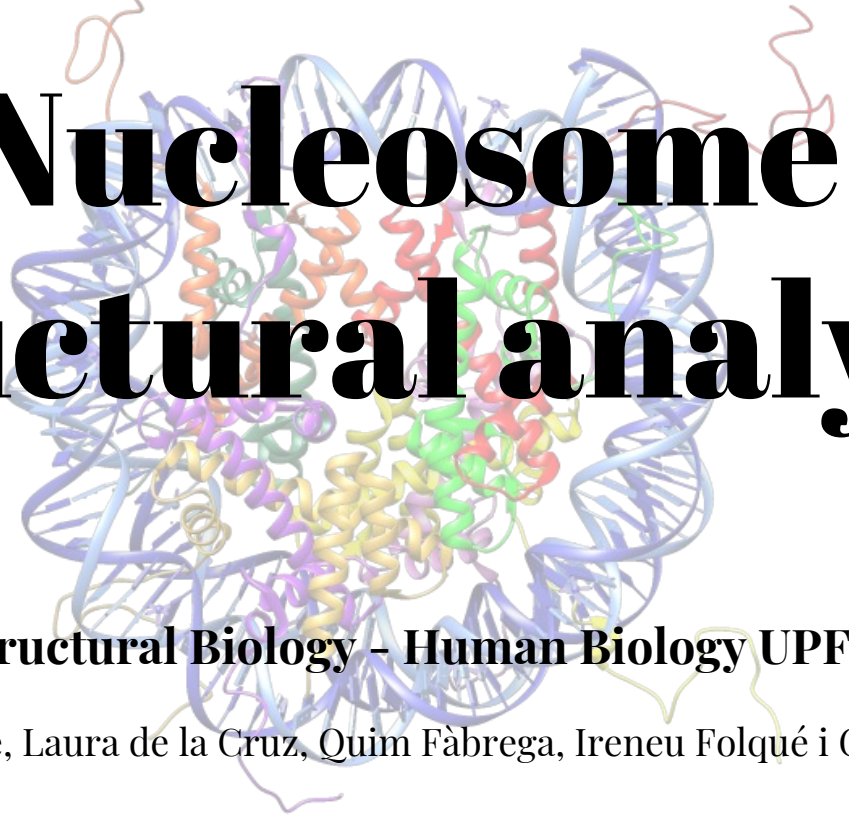
Nucleosome structural analysis

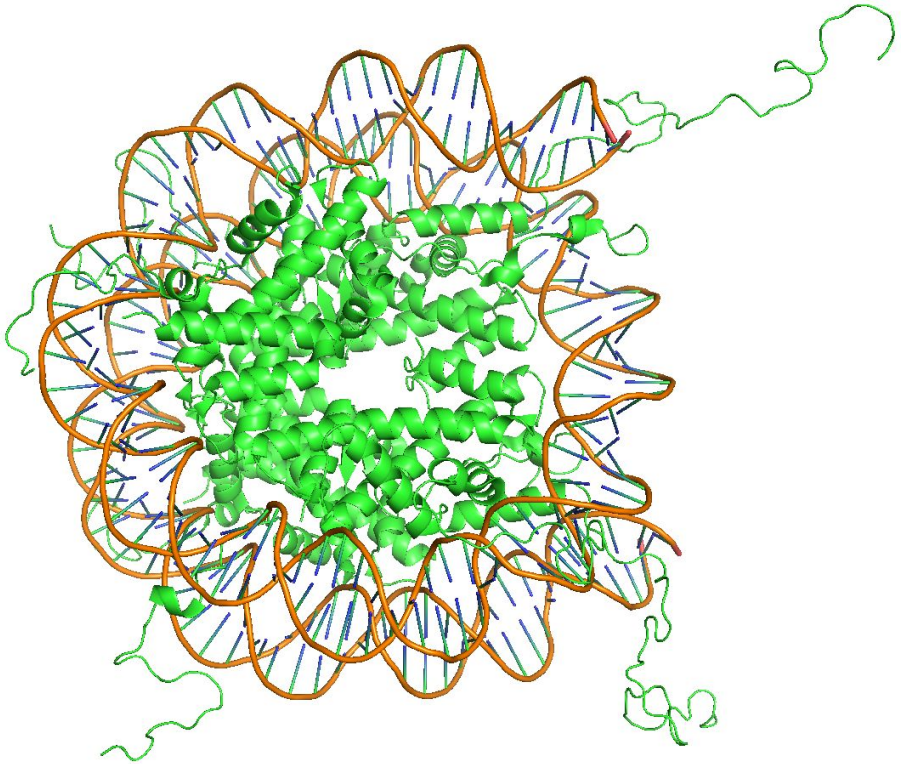


Structural Biology - Human Biology UPF

Arnau Cañabate, Laura de la Cruz, Quim Fàbrega, Ireneu Folqué i Gerard Llibre

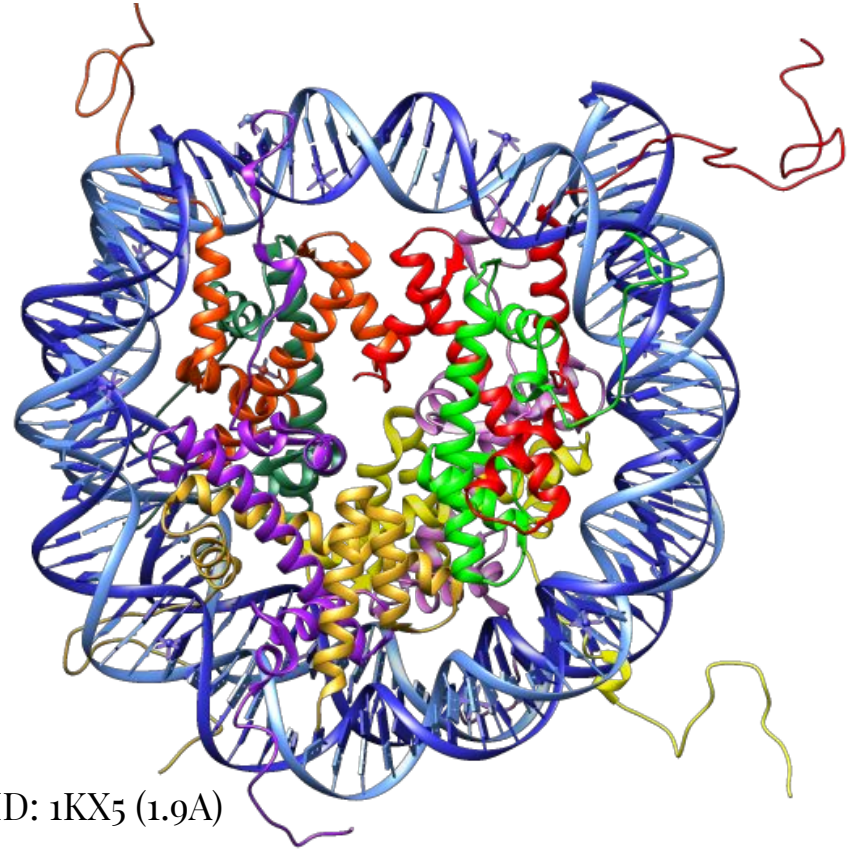
Index

Introduction
Interactions
Histone 1
DNA interaction
Modifications
Evolution
Conclusions



pdbID: 1KX5 (1.9A)

The nucleosome

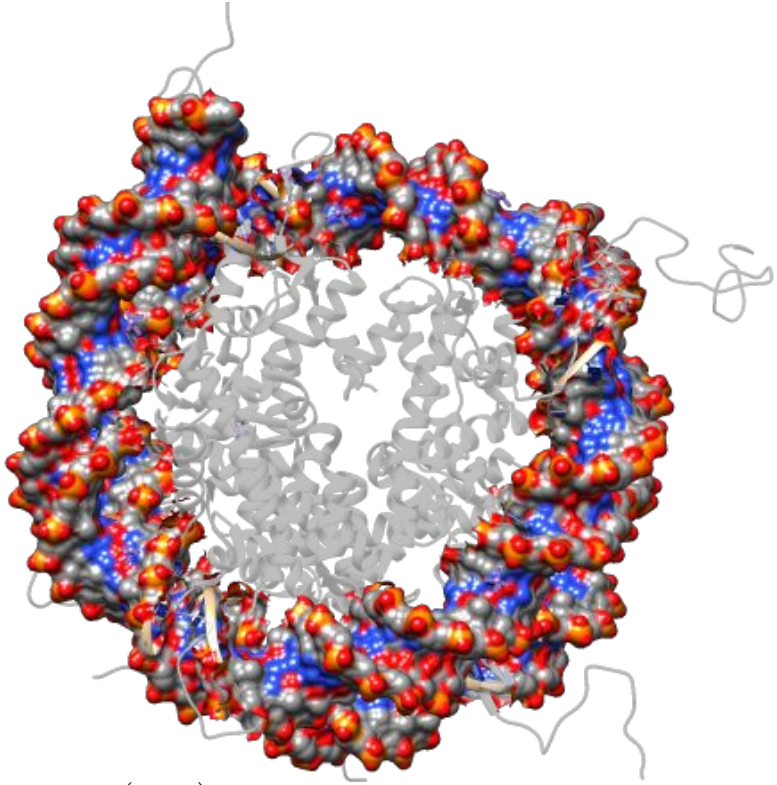


pdbID: 1KX5 (1.9A)

Nucleosomal DNA:
147 bp

Proteins:
-H2A and H2A'
-H2B and H2B'
-H3 and H3'
-H4 and H4'

The nucleosome: Functions



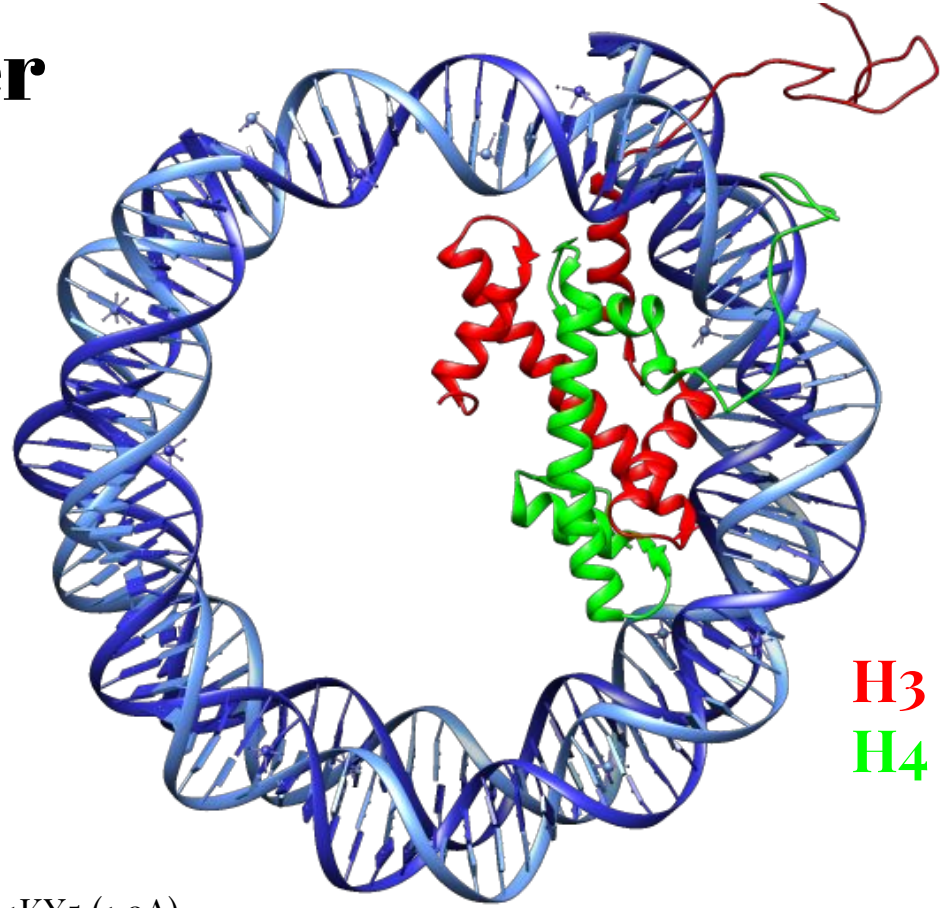
pdbID: 1KX5 (1.9A)

Assembles into higher order structures, leading to further compaction

Participates in the correct division of DNA in cells (mitosis & meiosis)

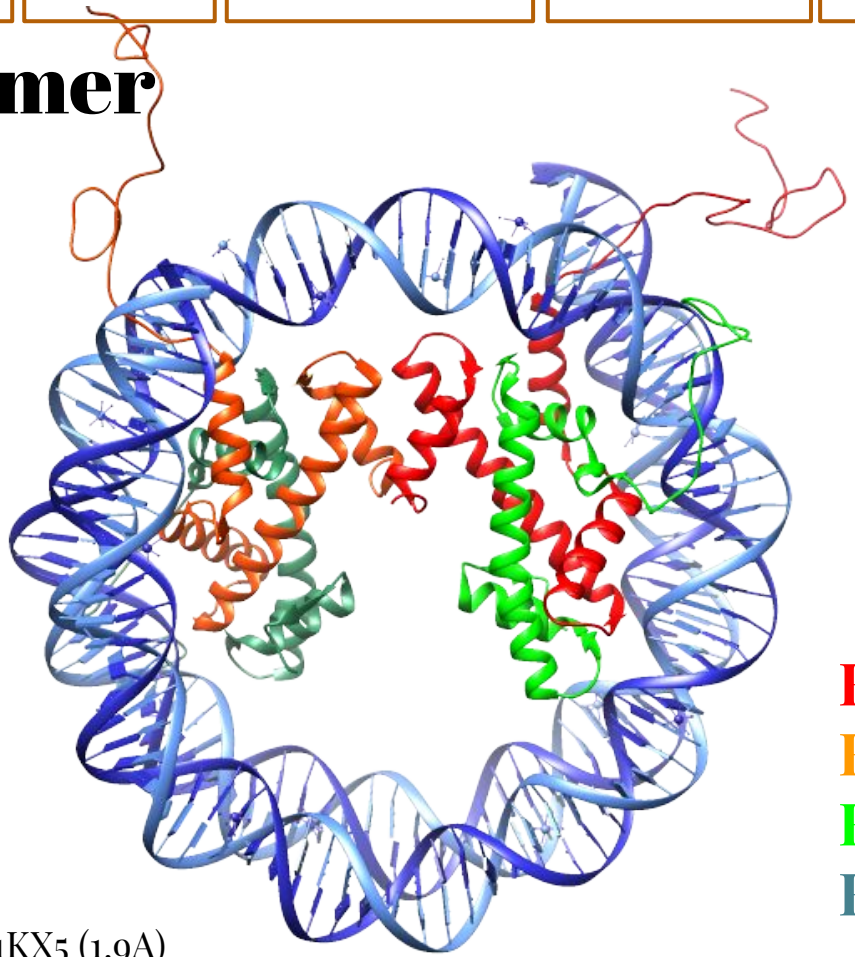
Important role in genetic regulation

H3-H4 dimer



pdbID: 1KX5 (1.9A)

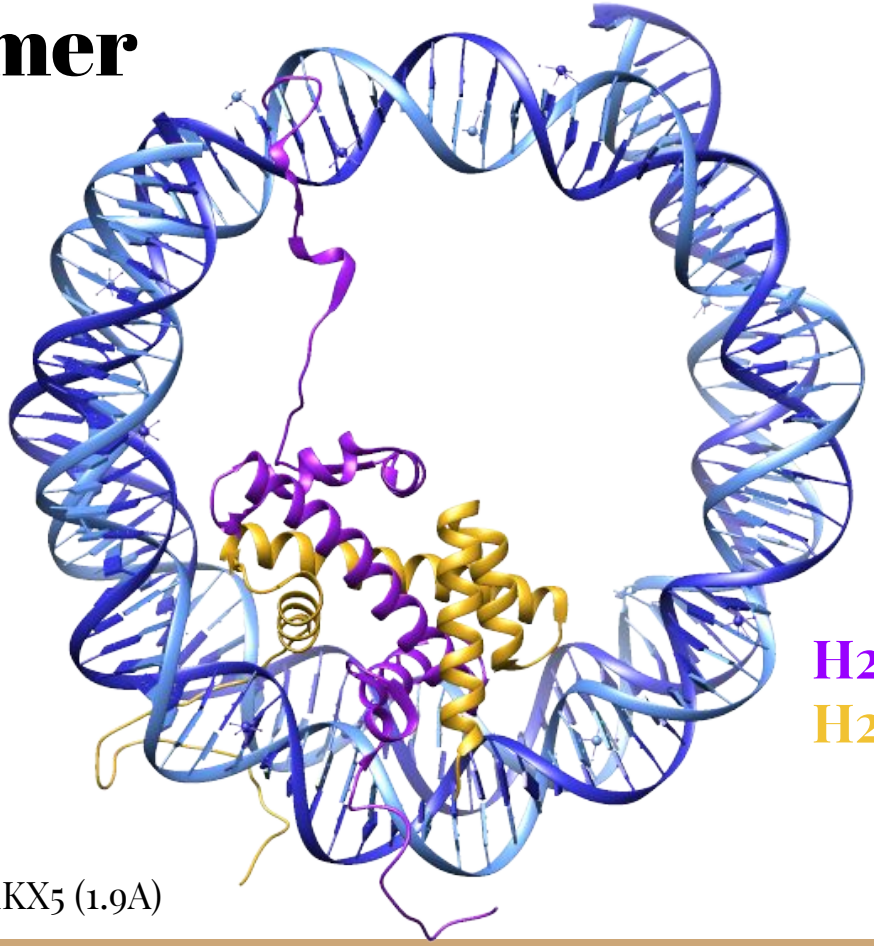
H3-H4 tetramer



H3
H3'
H4
H4'

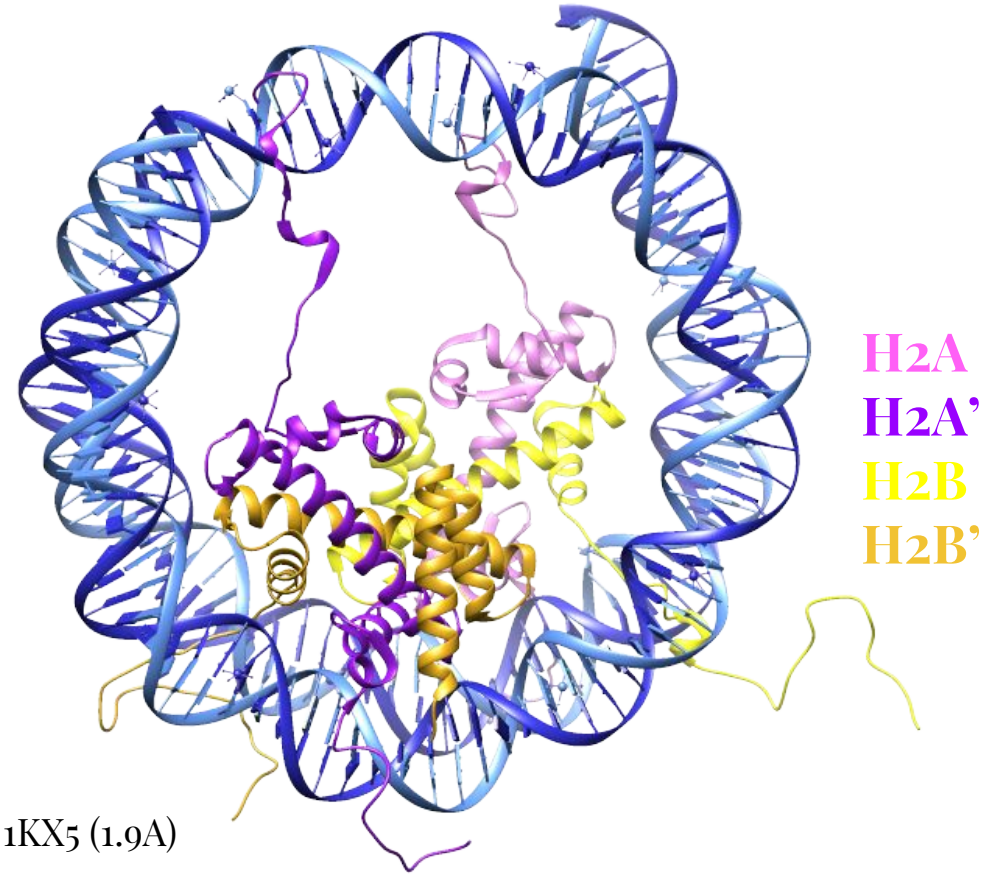
pdbID: 1KX5 (1.9A)

H2A-H2B dimer



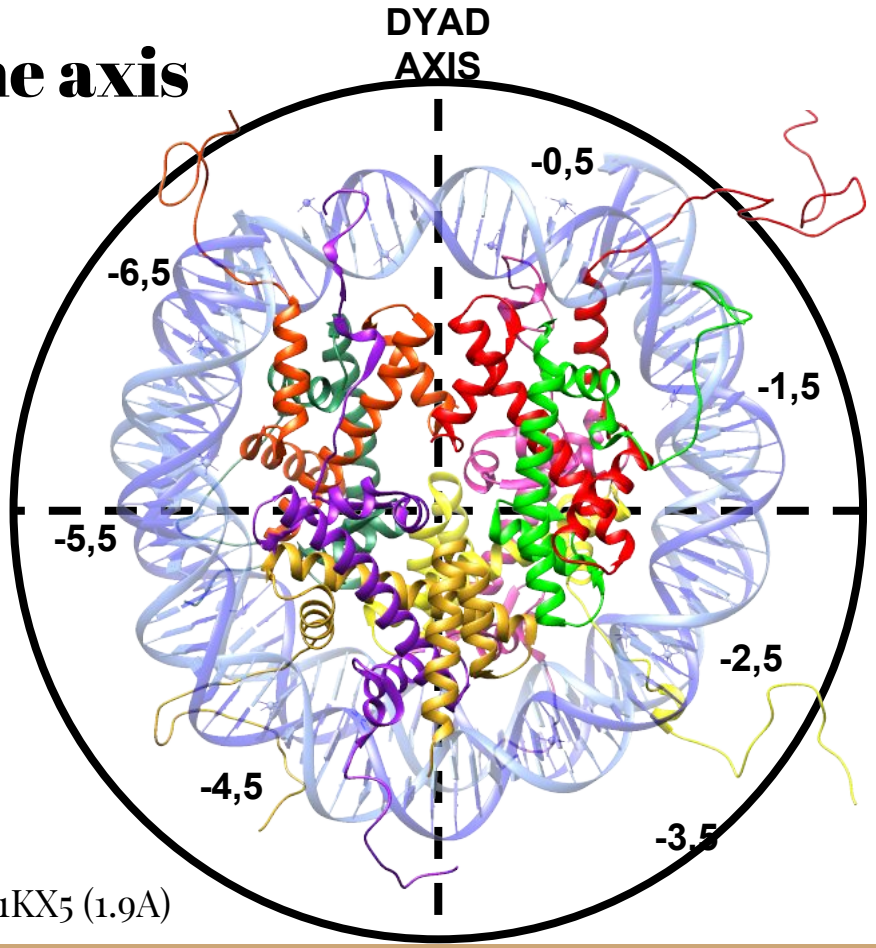
pdbID: 1KX5 (1.9A)

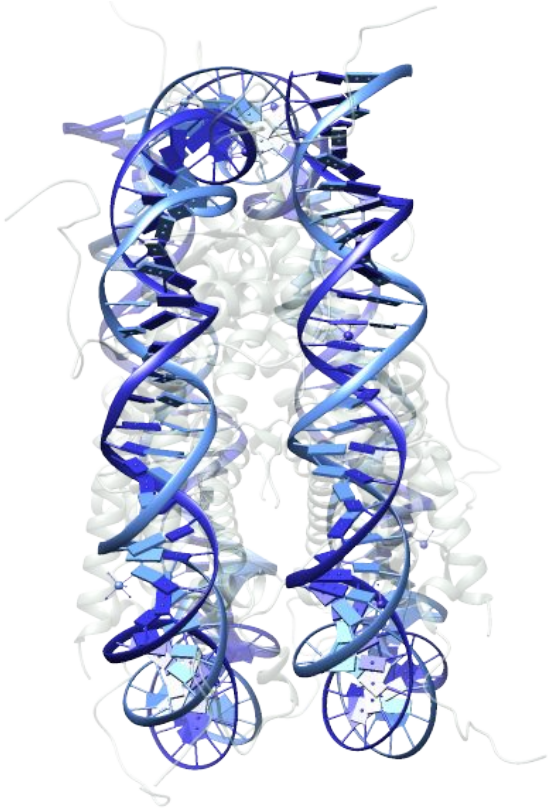
H2A-H2B



pdbID: 1KX5 (1.9A)

Nucleosome axis

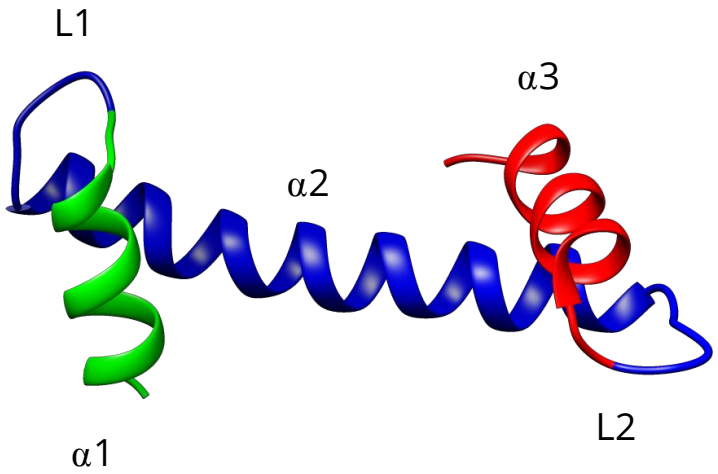




pdbID: 1KX5 (1.9A)

SCOP Classification: Histone core proteins

Class	All alpha proteins
Fold	Histone fold
Superfamily	Histone fold superfamily
Family	Nucleosome core histones



pdbID: 1KX5 (1.9A)

Histone fold

```
CLUSTAL W(1.60) multiple sequence alignment

H2A_Homo        ----------------RAKAKTRSSRAGLQF-PVGRVHRLLRKG-N-Y-S-E-RVGAGAP
H3_Homo         PHRYRPGTVALREIRRYQKSTE------LLI-RKLPFQRLVREIAQDF-KTDLRFQSSAV
H2B_Homo        ------------------KRSR------KE--SY--SVYVYKVLKQV-HPDTGISSKAM
H4_Homo         ------------------------------NIQGI--TKPAIRRLARRGGV-K-RISGLIY

H2A_Homo        VYLAAVLEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNK-LLGR-VT--I--
H3_Homo         MALQEACEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRI--R--G--ER---------
H2B_Homo        GIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRL--LLPGELAKHAVSEGTKA
H4_Homo         EETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKR--Q--G--RT--LY--G--


H2A_Homo        AQGGVLPNIQAVLLPK
H3_Homo         ----------------
H2B_Homo        VTKYTSAK--------
H4_Homo         FGG-------------
```

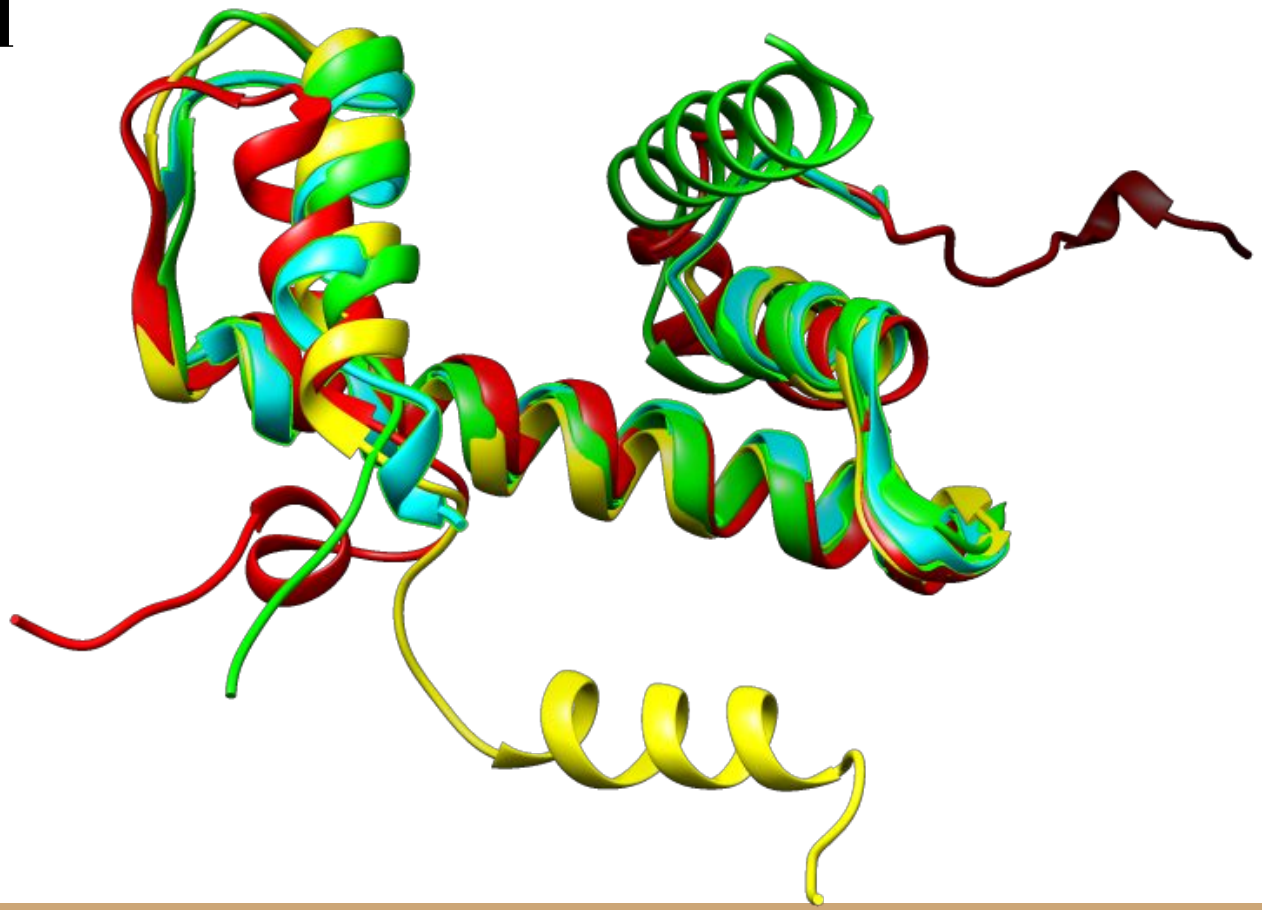
Histone fold

H2A

H3

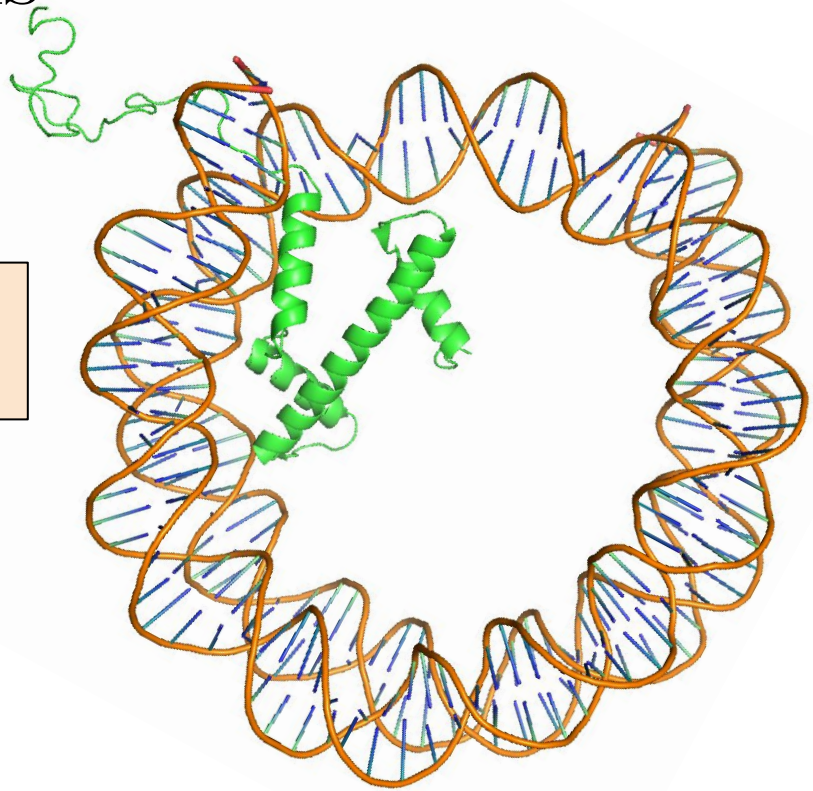
H2B

H4



Histone tails

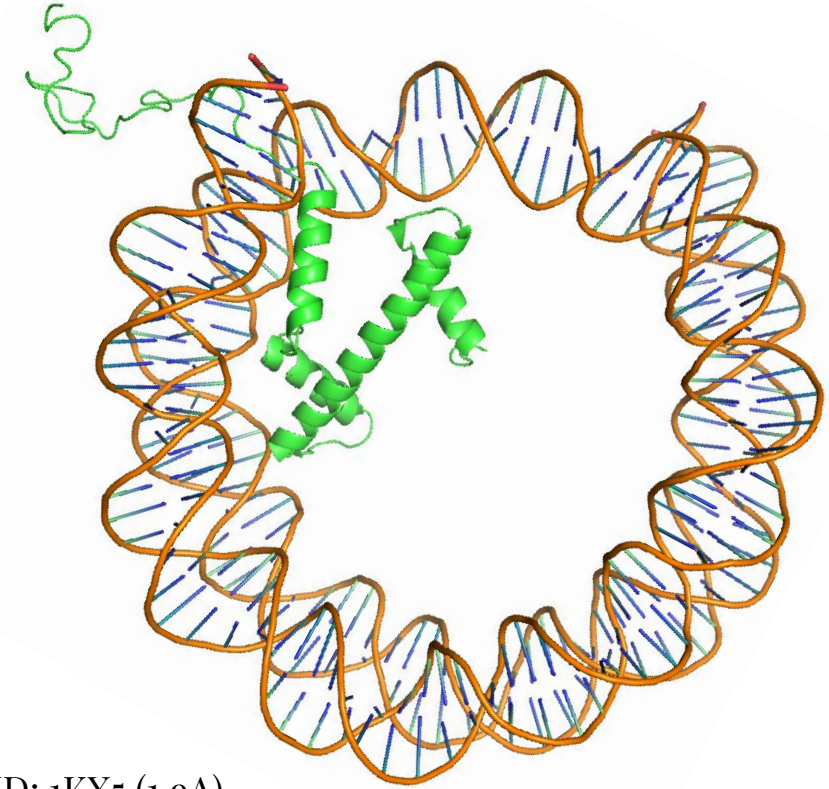
Genetic regulation



Nucleosome stabilization

pdbID: 1KX5 (1.9A)

Histone tails

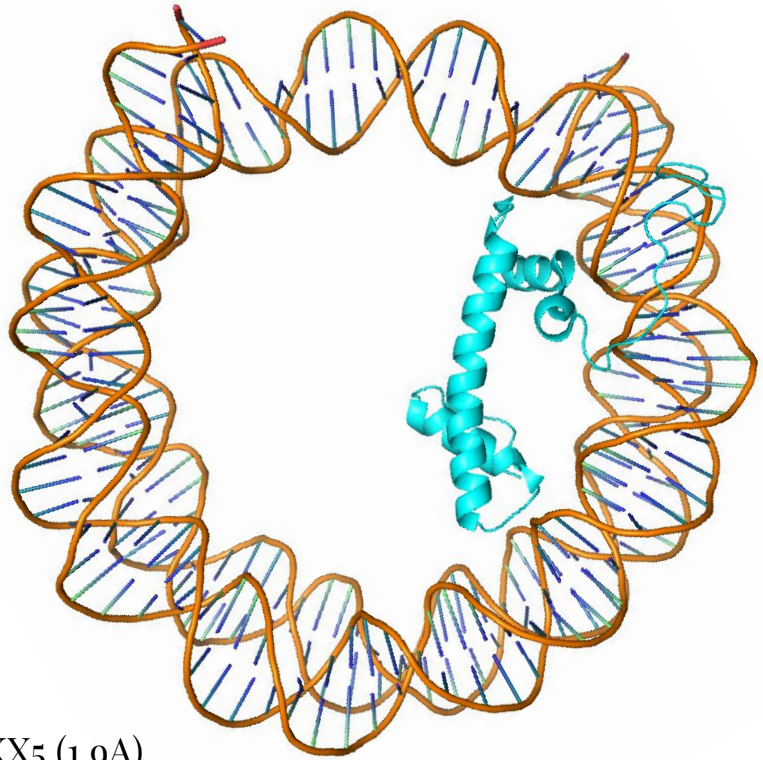


pdbID: 1KX5 (1.9A)

Type:
H3 N-terminal tail

Function:
DNA wrapping

Histone tails

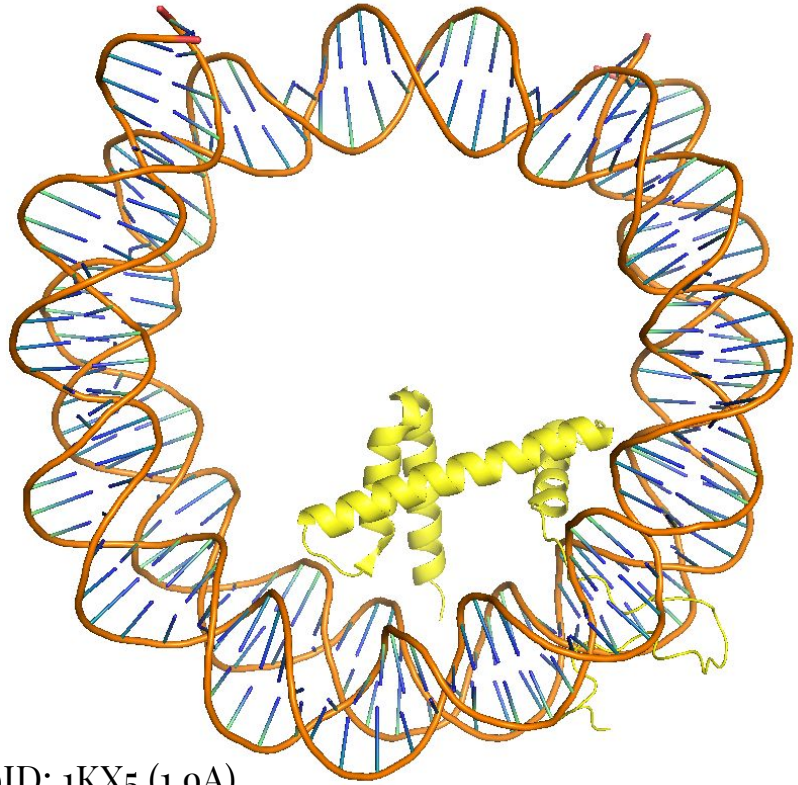


pdbID: 1KX5 (1.9A)

Type:
H4 N-terminal tail

Function:
Internucleosomal binding

Histone tails



pdbID: 1KX5 (1.9A)

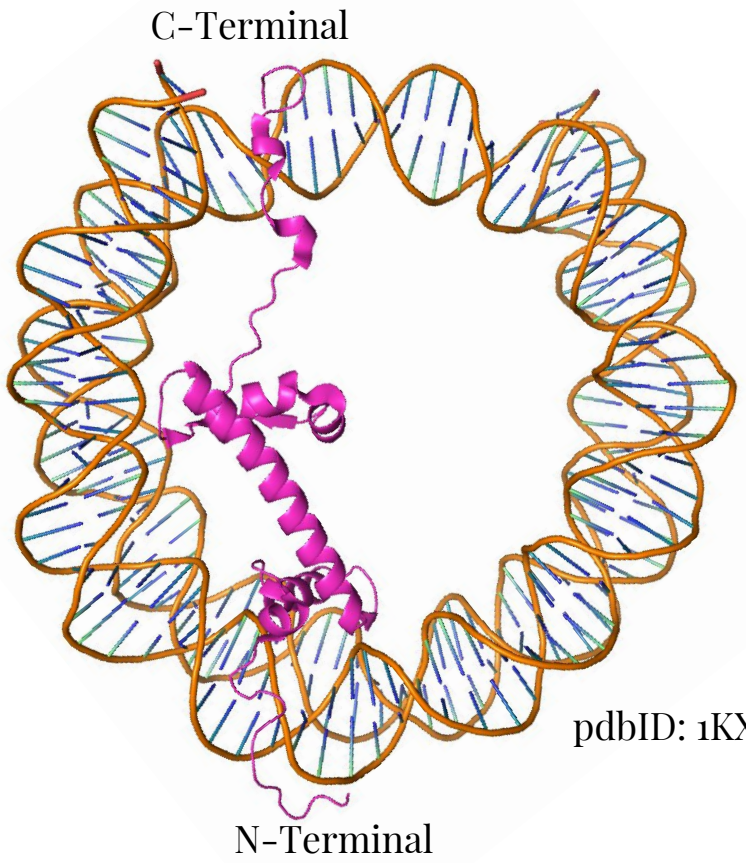
Type:
H2B N-terminal tail

Function:
Modulation of nucleosome position

Histone tails

Type:
H2A N-terminal tail

Function:
Internucleosomal binding



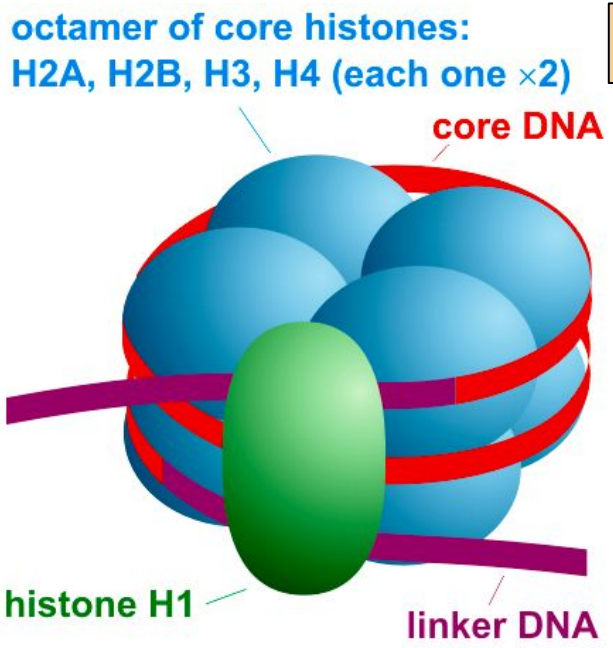
pdbID: 1KX5 (1.9A)

Type:
H2A C-terminal tail

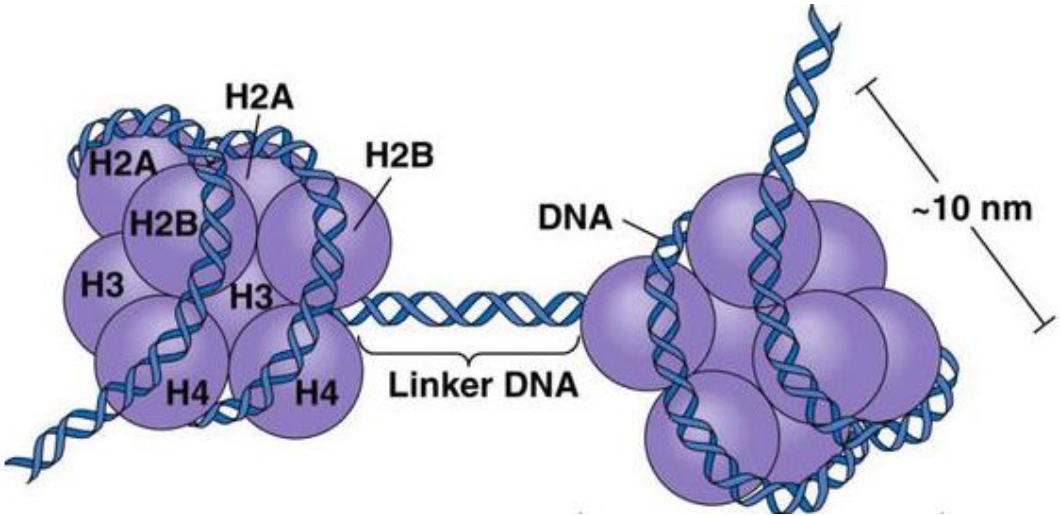
Function:
Binding to the linker DNA

Linker histone: H1

Stabilizes the nucleosome and does not take part of of the core



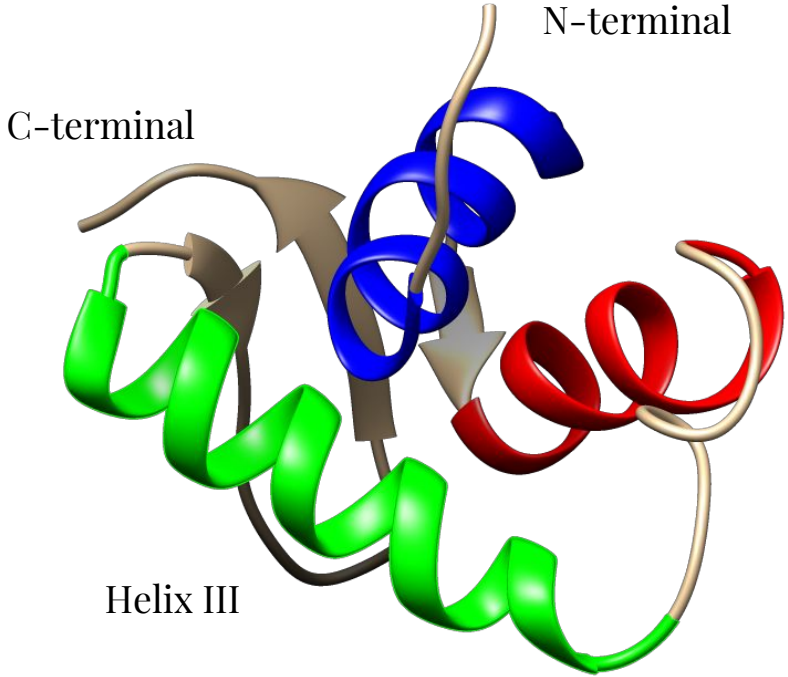
Source: https://upload.wikimedia.org/wikipedia/commons/4/45/Nucleosome_organization.png



Source: http://www.info-farmacia.com/bioquimica/nucleosomas

SCOP Classification: H1/H5

Class	All alpha proteins
Fold	DNA/RNA-binding 3-helical bundle
Superfamily	Winged helix DNA-binding domain
Family	Linker histone H1/H5
Protein	Histone H1/H5



pdbID: 1ghc

Methods

NodeId1	Interaction	NodeId2	Distance	Angle	Energy	Atom1	Atom2	Donor	Positive	Cation	Orientation
A:2:_:ARG	VDW:SC_SC	A:5:_:GLN	3.751	-999.9	6.000	CZ	CG				
A:9:_:LYS	VDW:SC_SC	A:17:_:ARG	3.301	-999.9	6.000	CE	CZ				
A:9:_:LYS	VDW:SC_SC	A:18:_:LYS	3.778	-999.9	6.000	CE	CE				
A:14:_:LYS	HBOND:MC_	A:17:_:ARG	2.895	56.437	17.000	O	NH1	A:17:_:ARG			
A:15:_:ALA	VDW:SC_SC	A:25:_:ALA	3.575	-999.9	6.000	CB	CB				
A:16:_:PRO	VDW:SC_MC	A:21:_:ALA	3.450	-999.9	6.000	CB	C				
A:16:_:PRO	VDW:SC_MC	A:22:_:THR	3.940	-999.9	6.000	CG	C				
A:25:_:ALA	VDW:SC_SC	A:30:_:PRO	3.789	-999.9	6.000	CB	CB				
A:35:_:VAL	VDW:SC_SC	A:38:_:PRO	3.093	-999.9	6.000	CG1	CG				
A:41:_:TYR	VDW:SC_SC	A:45:_:THR	3.926	-999.9	6.000	CD2	CG2				
A:41:_:TYR	VDW:SC_SC	A:46:_:VAL	3.858	-999.9	6.000	CB	CG2				
A:42:_:ARG	HBOND:MC_	A:45:_:THR	3.043	8.483	17.000	O	N	A:45:_:THR			
A:44:_:GLY	HBOND:MC_	A:47:_:ALA	3.450	40.329	17.000	O	N	A:47:_:ALA			
A:44:_:GLY	HBOND:MC_	A:48:_:LEU	2.983	18.438	17.000	O	N	A:48:_:LEU			
A:45:_:THR	HBOND:MC_	A:49:_:ARG	3.153	36.107	17.000	O	N	A:49:_:ARG			
A:46:_:VAL	HBOND:MC_	A:49:_:ARG	3.165	38.809	17.000	O	N	A:49:_:ARG			
A:46:_:VAL	VDW:SC_SC	A:49:_:ARG	4.004	-999.9	6.000	CG2	CZ				
A:46:_:VAL	HBOND:MC_	A:50:_:GLU	2.892	22.871	17.000	O	N	A:50:_:GLU			
A:47:_:ALA	HBOND:MC_	A:50:_:GLU	3.048	41.544	17.000	O	N	A:50:_:GLU			
A:47:_:ALA	HBOND:MC_	A:51:_:ILE	2.906	26.272	17.000	O	N	A:51:_:ILE			
A:48:_:LEU	HBOND:MC_	A:51:_:ILE	3.448	41.156	17.000	O	N	A:51:_:ILE			
A:48:_:LEU	VDW:SC_SC	A:51:_:ILE	3.915	-999.9	6.000	CD1	CD1				
A:48:_:LEU	HBOND:MC_	A:52:_:ARG	3.106	24.296	17.000	O	N	A:52:_:ARG			
A:49:_:ARG	HBOND:MC_	A:52:_:ARG	3.318	40.738	17.000	O	N	A:52:_:ARG			
A:49:_:ARG	HBOND:MC_	A:53:_:ARG	2.921	31.625	17.000	O	N	A:53:_:ARG			
A:50:_:GLU	HBOND:MC_	A:53:_:ARG	3.117	40.029	17.000	O	N	A:53:_:ARG			
A:50:_:GLU	HBOND:MC_	A:54:_:TYR	2.833	20.212	17.000	O	N	A:54:_:TYR			
A:50:_:GLU	VDW:MC_SC	A:54:_:TYR	3.916	-999.9	6.000	C	CD1				
A:51:_:ILE	HBOND:MC_	A:54:_:TYR	3.408	39.159	17.000	O	N	A:54:_:TYR			

Source:
http://protein.bio.unipd.it/ring/

Methods

Arpeggio

BBSRC bioscience for the future

UNIVERSITY OF CAMBRIDGE

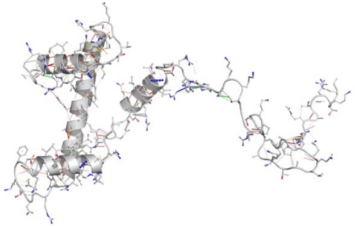
Overview [h3.pdb]

Mutually Exclusive Interactions	
Total number of contacts	3768
Of which VdW interactions	79
Of which VdW clash interactions	138
Of which covalent interactions	0
Of which covalent clash interactions	0
Of which proximal	3551

Polar Contacts	
Polar contacts	202
Water mediated polar contacts	0
Weak polar contacts	85
Water mediated weak polar contacts	0

Feature Contacts	
Hydrogen bonds	117
Water mediated hydrogen bonds	0
Weak hydrogen bonds	70
Water mediated weak hydrogen bonds	0
Halogen bonds	0
Ionic interactions	14
Metal complex interactions	0
Aromatic contacts	6
Hydrophobic contacts	118
Carbonyl interactions	3

Download All Results

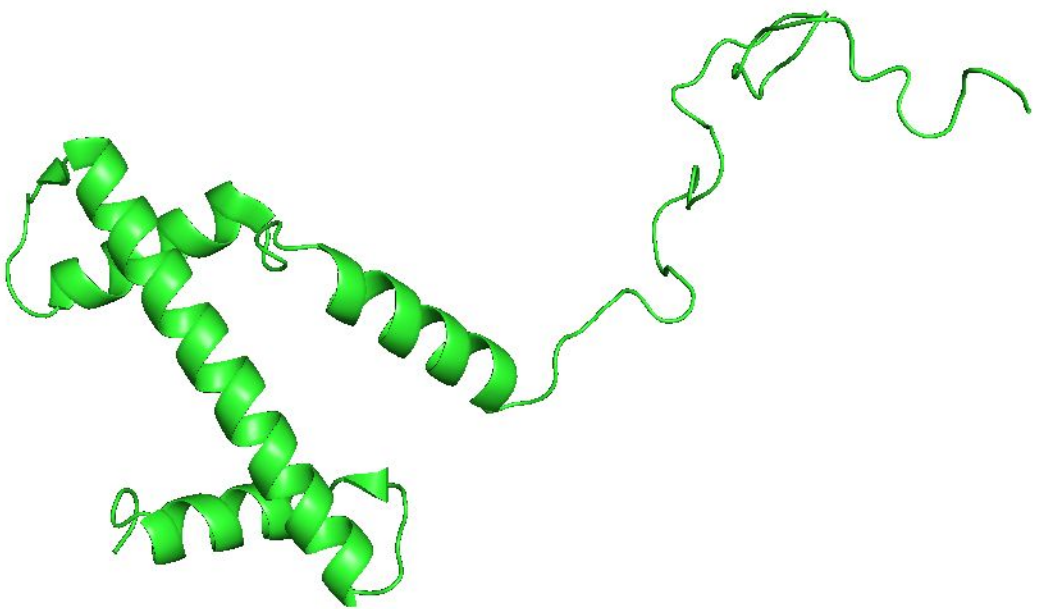


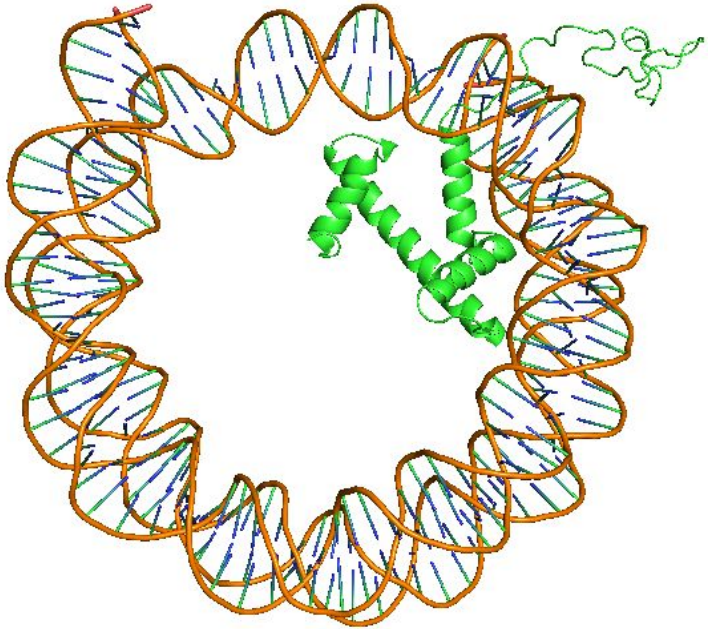
Download PyMOL Session

Source: http://biosig.unimelb.edu.au/arpeggioweb/result/pluto-oklahoma-lima/

Intra-histone interactions

Histone 3

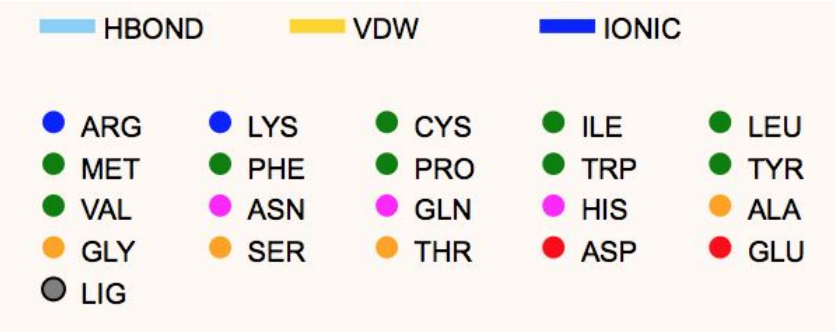


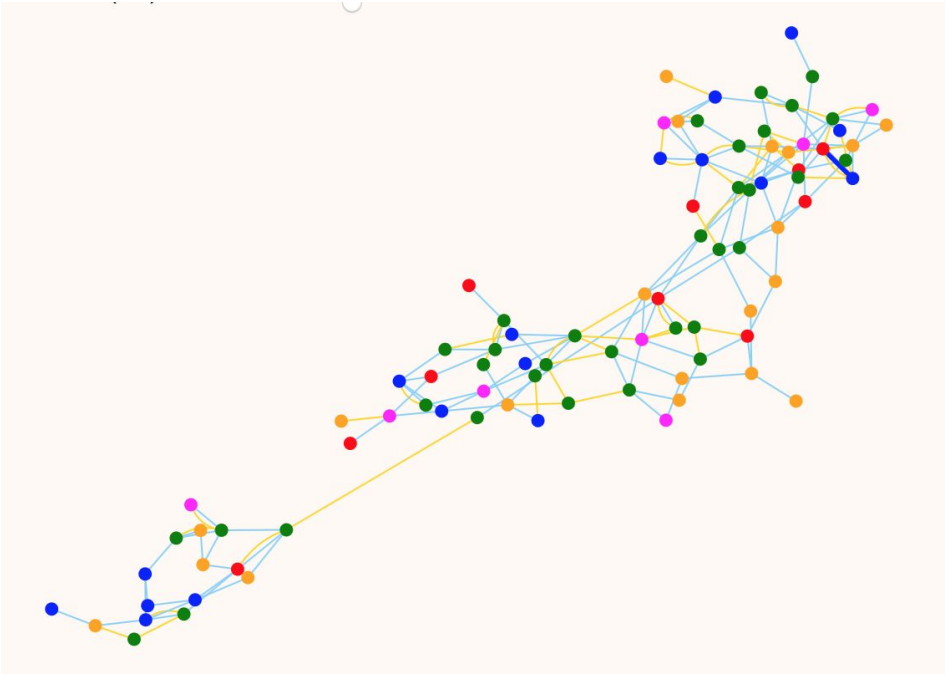


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 3



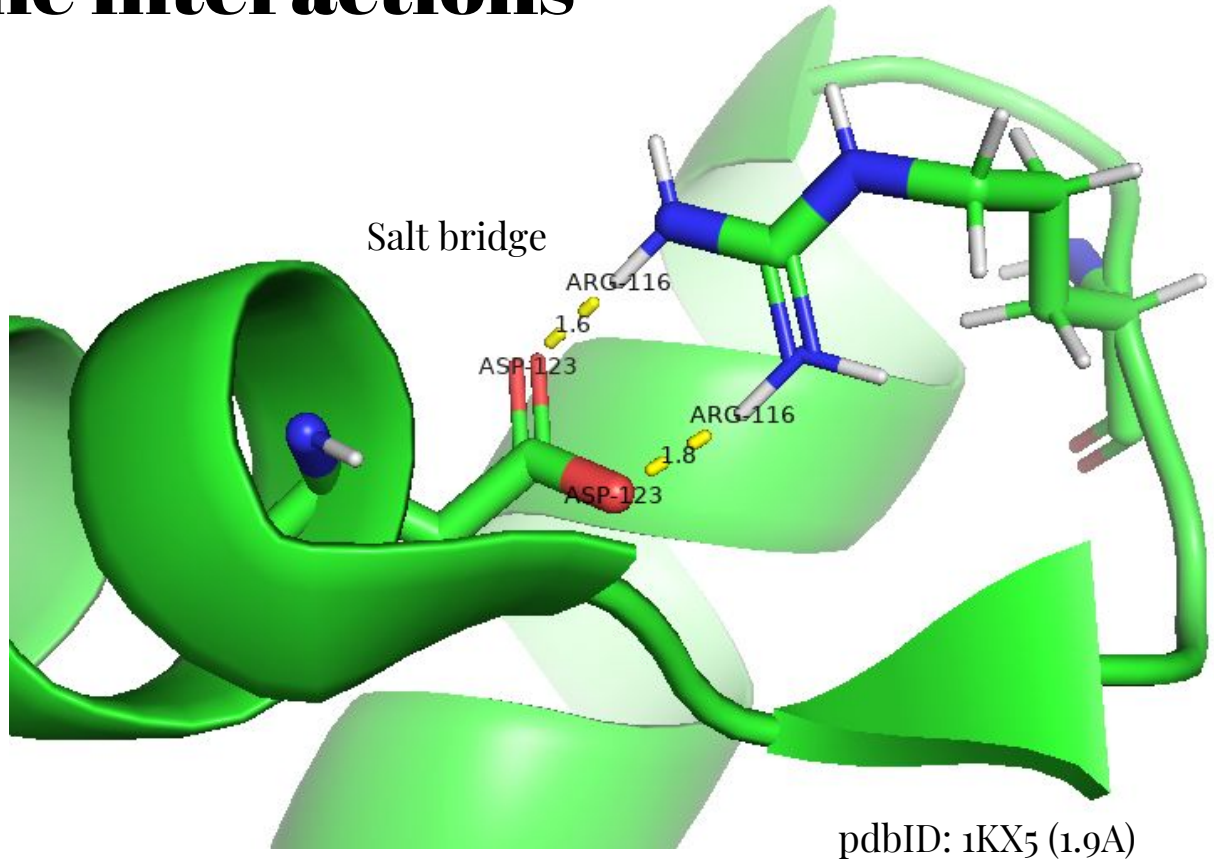


Source:
http://protein.bio.unipd.it/ring/

Intra-histone interactions

Histone 3

Arg 116	Asp 123
Met 120	Asp 123

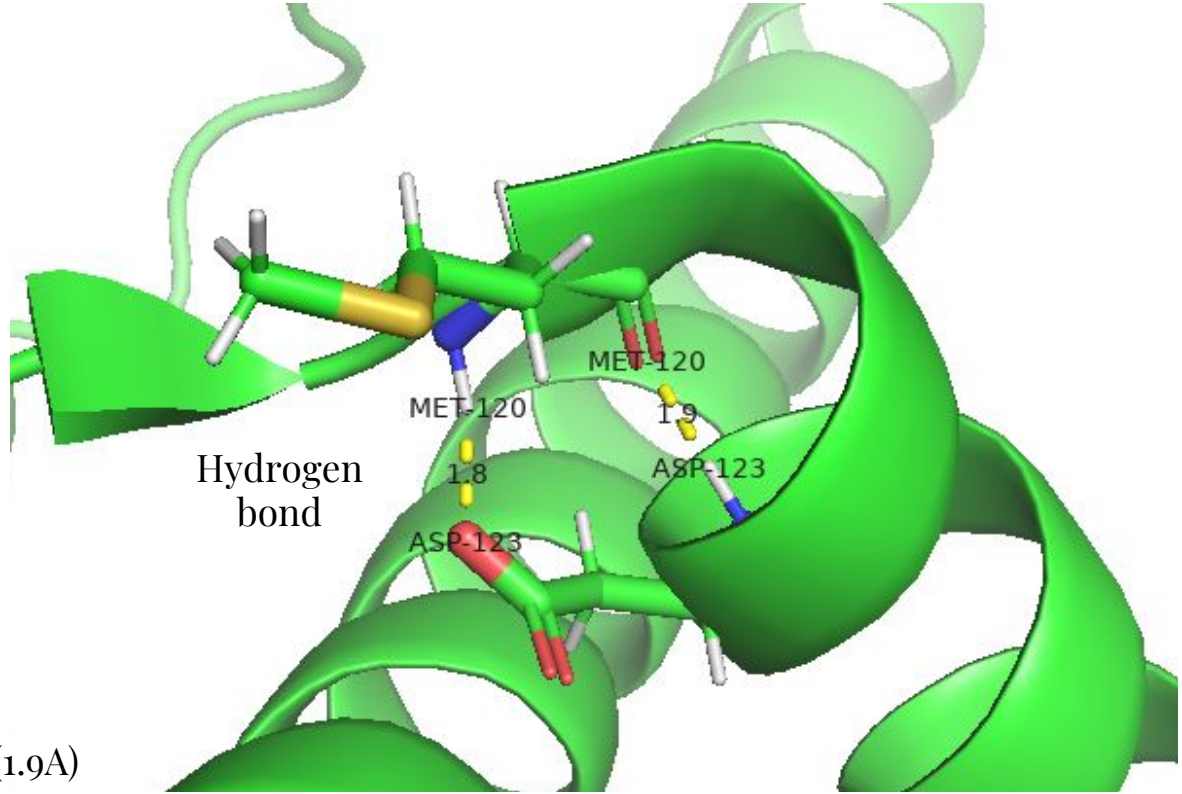


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 3

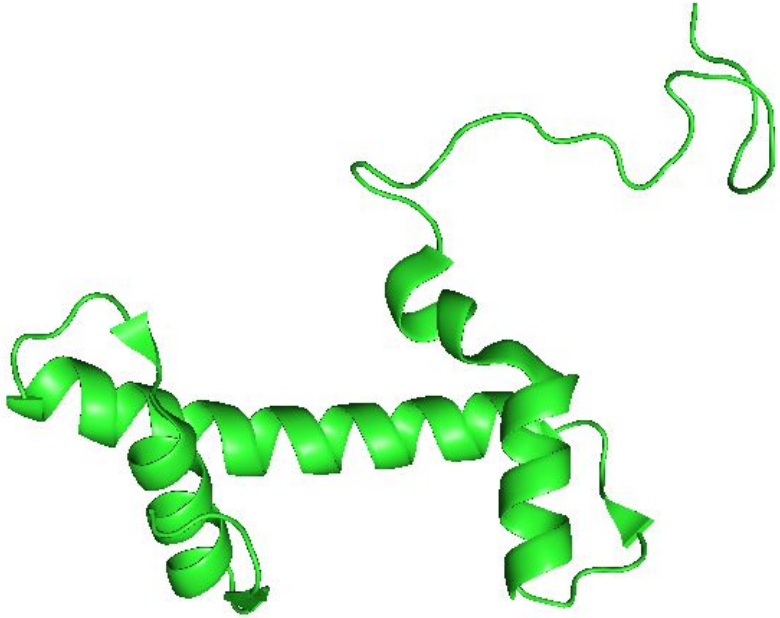
Arg 116	Asp 123
Met 120	**Asp 123**

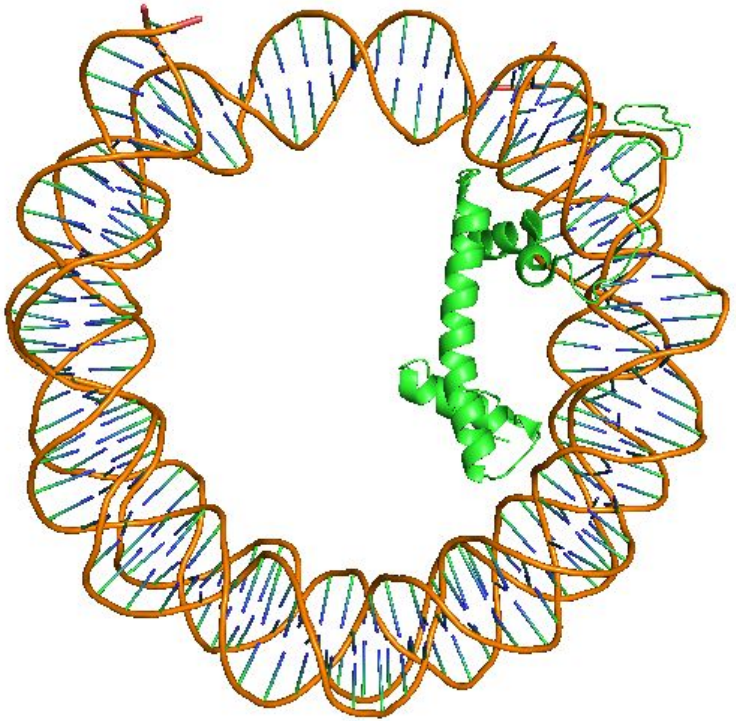


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 4

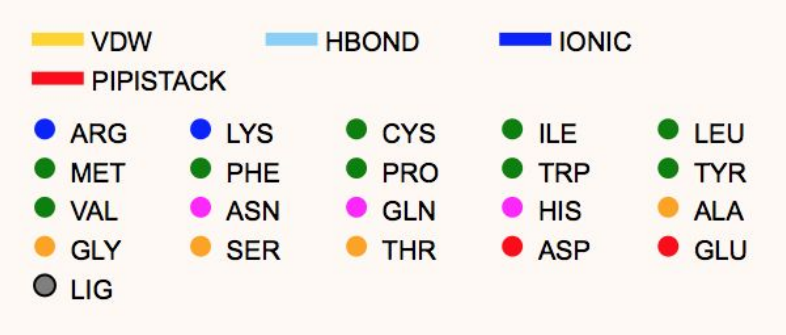


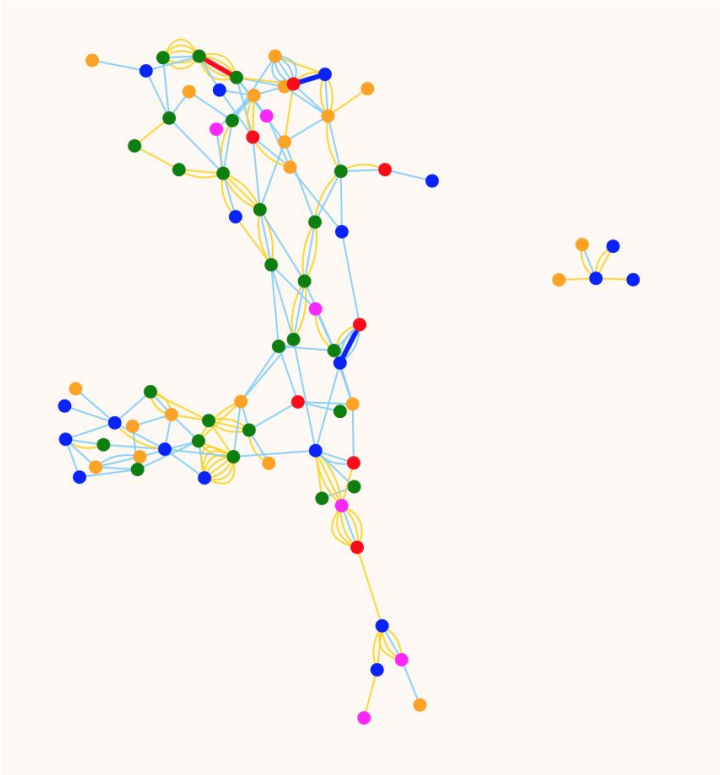


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 4



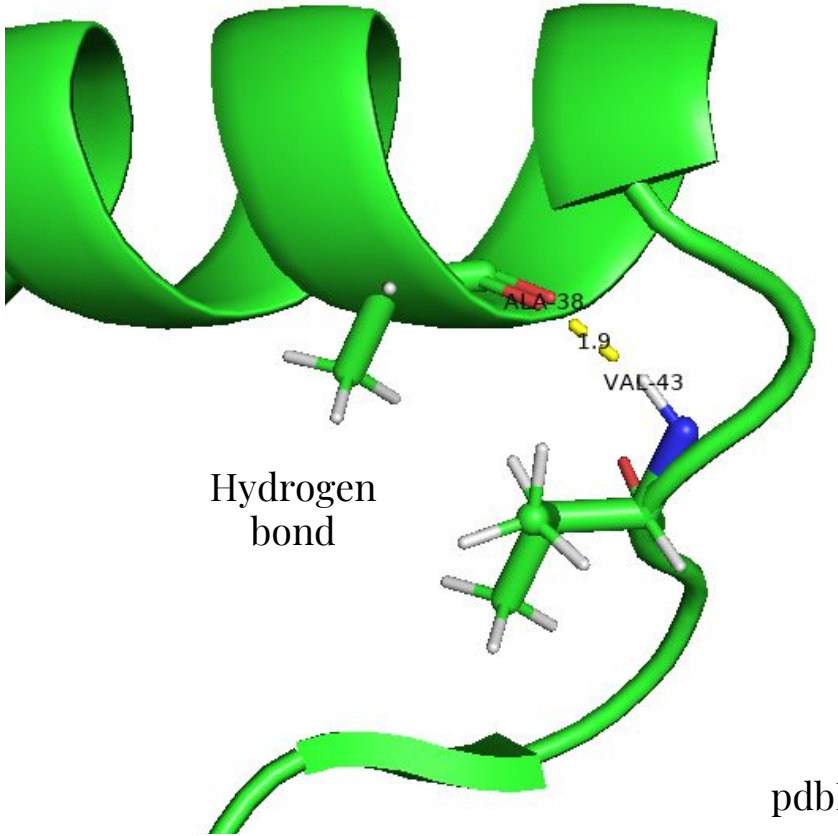


Source:
http://protein.bio.unipd.it/ring/

Intra-histone interactions

Histone 4

Ala 38	Val 43
Arg 78	Asp 85
Asp 68	Arg 92



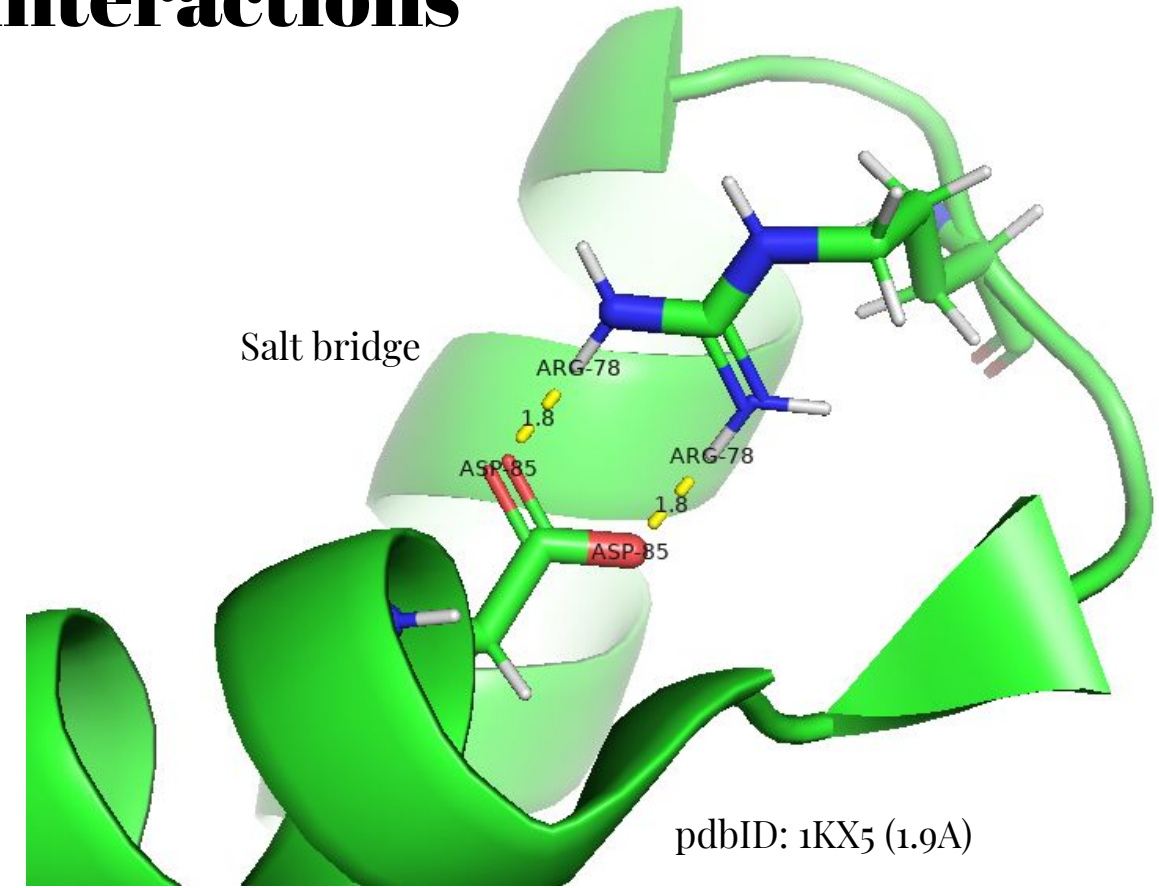
Hydrogen bond

pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 4

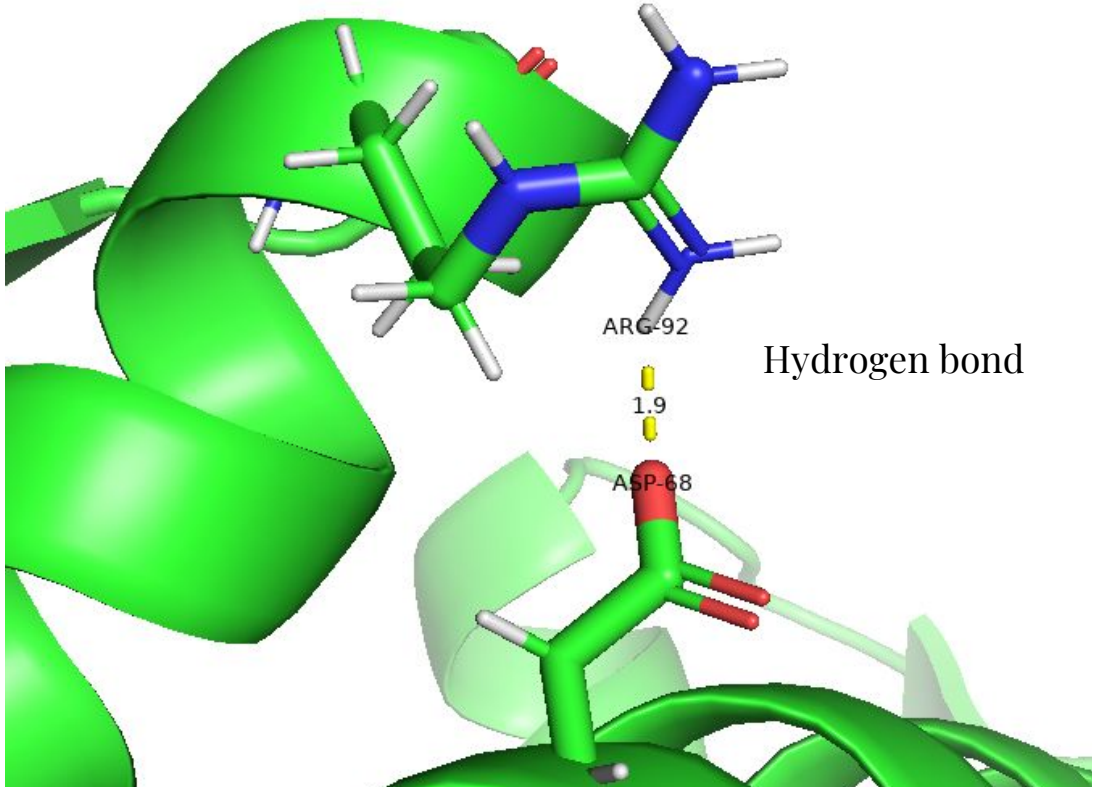
Ala 38	Val 43
Arg 78	**Asp 85**
Asp 68	Arg 92



Intra-histone interactions

Histone 4

Ala 38	Val 43
Arg 78	Asp 85
Asp 68	**Arg 92**

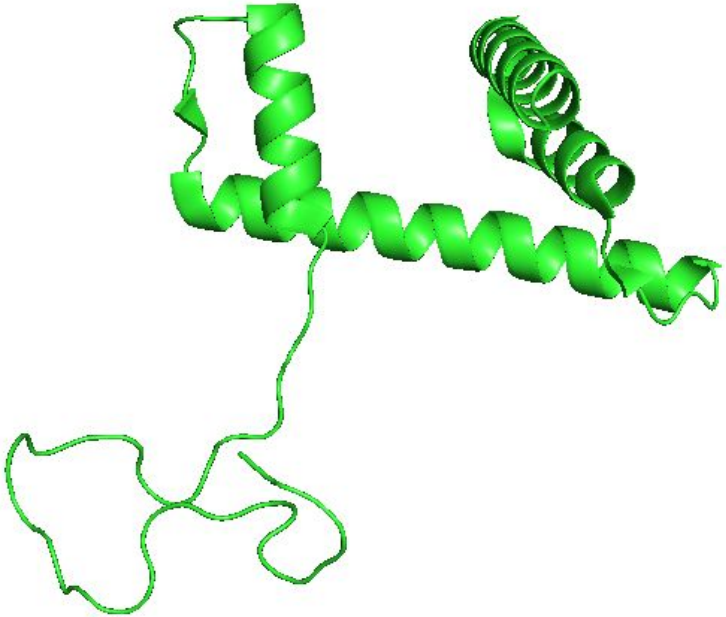


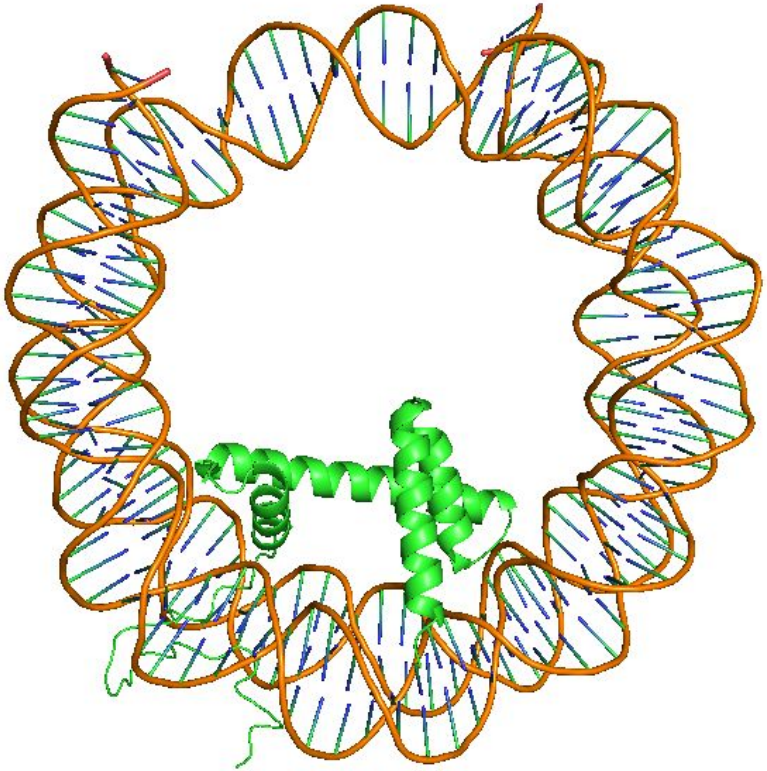
Hydrogen bond

pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 2B

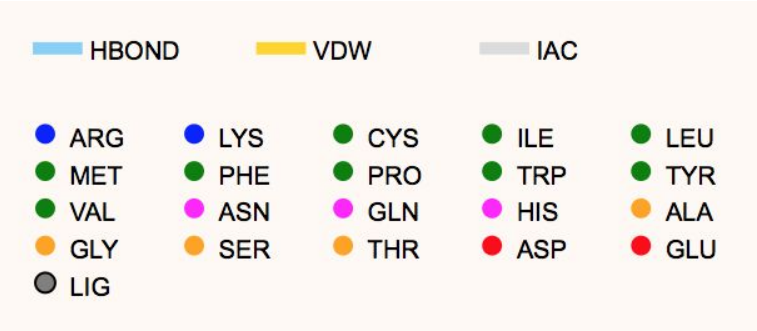


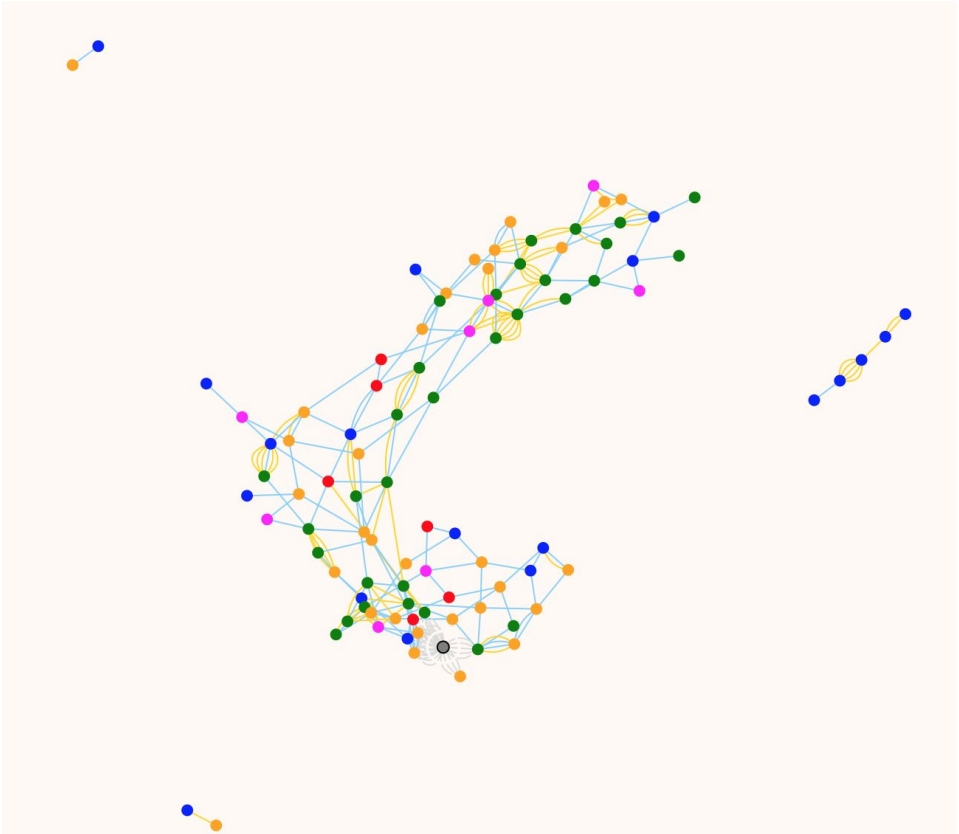


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 2B



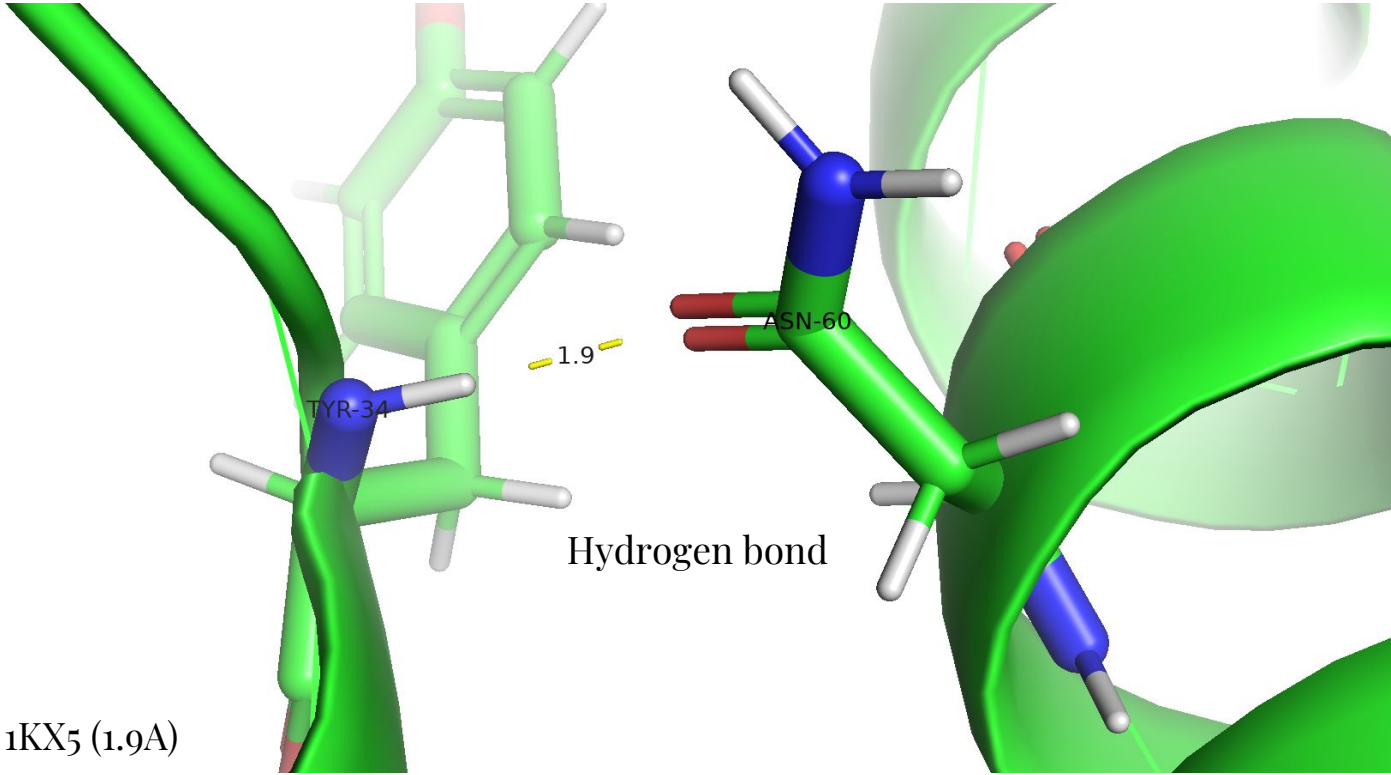


Source:
http://protein.bio.unipd.it/ring/

Intra-histone interactions

Histone 2B

Asn 60	Tyr 34
Glu 90	Thr 87



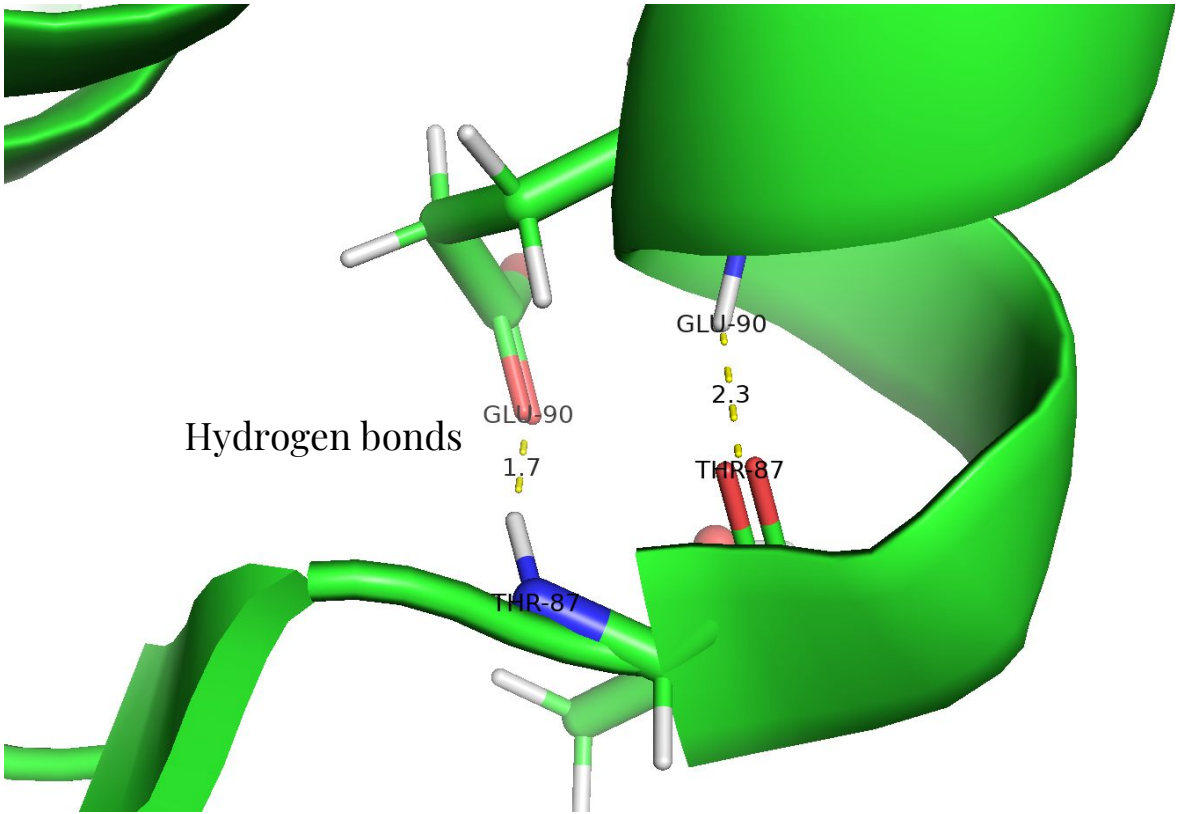
Hydrogen bond

pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 2B

Asn 60	Tyr 34
Glu 90	**Thr 87**

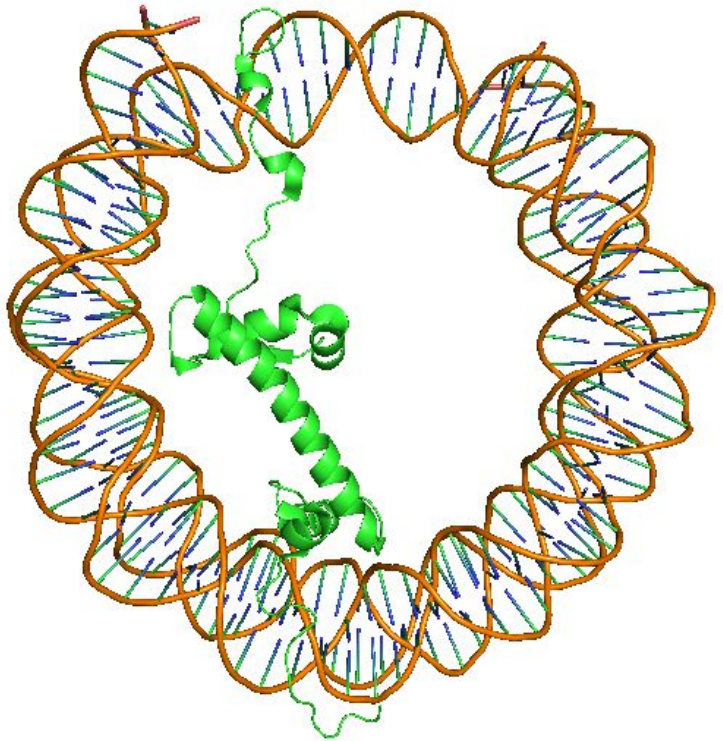


Hydrogen bonds

pdbID: 1KX5 (1.9A)

Intra-histone interactions

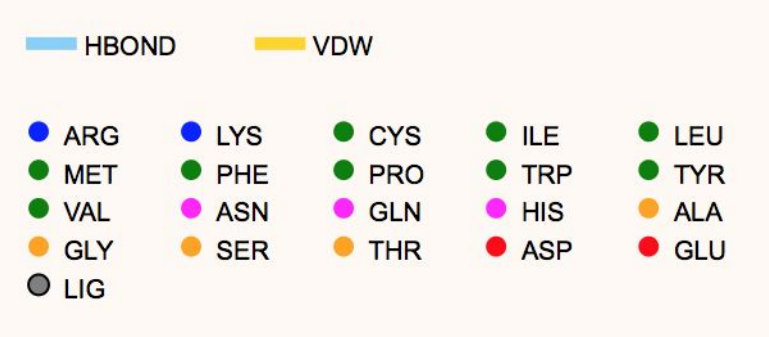
Histone 2A

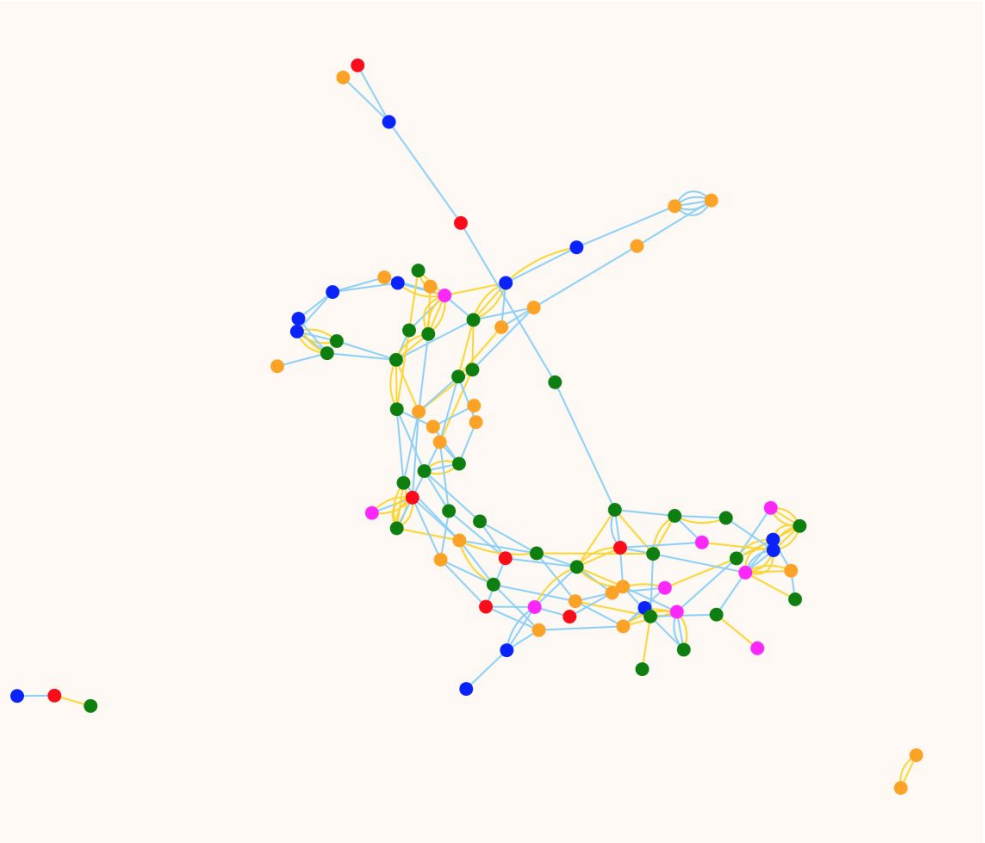


pdbID: 1KX5 (1.9A)

Intra-histone interactions

Histone 2A



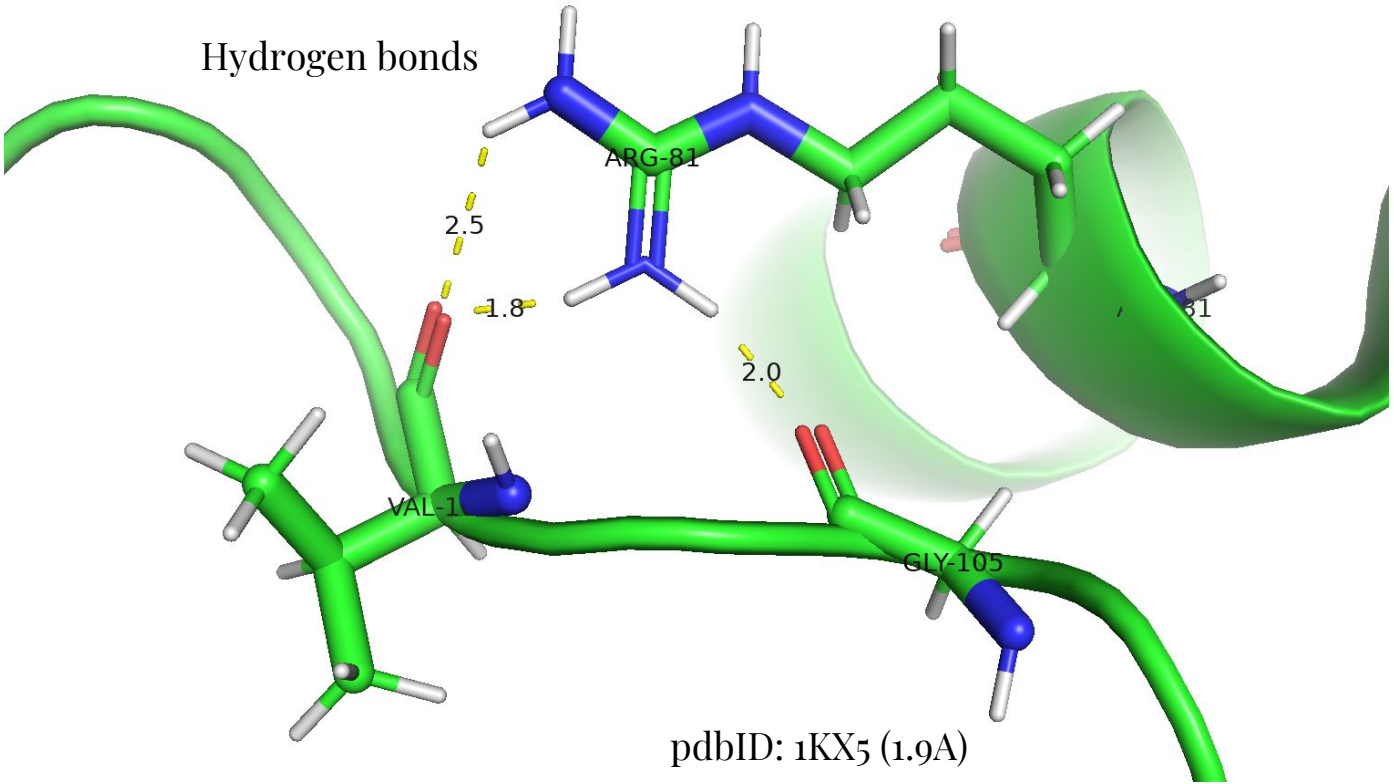


Source:
http://protein.bio.unipd.it/ring/

Intra-histone interactions

Histone 2A

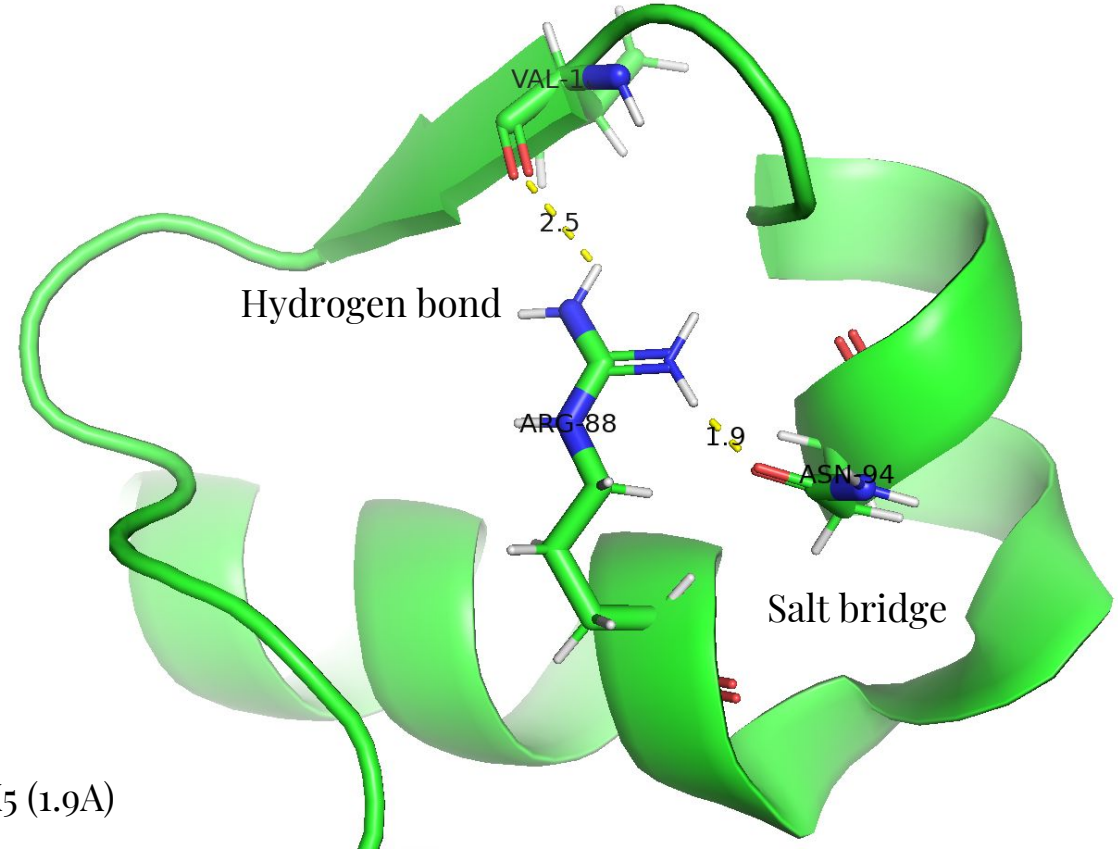
Gly 105	Arg 81	Val 107
Asn 94	Arg 88	Val 100



Intra-histone interactions

Histone 2A

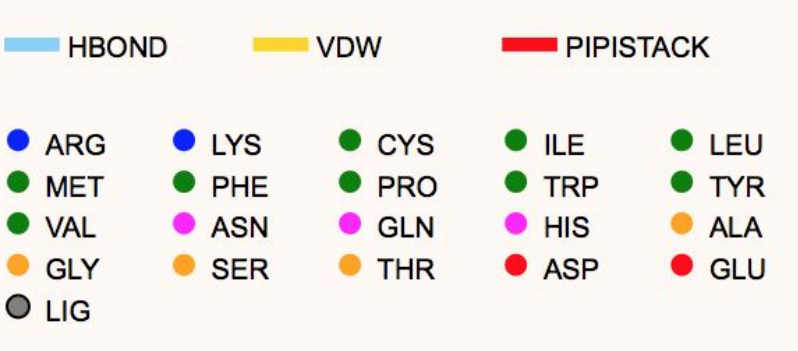
Gly 105	Arg 81	Val 107
Asn 94	**Arg 88**	**Val 100**

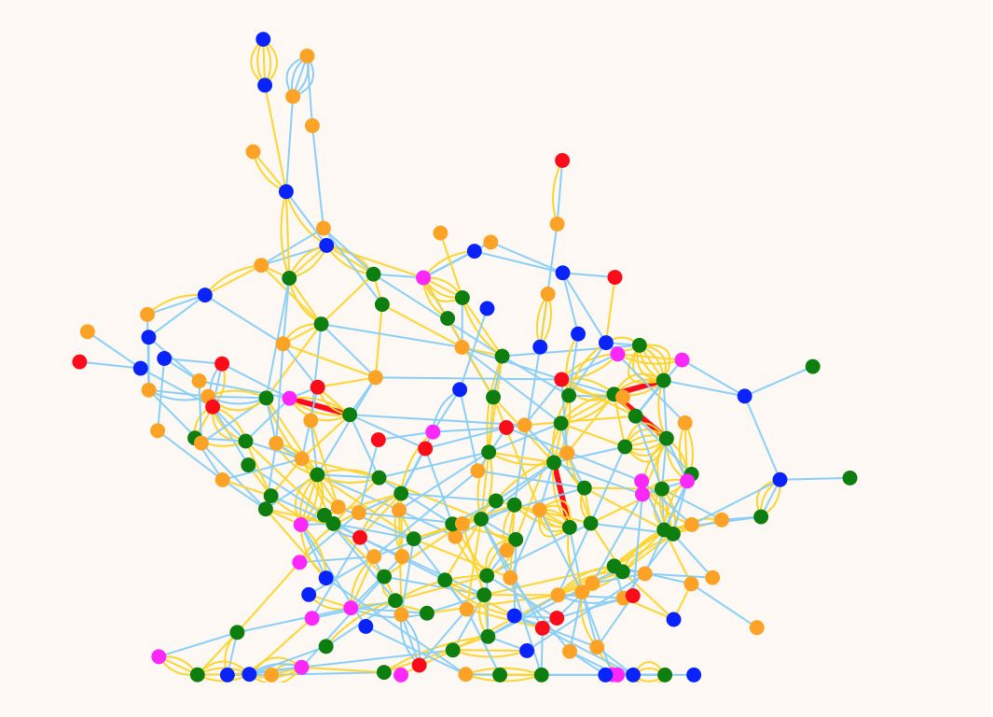


pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 2A - Histone 2B



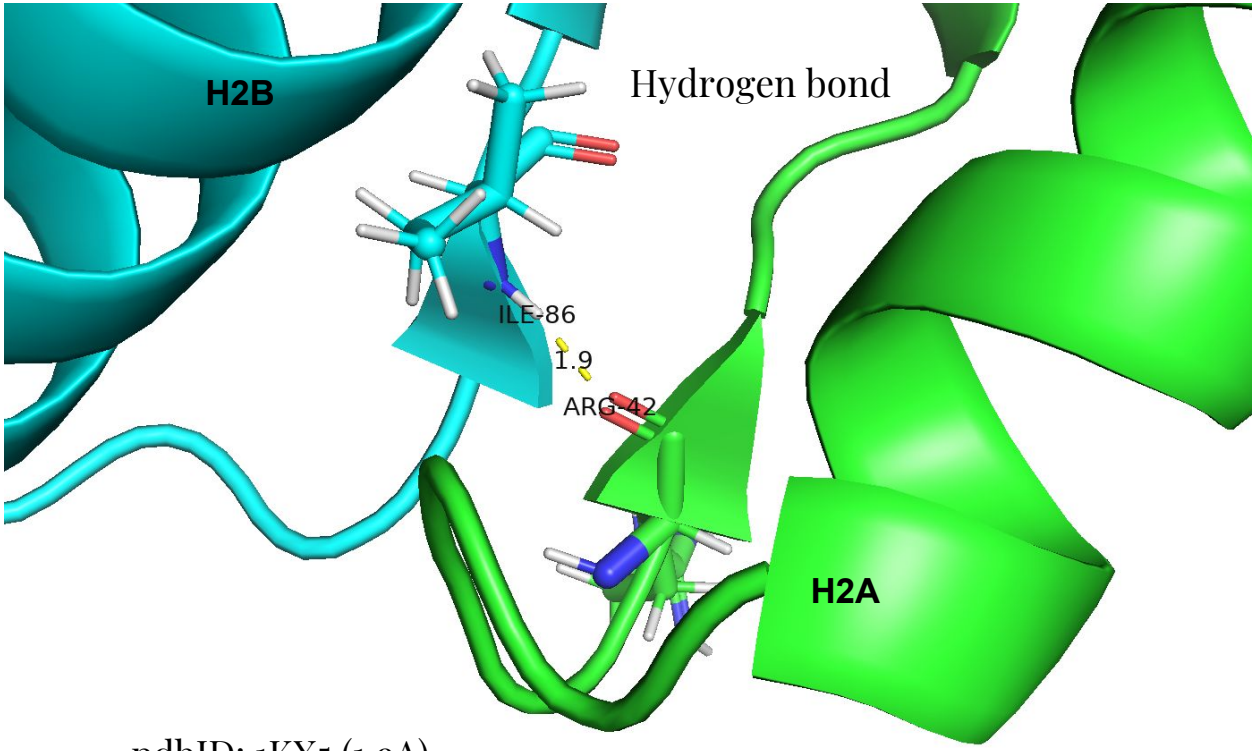


Source:
http://protein.bio.unipd.it/ring/

Dimer interactions

Histone 2A - Histone 2B

Arg 42	Ile 86
Gly 44	Ile 86
Ile 78	Ile 51

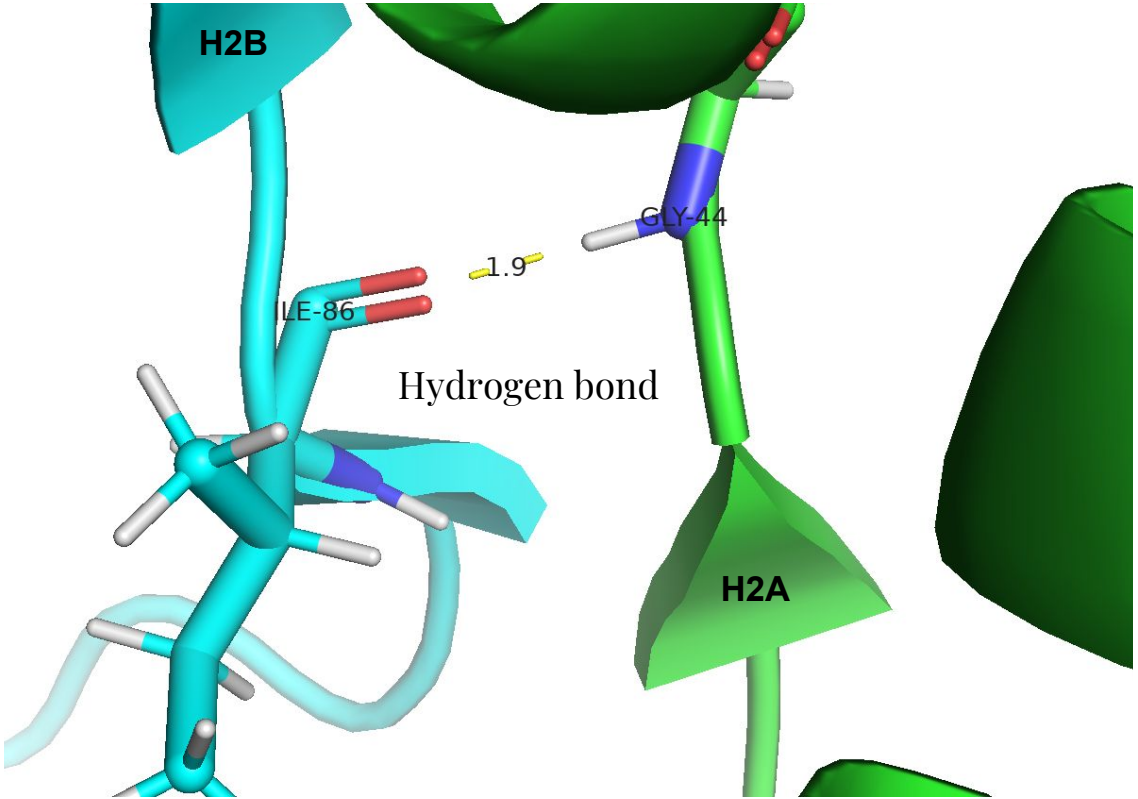


pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 2A - Histone 2B

Arg 42	Ile 86
Gly 44	**Ile 86**
Ile 78	Ile 51

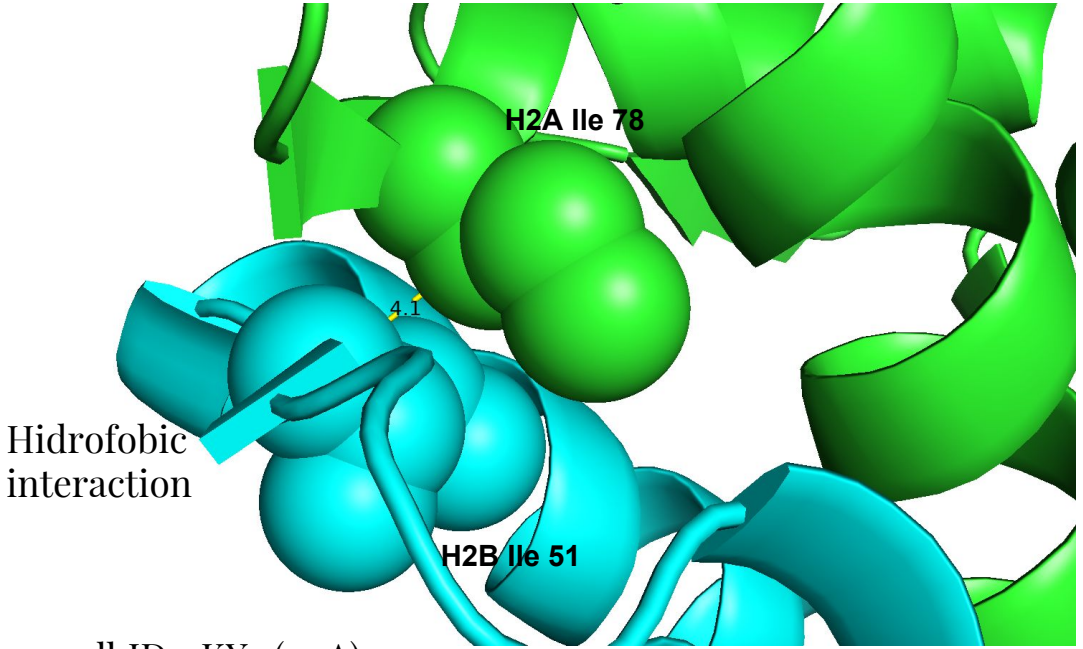


pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 2A - Histone 2B

Arg 12	Ile 86
Gly 44	Ile 86
Ile 78	**Ile 51**



Hidrofobic interaction

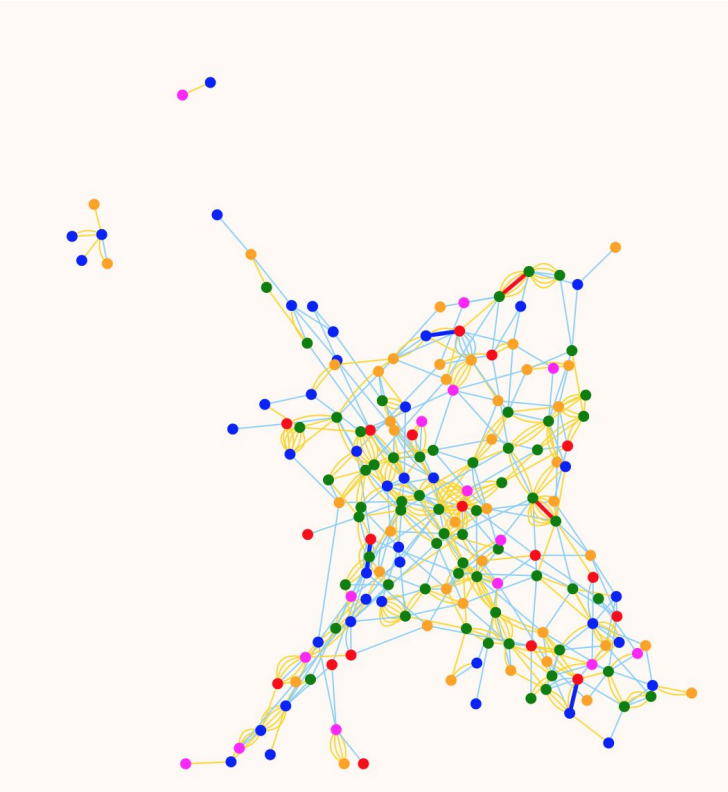
pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 3 - Histone 4

HBOND VDW IONIC
PIPISTACK

ARG LYS CYS ILE LEU
MET PHE PRO TRP TYR
VAL ASN GLN HIS ALA
GLY SER THR ASP GLU
LIG

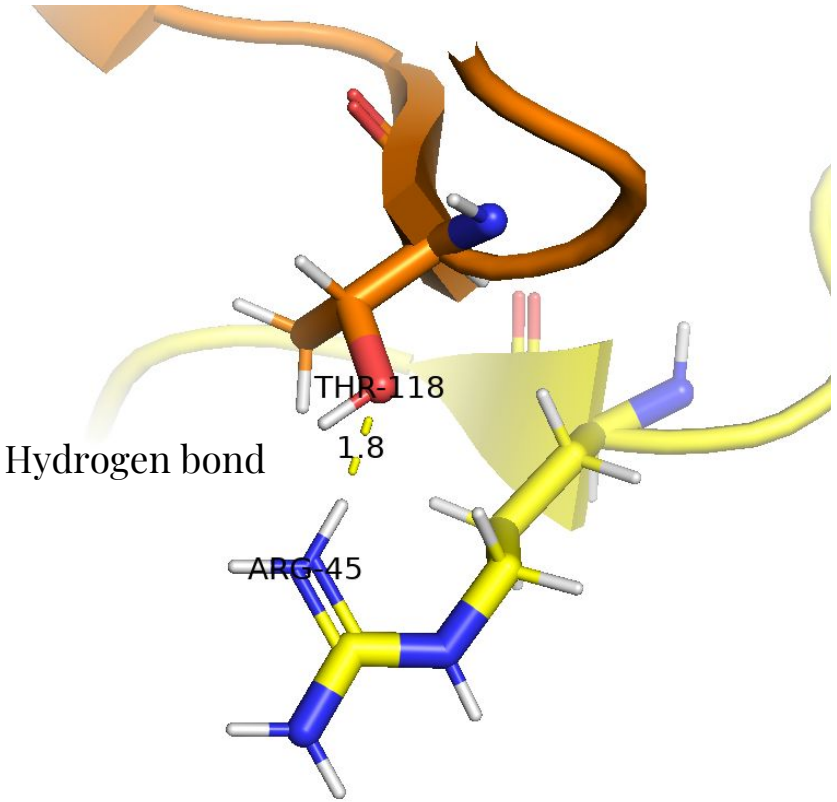


Source:
http://protein.bio.unipd.it/ring/

Dimer interactions

Histone 3 - Histone 4

Thr 118	Arg 45
Val 117	Arg 45
Ile 119	Ser 47



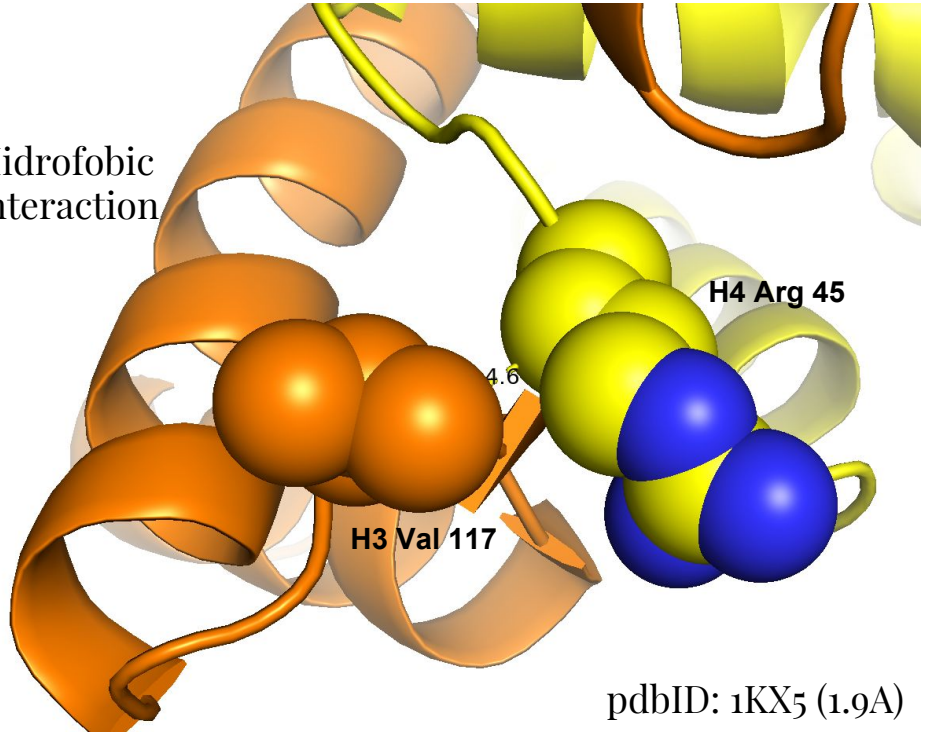
pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 3 - Histone 4

Thr 118	Arg 45
Val 117	**Arg 45**
Ile 119	Ser 47

Hidrofobic interaction

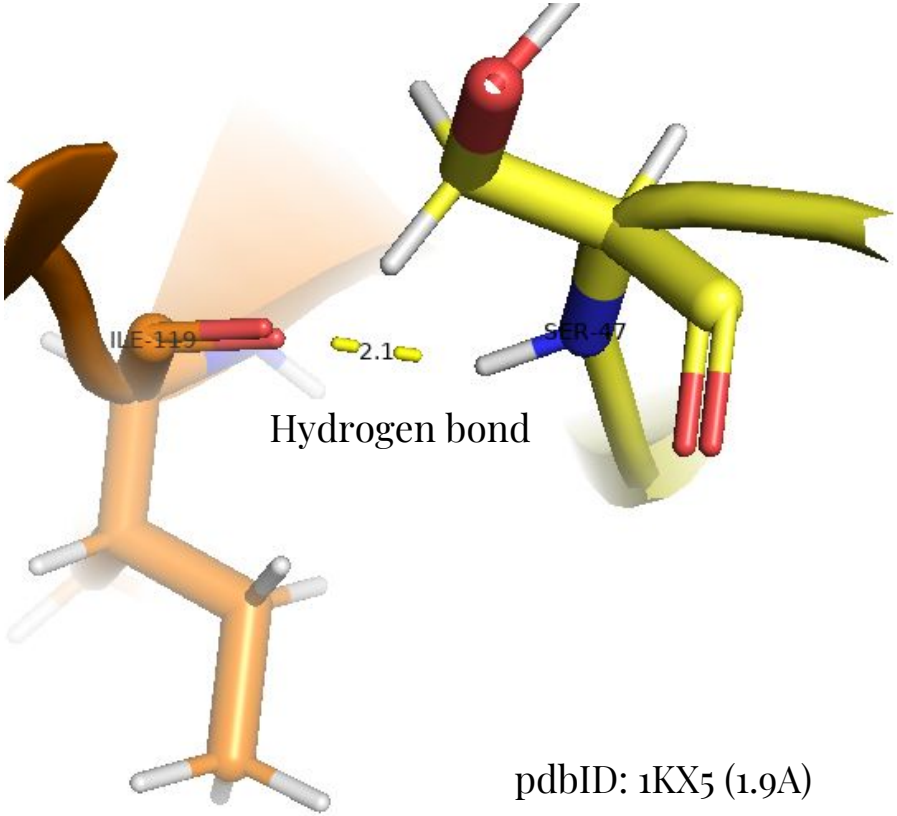


pdbID: 1KX5 (1.9A)

Dimer interactions

Histone 3 - Histone 4

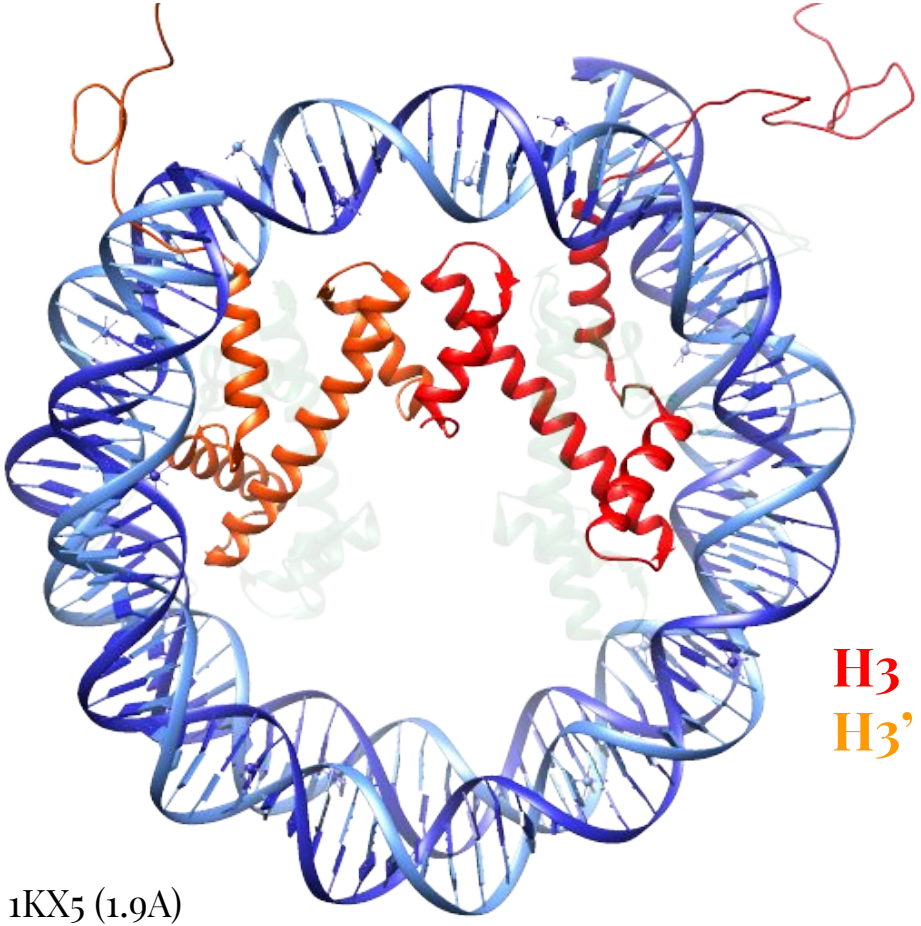
Thr 118	Arg 45
Val 117	Arg 45
Ile 119	**Ser 47**



Hydrogen bond

pdbID: 1KX5 (1.9A)

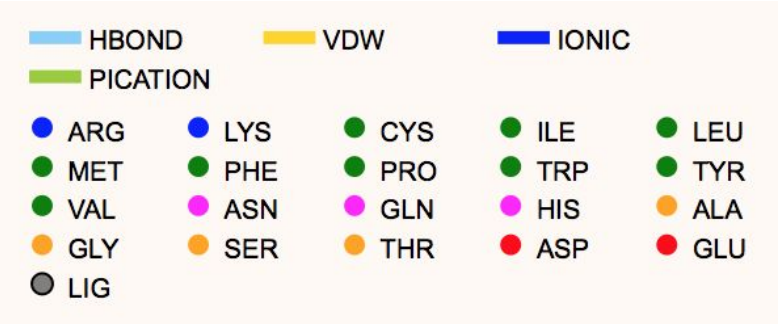
Tetramer H3-H4

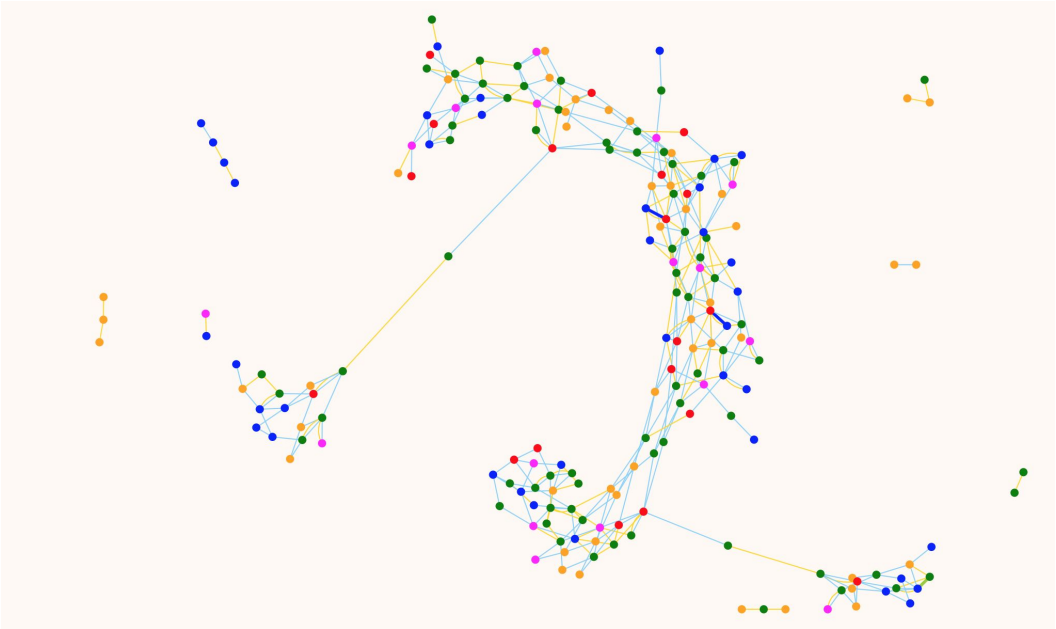


pdbID: 1KX5 (1.9A)

Tetramer

Histone 3 - Histone 3'



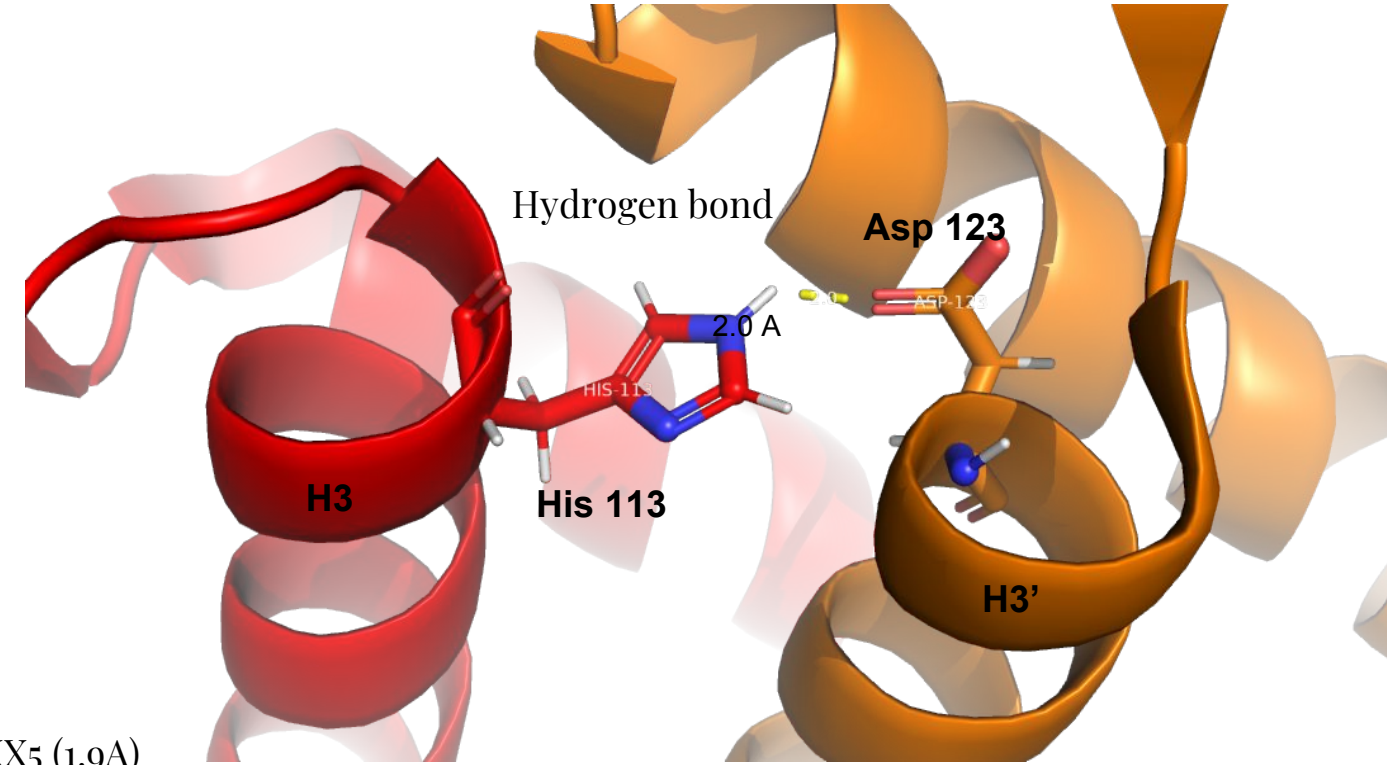


Source:
http://protein.bio.unipd.it/ring/

Tetramer

Histone 3 - Histone 3'

His 113	Asp 123
His 113	Cys 110

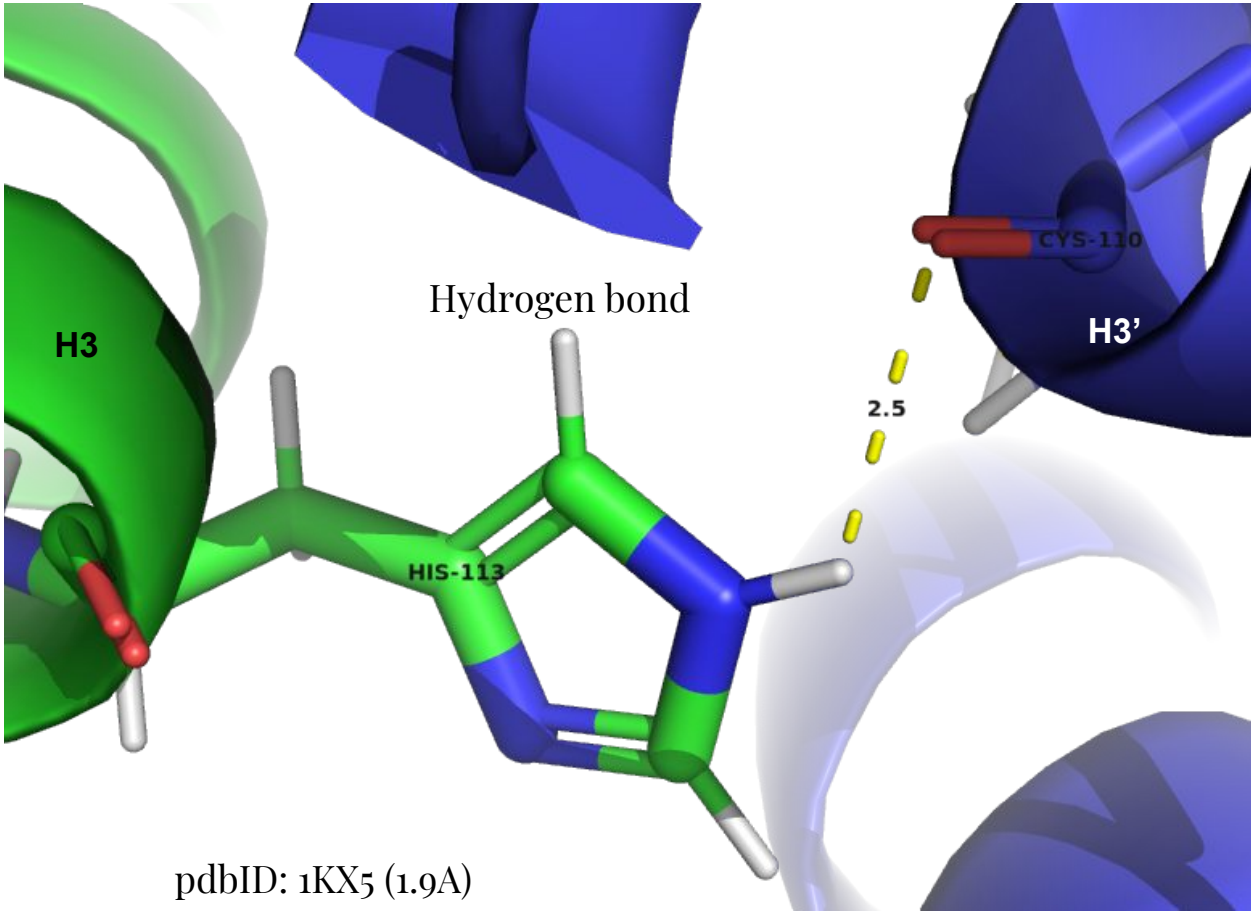


pdbID: 1KX5 (1.9A)

Tetramer

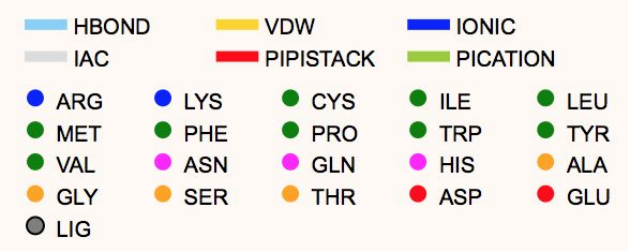
Histone 3 - Histone 3'

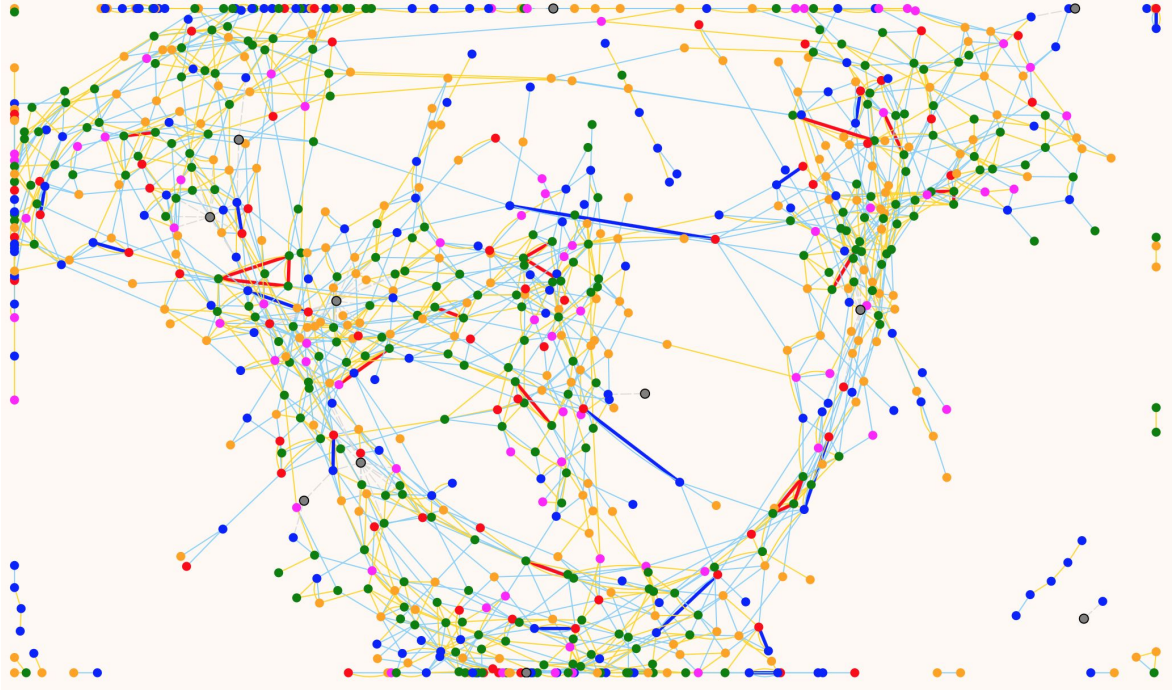
His 113	Asp 123
His 113	**Cys 110**



pdbID: 1KX5 (1.9A)

Octamer

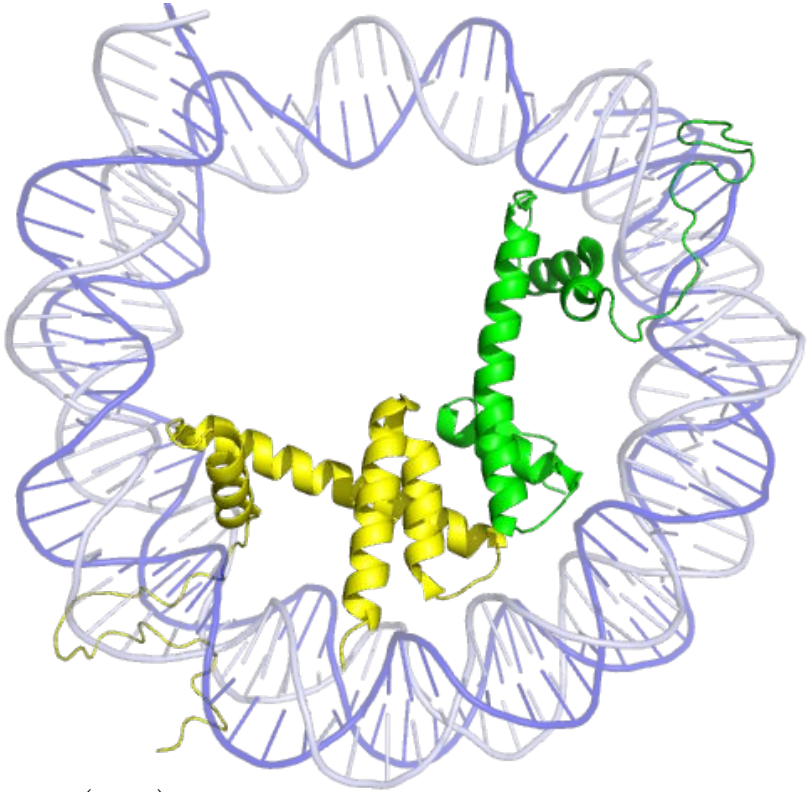




Source:
http://protein.bio.unipd.it/ring/

Octamer interactions

H4 – H2B

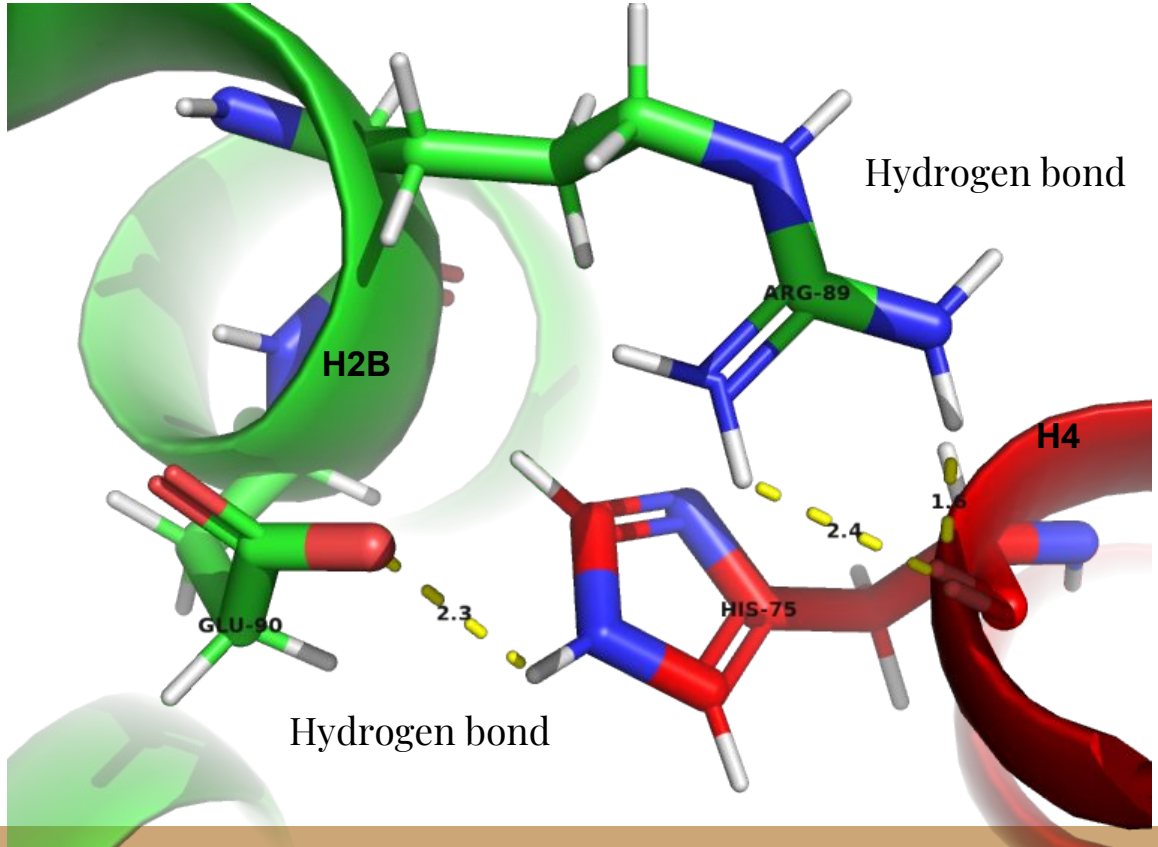


pdbID: 1KX5 (1.9A)

Octamer interactions

Histone 4 - Histone 2B

His 75	**Arg 89**
His 75	**Glu 90**
Arg 92	Glu 73

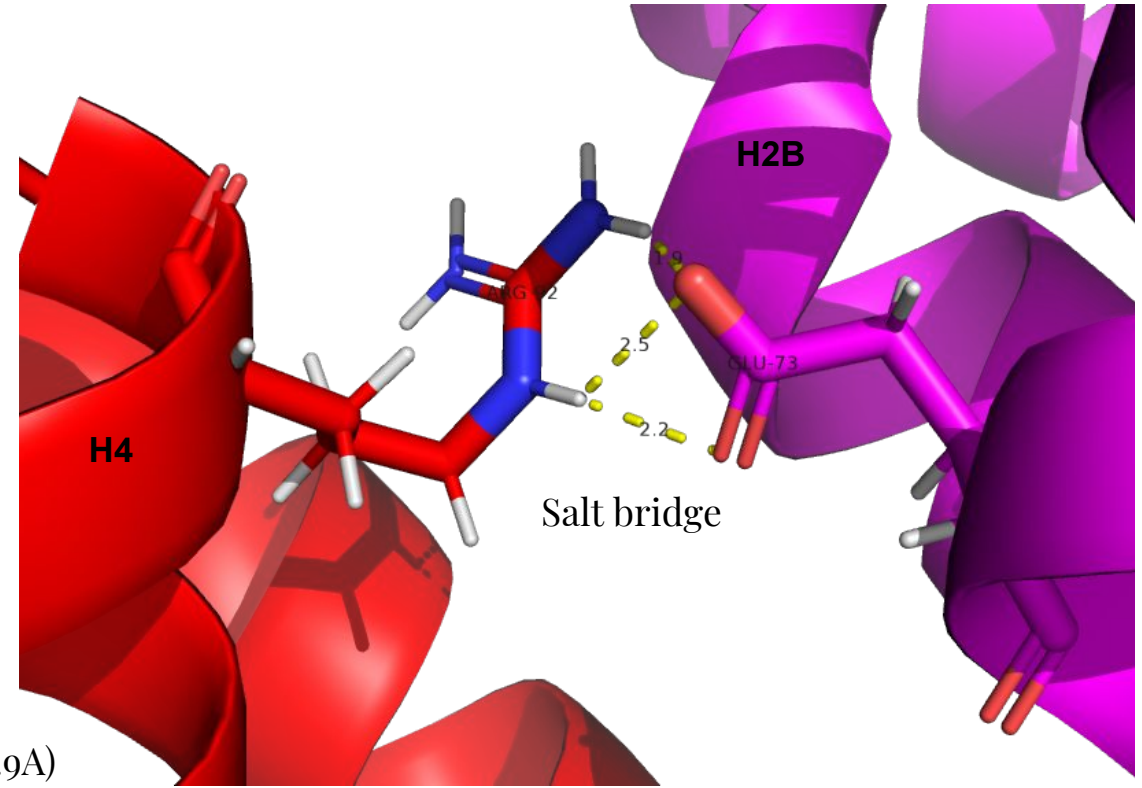


pdbID: 1KX5 (1.9A)

Octamer interactions

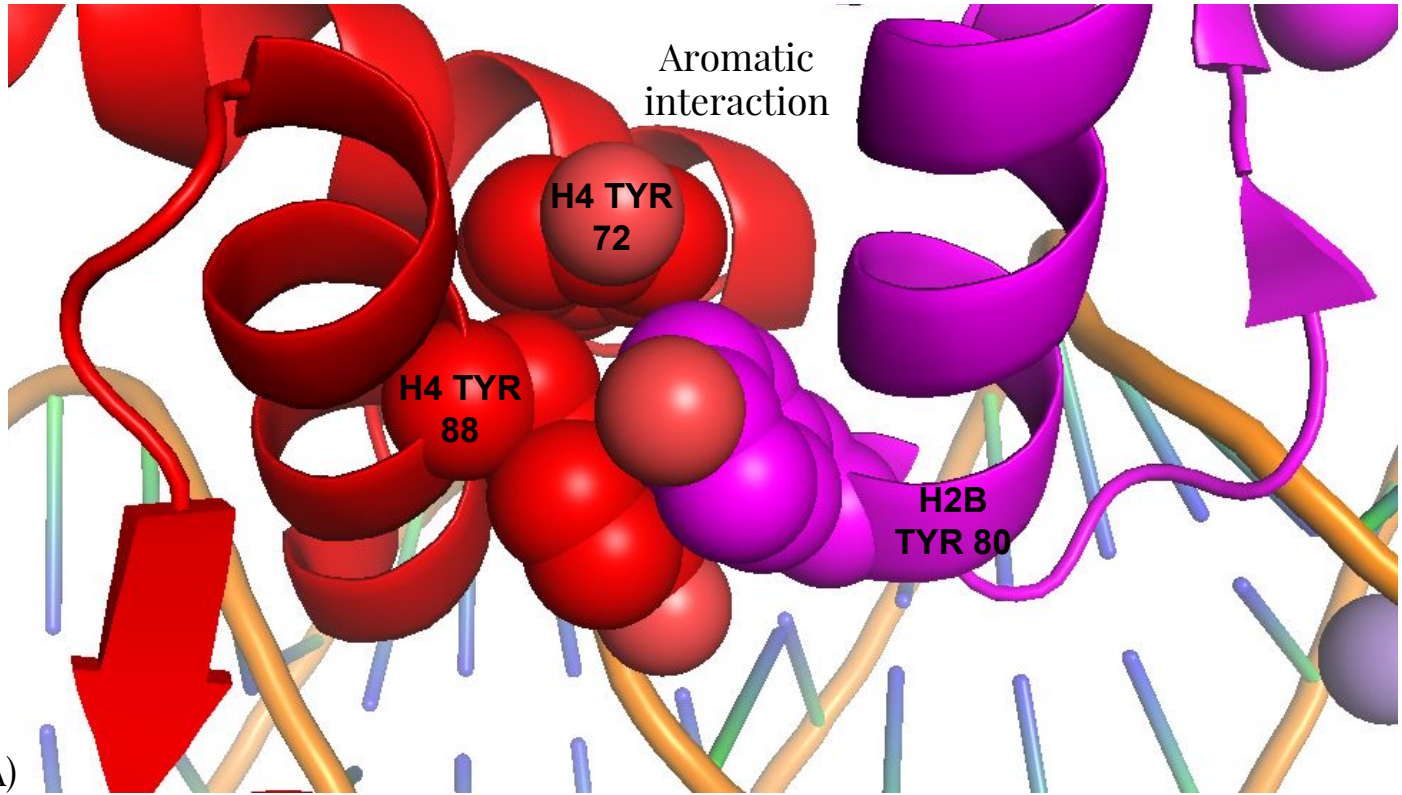
Histone 4 - Histone 2B

His 75	Arg 89
His 75	Glu 90
Arg 92	**Glu 73**



pdbID: 1KX5 (1.9A)

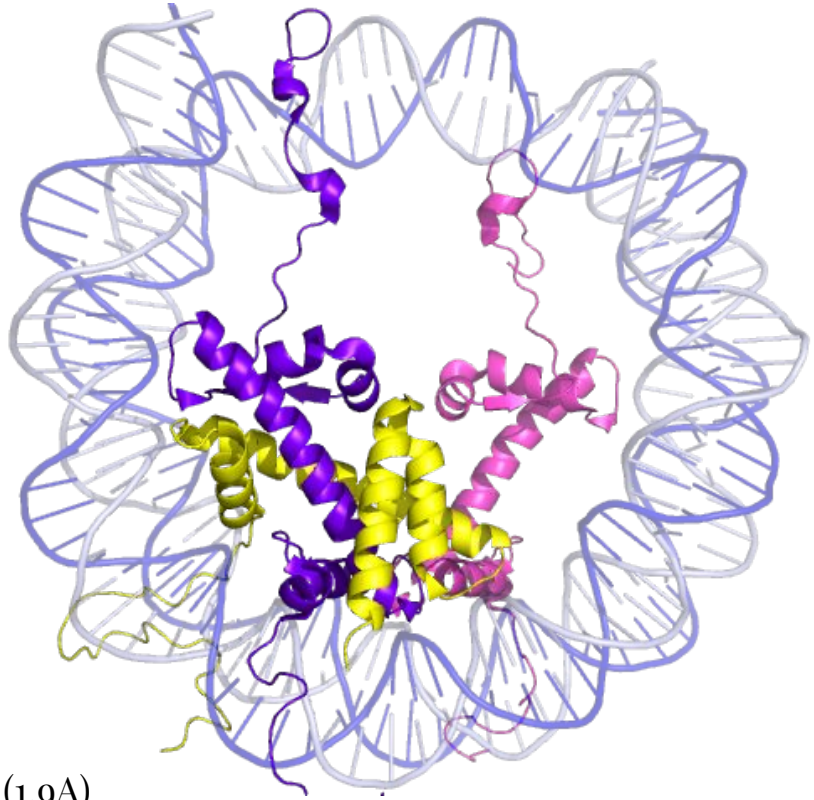
Octamer Hydrophobic Cluster



pdbID: 1KX5 (1.9A)

Octamer interactions

H2A-H2B-H2A'

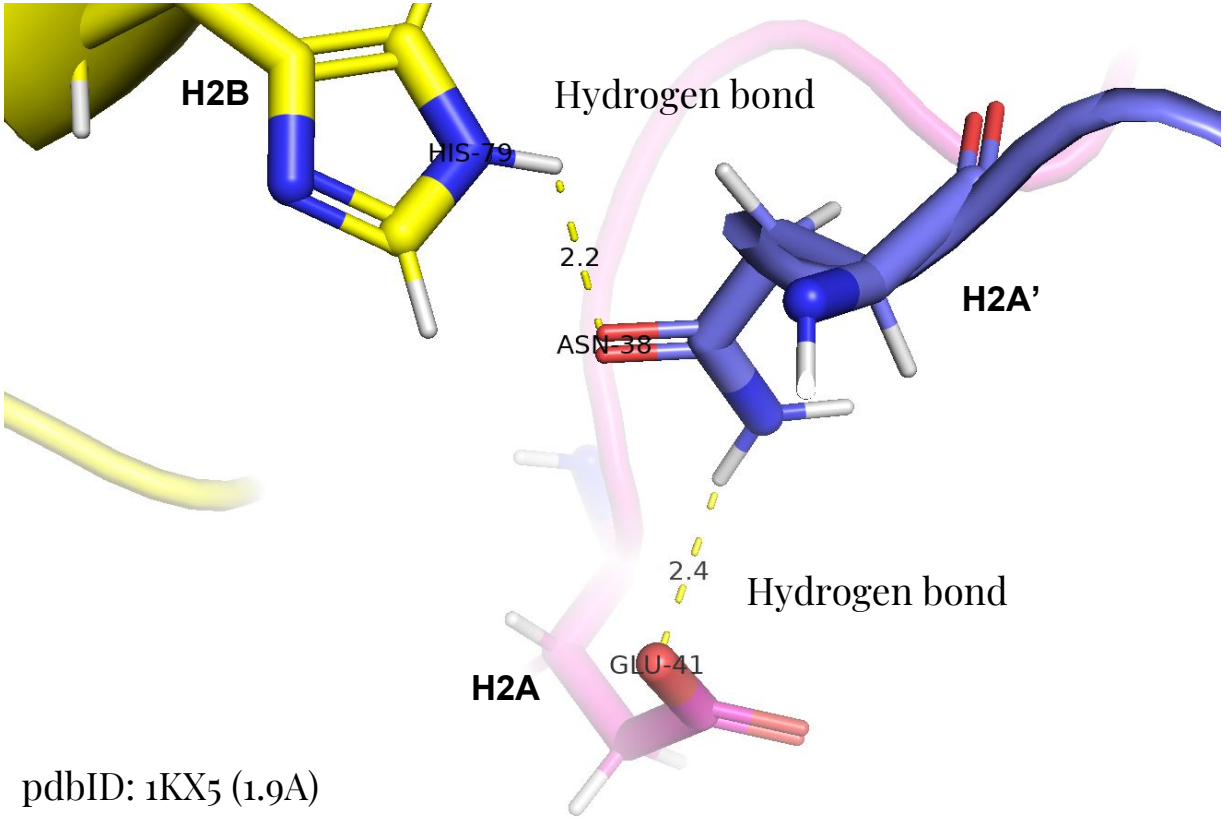


H2A
H2A'
H2B

pdbID: 1KX5 (1.9A)

Octamer interactions

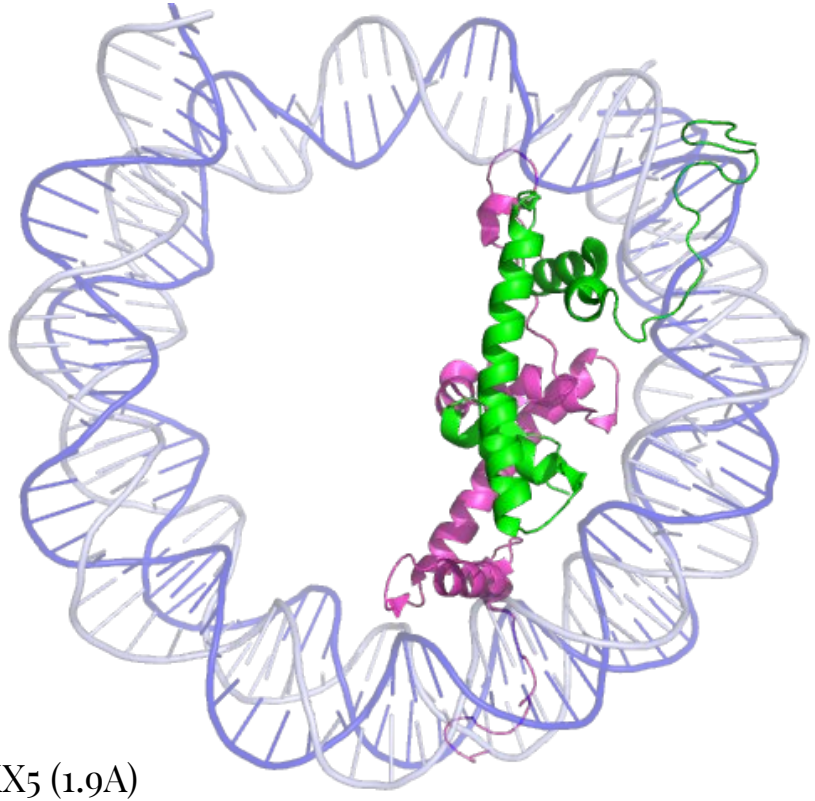
H2A 41 H2B 79 H2A' 38



pdbID: 1KX5 (1.9A)

Octamer interactions

H4-H2A'

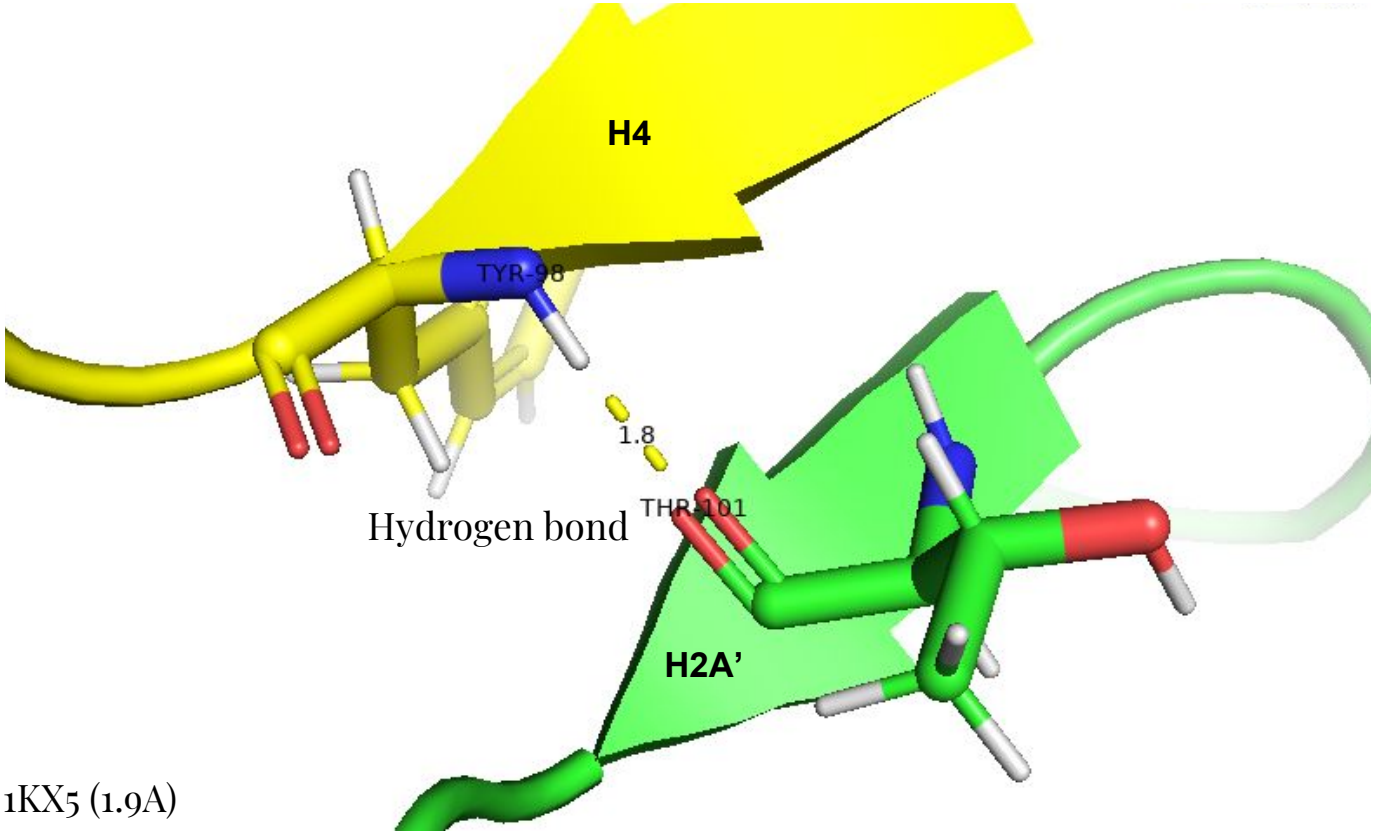


H2A'
H4

pdbID: 1KX5 (1.9A)

Octamer interactions

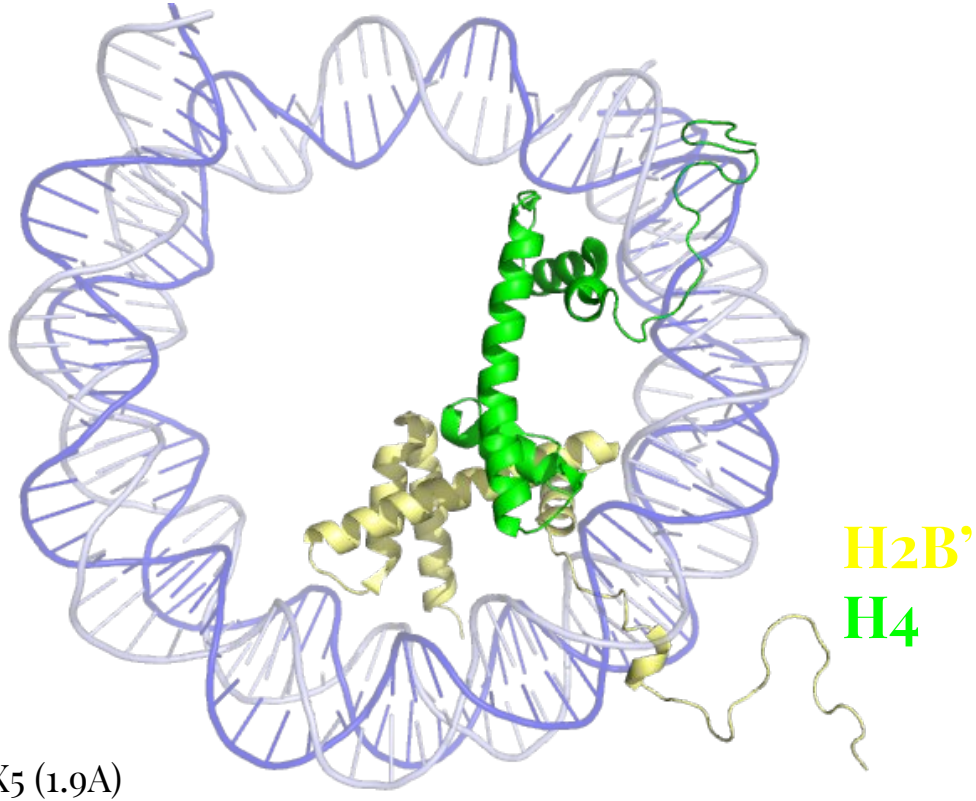
H2A' 101 H4 98



pdbID: 1KX5 (1.9A)

Octamer interactions

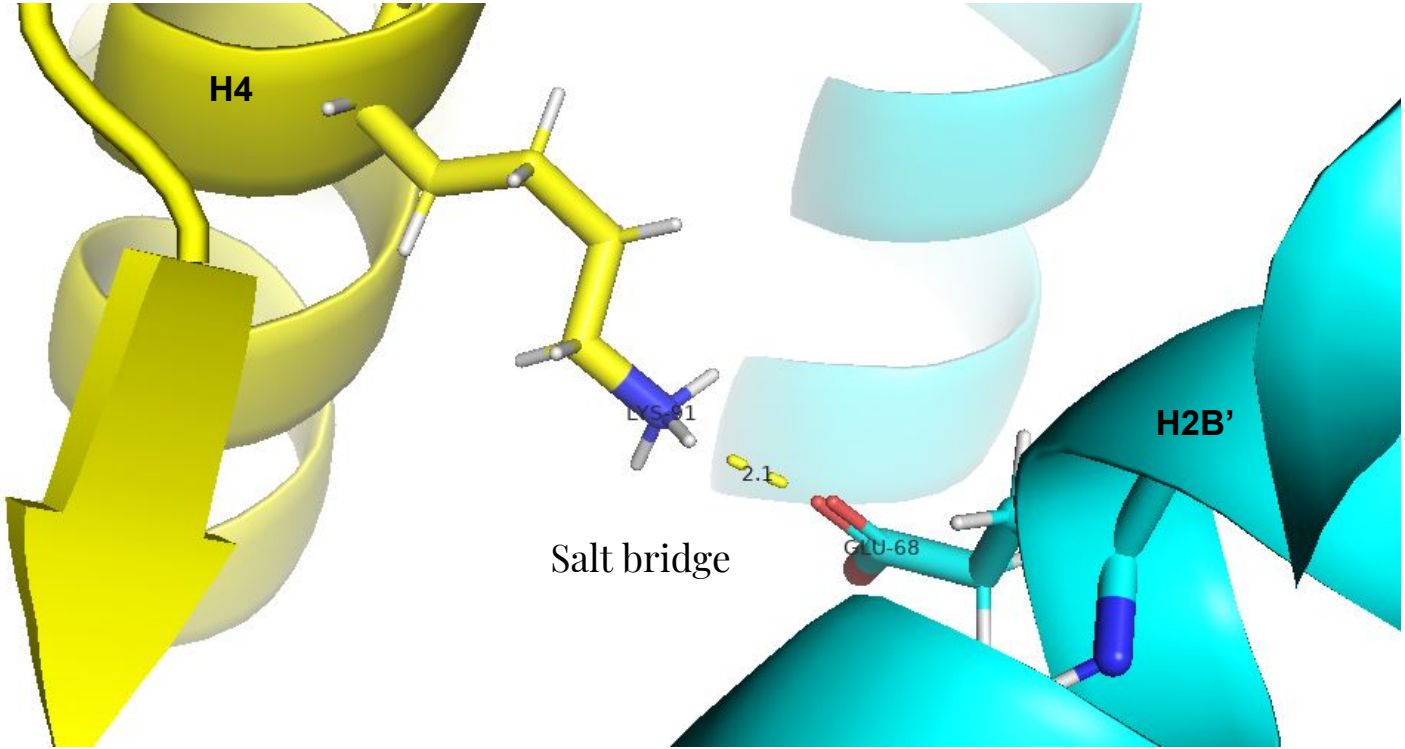
H4-H2B'



pdbID: 1KX5 (1.9A)

Octamer interactions

H2B' 68 H4 91

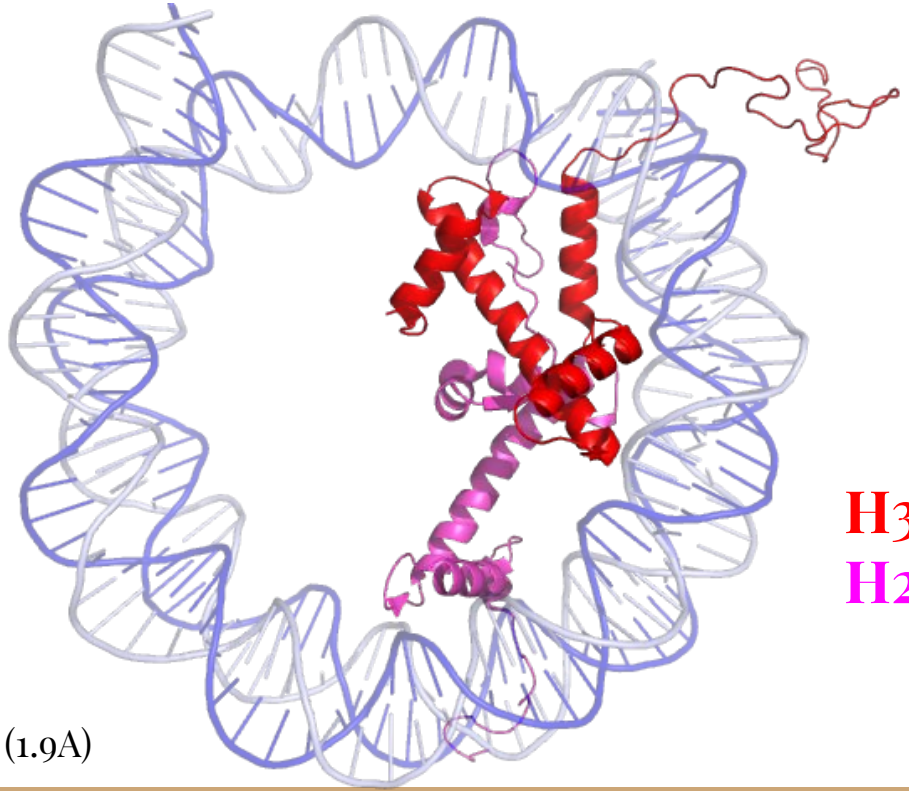


Salt bridge

pdbID: 1KX5 (1.9A)

Octamer interactions

H3-H2A'

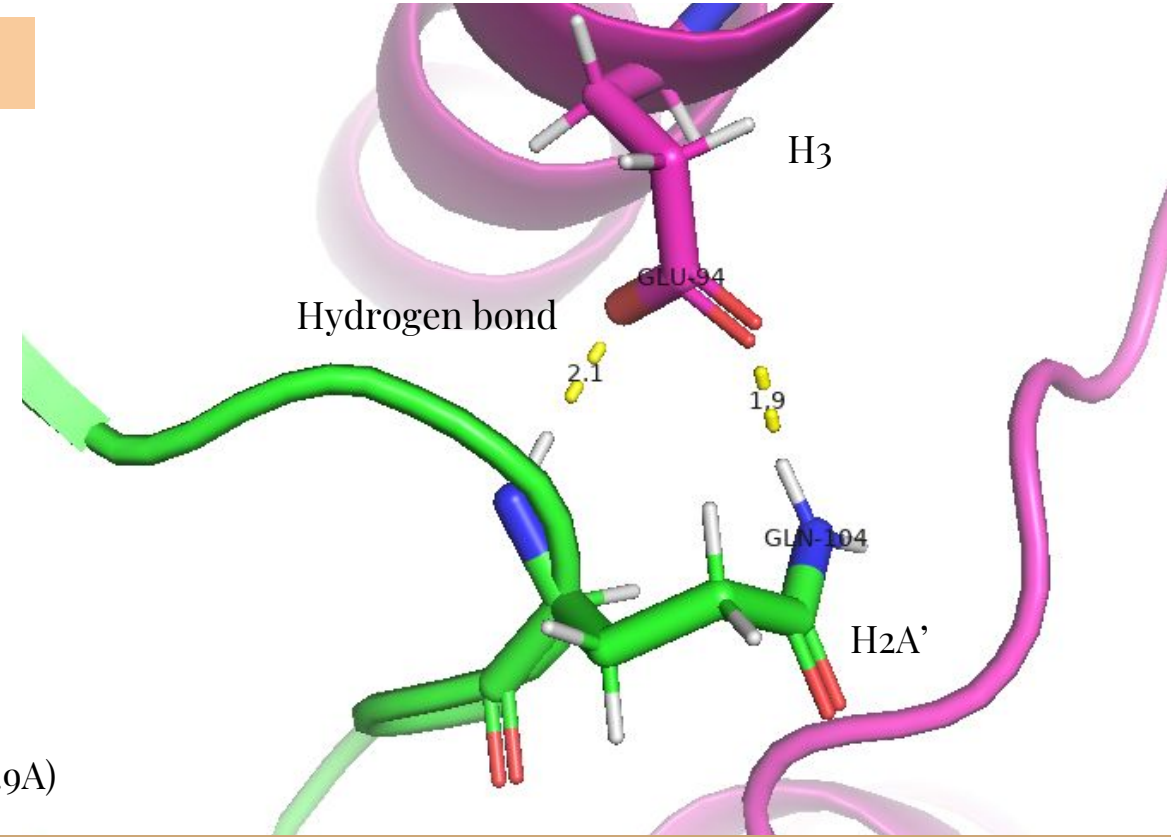


H3
H2A'

pdbID: 1KX5 (1.9A)

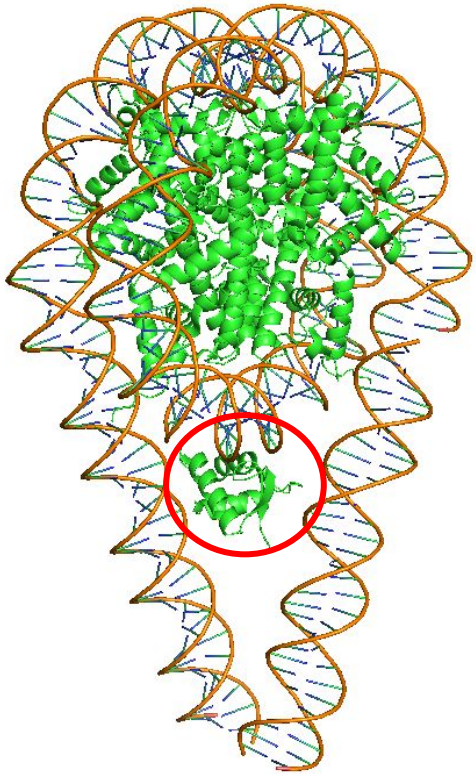
Octamer interactions

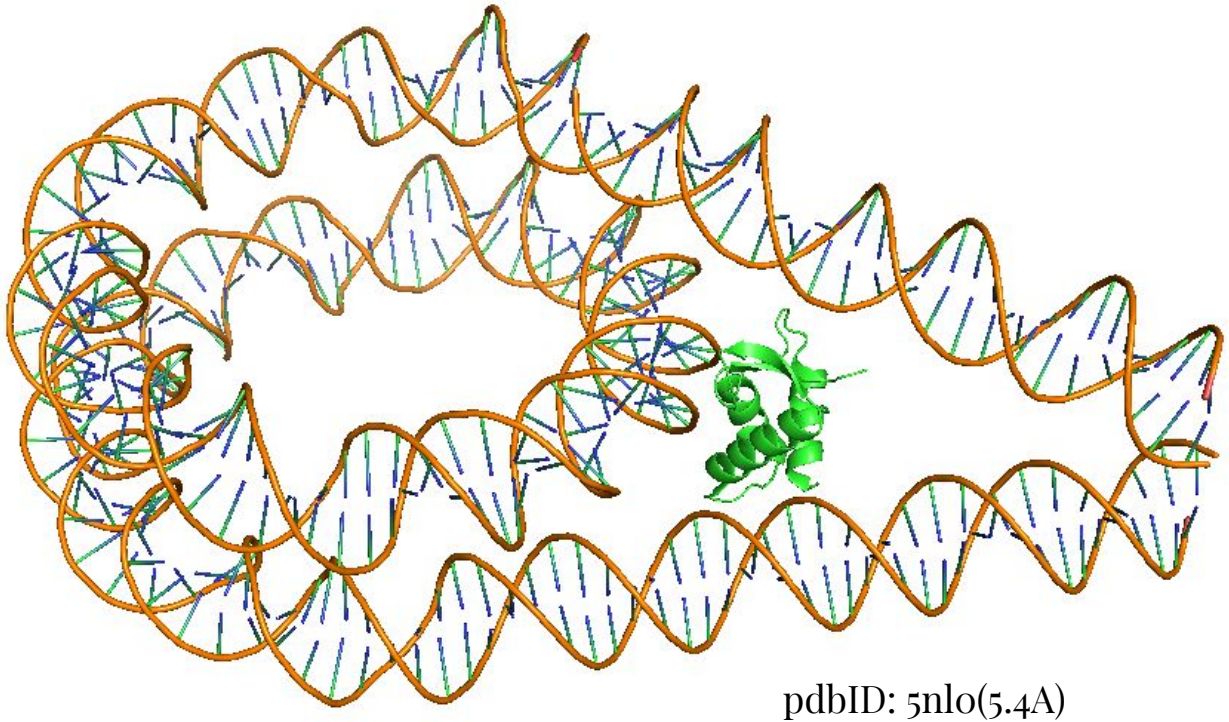
H3 94 H2A' 104



pdbID: 1KX5 (1.9A)

Linker histone H1





pdbID: 5nlo(5.4A)

H1 Site 1

His 25

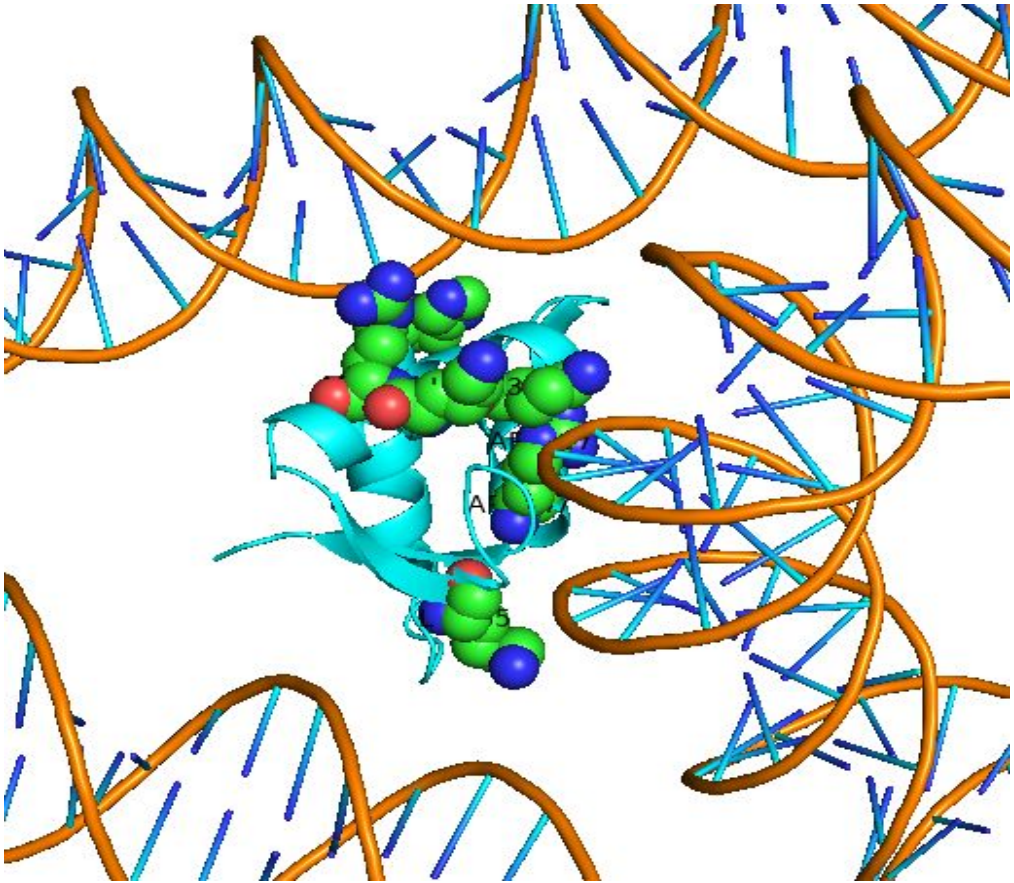
Arg 47

Lys 69

Lys 73

Arg 74

Lys 85



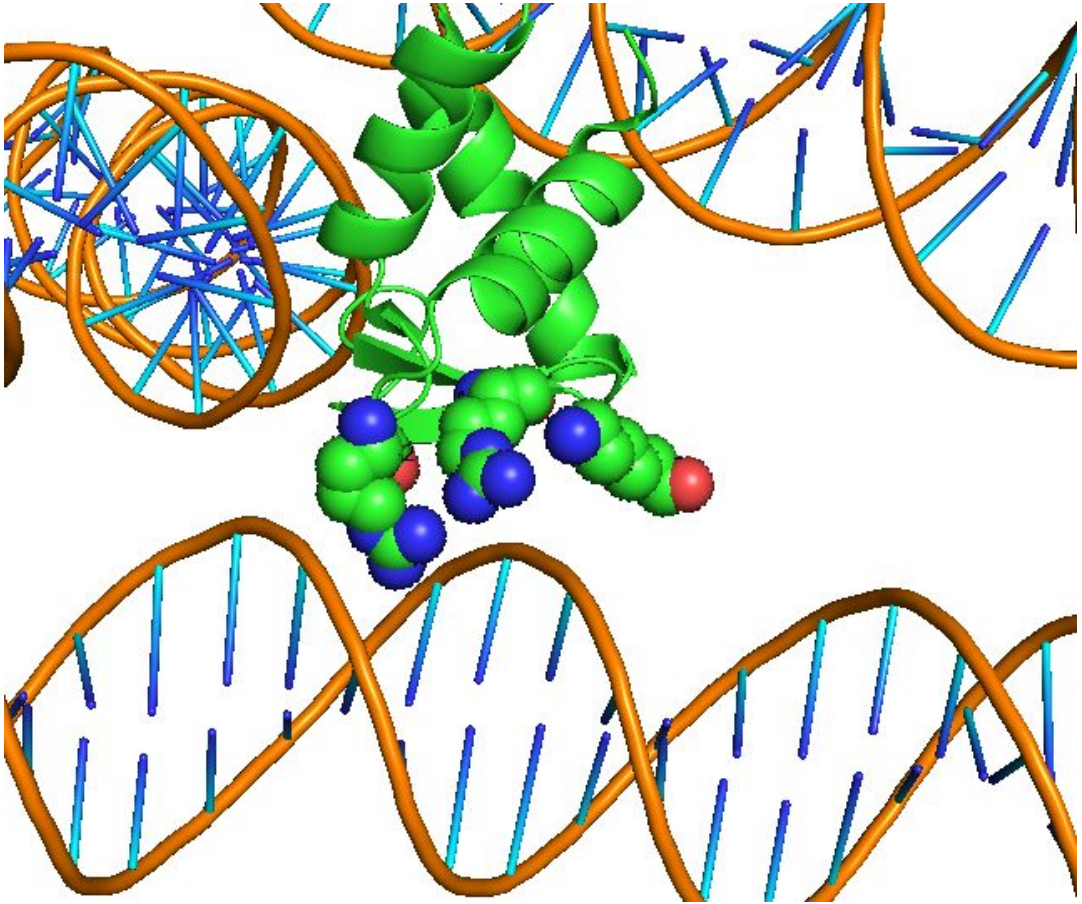
pdbID: 5nlo (5.4A)

H1 Site 2

Arg 42

Arg 94

Lys 97

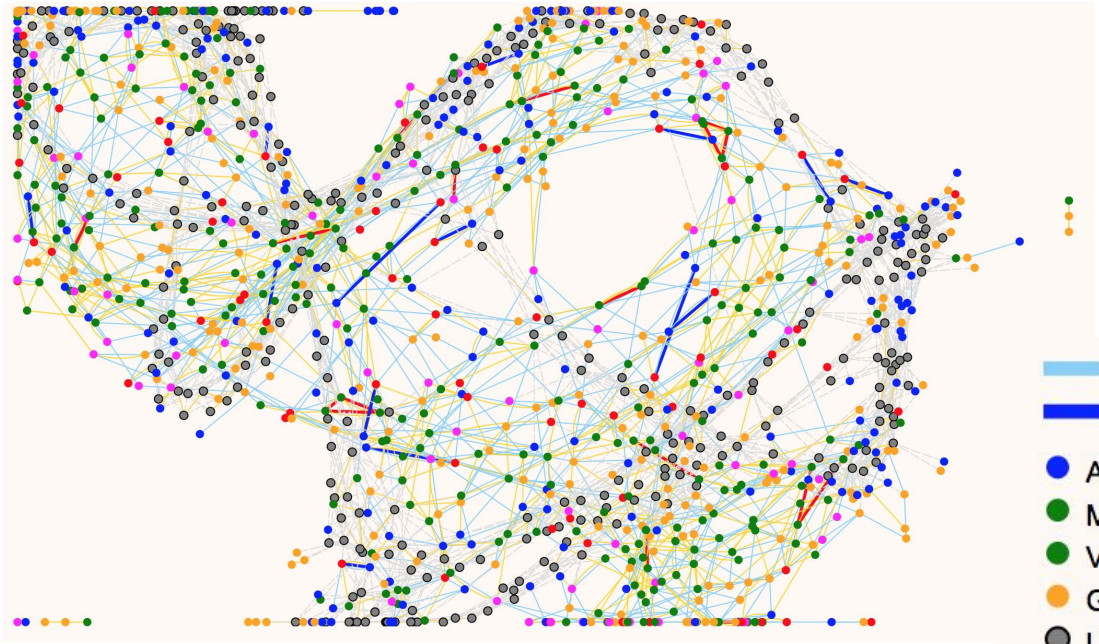


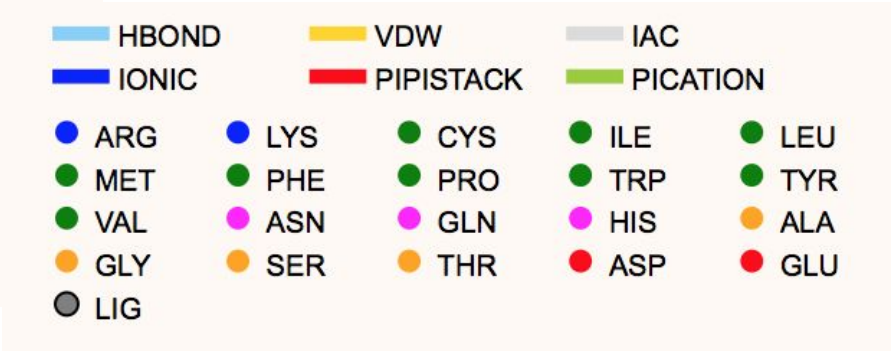
pdbID: 5nlo (5.4A)

Types of Histone-DNA interactions

- Salt bridge
- Hydrogen Bond
- Non-polar interactions
- Minor groove insertion
- AT rich regions interaction
- Helix dipoles

Types of DNA interactions

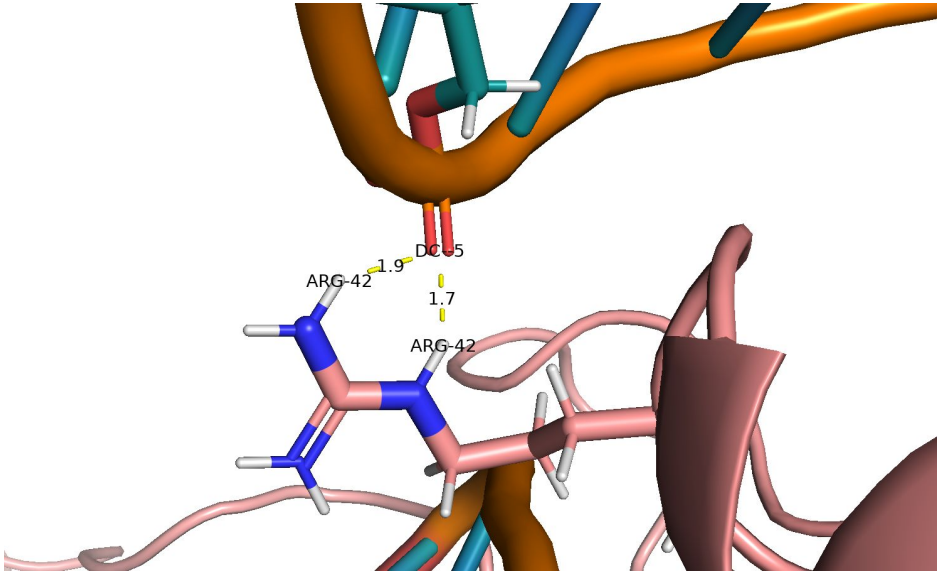


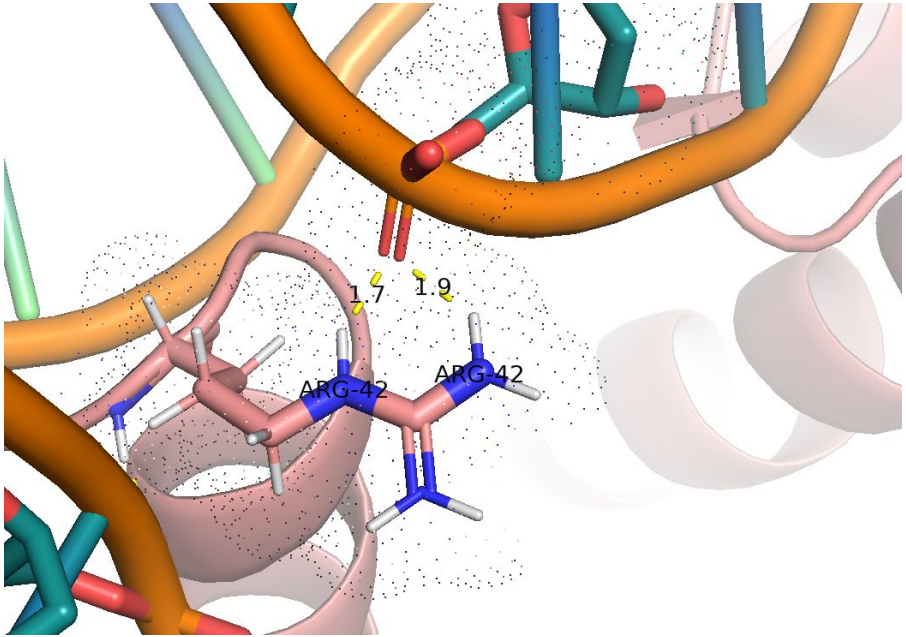


DNA interactions

Salt Bridge

H3’ Arg 42



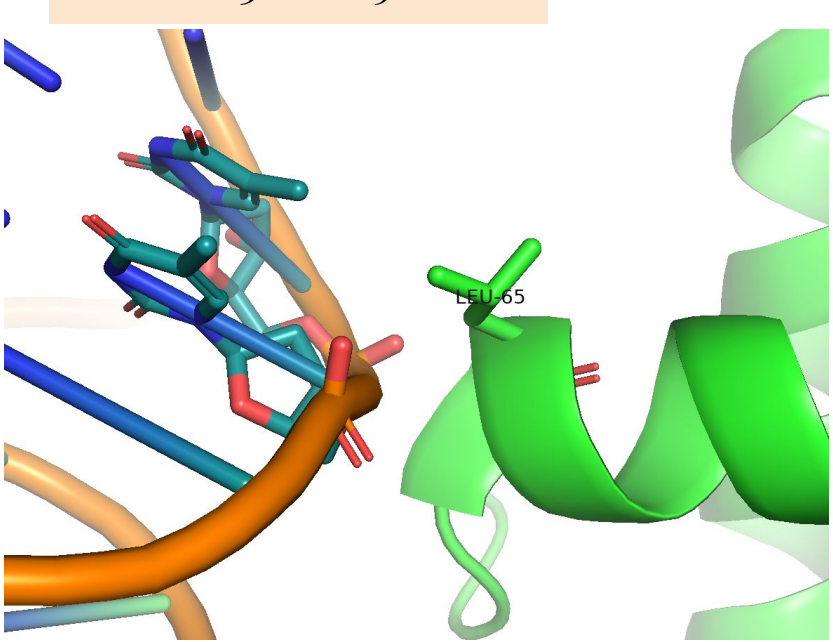


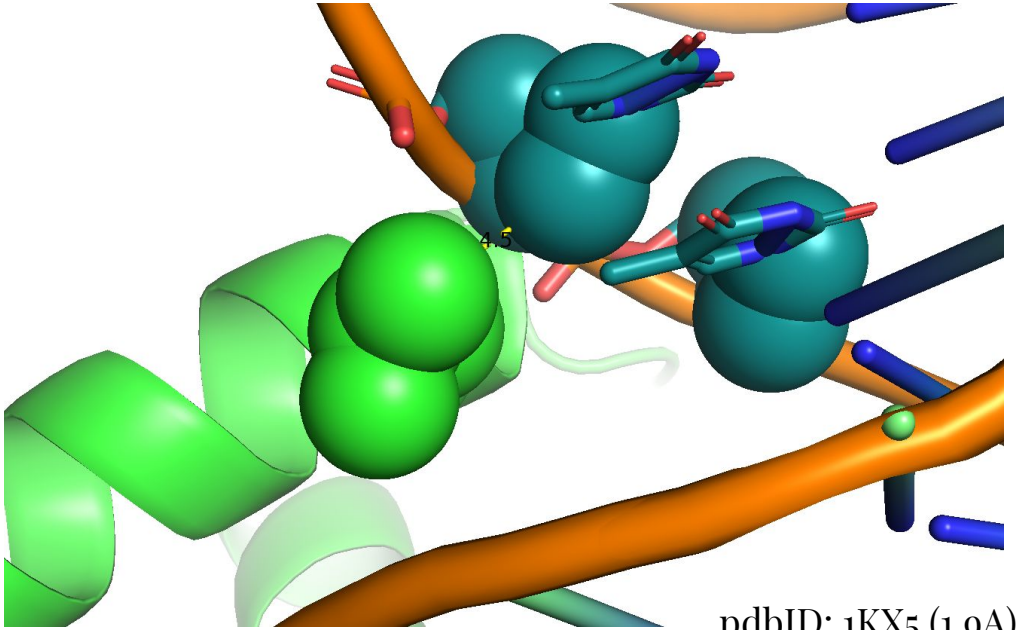
pdbID: 1KX5 (1.9A)

DNA interactions

Non-polar interactions

H3 Leu 65



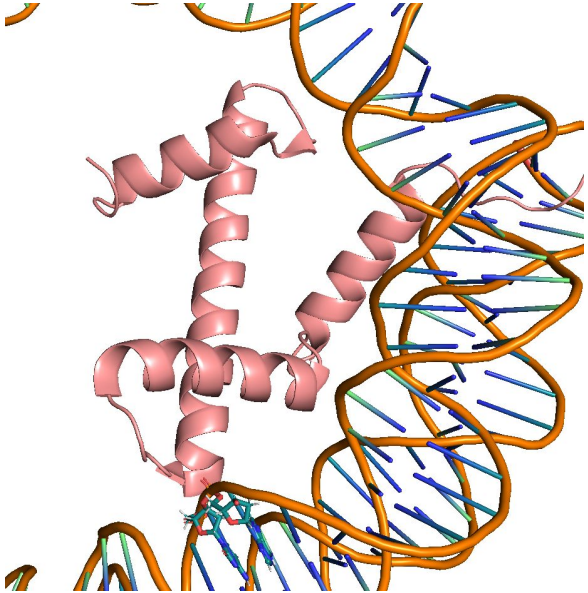


pdbID: 1KX5 (1.9A)

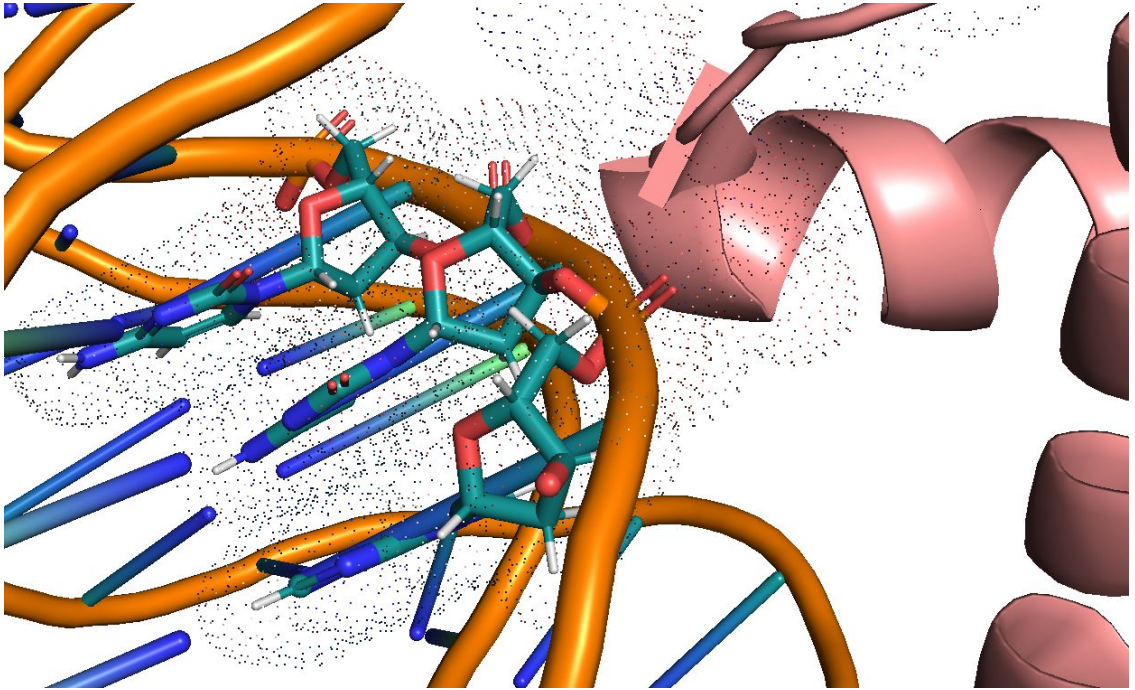
DNA interactions

Helix dipoles

H3’



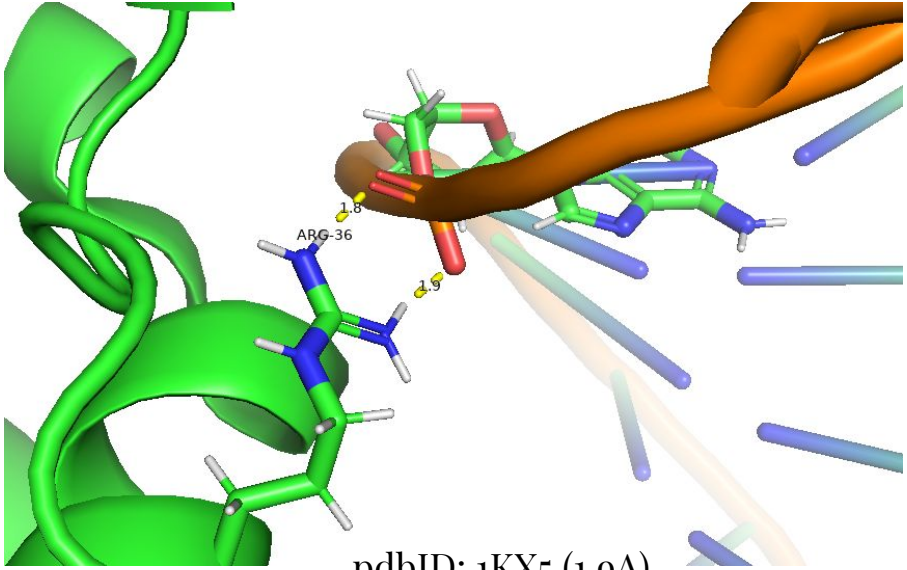
pdbID: 1KX5 (1.9A)



DNA interactions

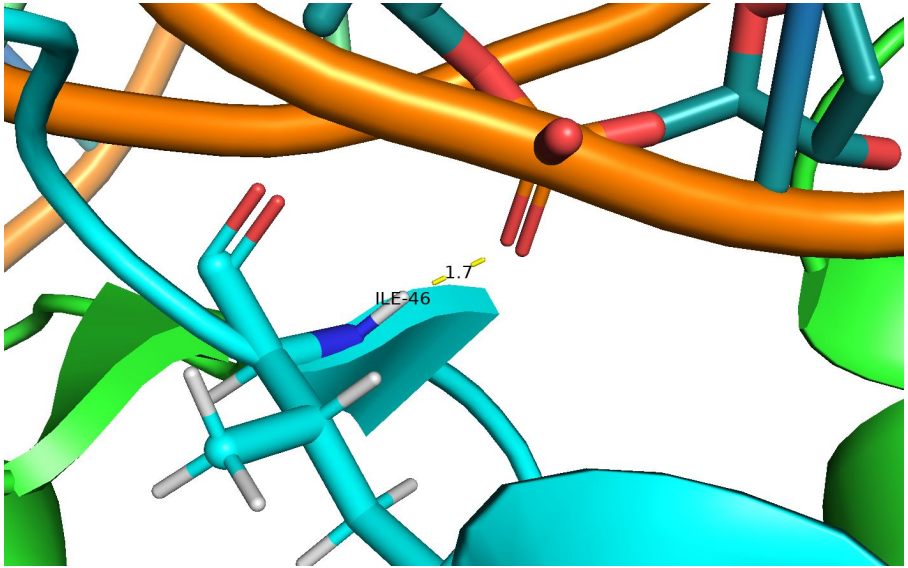
Hydrogen bond main and side chain - DNA

H3 Arg 36



pdbID: 1KX5 (1.9A)

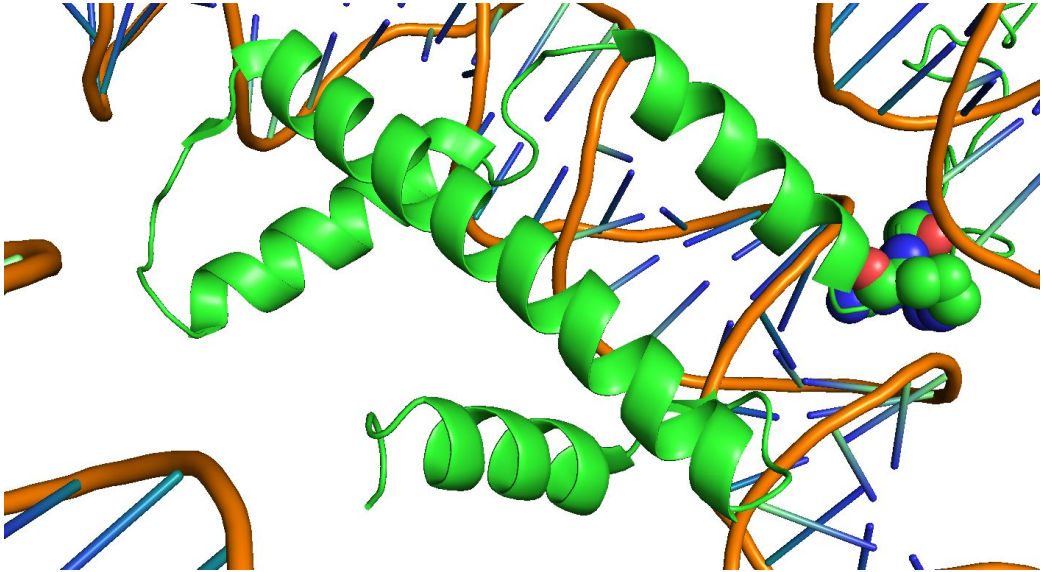
H4 Ile 46

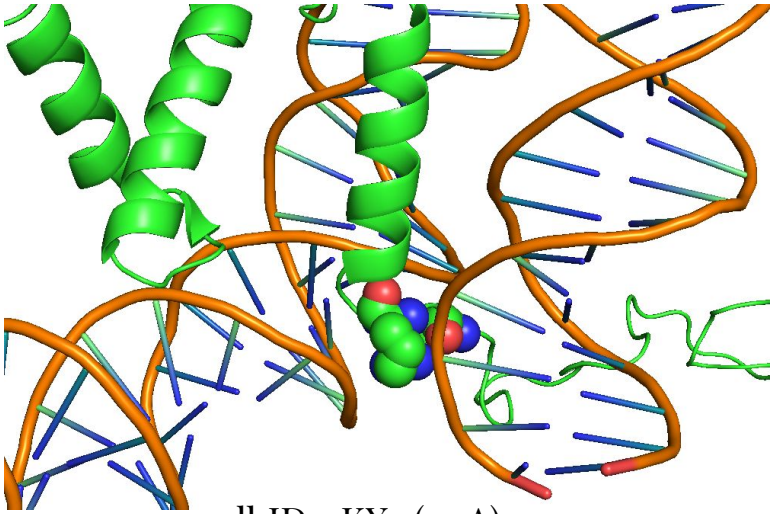


DNA interactions

Arginines minor groove insertion

H3 tail Arg 40-42



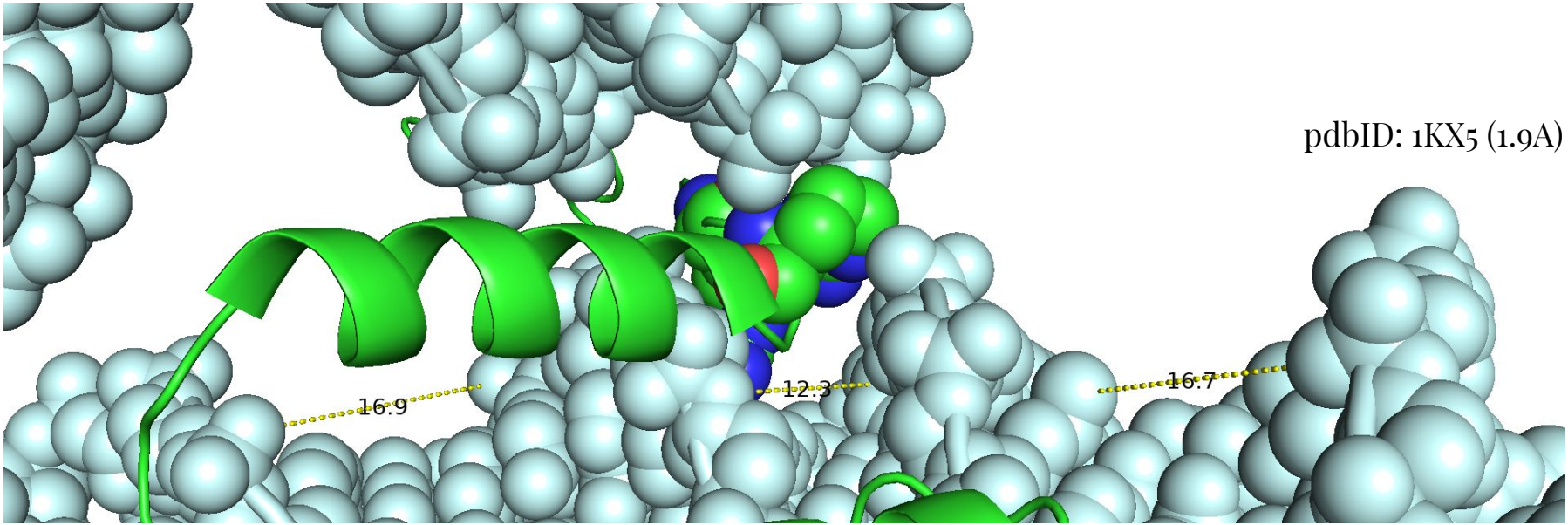


pdbID: 1KX5 (1.9A)

DNA interactions

Arginines minor groove insertion

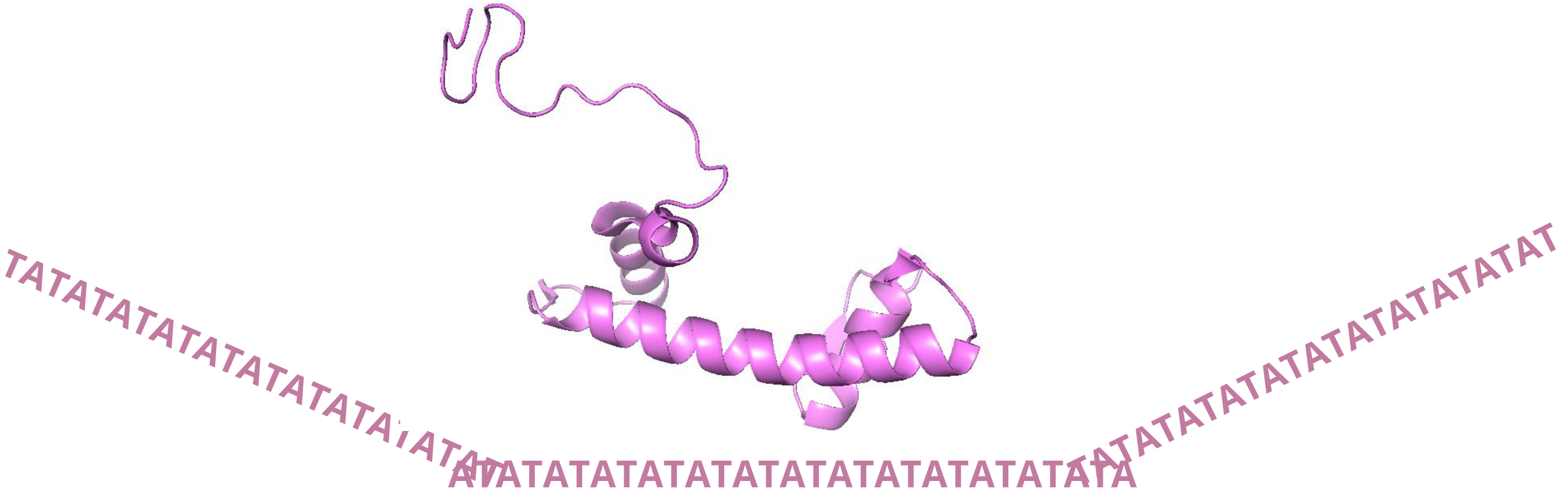
H3 tail Arg 40-42



pdbID: 1KX5 (1.9A)

DNA interactions

AT rich regions rigids and with curvature



Post-traductional modifications

ACETYLATION

Introduces negative charge which makes the chromatin more accessible.

METHYLATION

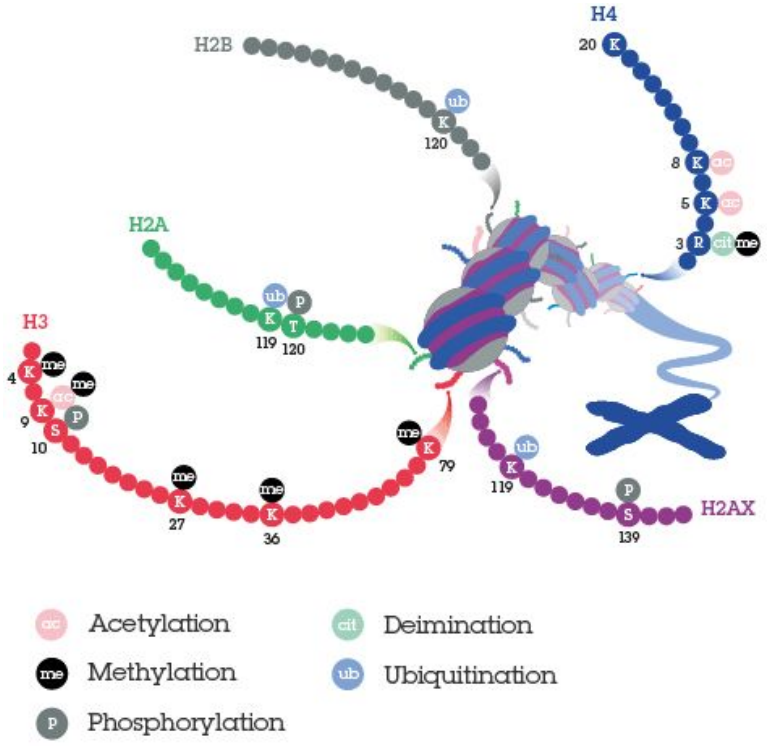
Maintains the positive charge evoking to the condensation of chromatin.

PHOSPHORYLATION

It appears in mitosis and it is thought to activate the DNA condensation.

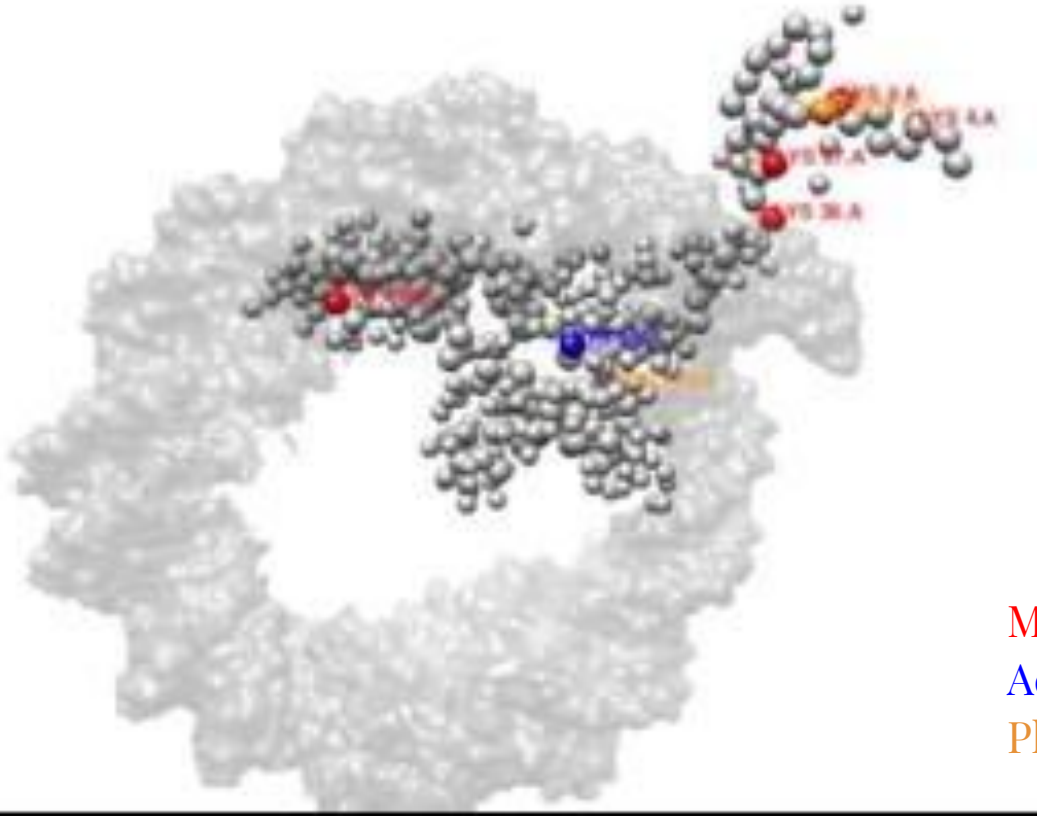
CITRUNILIZATION

Loss of positive charge and reduction in hydrogen-bonding ability.



Source: https://www.abcam.com/epigenetics/histone-modifications-a-guide

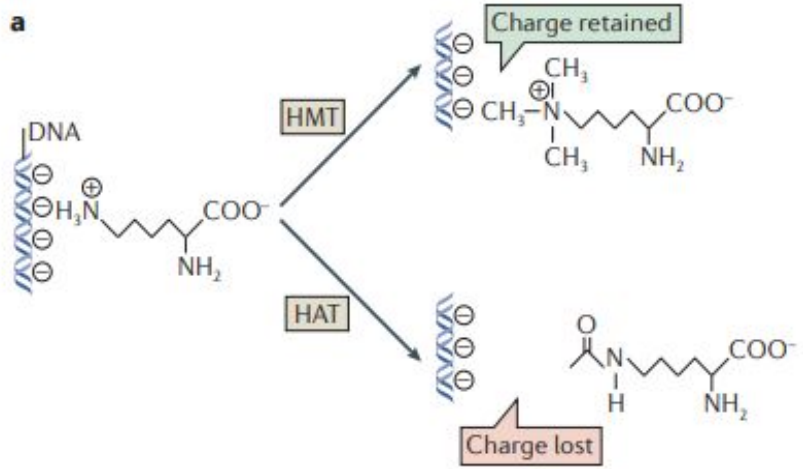
Histone H3 main post-traductional modifications



Methylations
Acetylations
Phosphorylations

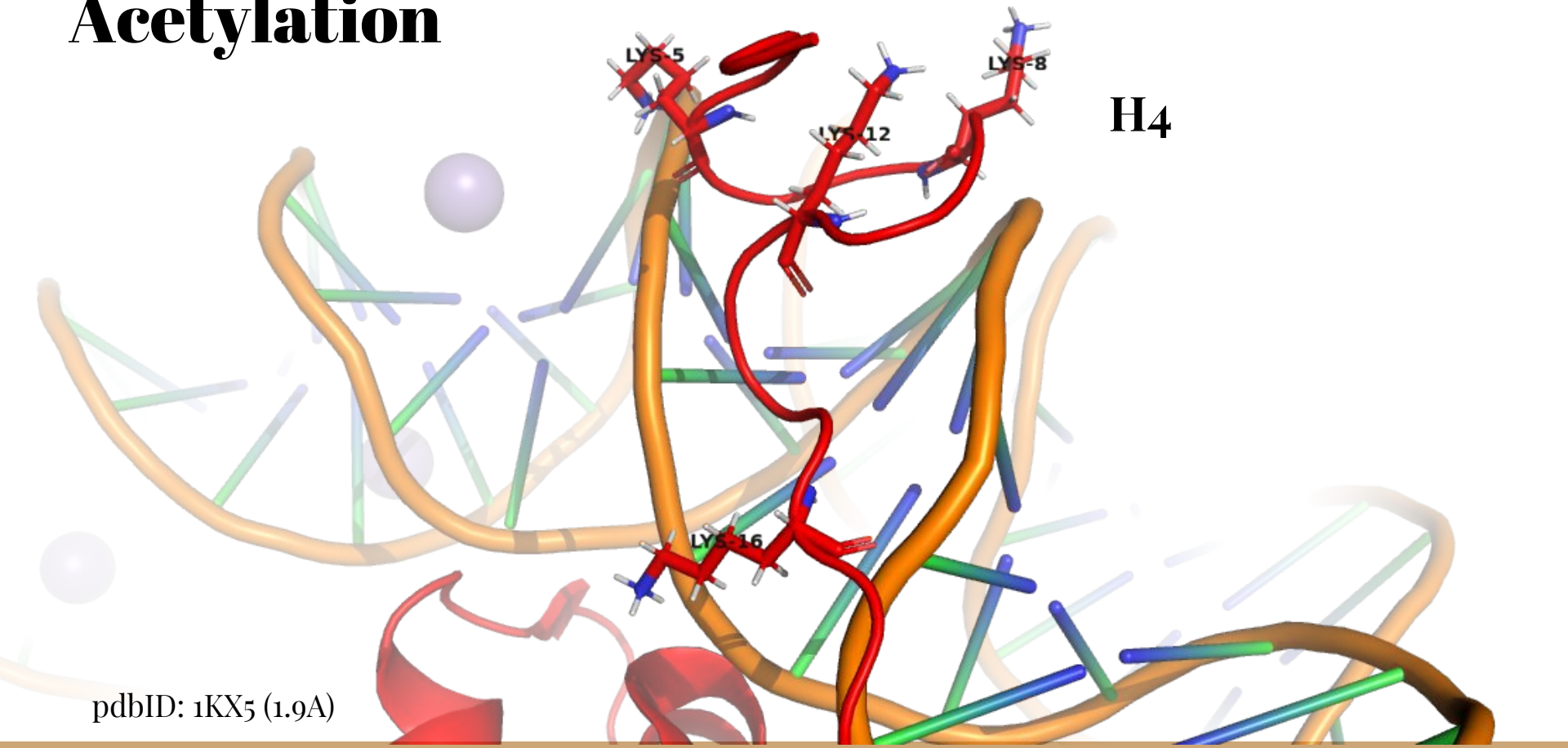
Acetylation

- **Neutralizes the positive** lysine charge, what makes that the electrostatic interactions of the tail with the DNA **weaker**
- This leads to a destabilization (euchromatin-like) of the nucleosome and respective **higher transcription activation**. It can also **attract transcription factors and chaperones** to promote transcriptio



Source: Tessarz P, Kouzarides T. Histone core modifications regulating nucleosome structure and dynamics. Nature Reviews Molecular Cell Biology. 2014;15(11):703-708.

Acetylation

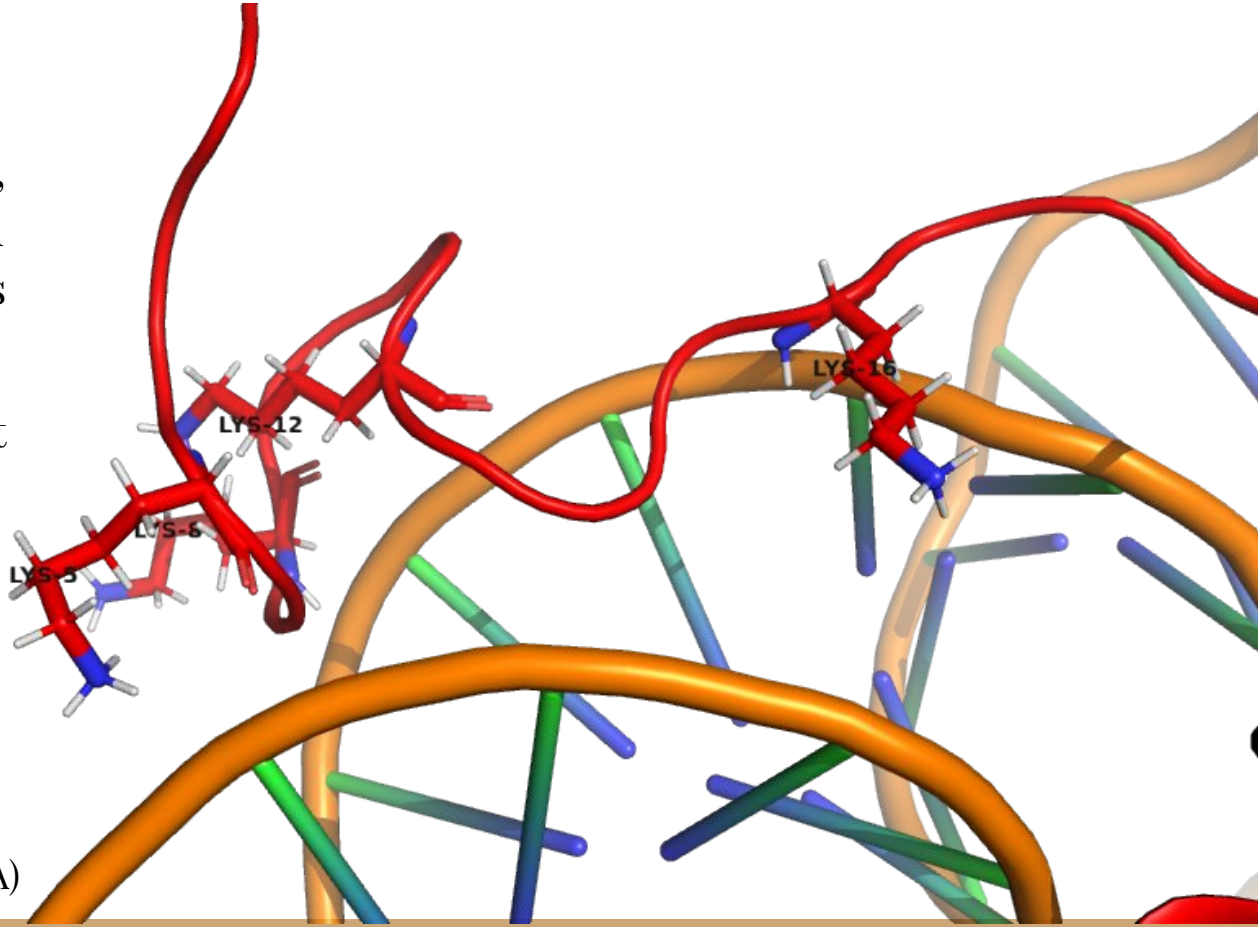


H4

pdbID: 1KX5 (1.9A)

Acetylation

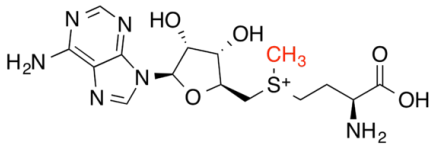
- N-terminal **H4K5, H4K8, H4K12, H4K16** are acetylated and are known to act as **transcriptional activators**
- They also play an important role in **epigenetic bookmarking**

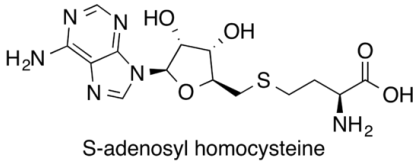


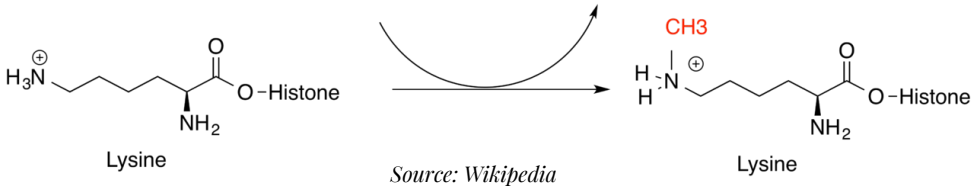
pdbID: 1KX5 (1.9A)

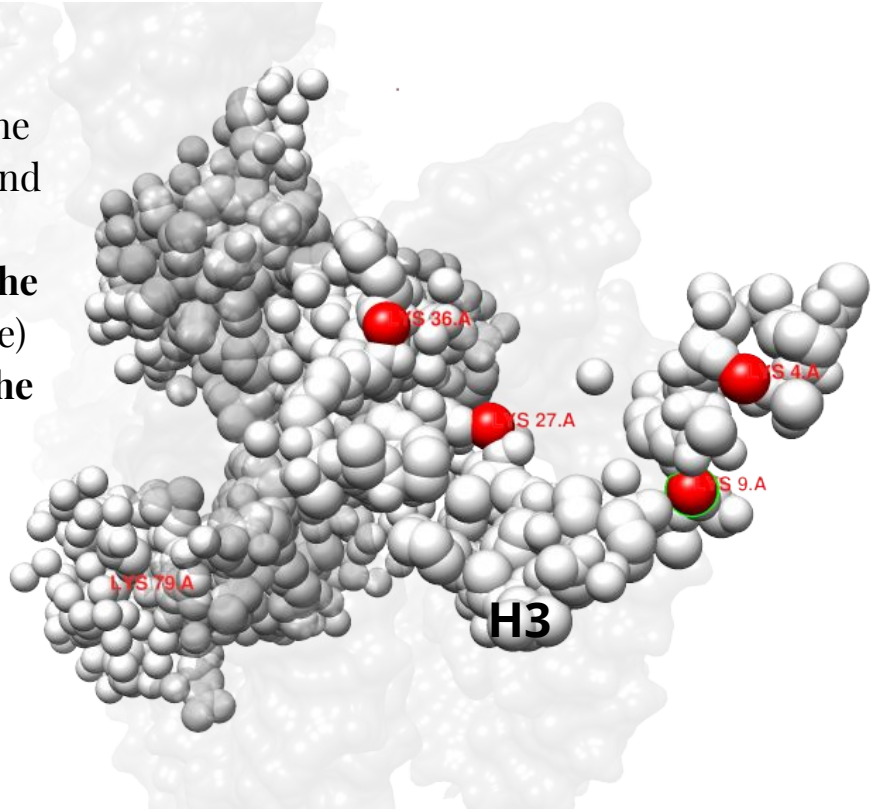
Methylation

- **Maintains the positive charge** and therefore the electrostatic interaction between the histone tail and the DNA.
- It generally **represses transcription by promoting the compaction of the chromatin** (heterochromatin-like) and by **inhibiting the binding of cofactors to the cromathin.**







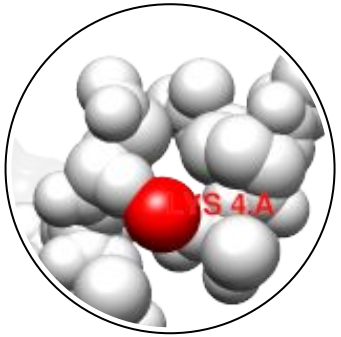


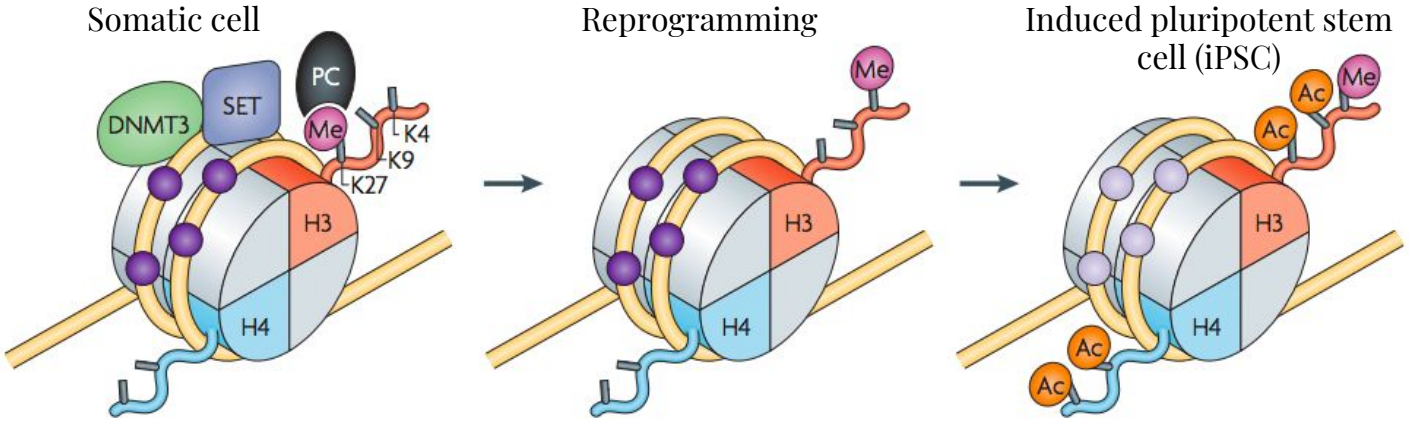
pdbID: 1KX5 (1.9A)

H3K4 methylation

- **H3K4 trimethylation acts as a transcriptional activator** by promoting the binding of positive transcription factors and blocking negative ones.
- It allows to **activate pluripotency genes** during the reprogramming of somatic cells into induced pluripotent stem cells

H3K4

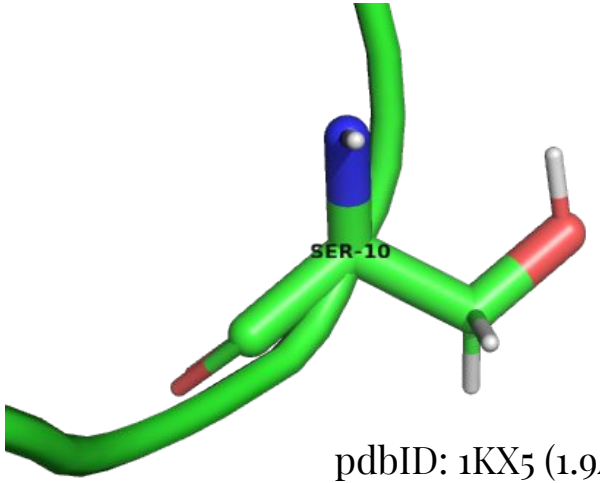




Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nature Reviews Genetics. 2009;10(5):295-304.

Phosphorylation

- It is a **transient** histone modification that becomes induced by extracellular signals, DNA damage or entry into mitosis. It is targeted to **serines (S), threonines (T) and tyrosines (Y).**
- It leads not only to the binding of specific reader proteins but also to changes in the affinity for readers or writers of other histone modifications.



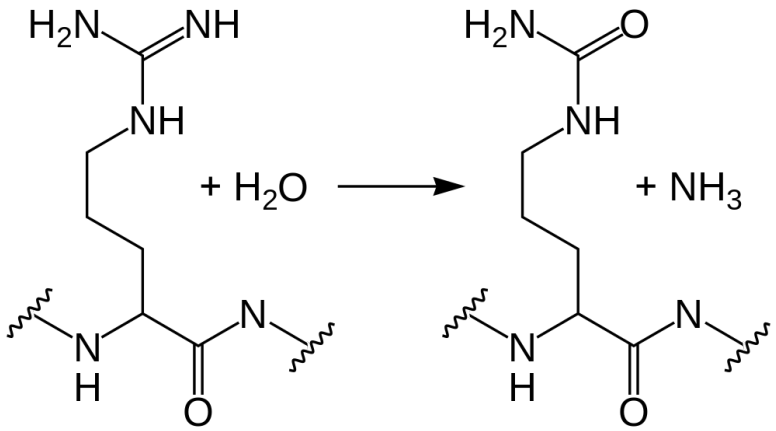
pdbID: 1KX5 (1.9A)

H3S10 is one of the most studied histone phosphorylations.

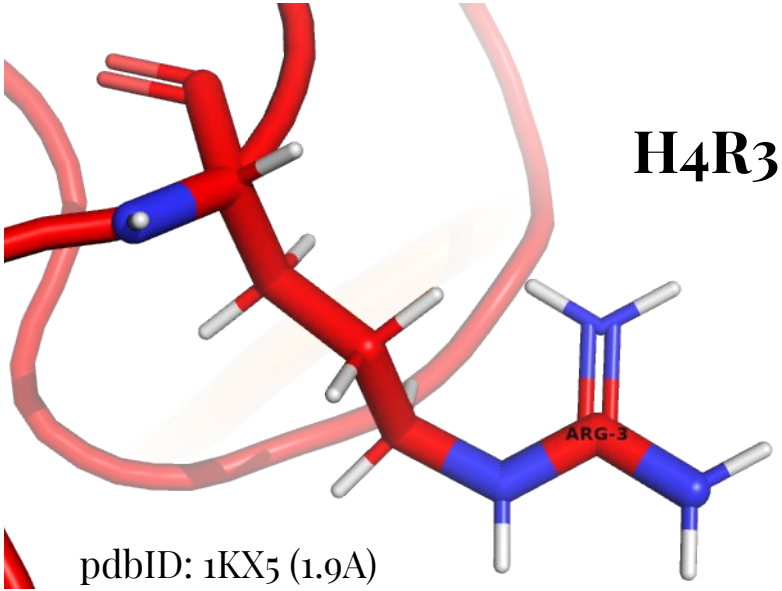
It is involved in both **transcription and cell division**, generally triggering DNA **condensation**.

Citrunilization

- It converts histone **arginine to citrulline**, and it is catalysed by peptidylarginine deiminases (**PADIs**)
- It leads to a **reduction in hydrogen-bonding and a looser chromatin structure**



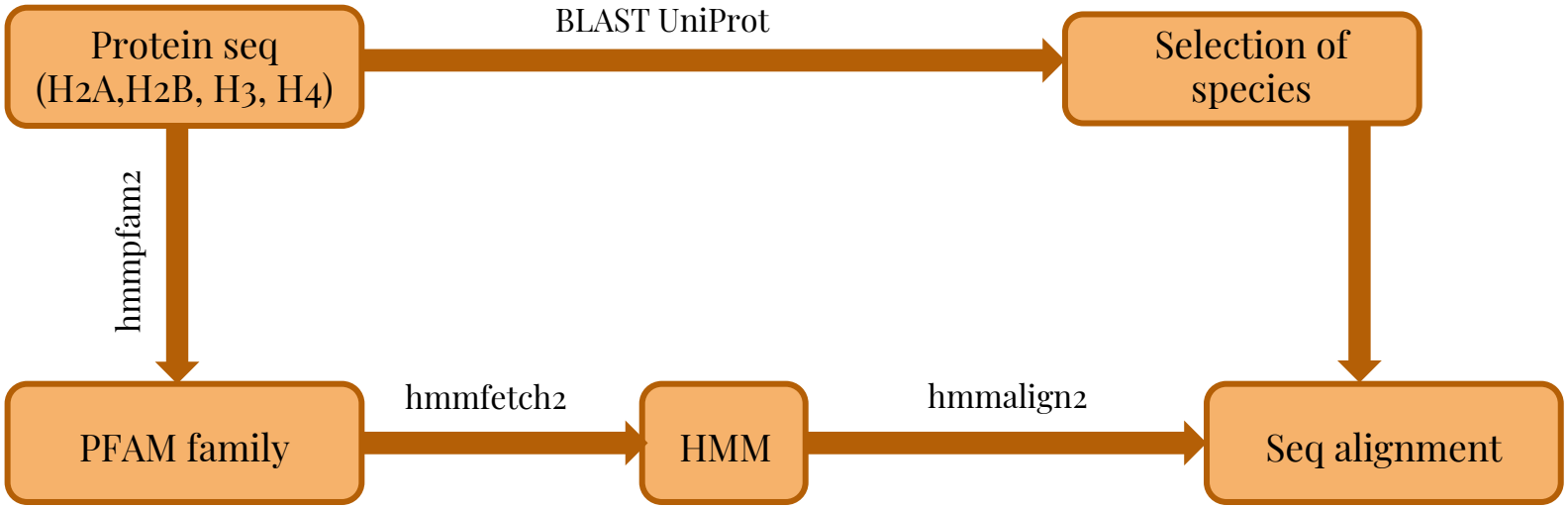
Source: Wikipedia



H4R3

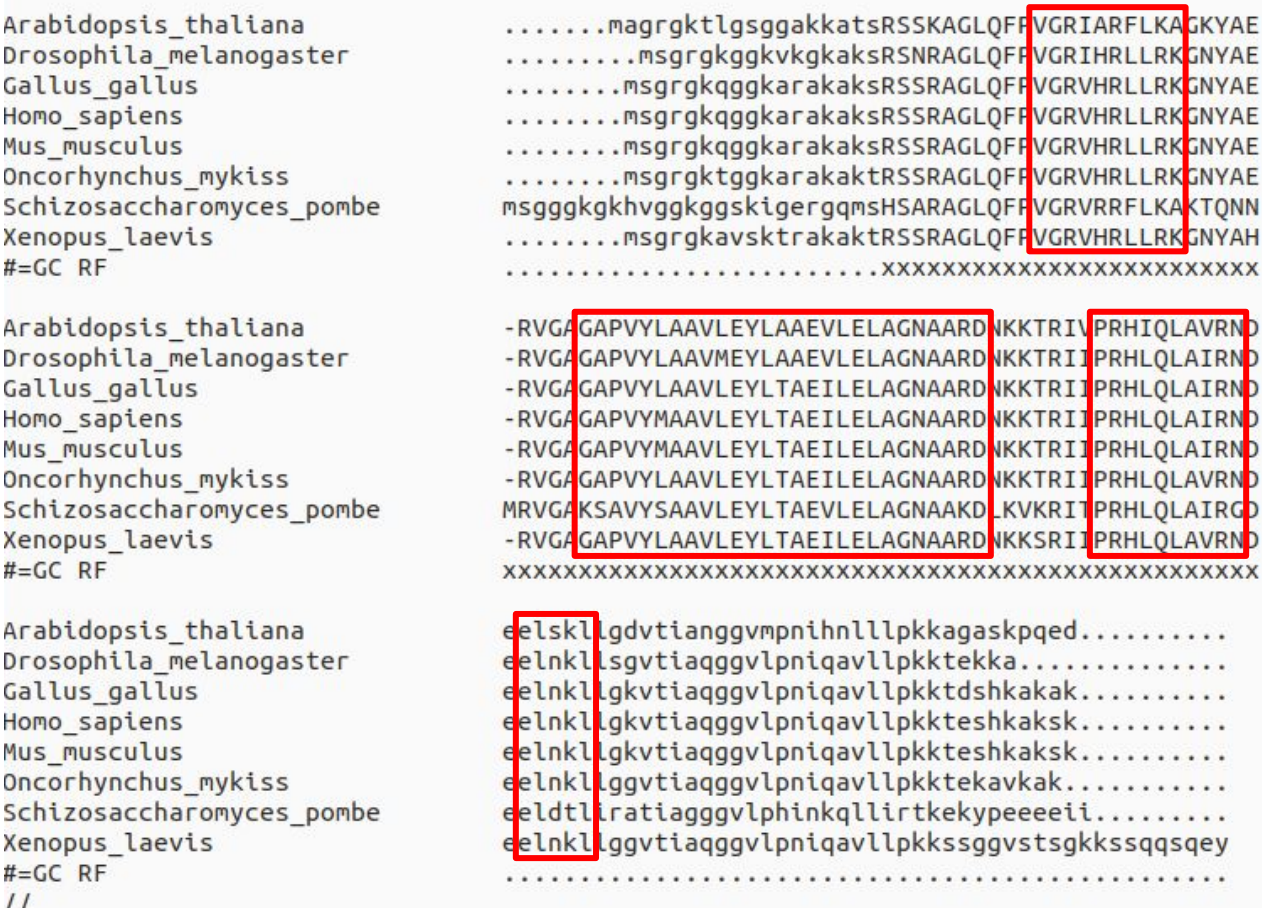
pdbID: 1KX5 (1.9A)

Evolution


Protein seq
(H2A,H2B, H3, H4)
BLAST UniProt
Selection of species
hmmpfam2
PFAM family
hmmfetch2
HMM
hmmalign2
Seq alignment

Evolution

H2A



Evolution

H2B

Evolution

H3

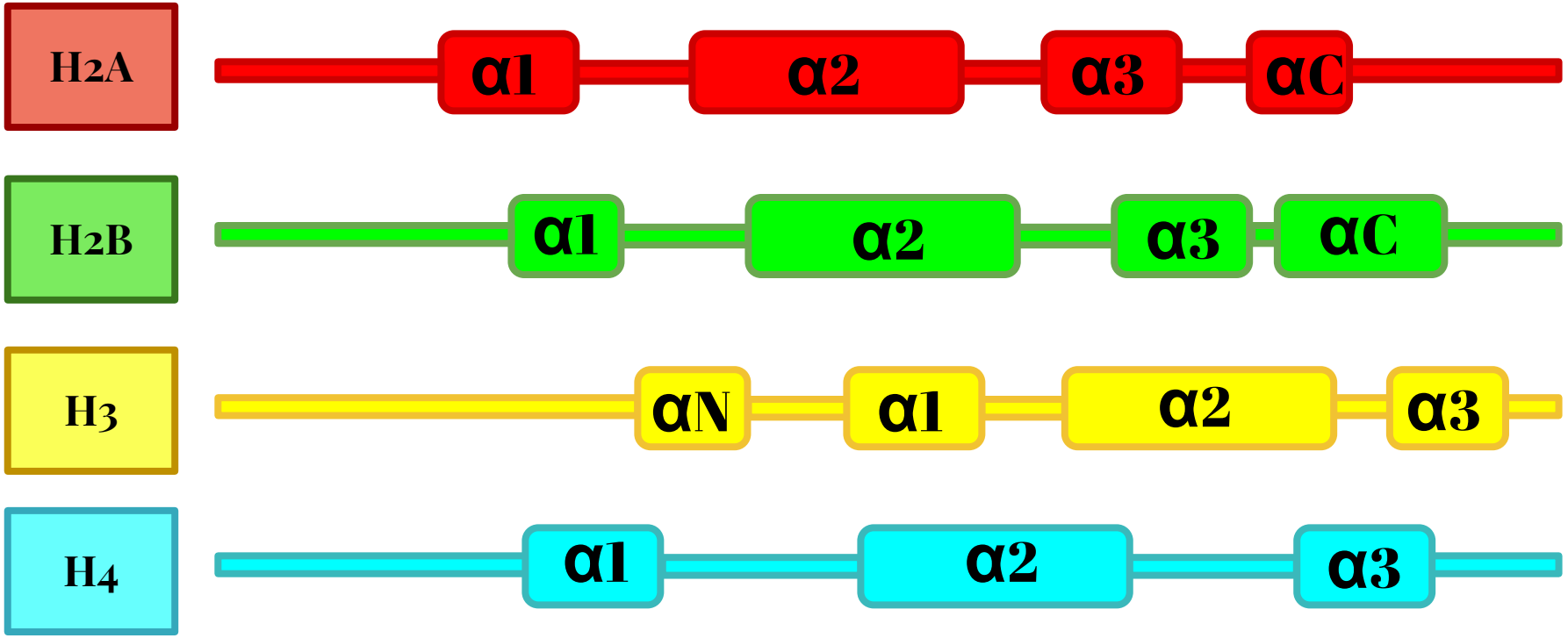
```
Arabidopsis_thaliana          martkqtarkstggkaprkqlatkaarksapatggvkkphrfrpgtvalr
Drosophila_melanogaster       martkqtarkstggkaprkqlatkaarksapstggvkkphryrpgtvalr
Gallus_gallus                 martkqtarkstggkaprkqlatkaarksapatggvkkphryrpgtvalr
Homo_sapiens                  martkqtarkstggkaprkqlatkaarksapatggvkkphryrpgtvalr
Mus_musculus                  martkqtarkstggkaprkqlatkaarksapatggvkkphryrpgtvalr
Oncorhynchus_mykiss           martkqtarkstggkaprkqlatkaarksapatggvkkphryrpgtvalr
Schizosaccharomyces_pombe     martkqtarkstggkaprkqlaskaarkaapatggvkkphryrpgtvalr
Xenopus_laevis                martkqtarkstggkaprkqlatkaarksapatggvkkphryrpgtvalr
#=GC RF                       ..................................................

Arabidopsis_thaliana          eirkyqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVAALQEAAEAY
Drosophila_melanogaster       eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSAAIGALQEASEAY
Gallus_gallus                 eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEASEAY
Homo_sapiens                  eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAY
Mus_musculus                  eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAY
Oncorhynchus_mykiss           eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEASEAY
Schizosaccharomyces_pombe     eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAIGALQEAVEAY
Xenopus_laevis                eirryqkSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEASEAY
#=GC RF                       .......xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

Arabidopsis_thaliana          LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Drosophila_melanogaster       LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Gallus_gallus                 LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Homo_sapiens                  LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Mus_musculus                  LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Oncorhynchus_mykiss           LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
Schizosaccharomyces_pombe     LVSLFEDTNLCAIHGKRVTIQPKDMQLARRLRgers
Xenopus_laevis                LVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRgera
#=GC RF                       xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx....
```

Evolution

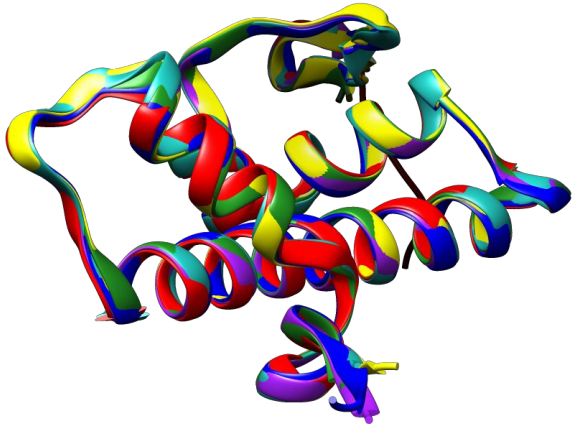
H4

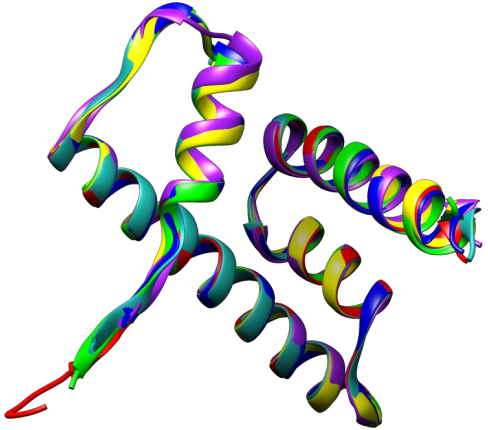
```
Arabidopsis_thaliana          msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Drosophila_melanogaster       mtgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Gallus_gallus                 msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Homo_sapiens                  msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Mus_musculus                  msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Oncorhynchus_mykiss           msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
Schizosaccharomyces_pombe     msgrgkggkglgkggakrhrkilrDNIQGITKPAIRRLARRGGV-----K
Xenopus_laevis                msgrgkggkglgkggakrhrkvlrDNIQGITKPAIRRLARRGGV-----K
#=GC RF                       .....................xxxxxxxxxxxxxxxxxxxxxxxxxx

Arabidopsis_thaliana          RISGLIYEETRGVLKIFLENVIRDAVTYTEHARRKTVTAMDVVYALKRQg
Drosophila_melanogaster       RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
Gallus_gallus                 RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
Homo_sapiens                  RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
Mus_musculus                  RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
Oncorhynchus_mykiss           RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
Schizosaccharomyces_pombe     RISALVYEETRAVLKLFLENVIRDAVTYTEHAKRKTVTSLDVVYSLKRQg
Xenopus_laevis                RISGLIYEETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQg
#=GC RF                       xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx.

Arabidopsis_thaliana          rtlygfgg
Drosophila_melanogaster       rtlygfgg
Gallus_gallus                 rtlygfgg
Homo_sapiens                  rtlygfgg
Mus_musculus                  rtlygfgg
Oncorhynchus_mykiss           rtlygfgg
Schizosaccharomyces_pombe     rtiygfgg
Xenopus_laevis                rtlygfgg
#=GC RF                       ........
//
```

Evolution



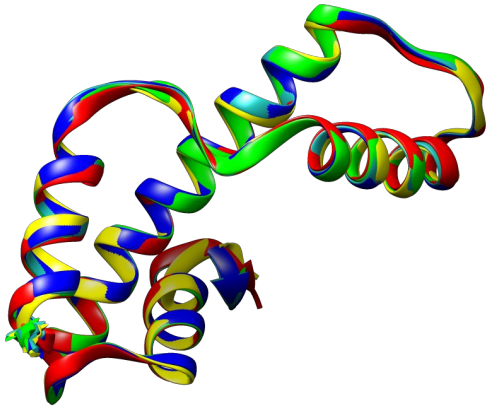
H2A
Sc 9,35
0,42

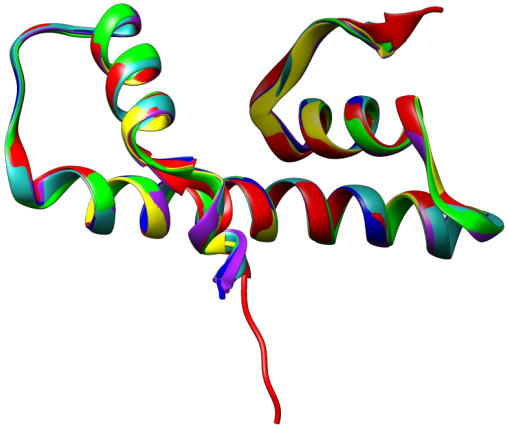




H2B
Sc 9,25
0,26

H3
Sc 9,39
0,41

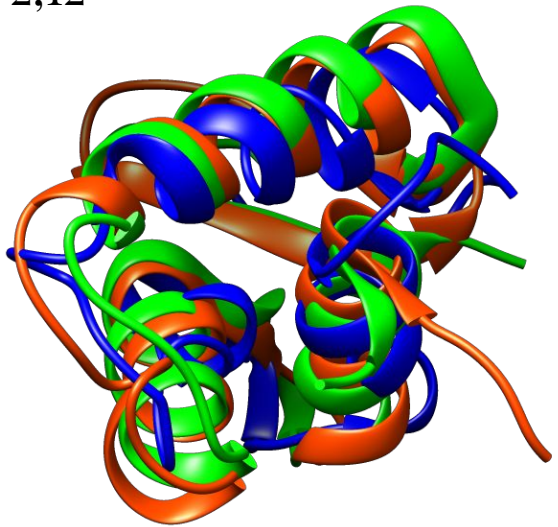




H4
Sc 8,96
0,35

Evolution

H1
Sc 7,32
2,12



```
Drosophila_melanogaster         msdsavatsaspvaappatvekkvvqkkasgsagtkakkasatpSHPPTQ
Gallus_gallus                   ........msetapvaapavsapgakaaakkpkkaaggakprkpAGPSVT
Homo_sapiens                    .........msetapaapaaappaekapvkkkaakkaggtprkaSGPPVS
Mus_musculus                    .........msetapaapaapapaektpvkkkarkaaggakrktSGPPVS
Oncorhynchus_mykiss             ................maevapapaaaapakapkkkaaakpkkAGPSVG
Xenopus_laevis                  ...maeaaesapapppaepaakkkkqqpkkaaaargaakskkpsSGPSVS
Saccharomyces_cerevisiae        ..mapkksttkttskgkkpatskgkekstskaaikkttakkeeaSSKSYR
#=GC RF                         ........................................xxxxxx

Drosophila_melanogaster         QMVDASIKNLKERGGSSLLAIKKYITA.TYKCDAQK--LAPFIKKYLKSA
Gallus_gallus                   ELITKAVSASKERKGLSLAALKKALAAgGYDVEKNN----SRIKLGLKSL
Homo_sapiens                    ELITKAVAASKERSGVSLAALKKALAAaGYDVEKNN----SRIKLGLKSL
Mus_musculus                    ELITKAVAASKERSGVSLAALKKALAAaGYDVEKNN----SRIKLGLKSL
Oncorhynchus_mykiss             ELIVKAVSASKERSGVSLAALKKSLAAgGYDVEKNN----SRVKIAVKSL
Xenopus_laevis                  EQIVTAVSASKERSGVSLAALKKTLAAgGYDVDKNN----SRLKLALKVT
Saccharomyces_cerevisiae        ELIIEGLTALKERKGSSRPALKKFIKE.NYPIVGSASNFDLYFNNAIKKG
#=GC RF                         xxxxxxxxxxxxxxxxxxxxxxxxxx.xxxxxxxxxxxxxxxxxxxx

Drosophila_melanogaster         VVNGKLIQTK-GKGASGSFKLSAsakkekdpkakskvlsaekkvqskkva
Gallus_gallus                   VSKGTLVQTK-GTGASGSFKLNKkpgetkakatkkkpaakpkkpaakkpa
Homo_sapiens                    VSKGTLVQTK-GTGASGSFKLNKkaasgeakpkvkkaggtkpkkpvgaak
Mus_musculus                    VSKGTLVQTK-GTGASGSFKLNKkaasgeakpkakragaakakkpagaak
Oncorhynchus_mykiss             VTKGTLVQTK-GTGASGSFKLNKkaveakkpakkaaapkakkvaakkpaa
Xenopus_laevis                  KE--TLLQVK-GSGASGSFKLNKkqlqskdkaaakkkaplaaeakkpaaa
Saccharomyces_cerevisiae        VEAGDFEQPK---GPAGAVKLAKkkspevkkekevspkpkqaatsvsata
#=GC RF                         xxxxxxxxxxxxxxxxxxxxxx.......................

Drosophila_melanogaster         skkigvsskktavgaadkkpkakkavatkktaenkktekakakdakktgi
Gallus_gallus                   aaakkpkkaaavkkspkkakkpaaaatkkaakspkkatkagrpkktaksp
Homo_sapiens                    kpkkaaggatpkksakktpkkakkpaaatvtkkvakspkkakvakpkkaa
Mus_musculus                    kpkkaagtatakkstkktpkkakkpaaaagakkakspkkakatkakkapk
Oncorhynchus_mykiss             akkpkkvaakkavaakkspkkakkpatpkkaakspkkvkkpaaaakkaak
Xenopus_laevis                  akktakspkkpkkvsaaakspkklkkpakaakspakktavkpkvaakspa
Saccharomyces_cerevisiae        skakaastklapkkvvkkksptvtakkasspssltykemilksmpqlndg
#=GC RF                         ..................................................

Drosophila_melanogaster         ikskpaatkakvtaakpkavvakaskakpavsakpkktvkkasvsatakk
Gallus_gallus                   akakavkpkaakskaakpkaakakkaatkkk...................
Homo_sapiens                    ksaakavkpkaakpkvvkpkkaapkkk.......................
Mus_musculus                    spakaktvkpkaakpktskpkaakpkktaakkk.................
Oncorhynchus_mykiss             spkkatkaakpkaakpkaakakkaapkkk.....................
Xenopus_laevis                  kakaakpkvakakkaapkkk...............................
Saccharomyces_cerevisiae        kgssrivlkkyvkdtfssklktssnfdylfnsaikkcvengelvqpkgps
#=GC RF                         ..................................................
```

Conclusions

- There is a **high conservation of the histones**, except Histone 1
- Despite H1 is not a histone core protein, its **function is indispensable** for chromatin organization
- There are **stable interactions between the histones**, however with the **DNA** the interactions are **dynamic and fluctuate**.
- **DNA interactions are nonspecific of sequence**, that allows them not to be so established and the ability to fluctuate
- Post-transcriptional modifications are able to modify not only **histone's affinity for chromatin**, but also the **binding of transcriptional cofactors**
- **Histone tails** play a fundamental role in **genetic regulation and inter/intra nucleosomal interactions**

Bibliography

- Wakamori, M., Fujii, Y., Suka, N., Shirouzu, M., Sakamoto, K., Umehara, T. and Yokoyama, S. (2015). Intra- and inter-nucleosomal interactions of the histone H4 tail revealed with a human nucleosome core particle with genetically-incorporated H4 tetra-acetylation. *Scientific Reports*, 5(1).
- Richmond, T. and Davey, C. (2003). The structure of DNA in the nucleosome core. *Nature*, 423(6936), pp.145-150.
- Luger, K. and Richmond, T. (1998). DNA binding within the nucleosome core. *Current Opinion in Structural Biology*, 8(1), pp.33-40.
- Luger, K., Mäder, A., Richmond, R., Sargent, D. and Richmond, T. (1997). Crystal structure of the nucleosome core particle at 2.8Å resolution. *Nature*, 389(6648), pp.251-260.
- Harp, J., Hanson, B., Timm, D. and Bunick, G. (2000). Asymmetries in the nucleosome core particle at 2.5 Å resolution. *Acta Crystallographica Section D Biological Crystallography*, 56(12), pp.1513-1534.
- Davey, C., Sargent, D., Luger, K., Maeder, A. and Richmond, T. (2002). Solvent Mediated Interactions in the Structure of the Nucleosome Core Particle at 1.9Å Resolution. *Journal of Molecular Biology*, 319(5), pp.1097-1113.
- Tessarz, P. and Kouzarides, T. (2014). Histone core modifications regulating nucleosome structure and dynamics. *Nature Reviews Molecular Cell Biology*, 15(11), pp.703-708.
- Ramakrishnan, V. (1997). HISTONE STRUCTURE AND THE ORGANIZATION OF THE NUCLEOSOME. *Annual Review of Biophysics and Biomolecular Structure*, 26(1), pp.83-112.

Bibliography

- Cutter, A. and Hayes, J. (2015). A brief review of nucleosome structure. *FEBS Letters*, 589(20PartA), pp.2914-2922.
- Bednar, J., Horowitz, R., Grigoryev, S., Carruthers, L., Hansen, J., Koster, A. and Woodcock, C. (1998). Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. *Proceedings of the National Academy of Sciences*, 95(24), pp.14173-14178.
- Biswas, M., Voltz, K., Smith, J. and Langowski, J. (2011). Role of Histone Tails in Structural Stability of the Nucleosome. *PLoS Computational Biology*, 7(12), p.e1002279.
- Yusufaly, T., Li, Y., Singh, G. and Olson, W. (2014). Arginine-phosphate salt bridges between histones and DNA: Intermolecular actuators that control nucleosome architecture. *The Journal of Chemical Physics*, 141(16), p.165102.
- Saha, C., Kumar, R. and Das, A. (2016). Understanding nucleosomal histone and DNA interactions: a biophysical study. *Journal of Biomolecular Structure and Dynamics*, 35(12), pp.2531-2538.
- Woodcock, C. (2005). A milestone in the odyssey of higher-order chromatin structure. *Nature Structural & Molecular Biology*, 12(8), pp.639-640.

PEM questions

1. Which of the following is true?
   a. H1 is important in fixing the nucleosome with the DNA linker
   b. H1 has only one important binding site
   c. a and b are correct
   d. H1 is part of the octamer
   e. All of them are correct
2. About histones interaction with DNA…
   a. There are concrete regions of the DNA with higher affinity than others with the histones.
   b. Histones bindings with DNA are dynamic and constantly moving
   c. A and B are correct
   d. Histones bind specifically with the DNA chain
   e. All of them are correct
3. Choose the right sentence:
   a. Histones are not under postranslational modifications
   b. There are two H1 on the histone core
   c. The histone core is formed by two H2A, two H2b, two H3 and two H4
   d. The histone fold is a common domain of all histons
   e. All the sentences are wrong
4. Related with the nucleosome structure:
   a. The hydrophobic core is not necessary for the structure stability
   b. H2A forms a dimer with H2A'
   c. H3 interacts with H3', H2B and H2B' for the tetramer
   d. H3 forms a dimer with H4
   e. Dimer stability only depends on hydrogen bonds
5. Related with the histone core proteins structure:
   a. All histone core proteins have a N-terminus alpha helix
   b. All histone core proteins have a C-terminus alpha helix
   c. All histone core proteins have four alpha helices
   d. H2A has only three alpha helices in its structure
   e. H4 has only three alpha helices in its structure
6. Related to the evolution of histones:
   a. Histone core proteins are really conserved through evolution
   b. The histone core proteins are conserved even in prokaryotes
   c. A and B are correct
   d. H1 is not really conserved through evolution so the function has changed
   e. All of them are correct

PEM questions

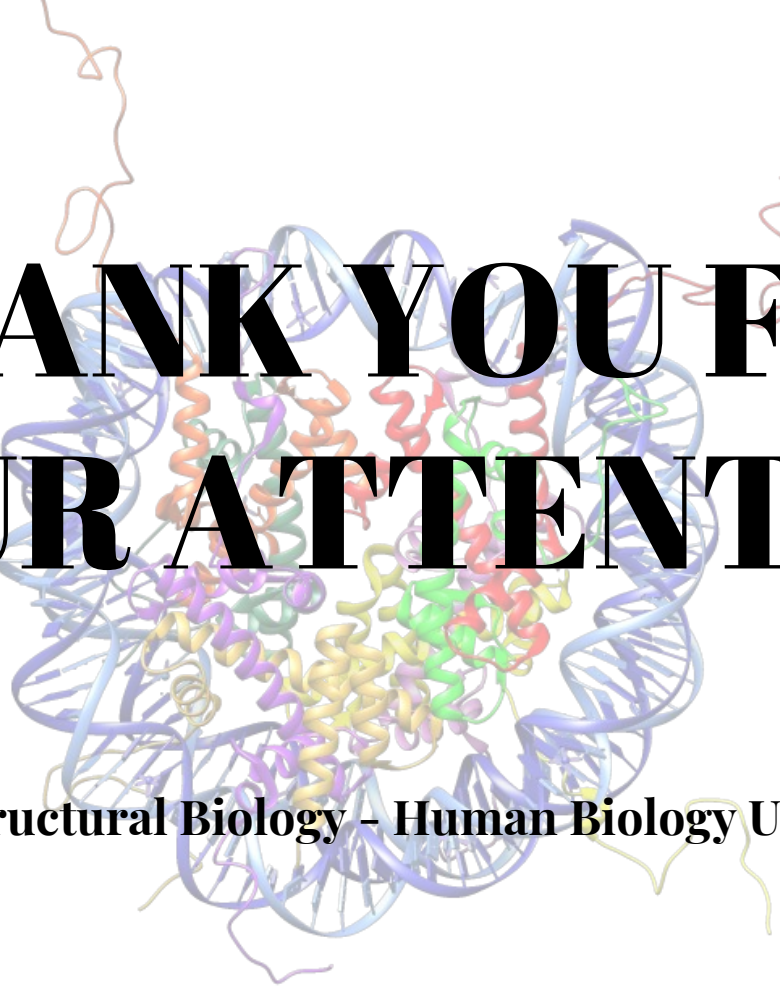
7. Related to the histone fold:
   a. The histone fold is found in all histones
   b. It is found only in histones
   c. A and B are correct
   d. It is composed by three alpha helices
   e. All of them are correct

8. Related with the histone tails:
   a. They are important to stabilize the binding with the DNA
   b. H2A has tails in N- and in C-terminus
   c. They contain mainly positively charged amino acids
   d. H1 has both N- and C-terminal tails
   e. All of them are correct

9. DNA-histone interactions are:
   a. Non-specific
   b. Dynamic
   c. A and B are correct
   d. Specific
   e. All the sentences are wrong

10. Related with the post translational modifications:
   a. Methylation always produces a repression of the transcription
   b. Acetylation induces a loss of charge in the histone, leading to a weaker DNA-histone binding
   c. Methylation and acetylation are the only two histone modifications described
   d. Methylation maintains the charge of the histone and its interaction with the DNA
   e. b and d are correct

THANK YOU FOR YOUR ATTENTION



Structural Biology - Human Biology UPF