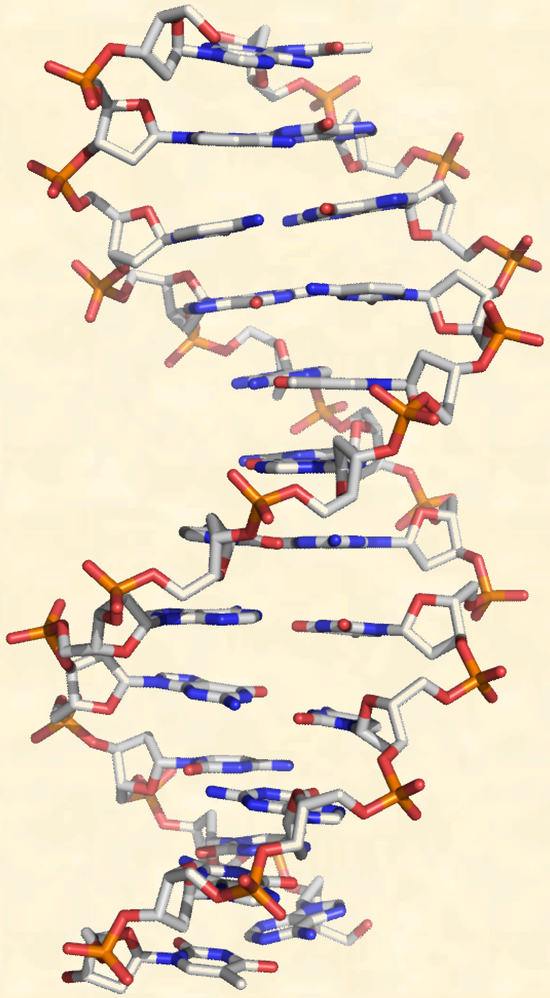
Reading the genetic code: The 3D version

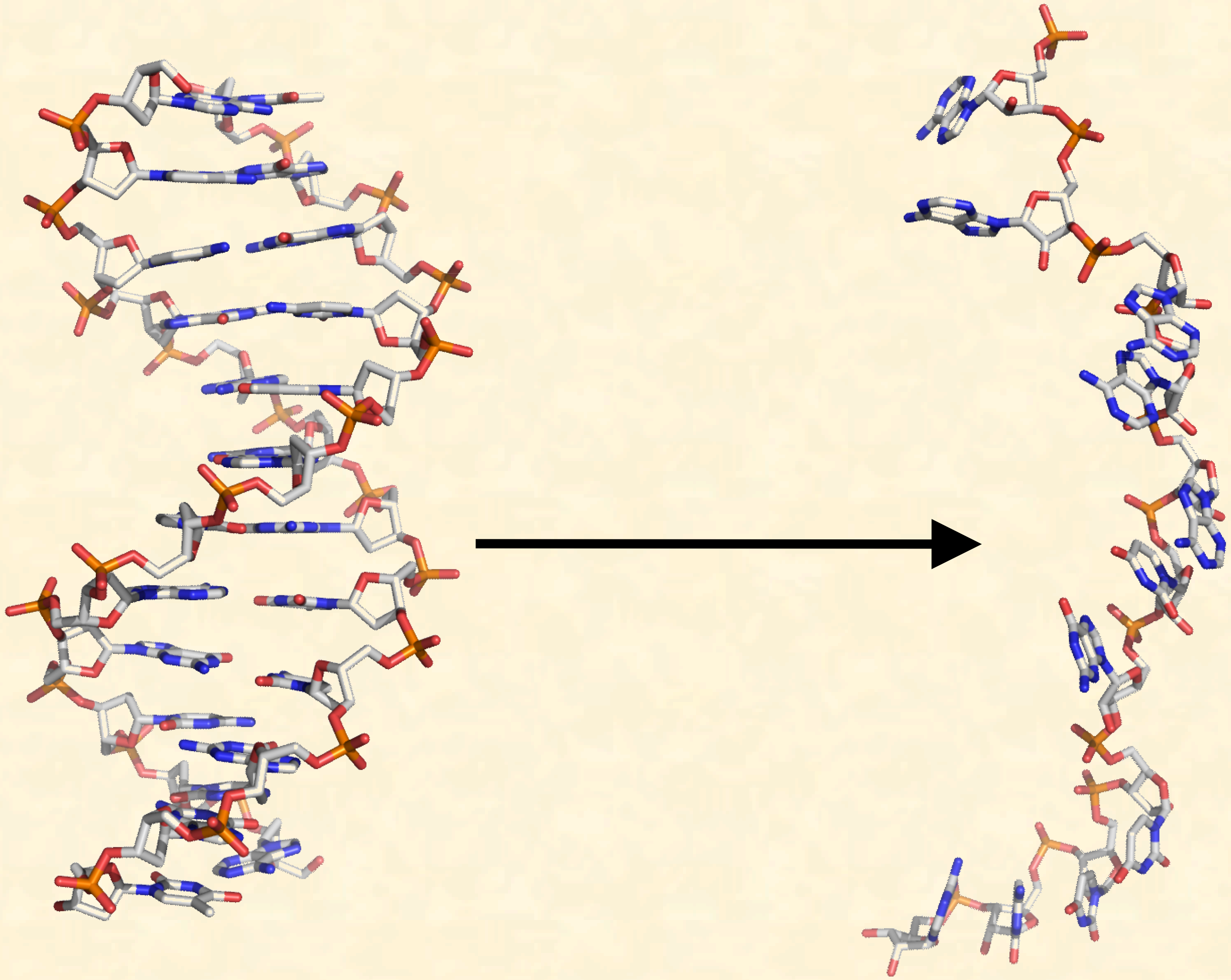
Venki Ramakrishnan
MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

The Central Dogma



DNA

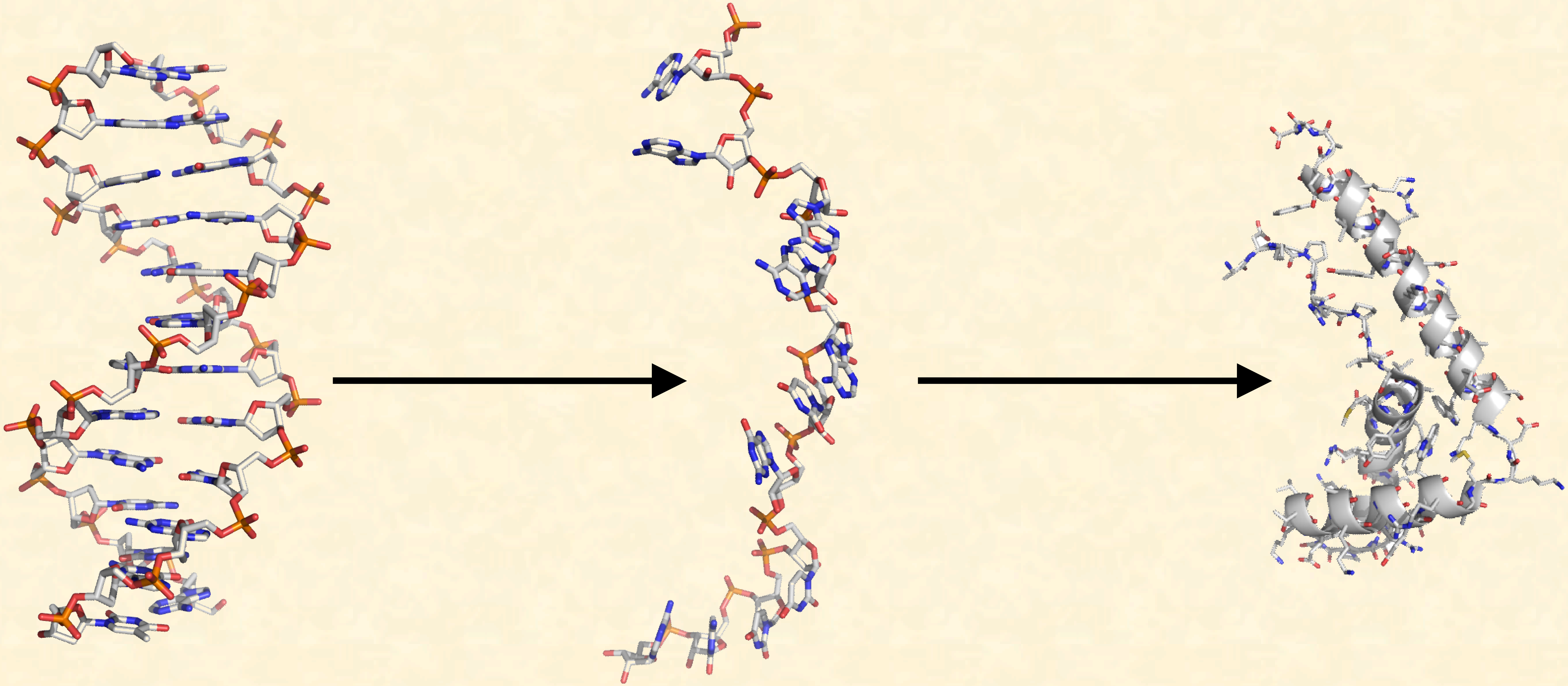
The Central Dogma



DNA

mRNA

The Central Dogma

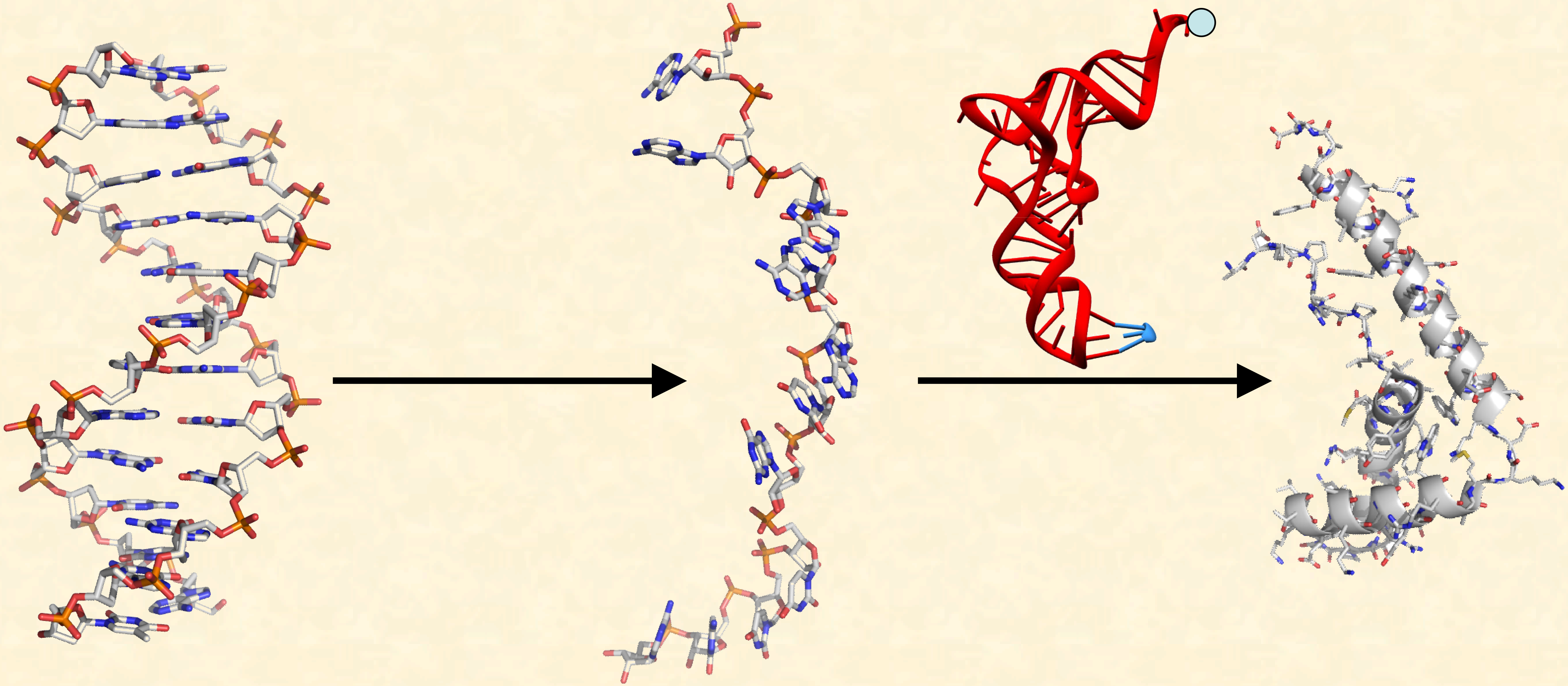


DNA

mRNA

Protein

The Central Dogma

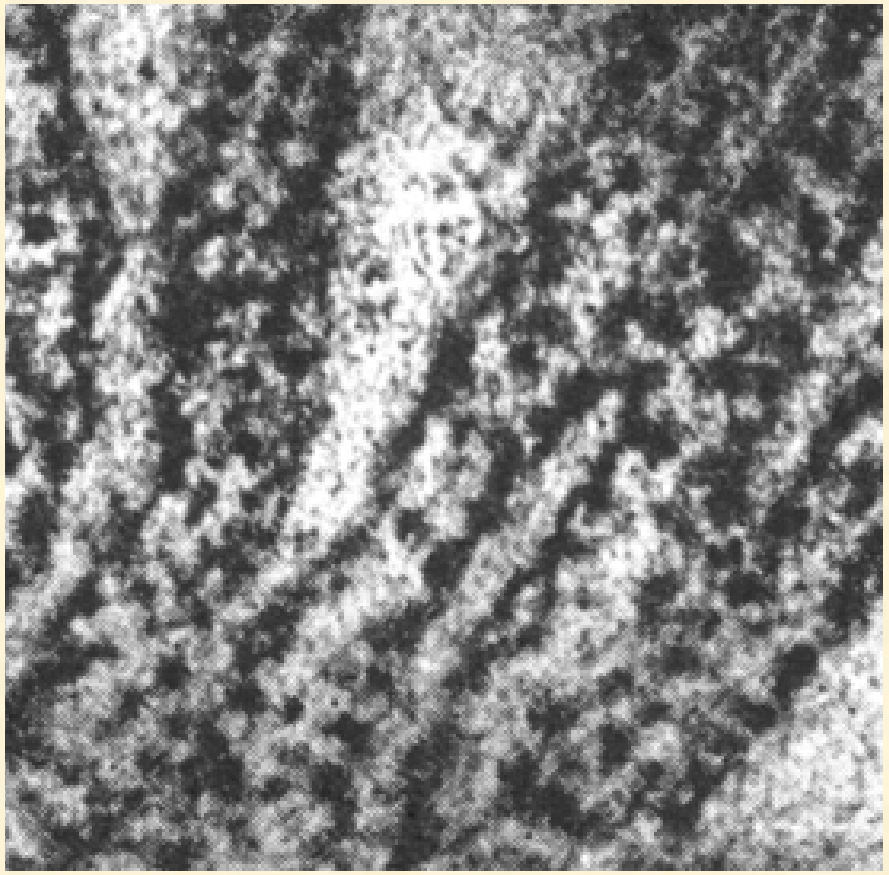


DNA

mRNA

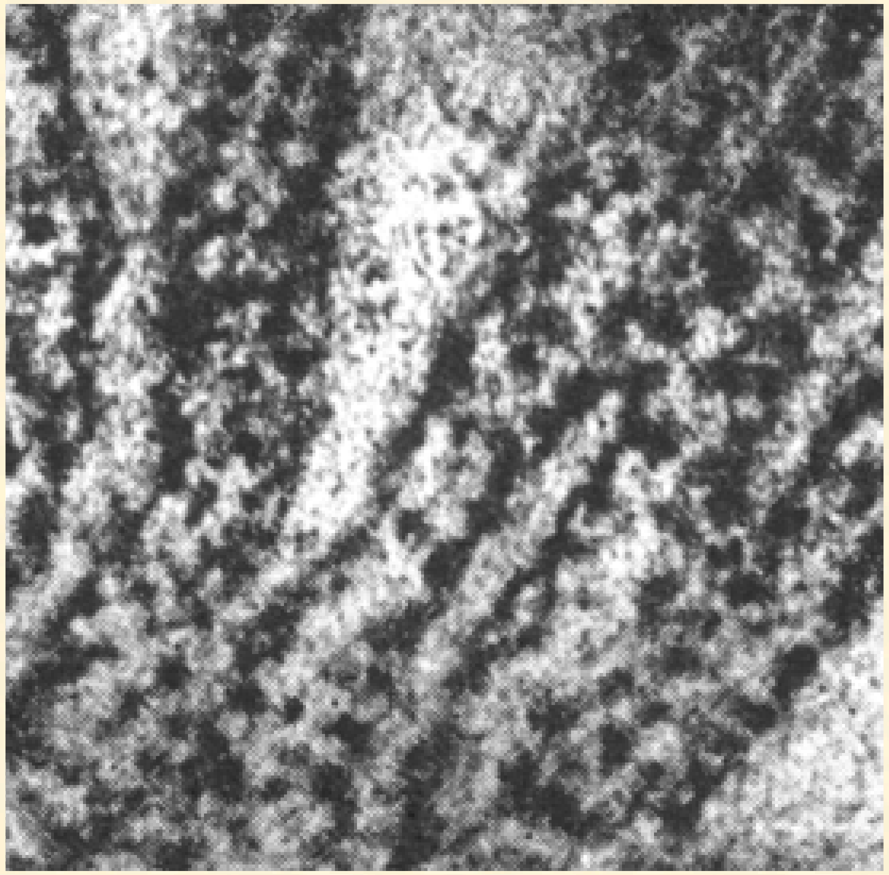
Protein

Sites of protein synthesis in cells

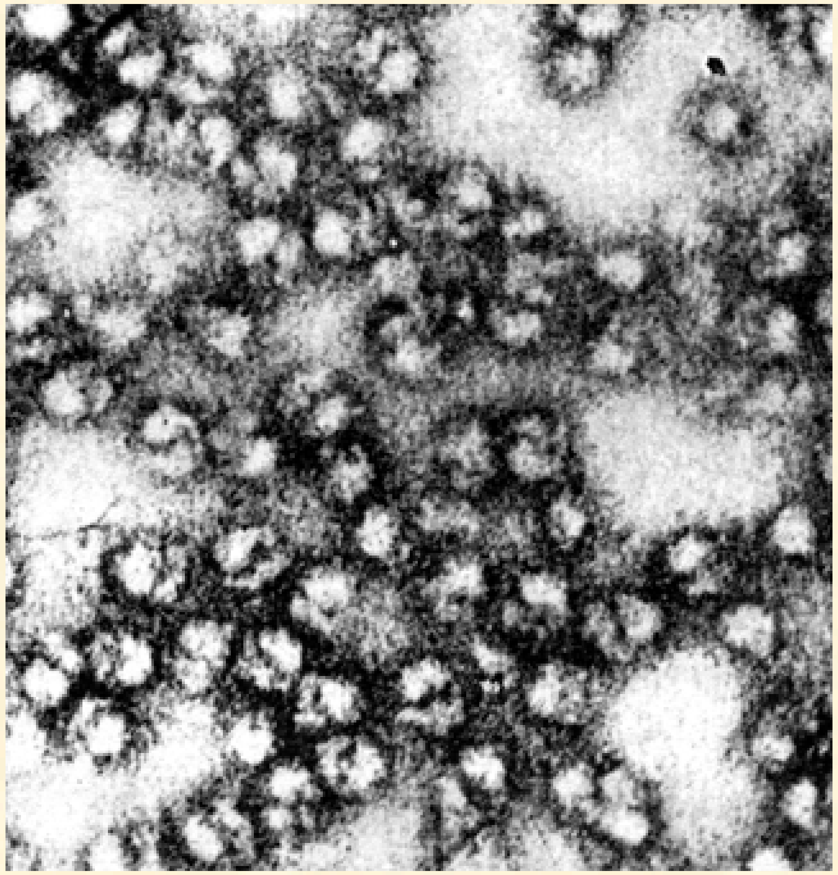


Palade, 1954

Sites of protein synthesis in cells

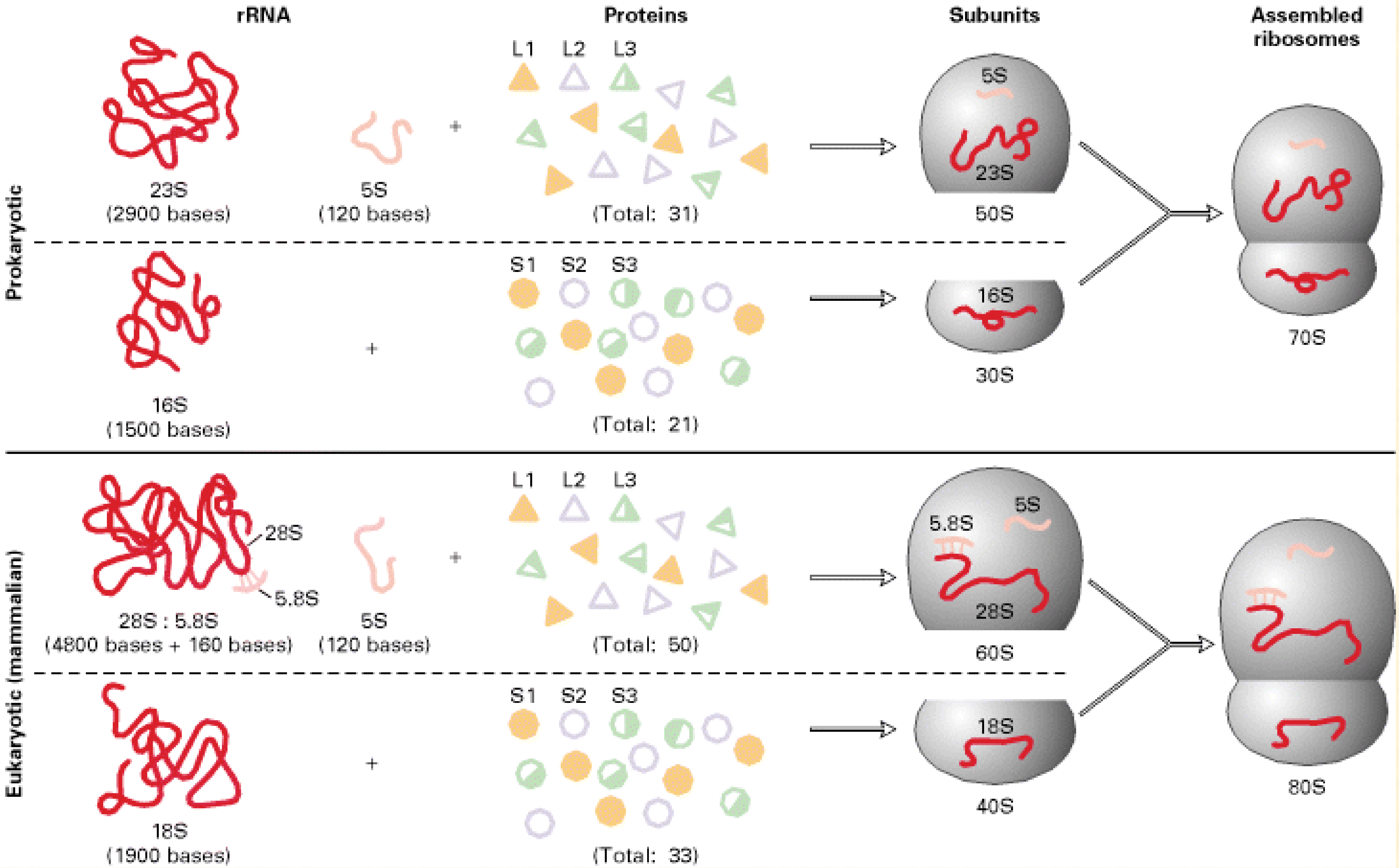


Palade, 1954



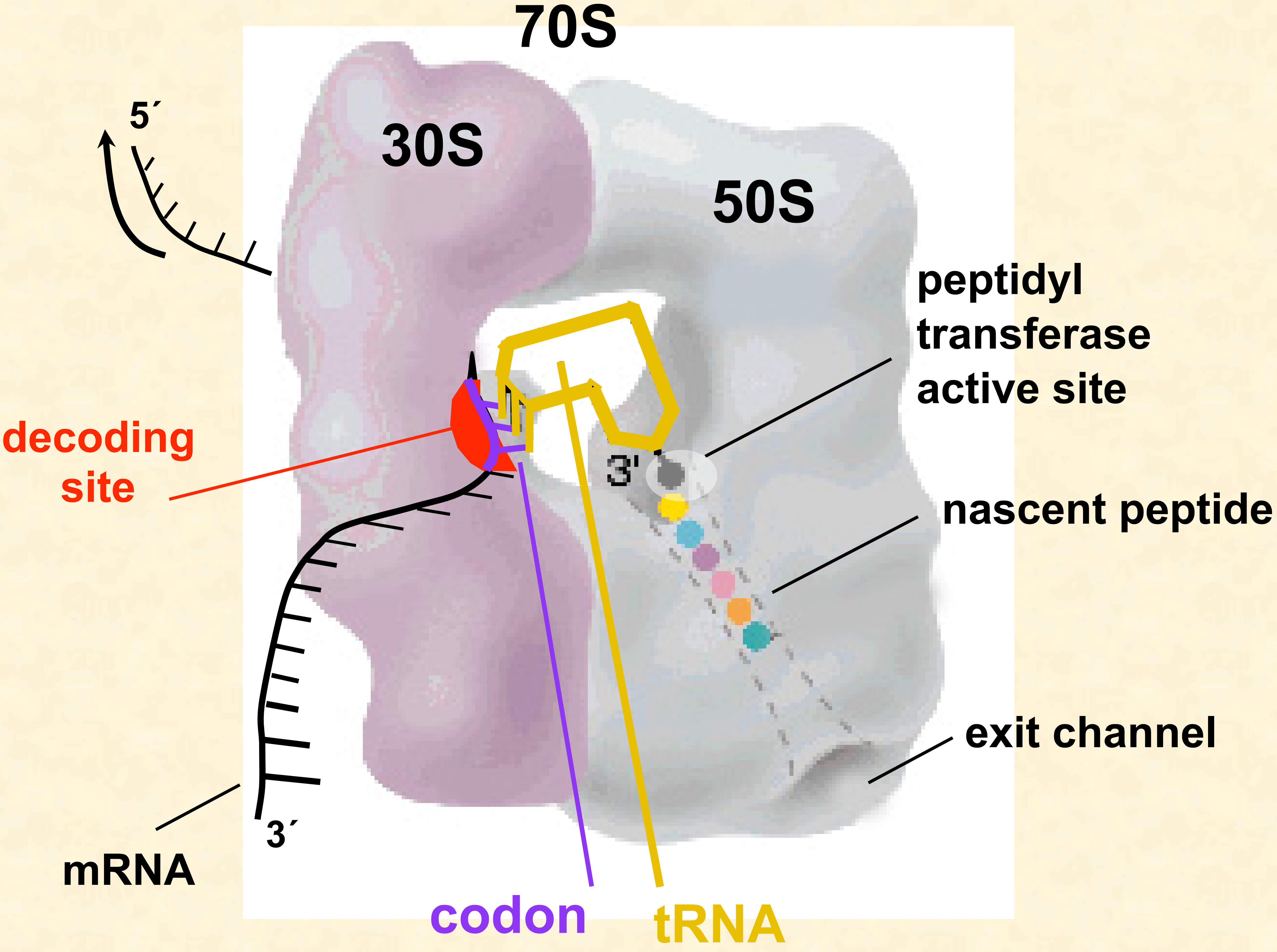
Huxley & Zubay, 1960

Components of ribosomes



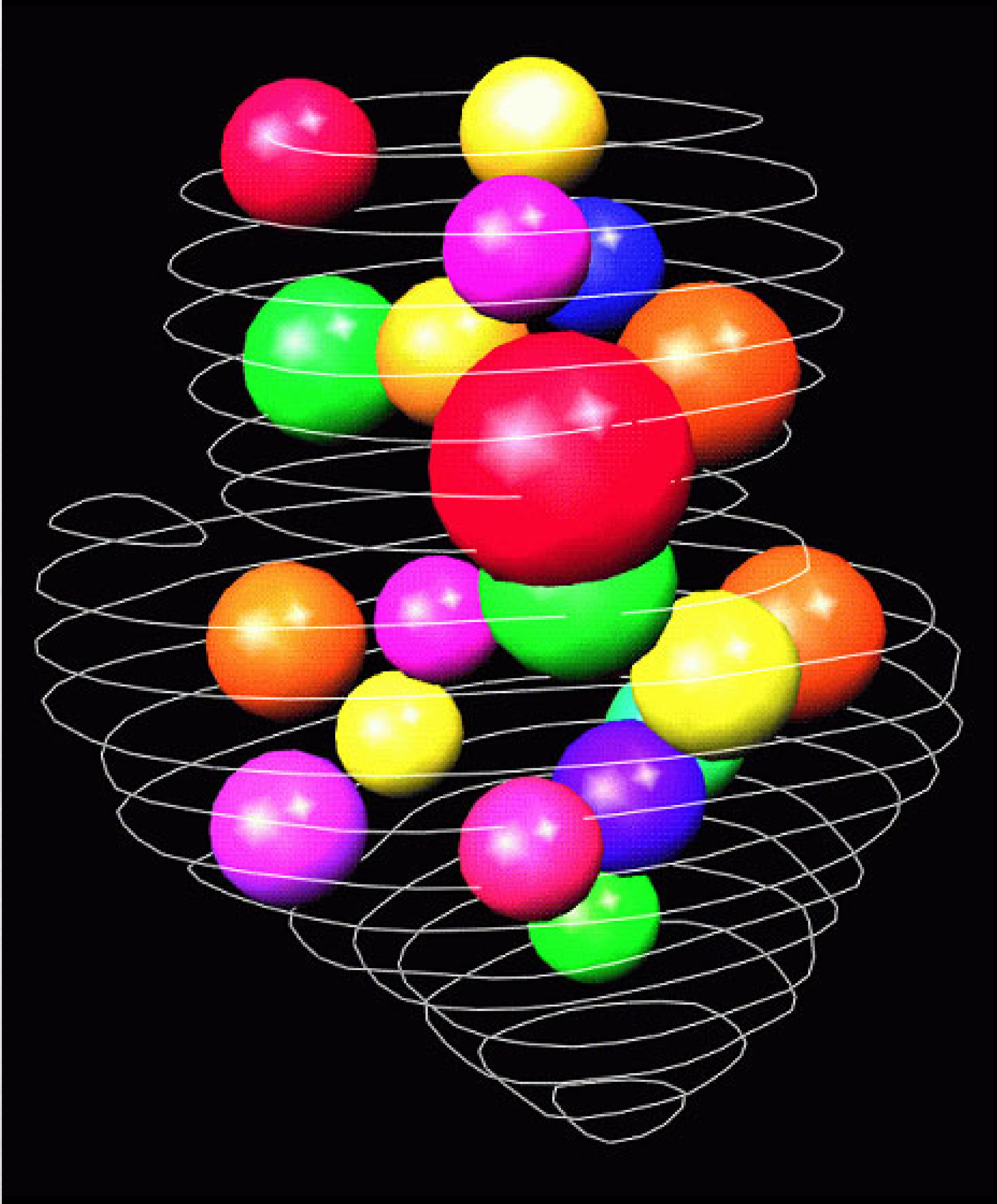
From Lodish *et al.* Molecular Cell Biology (2000)

The bacterial ribosome



adapted from A. Liljas, *Science* **285,** 2077-8 (1999)

Peter Moore



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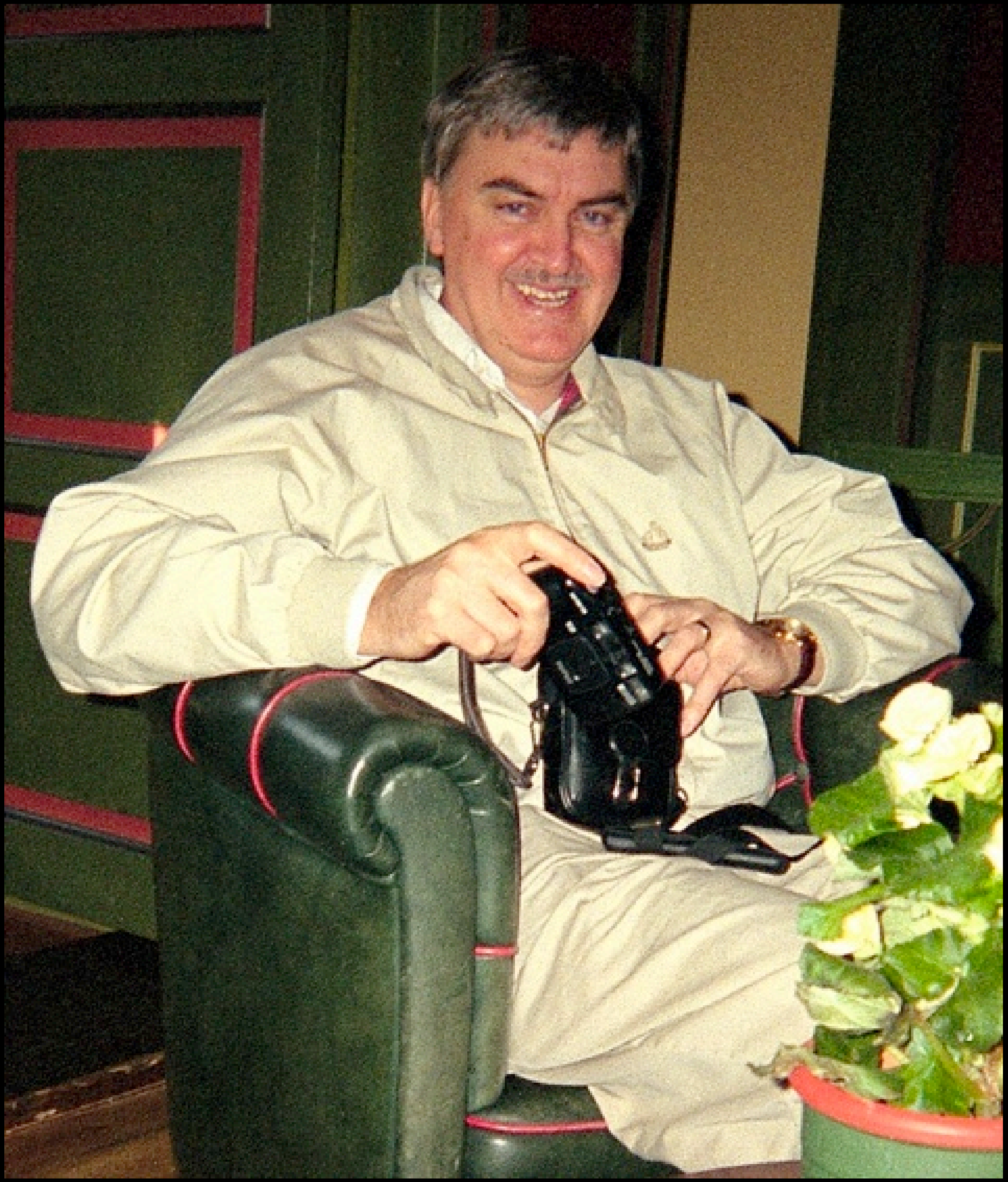
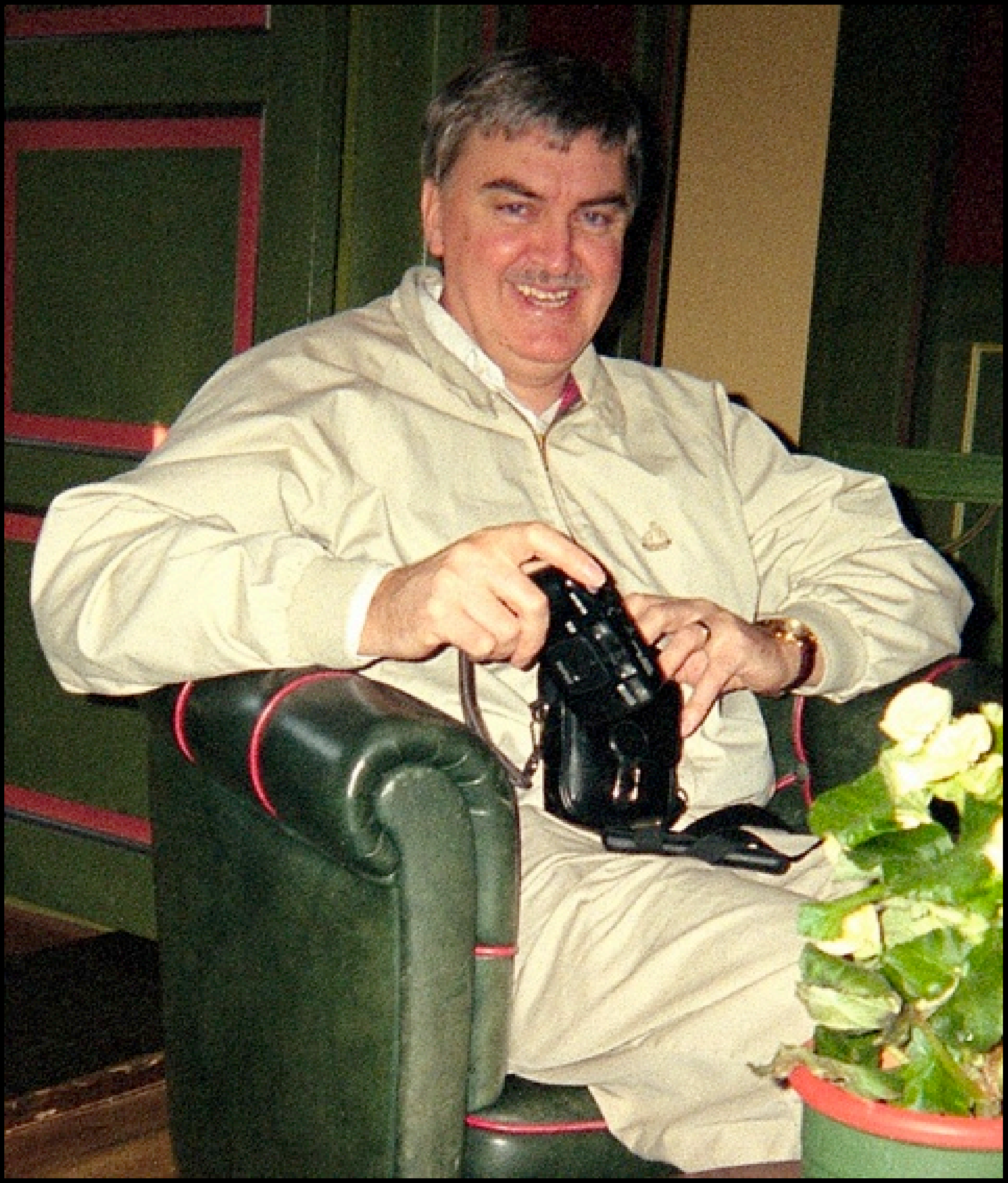


Stephen White



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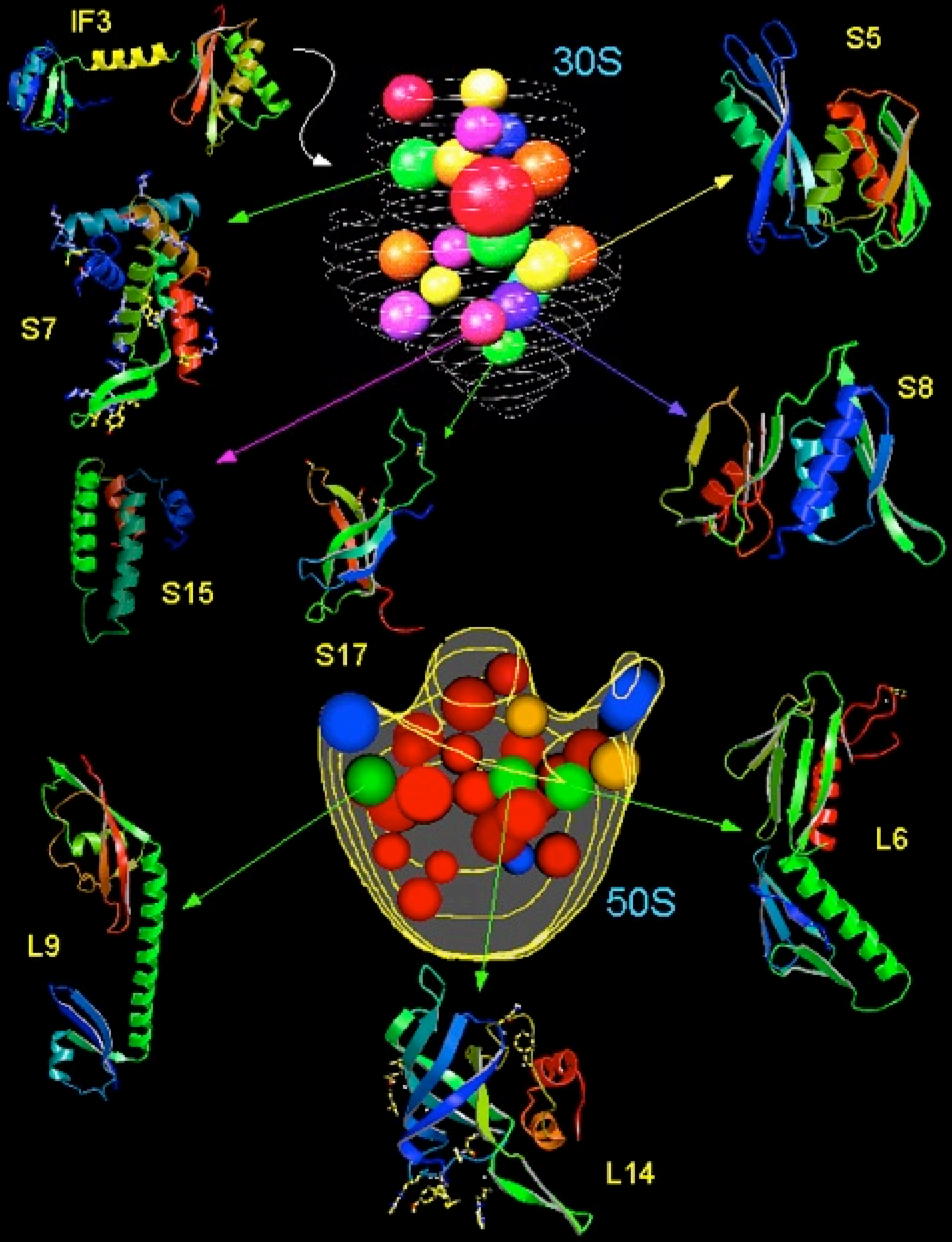
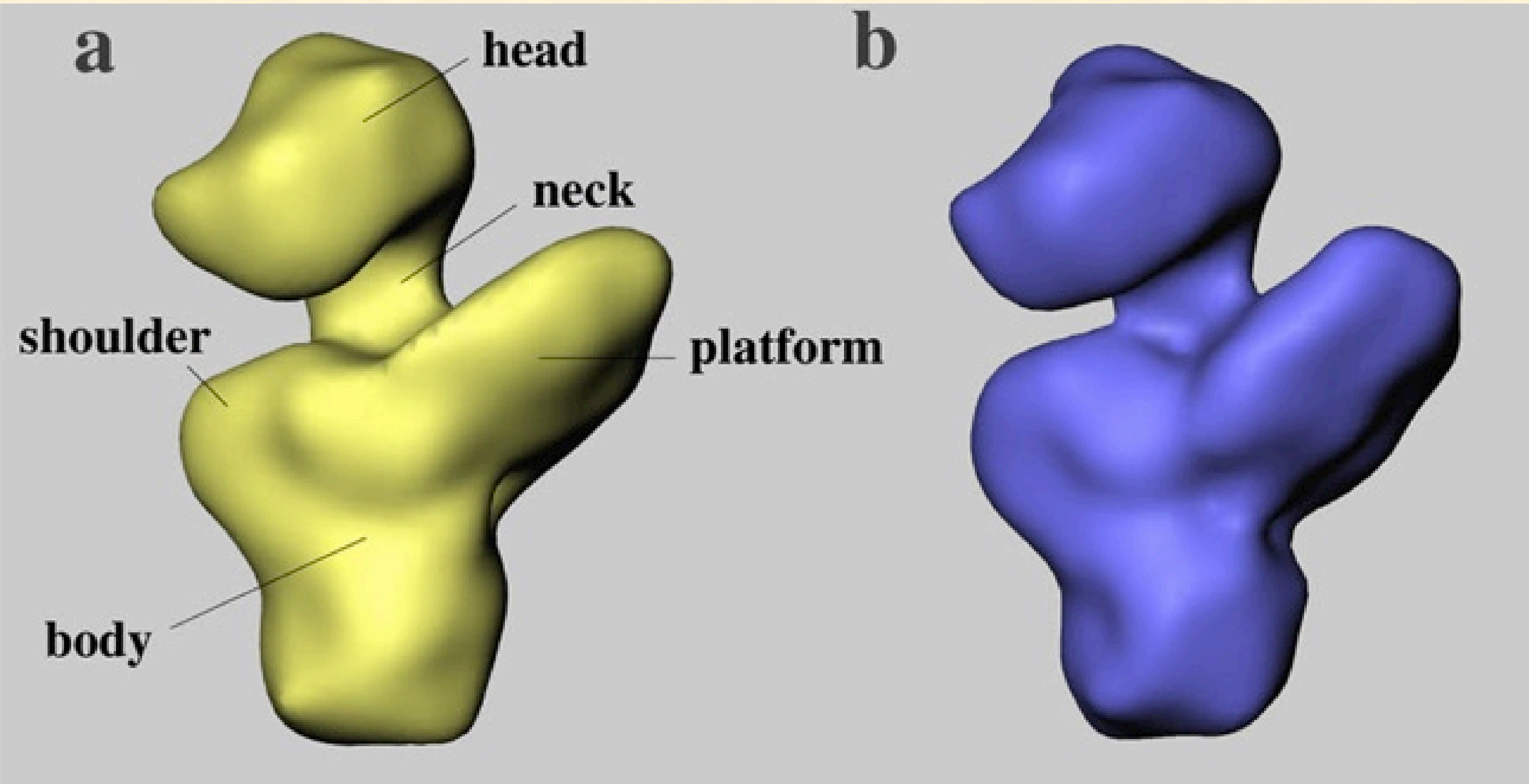
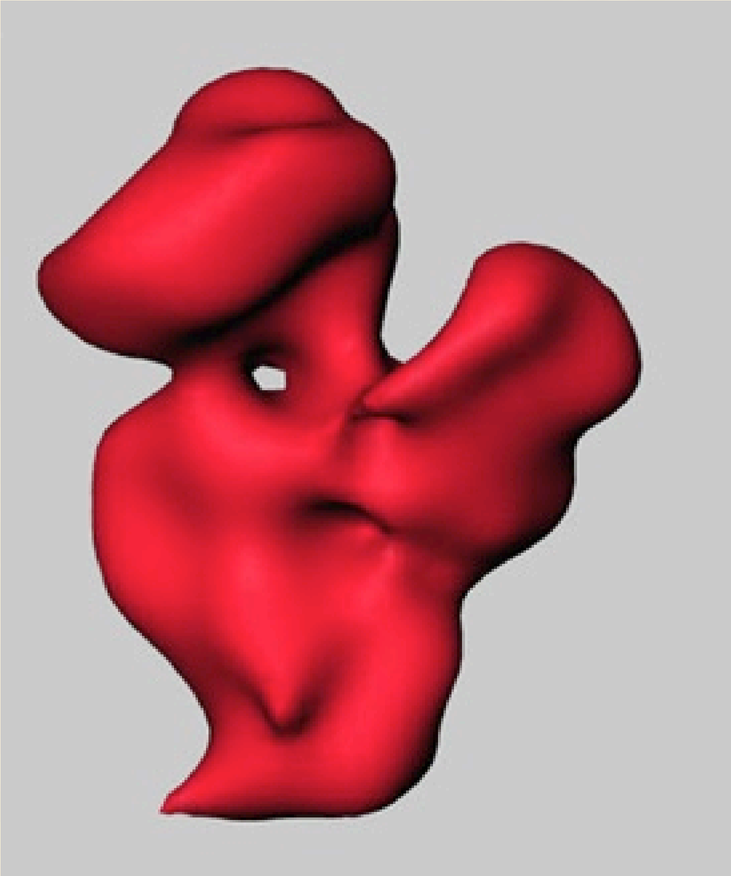


Cryo-EM views of the 30S subunit

Inactive Active 50S-Bound

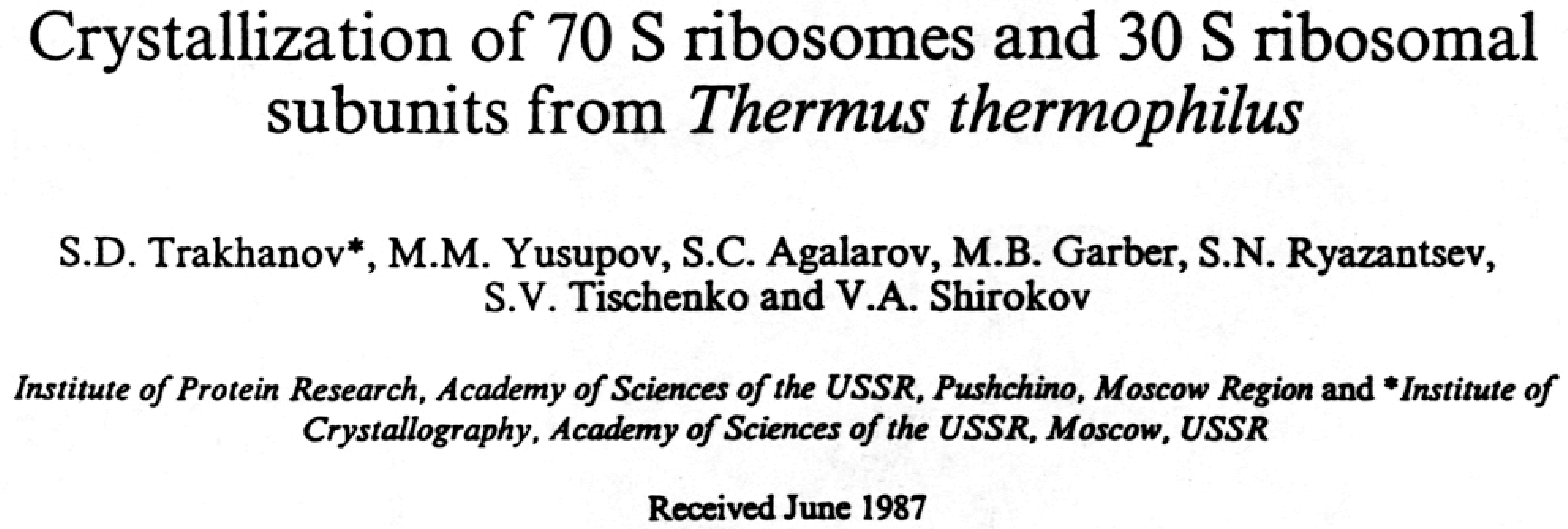




Agrawal, Lata & Frank (1995-6)

Starting the 30S project in Utah

Use of *Thermus thermophilus by* the Puschino group



Crystallization of 70 S ribosomes and 30 S ribosomal subunits from *Thermus thermophilus*

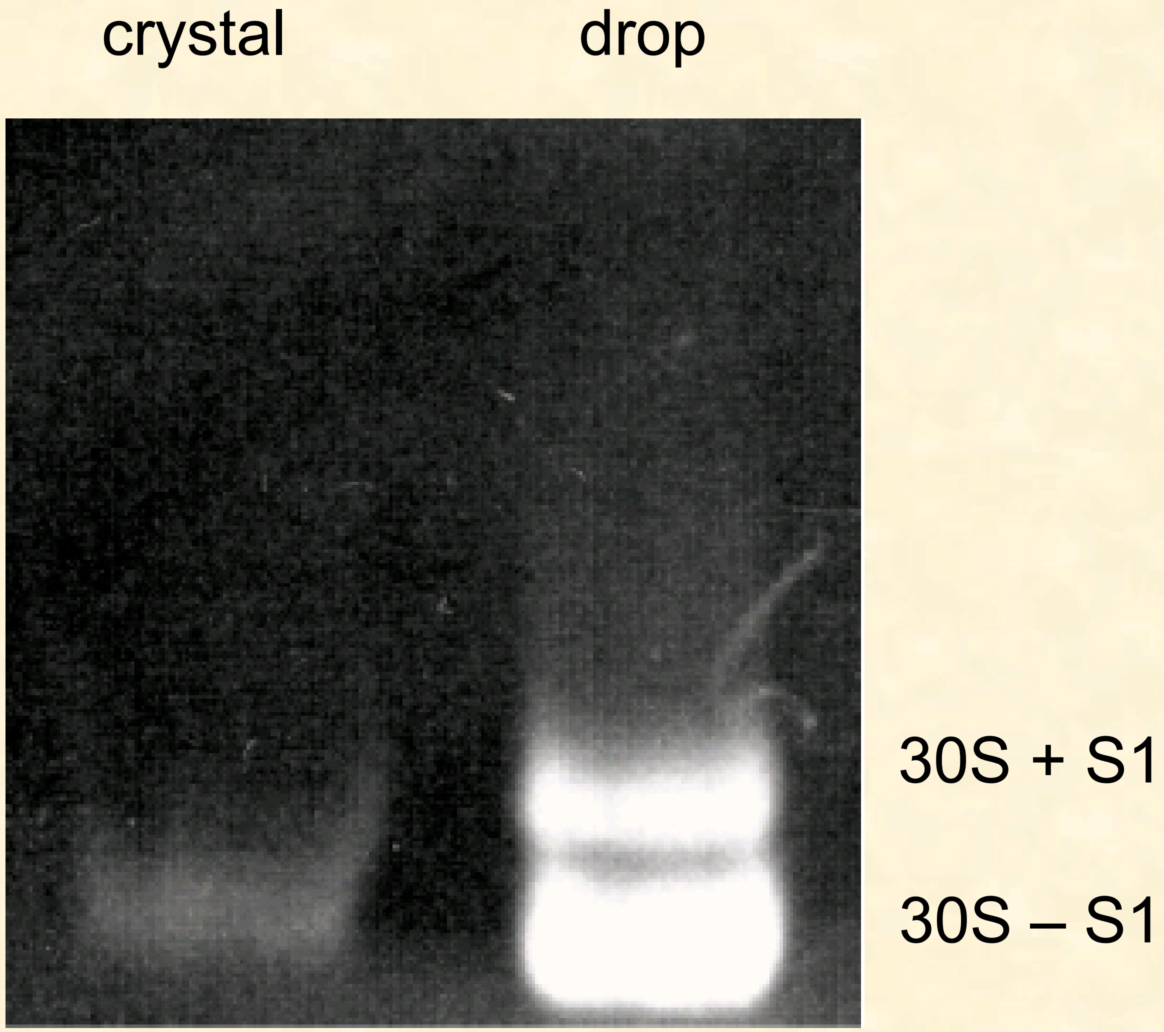
S.D. Trakhanov*, M.M. Yusupov, S.C. Agalarov, M.B. Garber, S.N. Ryazantsev,
S.V. Tischenko and V.A. Shirokov

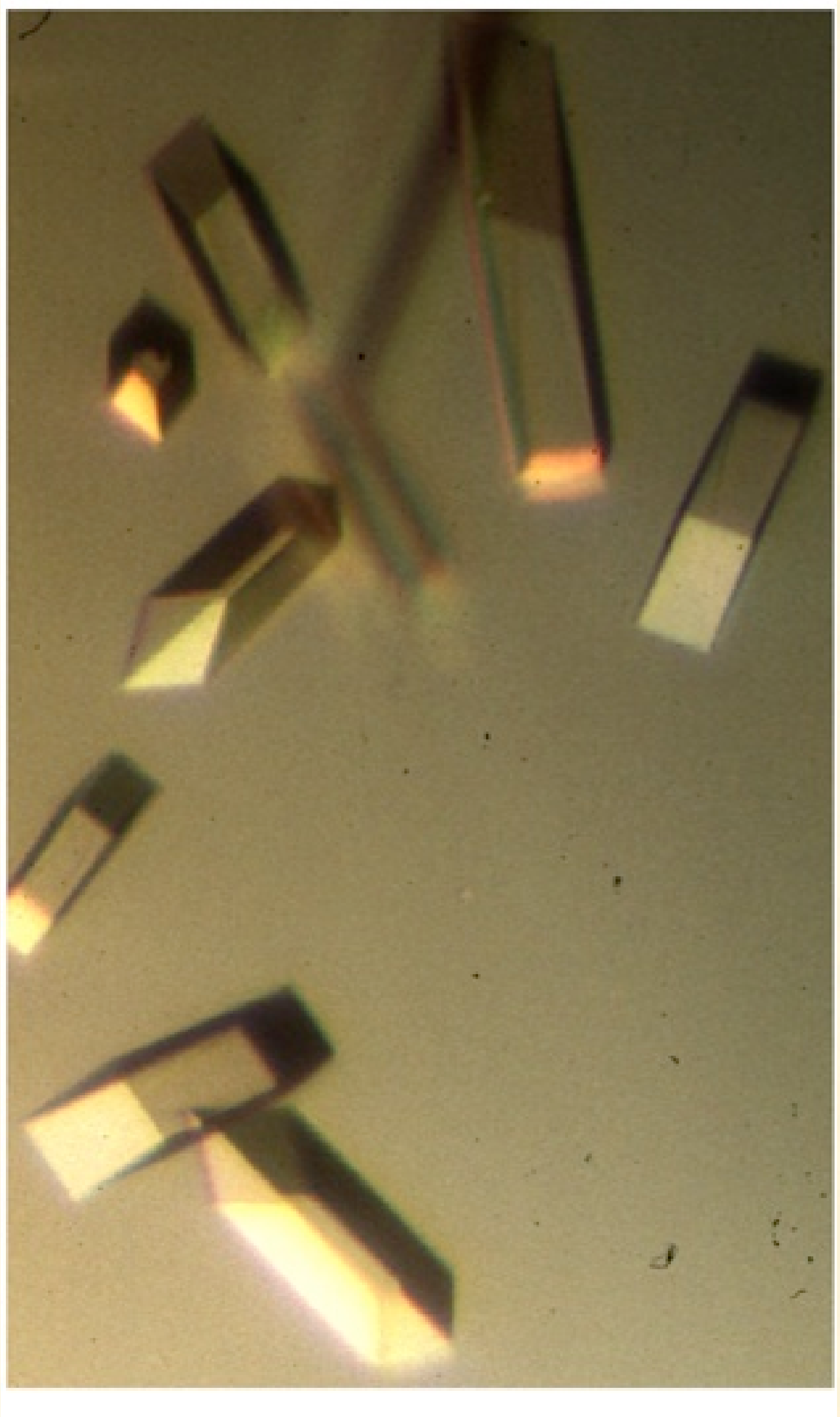
Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region and **Institute of Crystallography, Academy of Sciences of the USSR, Moscow, USSR*

Received June 1987

FEBS Letters (1987) **220**, 319-322

Crystals by removing a variable component



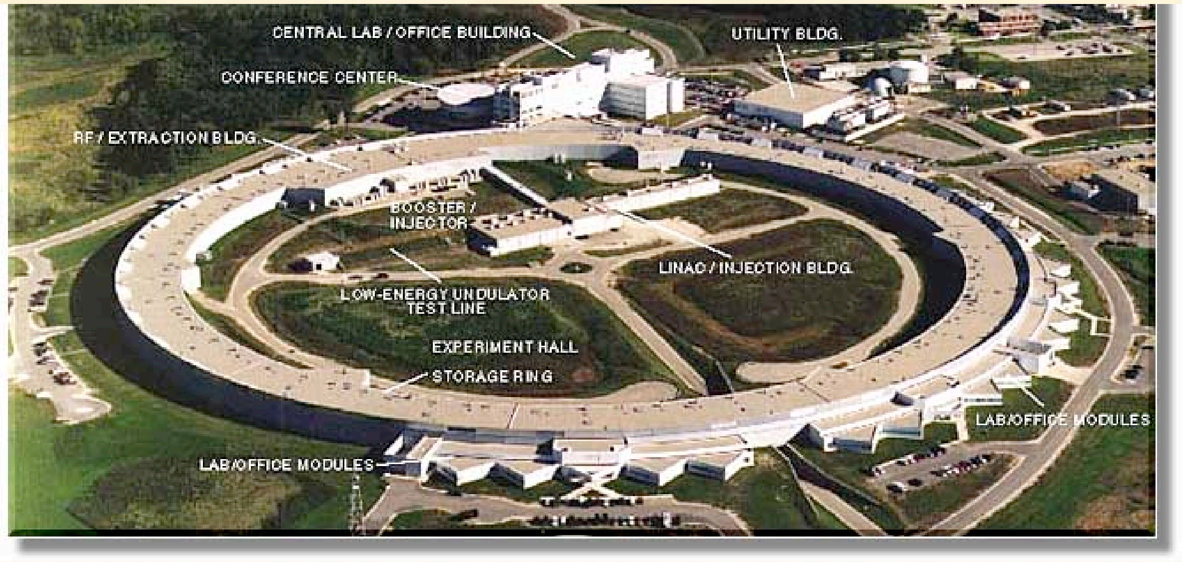


Synchrotrons: Powerful x-ray sources

ESRF, Grenoble, France

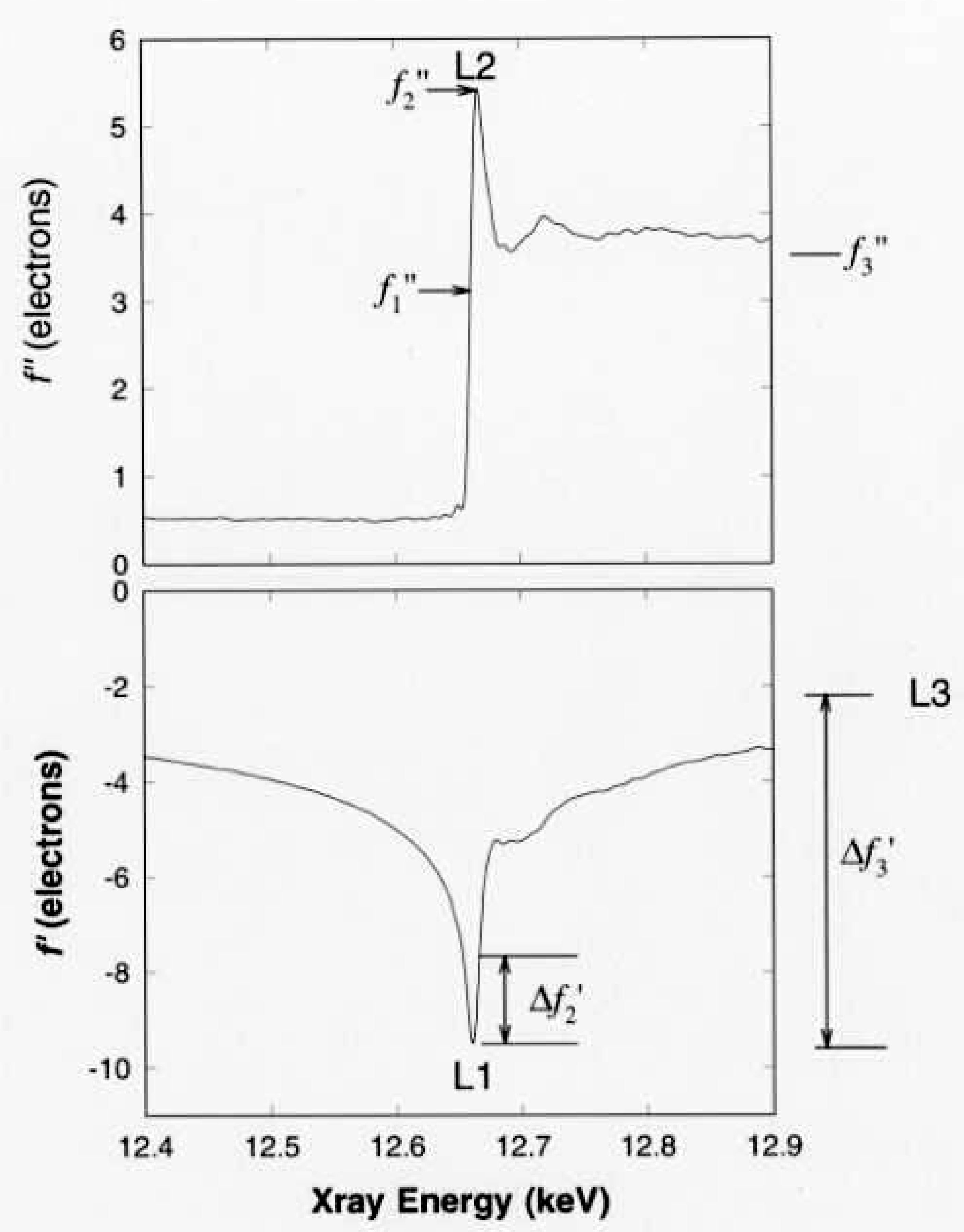
Swiss Light Source, Villigen

NSLS, Brookhaven (USA)



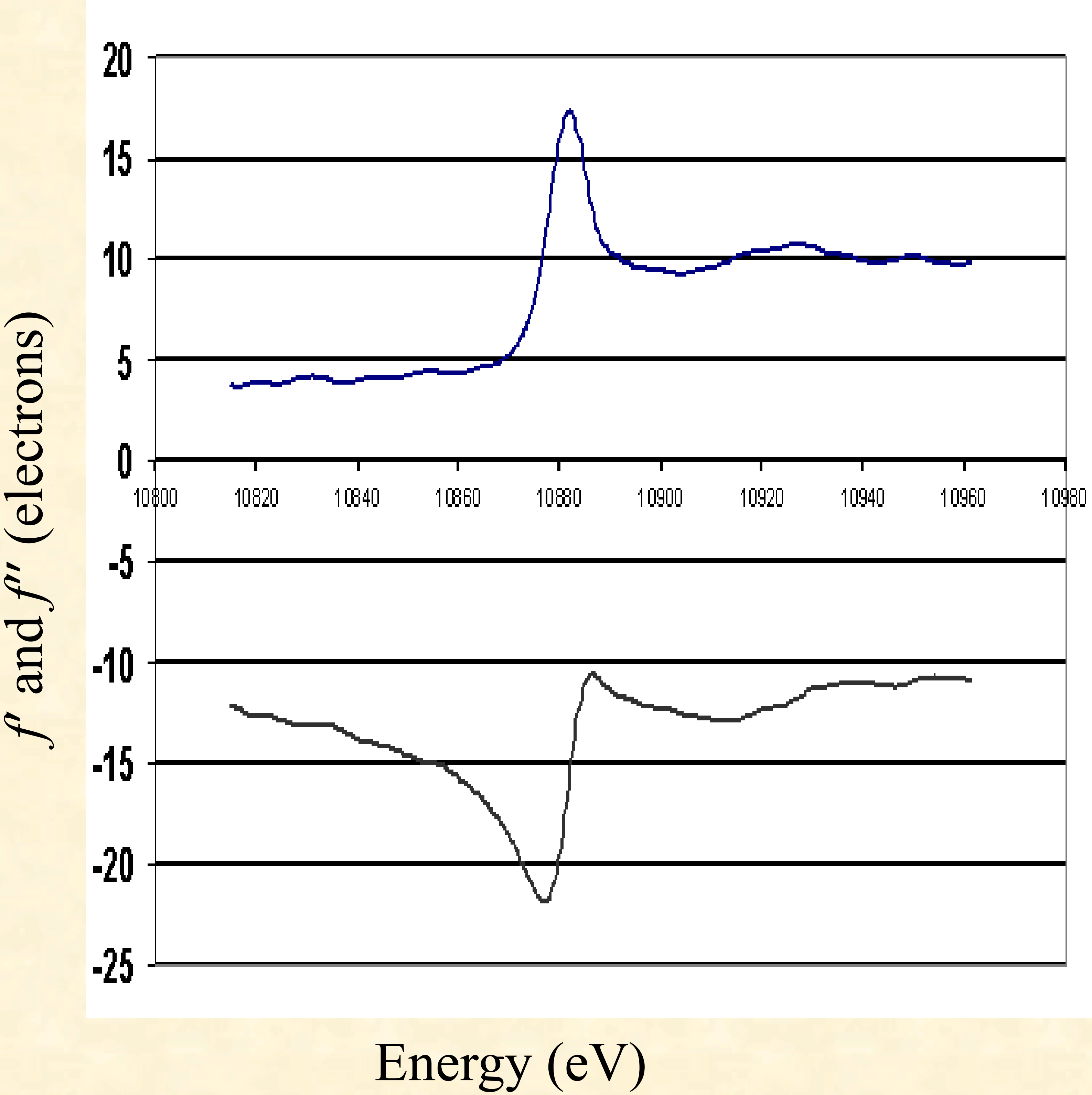
APS, Argonne (near Chicago)

MAD with selenomethionine



- Dispersive and anomalous terms only a few electrons
- Works because of minimal errors from scaling and non-isomorphism
- However, not enough methionines in the 30S

Anomalous scattering from LIII edges of lanthanides



Holmium used for mannose binding protein : Weis, Hendrickson *et al.* (1991)

LIII edges from hexammines

Crystal Structure of a Group I Ribozyme Domain: Principles of RNA Packing

Jamie H. Cate, Anne R. Gooding, Elaine Podell, Kaihong Zhou,
Barbara L. Golden, Craig E. Kundrot, Thomas R. Cech,*
Jennifer A. Doudna*

SCIENCE • VOL. 273 • 20 SEPTEMBER 1996

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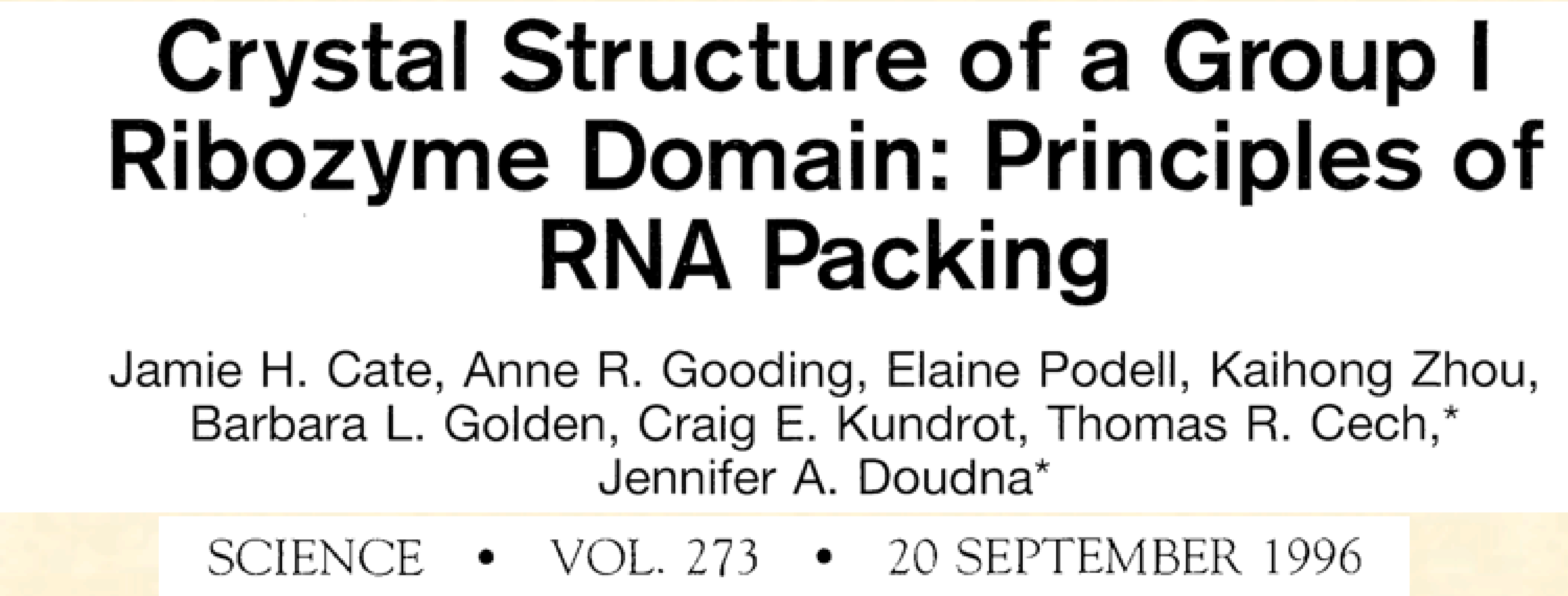

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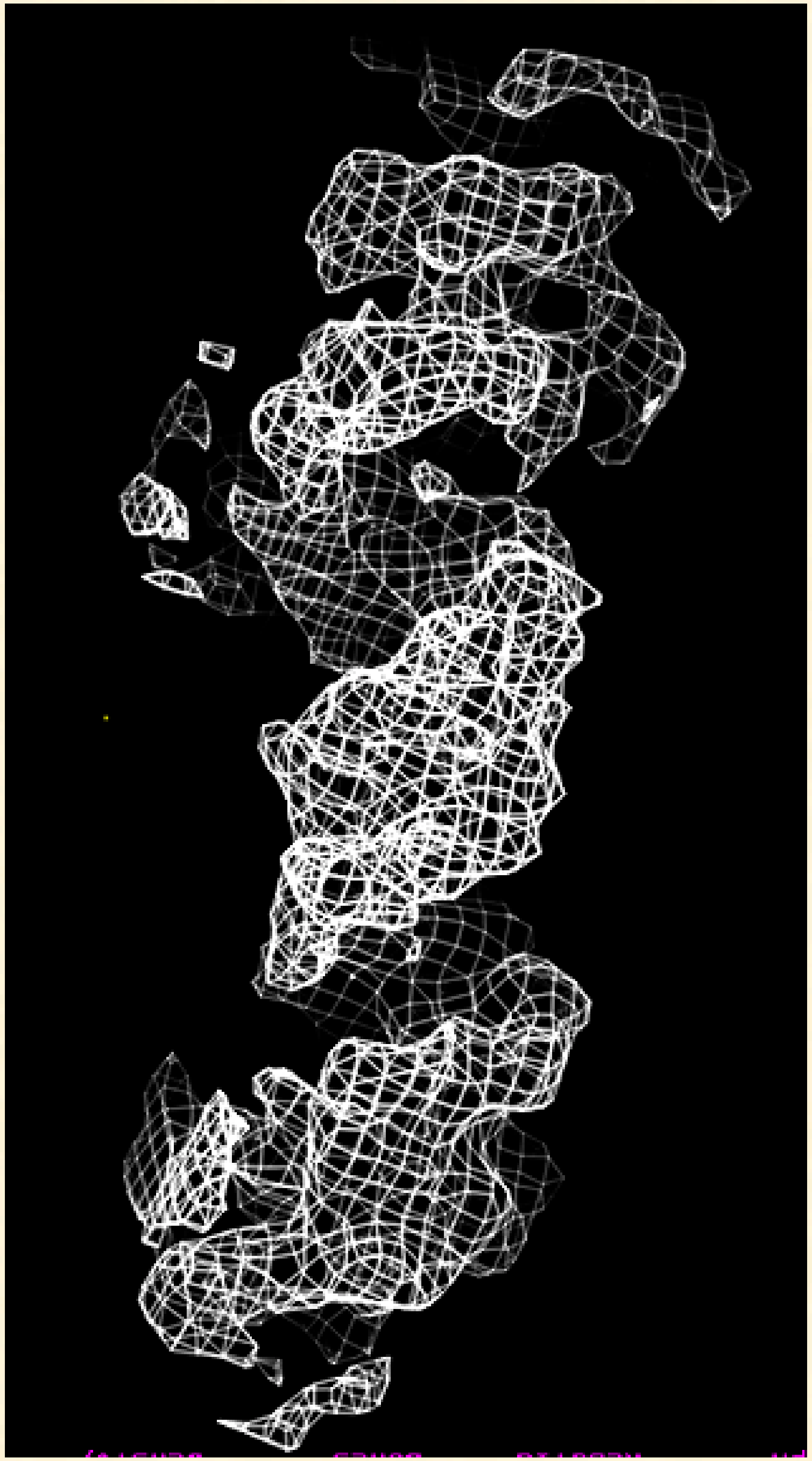
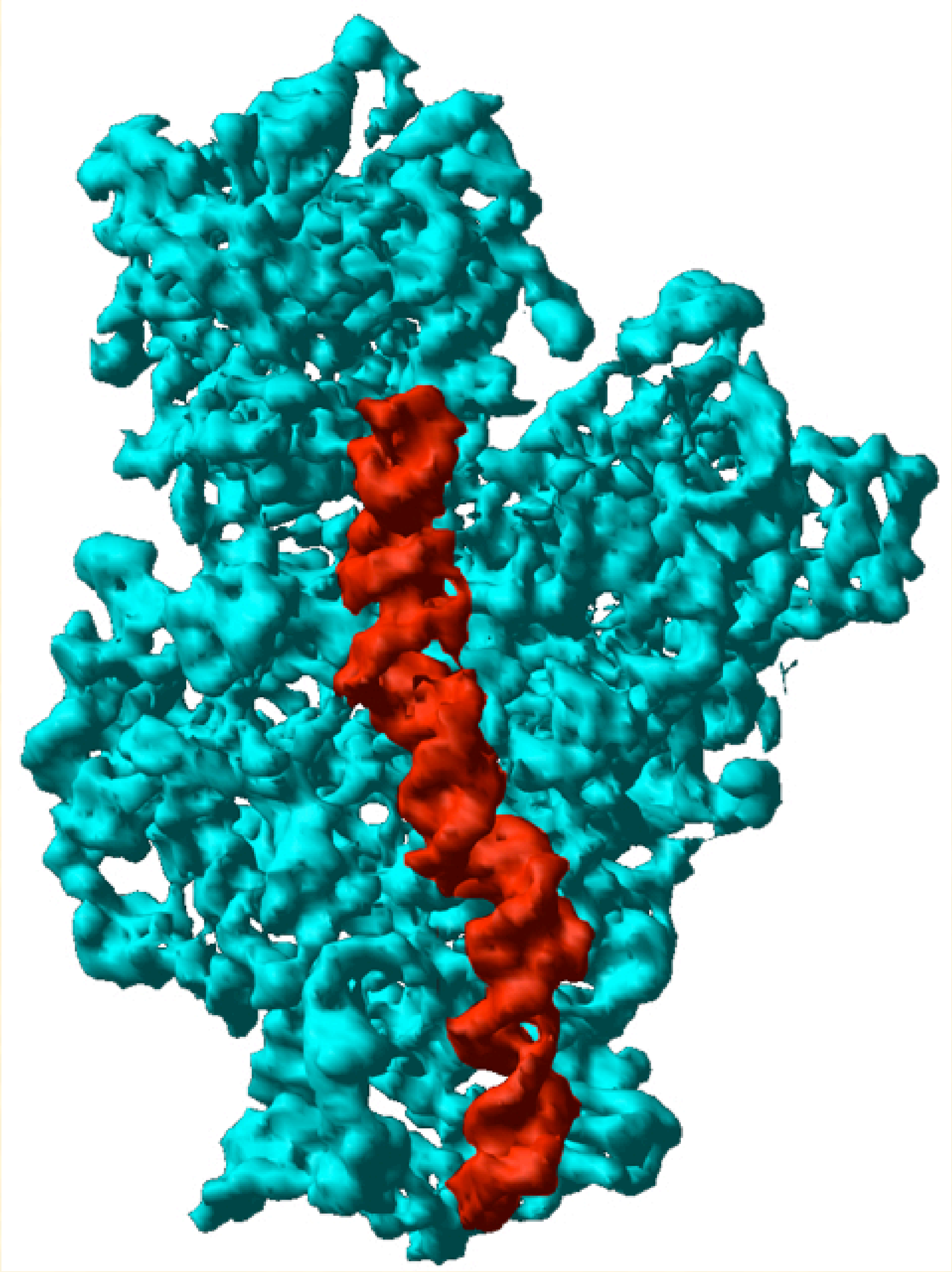
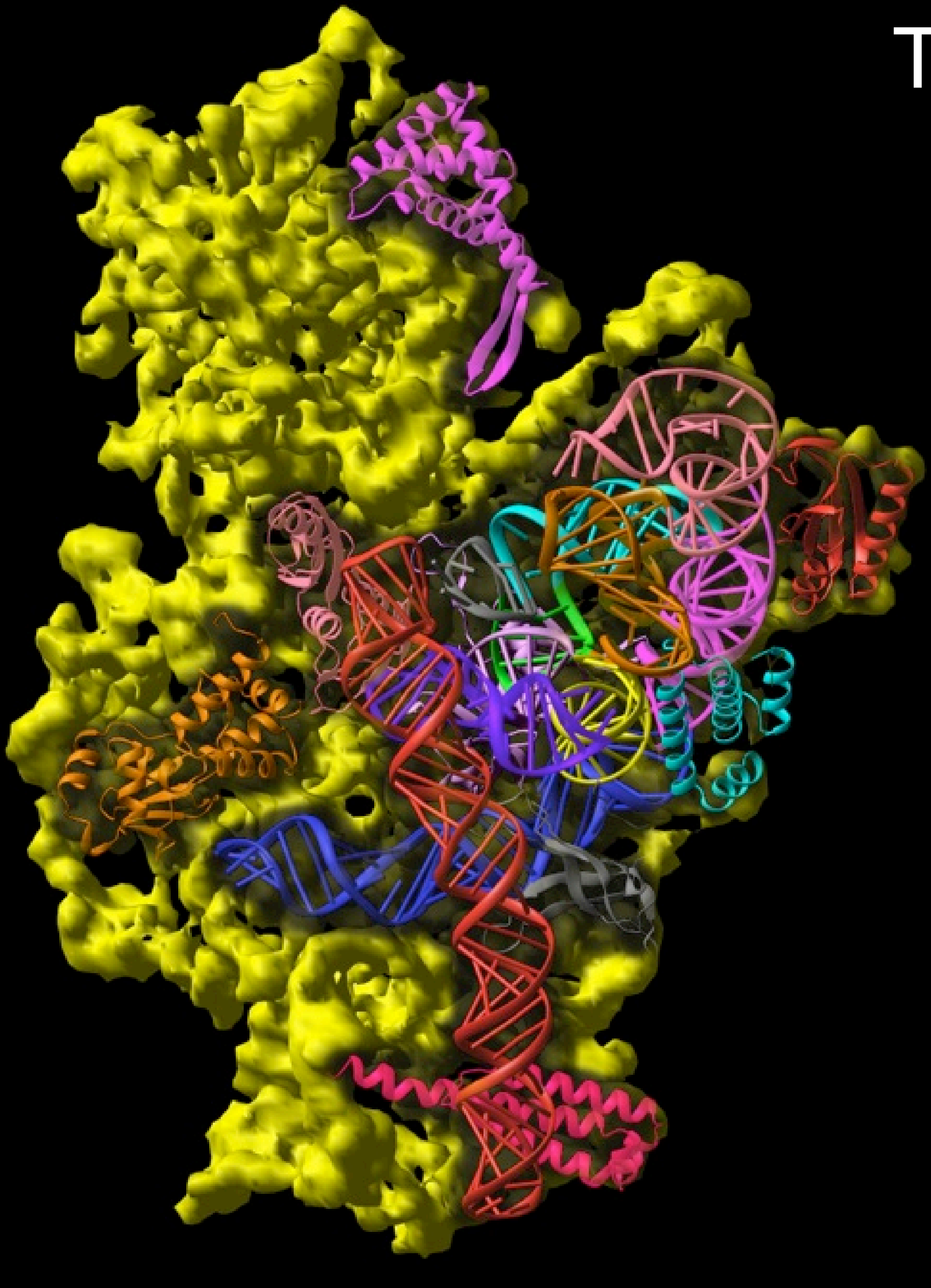
Bruce Brunschwig & Mei H. Chou

Double-stranded RNA helices at 5.5 Å





The 5.5 Å structure of the 30S subunit



Clemons *et al.*, Nature 1999

- 7 known proteins placed
- central domain of RNA
- penultimate H44 with decoding site
- S20

The 30S team

Brian
Wimberly

Bil
Clemons

Ditlev
Brodersen

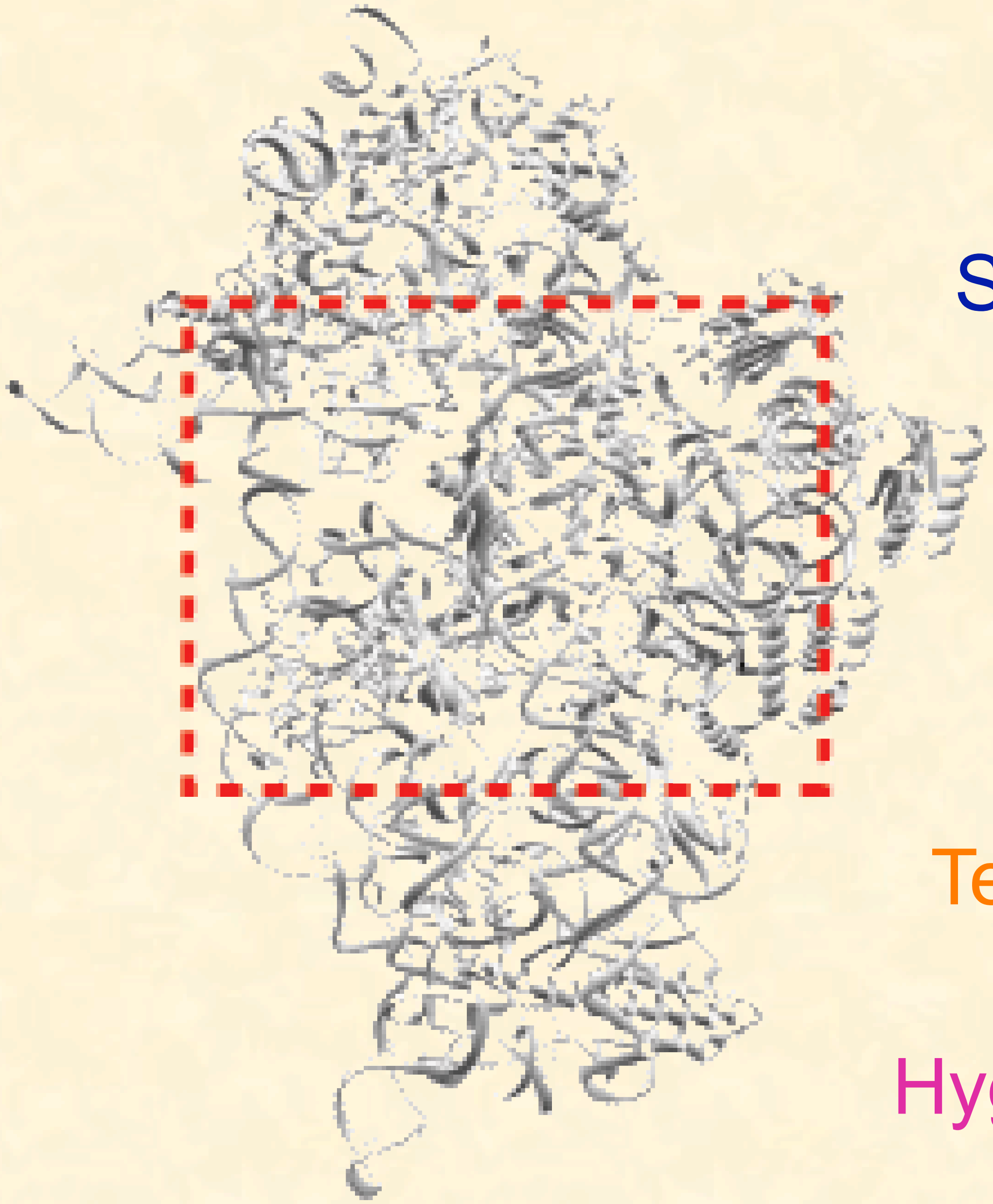
Andrew
Carter

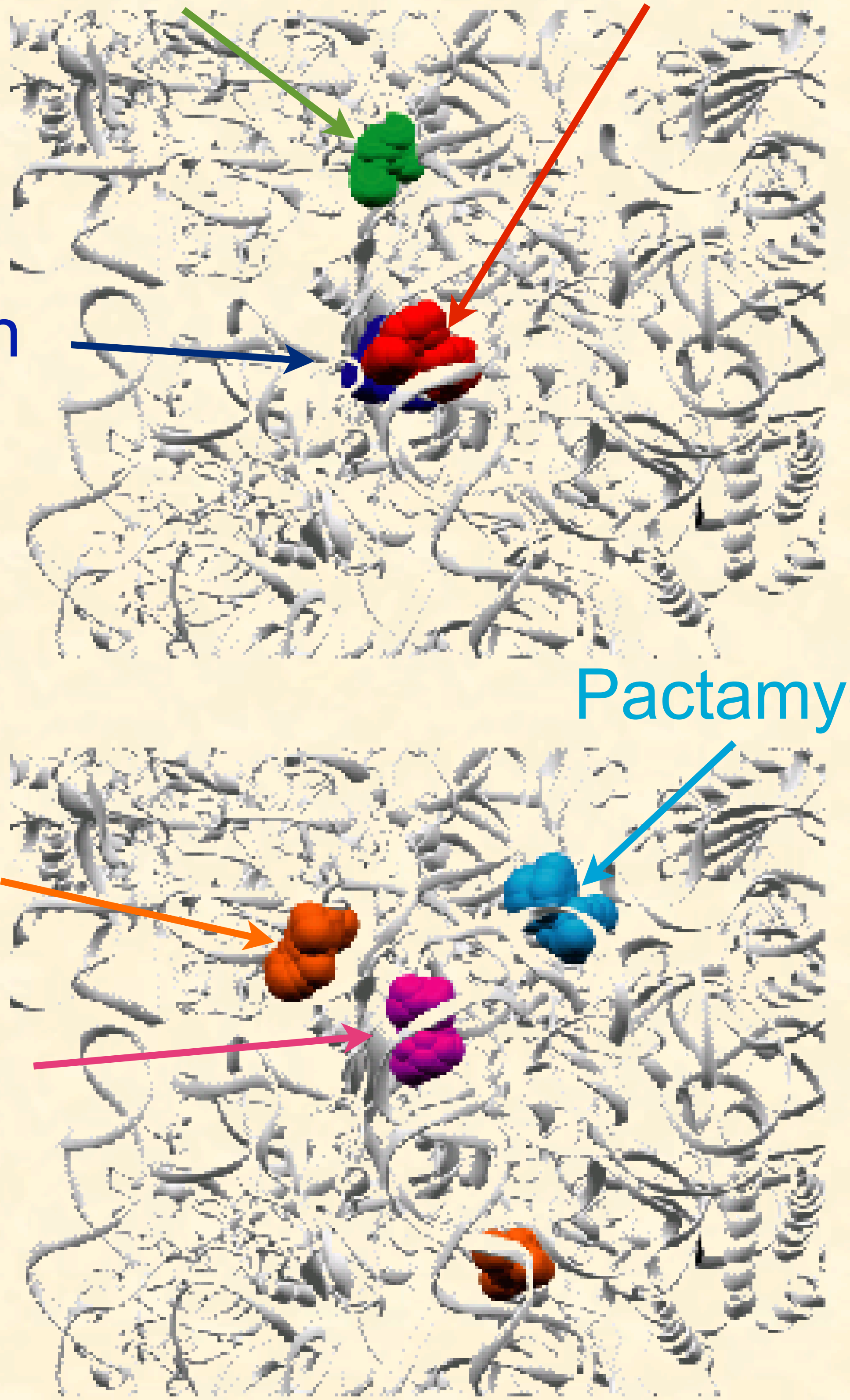
Robert
Morgan-Warren

The atomic structure of the 30S subunit

Wimberly *et al.*, *Nature,* 2000

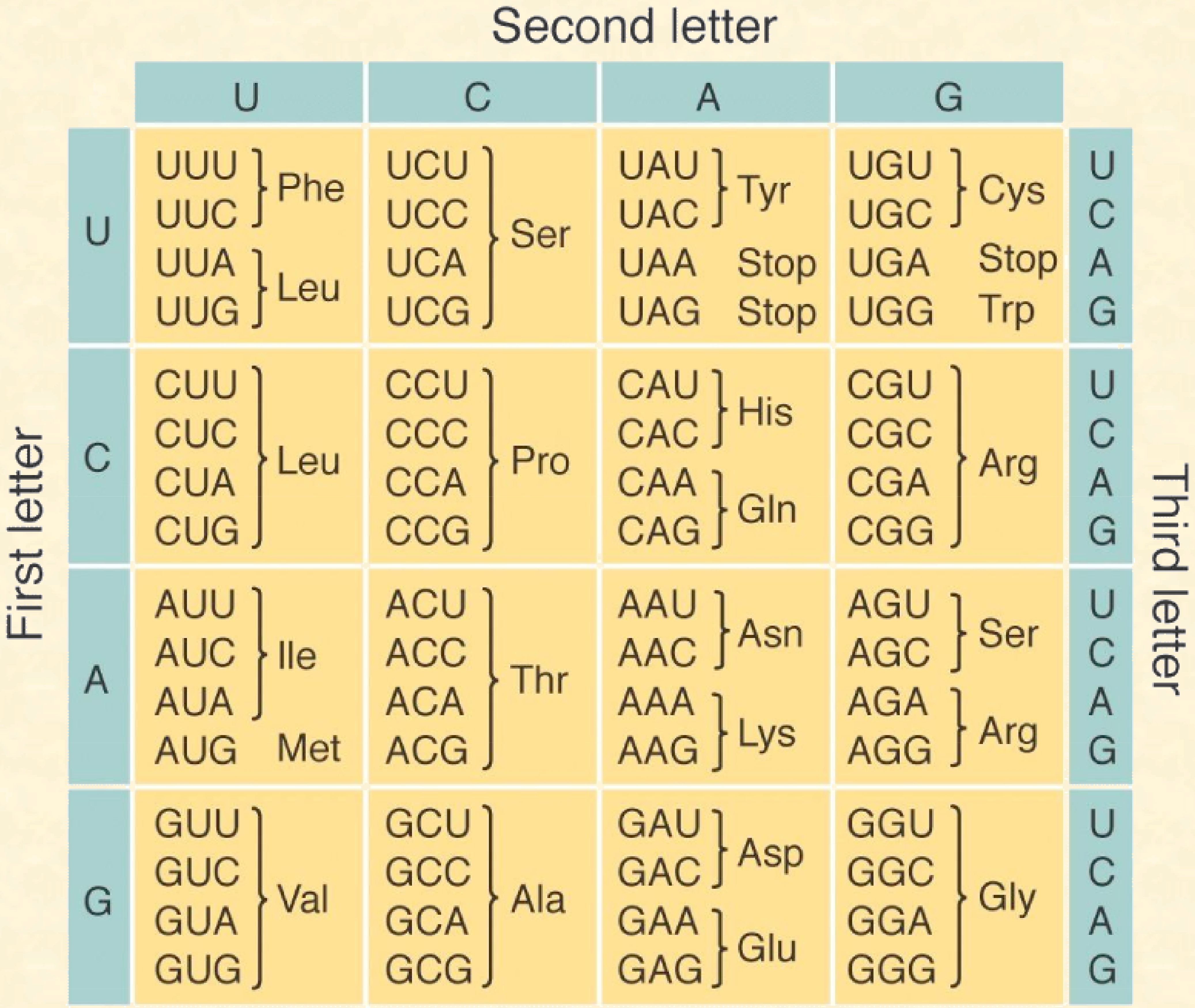
Direct Visualization of Antibiotics





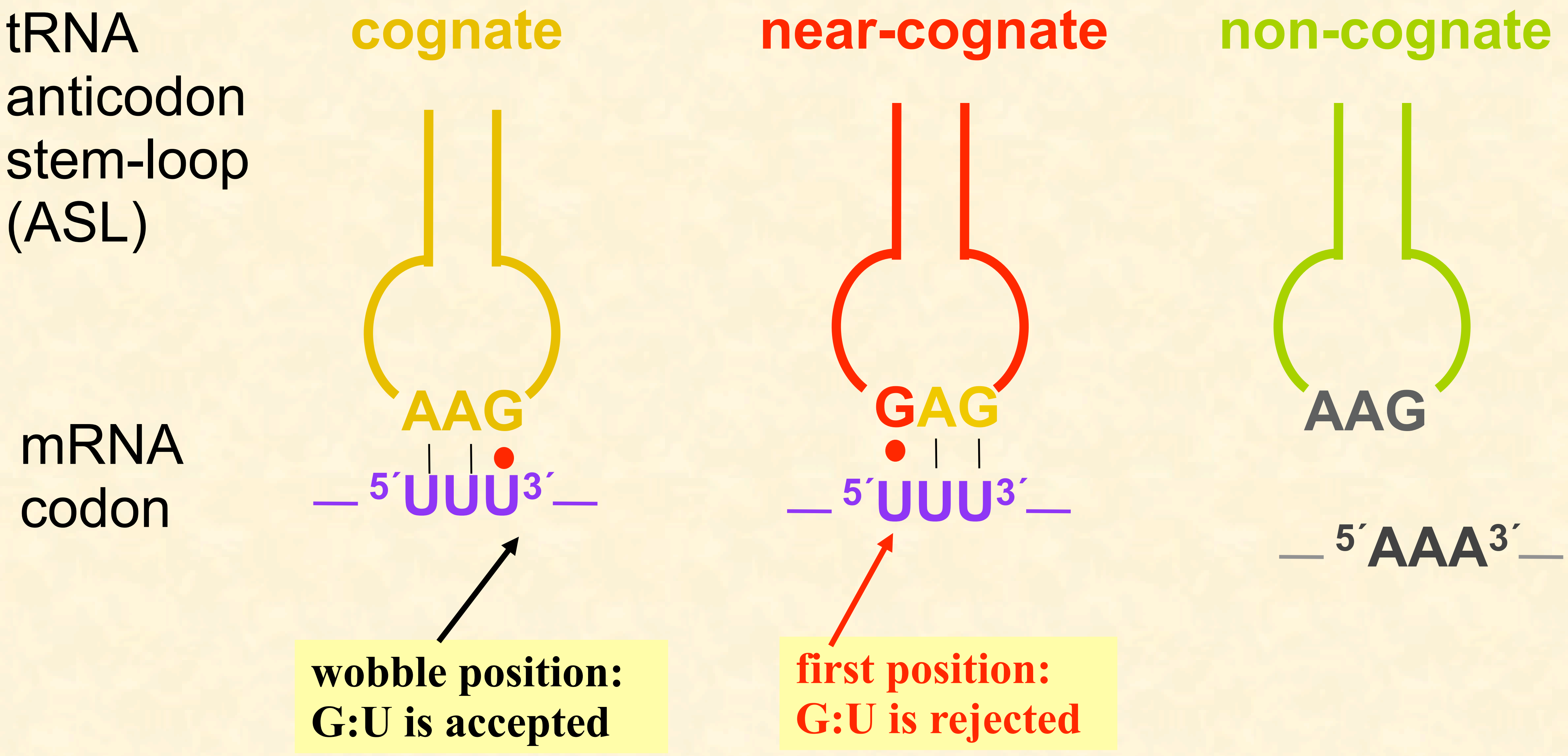
Carter *et al.* Nature, 2000
Brodersen *et al.* Cell, 2000

The Genetic Code



	Second letter								
First letter	U		C		A		G		Third letter
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Decoding: Selection of tRNA

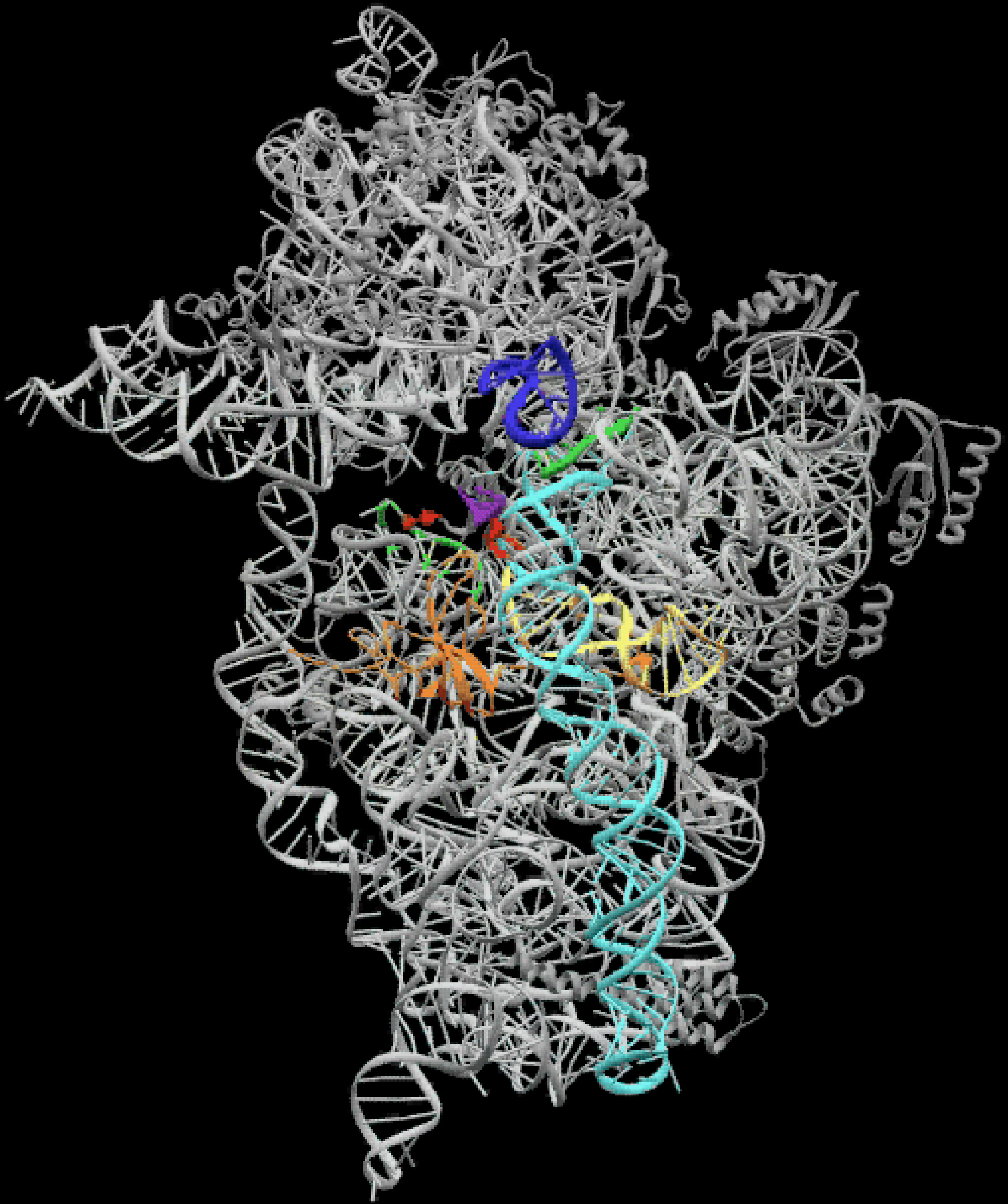


	cognate	near-cognate	non-cognate
tRNA anticodon stem-loop (ASL)	AAG	GAG	AAG
mRNA codon	$^{5'}$UUU$^{3'}$	$^{5'}$UUU$^{3'}$	$^{5'}$AAA$^{3'}$

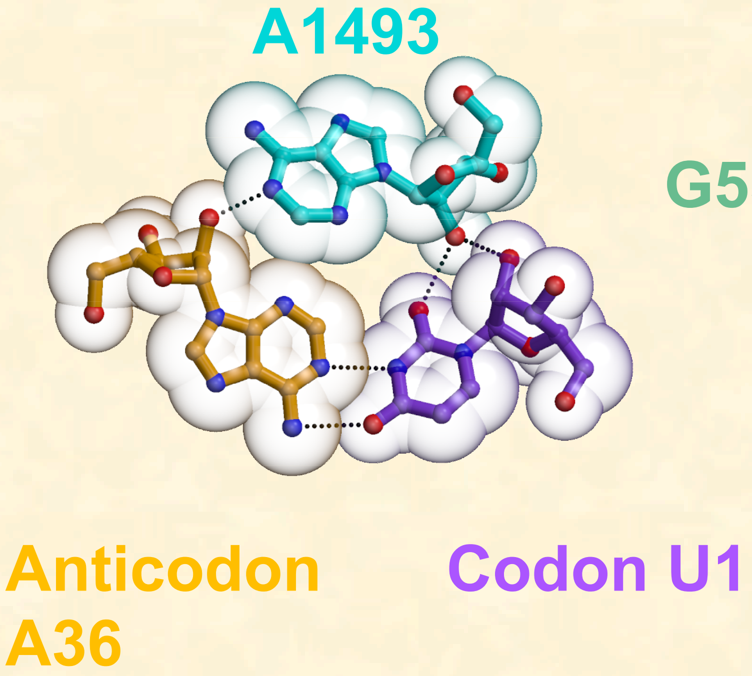
wobble position:
G:U is accepted

first position:
G:U is rejected

James
Ogle



Minor groove recognition of base-pairing geometry at first two positions



Ser50 (s12)

C518

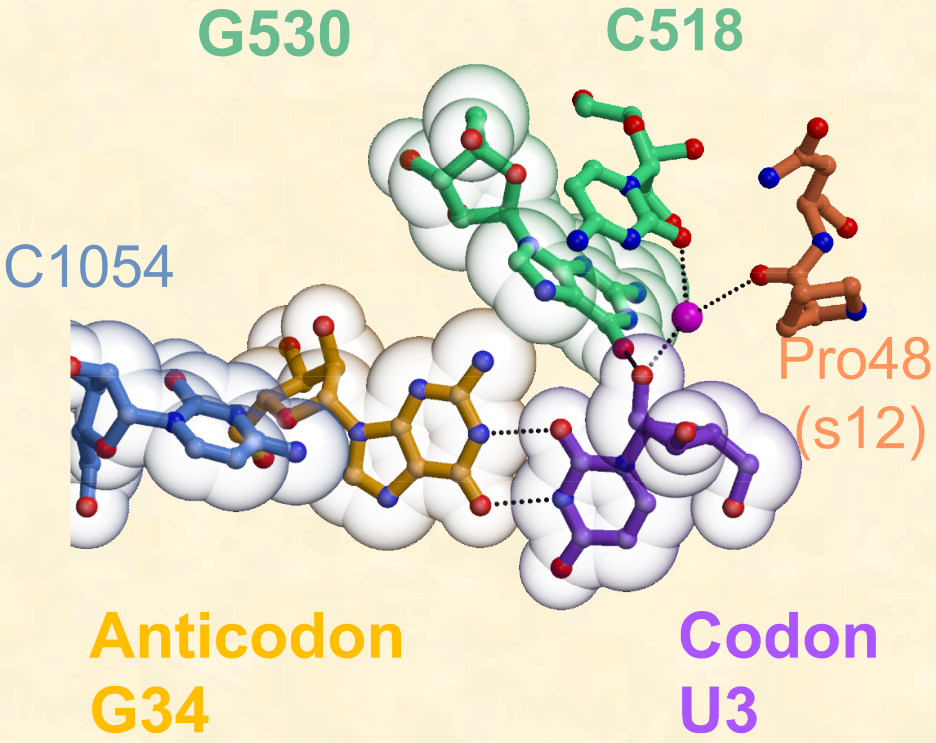
A1493

G530

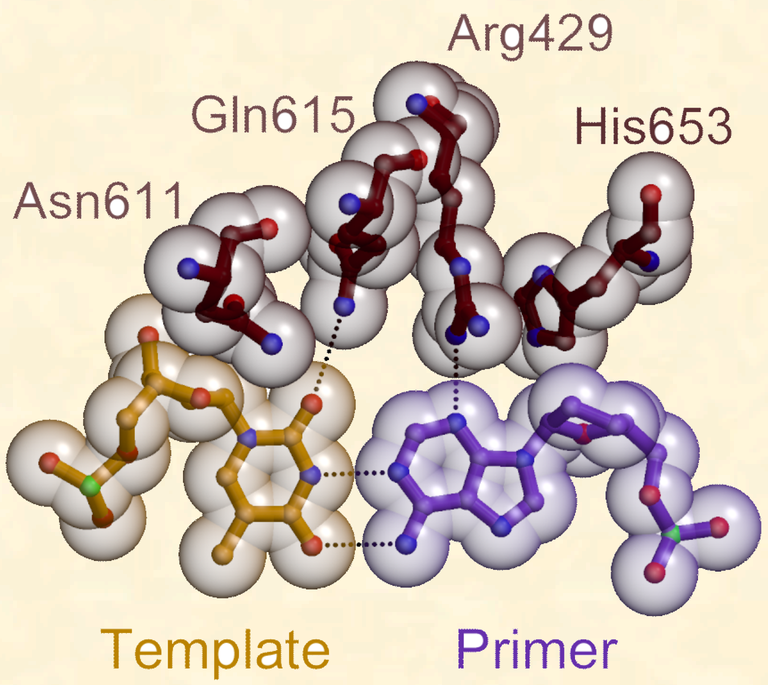
?

Anticodon A35

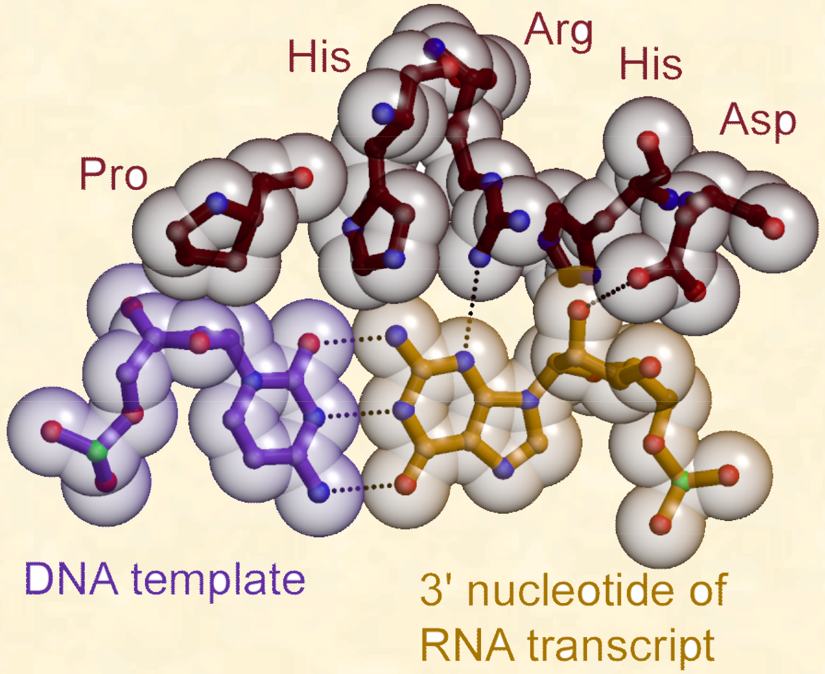
Codon U2



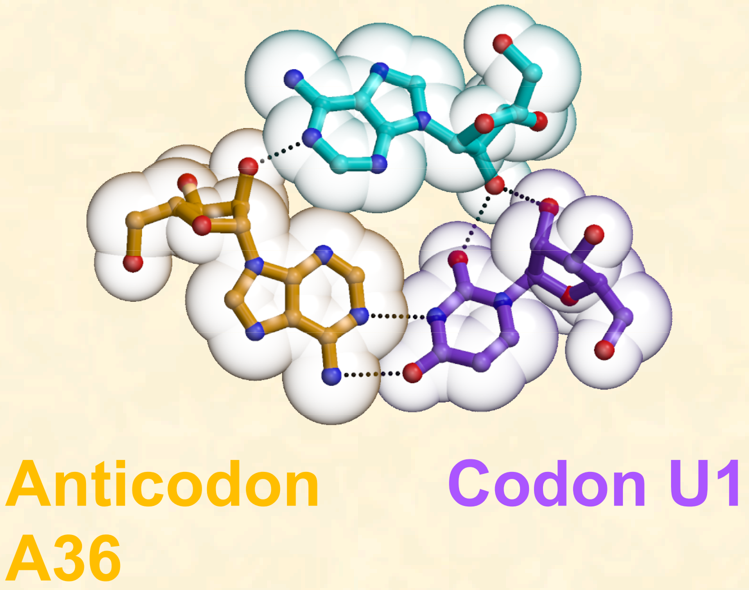
Base pairing and fidelity: Isosteric nature of Watson-Crick pairs



T7 DNA Polymerase: Doublie *et al.* Nature **391** (1998)



T7 RNA Polymerase: Cheetham and Steitz *Science* **286** (1999)



The ribosome: Shows that RNA contains within itself the ability to sense base pairing geometry and ensure fidelity

Delivery of tRNA by EF-Tu, a GTPase

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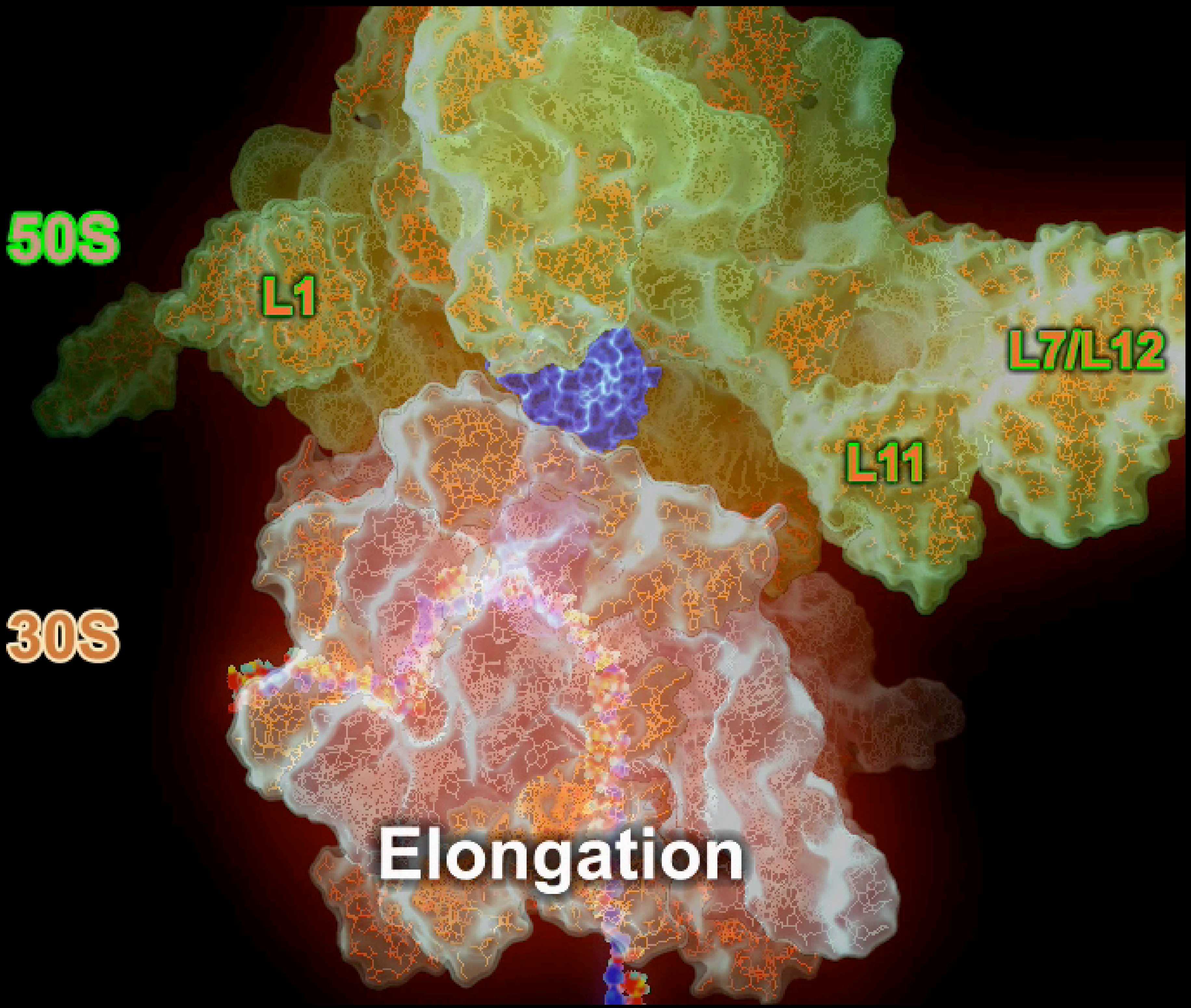
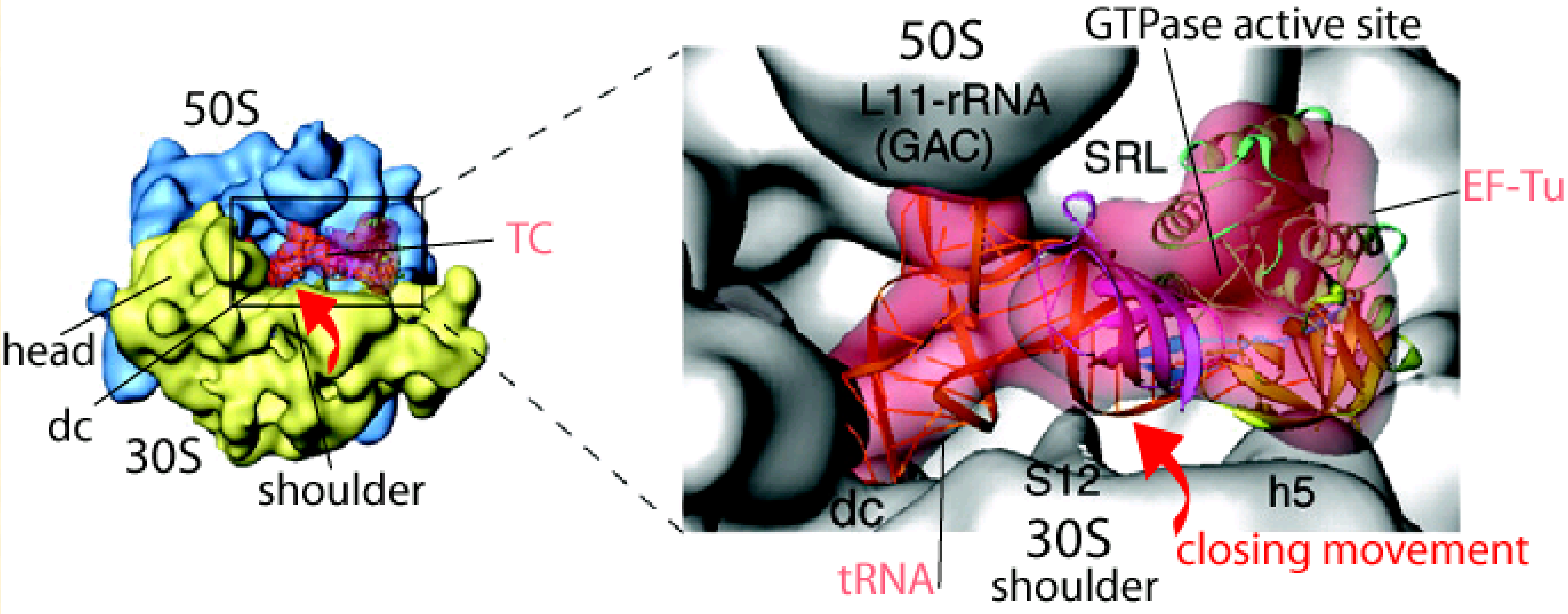


The ternary complex on the ribosome: Results from cryo-EM



“Frozen” on the ribosome after GTP-hydrolysis by the antibiotic kirromycin; about 11 Å resolution

Figure from Valle *et al.* (2002) *EMBO J* **21** 3557-3567
cf. also Stark *et al.* (2002) *Nat Struct Biol* **9**, 849-854

2001-2006: Towards high-resolution 70S structures

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- High quality preparations of ribosomes (**Frank Murphy, Mike Tarry**)

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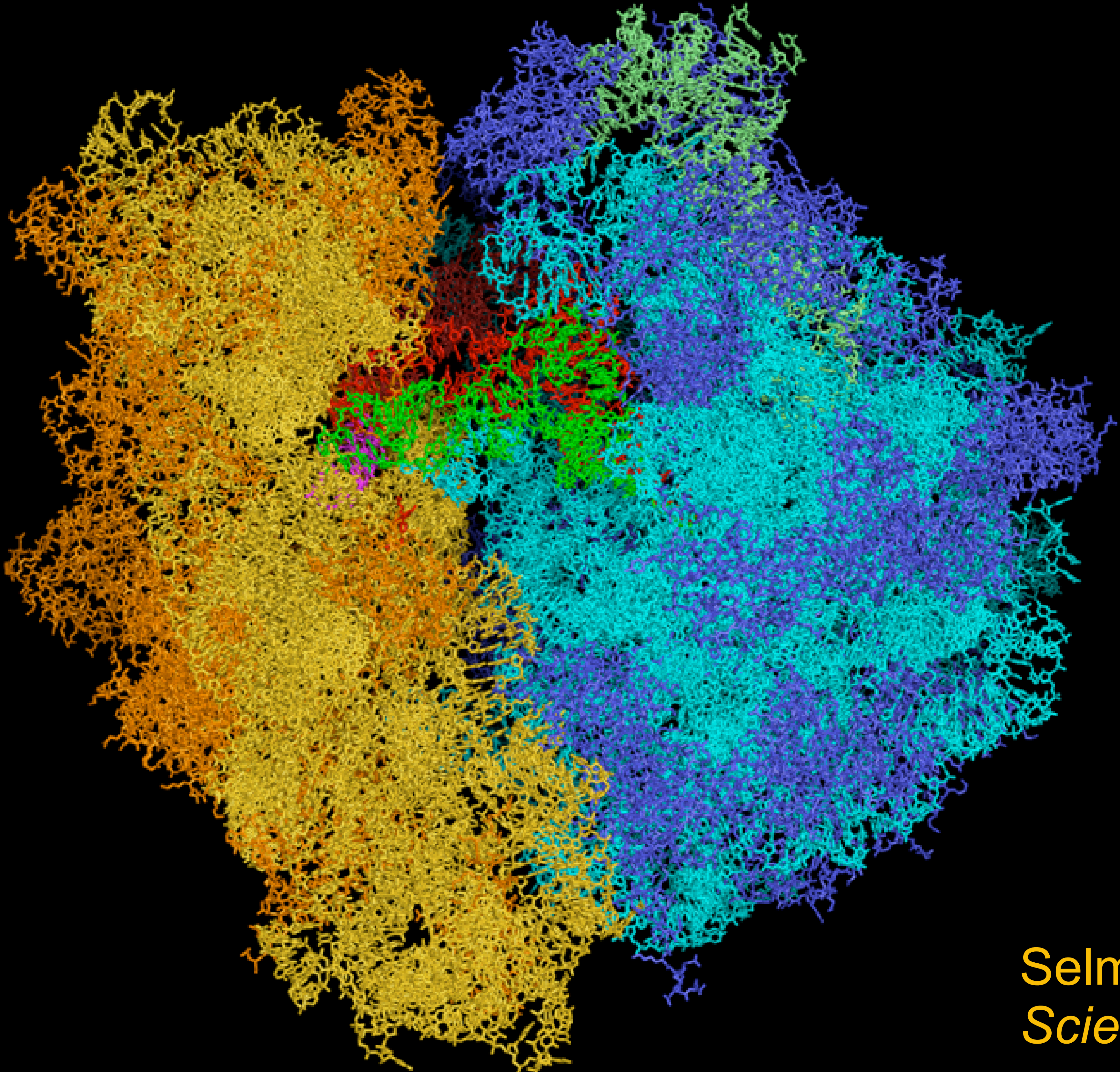
- High-resolution 70S structure with tRNA/mRNA (**Maria Selmer, Christine Dunham, Frank Murphy, Albert Weixlbaumer, Sabine Petry**)

The 70S ribosome with mRNA and tRNA at 2.8 Å resolution



Selmer *et al*.,
Science 2006

2001-2009: Crystal structure of the ribosome with EF-Tu

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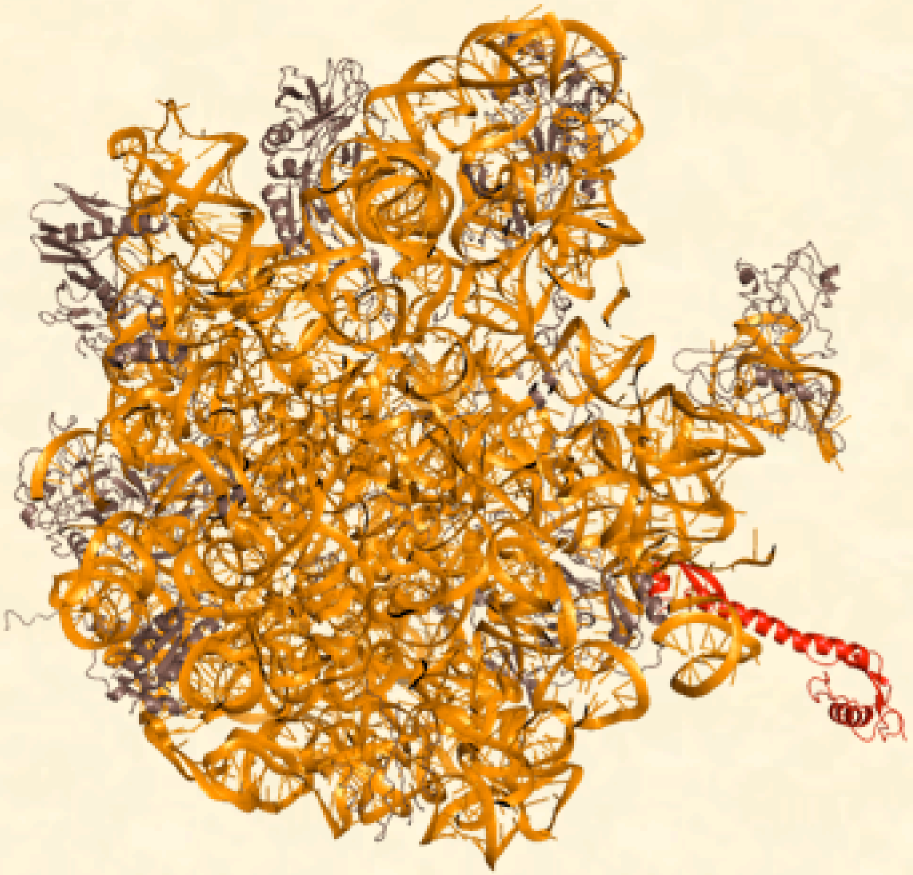

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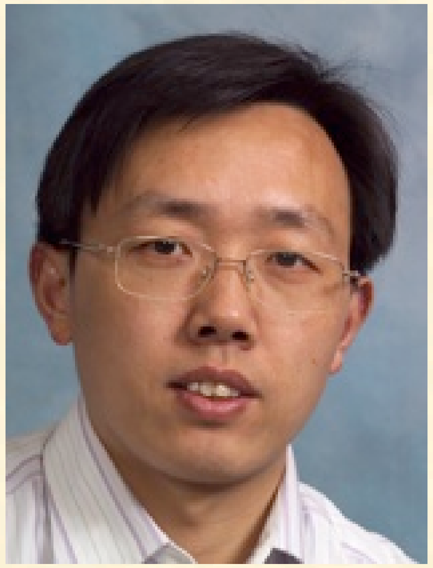

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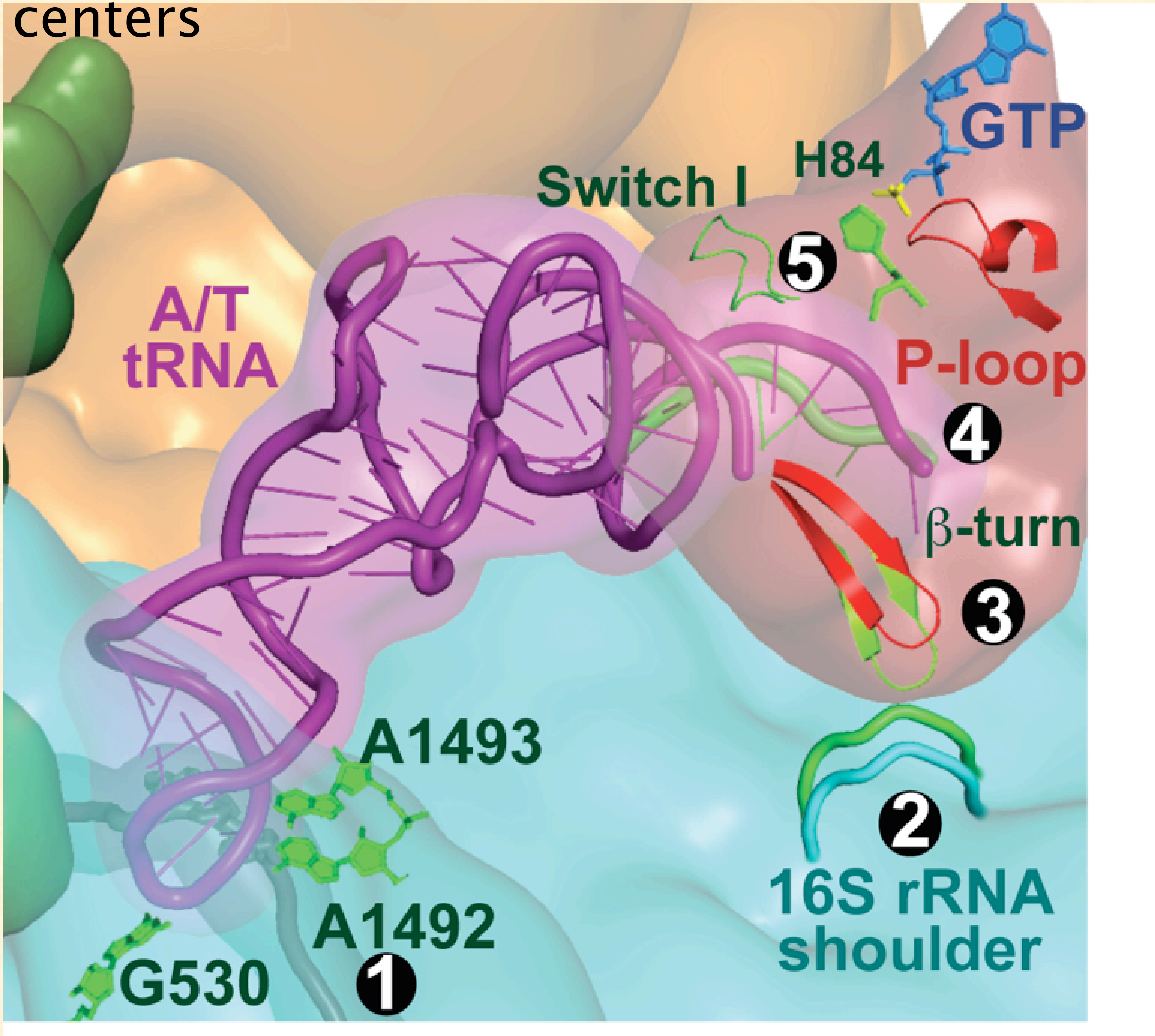
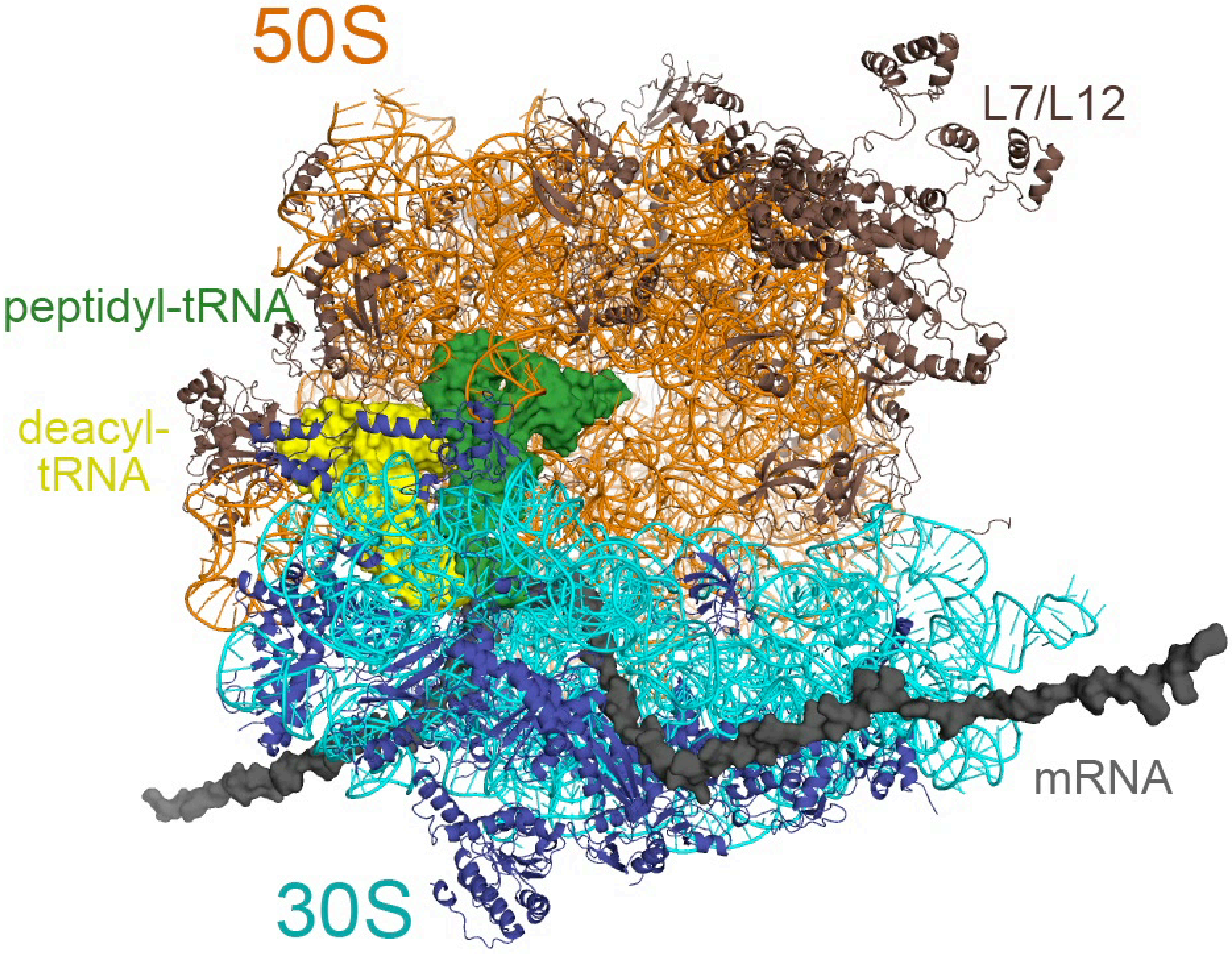
- EF-Tu: A lot of optimization of biochemistry and crystallization, ~ 1200 trays --> 1 data set from 12 crystals, to ~3.6 angstrom resolution (**Martin Schmeing, Rebecca Voorhees, Ann Kelley**)

Communication pathway between the decoding and GTPase centers




50S
L7/L12
peptidyl-tRNA
deacyl-tRNA
mRNA
30S