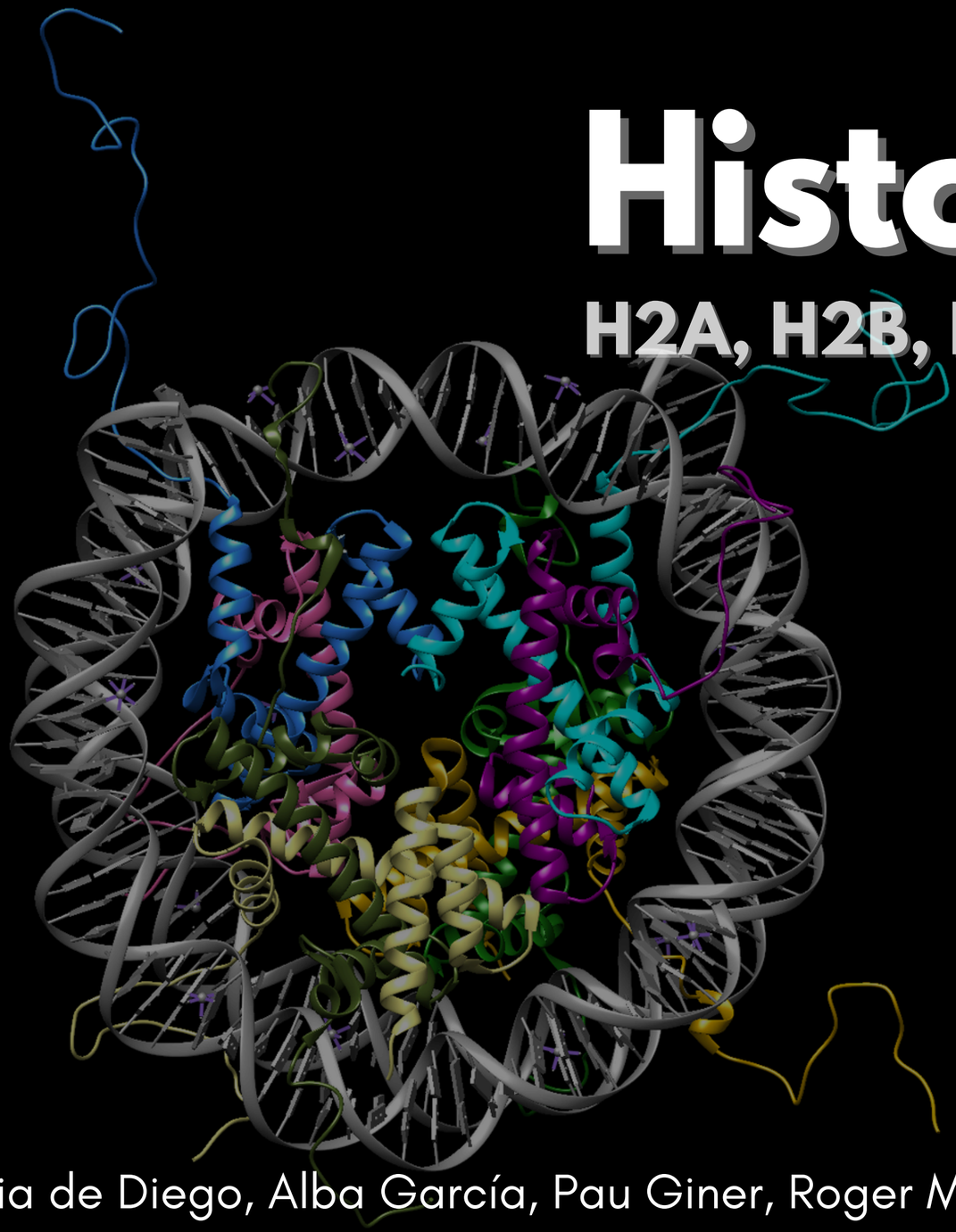
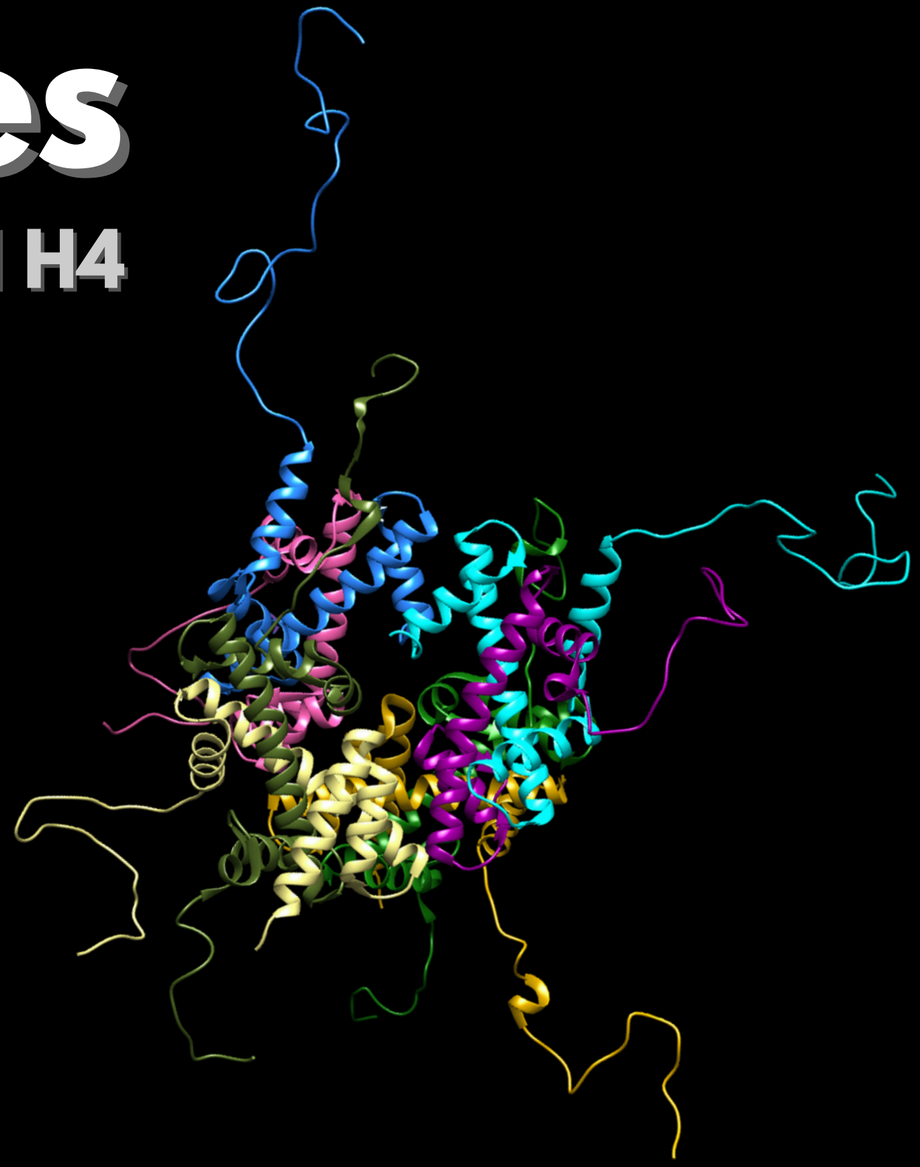
Histones

H2A, H2B, H3 and H4





Victoria de Diego, Alba García, Pau Giner, Roger Mayol

Structural Biology, UPF 25-26

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1 **Introduction**

2 **Structure**
Histone tail
Histone fold

3 **Core histones conservation**

4 **Octamer complex**
Core interactions
DNA interactions

5 **Post-translational modifications**

6 **Evolution**

Histones

- Basic proteins (Arg and Lys)
- Eukaryotic nucleus
- Highly conserved

FUNCTIONS:

- To form the nucleosome for packing DNA
- Gen regulation and DNA replication



1KX5 Homo sapiens, Xenopus laevis [1.9 Å]

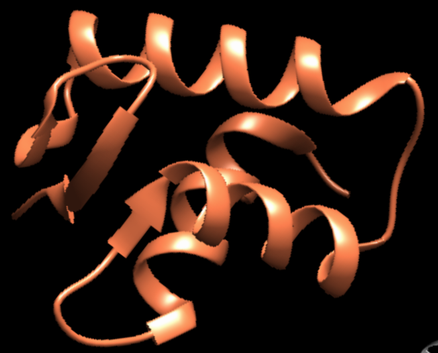
Histones

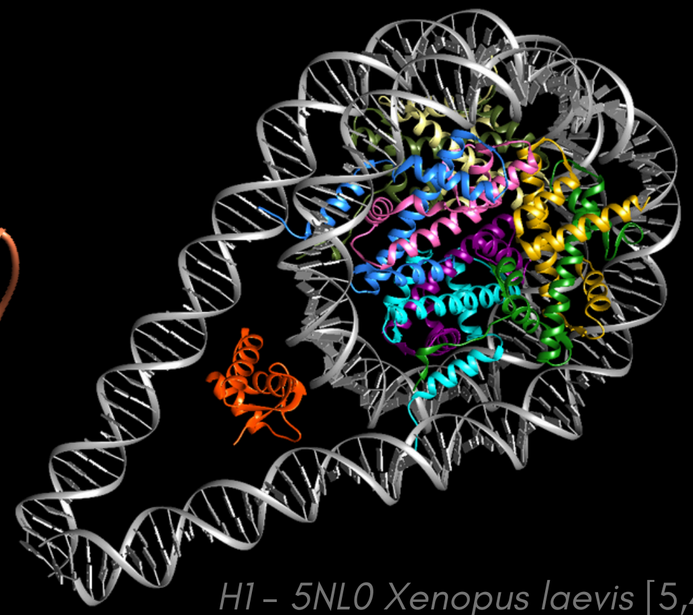
Typology

Linker histones

H1

H5 (birds and amphibians)





H1 – 5NL0 Xenopus laevis [5,4 Å]

Core histones

H2A

H2B

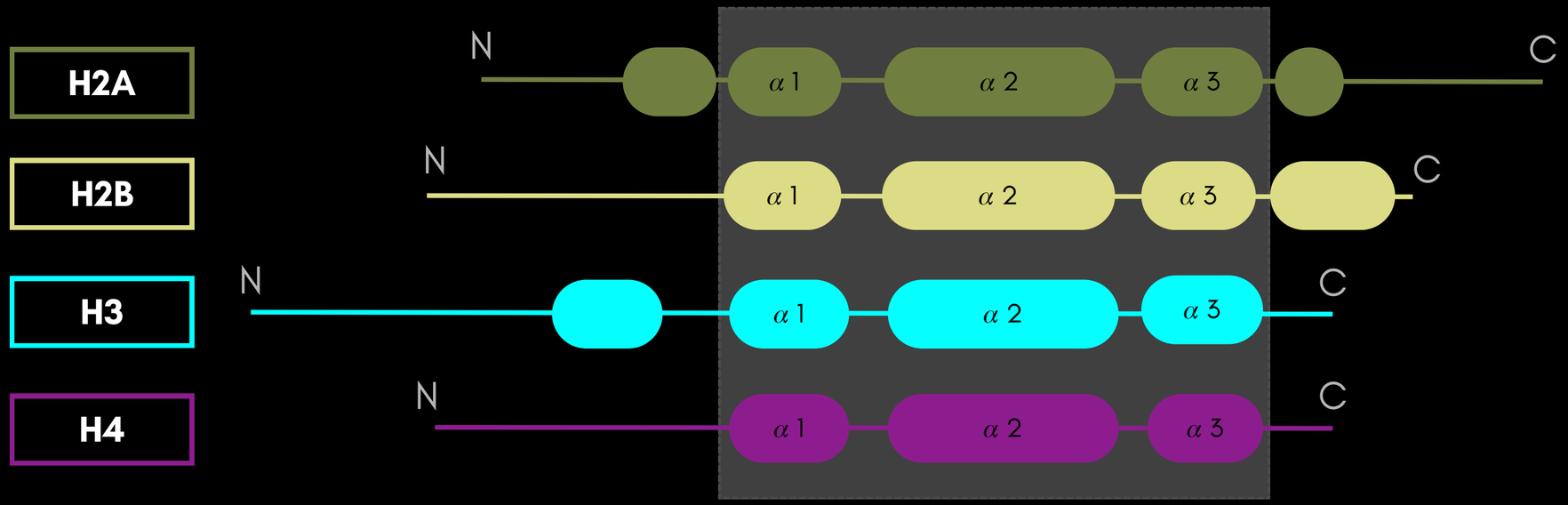
H3

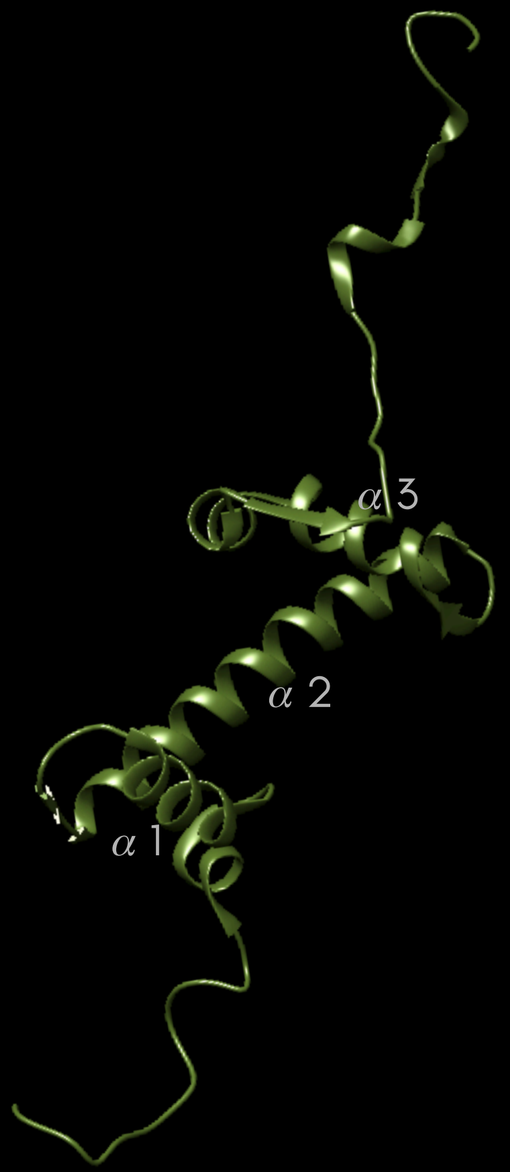
H4

Class	All alpha protein
Fold	Histone fold
Superfamily	Core histone-like
Family	Nucleosome core histone

Core histones

Preview of the structure





H2A – 1KX5 Homo sapiens, Xenopus laevis [1.9 Å]

Histone tails

- Unstructured in the absence of DNA
- Evolution conserved
- Predominance of lysines and arginine residues
- Significant amounts of glycine, alanine and threonine

DNA and Histones

H3 **N-tail**

H4 **N-tail**

H2B **N-tail**

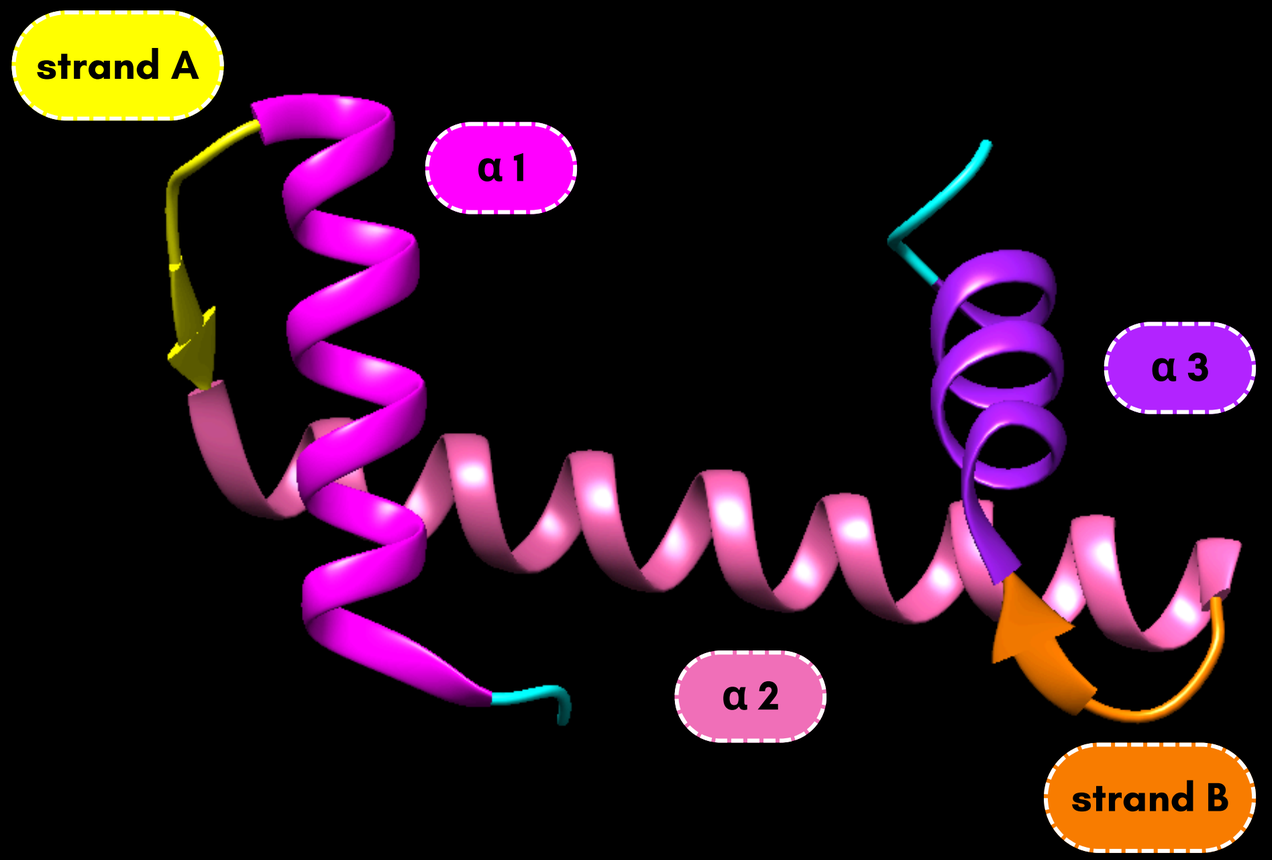
H2A **N-tail**

H2A **C-tail**

Histone fold

Secondary structure

- Short α-helix (α 1)
- Short loop and beta strand (strand A)
- Long α-helix (α 2)
- Short loop and beta strand (strand B)
- Short α-helix (α 3)



Histone fold, H3 - 1KX5 Homo sapiens, Xenopus laevis [1.9 Å]

Histone fold

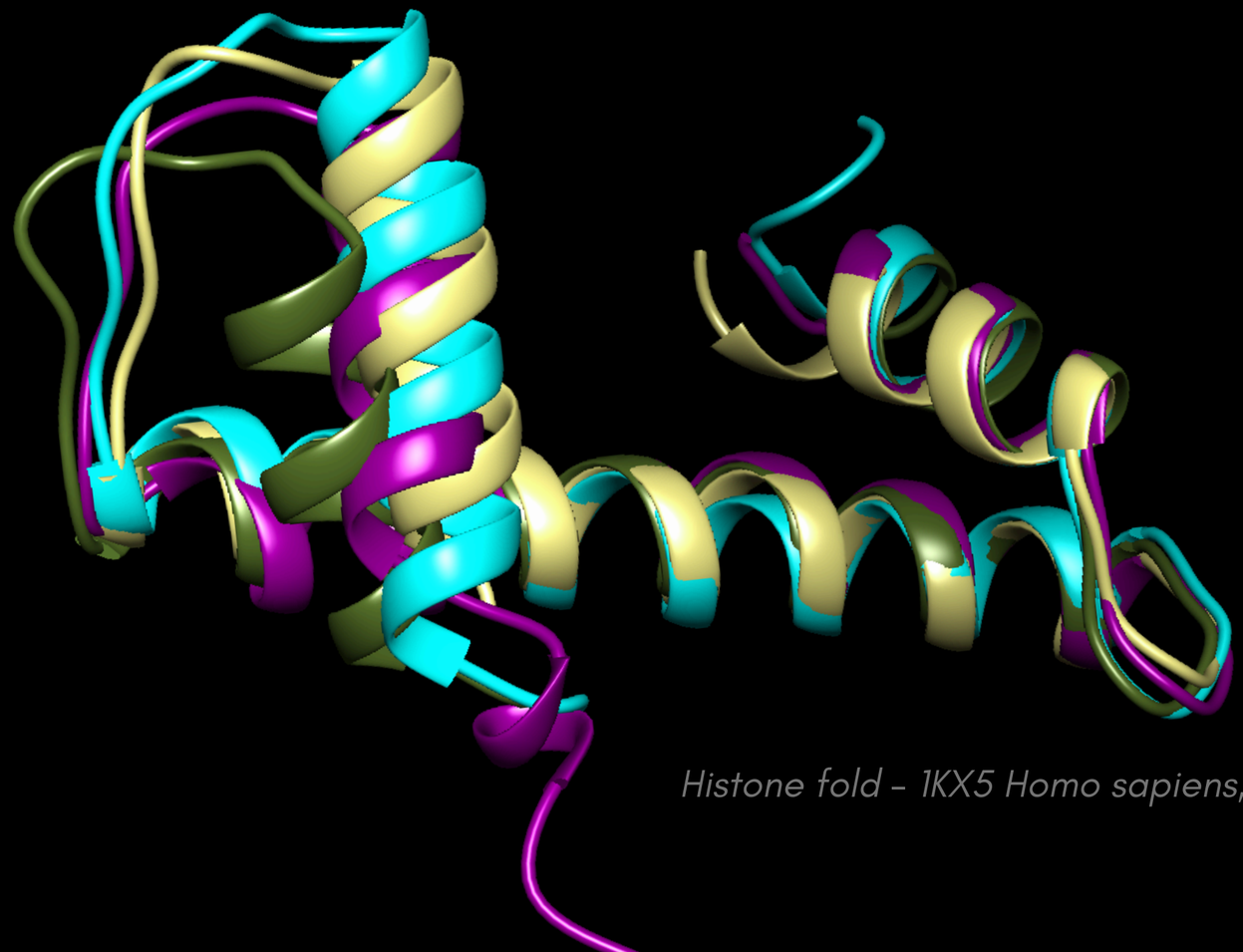
Comparison

H2A

H2B

H3

H4



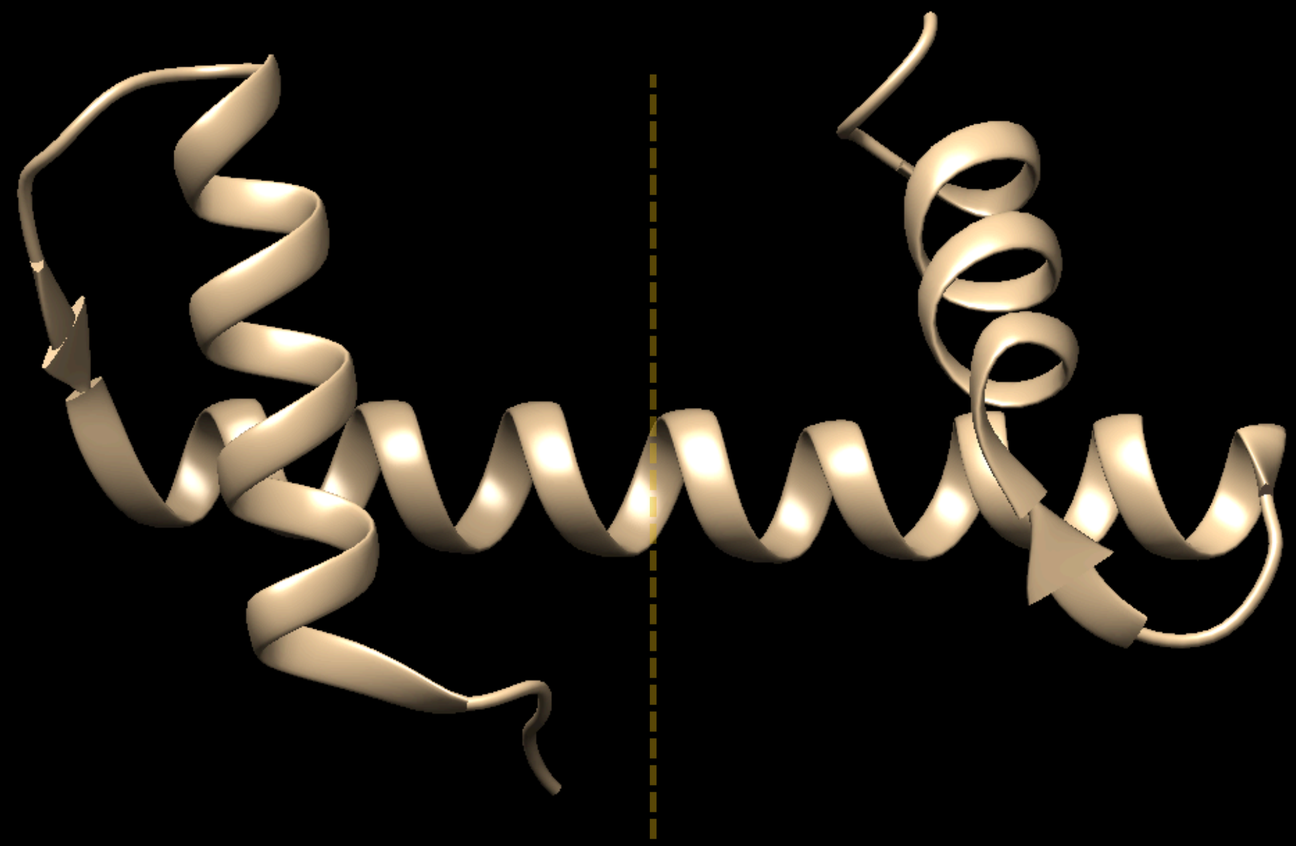
Histone fold – 1KX5 Homo sapiens, Xenopus laevis [1.9 Å]

Histone fold

HSH motifs

Helix-strand-helix

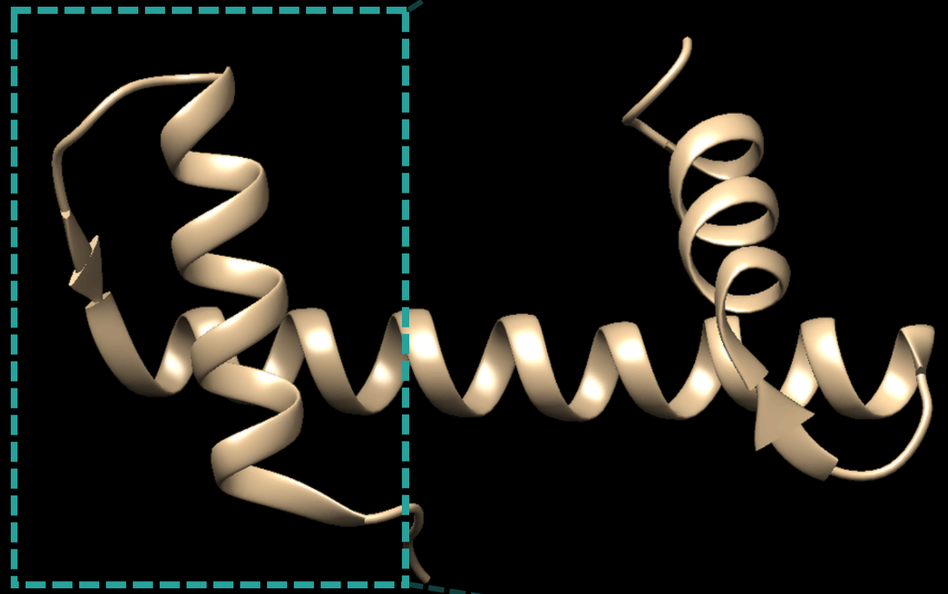
- Tandem duplication
- Gene duplication event



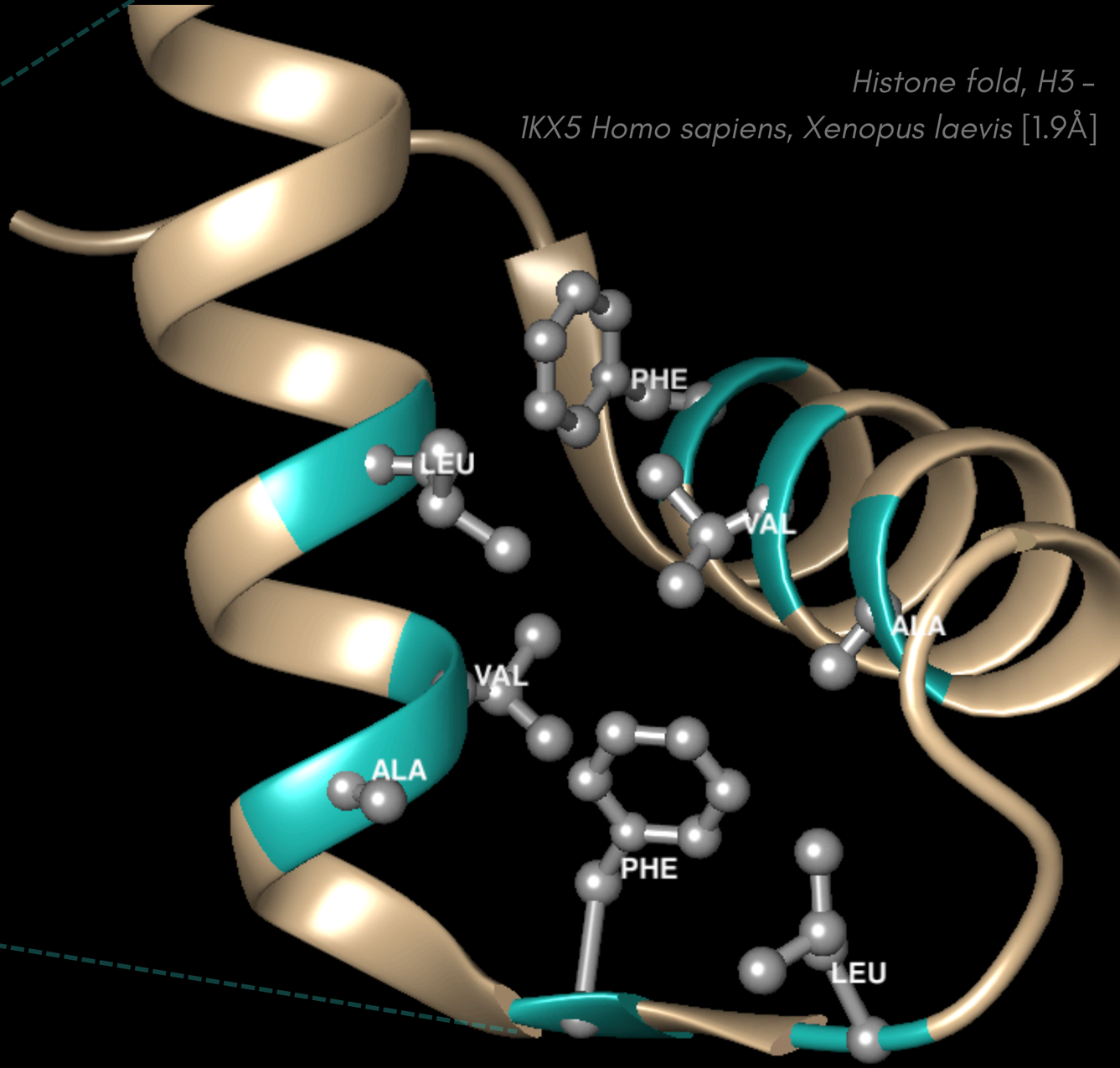
Histone fold - 1KX5 Homo sapiens, Xenopus laevis [1.9Å]

Histone fold

Spatial conformation
Hydrophobic interactions

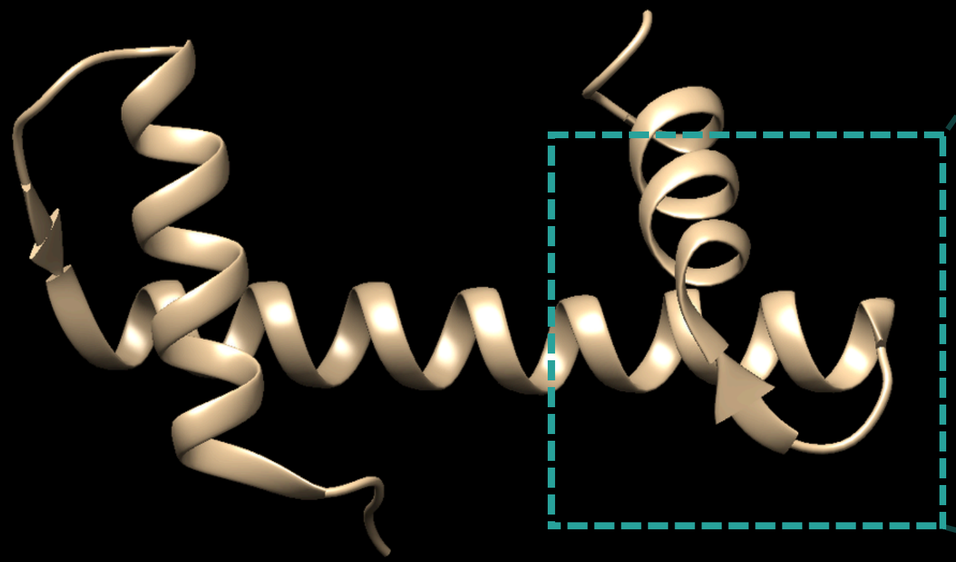


Histone fold, H3 -
1KX5 Homo sapiens, Xenopus laevis [1.9Å]

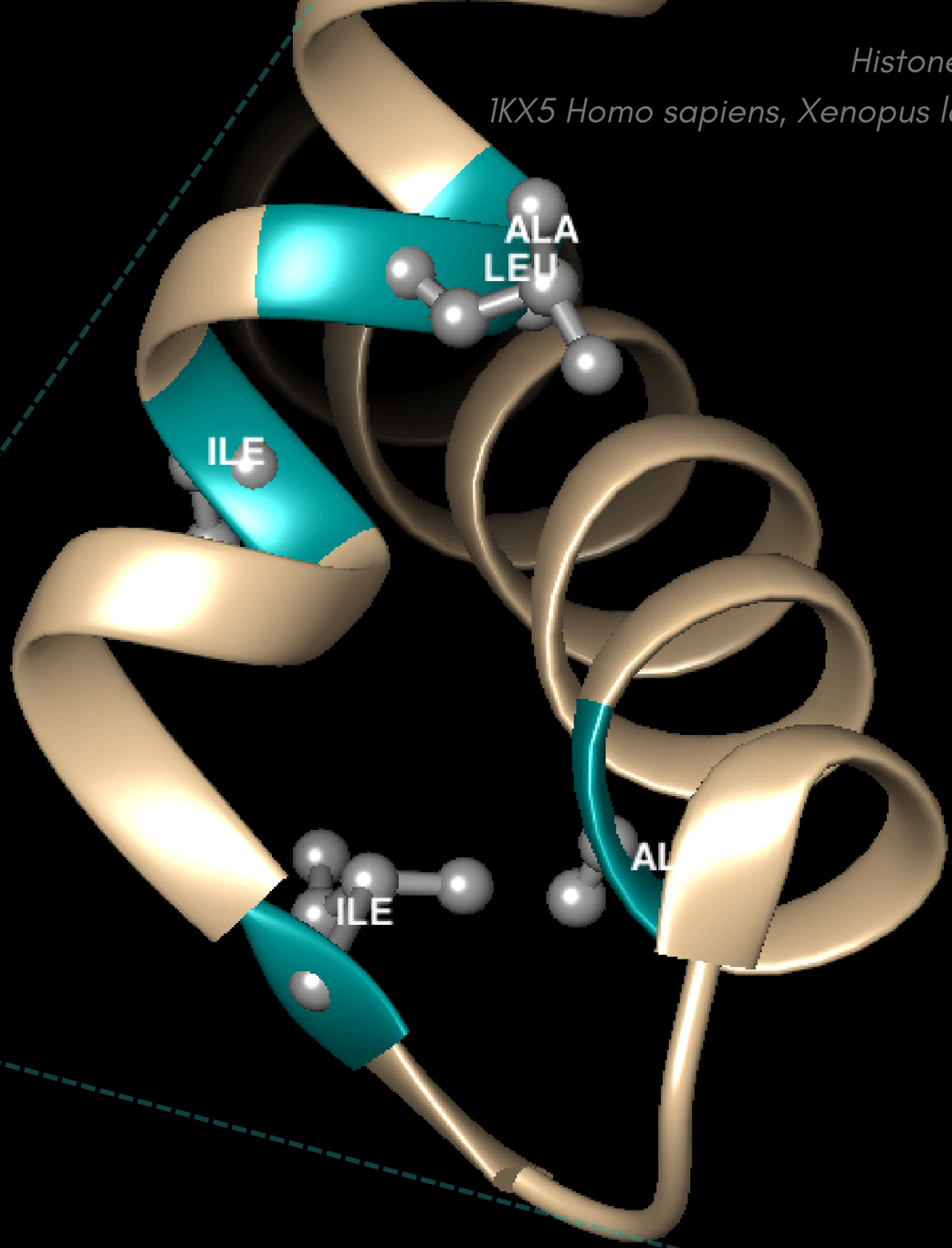


Histone fold

Spatial conformation
Hydrophobic interactions

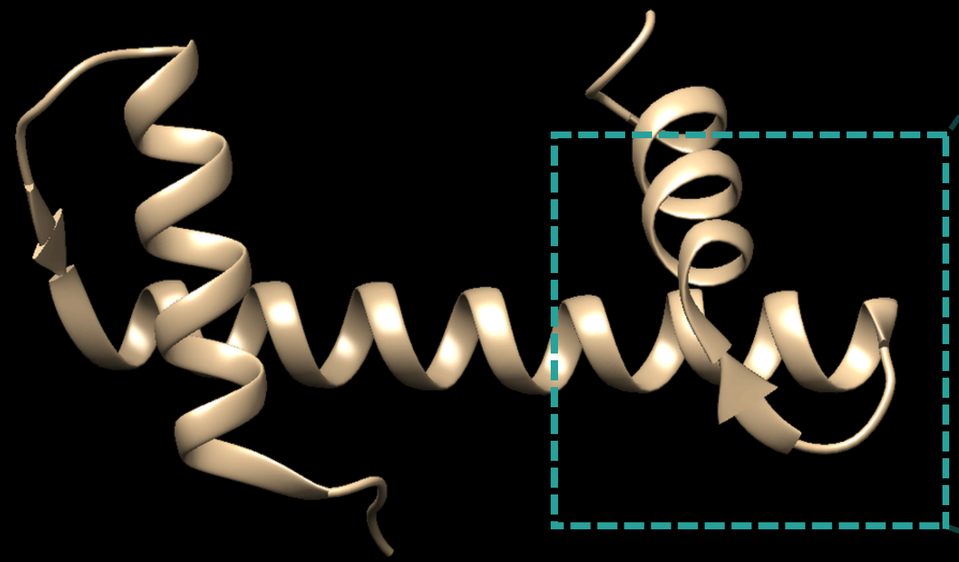


Histone fold, H3 - 1KX5 Homo sapiens, Xenopus laevis [1.9Å]

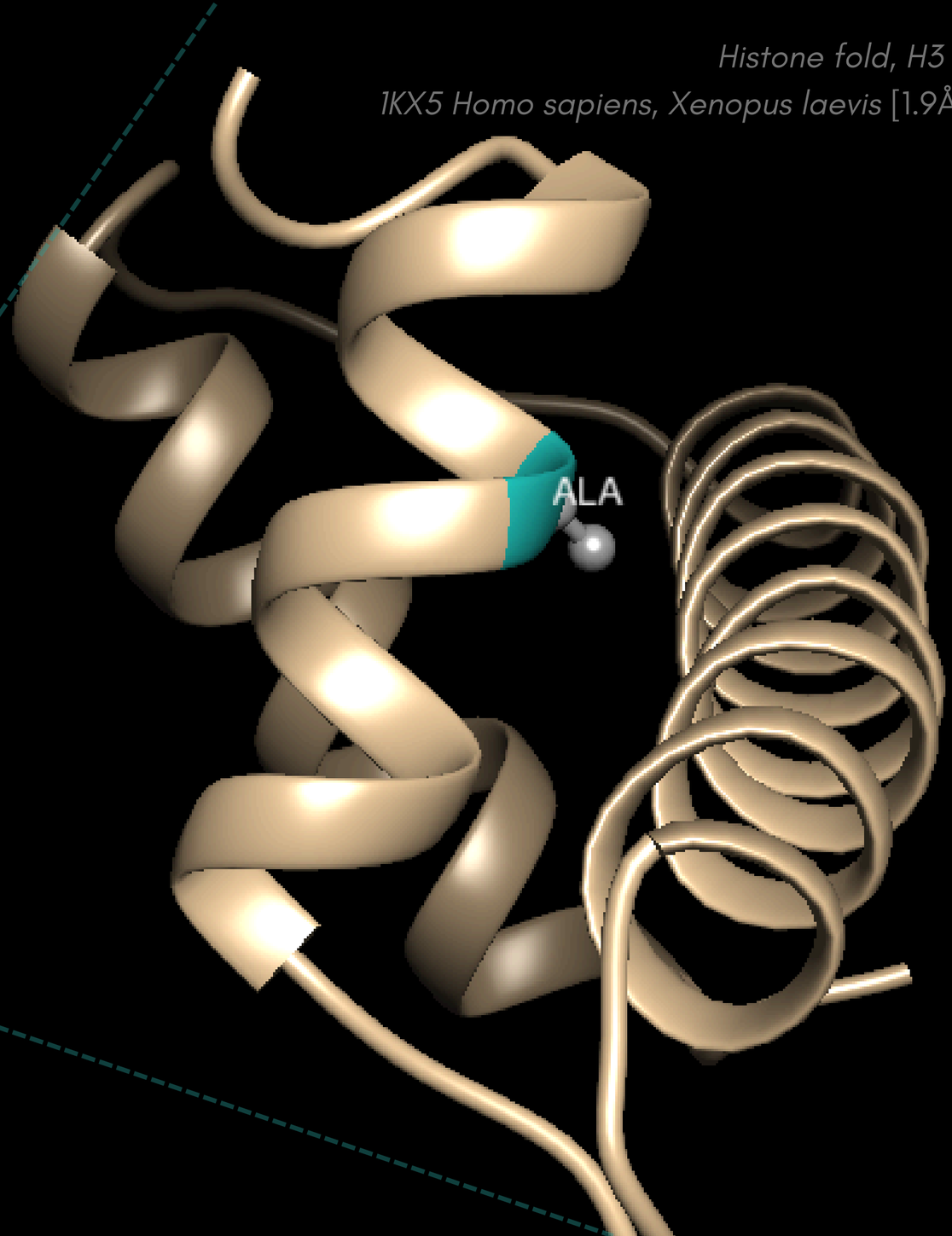


Histone fold

Conserved residues

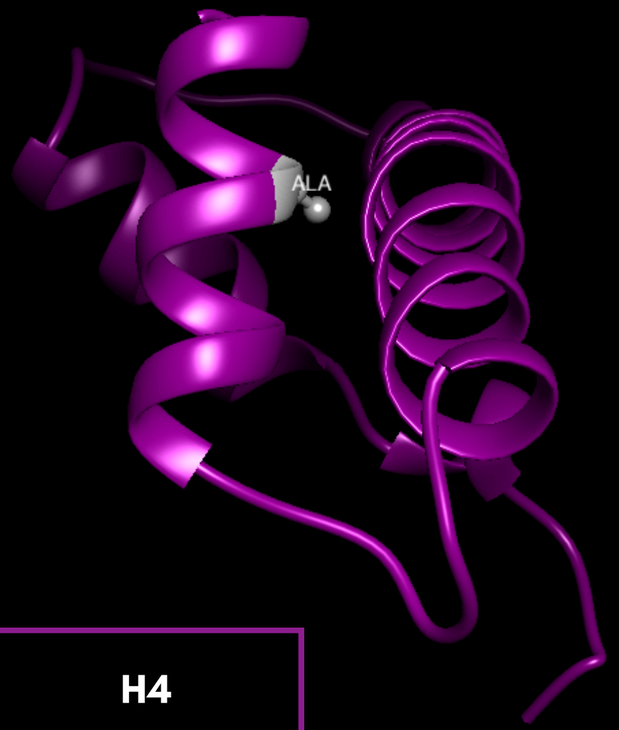


Histone fold, H3 –
1KX5 Homo sapiens, Xenopus laevis [1.9Å]

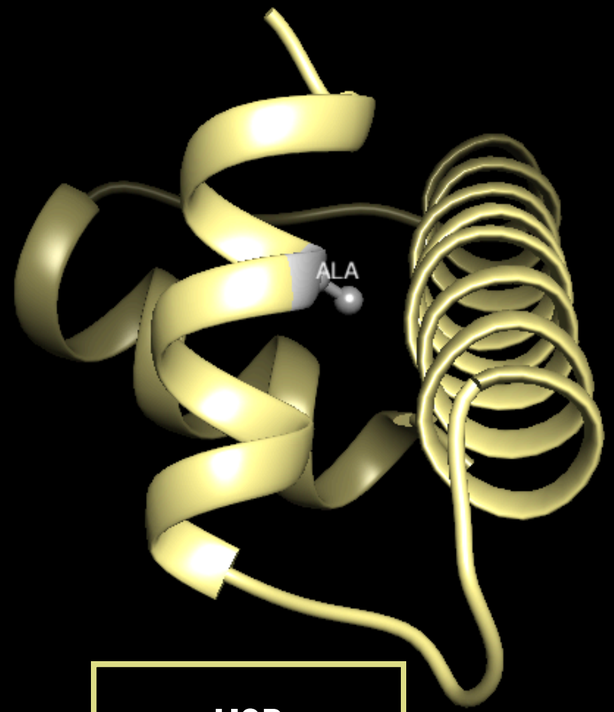


Histone fold

Conserved residues



H4



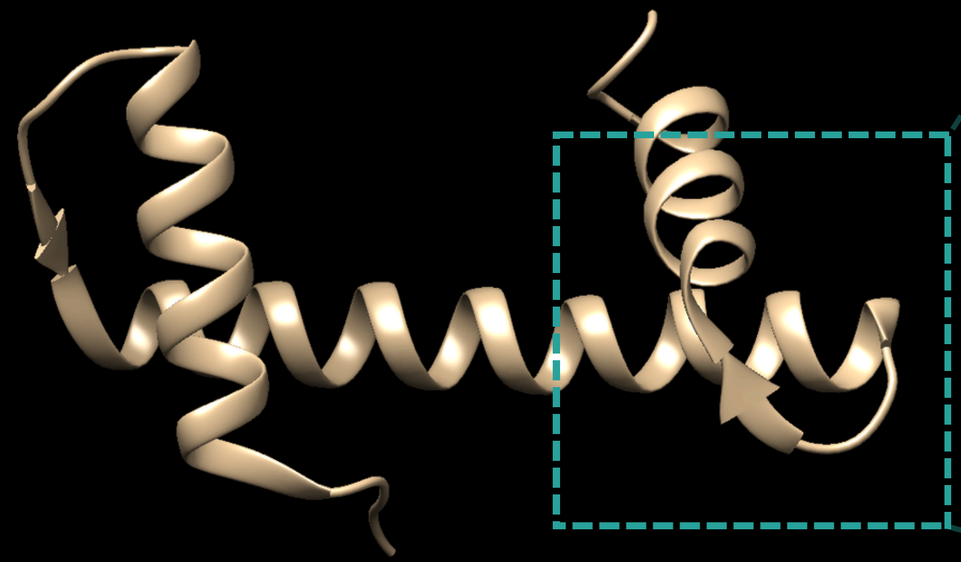
H2B



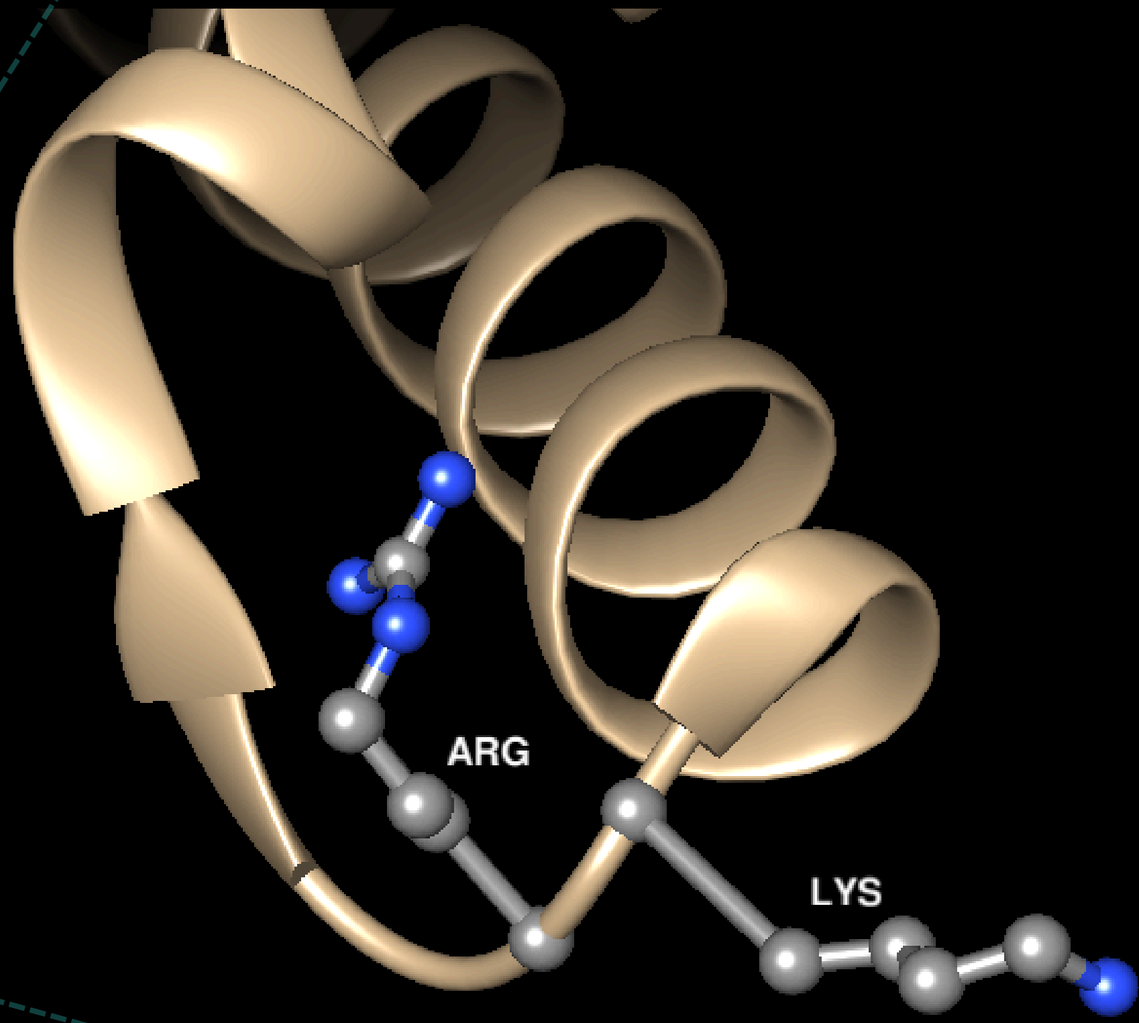
H2A

Histone fold

Conserved residues

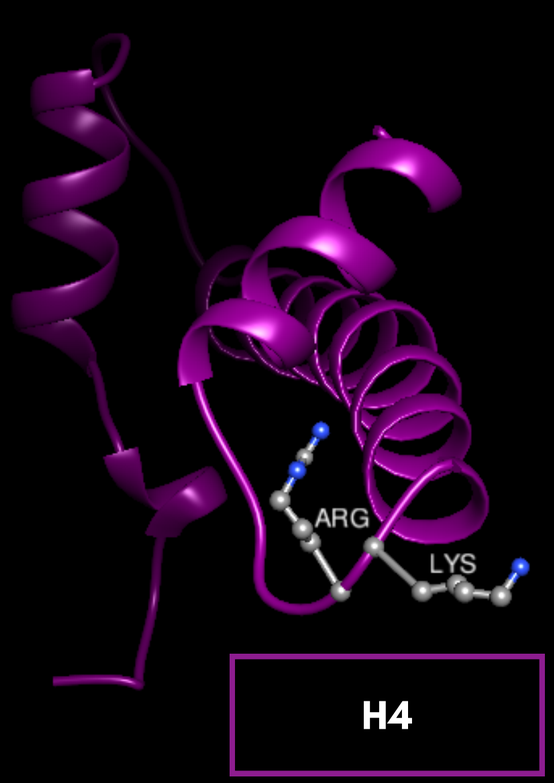


Histone fold, H3 –
1KX5 Homo sapiens, Xenopus laevis [1.9Å]



Histone fold

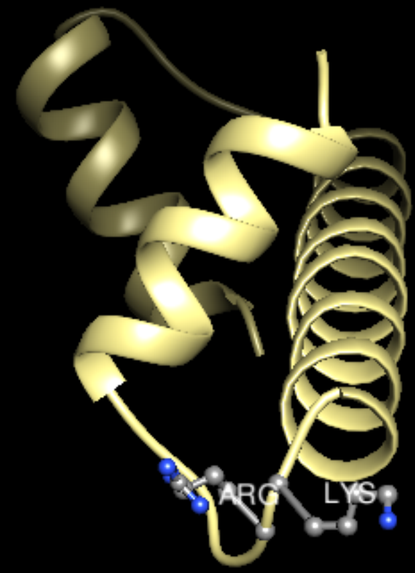
Important residues



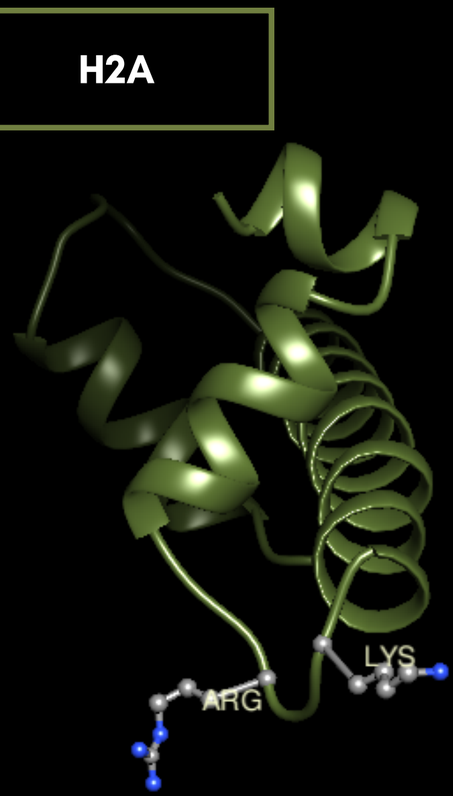
H4



H3



H2B



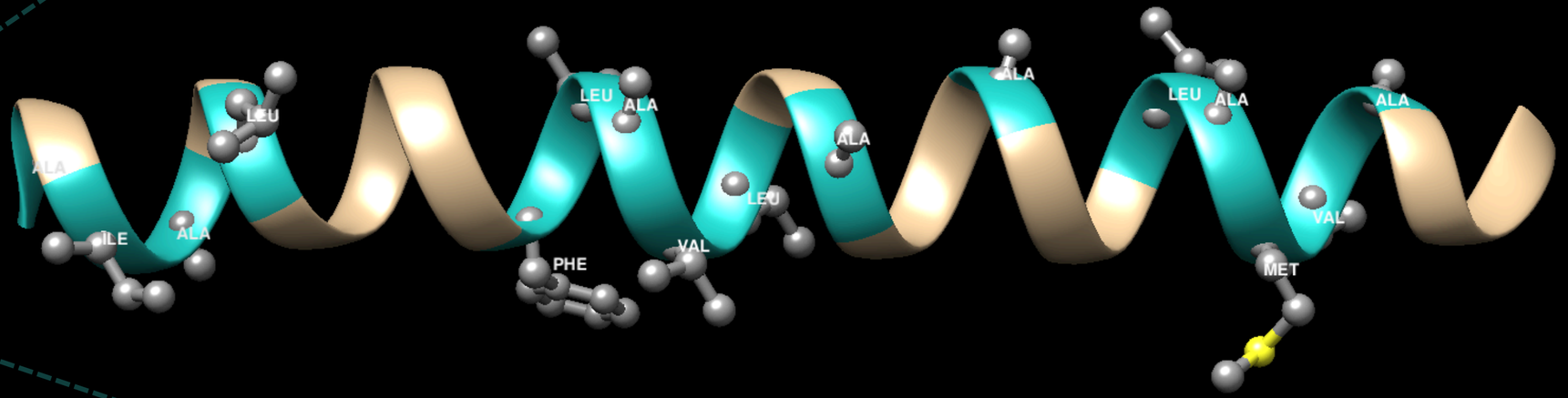
H2A

Histone fold, H3 –
1KX5 Homo sapiens, Xenopus laevis [1.9Å]

Histone fold

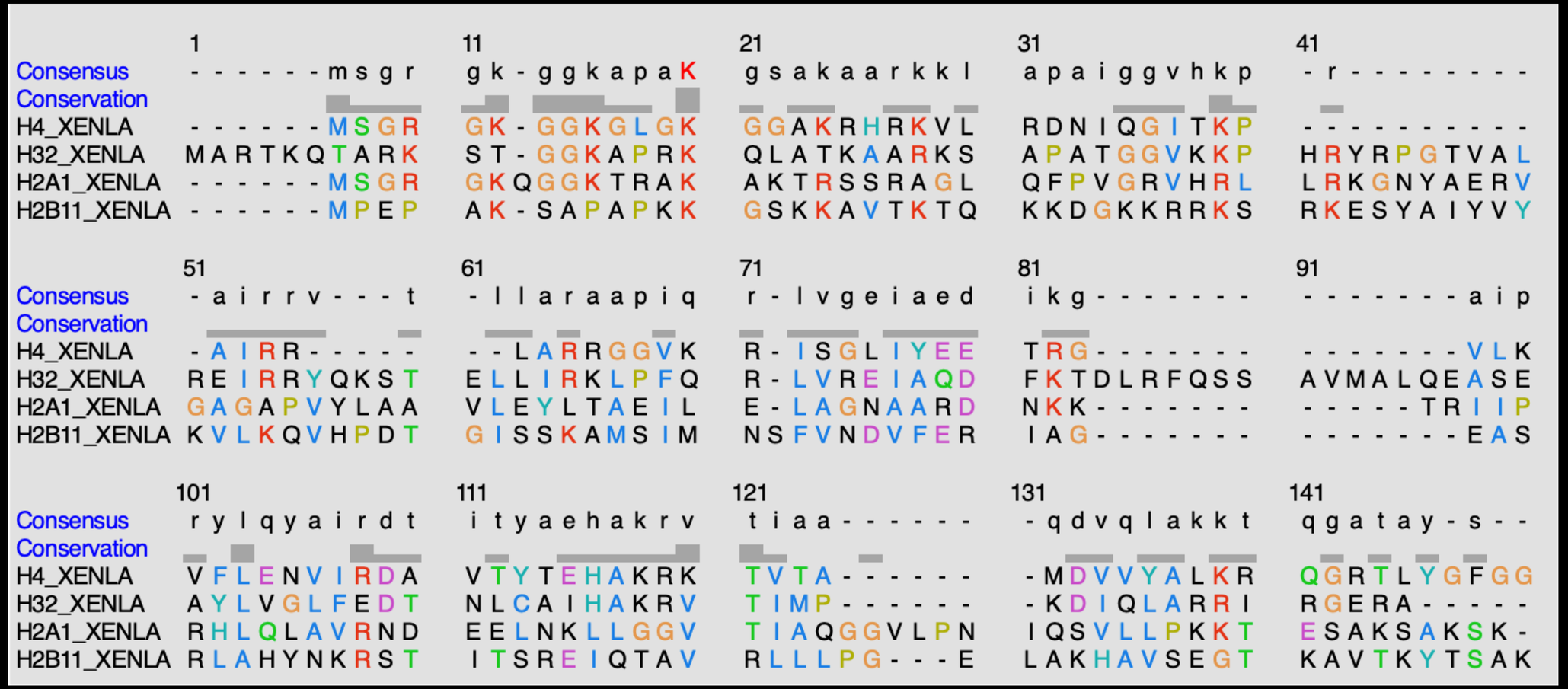
Spatial conformation
Hydrophobic interactions





Core histones

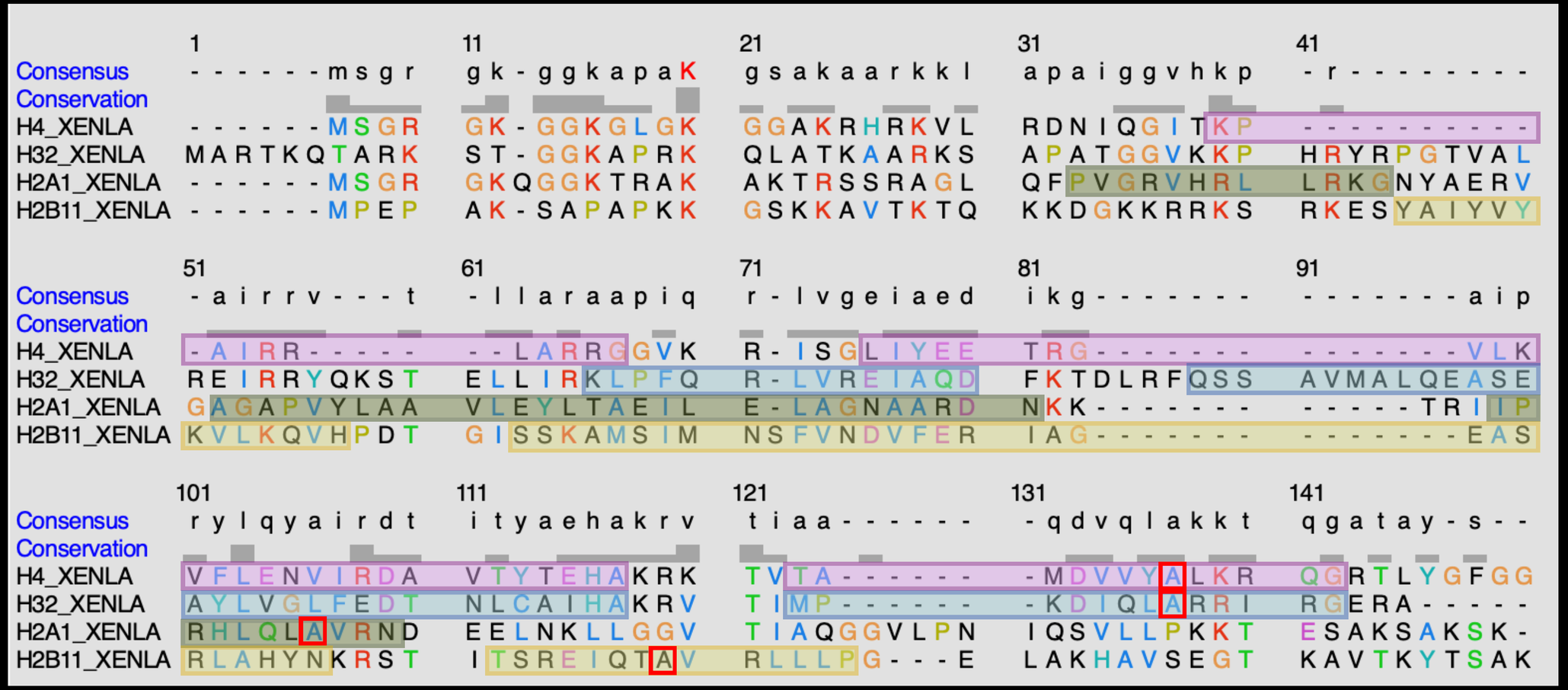
Sequence alignment



H2A, H2B, H3, H4 – 1KX5 Homo sapiens, Xenopus laevis [1.9Å]

Core histones

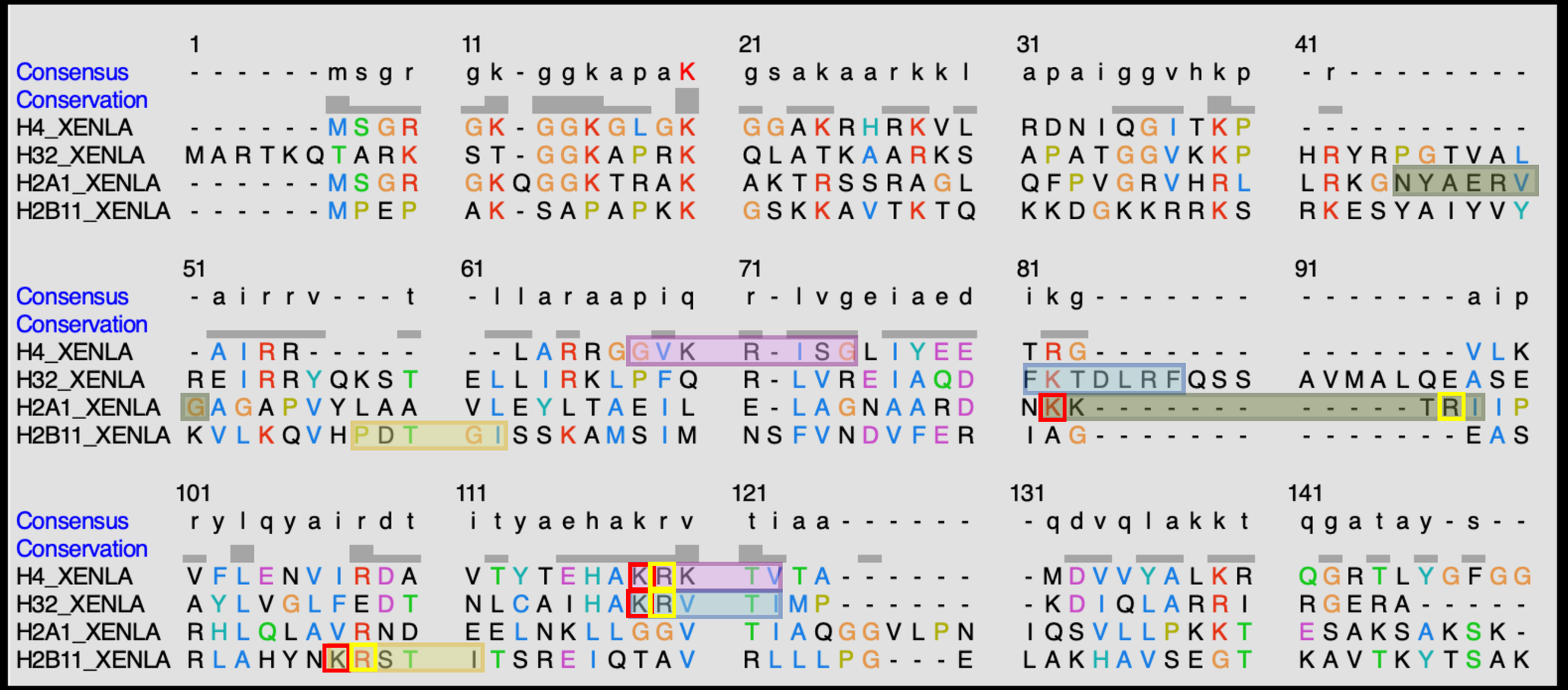
Sequence alignment (α helix)



H2A, H2B, H3, H4 – 1KX5 Homo sapiens, Xenopus laevis [1.9Å]

Core histones

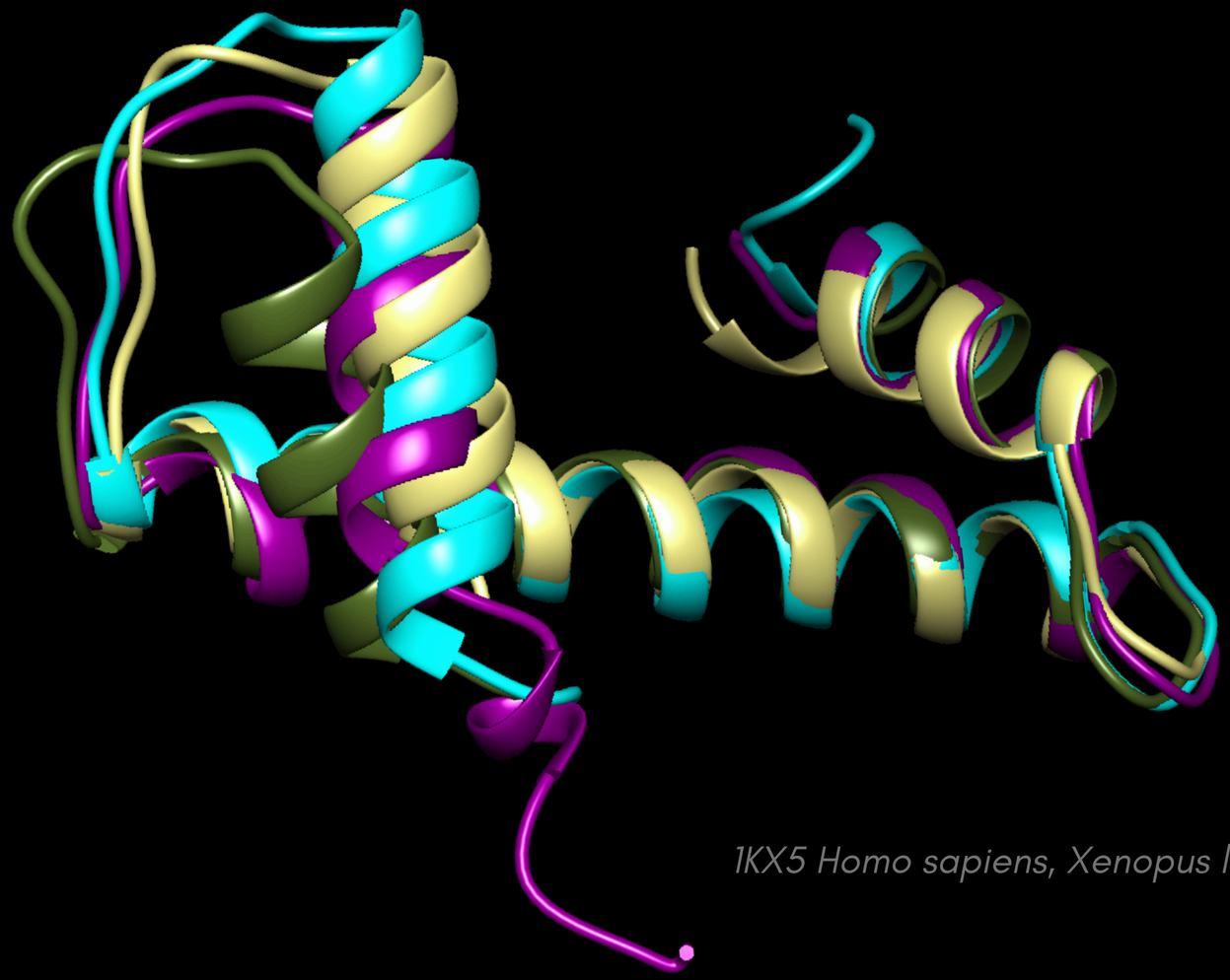
Sequence alignment (loops)



H2A, H2B, H3, H4 – 1KX5 Homo sapiens, Xenopus laevis [1.9Å]

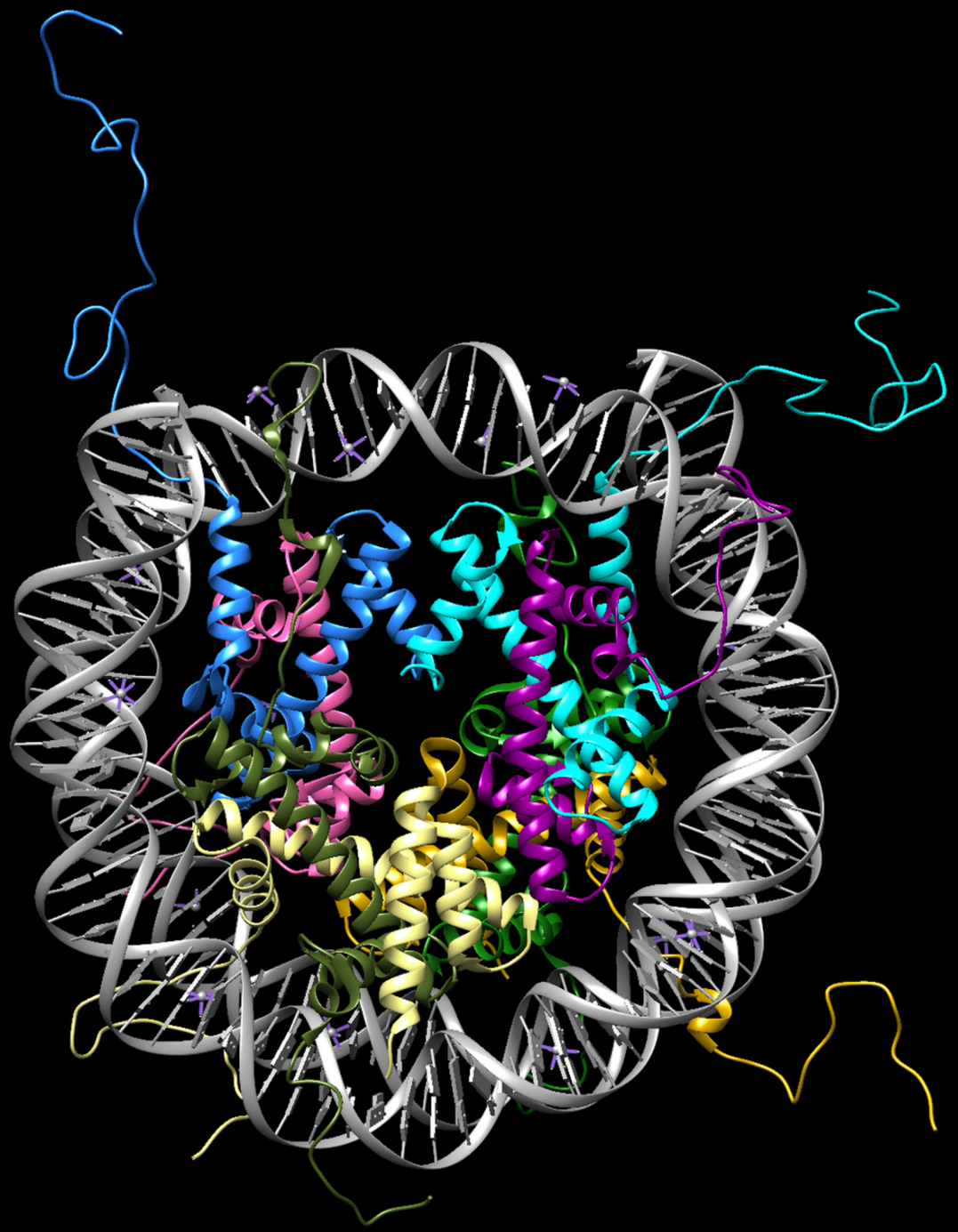
Core histones

Superimposition (MatchMaker)



1KX5 Homo sapiens, Xenopus laevis [1.9Å]

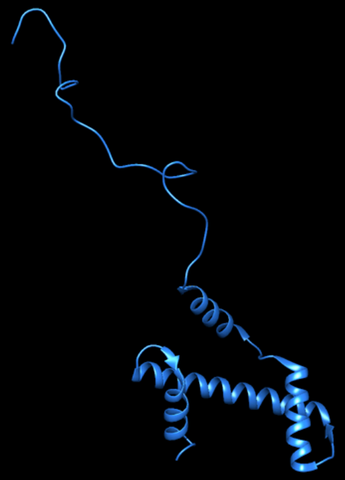
Octamer complex



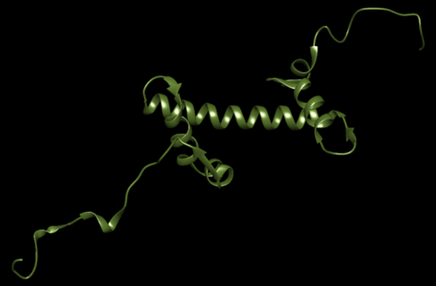
H3, H4, H2A, H2B, DNA - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]



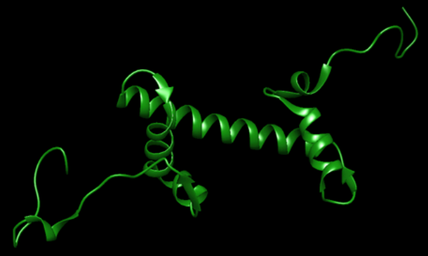
H3



H3



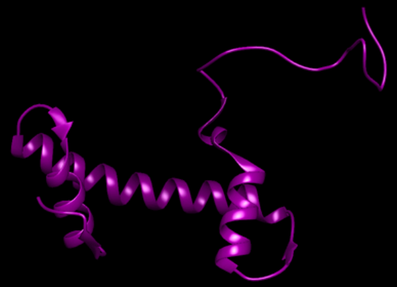
H2A



H2A



H1



H4



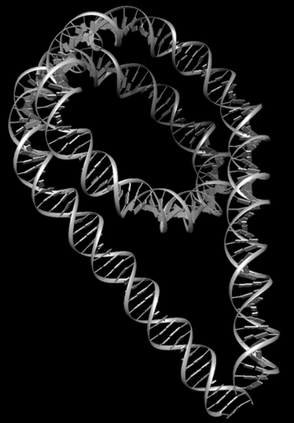
H4



H2B



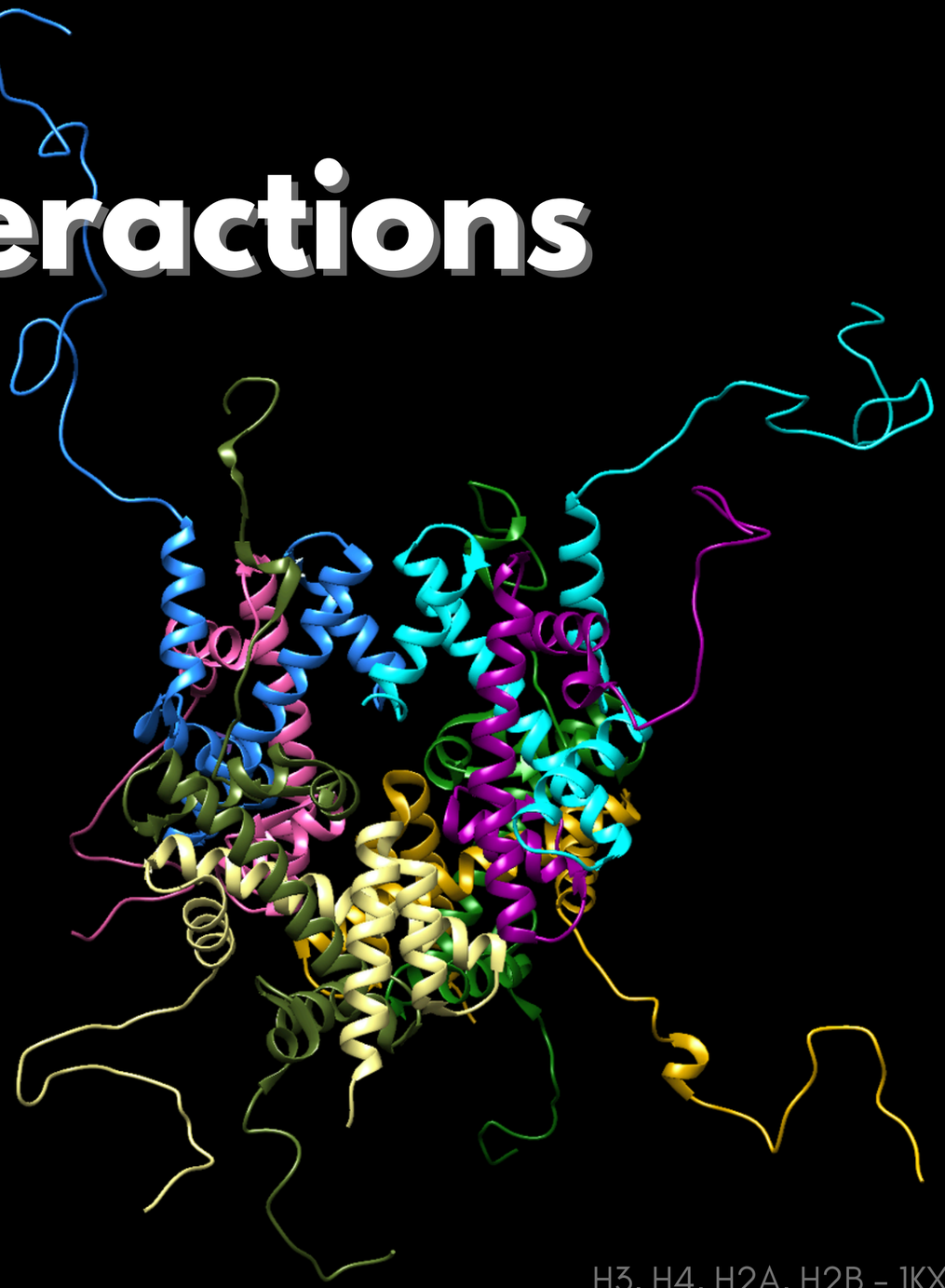
H2B



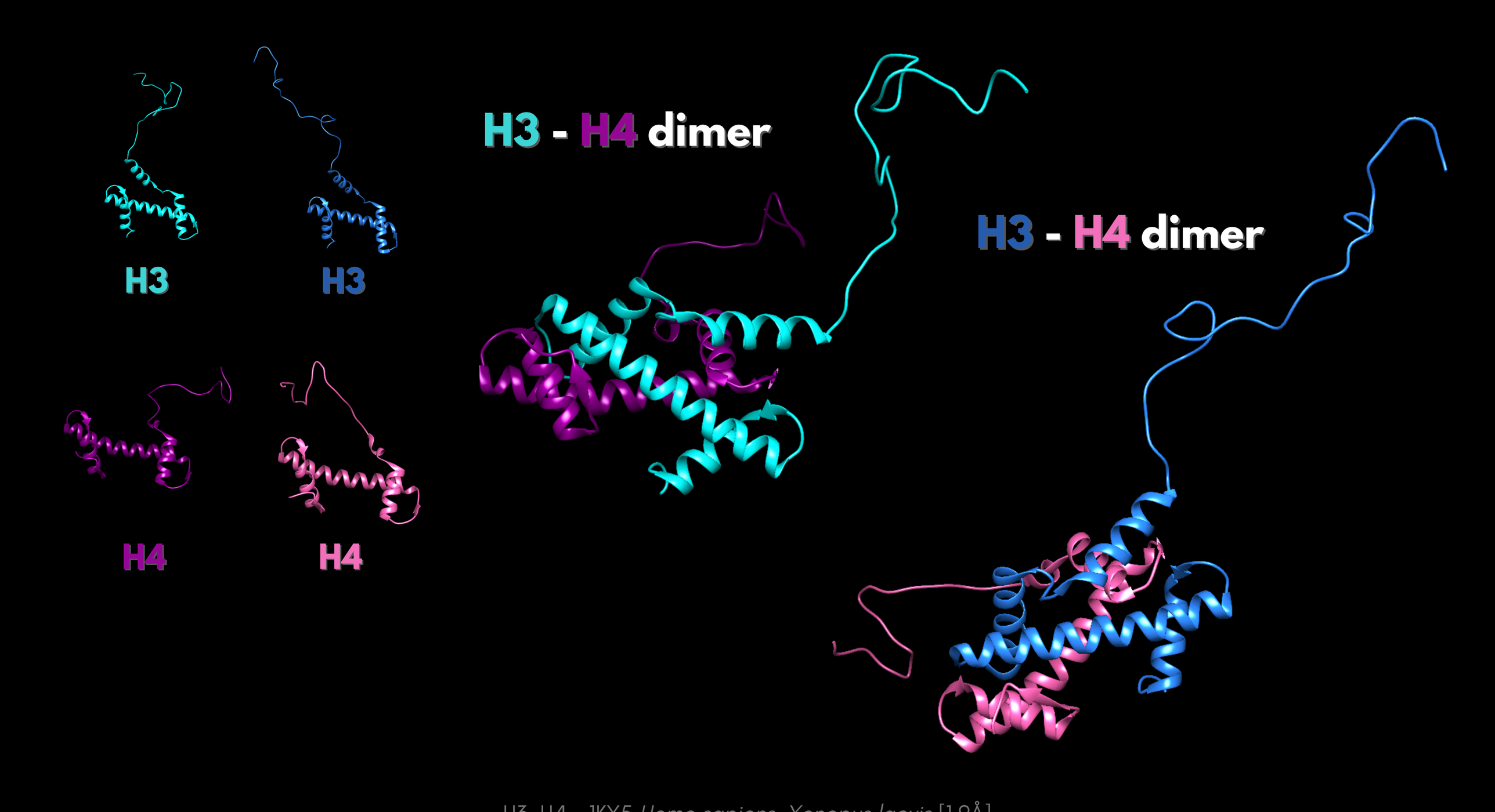
DNA

H3, H4, H2A, H2B - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å] / H1, DNA - 5NL0 *Xenopus laevis*, synthetic construct [5.4Å]

Core interactions



H3, H4, H2A, H2B - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

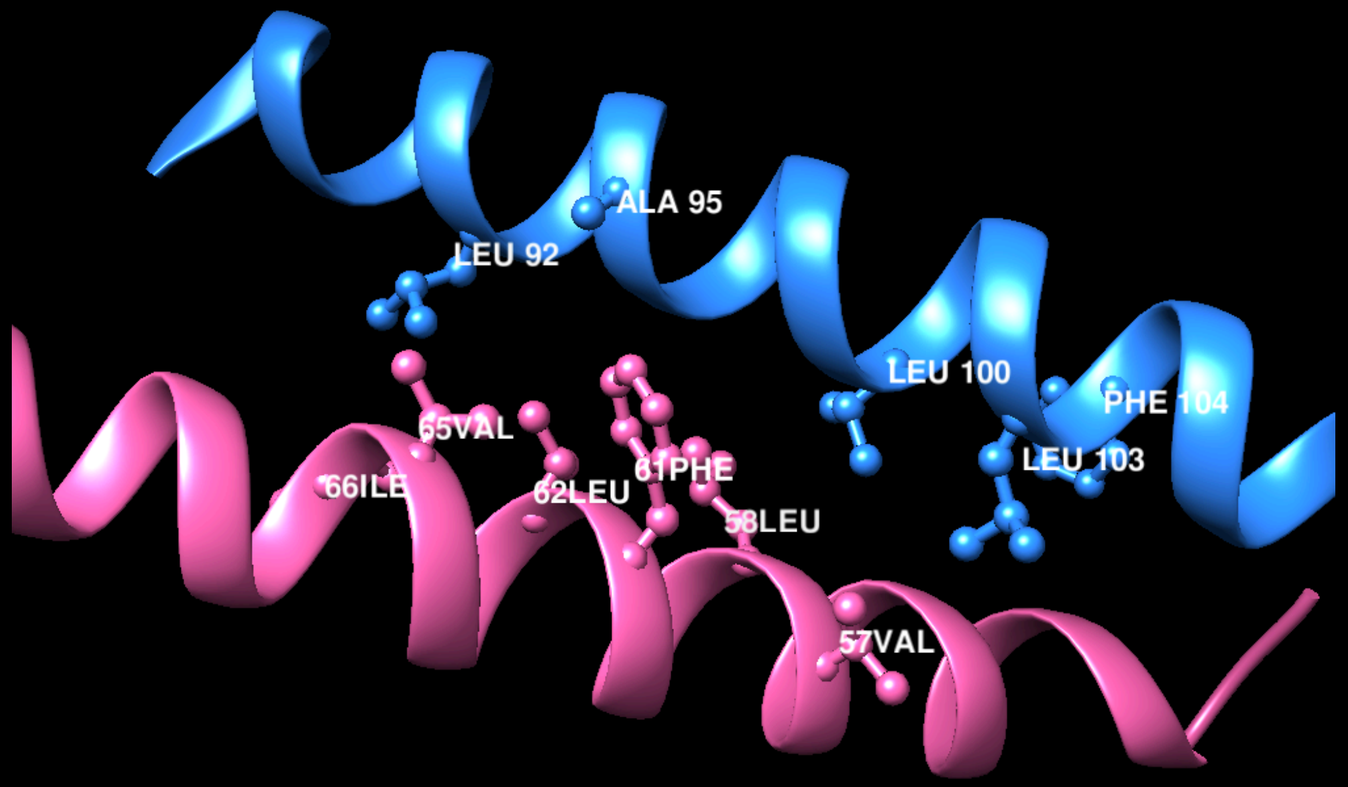


H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

H3 - H4 dimer



H3α2 - H4α2

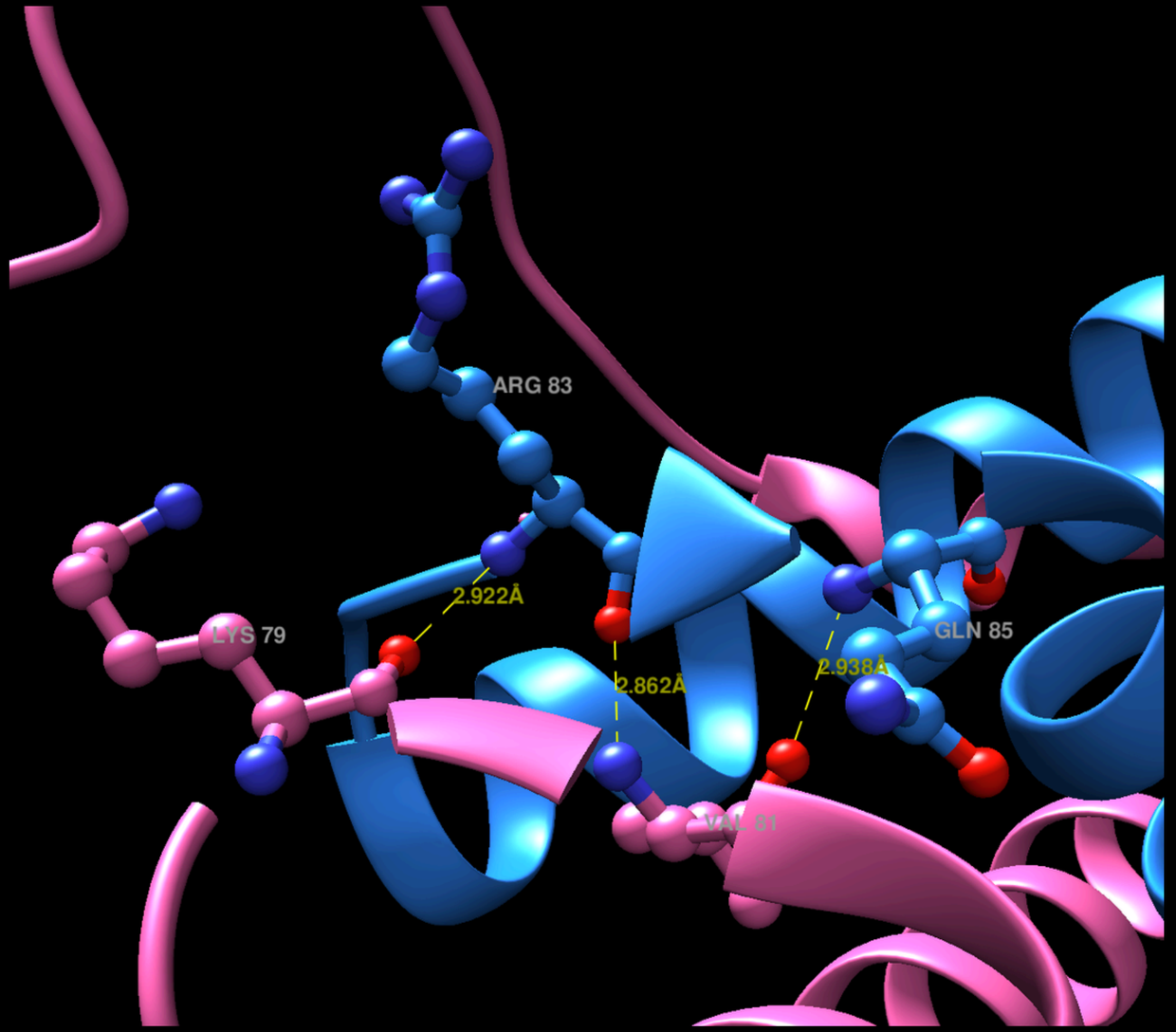


H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

H3 - H4 dimer

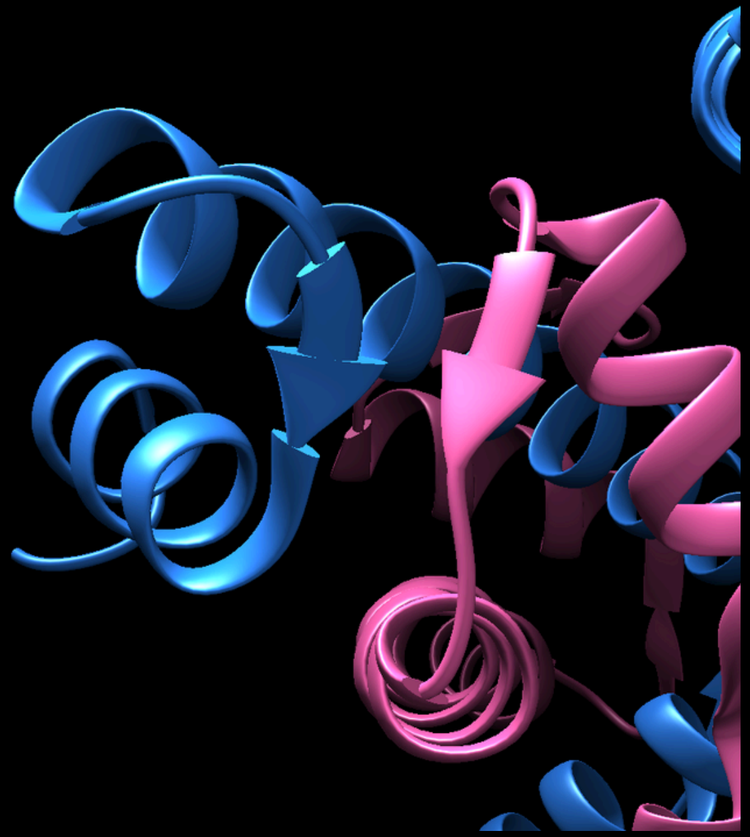


H3L1 - H4L2

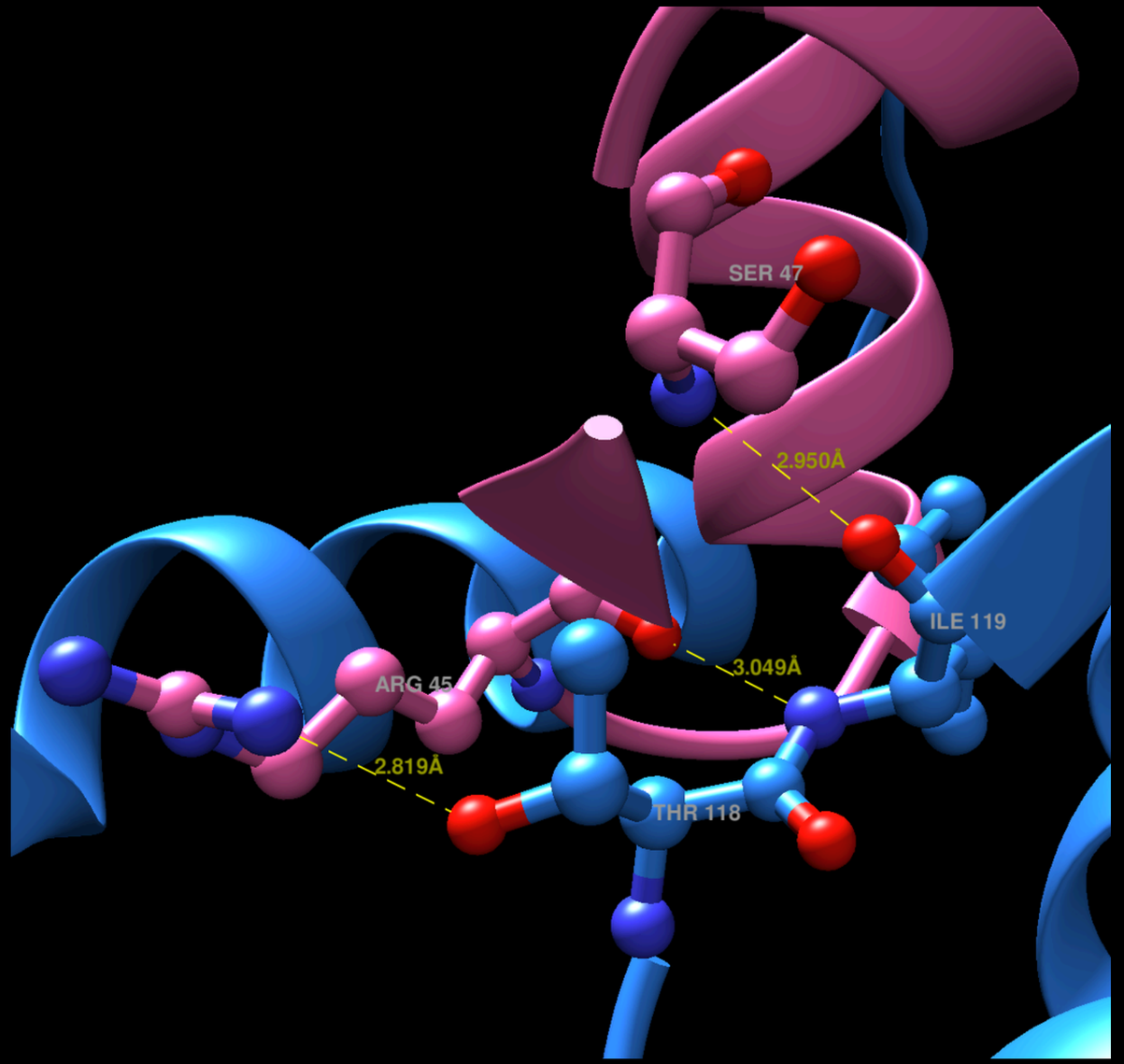


H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

H3 - H4 dimer



H3L2 - H4L1

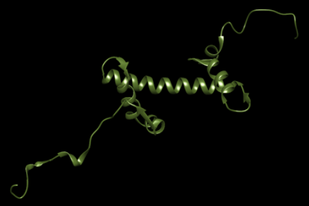


H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

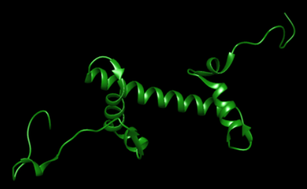
H3 - H4 dimer



H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]



H2A



H2A



H2B



H2B

H2A - H2B dimer



H2A - H2B dimer

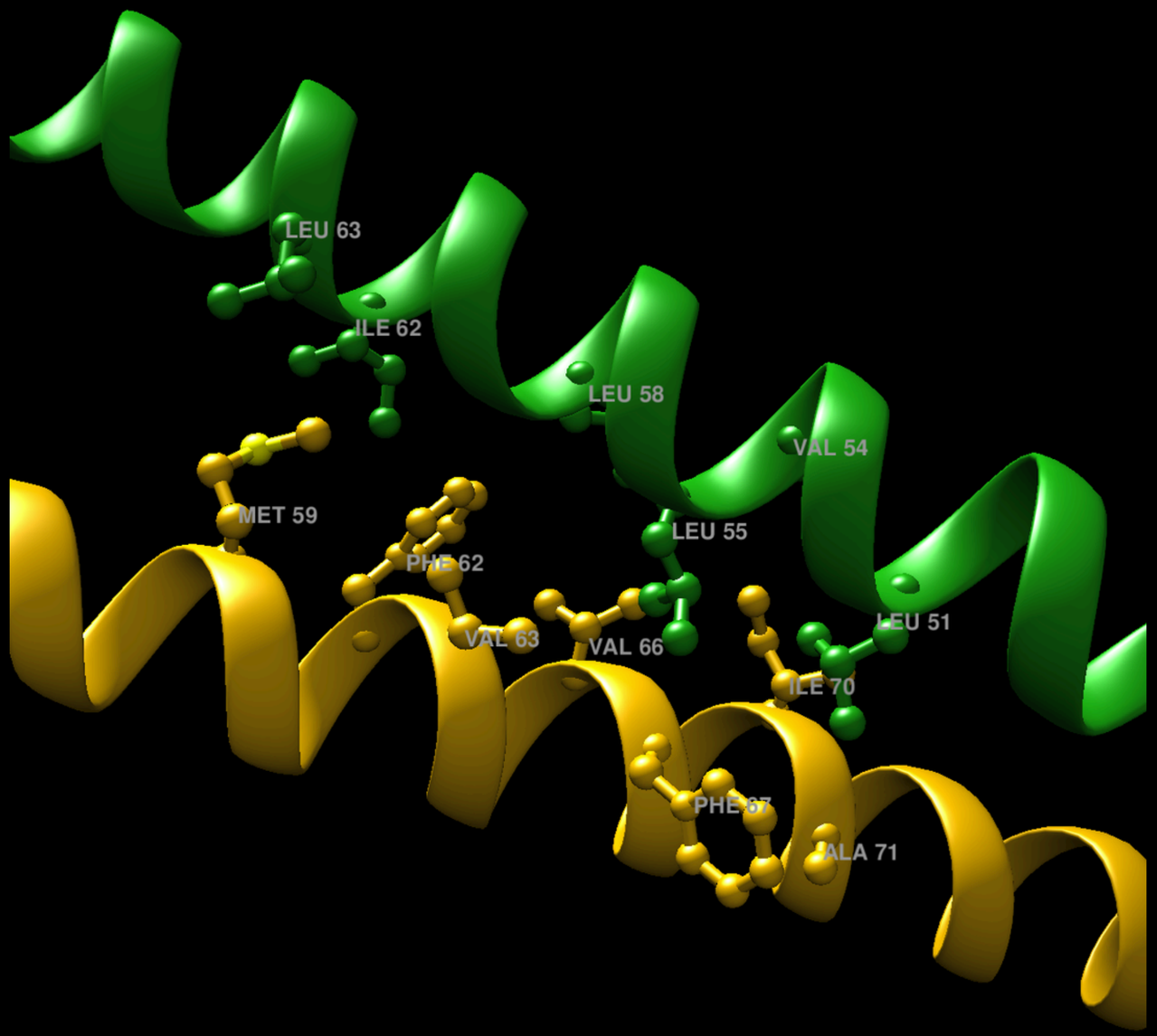


H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

H2A - H2B dimer

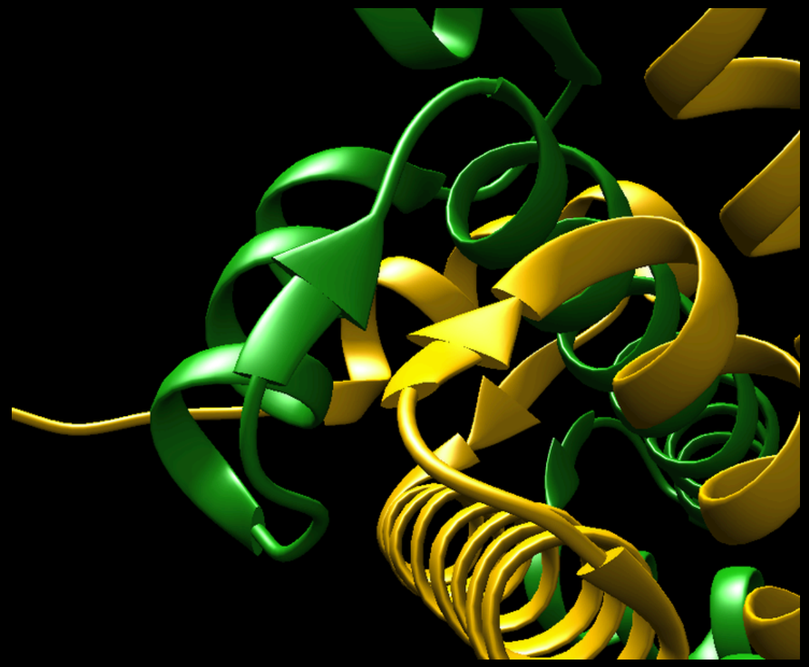


H2Aα2 - H2Bα2

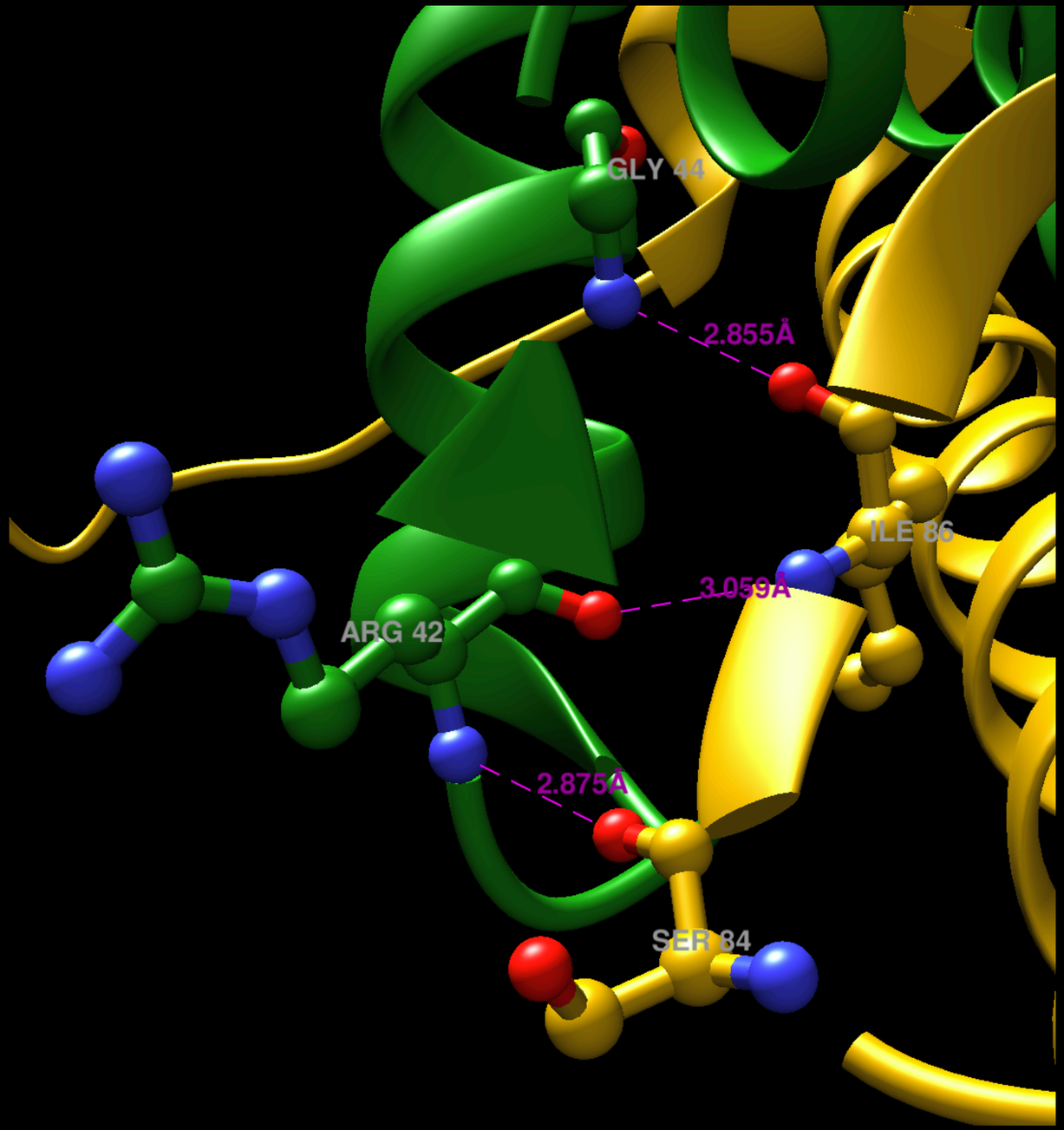


H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

H2A - H2B dimer

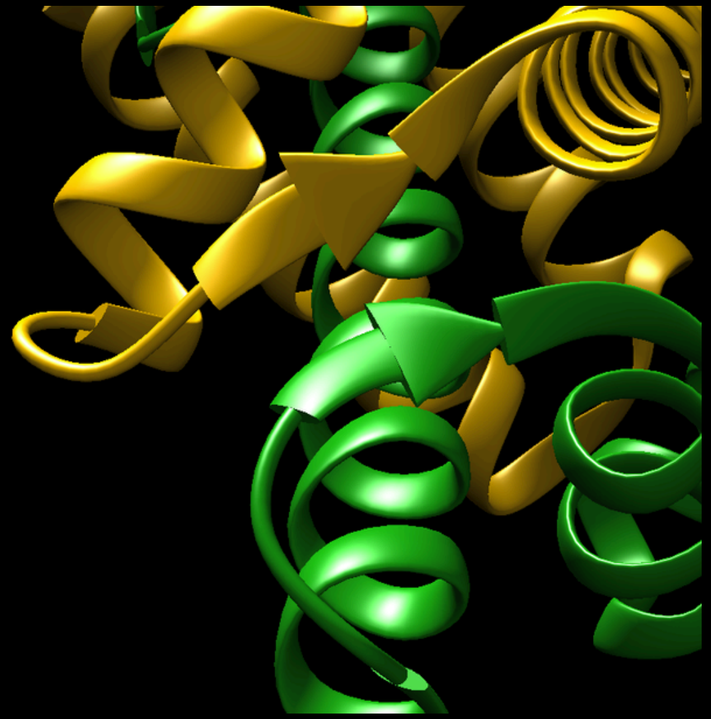


H2AL1 - H2BL2

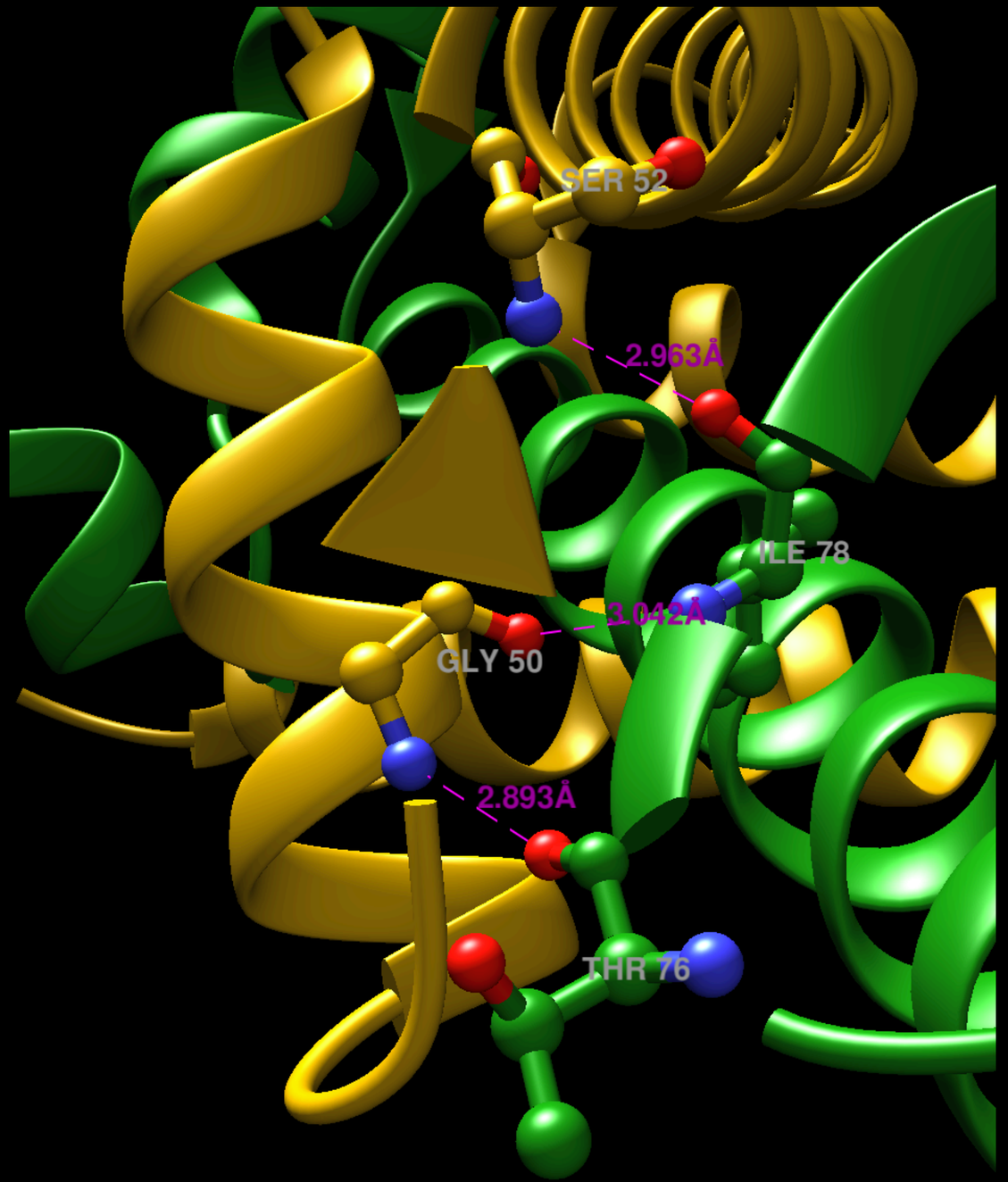


H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

H2A - H2B dimer



H2AL2 - H2BL1



H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

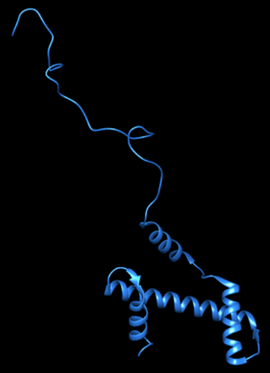
H2A - H2B dimer



H2A, H2B - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]



H3



H3

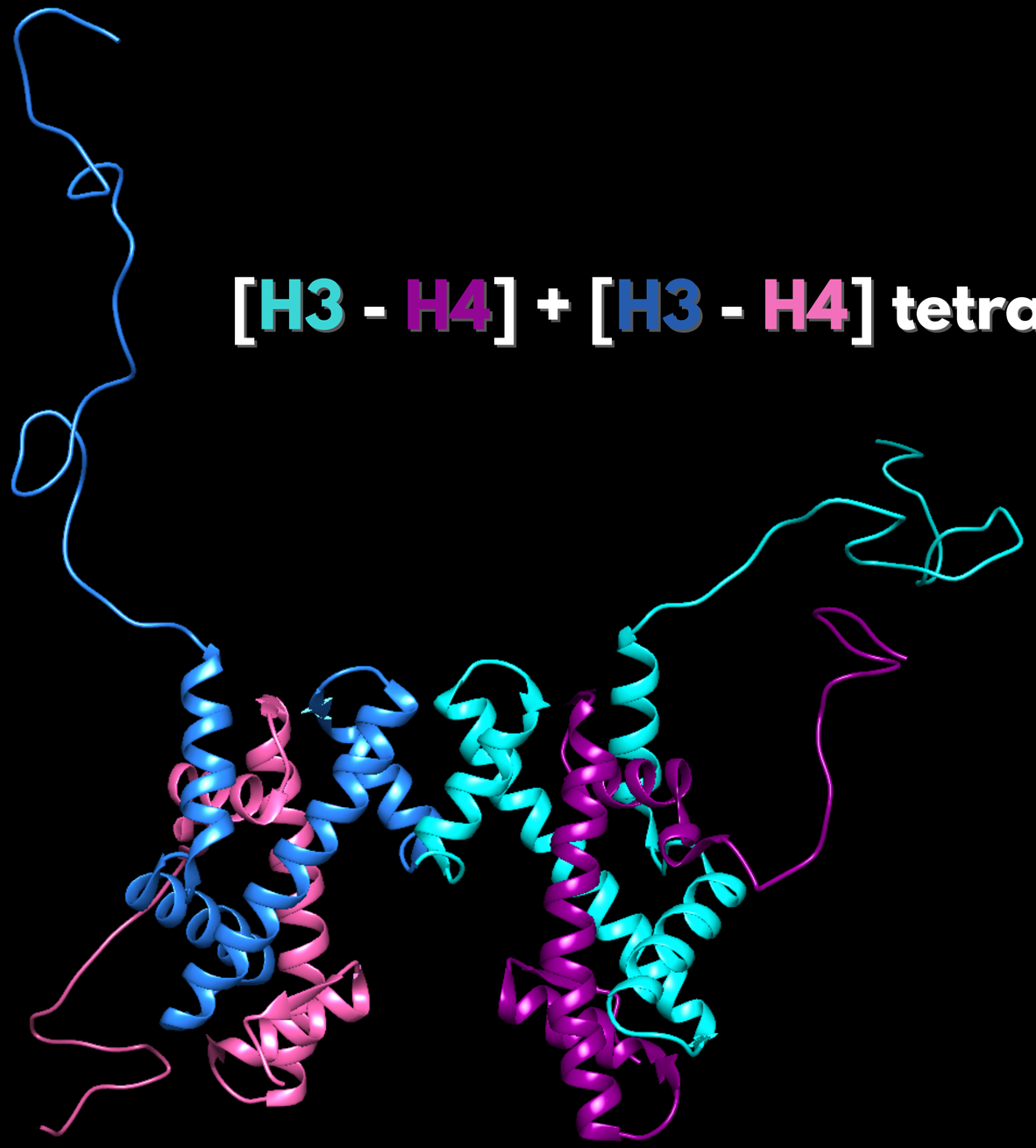


H4



H4

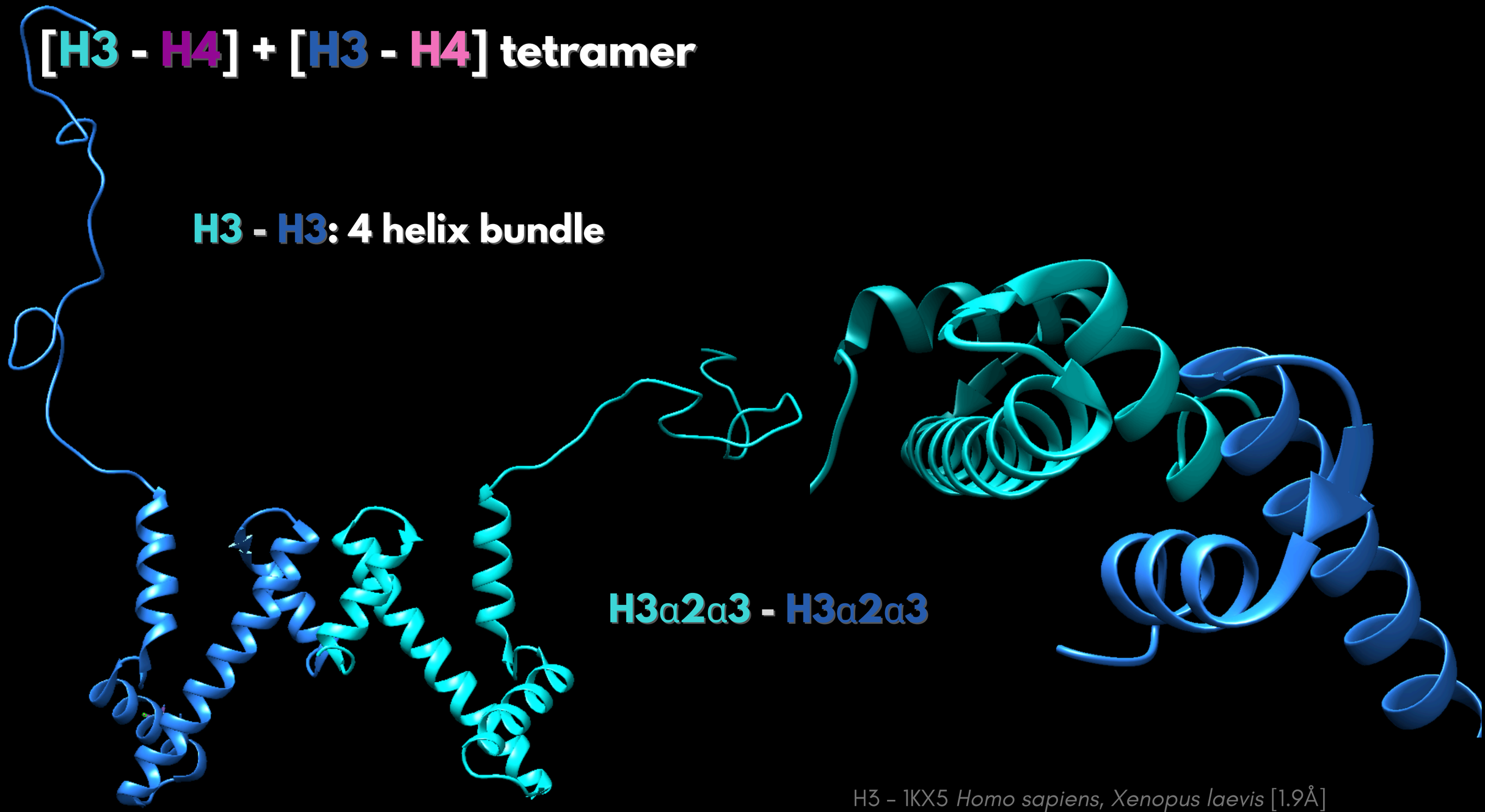
[H3 - H4] + [H3 - H4] tetramer



H3, H4 - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

[H3 - H4] + [H3 - H4] tetramer

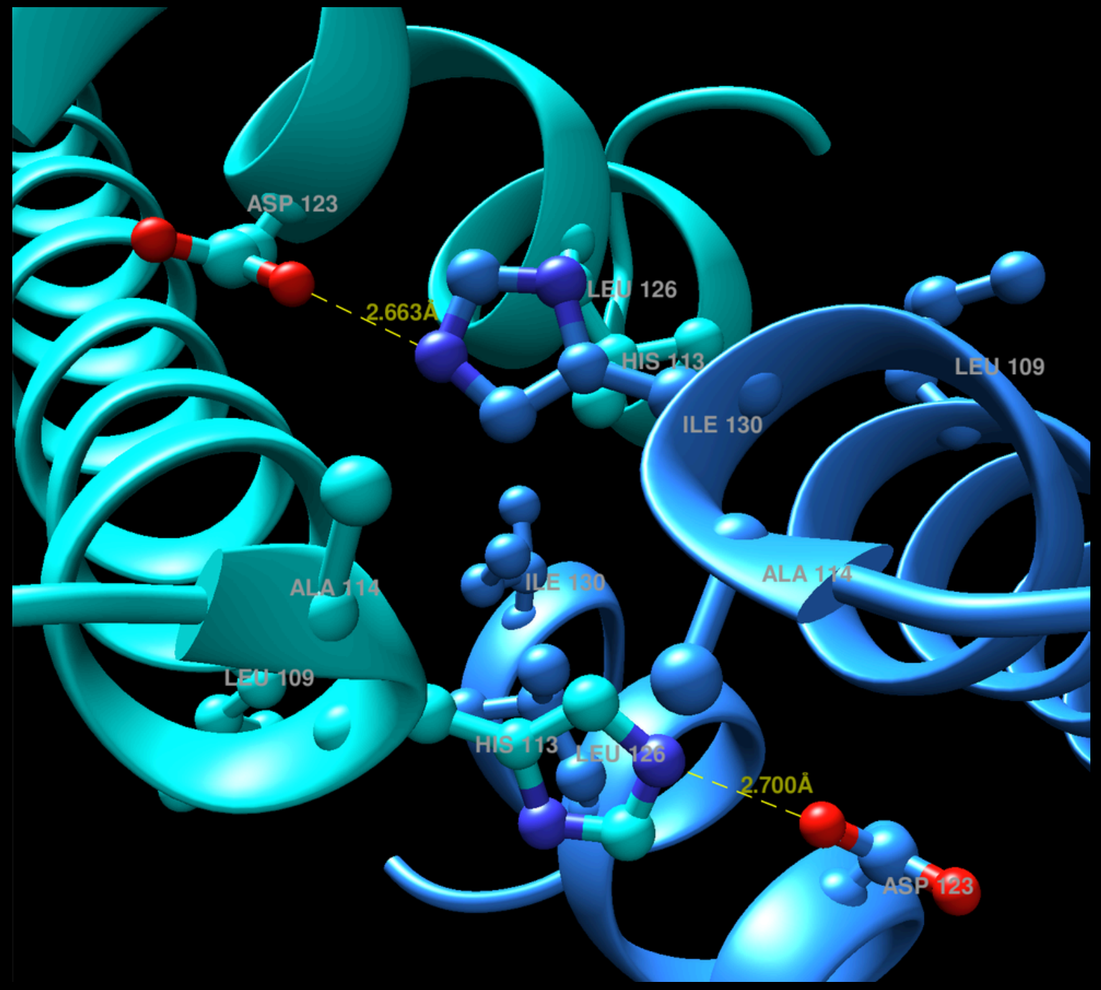
H3 - H3: 4 helix bundle

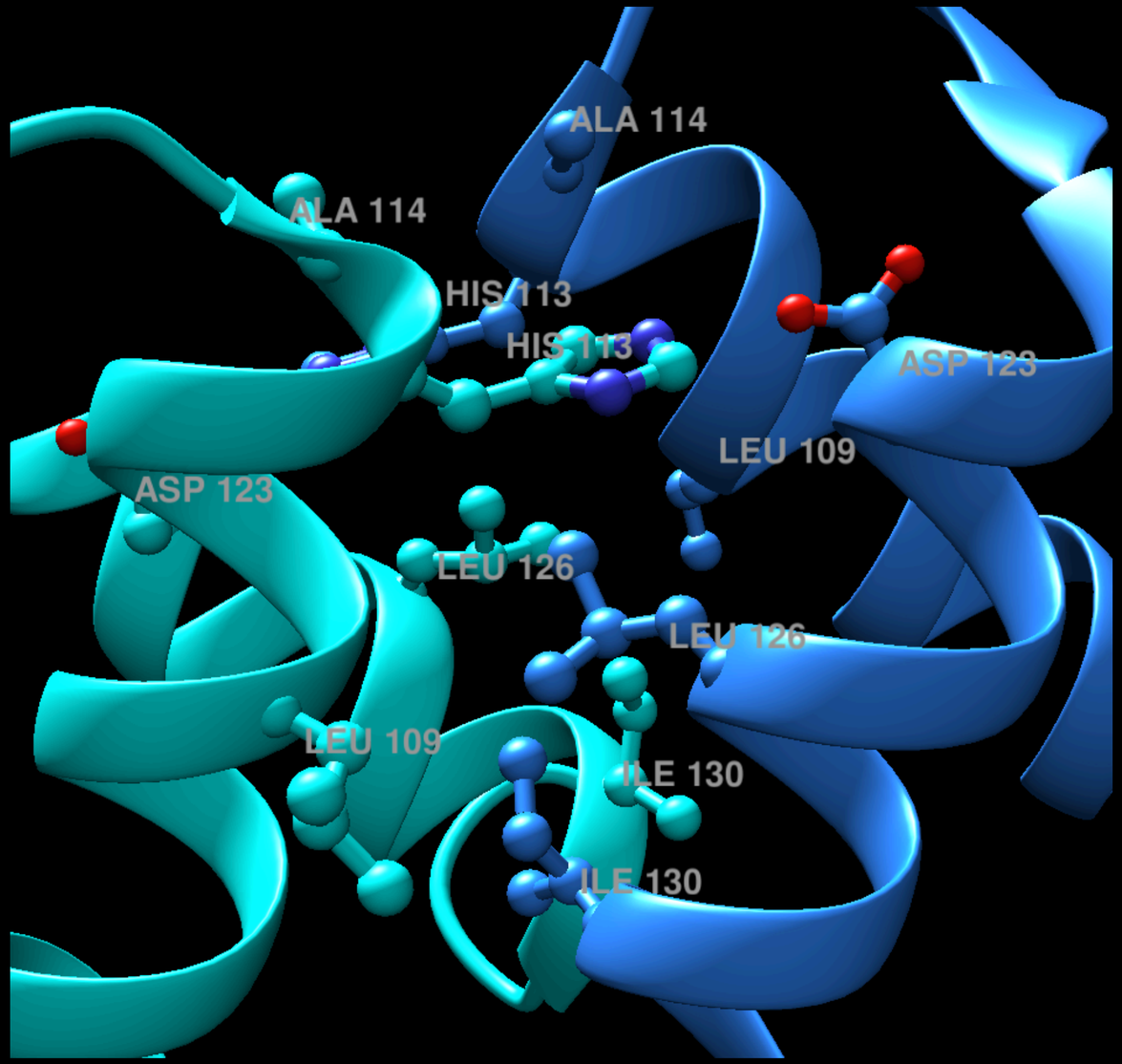


H3 - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

[H3 - H4] + [H3 - H4] tetramer

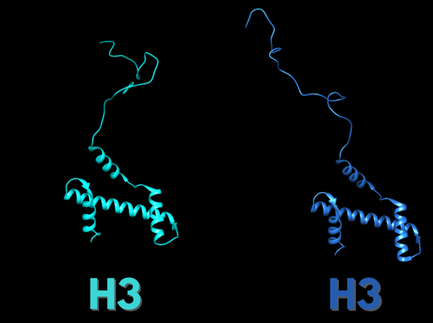
H3 - H3: 4 helix bundle





H3 - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

([H3 - H4] + [H3 - H4] tetramer) + [H2A - H2B dimer]





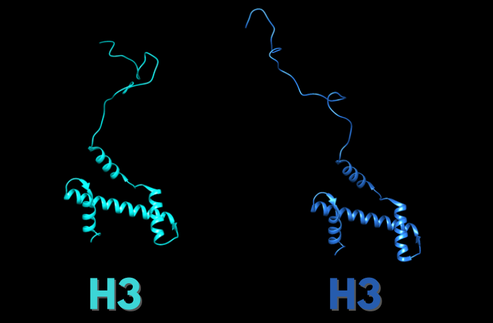






H3, H4, H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

([H3 - H4] + [H3 - H4] tetramer) + [H2A - H2B dimer]







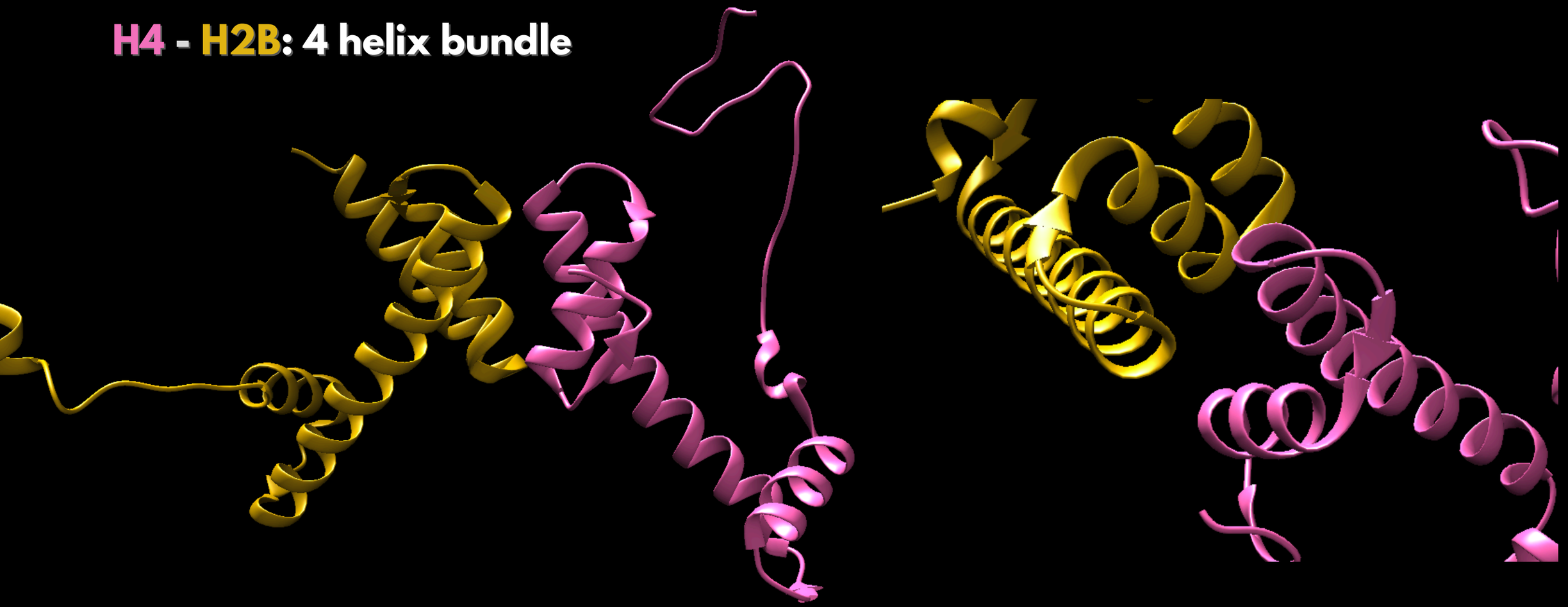




H3, H4, H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

([H3 - H4] + [H3 - H4] tetramer) + [H2A - H2B dimer]

H4 - H2B: 4 helix bundle

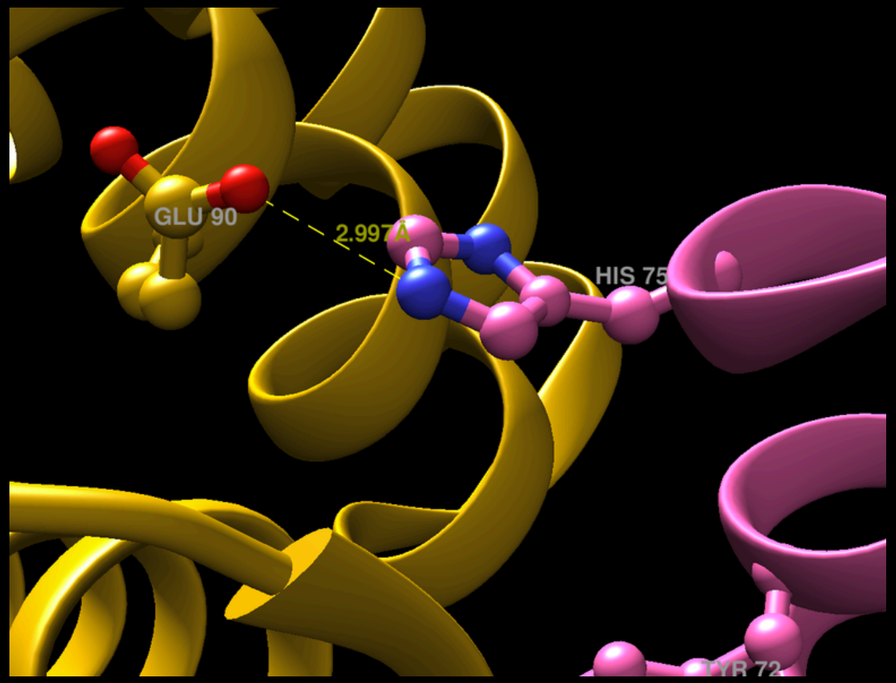


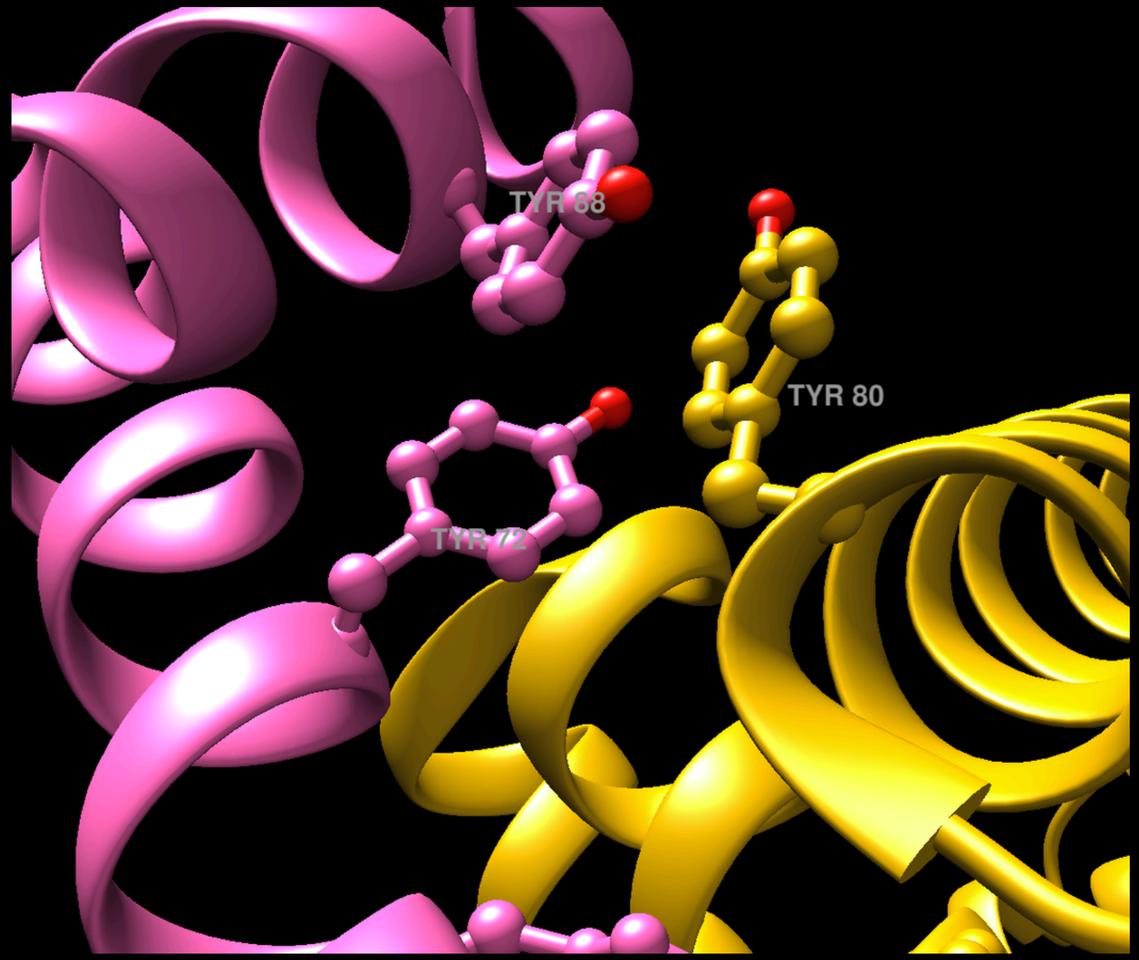
H4α2α3 - H2Bα2α3

H4, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

([H3 - H4] + [H3 - H4] tetramer) + [H2A - H2B dimer]

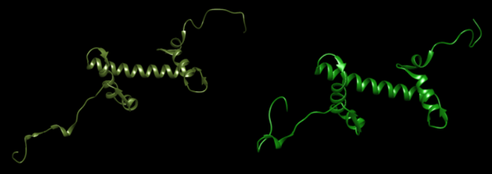
H4 - H2B: 4 helix bundle





H4, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

Octamer: ([H3 - H4] + [H3 - H4]) + [H2A - H2B] + [H2A - H2B]

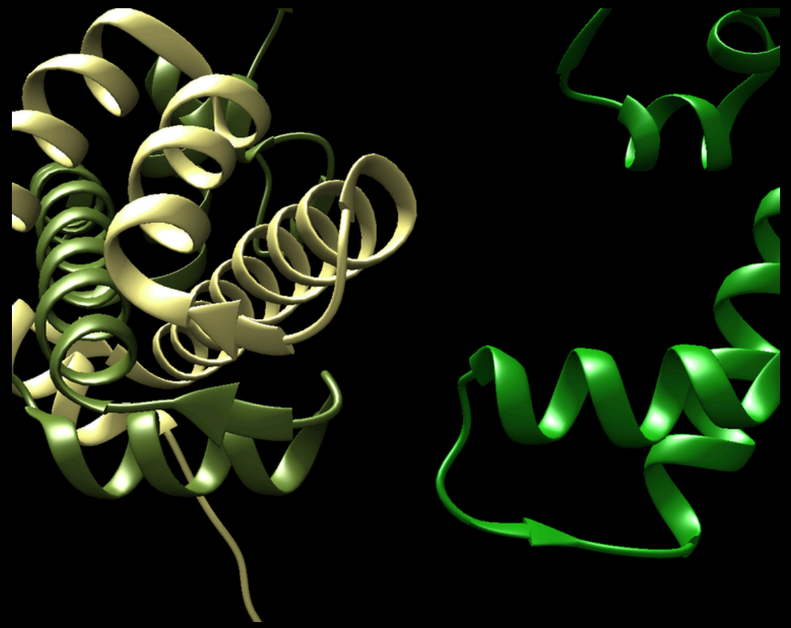


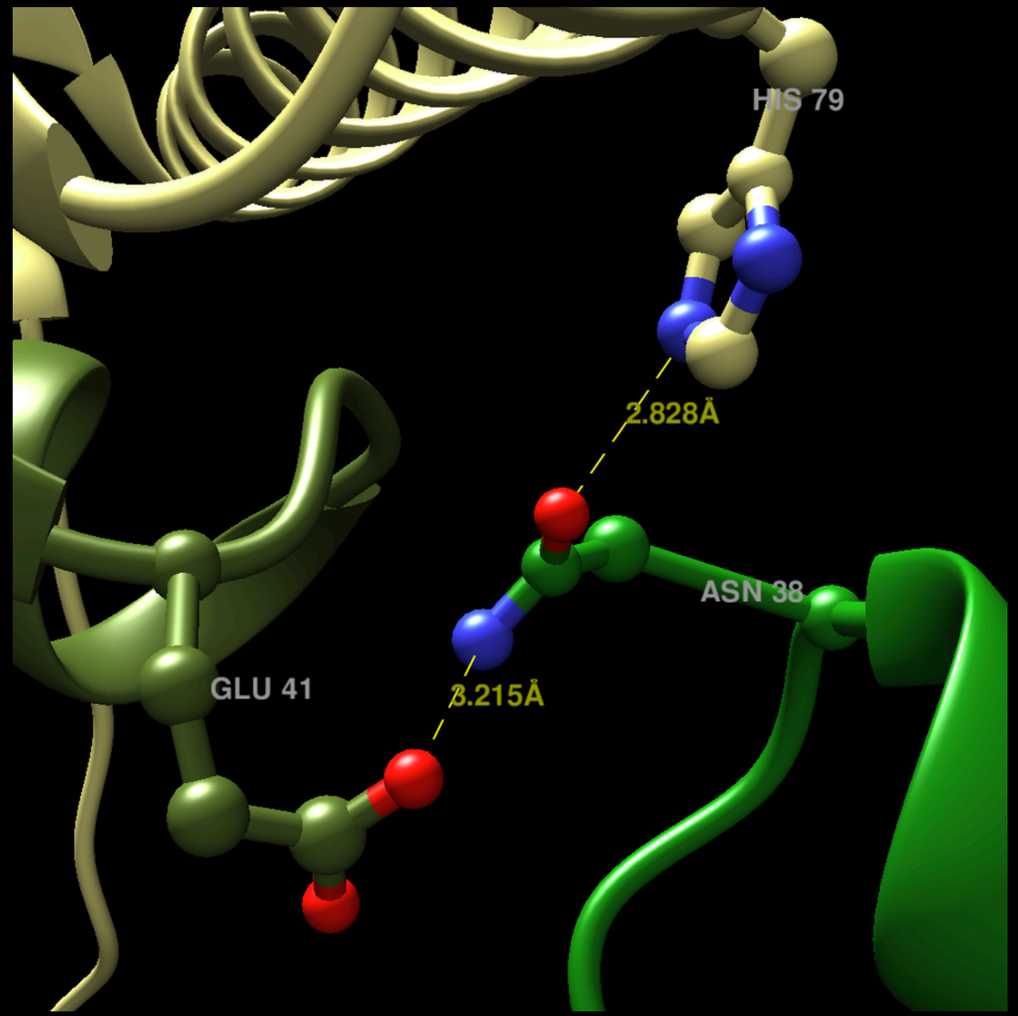
H2A

H2A

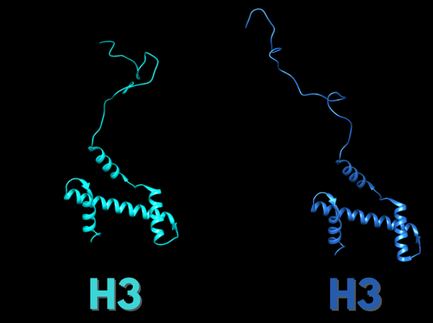


H2B

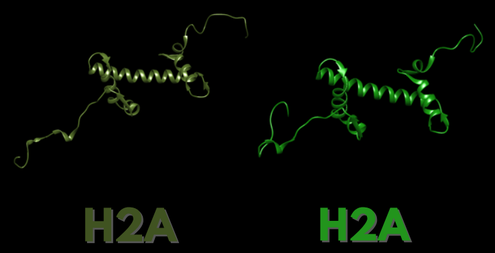




H2A, H2B - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

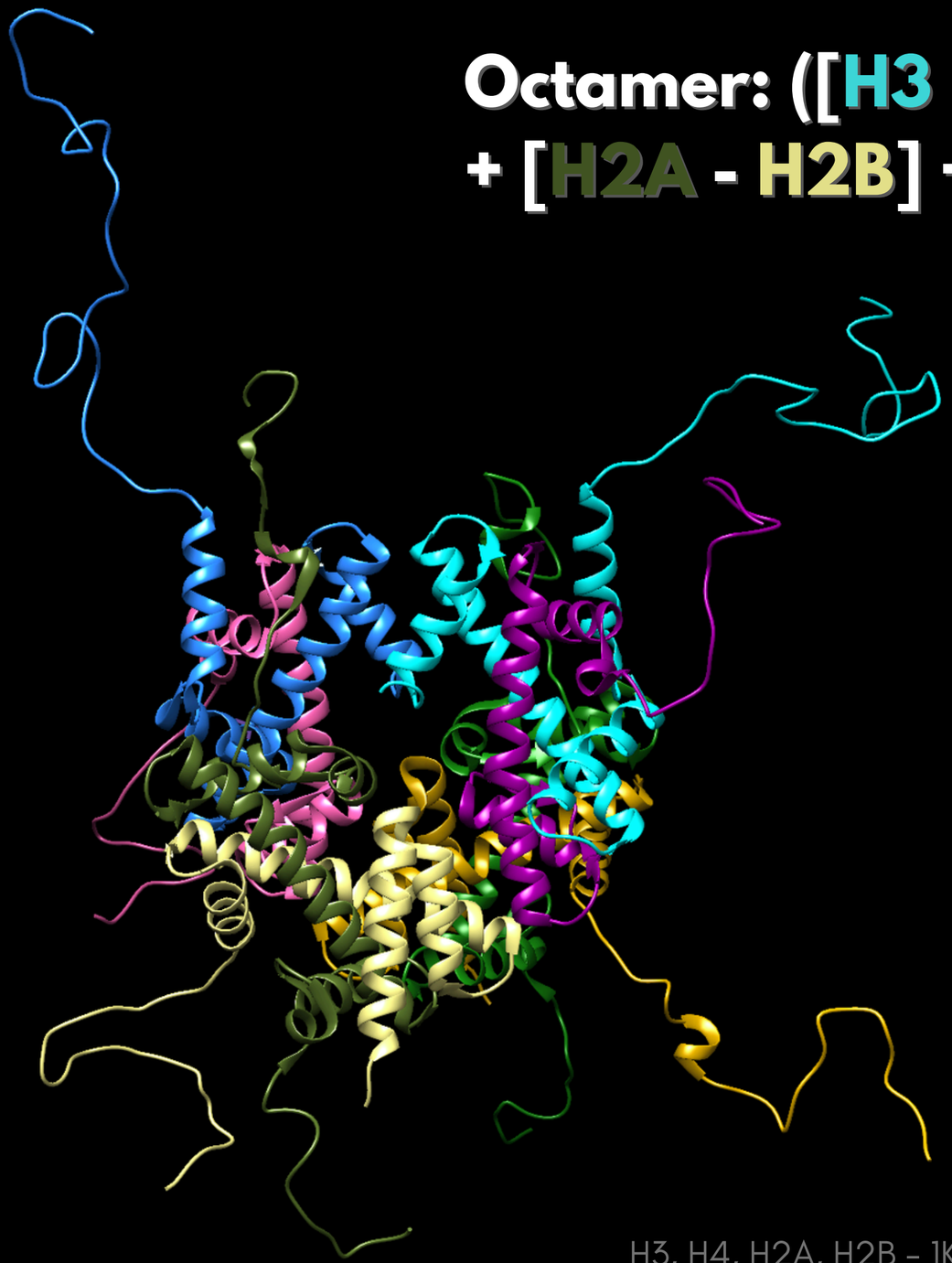






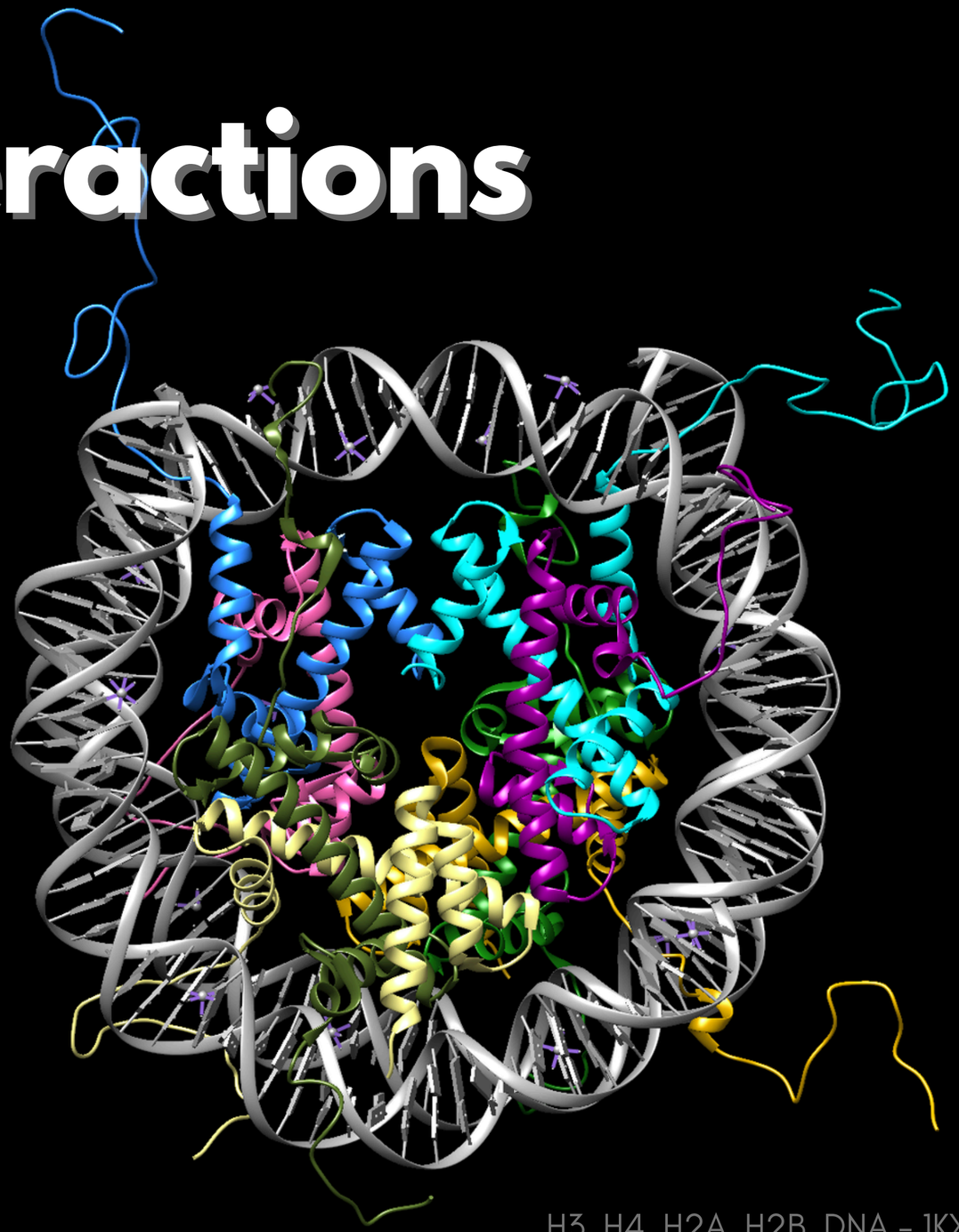


Octamer: ([H3 - H4] + [H3 - H4]) + [H2A - H2B] + [H2A - H2B]

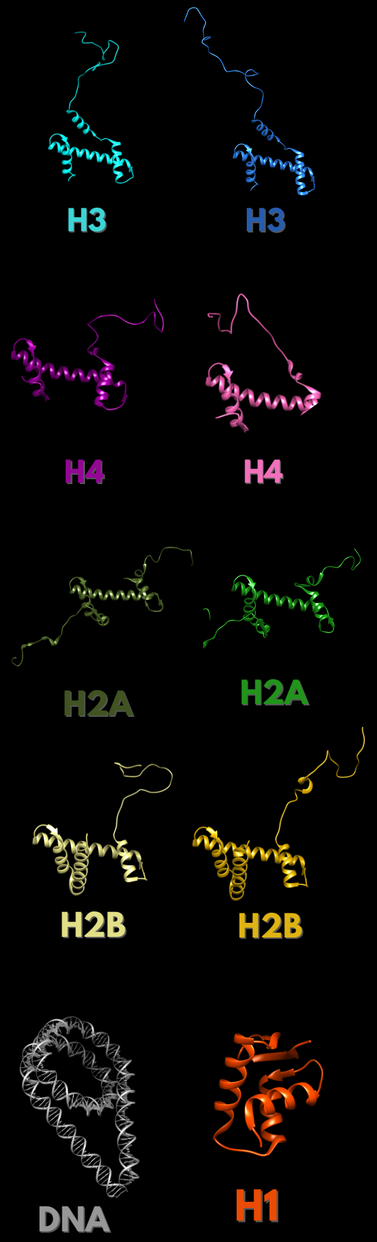


H3, H4, H2A, H2B - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]

DNA interactions



H3, H4, H2A, H2B, DNA – 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å]



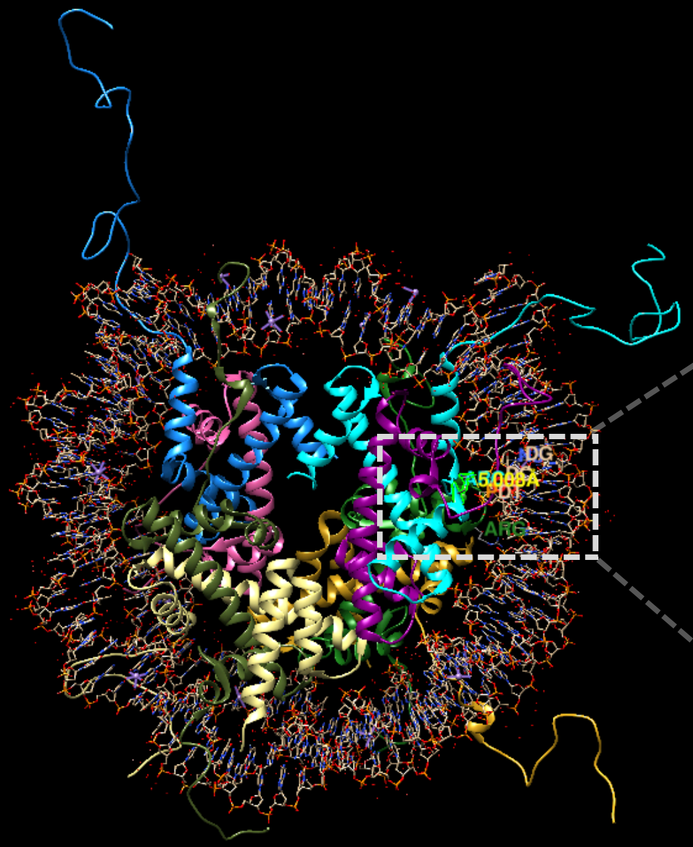
Octamer + DNA + H1

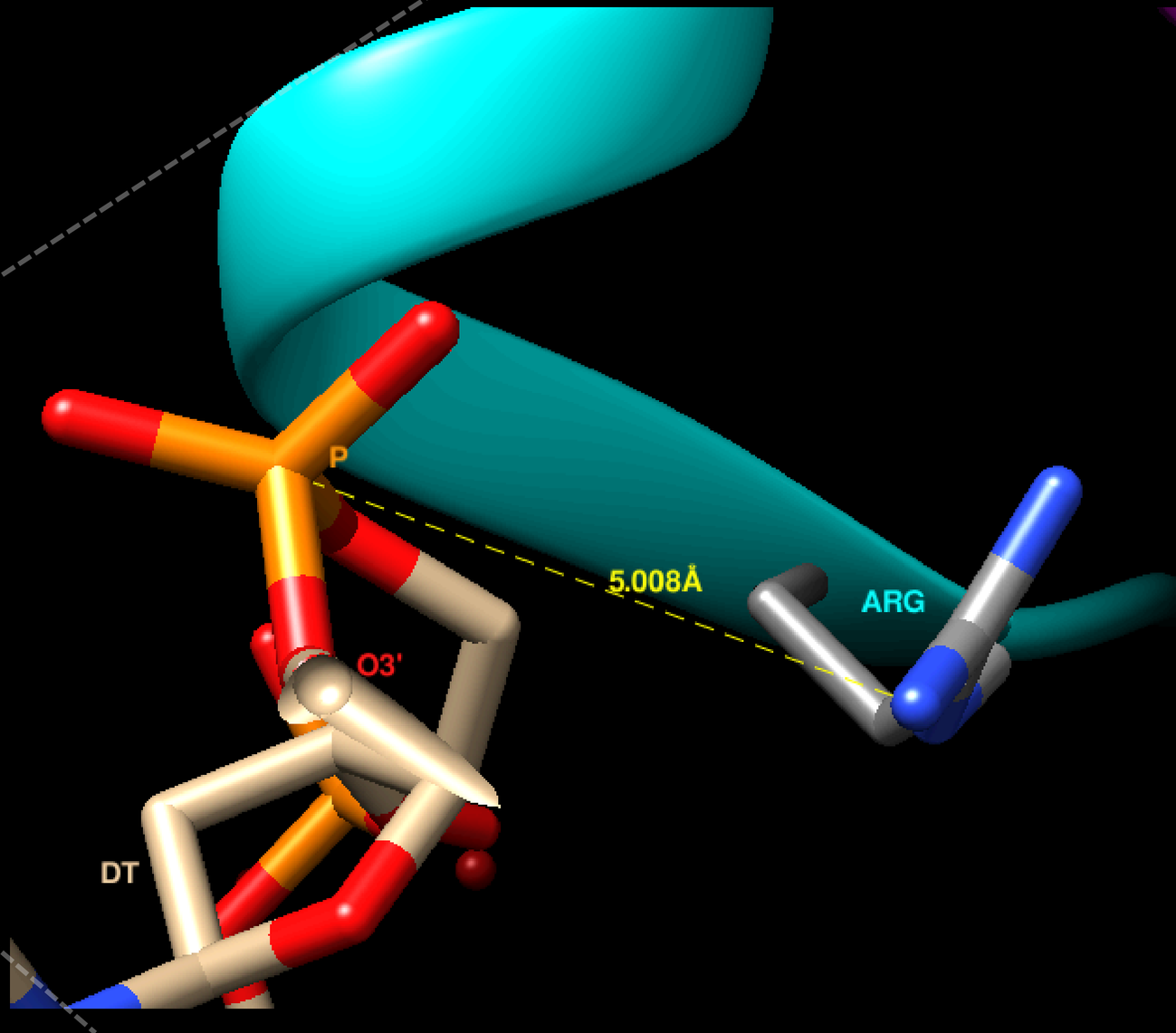
H3, H4, H2A, H2B - 1KX5 *Homo sapiens*, *Xenopus laevis* [1.9Å] / H3, H4, H2A, H2B, H1, DNA - 5NL0 *Xenopus laevis*, synthetic construct [5.4Å]

DNA interactions

Salt bridges

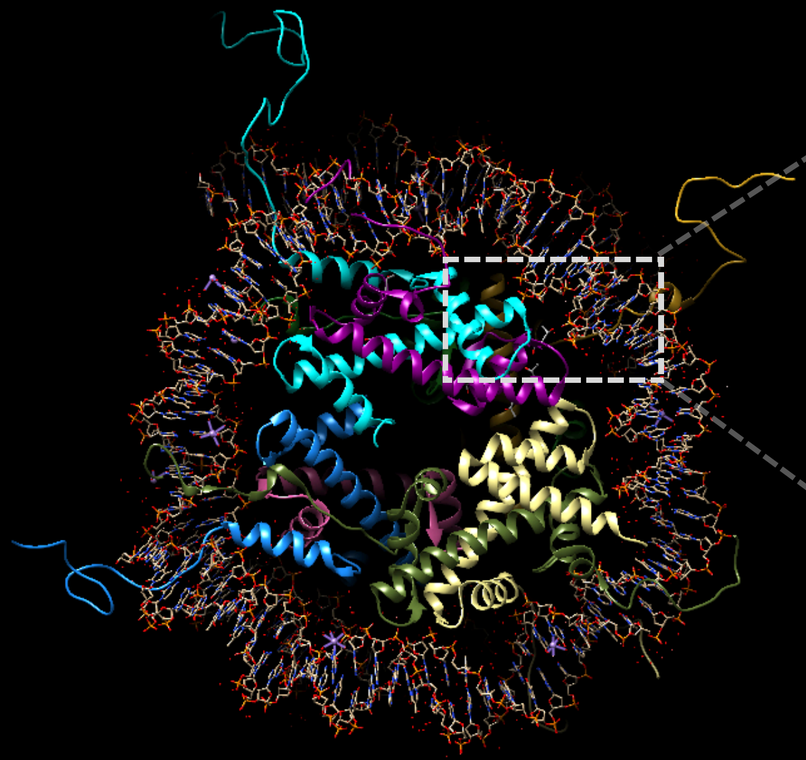
H3, ARG 63.A -
1KX5 Homo sapiens, Xenopus laevis [1.9Å]



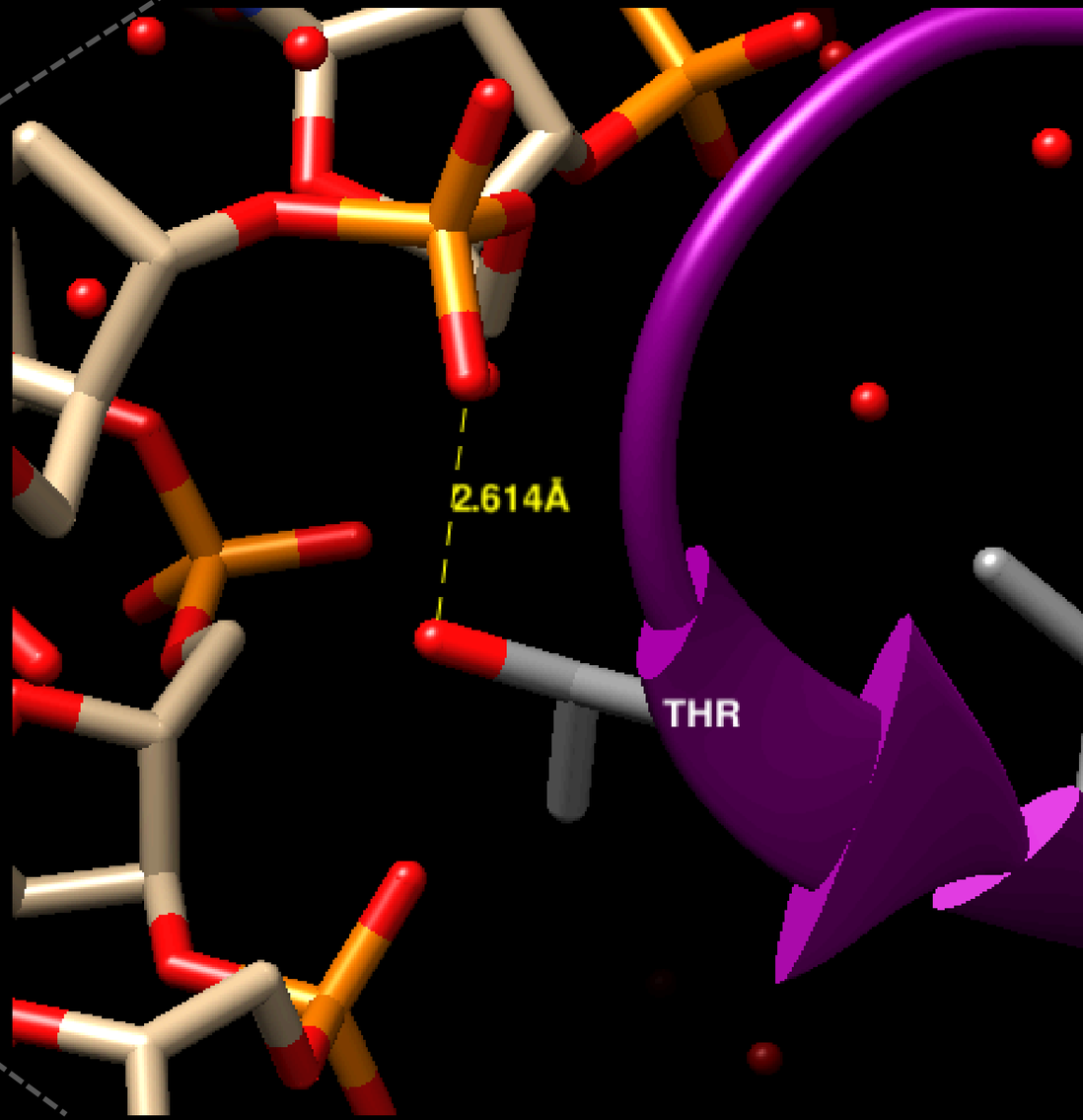


DNA interactions

Hydrogen bonds - direct interactions



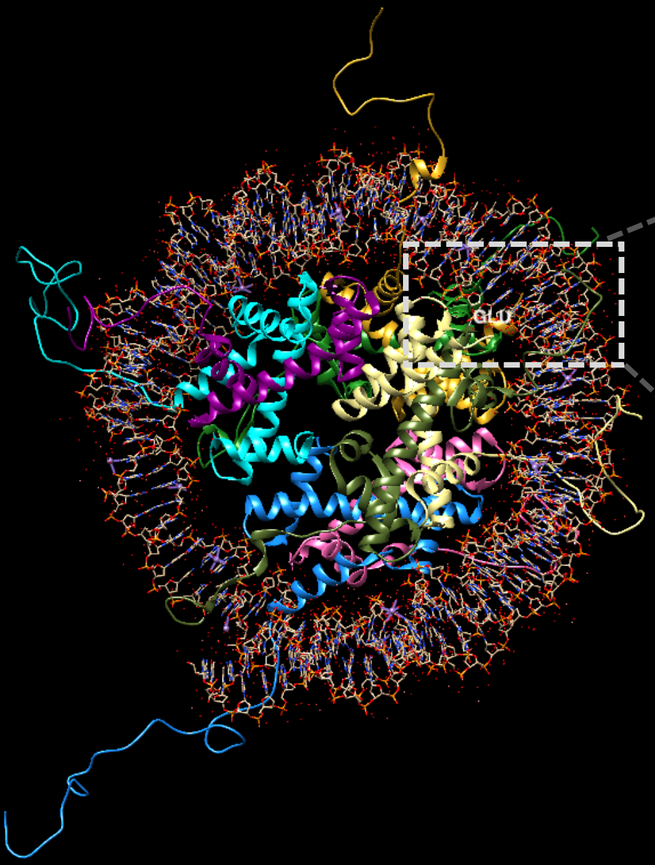
H4, THR 80.B -
1KX5 Homo sapiens, Xenopus laevis [1.9Å]

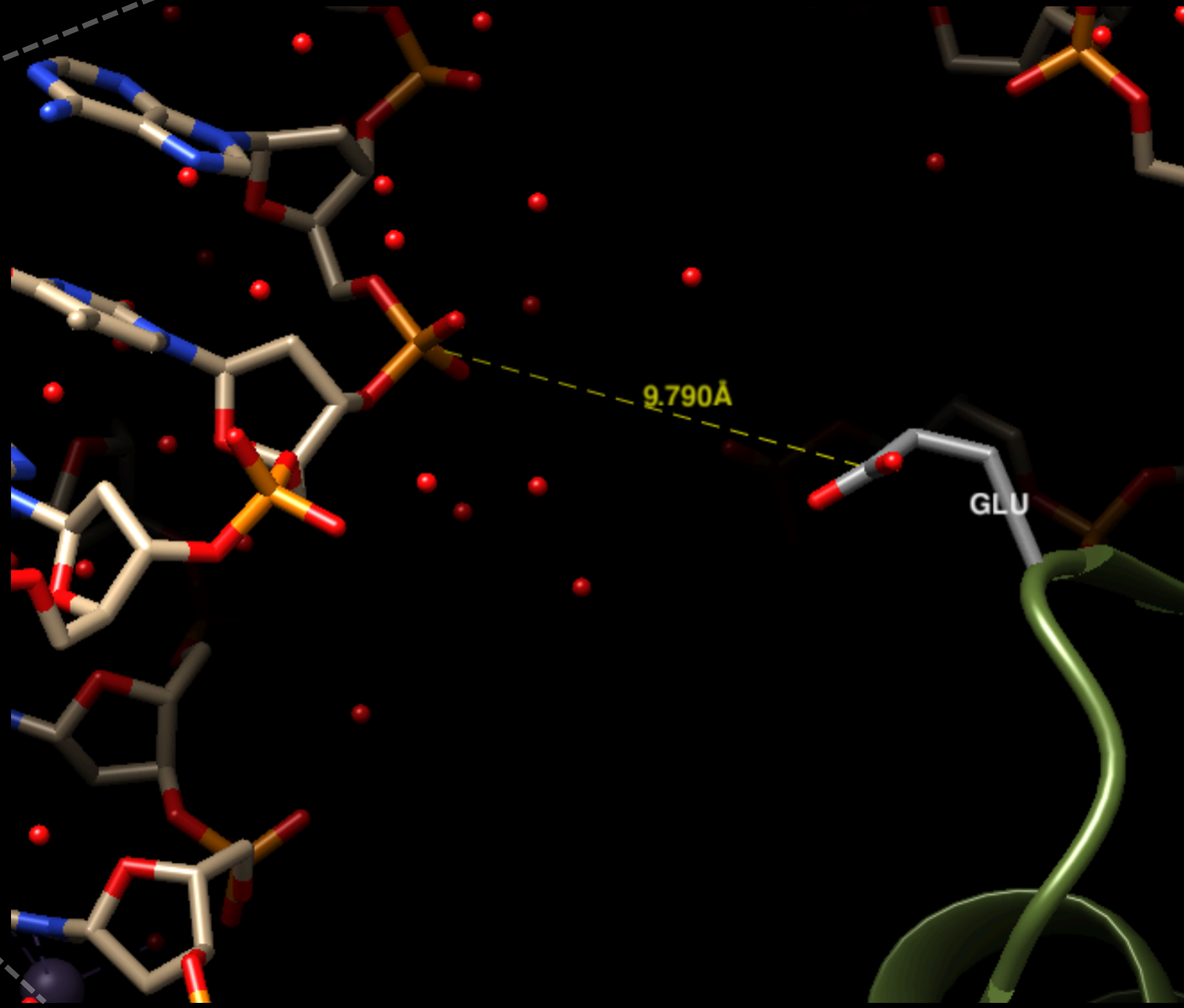


DNA interactions

Hydrogen bonds - Water-mediated interactions

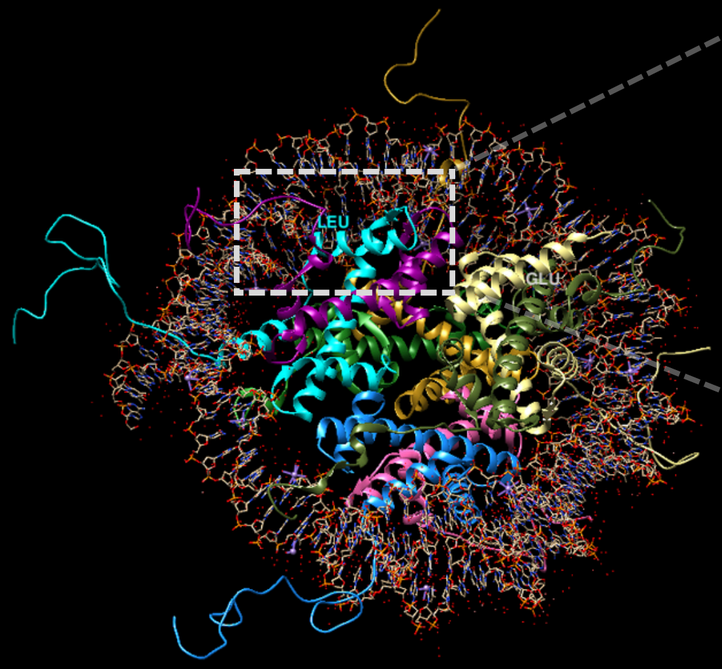
H2A, GLU 41.C -
1KX5 Homo sapiens, Xenopus laevis [1.9Å]



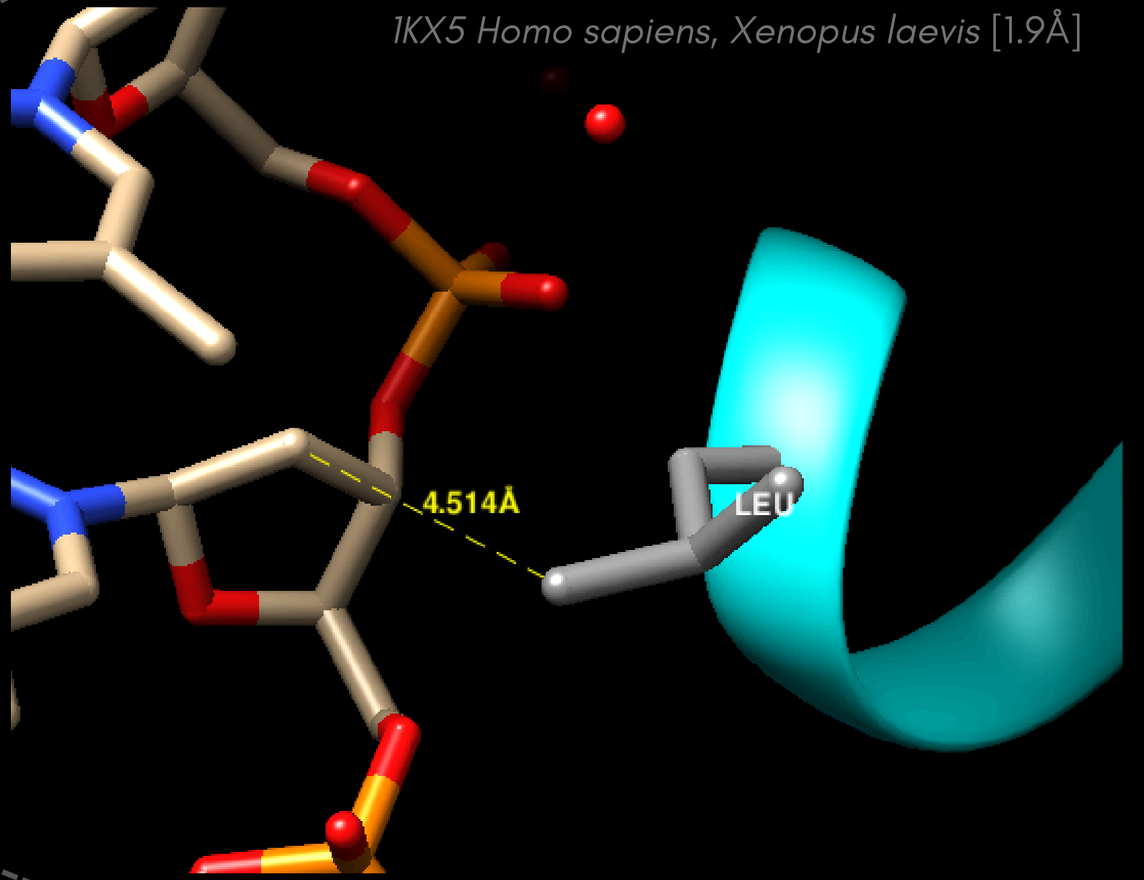


DNA interactions

Non-polar interactions



H3, LEU 65.A –
1KX5 Homo sapiens, Xenopus laevis [1.9Å]



Post-translational modifications

Gene regulation

PTMs

Methylation

The addition of a **methyl group** to a residue to mark as active or repress

Lysine, Arginine and Histidine residues

Acetylation

The addition of an **acetyl group** to a residue to reduce the positive charge

Lysine residues

Phsophorilation

The addition of a **phosphate group** to a residue increasing the negative charge

Serine, Tyrosine or Threonine residues

Histone methylation

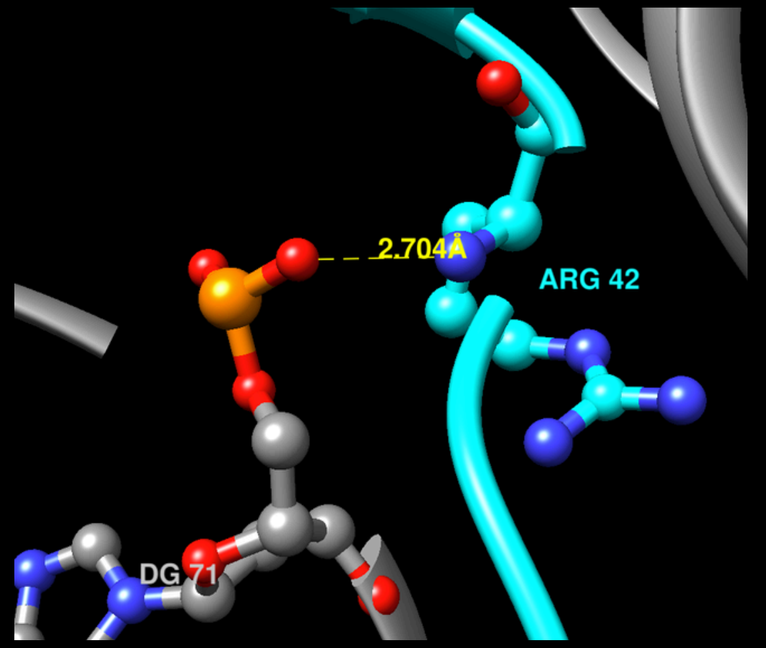
CONTEXT-DEPENDENT

- **Lysine (K)**, Arginine (R) and Histidine (H) of **H3** and **H4**
- Mono-, di-, and trimethylated (**me1**, **me2**, and **me3**)
  → H3K4me2 or H3K4me3

Active	**H3K4**, H3K36, and H3K79
Repress	H3K9, **H3K27**, and H4K20

H3K4me2 / H3K4me3 = active marks
H3K4me2 / H3K4me 3 + **ING2** = repress

H3K4me1 + **H3K27**me3 = enhancer repress



H3 - 1KX5 *Homo sapiens, Xenopus laevis* [1.9Å]

Histone methylation

- Lysine (K), Arginine (R) and Histidine (H) of **H3** and **H4**
- Mono-, di-, and trimethylated (**me1**, **me2**, and **me3**)
  → H3K4me2 or H3K4me3

Active — **H3K4**, H3K36, and H3K79

Repress — H3K9, **H3K27**, and H4K20

Histone acetylation

- Lysine (K) of **H3** and **H4**
  → **H3K4, H3K9, H3K27, H3K36, H3K79,** H4K5**,** H4K12**, H4K20** ...
- Inhibit the binding between histone tails and negatively charged DNA
- Antagonic histone marks with methylations

Reduce positive charge

H3 hyperacetylated → heavily transcribed regions
H3 hyposcetylated → silent regions (telomeres)

H3K4me1 + H3K27me3 = enhancer repress

H3K4me1 + **H3K27ac** = enhancer activated

Histone phosphorilation

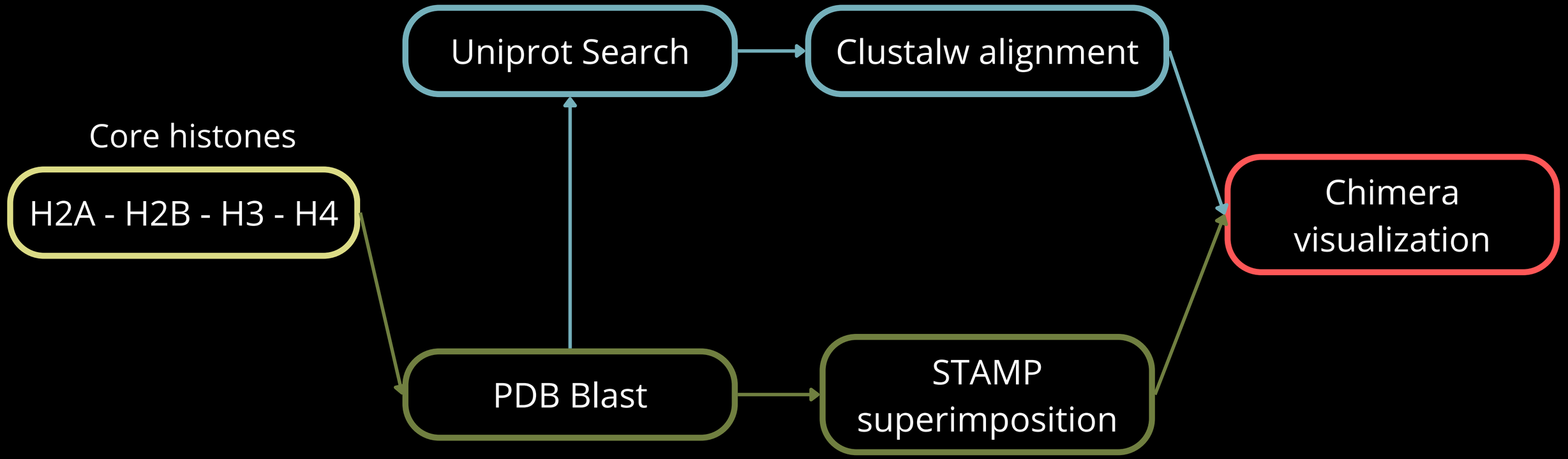
Increase negative charge

- Serine (S), Tyrosine (Y) and Threonine (T) residues
  → **H3S10, H3S28,** H3T3
- Gene expression, cell cycle regulation, DNA damage repair, and asymmetric cell division

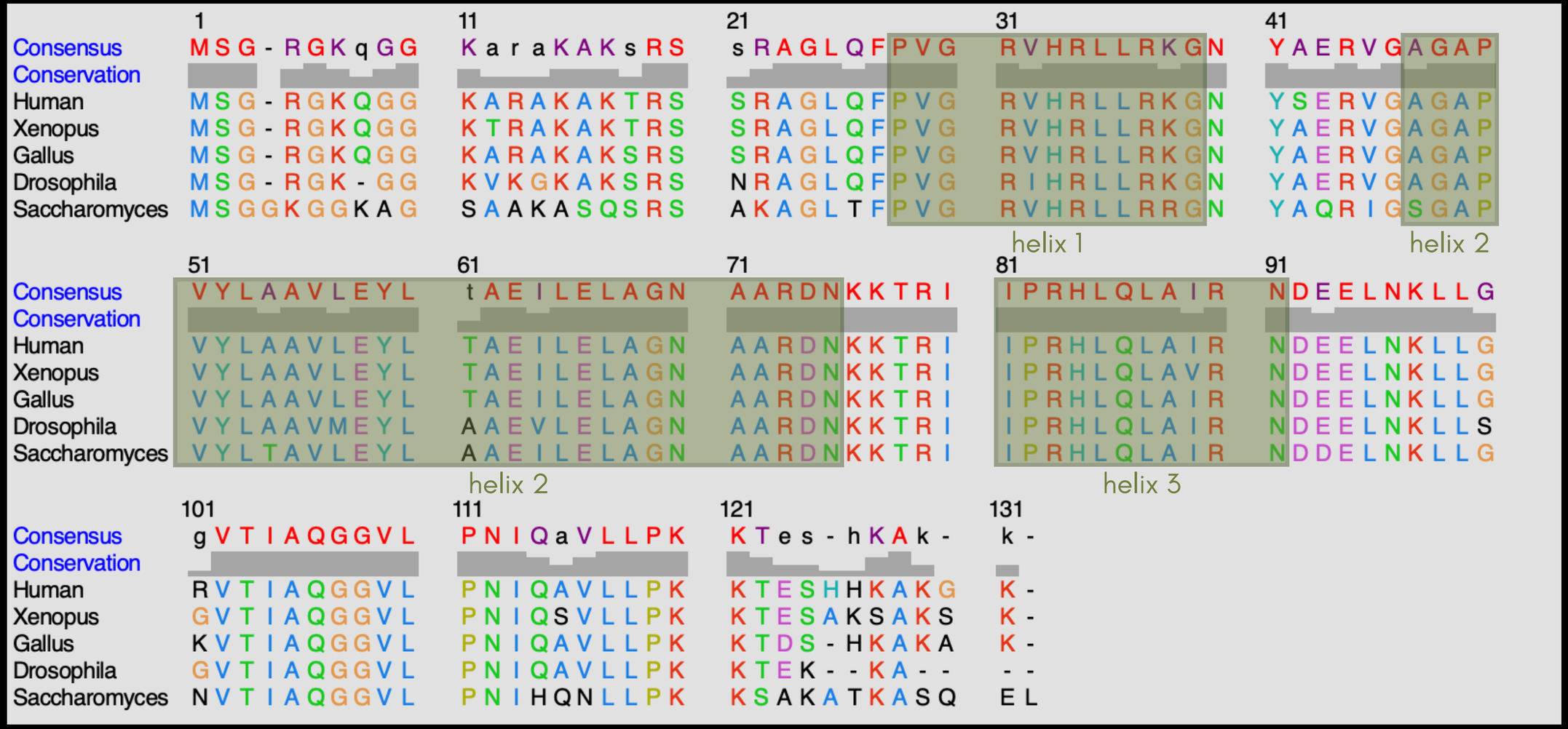


Evolution

Eukaryotic Evolution

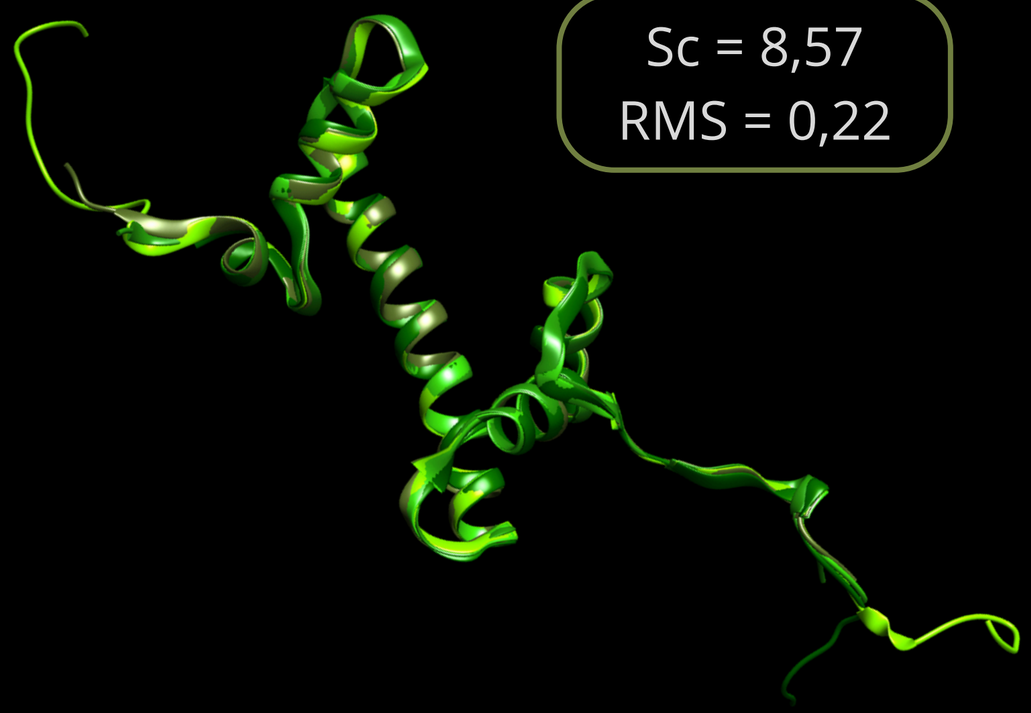


H2A

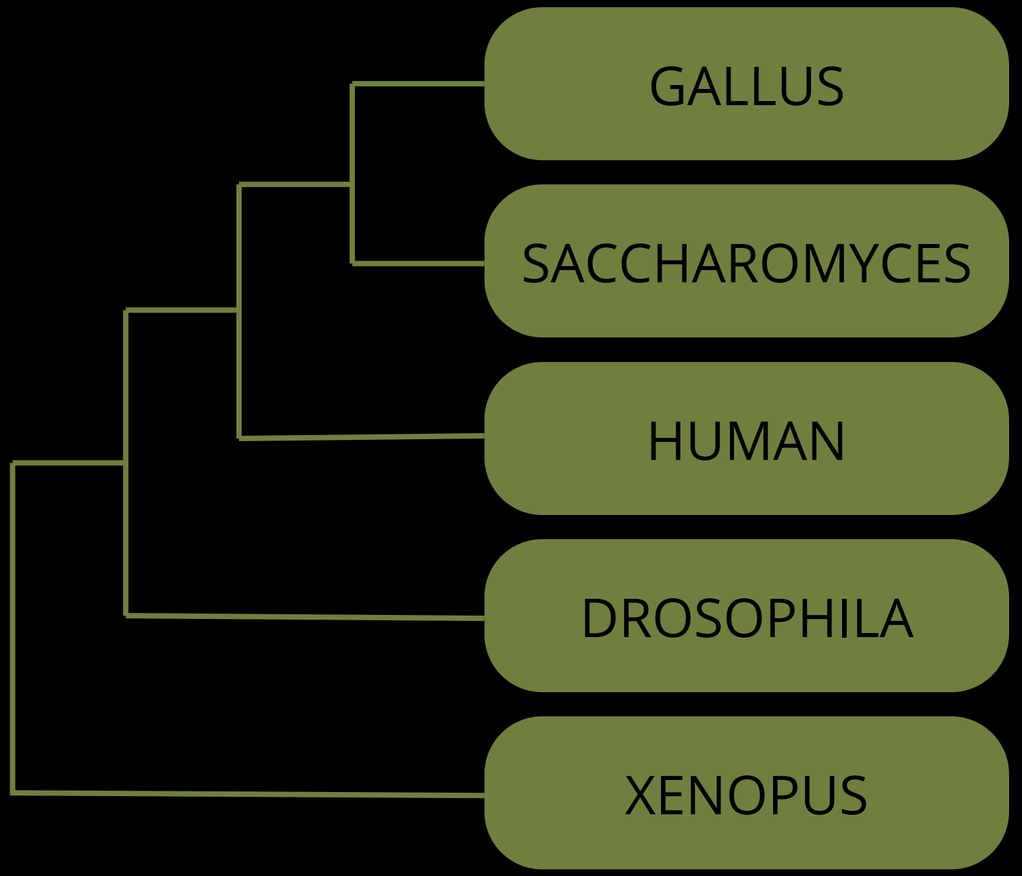


H2A (ClustalW&chimera) - UniProt - P04908 Homo sapiens, P06897 Xenopus laevis, P02263 Gallus gallus, P84051 Drosophila melanogaster, P04911 Saccharomyces Cerevisiae

H2A

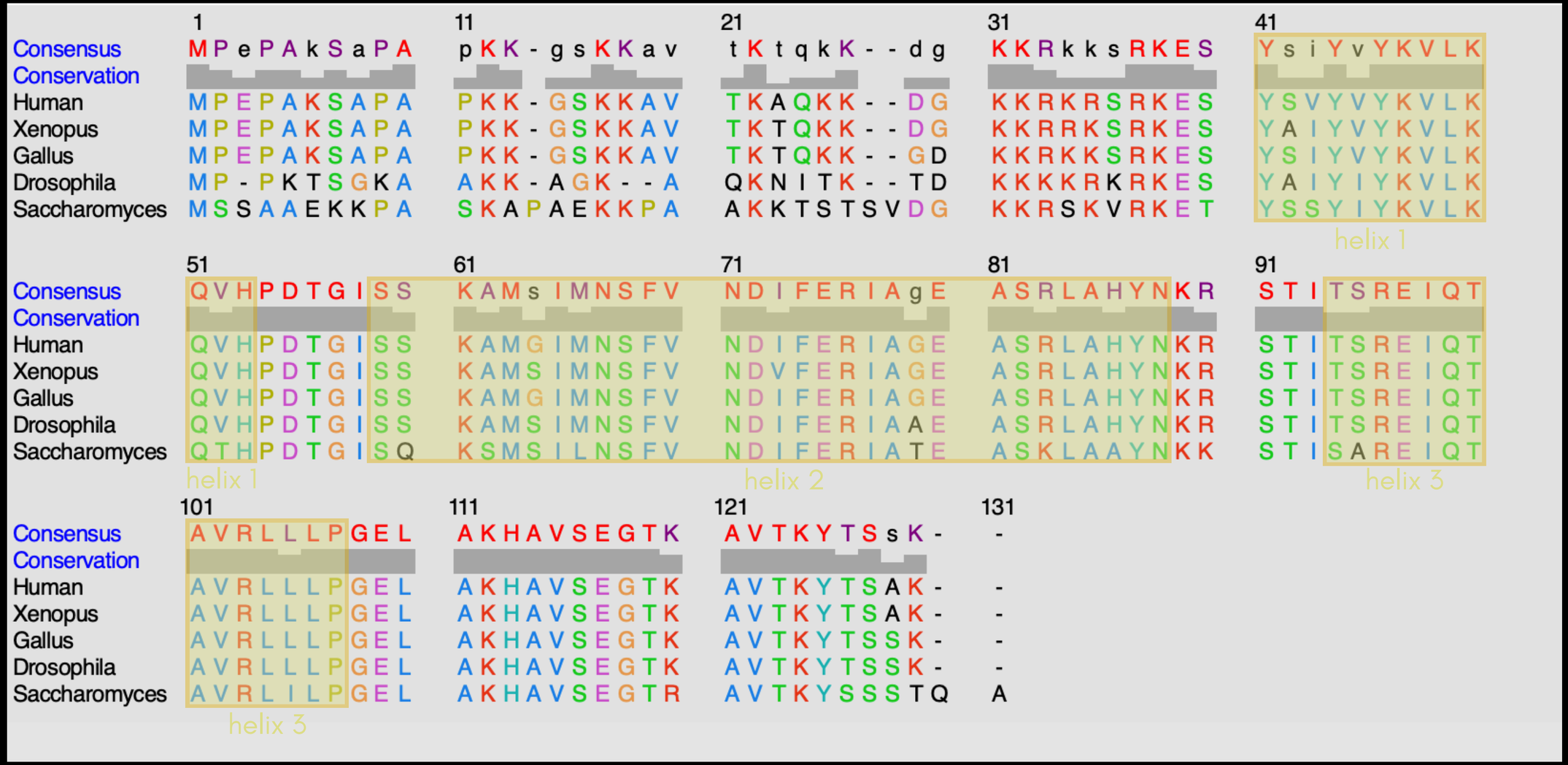


Sc = 8,57
RMS = 0,22



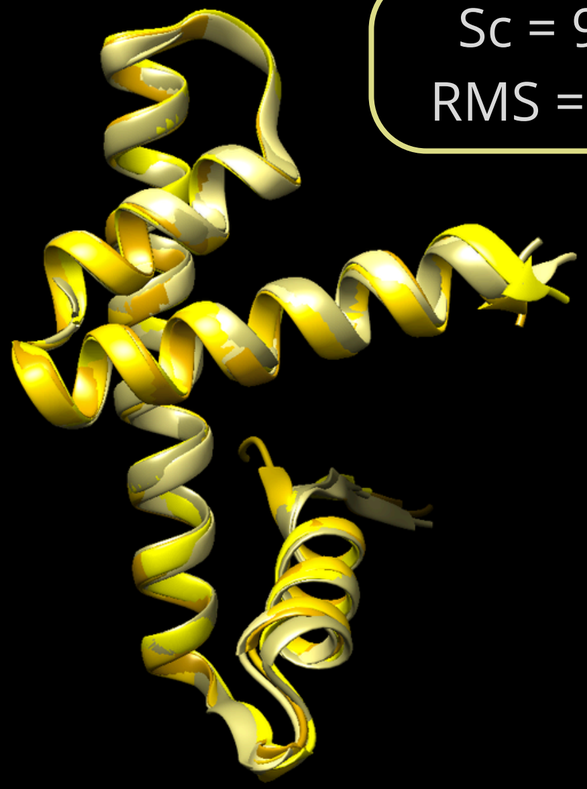
H2A (STAMP&chimera) - 2CV5 Homo sapiens [2,5 Å] , 2F8N Xenopus laevis [2,9 Å], 2ARO Gallus gallus [2,10 Å], 2NBQ Drosophila melanogaster [2,30 Å], 1ID3 Saccharomyces Cerevisiae [3,10 Å]

H2B

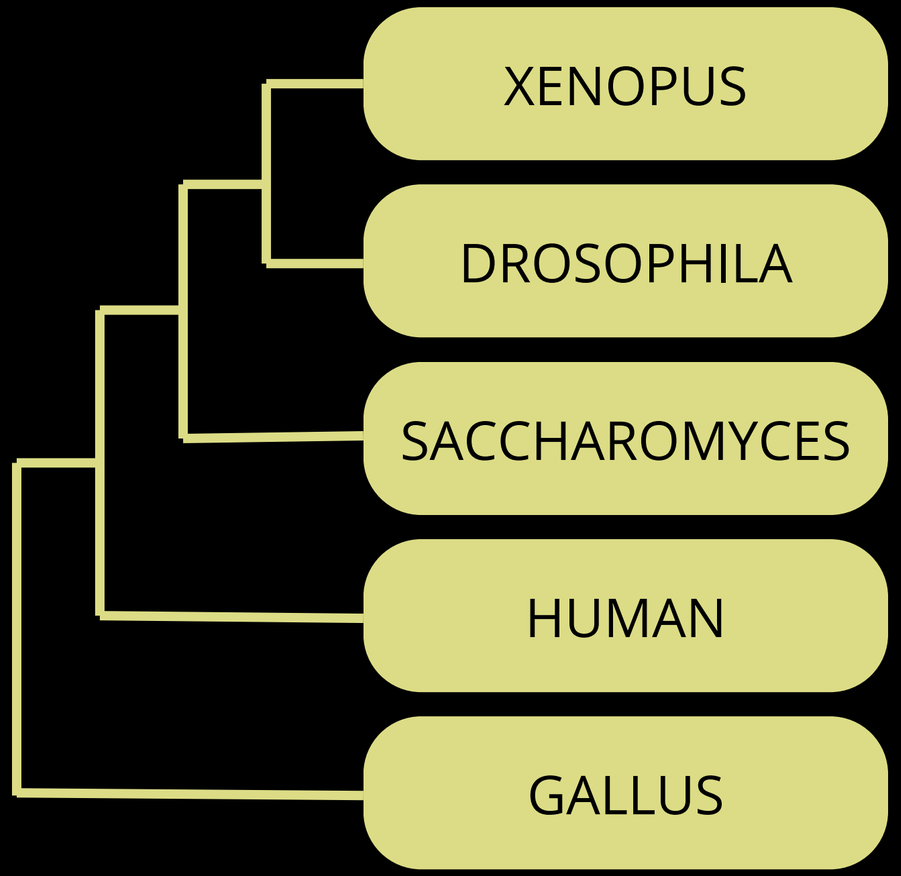


H2B (ClustalW&chimera) - UniProt -O60814 Homo sapiens, P02281 Xenopus laevis, P0C1H3 Gallus gallus, P02283 Drosophila melanogaster, P02294 Saccharomyces Cerevisiae

H2B

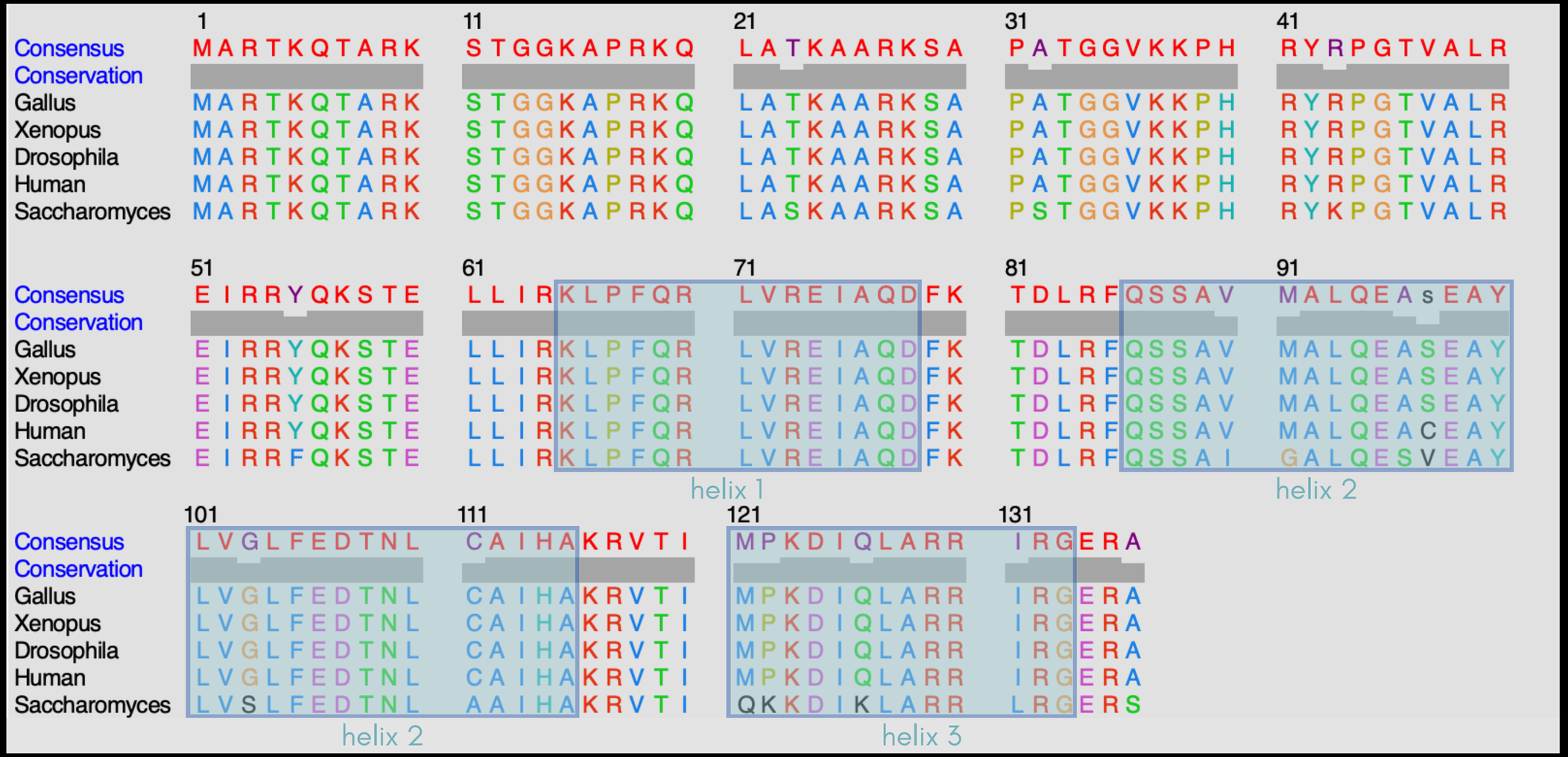


Sc = 9,12
RMS = 0,32



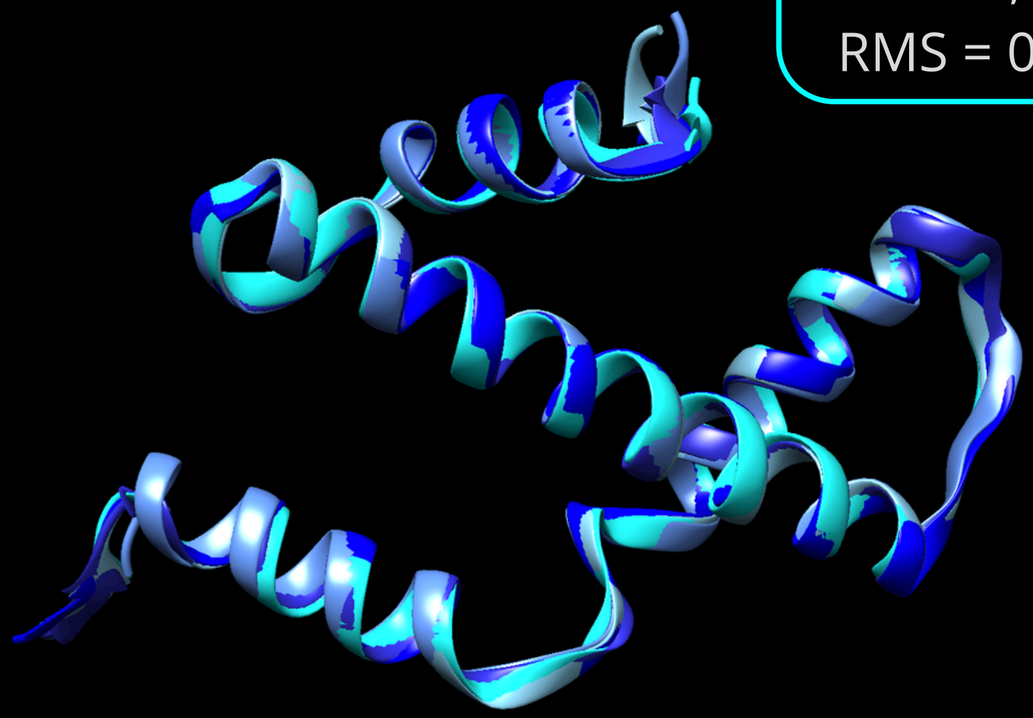
H2B (STAMP&chimera) - 2CV5 Homo sapiens [2,5 Å] , 2F8N Xenopus laevis [2,9 Å], 2ARO Gallus gallus [2,10 Å], 2NBQ Drosophila melanogaster [2,30 Å], 1ID3 Saccharomyces Cerevisiae [3,10 Å]

H3

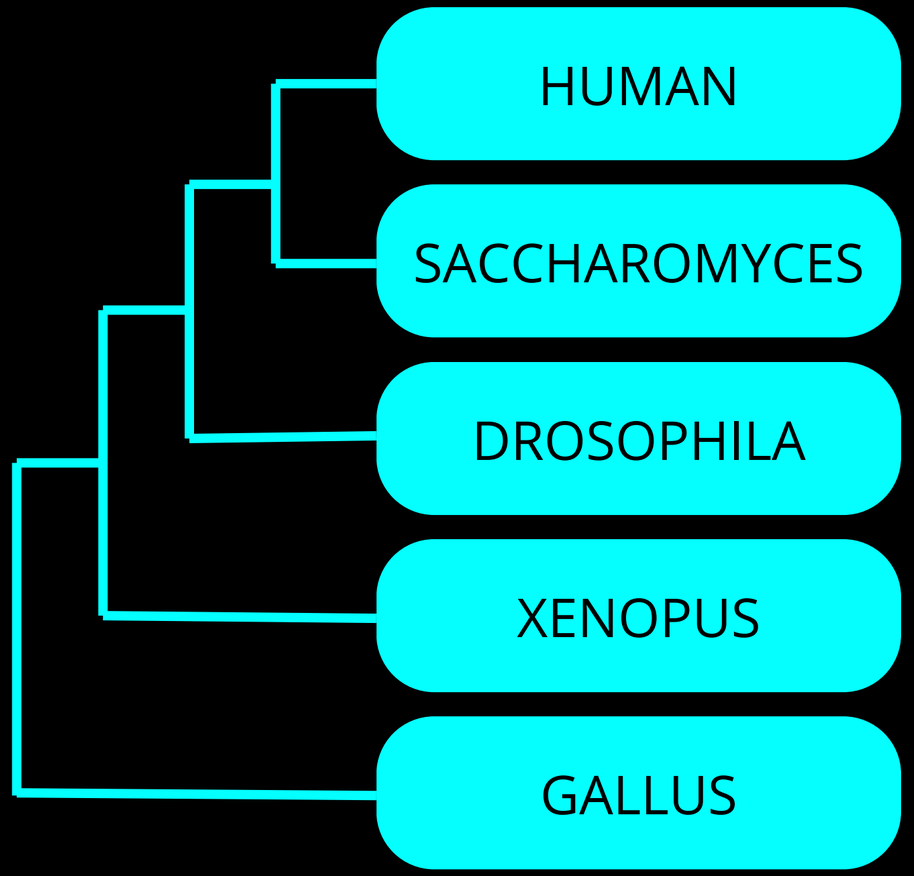


H3 (ClustalW&chimera) - UniProt - P68431 Homo sapiens, P84233 Xenopus laevis, P84229 Gallus gallus, P02299 Drosophila melanogaster, P61830 Saccharomyces Cerevisiae

H3

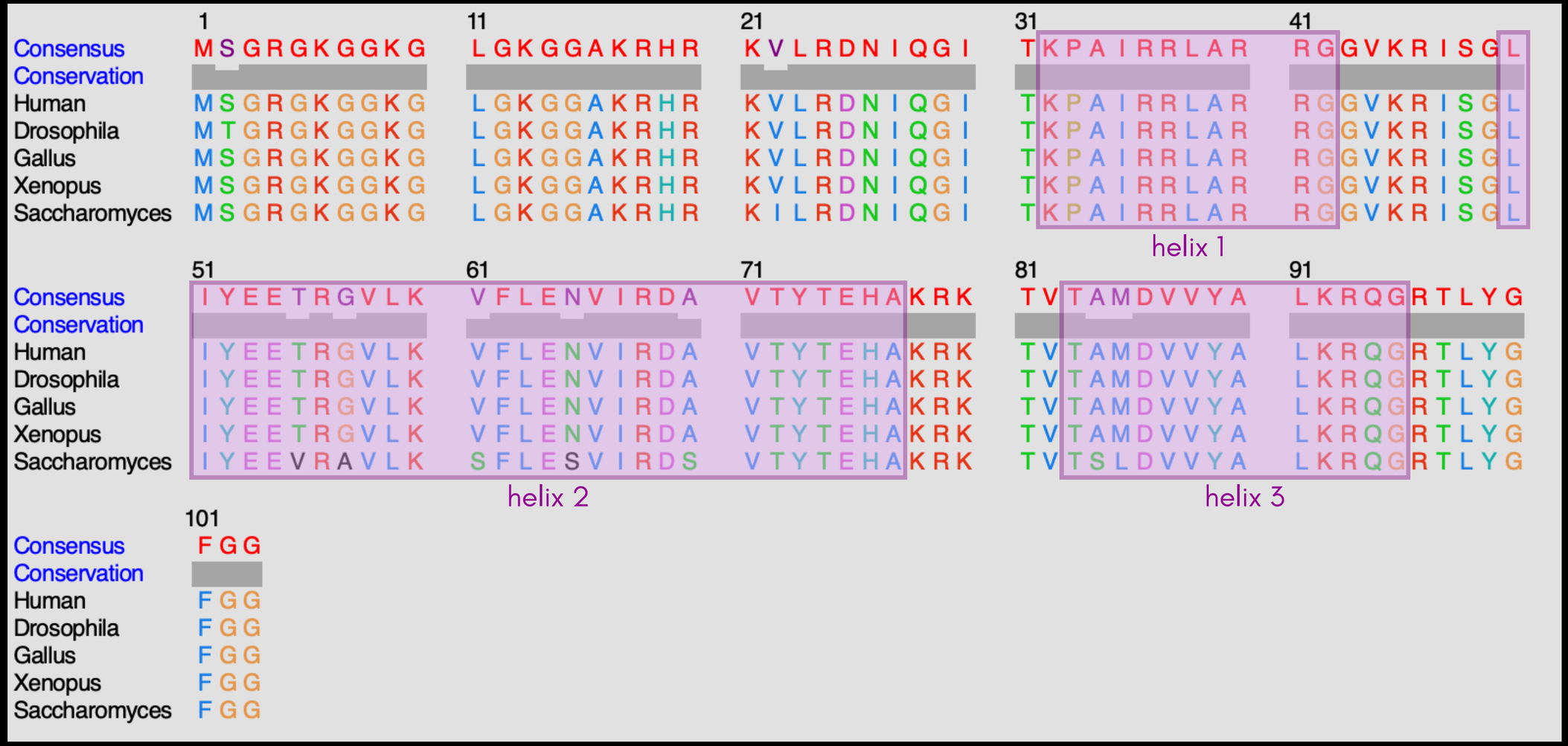


Sc = 9,40
RMS = 0,40



H3 (STAMP&chimera) - 2CV5 Homo sapiens [2,5 Å] , 2F8N Xenopus laevis [2,9 Å], 2ARO Gallus gallus [2,10 Å], 2NBQ Drosophila melanogaster [2,30 Å], 1ID3 Saccharomyces Cerevisiae [3,10 Å]

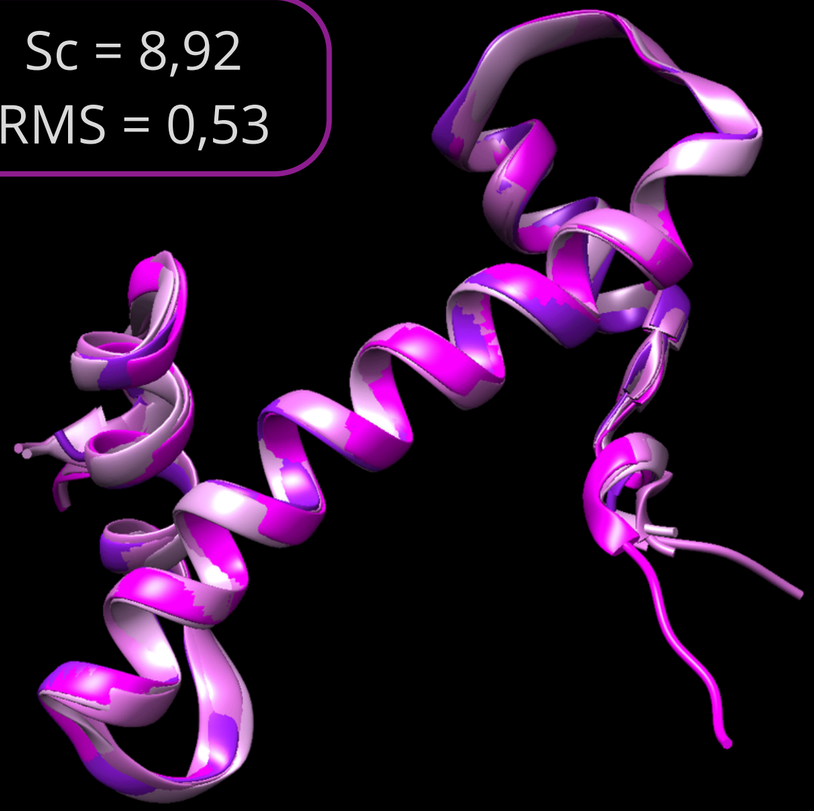
H4

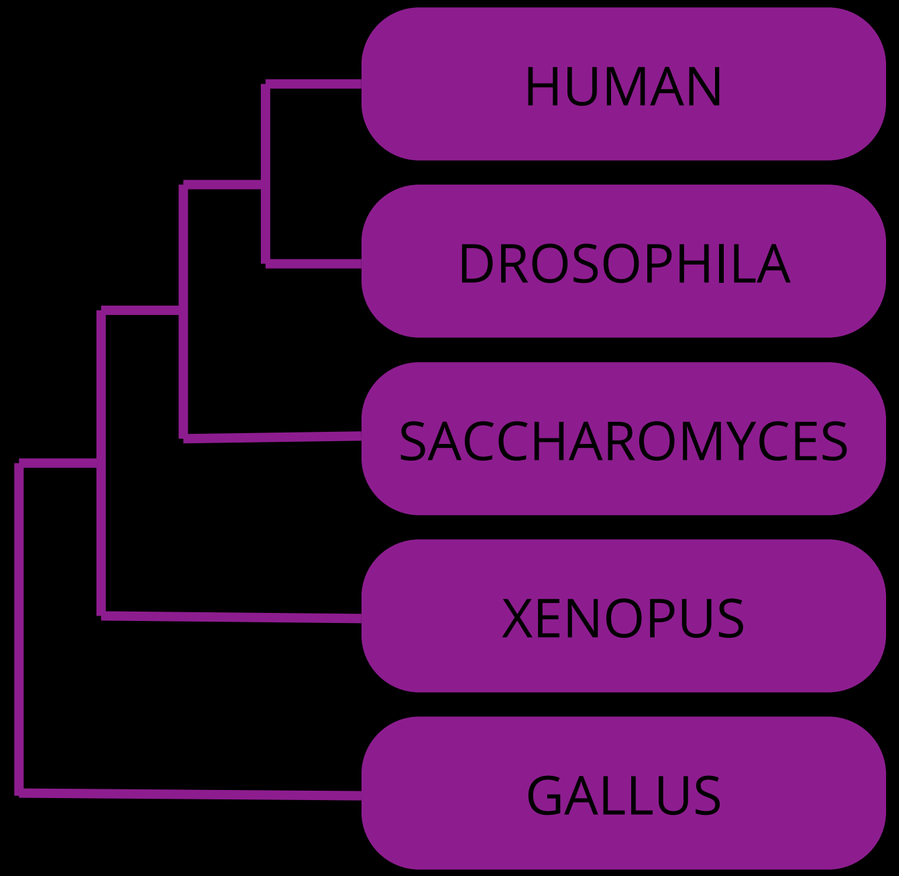


H4 (ClustalW&chimera) – UniProt – P62805 Homo sapiens, P62799 Xenopus laevis, P62801 Gallus gallus, P84040 Drosophila melanogaster, P02309 Saccharomyces Cerevisiae

H4

Sc = 8,92
RMS = 0,53

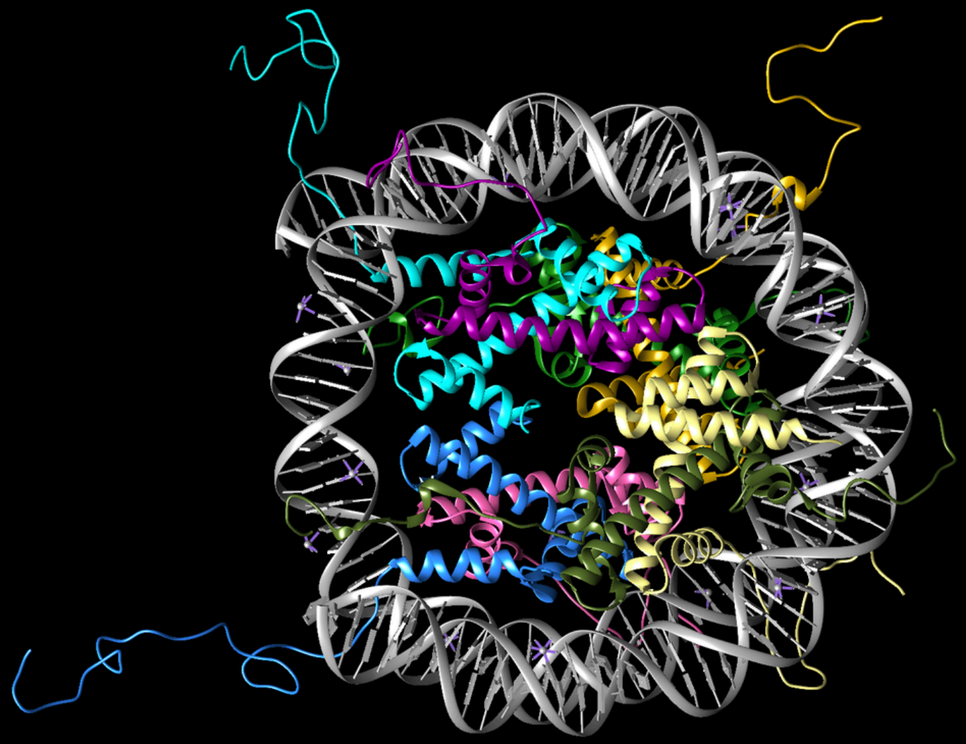




H4 (STAMP&chimera) - 2CV5 Homo sapiens [2,5 Å] , 2F8N Xenopus laevis [2,9 Å], 2ARO Gallus gallus [2,10 Å], 2NBQ Drosophila melanogaster [2,30 Å], 1ID3 Saccharomyces Cerevisiae [3,10 Å]

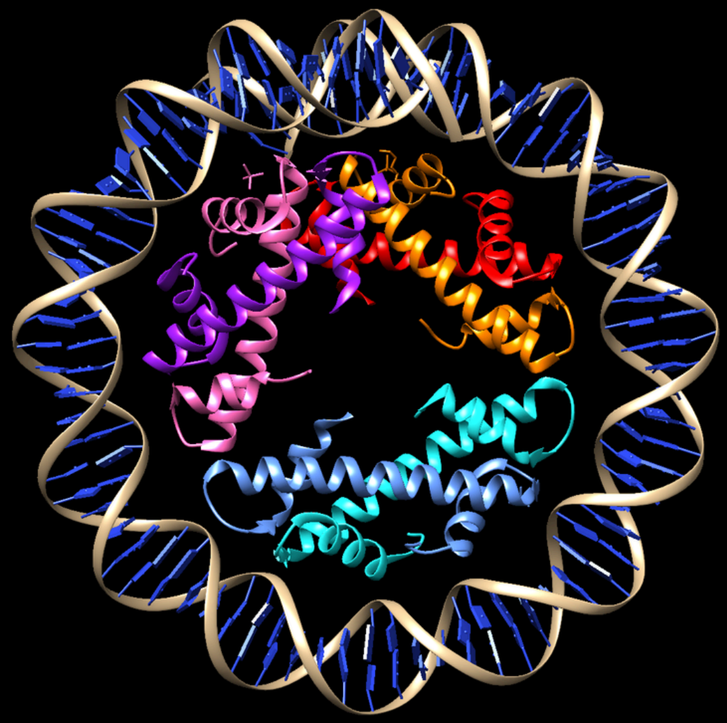
Histones across the tree of life

Octamer complex
(Eukaryotic lineage)



1KX5 - Homo sapiens, Xenopus laevis [1.9 Å]

Multidimer complex
(Archaeal lineage)



5T5K - Methanothermus fervidus [4.0 Å]

Histones across the tree of life

Dimer complex
(**Bacterial lineage**)

Protein HLp has
histone fold



9QT1 - Leptospira perolatii [2.10 Å]

Take home message

- Building of the structure:
  - Hydrophobic interactions between the central alpha helix
  - Hydrogen bonds between the loops

- PTMs are higly important in the functions of stability and the gene expression.

- Evolution: high level of conservation among eukaryotic species.

- Importance of their structural function.

Structural Biology, UPF 25-26

Thank you for your attention

Victoria de Diego, Alba García, Pau Giner, Roger Mayol

Bibliography

1. Arents, G., & Moudrianakis, E. N. (1995). The histone fold: A ubiquitous architectural motif utilized in DNA compaction and protein dimerization. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(24), 11170–11174. https://doi.org/10.1073/pnas.92.24.11170
2. Bednar, J., Garcia-Saez, I., Boopathi, R., Cutter, A. R., Papai, G., Reymer, A., Syed, S. H., Lone, I. N., Tonchev, O., Crucifix, C., Menoni, H., Papin, C., Skoufias, D. A., Kurumizaka, H., Lavery, R., Hamiche, A., Hayes, J. J., Schultz, P., Angelov, D., … Dimitrov, S. (2017). Structure and Dynamics of a 197 bp Nucleosome in Complex with Linker Histone H1. *Molecular Cell*, *66*(3), 384–397.e8. https://doi.org/10.1016/j.molcel.2017.04.012
3. Cutter, A. R., & Hayes, J. J. (2015). A brief review of nucleosome structure. *FEBS Letters*, *589*(20PartA), 2914–2922. https://doi.org/10.1016/j.febslet.2015.05.016
4. Davey, C. A., Sargent, D. F., Luger, K., Maeder, A. W., & Richmond, T. J. (2002). Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. *Journal of Molecular Biology*, *319*(5), 1097–1113. https://doi.org/10.1016/S0022-2836(02)00386-8
5. Greer, E. L., & Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. *Nature Reviews Genetics*, *13*(5), 343–357. https://doi.org/10.1038/nrg3173
6. Hu, Y., Schwab, S., Qiu, K., Zhang, Y., Bär, K., Reichle, H., Panzera, A., Lupas, A. N., Hartmann, M. D., Dame, R. T., Alva, V., & Alvarez, B. H. (2025). DNA Wrapping by a tetrameric bacterial histone. *Nature Communications*, *16*(1), 11108. https://doi.org/10.1038/s41467-025-67425-w
7. Ishibashi, T., Li, A., & Ausió, J. (2010). Histone variants. En *Elsevier eBooks* (pp. 2409–2425). https://doi.org/10.1016/b978-0-12-374145-5.00289-8
8. Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., & Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, *389*(6648), 251–260. https://doi.org/10.1038/38444
9. Luger, K., Mäder, A., Sargent, D. F., & Richmond, T. J. (2000). The Atomic Structure of the Nucleosome Core Particle. *Journal of Biomolecular Structure and Dynamics*, *17*(sup1), 185–188. https://doi.org/10.1080/07391102.2000.10506619
10. Mattiroli, F., Bhattacharyya, S., Dyer, P. N., White, A. E., Sandman, K., Burkhart, B. W., Byrne, K. R., Lee, T., Ahn, N. G., Santangelo, T. J., Reeve, J. N., & Luger, K. (2017). Structure of histone-based chromatin in Archaea. *Science*, *357*(6351), 609–612. https://doi.org/10.1126/science.aaj1849
11. McGinty, R. K., & Tan, S. (2015). Nucleosome Structure and Function. *Chemical Reviews*, *115*(6), 2255–2273. https://doi.org/10.1021/cr500373h
12. Onufriev, A. V., & Schiessel, H. (2019). The nucleosome: From structure to function through physics. *Current Opinion in Structural Biology*, *56*, 119–130. https://doi.org/10.1016/j.sbi.2018.11.003
13. Tessarz, P., & Kouzarides, T. (2014). Histone core modifications regulating nucleosome structure and dynamics. *Nature Reviews Molecular Cell Biology*, *15*(11), 703–708. https://doi.org/10.1038/nrm3890
14. Tsunaka, Y., Furukawa, A., & Nishimura, Y. (2022). Histone tail network and modulation in a nucleosome. *Current Opinion in Structural Biology*, *75*, 102436. https://doi.org/10.1016/j.sbi.2022.102436
15. Zhang, Y., Sun, Z., Jia, J., Du, T., Zhang, N., Tang, Y., Fang, Y., & Fang, D. (2021). Overview of Histone Modification. In D. Fang & J. Han (Eds.), *Histone Mutations and Cancer* (Vol. 1283, pp. 1–16). Springer Singapore. https://doi.org/10.1007/978-981-15-8104-5_1

Questions

1. **Which of the following statements is correct regarding the structure of the histone fold?**
   a. It consists of 4 helices, three loops, and one beta strand.
   b. It has a flexible structure that changes during the cell cycle.
   c. It differs greatly between eukaryotic species.
   d. It is conserved among eukaryotic species and also archaebacteria.
   e. None is correct.
2. **What is the function of conserved residues such as arginine and lysine in the interaction of the histone fold with DNA?**
   a. They facilitate electrostatic interactions with DNA.
   b. They allow the formation of covalent bonds between histones.
   c. They do not influence the binding between histones and DNA.
   d. They destabilize the three-dimensional structure of the nucleosome.
   e. They modulate DNA condensation.
3. **Why is alanine a conserved residue in the second turn of helix III in the histone fold?**
   a. Its negative charge contributes to interactions with DNA.
   b. Its small size allows helices II and III to be very close, stabilizing the structure.
   c. It facilitates the formation of disulfide bonds between histones.
   d. Its large size allows a precise fit between the helices.
   e. It has no functional role in the structure of the histone fold.
4. **What is the main difference between the histone fold of H2A and that of other histones like H3, H4, and H2B?**
   a. The histone fold of H2A has a different orientation of helix I.
   b. The histone fold of H2A lacks helices in its structure.
   c. The histone fold of H2A does not differ from the others.
   d. The histone fold of H2A has one more helix.
   e. None is correct.

Questions

5. **What is the main role of the H3-H4 dimers in the formation of the nucleosome?**
    a. They form the "handshake interaction" with H2A-H2B dimers.
    b. They interact to form a tetramer that binds with H2A-H2B dimers.
    c. They help in the formation of the H2A-H2B heterodimer.
    d. They stabilize the linker histone H1.
    e. They form a hydrophobic cluster with tyrosine residues.

6. What types of interactions occur between the H3 and H4 histones in the nucleosome core?
    a. Hydrogen bonds between the alpha 2 helix and the loops, and hydrophobic interactions between the loops.
    b. π-π stacking interaction the loops and non-polar interactions between the alpha 2 helix.
    c. Hydrogen bonds between the loops and electrostatic interactions between the alpha 2 helix.
    d. Hydrogen bonds between the loops and hydrophobic interactions between the alpha 2 helix.
    e. Electrostatic interactions between the H3 and H4 backbone.

7. Which of the following relations regarding the complexes created between histones in different lineages are correct?
    a. Multidimer complex - Archaeal lineage
    b. Dimer complex - Bacterial lineage
    c. Octamer complex - Eukaryotic lineage
    d. None of the above.
    e. All the answers are correct.

Questions

8. Regarding evolution, indicate the TRUE statement:

a. All core histones are 100% conserved.

b. For the different eukaryotic species, all four histones are highly conserved in terms of structure and sequence.

c. Histone tails are the most conserved regions of the sequence.

d. The four core histones have a high percentage identity.

e. All answers are correct

9. **Which of the following statements best describes histone methylation?**

a. Histone methylation always activates gene transcription.

b. Histone methylation always represses gene transcription.

c. Histone methylation is irreversible once it occurs.

d. The effect of histone methylation depends on the residue modified and the degree of methylation.

e. Histone methylation only occurs on lysine residues.

10. Which of the following statements correctly explains how histone acetylation influences chromatin structure?

a. It increases the positive charge of lysine residues, strengthening DNA-histone interaction.

b. It reduces the positive charge of lysine residues, weakening DNA-histone interaction and increasing DNA accessibility.

c. It adds methyl groups to arginine residues, leading to chromatin condensation.

d. It makes histone-DNA interactions irreversible.

e. It only occurs in transcriptionally silent regions of the genome