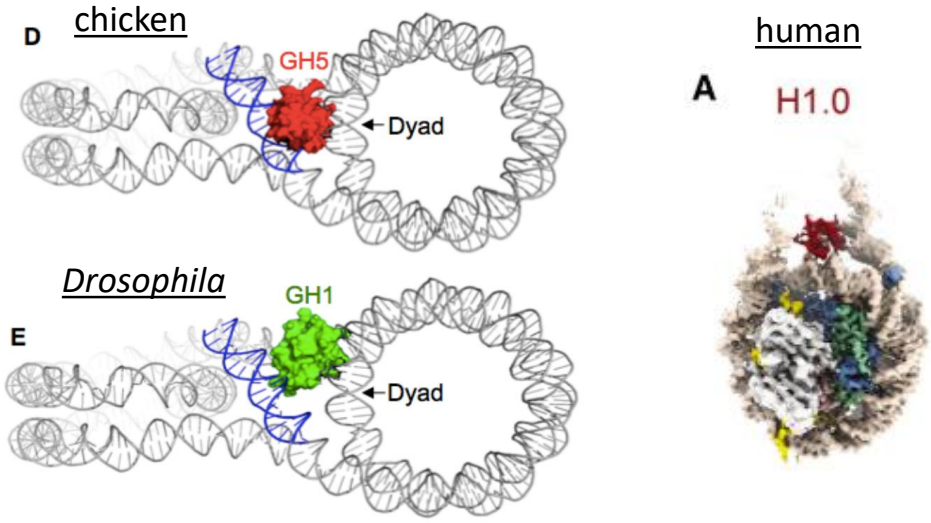
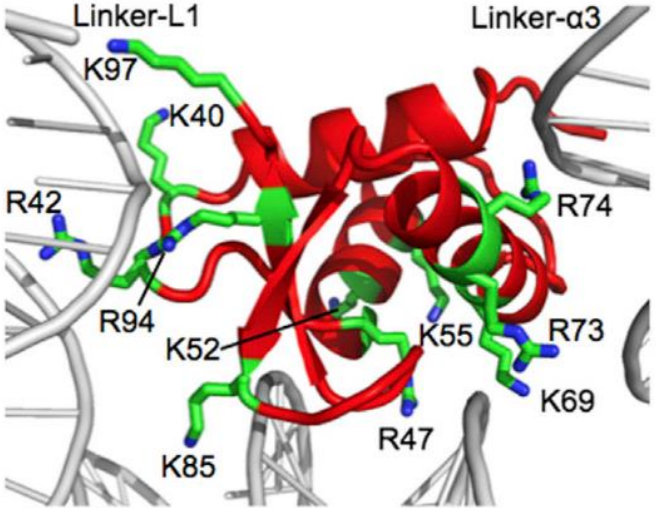
# Summary

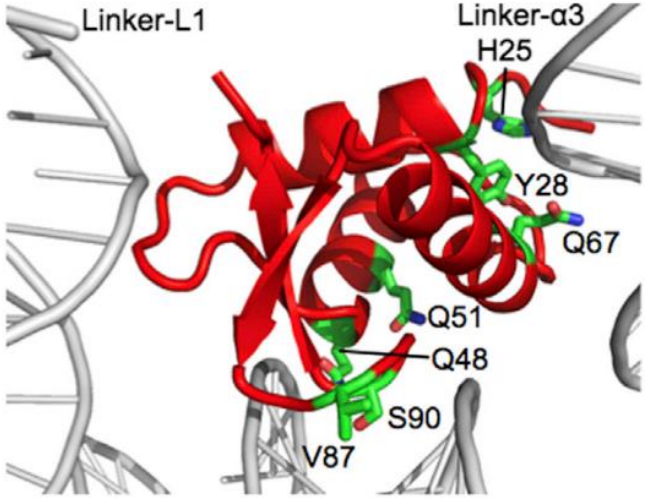



- **Different linker histone GH1 isoforms have different binding modes.**
- GH1 bind to the dyad DNA and linker-DNA.
- **GH1 can interact with both DNA linkers, one strongly and the other weakly.**
- **In addition to positively charged GD residues, Non-charged GD residues are also important to the interaction with GD and nucleosome**

## Positively charged GD residues (chicken)




## Non-charged GD residues (chicken)

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