Genomes packaged to perfection, in all domains of life

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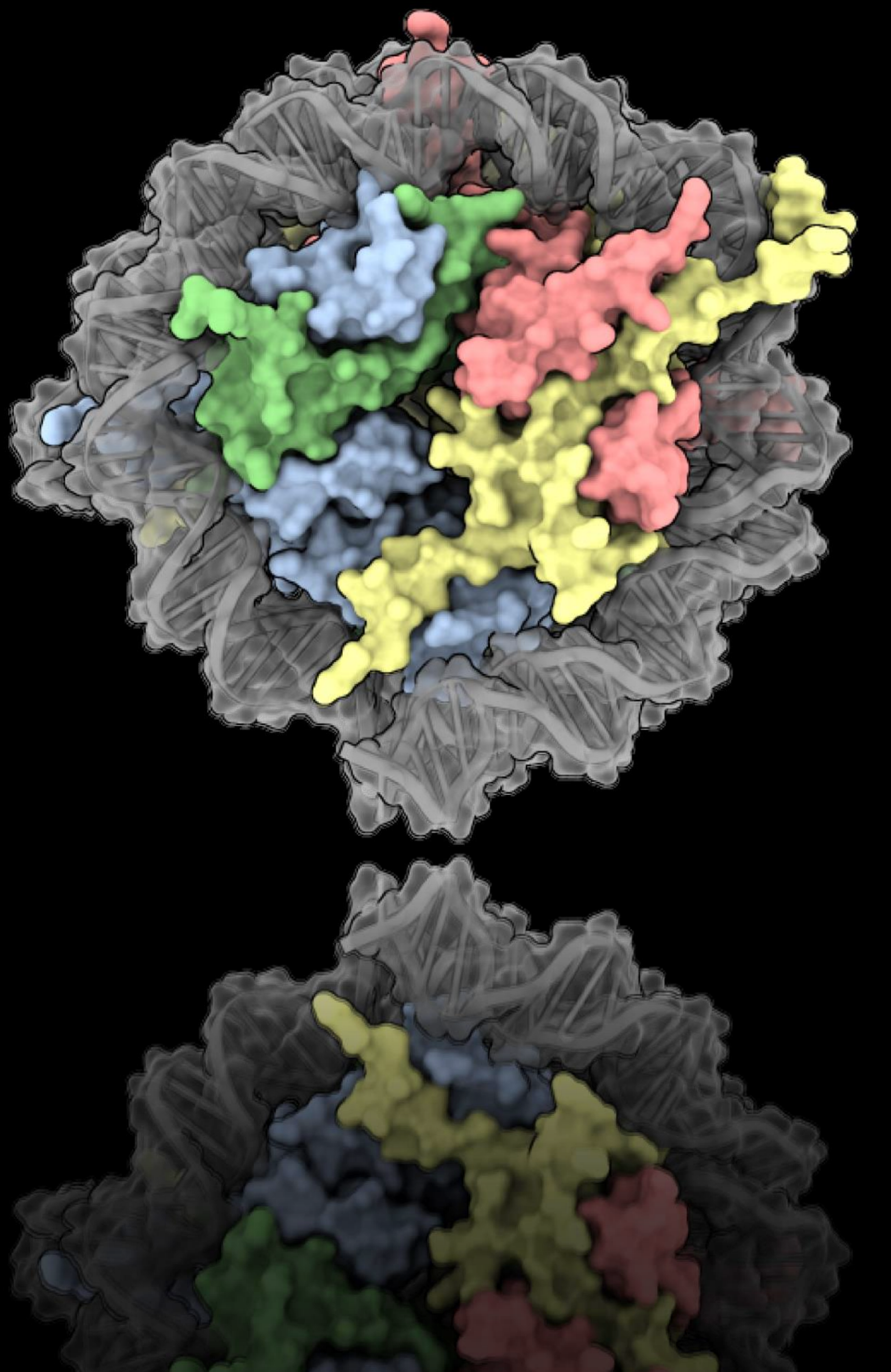
University of Colorado
Boulder | Colorado Springs | Denver | Anschutz Medical Campus



Three take-home points

1) Structural biology is essential to our understanding of cells

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2) Complex genomes are organized in complex structures

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1) Structural biology is essential to our understanding of cells

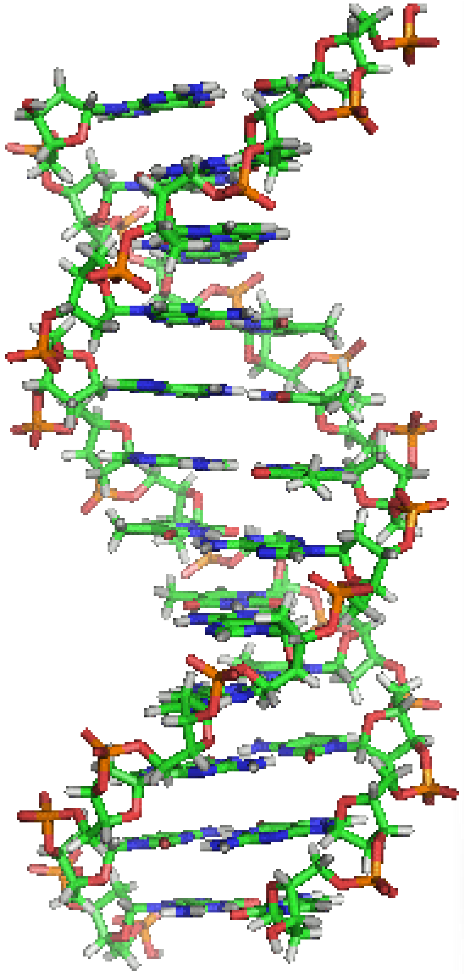
2) Complex genomes are organized in complex structures

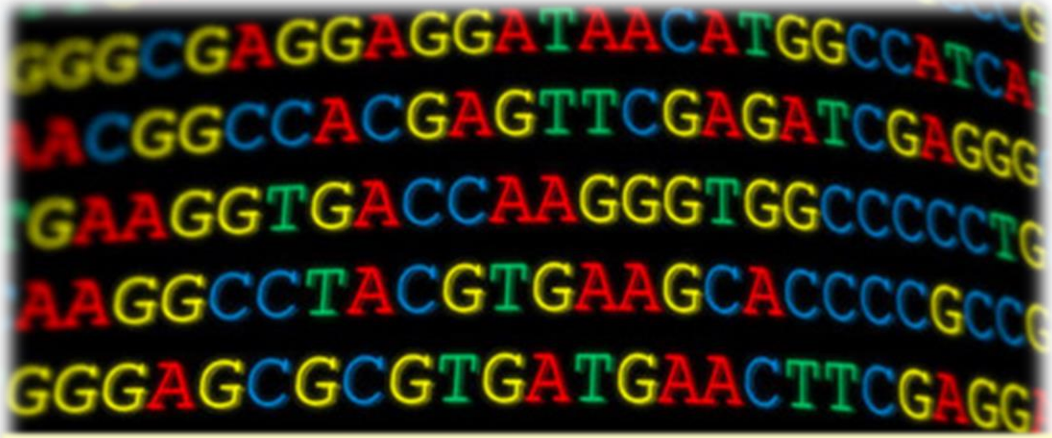
3) Embrace the 'weird'

Weird phenomena in weird organisms:
Antibiotics, restriction enzymes, ribozymes, genome editing, cancer drugs, aging….

Weird observations in day-to-day experiments
→ new research directions

Multicellular organisms have large and complex genomes





1000 phone books filled with combinations of four letters

Organization of information in linear form poses many challenges

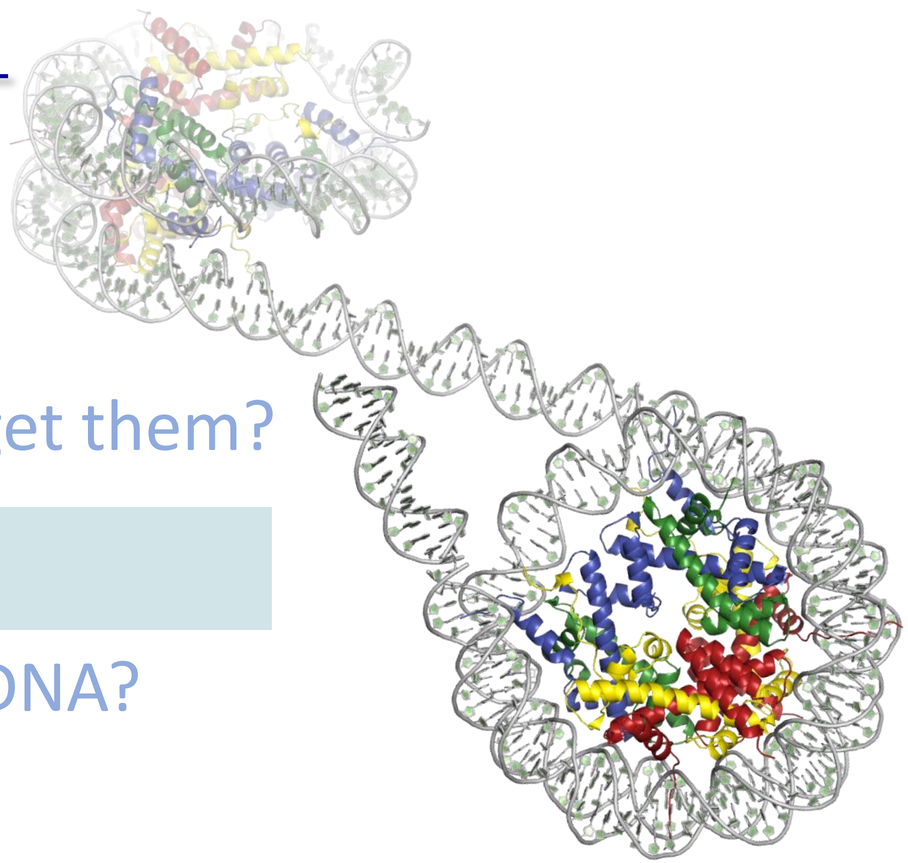
- Package information in a highly confined space (16 km thread in a golf ball) 
- Protect from physical damage and tangles
- Accurately duplicate the genome during cell division
- Find the required genes in a timely manner
- Physically access the stored information

Malfunction

⬇

Cancer, other diseases

Take-home points, ‘scientifically rephrased’



- **Structural biology of chromatin**

  Chromatin structure ‘101’

  Why do we need structures, how do we get them?

- **Decoding and navigating chromatin**

  How can we gain access to the wrapped DNA?

  ‘Epigenetics’

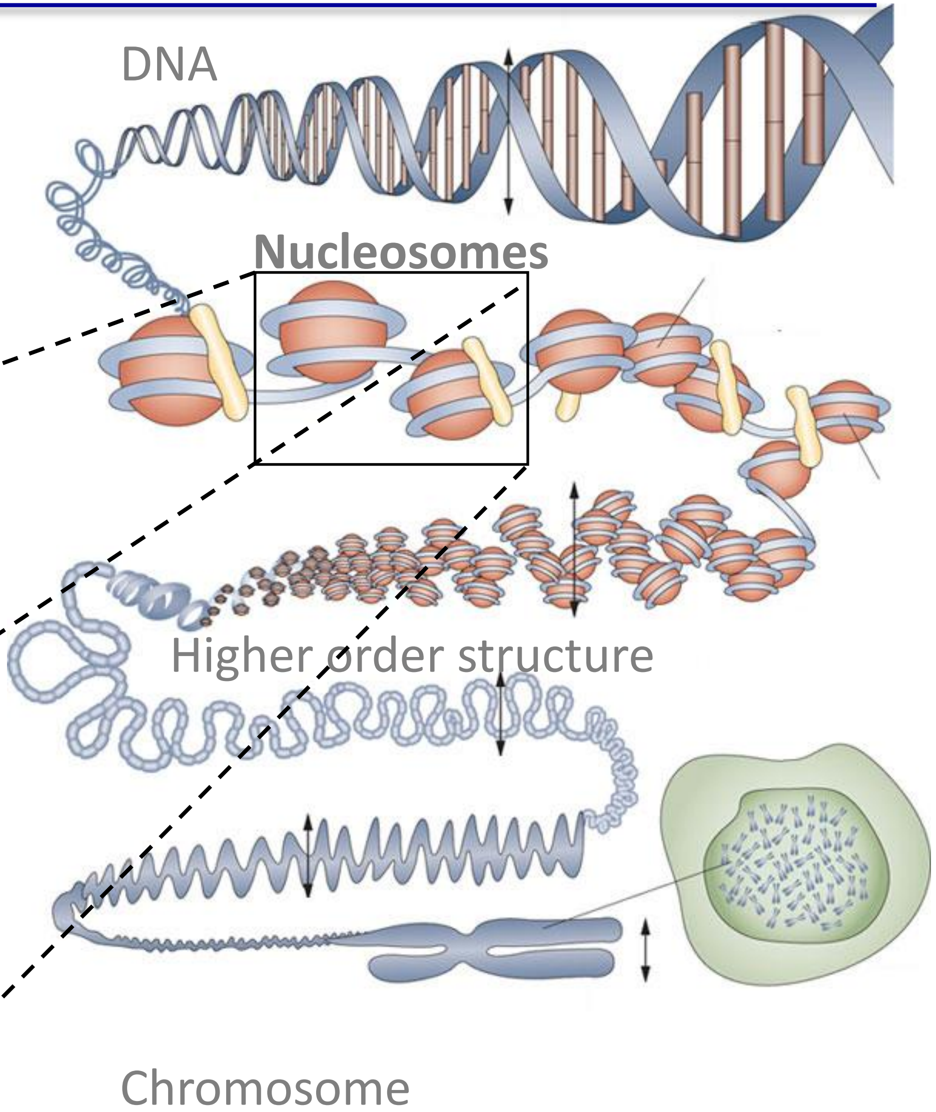
- **Where did the chromatin ‘starter kit’ come from?**

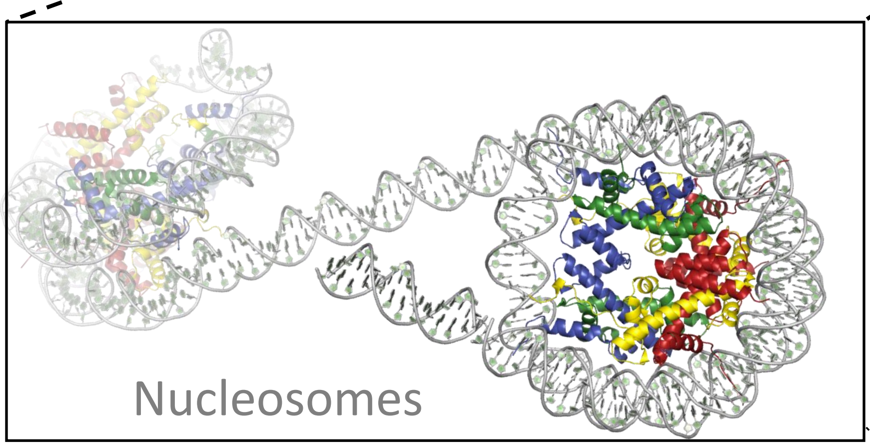
  What is the evolutionary origin of chromatin?

Eukaryotic DNA organization ‘101’

DNA forms a complex with an equal mass of proteins (‘chromatin’)

1st level: **‘Nucleosomes’**
Four histone proteins
H2A, H2B, H3, H4
bind 147 bp DNA

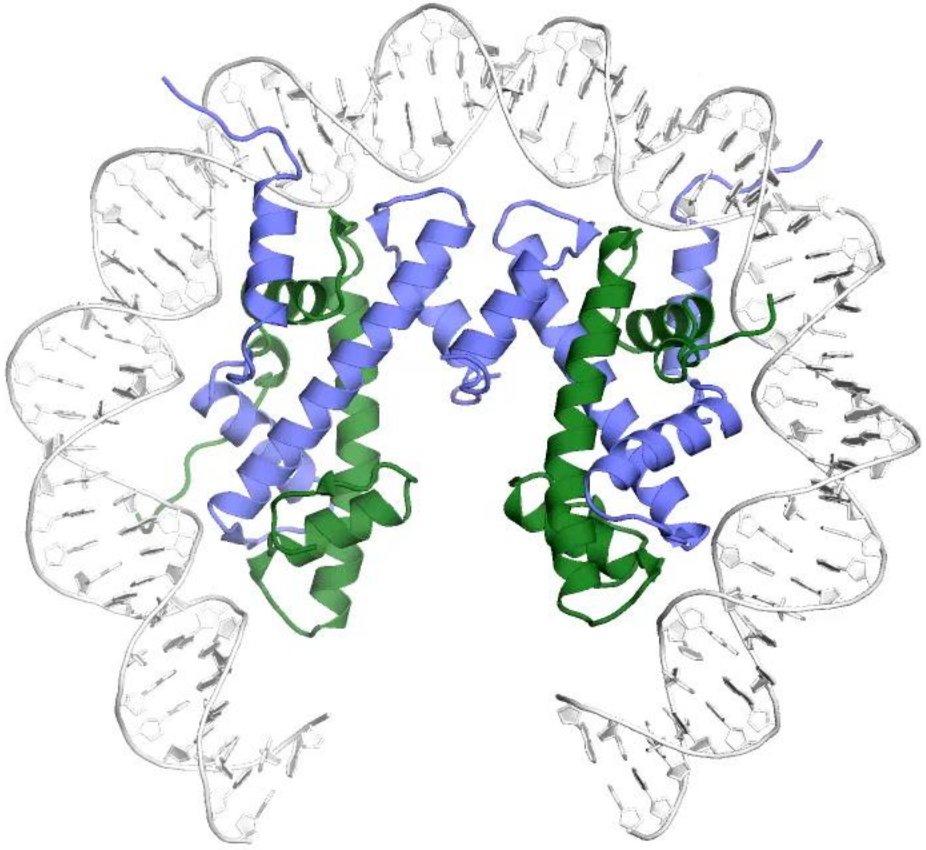




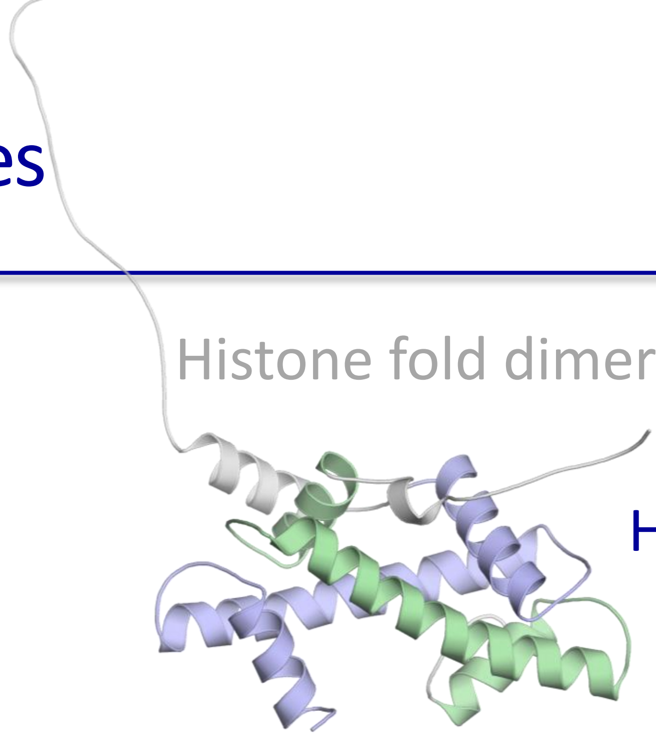
Tonna et al., 2010

Nucleosomes package all eukaryotic genomes

(H2A-H2B) • (H3-H4)$_2$ •(H2A-H2B)



Histone fold dimers



H3-H4

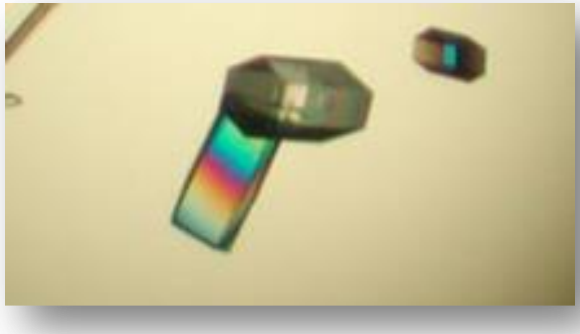
Histone extensions and tails

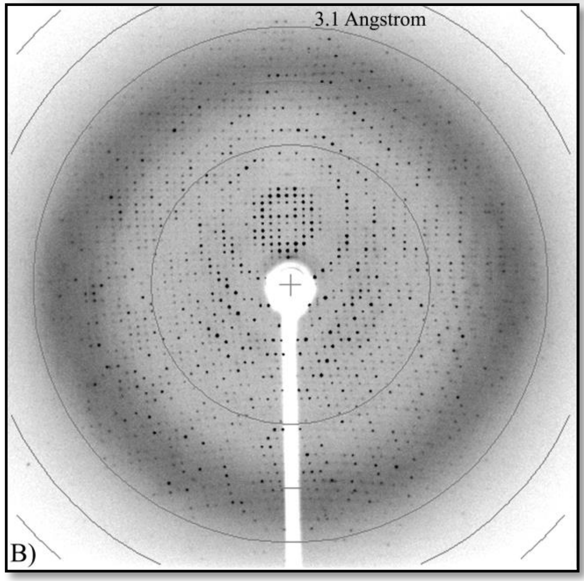
H2A-H2B

Yajie Gu

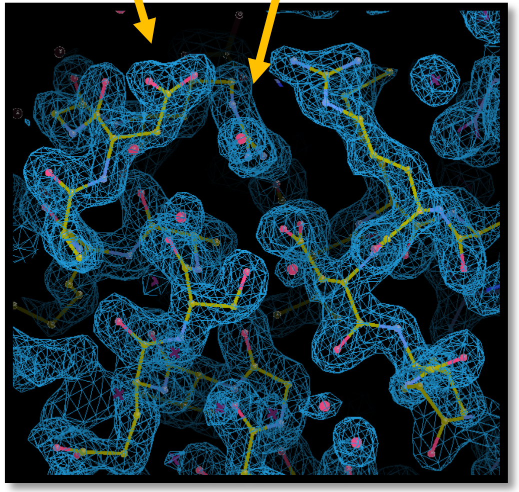
Two approaches to determine structures of biological samples

Xray crystallography

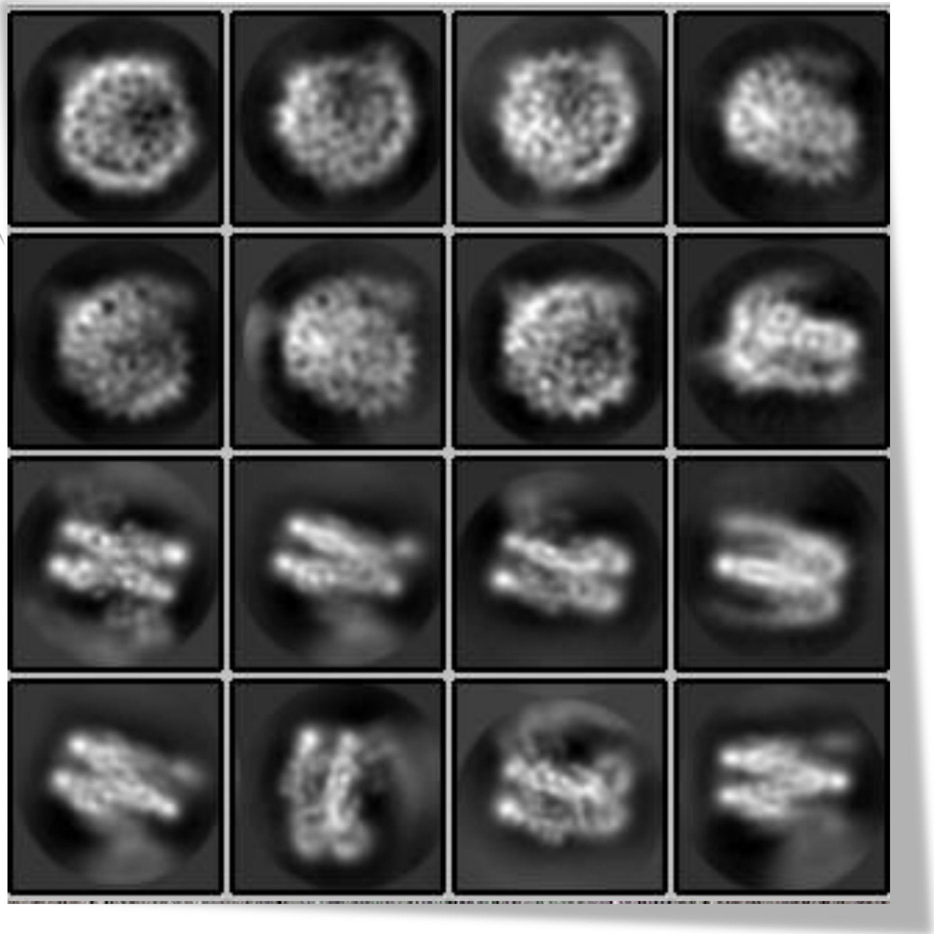




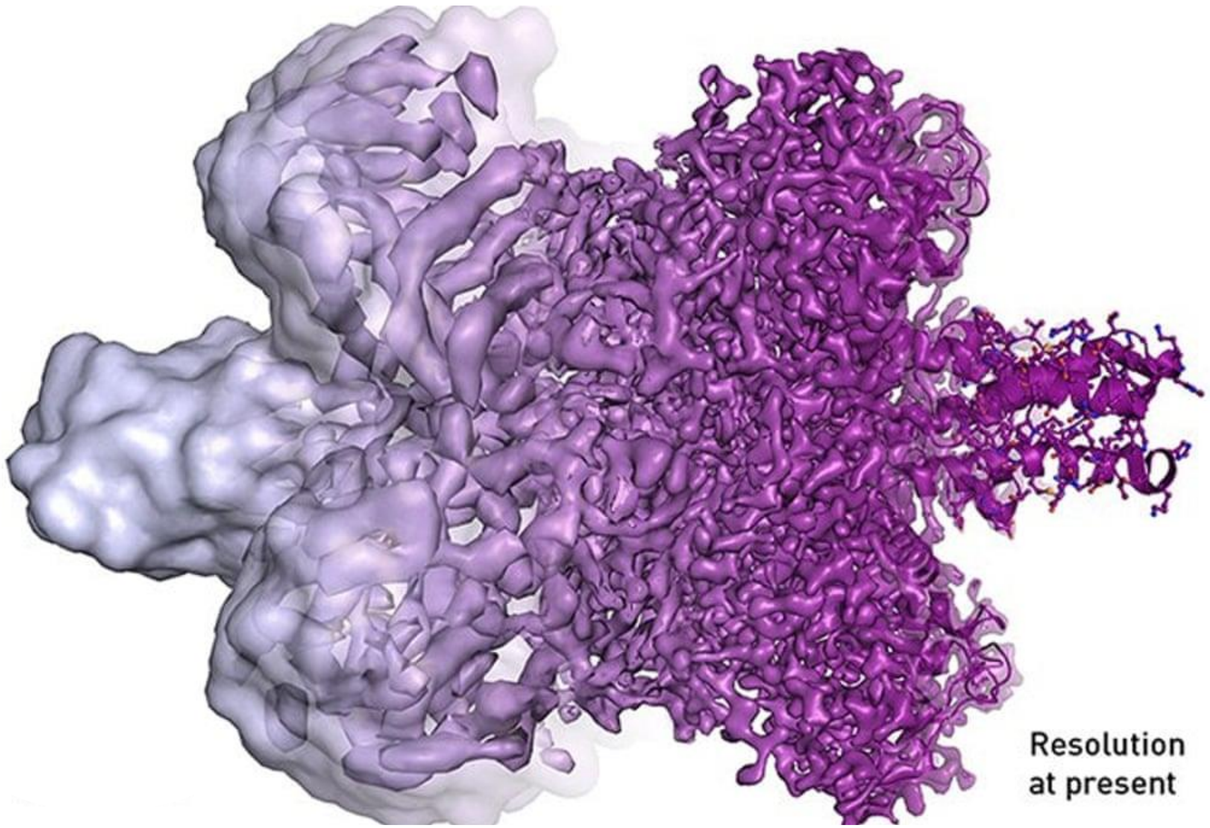
Visualize locations of atoms at ‘high resolution’



Cryo-electron microscopy

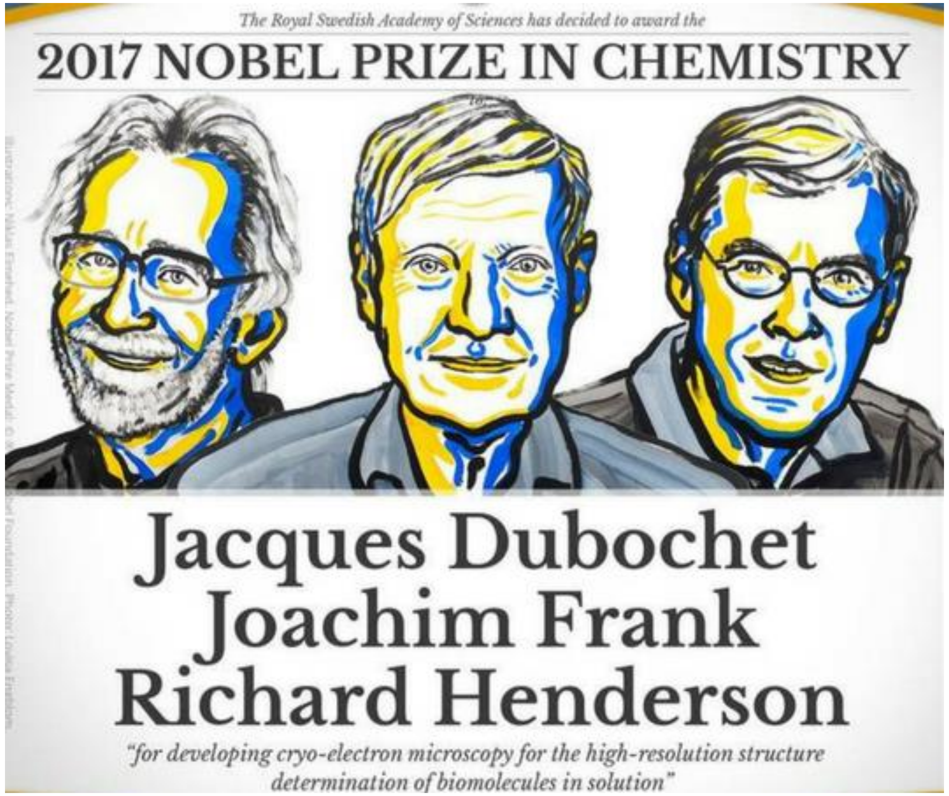


‘Resolution revolution’ in cryoEM



Resolution before 2013
‘blobology’

Resolution at present
‘atomic’



The Royal Swedish Academy of Sciences has decided to award the

2017 NOBEL PRIZE IN CHEMISTRY

Jacques Dubochet
Joachim Frank
Richard Henderson

“for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution”

- Microscopes
- Cameras / detectors
- Software and computing power

What’s with the obsession with ‘high resolution’ structures?

Experimental data: ‘electron density’ envelope

‘Low resolution’

‘Medium resolution’

‘High resolution’
(better than 2.5 Å)

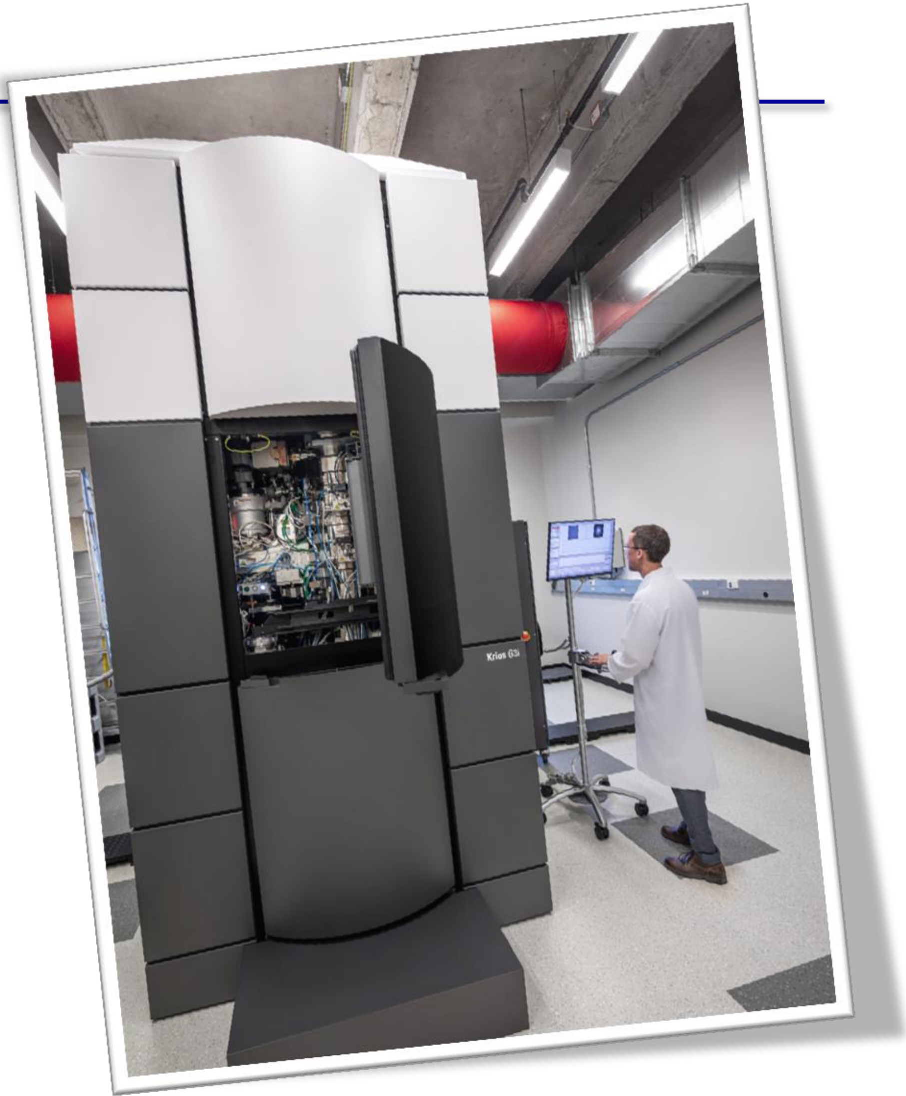
Structure informs function

CU Boulder joins the ‘resolution revolution’ in cryoEM:
Titan Krios G3i (aka Princess Krios)

October 2019

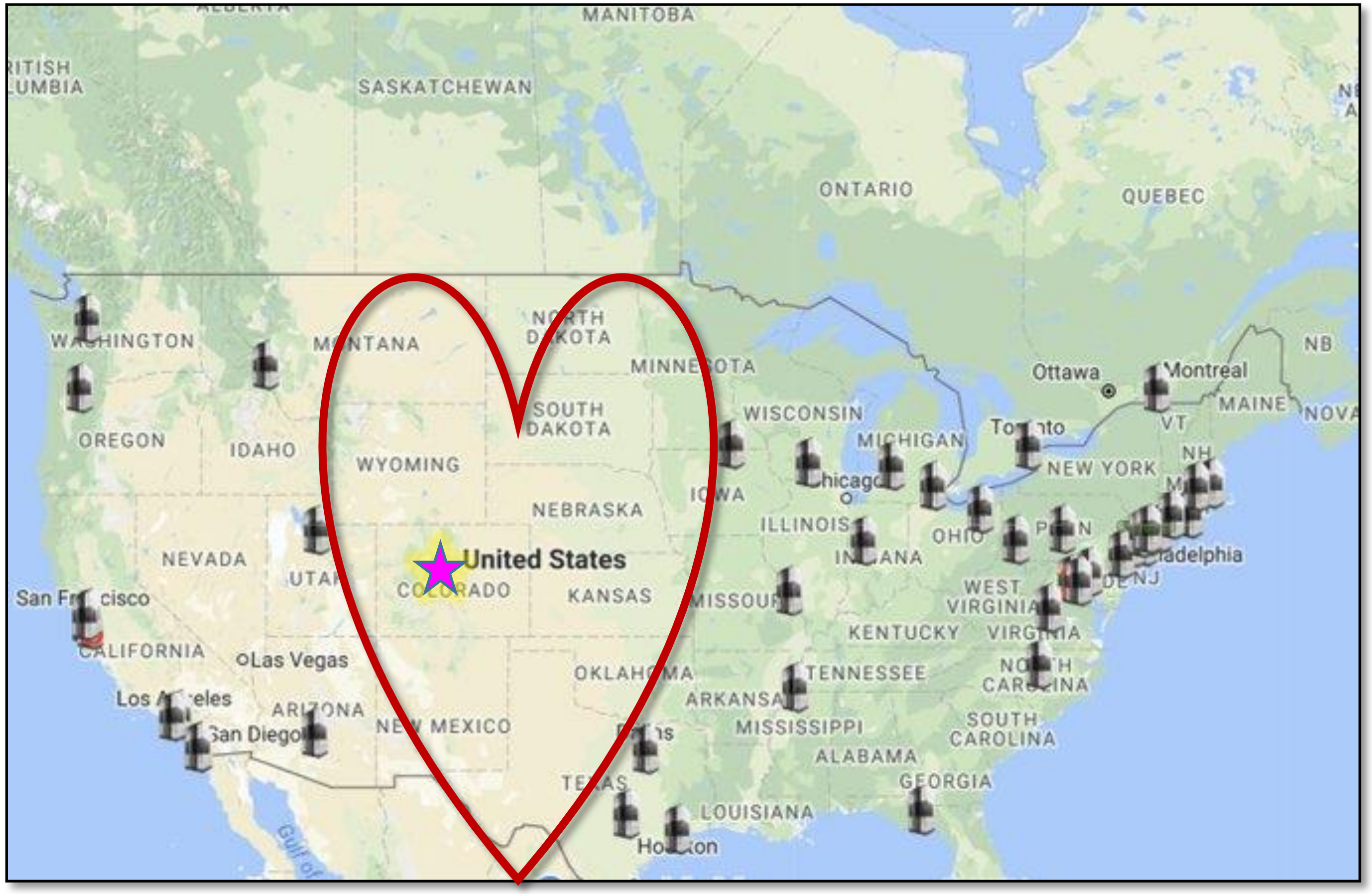
University of Colorado **Boulder**

hhmi Howard Hughes Medical Institute



‘First light’ April 2020
First data July 2020

The CU Boulder Titan Krios serves a vast geographic area



Titan Krios locations across the US

BioKEM facility at CU Boulder

- Current uptime ~90 %
- 14 CU Boulder user labs
- 9 'outside' user labs
- 7 industry users (25% time)

Soon to be joined by a **Glacios** 200 kV screening TEM

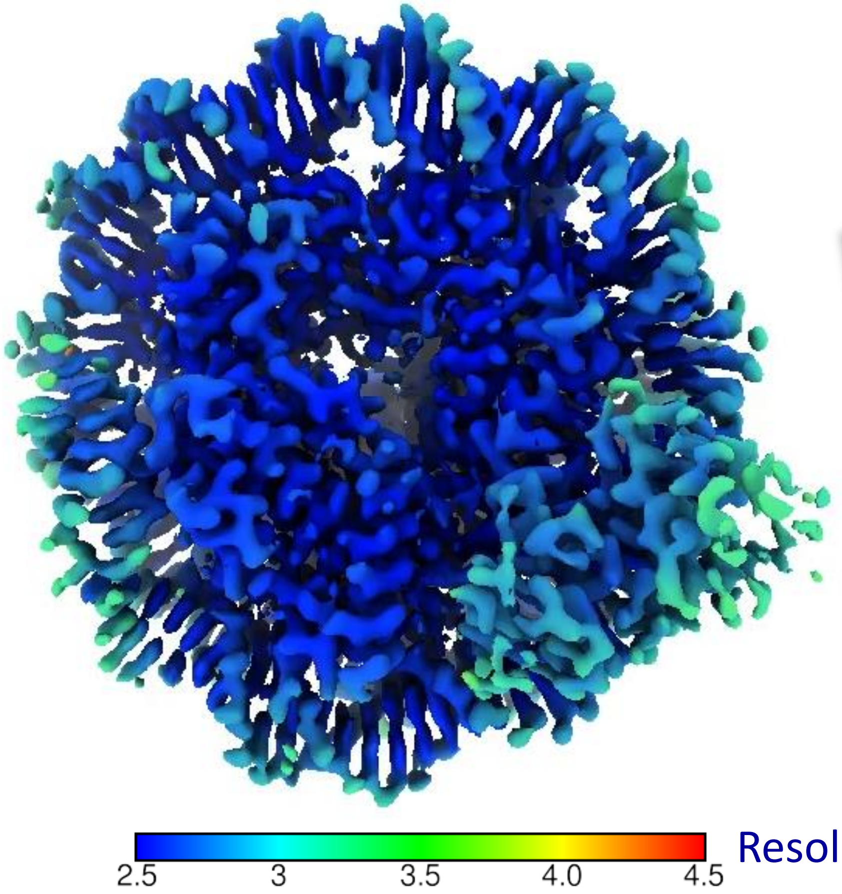
https://www.colorado.edu/facility/biokem/

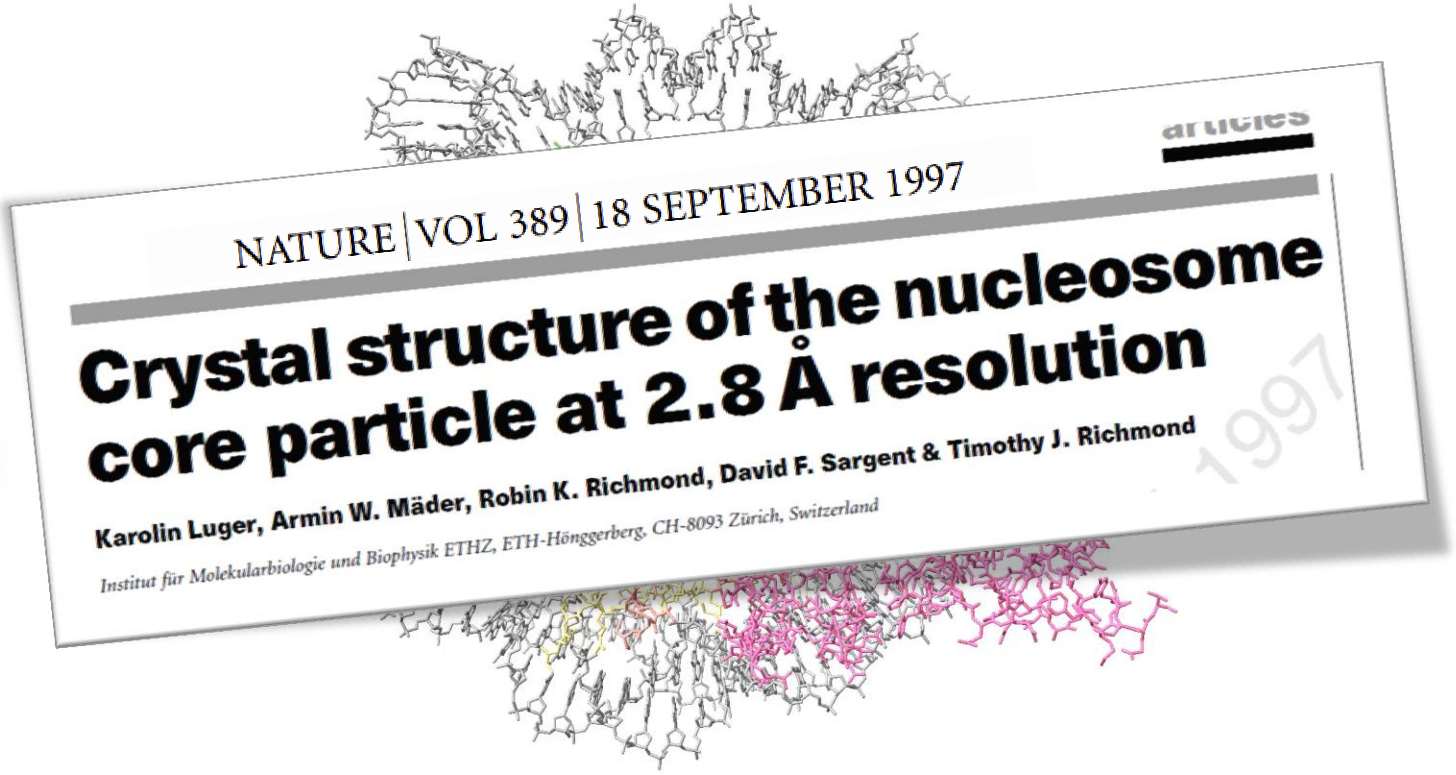
CU BOULDER CENTER FOR CRYO ELECTRON TOMOGRAPHY

Princess Krios at CU Boulder: 2.6 Å resolution nucleosomes

CENP-N promotes the compaction of centromeric chromatin

[Zhou….Luger, 2022, PMC9010303]




articles

NATURE | VOL 389 | 18 SEPTEMBER 1997

Crystal structure of the nucleosome core particle at 2.8 Å resolution

Karolin Luger, Armin W. Mäder, Robin K. Richmond, David F. Sargent & Timothy J. Richmond

Institut für Molekularbiologie und Biophysik ETHZ, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

1997

Tonight's talking points

- Structural biology of chromatin

  Chromatin structure '101'

  Why do we need structures, how do we get them?

- Decoding and navigating chromatin

  How can we gain access to the wrapped DNA?

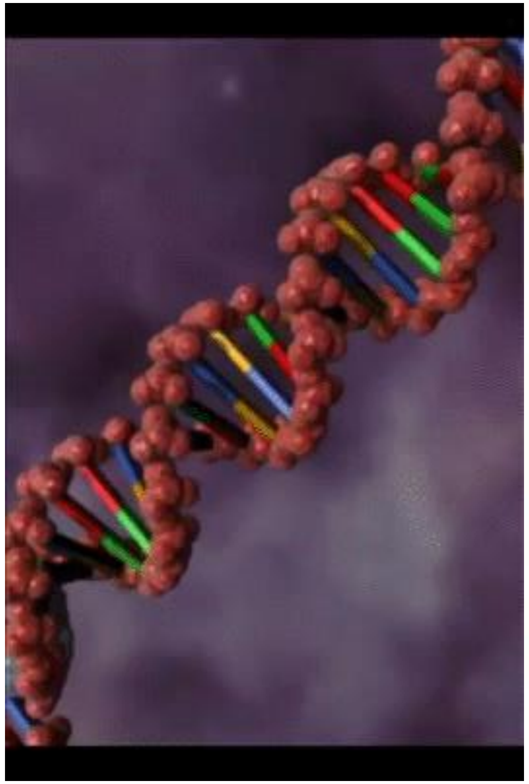
  Highly complex and complicated machinery is required

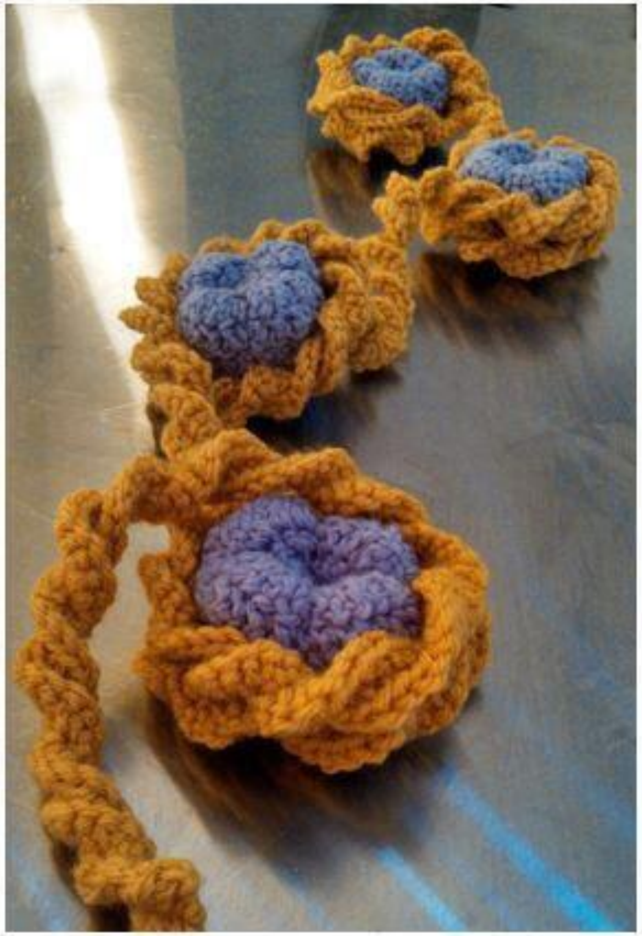
- Where did the chromatin 'starter kit' come from?

  What is the evolutionary origin of chromatin

Umm…. Unzipping the DNA double helix might be problematic!

https://www.ravelry.com/designers/jessica-polka





‘open chromatin’

‘closed chromatin’

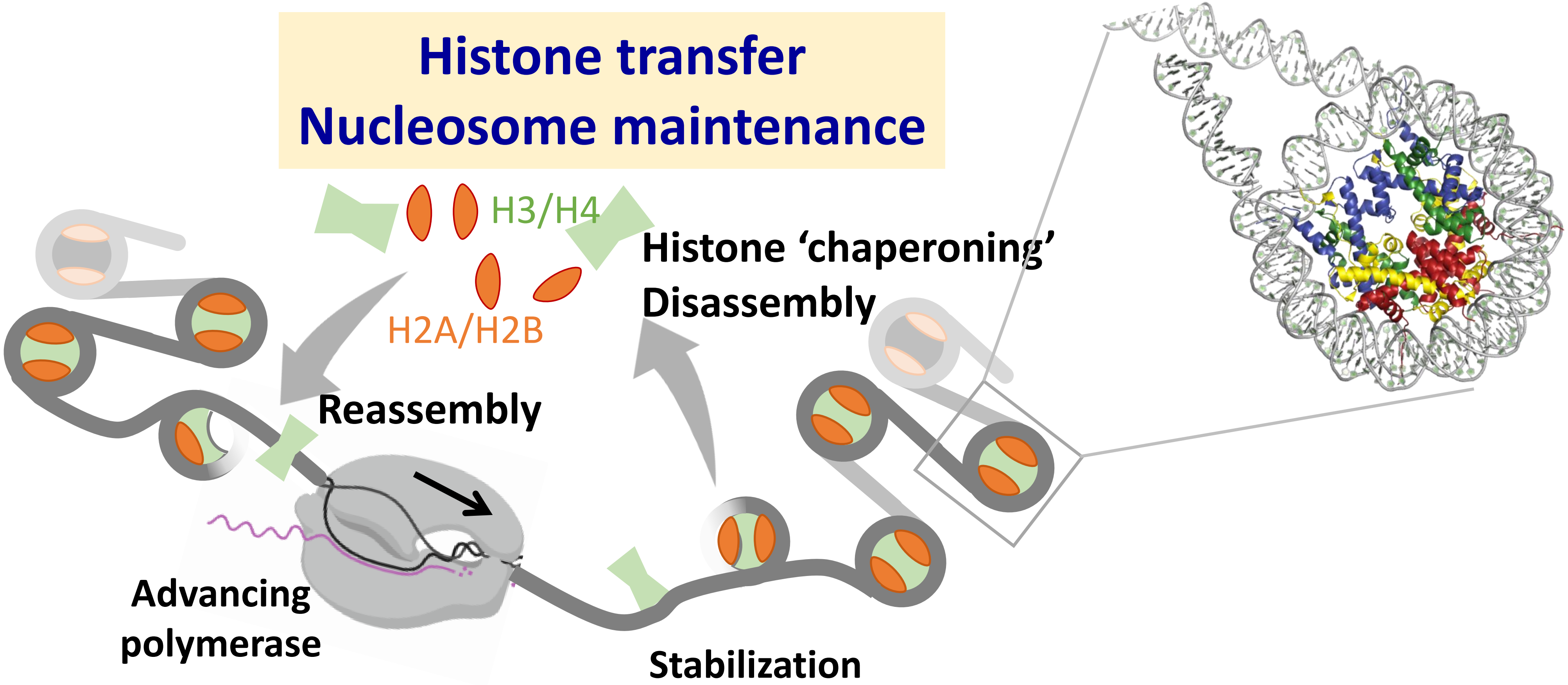
Nucleosome
Crochet work by
Jessica Polka

Navigating chromatin requires complex machinery

Histone chaperones

ATP-dependent chromatin remodelers

Histone transfer
Nucleosome maintenance



Histone 'chaperoning'
Disassembly

Reassembly

Advancing polymerase

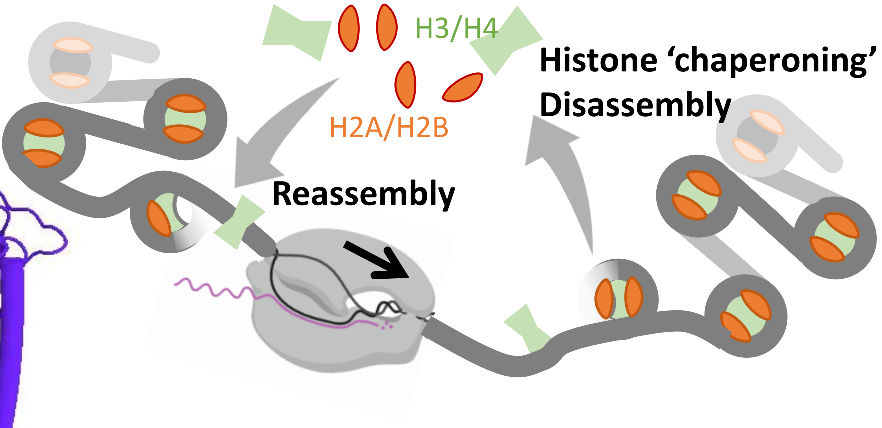
Stabilization
of partially dismembered nucleosomes

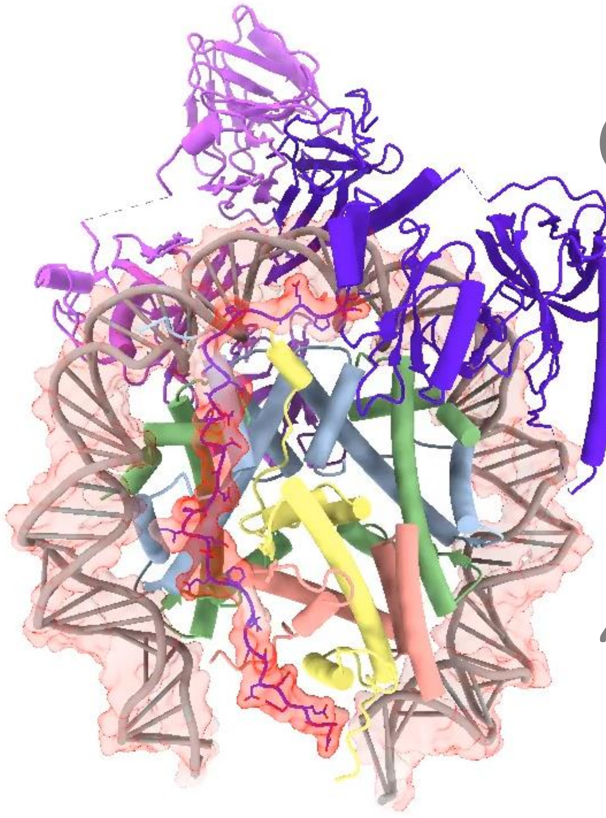
Navigating chromatin requires complex machinery

Histone chaperones

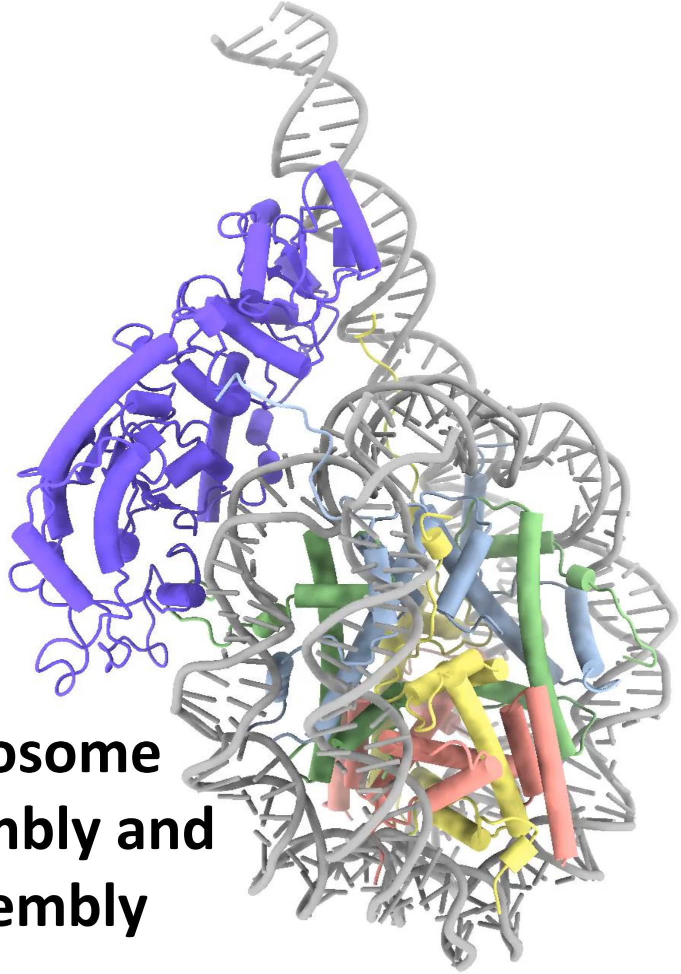
ATP-dependent chromatin remodelers

Histone transfer
Nucleosome maintenance



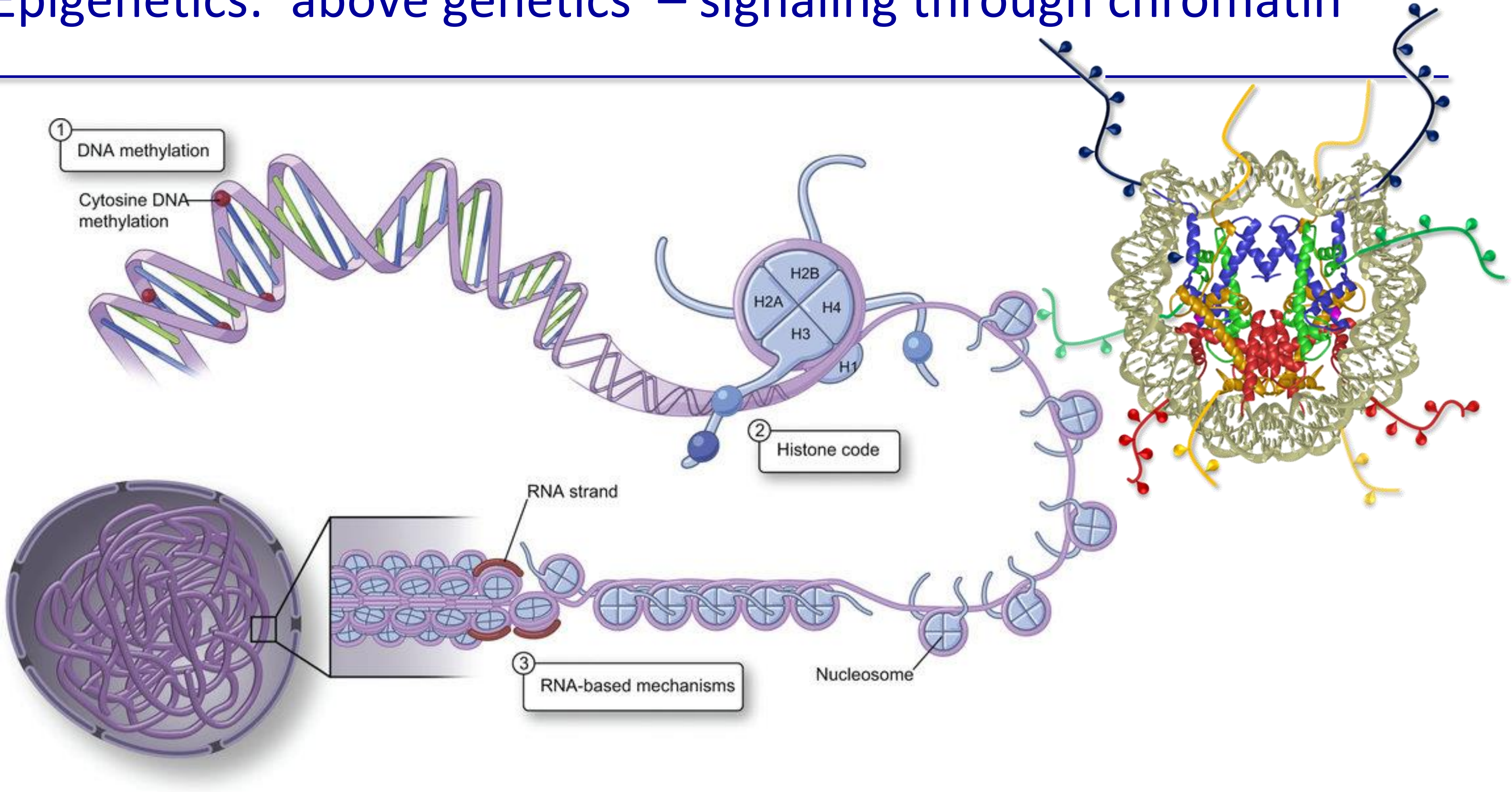


Stabilization of 'subnucleosomes'



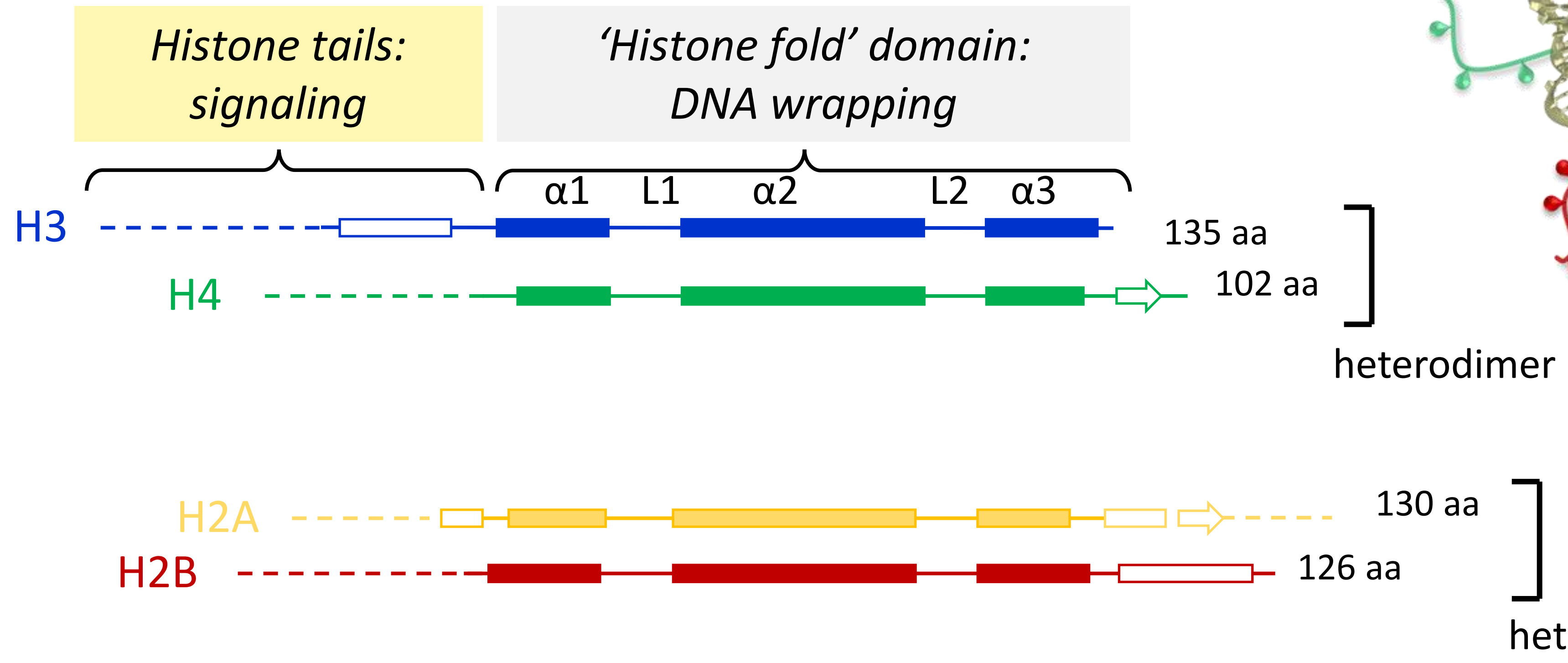
Nucleosome disassembly and reassembly

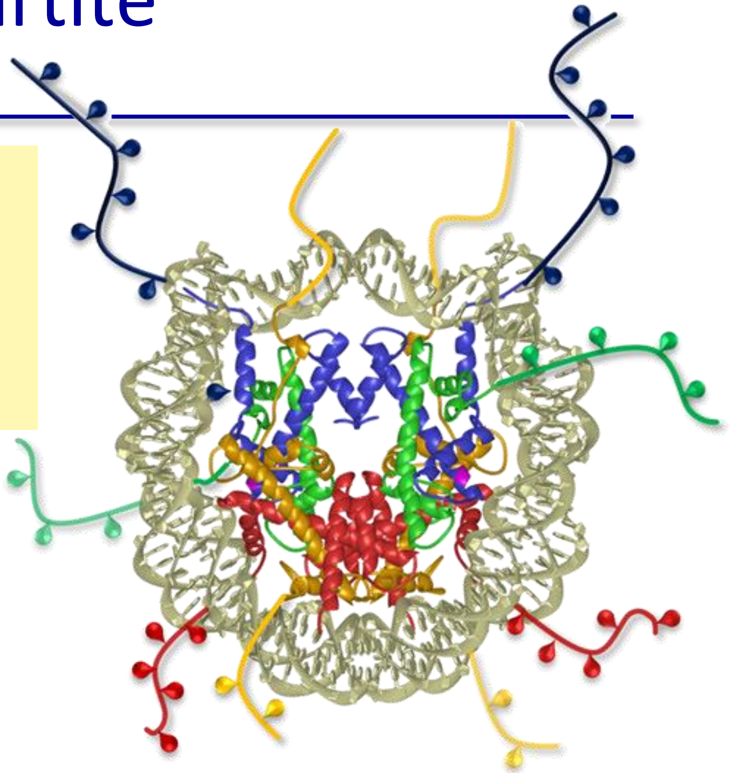
Epigenetics: ‘above genetics’ – signaling through chromatin



Histones are structurally and functionally bipartite

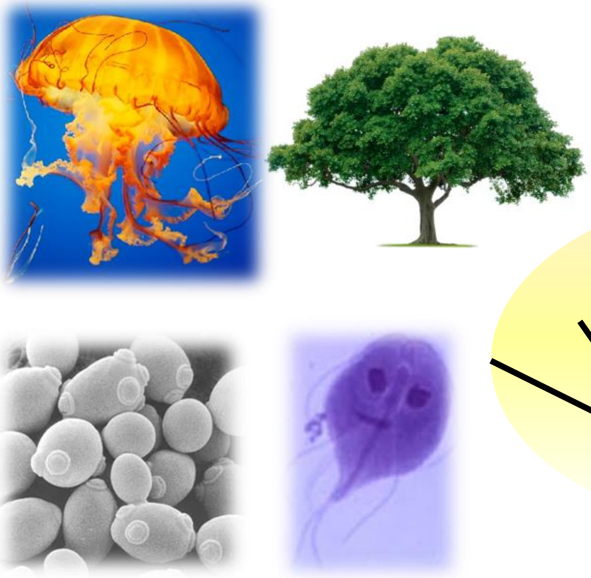
- **'Histone code':** combinations of small chemical modifications to recruit chromatin processing machinery
- 'Readers, writers, and erasers'

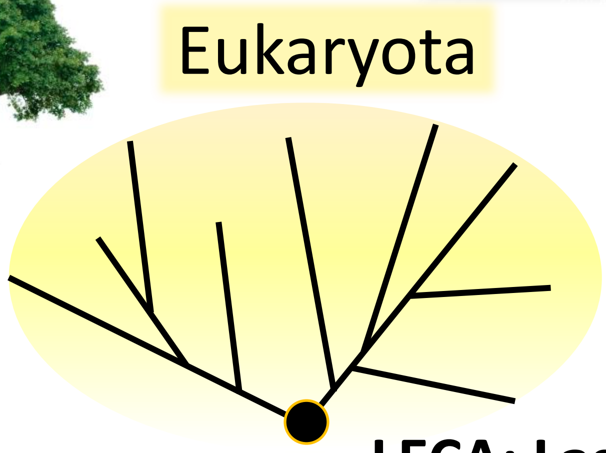




Who provided the chromatin ‘starter kit’?

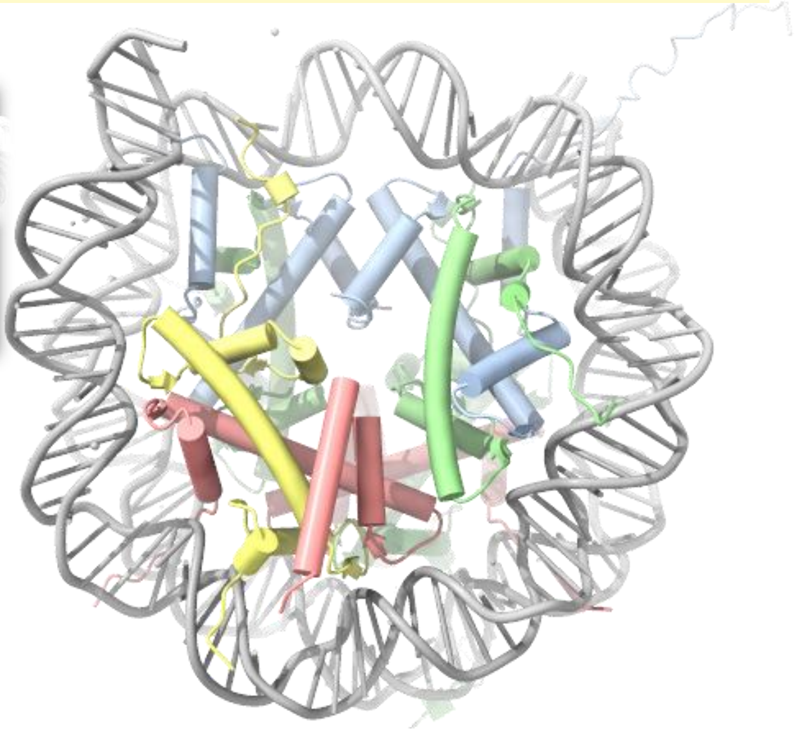
Histones, ‘histone code’, and chromatin maintenance machinery
are highly conserved across all eukaryotes



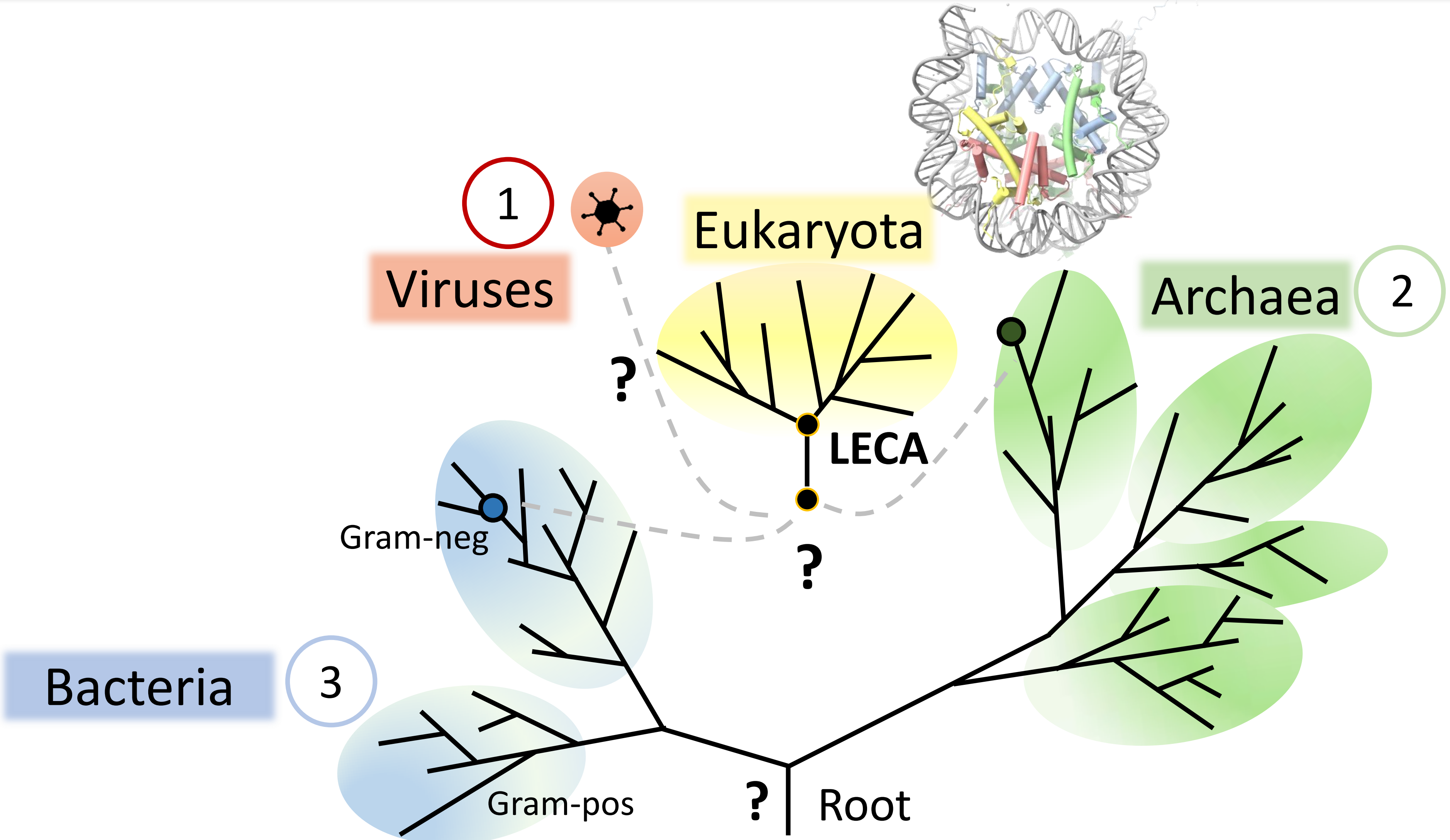


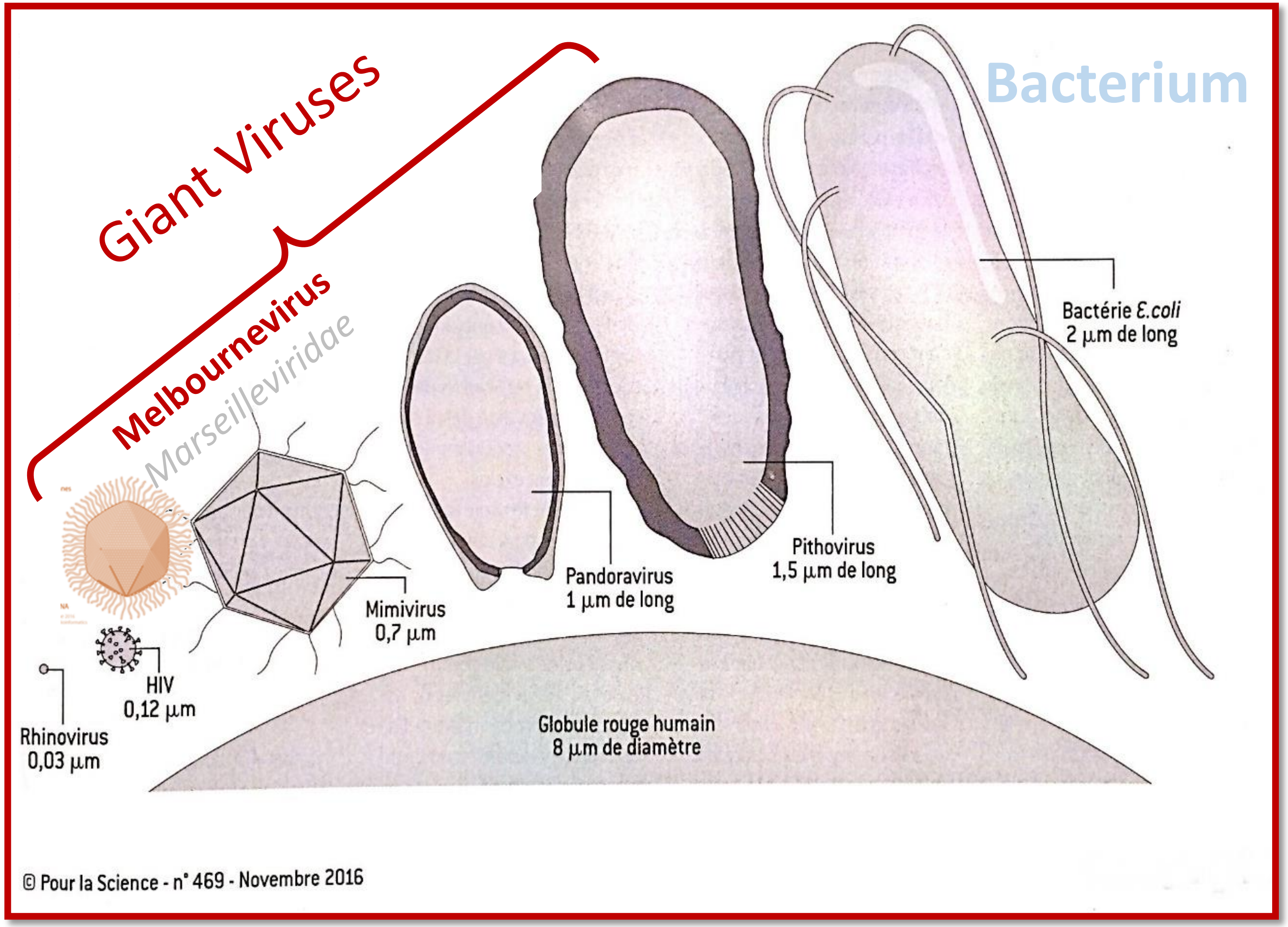
Eukaryota

LECA: Last Eukaryotic Common Ancestor



Searching for histones in all domains of life





© Pour la Science - n° 469 - Novembre 2016

Mostly infect Amoebae and other aquatic eukaryotes

①

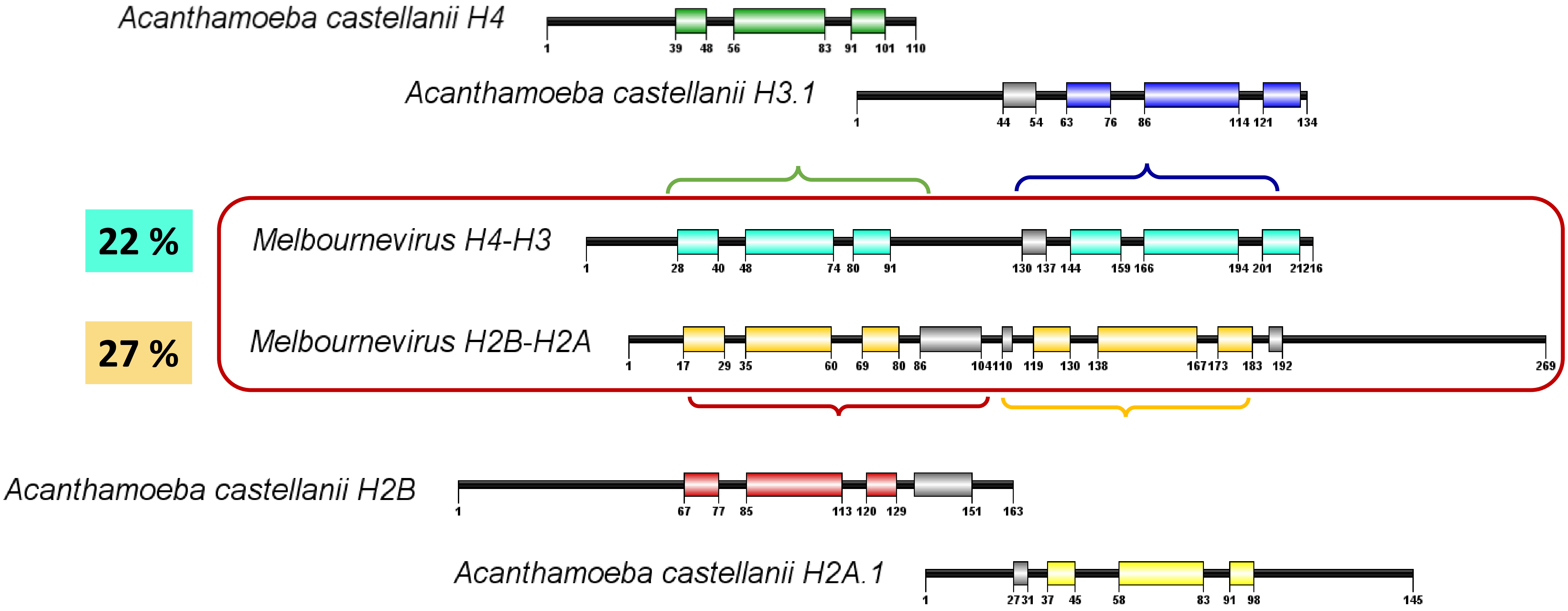
Introducing: Giant Viruses

- Large ds DNA genomes
- **‘Deep-branching’** in the tree of life

Marseilleviridae:

- Replicate in cytoplasm (amoeba)
- **Encode histones**

Melbournevirus histones are fused in doublets



Acanthamoeba castellanii H4

Acanthamoeba castellanii H3.1

22 %

Melbournevirus H4-H3

27 %

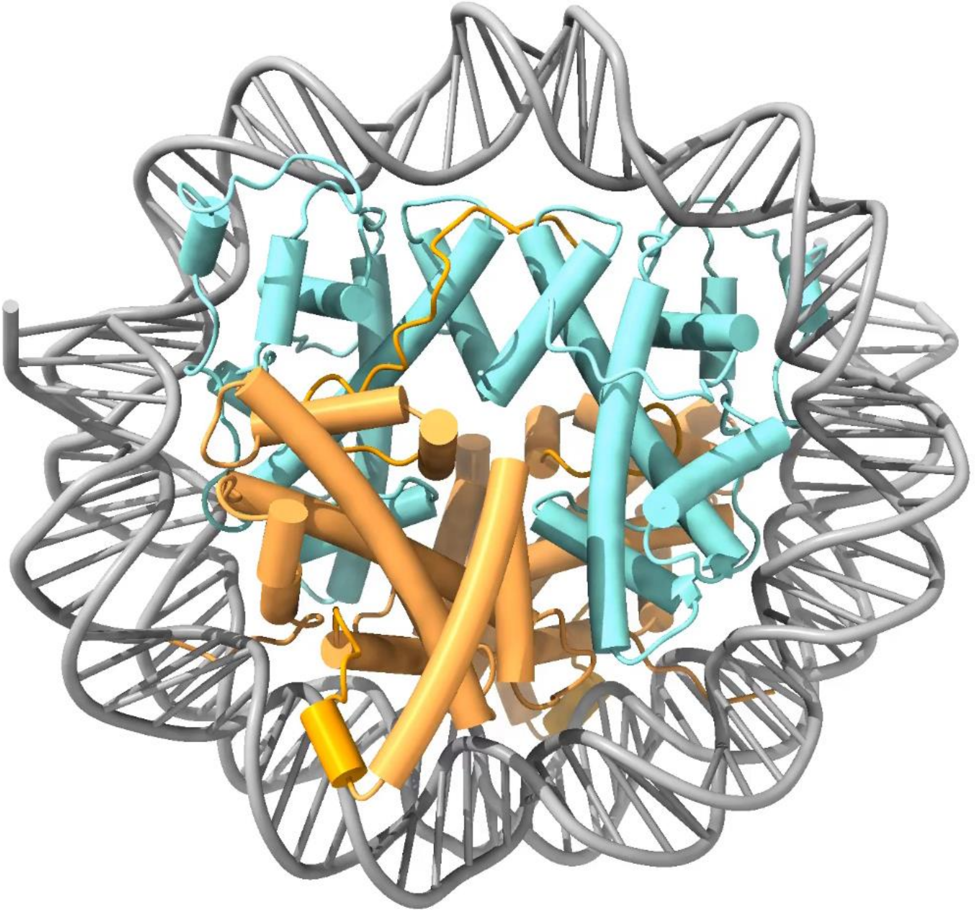
Melbournevirus H2B-H2A

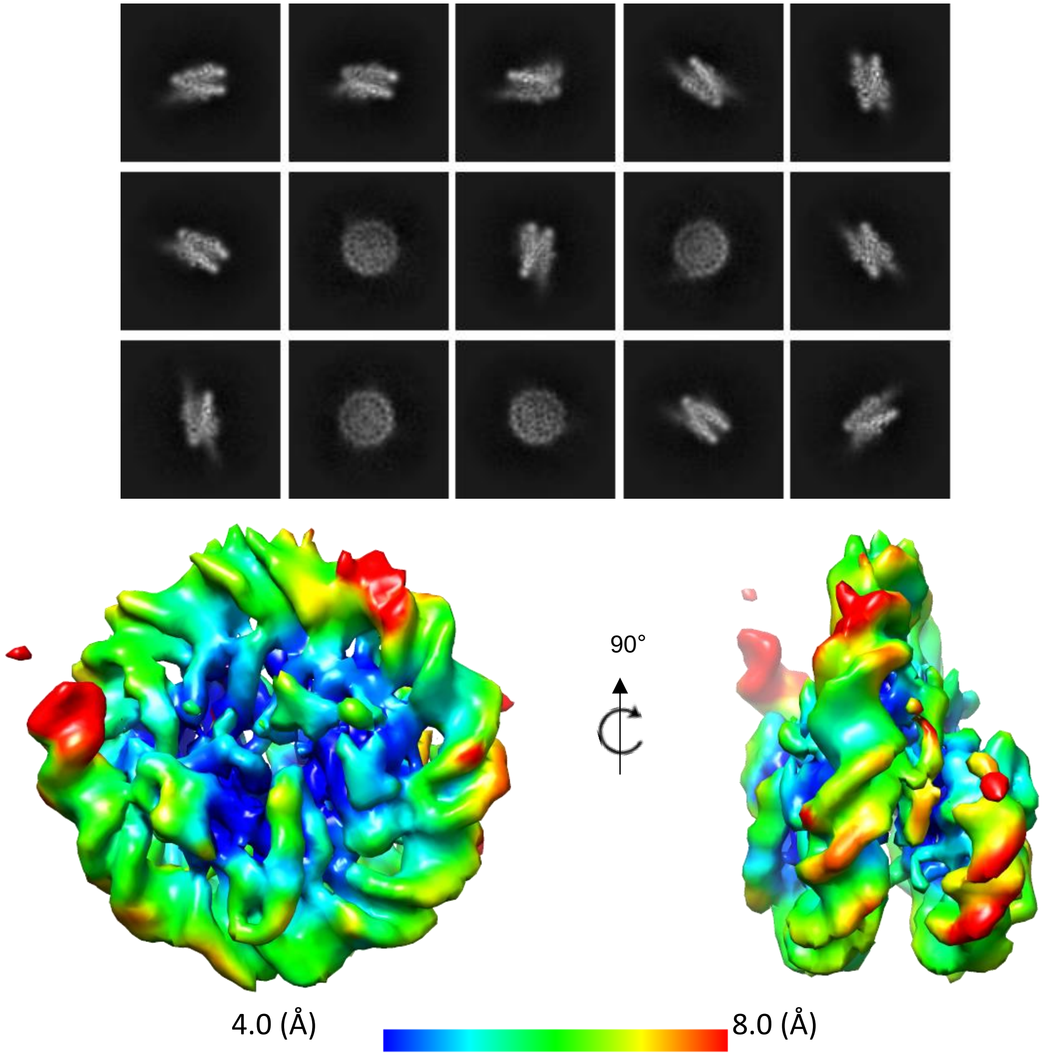
Acanthamoeba castellanii H2B

Acanthamoeba castellanii H2A.1

Melbournevirus nucleosomes: histones with ‘connections’

The first nucleosome outside the eukaryotic domain

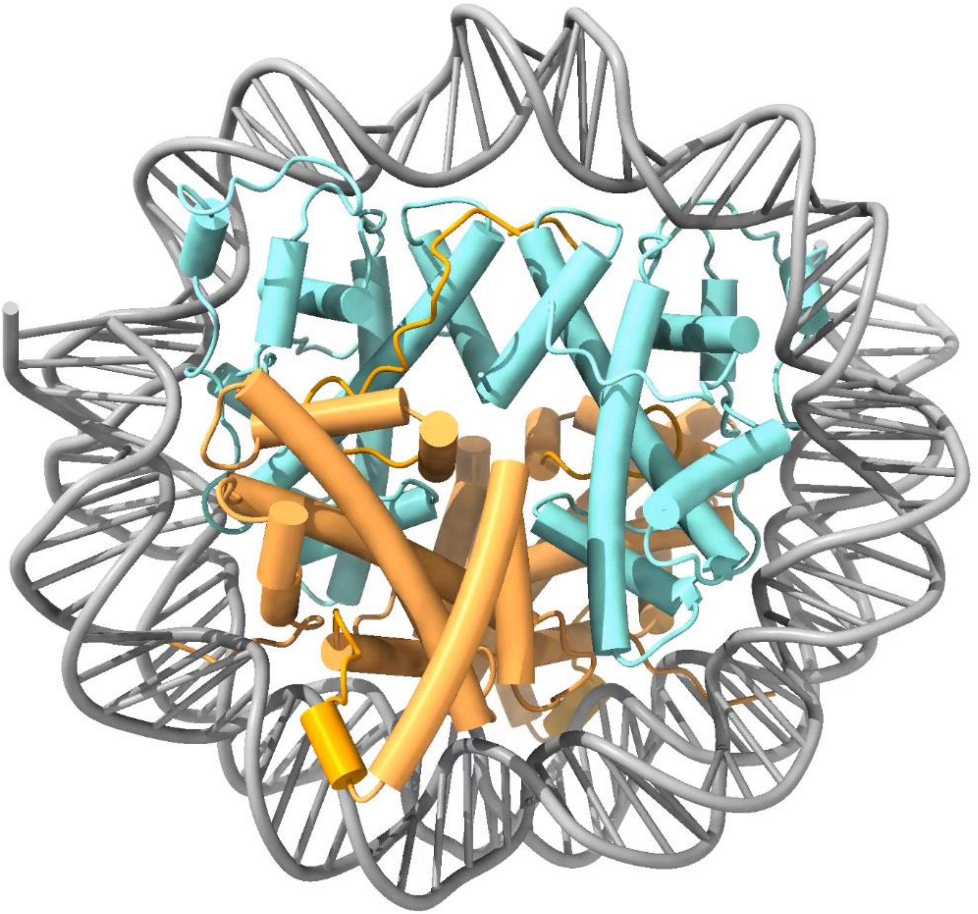




Histone tails and connectors shown as surfaces

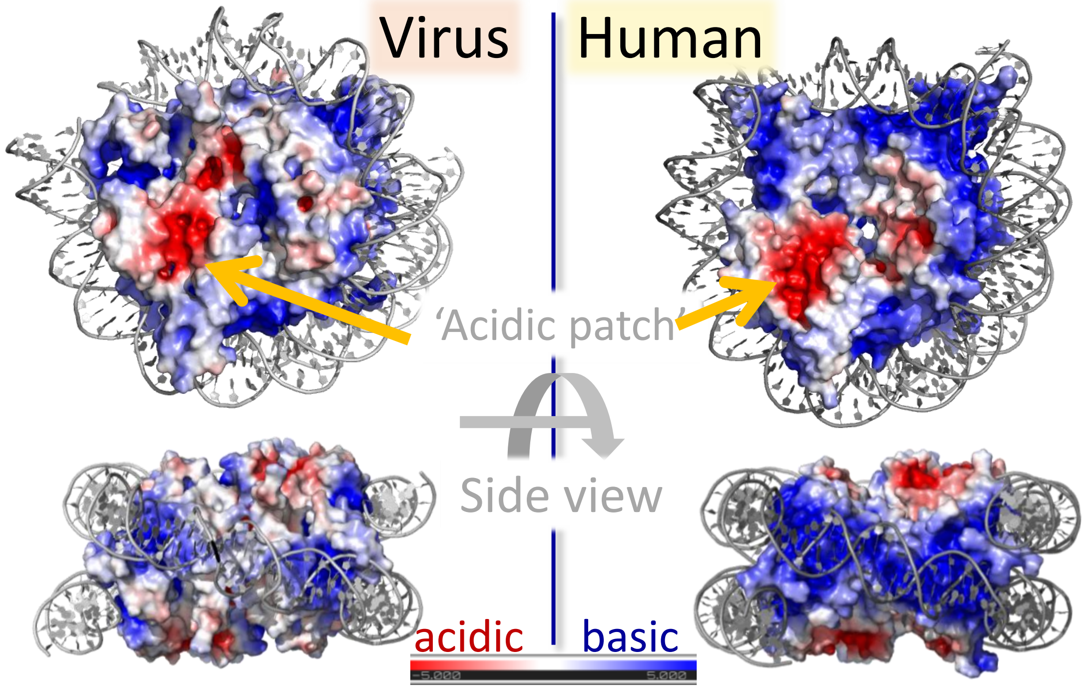
Liu et al, 2021, PMC8357426

Melbournevirus nucleosomes: histones with ‘connections’



- Destabilized nucleosomes
- Connectors and tails: structural functions

- Fewer positive charges
- Distinct surface (higher order structure?)



Histone tails and connectors shown as surfaces

Liu et al, 2021, Cell 184, PMC8357426

Viral histones are abundant in the mature virus

Table 1. Mass spectrometry proteomics of the purified MV virions. Histon
indicated in red.

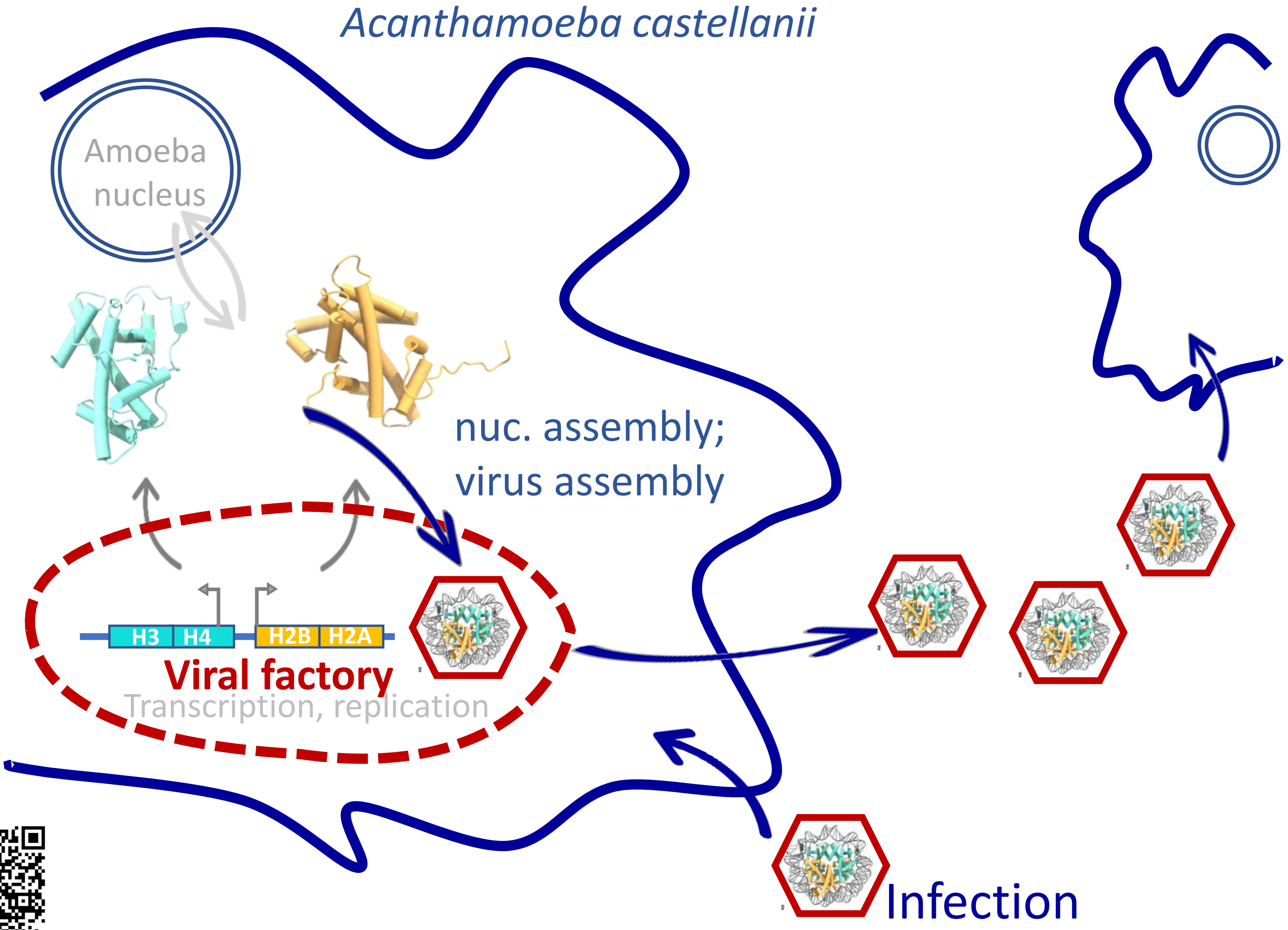
12/2022

Protein ID	Annotation	Final						Rank R2
MEL_236	hypothetical pro							1
MEL_342b	hyp							2
M								3
								4
M								5
M								
ME						8E+08	3812	7
MEL_				1862	8	5E+08	2429	8
MEL_			2,1E+08	1776	9	5E+08	2354	9
MEL_2		10	1,8E+08	1569	10	4E+08	2208	10
MEL_35	ical protein	11	1,4E+08	1211	11	3E+08	1454	11

Most abundant

**Fancy math:
enough viral histones to package the entire viral genome in nucleosomes**

Viral histones in the Melbournevirus infection cycle

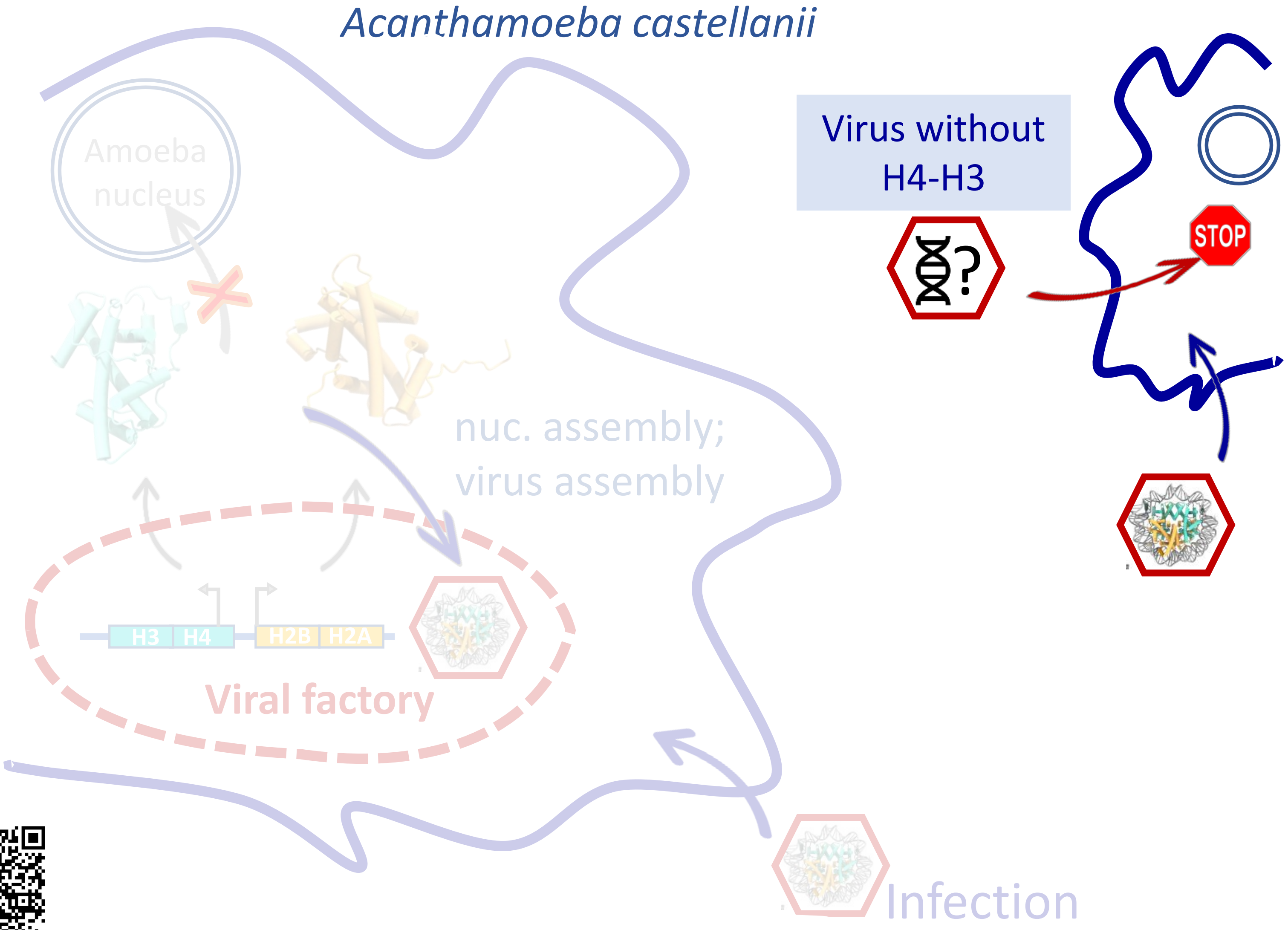


- Virus replicates in cytoplasm
- Never enters the nucleus

Liu et al, 2021, PMC8357426

Hugo Bisio / Chantal Abergel

Virally-encoded histones are essential to fitness and infectivity



Knockout of viral H4-H3:

- Virus particles assemble but are not infectious
- Outcompeted by wild type

Complement with viral H4-H3:

- Assembly of infectious virus
- Mutant virus persists

Liu et al, 2021, Cell, PMC8357426

Hugo Bisio / Chantal Abergel

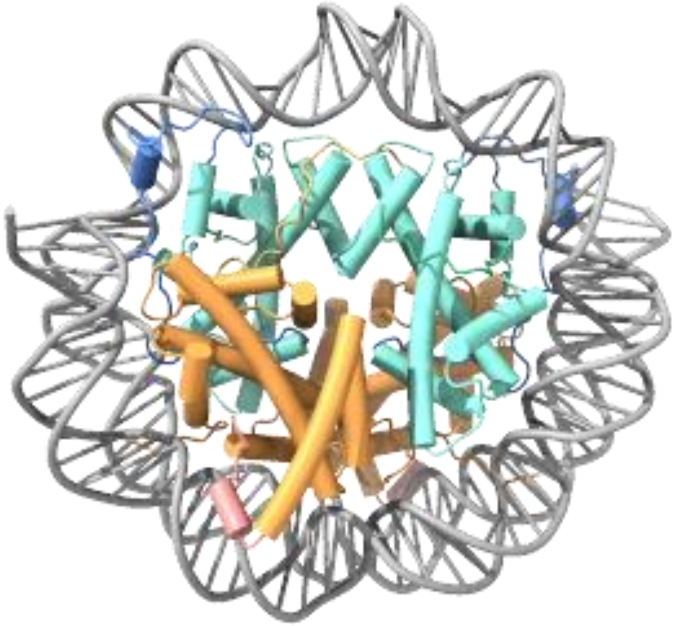
Virus-encoded histones in giant viruses: answers and questions

- Form nucleosomes with distinct structural features [a first for any virus] [Valencia-Sanchez, PMID 33927388]
- Localize to the viral factory, abundant in the virus
- **Required for viral fitness / infectivity**

But why, where? [in progress]

A fascinating streamlined system to study chromatin

- How do histones structure DNA in the virus?
- How / when are they assembled / disassembled?
  [60 % of the viral genome is ‘dark’]
- How wide-spread / diverse are histones in giant viruses, and where did they come from?

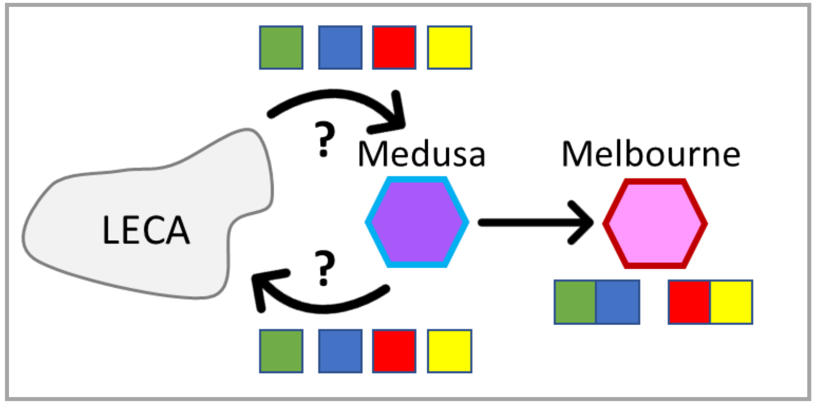


Giant virus genomes from the ice age

Rigou et al., 2022, PMC9546926

Medusavirus: a giant virus that replicates in the host nucleus

- Four separate histones, unusual tails
  [~20 % identity to host / other viral histones]
- Also encodes linker histone H1
- Predates Marseilleviridae

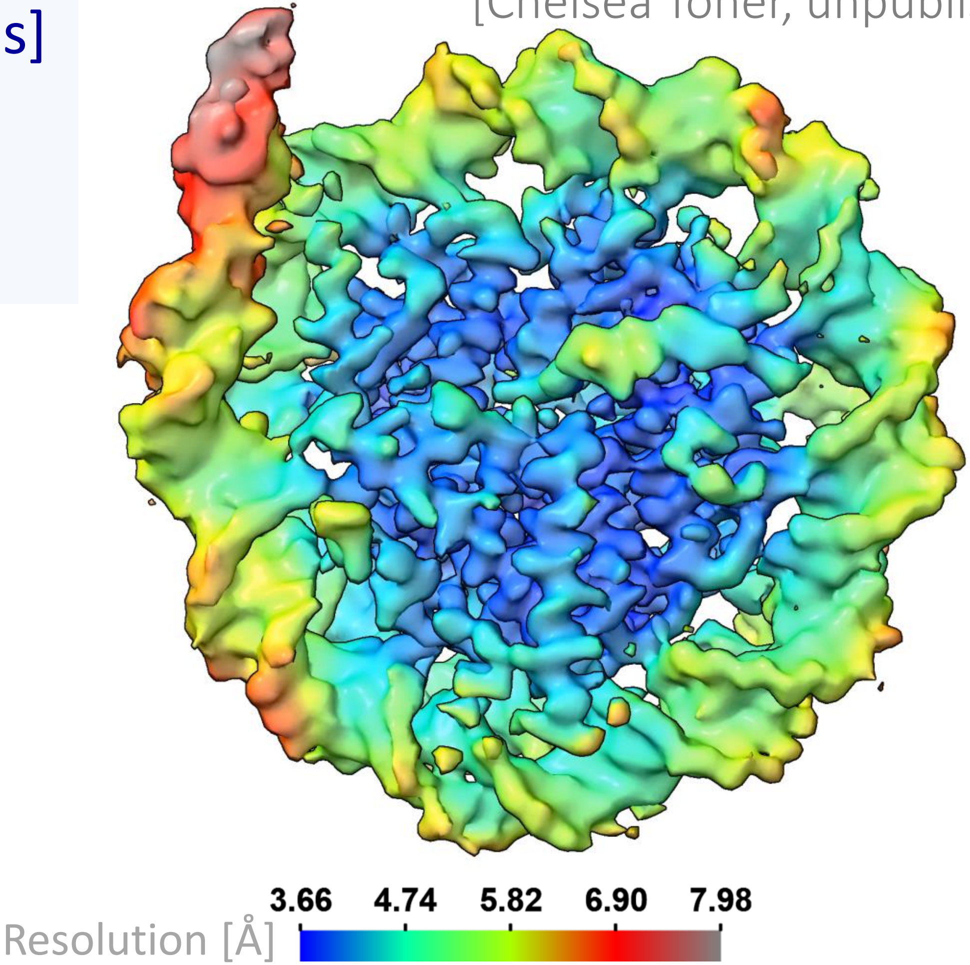


Adapted from Liu and Krupovic, 2022 (PMID 34657789)

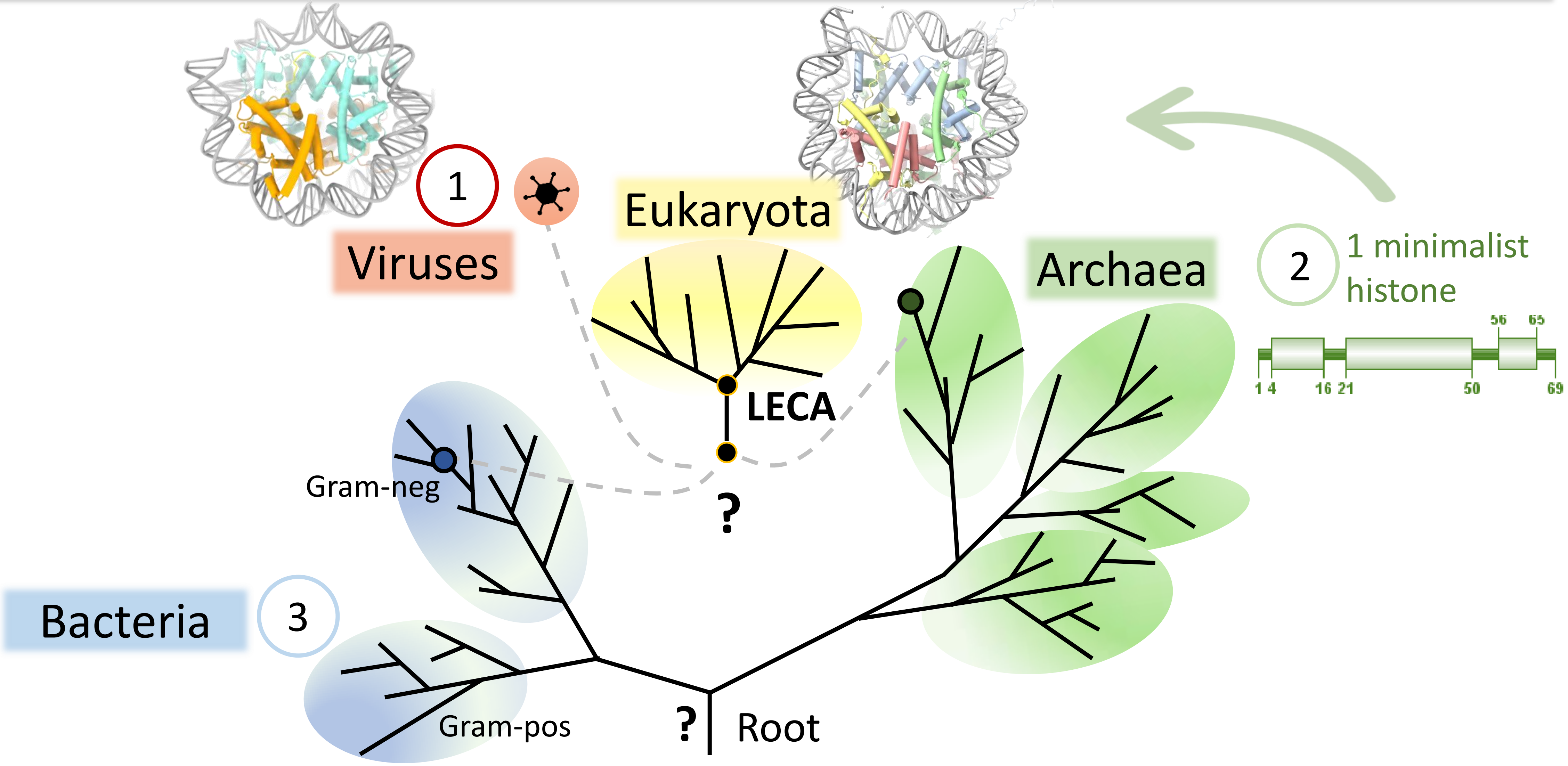
Yoshikawa et al, 2019, PMC6450098

Medusavirus NLP at 4.2 Å resolution

[Chelsea Toner, unpublished]



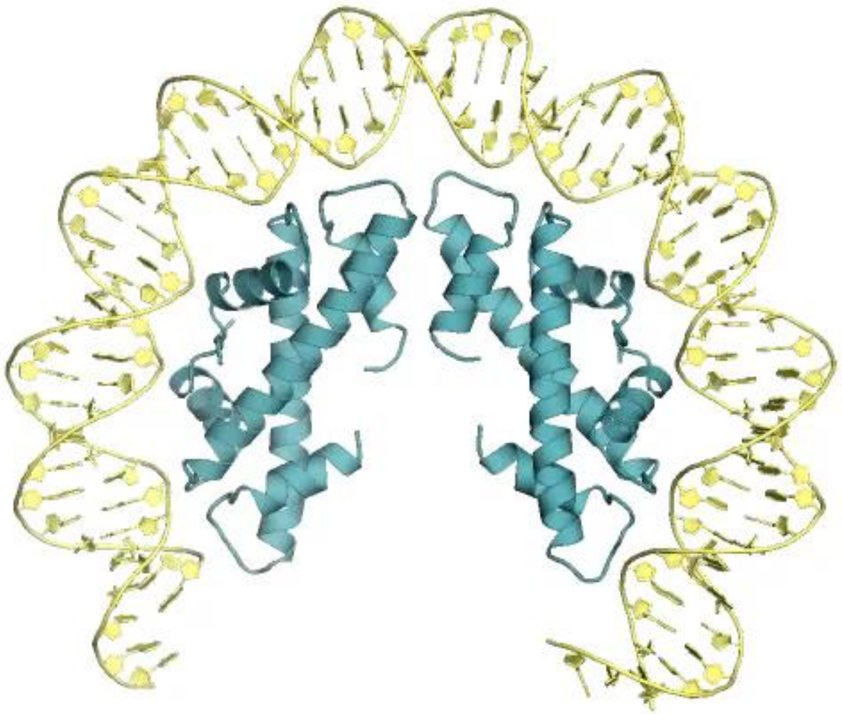
Most archaea encode a single minimalist histone



A single archaeal histone forms a continuous ‘hypernucleosome’

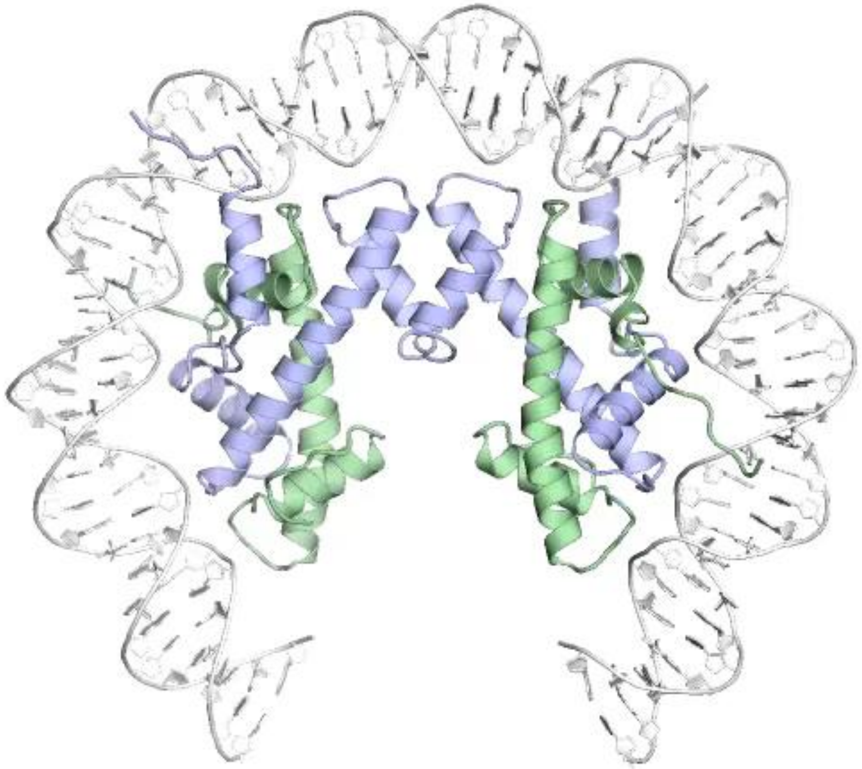
Archaeal chromatin

n•$(HMfB)_2$



Eukaryotic nucleosome

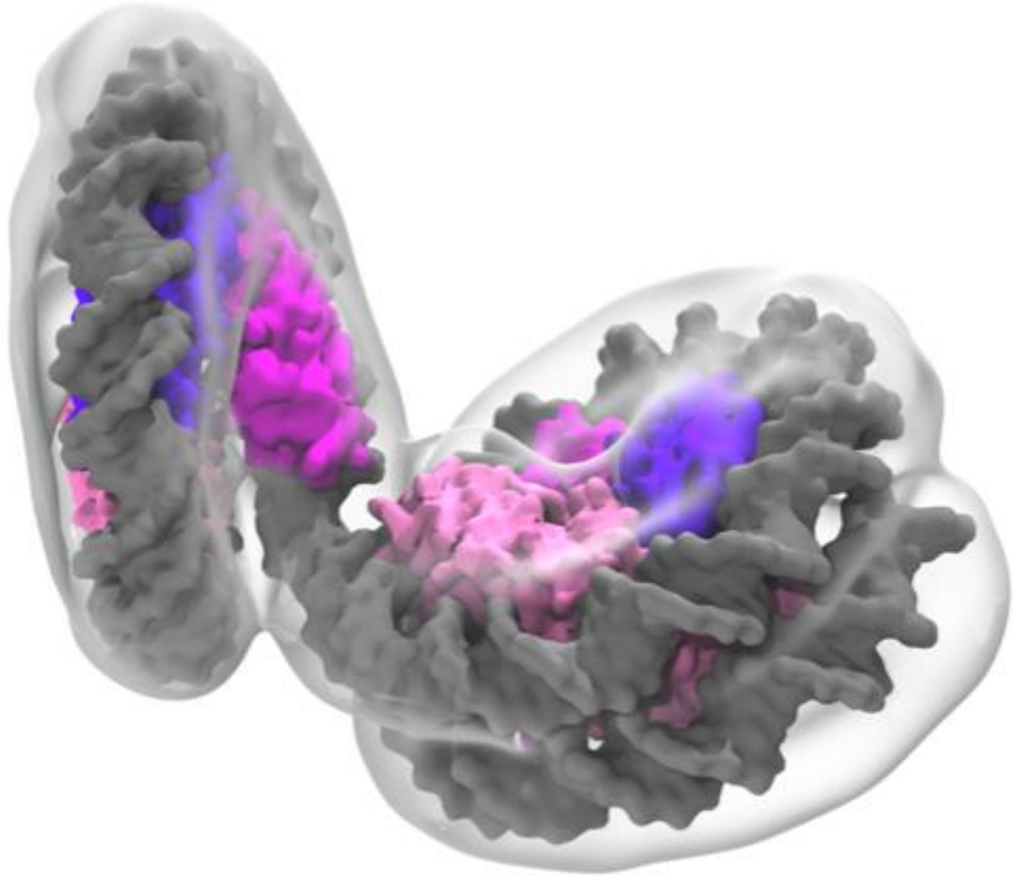
2 (H2A-H2B)•$(H3\text{-}H4)_2$



Mattiroli, F….& Luger, K, 2017, PMC5747315

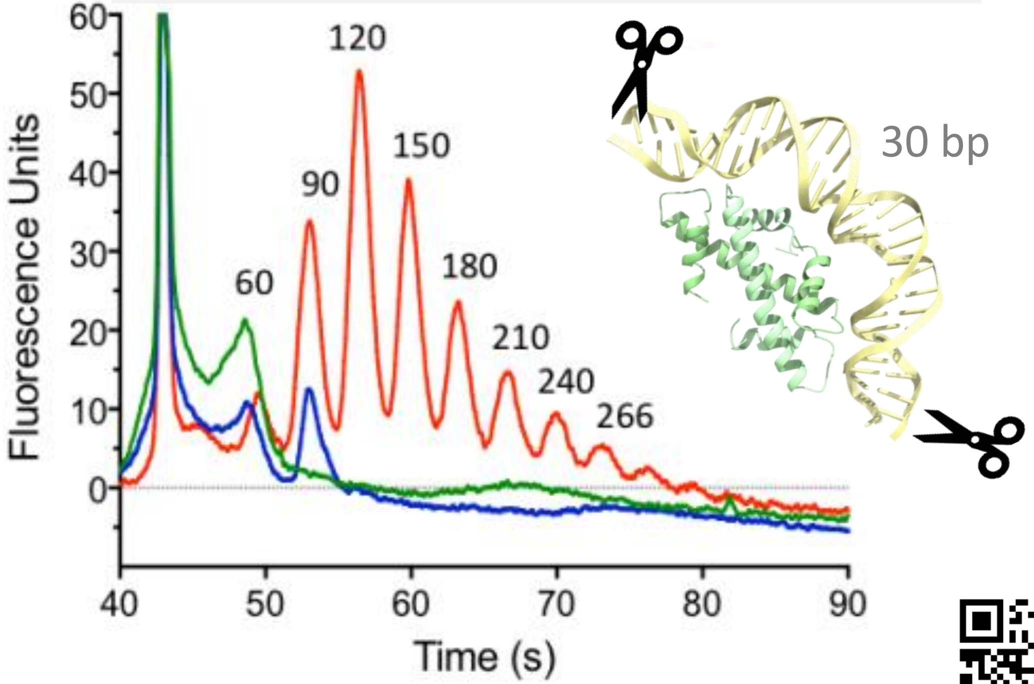
Archaeal hypernucleosomes flex and open stochastically

CryoEM structure of archaeal histone, 207bp DNA
Confirmed by analytical ultracentrifugation



In the cell:
MNase digestion of extracted archaeal chromatin: **30 bp ladder**

Bioanalyzer trace of DNA fragments



Bowerman, S.....& Luger, K, 2021, PMC7990501

Mattiroli, F....& Luger, K, 2017, PMC5747315

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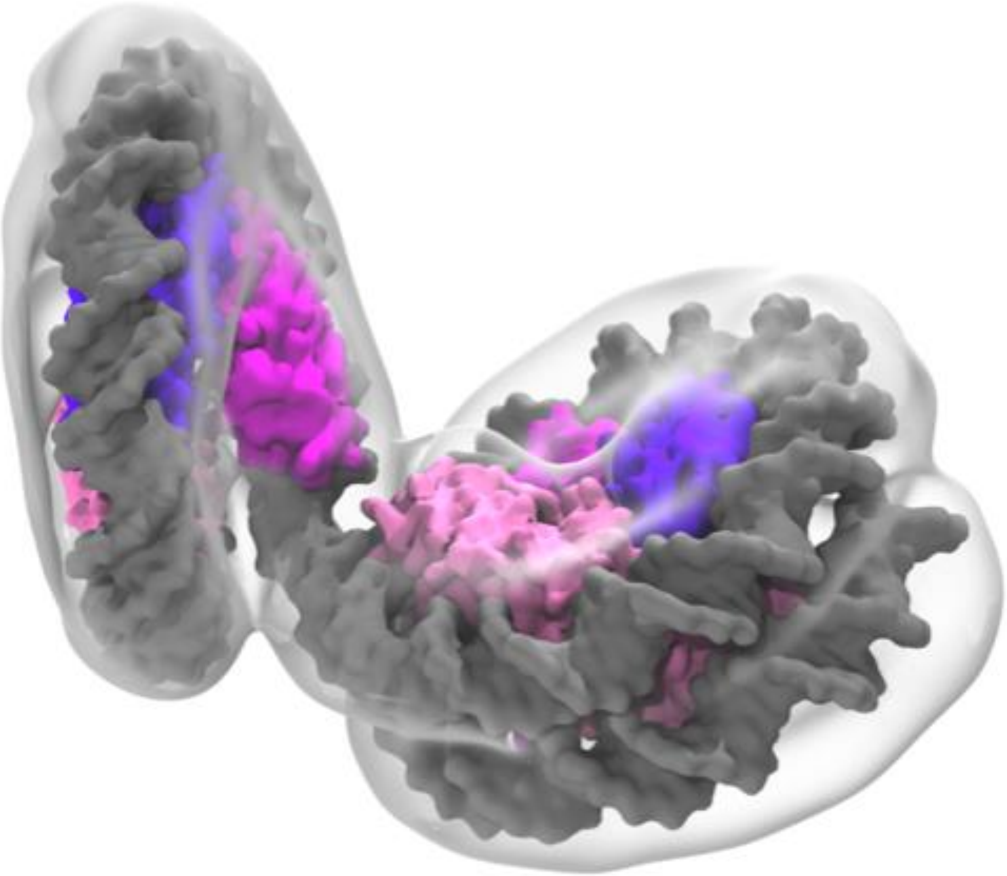


Stochastic opening provides a ‘low-tech’ way to permit access to the genome
(no remodelers or PTM in archaea)

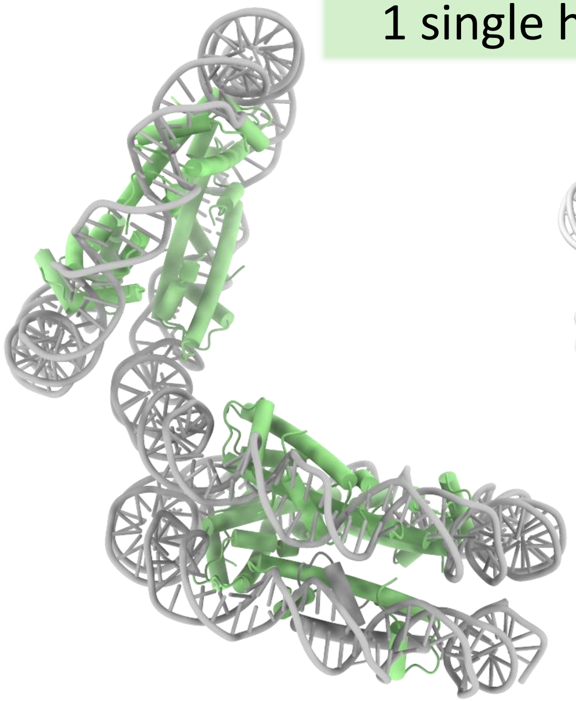
In eukaryotes (humans):

open and closed hypernucleosomes at the end of chromosomes (**telomeres)**
(Nordenskjold / Rhodes)

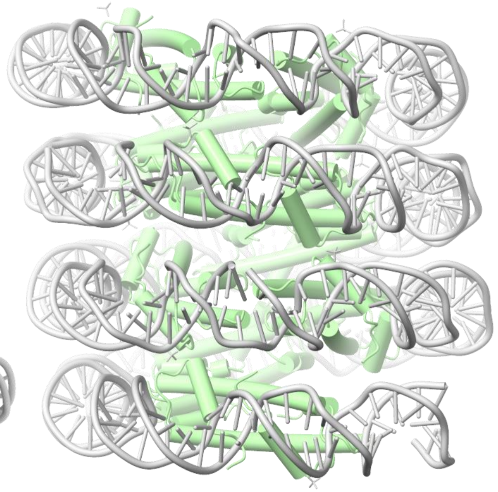
Bowerman, S…..& Luger, K, 2021, PMC7990501

Flexing hypernucleosomes are found at eukaryotic telomeres

Archaea:
1 single histone



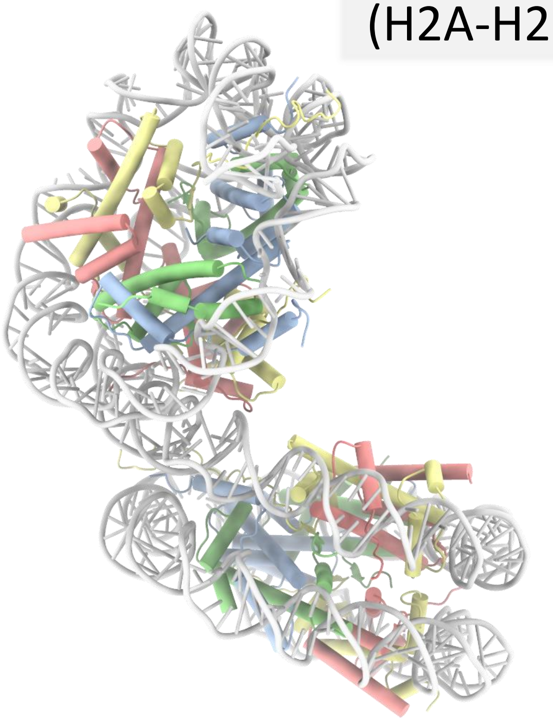
Open state



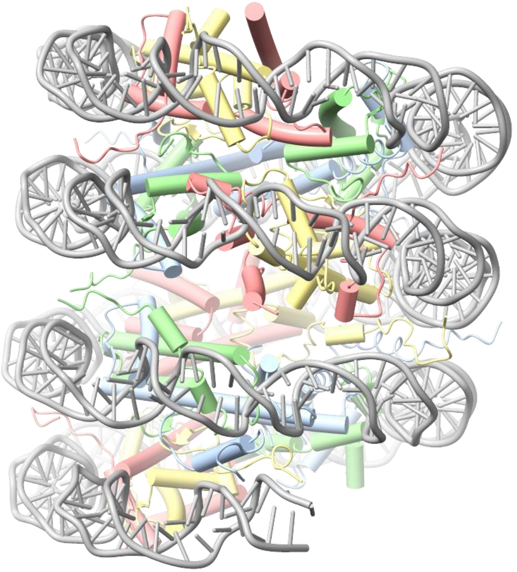
Closed state

Bowerman, S.....& Luger, K, 2021, PMC7990501

Eukaryotes:
(H2A-H2B-H3-H4)$_2$



Open state
7V9C



Closed state
7v96

Short nucleosome repeat length at telomere

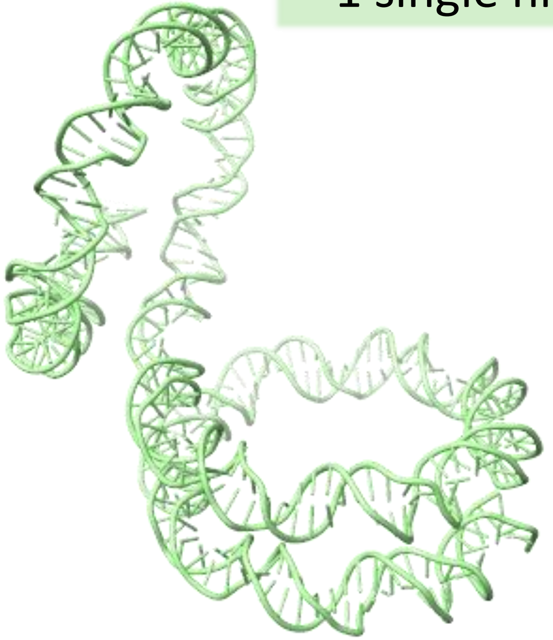
Soman, A...., Nordenskjold, 2022 PMID 36104563

Flexing hypernucleosomes are found at eukaryotic telomeres

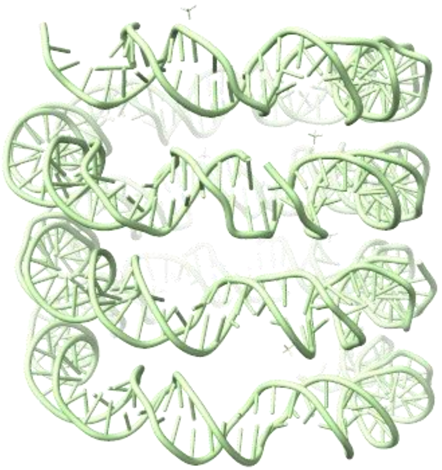
Archaea:
1 single histone

Histones removed for clarity

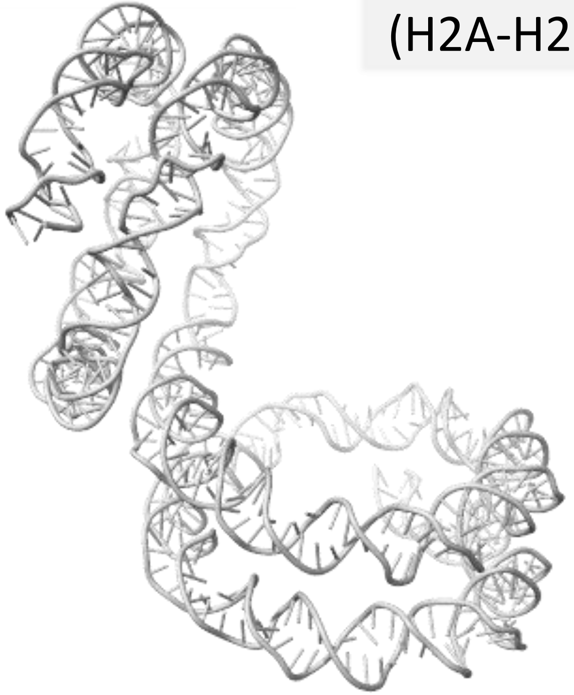
Eukaryotes:
$(H2A\text{-}H2B\text{-}H3\text{-}H4)_2$



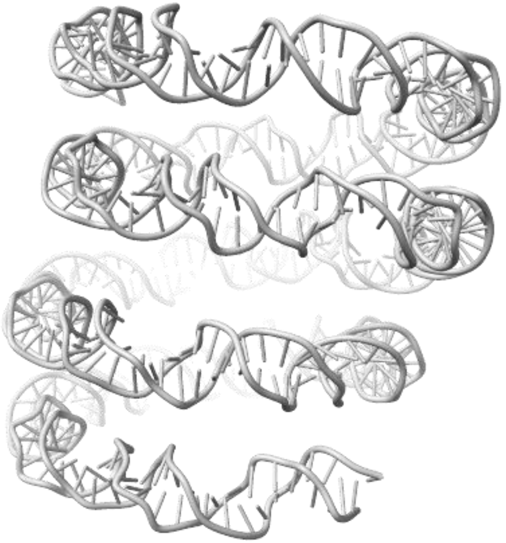
Open state



Closed state



Open state
7V9C



Closed state
7v96

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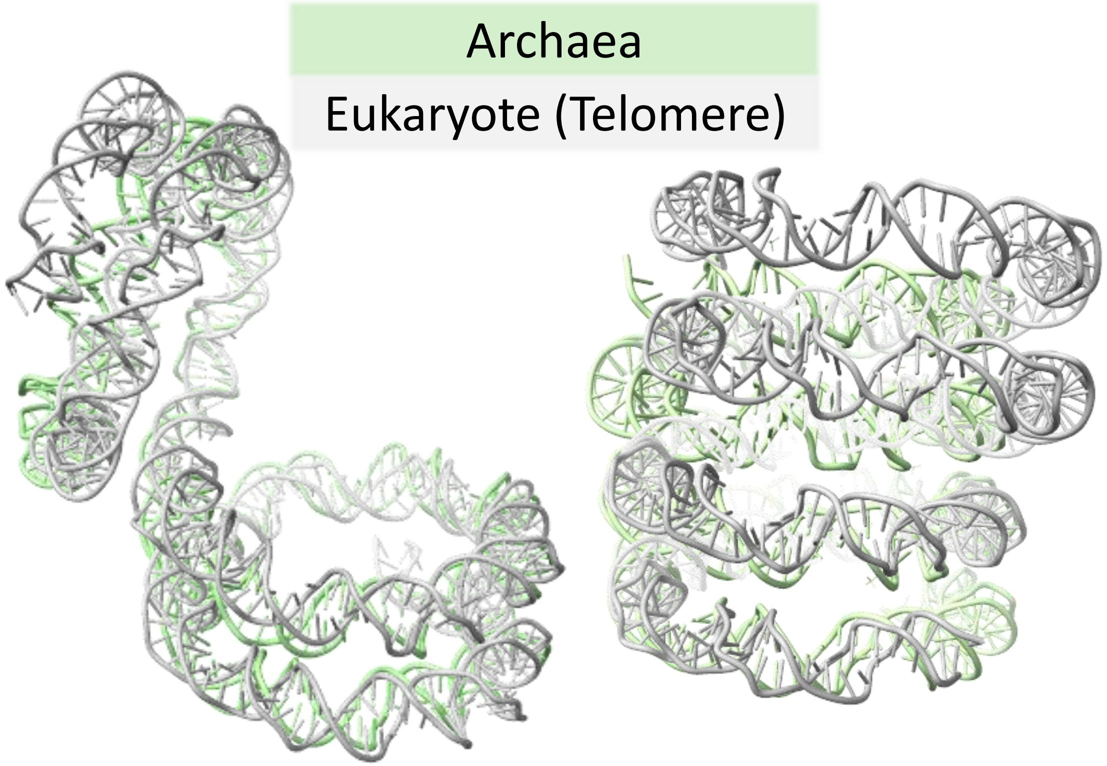


Open state
Open state
7V9C

Closed state
Closed state
7v96

Soman, A…., Nordenskjold, 2022 PMID 36104563
Bowerman, S…..& Luger, K, 2021, PMC7990501

Intriguing evolutionary link between archaeal and eukaryotic chromatin

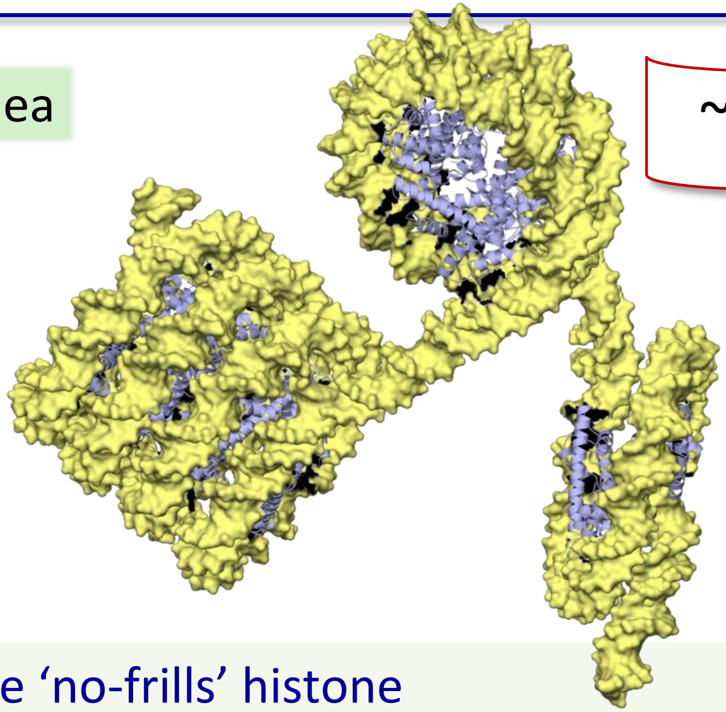
Back to archaea:

How common a phenomenon is this type of chromatin arrangement?

- Archaea inhabit diverse and extreme ecological niches
- Archaeal histone sequences are highly divergent

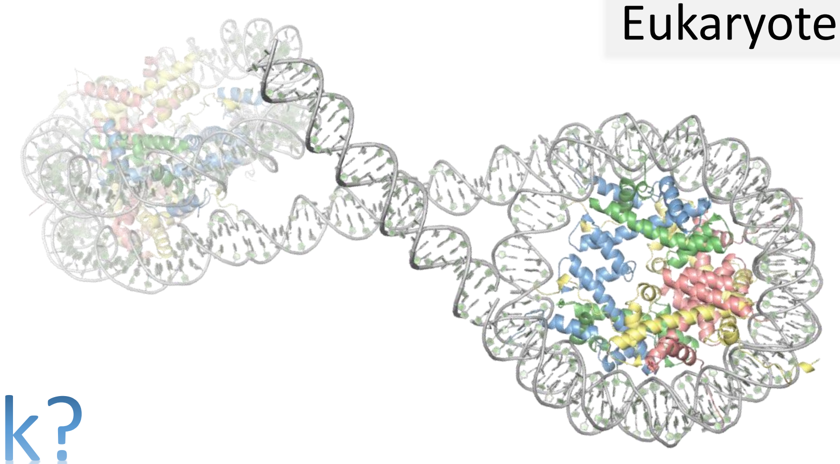
The expansion to four histones allows nucleosomes to assume complex regulatory functions

Archaea



~3 billion years

Eukaryotes



Missing link?

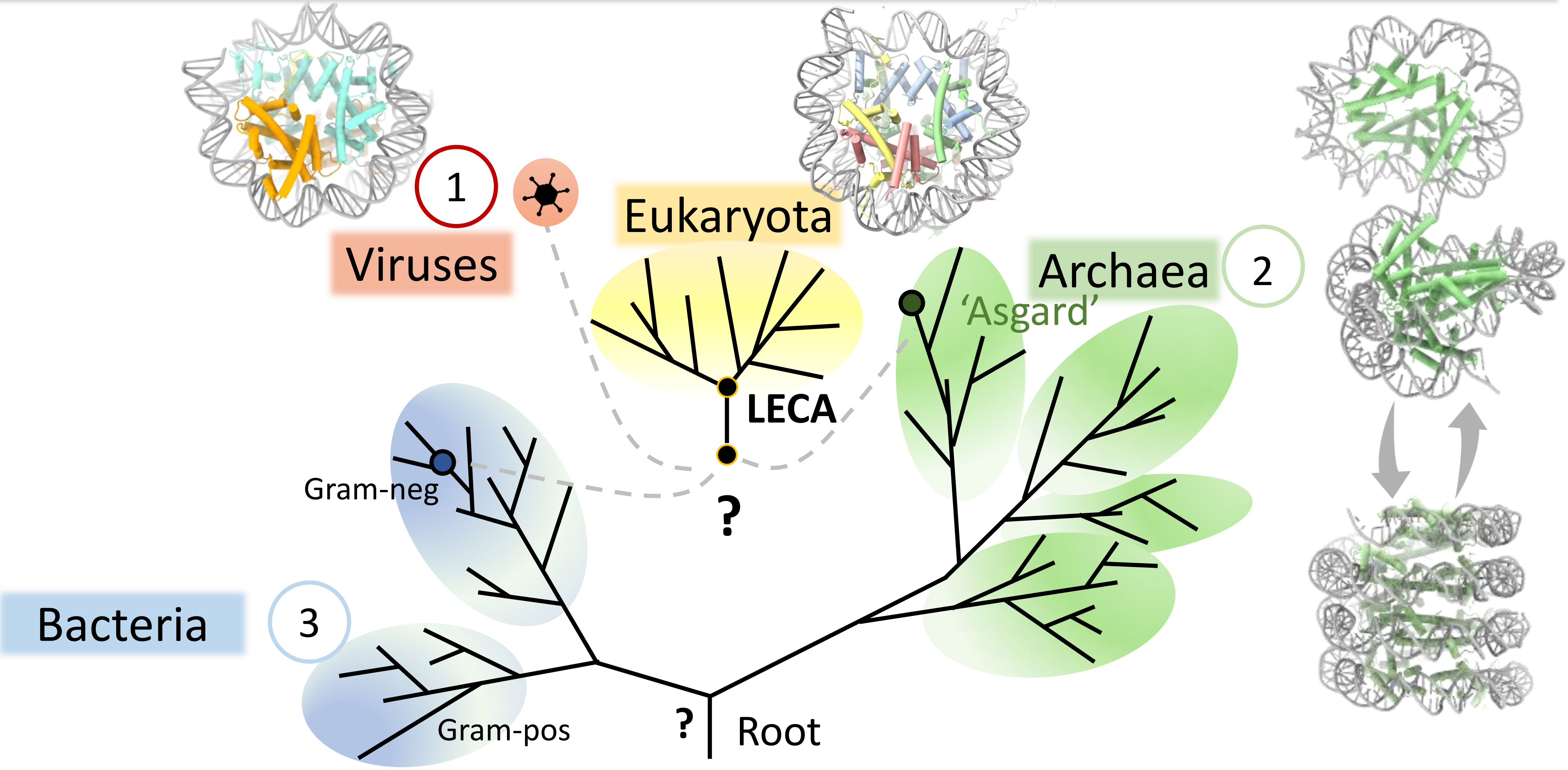
- Single ‘no-frills’ histone
- Semi-stable superhelix (‘slinky’)

- **Polymerases slowed, but not inhibited**
- No evidence for post-translational modifications, chromatin remodelers
- Self-assembling

- Four histones with tails; histone variants
- Discrete, stable particles from 2 building blocks

- **Profoundly inhibit DNA-dependent processes**
- Reliance on post-translational modifications; ATP-dependent chromatin remodelers
- Require histone chaperones for assembly

Bacteria: the last histone holdout



1

Viruses

Eukaryota

LECA

Archaea

2

'Asgard'

Gram-neg

?

Bacteria

3

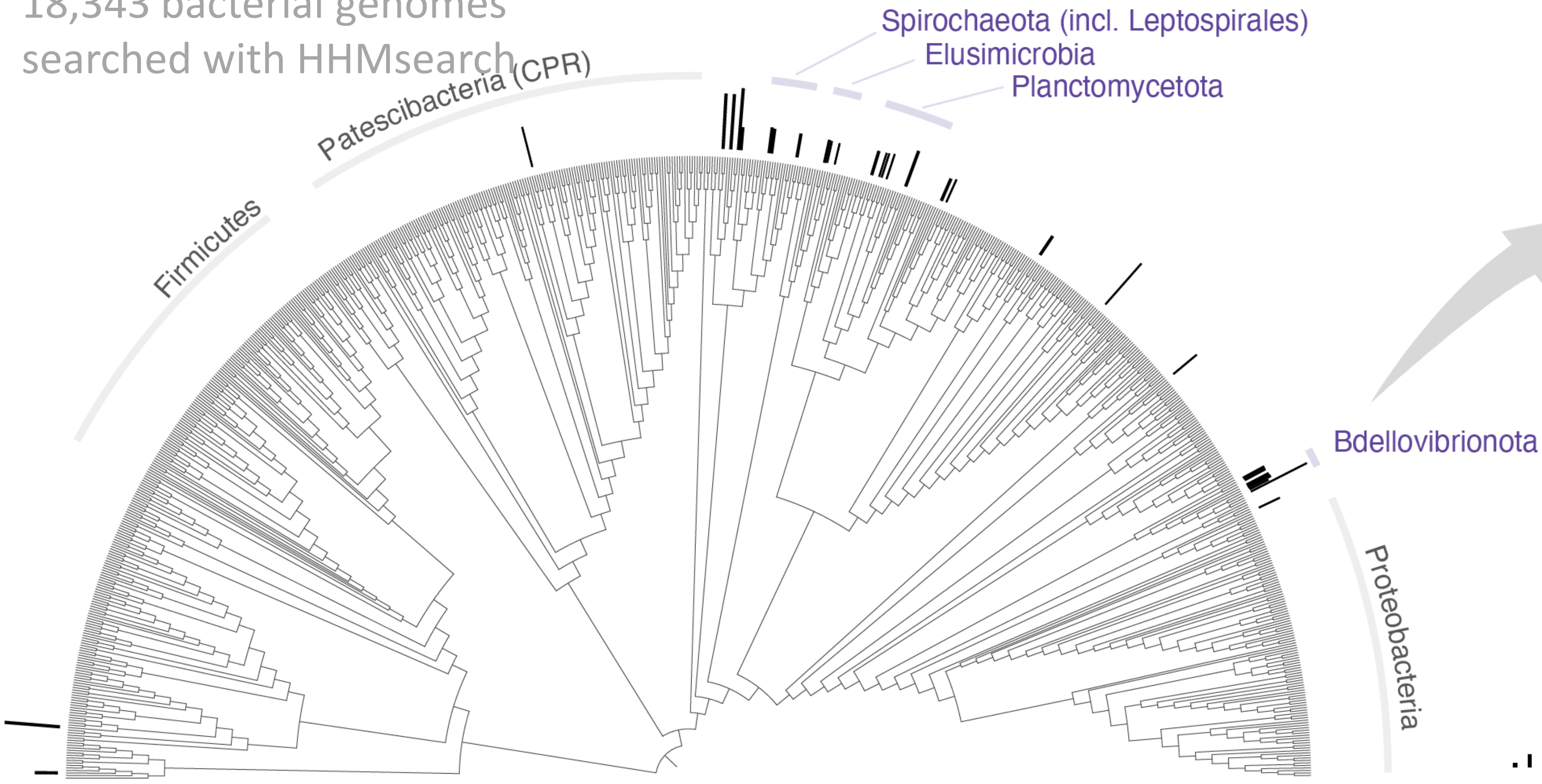
Gram-pos

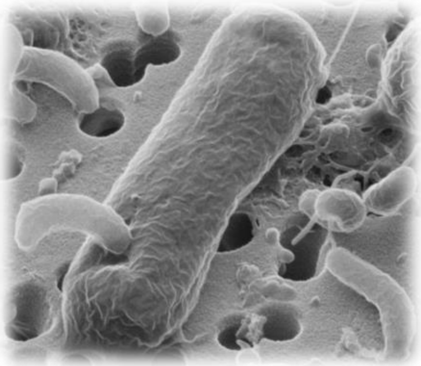
?

Root

Histones are sporadically present in bacterial genomes (2 %)

18,343 bacterial genomes
searched with HHMsearch





Bdellovibrio bacteriovorus
attacking *E.coli*

Putative histone fold proteins

Cladogram of bacteria
(abridged, GTDB)

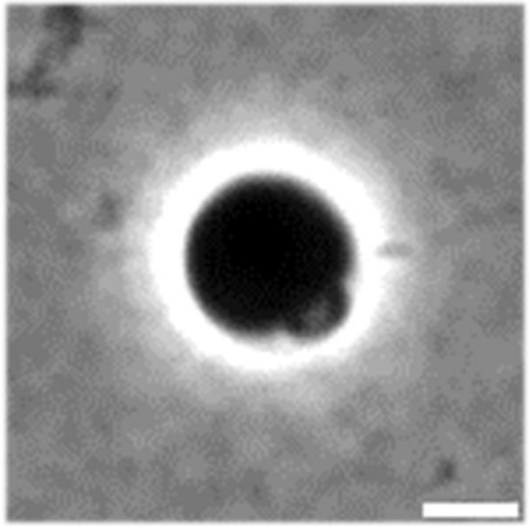
A. Hocher and T. Warnecke

B. bacteriovorus is a predator with a complex life cycle

- Free-swimming ‘attack phase’
- Growth phase in prey periplasm
- Non-binary division

Small size, large genome (3.8 Mb)

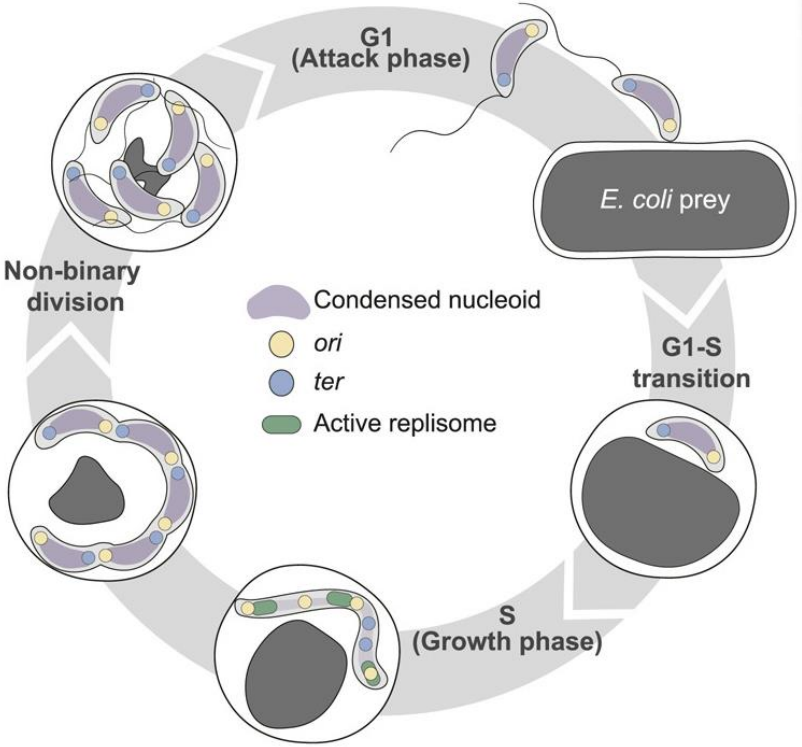
Single, highly expressed histone

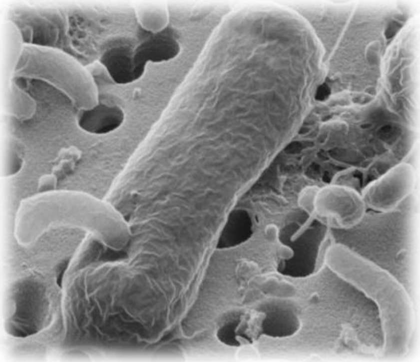


B. bacteriovorus feasting on *E.coli*

Movie by Yoann Santin (Laloux Lab)

Last-resort antibiotic





Bdellovibrio bacteriovorus

attacking *E.coli*

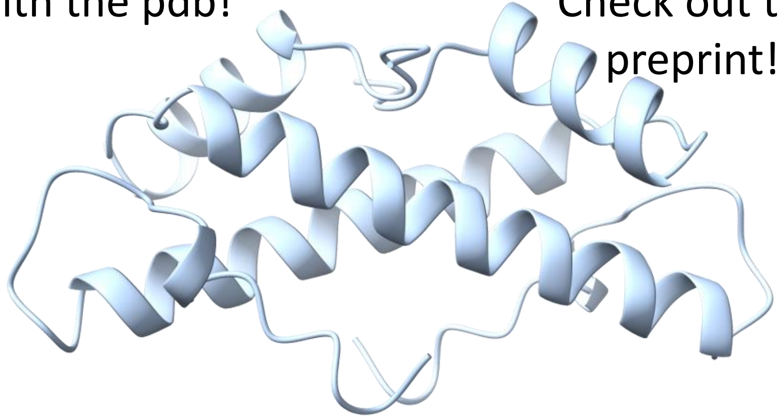
From Kaljevic et al., 2021, PMID 34256020

B. bacteriovorus encodes a highly expressed histone-like protein

Bacterial histone Bd0055: ~20% identical to archaeal and human histones; chromosome-associated

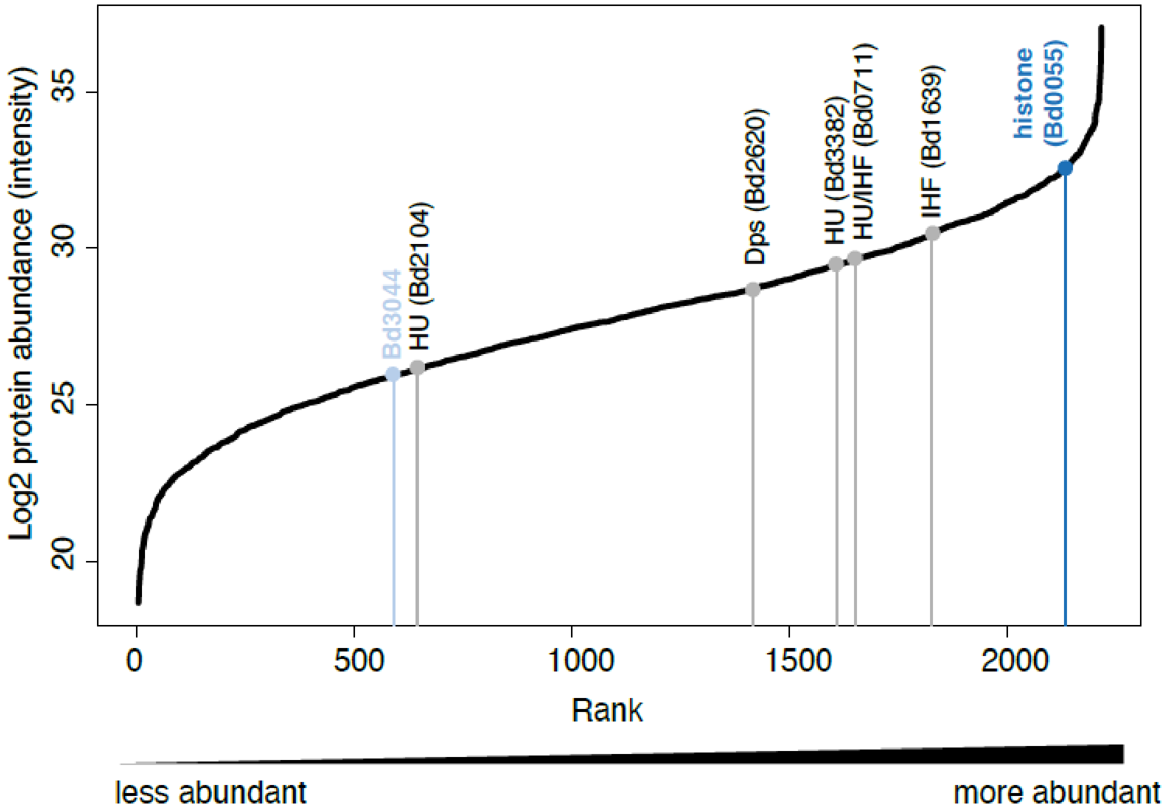
Play with the pdb!

Check out the preprint!



1.9 Å crystal structure of Bd0055

(Laursen; Biorxiv)



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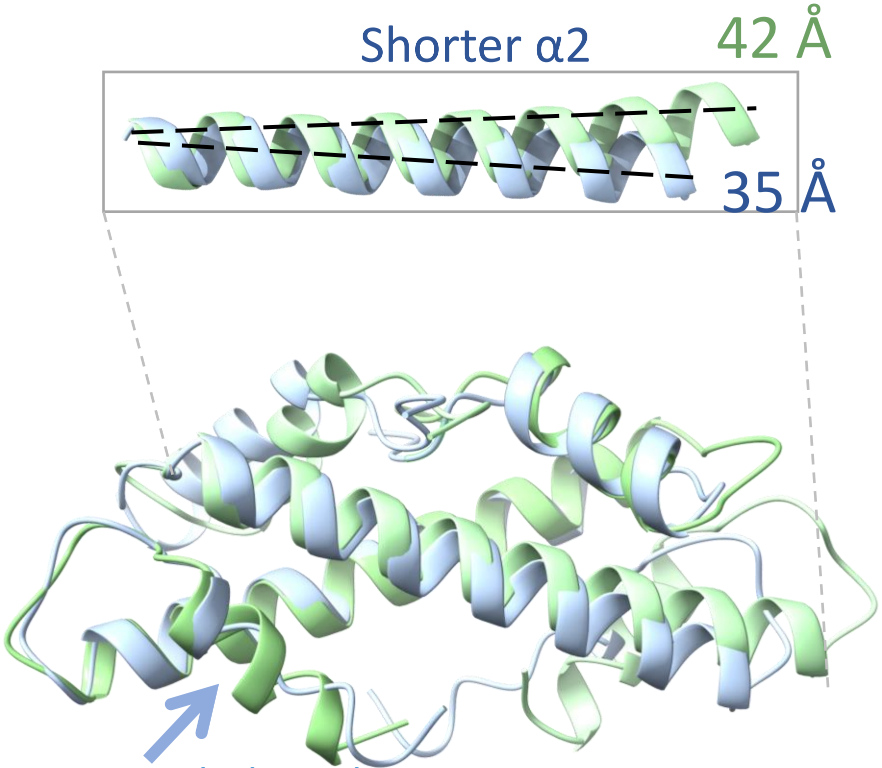
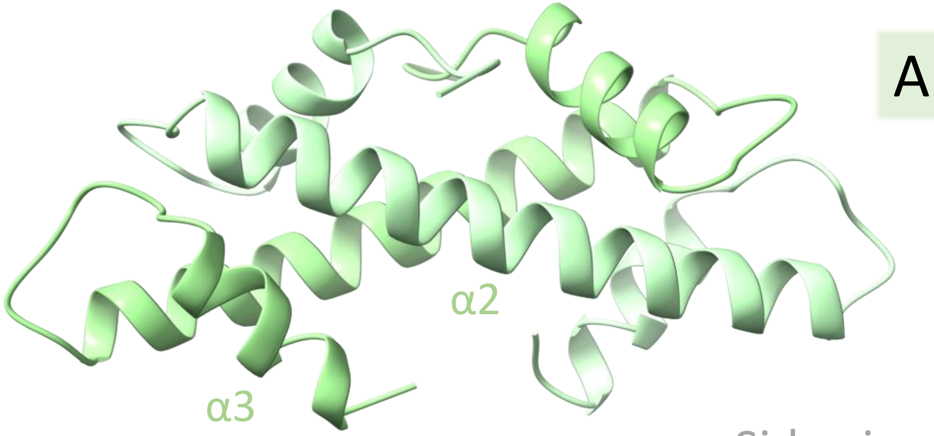


α3 helix only partially helical

Bacterial

Archaeal

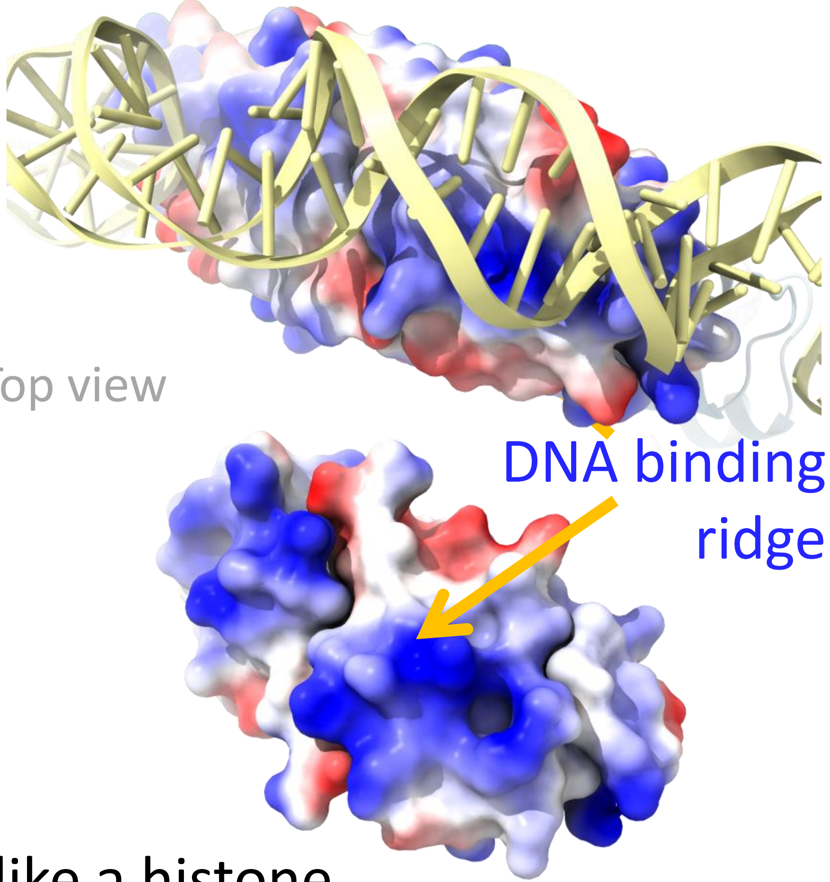
Bacterial histone has the 'DNA binding ridge' found in all histones



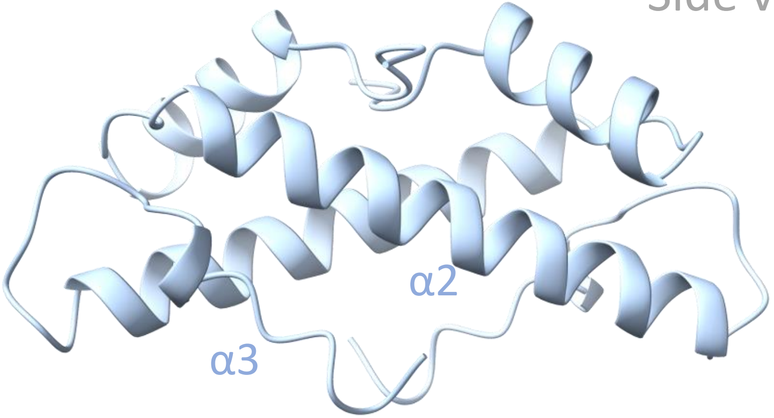
Archaeal

Side view

Top view



DNA binding ridge

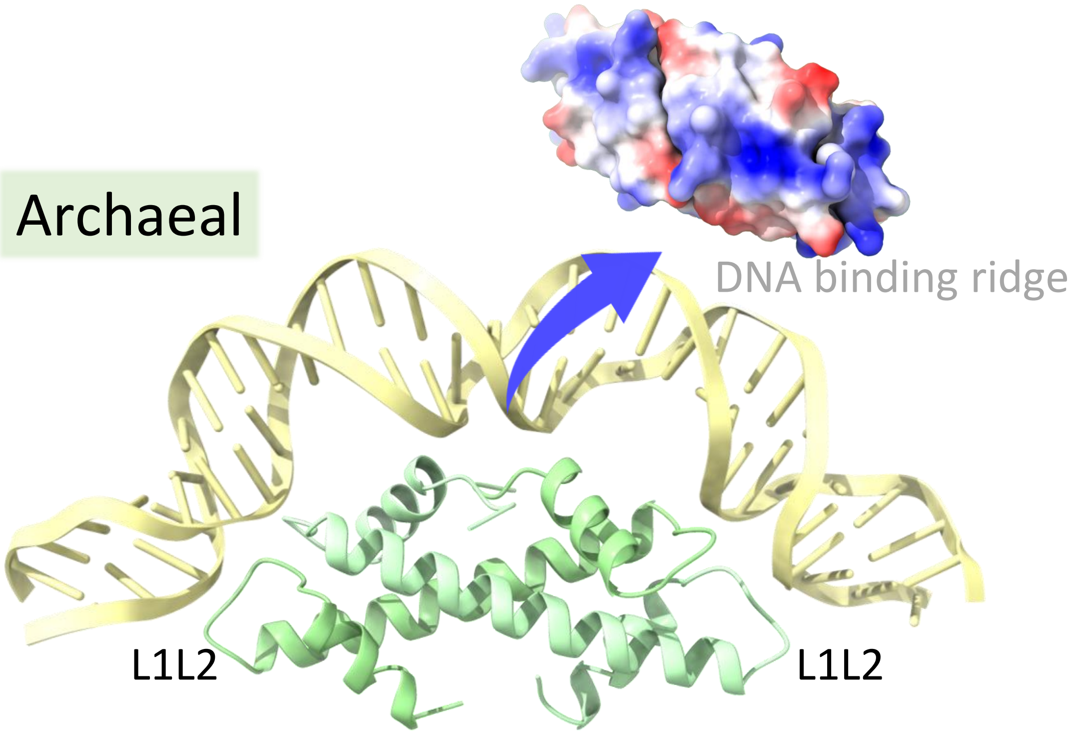


Bacterial

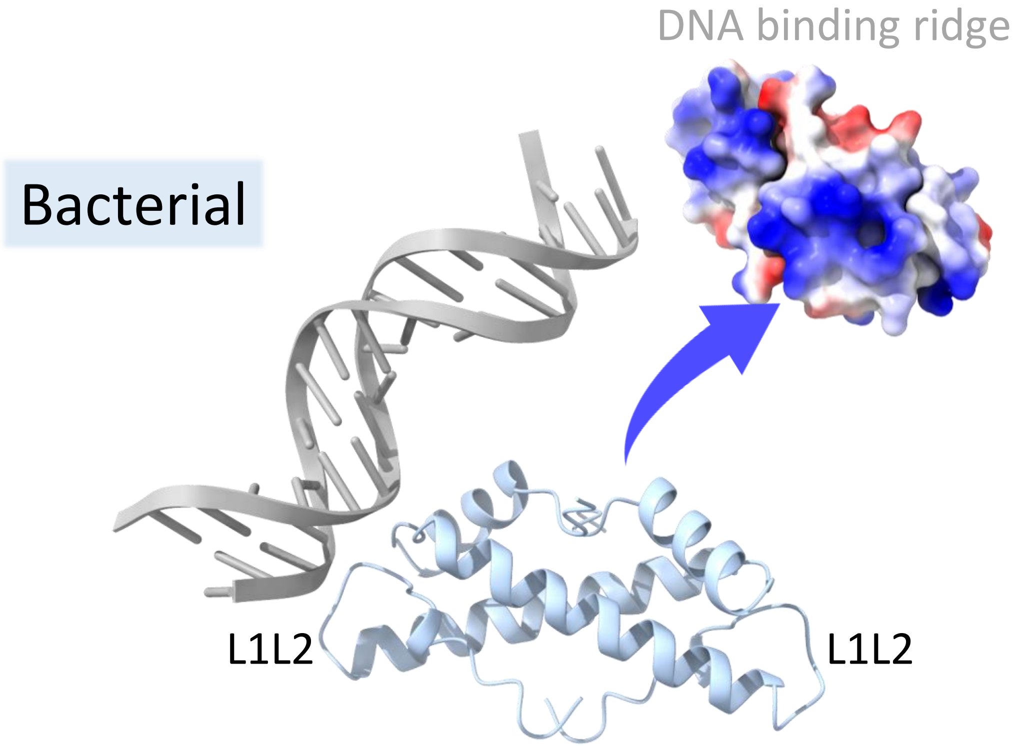
Shorter α2 helix,
α3 helix lost helical character

Looks like a histone,
Quacks like a histone…

Bacterial histone binds DNA end-on

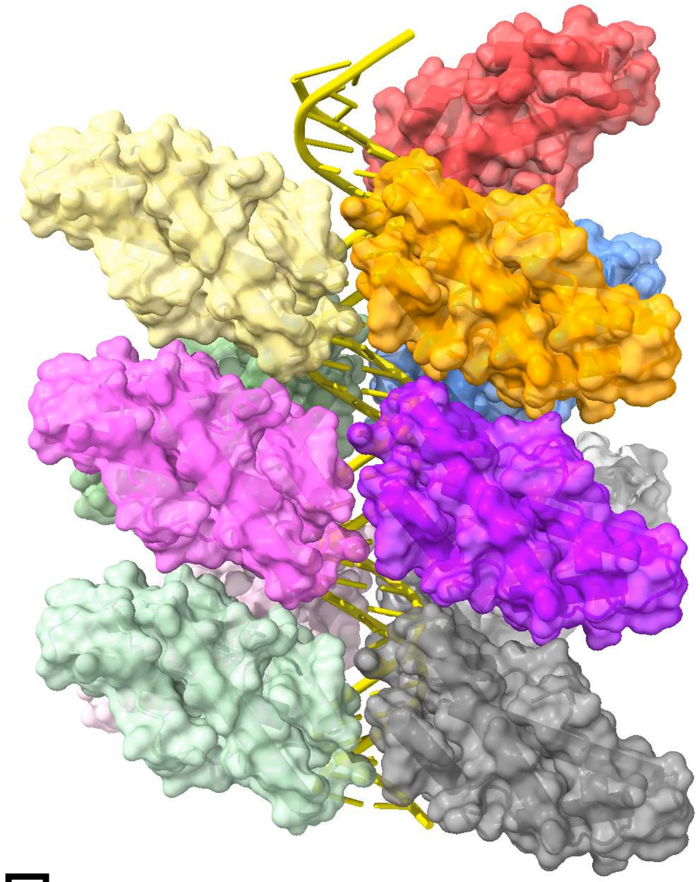


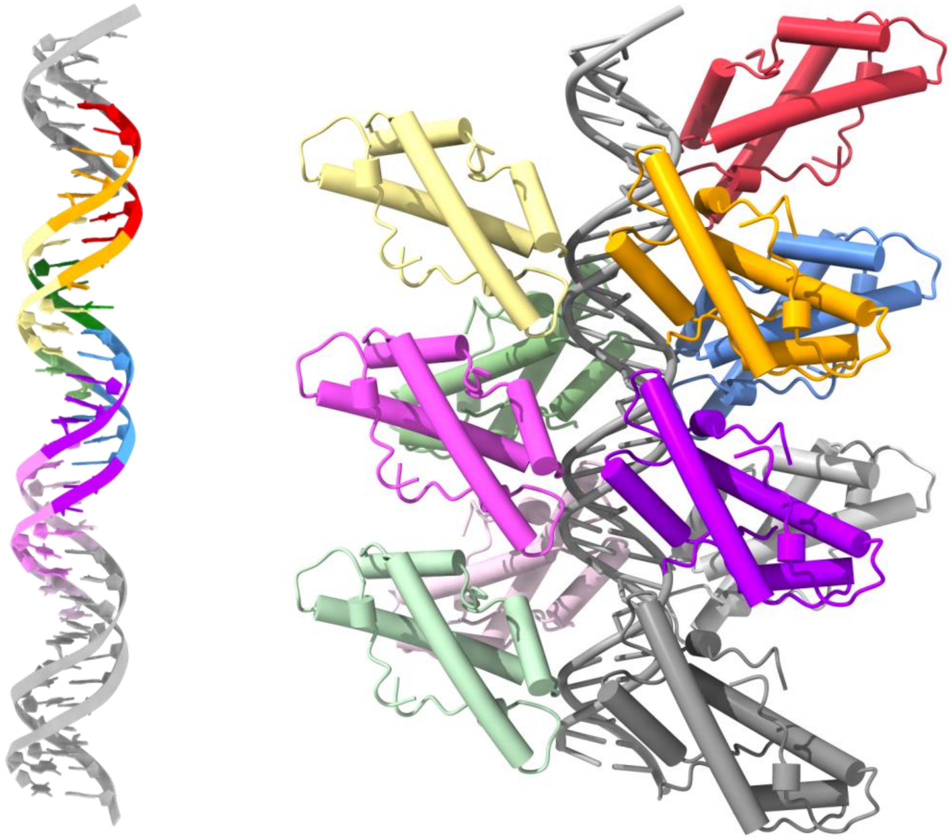
Crystal structure of HMf-DNA complex
Mattiroli, F….& Luger, K, 2017, PMC5747315



2.4 Å crystal structure of
Bd0055-DNA complex

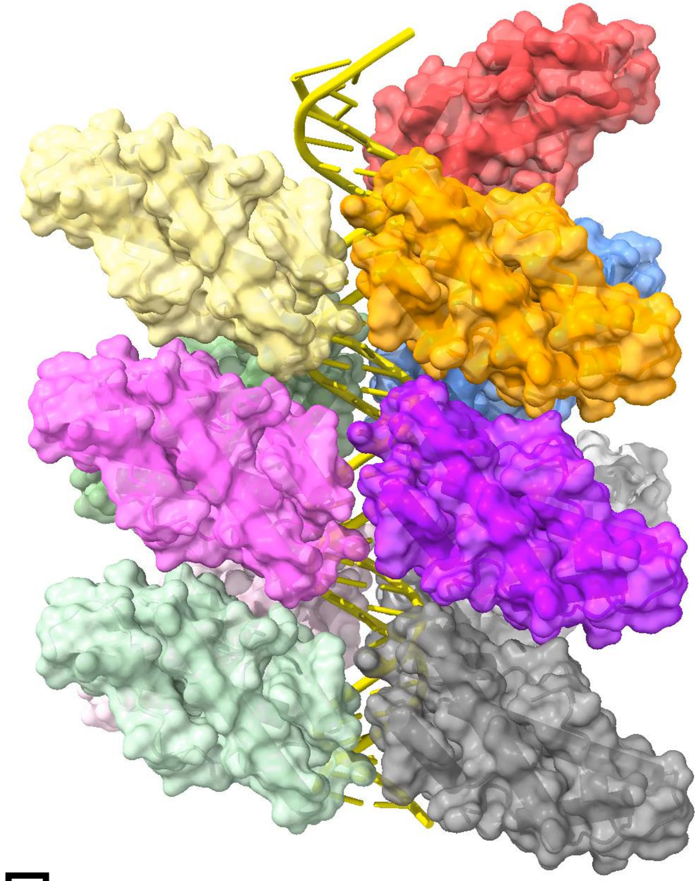
Bacterial histone coats and completely protects DNA

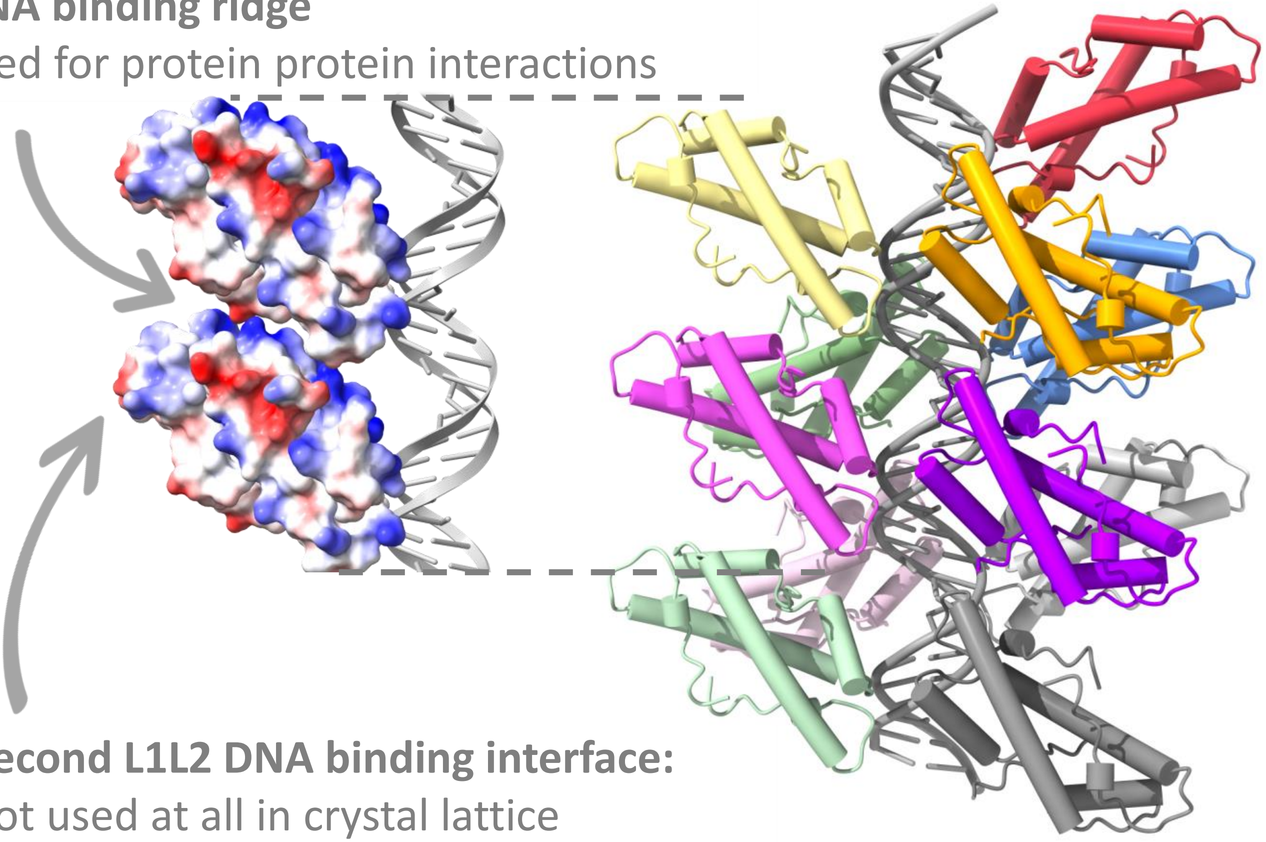




Every DNA phosphate is contacted by histone

Bacterial histone coats and completely protects DNA

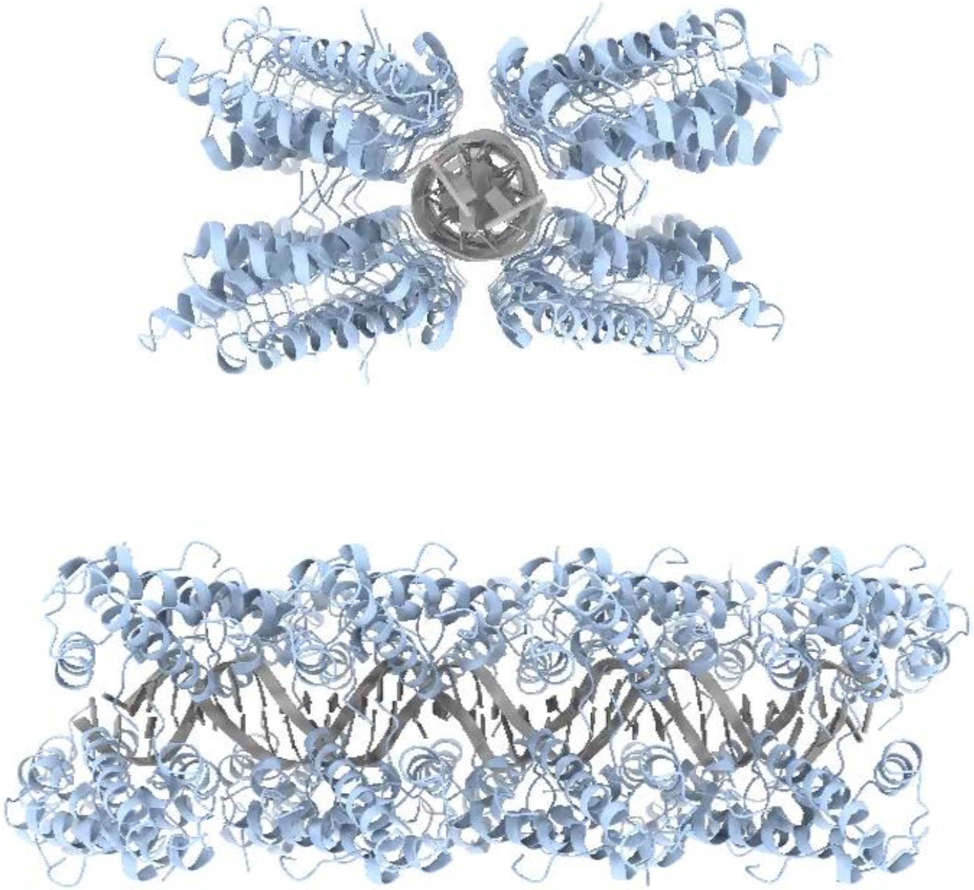


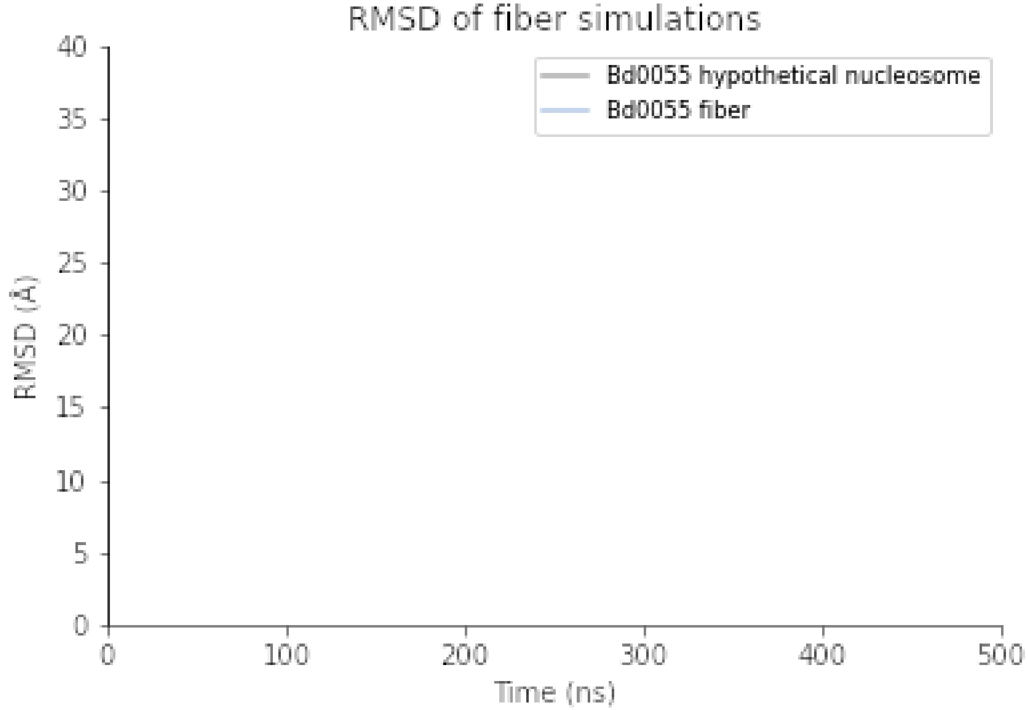


DNA binding ridge
used for protein protein interactions

Second L1L2 DNA binding interface:
not used at all in crystal lattice

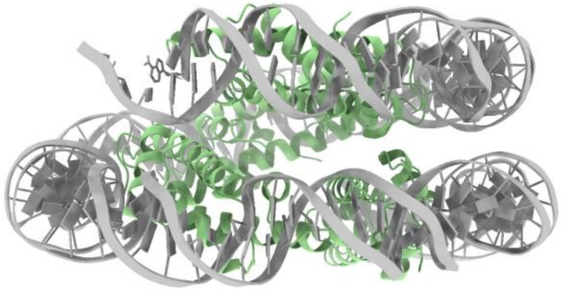
Bacterial histone nucleofilament remains stable *in silico*



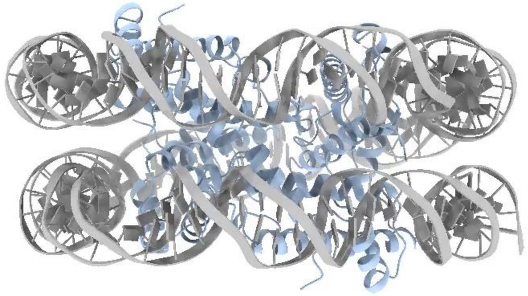


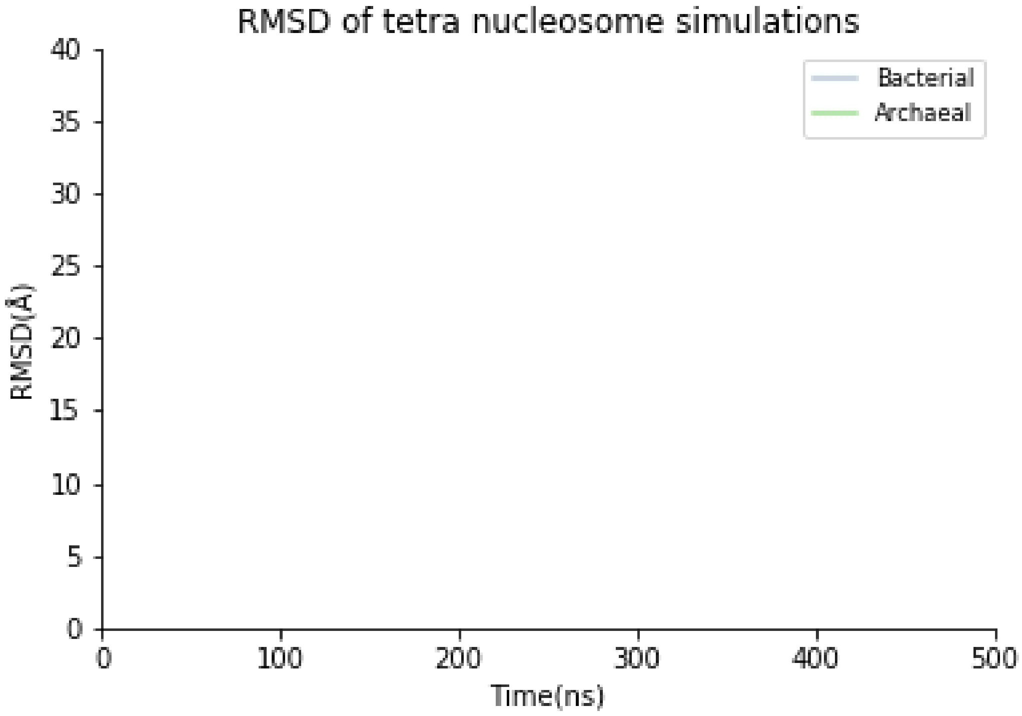
Bacterial histone ‘slinkies’ are unstable in MD simulations

Archaeal

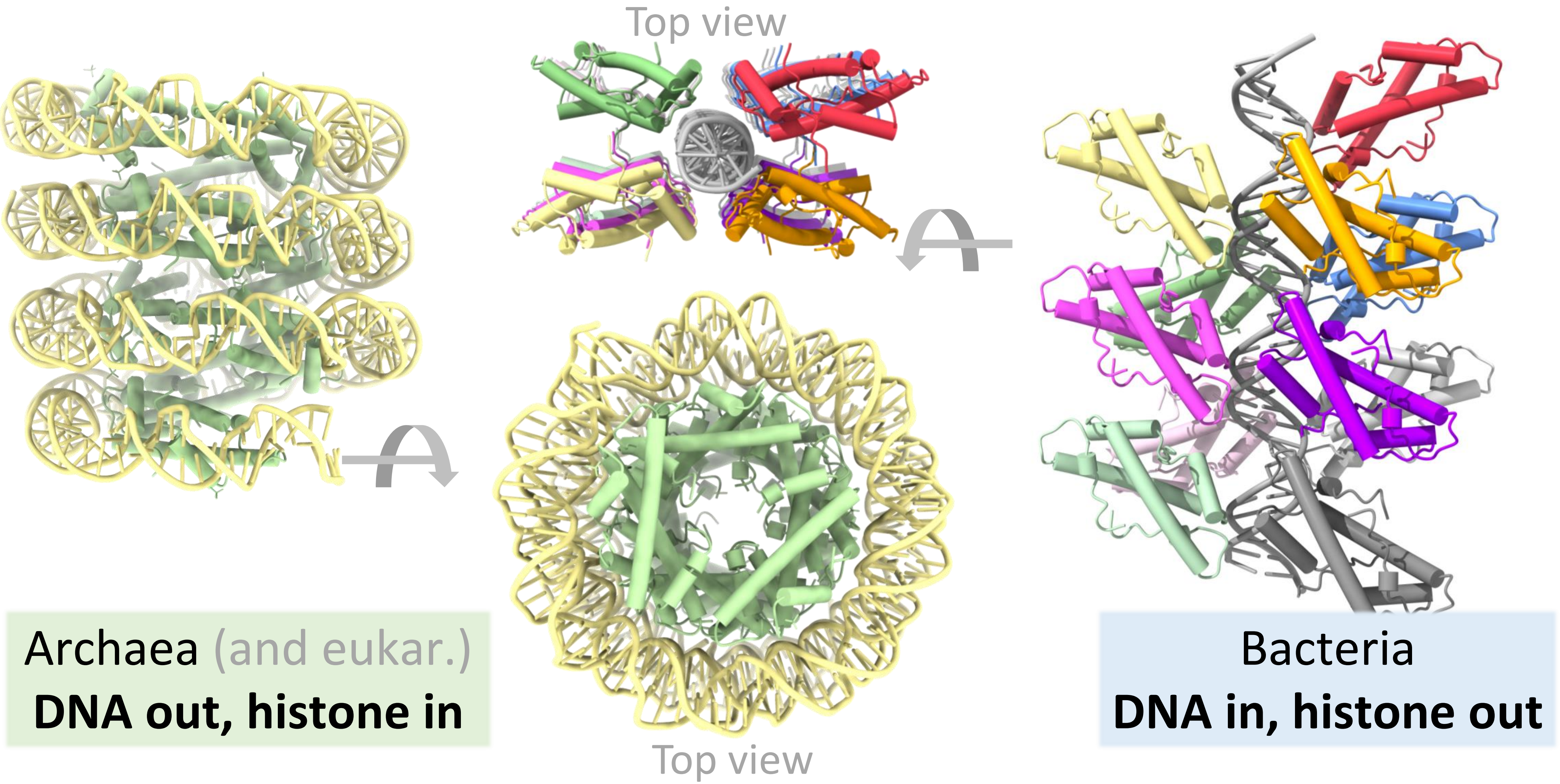


Bacterial





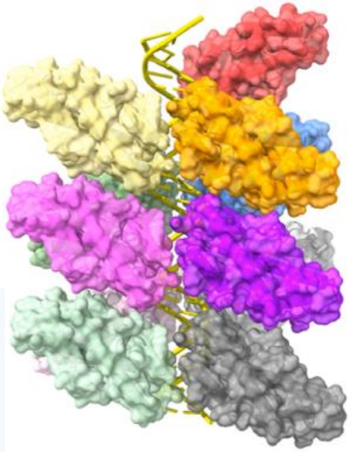
Bacterial 'nucleohistone filament' reverses histone logic



Archaea (and eukar.)
DNA out, histone in

Bacteria
DNA in, histone out

Does this structure exist in the cell?

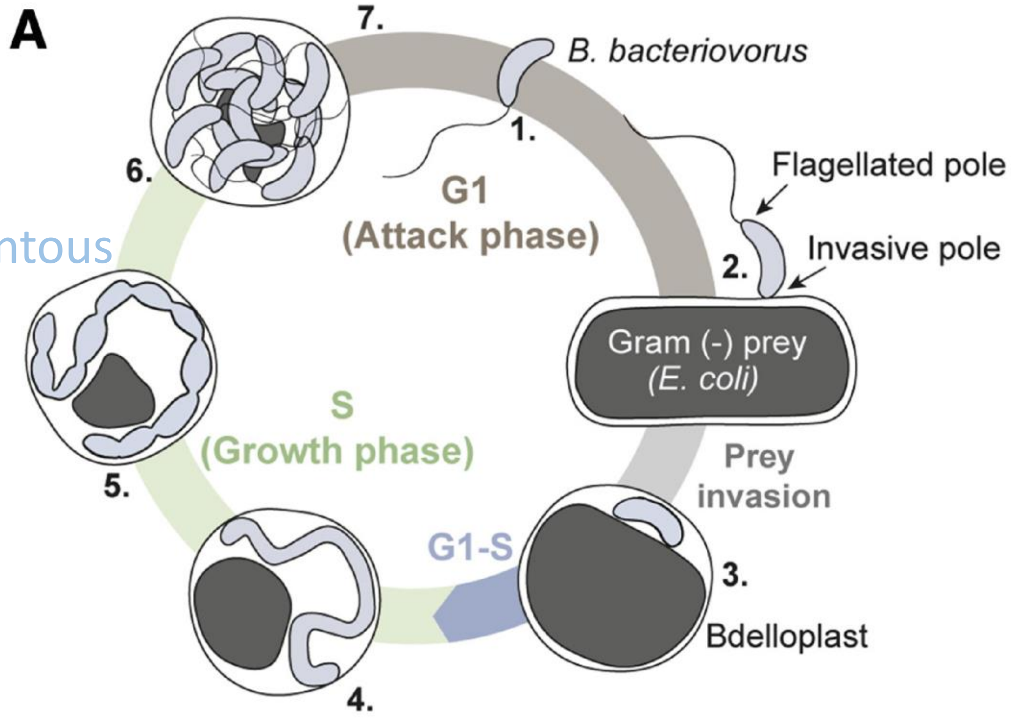


Nucleoid is inaccessible in attack phase

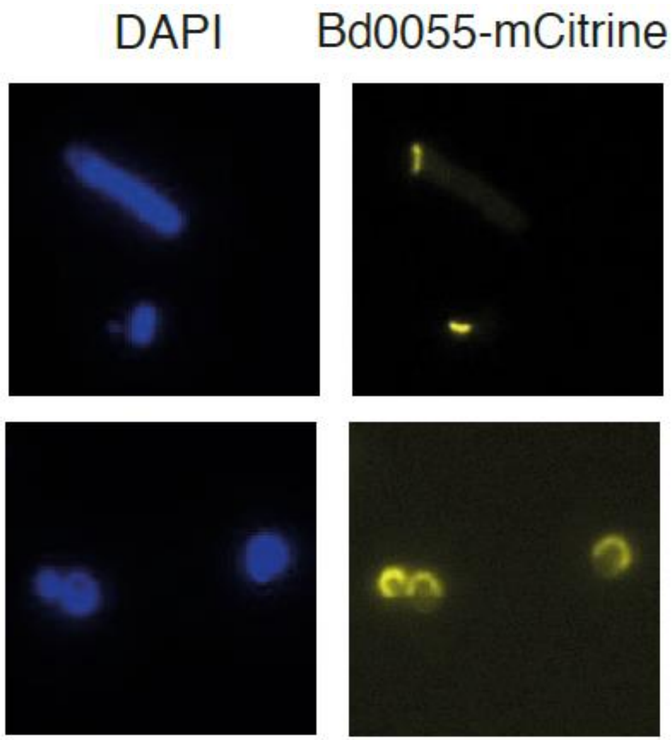
Kaljevic et al., 2021, PMID 34256020

With Sockett Lab:

- **Essential,** abundant
- Nucleoid-associated



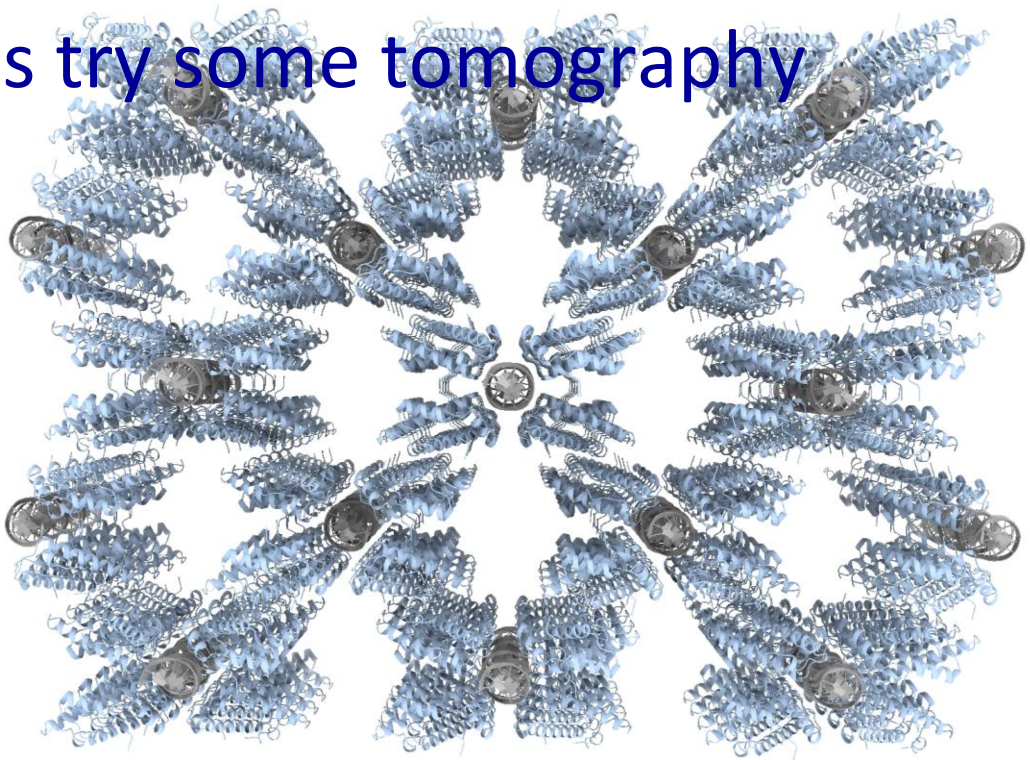
Filamentous growth

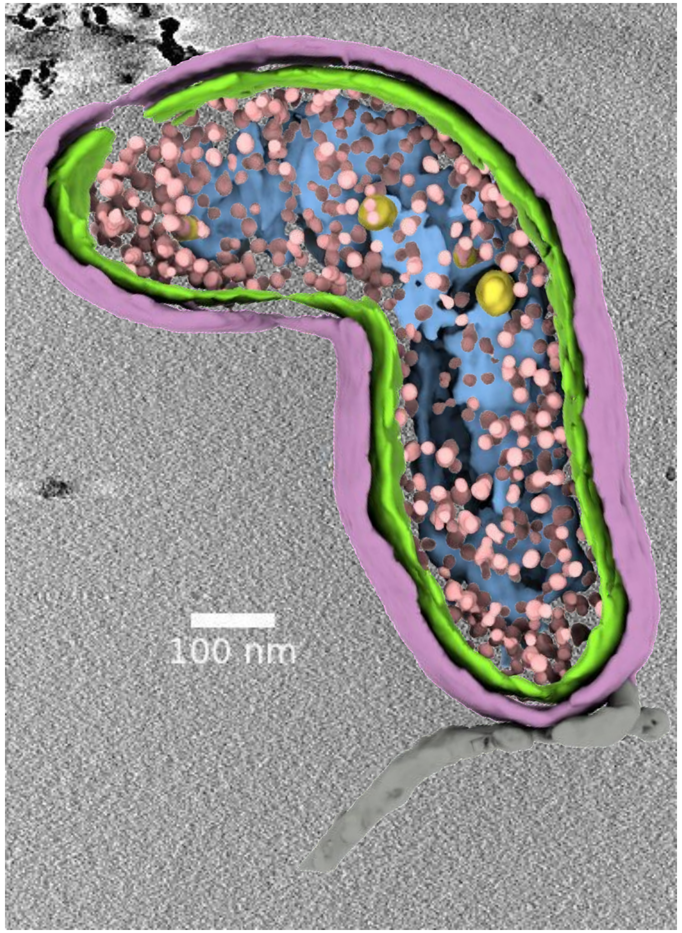


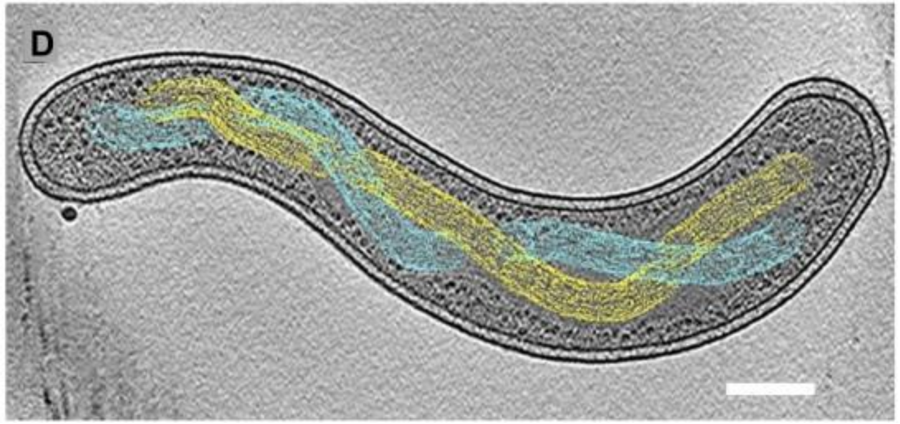
6 h PI: attack phase

16 h PI Filamentous growth

Does this fiber exist in the cell? Lets try some tomography

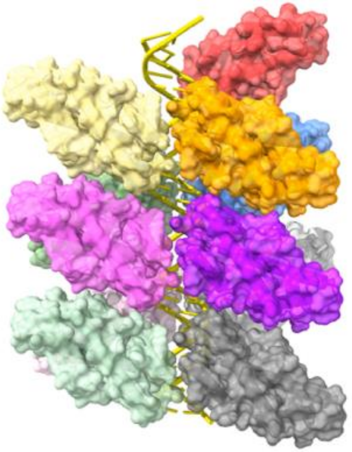






Butan et al., (2011) Spiral architecture of the nucleoid in *Bdellovibrio bacteriovorus* *J Bacteriol*, **193**, 1341-1350.

Why require a nucleohistone filament?



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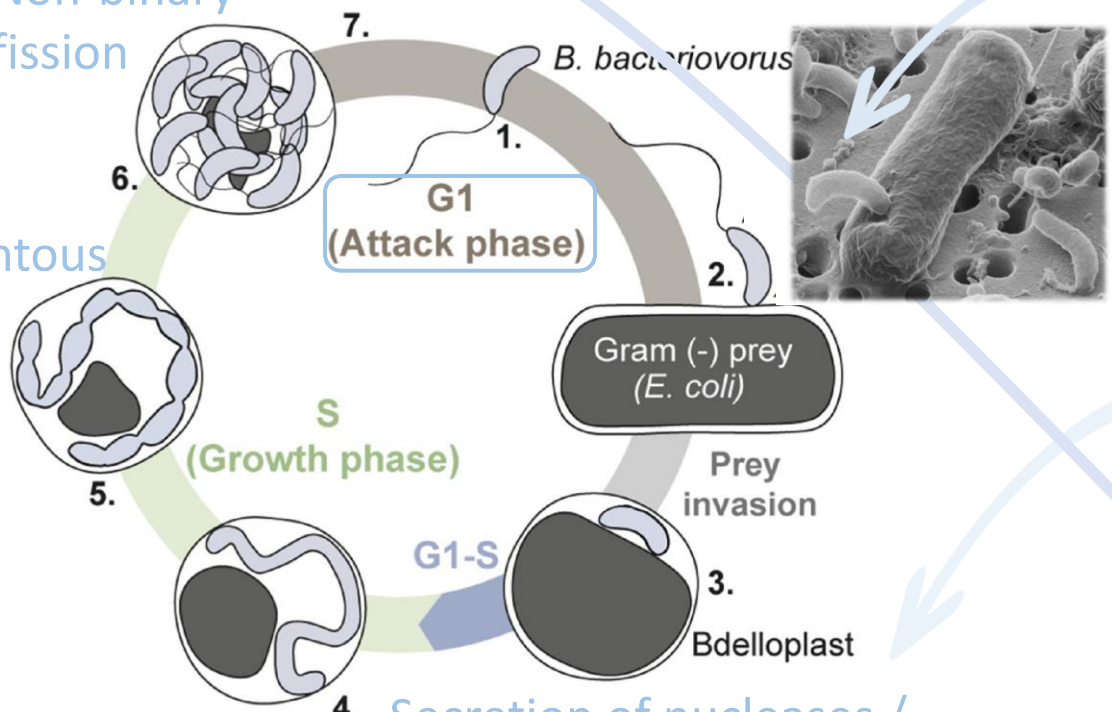


Non-binary fission

Filamentous growth

Secretion of nucleases / proteases into prey

- 20 x smaller vol. than *E.coli* prey
- Same genome size as *E.coli* prey

→ **Compaction required?**

- Growth phase in prey periplasm
  (protection against own nucleases?)

- Non-binary division
  (lining up the amplified nucleoids?)

Nucleohistone filament: curiosity or primordial use of histones?

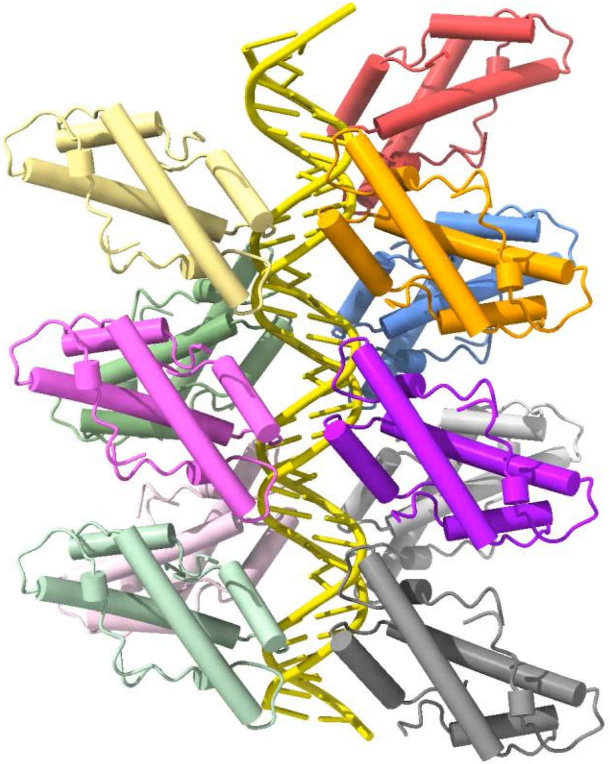
Ongoing / future work

- *In situ* nucleoid structure by cryo-tomography
- *In situ* chromatin accessibility (ATAC; ChIP; mutants…)
- Interacting proteins / regulation of accessibility?
- **Unrelated histone-encoding bacteria:** how general?

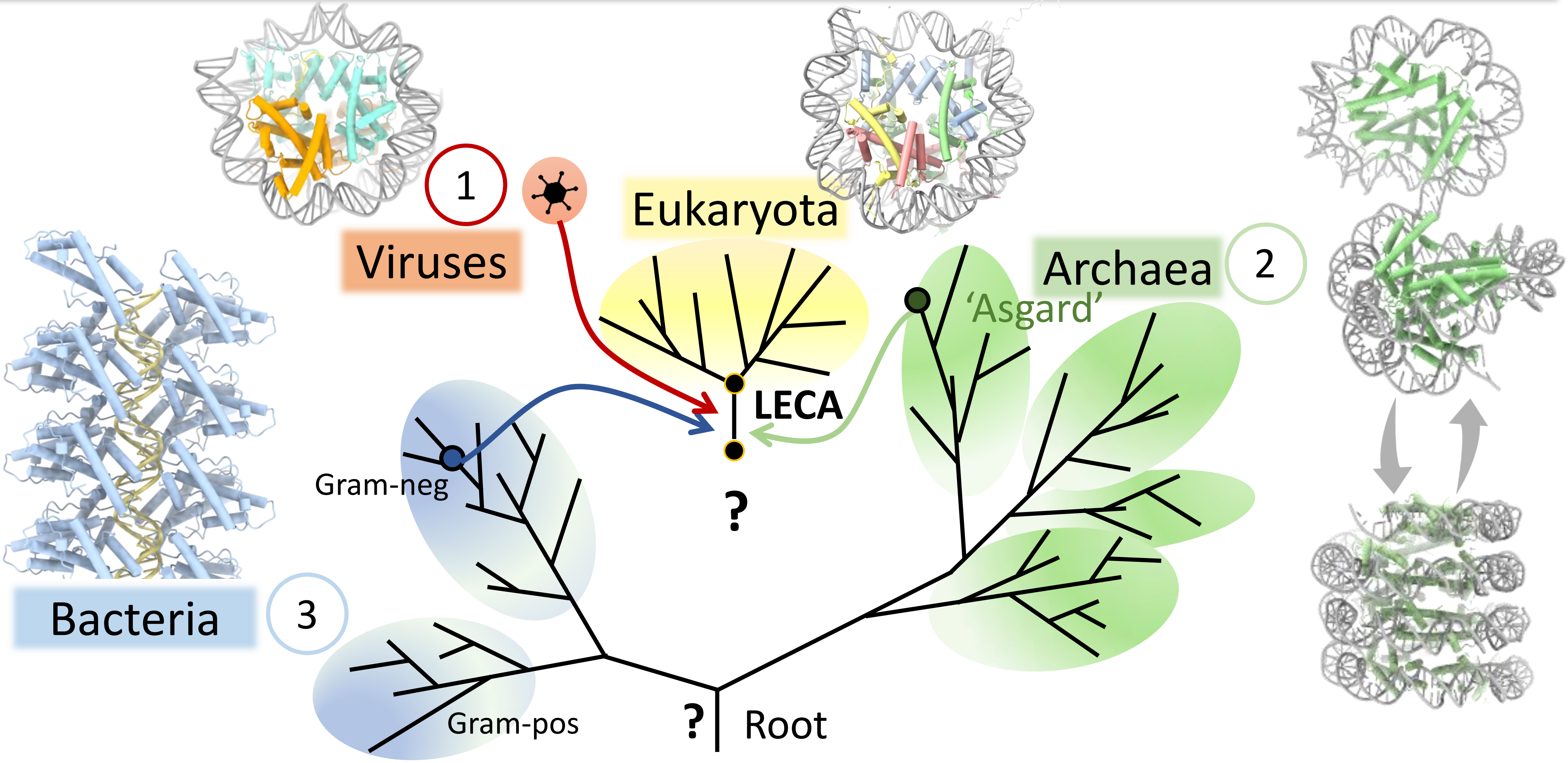
Why are we doing this?

- *B. bacteriovorus* explored as last resort antibiotic
- Paradigm shift in our perception of histones as DNA organizing agents

Unexpected insights often come out of 'left field'



Histones in all domains of life



Acknowledgements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Yang Liu
Keda Zhou
Fra Mattiroli

hhmi

CCET CU BOULDER CENTER FOR CRYO ELECTRON TOMOGRAPHY

BioChemistry Krios Electron Microscopy Facility


Shawn Laursen
@lugerlab


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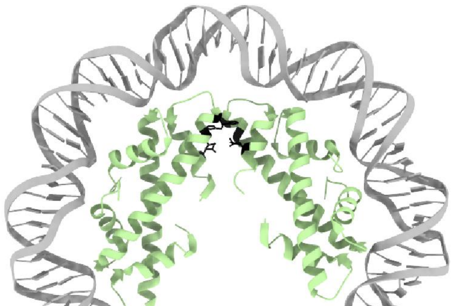
Hubrecht Inst.

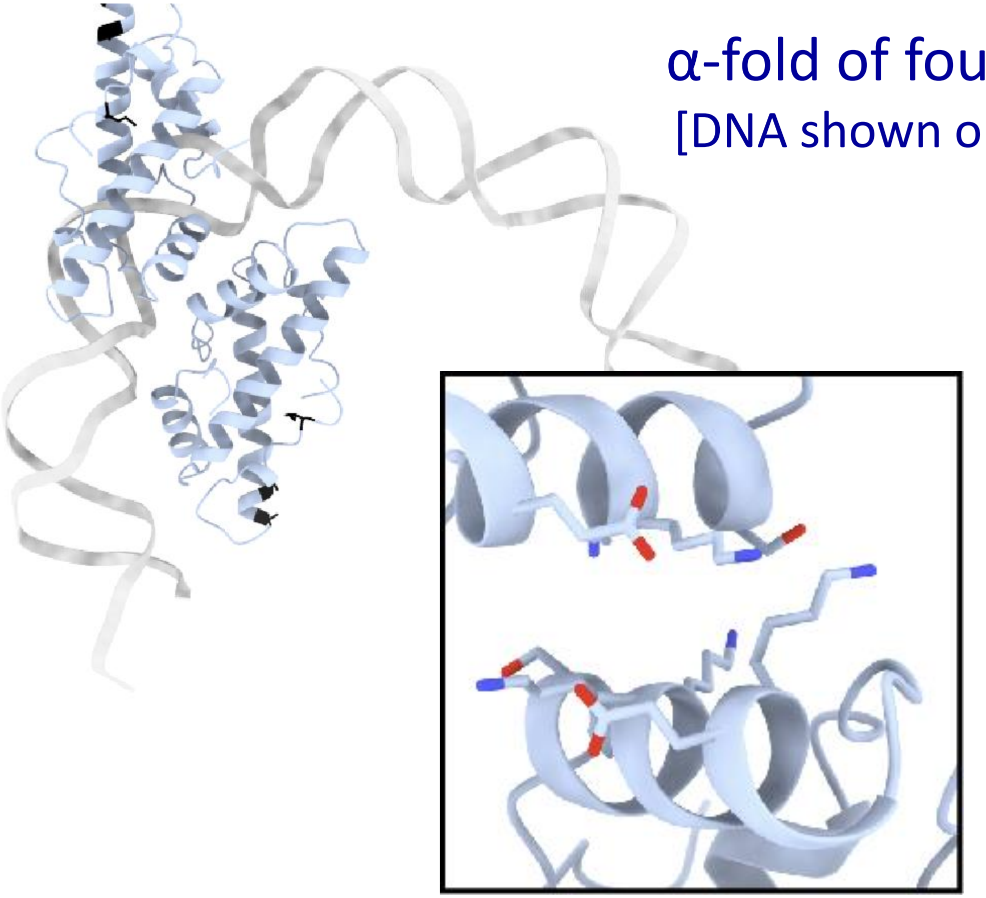

Keda Zhou
Uni. Hongkong


Yang Liu
Uni. Hongkong

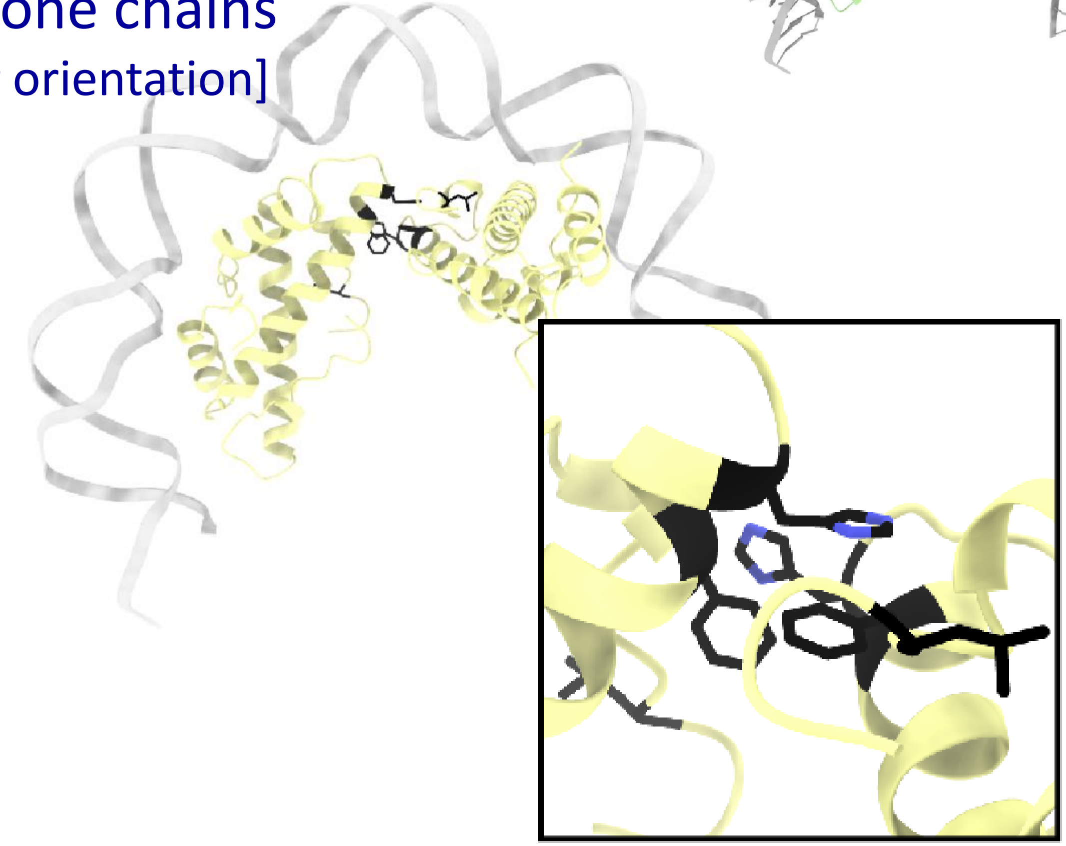
Restoring the four-helix bundle in bacterial histone



α-fold of four histone chains
[DNA shown only for orientation]

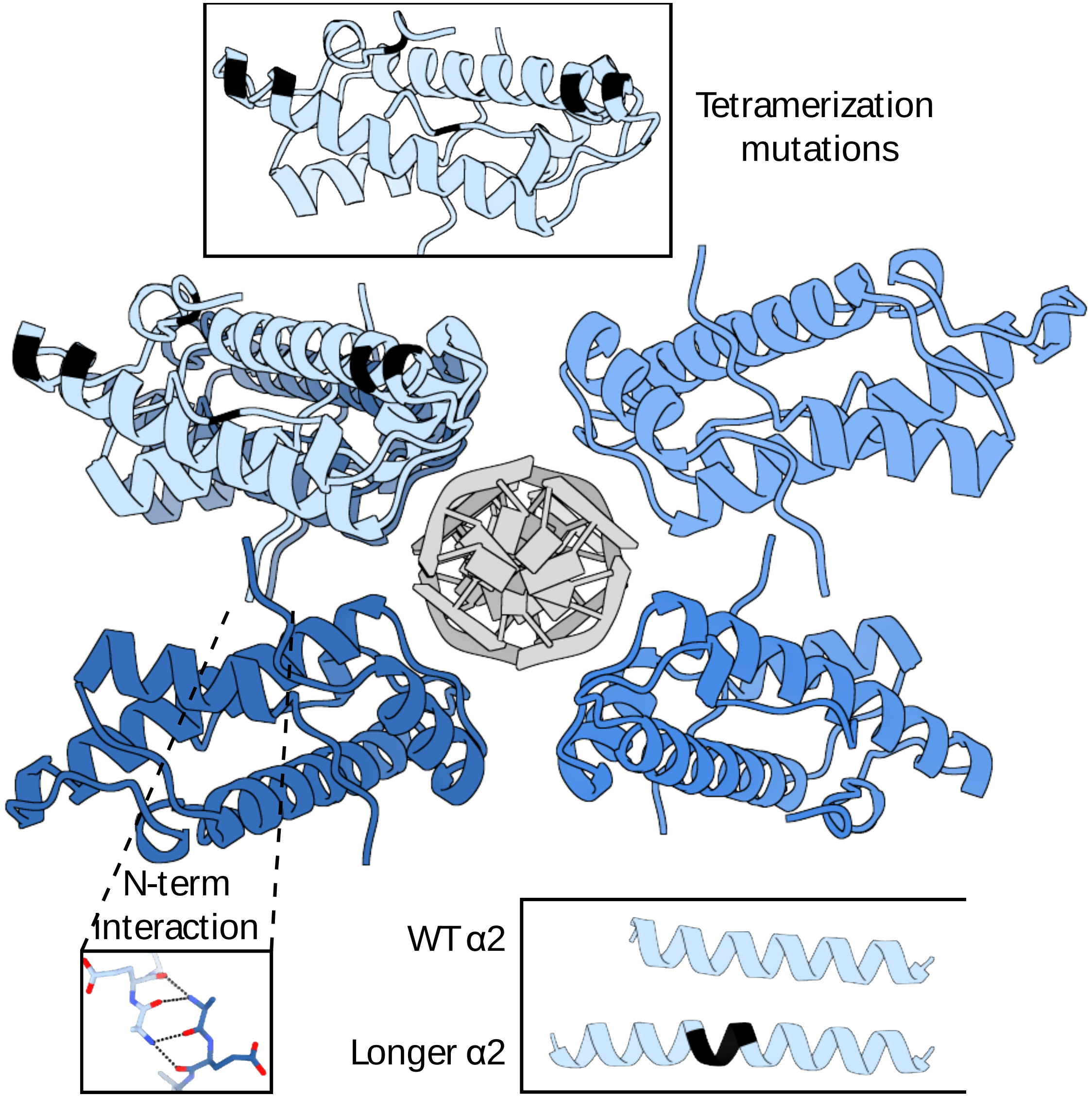


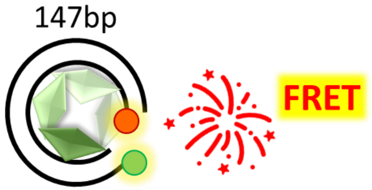
Wild type bacterial histone does not engage in four-helix bundle

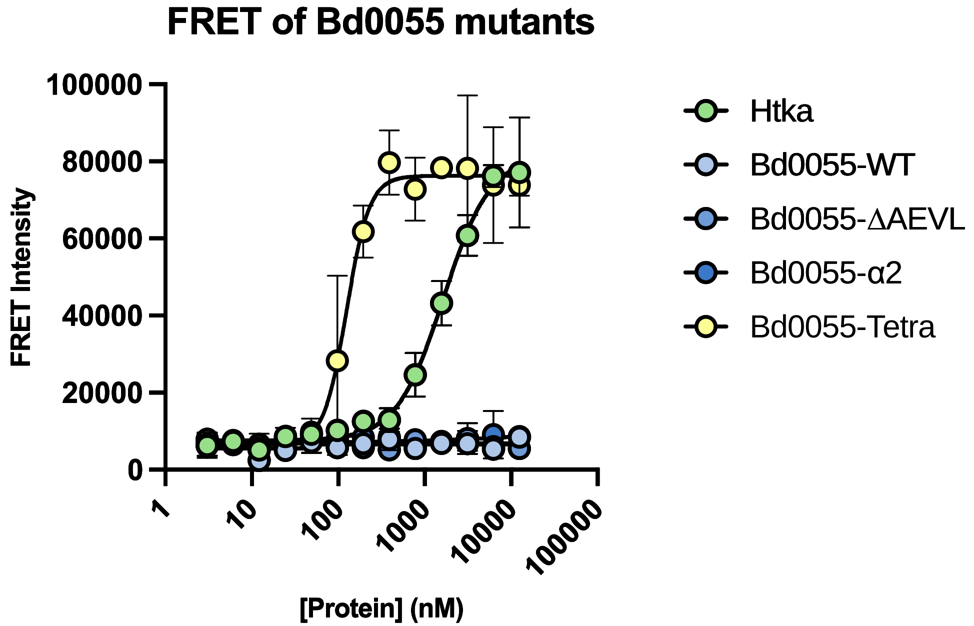


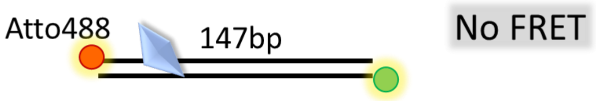
Mutating three residues allows tetramerization *in silico*

Reason for this unconventional binding mode









Shawn Laursen, with help from Nikhil Gupta

Nucleohistone filament: curiosity or primordial use of histones?

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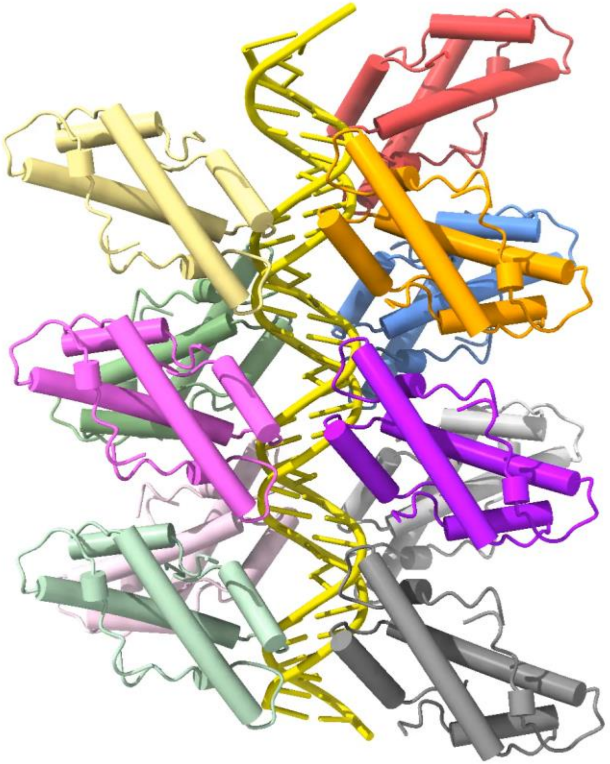
- Eukaryogenesis?



‘Nuc-evo’ group

Luger Lab (CU Boulder)

- **Francesca Mattiroli**
- **Sudipta Bhattacharayya**
- **Samuel Bowerman**
- Garrett Edwards
- Pamela Dyer
- Shawn Laursen
- Alison White
- **Yang Liu**
- **Chelsea Toner**
- **Keda Zhou**

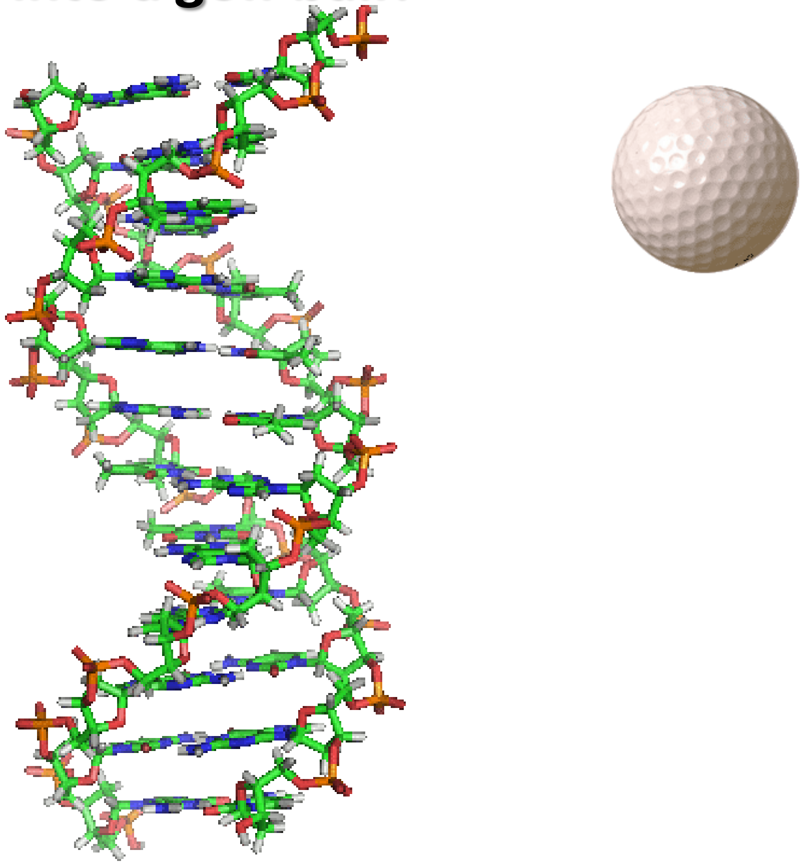
Santangelo lab (CSU)
Ahn Lab (CU Boulder)
John Reeve (OSU)
Abergel lab (Aix-Marseille Uni)
H. Bisio, S. Jeudy, N. Philippe


Chantal Abergel


Francesca Mattiroli
Hubrecht Institute

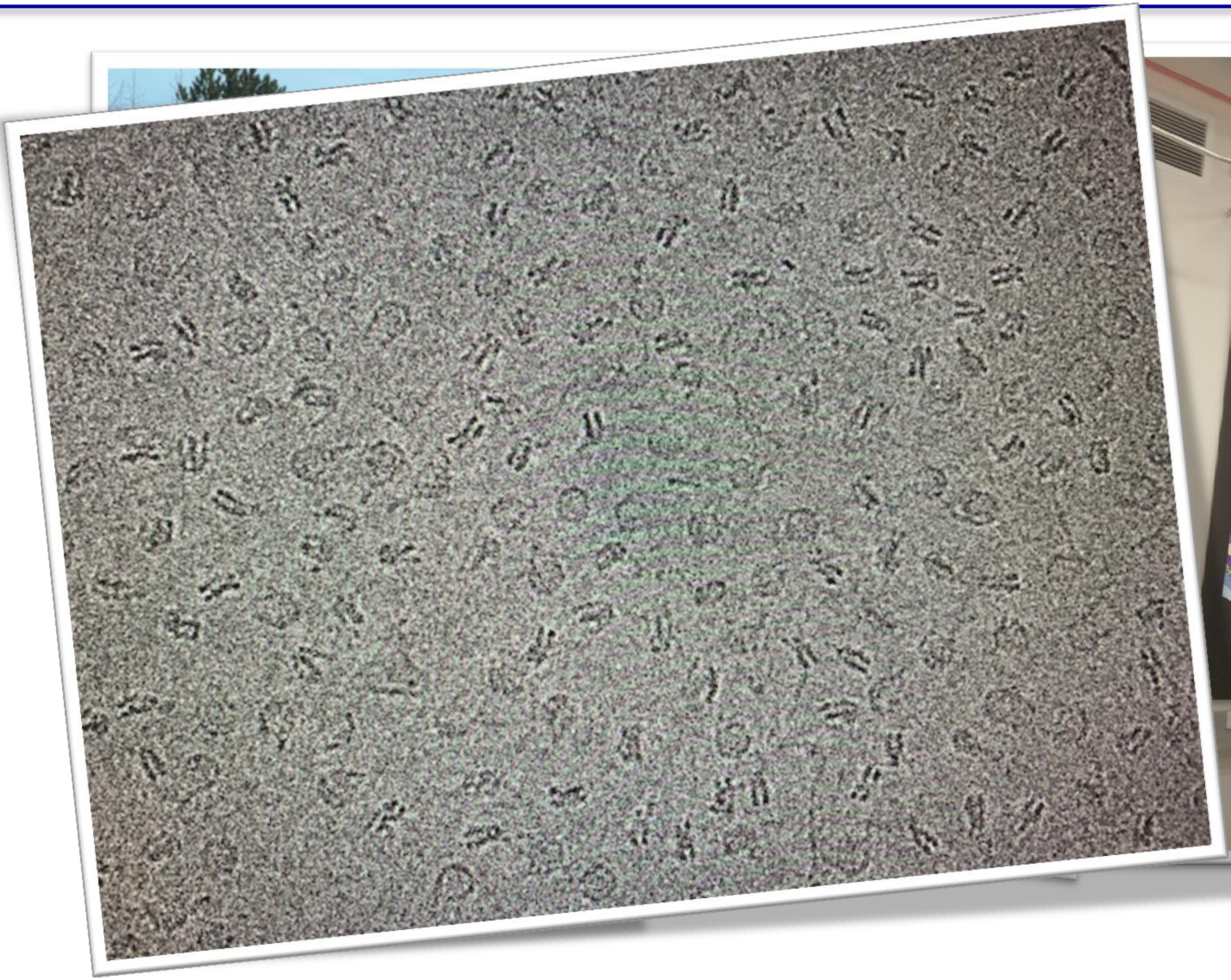
Questions you never knew you had…

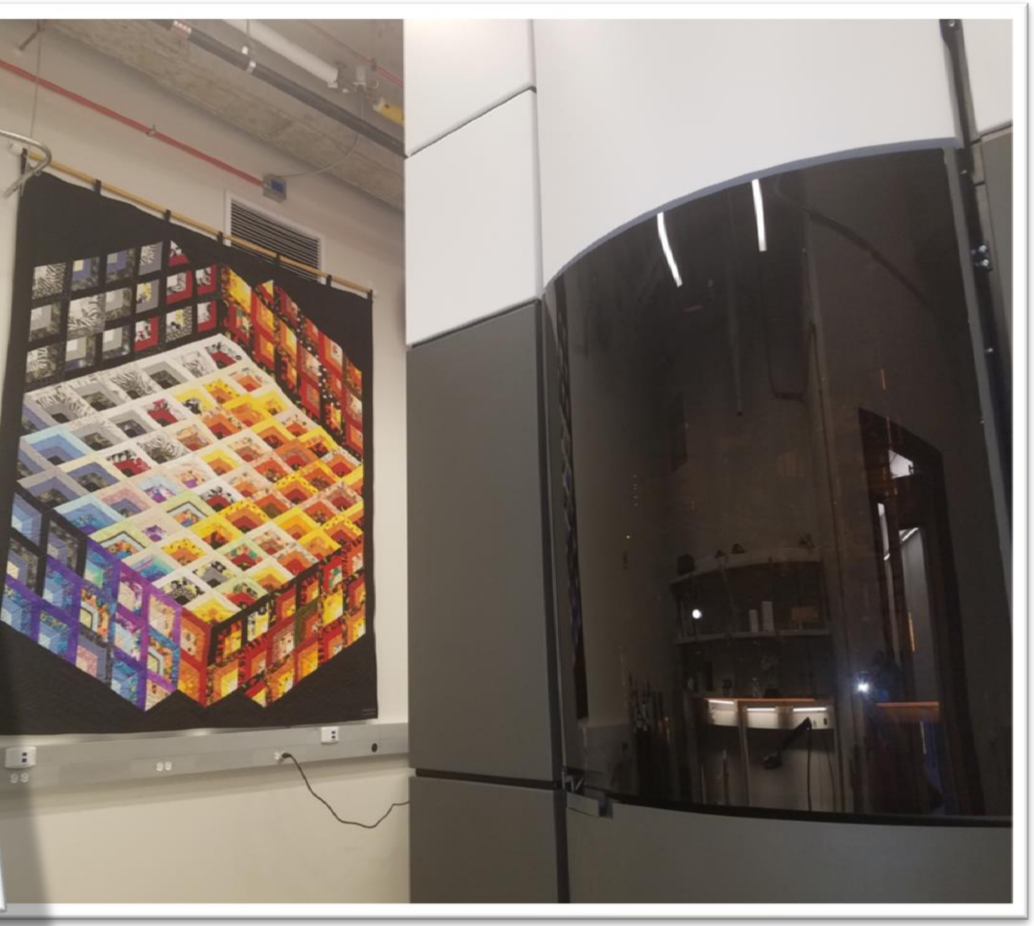
Could you pack 10 miles of DNA into a golf ball?



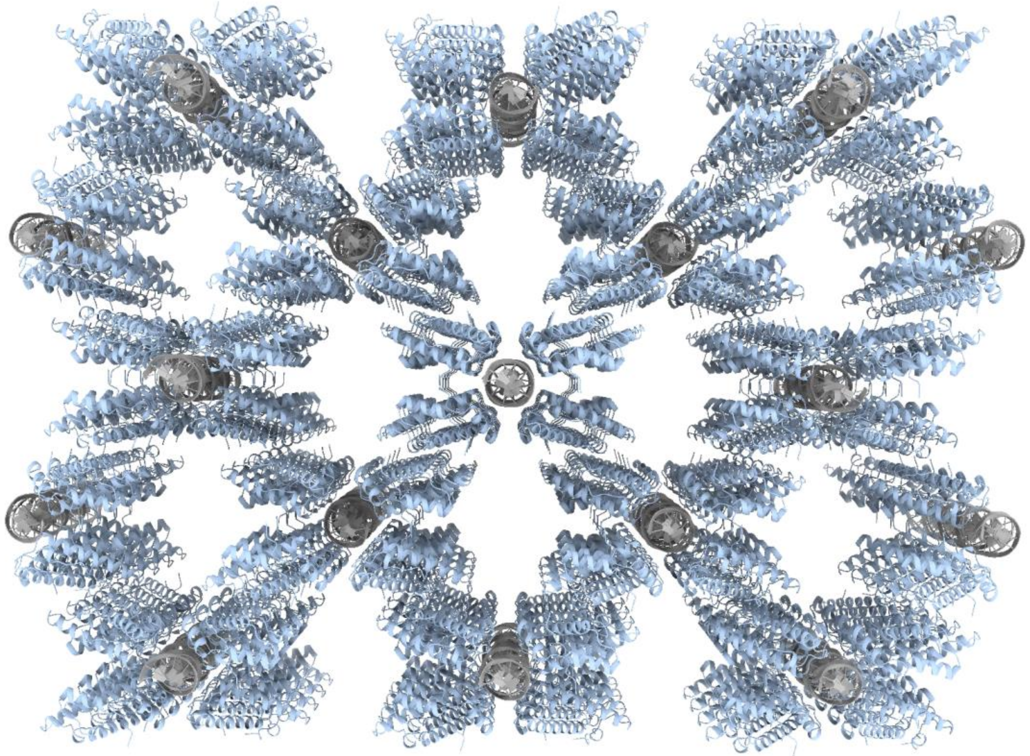
How would you find your favorite song on a cassette tape?

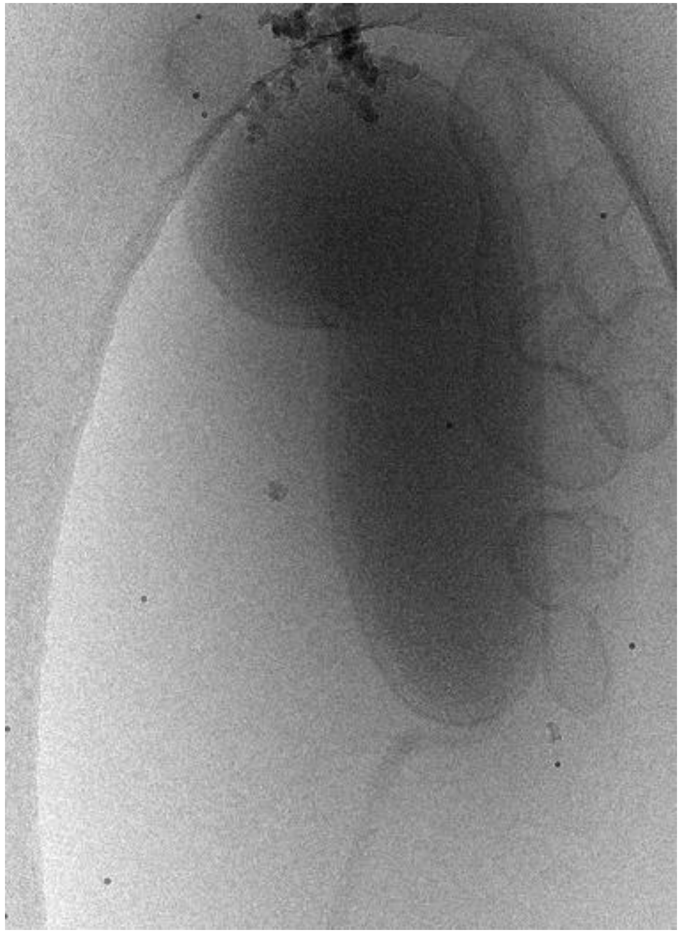
Titan Krios G3i [#PrincessKrios] at CU Boulder

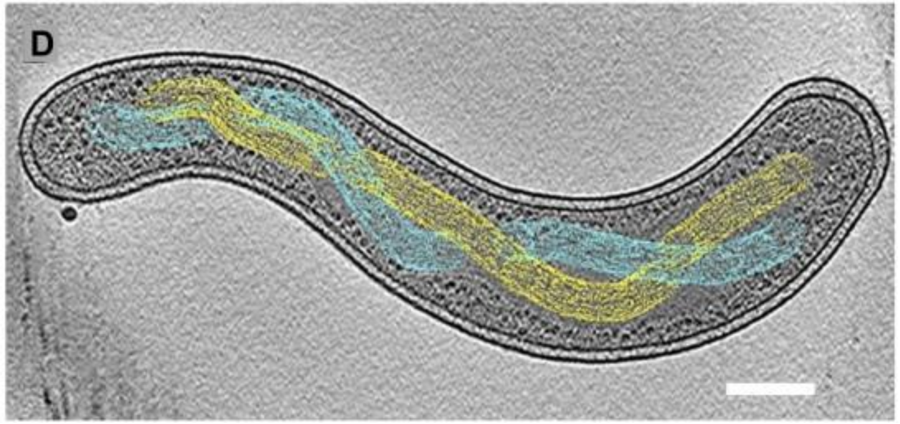




Does this fiber exist in the cell?

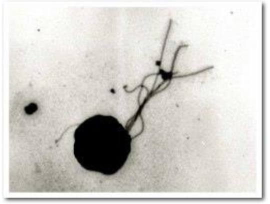






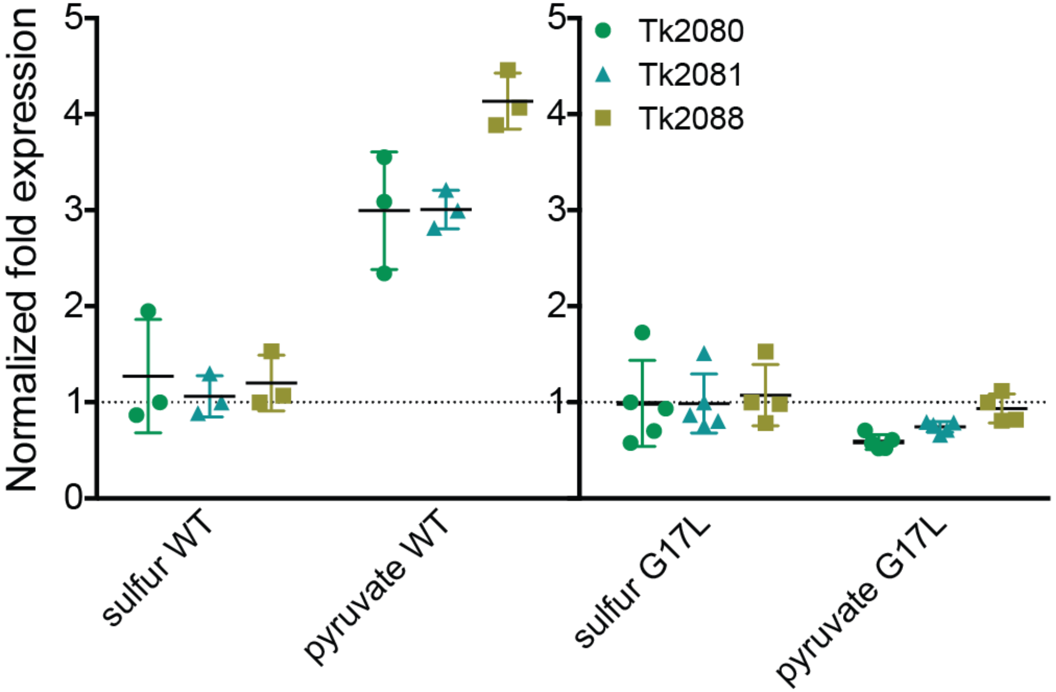
Butan et al., (2011) Spiral architecture of the nucleoid in *Bdellovibrio bacteriovorus* *J Bacteriol*, **193**, 1341-1350.

Mutating a close contact in the 'slinky' leads to defects in transcription regulation

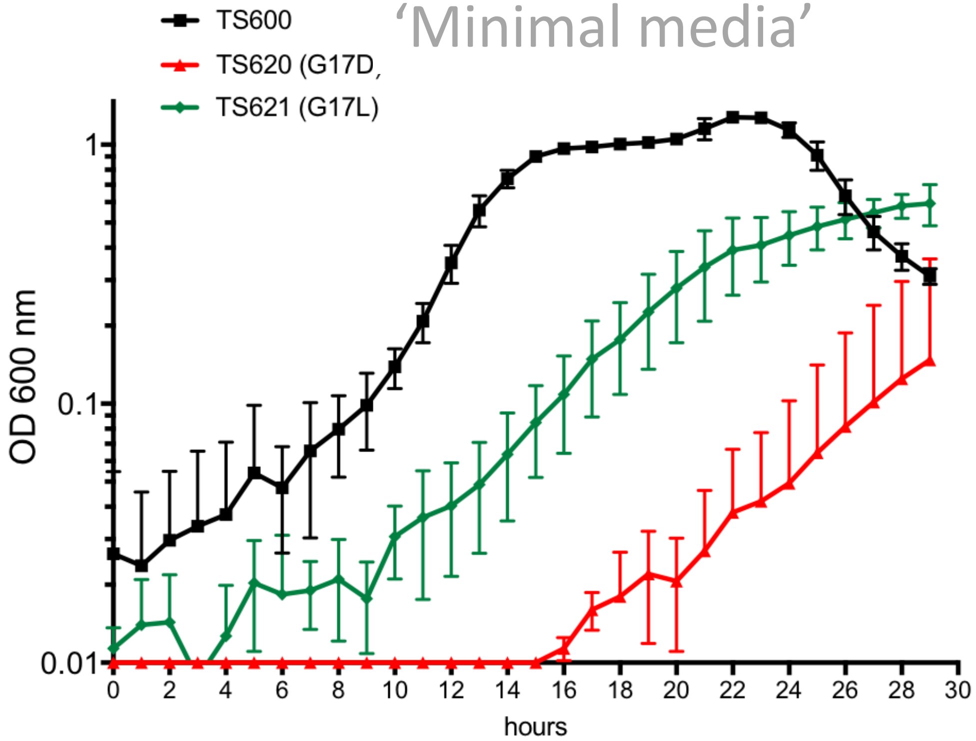


Wild type: upregulation of sulfur transporter genes

Mutant: no upregulation



Quantitative PCR of membrane-bound hydrogenase operon



'Minimal media'

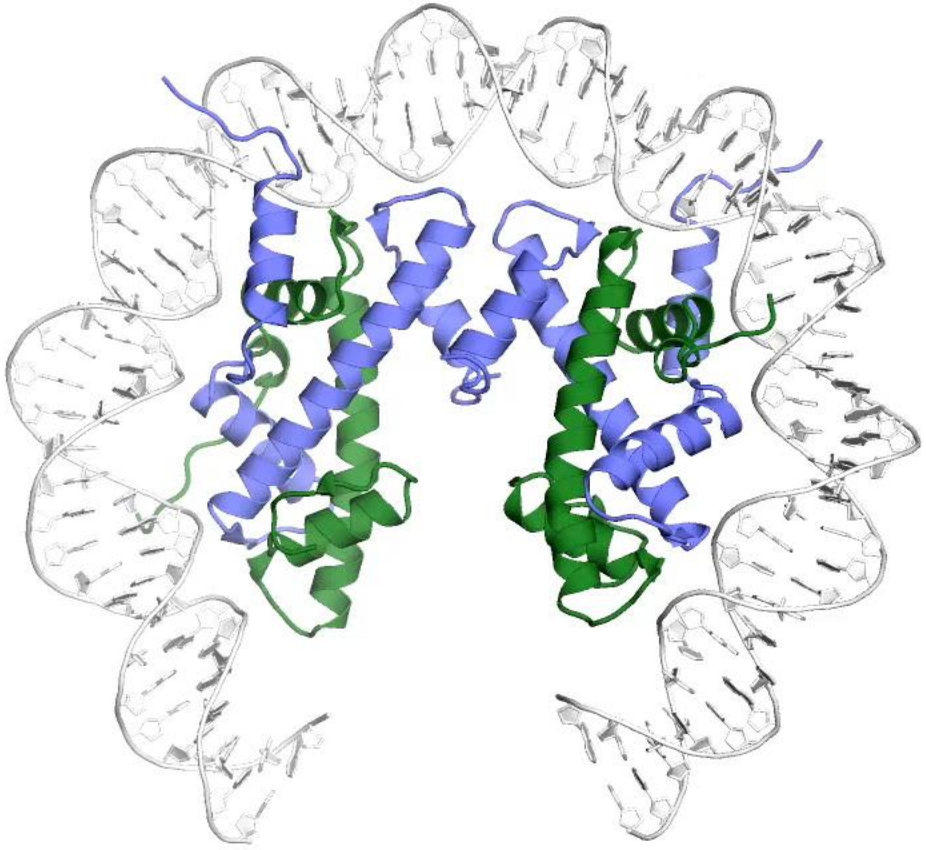
Severe growth defect under limiting growth conditions (no sulfur)

Francesca Mattiroli

Santangelo lab

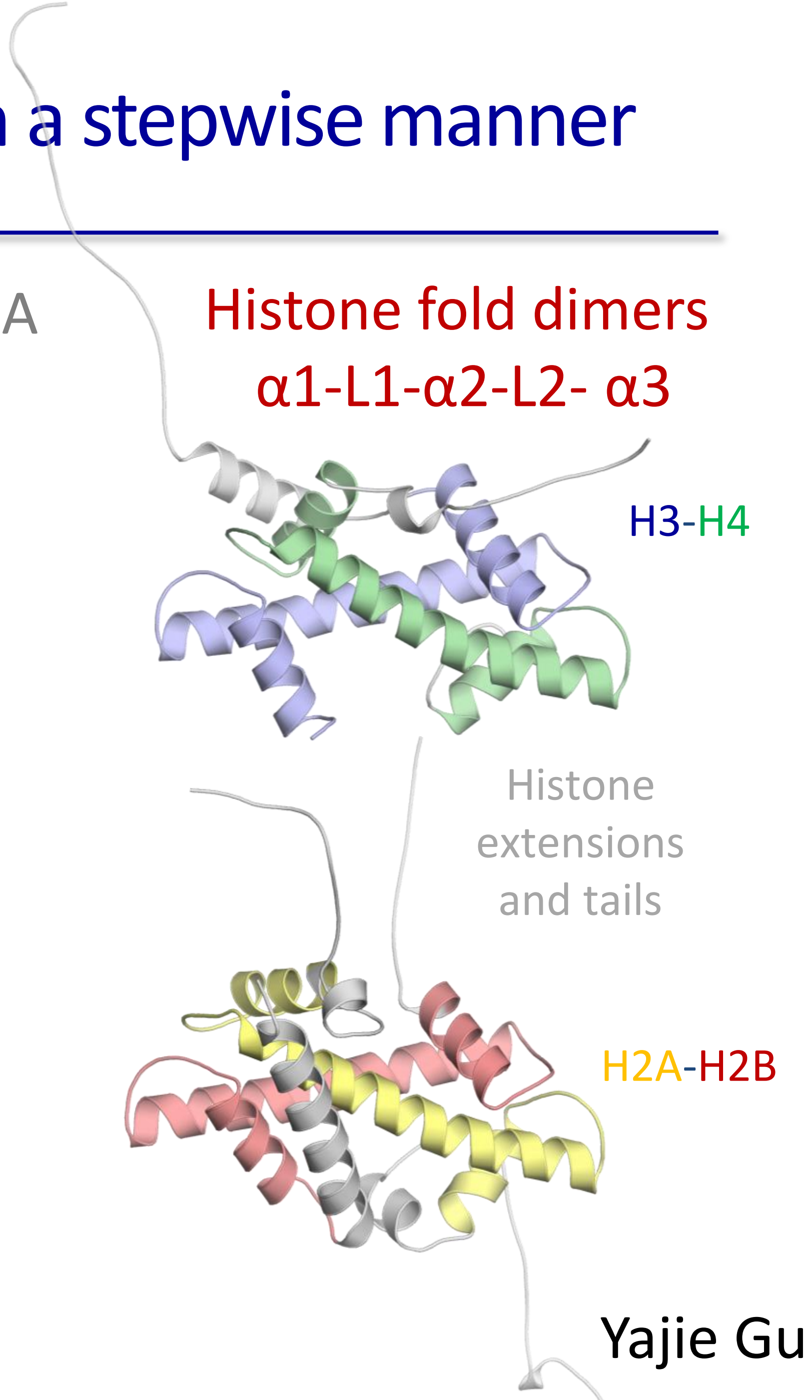
I. Nucleosomes assemble and disassemble in a stepwise manner

(H2A-H2B) • (H3-H4)$_2$ •(H2A-H2B) + 147 bp DNA



Histone fold dimers

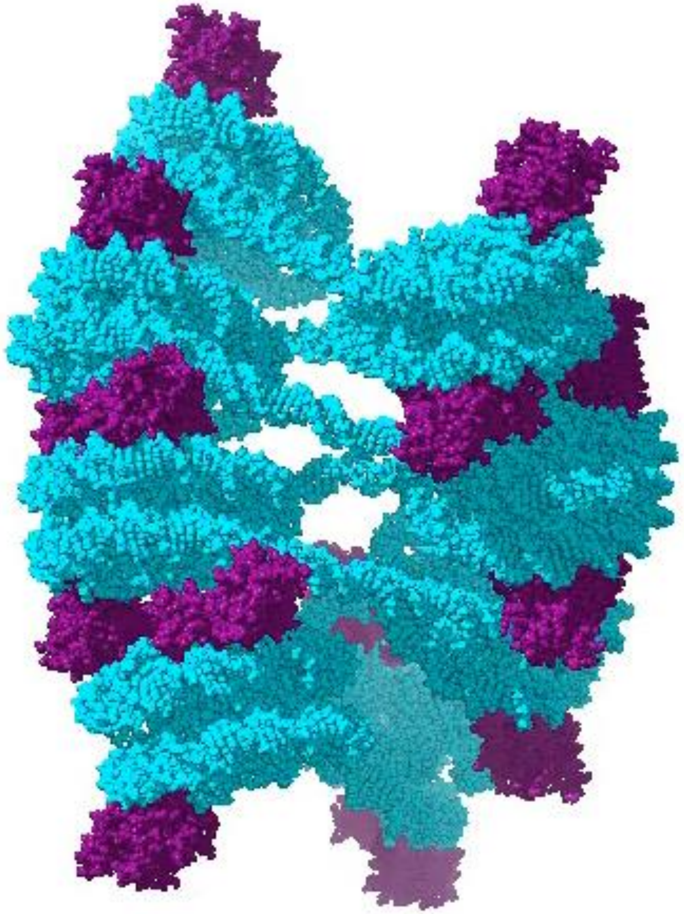
α1-L1-α2-L2- α3



Yajie Gu

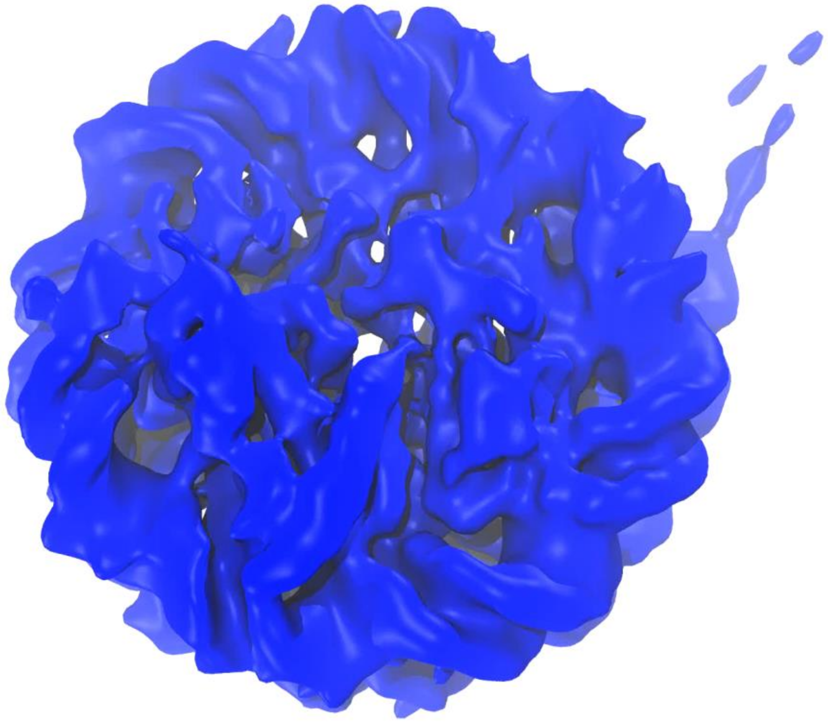
CENP-N: a centromeric ‘linker histone’?

- A new DNA interface on CENP-N
- Can bind CENP-A and H3-containing nucleosomes
- Promotes long-range and short-range nucleosome interactions
- Promotes the formation of compact chromatin at the centromere in cells
- CENP-A nucleosomes do NOT bind H1

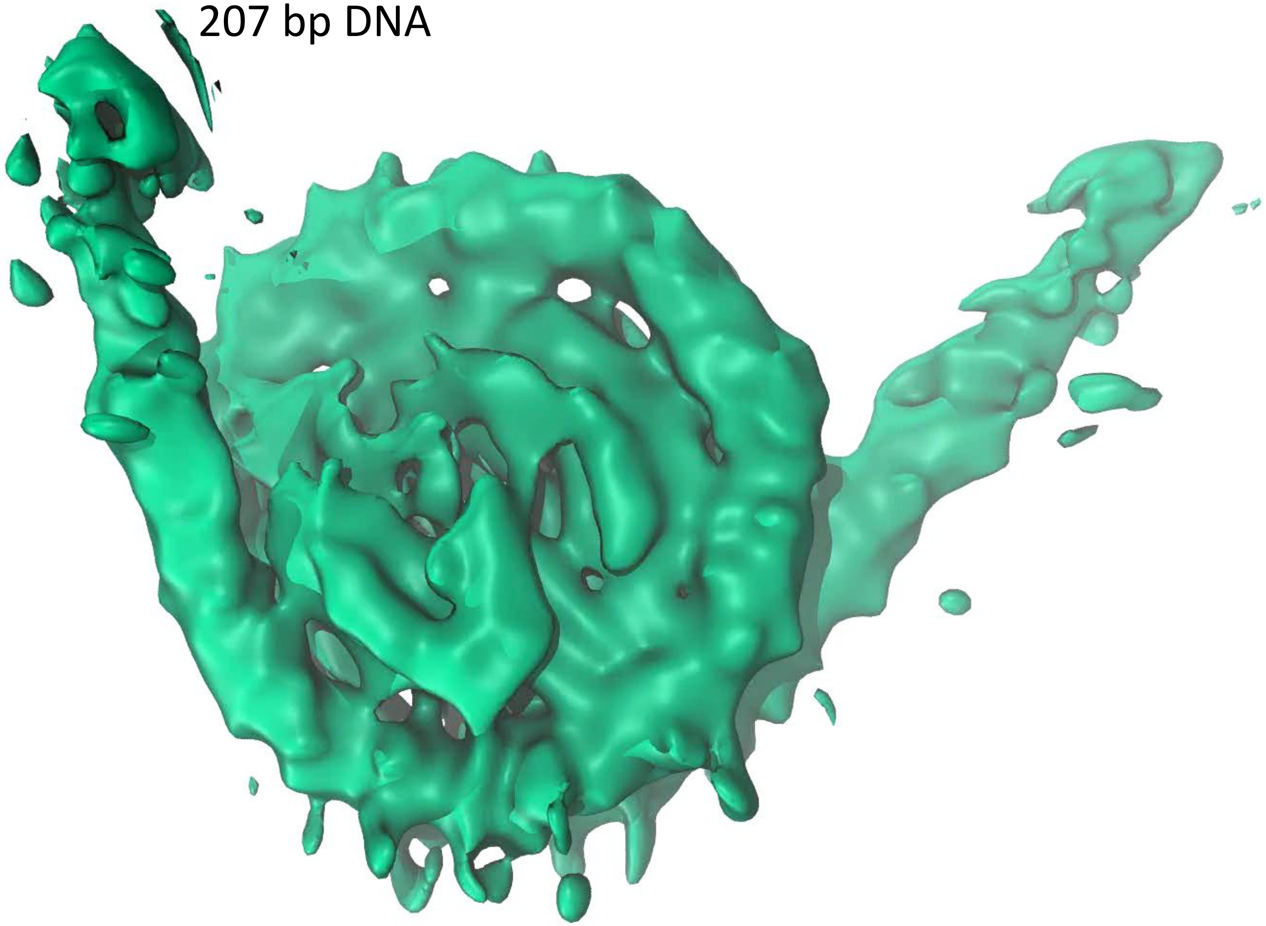


MV-nucleosomes bind less DNA than eukaryotic nucleosomes

The overall dimensions / path of the DNA is identical to eukaryotic nucleosomes



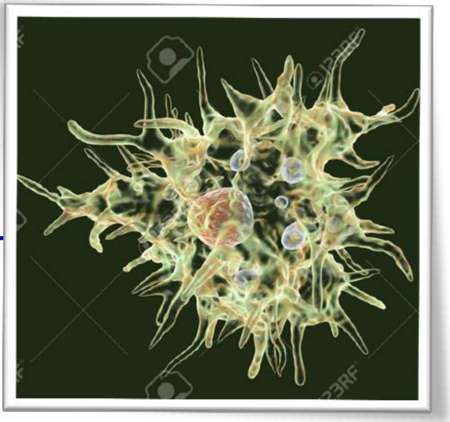
147 bp DNA

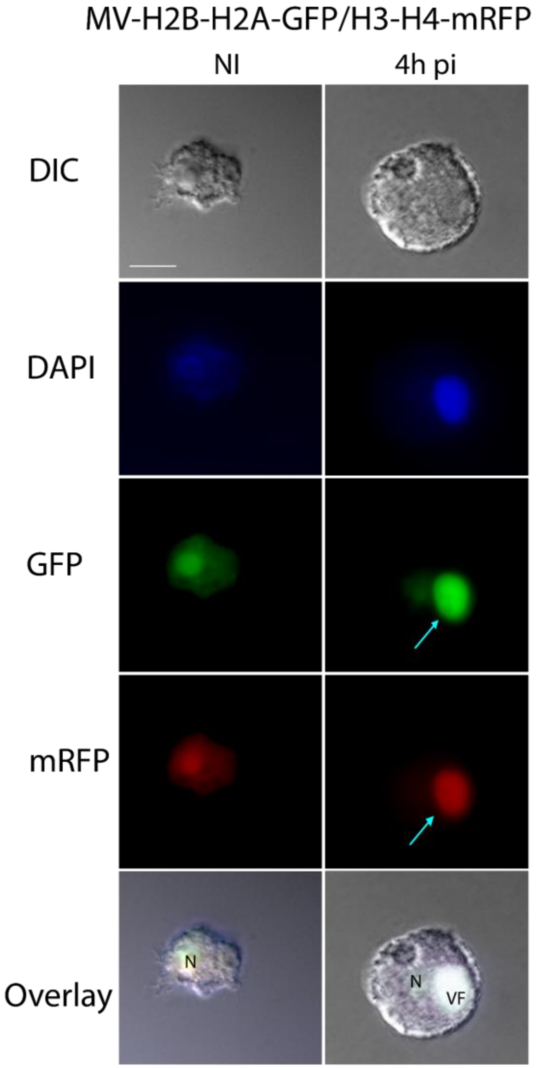


207 bp DNA

Yang Liu, Keda Zhou, Samuel Bowerman

Viral histone doublets **co-localize** to the viral factory



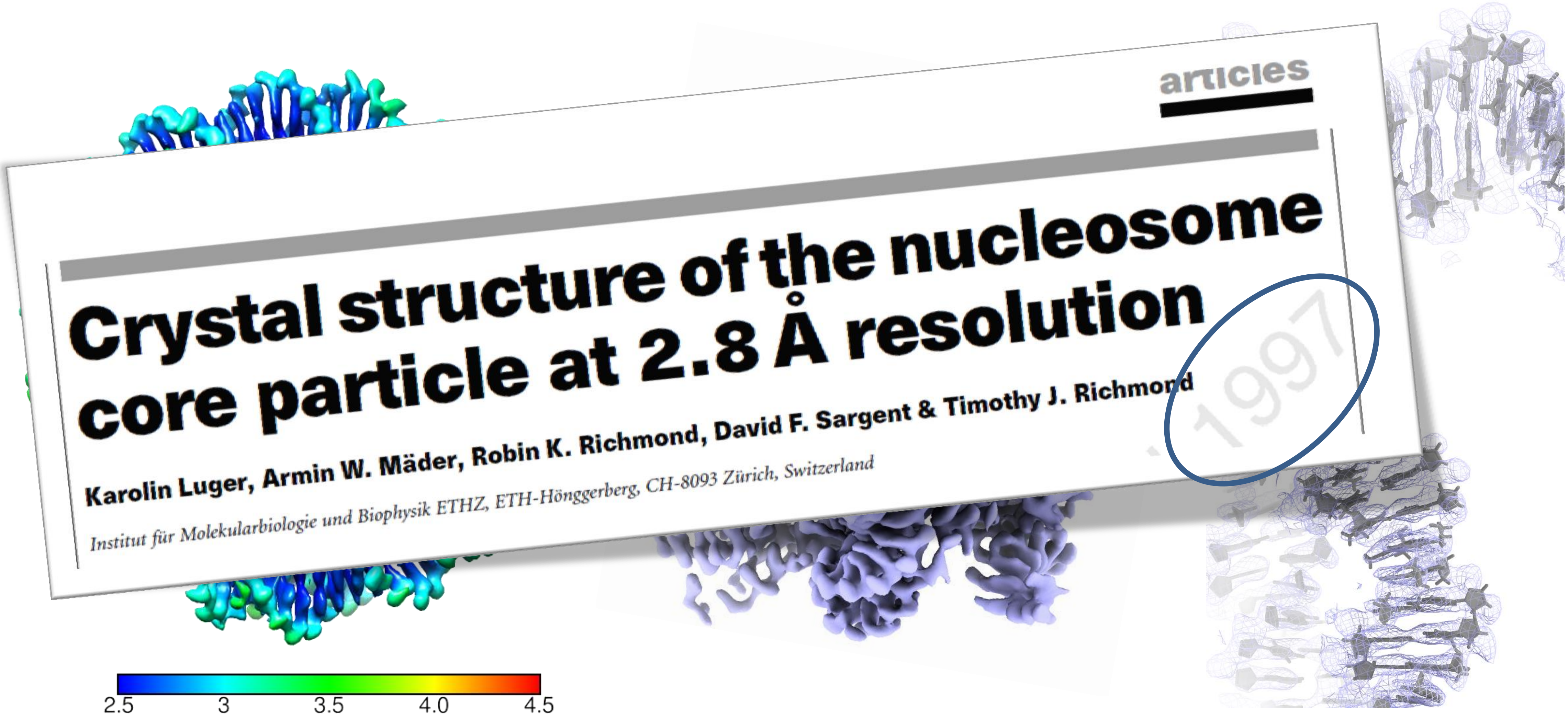


GFP-tagged viral H2B-H2A

RFP-tagged viral H4-H3

Nadege Philippe
Sandra Jeudy
Chantal Abergel

Princess Krios at CU Boulder: 2.6 Å nucleosomes!



articles

Crystal structure of the nucleosome core particle at 2.8 Å resolution

Karolin Luger, Armin W. Mäder, Robin K. Richmond, David F. Sargent & Timothy J. Richmond

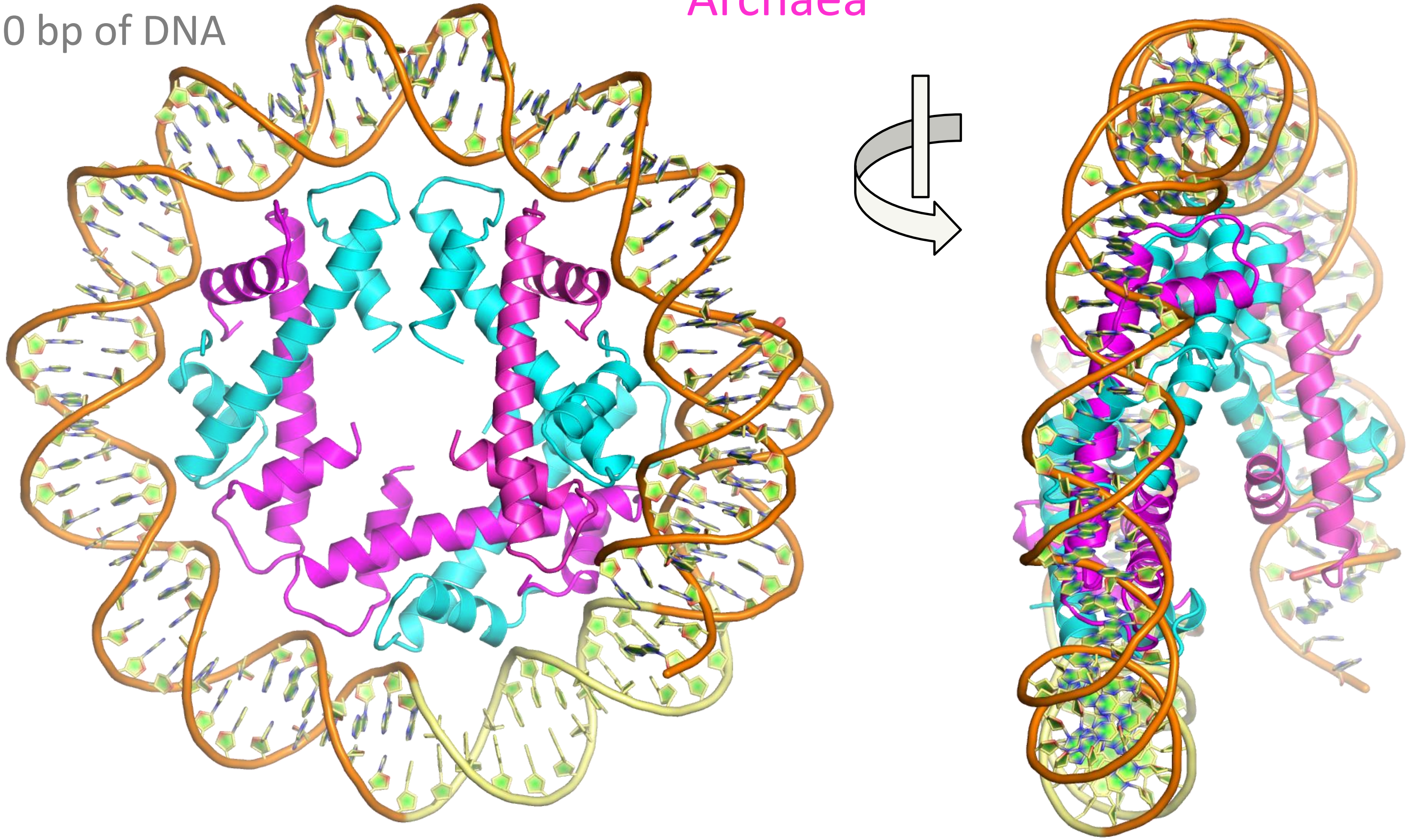
Institut für Molekularbiologie und Biophysik ETHZ, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

1997

Keda Zhou, Chuck Moe

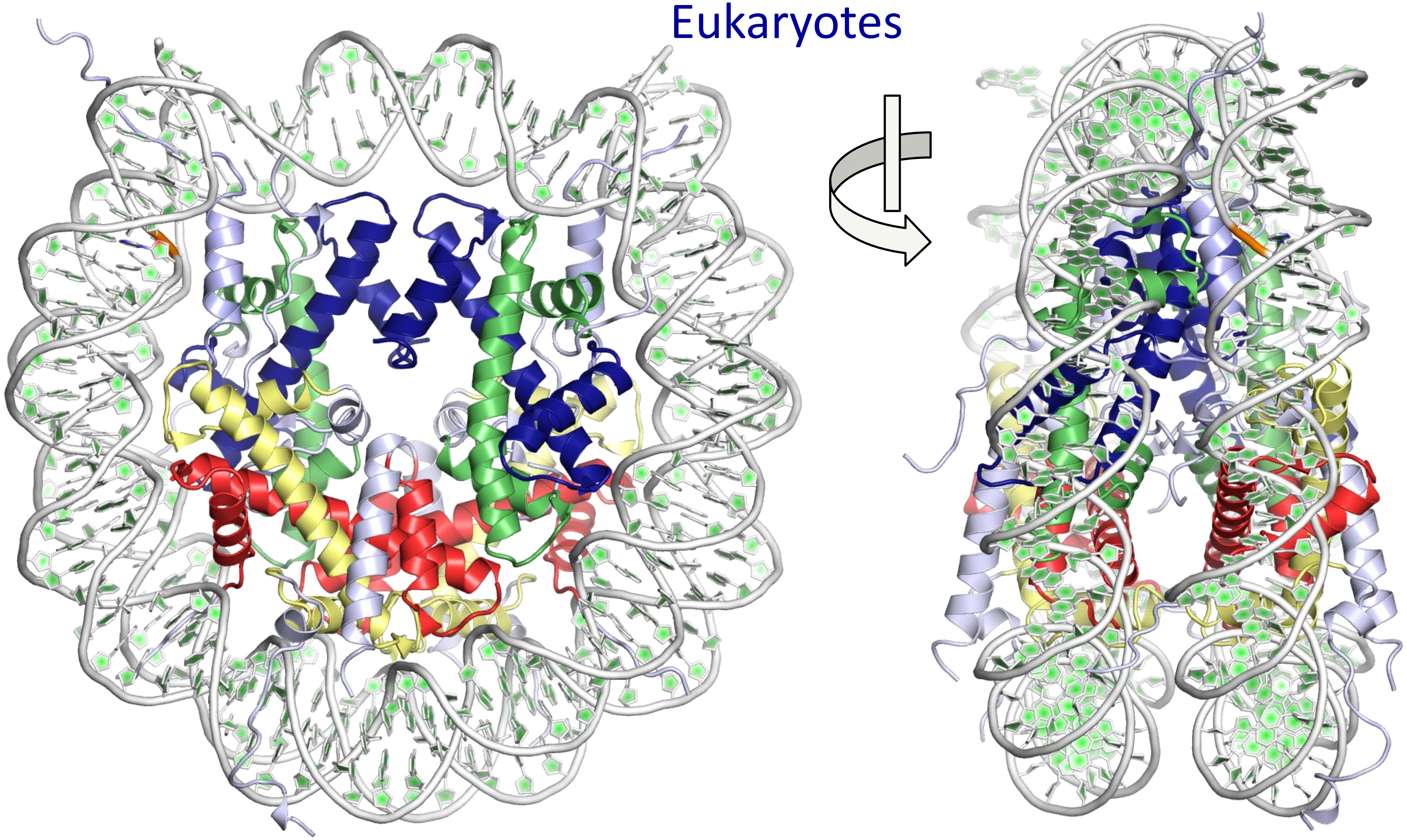
DNA organization in Archaea…

Crystal structure of archaeal histones bound to 90 bp of DNA



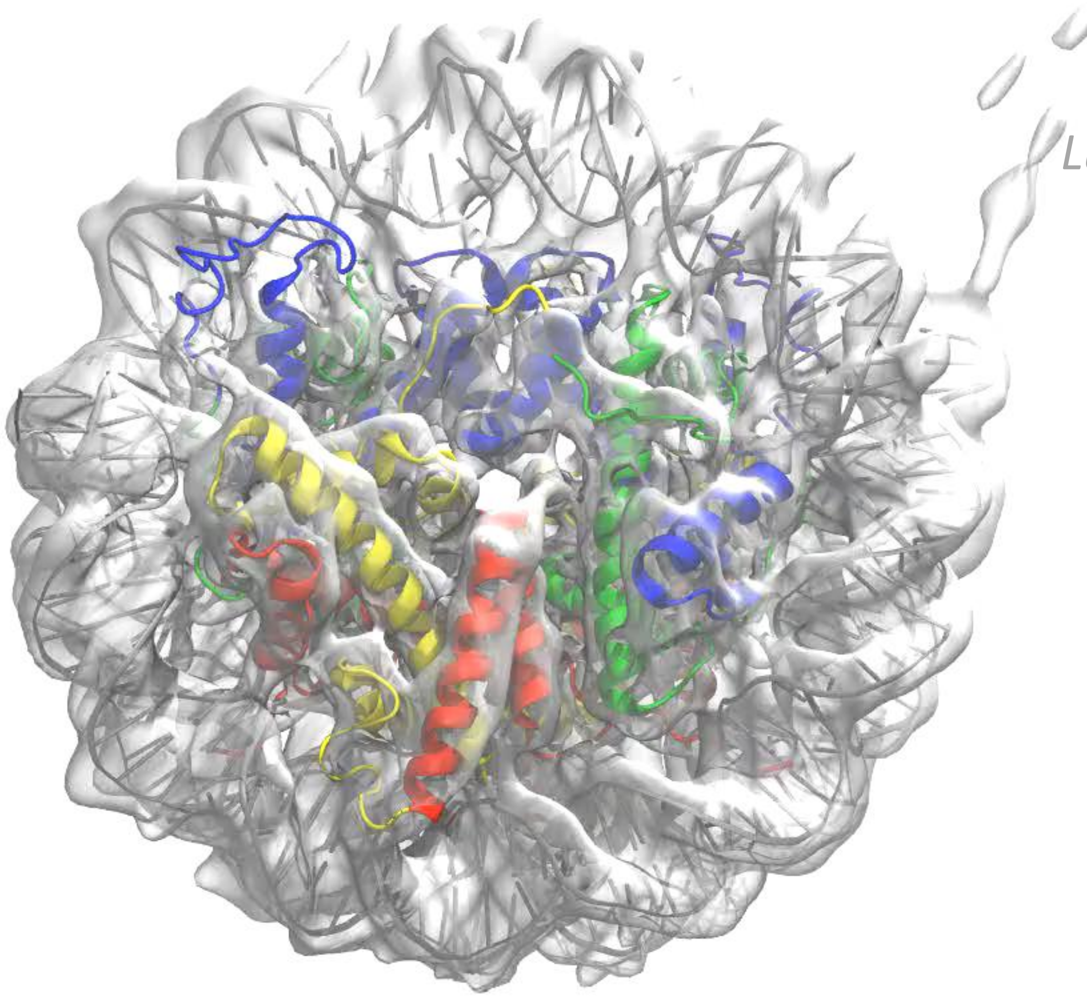
Francesca Mattiroli, Sudipta Bhattacharayya, Pamela Dyer, Kathy Sandman [*Science* **357**, 609-612 (2017)]

… and in all Eukaryotes



Eukaryotes

Melbournevirus nucleosome-like particles resemble destabilized eukaryotic nucleosomes

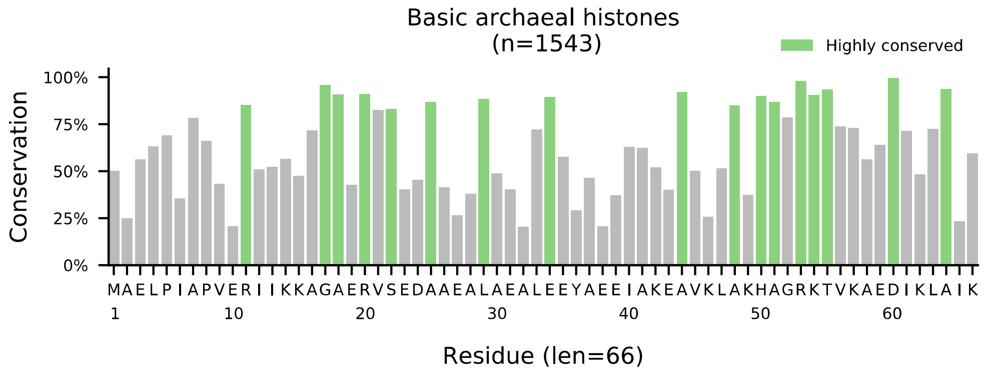


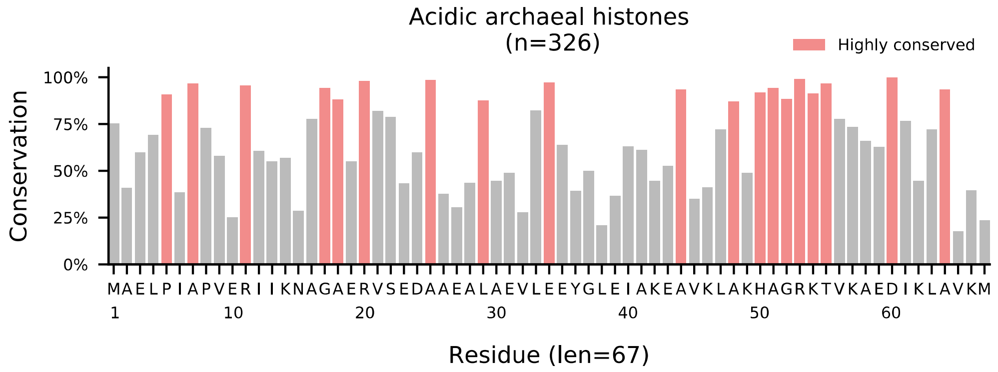
Liu, Bisio, Toner, Jeudy, Philippe, Zhou, Bowerman, White, Edwards, Abergel, Luger **[Cell, 2021,** 184(16):4237-4250**]**

Chelsea Toner, Samuel Bowerman

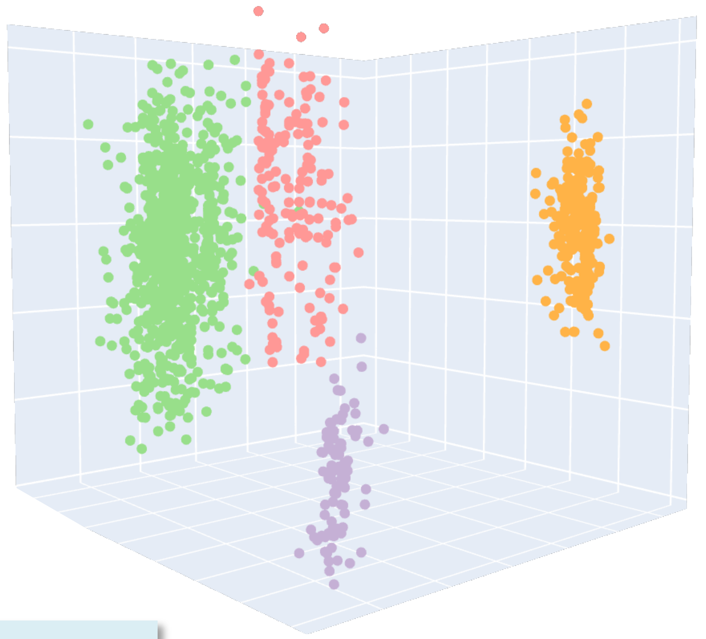
Archaeal histones cluster into four classes across the ‘domain’

Comprehensive sequence mining for putative histones across the archaeal domain of life





archaeal histone ‘space’

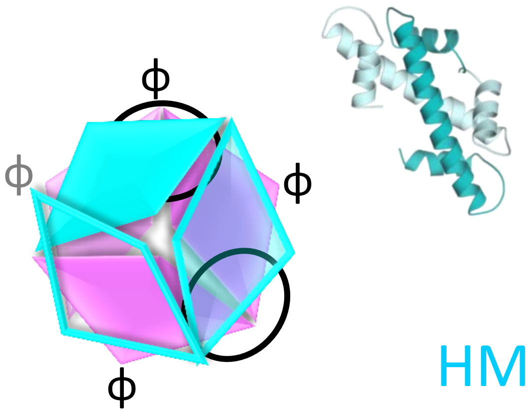


No diversification into H2A, H2B, H3, and H4

Shawn Laursen

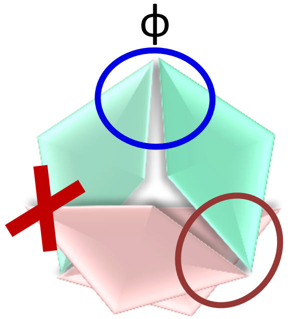
From variable-length solenoids to defined particles

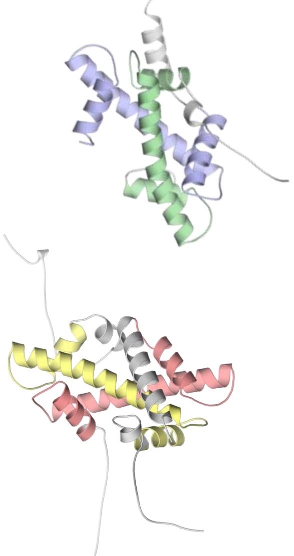
Archaeal histones



HMfB-HMfB

Eukaryotic histone octamer

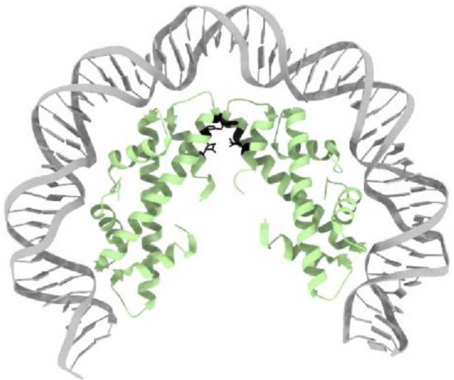




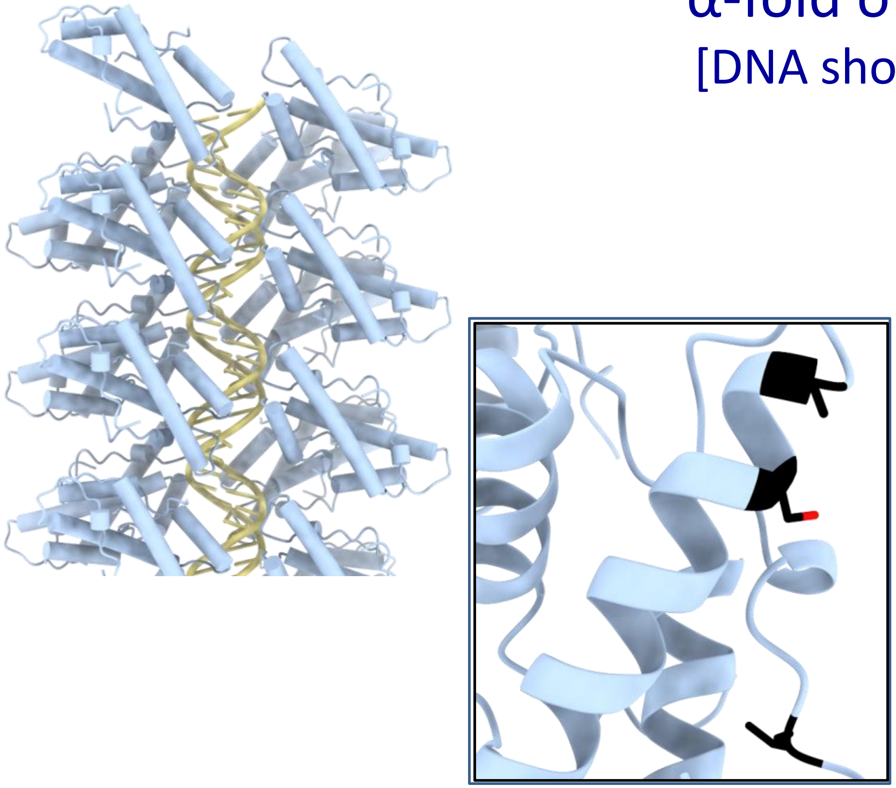
H2A-H2B dimer

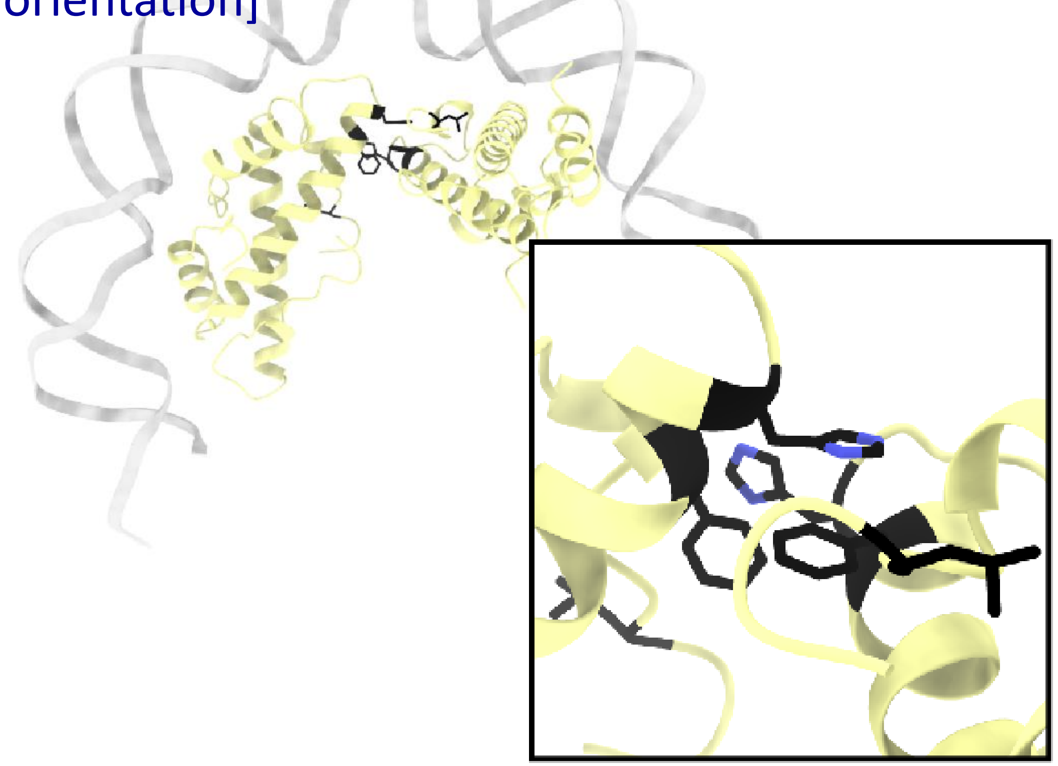
$(H3\text{-}H4)_2$ tetramer

Restoring the four-helix bundle in bacterial histone



α-fold of four histone chains
[DNA shown only for orientation]

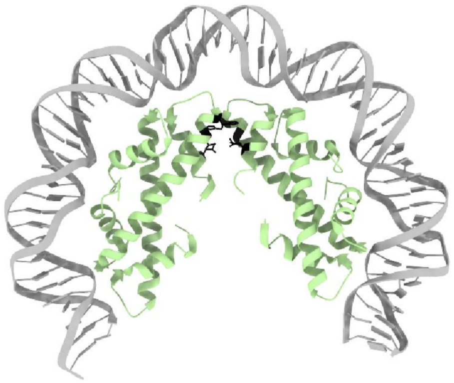


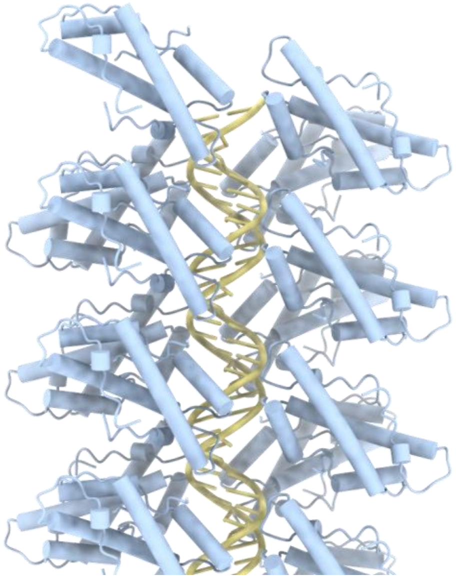


Wild type bacterial histone does not engage in four-helix bundle

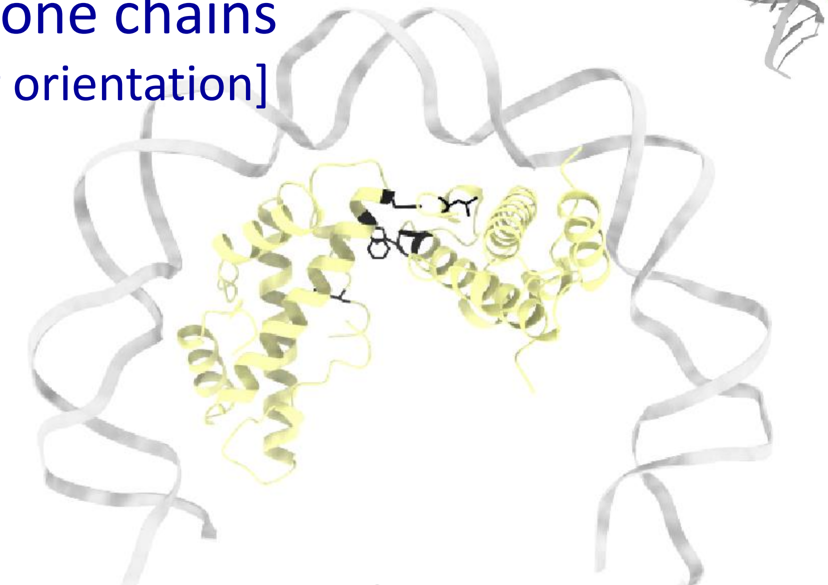
Mutating three residues allows tetramerization *in silico*

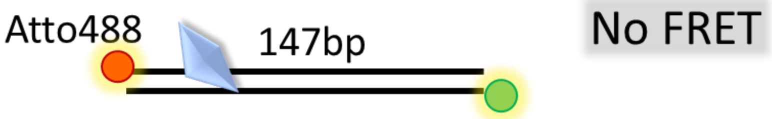
Restoring the four-helix bundle in bacterial histone

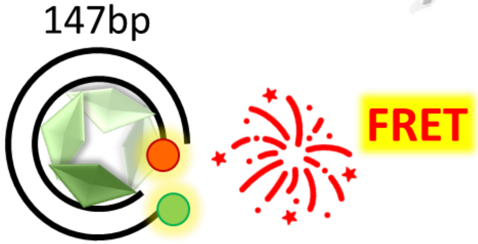




α-fold of four histone chains
[DNA shown only for orientation]







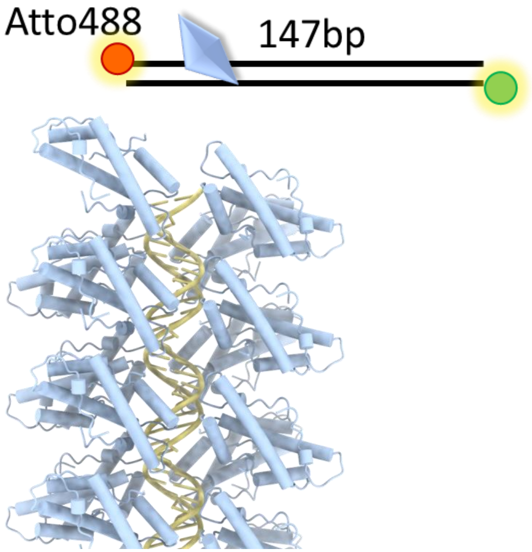
Wild type bacterial histone does not engage in four-helix bundle

Mutating three residues allows tetramerization *in silico*

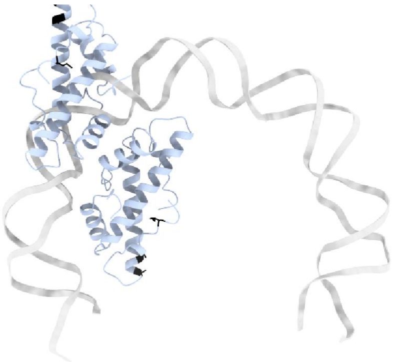
Reason for this unconventional binding mode

α-fold of four histone chains

[DNA shown only for orientation]



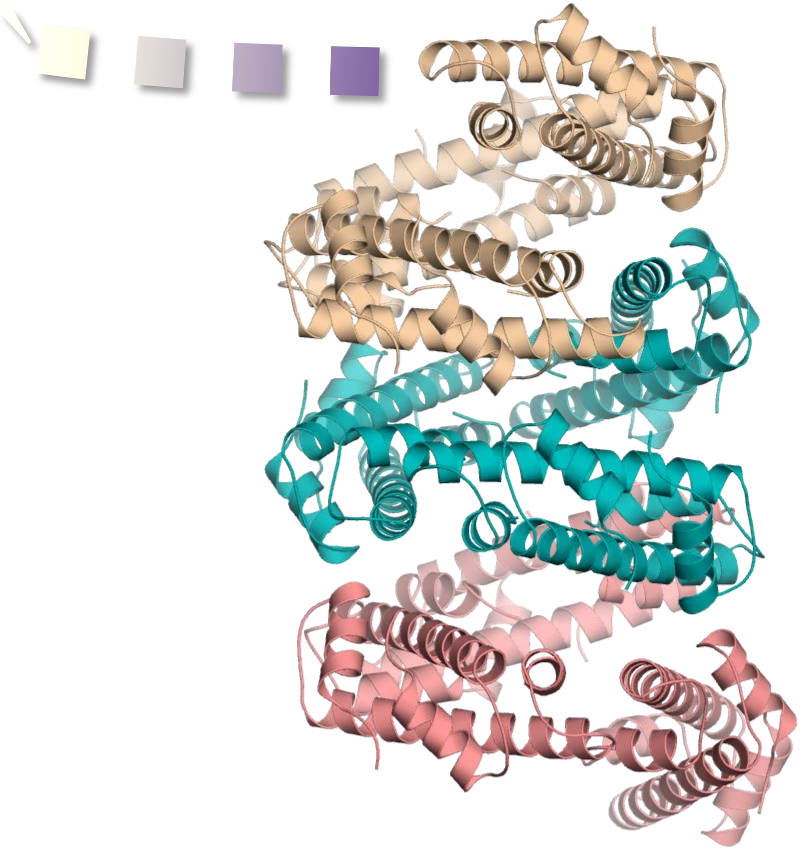
No FRET



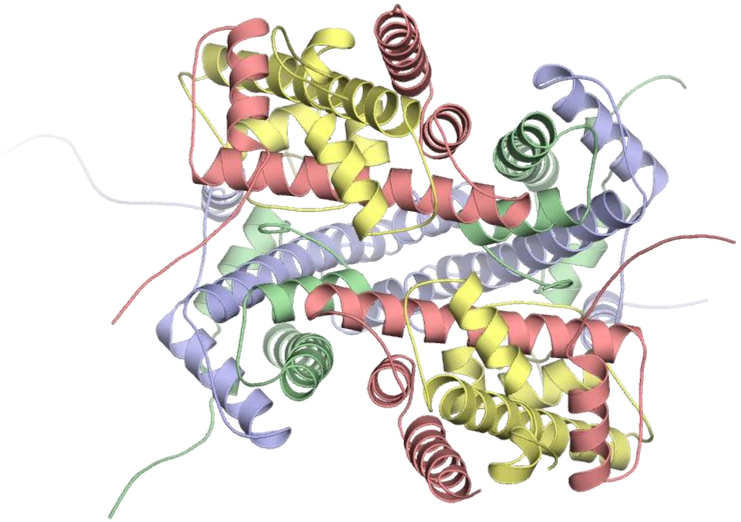
Shawn Laursen, with help from Nikhil Gupta

From variable-length solenoids to defined particles

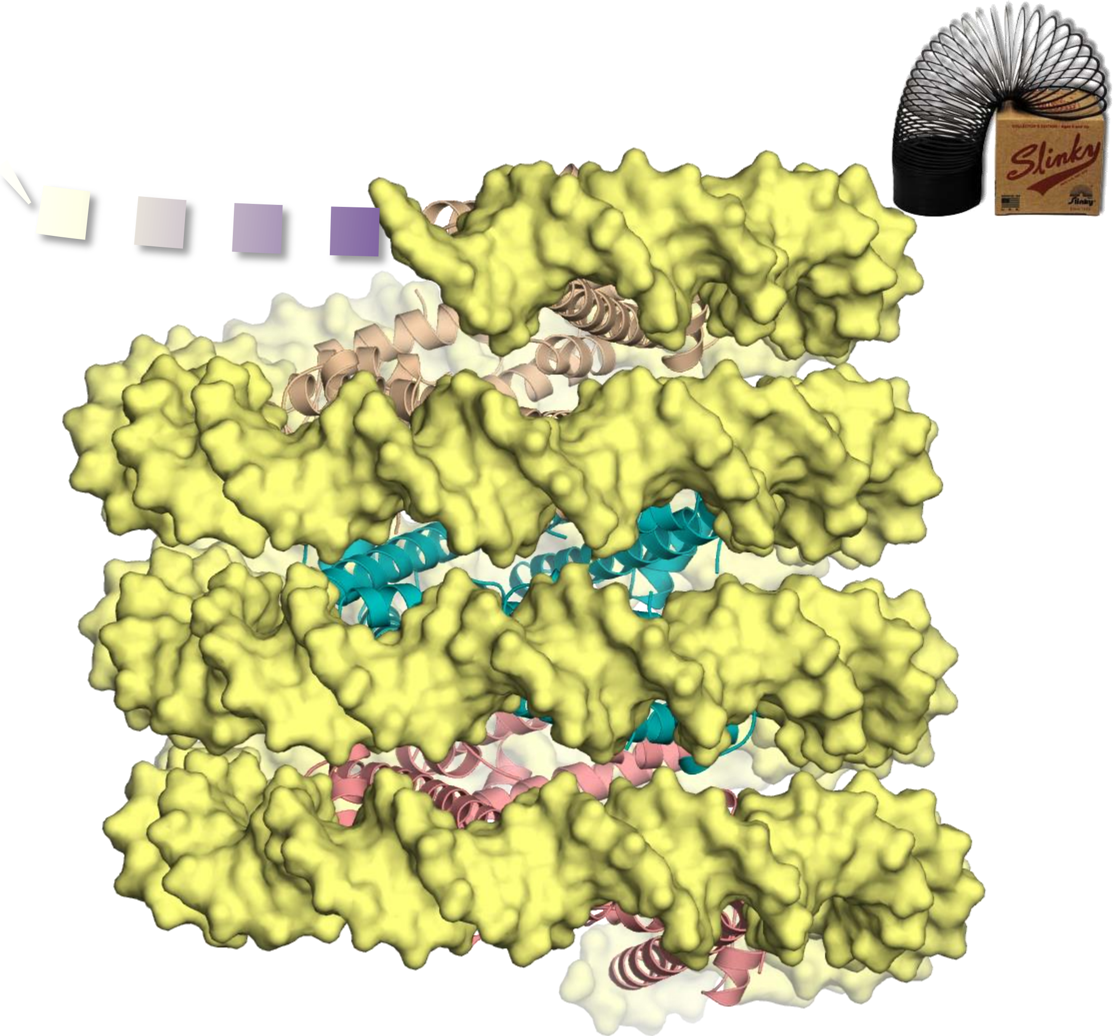
Archaeal histones

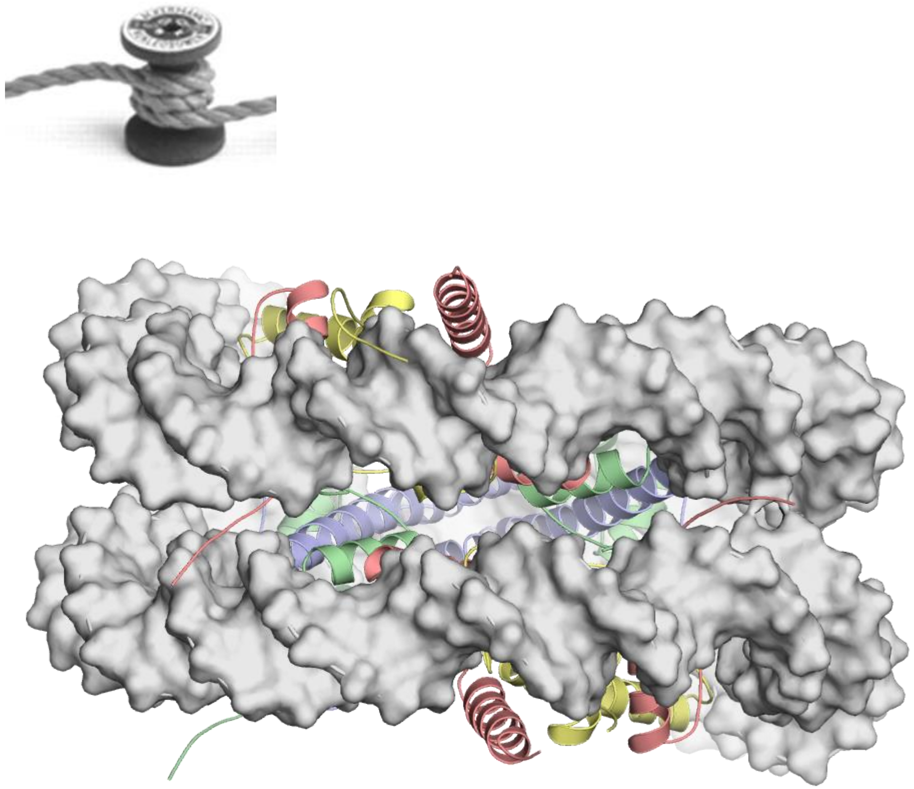


Eukaryotic histone octamer



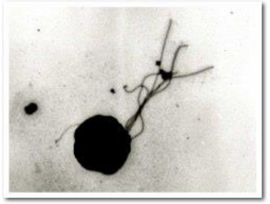
From variable-length solenoids to defined particles





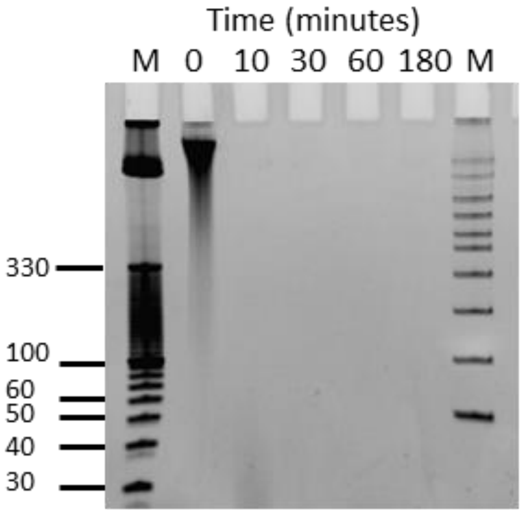
Francesca Mattiroli, Sudipta Bhattacharayya, Pamela Dyer, Kathy Sandman [*Science* **357**, 609-612 (2017)]

Mutant chromatin from archaea: rapid digestion to 60-90 bp, and profound transcriptional effects

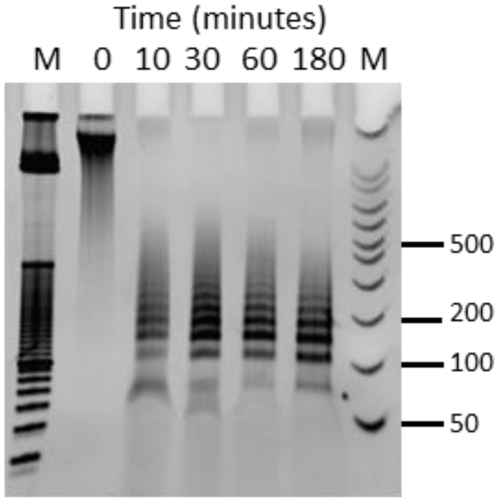


Chromatin extracted from wild type cells
Laddering (30 bp multiples)

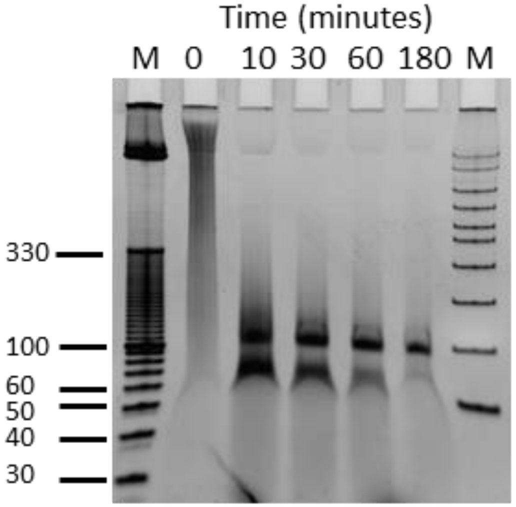
Chromatin extracted from mutant cells
Defined 60-90 bp stop



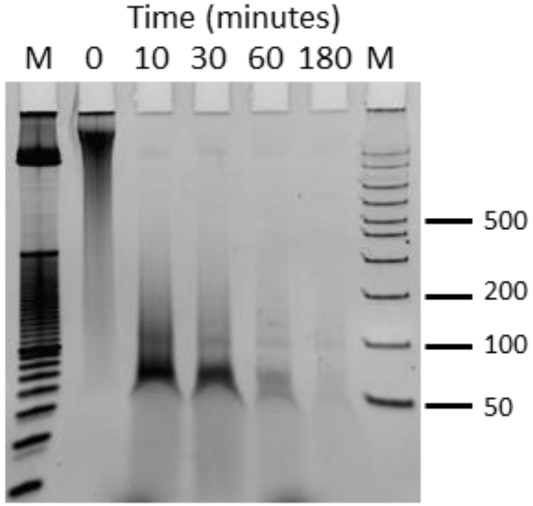
TS600 DNA



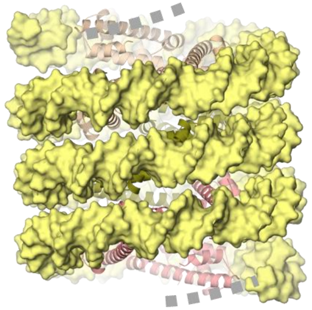
TS600 chromatin



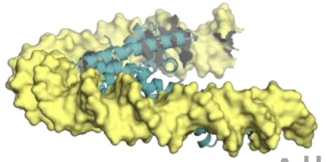
G17D chromatin



G17L chromatin

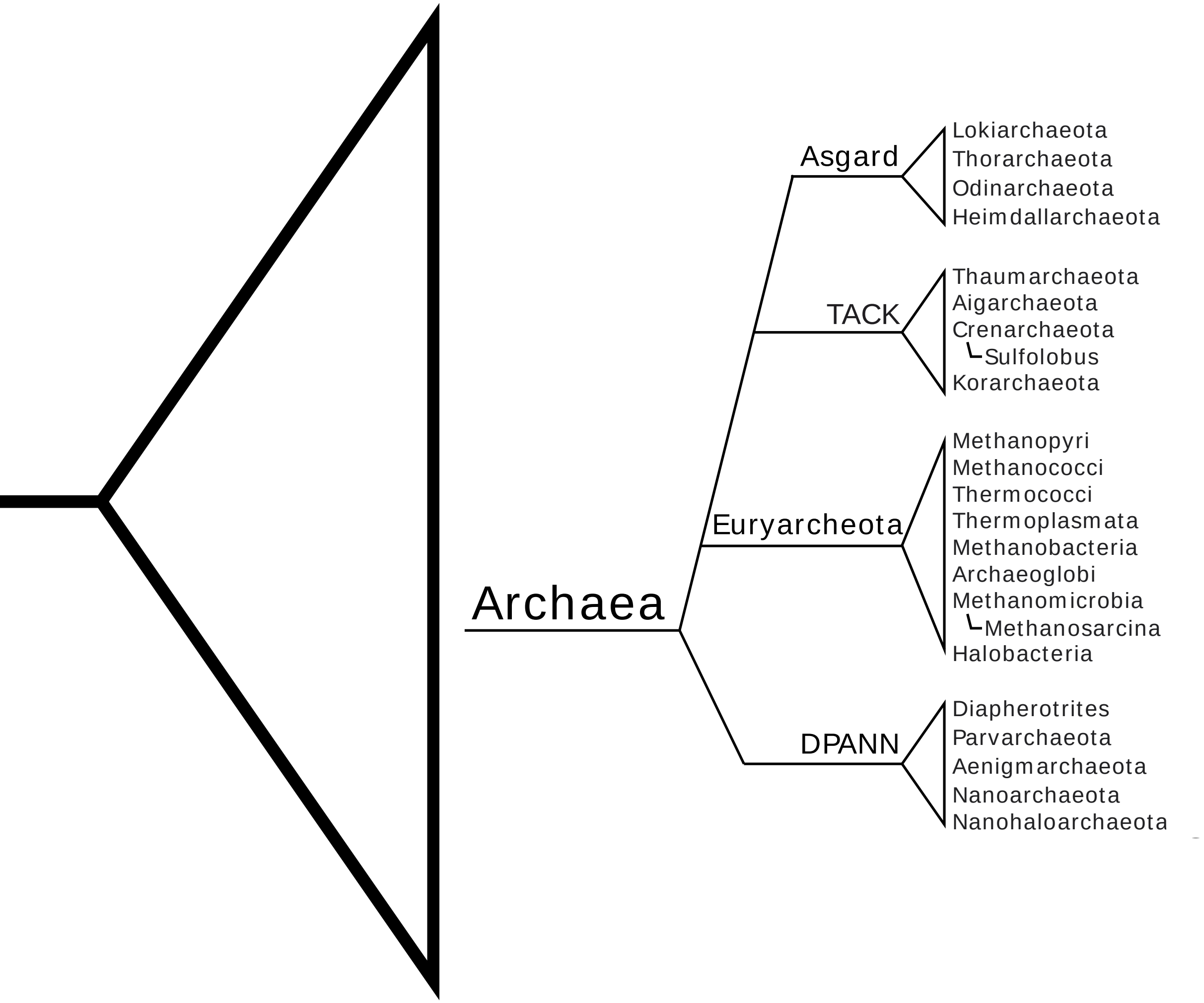


These structures exist in archaea and contribute to gene regulation



Allison White

Archaea use a mix of eukaryotic and bacterial strategies



Archaea

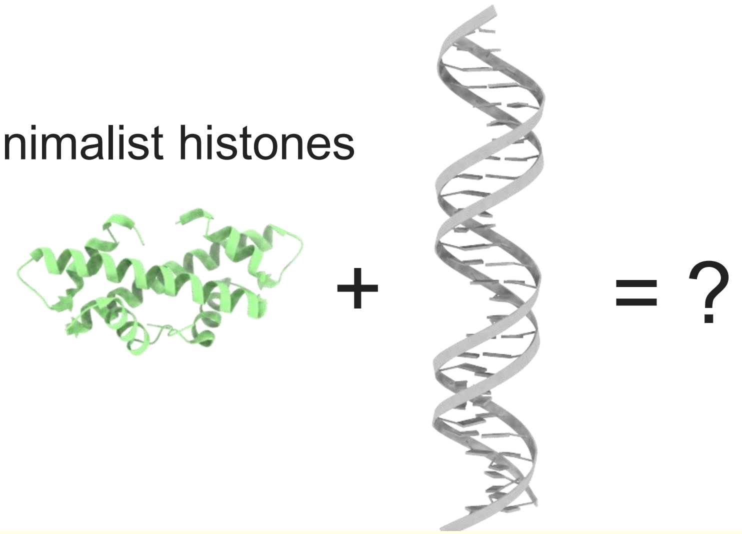
Asgard
- Lokiarchaeota
- Thorarchaeota
- Odinarchaeota
- Heimdallarchaeota

TACK
- Thaumarchaeota
- Aigarchaeota
- Crenarchaeota
  - Sulfolobus
- Korarchaeota

Euryarcheota
- Methanopyri
- Methanococci
- Thermococci
- Thermoplasmata
- Methanobacteria
- Archaeoglobi
- Methanomicrobia
  - Methanosarcina
- Halobacteria

DPANN
- Diapherotrites
- Parvarchaeota
- Aenigmarchaeota
- Nanoarchaeota
- Nanohaloarchaeota

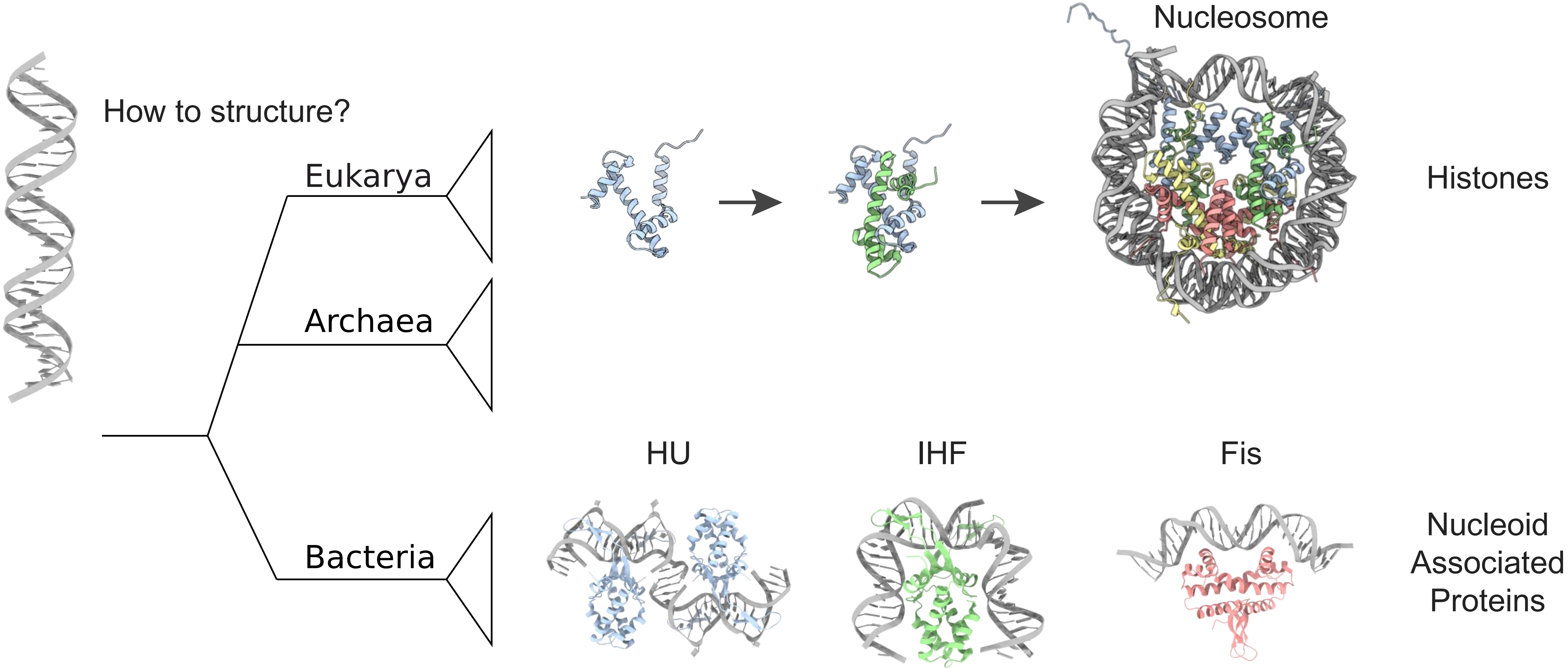
Minimalist histones



What can **Archaea** tell us about the origins of the nucleosome?

Laursen et. al JMB 2021.

Different strategies to organize and structure genomes in the three domains of life



How to structure?

Eukarya

Archaea

Bacteria

Nucleosome

Histones

HU

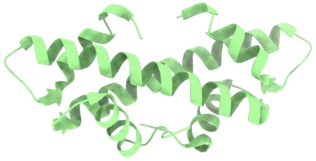
IHF

Fis

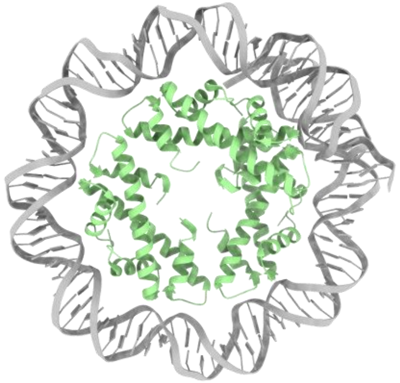
Nucleoid
Associated
Proteins

There are three main histone strategies in archaea

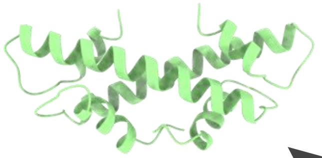
Thermophiles.



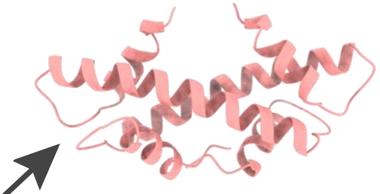
1 or more basic.



Some methanogens.



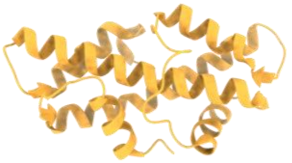
2 basic.



1 acidic.

Halophilies.

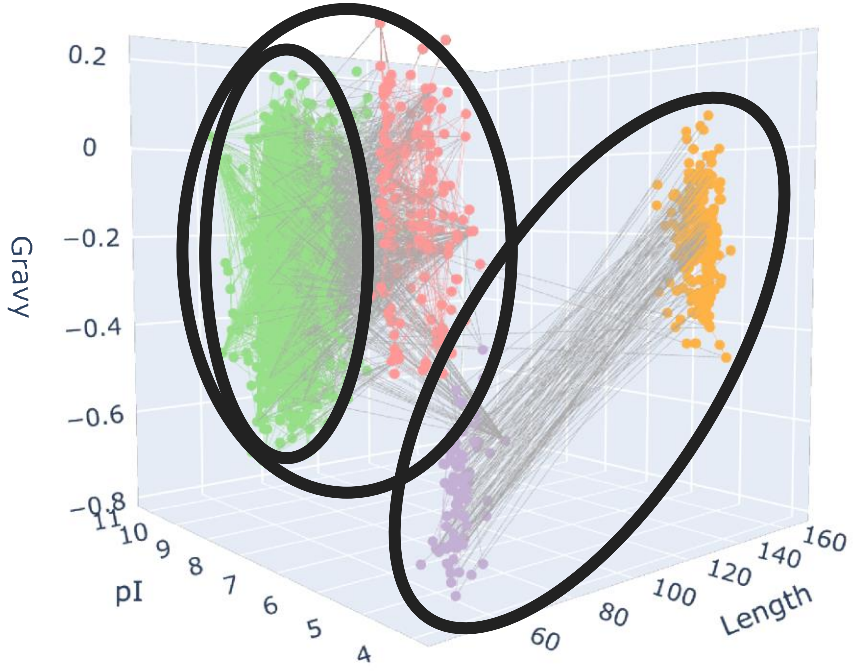
Predicted structures.



1 acidic
doublet.

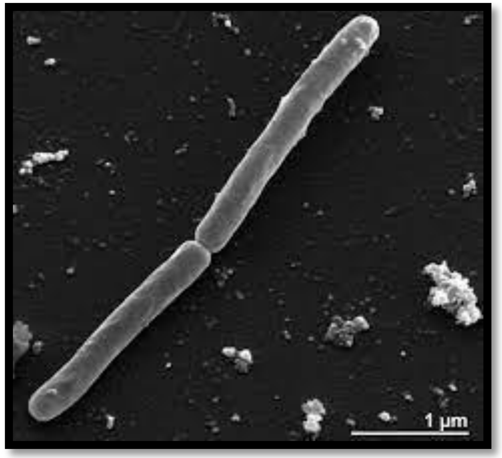
1 acidic
55aa.

Predicted archaeal histone properties

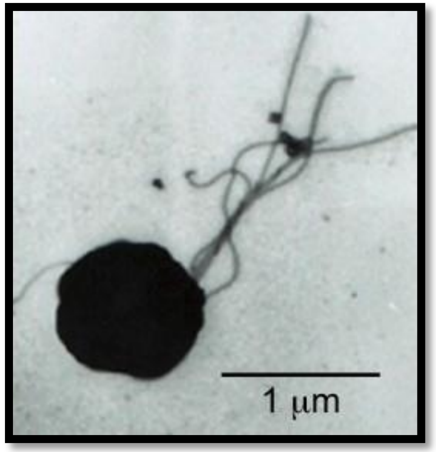


Some like it hot: Lets talk about thermophiles

Thermophiles: one or more basic histone-like proteins

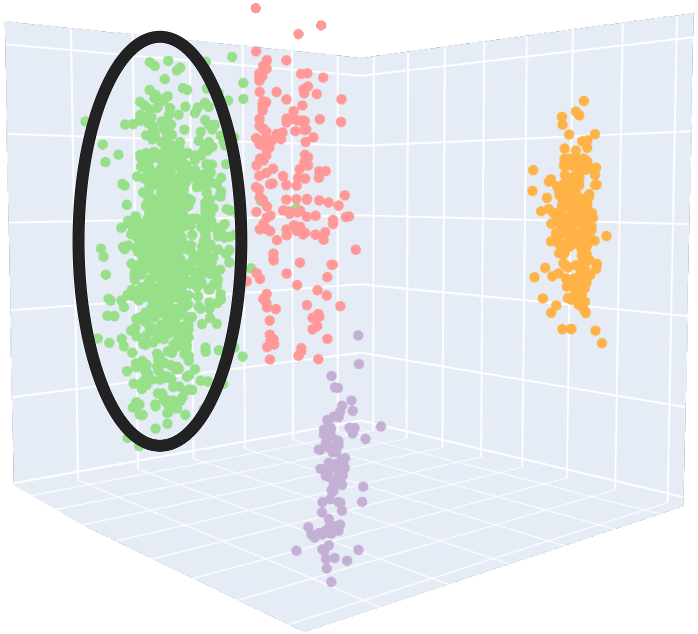


Methanothermus fervidus
~ 95 degrees Celsius



Thermococcus kodakarensis
~60-100 degrees Celsius

archaeal histone ‘space’



Shawn Laursen

'Histone fold dimers' are conserved across domains of life

Archaeal $(HMfB)_2$

~ 70 aa

Eukaryotic H3-H4

Histone extensions and tails

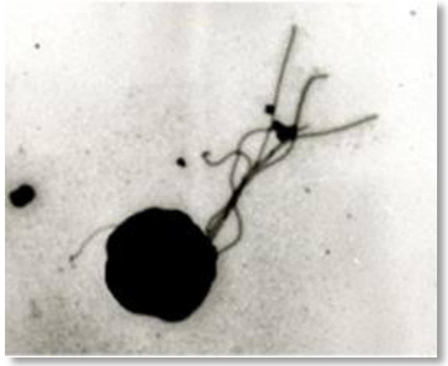
> 105 aa

Eukaryotic H2A-H2B

> 120 aa

Luger et al., Nature 1997; Decanniere et al., JMB 2000

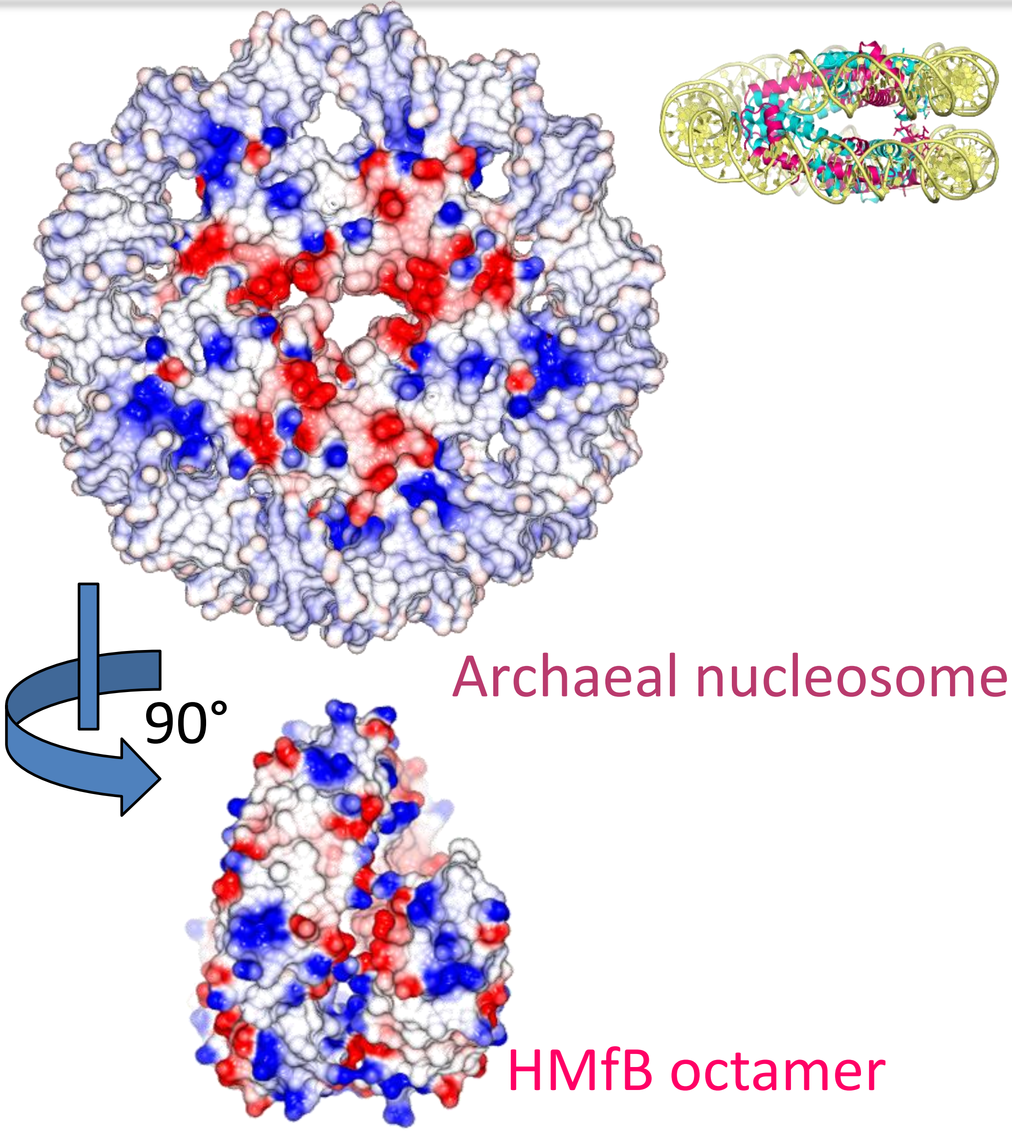
- How does a single tailless histone organize DNA?
- Why do these organisms need histones?



~1,2 x 10^6 bp

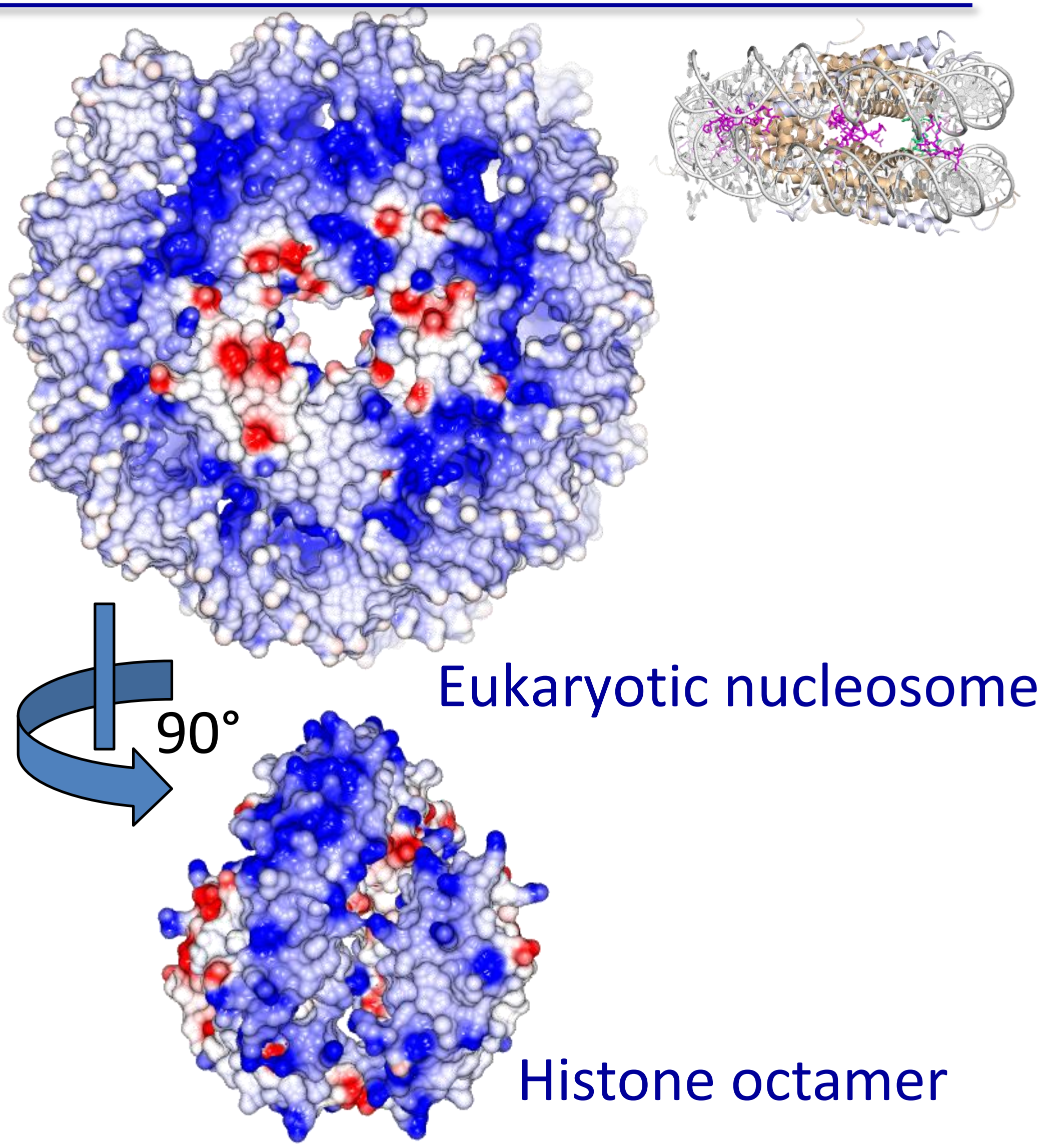
3.3 x 10^9 bp

More extreme charges and more ‘stuffing' stabilize the eukaryotic nucleosomes



Archaeal nucleosome

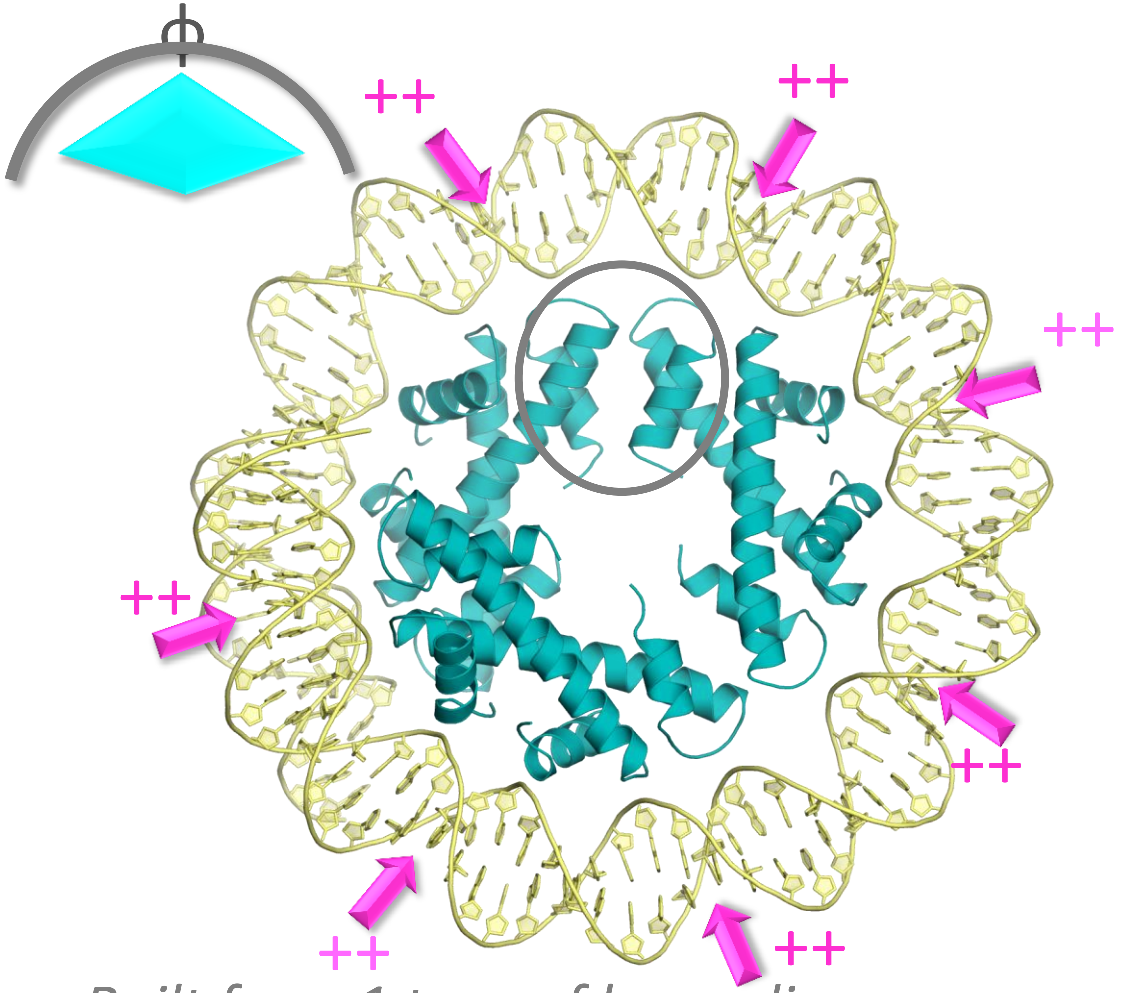
HMfB octamer



Eukaryotic nucleosome

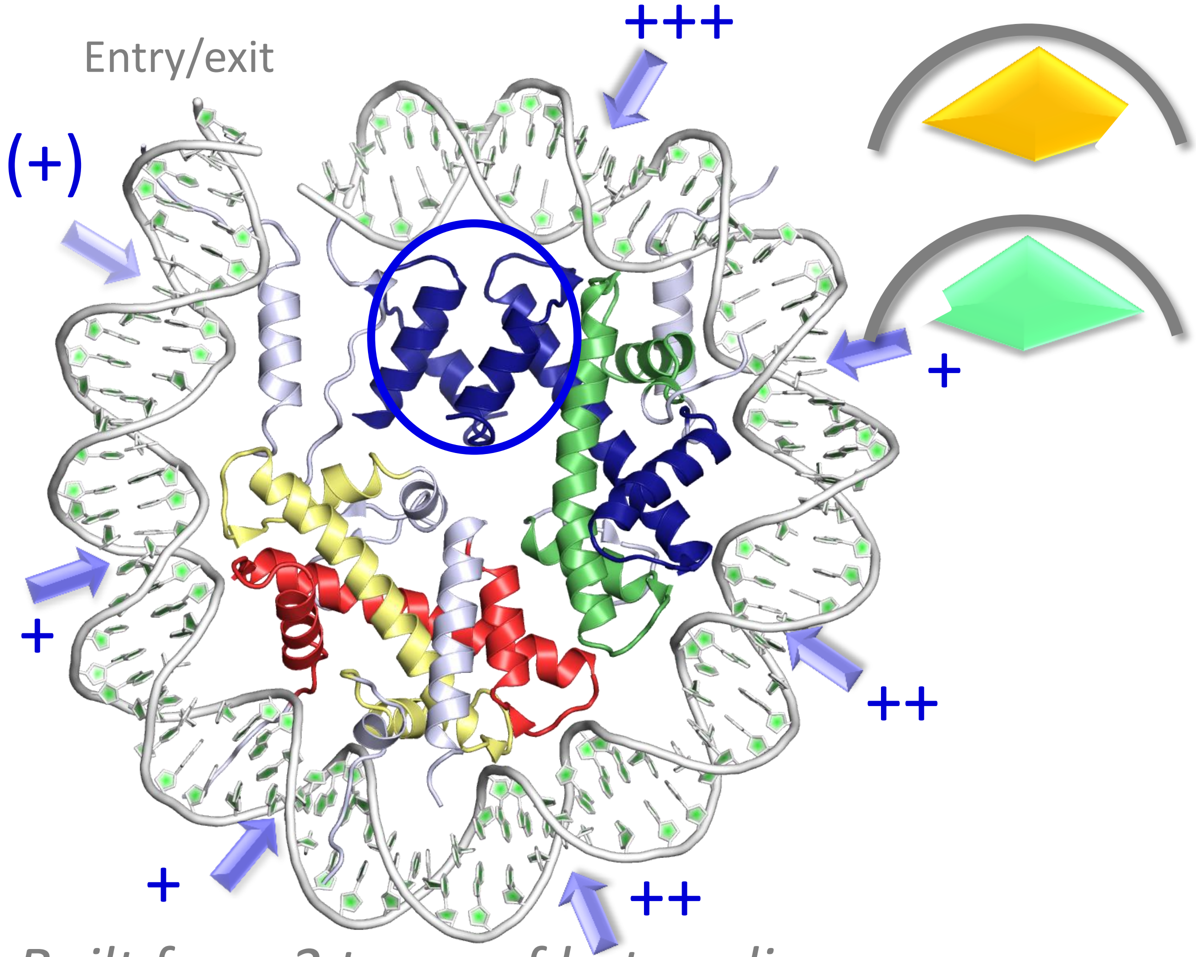
Histone octamer

Four histones allow modulation of the strength of DNA interactions



Built from 1 type of homodimer

Archaeal chromatin

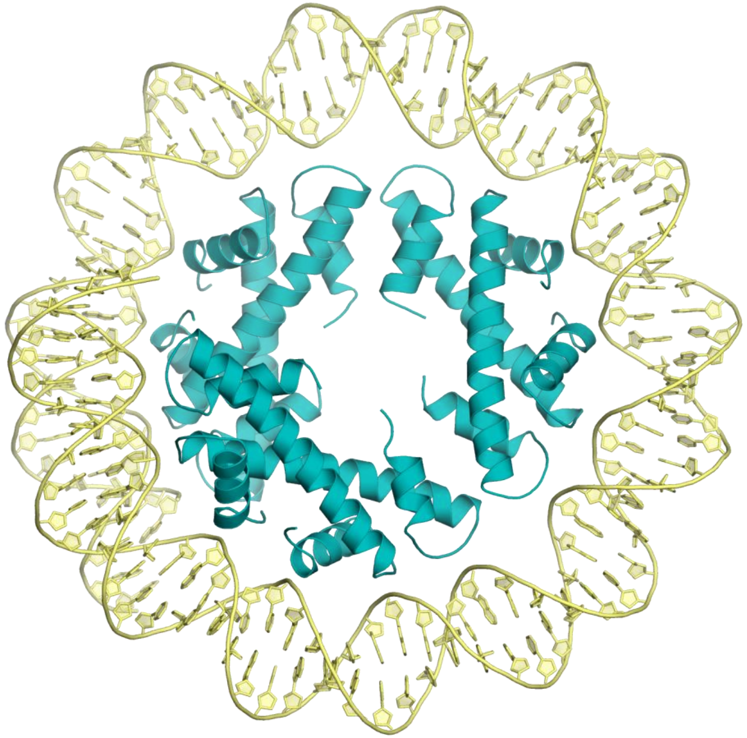


Built from 2 types of heterodimers

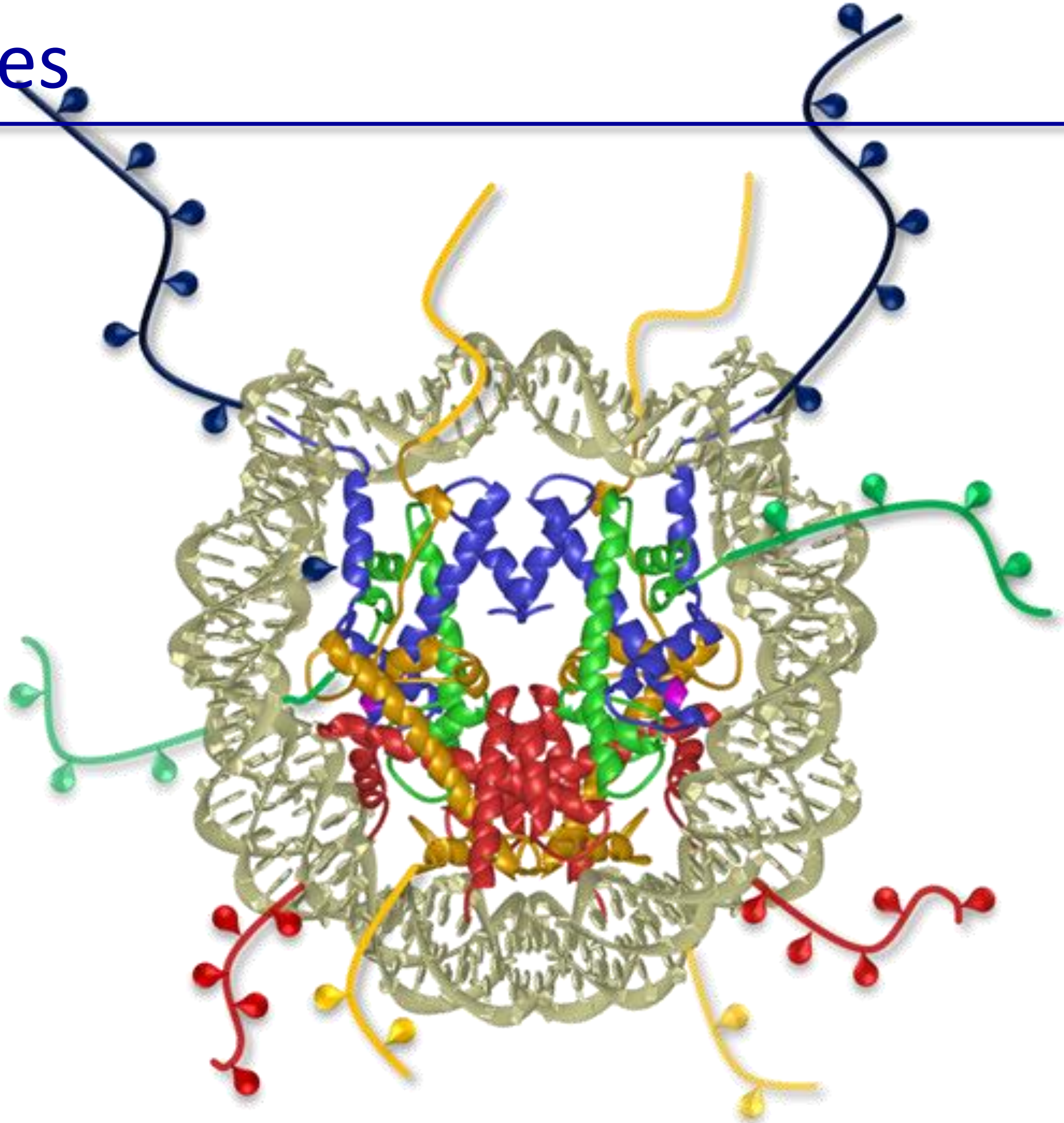
Eukaryotic nucleosome

Post-translational modifications of histone tails, and histone variants are unique to eukaryotes

No readers, writers, erasers

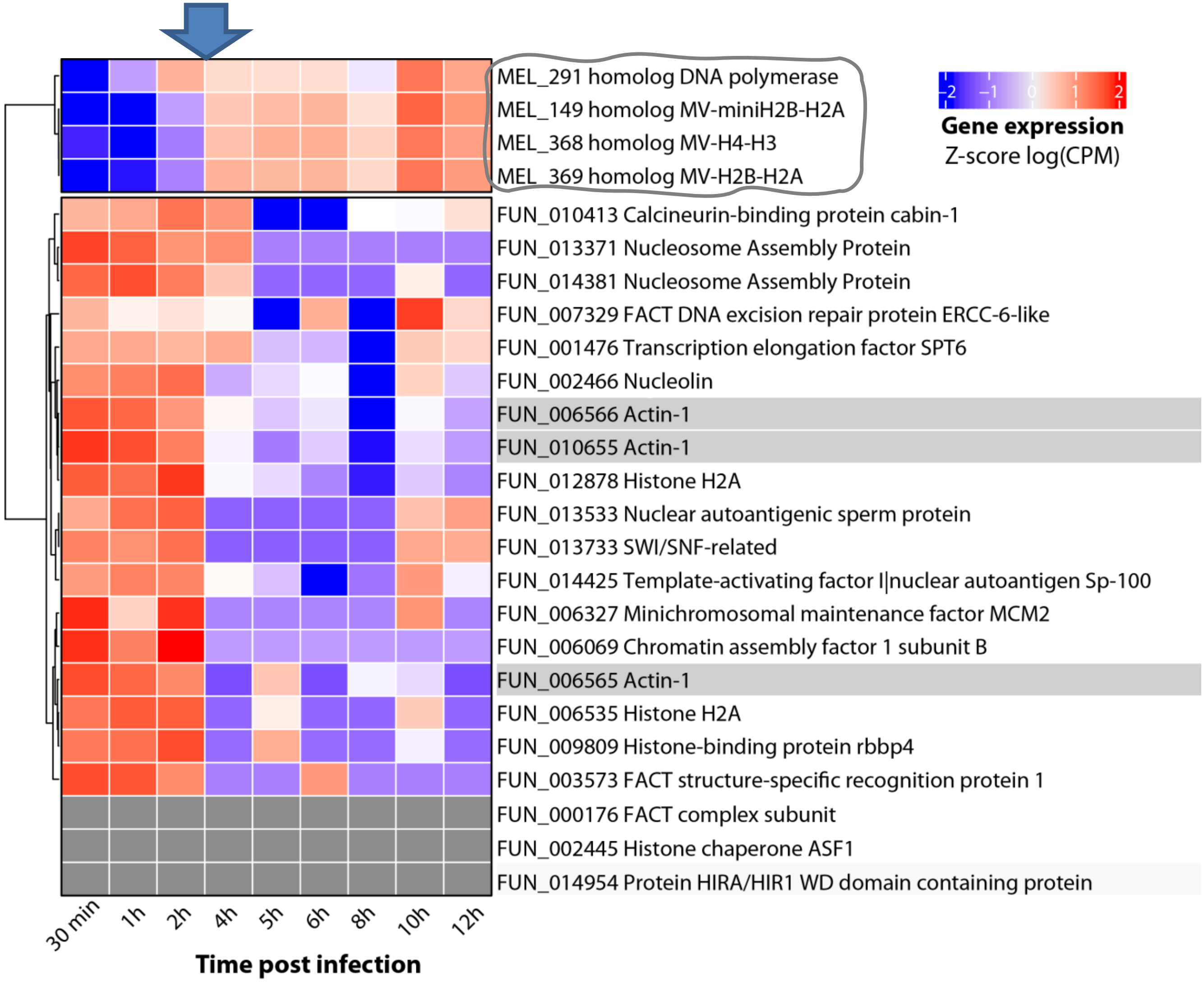


Archaeal nucleosome



Eukaryotic nucleosome

Virus histones (and polymerase) are expressed upon virus infection

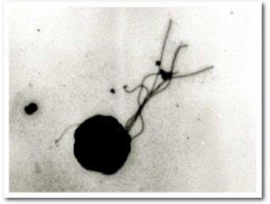


2 hours post infection:

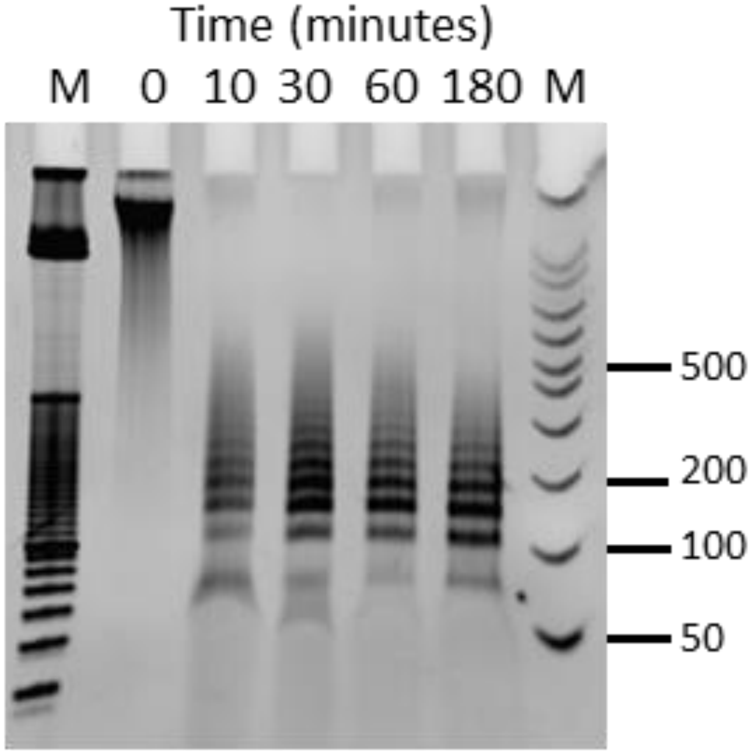
- Viral histone gene expression turned on
- Host gene expression turned off (including histones)
- No host histone chaperones upregulated

Transcriptome data from:
Rodrigues, R.A.L. et al. (2020). Analysis of a Marseillevirus Transcriptome Reveals Temporal Gene Expression Profile and Host Transcriptional Shift. Front Microbiol *11*, 651.

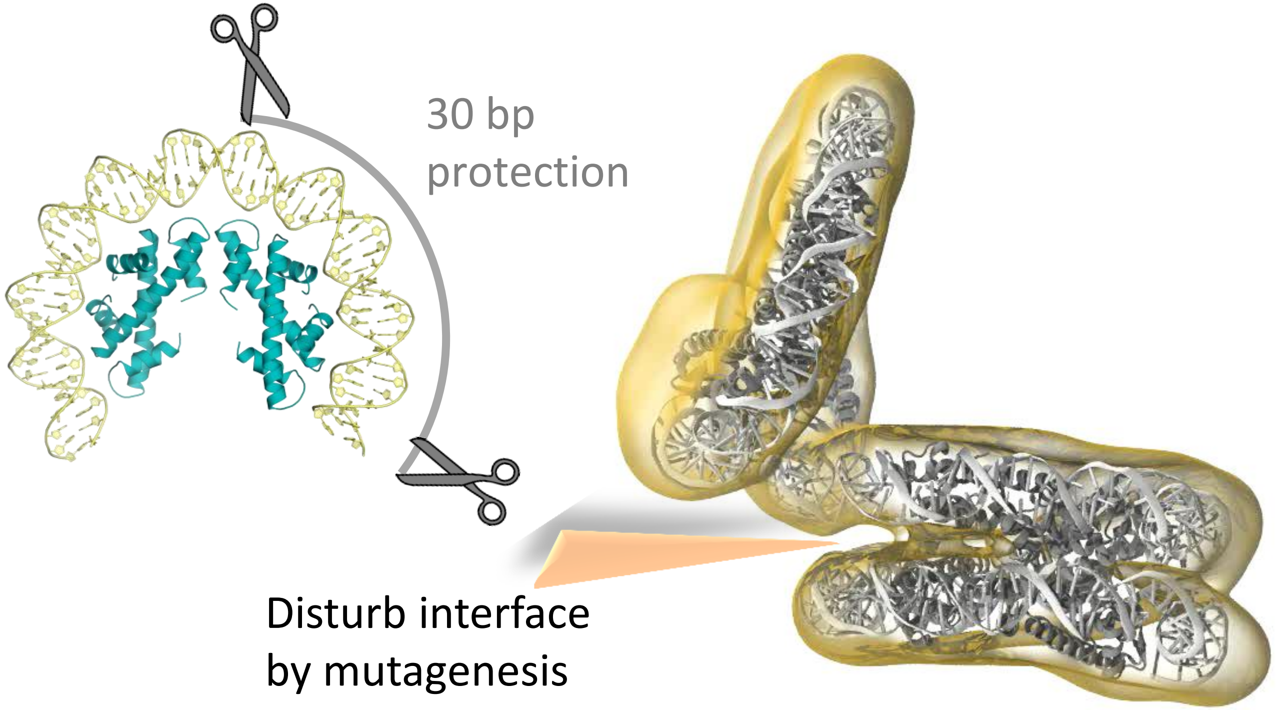
Archaeal histones oligomerize on DNA in the cell



MNase digestion of extracted archaeal chromatin shows a 30 bp ladder



Chromatin isolated from *Thermococcus kodakarensis*



Disturb interface by mutagenesis

Archaeal chromatin ‘slinky’

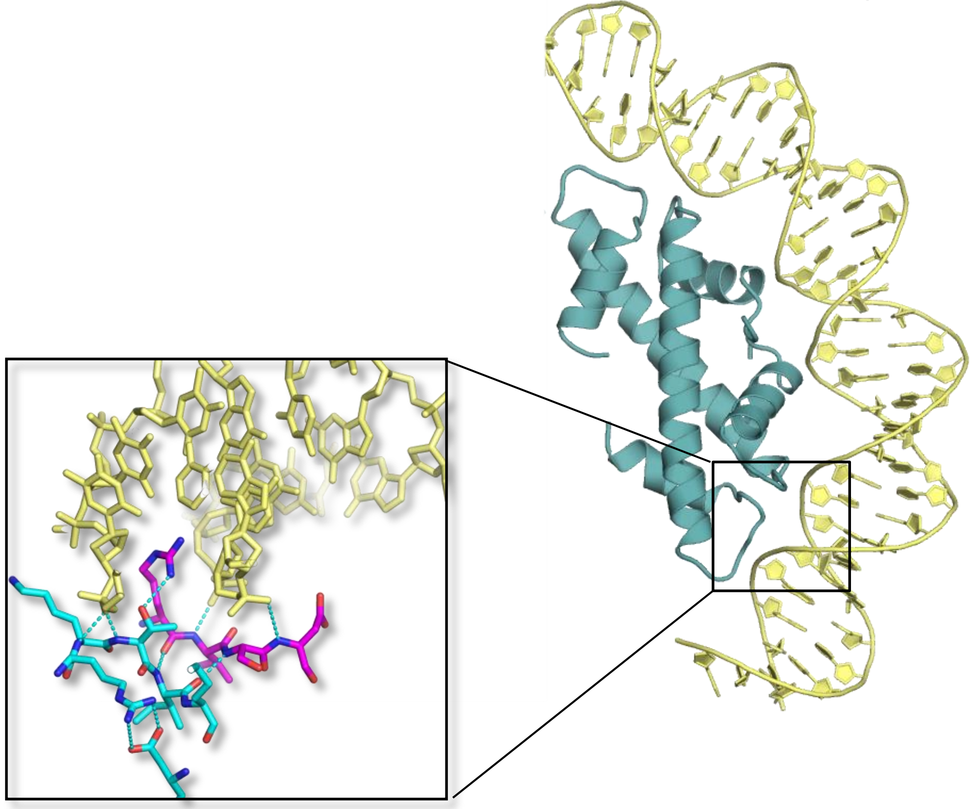
Opens stochastically

Allison White

DNA organization by the histone fold is very similar between archaea and eukaryotes

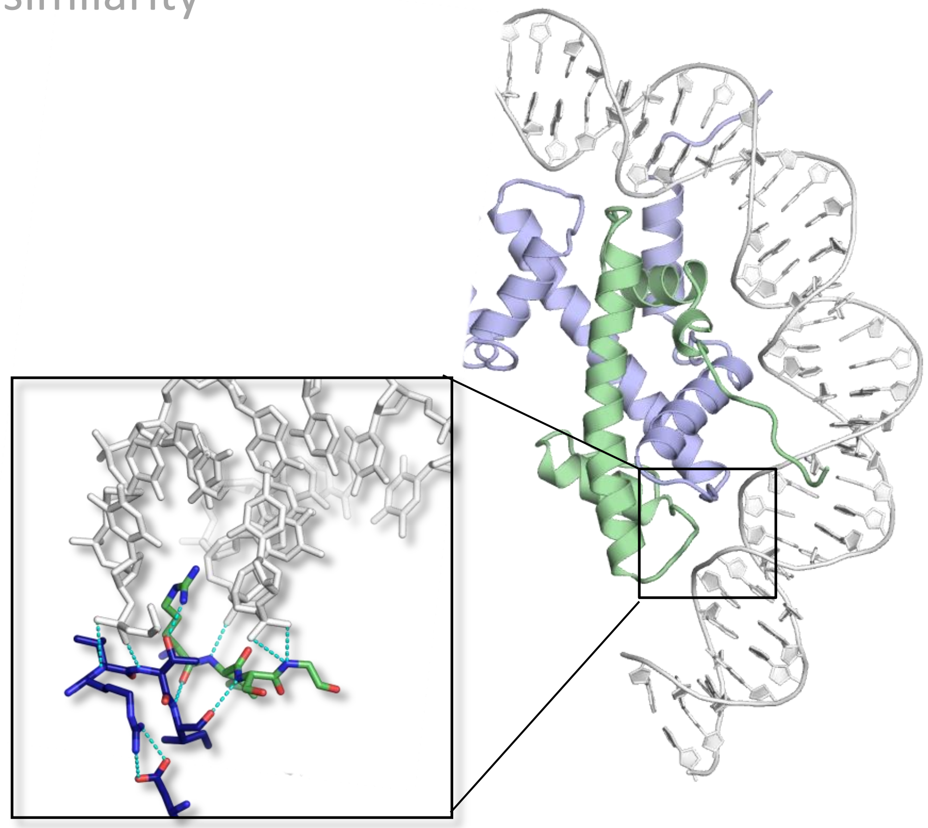
Crystal structure of 3 histone dimers with 90 bp of DNA

Archaeal chromatin



~ 40% amino acid sequence similarity

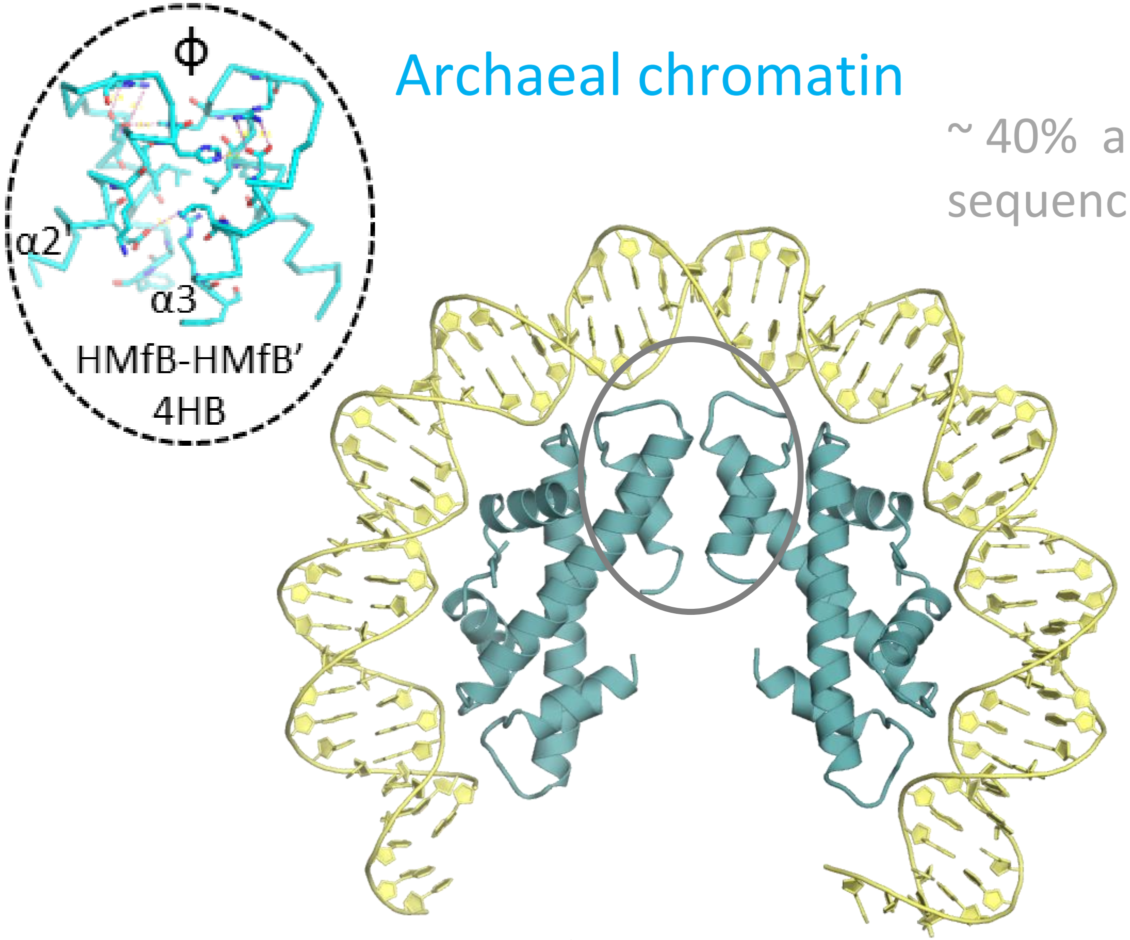
Eukaryotic nucleosome



Francesca Mattiroli, Sudipta Bhattacharayya, Pamela Dyer, Kathy Sandman [*Science* **357**, 609-612 (2017)]

Histone fold dimers interact through conserved ‘four-helix-bundle’ structures

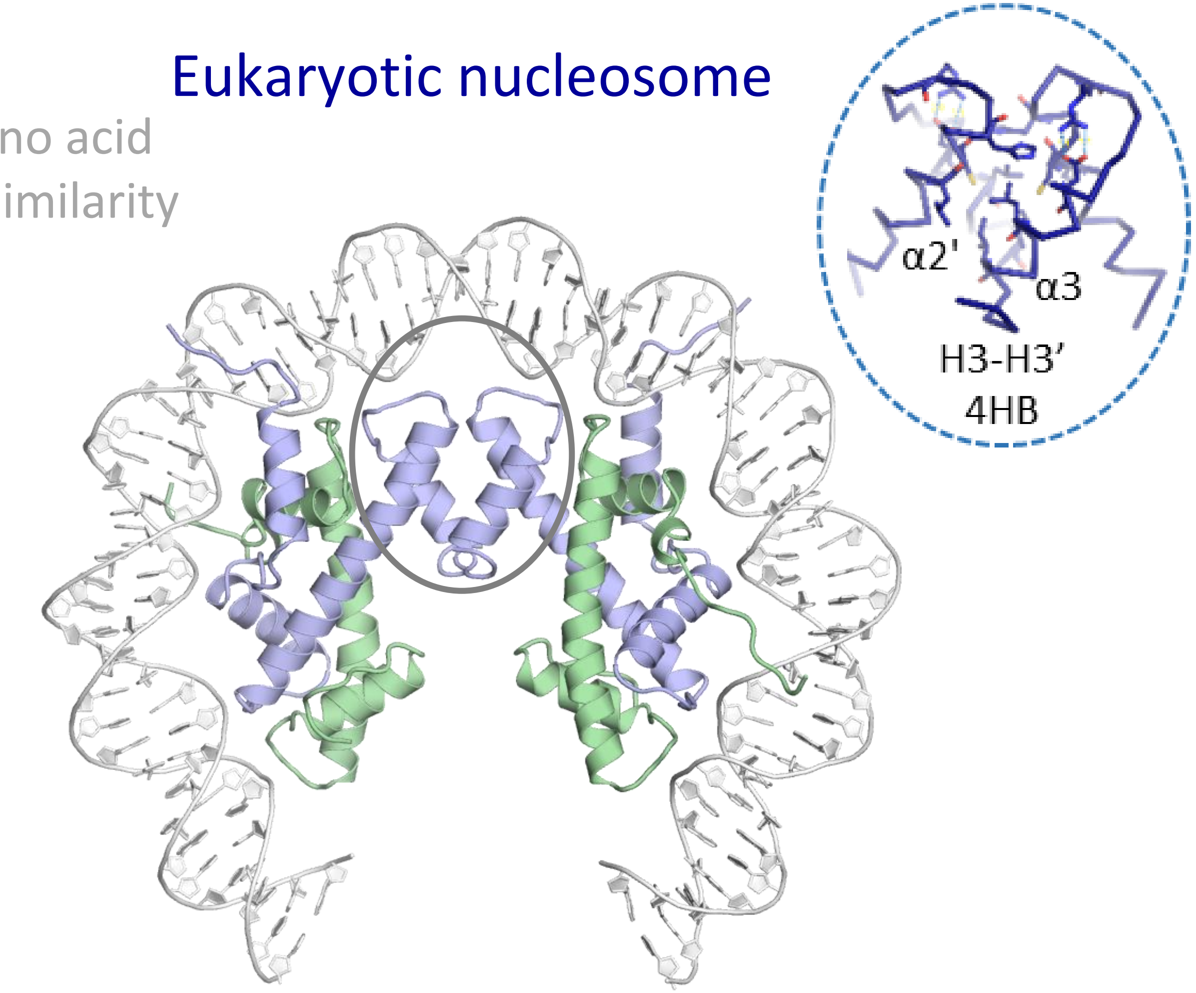
Archaeal chromatin



~ 70 base pairs DNA

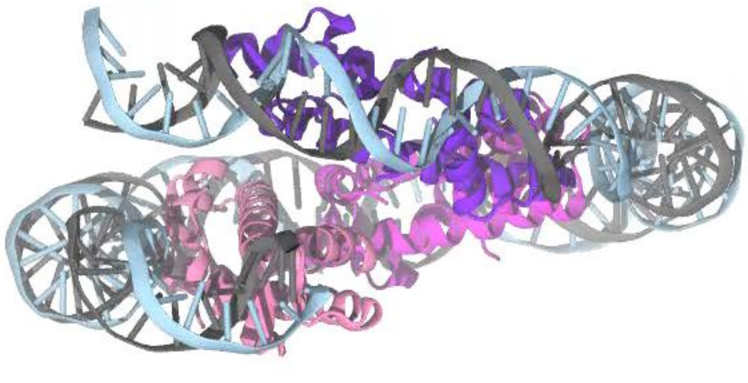
~ 40% amino acid sequence similarity

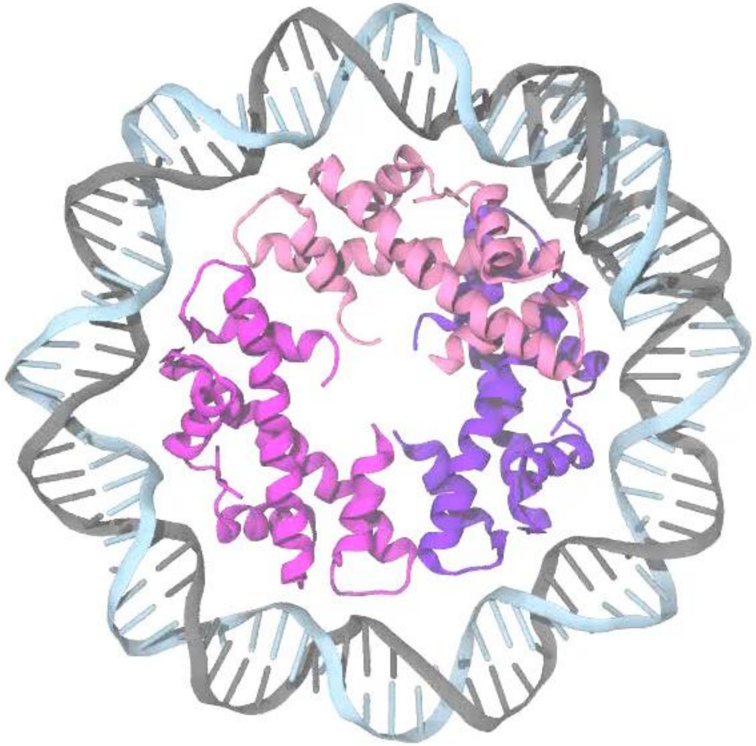
Eukaryotic nucleosome



The ‘Arc90 unit’ is highly dynamic

All atom MD, 300 ns per simulation,
3 independent simulations





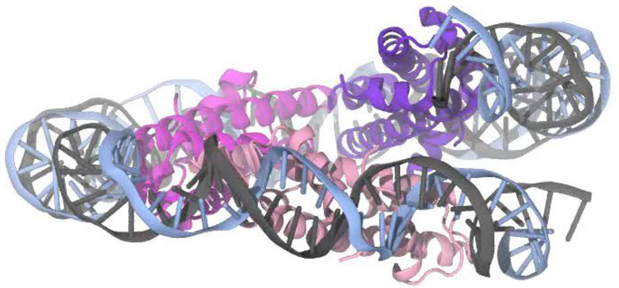
Arc90

90 bp of DNA

3 histone dimers

Bowerman et al., elife 10, 2021

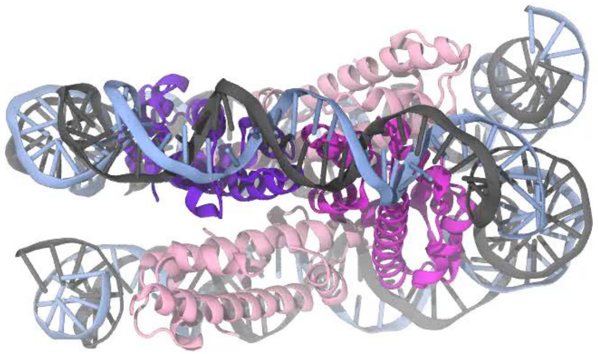
Introducing stacking interactions reduces DNA breathing



Arc90

90 bp of DNA

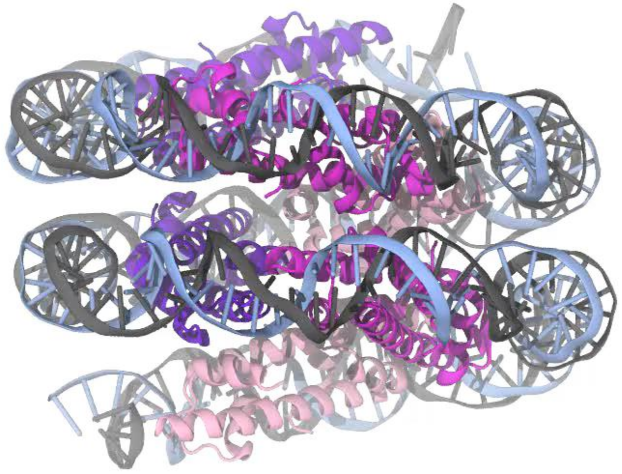
3 histone dimers



Arc120

120 bp of DNA

4 histone dimers



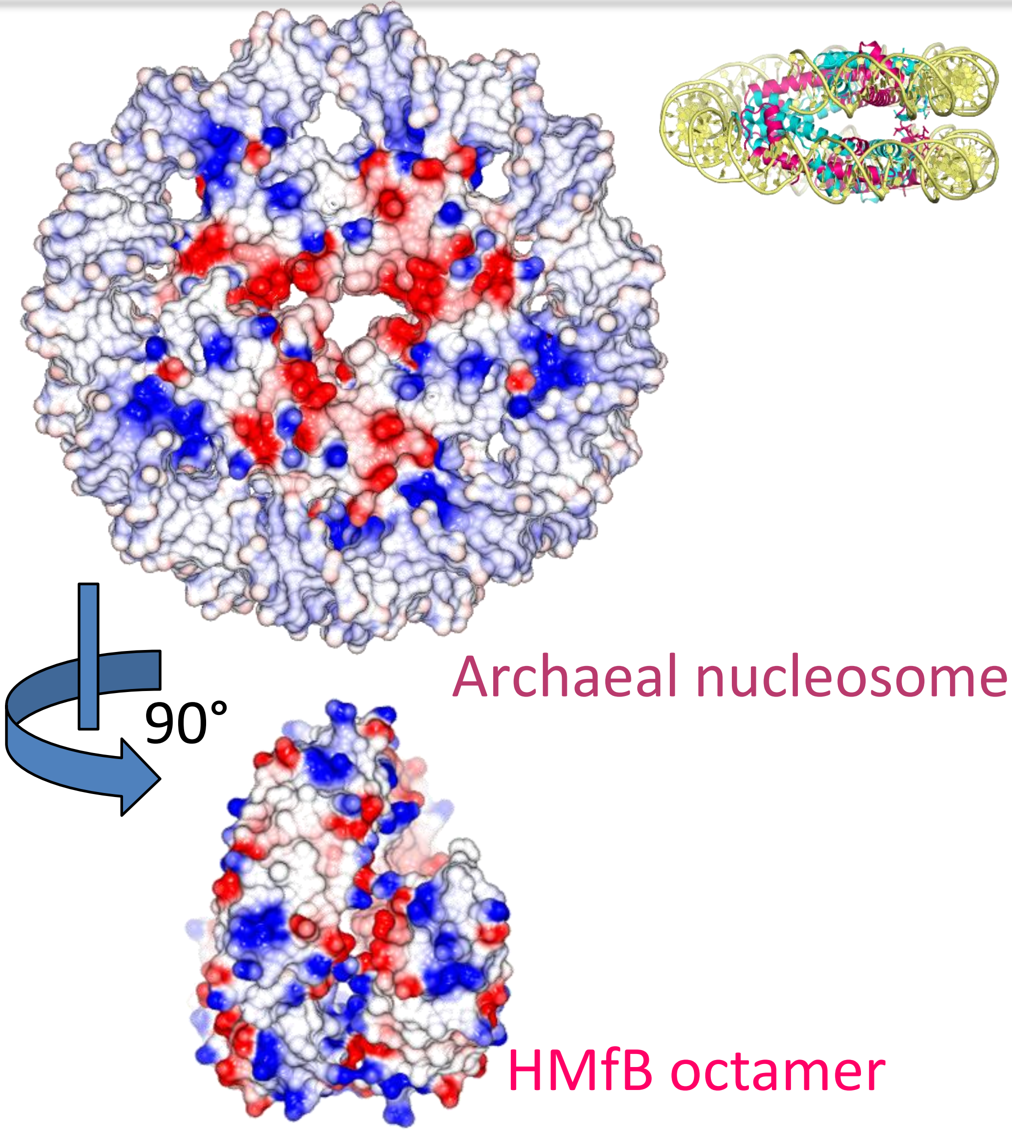
Arc180

190 bp of DNA

6 histone dimers

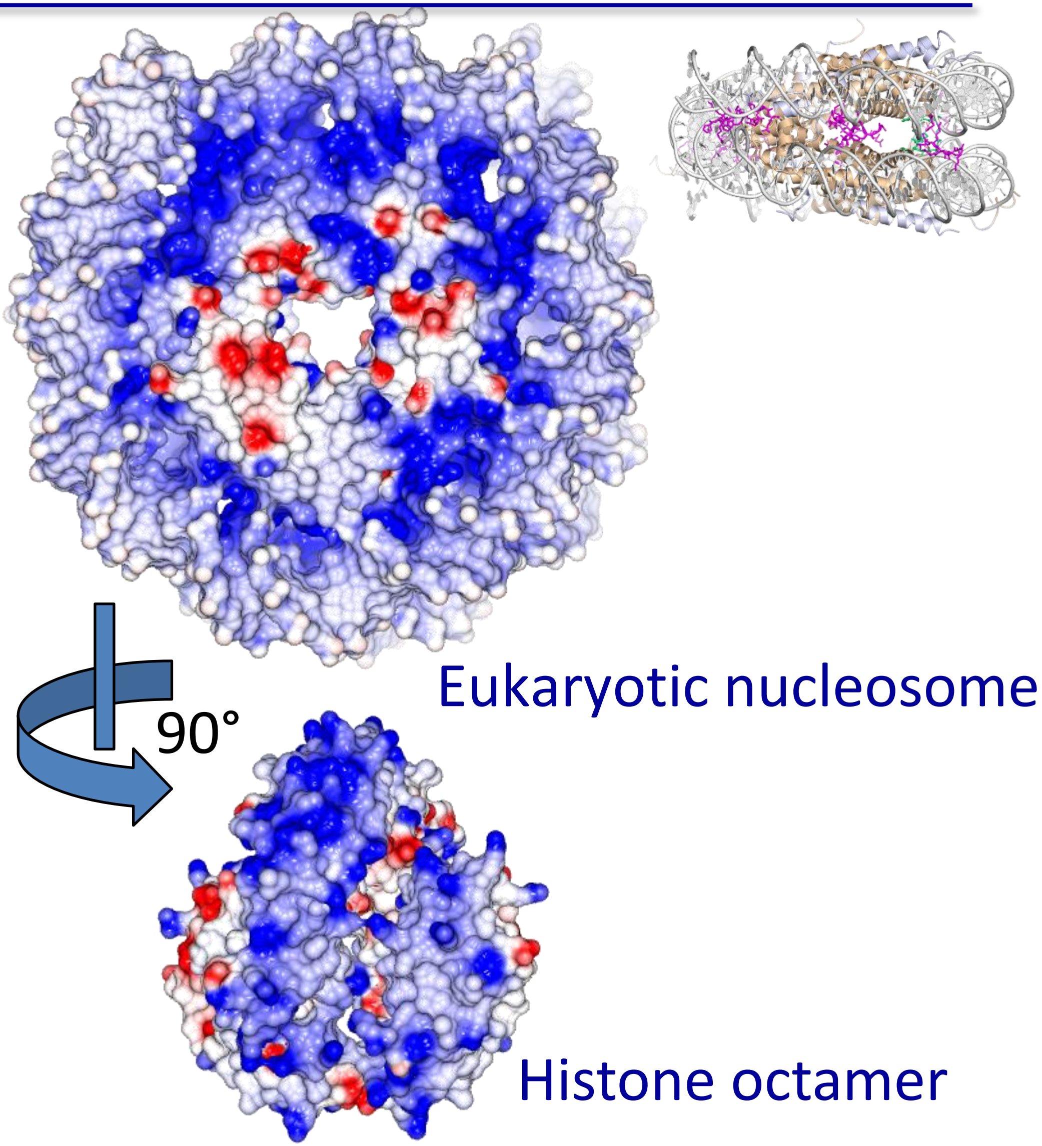
Bowerman et al., elife 10, 2021

More extreme charges and more ‘stuffing' stabilize the eukaryotic nucleosomes



Archaeal nucleosome

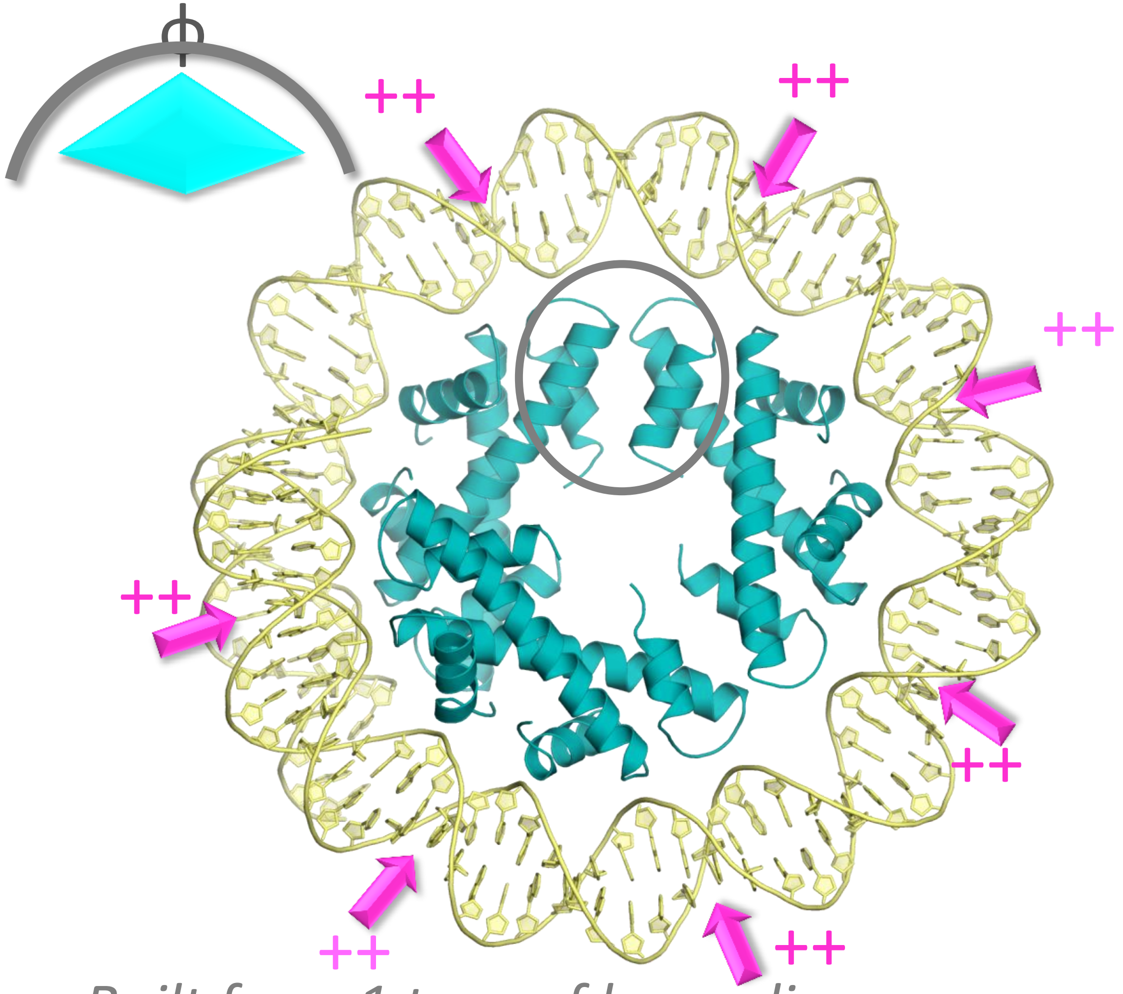
HMfB octamer



Eukaryotic nucleosome

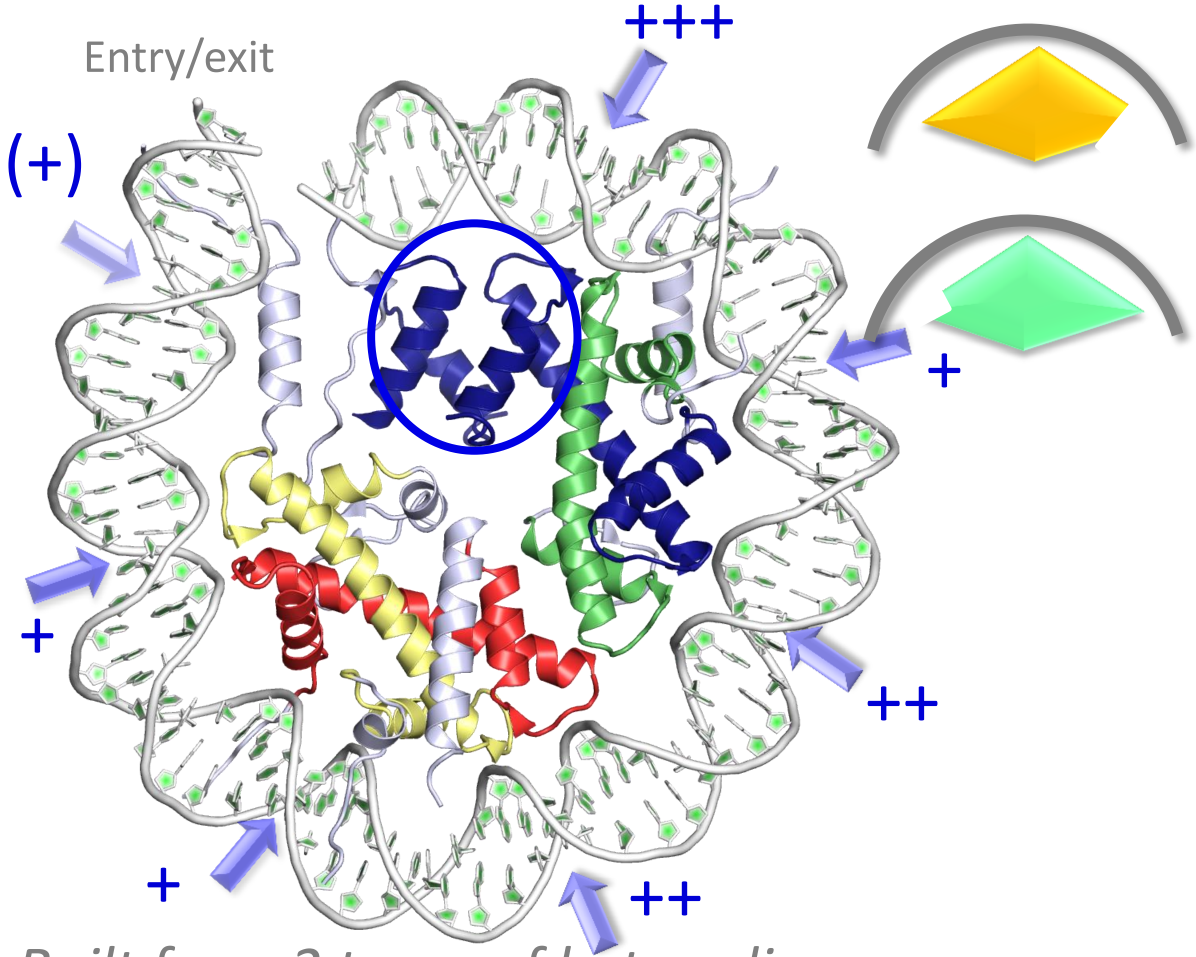
Histone octamer

Four histones allow modulation of the strength of DNA interactions



Built from 1 type of homodimer

Archaeal chromatin

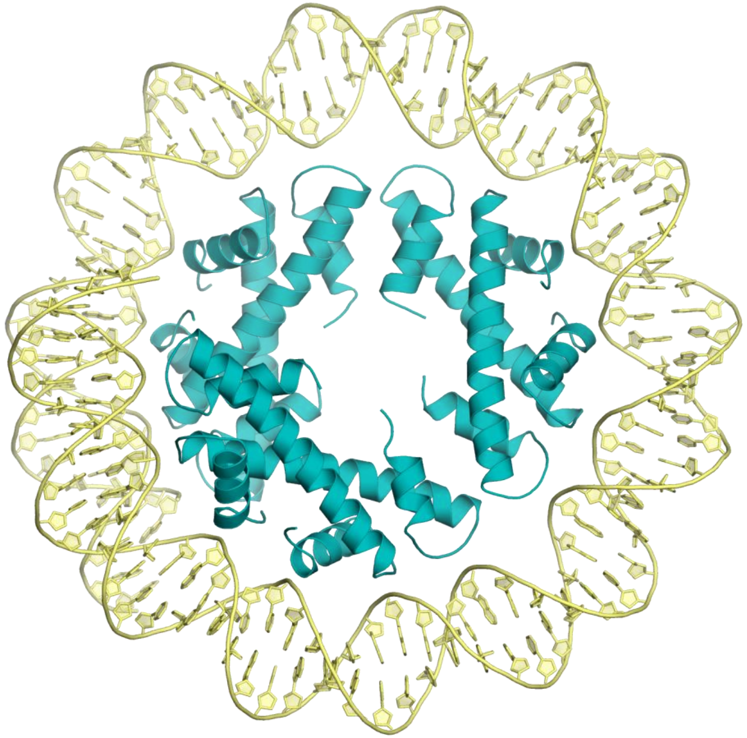


Built from 2 types of heterodimers

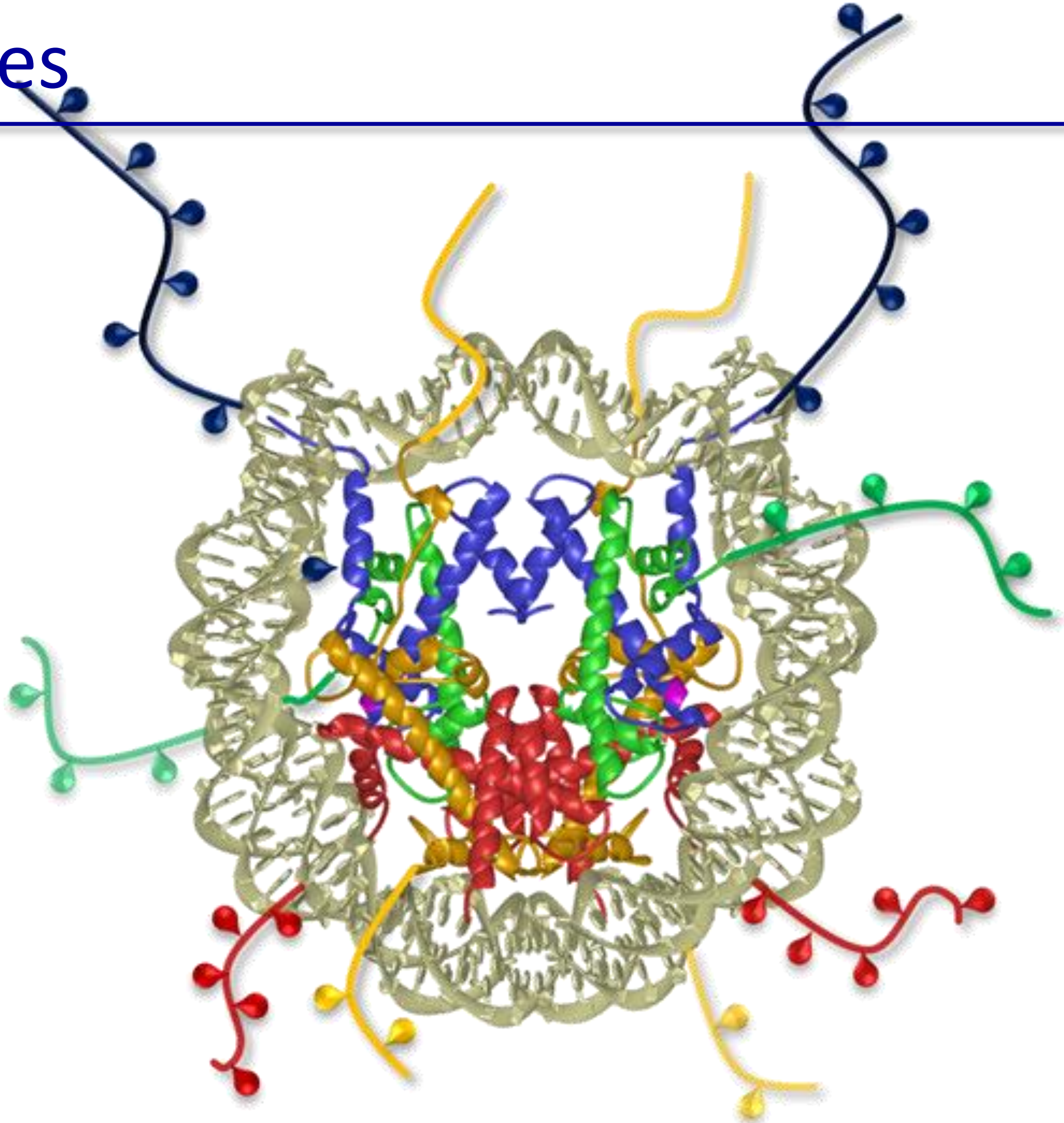
Eukaryotic nucleosome

Post-translational modifications of histone tails, and histone variants are unique to eukaryotes

No readers, writers, erasers

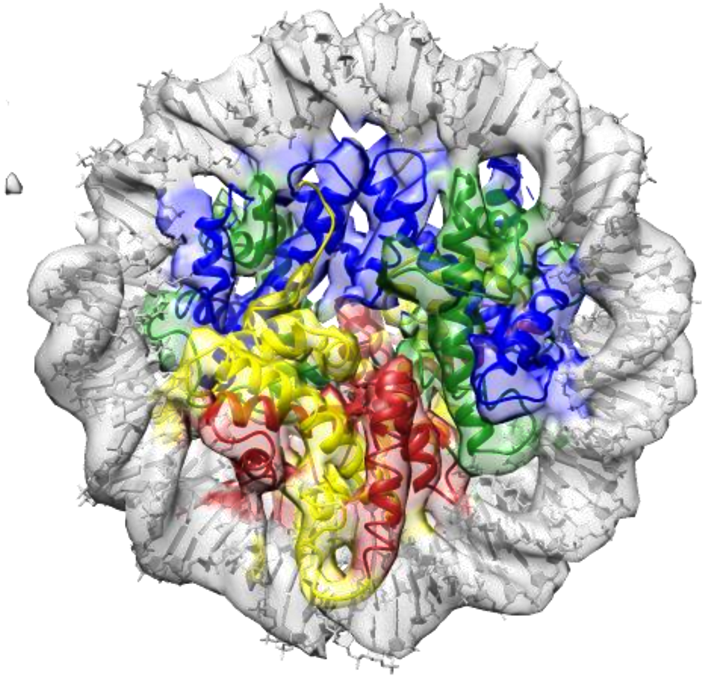


Archaeal nucleosome



Eukaryotic nucleosome

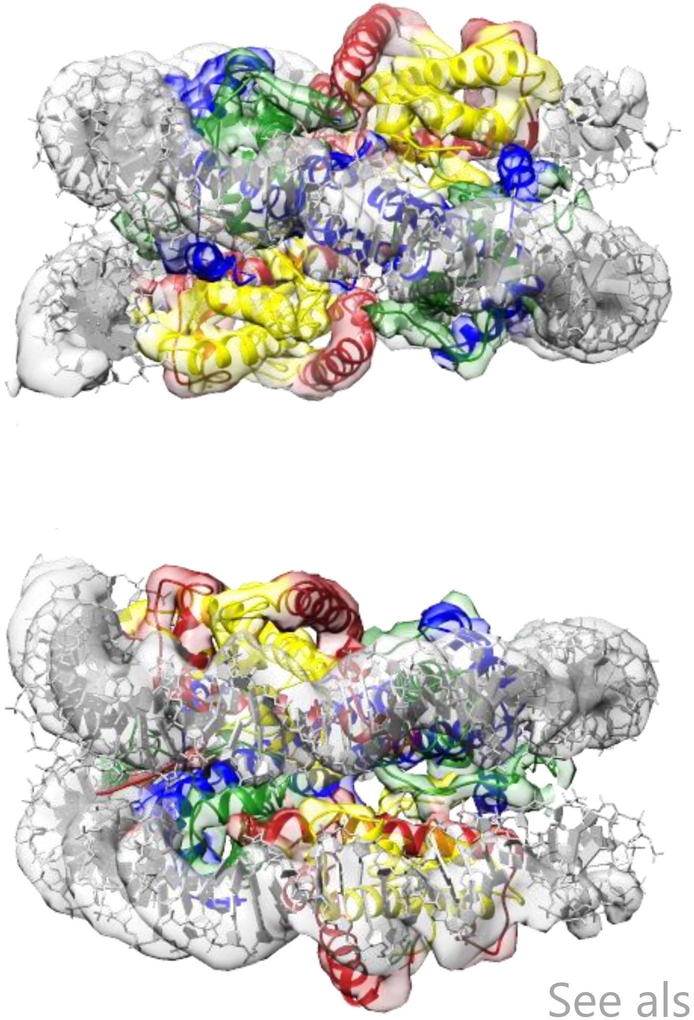
Overall architecture of viral nucleosomes is similar to eukaryotes

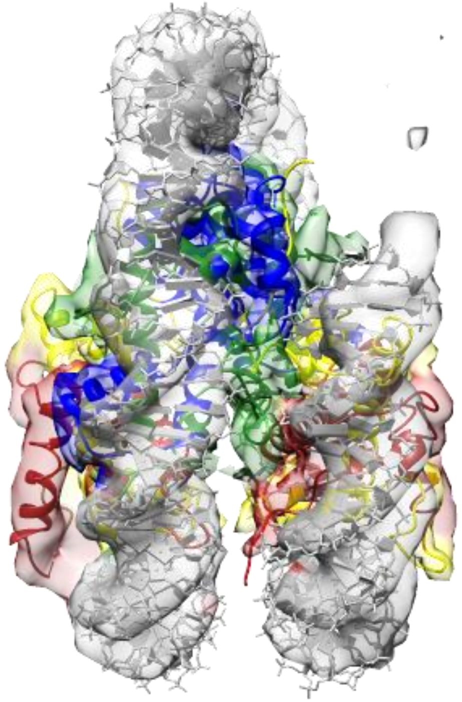


MV-H2B-H2A MV-H4-H3

Fit with homology-modeled histones

Connectors built ‘from scratch’



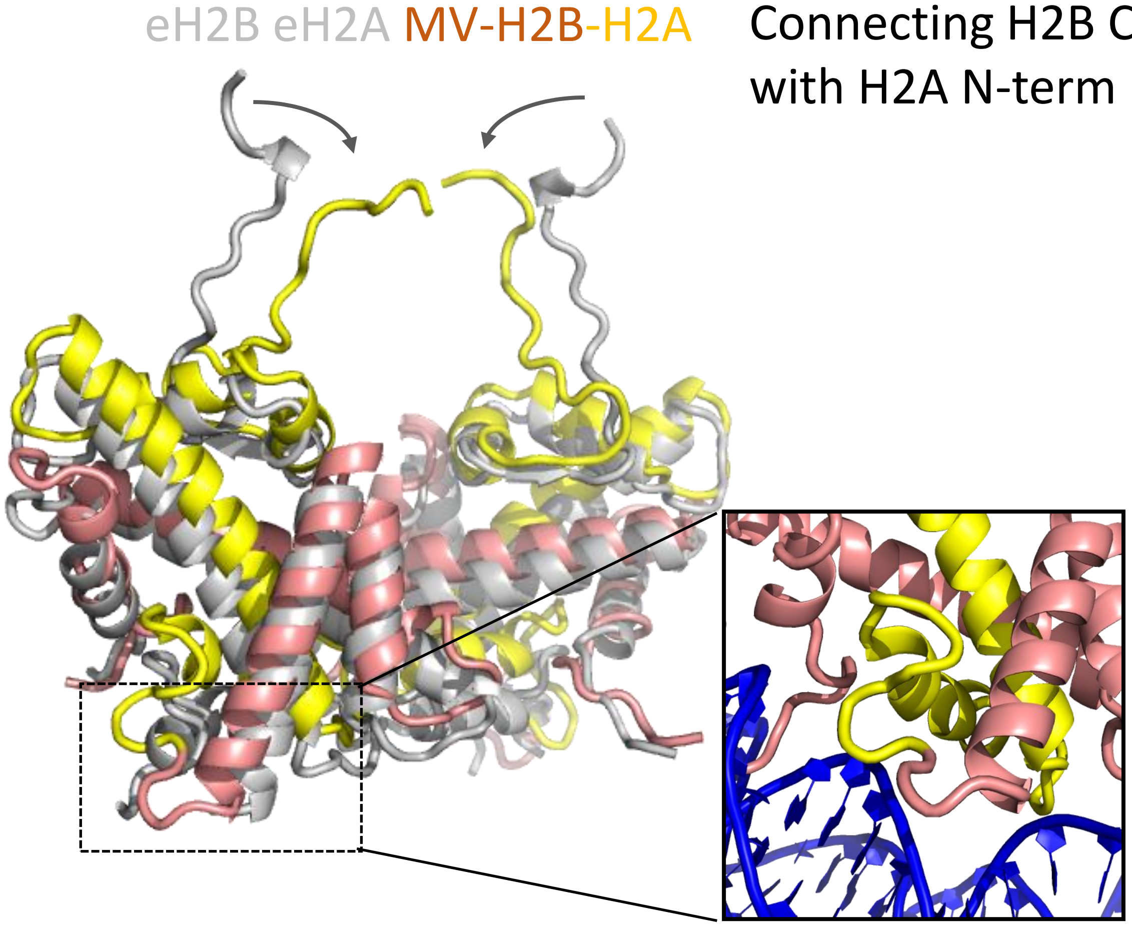


Liu, Toner, Zhou….. Cell, 2021

See also

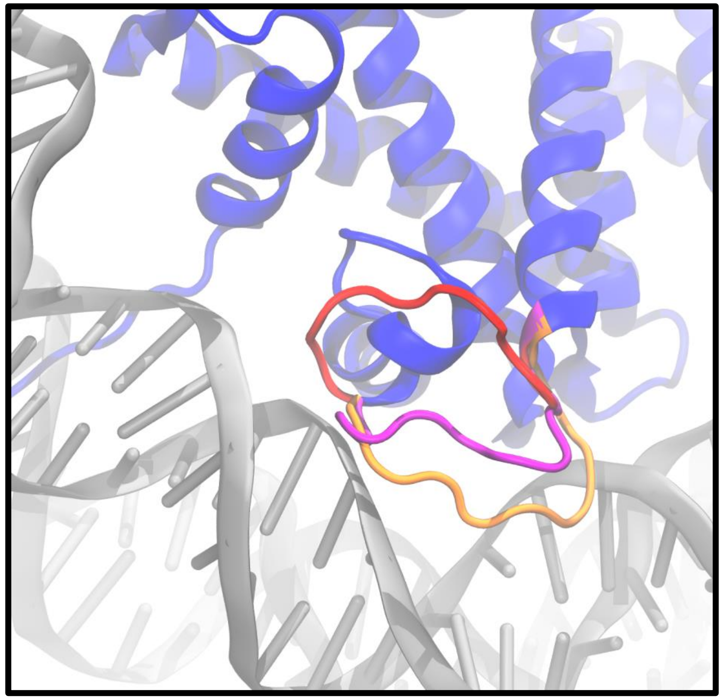
Valencia-Sanchez, (2021). Nat Struct Mol Biol.

Unique to viral histones: connectors to make the doublets: H2B-H2A



Connecting H2B C-term with H2A N-term is easy

MV-H2B-H2A doublet connector



De novo building and minimization
(not a lot of density)

Chelsea Toner, Samuel Bowerman

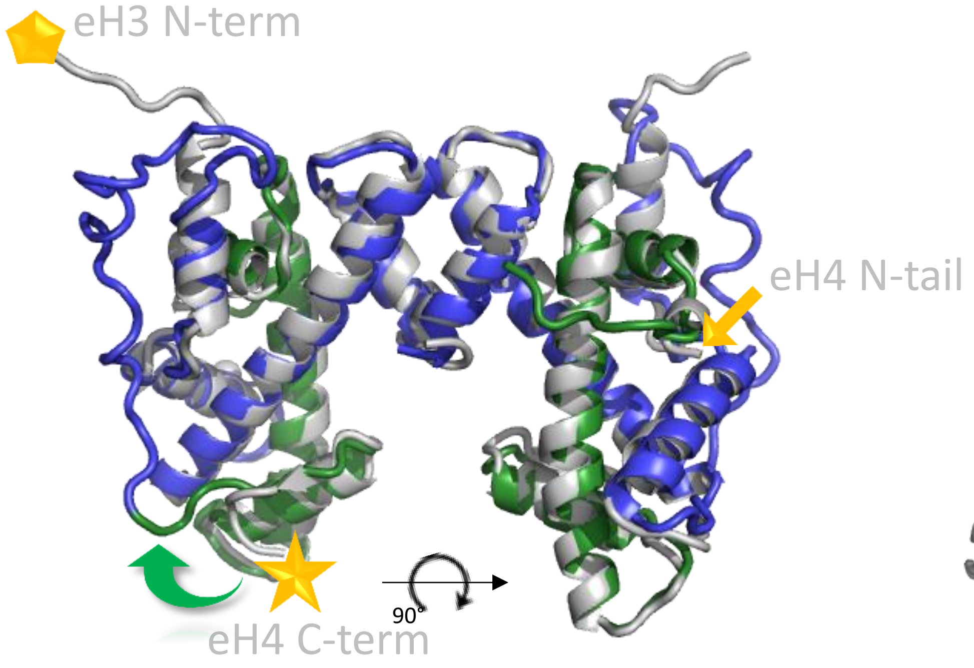
Connecting H4 C-term with the H3 N-terminal tail: structural roles

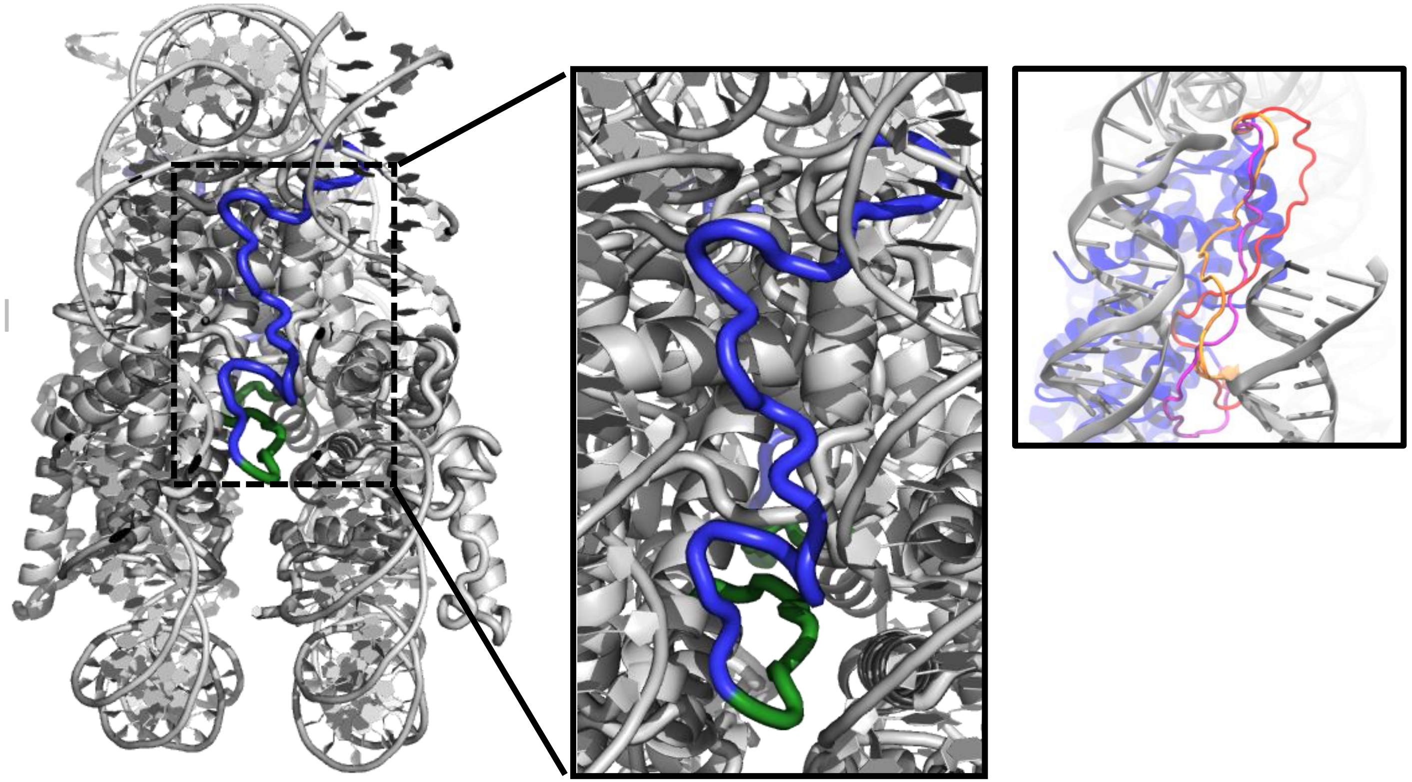
H4-H3 linker

MV-H4 extension

MV-H4-H3 doublet connector

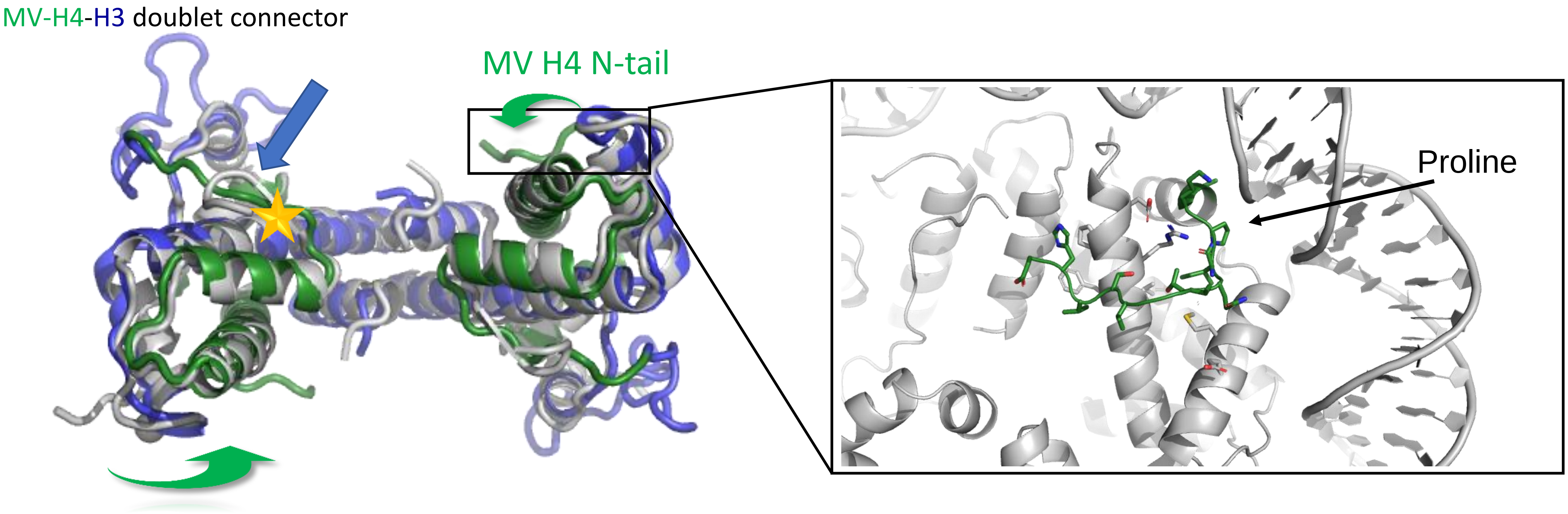
MV-H4-H3 doublet connector





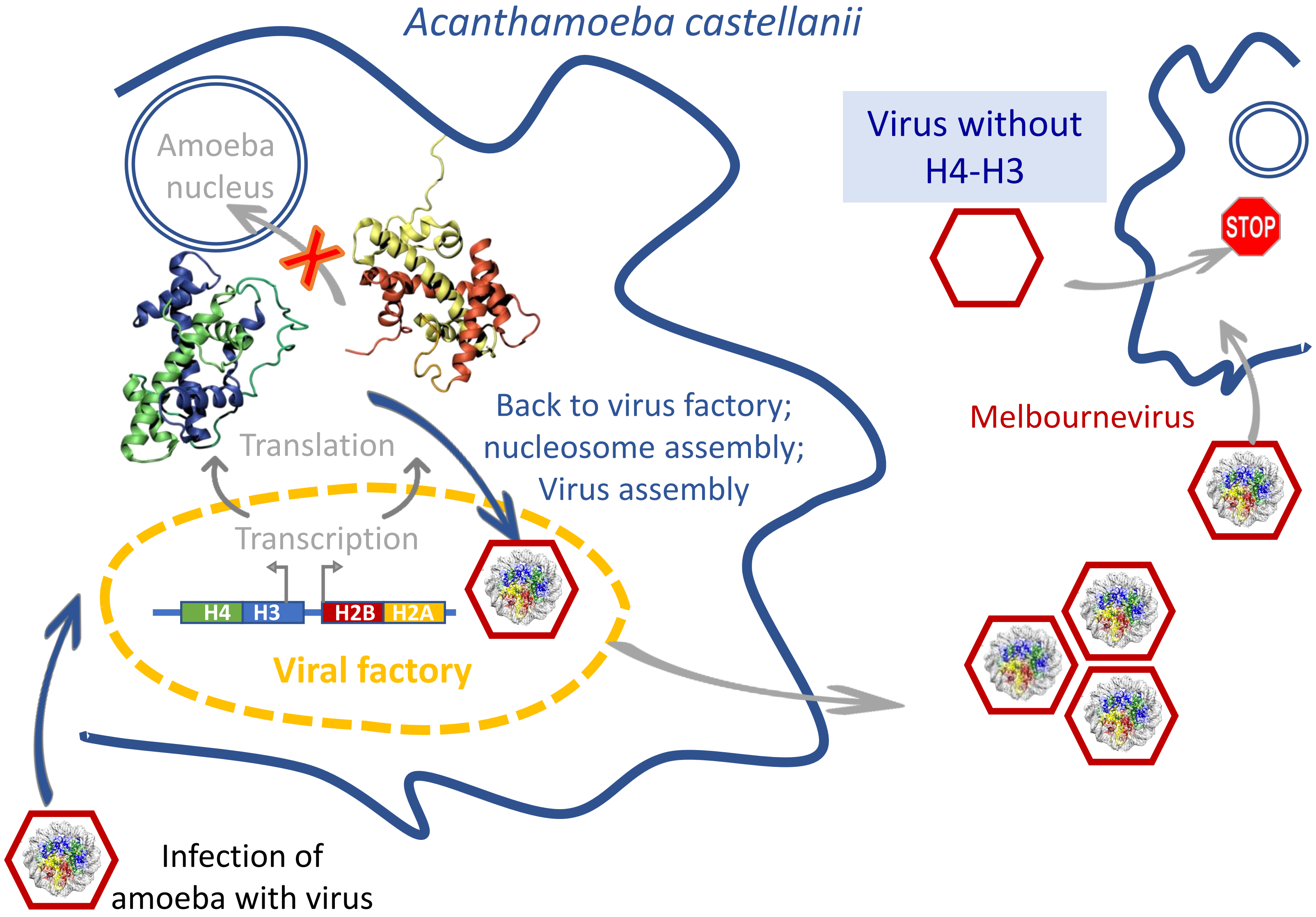
Chelsea Toner, Samuel Bowerman

The MV-H4 N-terminal tail interacts with the histone fold



Chelsea Toner, Samuel Bowerman

Are histones needed for virus fitness / infectivity?

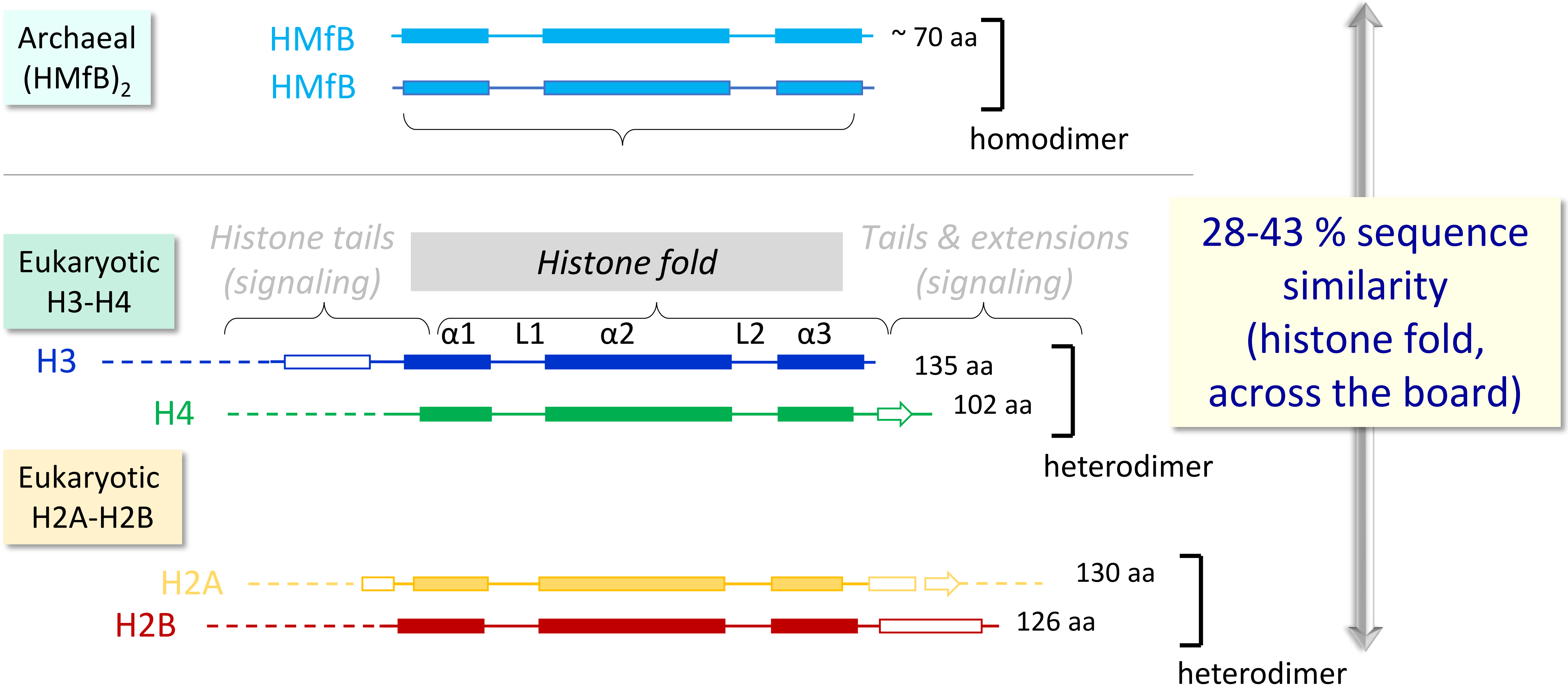


Knockout of H4-H3:
- One histone pair missing [GFP]
- Virus particles assemble but are not infectious
- Amoeba lives!

Complementation with transiently transfected viral H4-H3:
- Assembly of infectious virus particles
- Amoeba dies

Hugo Bisio / Chantal Abergel

‘Histone fold dimers’ are conserved across domains of life



Archaeal
(HMfB)$_2$

HMfB ~ 70 aa
HMfB

homodimer

Eukaryotic
H3-H4

Histone tails (signaling)

Histone fold

Tails & extensions (signaling)

α1 L1 α2 L2 α3

H3 135 aa
H4 102 aa

heterodimer

Eukaryotic
H2A-H2B

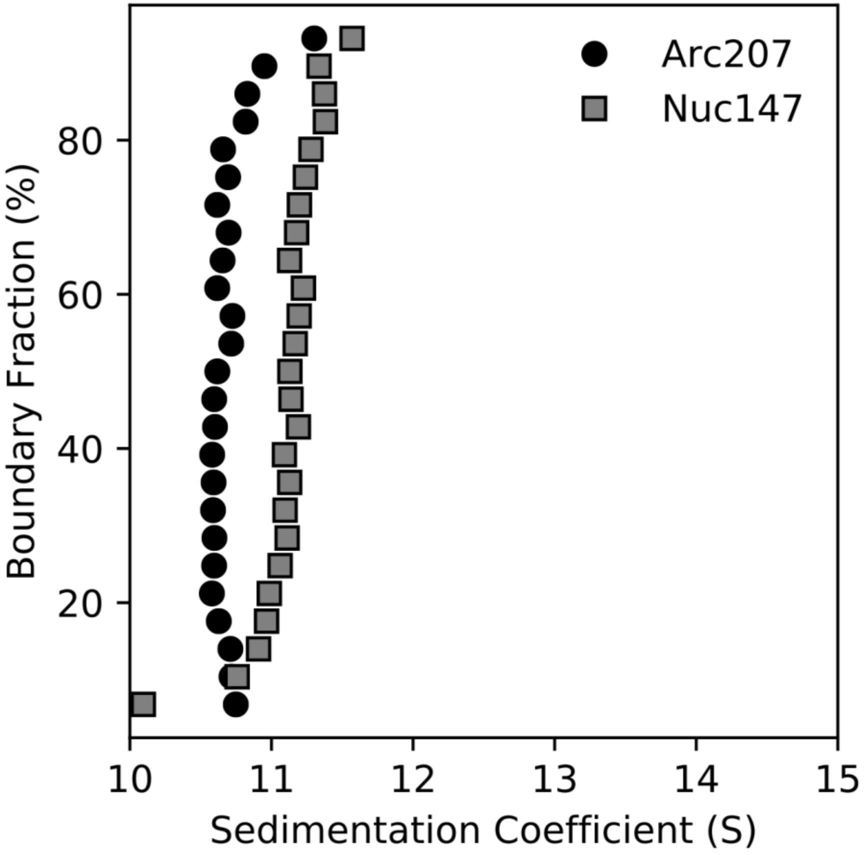
H2A 130 aa
H2B 126 aa

heterodimer

28-43 % sequence similarity
(histone fold, across the board)

Luger et al., Nature 1997; Decanniere et al., JMB 2000

Archaeosomes are ‘floppy’ in solution: Analytical Ultracentrifugation

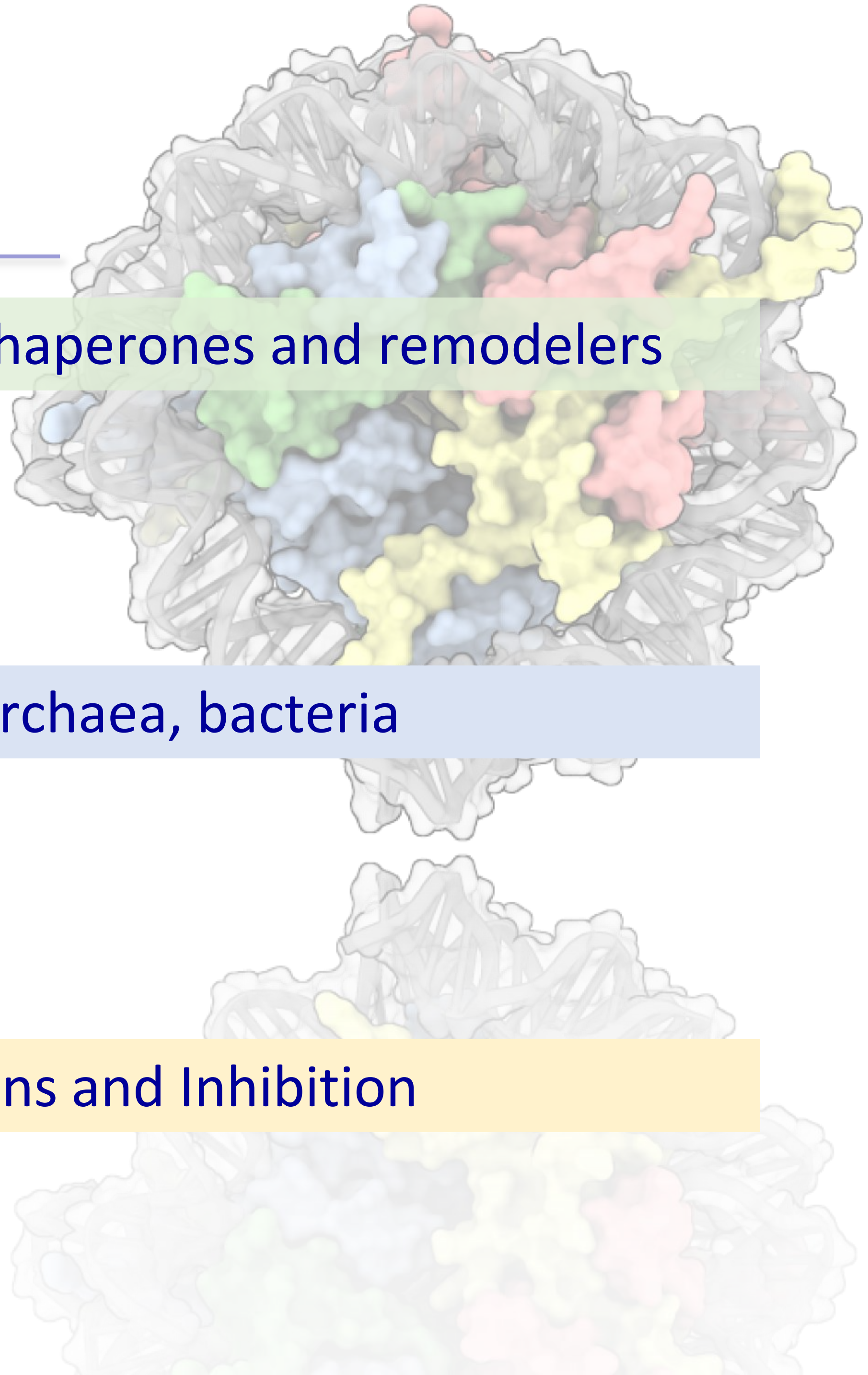


	Nuc147	Arc207	207 DNA
MW_{the} (kDa)	200.3	252.5	127.8
$MW_{GA\text{-}MC}$ (kDa)	218.6 (207.2, 229.9)	266.5 (256.9, 276.1)	156.6 (152.4, 160.1)
f/f_o	1.55 (1.49, 1.61)	1.94 (1.89, 1.99)	3.14 (3.08, 3.20)
Sedimentation Coefficient (S)	11.2 (11.1, 11.2)	10.6 (10.6, 10.7)	6.1 (6.1, 6.1)

Arc207 complex vs. nucleosome (Nuc147):

- Despite higher mass –
- Lower S-value
- Increased frictional coefficient

Three main areas of research

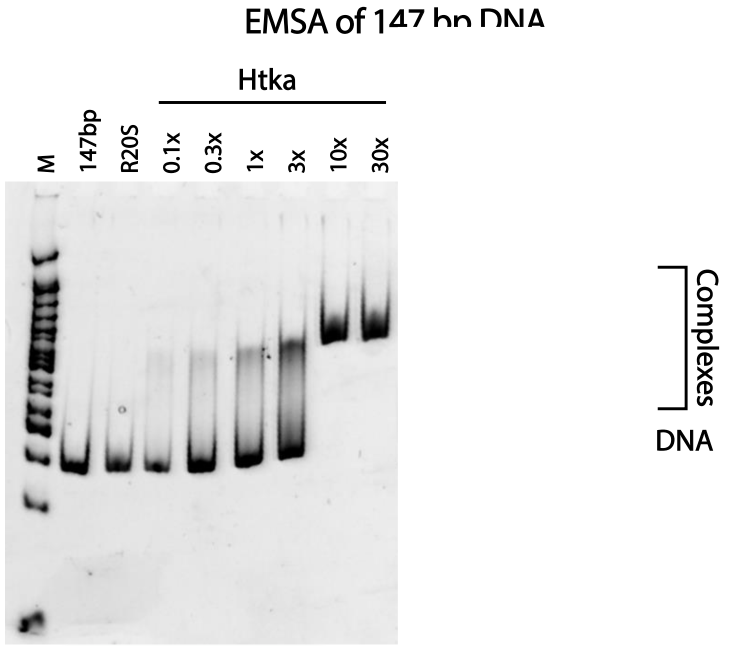


1) Regulators of genome accessibility: Histone chaperones and remodelers

2) Histones in all domains of life: Viruses, archaea, bacteria

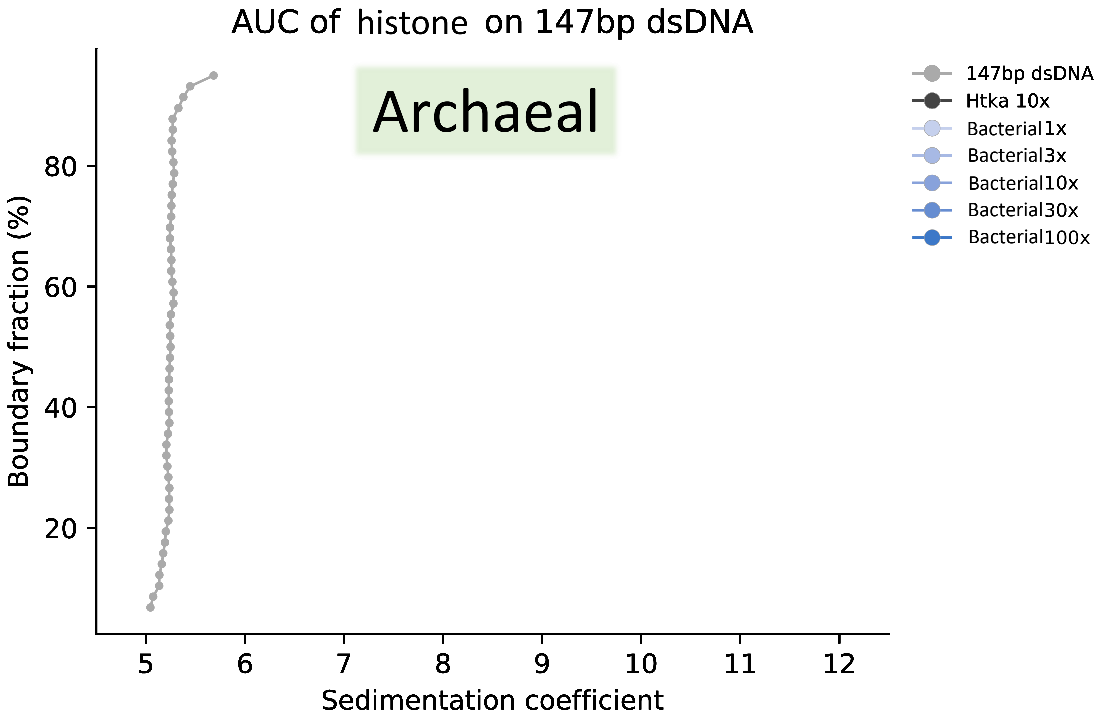
3) PARP and DNA damage repair: Interactions and Inhibition

Bacterial histone has a different DNA binding mode



Archaeal

Bacterial

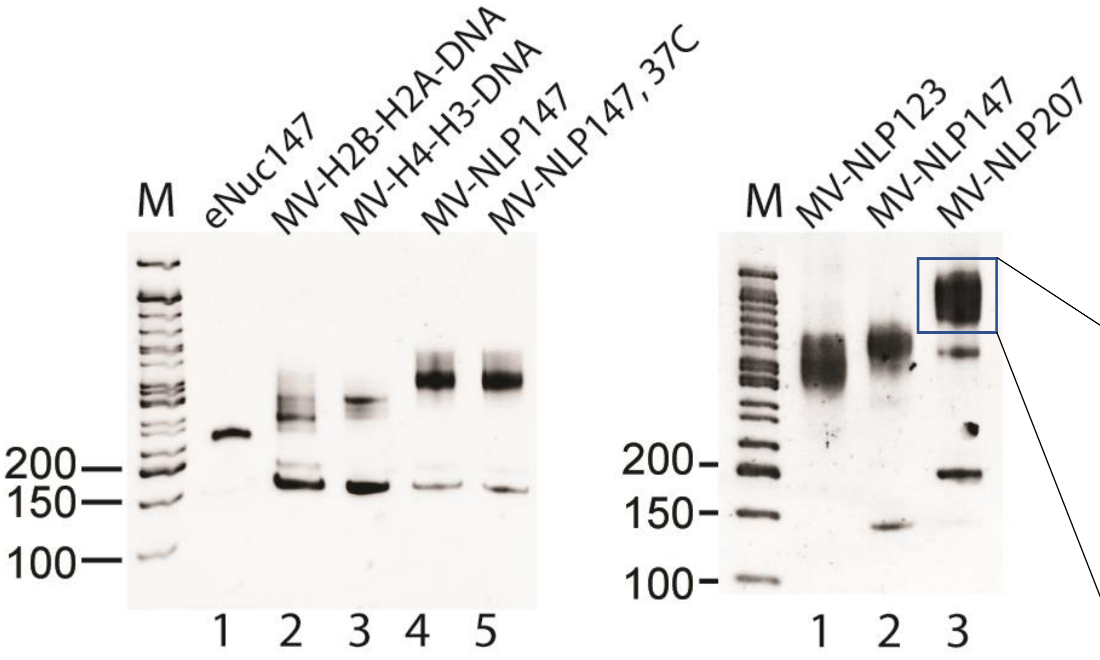


→

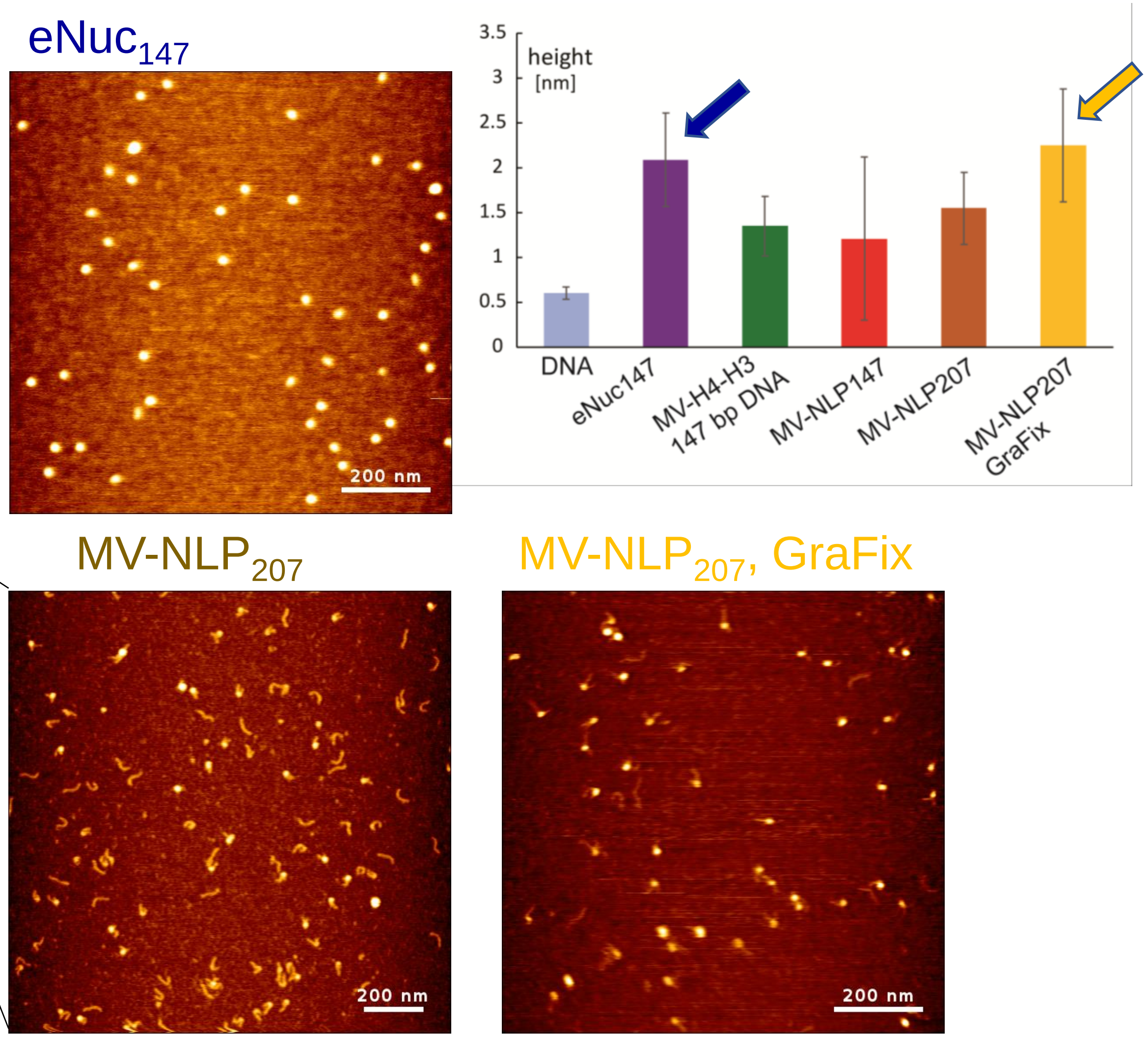
Larger

MV-histones assemble into unstable nucleosome-like particles

Nucleosomes assembled *in vitro* from purified histone doublets

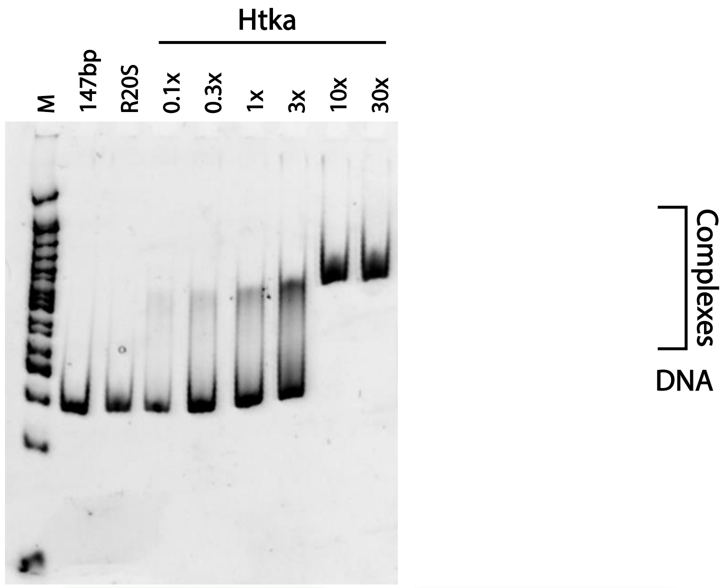


Native PAGE



Atomic Force Microscopy

Bacterial histone has a different DNA binding mode

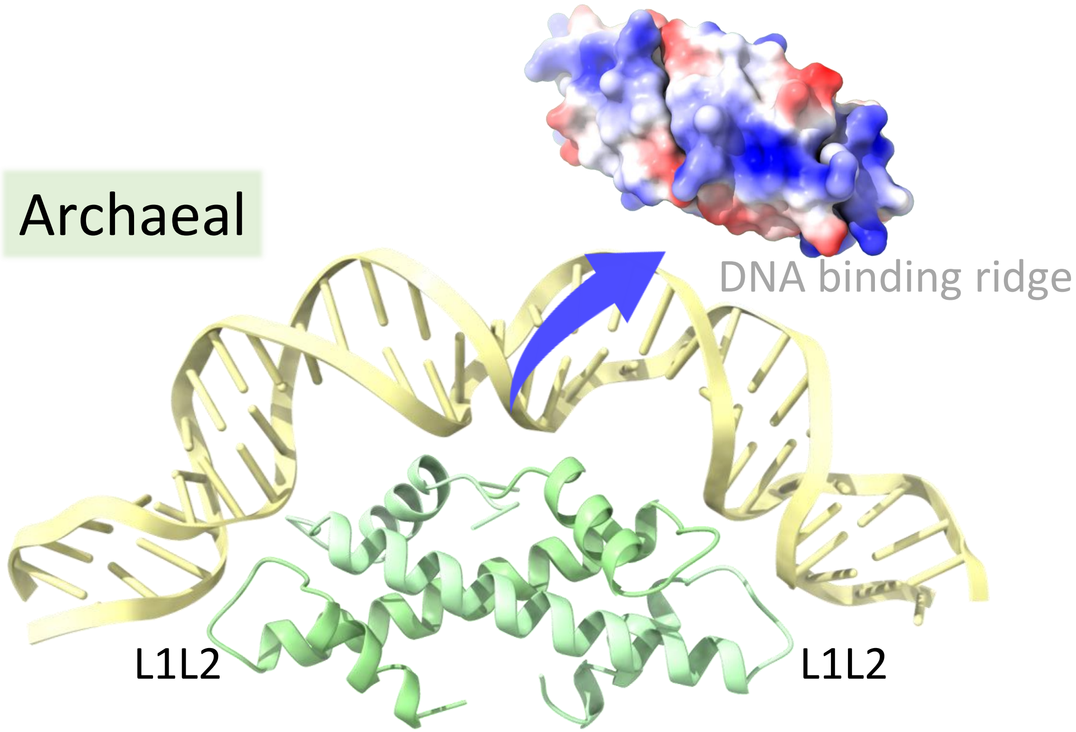


EMSA of 147 bp DNA

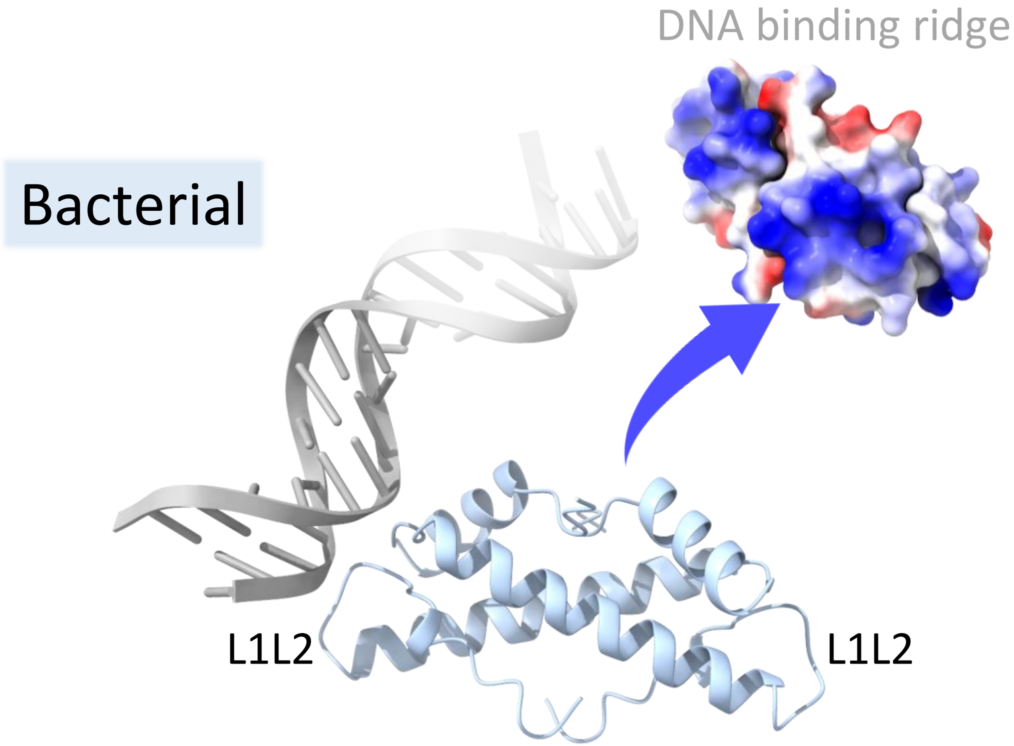
Archaeal

Bacterial

Reason for this unconventional binding mode

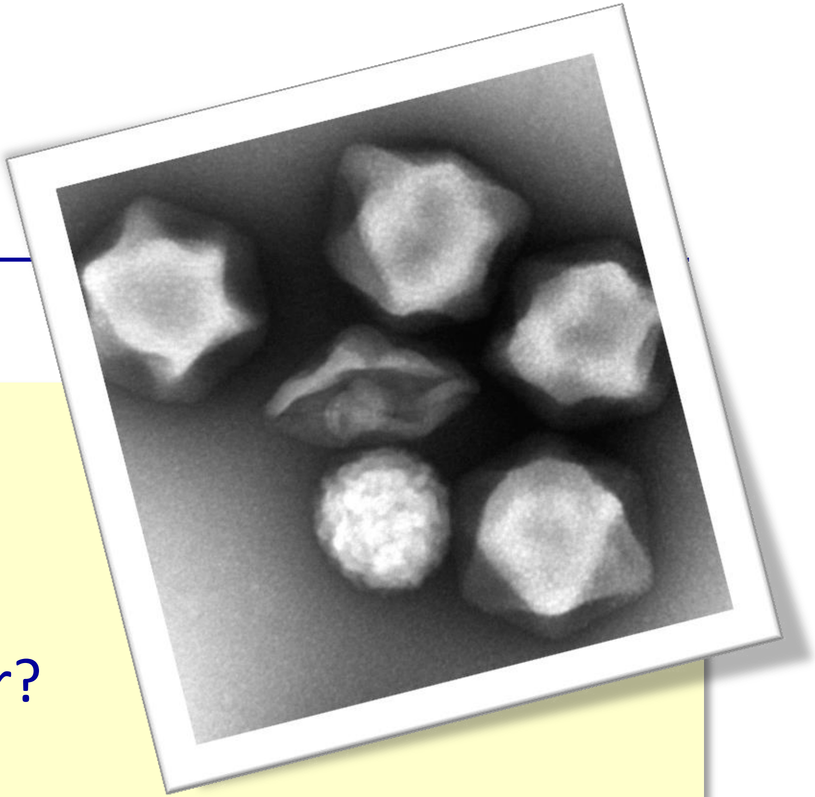


Crystal structure of HMf-DNA complex
Mattiroli, F.…& Luger, K, 2017, PMC5747315



2.4 Å crystal structure of
Bd0055-DNA complex

Viral histone doublets in Melbournevirus

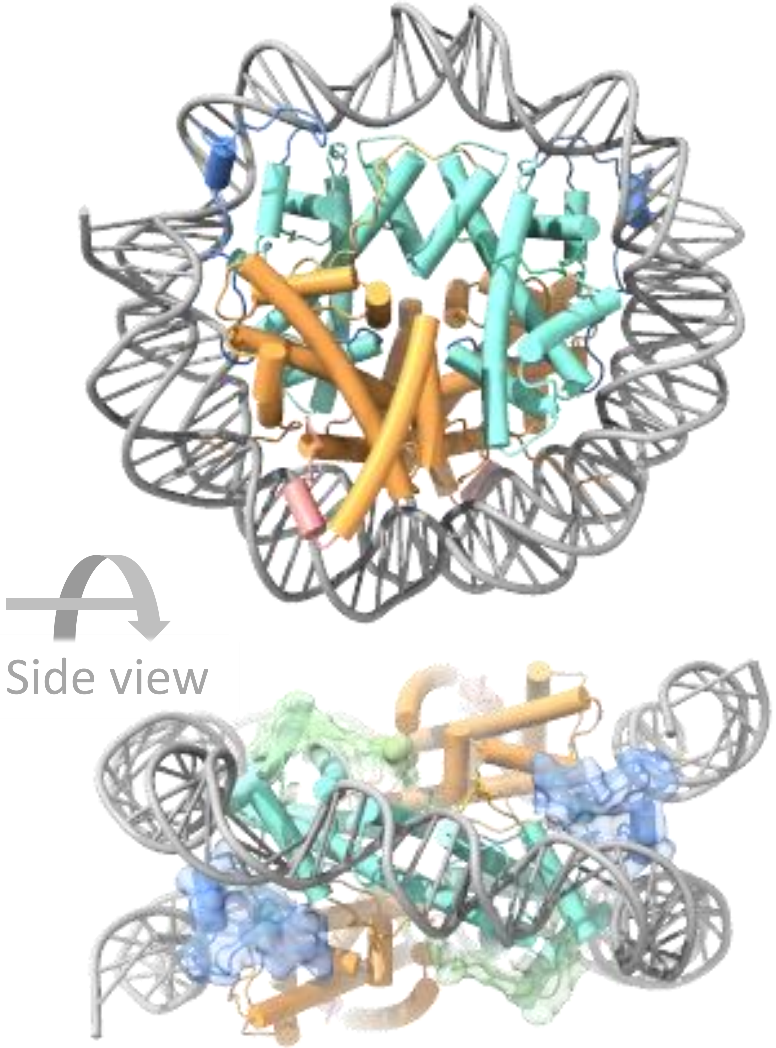


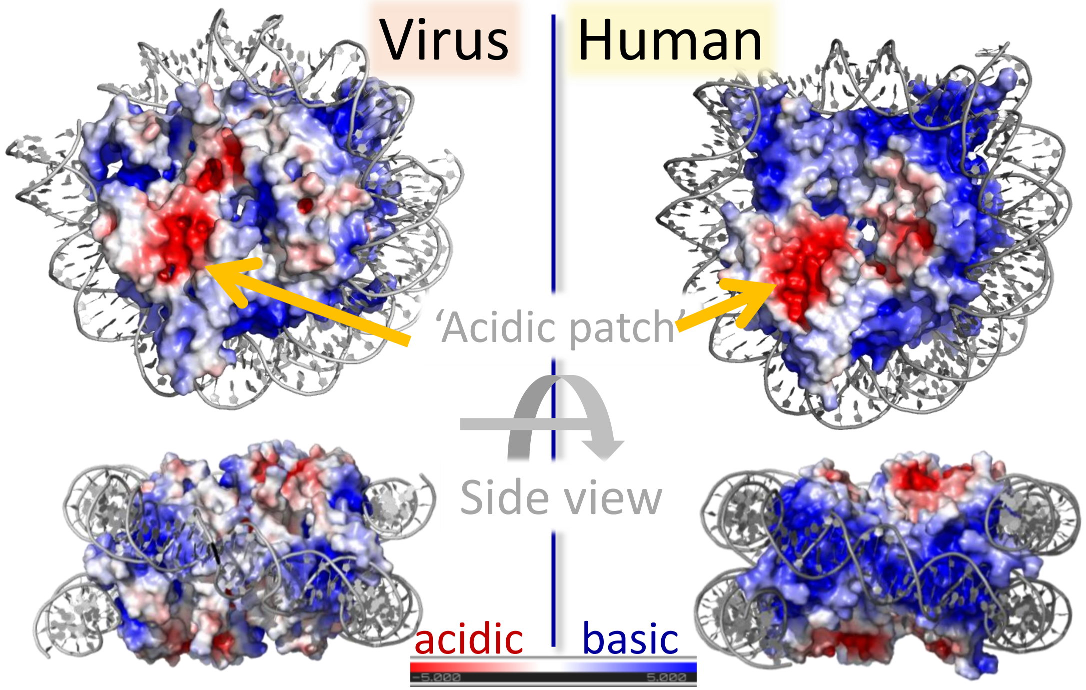
- Can they bind DNA / form nucleosomes?
- When / how do they arrive at the 'viral factory'?
- Does the virus <u>need</u> its own histones, and if yes, what for?
- Where did they come from? (virus ↔ eukaryote?)

Liu, Bisio, Toner, Jeudy, Philippe, Zhou, Bowerman, White, Edwards, Abergel, Luger
[Cell, 2021]

Melbournevirus nucleosomes: insights from the structure

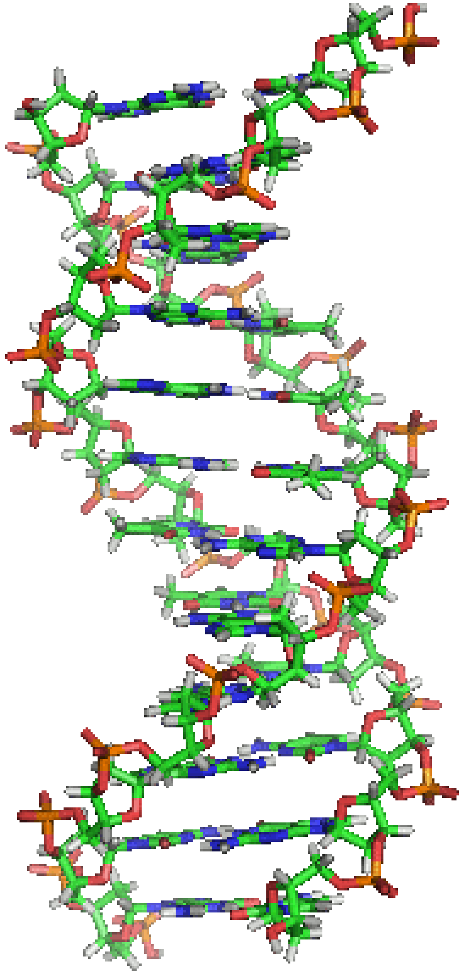
- Connectors and tails have structural functions
- Fewer positive charges
- Distinct surface (higher order structure?)
- Destabilized nucleosomes

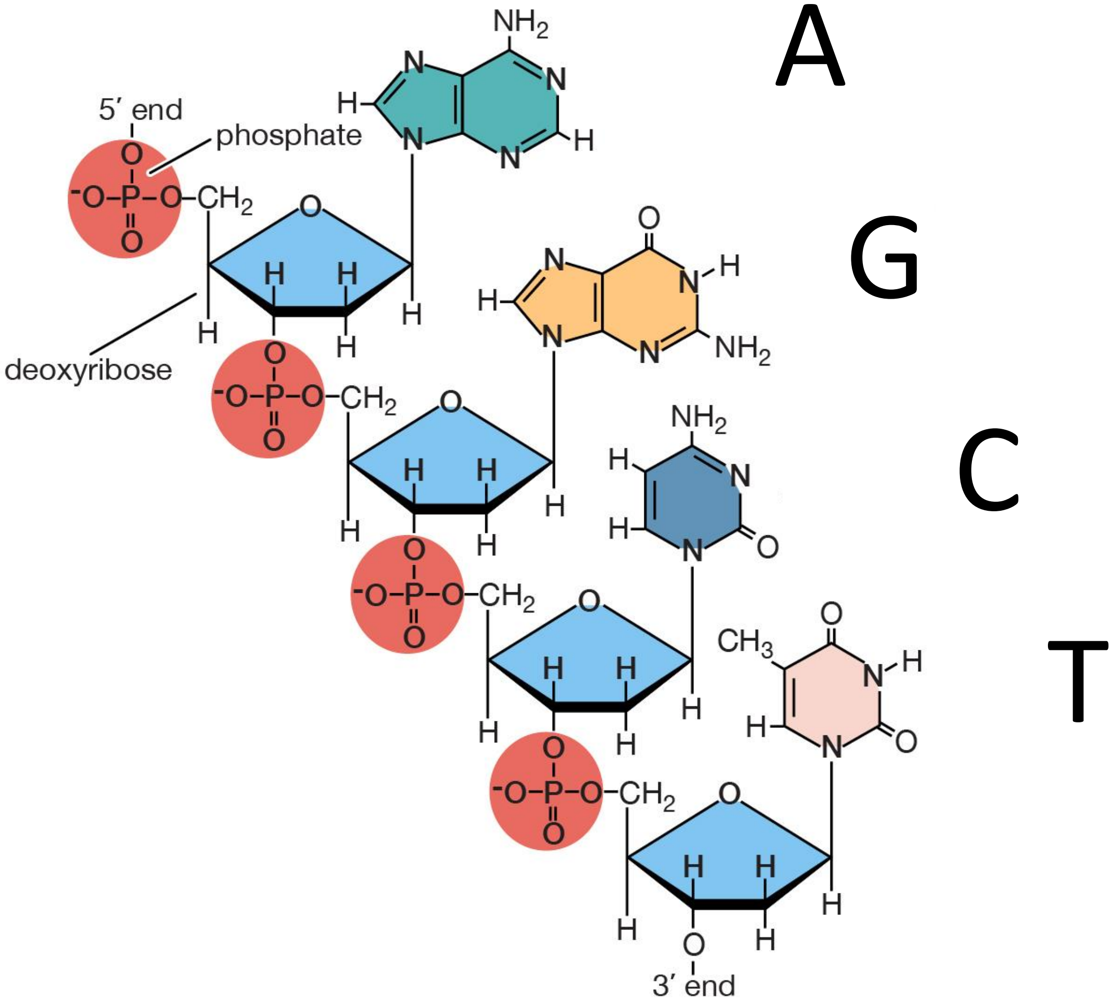


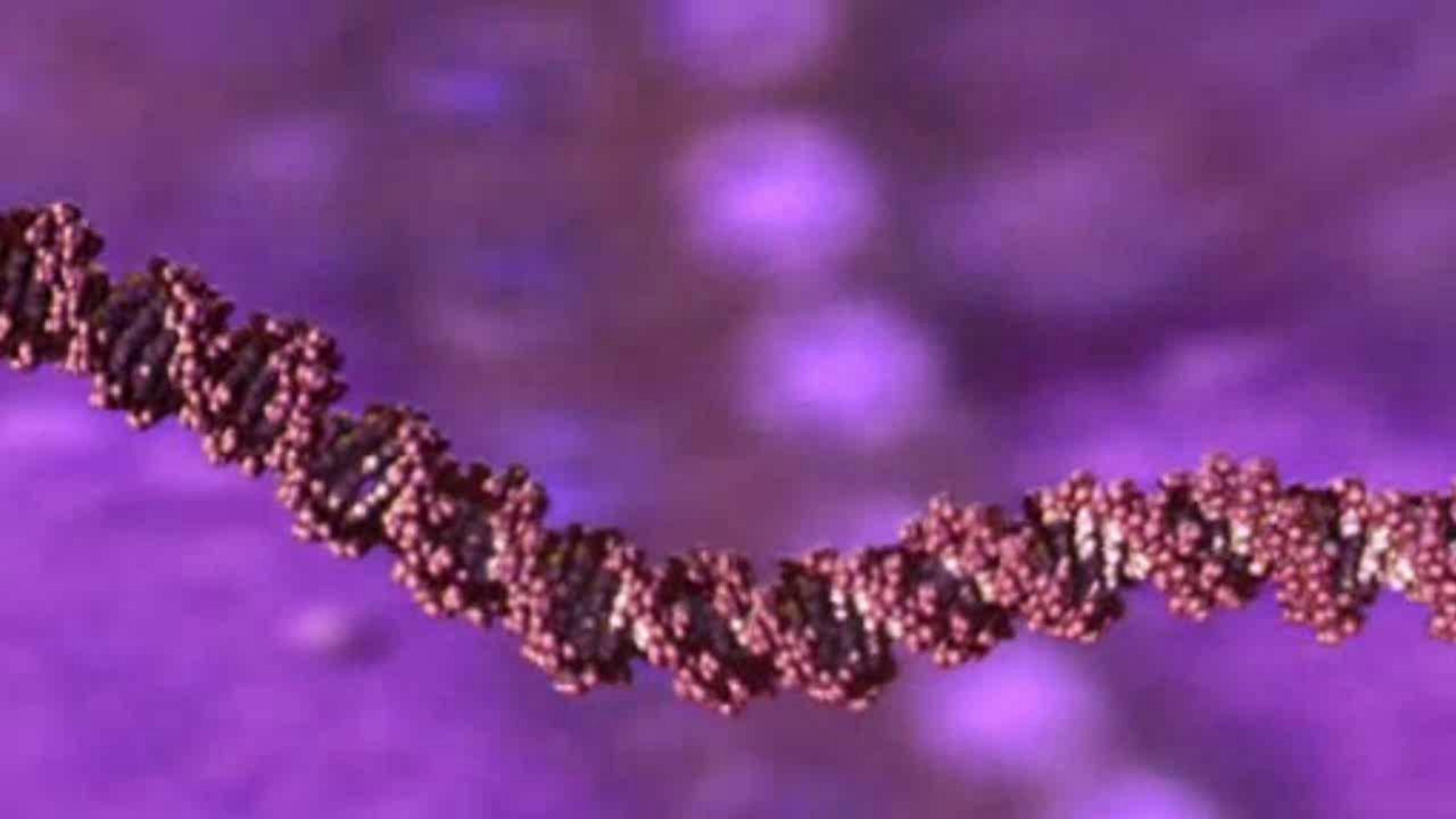


Liu et al, 2021, PMC8357426

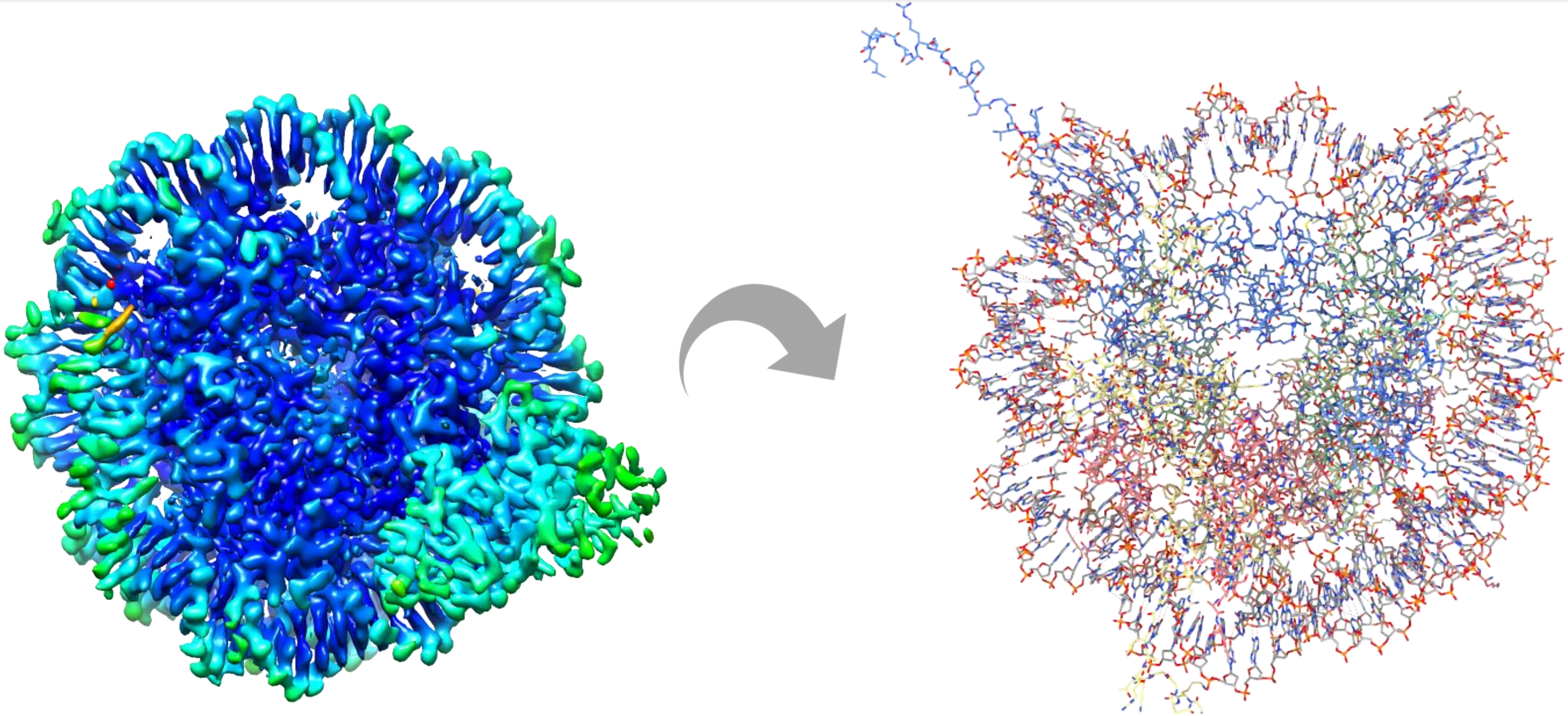
DNA encodes all information to build an organism



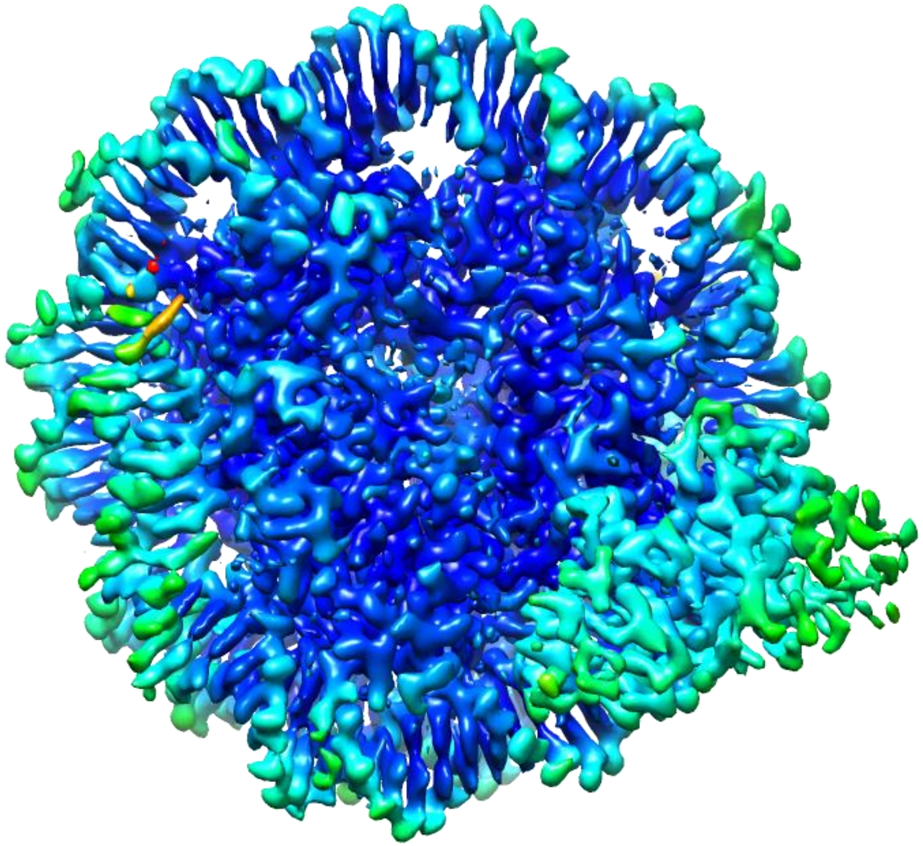


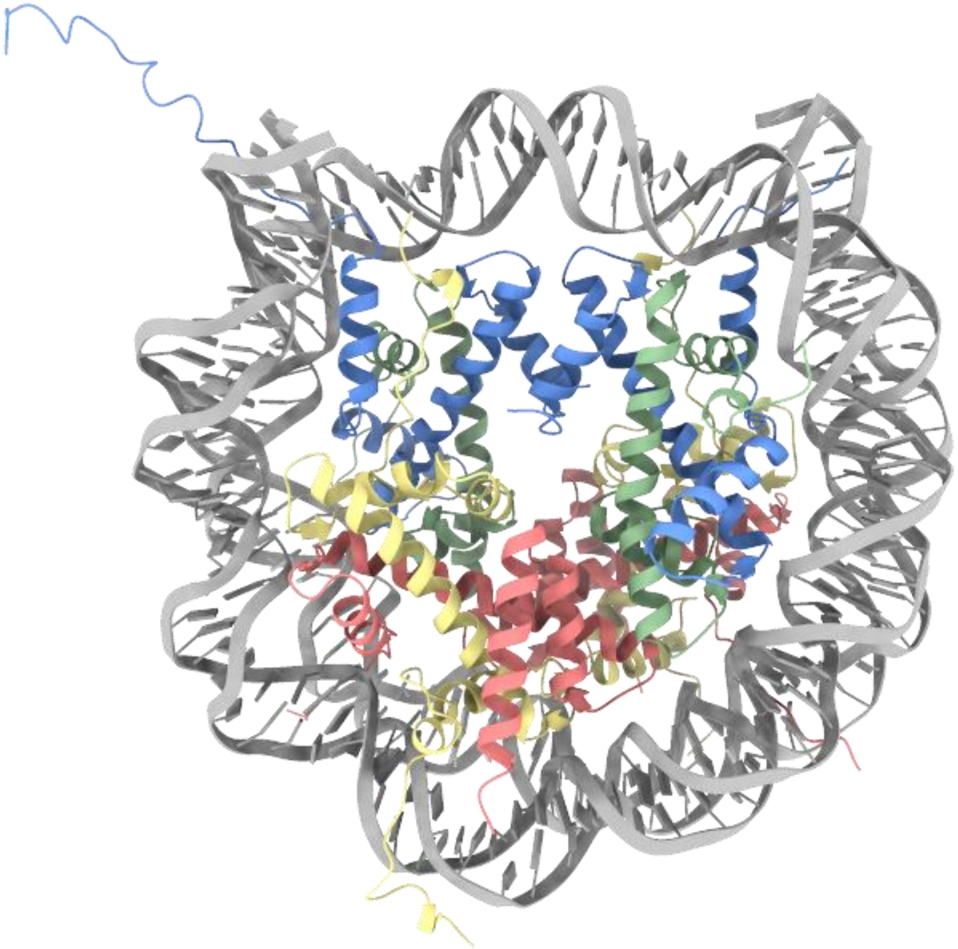


Electron densities allow us to build atomic models

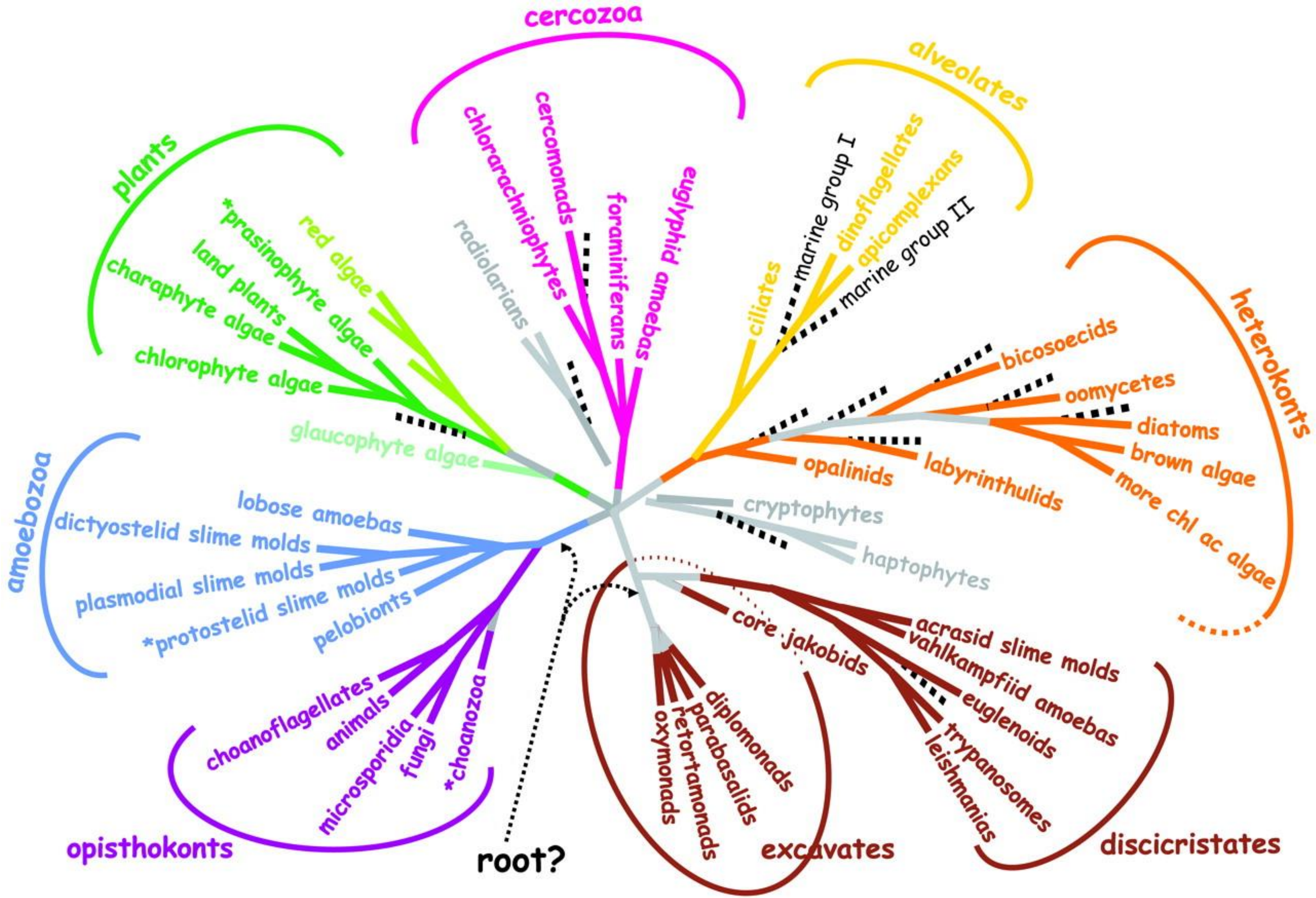


Electron densities allow us to build atomic models





A ‘stylized’ way to represent structures


cercozoa
chlorarachniophytes
cercomonads
foraminiferans
euglyphid amoebas
radiolarians
alveolates
ciliates
marine group I
dinoflagellates
apicomplexans
marine group II
plants
*prasinophyte algae
red algae
land plants
charaphyte algae
chlorophyte algae
glaucophyte algae
heterokonts
bicosoecids
oomycetes
diatoms
brown algae
more chl ac algae
opalinids
labyrinthulids
cryptophytes
haptophytes
amoebozoa
lobose amoebas
dictyostelid slime molds
plasmodial slime molds
*protostelid slime molds
pelobionts
opisthokonts
choanoflagellates
animals
microsporidia
fungi
*choanozoa
root?
excavates
core jakobids
diplomonads
parabasalids
retortamonads
oxymonads
discicristates
acrasid slime molds
vahlkampfiid amoebas
euglenoids
trypanosomes
leishmanias

‘Atomic resolution’: seeing every atom in proteins and DNA

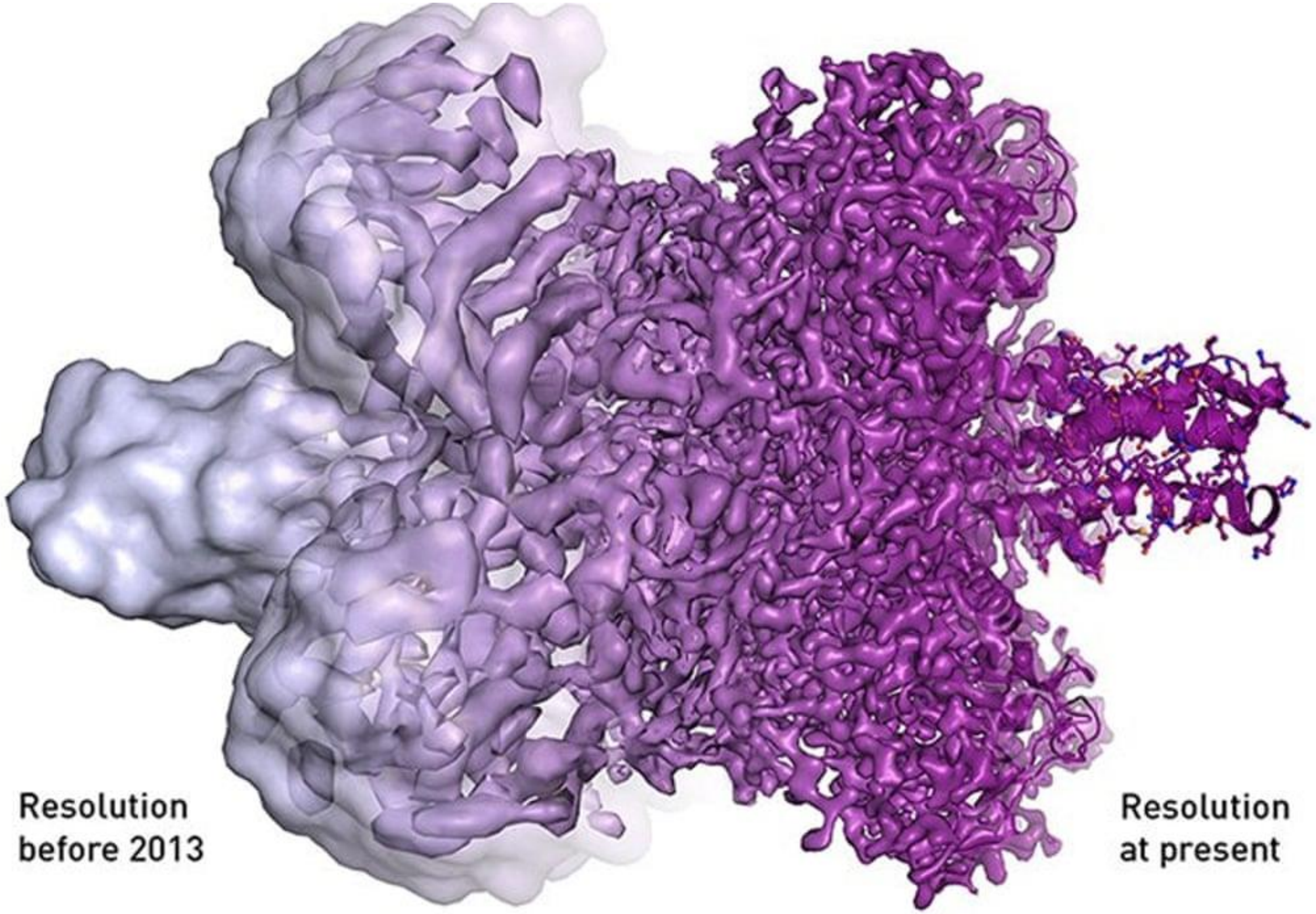
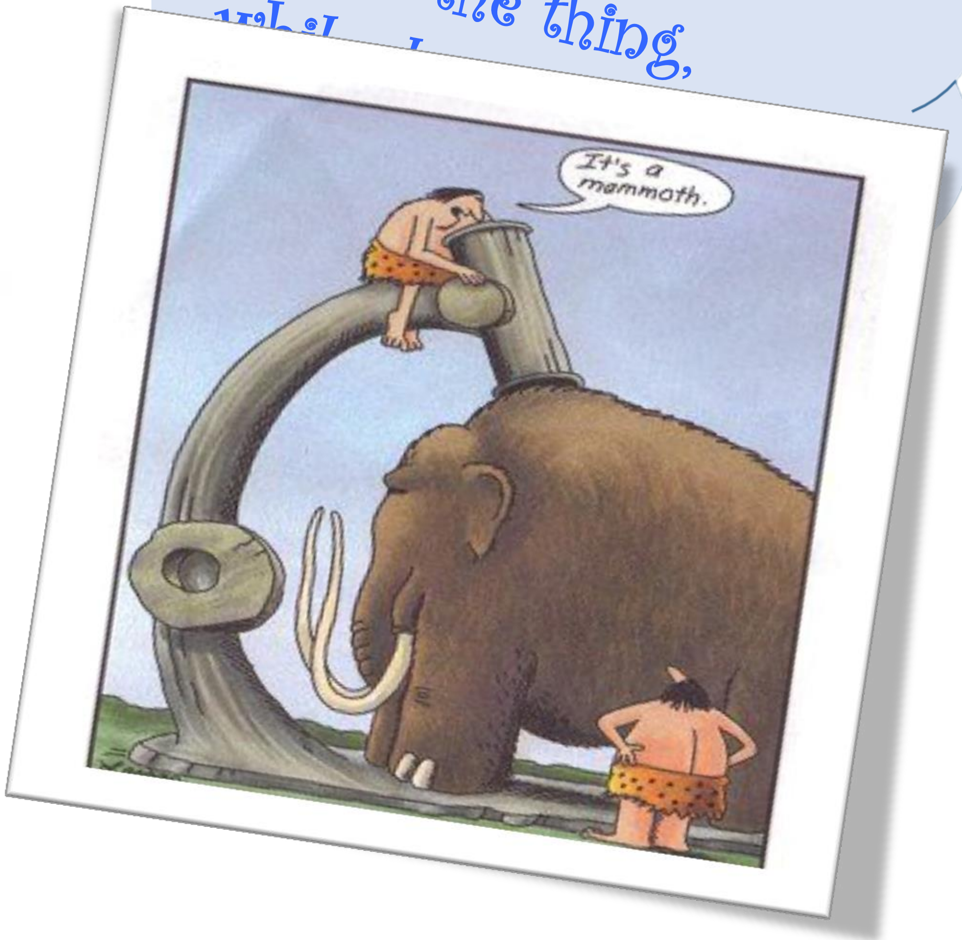
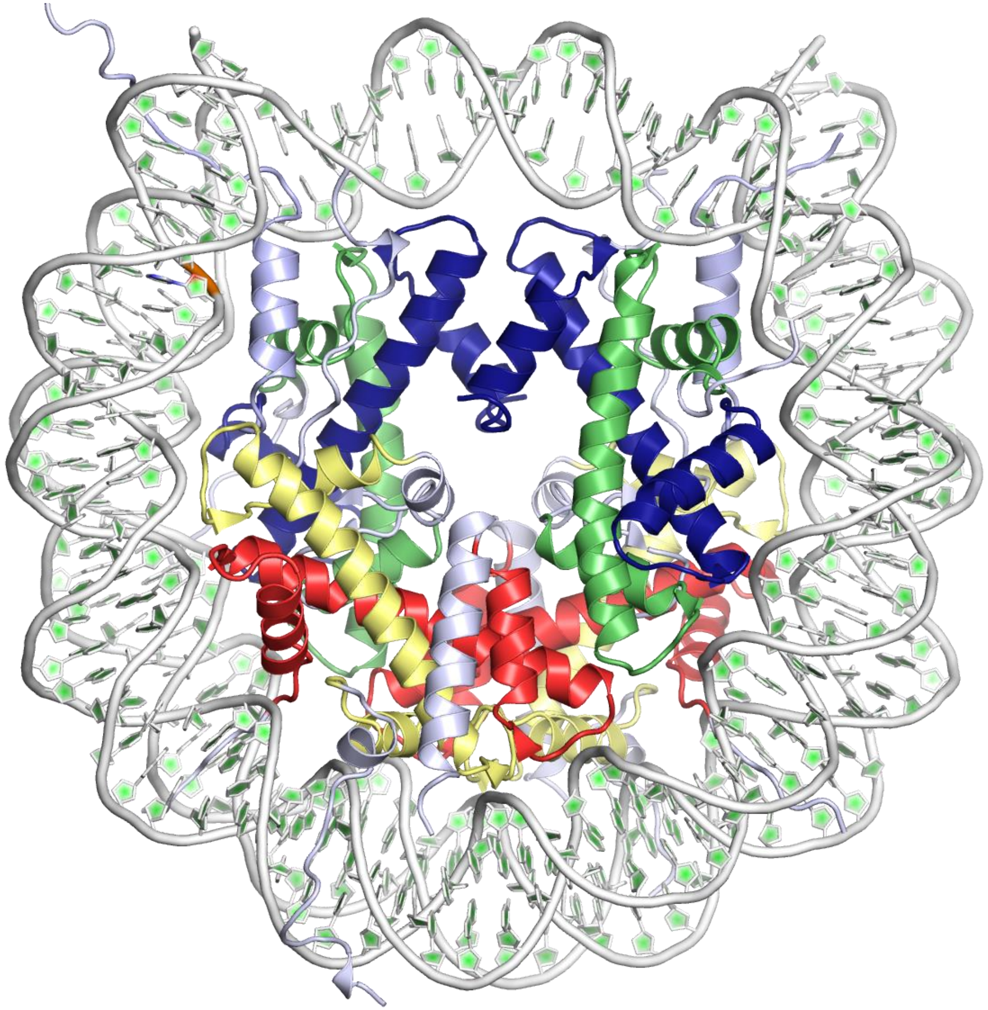


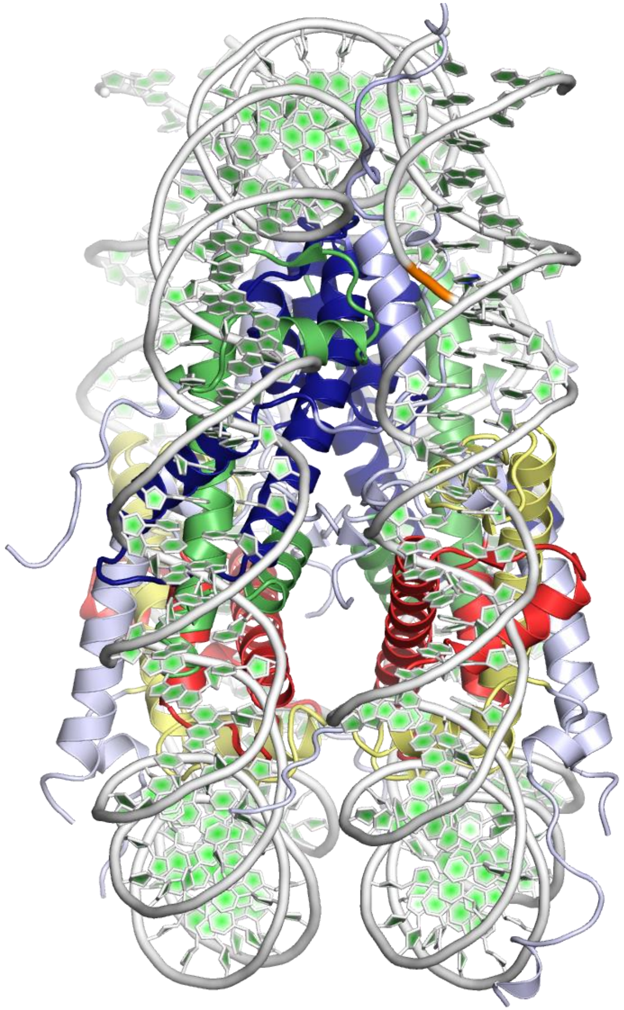
Illustration: ©Martin Högbom/The Royal Swedish Academy of Sciences

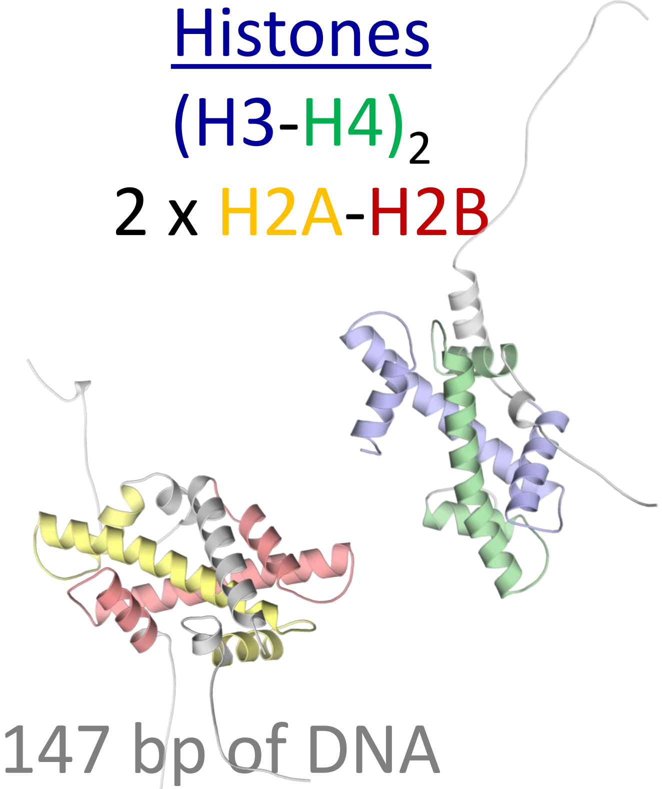
If only we could take
a look at the thing,



The crystal structure of the nucleosome







Histones

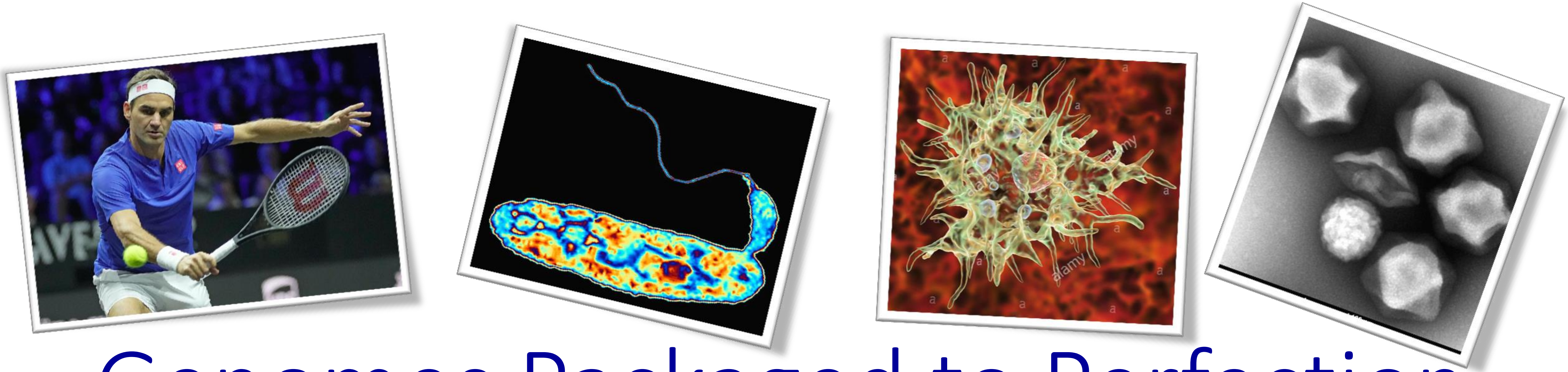
$(\mathrm{H3}\text{-}\mathrm{H4})_2$

2 x H2A-H2B

147 bp of DNA

Mw = 210 kDa

Luger et al., Nature 1997



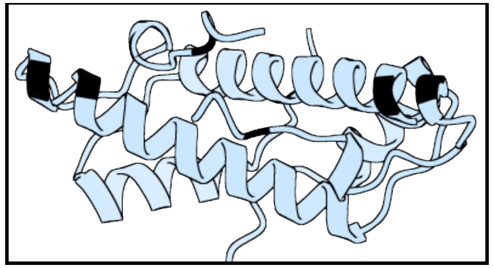
Genomes Packaged to Perfection, in All Domains of Life

Karolin Luger
Lugerlab.org
[@lugerlab]

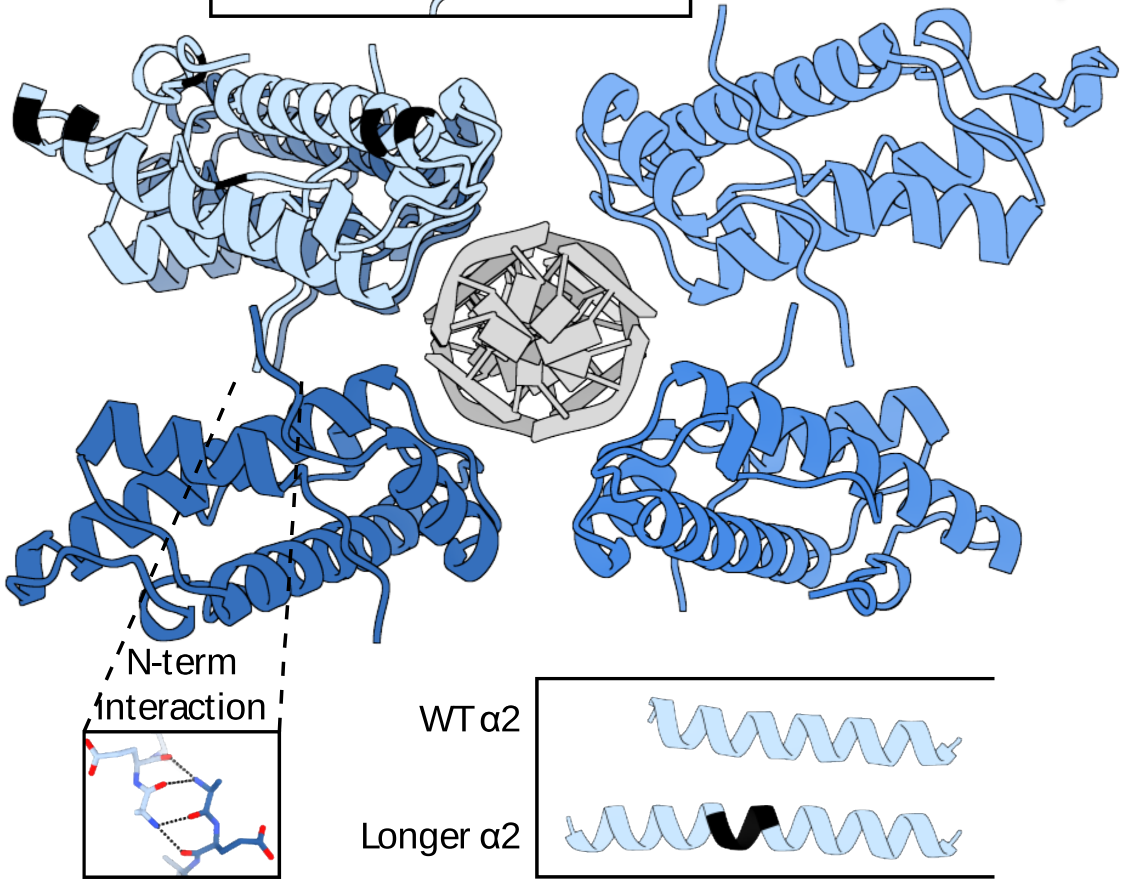
University of Colorado
Boulder

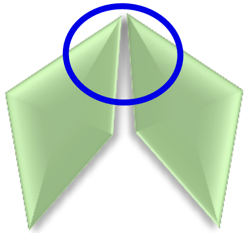
Howard Hughes
Medical Institute

Reason for this unconventional binding mode

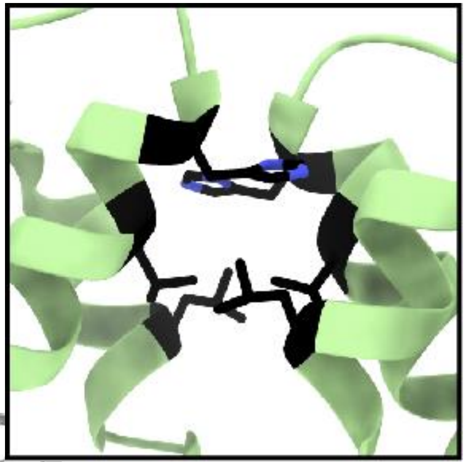


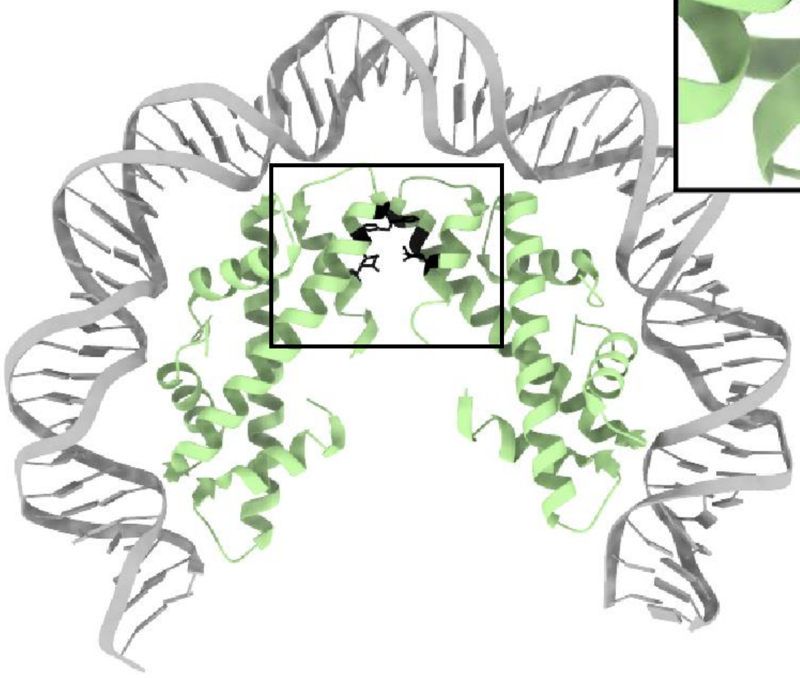
Inability to form tetramer?





Histone tetramer: minimal DNA binding unit?

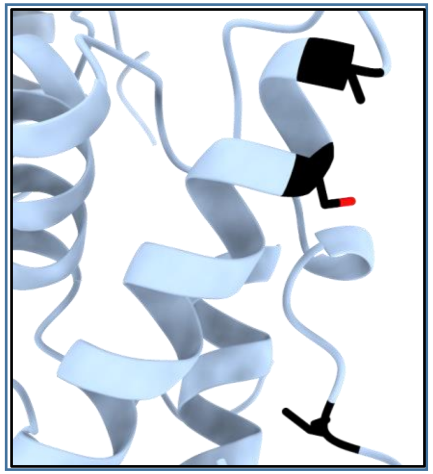




Crystal structure of HMf-DNA complex

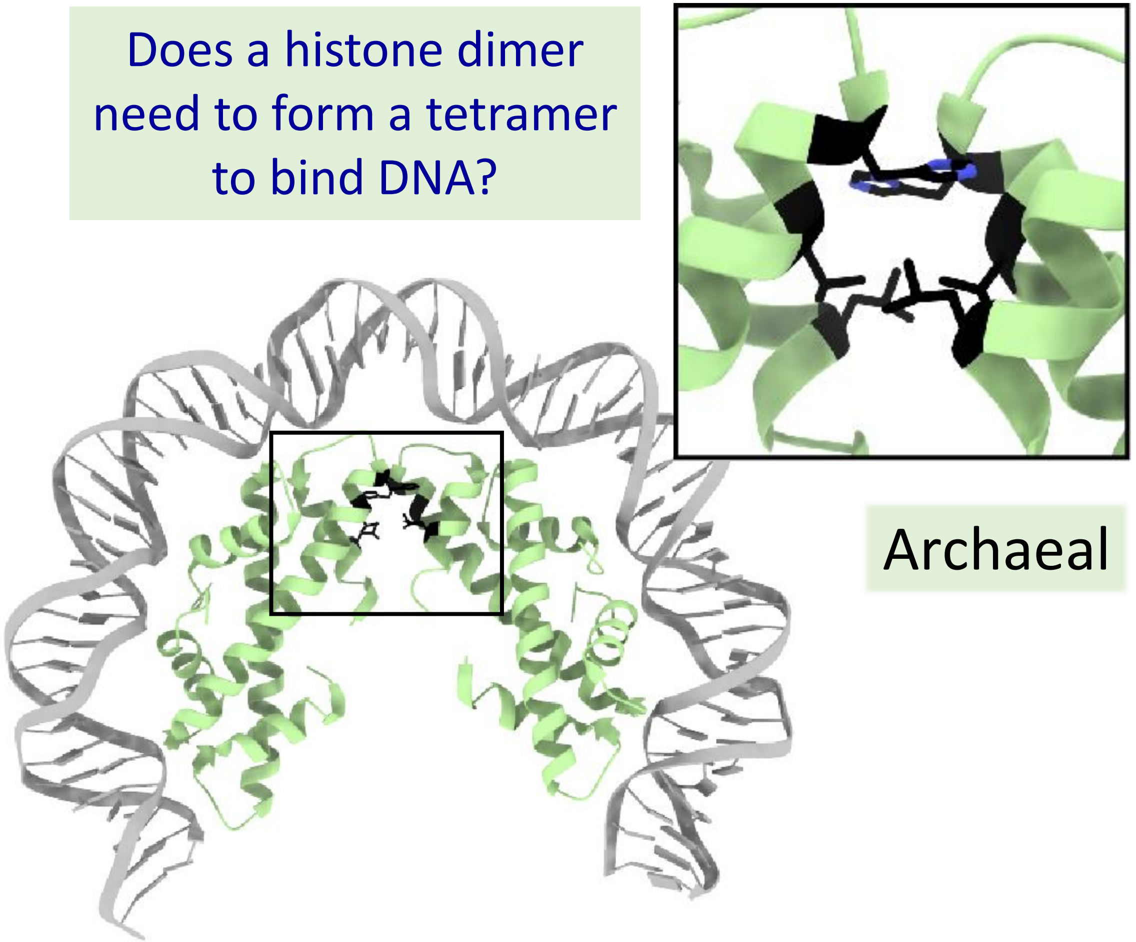
Mattiroli, F….& Luger, K, 2017, PMC5747315

Bacterial histone lacks tetramerization interface



Bacterial

What happens if we restore the tetramerization interface?

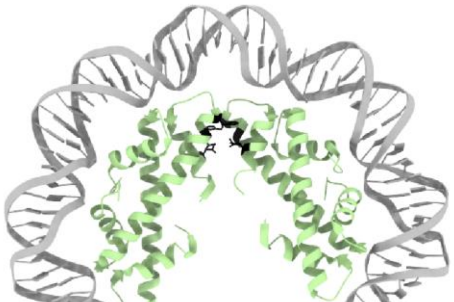


Does a histone dimer need to form a tetramer to bind DNA?

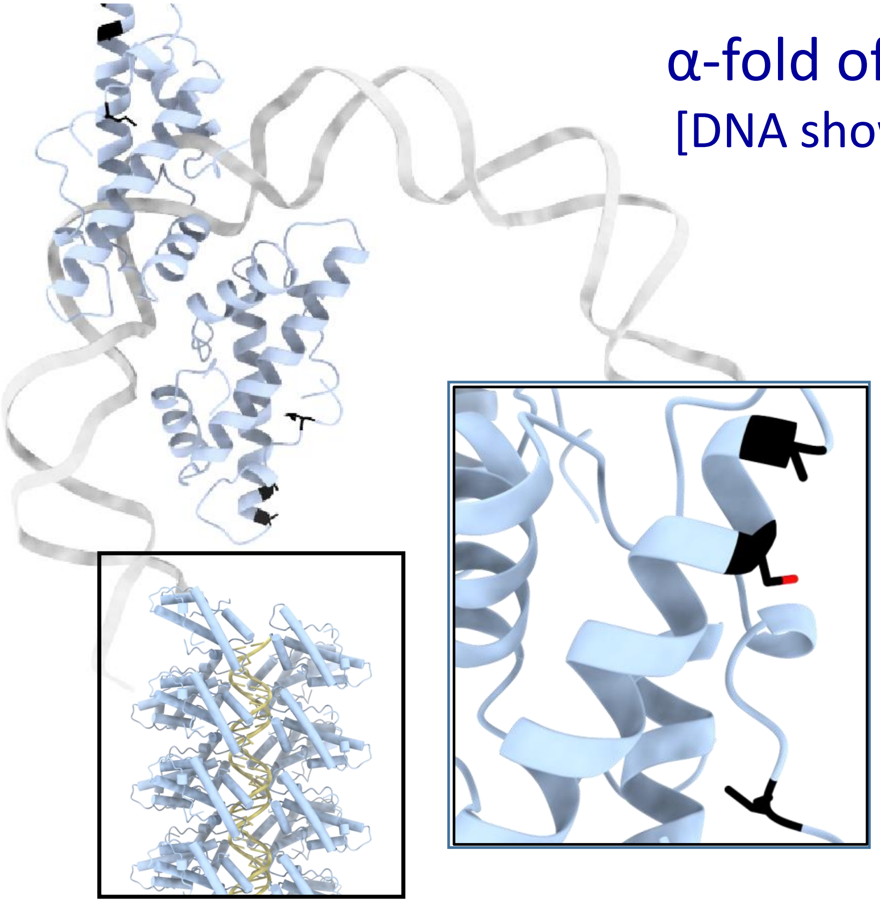
Archaeal

Crystal structure of HMf-DNA complex
Mattiroli, F….& Luger, K, 2017, PMC5747315

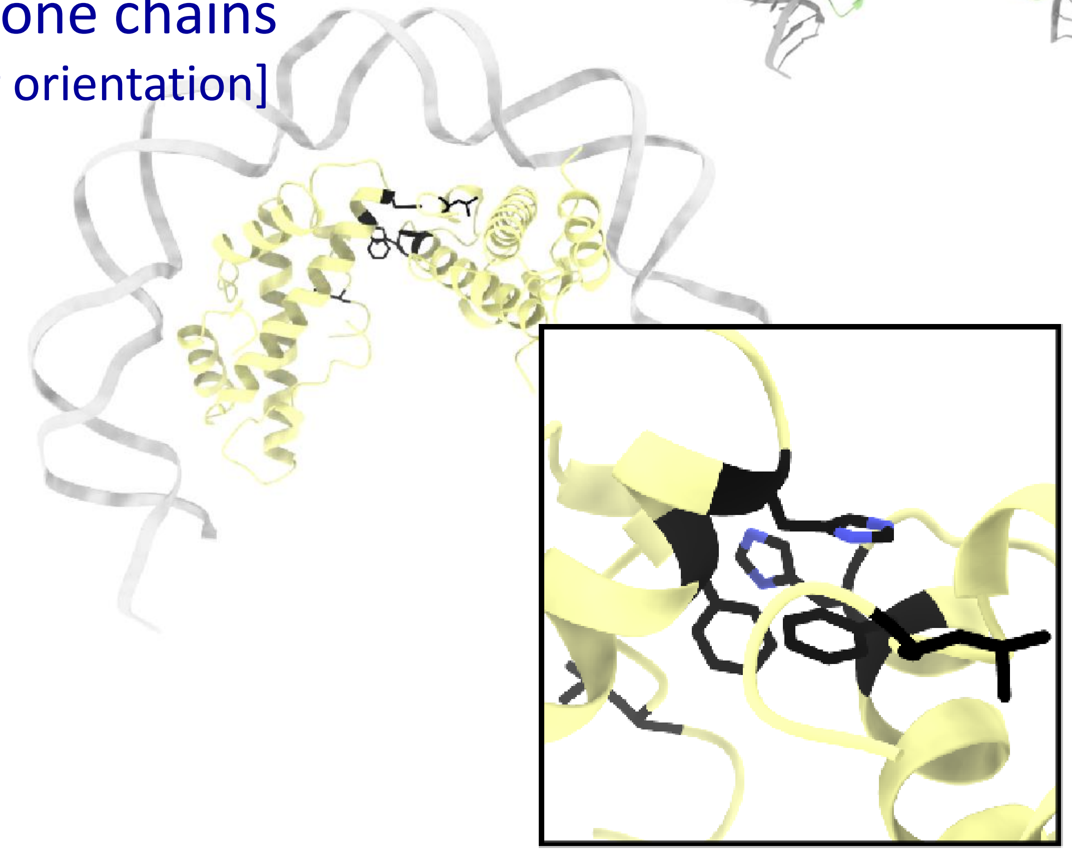
The awesome power of α-fold



α-fold of four histone chains
[DNA shown only for orientation]



A → H
S → F
I → L

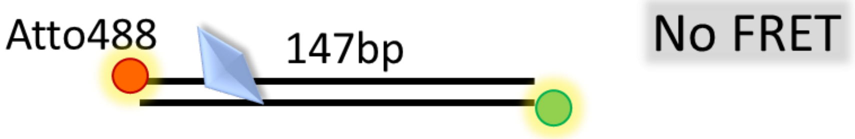


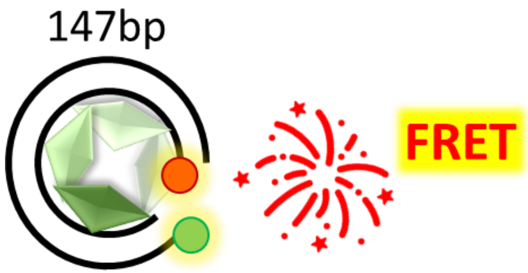
Wild type bacterial histone does not form a four-helix bundle

Mutating three residues allows tetramerization *in silico*

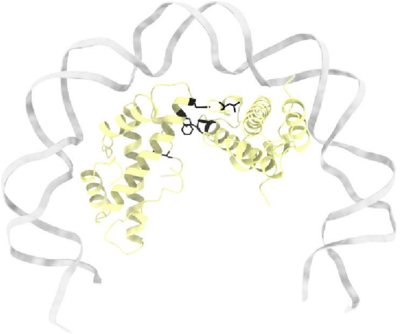
Reason for unconventional binding mode: **no tetramerization**

- Competing interaction of N-term?
- Shorter α2 helix?
- **Inability to form tetramer!**

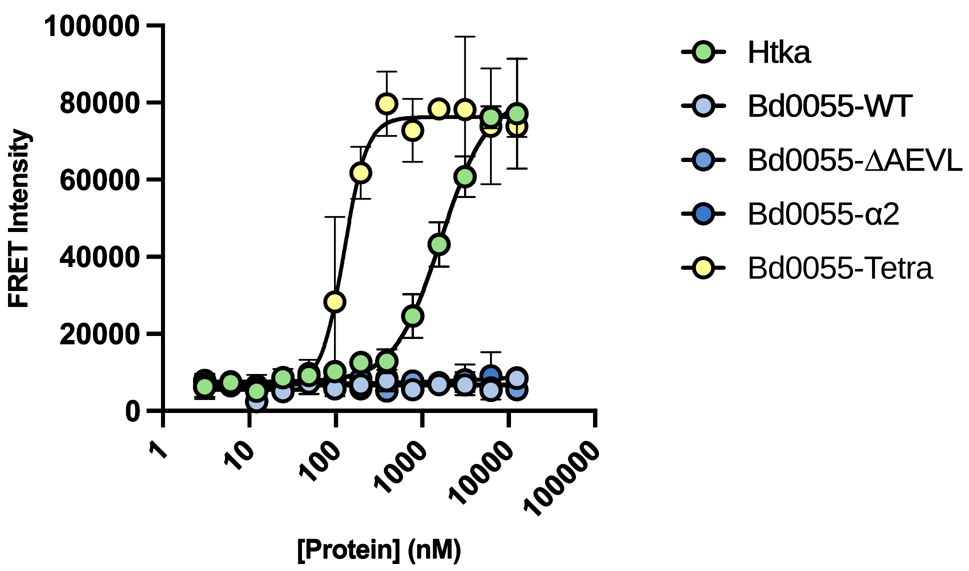




α-fold prediction;
structure needed

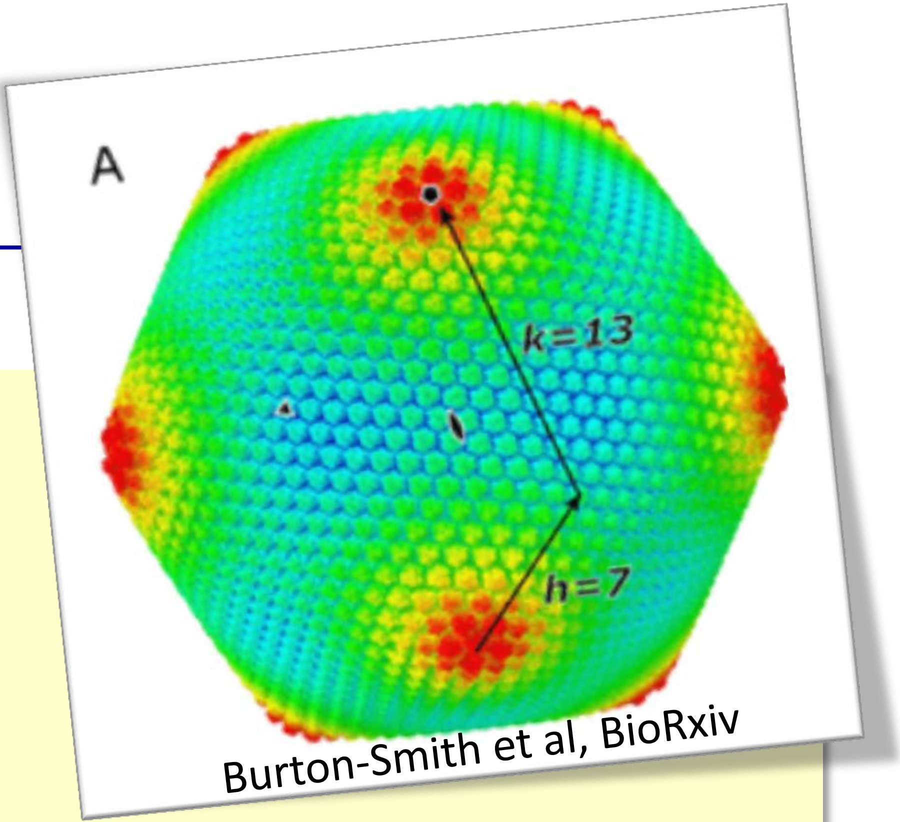


FRET of Bd0055 mutants



Shawn Laursen, with help from Nikhil Gupta

Viral histone doublets in Melbournevirus



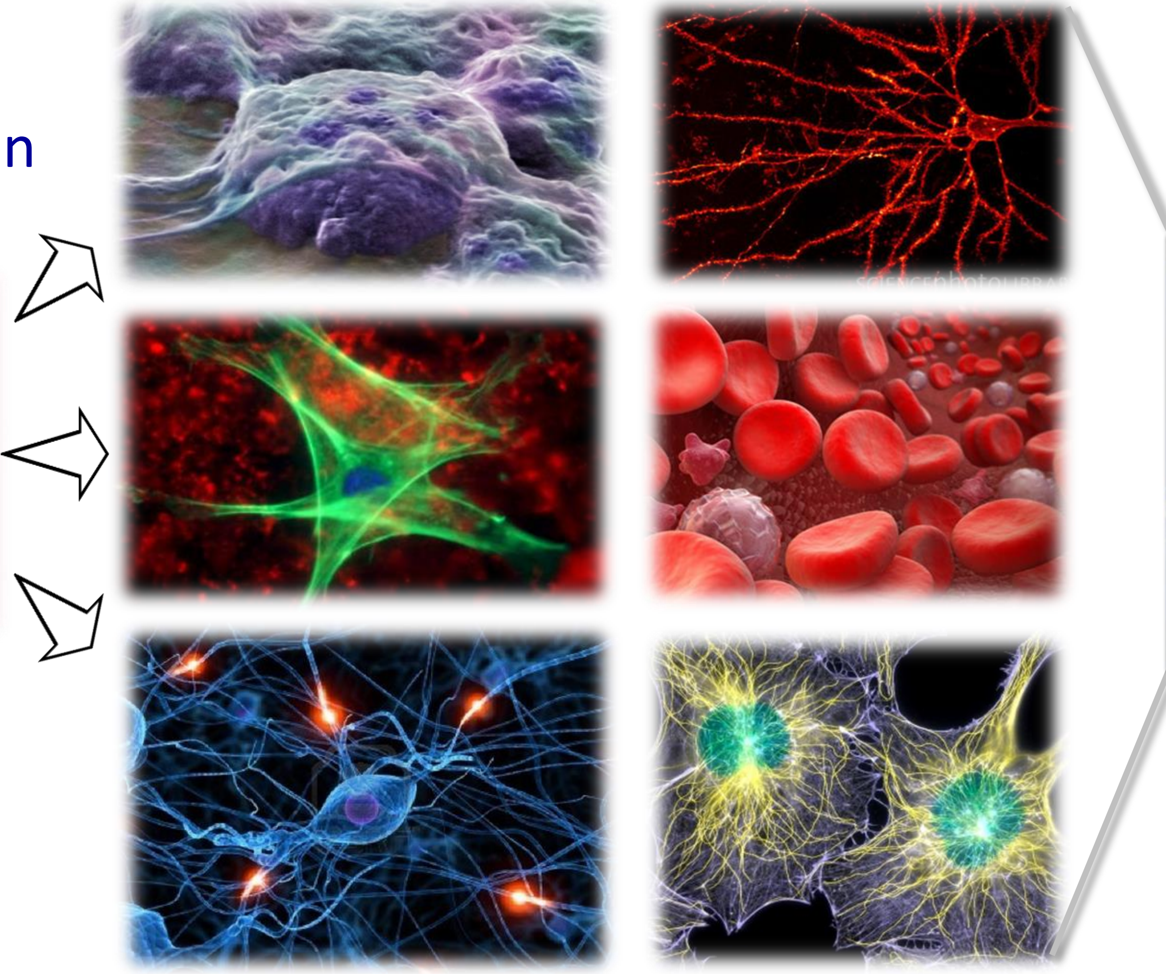
Burton-Smith et al, BioRxiv

- ➢ Can they bind DNA / form nucleosomes?
- ➢ If yes, what properties do they have?
- ➢ When / how do they arrive at the ‘viral factory’
- ➢ How are they assembled?
- ➢ Does the virus need its own histones, and if yes, what for?

Liu, Bisio, Toner, Jeudy, Philippe, Zhou, Bowerman, White, Edwards, Abergel, Luger
[Cell, 2021]

Every cell in our body has the same 'blueprint of life' (DNA)

Fertilized egg
contains all information
to build a human

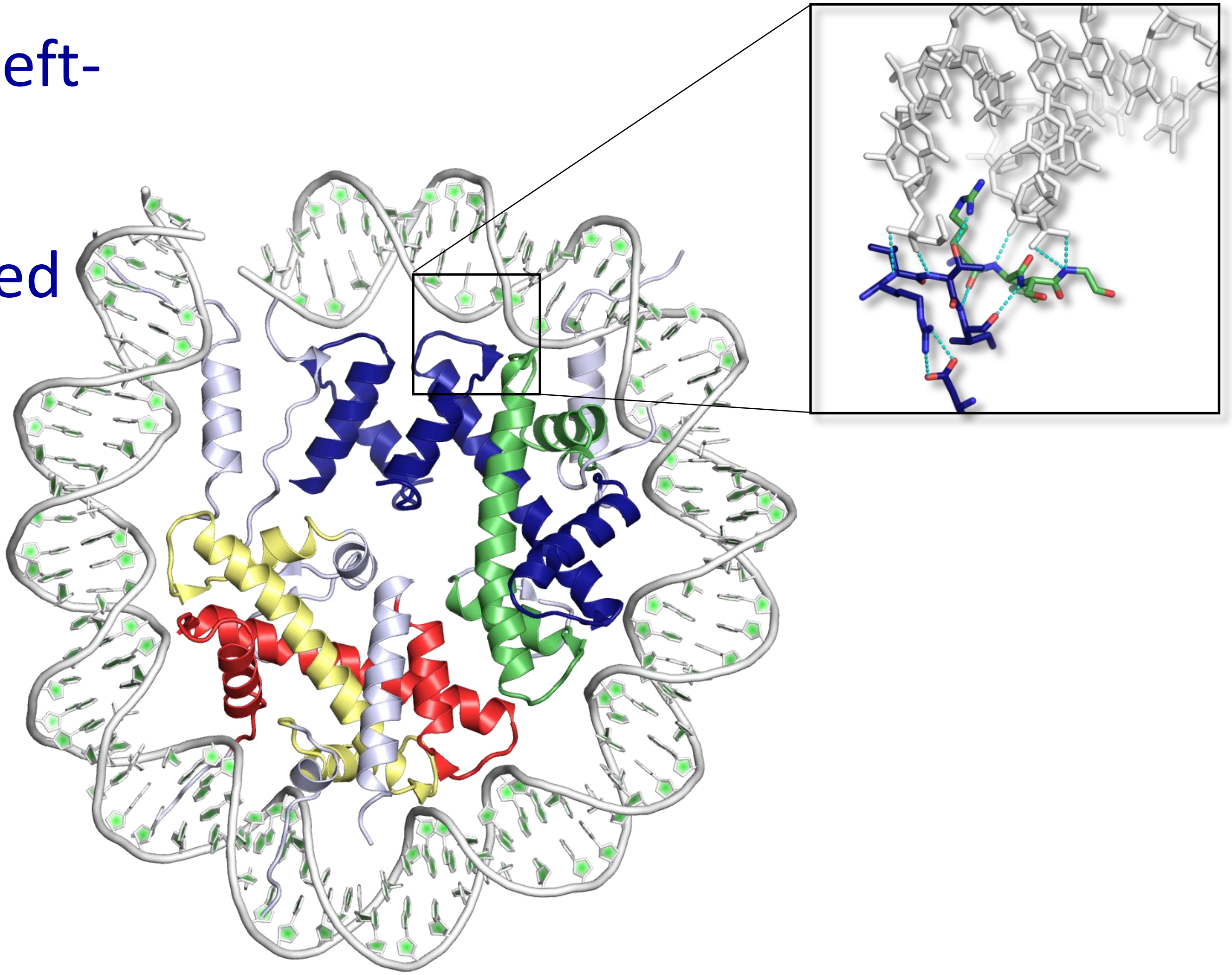


~ 30 trillion cells
~ 200 cell types

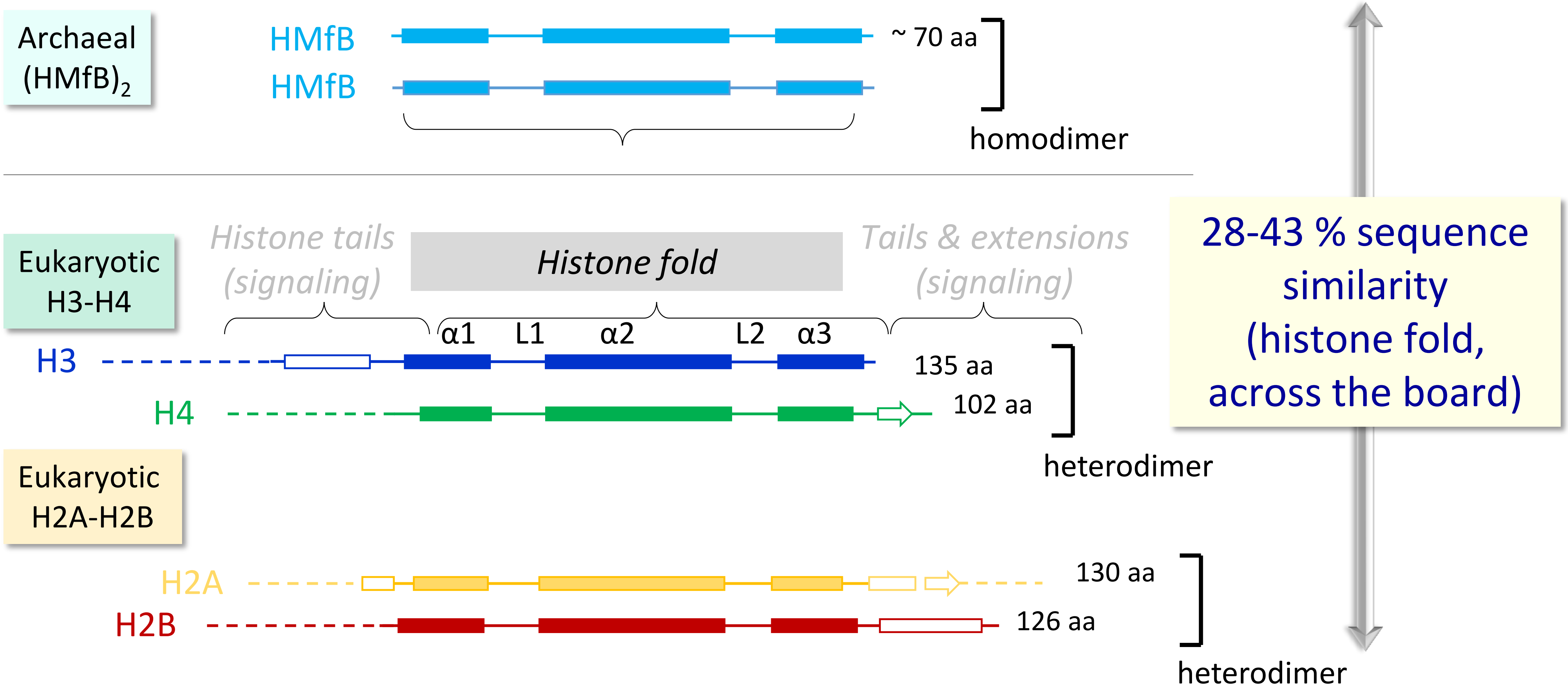
Cell fate and differentiation is determined by which parts of the blueprint are read

Nucleosomal DNA has unique features that profoundly affect how the genome is accessed

- DNA forms a highly distorted left-handed superhelix
- One face of the DNA is occluded over the entire 147 base pairs
- DNA is tightly bound via 14 contact points



‘Histone fold dimers’ are conserved across two domains of life



Archaeal $(HMfB)_2$

HMfB

HMfB

~ 70 aa

homodimer

Eukaryotic H3-H4

Histone tails (signaling)

Histone fold

Tails & extensions (signaling)

α1 L1 α2 L2 α3

H3

135 aa

H4

102 aa

heterodimer

Eukaryotic H2A-H2B

H2A

130 aa

H2B

126 aa

heterodimer

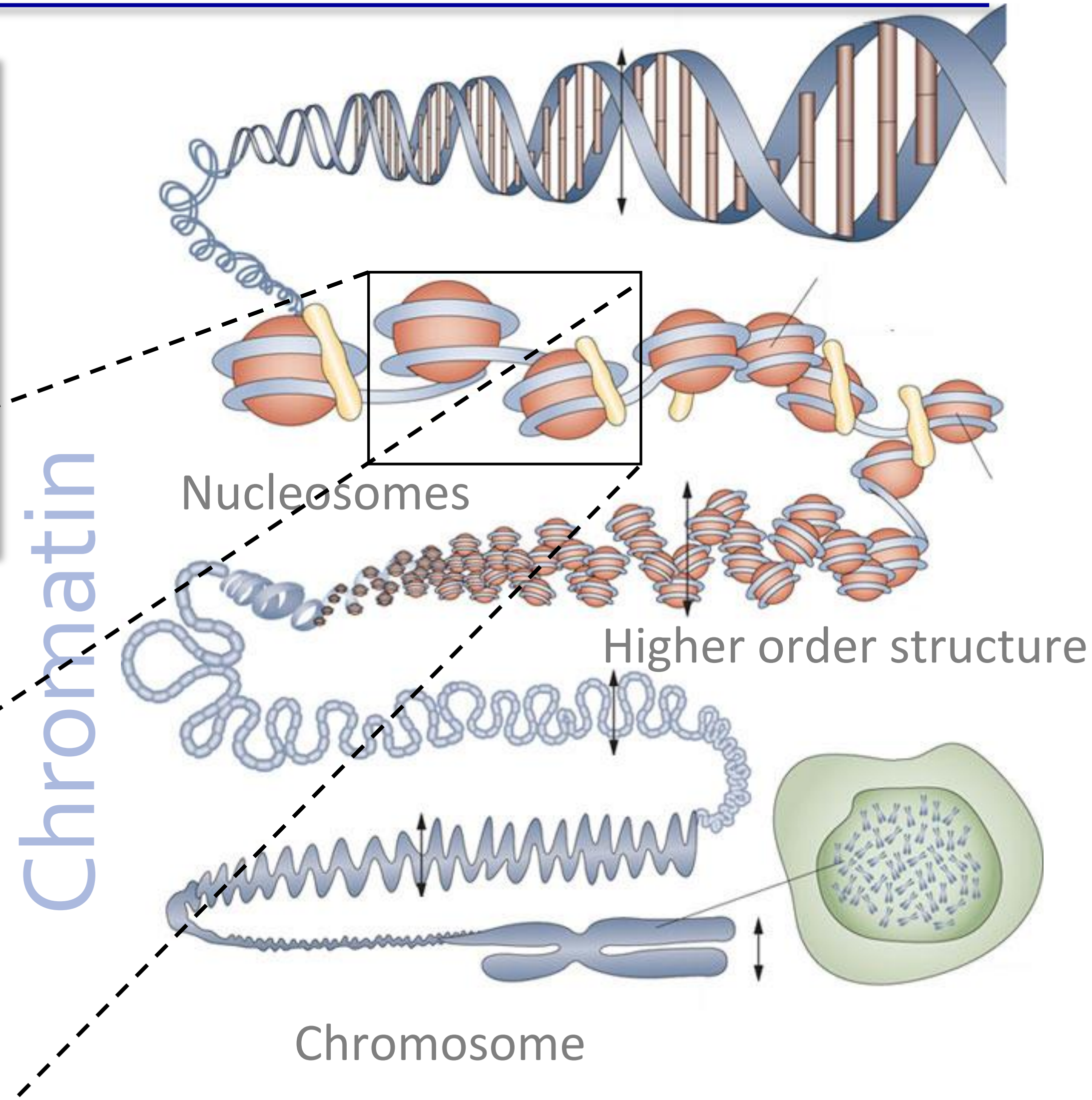
28-43 % sequence similarity (histone fold, across the board)

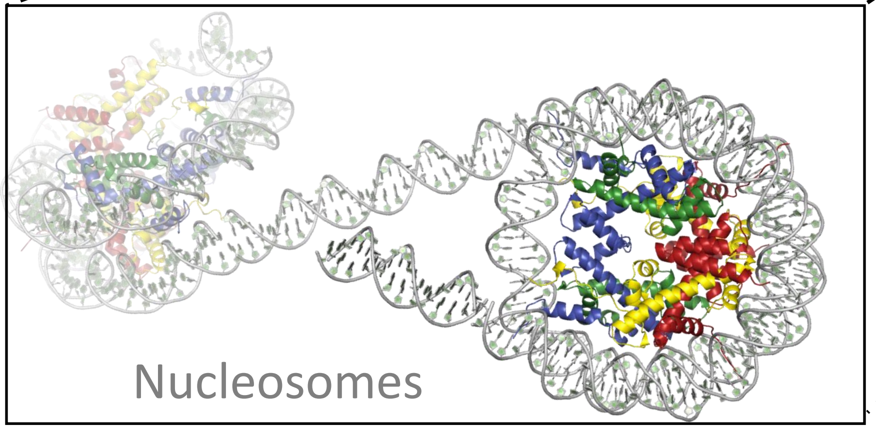
Luger et al., Nature 1997; Decanniere et al., JMB 2000

The entire eukaryotic genome is organized into nucleosomes

- Nucleosomes determine spatial and temporal **access to the genome**
- Targets of **epigenetic modifications**
- **Transcription, replication, and repair** in chromatin requires complex machinery

This holds true for all eukaryotes





Tonna et al., 2010

Three take-home points

1) Structural biology is essential to our understanding of cells

2) Complex genomes are organized in complex structures

Encoding (and manipulating) information in linear form: Large genomes pose many challenges

- Package information in a highly confined space (10 mile thread in a golf ball)
- Protect from physical damage and tangles
- Accurate duplication of the genome during cell division
- Find the required genes in a timely manner
- Physically access the stored information

Malfunction

⬇

Cancer, other diseases

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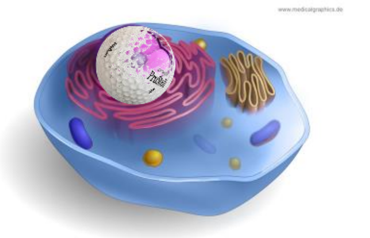

Malfunction

⬇

Cancer, other diseases