

Pokročilé metody v genomice a proteomice: analýza proteinových komplexů

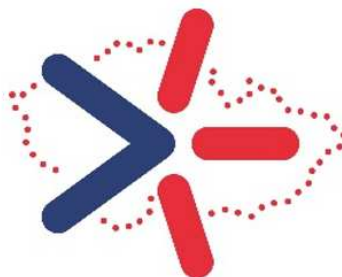
doc. Jan Paleček

jpalecek@sci.muni.cz

laboratoř Strukturních proteinů eukaryotních chromosomů
(<http://www.ncbr.muni.cz/SPEC/>)



Financováno
Evropskou unií
NextGenerationEU



Národní
plán
obnovy

MSMT
MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY

NPO_MUNI_MSMT-16606/2022

O čem už jste slyšeli ...

datum	čas	přednášející	přednáška
20.9.2024	12-14	dr. Petra Schrupfová-Procházková	Metody extrakce, purifikace a kvantifikace nukleových kyselin
	14-16	doc. Miloslava Fojtová	qPCR – princip, moderní přístupy, aplikace, především v klinice
27.9.2024	12-14	doc. Jan Hejátko	Analýza genové exprese a nástroje systémové biologie
	14-16	prof. Jiří Fajkus	Metody sekvenování DNA a RNA
4.10.2024	12-14	dr. Petr Fajkus	Příprava knihoven pro sekvenování nové (NGS) a třetí generace (TGS)
	14-16	dr. Vratislav Peška	Analýza sekvenčních dat
11.10.2024	12-14	doc. Miloslava Fojtová	Epigenetika – o co jde, jak to funguje a co se stane, když to nefunguje
	14-16	prof. Jiří Fajkus	Analýza epigenetických značek a struktury chromatinu
18.10.2024	12-14	doc. Ctirad Hofr	Pokročilé fluorescenční metody při detekci, analýze a vizualizaci biomolekul
	14-16	prof. Zbyněk Zdráhal	Nové trendy v proteomice pomocí hmotnostní spektrometrie

GENOMIKA

PROTOMIKA

... a o čem uslyšíte

- Co všechno se dozvíte o genu/proteinu z databází
 - 3D (terciární) struktura proteinů
 - Proteinové interakce
 - Proteinové komplexy (kvarterní struktura)
- Jak získat nové informace o proteinových komplexech experimentálně
 - Analýza komplexů
 - Vizualizace komplexů ...



NGS přináší nevídané množství dat

- rychlé sekvenování genomů poskytuje informace o proteinech v různých organismech
- proteinové databáze UNIPROT (SWISSPROT...): <https://www.uniprot.org/>
- potvrzuje představu evoluce proteinů/organismů – ukazuje na velkou konzervovanost většiny proteinů
- tyto podobnosti umožňují modelování proteinů i z málo charakterizovaných organismů (a jejich anotaci):
spolehlivé modelování pro homologie >30% (Rosetta, AlphaFold ...)

The Nobel Prize in Chemistry 2024



Ill. Niklas Elmehed © Nobel Prize Outreach
David Baker



Ill. Niklas Elmehed © Nobel Prize Outreach
Demis Hassabis



Ill. Niklas Elmehed © Nobel Prize Outreach
John M. Jumper

Základní alignment

- pokud pracujete s novou sekvencí (např. není v UNIPROT): BLAST – <https://blast.ncbi.nlm.nih.gov/>
- hledání příbuzných sekvencí vám napoví nakolik je protein evolučně konzervovaný, jaké má domény ... jakou mají funkci jemu podobné proteiny (např. UNIPROT databáze)

Web BLAST

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#)

Query subrange [?](#)

From

To

Or, upload file Soubor nevybrán. [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Choose Search Set

Databases Standard databases (nr etc.): **New** Experimental databases

Compare Select to compare standard and experimental database [?](#)

Standard

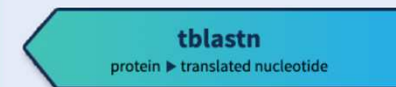
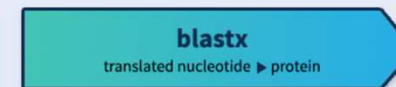
Database [?](#)

Organism Optional exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

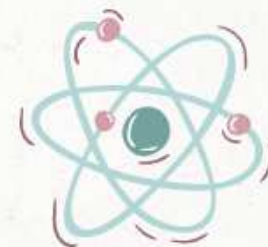
Exclude Optional Models (XM/XP) Non-redundant RefSeq proteins (WP) Uncultured/environmental sample sequences

Web BLAST



printscreen: autor

UniProt a jeho možnosti



Komplexní, vysoce kvalitní a volně přístupný zdroj sekvenčních a funkčních informací o proteinech

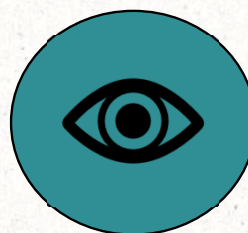
- Popis a funkce
- Zjištění přítomnosti domén daného proteinu
- Zjištění interakčních partnerů
- Struktura proteinu – PDB, Alphafold ... komplexy



**Hledání
proteinu**



Souvislosti
Funkce
Interaktom
Mutace



Vizualizace
Alphafold
Lokalizace proteinu



**Stahování
dat**

UniProt – vyhledávání

Histon H3

<https://www.uniprot.org/>

Browser address bar: <https://www.uniprot.org/uniprotkb?query=H3+cerevisiae>

UniProt navigation: BLAST Align Peptide search ID mapping SPARQL UniProtKB ▾ H3 cerevisiae Advanced | List Search

Status

Reviewed (Swiss-Prot)
(244)

Unreviewed (TrEMBL)
(308)

Popular organisms

S. cerevisiae (176)

Human (16)

Rat (7)



UniProtKB 552 results

BLAST Align Map IDs Download Add View: Cards ○ Table ● Customize columns Share ▾

Entry ▲	Entry Name ▲	Protein Names ▲	Gene Names ▲	Organism ▲	Length
<input type="checkbox"/> P61830	H3_YEAST	Histone H3	HHT1 , YBR010W, YBR0201, HHT2, SIN2, YNL031C, N2749	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	136 AA
<input type="checkbox"/> Q757N1	H3_ASHGO	Histone H3	HHT1 , ADL202C, HHT2, AER013W	Ashbya gossypii (strain ATCC 10895 / CBS 109.51 / FGSC 9923 / NRRL Y-1056) (Yeast) (Eremothecium gossypii)	136 AA

... i lidský

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Histon H3

Informace o proteinu - UNIPROT

- Function
- Names & Taxonomy
- Subcellular Location
- Phenotypes & Variants
- PTM/Processing
- Expression
- Interaction
- Structure
- Family & Domains
- Sequence
- Similar Proteins

Functionⁱ

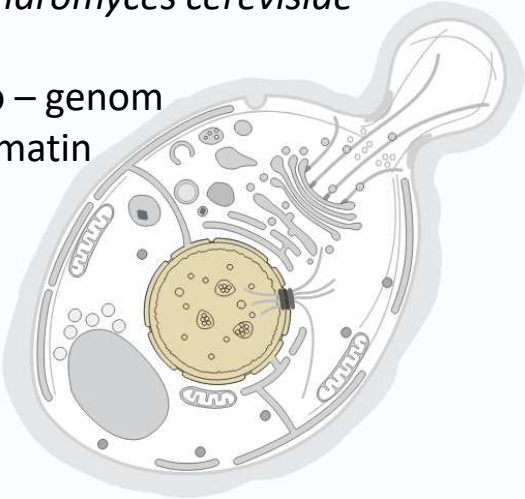
Core component of **nucleosome**. Nucleosomes wrap and compact DNA into **chromatin**, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. Component of the UAF (upstream activation factor) complex which interacts with the upstream element of the RNA polymerase I promoter and forms a stable preinitiation complex.

Subcellular Locationⁱ

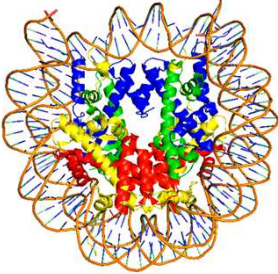
UniProt Annotation GO Annotation

Saccharomyces cerevisiae

jádro – genom
chromatin



- Nucleus Curated
- Chromosome Curated



Genová ontologie

ASPECT	TERM
Cellular Component	CENP-A containing nucleosome Source:SGD 1 Publication
Cellular Component	nucleosome Source:ComplexPortal 1 Publication
Cellular Component	nucleus Source:SGD 1 Publication
Cellular Component	replication fork protection complex Source:SGD 1 Publication
Cellular Component	RNA polymerase I upstream activating factor complex Source:ComplexPortal 1 Publication
Molecular Function	DNA binding Source:SGD 1 Publication
Molecular Function	protein heterodimerization activity Source:InterPro
Molecular Function	structural constituent of chromatin Source:InterPro
Biological Process	chromatin organization Source:SGD 1 Publication
Biological Process	global genome nucleotide-excision repair Source:SGD 1 Publication
Biological Process	nucleolar large rRNA transcription by RNA polymerase I Source:ComplexPortal 1 Publication

upozornění: informace nebývají kompletní (i chybné) – dohledat v člancích

printscreen: autor



Informace o proteinu - UNPROT



Function

Names & Taxonomy

Subcellular Location

Phenotypes & Variants



PTM/Processing

Expression

Interaction

Structure

Family & Domains

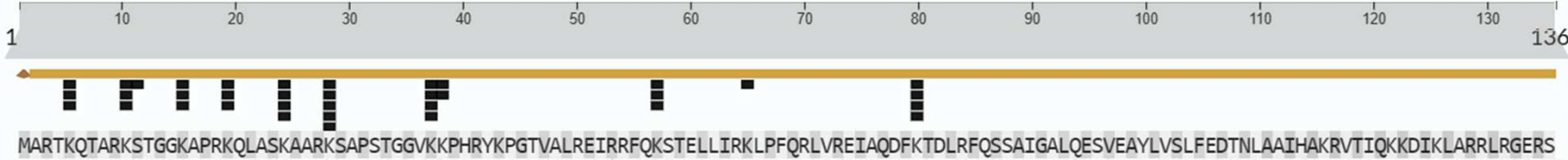
Sequence

Similar Proteins

Functionⁱ

Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called **histone code**, and nucleosome remodeling. Component of the UAF (upstream activation factor) complex which interacts with the upstream element of the RNA polymerase I promoter and forms a stable preinitiation complex.

PTM | #Acetylation
#Methylation
#Phosphoprotein



Acetylation of histone H3 leads to transcriptional activation. H3K14ac formation by GCN5, a component of the SAGA complex, is promoted by H3S10ph. Further acetylated by GCN5 to form **H3K9ac**, H3K18ac, H3K23ac, H3K27ac and H3K36ac. H3K14ac can also be formed by ESA1, a component of the NuA4 histone acetyltransferase (HAT) complex. **H3K56ac** formation occurs predominantly in newly synthesized H3 molecules during G1, S and G2/M of the cell cycle and may be involved in DNA repair. [13 Publications](#)

Histon H3

Informace o proteinu - UNIPROT



Function

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Subcellular Location

Phenotypes & Variants

PTM/Processing

Expression

Interaction

Structure



Family & Domains

Sequence

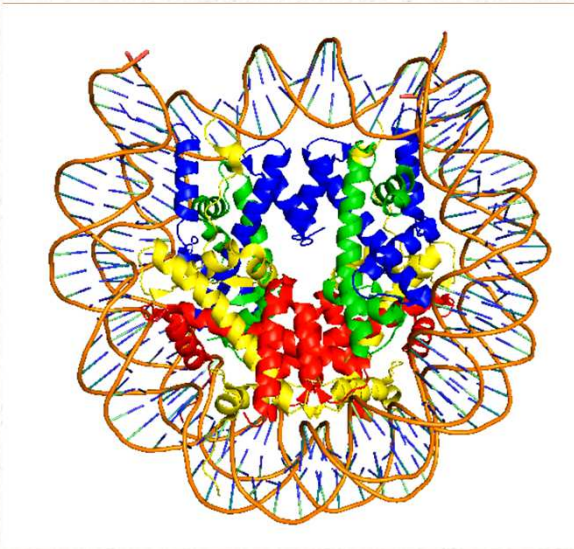
Similar Proteins

Functionⁱ

Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. Component of the UAF (upstream activation factor) complex which interacts with the upstream element of the RNA polymerase I promoter and forms a stable preinitiation complex.

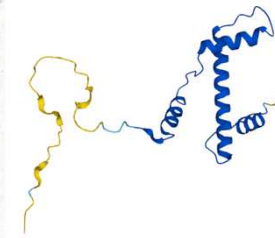
Family and domain databases

Gene3D	1.10.20.10 Histone, subunit A 1 hit
IDEAL	IID50143
InterPro	View protein in InterPro IPR009072 Histone-fold IPR007125 Histone_H2A/H2B/H3 IPR000164 Histone_H3/CENP-A
PROSITE	View protein in PROSITE PS00322 HISTONE_H3_1 1 hit PS00959 HISTONE_H3_2 1 hit
Pfam	View protein in Pfam PF00125 Histone 1 hit



printscreen: autor

Proteinové domény



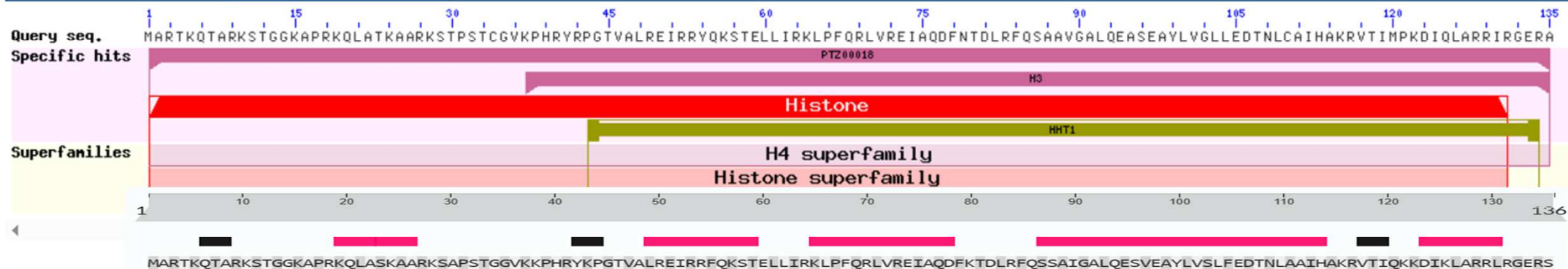
Protein Classification

histone H3 (domain architecture ID 10794185)

histone H3 is a core component of the nucleosome that wraps and compacts DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template

Graphical summary

Zoom to residue level show extra options >



List of domain hits

	Name	Accession	Description	Interval	E-value
[+]	PTZ00018	PTZ00018	histone H3; Provisional	1-135	1.16e-85
[+]	H3	smart00428	Histone H3;	37-135	5.99e-58
[+]	Histone	pfam00125	Core histone H2A/H2B/H3/H4;	1-131	5.89e-49
[+]	HHT1	COG2036	Archaeal histone H3/H4 [Chromatin structure and dynamics];	43-134	8.80e-30

<https://prosite.expasy.org/>, <https://www.ebi.ac.uk/interpro/entry/pfam>

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Proteinové domény

Family: *Histone* (PF00125)

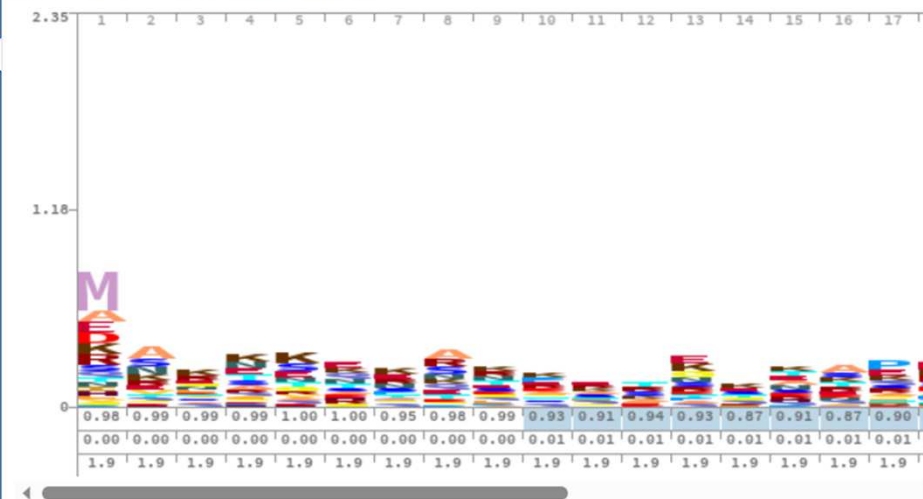
377 architecture



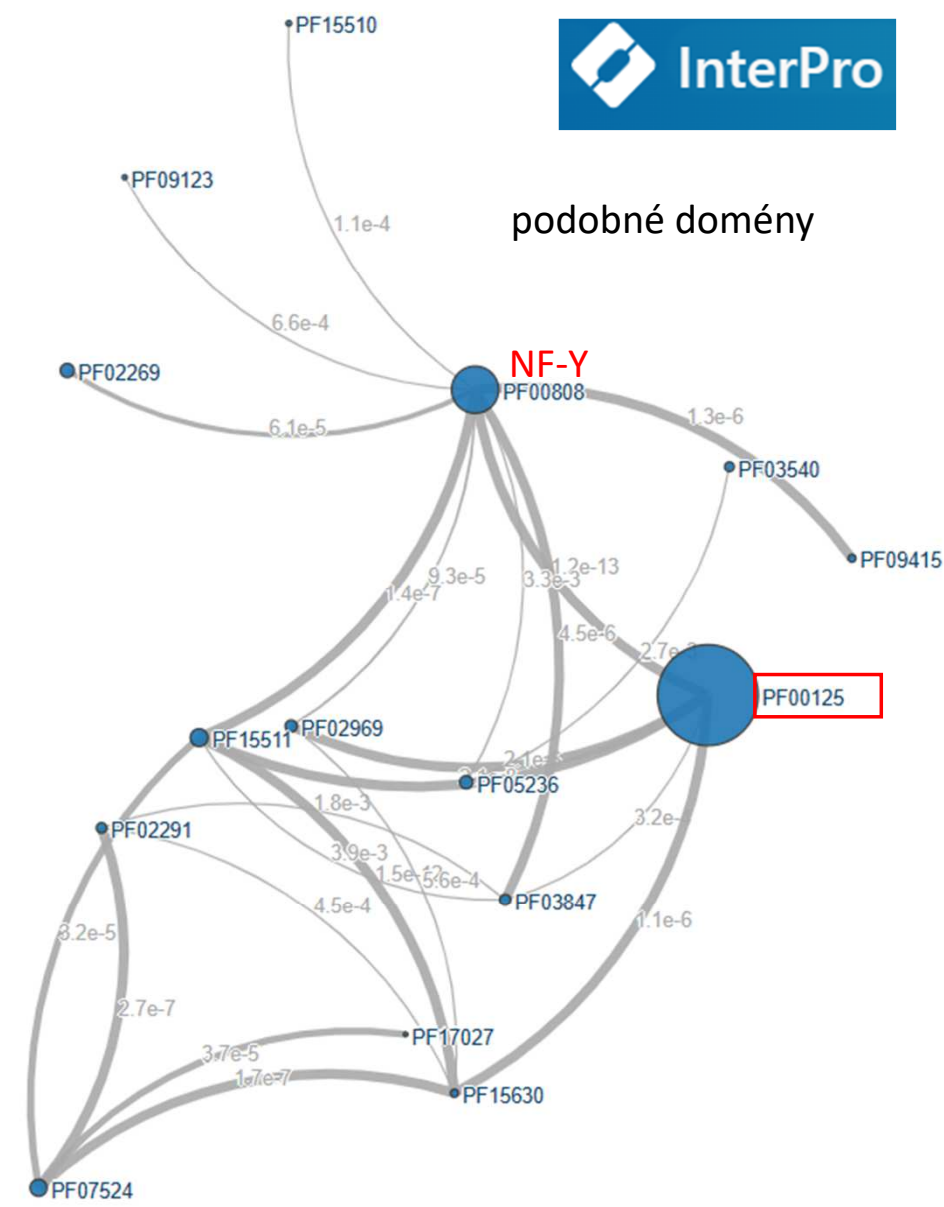
- Summary
- Domain organisation
- Clan
- Alignments
- HMM logo**
- Trees
- Curation & model
- Species
- Structures
- AlphaFold Structures
- trRosetta Structure

HMM logo

HMM logos is one way of visualising profile HMMs. Logos provide a quick overview of the properties you can interpret them [here](#). [More...](#)



printscreen: autor



Informace o proteinu - UNPROT

sekundární struktury

Histon H3

Function

Names & Taxonomy

Subcellular Location

Disease & Variants

PTM/Processing

Expression

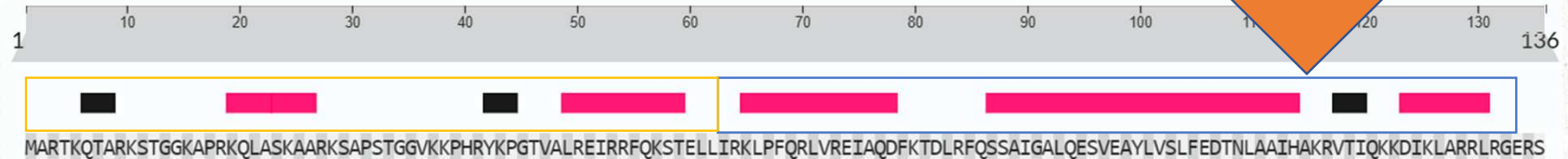
Interaction

Structure

Family & Domains

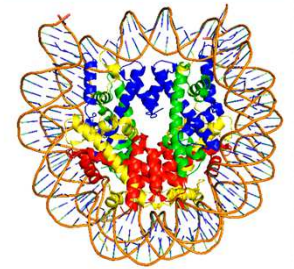
Sequence & Isoforms

Similar Proteins



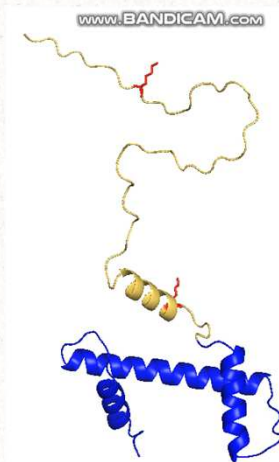
Family and domain databases

- Gene3D | 1.10.20.10 [↗](#) Histone, subunit A 1 hit
- IDEAL | IID50143 [↗](#)
- InterPro | [View protein in InterPro ↗](#)
- IPR009072 [↗](#) **Histone-fold**
- IPR007125 [↗](#) Histone_H2A/H2B/H3
- IPR000164 [↗](#) Histone_H3/CENP-A



Model Confidence:

- Very high (pLDDT > 90)
- Confident (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)



- PROSITE | [View protein in PROSITE ↗](#)
- PS00322 [↗](#) HISTONE_H3_1 1 hit
- PS00959 [↗](#) HISTONE_H3_2 1 hit
- Pfam | [View protein in Pfam ↗](#)
- PF00125 [↗](#) Histone 1 hit

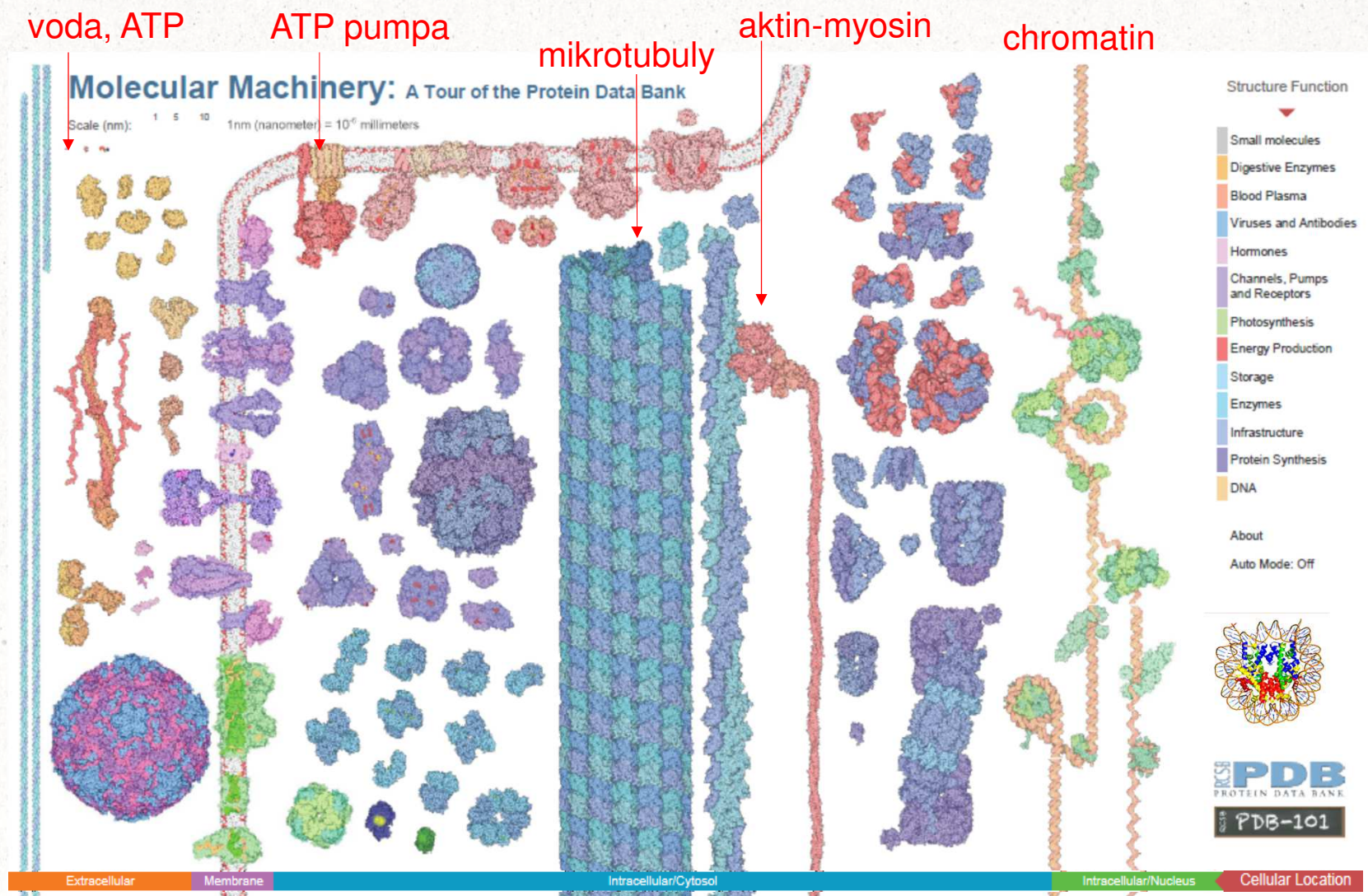
terciární a kvarterní struktura

Structureⁱ

PDB	7XAY	X-ray	3.30 Å	D	2-136	PDBe · RCSB-PDB · PDBj · PDBsum
PDB	7Z0O	EM	2.80 Å	A/C	1-136	PDBe · RCSB-PDB · PDBj · PDBsum
AlphaFold	AF-P61830-F1	Predicted			1-136	AlphaFold

video a printscreen: autor

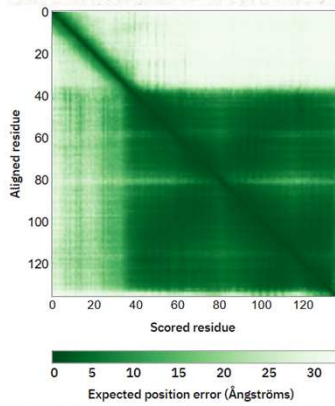
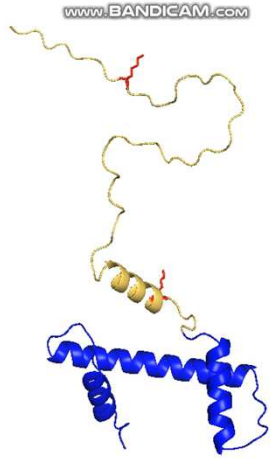
Primárním zdrojem strukturních informací = PDB



Interaktivní web PDB-101 (pro učitele): <https://pdb101.rcsb.org/>

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přes 200 000 struktur



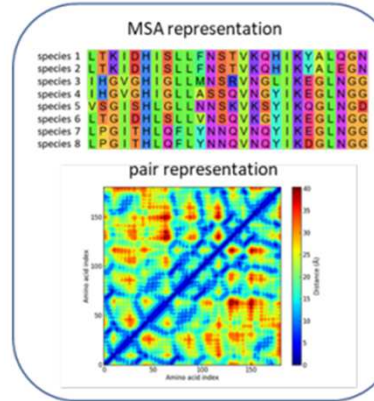
AlphaFold2

Nobelova cena
za chemii 2024

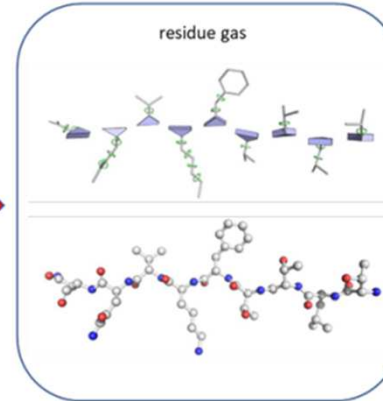


J.M. Jumper

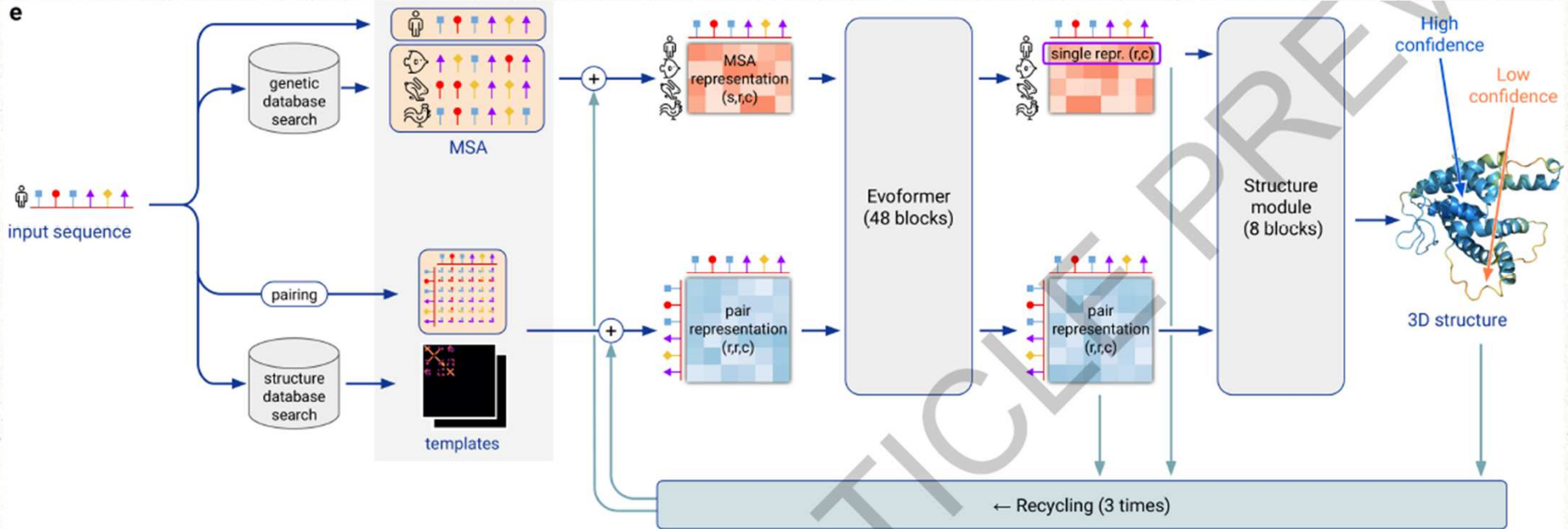
Evoformer



Structure module



EMBL databáze: <https://alphafold.ebi.ac.uk/> 200 000 000 struktur!



převzato z: Jumper et al., Nature, 2021; neural network: transformer architecture (attention mechanism for learning)

Informace o proteinu - UNPROT



Function

Names & Taxonomy

Subcellular Location

Phenotypes & Variants

PTM/Processing

Expression



Interaction

Structure

Family & Domains

Sequence

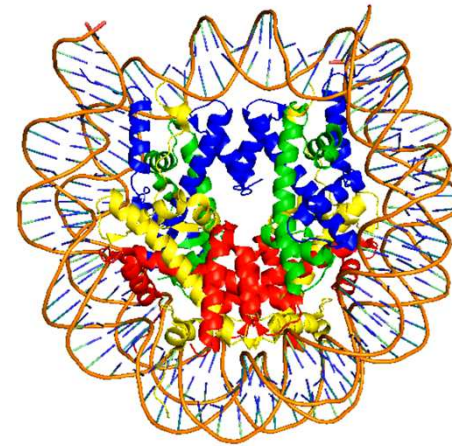
Similar Proteins

Functionⁱ

Core component of **nucleosome**. Nucleosomes wrap and compact DNA around histone cores, using DNA as a template. Histones thereby play a central role in transcription regulation. Histone H3 is regulated via a complex set of post-translational modifications of histone H3 (upstream activation factor) complex which interacts with the upstream

Subunitⁱ

The **nucleosome** is a histone octamer containing two molecules each of H2A, H2B, H3 and H4 assembled in one H3-H4 heterotetramer and two H2A-H2B heterodimers. The octamer wraps approximately 147 bp of DNA. Histone H3 is a component of the UAF (upstream activation factor) complex, which consists of UAF30, RRN5, RRN9, RRN10, and histones H3 and H4. 1 Publication



Histon H3

Similar machineries which require histone H3 for chromosomal stability. DNA accessibility is regulated by histone H3 acetylation. Component of the UAF (upstream activation factor) complex which acts as a stable preinitiation complex.

Protein-protein interaction databases

BioGRID	32711 ↗ 918 interactors	DIP	DIP-417N ↗
	35796 ↗ 705 interactors	IntAct	P61830 ↗ 153 interactors
ComplexPortal	CPX-1101 ↗ RNA polymerase I upstream activating factor complex	MINT	P61830 ↗
	CPX-1610 ↗ Nucleosome, variant HTA2-HTB2	STRING	4932.YBR010W ↗
	CPX-1611 ↗ Nucleosome, variant HTA2-HTB1		
	CPX-1612 ↗ Nucleosome, variant HTA1-HTB1		
	CPX-1613 ↗ Nucleosome, variant HTZ1-HTB1		
	More ComplexPortal links		

video a printscreen: autor

Informace o proteinu - UNIPROT

kvarterní struktura

Function

Names & Taxonomy

Subcellular Location

Disease & Variants

PTM/Processing

Expression

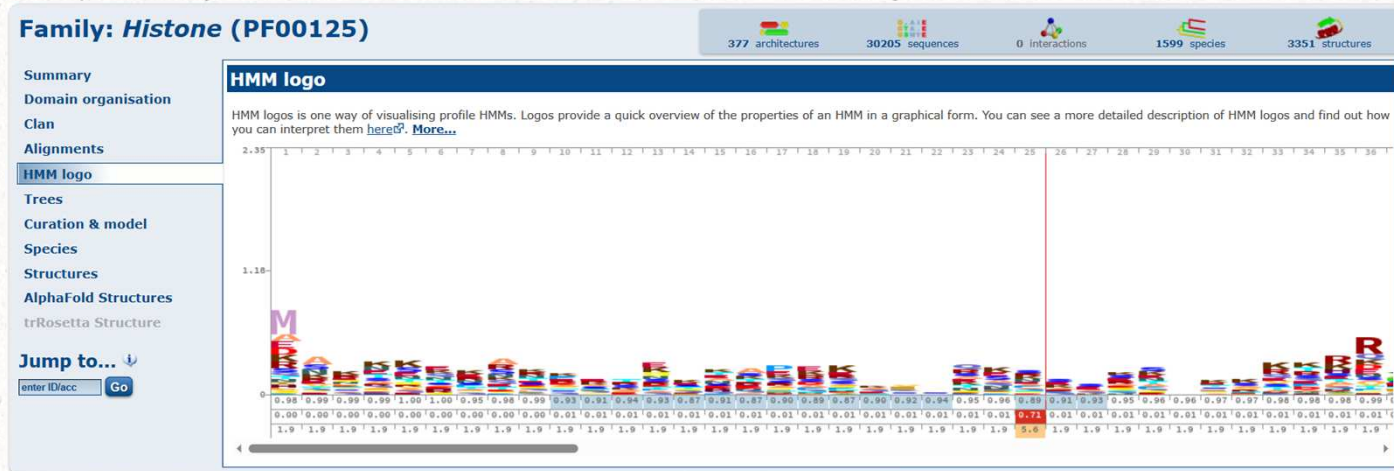
Interaction

Structure

Family & Domains

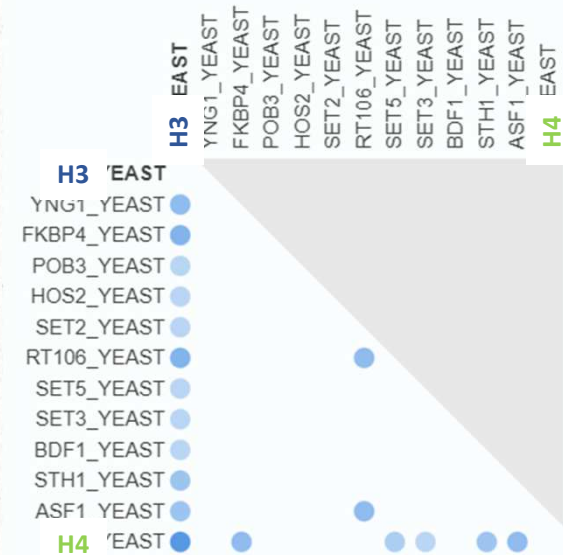
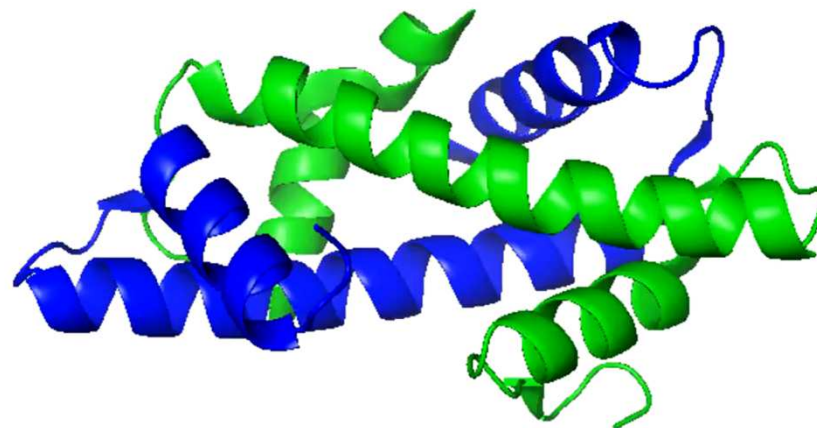
Sequence & Isoforms

Similar Proteins



Histon fold –
typicky P-P vazba
... vazba na DNA

H3-H4 dimer



video a printscreen: autor

Informace o proteinu – IntAct

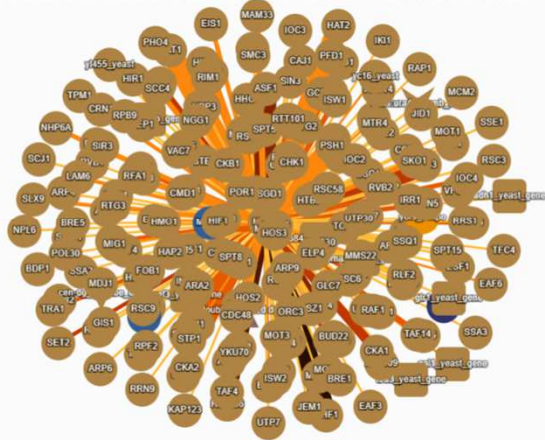


<https://www.ebi.ac.uk/intact>

Select	Molecule A	Molecule B	Identifier A	Identifier B	Type A	Type B	Species A	Species B	Host Organism	Positive interaction	Detection Method	Publication IDs	Interaction Type
<input type="checkbox"/>	H4	H3	UniProt P02309	UniProt P61830	protein	protein	Saccharomyces cerevisiae	Saccharomyces cerevisiae	Saccharomyces cerevisiae	✓	tap	16554755 10.1038/nature04670	physical association

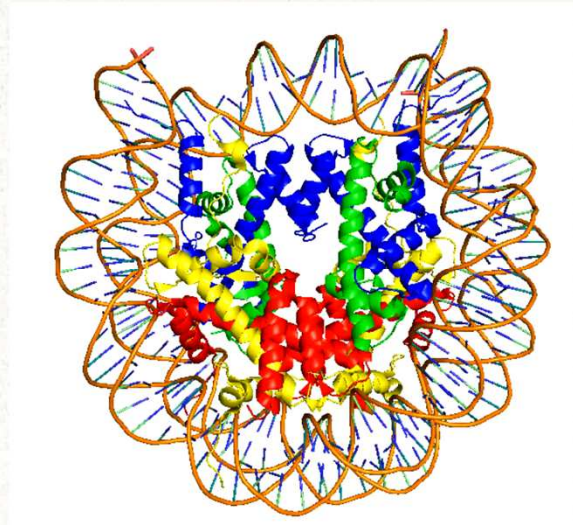
network ... i genetické (nefyzické), funkční ... interakce

sít'



- chaperon
- deacetylasa
- metyltransferasa
- chaperon
- metyltransferasa
- bromodoména
- chaperon
- histon H4

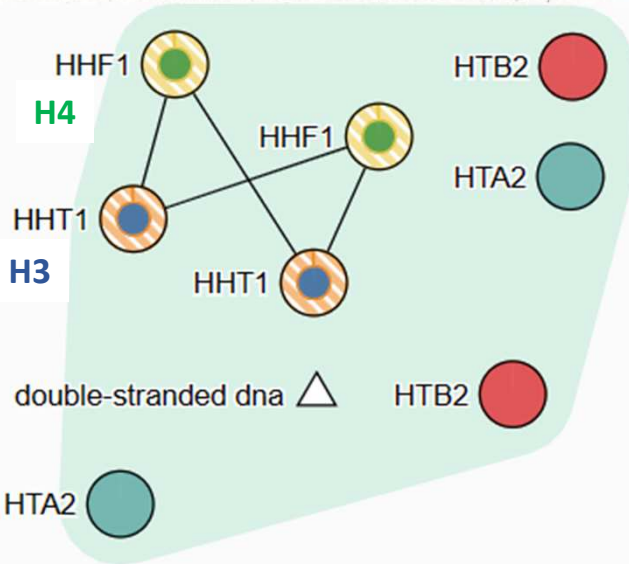
H3 YEAS	H4 YEAS
YNG1_YEAST	YNG1_YEAST
FKBP4_YEAST	FKBP4_YEAST
POB3_YEAST	POB3_YEAST
HOS2_YEAST	HOS2_YEAST
SET2_YEAST	SET2_YEAST
RT106_YEAST	RT106_YEAST
SET5_YEAST	SET5_YEAST
SET3_YEAST	SET3_YEAST
BDF1_YEAST	BDF1_YEAST
STH1_YEAST	STH1_YEAST
ASF1_YEAST	ASF1_YEAST
H4_YEAST	H4_YEAST



video a printscreen: autor



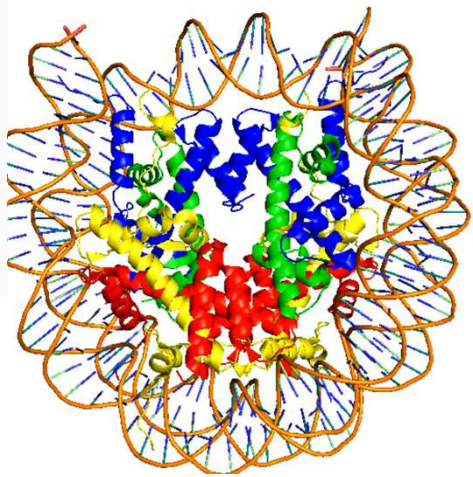
komplex - struktury



Protein-protein interaction databases

BioGRID	32711 ↗ 918 interactors 35796 ↗ 705 interactors
ComplexPortal	↗ CPX-1101 RNA polymerase I upstream activating factor complex ➔ ↗ CPX-1610 Nucleosome, ↗ CPX-1611 Nucleosome, ↗ CPX-1612 Nucleosome, ↗ CPX-1613 Nucleosome, ↗ More ComplexPortal links

DIP	↗ DIP-417N
IntAct	↗ P61830 153 interactors
MINT	↗ P61830



Legend	Description	Stoichiometry
	protein - HTA2 (unspecified role) P04912 ↗ Histone H2A.2	H2A 2
	protein - HTB2 (unspecified role) P02294 ↗ Histone H2B.2	H2B 2
	protein - HHT1 (unspecified role) P61830 ↗ Histone H3	H3 2
	protein - HHF1 (unspecified role) P02309 ↗ Histone H4	H4 2
	small molecule - double-stranded dna (unspecified role) CHEBI:4705 ↗ double-stranded DNA	1

video a printscreen: autor

AlphaFold 3 – predikce 3D struktury komplexů

- Online predikce AlphaFold: <https://golgi.sandbox.google.com/>

The screenshot displays the AlphaFold 3 web interface for creating a prediction job. It features five rows of input fields for different molecule types:

- Protein:** Molecule type: Protein, Copies: 1, Input: >Paste sequence or fasta Input
- DNA:** Molecule type: DNA, Copies: 1, Input: >Paste sequence or fasta 5'--3' single strand
- RNA:** Molecule type: RNA, Copies: 1, Input: >Paste sequence or fasta 5'--3' single strand
- Ligand:** Molecule type: Ligand, Copies: 1, Input: (empty)
- Ion:** Molecule type: Ion, Copies: 1, Input: (empty)

At the bottom of the interface, there are three buttons: "+ Add entity", "Save job", and "Continue and preview job".

Google DeepMind

About Google

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Terms

Output Terms

Privacy

Prohibited use policy

Release Updates

- Umožňuje i predikci 3D struktury proteinového komplexu (dimeru dvou proteinů – díky jejich **koevoluci**)

Histon H3

Informace o proteinu - UNPROT

Function

Names & Taxonomy

Subcellular Location

Disease & Variants

PTM/Processing

Expression

Interaction

Structure

Family & Domains

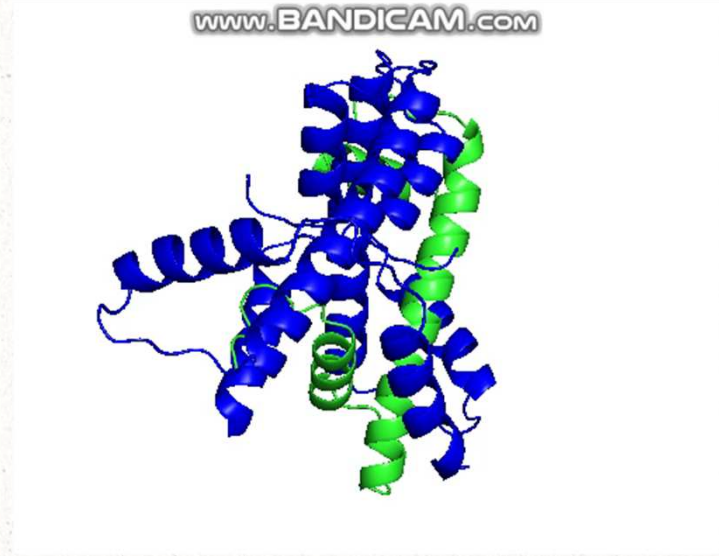
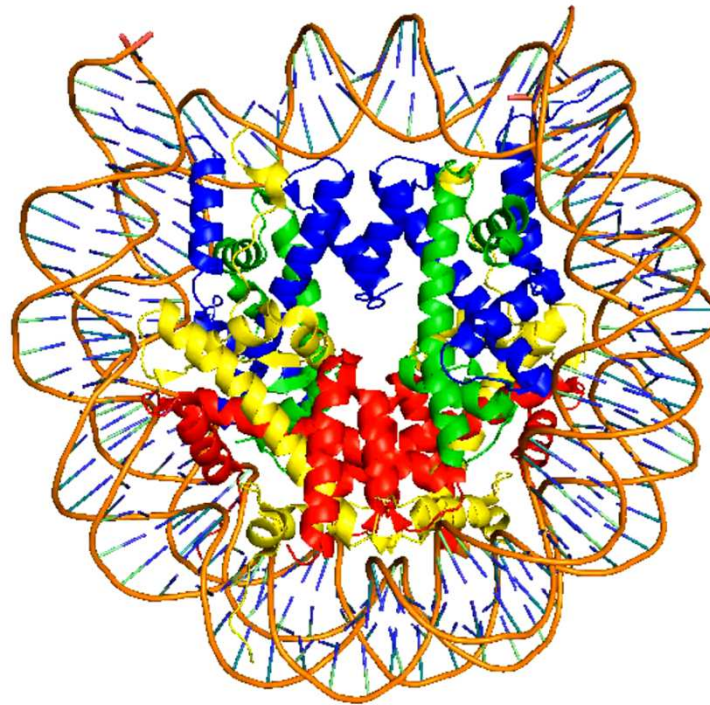
Sequence & Isoforms

Similar Proteins

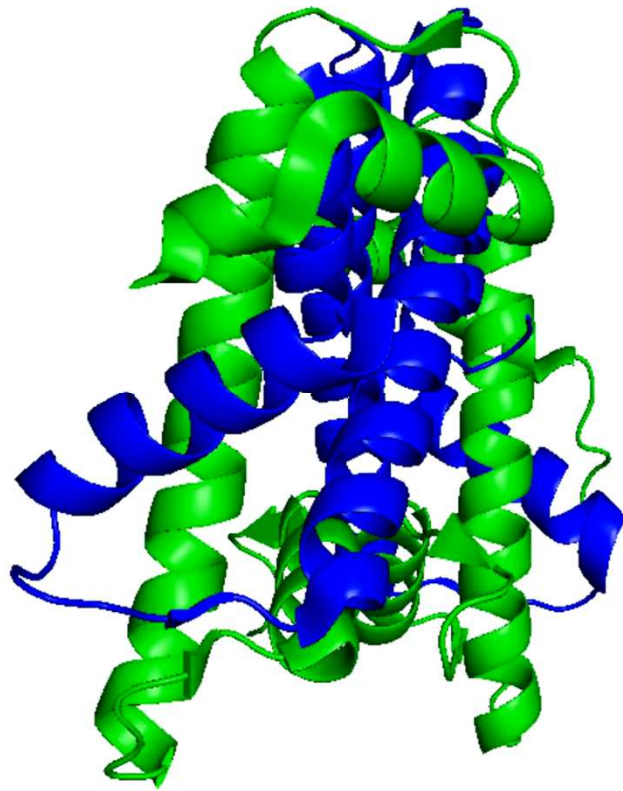
Structureⁱ

terciární a kvarterní struktura

PDB	7XAY	X-ray	3.30 Å	D	2-136	PDBe · RCSB-PDB · PDBj · PDBsum
PDB	7Z0O	EM	2.80 Å	A/C	1-136	PDBe · RCSB-PDB · PDBj · PDBsum
AlphaFold	AF-P61830-F1	Predicted			1-136	AlphaFold



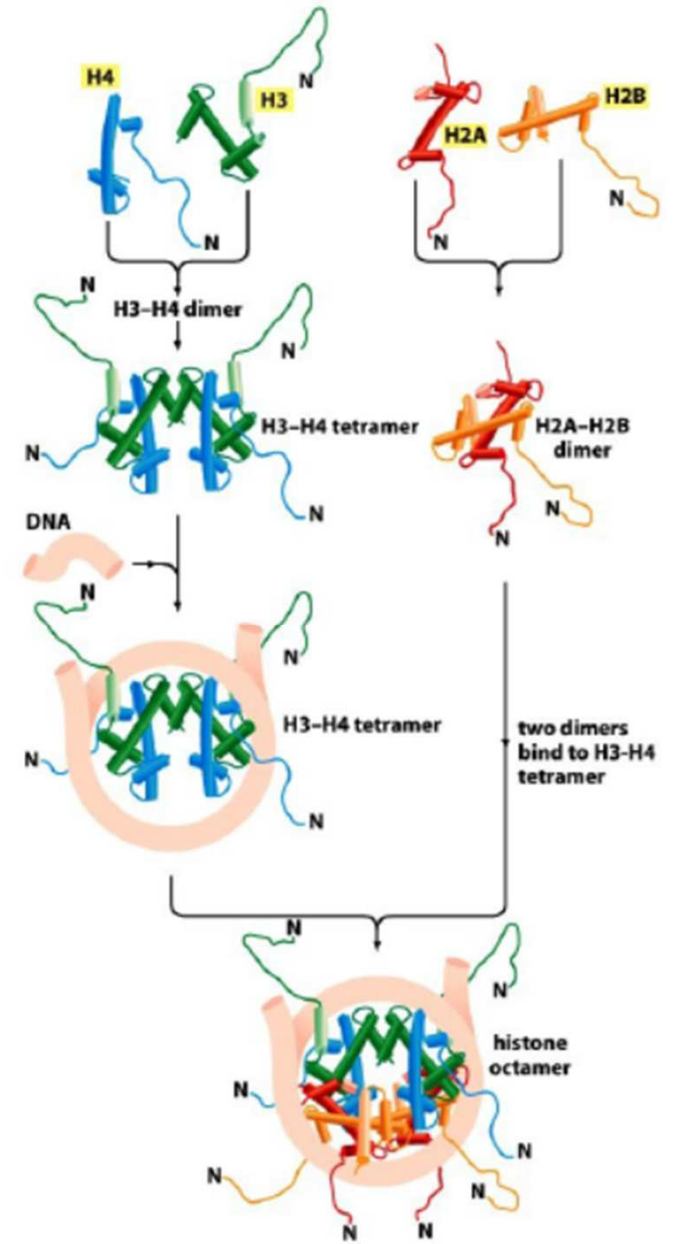
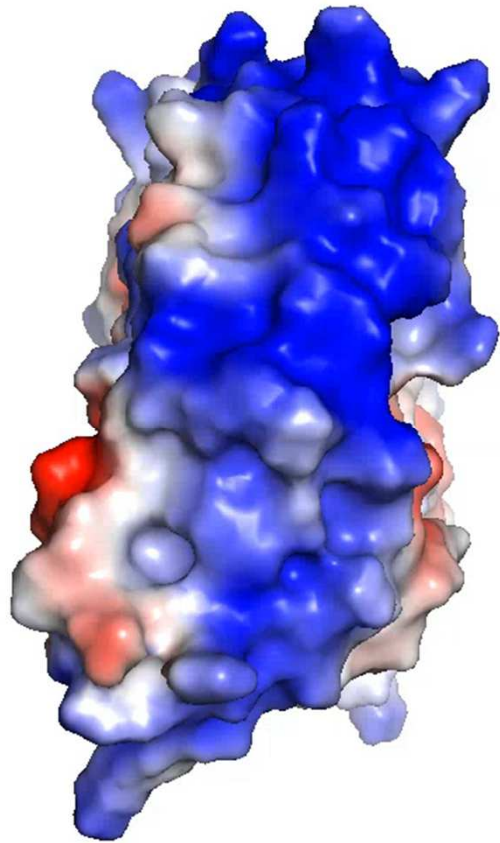
H3-H3 dimer vygeneroval alphafold jako H3-H4 dimer – až po použití H3-H4+H3 umístil H3-H3 dimer správně



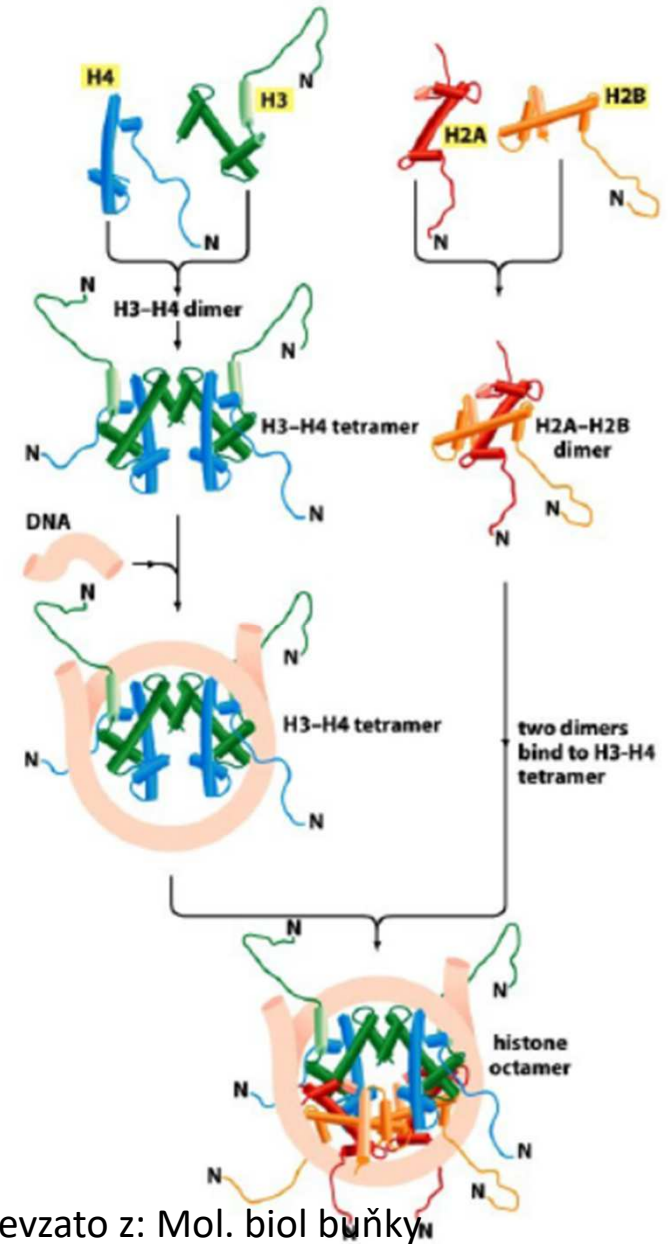
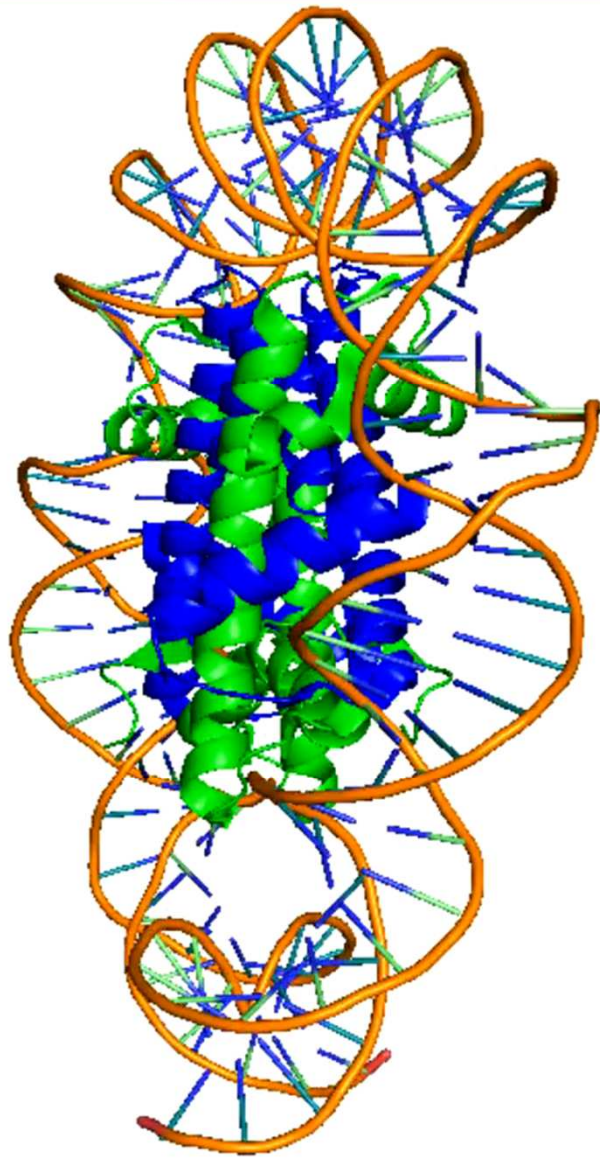
nukleosom se skládá na „začátku“ (replikace DNA – vznik nové DNA ...), ale i v průběhu života buňky (oprava DNA, transkripce) – odbalení a sbalení nukleosomu regulují chaperony, remodelační komplexy ...

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

video: autor



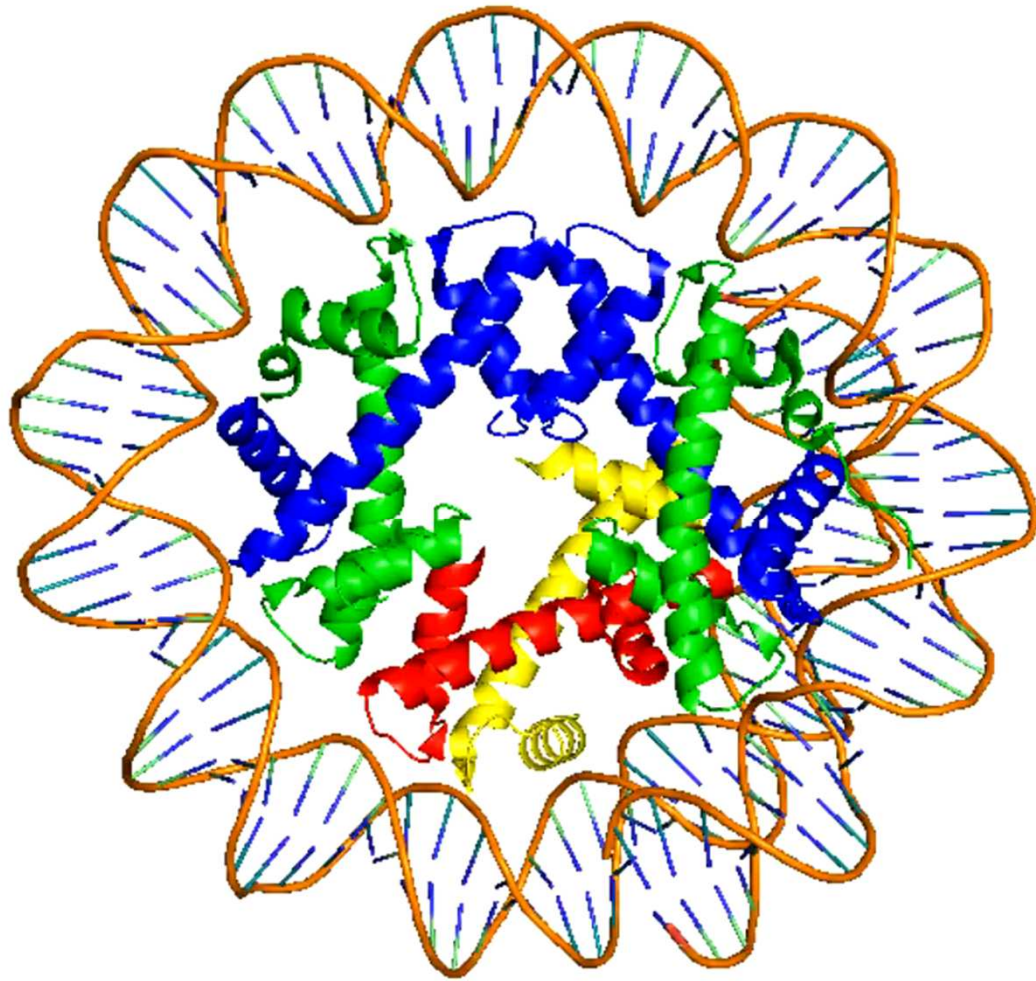
video: autor



video: autor

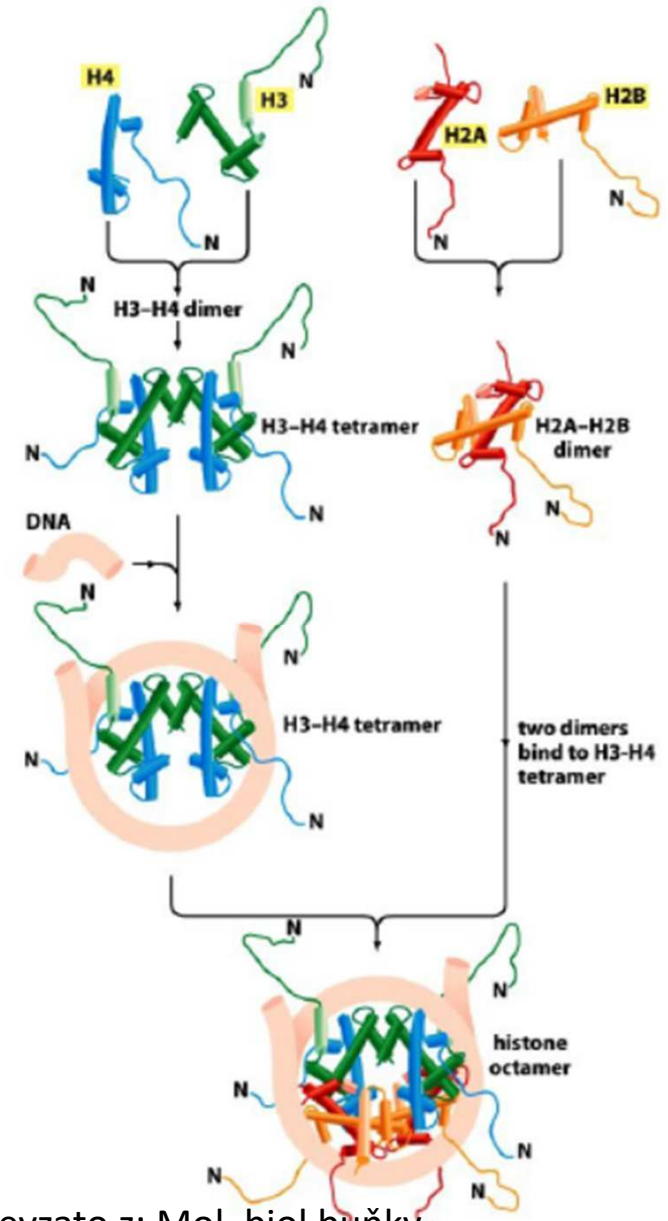
- H3-H3 interakce = (H3-H4)₂ tetramer – vazba na DNA

převzato z: Mol. biol buňky

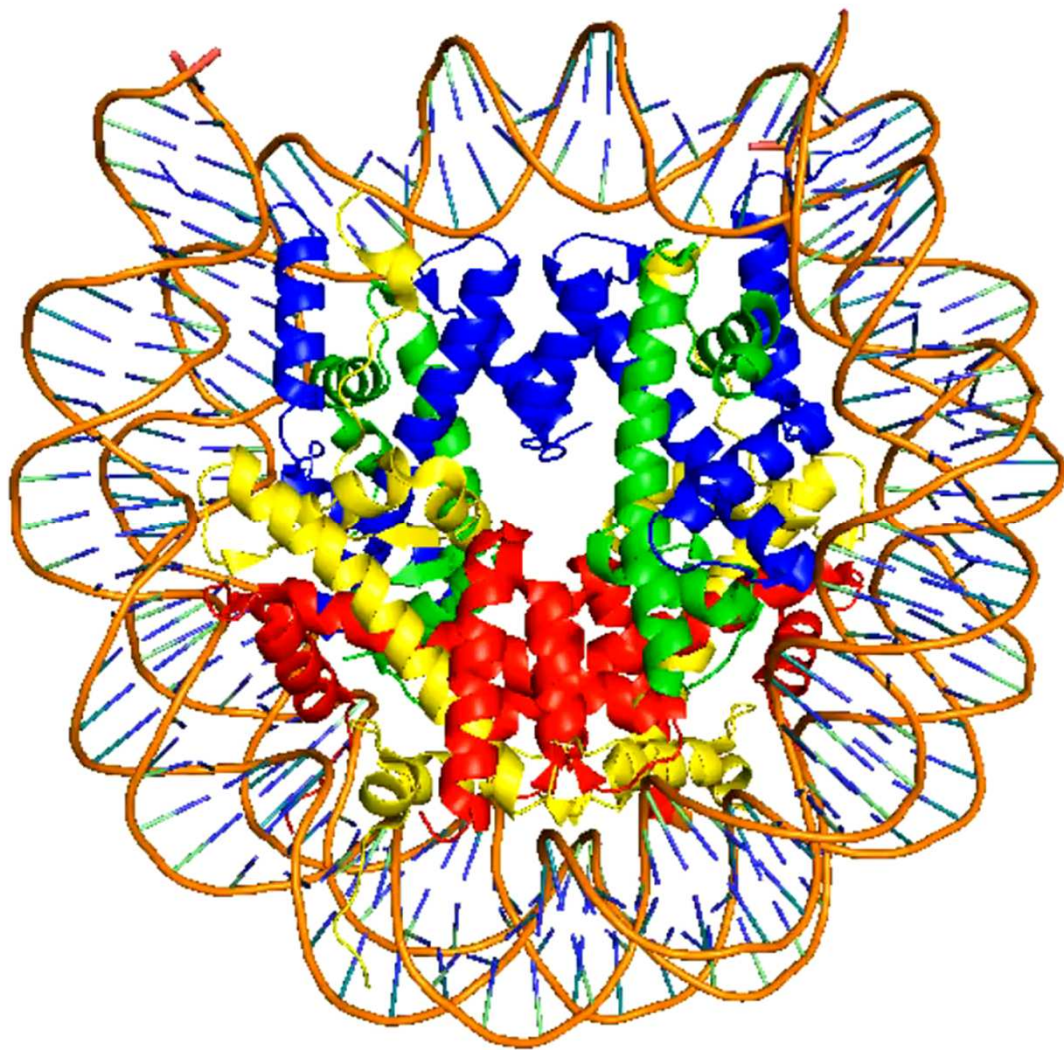


- H3-H3 interakce = (H3-H4)₂ tetramer
- dimery H2A-H2B (H2B – váže H4)

video: autor

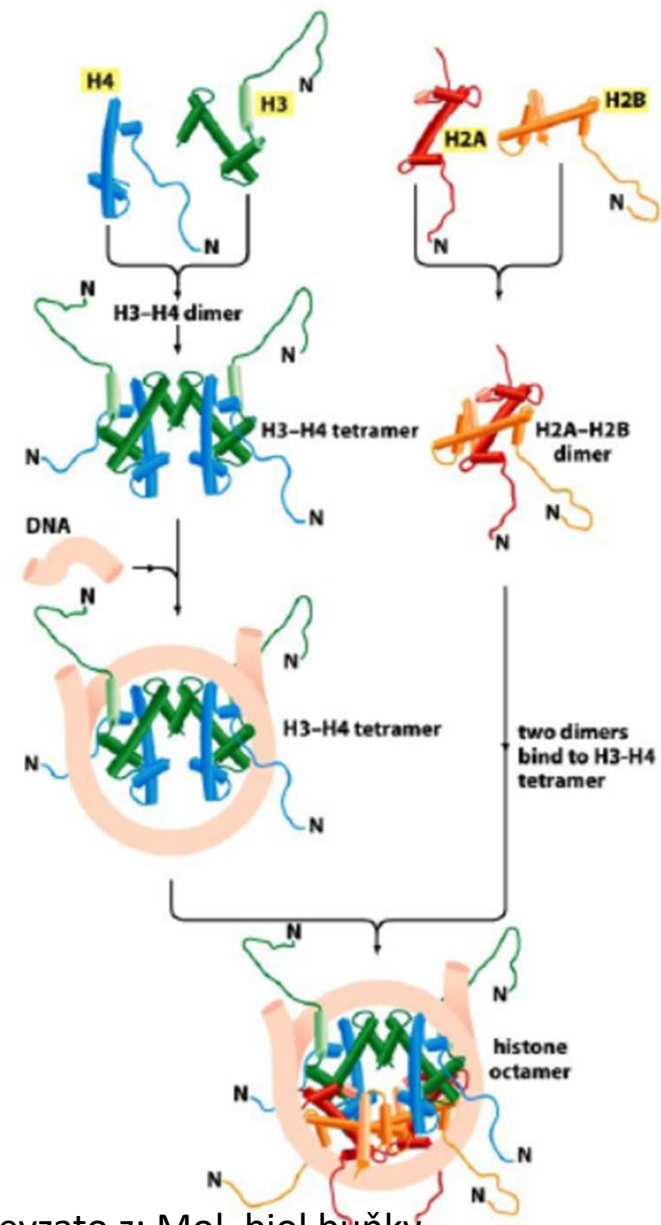


převzato z: Mol. biol buňky



- H3-H3 interakce = (H3-H4)₂ tetramer
- dimery H2A-H2B (H2B – váže H4)

video: autor



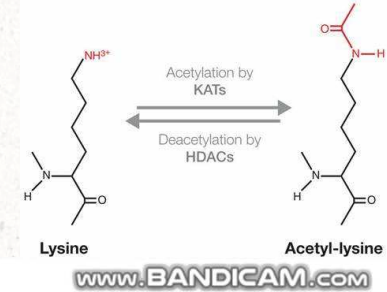
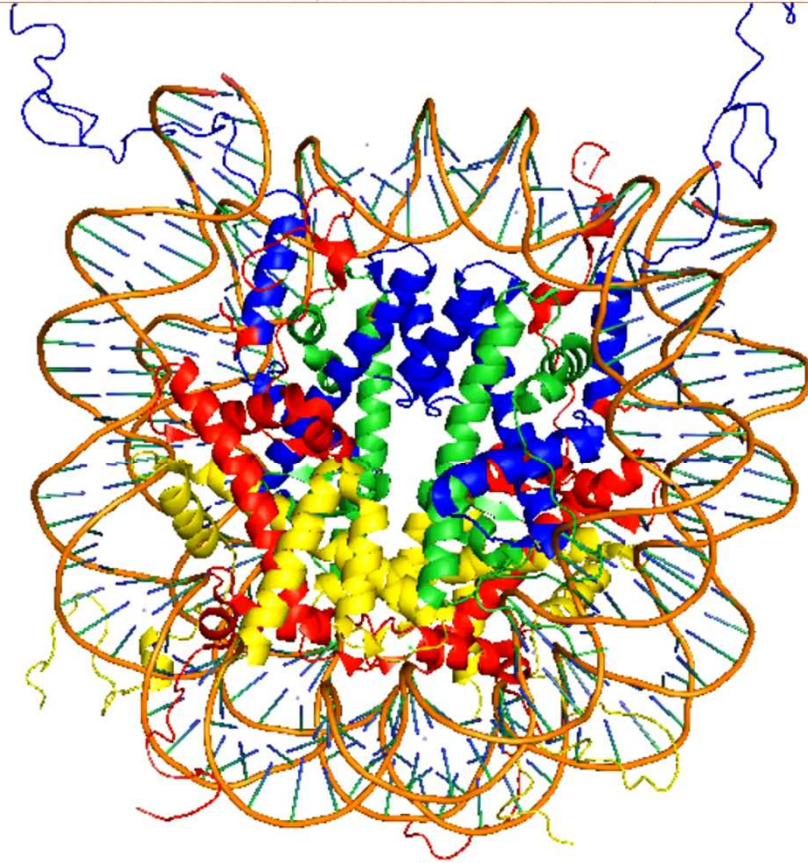
převzato z: Mol. biol buňky

Modifikace histonů – H3K56ac

- modifikace (např. acetylace) mohou: vázat jiné proteiny (změní tvar povrchu – váže specificky bromodoména) nebo oslabit interakce (změní náboj povrchu – oslabí vazbu s DNA a rozvolní nukleosom) – pozice PTM

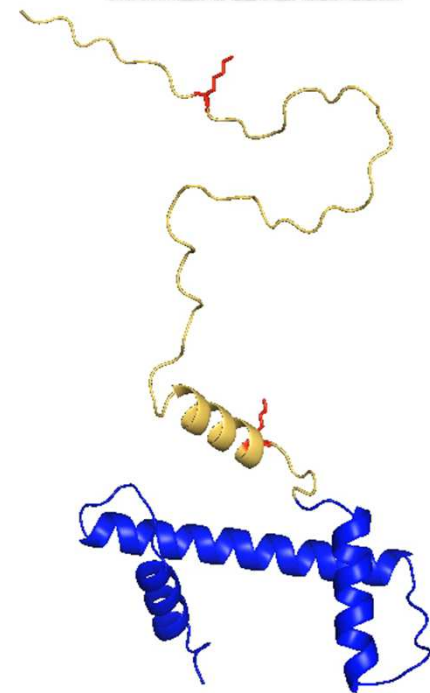
1KX5

Only



H3K9ac

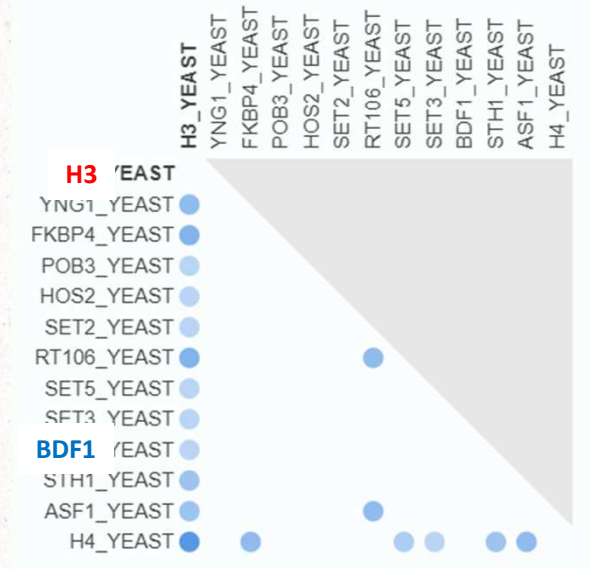
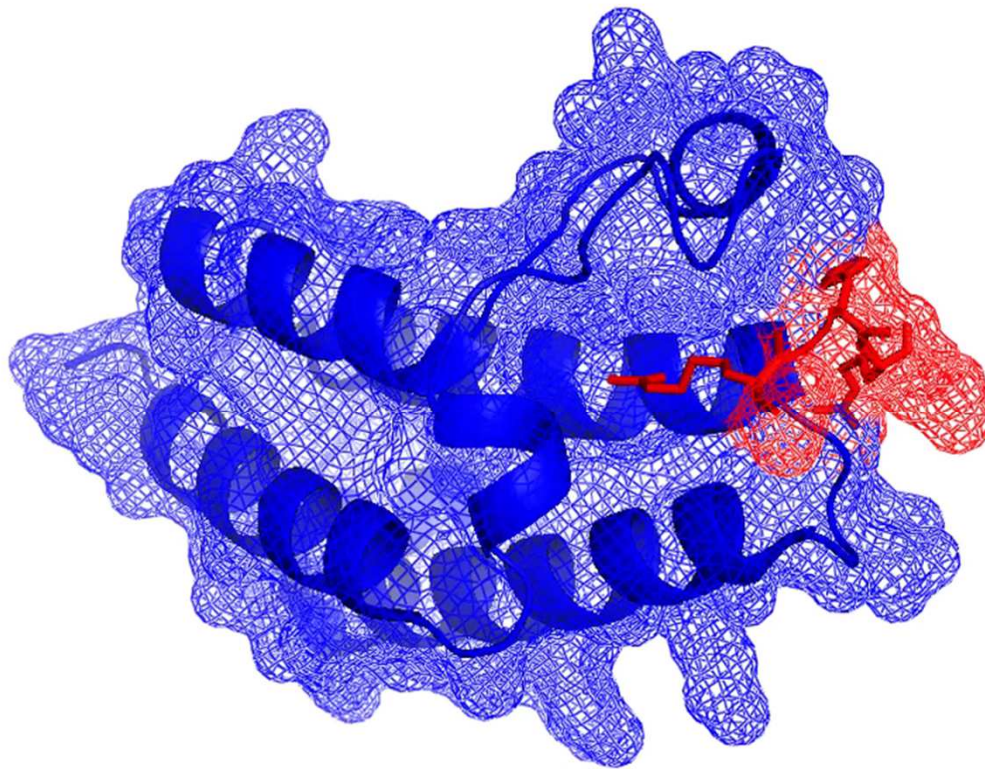
H3K56ac



video: autor

Modifikace histonů – H3K9ac

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bromodoména váže
acetylovaný **histon**

video a printscreen: autor

Docking - hra

Bioblox 2½D Game on the Topic of Protein Docking



https://www.youtube.com/watch?v=u_-8JyCWpEQ&t=7s

Bioblox 2½D is a free mobile game on the Topic of Protein Docking. Play the Proteins Docking game. Learn about the fascinating world of bio-molecules and their interactions. Drag, Rotate, Swipe and fit the chains together like the components of a mechanism.



or [PLAY ONLINE](#)

<https://www.doc.gold.ac.uk/bioblox/>

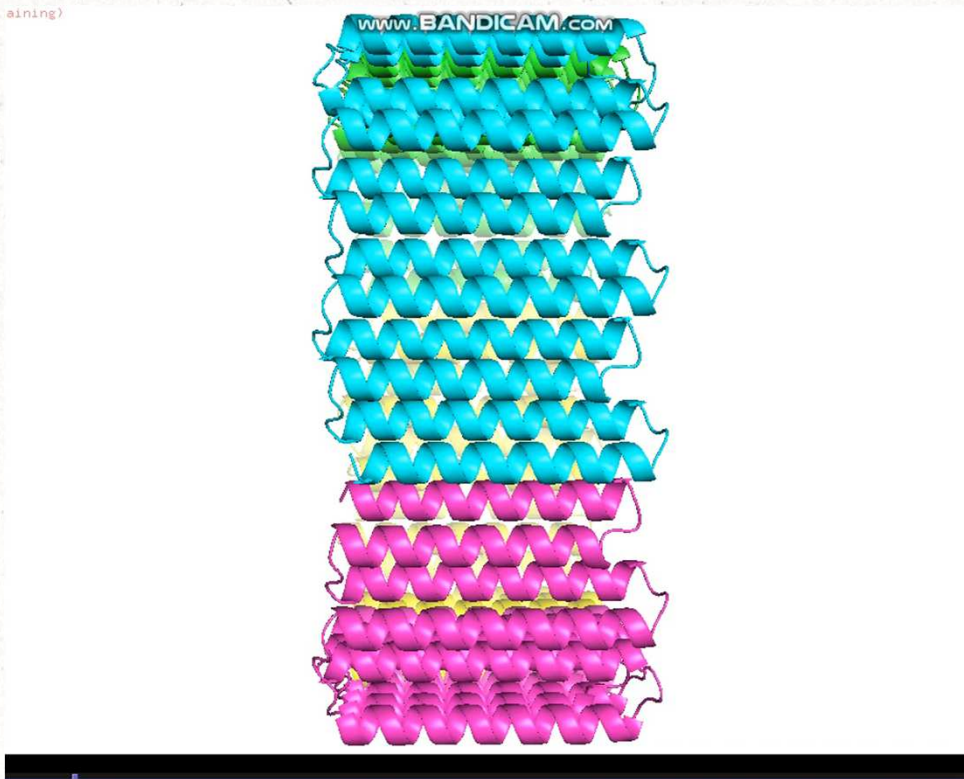
printscreen: autor

Predikce proteinů a komplexů - nanostruktury

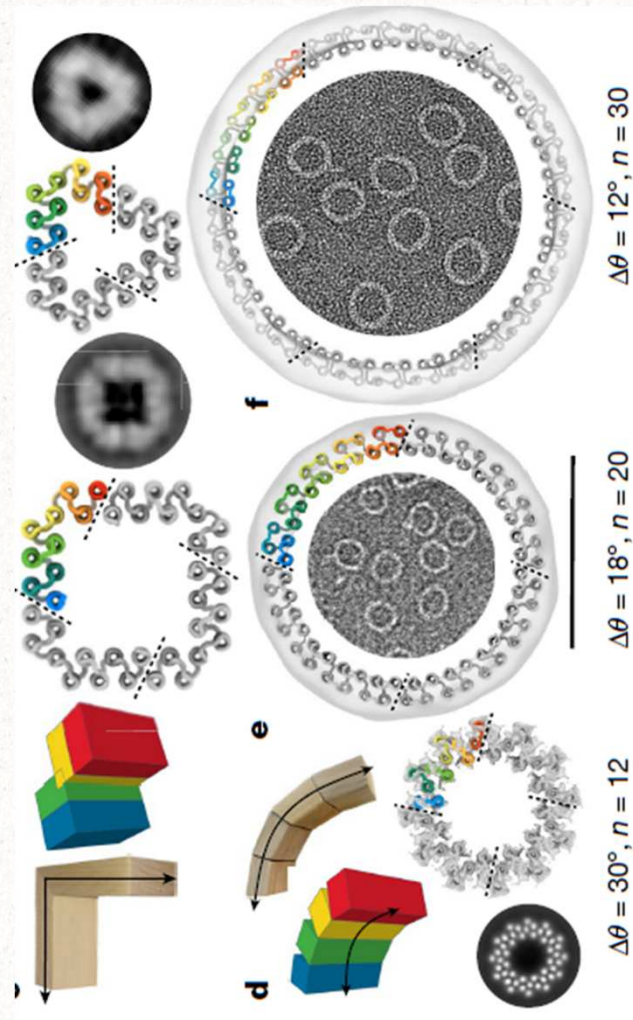
Nobelova cena
za chemii 2024



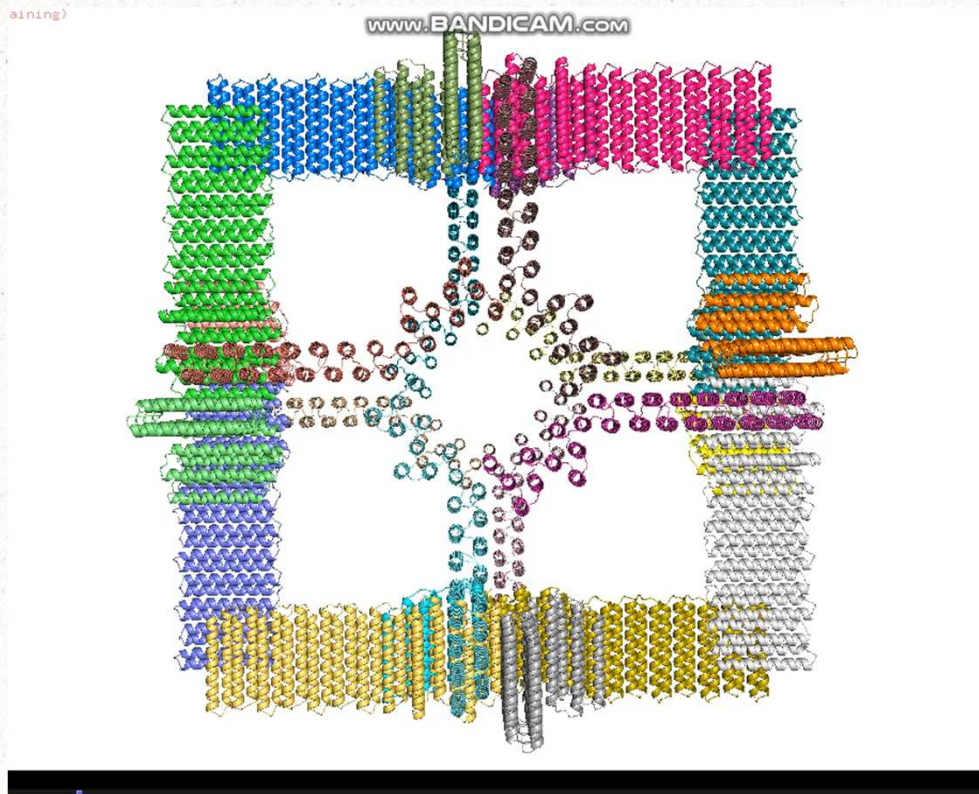
D. Baker



Huddy et al., Nature, 2024



Predikce proteinů a komplexů - nanoklece



Nobelova cena
za chemii 2024



D. Baker



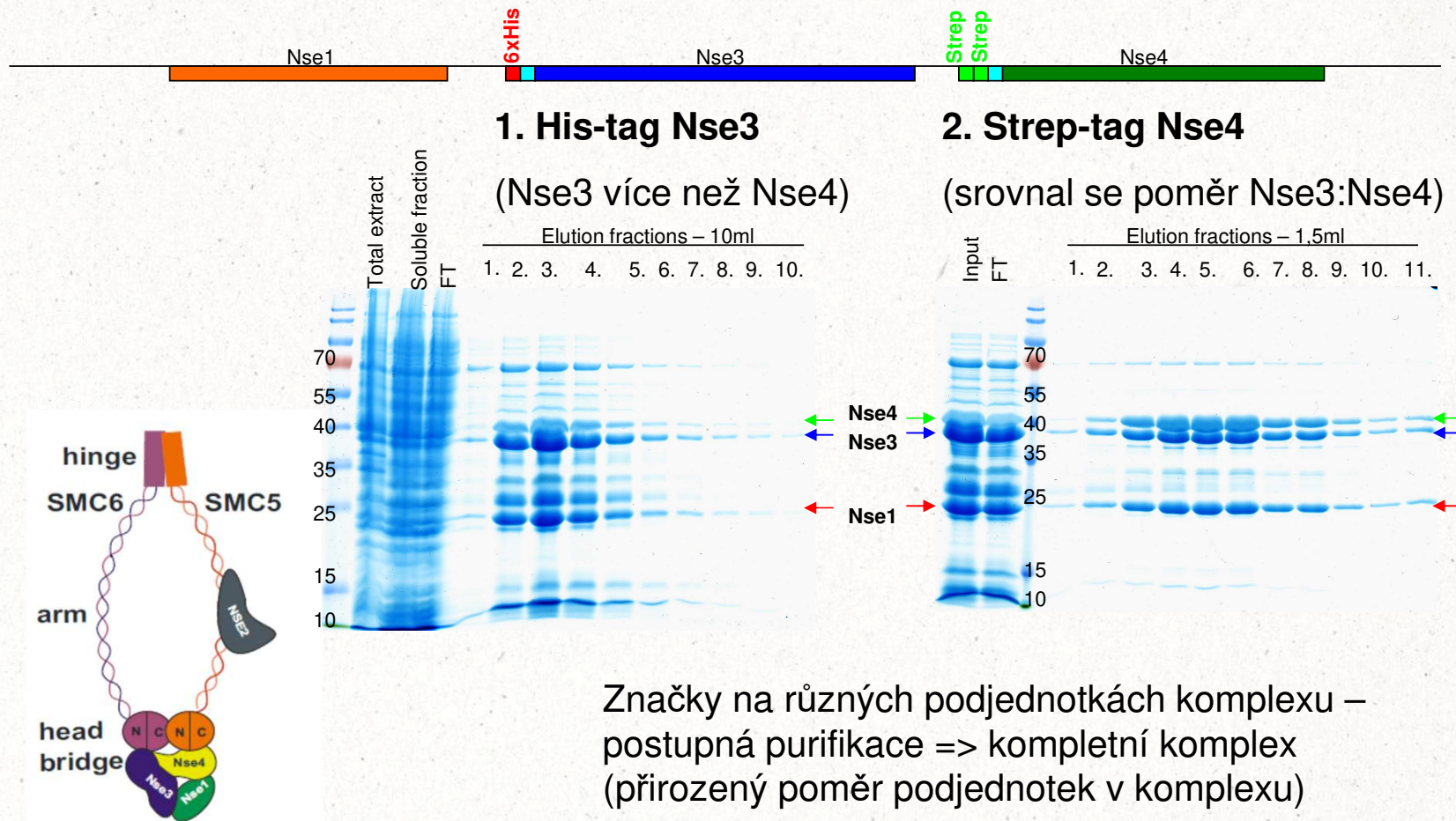
Huddy et al., Nature, 2024

Pokročilé metody v analýze proteinových komplexů

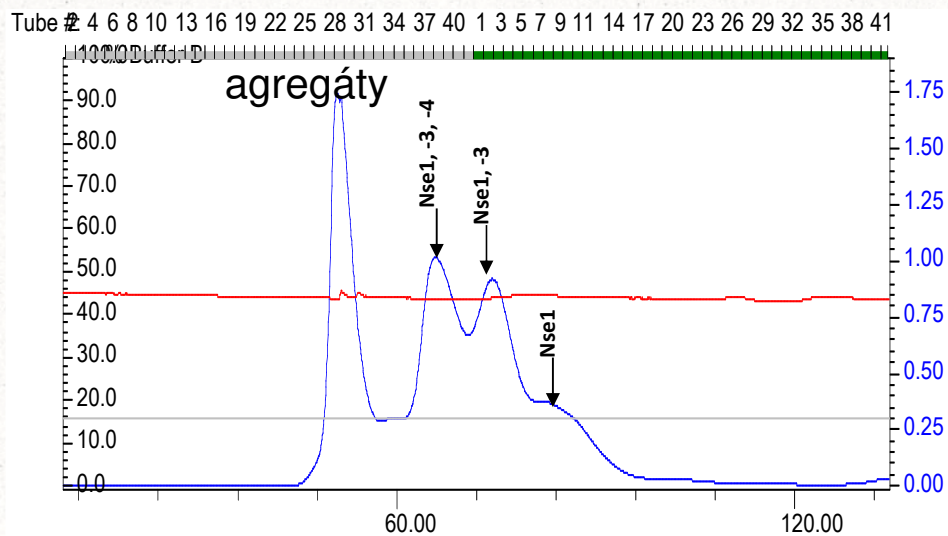
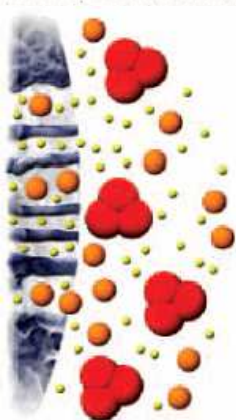
- Co všechno se dozvíte o genu/proteinu z databází
 - 3D (terciární) struktura proteinů
 - Proteinové interakce
 - Proteinové komplexy (kvarterní struktura)
- **Jak získat nové informace o proteinových komplexech experimentálně**
 1. - identifikace genů/proteinů ... jejich partnerů (PPI) ... izolace komplexu
 - funkce podjednotek (genetická analýza, lokalizace v buňce ...)
 2. - charakterizace komplexu
 - vzájemné PPI podjednotek – architektura komplexu
 3. - rekonstituce, struktura a analýza aktivit celého komplexu *in vitro*
 - struktura: crosslink MS, X-ray, (cryo) elektronová mikroskopie, modelování, integrativní analýza
 4. - vizualizace komplexů ...

Ko-purifikace - ověření

silné interakce/komplexy – proteiny lze ko-exprimovat a následně ko-purifikovat

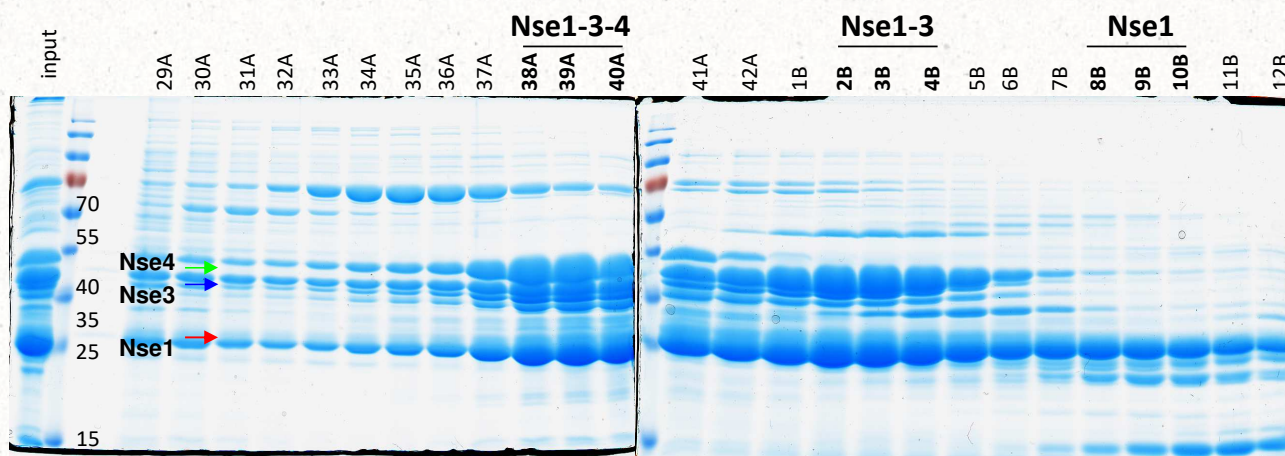
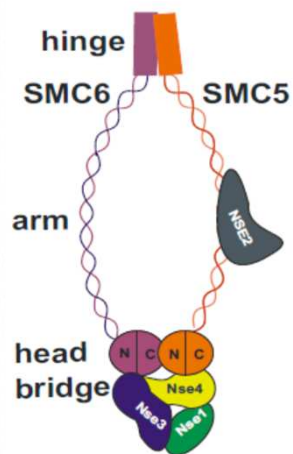


Ko-purifikace - příprava

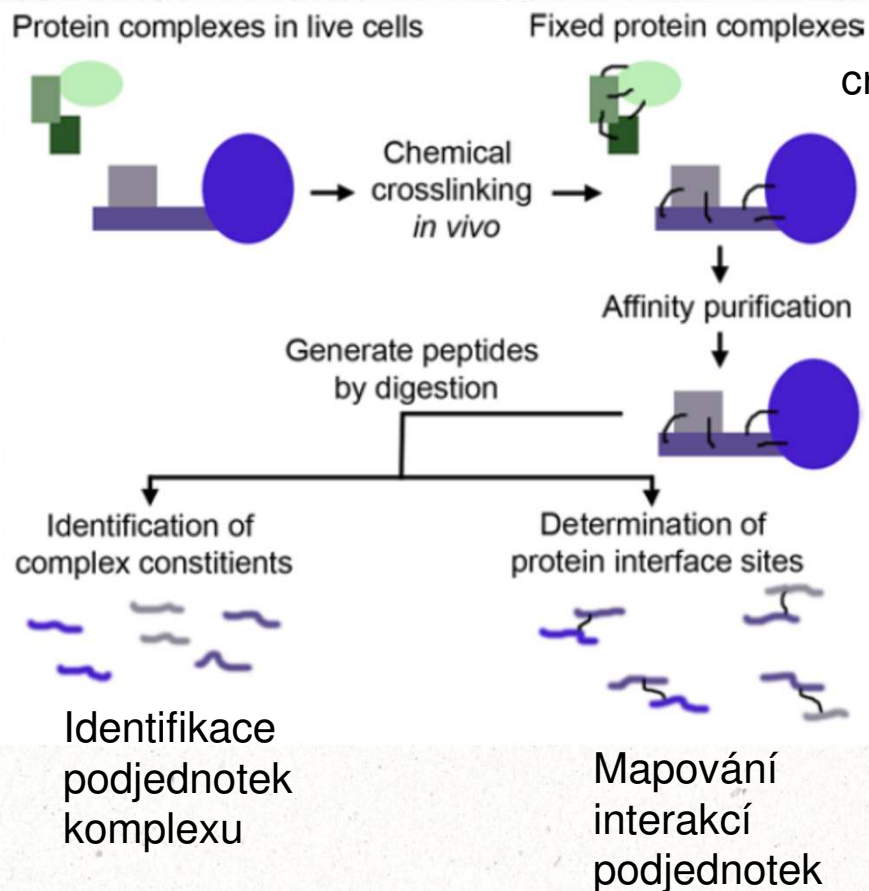


- analýza aktivit *in vitro*
- analýza struktury ...

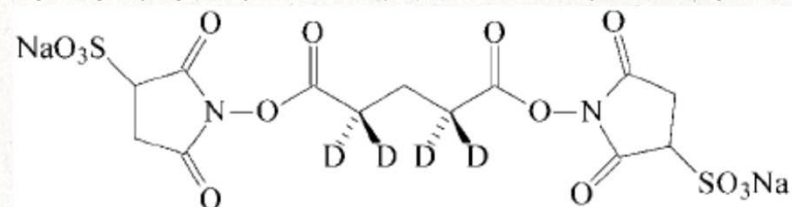
3. Gelová filtrace – lze ještě dočistit subpopulace komplexů



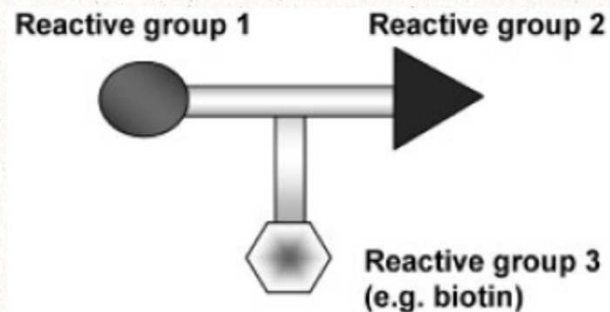
Detailní mapování komplexů - crosslinking



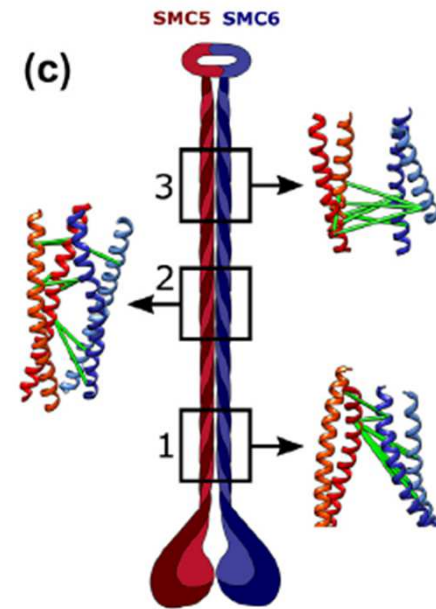
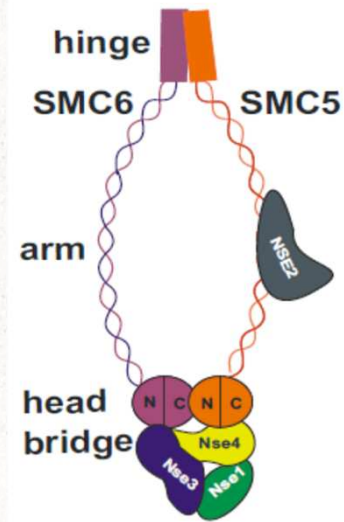
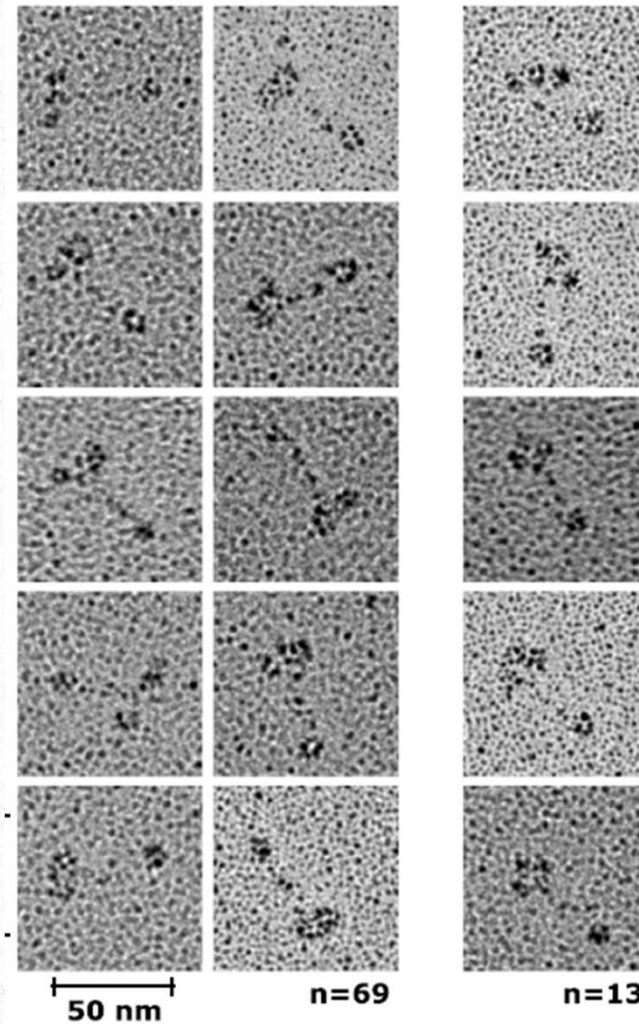
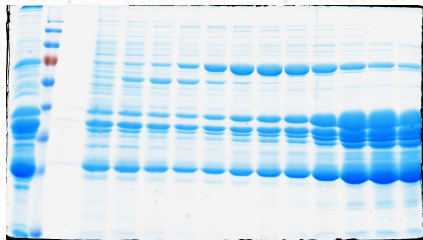
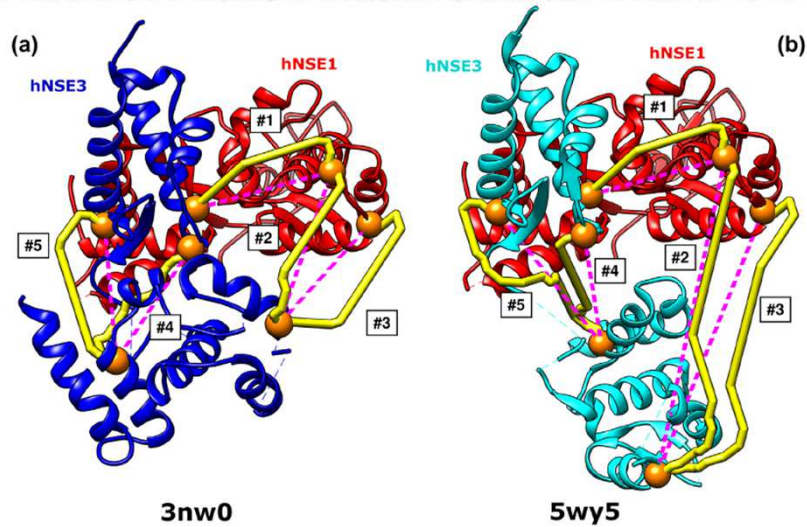
crosslink propojí podjednotky - stabilizuje komplex



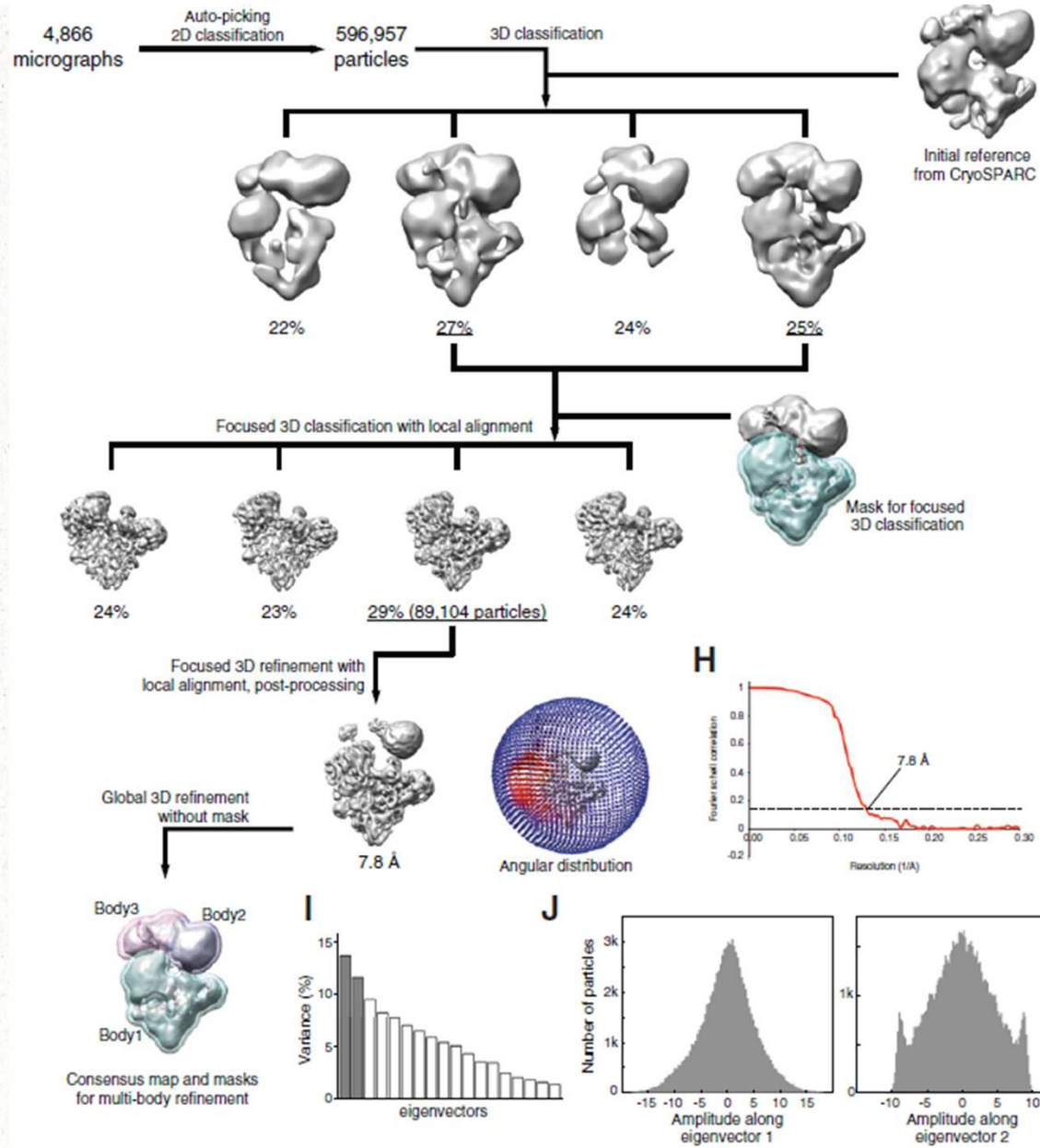
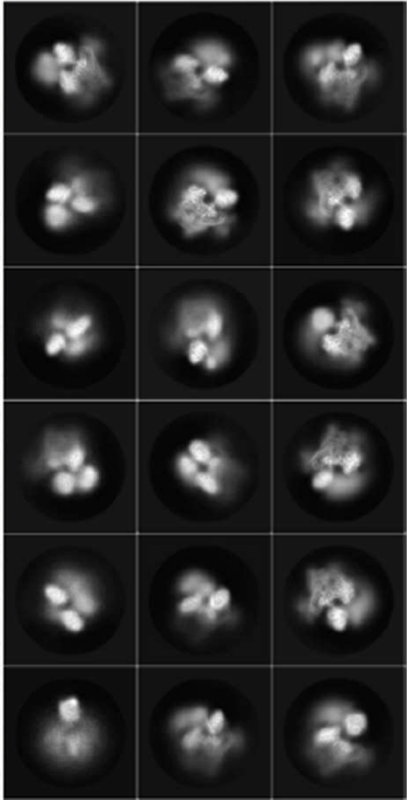
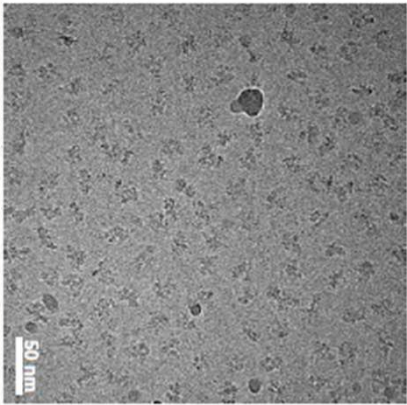
- estery reagují s Lys (ϵ -amin)
- homofunkční - spojí proteiny dohromady v jednom kroku
- heterofunkčních - postupná aktivace



- krosslink pomůže upřesnit strukturální model (Nse1-Nse3)
- ukázal jak jsou ramena Smc5-Smc6 vedle sebe umístěna



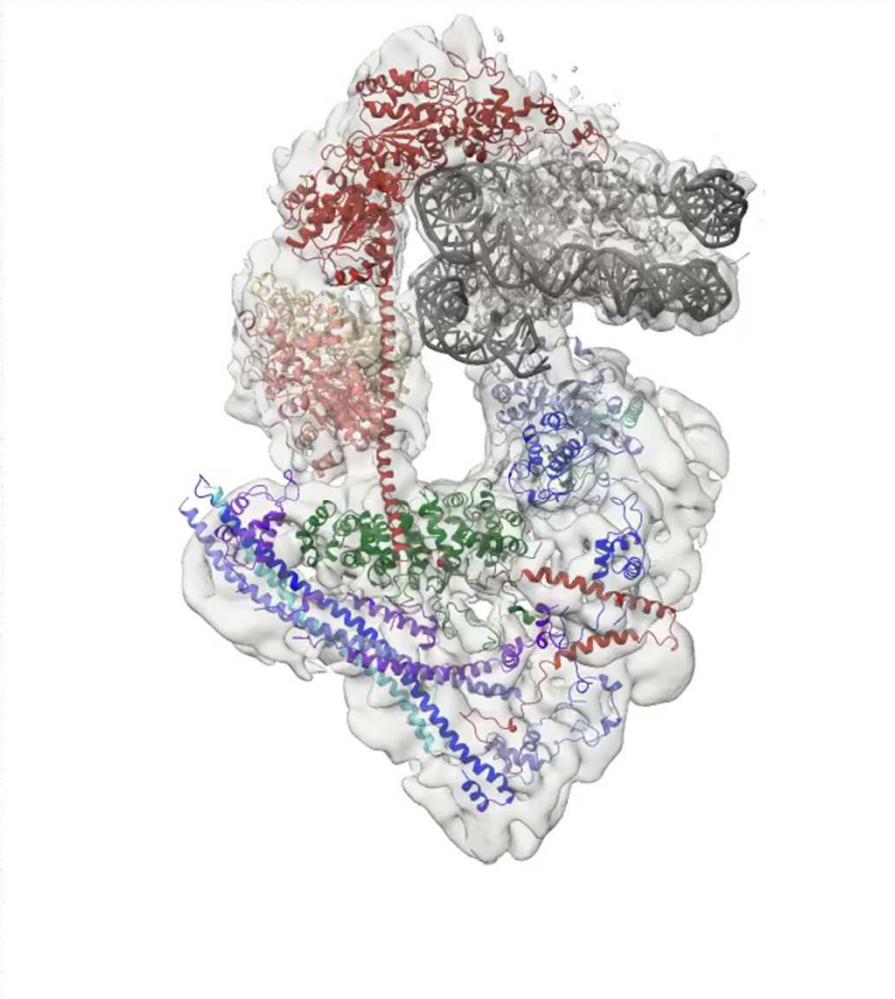
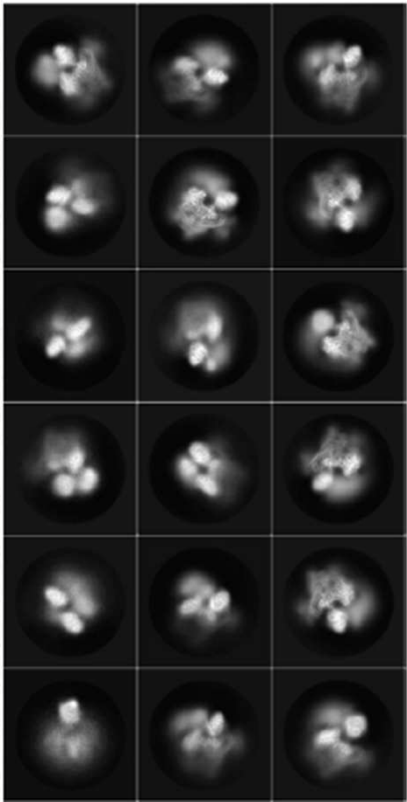
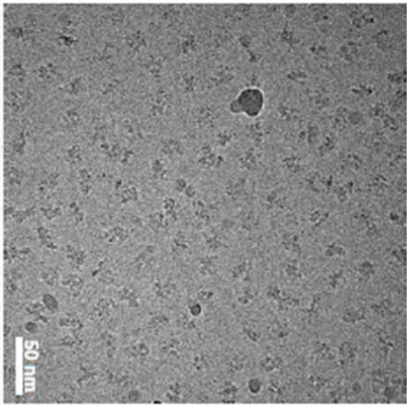
převzato z: Adamus et al, JMB, 2020



remodelační komplex

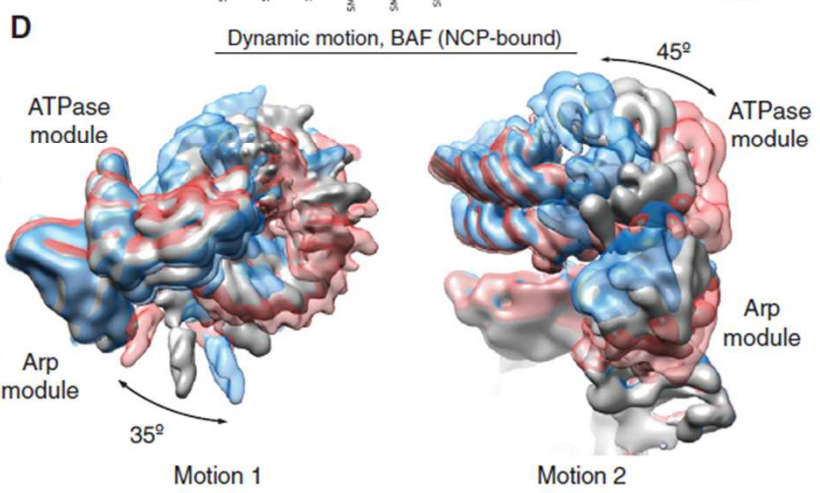
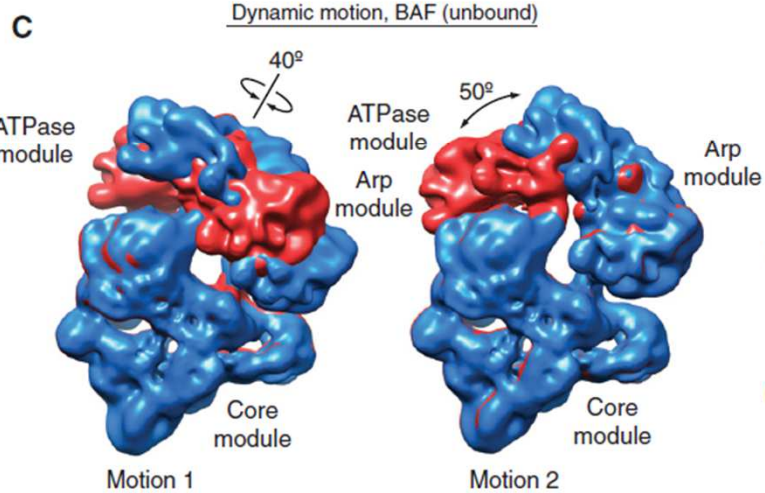
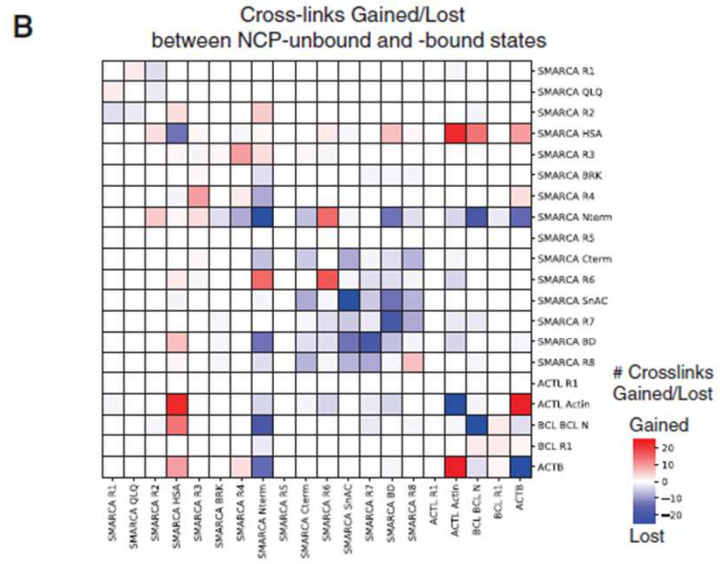
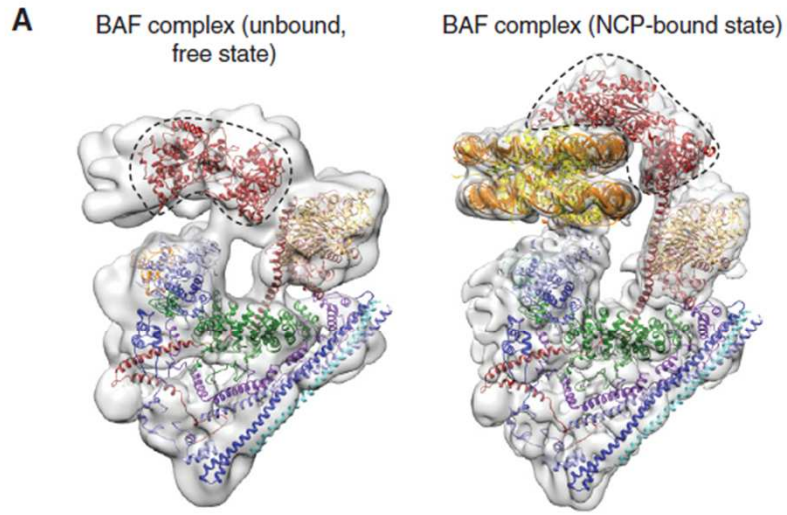
sběr velkého počtu částic, klasifikace a rekonstrukce struktury komplexu

převzato z: Mashtalir et al, Cell, 2020

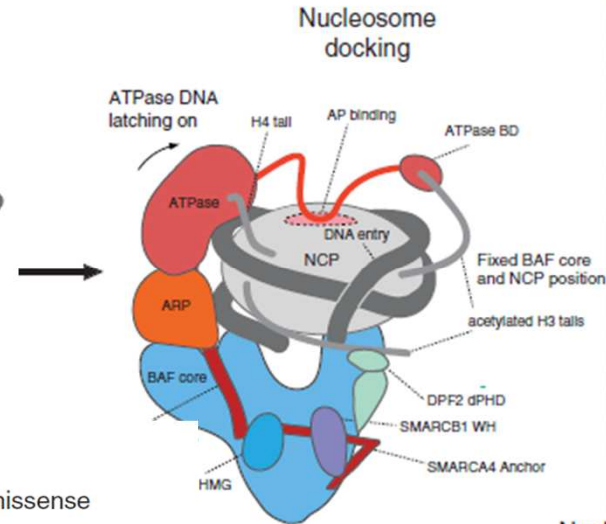
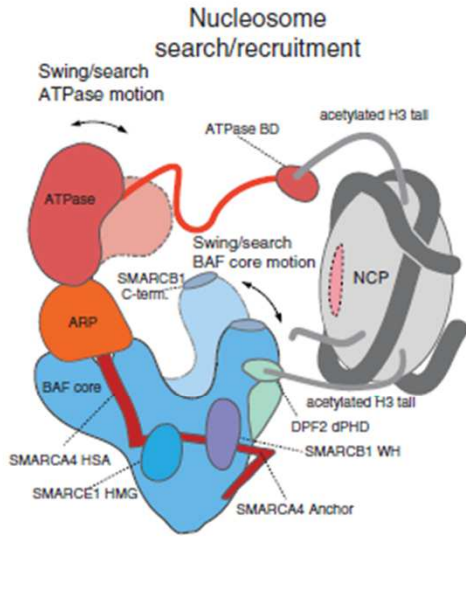
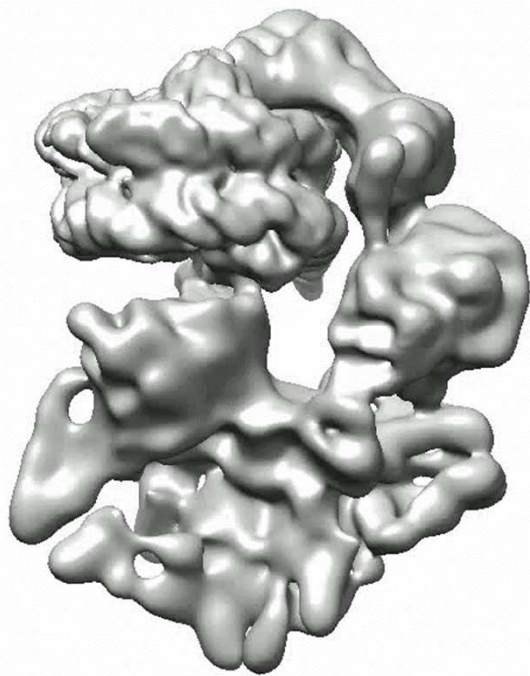


remodelační komplex

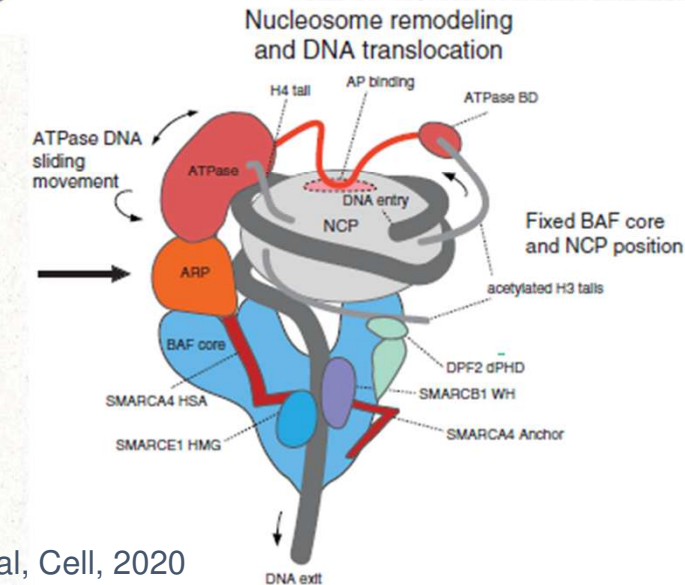
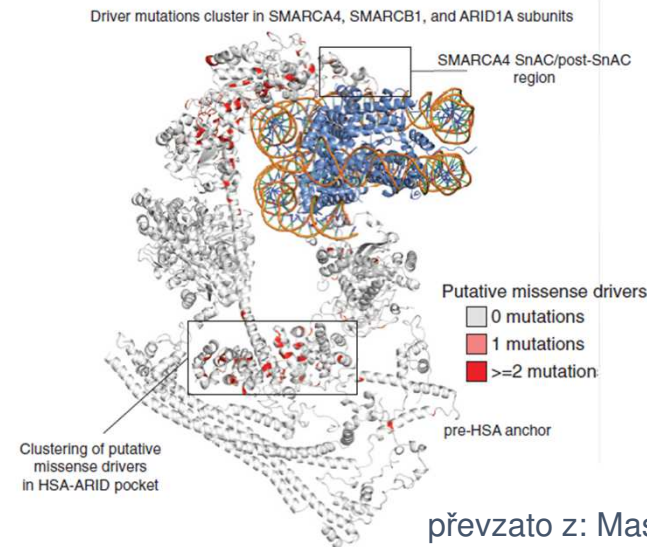
sběr velkého počtu částic, klasifikace a rekonstrukce struktury komplexu (crosslinky, krystaly – integrativní modelování)



remodelační komplex
 zachyceno několik konformací + rozdíly v krosslinku = dynamika



Distribution of Pan-cancer putative missense driver mutations



převzato z: Mashtalir et al, Cell, 2020

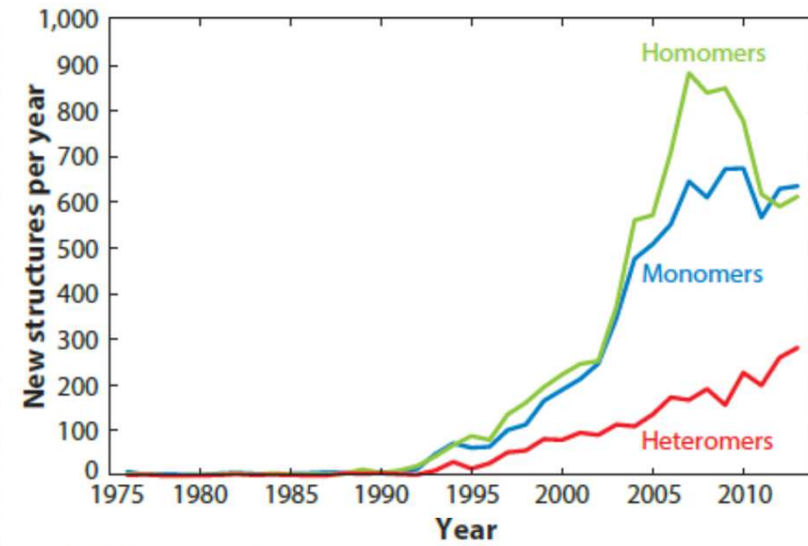
remodelační komplex

pomáhá v transaktivaci genové exprese – váže acetylované H3 (bromodomény) - uvolňuje nukleosom (volnější konce DNA)

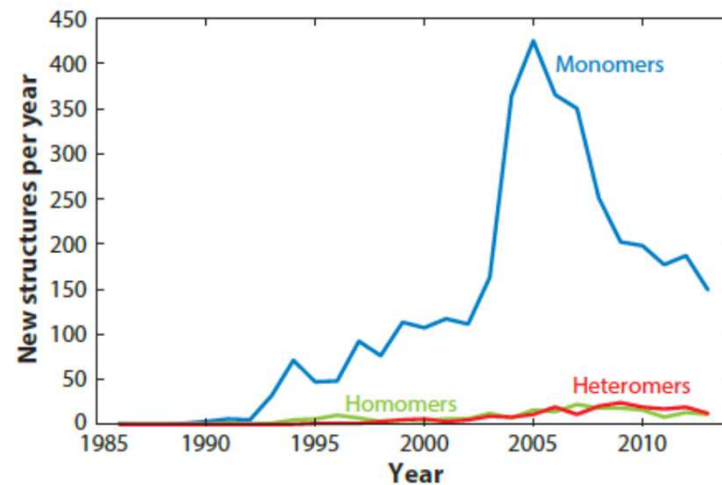
Strukturní metody

- krystalografie – nejvhodnější (boom v 1. dekádě díky sekvenačním projektům)
- NMR je limitována velikostí
- cryoEM je vhodná pro velké komplexy (boom v současnosti)

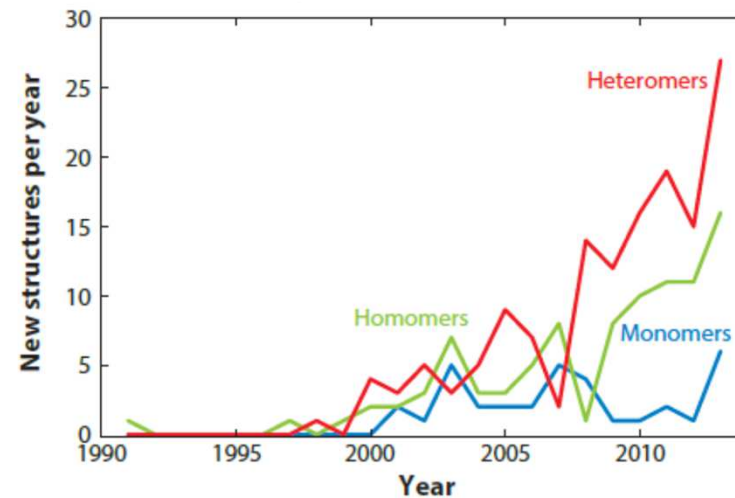
a X-ray crystallography



b NMR



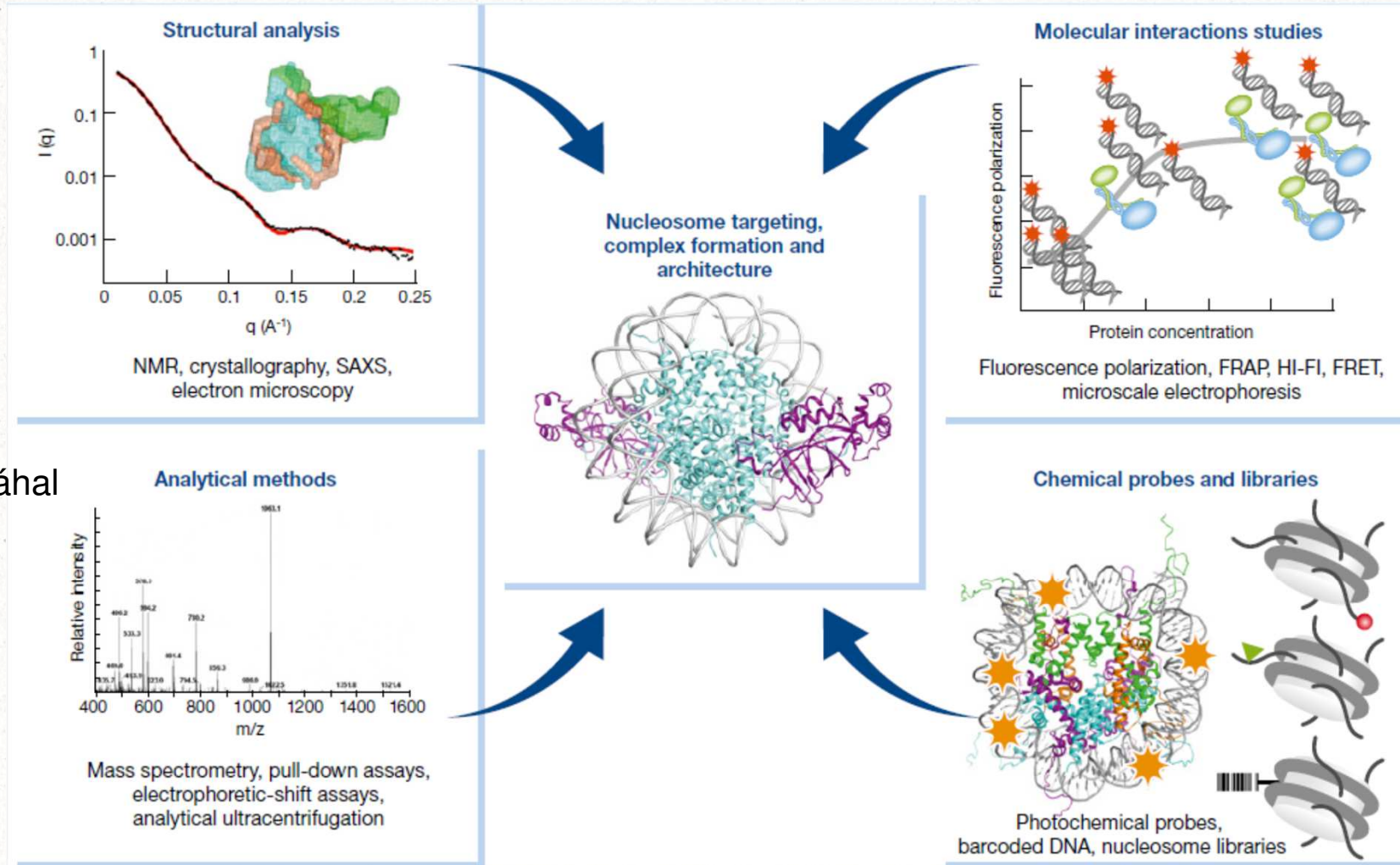
c Electron microscopy



převzato z: Marsh et al, ARB, 2015

Analýza proteinových komplexů

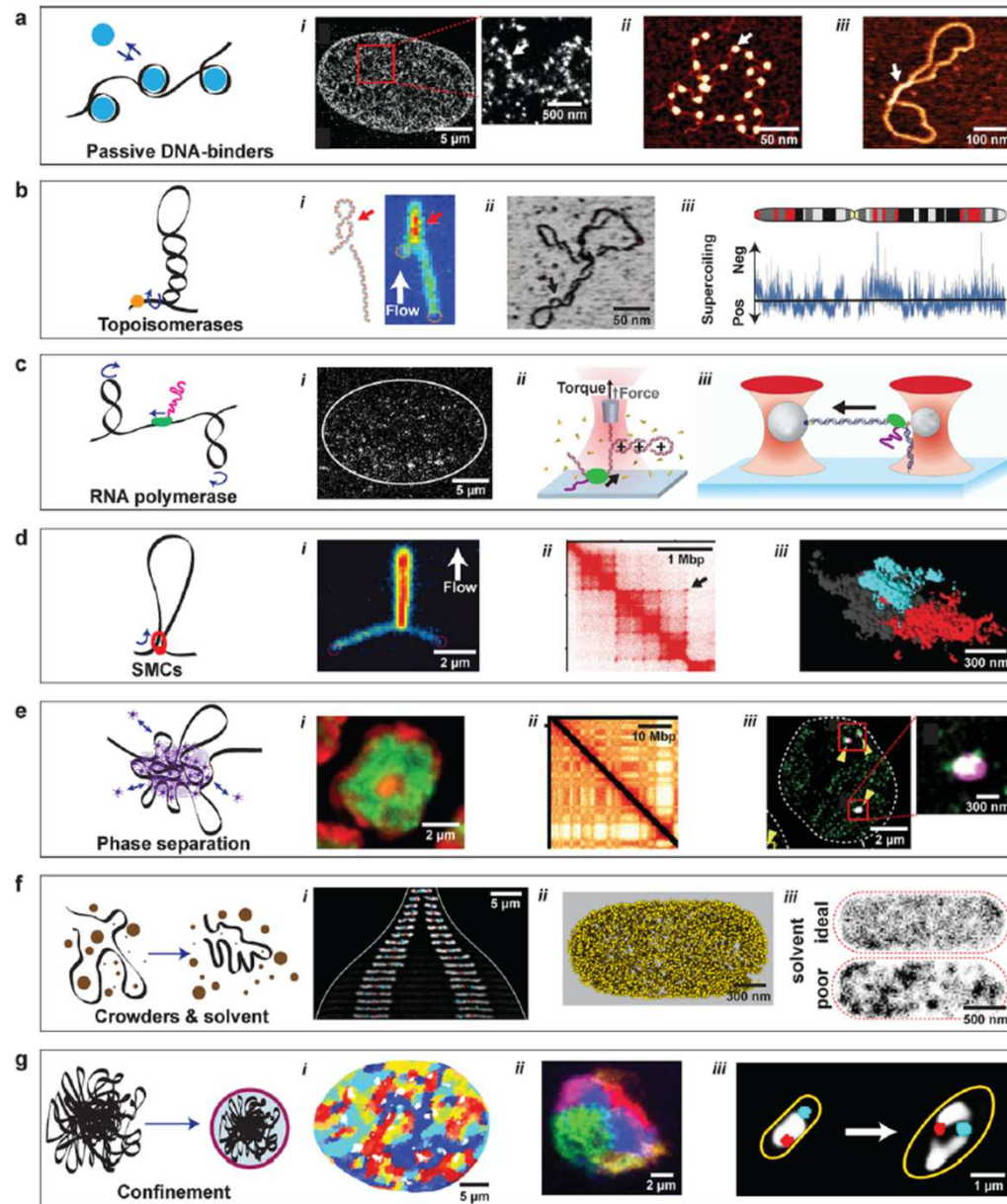
viz doc. Hofr



viz prof. Zdráhal

převzato z: Speranzini et al, EMBO J, 2016

... speciální biofyzikální metody ...

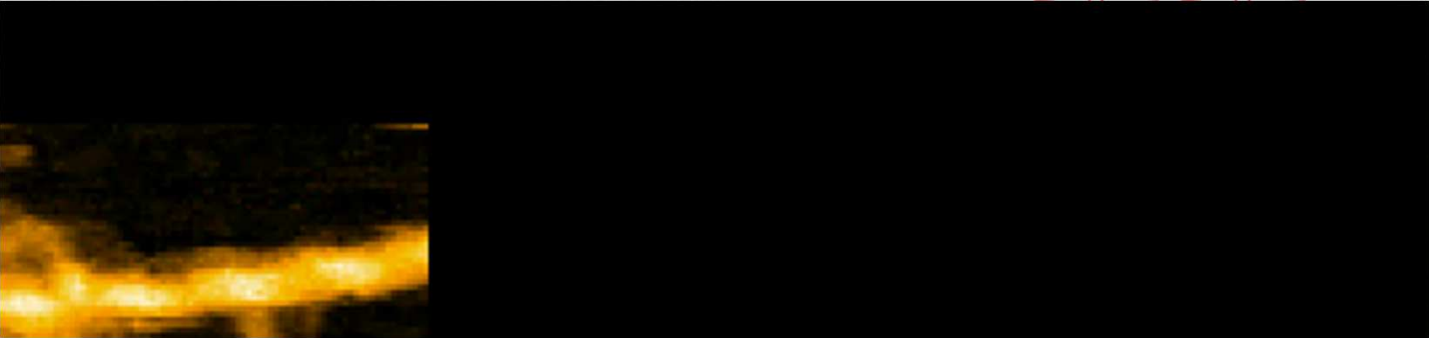
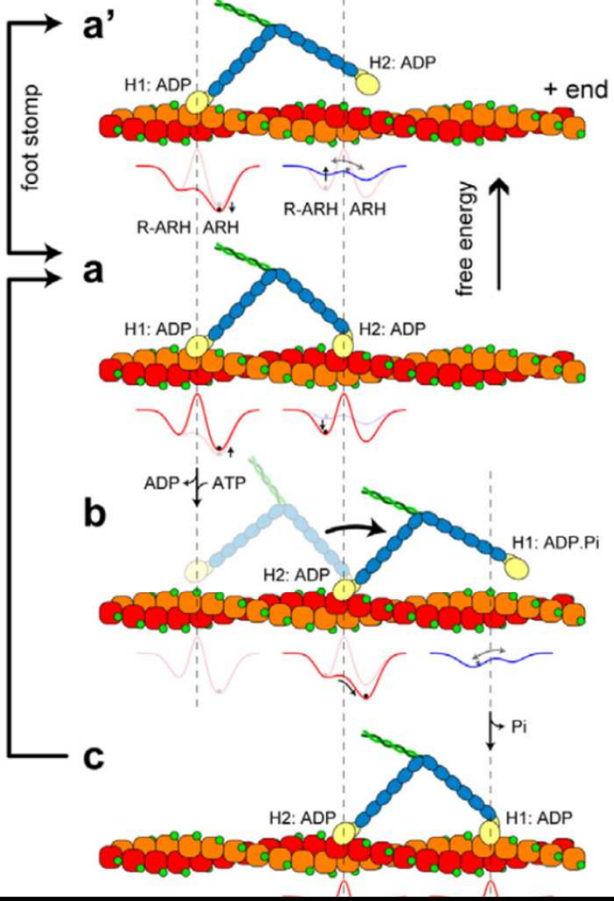
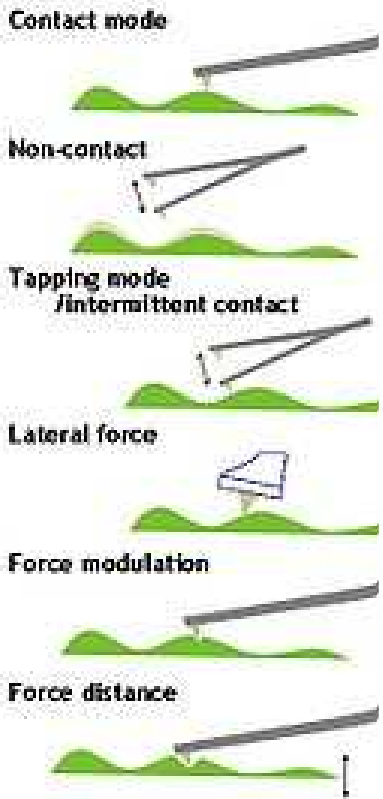
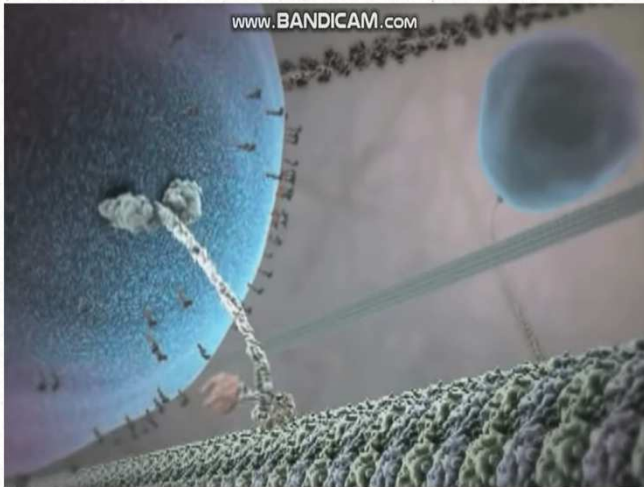


AFM

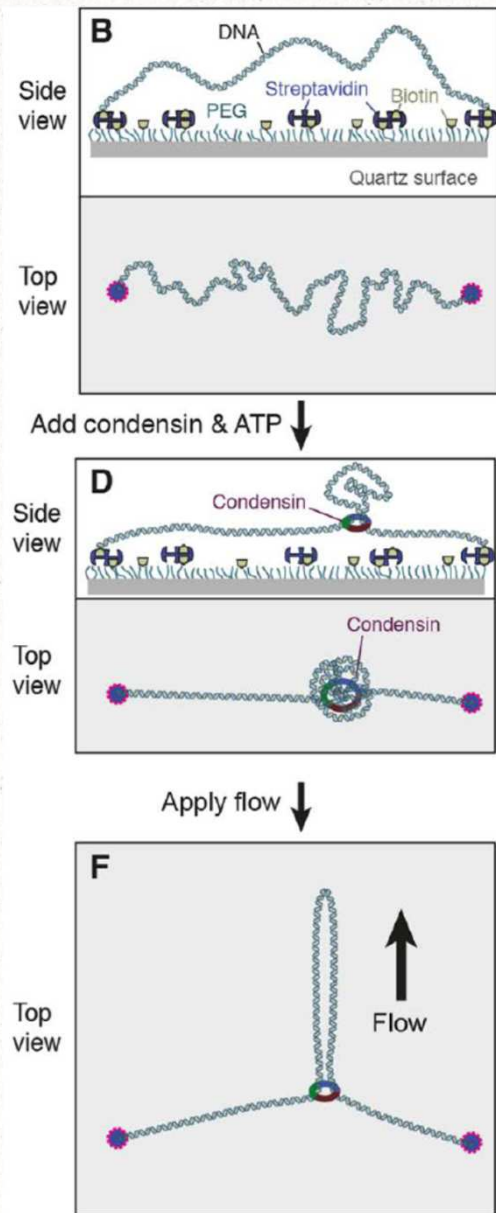
Optické pinzety

převzato z: Birnie et al, ACS nano, 2020

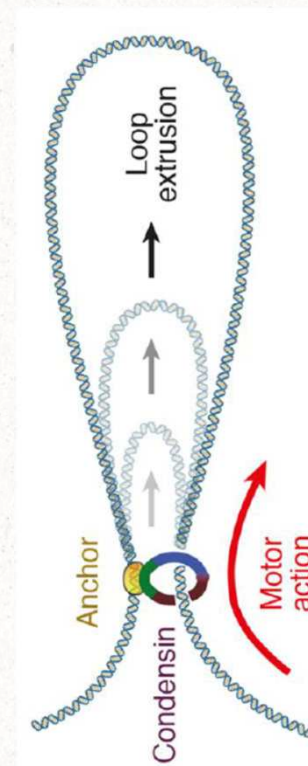
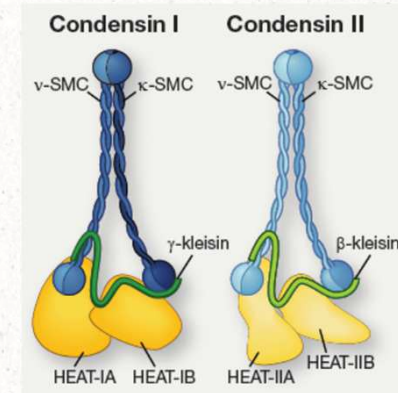
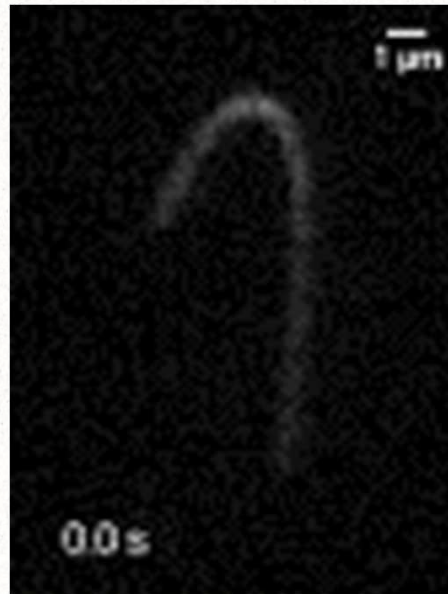
AFM (atomic force microscopy)



převzato z:
Ando et al, Annu Rev BioPhys, 2013



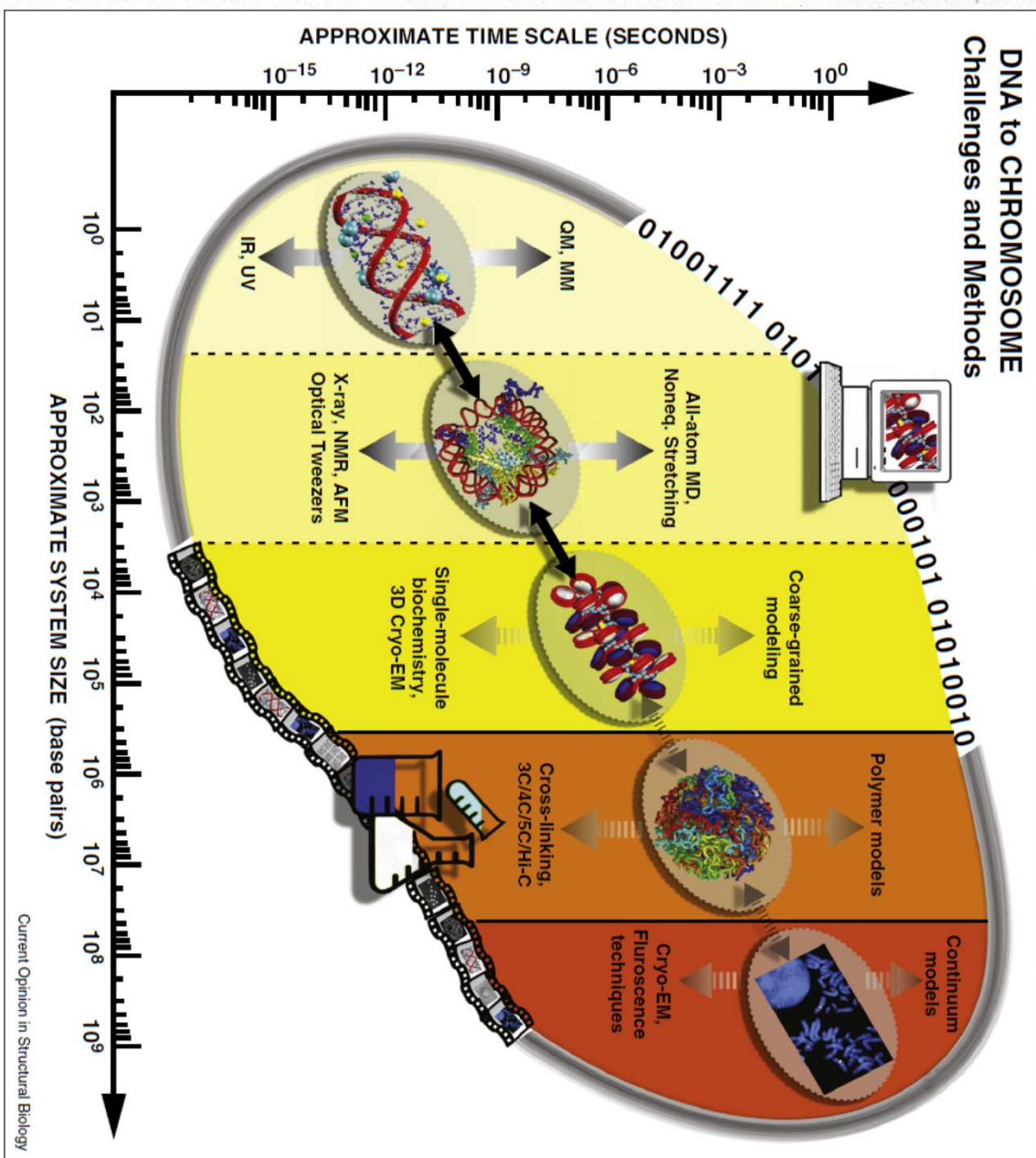
konce molekuly DNA uchyceny
 přidán SMC komplex + vazba a
 hydrolýza ATP – vytvoření
 smyčky („loop extrusion“)



převzato z: Ganji et al, Science, 2018; Takahashi, CO in CB, 2019

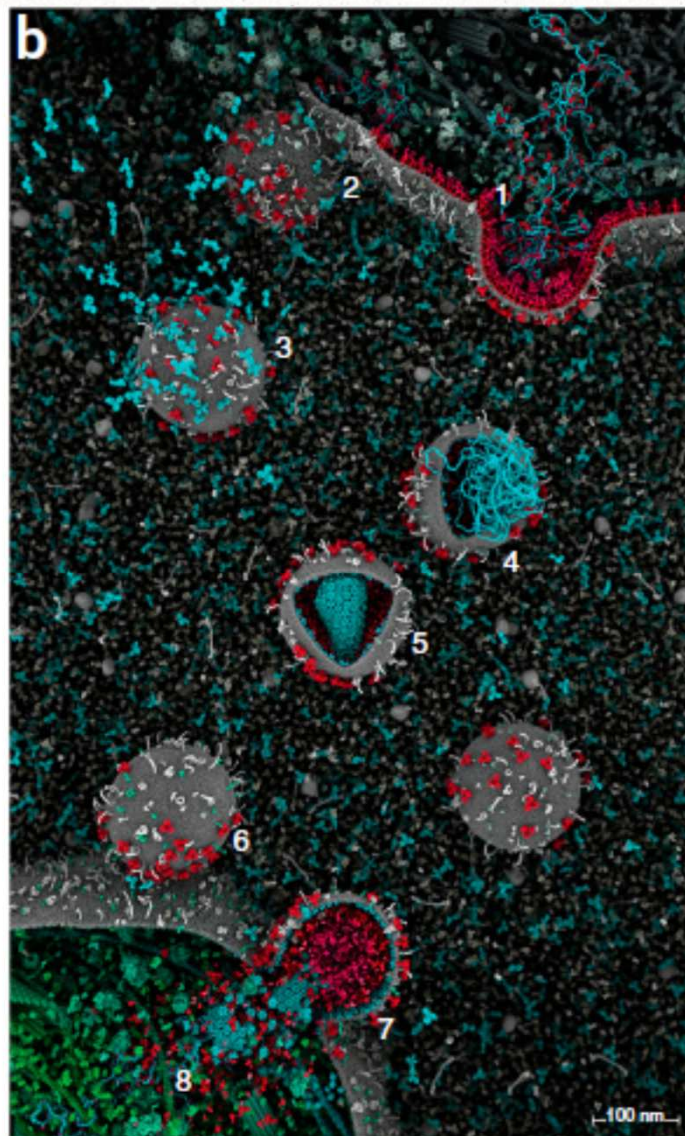
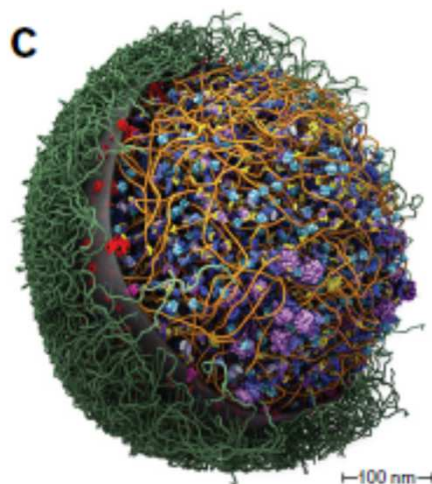
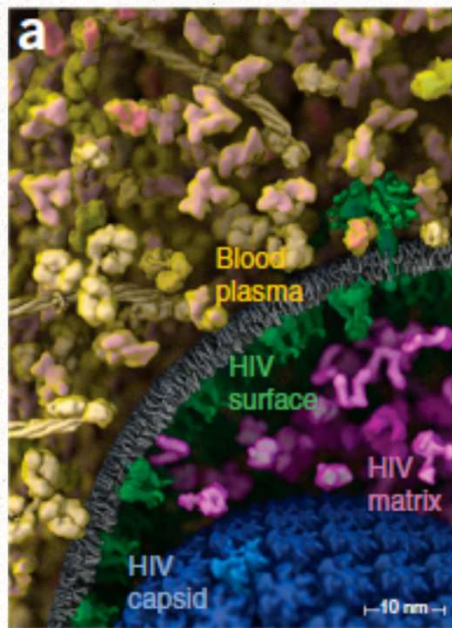
úrovně proteinových komplexů

DNA to CHROMOSOME Challenges and Methods



převzato z: Ozer et al, CO in SB, 2015

Visualizace proteinových komplexů



Existuje mnoho nástrojů na vizualizaci komplexů (i v buněčném prostředí)

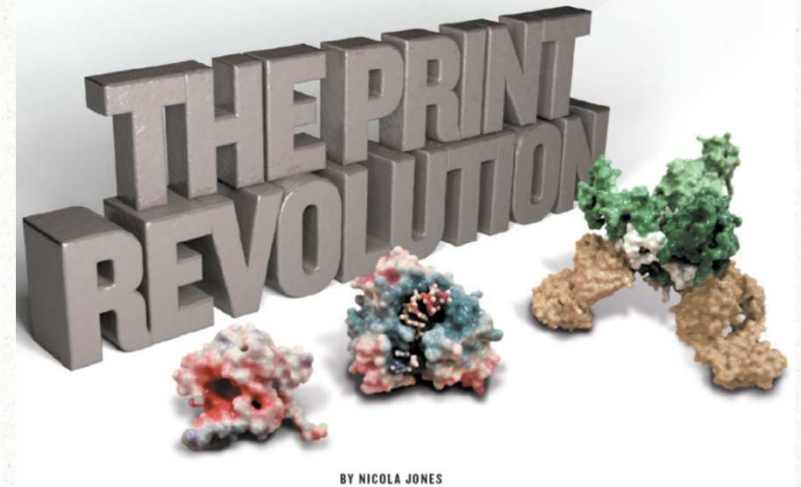
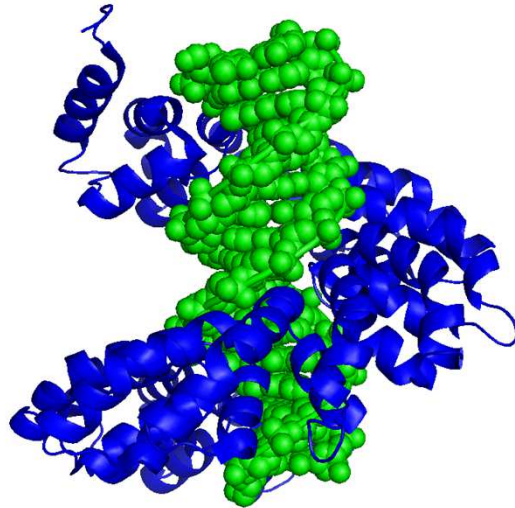
od **PyMOL** pro přímou vizualizaci krystalových struktur

... až po **CellPACK** pro interaktivní náhled do buňky a jejích procesů

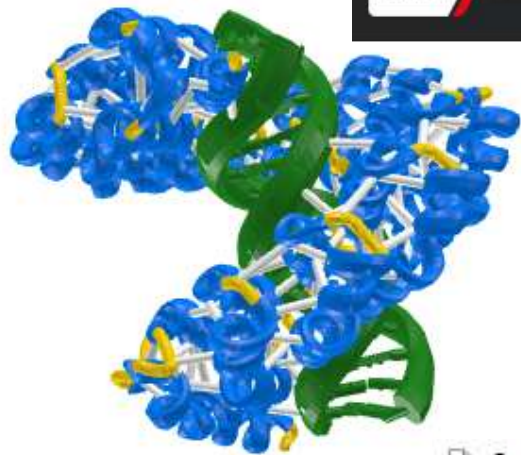
...vychází z herních a animačních algoritmu ...

převzato z: Johnson et al., Nat Meth, 2015

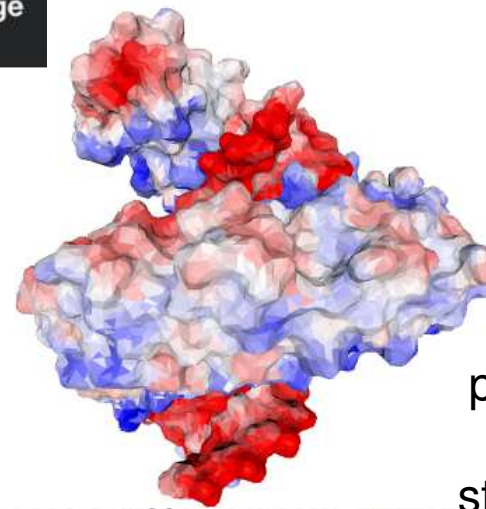
Visualizace proteinových komplexů



NIH NIH 3D Print Exchange



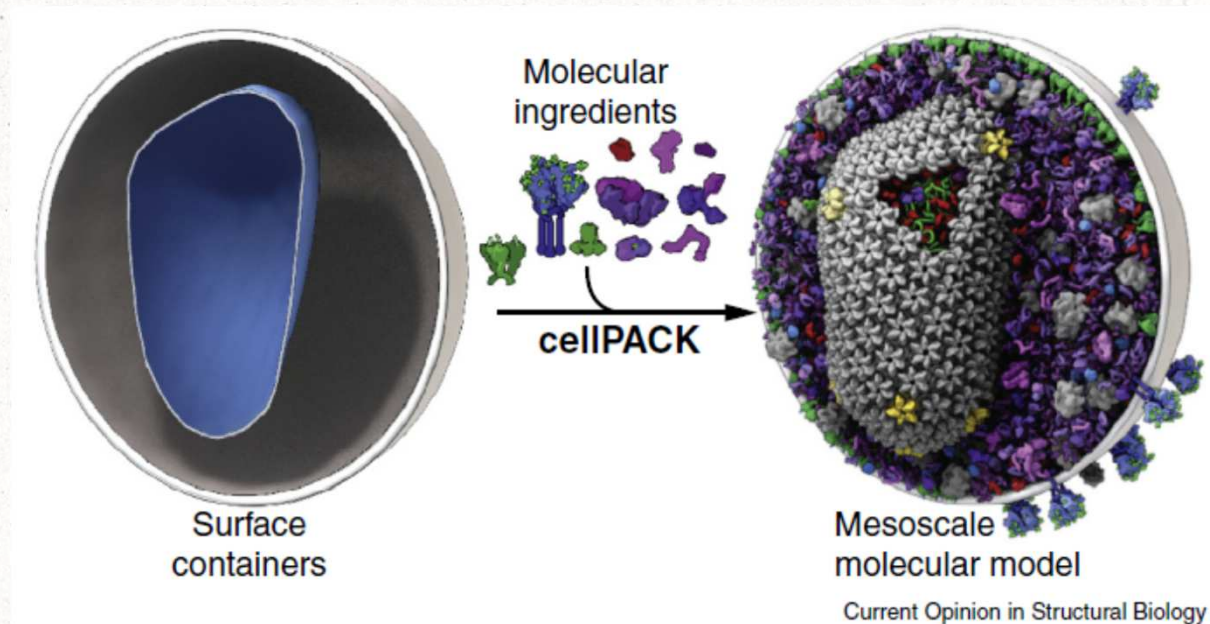
3dprint.nih.gov/



Existuje mnoho nástrojů na vizualizaci komplexů

od **PyMOL** pro přímou vizualizaci krystalových struktur ... **3D tisk**

Visualizace proteinových komplexů



Pro lepší představu (virové částice) se integrují ... (nakopírované) struktury, data z molekulární dynamiky (simulací), koordináty pohybu „objektu“ ve světelném mikroskopu ... animovat i buněčný kontext – namíchat v „reálných“ poměrech do „organel“ a na „membrány“ – **CellPack** ...

Lze použít k testování modelů ...

Malý průlet světem buňky



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The Molecular Machinery of Life

většina ... proteinové komplexy ...

... chromosomy

Jaké proteinové komplexy poznáte?

dostupné: <https://www.youtube.com/watch?v=FJ4N0iSeR8U>