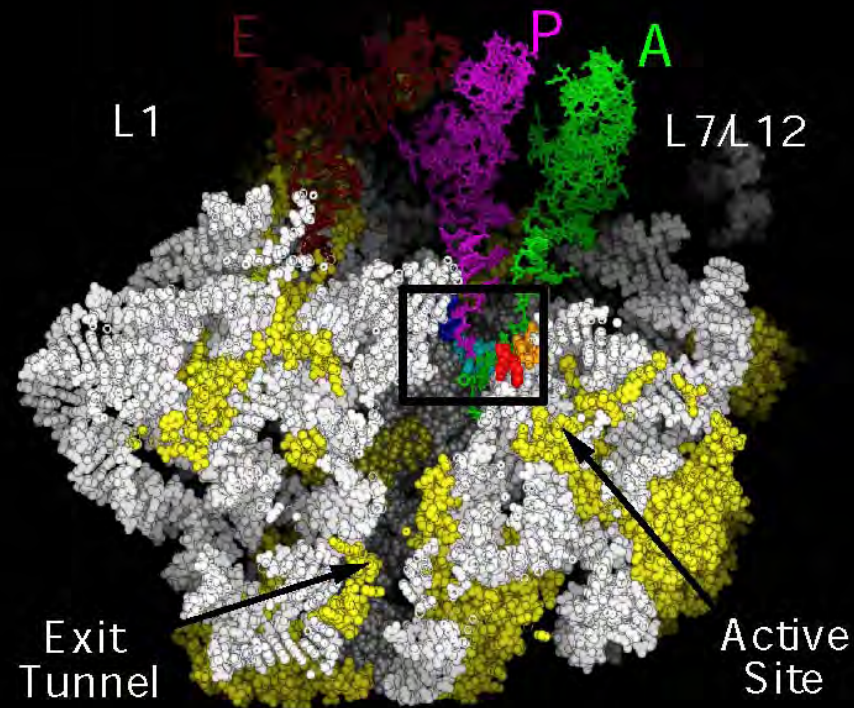
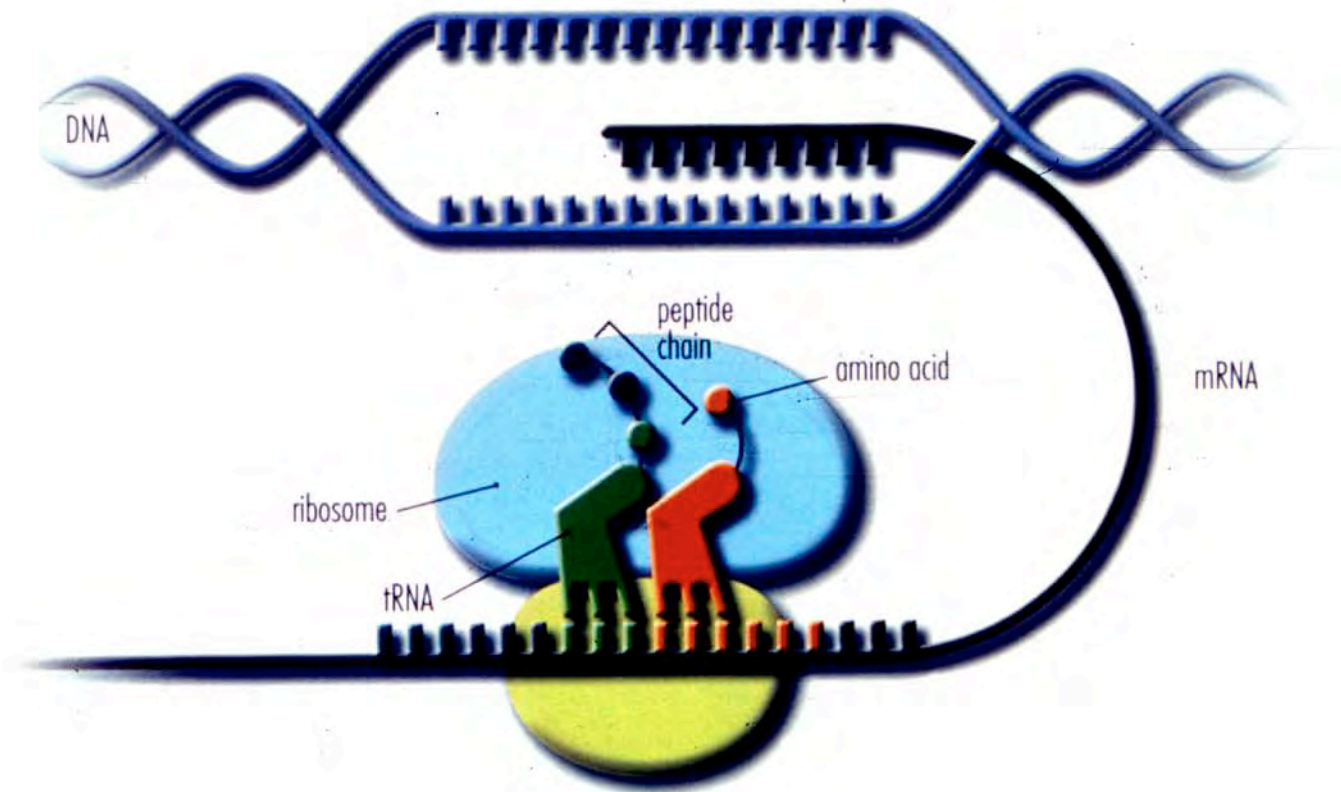
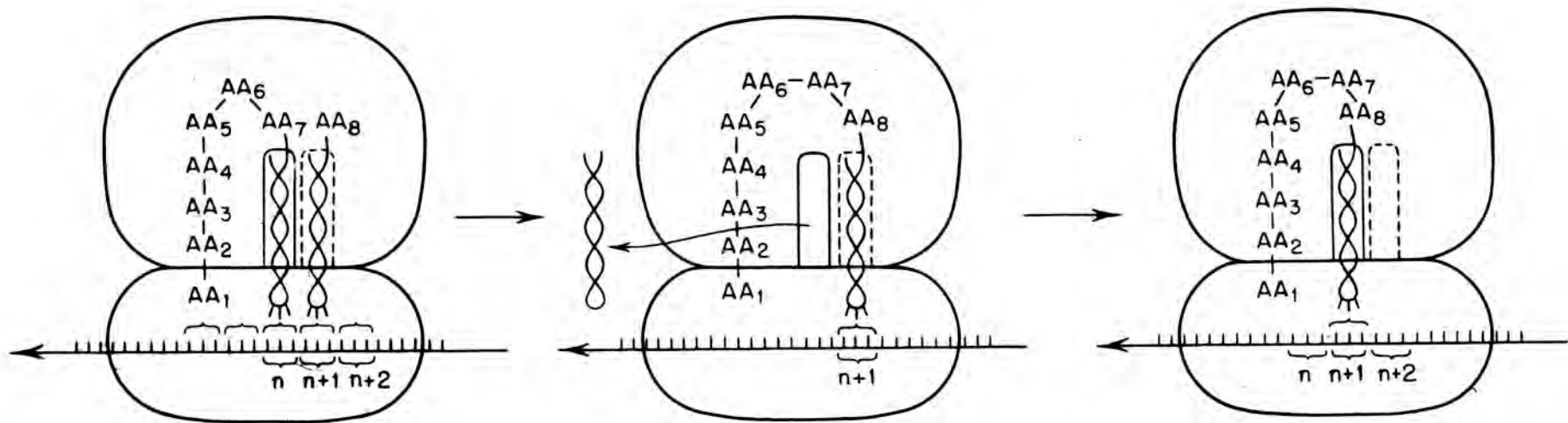


From the Structure and Function of the Ribosome to new Antibiotics



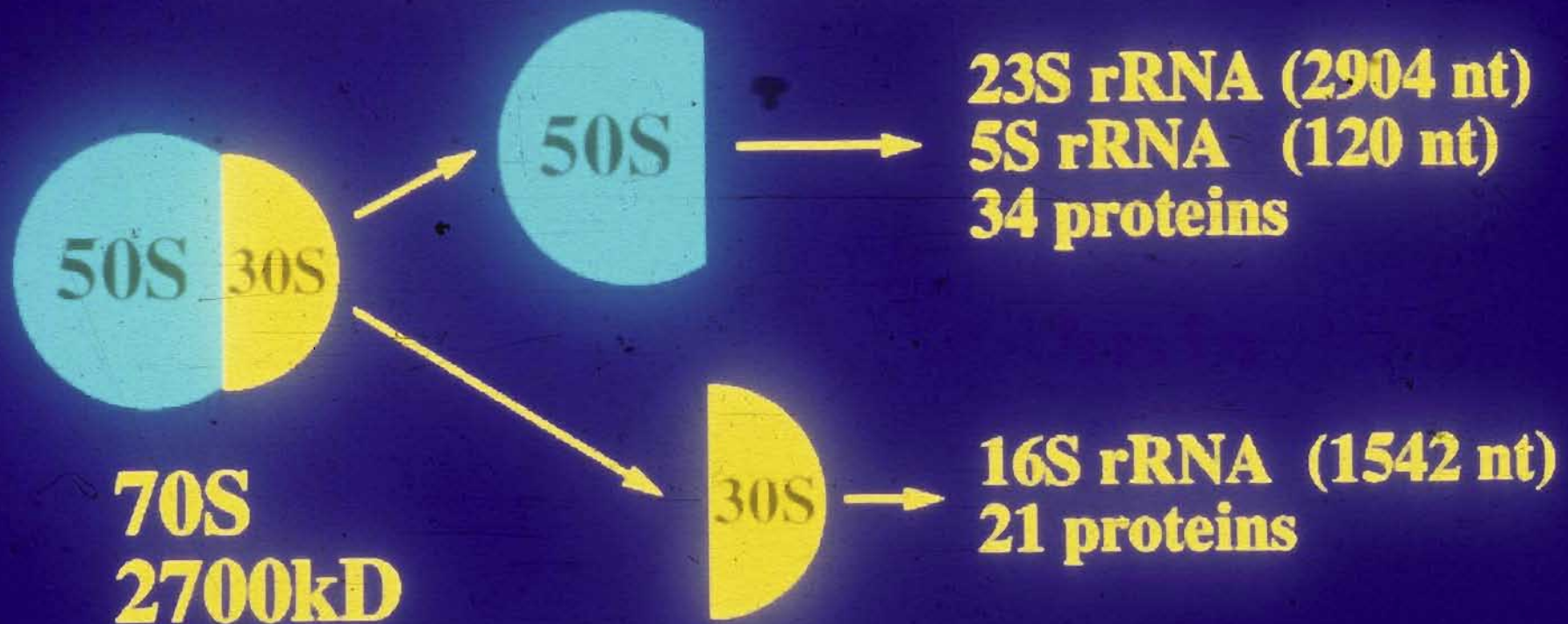
Crick's central dogma of molecular biology:
DNA makes DNA makes RNA makes protein



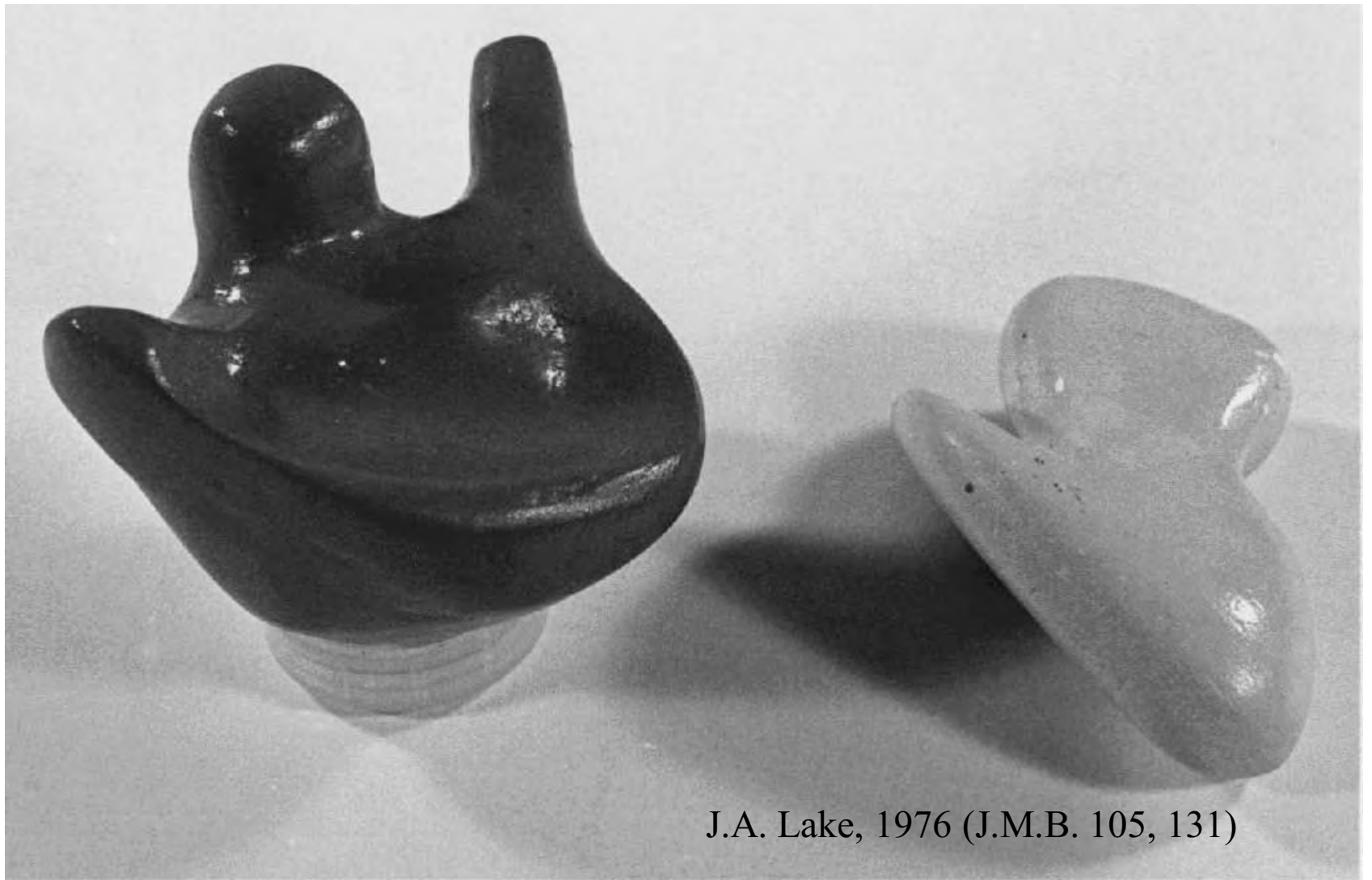


Jim Watson, 1964

Structural Components of the Ribosome from *E. coli*



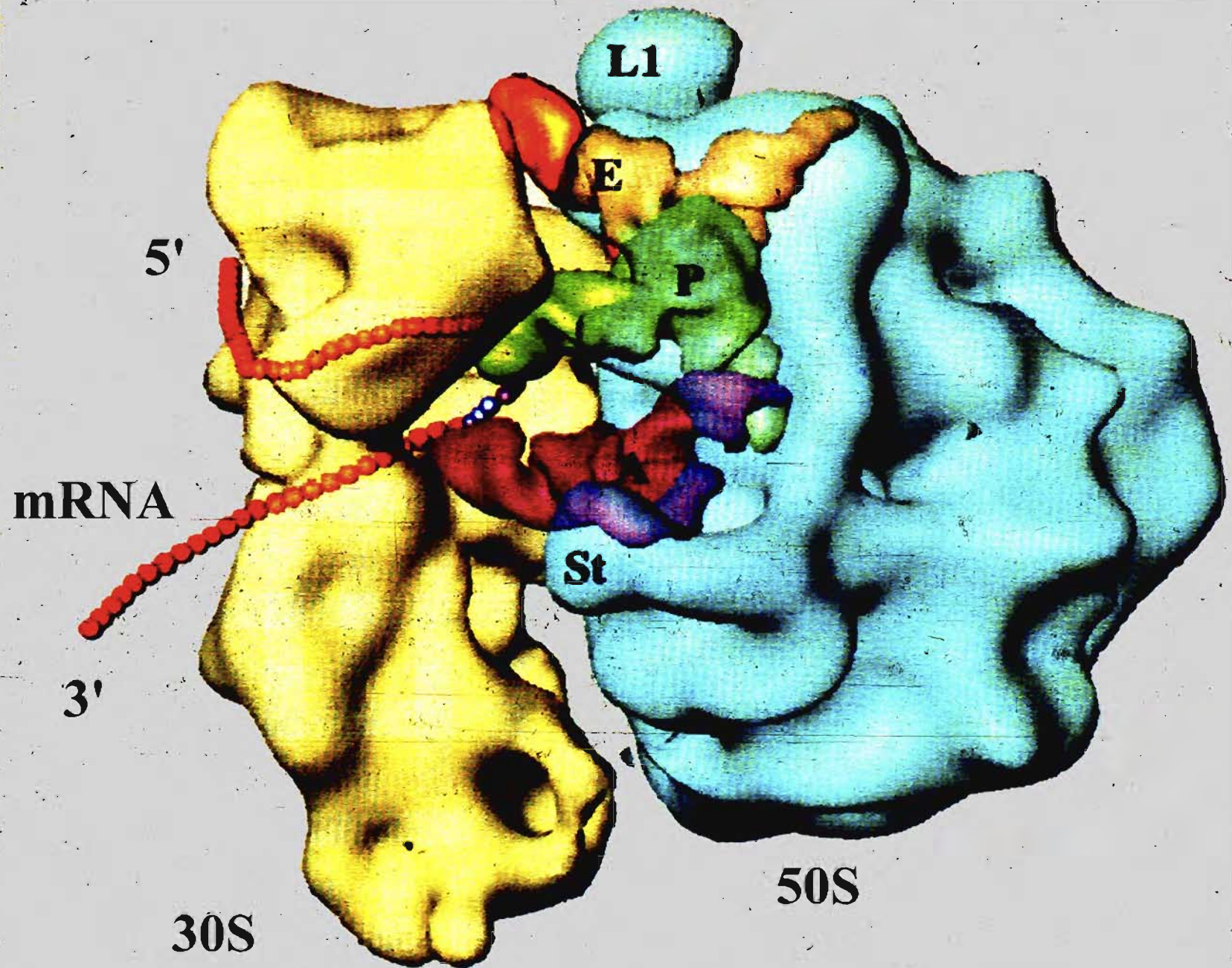
**Ribosome is 2/3 RNA by mass
and is 1/4 bacterial cell mass**



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



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



Joachim Frank, Current Opinion in Structural Biology (1997).

Nenad Ban,
1995-2000



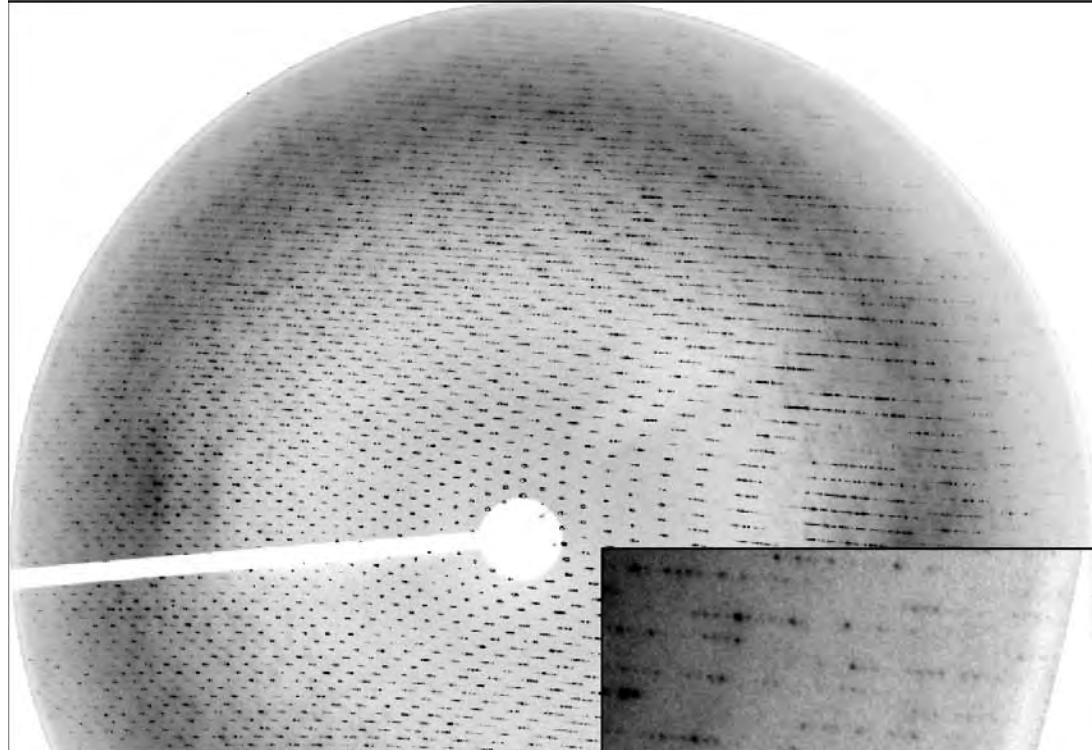
Peter Moore (and Striped Bass)





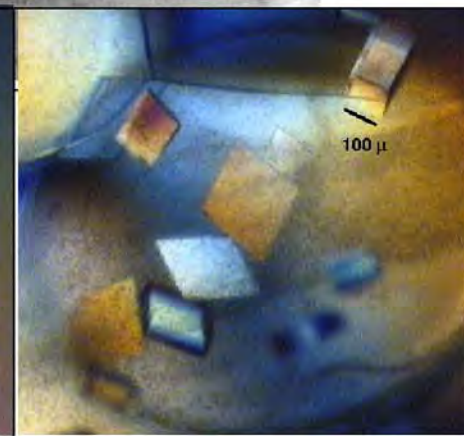
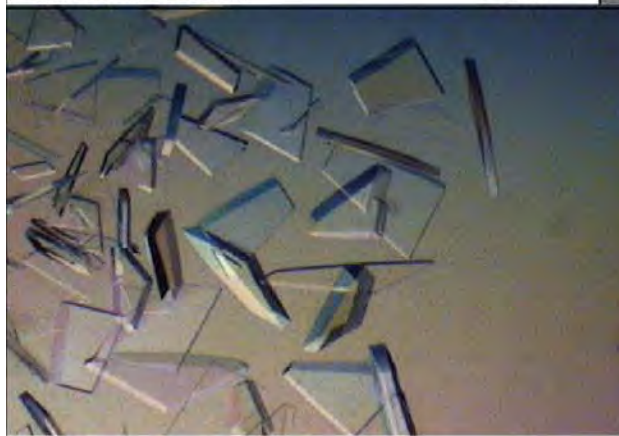
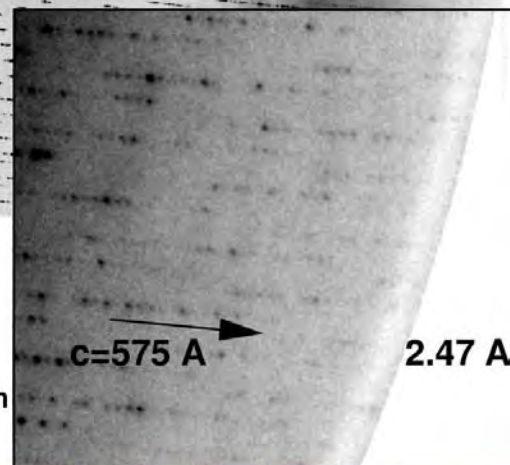
Poul Nissen, 1997-2000

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



Haloarcula marismortui 50S crystals
C2221 ($a=212$, $b=300$, $c=575$, $a,b,g=90^\circ$)

Brookhaven x25 (wiggler) Feb. 2000
Mar345 IP, 60 sec. exp, 1° osc.
100 m beam, $I=0.9$ A, dist=480 mm
spot separation: 0.75 mm
spot size (observed): 0.75 mm
orders resolved: ~460





Queen Mary



Queen Mary + Captain

Tungsten, 78 electrons

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



Sail Boat



Sail Boat + Captain

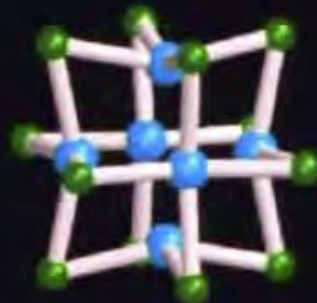
Tungsten, 78 electrons

Lysozyme,
14,600 dalton M.W.

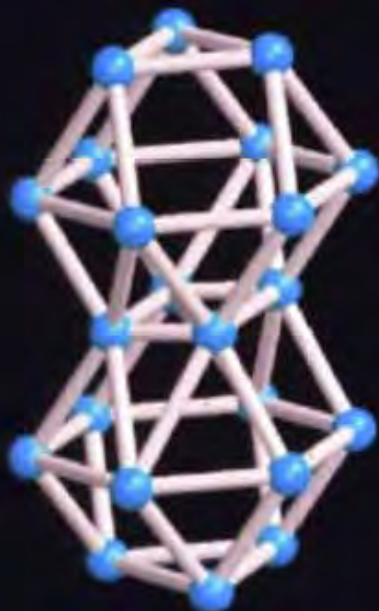
Heavy atom cluster derivatives



PIP 314 e⁻



Ta₆Br₁₂²⁺ 858 e⁻



W-18 ~2000 e⁻

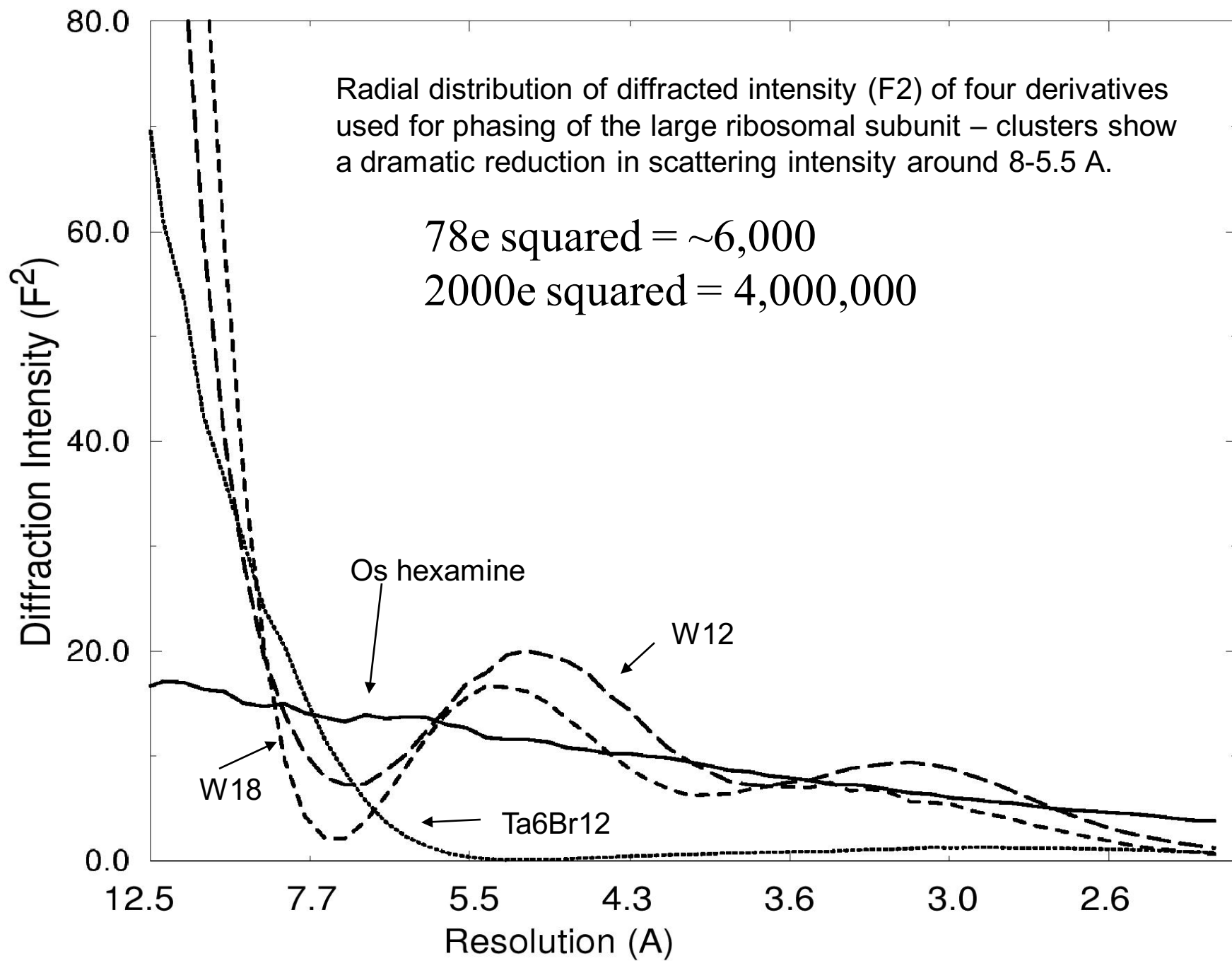


W-11 ~1250 e⁻

Radial distribution of diffracted intensity (F^2) of four derivatives used for phasing of the large ribosomal subunit – clusters show a dramatic reduction in scattering intensity around 8-5.5 Å.

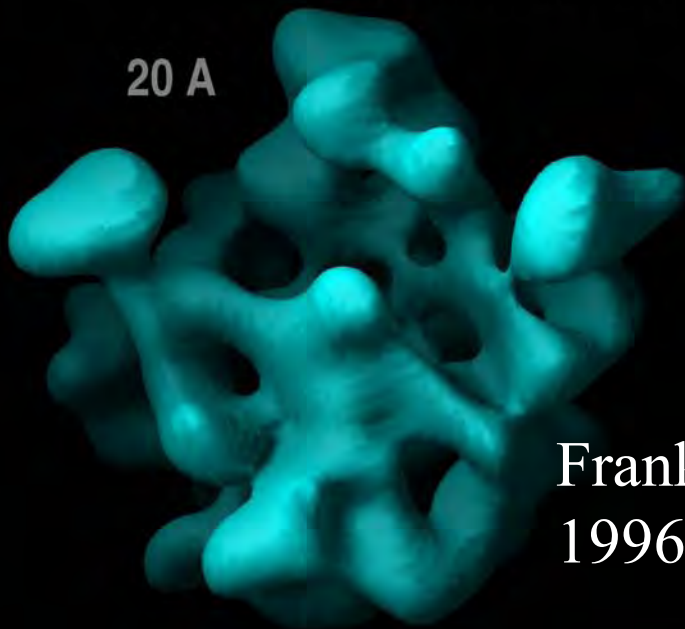
78e squared = ~6,000

2000e squared = 4,000,000



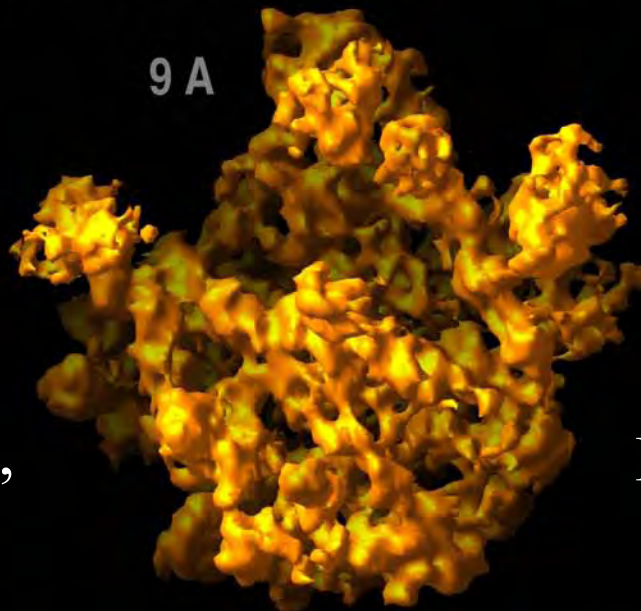
Experimental Electron Density Maps of 50S

20 Å



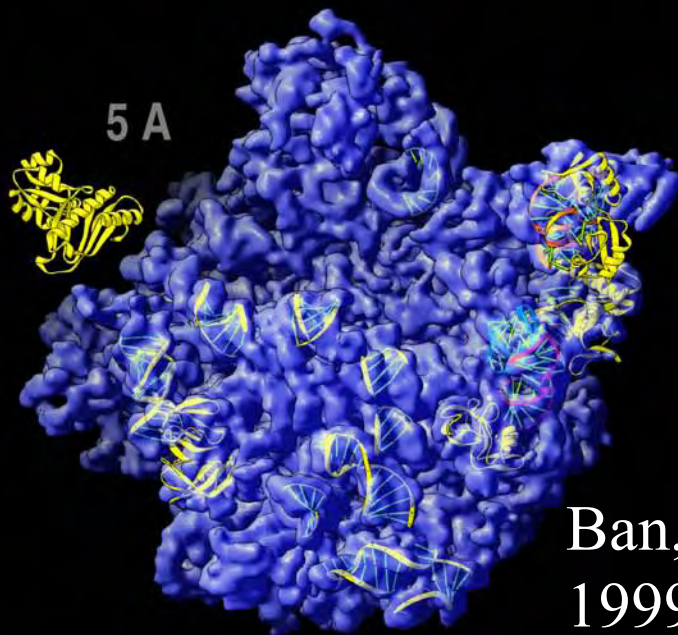
Frank,
1996

9 Å



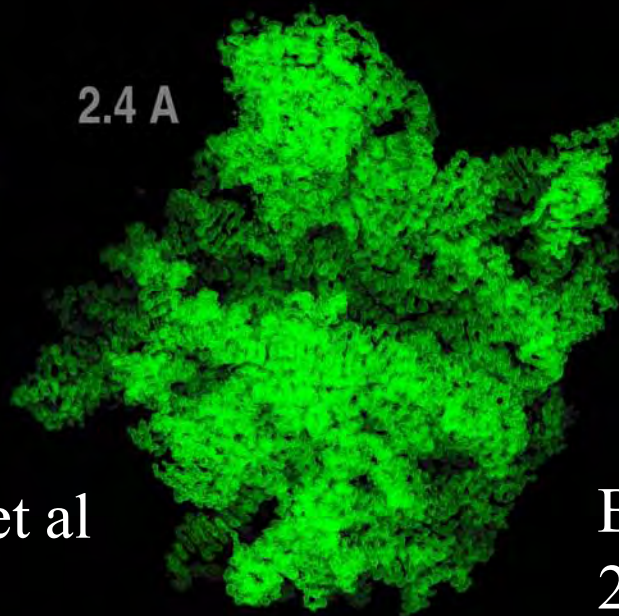
Ban, et al
1998

5 Å



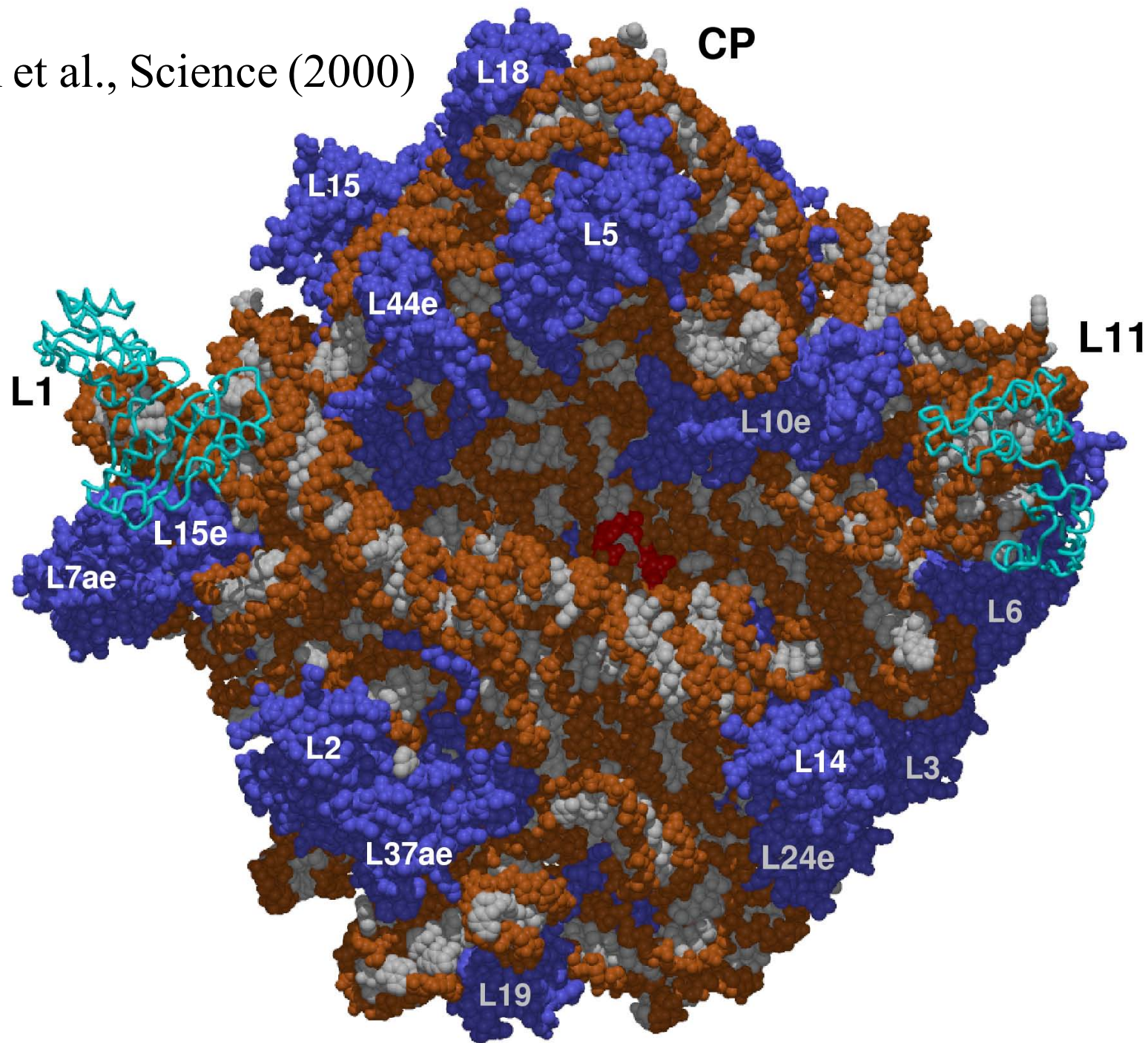
Ban, et al
1999

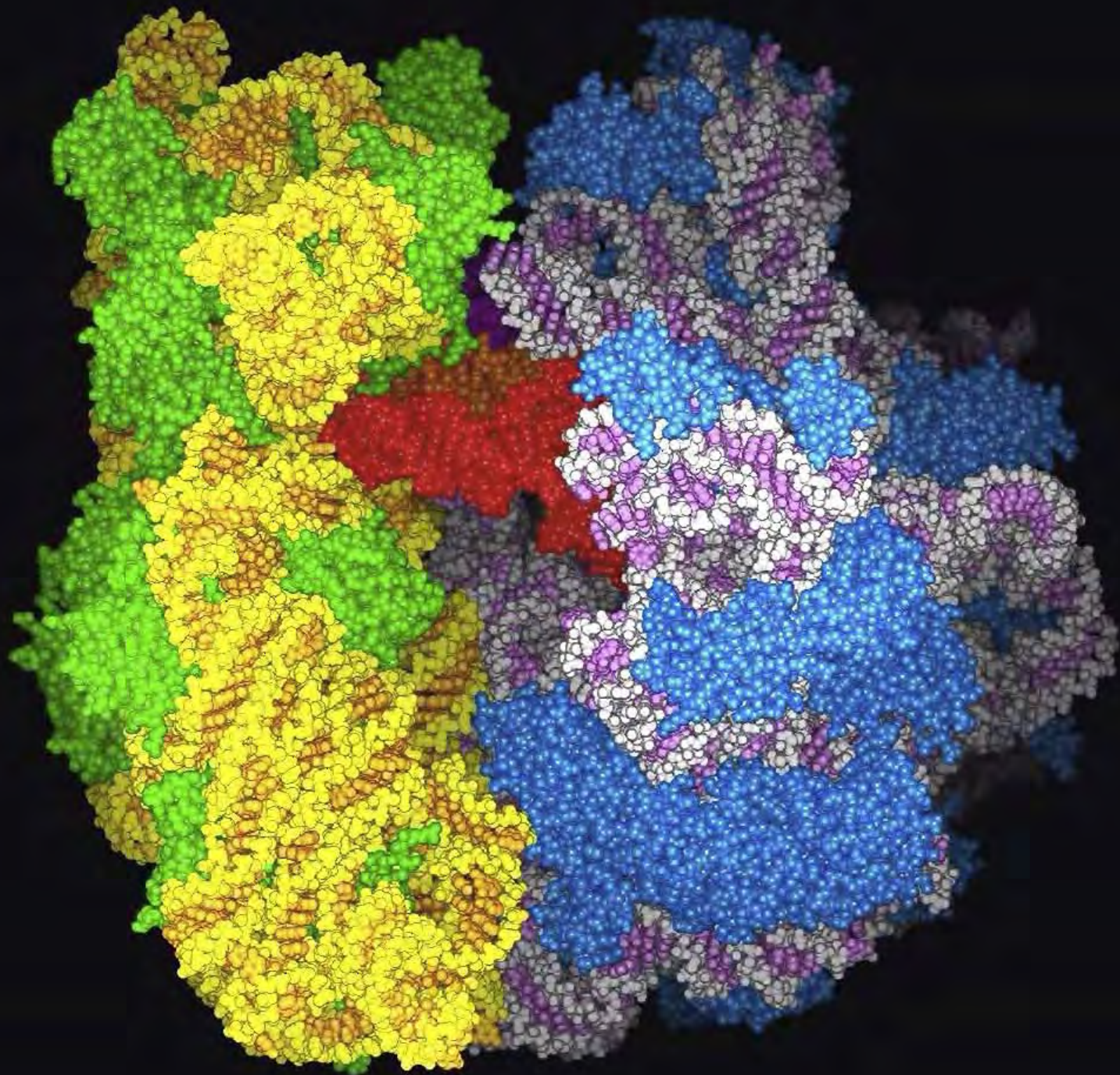
2.4 Å

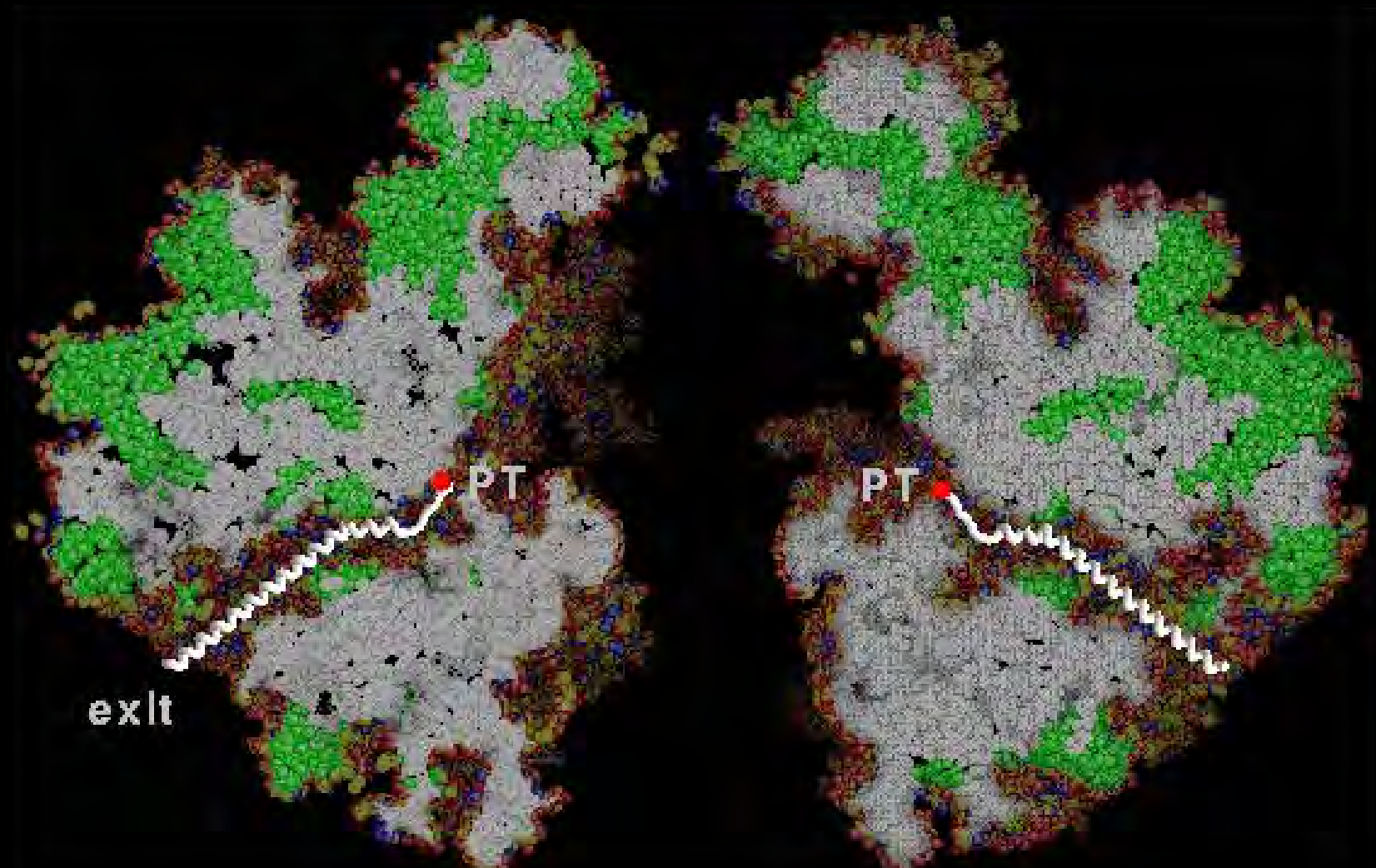


Ban, et al
2000

Nissen et al., Science (2000)

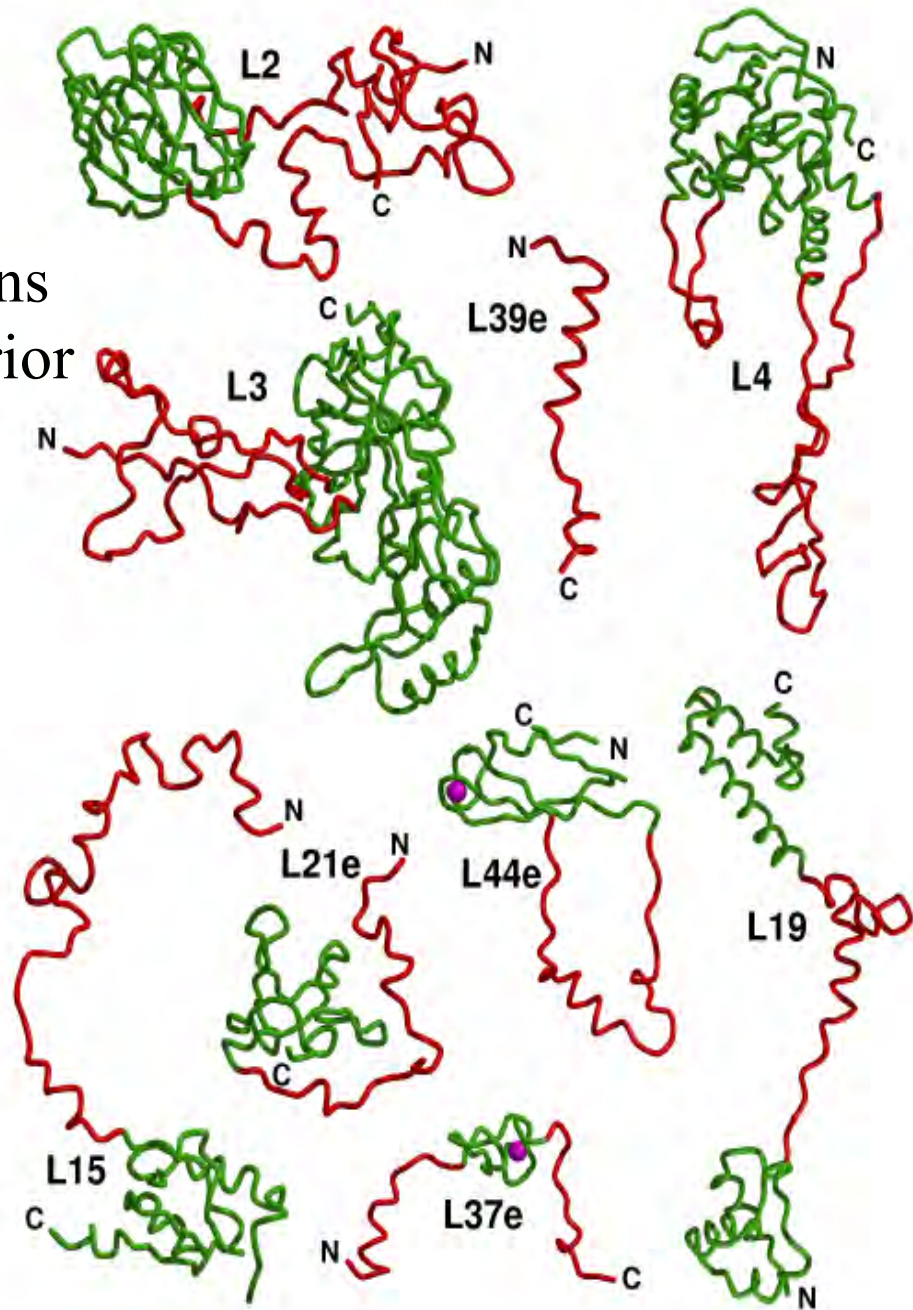






Nissen, et al. Science (2000)

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



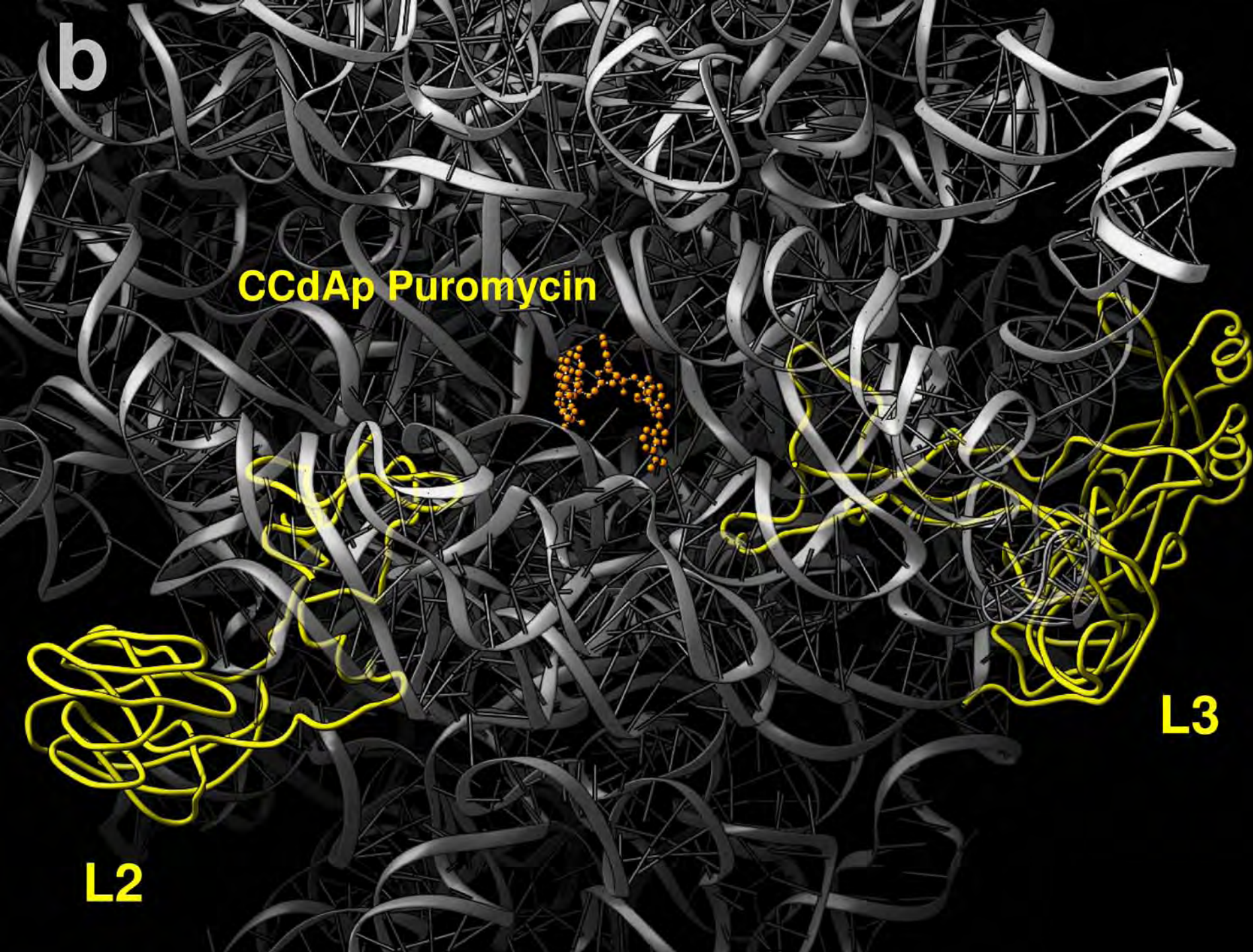
Ban et al., Science (2000)

b

CCdAp Puromycin

L3

L2



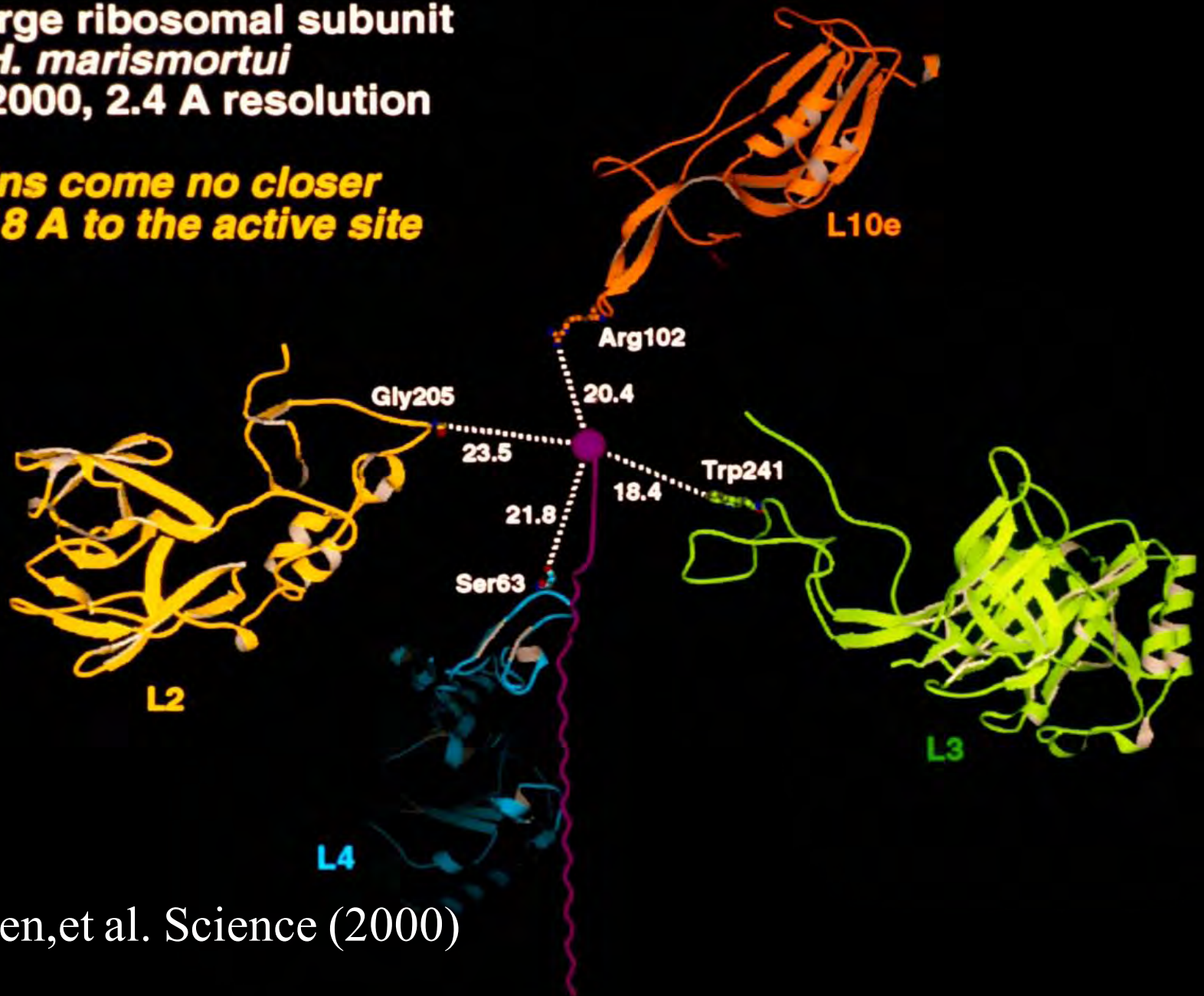
Crick recognized early that the ribosome should be a ribozyme



- “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)

The large ribosomal subunit
from *H. marismortui*
June 2000, 2.4 Å resolution

**Proteins come no closer
than 18 Å to the active site**



Nissen, et al. Science (2000)

THE RIBOSOME

IS

A RIBOZYME

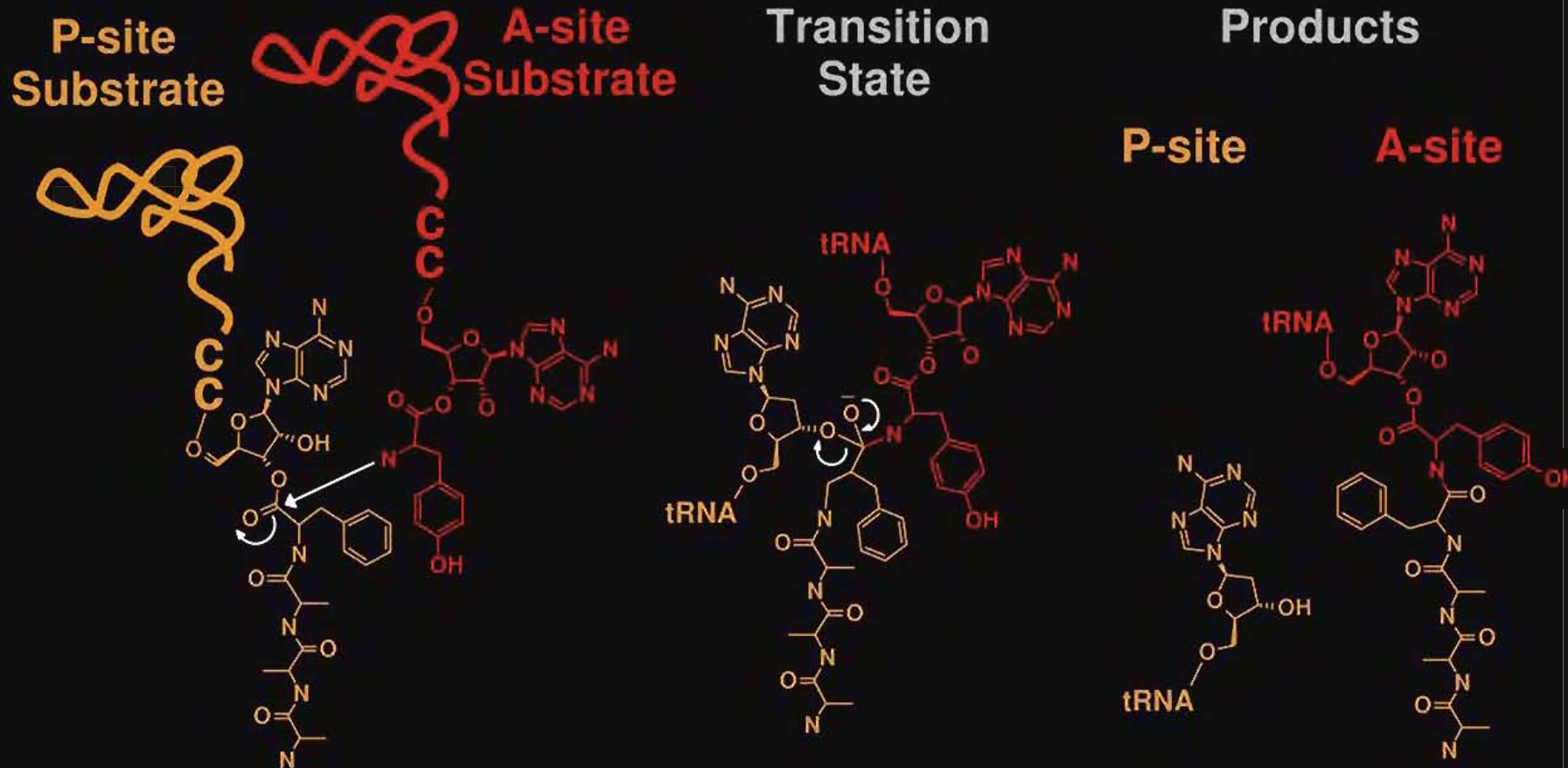
What is the source
of the
ribosome's catalytic
power
in peptide synthesis?

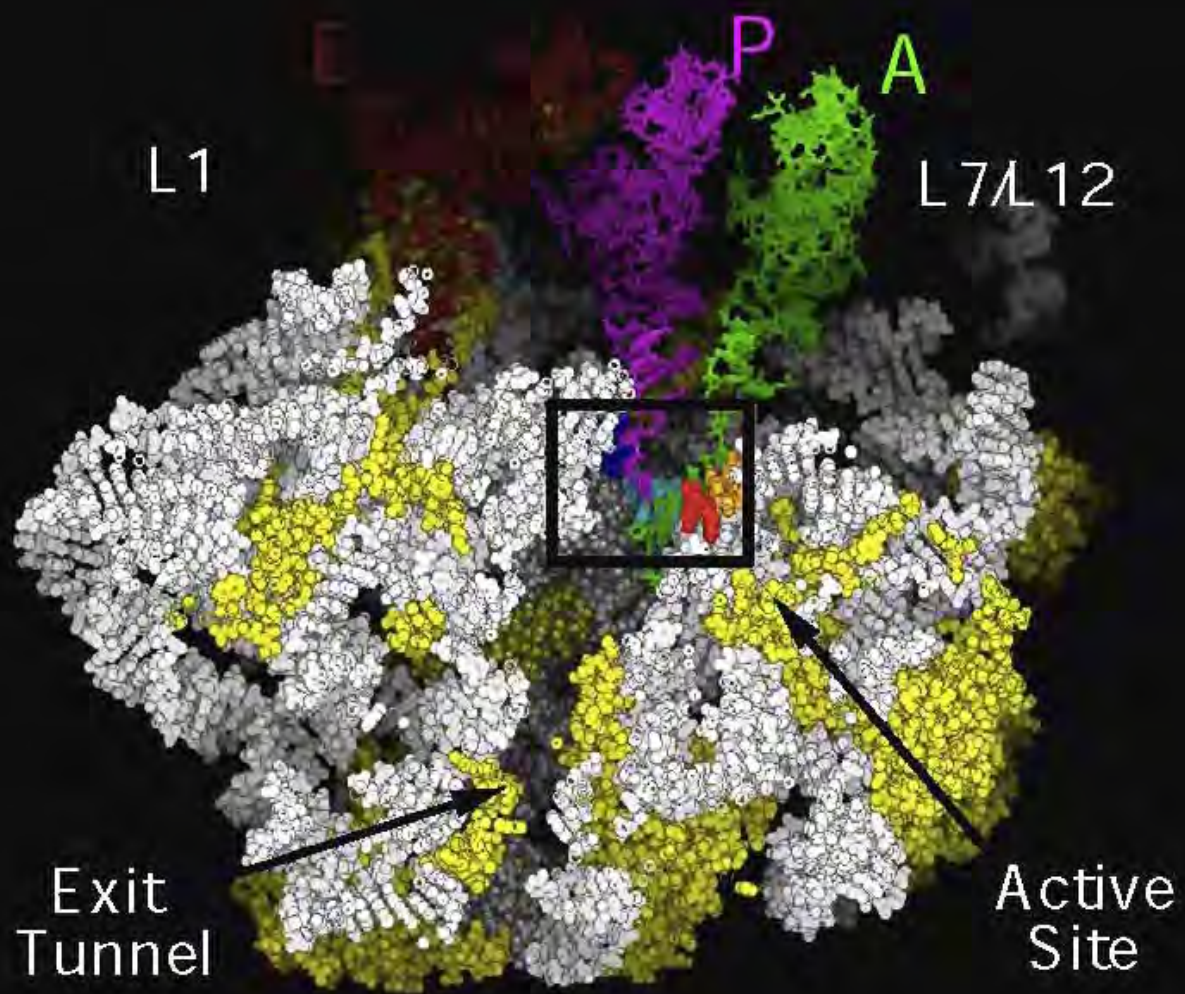
Martin Schmeing and Jeff Hansen

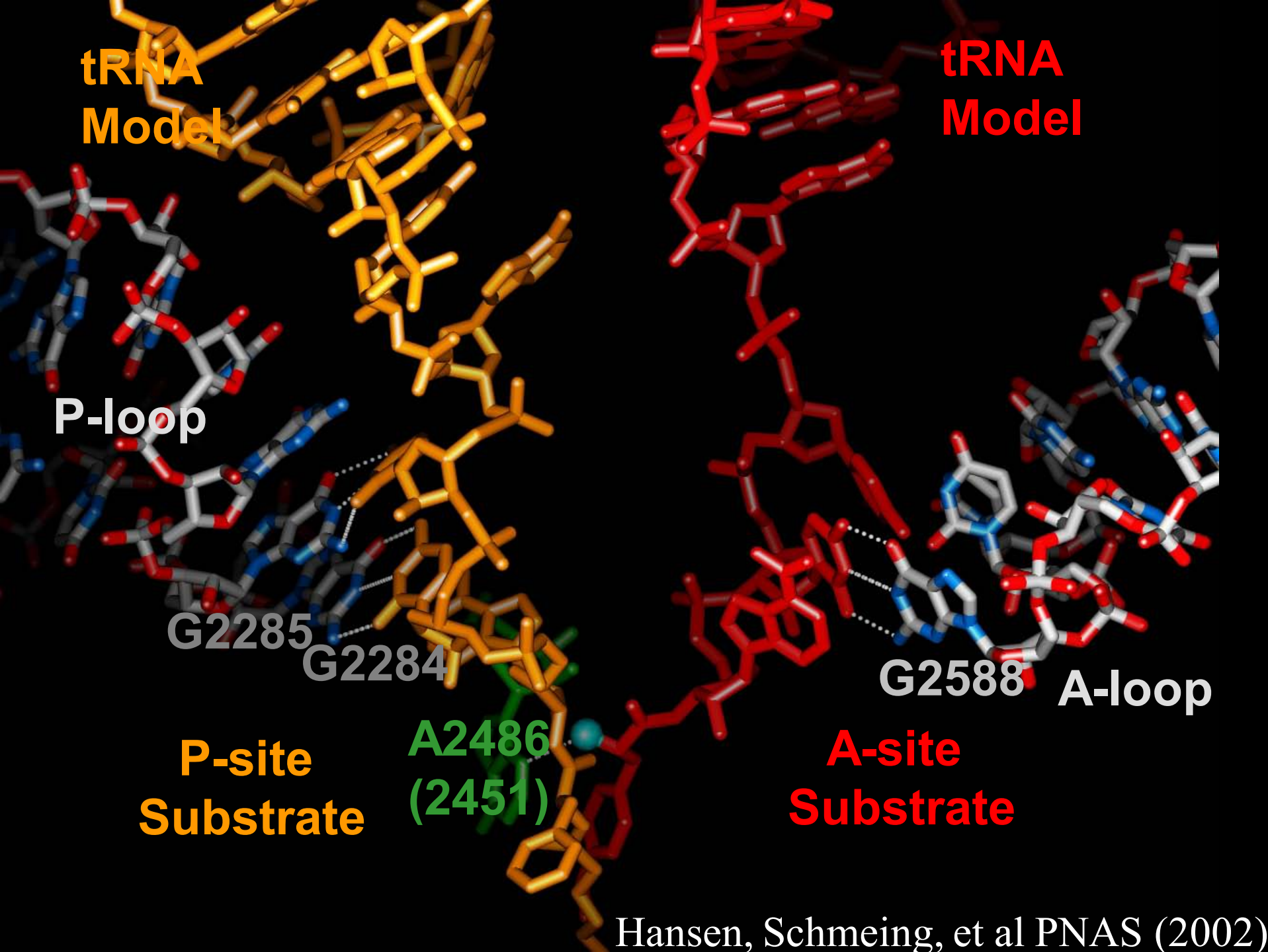


Martin Schmeing

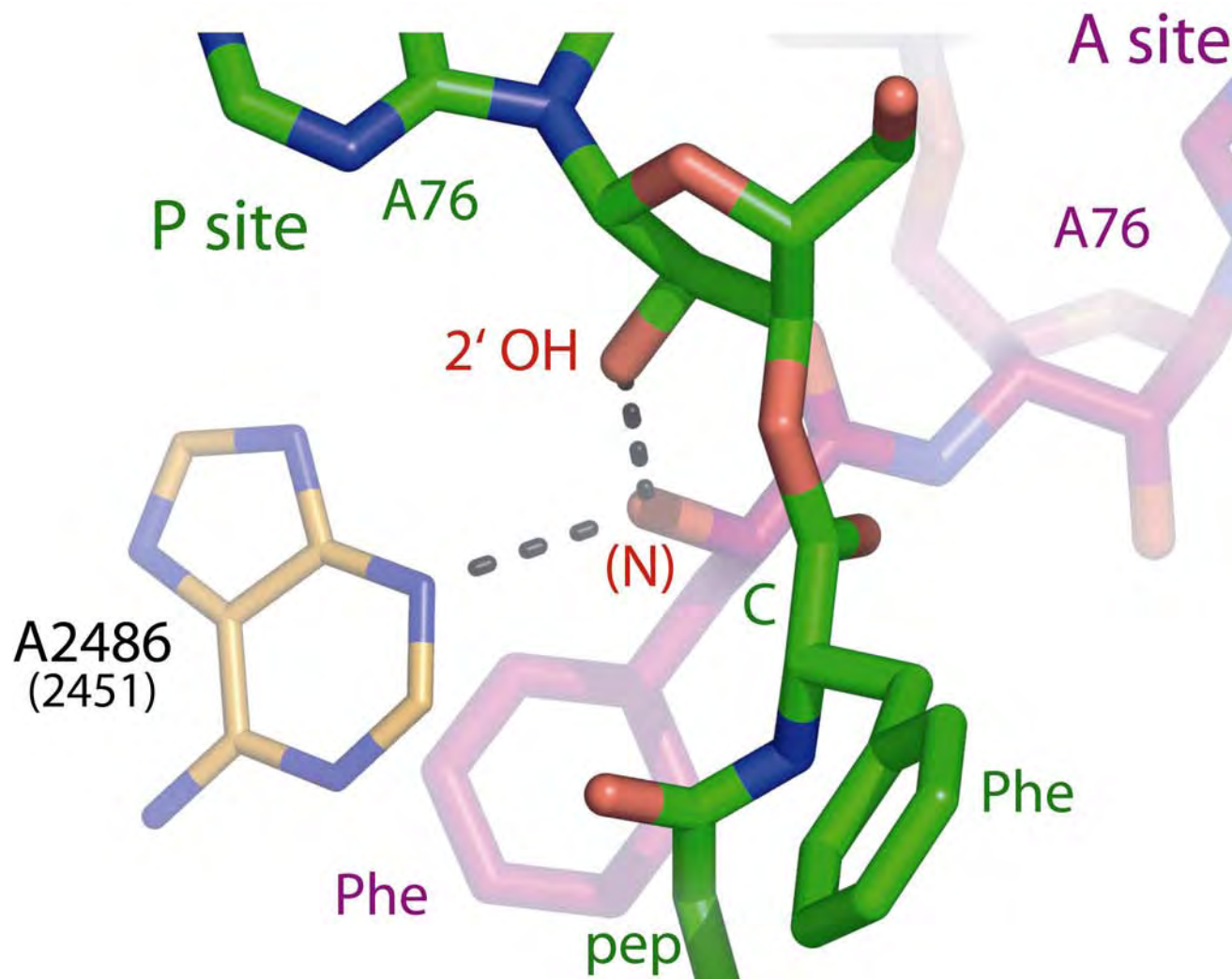
Peptidyl Transferase Reaction







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

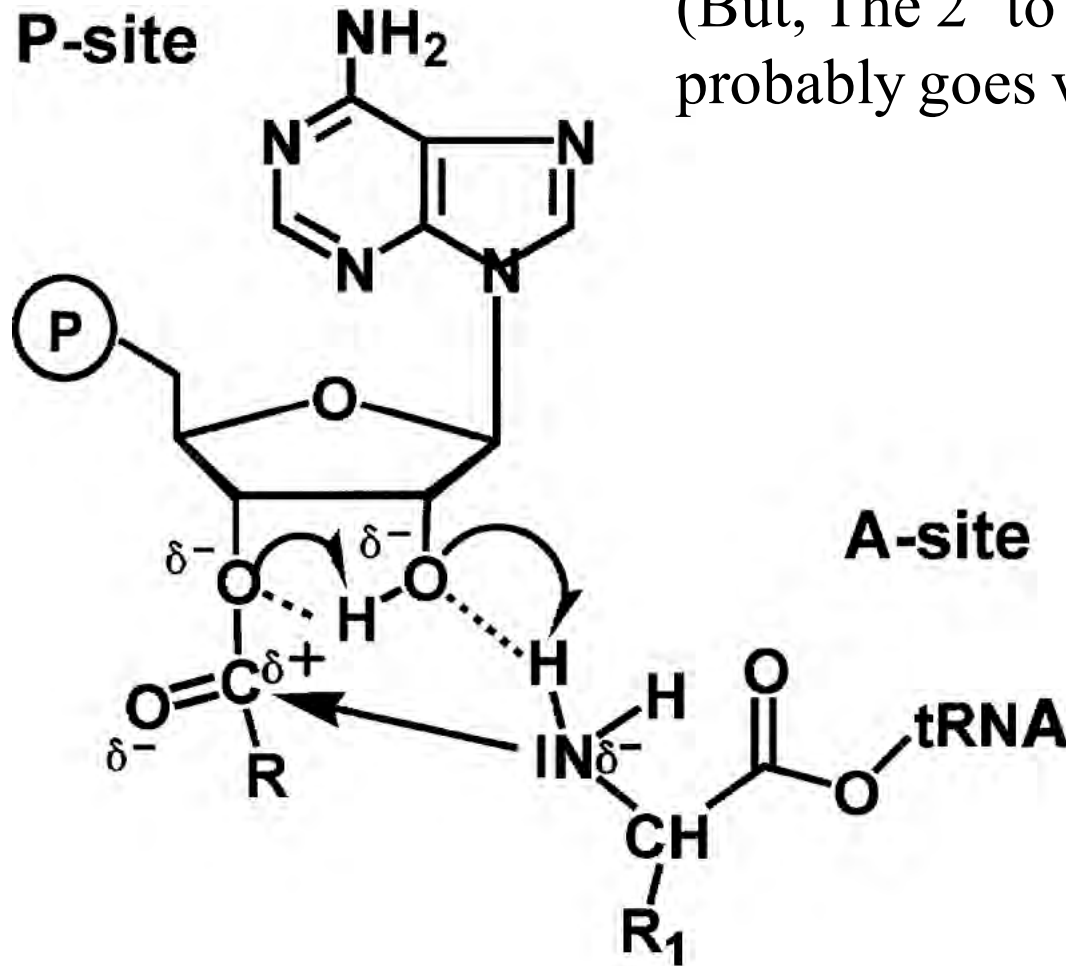
E.M. Youngman, J.L. Brunelle, A.B. Kochaniak, and
Rachel Green, Cell 117, 589-99 (2004)

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

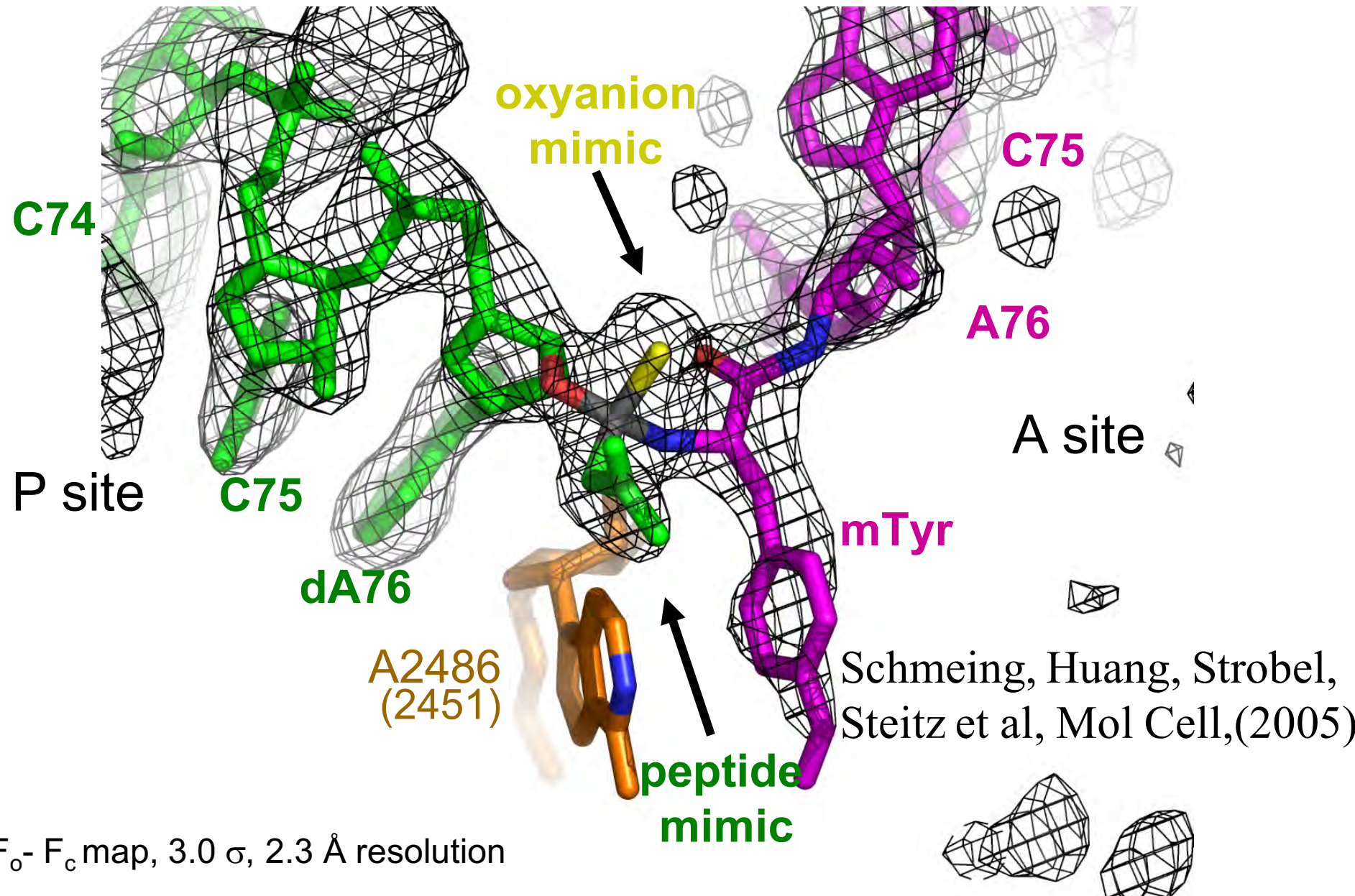
(But, The 2' to 3' transfer probably goes via a water).



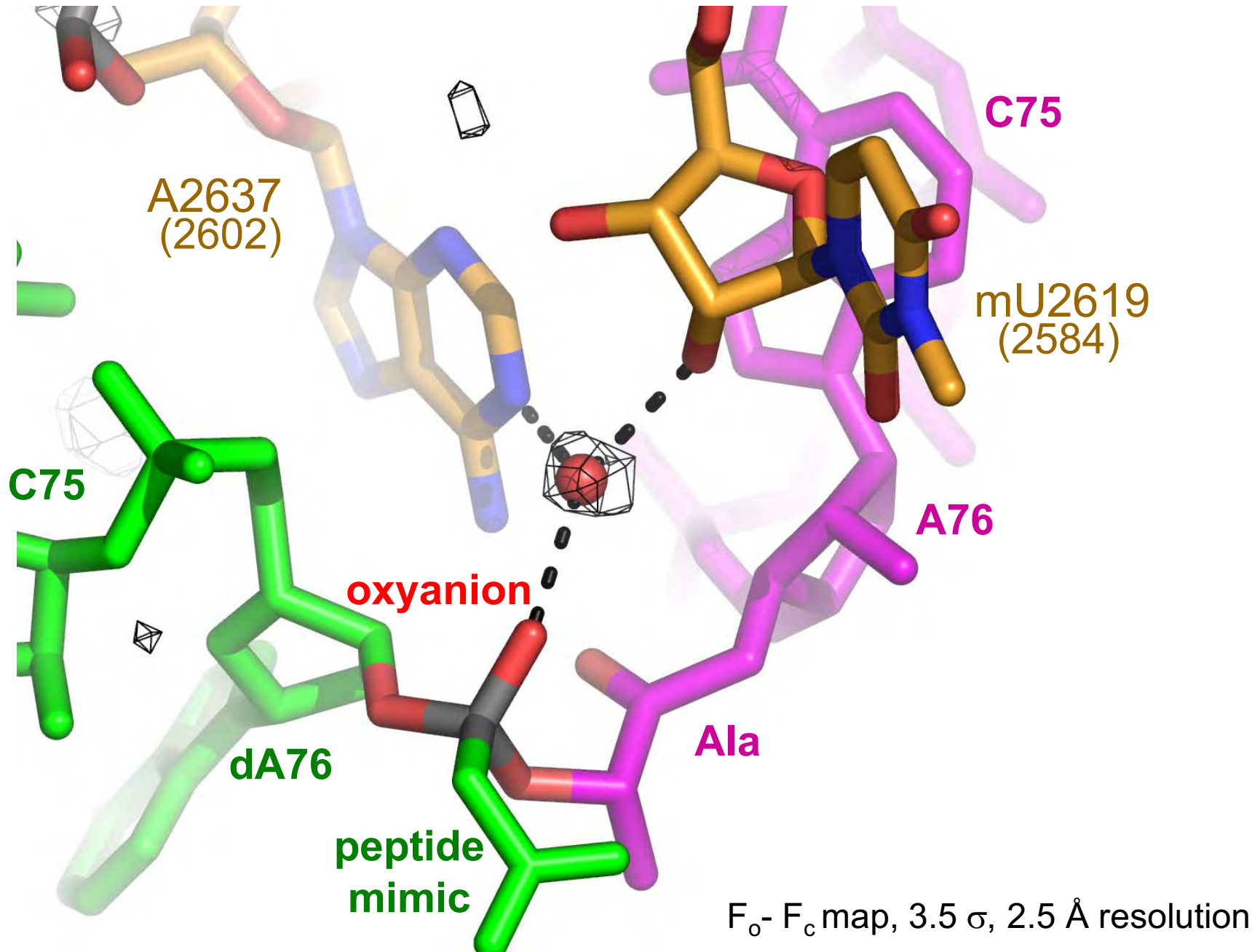
Dorner S, Polacek N, Schulmeister U, Panuschka C, Barta A. "Molecular aspects of the ribosomal peptidyl transferase." Biochem Soc Trans. 2002 Nov;30(Pt 6):1131-6.

Is the transition state being
stabilized?

The oxyanion of the transition state points away from A2486



The oxyanion hole is a water molecule



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

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

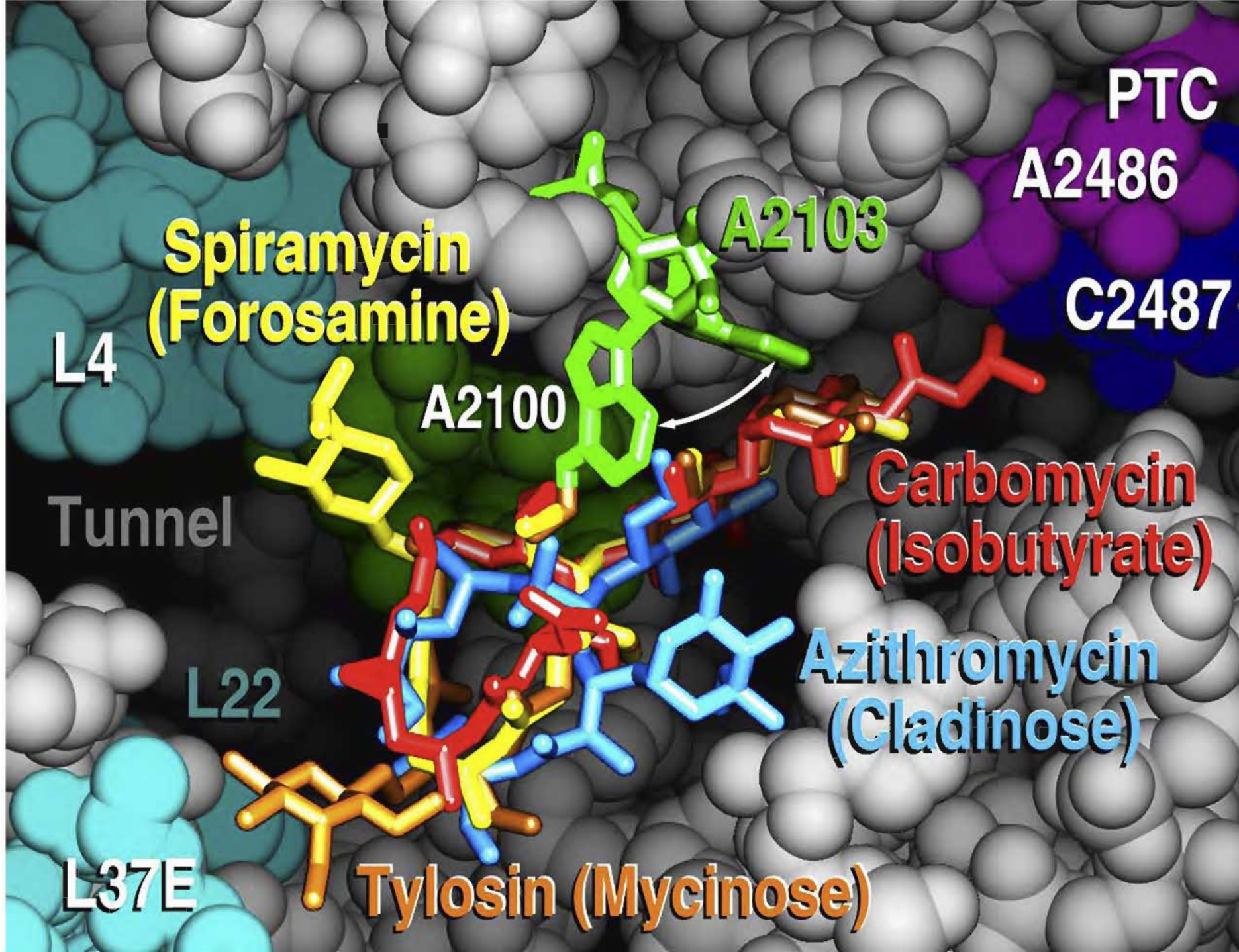
*An estimate that
fatalities could exceed
those for AIDS.*

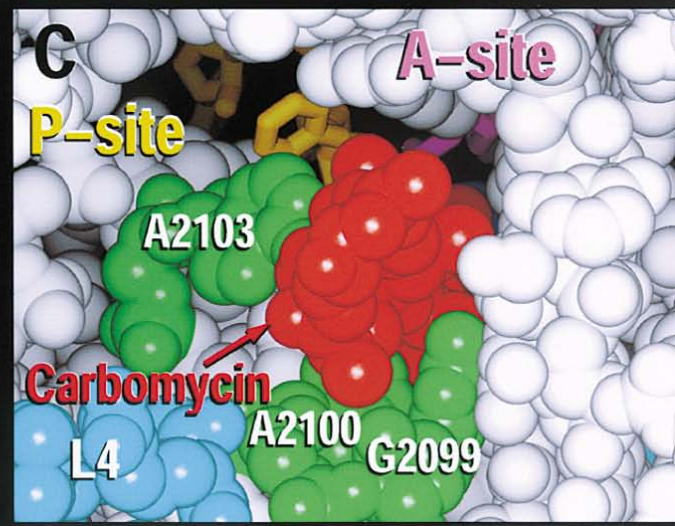
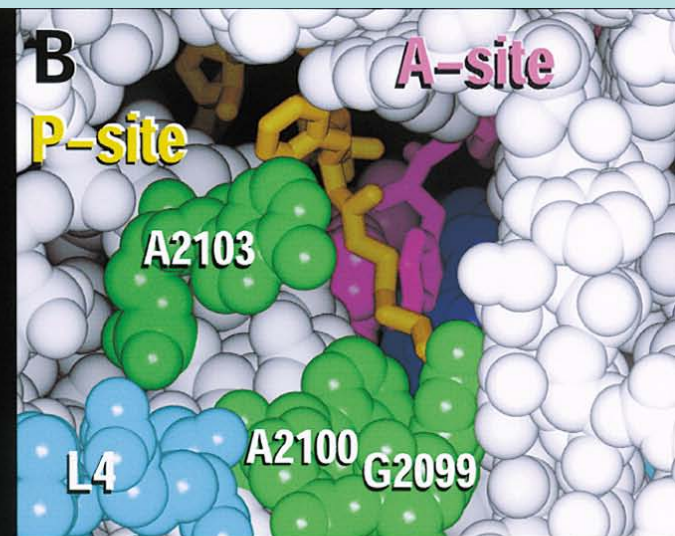
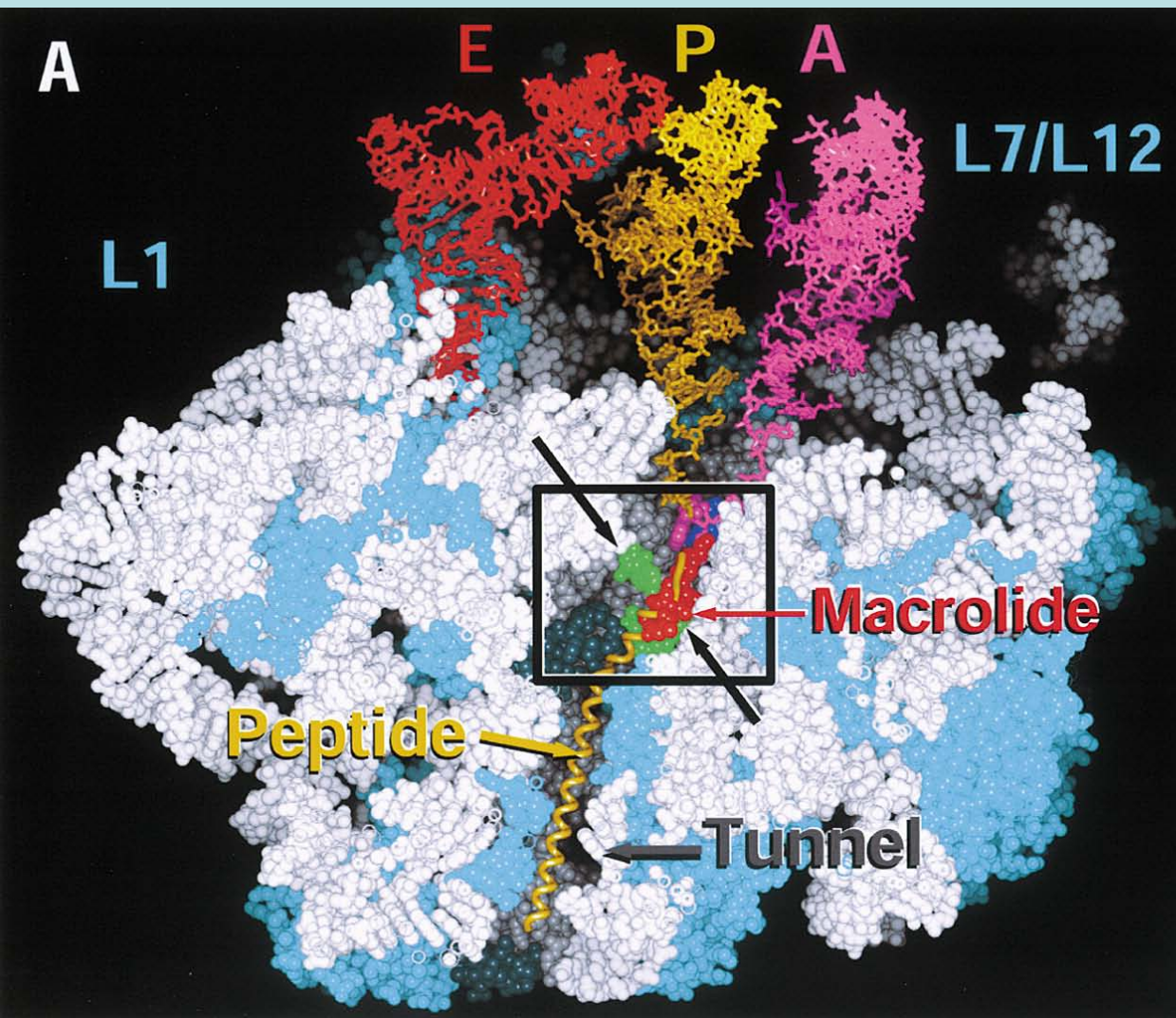
Gross sales of antibiotics amount to about \$30 billion per year worldwide. About half target the ribosome, mostly the large subunit.



Jeff Hansen 1998-2003

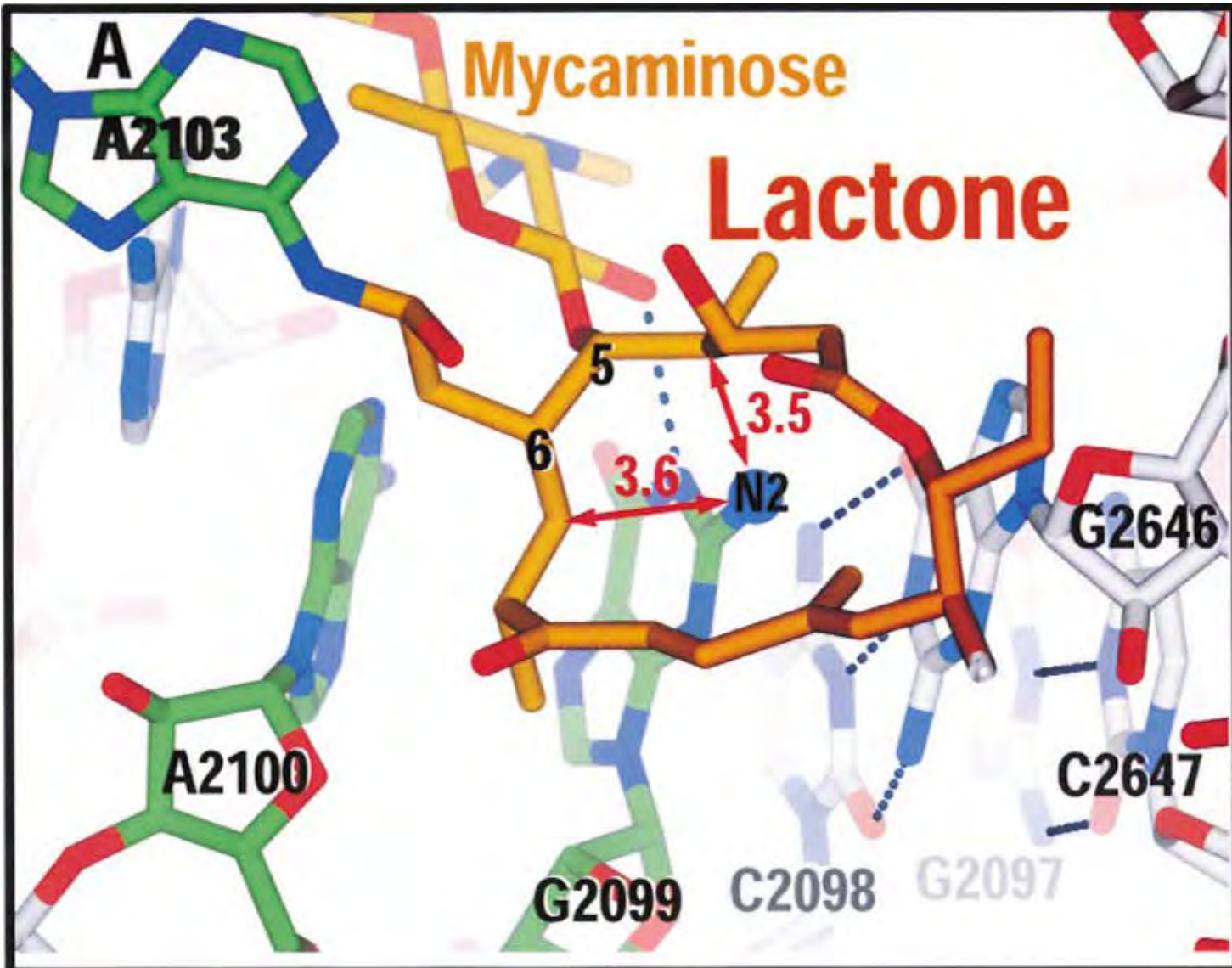
15- and 16-member macrolides
bind in the tunnel of the 50S
subunit





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

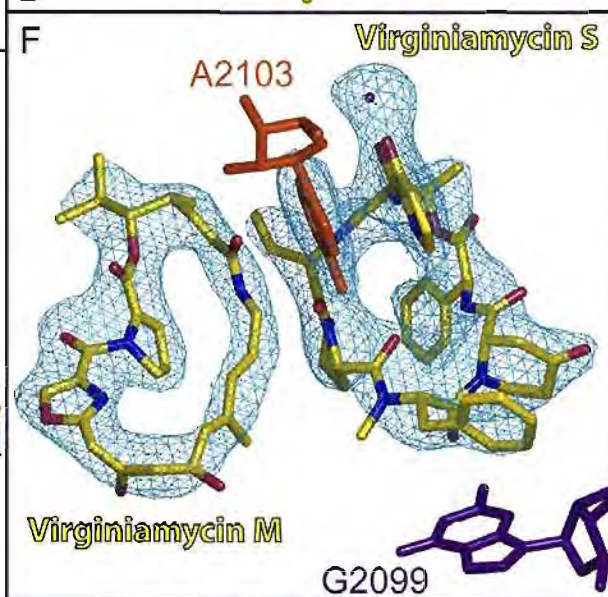
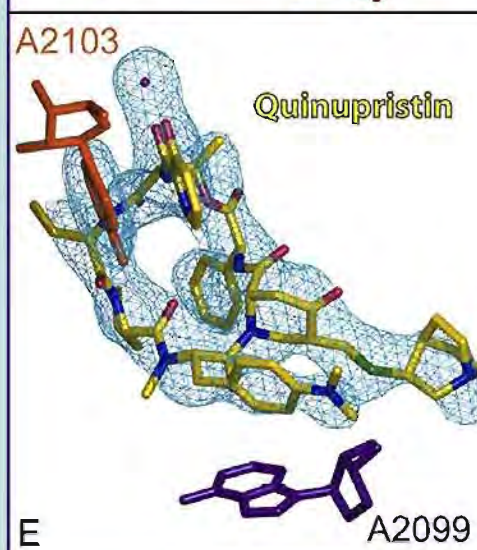
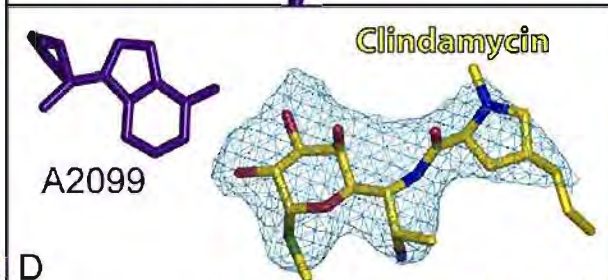
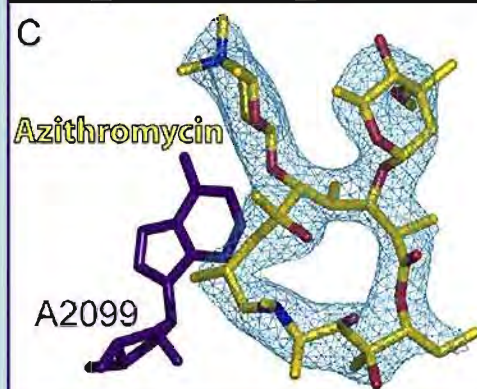
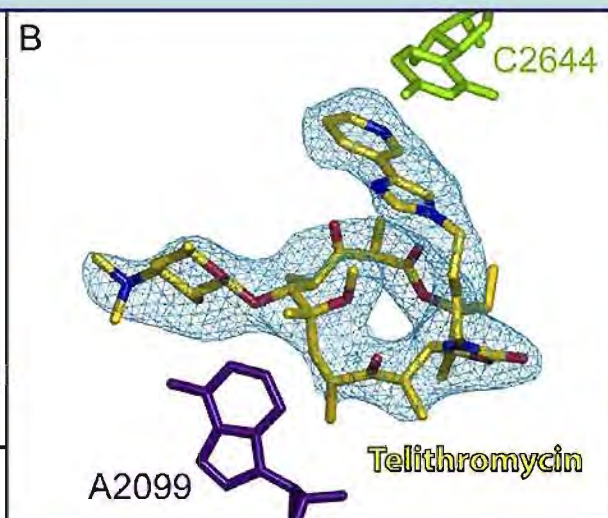
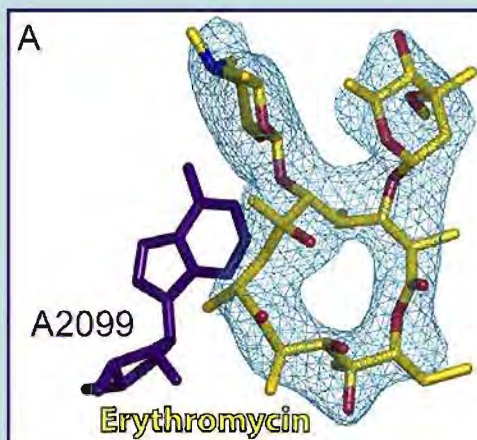
Since *E. coli* A2058 is G2099 in the *H. marismortui* 50S subunit, many MLSK antibiotics do not bind to this archeal subunit.



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

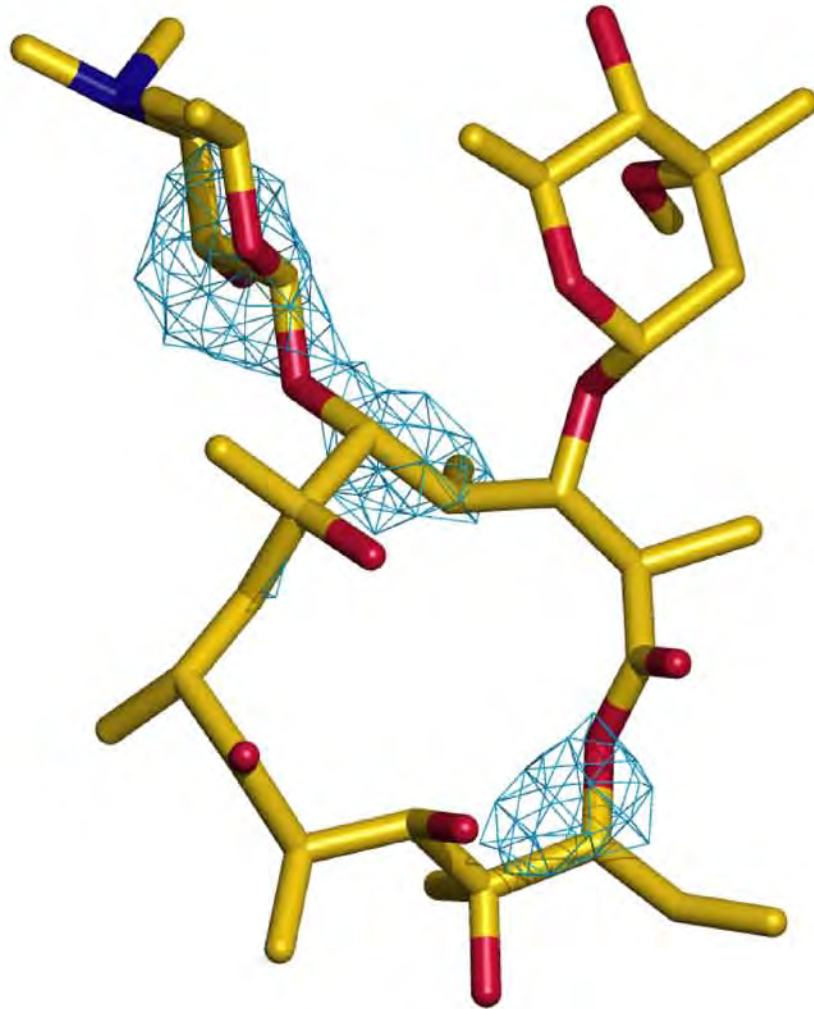
G2099 (A2058 *E. coli*) was
mutated to A2099 in one of the
three 23S rRNA genes

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



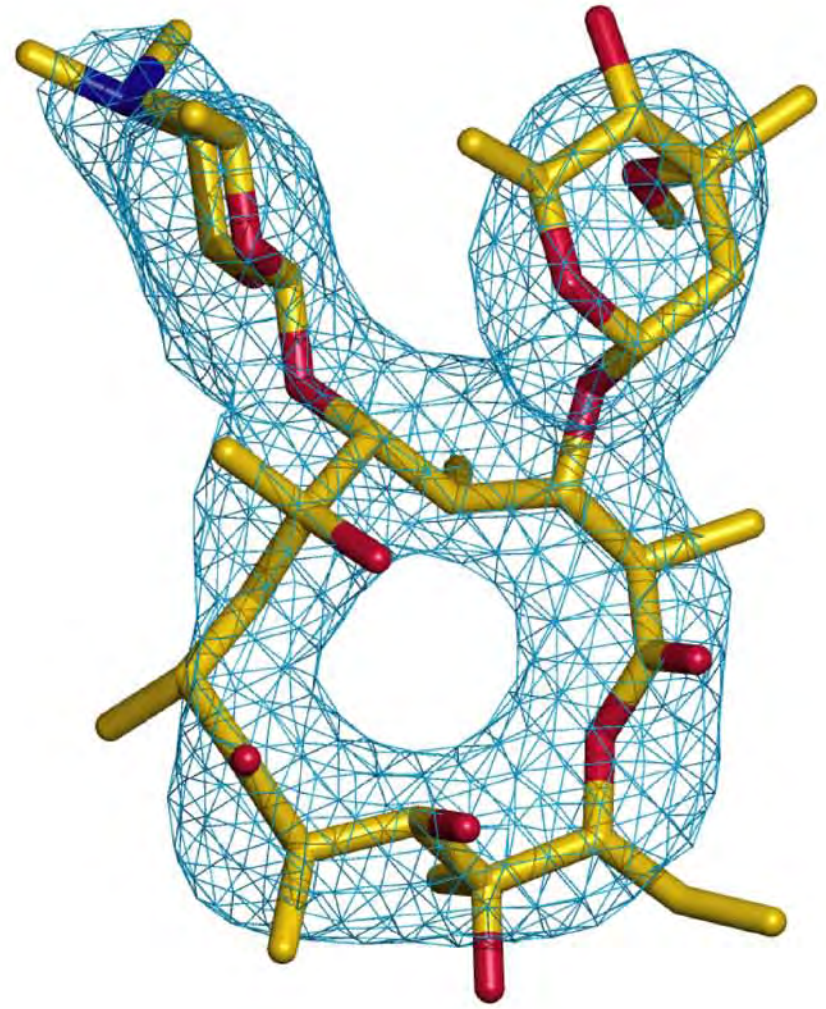
G2099A Mutation Increases Erythromycin Affinity >10,000 Fold

100% G2099



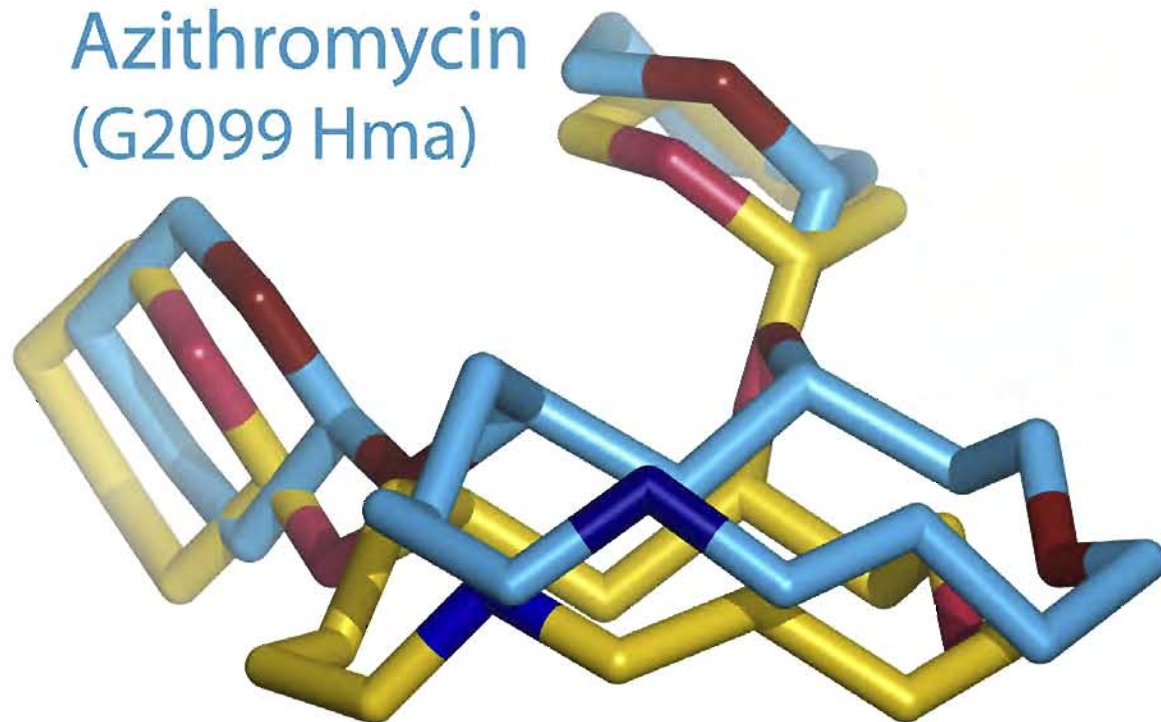
~ 3 mM erythromycin

33% G2099A

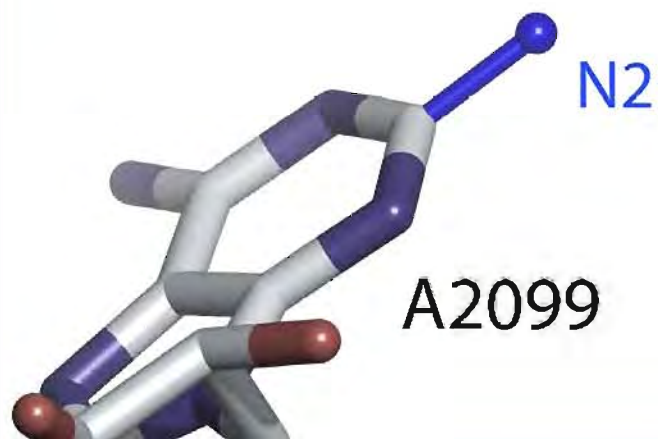


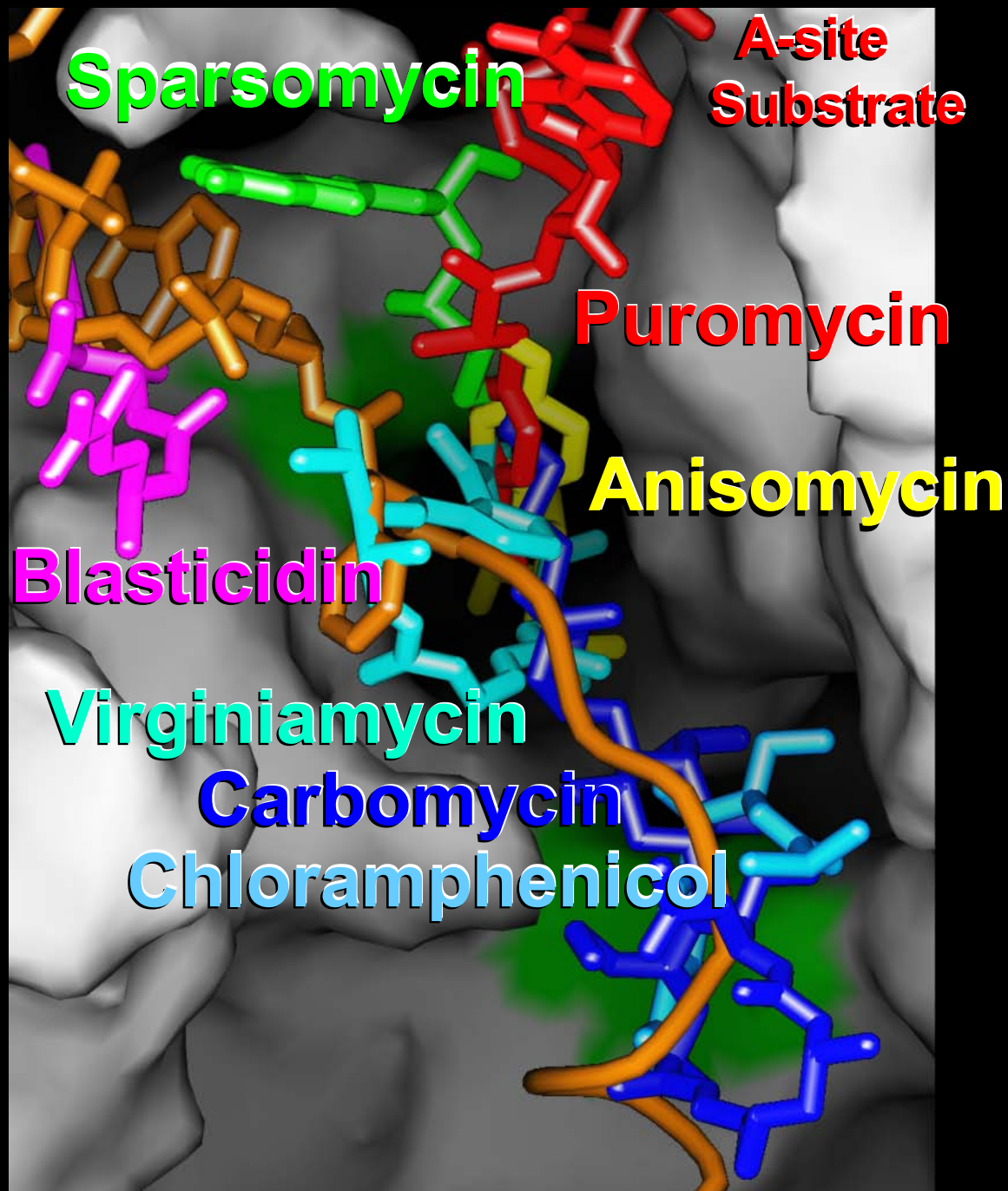
0.003 mM erythromycin

Azithromycin
(G2099 Hma)



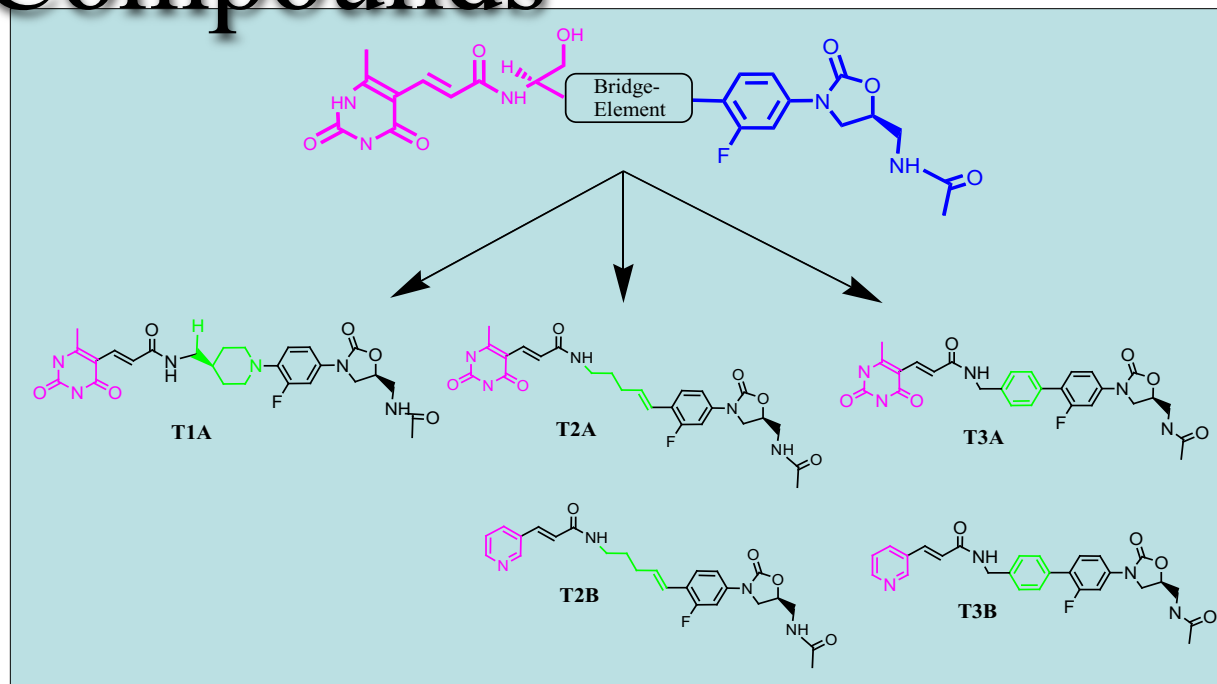
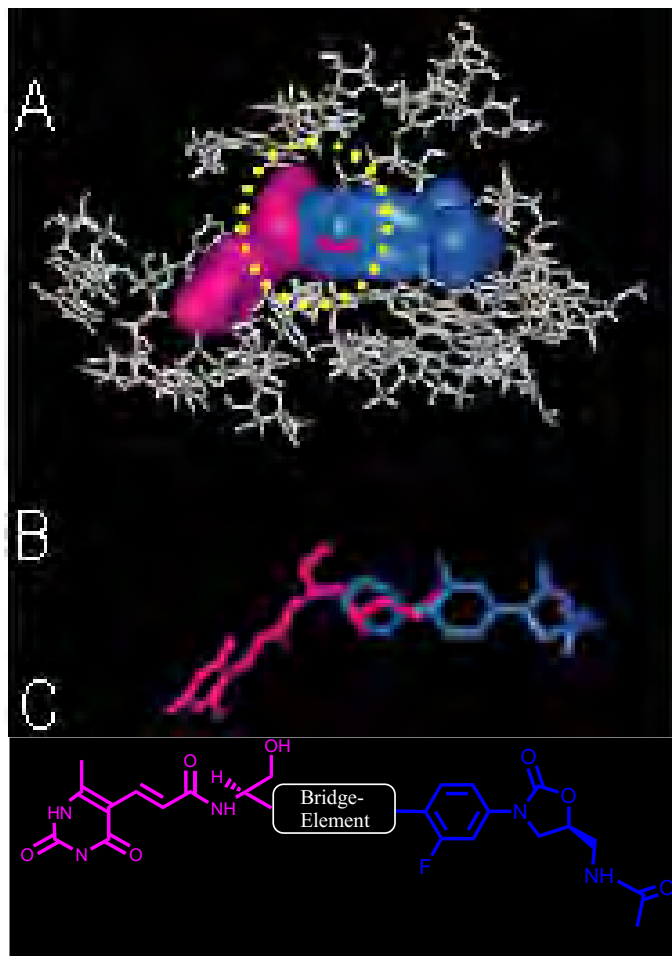
Azithromycin
(G2099A Hma)





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 Rx-01 Family of Compounds



	Linezolid	Sparsomycin	T1A	T2A	T2B	T3A	T3B
<i>E. coli</i> 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)						
<i>S. pneumoniae</i> 02J1175	2	2	4	1	8	≤0.25	0.5
<i>S. pyogenes</i> Msr610	1	2	4	1	4	≤0.25	0.5
<i>E. faecalis</i> P5 (lin ^R)	32	>128	>128	32	128	16	16
<i>H. Influenzae</i> RD1	16	8	>128	>128	>128	>128	>128

Iterative Cycle Yields Compounds to Treat Respiratory Tract Infections

Compound	Inhibition of Translation (μM)		MIC ($\mu\text{g/ml}$)	
	Prokaryote	Eukaryote	<i>S. pneumoniae</i>	<i>H. influenzae</i>
RX-A ₁	0.92	0.23	1	>128
RX-A ₂	14.6	>200	8	>128
RX-A ₇	<0.2	1.5	0.25	>128
RX-A ₈	6.8	>100	0.5	>128
RX-A ₈₄	0.083	>100	0.25	2
RX-A ₈₉	0.049	>100	0.25	16
RX-A ₁₈₈	<0.02	1.01	0.06	2
RX-A ₂₅₈	<0.02	20	0.25	2

Rib - X Pharmaceuticals, Inc.

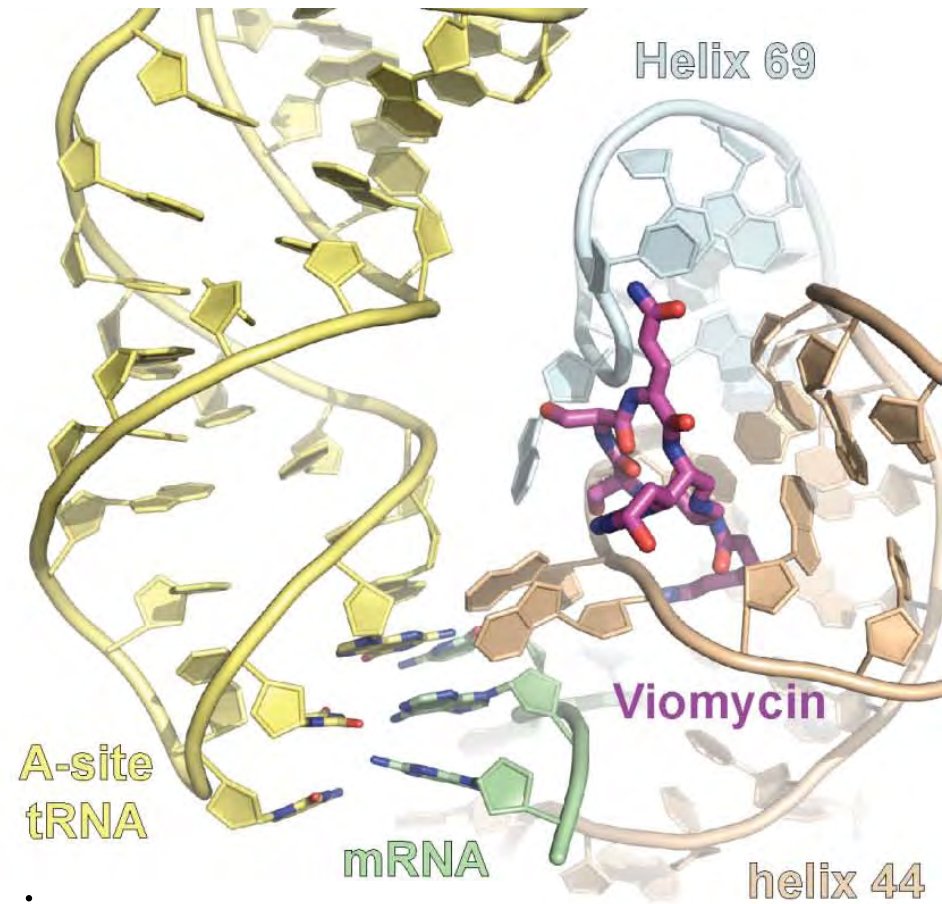
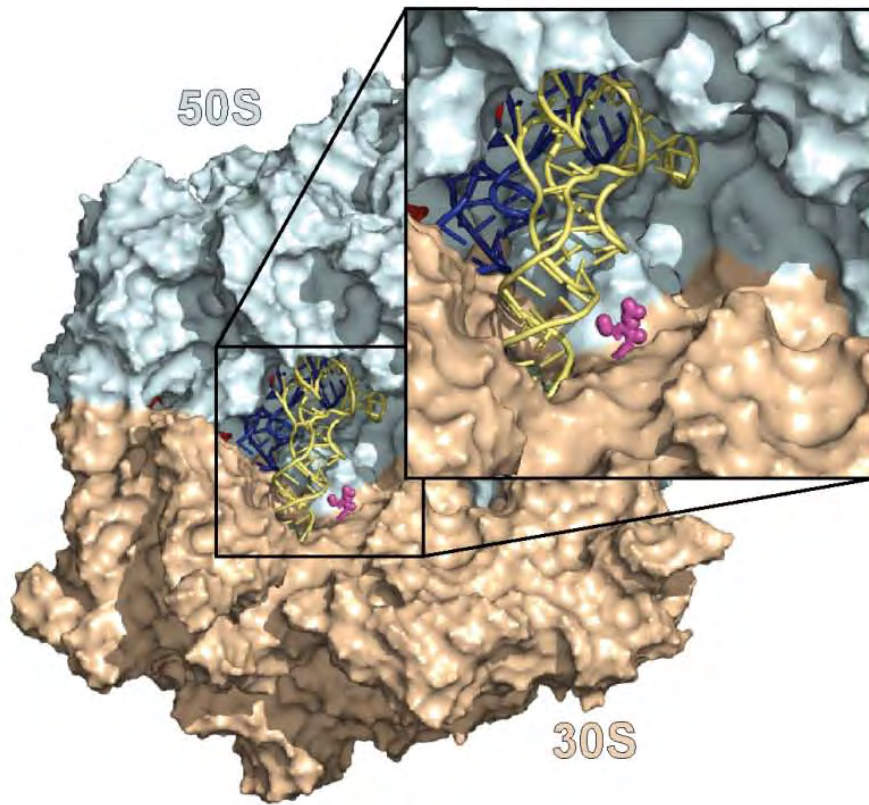
Superior compounds obtained

	MIC (µg/mL); Target ≤4		
Bacterial Strains	Zithromax	RX-A	RX-B
<i>Streptococcus pneumoniae:</i>			
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
<i>Streptococcus pyogenes:</i>			
Methylase of 23S delivering macrolide resistance	>128	≤0.25	≤0.25
<i>Haemophilus influenzae:</i>			
Tough clinical strain	1	4	4
<i>Enterococcus faecalis:</i>			
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 ($\mu\text{g/ml}$)		
	Radezolid	Linezolid (Zyvox)	Vancomycin
<i>E. faecalis</i> ATCC 29212	≤ 0.25	4	2
<i>E. faecalis</i> A5962	1	32	8
<i>E. faecalis</i> A7789	4	64	1
<i>E. faecium</i> A5959	4	32	>128
<i>E. faecium</i> A5960	4	64	>128
<i>E. faecium</i> A8130	2	32	128
<i>E. faecium</i> A9650	0.5	16	>128
<i>E. faecium</i> A8948	≤ 0.25	8	>128
<i>E. faecium</i> 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

