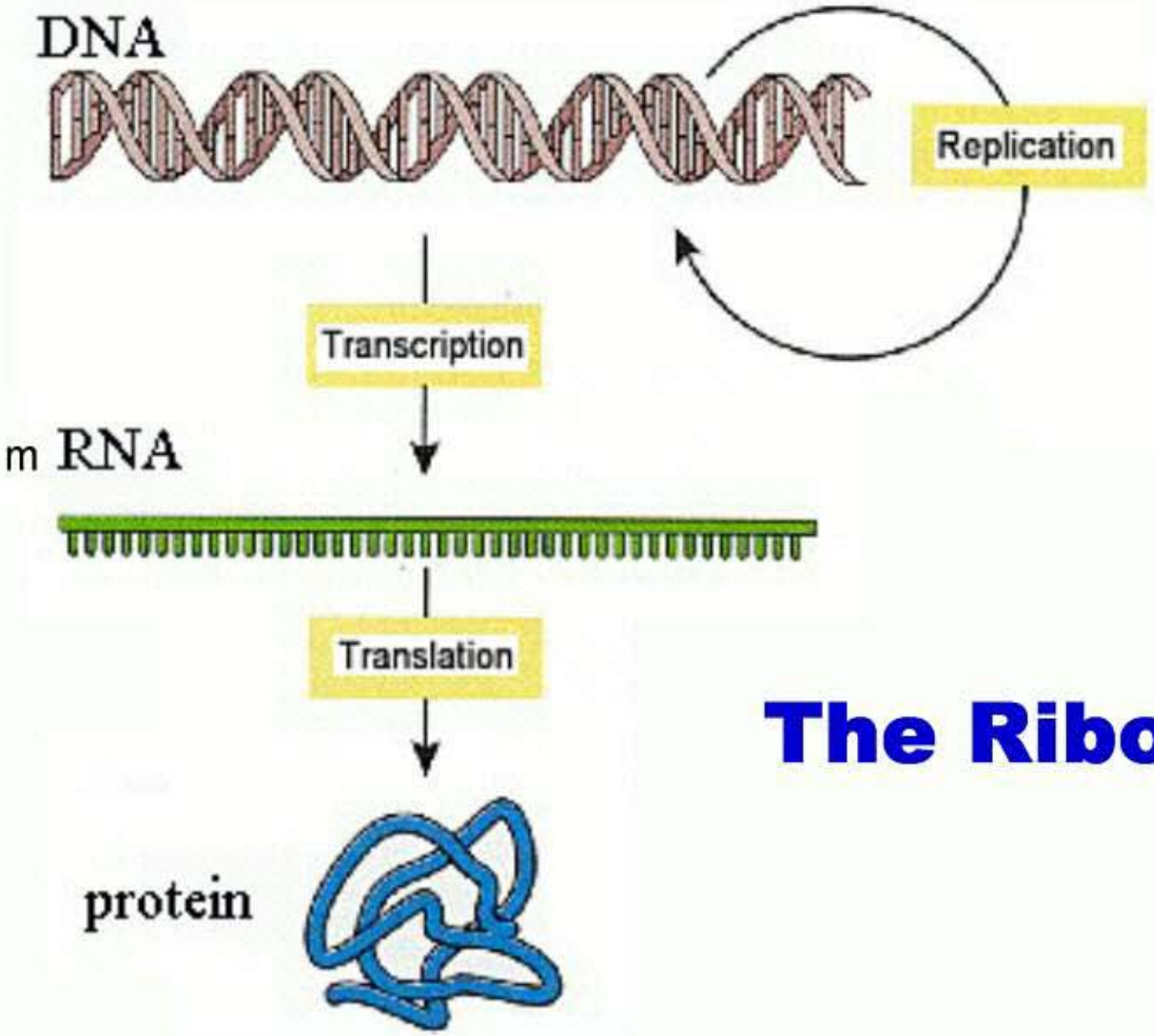
Polar bears, Unpaved roads, Everest Climbing and Ribosomes in Action

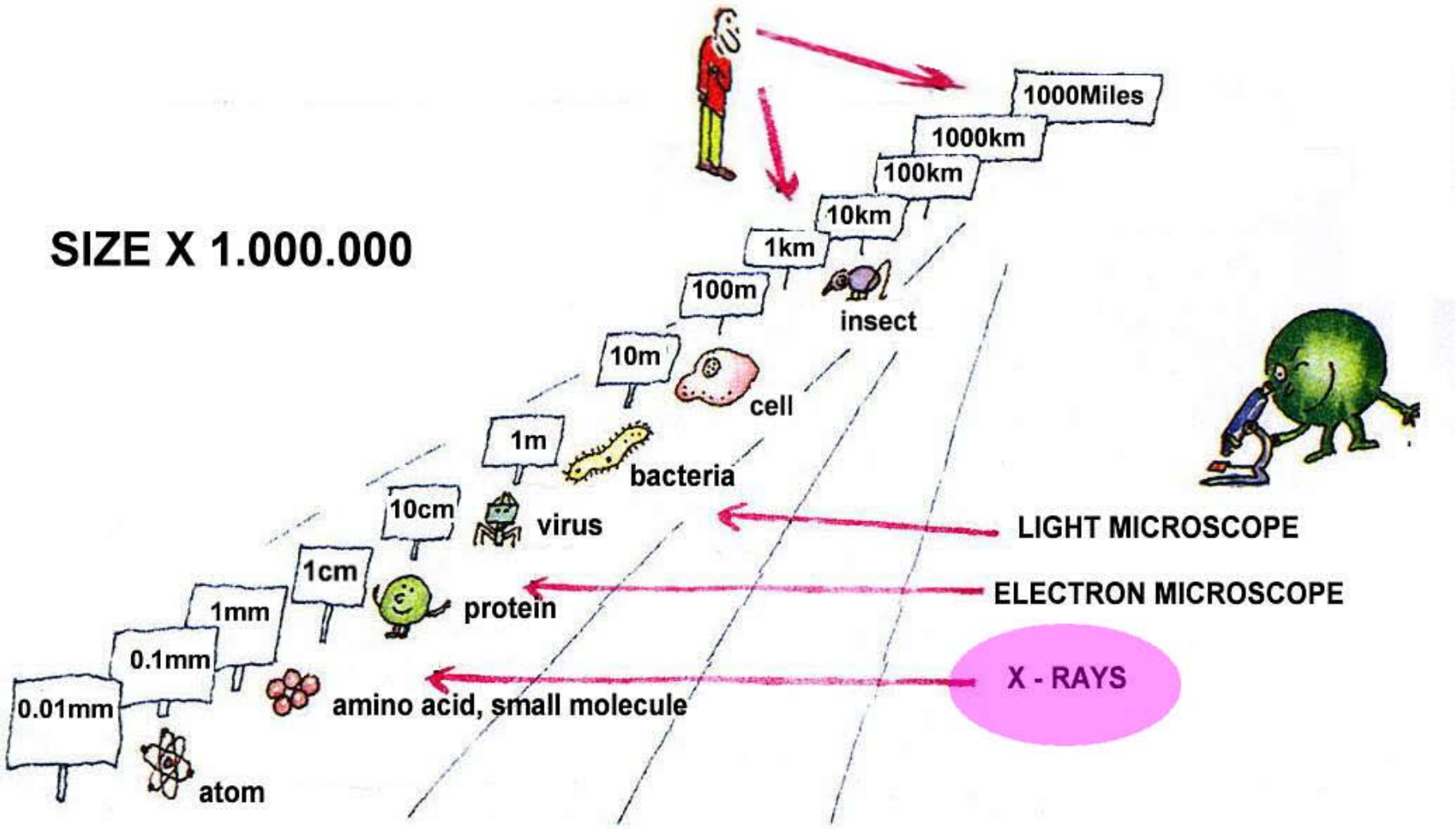
Ada Yonath

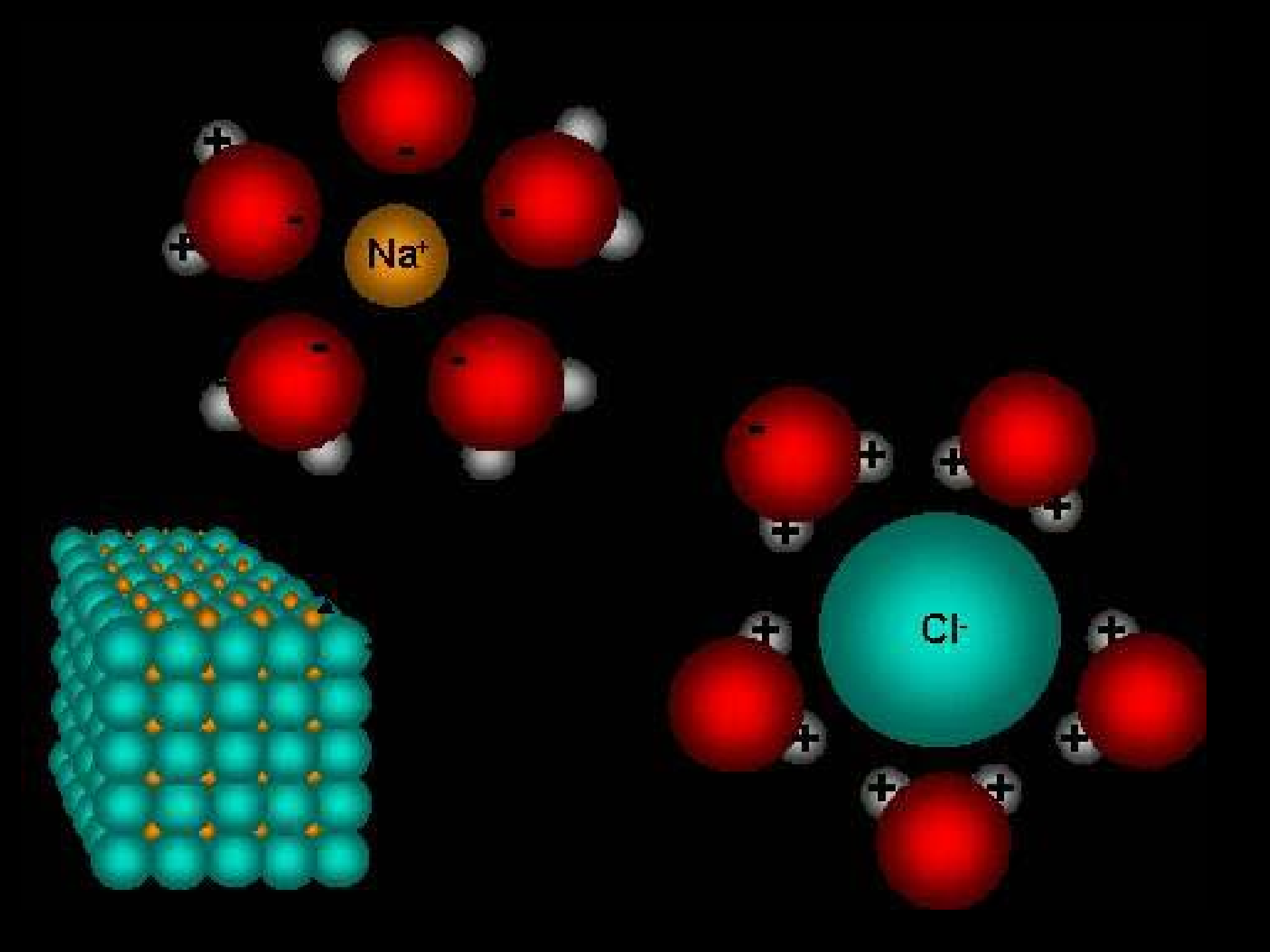
Weizmann Institute, Israel

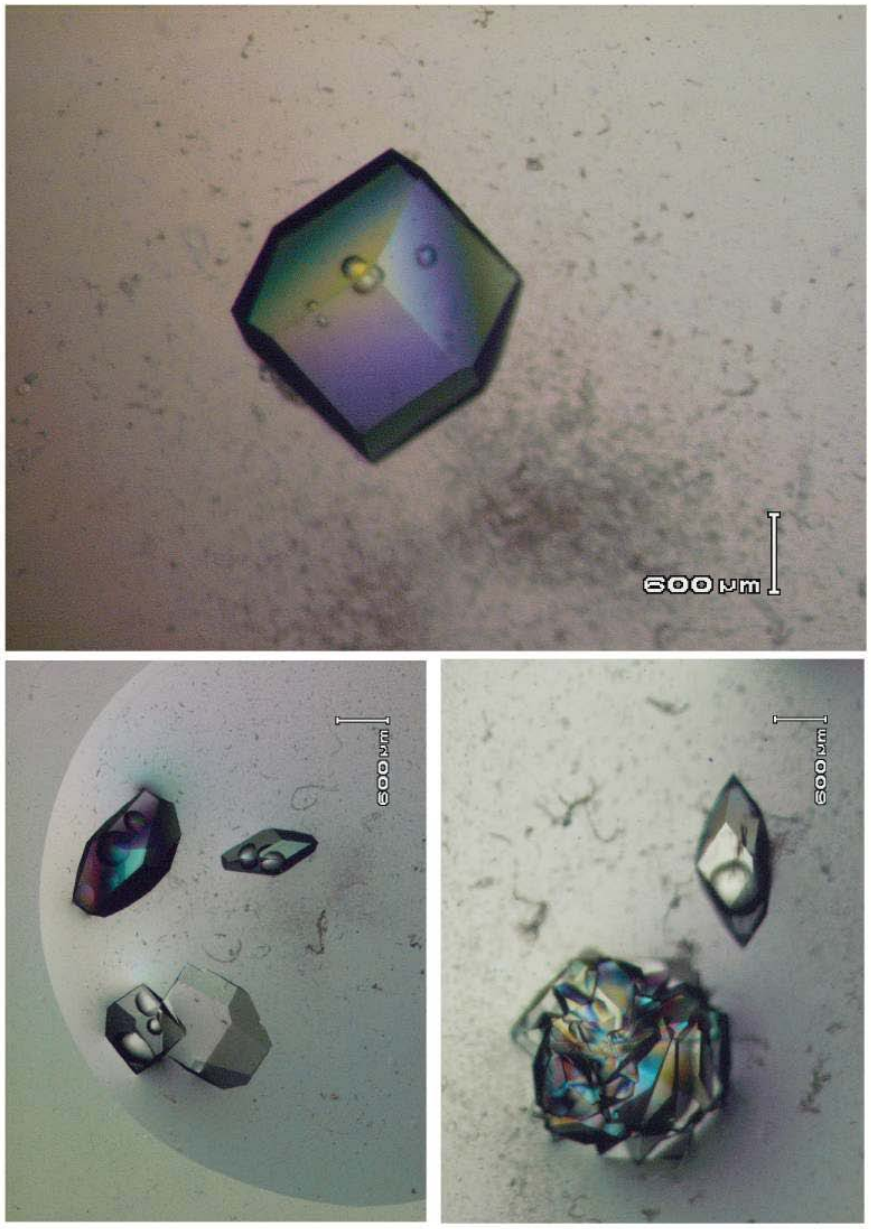


The Ribosome

Why X-ray crystallography?




Na+
Cl-

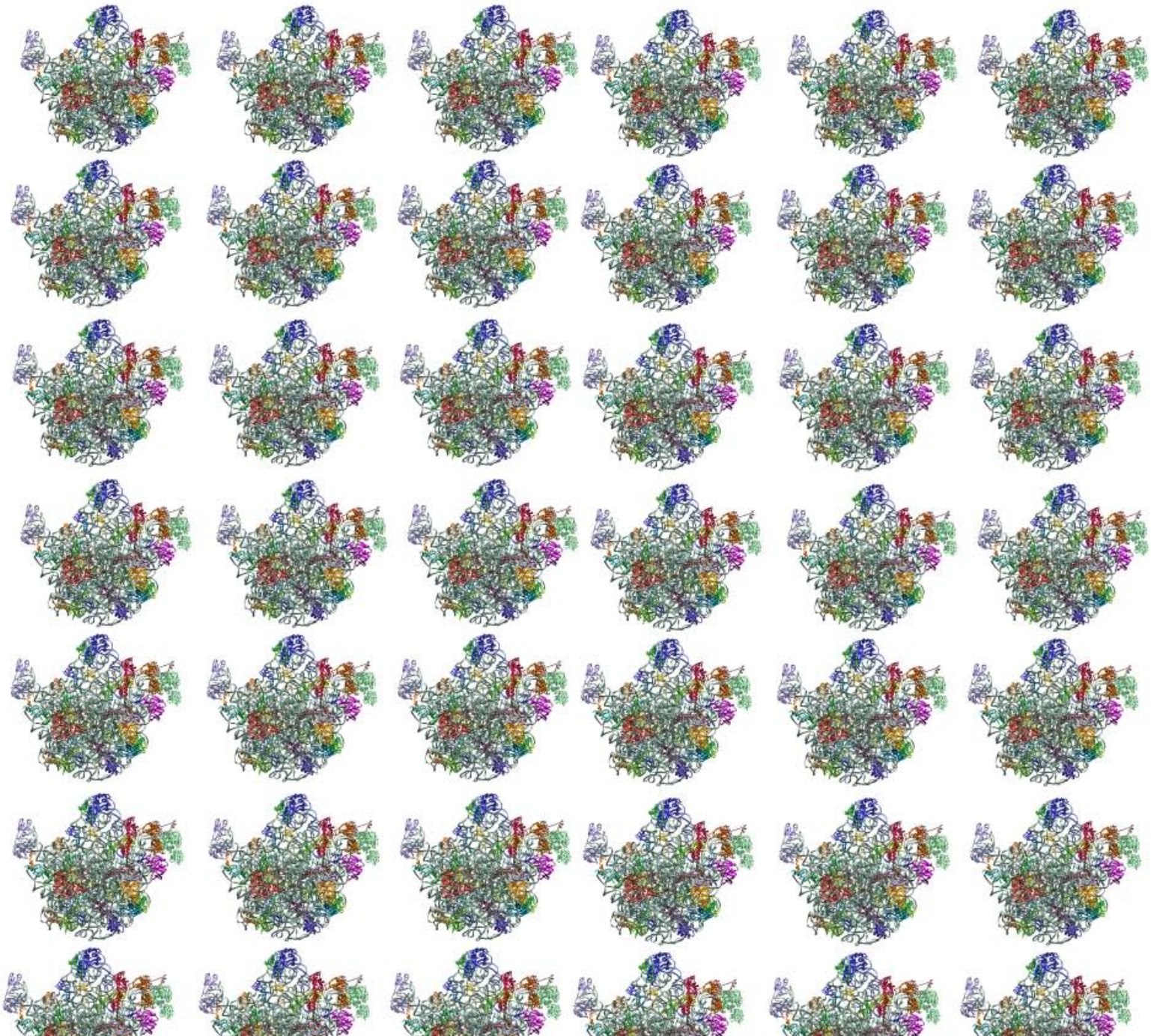


Crystals of lysozyme

Grown by Noa Yonath Comarov

6th grade

Dec 2007



Lessons from POLAR BEARS

Hibernating polar bears pack their ribosomes orderly just before their winter sleep, indicating that:

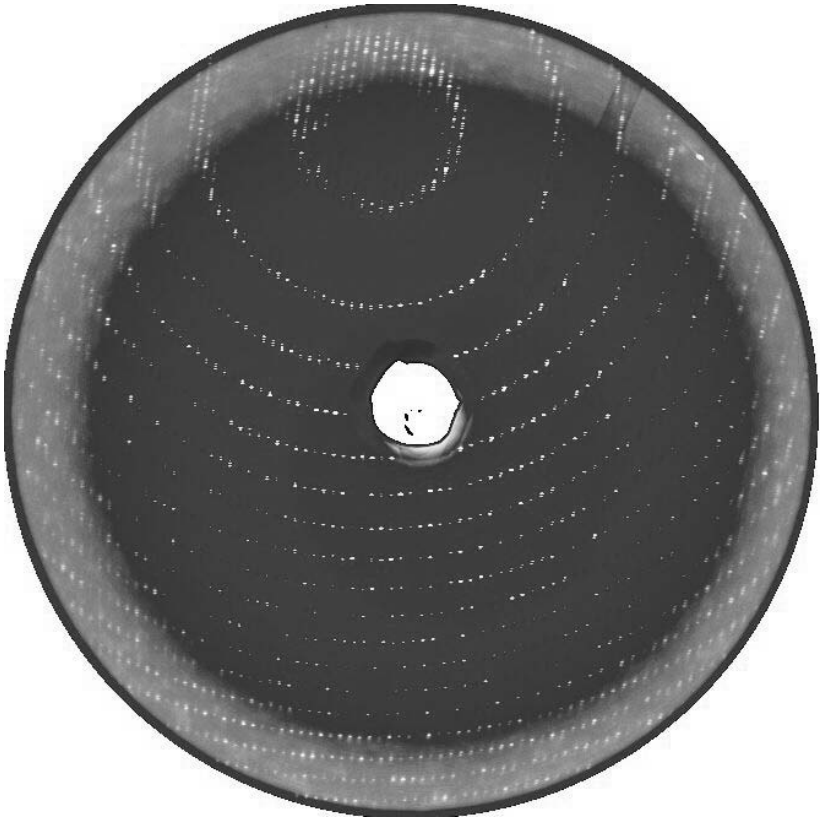
Ribosomes can be orderly packed

While densely packed, ribosomes can maintain their integrity and functional activity for months, despite their natural tendency to deteriorate within days


The Dead Sea


Haloarcula marismortui

Haloarcula marismortui

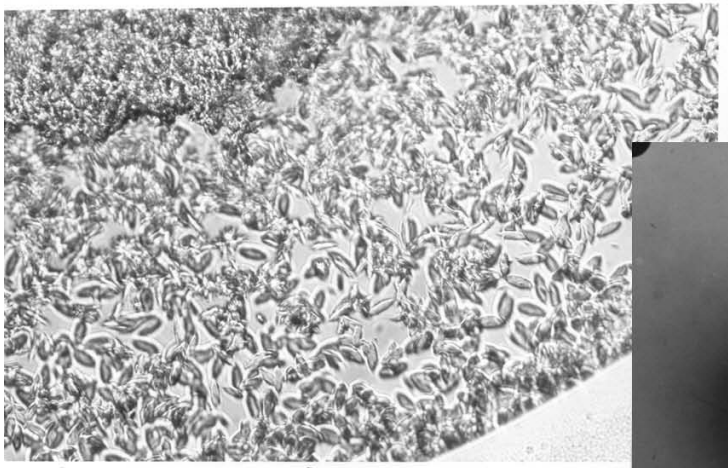


Aimed

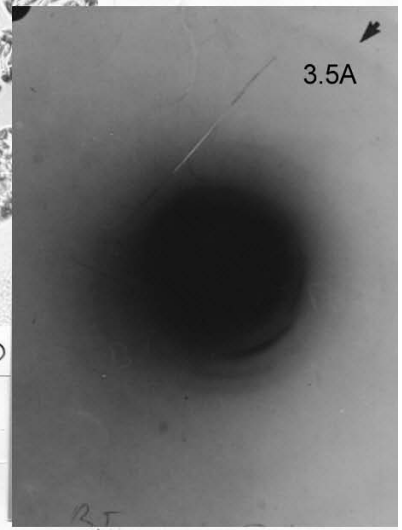
The first ribosomal (B50S) microcrystals

and their powder diffraction pattern

1980



Exp. 30 B50 Tohen pH 6,2 ⊖

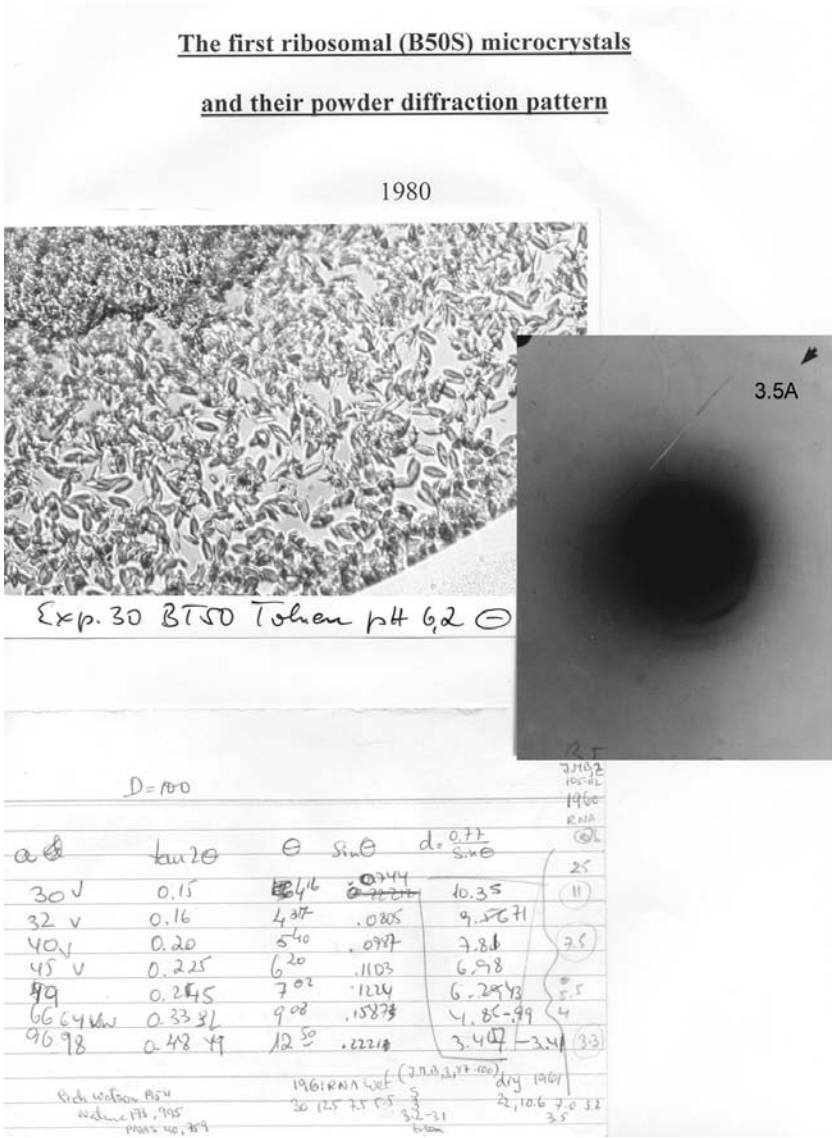


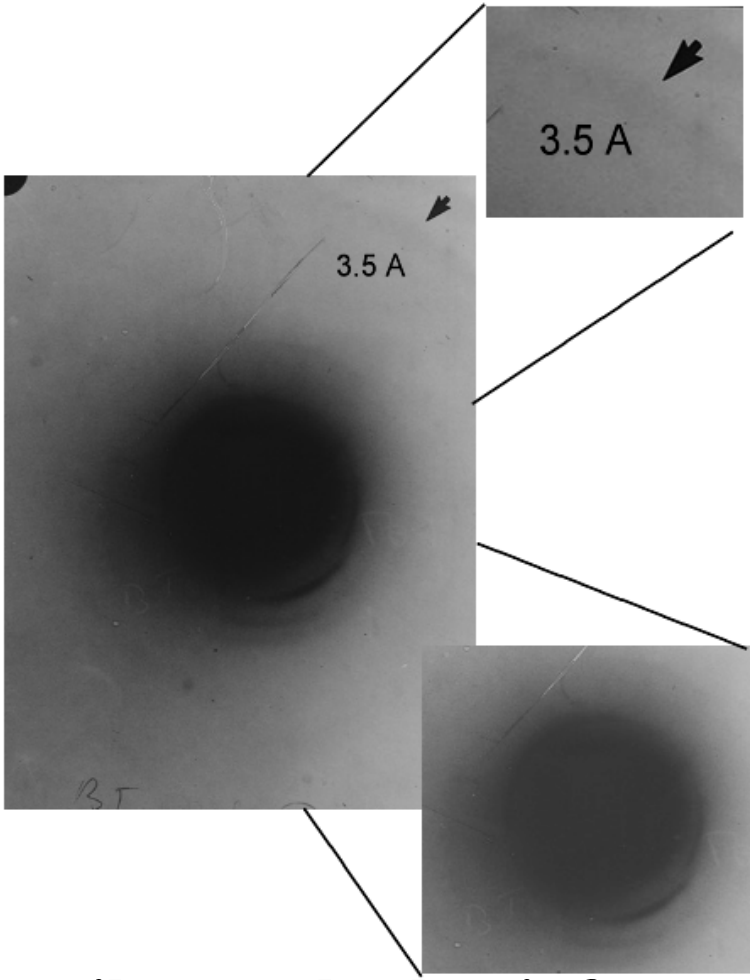
D=100

a	tau2θ	θ	Sinθ	d = 0.77/Sinθ
30 V	0.15	4.16	.0744	10.35
32 v	0.16	4.37	.0805	9.5671
40 V	0.20	5.40	.0987	7.86
45 V	0.225	6.20	.1103	6.98
99	0.245	7.02	.1224	6.2943
66 64 VW	0.333	9.08	.1587	4.85-.99
96 98	0.48 99	12.50	.2221	3.407 — 3.41

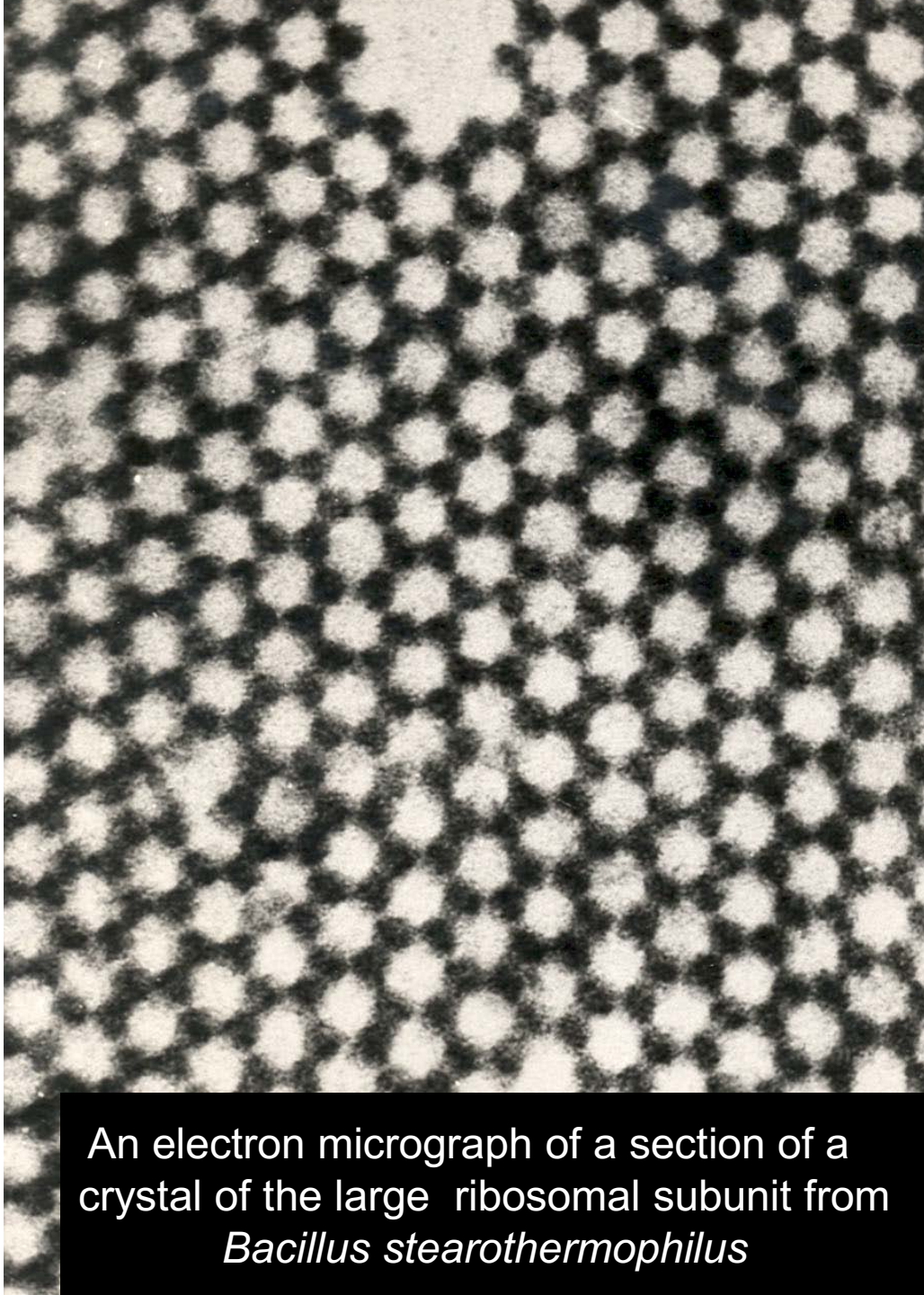
Rich Watson PNAS
Nature 173, 995
PNAS 40, 759

1961 RNA wet (J.M.B. 3, 87 (1961)) dry 1961
30 12.5 7.5 5.5 3.2-3.1 22, 10.6 7.0 5.2 3.5





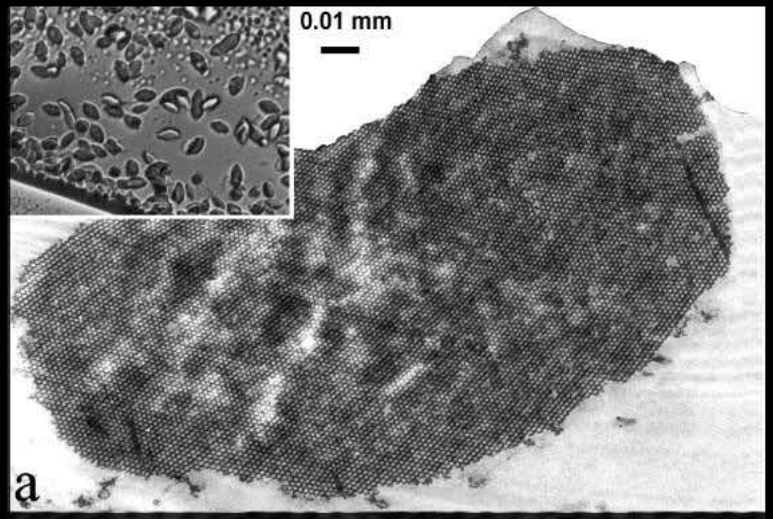
1980 - the first microcrystals of the large ribosomal subunit from *Bacillus stearothermophilus*

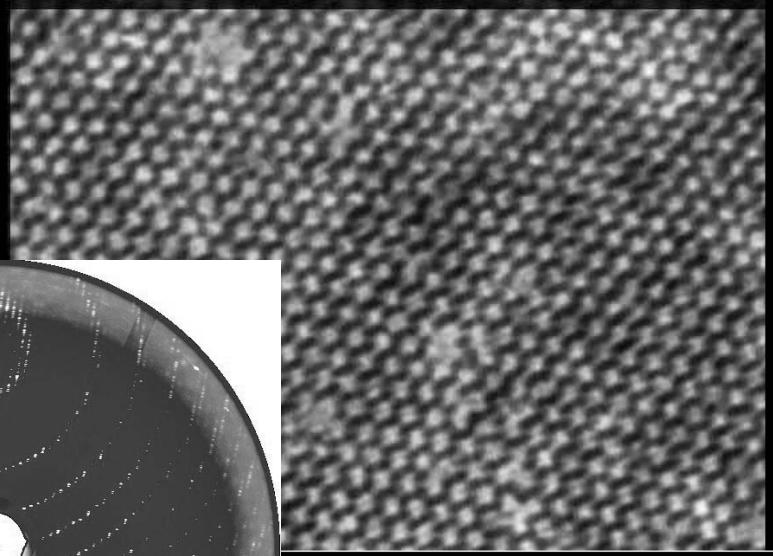


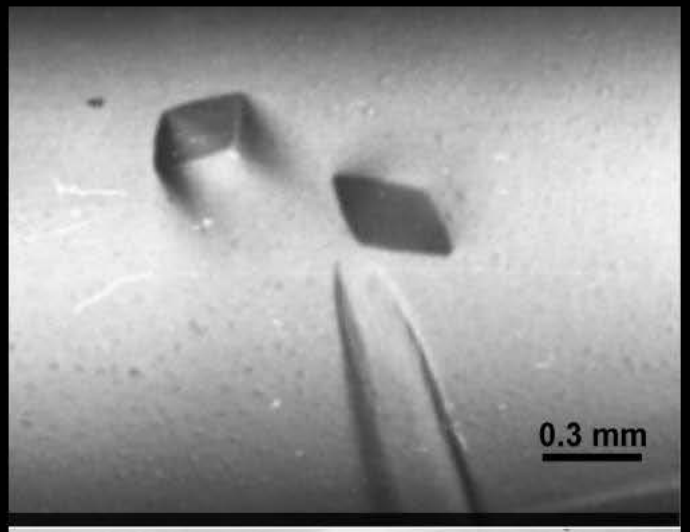
An electron micrograph of a section of a crystal of the large ribosomal subunit from *Bacillus stearothermophilus*

"Powder" diffraction to 3.5A Spacings match solution scattering.

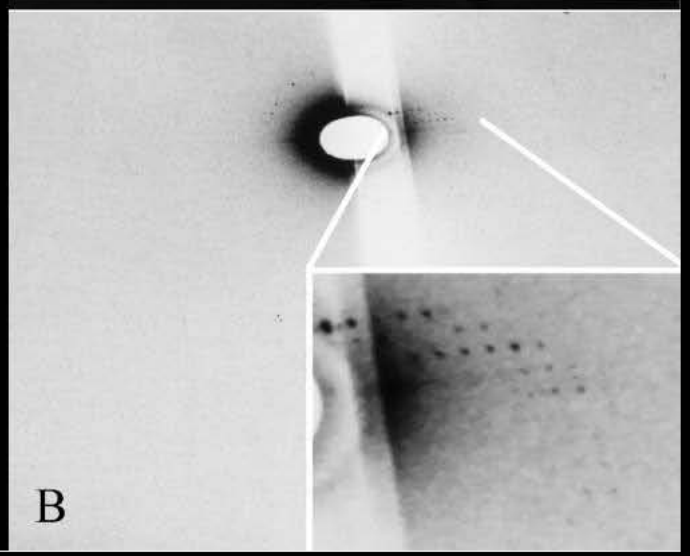
1980



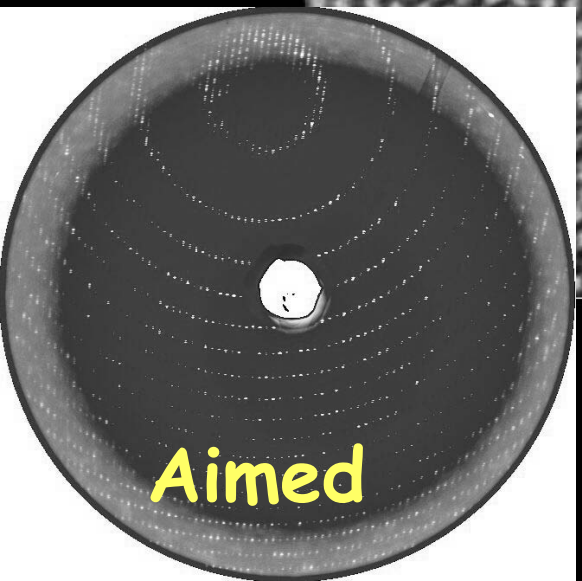


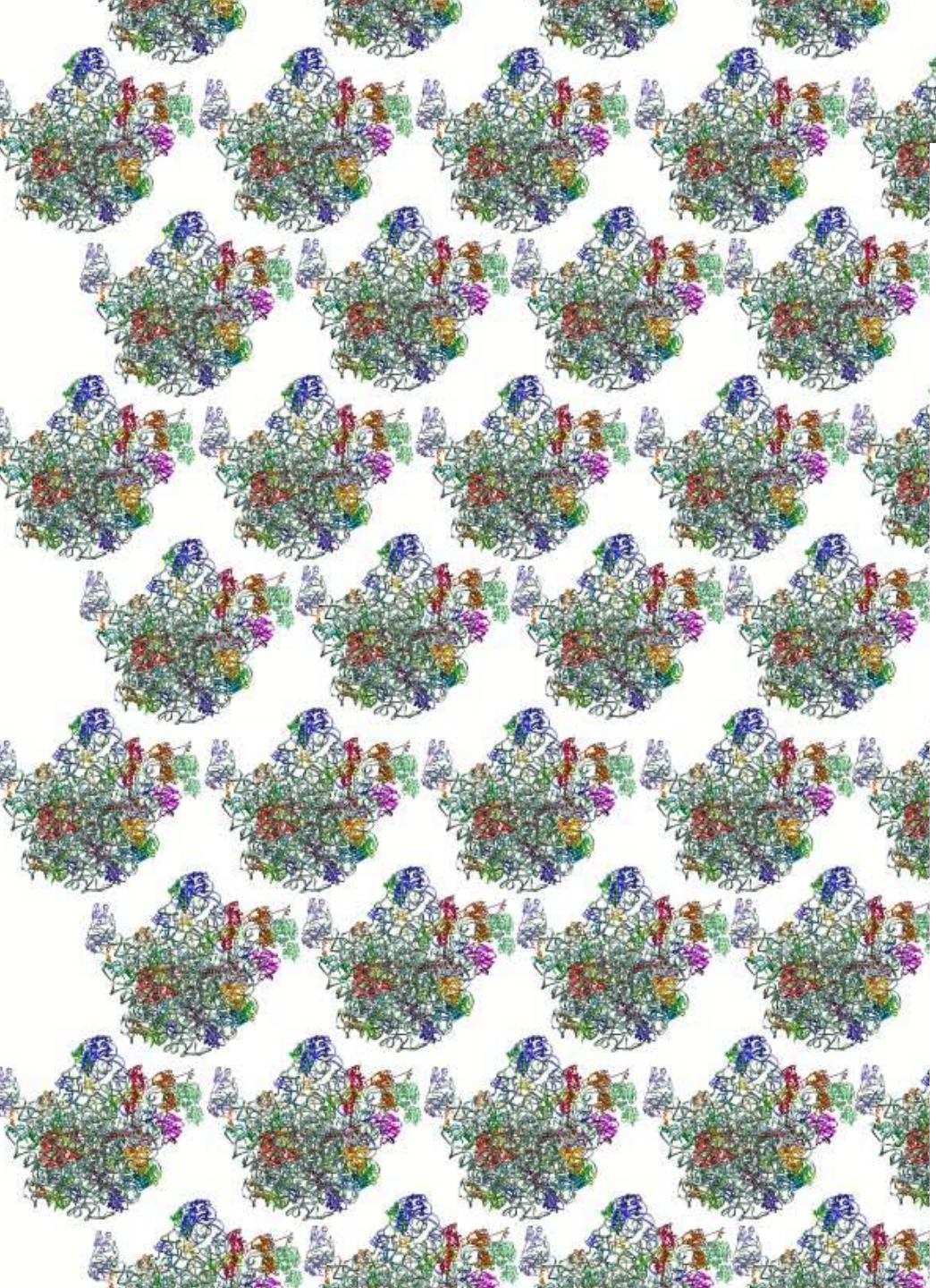


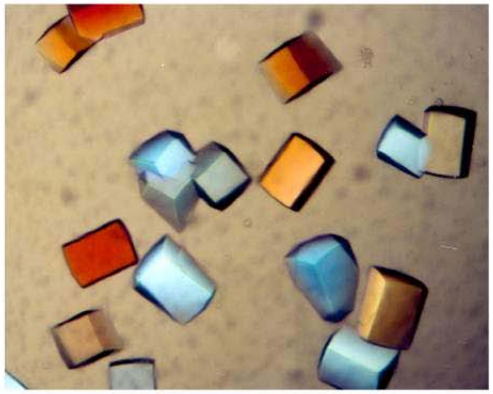
1984

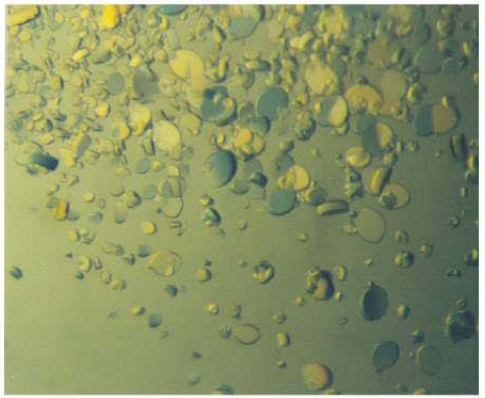


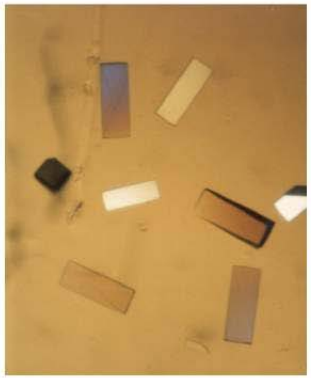
Publish in J. Mol. Bio

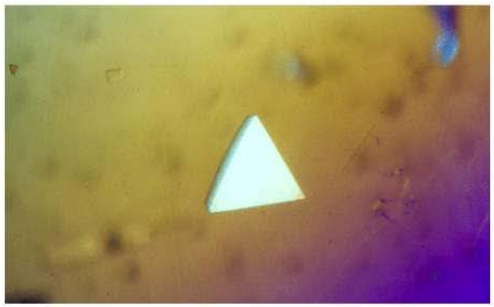


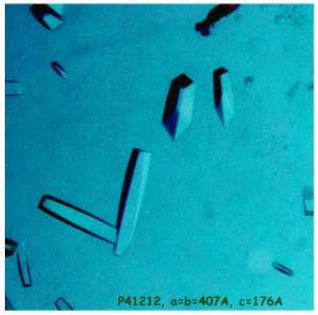




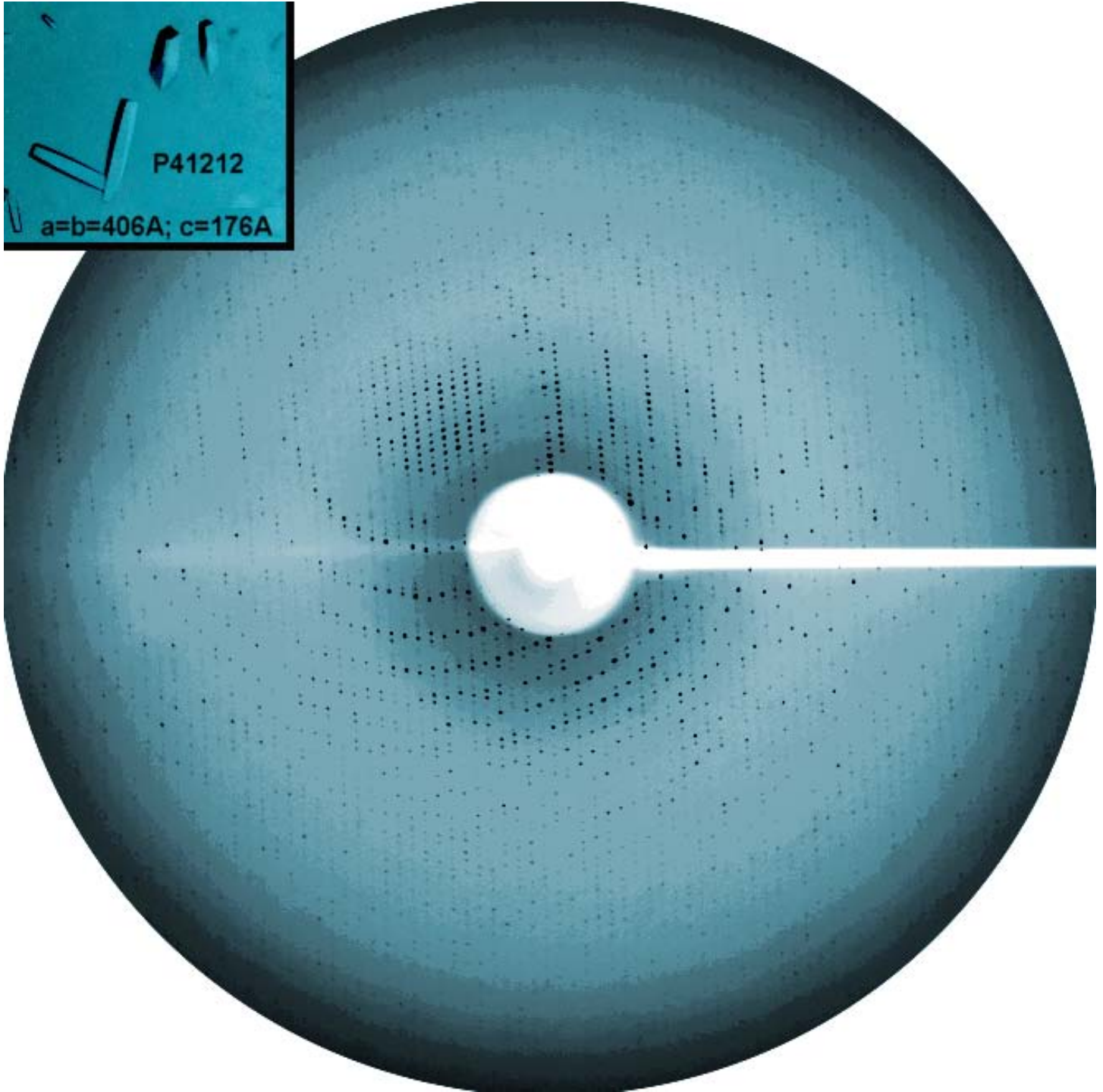








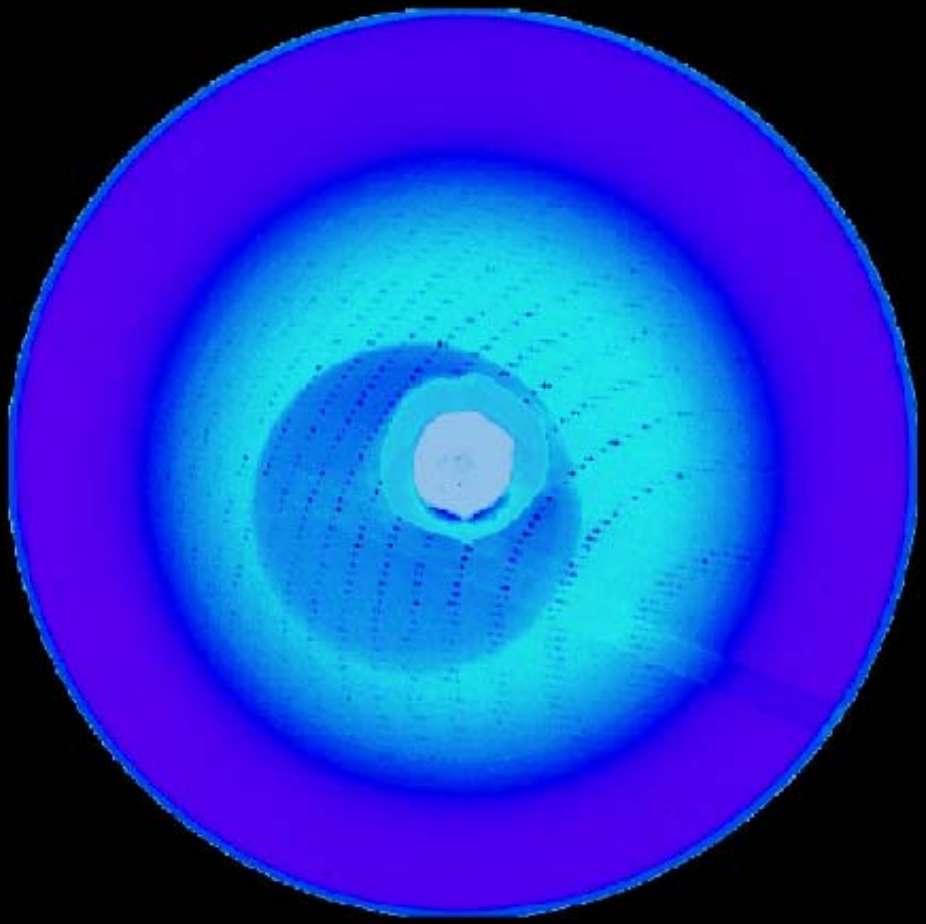
Crystals of ribosomes

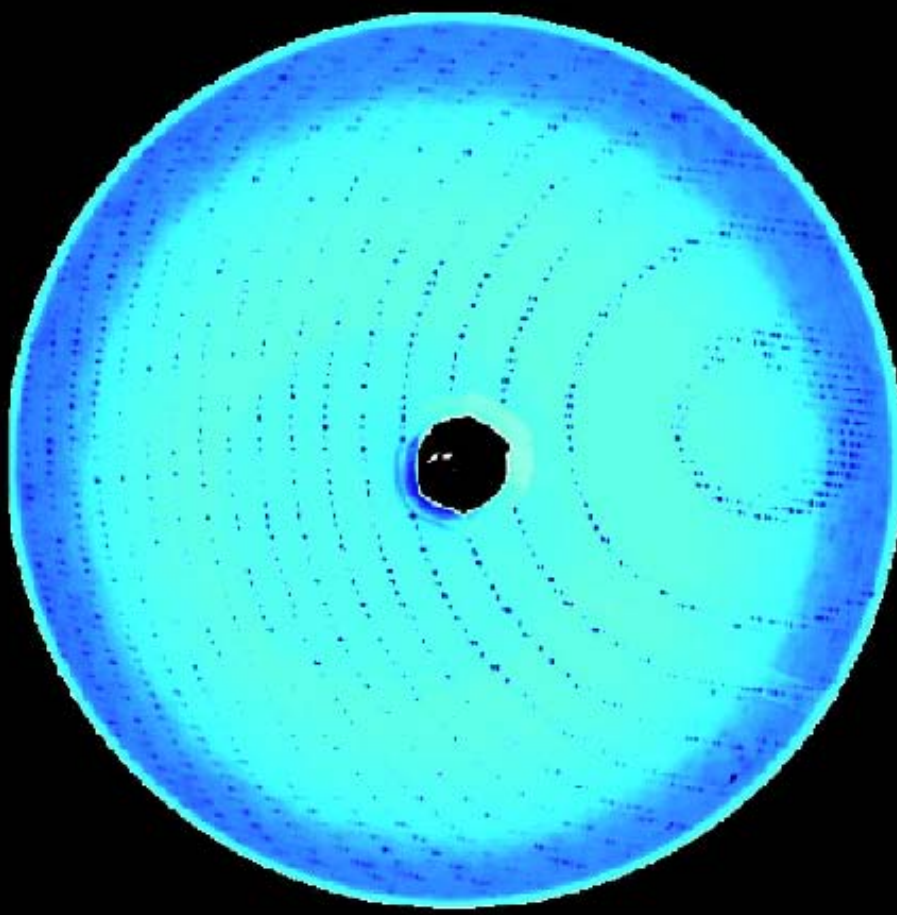

P41212
a=b=406A; c=176A

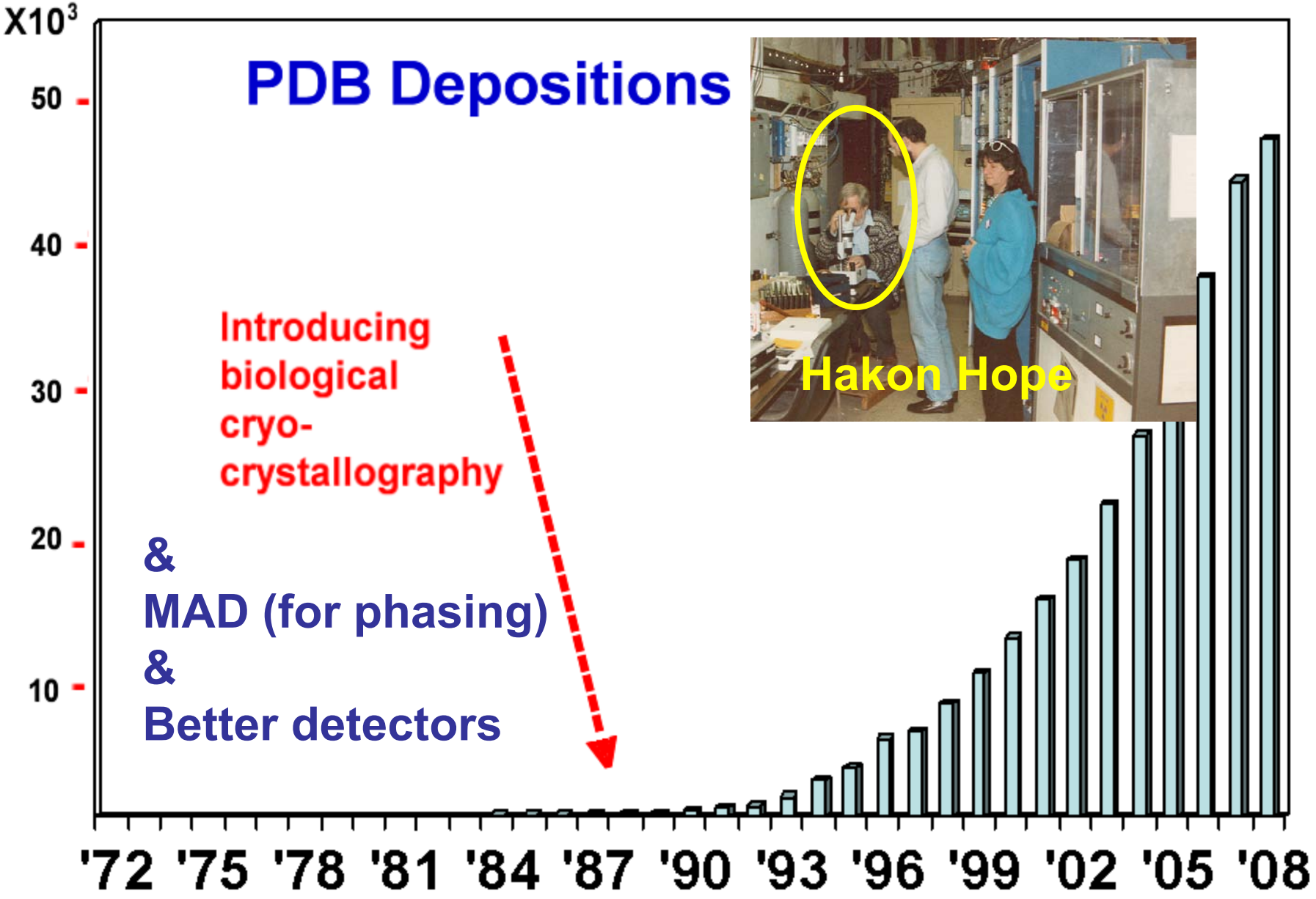
ESRF synchrotron in Grenoble

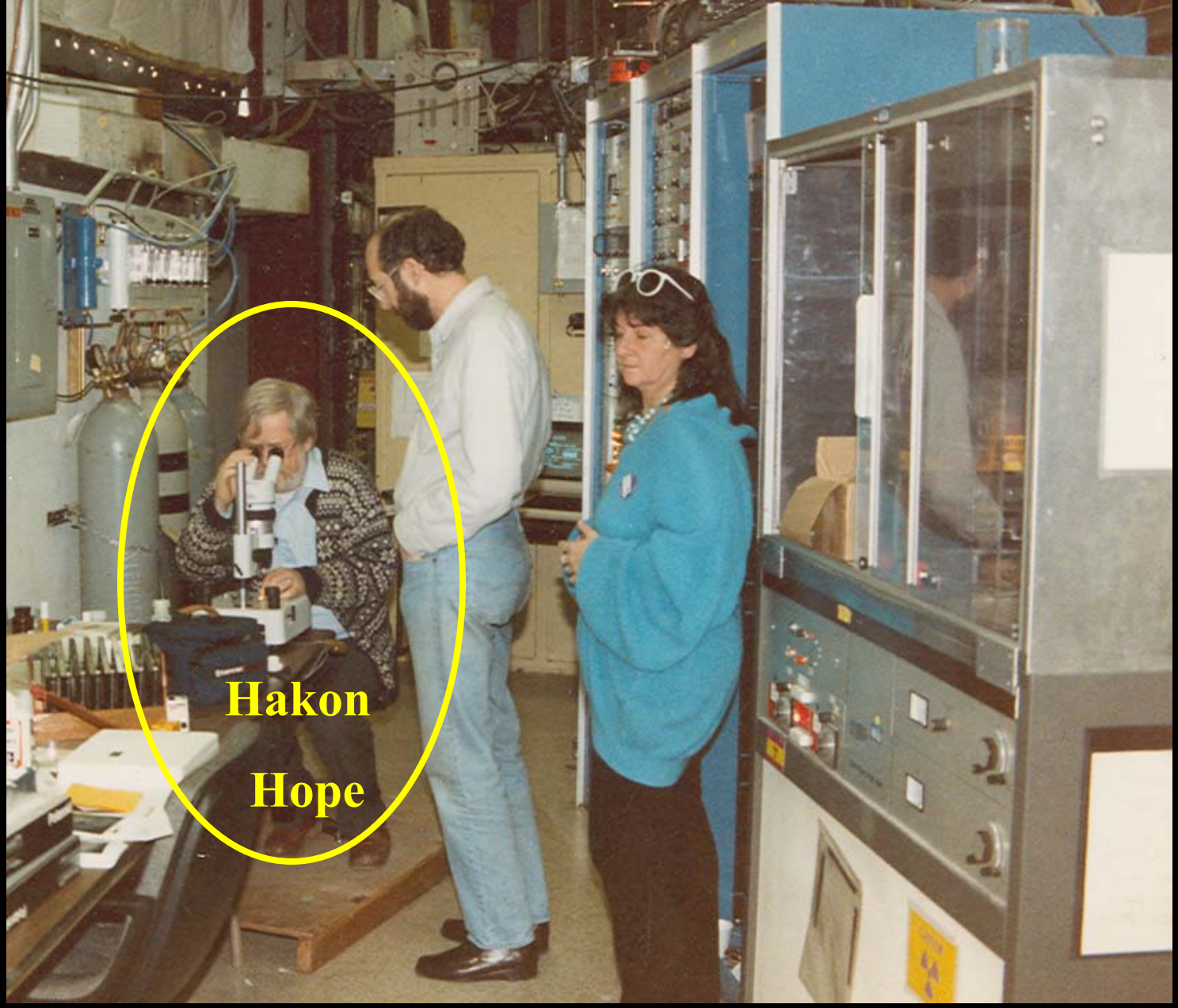
Crystal decay by irradiation

after 0.1 seconds at -10 deg C






X10^3
50
40
30
20
10
PDB Depositions
Introducing
biological
cryo-
crystallography
&
MAD (for phasing)
&
Better detectors
Hakon Hope
'72 '75 '78 '81 '84 '87 '90 '93 '96 '99 '02 '05 '08



The first cryo bio crystallography experiment, SSRL, Stanford 1986

The way to structure determination was long and demanding, we frequently felt as is we are climbing high mountains, just for discovering that a higher Everest is still in front of us

We hardly felt like Archimedes discovering the "bath principle" and rushing out shouting "Eureka! Eureka! (I've found it! I've found it!)"

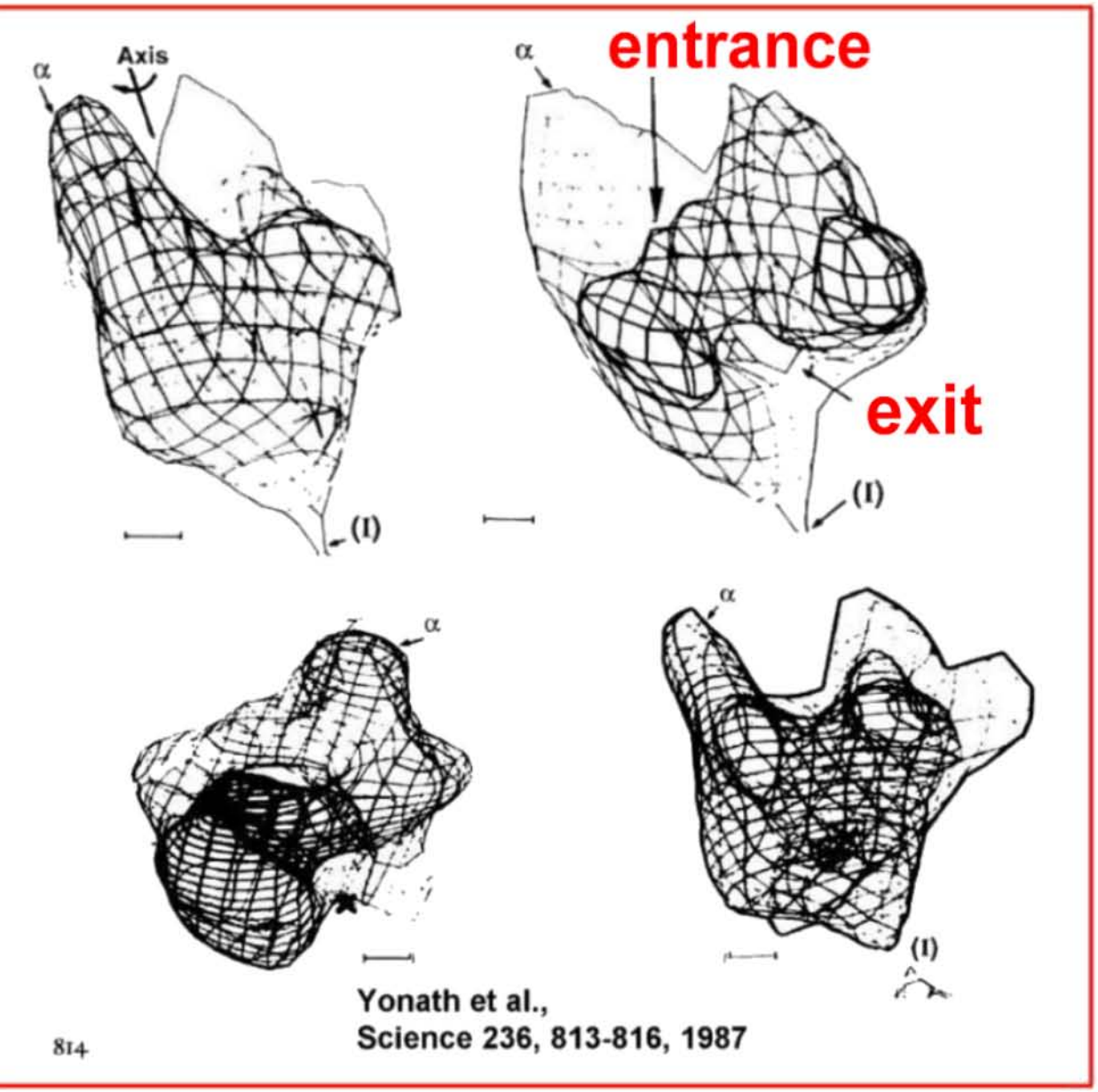
Introducing cryo bio crystallography and discovering that crystals can acquire almost eternal life, was one of these rare moments

NUMBER OF EXPOSED CRYSTALS

		UNTIL CONDITIONS FOR HIGH RESOLUTION WERE FOUND		AFTER CONDITIONS FOR HIGH RESOLUTION WERE FOUND
HALOARCULA MARISMORTUI	50S	>2500	Pioneering CRYO - bio-crystallography	>2500
THERMUS THERMOPHILUS	30S	>1300	Crystal Stabilization	>1200
Deinococcus Radiodurans	50S	75	Freezing conditions	170

Ribosome in Action

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



Using three dimensional image reconstruction of tilt series of two-dimensioal sheets we* found that the PTC is situated above the entrance to an internal tunnel.

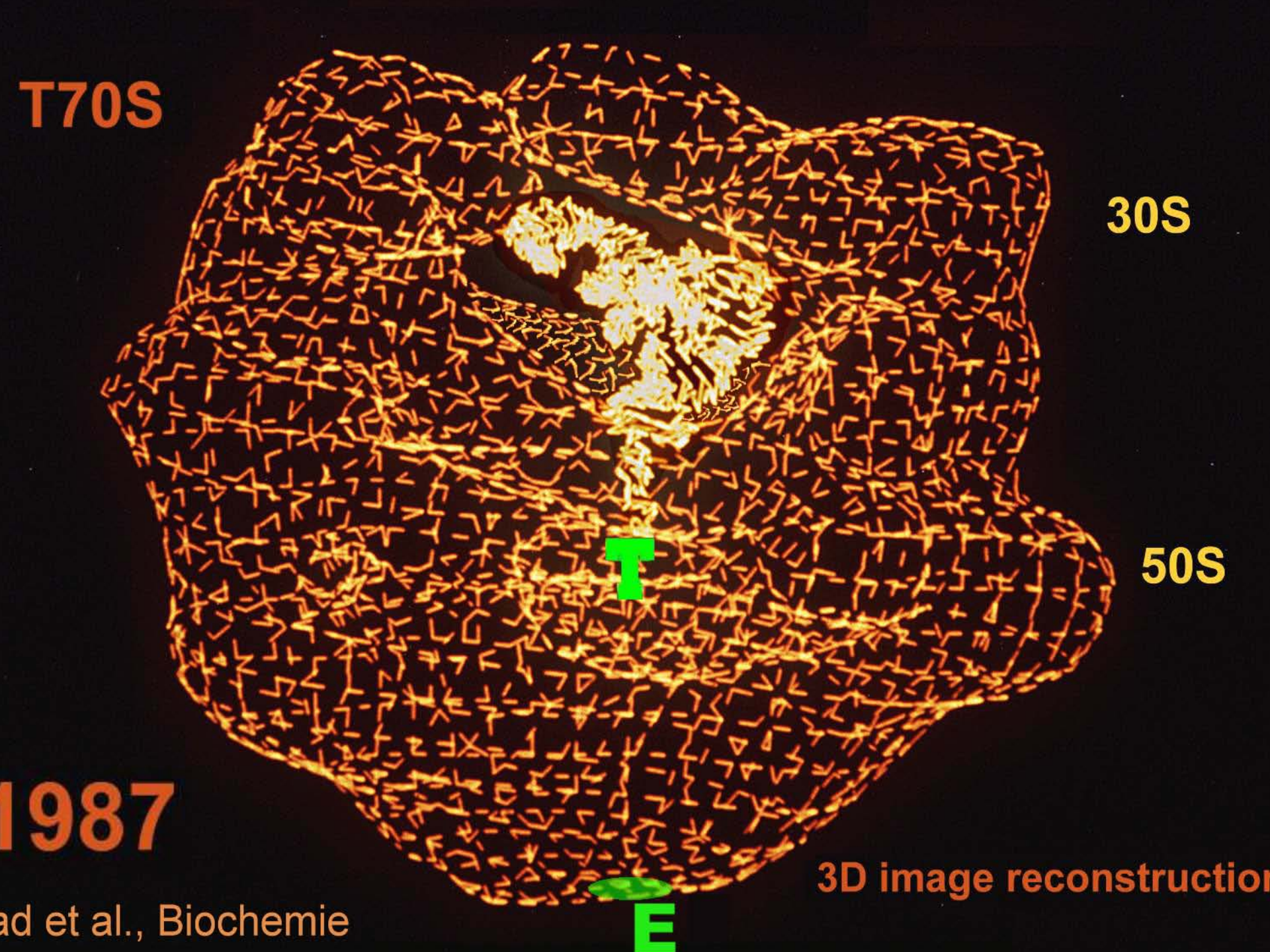
Based on previous biochemical studies,

Malkin & Rich, 1967

Sabatini & Blobel, 1970

we suggeted that this is the nascent protein exit tunnel.

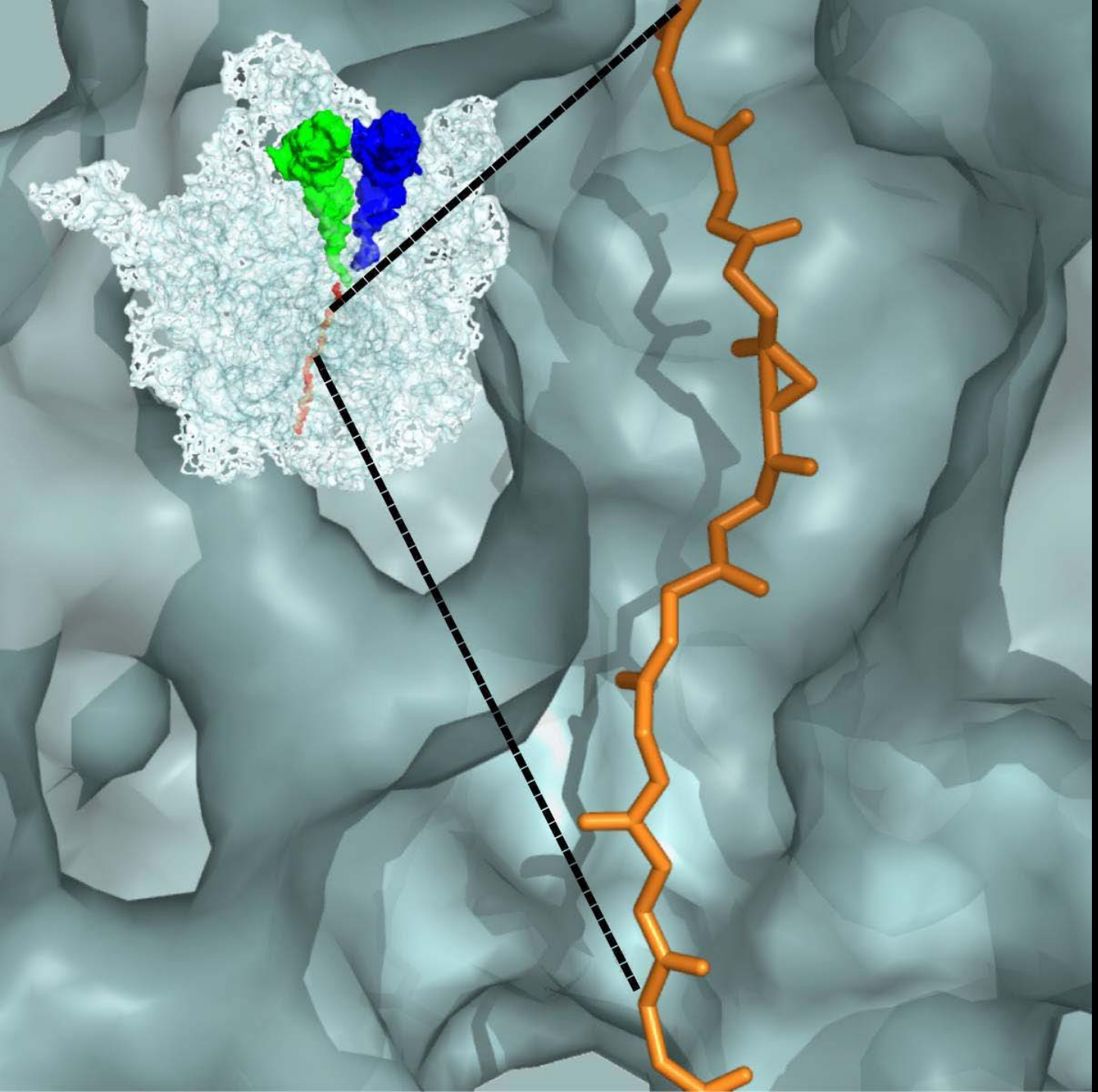
* (Milligan and Unwin, 1986; Yonath et al., 1987).


T70S
30S
50S
T
E
1987
3D image reconstructio
ad et al., Biochemie

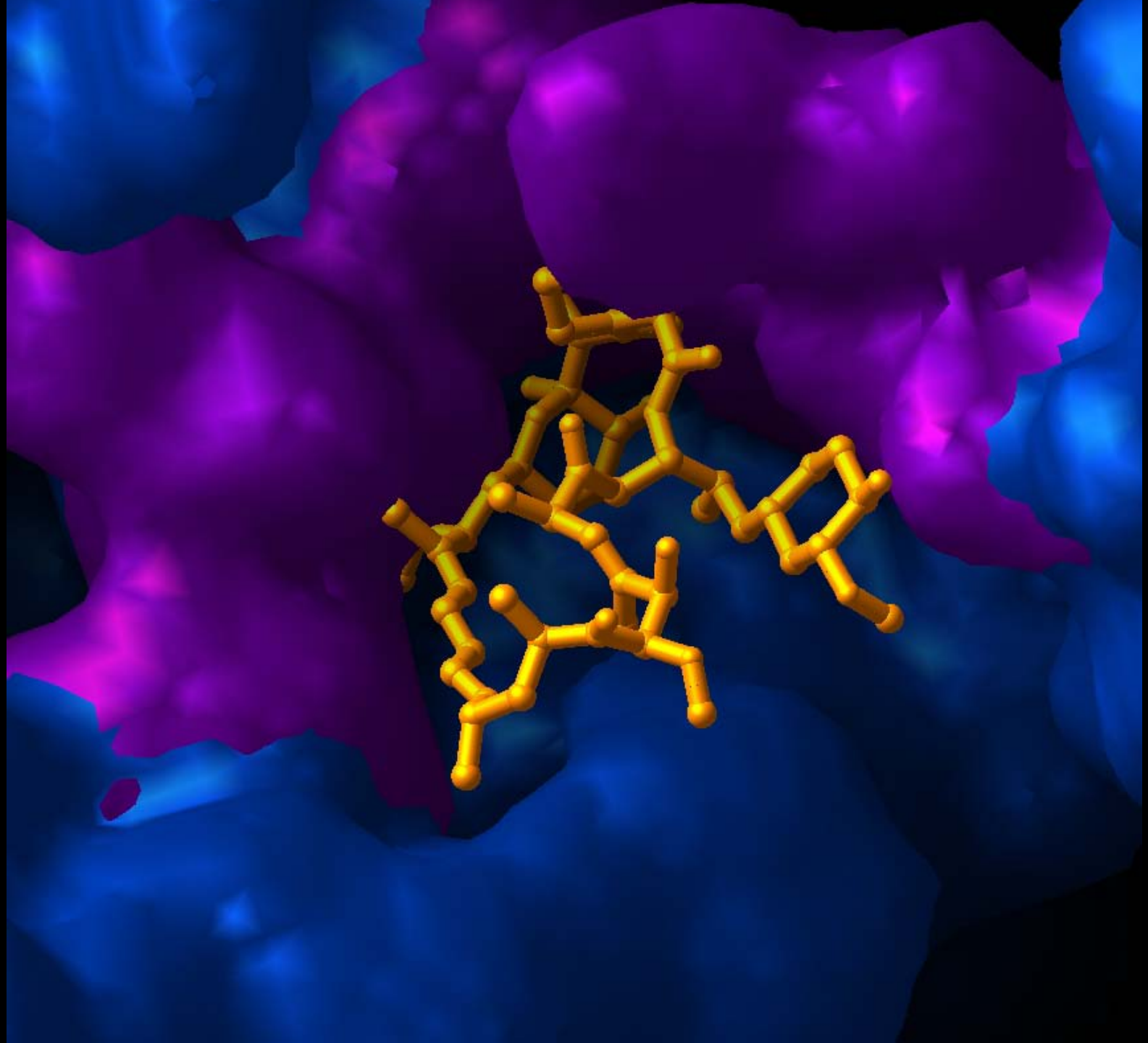
The ribosome returns

Peter B. Moore

In the longer term, however, the need for high resolution information about ribosome structure is clear. The progress made by Yonath and collaborators in their crystallographic investigations in the past year is encouraging in this respect. They recently proposed a startling model for the 50S ribosomal subunit in which they postulate a large channel through the centre of the particle[30]. This model was based on a three-dimensional reconstruction from electron micrographs of crystalline sheets of ribosomal subunits from *Halobacterium marismortui*, a bacterium that lives only in saturated, or near-saturated salt solutions. It would be interesting to know whether this radical model is consistent with, for example, the solution-scattering data available for large subunits from *E. coli*. More significant than this model, however, is the fact that Yonath and co-



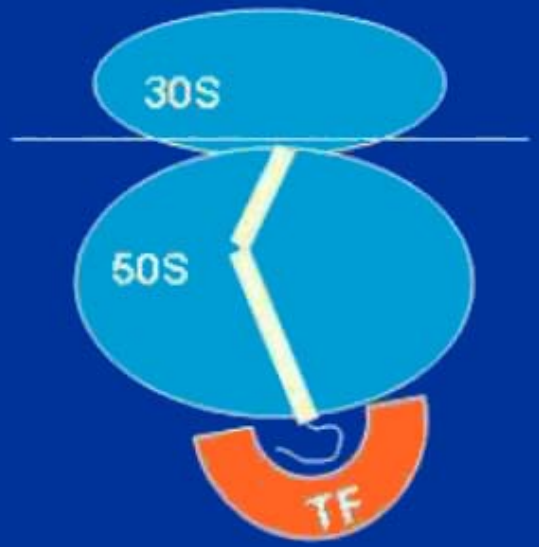
The nascent protein exit tunnel



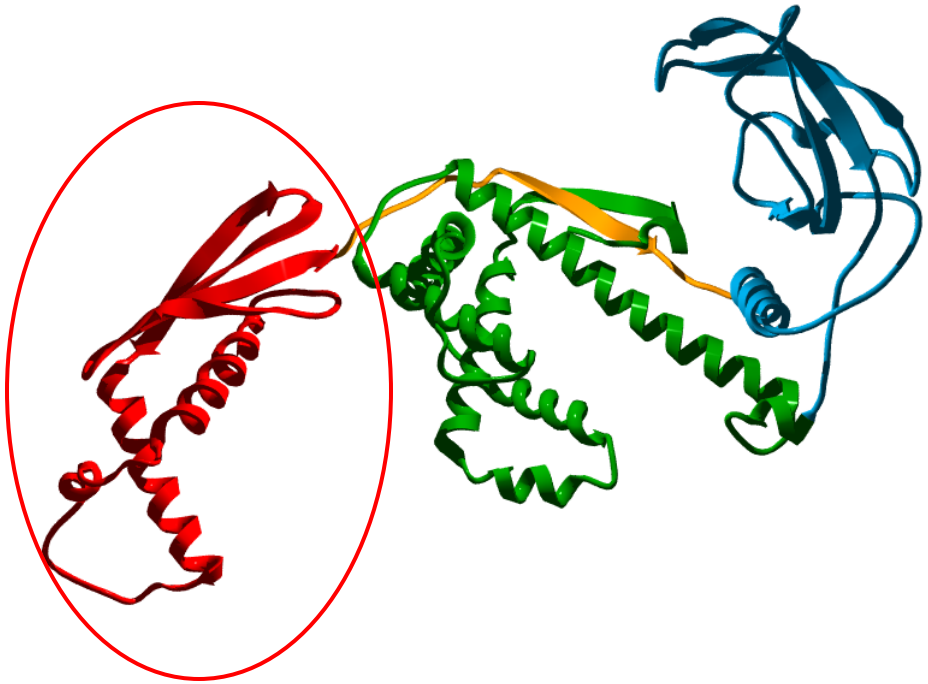
Trigger Factor

- Unique to eubacteria and chloroplasts
- First chaperone to encounter nascent chains
- Prevents aggregation of cytosolic proteins
- Acts co-translationaly
- More?

Trigger Factor



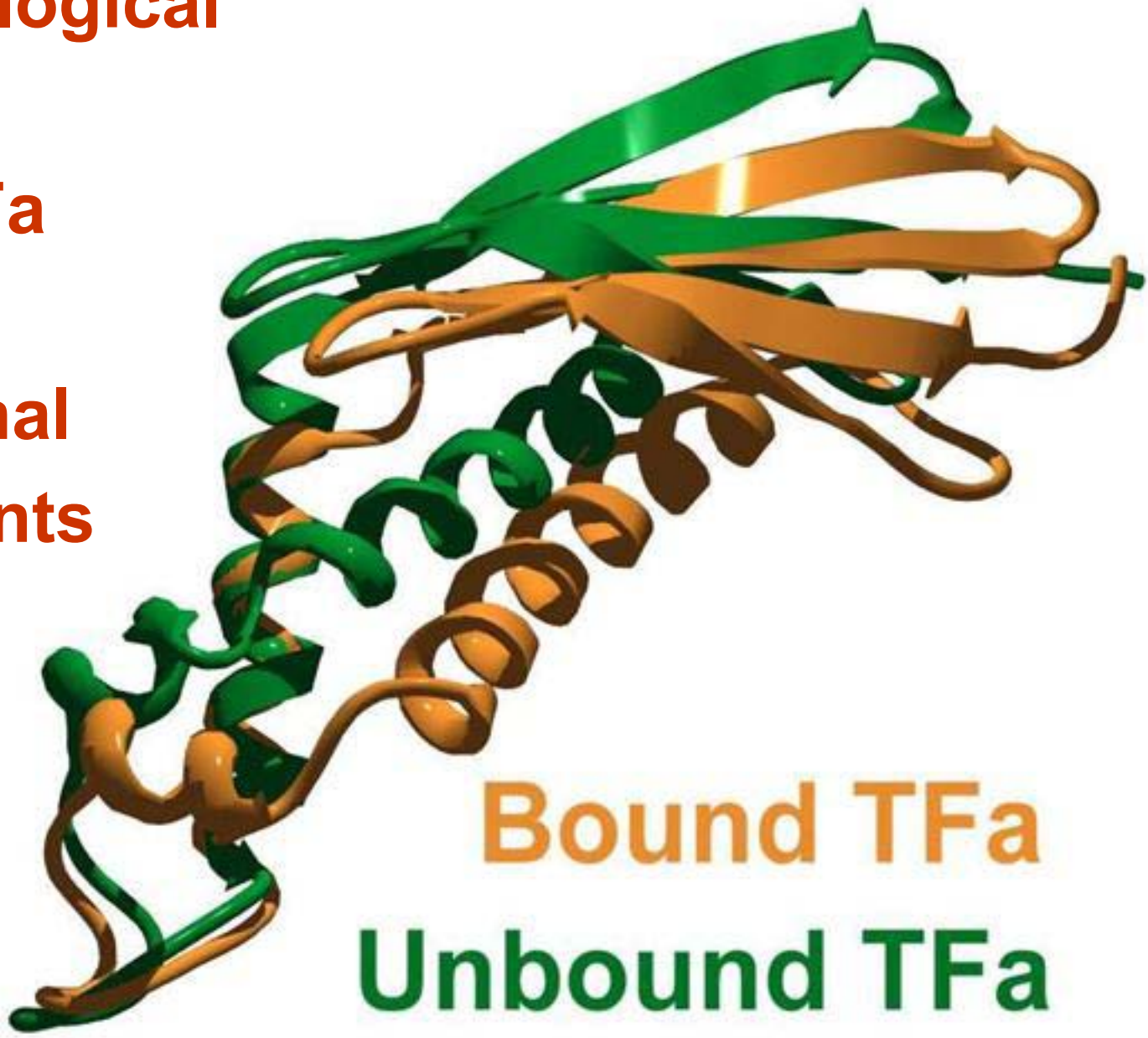
binds to ~ 90% of the translating ribosomes



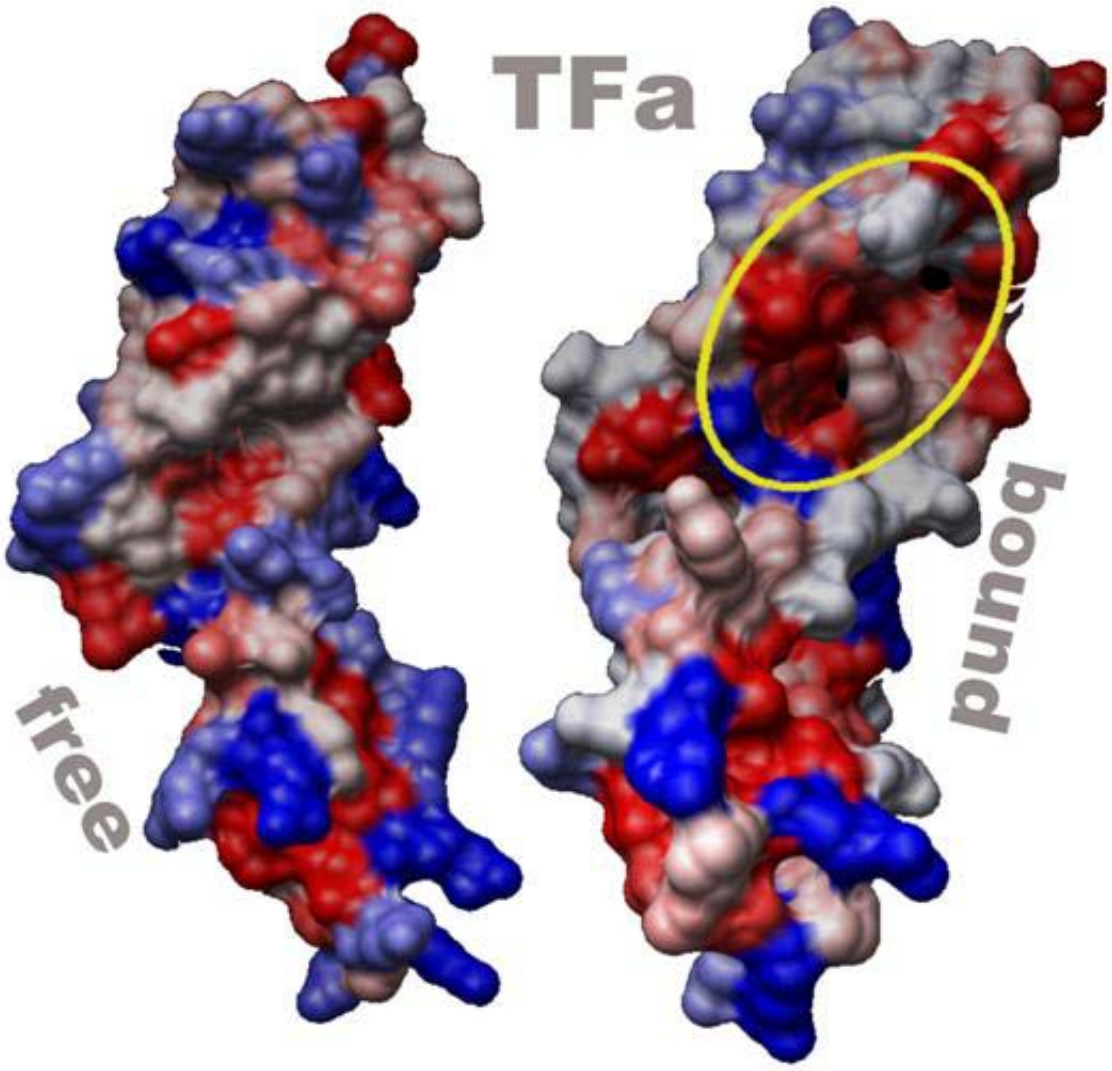
N-terminal (TFa)

Ferbitz et al. *Nature* 2004

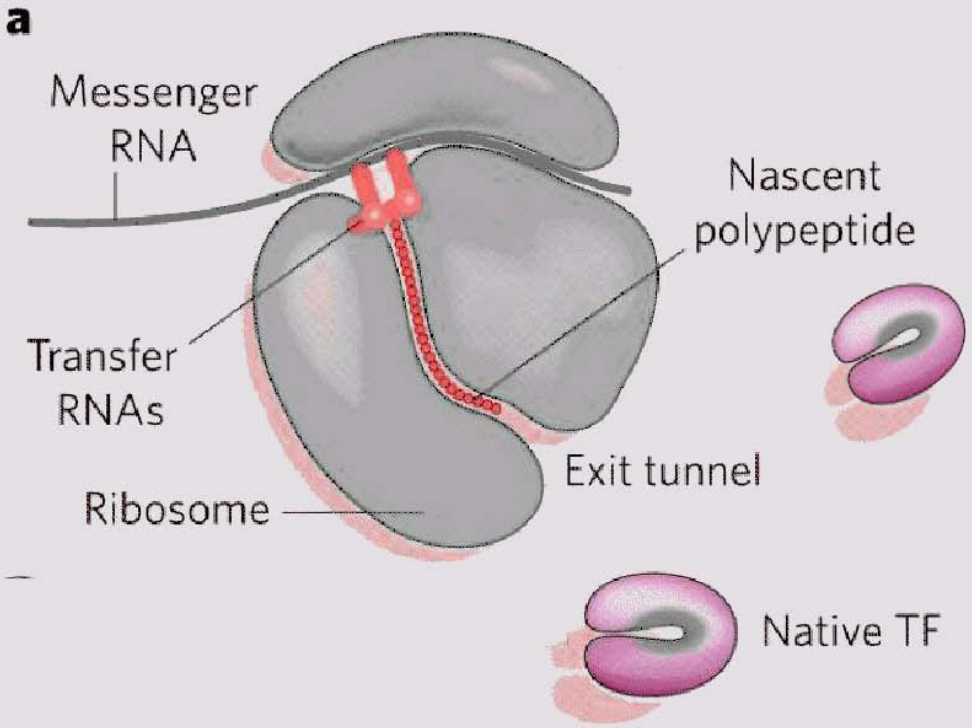
In the physiological complex the bound TFa undergoes conformational rearrangements

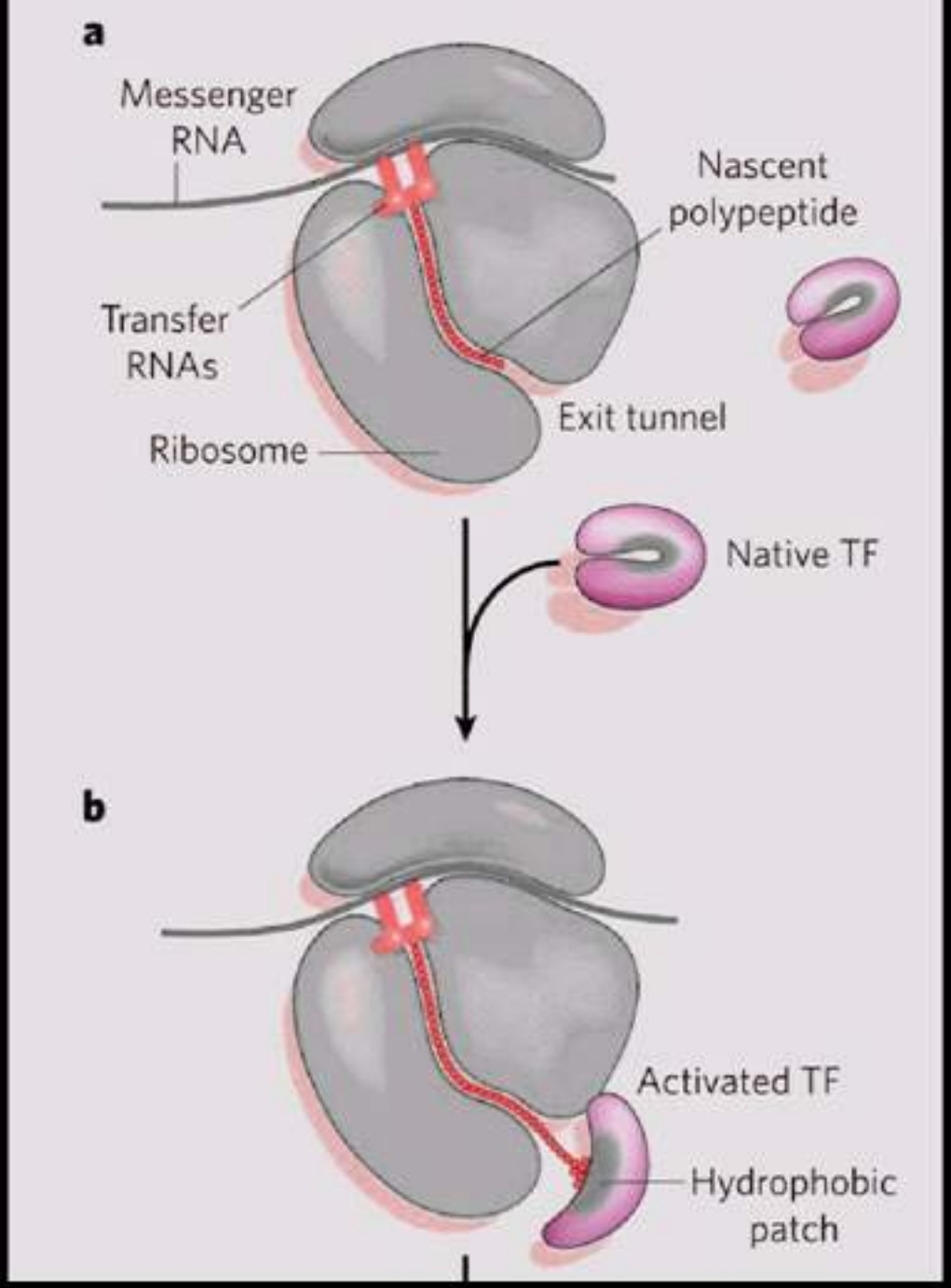


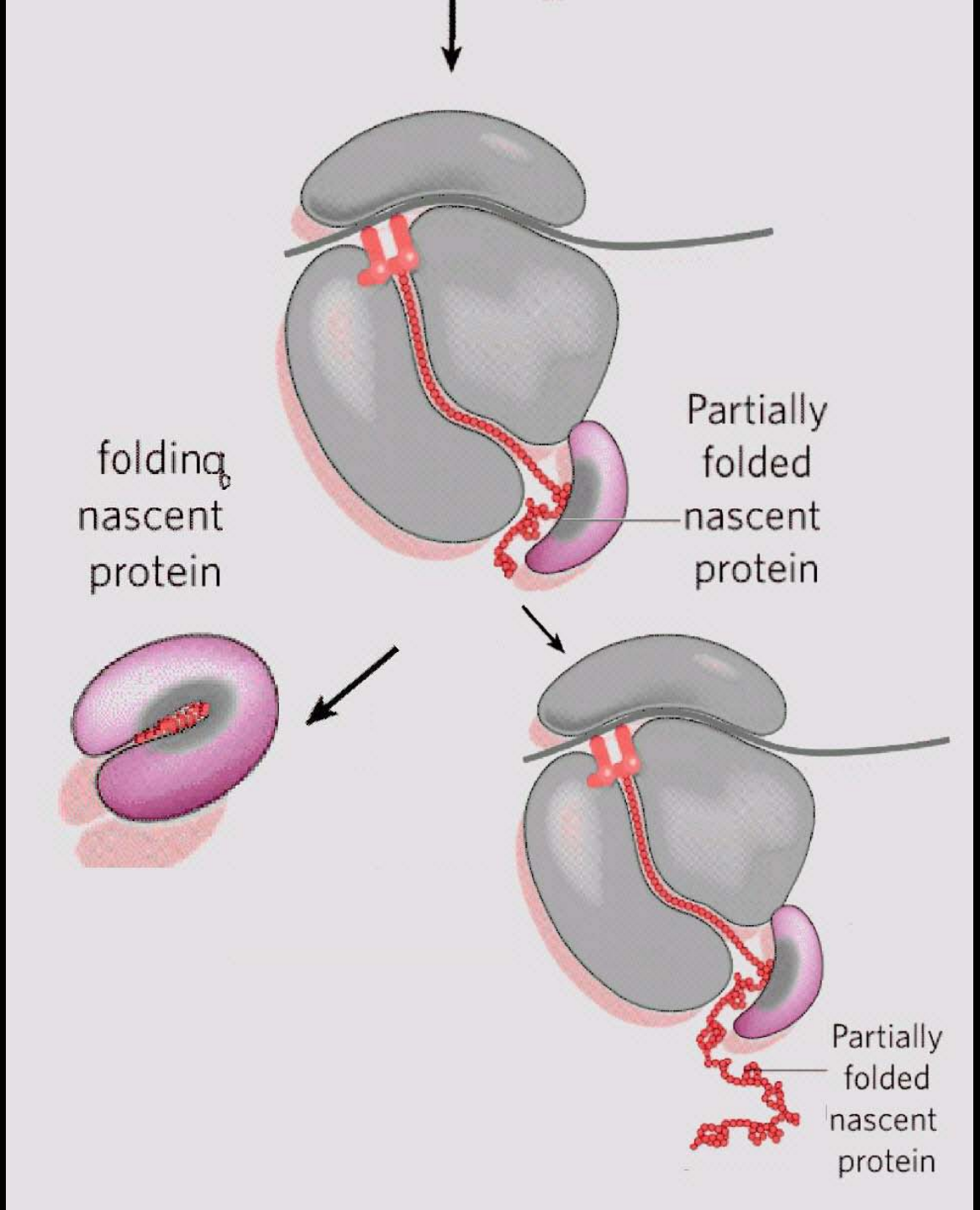
A hydrophobic Pocket Opens upon Binding to the Ribosome

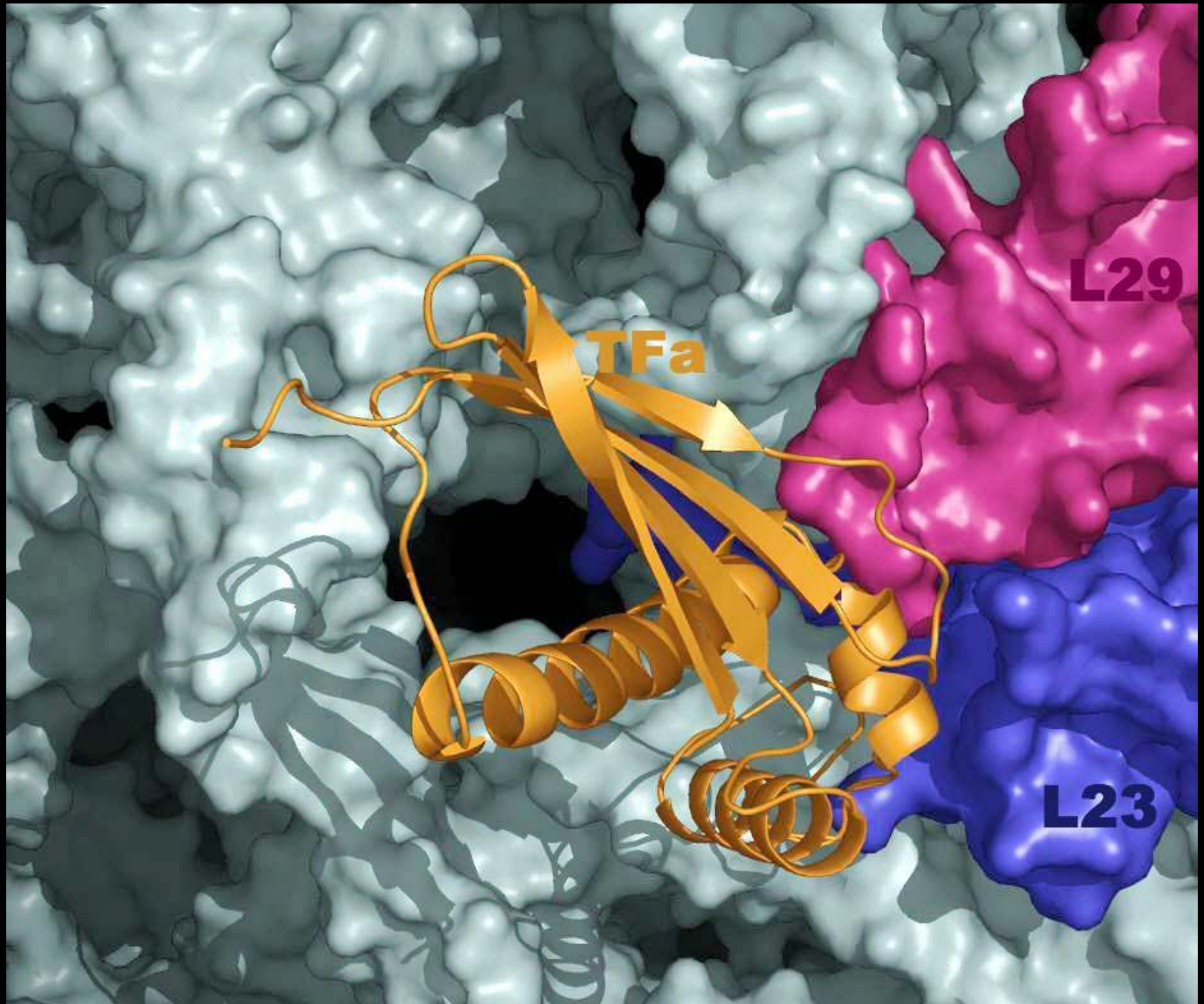


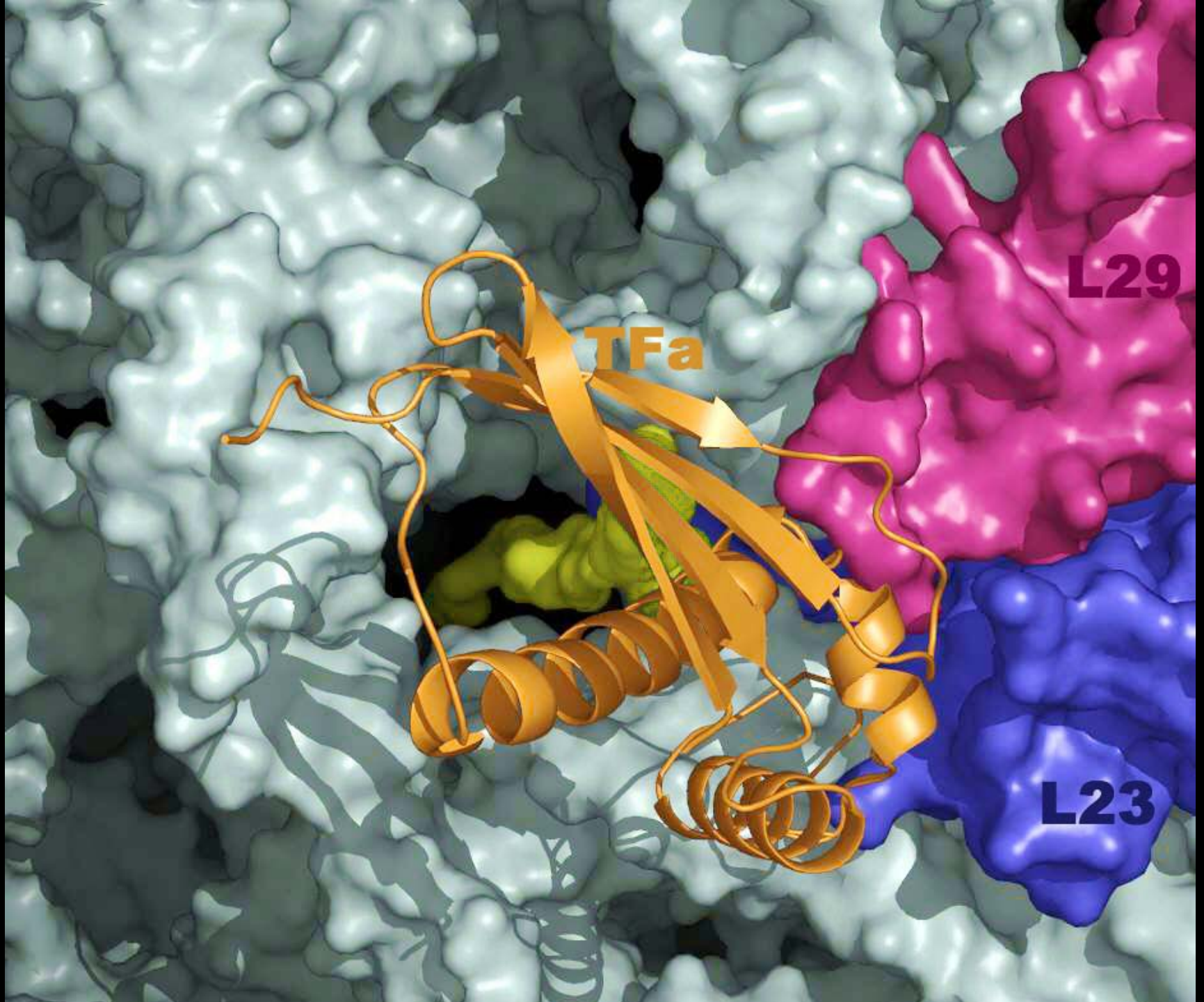
Combination of real-time experiments (Kaiser et al., 2006) & crystallographic analysis (Baram et al., 2005)

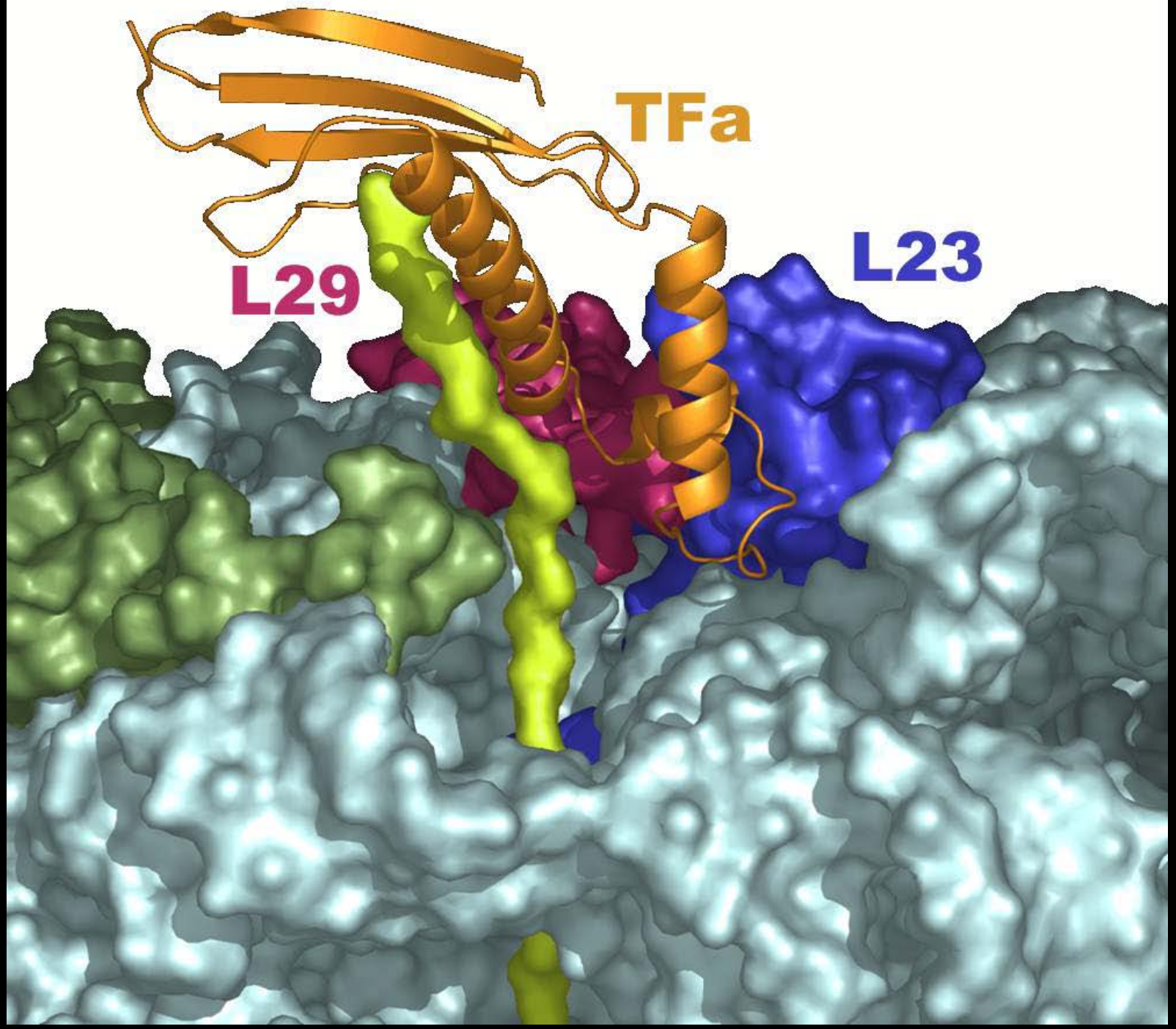



a
Messenger RNA
Nascent polypeptide
Transfer RNAs
Exit tunnel
Ribosome
Native TF
b
Activated TF
Hydrophobic patch

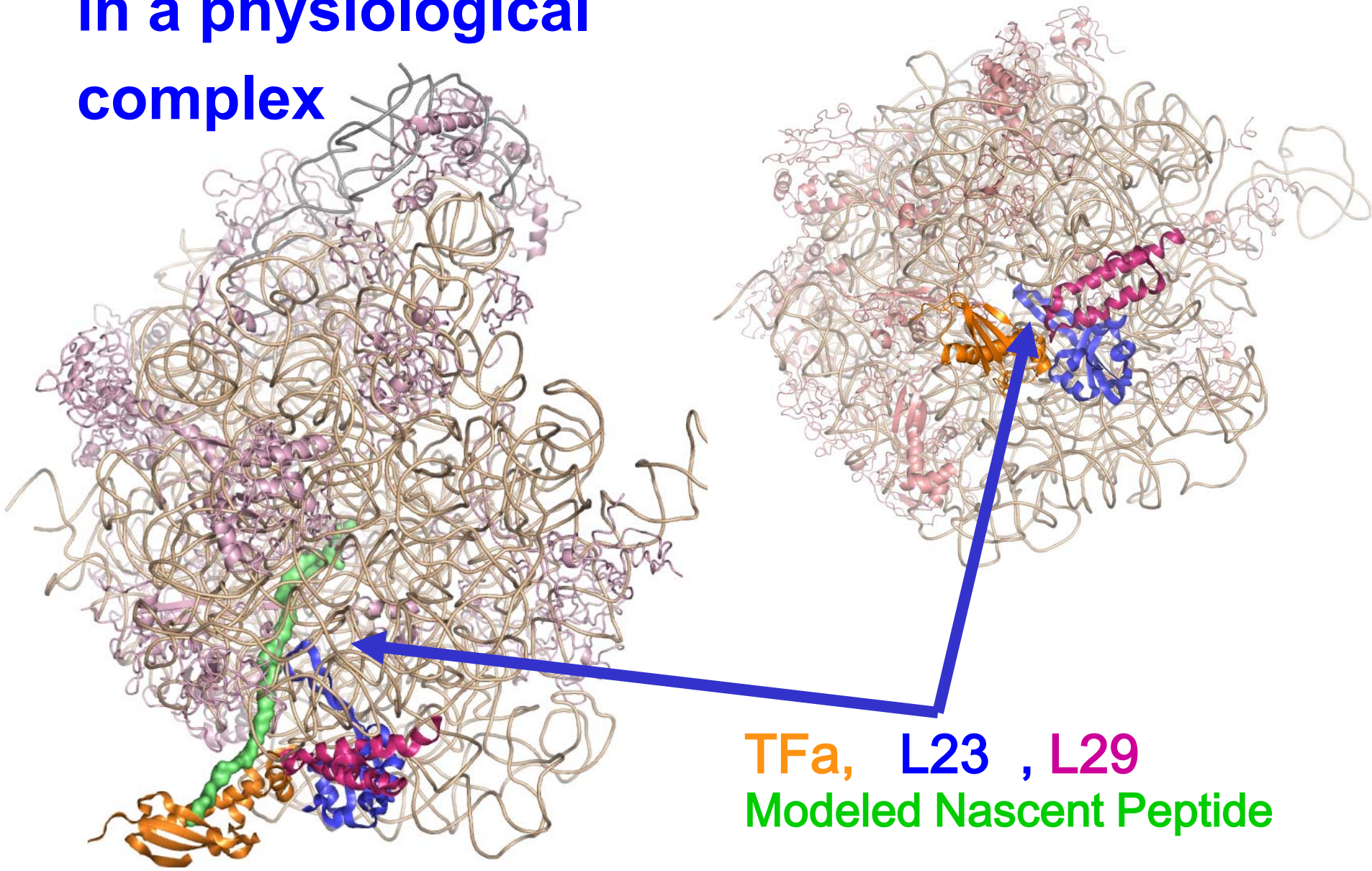

folding
nascent
protein
Partially
folded
nascent
protein
Partially
folded
nascent
protein


L29
TFa
L23


TFa
L29
L23

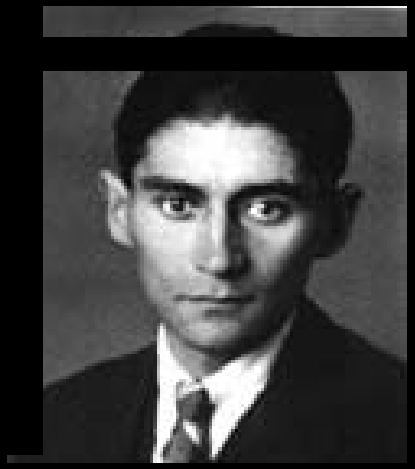

TFa
L29
L23

Binding of TFa to D50S in a physiological complex

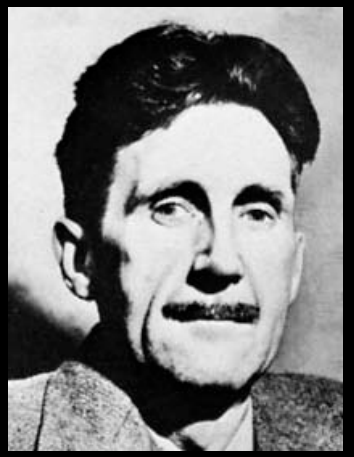


Early deaths caused by infectious diseases and no antibiotics

Keats (1795-1821)



Kafka (1883-1924)



Orwell (1903-1950)

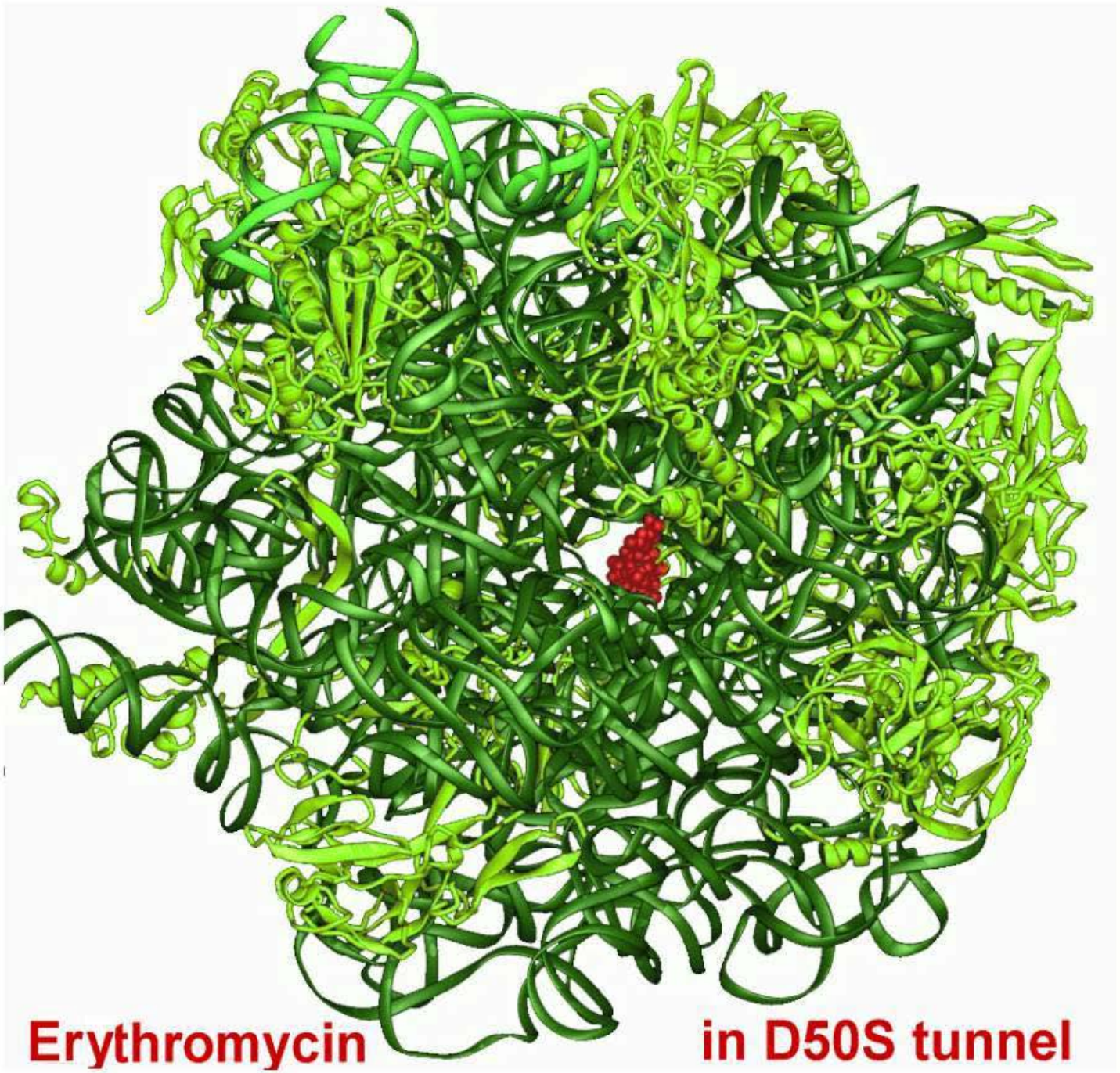
Mozart (1756-1791)

Schubert (1797-1828)

Chopin (1810-1849)

Adapted from V. Ramakrishnan

Because of the fundamental role played by the ribosomes, many antibiotics target them


Erythromycin
in D50S tunnel

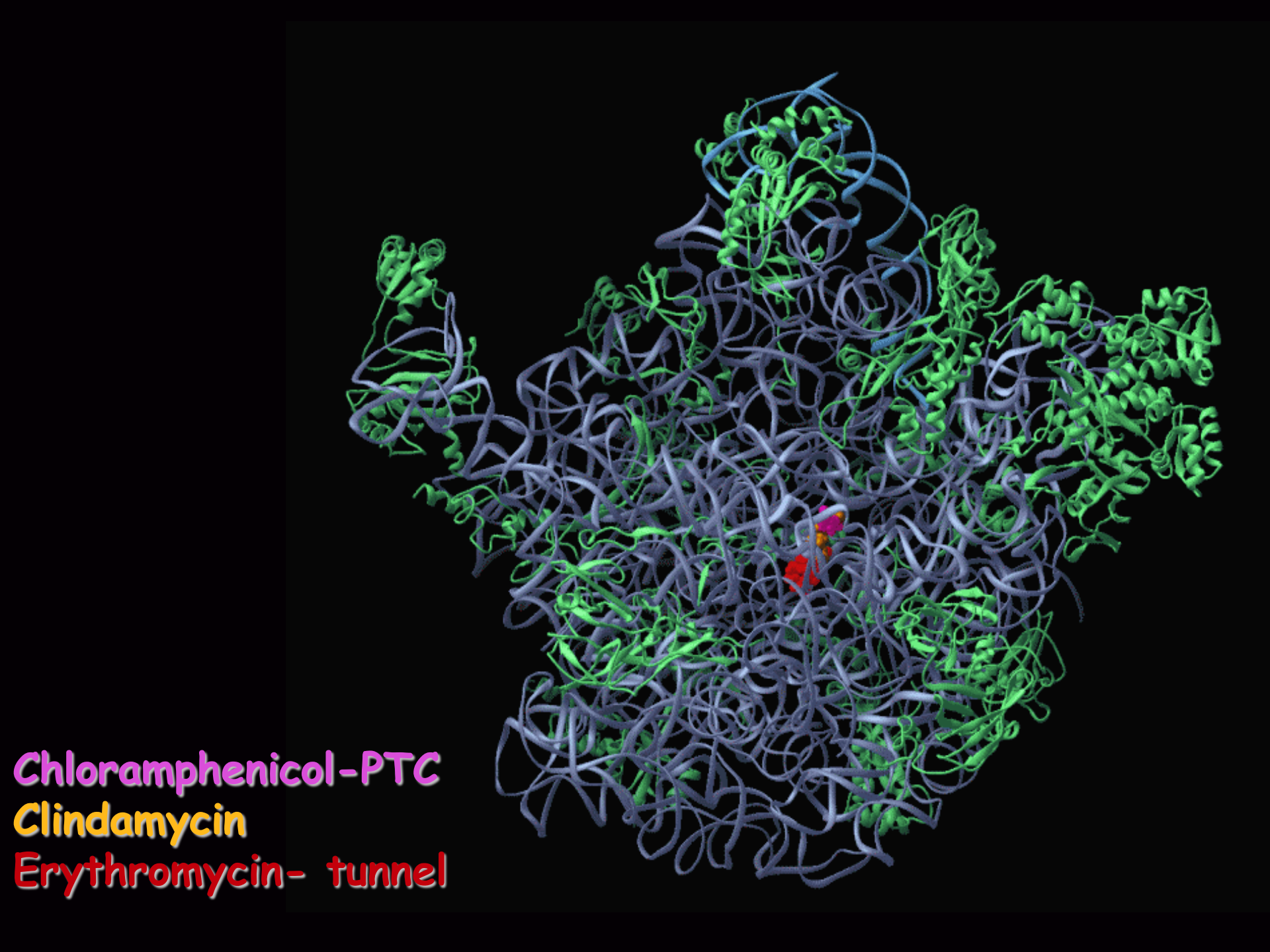
Over 40% of the antibiotics inhibit protein biosynthesis.

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.

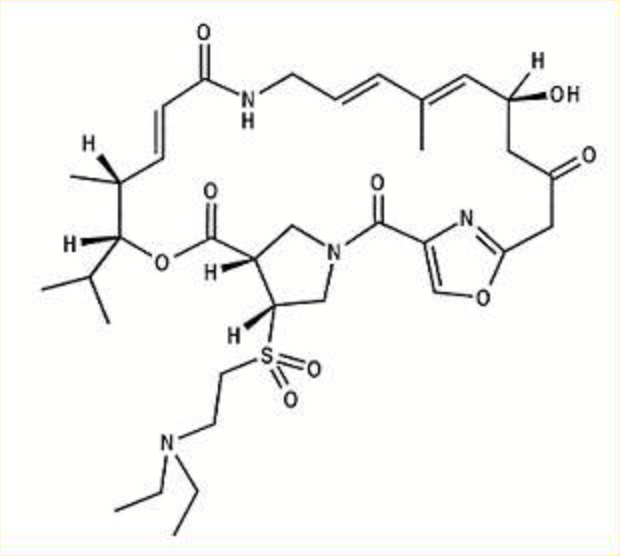
Most antibiotics are not fully selective.


Chloramphenicol-PTC
Clindamycin
Erythromycin- tunnel

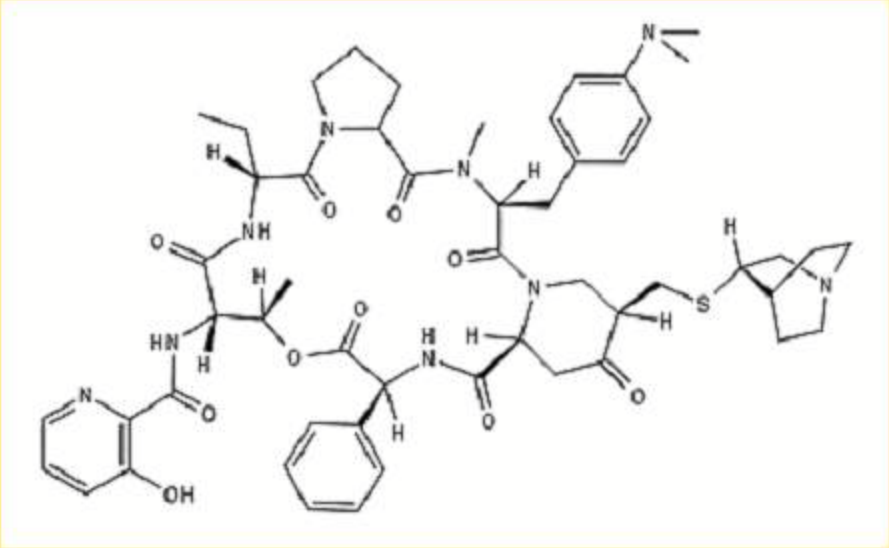
Synergism of ribosomal antibiotics:

The combination of two antibiotics drugs that can interact with each other and enhance activity

Synercid

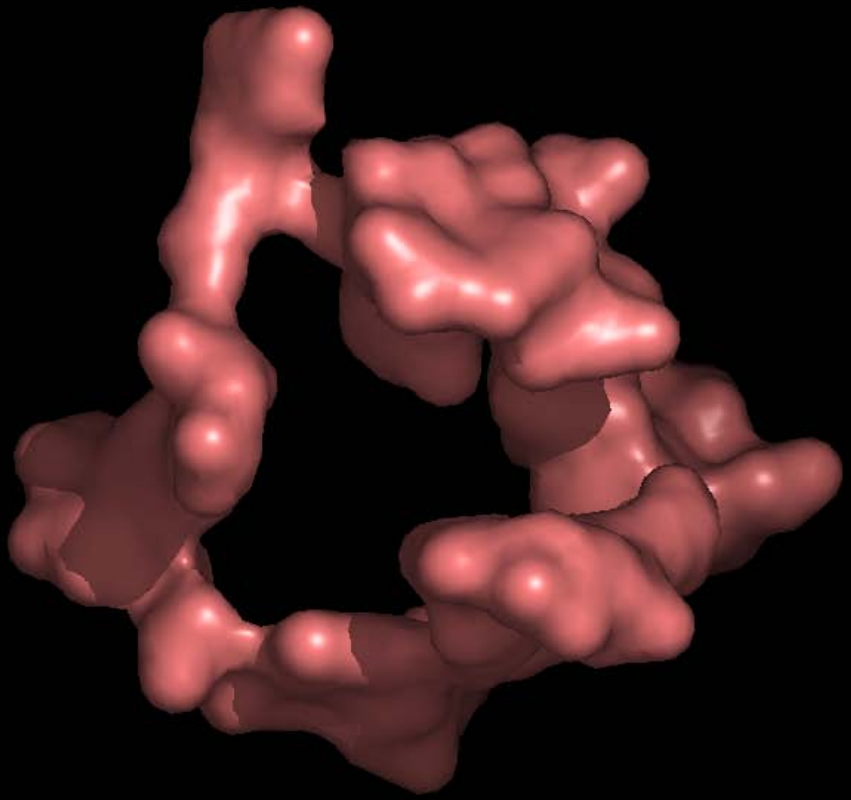


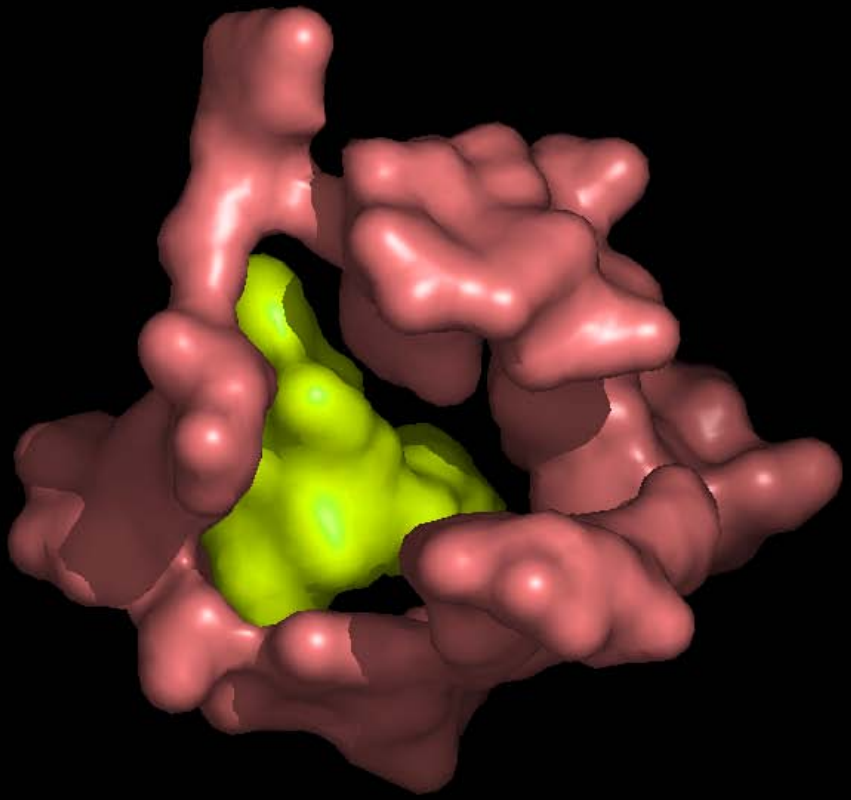
Streptogramin $_{A}$
dalfopristin

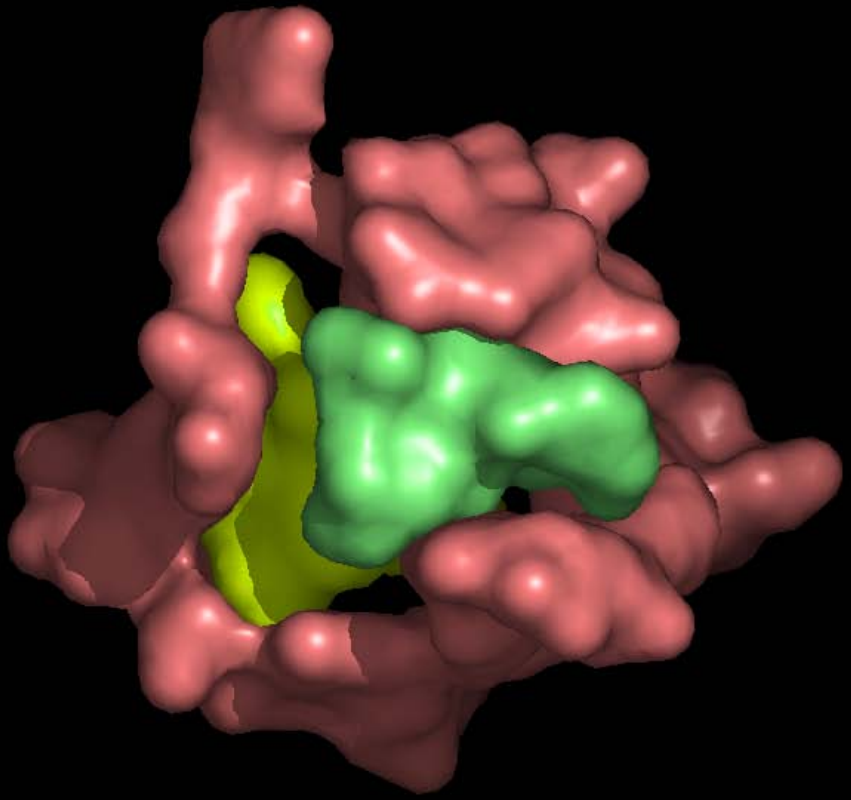


Streptogramin $_{B}$
quinpristin

syn·er·gid Function: *noun*, Etymology: New Latin *synergida*, from Greek *synergos* working together



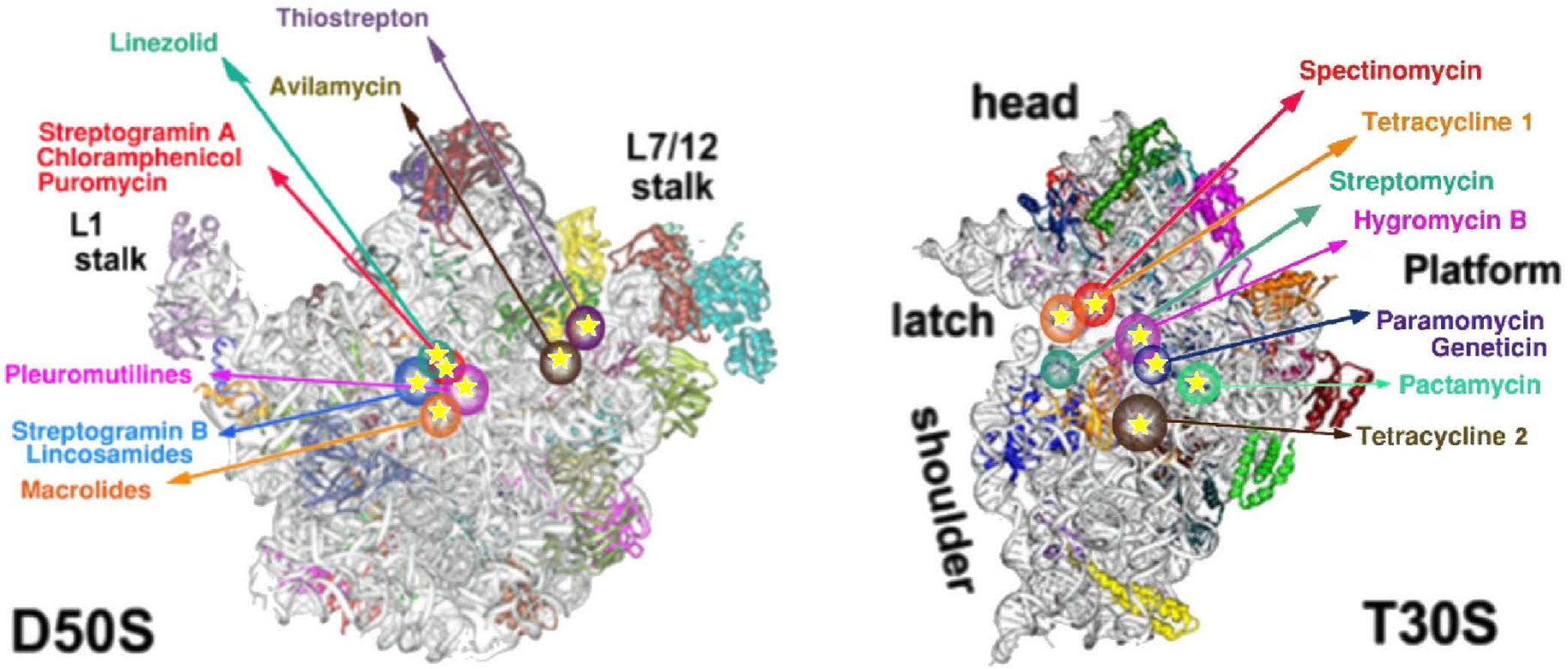




Synergism opens the gates for:

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

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



All antibiotic binding sites on the ribosome are of functional relevance

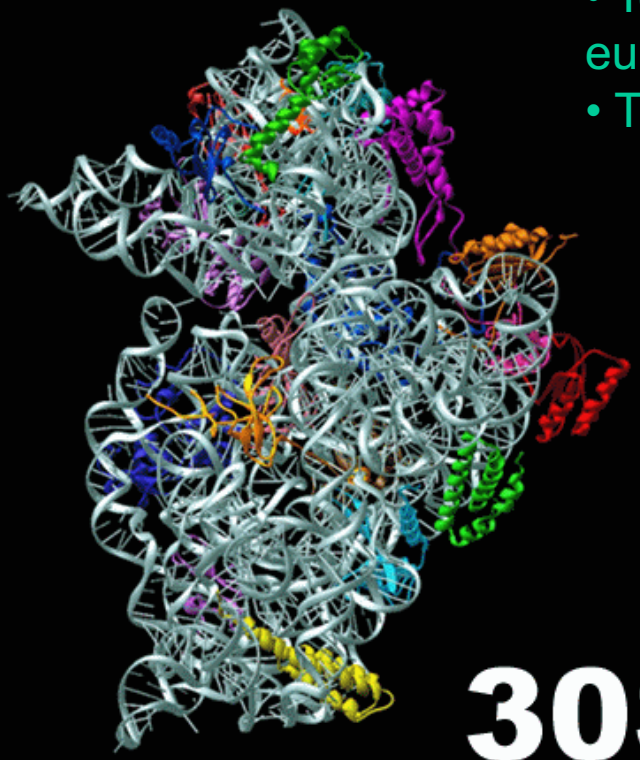
David and Goliath:
How do the tiny antibiotics paralyze the giant ribosome?

Antibiotics Targeting Ribosomes

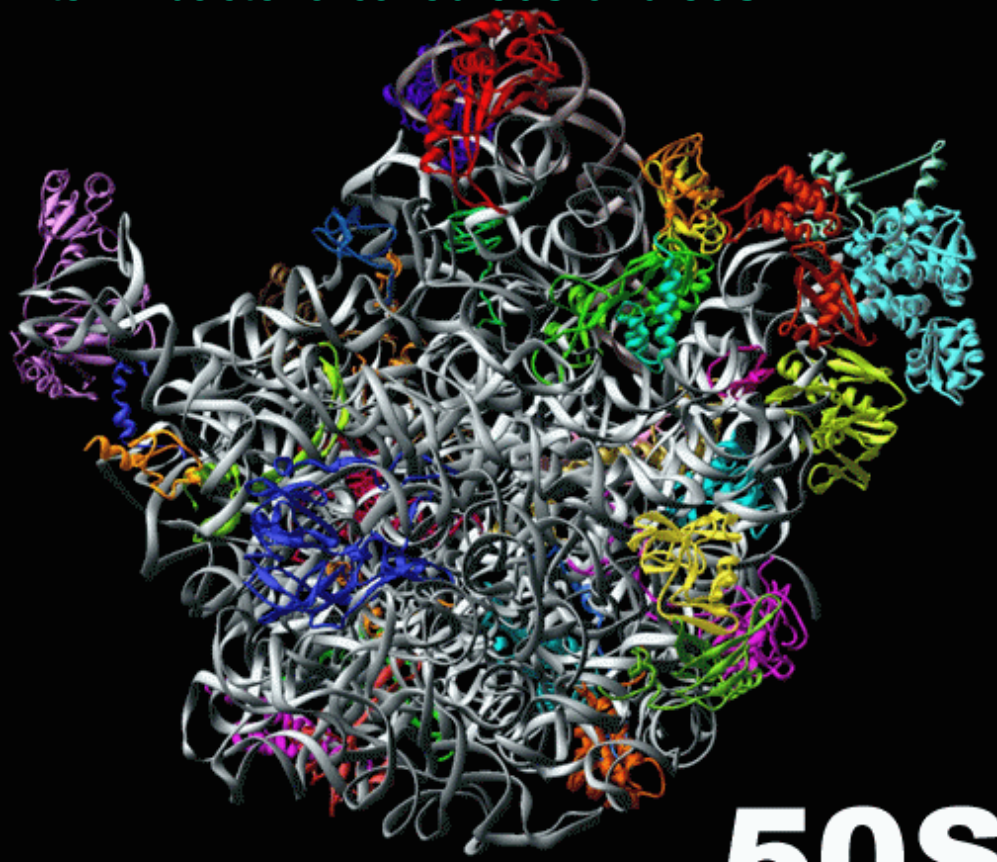
Based on crystallographic studies, Yonath's group,
The Weizmann Institute, Rehovot, Israel,
and Max-Planck research Unit, Hamburg, Germany

The ribosome translates the genetic code into proteins

- Universal cellular assembly of rRNA and r-proteins
- Total mol weight 2.5 - 4 Mega Dalton (for prokaryotes & eukaryotes, respectively)
- Two subunits: in bacteria called 30S and 50S



30S

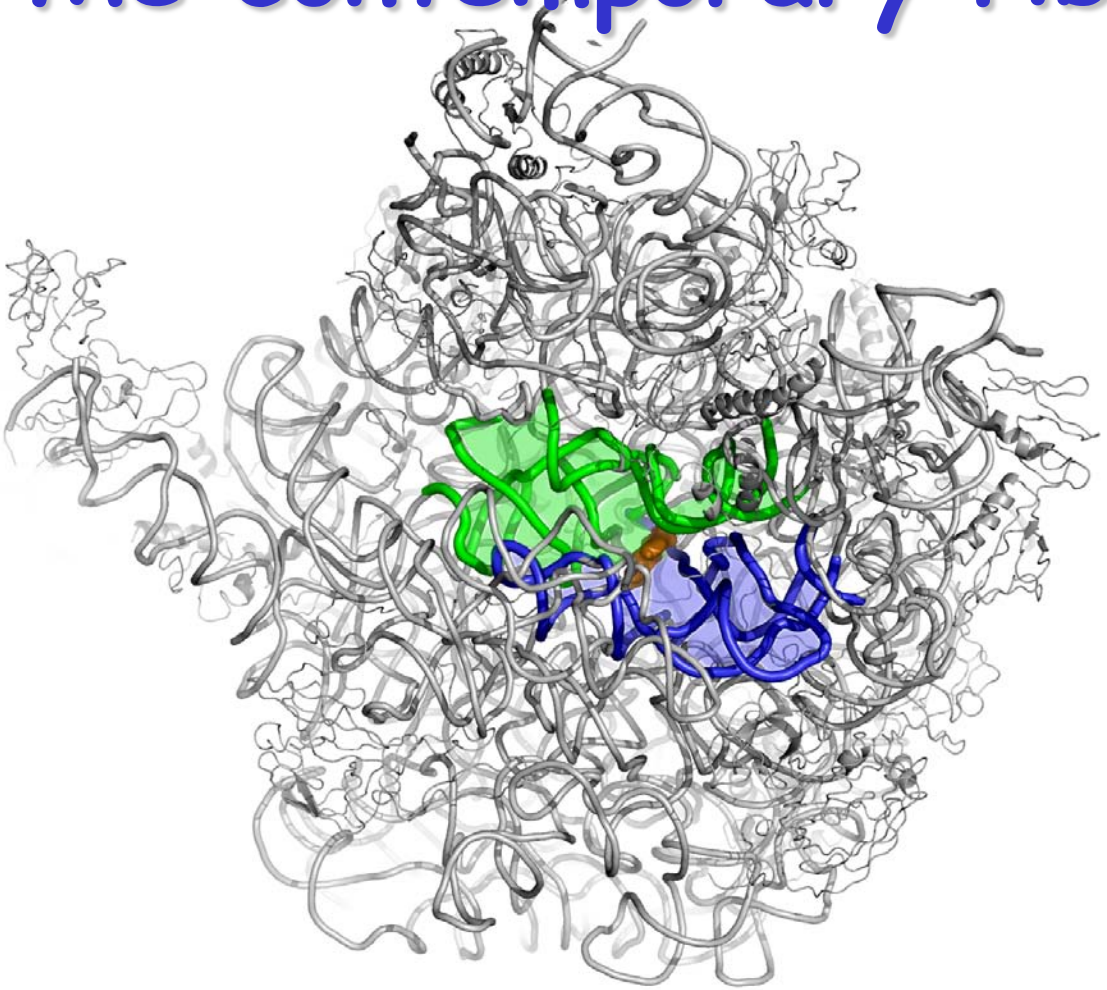


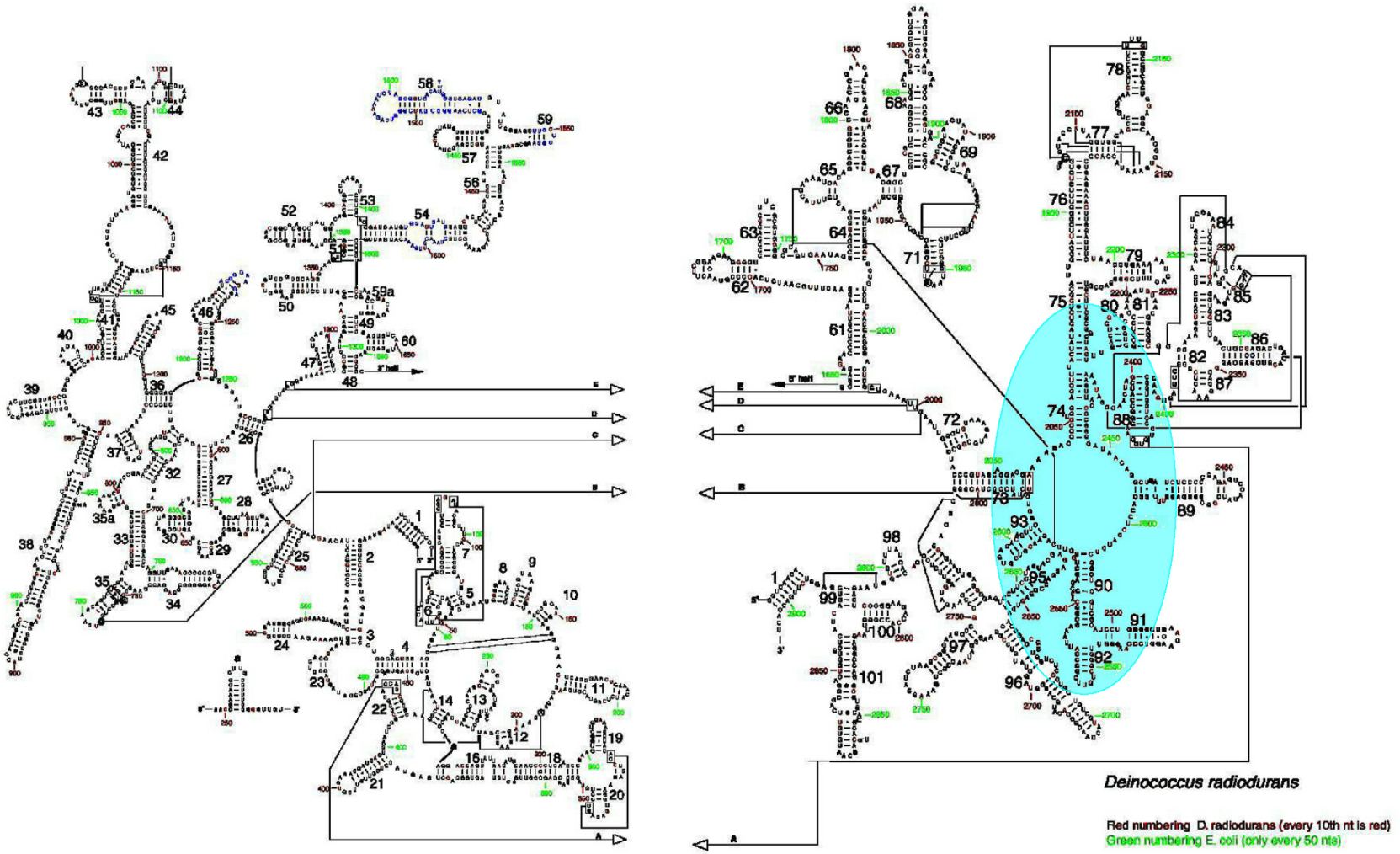
50S

Total mol weight 2.5 Mega dalton in prokaryotes: One RNA chain of ~1600 nucleotides (16S RNA) and ~ 21 different proteins (called S1….S21)

Total mol weight 1,5 Mega dalton in prokaryotes Two RNA chain of total ~3000 nucleotides (5S RNA & 23S RNA) and ~ 34 different proteins (called L1….L34

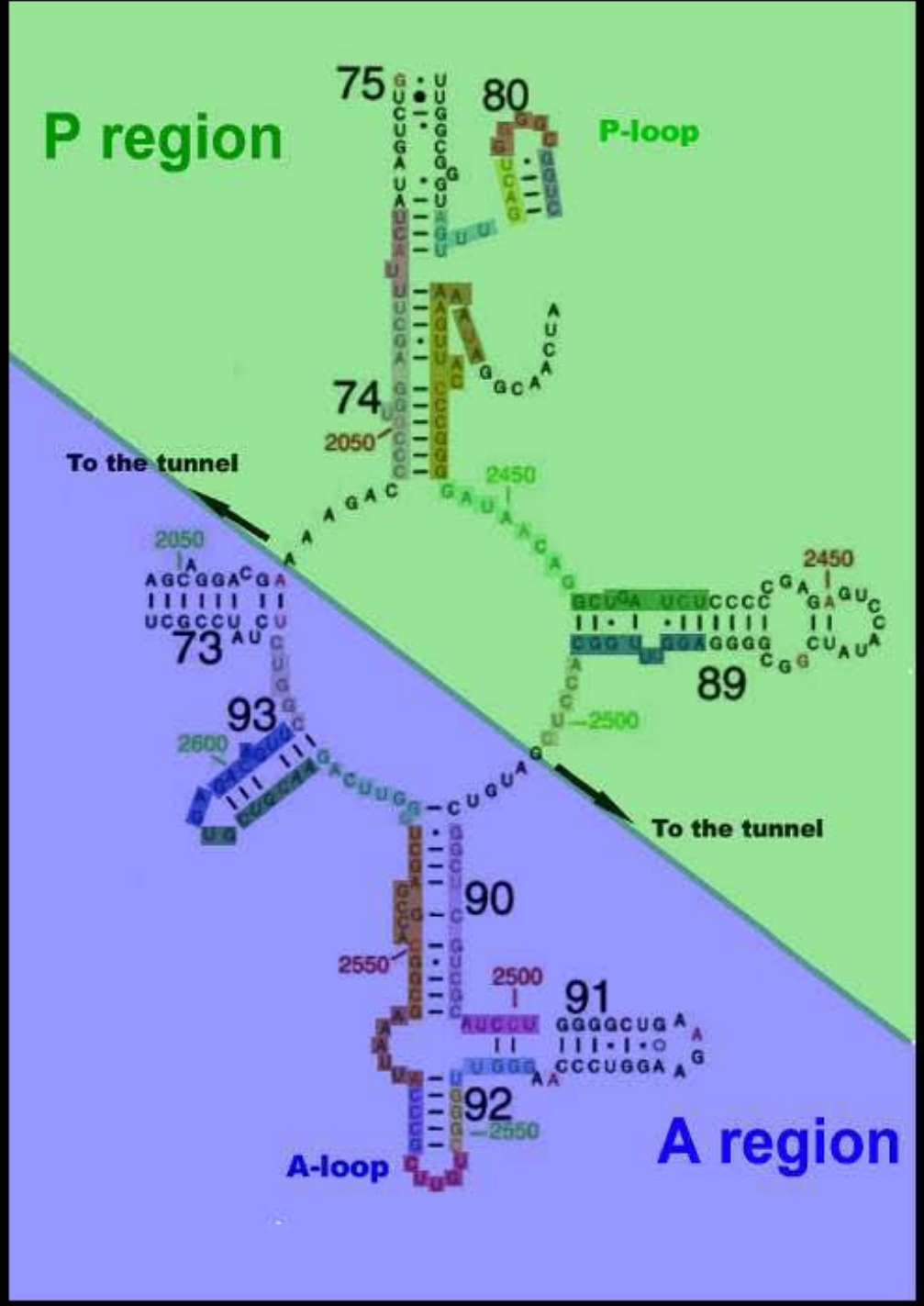
We identified the ancient (prebiotic) translation apparatus within the contemporary ribosome



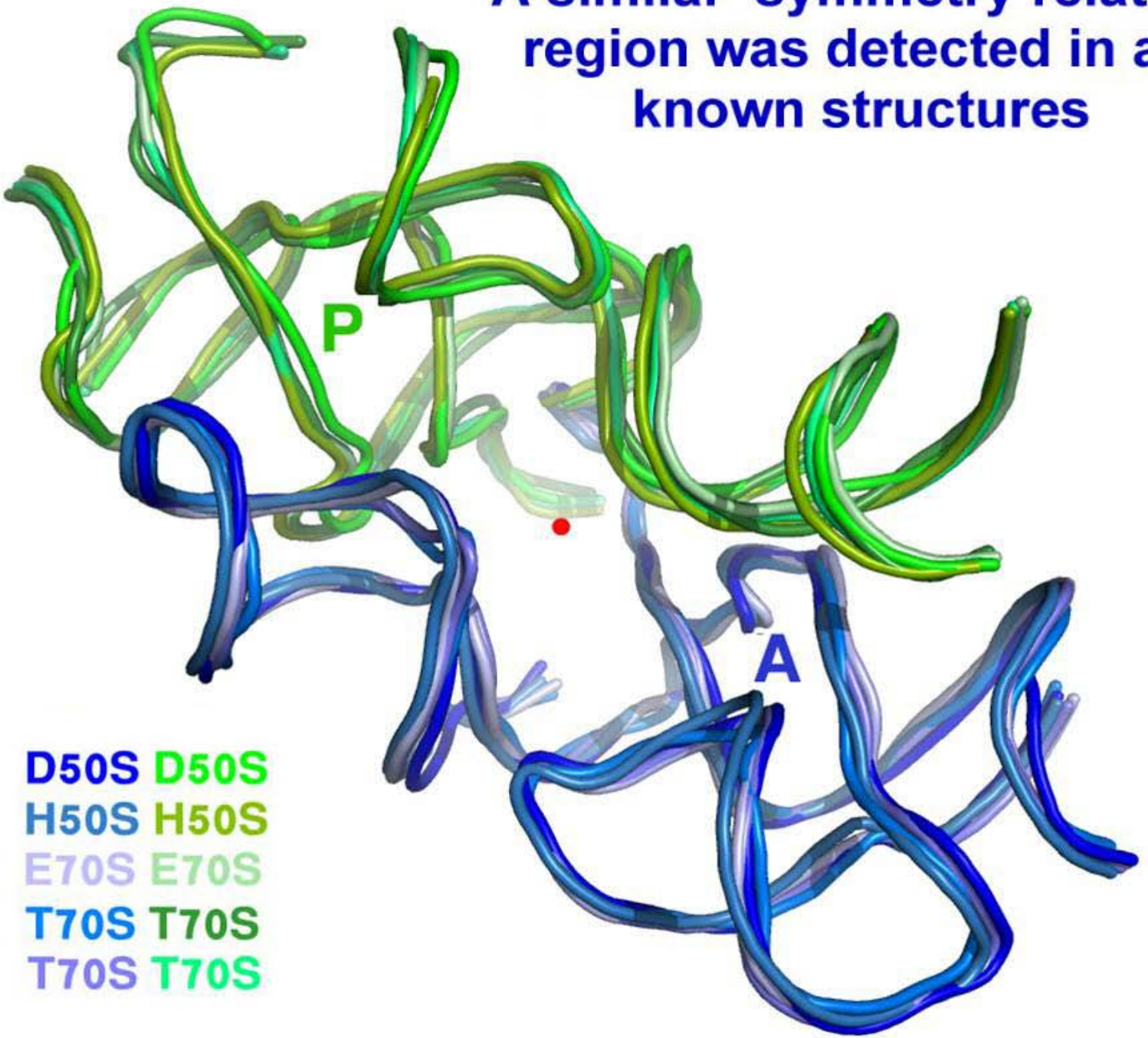


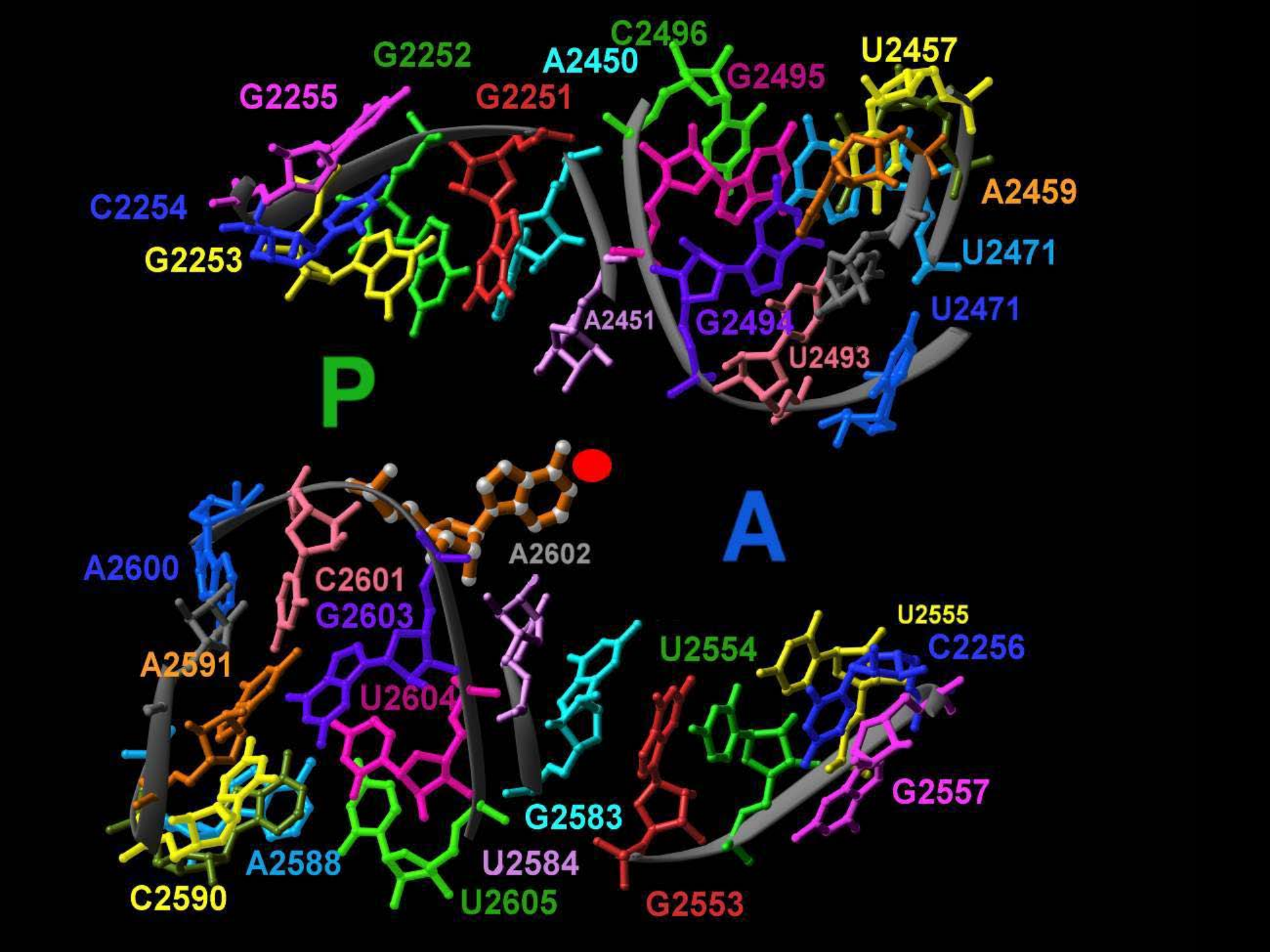
2D representation of
the 23S RNA from
D. rediodurans

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

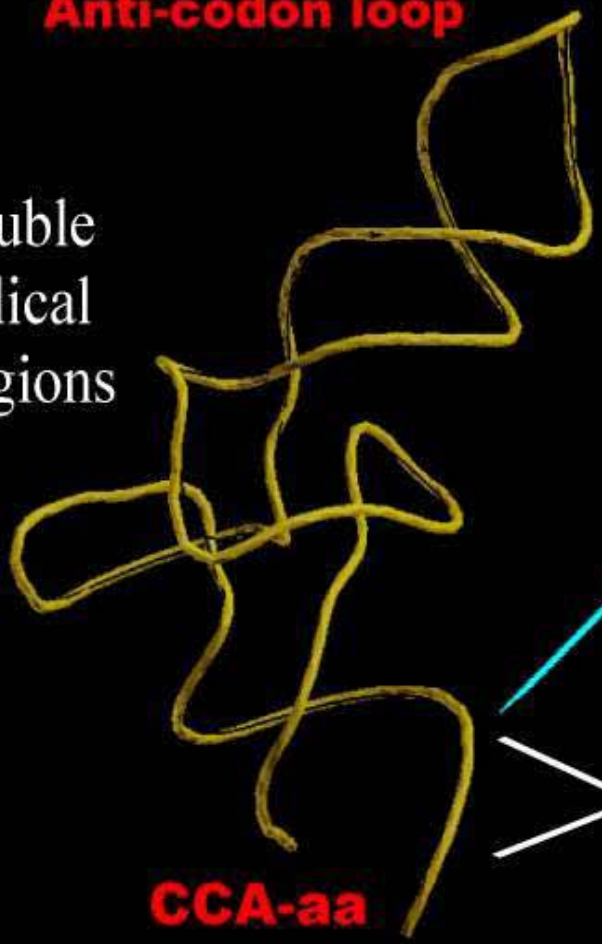


A similar symmetry related region was detected in all known structures




C2496
G2252
A2450
U2457
G2255
G2251
G2495
A2459
C2254
U2471
G2253
A2451
G2494
U2471
U2493
P
A
A2600
A2602
C2601
G2603
U2555
U2554
C2256
A2591
U2604
G2557
G2583
A2588
U2584
C2590
U2605
G2553

The tRNA molecule

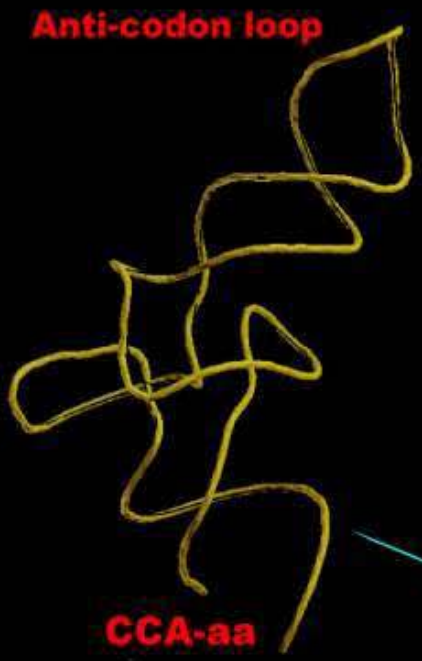


Double
Helical
Regions

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)

tRNA motions

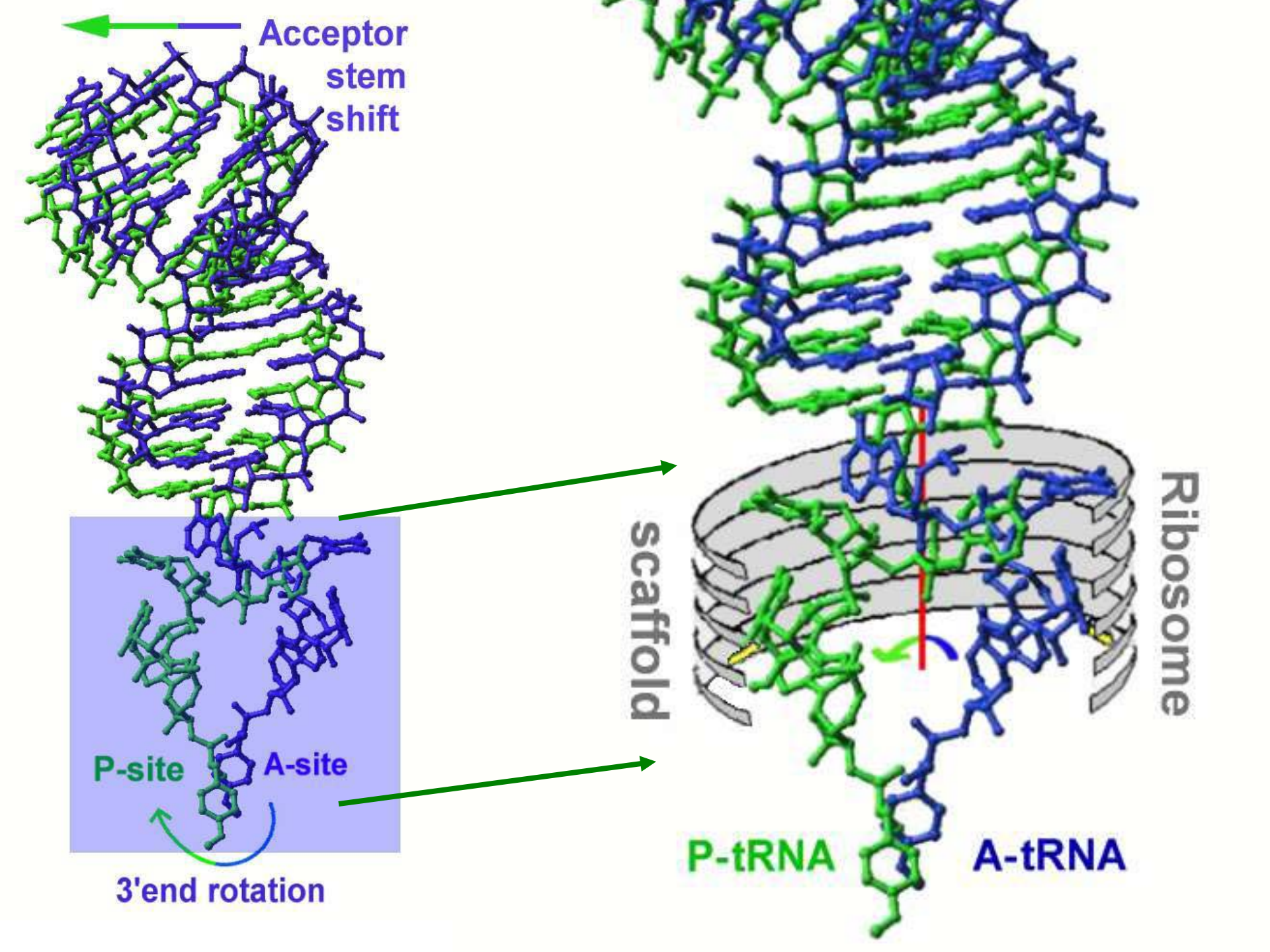


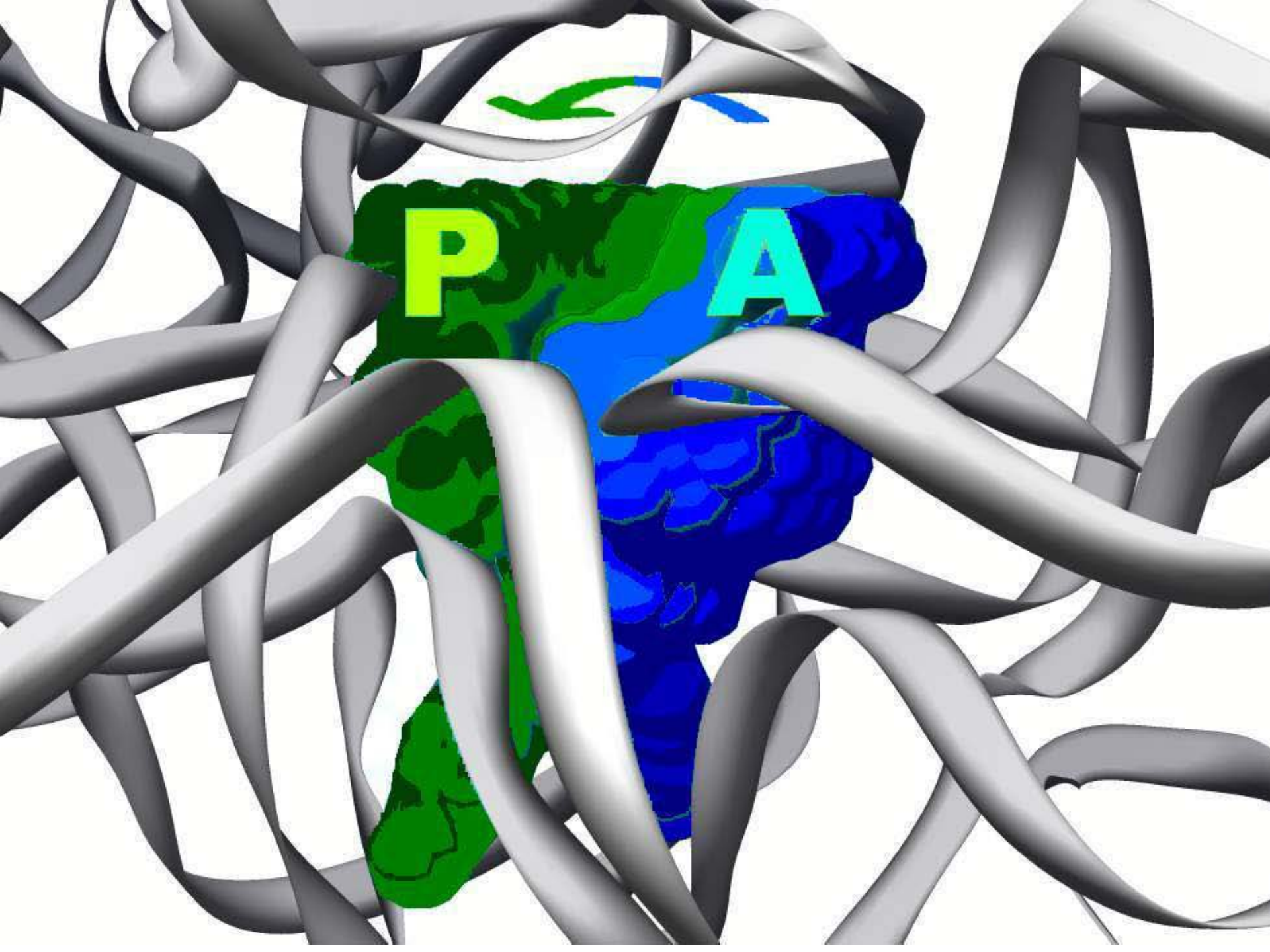
The independent-correlated motions:

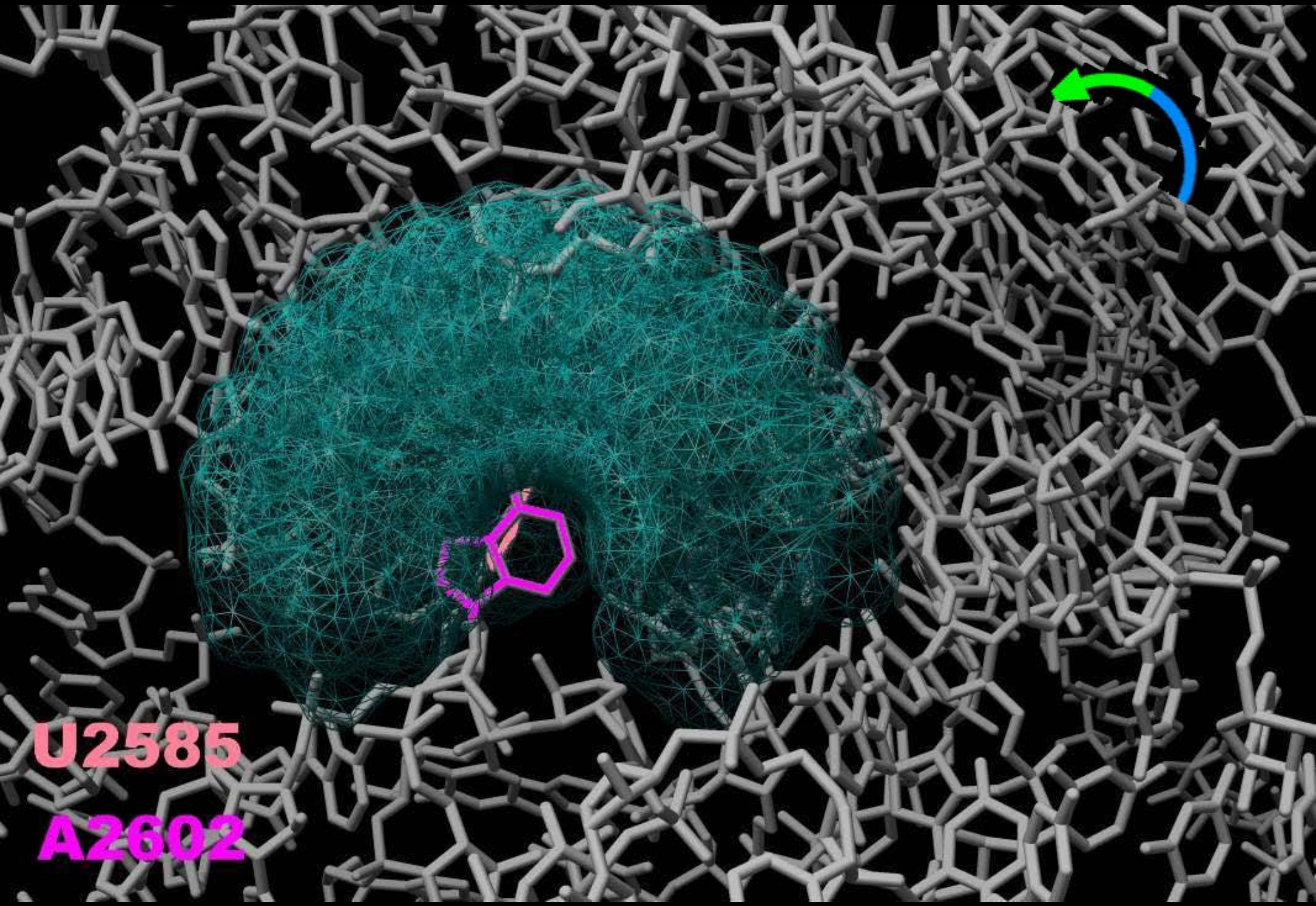
1. **A shift of the helical part**

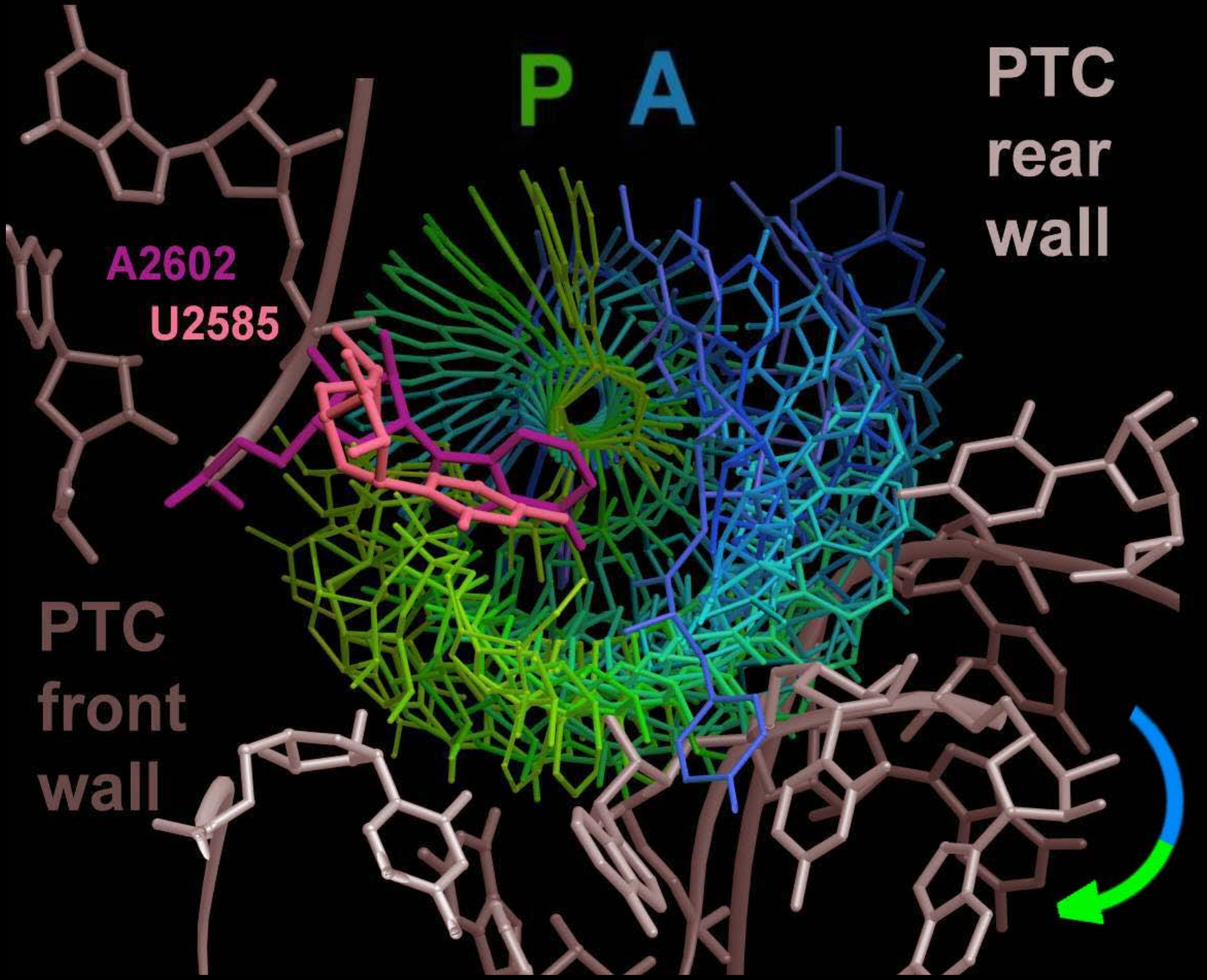
2. **Rotation of the 3'-end**

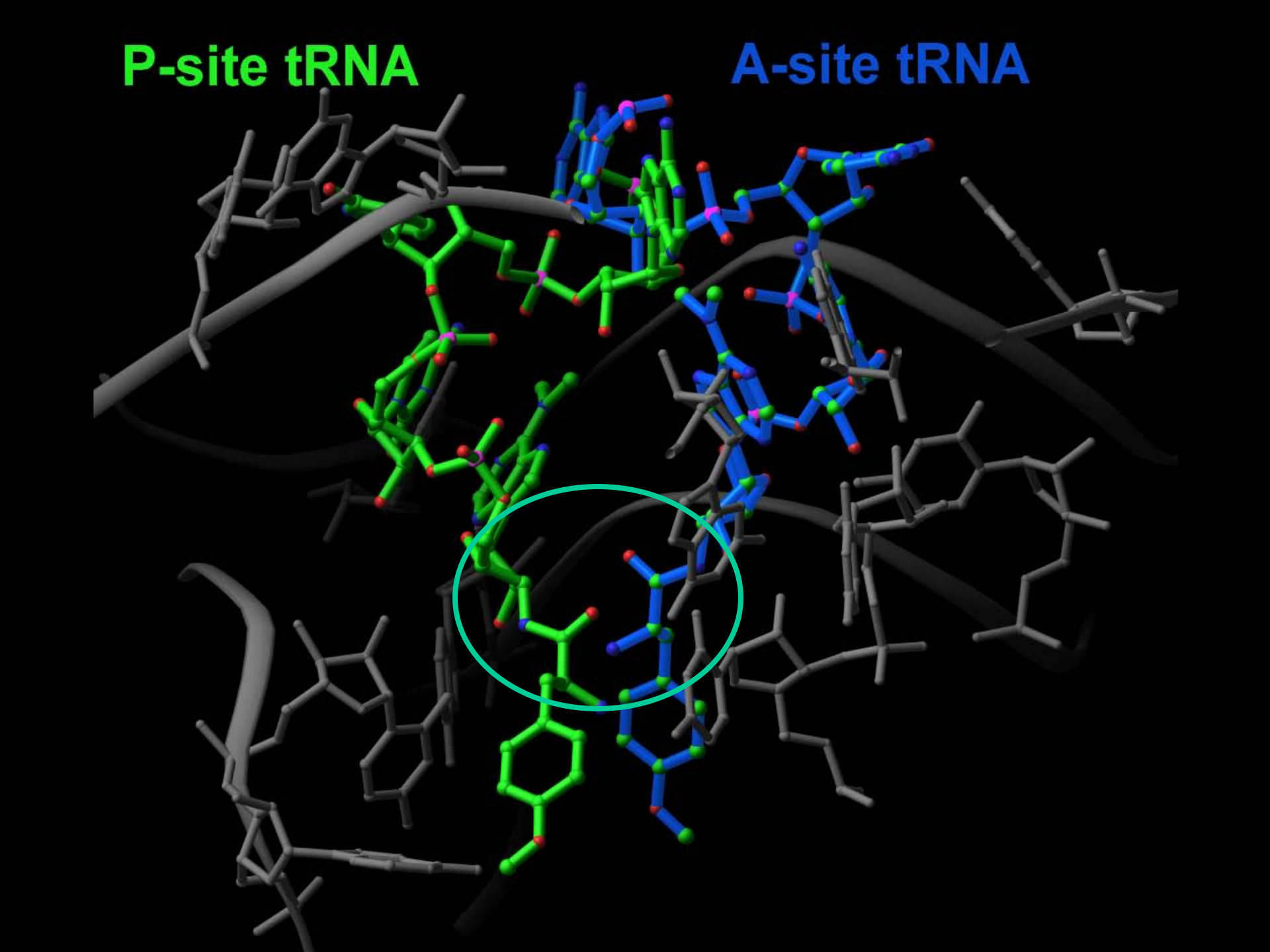
around the bond connecting it with the tRNA acceptor stem, which coincides with the PTC 2-fold axis


Acceptor
stem
shift
P-site
A-site
3'end rotation
scaffold
Ribosome
P-tRNA
A-tRNA


P
A


U2585
A2602


P A
PTC
rear
wall
A2602
U2585
PTC
front
wall


P-site tRNA
A-site tRNA

THE RIBOSOME IS A POLYMERASE,

HENCE IT HAS TWO CATALYTIC TASKS:

1. The creation of the peptide bond

2. The elongation of the nascent protein

The ribosome architecture provides a symmetrical the frame for the tRNA 3'end end (CCA-aminoacid), which accords with the early finding of symmetrical relationship of the ribosome substrates*

* (Nissen et al., 2000)

The ribosome architecture provides a symmetrical the frame for the tRNA 3'end end (CCA-aminoacid), which accords with the early finding of symmetrical relationship of the ribosome substrates*

The tRNA 3'end rotatory motion is part of the mRA/tRNA translocation and can occur regardless of the nature of this motion (simple sideways shift, hybrid motion, etc) It also does not depend on global ratcheting.

* (Nissen et al., 2000)

The symmetrical region is highly conserved

FREQUENT nucleotides: same in > 95% of ALL sequences

In the symmetrical region: **98%**

In the ribosomal 23S RNA excluding symmetrical region: 36%

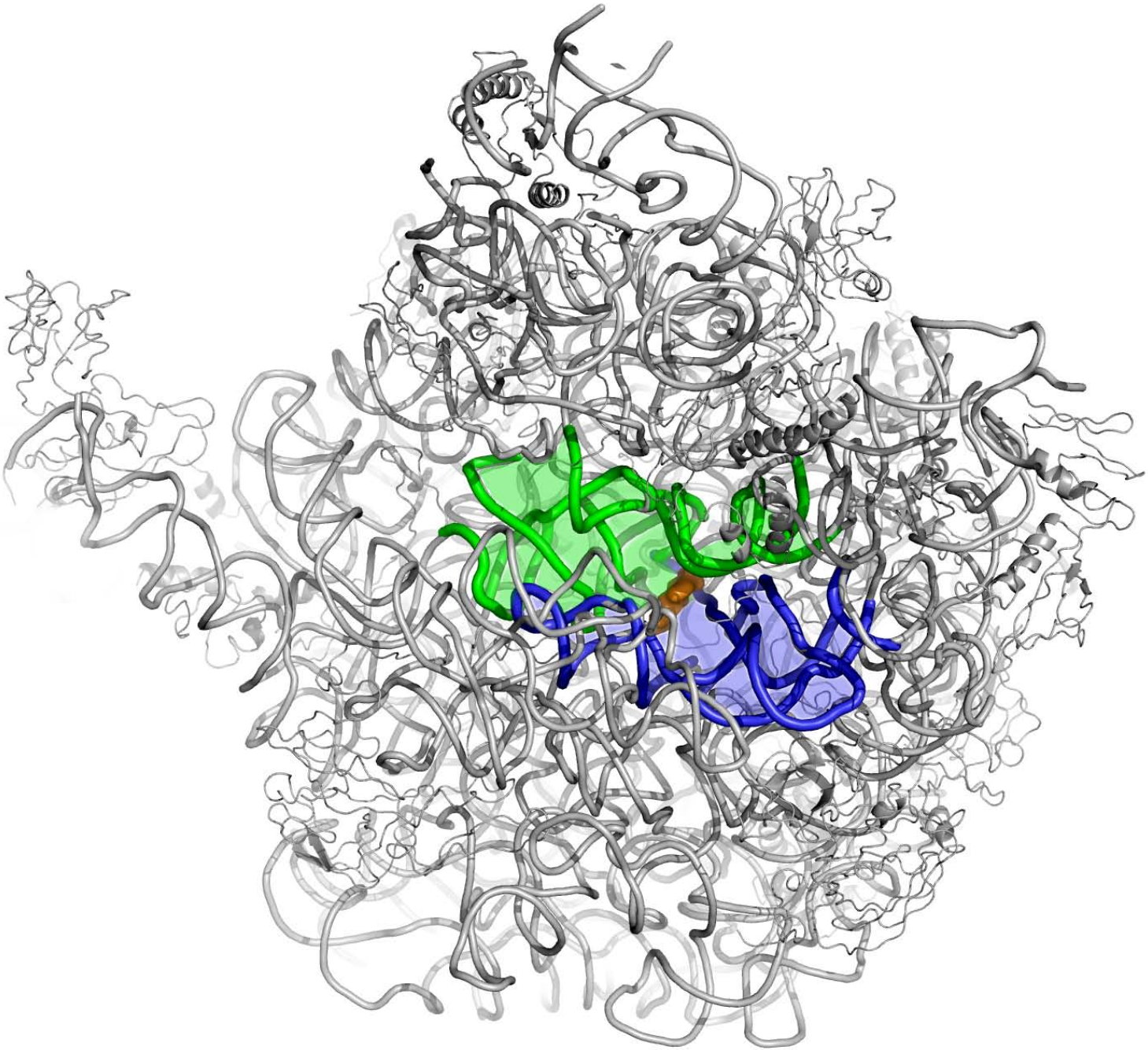
(based on 930 species from all phylogenetic domains)

The high conservation of the symmetrical region indicates that its existence is beyond environmental conditions

Suggesting that

The proto-ribosome, which was a simple dimeric RNA enzyme,

is still embedded in the core the contemporary ribosome



Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions

Powner, Be´atrice Gerland & Sutherland
nature 459, 14 May 2009

Systems chemistry on early Earth

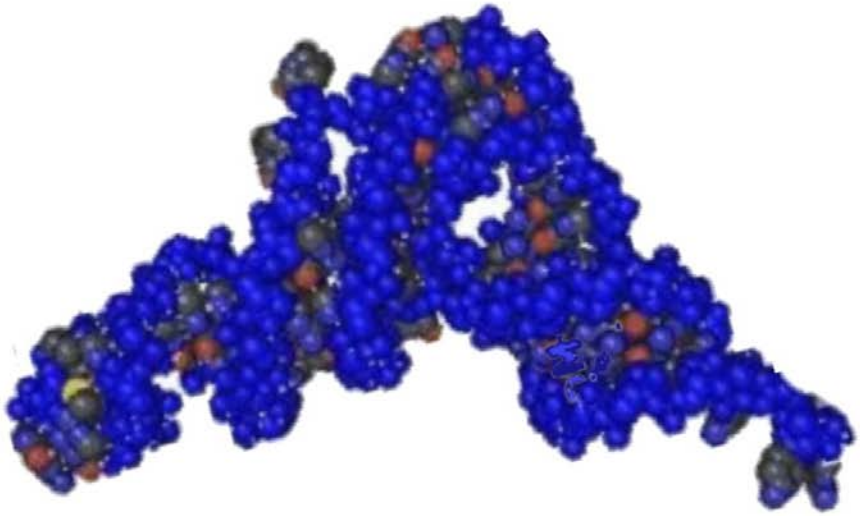
Jack W. Szostak

A new way of looking at the synthesis of RNA

Self-Sustained Replication of an RNA Enzyme

Lincoln and Joyce
SCIENCE, 323, 2009

The SES motif (Stem-Elbow-Stem)



The symmetrical region of the ribosome core contains the SES fold. This motif, which has a tendency to dimerize, seems to be an ancient form of RNA.

Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions

Powner, Be´atrice Gerland & Sutherland
nature 459, 14 May 2009

Systems chemistry on early Earth

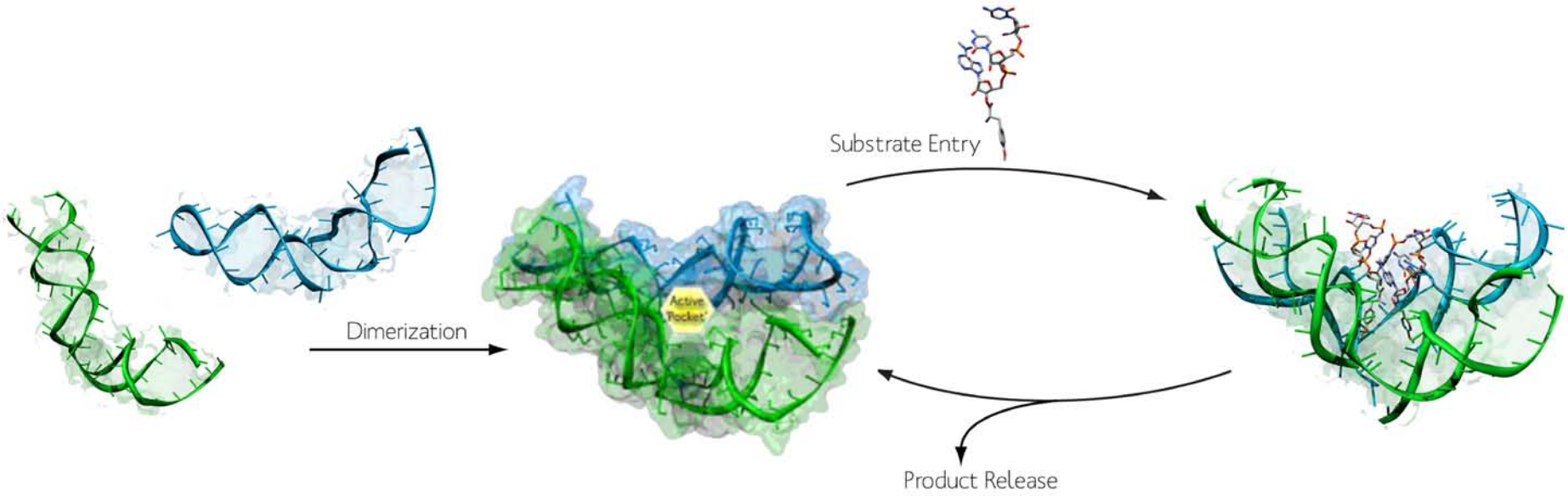
Jack W. Szostak

A new way of looking at the synthesis of RNA

Self-Sustained Replication of an RNA Enzyme

Lincoln and Joyce
SCIENCE, 323, 2009

Our hypothesis

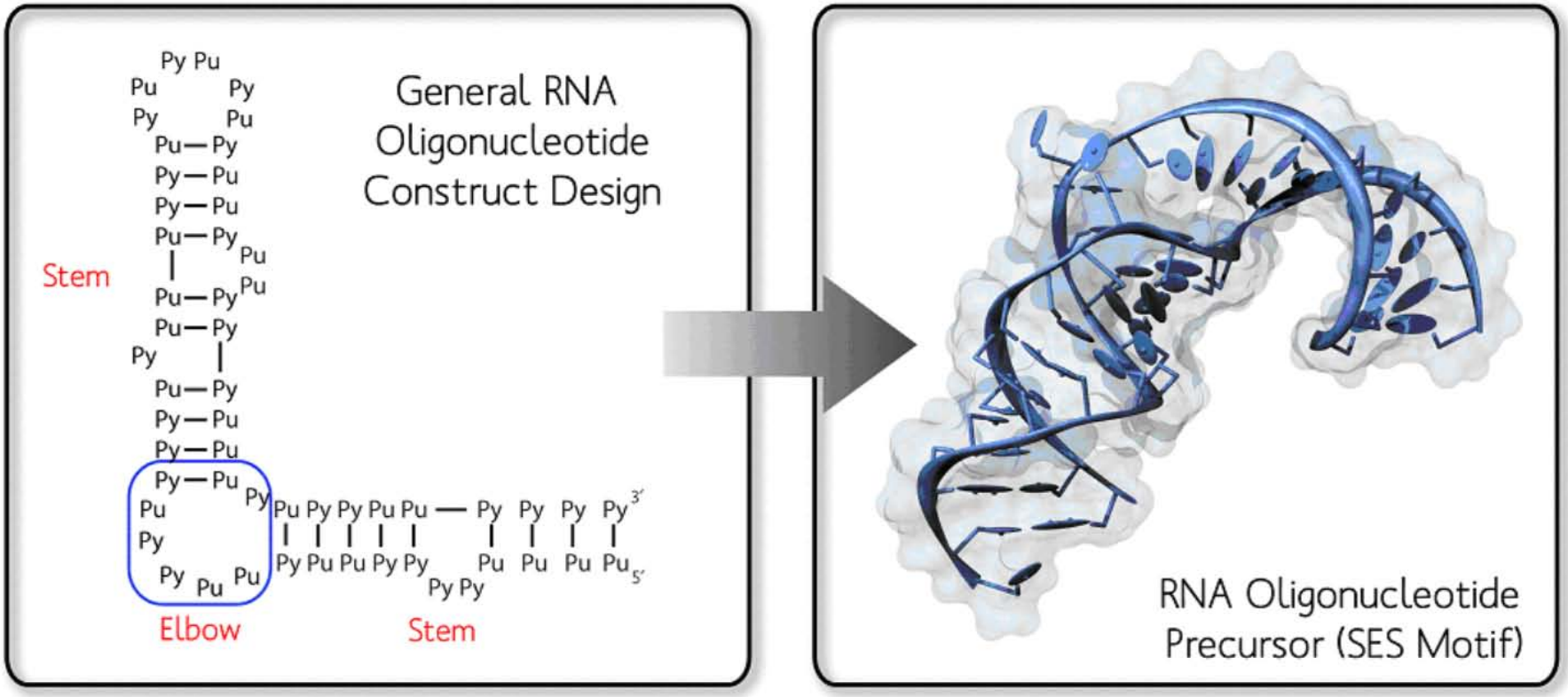


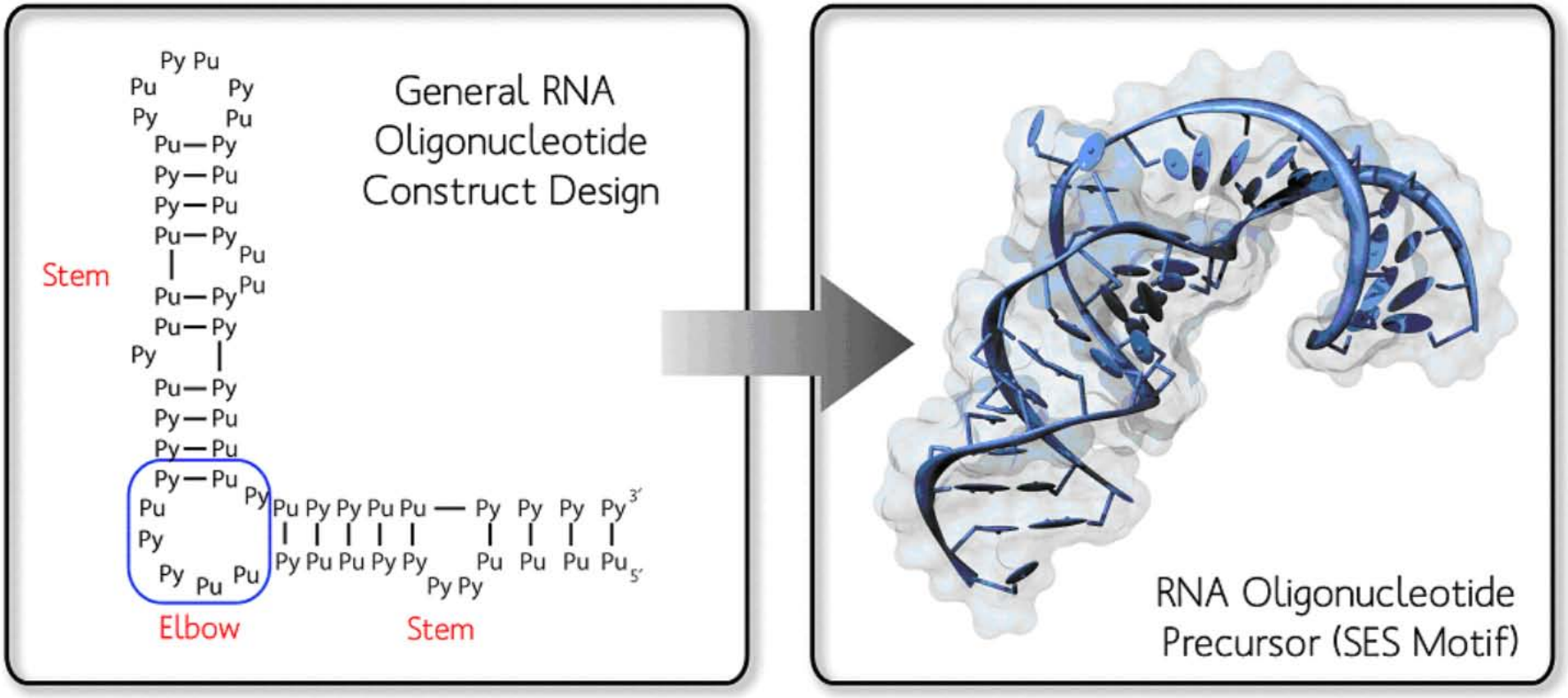
The proto-ribosome was a dimer of RNA chains resembling the contemporary PTC

Agmon et al.,(2005), Biol Chem 386, 833-44
Agmon et al.,(2006), Isr J Ecol Evol, 52, 359-74
Agmon et al.,(2009), http://proceedings. nature.com/documents/2921/
Bokov et al., (2009), Nature 457, 977-980

We suggest a chemical prebiotic process, originating from an oligonucleotide and proceeding via a self folded unit into a self-assembled dimer,
thus producing proto-ribosome pockets.

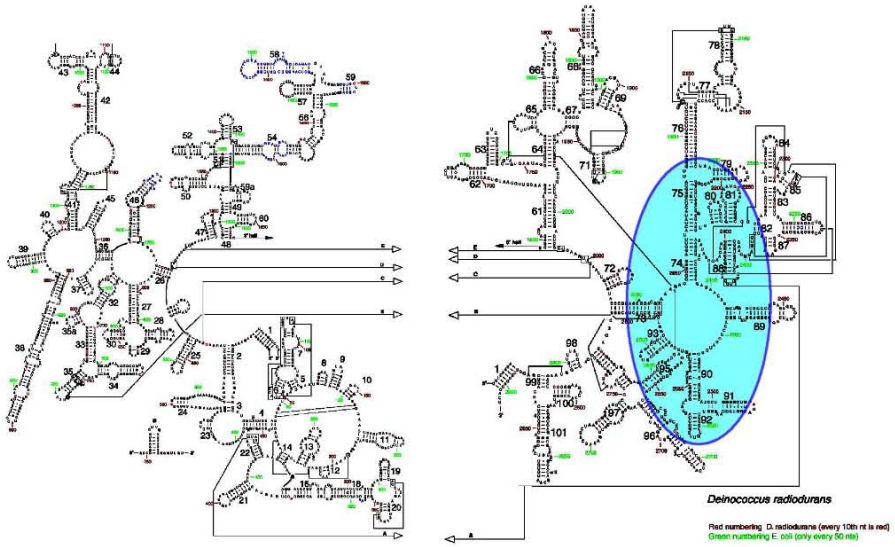
As RNA can be its own template for replication, the proto ribosome could have evolved by gene fusion or gene duplication.


General RNA
Oligonucleotide
Construct Design
Stem
Elbow
Stem
RNA Oligonucleotide
Precursor (SES Motif)



Proto-ribosome constructs:

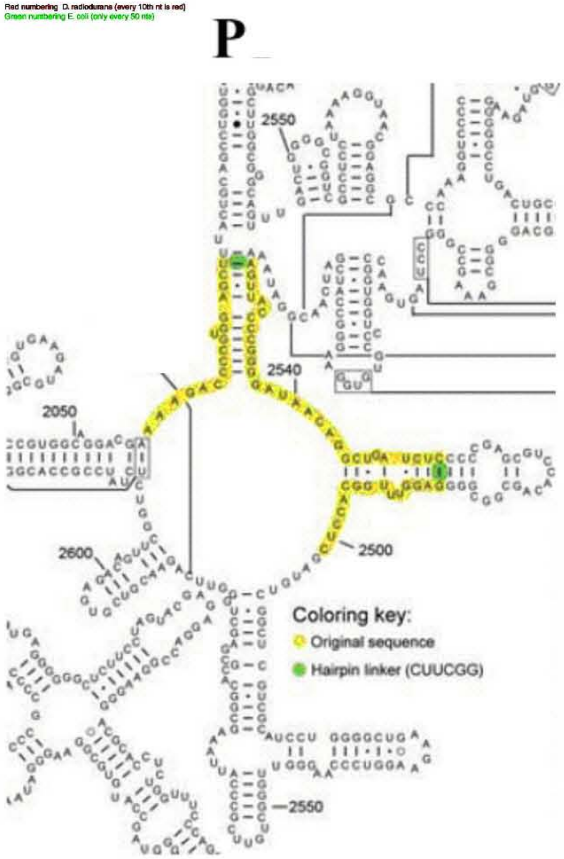
An example for a “primitive” version

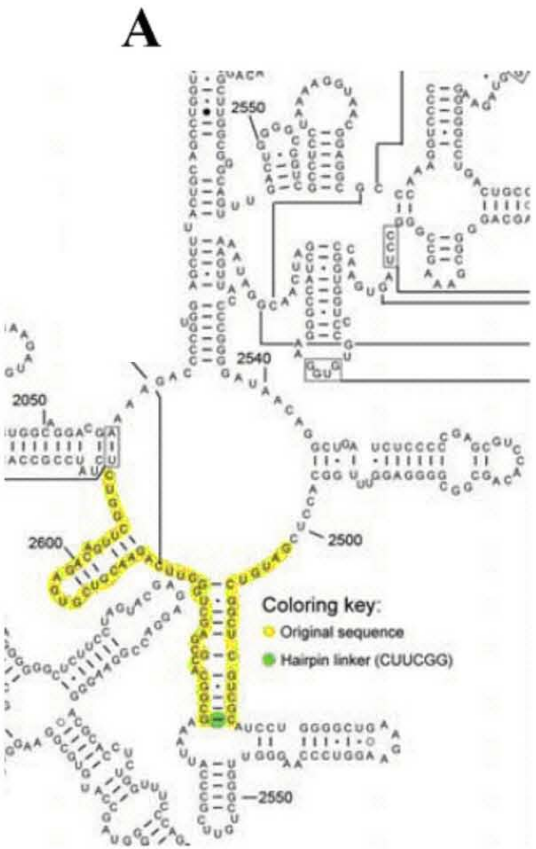


the two dimensional diagram of D50S

Proto-ribosome constructs:

An example for a "still existing" version





Non uniform tendency to dimerize.

The preference of selected sequences over very similar, albeit not identical ones, indicate that:

survival of the fittest and natural selection seem to play a major role in the prebiotic world, although these properties are commonly related to the evolution of species

nature Vol 457 | 19 February 2009

HYPOTHESIS

A hierarchical model for evolution of 23S ribosomal RNA

Konstantin Bokov[1] & Sergey V. Steinberg[1]

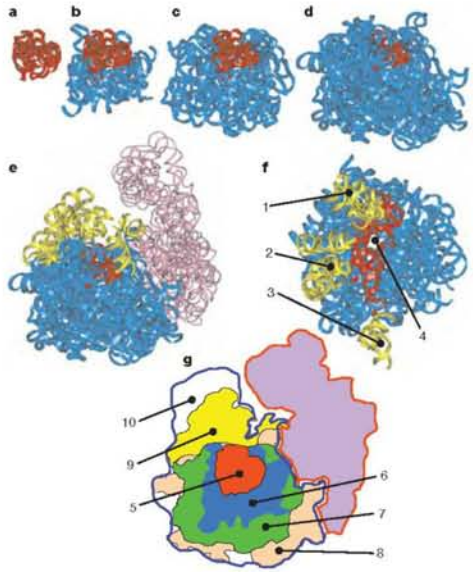
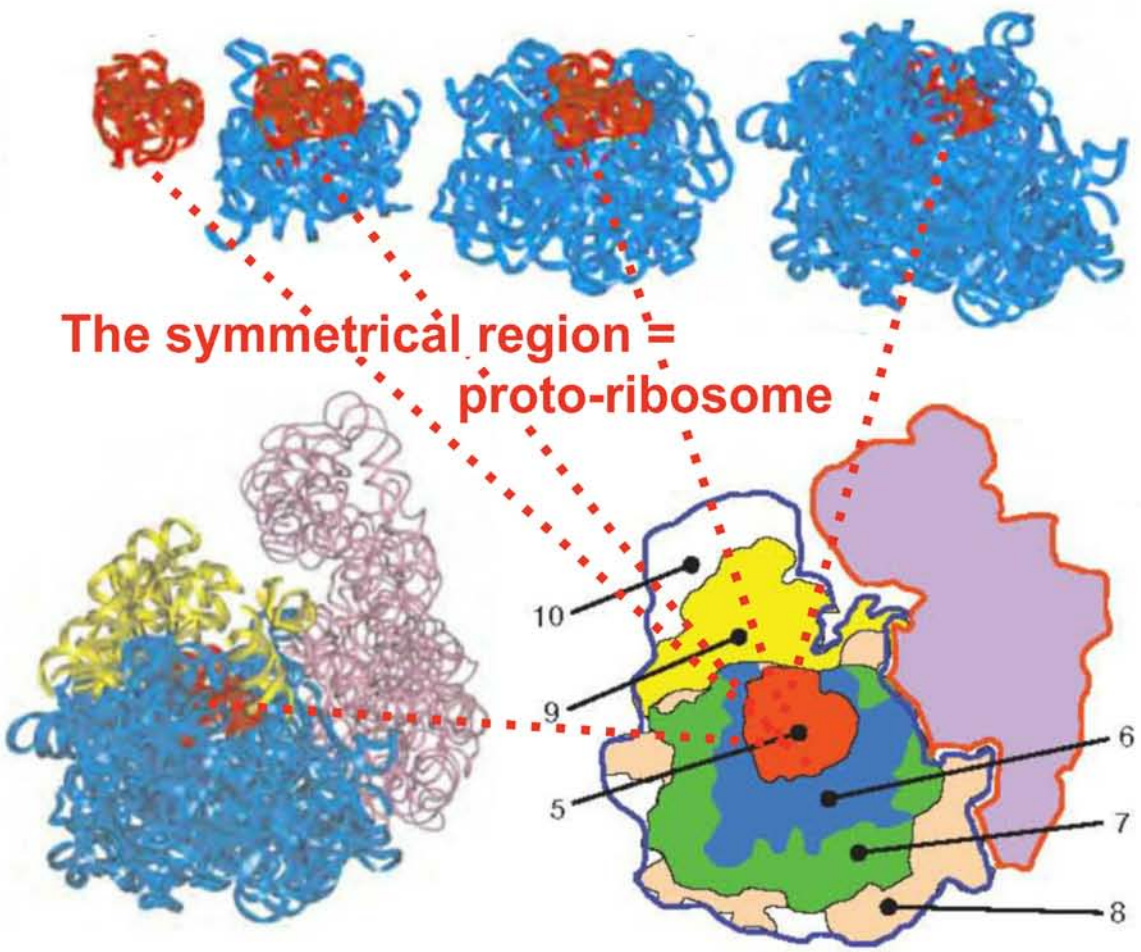
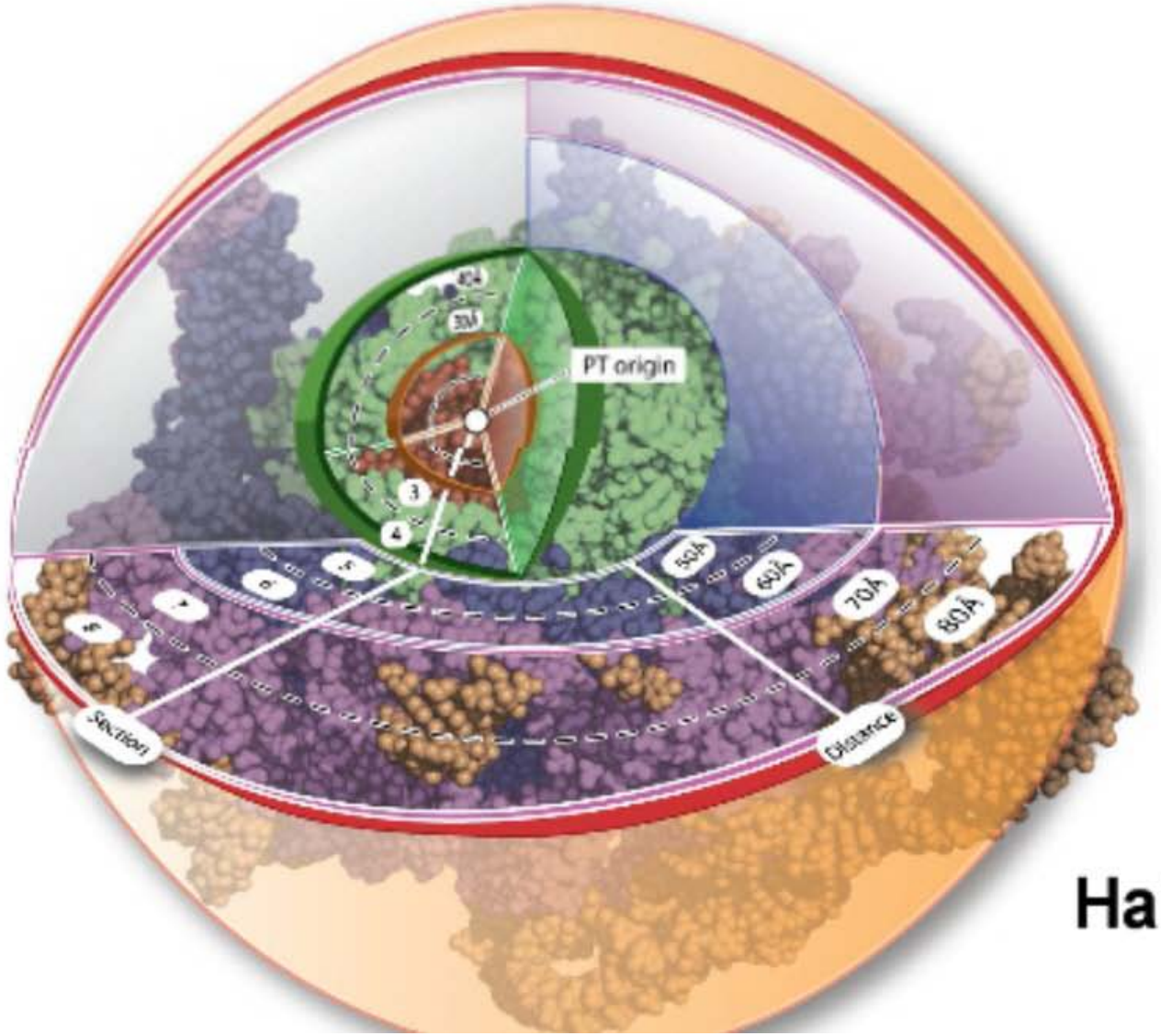


Figure 3 | The aggrandizement of the 23S rRNA structure during its evolution. **a**–**e**, the proto-ribosome with 0 (**a**), 8 (**b**), 20 (**c**), 50 (**d**) and all 59 (**e**) elements added. The proto-ribosome is red, elements forming the proto-ribosome foundation are blue, the protuberances are yellow, and 16S rRNA is purple. The complete list of the elements forming structures **a**–**e** is given in Supplementary Fig. 3. **f**, The top view of the 23S rRNA structure shown in **e**. **g**, The positions of the parts of 23S rRNA shown in **a**–**e** in the context of the whole ribosome. The structures of the 50S and 30S subunits are contoured by the blue and red line, respectively. 1–3 are the L7/L12, central and L1 protuberances, respectively; 4 is the exit channel; 5–9 are the structures shown in **a**–**e**, respectively; 10 is the part of 50S subunit that does not include 23S rRNA. This part is formed by ribosomal proteins and 5S rRNA.



Based on the pattern of A-minor interactions within the 23S RNA

Peeling the union:
Ribosomes are ancient molecular fossiles



Based on the pattern of the RNA tetra-loops within the 23S RNA

Haiso et al., 2009

Conclusions

Analysis of the early steps in protein biosynthesis enabled visualization of a continuous path from the primordial world to contemporary genetic translation.

It also indicates that the ribosome is a naturally occurring ribozyme that outlived the transition from the presumed pre-biotic 'RNA World' to contemporary life.

Still open questions (a selected list)

Was there an RNA world?

Why should RNA produce machine for making proteins? Or: Was the proto-ribosome an RNA machine, performing RNA needs, prior to the appearance of amino acids?

What was first: the genetic code or its products?

Nonenzymatic RNA Ligation in Water

Pino, Ciciriello, Costanzo and Di Mauro
J. Biol. Chem. 2008

Efficiency of a self-aminoacylating ribozyme: Effect of the length and base-composition of its 3' extension

Lehmann, Reichel, Buguin and Libchaber
RNA (2007), 13:1191–1197

Charging of tRNA with non-natural amino acids at high pressure

Giel-Pietraszuk Jan Barciszewski
FEBS Journal 273 (2006) 3014–3023

Aminoacyl-RNA Synthesis Catalyzed by an RNA

Ilangasekare, Sanchez, Nickles and Yarus
Science 267 (1995) 643-647

The existence of

well performing polypeptides

catalyzing fundamental reactions

and/or

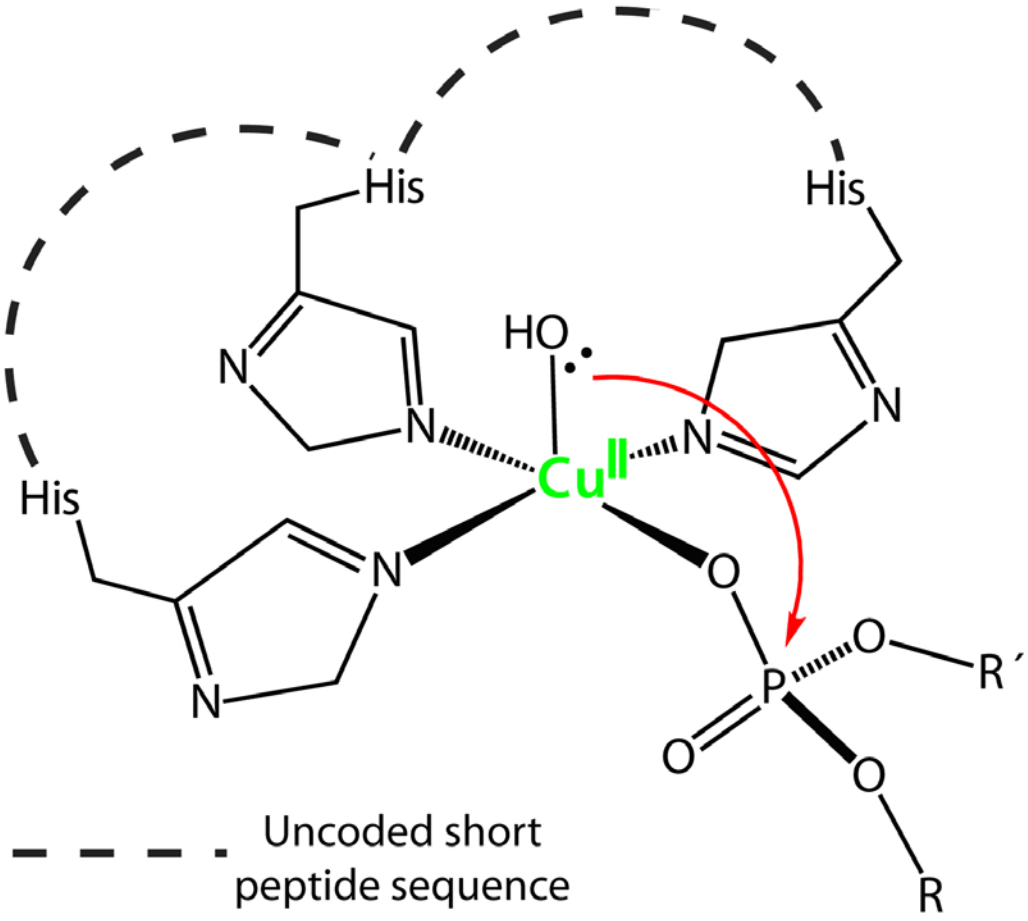
stabilizing the machines producing them

may have led to the

emergence of the genetic code

A hypothetical non-coded "enzyme" that can be useful for RNA metabolism

Active site formed by enhanced local concentration of imidazole rings



First amino acid?

Glycine?

Lysine and arginine?

Histidine?

First amino acid?

Glycine? Because it is the simplest

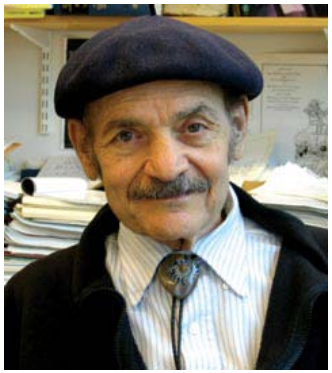
Lysine and arginine? Because they are basic, thus interacting with RNA

Histidine? Because of its imidazole that is synthesized in a pathway similar to RNA bases; and because imidazole can act as a catalyst

Those who believed in us

H.G. Wittmann, & later, Franceschi , MPI, Berlin

Michel Sela, Weizmann inst.



Alexander Rich MIT

Christian Anfinsen, NIH

Sir John Kendrew EMBL

Thanks are due to

he Weizmann Institute

for keeping up with me

IH, Max Planck Society and the WI Kimmelman Center

for financing my "dreams"

he members of my group

for their devotion and enthusiasm

in good and bad times

Ms. Helen Kimmel, who paved the way with us

Thanks to the Max-Planck Ribosome Research Unit at the DESY, Hamburg

X-ray data were collected initially at BW6/EMBL/DESY, KEK CHESS/Cornell ans SSRL/Stanford

High resolution data were collected at ID14/4 and ID29 ESRF/EMBL, Grenoble, France and 19ID/APS/ Argonne Nat lab, USA

Funding throughout 1980-2009: NIH, MPG, BMBF, GIF, HFSP, BSF, MINERVA, ESA, AFIRST

and, more than to anybody else,

to MY FAMILY

who supported me throughout, with
no questions or complains
despite my frequent "disappearances"
and although, at times,
my mind was not solely with them....