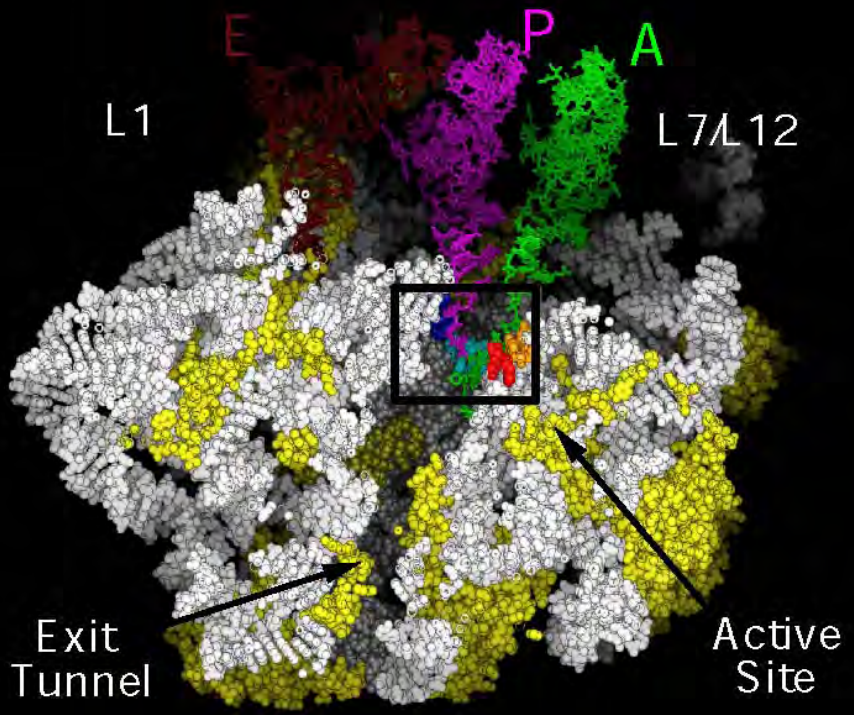
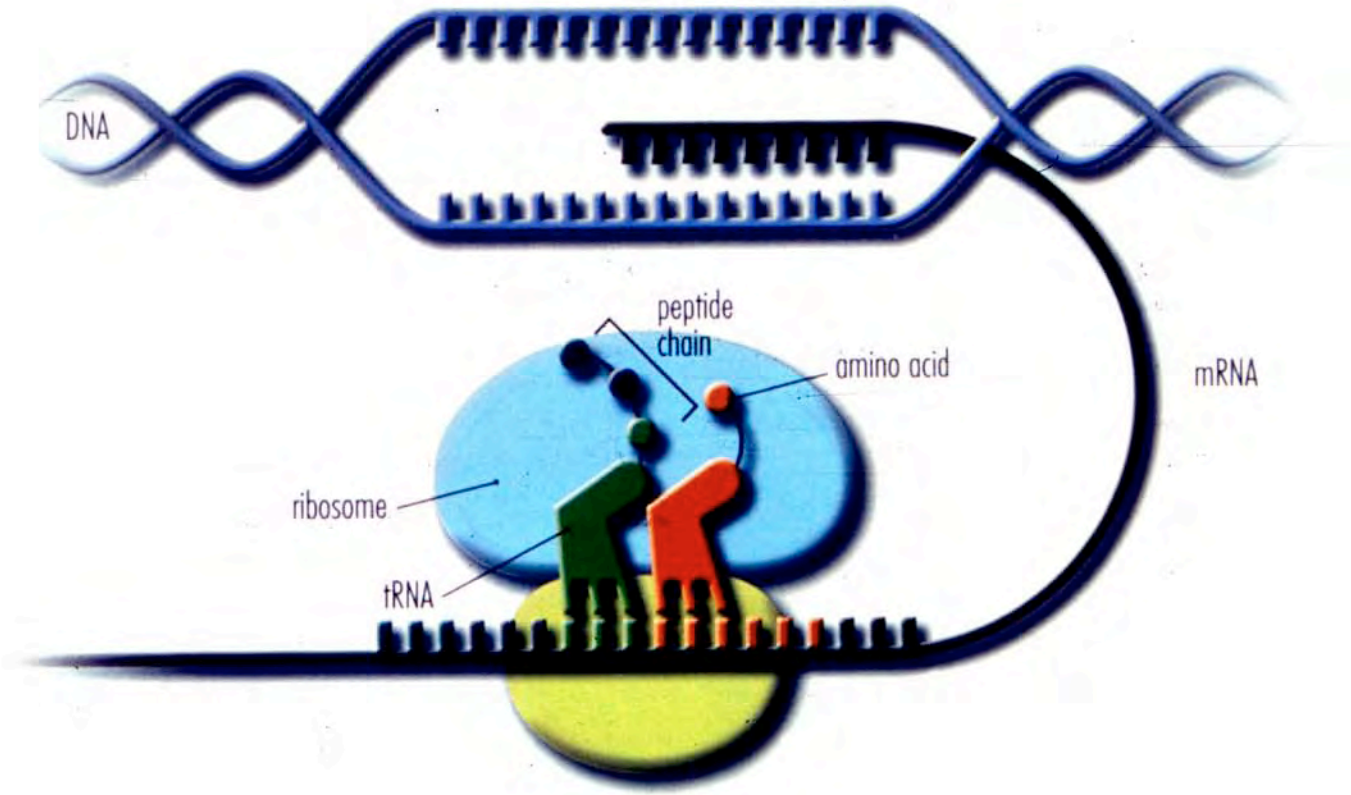
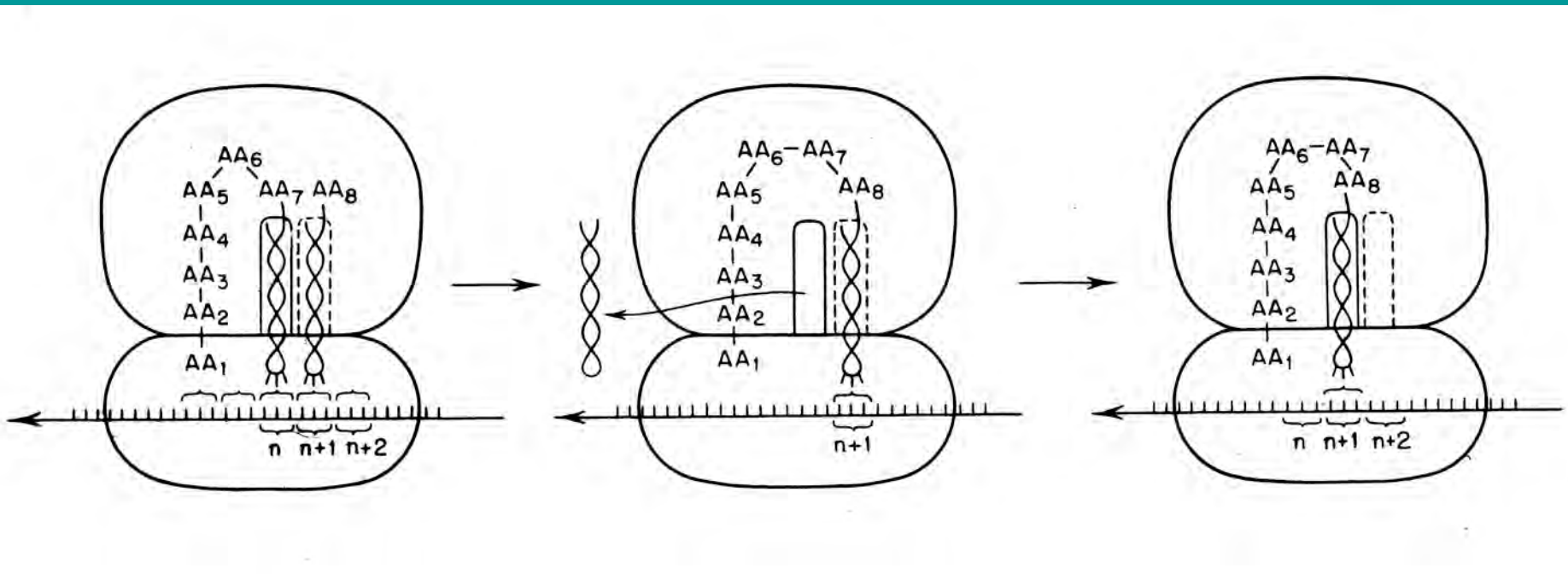
From the Structure and Function of the Ribosome to new Antibiotics



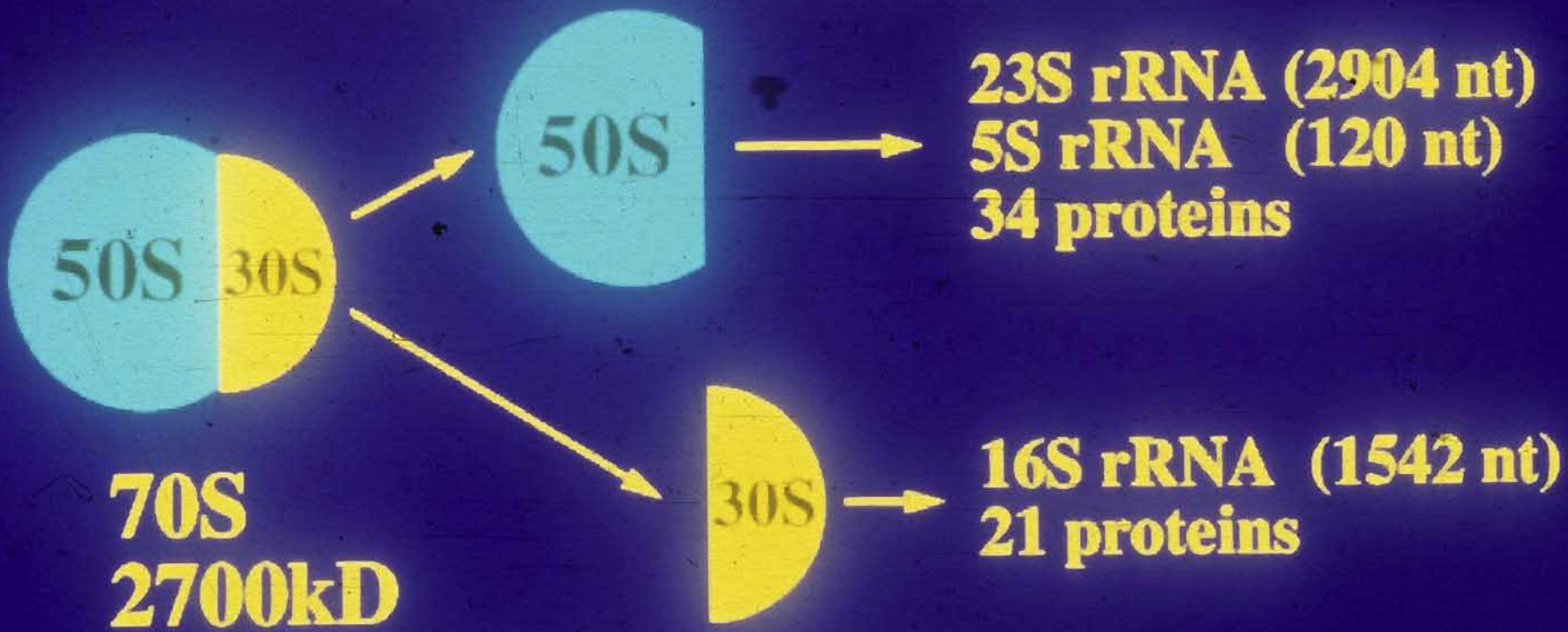
Crick's central dogma of molecular biology:
DNA makes DNA makes RNA makes protein



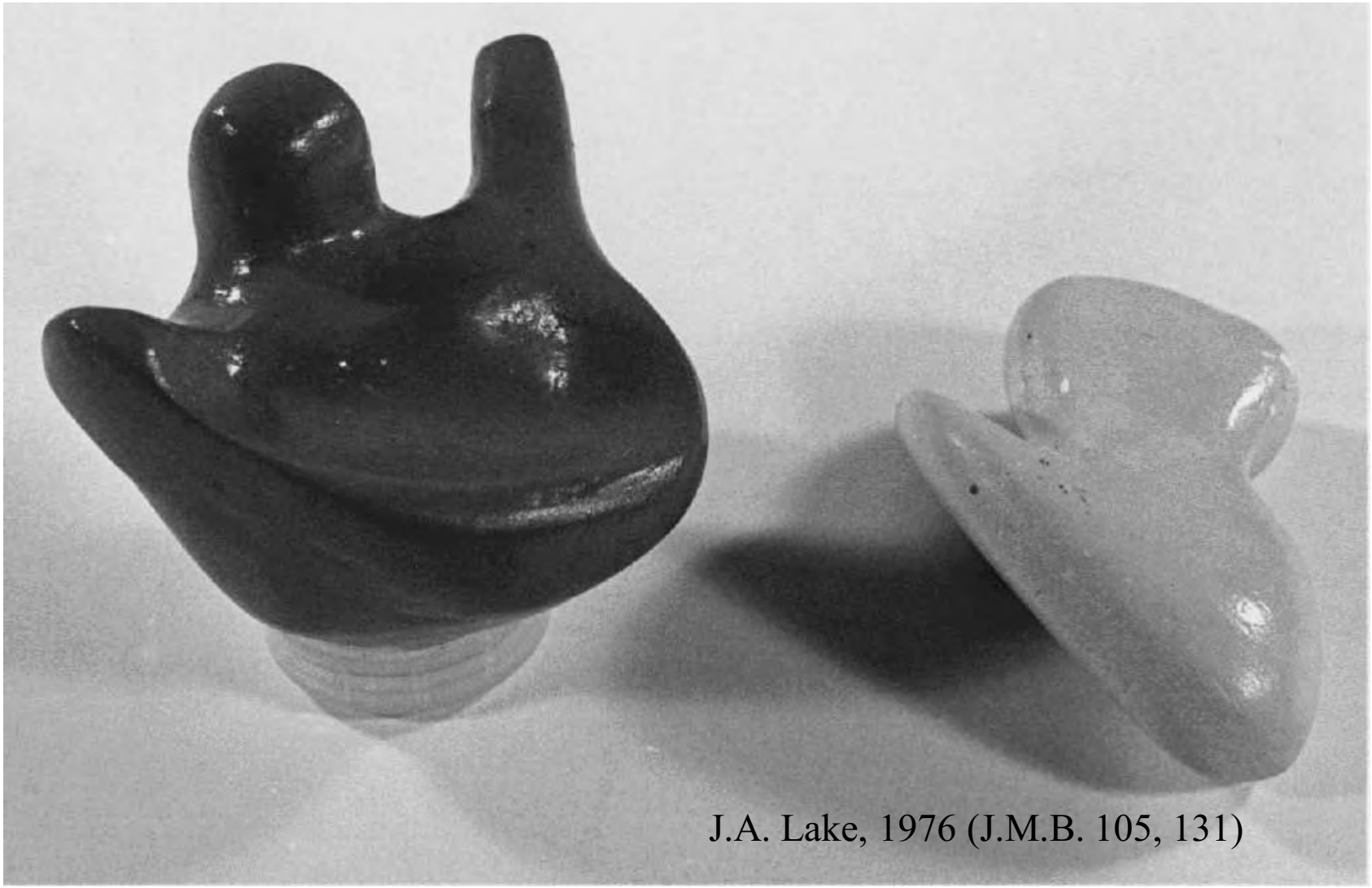


Jim Watson, 1964

Structural Components of the Ribosome from *E. coli*

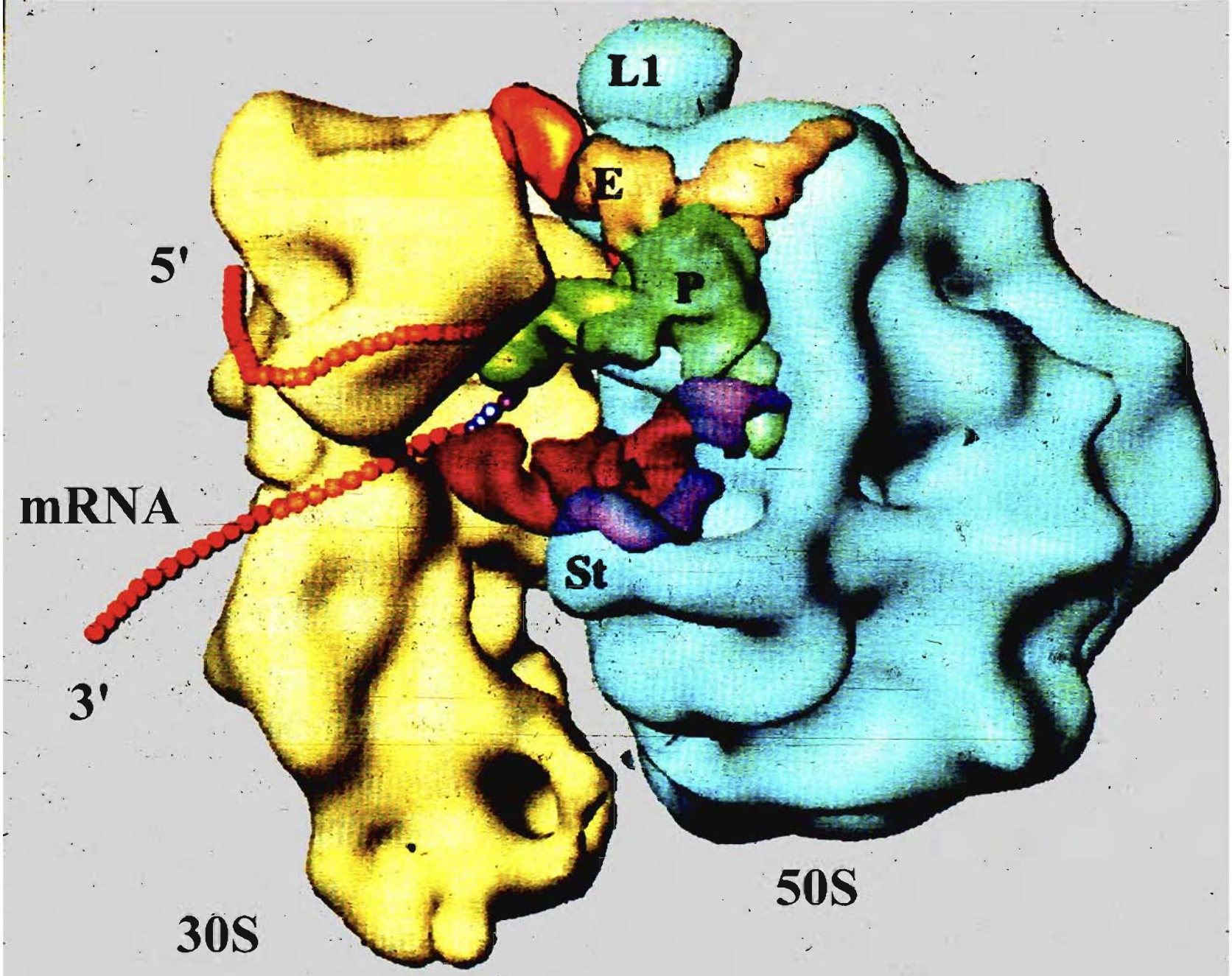


Ribosome is 2/3 RNA by mass and is 1/4 bacterial cell mass



J.A. Lake, 1976 (J.M.B. 105, 131)

J.A. Lake, 1976 (J.M.B. 105, 131)



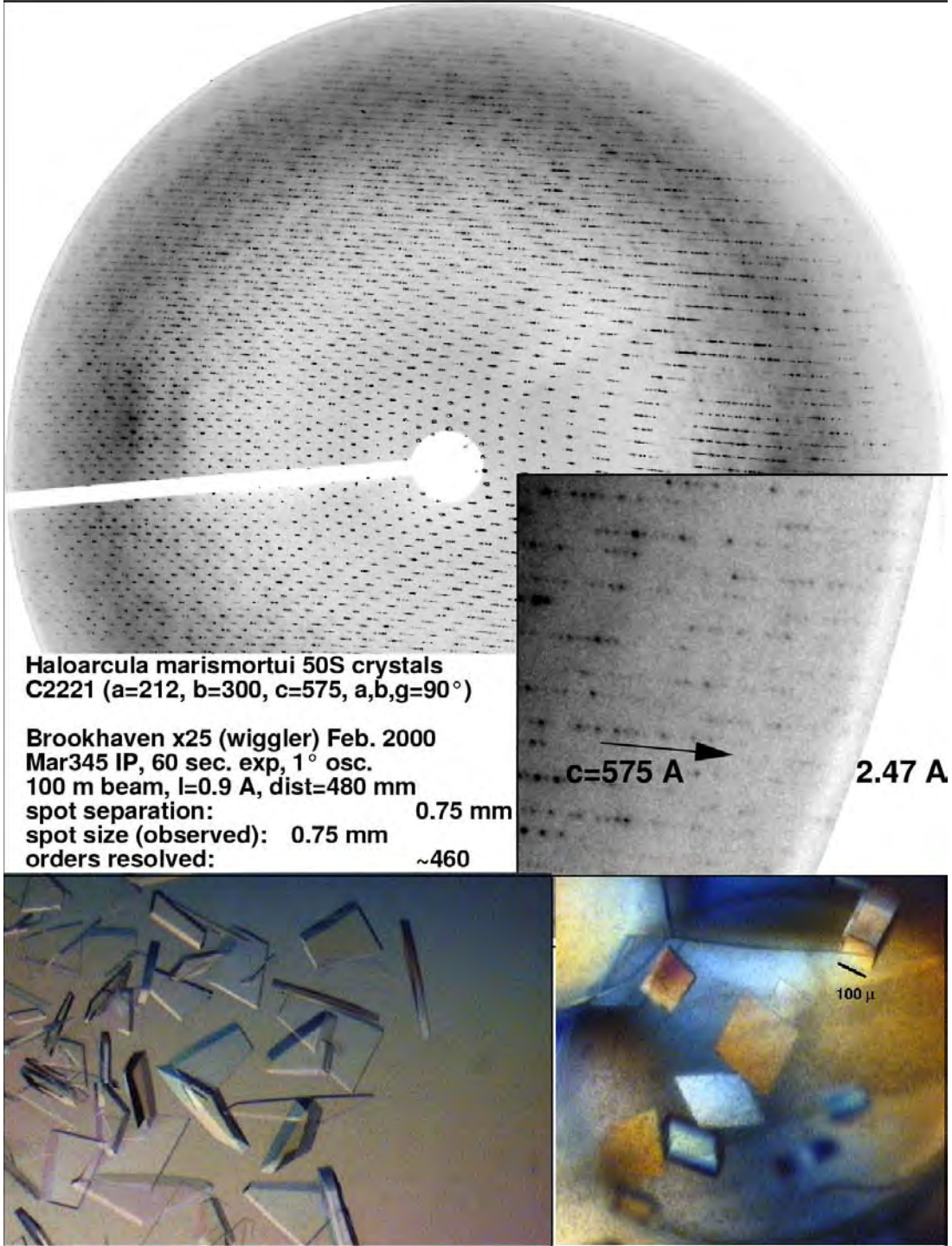
Joachim Frank, Current Opinion in Strucural Biology (1997)

Nenad Ban,
1995-2000

Peter Moore (and Striped Bass)

Poul Nissen, 1997-2000

Seeding and reverse extraction procedures yielded more isometric and reproducible crystals with excellent diffraction properties.



Queen Mary

Queen Mary +Captain

Tungsten, 78 electrons

Ribosome 50S Subunit,
1,600,000 dalton M.W.

Sail Boat

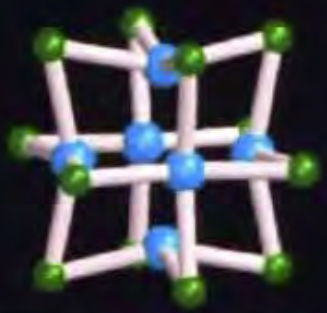
Sail Boat + Captain

Tungsten, 78 electrons

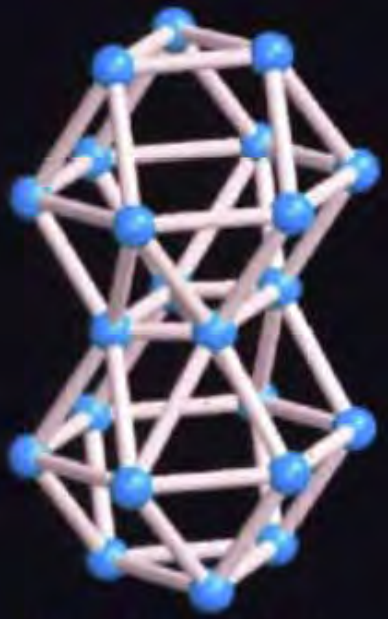
Lysozyme,
14,600 dalton M.W.

Heavy atom cluster derivatives

PIP 314 e–

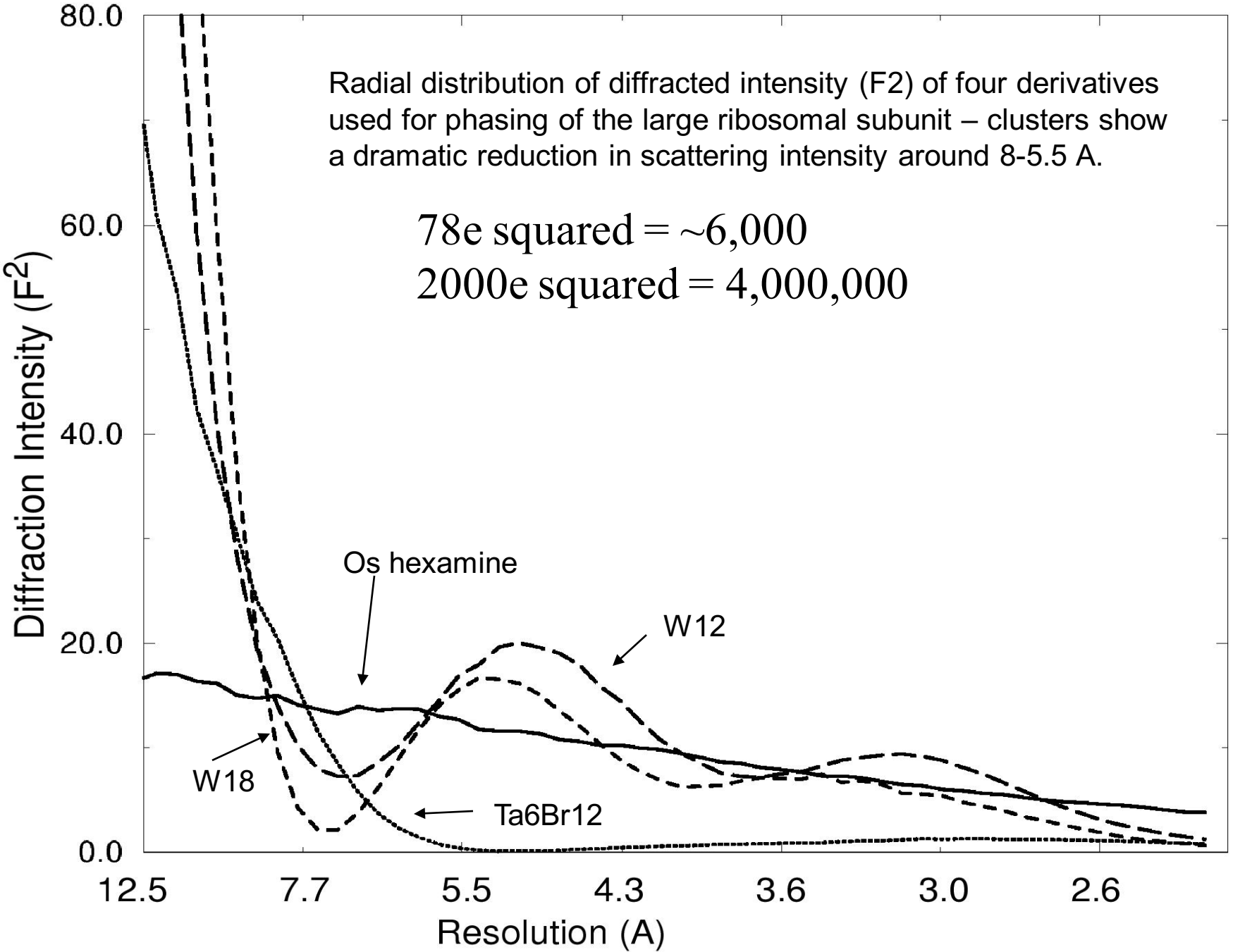


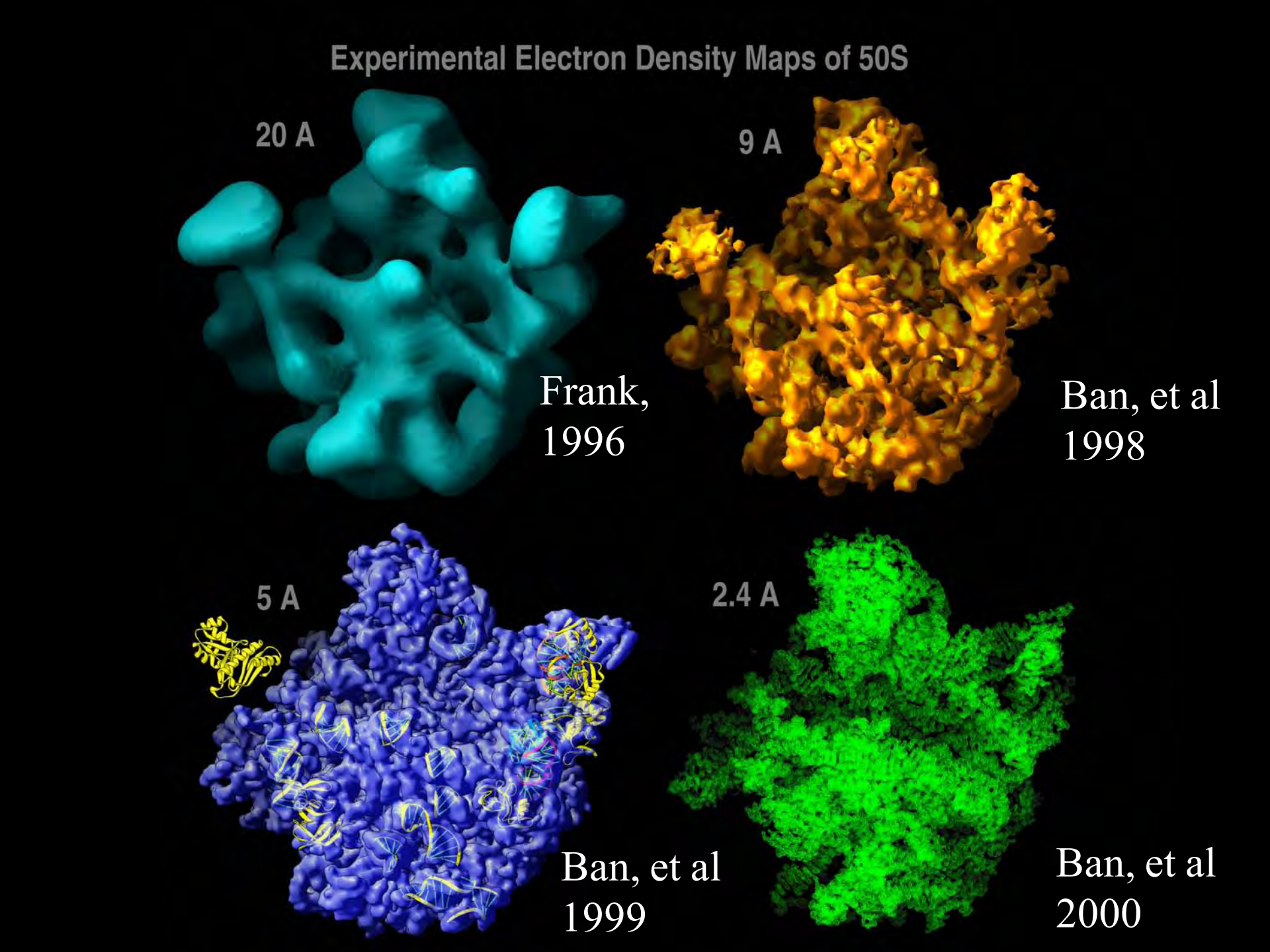
$Ta_6Br_{12}^{2+}$ 858 e–



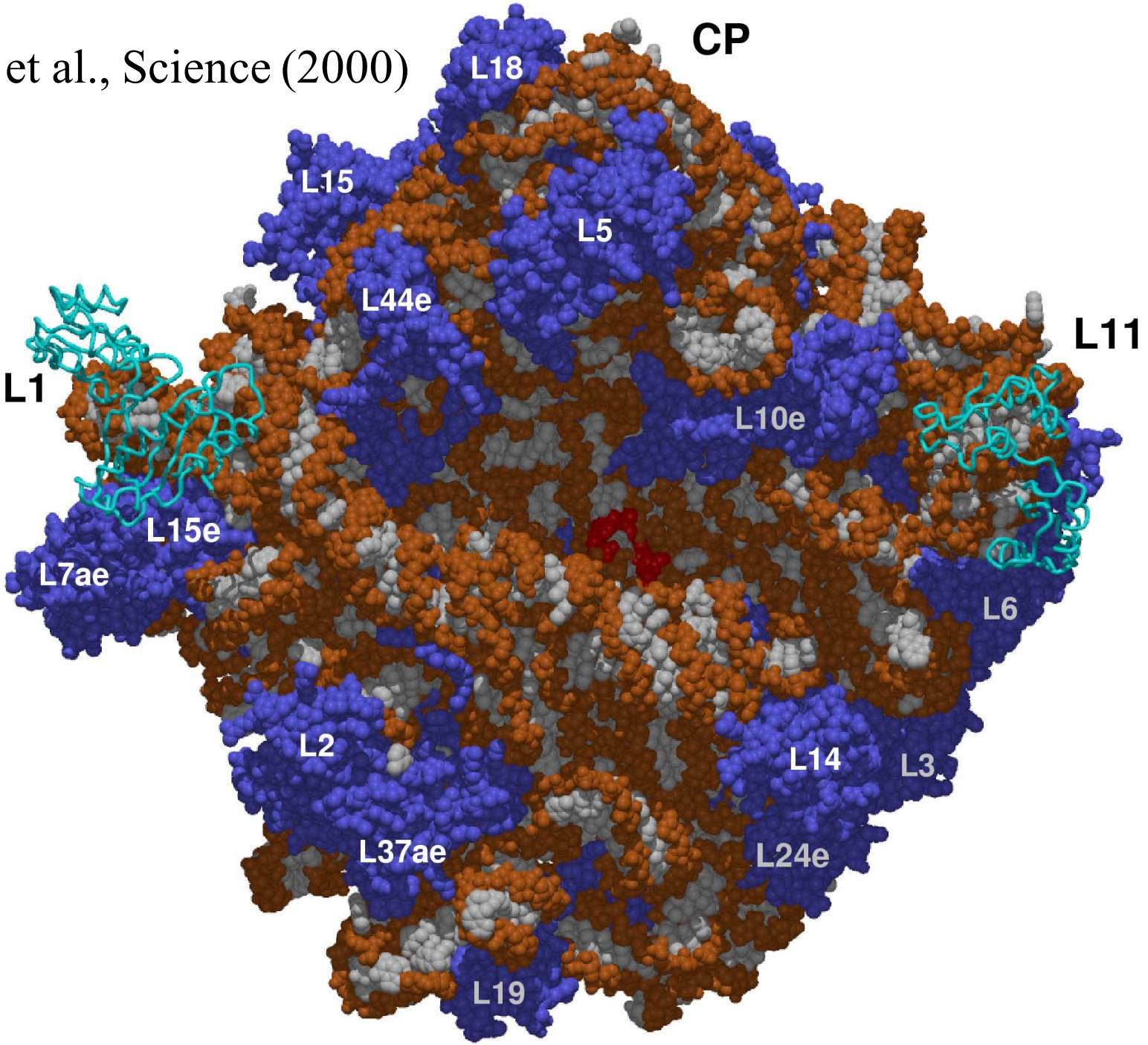
W–18 ~2000 e–

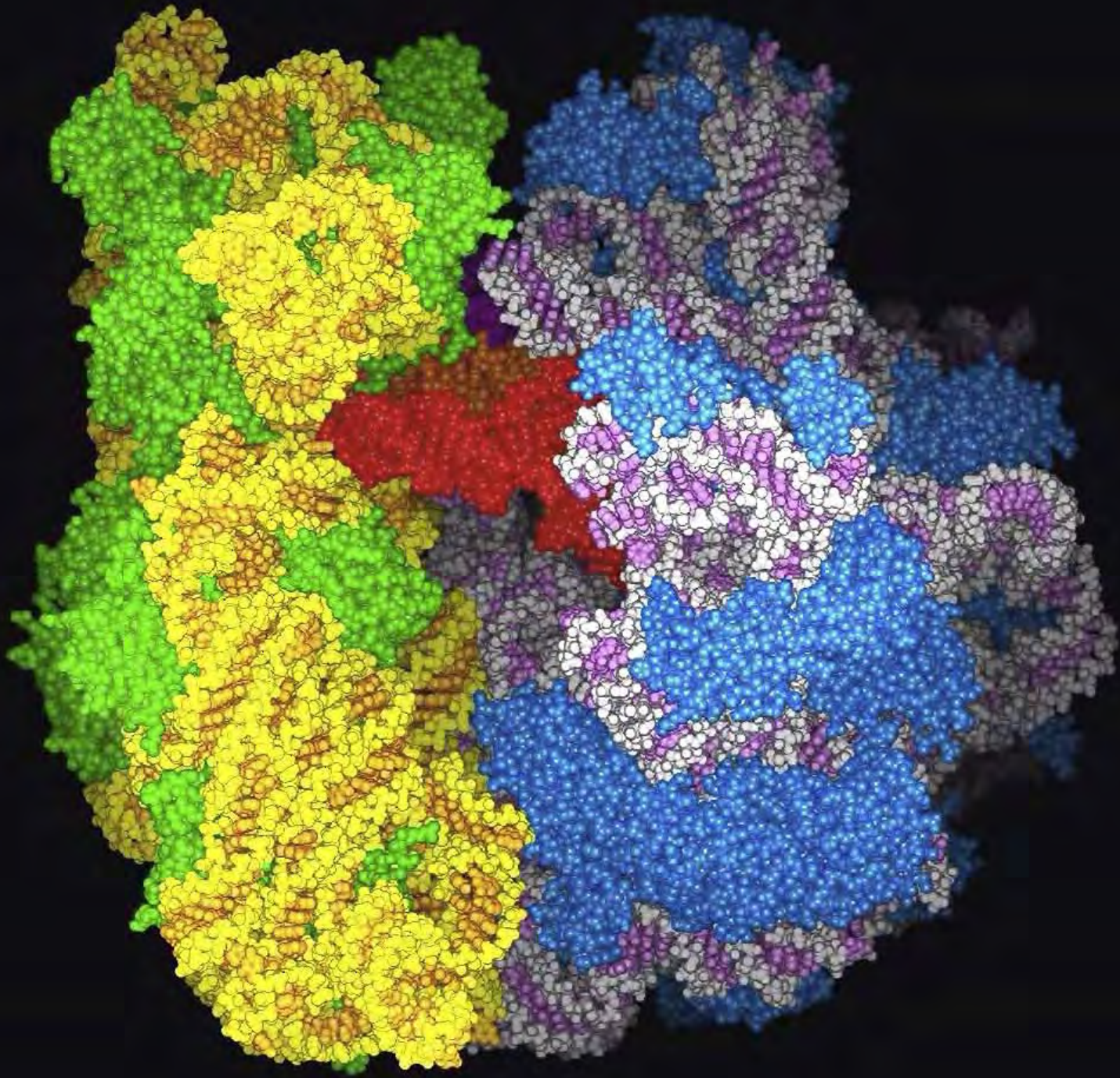
W–11 ~1250 e–

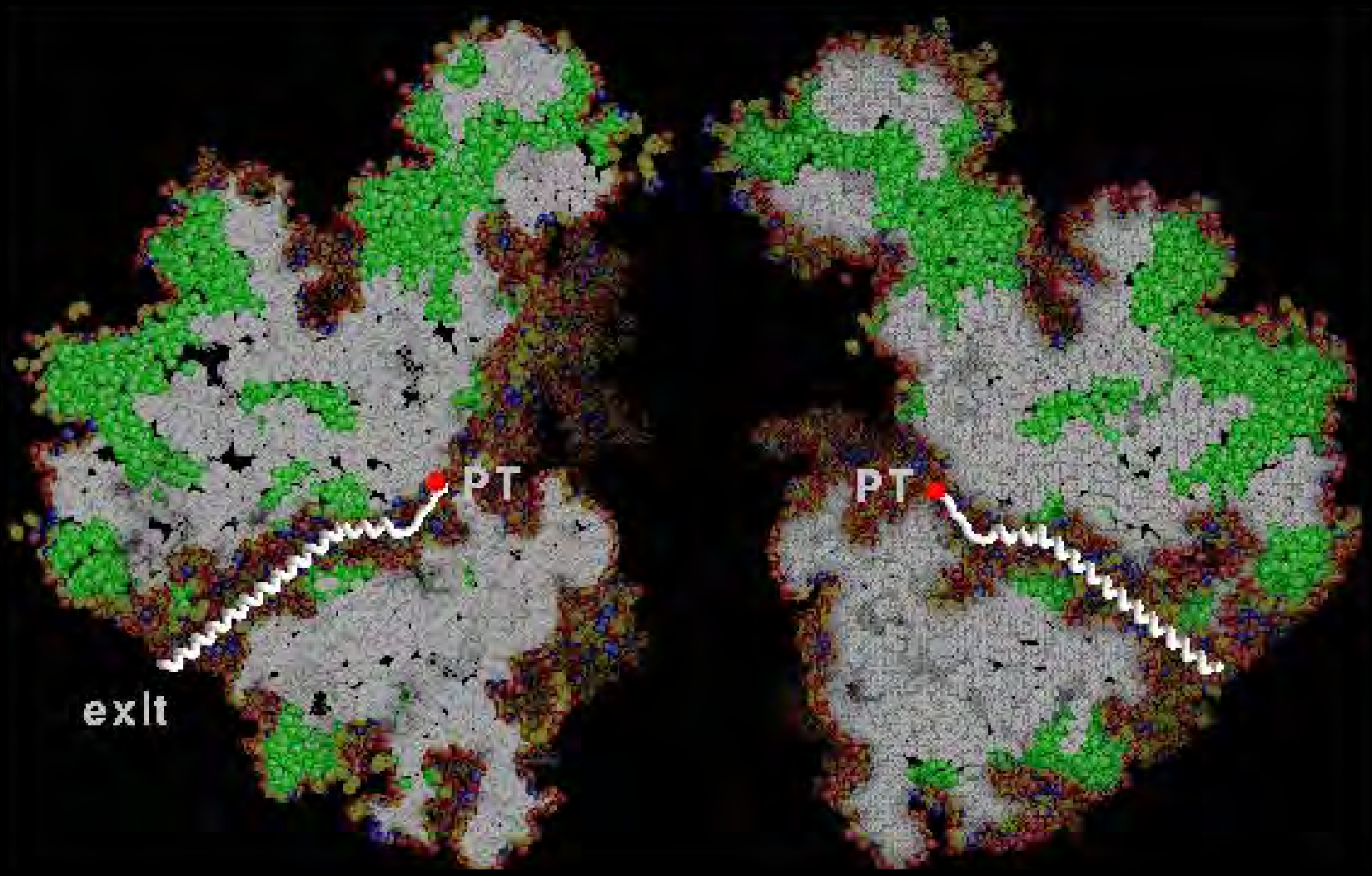

Radial distribution of diffracted intensity (F2) of four derivatives used for phasing of the large ribosomal subunit – clusters show a dramatic reduction in scattering intensity around 8-5.5 A.
78e squared = ~6,000
2000e squared = 4,000,000
Os hexamine
W12
W18
Ta6Br12
Diffraction Intensity (F2)
Resolution (A)
80.0
60.0
40.0
20.0
0.0
12.5
7.7
5.5
4.3
3.6
3.0
2.6


Experimental Electron Density Maps of 50S
20 A
Frank,
1996
9 A
Ban, et al
1998
5 A
Ban, et al
1999
2.4 A
Ban, et al
2000

Nissen et al., Science (2000)

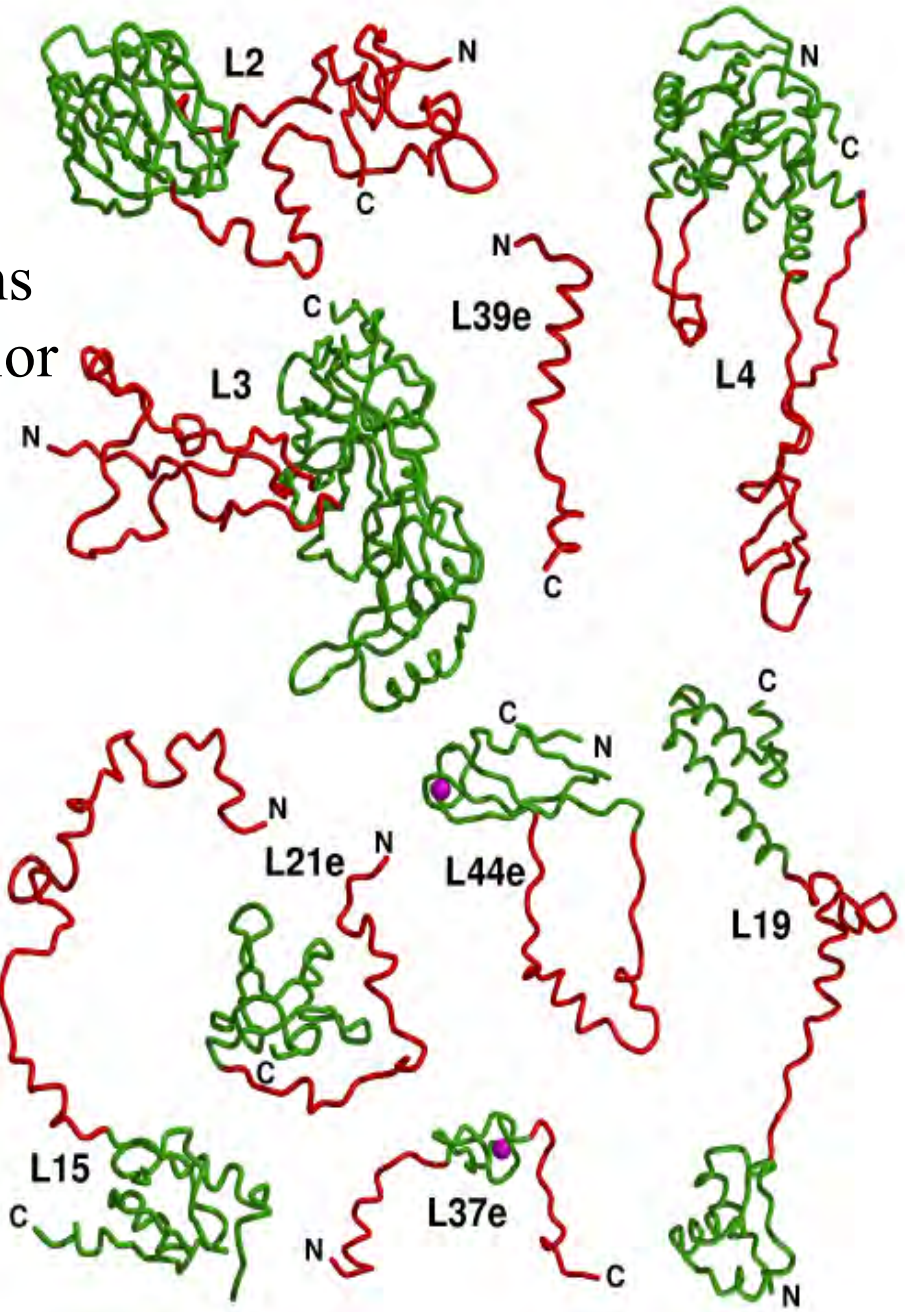




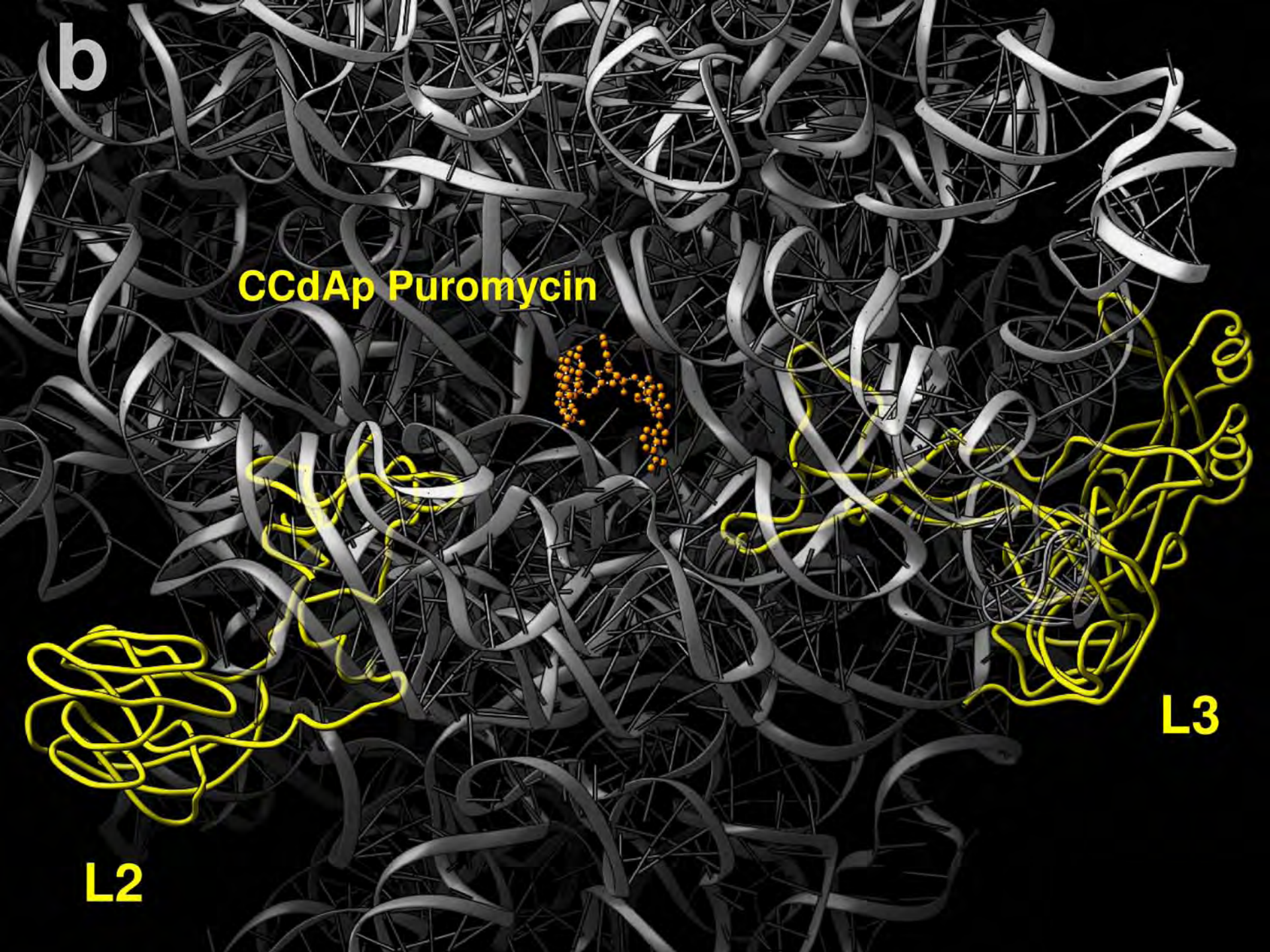


Nissen, et al. Science (2000)

Many ribosomal proteins have extended, basic regions that penetrate into the interior of the 23S rRNA

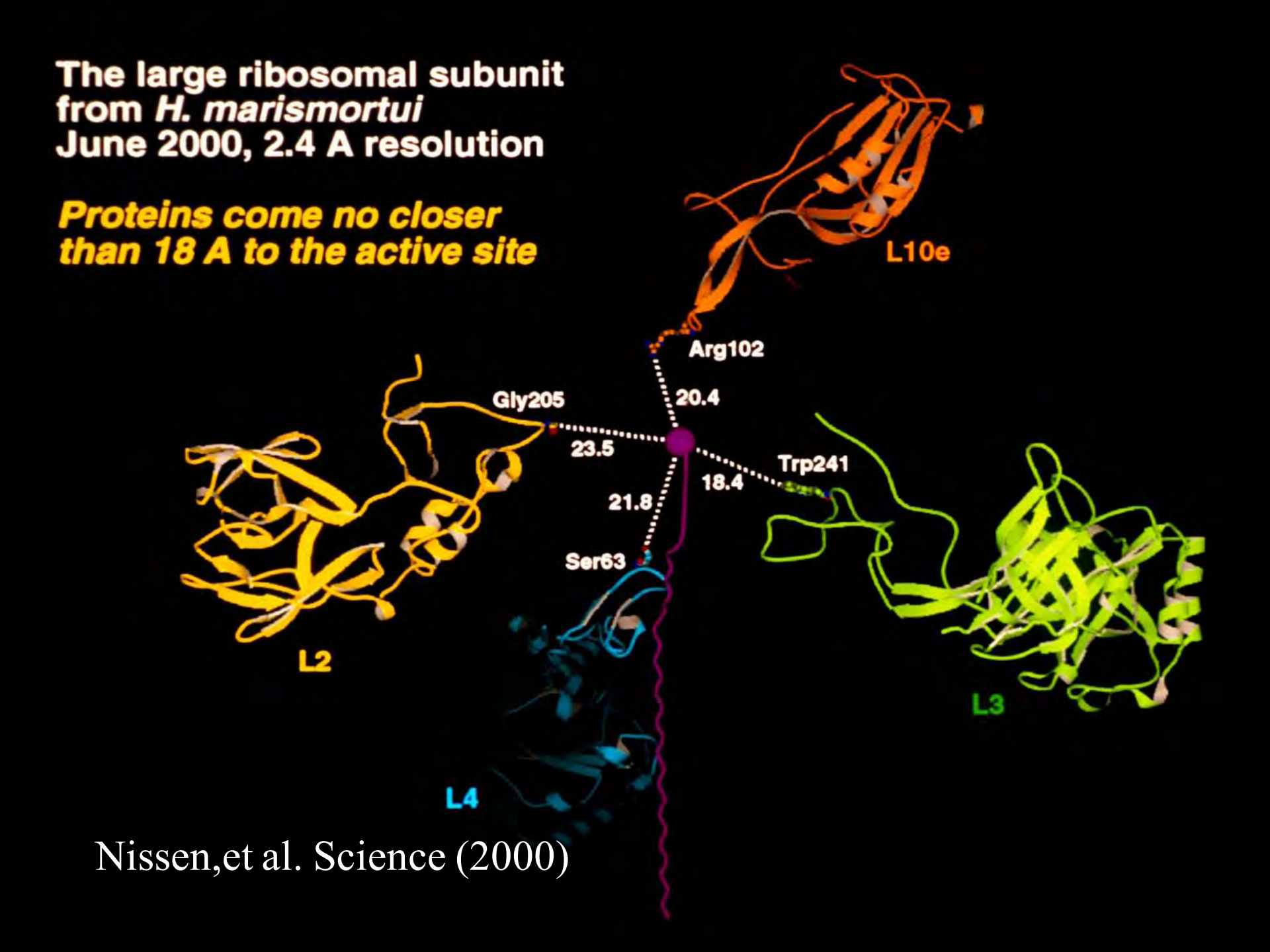


Ban et al., Science (2000)


b
CCdAp Puromycin
L3
L2

Crick recognized early that the ribosome should be a ribozyme

- "It is tempting to wonder if the primitive ribosome could have been made entirely of RNA"
- F. H. C. Crick, JMB, 38, 367-379 (1968)



Nissen,et al. Science (2000)

THE RIBOSOME

IS

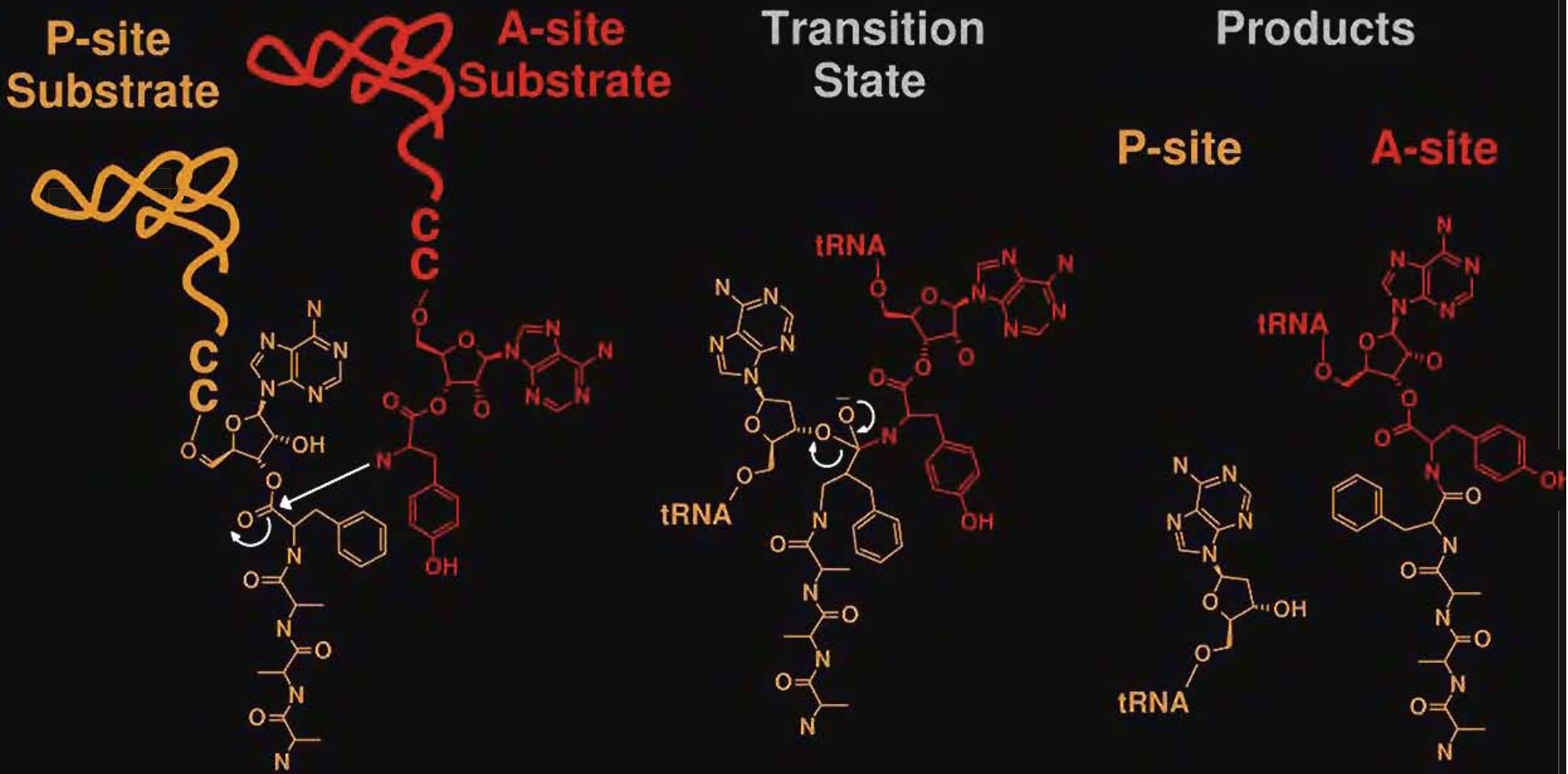
A RIBOZYME

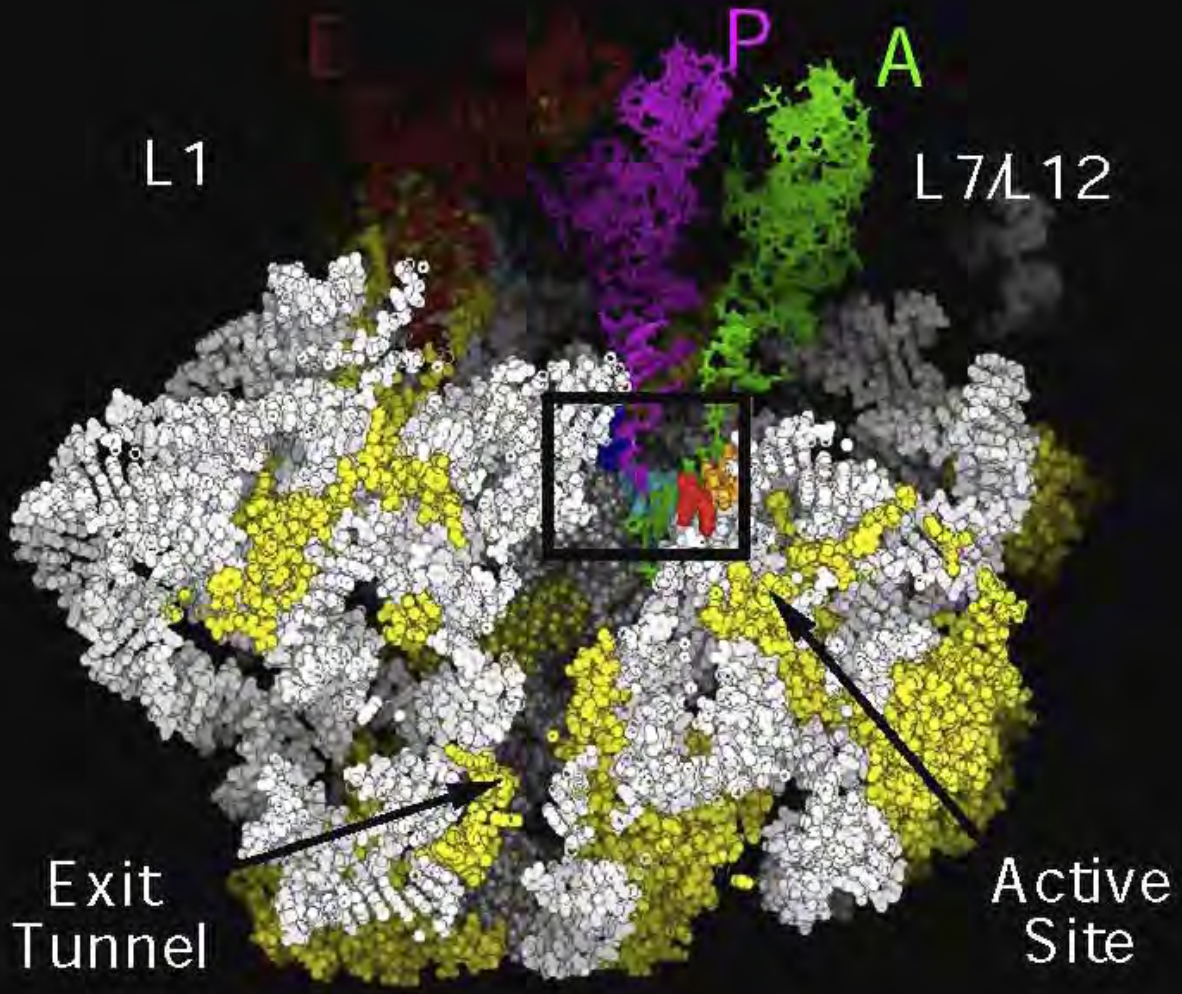
What is the source of the ribosome's catalytic power in peptide synthesis?

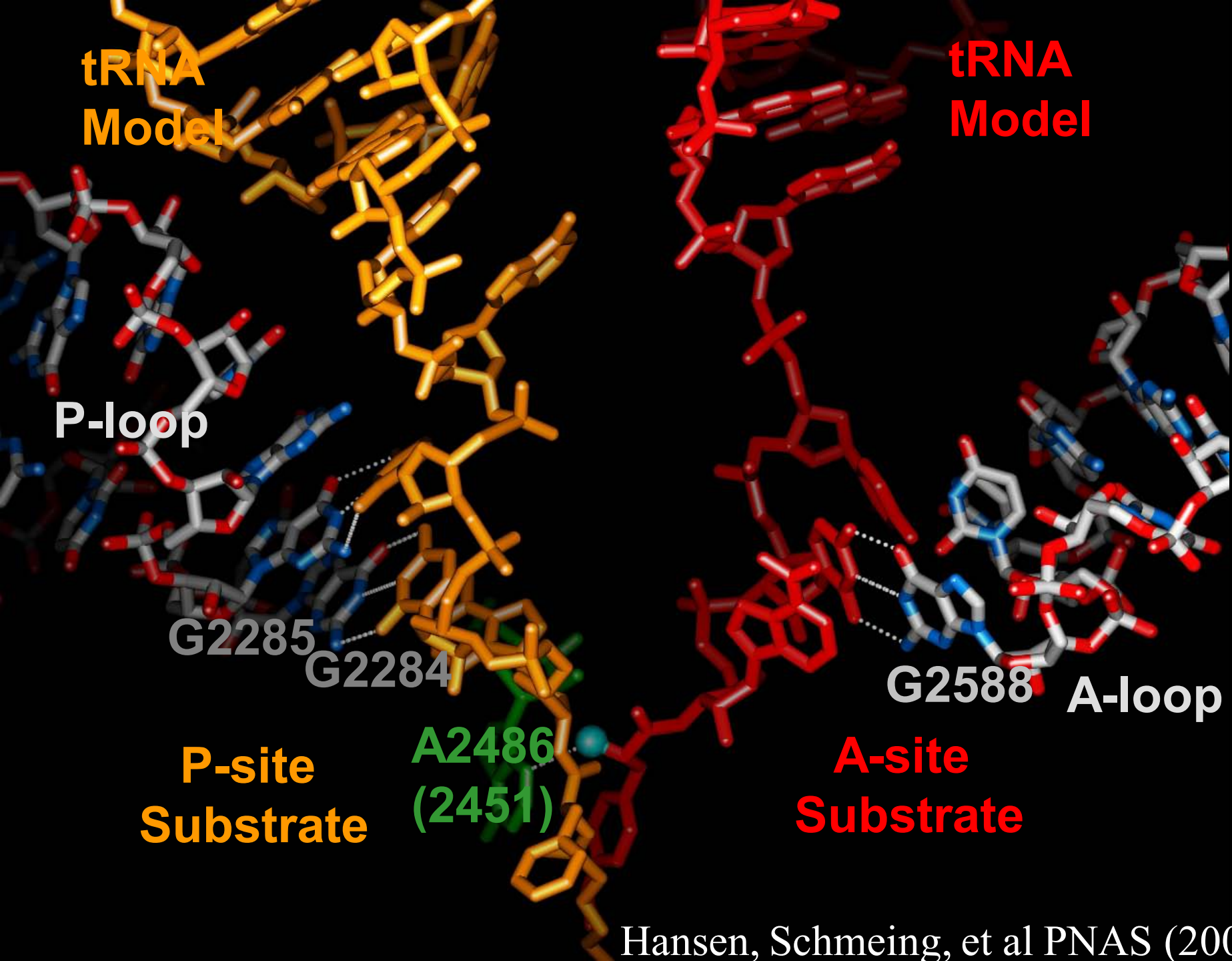
Martin Schmeing and Jeff Hansen


Martin Schmeing

Peptidyl Transferase Reaction

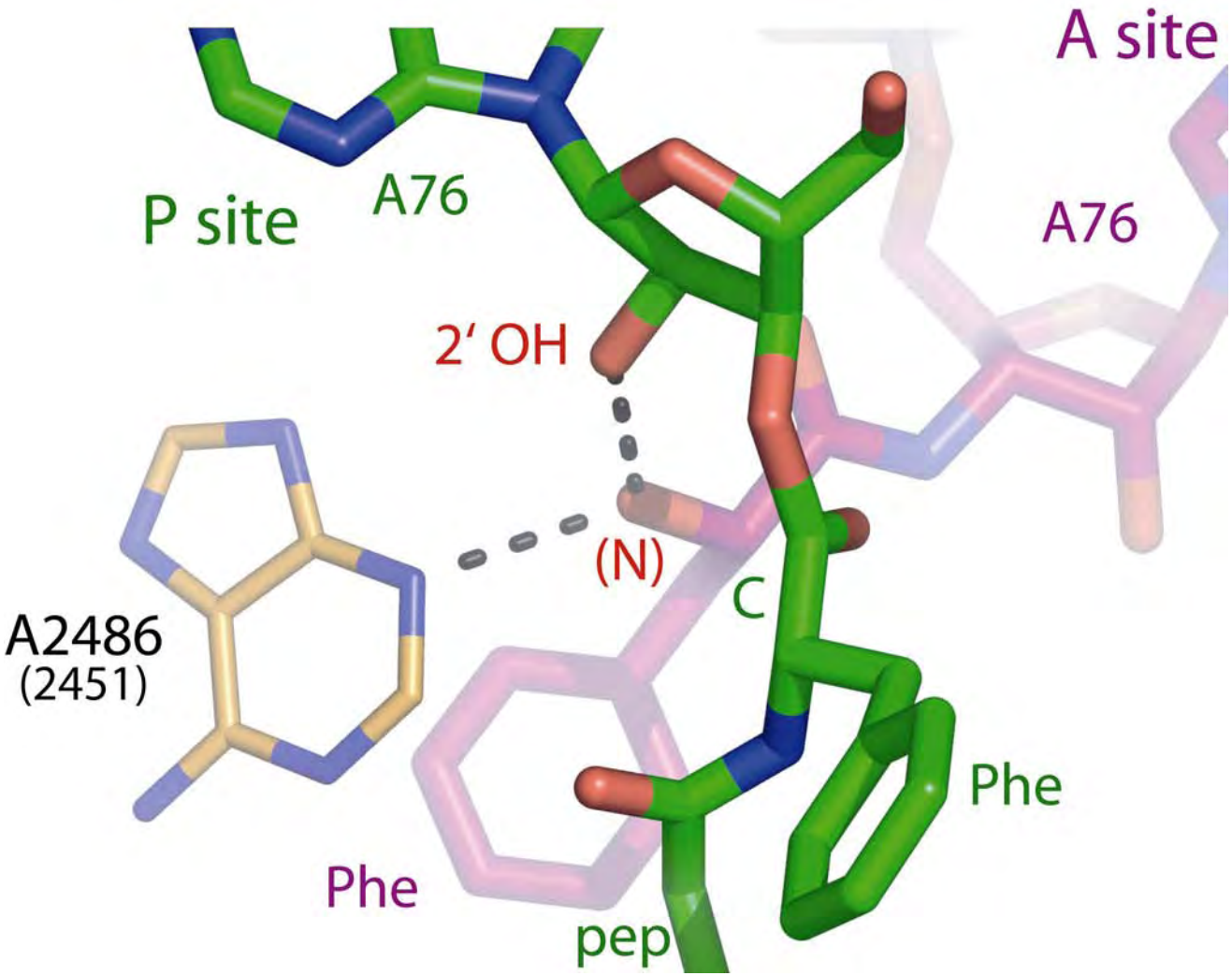



P
A
L1
L7/L12
Exit
Tunnel
Active
Site



Hansen, Schmeing, et al PNAS (2002)

The pre-reaction ground state



Schmeing, et al, Nature (2005)

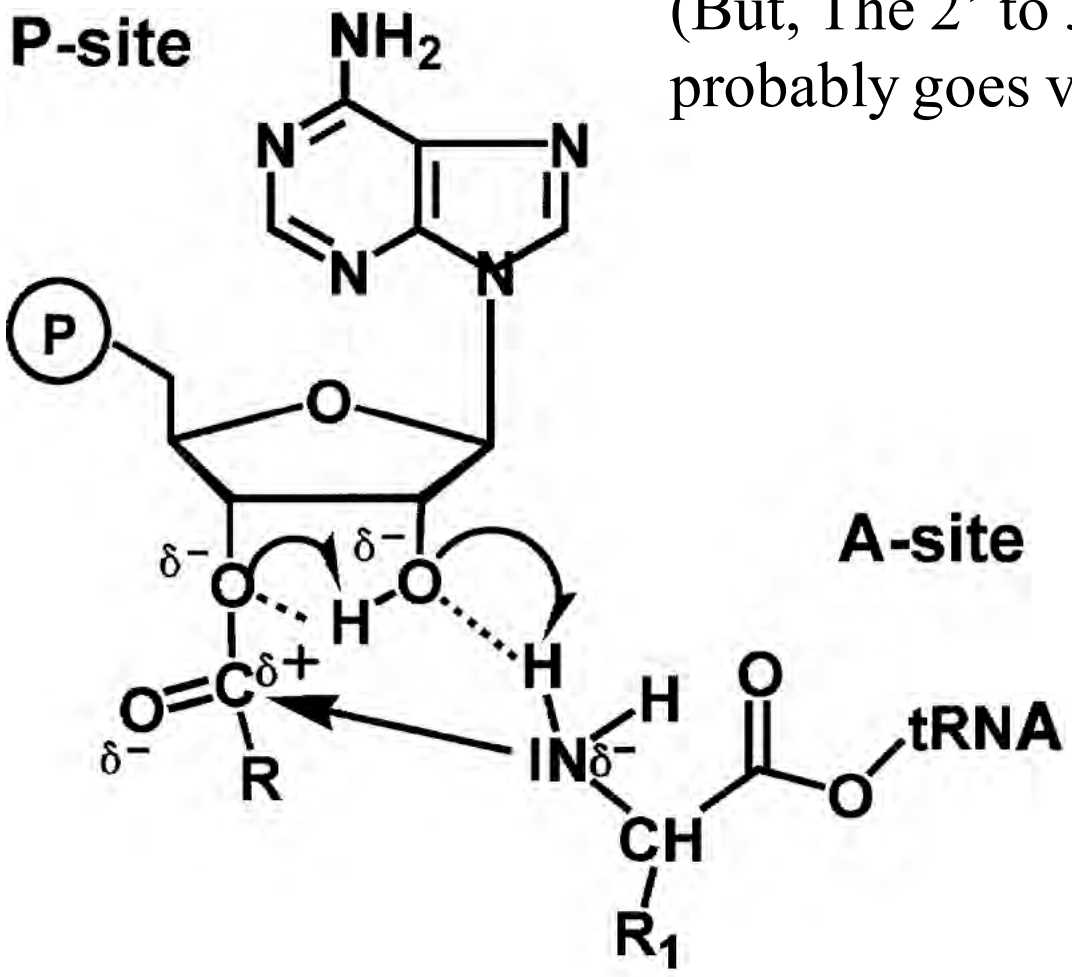
Mutation of A2486 (2451) does not affect the rate of peptide bond formation when the A-site substrate is aminoacyl-tRNA

E.M. Youngman, J.L. Brunelle, A.B. Kochaniak, and Rachel Green, Cell 117, 589-99 (2004)

Removal of the 2'OH of the P-site A76 reduces the peptidyl-transferase rate by more than 10,000 fold.

J.S. Weinger, K.M. Parnell, S. Dorner, R.Green, and Scott Strobel, Nature Struct Mol Biol 330,11,1101-6(2004)

A possible role for 2’ OH on A76 of the P-site in chemical catalysis

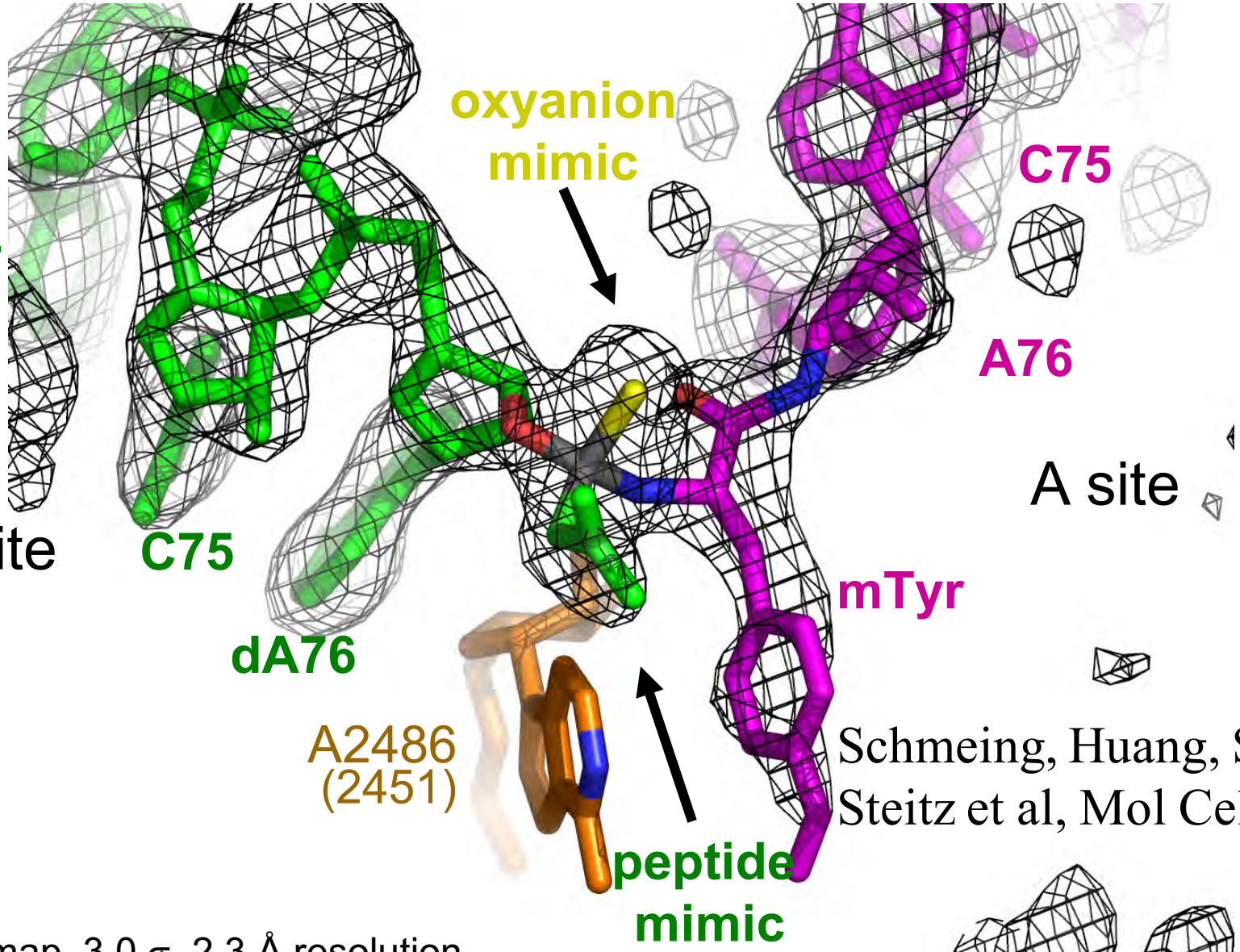


(But, The 2’ to 3’ transfer probably goes via a water).

Dorner S, Polacek N, Schulmeister U, Panuschka C, Barta A. **“Molecular aspects of the ribosomal peptidyl transferase.”** Biochem Soc Trans. 2002 Nov;30(Pt 6):1131-6.

Is the transition state being stabilized?

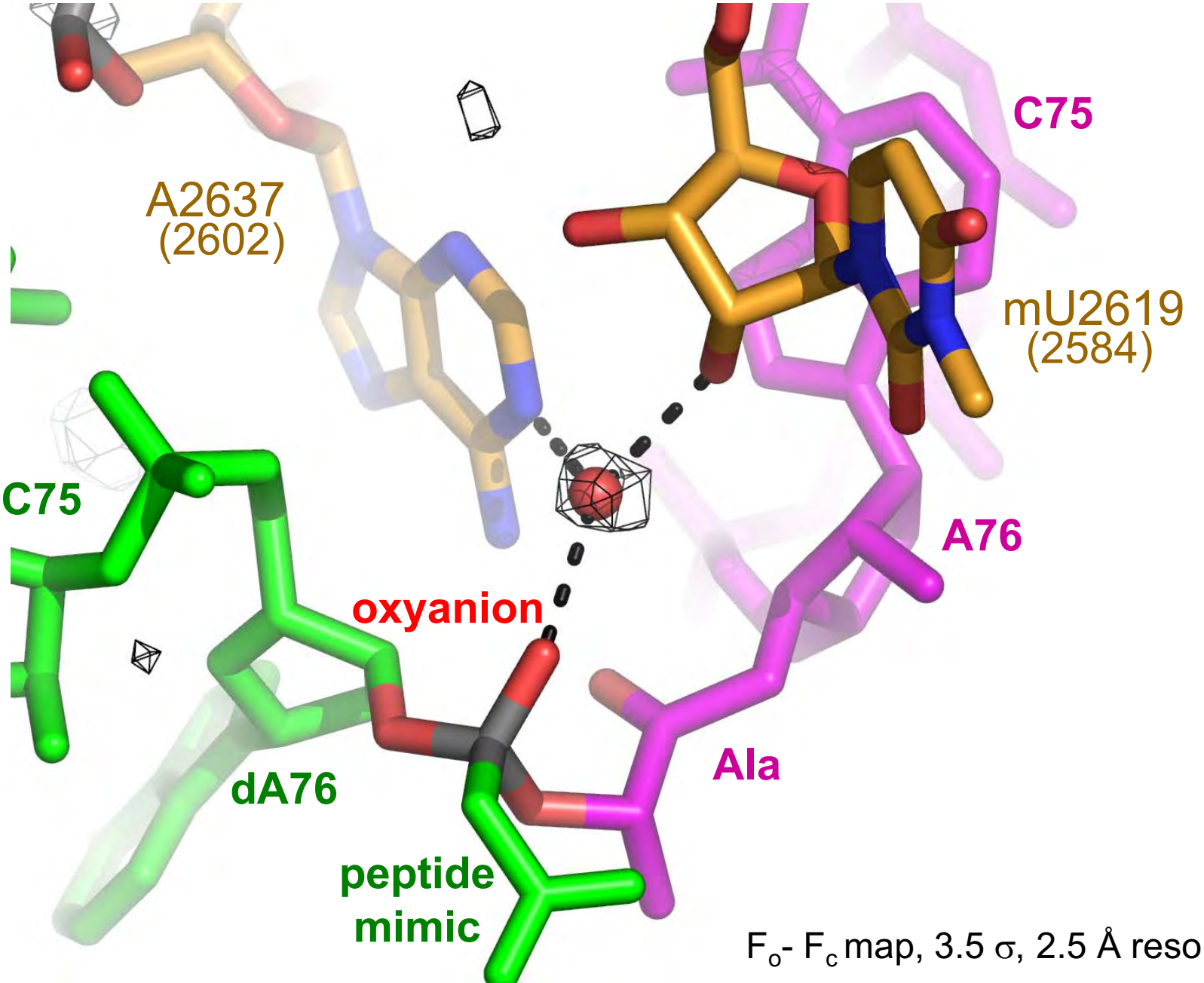
The oxyanion of the transition state points away from A2486



Schmeing, Huang, Strobel, Steitz et al, Mol Cell,(2005)

F_o- F_c map, 3.0 σ, 2.3 Å resolution

The oxyanion hole is a water molecule



F_o- F_c map, 3.5 σ, 2.5 Å resolution

Contributors to the ribosome's catalytic power

- Substrate orientation by the 23S rRNA
- Proton shuttle from alpha-amino to the 3'OH by the 2'OH of A76 of the peptidyl-tRNA
- Transition state stabilization by a water molecule bound to the oxyanion of the intermediate

The Peptidyl Transferase Reaction

Martin Schmeing
T. Steitz lab

WEDNESDAY, OCTOBER 17, 2007

National Report

The New York Times

Lethal Bacterial Infections Are Found More Common

Study Links 19,000 Deaths to Germ in 2005

By KEVIN SACK

ATLANTA, Oct. 16 — Nearly 19,000 people died in the United States in 2005 after being infected with virulent drug-resistant bacteria that have spread rampantly through hospitals and nursing homes, according to the most thorough study of the disease's prevalence ever conducted.

vasive MRSA infections by 60 percent after it began screening all patients in 2005.

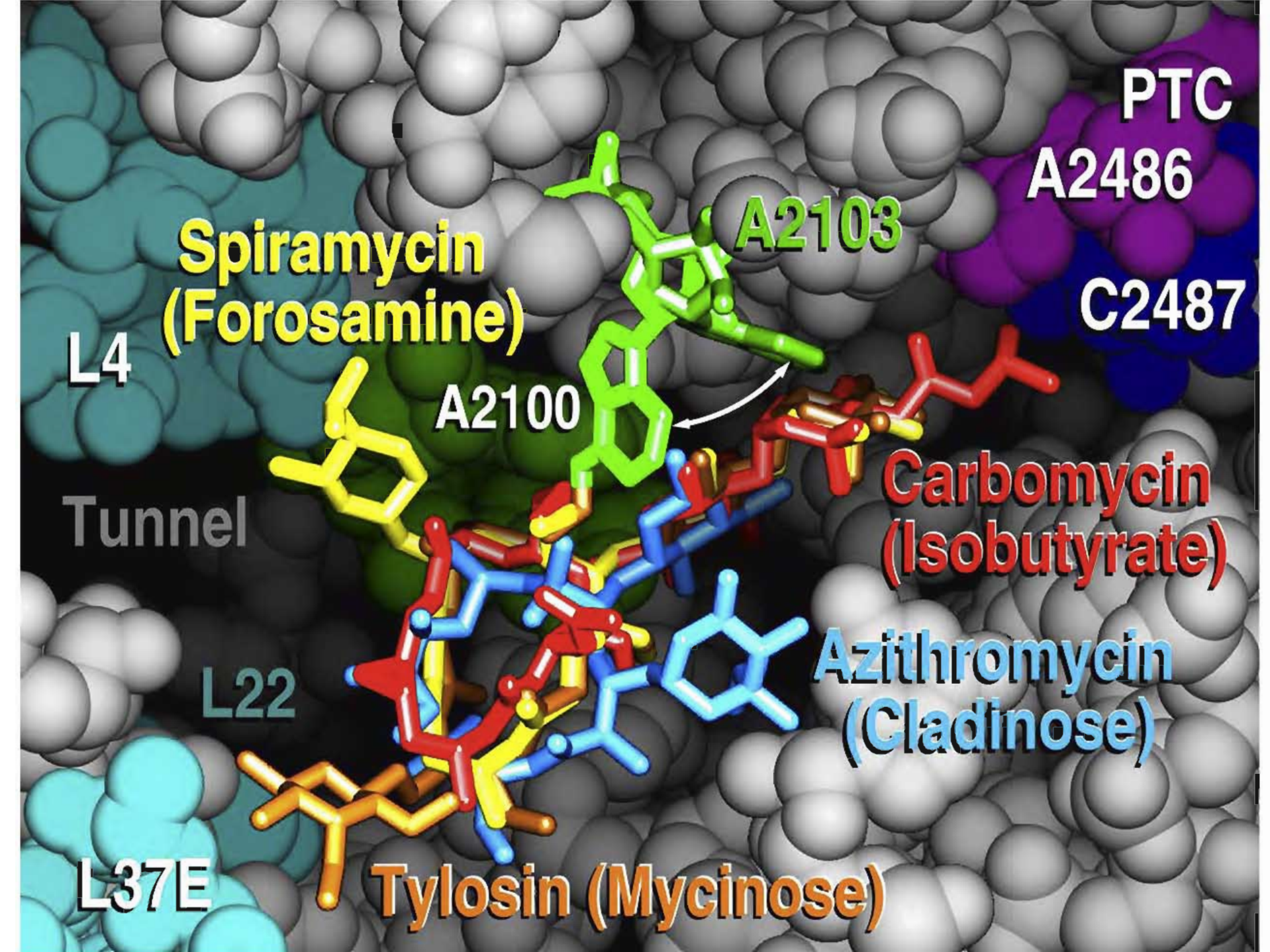
"This study puts more onus on organizations that don't do active surveillance to demonstrate that they're reducing their MRSA infections," Dr. Peterson said. "Other things can work, but nothing else has been demonstrated to have this kind of impact

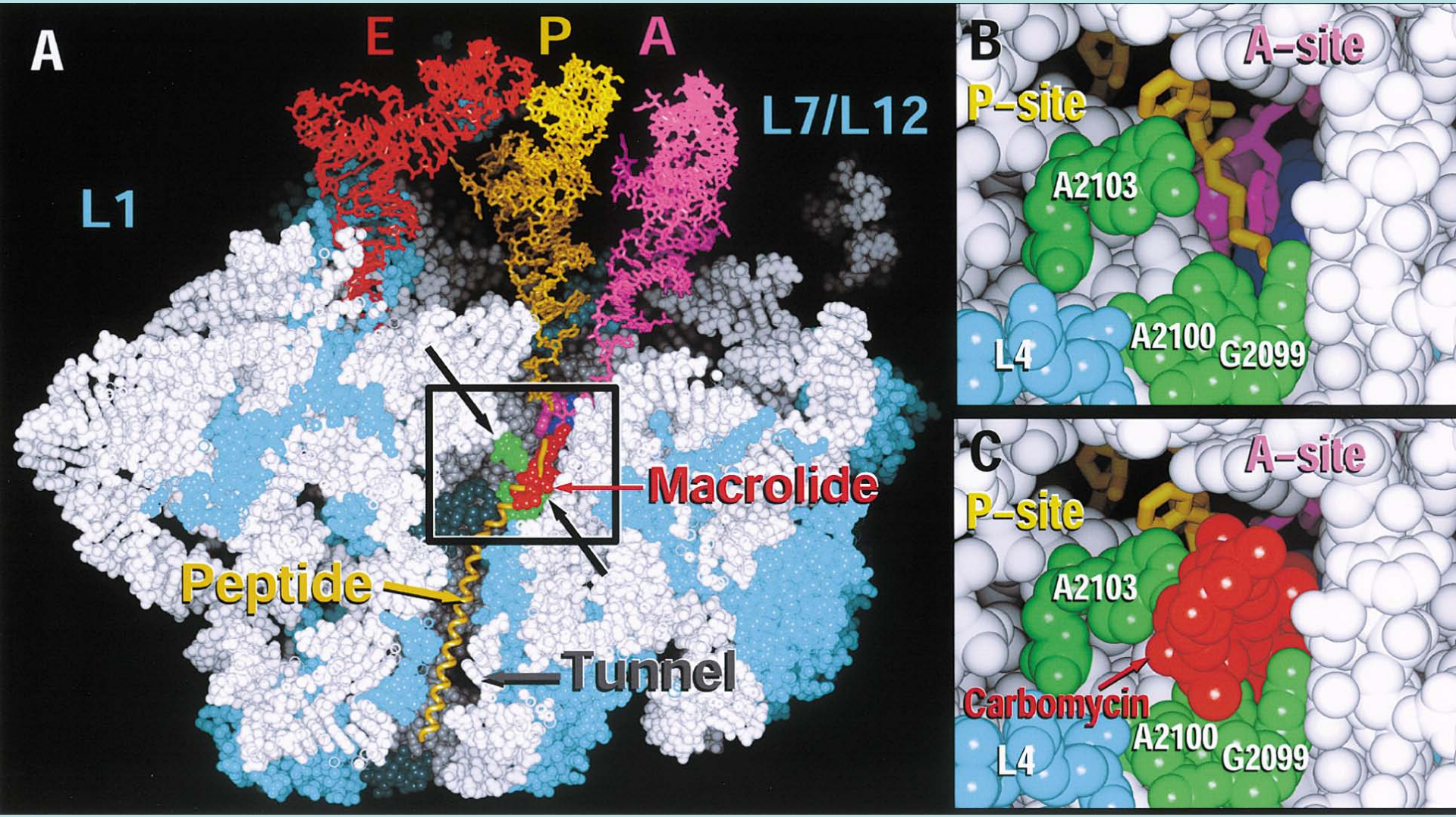
An estimate that fatalities could exceed those for AIDS.

Gross sales of antibiotics amount to about $30 billion per year worldwide. About half target the ribosome, mostly the large subunit.


Jeff Hansen 1998-2003

15- and 16-member macrolides bind in the tunnel of the 50S subunit

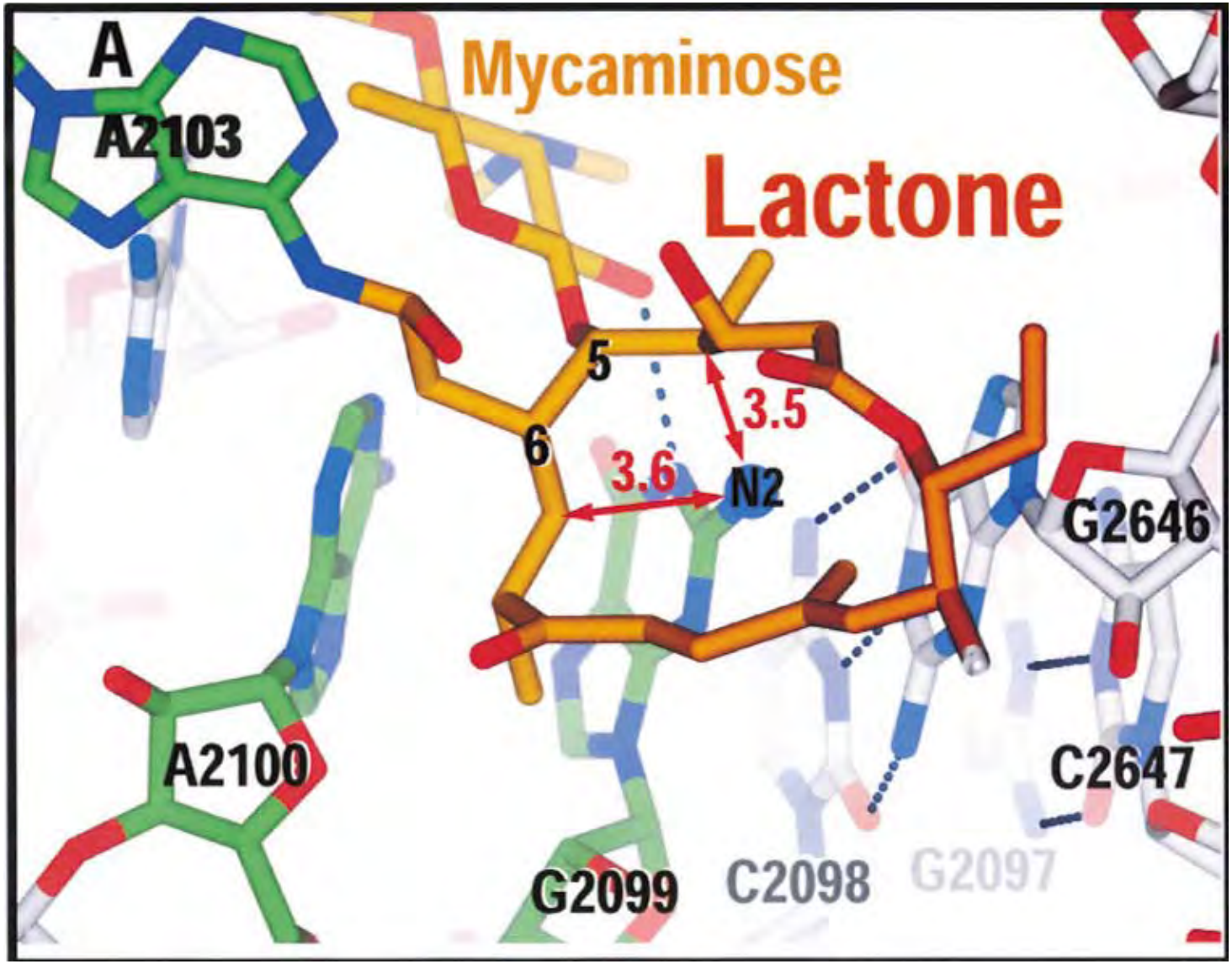

PTC
A2486
C2487
A2103
Spiramycin
(Forosamine)
L4
A2100
Tunnel
Carbomycin
(Isobutyrate)
Azithromycin
(Cladinose)
L22
L37E
Tylosin (Mycinose)



Hansen et al Mol. Cell, 2002

Mutation of A2058 to G in E. coli reduces the binding constant for erythromycin by 10,000 fold

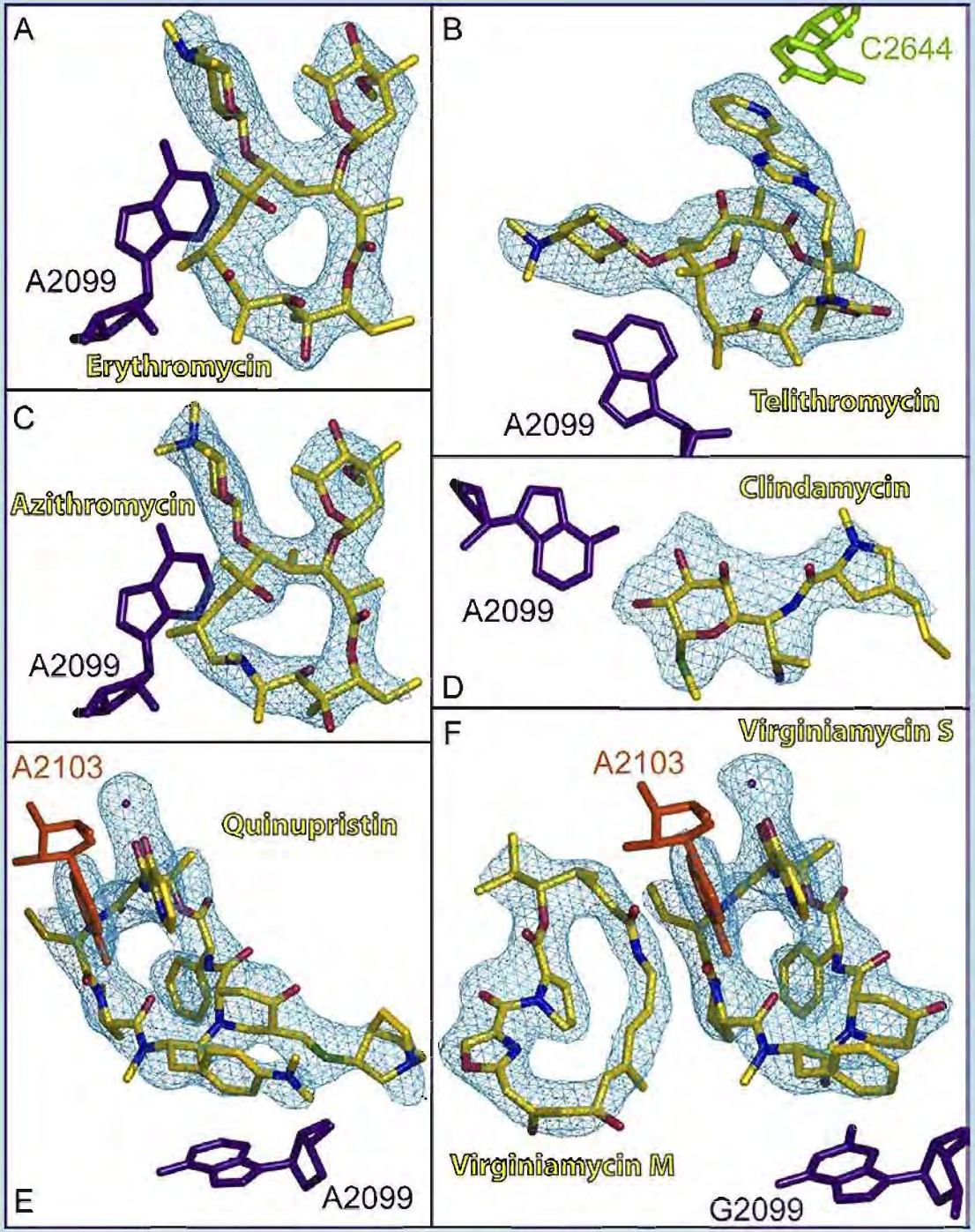
Since E. coli A2058 is G2099 in the H. marismortui 50S subunit, many MLSK antibiotics do not bind to this archeal subunit.



G2099 is A2058 in E. coli Hansen et al Mol. Cell, 2002

G2099 (A2058 E. coli) was mutated to A2099 in one of the three 23S rRNA genes

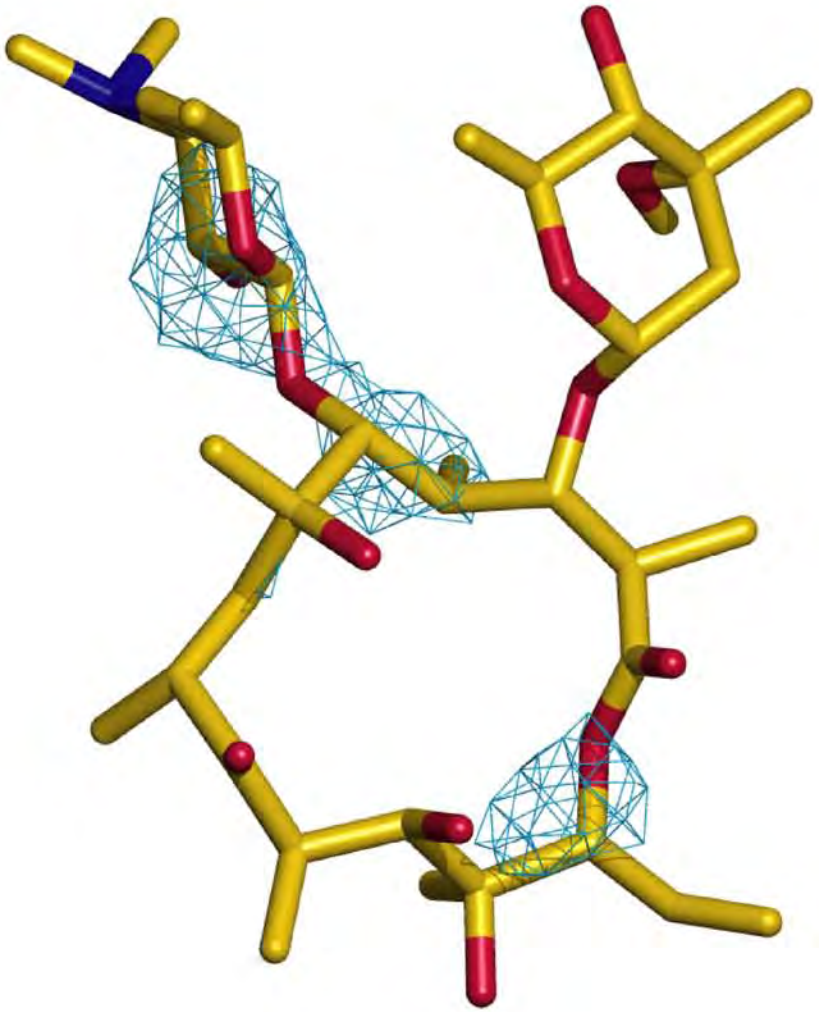
Daqi Tu, Gregor Blaha, Peter Moore & Tom Steitz, Cell, 2005.

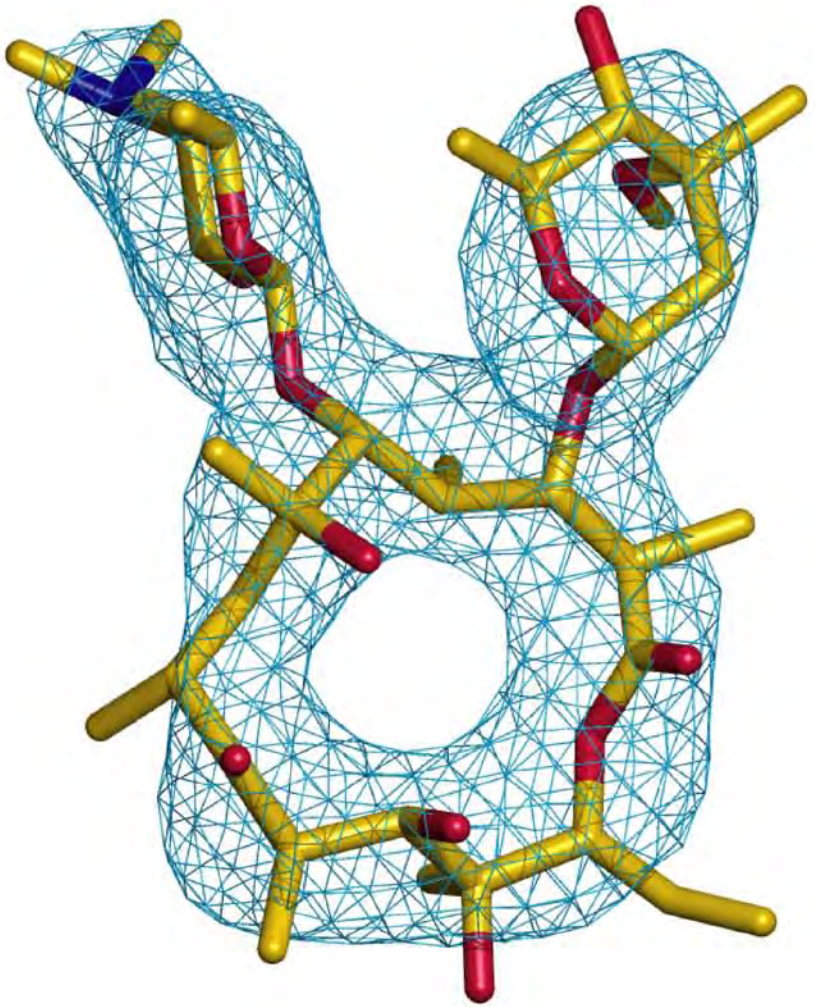

A
A2099
Erythromycin
B
C2644
Telithromycin
A2099
C
Azithromycin
A2099
D
Clindamycin
A2099
E
A2103
Quinupristin
A2099
F
Virginiamycin S
A2103
Virginiamycin M
G2099

G2099A Mutation Increases Erythromycin Afinity >10,000 Fold

100% G2099

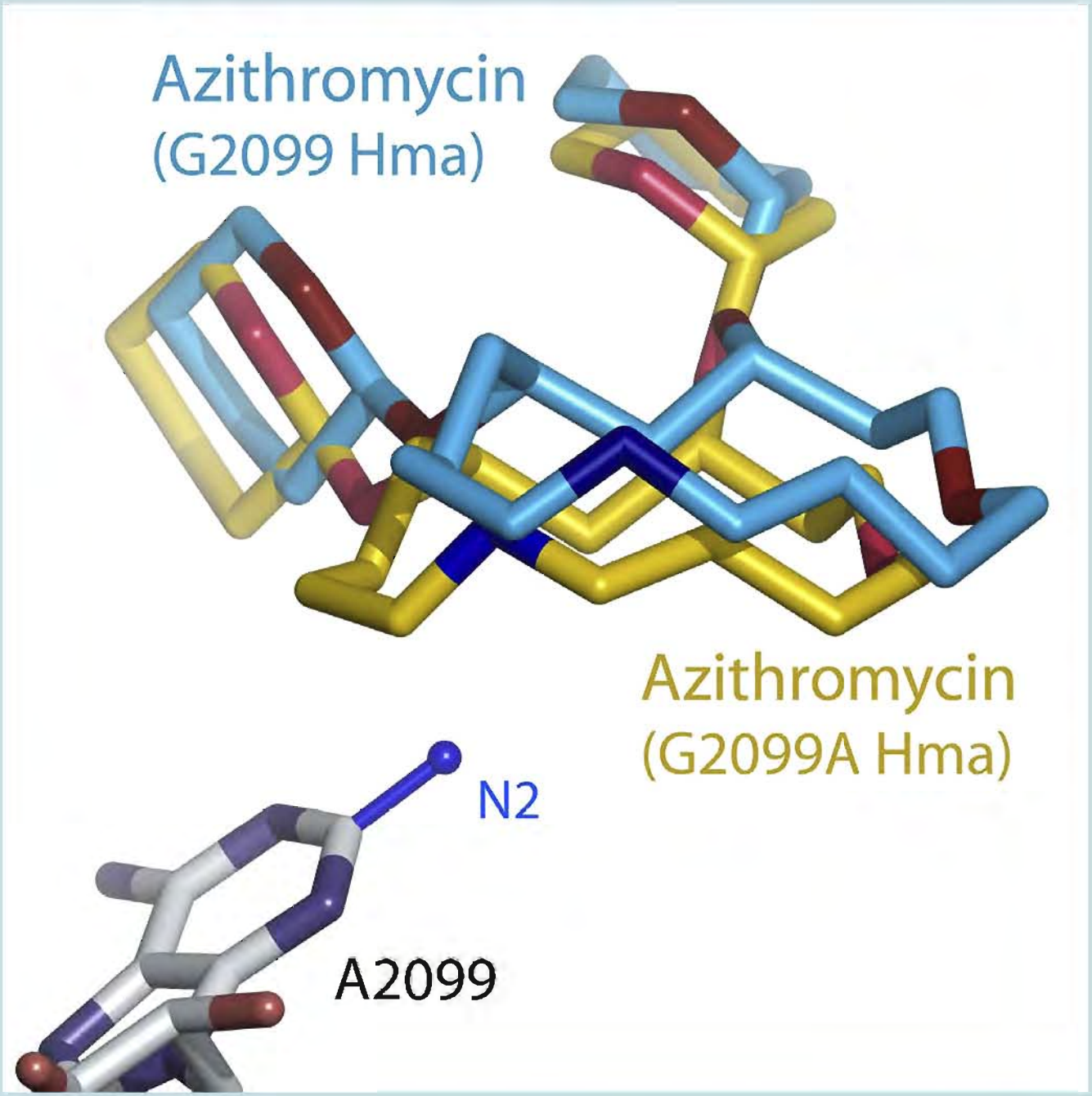
33% G2099A

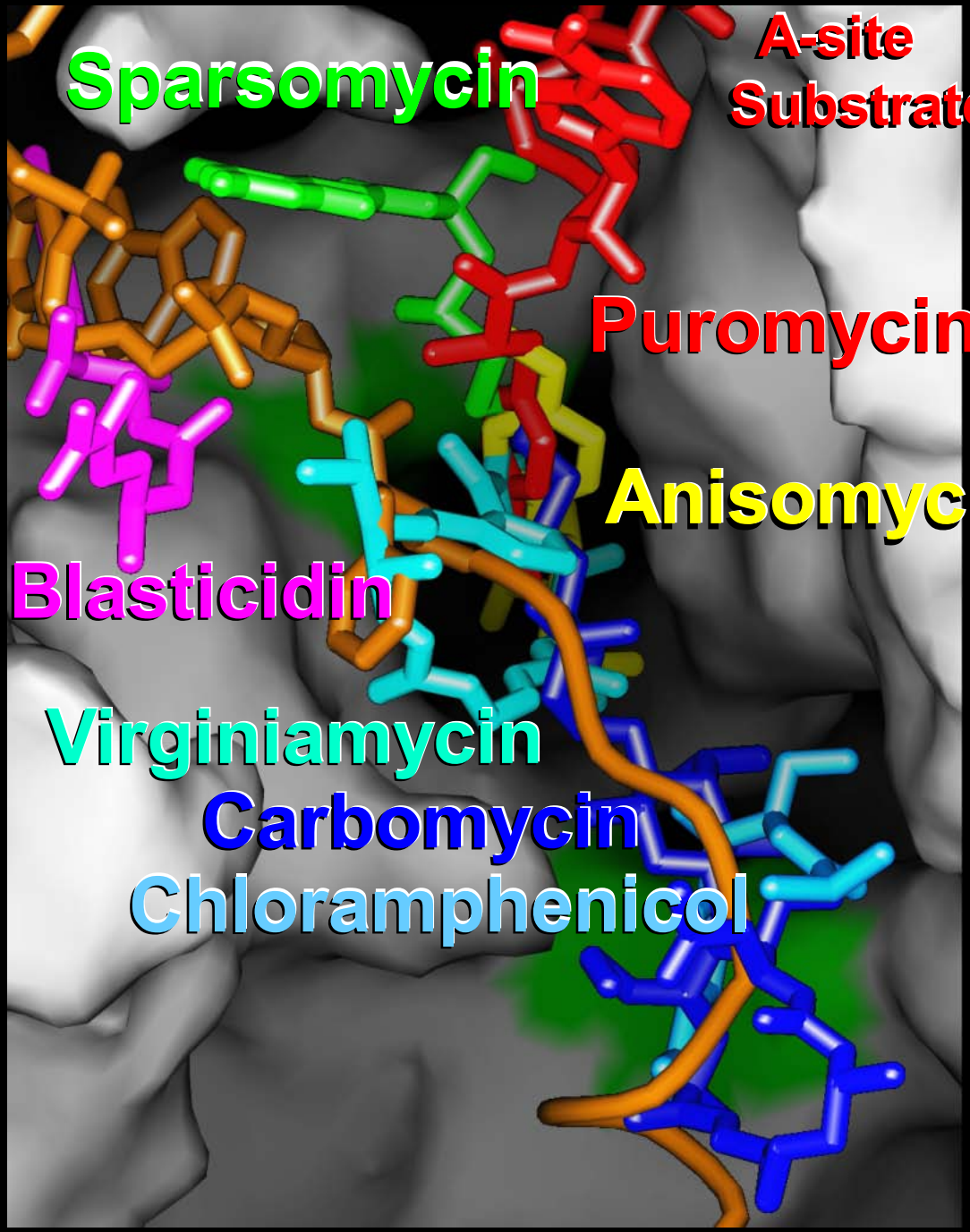




~ 3 mM erythromycin

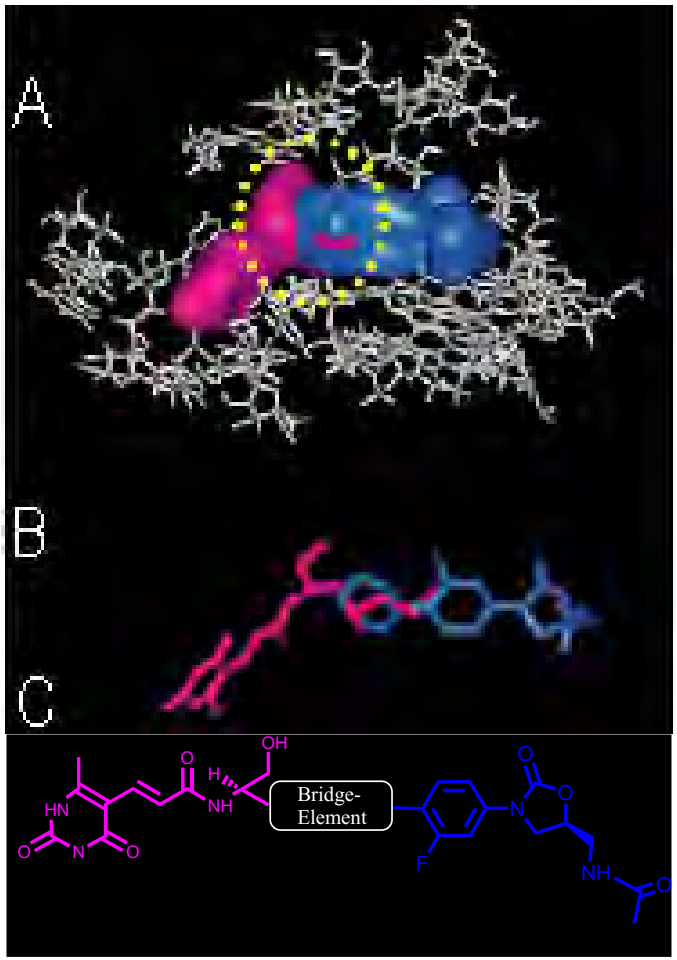
0.003 mM erythromycin

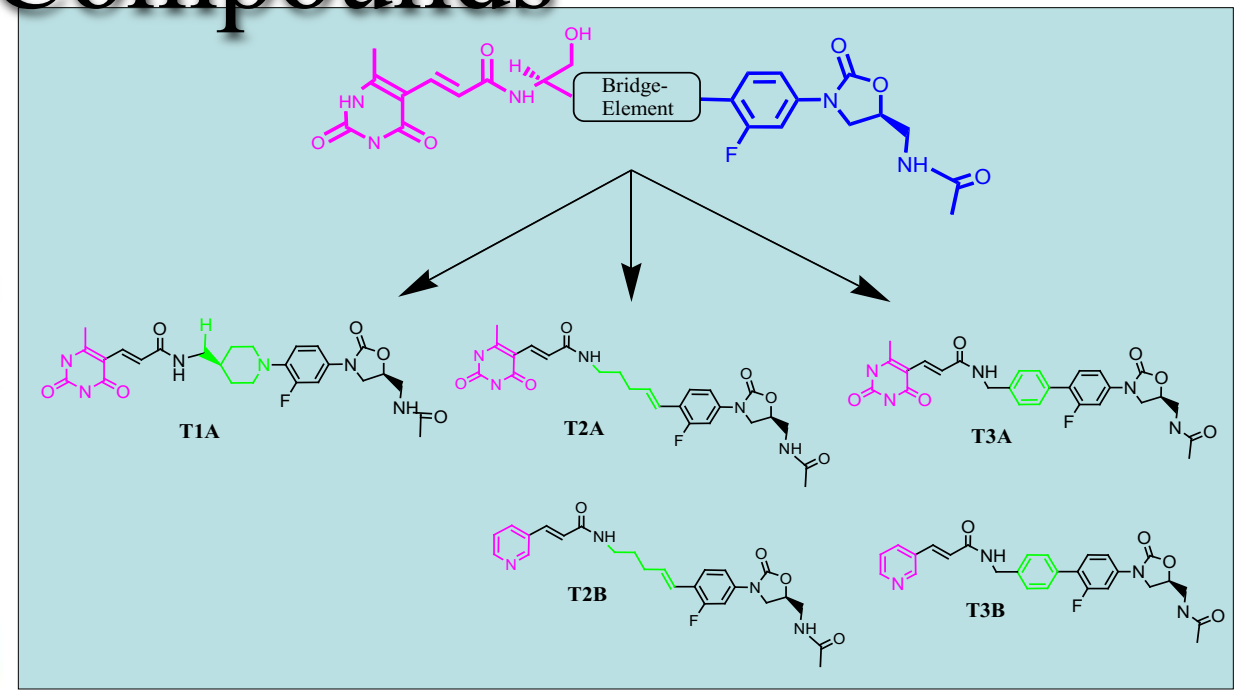

Azithromycin
(G2099 Hma)
Azithromycin
(G2099A Hma)
N2
A2099


Sparsomycin
A-site
Substrate
Puromycin
Anisomycin
Blasticidin
Virginiamycin
Carbomycin
Chloramphenicol

The structures of the antibiotic complexes with the H. marismortui large subunit are being used by Rib-X Pharmaceuticals to design new antibiotics effective against resistant strains.

Genesis of Rχ-01 Family of Compounds





	Linezolid	Sparsomycin	T1A	T2A	T2B	T3A	T3B
E. coli Translation IC_{50} (μM)	Intrinsic Affinity						
	4.6	≤0.02	0.26	0.03	16	0.03	0.58
Selectivity	Y	N	N	N	Y	N	Y
	MIC (μg/ml)						
S. pneumoniae 02J1175	2	2	4	1	8	≤0.25	0.5
S. pyogenes Msr610	1	2	4	1	4	≤0.25	0.5
E. faecalis P5 (lin^R)	32	>128	>128	32	128	16	16
H. Influenzae RD1	16	8	>128	>128	>128	>128	>128

Iterative Cycle Yields Compounds to Treat Respiratory Tract Infections

	Inhibition of Translation (μM)		MIC (μg/ml)	
Compound	Prokaryote	Eukaryote	*S. pneumoniae*	*H. influenzae*
$RX\text{-}A_1$	0.92	0.23	1	>128
$RX\text{-}A_2$	14.6	>200	8	>128
$RX\text{-}A_7$	<0.2	1.5	0.25	>128
$RX\text{-}A_8$	6.8	>100	0.5	>128
$RX\text{-}A_{84}$	0.083	>100	0.25	2
$RX\text{-}A_{89}$	0.049	>100	0.25	16
$RX\text{-}A_{188}$	<0.02	1.01	0.06	2
$RX\text{-}A_{258}$	<0.02	20	0.25	2

Rib - X Pharmaceuticals, Inc.

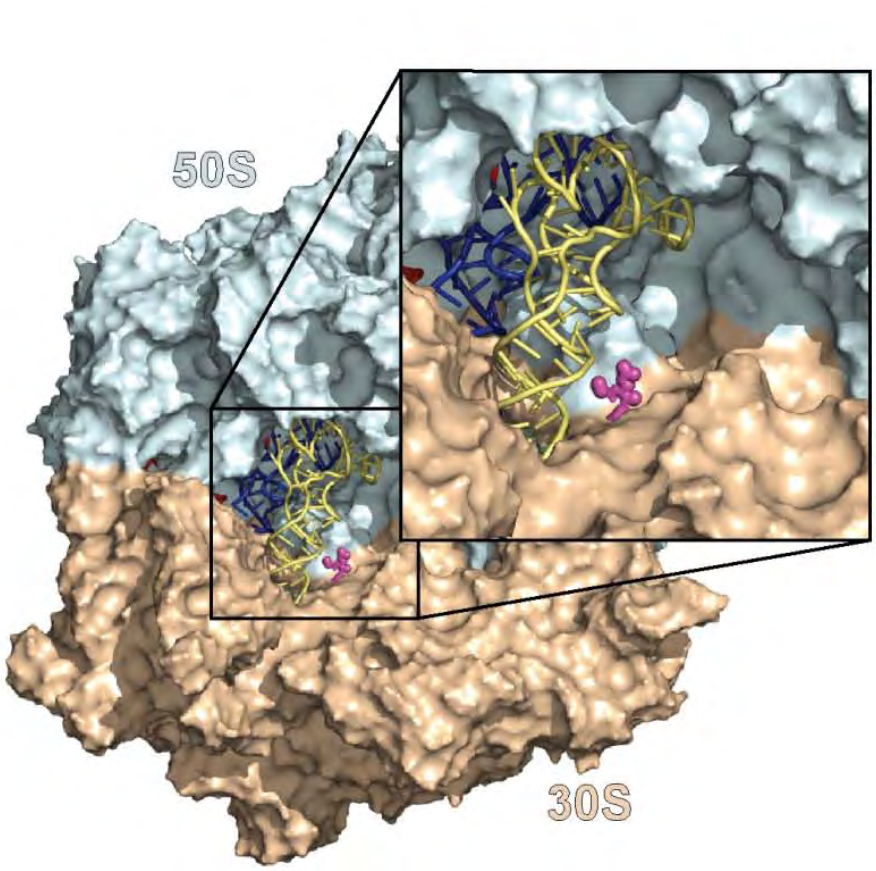
Superior compounds obtained

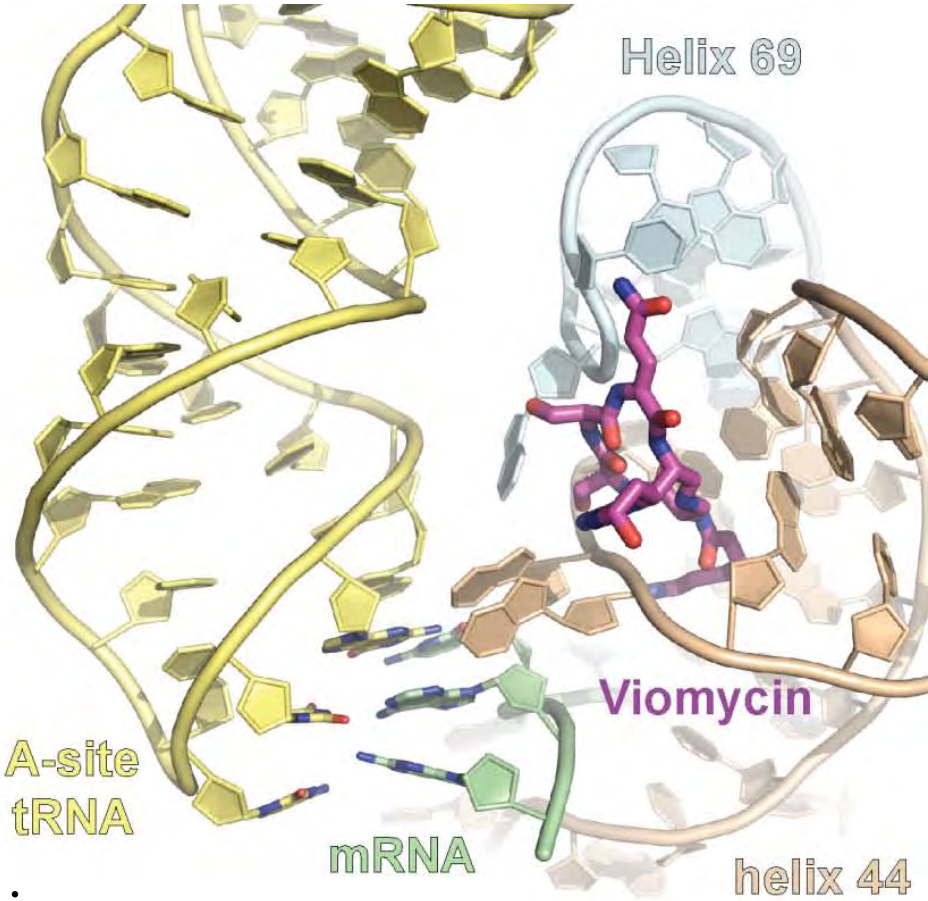
	MIC (μg/mL); Target ≤4		
Bacterial Strains	**Zithromax**	**RX-A**	**RX-B**
Streptococcus pneumoniae:			
Point mutation in 23S delivering macrolide resistance	>128	≤0.25	≤0.25
Methylase of 23S + ribosomal protein mutation with resistance to macrolides	>128	≤0.25	1
Acquired efflux pump delivering 14,15-membered macrolide resistance	16	≤0.25	≤0.25
Streptococcus pyogenes:	>128		
Methylase of 23S delivering macrolide resistance		≤0.25	≤0.25
Haemophilus influenzae:			
Tough clinical strain	1	4	4
Enterococcus faecalis:			
Point mutation in 23S delivering linezolid resistance	4	2	≤0.25
Vancomycin & linezolid resistance	>128	1	≤0.25
Vancomycin resistance	>128	≤0.25	≤0.25

Radezolid: Antimicrobial Activity Against Zyvox-Resistant Enterococci

Isolate	MIC (μg/ml)		
	Radezolid	Linezolid (Zyvox)	Vancomycin
E. faecalis ATCC 29212	≤0.25	4	2
E. faecalis A5962	1	32	8
E. faecalis A7789	4	64	1
E. faecium A5959	4	32	>128
E. faecium A5960	4	64	>128
E. faecium A8130	2	32	128
E. faecium A9650	0.5	16	>128
E. faecium A8948	≤0.25	8	>128
E. faecium A9621	4	64	>128

Viomycin binds between subunits, interacting with B2A bridge & tRNA





Stanley, Blaha, et al., NSMB, in press

Viomycin, hygromycin & paromomycin bind to adjacent sites