Can structures lead to improved (or newly designed) drugs?

Lessons from ribosomal crystallography

Ada Yonath, Weizmann Inst. Israel

Ribosome in Action

Based on crystallographic studies, Yonath's group,
The Weizmann Institute, Rehovot, Israel,
and Max-Planck research Unit, Hamburg, Germany

Over 40% of the antibiotics inhibit

protein biosynthesis.

Most of them bind to the ribosome.

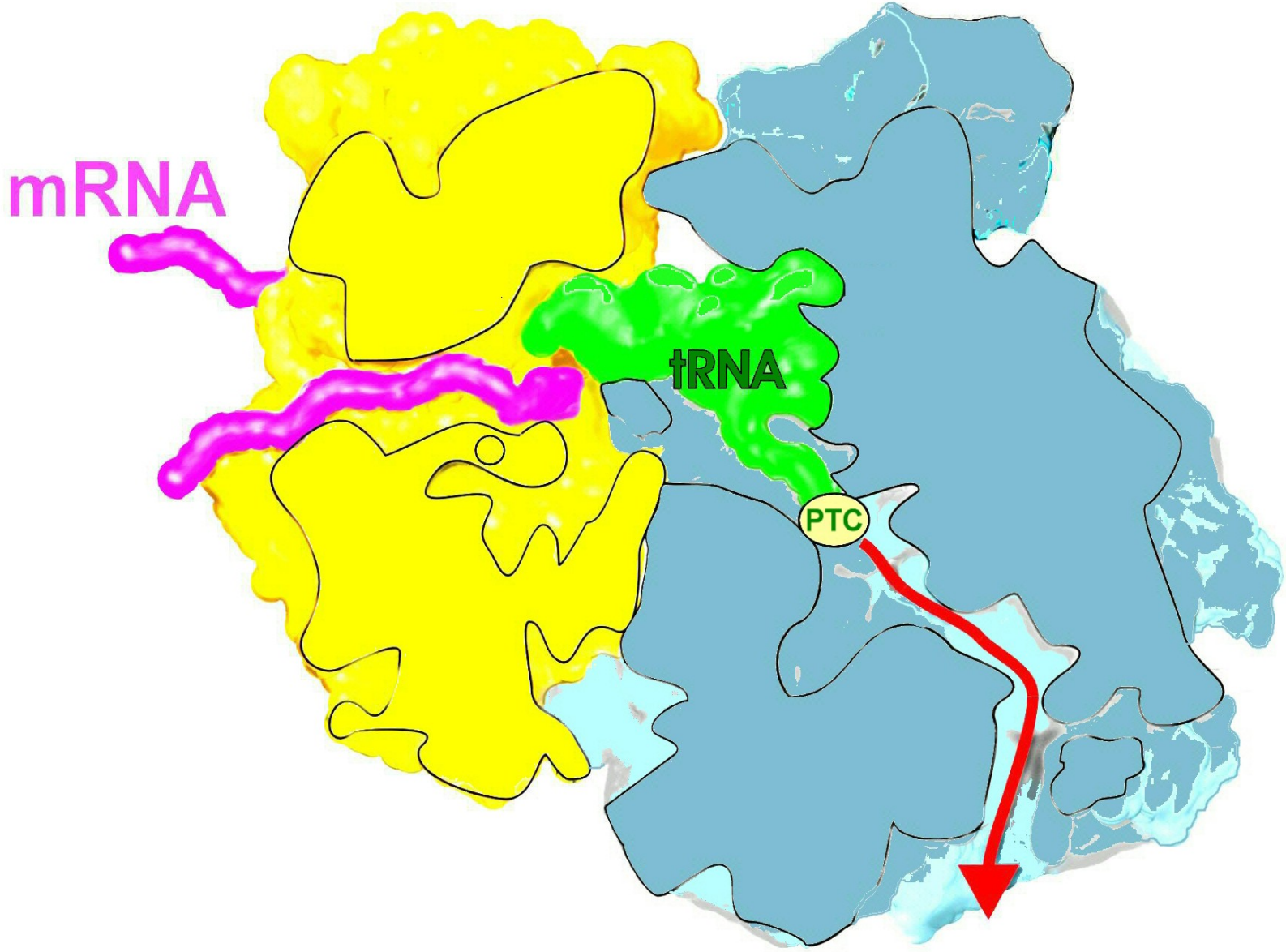
The main problems in the

clinical use of the antibiotics

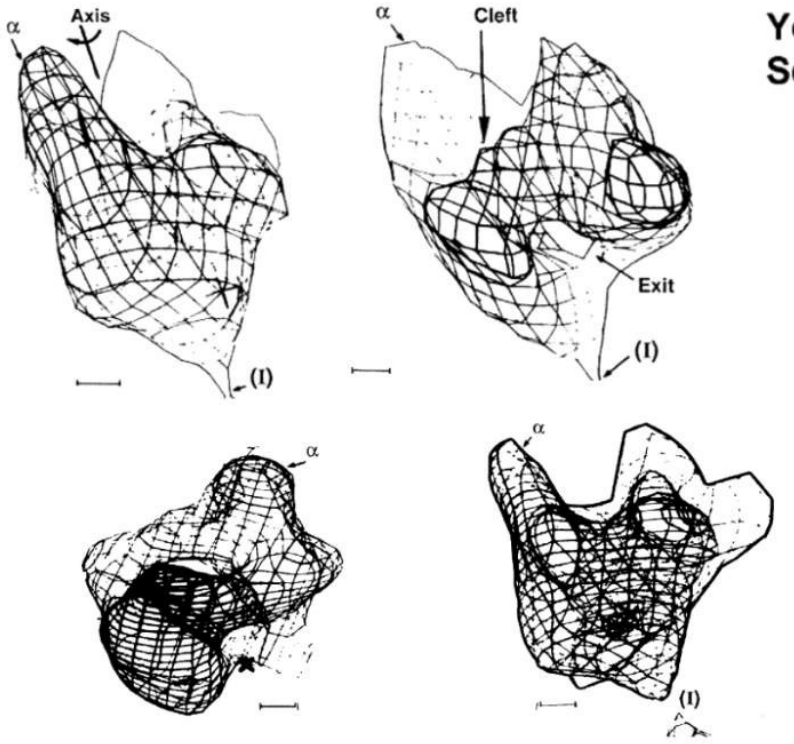
are selectivity and resistance.

All antibiotics induce resistance.

Most antibiotics are not fully selective.


mRNA
tRNA
PTC

The peptidyl transferase center (PTC) is situated above the entrance to the protein exit tunnel* that was detected first by conventional electron microscopy at low resolution (Milligan and Unwin, 1986; Yonath et al., 1987).



Yonath et al.,
Science 236, 813-816, 1987

Fig. 3. Computer graphic display of the outline of the reconstructed model of the 50*S* ribosomal subunit at 30 Å resolution. The front is contoured with heavy lines, the back with lighter ones. Bar length, 20 Å. The asterisk marks the exit from the tunnel on the other side of the ribosome. Alpha (α) marks the longest projecting arm. The contour level for the particle boundary was chosen to be that at which the interparticle contacts could be resolved. For real-time display we used the computer program FRODO (*24*) combined with the GHC650 (written by G. H. Cohen, NIH) for Fourier synthesis, and FRODOMAP (written by J. L. Bhat, NIH) as well as the version, written by H. Bosshard, EMBL, Heidelberg. Views (A) to (C) were obtained from one reconstruction, and views (D) and (E) from another. (**A**) A side view of the model. The entire particle and part of a second one are shown. The arrow (I) points at the crystal contact between the two particles. (**B**) The model in (A) was rotated about the axis shown to generate the model in (B). (**C**) The model shown in (A and B) viewed into the branch of the tunnel from the exit point. (**D**) A view into the tunnel from the cleft. (**E**) The model viewed in a projection that resembles models derived from electron microscopy of single particles. The contour level is 2 to 5% higher than in previous views.

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SCIENCE, VOL. 236

* Based on biochemical studies
(Malkin and Rich, 1967; Bloble and Sabatini (1970)

Ribosomes are universal, yet subtle differences in their chemical composition allow discrimination by

Antibiotics

THE BASIS FOR CLINICAL UTILIZATION

Crystallographic Structures of Ribosomal Particles

Published by the end of 2001

Eubacteria - resembling *E.coli*

hence can be directly correlated with the vast volume of biochemical observations

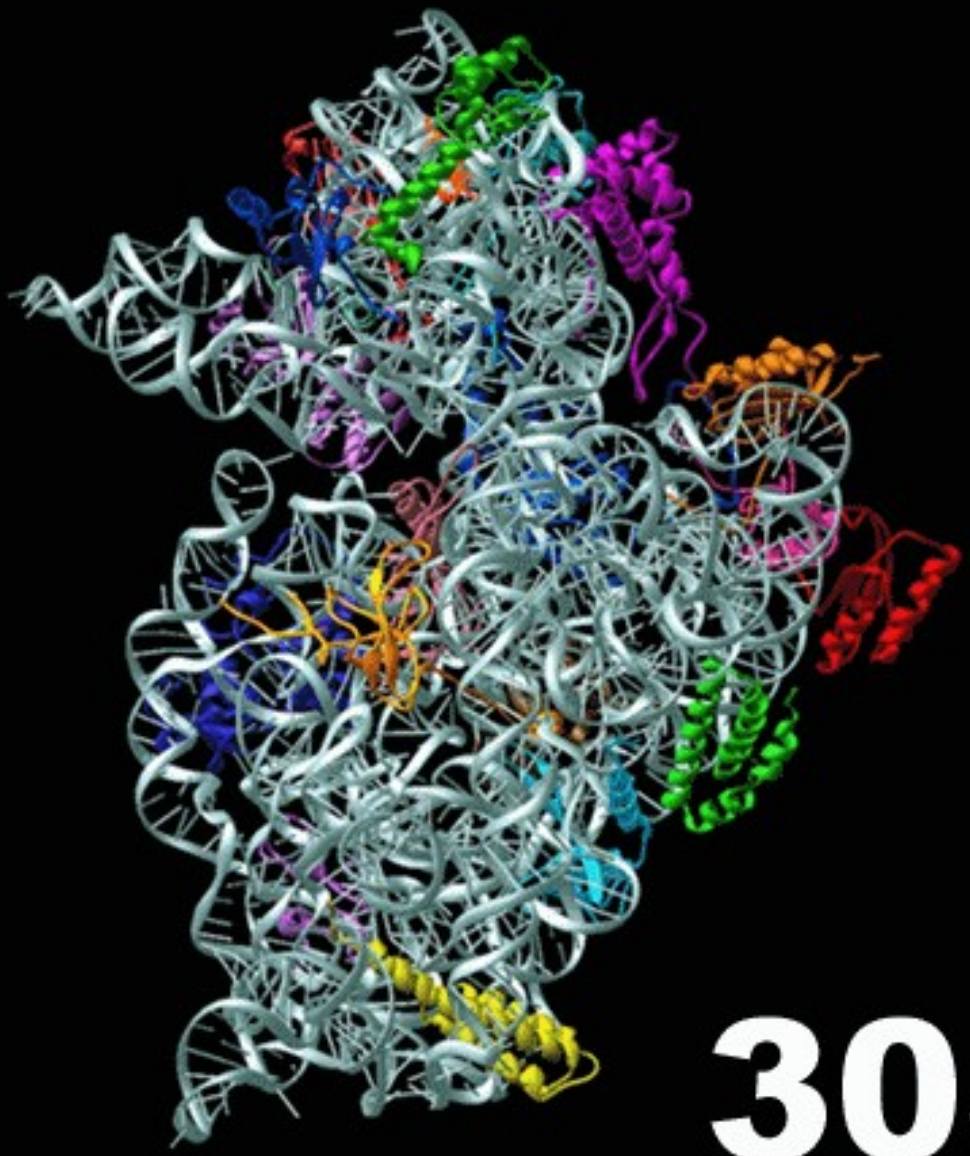
3.0 A The small ribosomal subunit from Thermus thermophilus (T30S)
Schluenzen et al., 2000 (Weizmann-MaxPlanck)
Wimberly et al., 2000 (MRC)

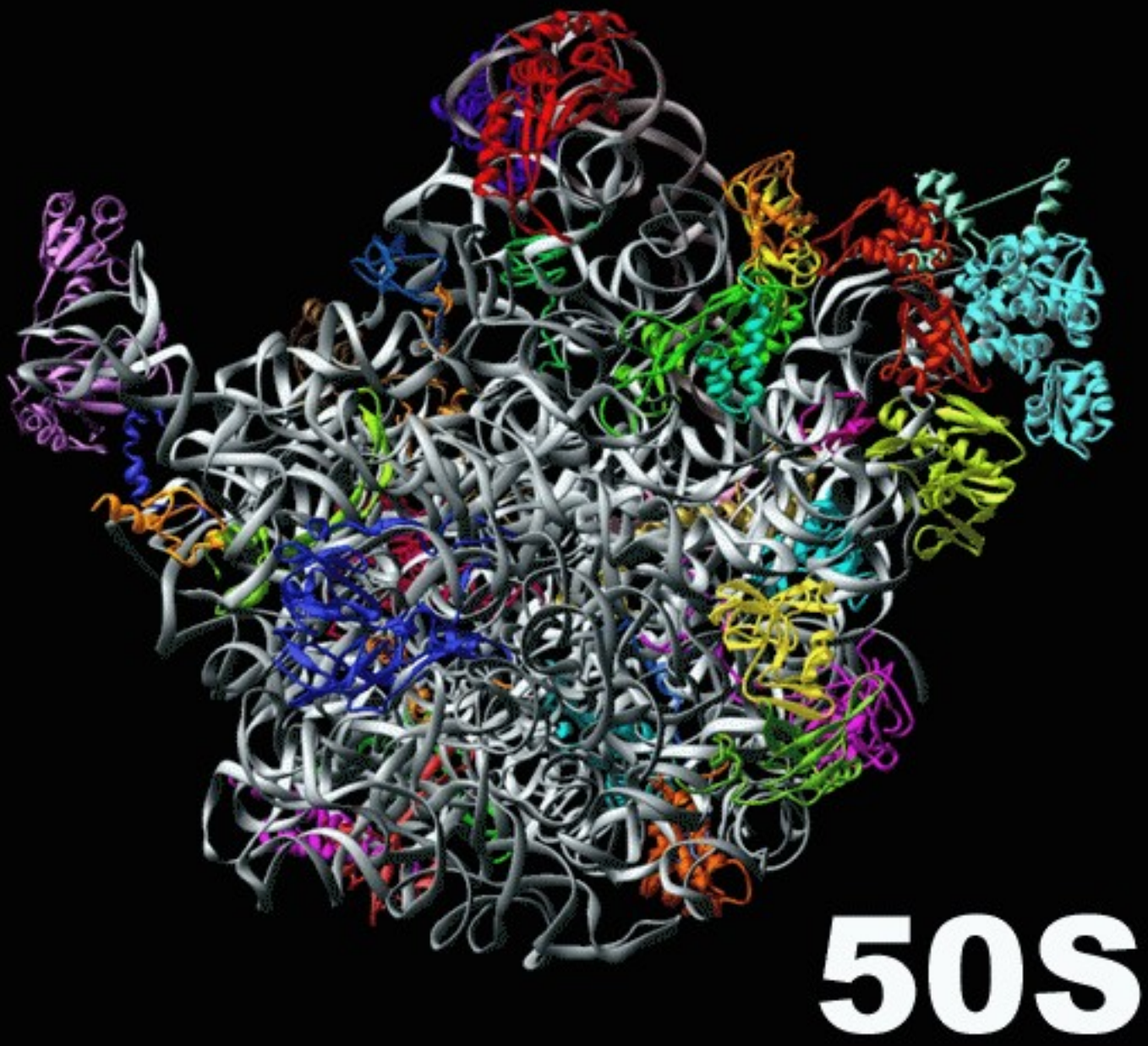
3.0 A The large ribosomal subunit from Deinococcus radiodurans (D50S)
Harms et al., 2001 (Weizmann-MaxPlanck)

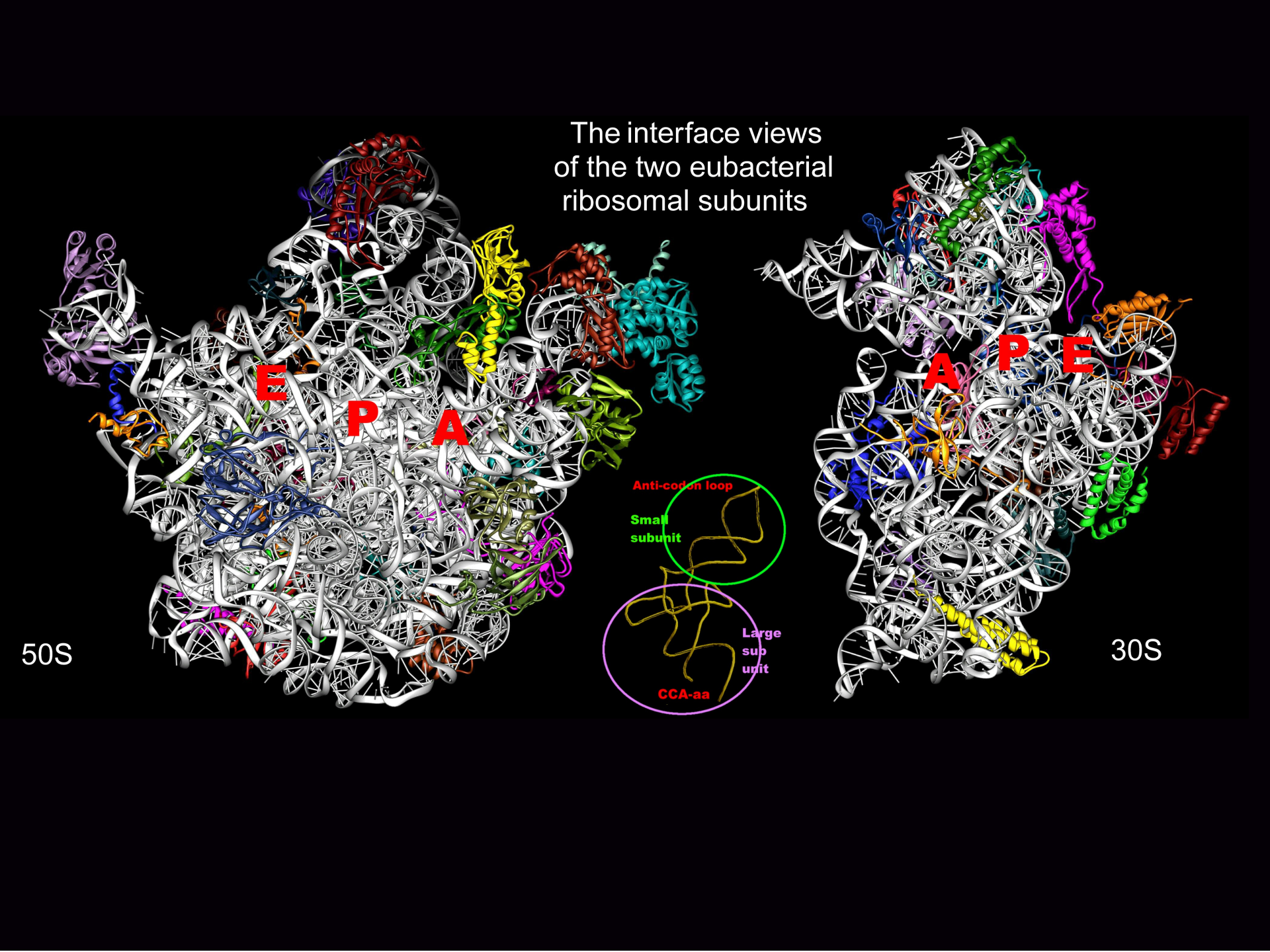
5.5 A A complex of the whole ribosome from Thermus thermophilus (T70S) with tRNAs
Yusupov et al., 2001 (Santa Cruz)

Archaea - sharing properties with prokaryotes and eukaryotes

2.4 A The large ribosomal subunit from Haloarcula marismortui (H50S)
Ban et al., 2000 (Yale Uni.)

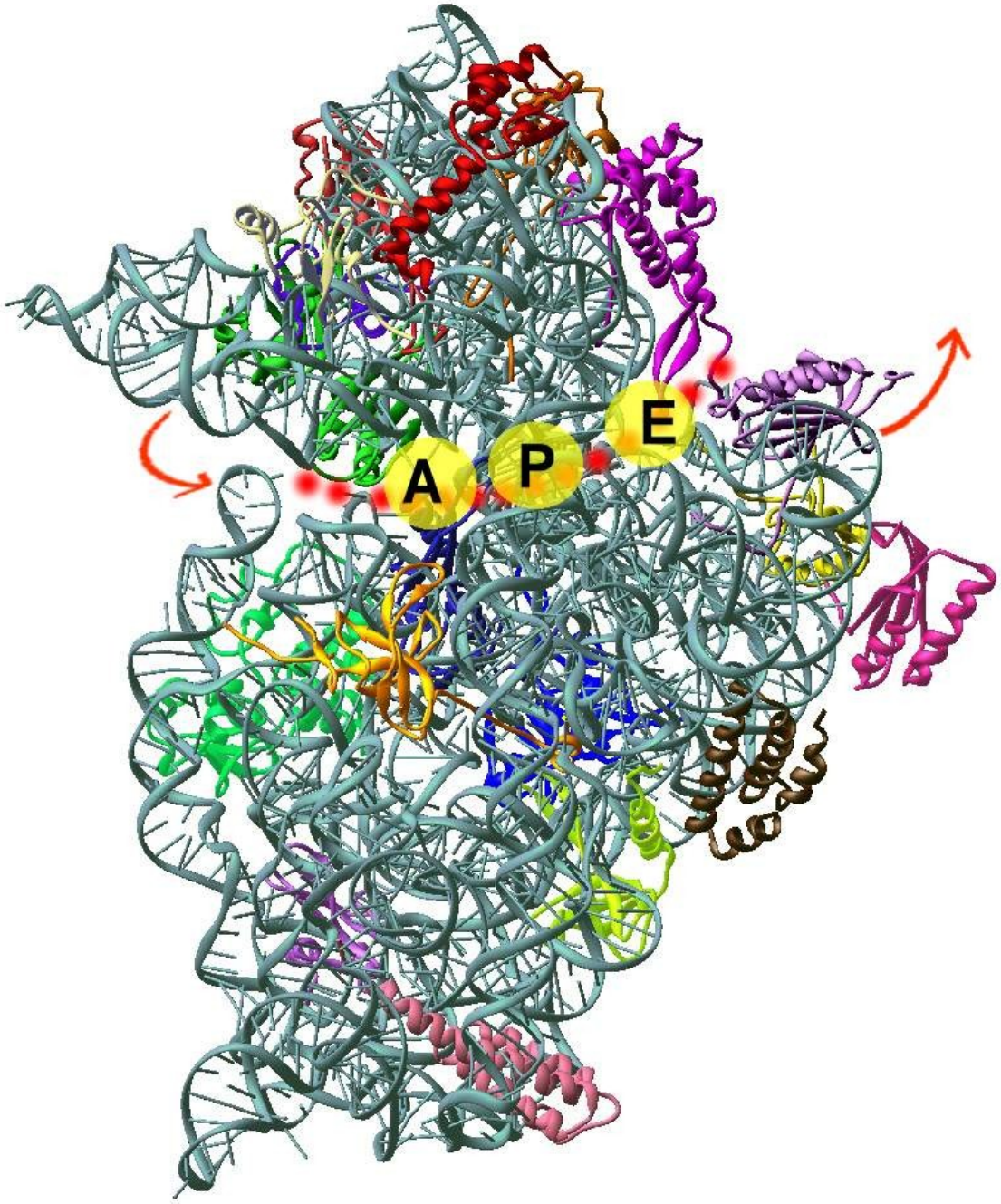

30S

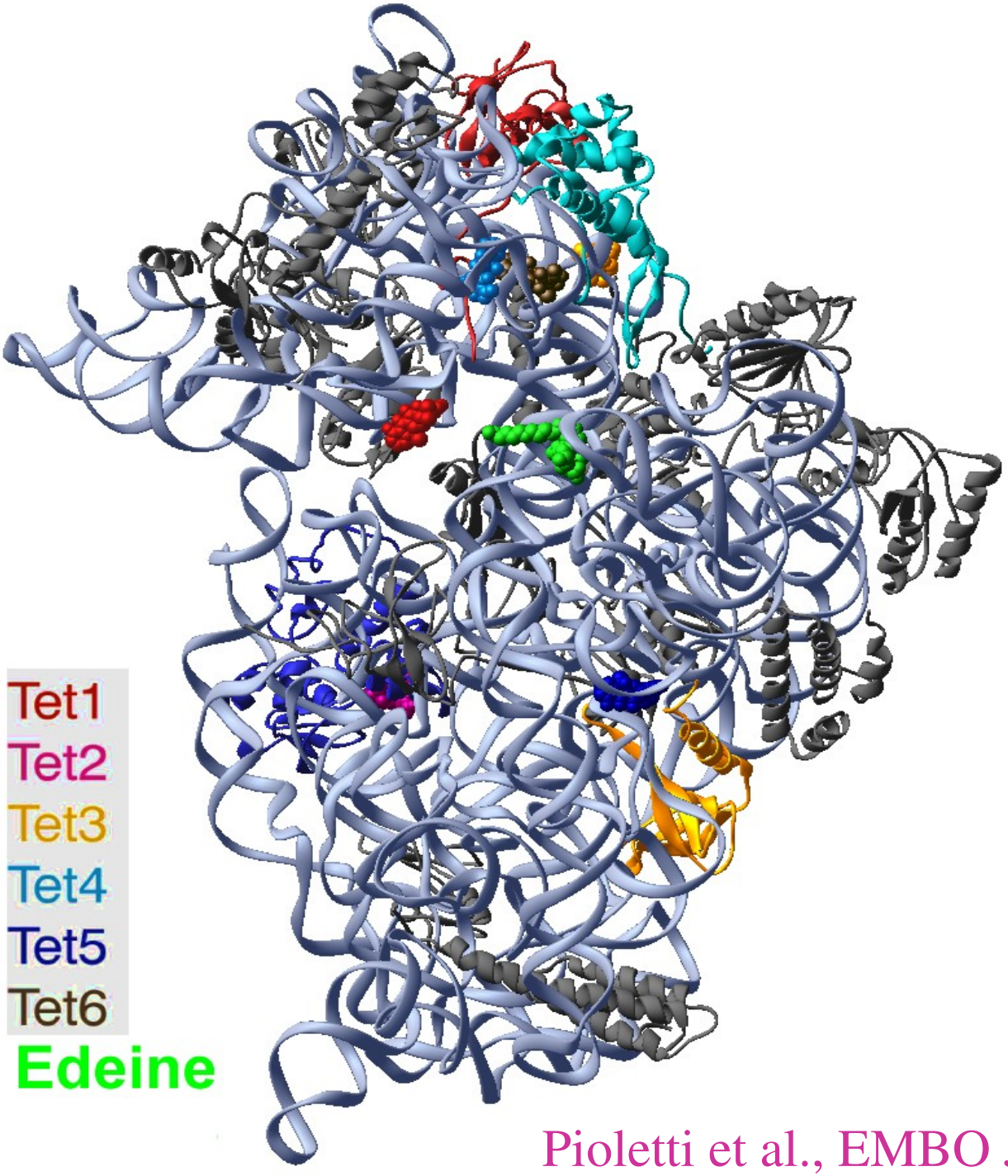

50S


The interface views
of the two eubacterial
ribosomal subunits
E
P
A
50S
Anti-codon loop
Small
subunit
Large
sub
unit
CCA-aa
A
P
E
30S

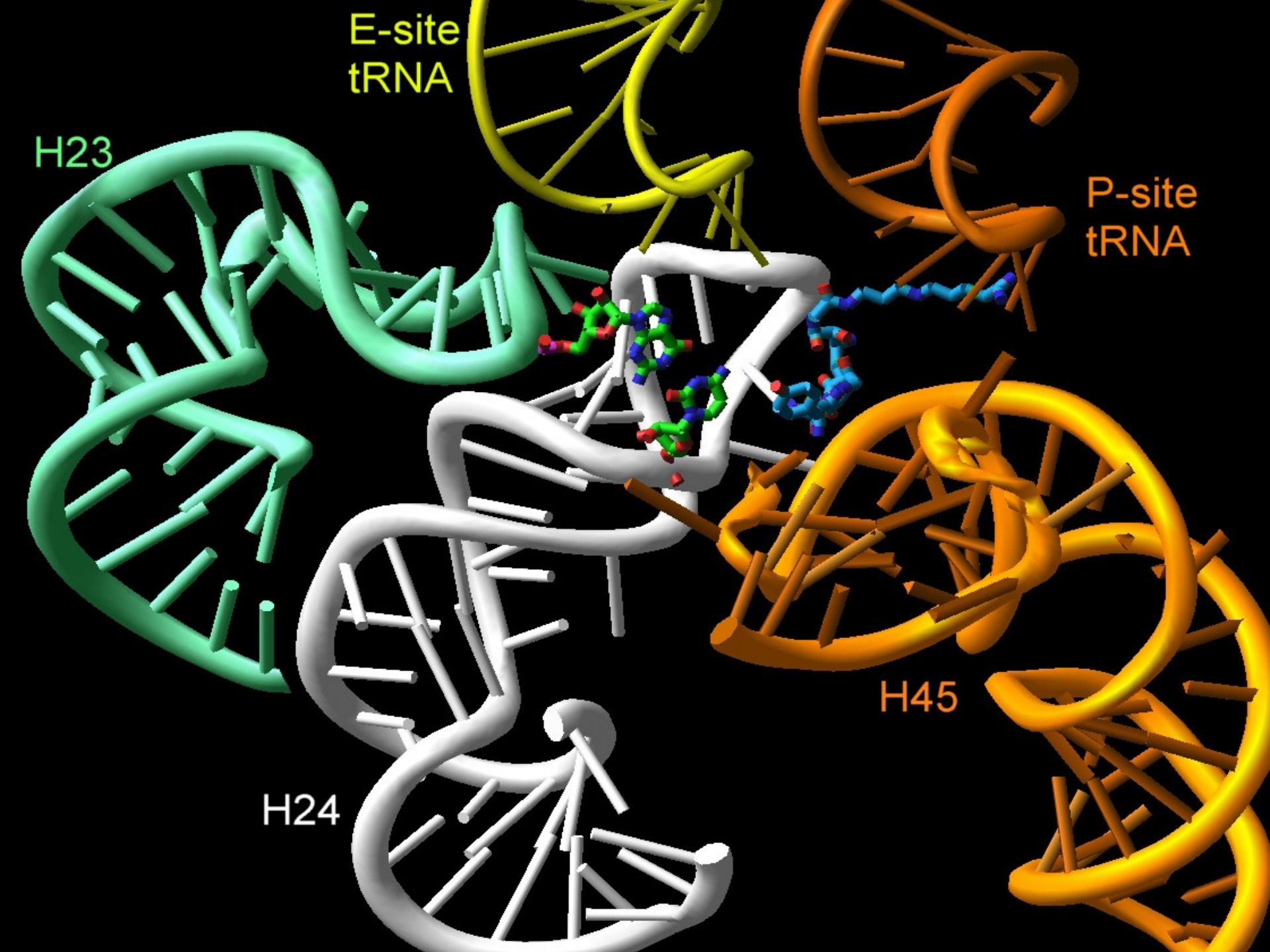
Antibiotics Targeting Ribosomes

Based on crystallographic studies, Yonath's group,
The Weizmann Institute, Rehovot, Israel,
and Max-Planck research Unit, Hamburg, Germany

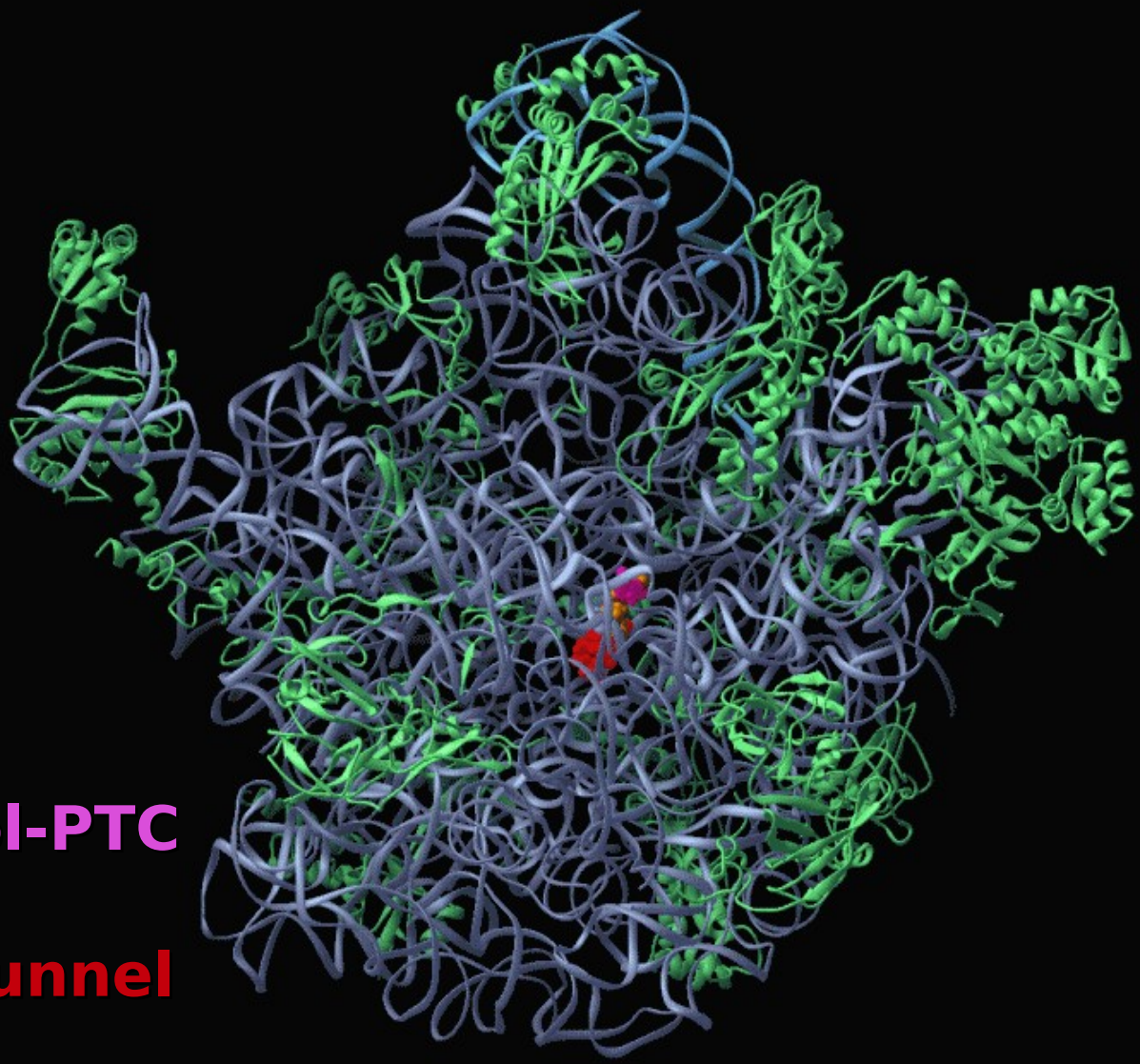

A
P
E



Pioletti et al., EMBO Journal 2001

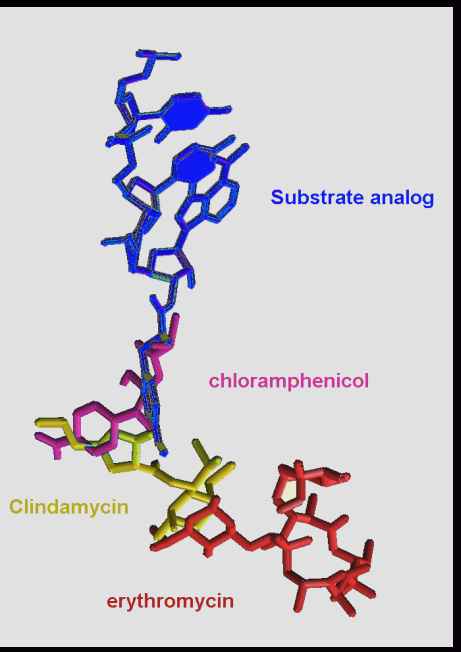

E-site
tRNA
H23
P-site
tRNA
H45
H24

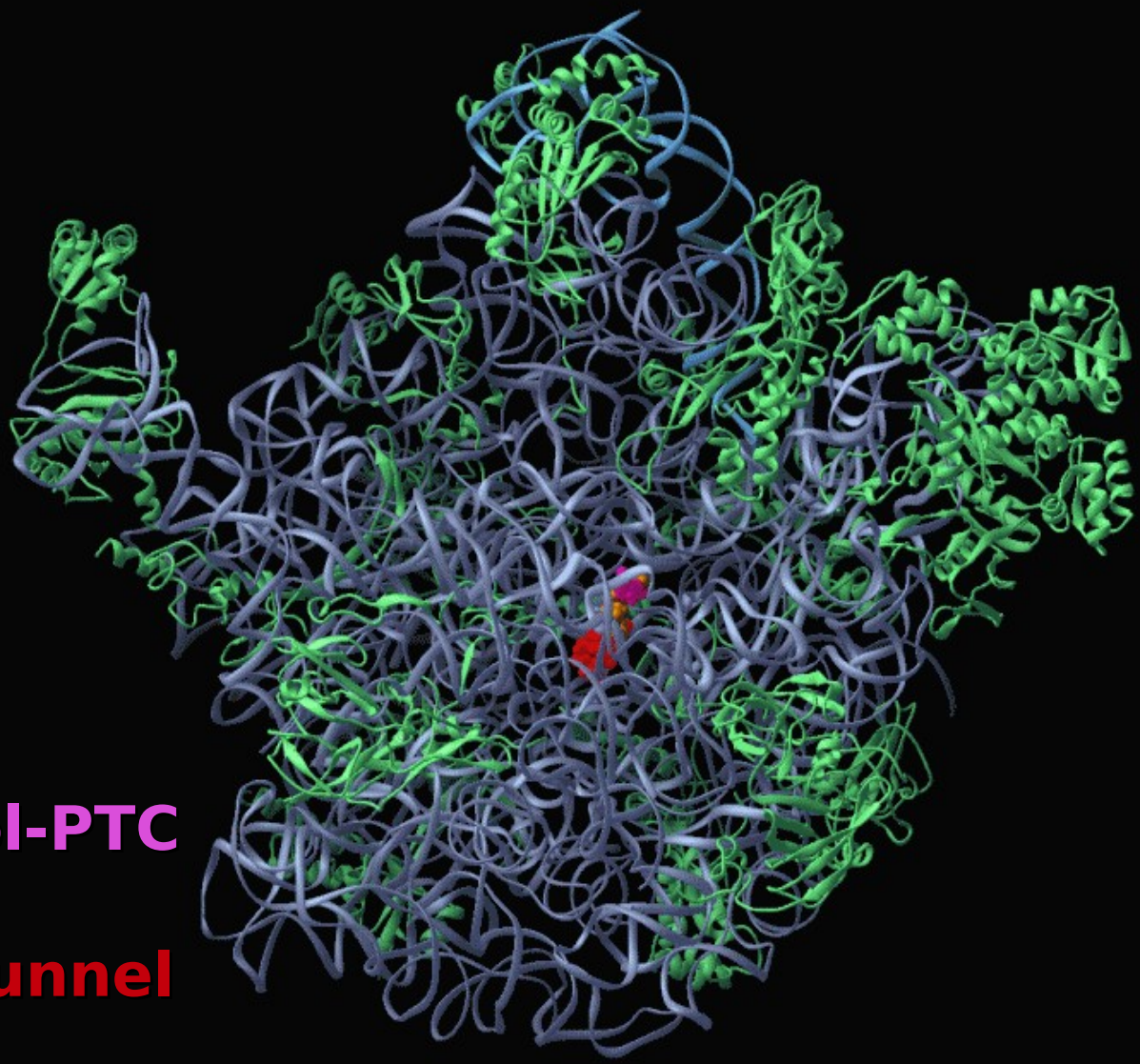
From PTC into the tunnel



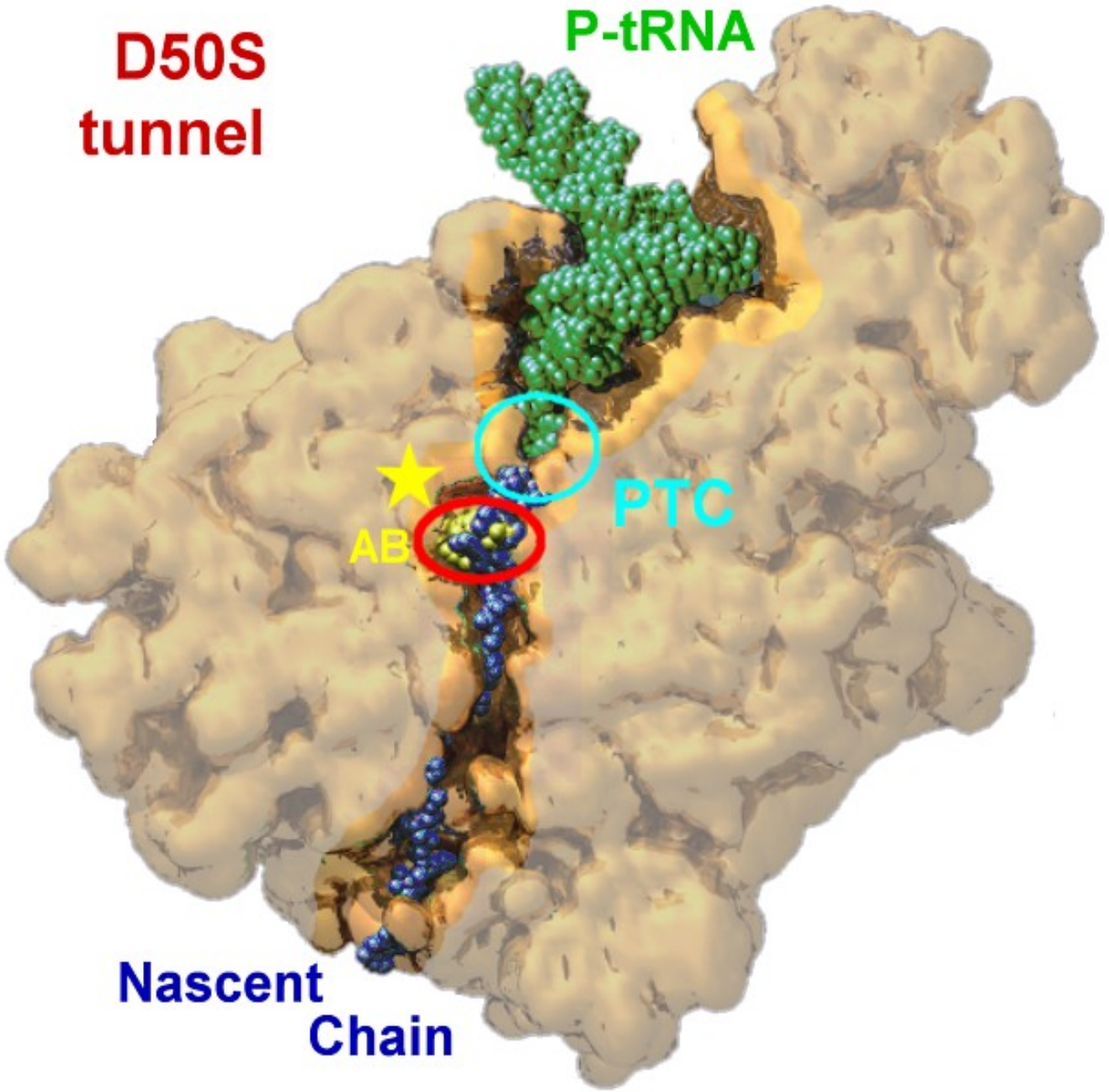
Chloramphenicol-PTC
Clindamycin
Erythromycin- tunnel

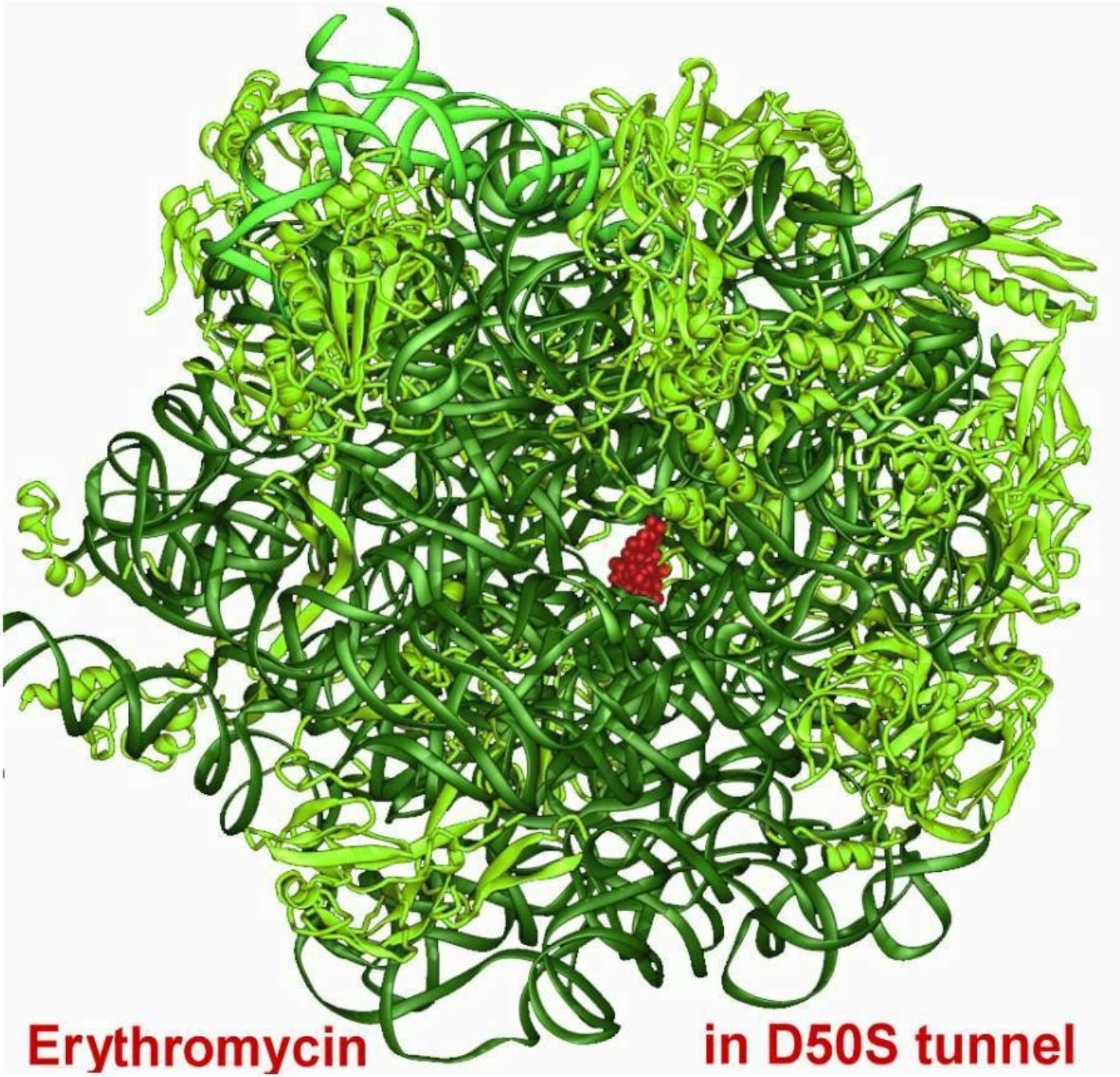
From PTC into the tunnel

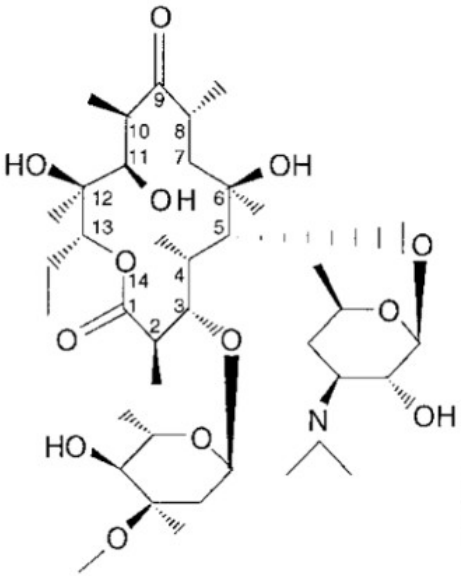




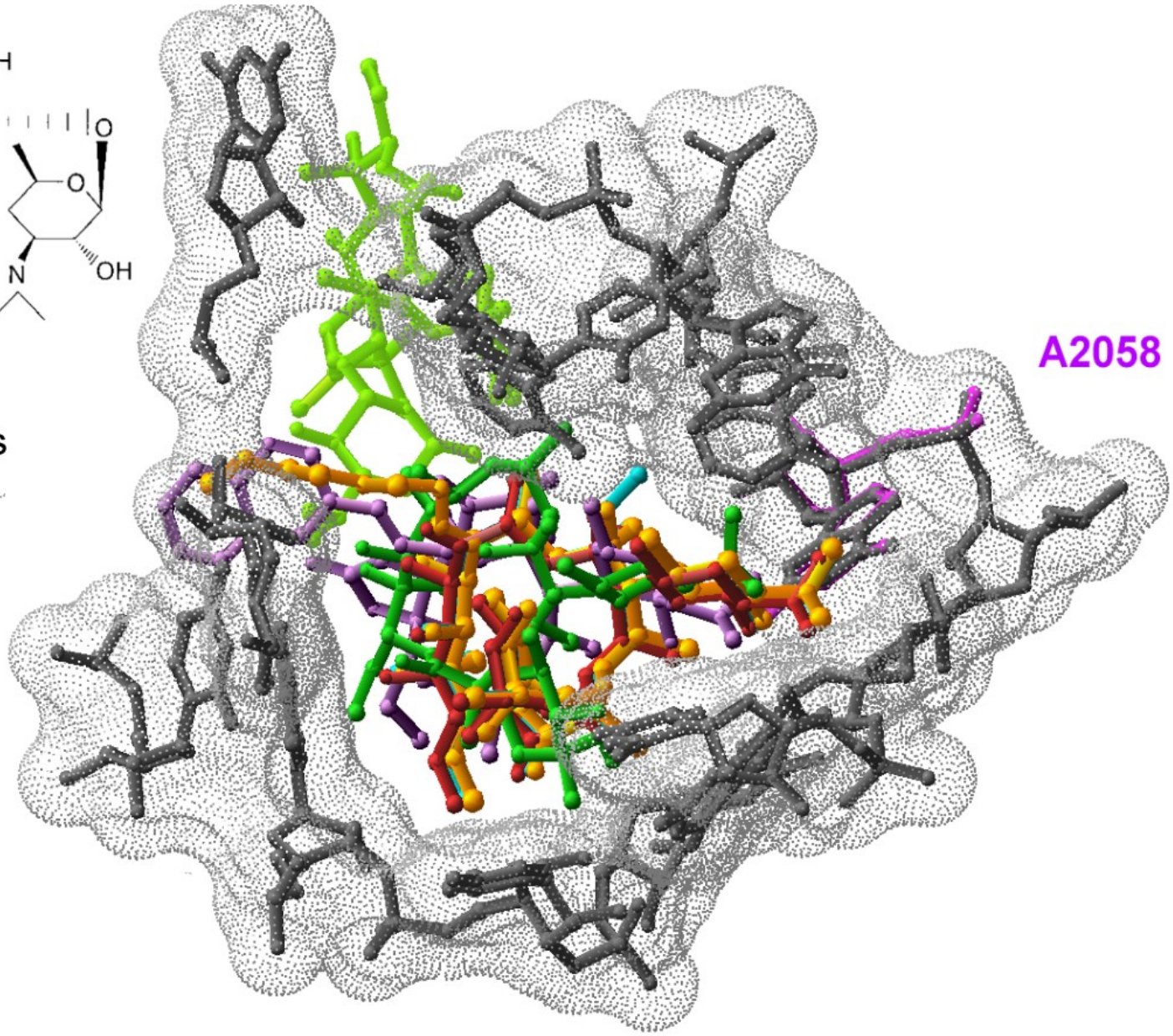
Chloramphenicol-PTC
Clindamycin
Erythromycin- tunnel


D50S
tunnel
P-tRNA
PTC
AB
Nascent
Chain

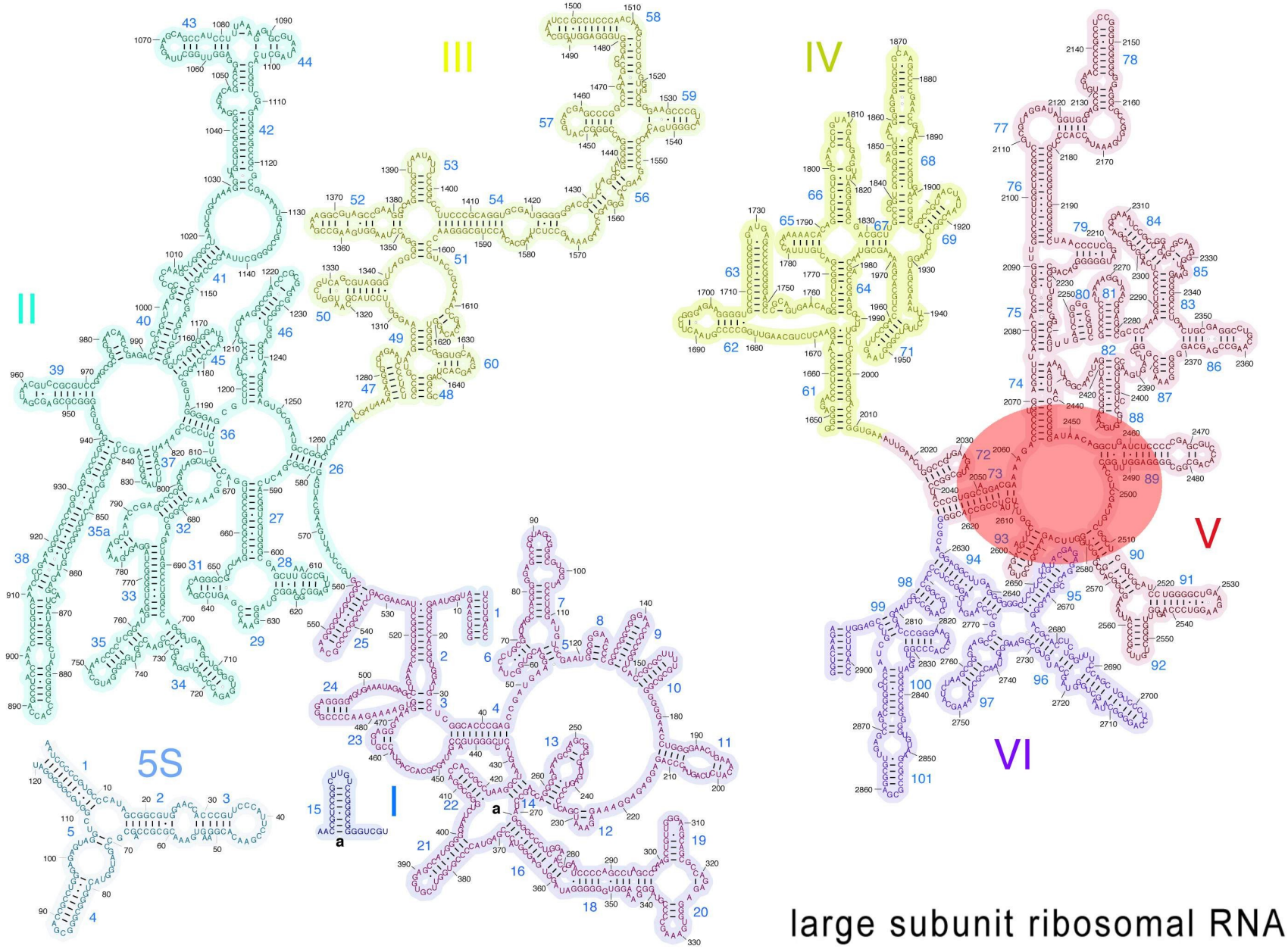

Erythromycin
in D50S tunnel



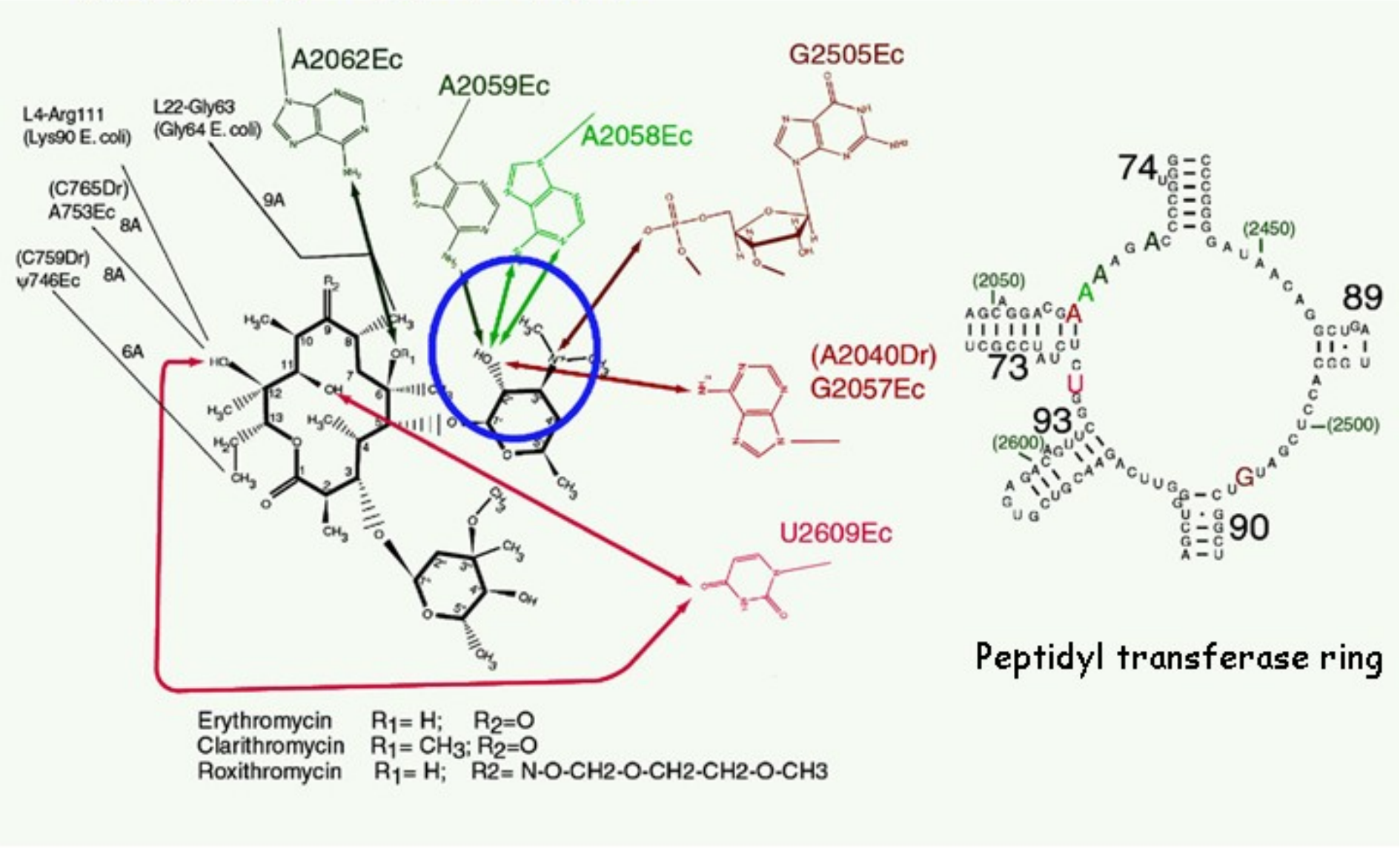
Macrolides



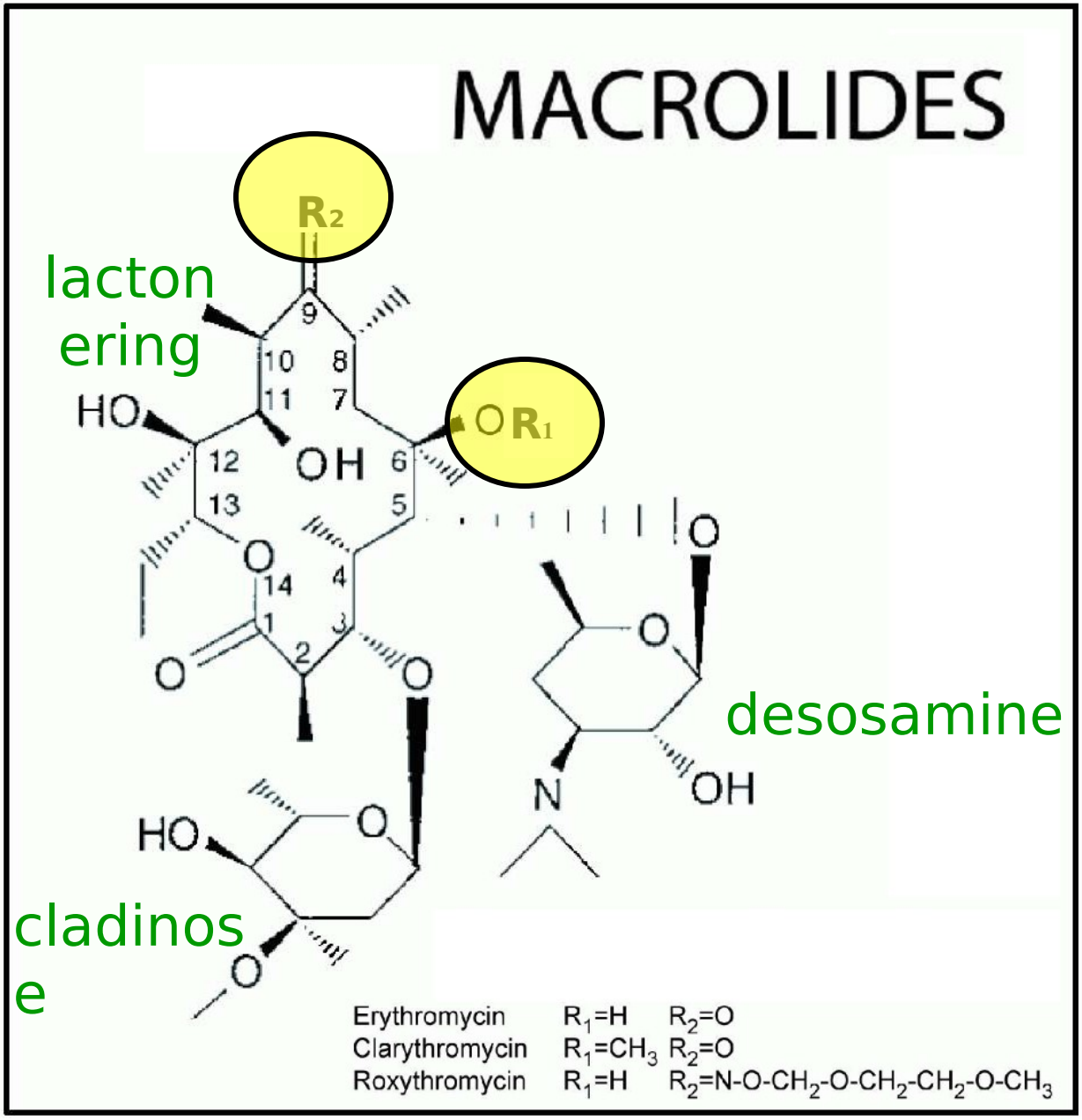
A2058


I
II
III
IV
V
VI
5S
large subunit ribosomal RNA

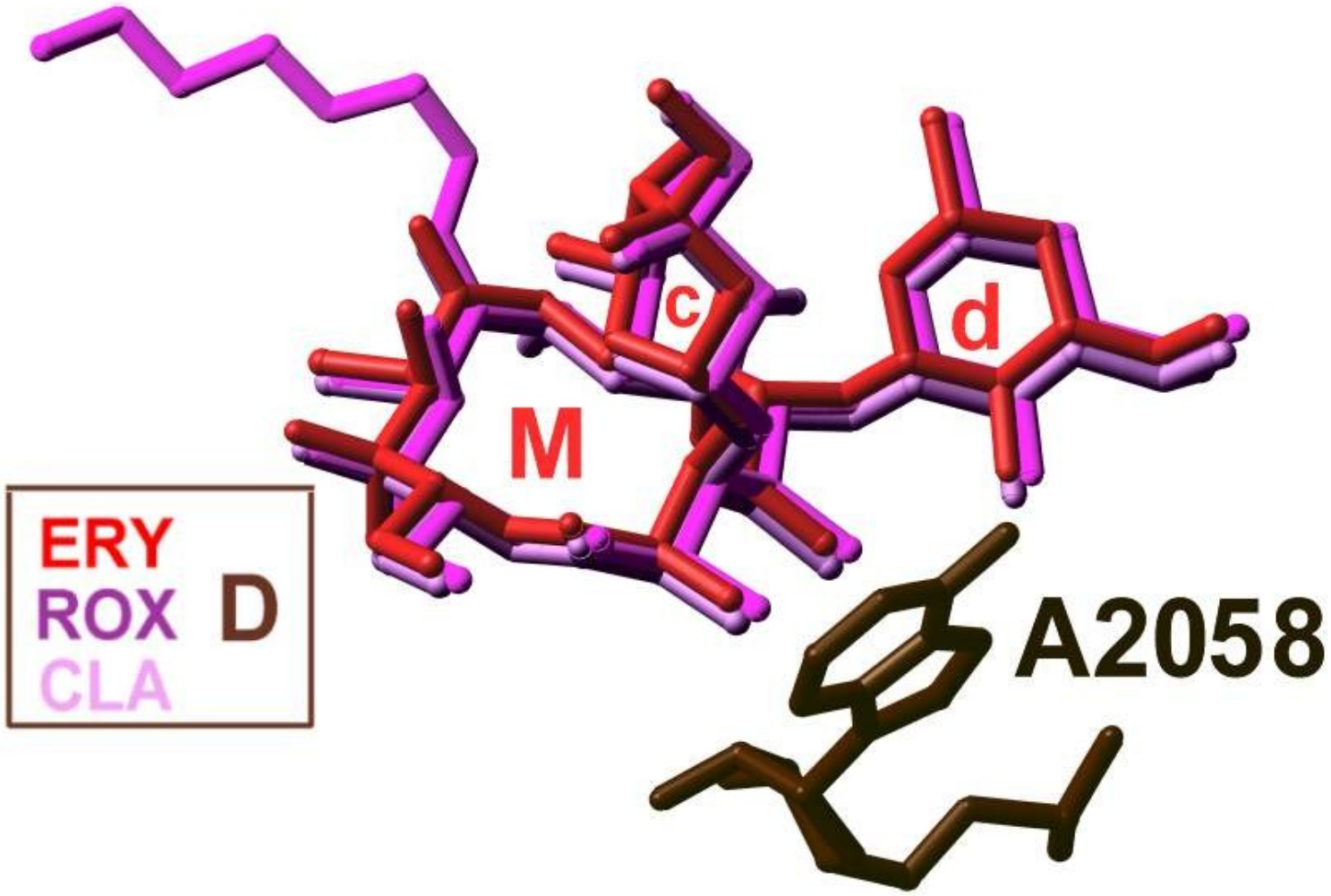
Contacts between erythromycin and nucleotides from the 23S rRNA:

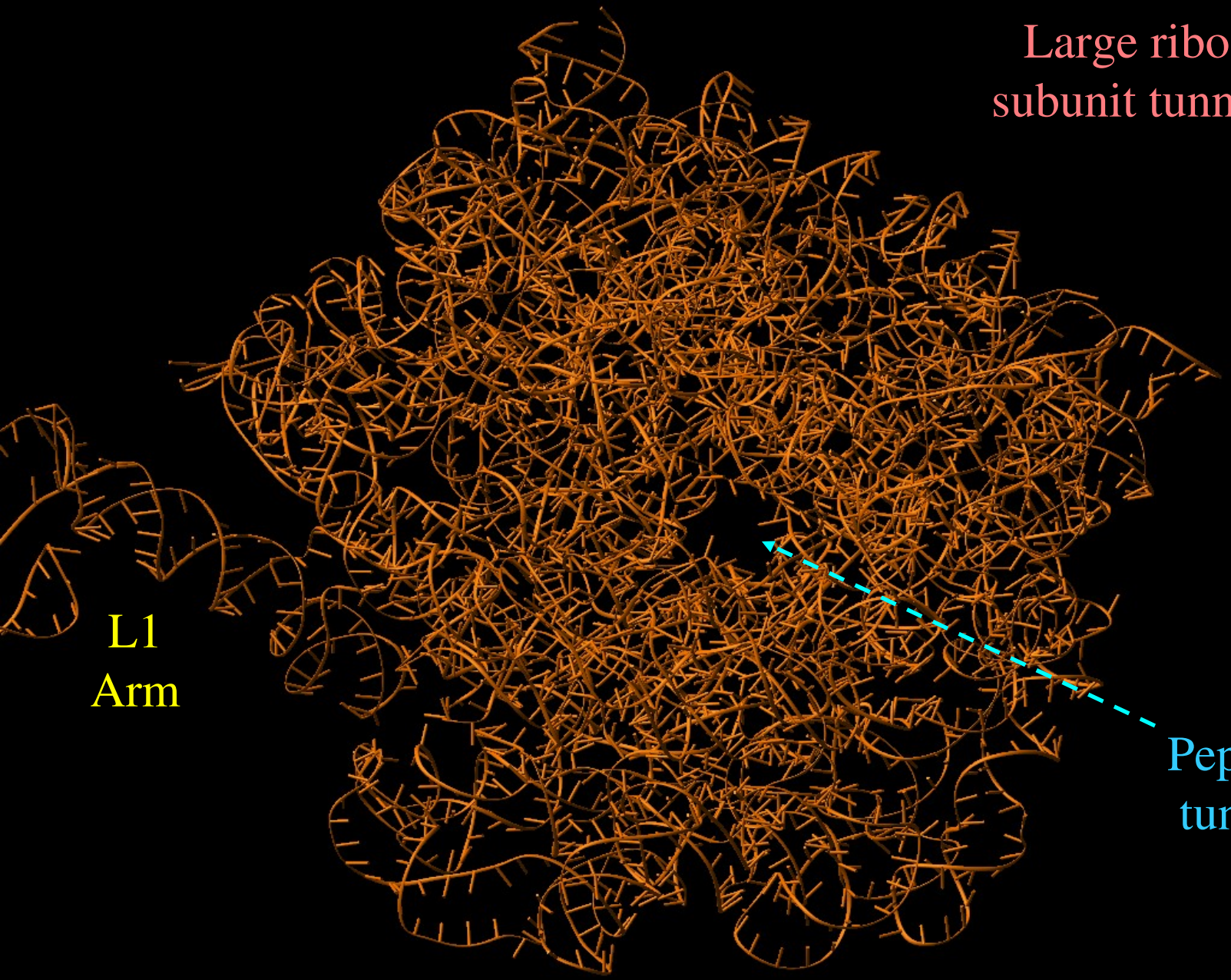


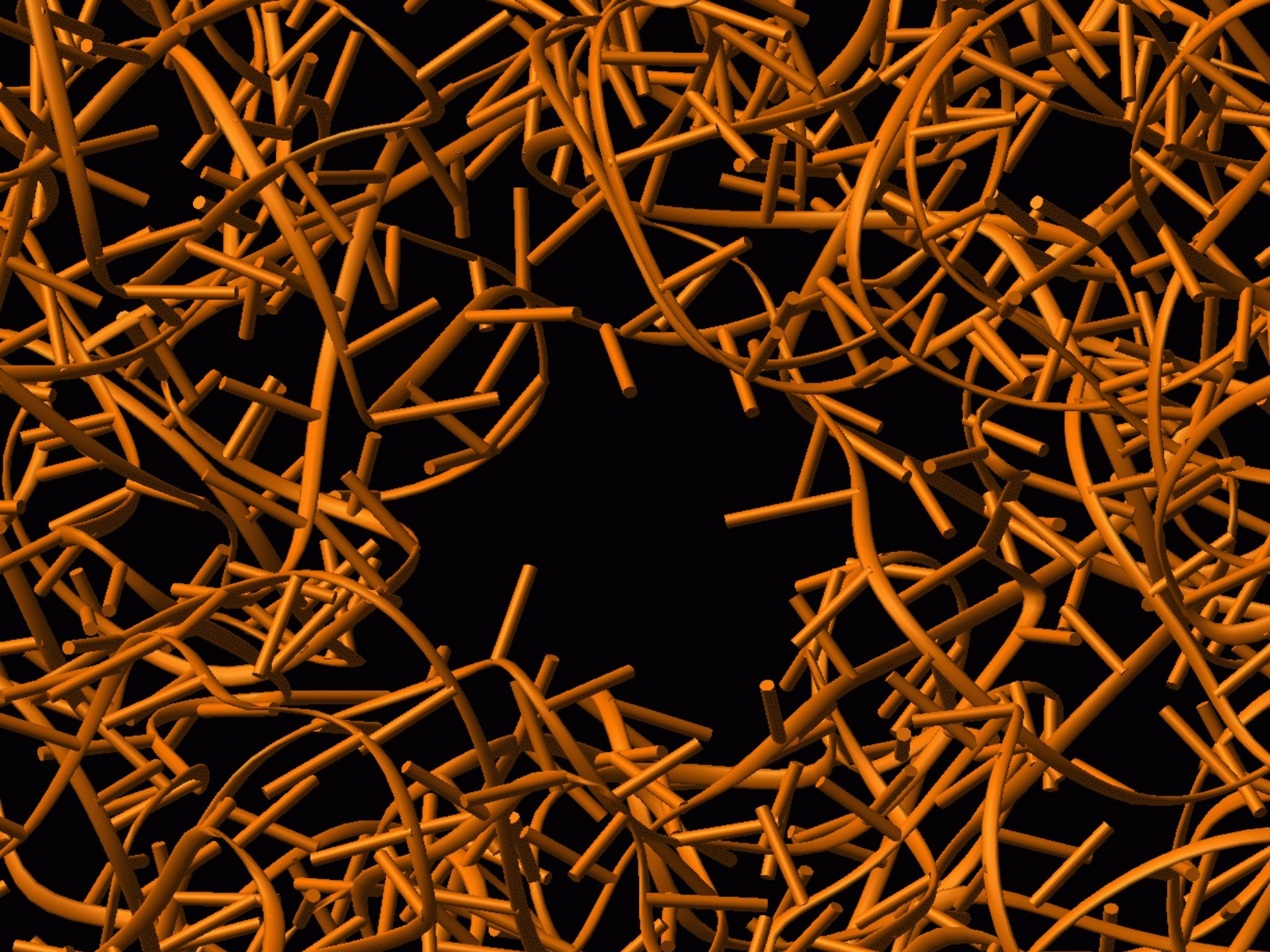
Peptidyl transferase ring

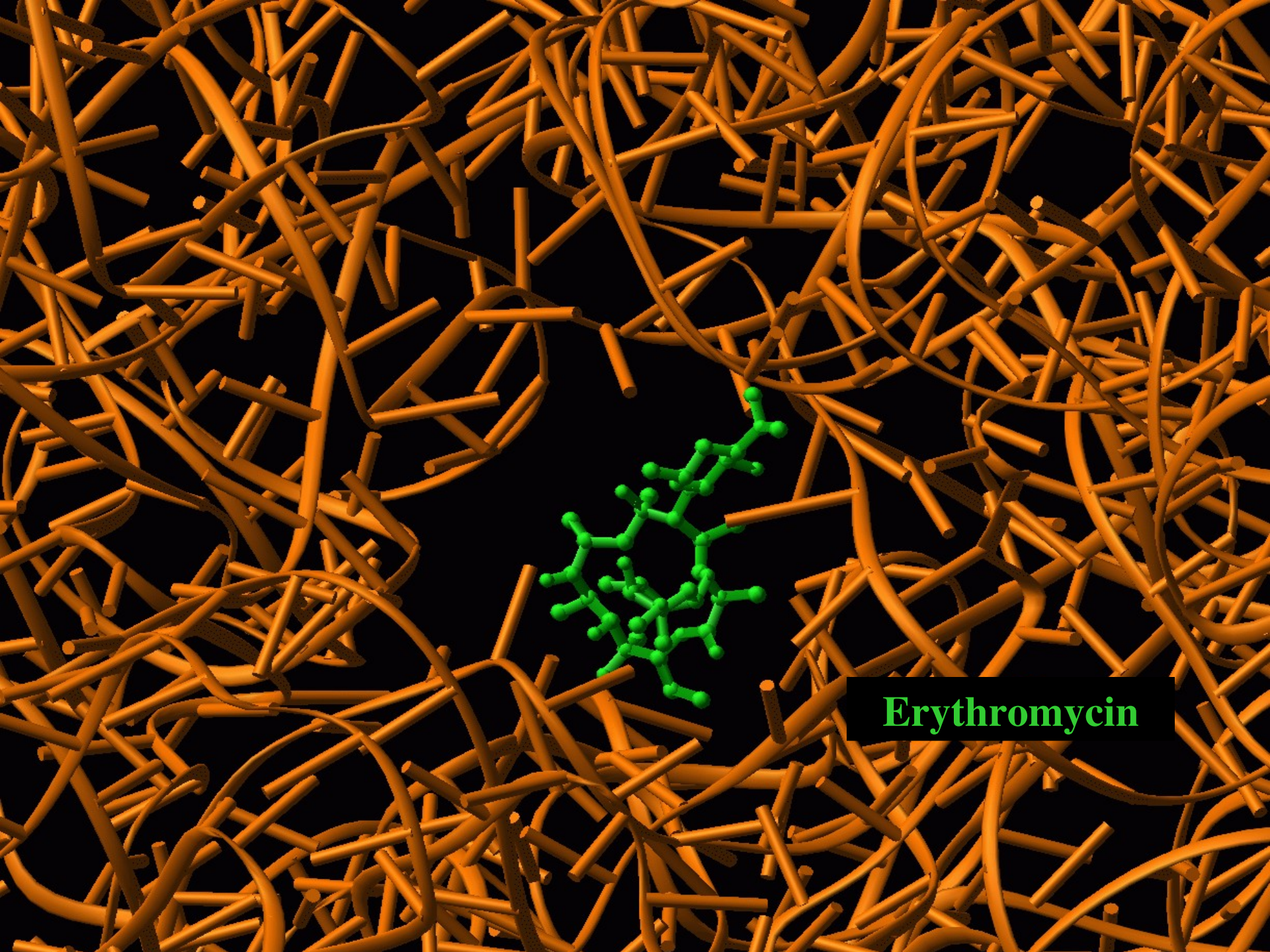


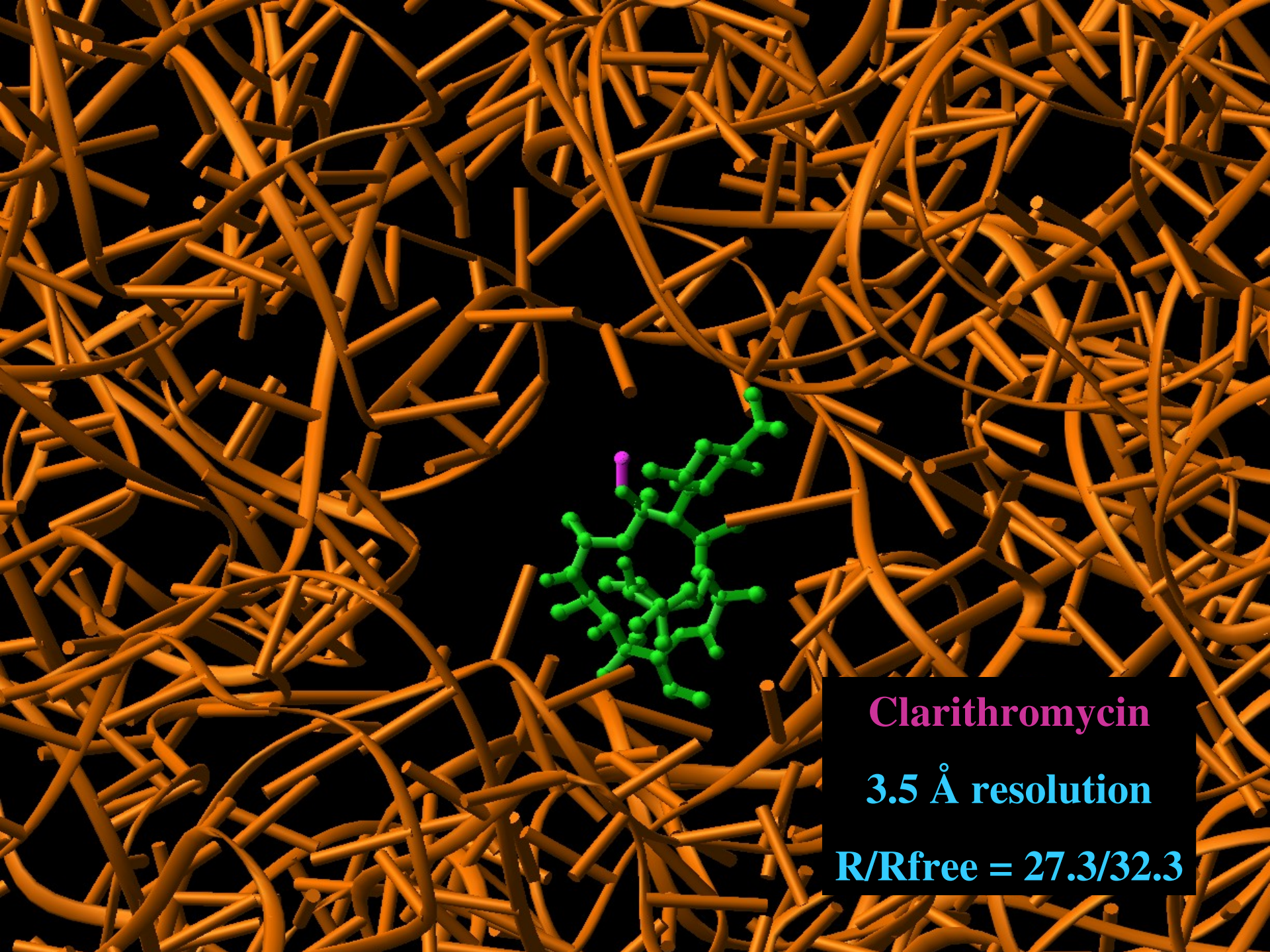
Schluenzen et al., Nature 2001

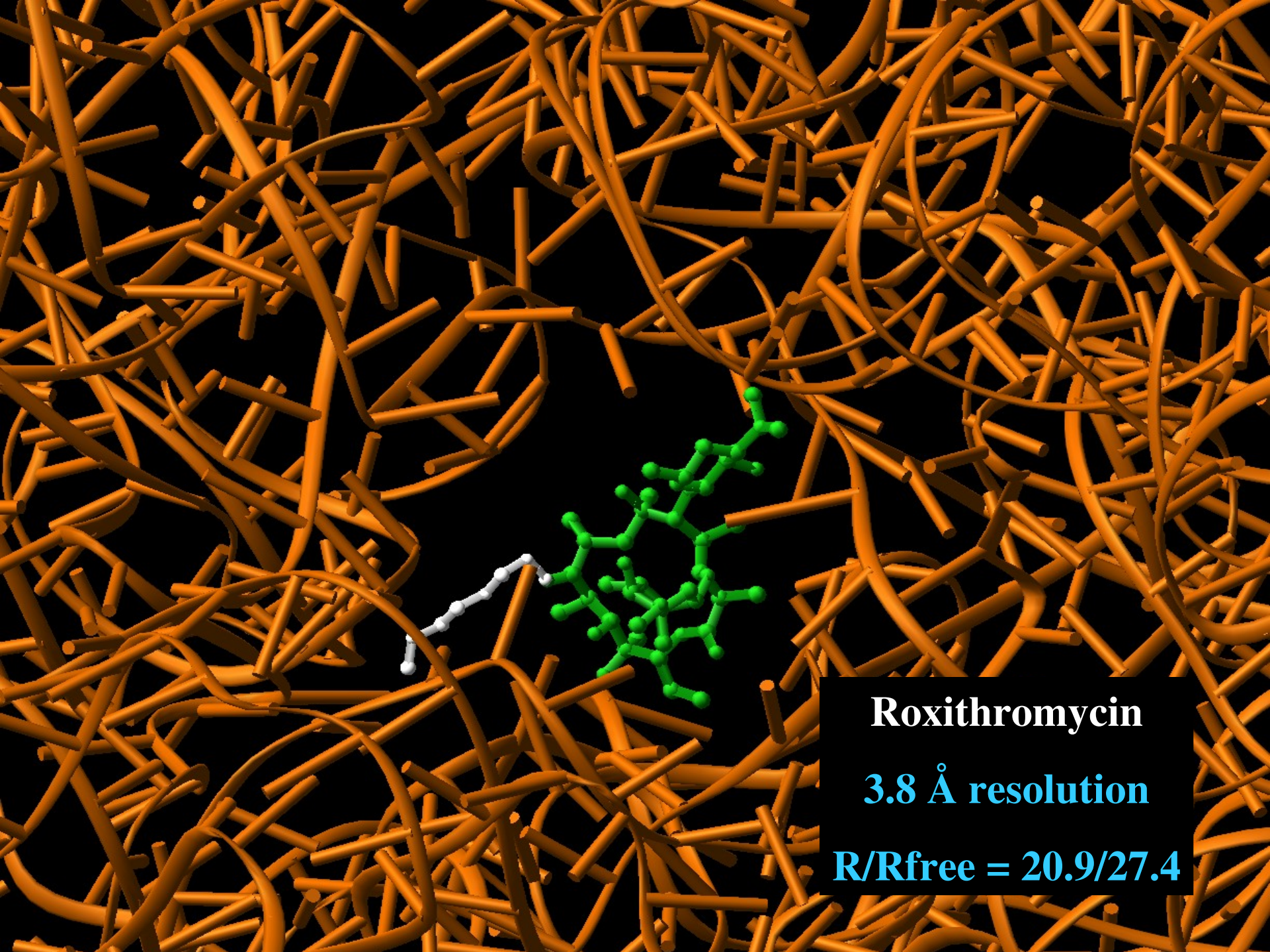

ERY
ROX
CLA
D
M
c
d
A2058


Large ribosomal
subunit tunnel view
L1
Arm
Peptide
tunnel
Small subunit interface




Erythromycin

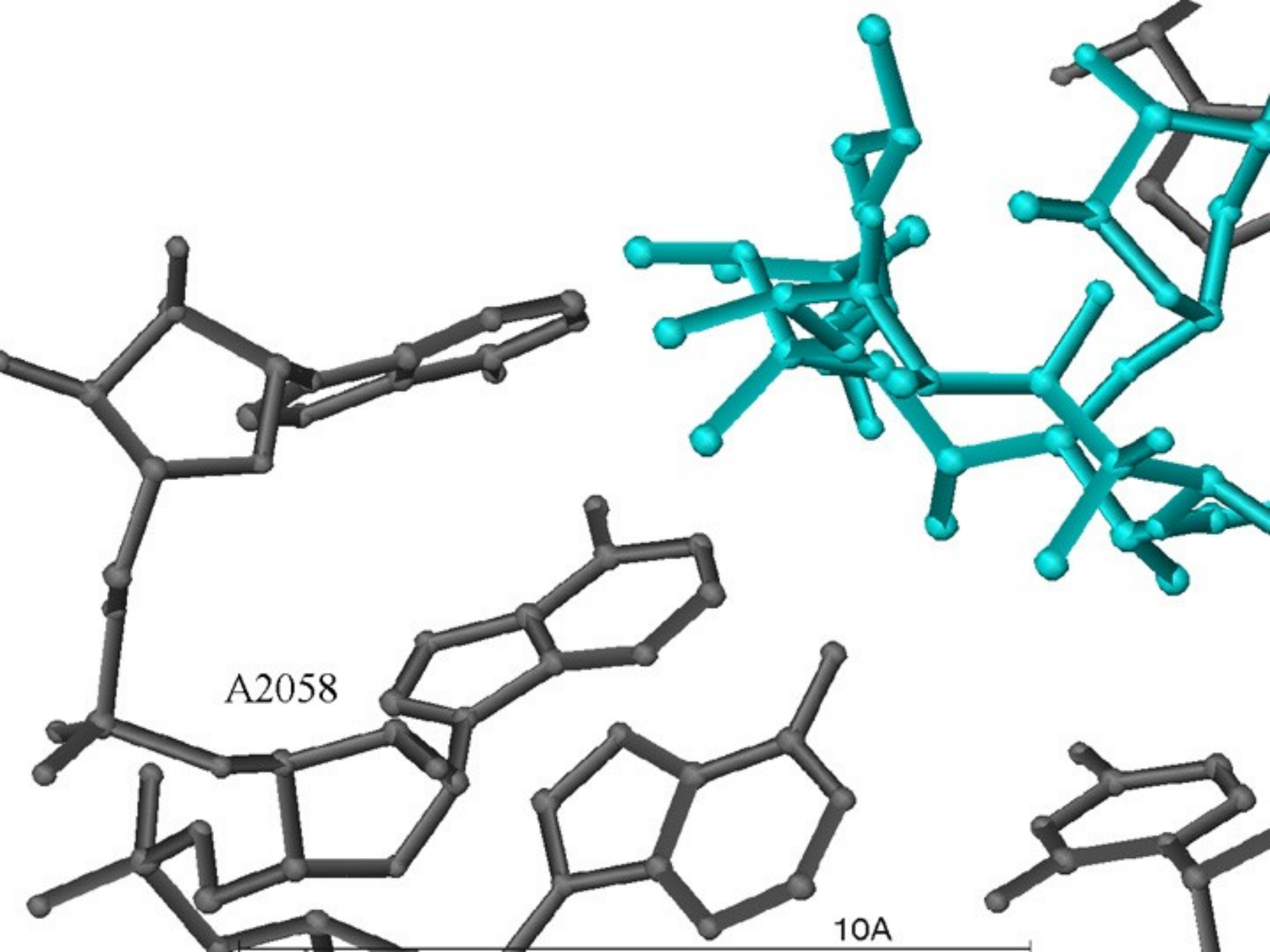

Clarithromycin
3.5 Å resolution
R/Rfree = 27.3/32.3


Roxithromycin
3.8 Å resolution
R/Rfree = 20.9/27.4

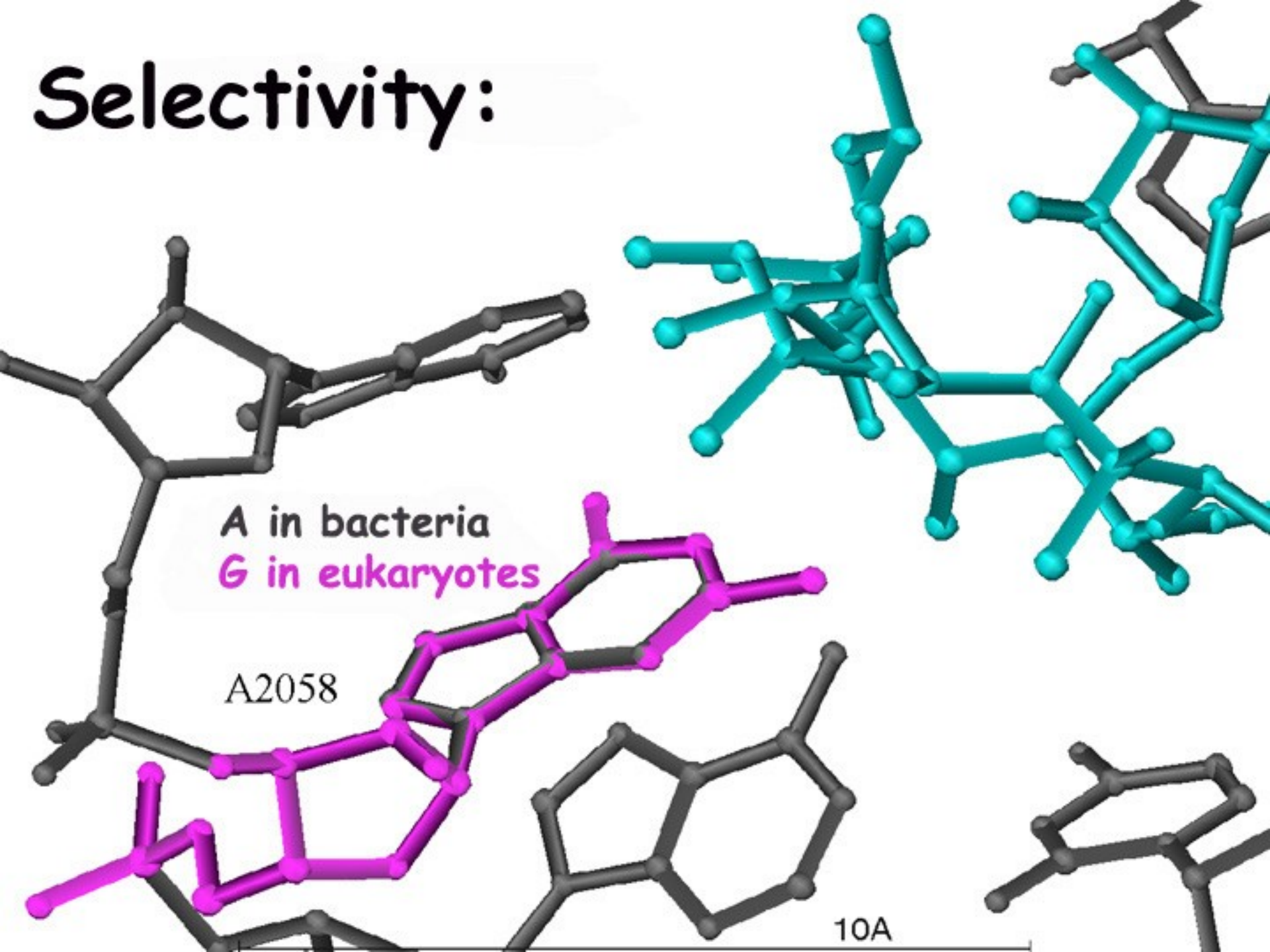
treatment of pneumonia

Erythromycin 500 mg x 4 times daily

Roxithromycin 150 mg x twice daily


A2058
10A

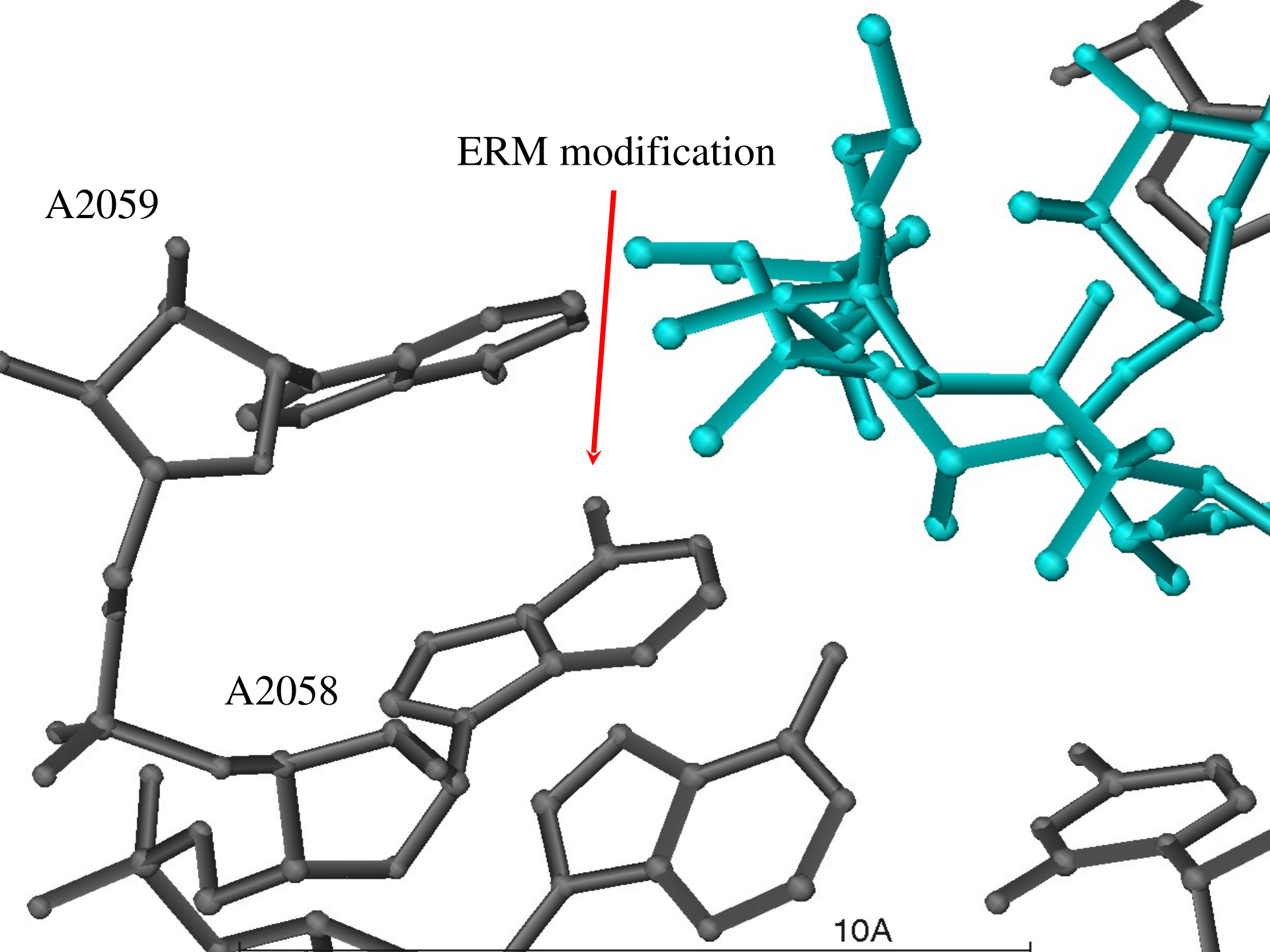
Selectivity:

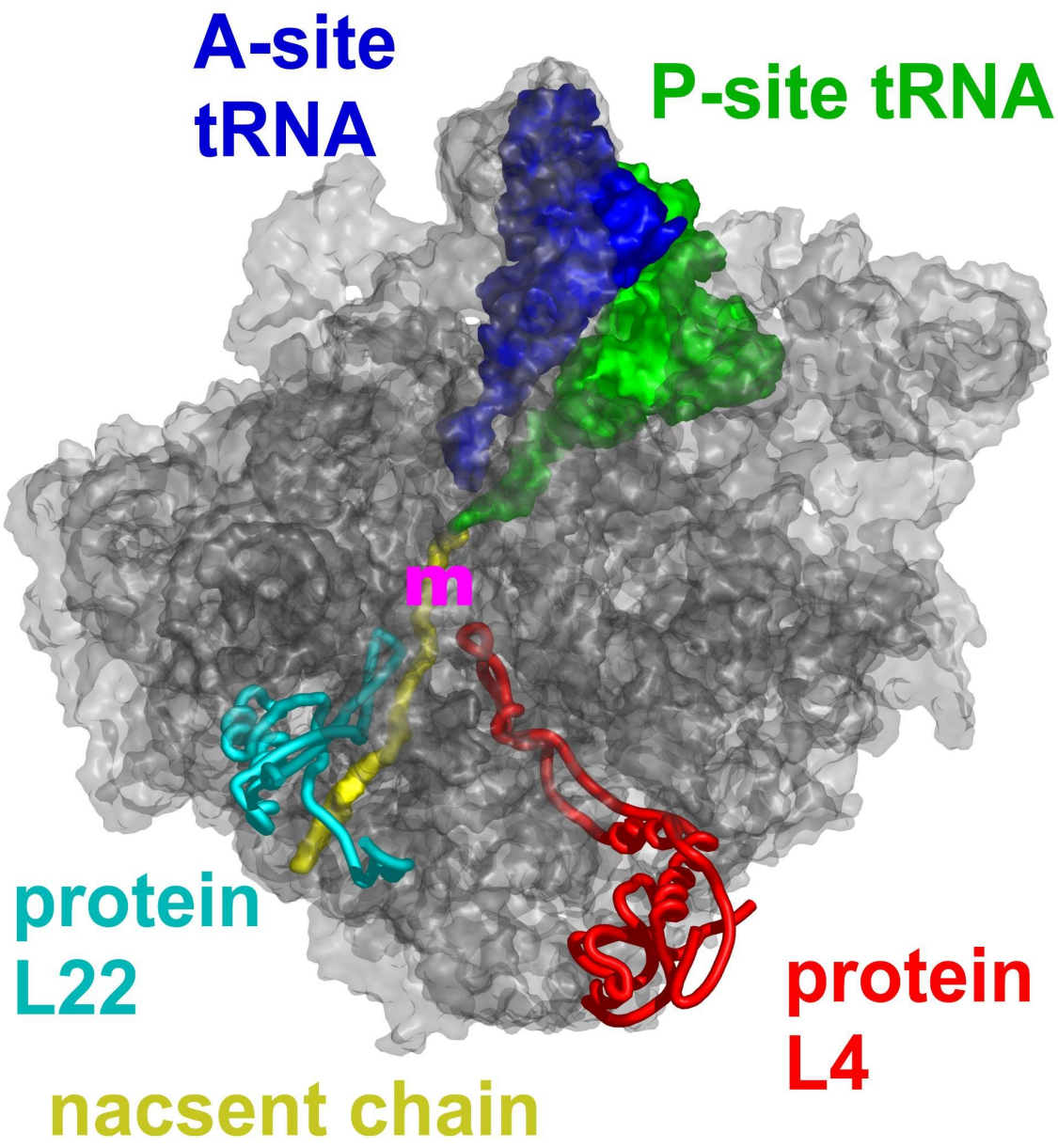


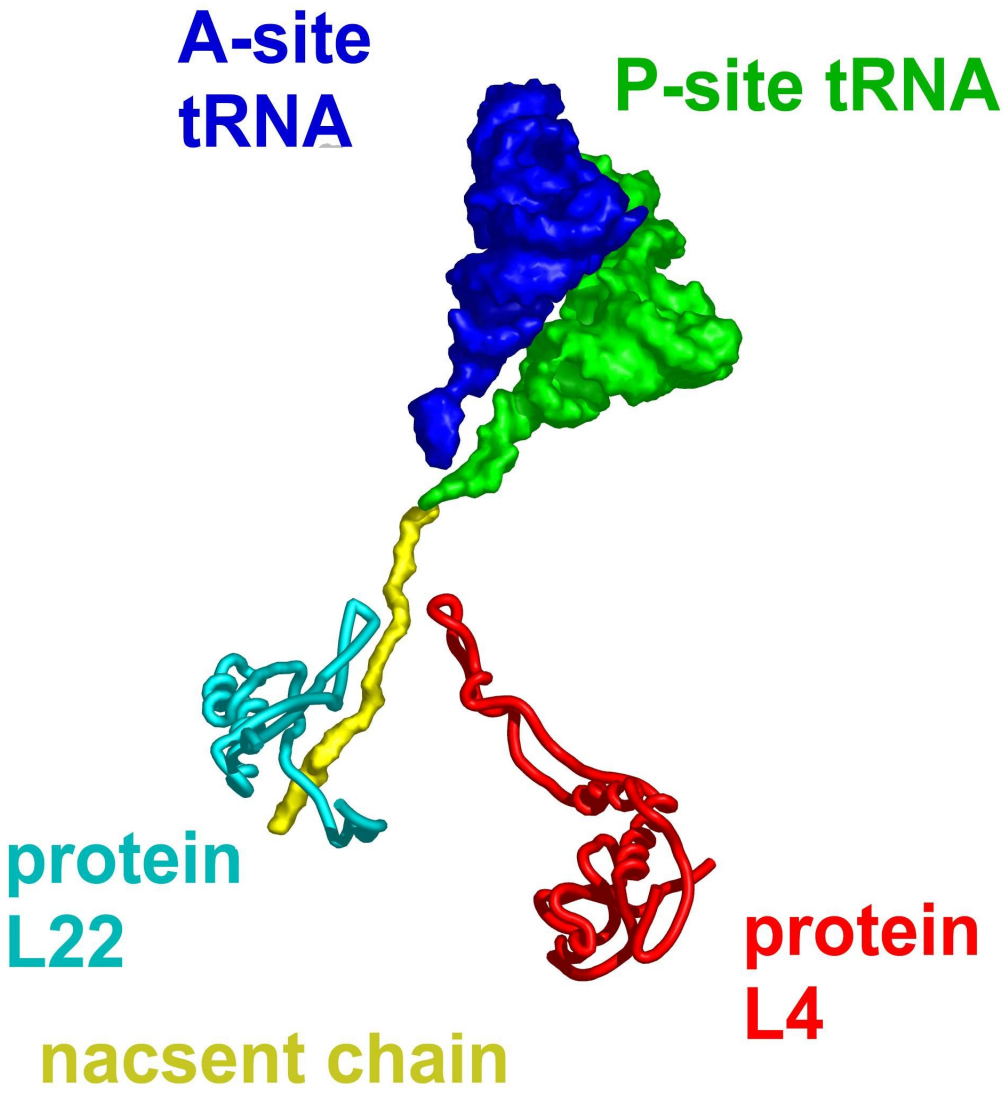
Resistance mechanisms exploiting 2058

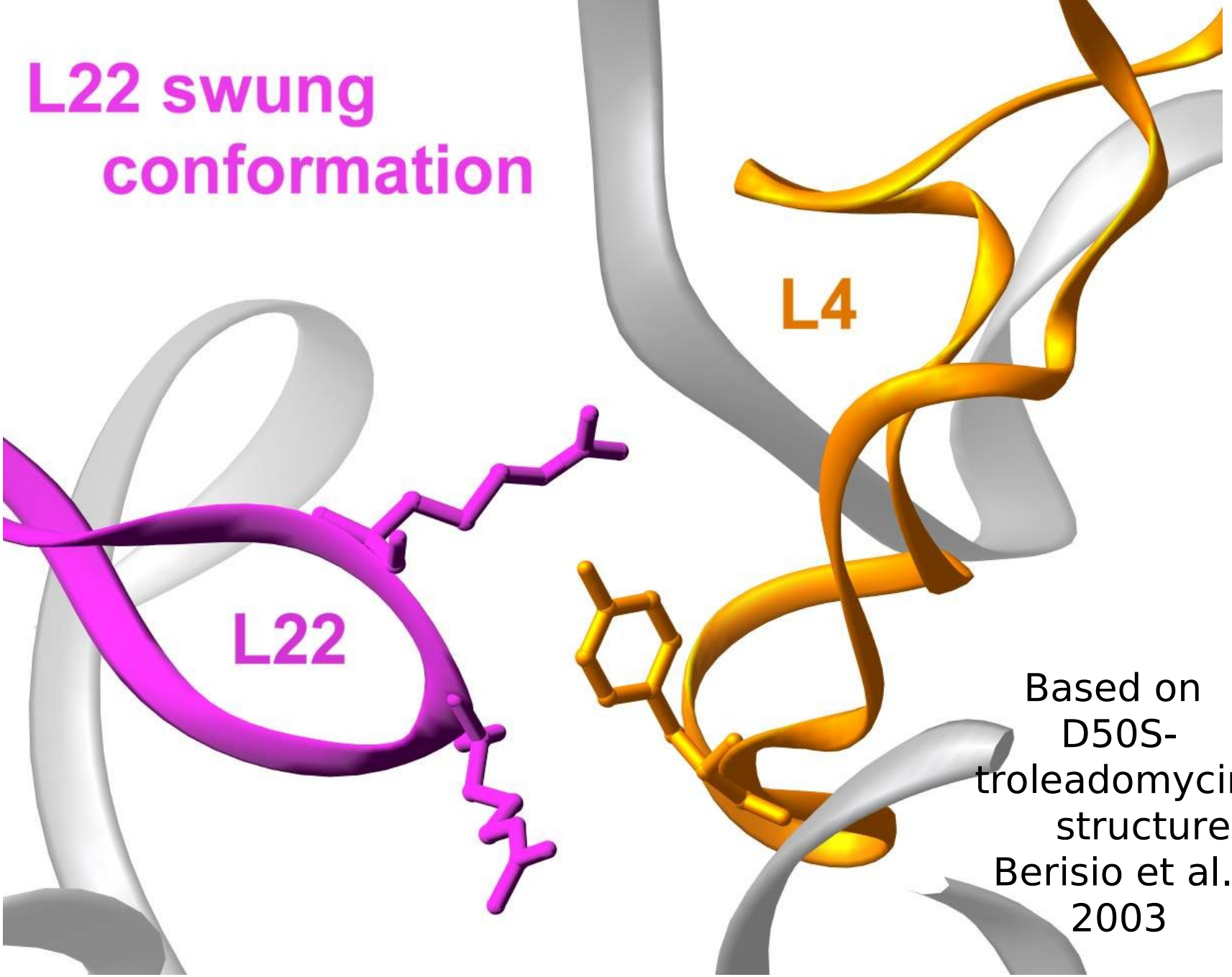
Erm methylation

A→ G mutation


ERM modification
A2059
A2058
10A

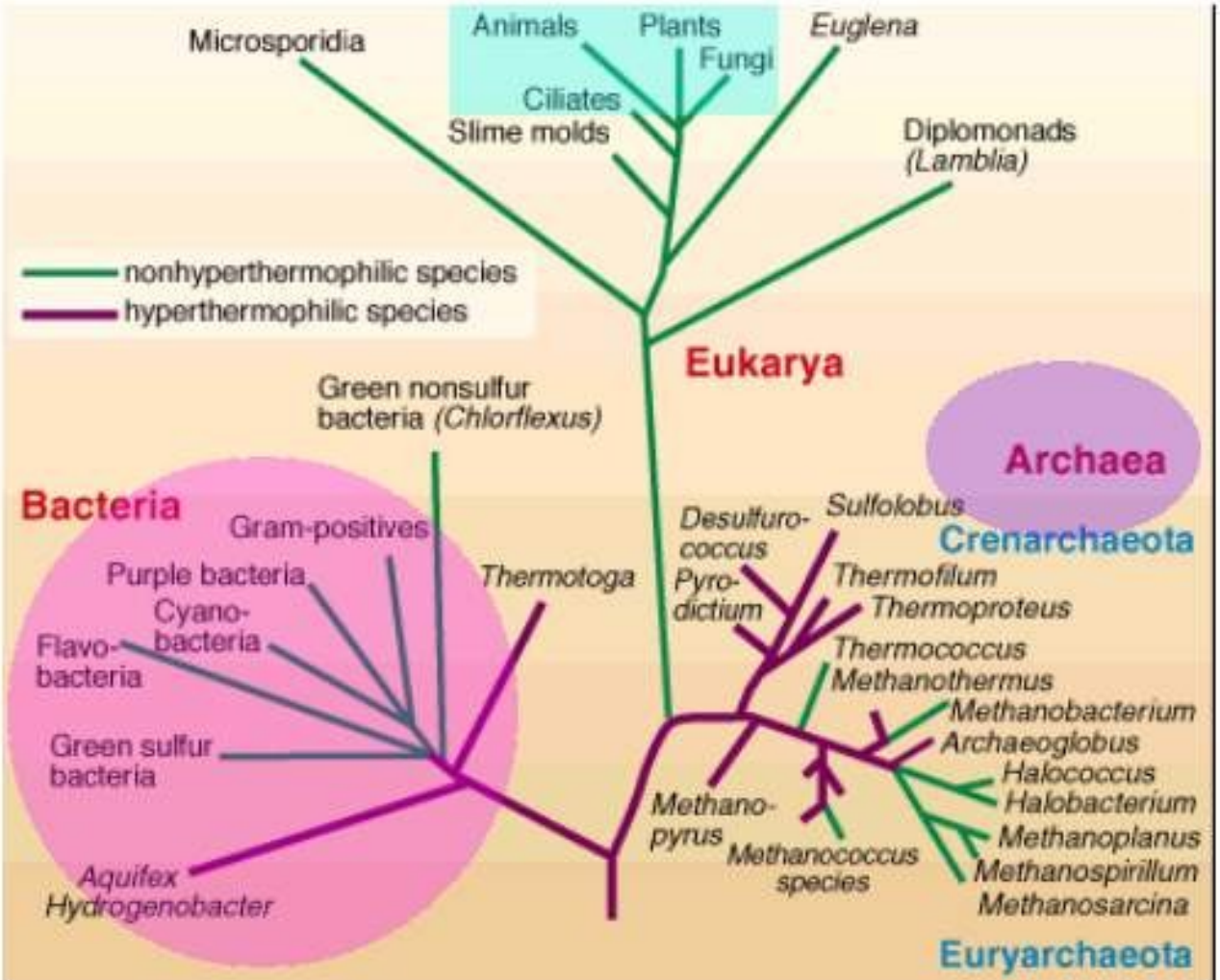

A-site
tRNA
P-site tRNA
m
protein
L22
protein
L4
nacsent chain


A-site
tRNA
P-site tRNA
protein
L22
protein
L4
nacsent chain



Based on D50S-troleadomycin structure, Berisio et al., 2003

The Tree of Life



Archaea - share properties with prokaryotes and eukaryotes

II

Bacteria

II

The large ribosomal subunit from Deinococcus radiodurans (D50S)

Harms et al., 2001

(Weizmann-MaxPlanck)

II

Human-beings

II

The large ribosomal subunit from Haloarcula marismortui (H50S)

Ban et al., 2000 (Yale Uni.)

Archaea - share properties with prokaryotes and eukaryotes

||

Bacteria

||

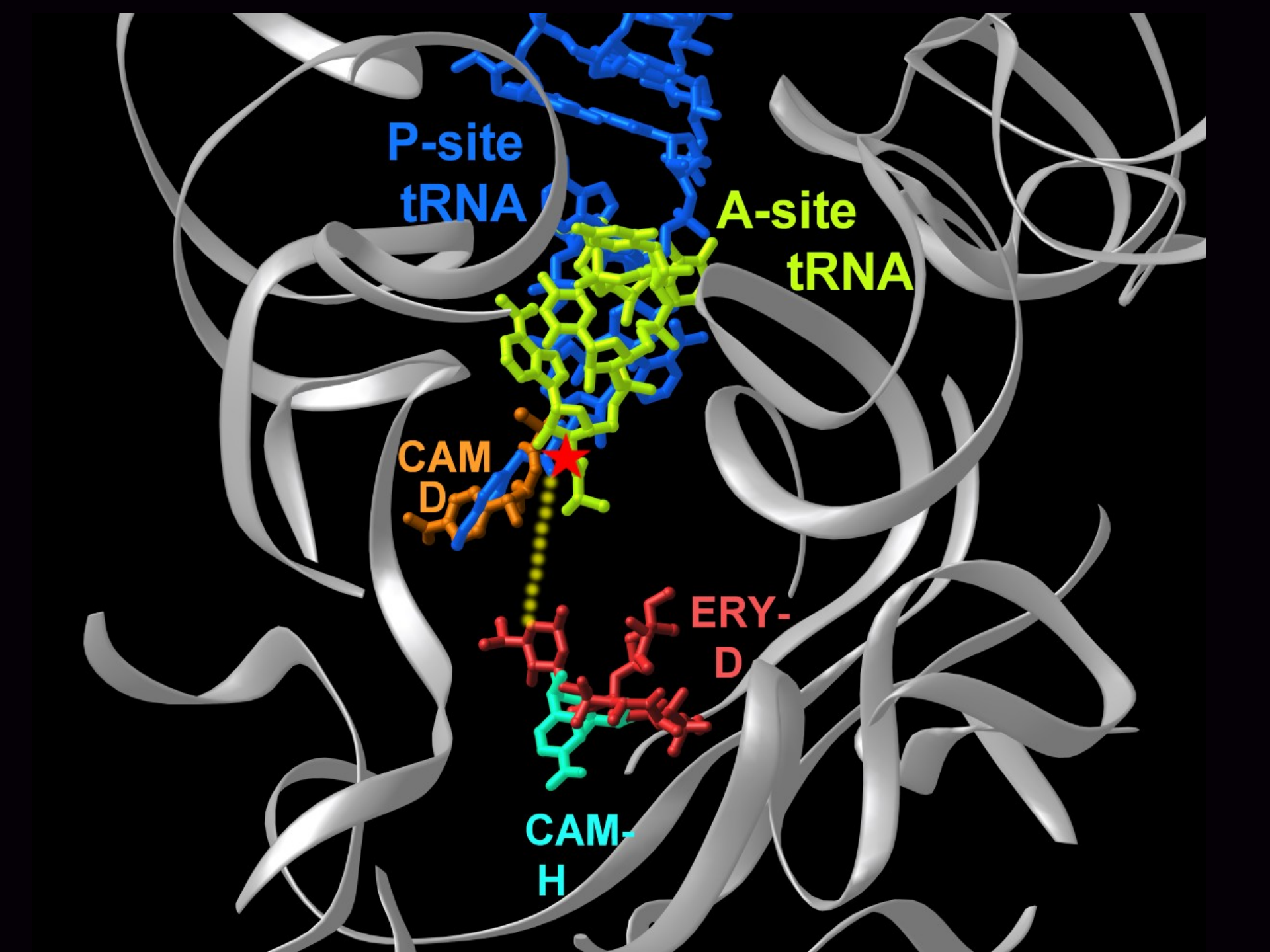
D (WI)

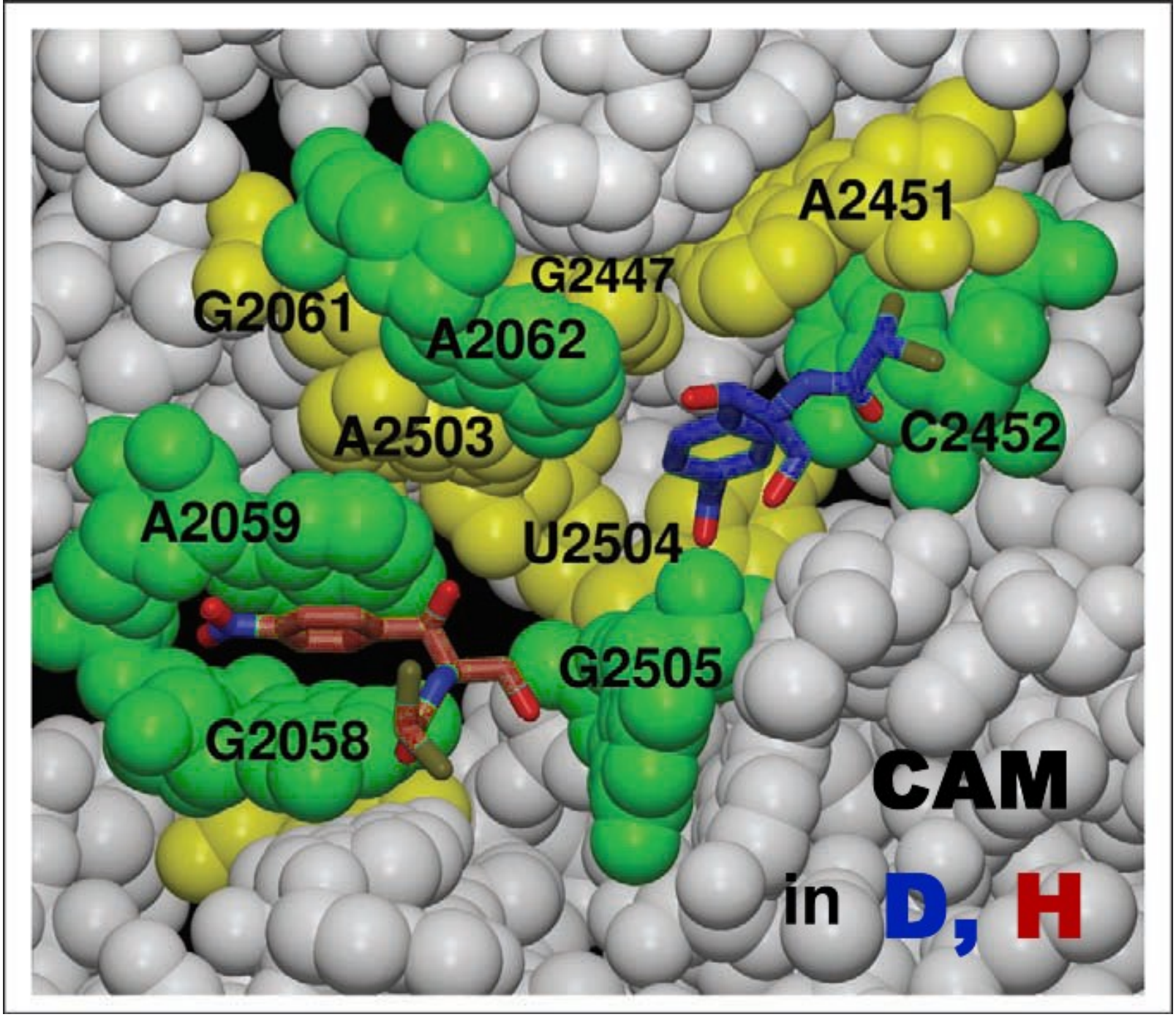
||

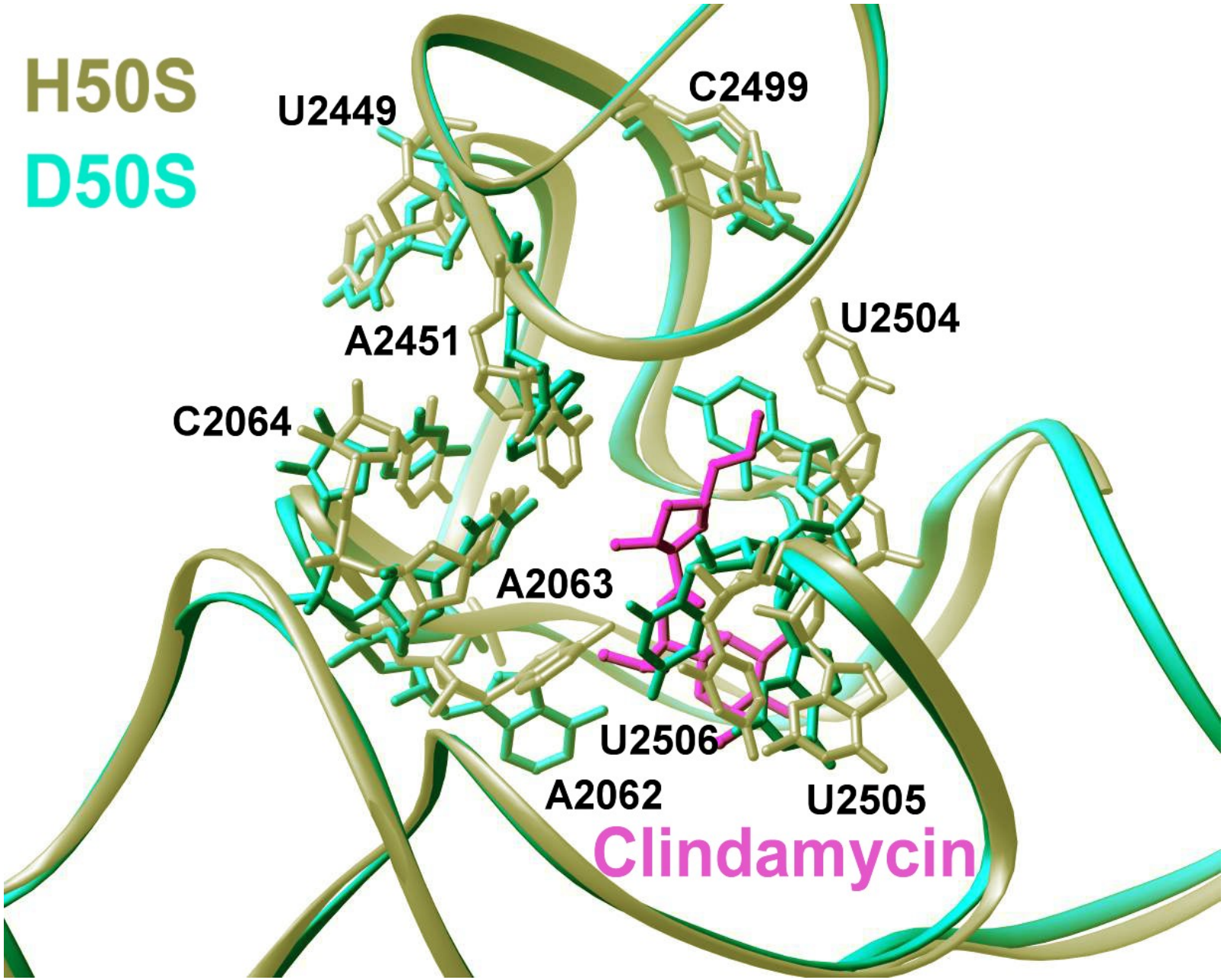
Human-beings

||

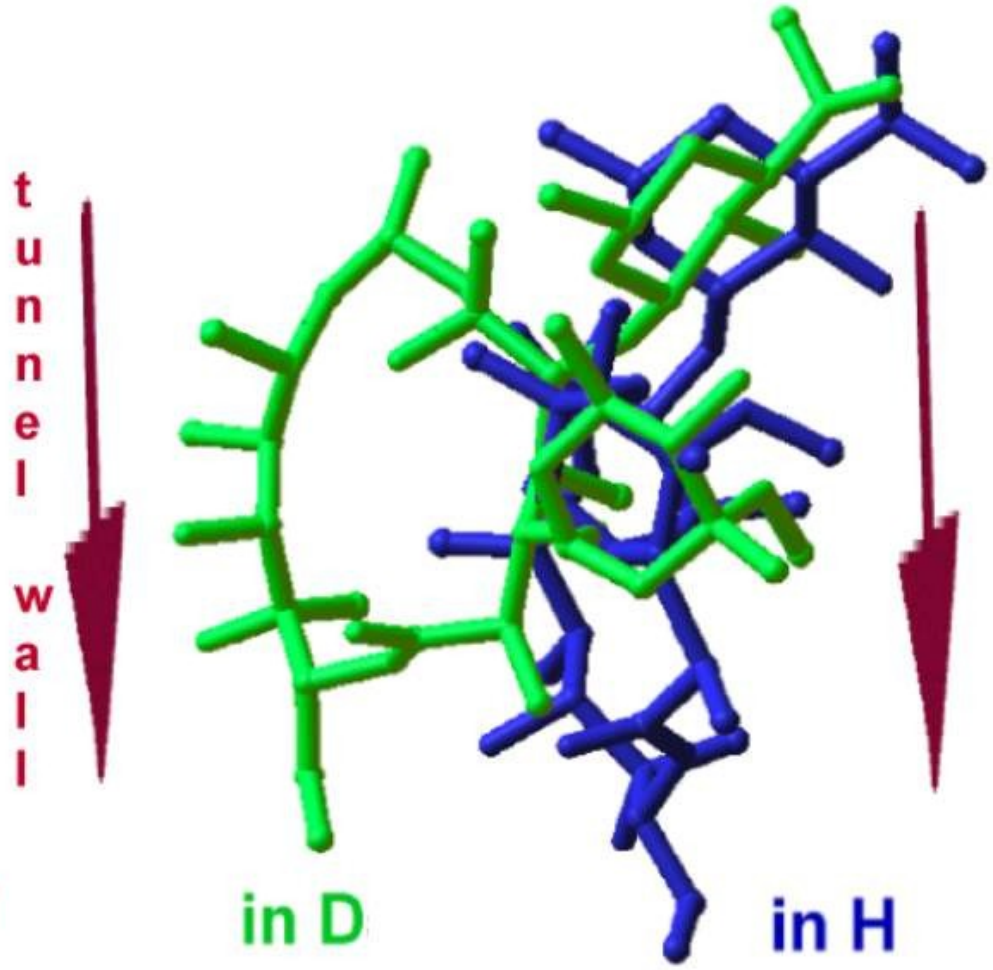
H(YALE)


P-site
tRNA
A-site
tRNA
CAM
D
ERY-
D
CAM-
H


A2451
G2447
G2061
A2062
A2503
C2452
A2059
U2504
G2505
G2058
CAM
in D, H

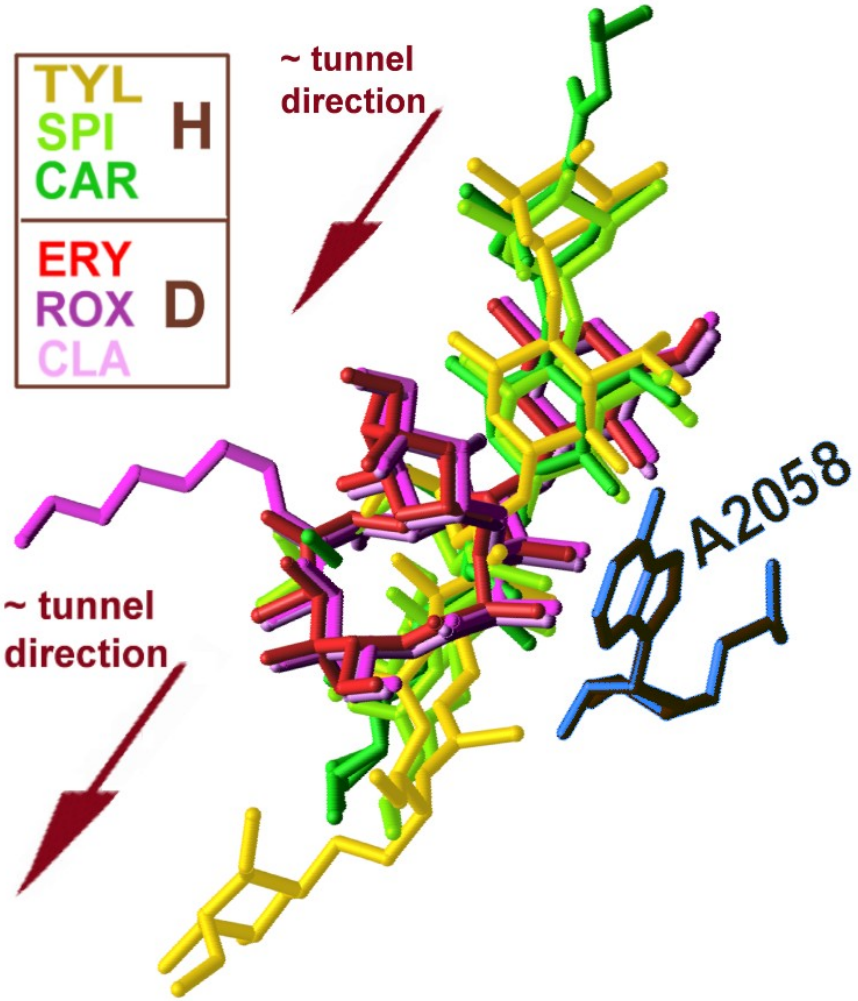

H50S
D50S
U2449
C2499
A2451
U2504
C2064
A2063
U2506
A2062
U2505
Clindamycin

Macrolides having a lactone ring of 15 or 16 members can bind to ribosomes with G at position 2058, when applied at higher than clinically relevant concentrations

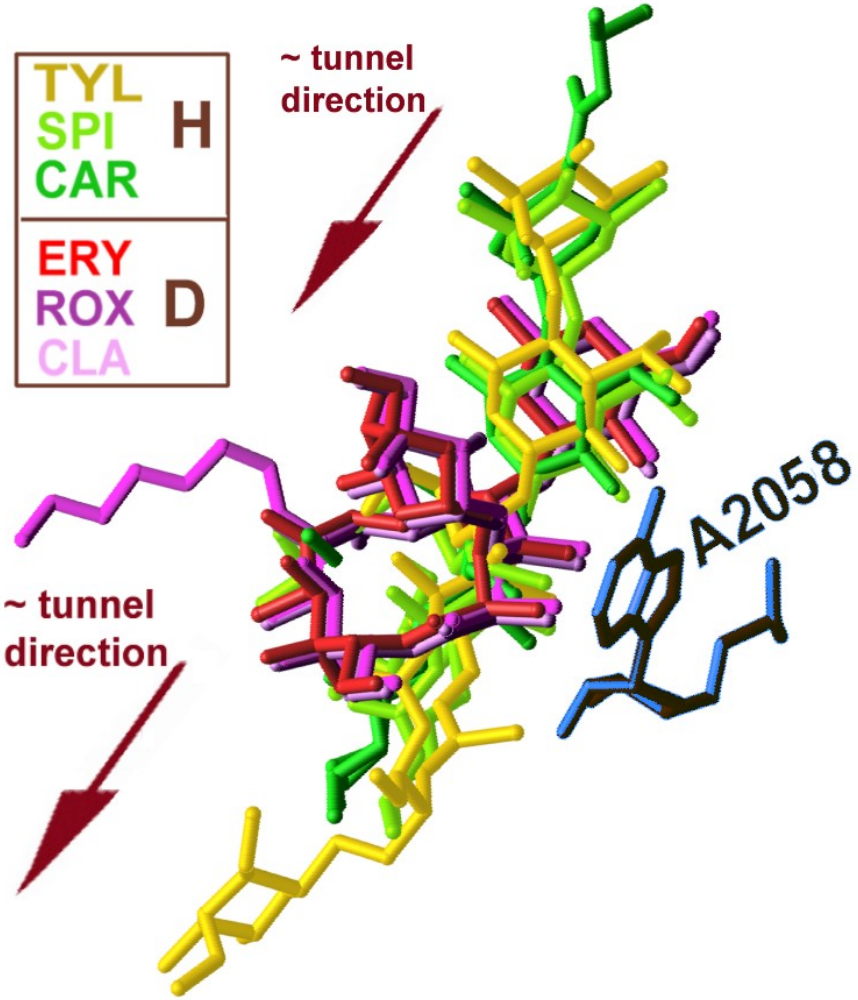


16-member-ring macrolides bind also to ribosomes with G in position 2058

.



16-member-ring macrolides bind also to ribosomes with G in position 2058, albeit in a fashion that hardly block the tunnel



Binding is not synonymous to inhibitory activity

2058 A/G determines **If** binding occurs

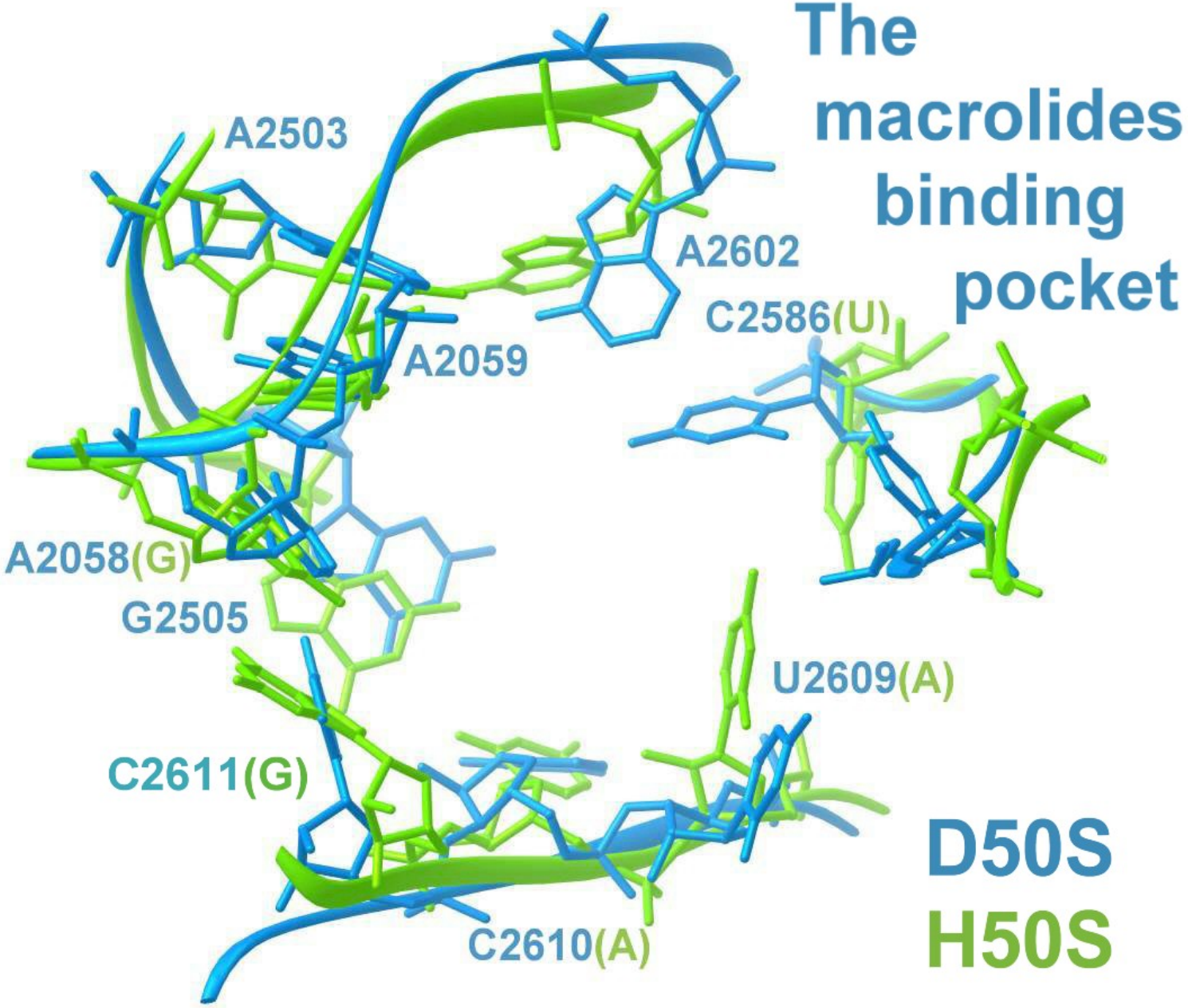
may be overcome

Pocket constituents determines **how**

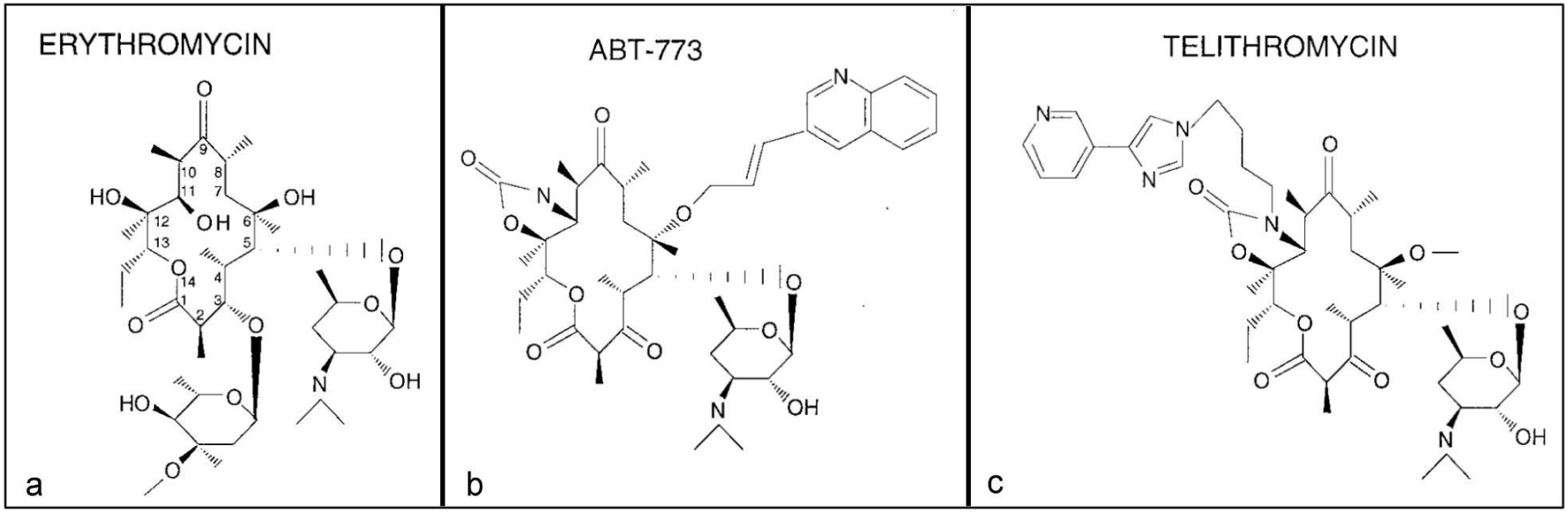
= binding mode

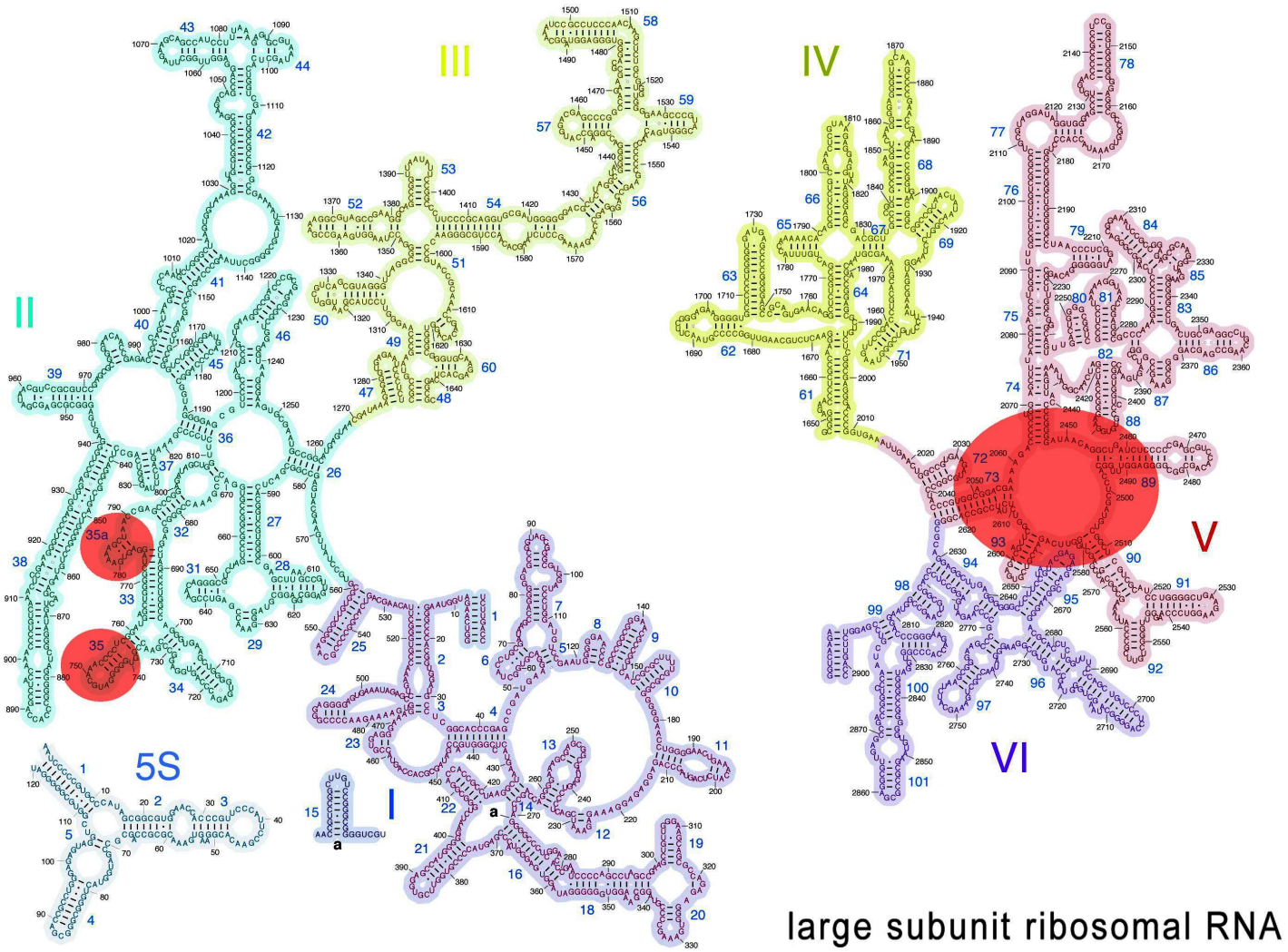
= efficiency of inhibition

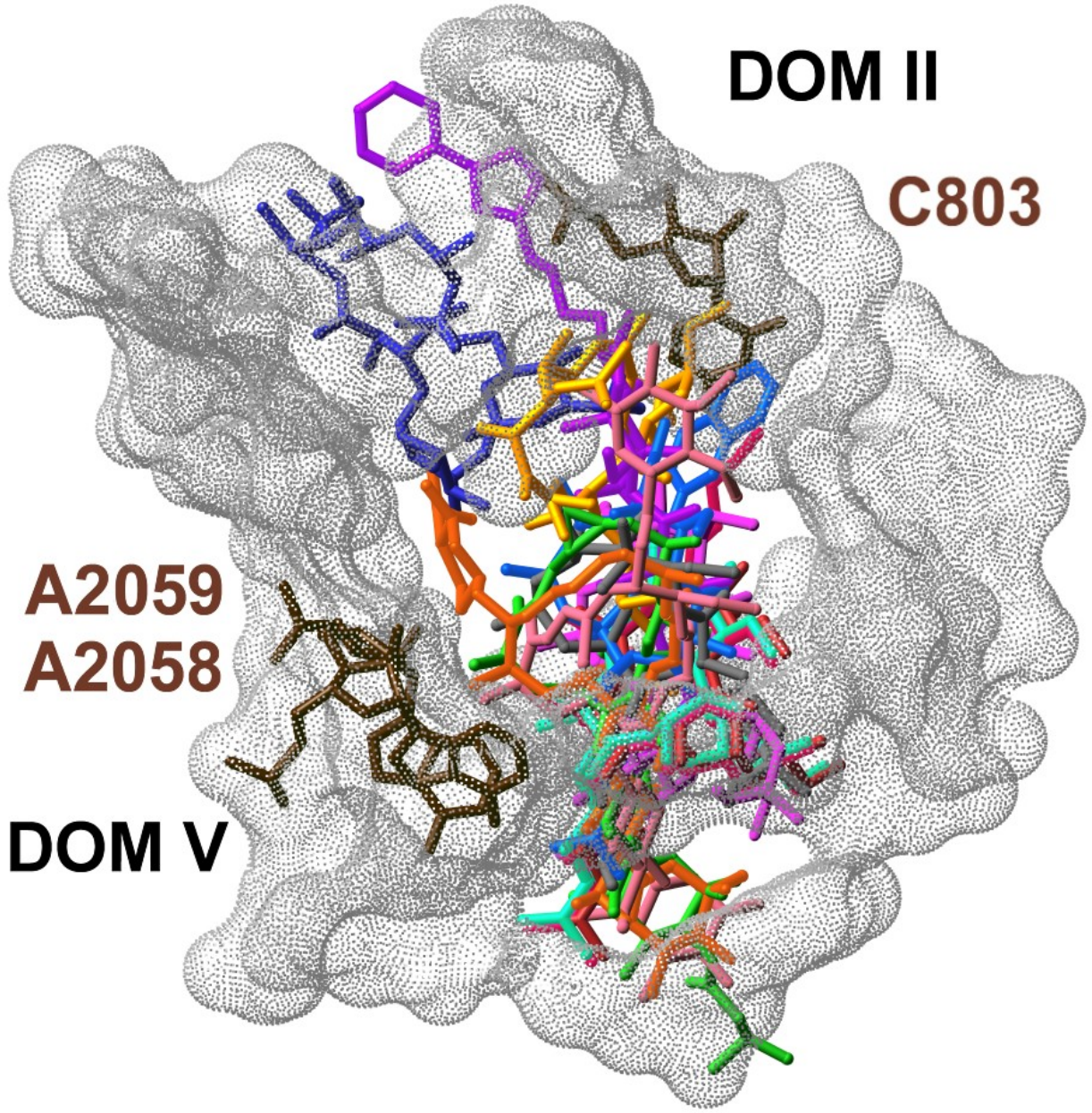
= clinical usefulness

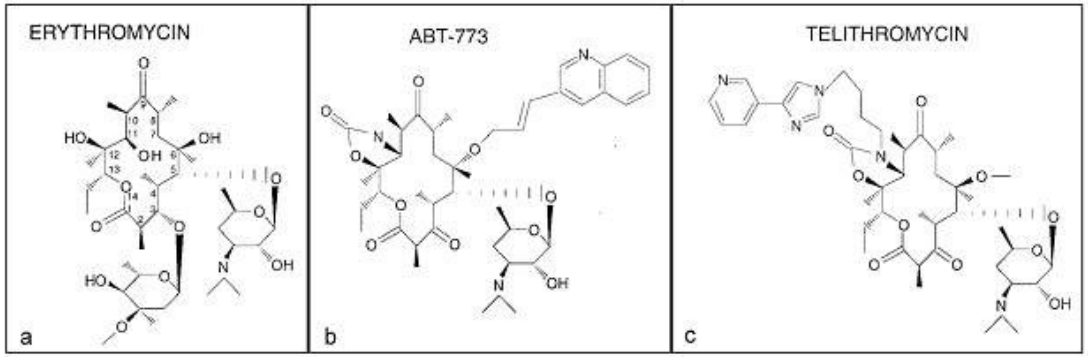

The macrolides binding pocket
A2503
A2602
C2586(U)
A2059
A2058(G)
G2505
U2609(A)
C2611(G)
C2610(A)
D50S
H50S

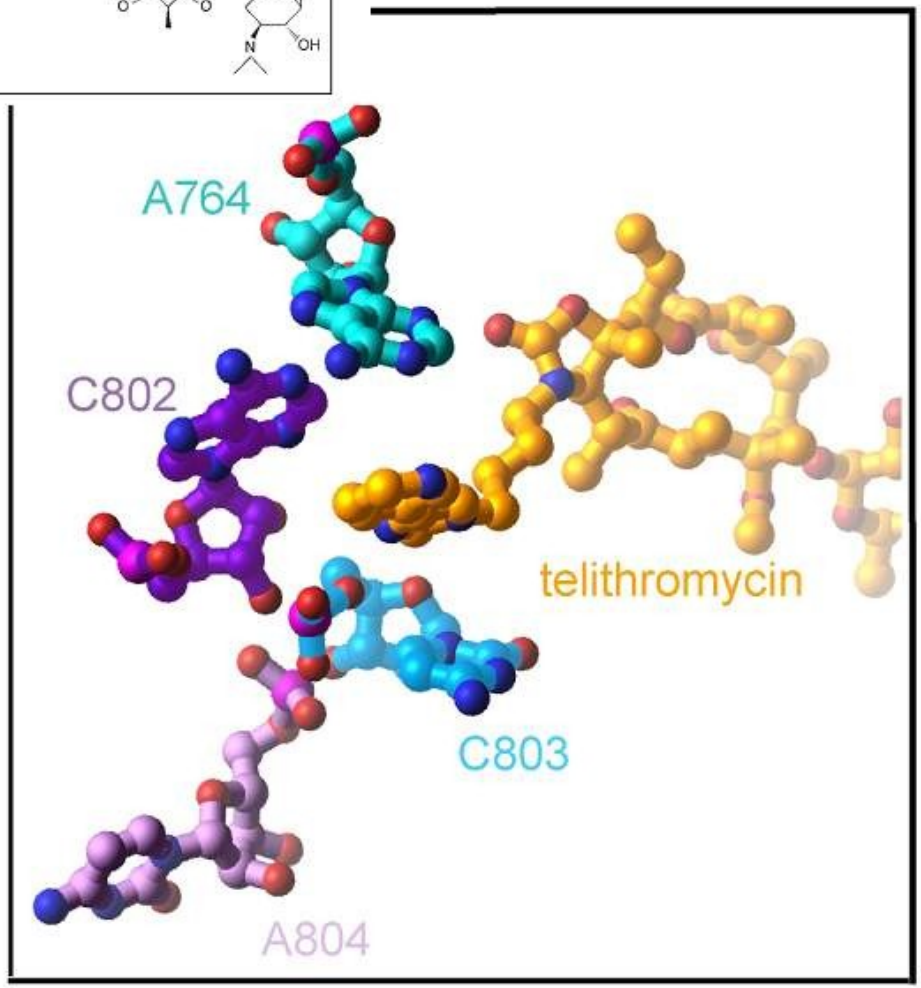
Ketolides – mono-sugar macrolides with extended arms and a keto group




I
II
III
IV
V
VI
5S
large subunit ribosomal RNA


DOM II
C803
A2059
A2058
DOM V

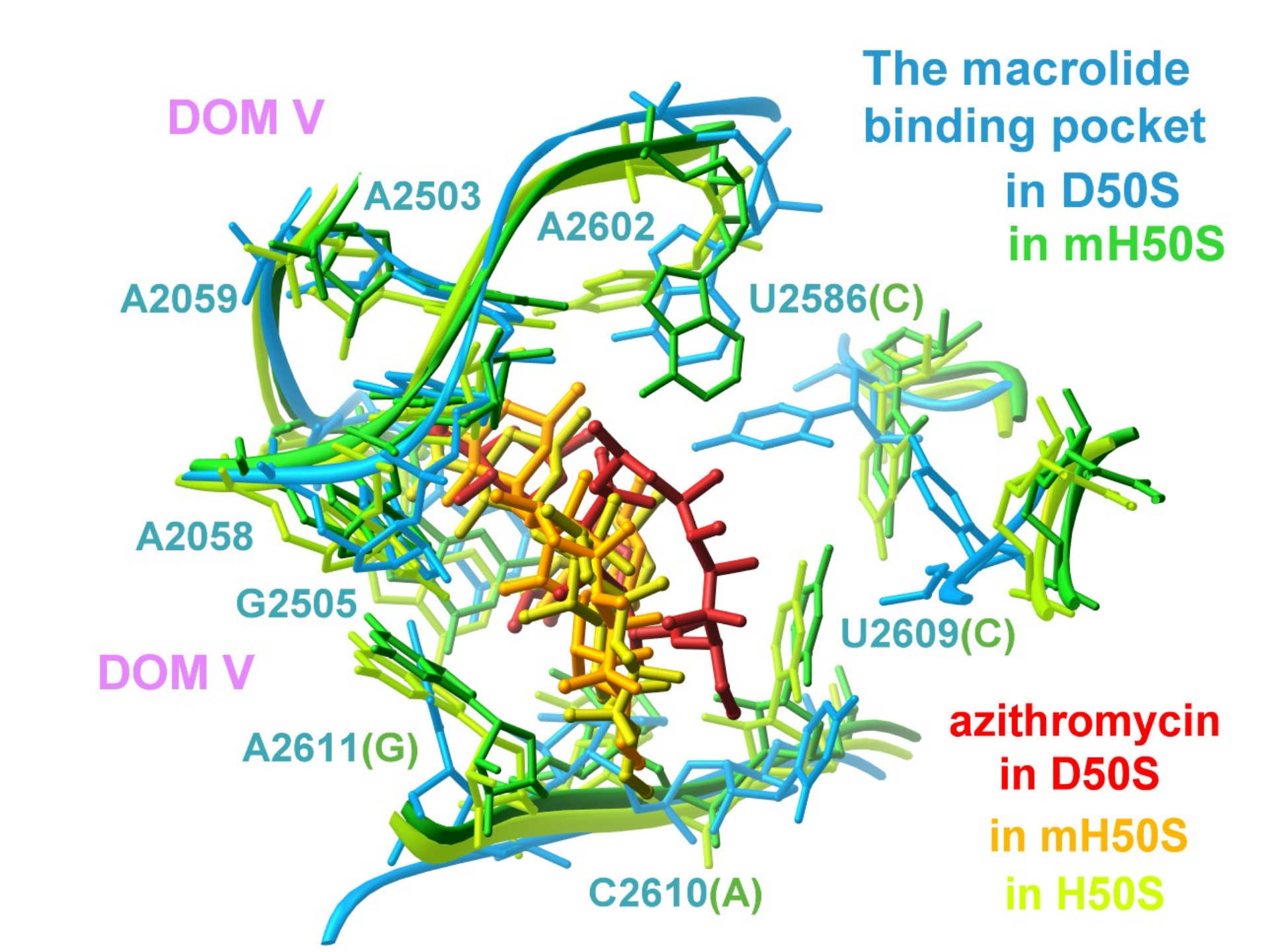

ERYTHROMYCIN
ABT-773
TELITHROMYCIN
a
b
c

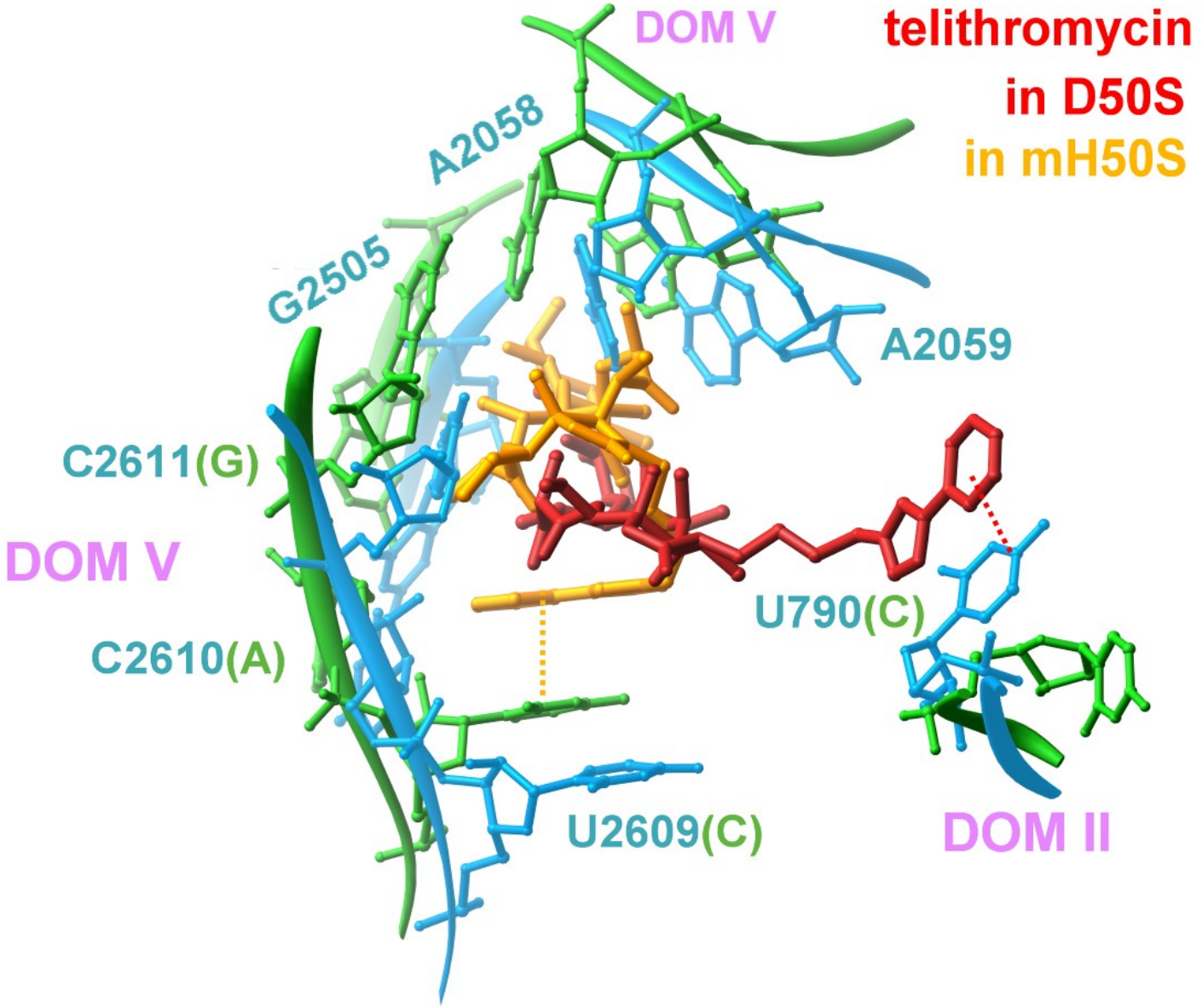

A764
C802
telithromycin
C803
A804

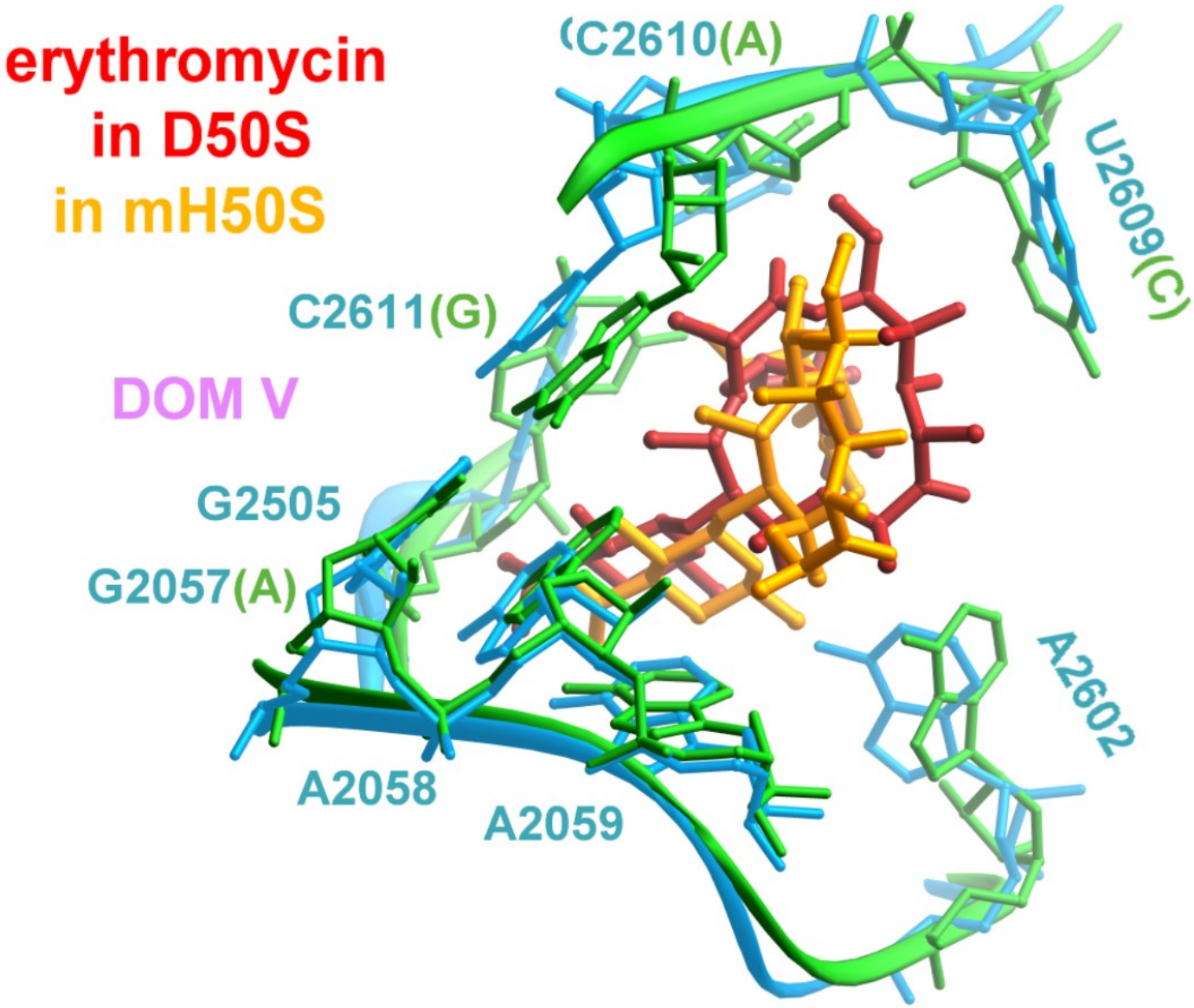
Puzzling facts

- **The impressive gain in binding affinity obtained by G->A in nt. 2058 in *H. marismortui* (H50S), was not accompanied by differences in binding mode.**

- **In H50S complexes, all macrolide antibiotics maintain their 3D structure, as determined in isolation (namely in ribosome-free environment).**


The macrolide binding pocket
in D50S
in mH50S
DOM V
A2503
A2602
A2059
U2586(C)
A2058
G2505
U2609(C)
DOM V
A2611(G)
azithromycin
in D50S
in mH50S
in H50S
C2610(A)


DOM V
telithromycin
in D50S
in mH50S
A2058
G2505
A2059
C2611(G)
DOM V
U790(C)
C2610(A)
U2609(C)
DOM II


erythromycin
in D50S
in mH50S
C2610(A)
U2609(C)
C2611(G)
DOM V
G2505
G2057(A)
A2602
A2058
A2059

G2058-> A mutation

The G2058A mutation in *Haloarcula marismortui* ribosome was found to be most beneficial because it:

- confirmed that 2058 is the key player in macrolide binding

- clarified the distinction between mere binding and antibiotics' inhibitory effectiveness

The sole A→G Mutation of 2058

Provided structural insight into an intriguing question, of utmost importance for drug development:

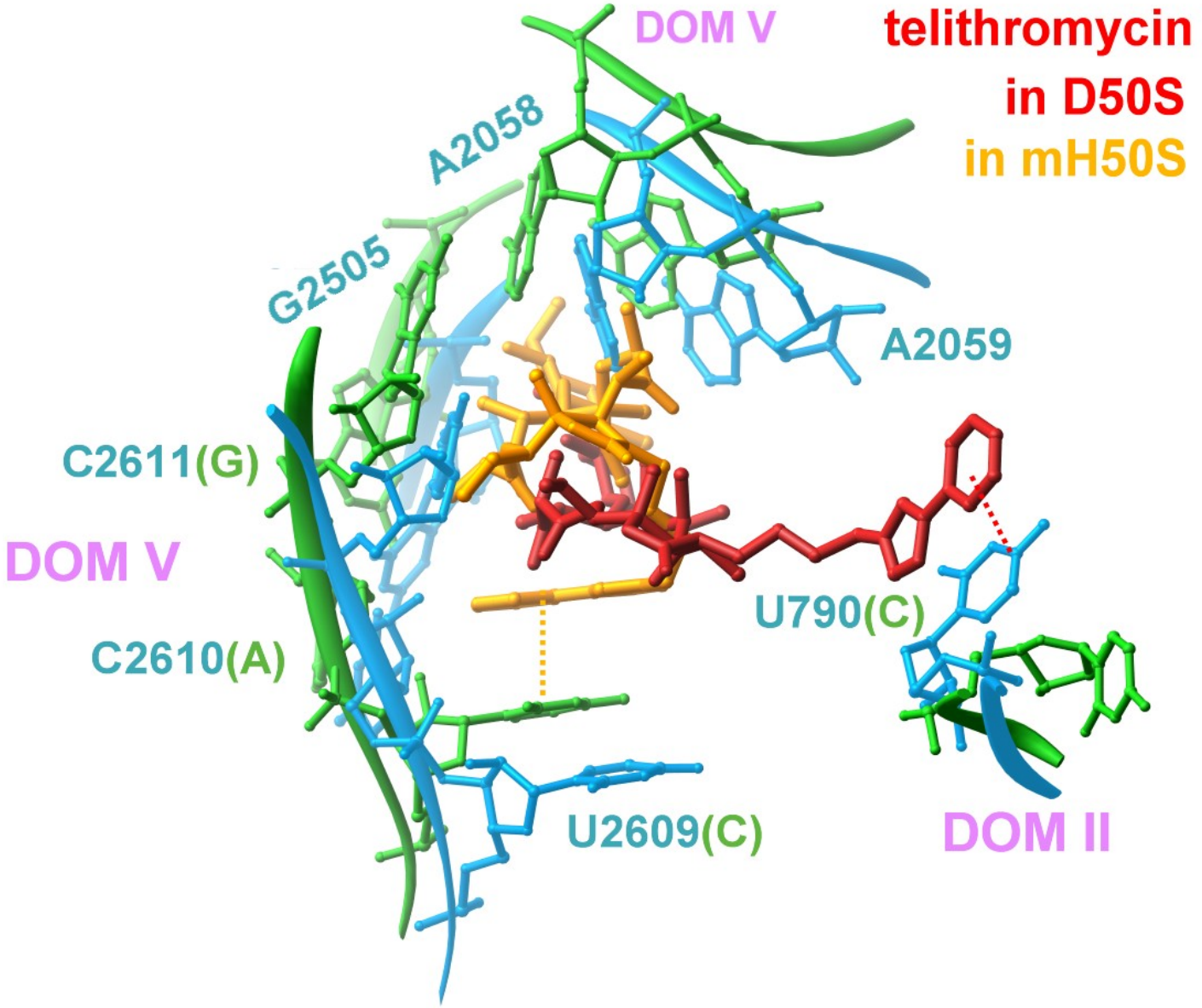
What is the correlation between antibiotics' "minimum free-energy conformation", determined in ribosome-free environment, and its therapeutic effectiveness?

Our results show that the ribosome environment influences the antibiotics conformation

Solvent affects the conformation of virginiamycin M1

Dang, J., Bergdahl, M., Separovic, F., Brownlee, R. T., and Metzger, R. P. (2004) Org Biomol Chem. 2, 2919

We now report the results of high resolution 2D NMR experiments that show that the conformation of VM1 in dimethyl sulfoxide and methanol differs from both that in chloroform solution and in the bound form.


DOM V
telithromycin
in D50S
in mH50S
A2058
G2505
A2059
C2611(G)
DOM V
U790(C)
C2610(A)
U2609(C)
DOM II

More puzzling facts

- **A “permitted” double mutation A--U -> C--G led to differences in drug susceptibility.**

- **These differences were observed in several species, for telithromycin and not for erythromycin, thus implying differences in binding modes.**

More puzzling facts

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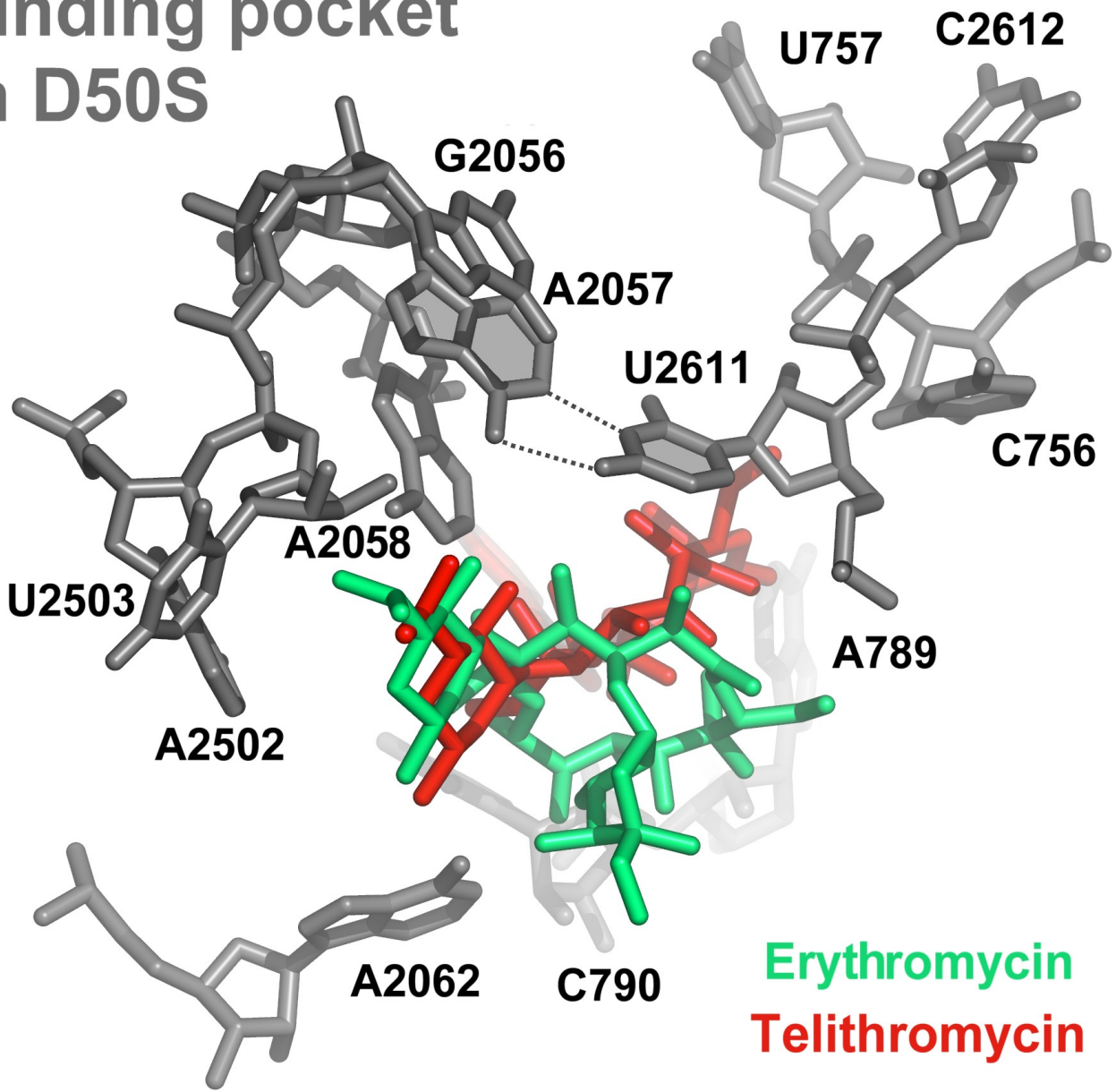
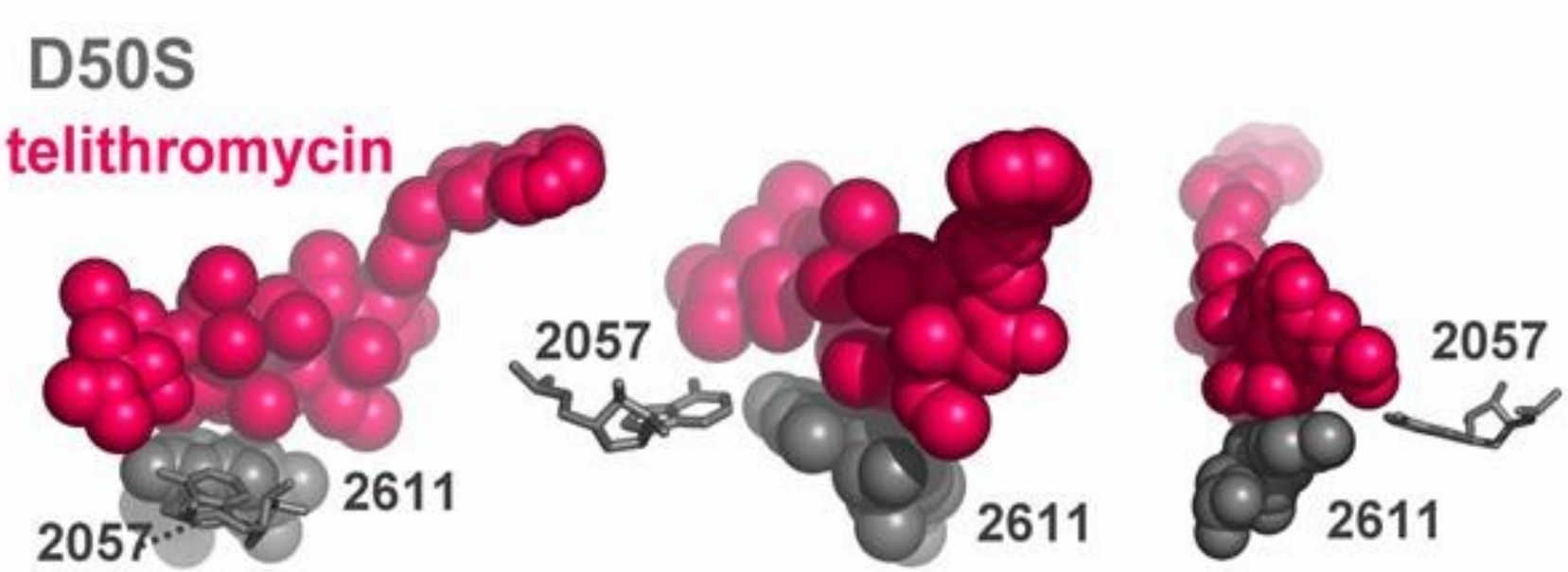
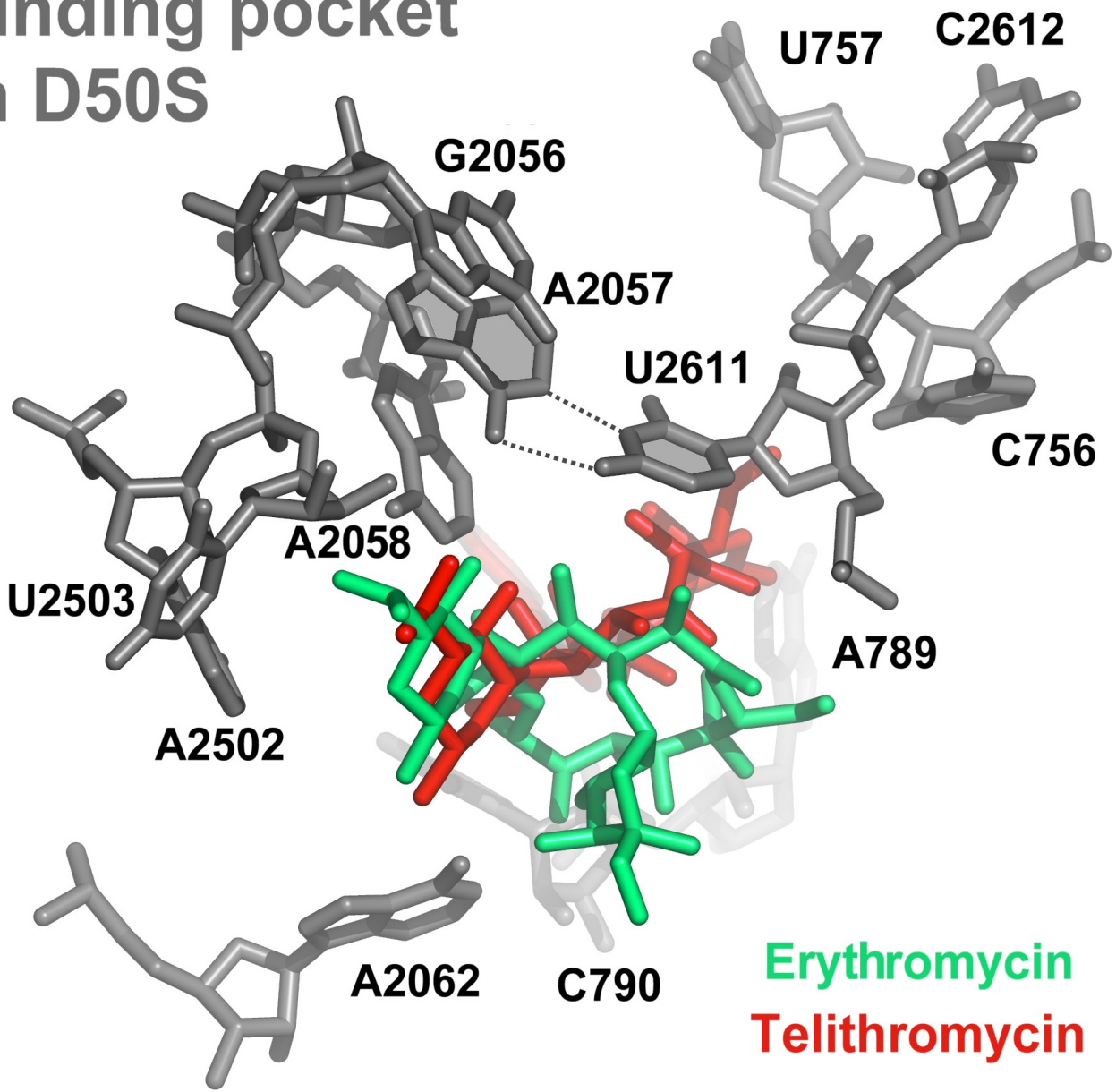
- **These differences were observed in several species, for telithromycin and not for erythromycin, thus implying differences in binding modes.**

The macrolide binding pocket in D50S




D50S
telithromycin
2057
2611
2057
2611
2057
2611

The macrolide binding pocket in D50S



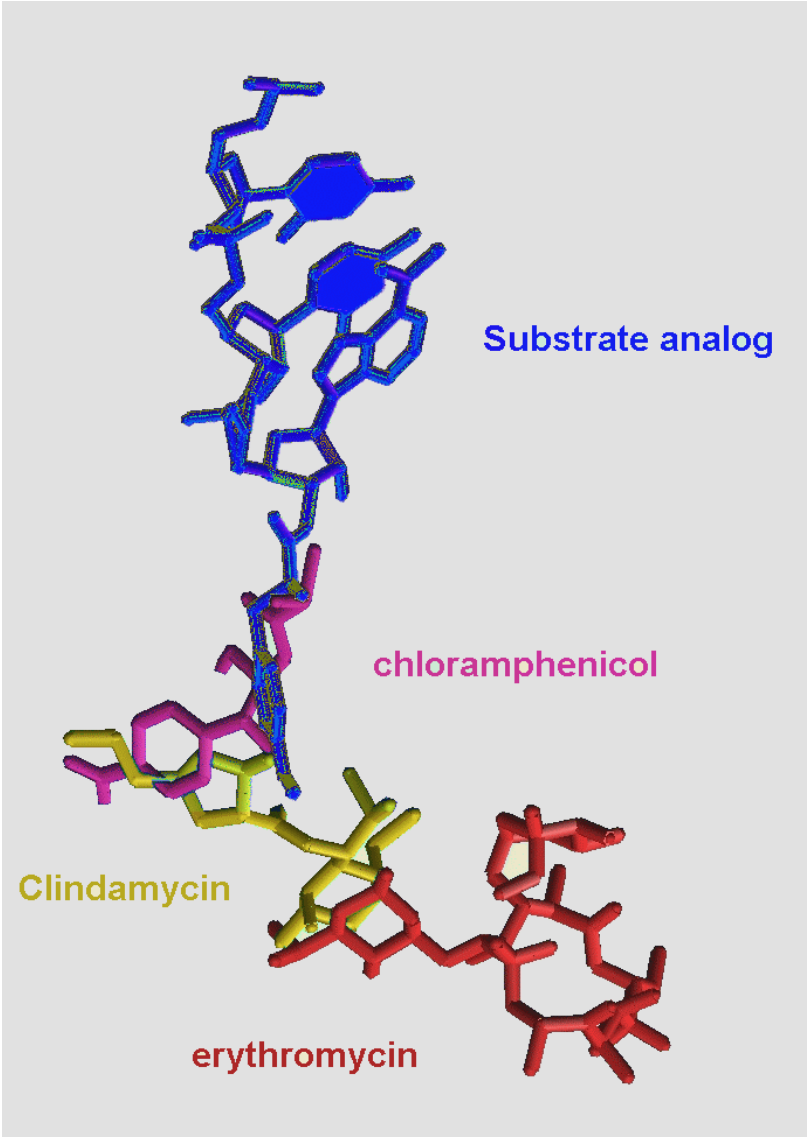
Species specificity

- These results explain the variability is susceptibility of different pathogens to antibiotics, even when seemingly identical resistance mechanisms are acquired.

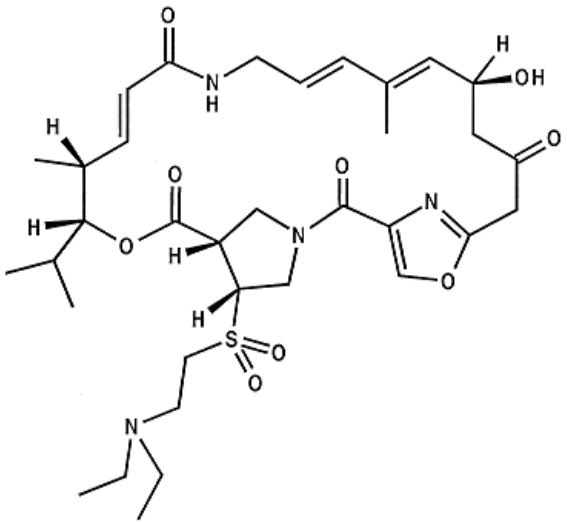
- Hence, manifesting the need to investigate various pathogens.

From PTC into the tunnel

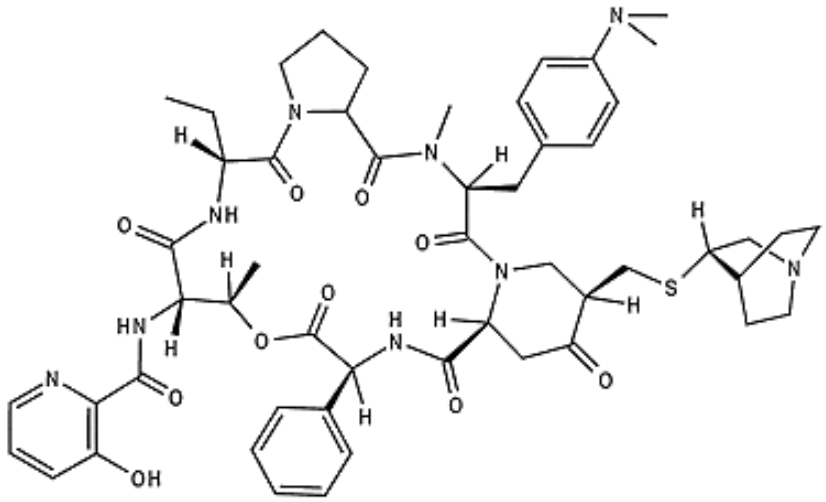
Chloramphenicol-PTC
Clindamycin
Erythromycin- tunnel



Synercid



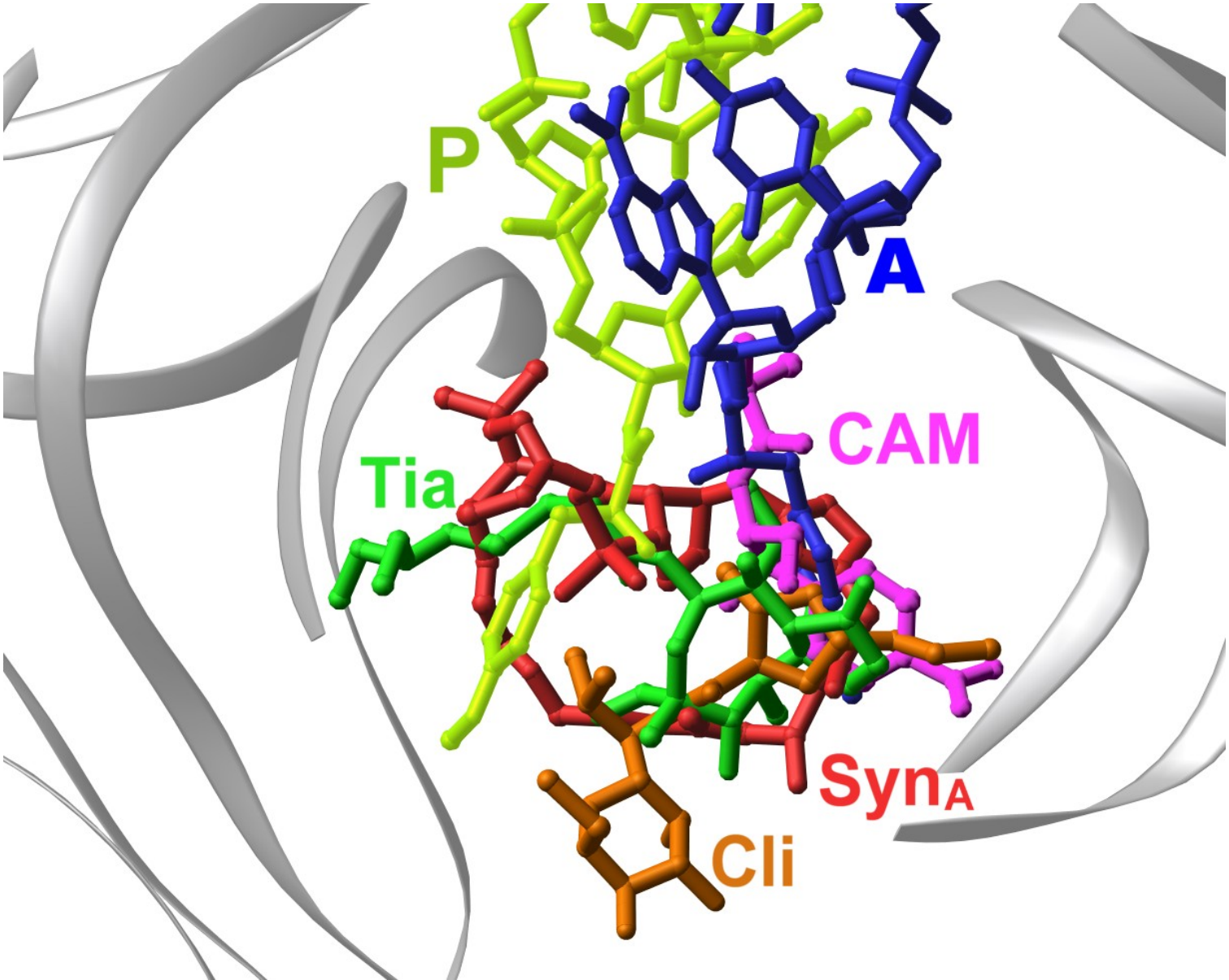
Streptogramin $_{A}$
dalfopristin

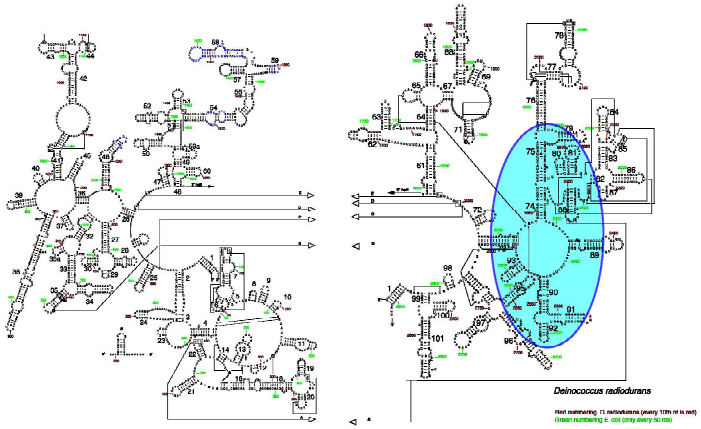


Streptogramin $_{B}$
quinpristin

Etymology: New Latin *synergida,* from Greek *synergos* working together

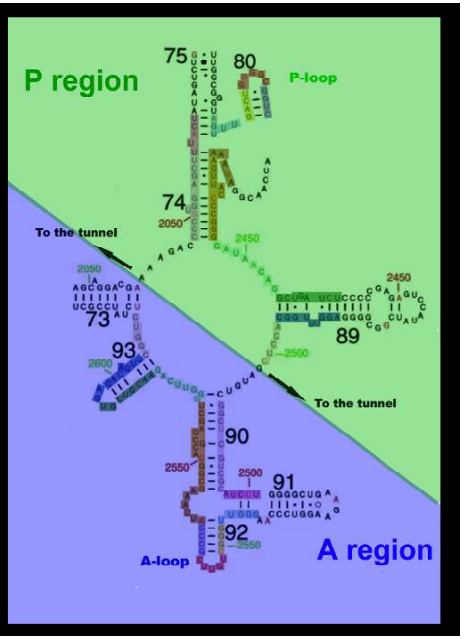
PTC antibiotics



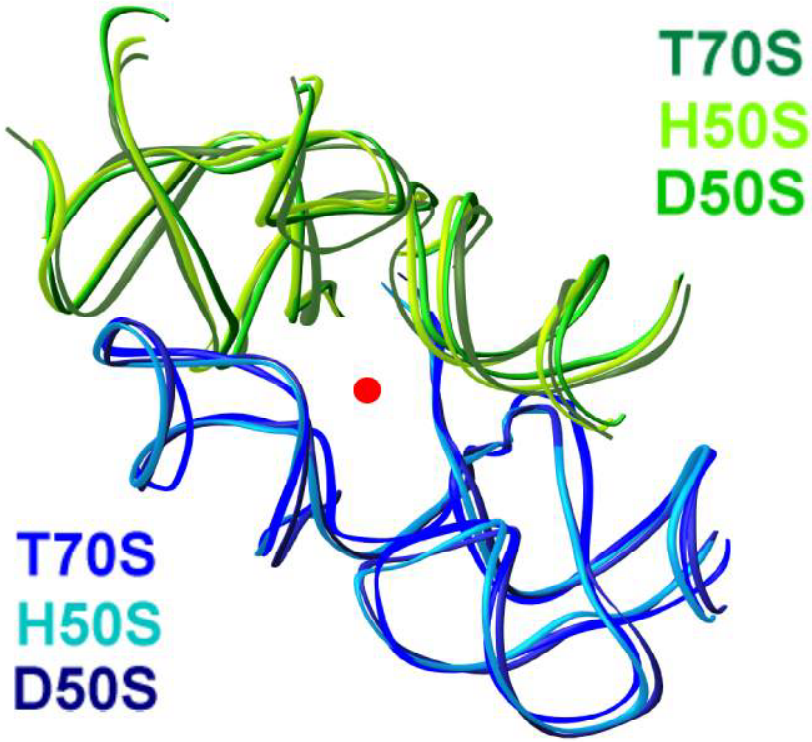


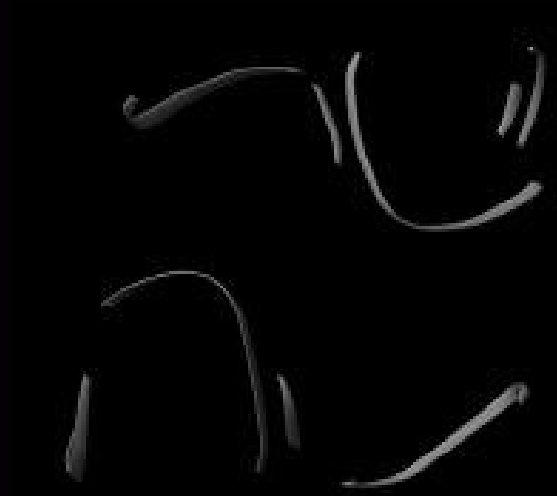
the two dimensional diagram of D50S

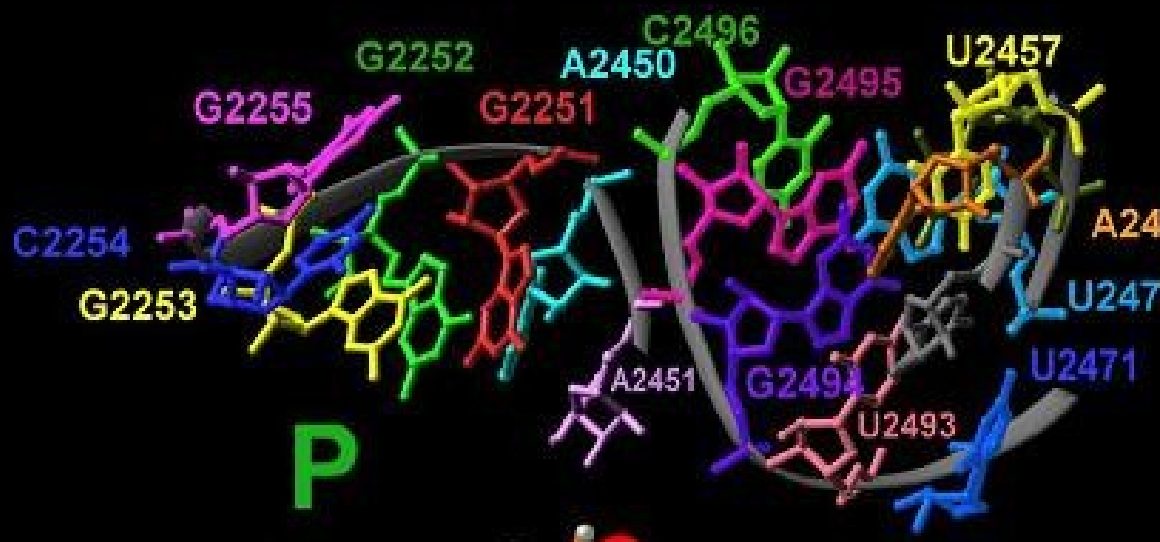
The sizable symmetry related region (180 nucleotides) within the large ribosomal subunit



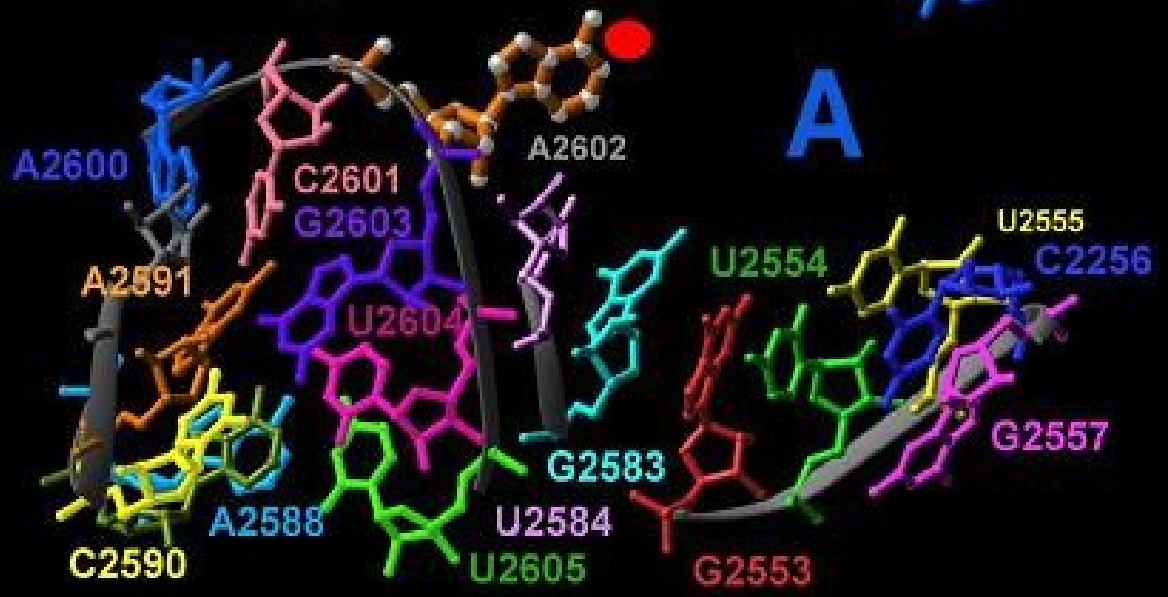
A similar symmetry related region was detected in all known structures of the large ribosomal subunit



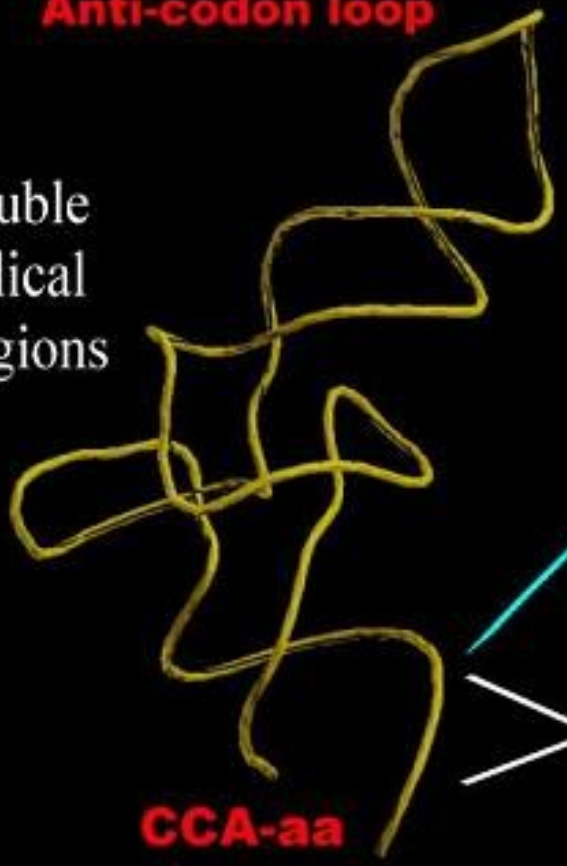




Superposition of PTC symmetry related nucleotides

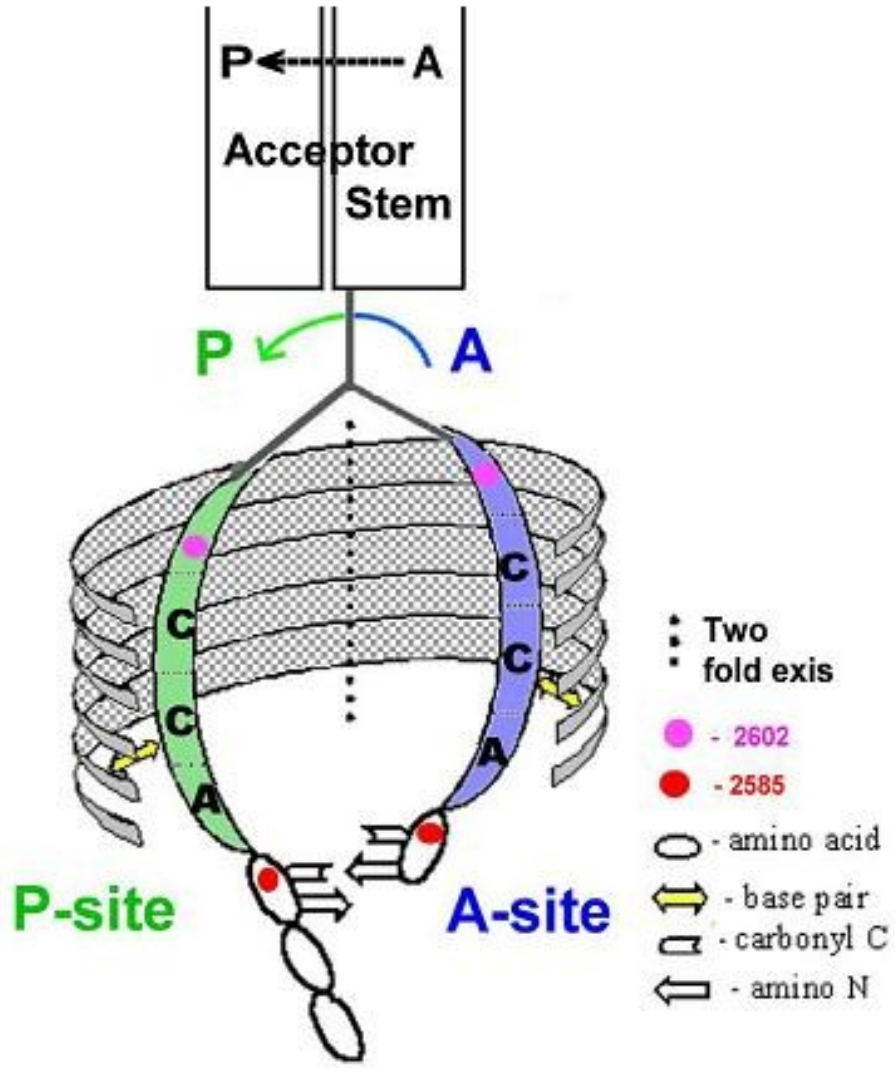


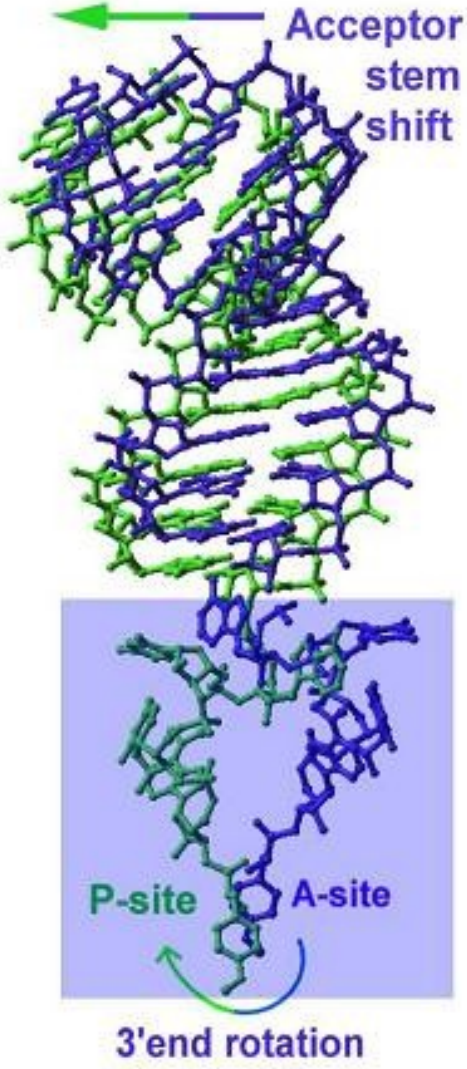
The tRNA molecule

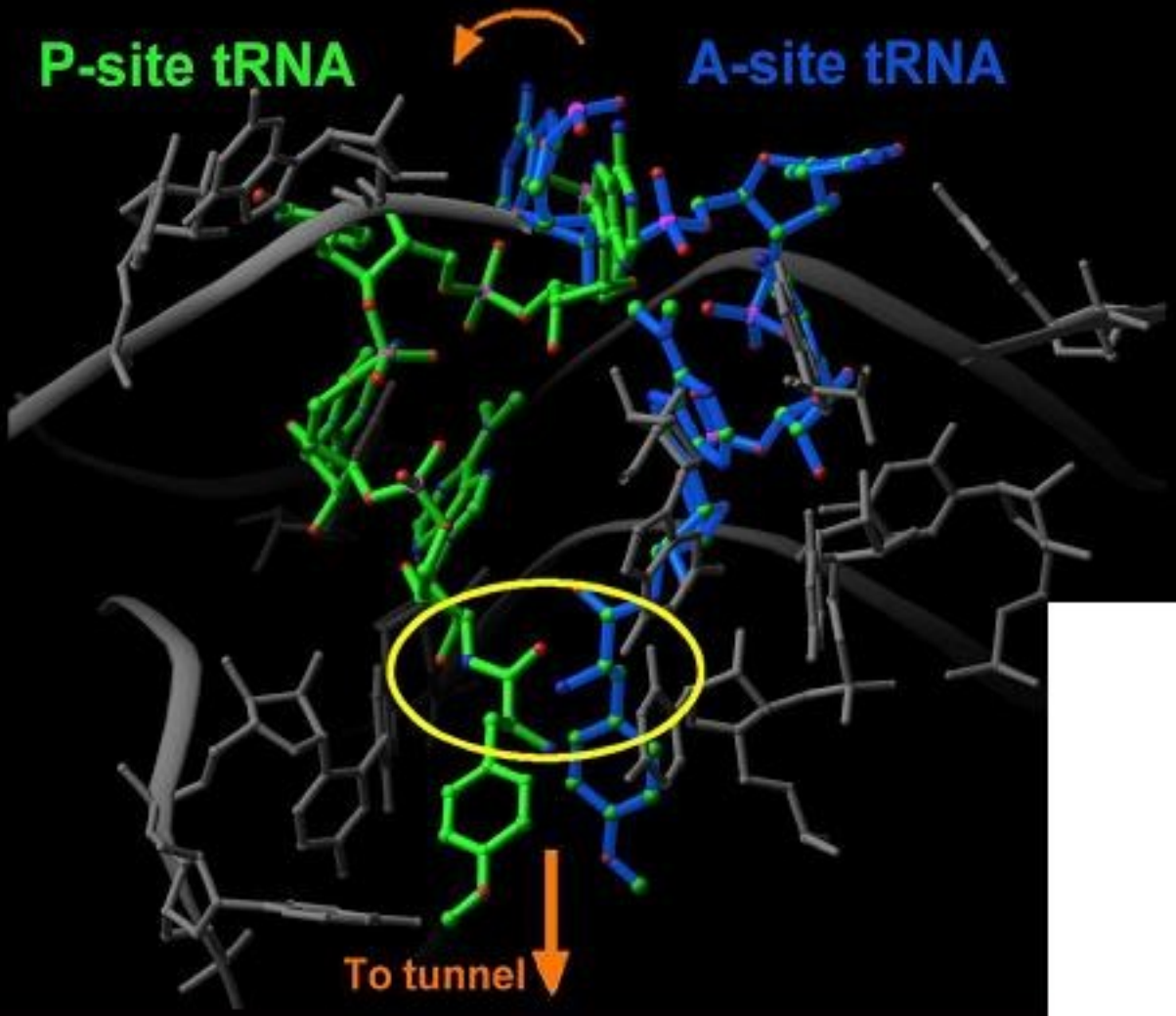


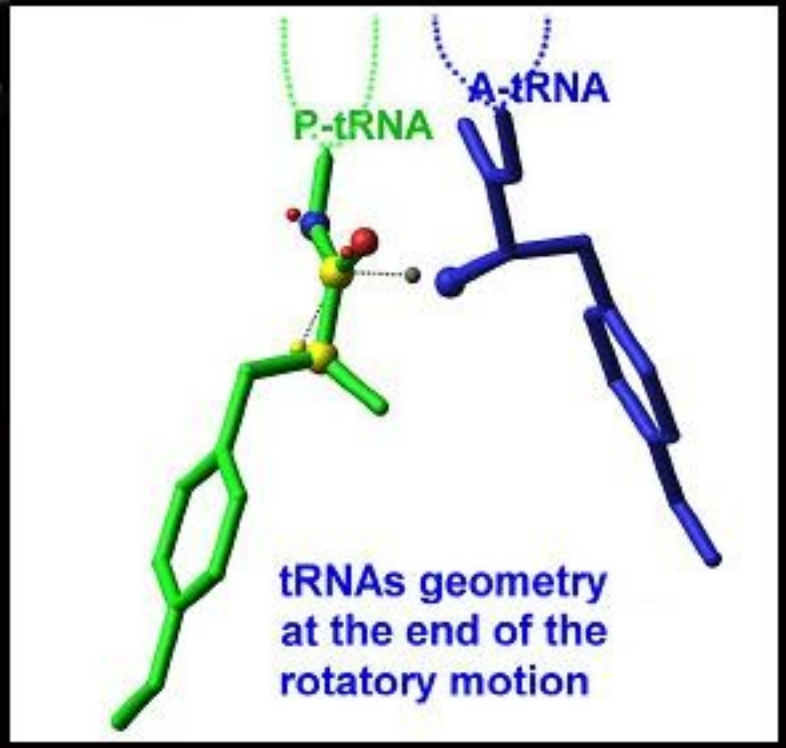
The bond connecting the helical region and the single strand almost overlaps with the two fold axis

The Universal Single Strand CCA (3'end) carries the amino-acid (A-site) or the nascent peptide (P-site)

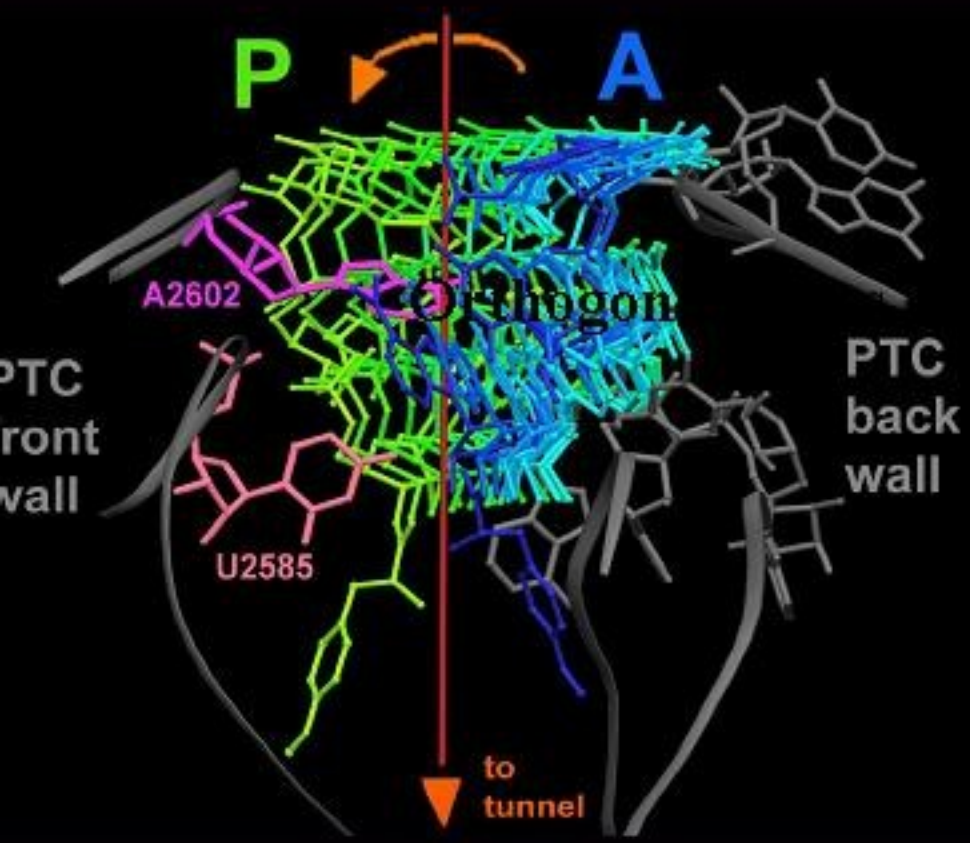

P
A
Acceptor
Stem
P
A
C
C
C
C
A
A
P-site
A-site
Two
fold exis
- 2602
- 2585
- amino acid
- base pair
- carbonyl C
- amino N

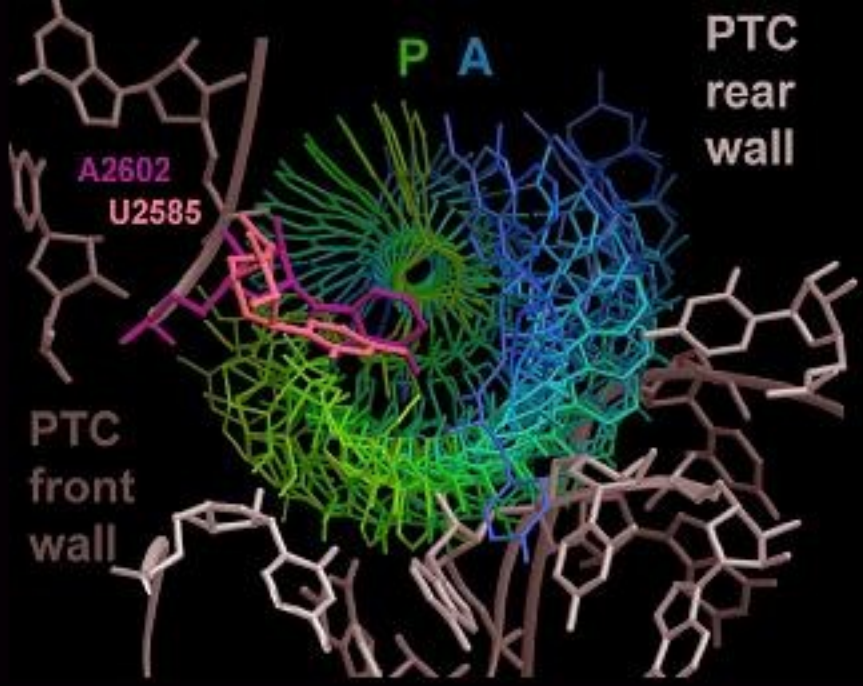

Acceptor
stem
shift
P-site
A-site
3'end rotation

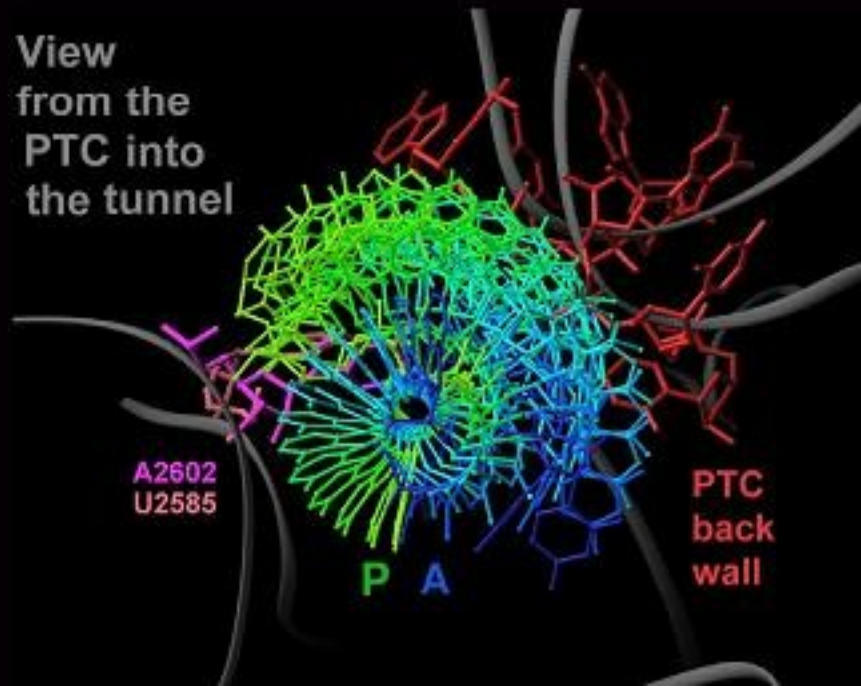

P-site tRNA
A-site tRNA
To tunnel

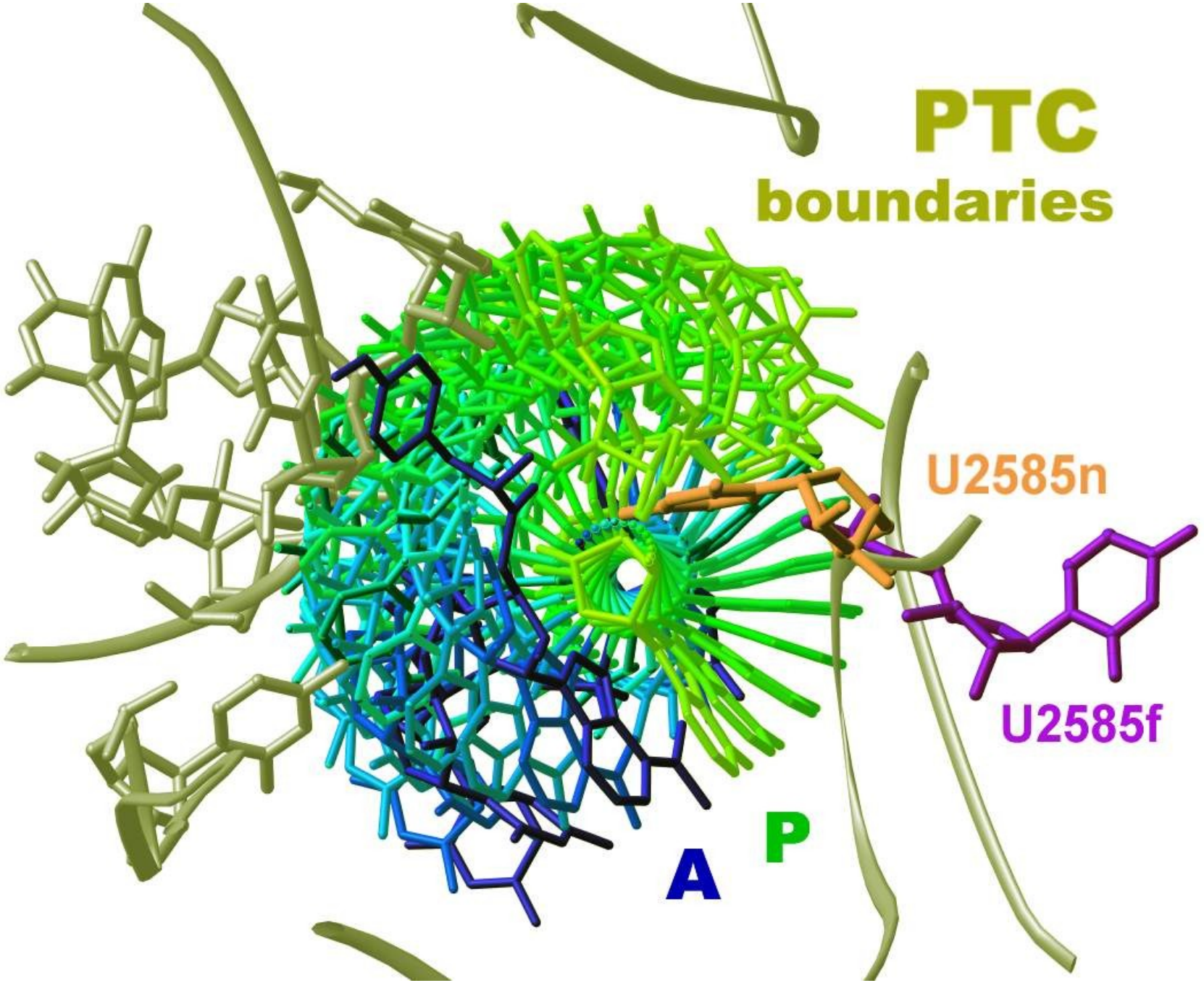

P-tRNA
A-tRNA
tRNAs geometry
at the end of the
rotatory motion

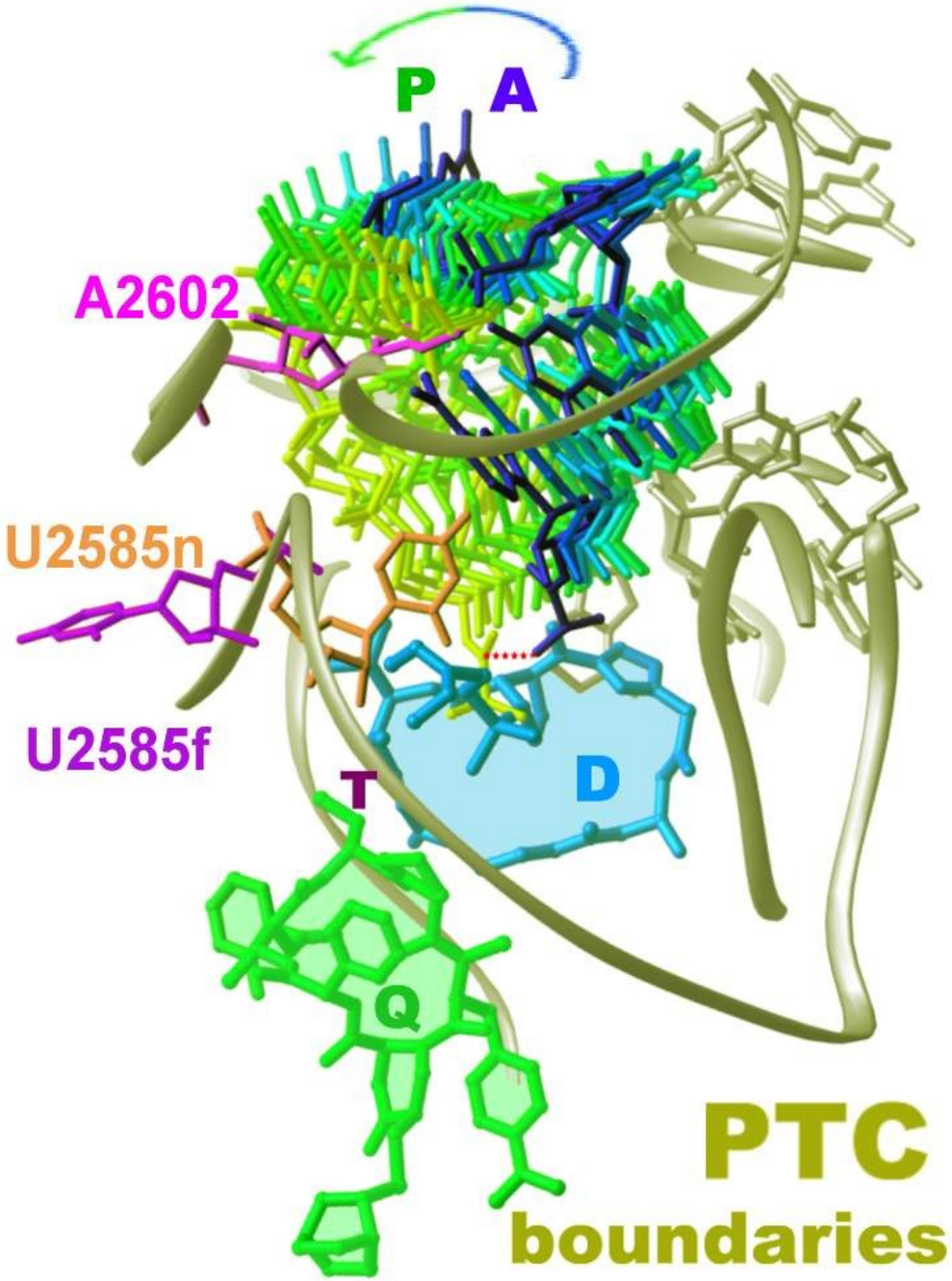
Orthogonal views of the rotatory motion








PTC
boundaries
U2585n
U2585f
A
P


P
A
A2602
U2585n
U2585f
T
D
Q
PTC
boundaries

Synergism = antibiotics future

Synercid® is a powerful antibiotic agent, designed to benefit from synergism of two components

Although each of its components is a weak drug, their combination is powerful.

Synercid® targets two functionally important regions of the ribosome: the PTC and the exit tunnel. Both are sensitive to antibiotics.

It has many anchors since each of its components is larger than the "normal" macrolides or PTC antibiotics.

This opens the gates for:

(a) Introduction of further species specific anchors, thus increasing selectivity

(b) Providing alternative interactions, thus reducing the rate of the appearance of resistance

(c) Chemical combination between the two

Can structures lead to improved and/or advanced drugs?

Can structures lead to design of new drugs?

Most promising

Sinergism

Investigate real pathogens


Tamar Auerbach Nevo

Raz Zarivach

Anat Bashan


Chen Davidovitch

Ada Yonath


Maggie Kessle


Moran Grossman


Miriam Laschever

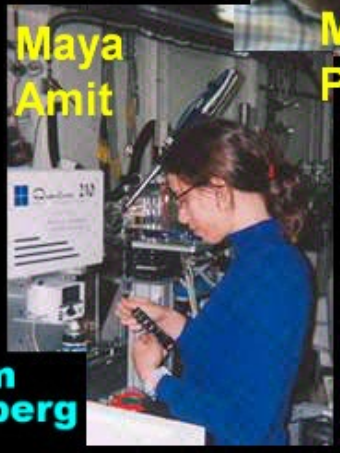

Ilana Agmon


Moshe Peretz


Inbal Greenberg


David Baram


Haim Rozenberg


Maya Amit

Dina Levy


Erez Pyetan


Assa Sittner

Rita Berisio

Collaborations relevant to this work

*** Max Planck Society: Hamburg Ribosome Research Unit, and the Ribosome Group in Berlin**

R. Berisio, Uni Napoly

E. Boettger, Uni Zurich, Medical School

Pfizer USA and GlaxoSmithKline provided compounds

Data were collected at ID14-4/ESRF/EMBL, Grenoble, France and 19ID/SBC/ANL/Il,USA

Funds were provided by NIH, *Max Planck Society, Human Frontiers, Pfizer, and the Kimmelman Center of the Weizmann Inst.

*** terminated in 2004**