Understanding the initial steps of eukaryotic protein synthesis and its regulation

TANWEER HUSSAIN
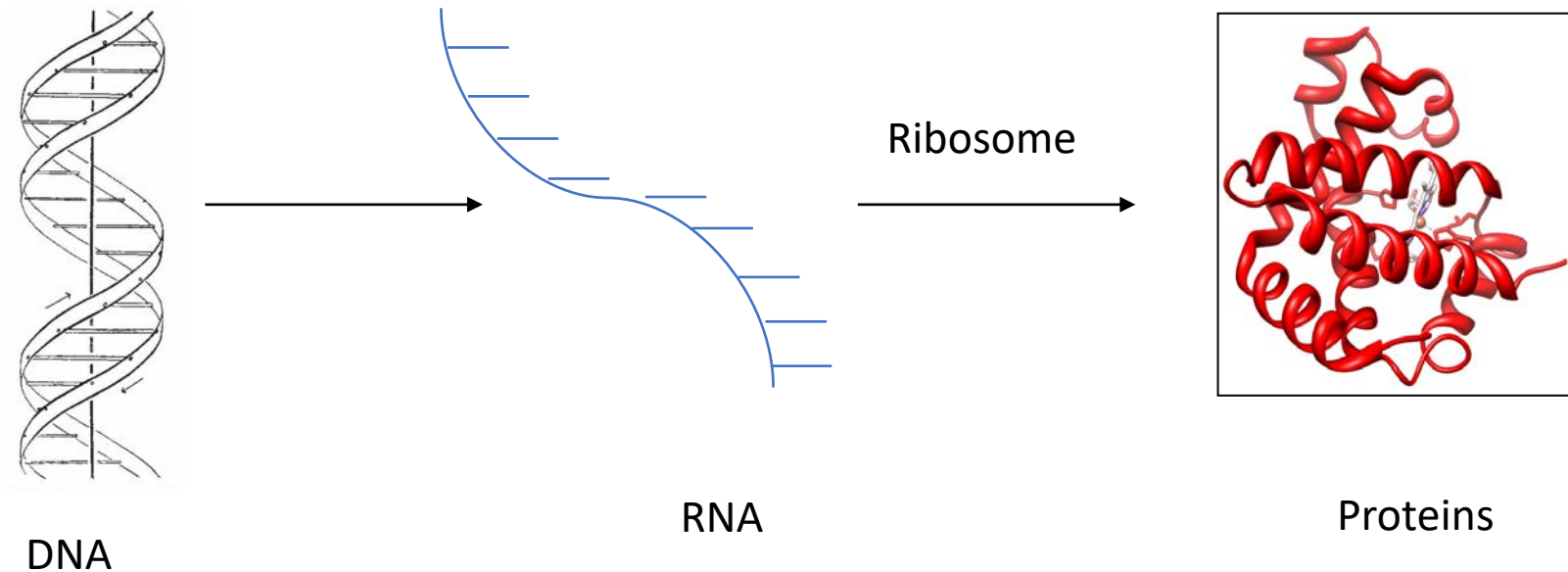
Molecular Reproduction Development and Genetics (MRDG)

Indian Institute of Science, Bangalore

STATISTICAL BIOLOGICAL PHYSICS: FROM SINGLE MOLECULE TO CELL (ONLINE), DECEMBER 14, 2020

Ribosome:
a sophisticated molecular machine

reads the genetic code (nucleotide codons in the mRNA) to form the cognate polypeptide
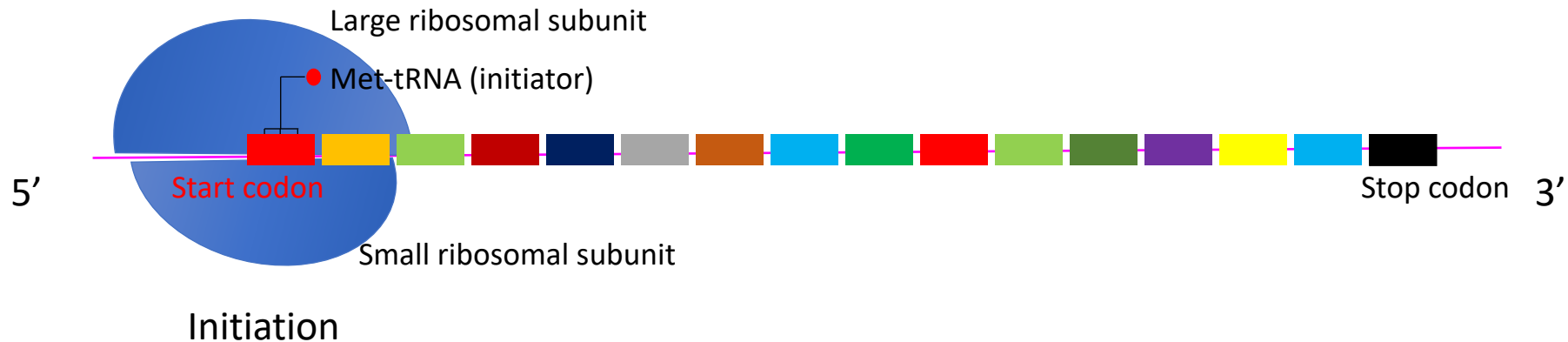

DNA

RNA

Ribosome

Proteins

Translation: Protein synthesis

- Three steps:

1. **Initiation**
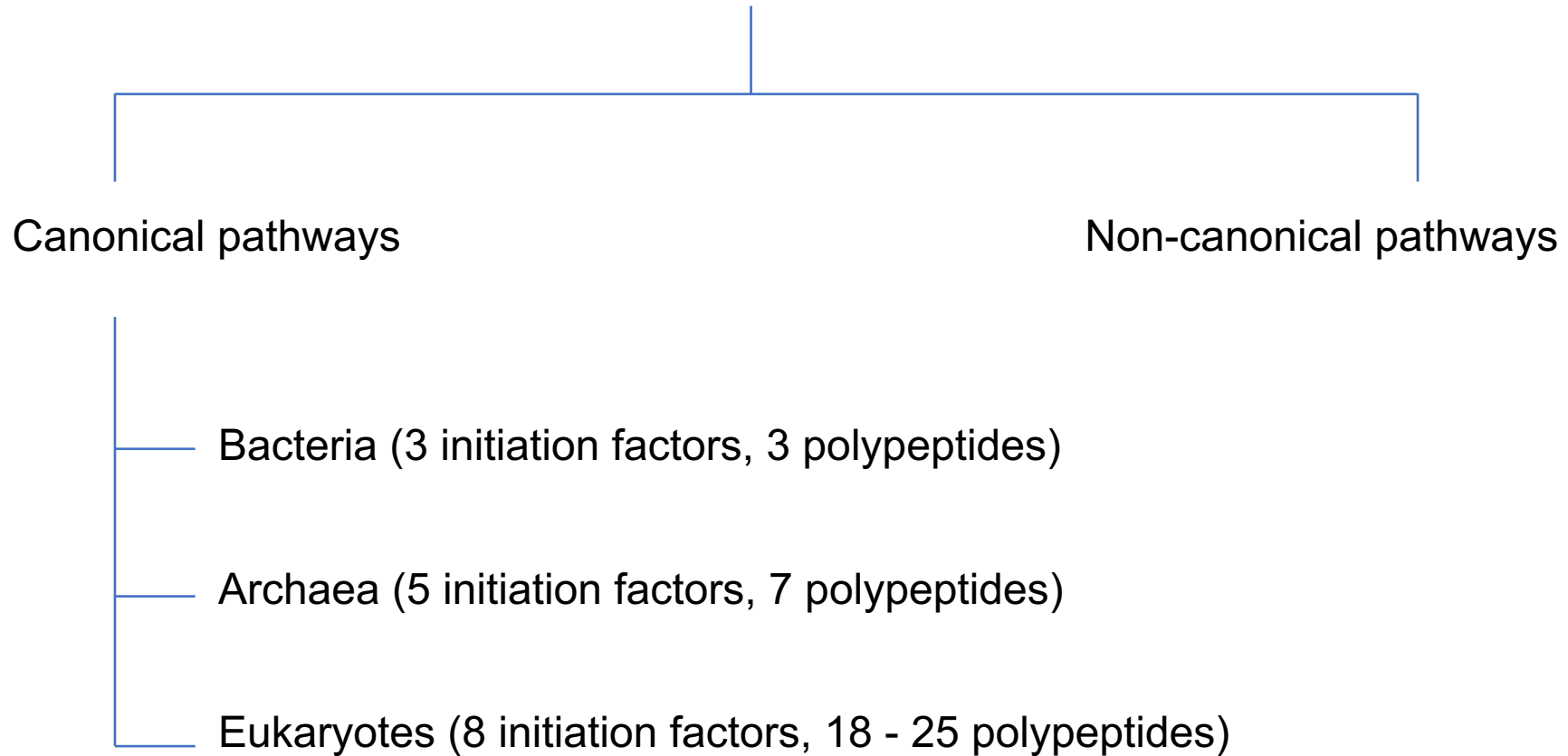
2. Elongation

3. Termination (and recycling)

Step-wise assembly of the ribosome at the start codon

Diversity in Initiation Pathways

Canonical pathways

Non-canonical pathways

Bacteria (3 initiation factors, 3 polypeptides)

Archaea (5 initiation factors, 7 polypeptides)

Eukaryotes (8 initiation factors, 18 - 25 polypeptides)

Translation initiation in eukaryotes is more complex

Schematic of Eukaryotic translation initiation

head

A   P   E

body

Free 40S
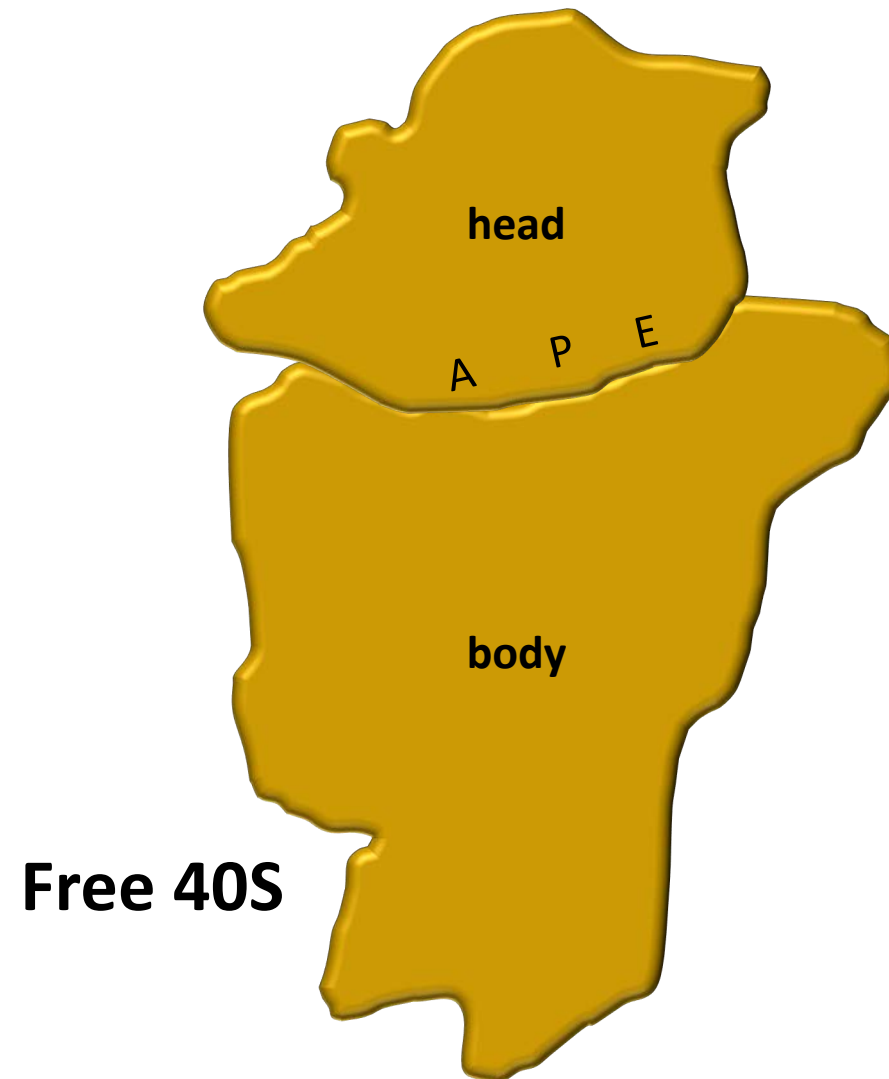
40S-eIF1-eIF1A-eIF3 complex

head

A   P   E

1A   1
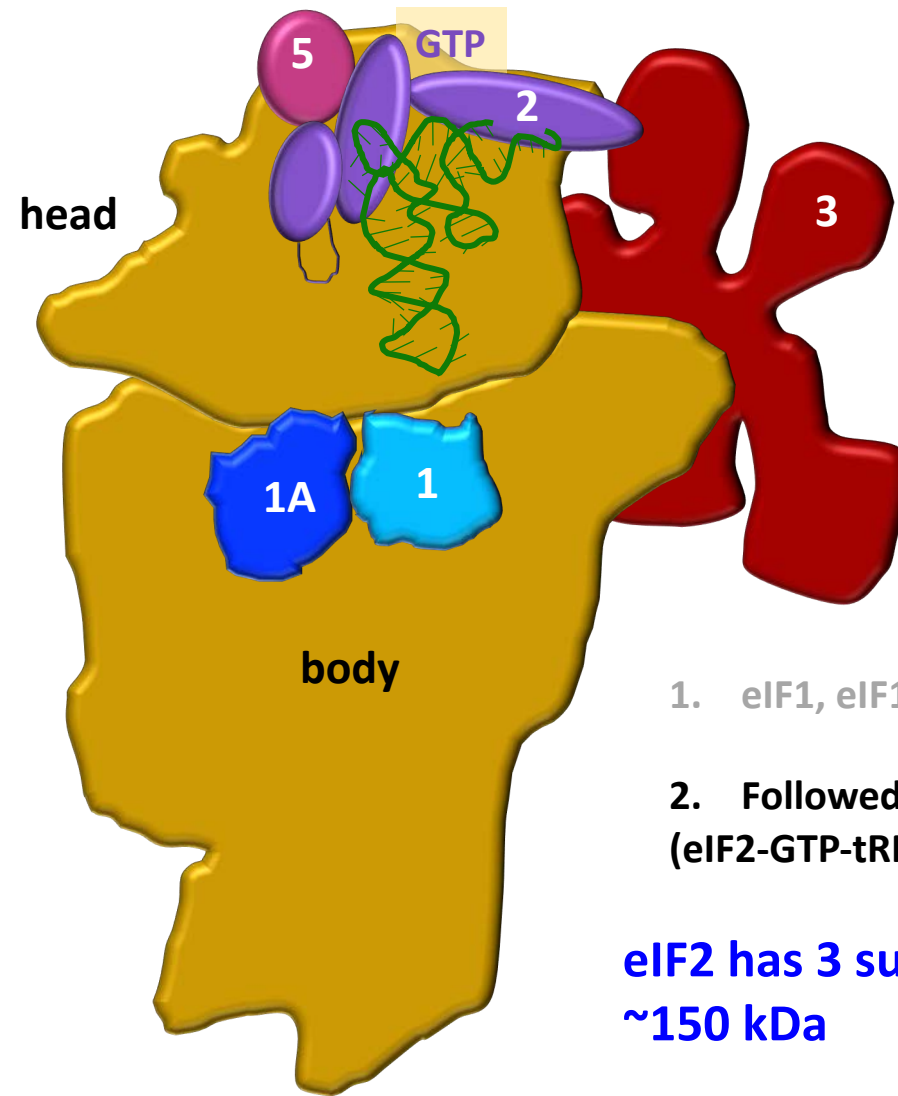
body

3
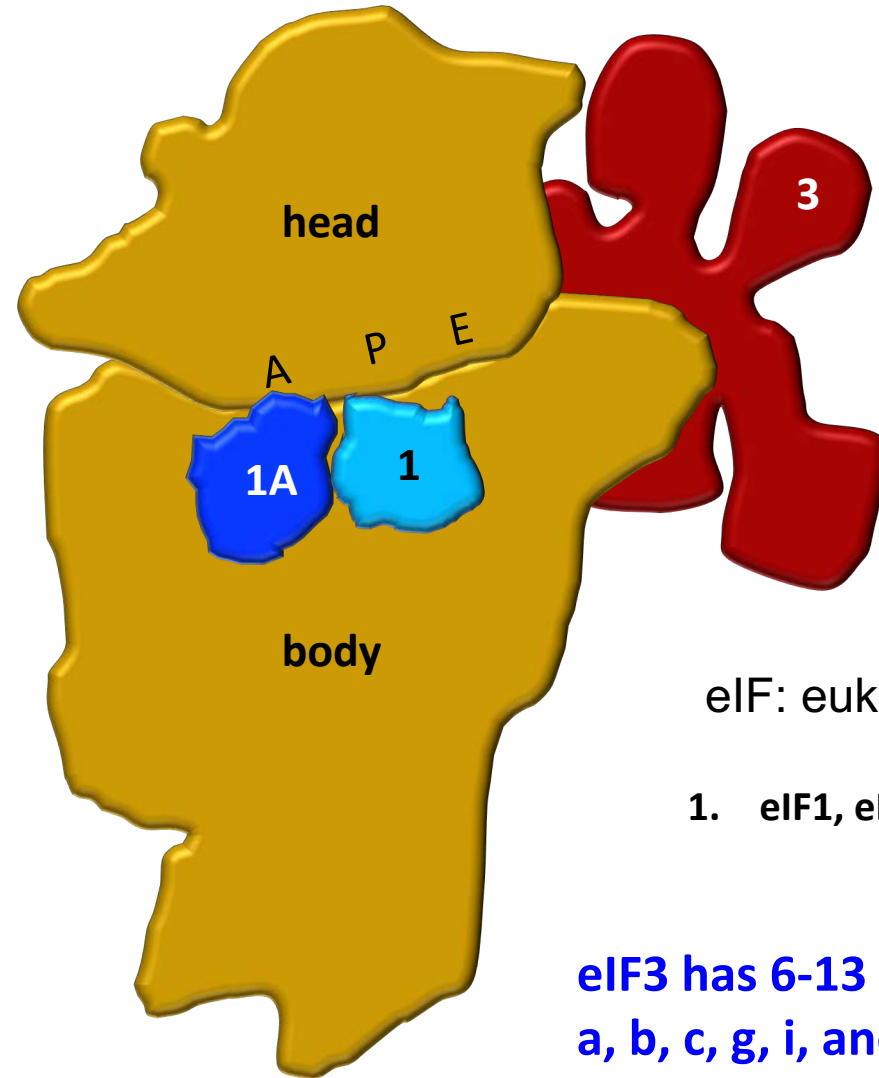
eIF: eukaryotic Initiation Factor

1. eIF1, eIF1A and eIF3 binds to 40S

eIF3 has 6-13 subunits:
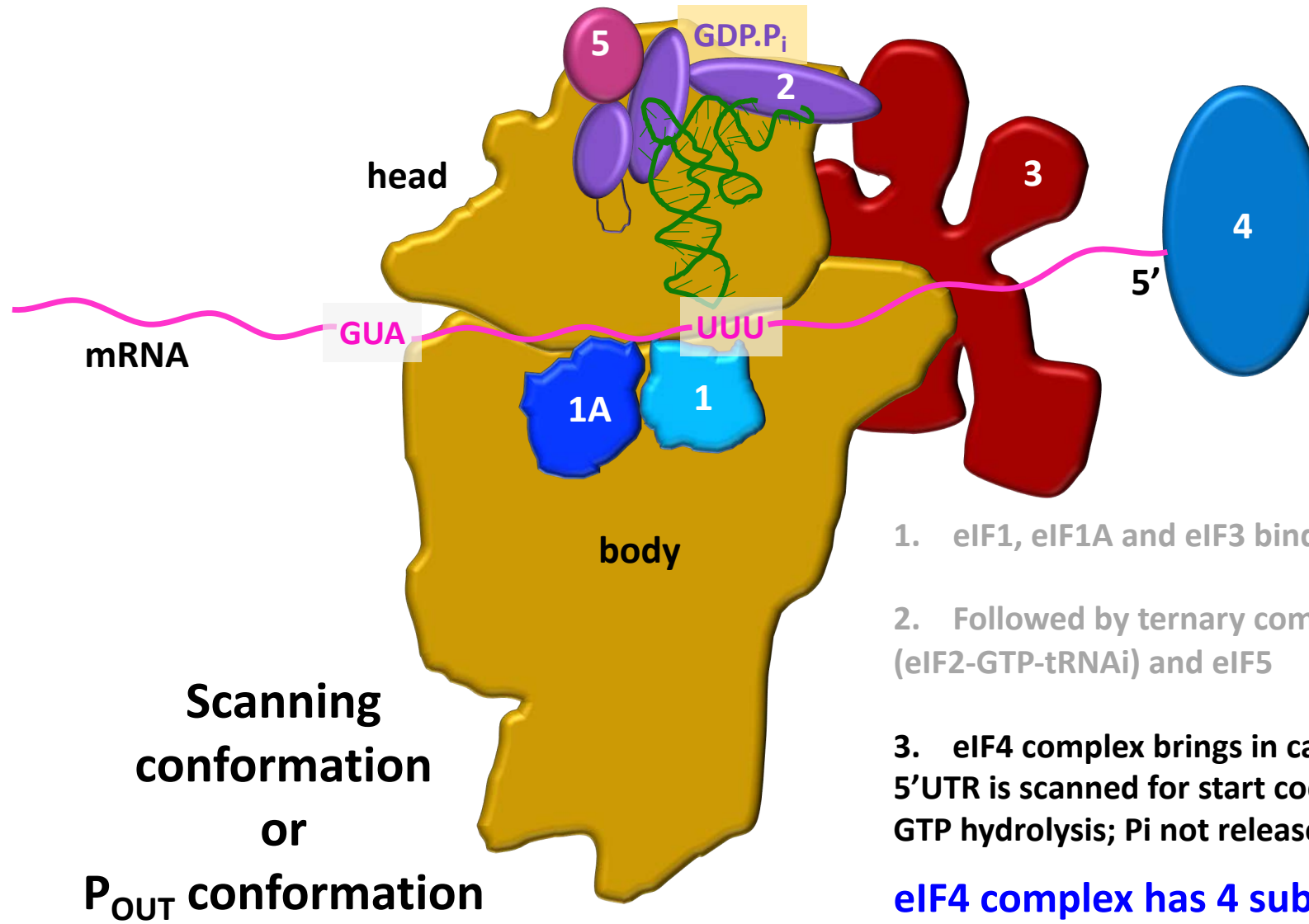a, b, c, g, i, and j (in *S. cerevisiae*)
400 - 800 kDa

Recruitment of ternary complex (eIF2-tRNA$_i$-GTP); 43S complex

head

GTP

5

2

3

1A

1

body

1. eIF1, eIF1A and eIF3 binds to 40S

2. Followed by ternary complex (eIF2-GTP-tRNAi) and eIF5

eIF2 has 3 subunits: α, β and γ ~150 kDa

Binding of mRNA-eIF4 complex; 48S (P$_{OUT}$) scanning complex

head

body

GDP.P$_i$

5

2

3

4

5'

GUA

UUU

mRNA

1A

1

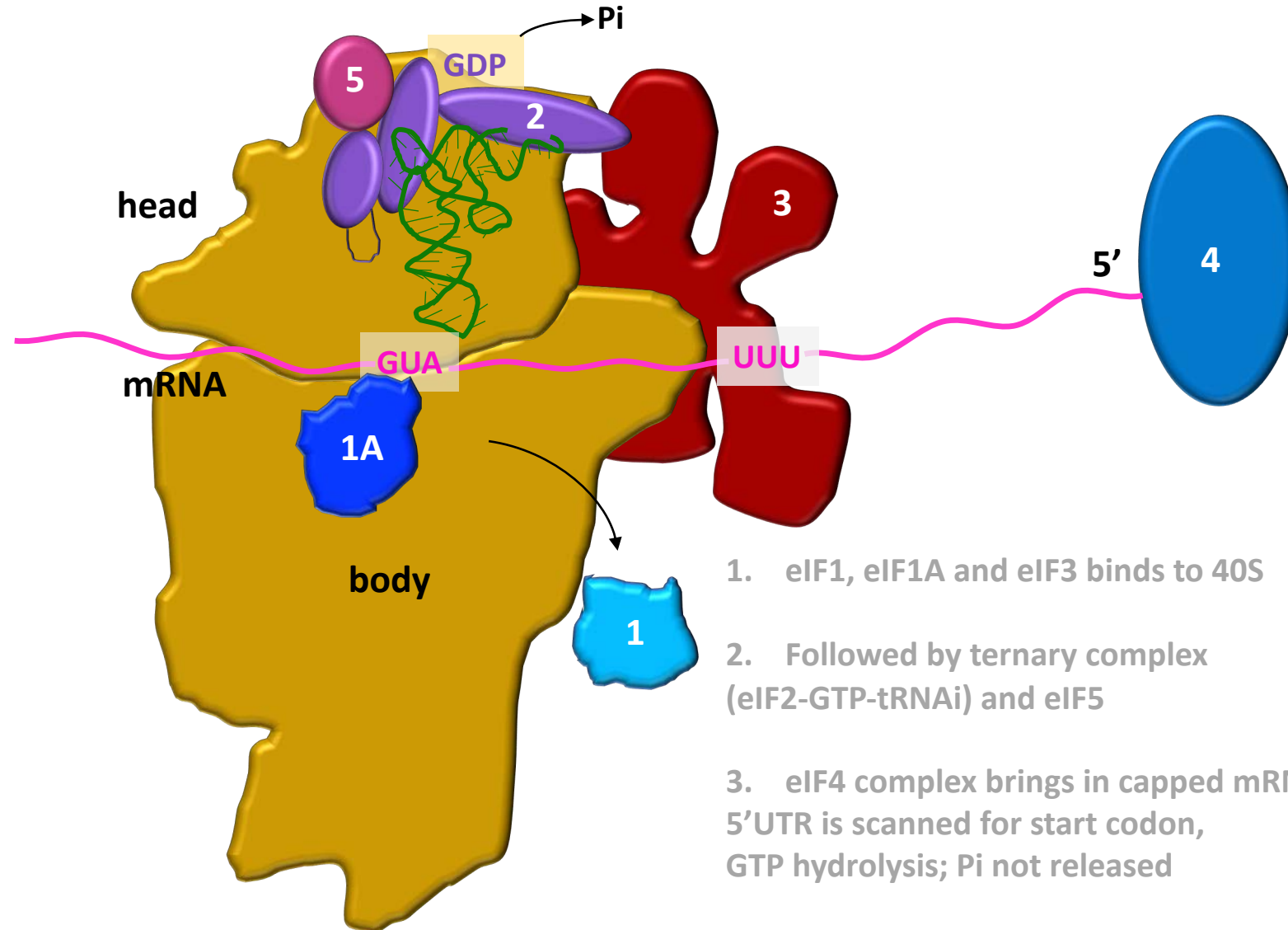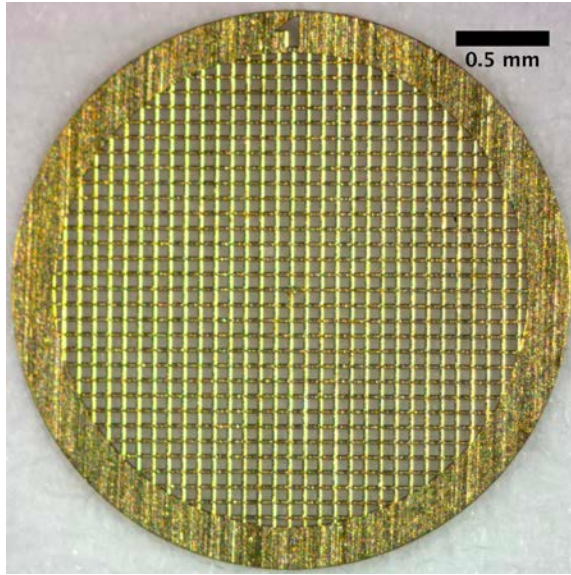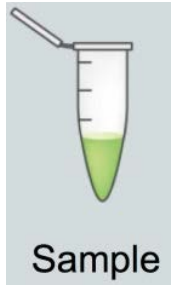**Scanning
conformation
or
P$_{OUT}$ conformation**

1.   eIF1, eIF1A and eIF3 binds to 40S

2.   Followed by ternary complex
(eIF2-GTP-tRNAi) and eIF5

3.   eIF4 complex brings in capped mRNA
5'UTR is scanned for start codon
GTP hydrolysis; Pi not released

eIF4 complex has 4 subunits:
E, G, A and B
~225 kDa

Recognition of start codon; 48S (P_{IN}) scanning-arrested complex

Pi

GDP

5

2

head

3

5'

4

mRNA

GUA

UUU

1A

body

1

P_{IN} conformation of 48S

1. eIF1, eIF1A and eIF3 binds to 40S

2. Followed by ternary complex (eIF2-GTP-tRNAi) and eIF5

3. eIF4 complex brings in capped mRNA 5'UTR is scanned for start codon, GTP hydrolysis; Pi not released

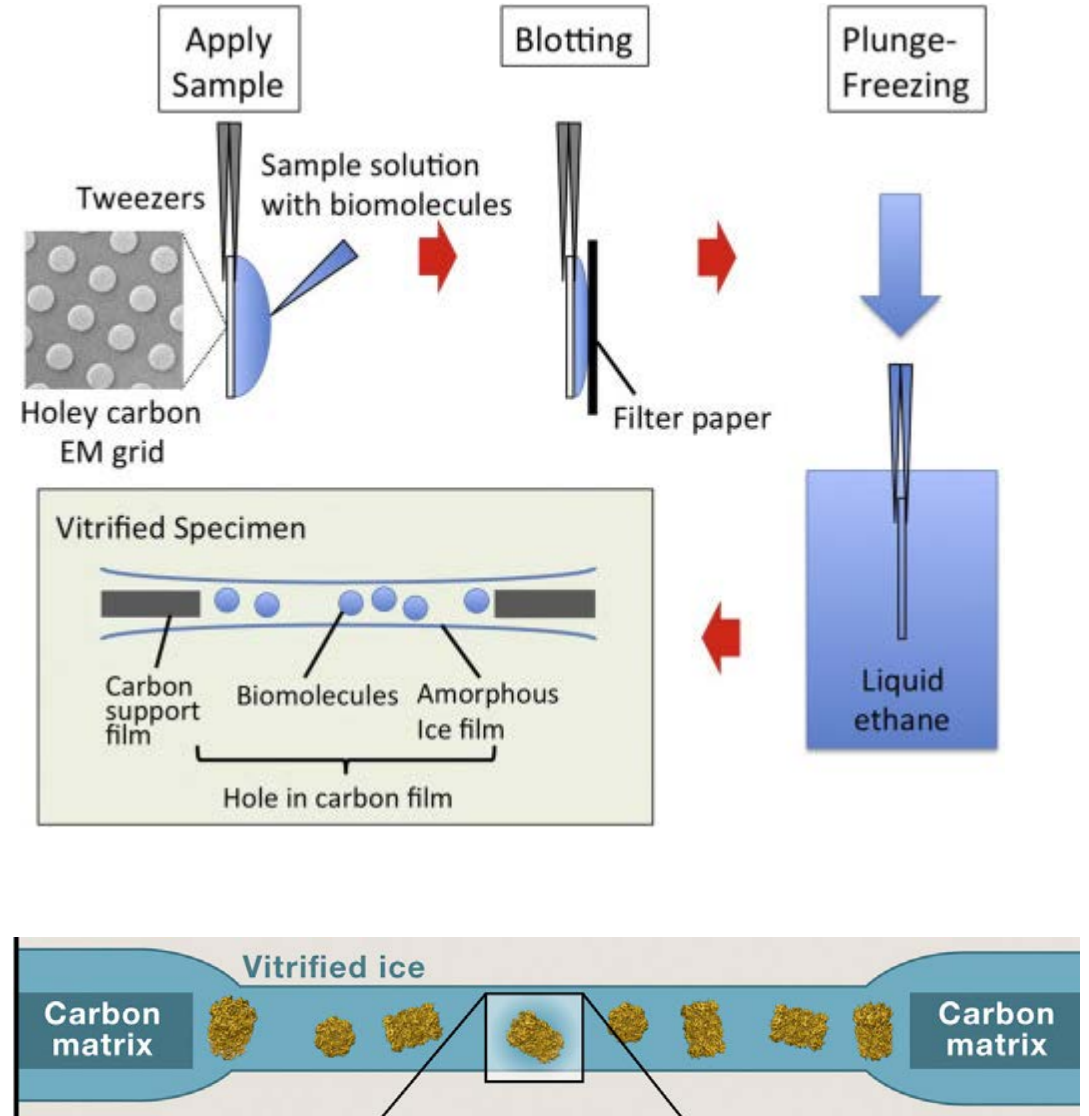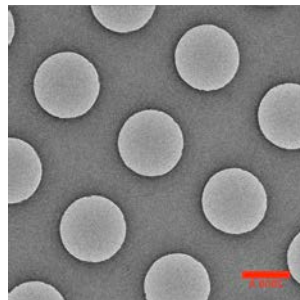4. **Start codon is recognized by tRNAi eIF1 dissociates, Pi is released**

Dissociation of eIF1 triggers downstream steps of release of factors and joining of 60S ribosomal subunit with the help of eIF5B to form elongation competent 80S
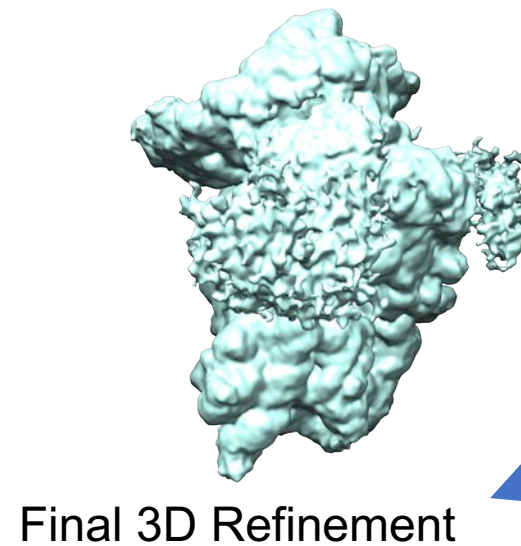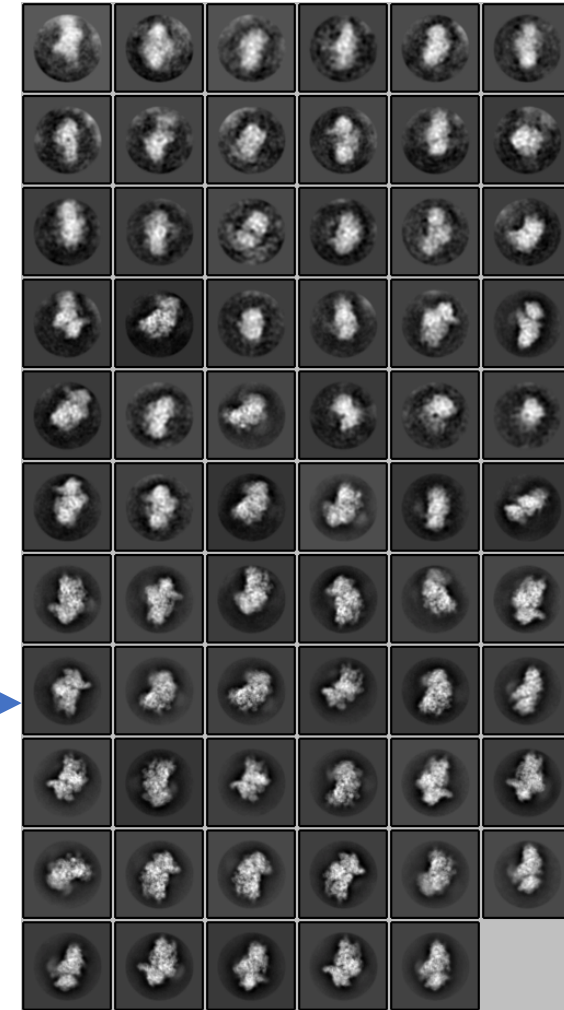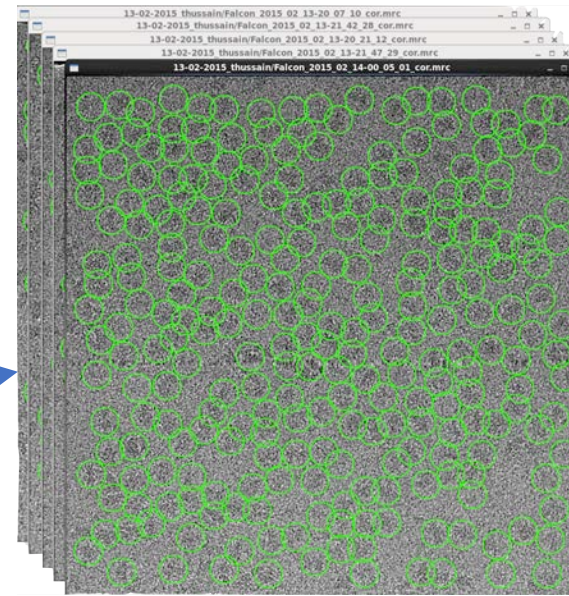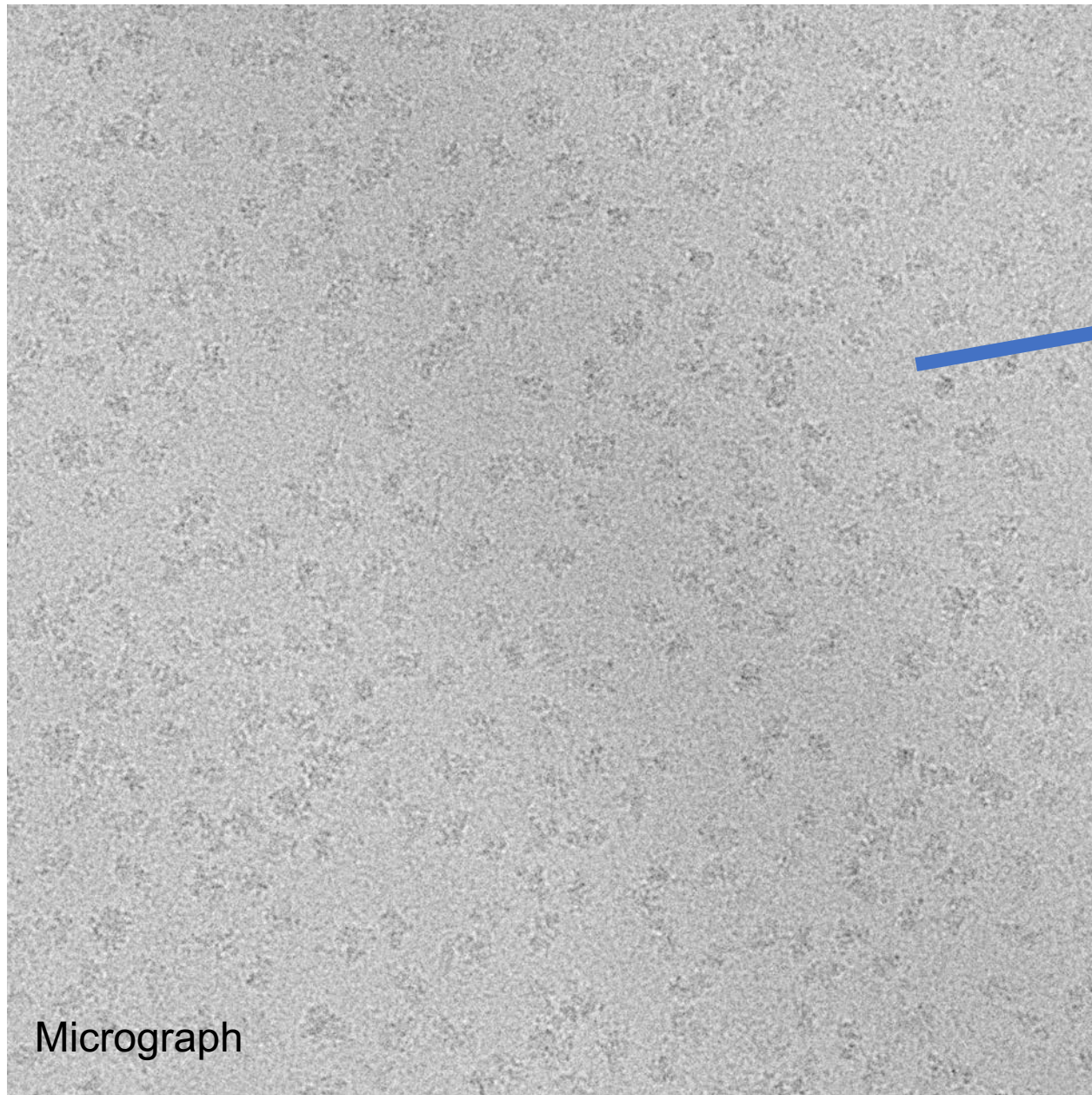
Cryo-EM grid preparation

Cryo-EM grid

Randomly oriented macromolecules

Cryo-EM pipeline from data to map

Micrograph

Image processing
(Particle picking)

2D Class averaging

Final 3D Refinement

Strategy used to capture a 48S P$_{IN}$ conformation

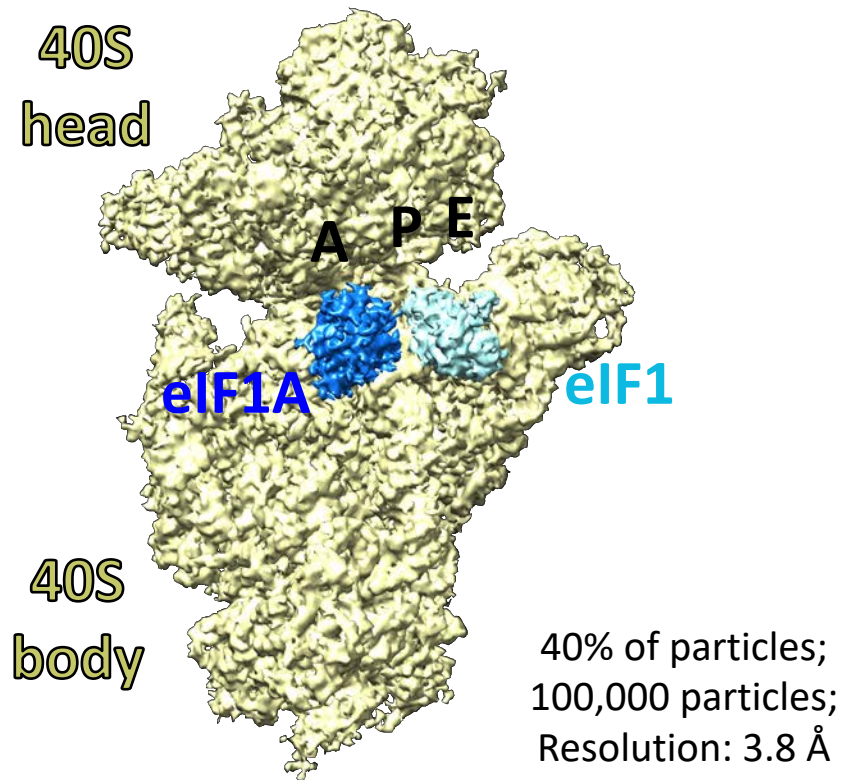| To use	Rationale
Yeast system	Simpler system; Unstructured mRNA (with AUG) without 5' cap can assemble 48S without eIF4 and eIF3
K. lactis	to make 48S at slightly lower pH (6.5) to minimize deacylation of tRNAi
Sui3-2 mutant of eIF2 (eIF2β-S264Y)	stabilizing P$_{IN}$ state
tRNAi variant (U31•A39)	favouring P$_{IN}$ state
eIF5	shifts the equilibrium towards P$_{IN}$ state

Reconstitution of the yeast 48S complex:

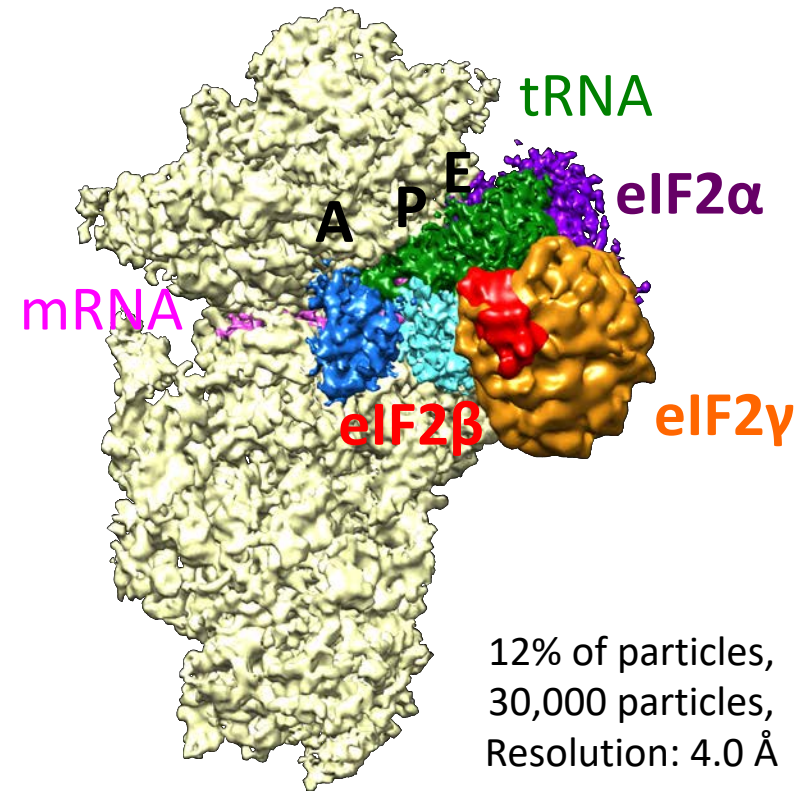40S + eIF1 + eIF1A + eIF3 + TC* + eIF5 + mRNA(AUG)

*TC= eIF2 (Sui3-2 variant) + GDPCP + tRNAi (U31•A39)

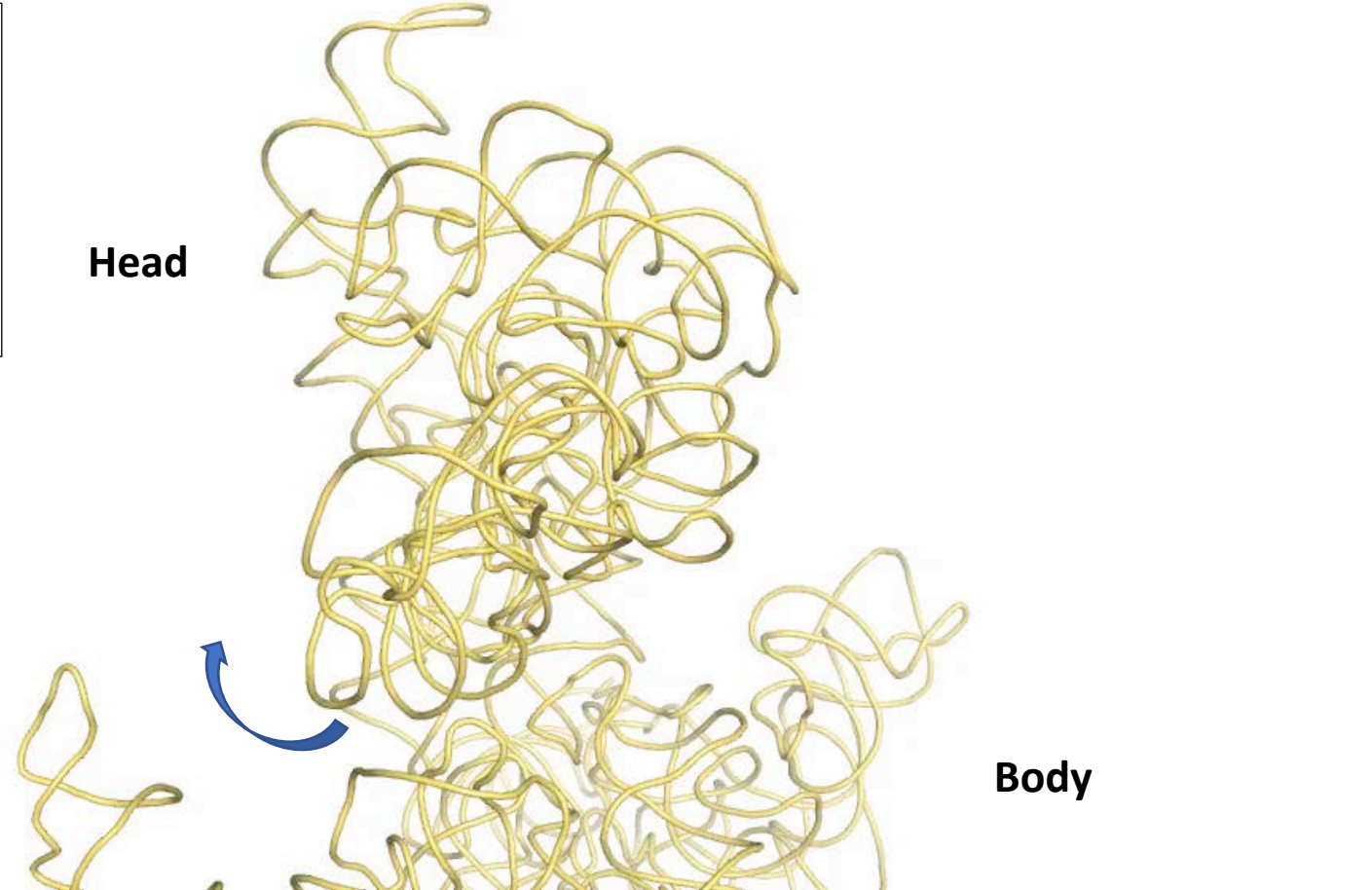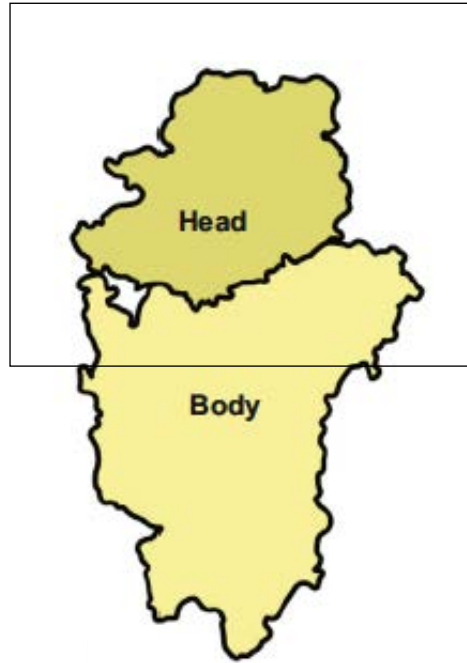# Two complexes of interest was obtained upon 3D classification

40S-eIF1-eIF1A complex

py48S: 'partial yeast' 48S (lacks eIF3)

40S head

40S body

A P E

eIF1A

eIF1

40% of particles;
100,000 particles;
Resolution: 3.8 Å

tRNA

A P E

mRNA

eIF2α

eIF2β

eIF2γ

12% of particles,
30,000 particles,
Resolution: 4.0 Å

Rotation of 40S head between the 40S-eIF1-eIF1A complex and py48S

Head

Body

Consequence of rotation of 40S head

40S
40S-eIF1-eIF1A
py48S
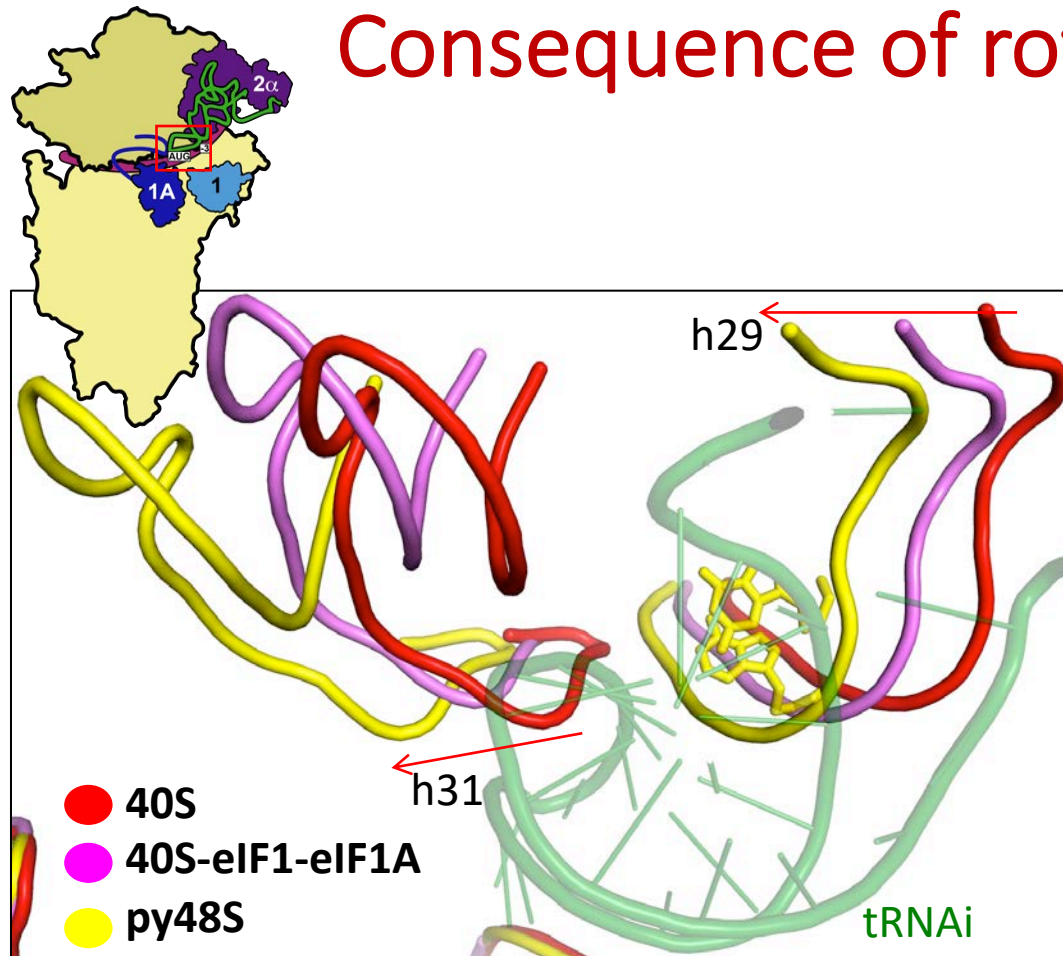
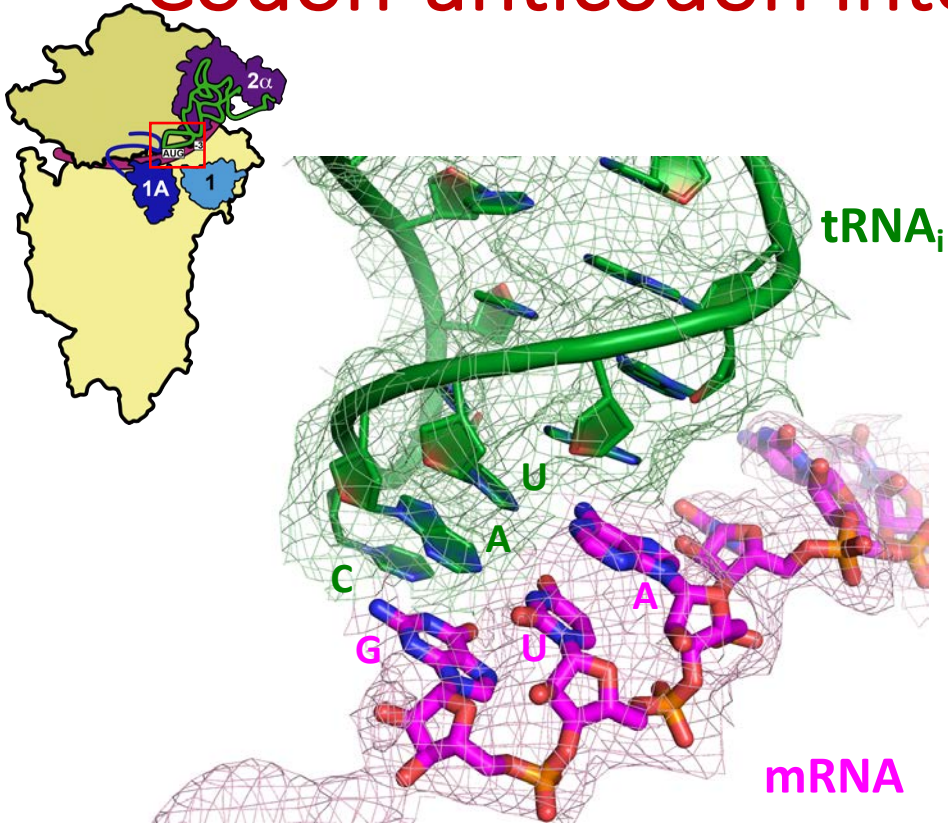**Conformational rearrangement of the P site**
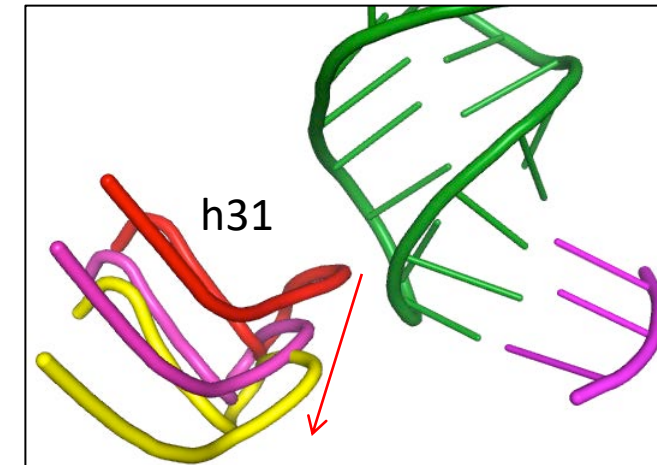
tRNAi can be accommodated deeper in the P site
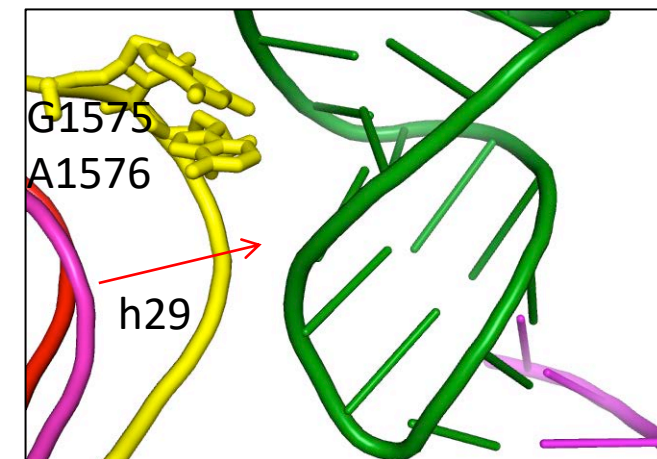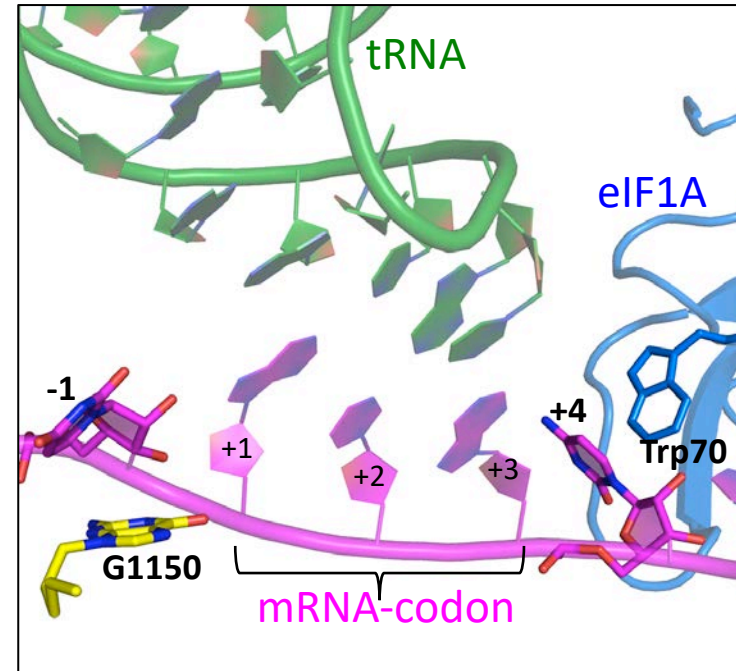
Recognition of conserved GC bps in ASL

Codon-anticodon interaction (P$_{IN}$ conformation)


tRNAi is bound deep in P site recognizing start codon

-1 and +4 interactions may allow scanning to pause

N-terminal tail (NTT) of eIF1A is conserved

NTT

S_cerevisiae	MGKKNTKGGKKGRRGKNDSDGPKRELIYKEEGQEYAQIT
K_lactis	MGKKNTKGGKKGRRGKNDSDGPKRELIYKEEGQEYAQIT
S_pombe	MPKNKGKGGKNRRGKNENENEKRELTYAEEGQMYAQVT
A_oryzae	MPKNKGKGGKNRRGKNESDKEKRELVFKEEGQEYAQVV
C_elegans	MPKNKGKGGKNRRGKNENDFMKRELDLKEEGQEYGQVS
B_taurus	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
X_laevis	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
H_sapiens	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
G_gallus	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
M_musculus	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
R_norvegicus	MPKNKGKGGKNRRGKNENEPEKRELVFKEDGQEYAQVI
D_rerio	MPKNKGKGGKNRRGKNENESEKRELVFKEDGQEYAQVI
D_discoideum	MPKNKGKGGKNRRGKNENE-QKRELQFKEEGQEYAQVL
D_melanogaster	MPKNKGKGGKNRRGKNENEFEKRELIFKEDQQEYAQVT
A_thaliana	MPKNKGKGGKNRKRGKNEADDEKRELIFKEDGQEYAQVL
T_thermophila	MPKNKGRGGKNYRRGKNENE-TKRELVFKEEGMEYAQVI
P_tetraurelia	MPKNKGRGGKNYRRGKNENL-TKRQLETKEDGQDYAQVI
	* *:: :***: :****: **:* *: *.*:

- NTT of eIF1A enhances start codon recognition
- Mechanism unknown
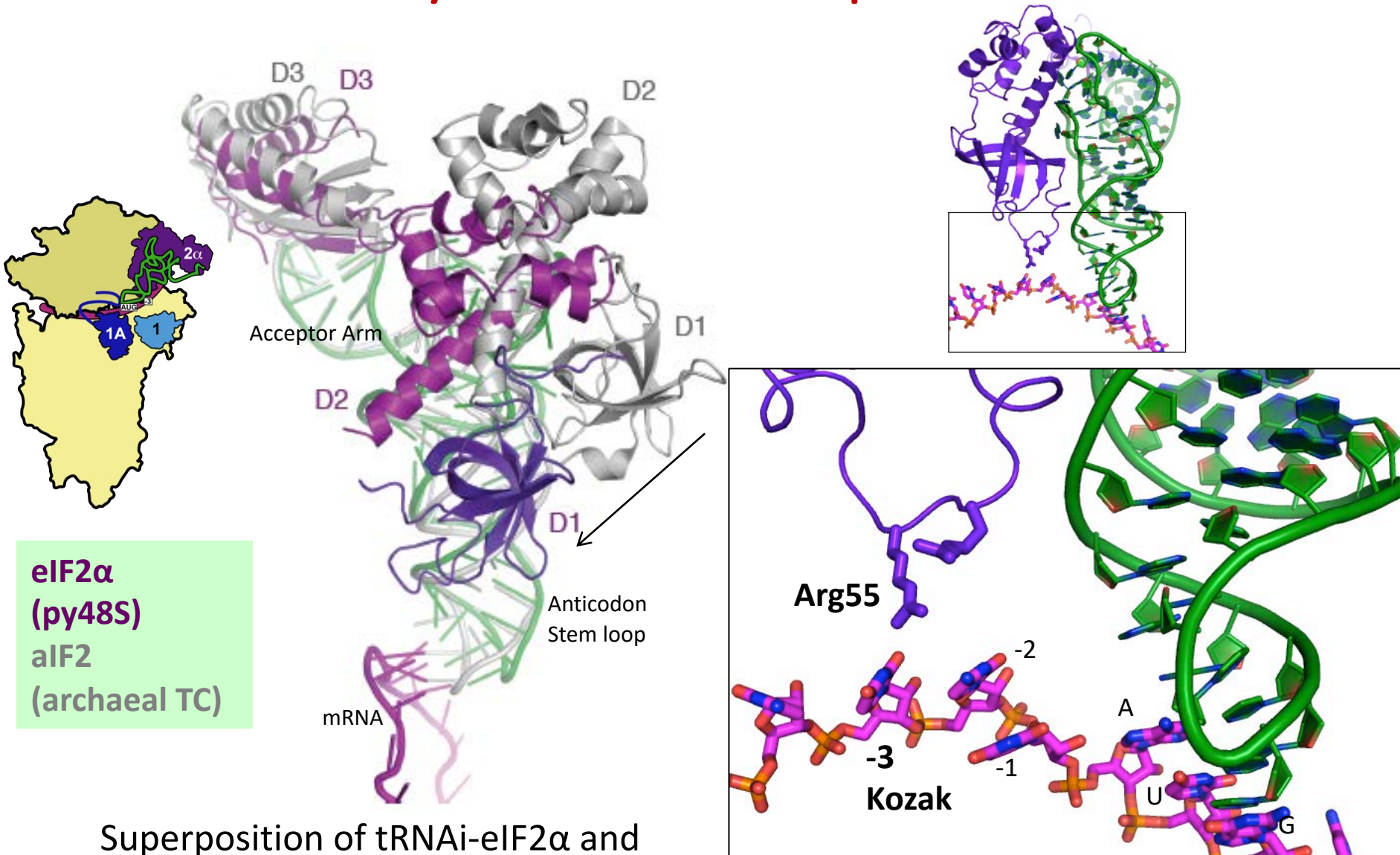- NTT of eIF1A not observed in any structure

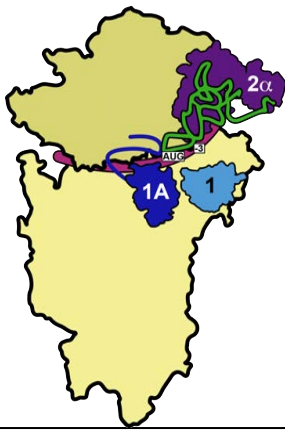# NTT of eIF1A stabilizes the codon-anticodon interaction
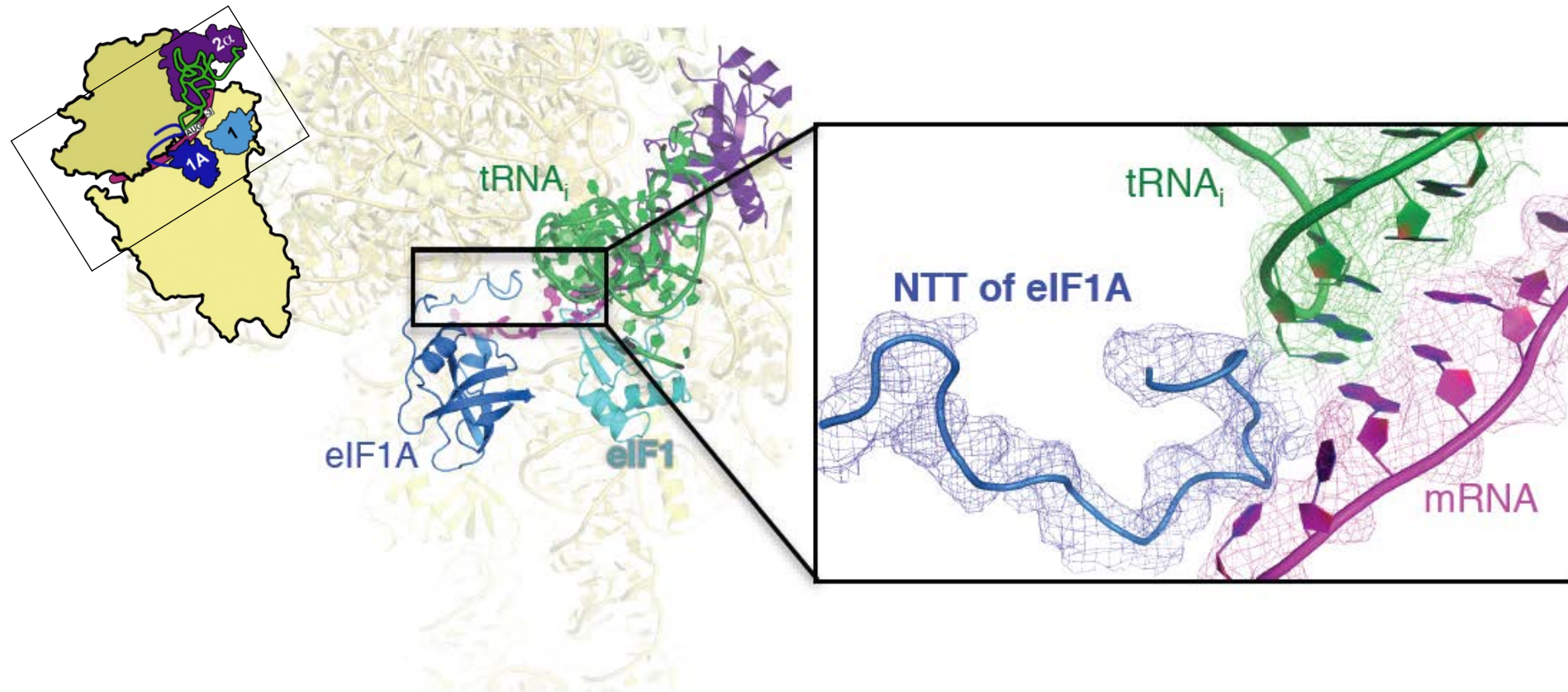
The NTT of eIF1A is observed for the first time in this complex

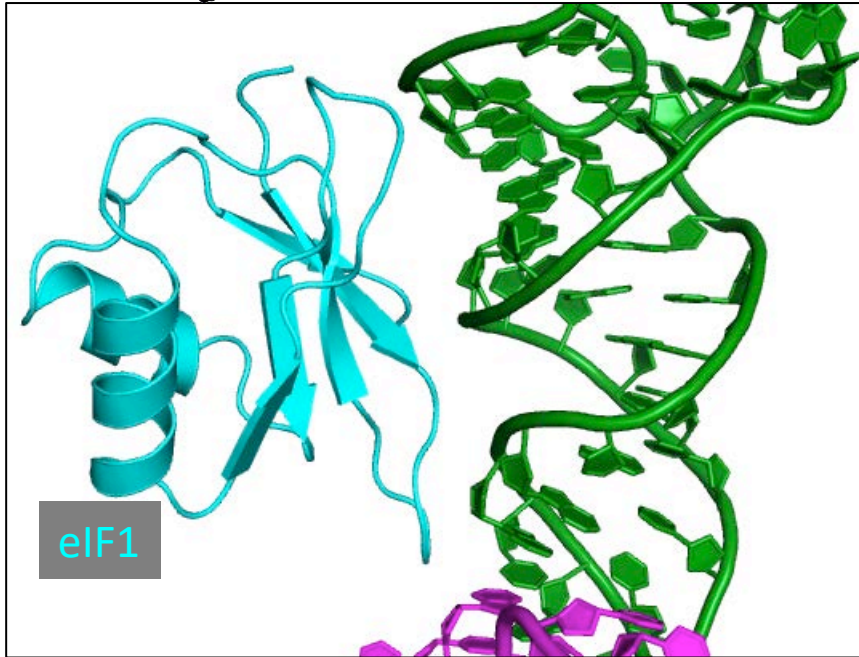Conserved glycines help to bend the NTT at the codon-anticodon
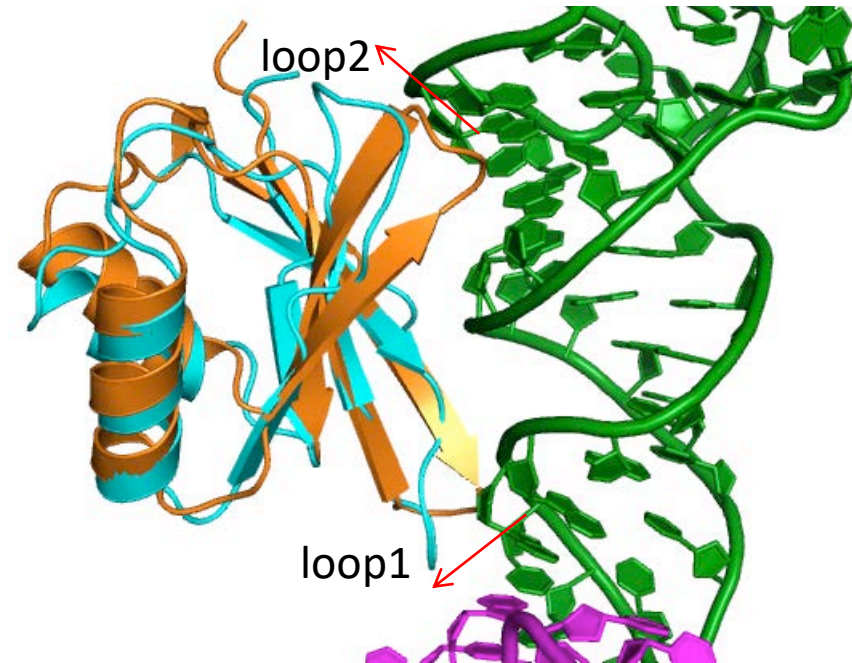
eIF2α makes key contact at -3 position of mRNA

eIF2α
(py48S)
aIF2
(archaeal TC)

Superposition of tRNAi-eIF2α and
Archaeal tRNA-aIF2α (gray)

Conformational change in eIF2α makes
it possible to contact mRNA

eIF1 captured prior to release from 48S

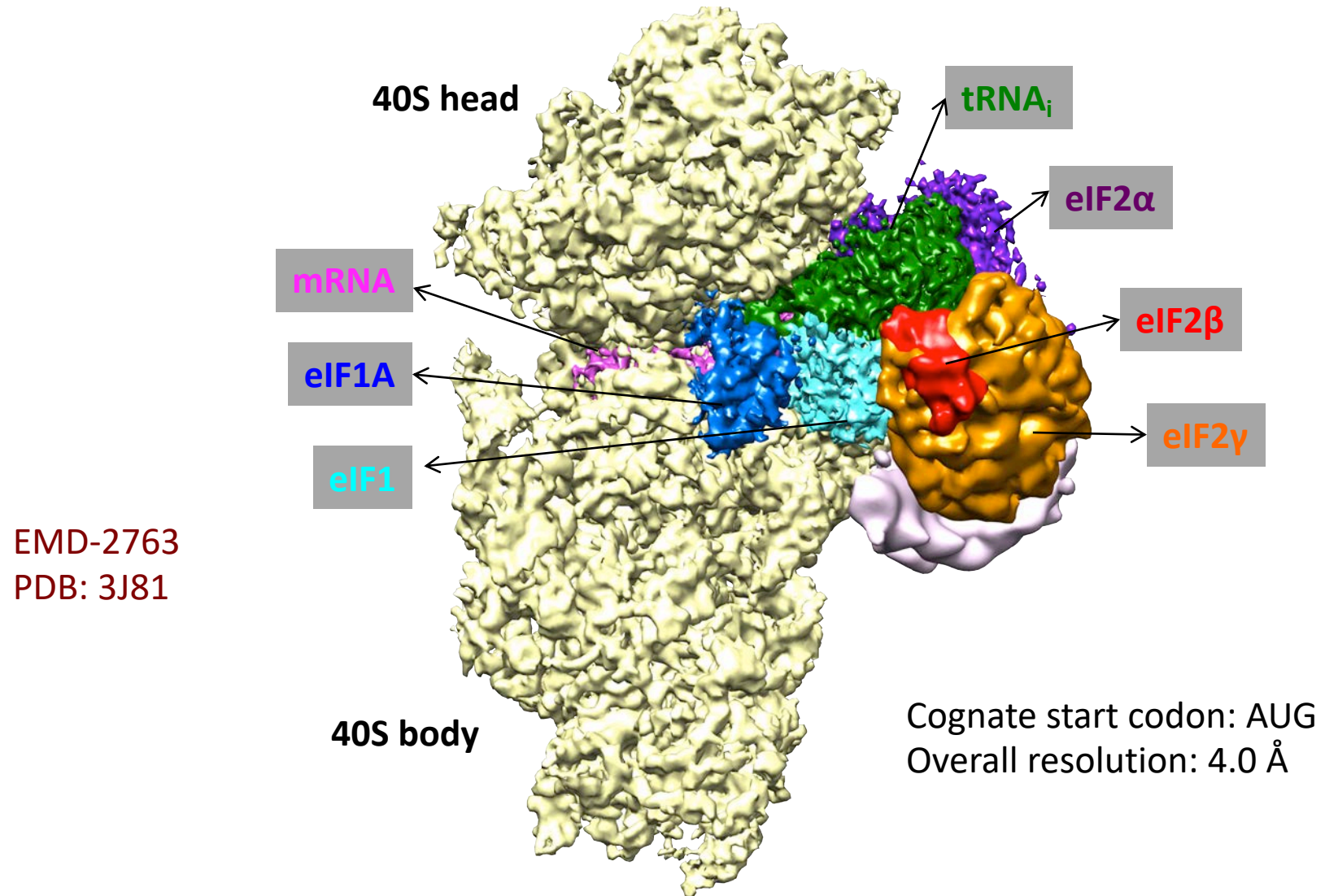eIF1 in 40S-eIF1-eIF1A complex

loop2
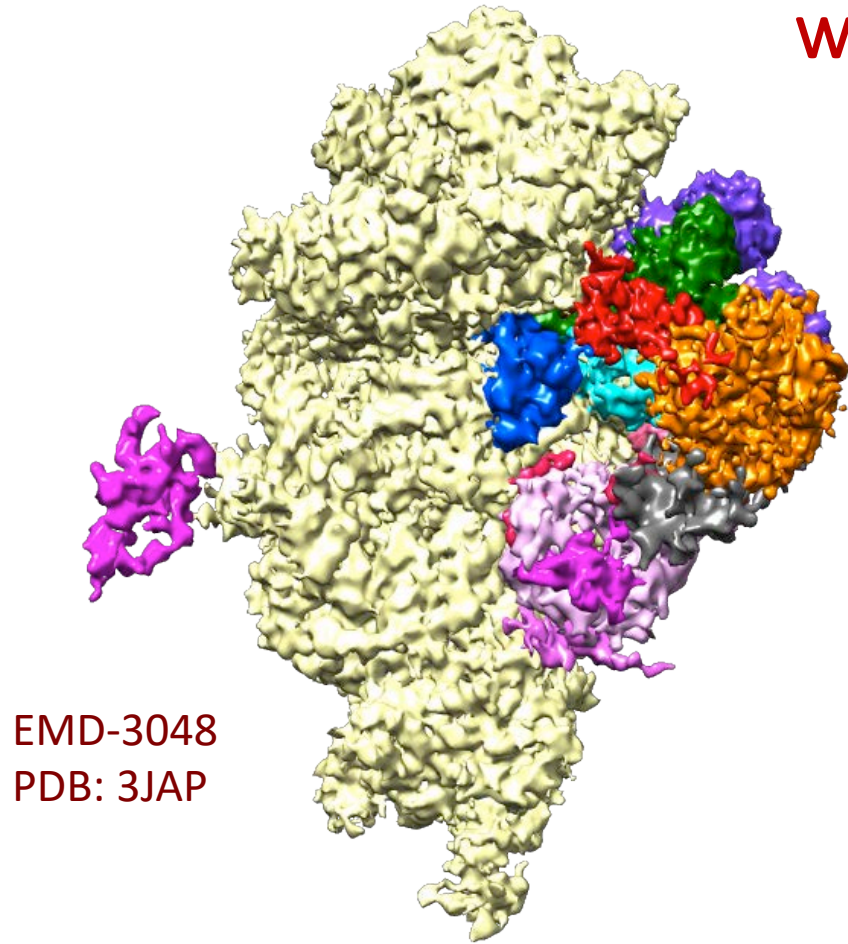
loop1

eIF1

eIF1 in py48S PIC

Movement of β-hairpin loops of eIF1
away from tRNAi in 48S

eIF1 may promote fidelity of AUG recognition by destabilizing PIN

Partial 48S complex (without eIF3) from yeast

40S head

tRNA$_i$

eIF2α

mRNA

eIF2β

eIF1A

eIF1

eIF2γ

EMD-2763
PDB: 3J81

40S body

Cognate start codon: AUG
Overall resolution: 4.0 Å

Hussain*, Llácer* et al. Cell (2014) 159: 597–607

Yeast 48S complexes with cognate and non-cognate start codon with eIF3

eIF1
eIF1A
tRNAi
eIF2α
eIF2β
eIF2γ
eIF3

EMD-3048
PDB: 3JAP

EMD-3049
PDB: 3JAQ

Cognate start codon: AUG
Overall resolution: 4.9 Å
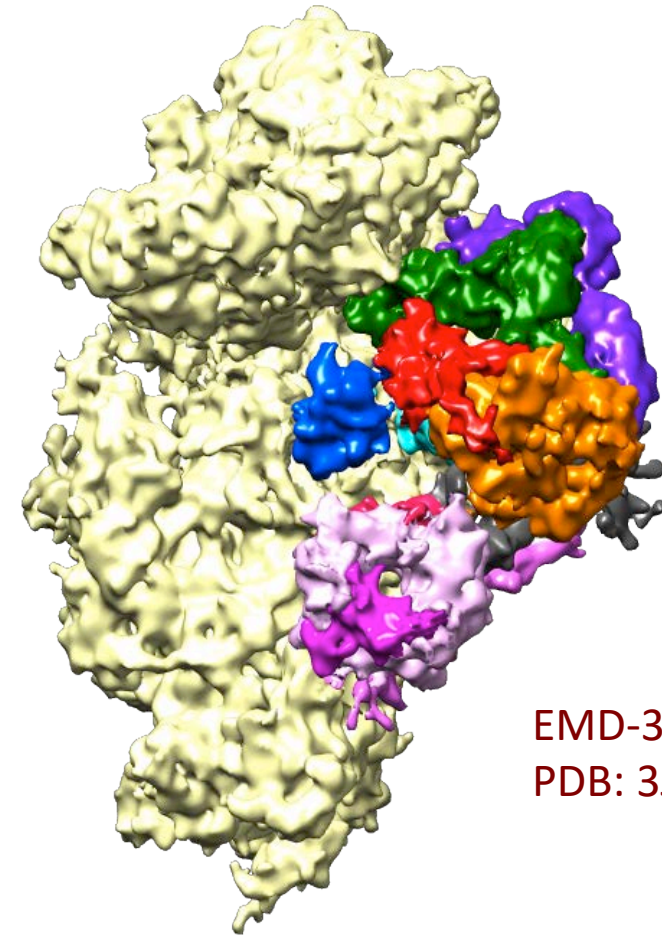
Non-Cognate start codon: AUC
Overall resolution: 6.0 Å

Llácer*, Hussain* et al. Mol Cell (2015) 59: 399–412

New dataset: 48S-AUG (cognate codon)

- Wild type version of eIF2

- Again py48S, ~15% of the particles

- But, after further 3D classification, only 2% of particles

48S-AUG PIC with eIF3
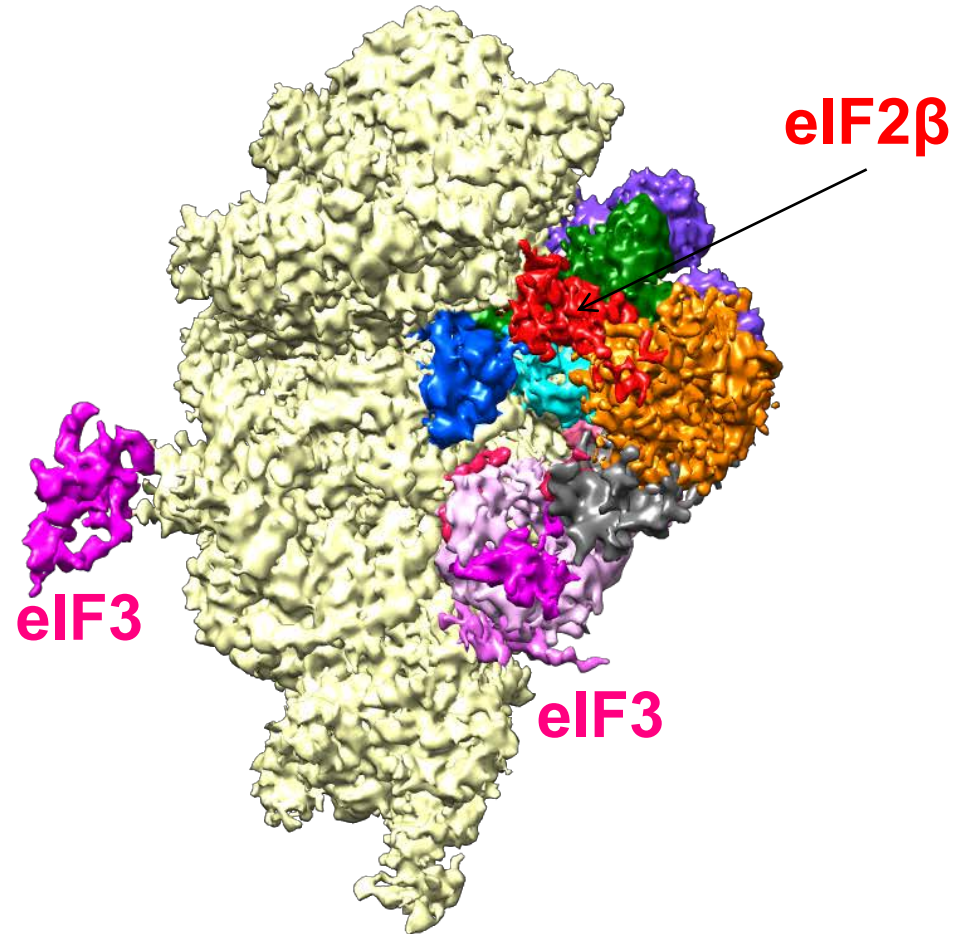
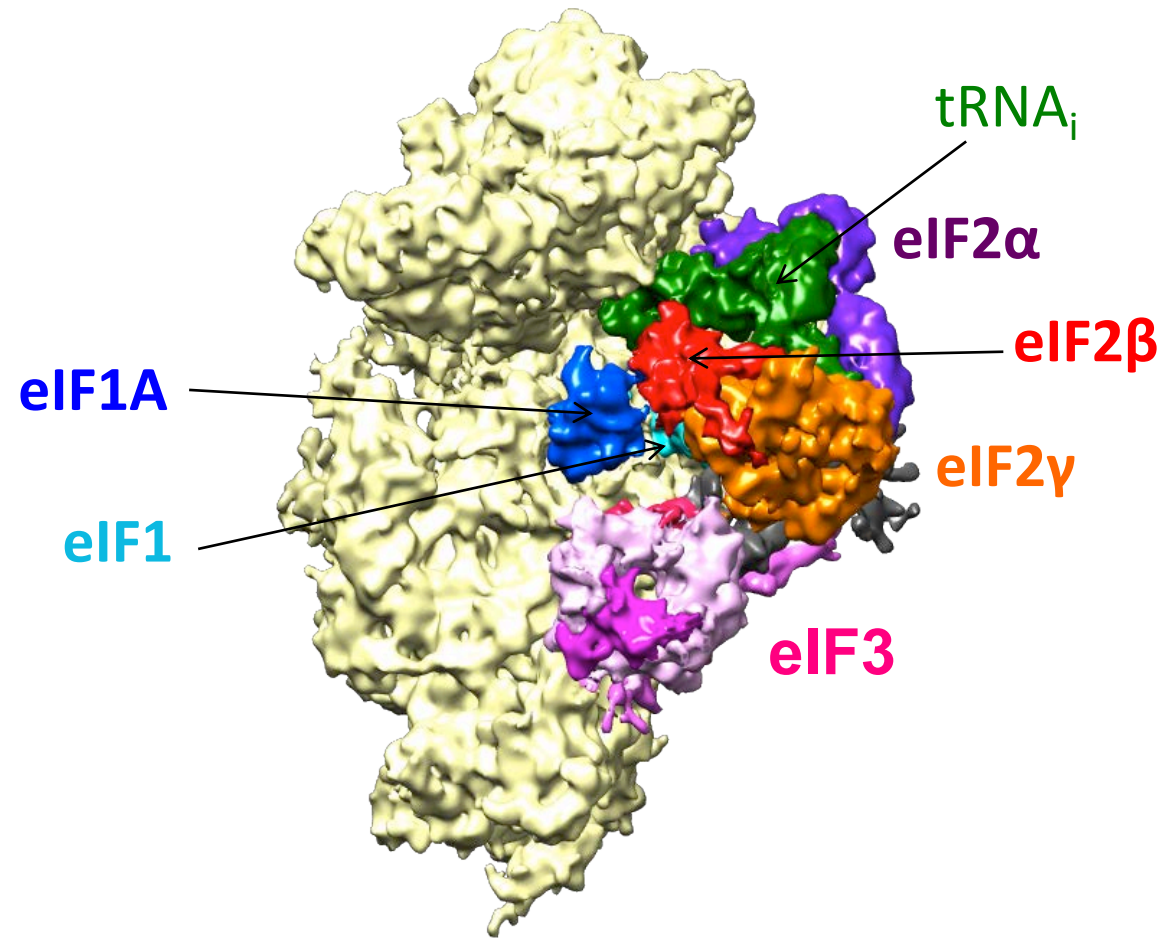# Different components used to reconstitute 48S complex with non-cognate start codon

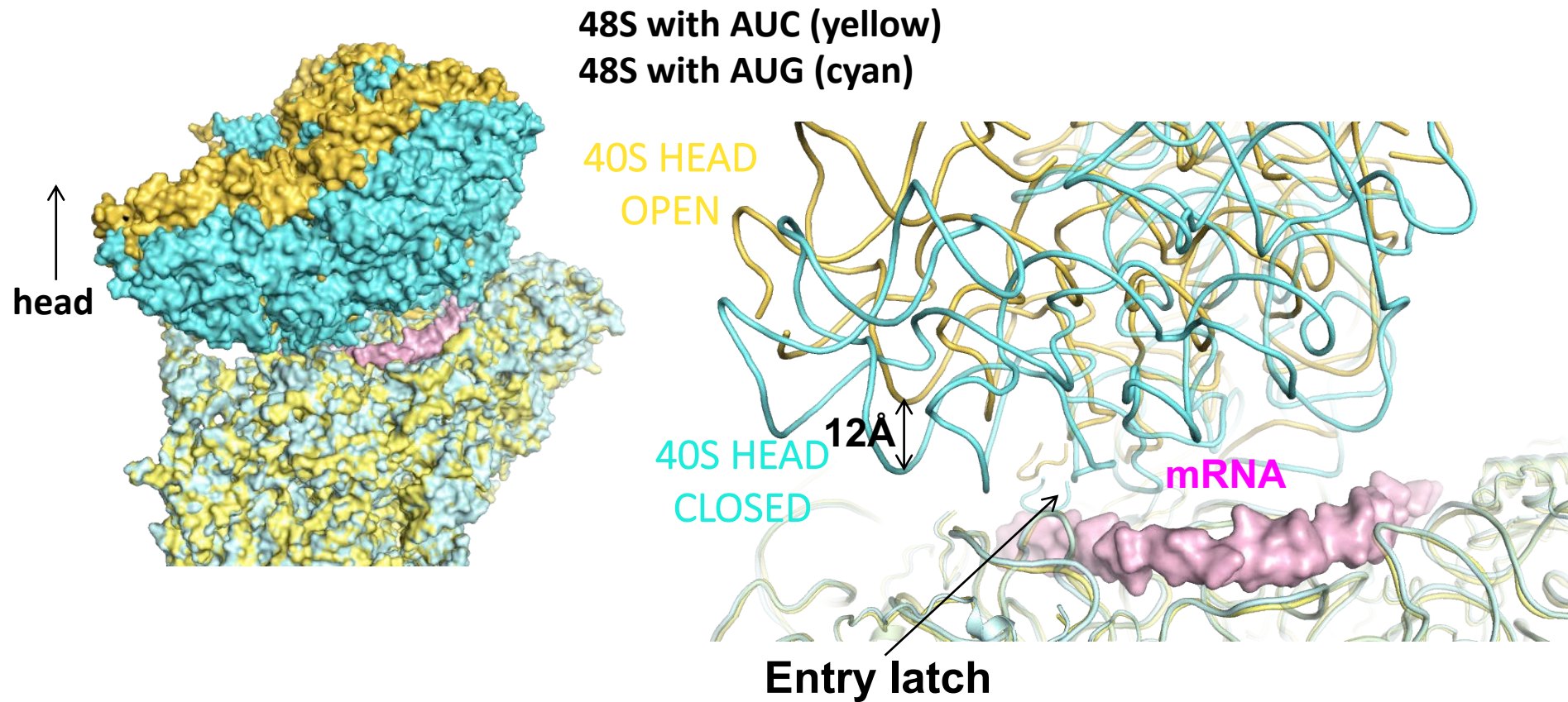| Component	Non-cognate	Cognate
mRNA	Unstructured mRNA with AUC	Unstructured mRNA with AUG
tRNAi	Wild type tRNAi	tRNAi variant (U31•A39) (favouring P_{IN} state)
eIF5	NONE	YES (shifts the equilibrium towards P_{IN} state)
eIF3	Recombinant eIF3 expressed in bacteria (hence free of eIF5)	eIF3 expressed in yeast (may contain co-purified eIF5)

Reconstitution of the yeast 48S-AUC complex:

40S + eIF1 + eIF1A + eIF3 + TC + mRNA(AUC)
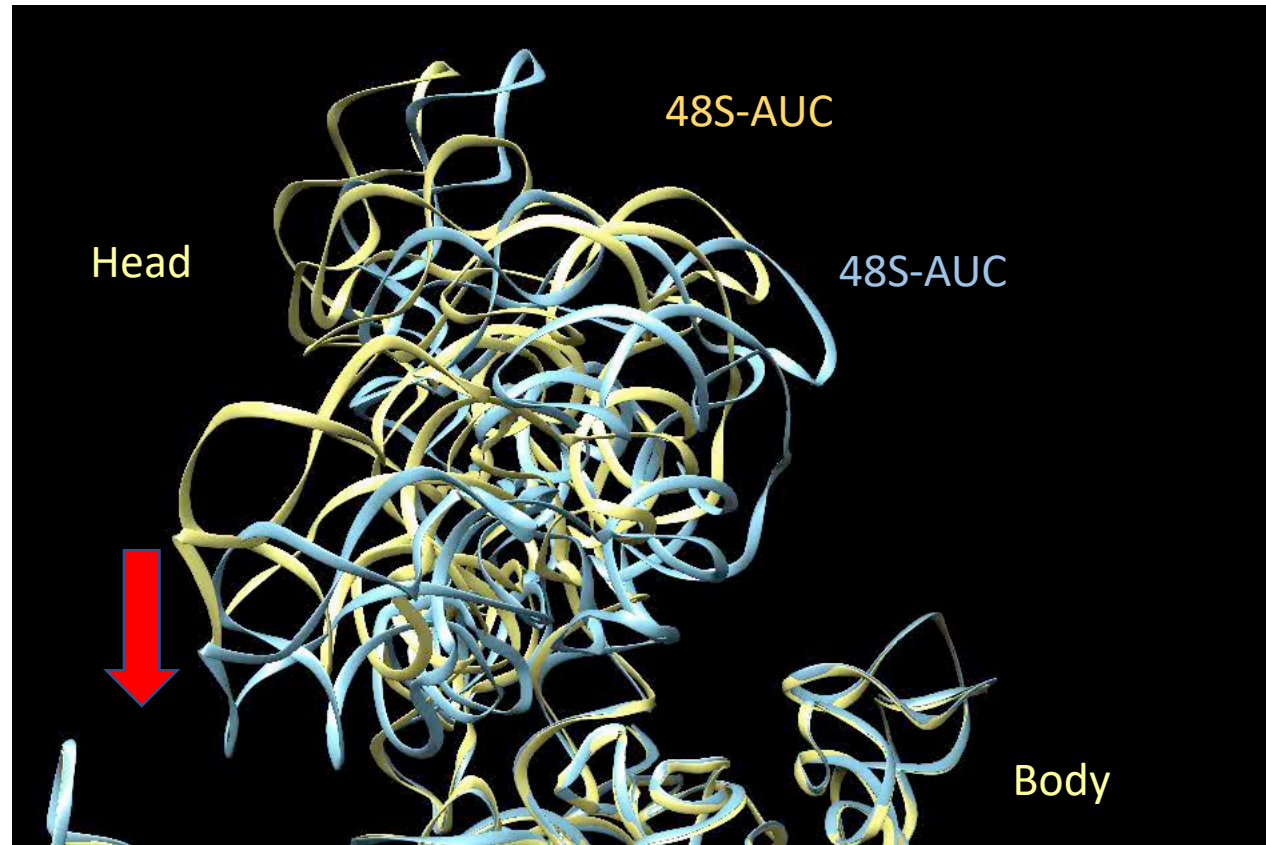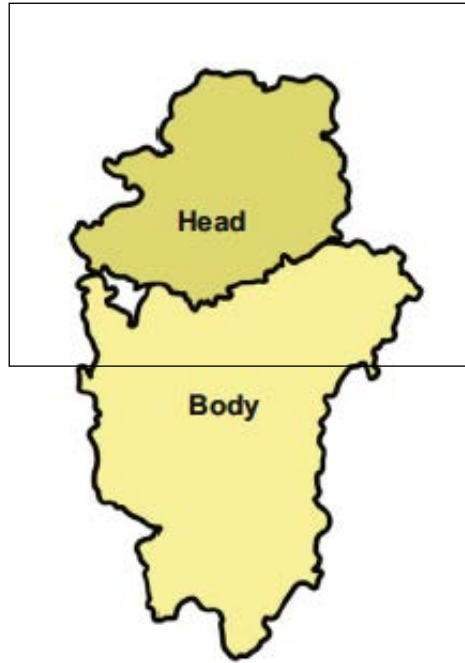
TC= eIF2 + GDPCP + tRNAi
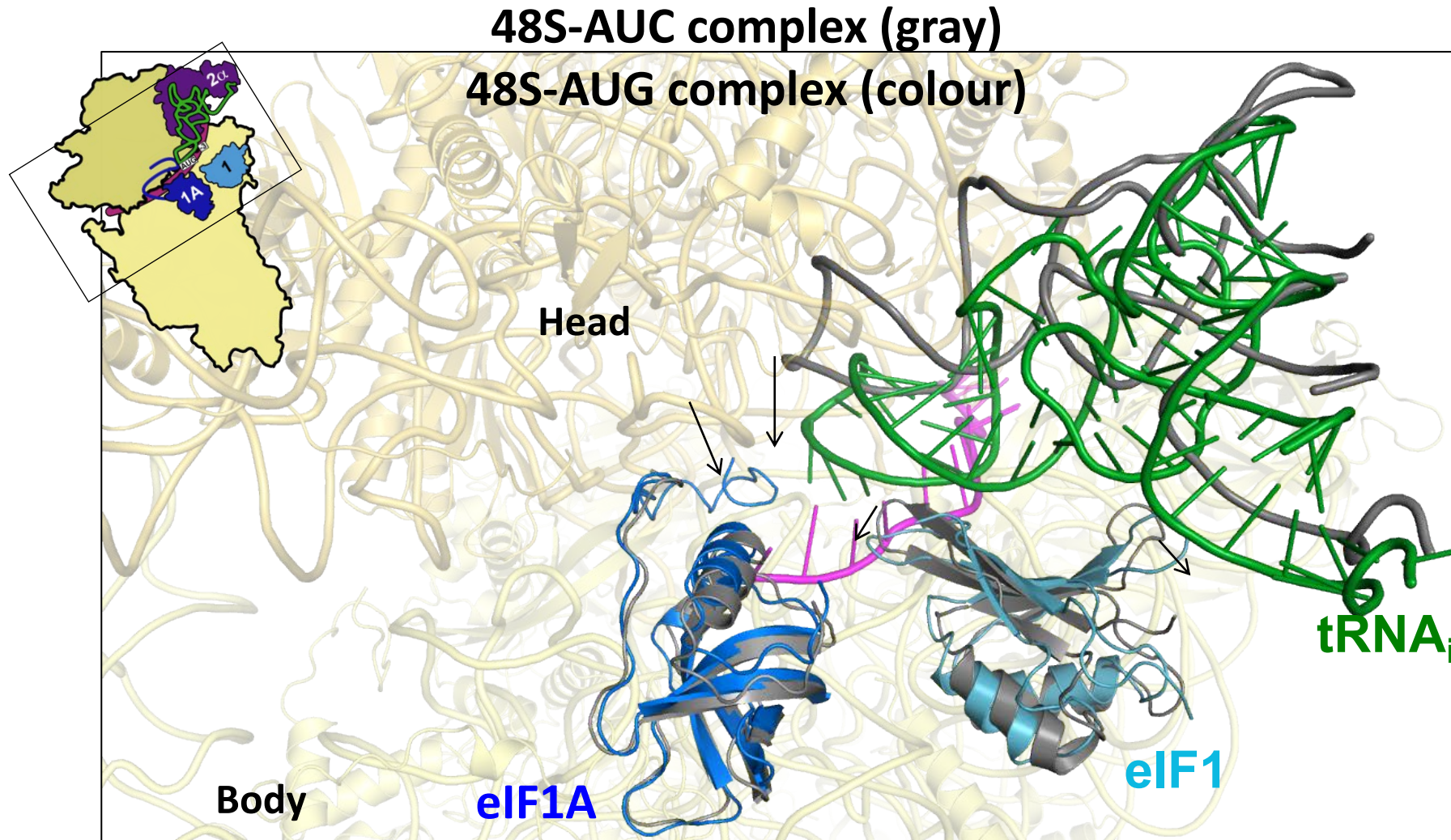
48S complex with non-cognate AUC codon at 6.0 Å

tRNA_i
eIF2α
eIF2β
eIF1A
eIF2γ
eIF1
eIF3

Comparison between 48S- AUG and AUC complexes

48S with AUC (yellow)
48S with AUG (cyan)

40S HEAD OPEN

head

12Å

40S HEAD CLOSED

mRNA

Entry latch

40S head movement from 48S- AUC to AUG complex

eIF1 and eIF1A movements

48S-AUC complex (gray)
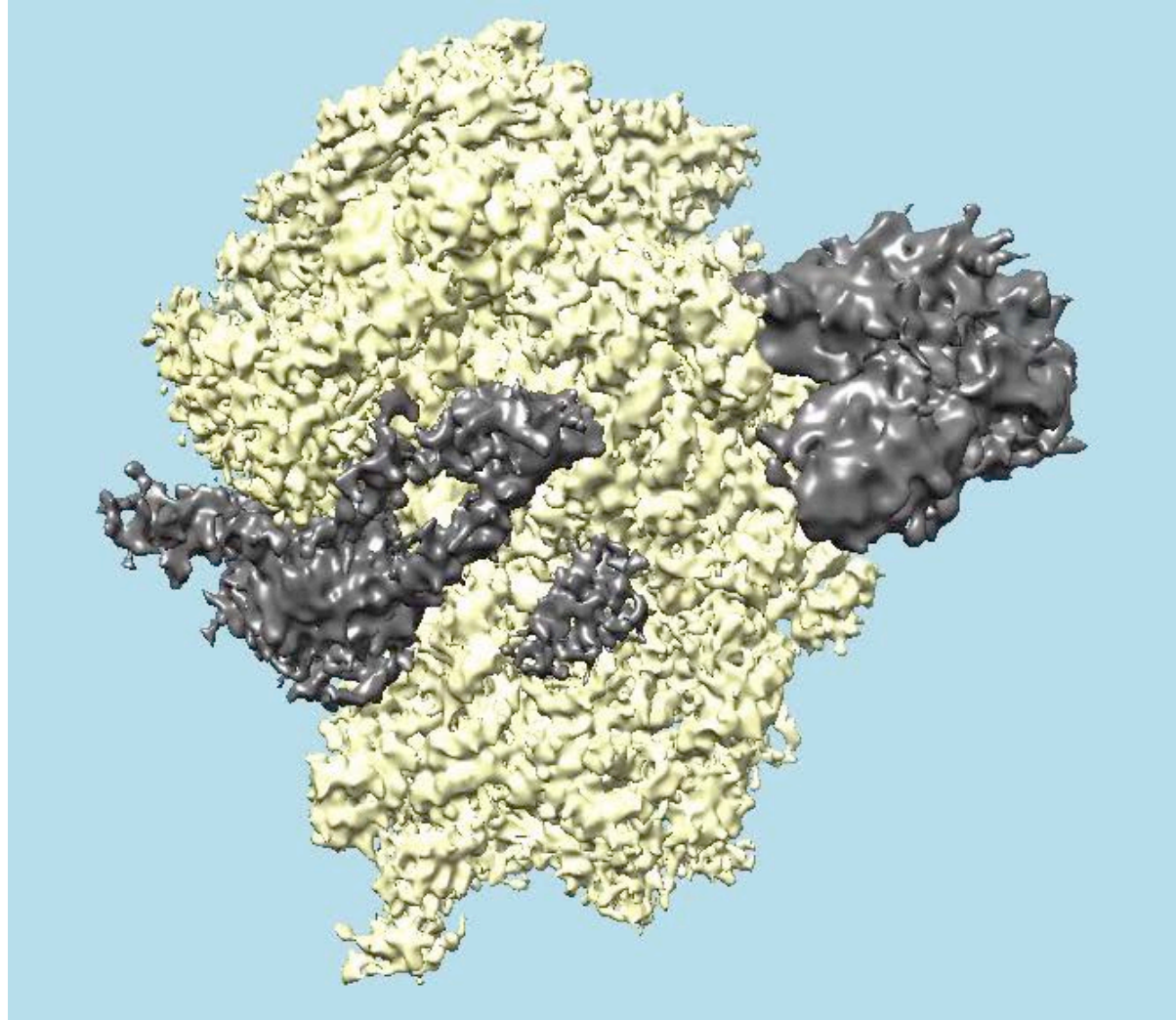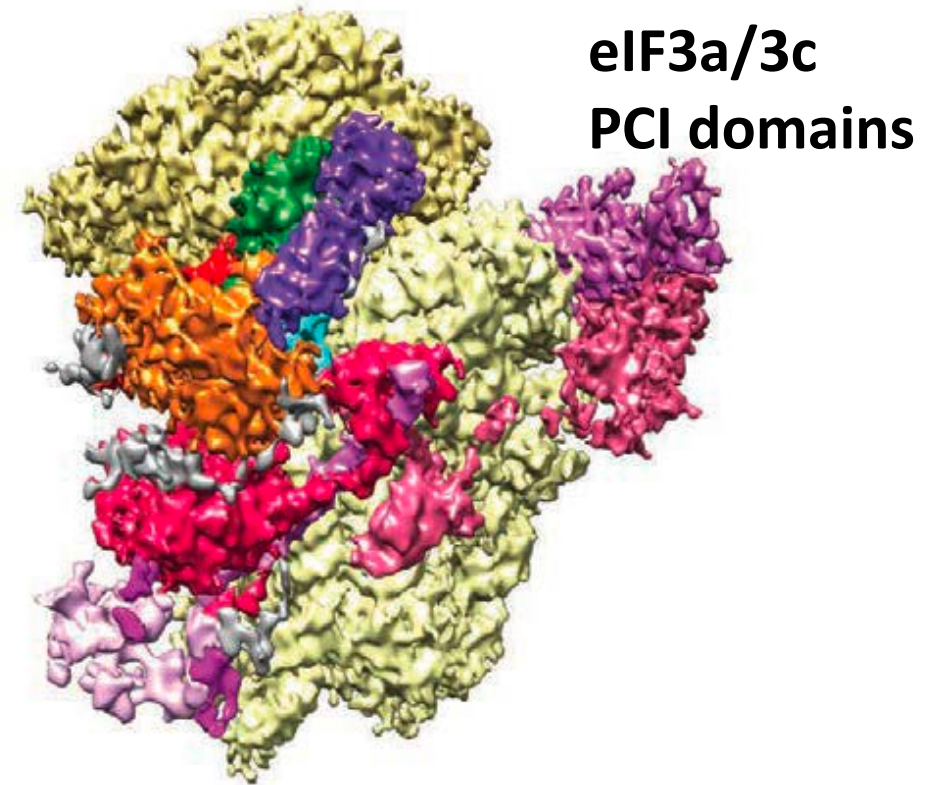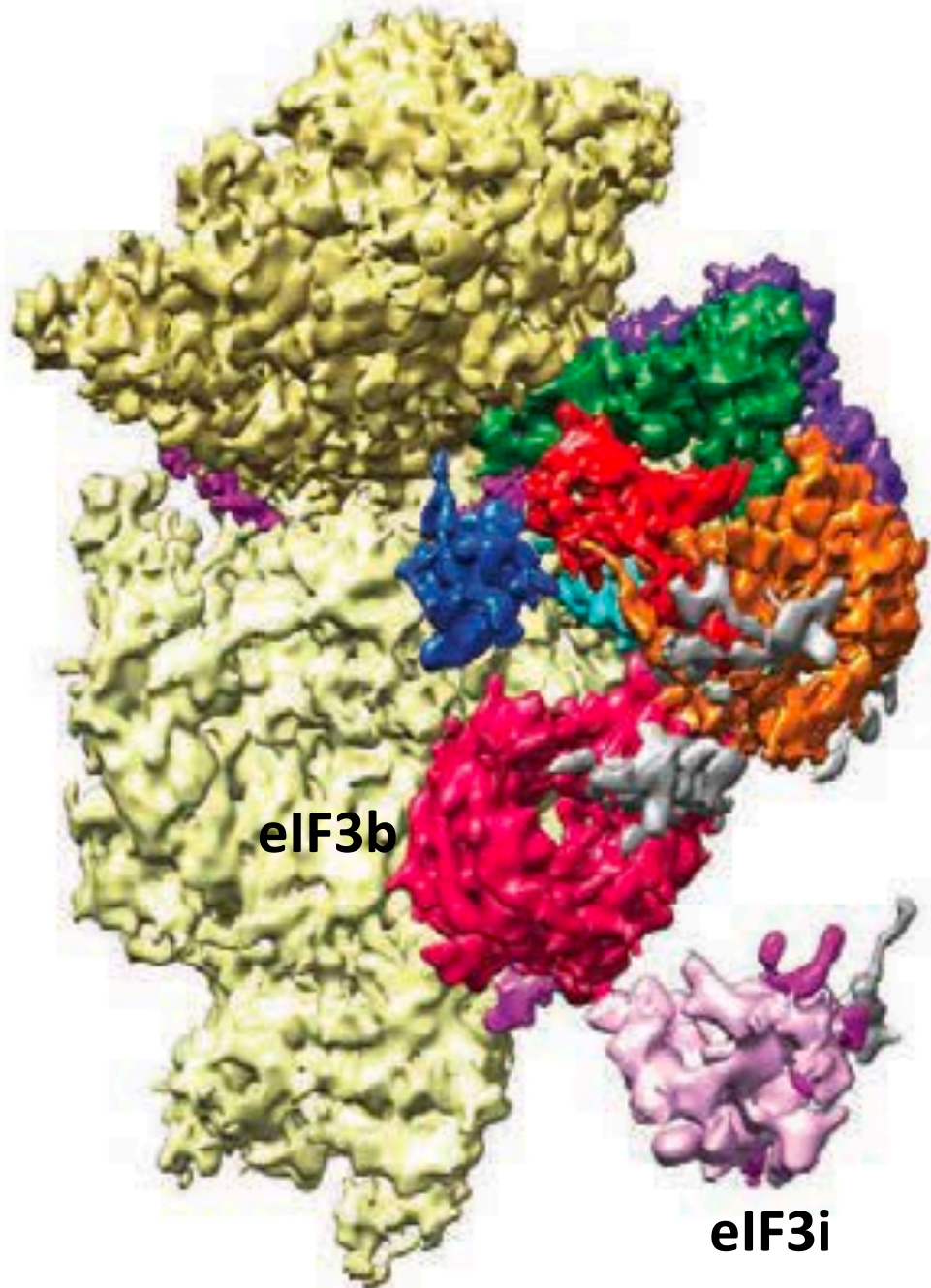**48S-AUG complex (colour)**

The NTT of eIF1A is only present in the 48S-AUG complex

Major conformational changes between 48S-AUC and 48S-AUG complexes
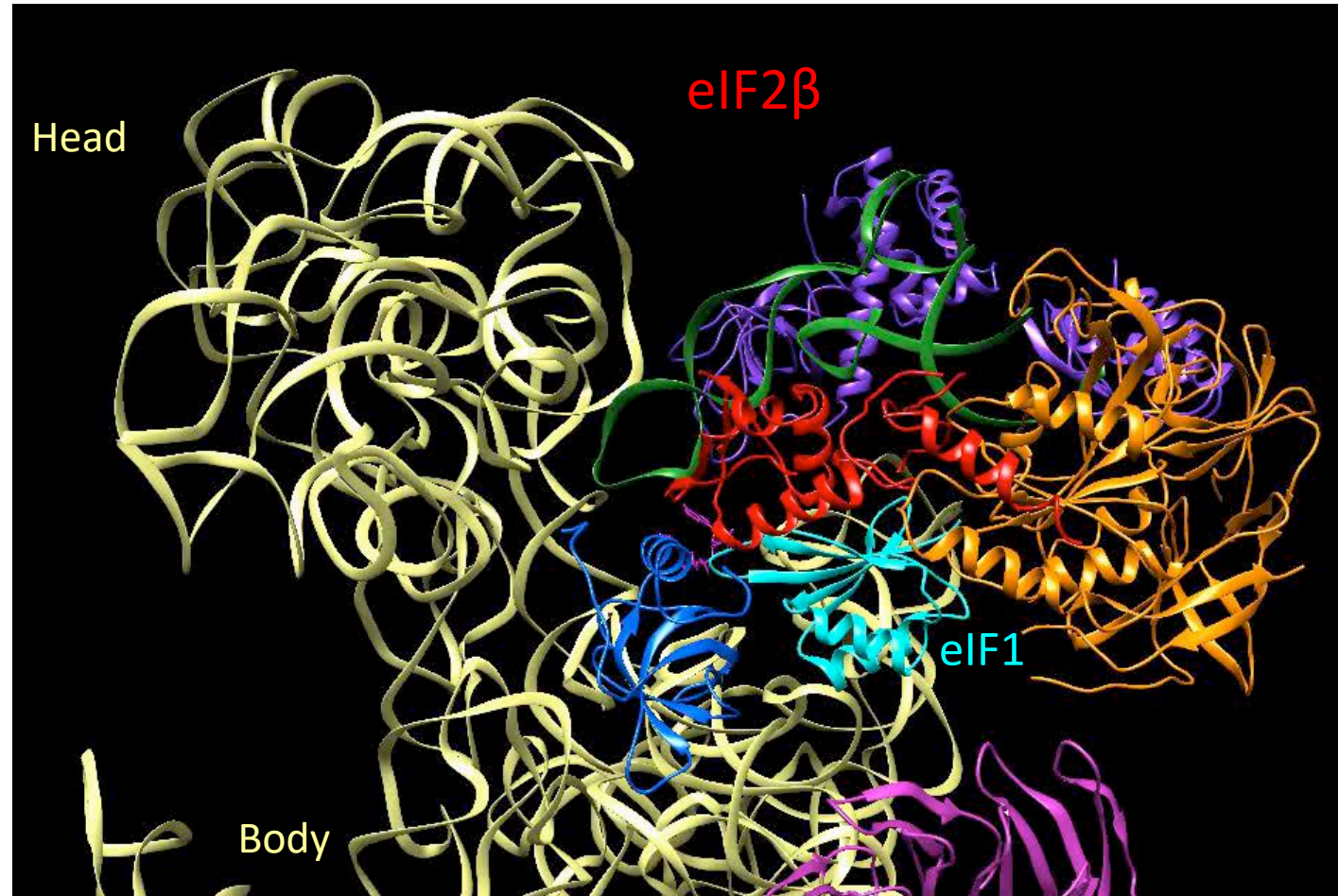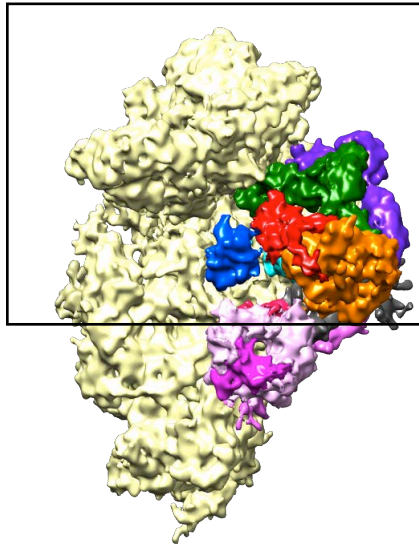
eIF3 seems to encircle the 40S

eIF3a/3c
PCI domains

eIF3b
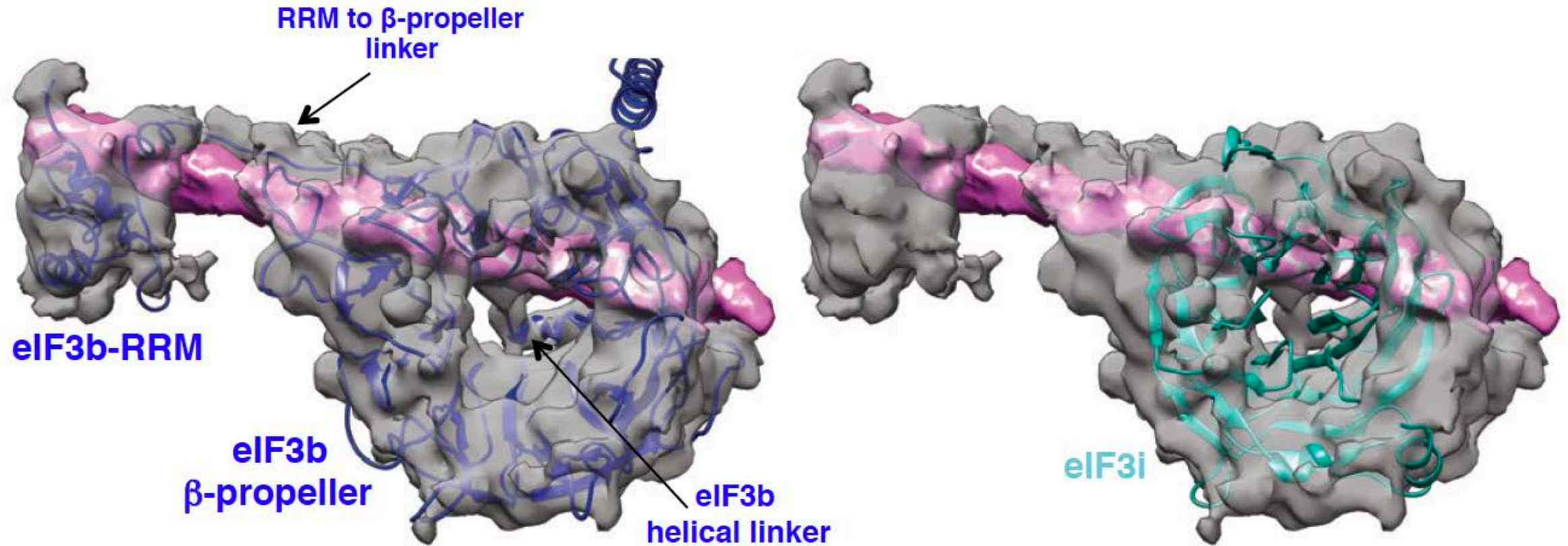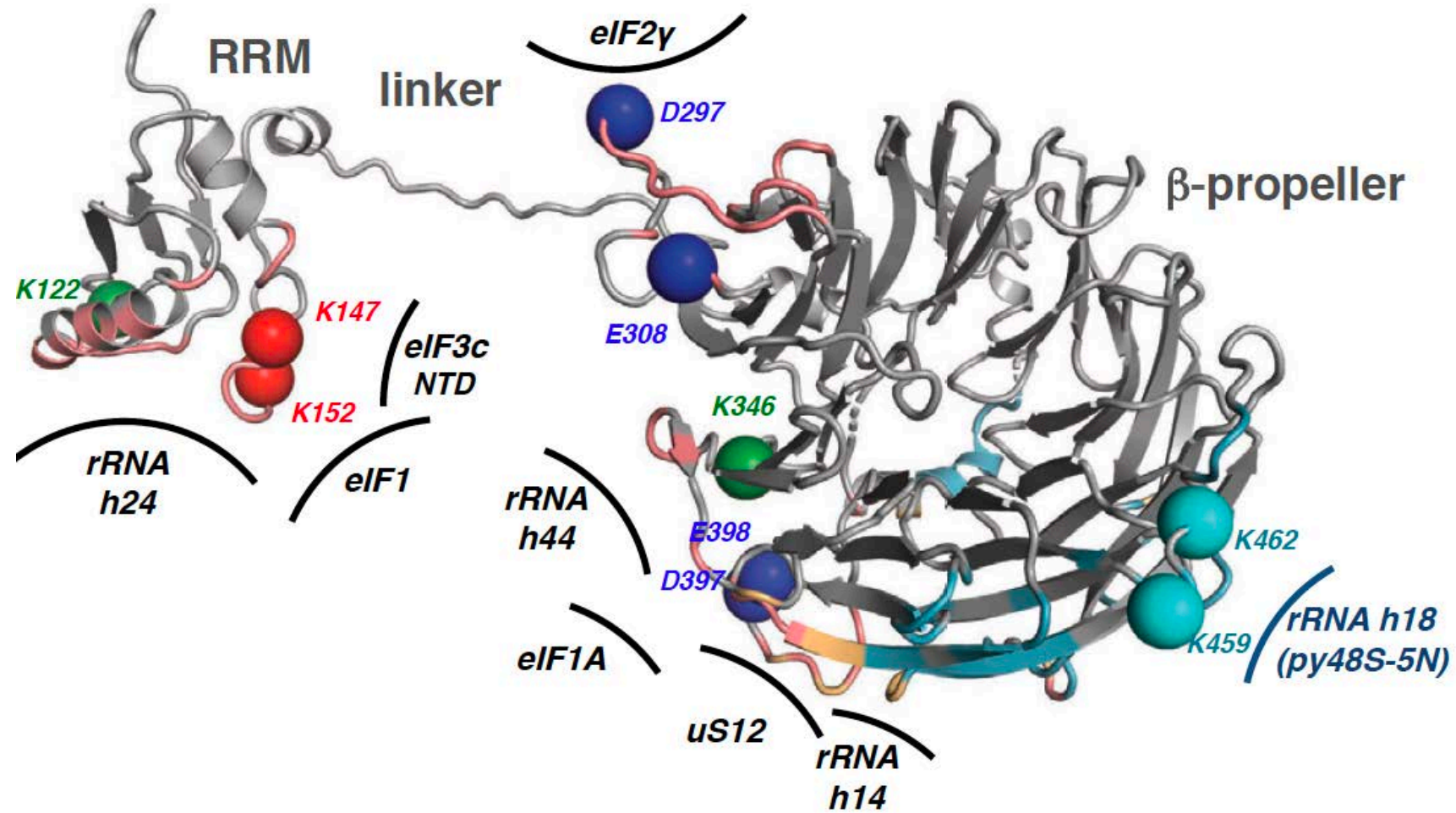
eIF3i

eIF3b and eIF3i relocate together to the ribosomal subunit interface during translation initiation and modulate start codon selection

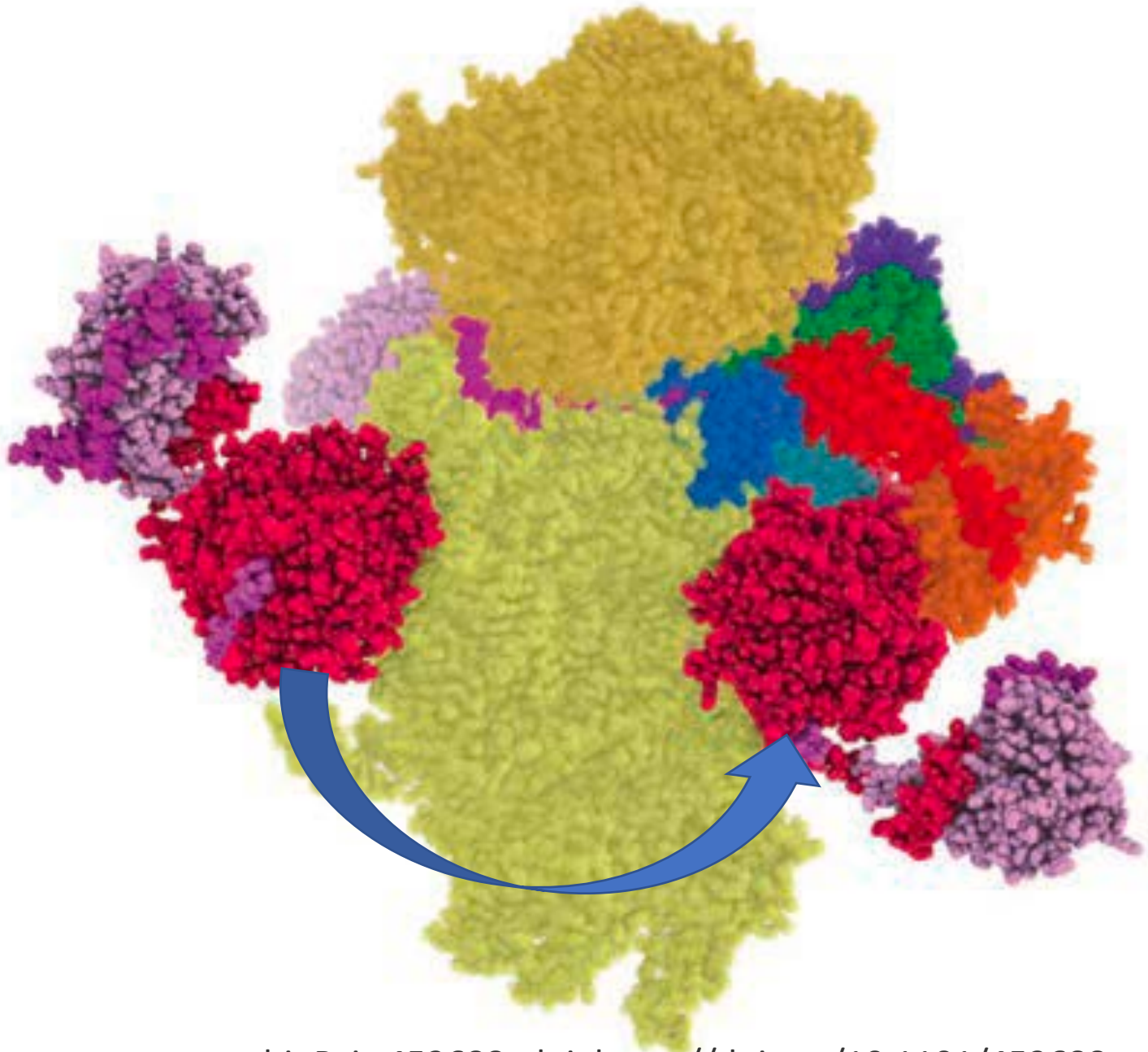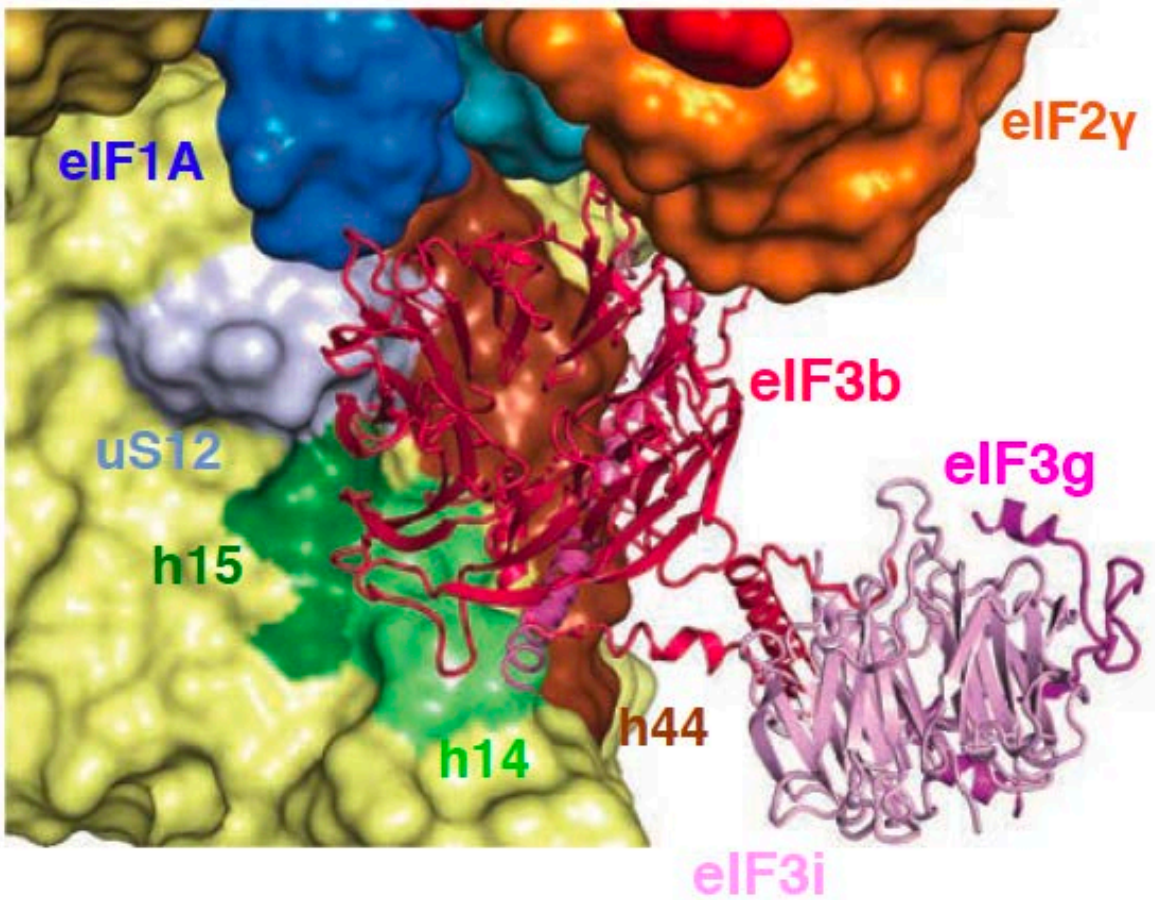# eIF3b beta-propeller in density

RRM to β-propeller linker

eIF3b-RRM

eIF3b β-propeller

eIF3b helical linker

eIF3i

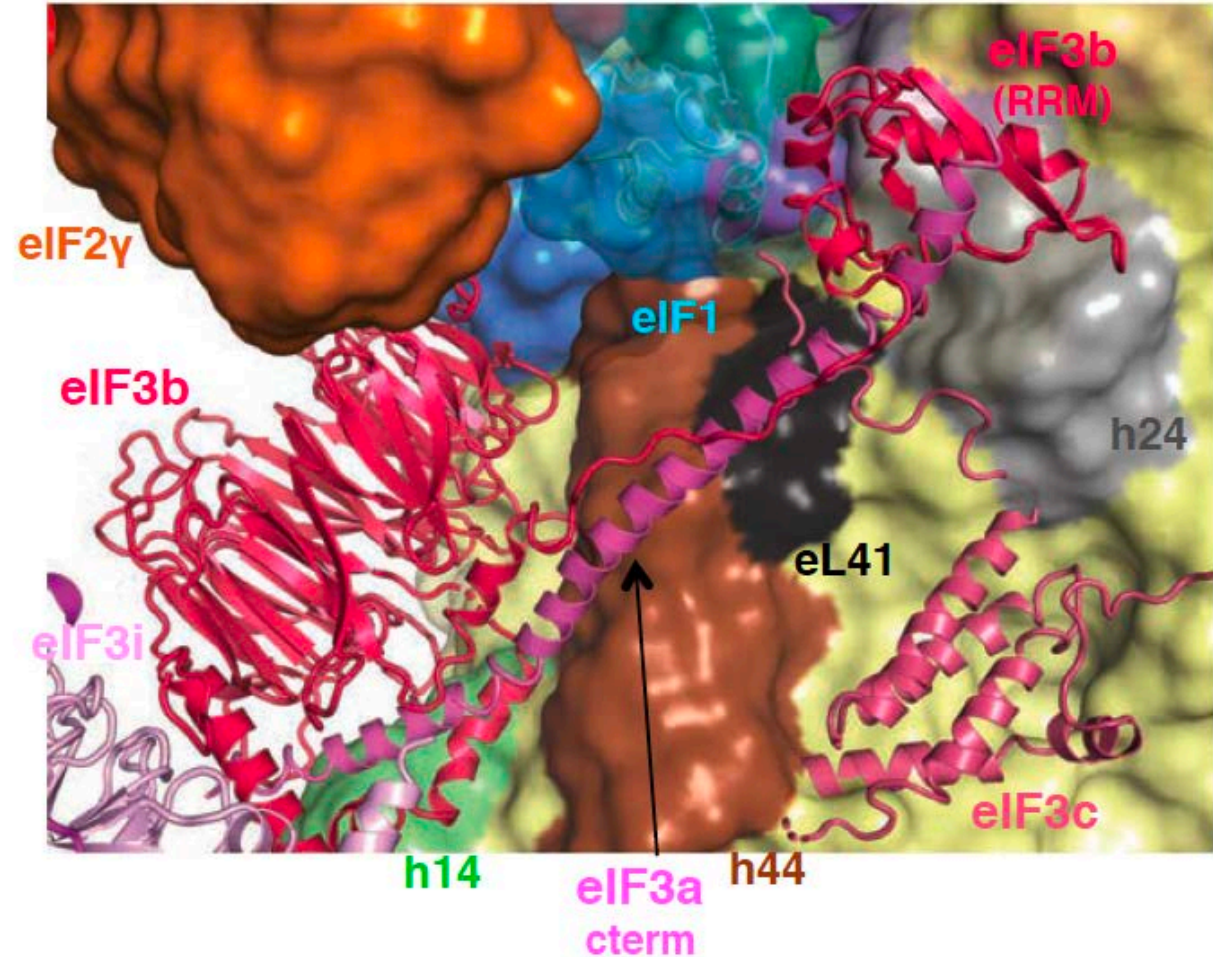# Mutational analysis of eIF3b ressidues

eIF3b/3i relocates to subunit interface from its position on solvent interface

How eIF3 interacts with eIF1 and eIF2?

eIF3 subunit binds close to eIF2γ

N-terminal region of eIF3c in contact with eIF1

Can the OPEN conformation of 48S discriminate between cognate, near-cognate and non-cognate codons?

In collaboration with Prof. Prabal Maiti
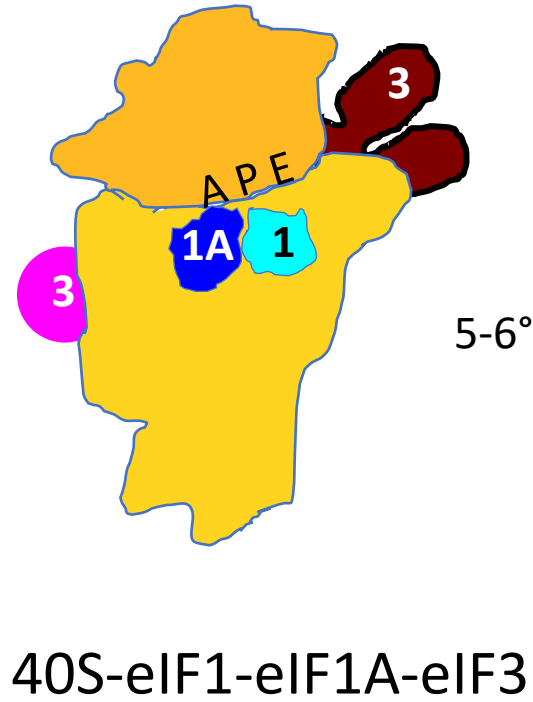Department of Physics, IISc

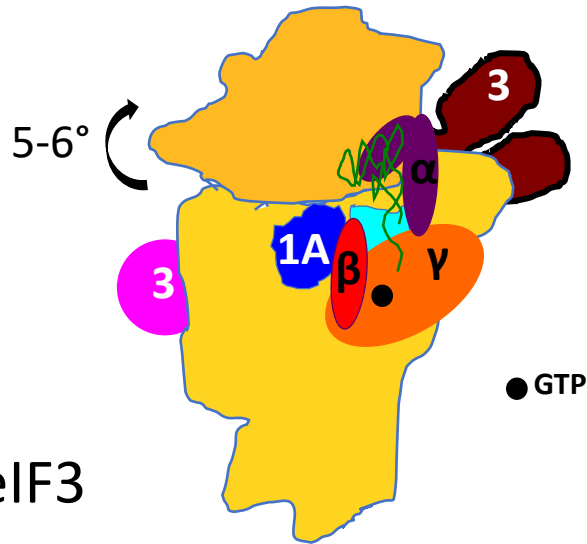Selection of start codon during mRNA scanning in eukaryotic translation initiation

Ipsita Basu, Biswajit Gorai, Thyageshwar Chandran, Prabal K. Maiti, Tanweer Hussain

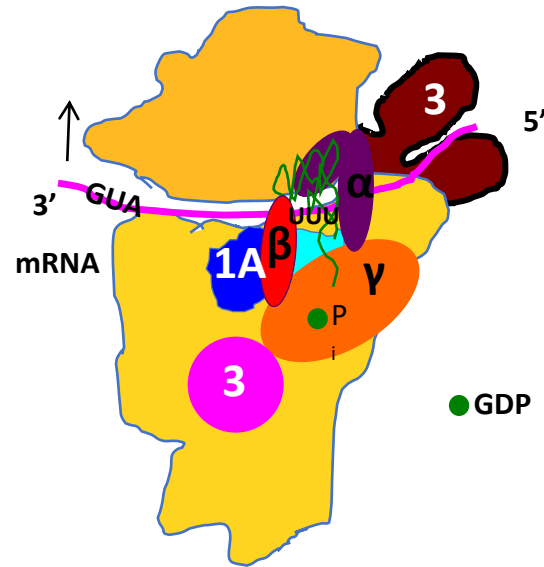bioRxiv 2020.11.06.371484; doi: https://doi.org/10.1101/2020.11.06.371484

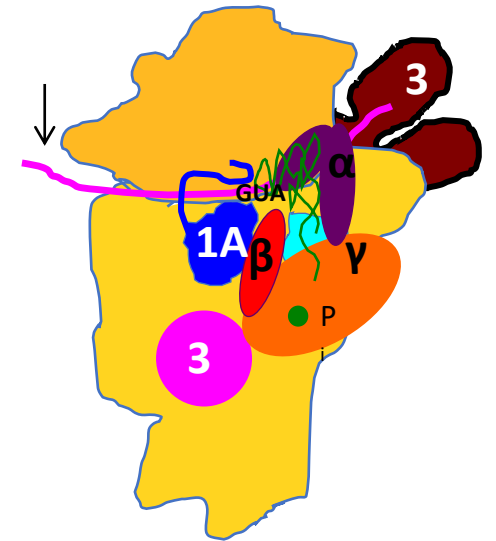# What these structures have revealed about the mechanism of initiation

40S-eIF1-eIF1A-eIF3

43S (P$_{OUT}$)

48S (open, scanning)

48S (P$_{IN}$, closed, Scanning-arrested)

Where does eIF5 bind?

- eIF5 is a GTPase activating protein (GAP)
- Stimulates the hydrolysis of GTP in eIF2 complex
- Contains two domains: N and C-terminus domains (NTD and CTD)
- Arg15 is important for GAP activity
- CTD interacts with eIF1

48S PIC with eIF5 (NTD) at 3 Å

Head

eIF3i

mRNA

eIF1A

eIF3b

Body

eIF2α

tRNA$_i$

eIF2β

eIF2γ

eIF5 NTD

eIF3a

eIF3c

eIF3c
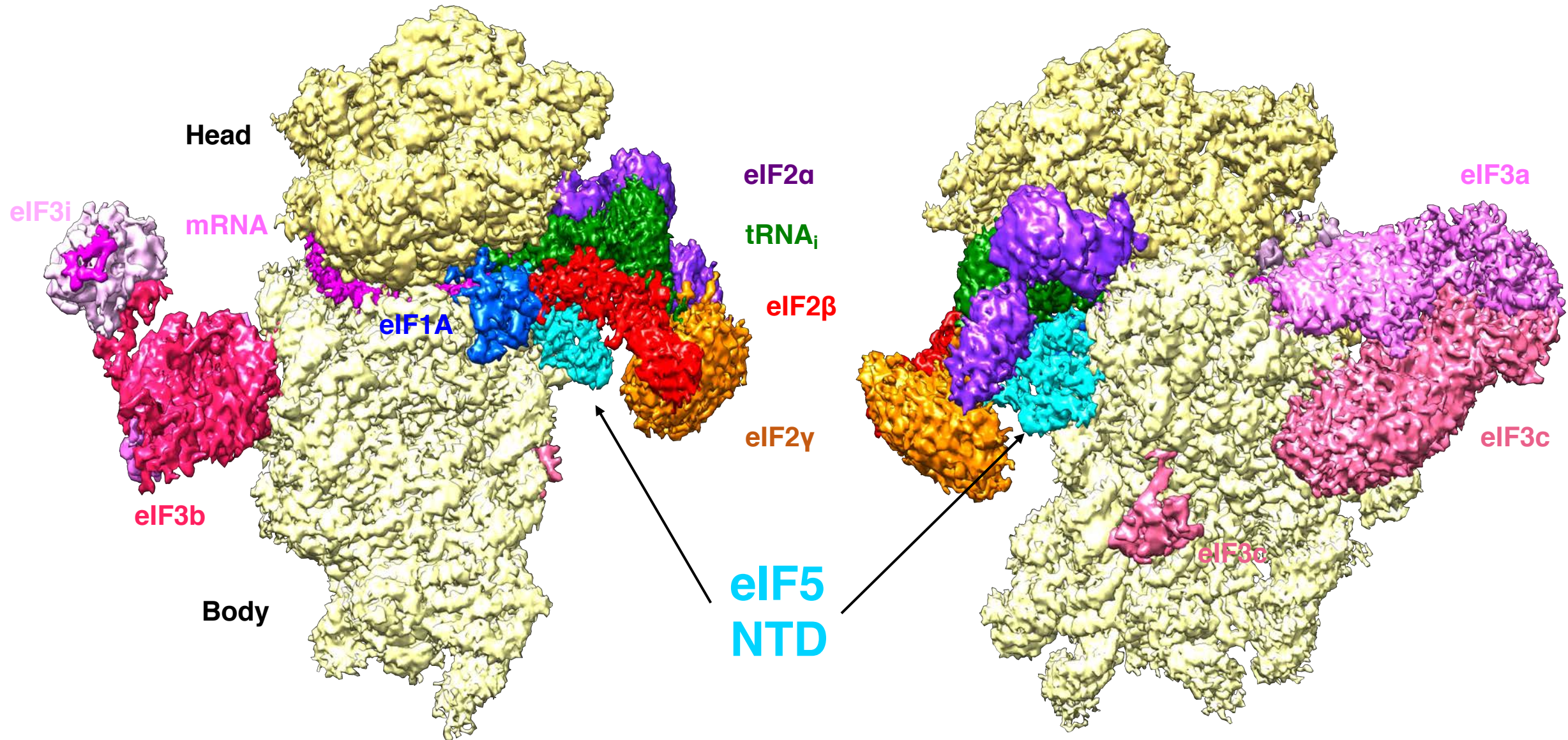
48S PIC with eIF5 (NTD)
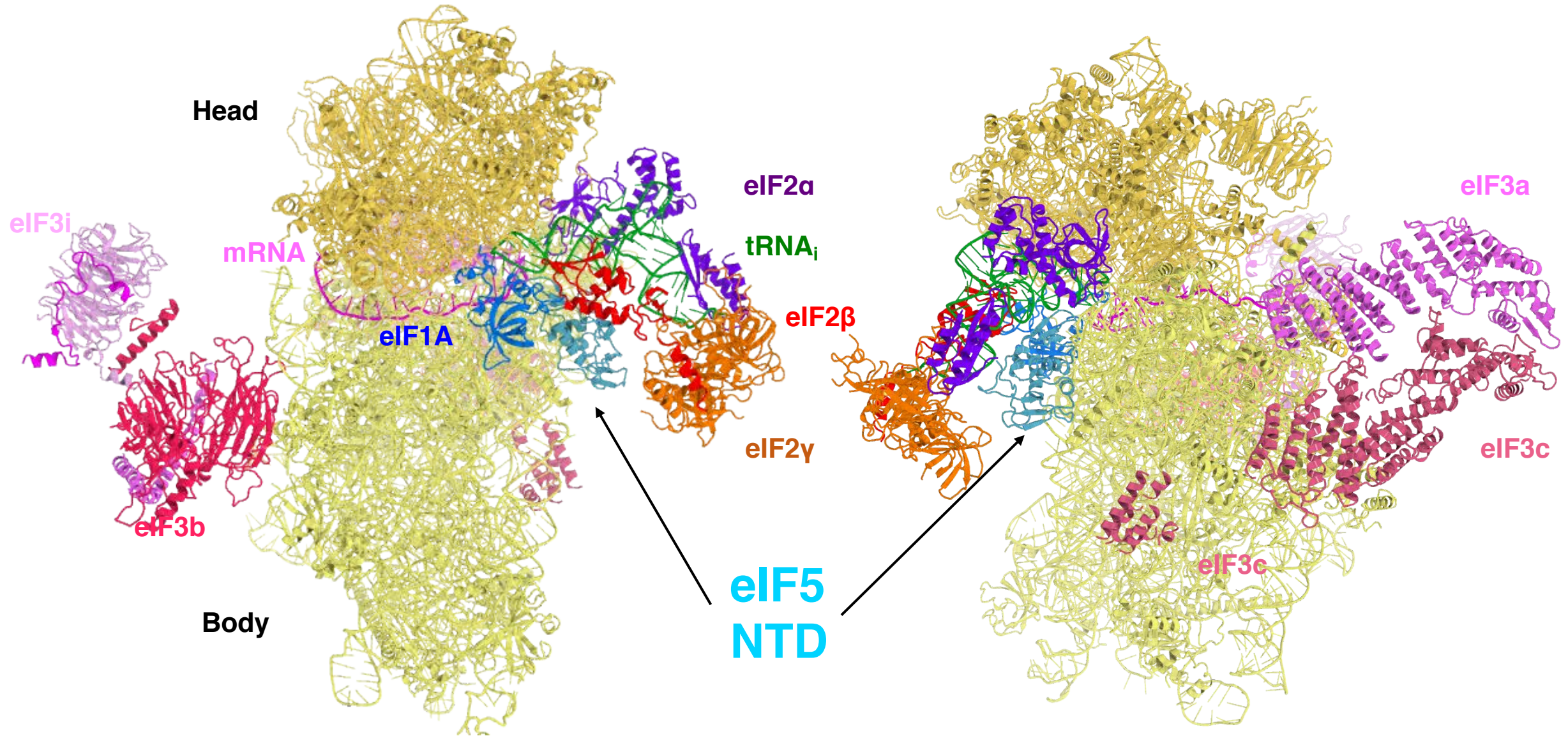
Fitting of eIF5-NTD in map

tRNA_i

mRNA

eIF5

eIF1A

α3

ZBD

eIF5-NTD

eIF1

Llácer, Hussain et al. eLife 2018;7:e39273.
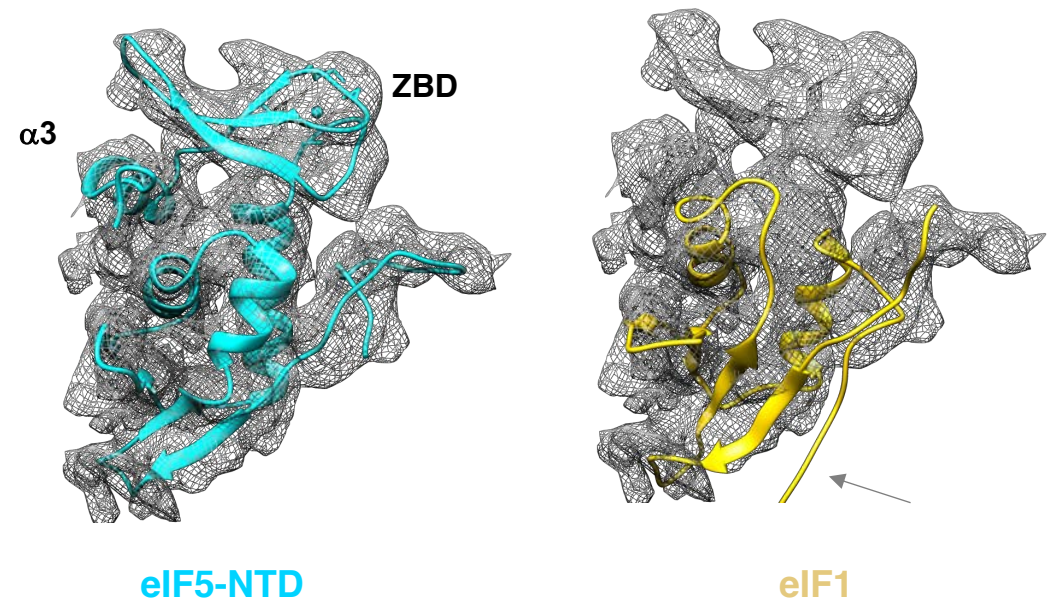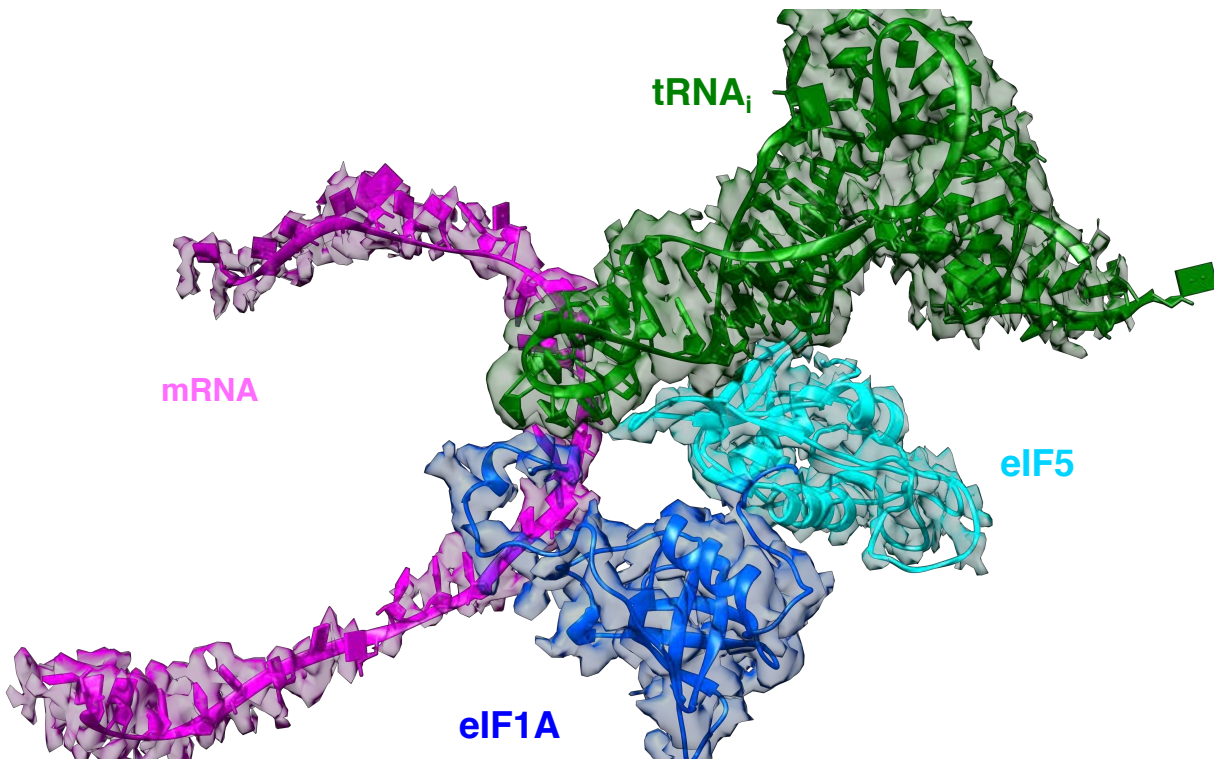
eIF5-NTD binds near P site in P$_{IN}$ state of 48S
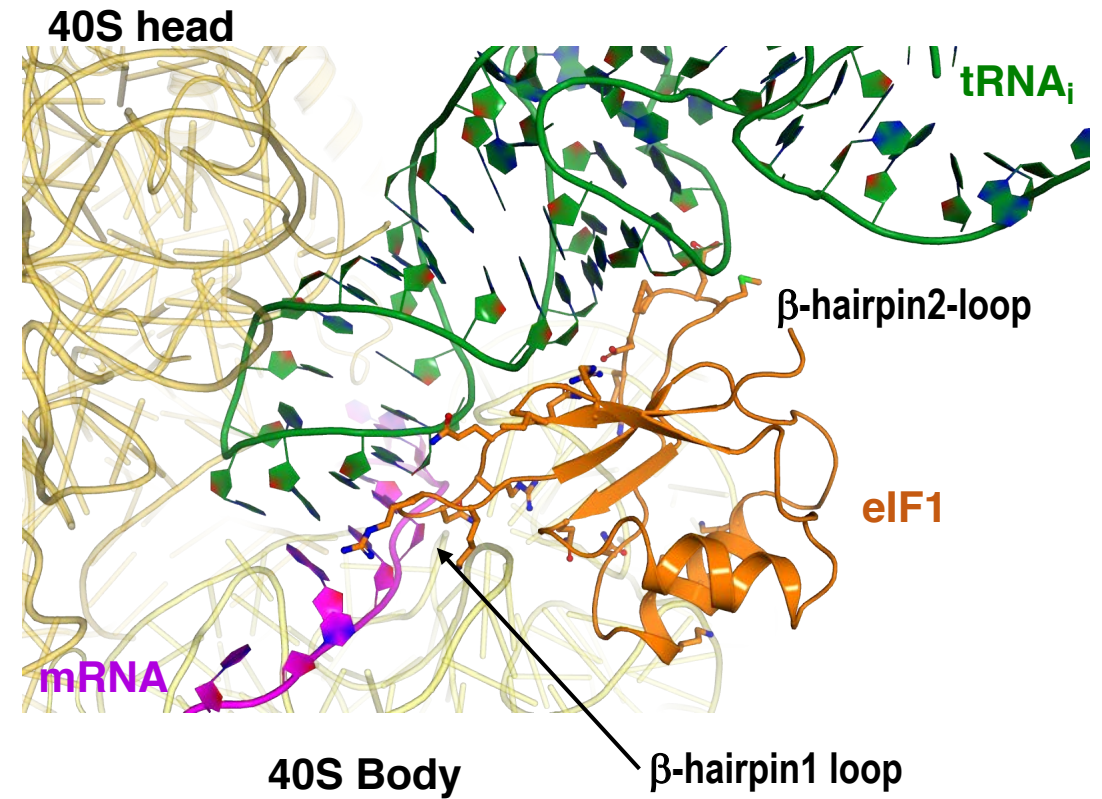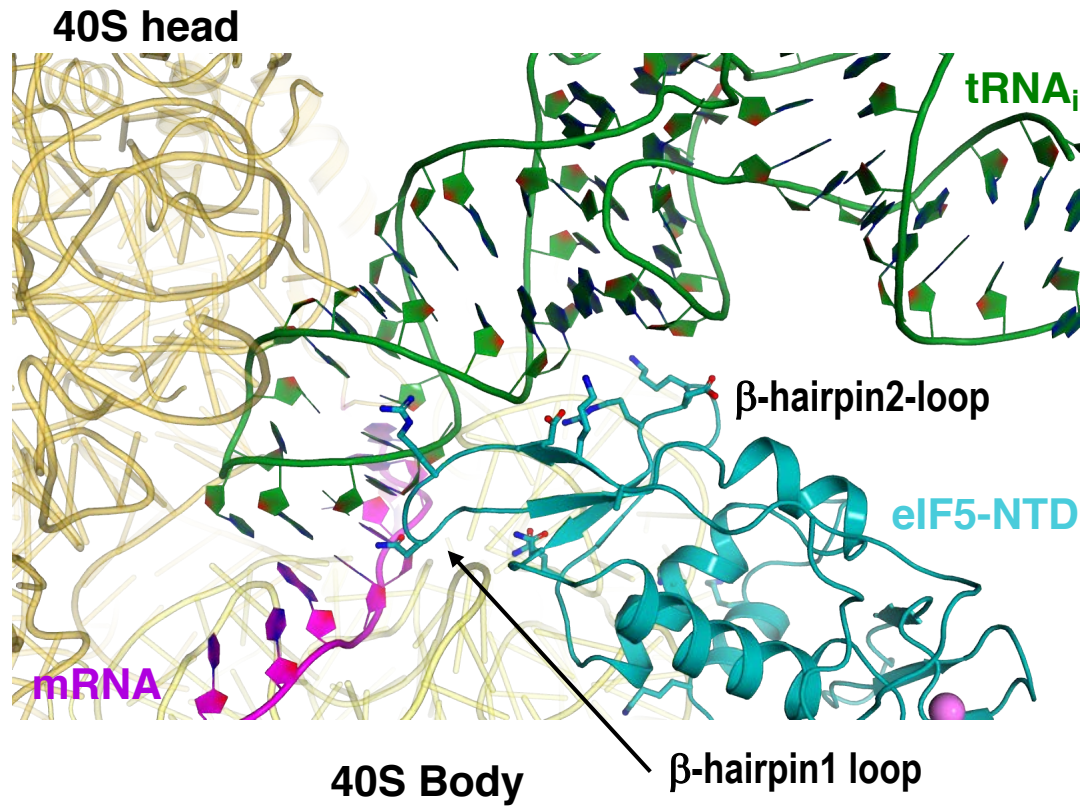
eIF5-NTD occupies the position left vacant by eIF1 after start codon recognition

tRNA_i (py48S)

tRNA_i

eIF1

eIF5-NTD

β-hairpin loop 2

eIF2γ

GDPCP

13Å

Arg15

eIF5-NTD

Arg15, which is important for GAP activity, is away from GTP analog
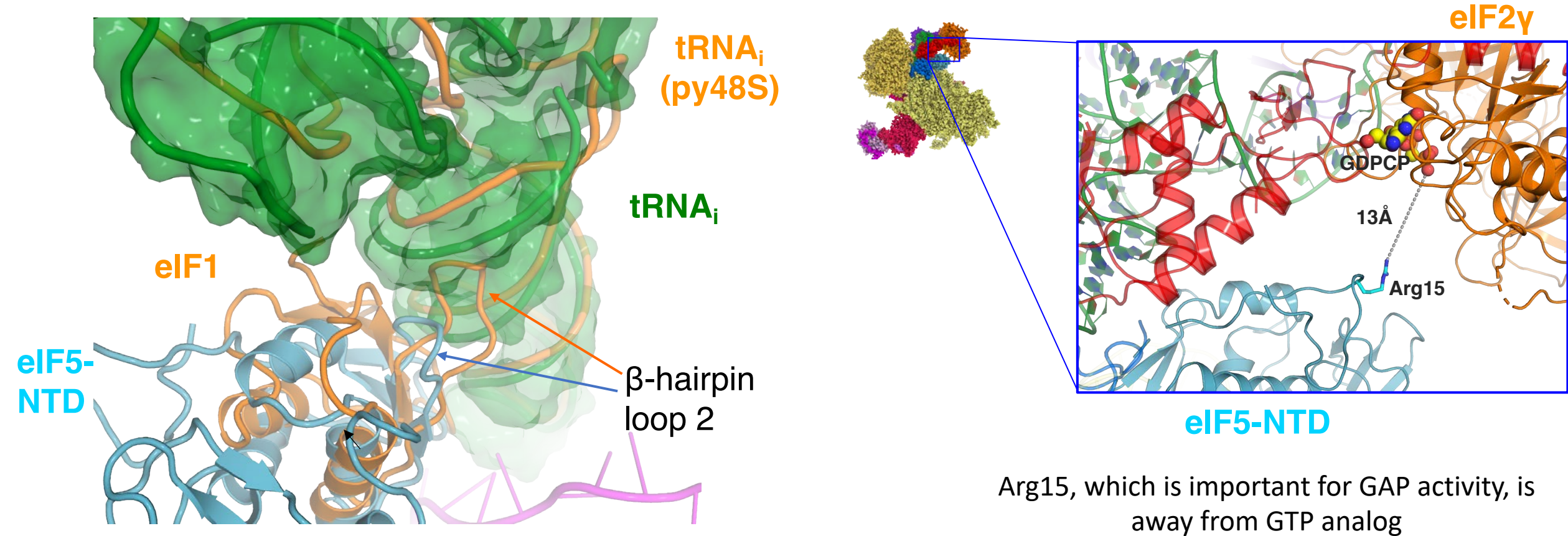
eIF3 leaves the subunit interface and returns to solvent interface
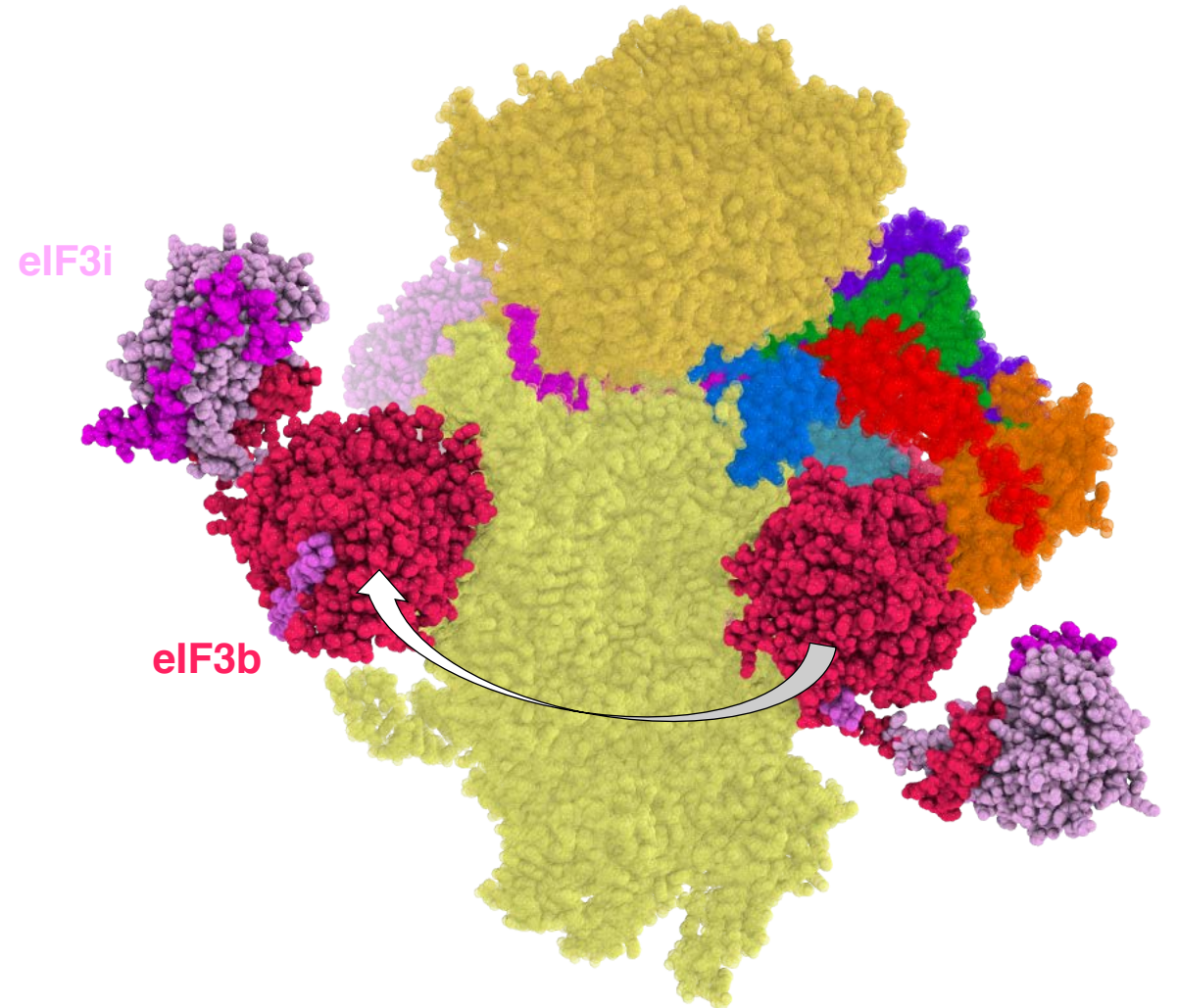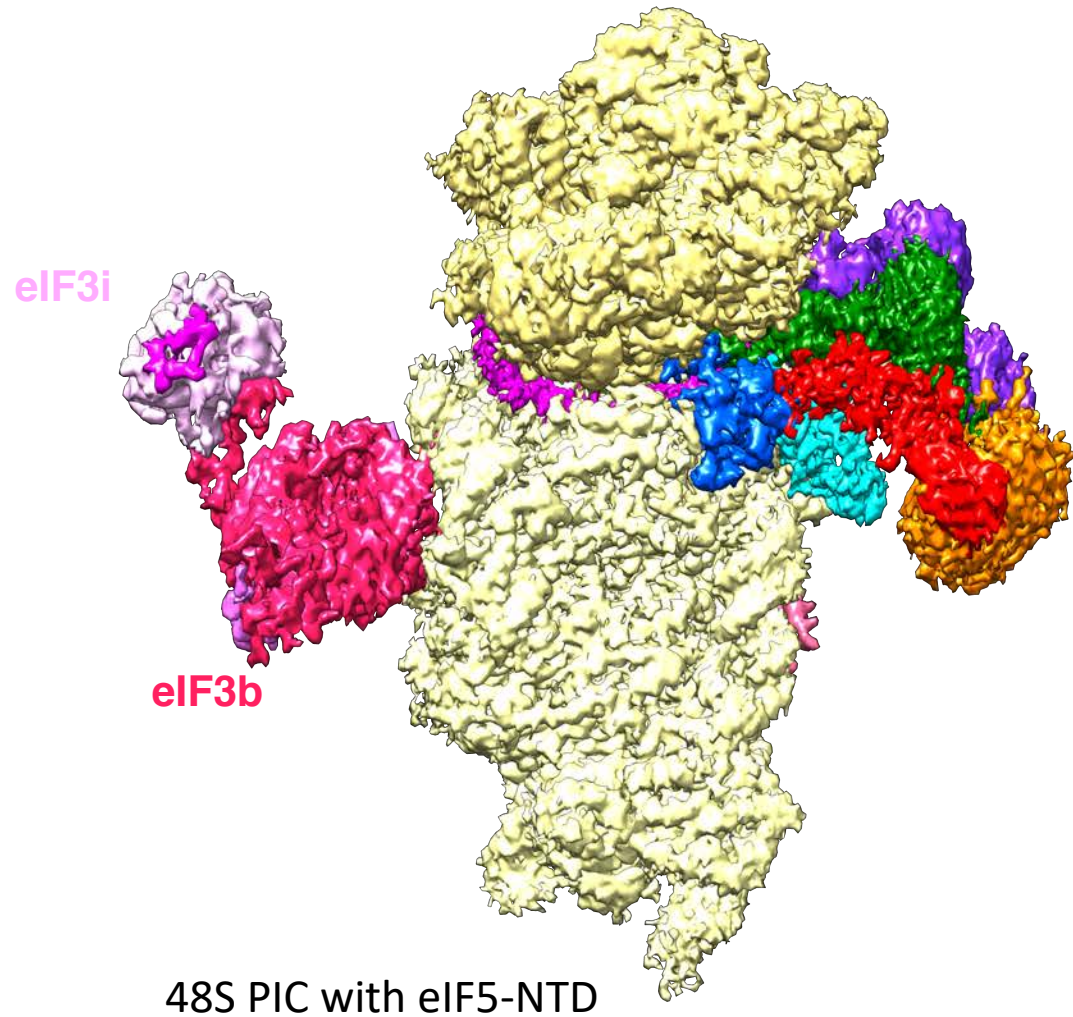
eIF3i

eIF3b

48S PIC with eIF5-NTD

eIF3i

eIF3b

What these structures have revealed about the mechanism of initiation

40S-eIF1-eIF1A-eIF3

43S (P_OUT)

48S (open, scanning)

48S (P_IN, closed, Scanning-arrested)

48S (P_IN, closed, Scanning-arrested)

What these structures have revealed about the mechanism of initiation

40S-eIF1-eIF1A-eIF3

43S (P$_{OUT}$)

48S (open, scanning)

48S (P$_{IN}$, closed, Scanning-arrested)

48S (P$_{IN}$, closed, Scanning-arrested)
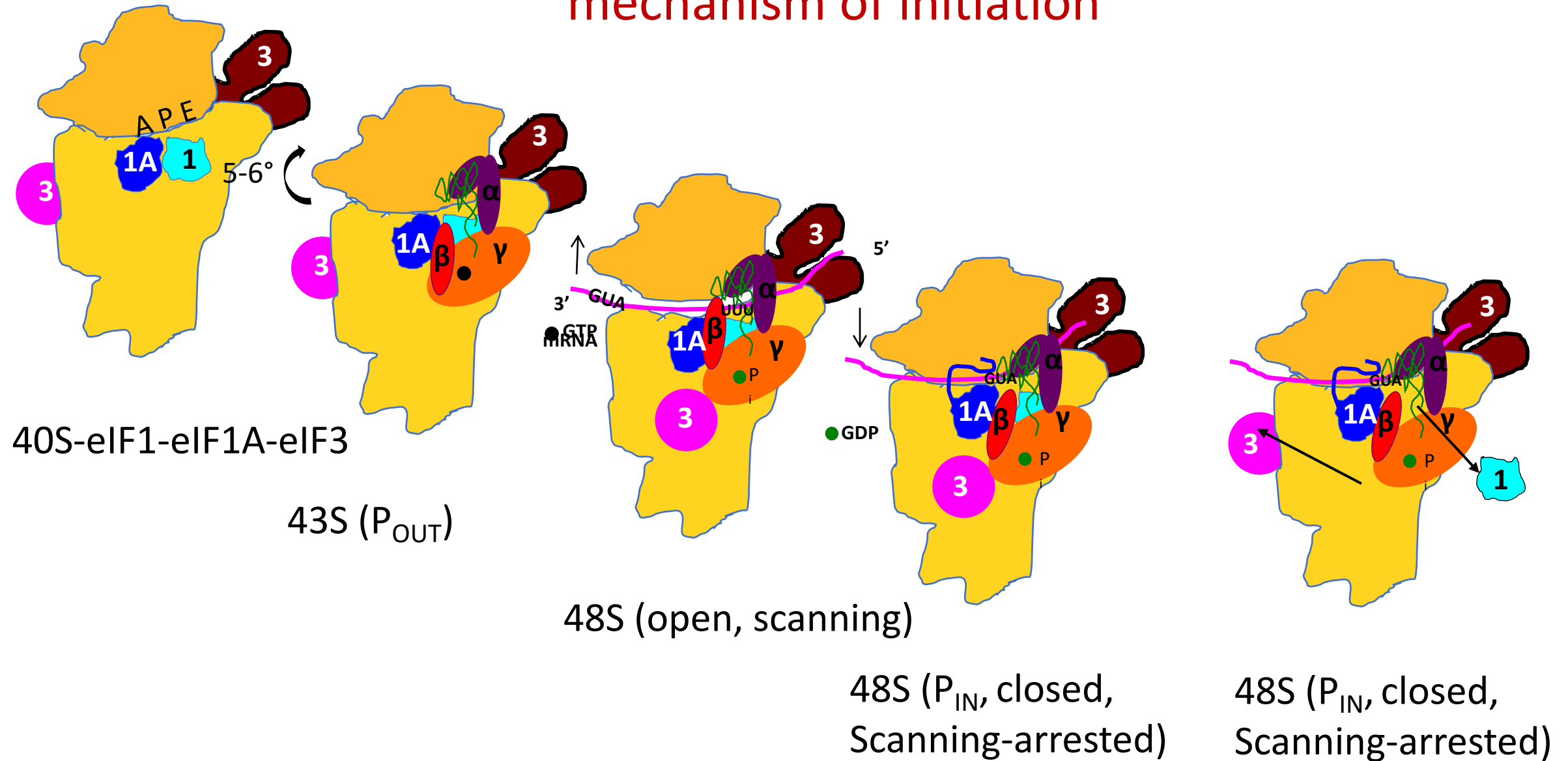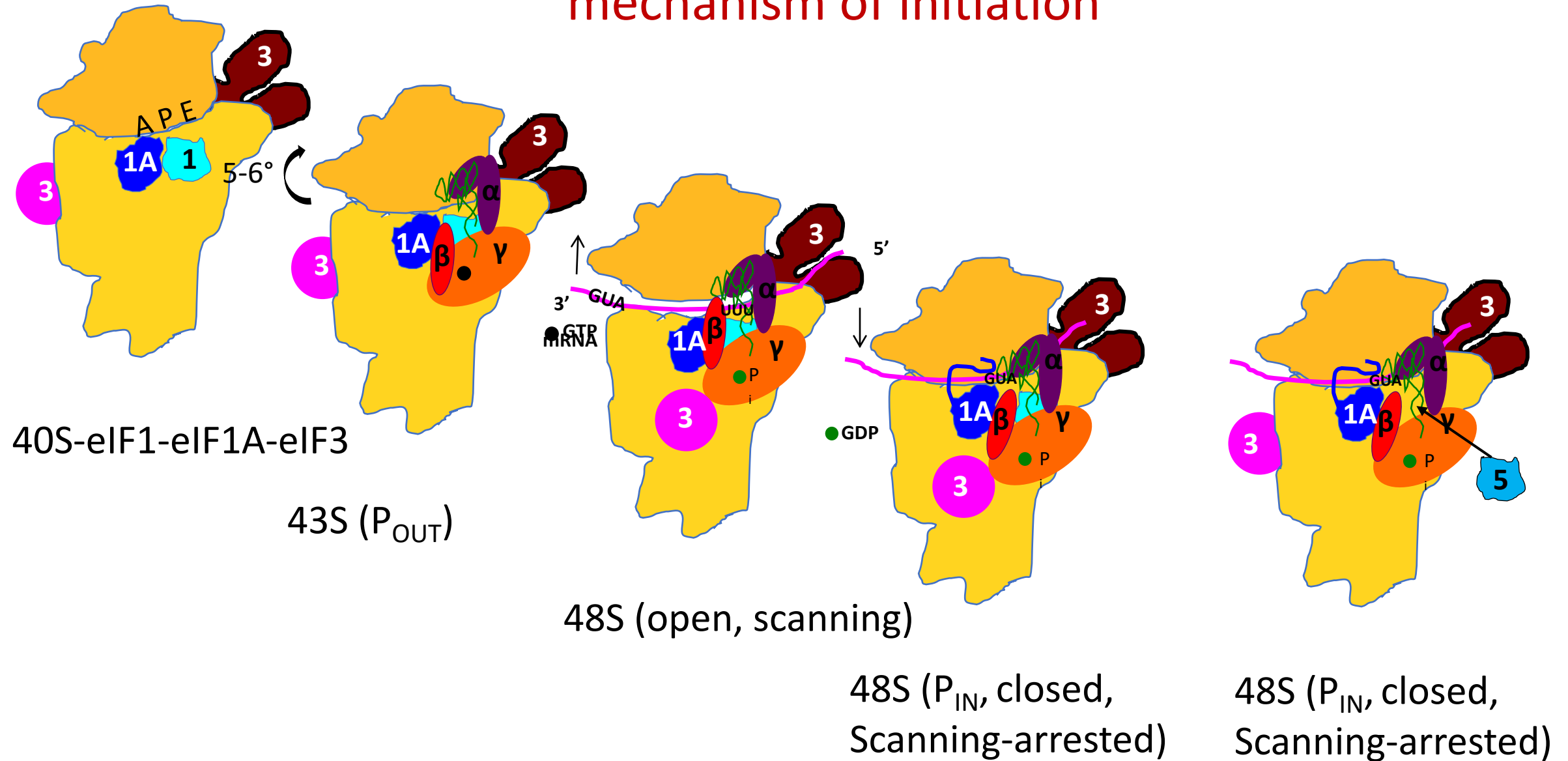
Translational control

Fundamental to all biological processes

Development and Differentiation

Learning and memory

Ageing and diseases

Response to various cellular stress

Translation Initiation is the target of most regulation

Understanding the detailed mechanism of regulation of translation initiation is essential

Eukaryotic Translation Initiation

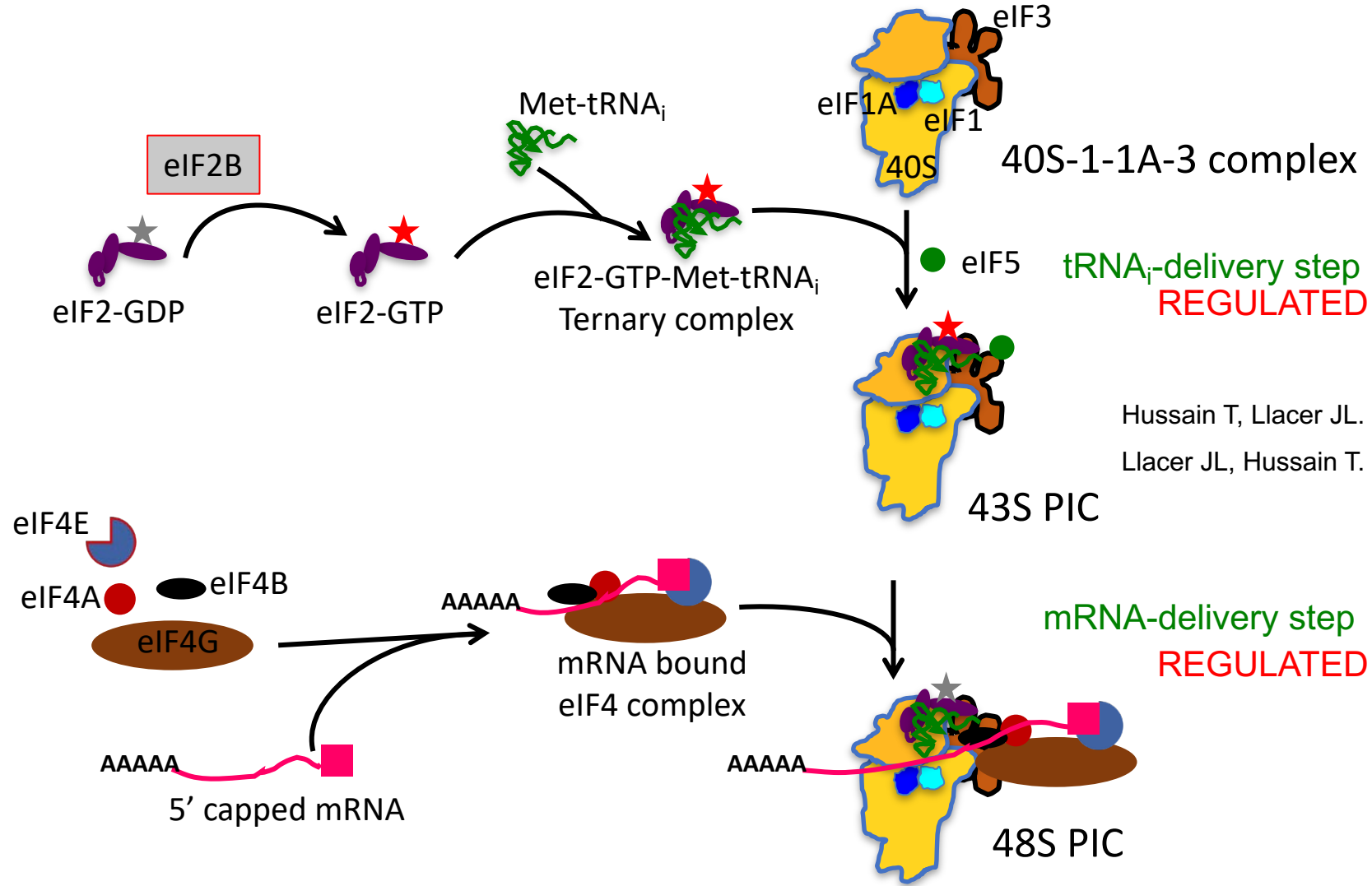eIF3

eIF1A

eIF1

40S

40S-1-1A-3 complex

Met-tRNA$_i$

eIF2B

eIF2-GDP

eIF2-GTP

eIF2-GTP-Met-tRNA$_i$
Ternary complex

eIF5

tRNA$_i$-delivery step
REGULATED

Hussain T, Llacer JL. et al. 2014

Llacer JL, Hussain T. et al. 2015

43S PIC

eIF4E

eIF4A   eIF4B

eIF4G

5' capped mRNA

AAAAA

mRNA bound
eIF4 complex

AAAAA

mRNA-delivery step
REGULATED

AAAAA

48S PIC

The mRNA recruiting eIF4 factors involved in protein synthesis and its regulation

Rishi Kumar Mishra, Ayushi Datey, and Tanweer Hussain

Take home message

- Large scale conformational changes guide the ribosomal PIC along the initiation pathway

Advanced Center for Cryo-Electron Microscopy Facility (ACCEM-IISc) at IISc Bengaluru

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MRDG &
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Thank you