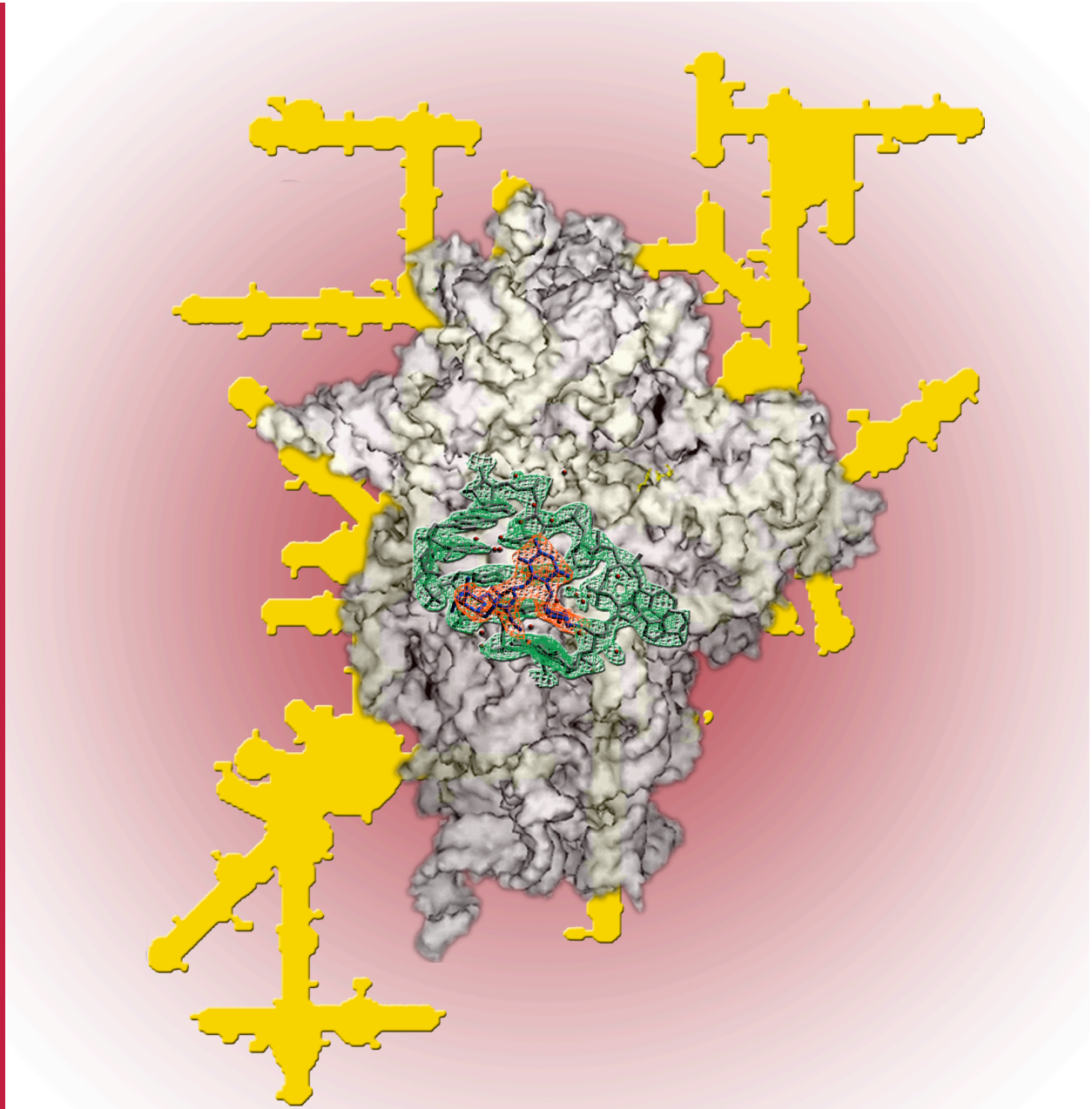
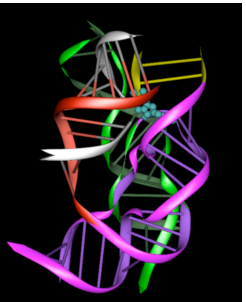
The RNA World -V

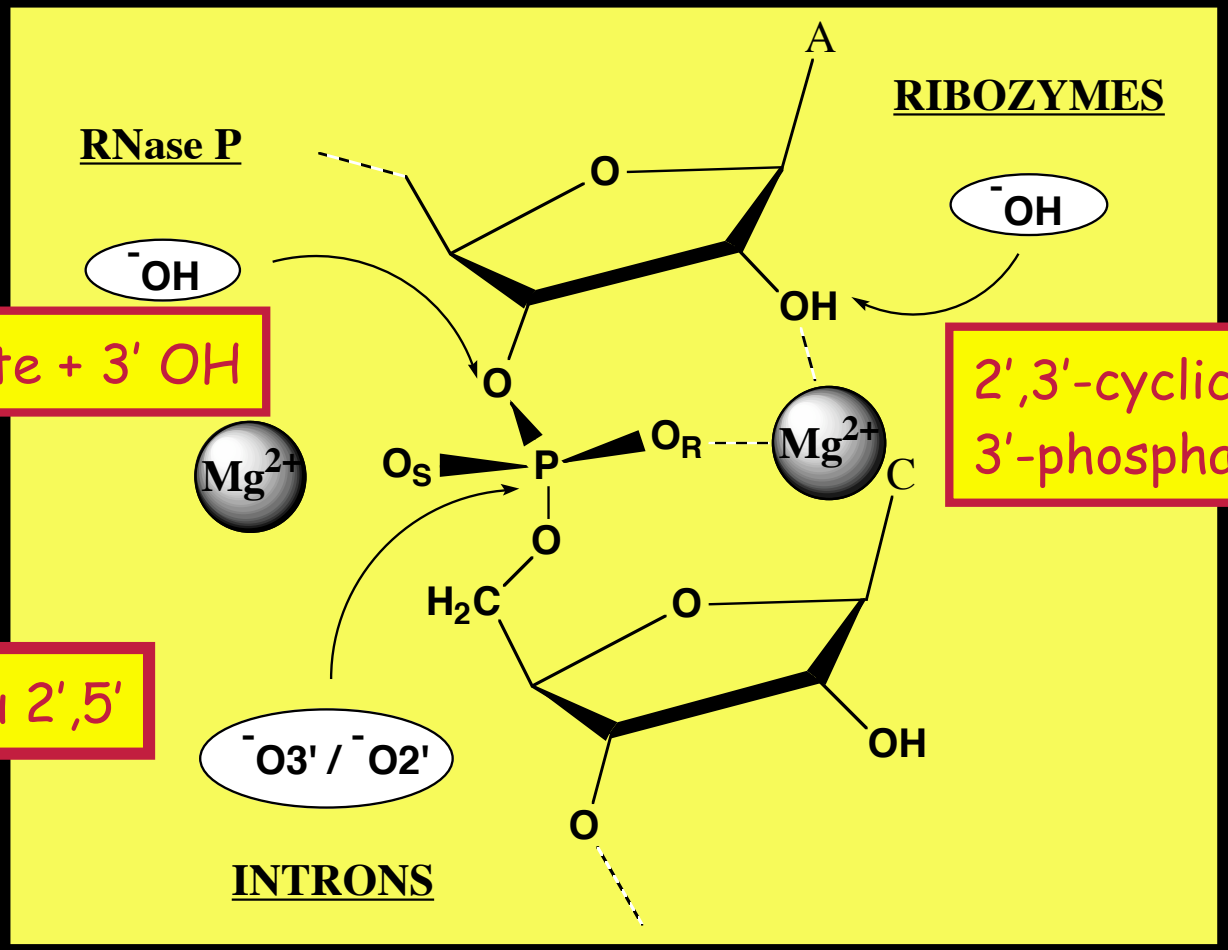


E. Westhof

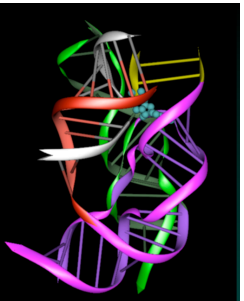
http://www-ibmc.u-strasbg.fr/upr9002/westhof/

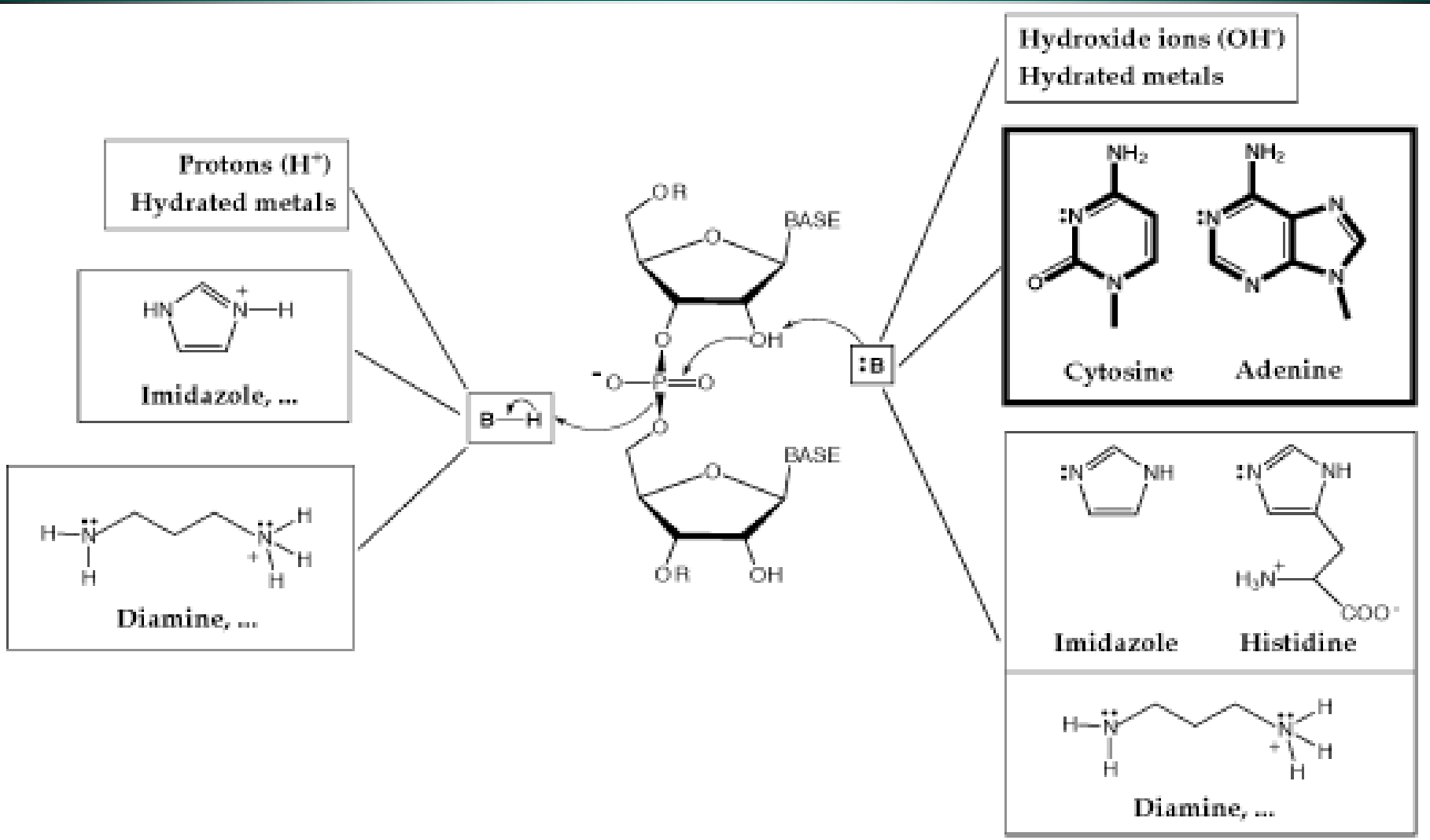
Three families of catalytic RNAs



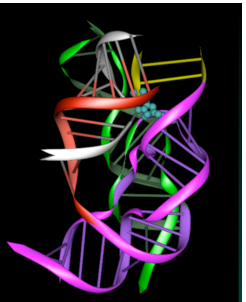


General acid-base catalysis





Catalytic RNA



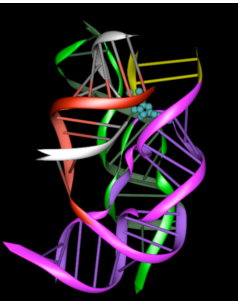
Molecular objects autonomous for 3D folding & catalysis

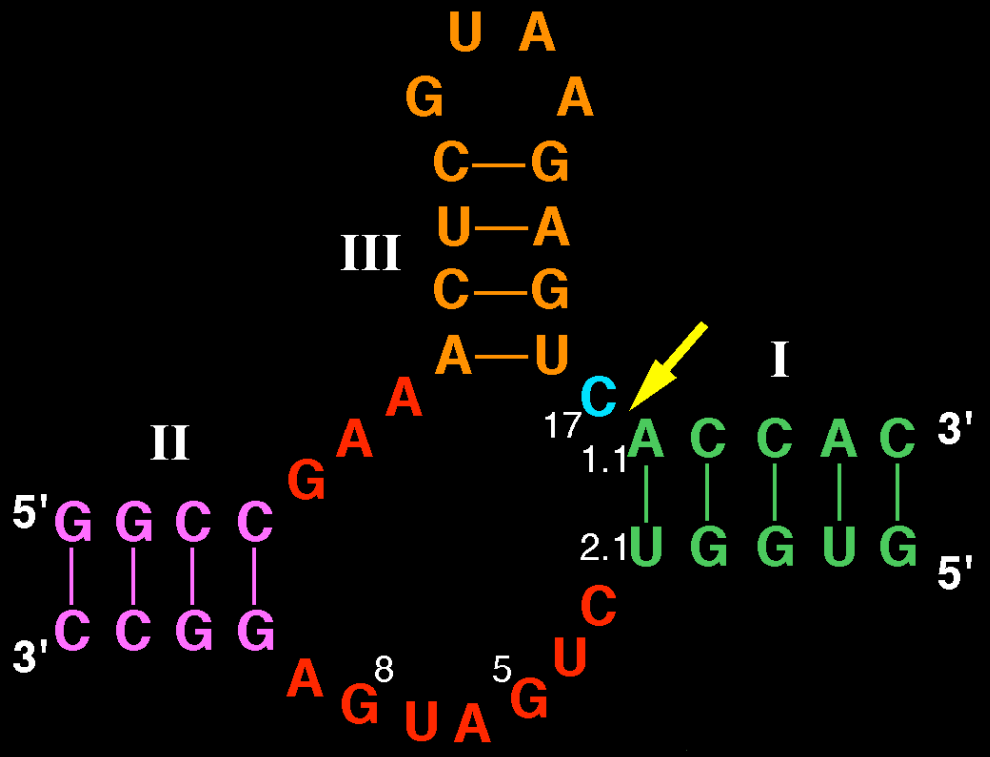
AUTO-ASSEMBLY

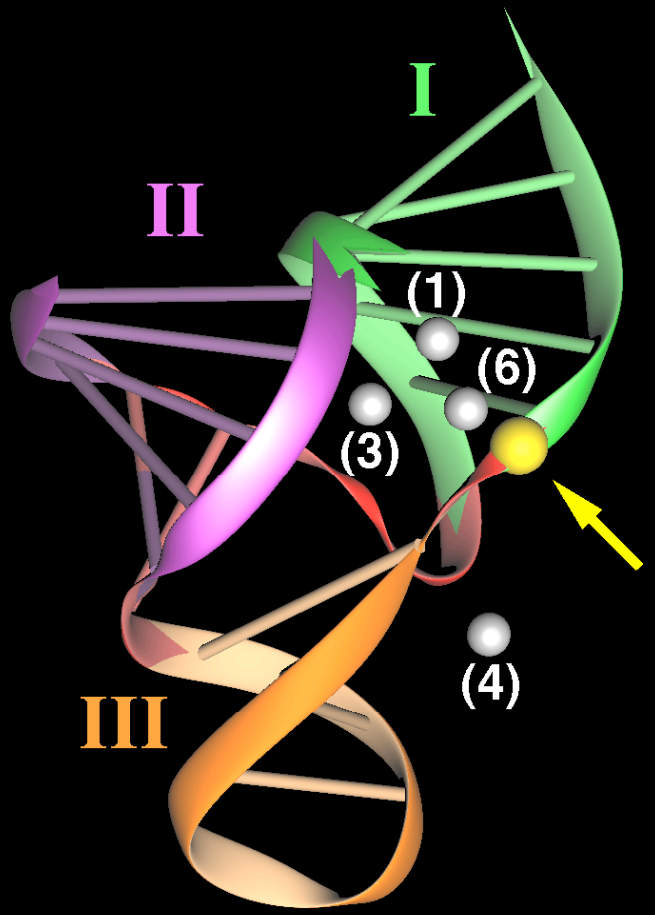
↓

AUTO-CATALYSIS

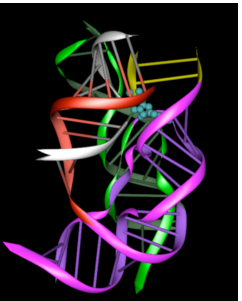
Hammerhead Ribozyme

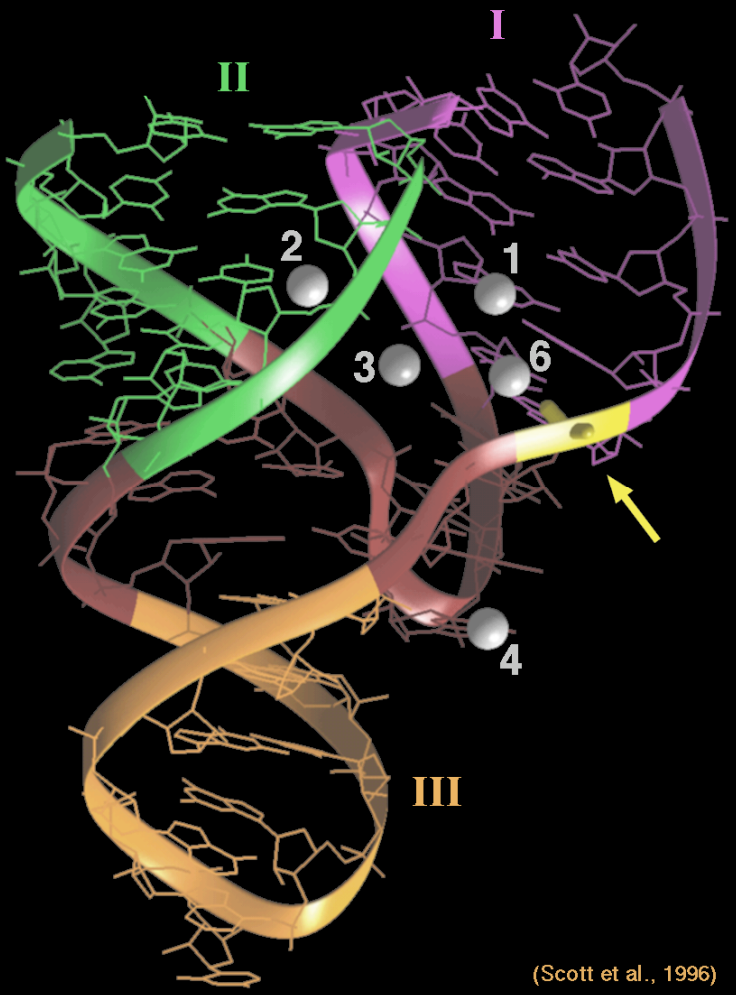




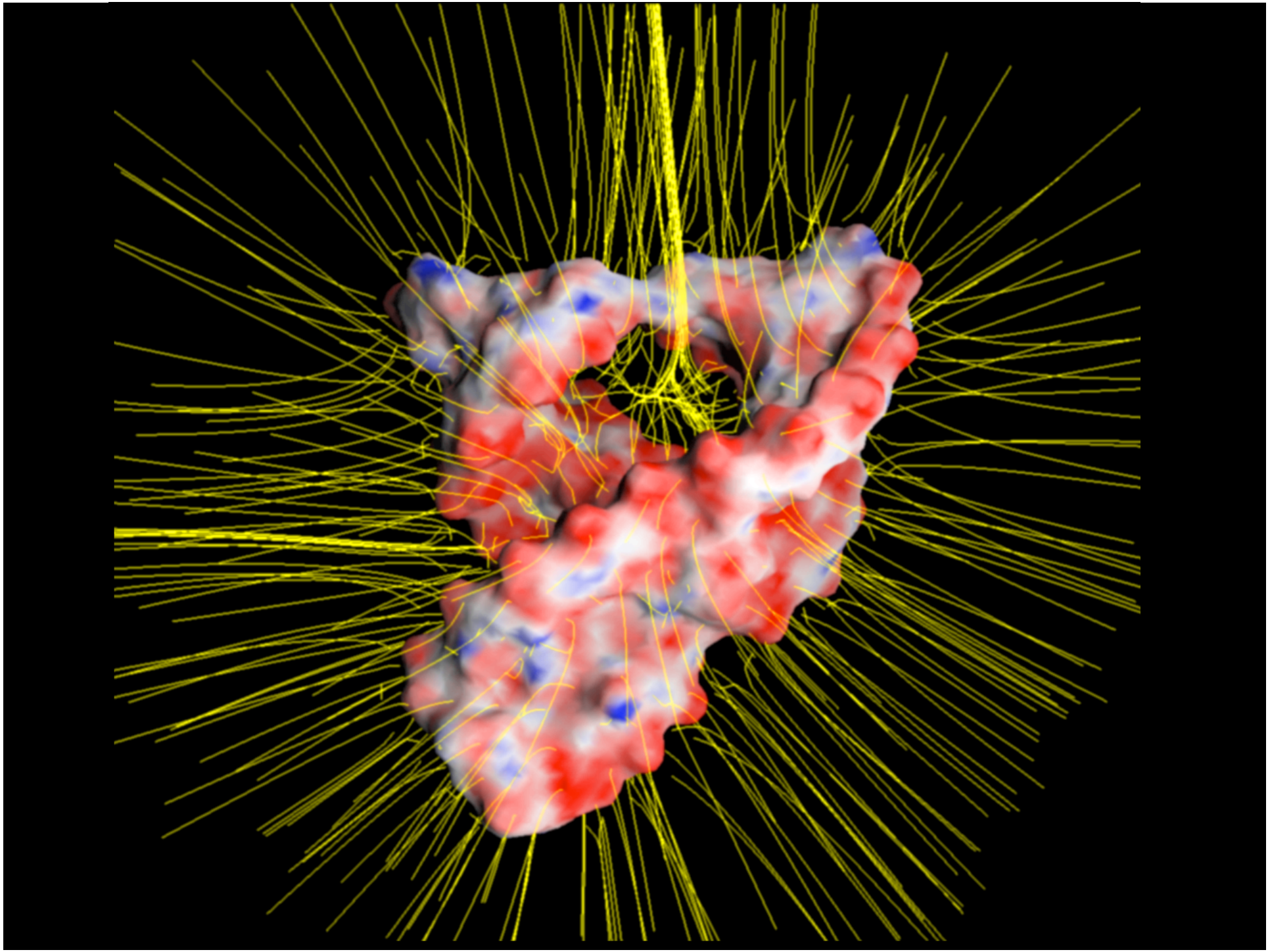


Hammerhead ribozyme

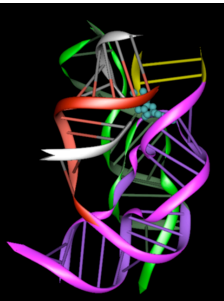


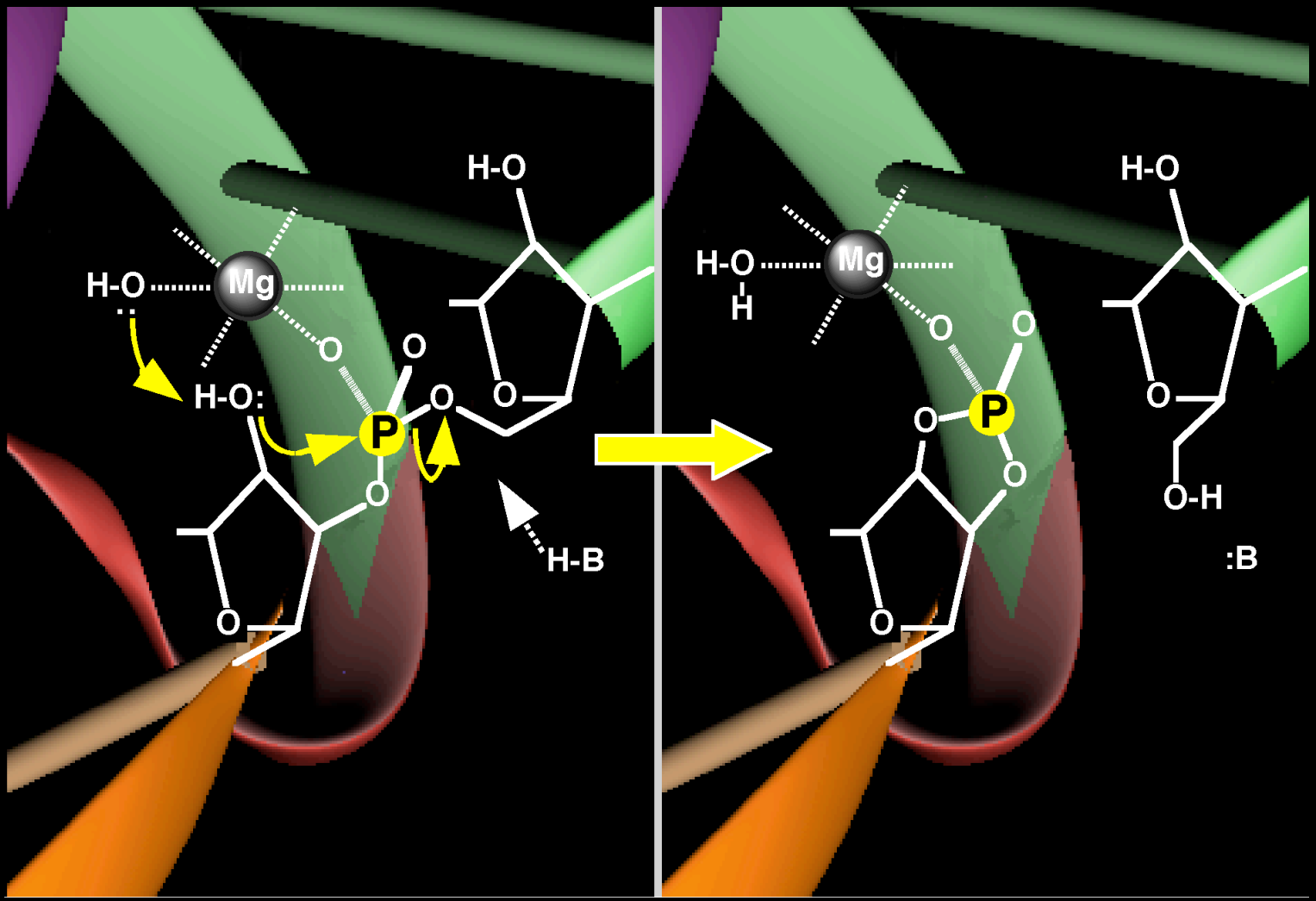


(Scott et al., 1996)

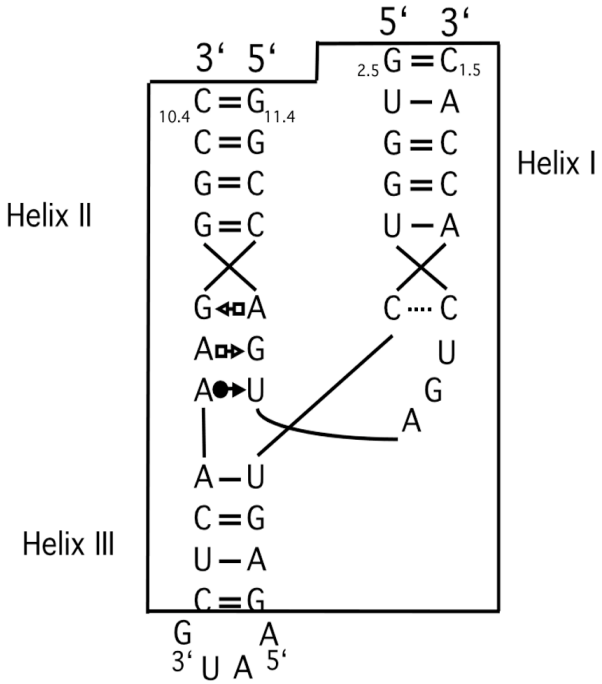


Catalysis of RNA

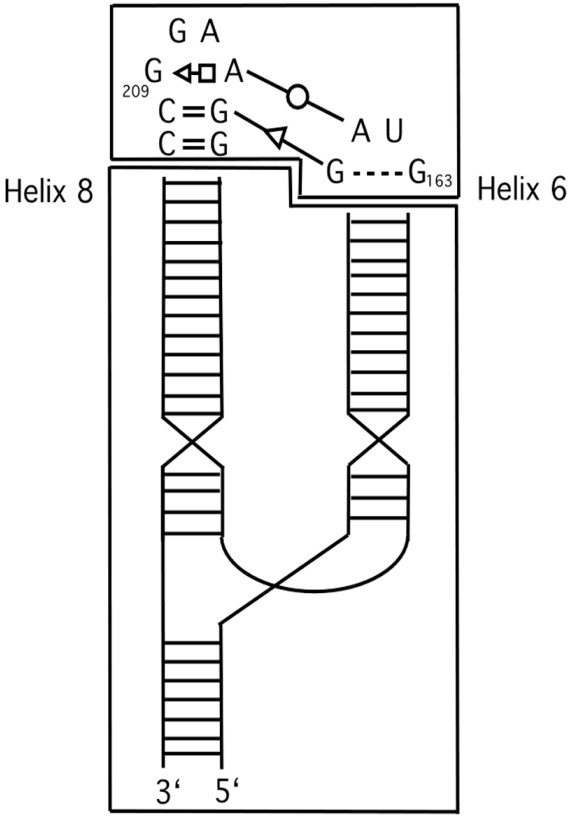




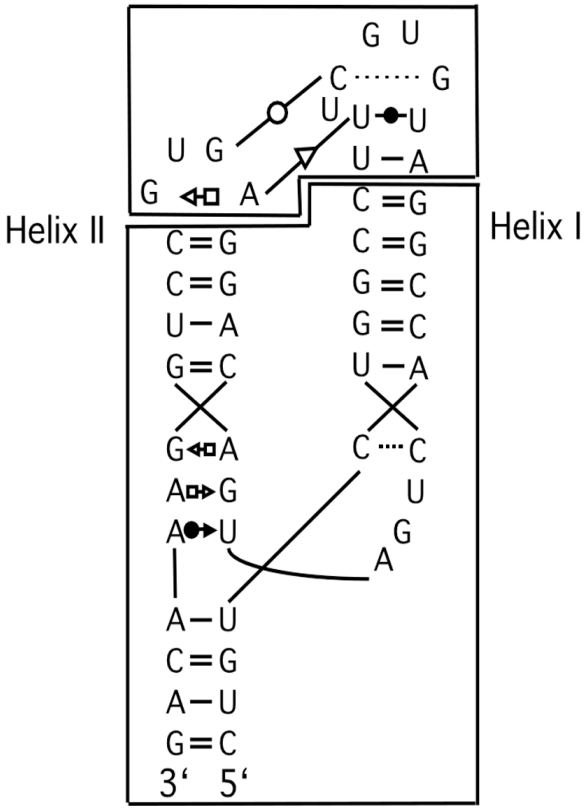
HH

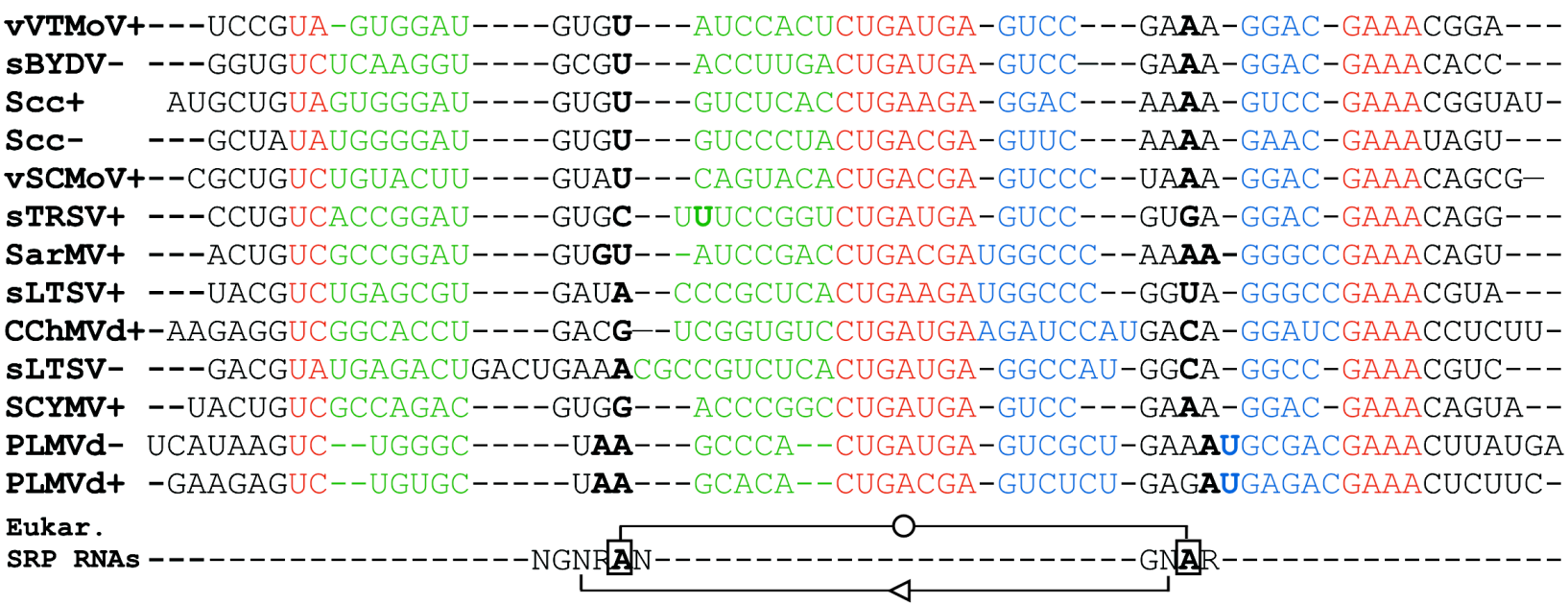

5‘ 3‘
3‘ 5‘
2.5 G = C 1.5
10.4 C = G 11.4
U – A
G = C
G = C
U – A
C ···· C
U
G
A
C = G
G = C
G = C
G ◂□ A
A □▸ G
A ●▸ U
A – U
C = G
U – A
C = G
Helix I
Helix II
Helix III
G A
3‘ U A 5‘

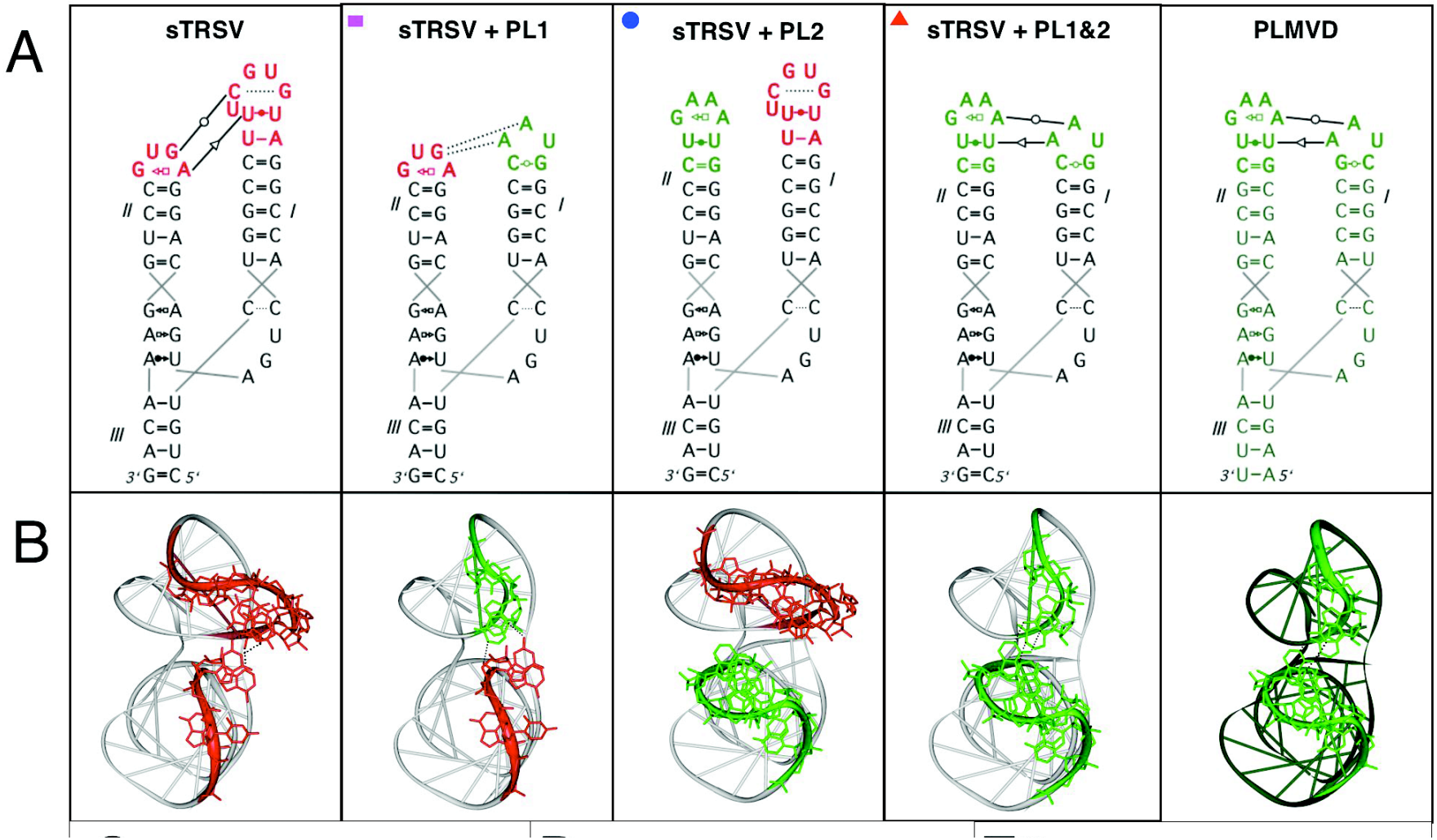
SRP

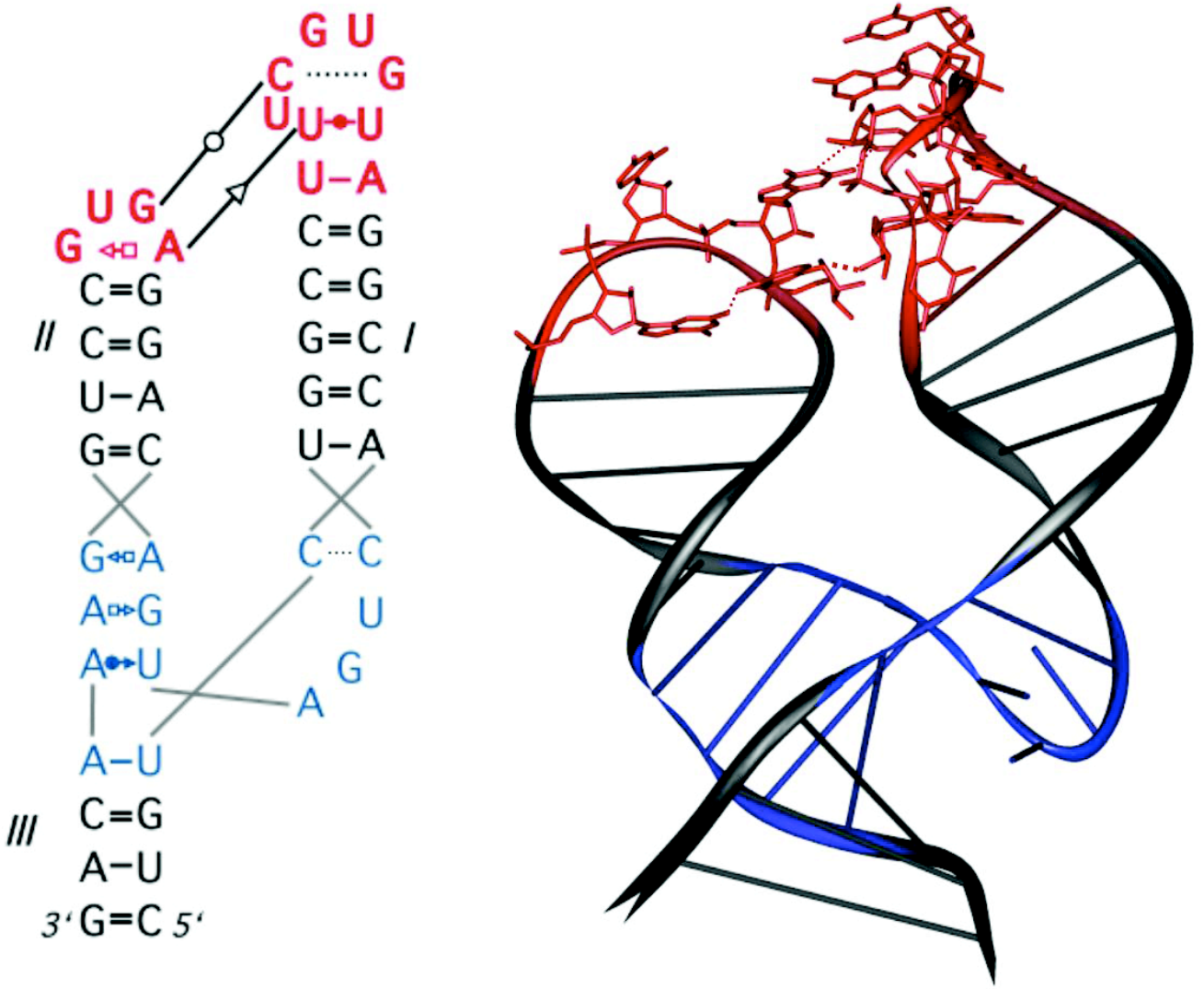

G A
G ◂□ A
209
C = G
C = G
A U
G ---- G 163
Helix 8
Helix 6
3‘ 5‘

sTRSV+

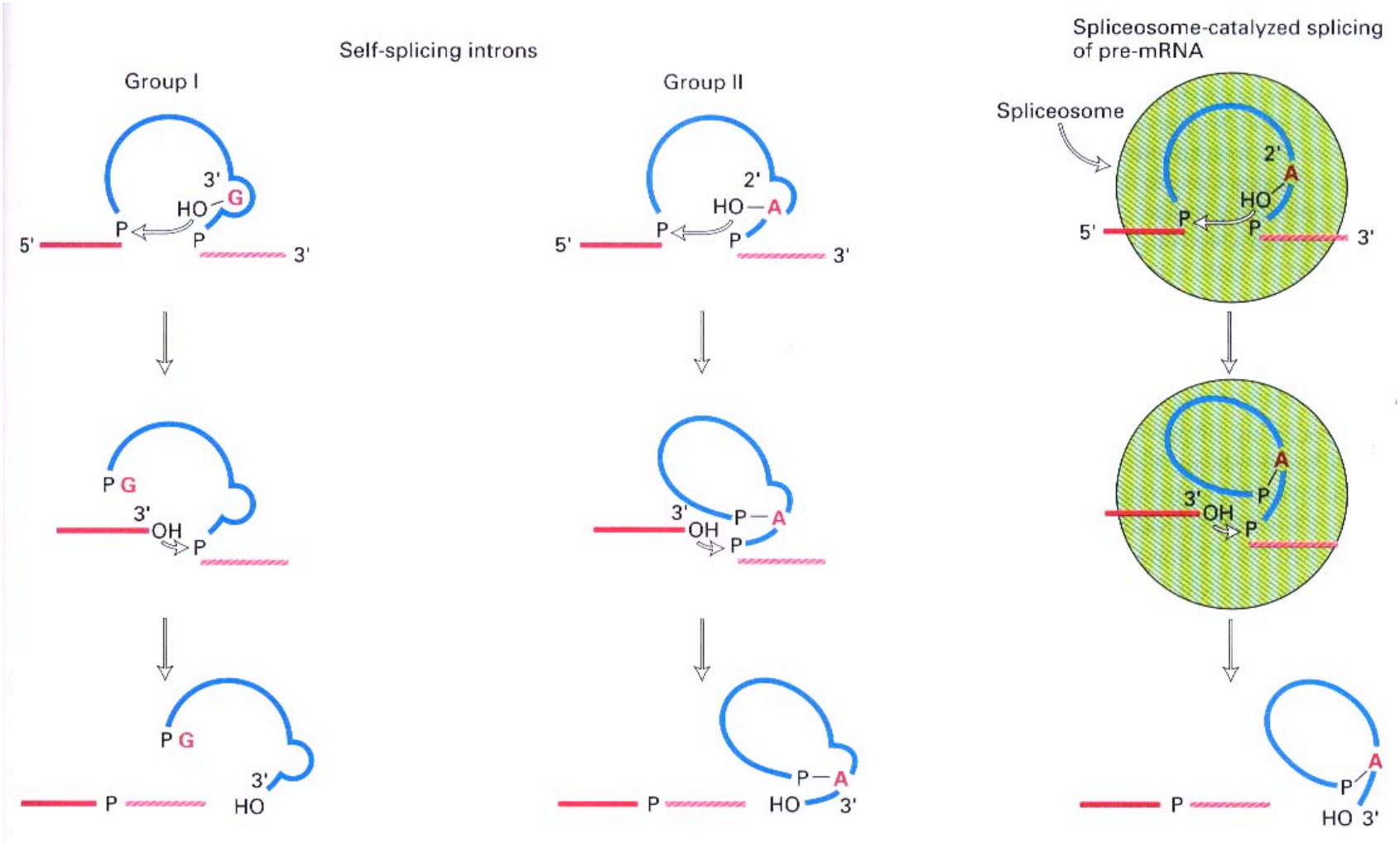

G U
C ····· G
U U ●– U
U G
U – A
G ◂□ A
C = G
C = G
G = C
G = C
U – A
C ···· C
U
G
A
Helix II
Helix I
C = G
C = G
U – A
G = C
G ◂□ A
A □▸ G
A ●▸ U
A – U
C = G
A – U
G = C
3‘ 5‘


vVTMoV+---UCCGUA-GUGGAU----GUGU---AUCCACUCUGAUGA-GUCC---GAAA-GGAC-GAAACGGA---
sBYDV- ---GGUGUCUCAAGGU----GCGU---ACCUUGACUGAUGA-GUCC---GAAA-GGAC-GAAACACC---
Scc+ AUGCUGUAGUGGGAU----GUGU---GUCUCACCUGAAGA-GGAC---AAAA-GUCC-GAAACGGUAU-
Scc- ---GCUAUAUGGGGAU----GUGU---GUCCCUACUGACGA-GUUC---AAAA-GAAC-GAAAUAGU---
vSCMoV+--CGCUGUCUGUACUU----GUAU---CAGUACACUGACGA-GUCCC--UAAA-GGAC-GAAACAGCG—
sTRSV+ ---CCUGUCACCGGAU----GUGC--UUUCCGGUCUGAUGA-GUCC---GUGA-GGAC-GAAACAGG---
SarMV+ ---ACUGUCGCCGGAU----GUGU--AUCCGACCUGACGAUGGCCC--AAAA-GGGCCGAAACAGU---
sLTSV+ ---UACGUCUGAGCGU----GAUA--CCCGCUCACUGAAGAUGGCCC--GGUA-GGGCCGAAACGUA---
CChMVd+-AAGAGGUCGGCACCU----GACG—UCGGUGUCCUGAUGAAGAUCCAUGACA-GGAUCGAAACCUCUU-
sLTSV- ---GACGUAUGAGACUGACUGAAACGCCGUCUCACUGAUGA-GGCCAU-GGCA-GGCC-GAAACGUC---
SCYMV+ --UACUGUCGCCAGAC----GUGG---ACCCGGCCUGAUGA-GUCC---GAAA-GGAC-GAAACAGUA--
PLMVd- UCAUAAGUC--UGGGC-----UAA---GCCCA--CUGAUGA-GUCGCU-GAAAUGCGACGAAACUUAUGA
PLMVd+ -GAAGAGUC--UGUGC-----UAA---GCACA--CUGACGA-GUCUCU-GAGAUGAGACGAAACUCUUC-
Eukar.
SRP RNAs----------NGNRAN----------GNAR----------


A
B
sTRSV
sTRSV + PL1
sTRSV + PL2
sTRSV + PL1&2
PLMVD

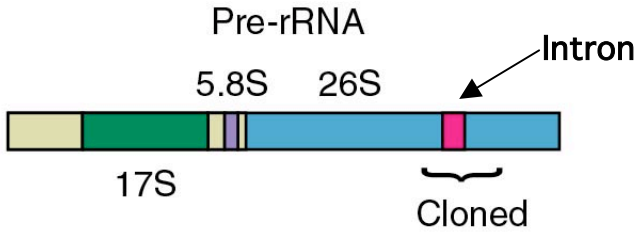

G U
C G
U U U
U–A
U G
G A
C=G
C=G
II C=G
G=C I
U–A
G=C
G=C
U–A
G A
C C
A G
U
A U
G
A
A–U
III C=G
A–U
3' G=C 5'

GROUP I AND GROUP II (SELF-SPLICING) INTRONS

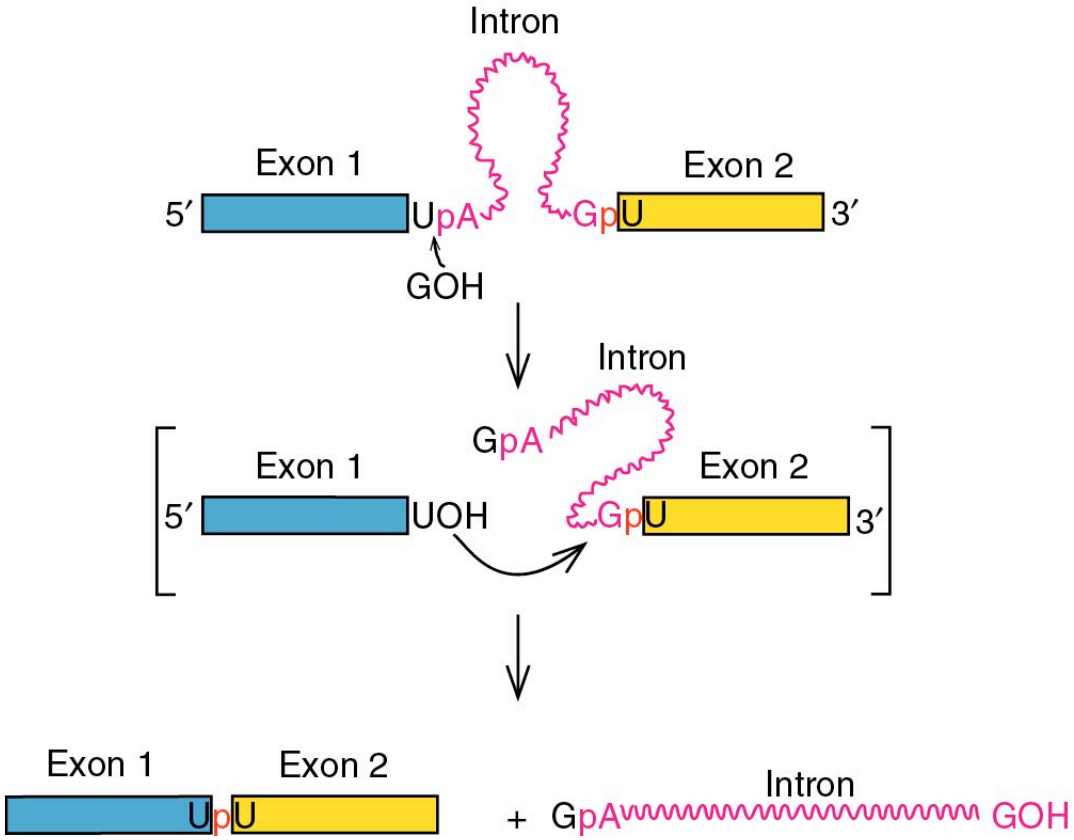


COMPARISON OF SPLICING REACTIONS IN PRE-rRNA AND PRE-mRNA

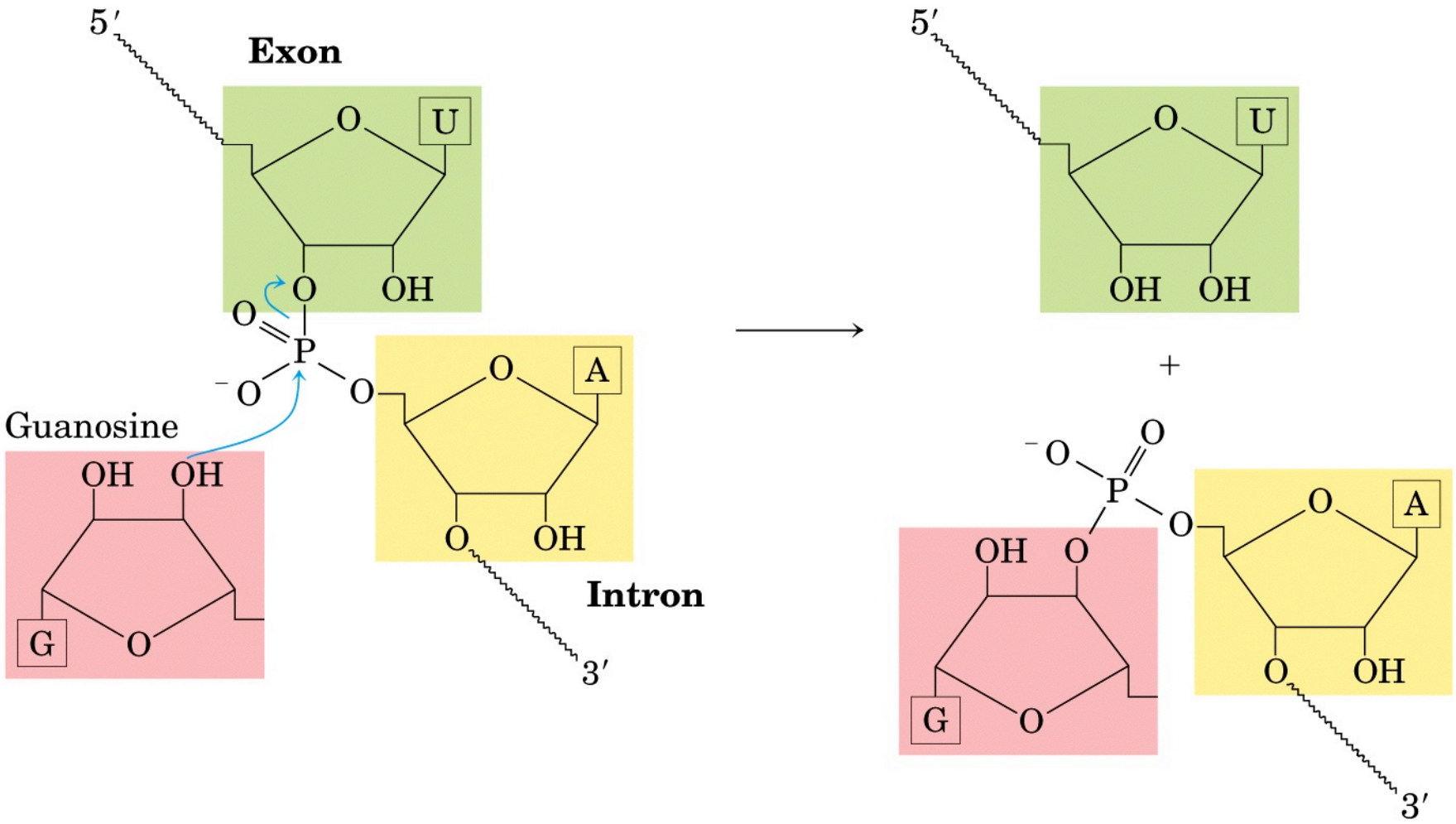
ADAPTED FROM SHARP (1987), *SCIENCE* **235**, 769



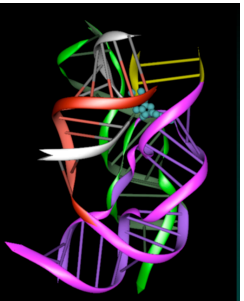
STRUCTURE OF *TETRAHYMENA* rRNA PRECURSOR



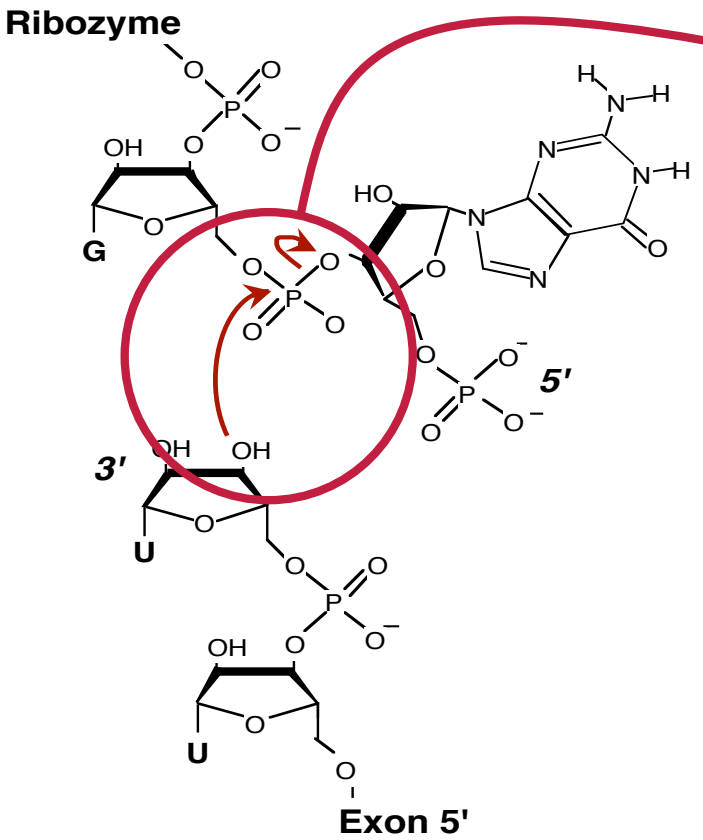
SELF-SPLICING OF *TETRAHYMENA* rRNA PRECURSOR

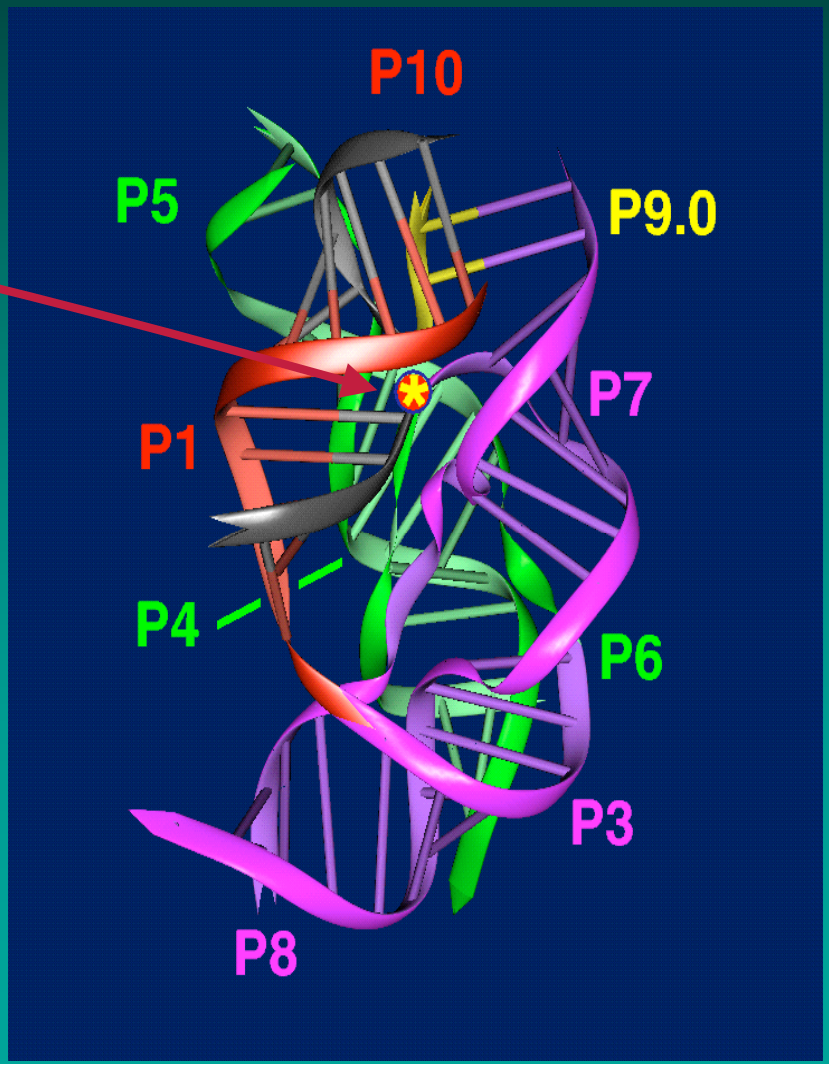

5′
Exon
U
O
OH
O
O
P
⁻O
O
A
O
OH
Guanosine
OH
OH
G
O
Intron
3′
5′
U
O
OH
OH
+
⁻O
O
P
O
A
O
OH
O
OH
G
O
3′

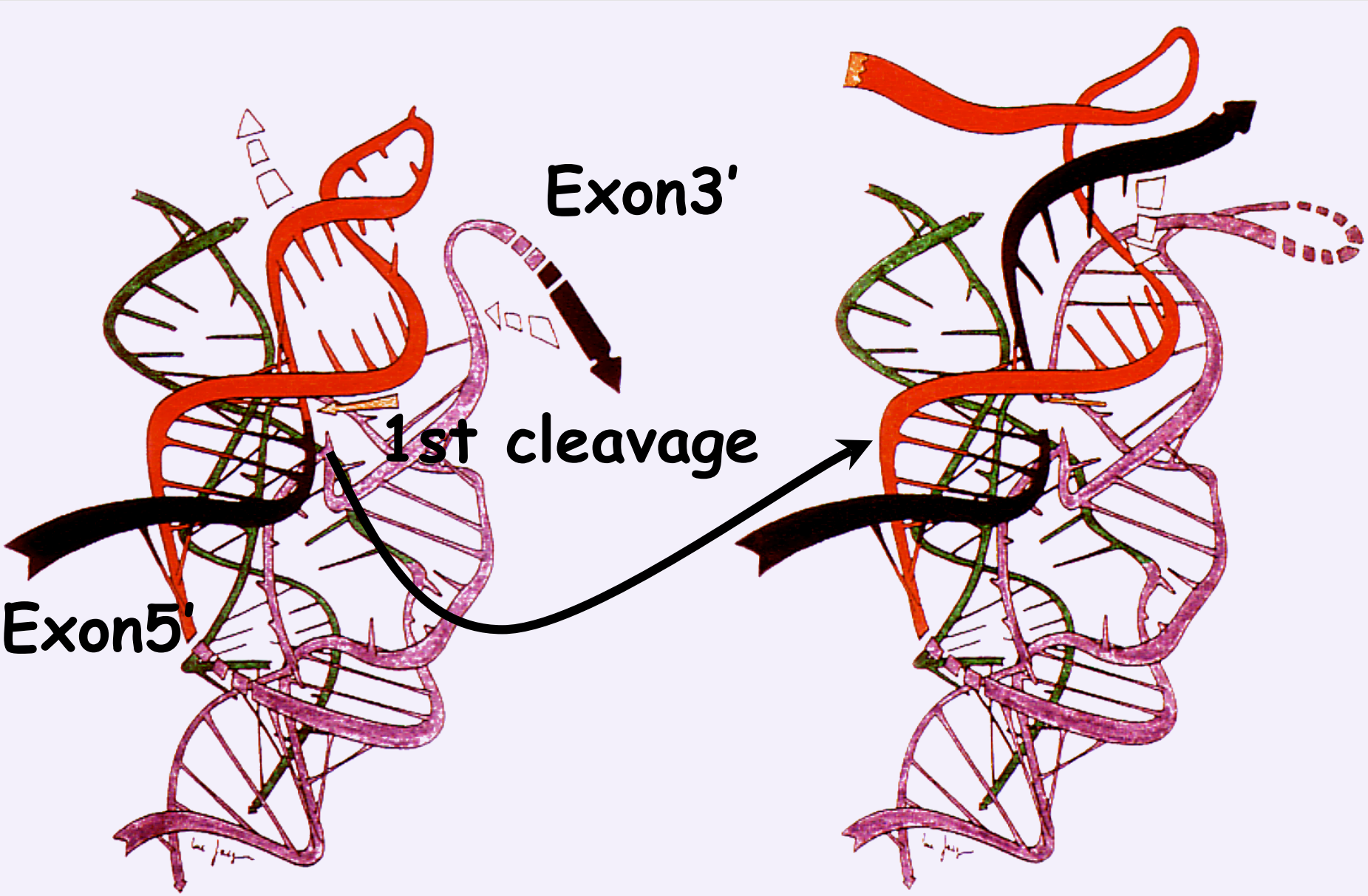
Catalytic Core of Group I introns

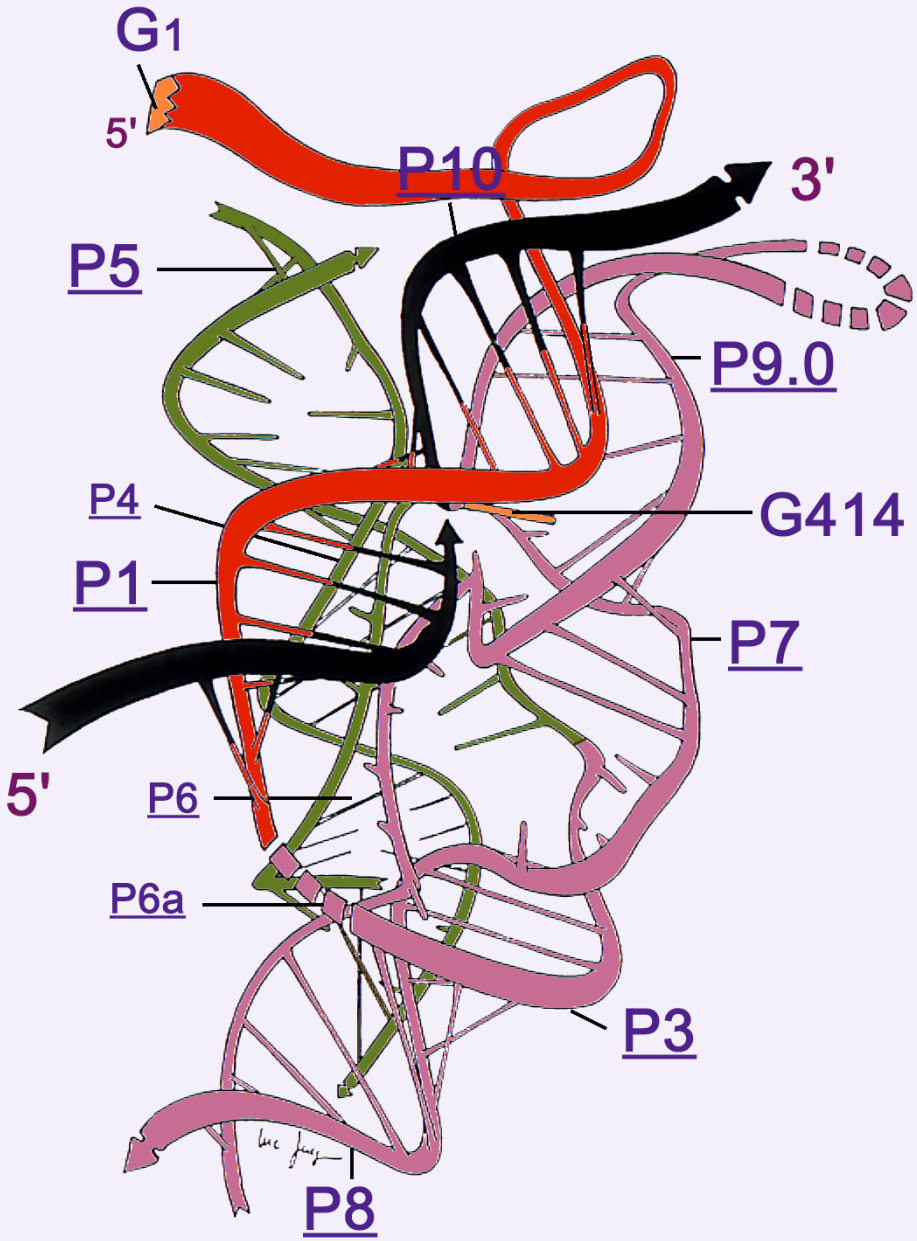


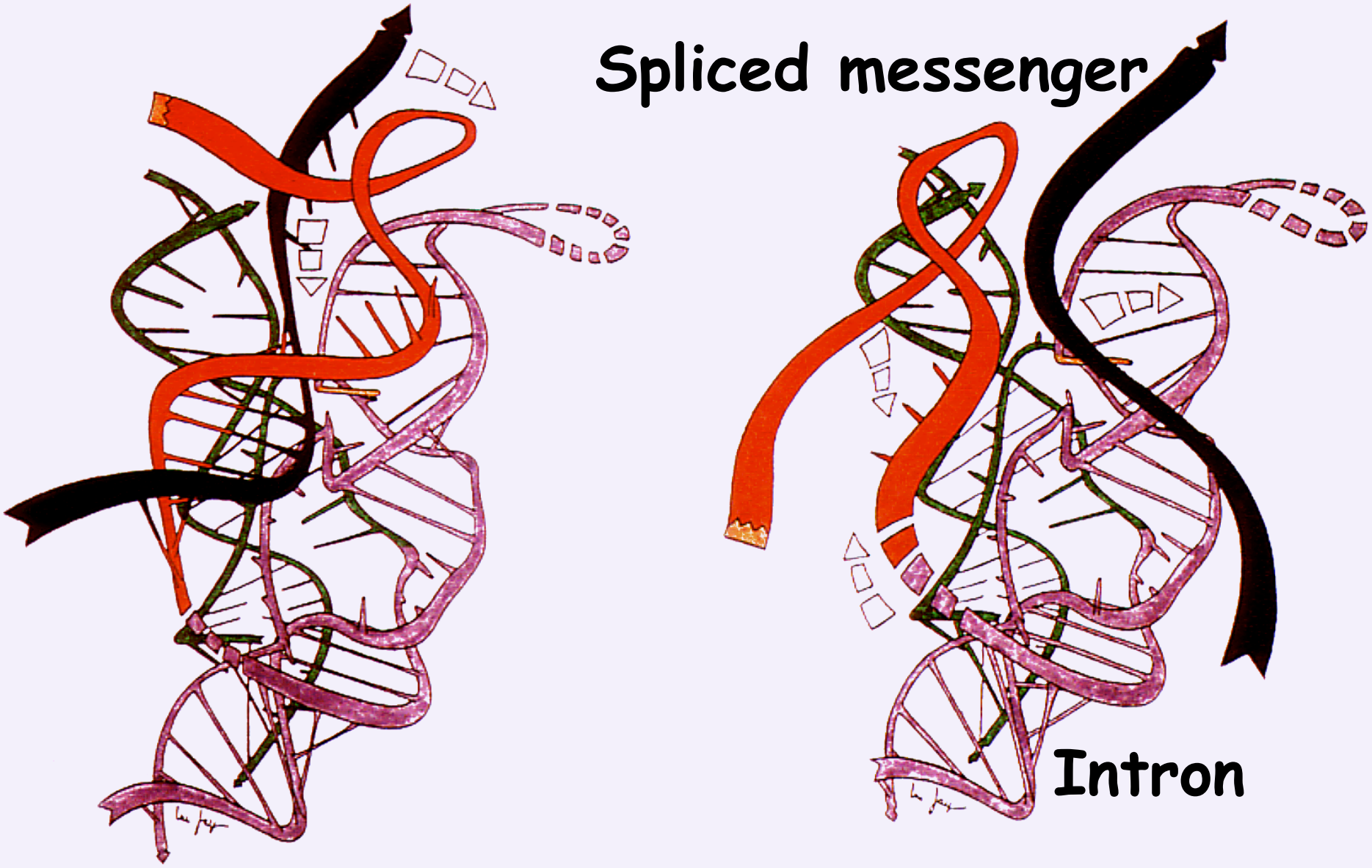
Transesterification



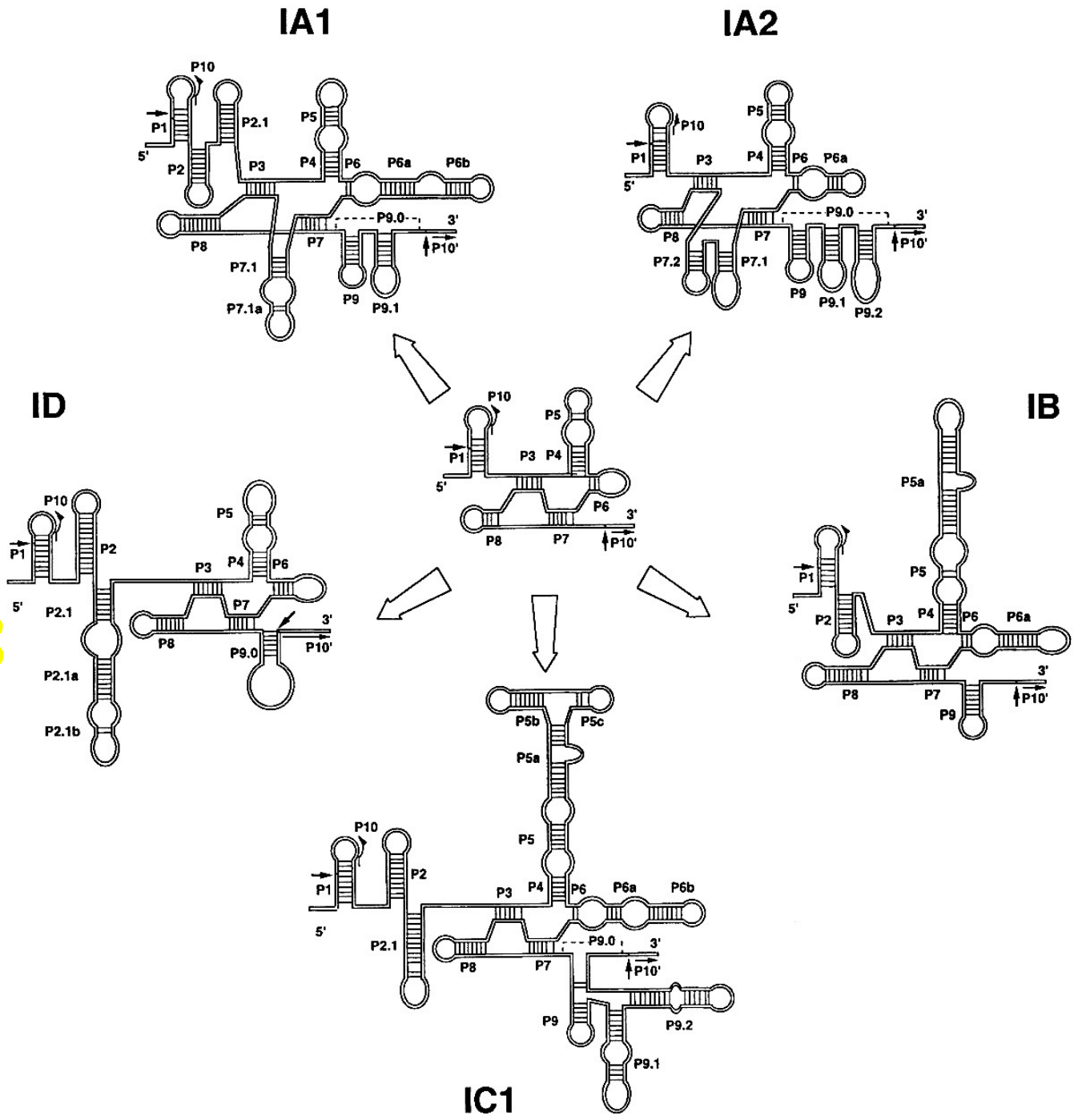



Exon3'
1st cleavage
Exon5'

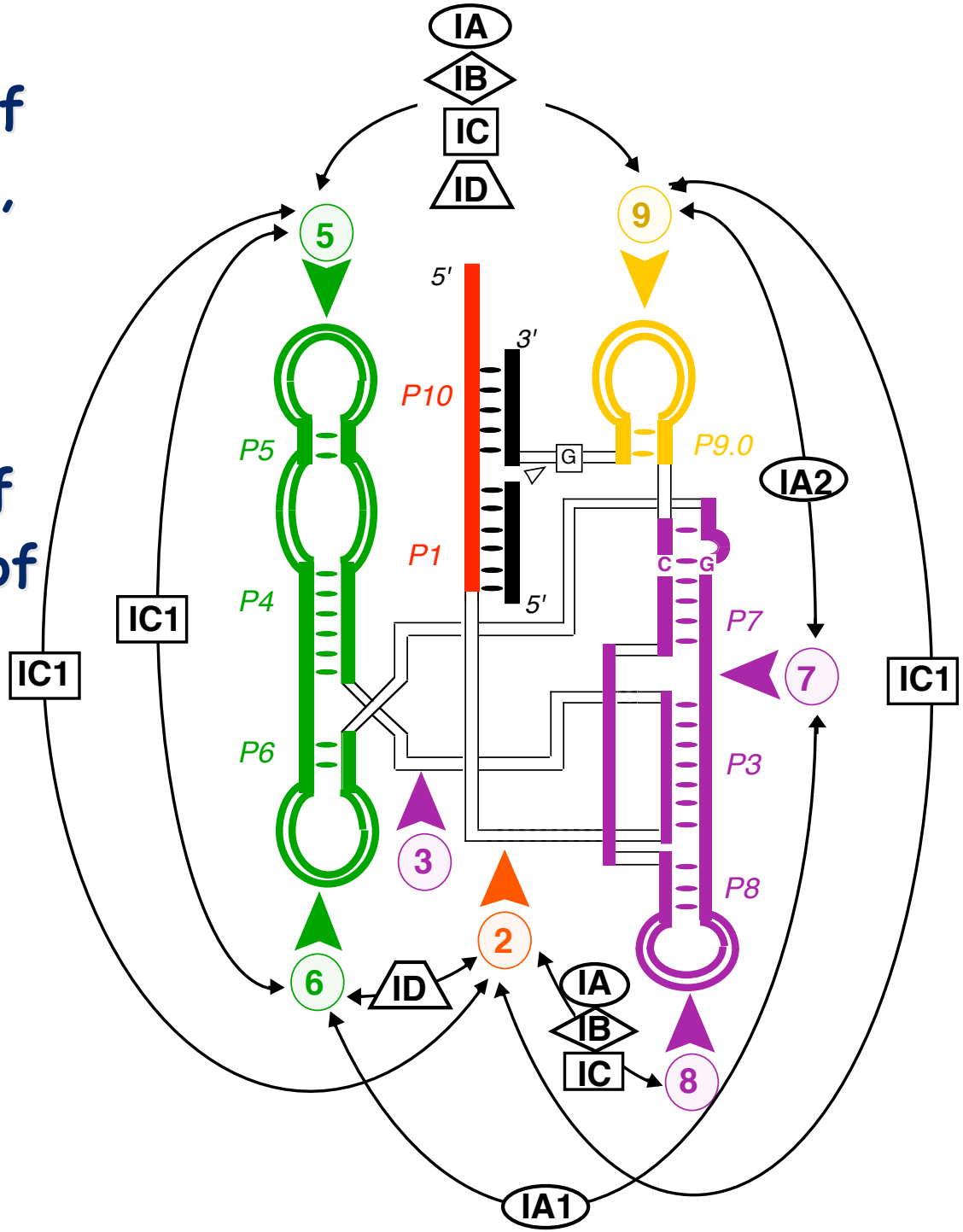

G1
5'
P10
3'
P5
P9.0
P4
G414
P1
P7
5'
P6
P6a
P3
P8

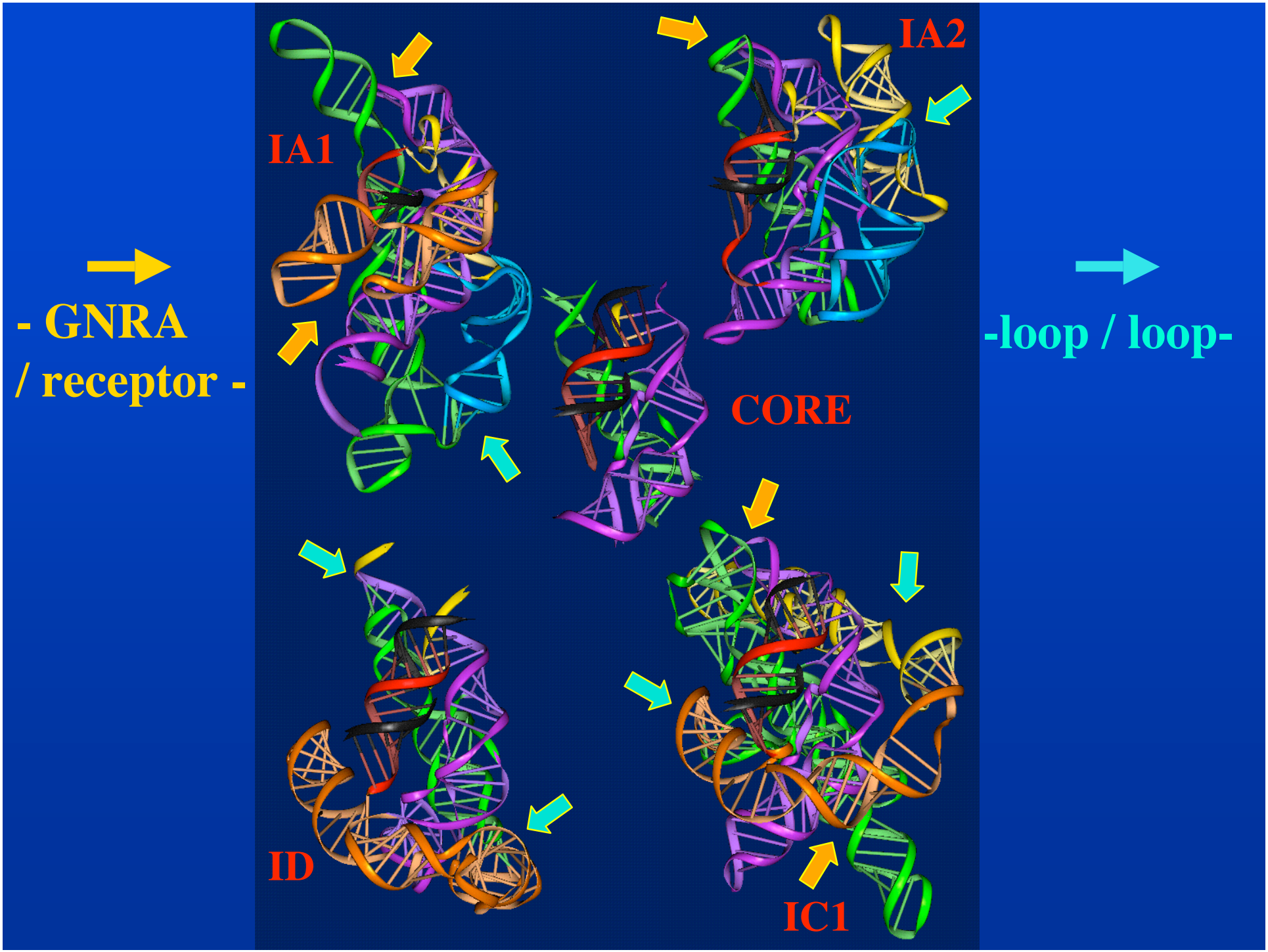

Spliced messenger
Intron

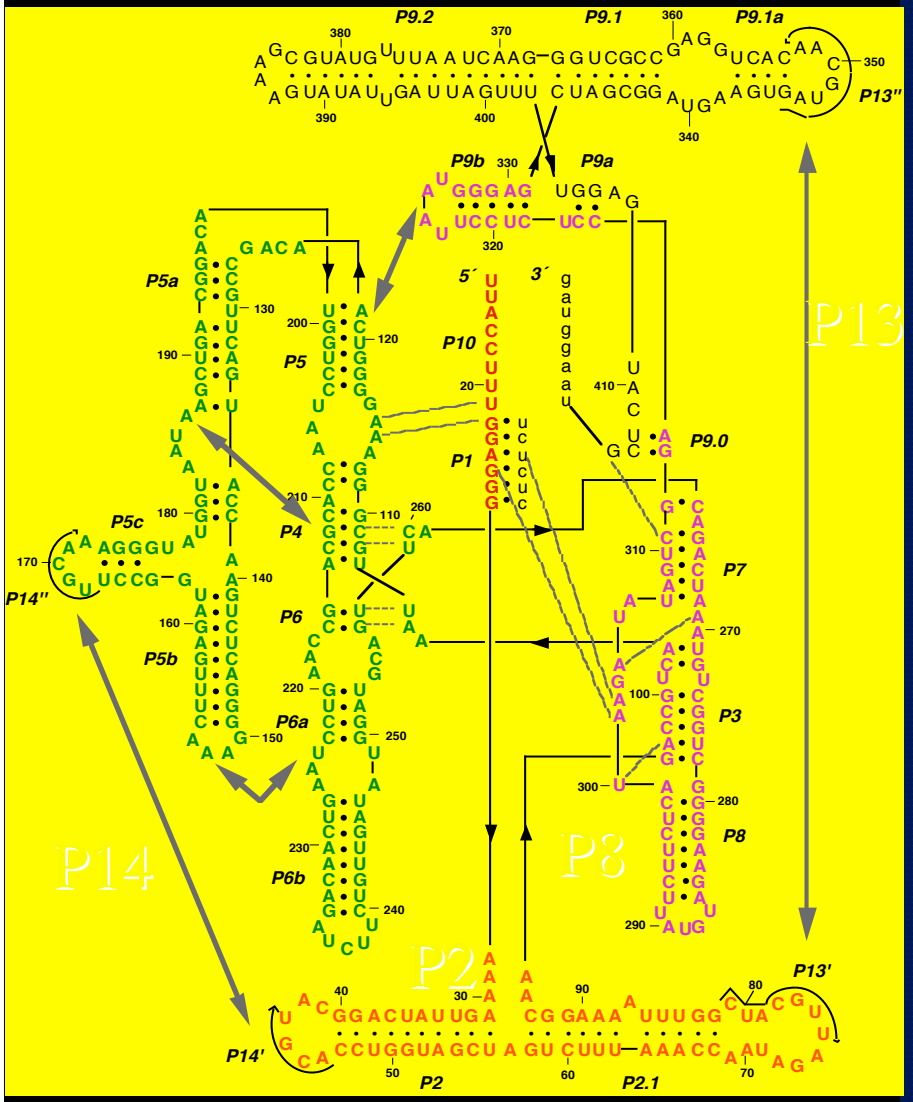
How do RNA molecules Evolve ?

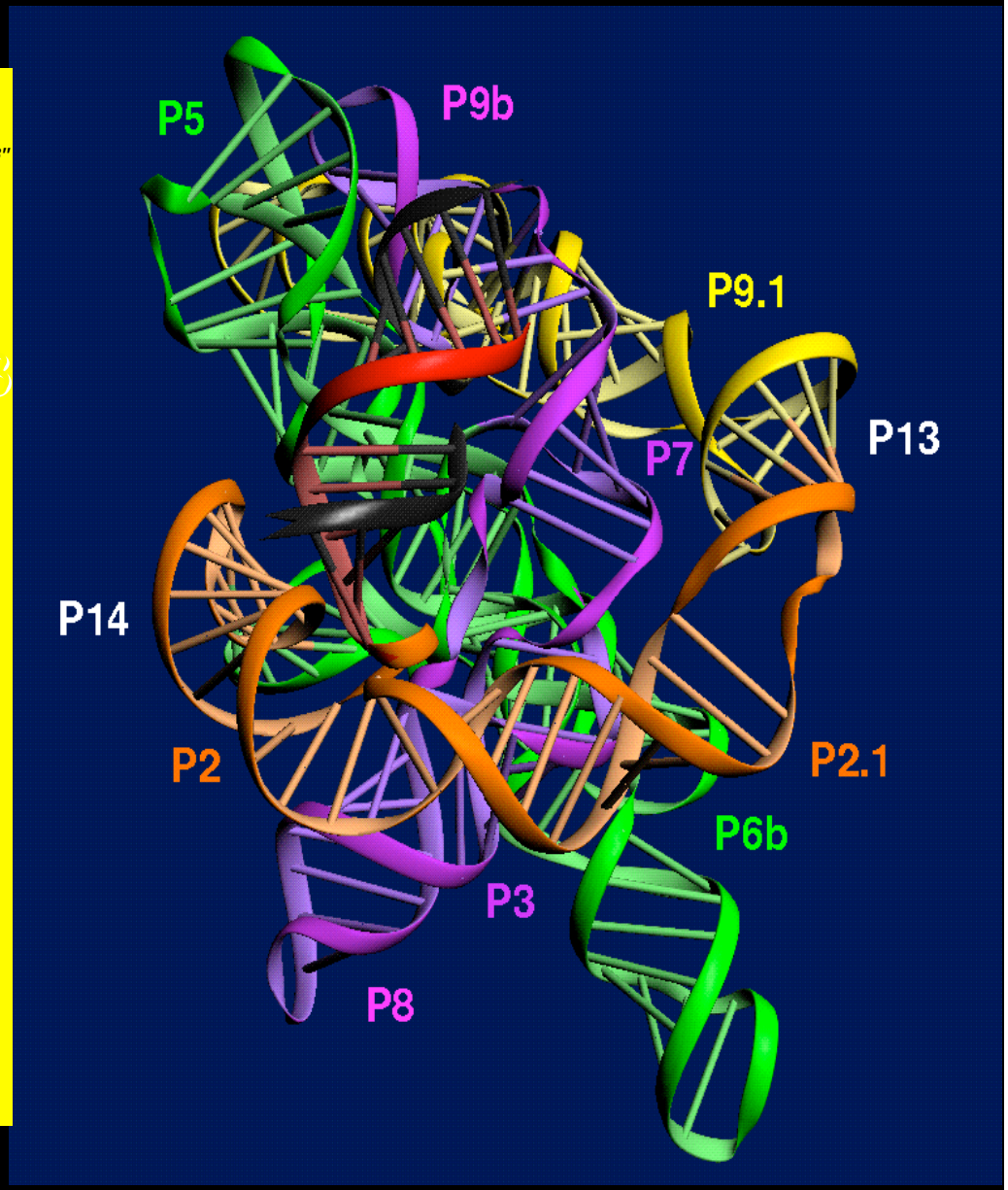


Several sub-groups of Group I introns exist, all with a unique common core.
Each of them uses a different sub-sets of the same two types of 3D motifs.

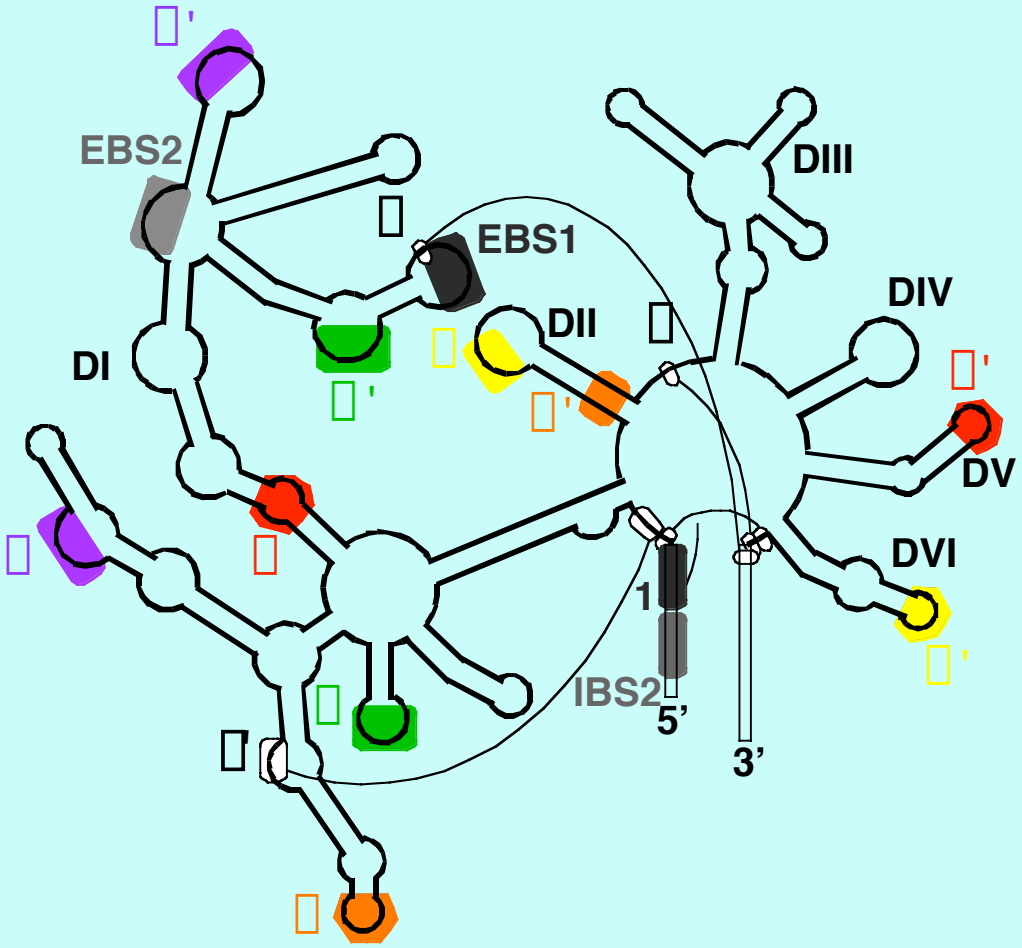


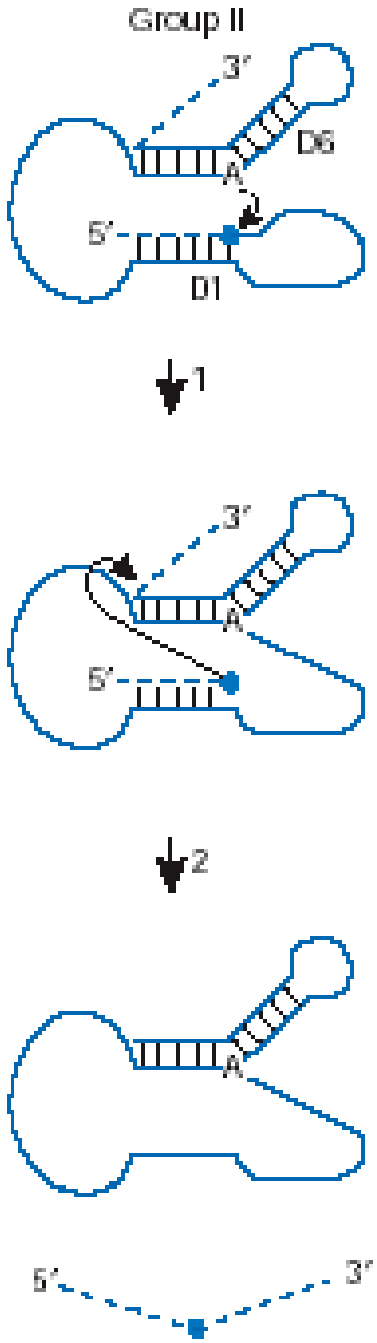

- GNRA
/ receptor -
IA1
IA2
CORE
-loop / loop-
ID
IC1


P9.2
P9.1
P9.1a
P13''
P9b
P9a
P5a
P5
P10
P1
P13
P5c
P4
P14''
P7
P9.0
P5b
P6
P6a
P3
P14
P8
P6b
P2
P13'
P14'
P2
P2.1

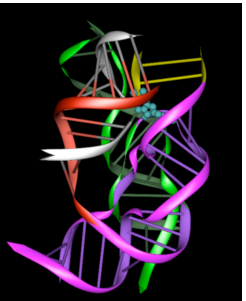

P5
P9b
P9.1
P13
P7
P14
P2
P2.1
P6b
P3
P8

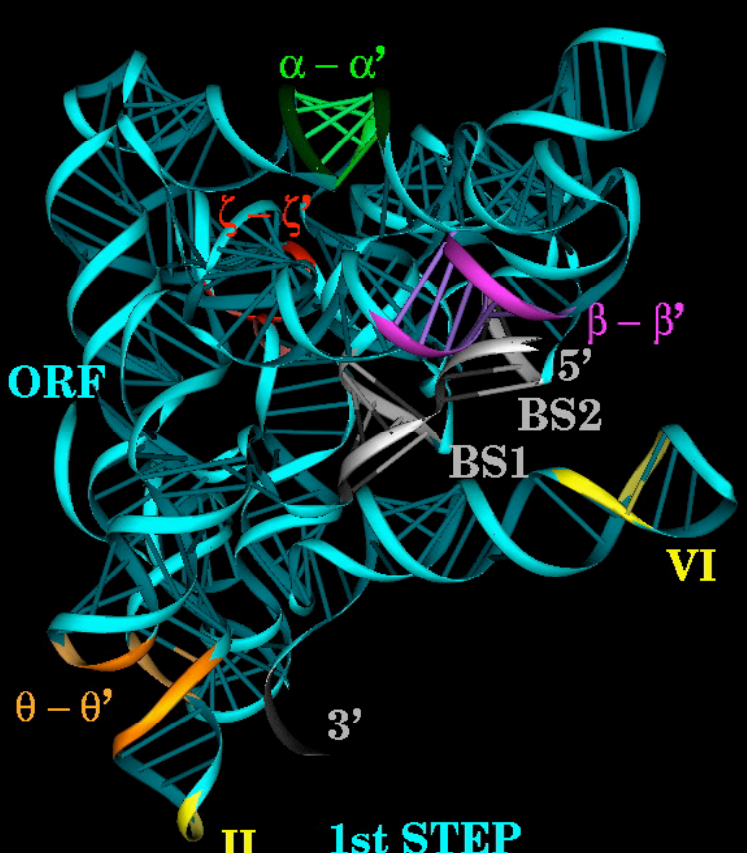
Group II introns

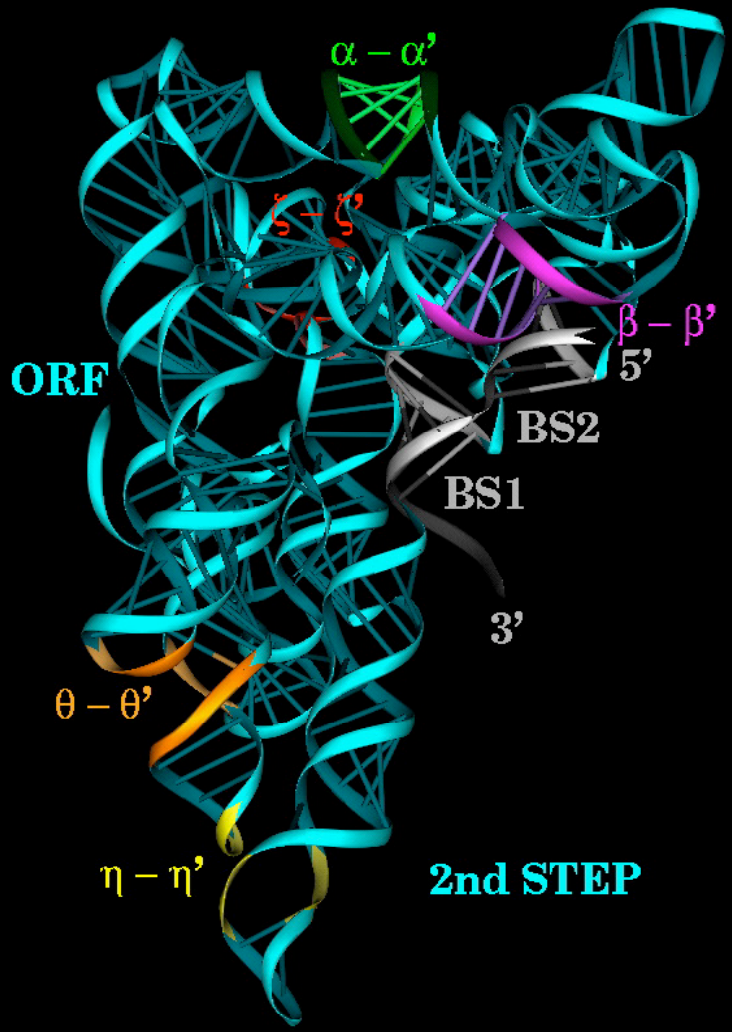


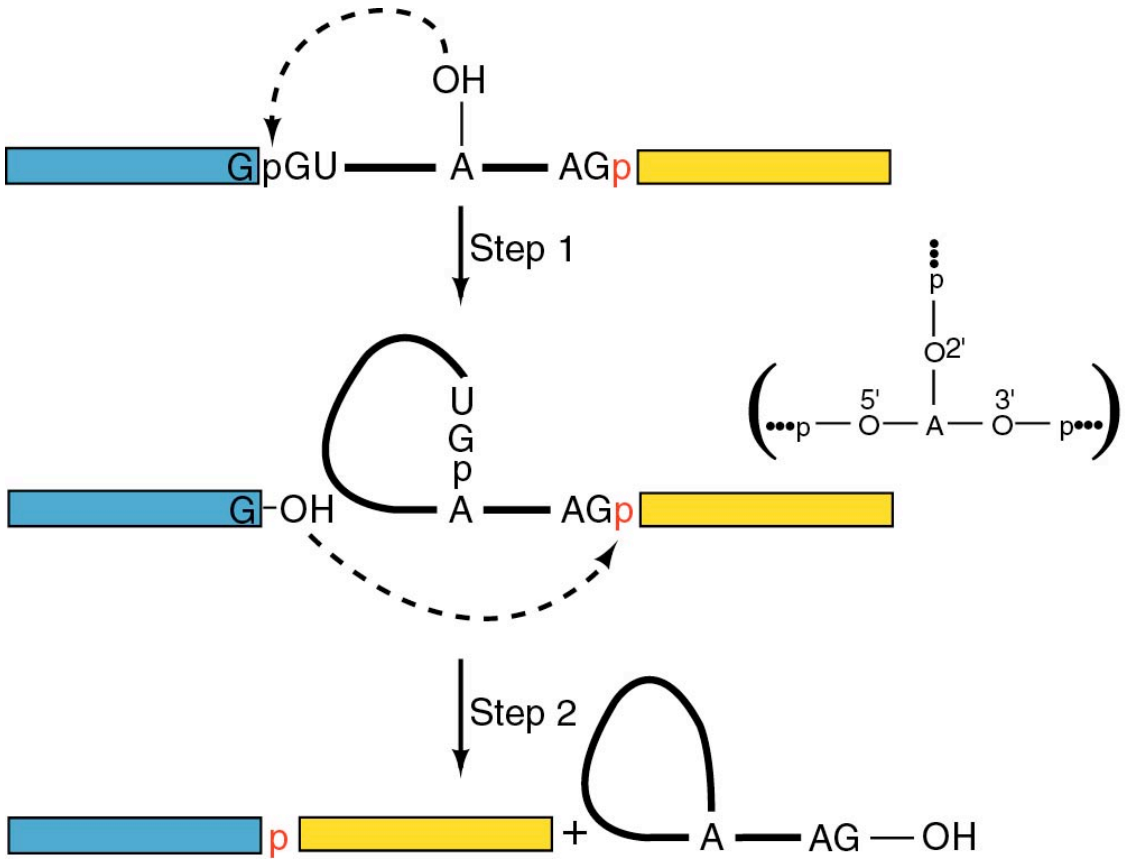


Group II introns : 3D models









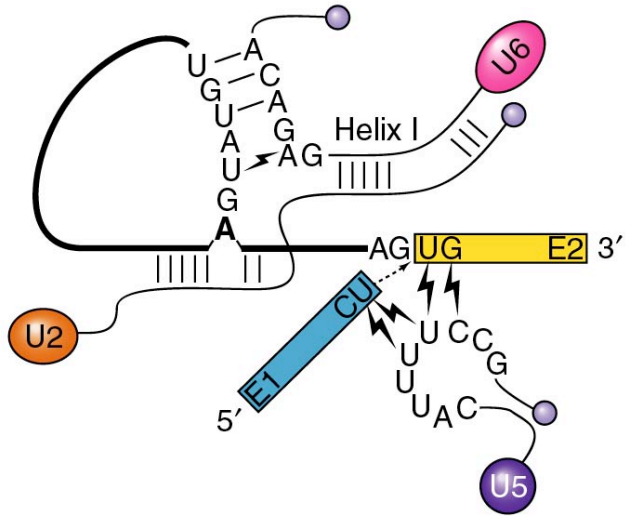
MECHANISM OF SPLICING OF EUKARYOTIC PRE-mRNA: AN OUTLINE

VERTEBRATES: 5'-AG/GUAAGU---intron---YNCURAC--$(Y)_n$--NAG/G-3'
YEAST: 5'-NN/GUAUGU---intron---UACUAAC---------YAG/G-3'

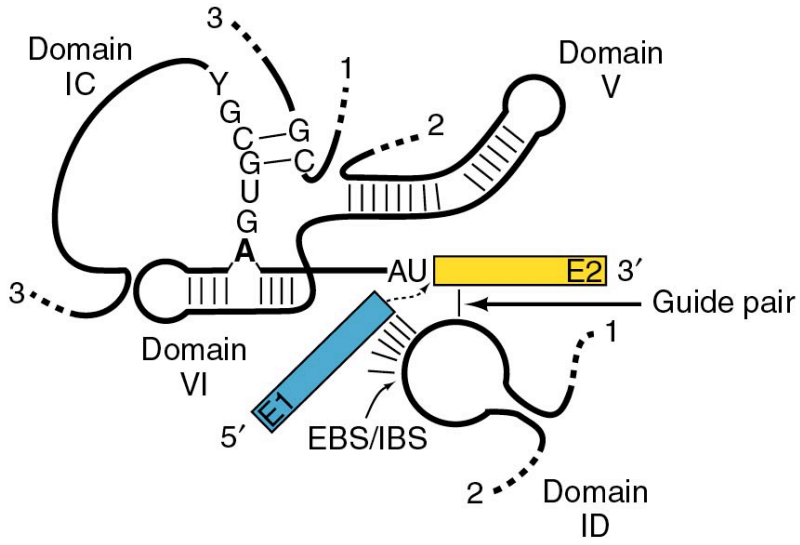
CONSENSUS SEQUENCES AT 5' SPLICE SITE, BRANCH SITE AND 5' SPLICE SITE

WEAVER: FIG. 14.4

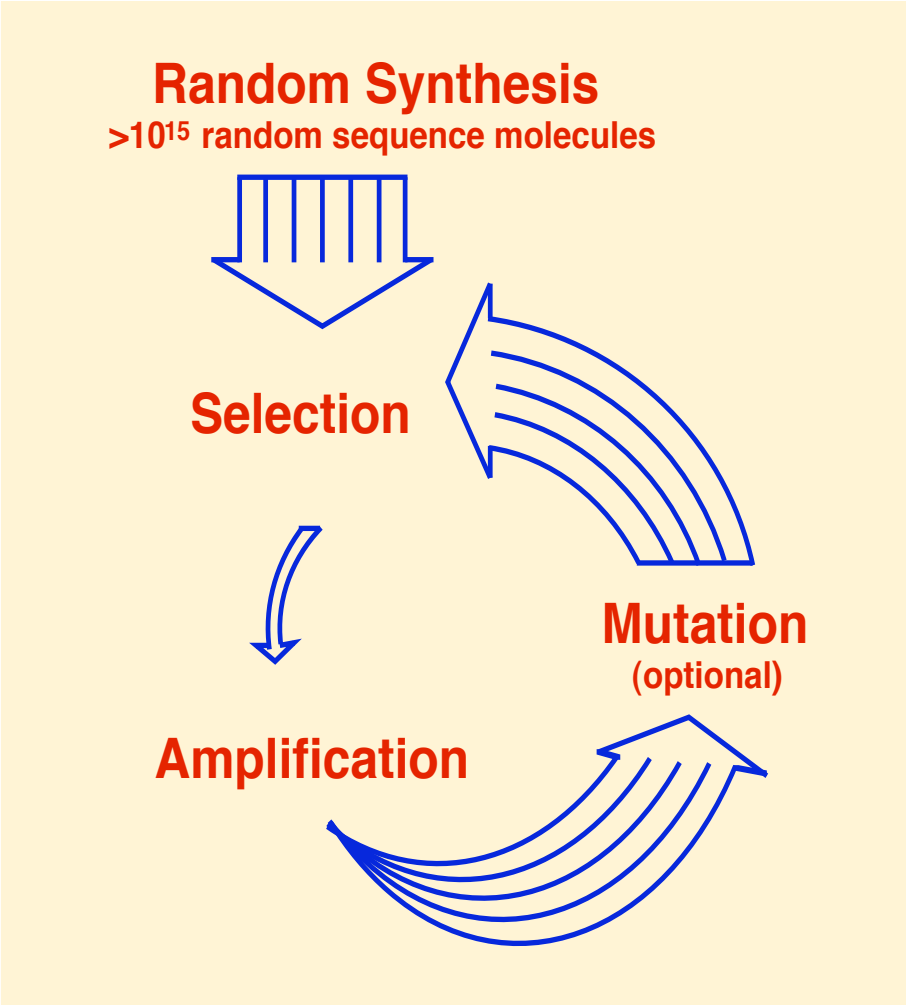
Spliceosomal
pre-mRNA

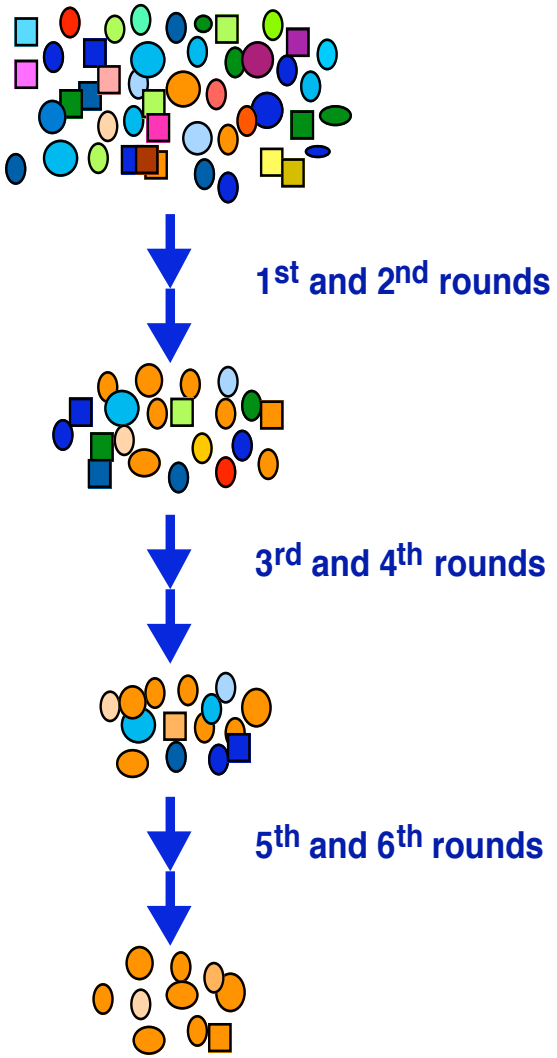


Group II intron



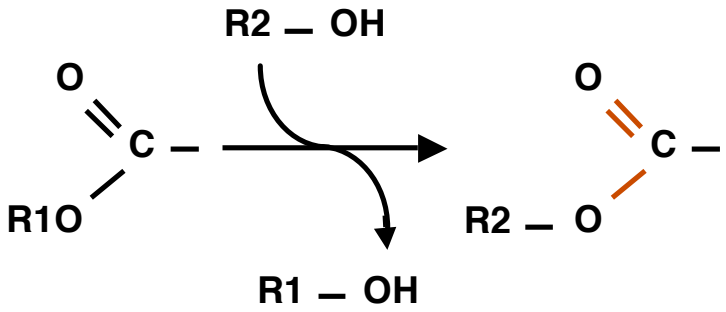
In vitro selection and evolution techniques



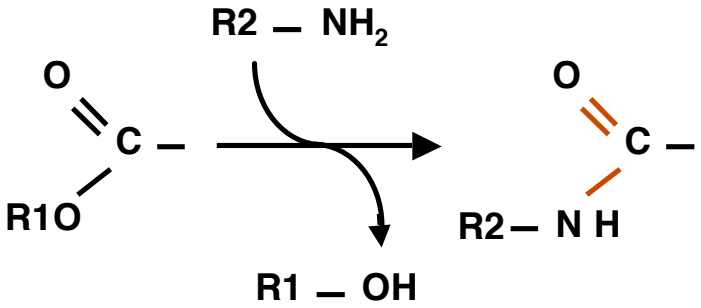


Ellington & Szostak (1990), Tuerk & Gold (1990), Beaudry & Joyce (1992)

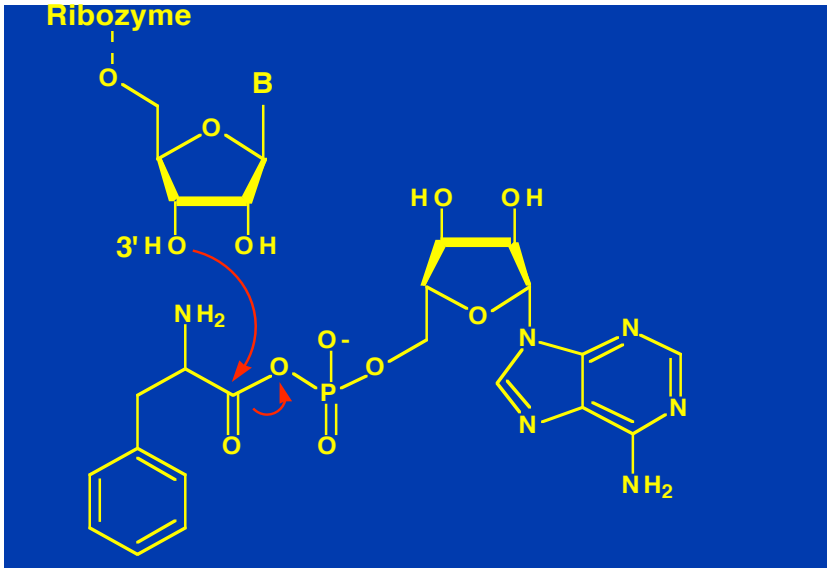
Ester bond formation



Amide bond formation

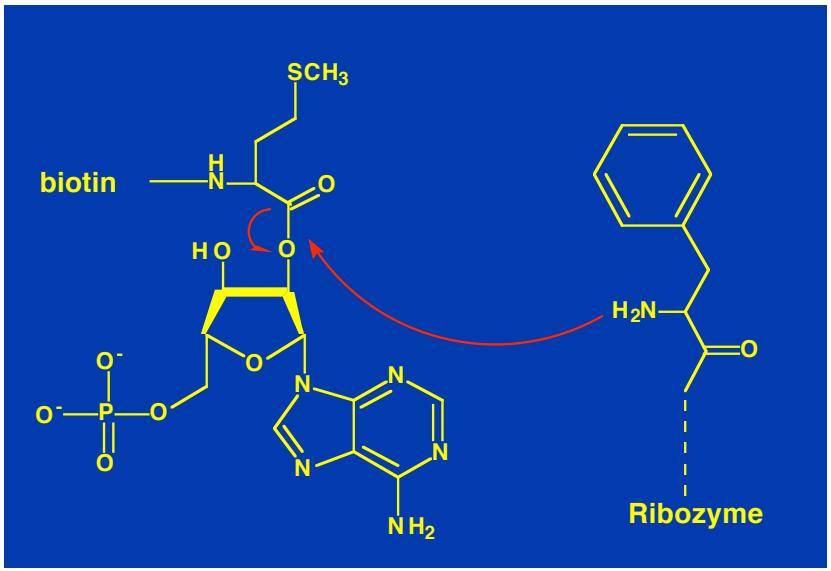


Aminoacylation



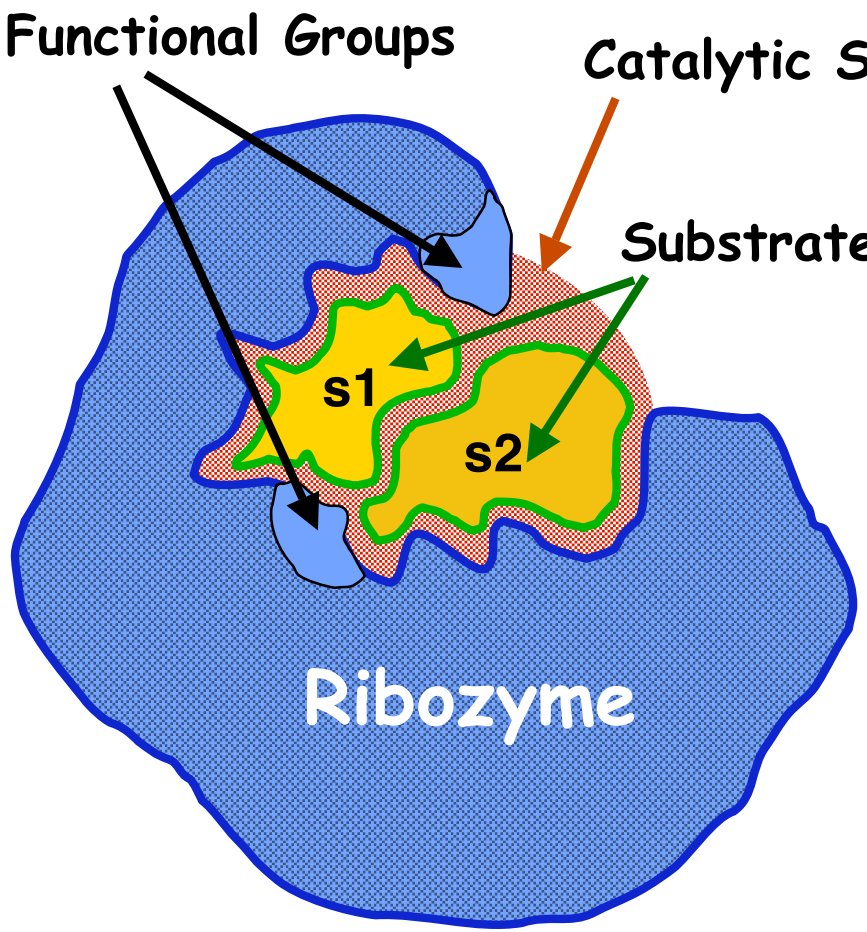
(Illangasekare *et al.*, 1995; Jenne & Famulok, 1998)

Peptidyl transferase



(Zhang & Cech,1997, 1998; Lee *et al.* ,2000)

Contributions to the catalysis by ribozymes



Proximity

Orientation

Structural Complementarity

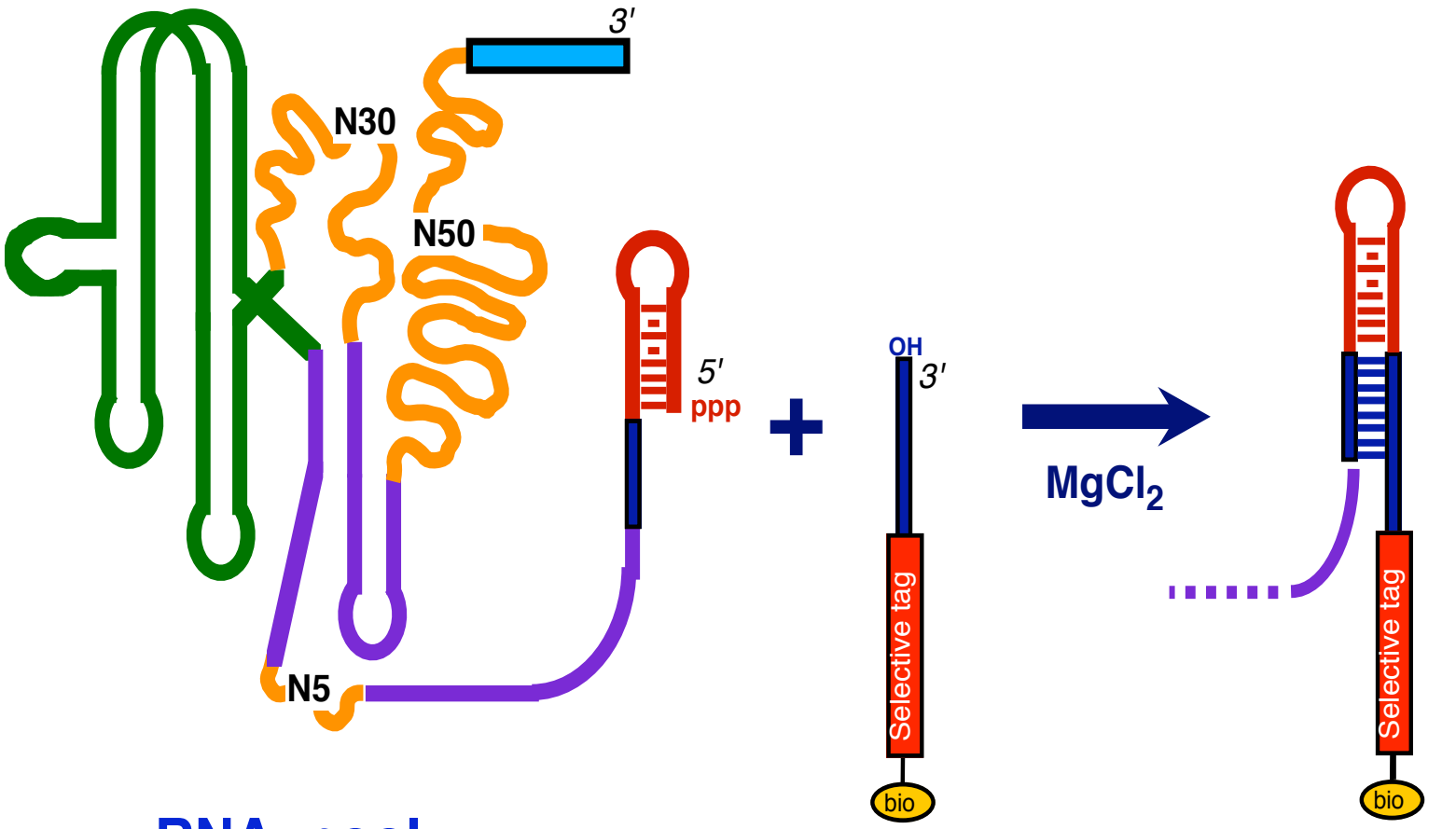
Polarizable Groups

Ions

Covalent catalysis

Acceleration of reaction velocities : 10^3-10^{11}

Selection criterion

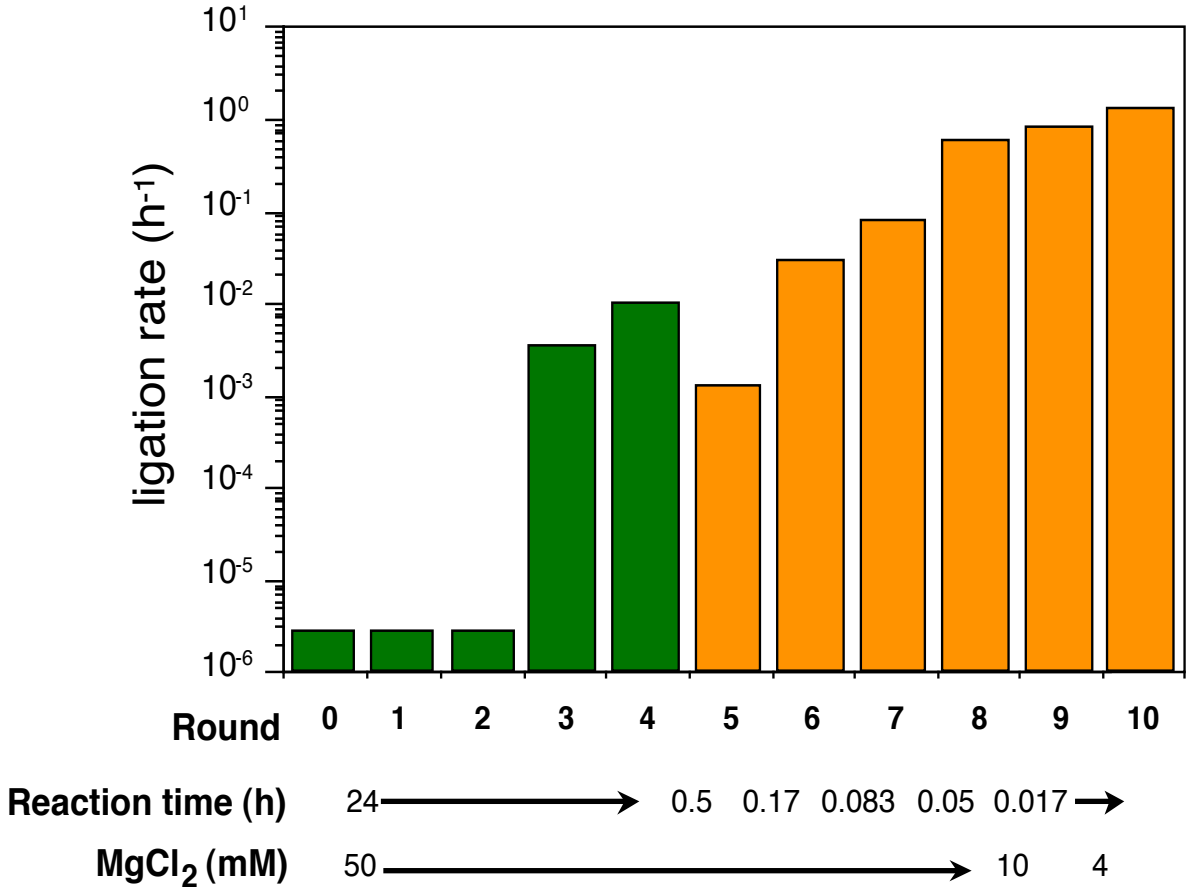


RNA pool
(10^{16} variants)

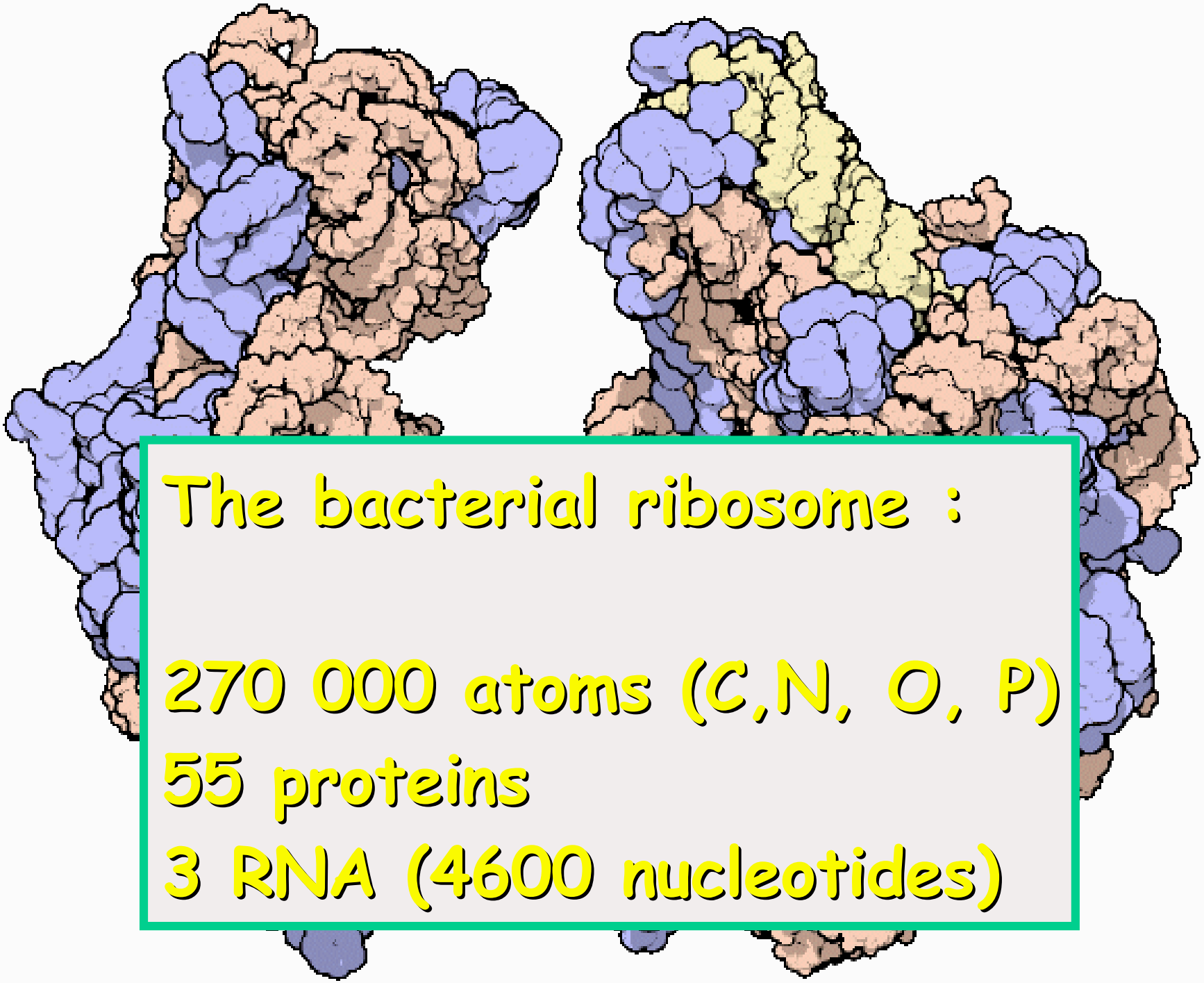
Substrate

Ligated product

Enhancement of ligation activity with successive rounds of selection



Jaeger, Wright & Joyce (1999) *Proc. Natl. Acad. Sci.* **96**, 14712



The bacterial ribosome :

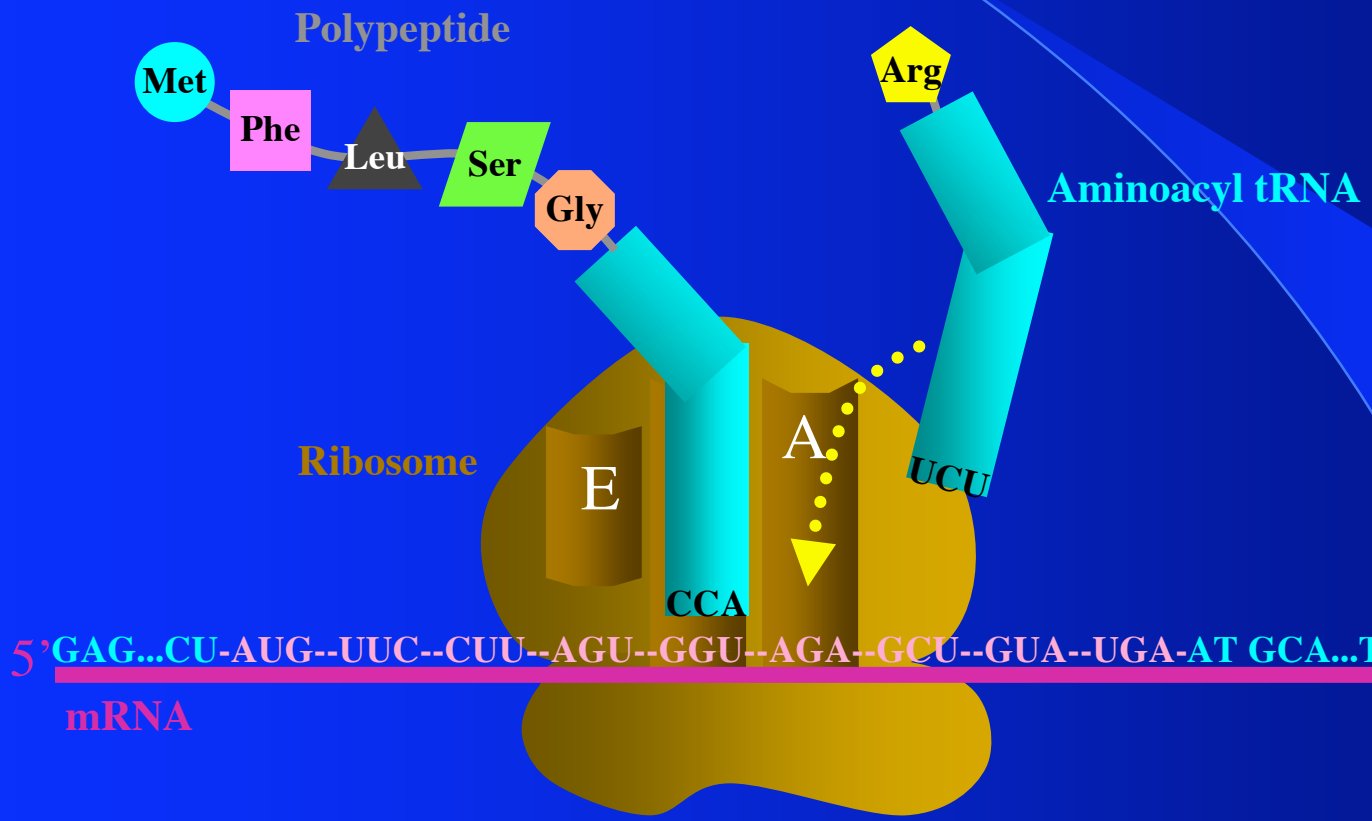
270 000 atoms (C,N, O, P)

55 proteins

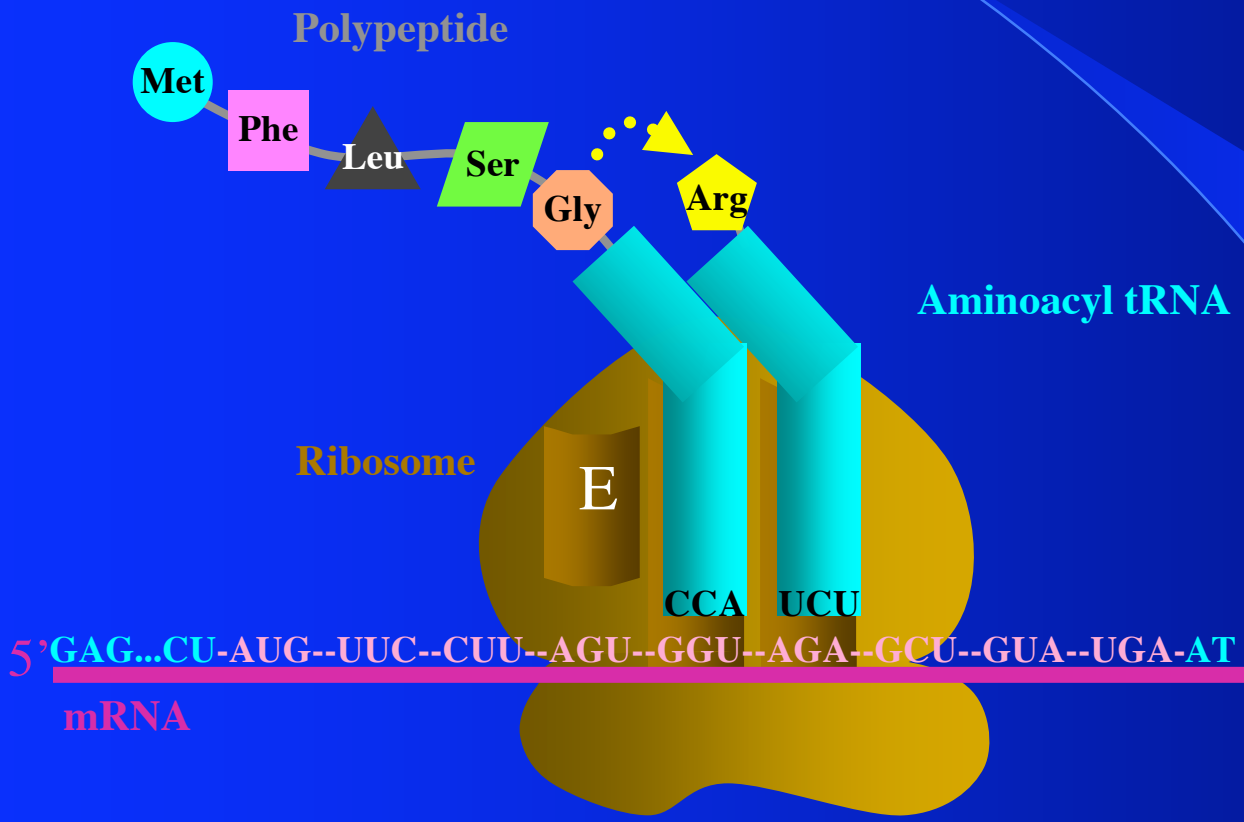
3 RNA (4600 nucleotides)

Translation - Initiation

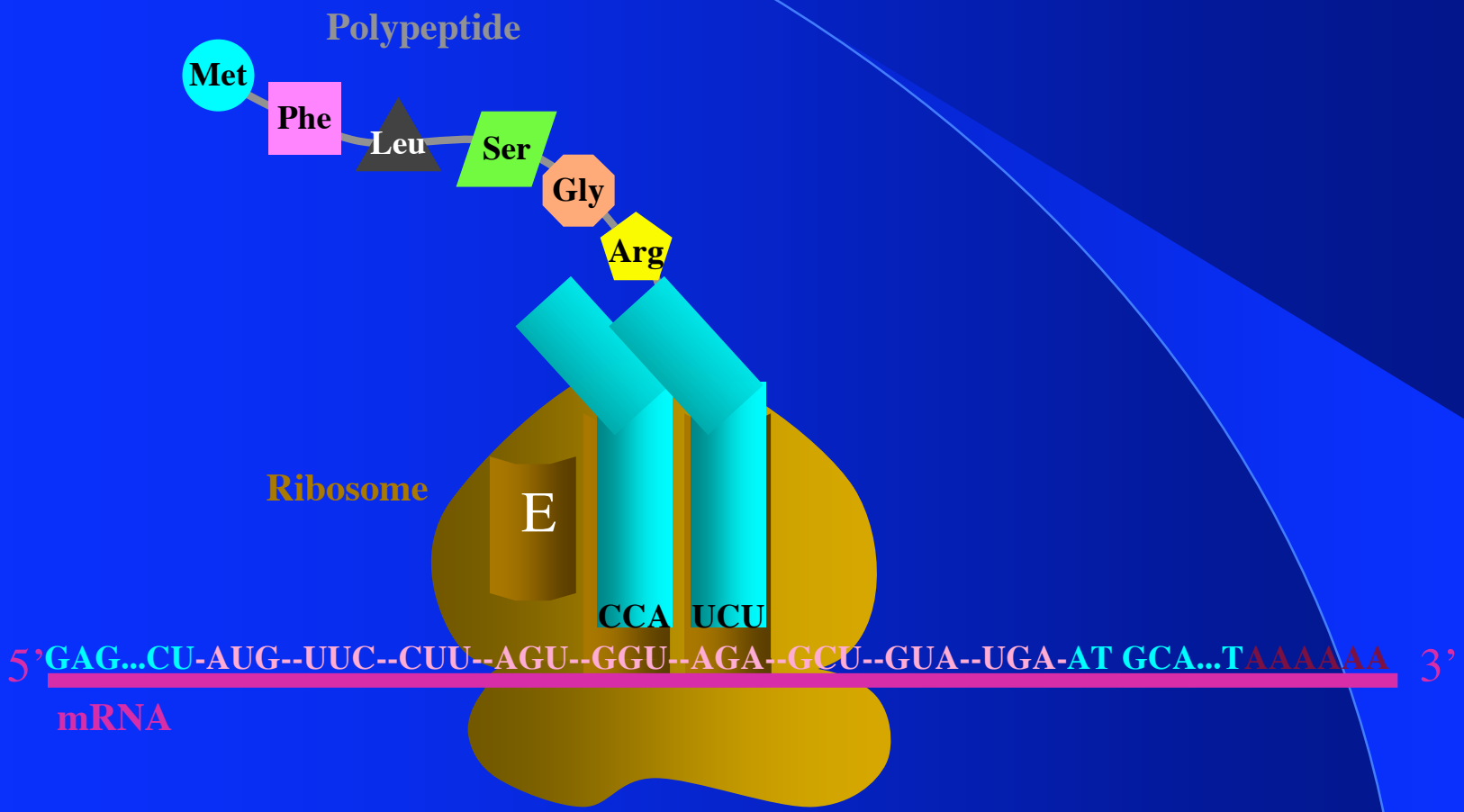
Translation - Elongation



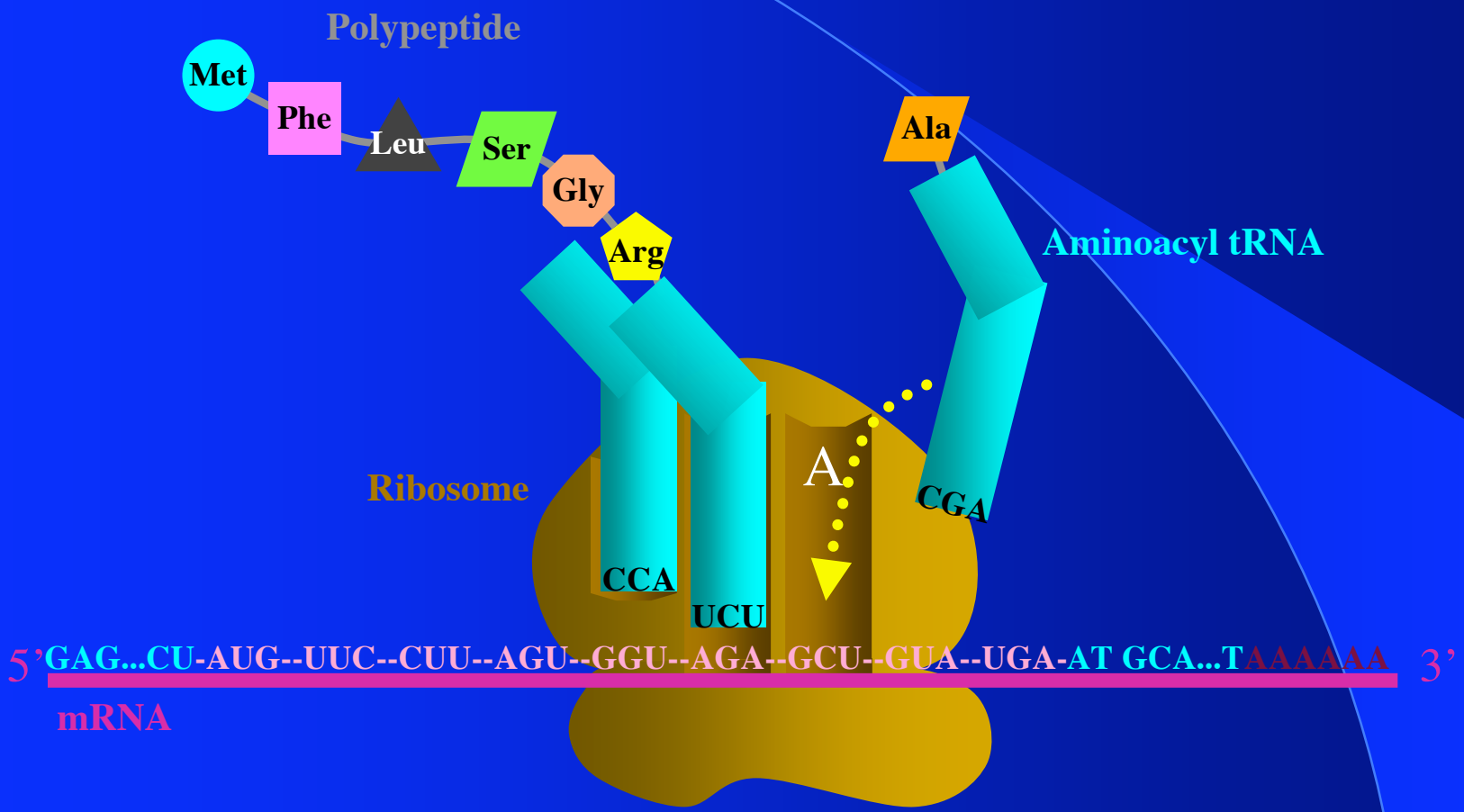
Translation - Elongation



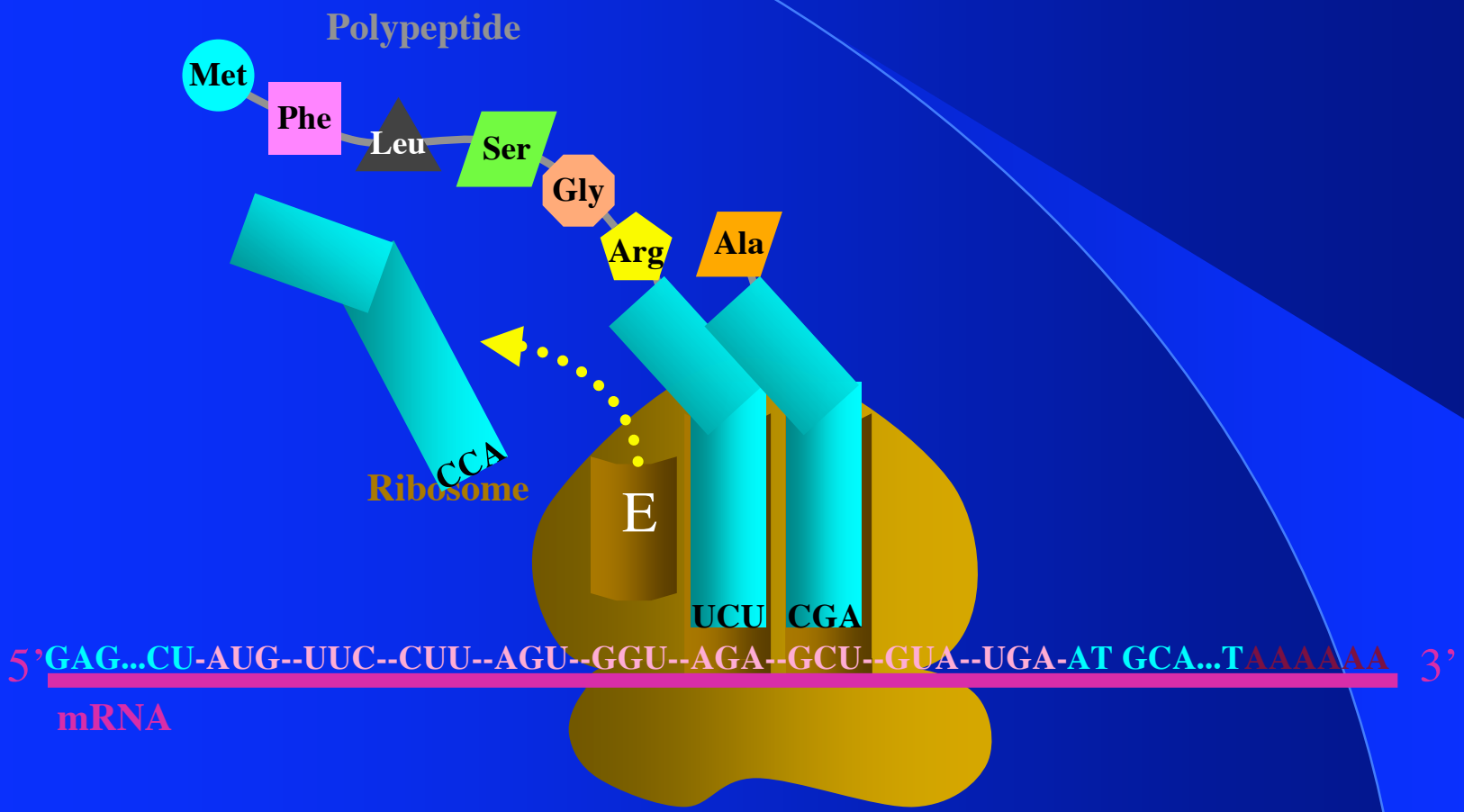
Translation - Elongation



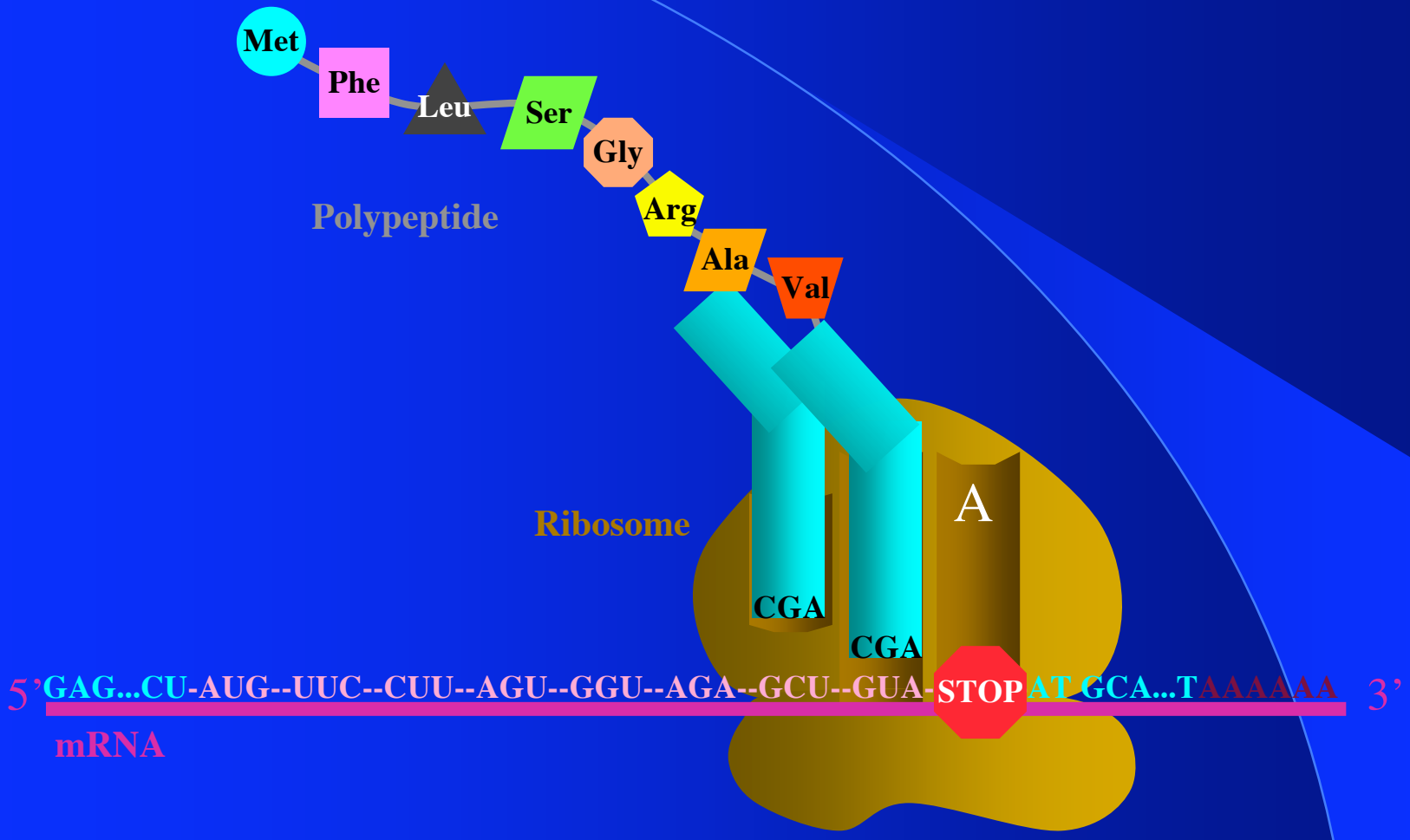
Translation - Elongation



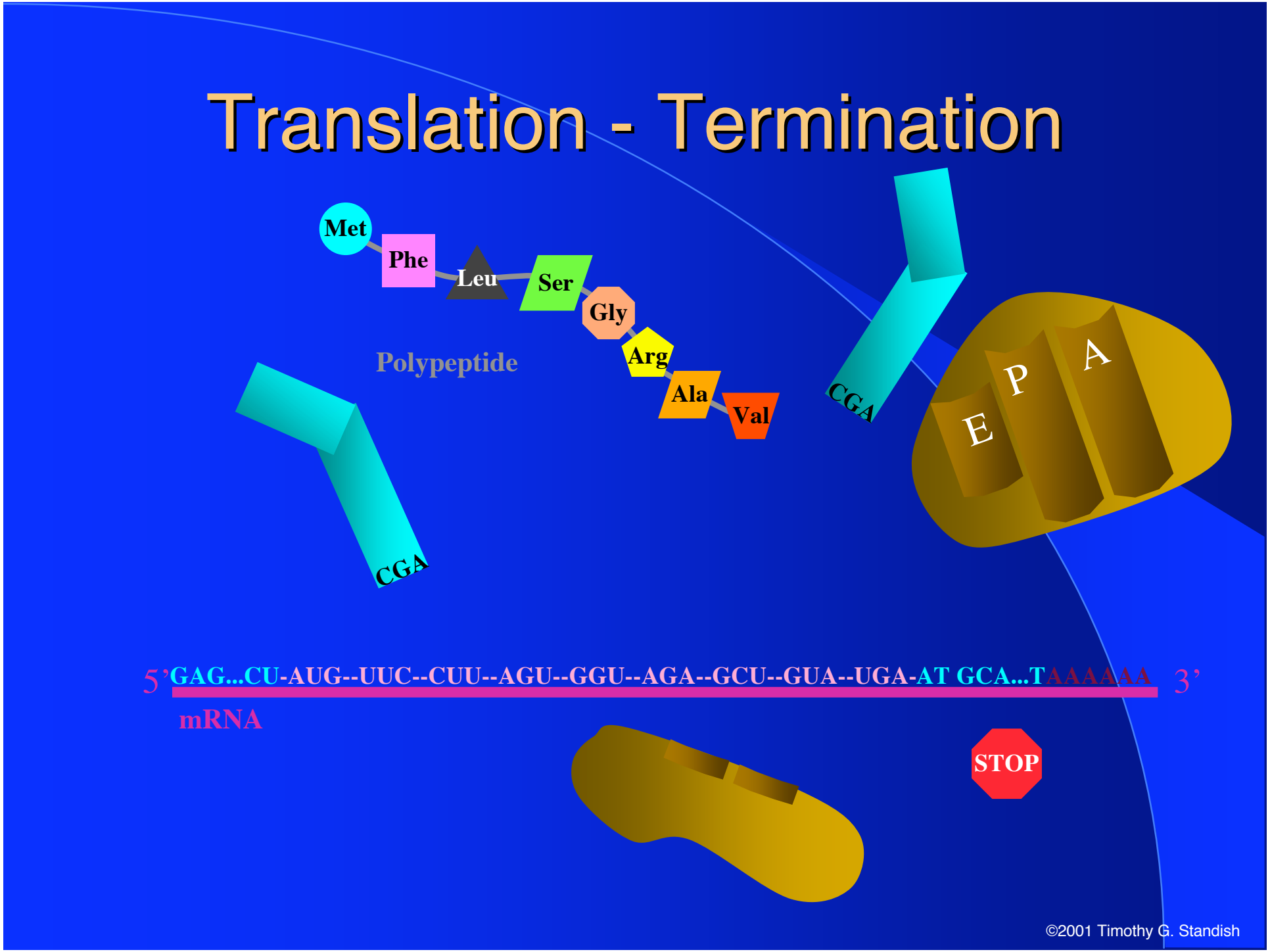
Translation - Elongation

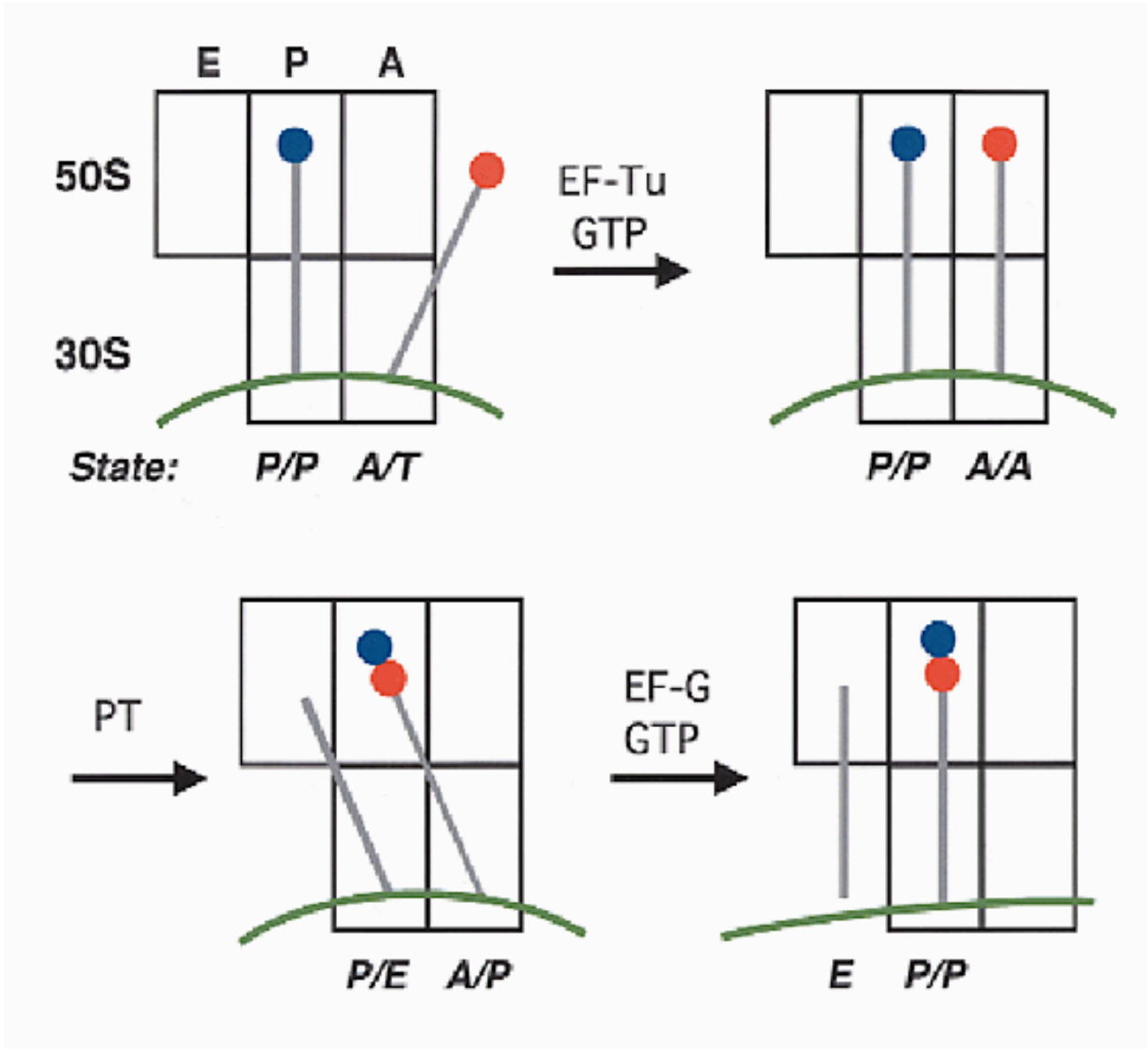


Translation - Termination



Translation - Termination




E
P
A
50S
30S
State:
P/P
A/T
EF-Tu
GTP
P/P
A/A
PT
P/E
A/P
EF-G
GTP
E
P/P

Kinetic scheme for the binding of cognate tRNA at the A site

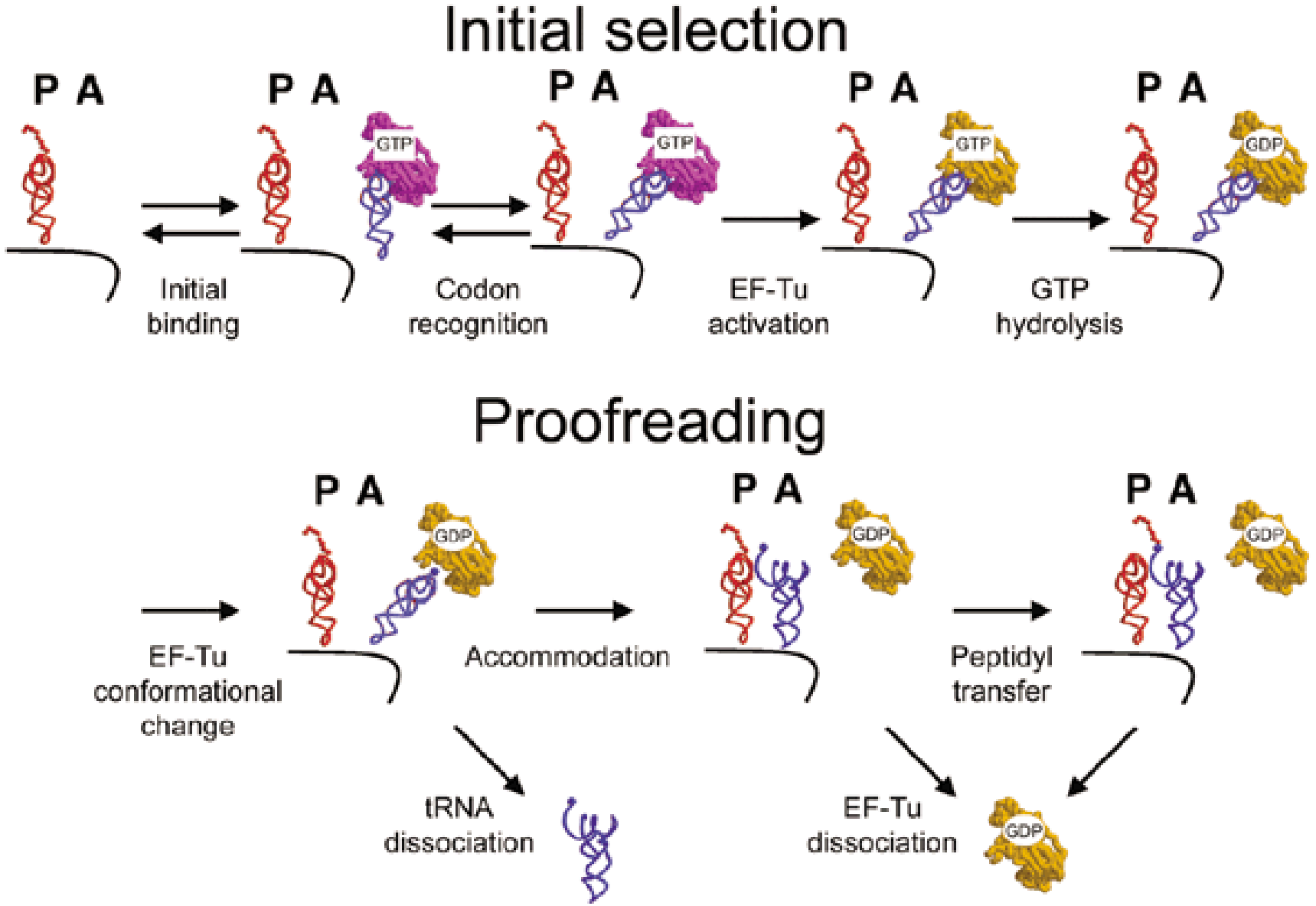
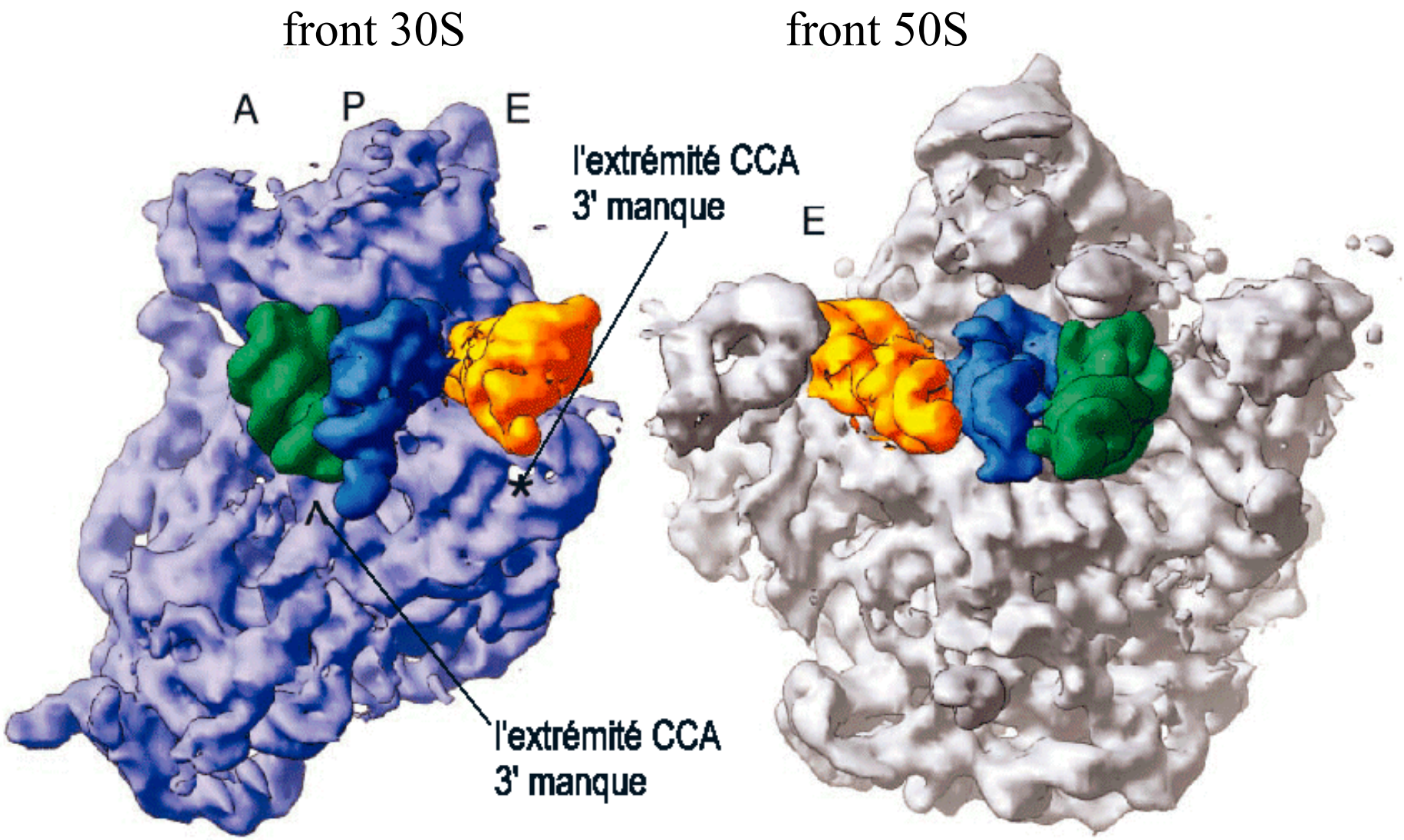


Table. Elemental rate constants of near-cognate (Leu) and cognate (Phe)[a] aa-tRNA binding to the A site according to the model presented in Figure 1

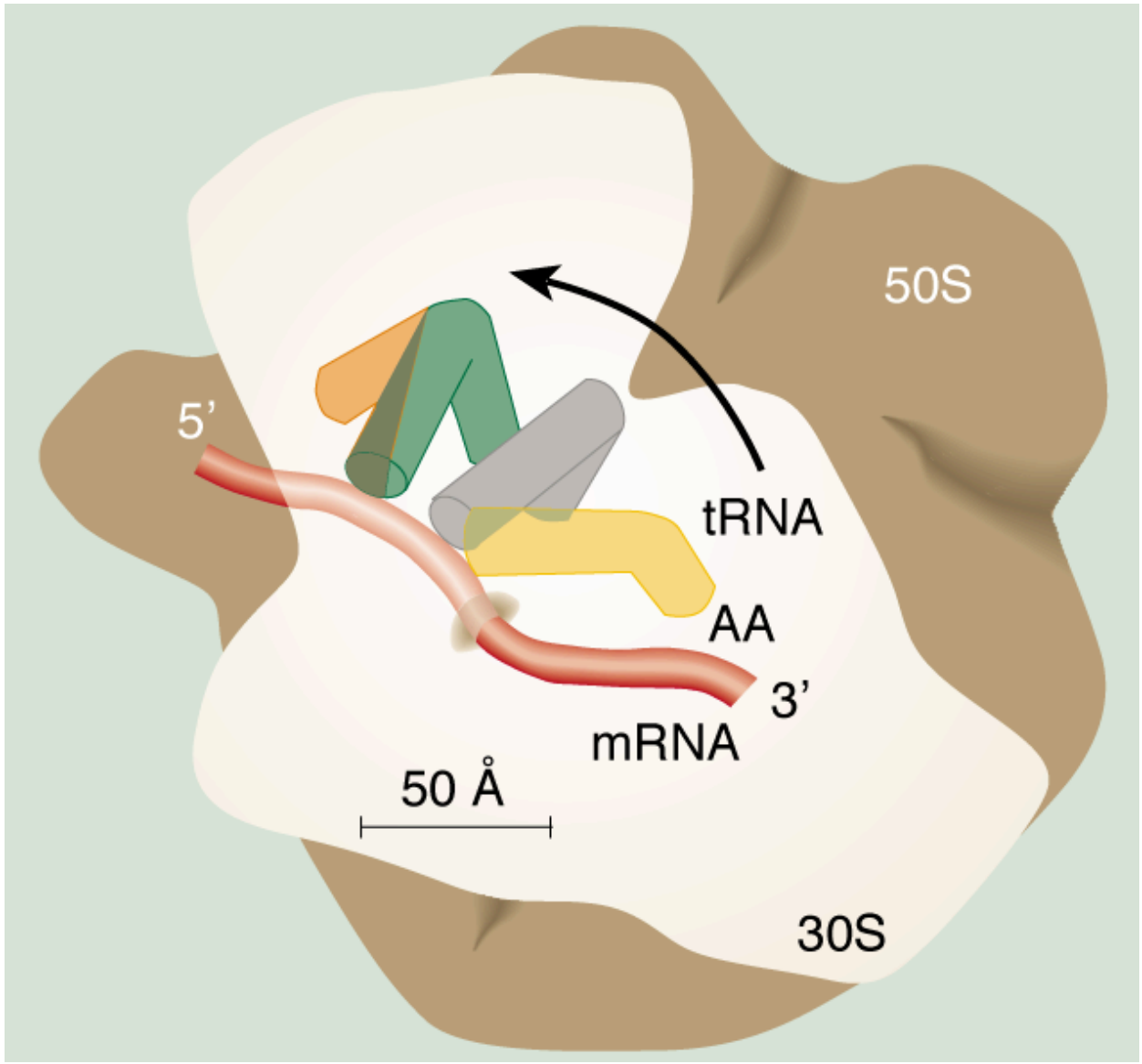
Step	Rate constant (per s)	Near-cognate	Cognate
Initial binding	k_1	110 ± 10[b]	110 ± 20[b] (non-cognate 60)
	k_{-1}	25 ± 5	25 ± 5 (non-cognate 25)
Codon recognition			
	k_2	100 ± 20	100 ± 15
	k_{-2}	17 ± 8[d]	0.2 ± 0.1
GTPase activation and GTP hydrolysis (e)			
	k_3	50 ± 20	500 ± 100 (non-cognate 0.005)
GTP-GDP conformation change of EF-Tu			
	k_4	50 ± 20	60 ± 20
aa-tRNA accommodation and peptide bond formation (e)			
	k_5	0.1 ± 0.03	7 ± 2
Dissociation of EF-Tu			
	k_6	2 ± 1	3 ± 1
aa-tRNA rejection			
	k_7	6 ± 1	<0.3

[b] per μM/s.
[e] Grouped for analysis, because the former reaction is rate limiting.

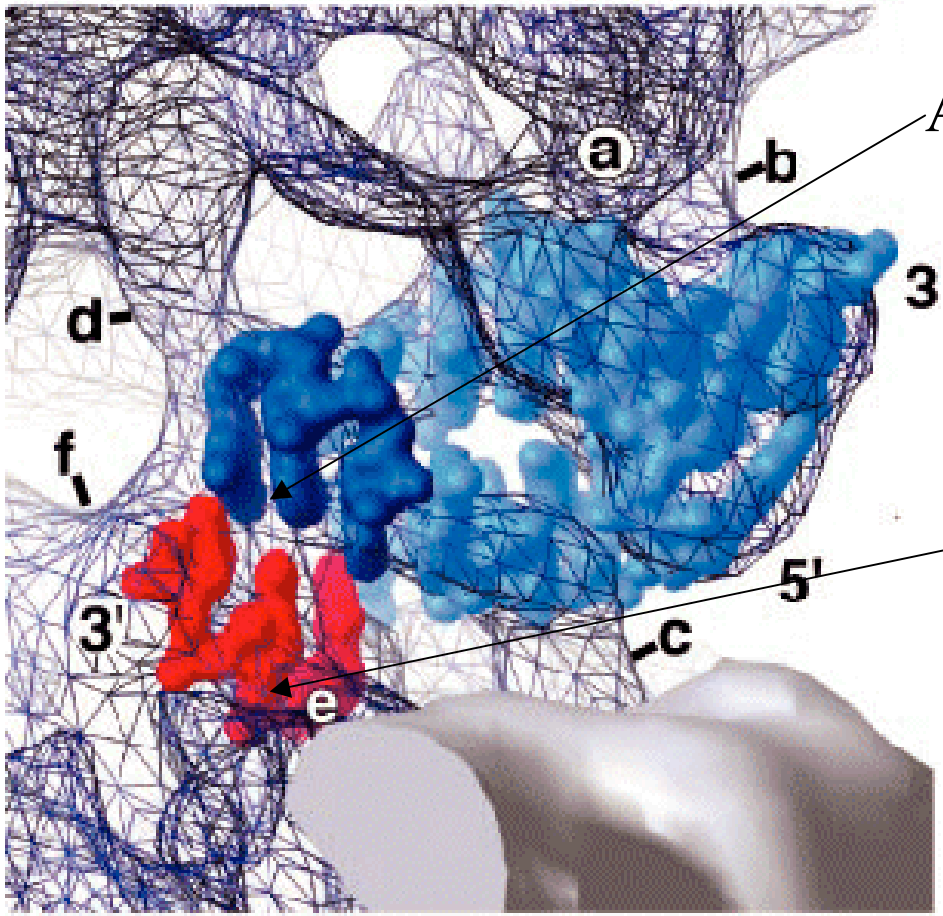
70S: ARNt (*Thermus thermophilus*)



J.H.Cate et al. (1999) Science 285, 2095-2104


50S
5’
tRNA
AA
3’
mRNA
50 Å
30S

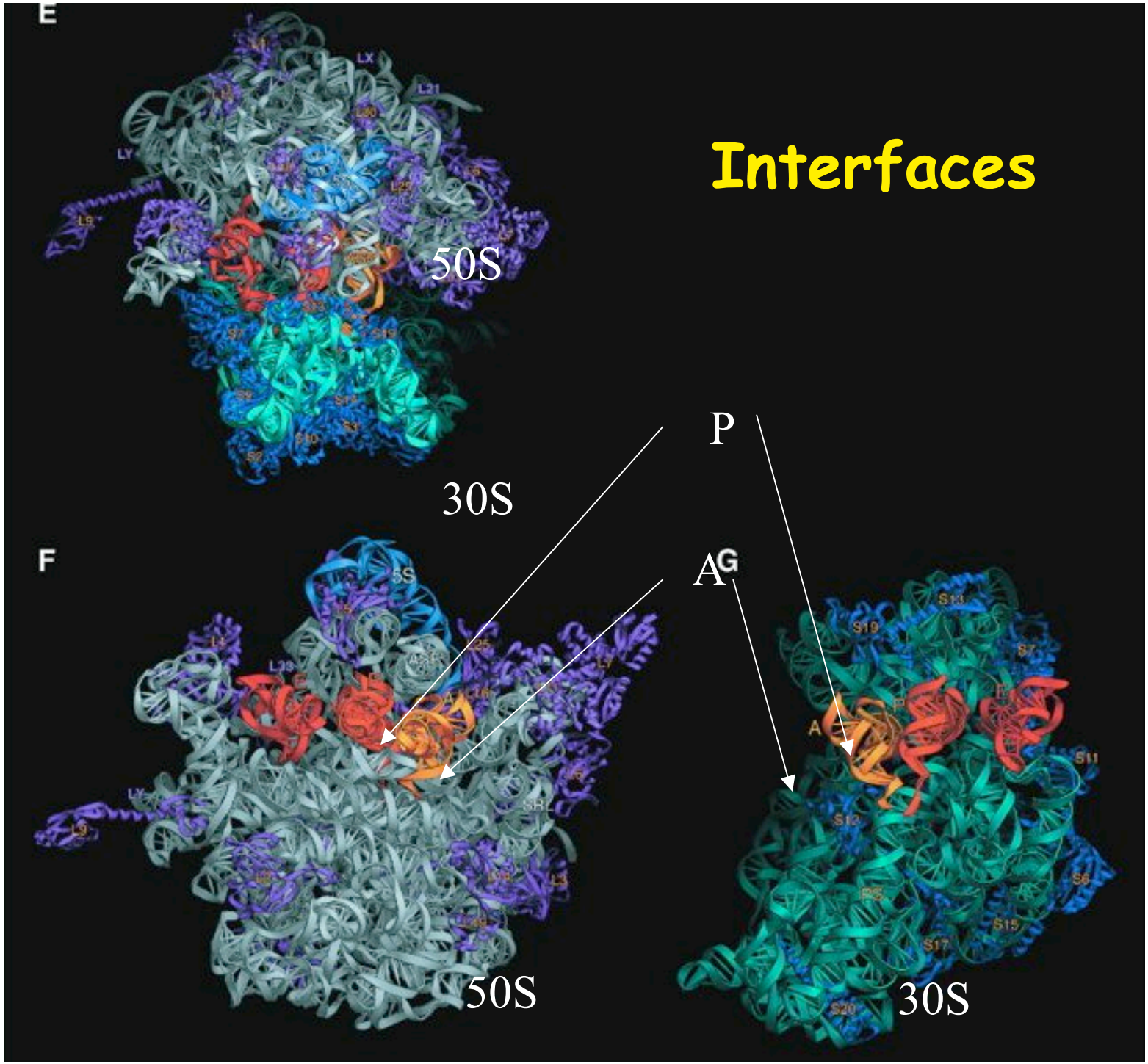
70S: tRNA, contacts codon-anticodon in the P site

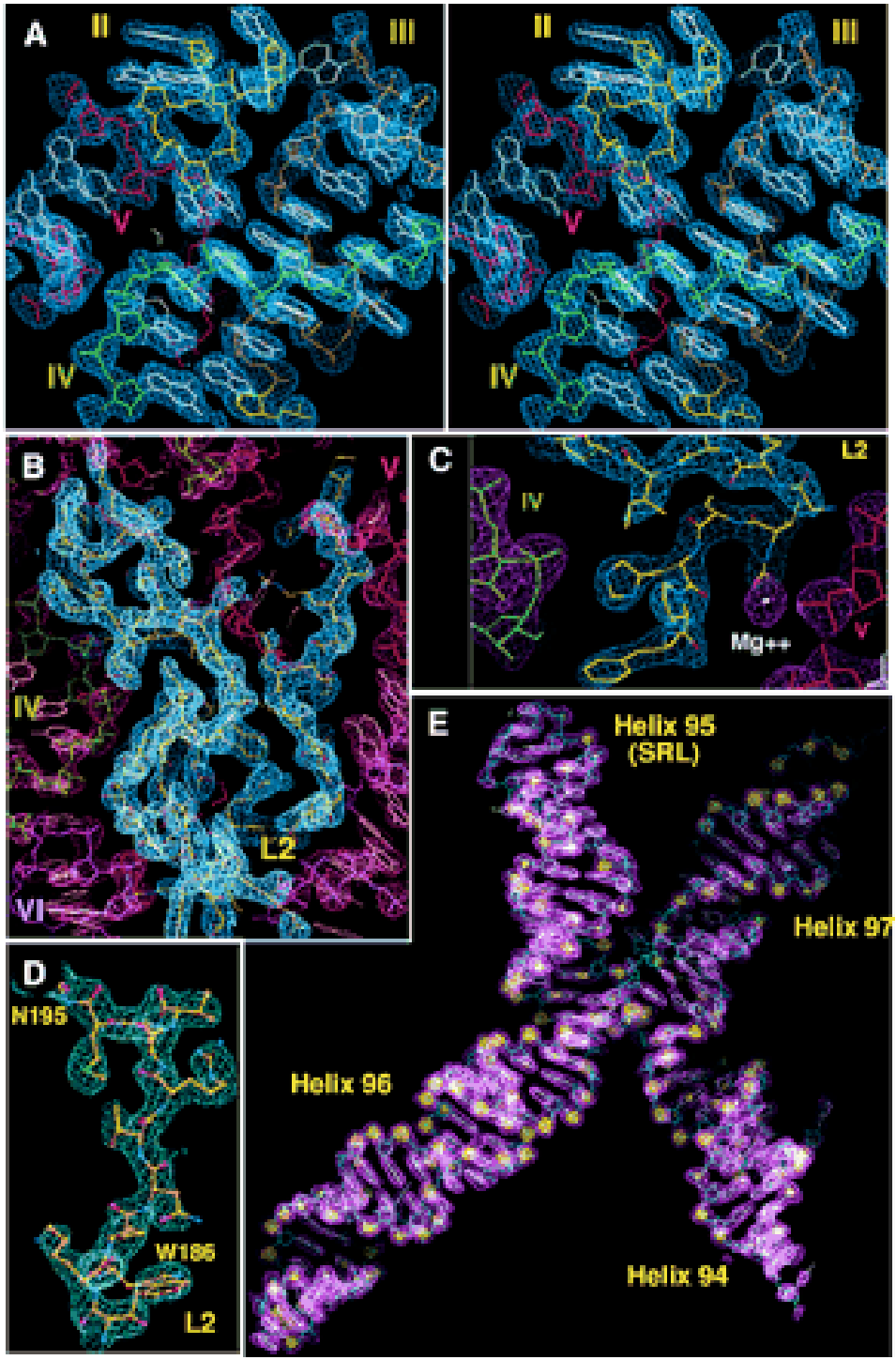


Anticodon

Codon at the P site

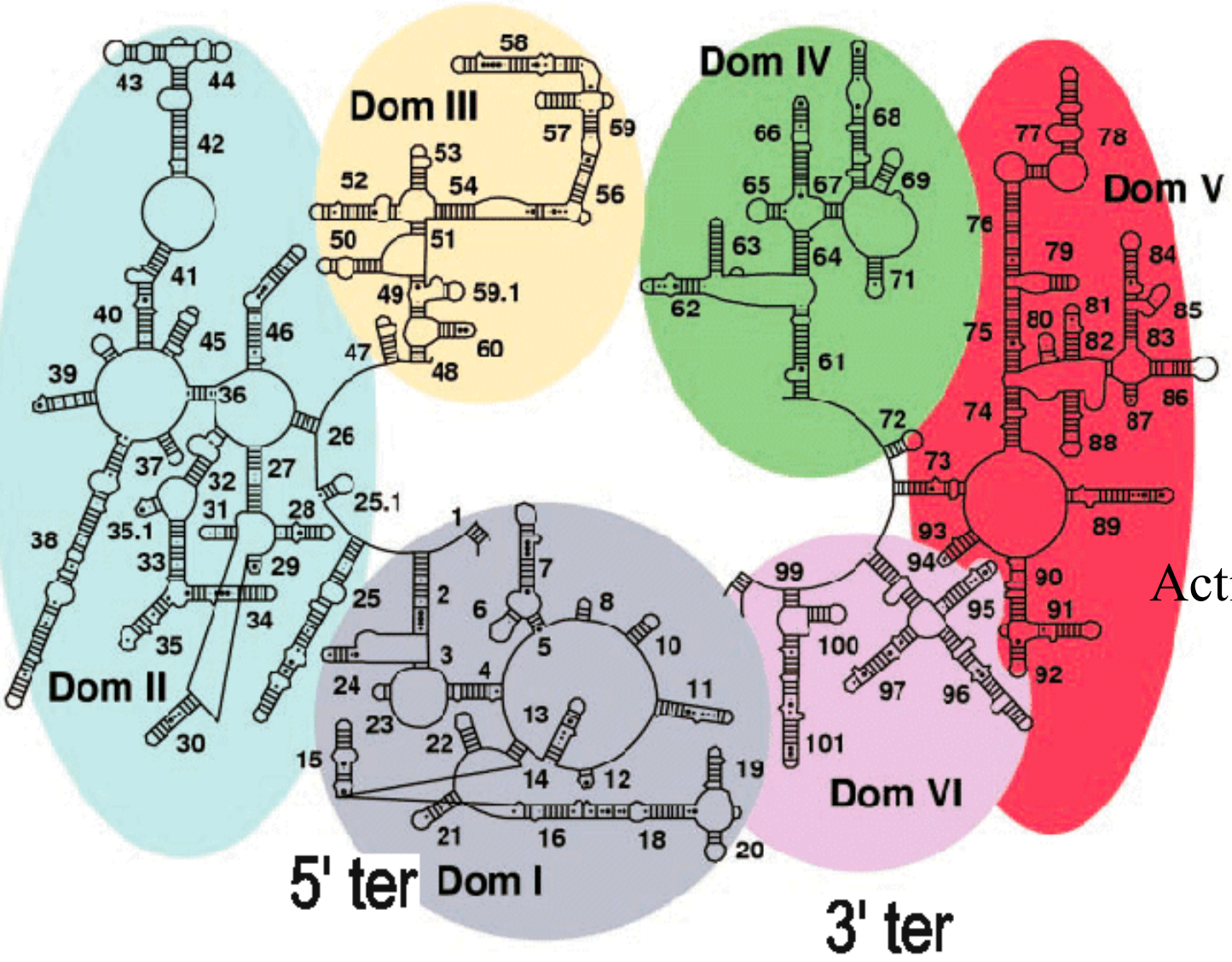
J.H.Cate et al. (1999) Science 285, 2095-2104


E
50S
Interfaces
P
30S
A
F
5S
50S
30S



2.4 Å resolution for the 50S

ARNr 23S (*H. marismortui*)

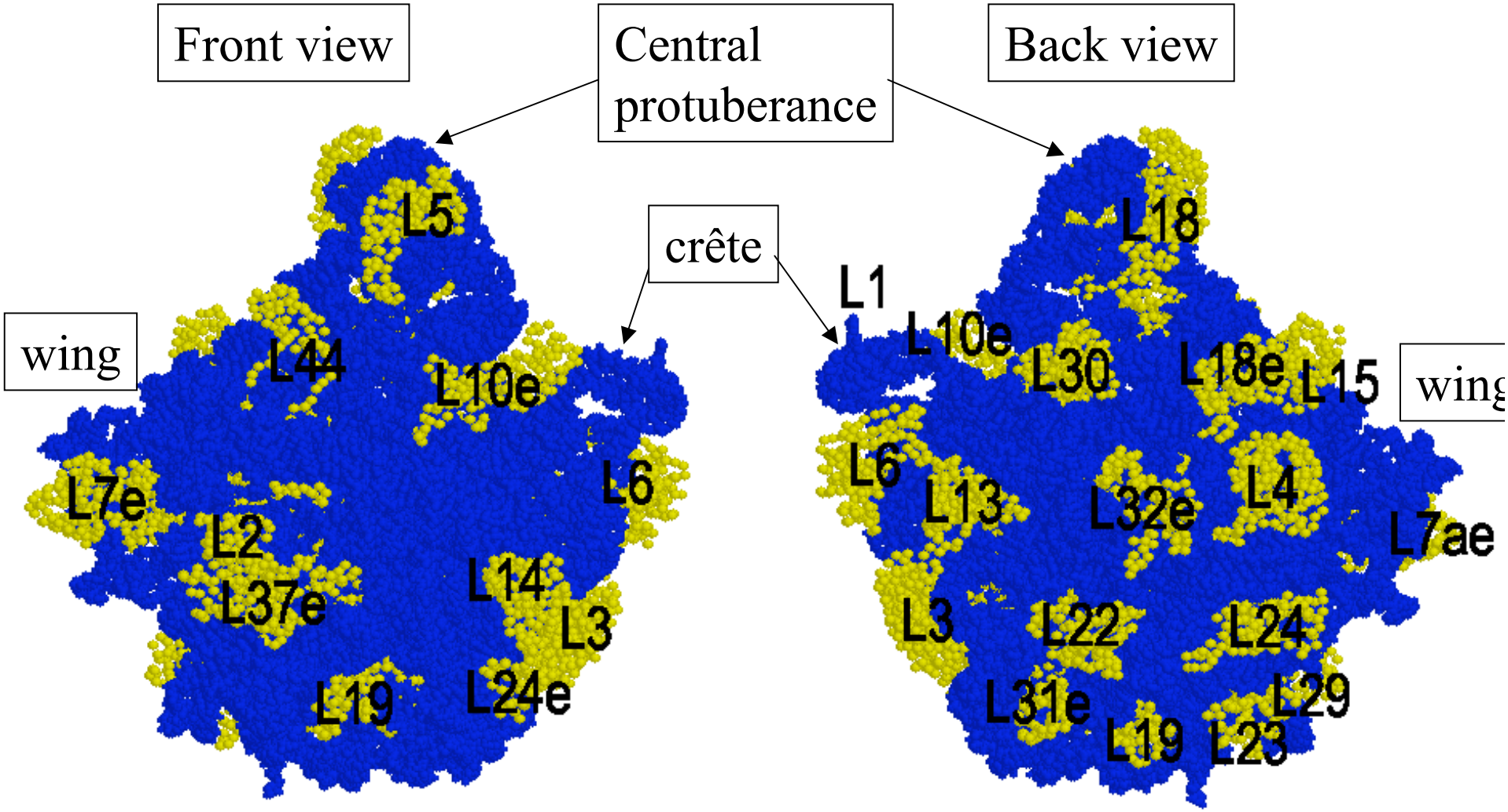


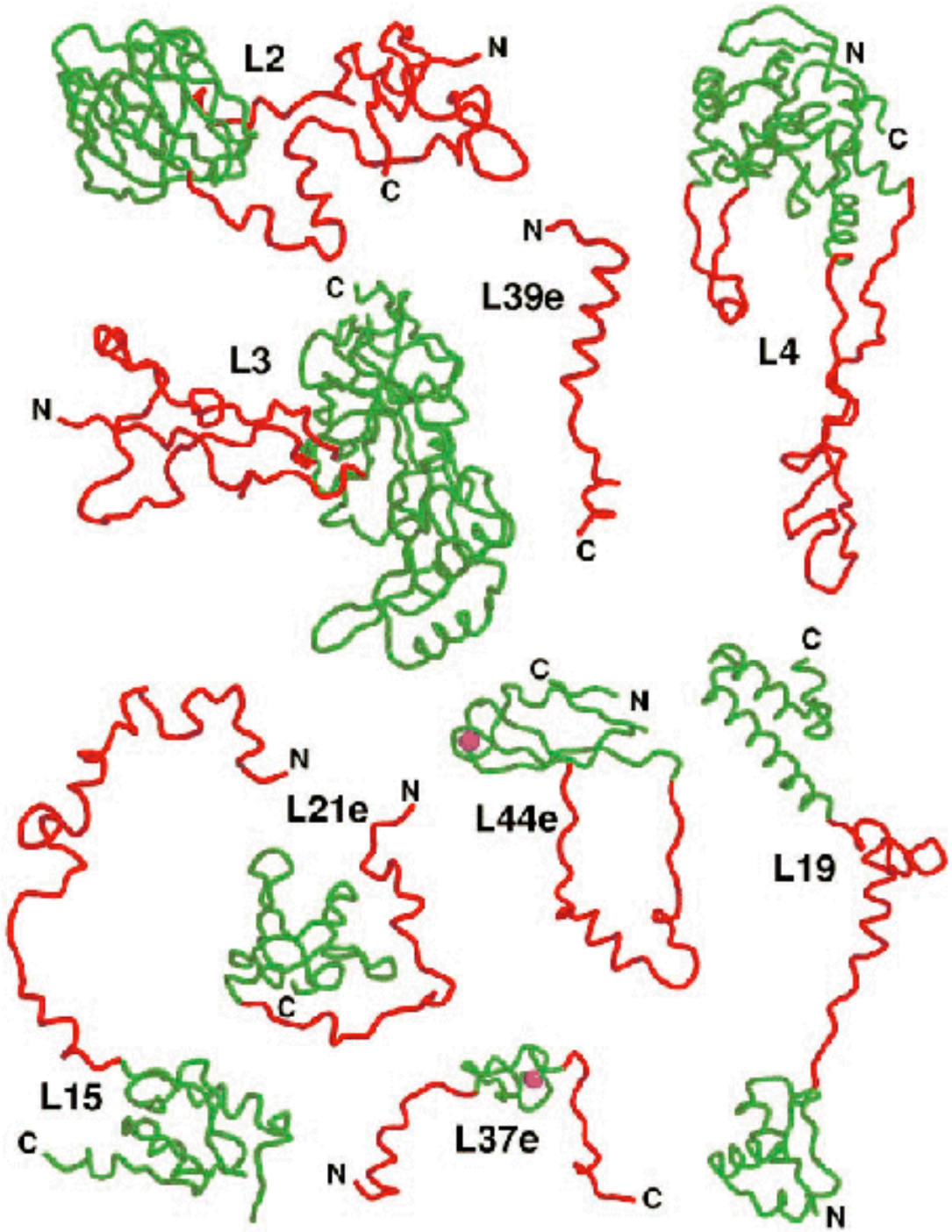
Active site

N.Ban et al., (2000) Science, 289, 905-920

50S: localisation of the peptides (*H. marismortui*)

N.Ban et al., (2000) Science, 289, 905-920





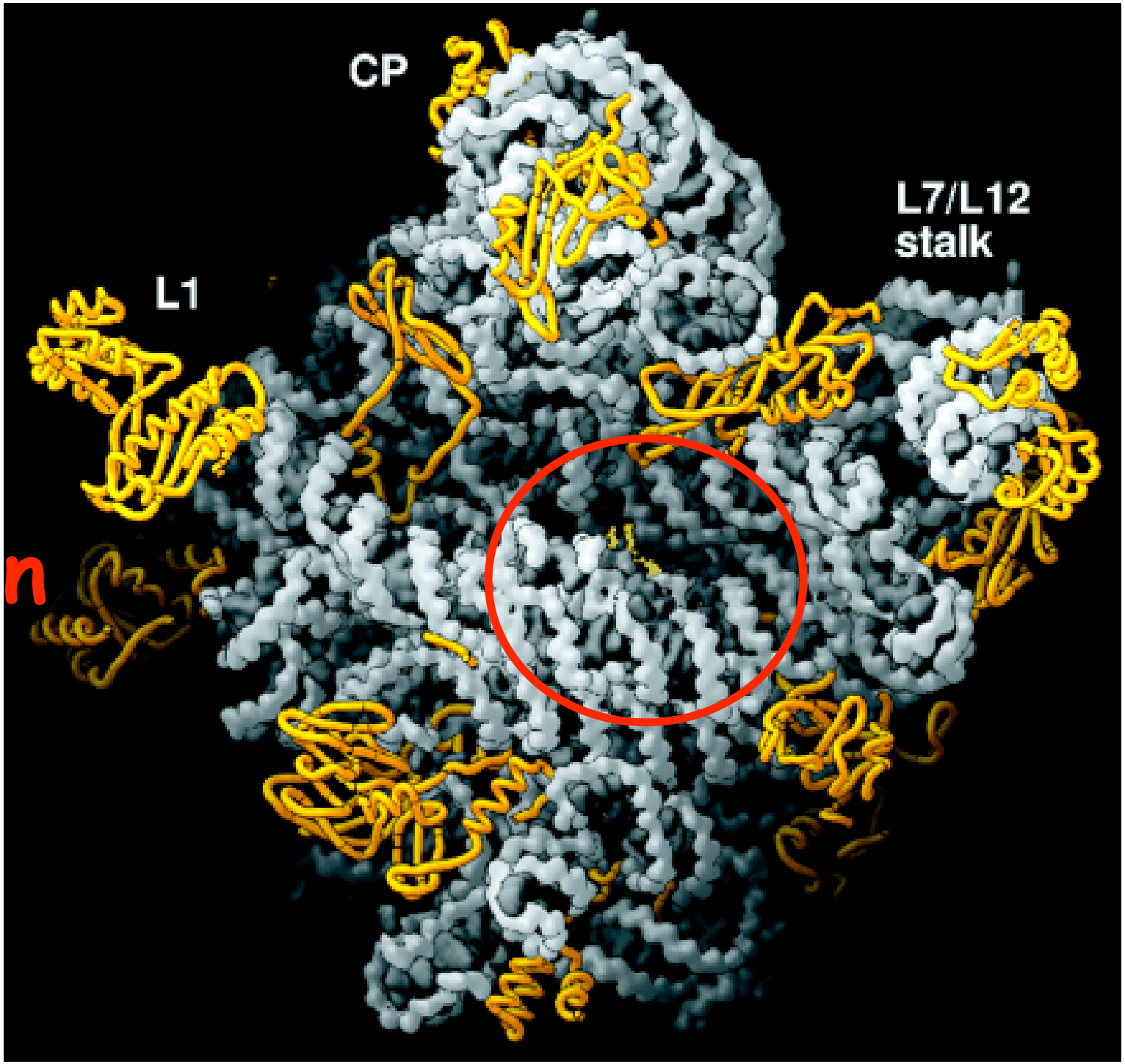
50S: structure of proteins (*H. marismortui*)

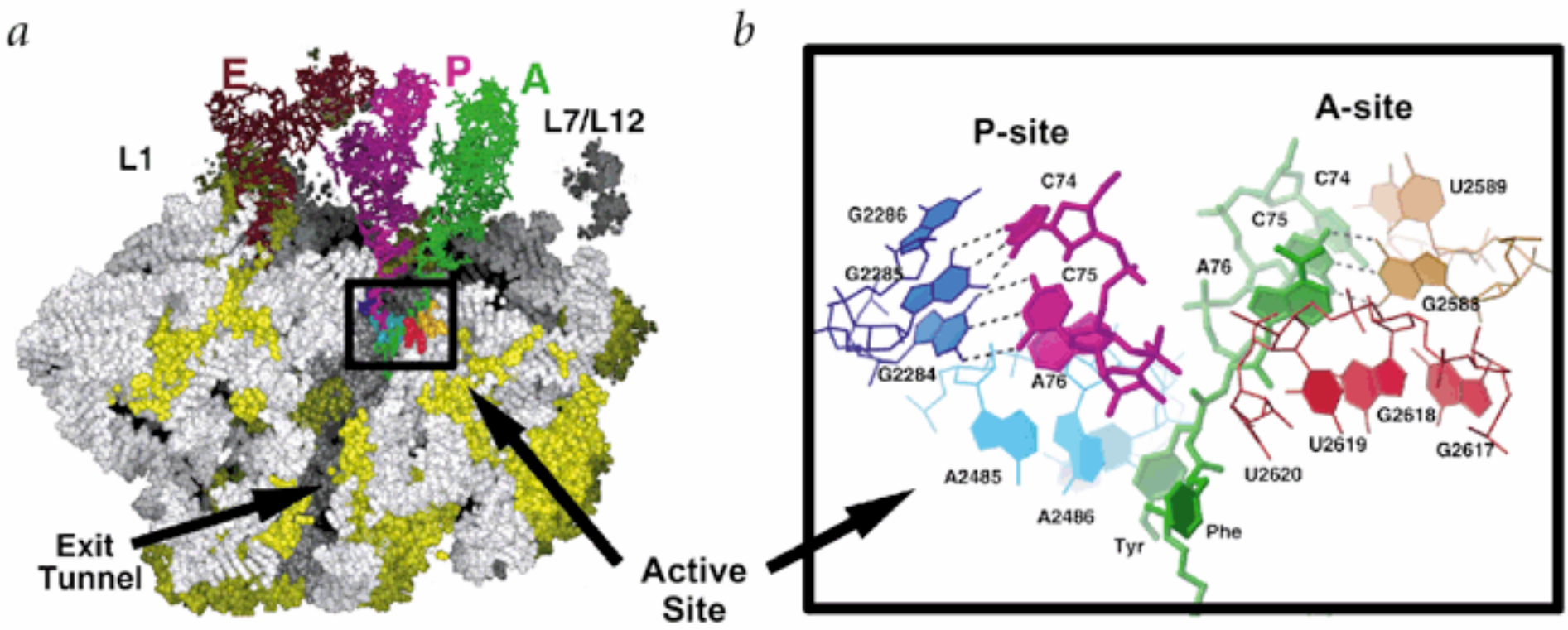
17 globular
13 partly or completely stretch

Red :stretched
Green : globular

N.Ban et al., (2000) Science, 289, 905-920

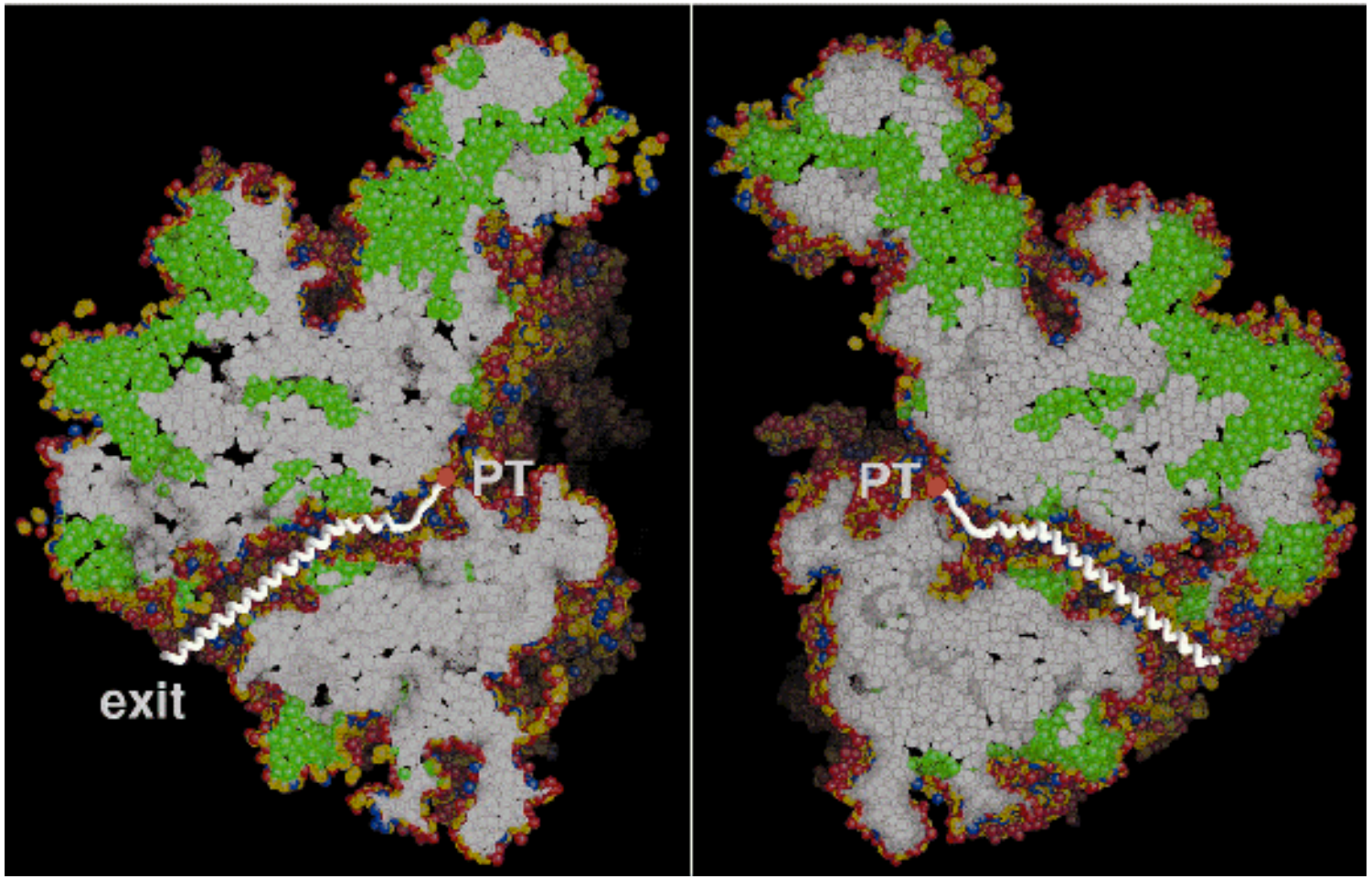
Only RNA in the reaction site




a
E
P
A
L7/L12
L1
Exit
Tunnel
Active
Site
b
P-site
A-site
C74
G2286
G2285
C75
G2284
A76
A2485
A2486
Tyr
Phe
C74
C75
A76
U2589
G2588
G2618
U2619
G2617
U2620

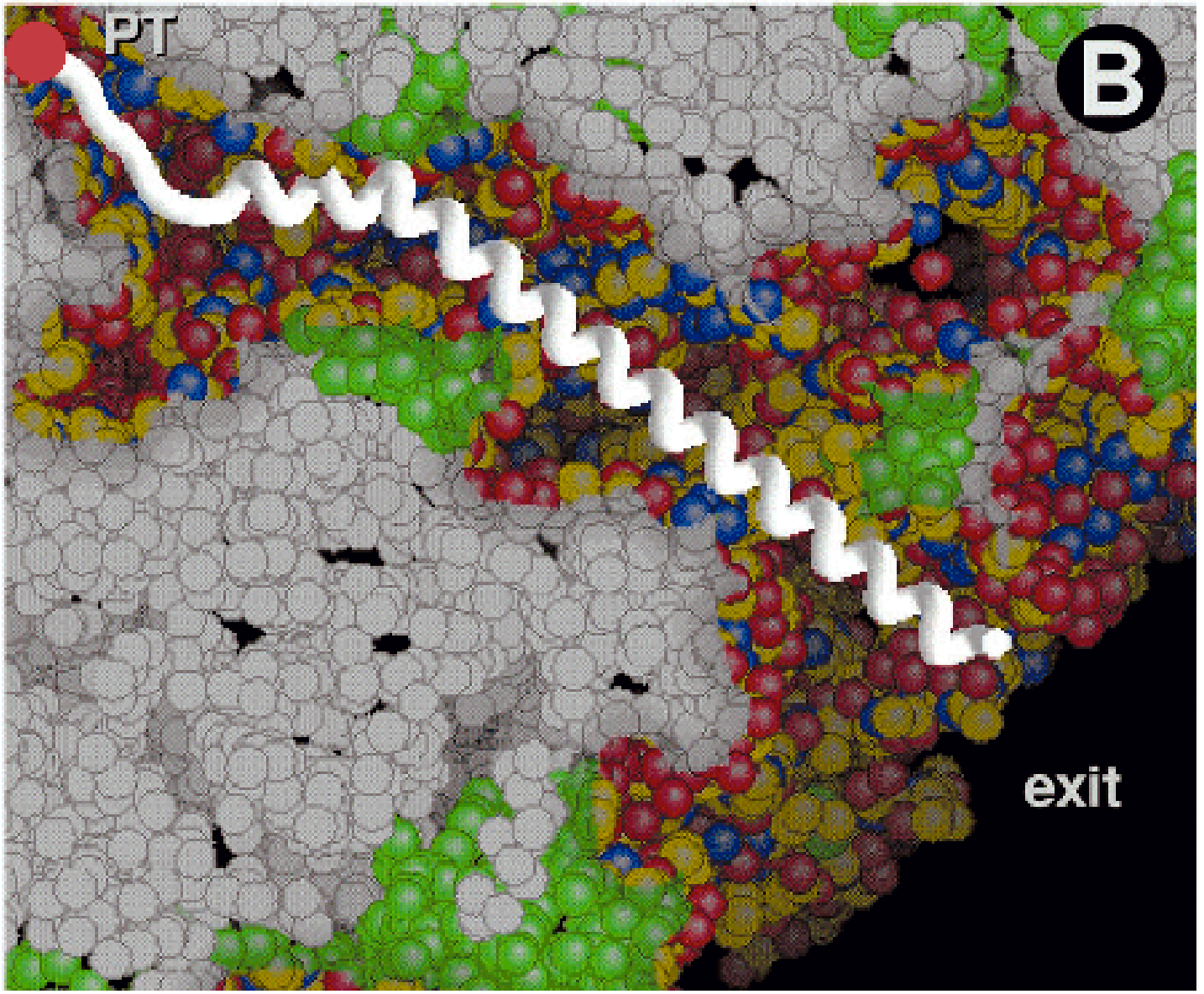
Tunnel for nascent peptide

Diameter18 à 25 Å: no helical formation



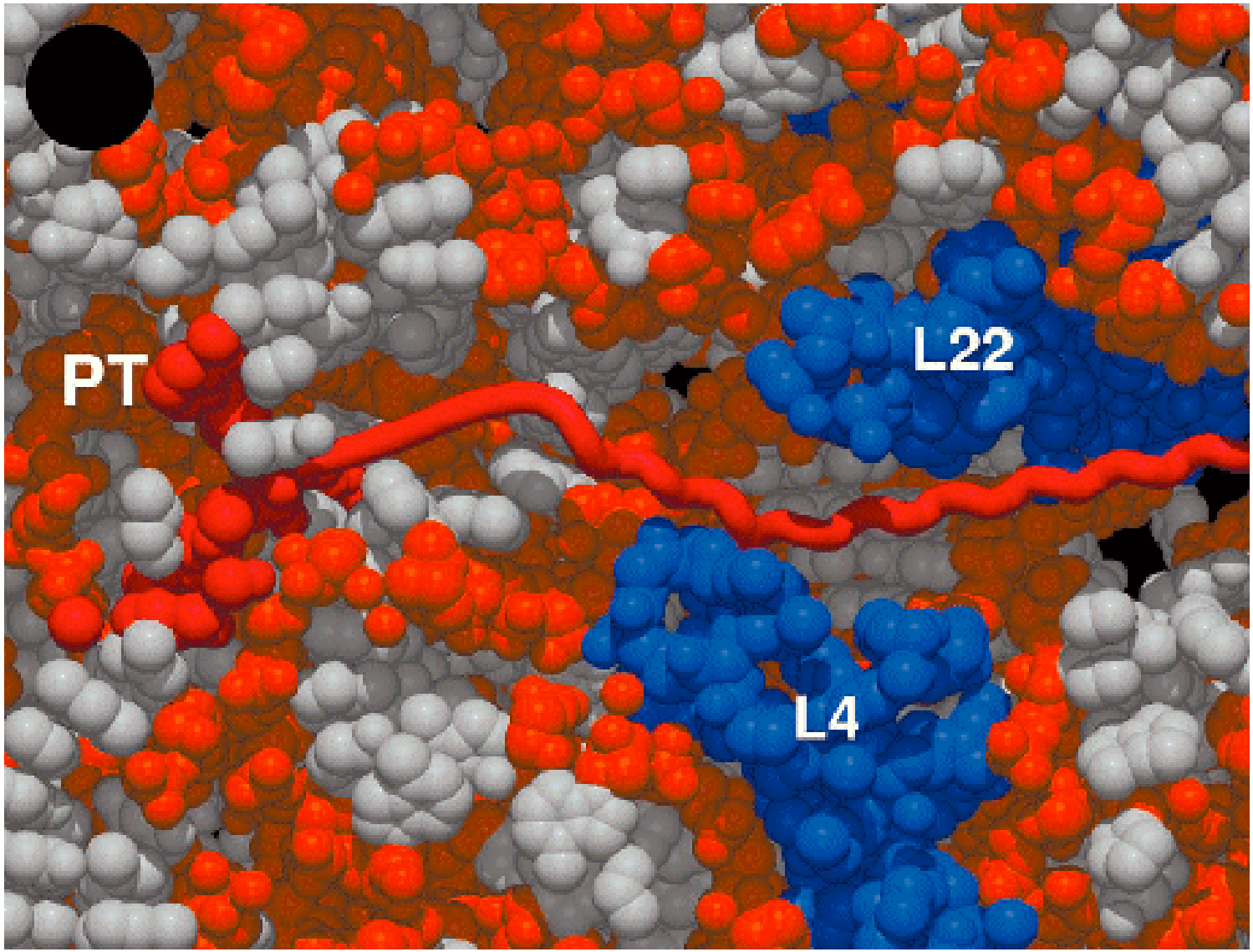
Nissen et al. (2000) Science,289, 920-930

Tunnel of nascent peptide



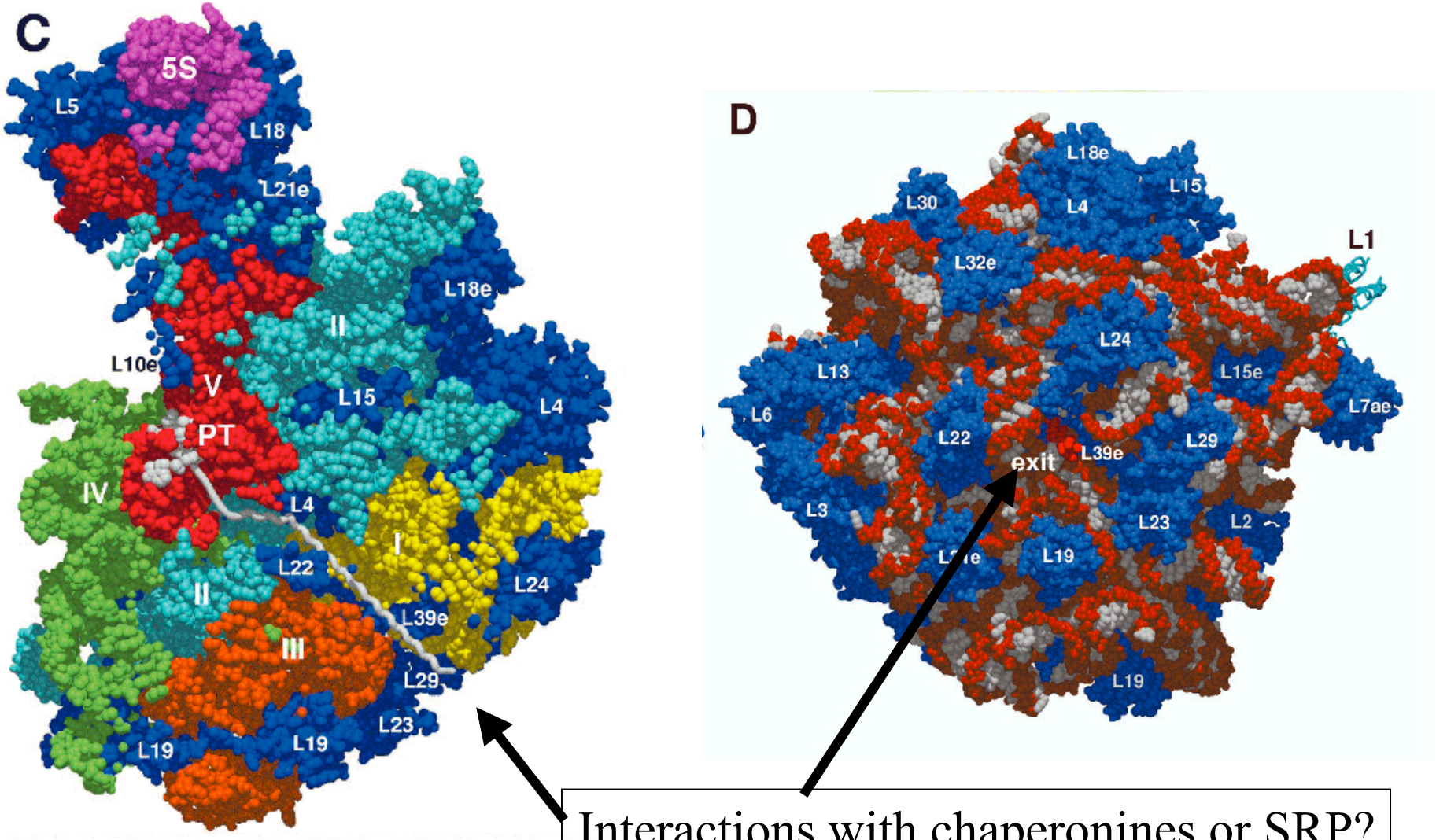
Nissen et al. (2000) Science,289, 920-930

Tunnel for nascent peptide



Nissen et al. (2000) Science,289, 920-930

Tunnel of nascent peptide

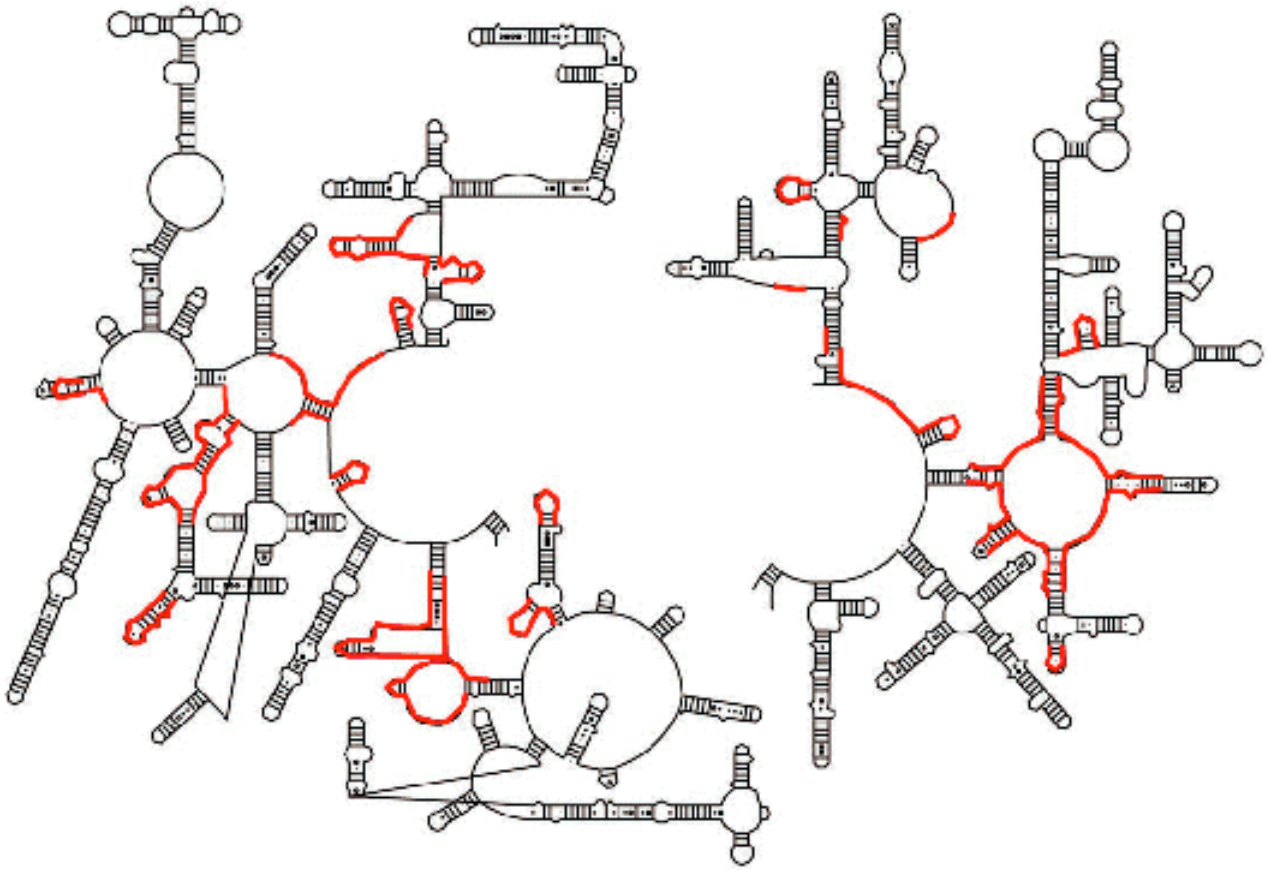


Interactions with chaperonines or SRP?

Nissen et al. (2000) Science,289, 920-930

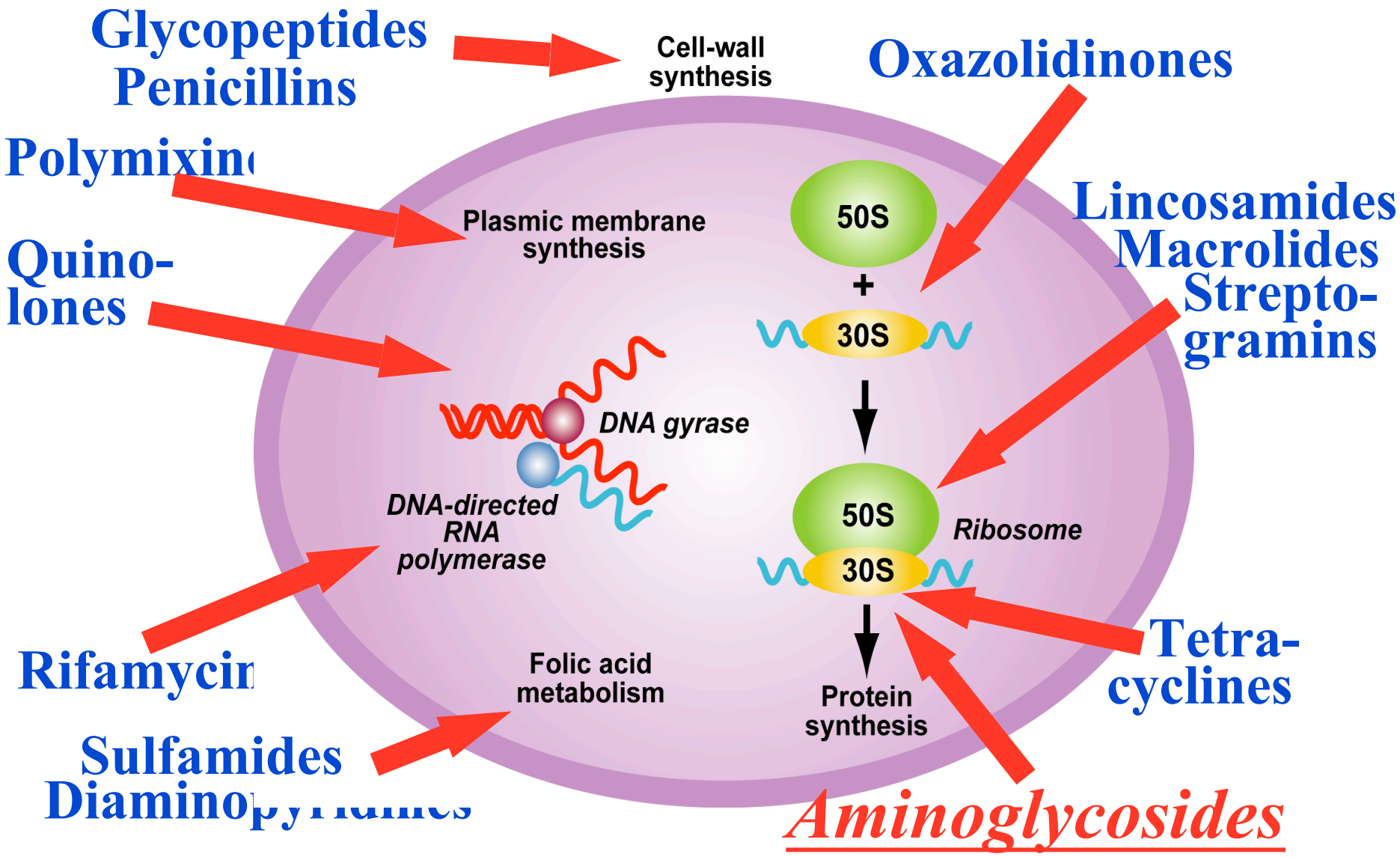
Tunnel for nascent peptide

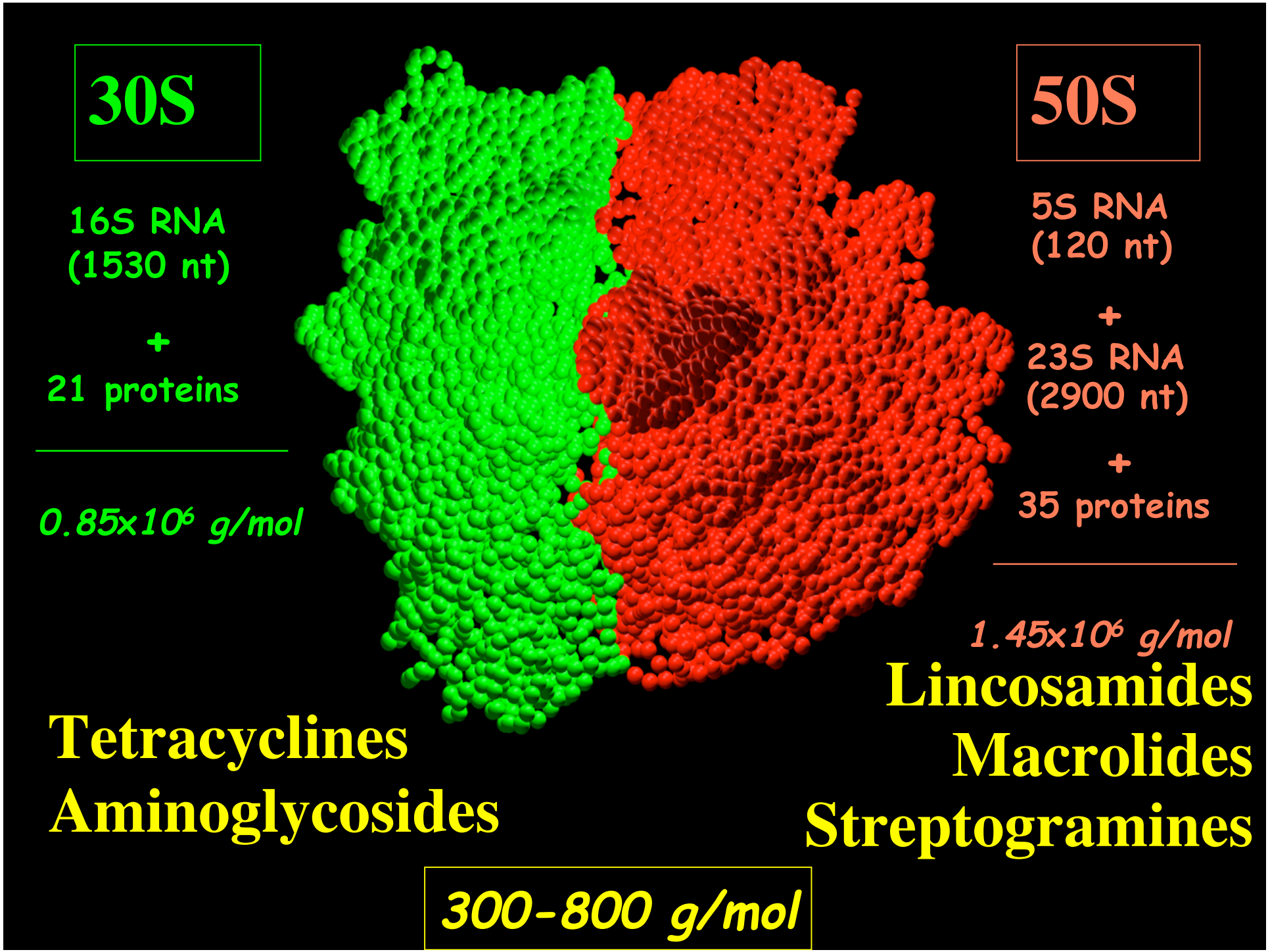
Rôle of 23S rRNA (in red)



Nissen et al. (2000) Science,289, 920-930

Antibiotic targets




30S
16S RNA
(1530 nt)
+
21 proteins
0.85x10⁶ g/mol
50S
5S RNA
(120 nt)
+
23S RNA
(2900 nt)
+
35 proteins
1.45x10⁶ g/mol
Tetracyclines
Aminoglycosides
Lincosamides
Macrolides
Streptogramines
300-800 g/mol

Where do they bind ?

Mainly at the rRNA

- At tRNA or mRNA binding sites
- In the exit tunnel (50S particle)
- At pivot points, key for structural rearrangements during mechanical steps of the ribosomes

How do they bind ?

- Mainly with Bases (Hygromycin)
- Only with Phosphates (Streptomycin)
- With bases & Phosphates (Aminoglycosides)

The ribosome is a ribozyme

and,

like all other known ribozymes,

the ribosome uses RNA-based recognition motifs

not only for catalysis

but also

for decoding processes.

The ribosome as a molecular machine

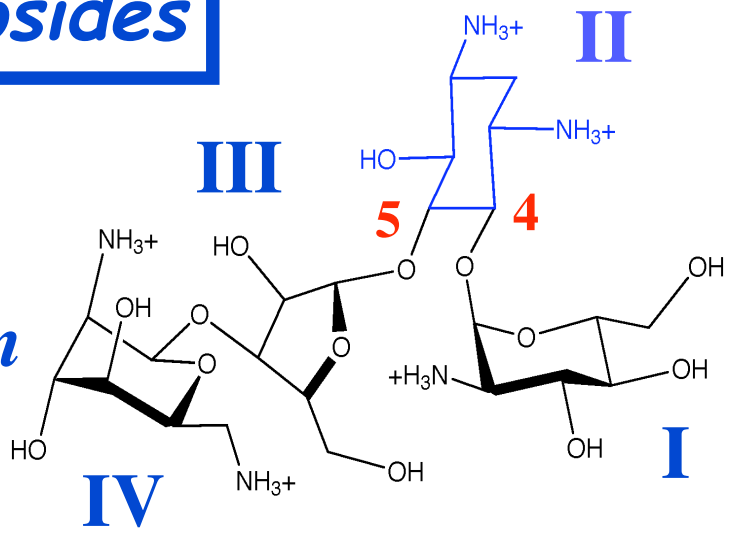
This requires the presence of

- Relative mouvements of parts
- Alternative conformations (2D/3D)
- Molecular switches
- ….

Some aminoglycosides

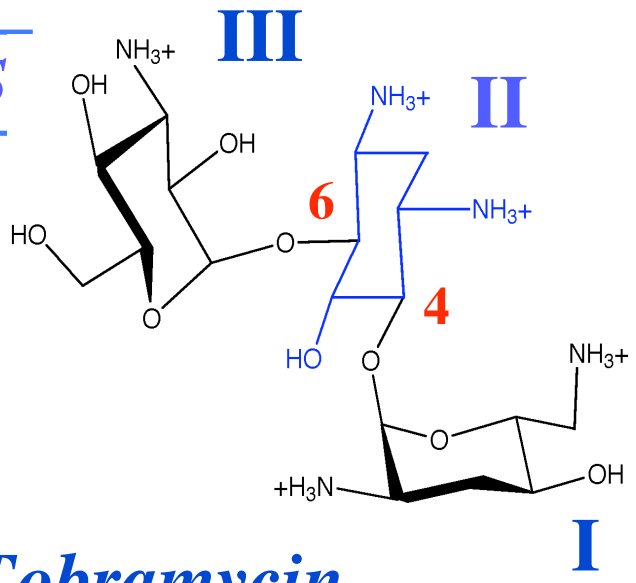
4,5 - DOS

Paromomycin

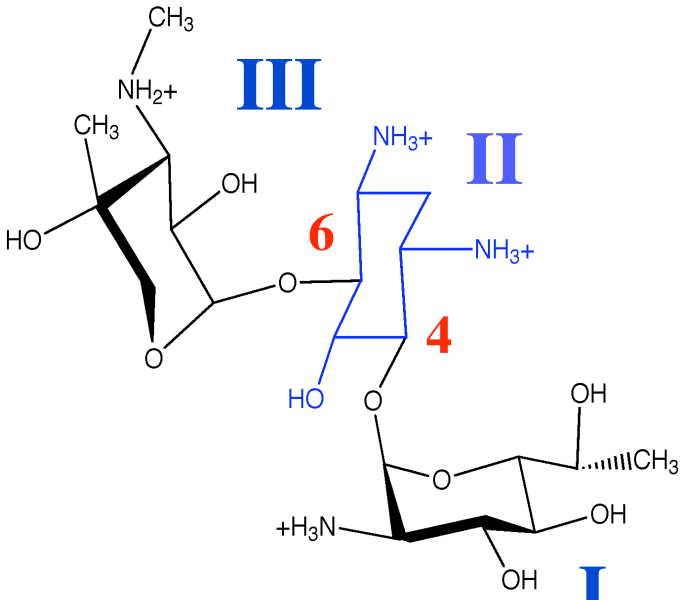


4,6 - DOS

Tobramycin

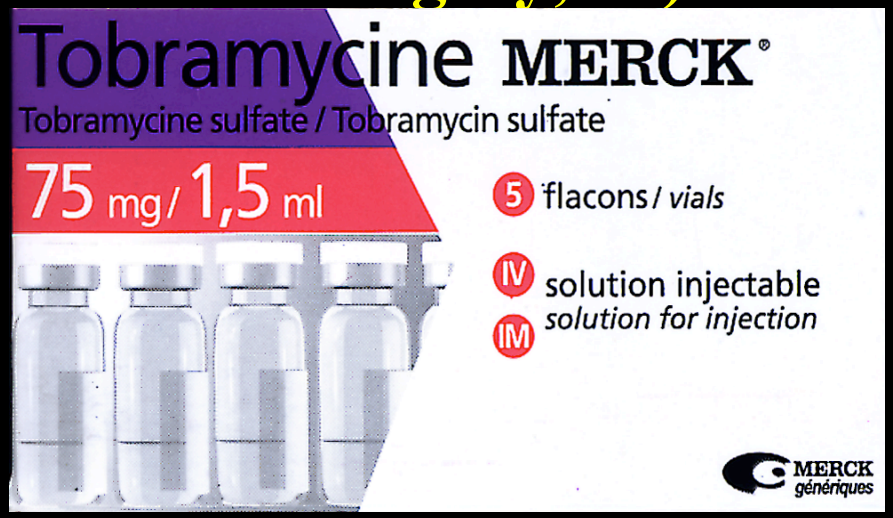


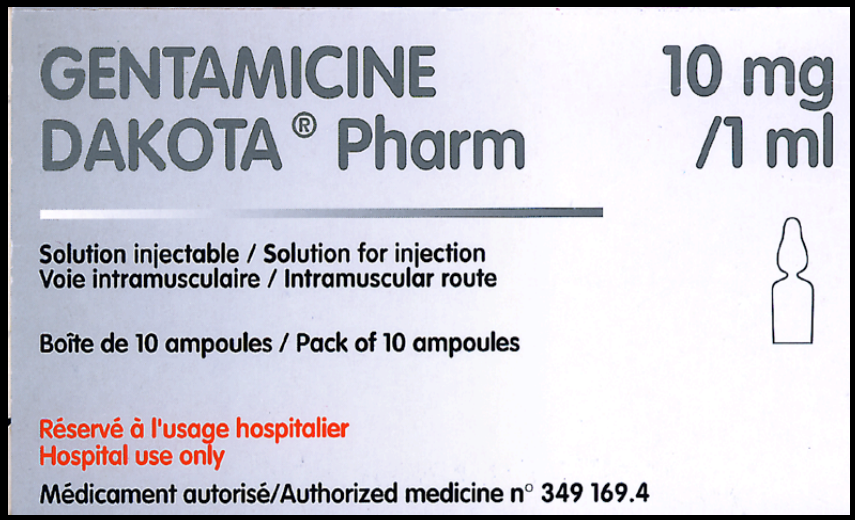
Geneticin (G418)



The aminoglycosides

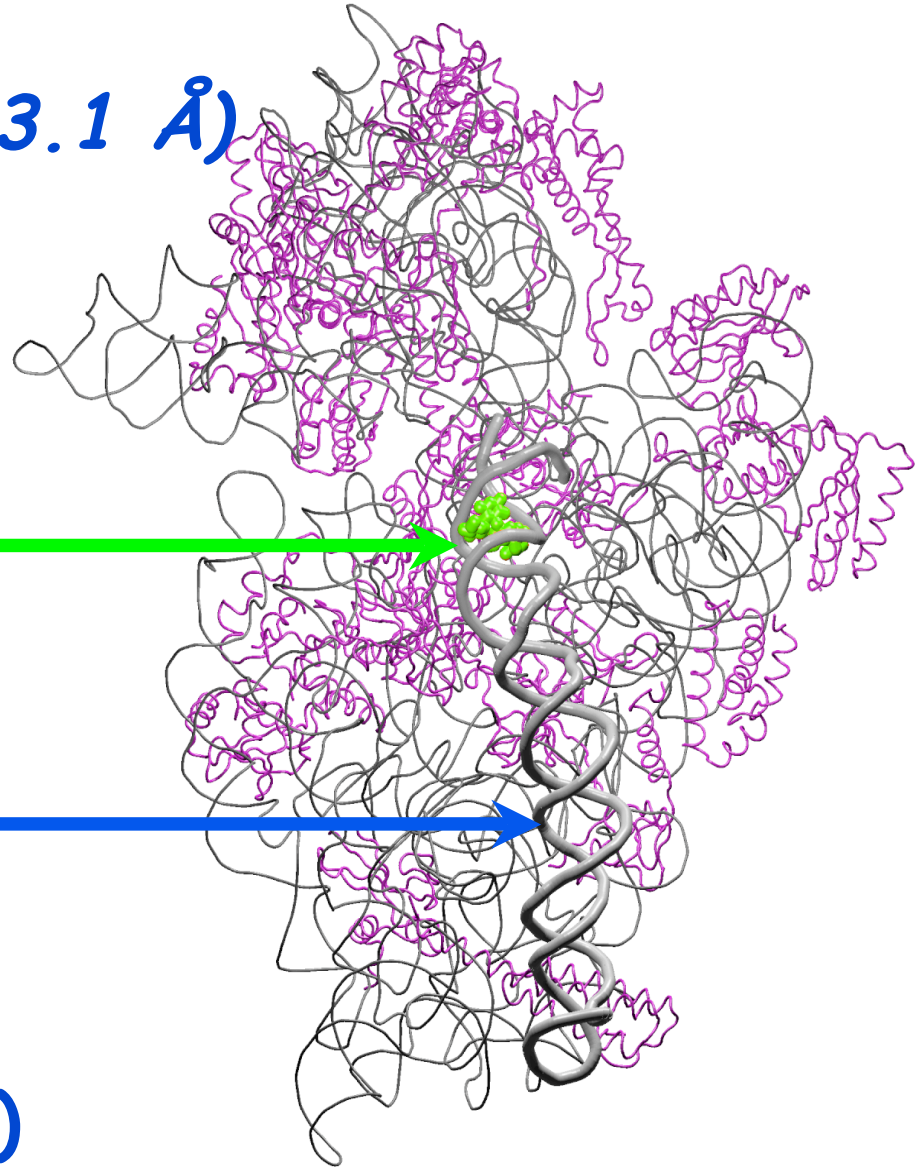
- **Antibiotics (*Actinomycetes)* discovered in the 1940's and used against:**
  - **— Tuberculosis**
  - **— Conjunctivitis**
  - **— Strong infections (intestinal, after surgery, …)**





Aminoglycoside target : the A site

30S particle *(X-ray: 3.1 Å)*

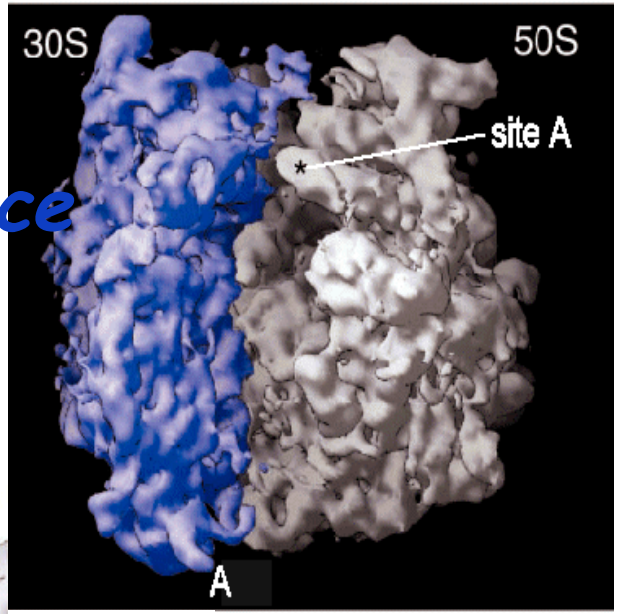


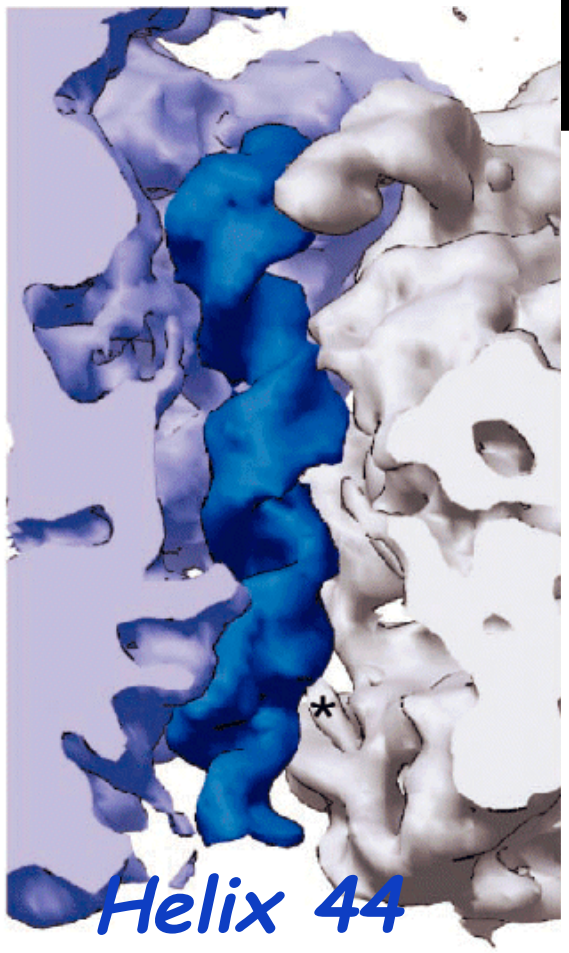
Aminoglycosides

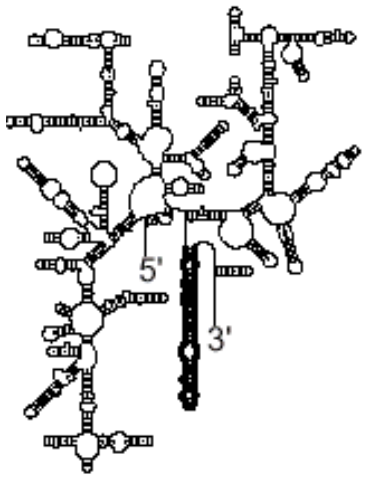
Helix 44

Carter *et al.*, *Nature* (2000)

No protein
At the interface
Between 30S
and 50S

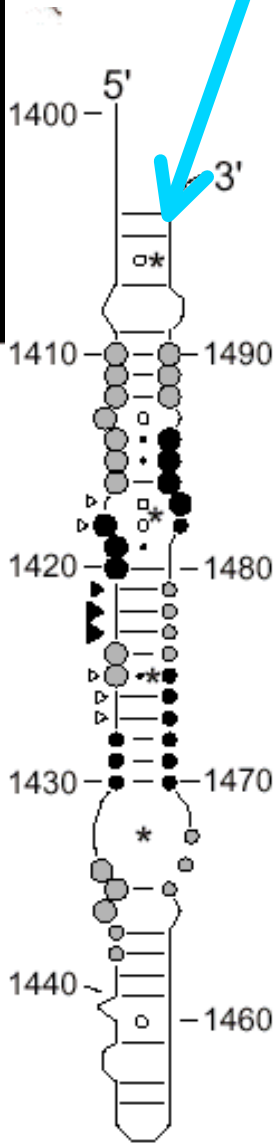


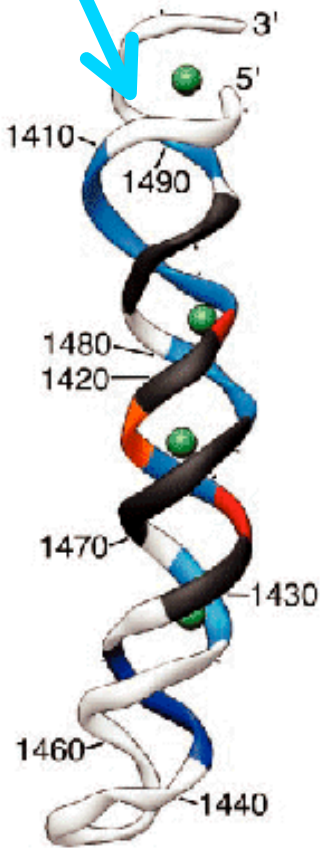


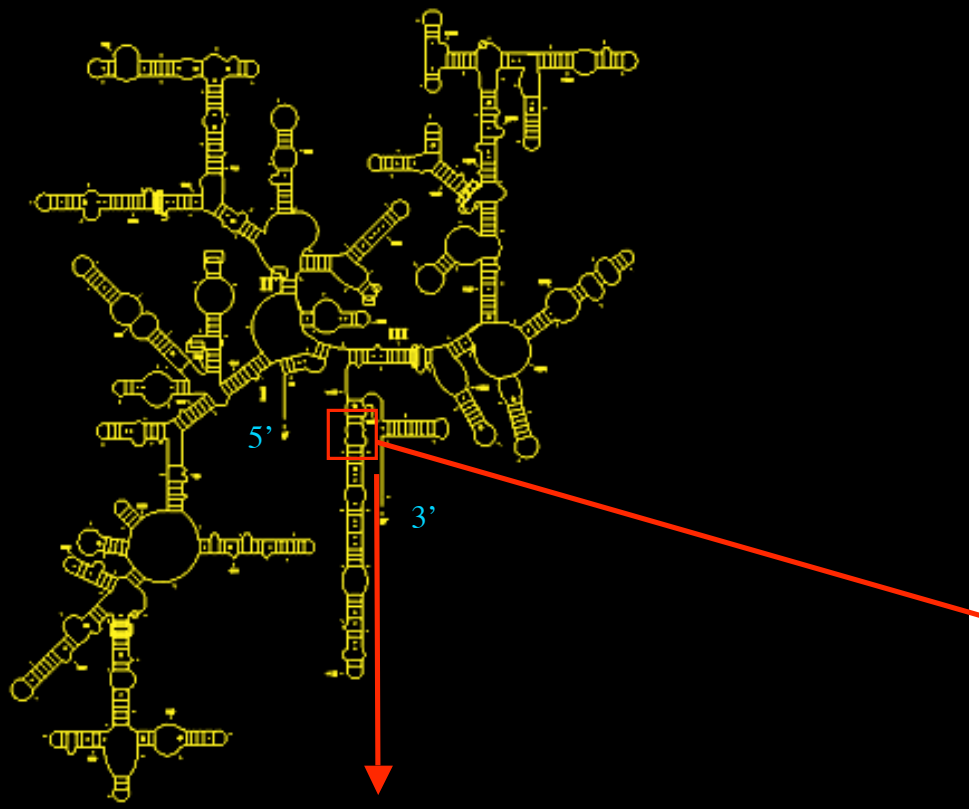


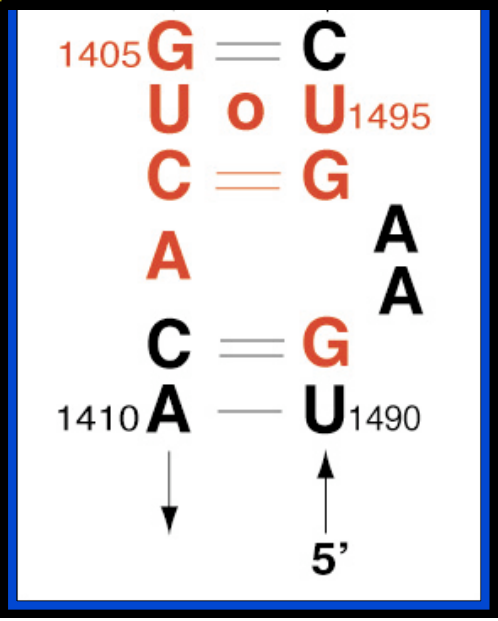
RNA based
recognition

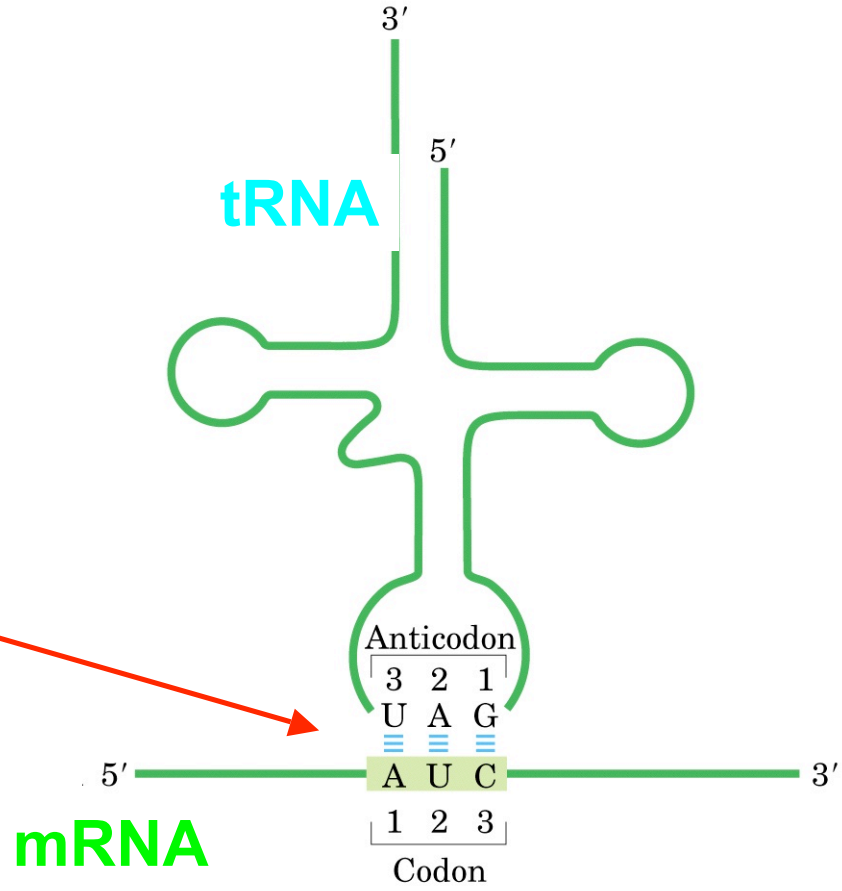
A site







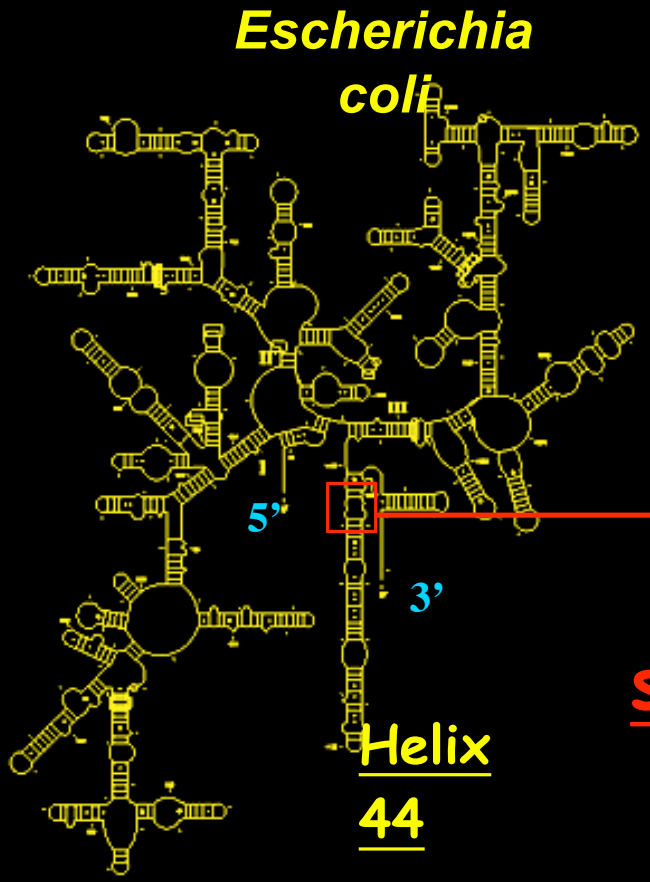


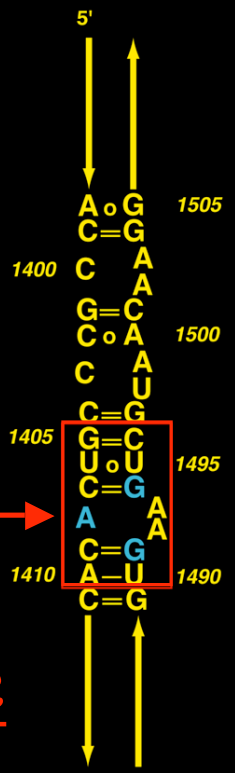


A site : recognition of the codon-Anticodon helix.
Central to the fidelity of the Decoding process of the ribosome

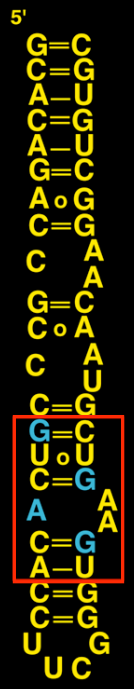
16S rRNA 2D structure

Protections induced by aminoglycoside binding

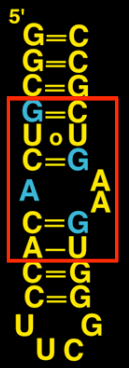




Moazed & Noller *Nature* (1987)



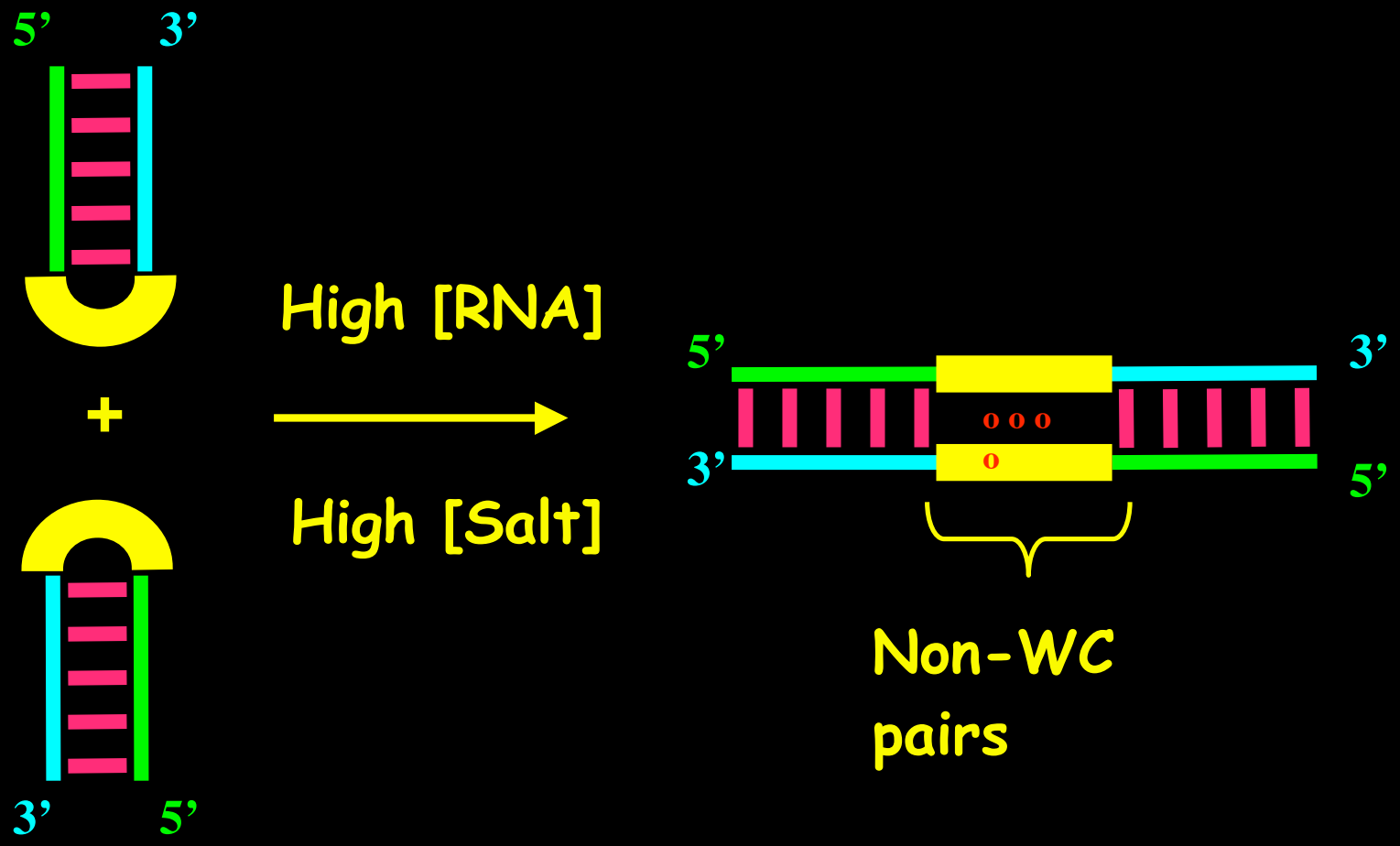
Purohit & Stern, *Nature* (1994)



Recht *et al., JMB* (1996)

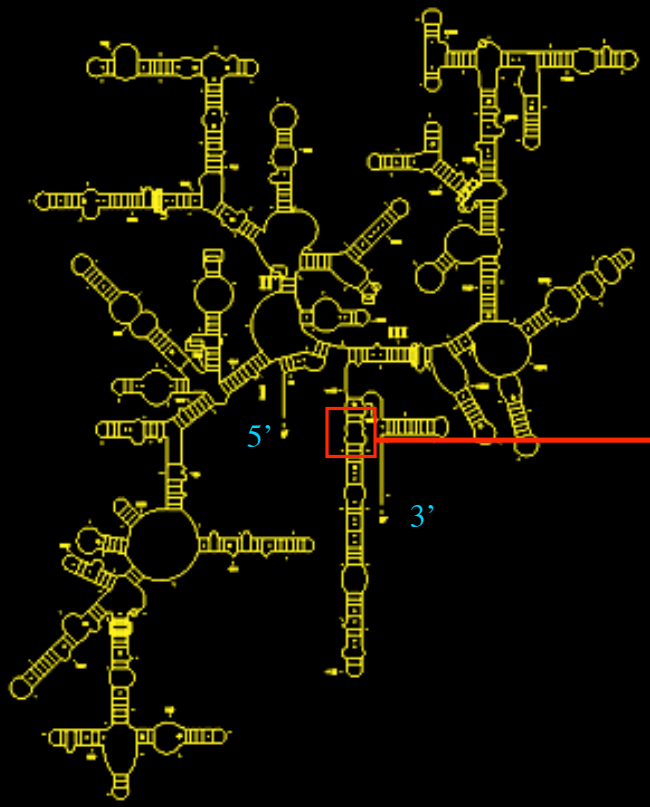
NMR Structures

Crystallization of stem-loops

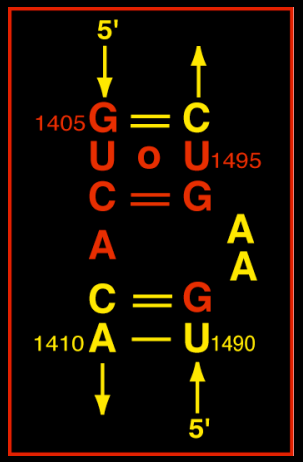


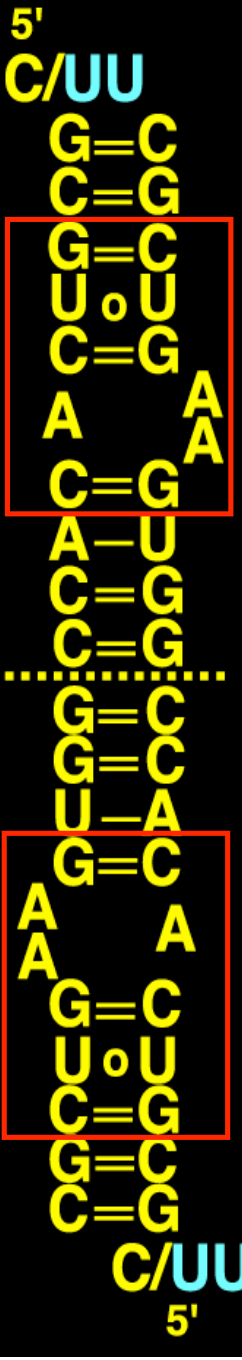
2D Structure of 16S rRNA

rRNA used for co-crystallization



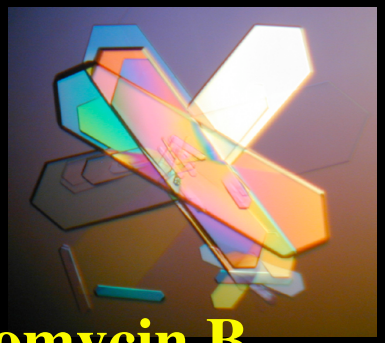
A Site



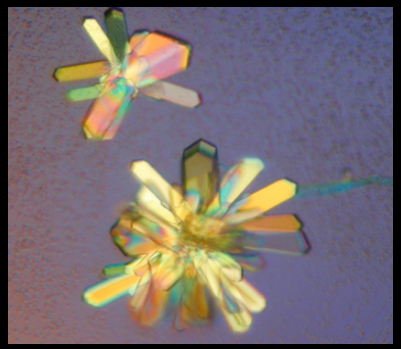


4,5

Paromomycin



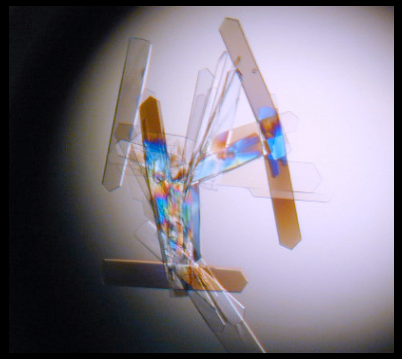
Neomycin B



Lividomycin B

Ribostamycin

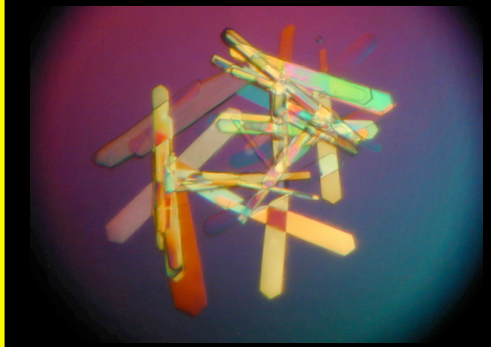
4,6



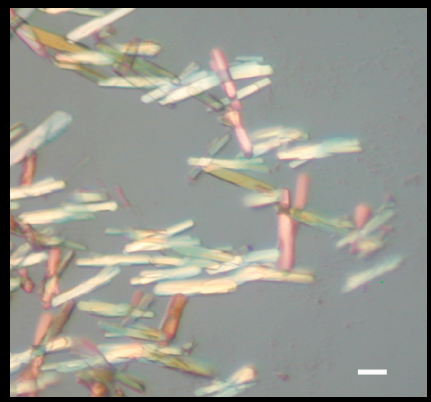
Tobramycin

Kanamycin

4,6



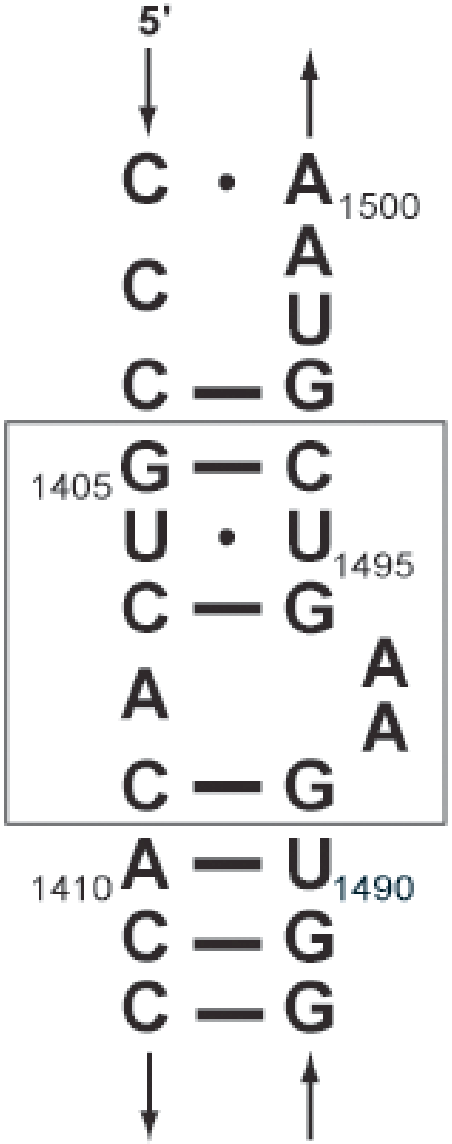
Geneticin



Gentamicin C1a

4, Neamine

High sequence conservation > conservation of contac



Conservation (%)

99.5

98.7

99.7

98.9

99.0

99.0

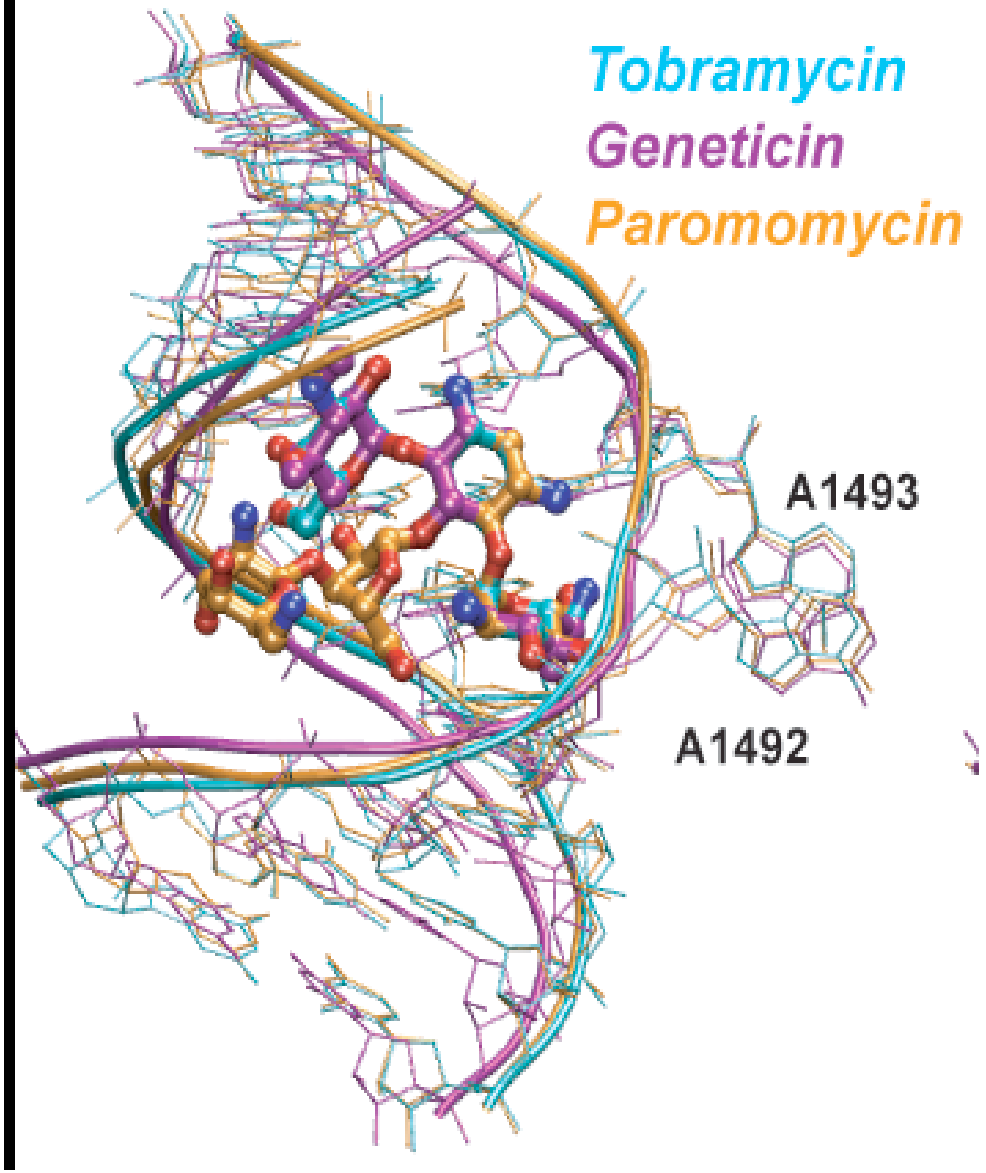
96.5

83.7 (A-U: 13.6)

66.5 (G=C: 26.4)

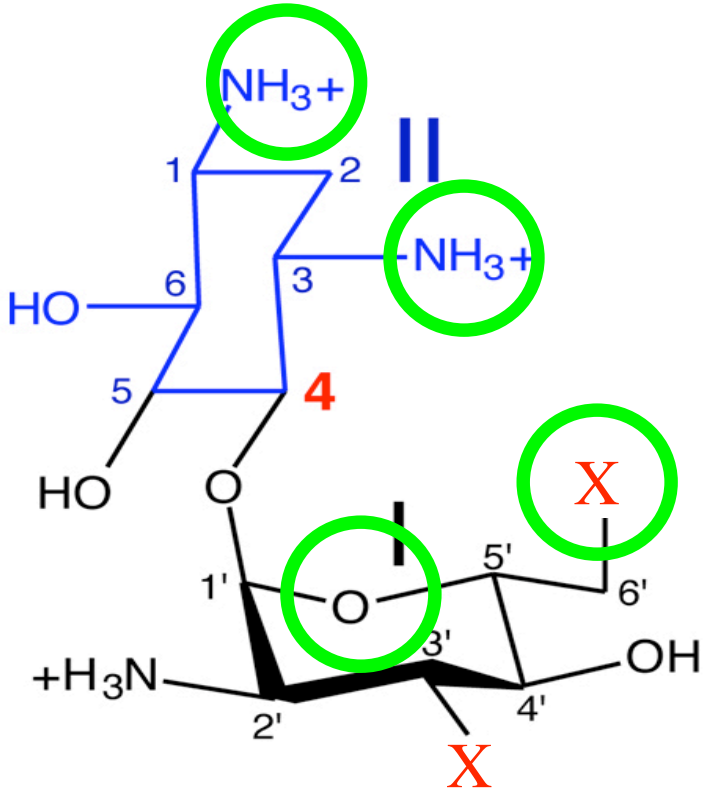
79.4 (U-A: 17.8)

96.5 (U-A: 2.7)



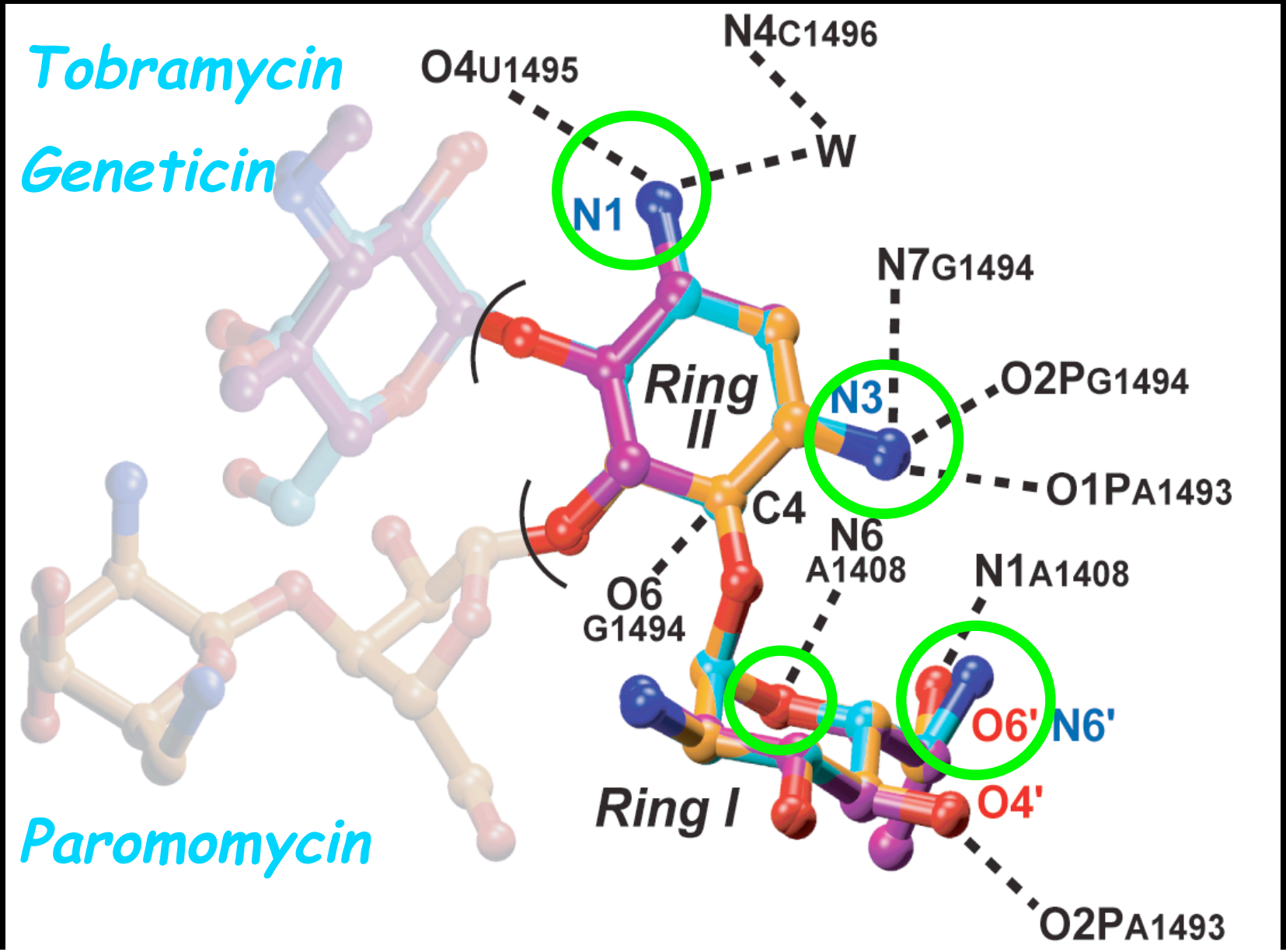
Aminoglycoside antibiotics

(2-DOS) ring substituted at position 4



Neamine ring

Conserved interactions: the neamine ring

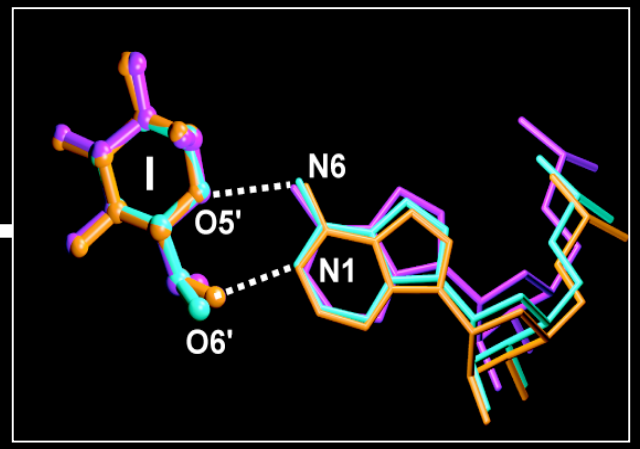


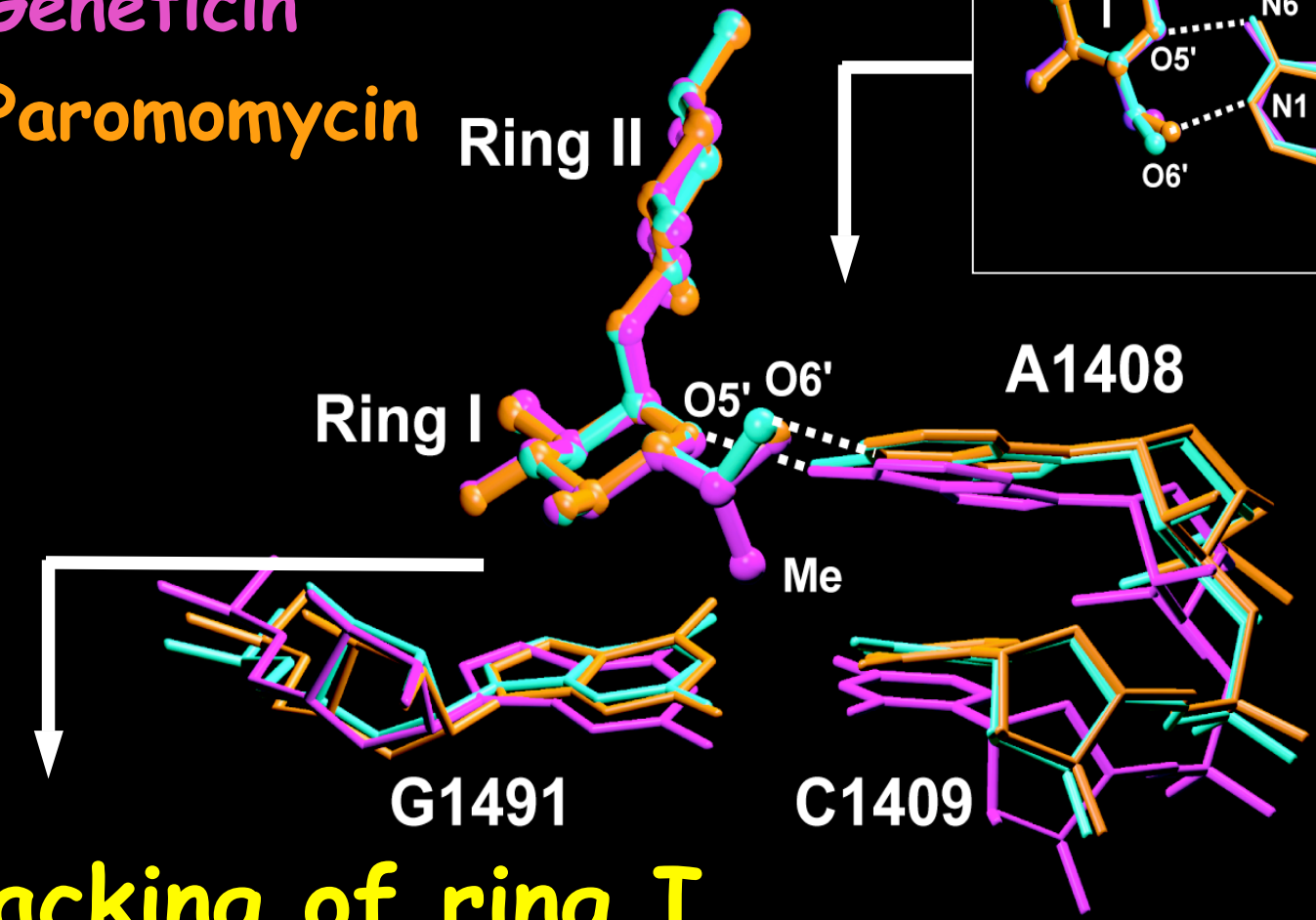
Pseudo-base pair formation

Tobramycin

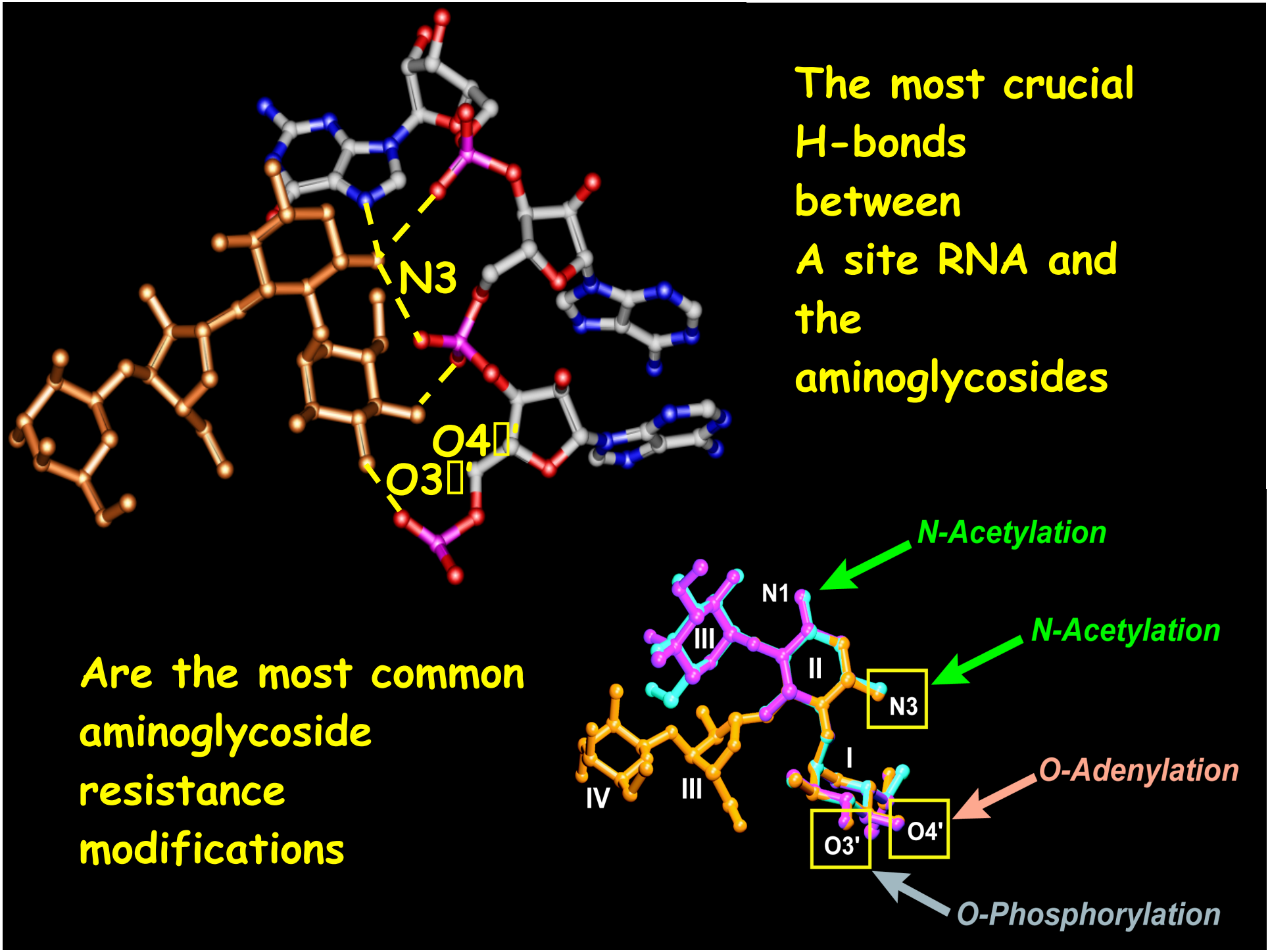
Geneticin

Paromomycin



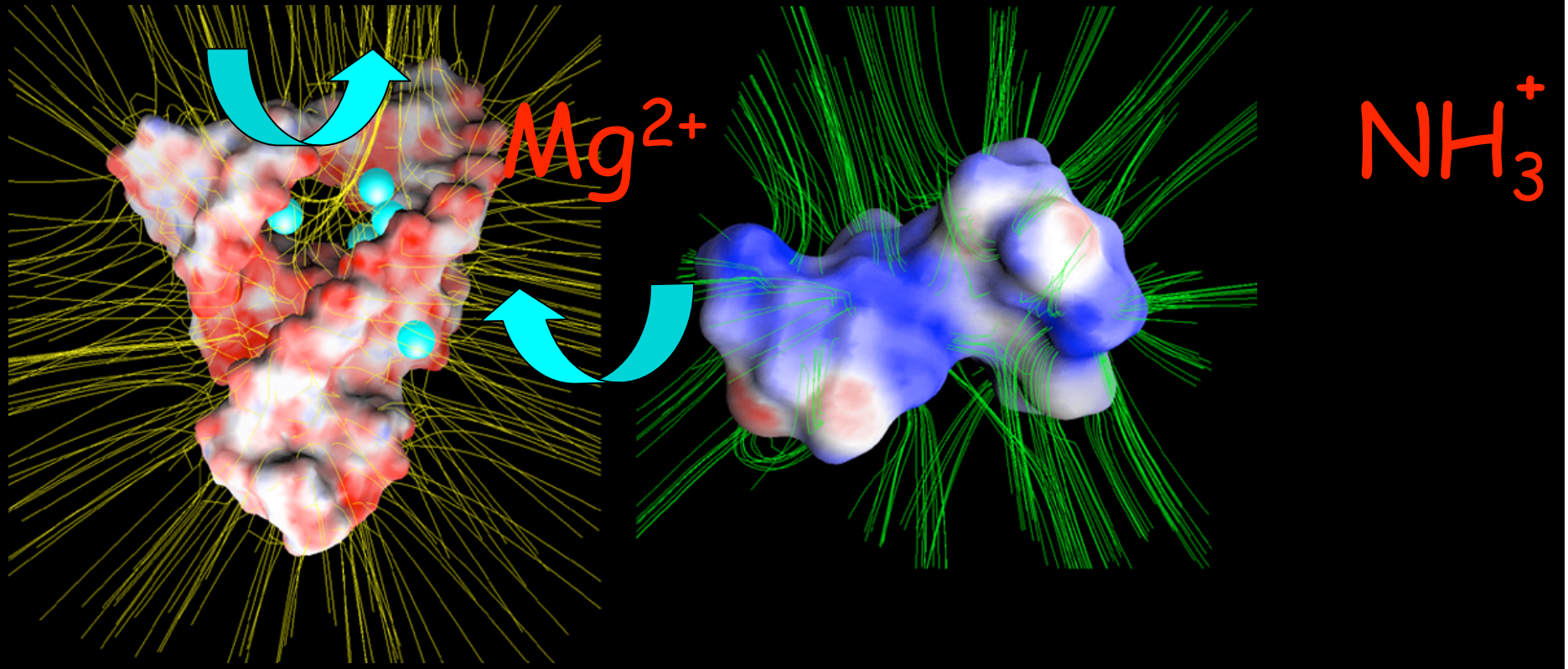


Stacking of ring I

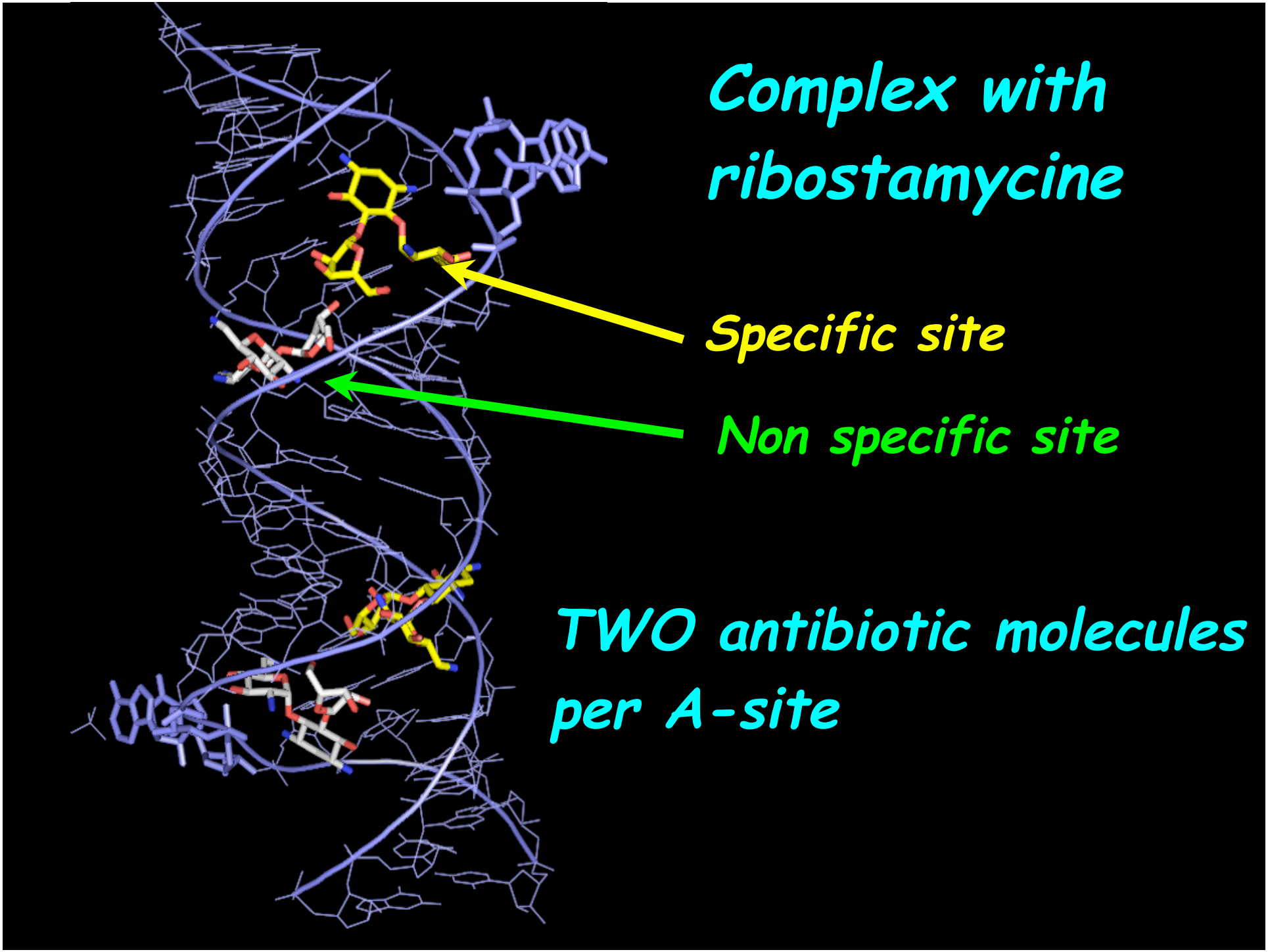

N3
O4'
O3'
The most crucial H-bonds between A site RNA and the aminoglycosides
Are the most common aminoglycoside resistance modifications
N-Acetylation
N1
III
II
N-Acetylation
N3
I
O-Adenylation
IV
III
O3'
O4'
O-Phosphorylation

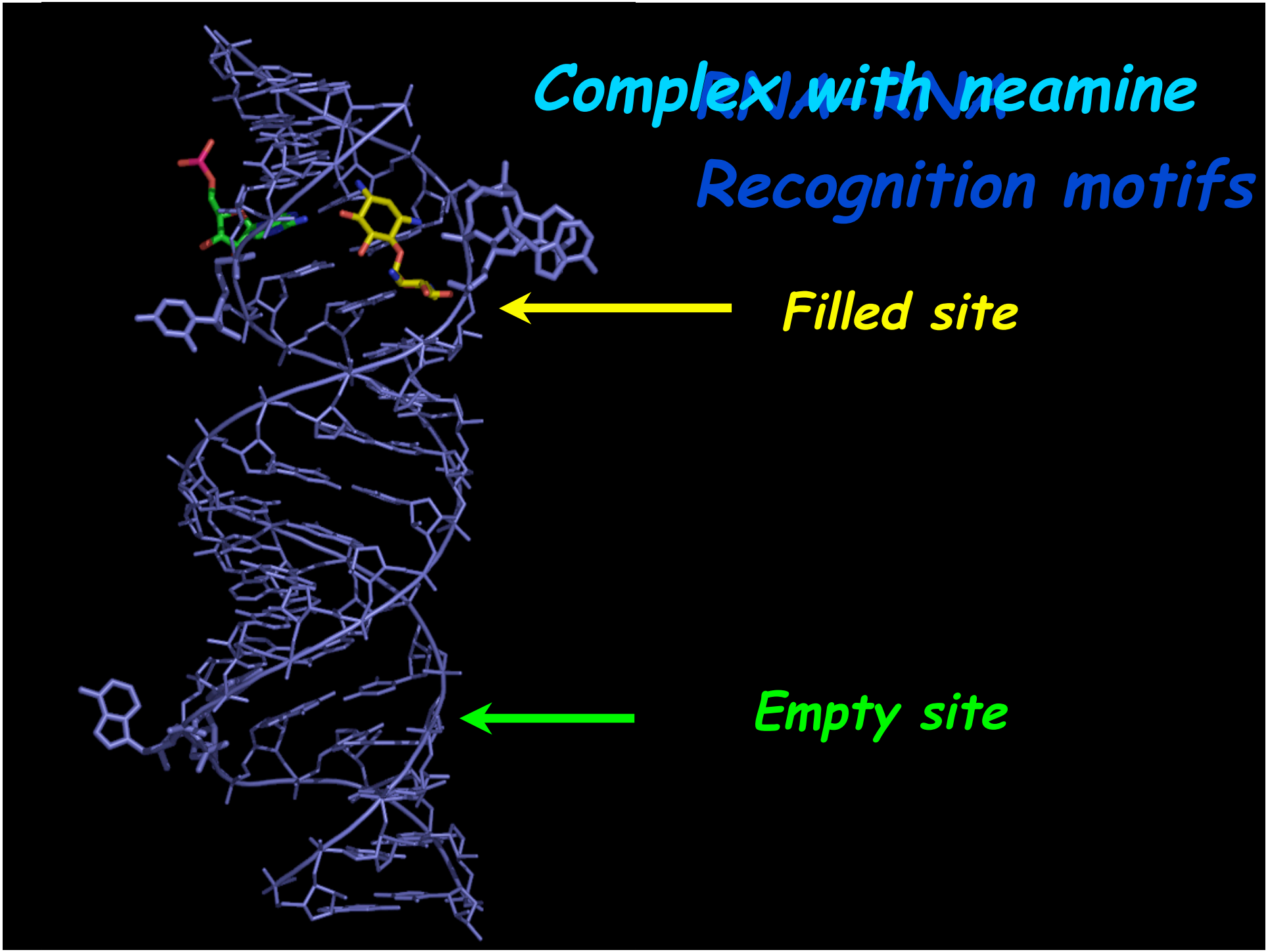
Origins of the binding force ?

Displacements of ions, waters… ?

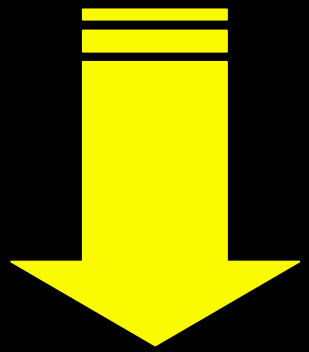


If the main driving force for binding is electrostatics what about specificity of binding ?


Complex with ribostamycine
Specific site
Non specific site
TWO antibiotic molecules per A-site


Complex with neamine
RNA-RNA
Recognition motifs
Filled site
Empty site

Differential occupations of the sites



The A site is in a dynamic Equilibrium

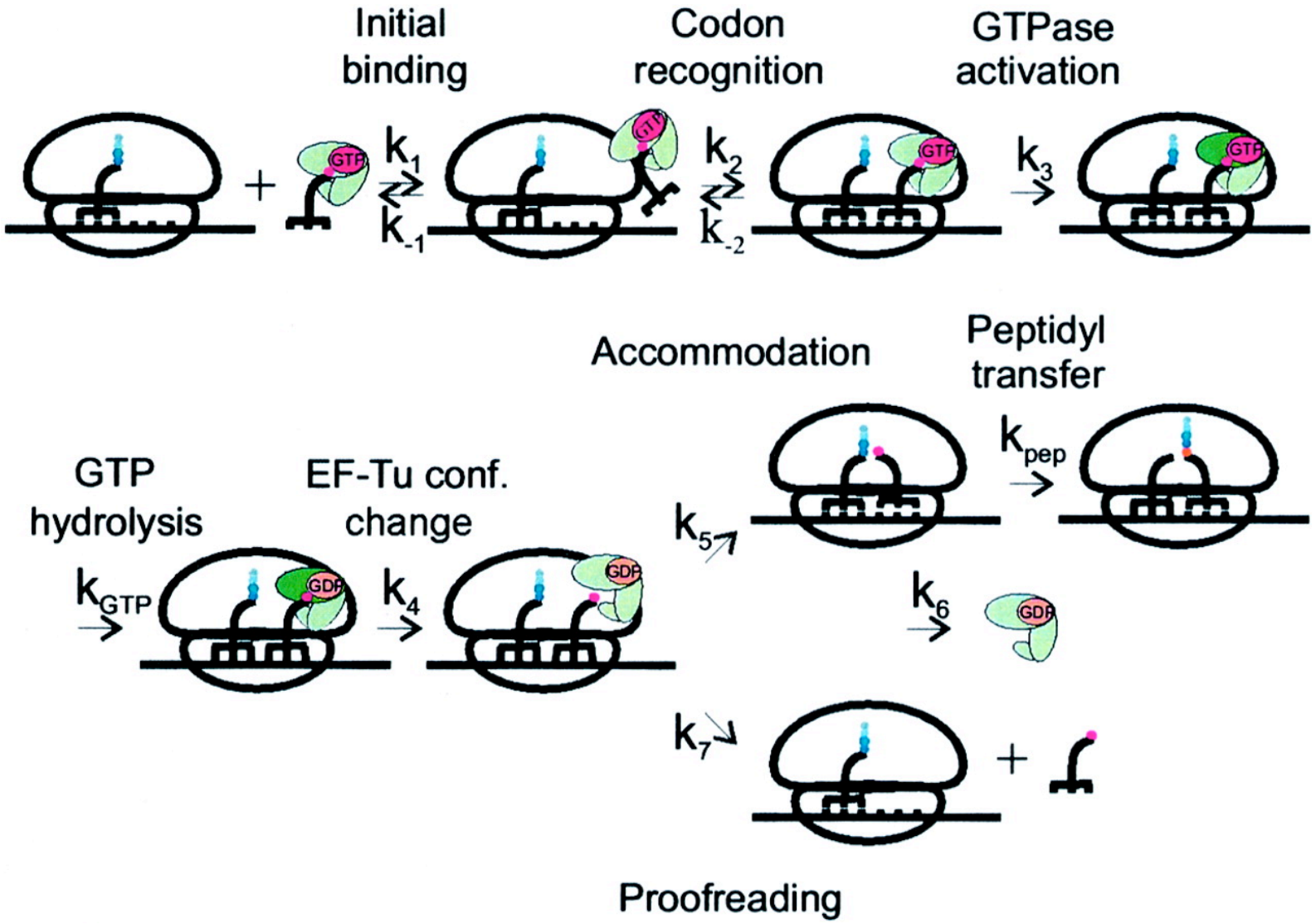
How to reconcile these results with biological efficiency ?

Fidelity of decoding

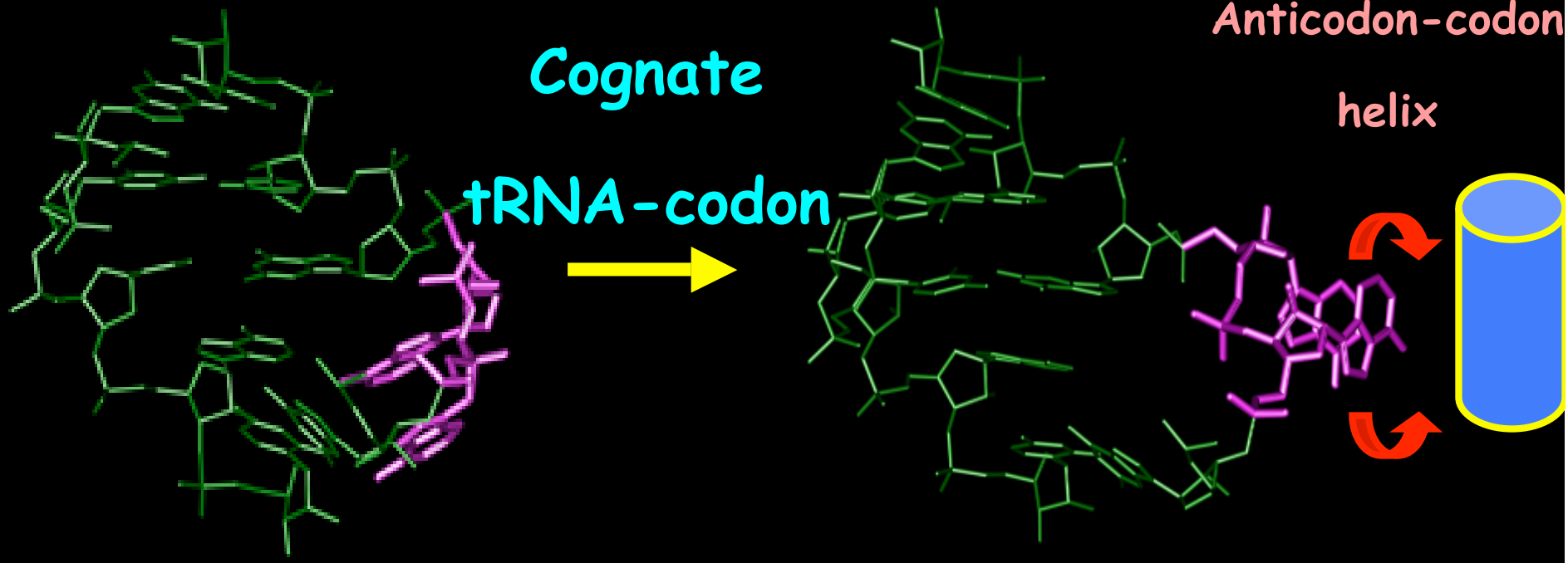
Decoding = Discrimination between cognate and near- or non-cognate aa-tRNA

- Decoding performed at the A site
- Selection depending on the base complementarity between codon (mRNA) and anticodon (aa-tRNA)
- Conformational change of the A site induced by cognate codon-anticodon duplex formation

H. Noller, V. Ramakrishnan


Initial binding
Codon recognition
GTPase activation
GTP
k_1
k_{-1}
k_2
k_{-2}
k_3
Accommodation
Peptidyl transfer
k_{pep}
k_5
GTP hydrolysis
EF-Tu conf. change
k_{GTP}
GDP
k_4
k_6
k_7
Proofreading

The A-site is a Molecular switch



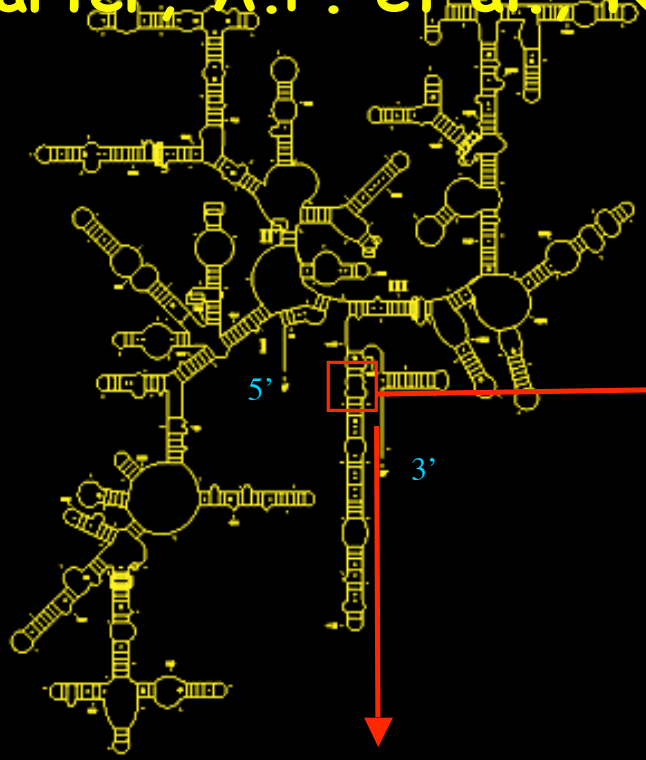
CLOSED FORM

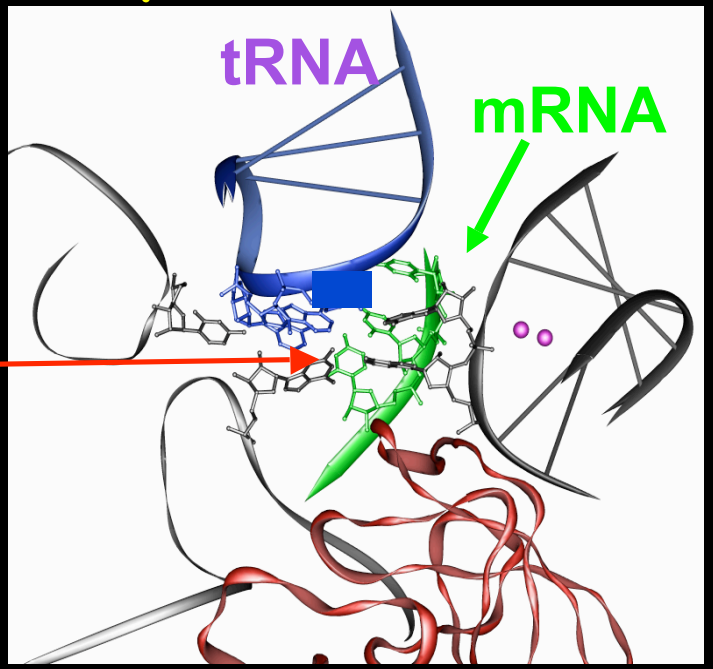
OFF STATE

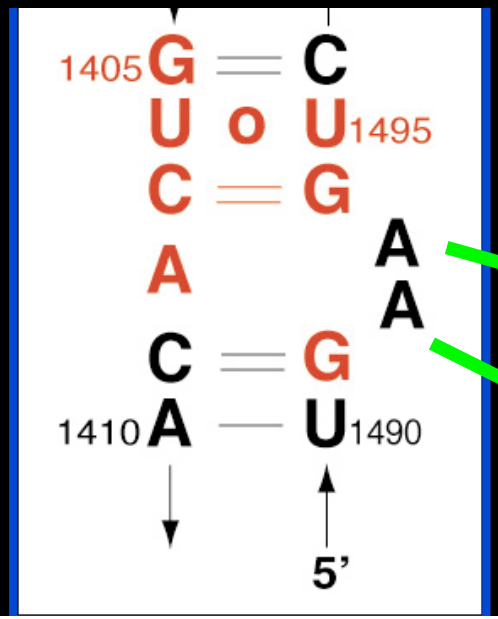
OPEN FORM (Bulging Adenines)

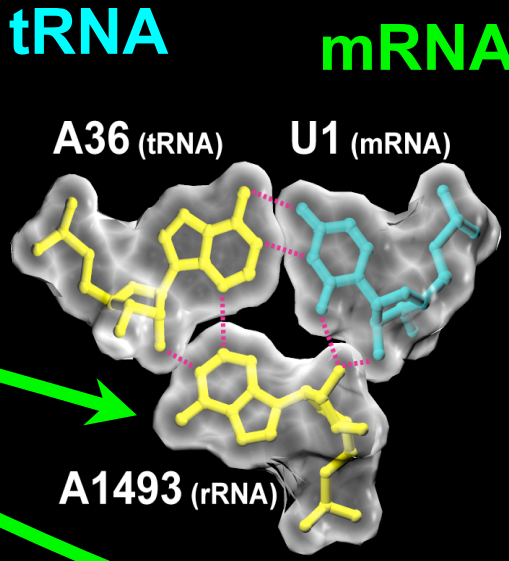
ON STATE

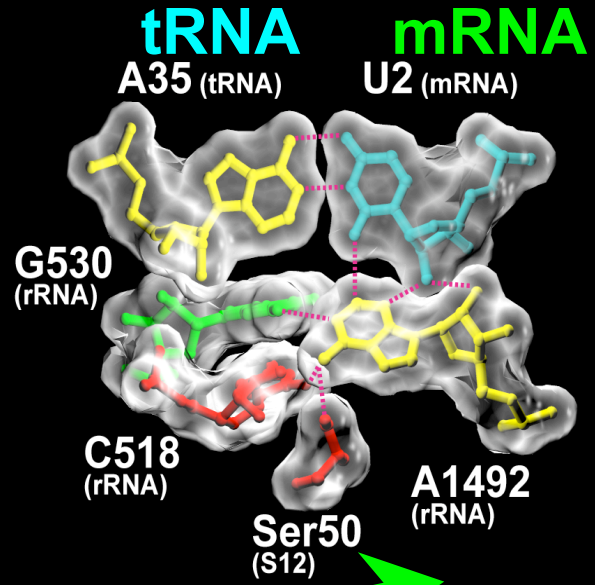
Carter, A.P. et al. Nature (2000), 407, 340.







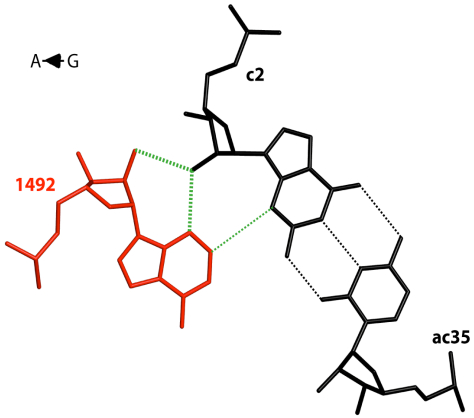


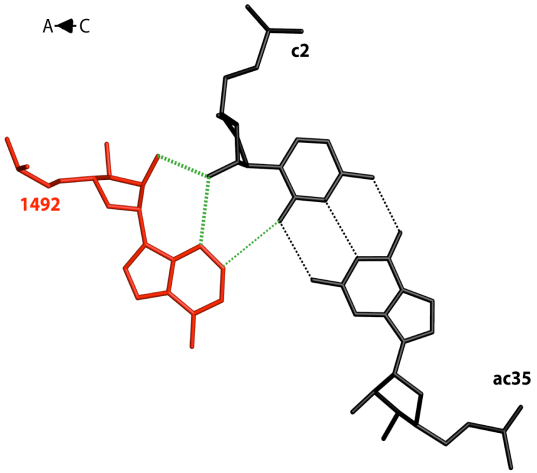


A1492
2nd codon base pair

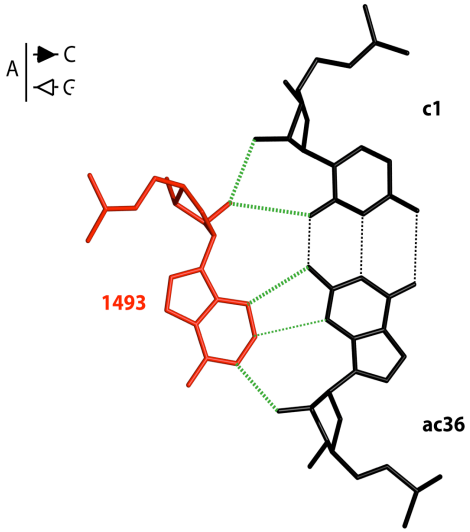
A1493
1st codon base pair

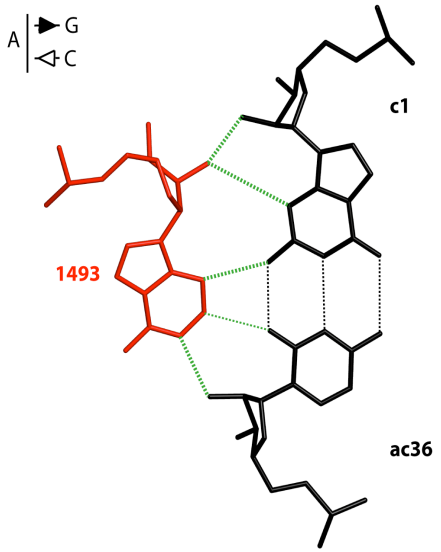
Type II : cis Sugar-edge/Sugar-edge



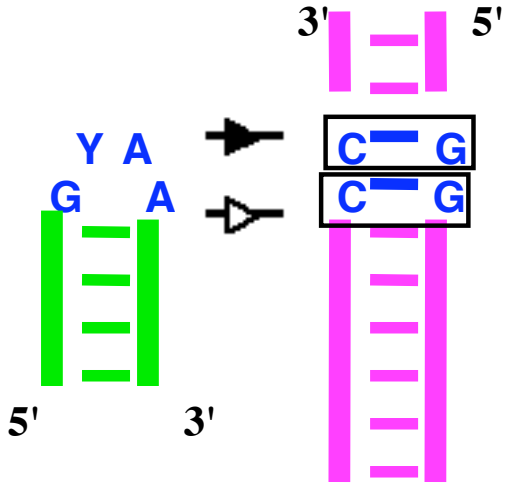


Type I : trans Sugar-edge/Sugar-edge

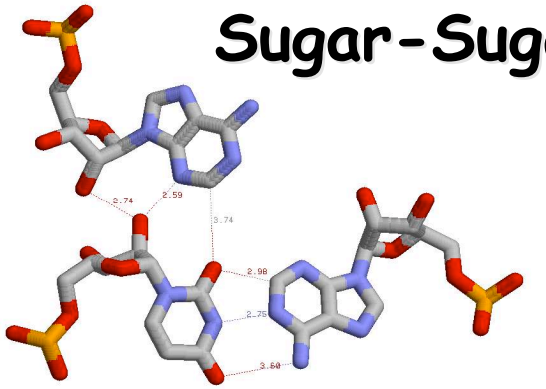




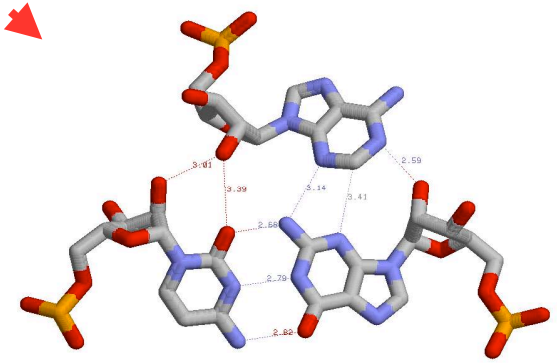
The most frequent RNA-RNA Recognition motifs

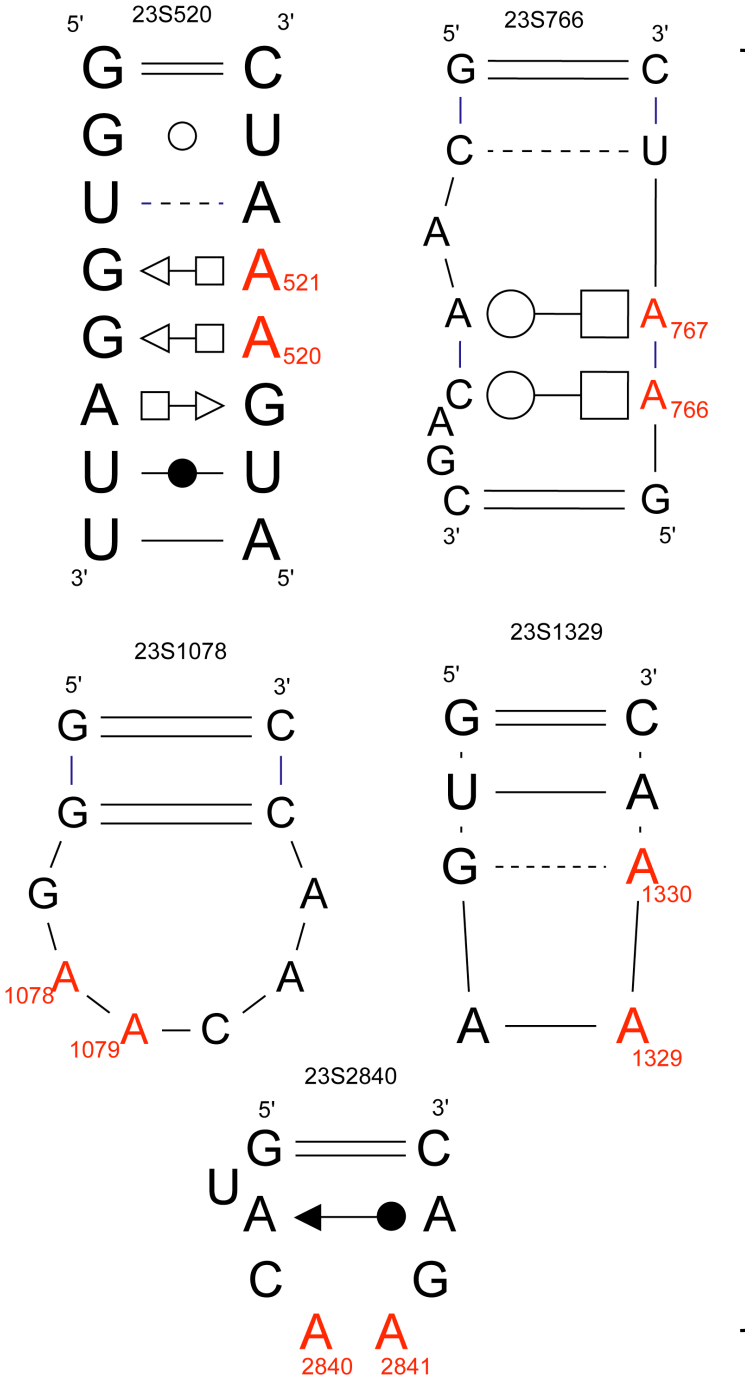


Type II
Sugar-Sugar Cis

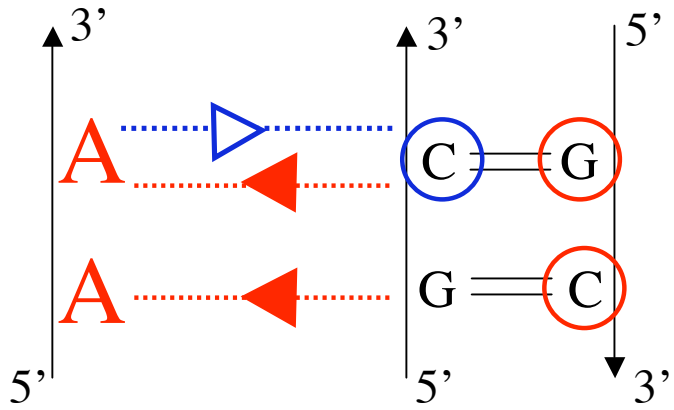


Type I
Sugar-Sugar Trans





Always the same base pairs with two adenines presented by various motifs



Origins of the aminoglycoside-induced miscoding

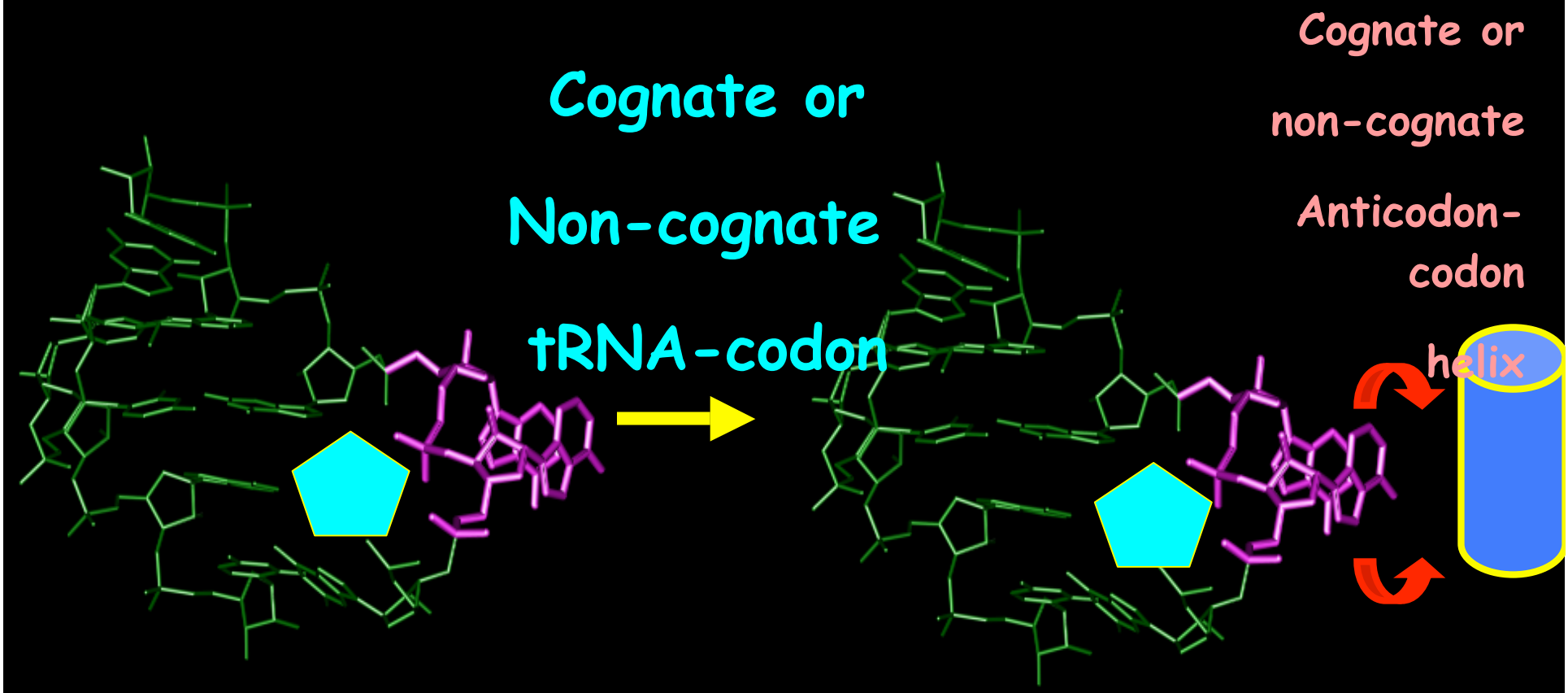
Loss of control at the decoding site

by mimicking correct cognate codon-anticodon interactions

Pape *et al.*, *NSB* (2000); Rodnina *et al.*, *Biochimie* (2002)

Ogle *et al.*, *Science* (2001)

The A-site Molecular switch is always 'on' in presence of aminoglycosides



OPEN FORM (Bulging Adenines)

ON STATE

OPEN FORM (Bulging Adenines)

ON STATE

RNA as a molecular switch

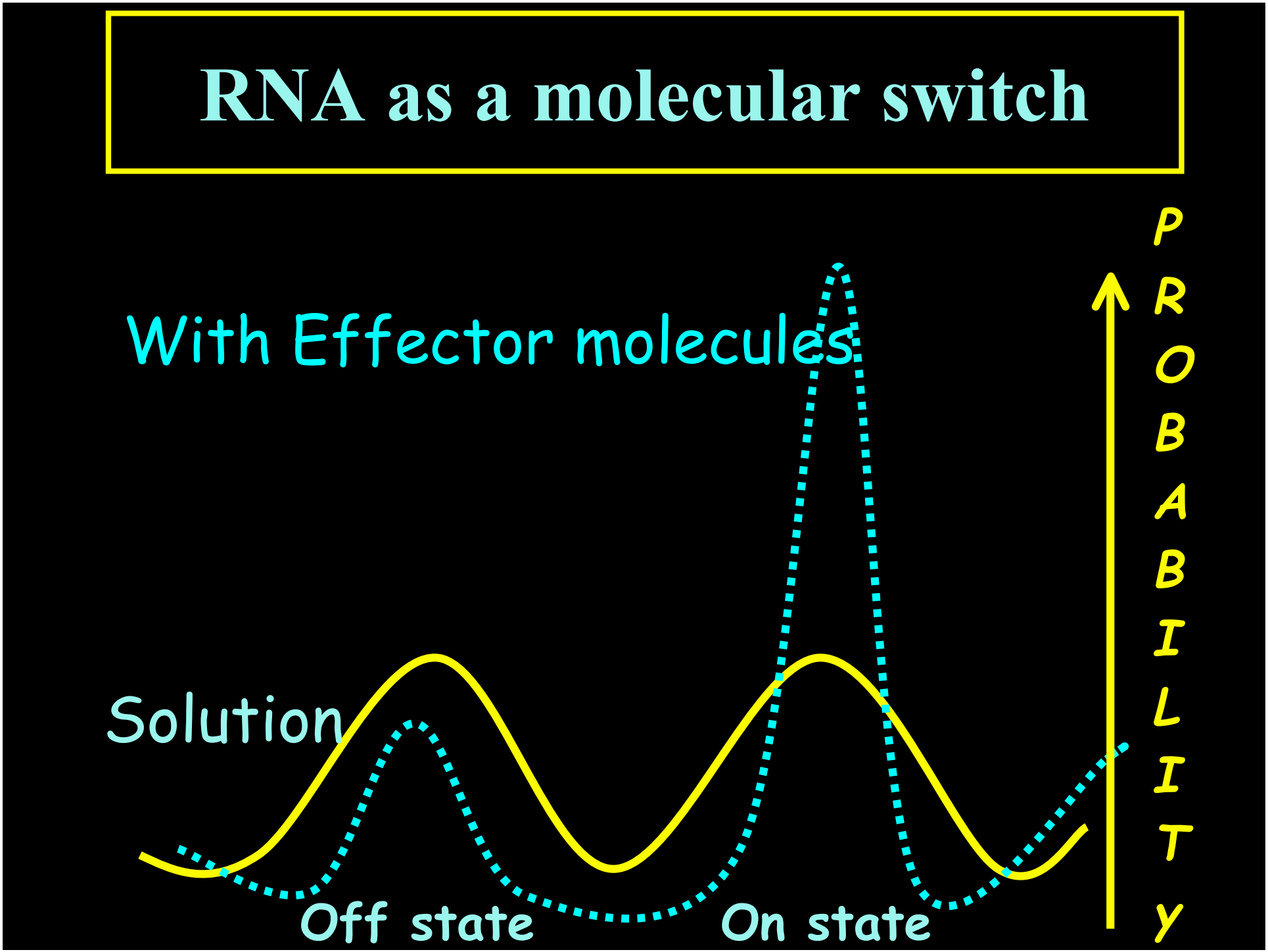
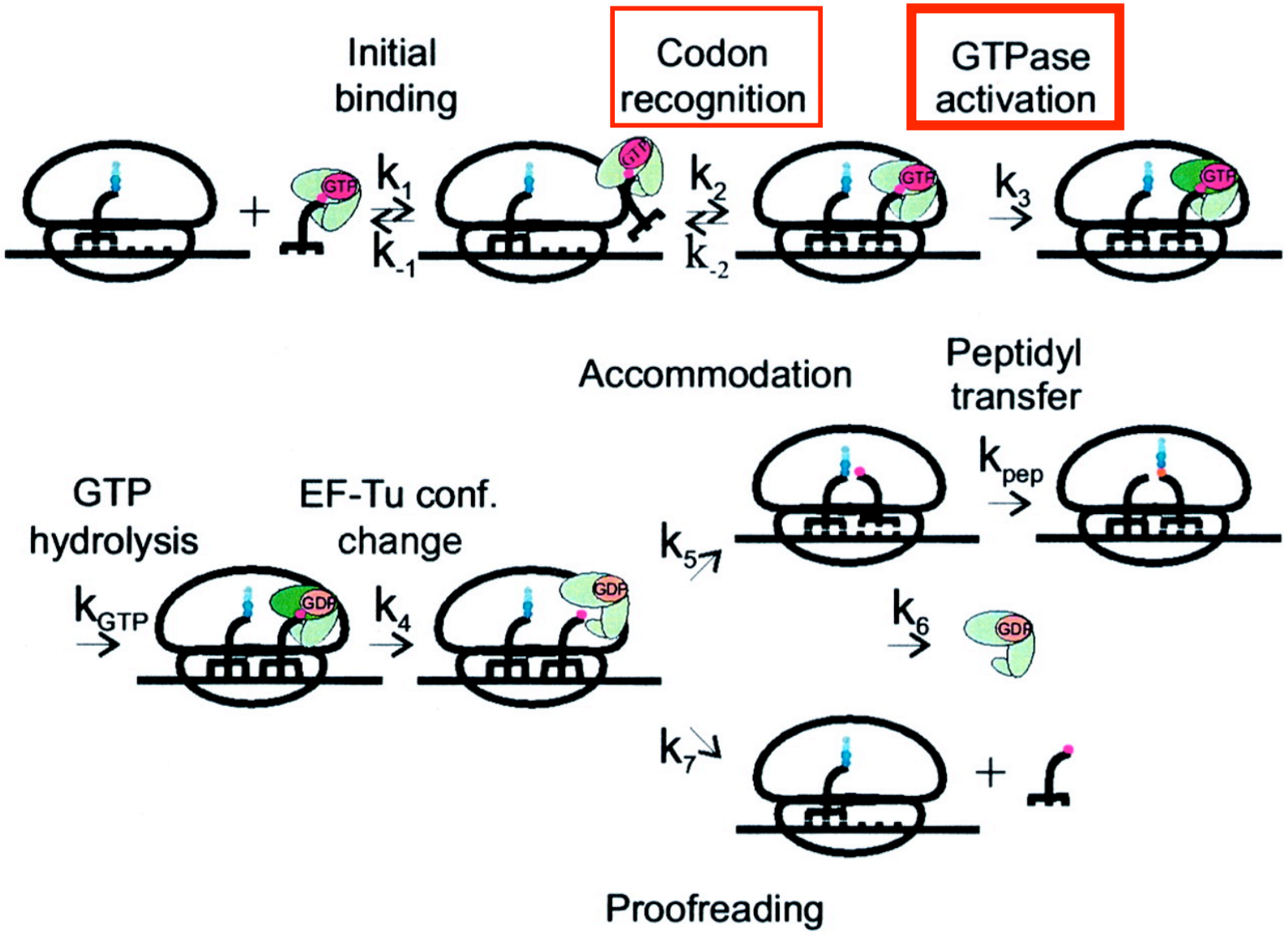


Table. Effects of paromomycin on the elemental rate constants of near-cognate (Leu) (Phe)[a] aa-tRNA binding to the A site.

Step	Rate constant (per s)	Near-cognate	Near-cognate + Paromomycin
Initial binding	k_1	110 ± 10[b]	140 [b]
	k_{-1}	25 ± 5	25
Codon recognition			
	k_2	100 ± 20	37
	k_{-2}	17 ± 8[d]	3.5
GTPase activation and GTP hydrolysis (e)			
	k_3	50 ± 20	>500
GTP-GDP conformation change of EF-Tu			
	k_4	50 ± 20	6
aa-tRNA accommodation and peptide bond formation (e)			
	k_5	0.1 ± 0.03	1
Dissociation of EF-Tu			
	k_6	2 ± 1	-
aa-tRNA rejection			
	k_7	6 ± 1	0.9

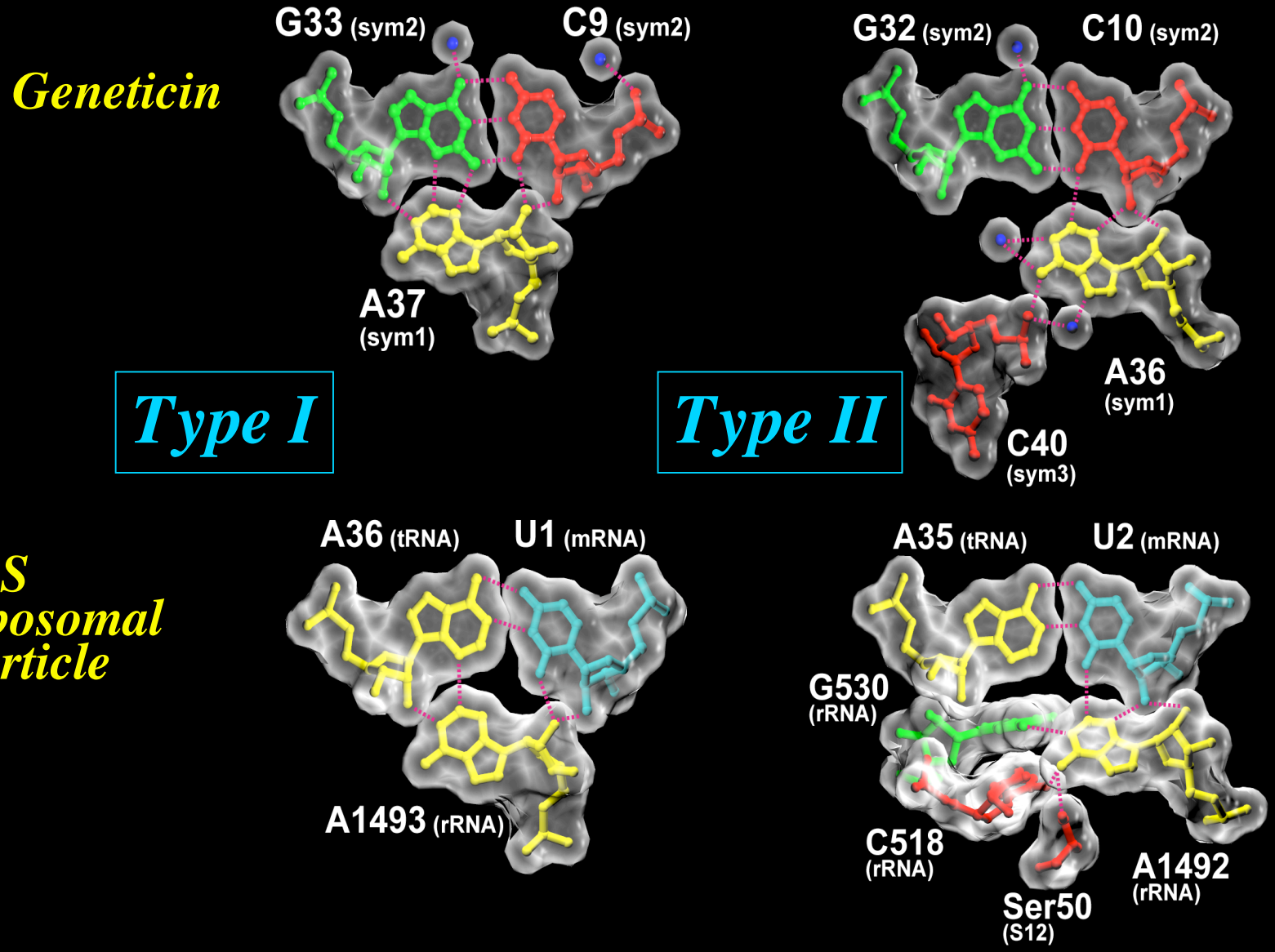
[b] per μM/s.

[e] Grouped for analysis, because the former reaction is rate limiting.

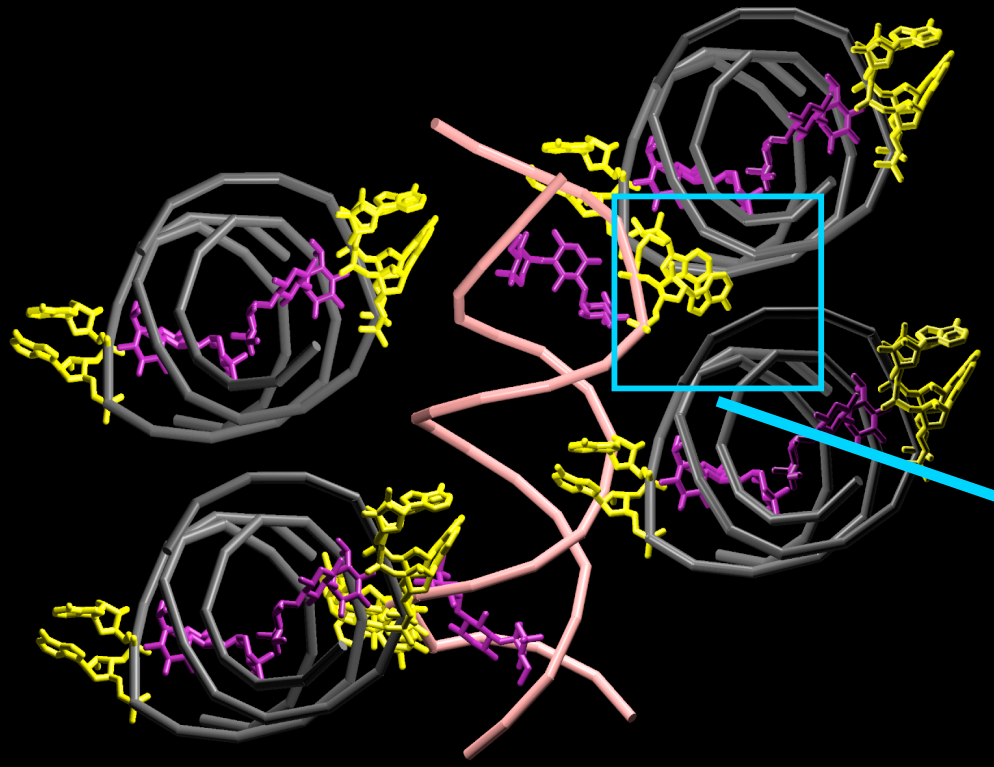

Initial binding
Codon recognition
GTPase activation
GTP
k_1
k_{-1}
k_2
k_{-2}
k_3
Accommodation
Peptidyl transfer
k_{pep}
k_5
GTP hydrolysis
EF-Tu conf. change
k_{GTP}
GDP
k_4
k_6
k_7
Proofreading

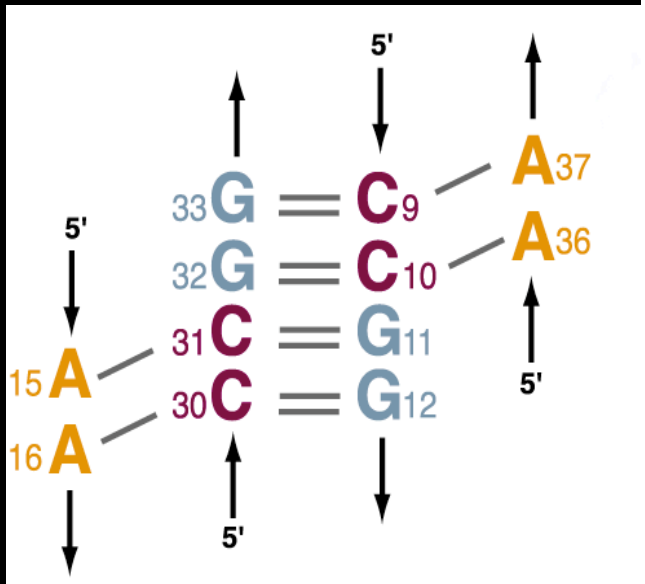
Can we develop new antibiotics less prone to resistance and toxicity ?

Mimicry of the codon-anticodon recognition by the rRNA



Crystal packing interactions

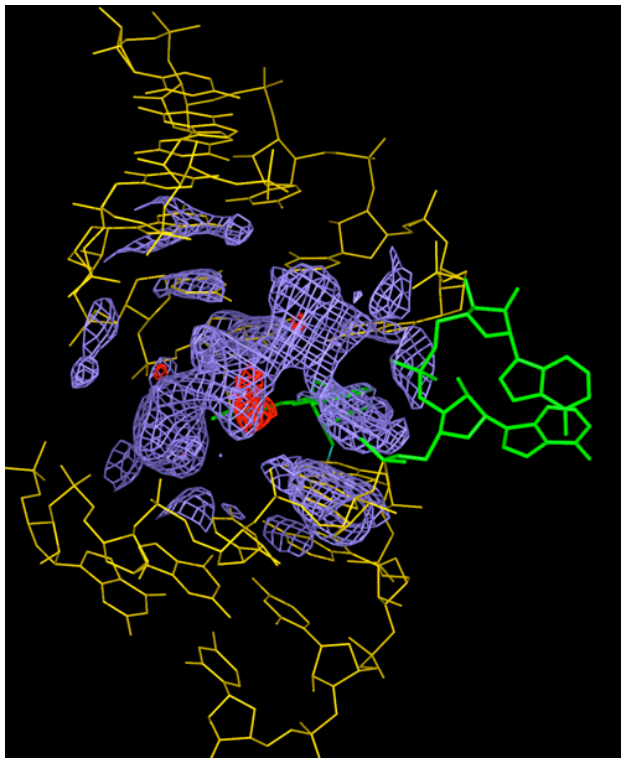




Use of crystallization as a drug screening process

TOWARDS NON-NATURAL ANTIBIOTICS

A Site - Antibiotics Structures : Molecular Recognition



Crystallizatio n of new complexes

Synthesis of new antibiotics

Resistance Mechanisms + Comparative Genomics + Molecular Evolution