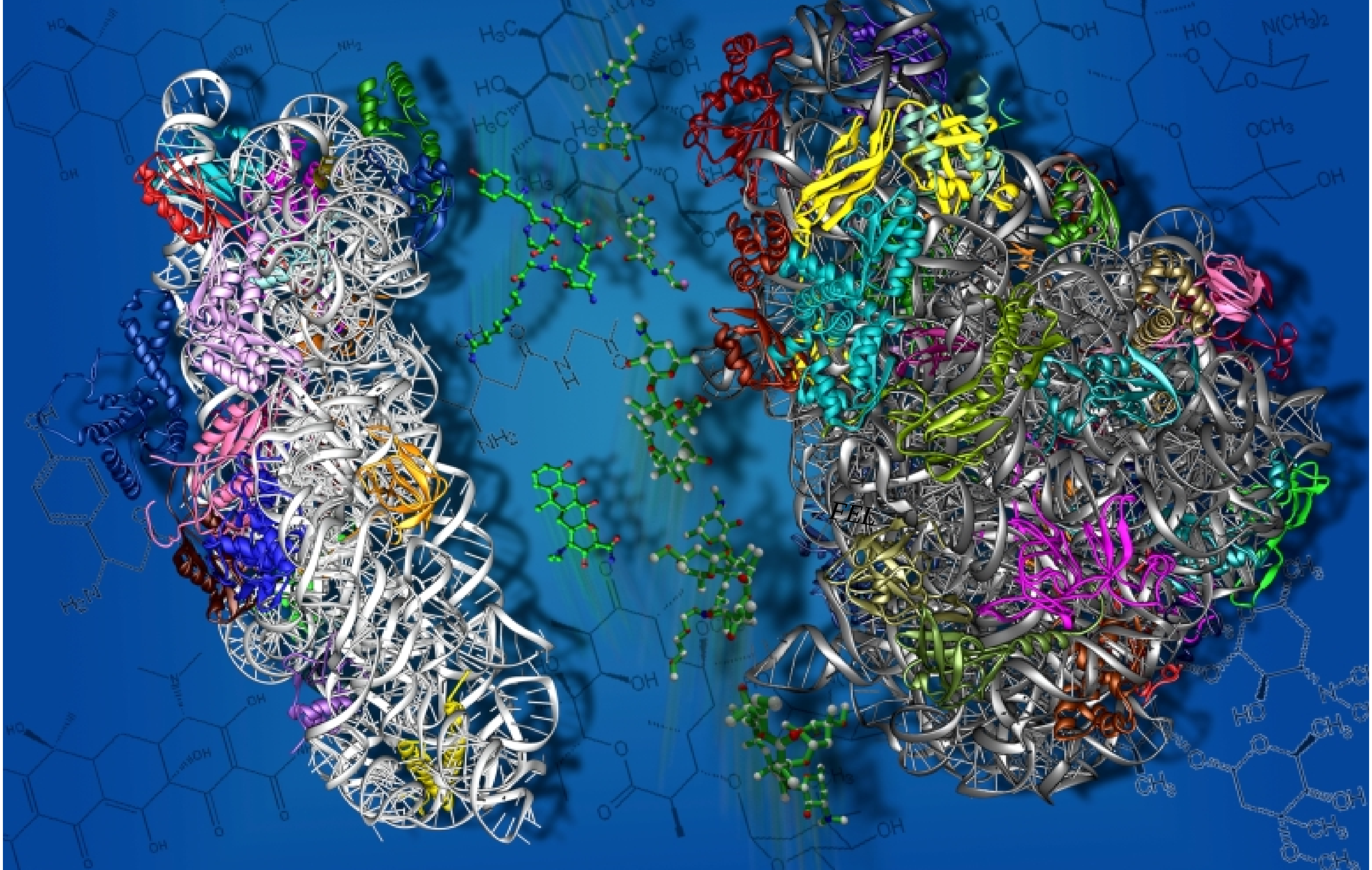
Structure of the ribosome



Frank Schluenzen - MPG-ARS/Hamburg

Ribosomes ...

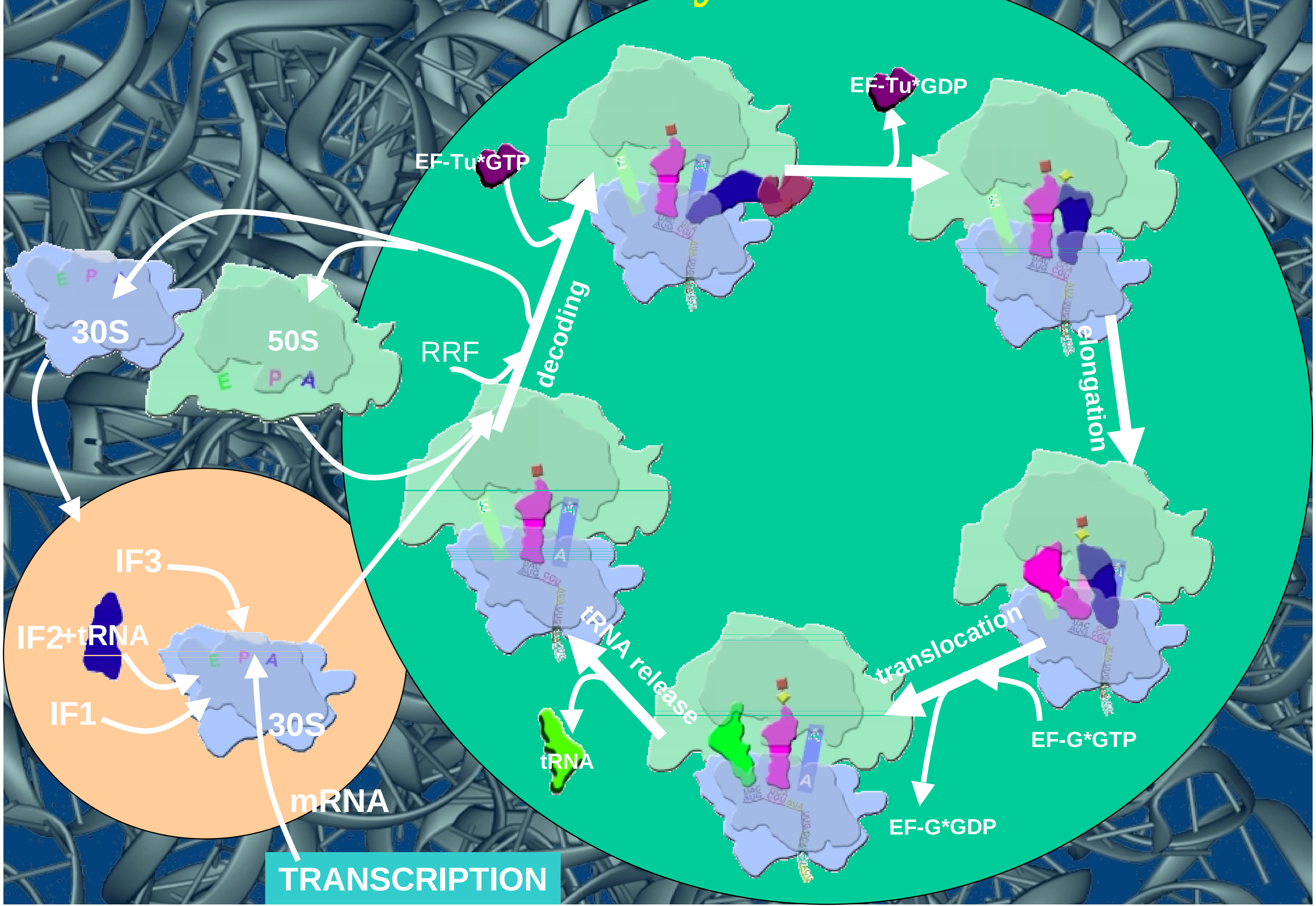
- are universal cell organelles

- Protein-Biosynthesis: translate the transcribed copy of the genetic code contained on the mRNA into the composition of proteins

- produce (almost) all active cell constituents

- comprise ~25% of cell mass

Protein-Biosynthesis



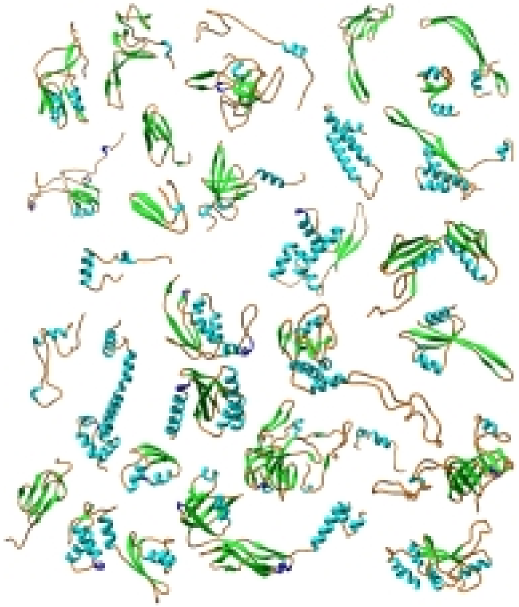
30S
E P A
50S
E P A
RRF
decoding
EF-Tu*GTP
EF-Tu*GDP
elongation
translocation
EF-G*GTP
EF-G*GDP
tRNA release
tRNA
IF3
IF2+tRNA
IF1
30S
mRNA
TRANSCRIPTION

70S Ribosome

=

50S

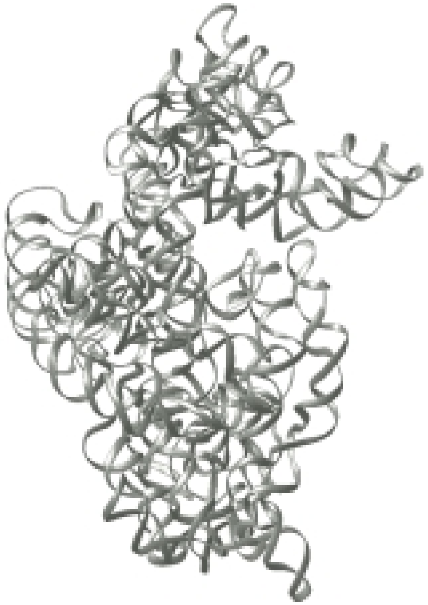
23S+5S rRNA



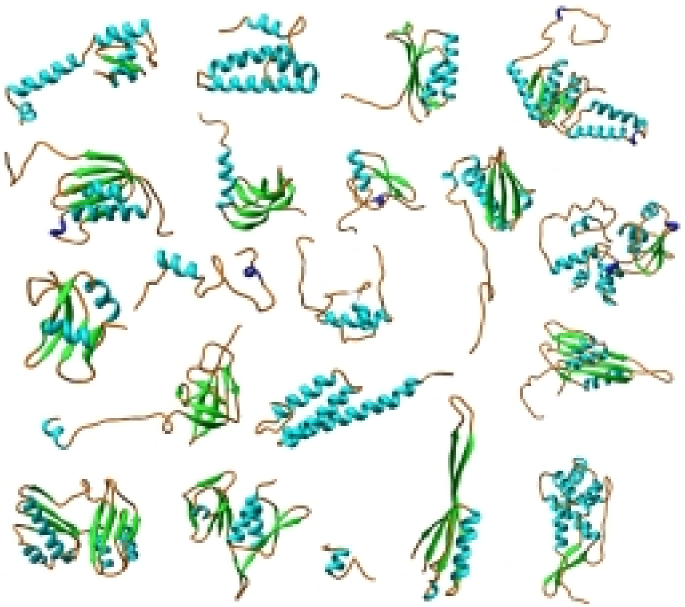
~30 proteins

+

30S



16S rRNA

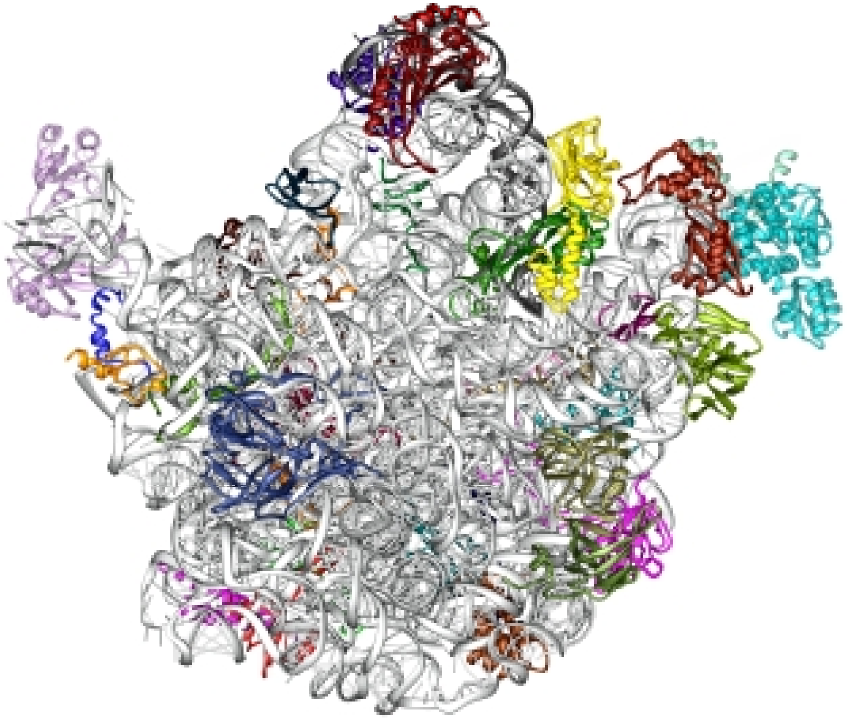


~20 proteins

70S Ribosome

||

50S



Peptide-Bond
GTPase
Exit-Tunnel

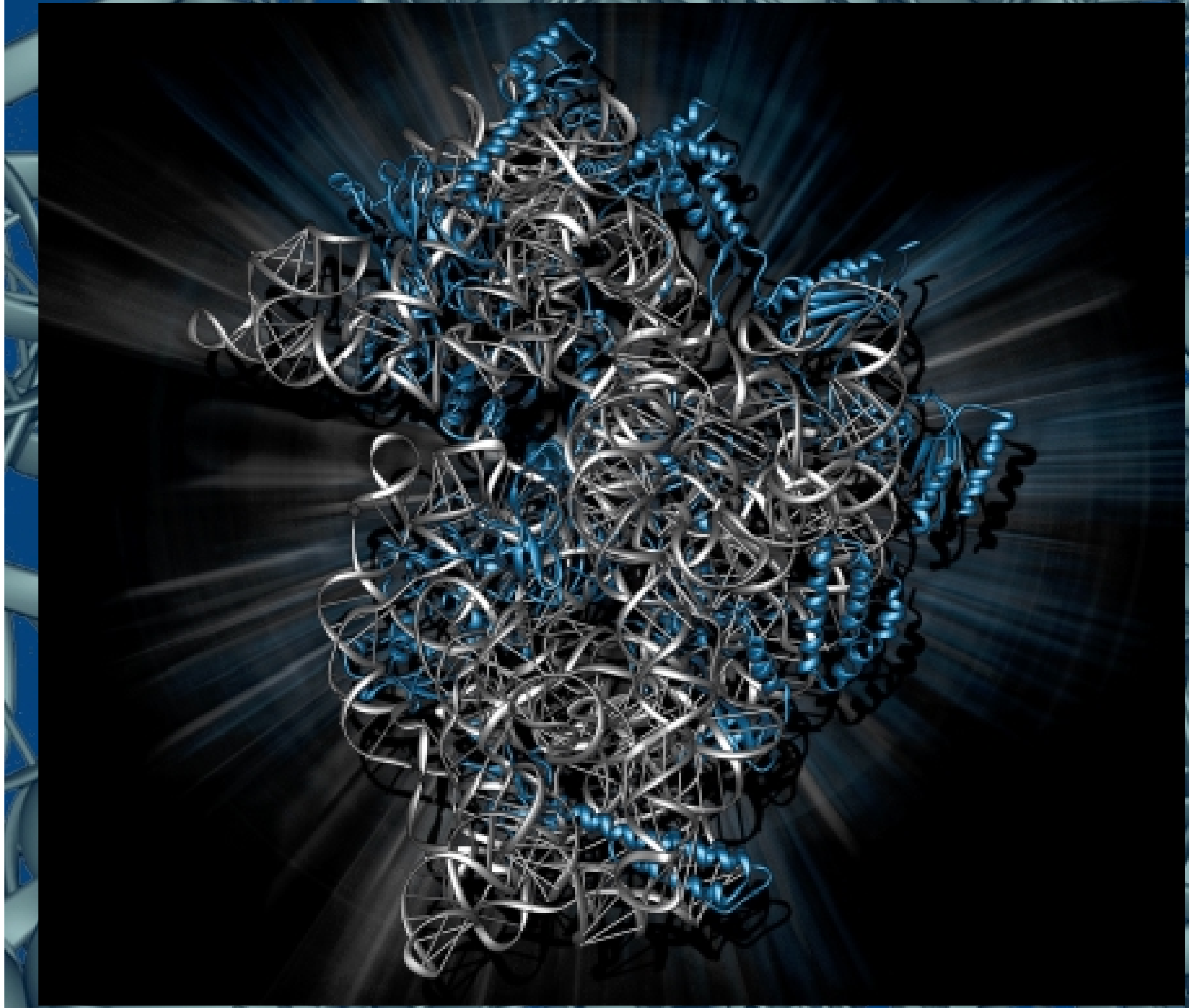
+

30S

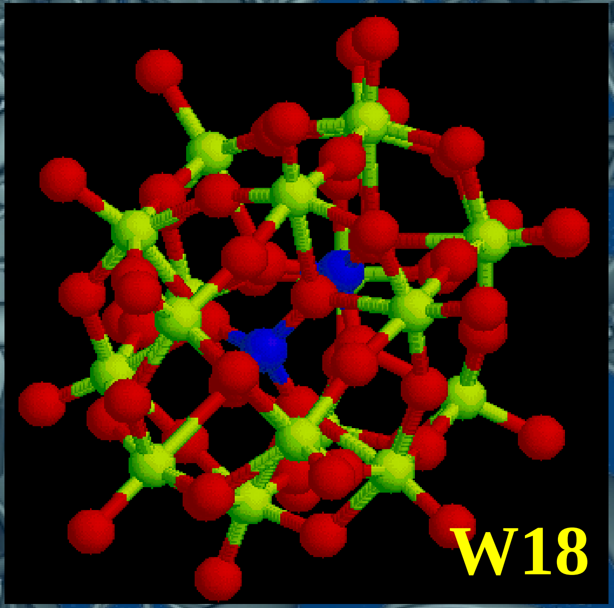
Decoding
Initiation
Termination
Recycling

Structure of the 30 ribosomal subunit

from *Thermus thermopilus*

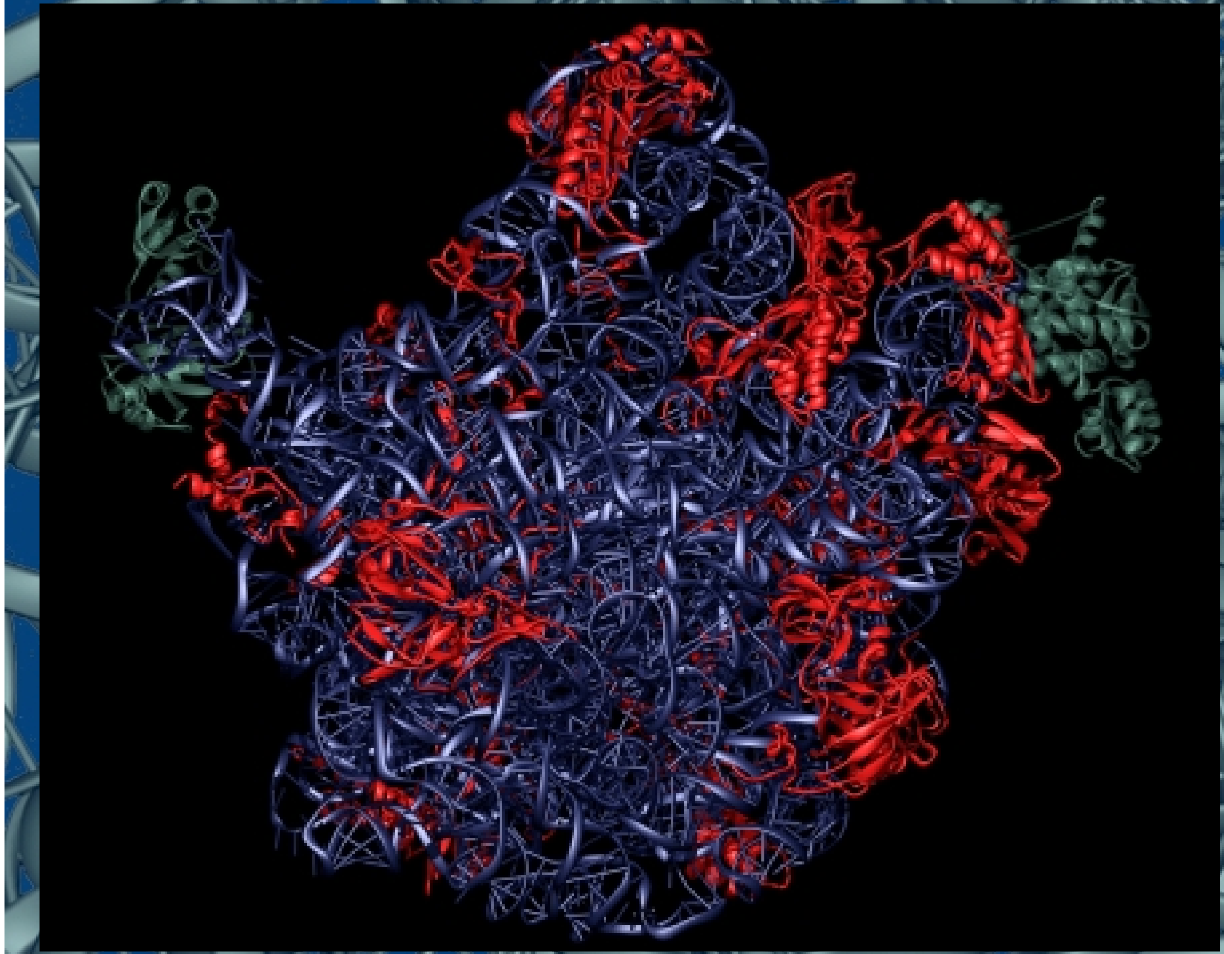


- **16S rRna = 1500 bases**
- **20 ribosomal proteins**
- **Resolution: 3.0Å**
- **Phasing:** SIRAS with W18



Structure of the 50 ribosomal subunit

from *Deinococcus radiodurans*



- **23S rRna = 2800 bases**
- **5S rRna =   120 bases**
- **30 ribosomal proteins**

- **Resolution: 3.0Å**

- **Phasing:** MR

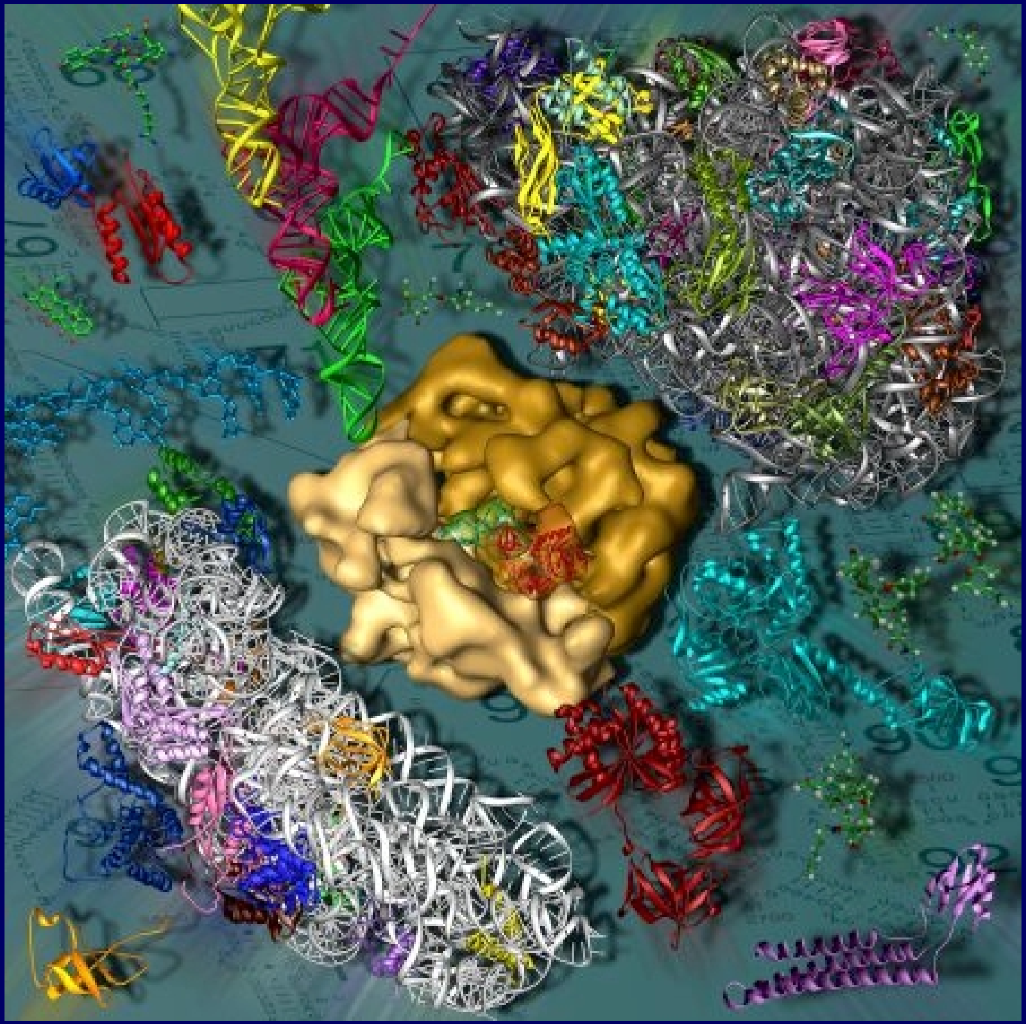
Infrastructure

Biophysical

NMR
Exafs
SAS
CD
MS
TFM
…

Biochemical

Labeling
Mutants
Antibiotics
Antibodies
PhotoCrosslink
Maldi
…



Neutron Scattering

Electron Microscopy

Xray-Crystallography

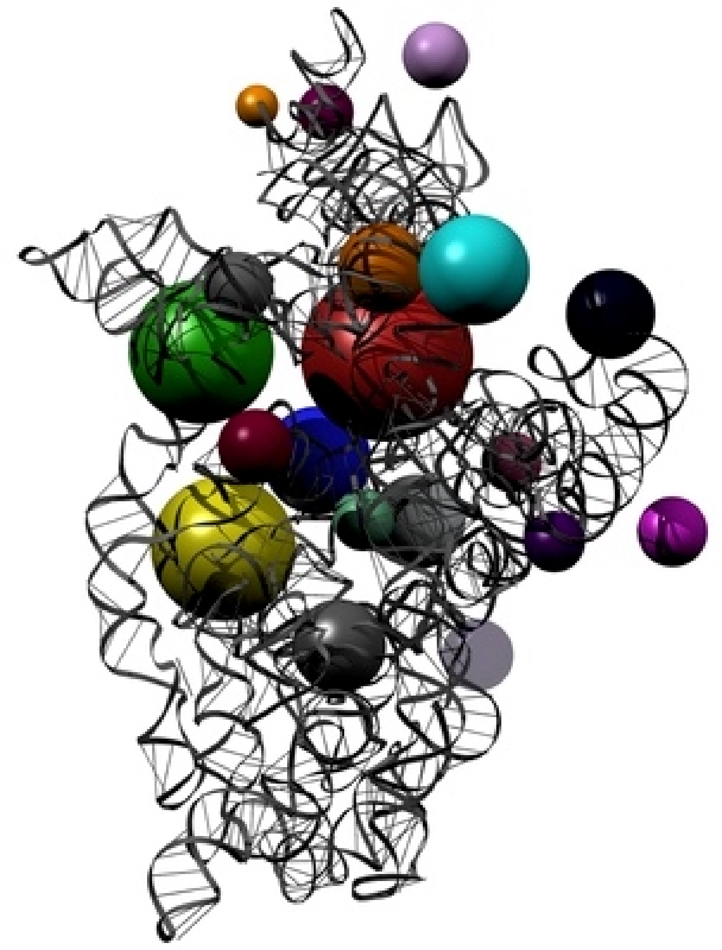
Neutron Diffraction

- **Pairwise deuteration**
- **Reconstitution of ribosomes**
- **Relative distance by N.D.**
- **c.m. and radii of gyration**

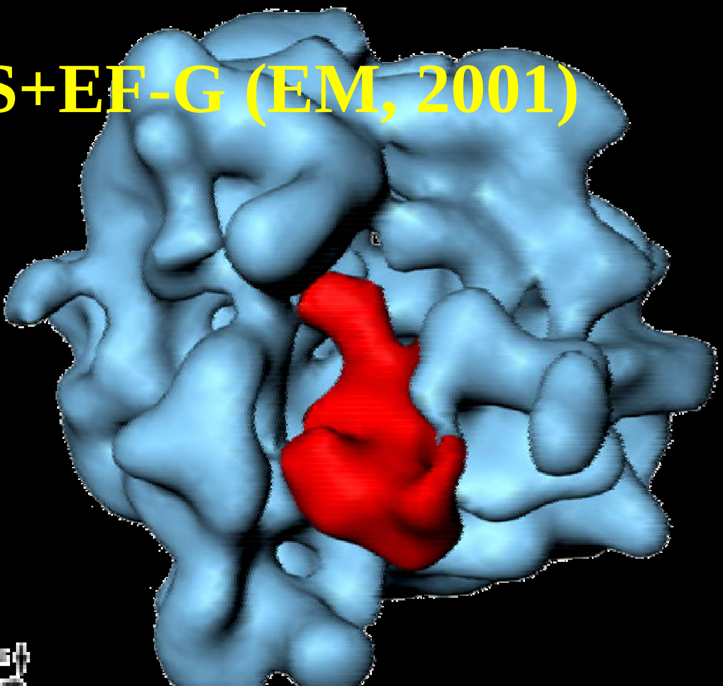
Ramakrishnan & Moore, JMB 1981

More recent & more accurate: Spin-Contrast-Variation yields precise distribution of ribosomal proteins in the 50S subunit

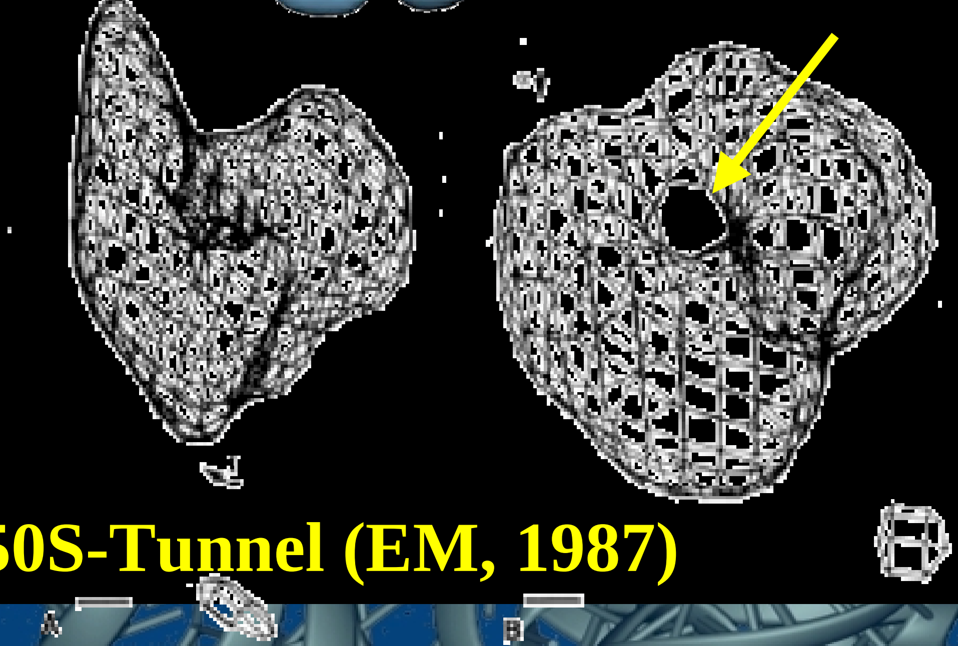
Willumeit et al., BBA, 2001



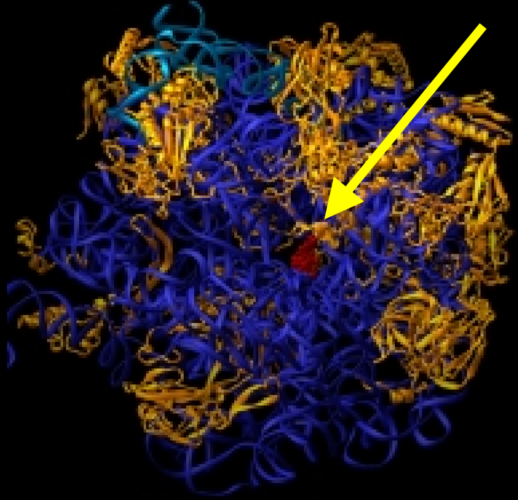
Electron Microscopy



50S+EF-G (EM, 2001)

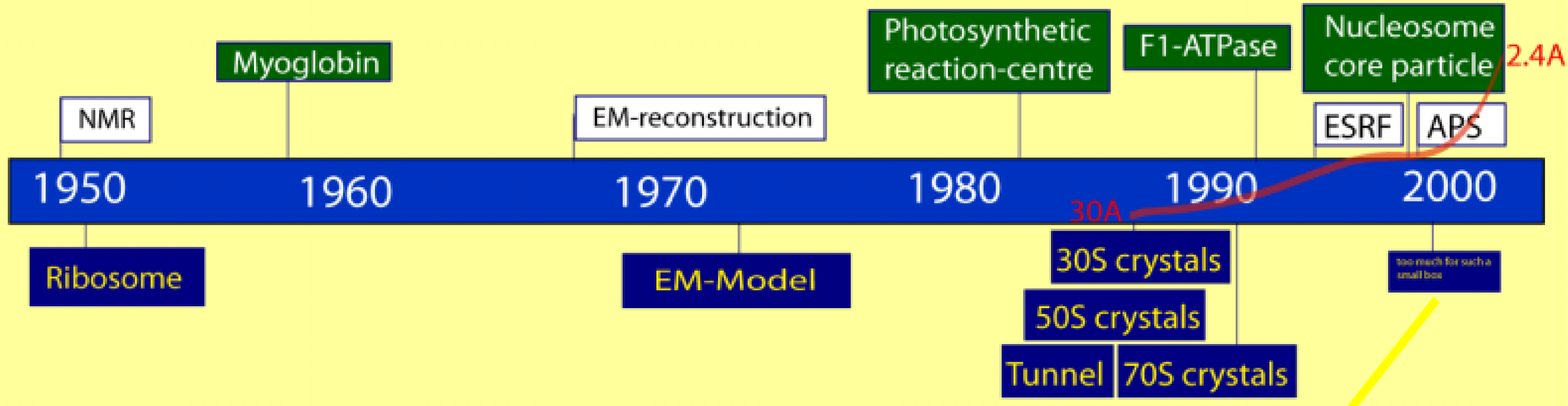


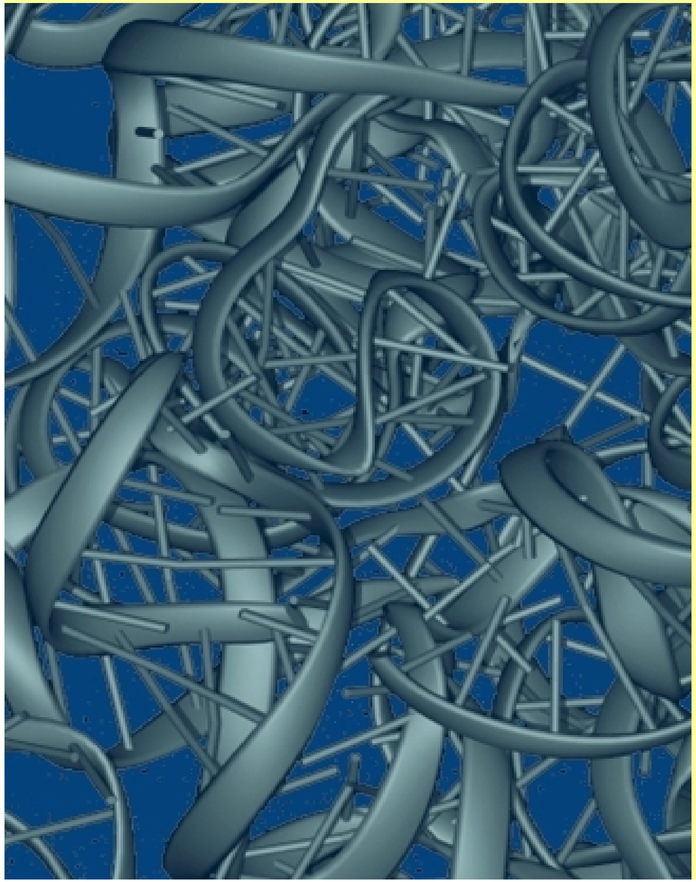
50S-Tunnel (EM, 1987)



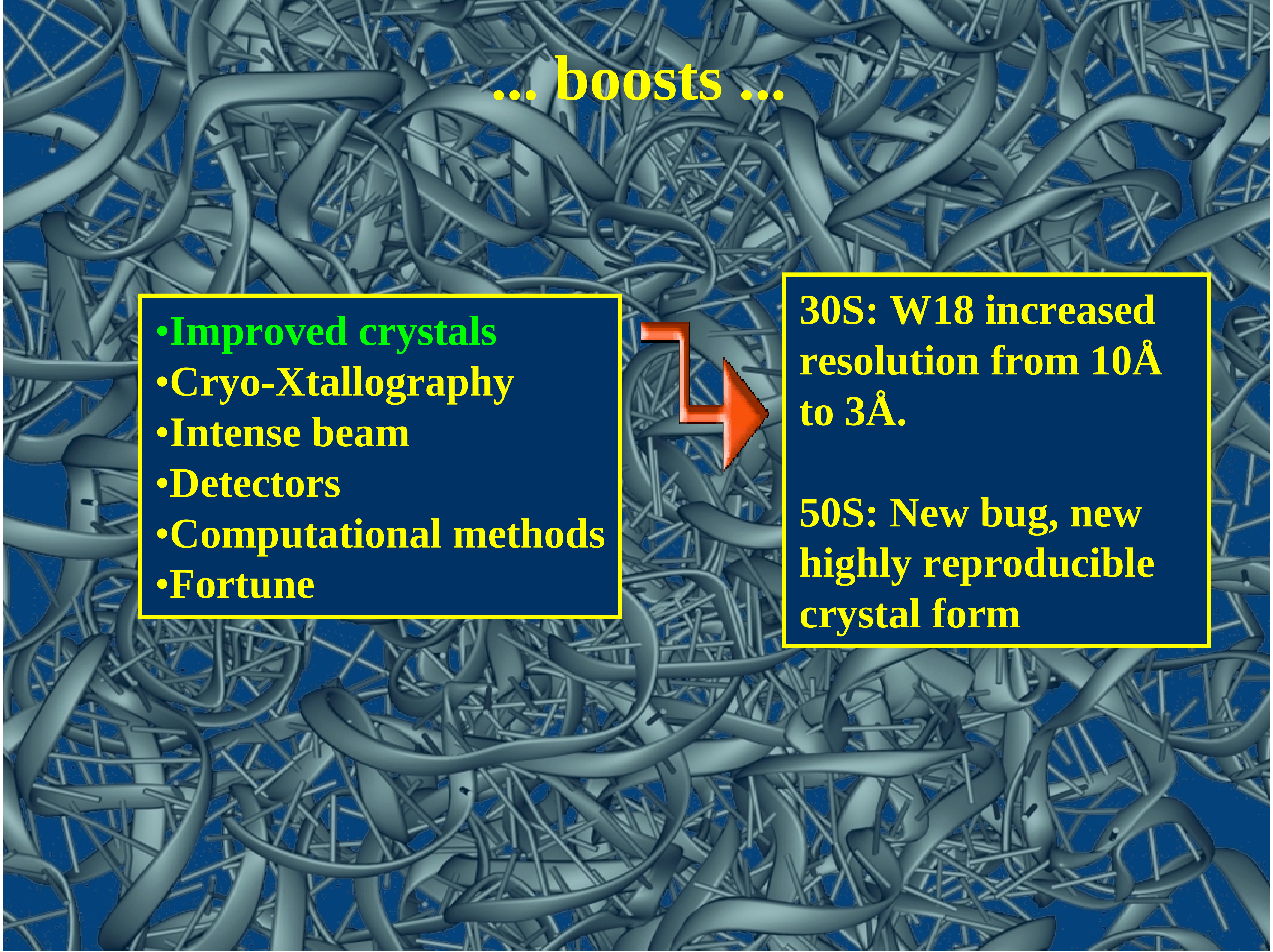
50S-Tunnel (Xray, 2001)

Timeline





1997: First experiment at ESRF.
First high resolution data of 30S.
1999: Structure of 30S.
1999: First experiment at APS.
2000: Structure of 30S + IF3
Structure of 30S + tetracycline
Structure of 30S + edeine
2001: First high resolution data of 50S.
2001: Structure of 50S.
Structure of 50S + Macrolides, Clindamycin, Chloramphenicol
2002: Structure of 50S + tRNA-analogs
Structure of 50S + 32 different antibiotics.



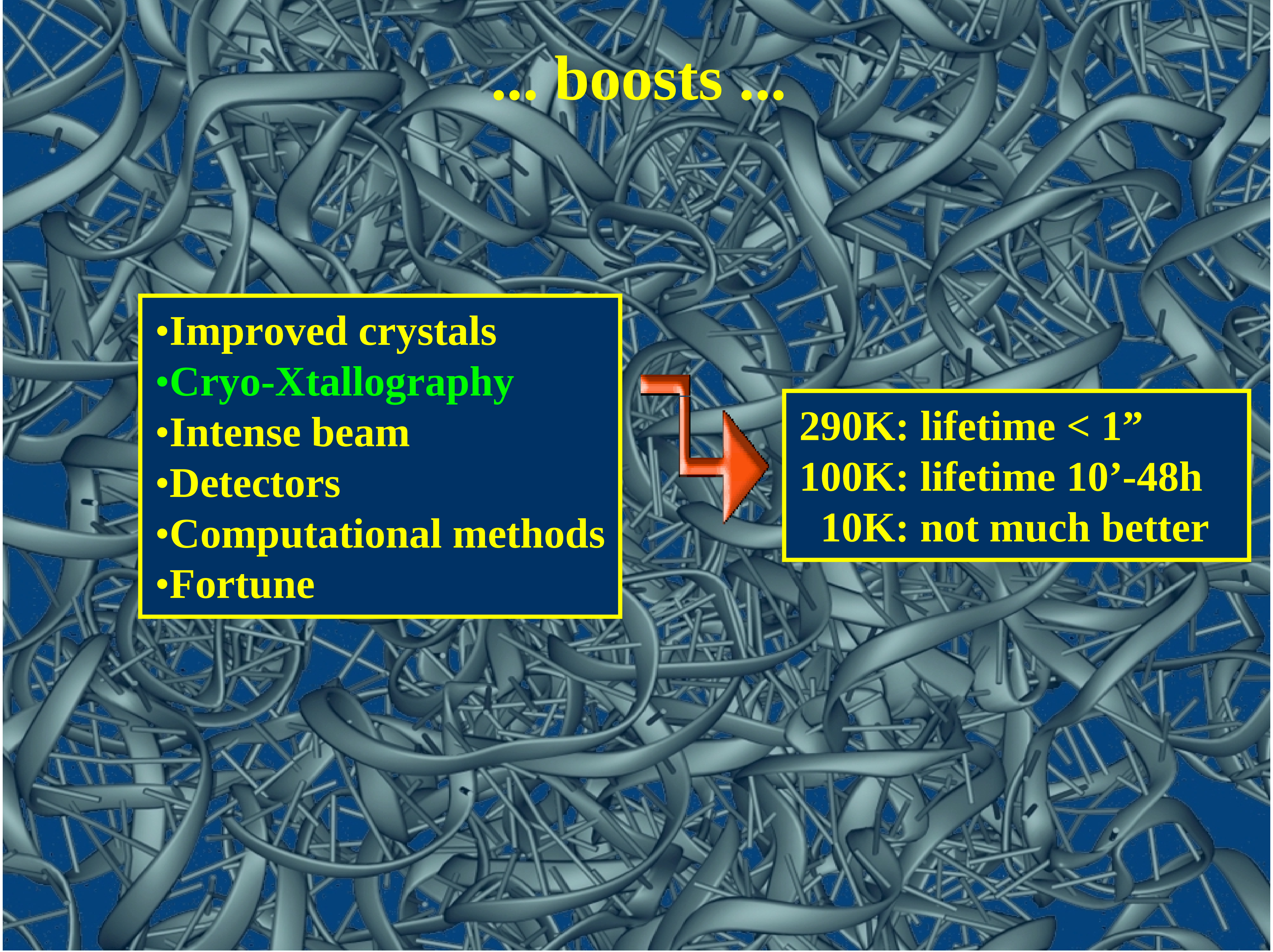
... boosts ...

- **Improved crystals**
- **Cryo-Xtallography**
- **Intense beam**
- **Detectors**
- **Computational methods**
- **Fortune**

30S: W18 increased resolution from 10Å to 3Å.

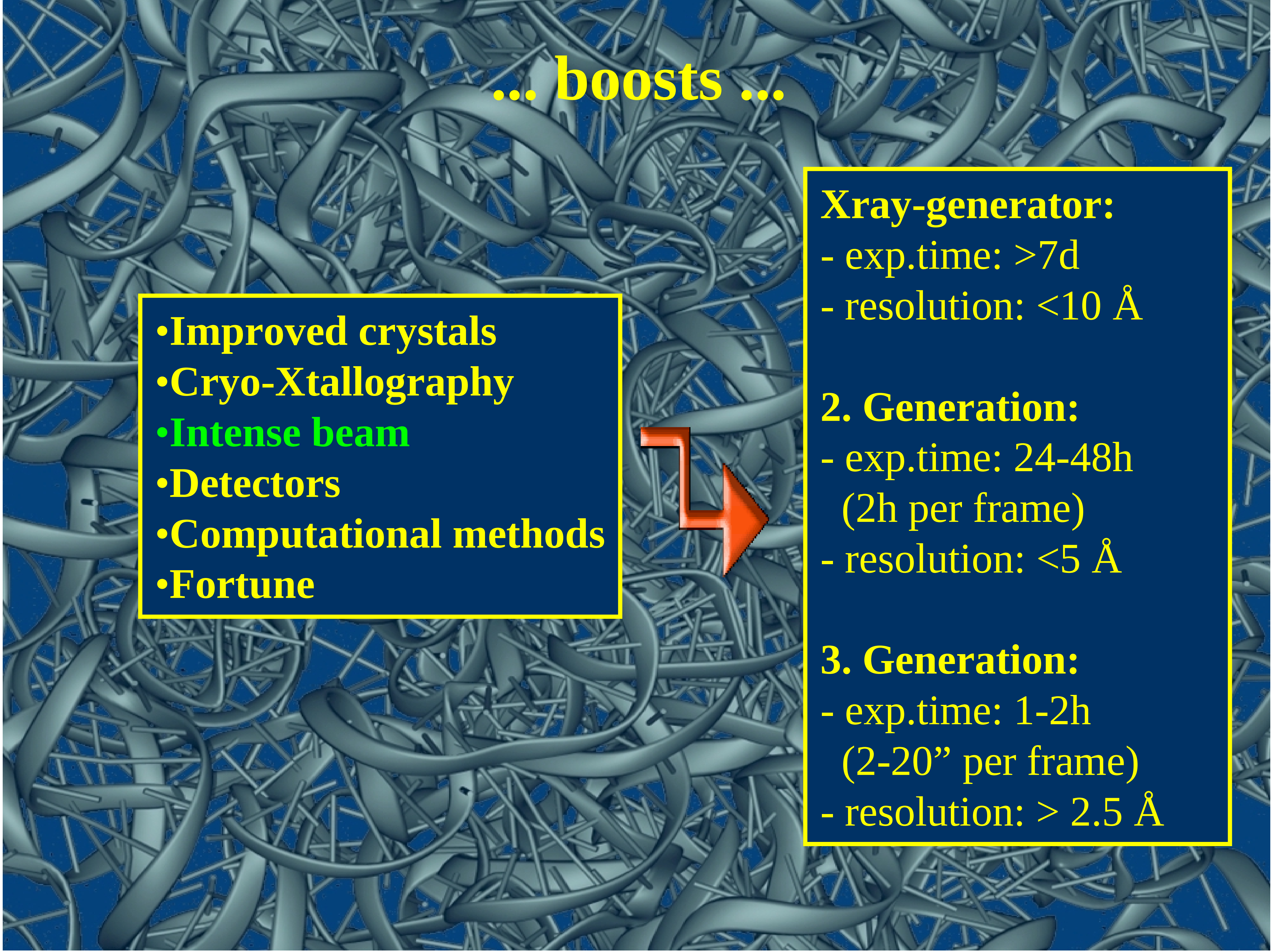
50S: New bug, new highly reproducible crystal form

... boosts ...



- •Improved crystals
- •Cryo-Xtallography
- •Intense beam
- •Detectors
- •Computational methods
- •Fortune

290K: lifetime < 1”
100K: lifetime 10’-48h
10K: not much better



... boosts ...

- •Improved crystals
- •Cryo-Xtallography
- •Intense beam
- •Detectors
- •Computational methods
- •Fortune

Xray-generator:
- exp.time: >7d
- resolution: <10 Å

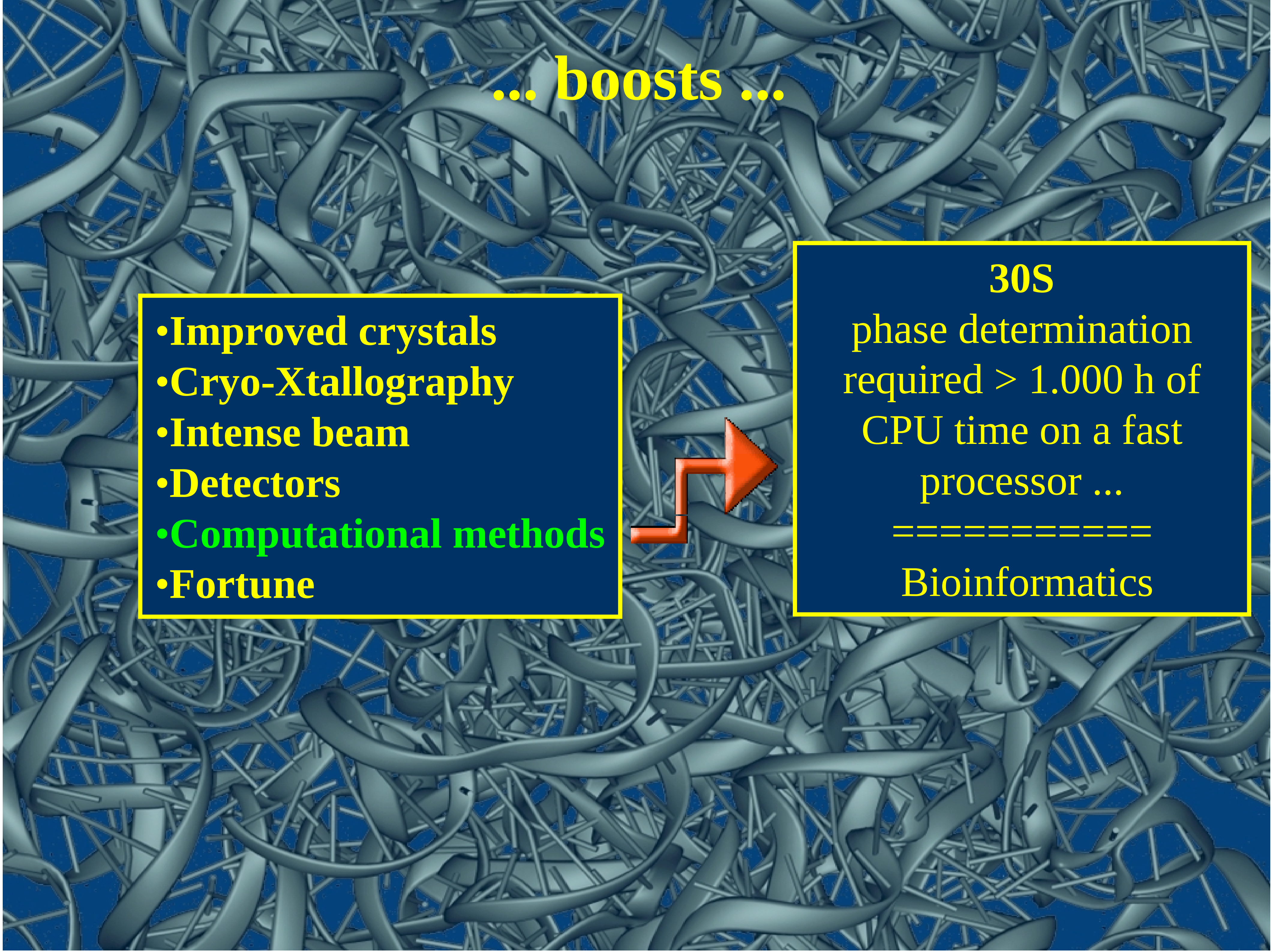
2. Generation:
- exp.time: 24-48h
  (2h per frame)
- resolution: <5 Å

3. Generation:
- exp.time: 1-2h
  (2-20” per frame)
- resolution: > 2.5 Å

... boosts ...

- Improved crystals
- Cryo-Xtallography
- Intense beam
- Detectors
- Computational methods
- Fortune

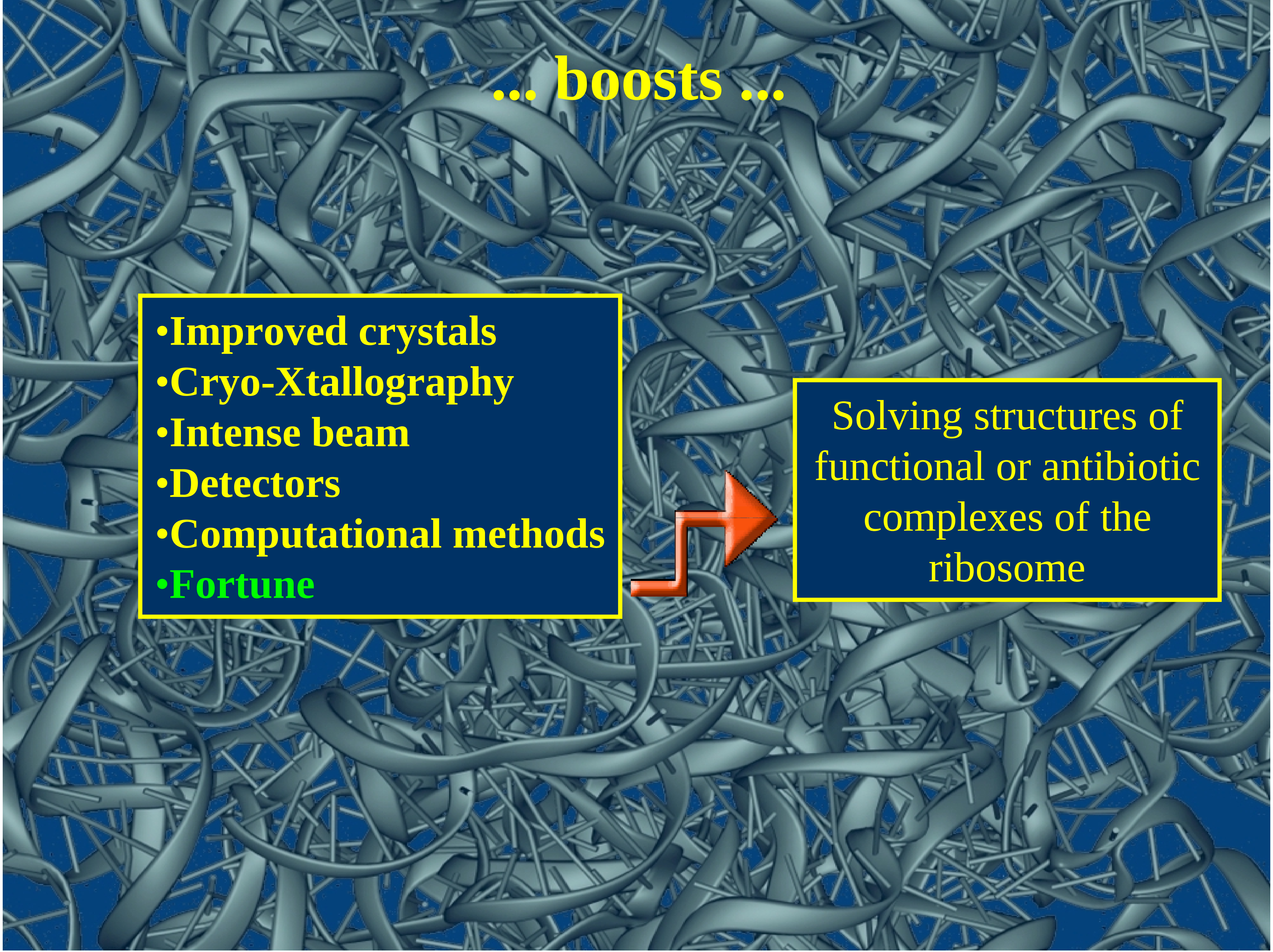
- **xray film**
  - spatial resolution <10µm
  - *unlimited* size
  - dynamic range
  - slow, nonlinear response
  - error prone
- **offline/online IP**
  - large area
  - large readout error
  - limited exposure time
  - bit/very slow
  - great dynamic range
- **CCD**
  - limited area
  - good dynamic range
  - fast & efficient
  - very limited exp.time



... boosts ...

- **Improved crystals**
- **Cryo-Xtallography**
- **Intense beam**
- **Detectors**
- **Computational methods**
- **Fortune**

30S
phase determination
required > 1.000 h of
CPU time on a fast
processor ...
============
Bioinformatics

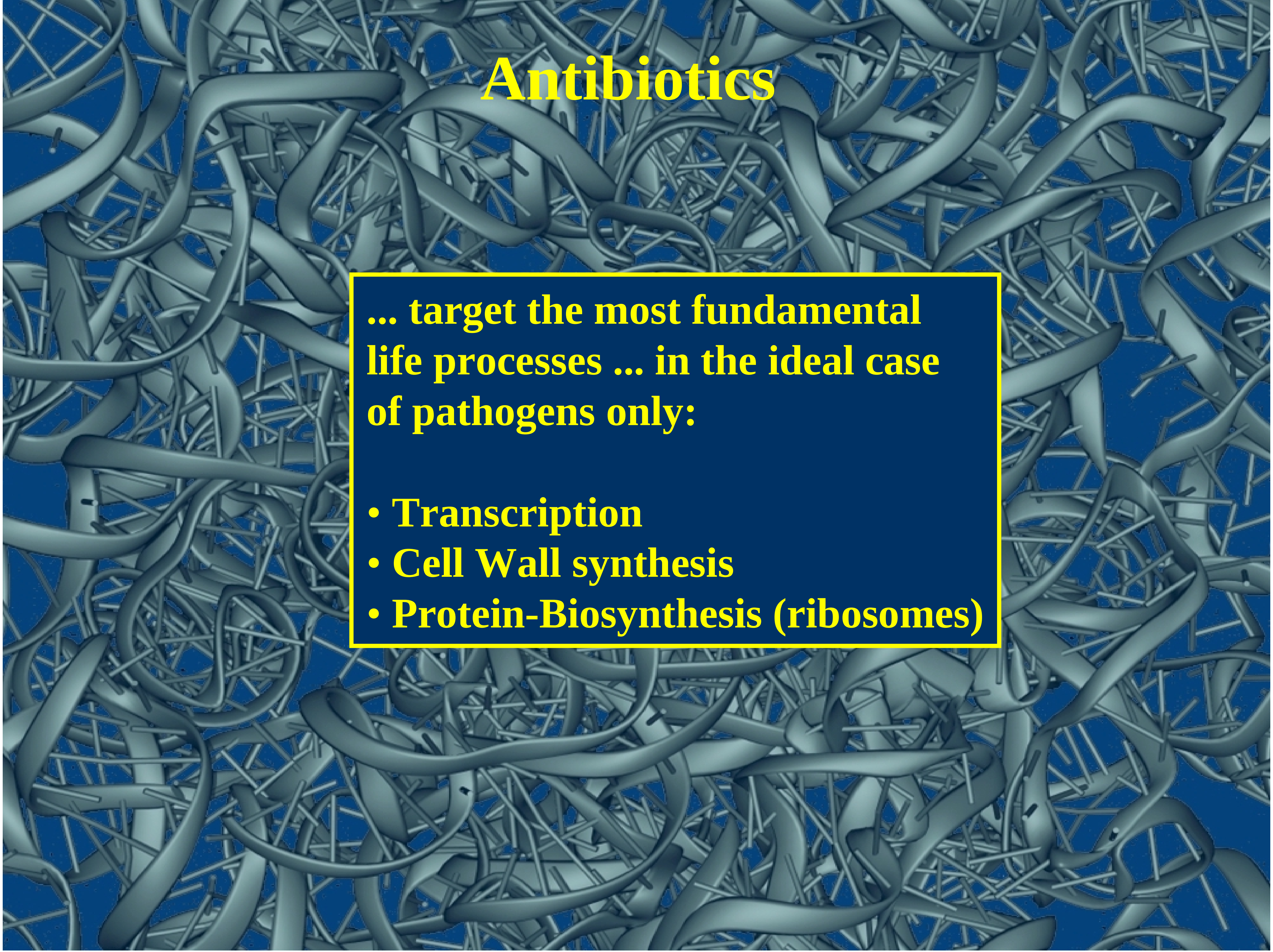


... boosts ...

- **Improved crystals**
- **Cryo-Xtallography**
- **Intense beam**
- **Detectors**
- **Computational methods**
- **Fortune**

Solving structures of functional or antibiotic complexes of the ribosome

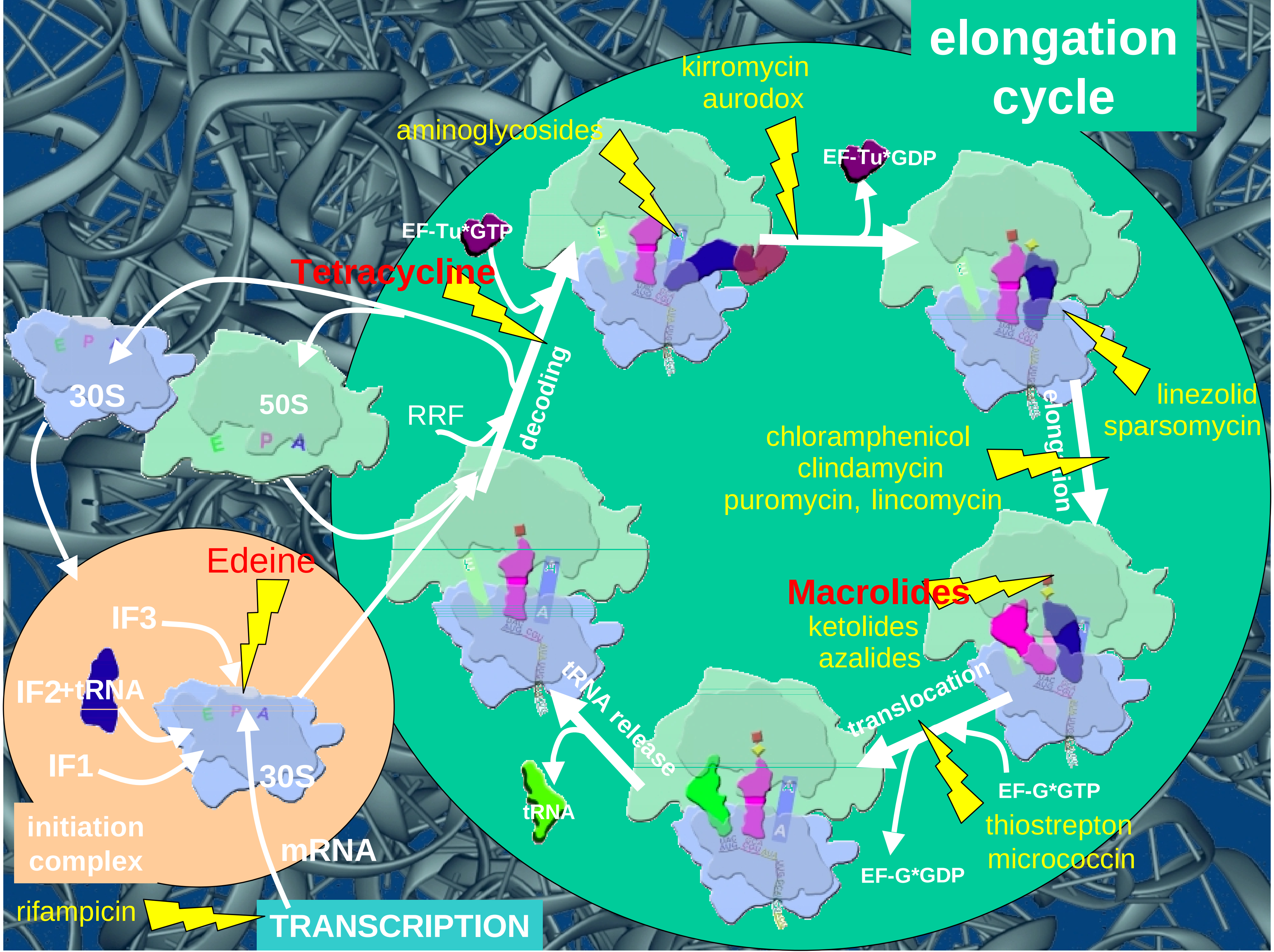
Antibiotics



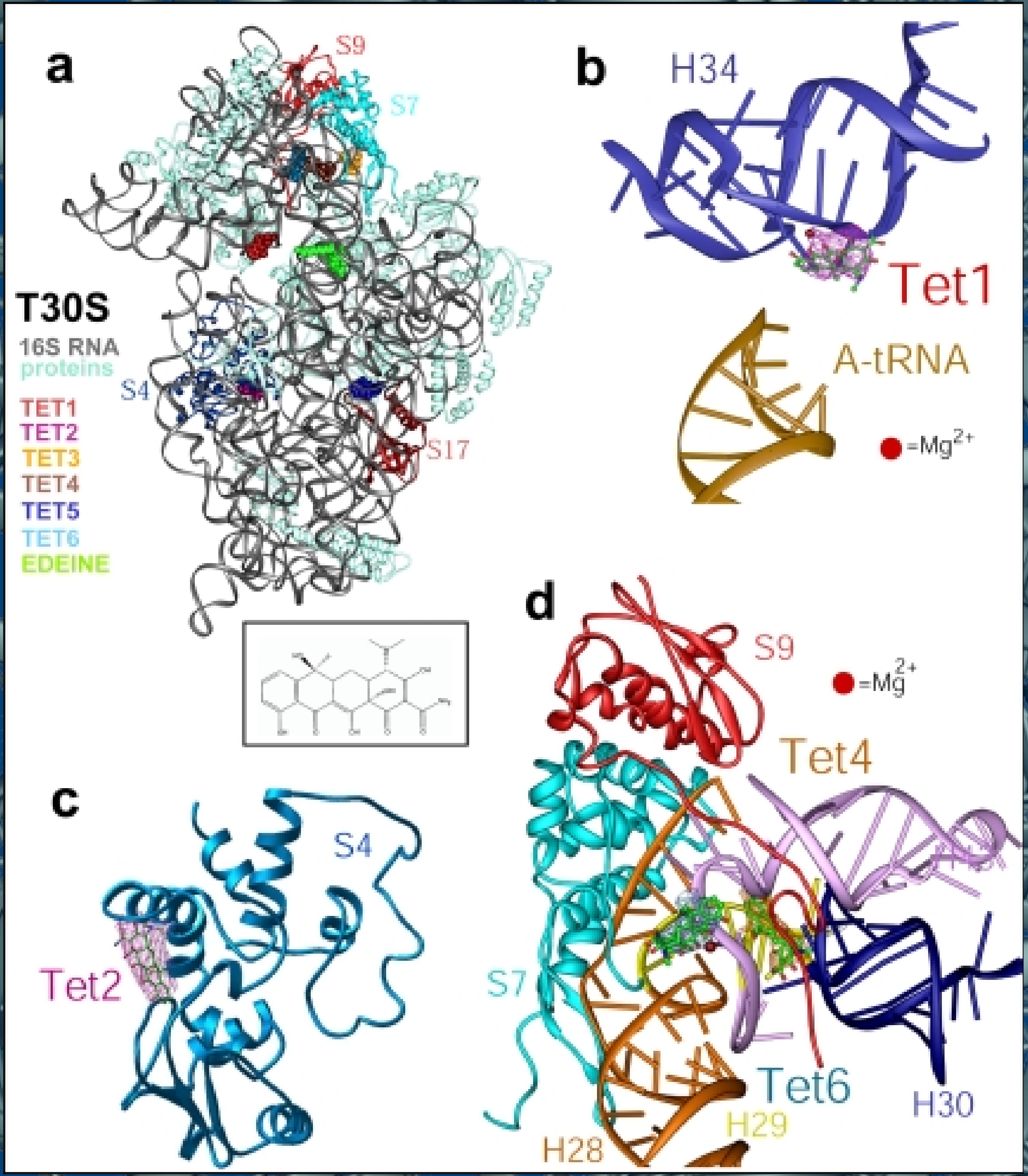
... target the most fundamental life processes ... in the ideal case of pathogens only:

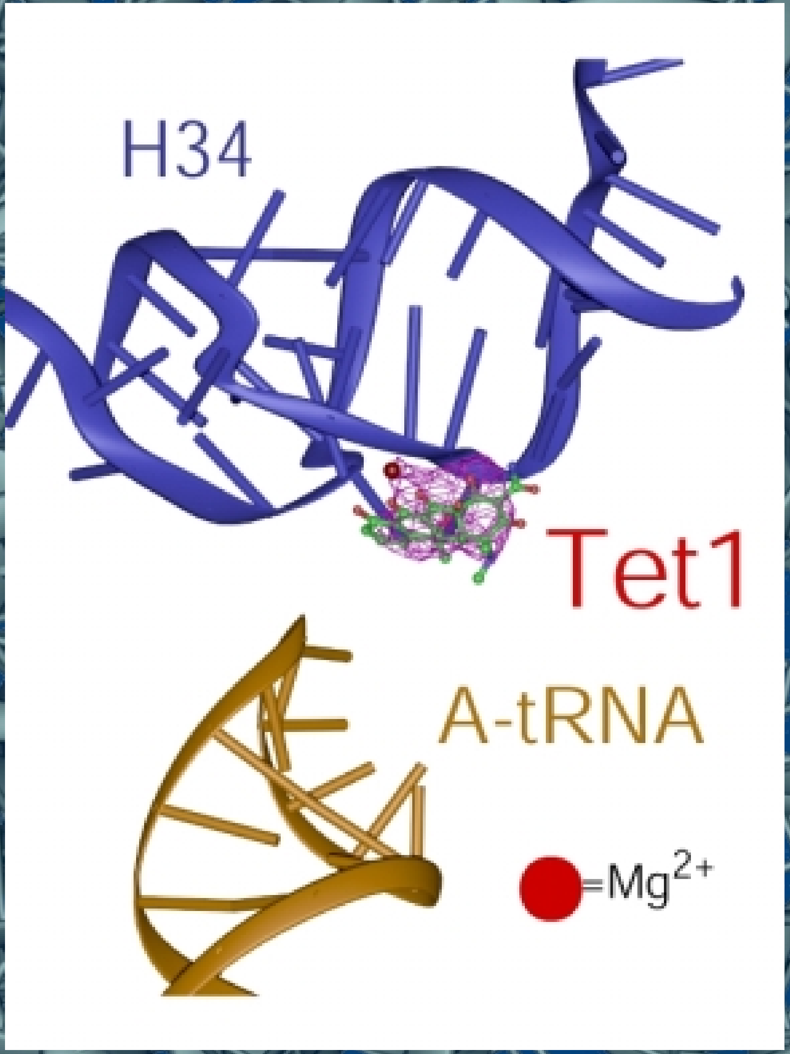
- Transcription
- Cell Wall synthesis
- Protein-Biosynthesis (ribosomes)

elongation cycle



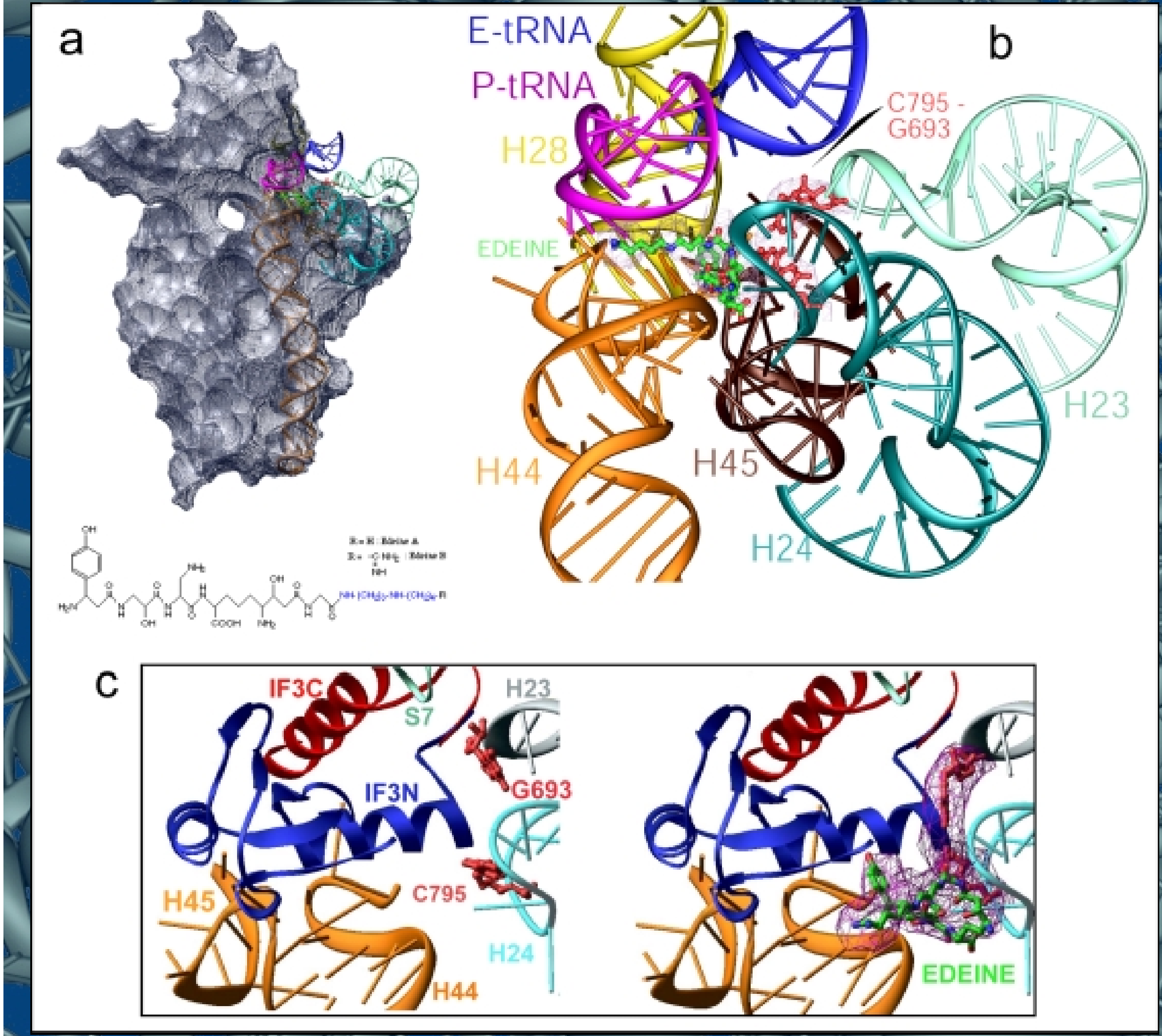
Tetracycline





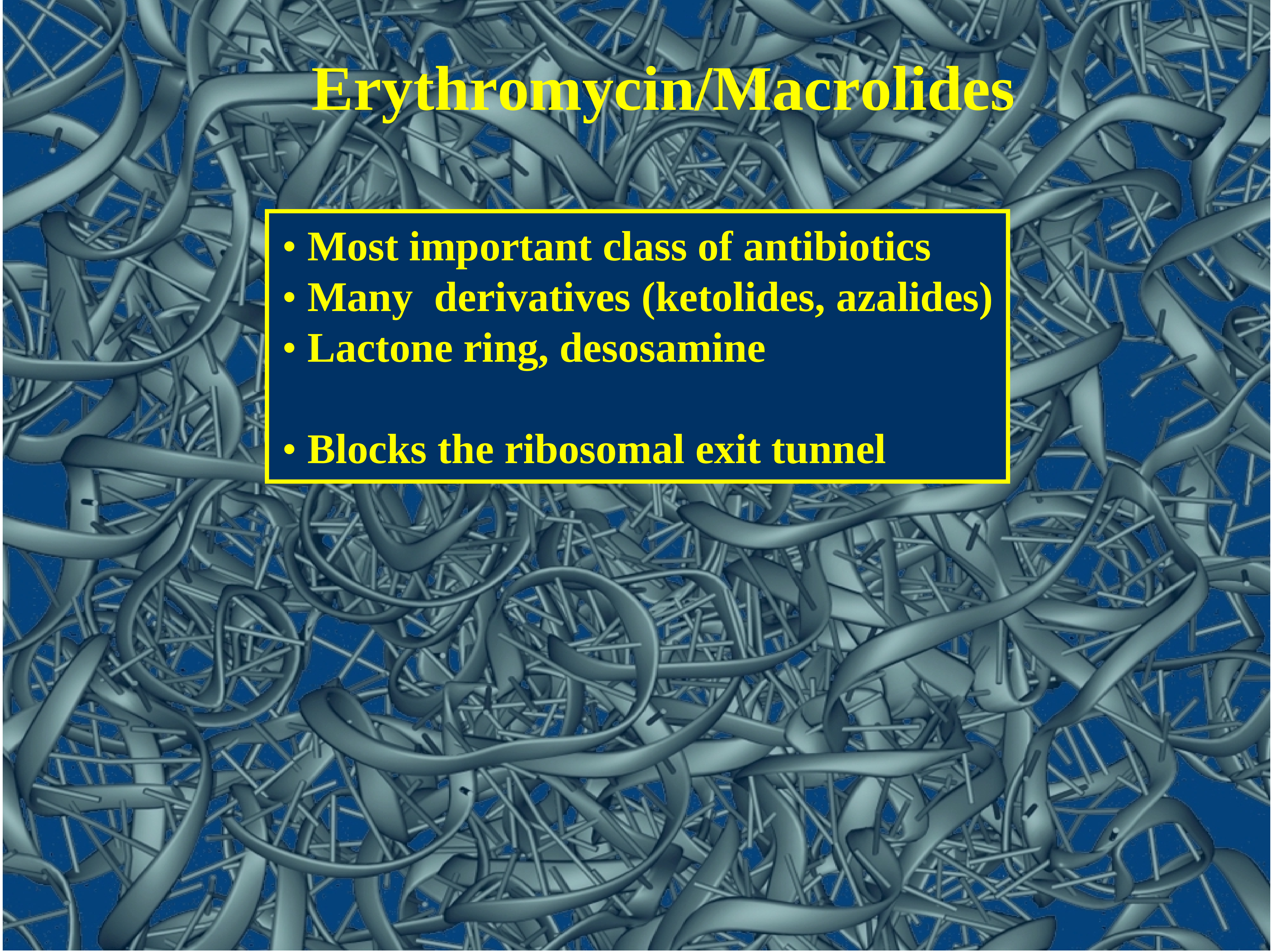
- **Many binding sites**
- **1 functional site**
- **blocks A-site**

Edeine



- •Universal drug
- •inhibits initiation
- •hampsers P-site
- •blocks mRNA

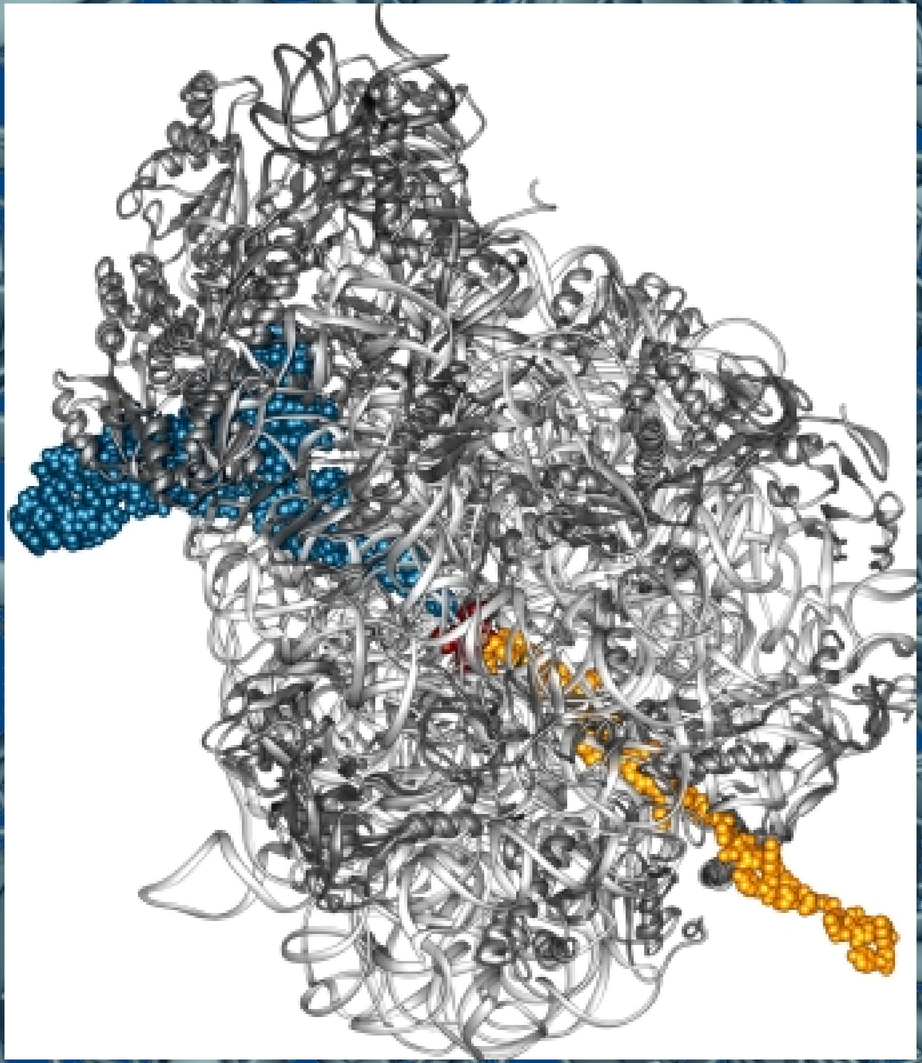
Erythromycin/Macrolides

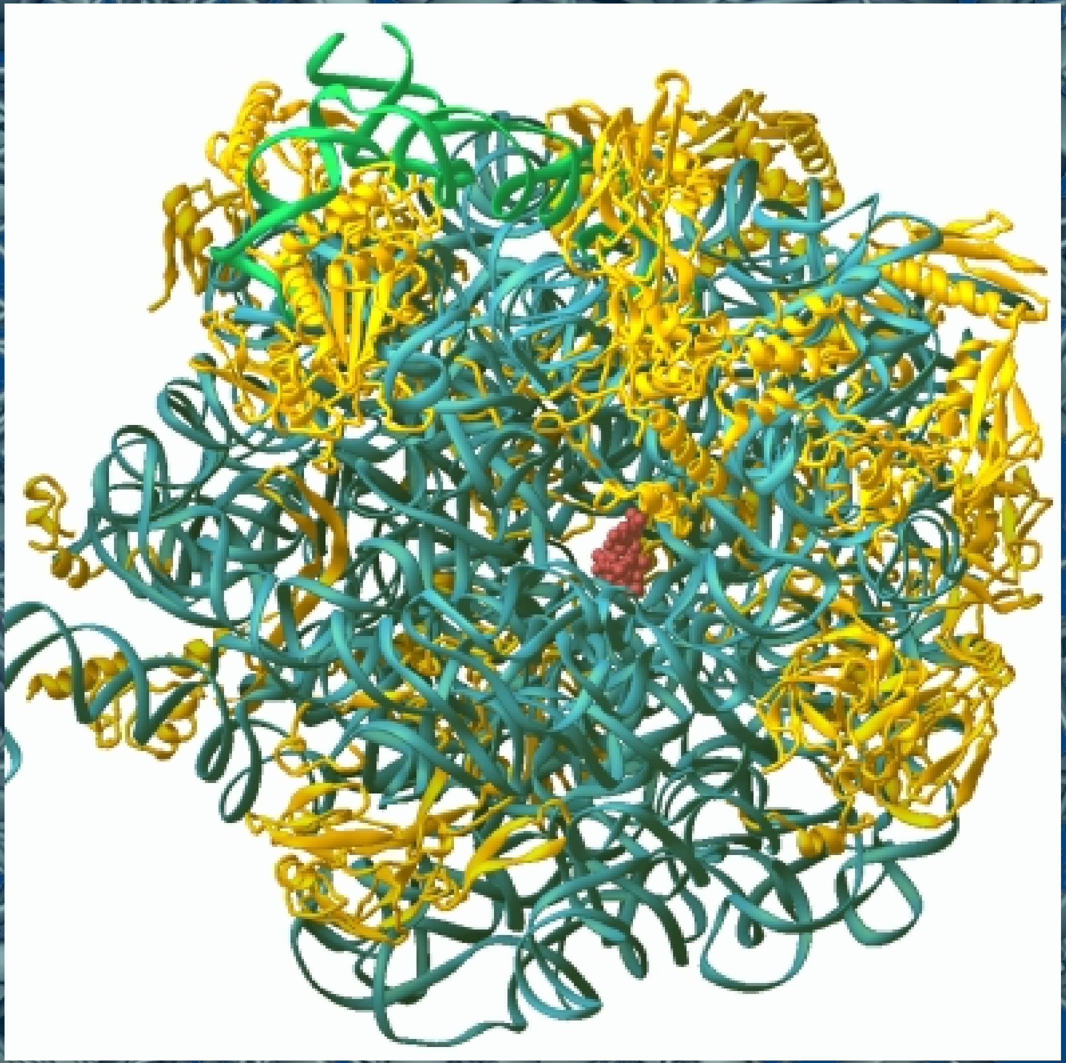


- Most important class of antibiotics
- Many derivatives (ketolides, azalides)
- Lactone ring, desosamine

- Blocks the ribosomal exit tunnel

Erythromycin/Macrolides

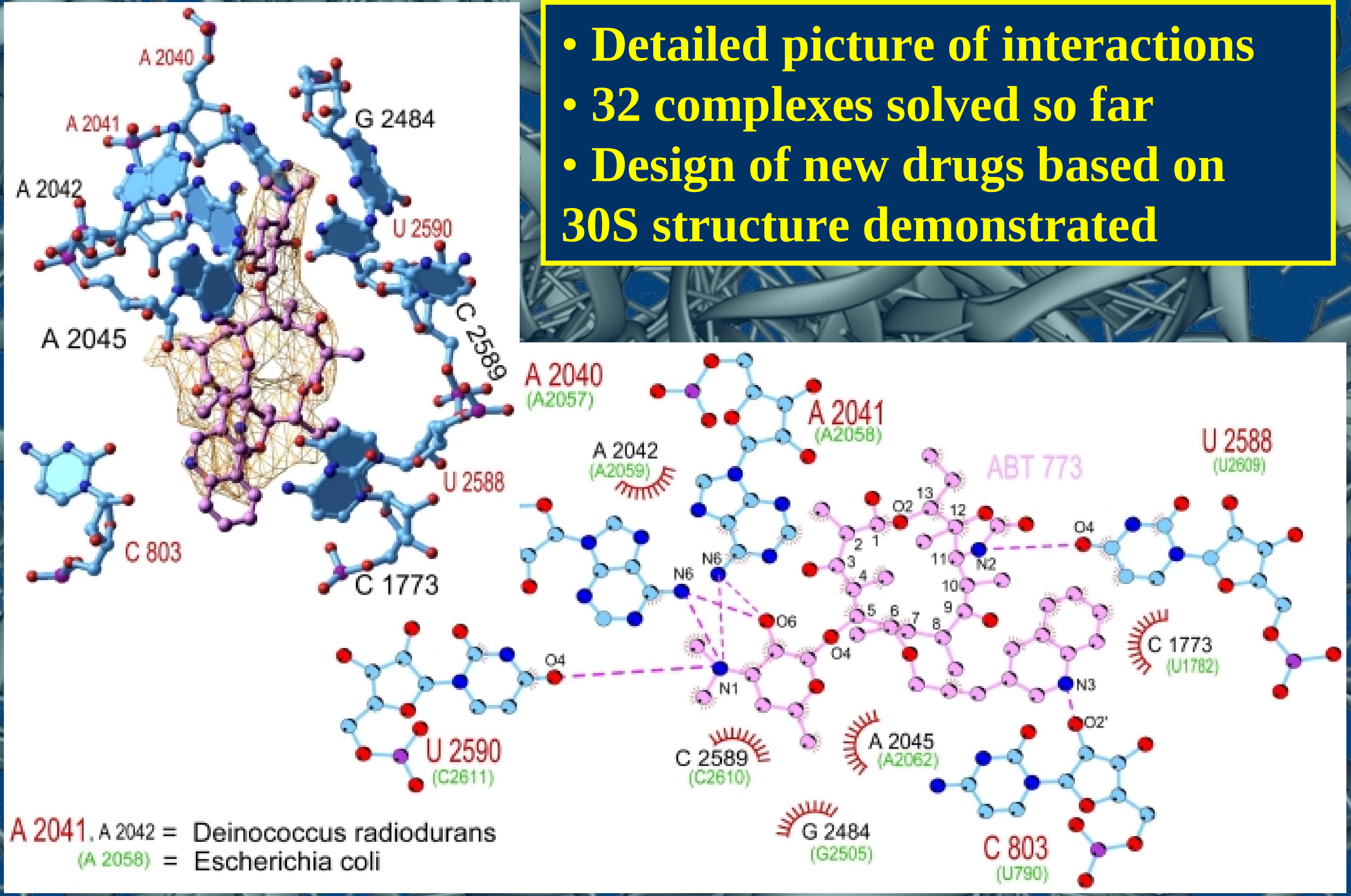




Exit tunnel ... ... blocked by macrolides

Structural Antibiomics

- Detailed picture of interactions
- 32 complexes solved so far
- Design of new drugs based on 30S structure demonstrated



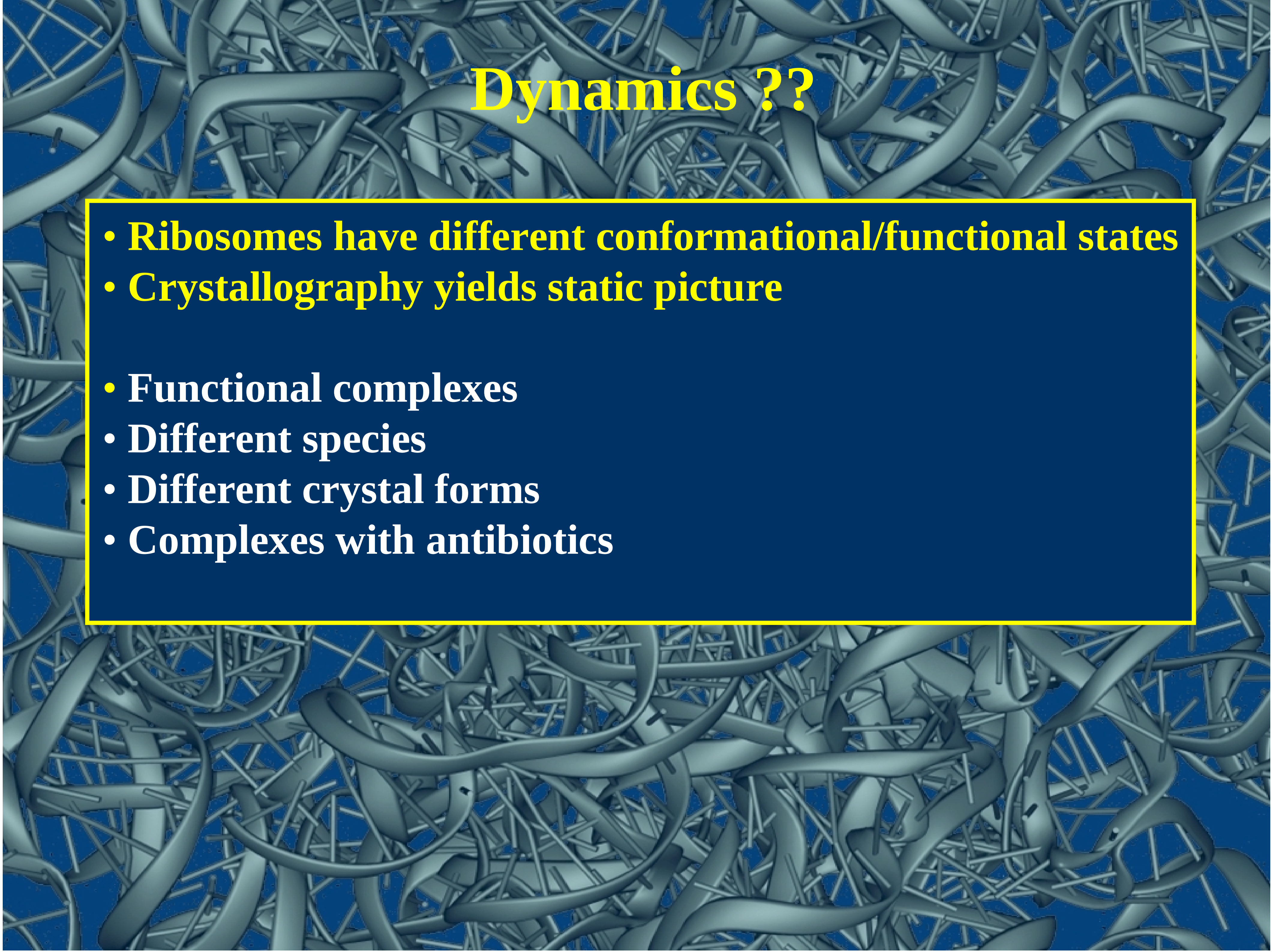
Needs & Benefits ??

- Only 1 new class of antibiotics during last 30 years
- Pharmaceutical industry reduced research
- Many drugs removed from clinical trials (costs, resistance)

- Rising levels of antibiotic resistance
- 50-80% used in food production/vet. medicine

- 1500 dead/yr in NY as a consequence of resistance
- Costs estimated to 200-800 m$/yr in US

- Structure aided drug design might be a way out ...

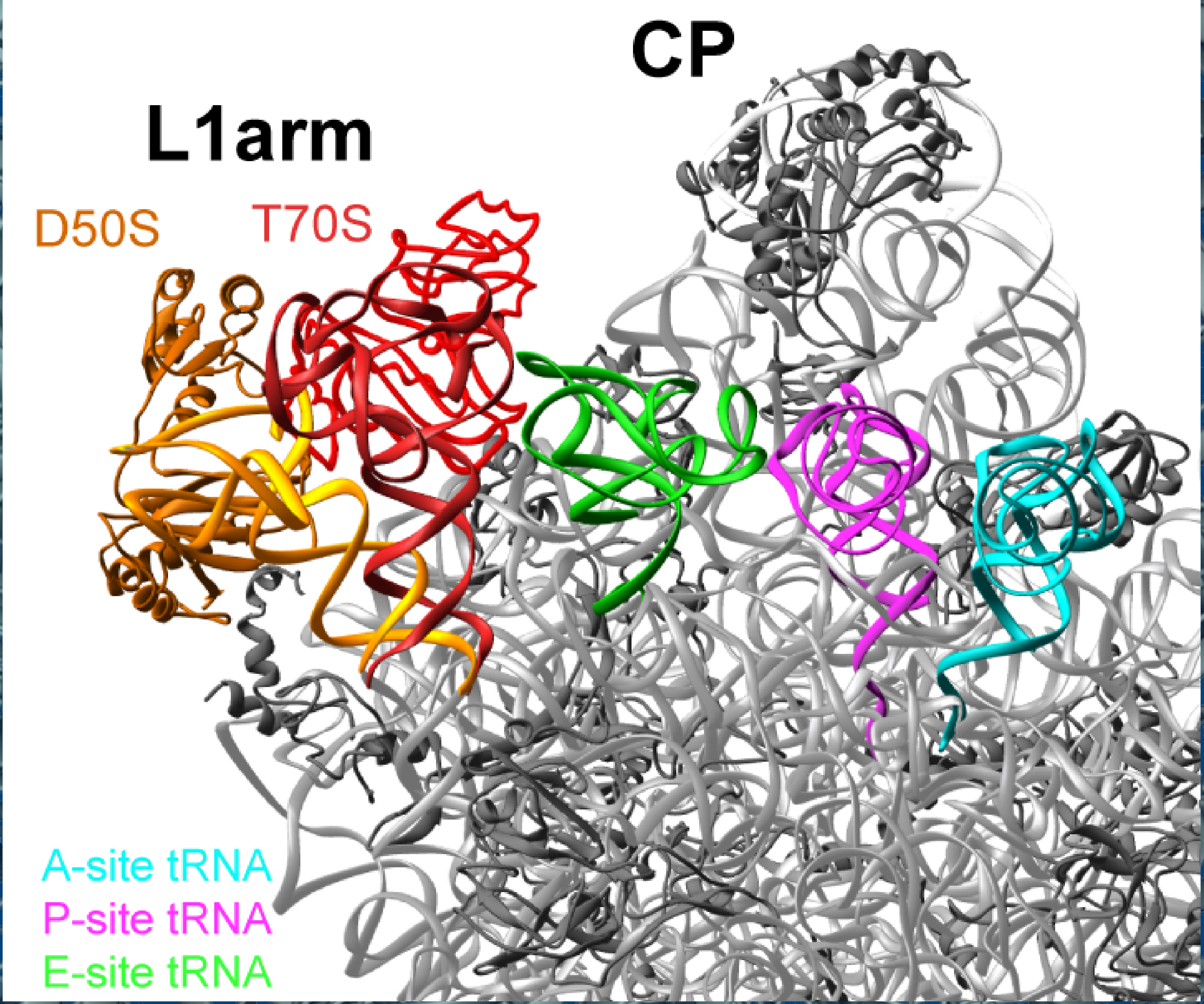


Dynamics ??

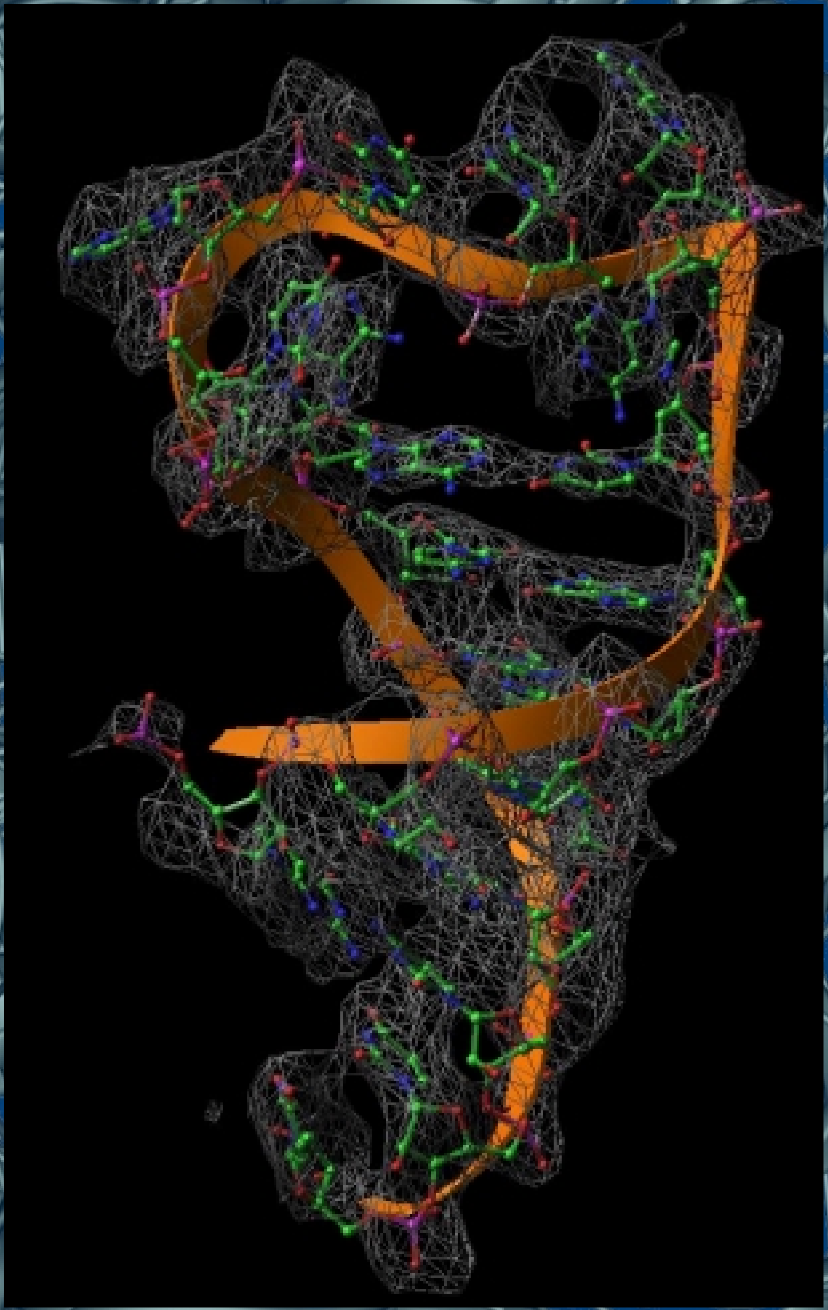
- **Ribosomes have different conformational/functional states**
- **Crystallography yields static picture**

- **Functional complexes**
- **Different species**
- **Different crystal forms**
- **Complexes with antibiotics**

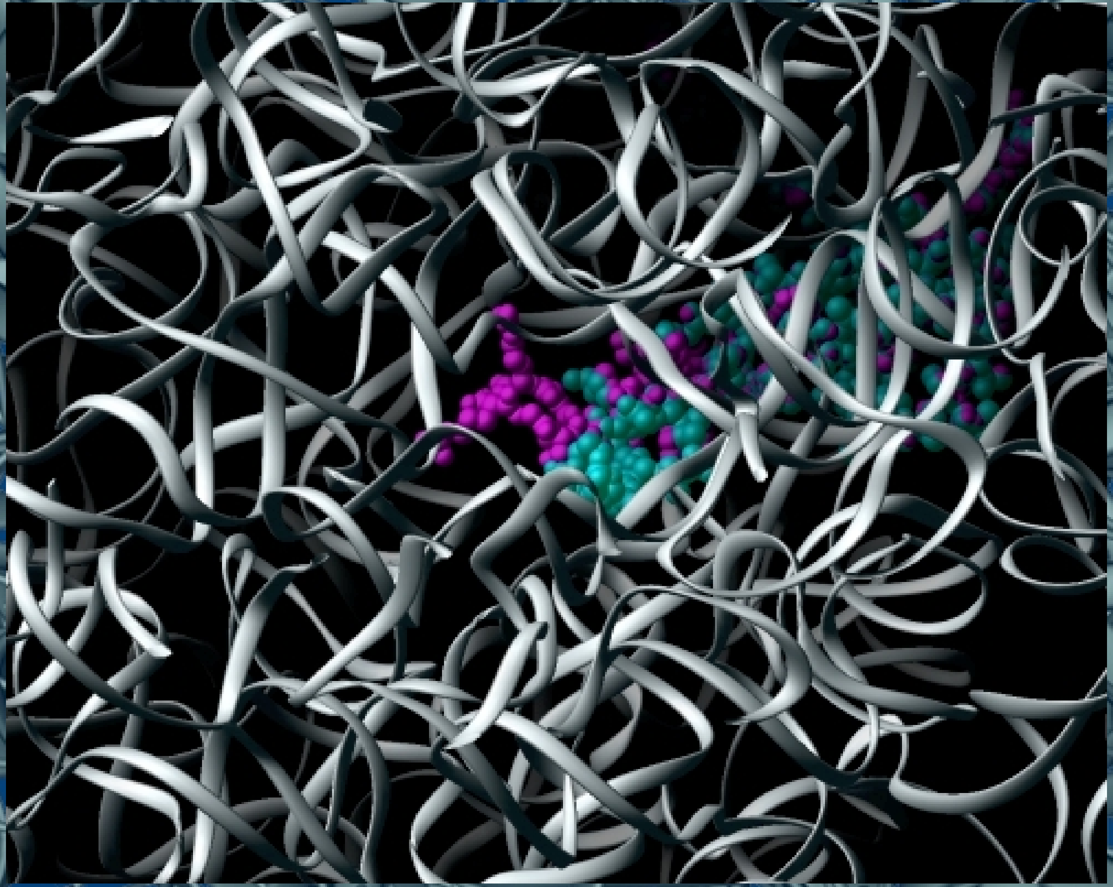
L1-arm



30S-50S Interface



Special Macrolide



Induces conformational change: important regulatory mechanism

Acknowledgement

- **BW6 MPG/Doris/DESY** - **H.**Bartunik, G.Bourenkov, ...
- **BW7 EMBL/Doris/DESY** - E.Pohl, C.Hermes, ...
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- **SBC APS/Chicago** - R. Alkire, S. Ginell, ...
- **ID14 ESRF/Grenoble** - E. Mitchell, R. Ravelli, ...

... and J. Harms for many of the figures

Work done by ...

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- **MPI for Genetics - Group Franceschi**
- Weizmann Institute - Structural Biology