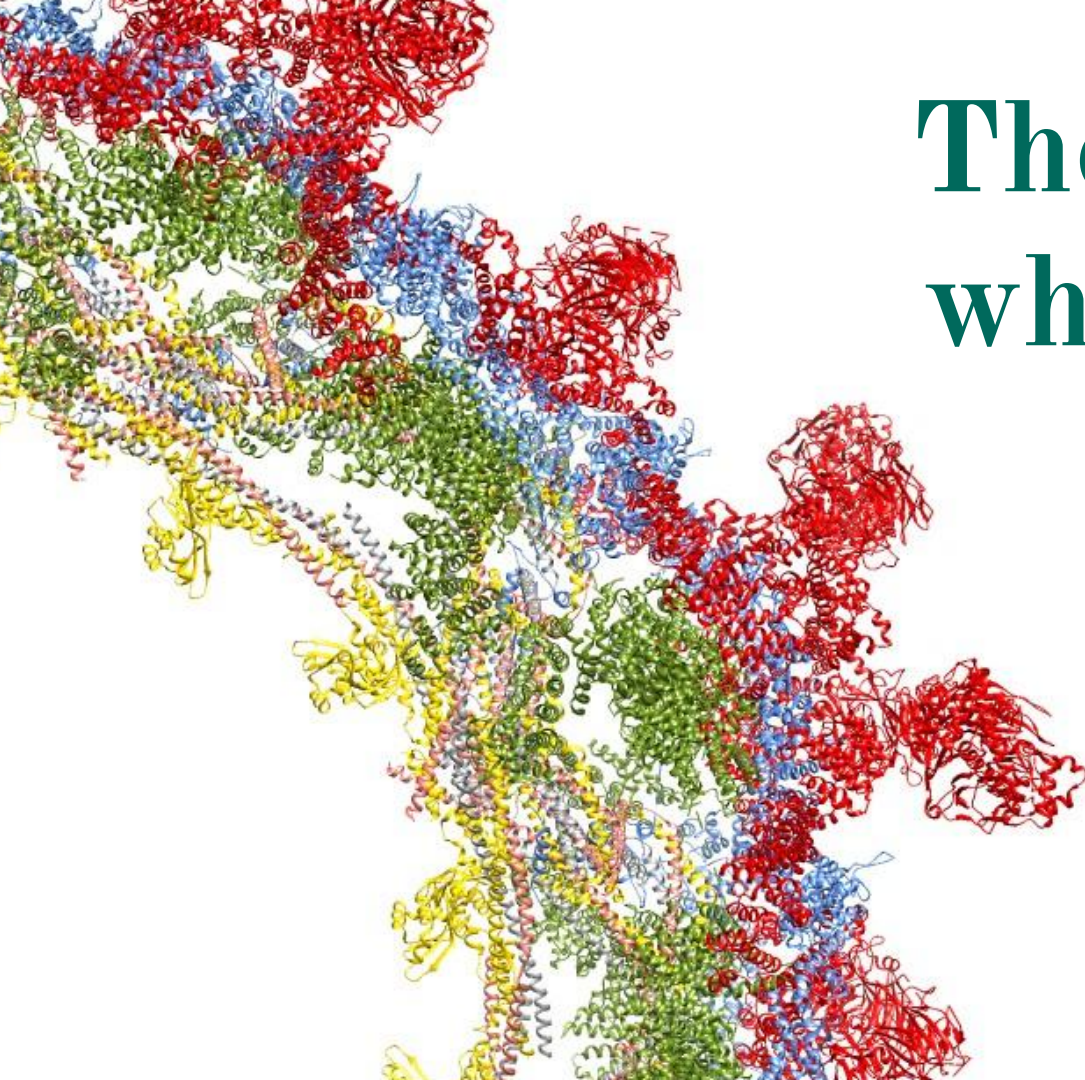
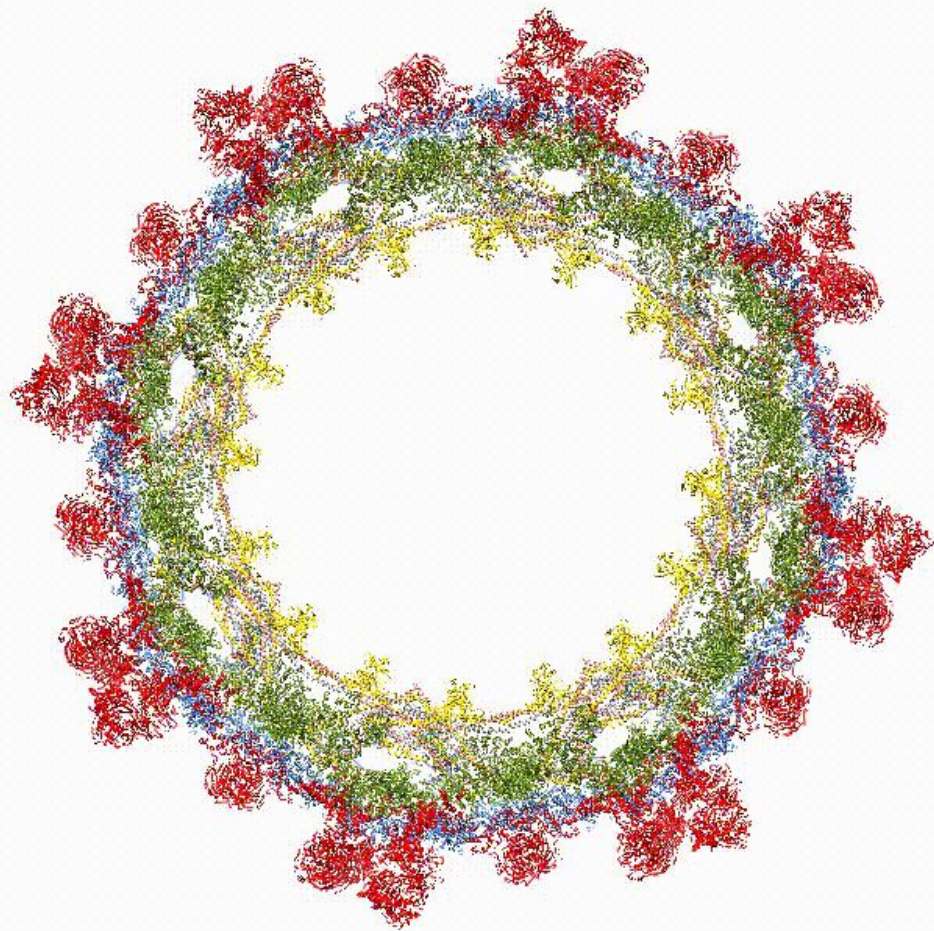




The nucleopore: what a journey!

Bea Calvo
Paula Casajust
Carolina Frías
Laia Gutierrez
Ana Portillo



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2. Scaffold Nups

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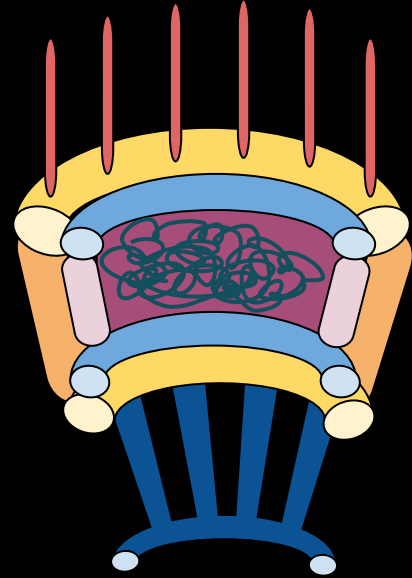
3. Barrier Nups

3.1 Nup54-Nup58-Nup62 complex

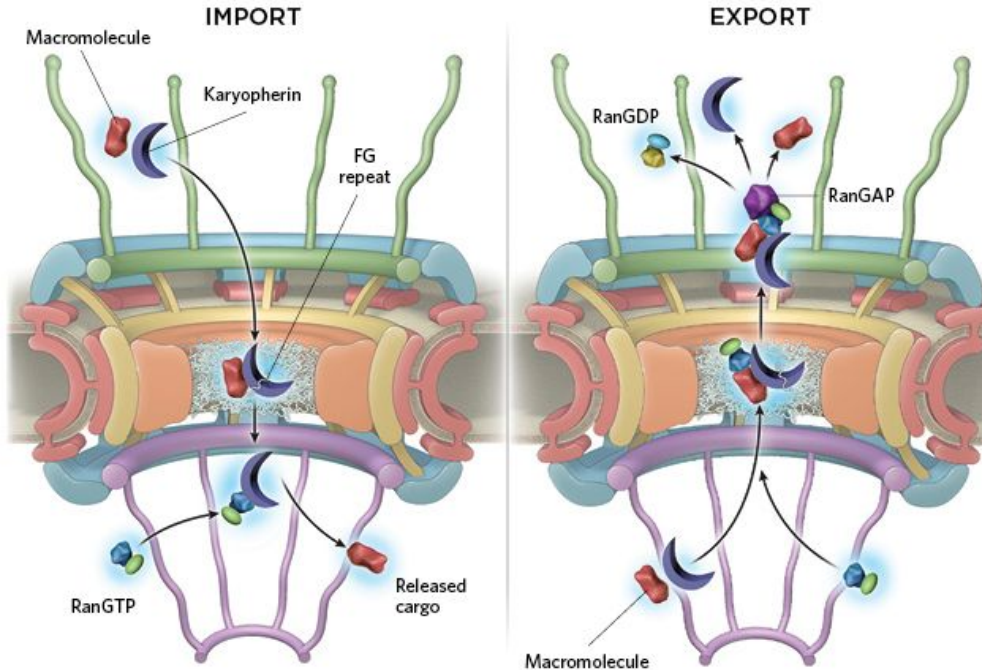
3.2 Karyopherins and Barrier Nups

4. Conclusions

1. Introduction to the Nucleopore Complex



1.1 The Nucleopore complex



IMPORT: histones, DNA, RNA polymerases...

EXPORT: tRNA, mRNA...

OUTER MEMBRANE

INNER MEMBRANE

NUCLEAR
ENVELOPE

1.2 Some characteristics

- Fundamental component of all eukaryotic cells
- Sole gateway for import and export to and from the nucleus
- Small molecules & ions vs large molecules
- 110 MDa structure
- Central aqueous translocation channel of ~60 nm in diameter
- Octagonal symmetry

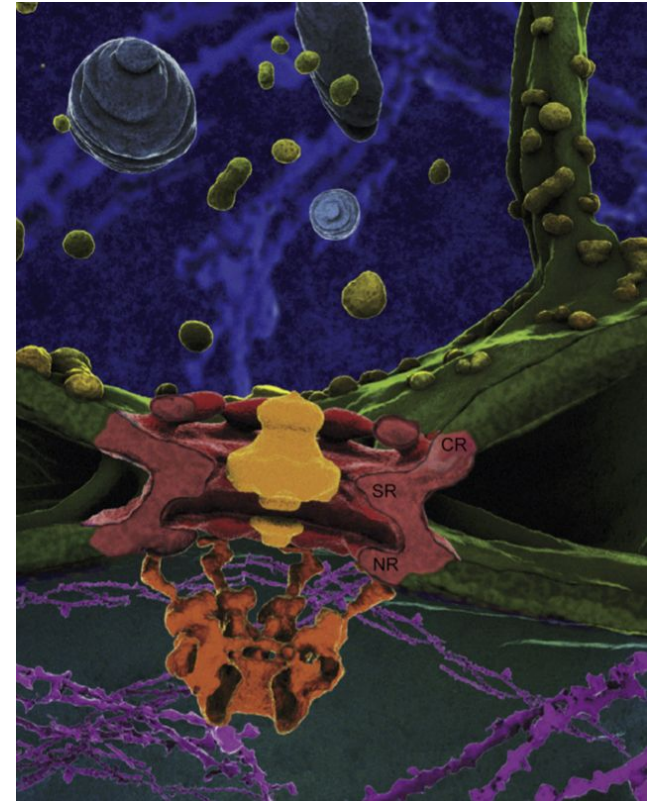
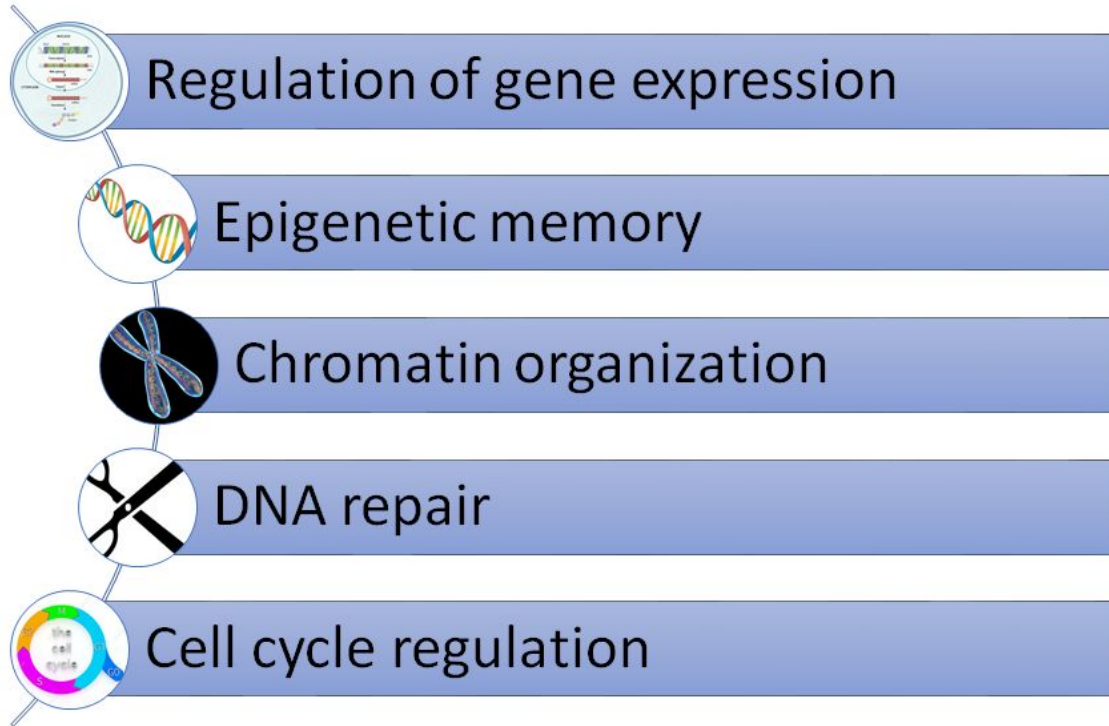
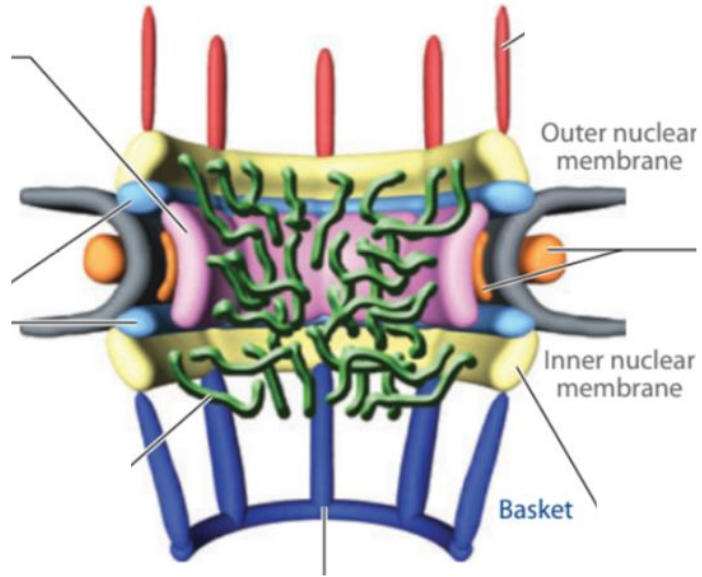


Image: Elad N, et al. (2009)

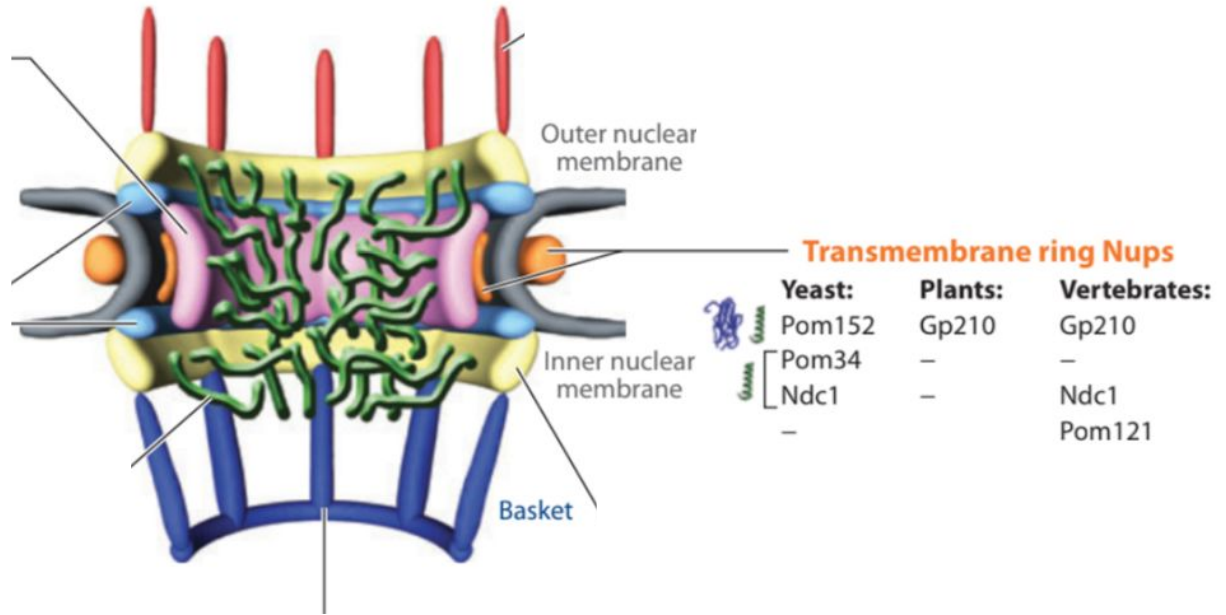
1.3 Other functions



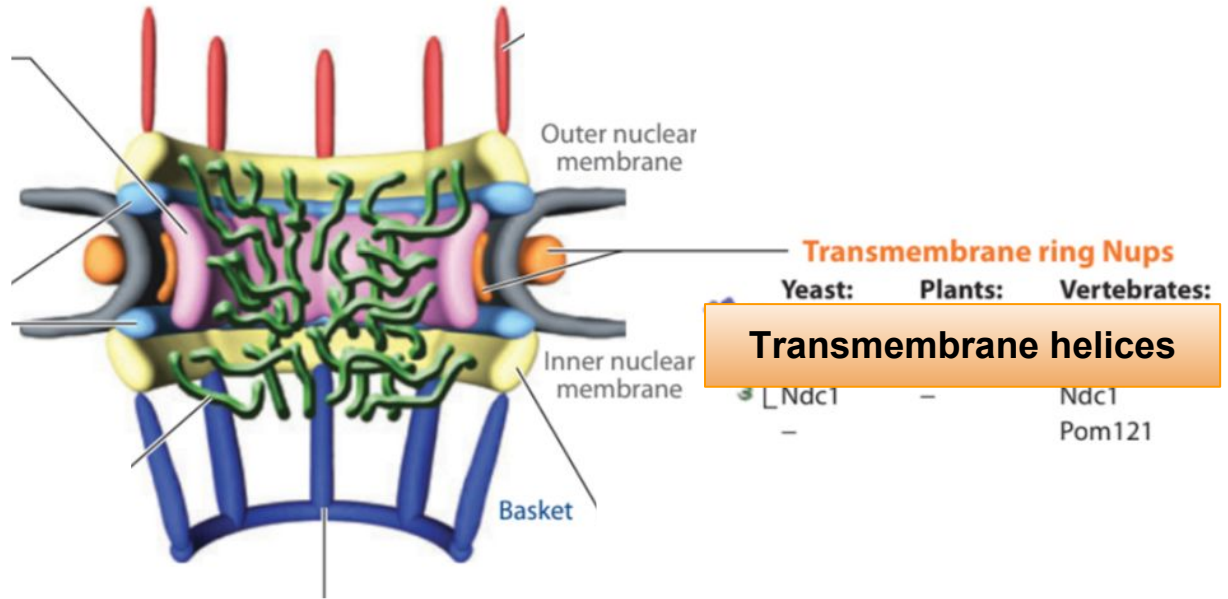
1.4 Nucleopore structure



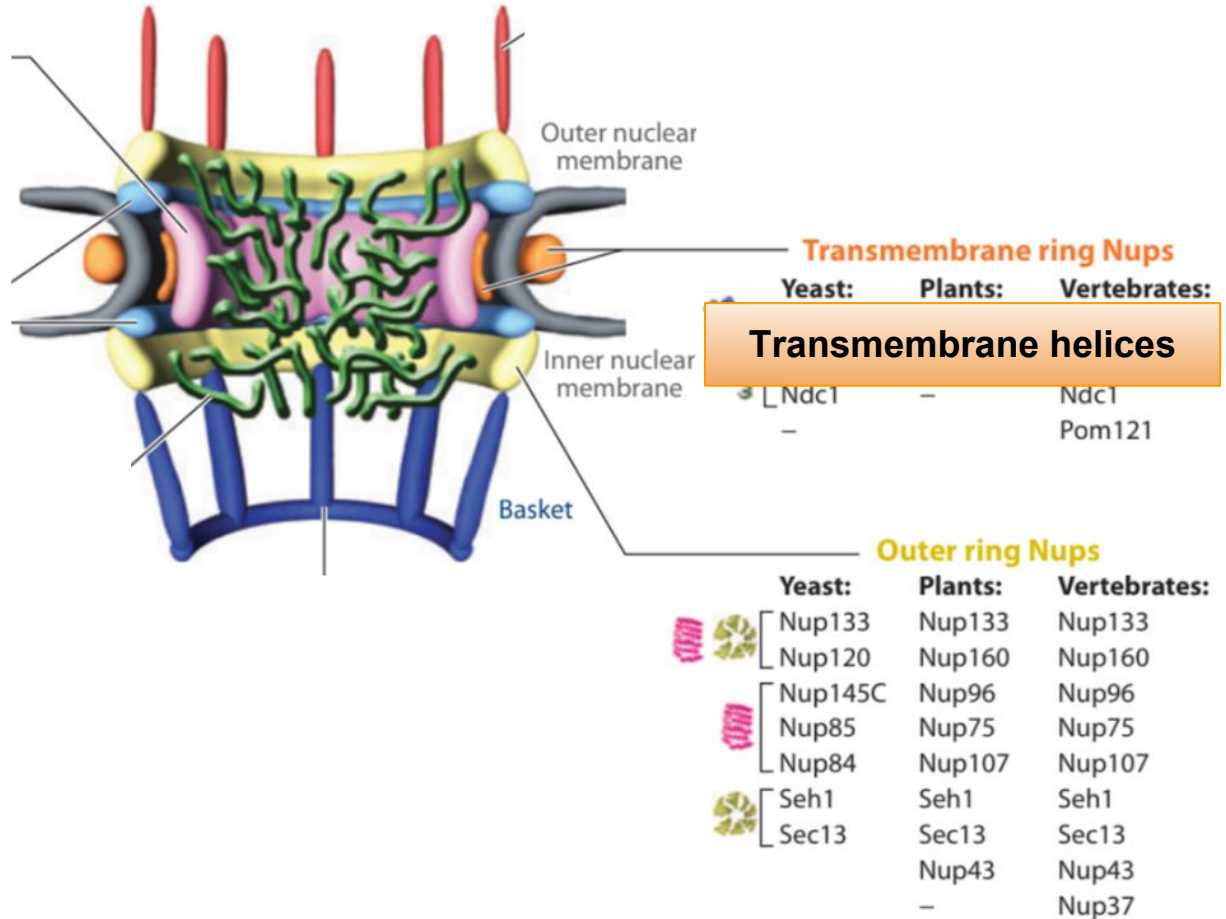
1.4 Nucleopore structure



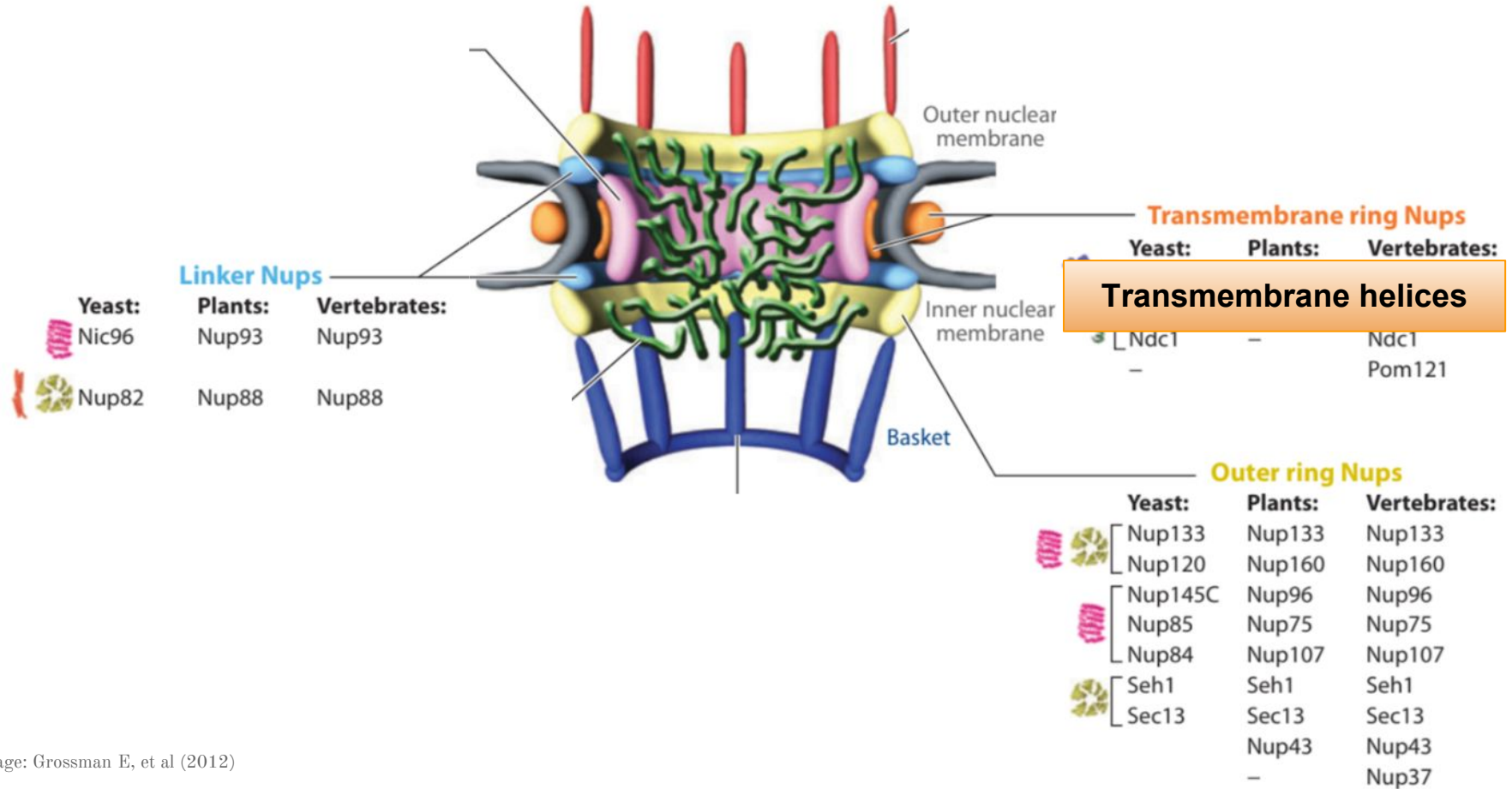
1.4 Nucleopore structure



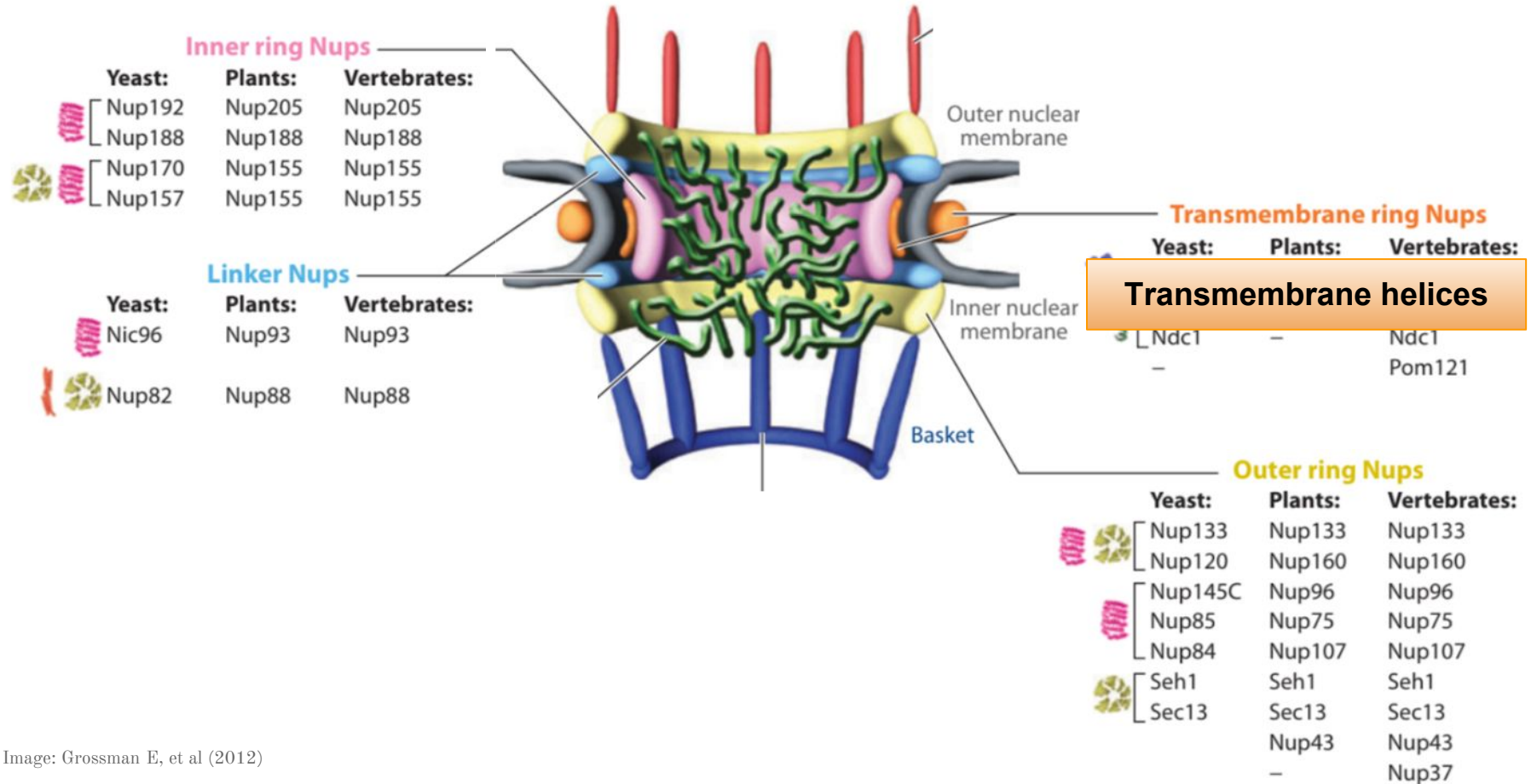
1.4 Nucleopore structure



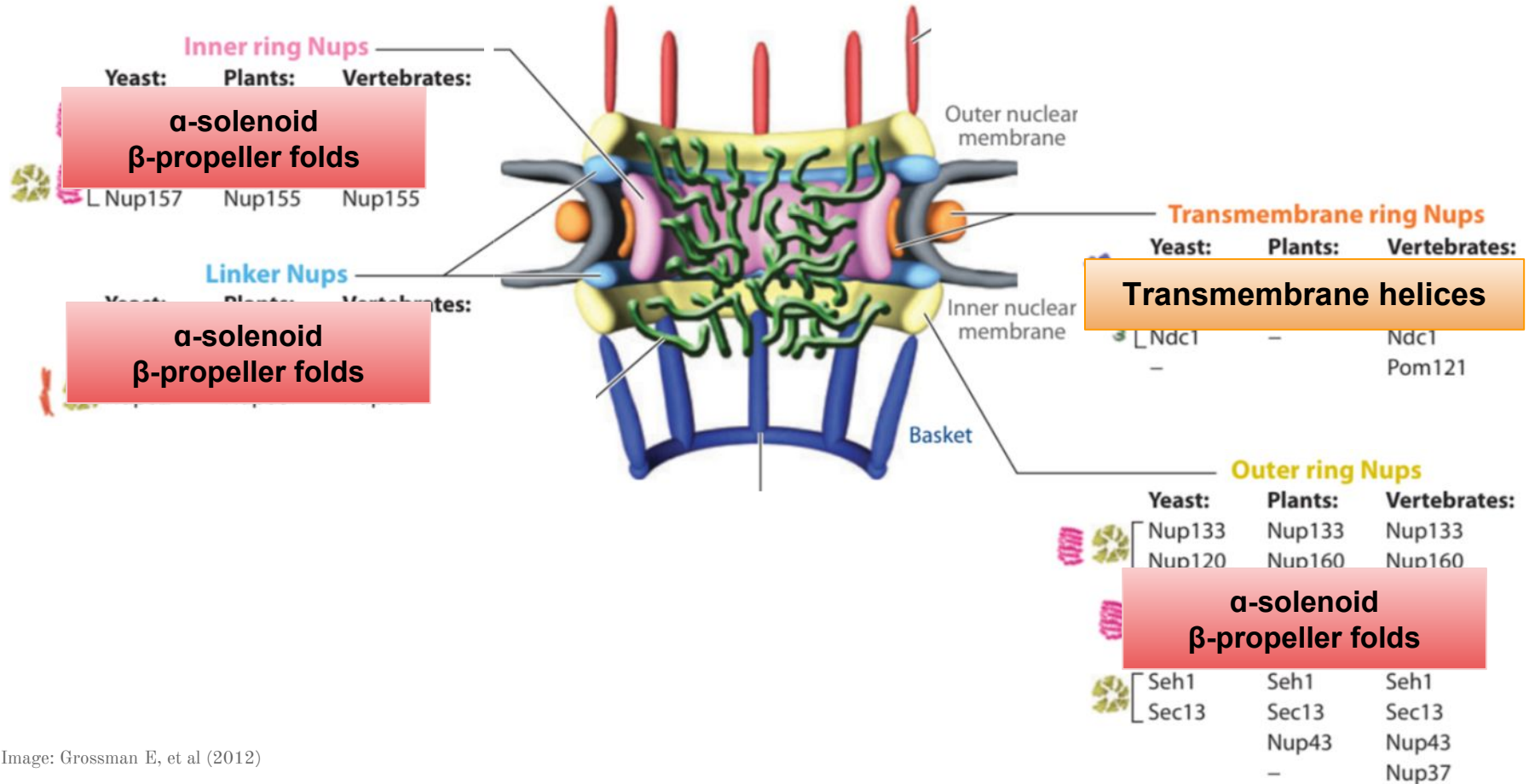
1.4 Nucleopore structure



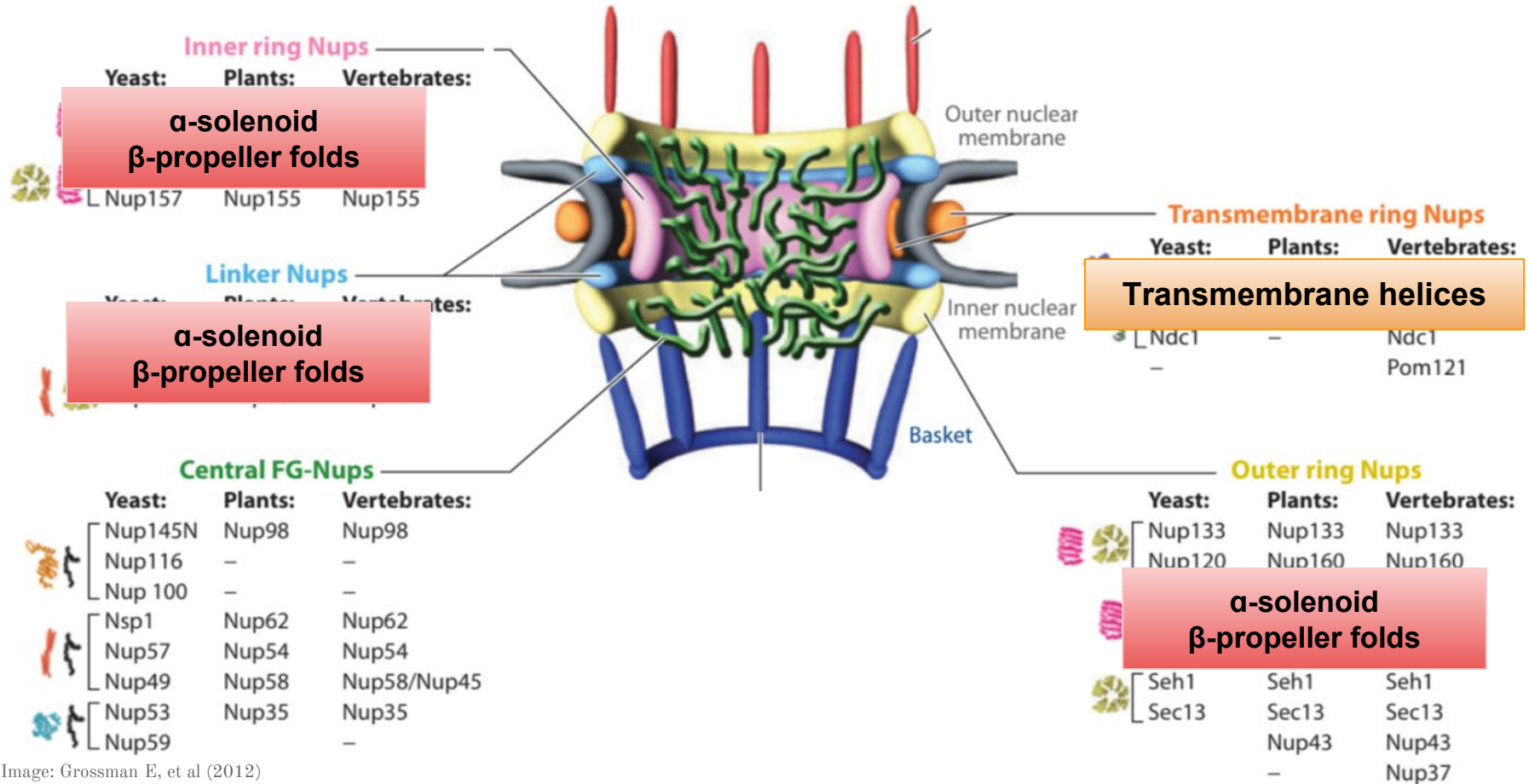
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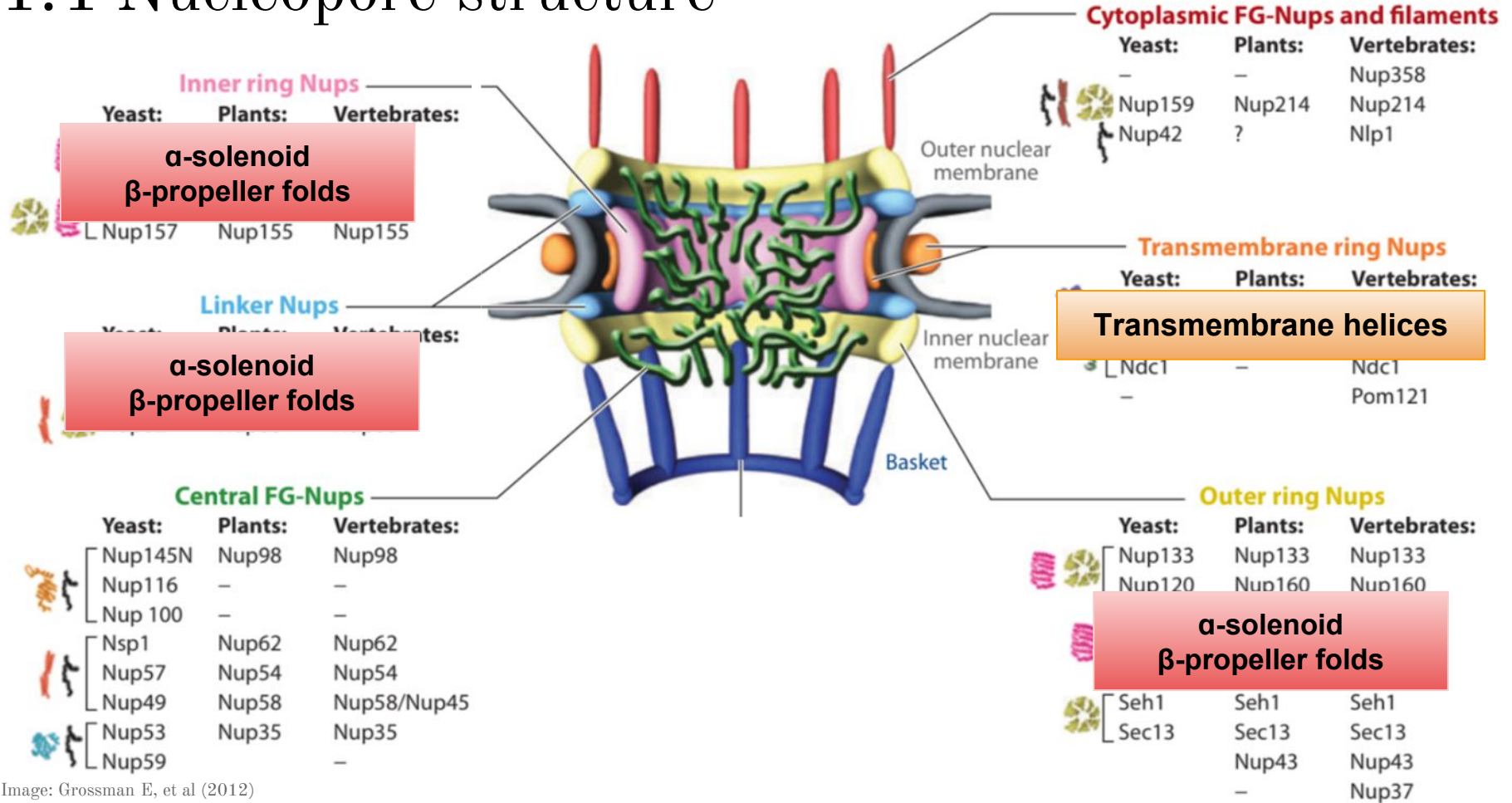
1.4 Nucleopore structure



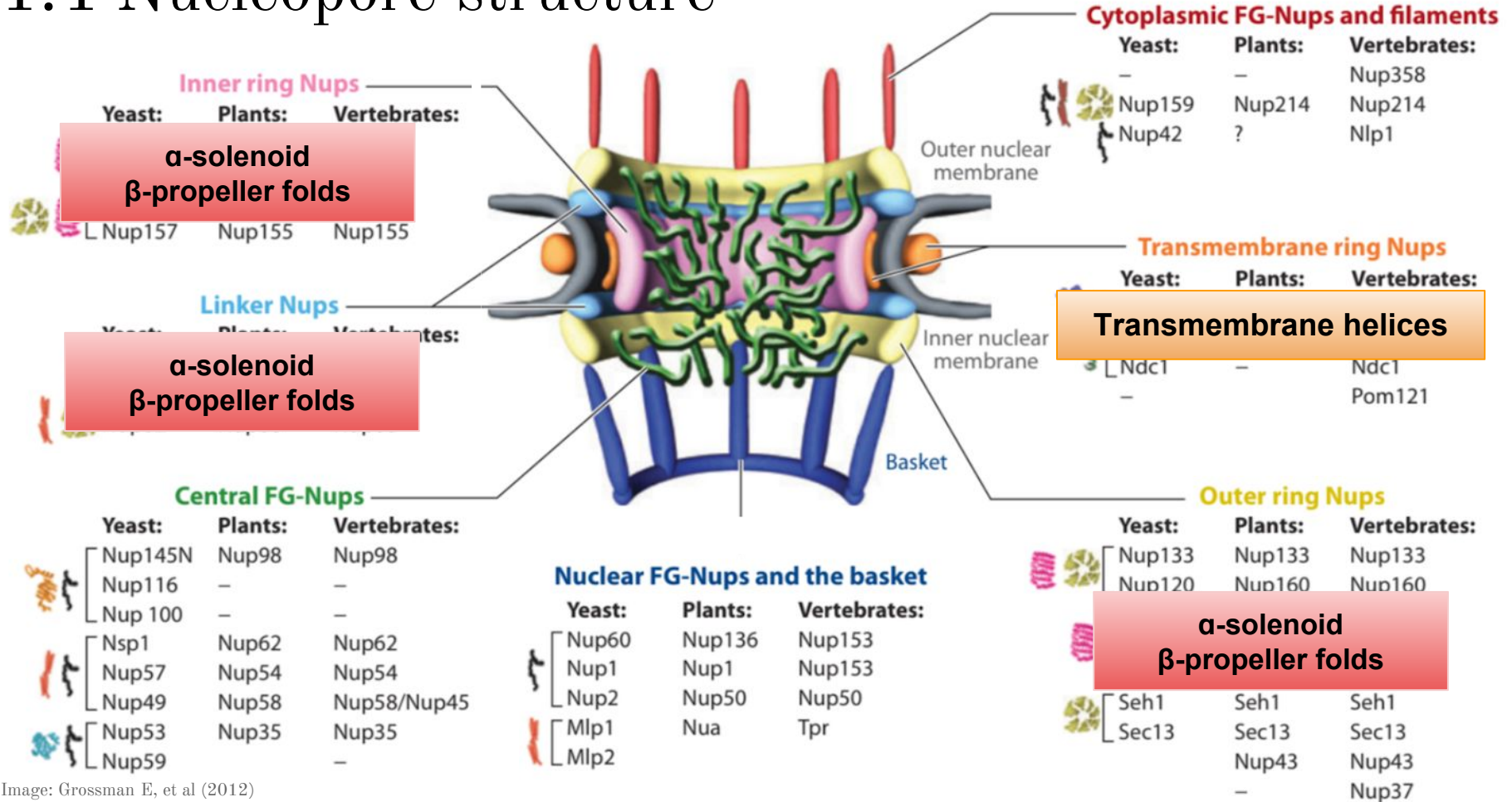
1.4 Nucleopore structure



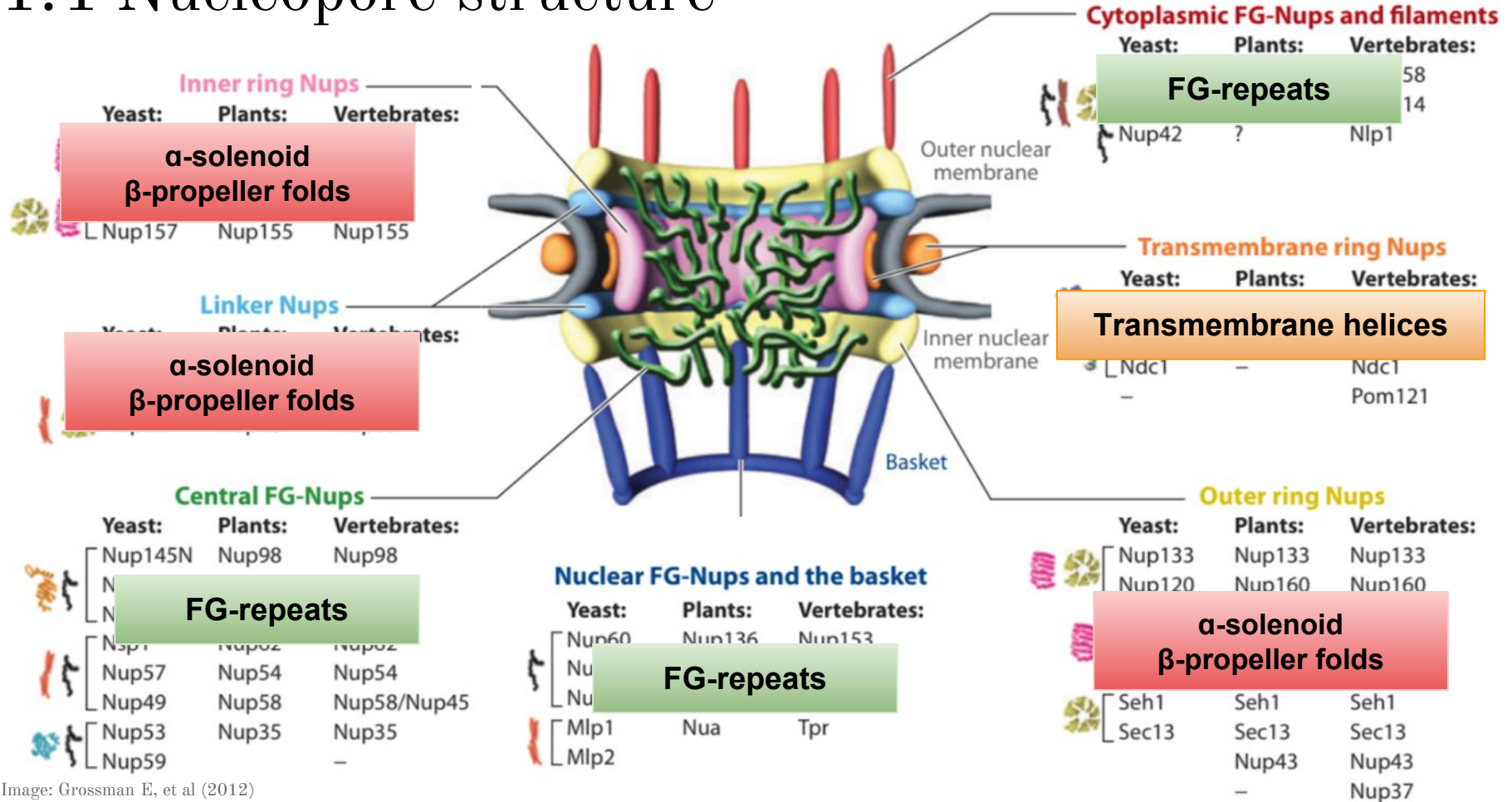
1.4 Nucleopore structure



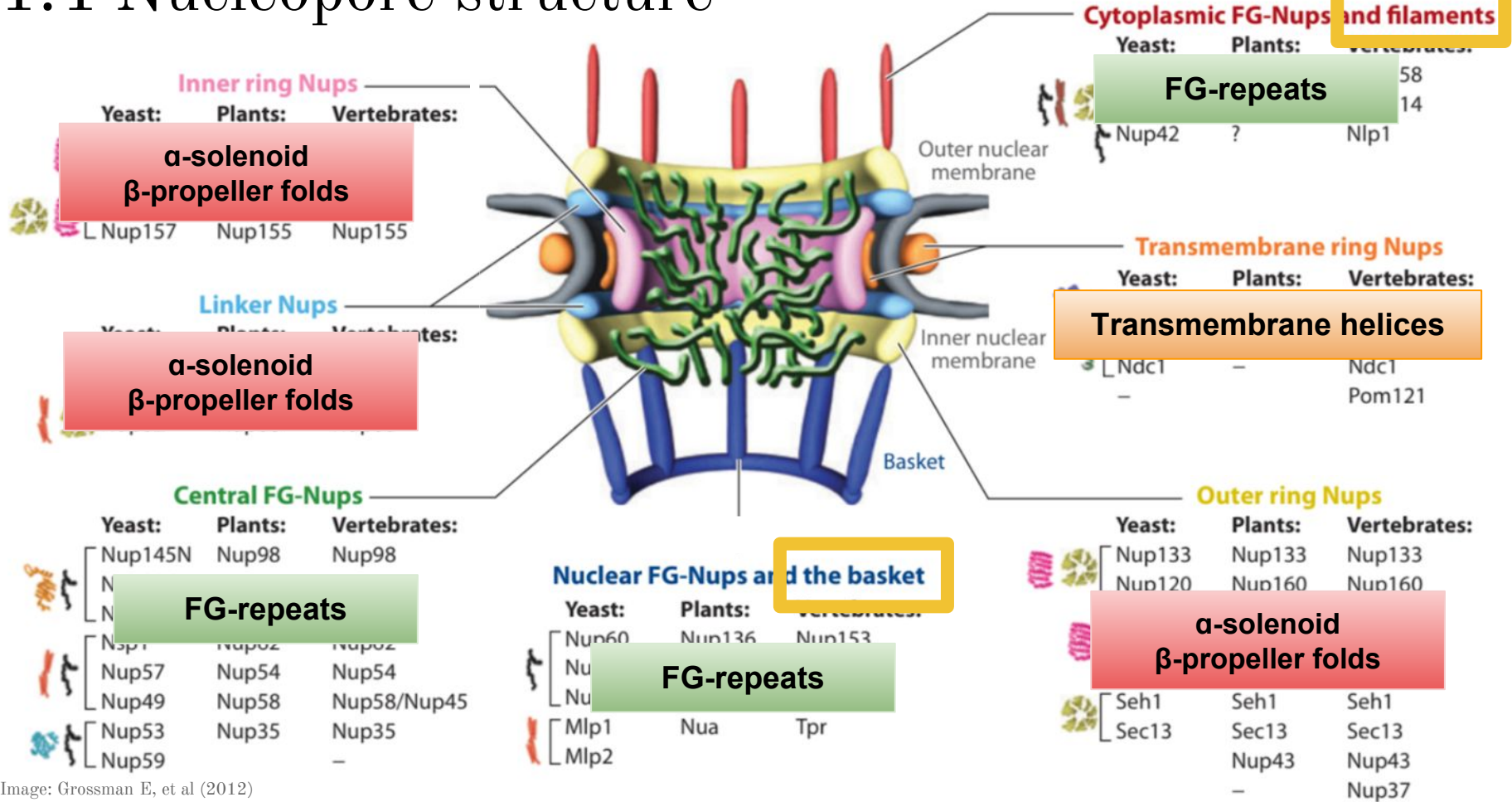
1.4 Nucleopore structure



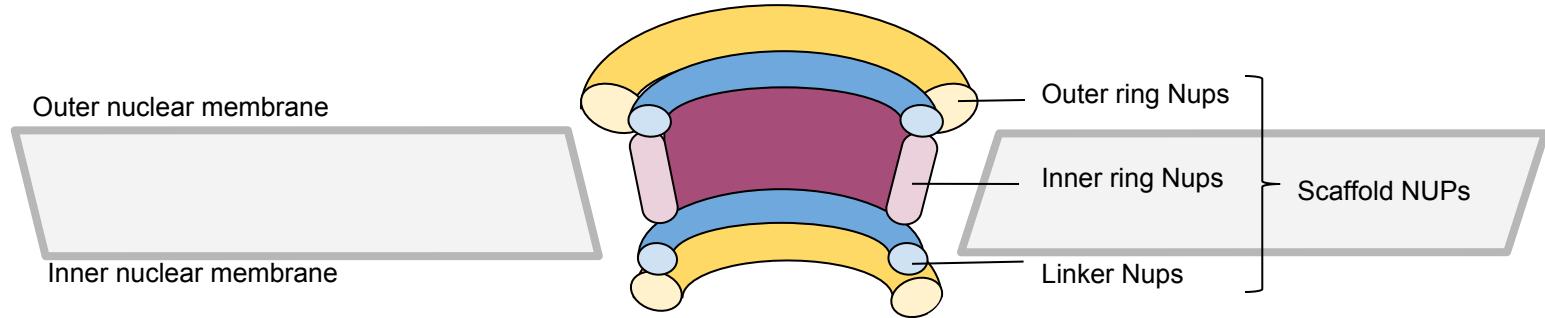
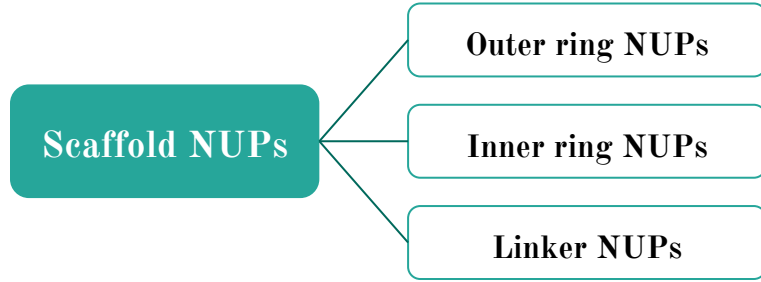
1.4 Nucleopore structure



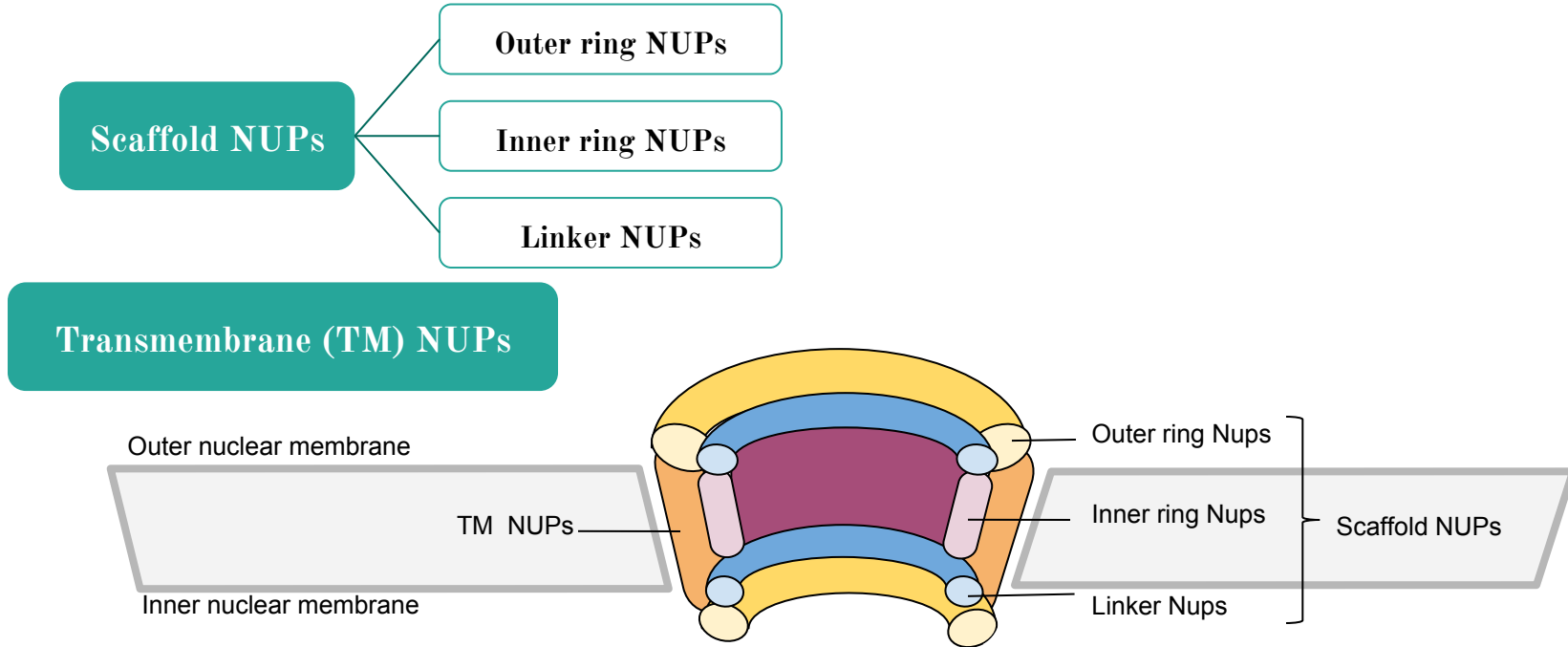
1.4 Nucleopore structure



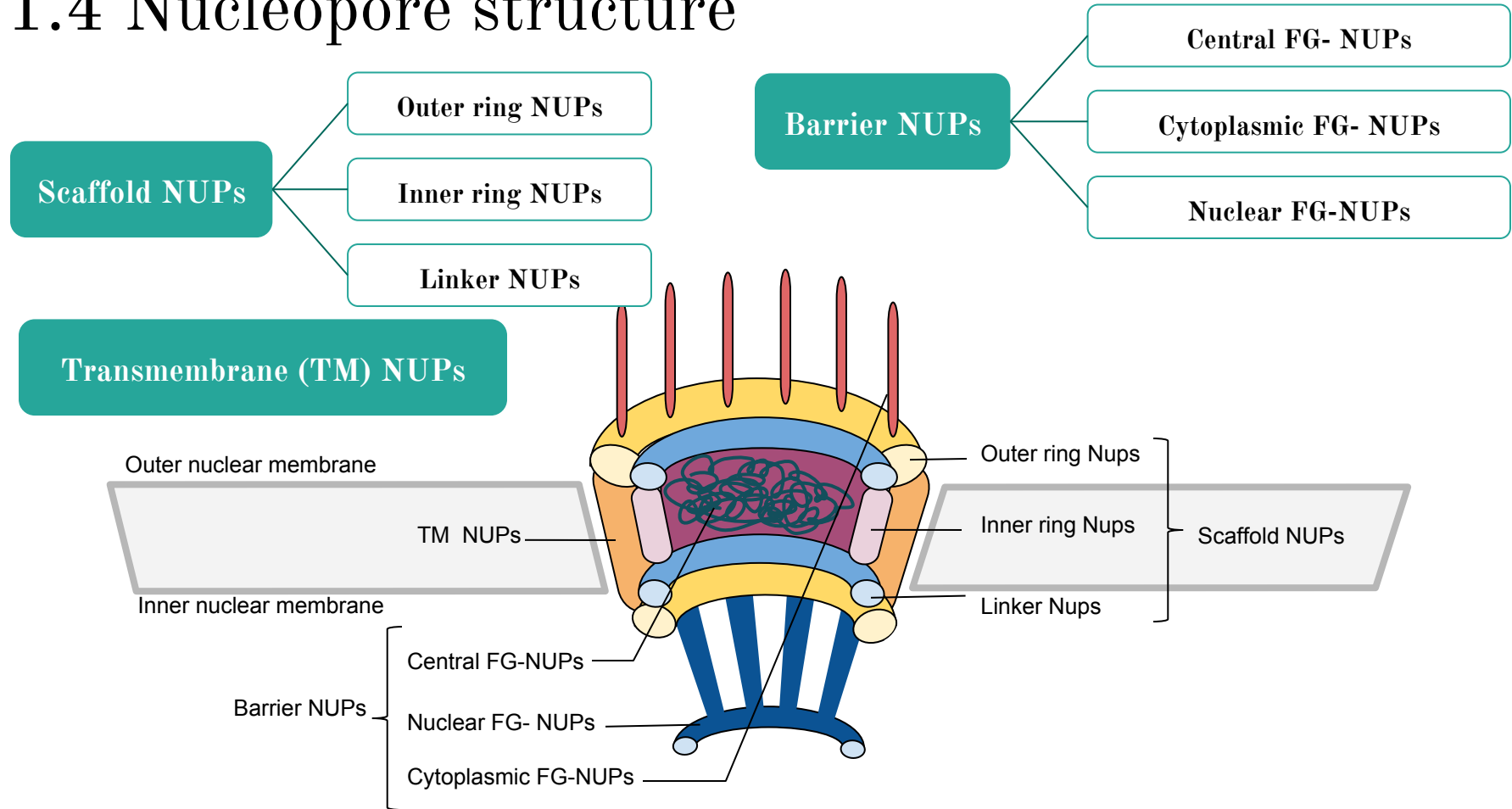
1.4 Nucleopore structure



1.4 Nucleopore structure

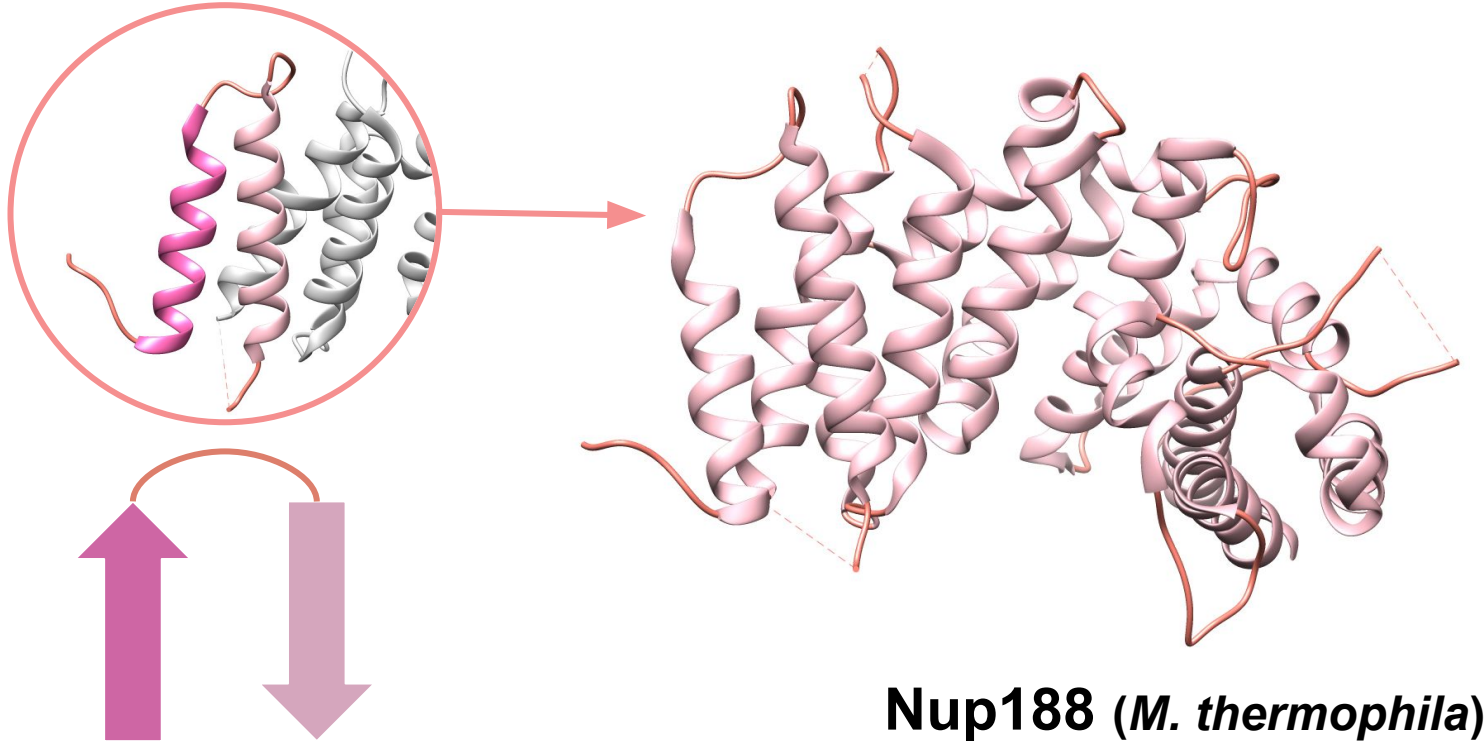


1.4 Nucleopore structure



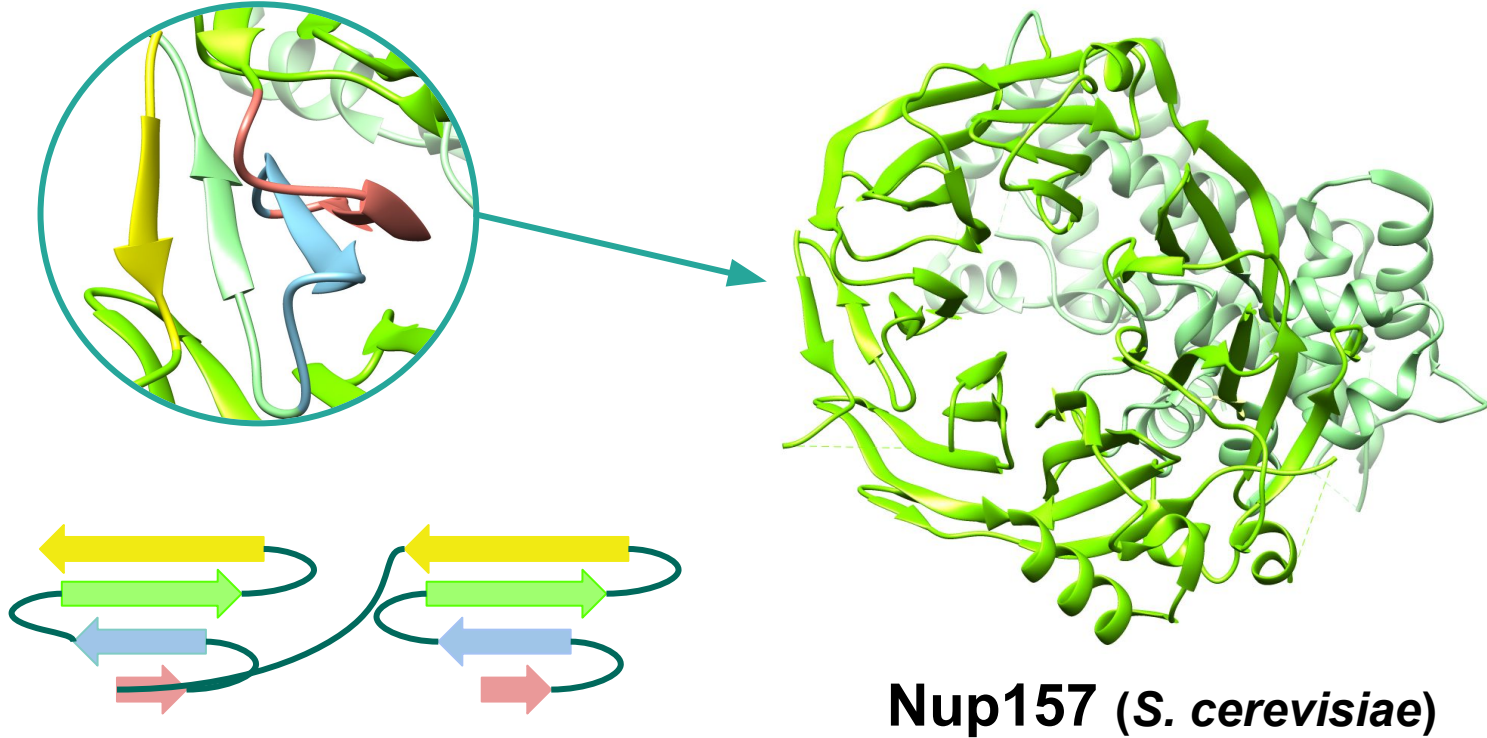
1.5 Main domains

α -solenoid



1.5 Main domains

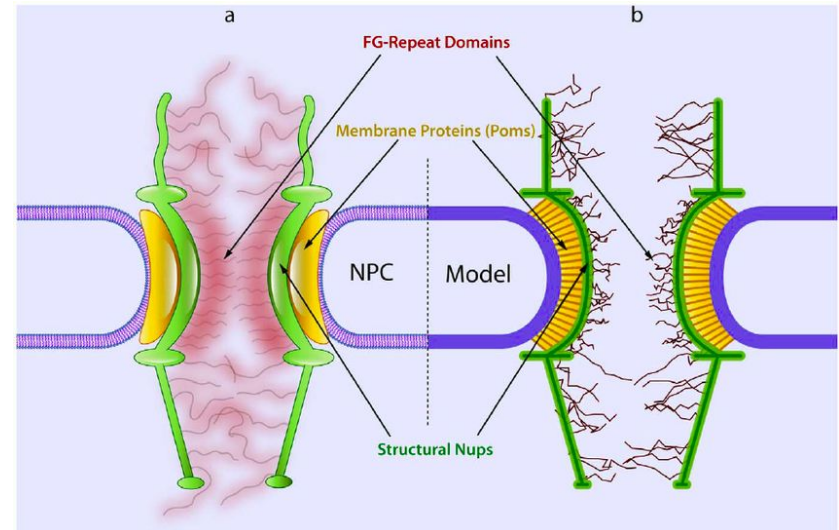
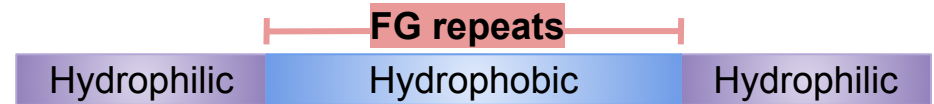
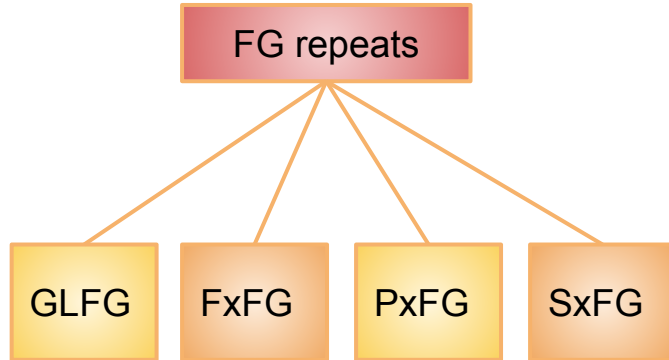
β -propeller



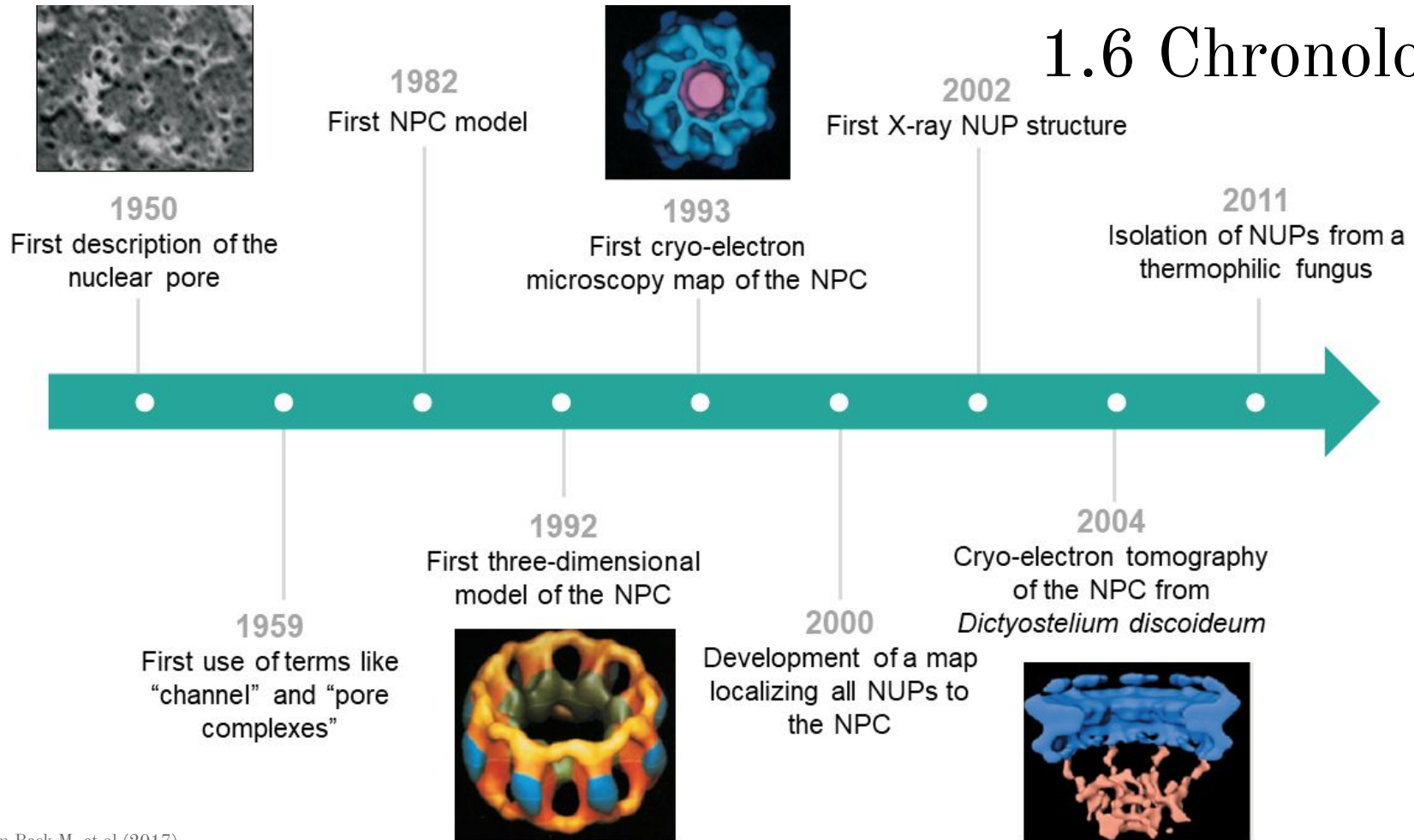
1.5 Main domains

FG repeats

- Do not have a secondary structure



1.6 Chronology



1.7 Difficulties in characterization

- Expression in model organisms
- Large, complex and flexible structure
- Membrane localization
- Conformation depending on exact region localization
- Not all the layers were predicted

Index

1. Introduction to Nucleopore



2. Scaffold Nups

2.1 Outer Ring: Y-complex

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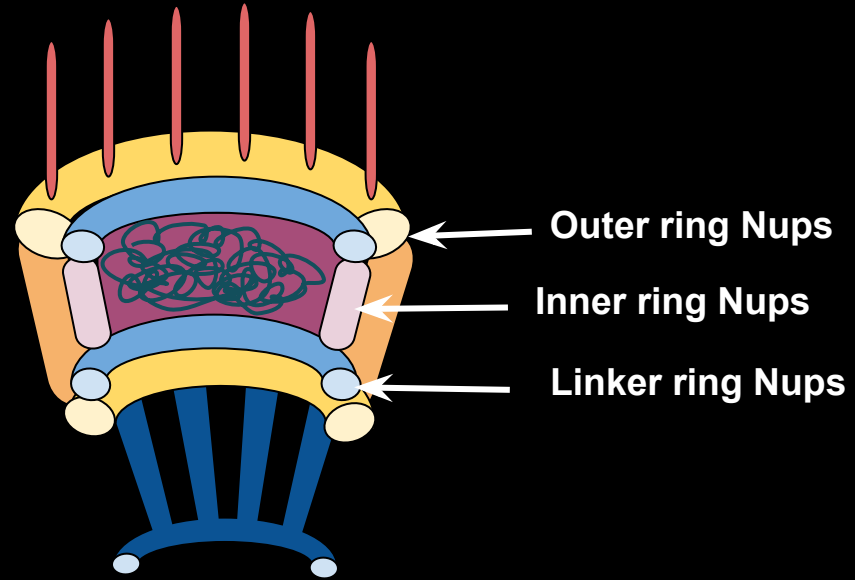
2. Scaffold Nups

Scaffold Nups

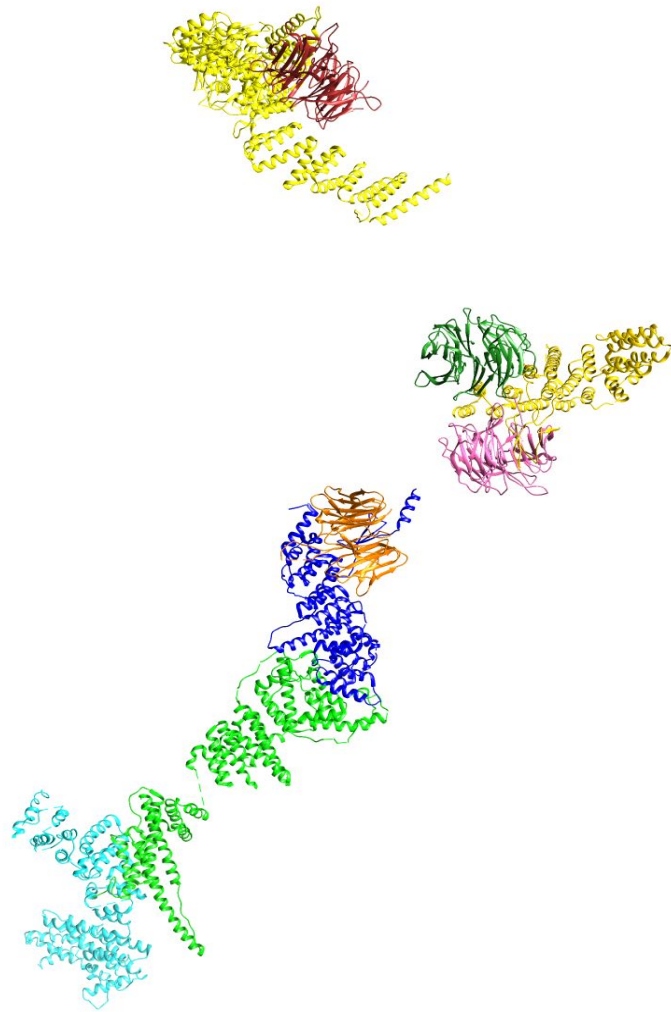
Outer ring Nups

Inner ring Nups

Linker Nups

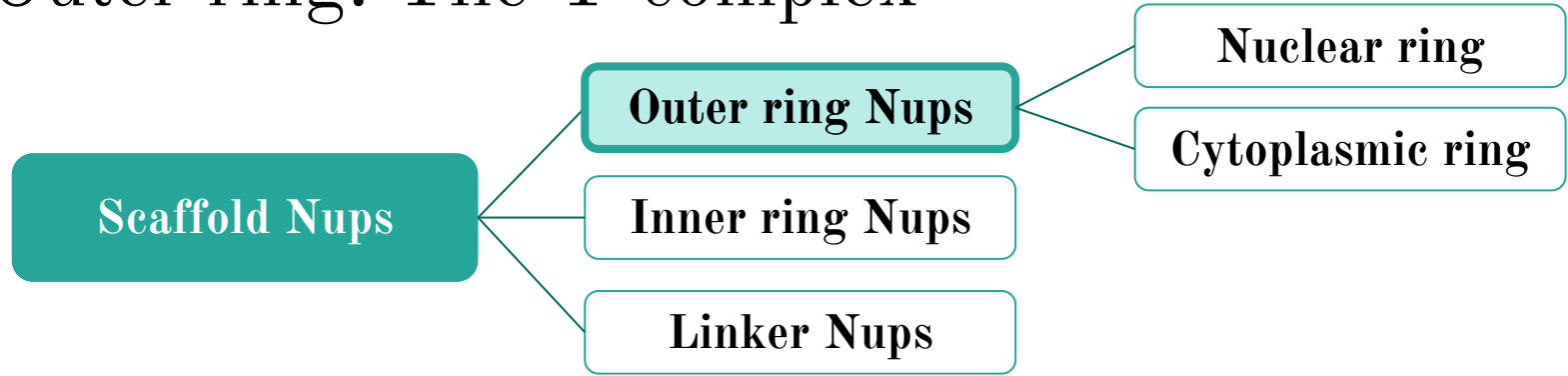


2.1 Outer ring: Y-complex





2.1 Outer ring: The Y-complex



- Building block of the outer ring (scaffold Nups)
- Found both in the nuclear and cytoplasmic outer rings
- Composed of:
 - 7 different Nups in yeast
 - 9 different Nups in vertebrates

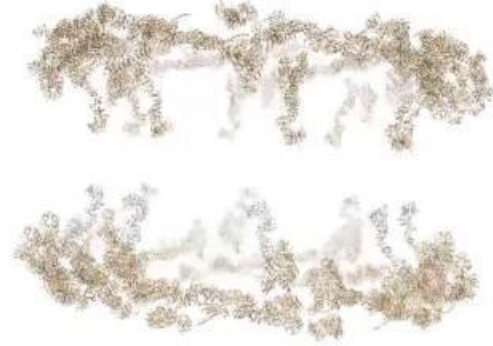


2.1 The Y-complex: Outer ring wide view

- The Outer ring is formed by the repetitions in tandem of Y-complexes

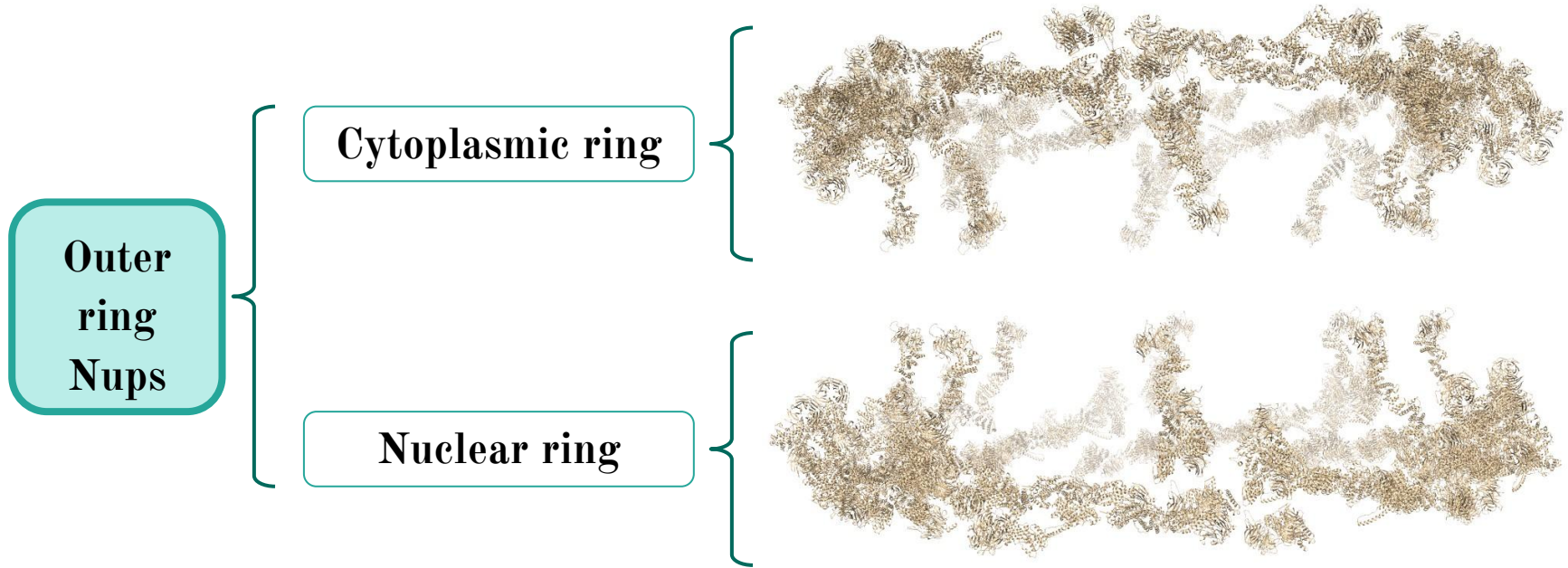


PDBid = 5A9Q





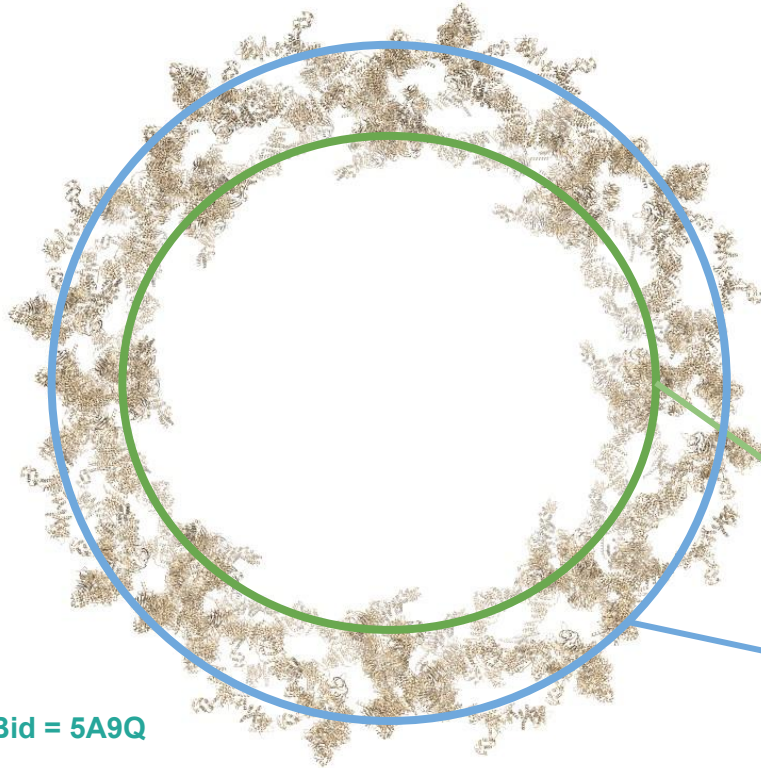
2.1 The Y-complex: Outer ring wide view



PDBid = 5A9Q



2.1 The Y-complex: Outer ring wide view



PDBid = 5A9Q

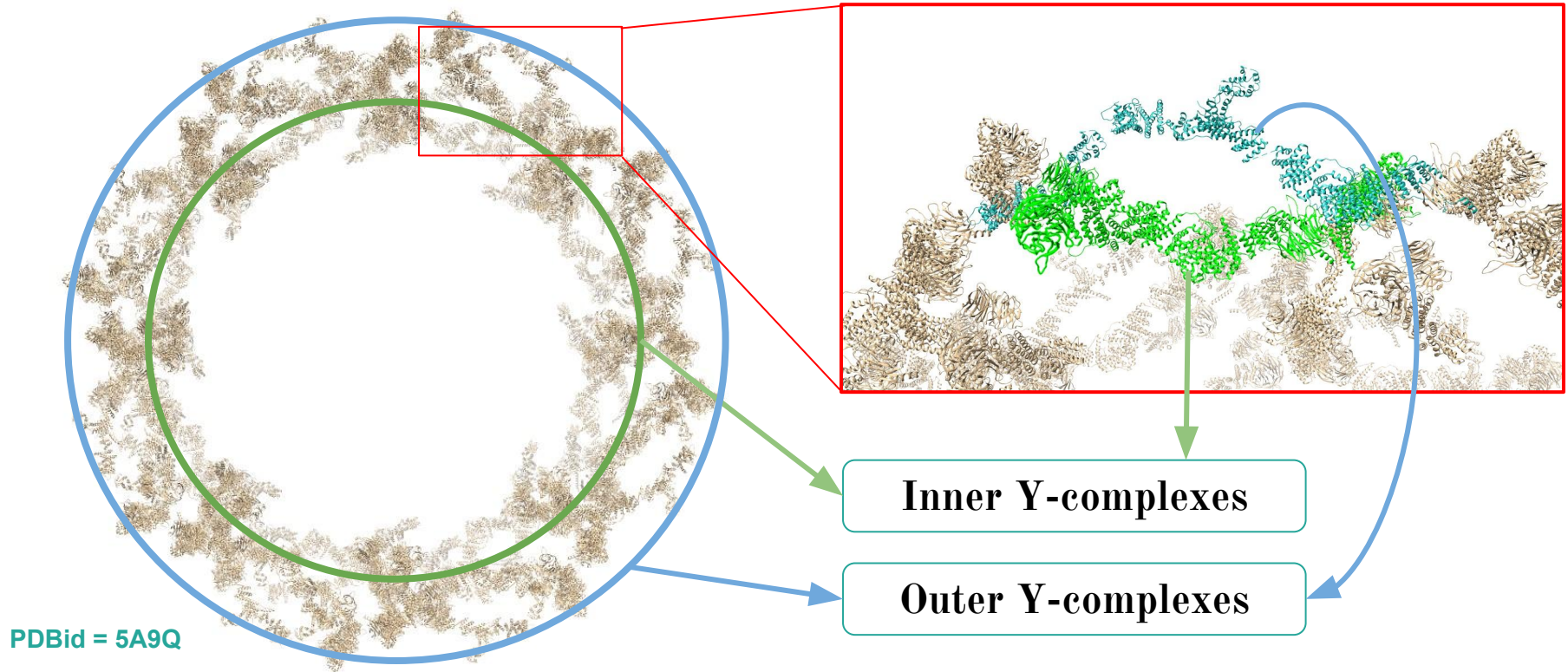
- Both the cytoplasmic and the nuclear ring are composed by **two concentric** rings made by 32 repetitions of the Y-complex
- Therefore, both cytoplasmic and nuclear rings are formed, in turn, by **inner** and **outer Y-complexes**

Inner Y-complexes

Outer Y-complexes



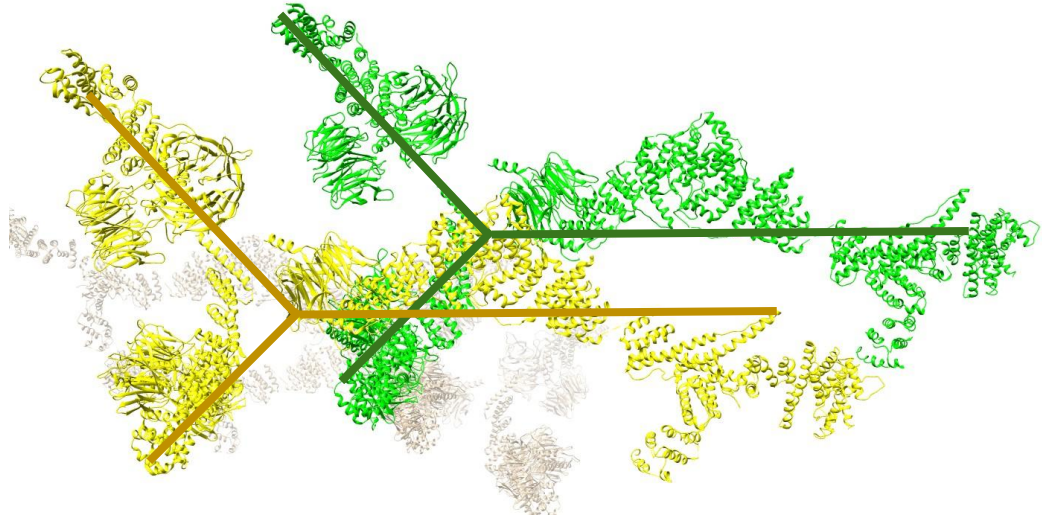
2.1 The Y-complex: Outer ring wide view





2.1 The Y-complex

- The proteins form an Y-shaped arrangement
- Iso-stoichiometric
- The two Y-complexes forming the dimers are slightly shifted

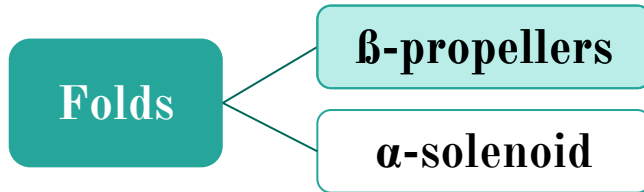


Inner Y-complex
Outer Y-complex

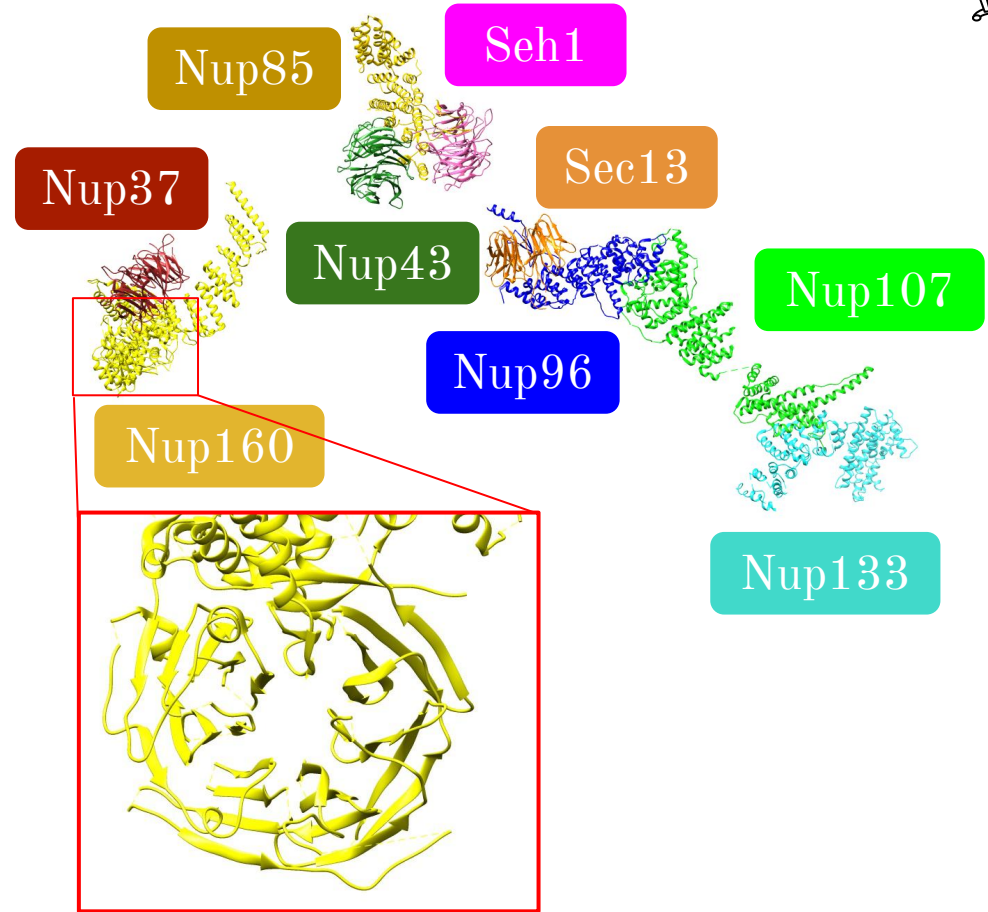


2.1 The Y-complex

- Proteins in vertebrates:
 - Nup133, Nup107, Nup96, Sec13, Nup85, Seh1, Nup160, Nup37, and Nup43



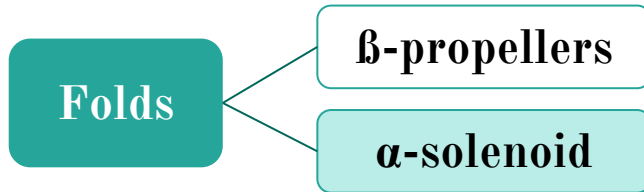
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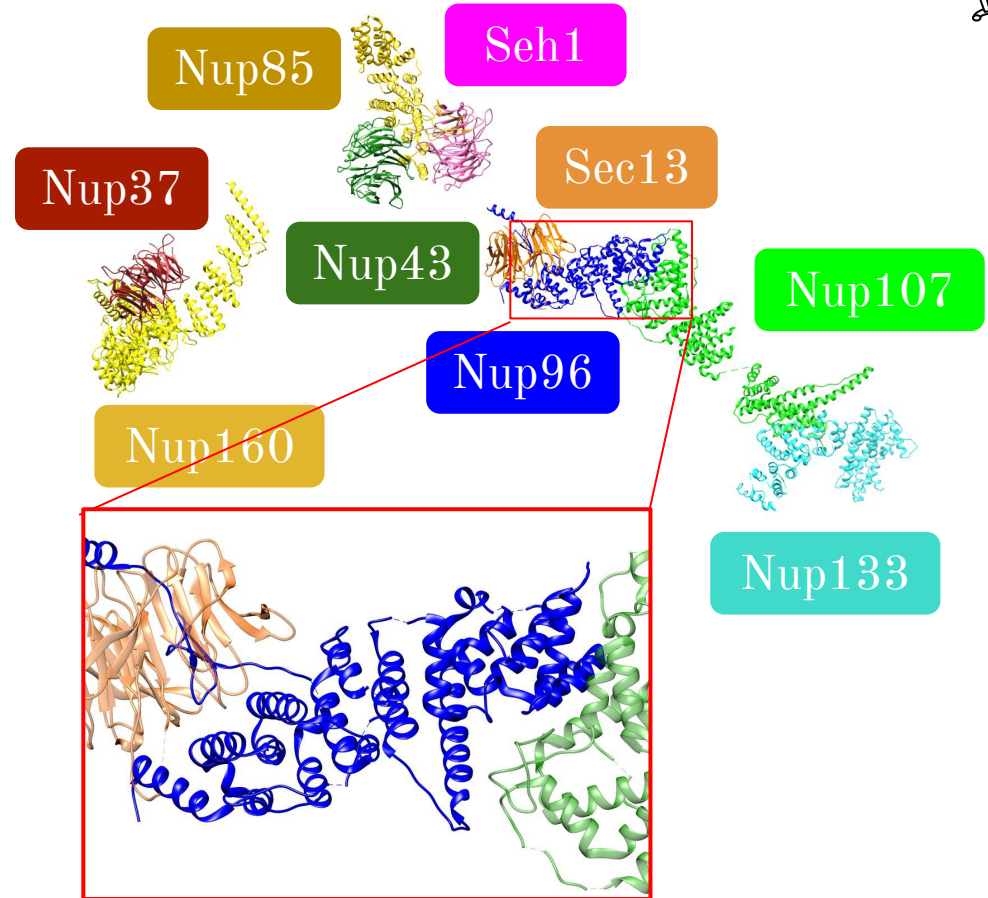


2.1 The Y-complex

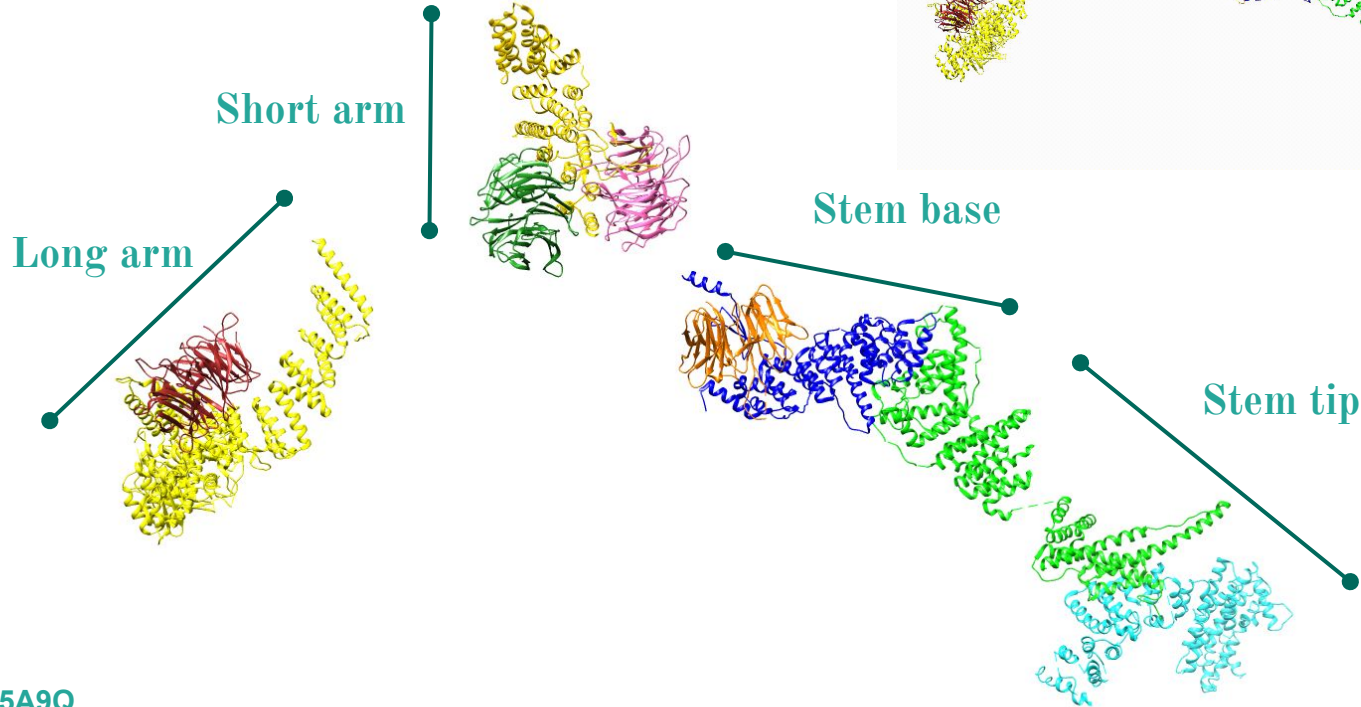
- Proteins in vertebrates:
 - Nup133, Nup107, Nup96, Sec13, Nup85, Seh1, Nup160, Nup37 and Nup43



PDBid = 5A9Q



2.1 The Y-complex: regions

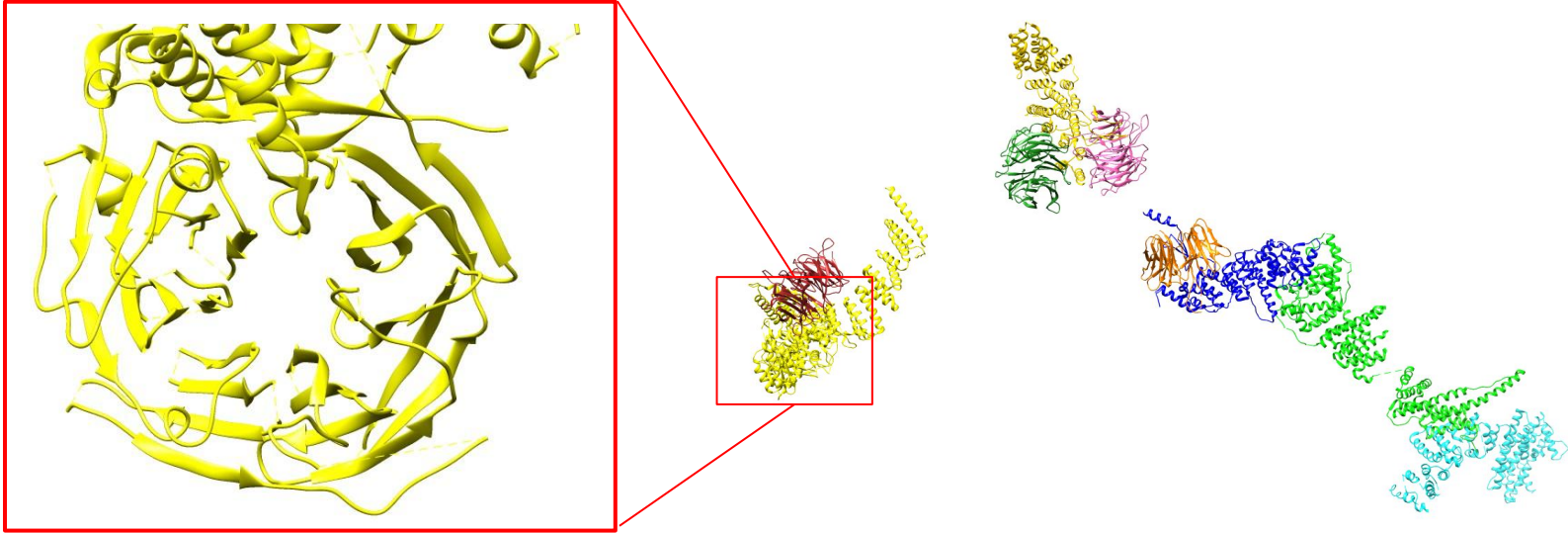


PDBid = 5A9Q



2.1 The Y-complex: Nup160, Nup155 and Nup133

- **Nup160**, Nup155 and Nup133 contain β -propellers which bind to the membrane



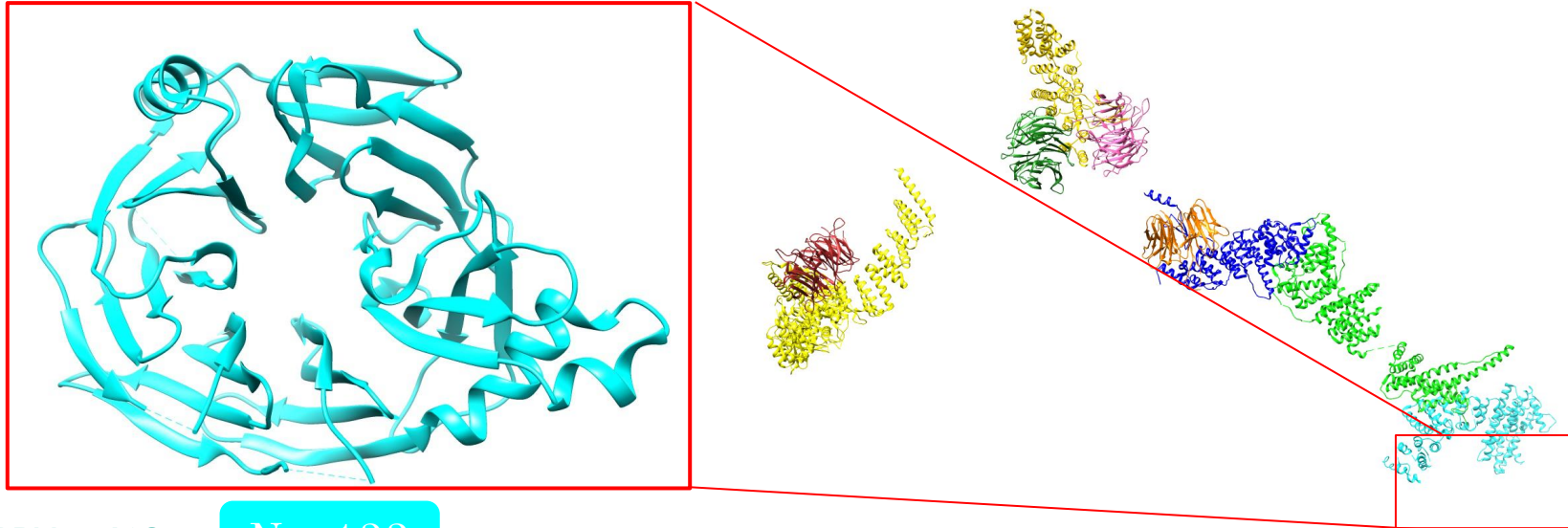
PDBid = 5A9Q

Nup160

2.1 The Y-complex: Nup160, Nup155 and Nup133



- Nup160, Nup155 and **Nup133** contain β -propellers which bind to the membrane



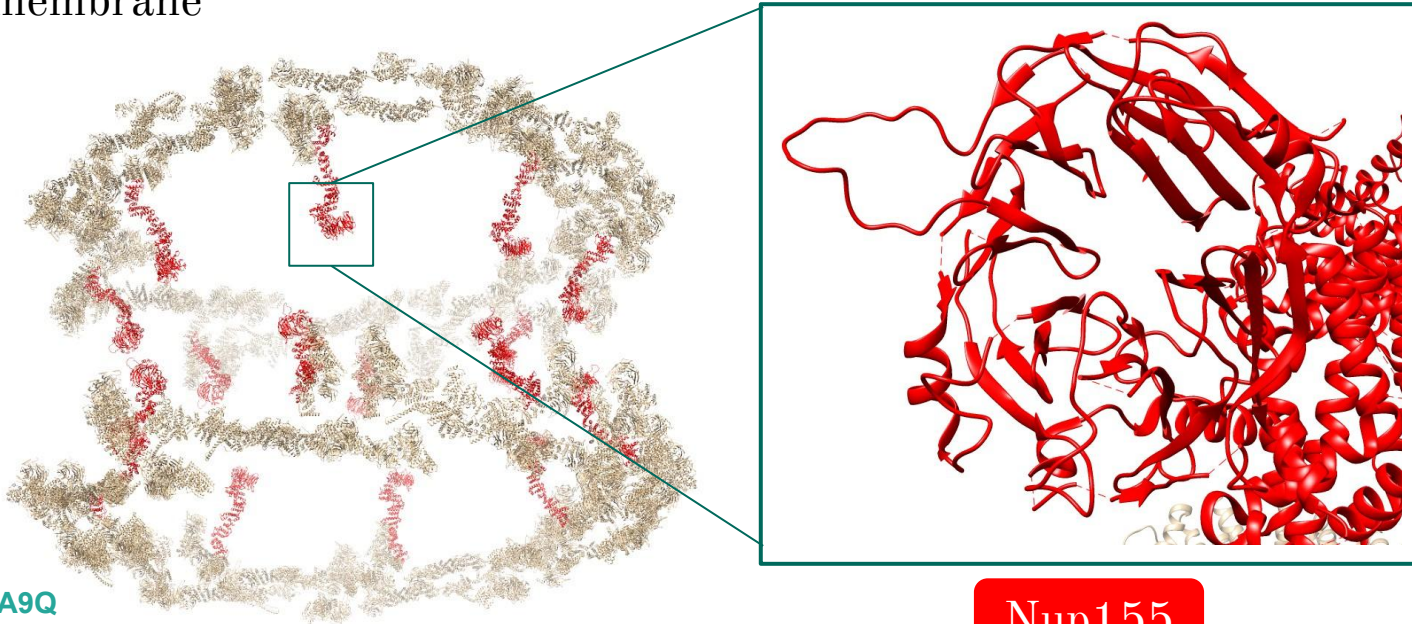
PDBid = 5A9Q

Nup133

2.1 The Y-complex: Nup160, Nup155 and Nup133



- Nup160, **Nup155** and Nup133 contain β -propellers which bind to the membrane



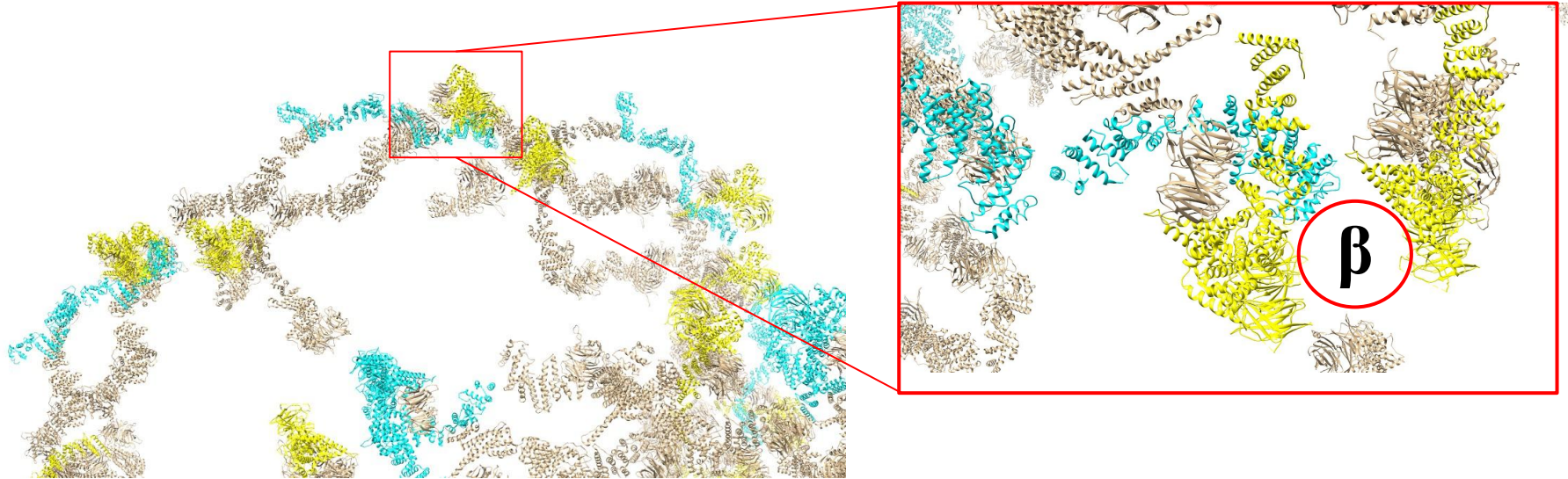
PDBid = 5A9Q

Nup155



2.1 The Y-complex: interactions

- N-terminal β -propeller **Nup133** vs posterior **Nup160** → **Head-to-tail contact**

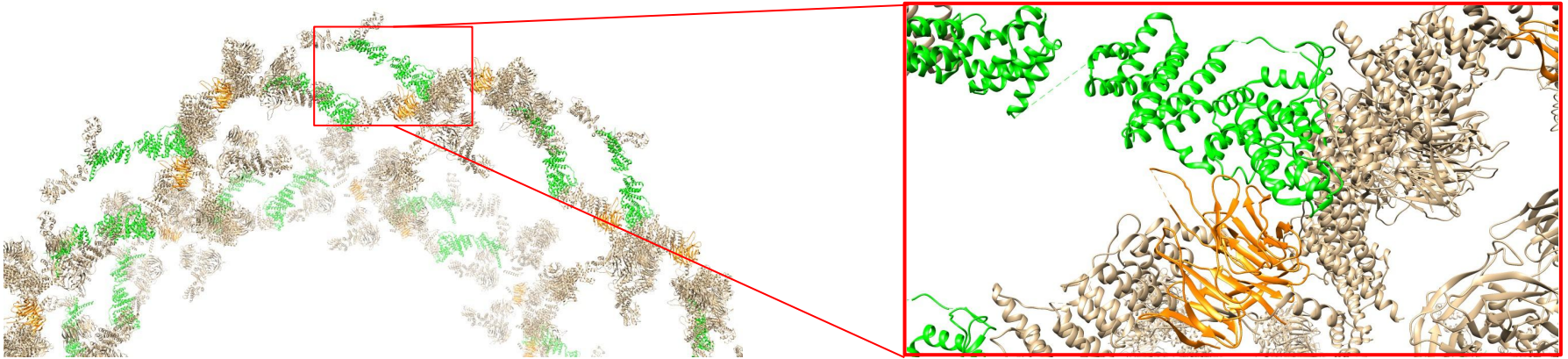


PDBid = 5A9Q



2.1 The Y-complex: interactions

- Outer **Sec13** vs inner N-terminus **Nup107**

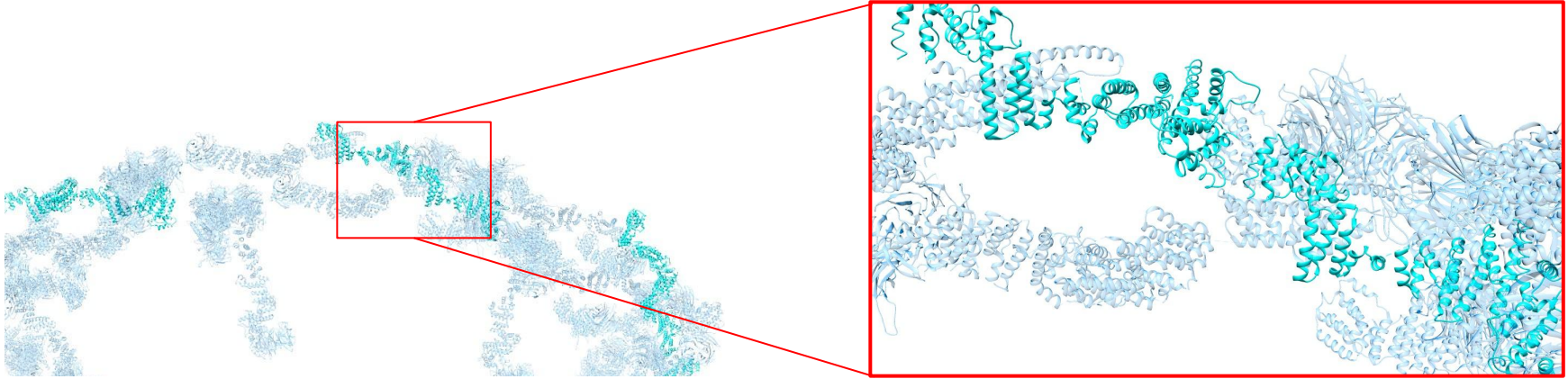


PDBid = 5A9Q



2.1 The Y-complex: interactions

- Inner C-terminus **Nup133** vs outer **Nup133**

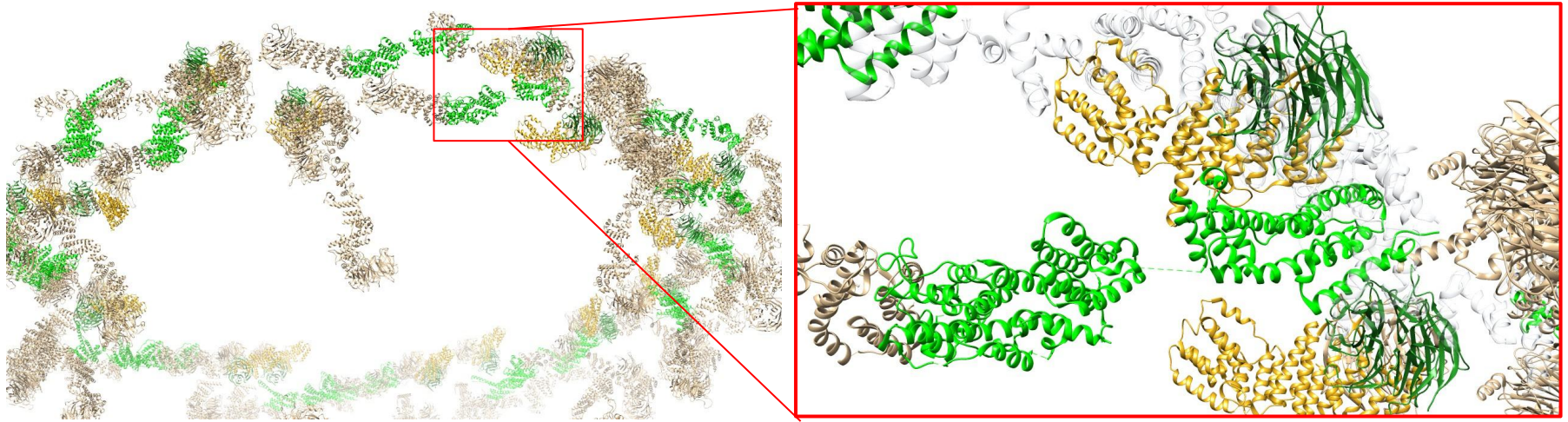


PDBid = 5A9Q



2.1 The Y-complex: interactions

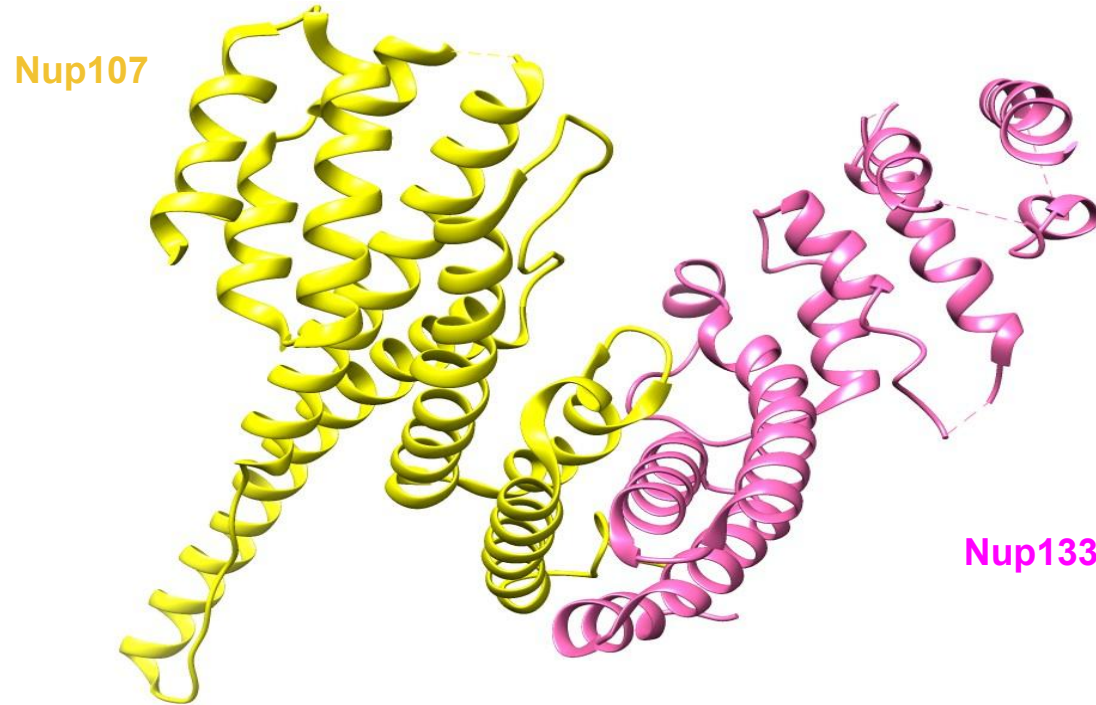
- Outer **Nup85** and **Nup43** vs inner C-terminus **Nup107**



PDBid = 5A9Q



2.1.1 The Y - complex: Nup107 - Nup133 interaction



- DOMAIN interaction
- α -helices
- L-shaped arrangement



2.1.1 The Y - complex: Nup107

Nup107: PSI-BLAST

Query= NU107_HUMAN

Length=925

Sequences producing significant alignments:				Score (Bits)	E Value
3cqc_A	mol:protein	length:270	Nuclear pore complex protein Nup107	421	6e-141
3cqq_A	mol:protein	length:246	Nuclear pore complex protein Nup107	363	4e-119
1vz8_D	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85
1vz8_C	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85
1vz8_B	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85
1vz8_A	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85
1vz7_D	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85
1vz7_C	mol:protein	length:393	ORNITHINE ACETYL-TRANSFERASE	280	2e-85

2.1.1 The Y - complex: Nup107



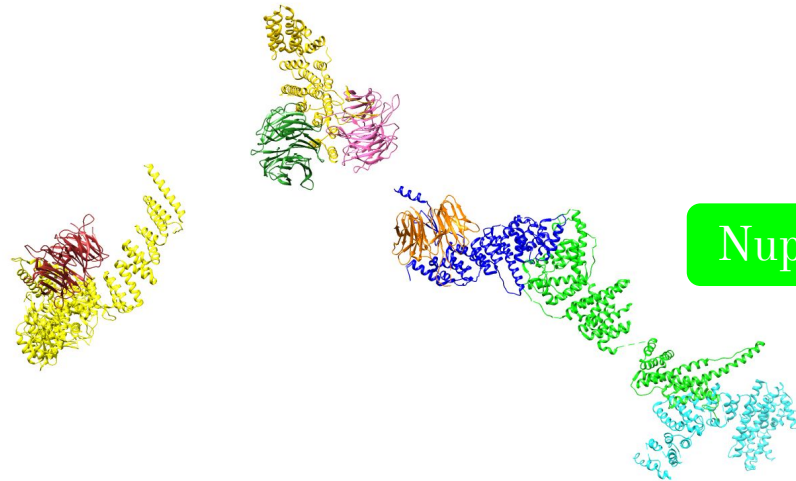
CATH: Nup107 vs. Ornithine Acetyltransferase

Ornithine
Acetyltransferase

CATH Classification

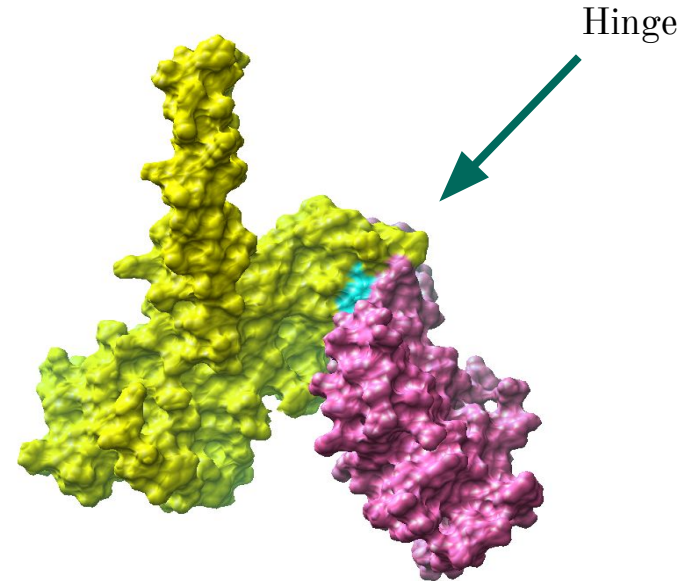
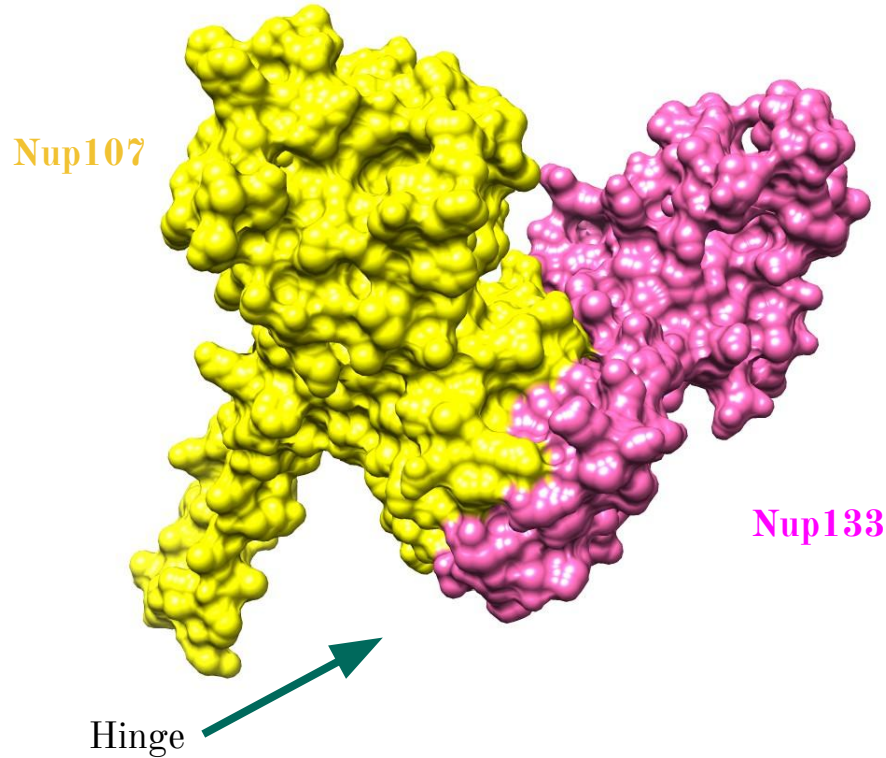
Level	CATH Code	Description
	3	Alpha Beta

Nup107



Nup107

2.1.1 The Y - complex: Nup107 - Nup133

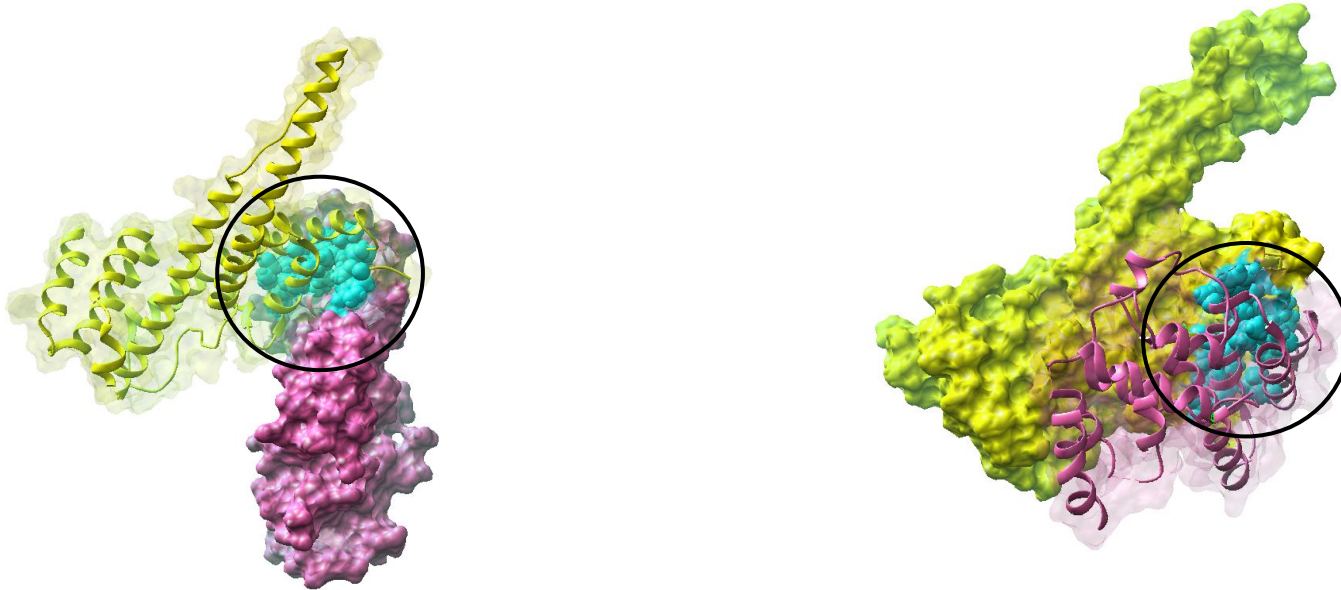


PDBid = 3CQC



2.1.1. The Y - complex: Nup107 - Nup133

Van der Waals interactions

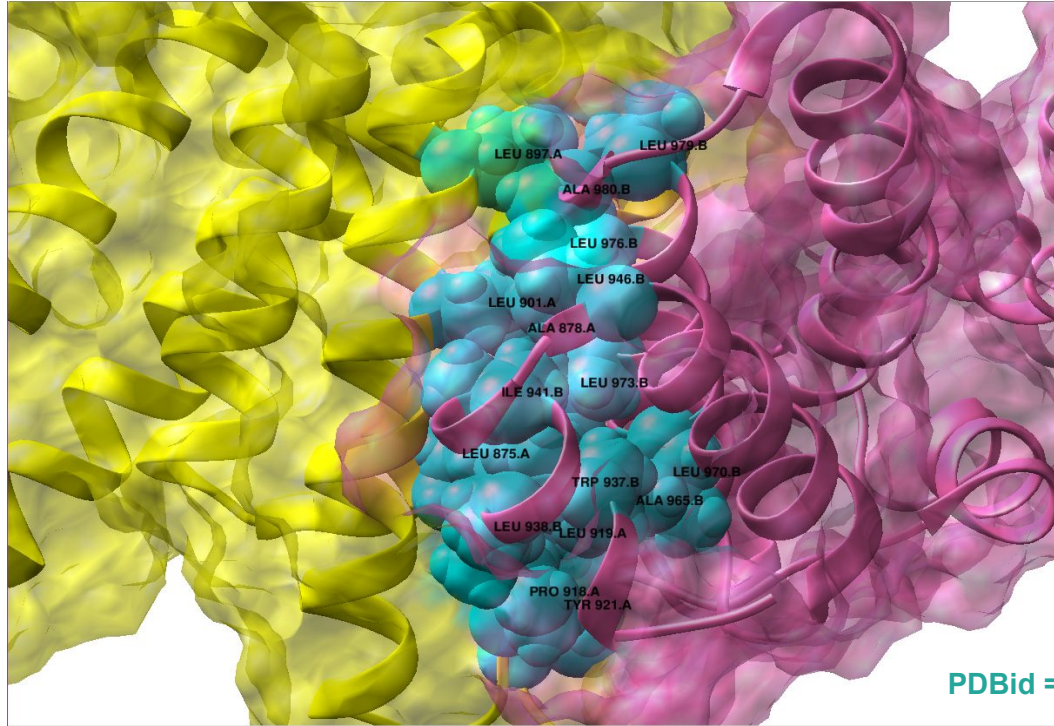


PDBid = 3CQC



2.1.1 The Y - complex: Nup107 - Nup133

Van der Waals interactions



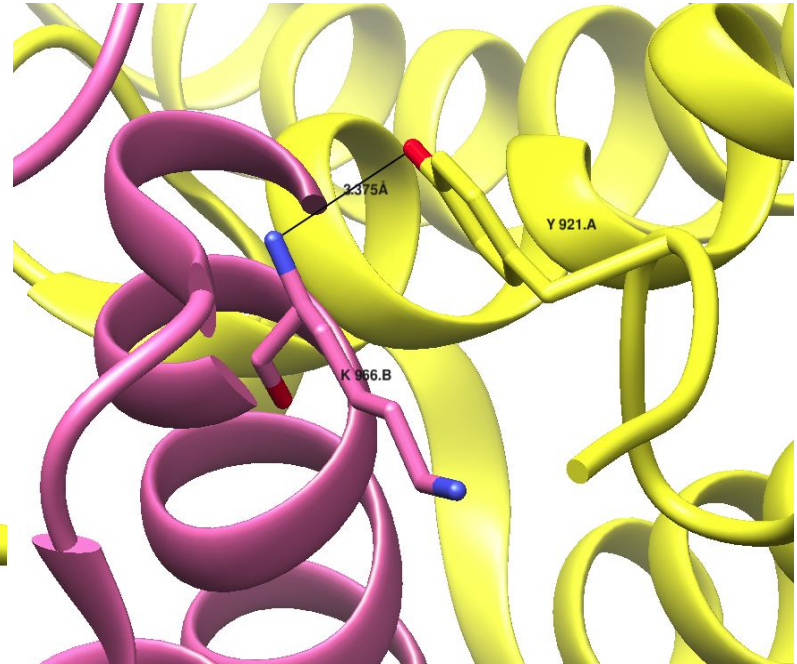
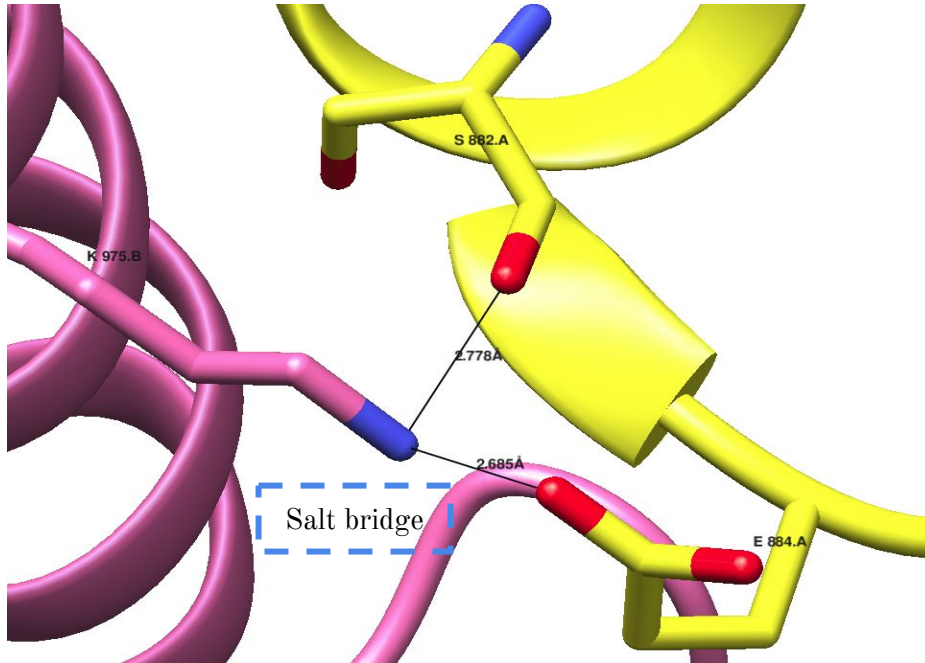
PDBid = 3CQC

Nup107	Nup133
L875	W937
A878	L938
L897	I941
L901	L946
P918	A965
L919	L970
Y921	L973
	L976
	L979
	A980

2.1.1 The Y - complex: Nup107 - Nup133



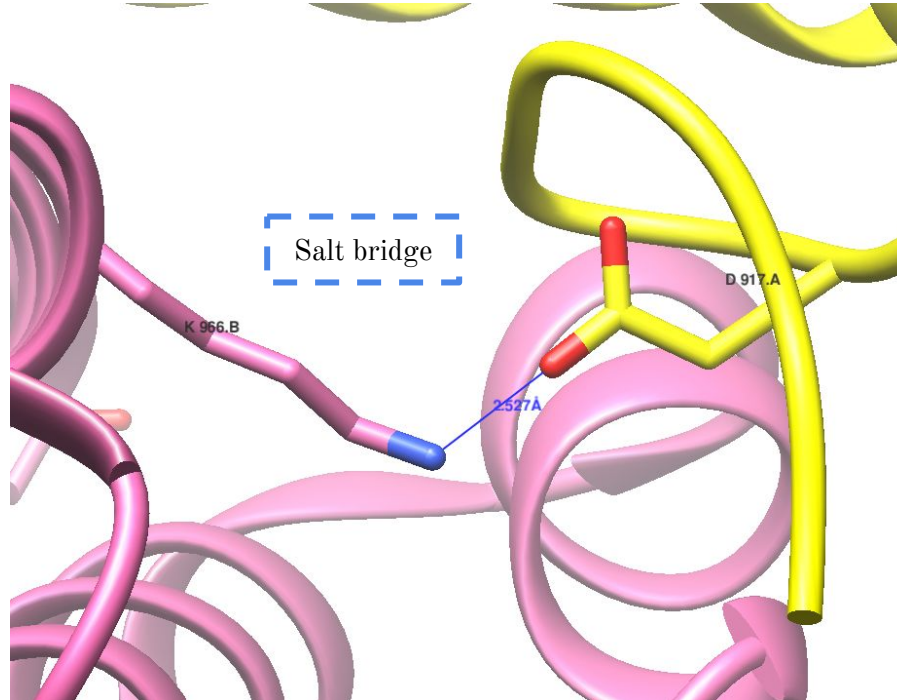
Hydrogen bonds / Salt bridges



2.1.1 The Y - complex: Nup107 - Nup133



Hydrogen bonds / Salt bridges



PDBid = 3CQC



2.1.1 The Y - complex: Nup107 - Nup133

Nup107: PFAM family and MSA

C-terminus

		E884	K894
NU107_HUMAN	-ILHSTG-----QYQECLQLADMV	SSERHKLYLVF-S	KEELRKLLQKLRESSLMlldqgl
NU107_DROSO	-VMFKSG-----DFAGCVRLADEI	SSENRQLYKVY-T	KHKLAELLAIAKIDASL-ellnsk
NU107_RAT	-ILHSTG-----QYQECLQLADMV	SSERHKLYLVF-S	KEELRKLLQKLRESSLMlldqgl
NU107_MOUSE	-ILHSTG-----QYQECLQLADMV	SSERHKLYLVF-S	KEELRKLLQKLRESSLMlldqgl
NUP84_YEAST	lVEAAKLLKIPKFISEALFTSLVAN	ENDKIYLLFQS	SGKLKEYLDLVARTAT-lsn---
		S882	
NU107_HUMAN	dplgyeiql-		
NU107_DROSO	ldpwgypitt		
NU107_RAT	dplgyeiqs-		
NU107_MOUSE	dplgyeiqs-		
NUP84_YEAST	-----		

- **PFAM Family:** Nup84_Nup100 (PF04121)



2.1.1 The Y - complex: Nup107 - Nup133

Nup133: PFAM family and MSA

C-terminus

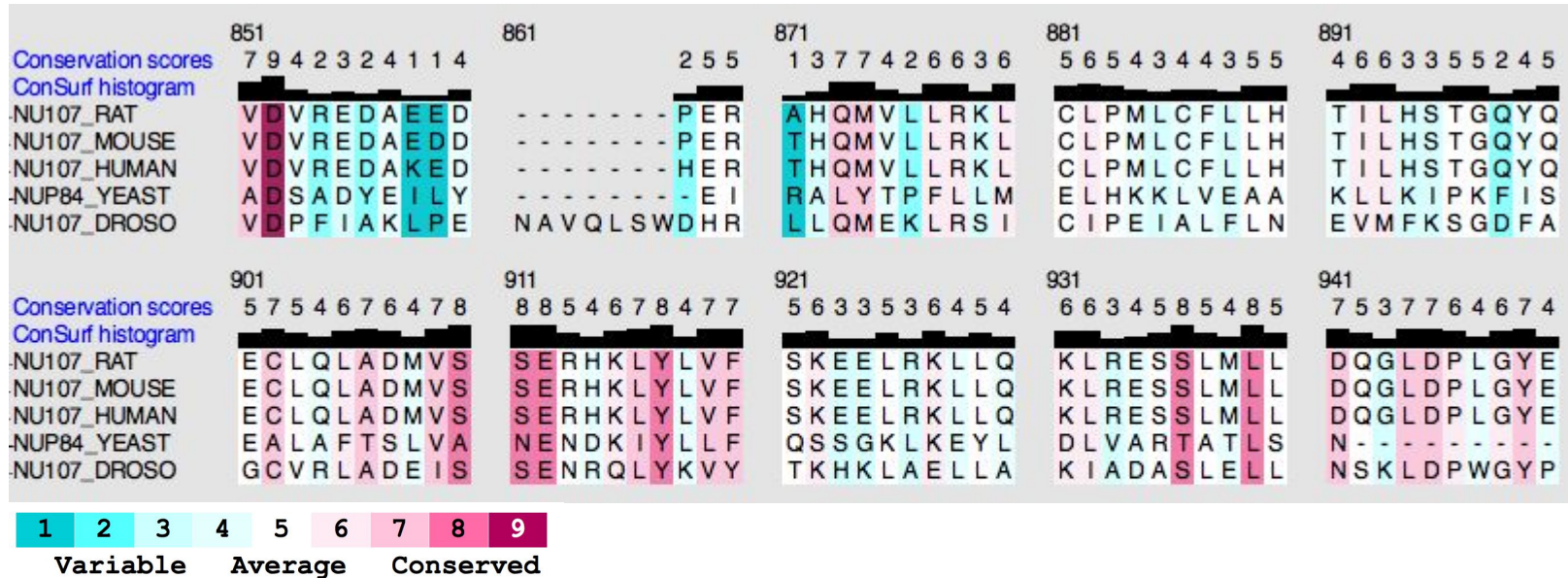
		K966	K975
NU133_Cterm_HUMAN	LQA----HEHLSWLHEINSQELEKAHATLLGLANMETRYF---AKKKTLLGLSKLAALAS		
NU133_Cterm_YEAST	FQESAPKYGHVAWIQQILDGSYADAMNTLKNITVDDSKKGESLSECELHLNVAKLSSLLV		
NU133_Cterm_MOUSE	LQA----HEHLSWLHEINSQELEKAHTTLLGLANMETRYF---AKKKTLLGLSKLAALAS		
NU133_Cterm_CELEG	LDN----FKELRYSREIANKQFGKAALTMLSLADAETKS----FSKFVEFLTRAYYCACS		
NU133_Cterm_DROSO	MRD----HPSLGWIQLIFNGDFERAQVLYELAQCETEFV---ARKKSMLSLAKLAFAA		
NU133_Cterm_HUMAN	DFsE-dM---LQEKIEEMAEQERFLLHQETLPEQLLAEKQLN-----		
NU133_Cterm_YEAST	EKdN--L---DINTLRKIQYNLDTIDAENISNKLKKGEVQICKR-FK--NGSIREVFNI		
NU133_Cterm_MOUSE	DI sE-dR---LQEKIEEMAEQERFLLHQETLPEQLLTERQLS-----		
NU133_Cterm_CAEEL	SI-DgtD---VSEVLDFYKRRYPPEMKHRKRIPTTEILKICFGNDLDAMMsVEDMLEW----		
NU133_Cterm_DROSO	AE-S--D---LTAQVEKINADLTLEVYQSQLGHDVLESFGFD-----		

- PFAM Family: Nucleoporin_C (PF03177)

2.1.1 The Y - complex: Nup107 - Nup133



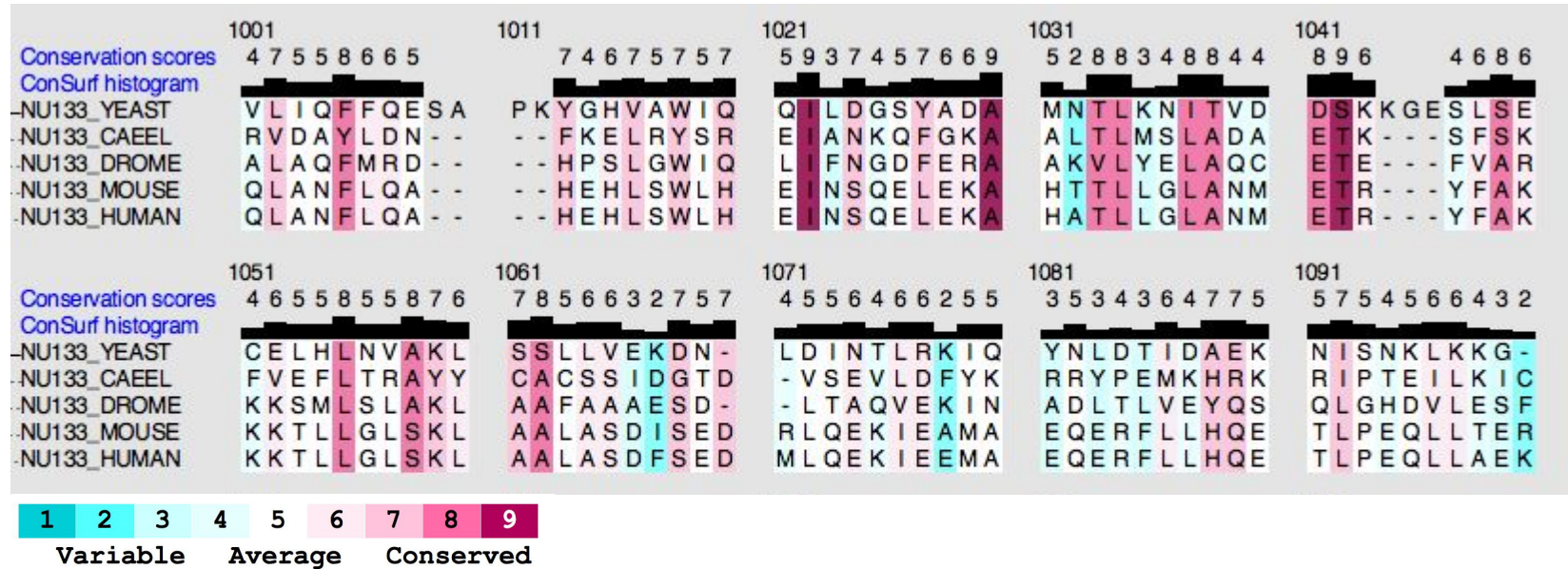
Nup107: ClustalW sequence alignment with ConSurf



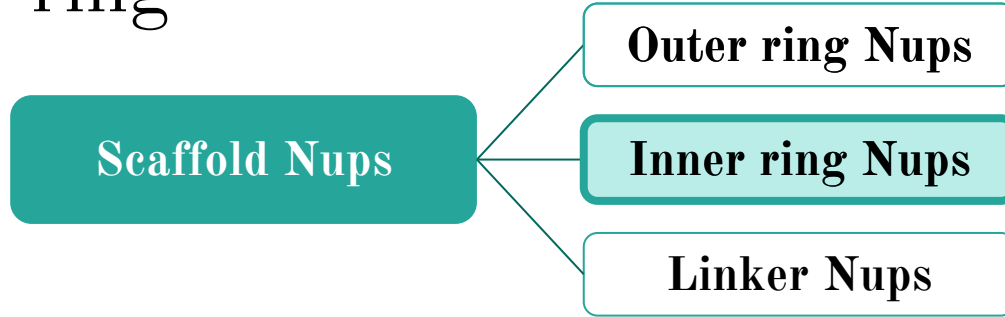


2.1.1 The Y - complex: Nup107 - Nup133

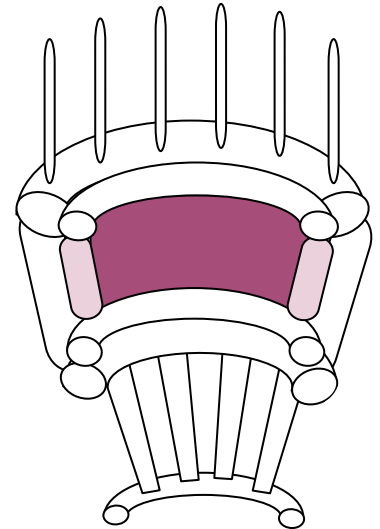
Nup133: ClustalW sequence alignment with ConSurf



2.2 Inner ring



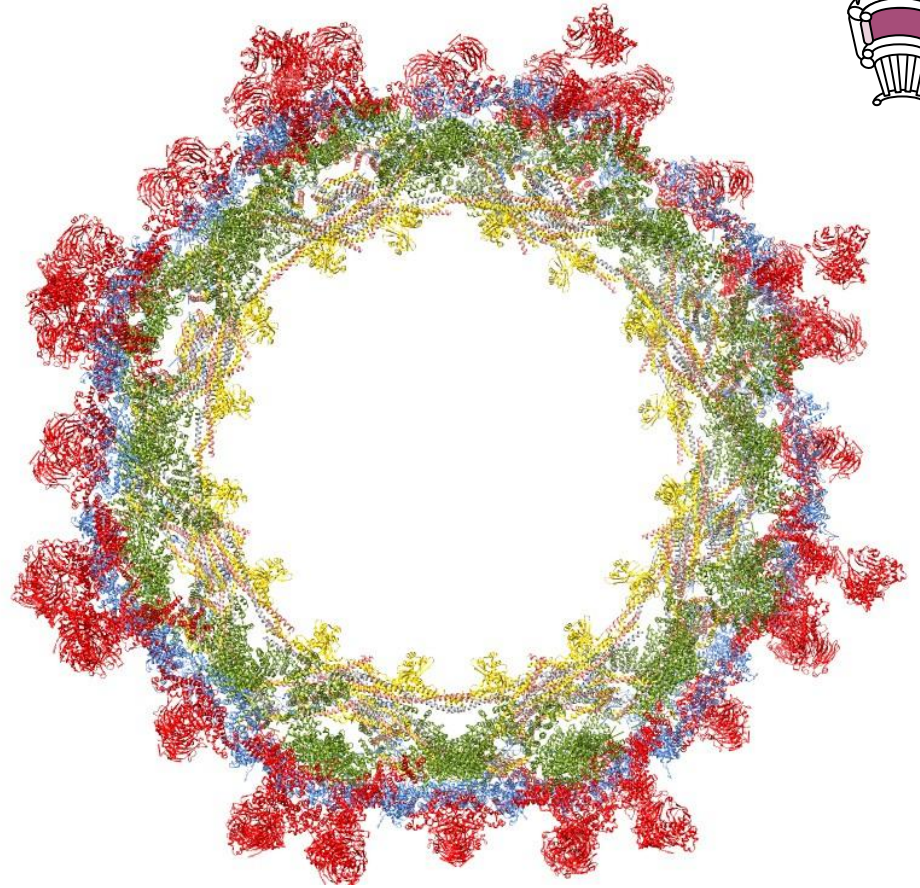
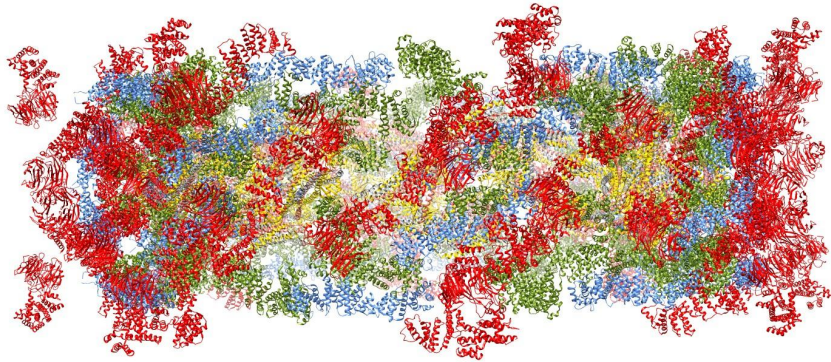
- Located in the central part of the main channel
- Anchor the Nucleopore complex to the membrane by interacting with membrane proteins

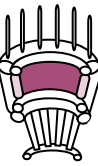




2.2 Inner ring

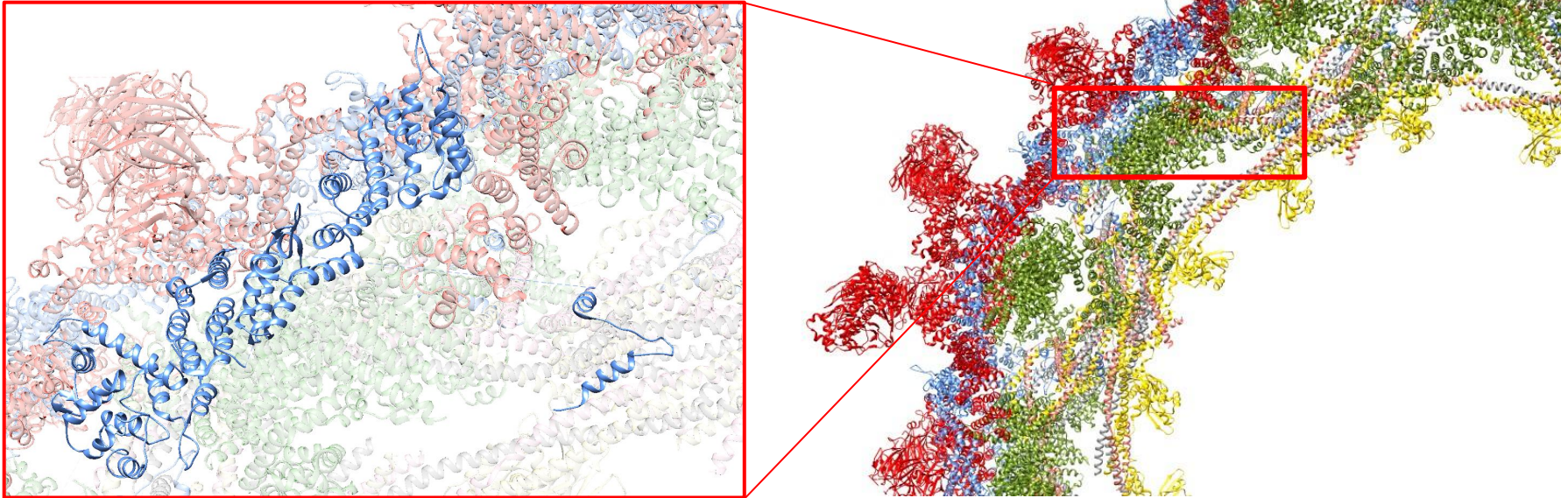
- Formed by Nup205, Nup188, Nup93 and Nup35.
- Iso-stoichiometric

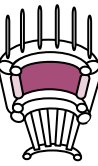




2.2 Inner ring

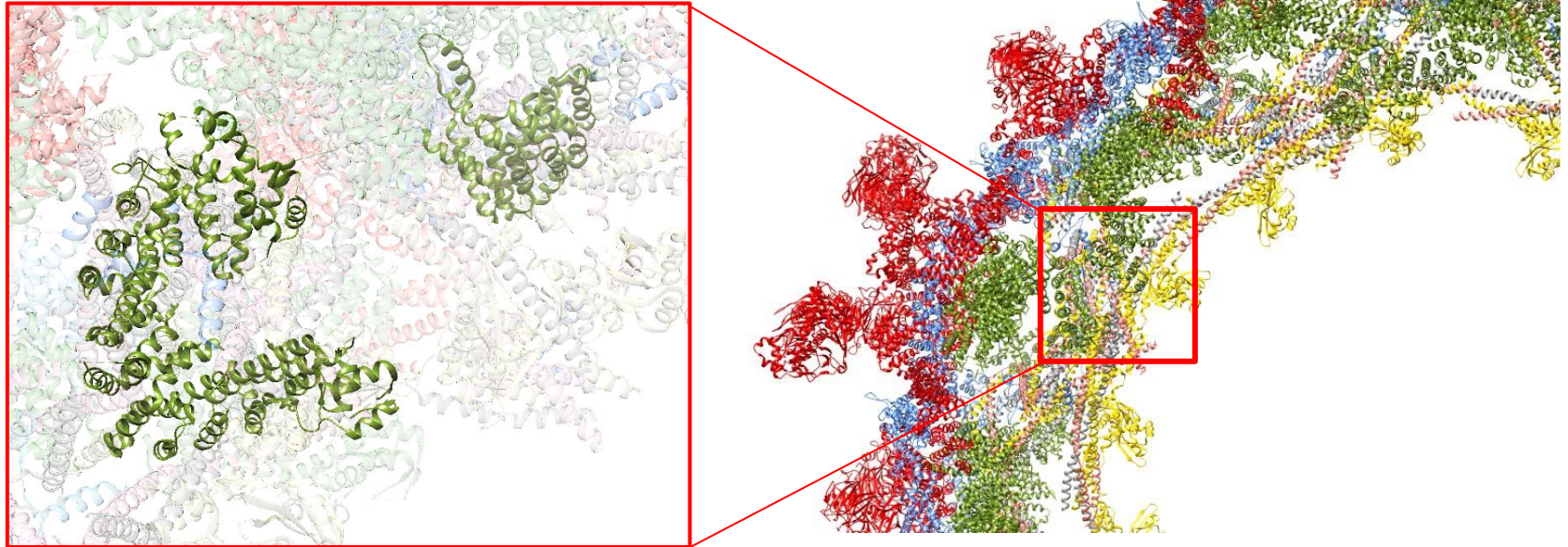
- Formed by **Nup93**, Nup205, Nup188 and Nup35





2.2 Inner ring

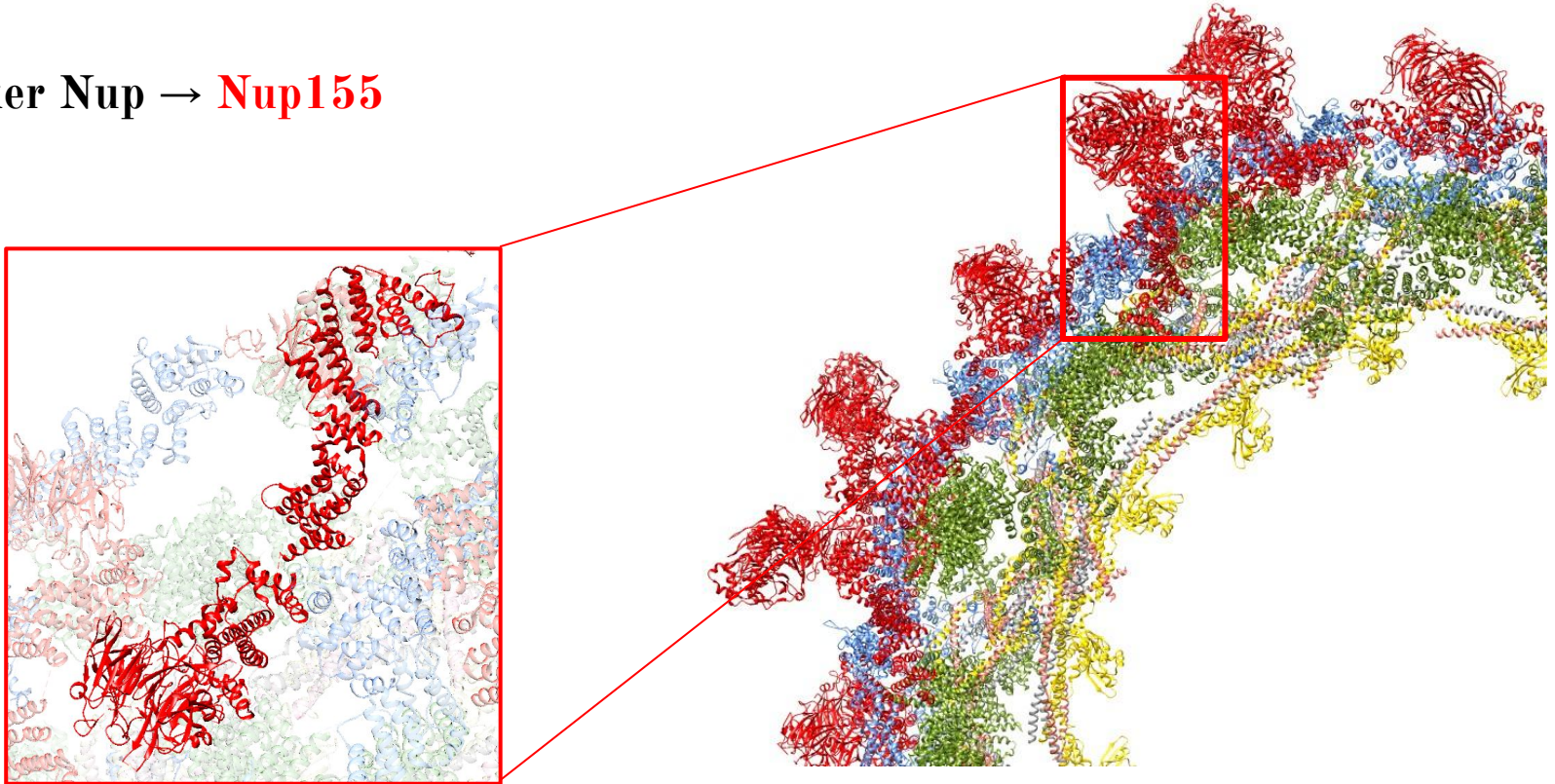
- Formed by Nup93, **Nup205**, Nup188 and Nup35



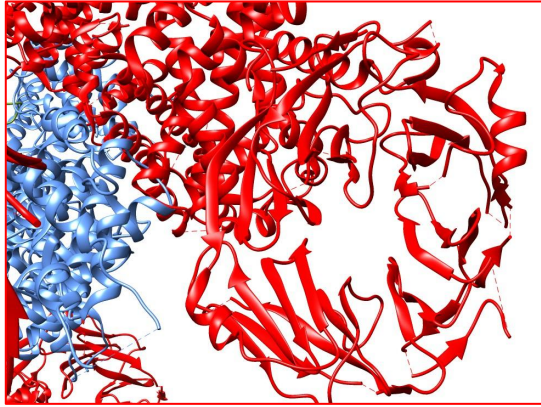


2.2 Inner ring

- Linker Nup → **Nup155**



2.2 Inner ring

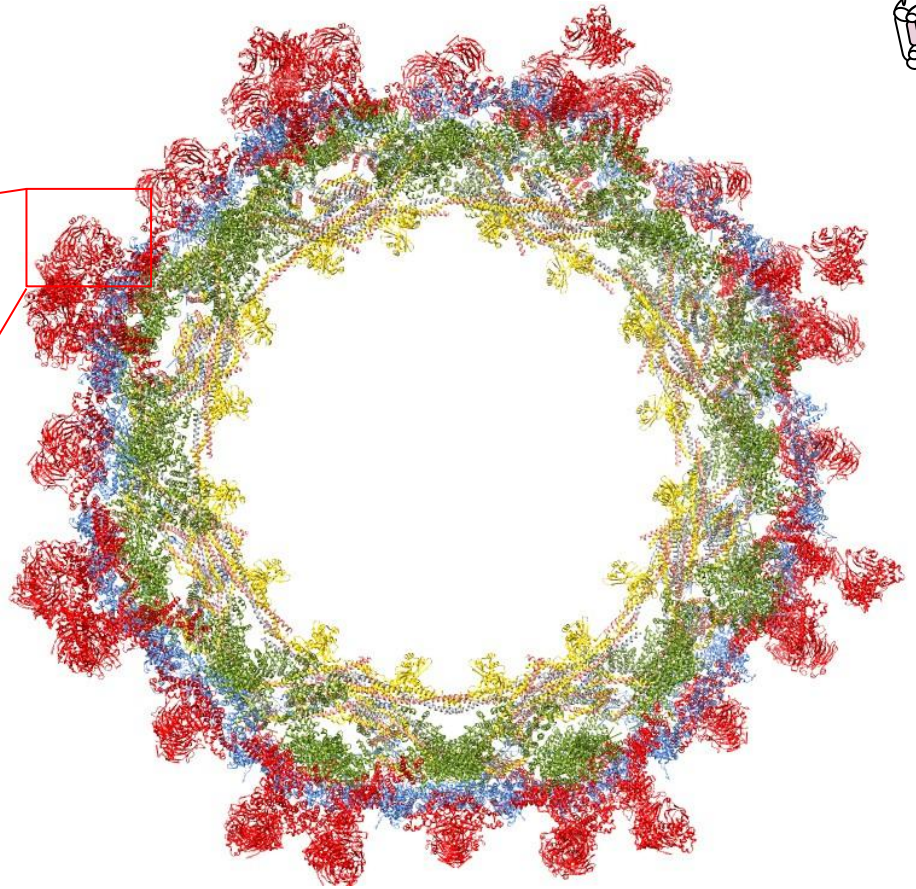


Nup155

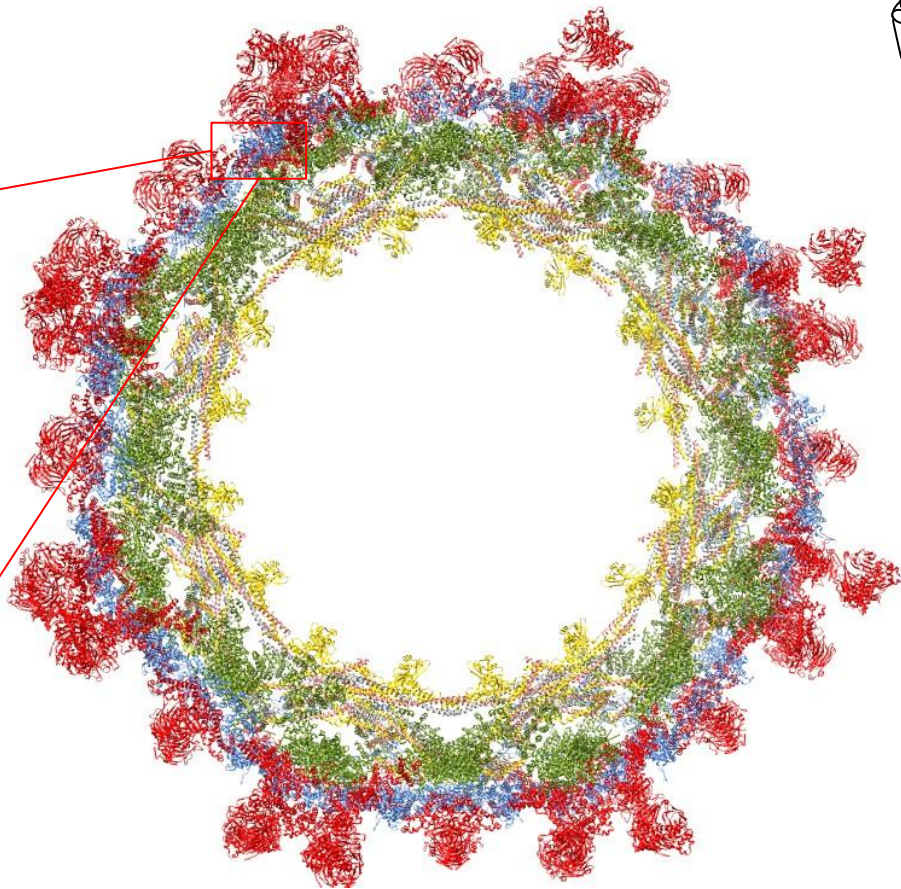
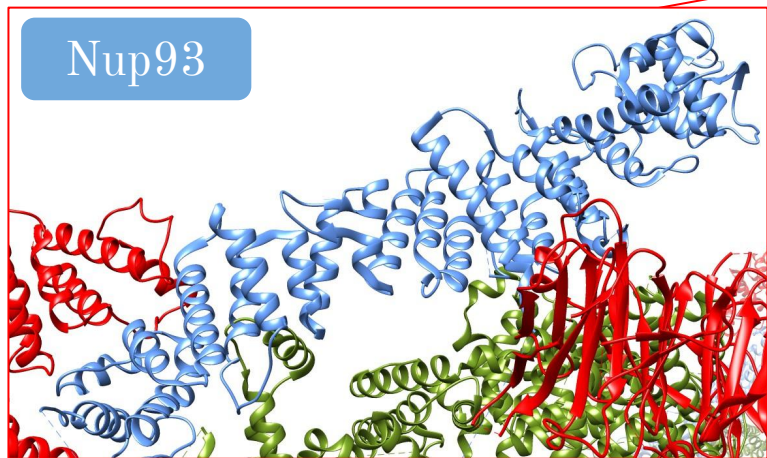
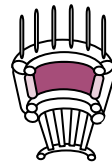
Folds

β -propellers

α -solenoid



2.2 Inner ring



Folds

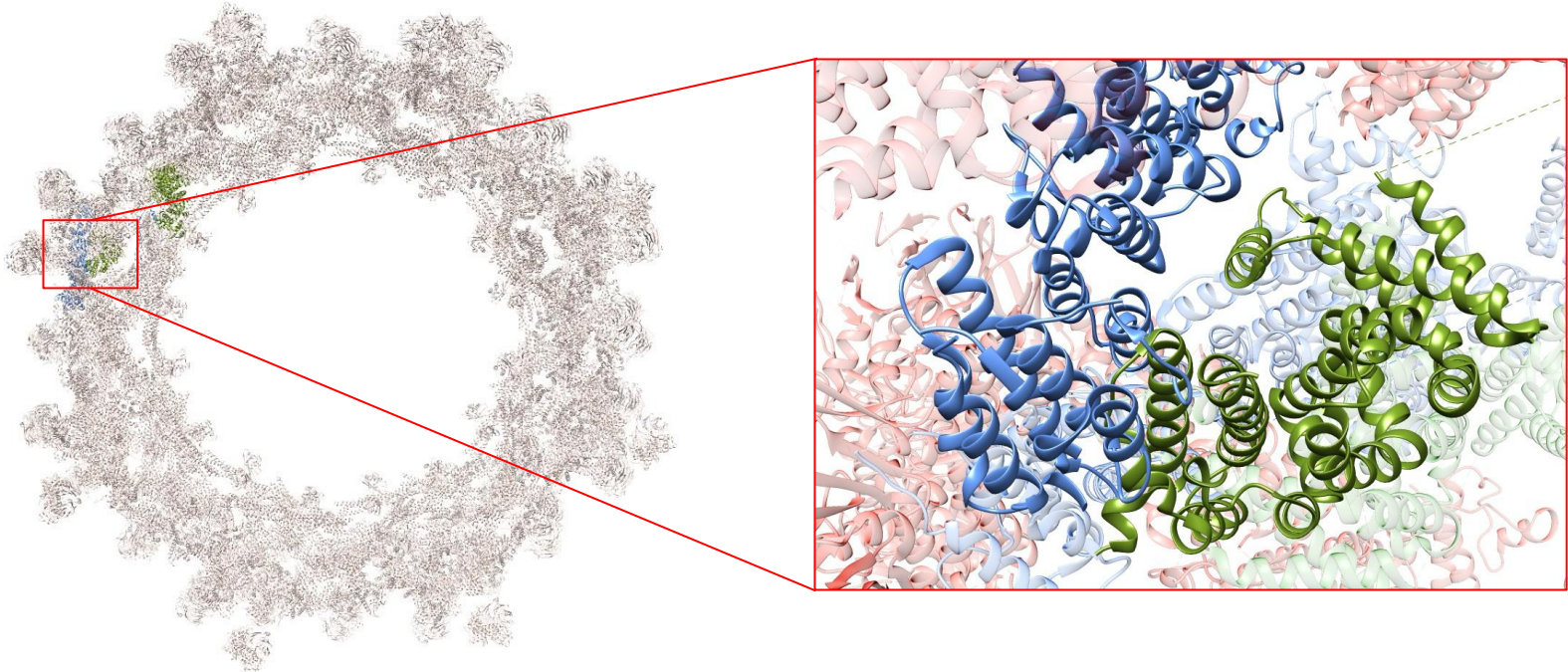
β -propellers

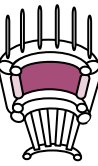
α -solenoid



2.2 Inner ring

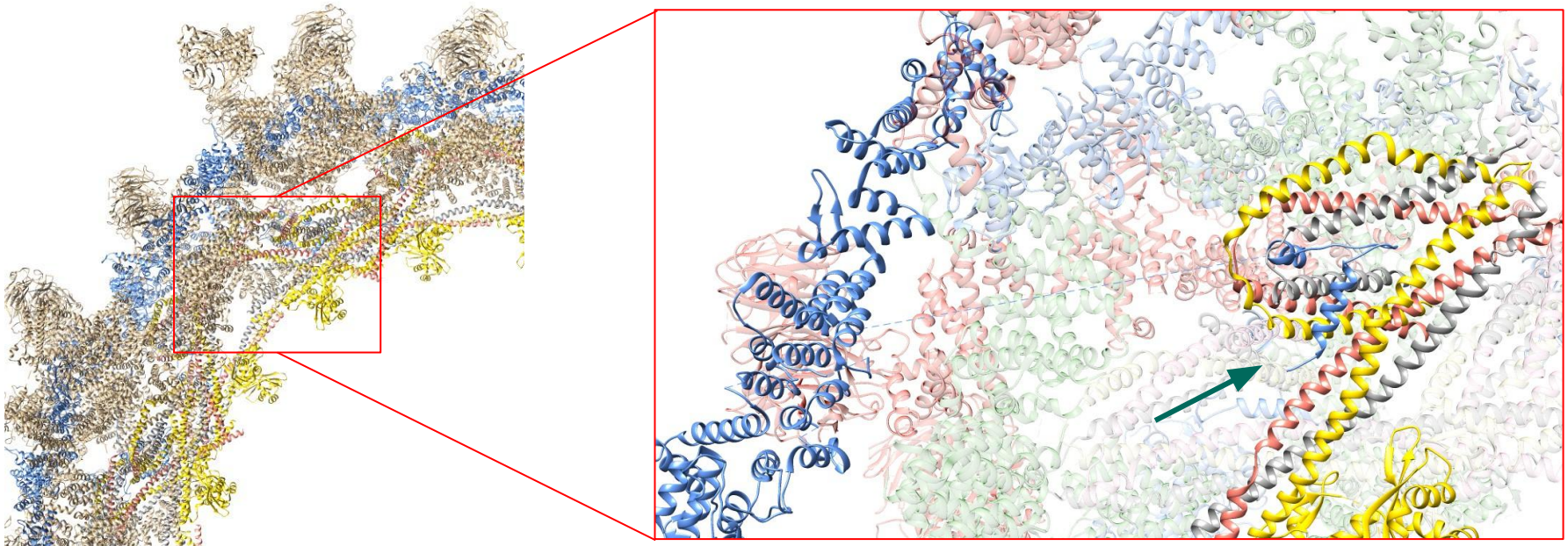
- Interaction between **Nup93** and **Nup205**

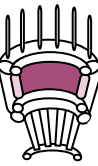




2.2 Inner ring

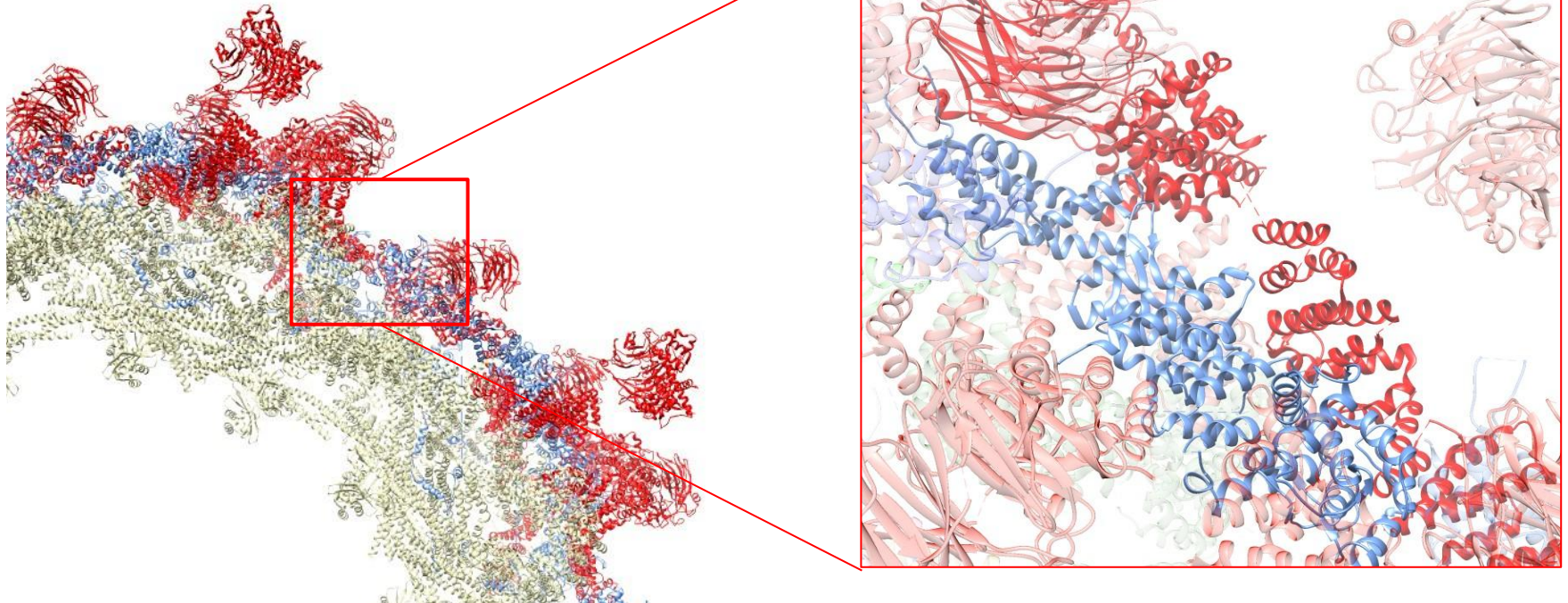
- Interaction between **Nup93** and the barrier proteins (**Nup54**, **Nup58**, **Nup62**)

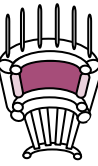




2.2 Inner ring

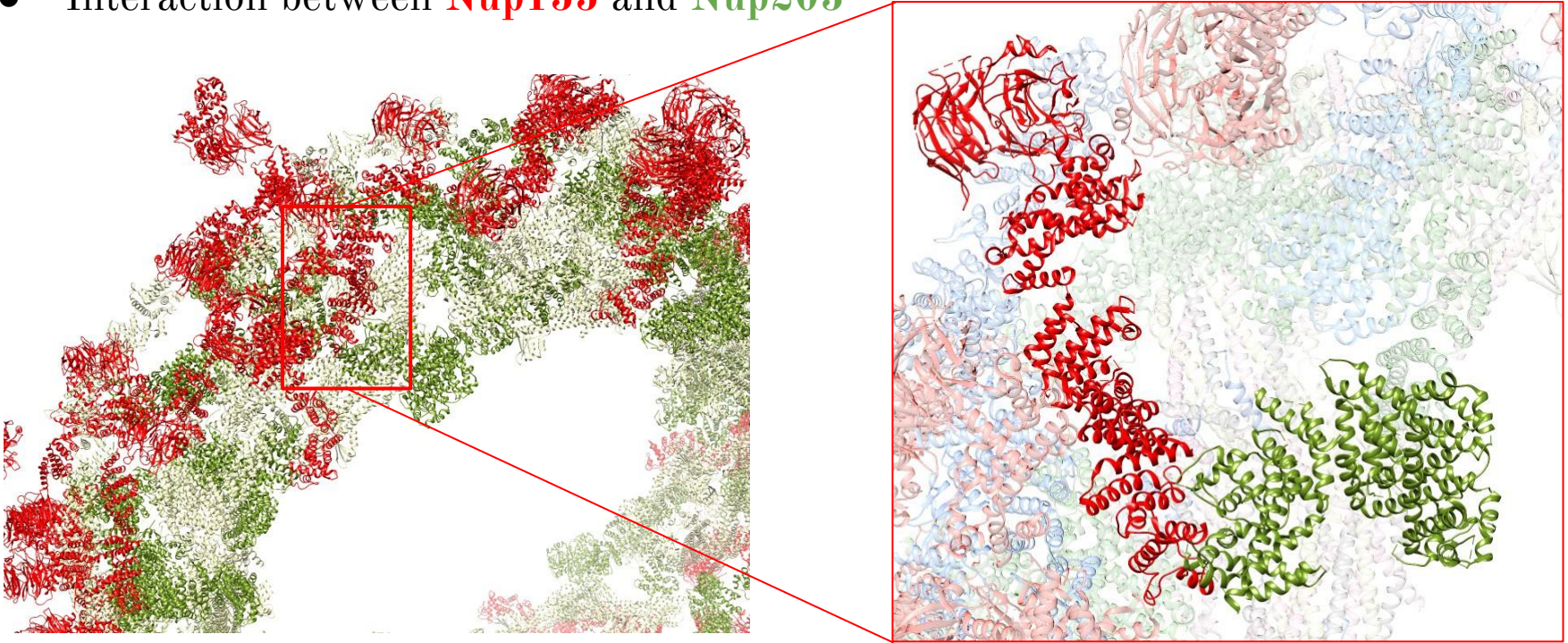
- Interaction between **Nup93** and **Nup155**





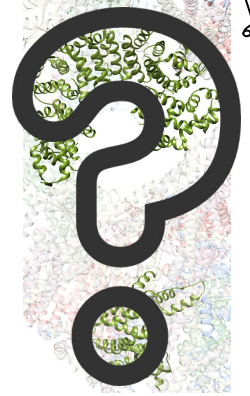
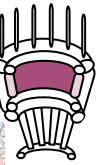
2.2 Inner ring

- Interaction between **Nup155** and **Nup205**

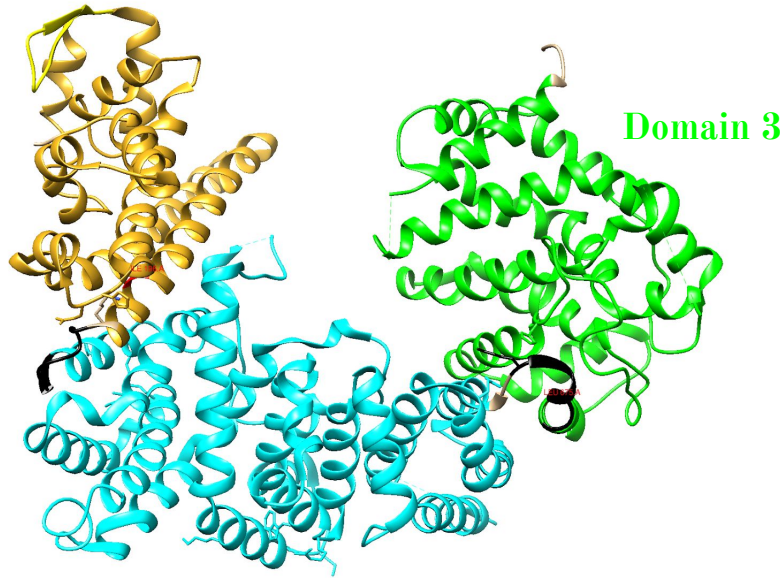


2.2.1 Nup192 (h205)

Crystallization of the “curved” part of the



Domain 1

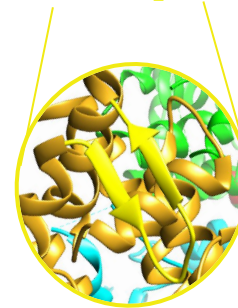


Domain 3

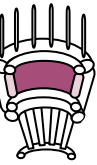
Domain 2

PDBid = 4IFQ

Alpha solenoid-like structure with stacked pairs of alpha helices and a short **β-hairpin** near the N-terminus



2.2.2 Nup192's flexibility



D1 is more mobile than the D2 and D3 domains



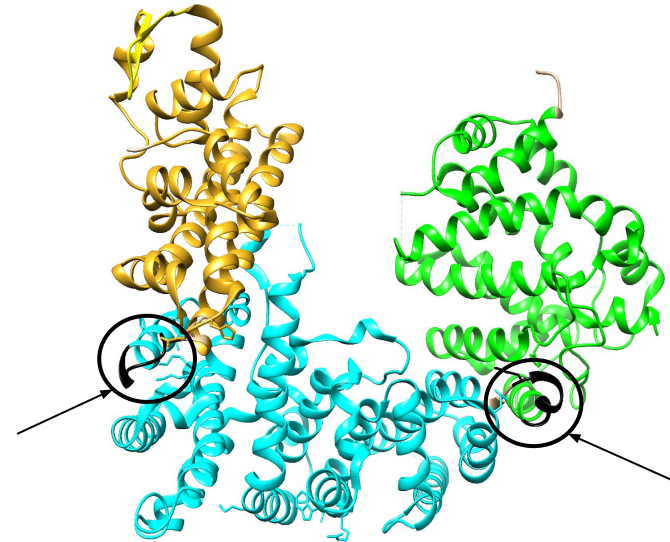
Possible conformational flexibility



Average B-factor:

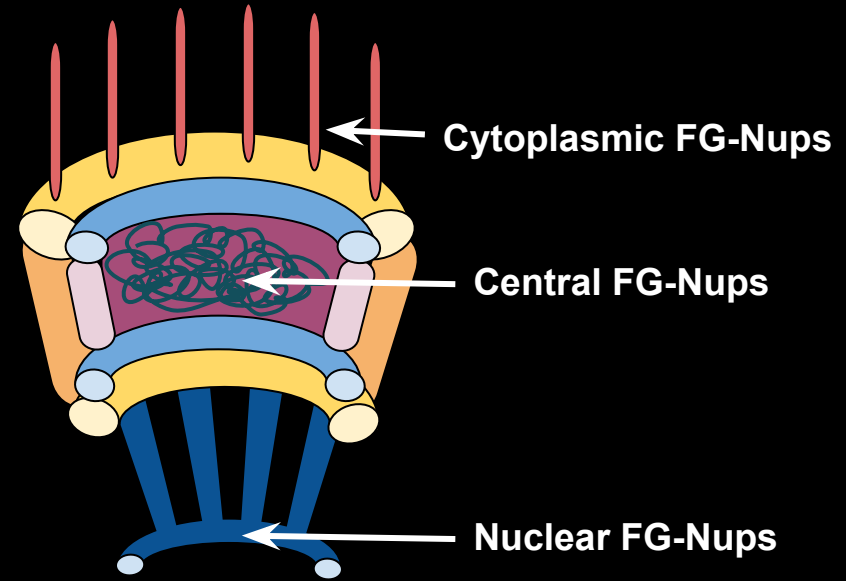
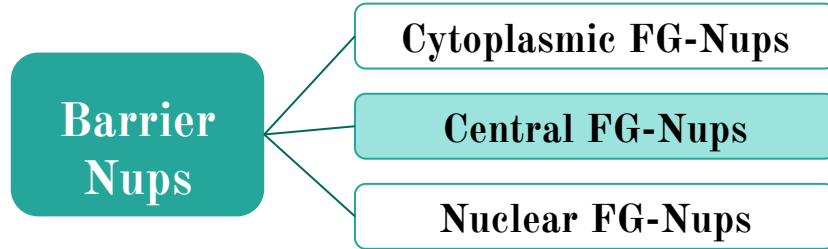
D1 $\Rightarrow$ **104 Å²** vs **74 Å²** of **D2** and **78 Å²** of **D3**

Hinge
region

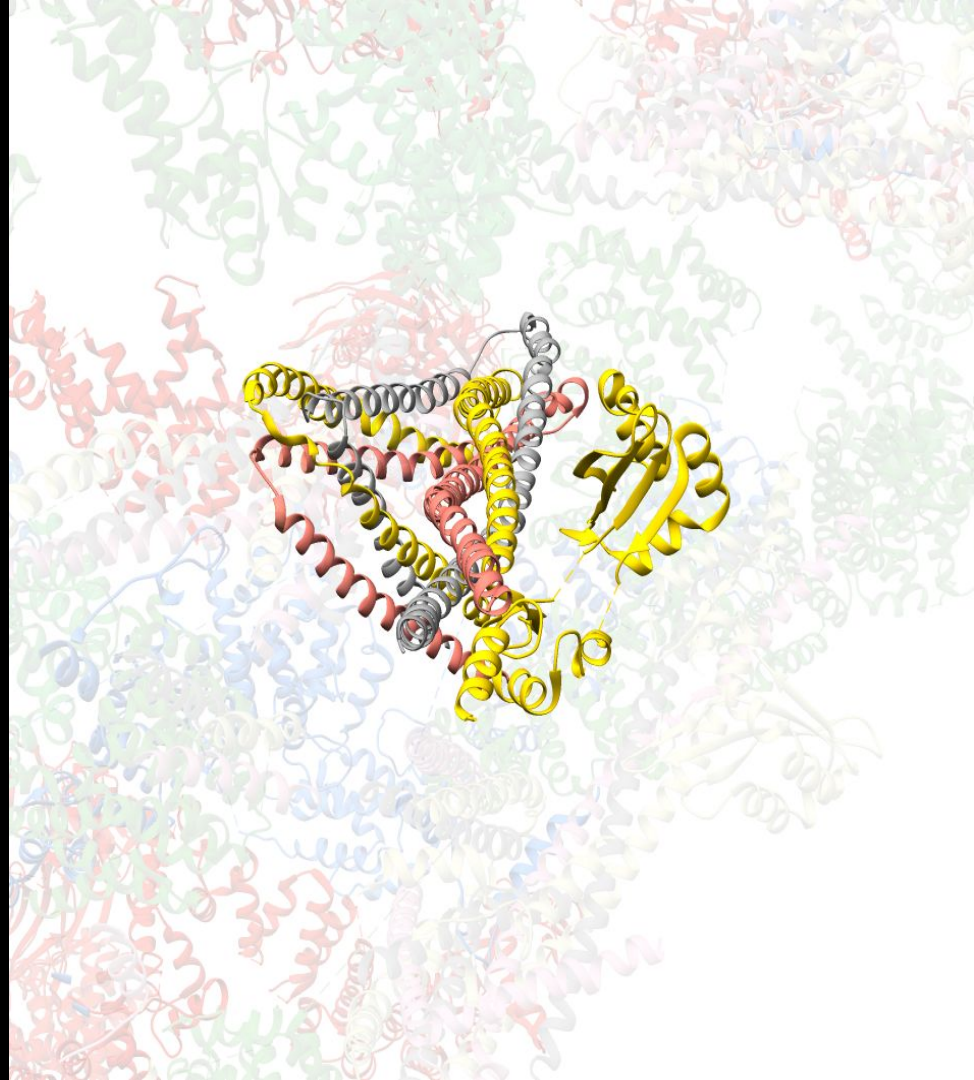


Hinge
region

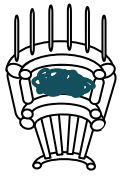
3. Barrier Nups



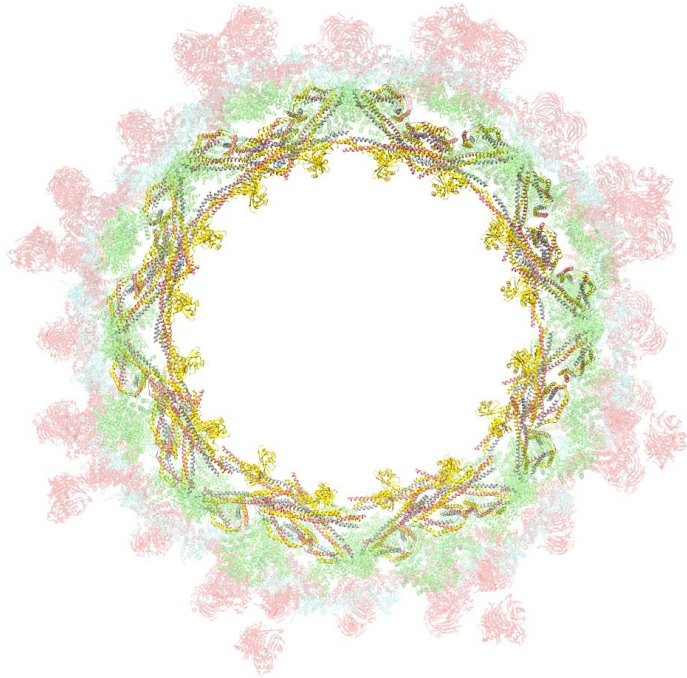
3.1 Nup54/58/62 complex



3.1.1 Central Transport Channel (CTC)



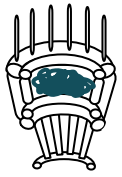
The CTC is one complex located in the central region of the NPC and is an important site for shuttling of specific cargoes across the NPC



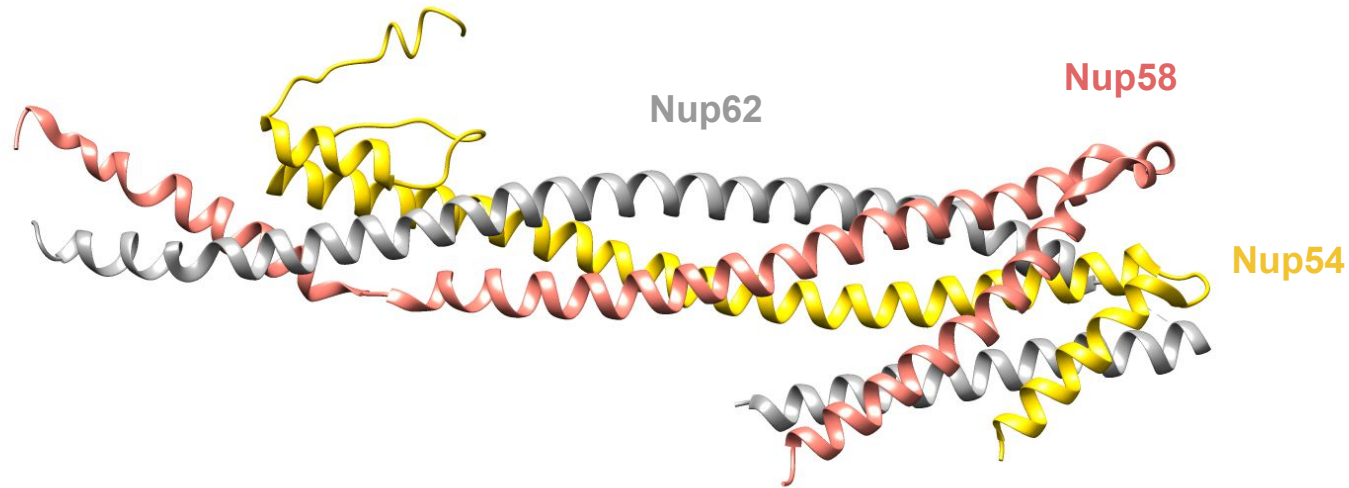
Nup54

Nup58 / (Nup45)

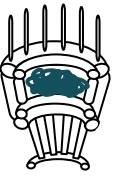
Nup62



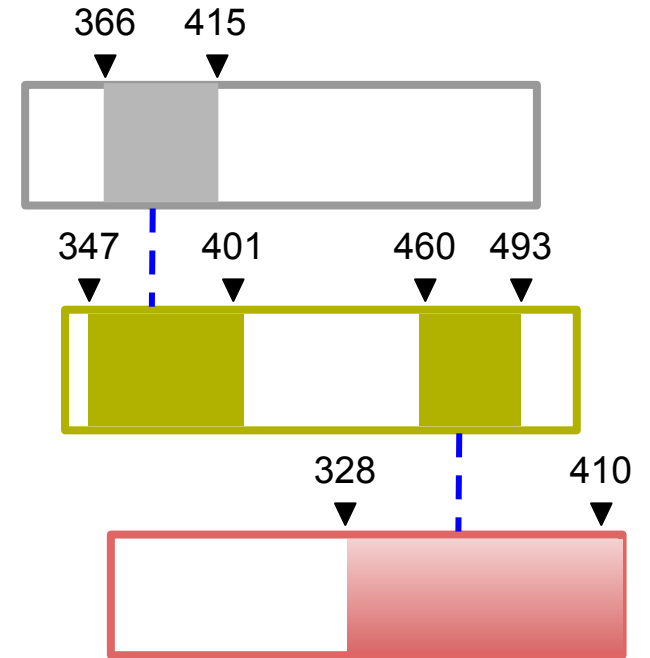
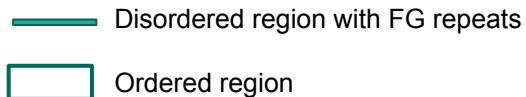
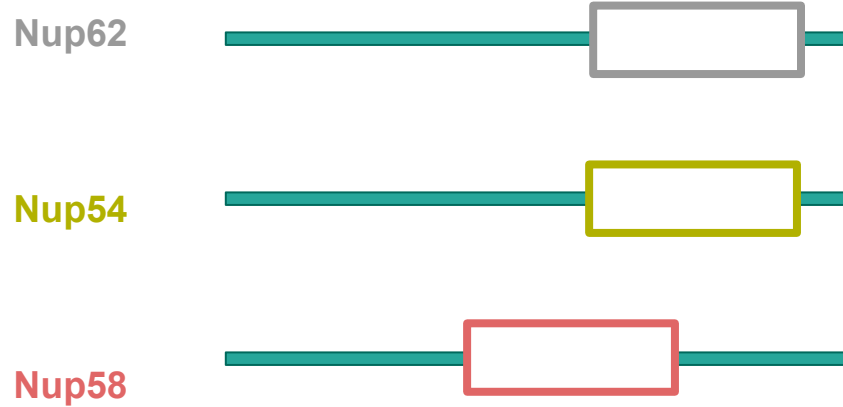
3.1.2 Nup54-Nup58-Nup62 complex



PDBid = 5C3L



3.1.3 FG-repeats



>Nup62: (*Rattus norvegicus*)

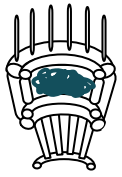
MSG**FNFG**GTGAPAGG**FTFG**TAKTATTTTATG**FSF**SASGTGTGG**FNFG**TPSQPAATTPSTSLFSLATQTSTTQTPG**FNFG**TTPASGGTGFS
LGISTPKLSLSSTAATPATANTGS**FG**LGSSTLTNAISGASTSSQGTAPT**G**FVFGSSTTSAPSTGTTG**FSF**TSGSASQPGASGFNIGSVGS
LAQPTALSGSPFTPATLATTTAGATQPAAATPTAATTSAGSTLFASIAAAPASSSTTVLSLSAPATTAATPTAGTLGFSLKAPGAAPGAS
TTSTTTTTTTTTTTASTSSSTTTTG**F**ALS**L**KPLVPAGPSSVAATALPASSTAVGTTTG**P**AMTYAQ**L**ESLINKWSLELEDQERHFLQ**Q**ATQ
V**NAWDR**TL**LIENGEKITS**LHREVEK**V**KLD**Q**KRLD**Q**ELD**F**IL**S**Q**Q**KELED**LLSP**LEESVKEQSGTIY**L**QHADAEREKTY**K**LAENIDA**Q**LKRM
AQDLKDII**E**HLNMAGGPADTSDPL**Q**QICKILNAHMD**S**LQWVDQSSALLQRRVEEASRVCE**S**RRKEQERS**L**RIAFD

>Nup54: (*Rattus norvegicus*)

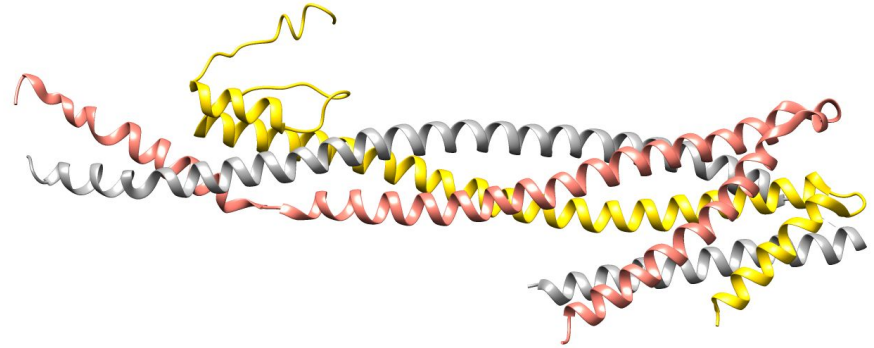
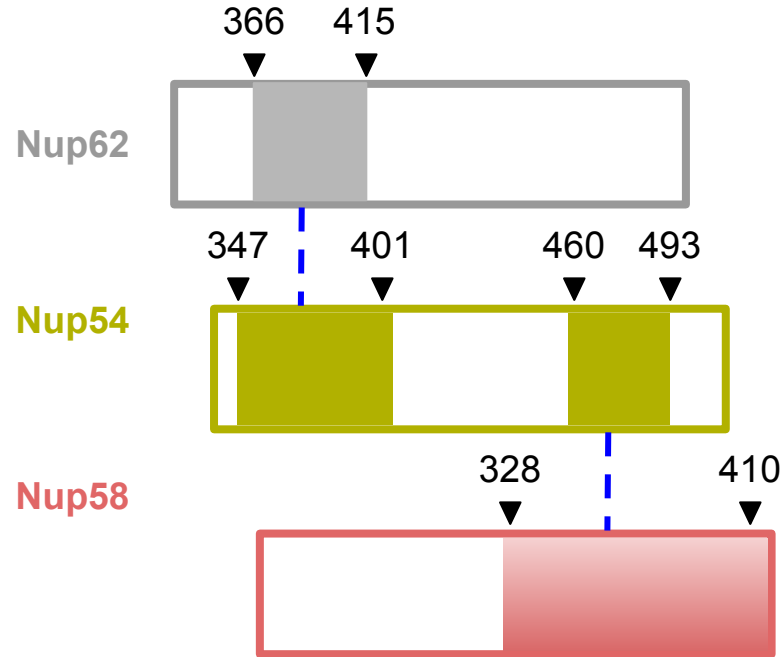
MA**FNFG**APSGTSGTSTATAAPAGG**FGG****FG**TTTTTTAGSA**FSF**SAPTNTGSTGLLG**T**QNK**G****FGFG**T**G****FG**TSTGTGTGLGTGLGTGL**FG**
GFNTQQQQQQQ**Q**TS**L**GG**L**FSQ**P**AQ**A**PAQ**S**N**Q**LINTASALSAPTLLGDERDAILAKWN**Q**LQAFWGTGKG**Y**FNNNI**P**PVEFTQEN**P**FCRF
KAVGYSCMPNNKDEDGLV**V**LI**F**NK**K**ETDIRSQQQ**Q**L**V**ESLHK**V**LGG**N**QTLTVN**V**EGIK**T**LPDDQTE**V**VIY**I**VERS**P**NGTSRRVPATTL
YAHFEQ**A**NIKT**Q**L**Q**Q**L**GV**T**LSM**T**RELS**P**AQ**I**K**Q**LLQ**N**PPAGVD**P**I**I**WEQAKVD**N**PDSE**K**L**I**P**V**PMVG**F**KELLRRLKV**Q**D**Q****MTKQHQT**
RLD**I**ISED**I**SEL**Q**KN**Q**TT**T**MA**K**IA**Q**Y**K**R**K**LM**D**LS**H**RTL**Q**V**L**IK**Q**E**I**Q**R**K**S**GY**A**I**Q**AE**E**EQ**L**RV**Q**LD**T**I**Q**GEL**N**APT**Q**F**K**GR**L**NEL**M**S**Q**
IRM**Q****N**H**F**GA**V**K**S**E**E**K**Y**Y**I**D**A**DL**L**RE**I**K**Q**HL**K****Q****Q**EG**L**SH**L**IS**I**IK**D**D**L**ED**I**KL**V**EHGLNET**I**HSRGG**V**FS

>Nup58: (*Rattus norvegicus*)

MAT**G****FSF**GS**G**TLG**S**TTVAPGGTGTG**S****G****FSF**GASSTPSVGL**N****FG**TLGSSATPASTSTSAS**G****FG**TGL**FG**SKPGTGFTLGGTSAGTTATTS**A**
STTG**F**SLG**F**SK**P**AASATPFALPVTSTTASGLTLSSALTSAPAASTGFTLNNLGATPATTTAASTGLSLGGALAGLGGS**L**FQSGNTATSG
LGQ**N**ALSLSLGTATPTSAASSEGLGGIDFSTSSDKKSDKTGTRPEDSKALKDENLPPVICQDVENLQKFVKEQ**Q**VQ**E**EISRMSSK**A**ML
KVQEDIKALK**Q**LLSLAASGLQ**R**NTLNIDKLK**L**ETAQELKNAEIALRTQKT**P**PGLQHENT**A****PADYFRVLVQQFEVQLQQYRQQIEELENH**
LATQANN**S**HIT**P**Q**D**LSM**A**M**Q**K**I**Y**Q**TF**V**ALAA**Q**LS**I**HENV**K**VLKE**Q**YLSY**R**K**M**FLGD**A**G**D**VF**E**ARRTEAKKWQ**N**APRVTTGTP**F**ST**M**P
NAAVAMAATLTQQQQPATGPQPSLGVS**FG**TP**FG**SGIGTGLQSSGLGSSNLGG**FG**TSS**G****FG**CGTTGAST**FGFG**T**D**K**P**SGSL**S**AG**FG**SS
STSG**FN****F**SNPGITASAGLT**FG**VSNPASAG**FG**TGGQLLQLKRPPAGNKR**G**KR

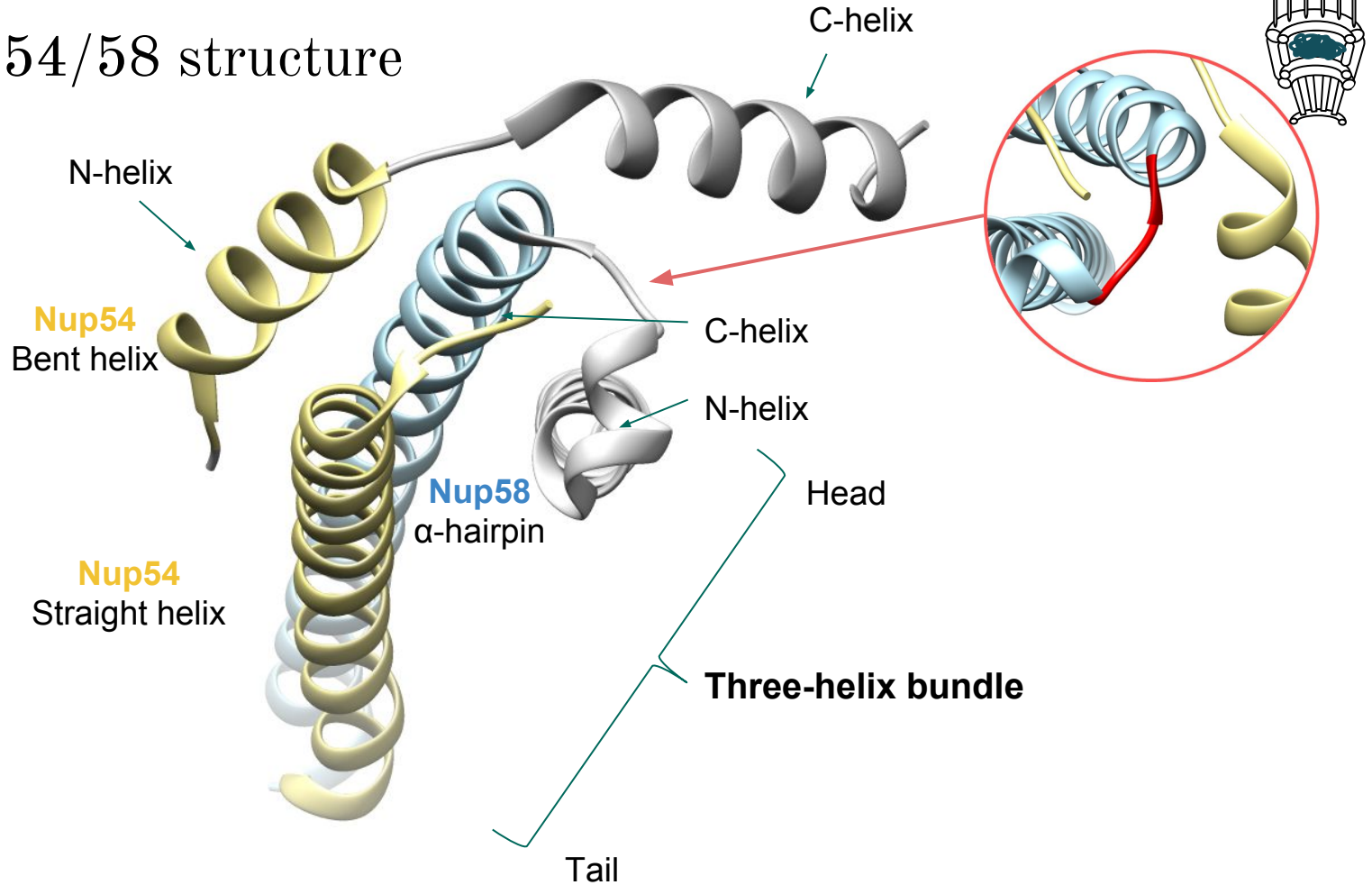


3.1.4 Nup54-Nup58-Nup62 complex

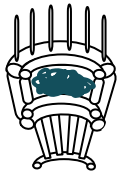
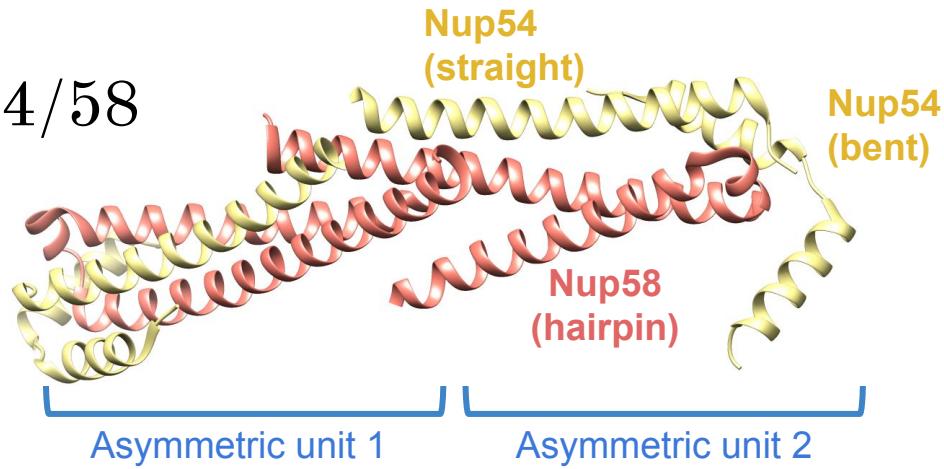


PDBid = 5C3L

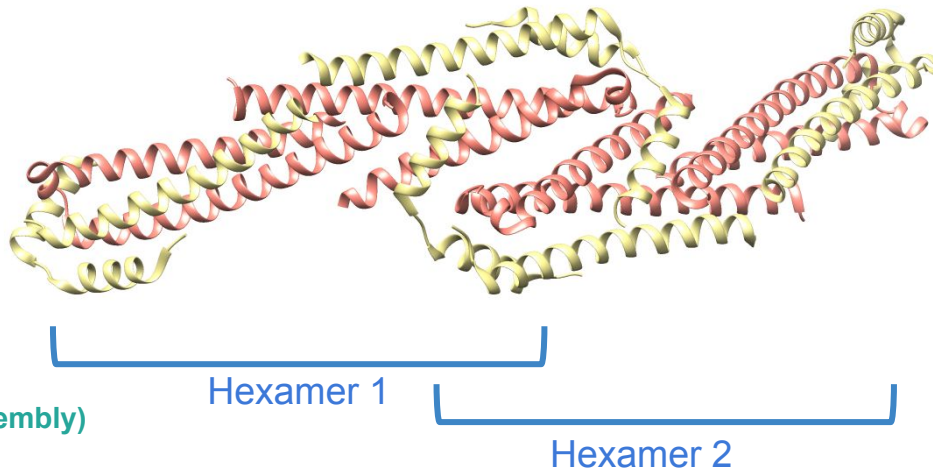
3.1.5 Nup54/58 structure



3.1.6 Nup54/58

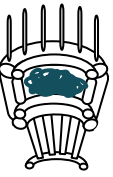


HEXAMER

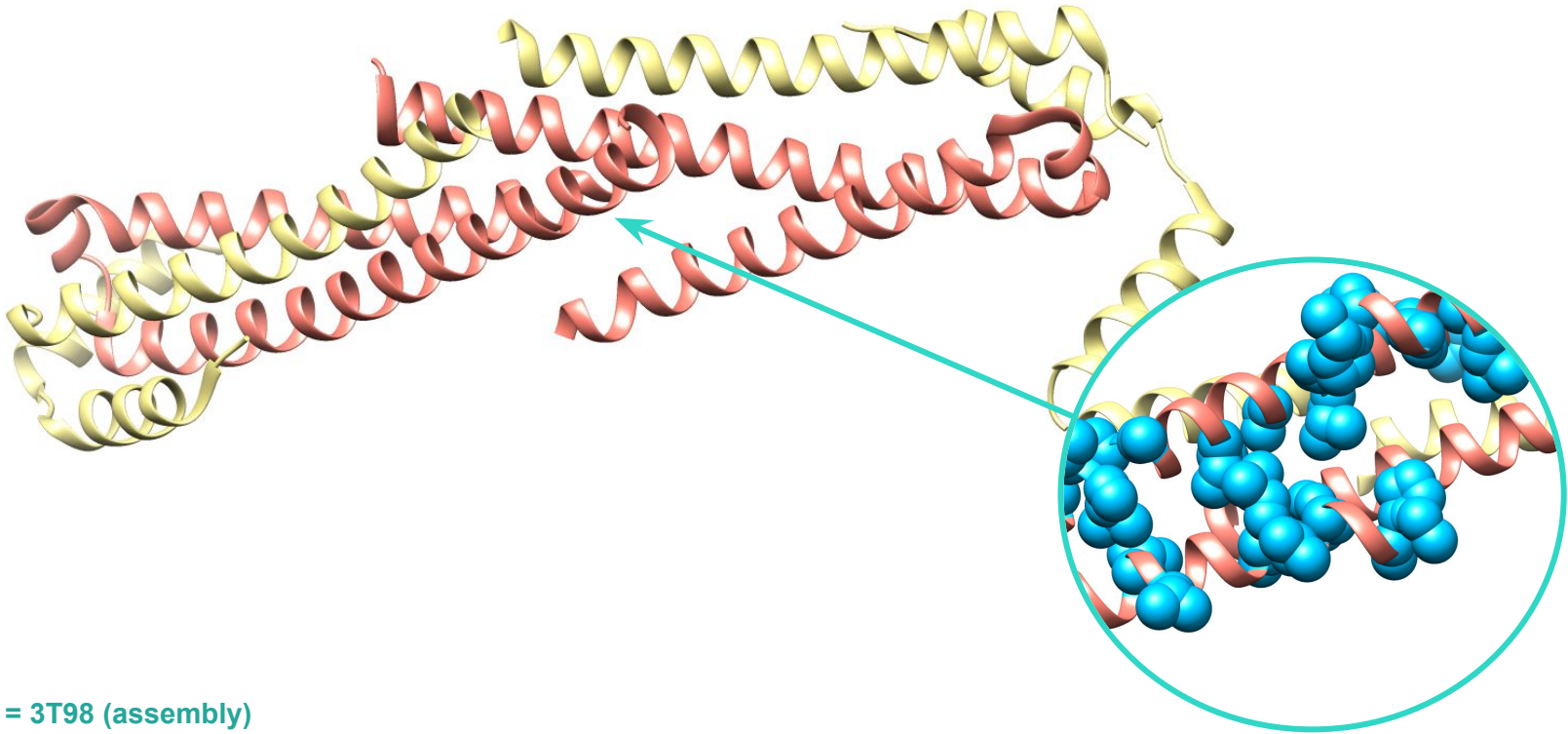


DODECAMER

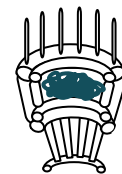
PDBid = 3T98 (assembly)



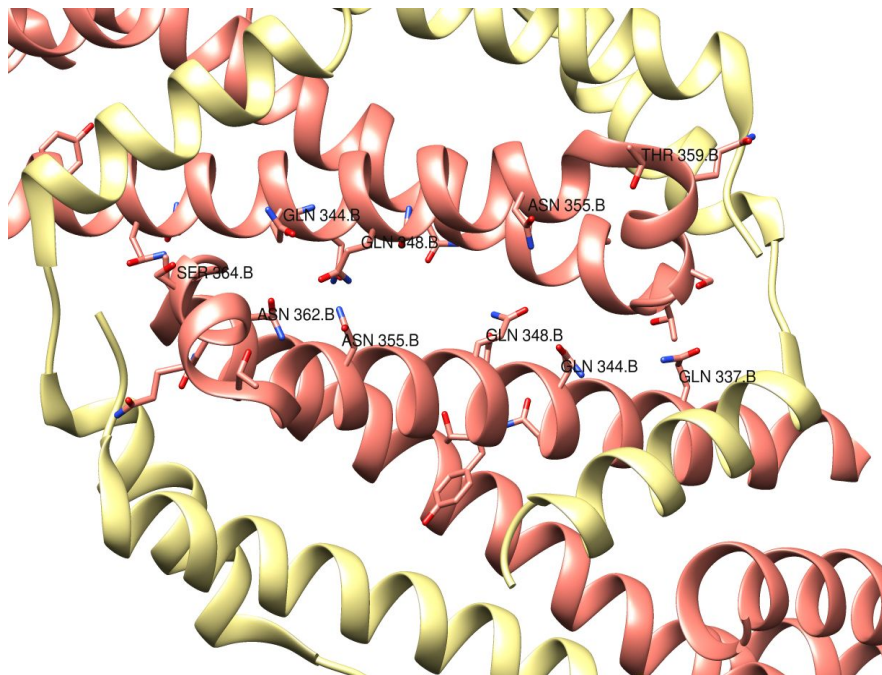
3.1.7 Hexamer interactions



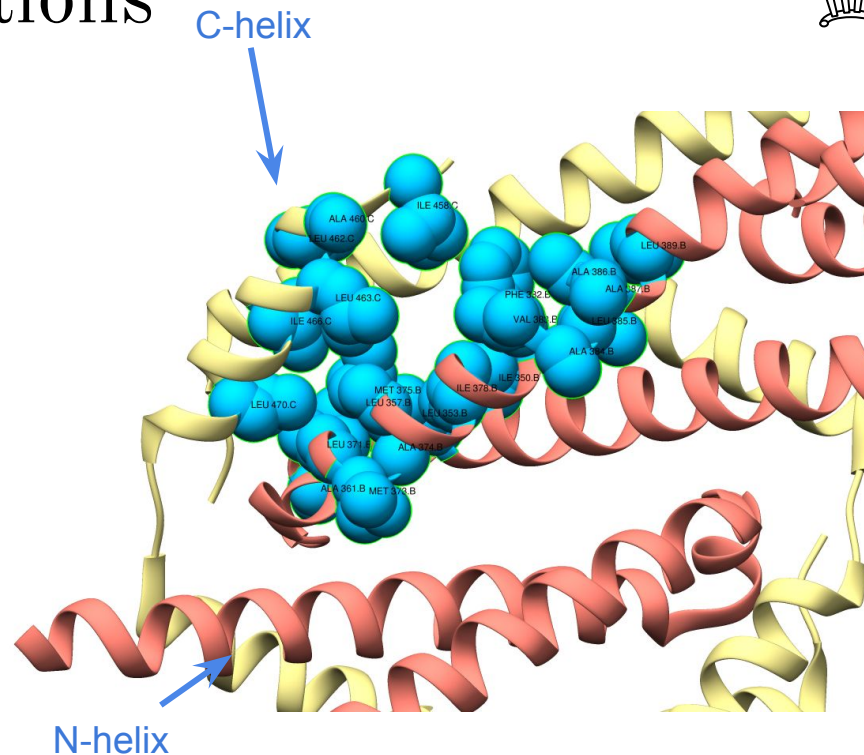
PDBid = 3T98 (assembly)



3.1.8 Dodecamer interactions

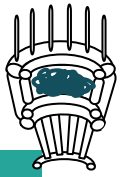


Polar interactions

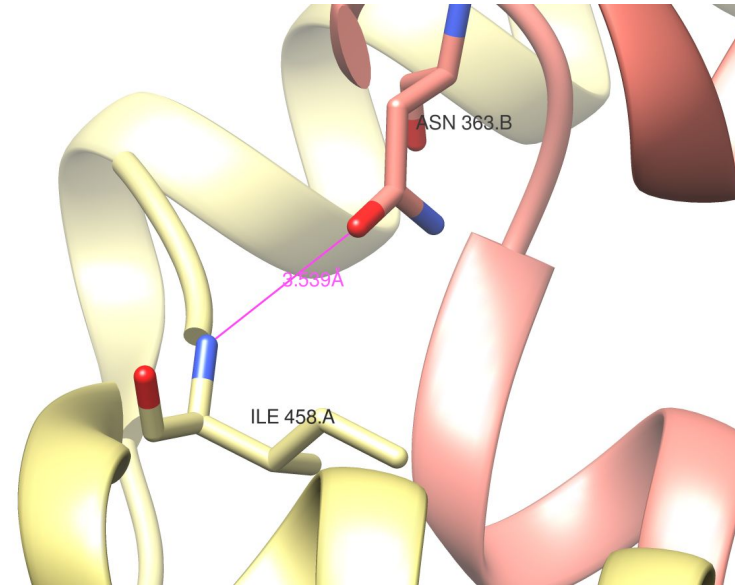
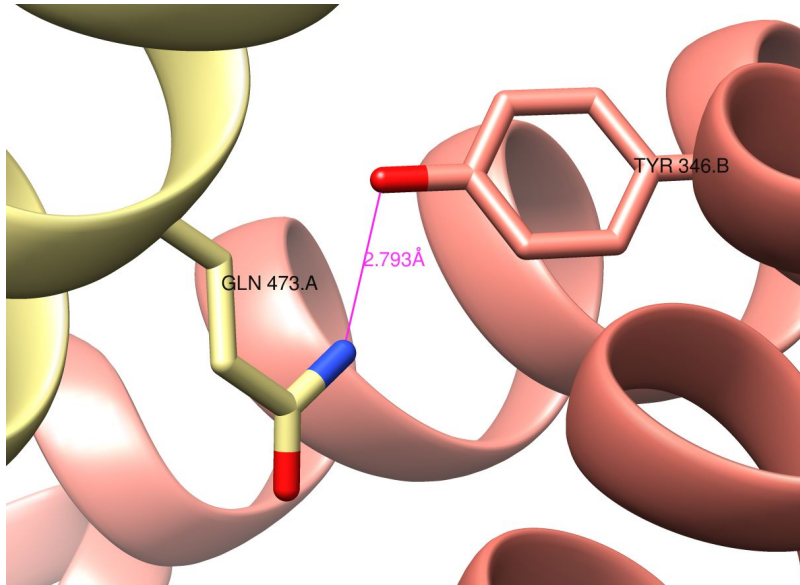


Non-polar interactions

3.1.9 **Nup54** - **Nup58** interaction



Hydrogen bonds / Salt bridges

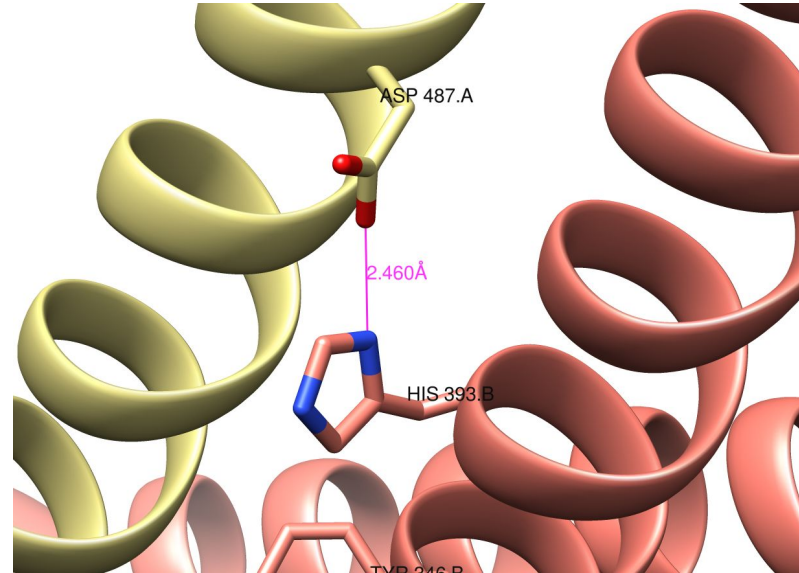
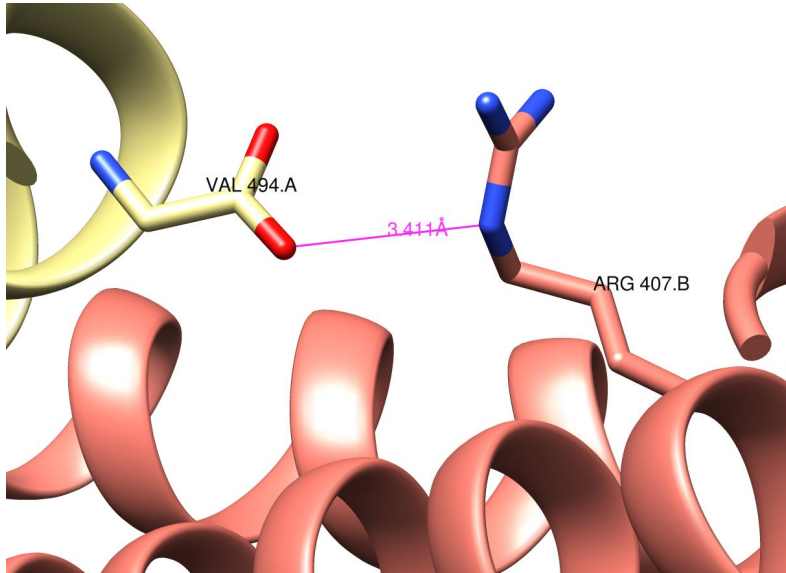


PDBid = 3T98

3.1.9 Nup54 - Nup58 interaction



Hydrogen bonds / Salt bridges

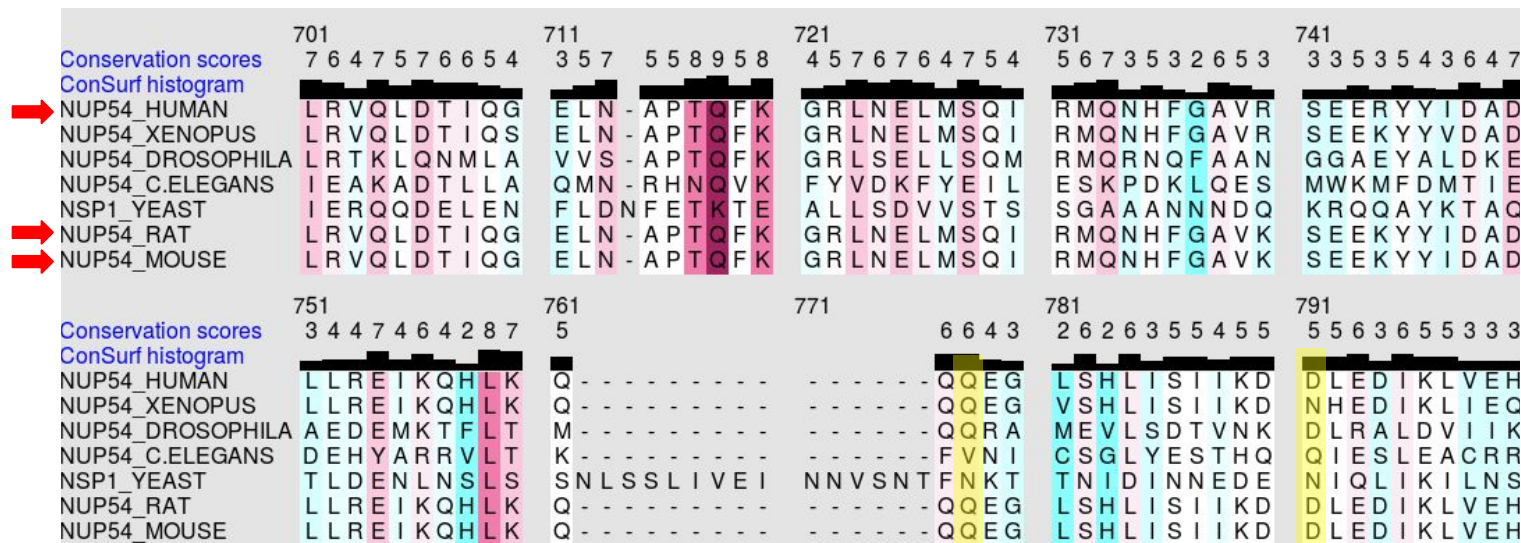


PDBid = 3T98

3.1.9 Nup54 - Nup58 interaction

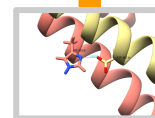
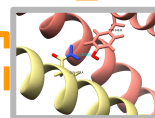


Nup54: ClustalW sequence alignment with ConSurf (all species)



1 2 3 4 5 6 7 8 9
Variable Average Conserved

GLN 473



Salt bridge

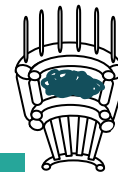
ASP 487



NUP54_MOUSE	TEVVIYVVVERSPNGTSRRVPATTLYAHFEQANIKAQQLQQLGVTL SMTRTELSPAQIKQLL
NUP54_RAT	TEVVIYIVERSPNGTSRRVPATTLYAHFEQANIKTQLQQLGVTL SMTRTELSPAQIKQLL
NUP54_HUMAN	TEVVIYVVVERSPNGTSRRVPATTLYAHFEQANIKTQLQQLGVTL SMTRTELSPAQIKQLL
	*****:*****:*****
NUP54_MOUSE	QNPPAGVDPIIWEQAKVDNPDSEKLIPVPMVGFKELLRRLKVQDQMTKQHQT RLDIISED
NUP54_RAT	QNPPAGVDPIIWEQAKVDNPDSEKLIPVPMVGFKELLRRLKVQDQMTKQHQT RLDIISED
NUP54_HUMAN	QNPPAGVDPIIWEQAKVDNPDSEKLIPVPMVGFKELLRRLKVQDQMTKQHQT RLDIISED

NUP54_MOUSE	ISELQKNQTTTMAKIAQYKRKLMDLSHRTLQVLIKQEIQRKSGYAIQADEEQ LRVQLDTI
NUP54_RAT	ISELQKNQTTTMAKIAQYKRKLMDLSHRTLQVLIKQEIQRKSGYAIQAE EEQ LRVQLDTI
NUP54_HUMAN	ISELQKNQTTSVAKIAQYKRKLMDLSHRTLQVLIKQEIQRKSGYAIQADEEQ LRVQLDTI
	*****: :*****:*****:*****
NUP54_MOUSE	QGELNAPTQFKGRNLNELMSQIRMQNHFGAVKSEEKYYIDADLLREIKQHLKQQQ EGLSHL
NUP54_RAT	QGELNAPTQFKGRNLNELMSQIRMQNHFGAVKSEEKYYIDADLLREIKQHLKQQQ EGLSHL
NUP54_HUMAN	QGELNAPTQFKGRNLNELMSQIRMQNHFGAVRSEERYIDADLLREIKQHLKQQQ EGLSHL
	*****.***.*****

3.1.9 Nup54 - Nup58 interaction



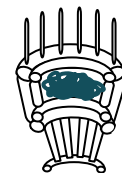
Nup58: PFAM family?

```
Query:          NUP58_HUMAN [L=599]
Scores for complete sequence (score includes all domains):
--- full sequence ---   --- best 1 domain ---   -#dom-
E-value  score  bias    E-value  score  bias    exp  N  Model          Description
-----
----- inclusion threshold -----
0.059    12.9    4.4      0.093    12.2    3.1      1.3  1  PBC              PBC domain
0.21     10.6    10.9     0.32     10.0    7.5      1.2  1  Exonuc_VII_L    Exonuclease VII, large subunit
1.4      8.6     7.5       3.6      7.3     0.9      2.8  3  COG2            COG (conserved oligomeric Golgi) complex compon
4.8      6.8     8.1       14       5.3     0.5      2.4  2  DUF1664         Protein of unknown function (DUF1664)
7.2      4.7    14.8       6       5.0     6.9      2.1  2  DUF342         Protein of unknown function (DUF342)
```



E-value too
high!

3.1.10 Nup62



Nup62: PFAM family and MSA

NPP11_CELEGANS	tlseeeikaglggndtkaff-----TALQEVVNSYHSEIAKQERVFH
NSP1_YEAST	eesstgkstadvkssdslklnSKPVE-LKPVS---LDNKTLDLVTWKTNQLTESASHFE
NUP62_DROSOPHILA	qgaapaptlstggafanlttETKTtDsSAVSTASQLSYHQLEEHINKWTFEEQEKVFT
NUP62_HUMAN	ipsntaaavtappgpaaag-----AAASSAMTYAQLES LINKWSLELEDQERHFL
NUP62_MOUSE	kplvsagpssvaatalpass---TA--AGTATGPAMTYAQLES LINKWSLELEDQERHFL
NUP62_RAT	kplvpagpssvaatalpass---TA-VGTTTGPAMTYAQLES LINKWSLELEDQERHFL
NUP62_XENOPUS	kaplagtivqanavgsaaat-----G-IS--TATAMTYAQLEN LINKWSLELEDQEKHFL
NPP11_CELEGANS	NKMLELNAYDRELITLPEKVLGLYNEMDDLSGSCKLHFNVASMTSVLNDIEQNVVELEN
NSP1_YEAST	QYTKKINSWDQVLVKGGEQISQLYSDAVMAEHSQNKIDQSLQYIERQQDELENFLDNFET
NUP62_DROSOPHILA	EQATQINAWDKLLISNNGKIVELNDVAVKKVKTDDQVLDQELEFIATQQKELED SLGPLEK
NUP62_HUMAN	QQATQVNAWDRTLIENGEKITS LHREVEKVKLDQKRLDQELDFILSQQKELEDLLSPLEE
NUP62_MOUSE	QQATQVNAWDRTLIENGEKITS LHREVEKVKLDQKRLDQELDFILSQQKELEDLLSPLEE
NUP62_RAT	QQATQVNAWDRTLIENGEKITS LHREVEKVKLDQKRLDQELDFILSQQKELEDLLSPLEE
NUP62_XENOPUS	QQATQVNAWDRTILMQNGERITTLHREMEKVKLDQKRLDQELDFILSQQKELEDLLTPLEE
NPP11_CELEGANS	KLS-----lpewhtldykfpldsrffasrhdvqrvqiaqmm lnvdsqmkcad
NSP1_YEAST	KTEALLSDVVSTSS---gaaannndqkrqqayktaqtldenlnslssnlssliveinnvs
NUP62_DROSOPHILA	EFVNLPR-----vdmersqtylmvenldtqlkqmsedlkeiidnlneankgqdt
NUP62_HUMAN	LVKEQSGTIY LQHADEErektyklaenidaqlkrmaqdlkdiiehlntsgapadtsdplq
NUP62_MOUSE	SVKEQSGTIY LQHADEErektyklaenidaqlkrmaqdlkdiiehl nmaggp adtsdplq
NUP62_RAT	SVKEQSGTIY LQHADEErektyklaenidaqlkrmaqdlkdiiehl nmaggp adtsdplq
NUP62_XENOPUS	SVKEQSGTIY LQHADEErektyklaenidaqlkrmaqdlkeviehlnt sagpg dasnplq

 Coiled coil Nup62 available in PDB

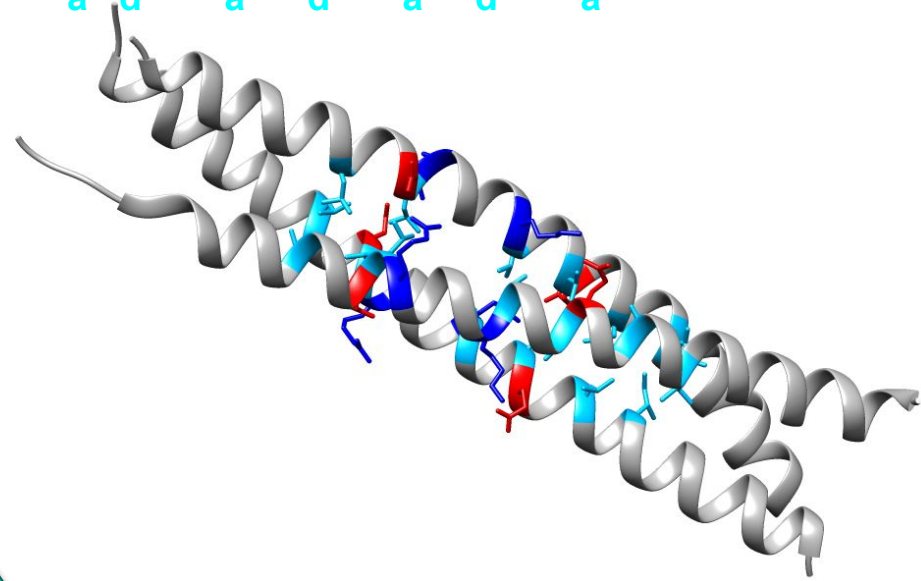
- PFAM Family: Nsp1_C (PF05064.8)



3.1.11 Nup62 Homotrimer

>5H1X: (*Rattus norvegicus* Nup62 coiled-coil motif)

NAWDRTLIEGKITS^aLHRE^dVEK^aVKLDQKRL^dDQEL^aDFILSQQKELEDLLSP



SALT BRIDGES	
- charge	+ charge
E381	K386
D393	R391

NON-POLAR RESIDUES	
Position "a"	Position "d"
I375	L378
V382	V385
L396	L392



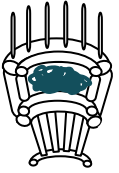
Interface of hydrophobic residues

- Exception of one glutamine (Q389)

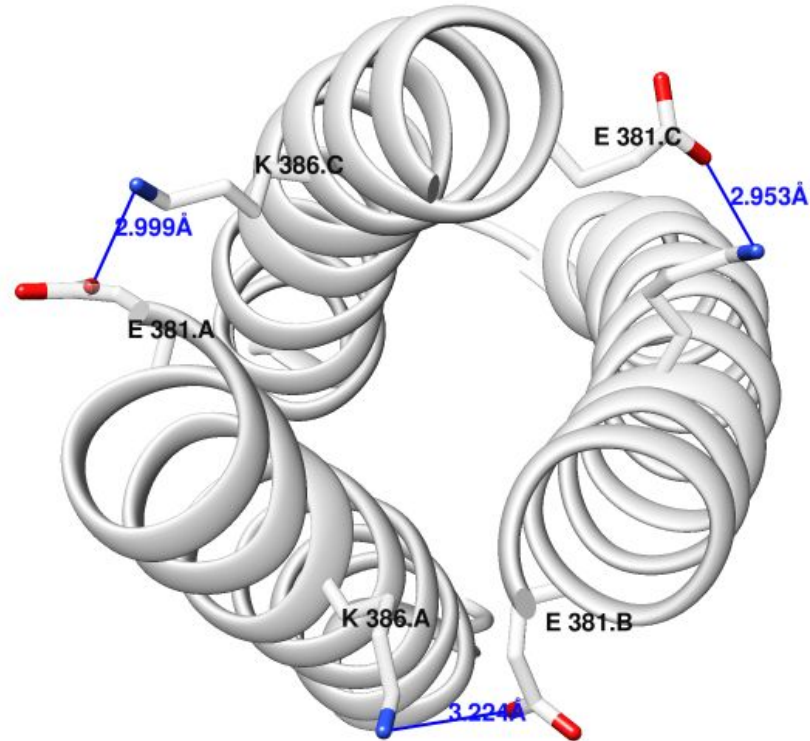
3.1.11 Nup62 Homotrimer

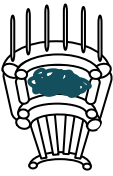
>5H1X: (*Rattus norvegicus* Nup62 coiled-coil motif)

NAWDRTLIEGKITSLSLHREVEKVKLDQKRLDQELDFILSQQKELEDLLSP



SALT BRIDGES	
- charge	+ charge
E381	K386
D393	R391

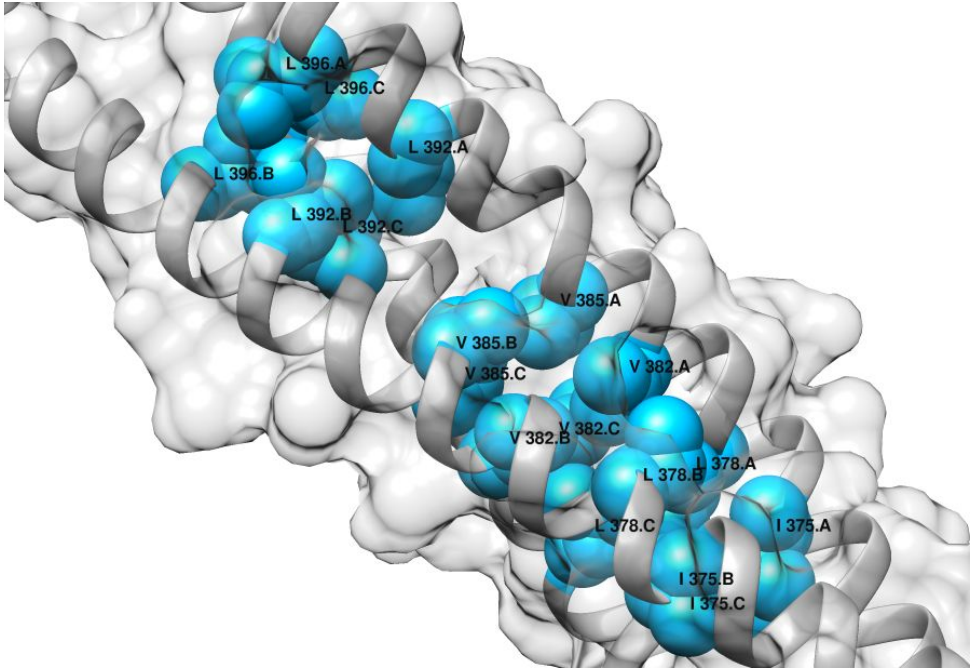




3.1.11 Nup62 Homotrimer

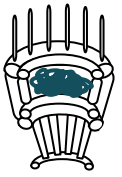
>5H1X: (*Rattus norvegicus* Nup62 coiled-coil motif)

NAWDRTLIEGKELITSLHREVEKVKLDQKRLDQELDFILSQQKELEDLLSP
a d a d a d a

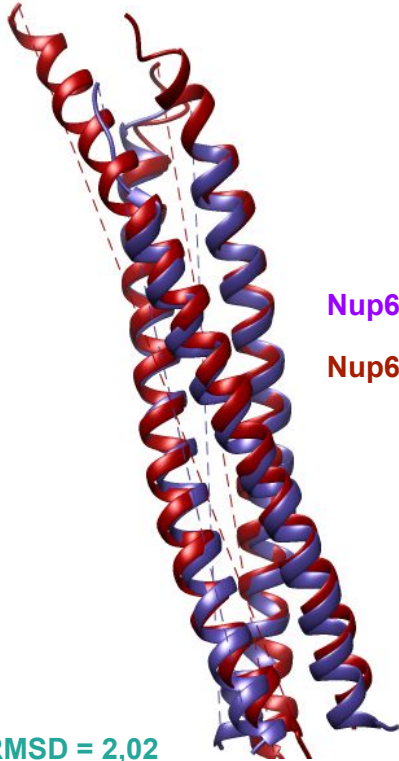


NON-POLAR RESIDUES	
Position "a"	Position "d"
I375	L378
V382	V385
L396	L392

3.1.12 Nup62 homotrimers and heterotrimers

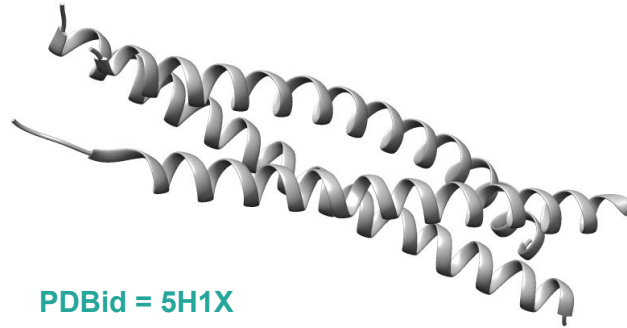


- The coiled-coil motif present in Nup62 imparts the ability to form a homotrimer and heterotrimers

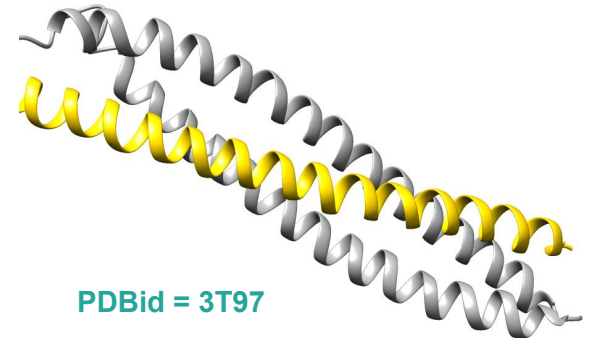


Nup62 homotrimer (5h1x)

Nup62/Nup54 heterotrimer (3t97)



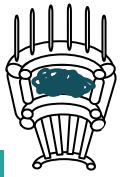
PDBid = 5H1X



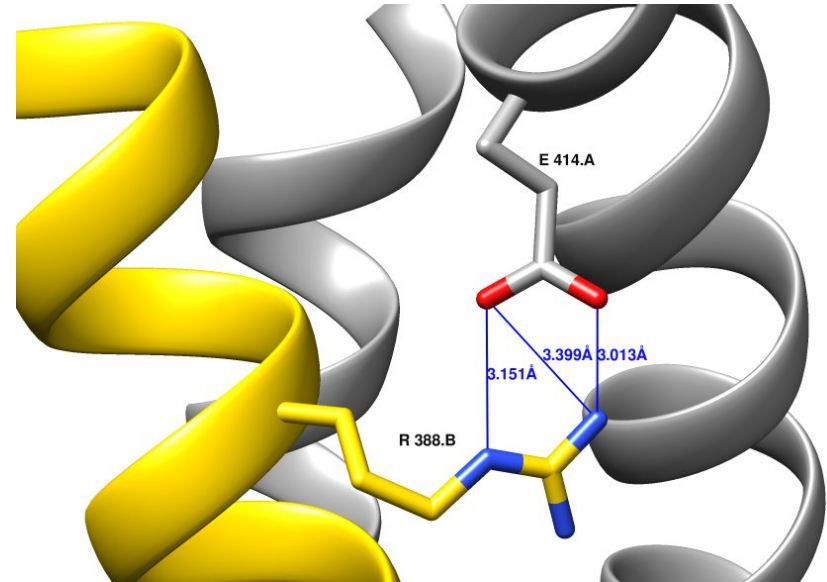
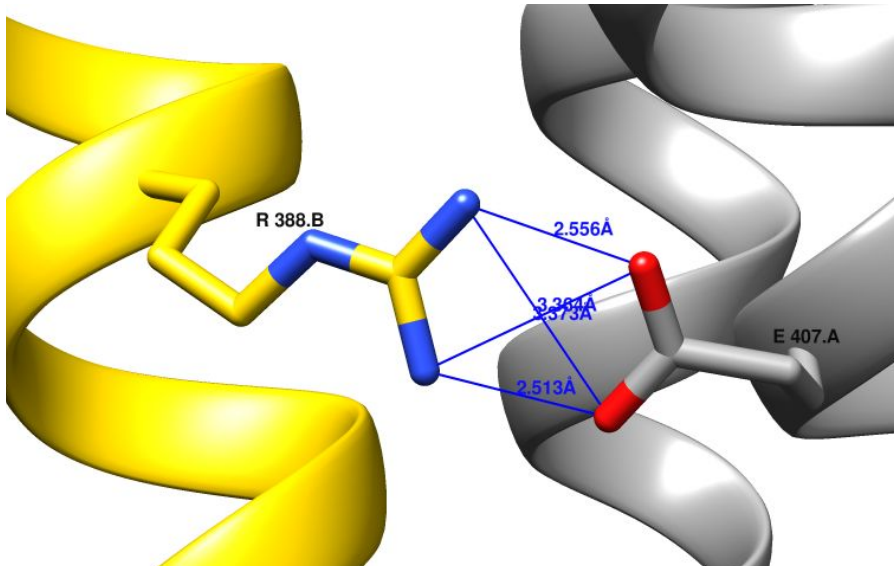
PDBid = 3T97

RMSD = 2,02

3.1.13 Nup54 - Nup62 interaction

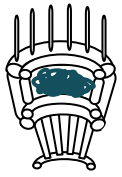


Hydrogen bonds / Salt bridges

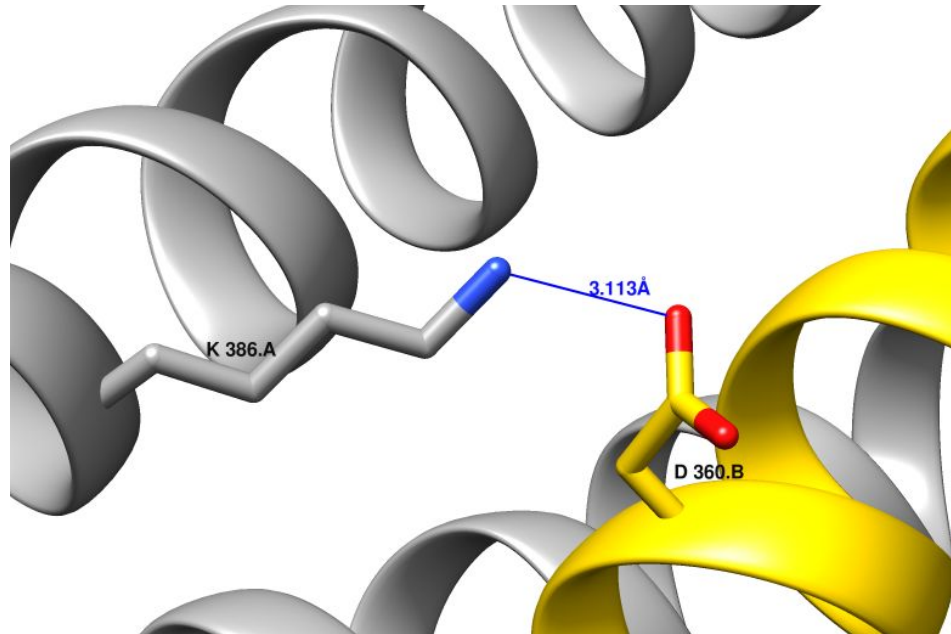


PDBid = 3T97

3.1.13 **Nup54** - Nup62 interaction

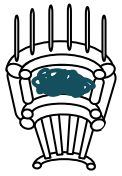


Hydrogen bonds / Salt bridges



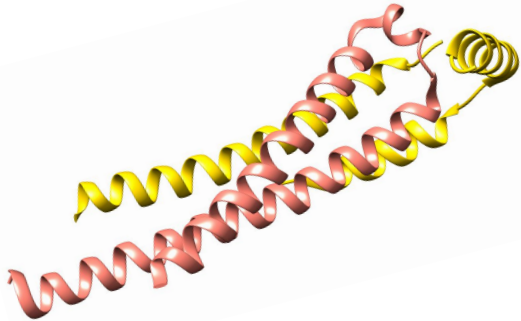
PDBid = 3T97

3.1.14 Nup54-Nup58-Nup62 complex



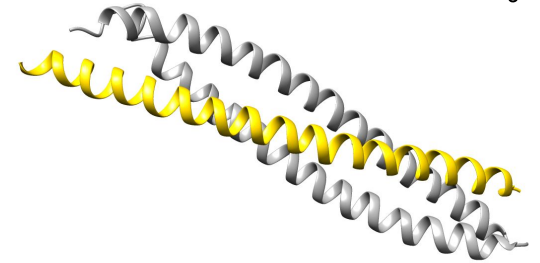
4:2:1
Nup62 /Nup54 /Nup58

Nup54 - Nup58:
mid-plane ring

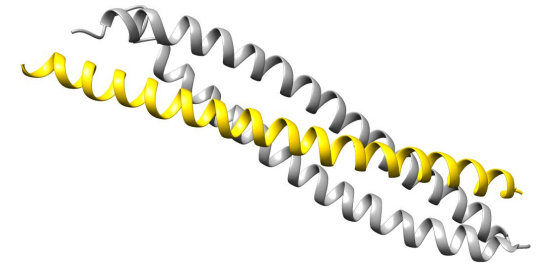


PDBid = 3T98

Ring
cycle
model

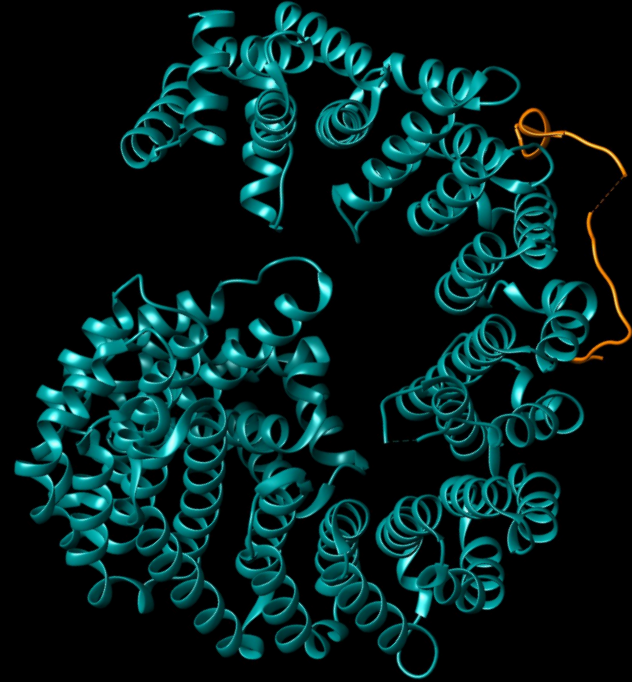


Nup54 - Nup62 :
fingers



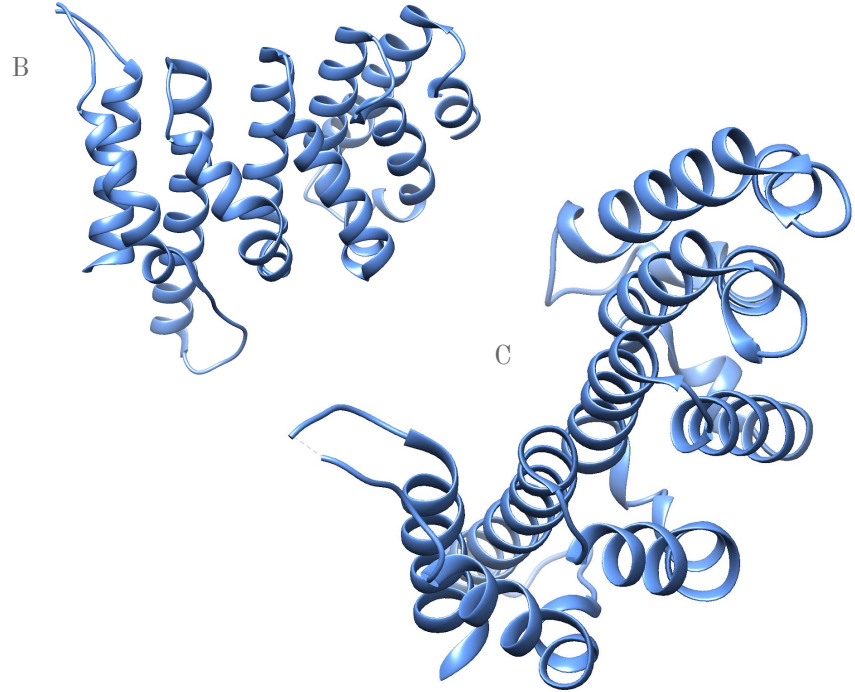
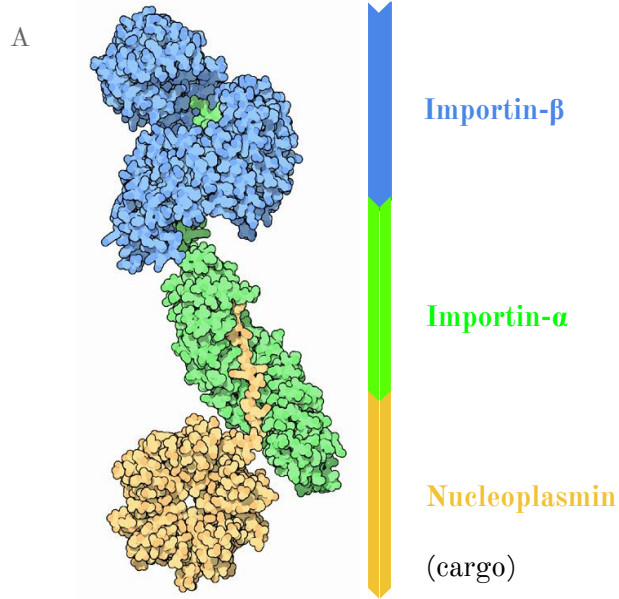
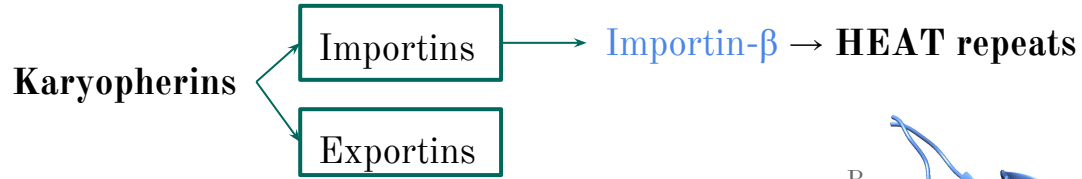
PDBid = 3T97

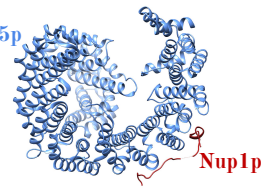
3.2 Karyopherins & Barrier Nups



—

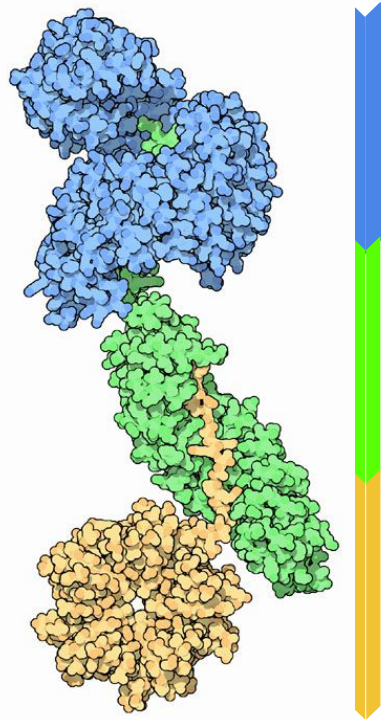
3.2 Barrier Nup and Karyopherin





3.2 Kap95p and Nup1p (*Saccharomyces cerevisiae*)

A

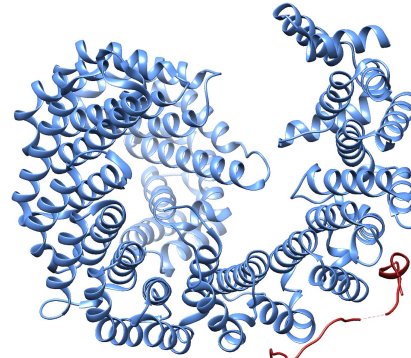


Importin-β →

Importin-α

Nucleoplasmin (cargo)

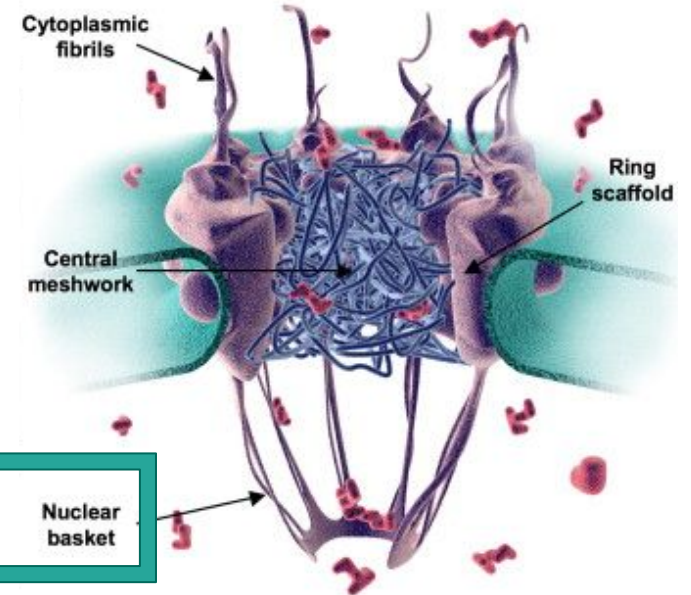
B

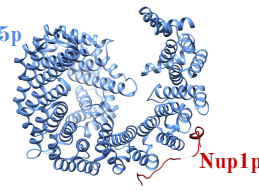


PDBid = 5OWU

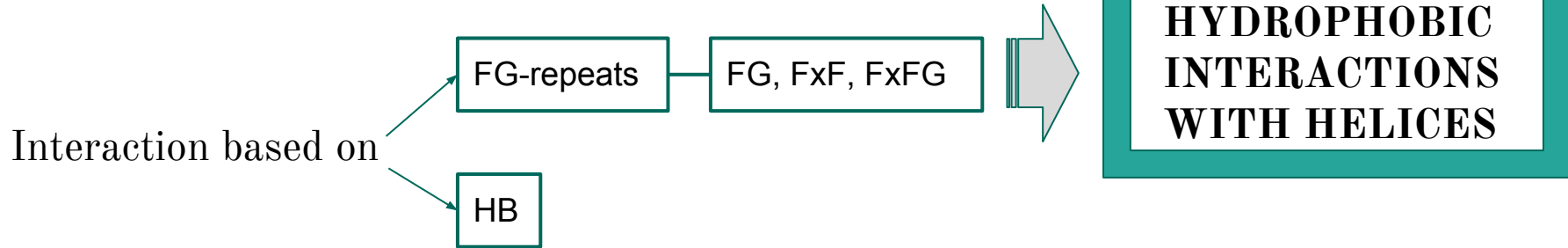
Nup1p →

C

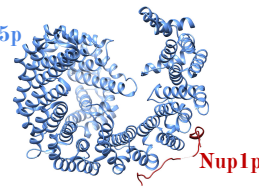




3.2 Kap95p and Nup1p (*Saccharomyces cerevisiae*)



LLLKGPASTVAKTAPIQESFVPNSERSGTPTLKKNIEPKKDKESIVLPTV**EDF**IKDNETPSKKTSPKATSSAGAVFKSSVEMGKTD
 KSTKTAEAPTLS**FNFS**QKANKTKAVDNTVPSTTI**FNFG**GKSDTVTSASQ**FKFG**KTSEKSENHTESDAPPKSTAP**FSFG**KQEENG
 EGDDENEPKRKRRLPVSEDTNTKPI**FDFG**KTGDKQETKKGESEKDASGKPS**FVFG**ASDKQAEGTPI**FTFG**KKADVTSNIDSSAQ**FTF**
GKAATAKETHTKPSETPATIVKKPI**FTFG**QSTSENKISEGSAKPI**FSF**SKSEEERKSSPISNEAAKPS**FSF**PGKPVQAPTDKTL
 KPT**FSF**TEPAQKDSSVSEPKKPS**FTF**ASSKTSQPKPI**FSFG**KSDAAKEPPGSNTS**FSF**TKPPANETDKRPTPPS**FTFG**GSTTNNTT
 TTSTKPS**FSFG**APESMKSTASTAAANTEKLSN**FSF**TKFNHNKEKSNSPTSFFDGSASSTPIPVLGKPTDATGNTTSKS**FSFG**TAN
 TNGTNASANSTS**FSF**NAPATGNGTTTTNTSGTNIAGTFNVGKPDQSIASGNTNGAGS**EGF**SSSGTAATGAASNQSS**FNFG**NNGAG
 GLNPFTSATSSTNANAGLFNKPPSTNAQNVNPS**FNFT**GNNSTPGGGSVFNMNGNTNANTVFAGSNNQ**F**⁹⁶⁴**HQSQT**PSFNTNSSFT
 PSTVPNINFSGLNGGITNTATNALRPSD**FG**ANAASGSNSNVTNPSS**IFG**GAGGVPTTS**FG**QPQSAPNQMGMTNNGMSMGGMAN
 RKIARMRHSKR¹⁰⁷⁶

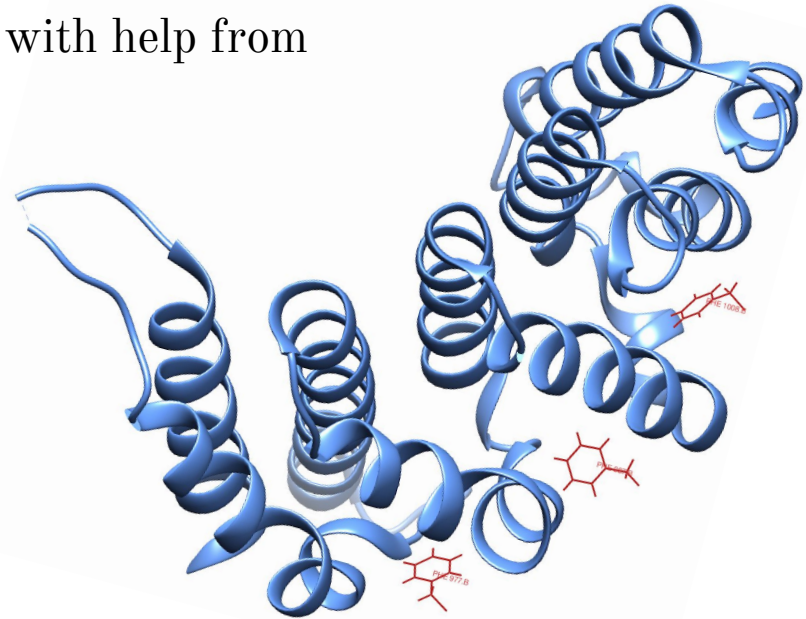


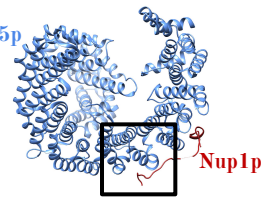
3.2 Hydrophobic Interaction

Phe conforms the “core” of the interaction with help from friends **Pro** or **Ile**.

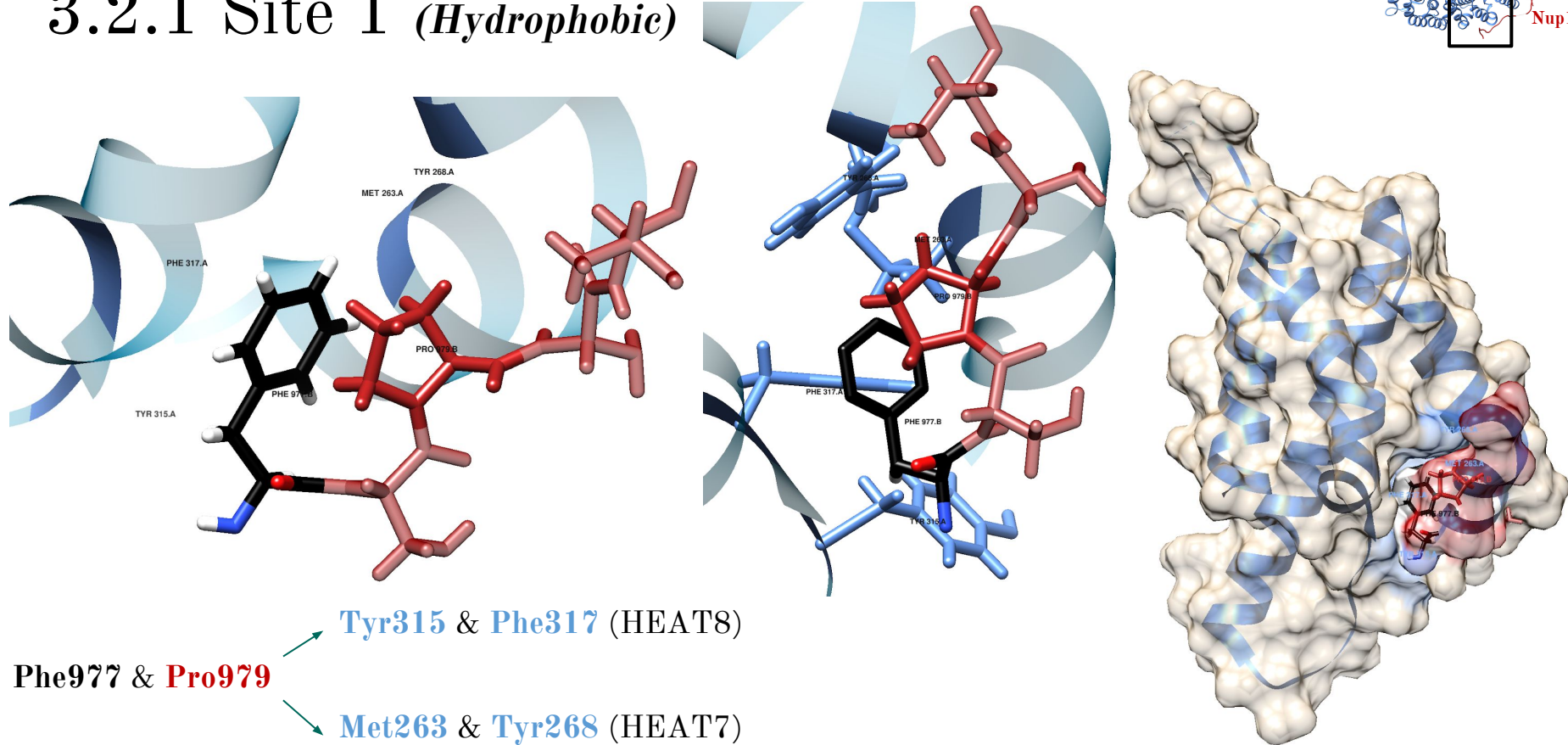
HEAT repeat define **3** interaction sites:

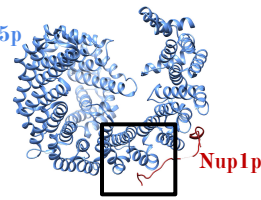
- Site 1: HEAT 7-8
- Site 2: HEAT 6-7
- Site 3: HEAT 5-6



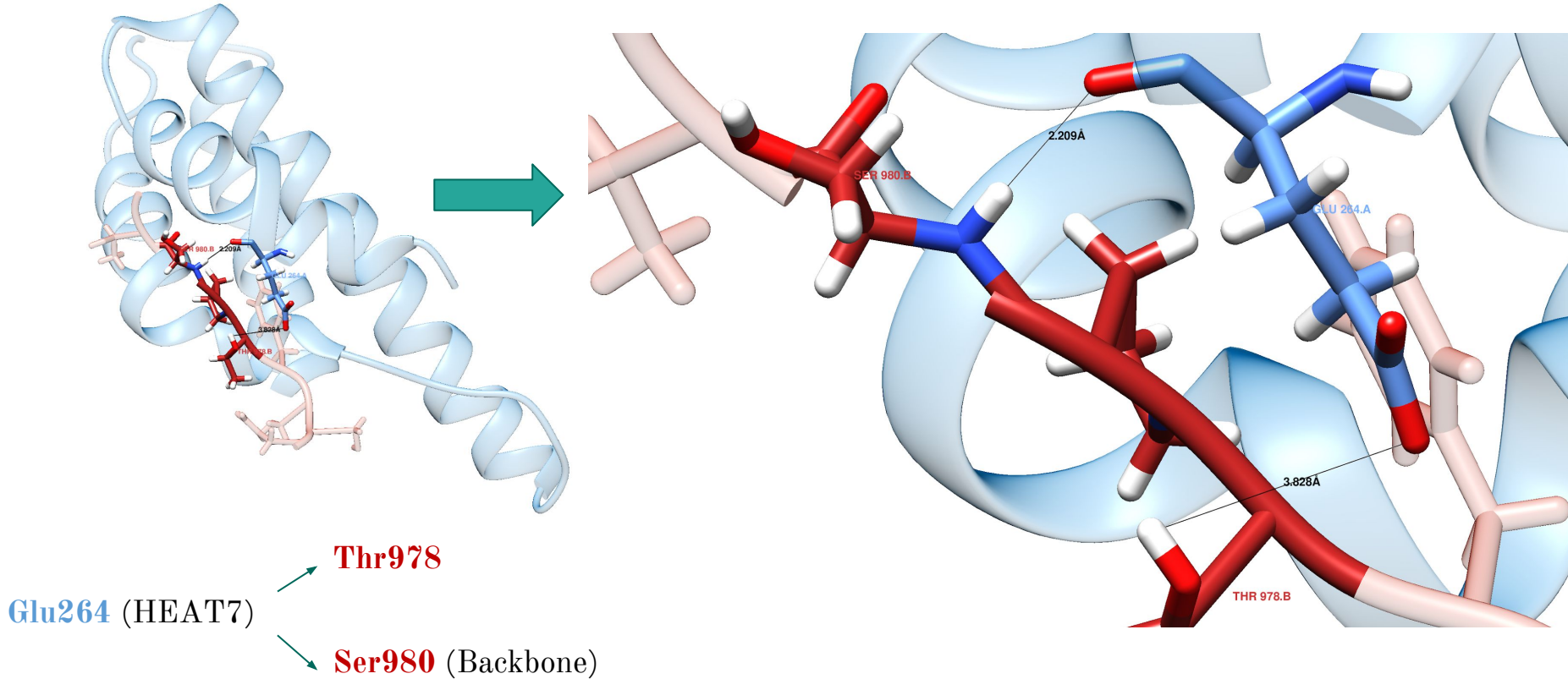


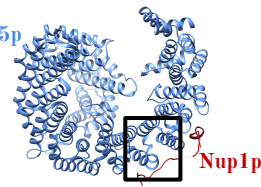
3.2.1 Site 1 (*Hydrophobic*)



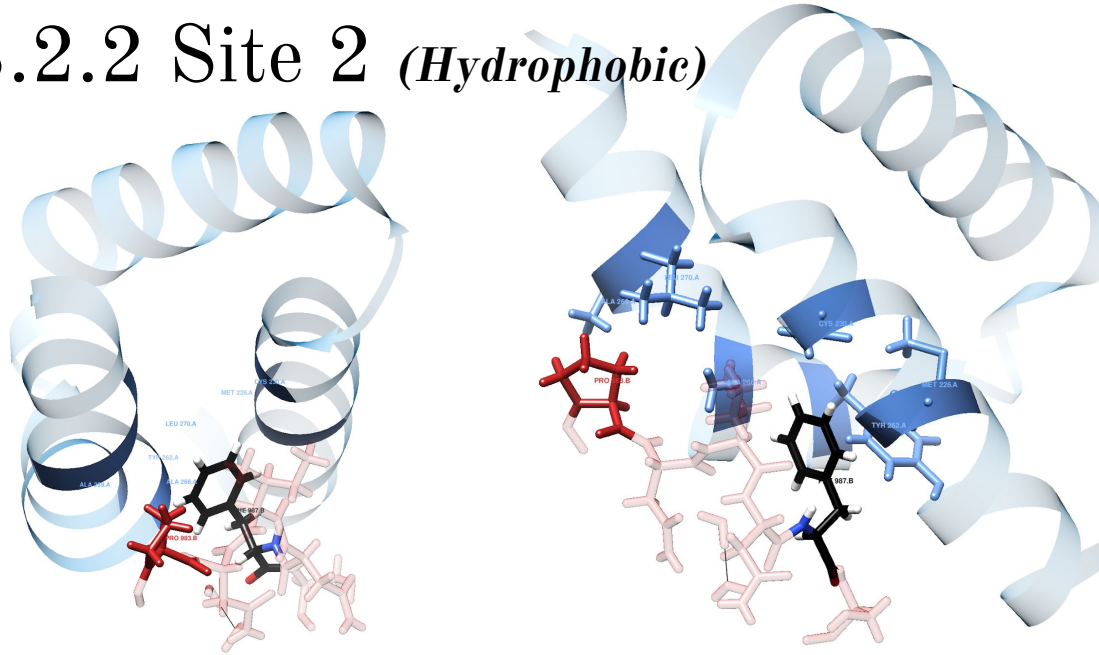


3.2.1 Site 1 (*H-Bonds*)





3.2.2 Site 2 (*Hydrophobic*)

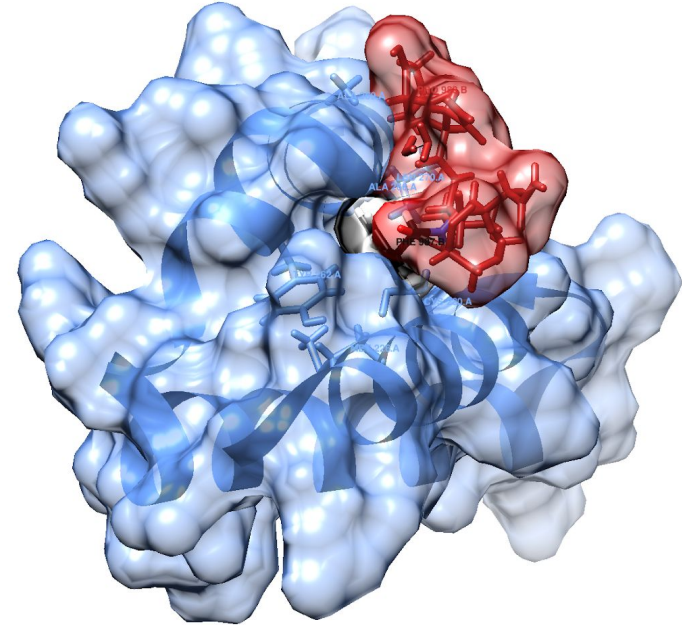


Pro983 → **Ala269** & **Leu270** (HEAT7)

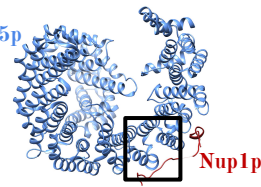
Tyr262 & **Ala266** (HEAT7)

Phe987

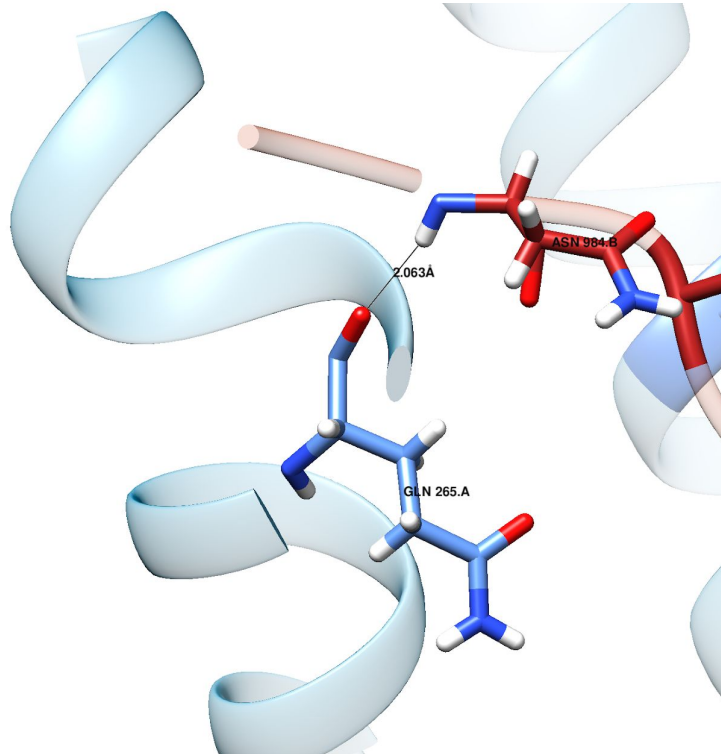
Met226 & **Gln227** & **Cys230** (HEAT6)



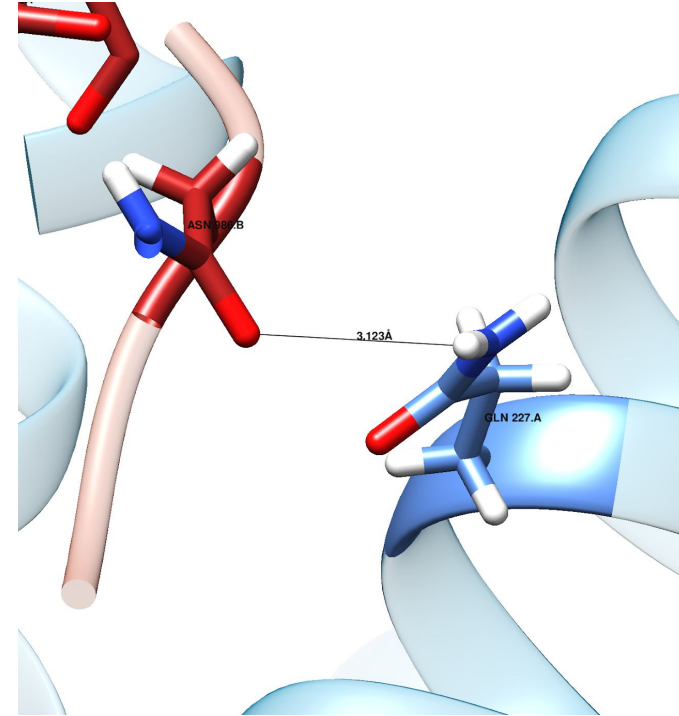
Surface bottom-view



3.2.2 Site 2 (*H-Bonds*)

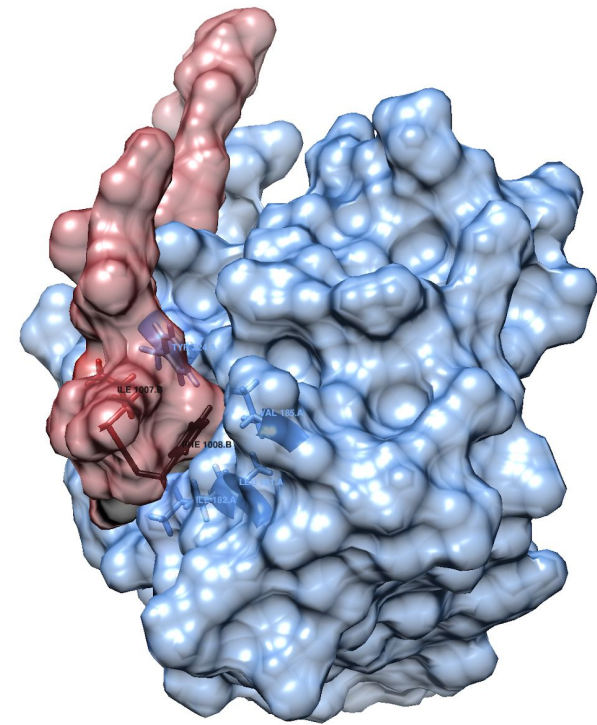
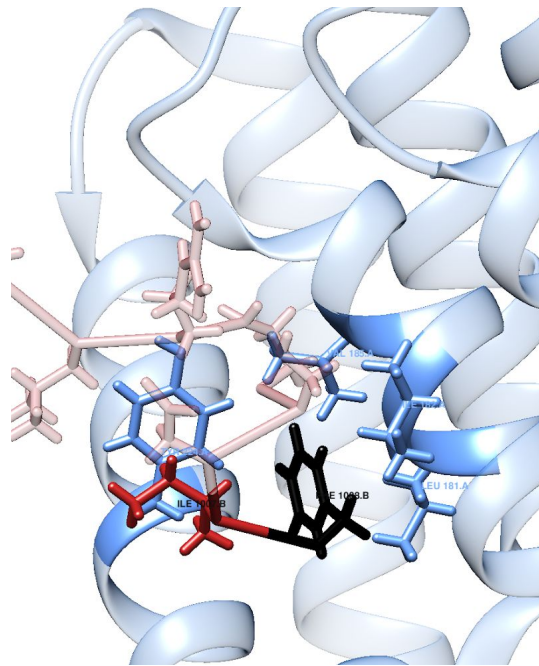
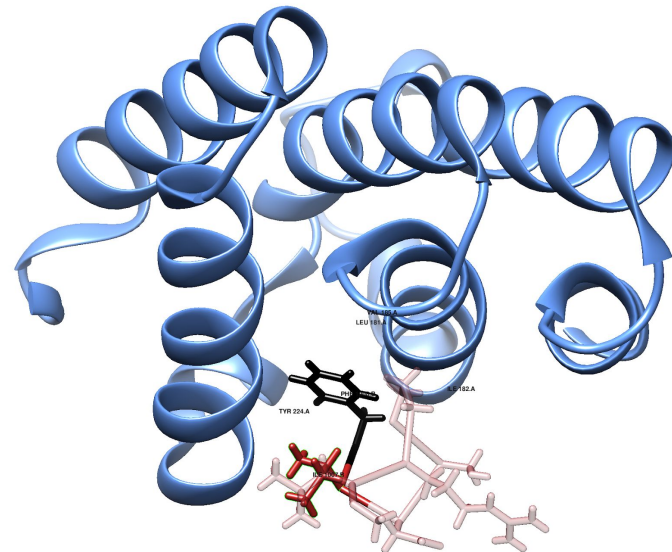
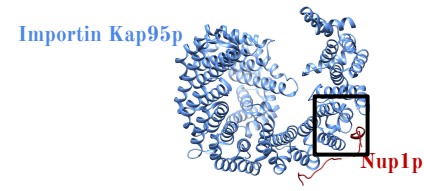


Gln265 (HEAT7) → Asn984



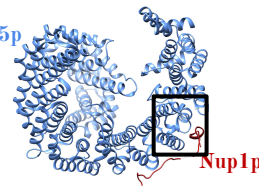
Gln227 (HEAT6) → Asn986

3.2.3 Site 3 (*Hydrophobic*)

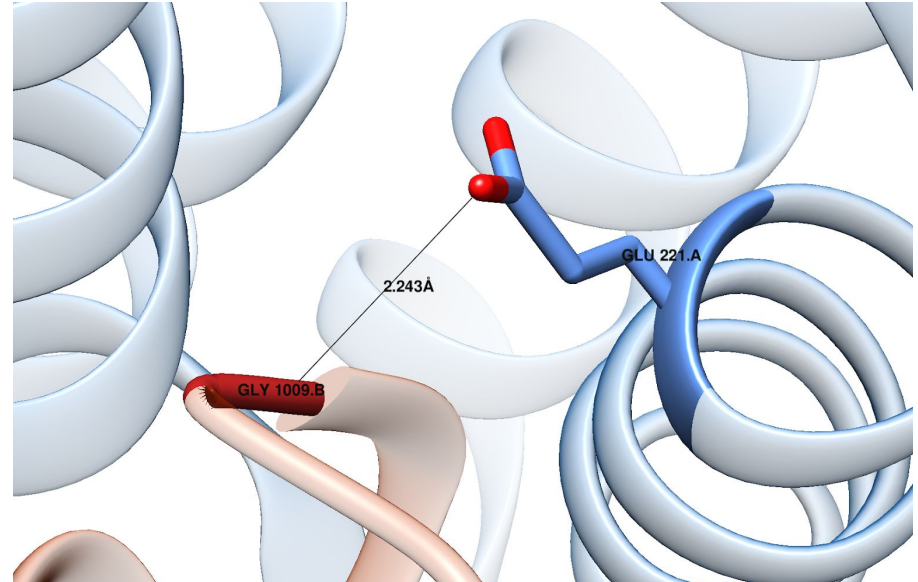
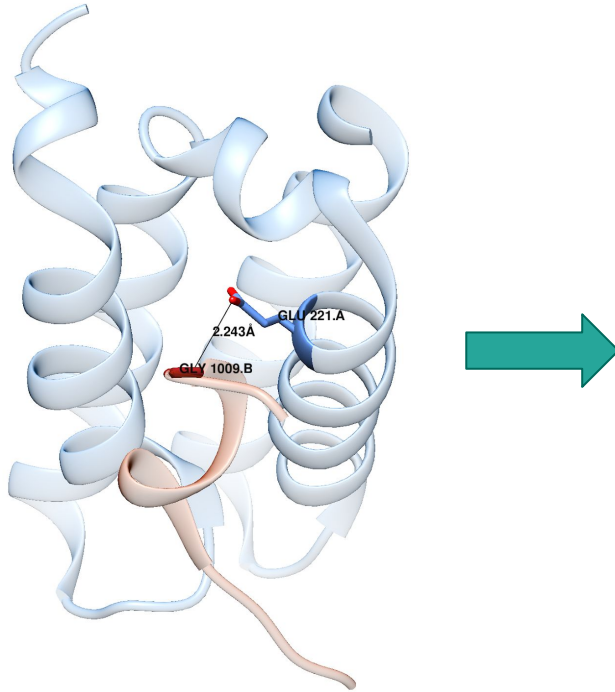


- Ile1007** → **Tyr224** (HEAT6)
- Phe1008** → **Leu181, Ile182 & Val185** (HEAT5)

Surface view from the top

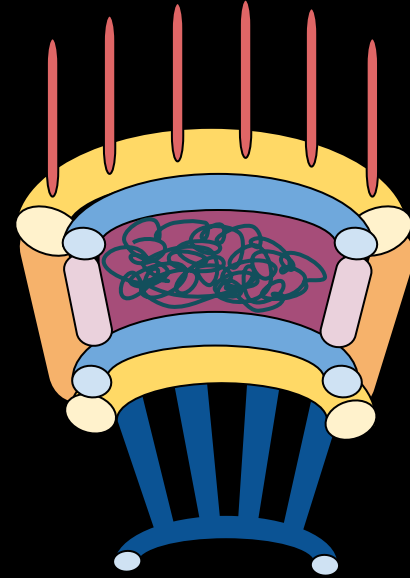


3.2.3 Site 3 (*H-Bonds*)



Glu221 (HEAT6) → **Gly1009** (Backbone)

4. Conclusions



4. Conclusions

- Sequence is overall not conserved
- Its function is essential for the existence of eukaryotes
- Complex with more than 30 different NUPs, each Nup with its own structure
- Its characterization represents a great milestone
- Difficulties in characterization
- Further research is needed in order to define the interactions and the organisation of the Nucleopore complex

5. PEM questions

1) How many proteins form the nucleopore complex?

- A. 1
- B. 2
- C. 3
- D. 4
- E. >30

2) What's the main function of the nucleopore complex?

- A. Transport
- B. RNA synthesis
- C. Stability
- D. Osmosis
- E. Excretion of toxics

3) Regarding to the structure of the nucleopore, the Y-complex is:

- A. The basic structural element of the outer ring
- B. An homotrimer
- C. A shuttle protein that mediates the nucleocytoplasmic transport
- D. A and C are correct
- E. All options are correct

4) The main fold/s found in the Y-complex of the nucleopore are:

- A. α -solenoid
- B. β -propeller
- C. A and B are correct
- D. TIM barrel
- E. Jelly roll

5. PEM questions

5) Which supersecondary structure (motif) is the most important in the interactions between the proteins of the Nup62 complex?

- A. β - β hairpin
- B. Greek key
- C. Coiled coil
- D. α hairpin
- E. β -meander

6) In which group of NUPs should the proteins that form the Nup62 complex be included?

- A. Inner ring NUPs
- B. Outer ring NUPs
- C. Linker NUPs
- D. Central FG-NUPs
- E. Transmembrane NUPs

7) Mark the correct answer:

- A. Karyopherins bind to Barrier Nups allowing the transport of cargo through the Nucleopore.
- B. Barrier Nups have FG repeats.
- C. A and B are correct
- D. Importin-beta is a type of Karyopherin.
- E. All of the above are correct.

8) Which of the following sentence is not correct?

- A. The Nucleopore Complex is difficult to crystallize due to its large size and membrane location.
- B. Karyopherins contain HEAT repeats.
- C. Barrier Nups can be found in the Basket of the Nucleopore.
- D. Karyopherins have FG repeats that allow them to bind to Barrier Nups.
- E. The Nucleopore is the only gateway between the nucleus and the cytoplasm.

5. PEM questions

9) Mark the correct answer.

- A. The nucleopore is a small complex
- B. The structure of the nucleopore complex is completely characterised
- C. The nucleopore is composed by three main types of nups: transmembrane, scaffold and barrier nups
- D. The nucleopore is found both in the cytoplasmic and the nuclear membranes
- E. The nucleopore complex is also found in bacteria

10) The hinge region in the Y-complex involves proteins:

- A. Nup133 and Nup107
- B. Nup85 and Nup43
- C. Nup160 and Nup37
- D. Nup107 and Nup96
- E. Nup1 and Nup2

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Elad, N., Maimon, T., Frenkiel-Krispin, D., Lim, R. Y., & Medalia, O. (2009). Structural analysis of the nuclear pore complex by integrated approaches. *Current opinion in structural biology*, 19(2), 226-232.

F. (n.d.). Retrieved March 03, 2018, from <http://flybase.org/reports/FBgg0000146.html>

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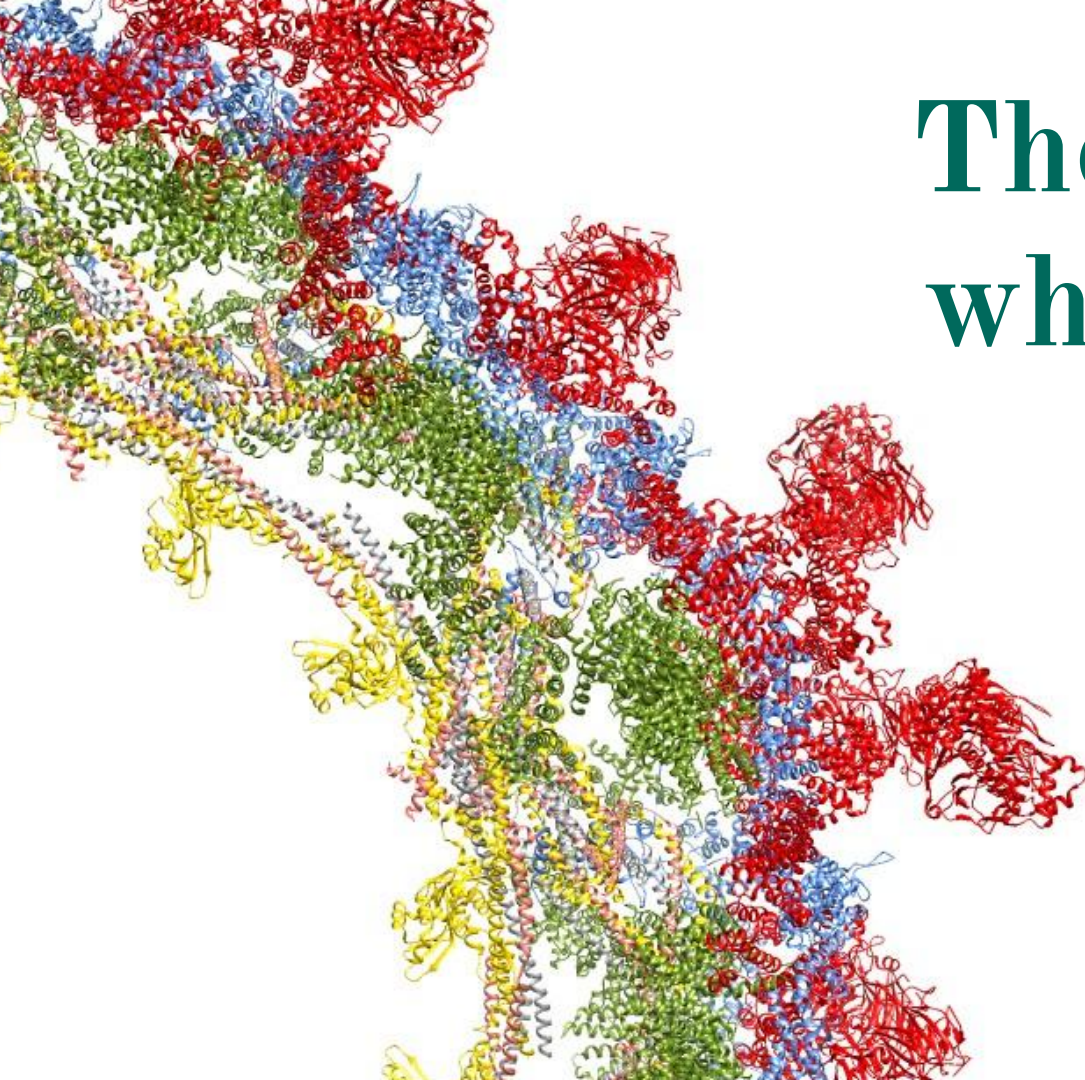
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