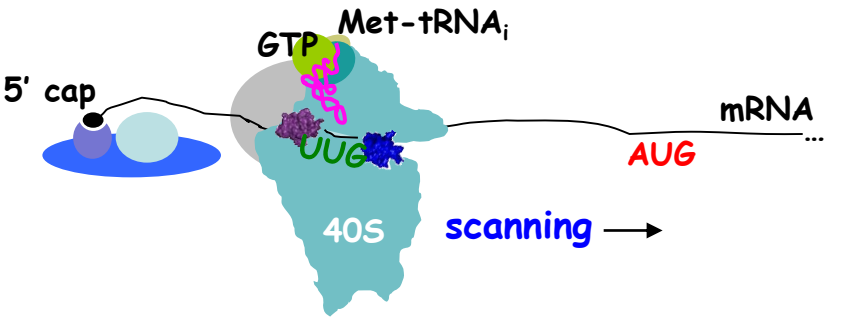
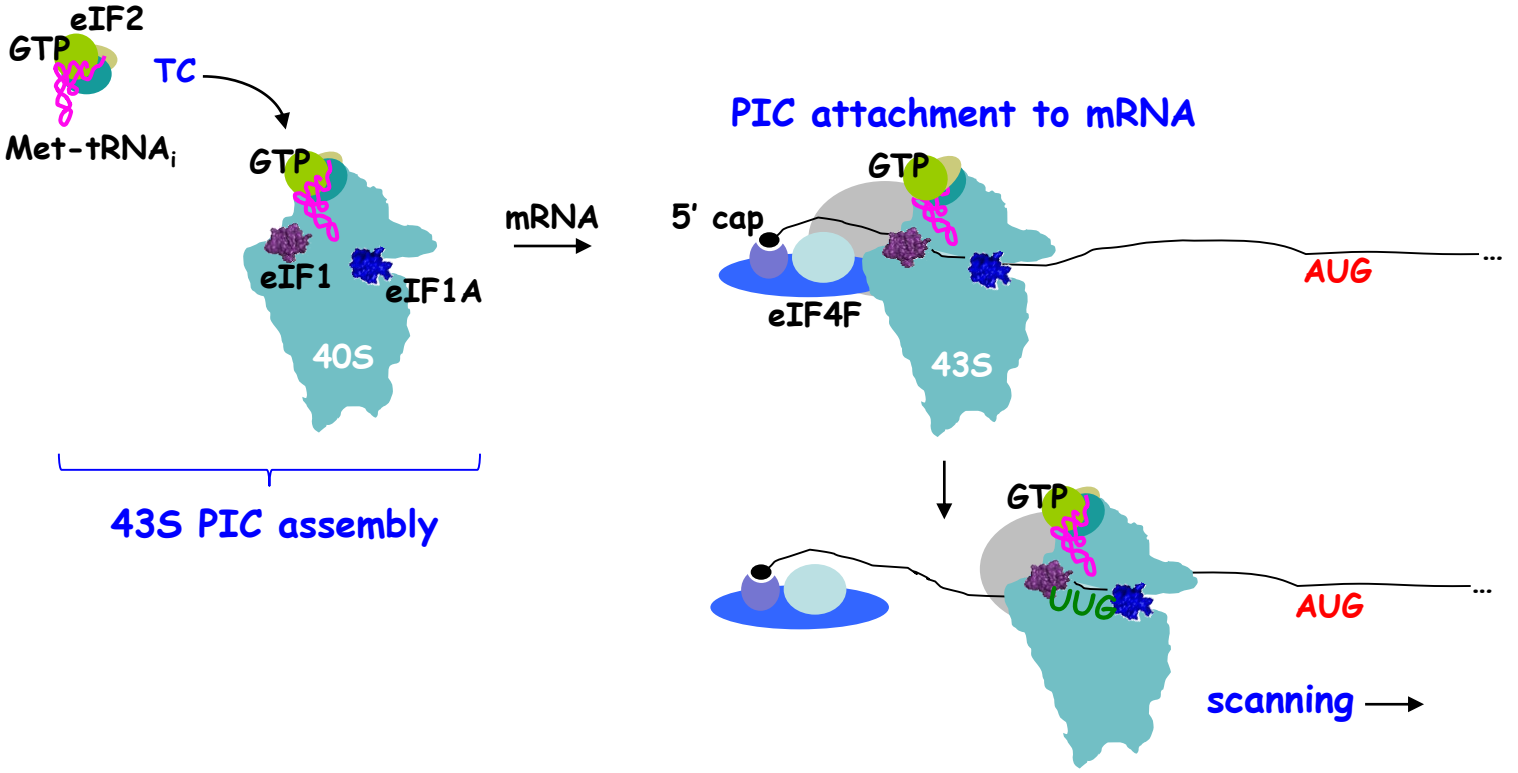
Molecular Determinants of Accurate Translation Initiation

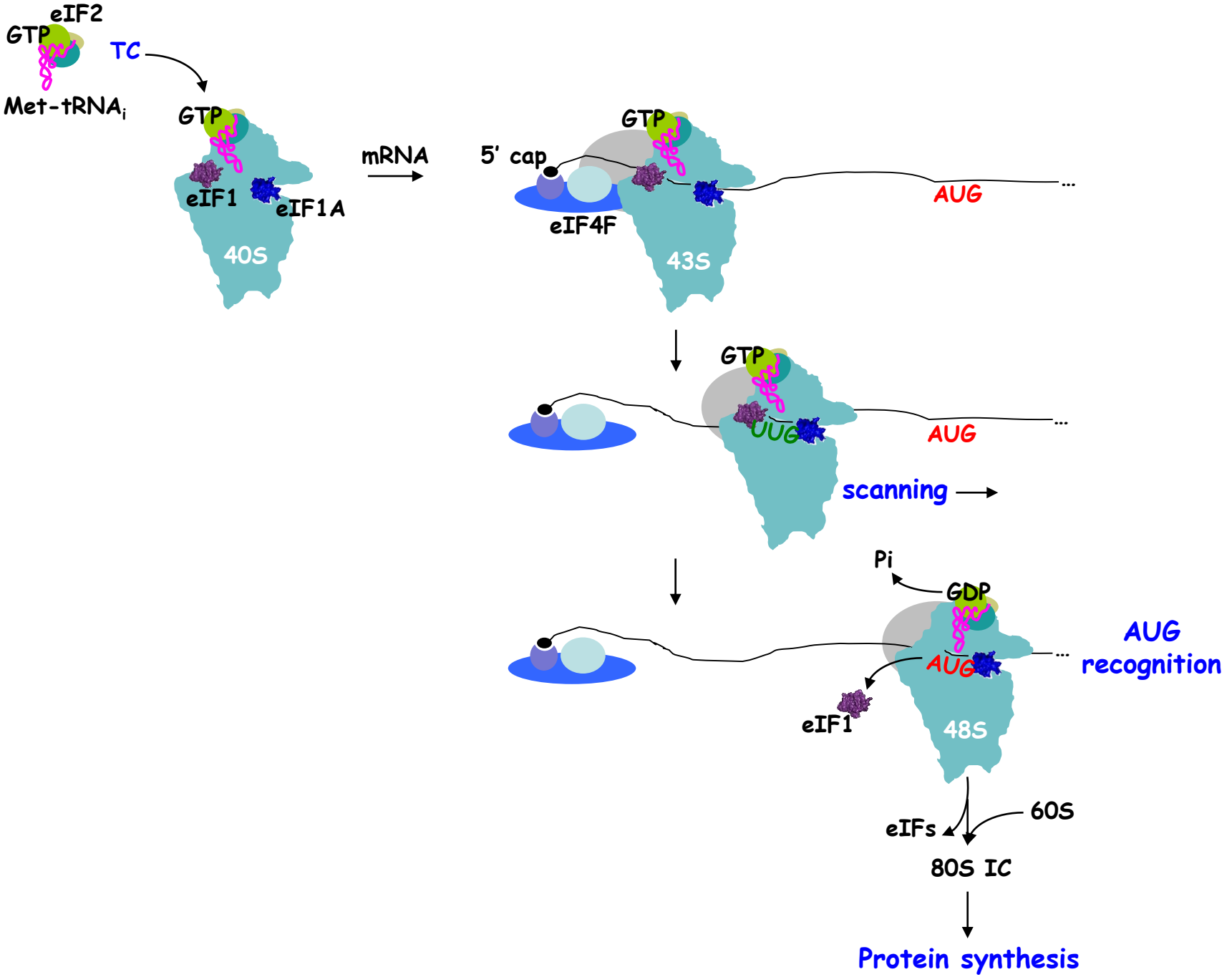
How do ribosomes identify the correct translation initiation codons in mRNAs?

Hinnebusch Lab (NICHD)
Lorsch Lab (NIGMS/NICHD)
Ramakrishnan Lab (MRC, U.K.)

Translation initiation by the scanning mechanism




eIF2
GTP
TC
Met-tRNA$_i$
GTP
eIF1
eIF1A
40S
mRNA
43S PIC assembly
PIC attachment to mRNA
GTP
5' cap
eIF4F
43S
AUG
GTP
UUG
AUG
scanning


eIF2
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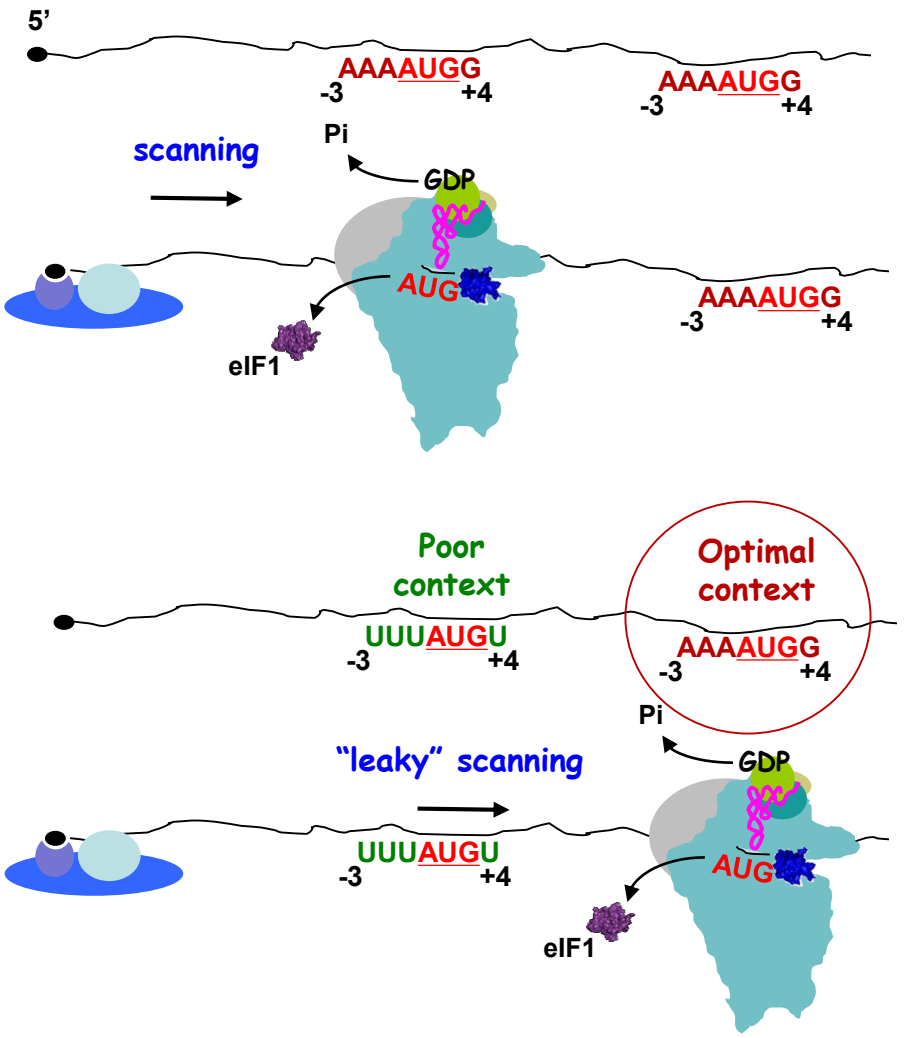
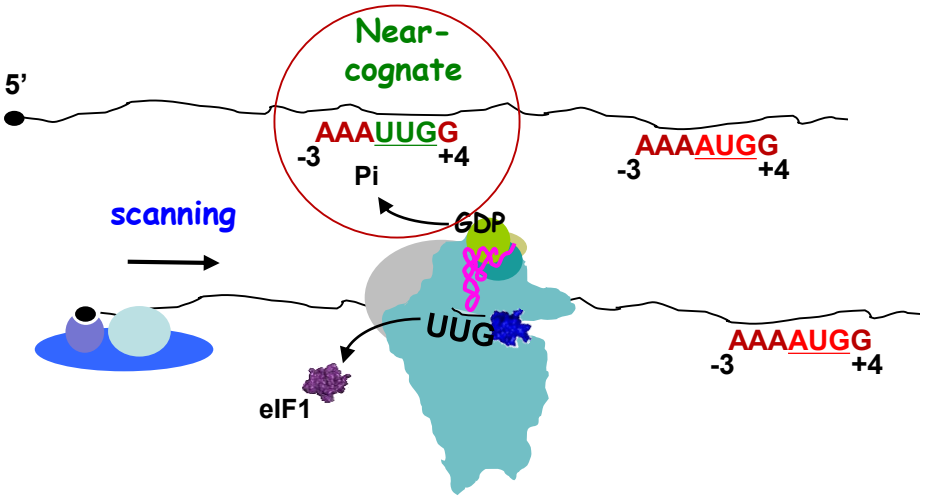
AUG
GTP
UUG
AUG
scanning
Pi
GDP
AUG
eIF1
48S
AUG
recognition
eIFs
60S
80S IC
Protein synthesis

Scanning favors initiation at 5'-proximal AUGs



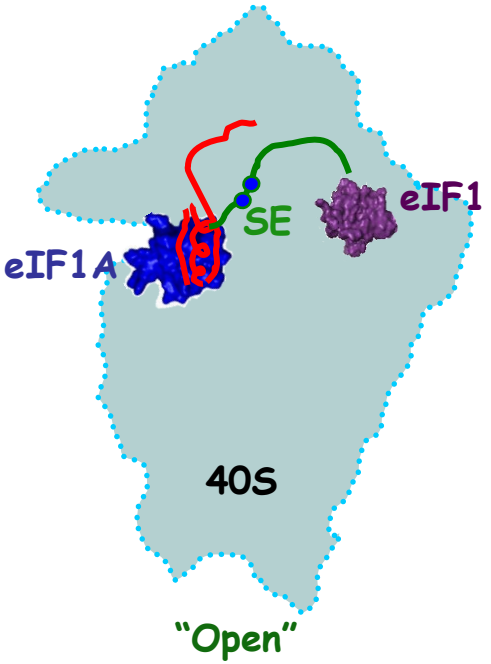
…and near-cognate triplets in good context can be used instead



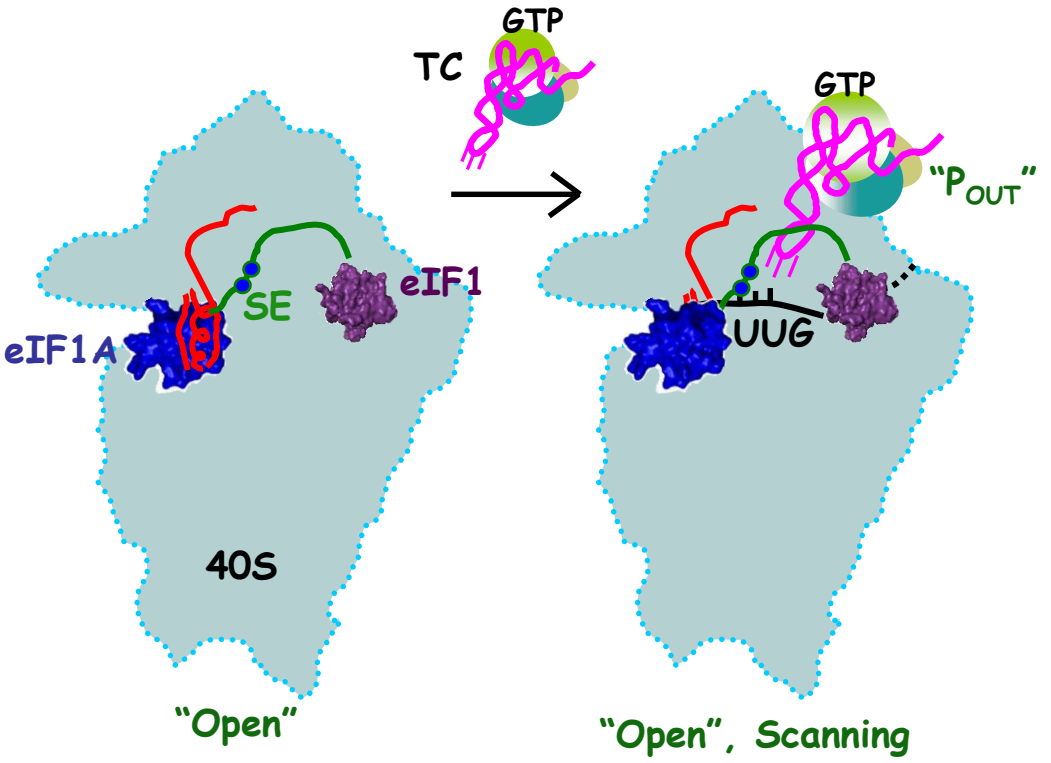
Translation initiation defects in human disease

- Mutations adding or removing upstream AUGs or changing AUG context: melanoma, breast cancer, thalassemia, thrombocytemia, hereditary pancreatitis, familial hypercholesterolemia
- Overexpression of eIFs: malignant transformation.
- Mutations affecting eIF2B, the GEF for eIF2: leukoencephalopathy with vanishing white matter.
- eIF2γ mutation: intellectual disability
- eIF1A mutations: uveal melanoma (UM) and thyroid carcinomas

eIF1 and eIF1A promote "open" conformation of the 40S



eIF1 and eIF1A promote "open" conformation of the 40S conducive to TC loading and scanning…



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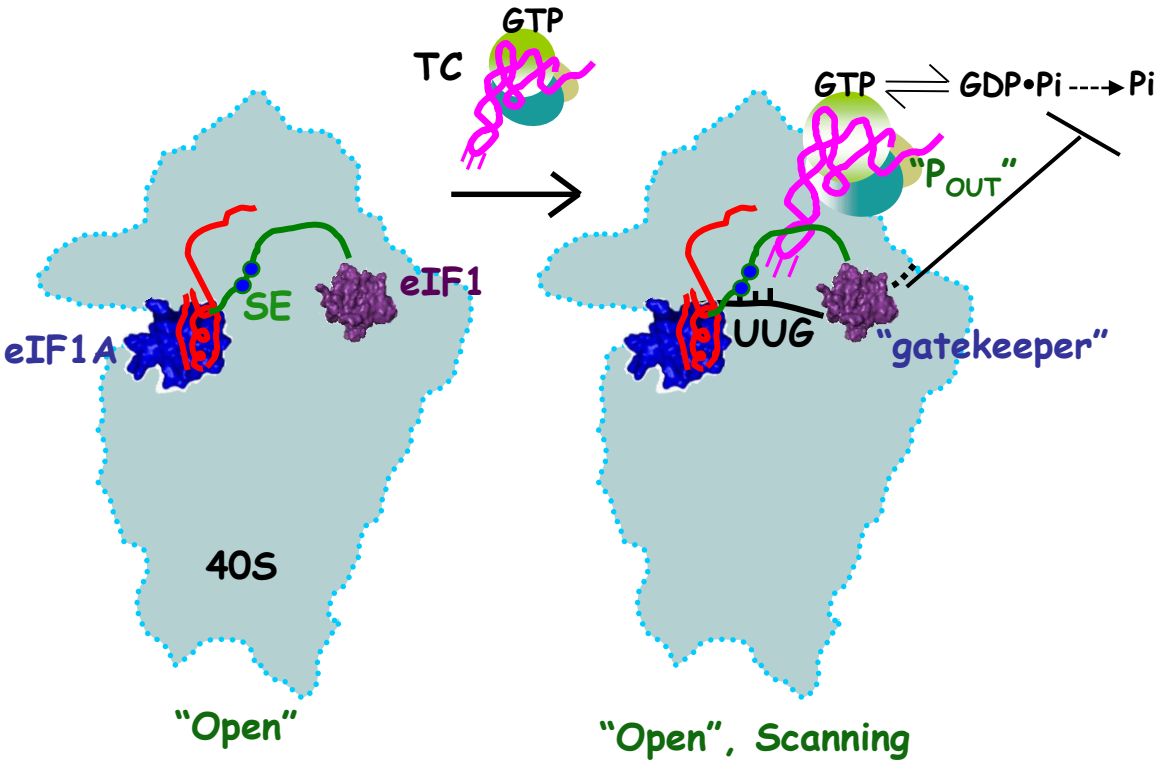
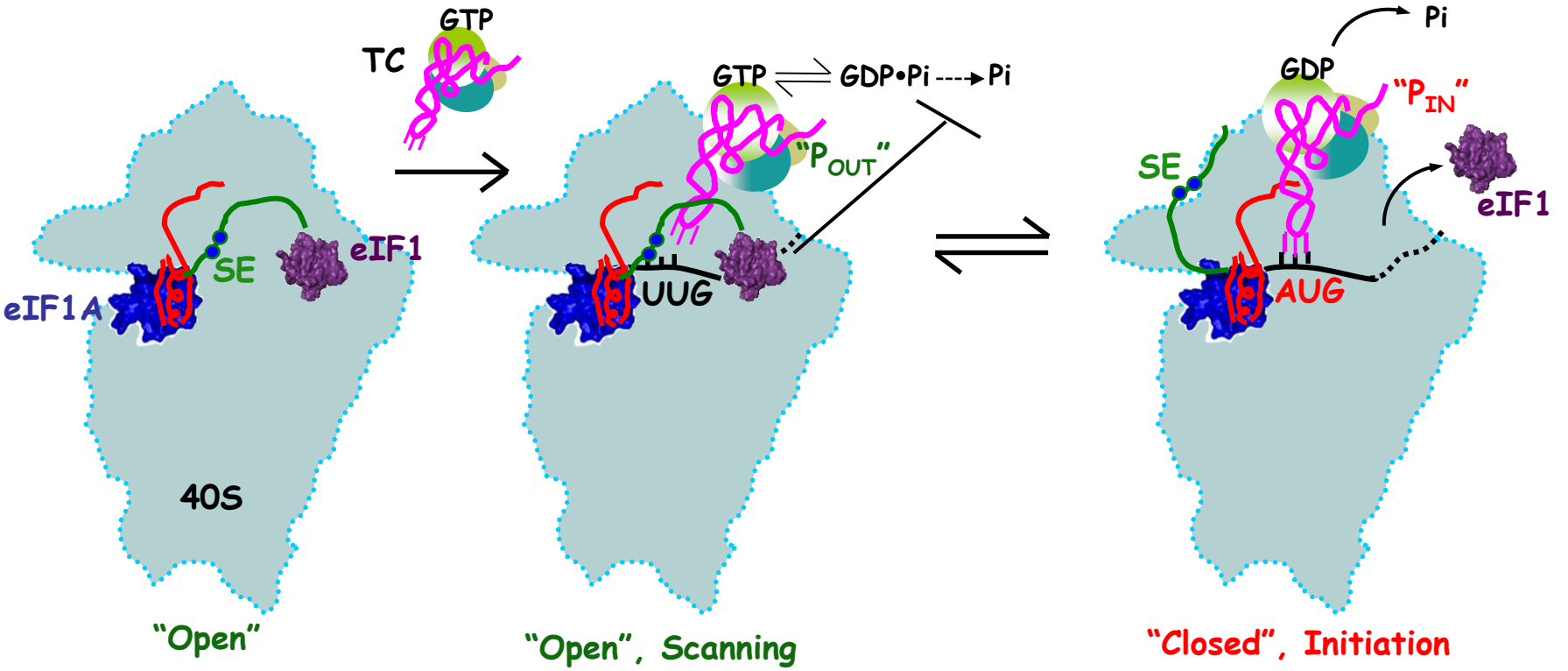
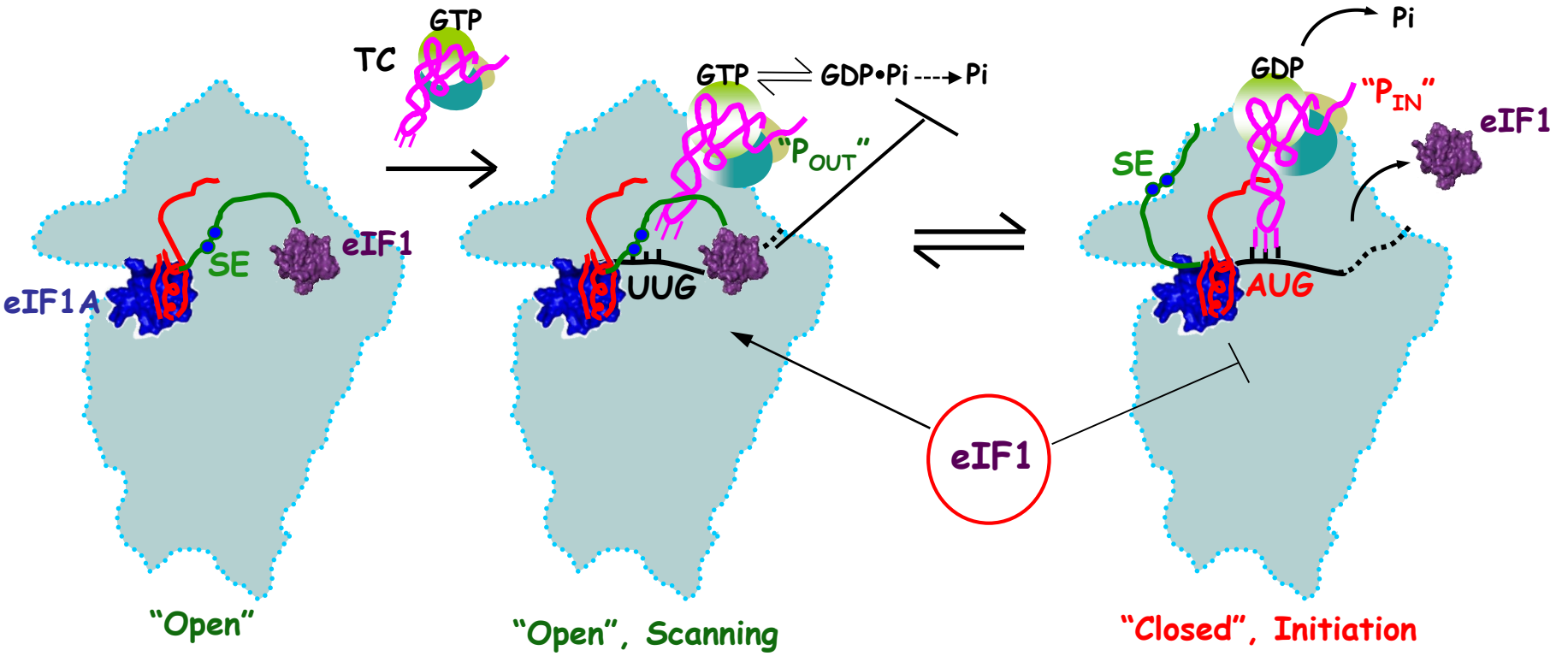


…but eIF1 must be ejected to allow Pi release and stabilize TC binding in P_{IN} state

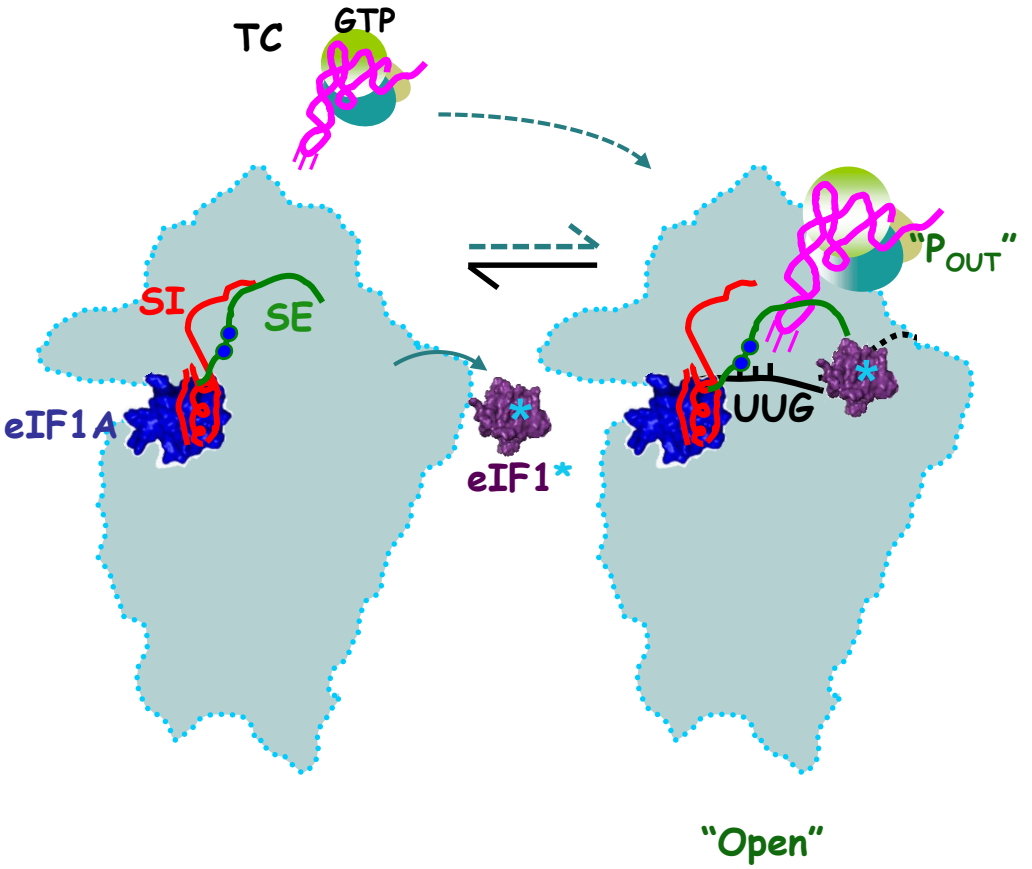


eIF1 promotes P_{OUT} for scanning and blocks P_{IN} at non-AUG codons…

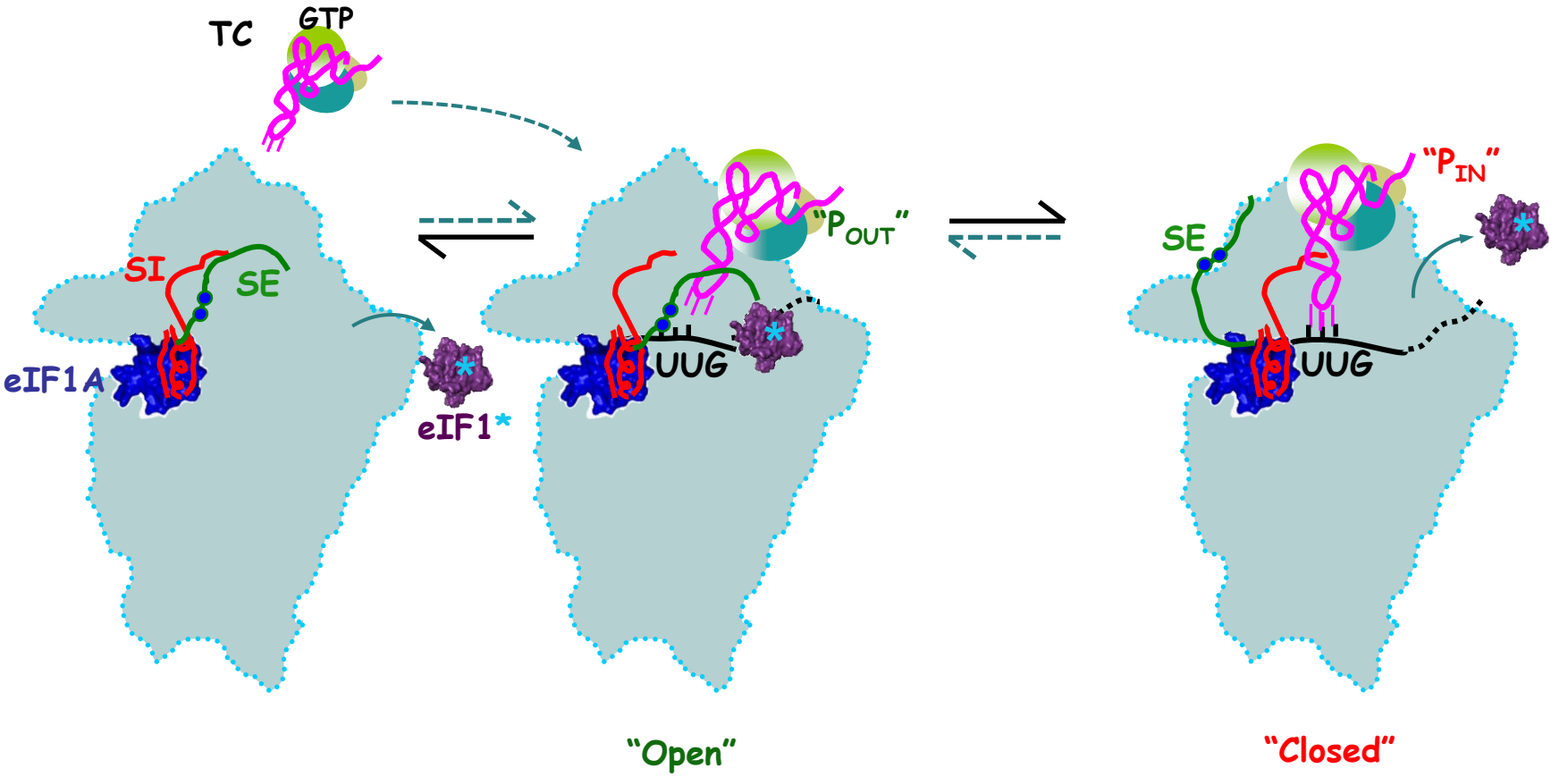


…requiring eIF1 release for AUG selection

Prediction: eIF1 mutations that weaken 40S binding should reduce TC binding to open complex in P_{OUT} state…

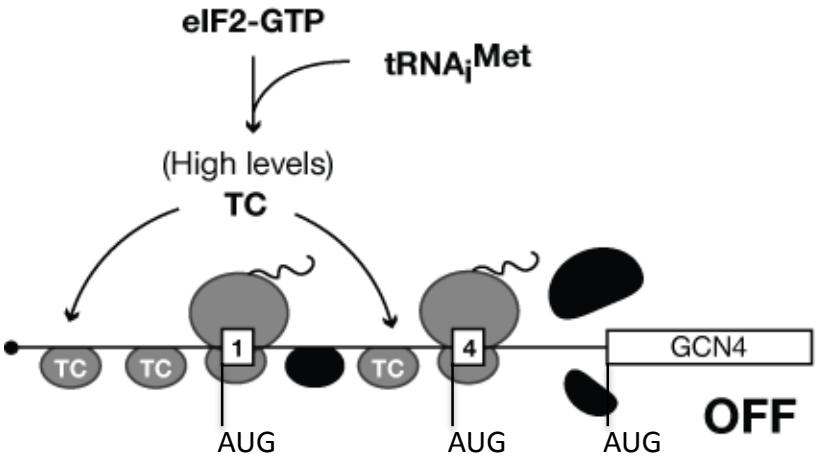


Prediction: eIF1 mutations that weaken 40S binding should reduce TC binding to open complex in P_{OUT} state…



…but allow transition to P_{IN} at UUG codons

Translational Control of *GCN4* by phosphorylation of eIF2



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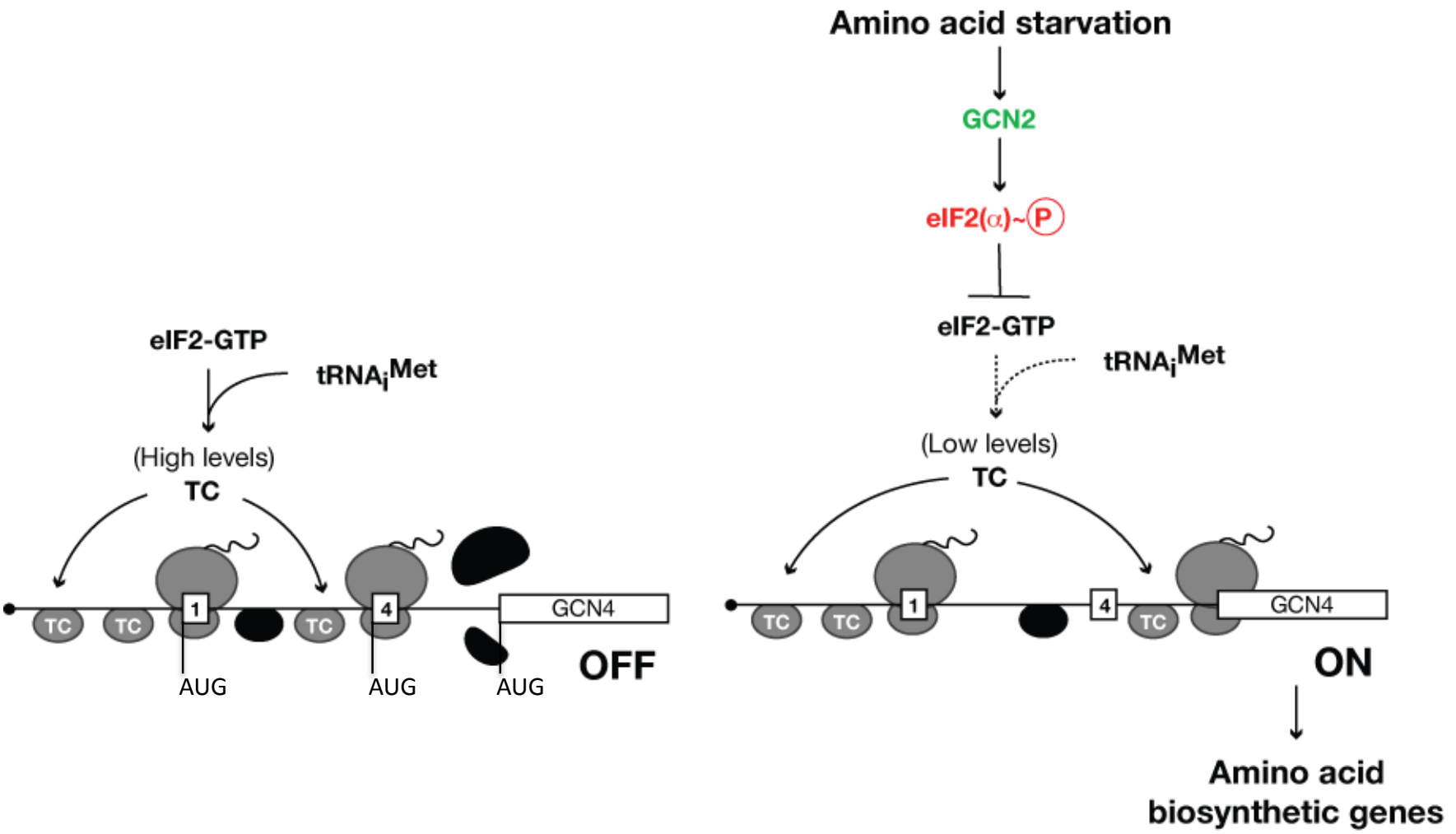
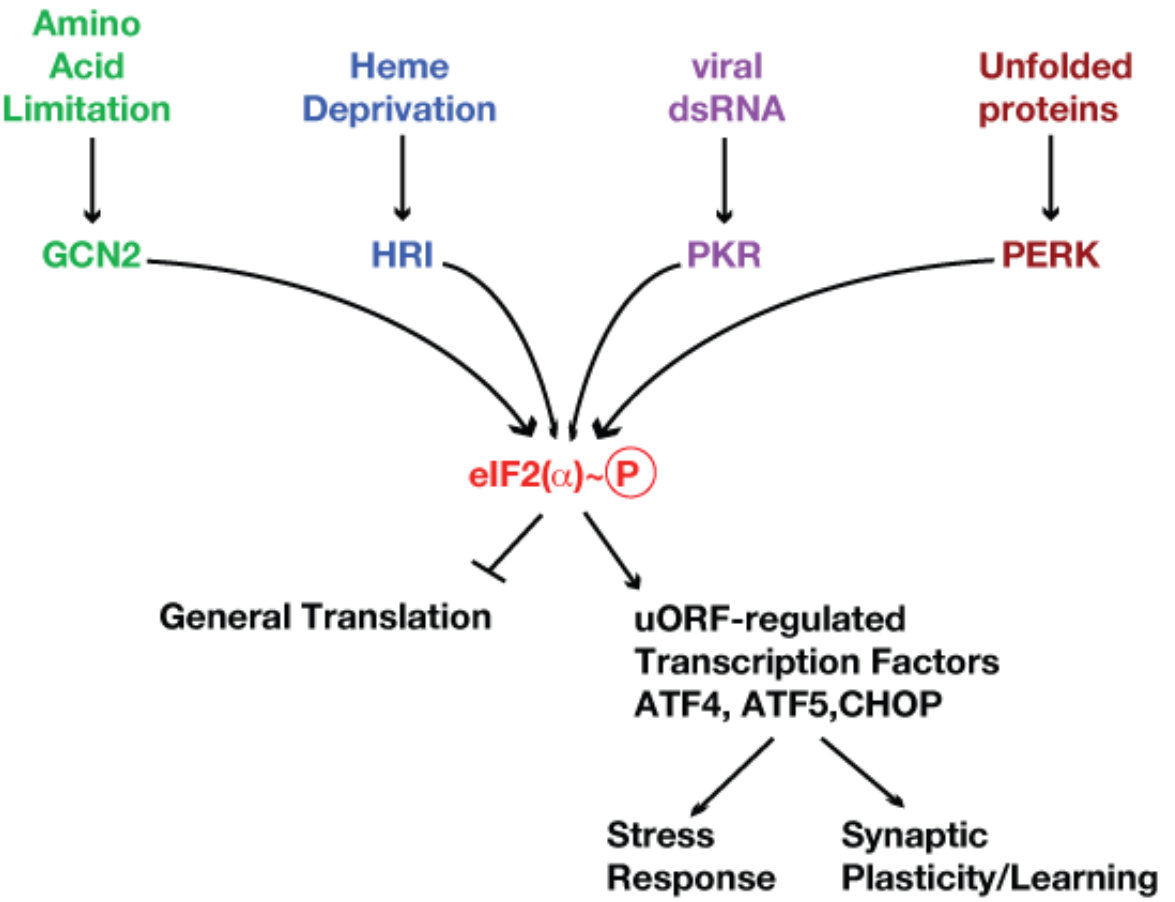
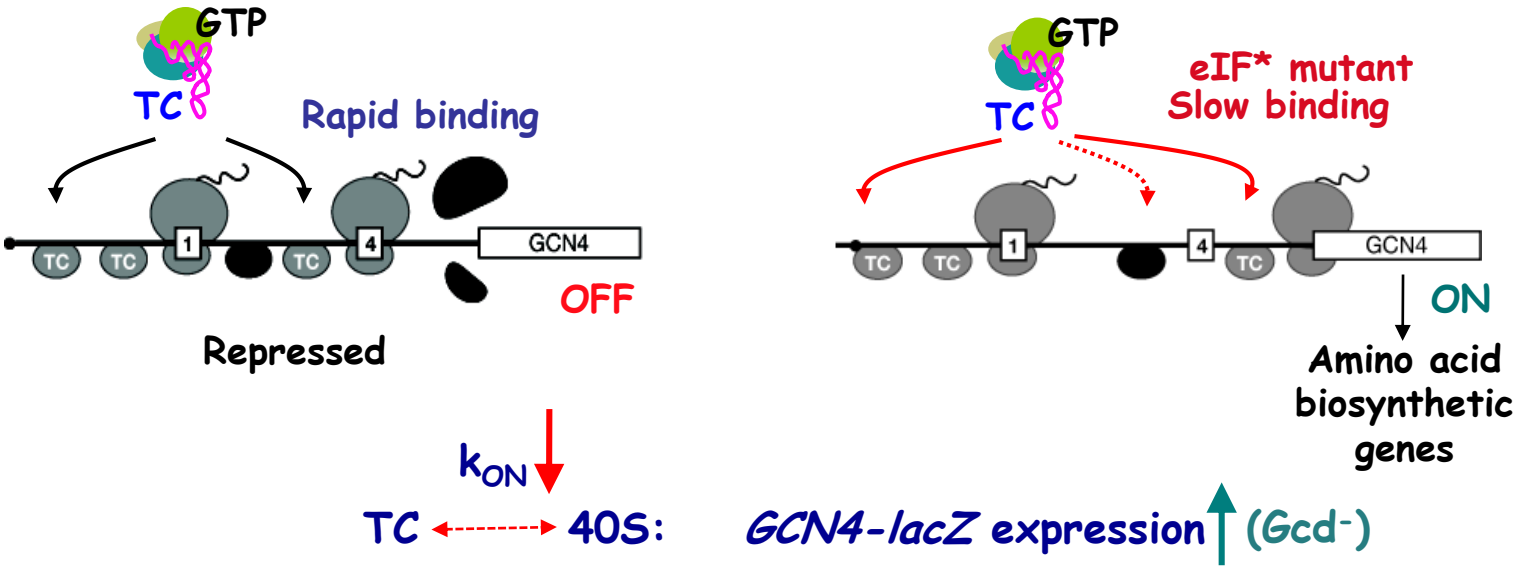


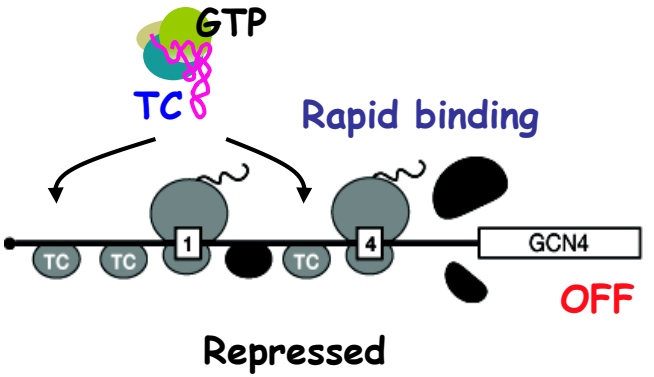
Integrated Stress Response by phosphorylation of eIF2

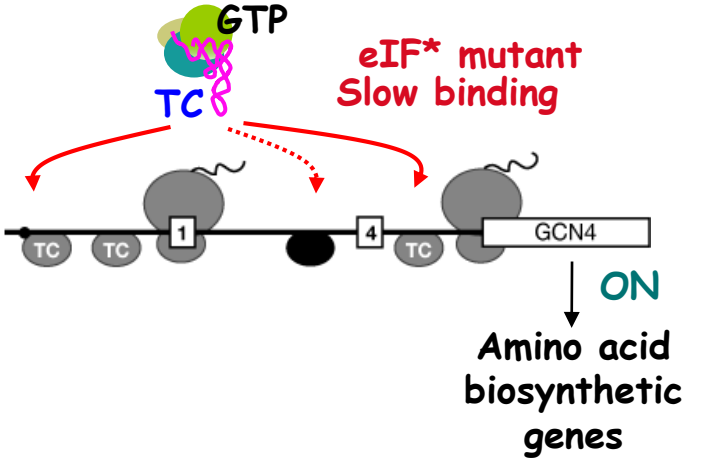


GCN4 translation: *in vivo* reporter of defective TC loading on 40S subunits



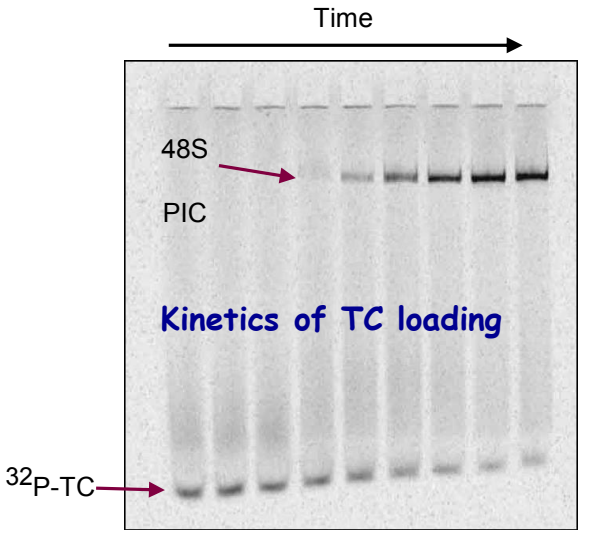
GCN4 translation: *in vivo* reporter of defective TC loading on 40S subunits

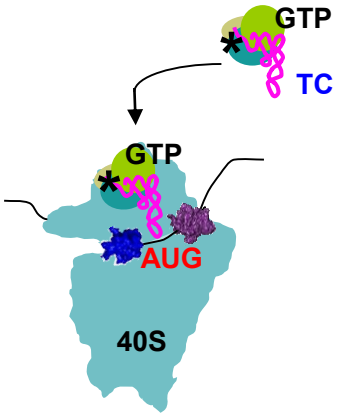




k_{ON}

TC ⇢ 40S: *GCN4-lacZ* expression ↑ (Gcd⁻)



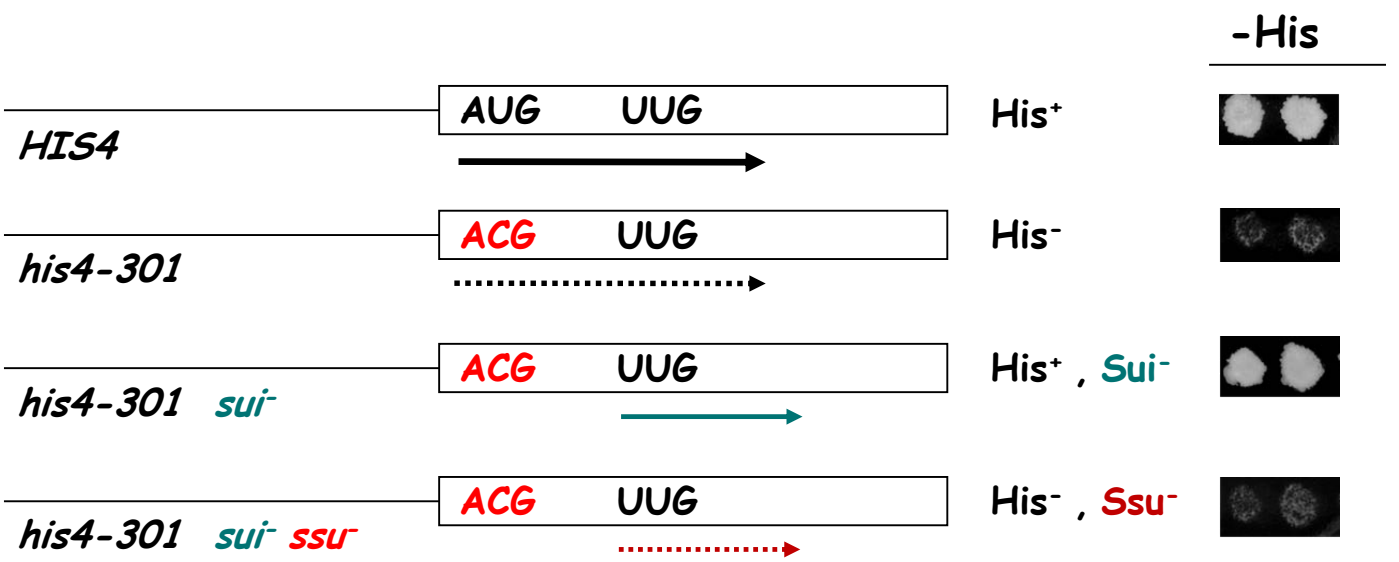


Gcd⁻ mutations:

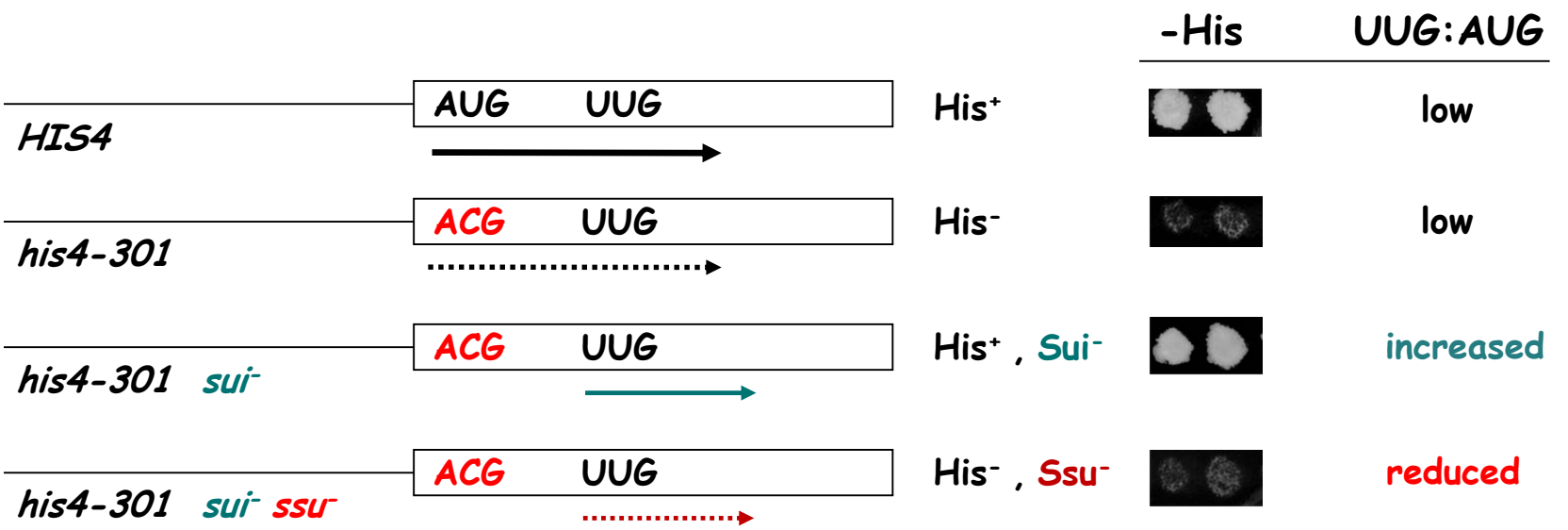
- eIF1 (*sui1*)
- eIF1A (*tif11*)
- 18S rRNA
- tRNAi

Lorsch et al

Sui⁻ and Ssu⁻ mutations alter accuracy of start codon selection

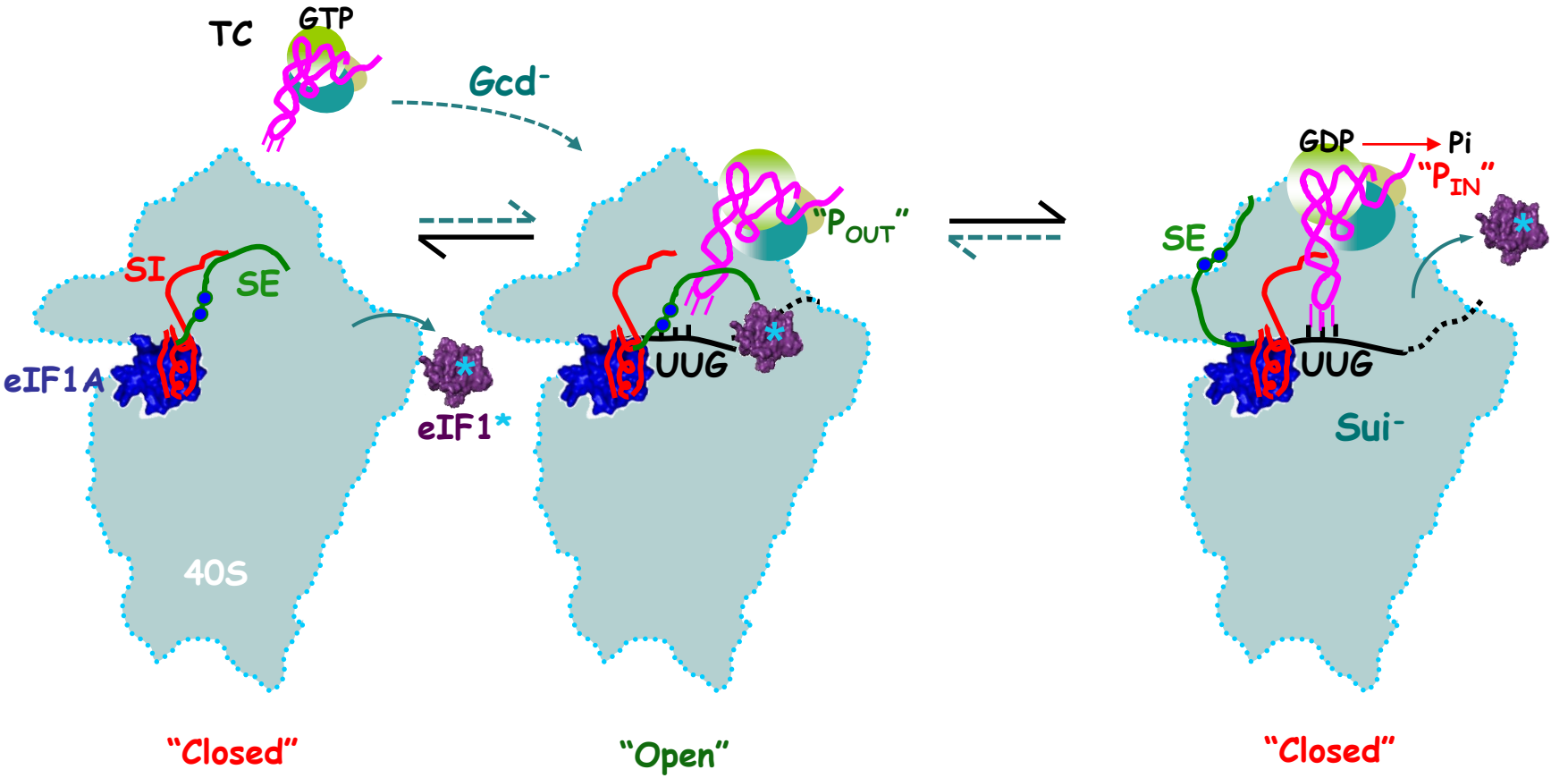


Sui⁻ and Ssu⁻ mutations alter accuracy of start codon selection



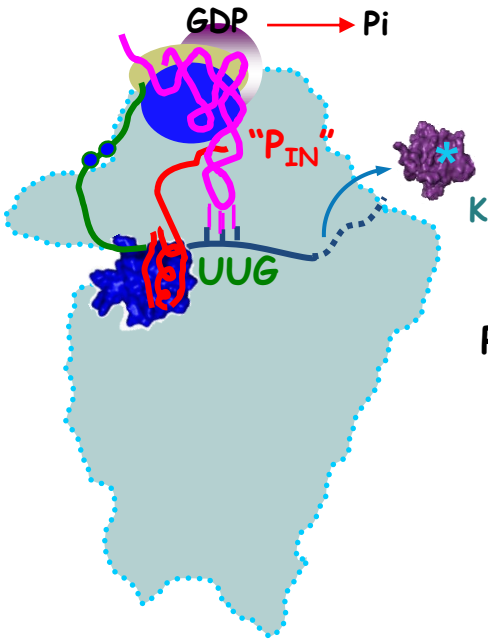
Quantify UUG/AUG initiation ratio:

Prediction: eIF1 mutations that weaken 40S binding should reduce TC loading rate (Gcd⁻ phenotype)…



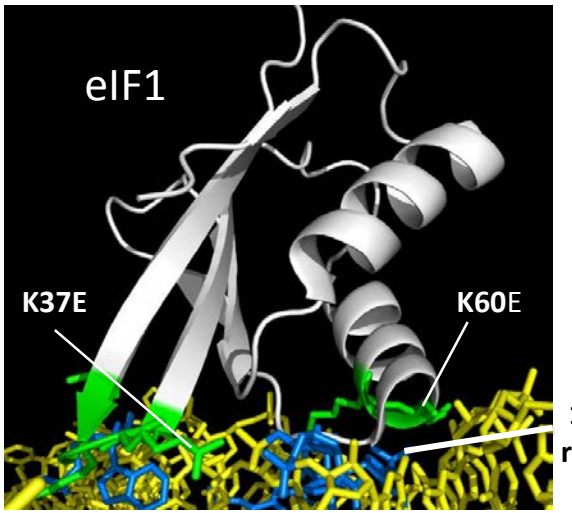
…and elevate UUG initiation (Sui⁻ phenotype)

eIF1 affinity for 40S dictates TC loading and initiation accuracy



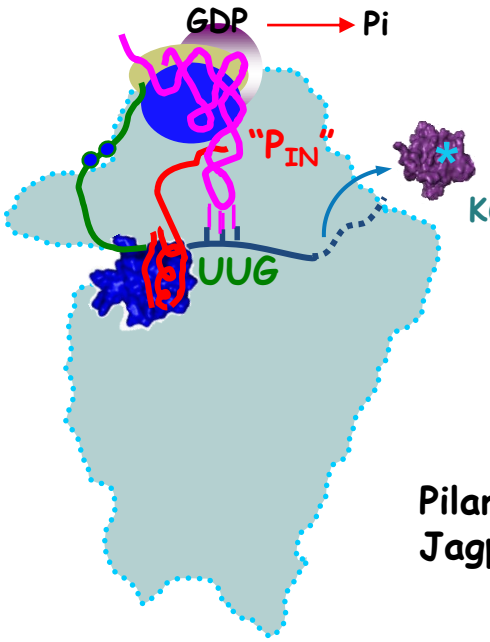
Pilar Martin-Marcos

40S-eIF1 crystal structure



Rabl et al (Ban N.) *Science* 2011

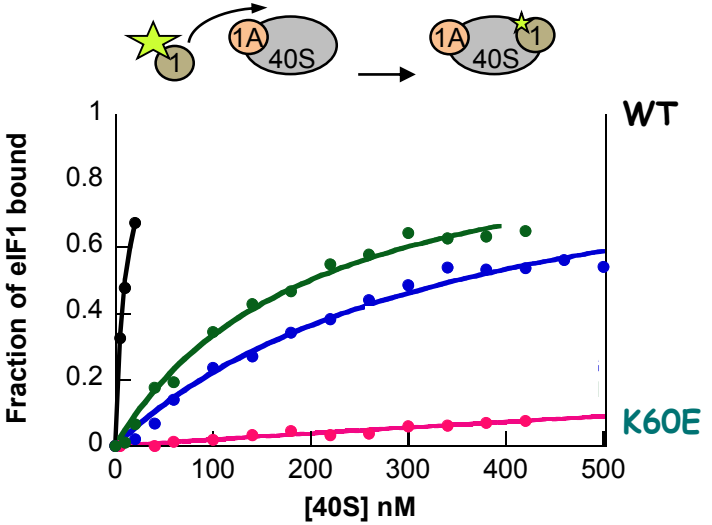
eIF1 affinity for 40S dictates TC loading and initiation accuracy



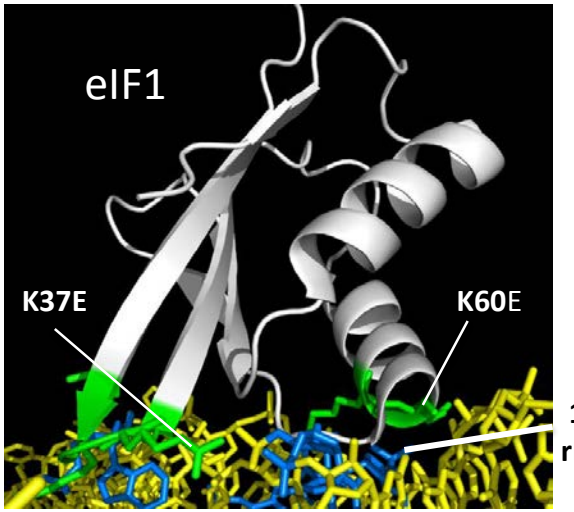
Gcd⁻: ↑ *GCN4-lacZ* : ↓ TC loading

Sui⁻: ↑ UUG/AUG initiation

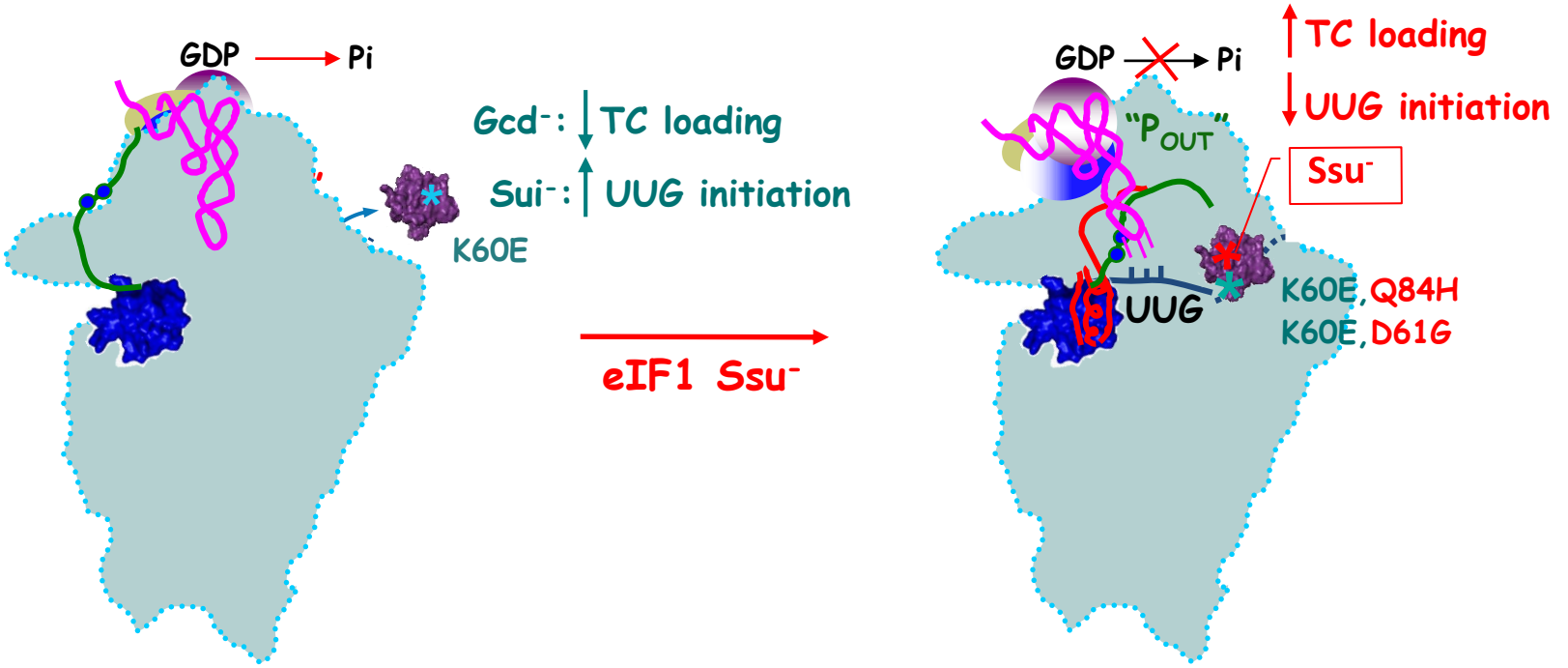
Pilar Martin-Marcos &
Jagpreet Nanda

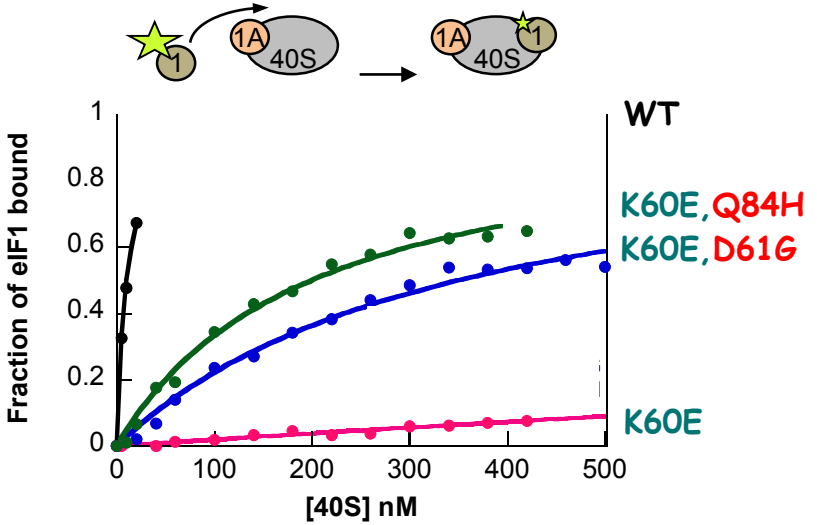


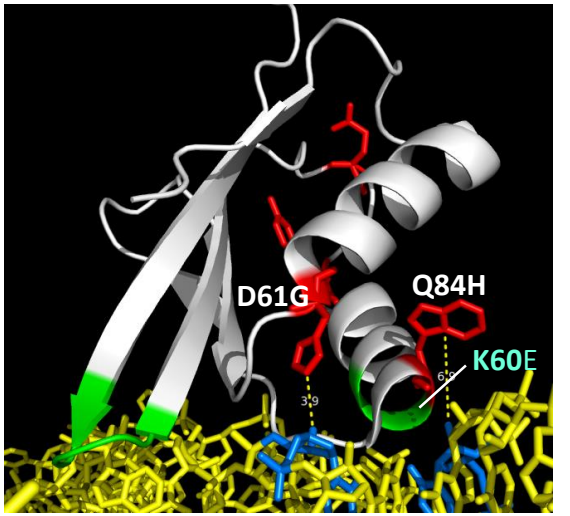
40S-eIF1 crystal structure



eIF1 affinity for 40S dictates TC loading and initiation accuracy







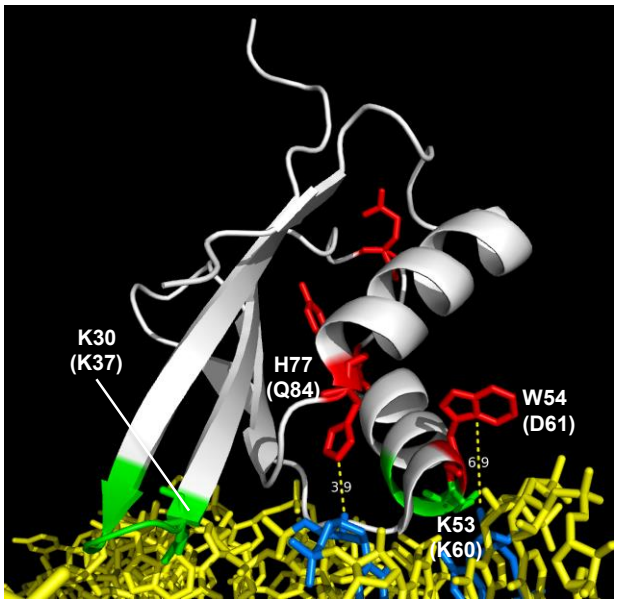
➢ eIF1 affinity for 40S subunit is finely tuned for optimum initiation accuracy

Sui⁻

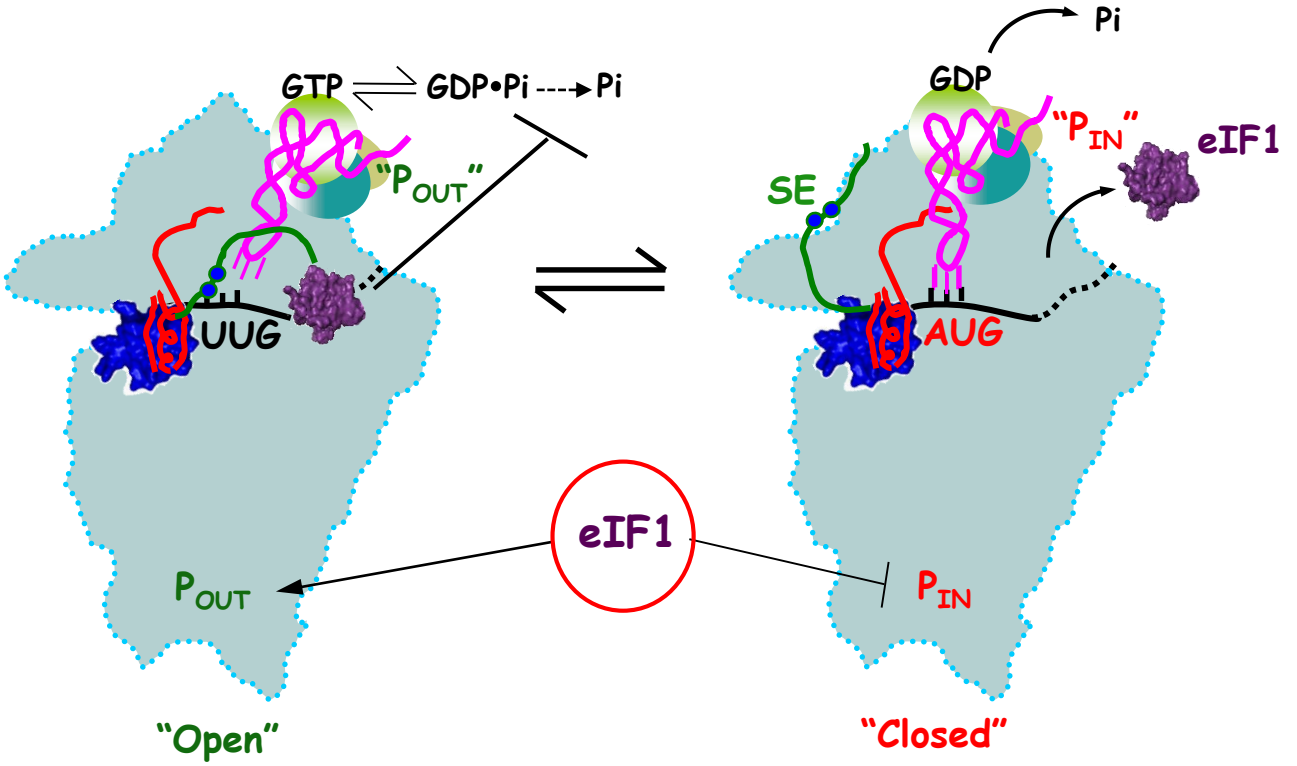
eIF1 ⇢ 40S: ↑ UUG:AUG

Ssu⁻

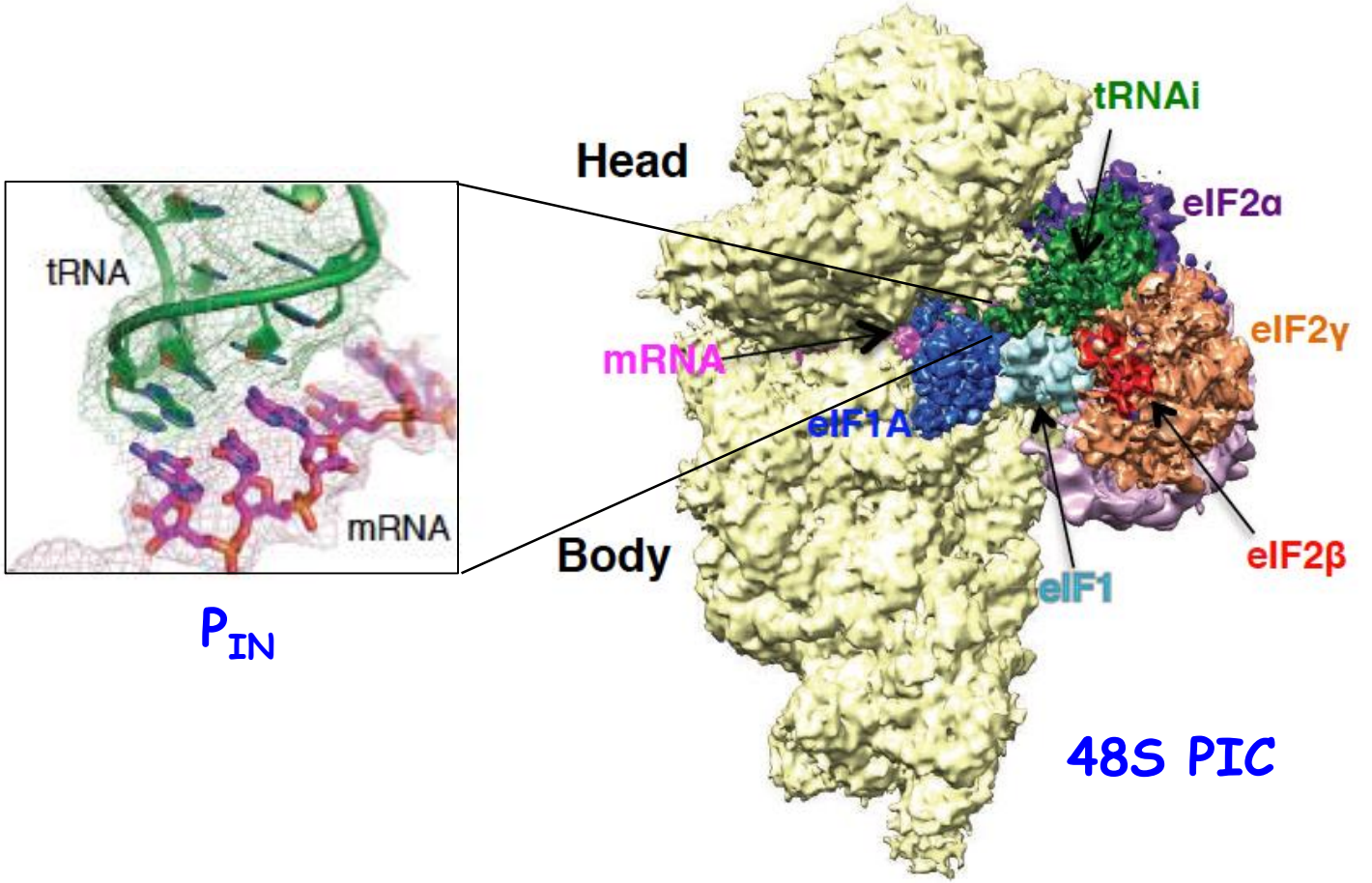
eIF1 ⟷ 40S: ↓ UUG:AUG



eIF1 blocks transition to P_{IN} at non-AUG codons…



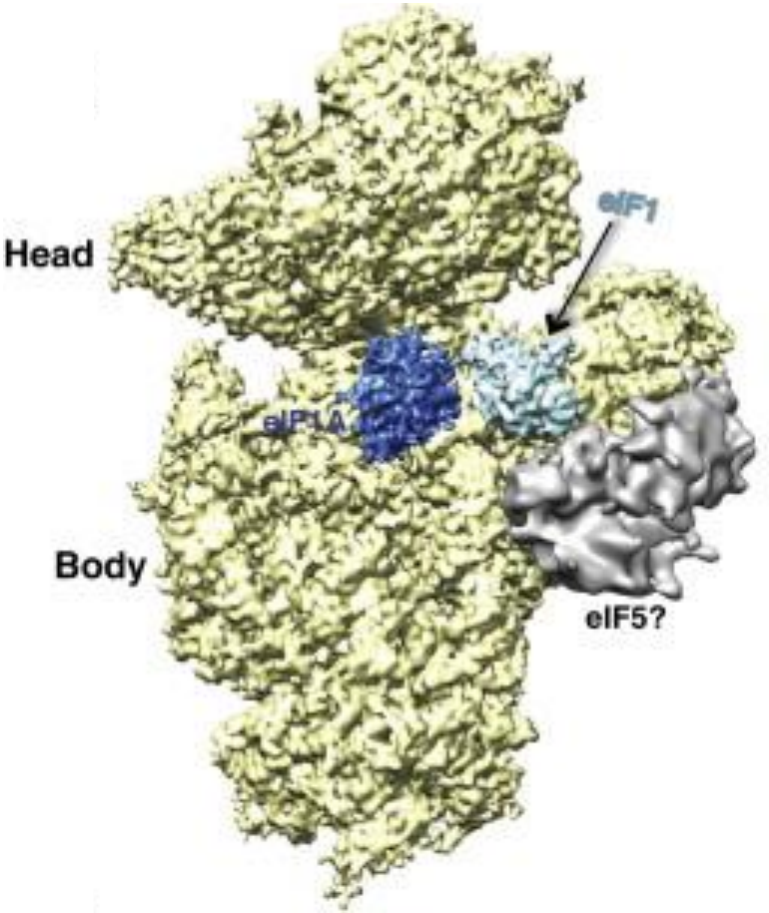
Cryo-EM structures of yeast PICs at 4.0 Å



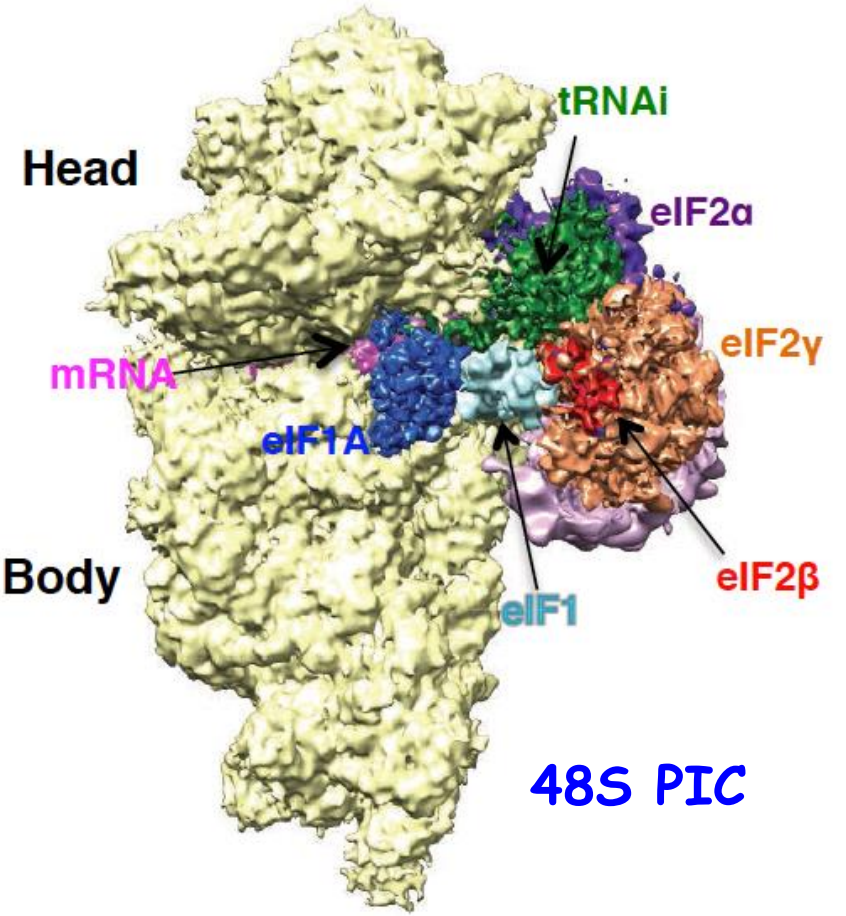
- Assembled using Sui$^-$ mutants of $tRNA_i$ and eIF2 characterized at NIH

Hussain & Llacer et al (Ramakrishnan)

Cryo-EM structures of yeast PICs at 4.0 Å



40S•eIF1•eIF1A

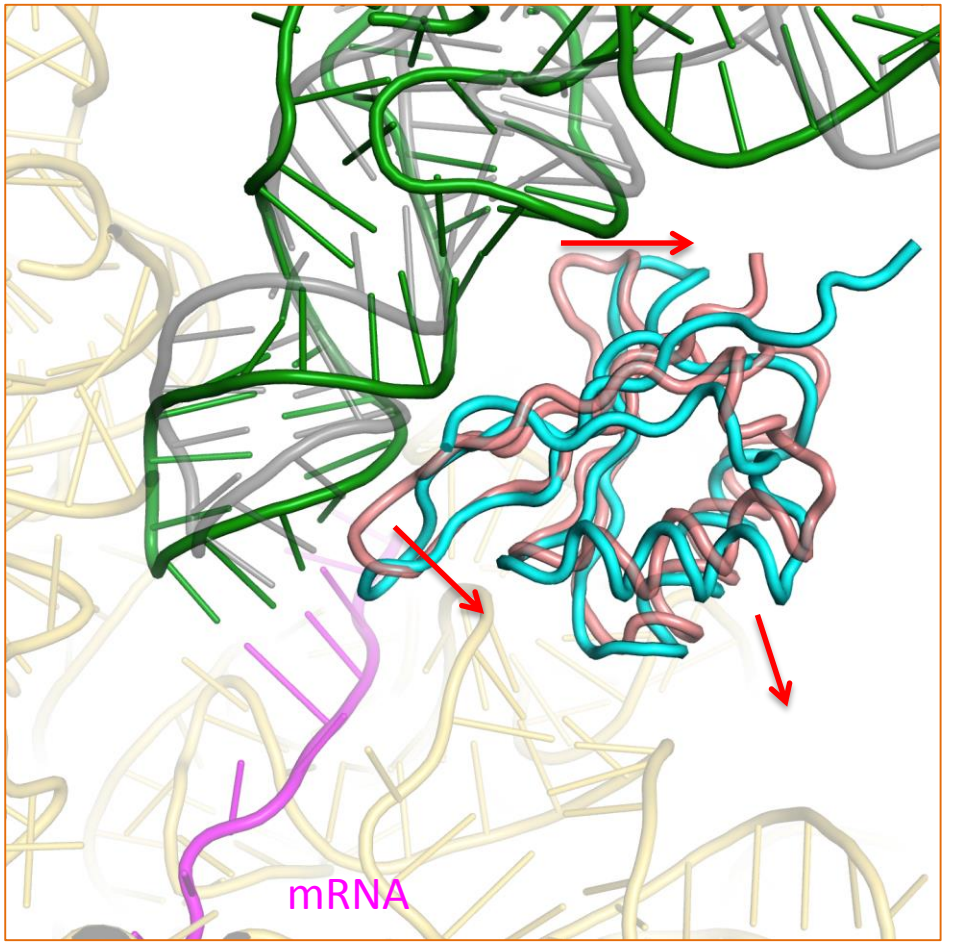


48S PIC

- Assembled using Sui$^-$ mutants of tRNA$_i$ and eIF2 characterized at NIH

Hussain & Llacer et al (Ramakrishnan)

Transition to P_{IN} alters eIF1 location to alleviate clash with $tRNA_i$



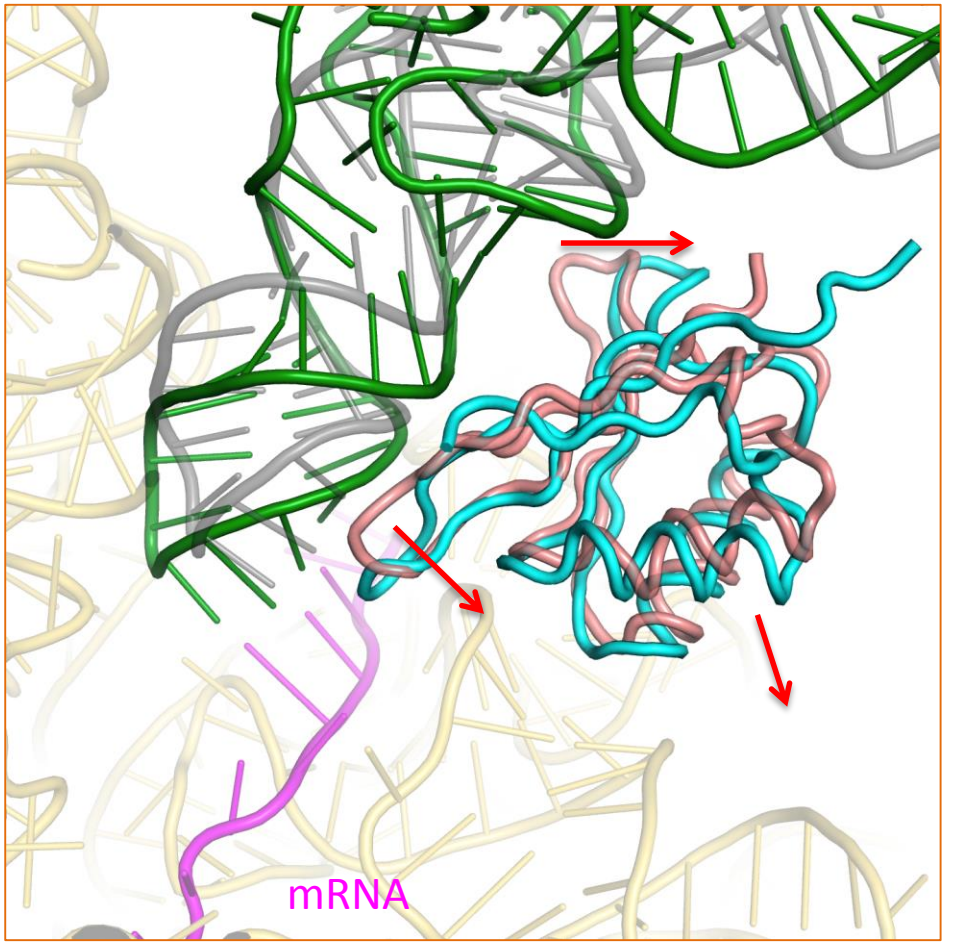
tRNAi (P_{IN})

tRNAi (P_{OUT}): Hashem et al. (Frank)

eIF1 in 40S•eIF1•eIF1A
eIF1 in 48S PIC (P_{IN})

- likely facilitates eIF1's dissociation for *AUG* selection

Transition to P_{IN} alters eIF1 location to alleviate clash with $tRNA_i$



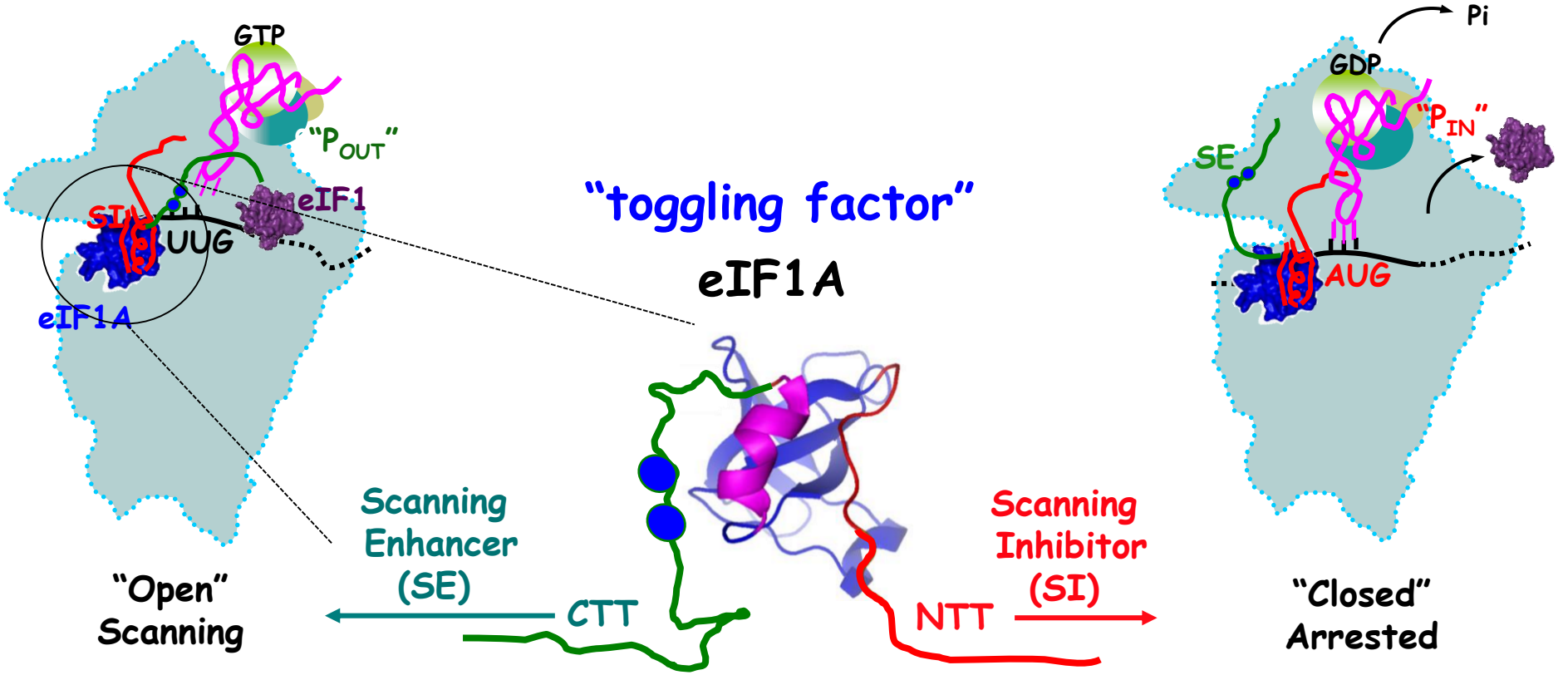
tRNAi (P_{IN})

tRNAi (P_{OUT}): Hashem et al. (Frank)

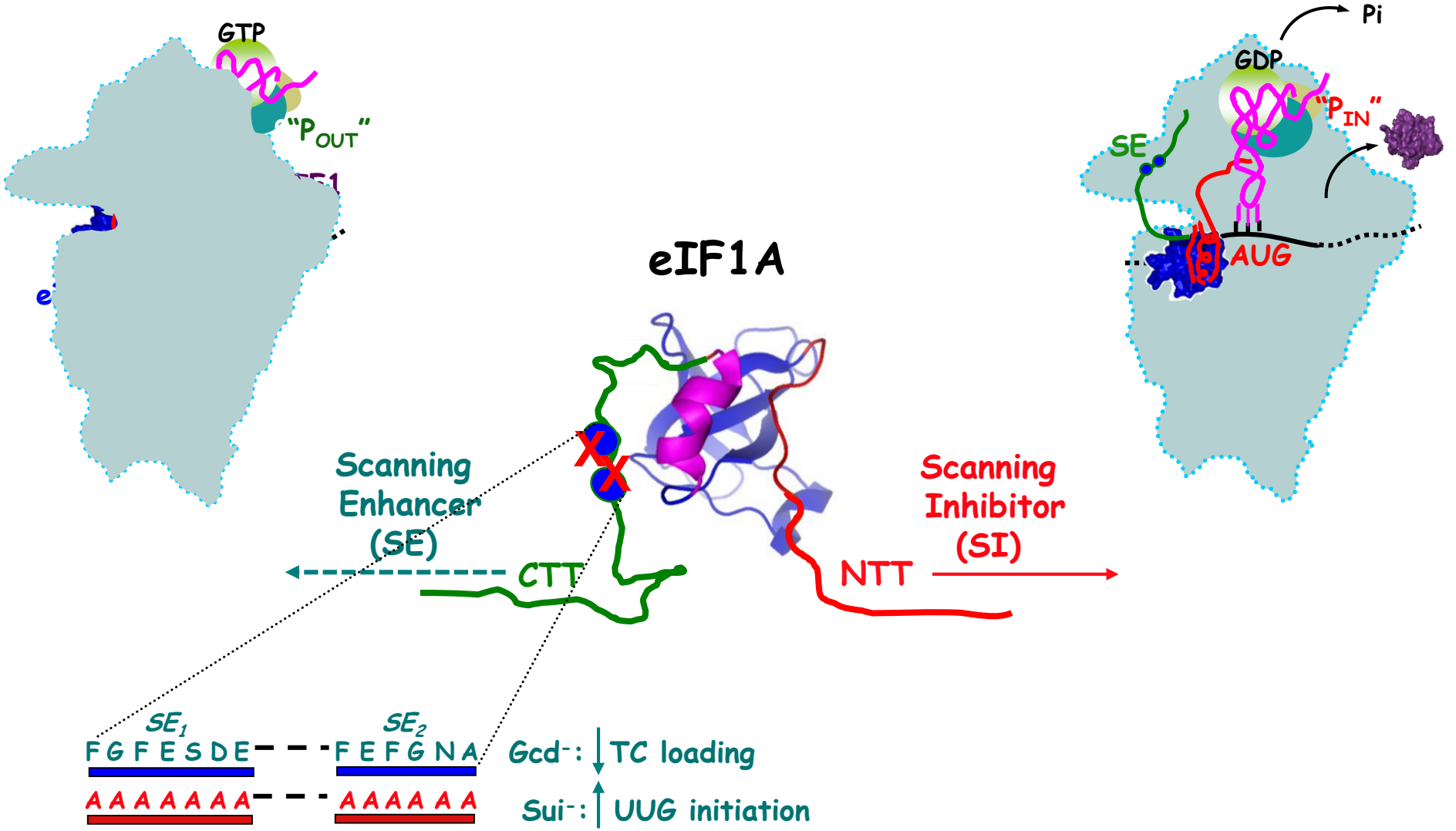
eIF1 in 40S•eIF1•eIF1A
eIF1 in 48S PIC (P_{IN})

- Anil Thakur: mutations in eIF1 loops that should diminish the clash stabilize P_{IN} at UUG codons (Sui^-)

Tails of eIF1A regulate transition from open to closed conformation

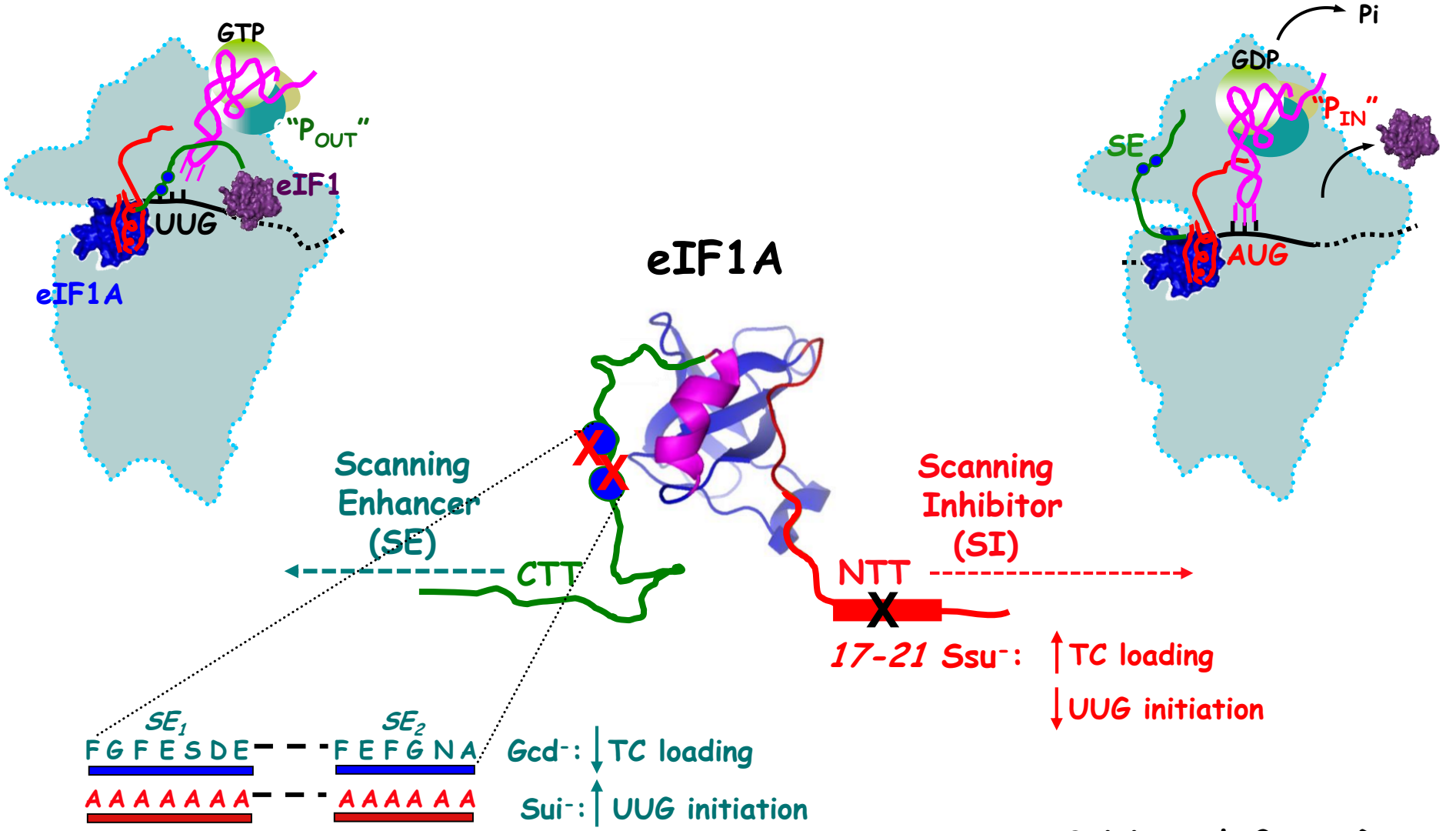


Mutating SE elements in eIF1A CTT decreases accuracy and impairs TC loading



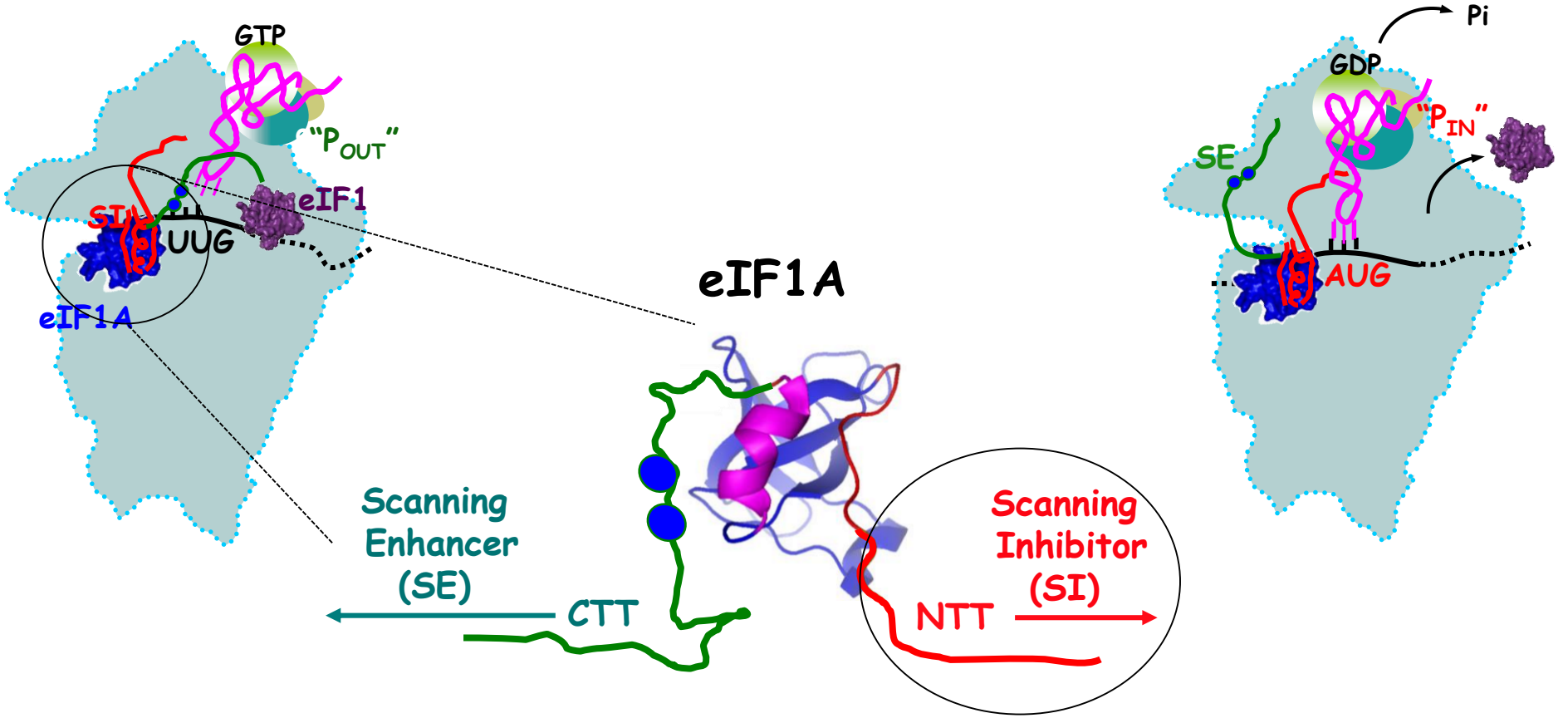
Saini et al *Genes Dev*

Mutating SI elements in eIF1A NTT restores accuracy and rapid TC loading

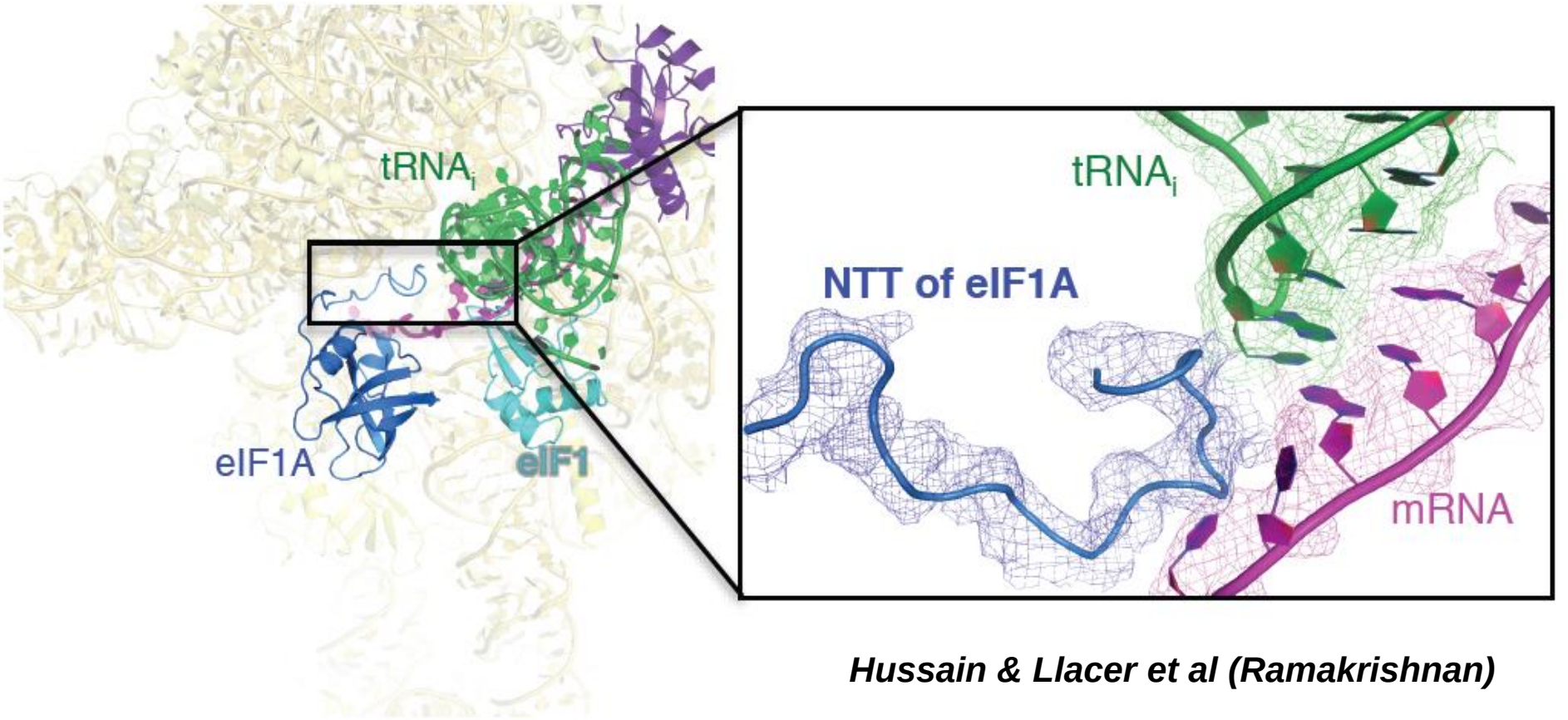


Saini et al *Genes Dev*

eIF1A NTT promotes the P_{IN} state



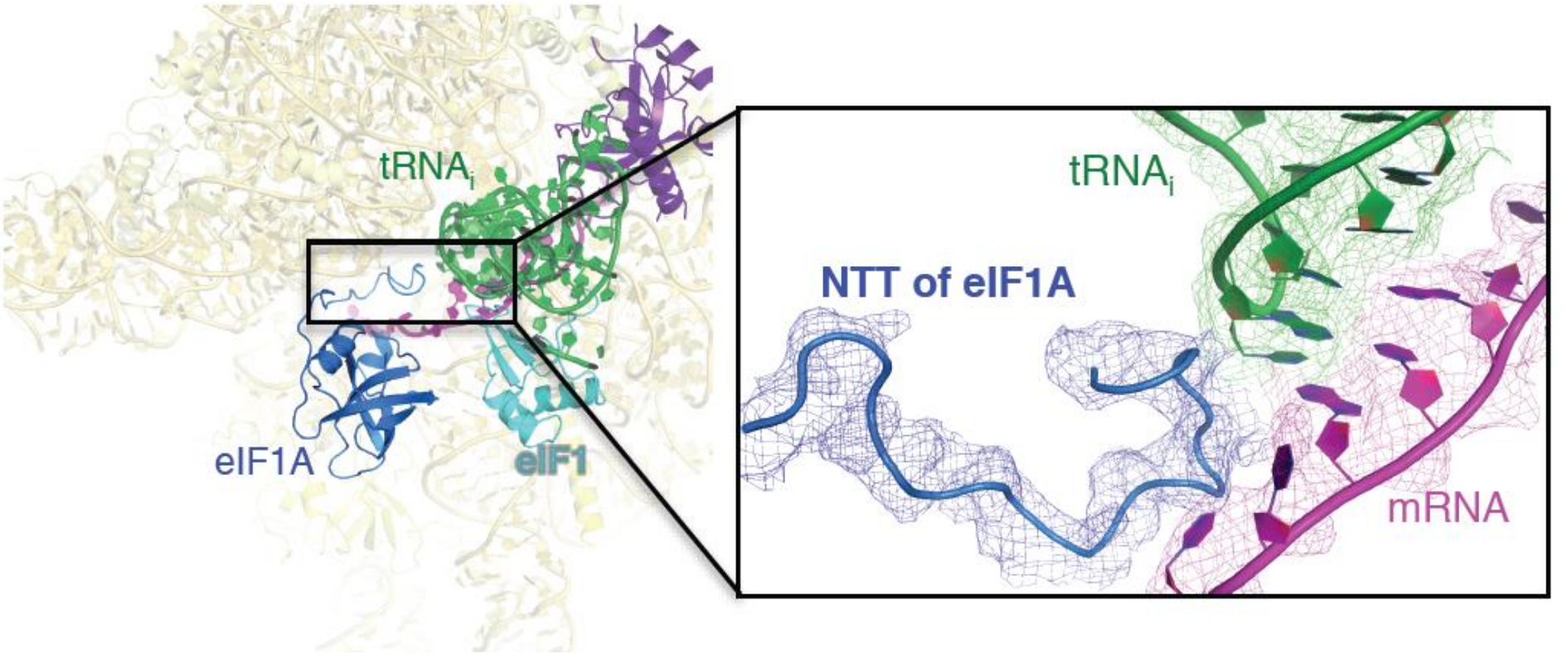
eIF1A NTT interacts with AUG-anticodon helix



Hussain & Llacer et al (Ramakrishnan)

- Ssu$^-$ mutations in the eIF1A NTT impede start codon recognition

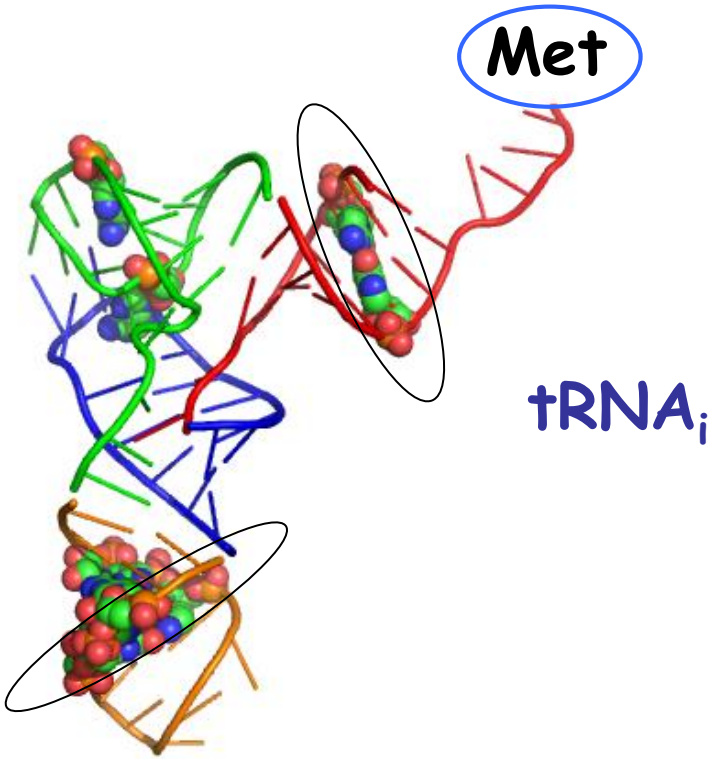
eIF1A NTT interacts with AUG-anticodon helix



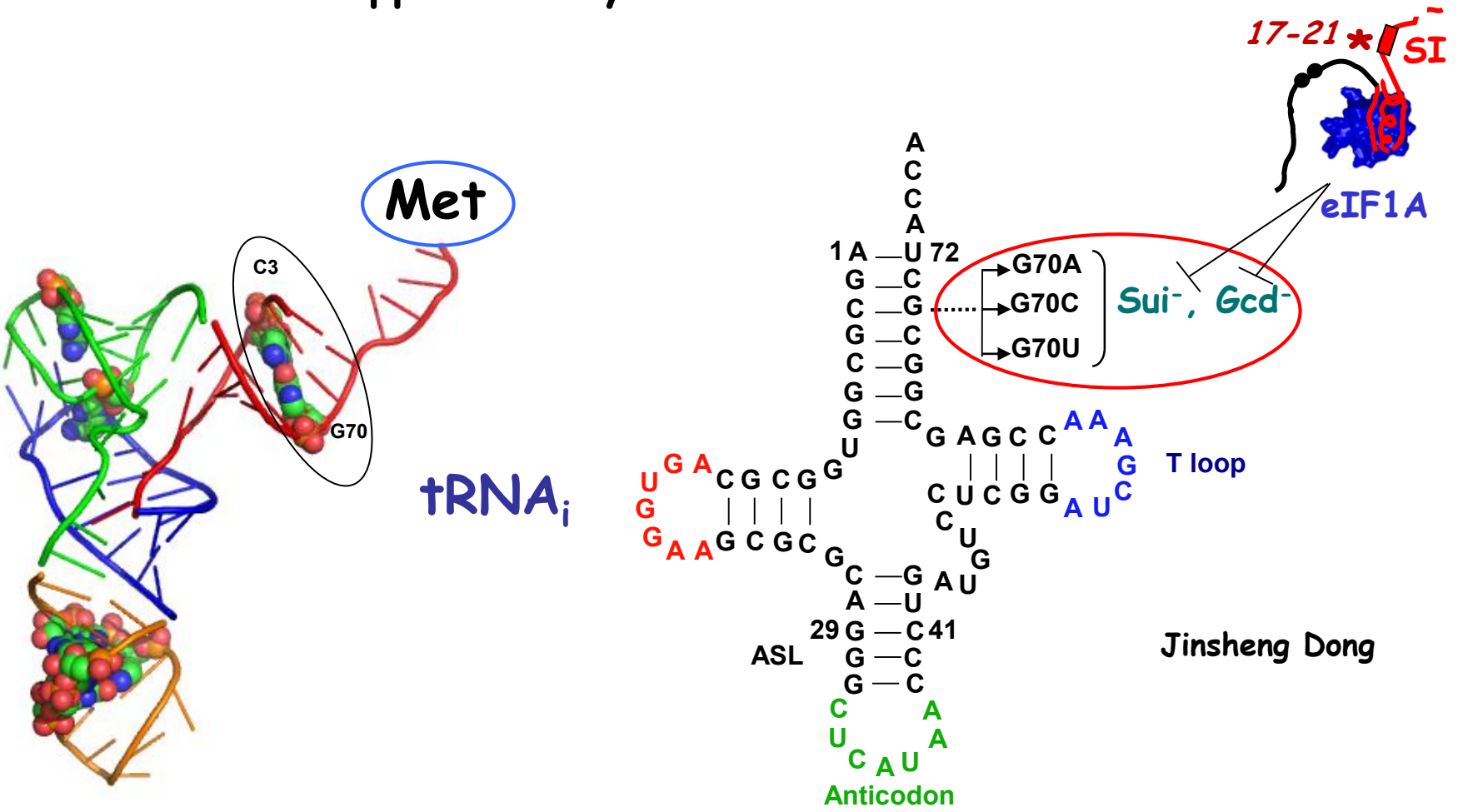
Exome sequencing identifies recurrent somatic mutations in *EIF1AX* and *SF3B1* in uveal melanoma with disomy 3

Marcel Martin[1,2], Lars Maßhöfer[3], Petra Temming[4], Sven Rahmann[1], Claudia Metz[5], Norbert Bornfeld[5], Johannes van de Nes[6], Ludger Klein-Hitpass[7], Alan G Hinnebusch[8], Bernhard Horsthemke[3], Dietmar R Lohmann[3,9] & Michael Zeschnigk[3,9]

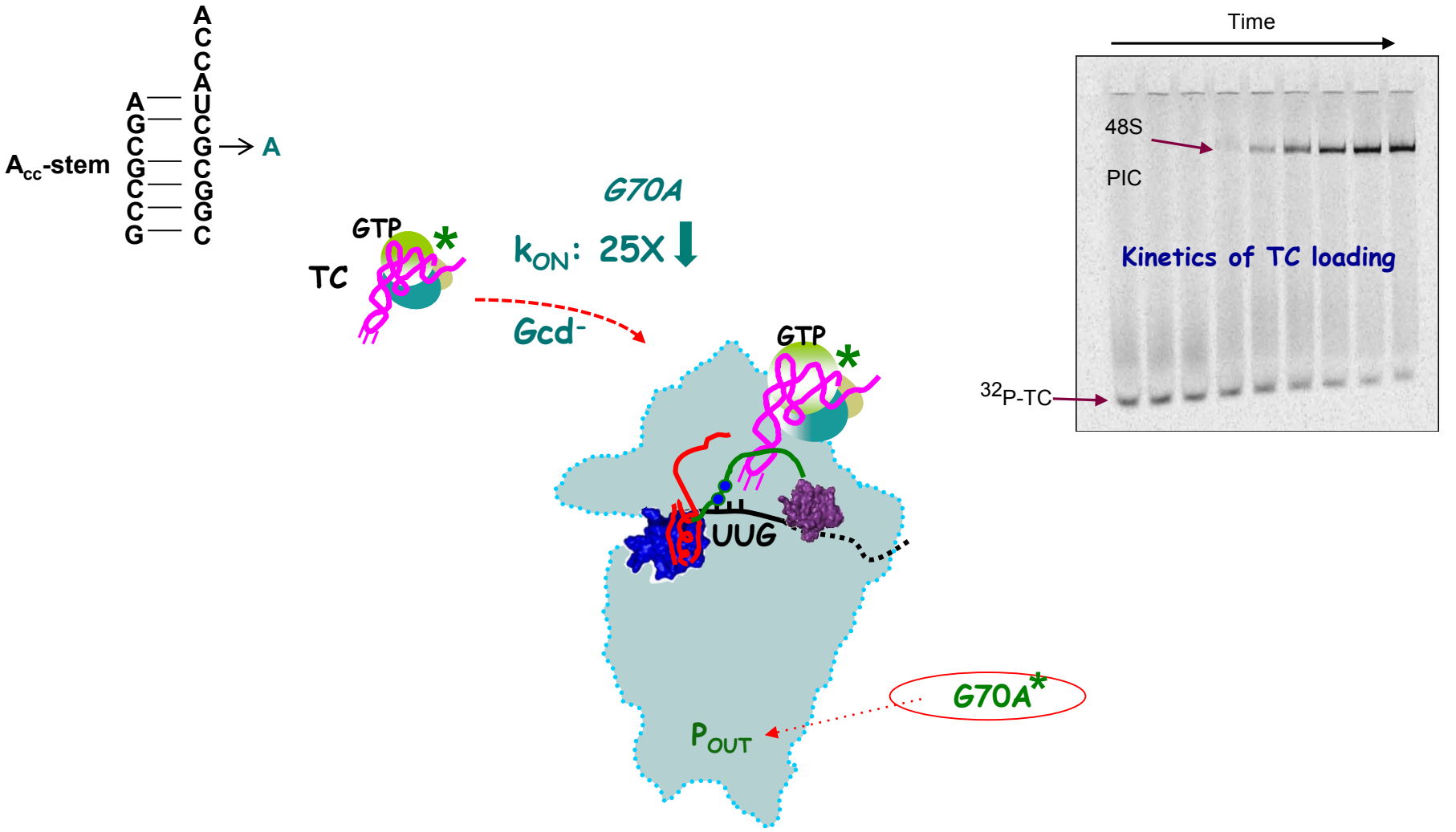
Conserved bases in $tRNA_i$ play distinct roles in the accuracy of AUG selection



Disruption of C3-G70 confers Sui⁻ and Gcd⁻ phenotypes co-suppressed by eIF1A NTT mutation

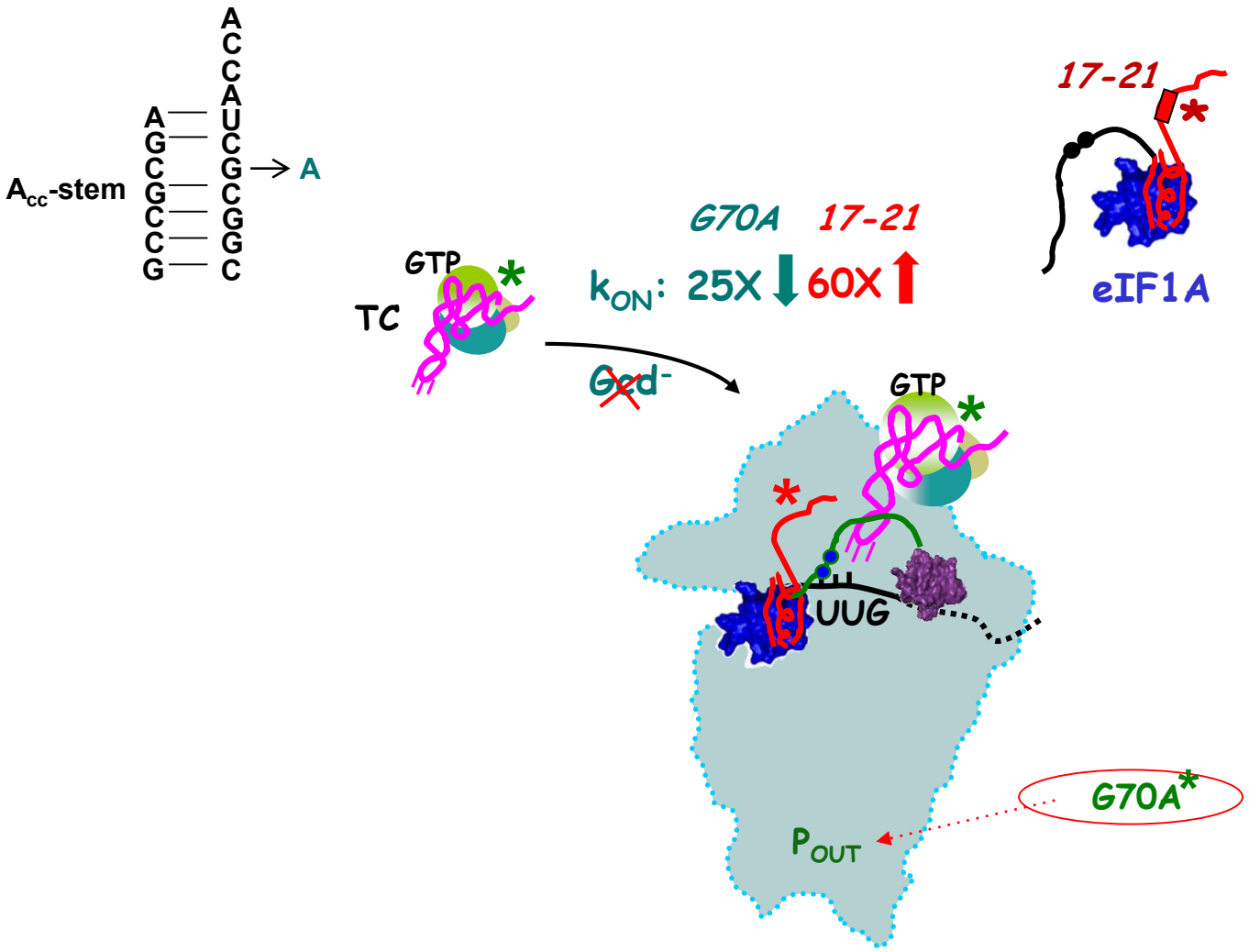


G70A mutation decreases rate of TC binding in vitro…



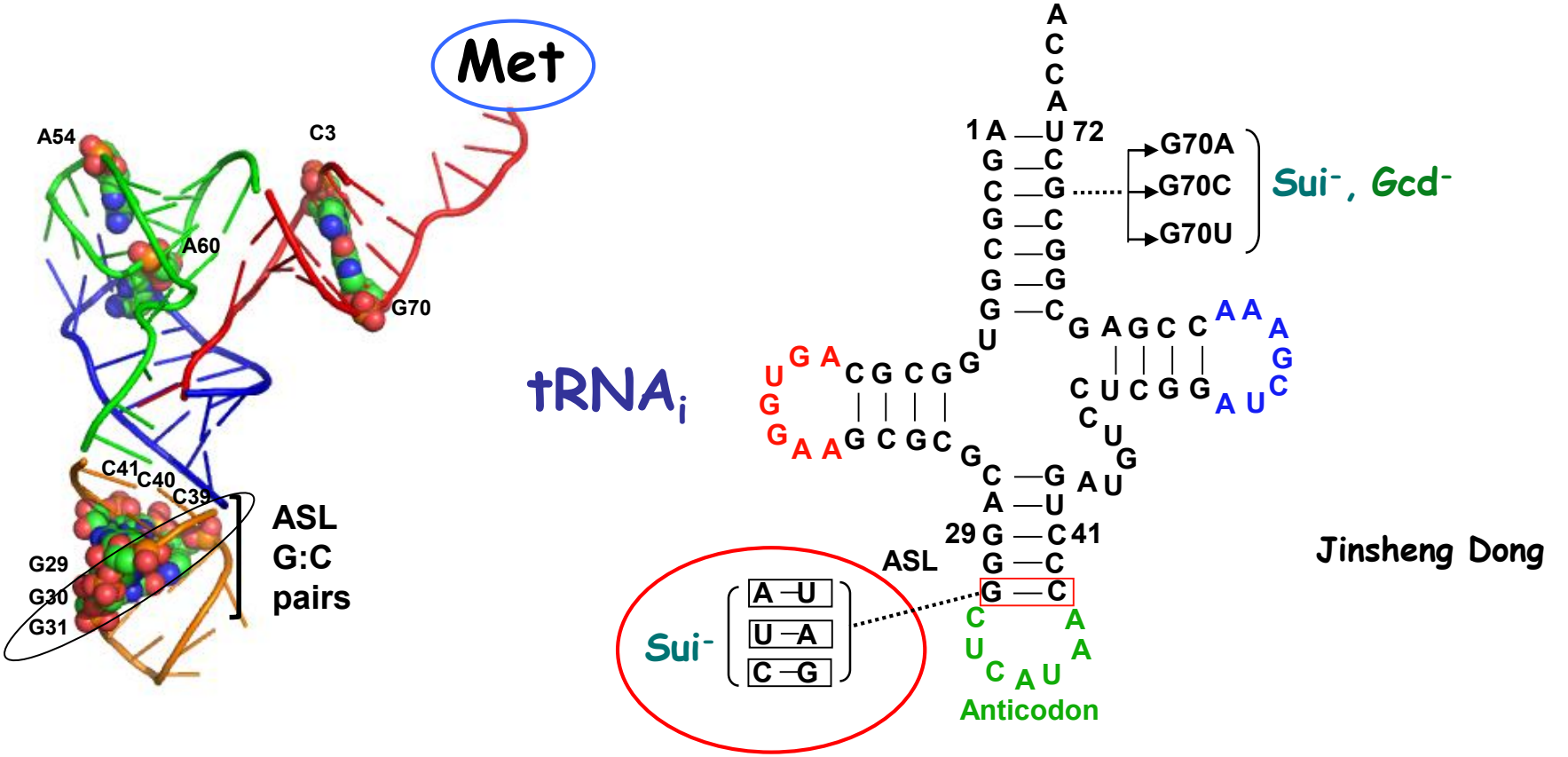
Tony Munoz (Lorsch lab)

…in a manner reversed by eIF1A NTT mutation *17-21*

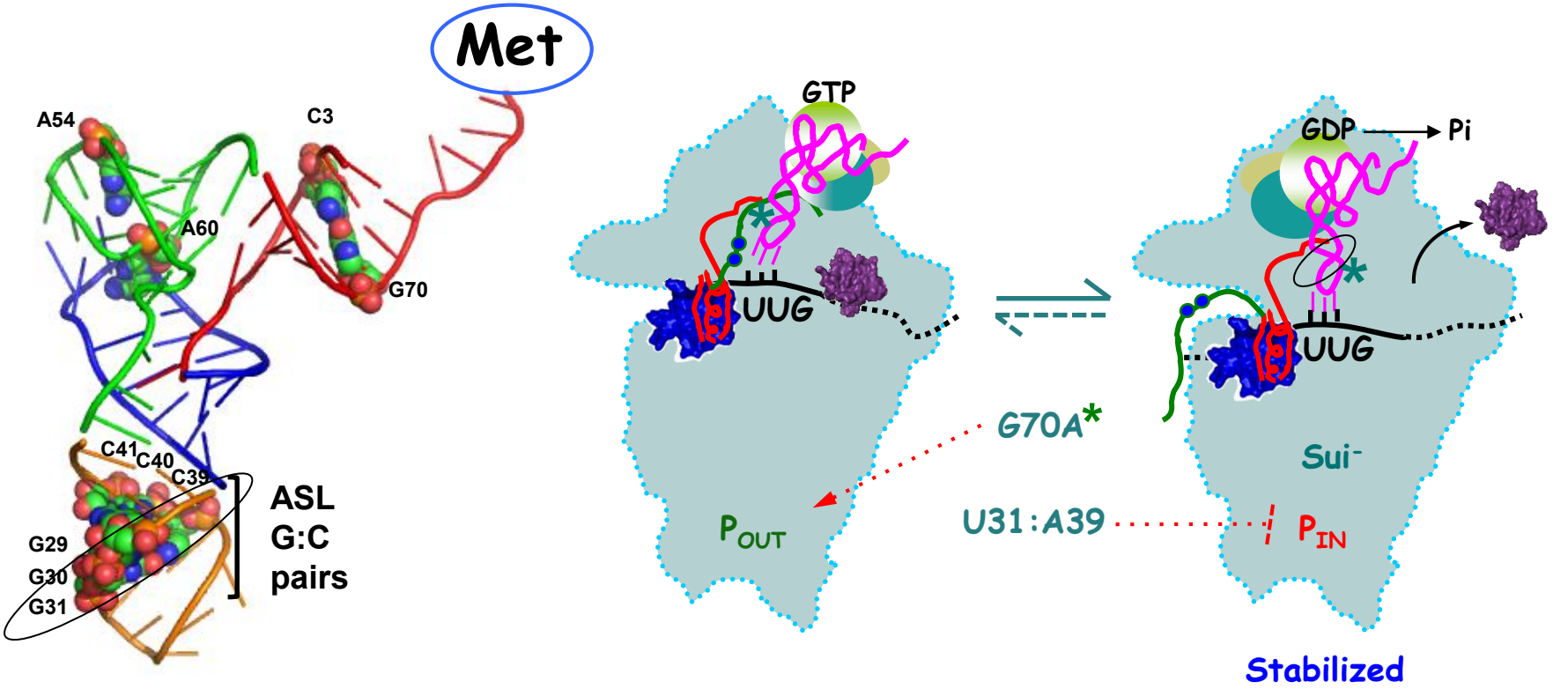


Tony Munoz (Lorsch lab)

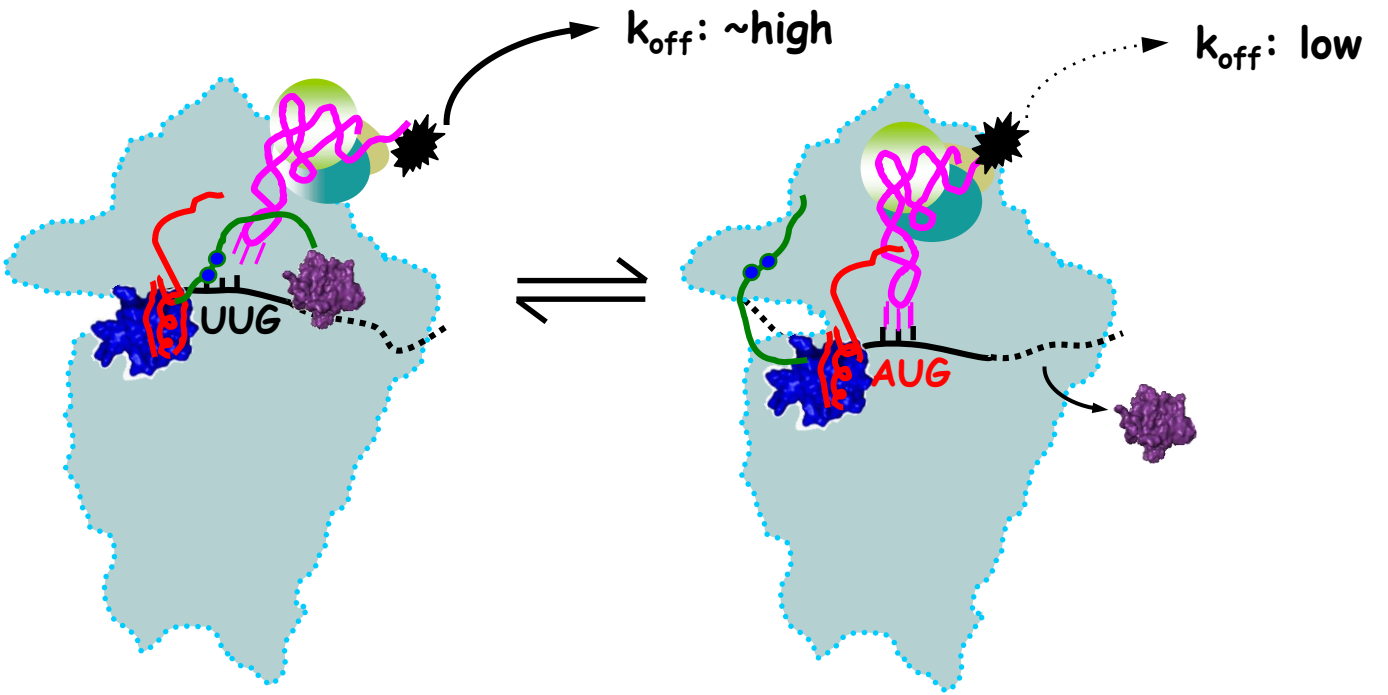
Base-pair substitutions of G31-C39 confer Sui⁻ but not Gcd⁻ phenotypes



Hypothesis: U31:A39 substitution in ASL removes barrier to P_{IN}

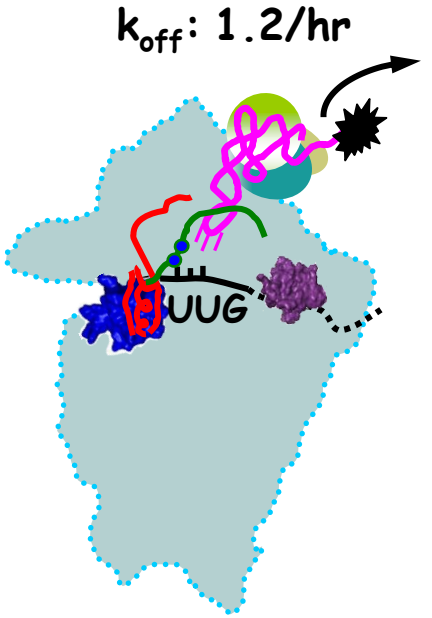


TC is less tightly bound to the PIC at UUG codons

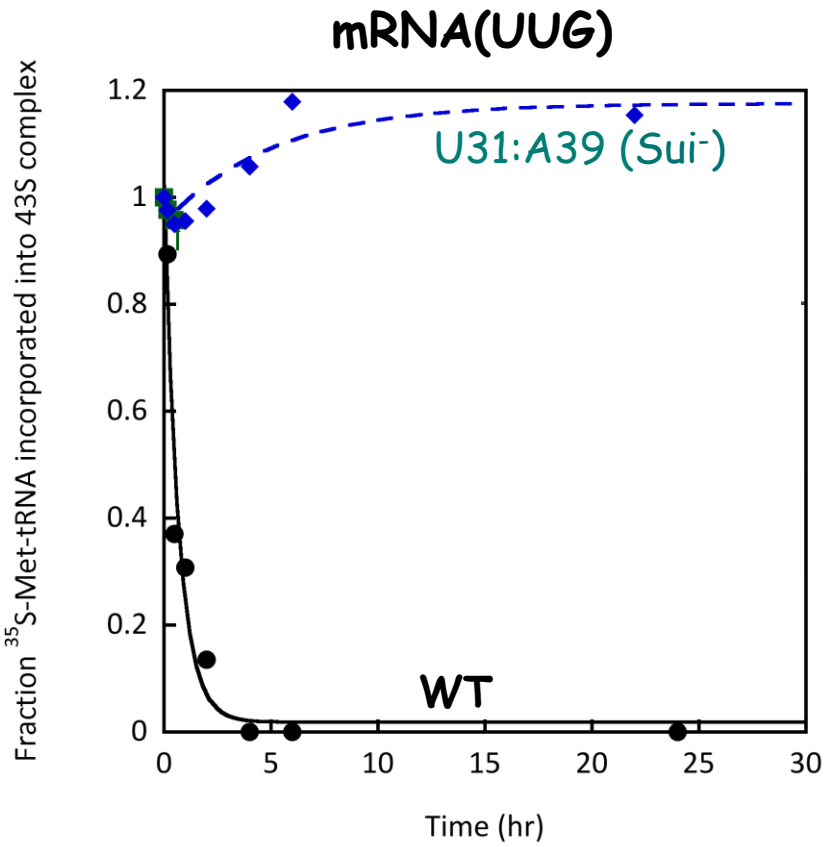


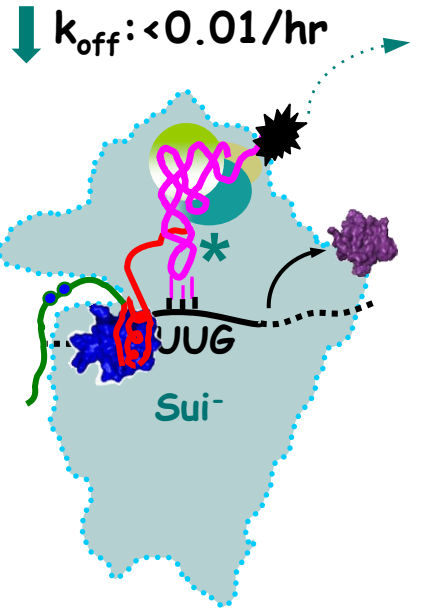
	k_{off} (h^{-1})	
	AUG	UUG
WT	<0.4	1.2

U31:A39 replacement stabilizes P_{IN} at UUG codons

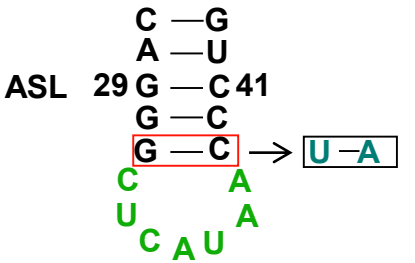


WT



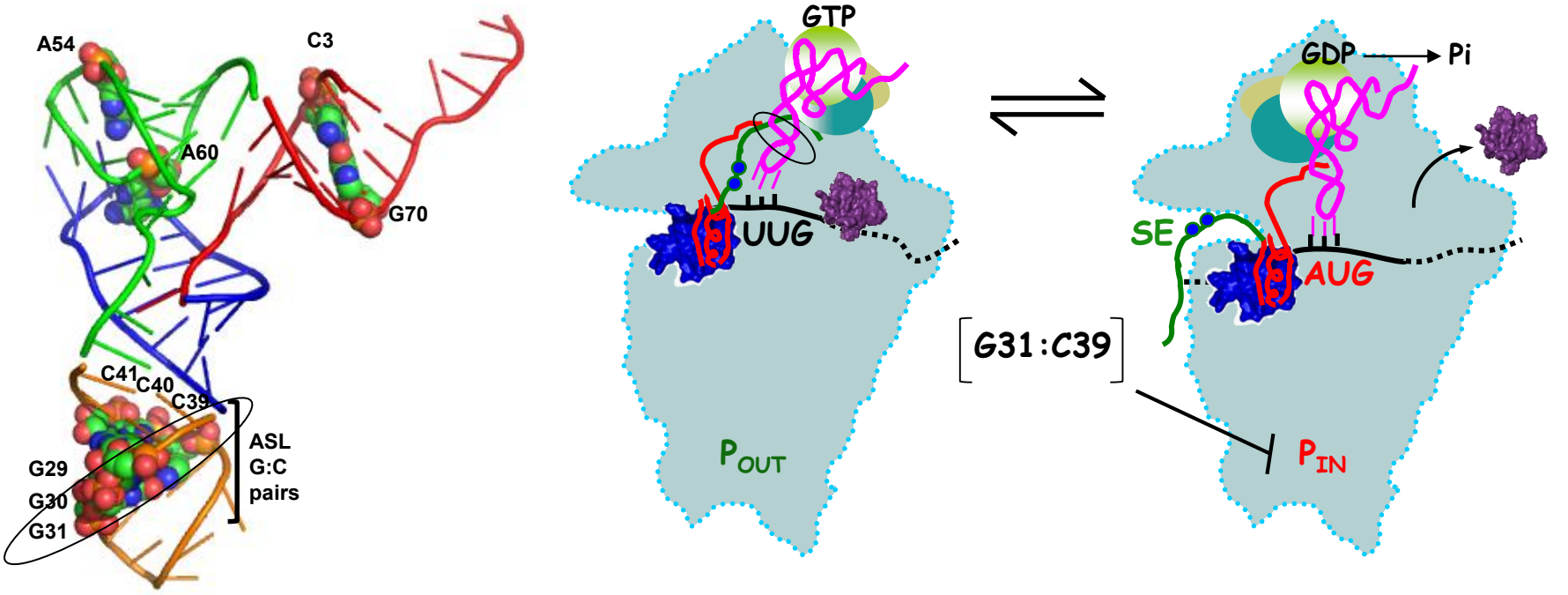


U31:A39



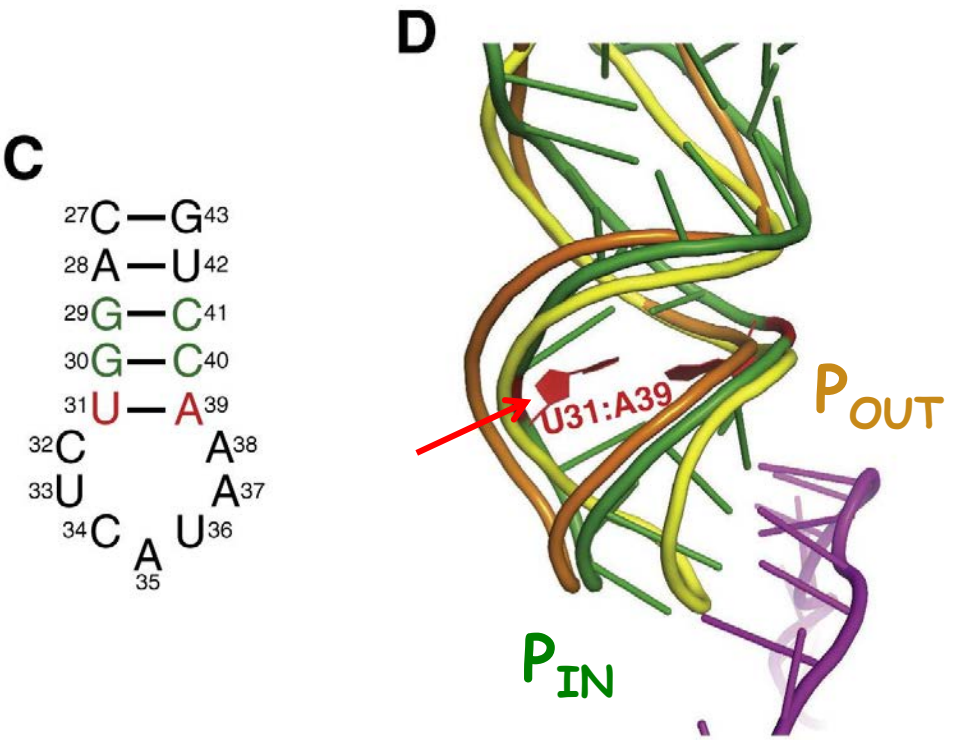
Tony Munoz (Lorsch lab)

G31:C39 impedes P_{IN} state & demands perfect AUG-anticodon duplex



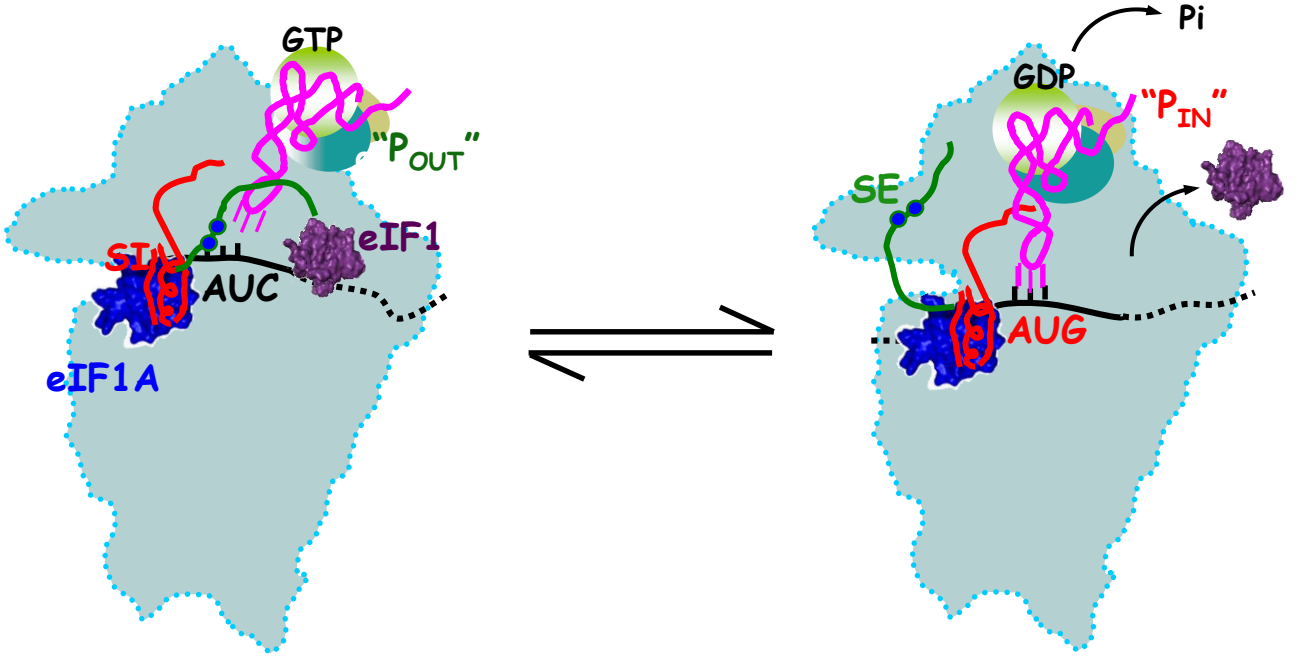
...

$tRNA_i$ anticodon stem is distorted in P_{IN} state

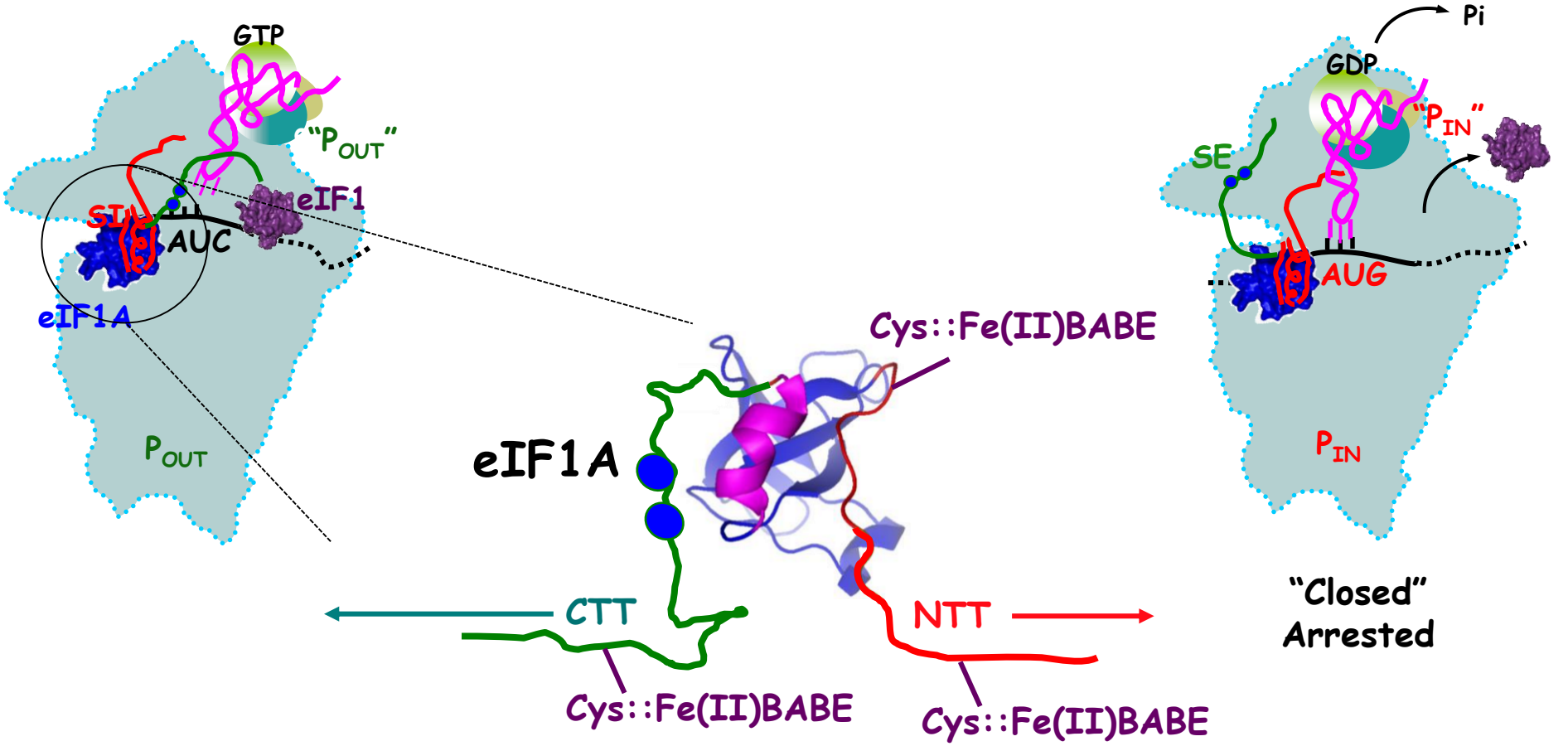


Hussain & Llacer et al (Ramakrishnan)

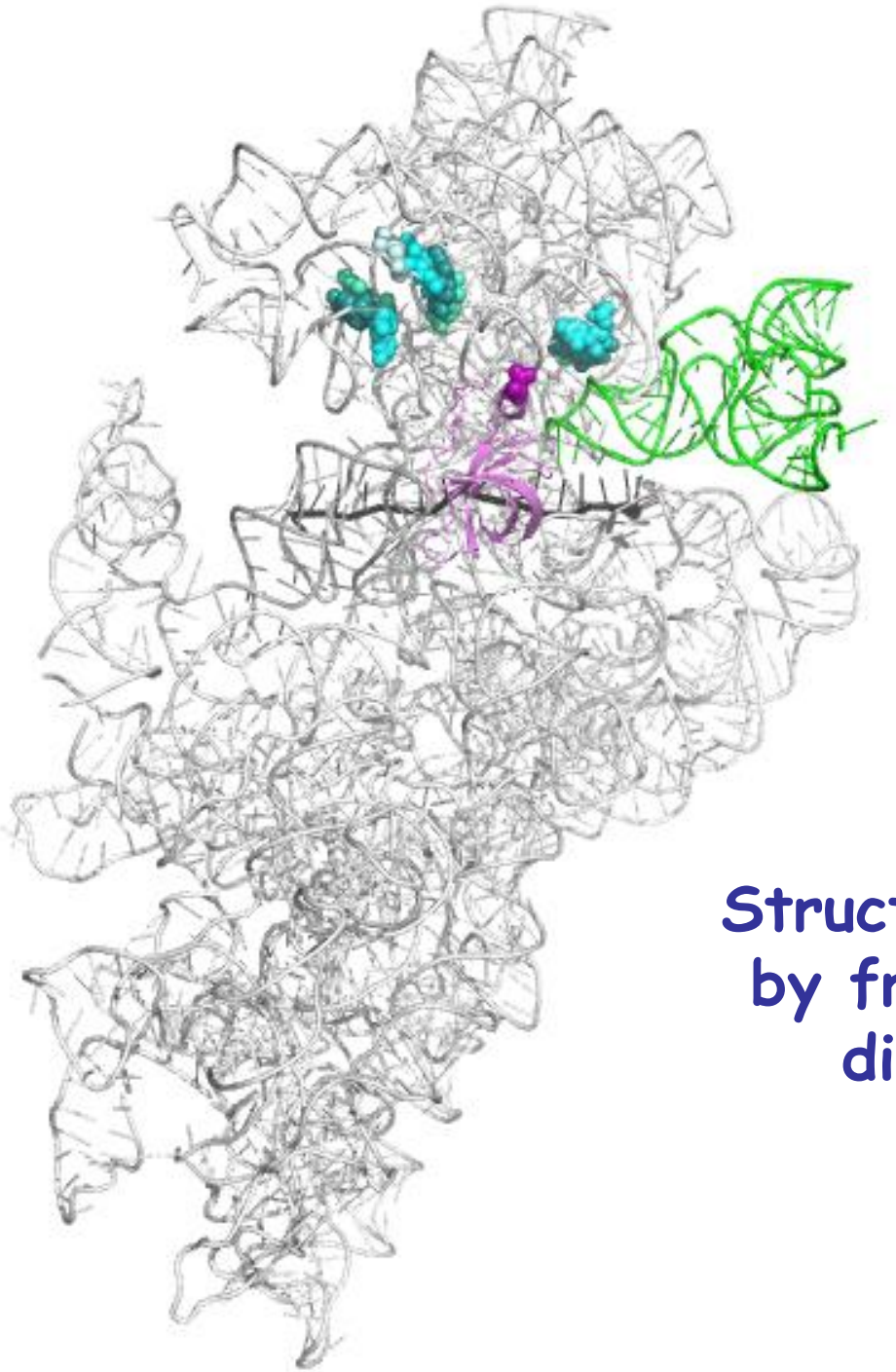
Evidence for 40S conformational changes was lacking



Structural probing of PICs by free-radical cleavage directed by eIF1A



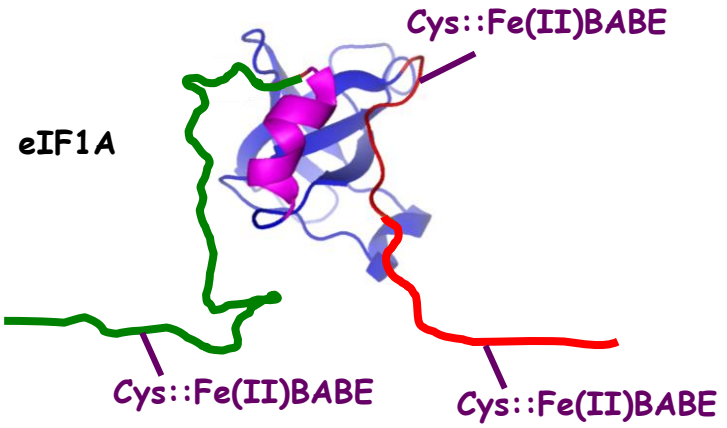
Fan Zhang & Adesh Saini

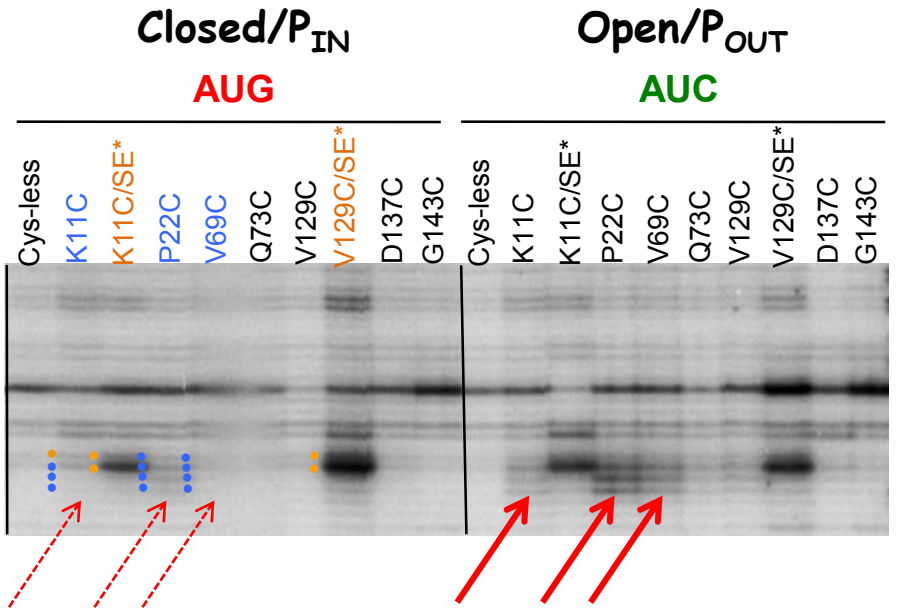


Structural probing of PICs by free-radical cleavage directed by eIF1A

Fan Zhang & Adesh Saini

Greater cleavage of P-site residues in "open" (AUC) versus "closed" (AUG) complex

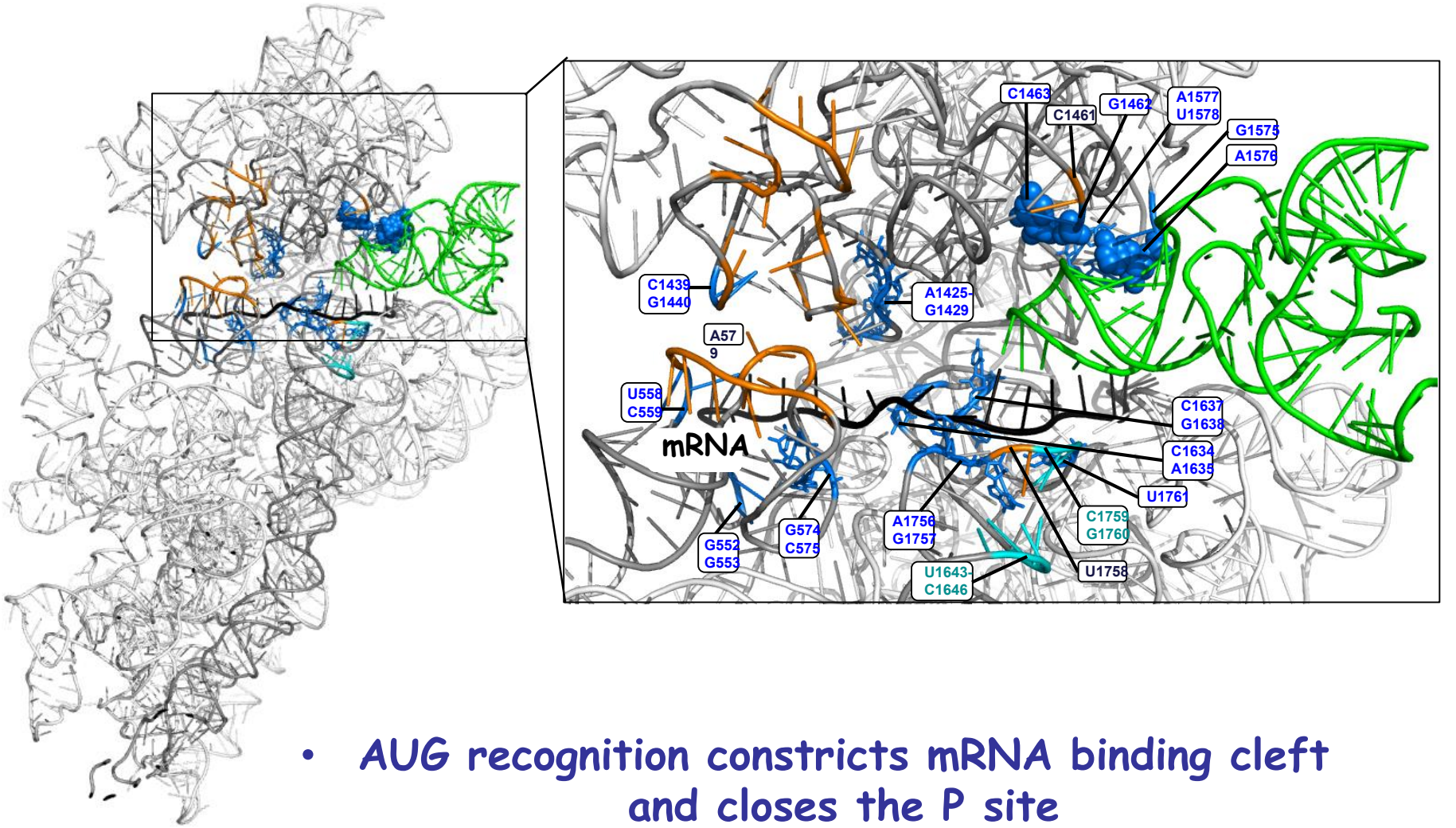




Fan Zhang & Adesh Saini

AUG recognition evokes closure of P site (P_{IN})

Cleavages in P-site and mRNA binding cleft suppressed in AUG vs AUC complex



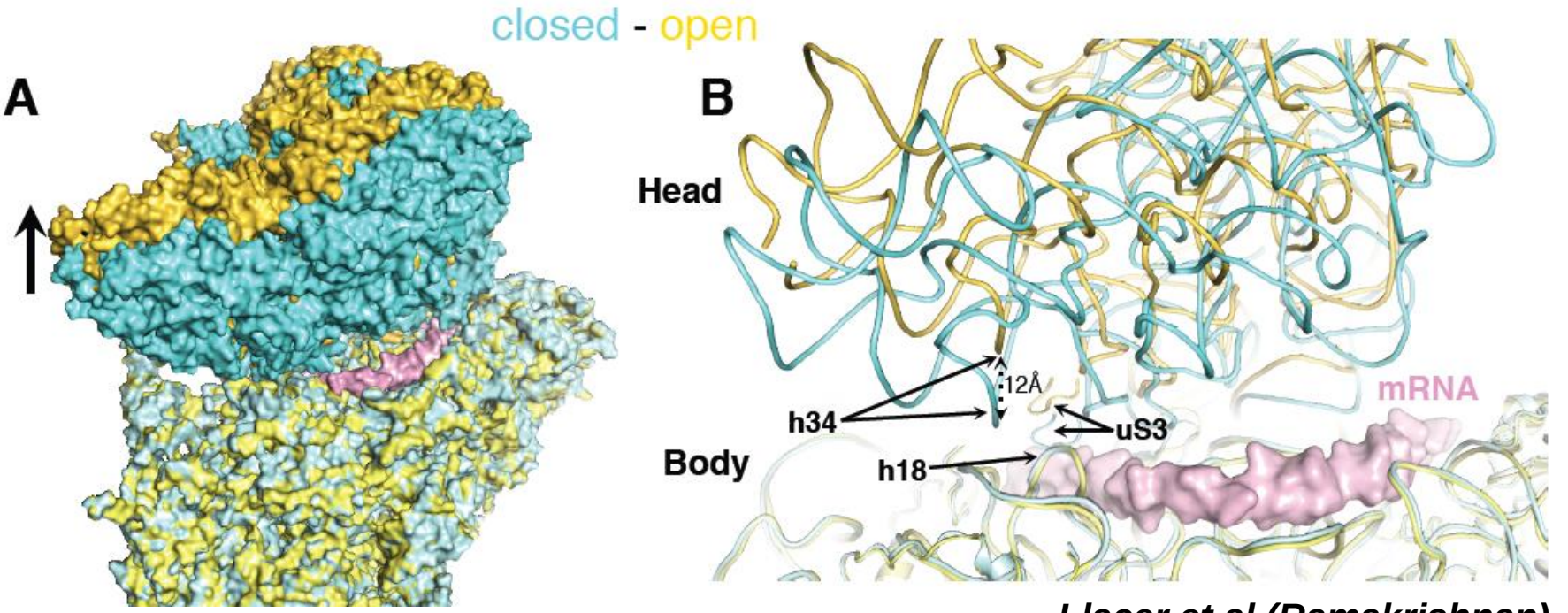
- AUG recognition constricts mRNA binding cleft and closes the P site

Open PIC conformation at AUC shows upward movement of 40S head

py48S-open: (AUC)mRNA

py48S-closed: (AUG)mRNA

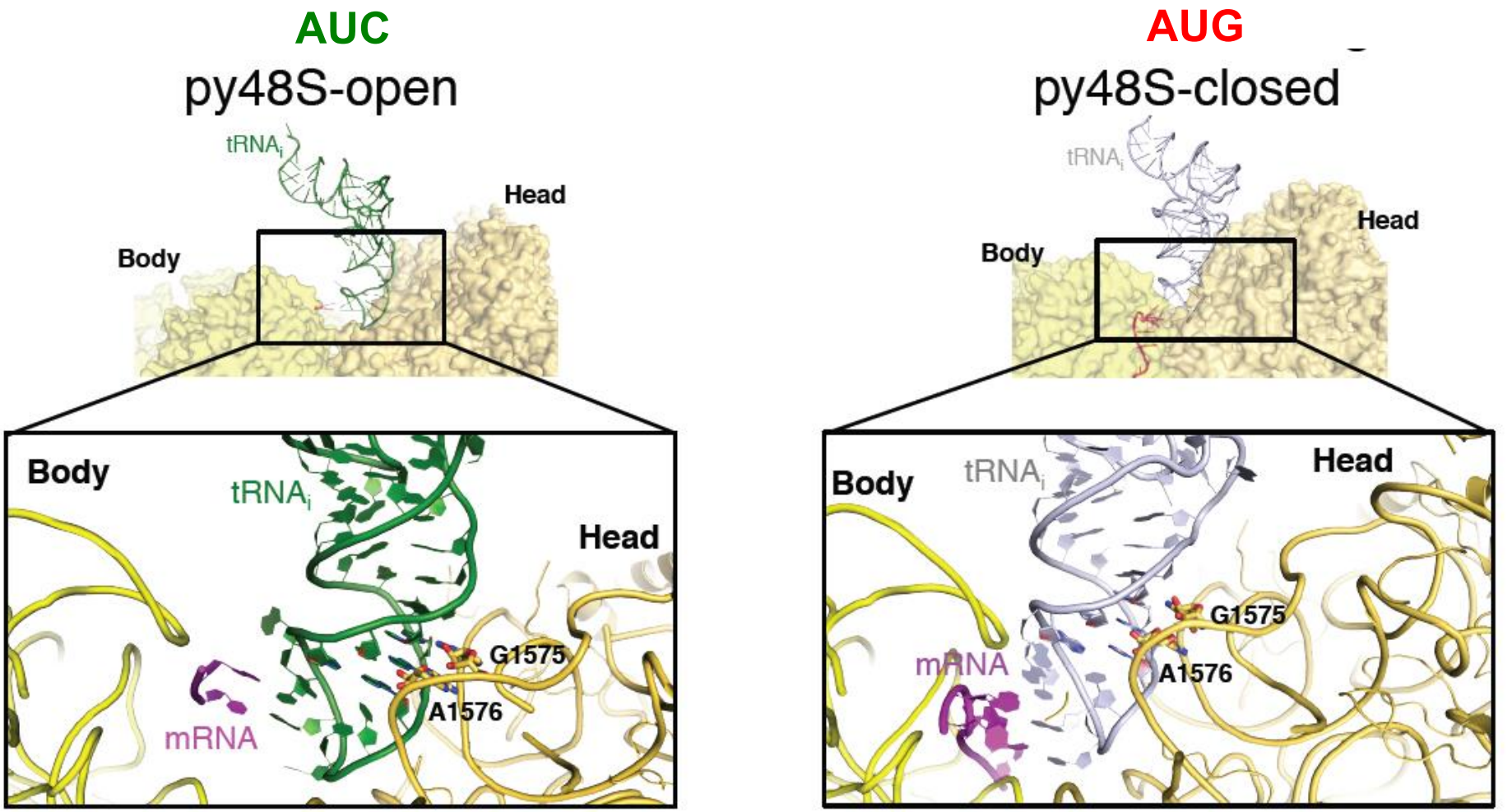
AUC



Llacer et al (Ramakrishnan)

- Conducive for mRNA recruitment & scanning

Open PIC conformation at AUC shows widened P-site

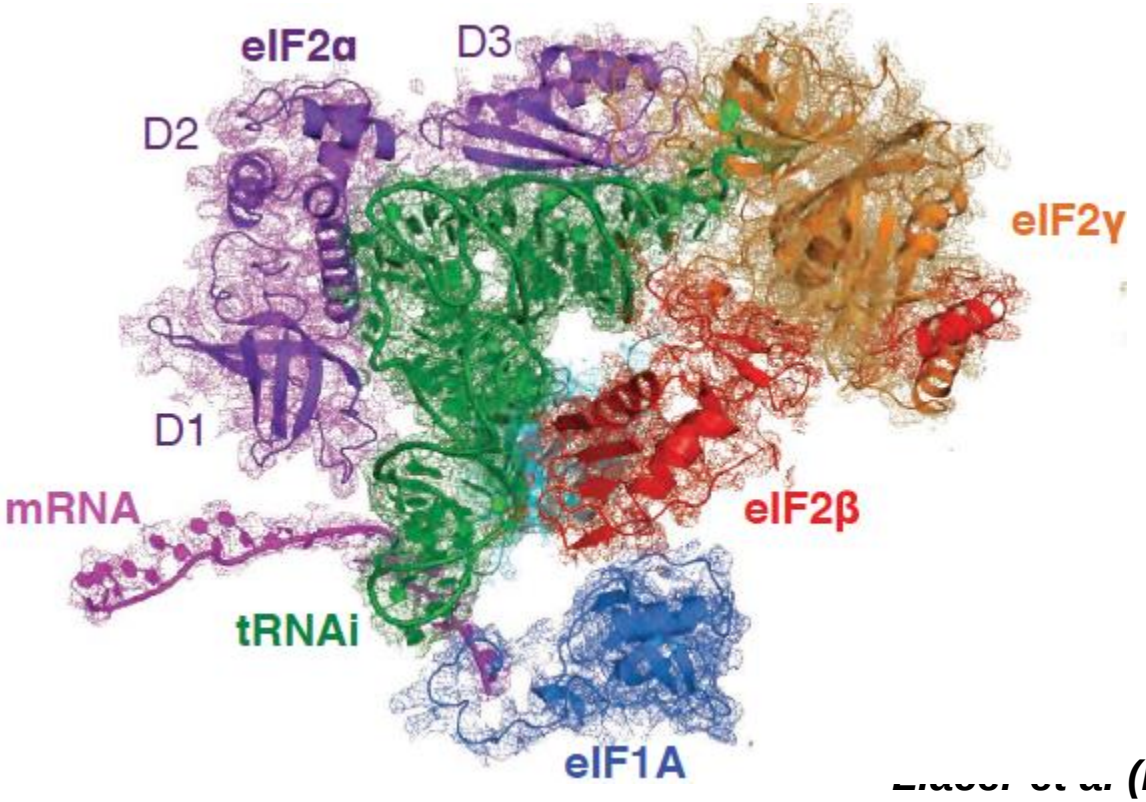


Llacer et al (Ramakrishnan)

- Compatible with triplet sampling by $tRNA_i$ during scanning

eIF2β contacts tRNA$_i$, eIF1, and eIF1A in open complex

py48S-open

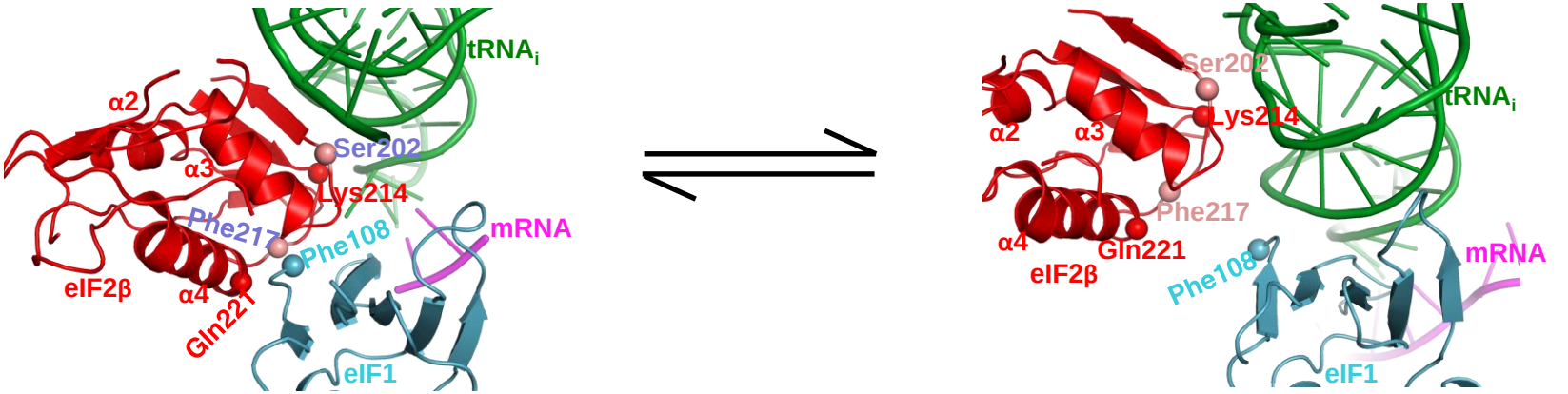


Llácer et al. (Ramakrishnan)

eIF2β contacts eIF1 exclusively in open complex

open (AUC): scanning

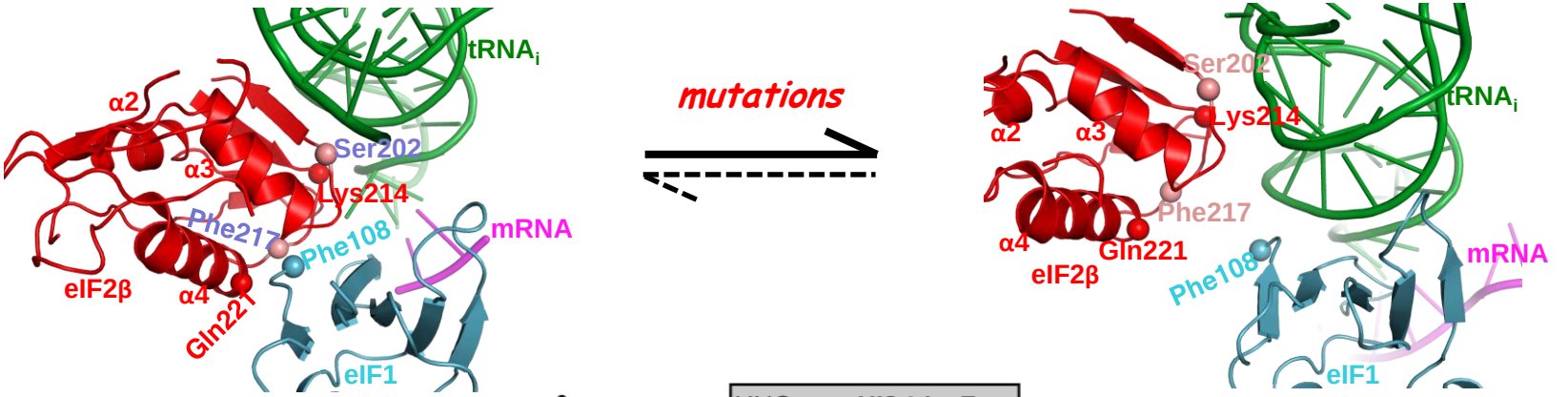
Closed (AUG) initiation

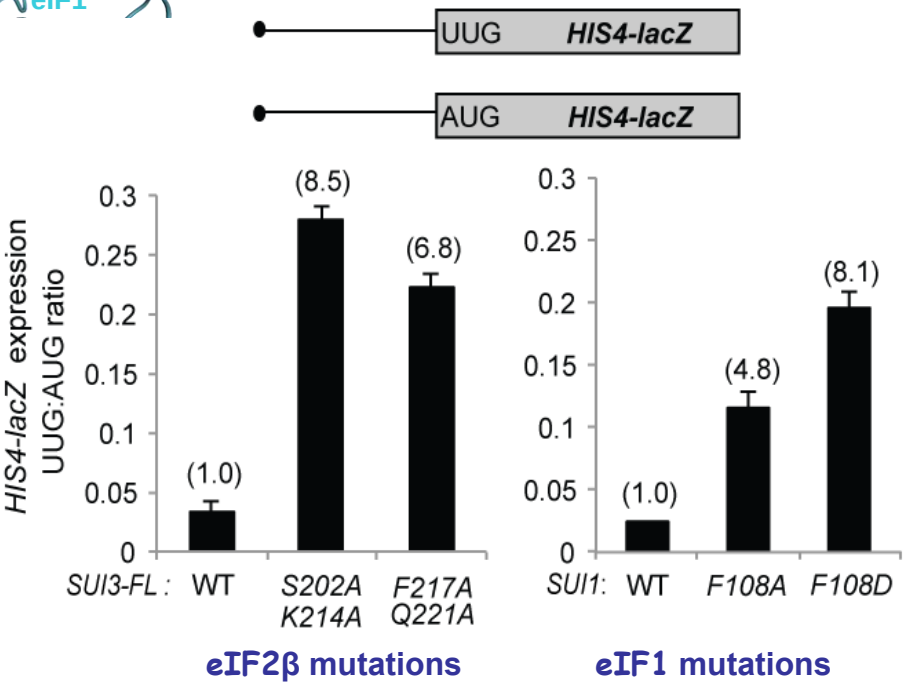


eIF2β contacts with eIF1 promote scanning and impede UUG initiation

open (AUC): scanning

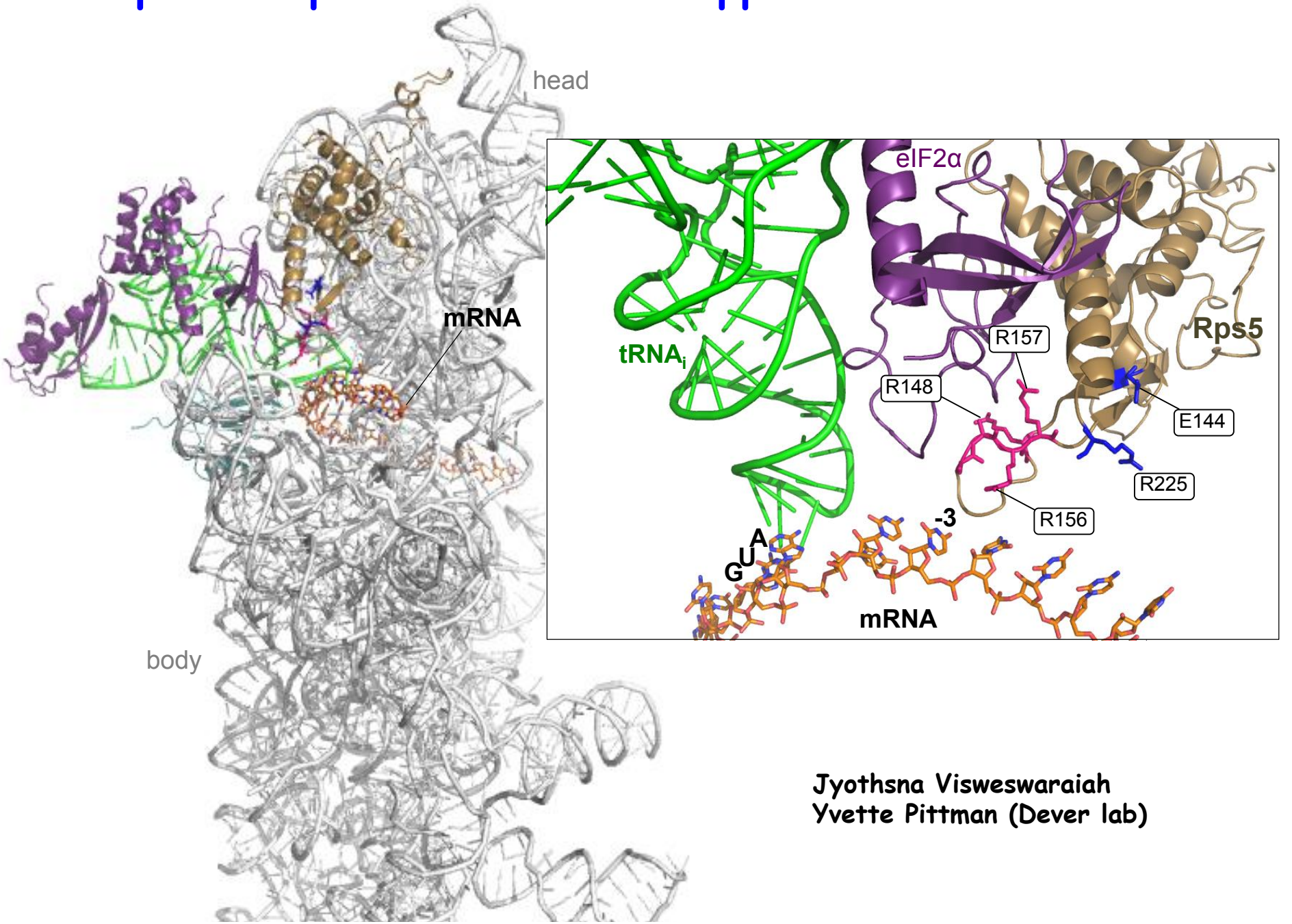
Closed (AUG) initiation





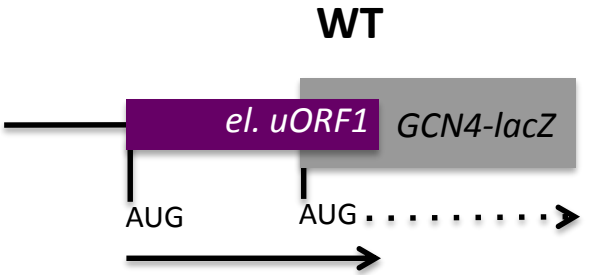
Laura Marler
Anil Thakur

Rps5 hairpin substitutions suppress UUG initiation



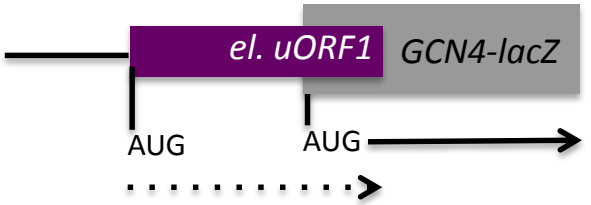
Jyothsna Visweswaraiah
Yvette Pittman (Dever lab)

rps5-E144R impairs AUG recognition by the scanning PIC

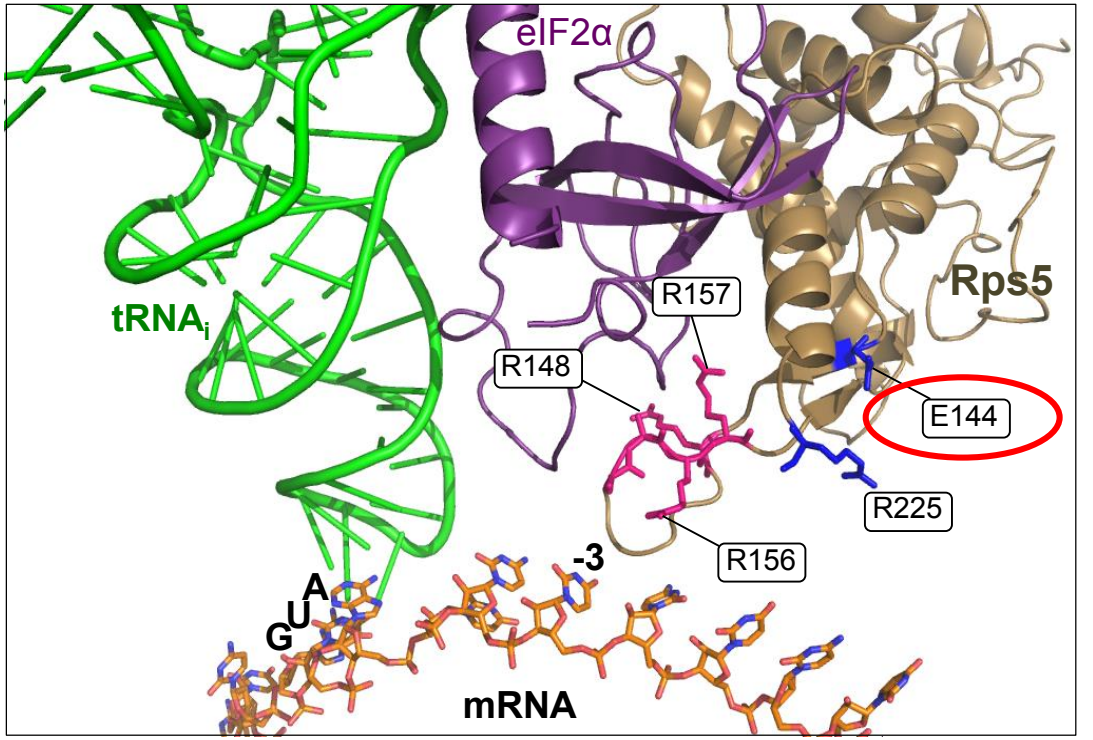


1st AUG blocks initiation downstream

rps5-E144R

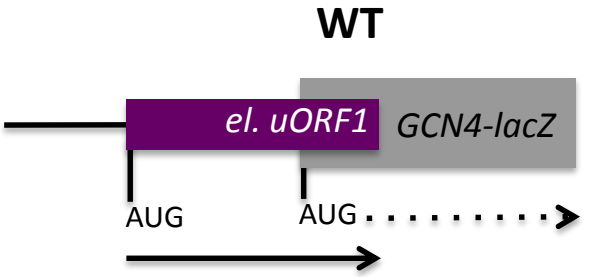


Bypass of 1st AUG enables initiation downstream



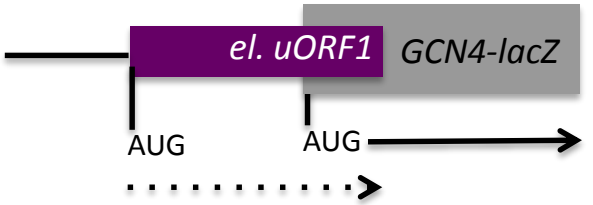
Jyothsna Visweswaraiah
Yvette Pittman (Dever lab)

rps5-E144R impairs AUG recognition by destabilizing P_{IN} state

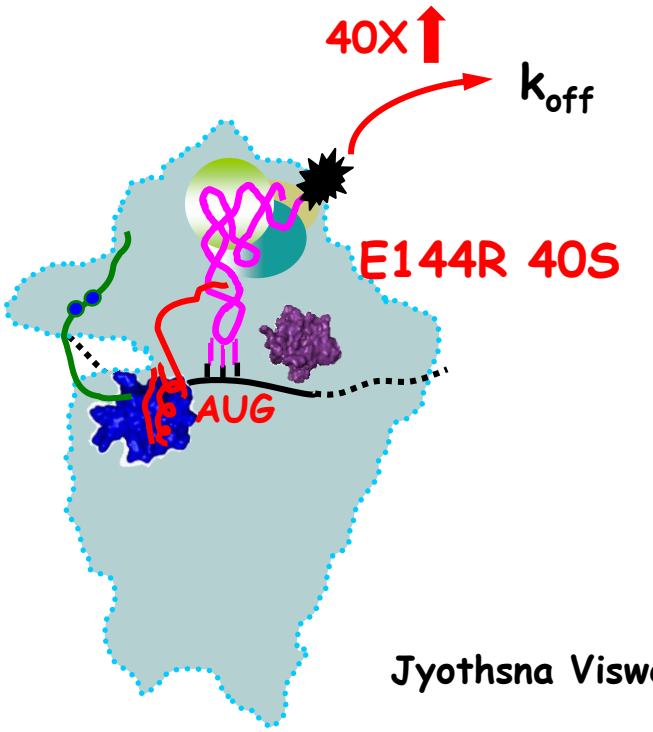


1st AUG blocks initiation downstream

rps5-E144R



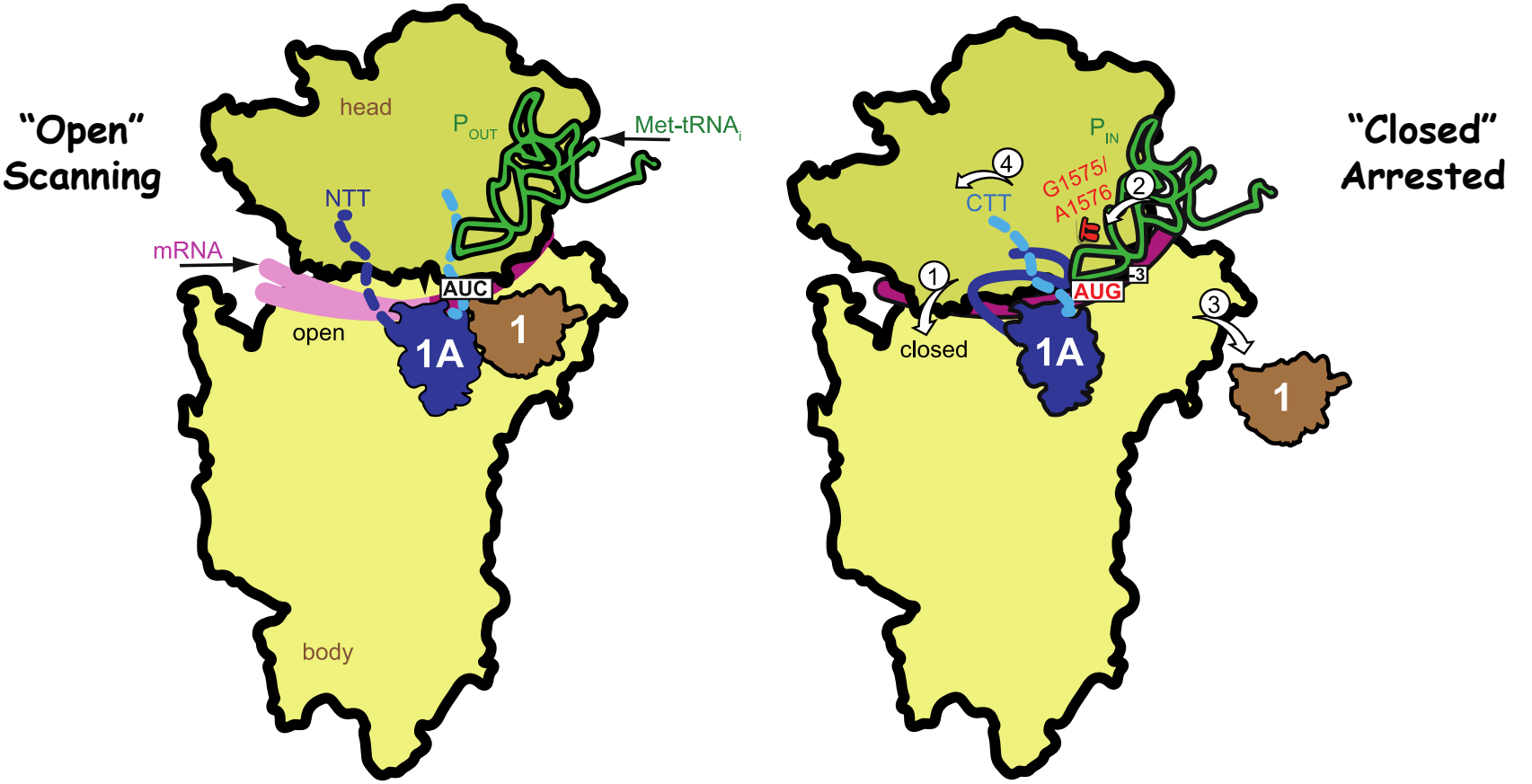
Bypass of 1st AUG enables initiation downstream



Jyothsna Visweswaraiah

- Rps5 is on par with eIFs in controlling AUG recognition

Conformational rearrangements in transition from scanning to AUG selection



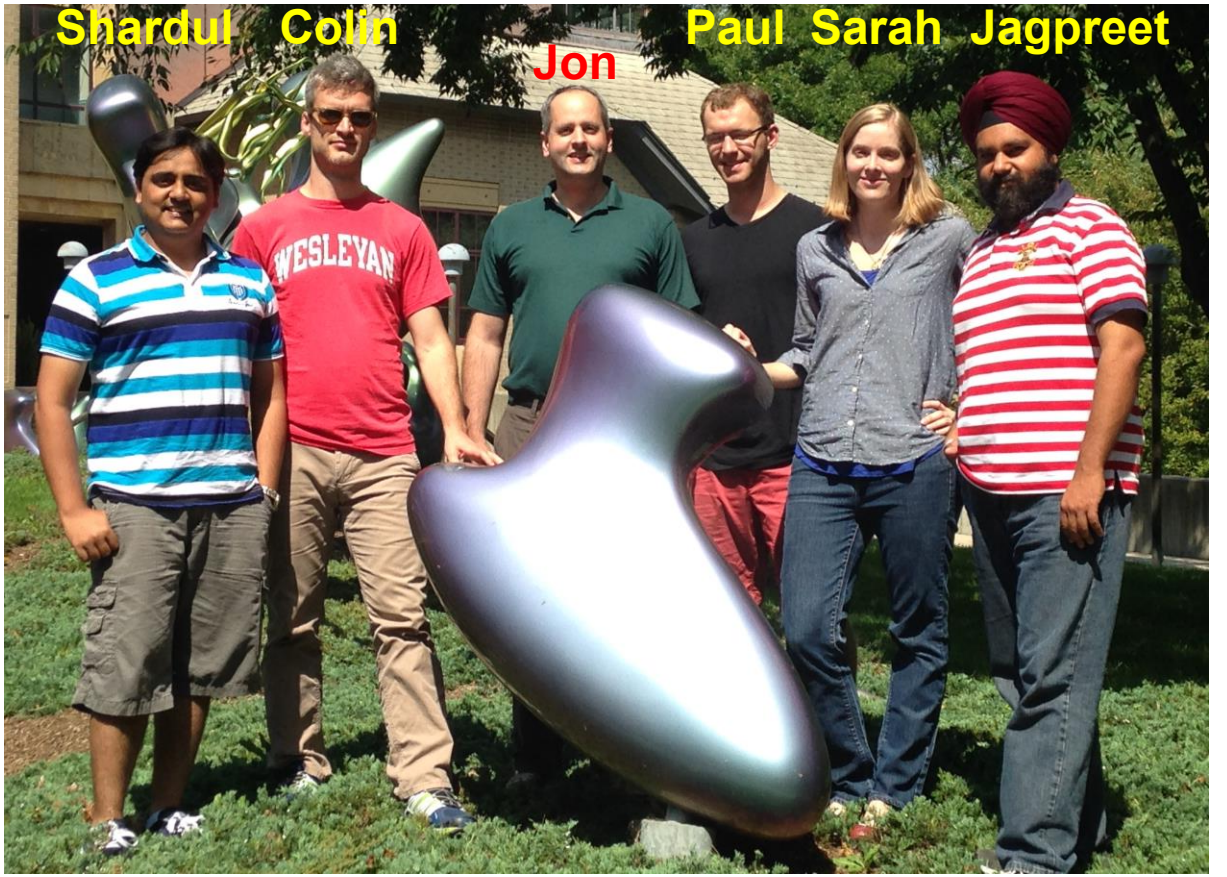
- Downward head movement constricts mRNA cleft
  - P site closes around $tRNA_i$
- eIF1A NTT interacts with codon:anticodon duplex
  - eIF1 displaced by $tRNA_i$ from P site
- eIF1 dissociates to allow P_i release from eIF2

MRC Laboratory of Molecular Biology, University of Cambridge, UK

Not shown:
Israel Fernandez

Venki Ramakrishnan

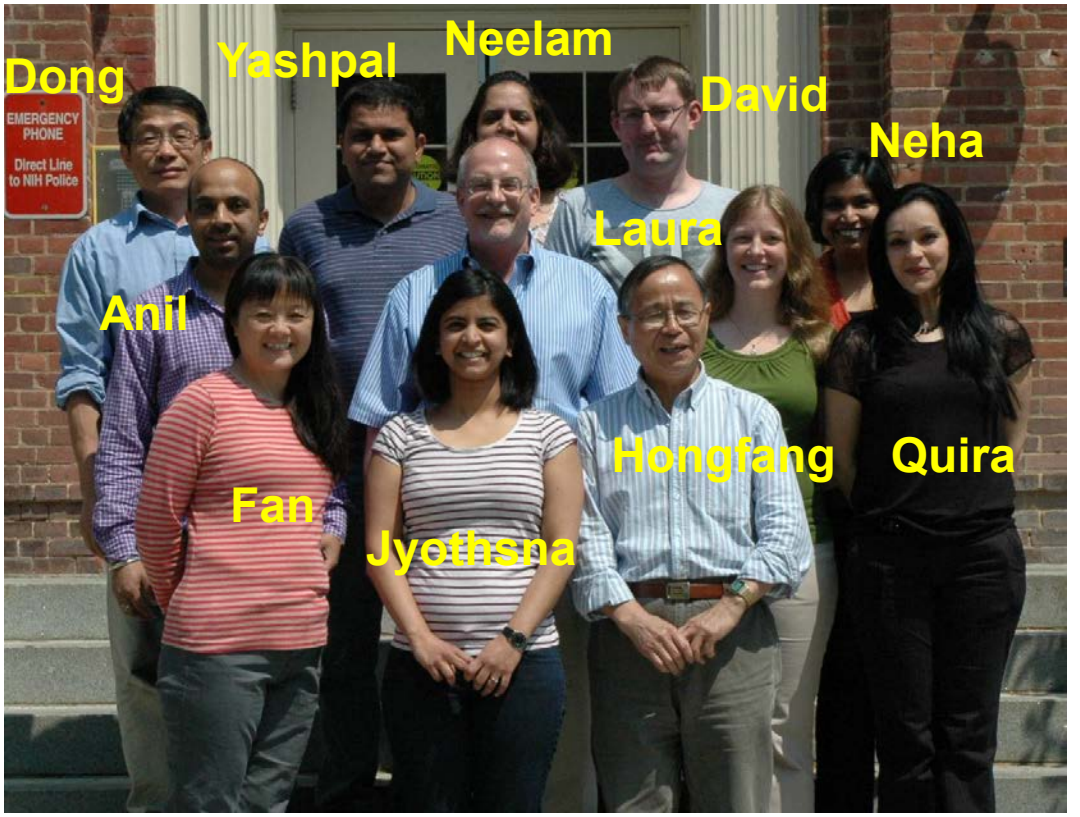
Lorsch Lab



Not shown: Tony Munoz & Fujun Zhou

Funding: NIH

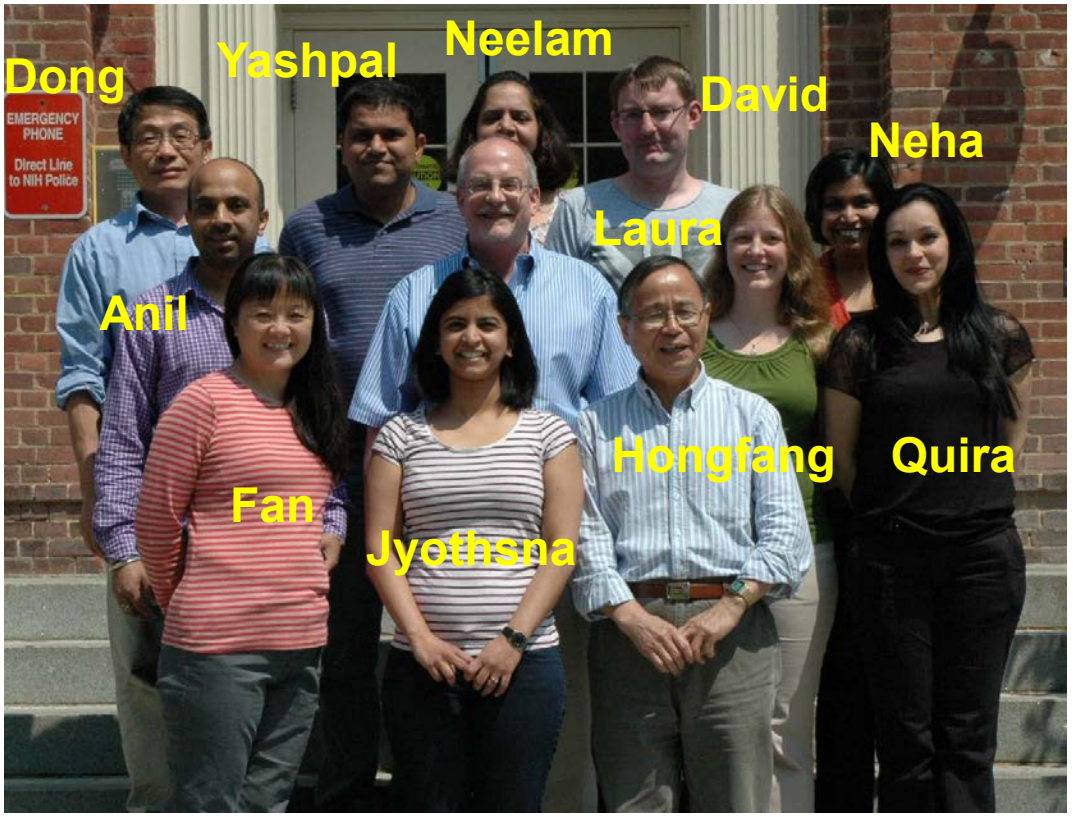
Alan's Lab



Not shown: Suna Gulay,
Pilar Martin-Marcos, Adesh Saini

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

Alan's Lab



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NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development