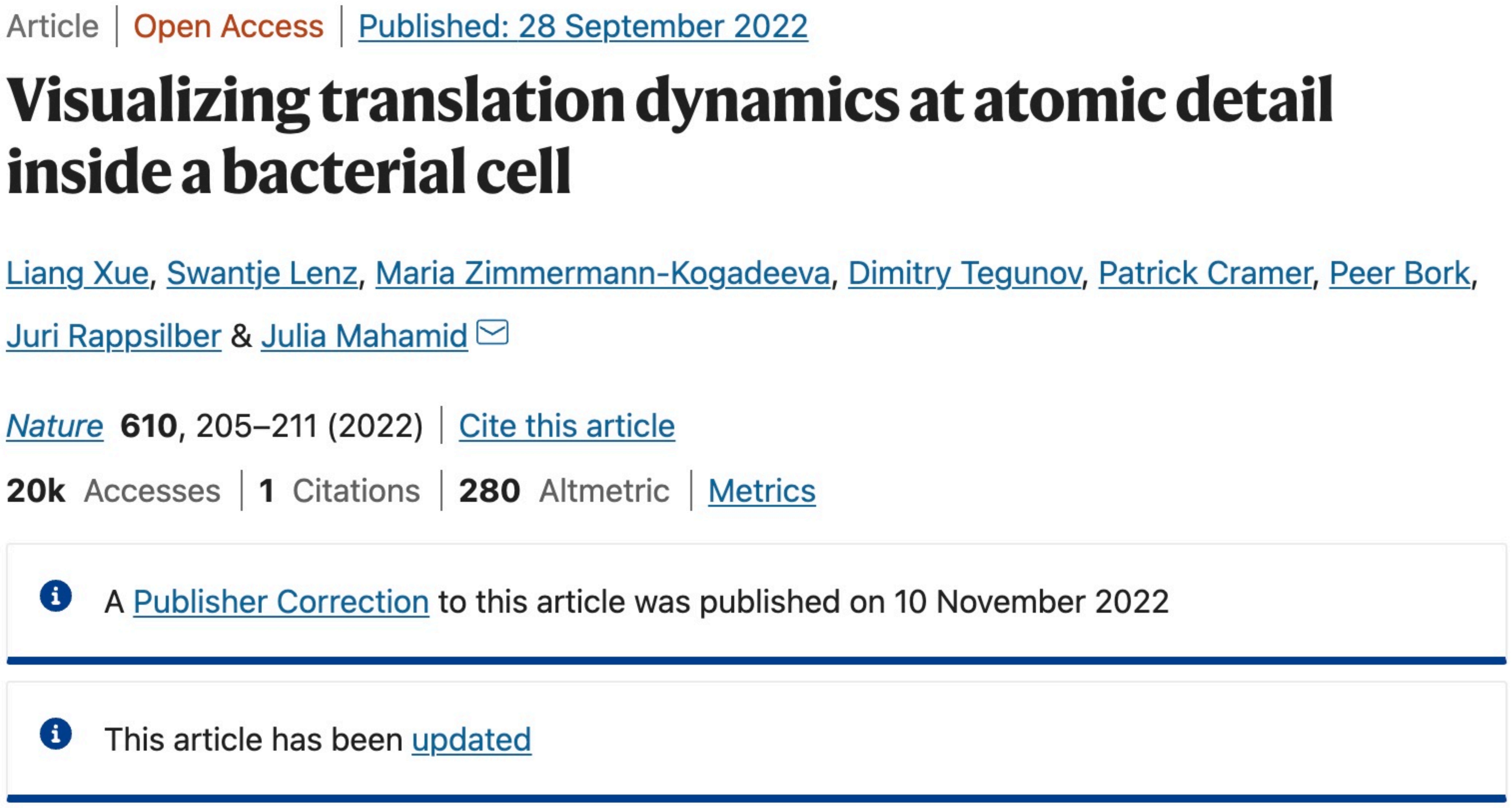

Article | Open Access | Published: 28 September 2022

Visualizing translation dynamics at atomic detail inside a bacterial cell

Liang Xue, Swantje Lenz, Maria Zimmermann-Kogadeeva, Dimitry Tegunov, Patrick Cramer, Peer Bork, Juri Rappsilber & Julia Mahamid

Nature **610**, 205–211 (2022) | Cite this article

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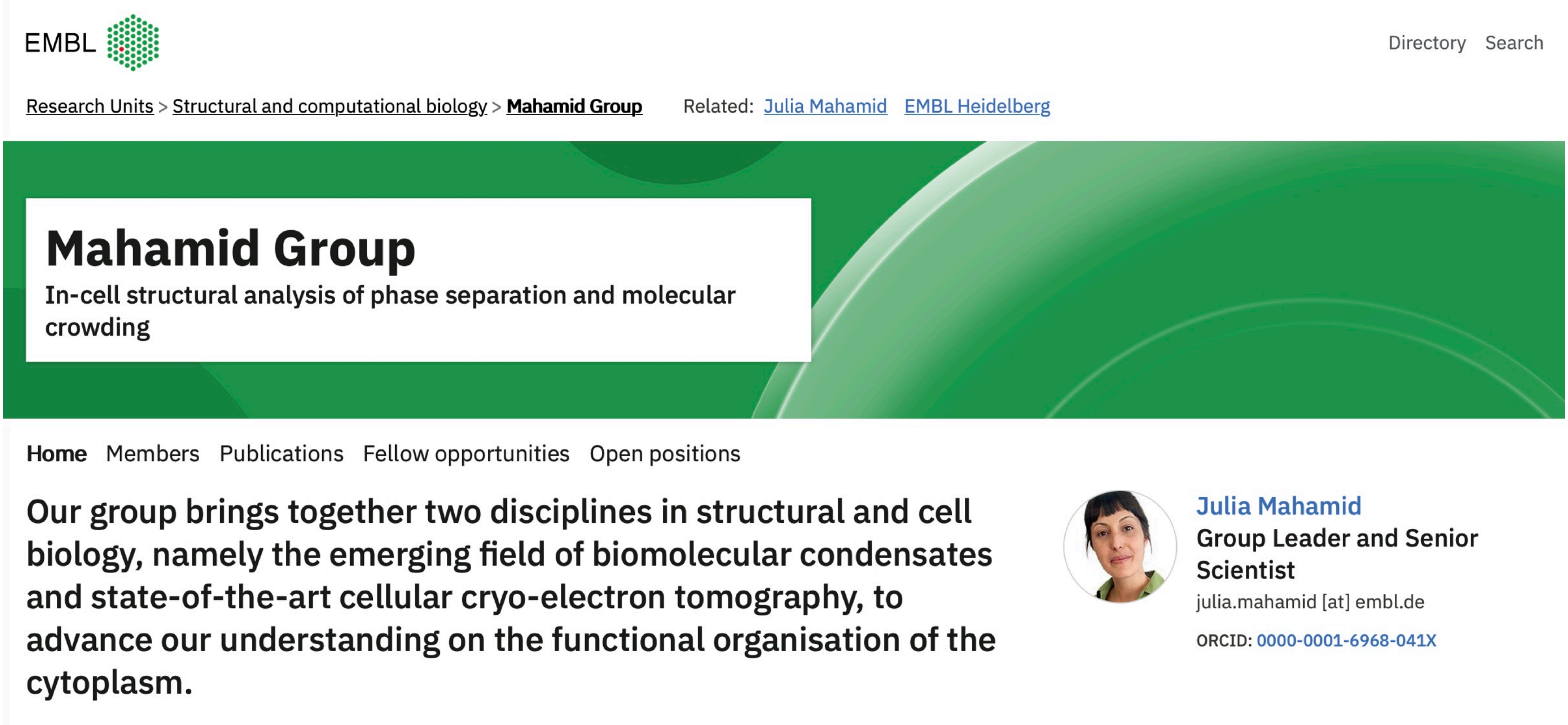
A Publisher Correction to this article was published on 10 November 2022

This article has been updated

黄振 2023.2

Authors



Mahamid Group

In-cell structural analysis of phase separation and molecular crowding

Our group brings together two disciplines in structural and cell biology, namely the emerging field of biomolecular condensates and state-of-the-art cellular cryo-electron tomography, to advance our understanding on the functional organisation of the cytoplasm.

Julia Mahamid
Group Leader and Senior Scientist
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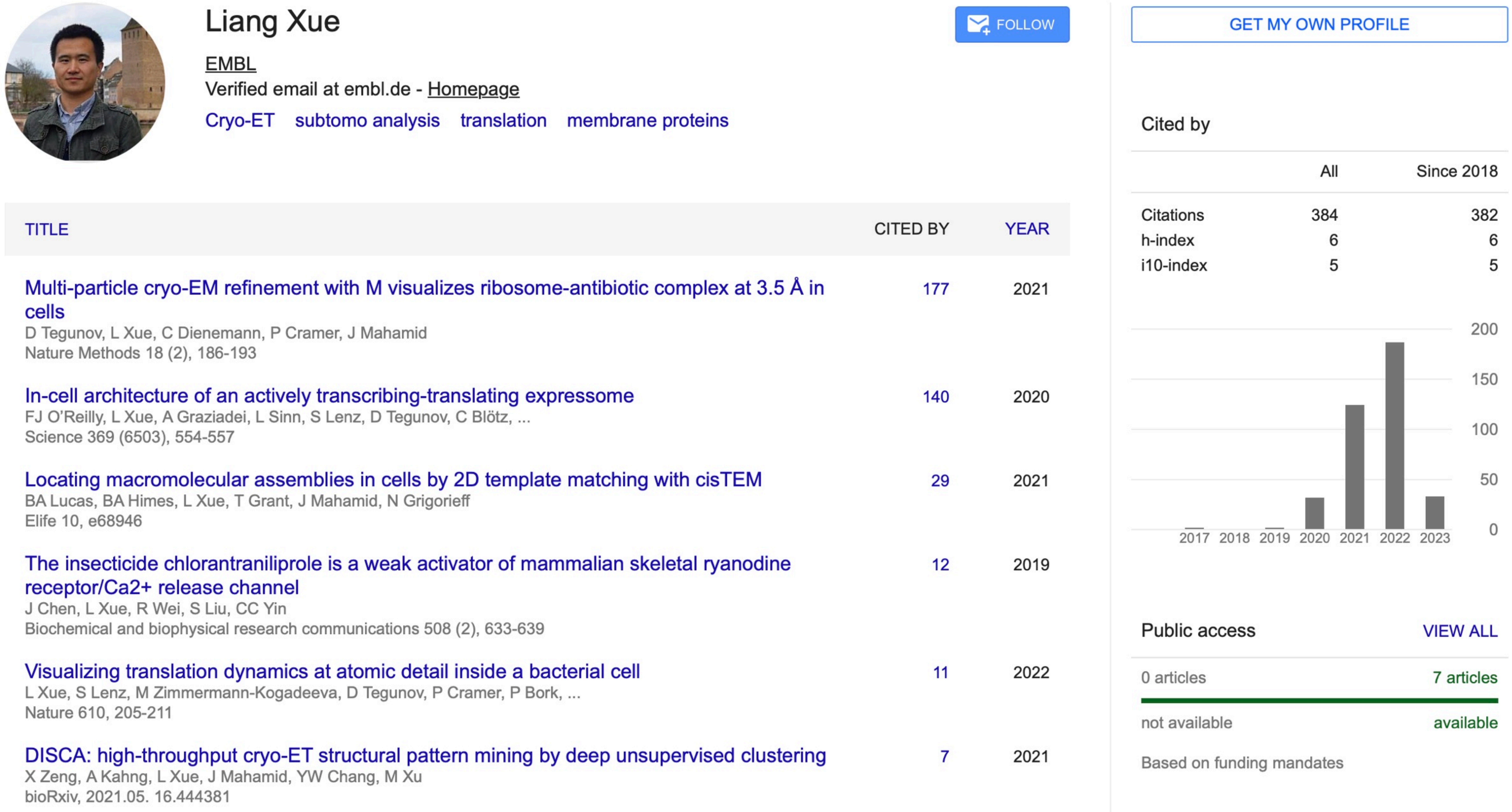
Authors



Liang Xue

EMBL

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Cryo-ET subtomo analysis translation membrane proteins

TITLE	CITED BY	YEAR
Multi-particle cryo-EM refinement with M visualizes ribosome-antibiotic complex at 3.5 Å in cells D Tegunov, L Xue, C Dienemann, P Cramer, J Mahamid Nature Methods 18 (2), 186-193	177	2021
In-cell architecture of an actively transcribing-translating expressome FJ O'Reilly, L Xue, A Graziadei, L Sinn, S Lenz, D Tegunov, C Blötz, ... Science 369 (6503), 554-557	140	2020
Locating macromolecular assemblies in cells by 2D template matching with cisTEM BA Lucas, BA Himes, L Xue, T Grant, J Mahamid, N Grigorieff Elife 10, e68946	29	2021
The insecticide chlorantraniliprole is a weak activator of mammalian skeletal ryanodine receptor/Ca2+ release channel J Chen, L Xue, R Wei, S Liu, CC Yin Biochemical and biophysical research communications 508 (2), 633-639	12	2019
Visualizing translation dynamics at atomic detail inside a bacterial cell L Xue, S Lenz, M Zimmermann-Kogadeeva, D Tegunov, P Cramer, P Bork, ... Nature 610, 205-211	11	2022
DISCA: high-throughput cryo-ET structural pattern mining by deep unsupervised clustering X Zeng, A Kahng, L Xue, J Mahamid, YW Chang, M Xu bioRxiv, 2021.05. 16.444381	7	2021

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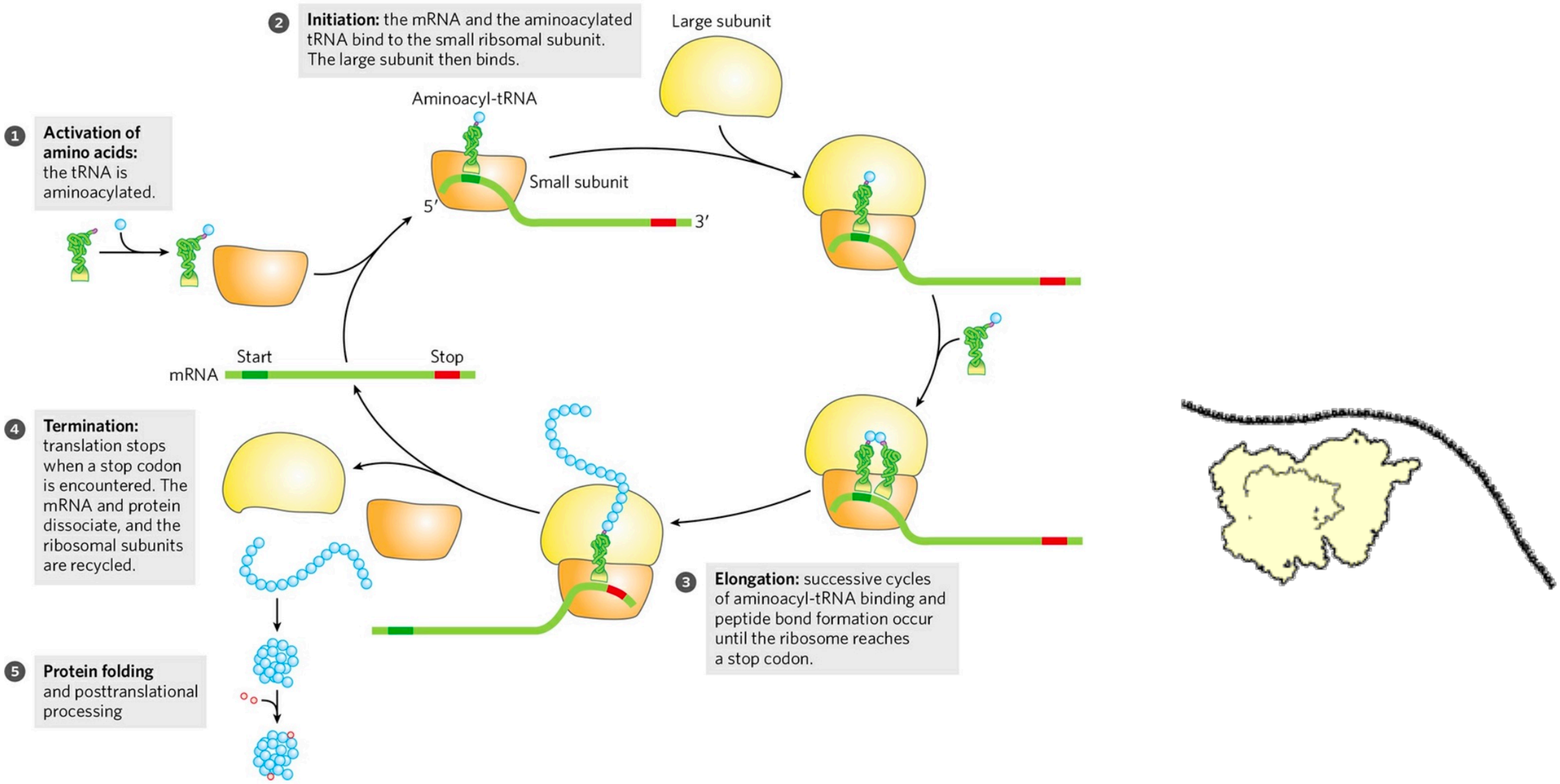
Background

Ribosome and translation process



① **Activation of amino acids:** the tRNA is aminoacylated.

② **Initiation:** the mRNA and the aminoacylated tRNA bind to the small ribsomal subunit. The large subunit then binds.

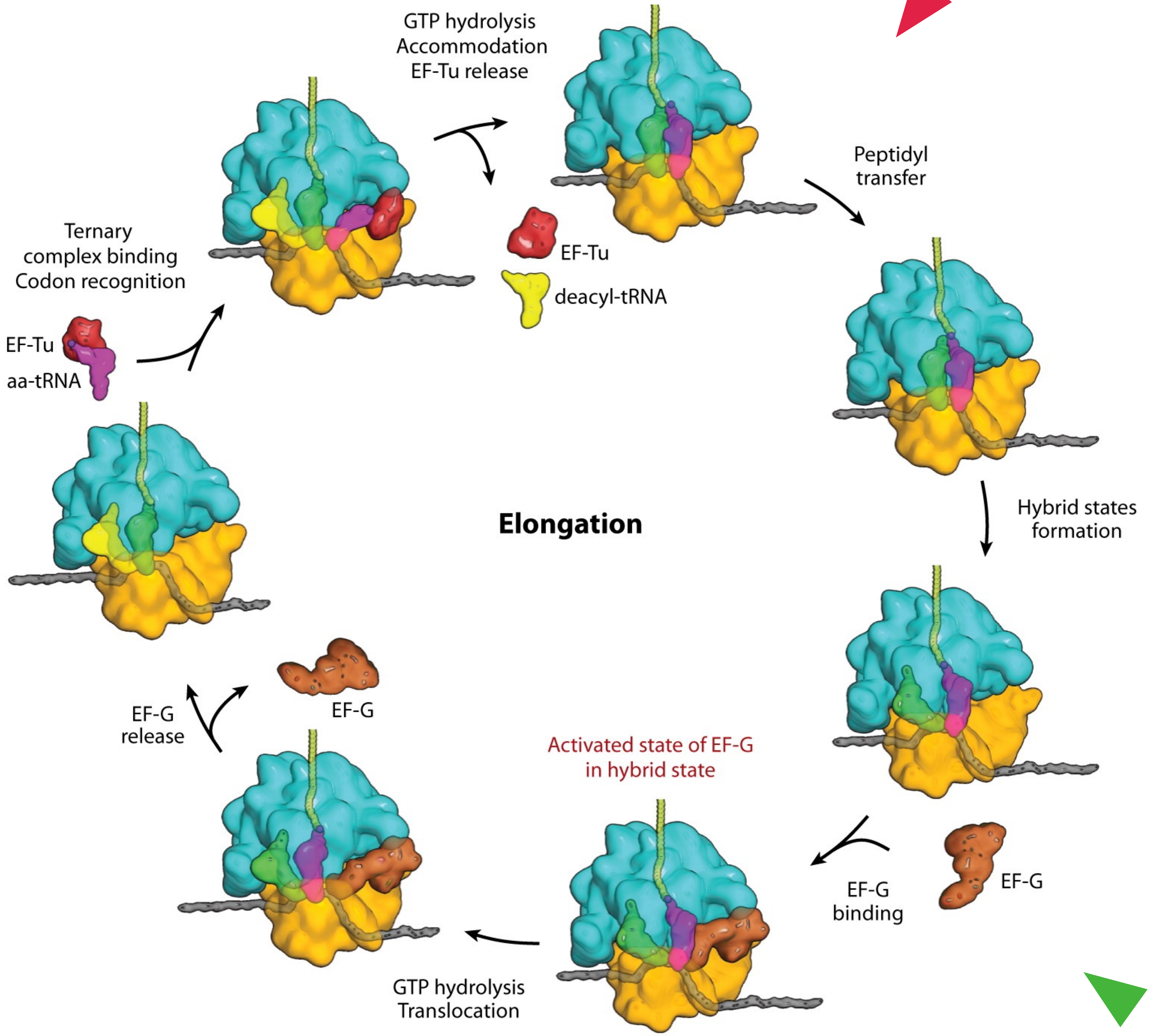
③ **Elongation:** successive cycles of aminoacyl-tRNA binding and peptide bond formation occur until the ribosome reaches a stop codon.

④ **Termination:** translation stops when a stop codon is encountered. The mRNA and protein dissociate, and the ribosomal subunits are recycled.

⑤ **Protein folding** and posttranslational processing

An overview of the five stages of protein synthesis.
(Lehninger Principles of Biochemistry, 7th edition)

Ribosome and translation process



Voorhees RM, Ramakrishnan V. 2013.
Annu. Rev. Biochem. 82:203–36

Unsolved problems ?

Q1: The maps generated in previous studies were limited to resolutions on the nanometre scale

- The resolution is not high enough to see the details in translating ribosomes

Q2: A detailed structural description of the translation process within the native cellular context is lacking

- Most available structures are resolved from **model bacteria**
- Often **trapped in specific states** by antibiotics/GTP analogues/mutations

Objects and Subjects of this study

Q1: The maps generated in previous studies were limited to resolutions on the nanometre scale

- The resolution is not high enough to see the details in translating ribosomes

→ **Build an in-cell native atomic model for the translating ribosomes**

Q2: A detailed structural description of the translation process within the native cellular context is lacking

- Most available structures are resolved from **model bacteria**
- Often **trapped in specific states** by antibiotics/GTP analogues/mutations

→ **Analyze the native cellular translation landscape(different states and organization of translating ribosomes)**

Objects and Subjects of this study

Build an in-cell **native atomic** model for the translating ribosomes → cryo-electron microscopy
cryo-ET & Subtomogram Average(STA)

Analyze the **native** cellular translation landscape(**different states** and **organization** of translating ribosomes) → cryo-ET classification methods
use antibiotics to reshape the landscape
project back the structure into the tomogram
...

Objects and Subjects of this study

Build an in-cell **native atomic** model for the translating ribosomes →

cryo-electron microscopy
cryo-ET & Subtomogram Average(STA)

Analyze the cellular translation landscape(**different states** and **organization** of translating ribosomes) →

cryo-ET classification methods
use antibiotics to reshape the landscape
project back the structure into the tomogram
…

How to choose prokaryotic cell model ?

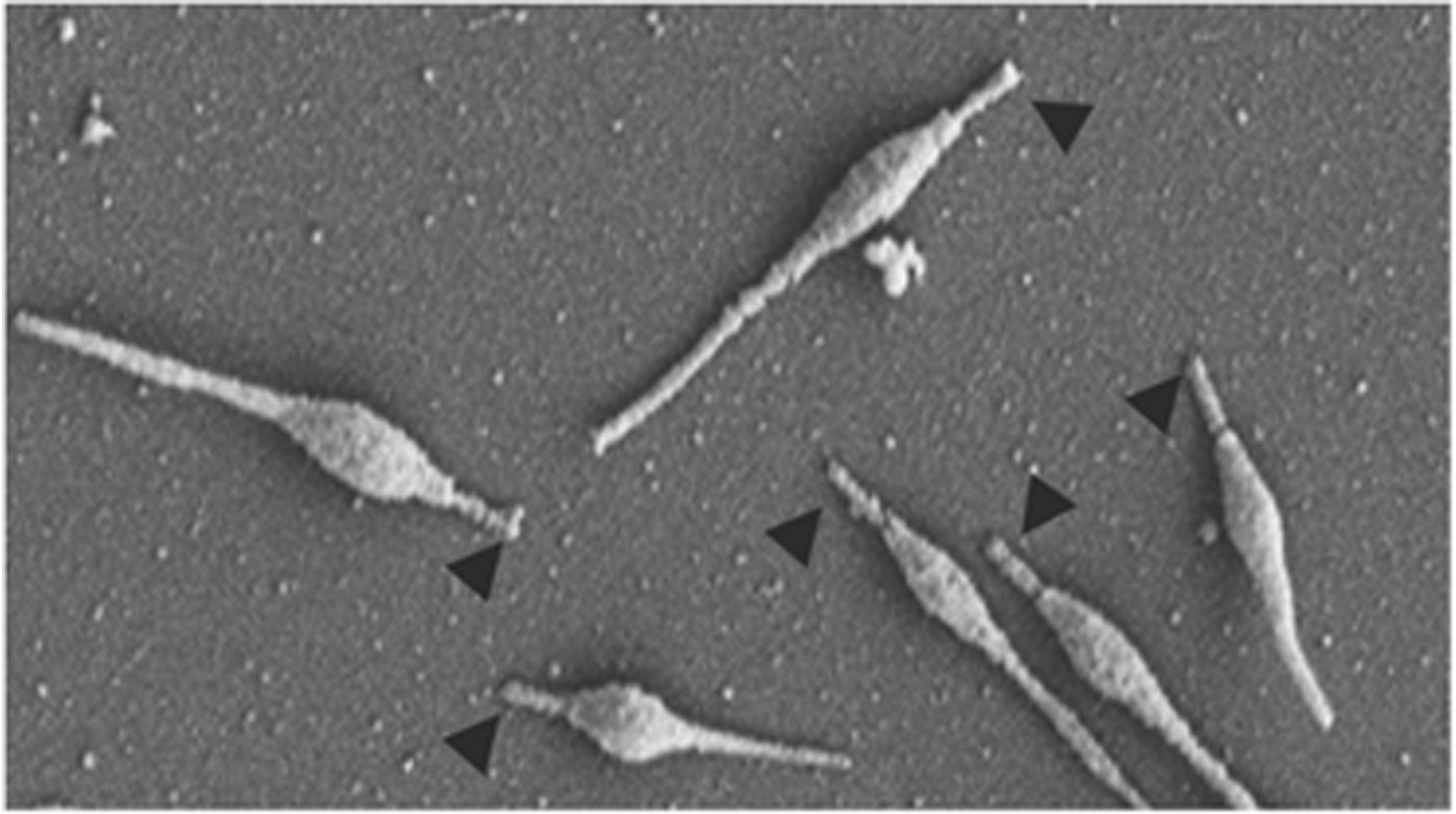
- ○ Cell size - small enough
- ○ Genome - reduced enough

Objects and Subjects of this study

How to choose prokaryotic cell model ?

- Cell size - small enough
- Genome - reduced enough

Mycoplasma pneumoniae（肺炎枝原体）



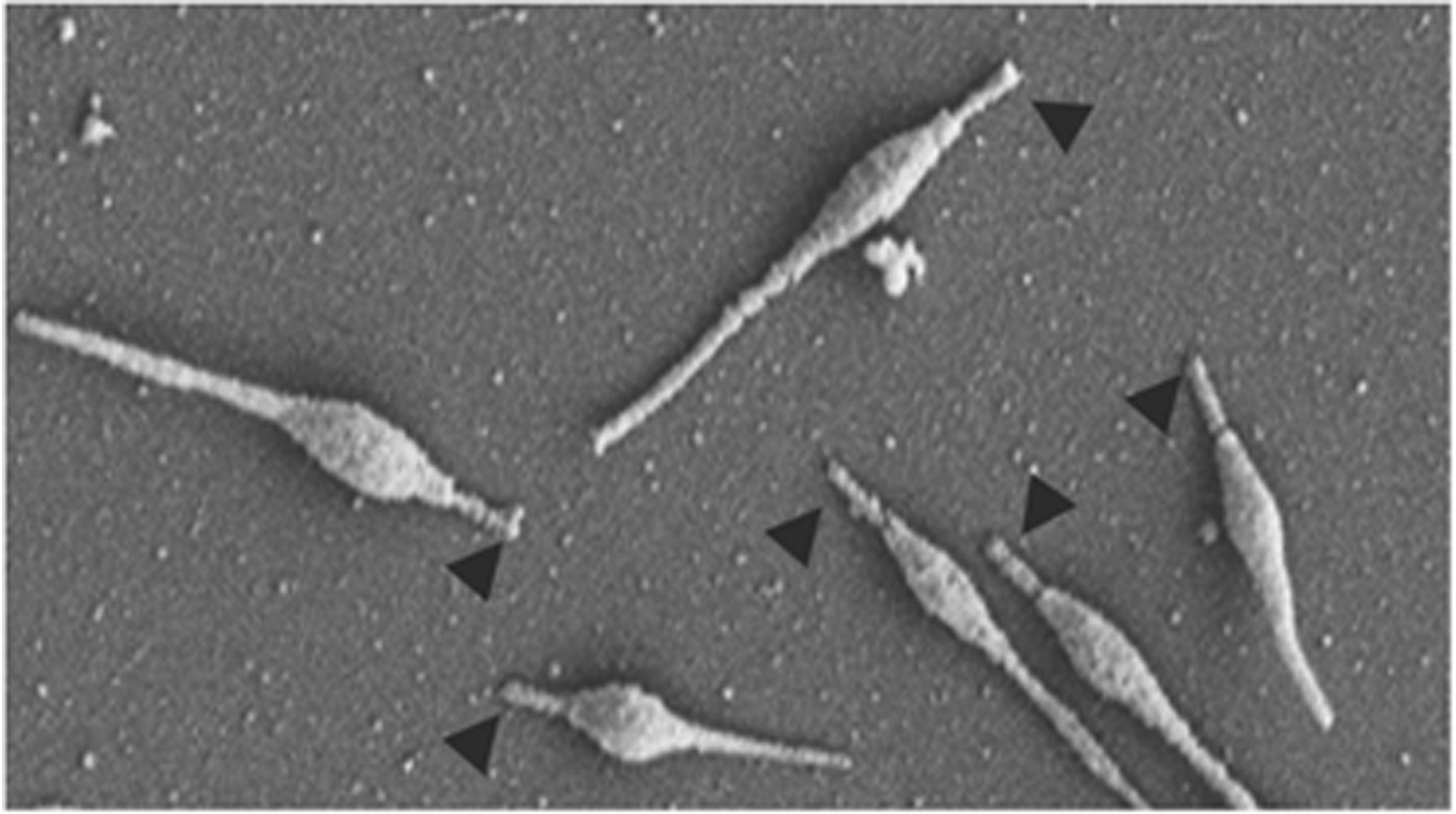
(FEMS Microbiology Reviews, 2008)

Objects and Subjects of this study

How to choose prokaryotic cell model ?

- Cell size - small enough
- Genome - reduced enough

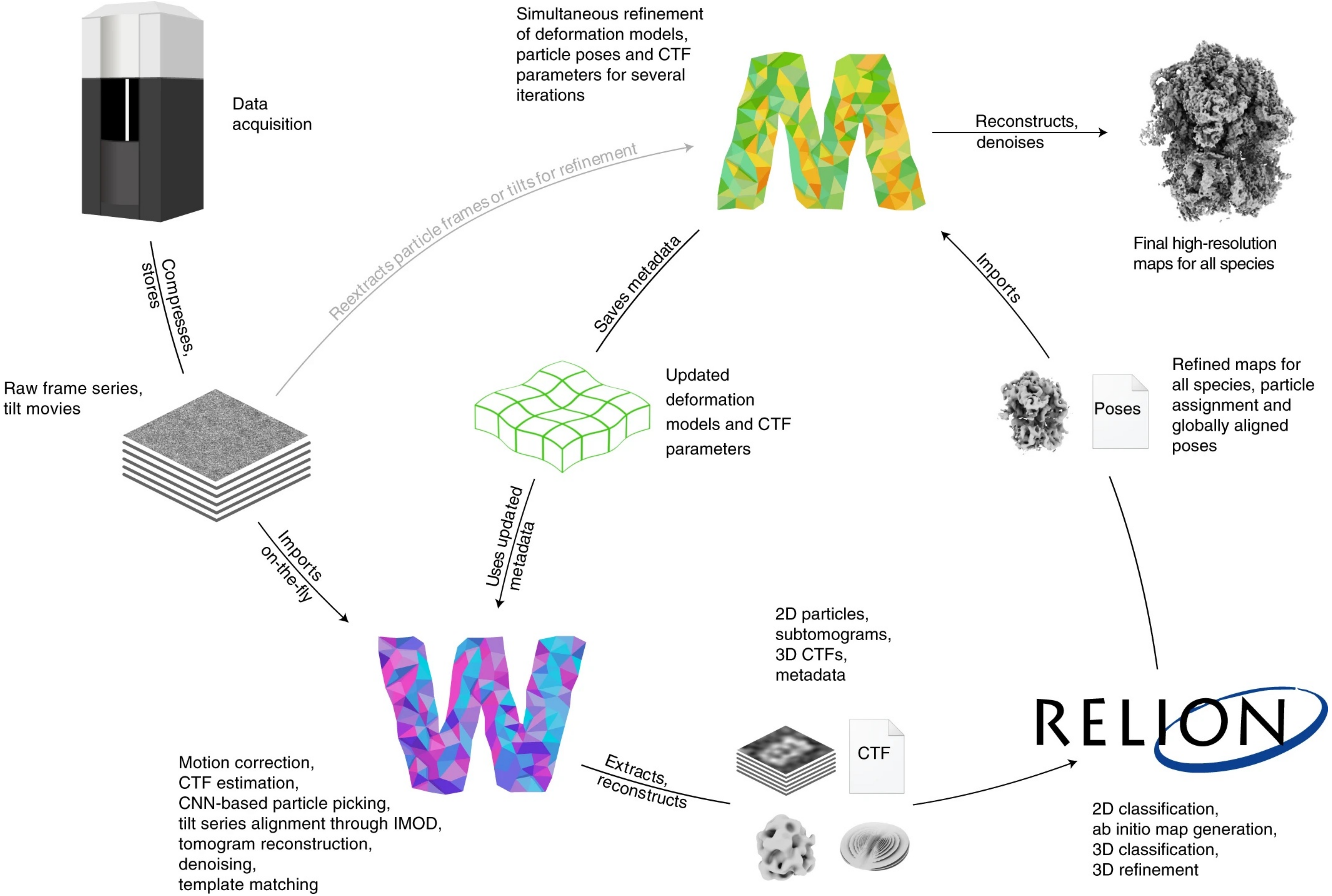
Mycoplasma pneumoniae（肺炎枝原体）



- among the **smallest** self-replicating organisms
- Parasitic
- no cell wall and periplasmic space
- reduced genomes
- limited metabolic activity

(FEMS Microbiology Reviews, 2008)

Materials & Methods



Data acquisition

Compresses, stores

Raw frame series, tilt movies

Reextracts particle frames or tilts for refinement

Simultaneous refinement of deformation models, particle poses and CTF parameters for several iterations

Reconstructs, denoises

Final high-resolution maps for all species

Saves metadata

Updated deformation models and CTF parameters

Imports

Poses

Refined maps for all species, particle assignment and globally aligned poses

Uses updated metadata

Imports on-the-fly

2D particles, subtomograms, 3D CTFs, metadata

CTF

RELION

Motion correction,
CTF estimation,
CNN-based particle picking,
tilt series alignment through IMOD,
tomogram reconstruction,
denoising,
template matching

Extracts, reconstructs

2D classification,
ab initio map generation,
3D classification,
3D refinement

(Tegunov, D., Xue, L., Dienemann, C. et al. 2021)

Results

1 In-cell structure of the *M.pnuemoniae* ribosome

1.1 High-resolution in-cell consensus maps

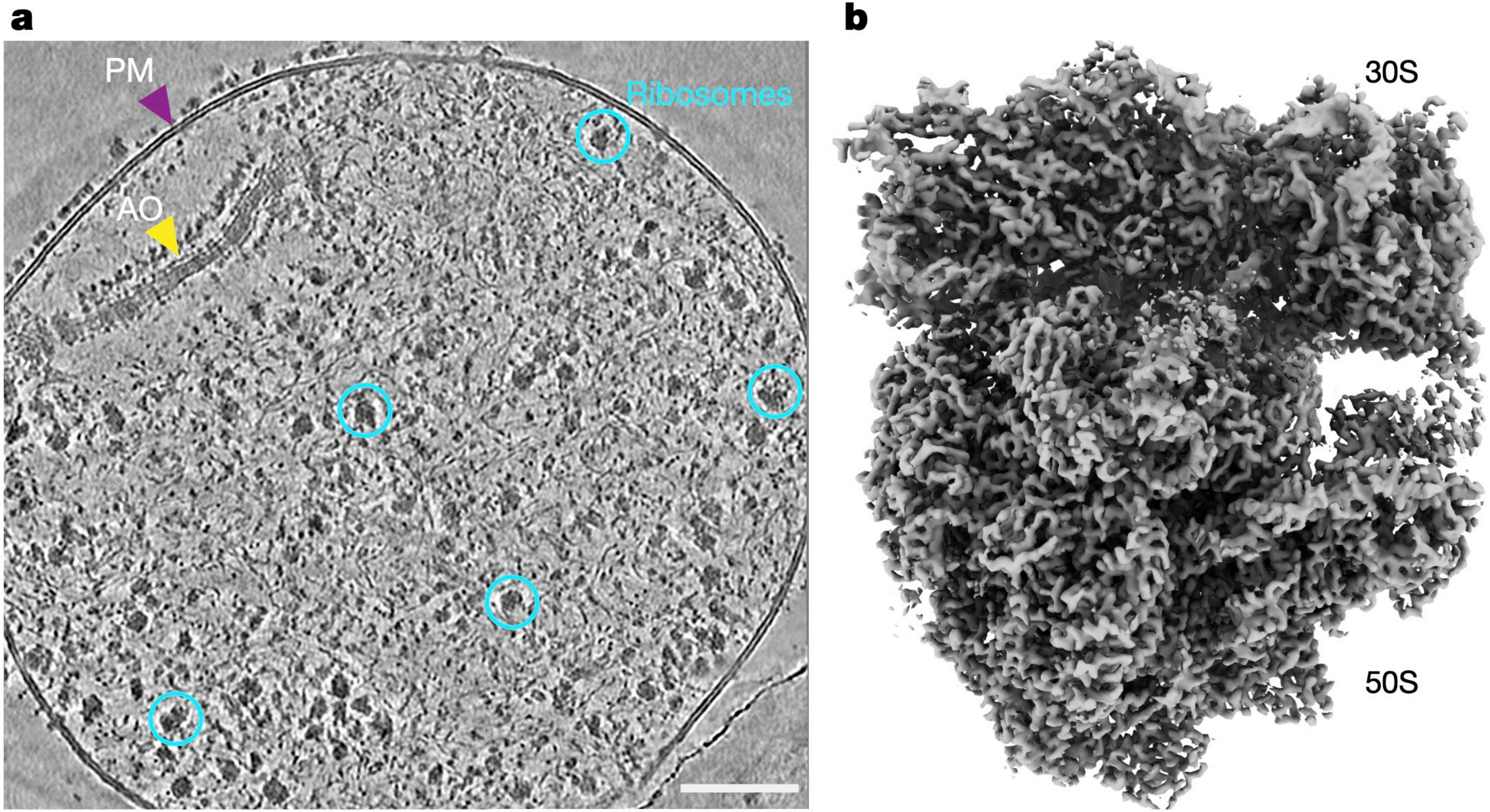
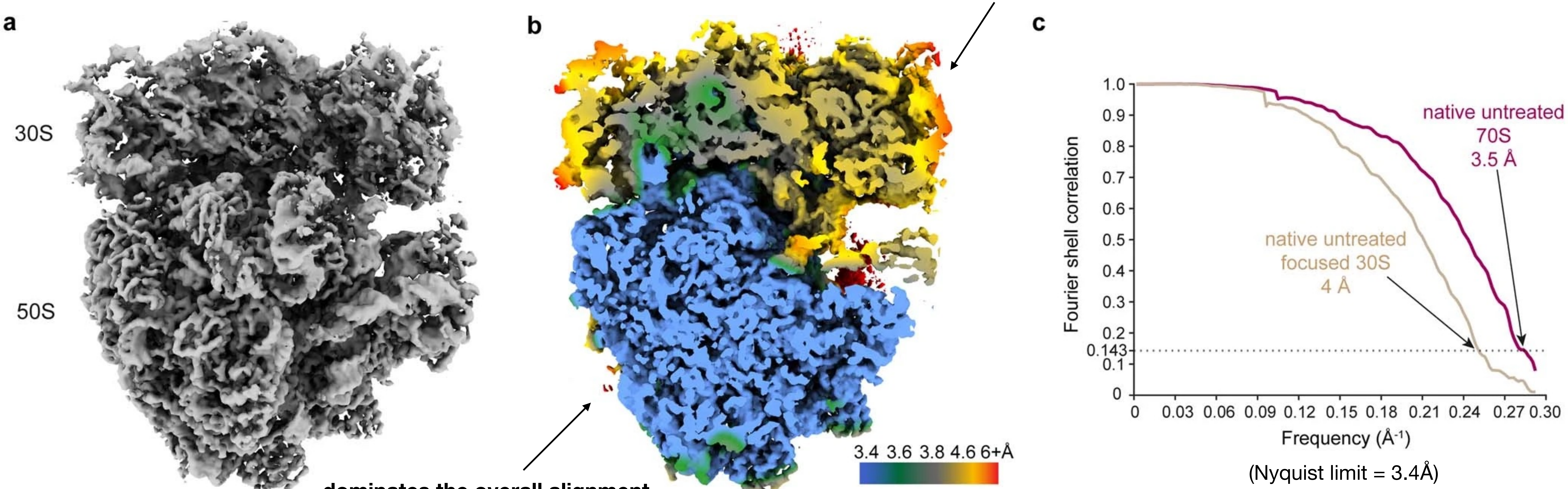


Figure 1

1 In-cell structure of the *M.pnuemoniae* ribosome

1.1 High-resolution in-cell consensus maps



high flexibility during active translation

dominates the overall alignment

(Nyquist limit = 3.4Å)

77,539 sub-tomograms
from 356 untreated *M. pneumoniae* cells

coloured by local resolutions

FSC curves
for global 70S and focused 30S refinement

Extended Data Figure 1

1 In-cell structure of the *M.pnuemoniae* ribosome

1.1 High-resolution in-cell consensus maps

18,987 sub-tomograms
from 65 Cm-treated cells

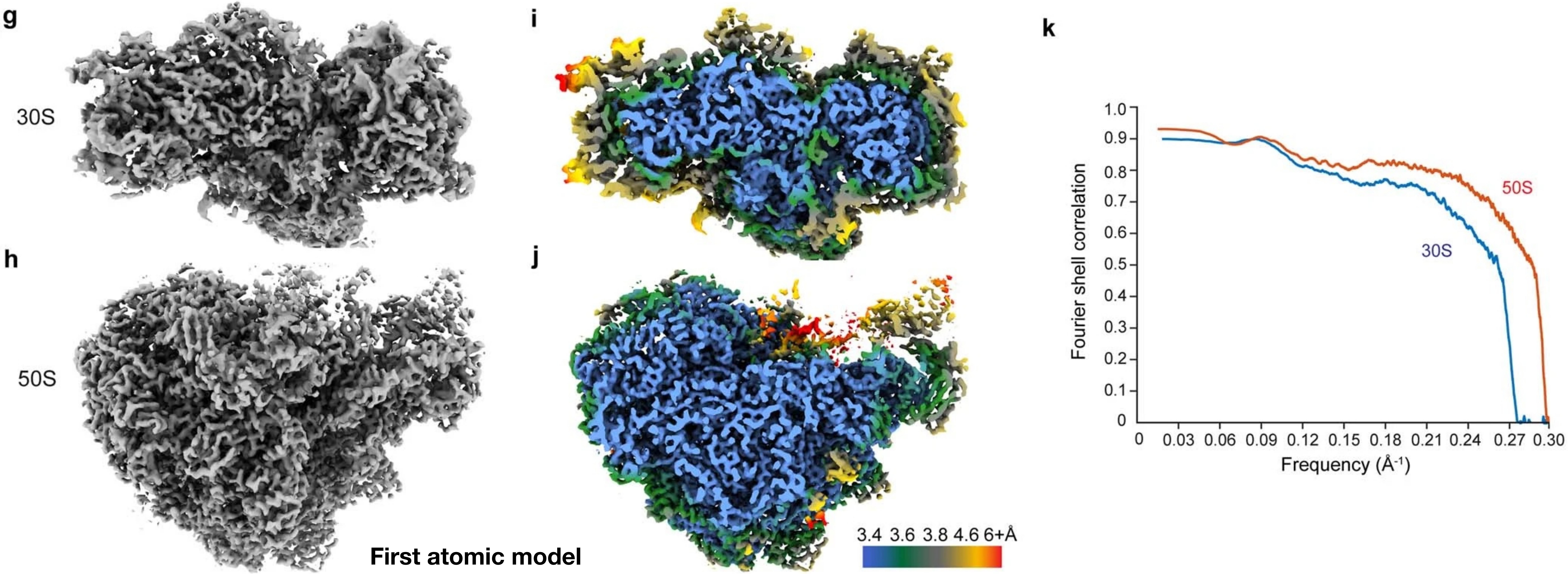
coloured by local resolutions

FSC curves
for global 70S and focused 30S refinement

Extended Data Figure 1

1 In-cell structure of the *M.pnuemoniae* ribosome

1.1 High-resolution in-cell consensus maps



First atomic model

Focused refined 30S and 50S maps from the Cm-treated dataset

coloured by local resolutions

FSC curves for global 70S and focused 30S refinement

Extended Data Figure 1

1 In-cell structure of the *M.pnuemoniae* ribosome

1.2 Focused refinements and atomic model

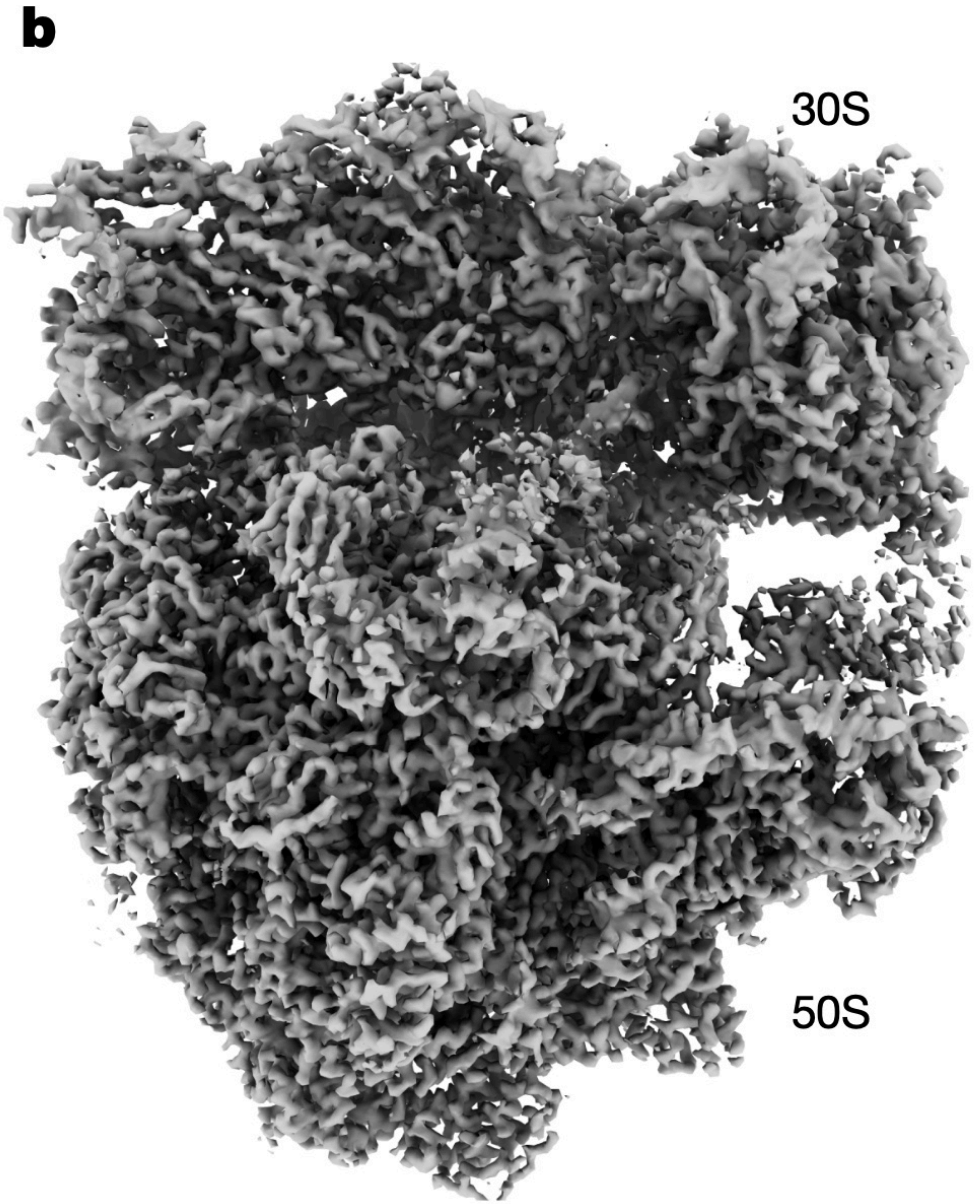
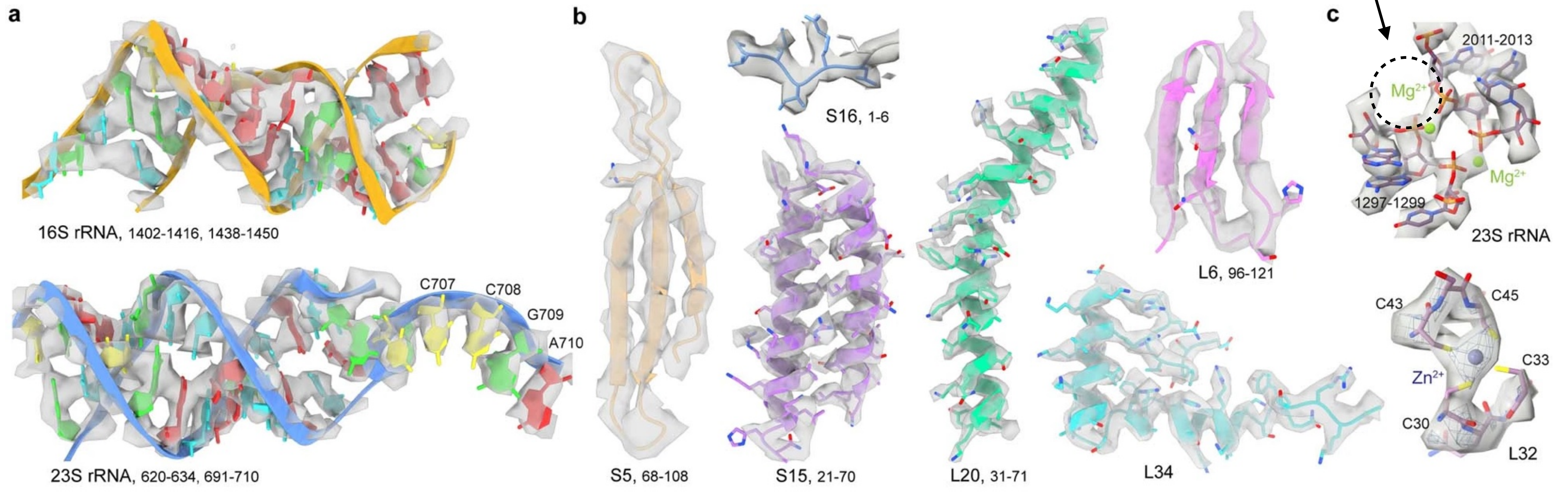


Figure 1

Densities corresponding to ions

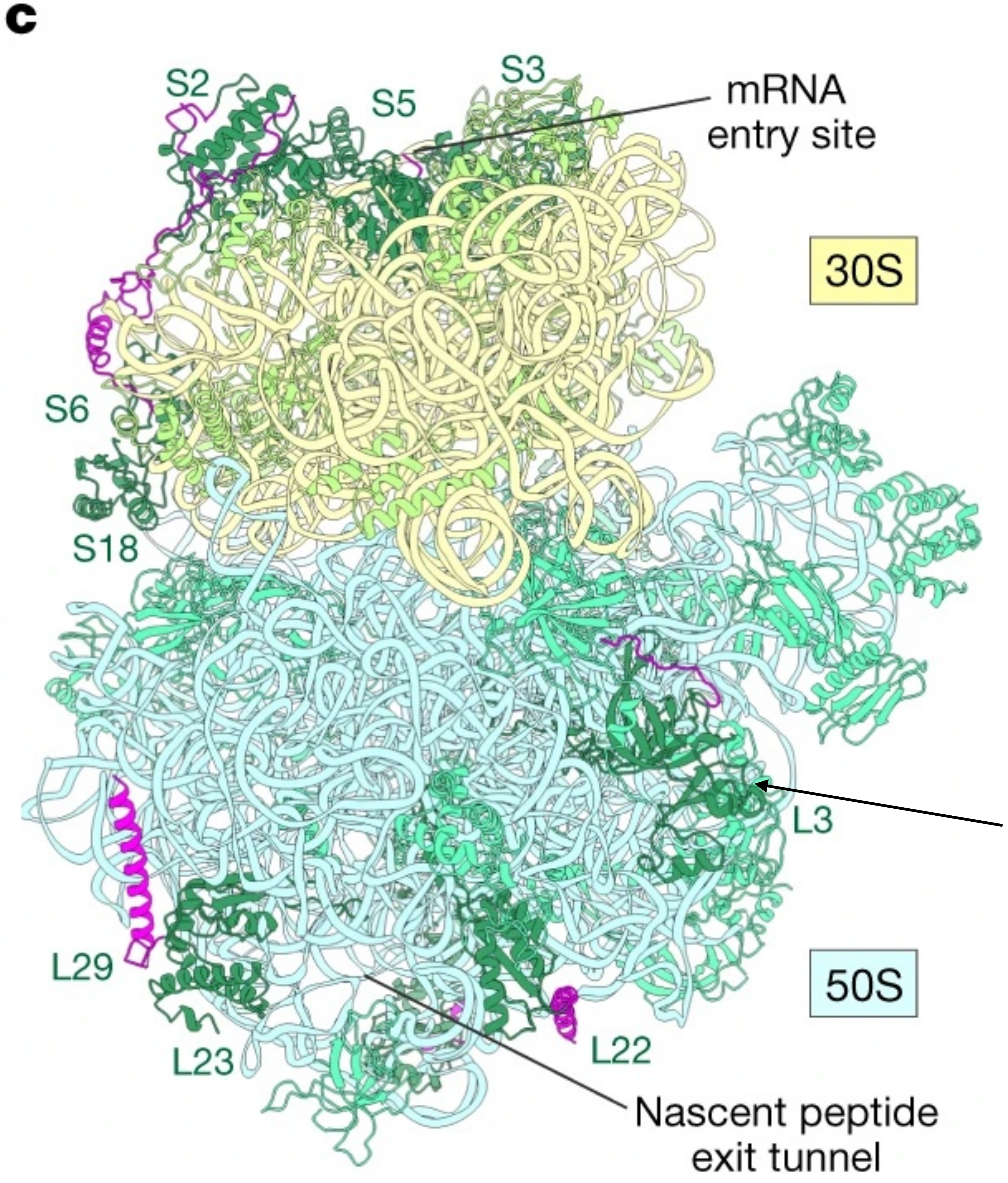


Extended Data Figure 2

Focused refinements revealed rRNA bases and AA side chains

1 In-cell structure of the *M.pnuemoniae* ribosome

1.3 Ribosomal protein extensions

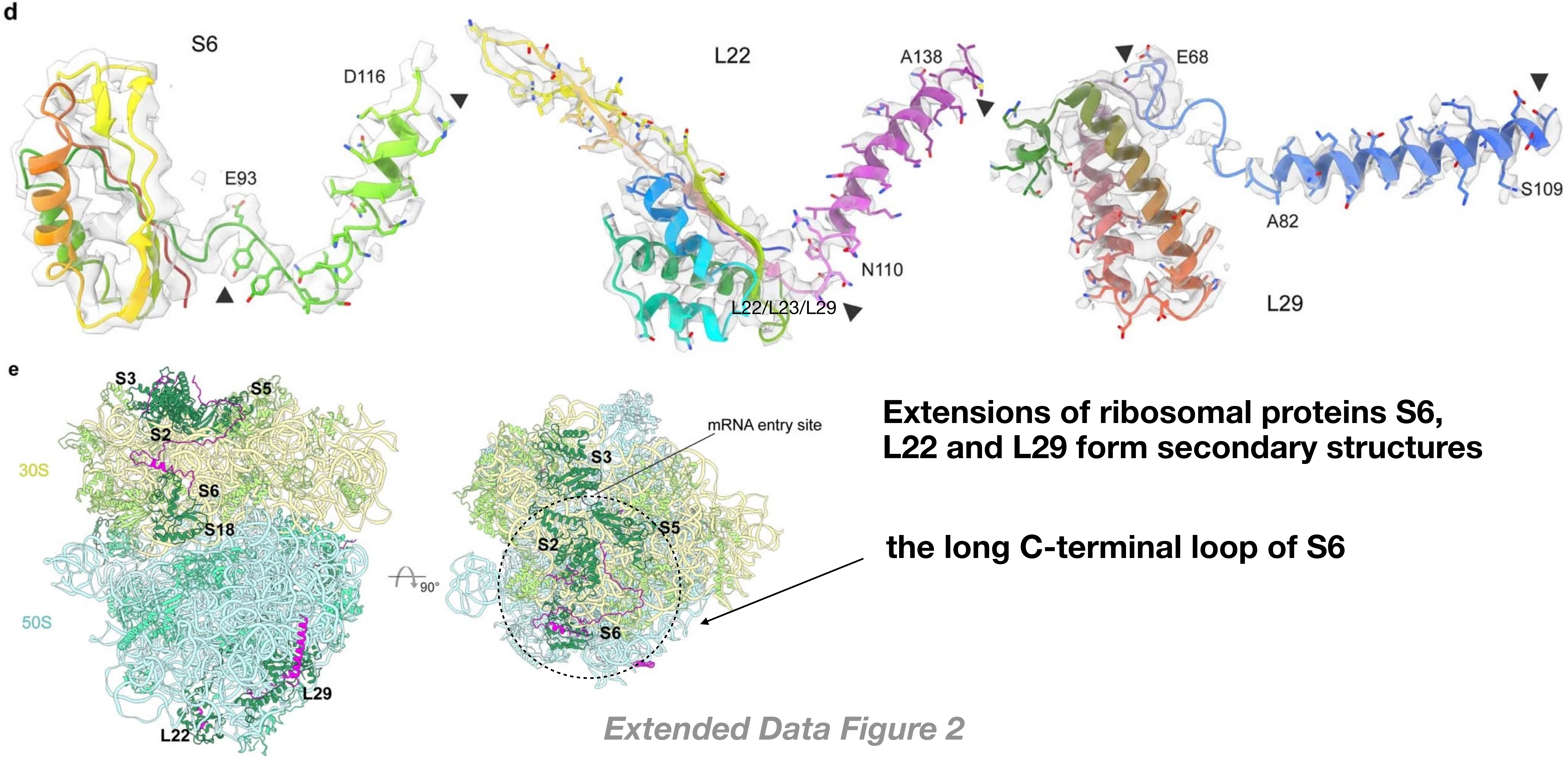


Have sequence extensions

Figure 1

1 In-cell structure of the *M.pnuemoniae* ribosome

1.3 Ribosomal protein extensions



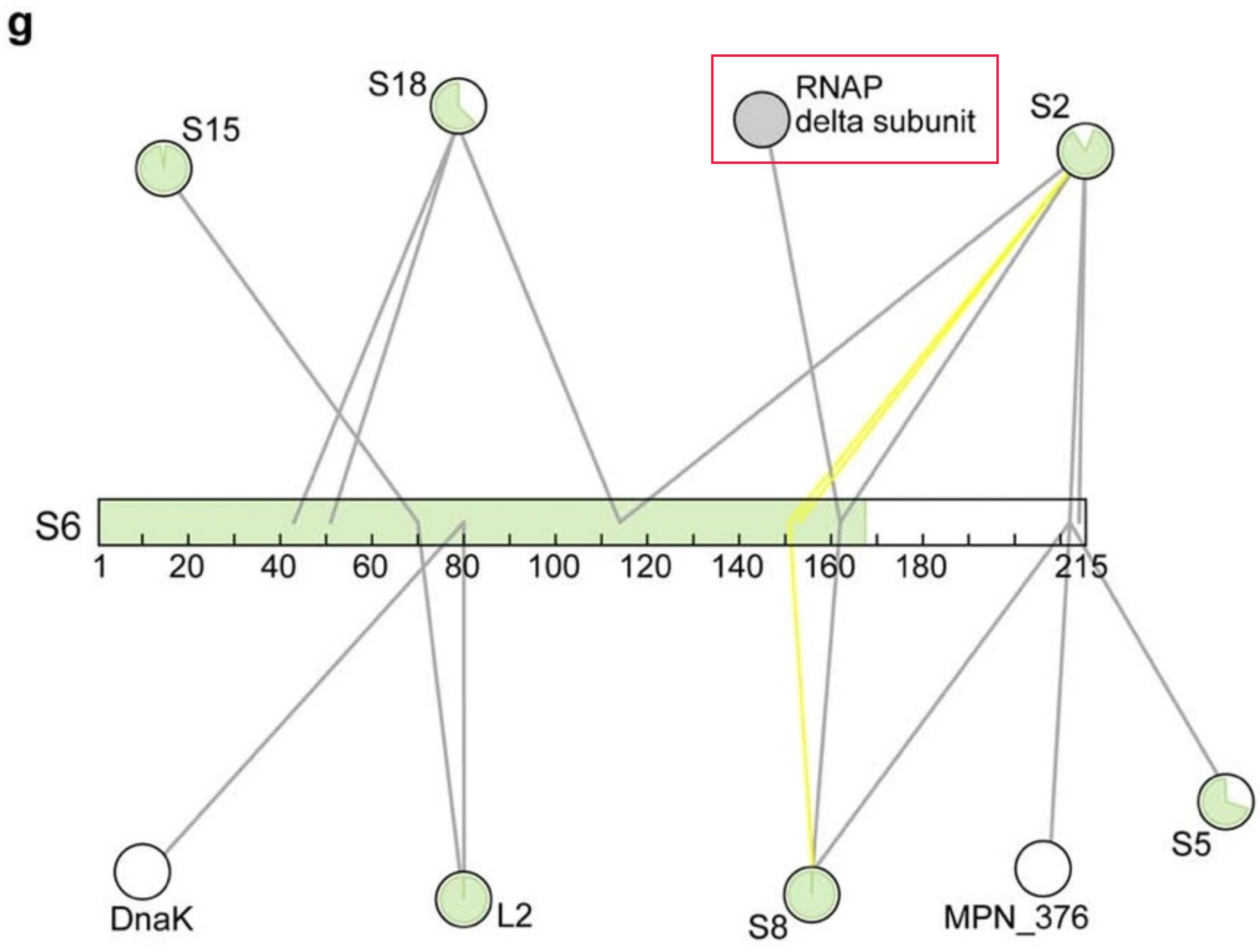
Extensions of ribosomal proteins S6, L22 and L29 form secondary structures

the long C-terminal loop of S6

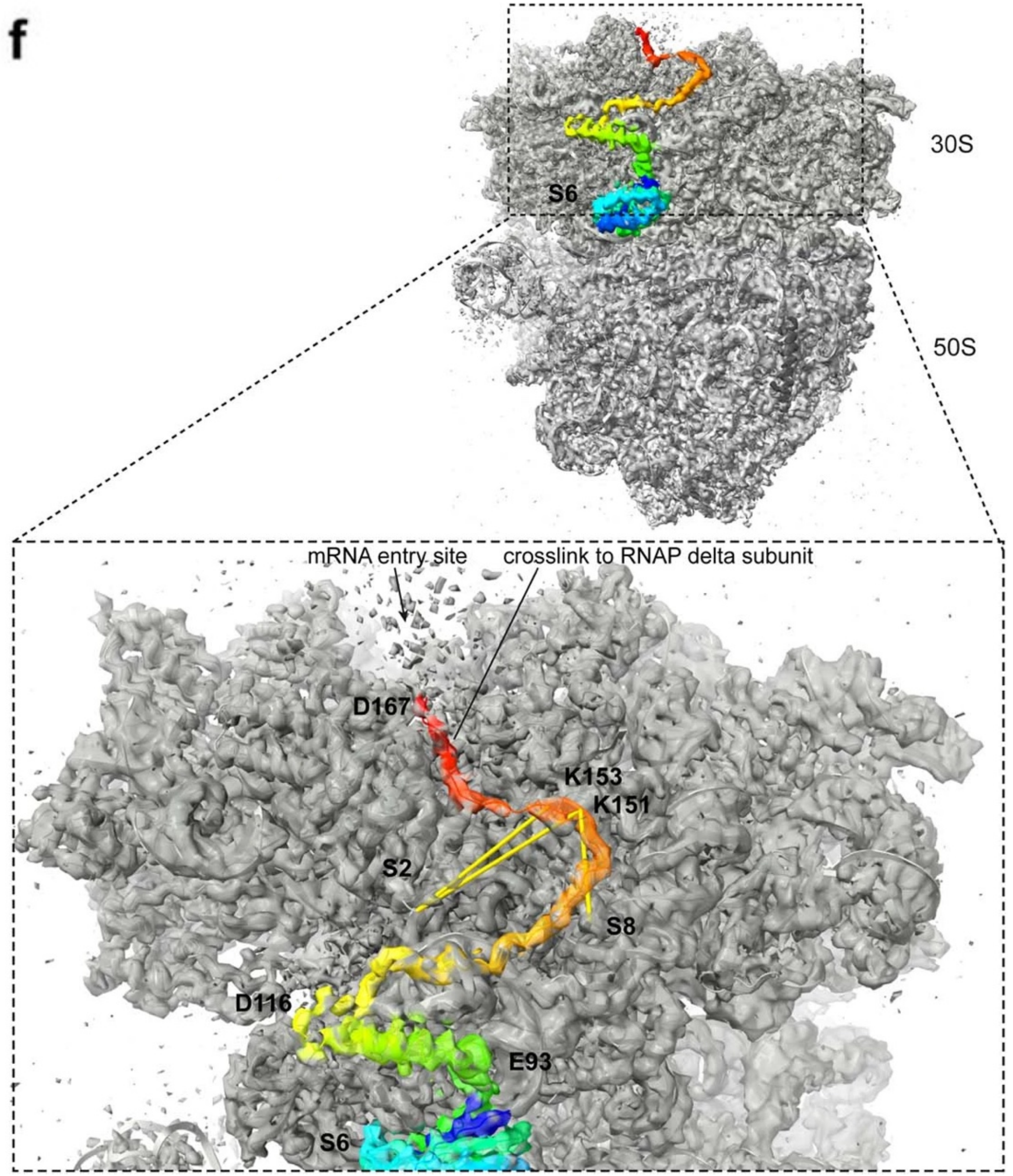
Extended Data Figure 2

1 In-cell structure of the *M.pnuemoniae* ribosome

1.3 Ribosomal protein extensions



Whole-cell cross-linking mass spectrometry(CLMS)



The cross-linking network of S6

Extended Data Figure 2

1 In-cell structure of the *M.pnuemoniae* ribosome

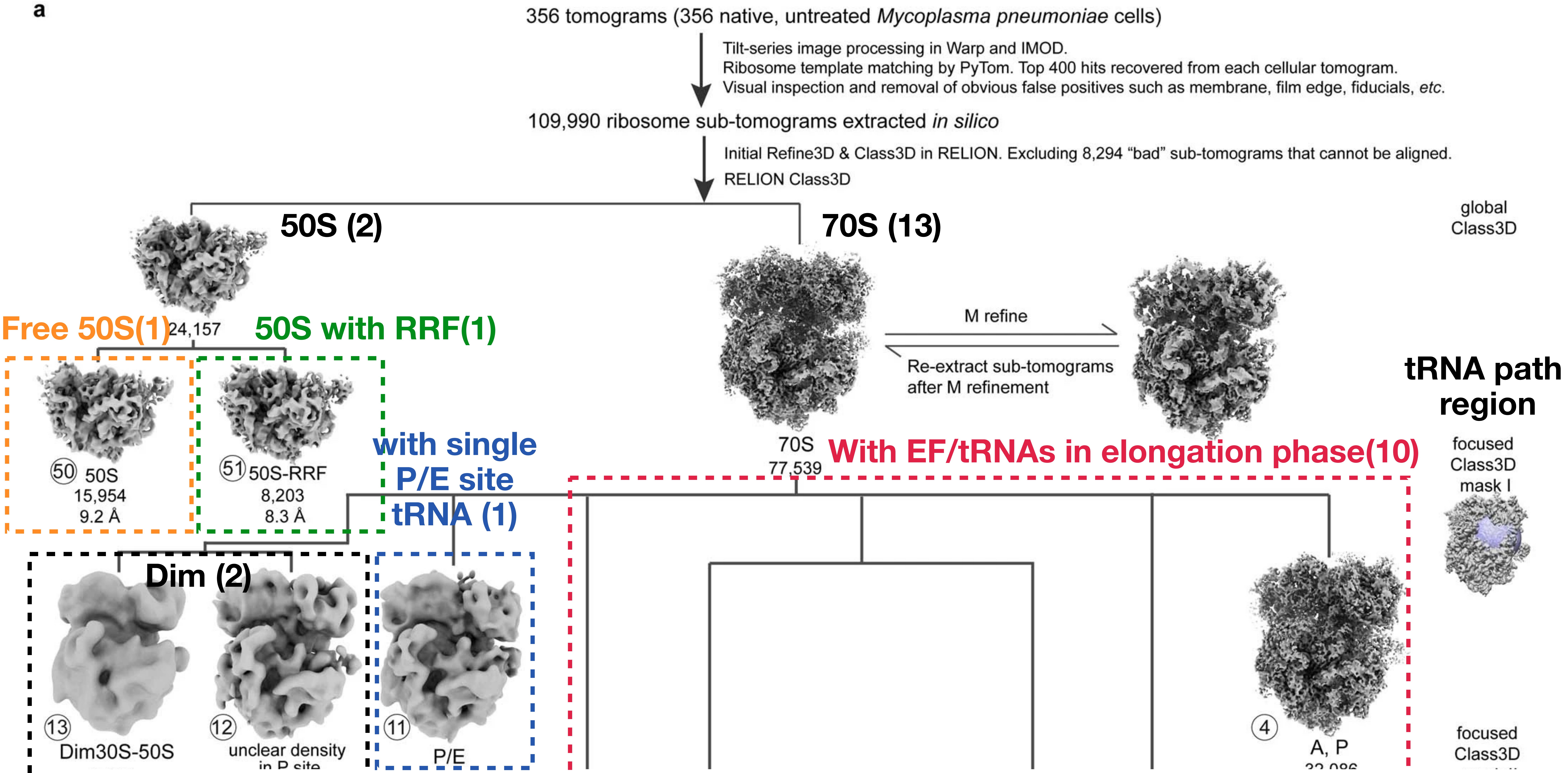
1.3 Ribosomal protein extensions

Ribosome diversity possibly in adaption to different environments and lifestyles

Extended Data Figure 3

2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells



a

356 tomograms (356 native, untreated *Mycoplasma pneumoniae* cells)

Tilt-series image processing in Warp and IMOD.
Ribosome template matching by PyTom. Top 400 hits recovered from each cellular tomogram.
Visual inspection and removal of obvious false positives such as membrane, film edge, fiducials, *etc.*

109,990 ribosome sub-tomograms extracted *in silico*

Initial Refine3D & Class3D in RELION. Excluding 8,294 "bad" sub-tomograms that cannot be aligned.
RELION Class3D

50S (2)

70S (13)

global Class3D

24,157

Free 50S(1)

50S with RRF(1)

M refine

Re-extract sub-tomograms after M refinement

tRNA path region

focused Class3D mask I

70S
77,539

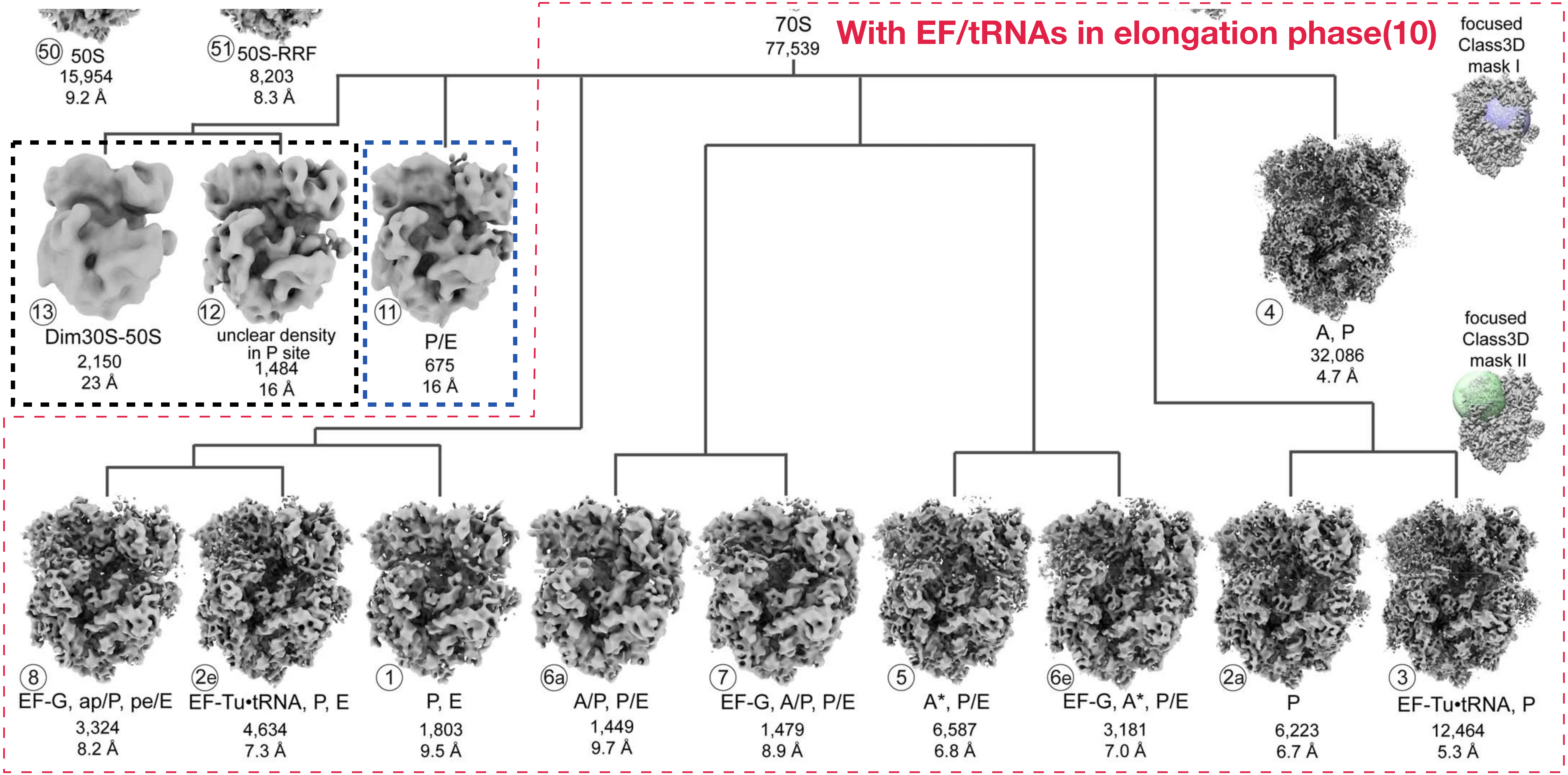
With EF/tRNAs in elongation phase(10)

with single P/E site tRNA (1)

focused Class3D

2 Structure dynamics of translation in cells

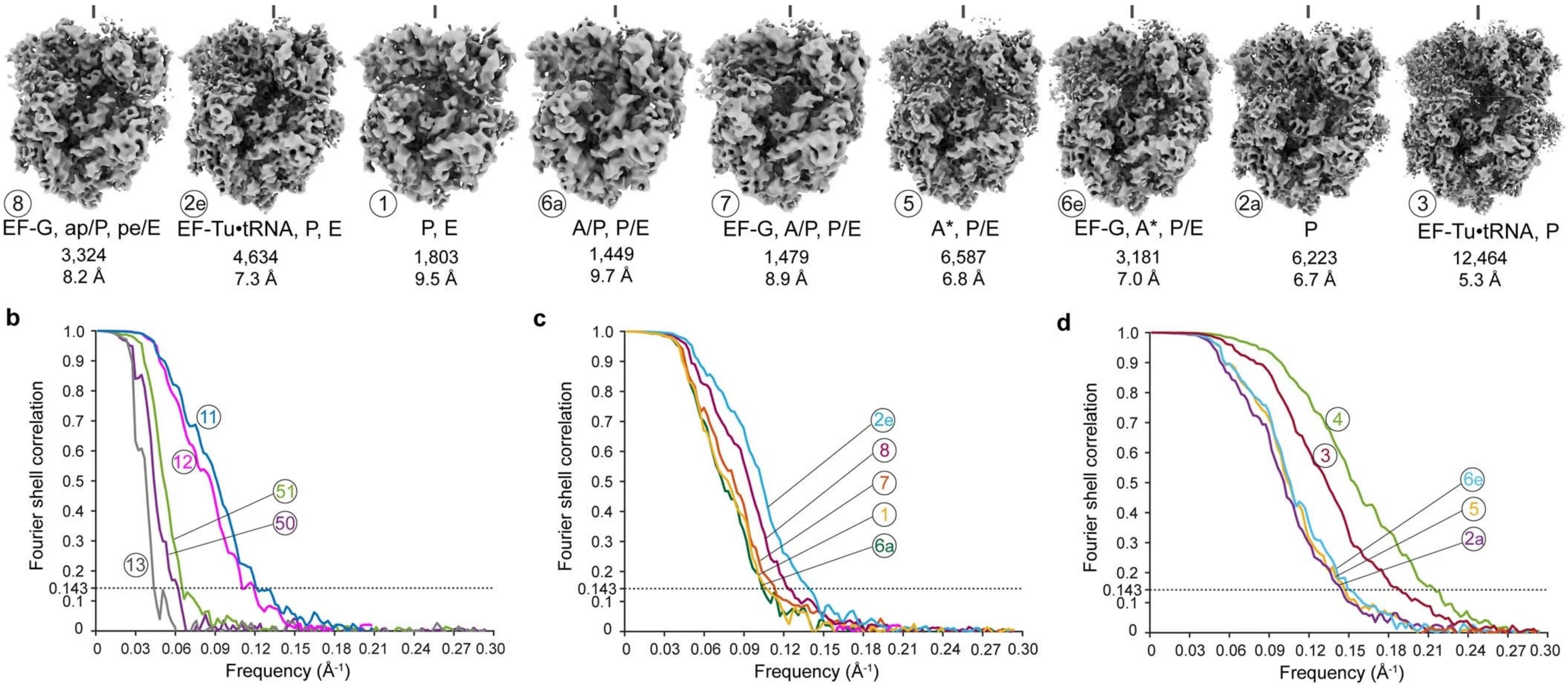
2.1 Classification and refinement of ribosomes in native untreated cells



With EF/tRNAs in elongation phase(10)

2 Structure dynamics of translation in cells

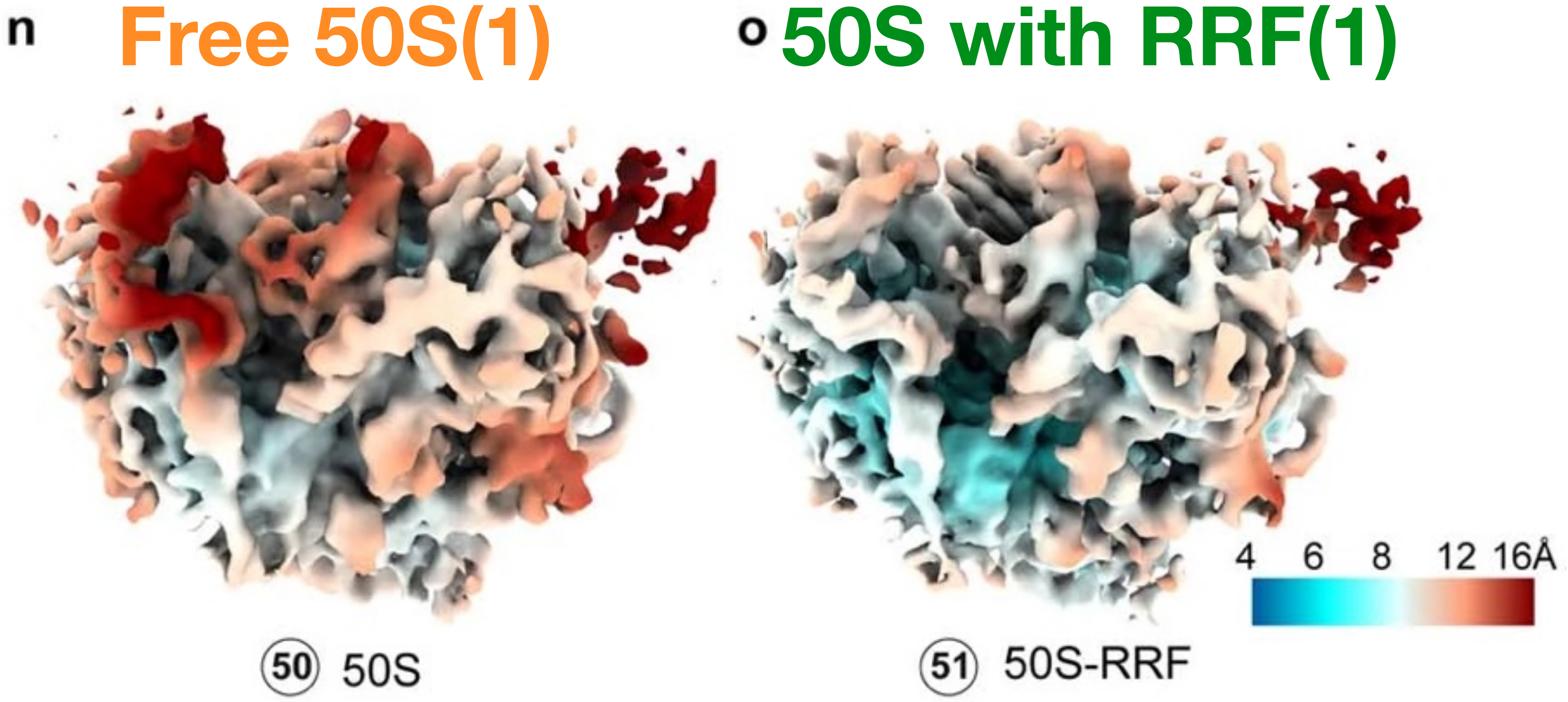
2.1 Classification and refinement of ribosomes in native untreated cells



Extended Data Figure 4

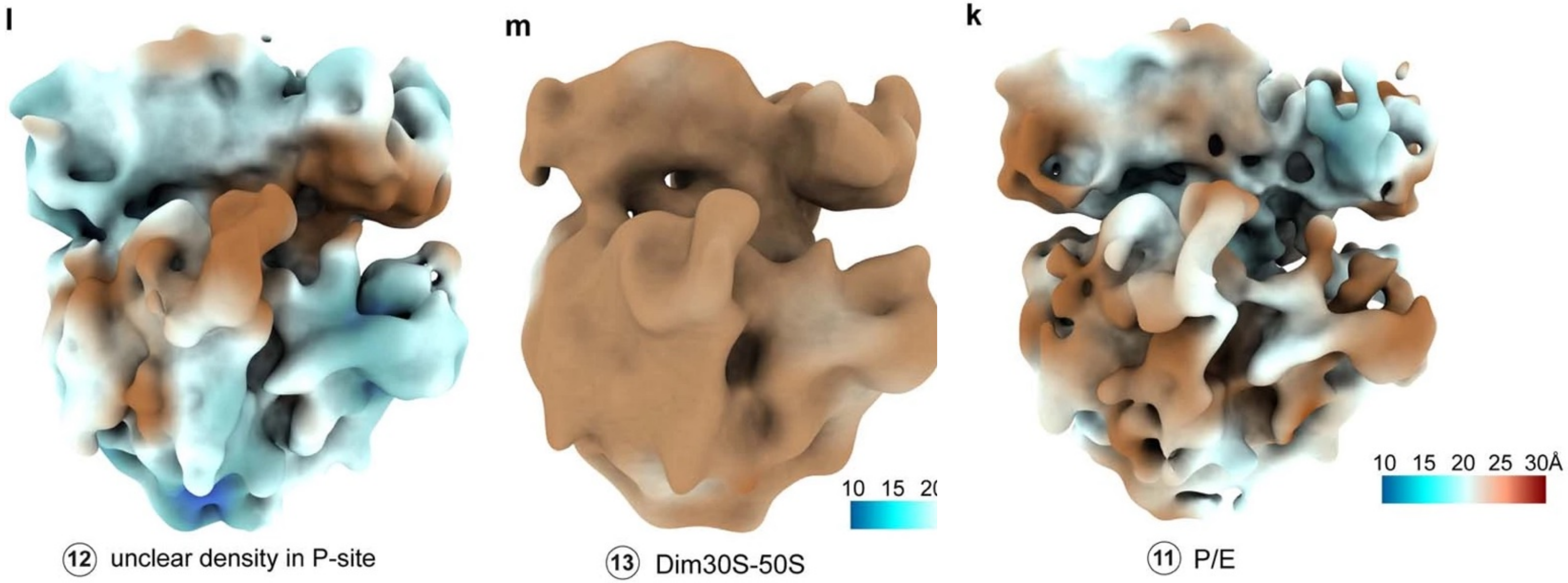
2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells



Free 50S(1)

50S with RRF(1)



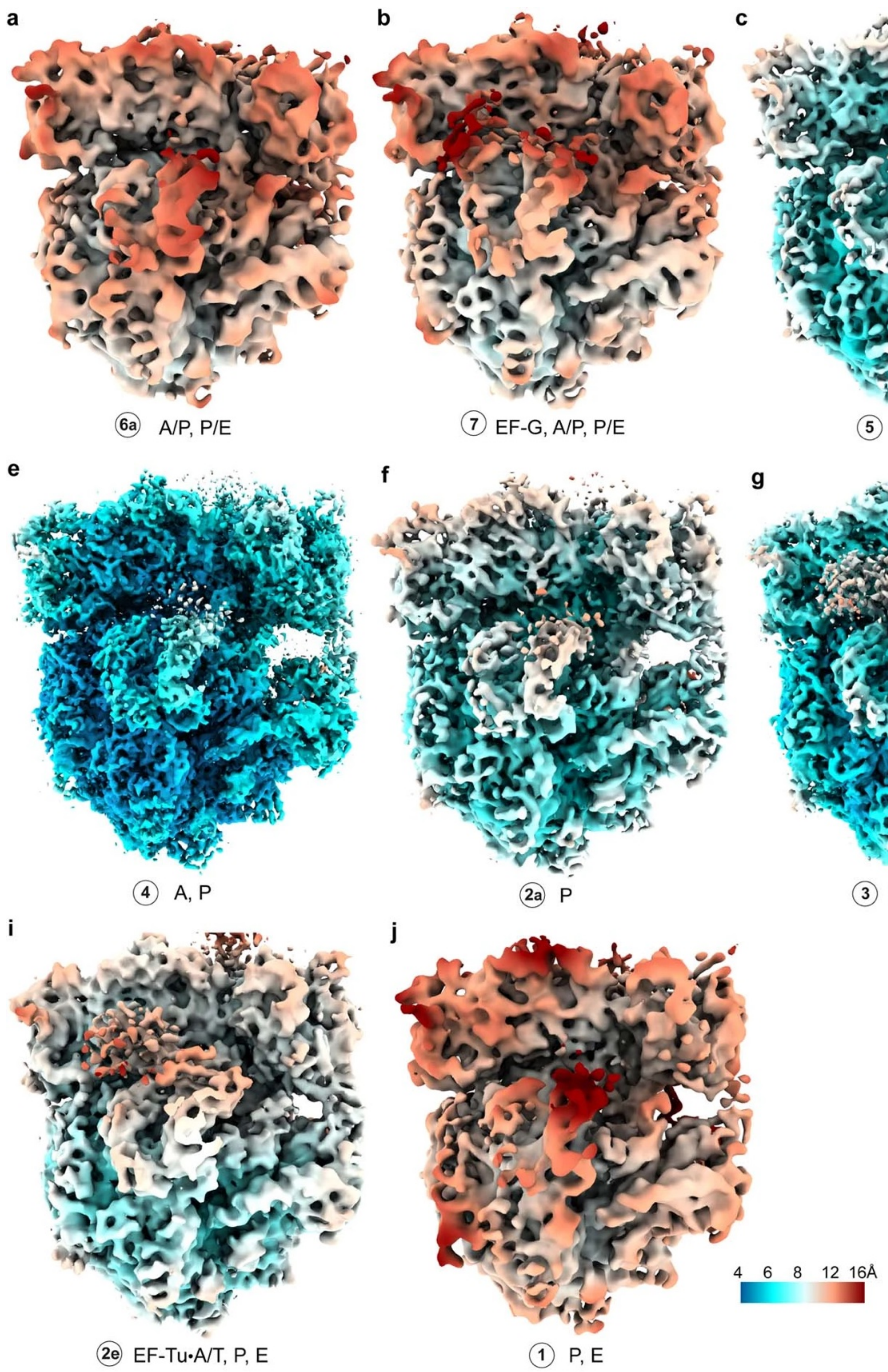
Dim (2)

with single P/E site tRNA (1)

Extended Data Figure 6

2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells

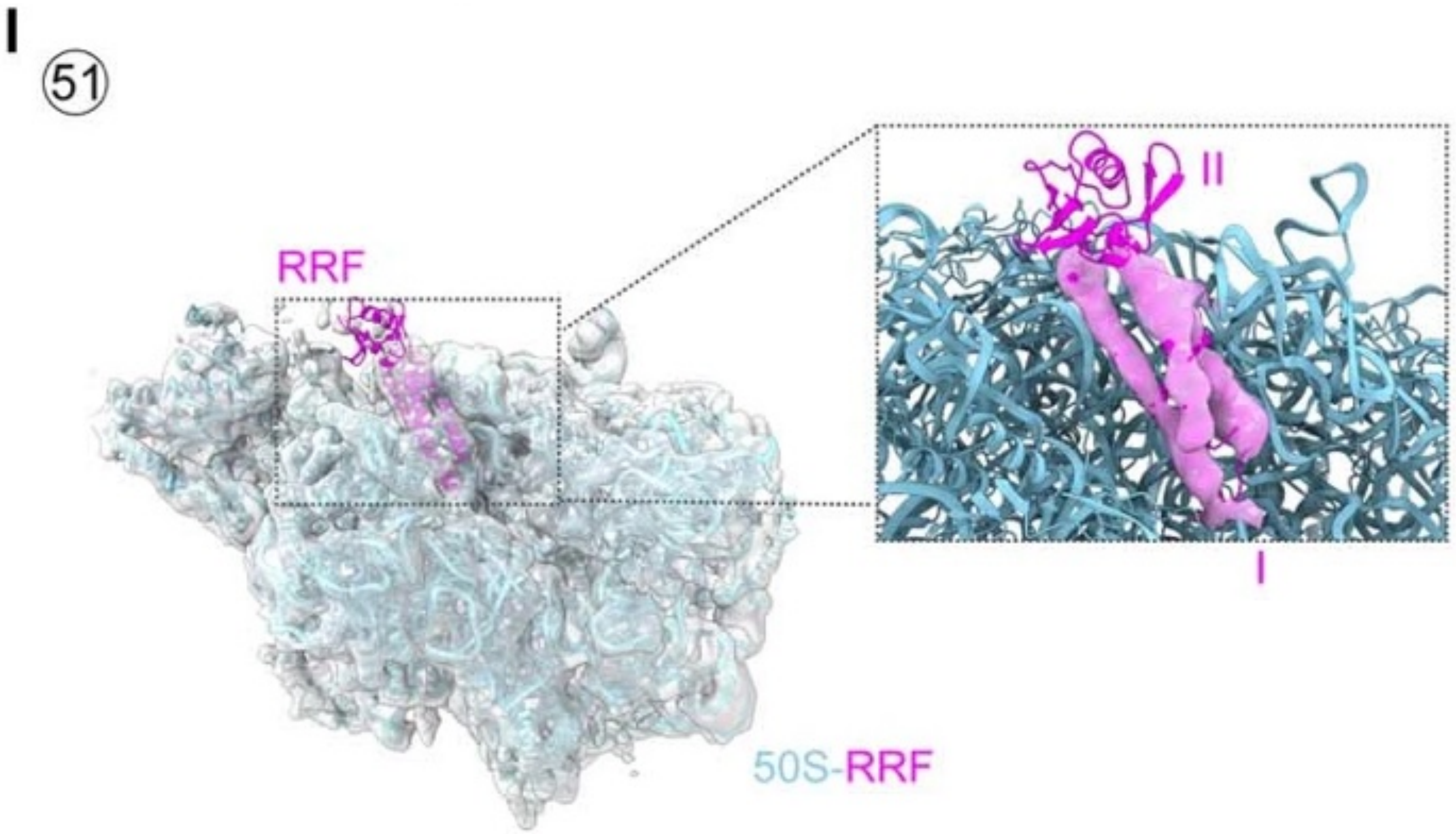


With EF/tRNAs in elongation phase(10)

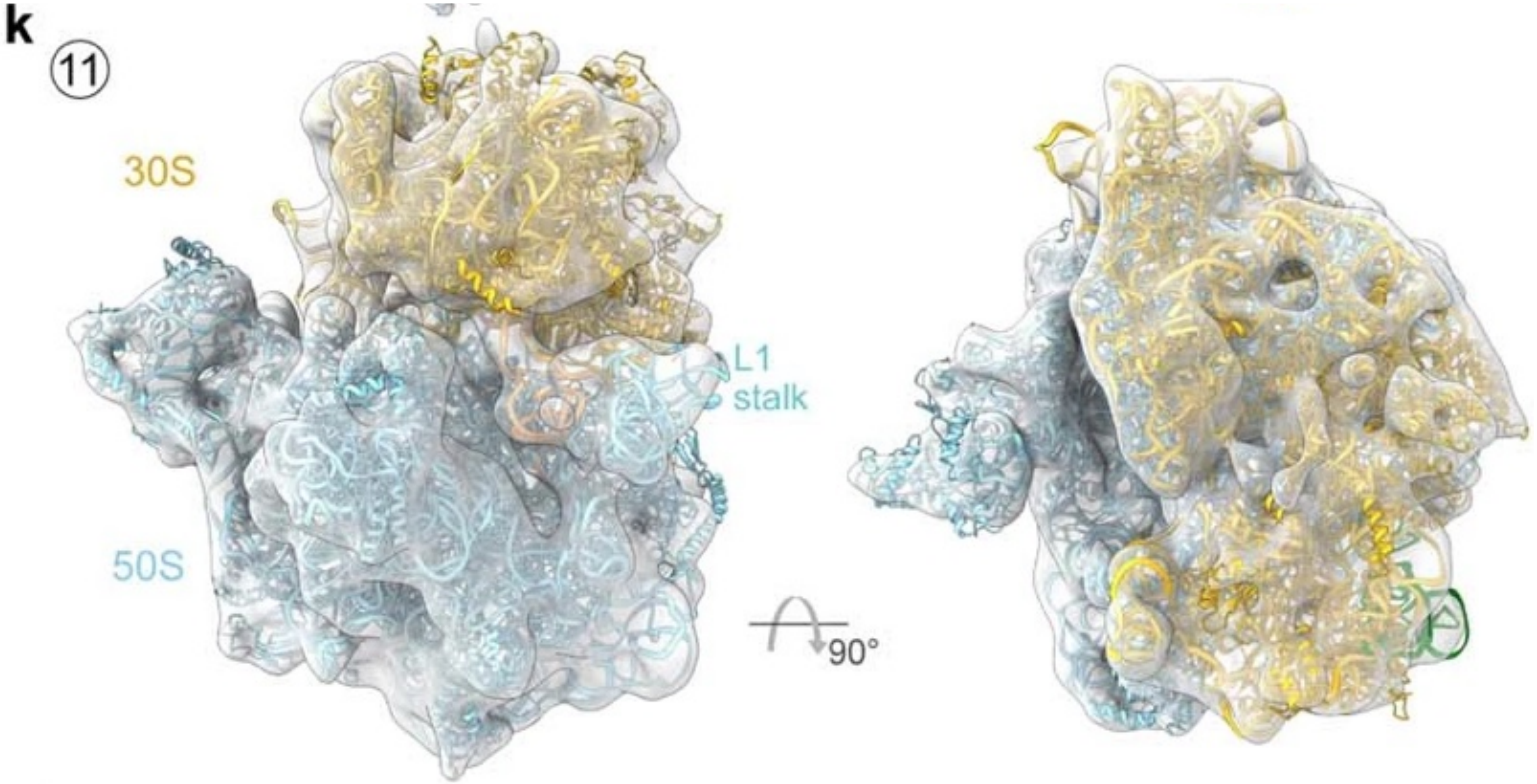
Extended Data Figure 6

2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells



50S with RRF(1)



with single P/E site tRNA (1)

Models constructed by flexible fitting

Extended Data Figure 7

2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells

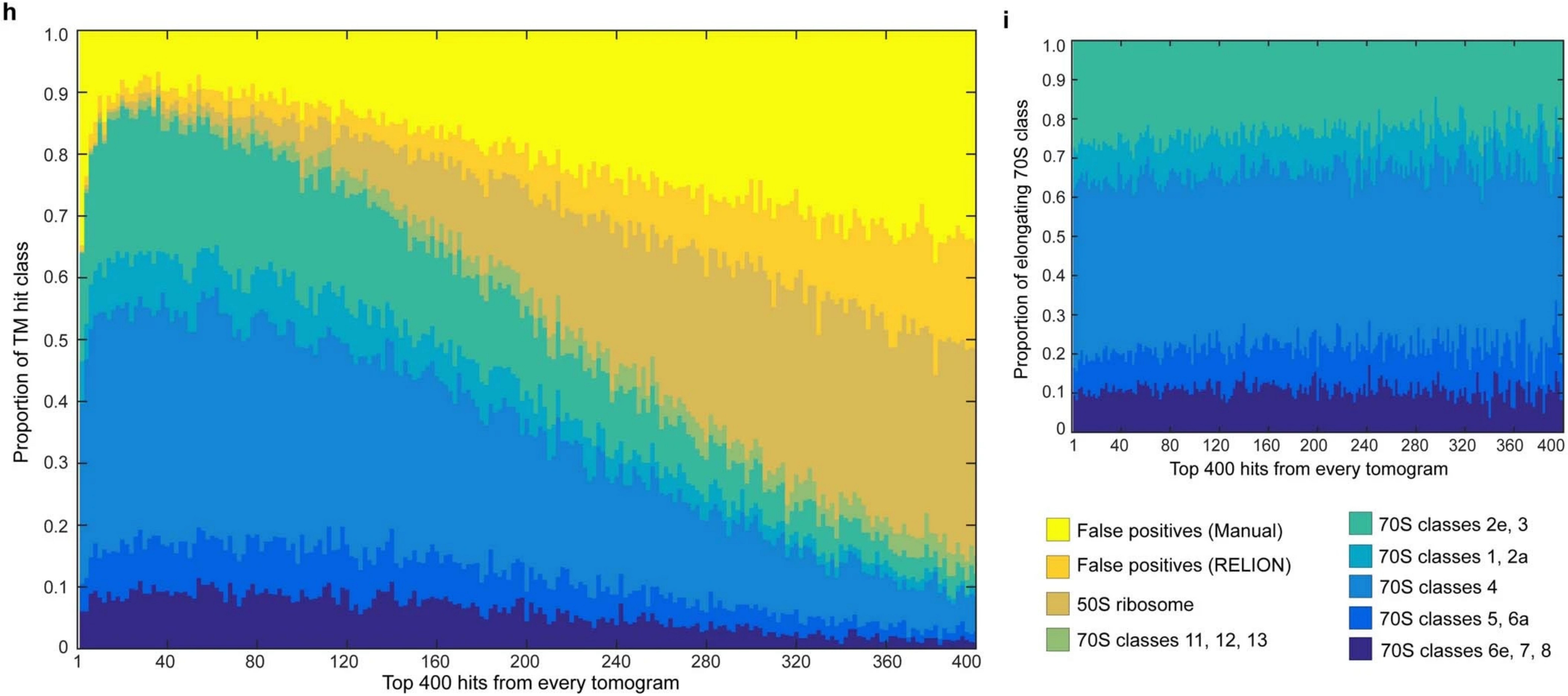
With EF/tRNAs in elongation phase(10)

Models constructed by flexible fitting

Extended Data Figure 7

2 Structure dynamics of translation in cells

2.1 Classification and refinement of ribosomes in native untreated cells

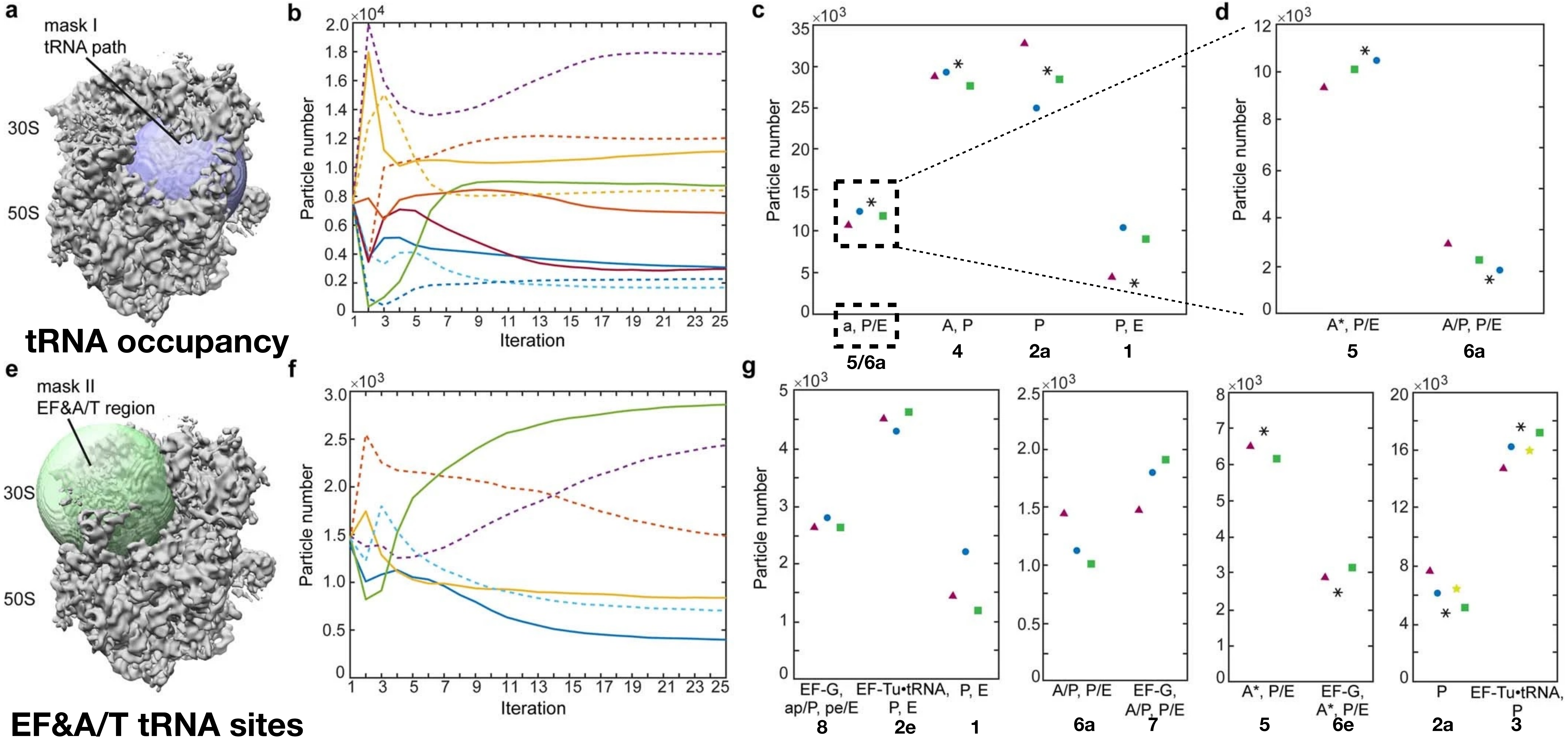


the classification results are not biased by the ribosome picking

Extended Data Figure 5

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle



tRNA occupancy

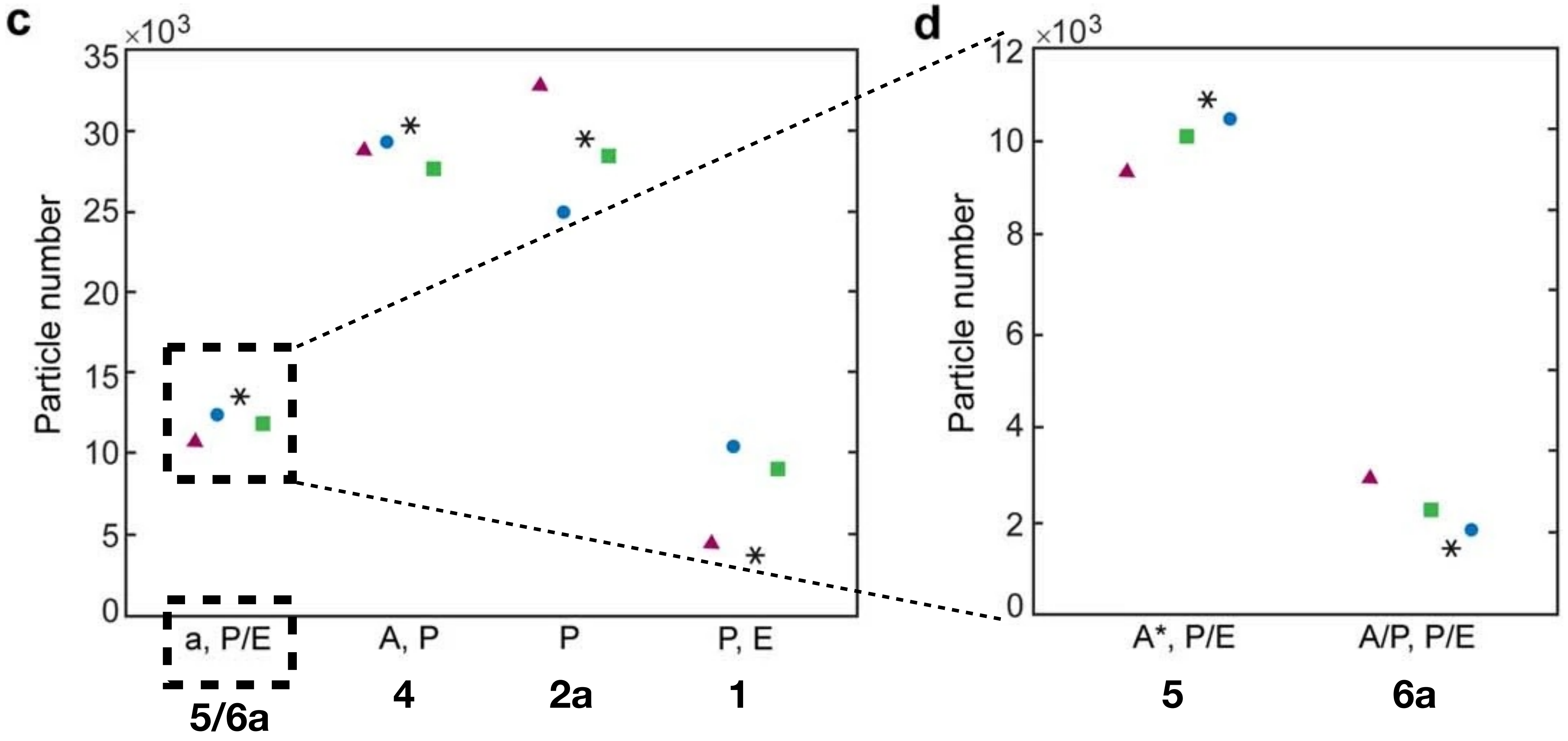
EF&A/T tRNA sites

Extended Data Figure 5

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle

tRNA occupancy



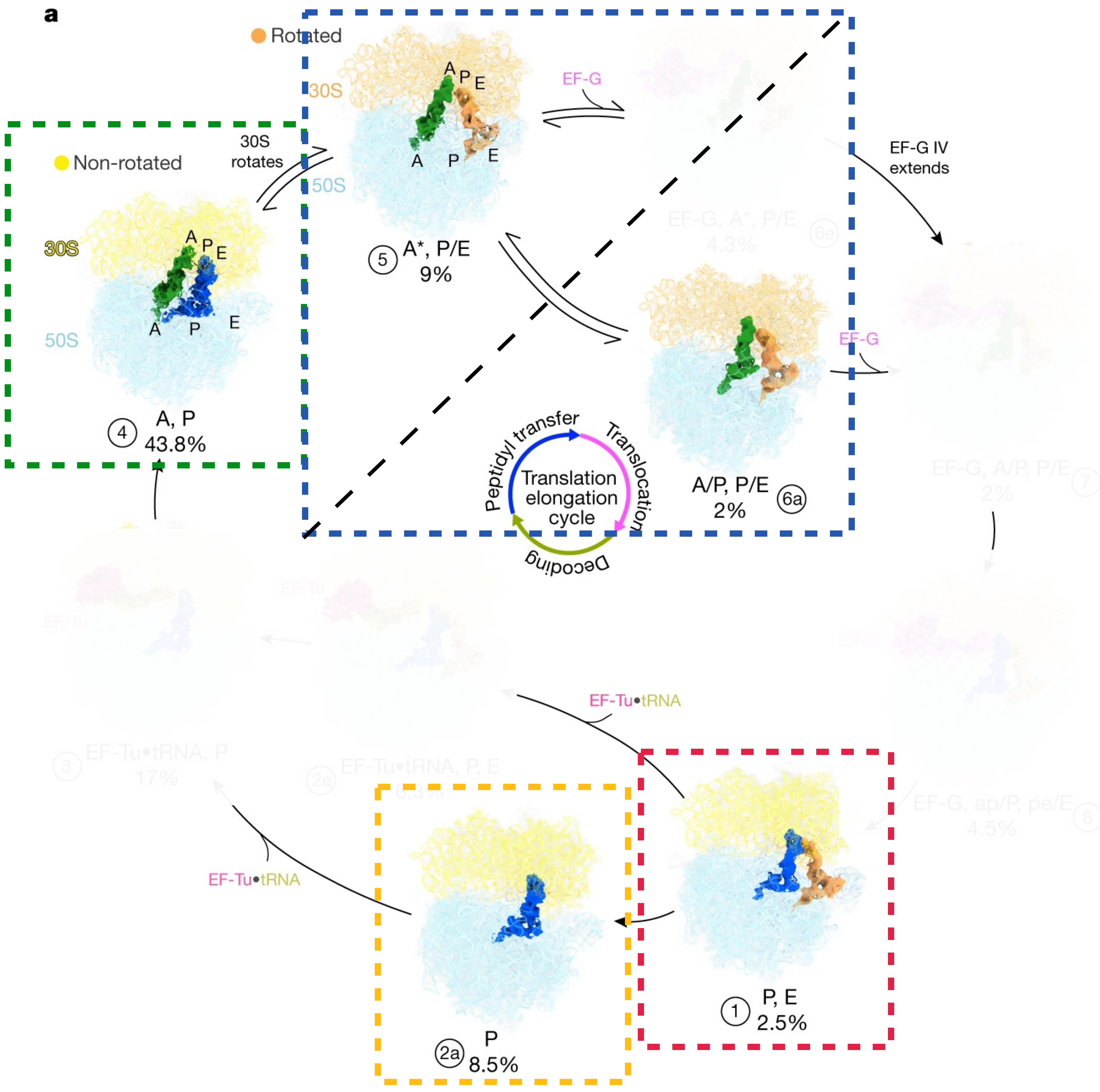
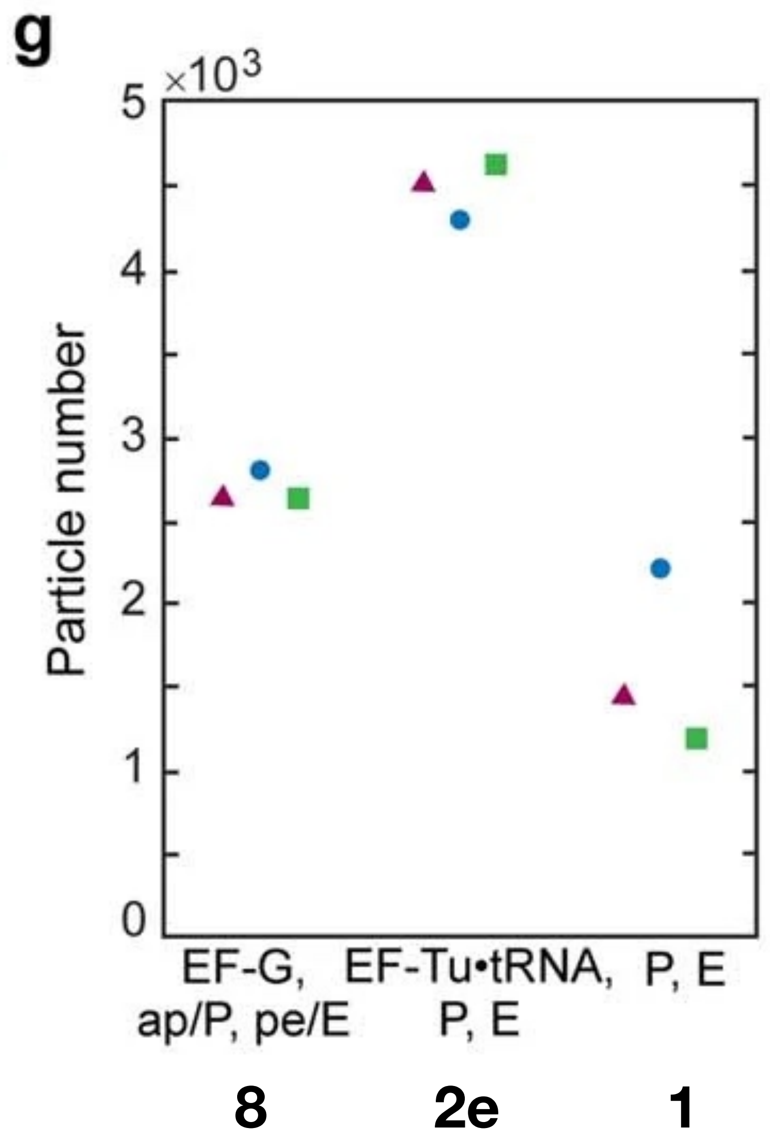


Figure 2

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle

EF&A/T tRNA sites



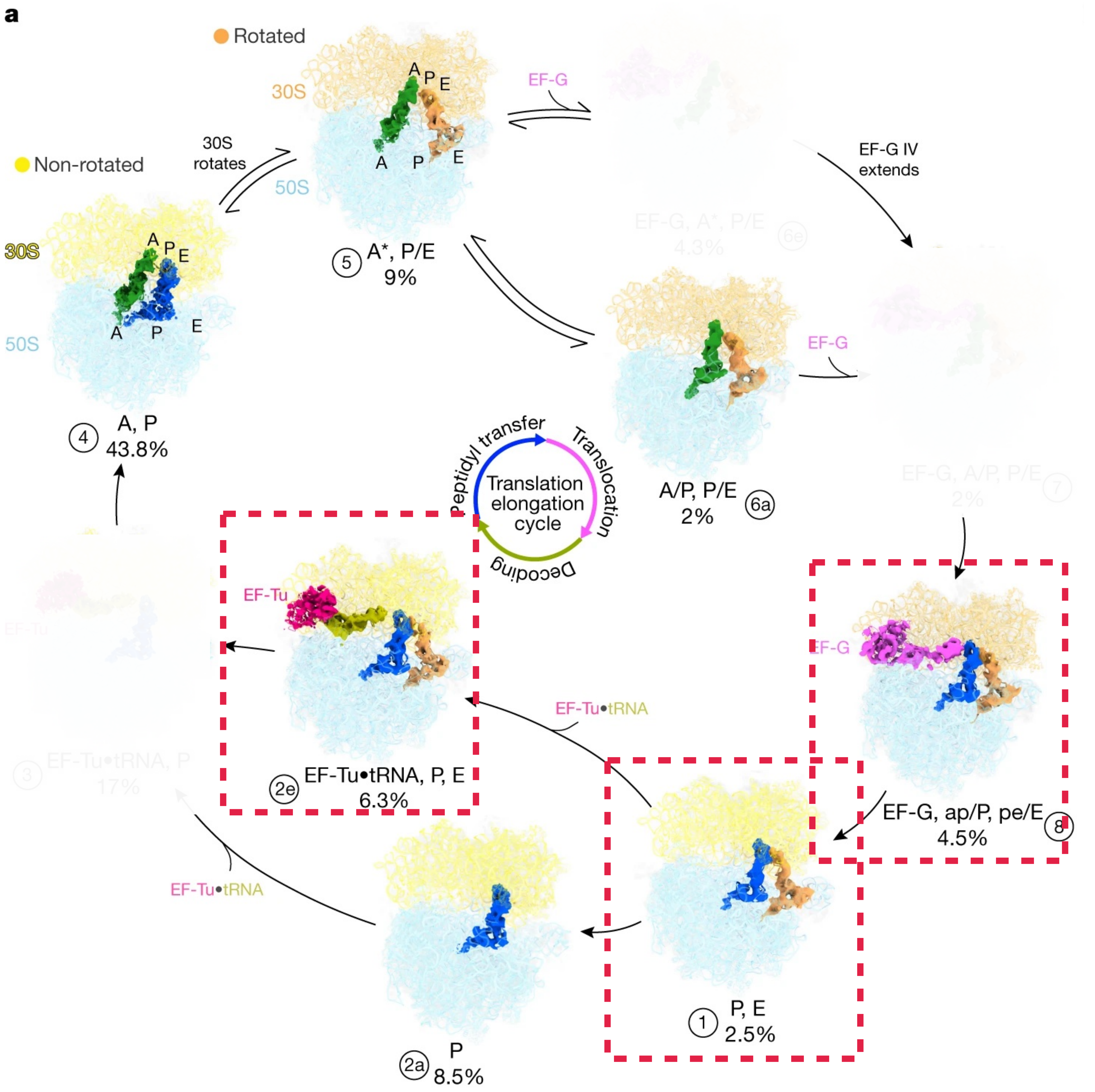
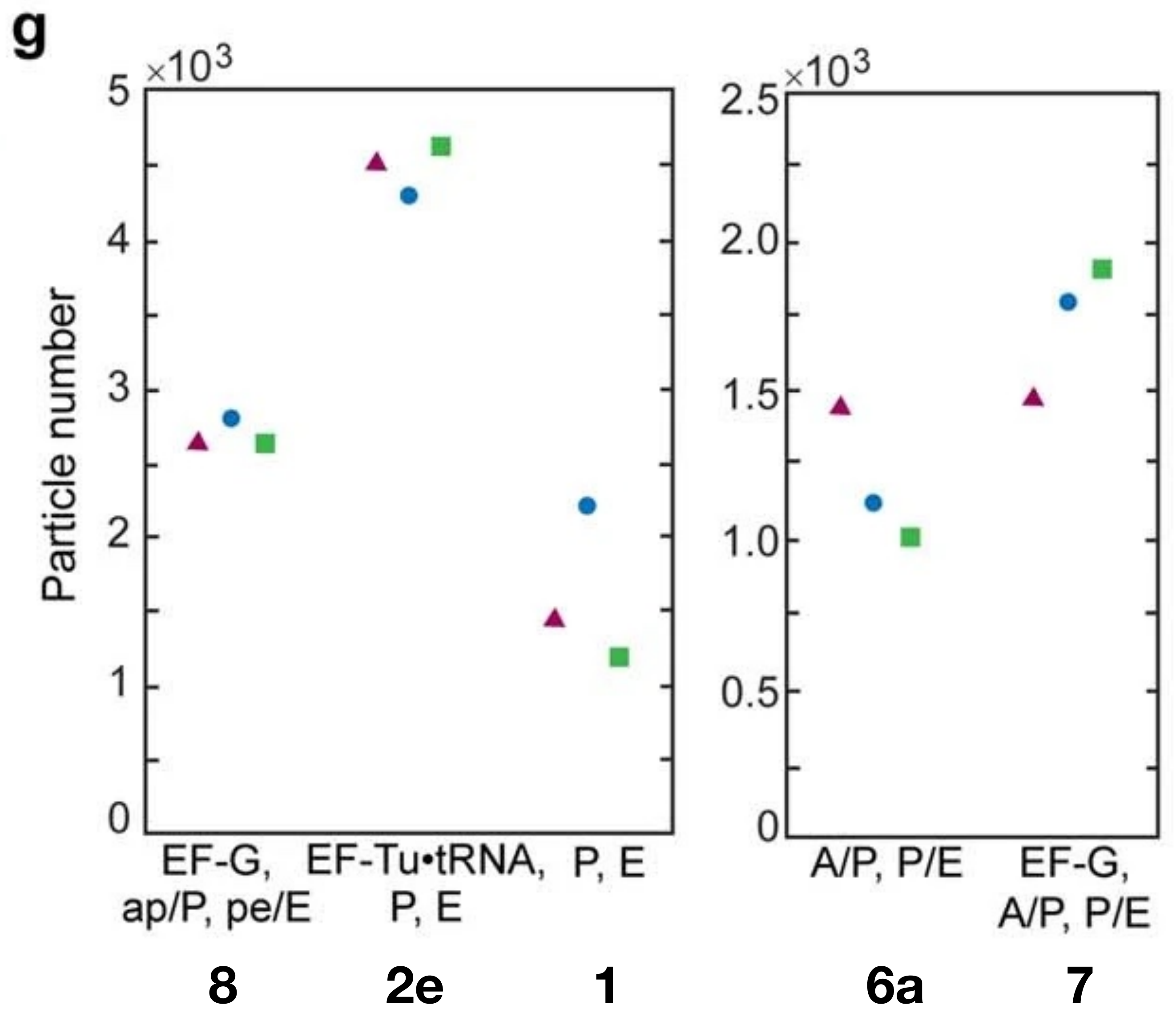


Figure 2

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle

EF&A/T tRNA sites



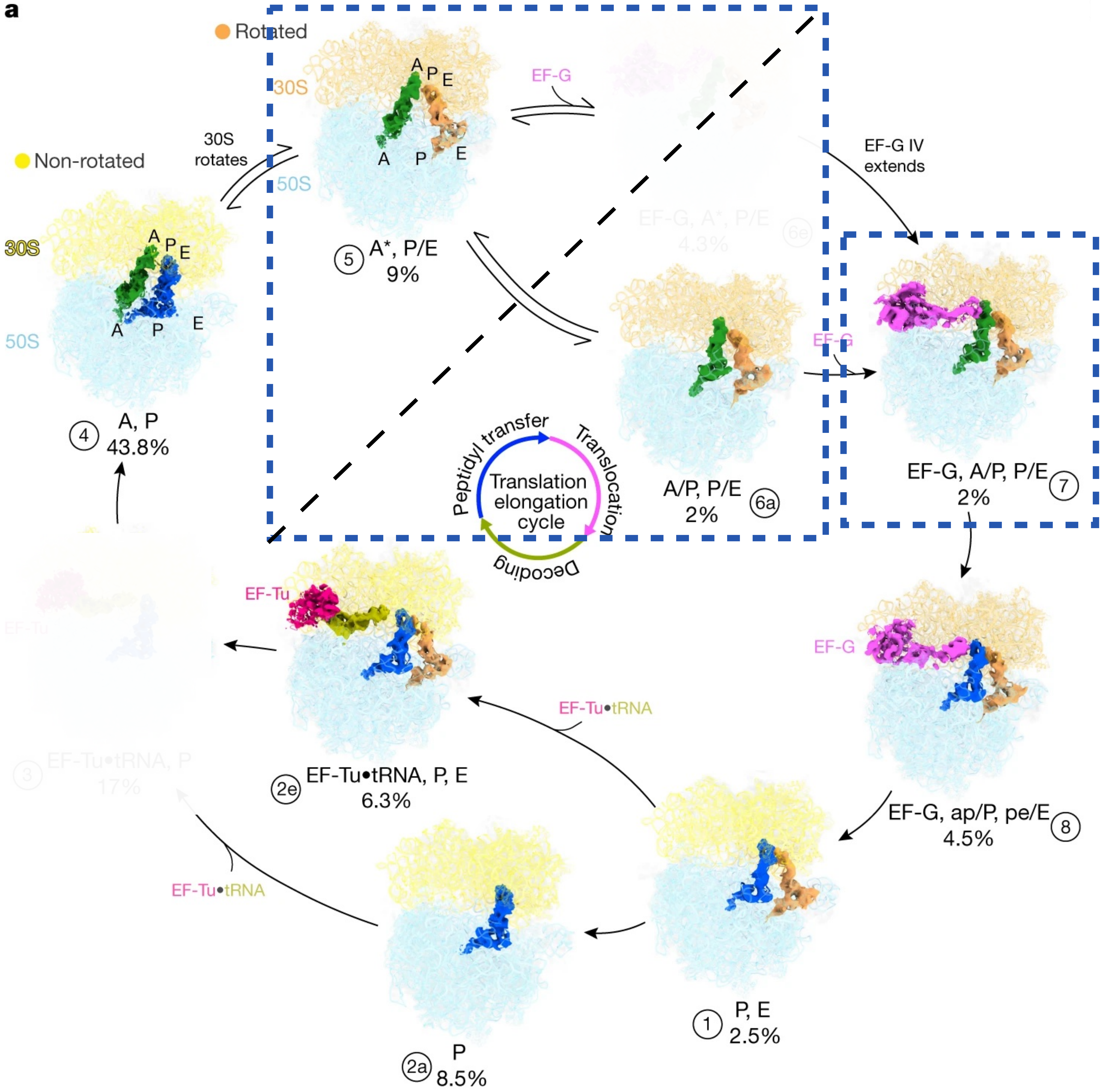
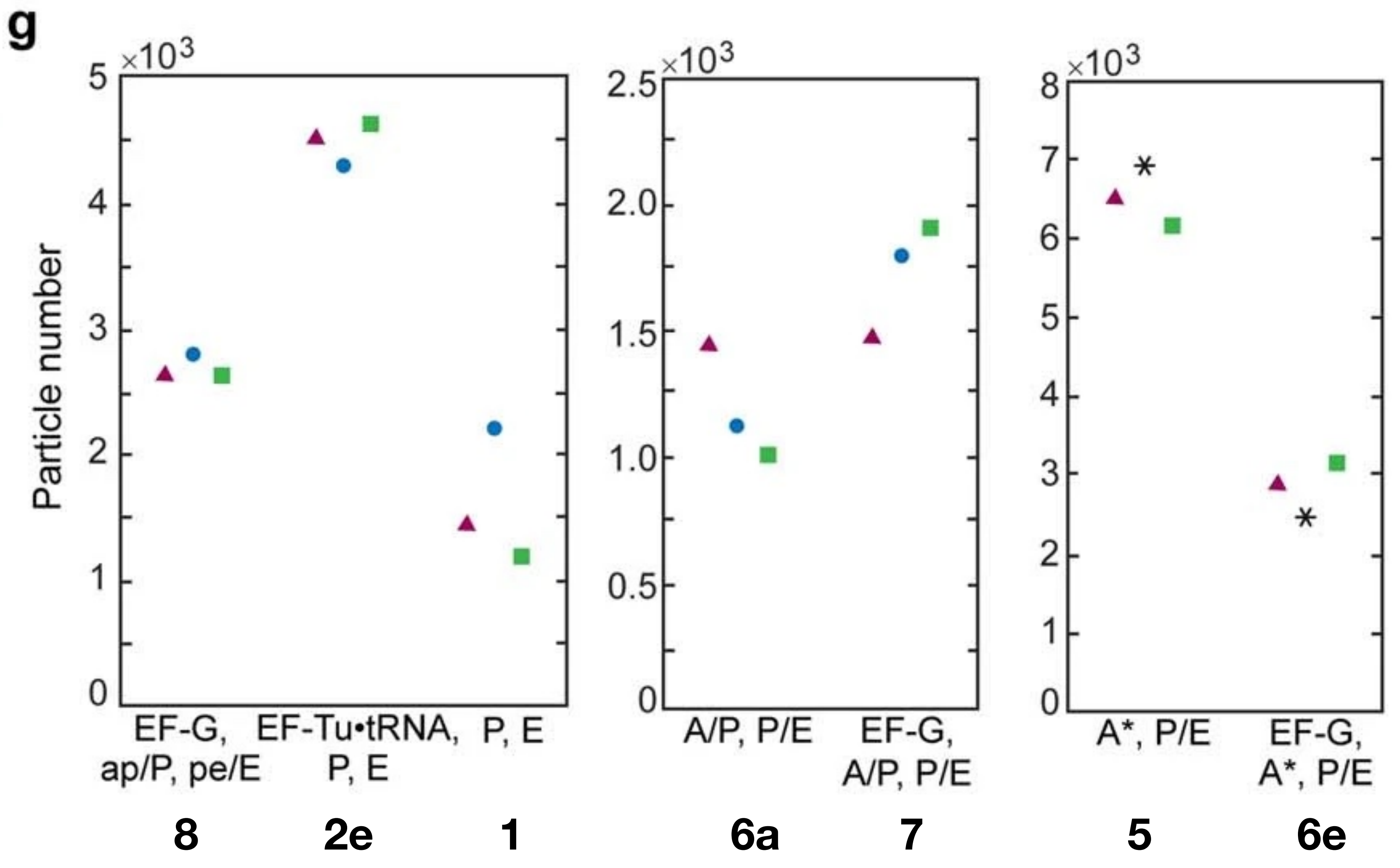


Figure 2

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle

EF&A/T tRNA sites



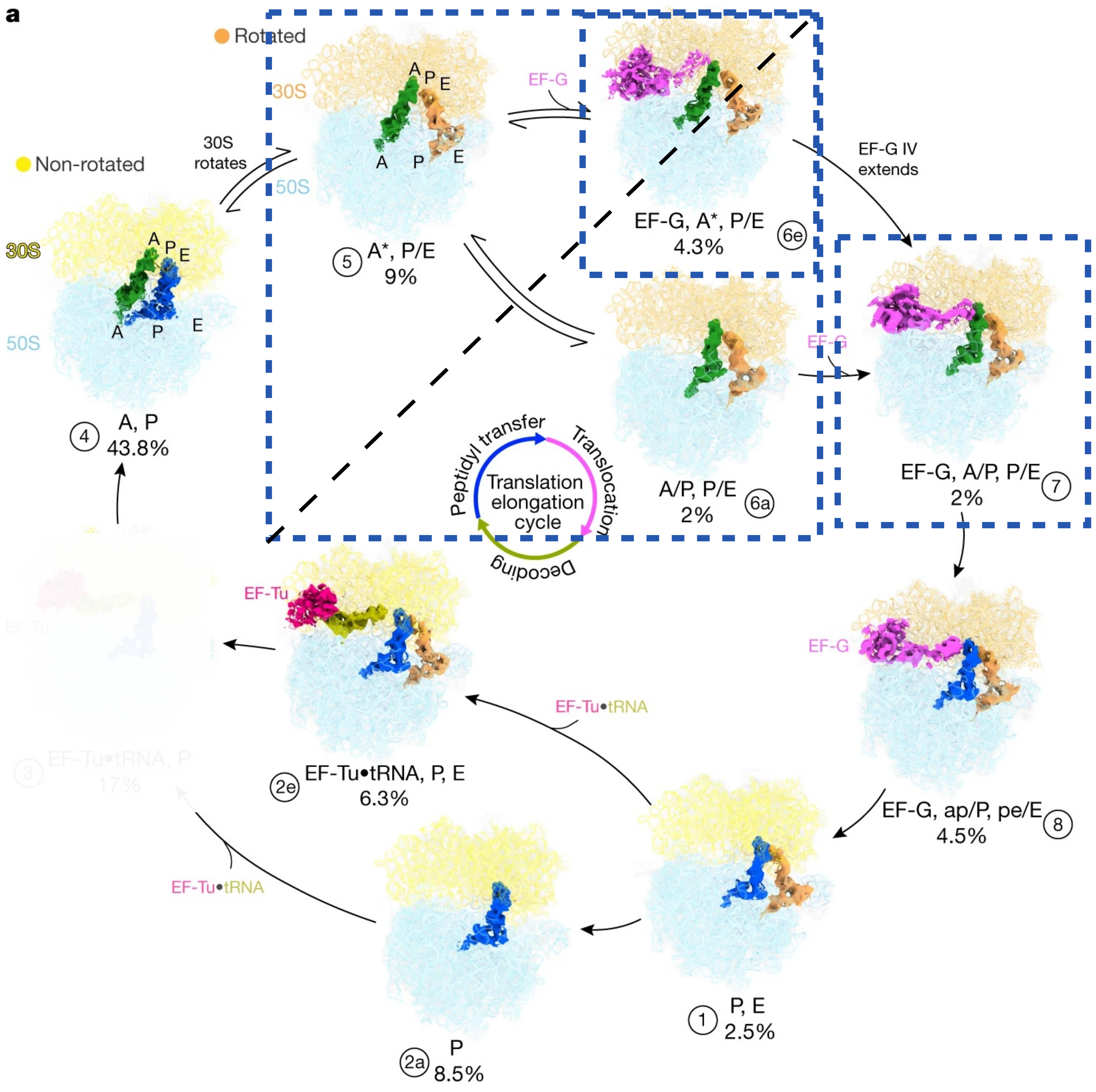
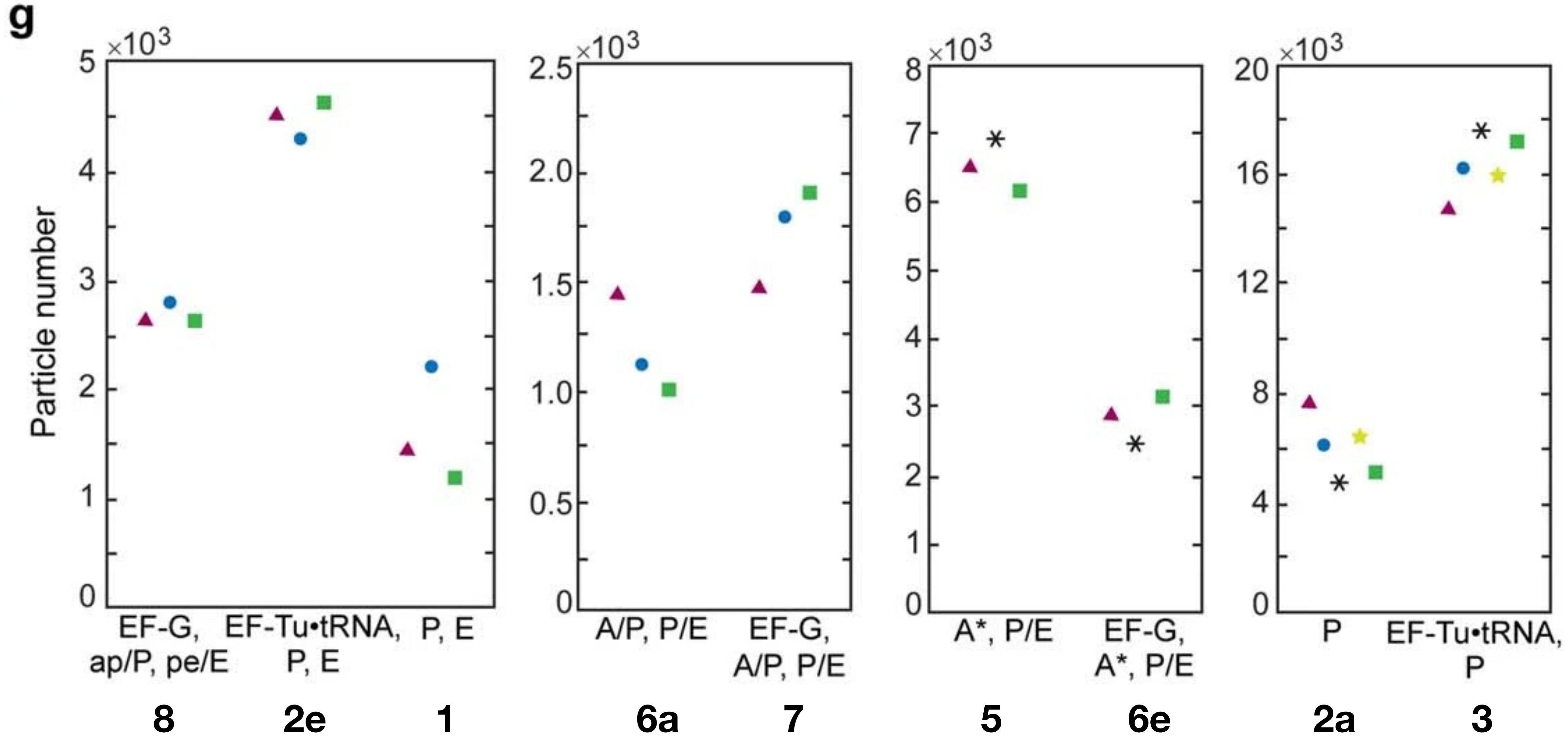


Figure 2

2 Structure dynamics of translation in cells

2.2 Ribosome classification reconstructs the elongation cycle

EF&A/T tRNA sites



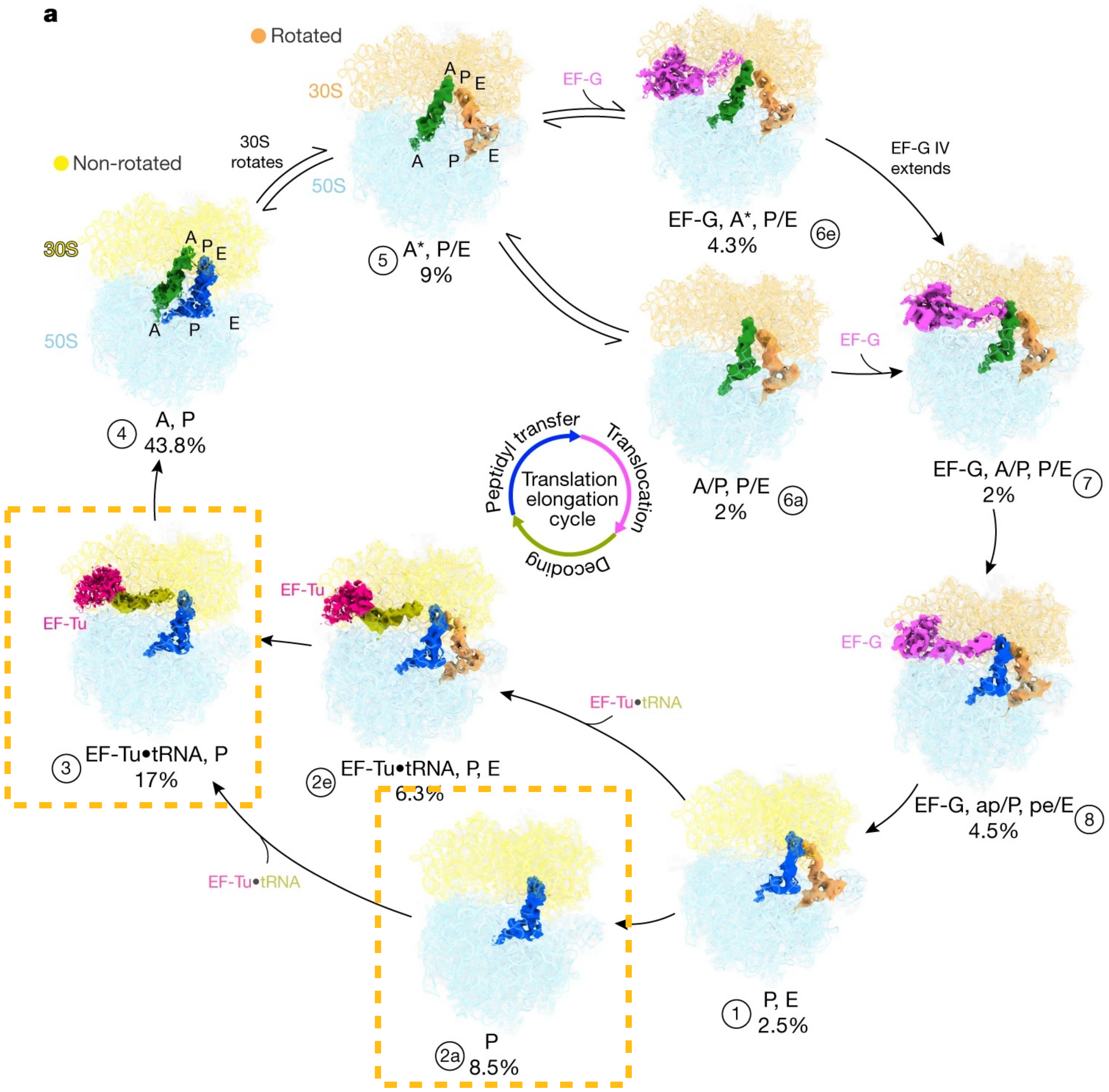
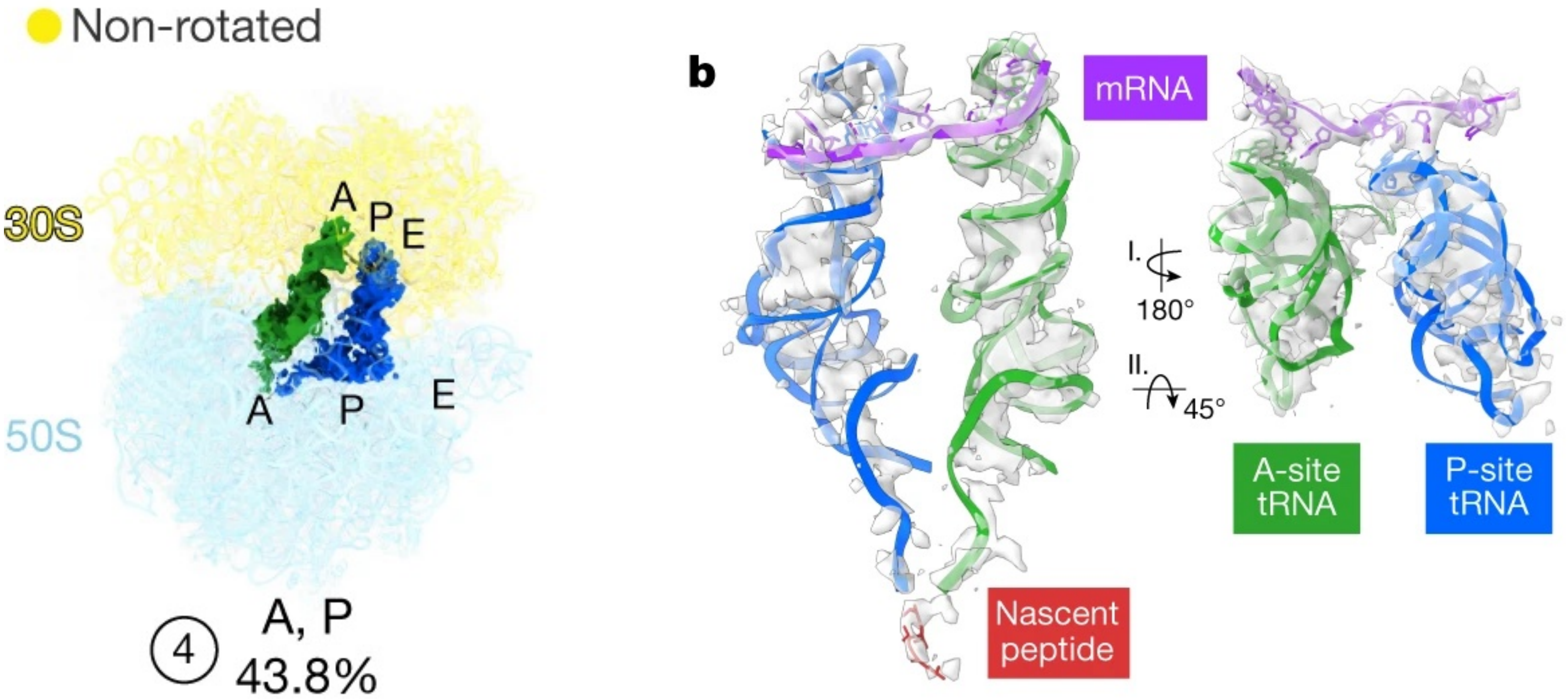


Figure 2

2 Structure dynamics of translation in cells

2.3 ‘A, P’ state was the most populated

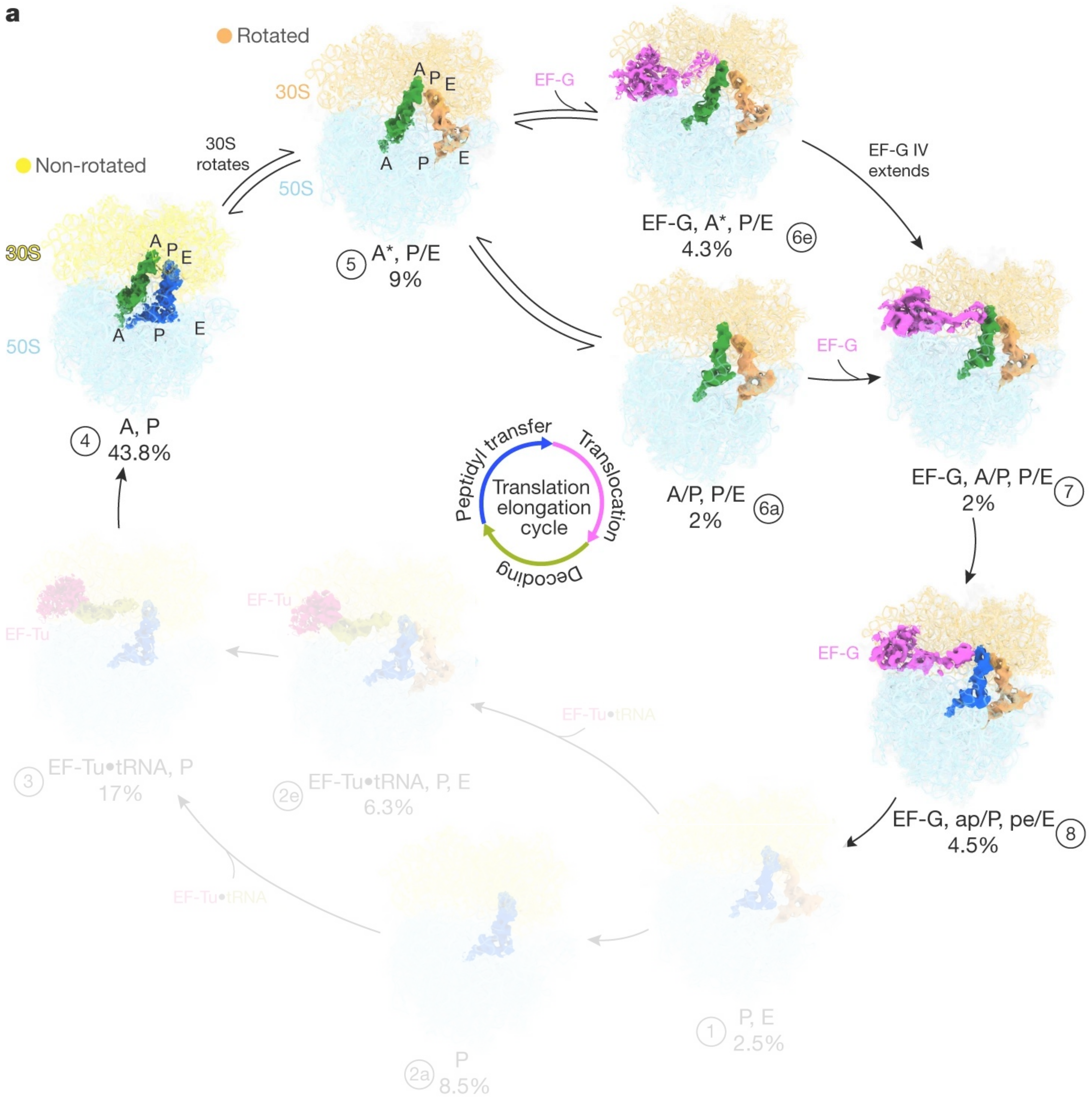


At the highest resolution and showing mRNA, tRNAs and the nascent peptide

Figure 2

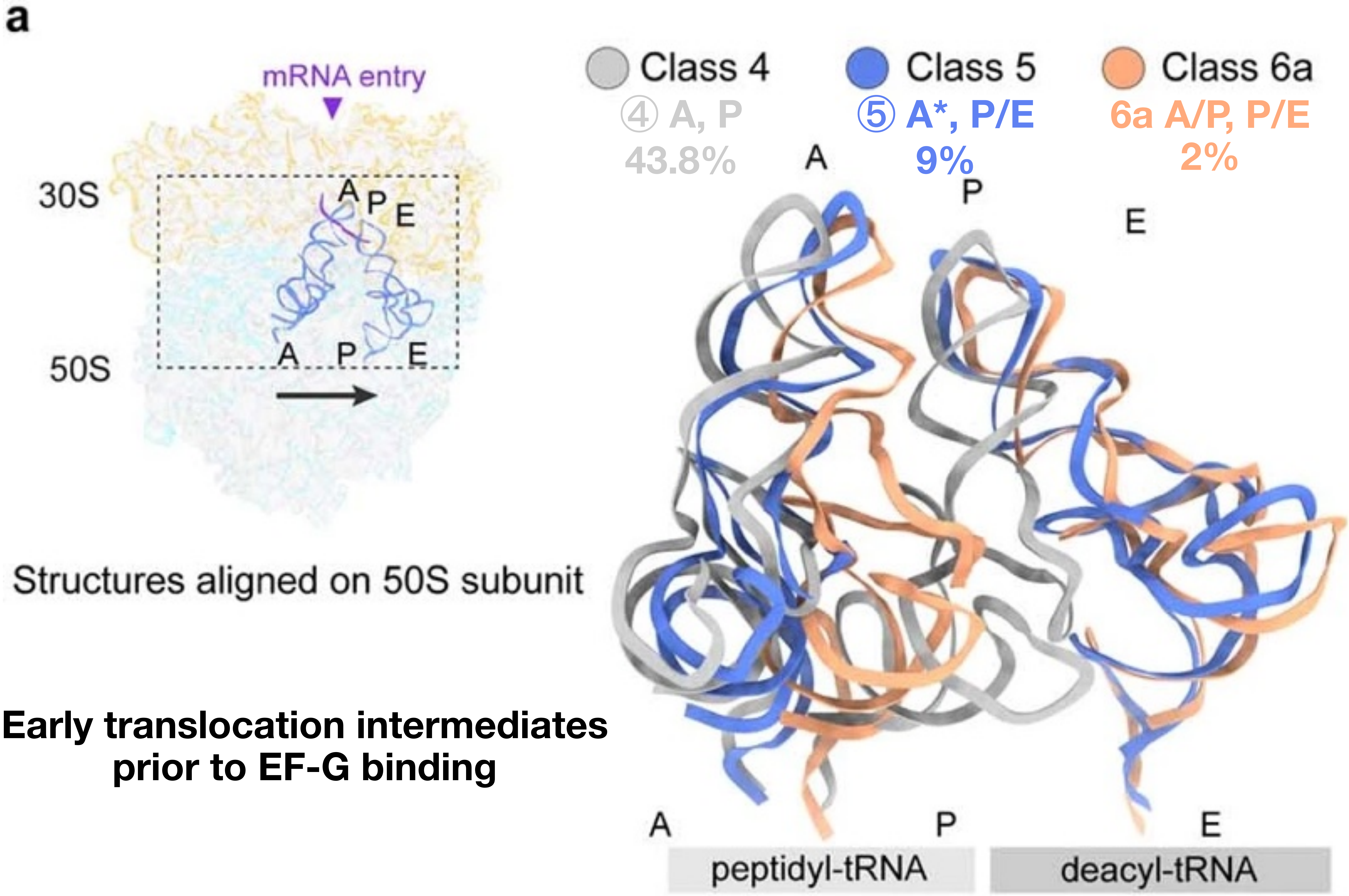
2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates



2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates



Early translocation intermediates prior to EF-G binding

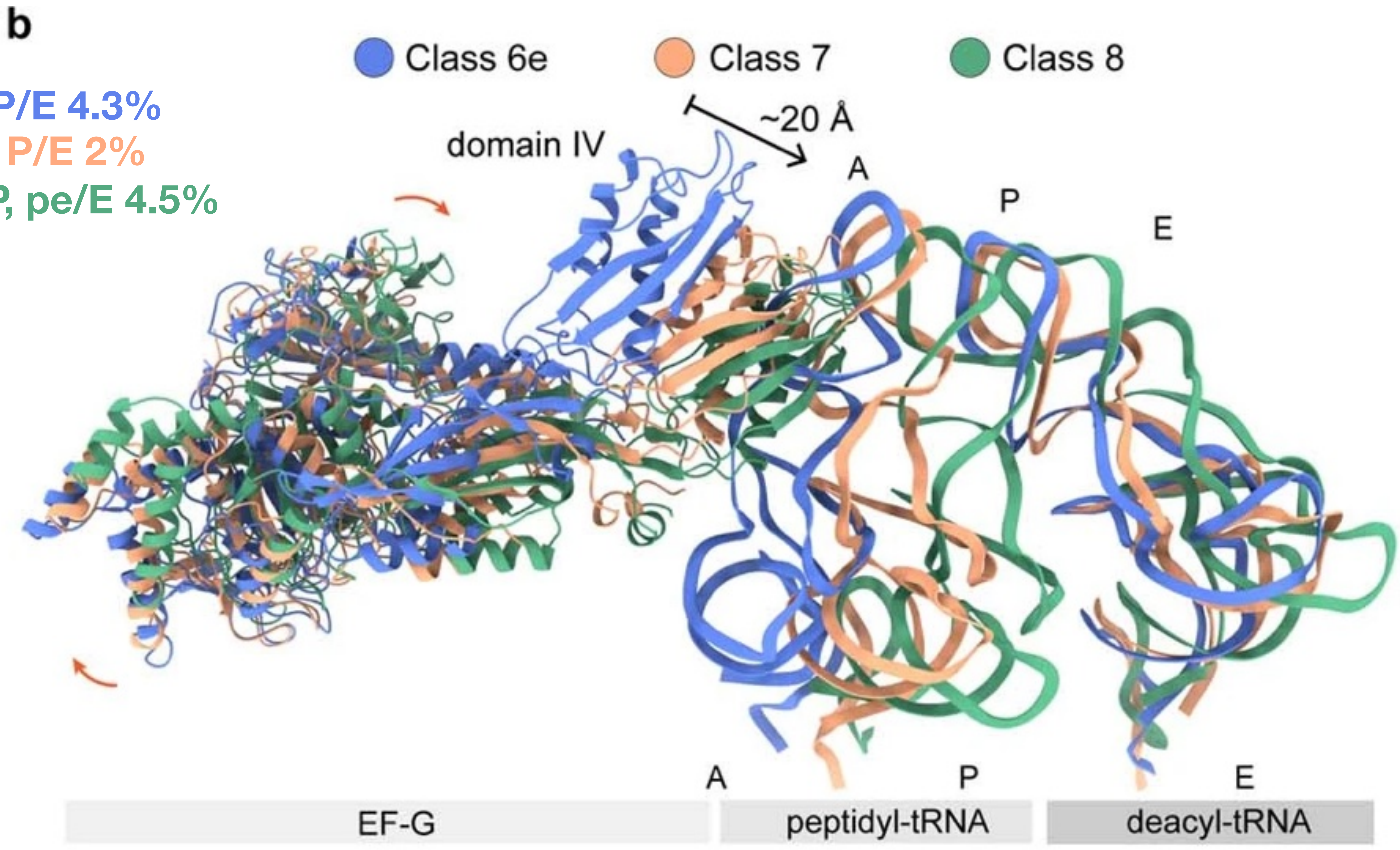
2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates

6e EF-G, A*, P/E 4.3%

⑦ EF-G, A/P, P/E 2%

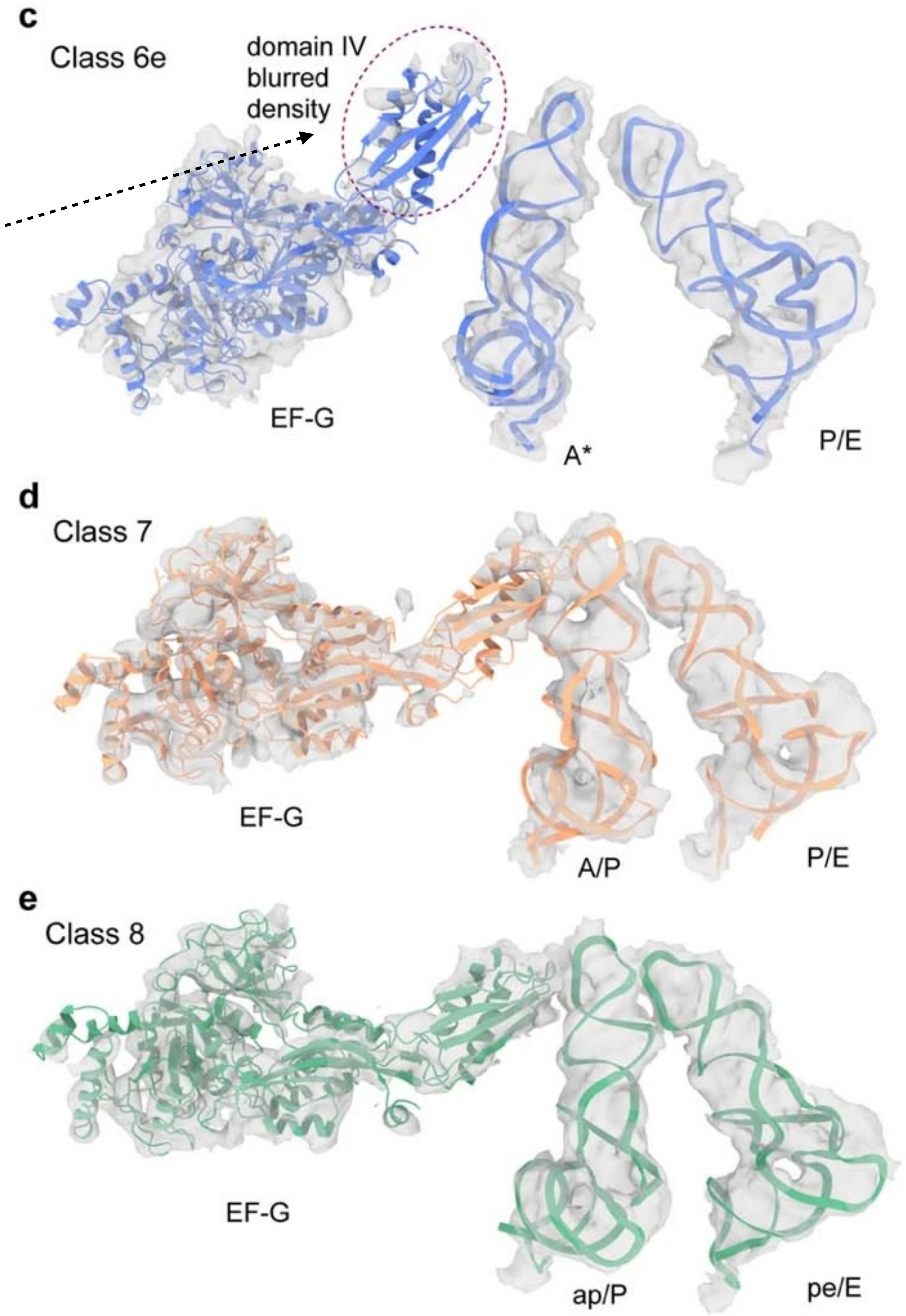
⑧ EF-G, ap/P, pe/E 4.5%



Early-to-late translocation intermediates in the presence of EF-G

2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates



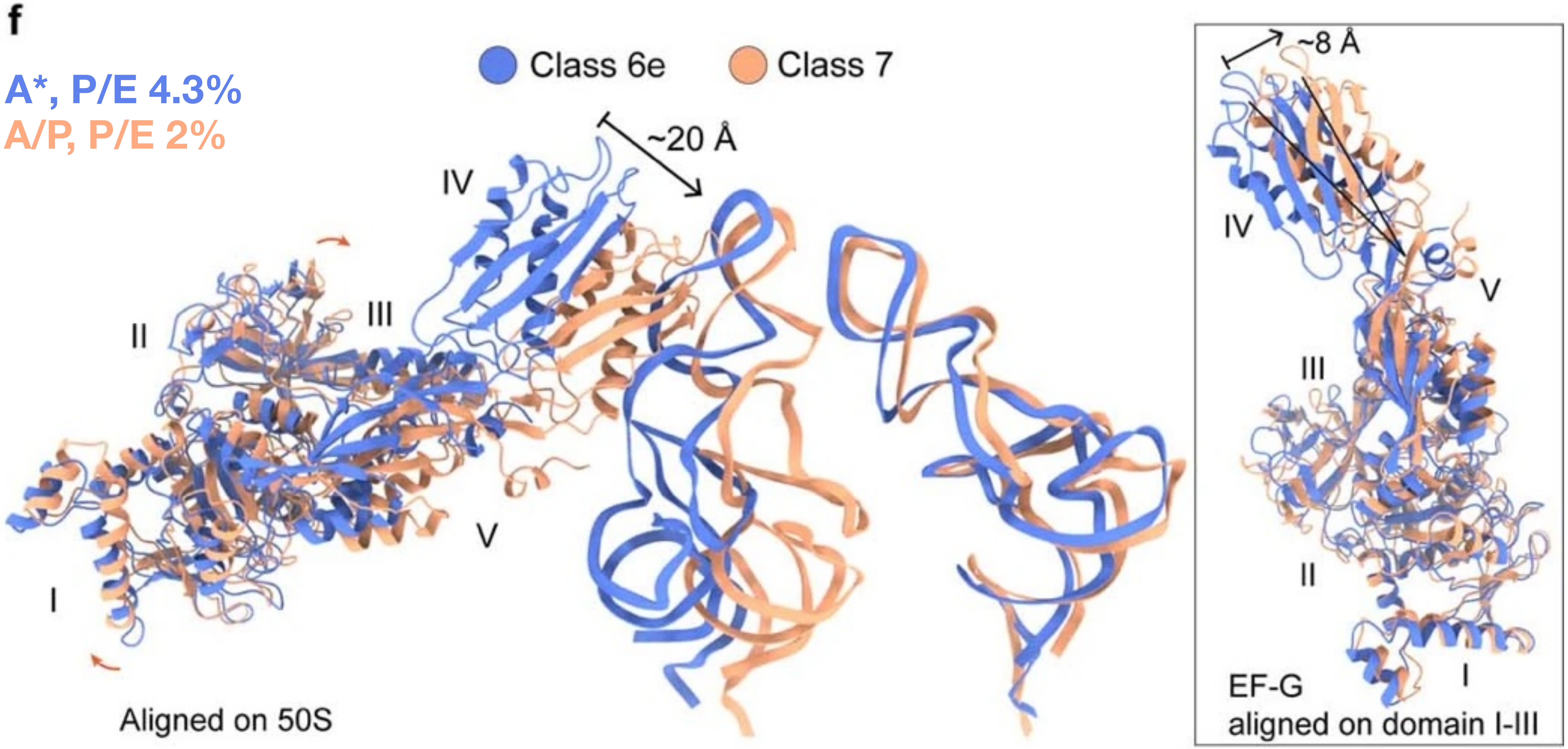
may contain a mixture of intermediate states with domain IV moving toward the A site

2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates

6e EF-G, A*, P/E 4.3%

⑦ EF-G, A/P, P/E 2%



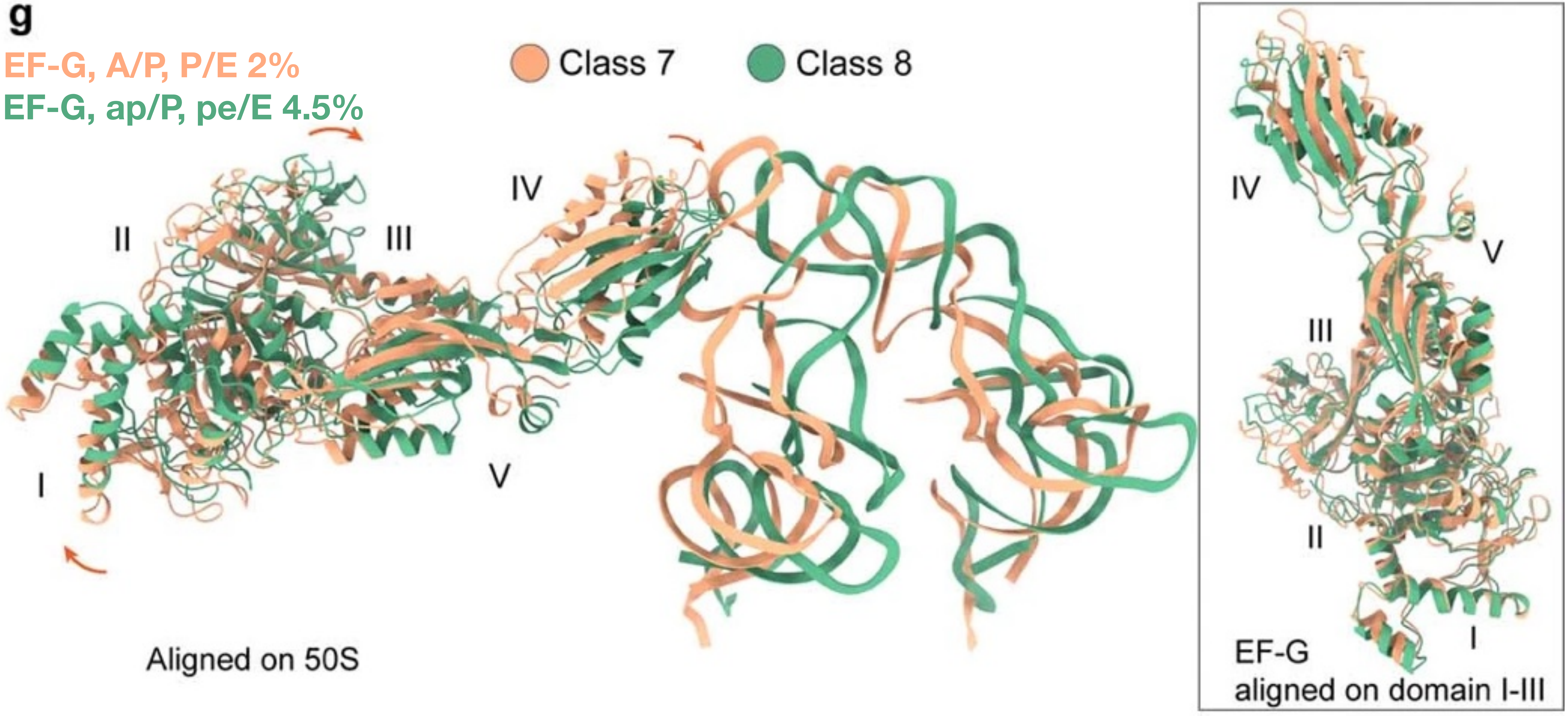
- ○ a small overall rotation of EF-G
- ○ an inter-domain rearrangement

2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates

⑦ EF-G, A/P, P/E 2%

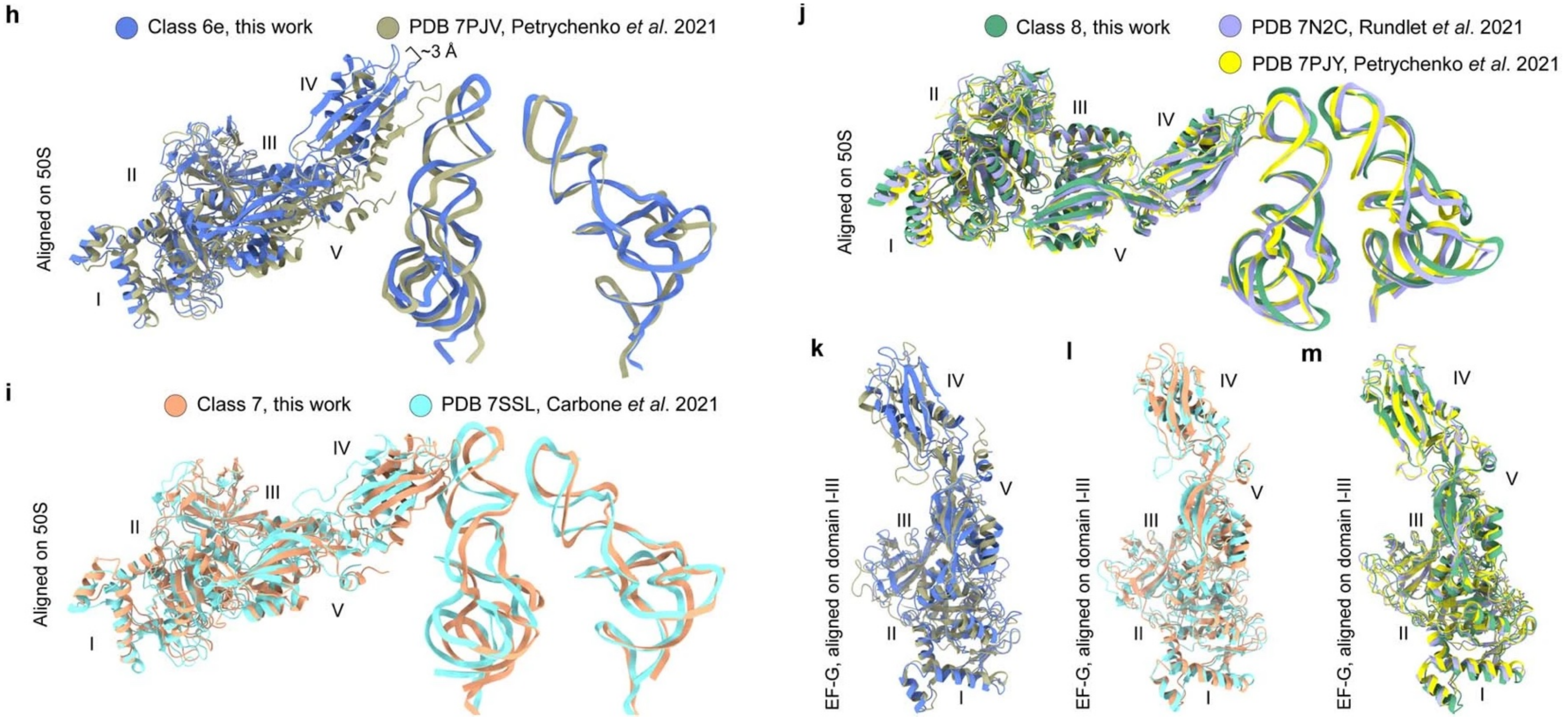
⑧ EF-G, ap/P, pe/E 4.5%



- EF-G rotates as one body without significant inter-domain rearrangement

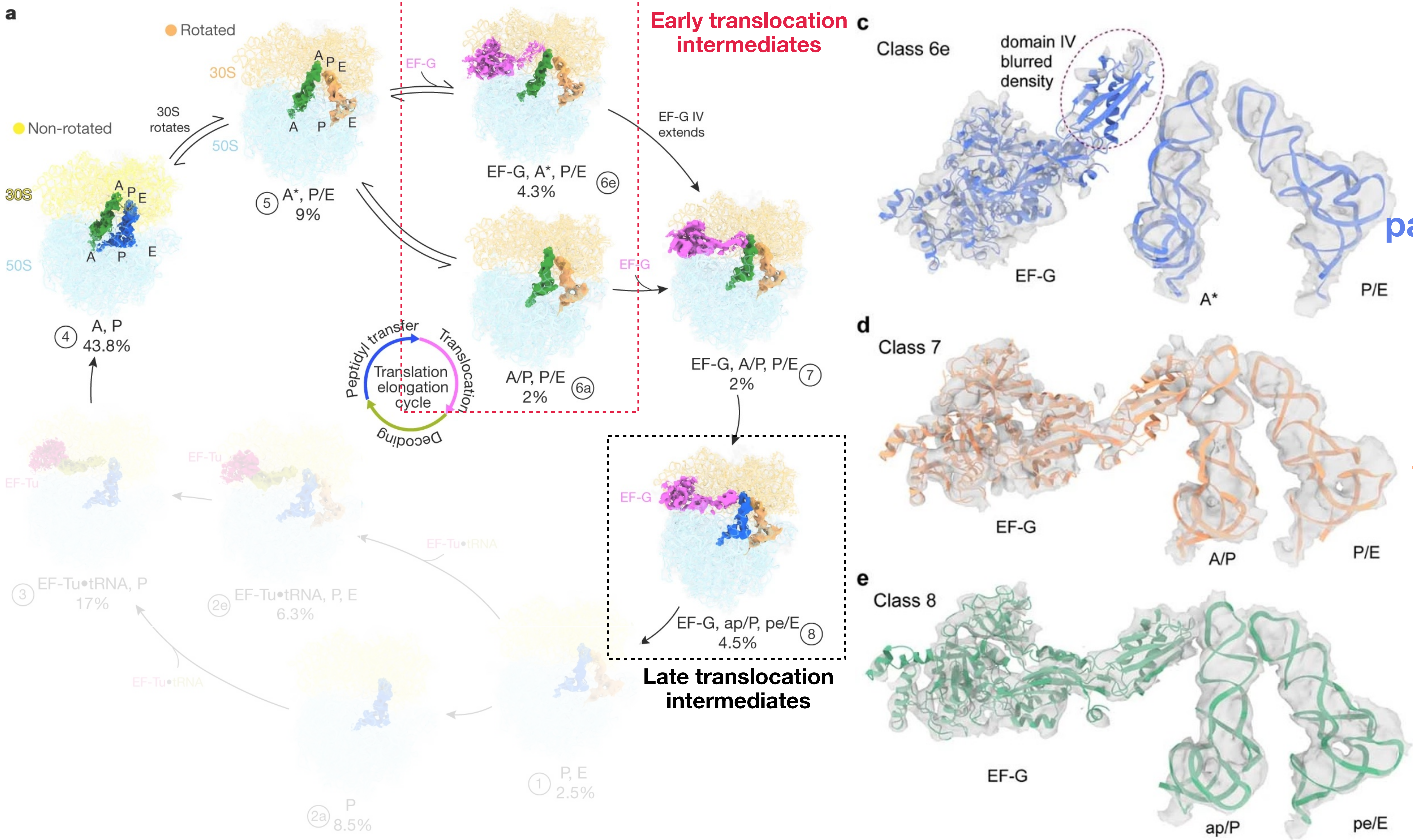
2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates



2 Structure dynamics of translation in cells

2.4 Early-to-late translocation intermediates



Early translocation intermediates

Late translocation intermediates

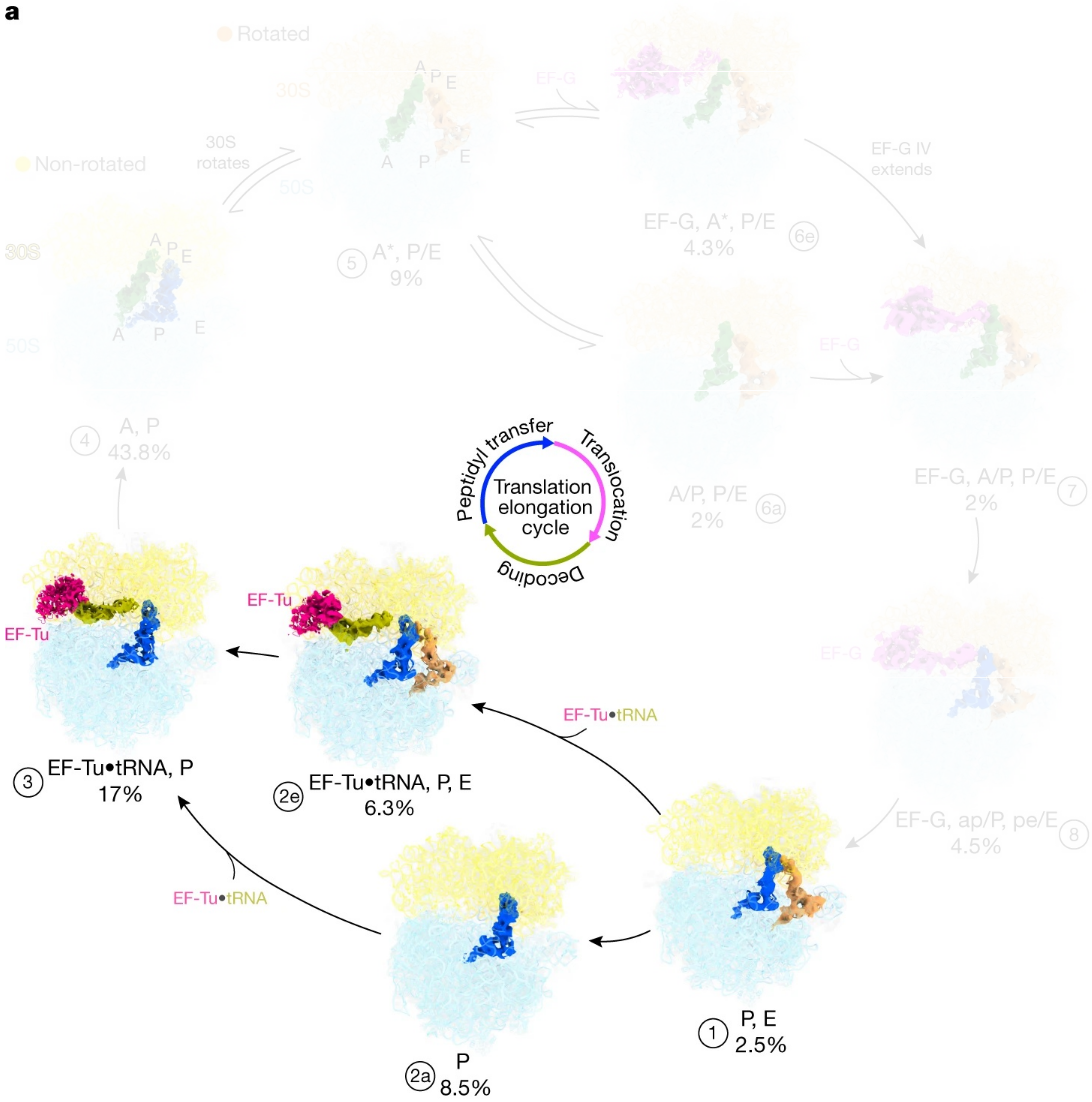
EF-G-bound partial hybrid state

EF-G-bound full hybrid state

The fully extended EF-G

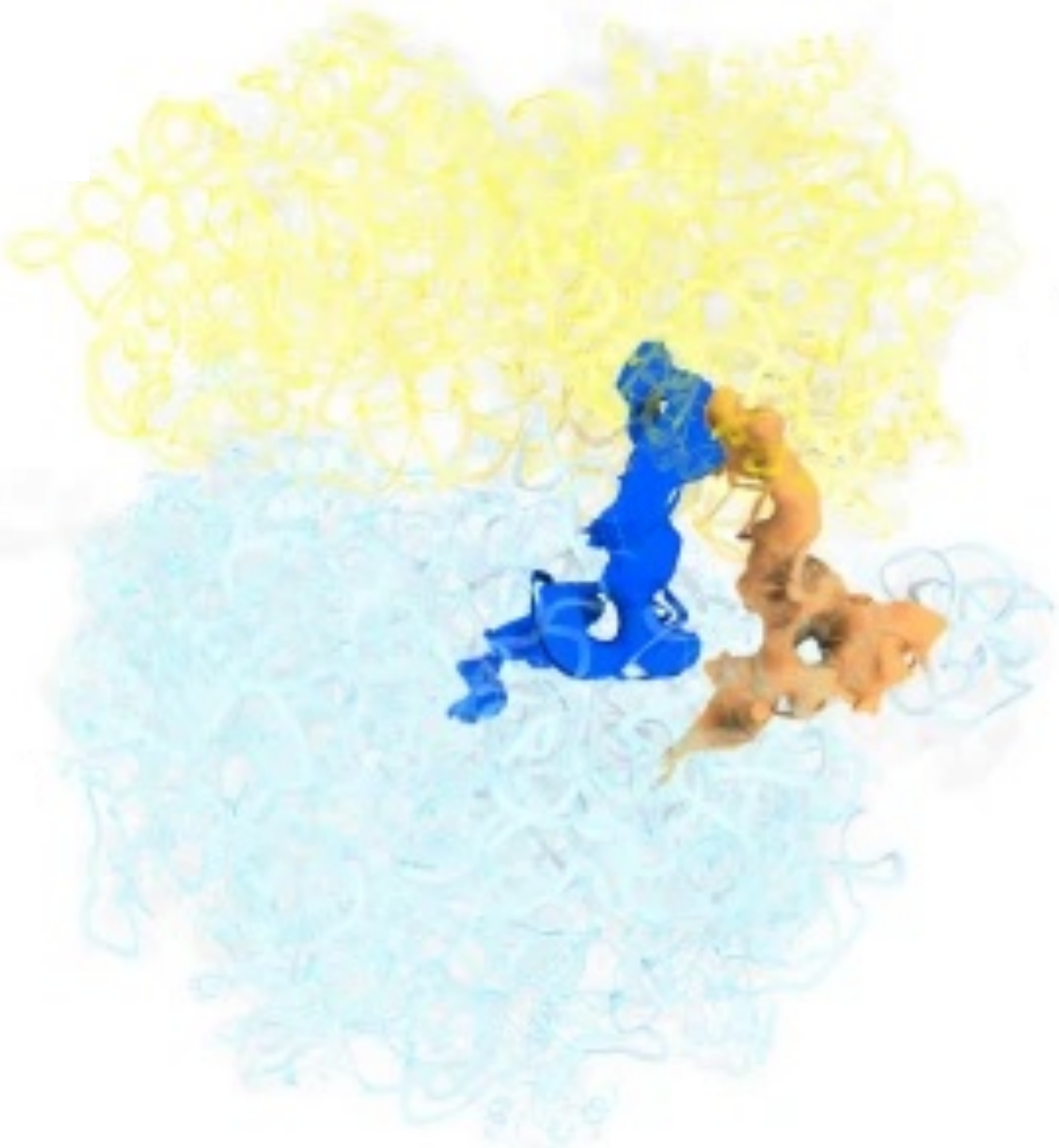
2 Structure dynamics of translation in cells

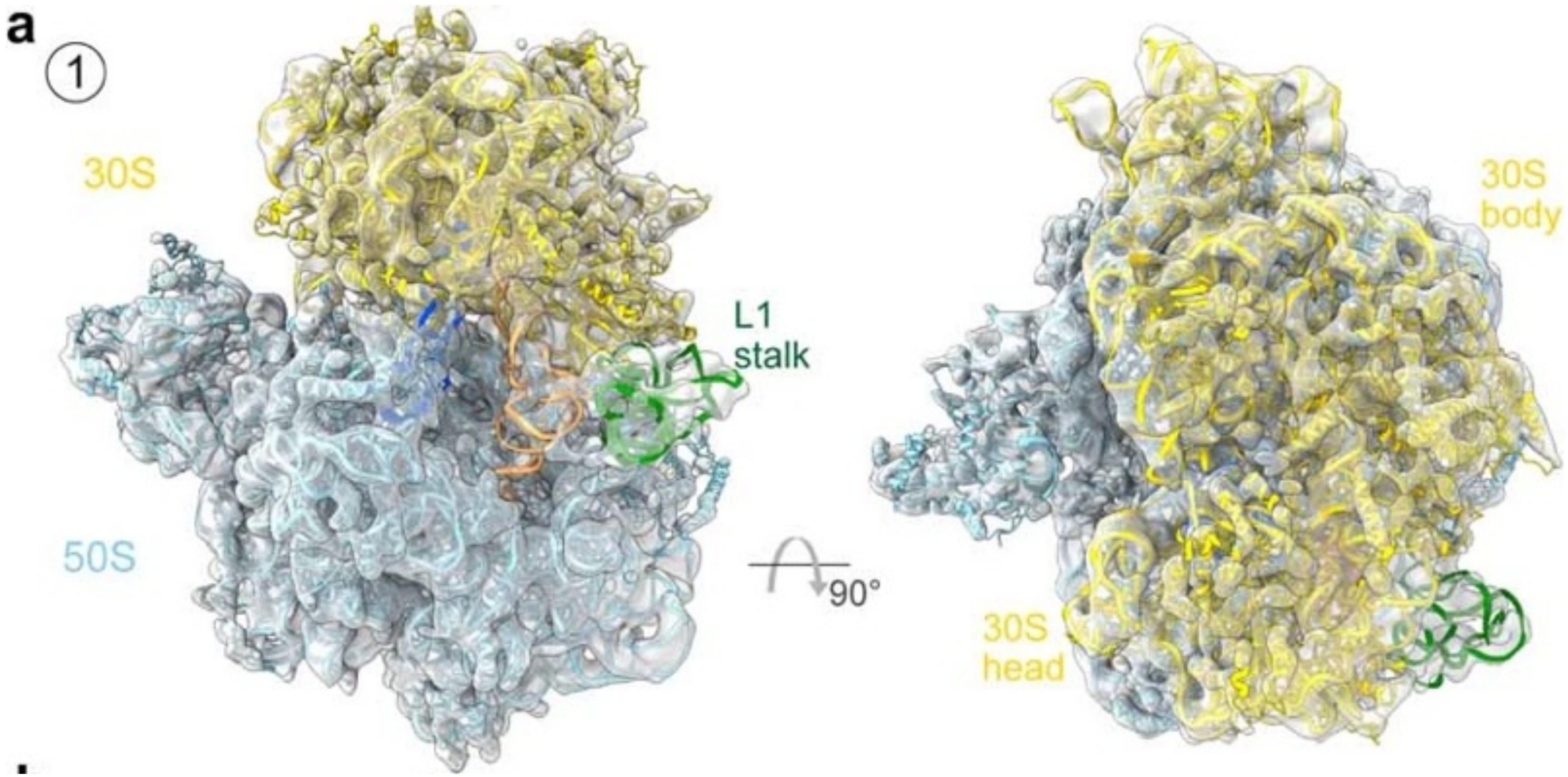
2.5 Decoding and EF-Tu-associated structures



2 Structure dynamics of translation in cells

2.5 Decoding and EF-Tu-associated structures





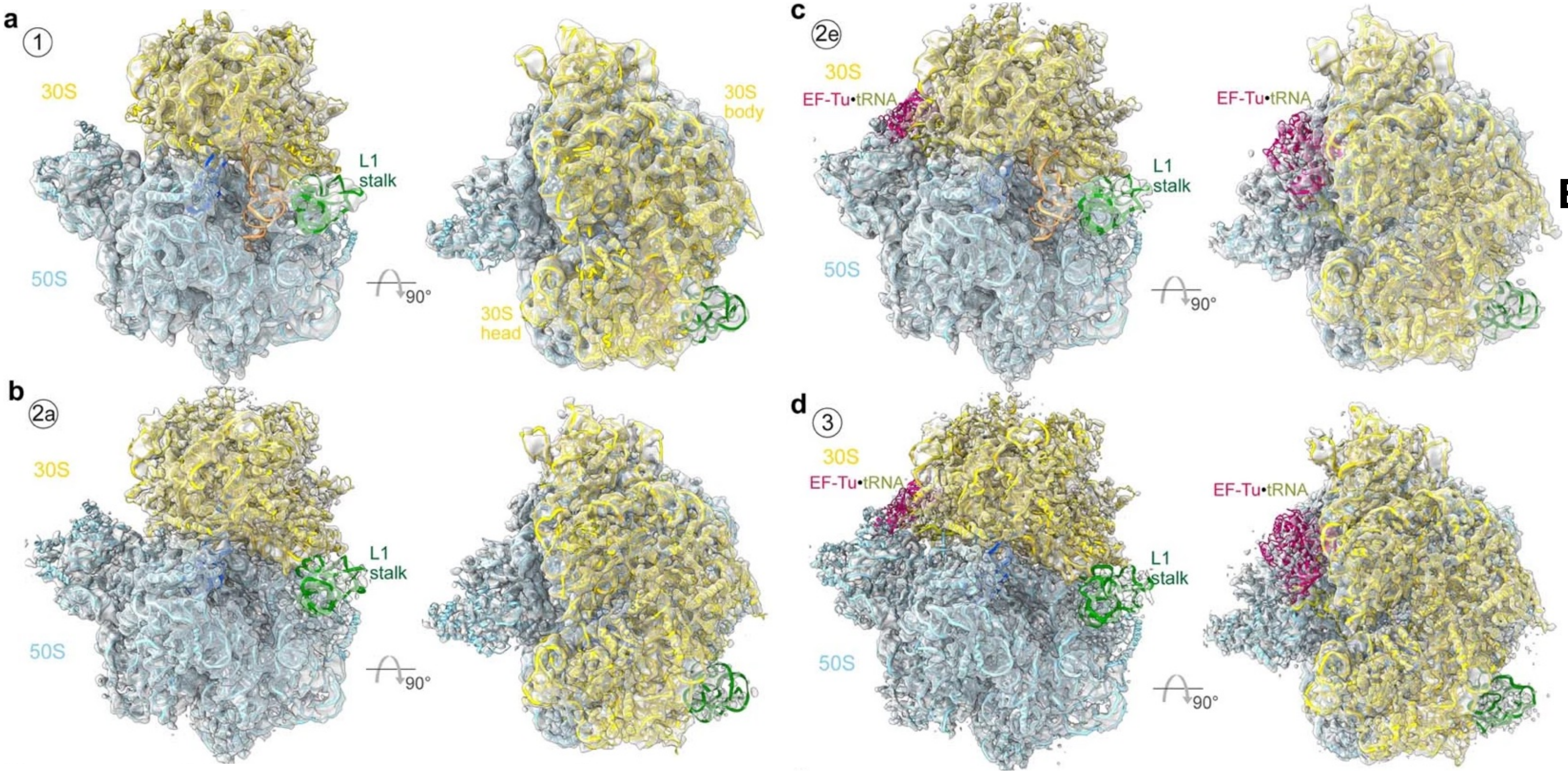
① P, E
2.5%

→

E-site tRNA is not stable and tend to disassociate quickly after translocation

2 Structure dynamics of translation in cells

2.5 Decoding and EF-Tu-associated structures



P, E
2.5%

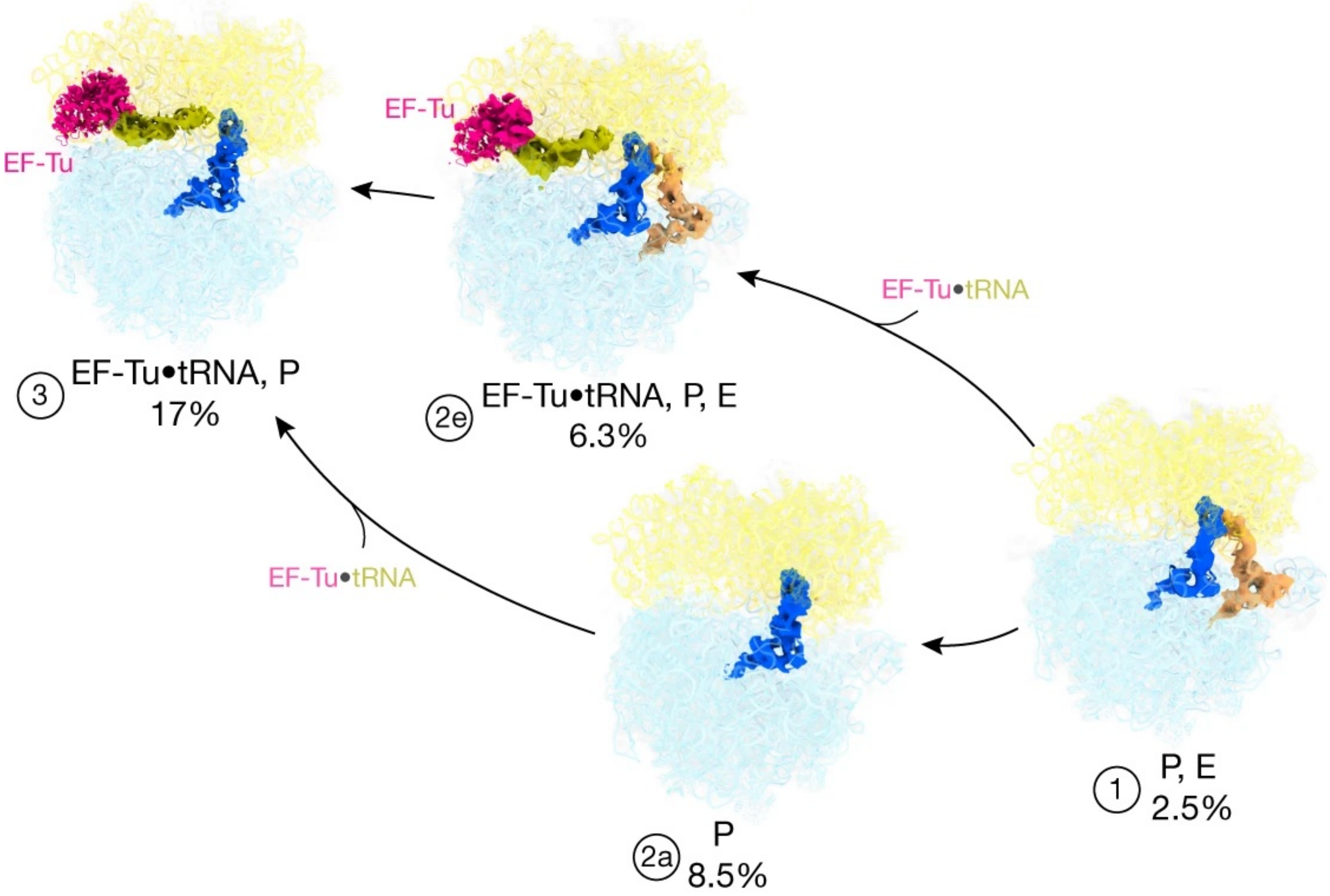
EF-Tu·tRNA, P, E
6.3%

P
8.5%

EF-Tu·tRNA, P
17%

2 Structure dynamics of translation in cells

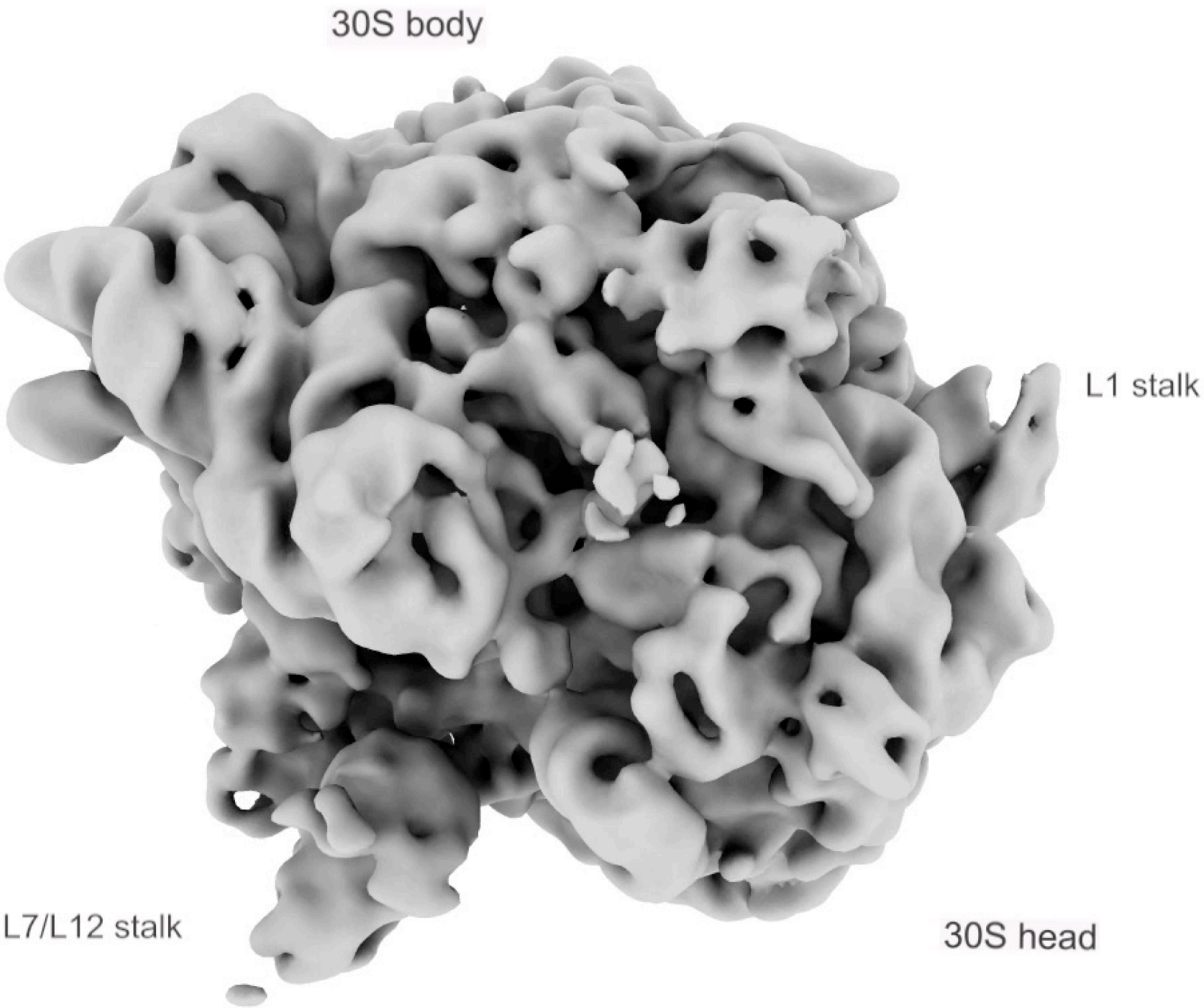
2.5 Decoding and EF-Tu-associated structures

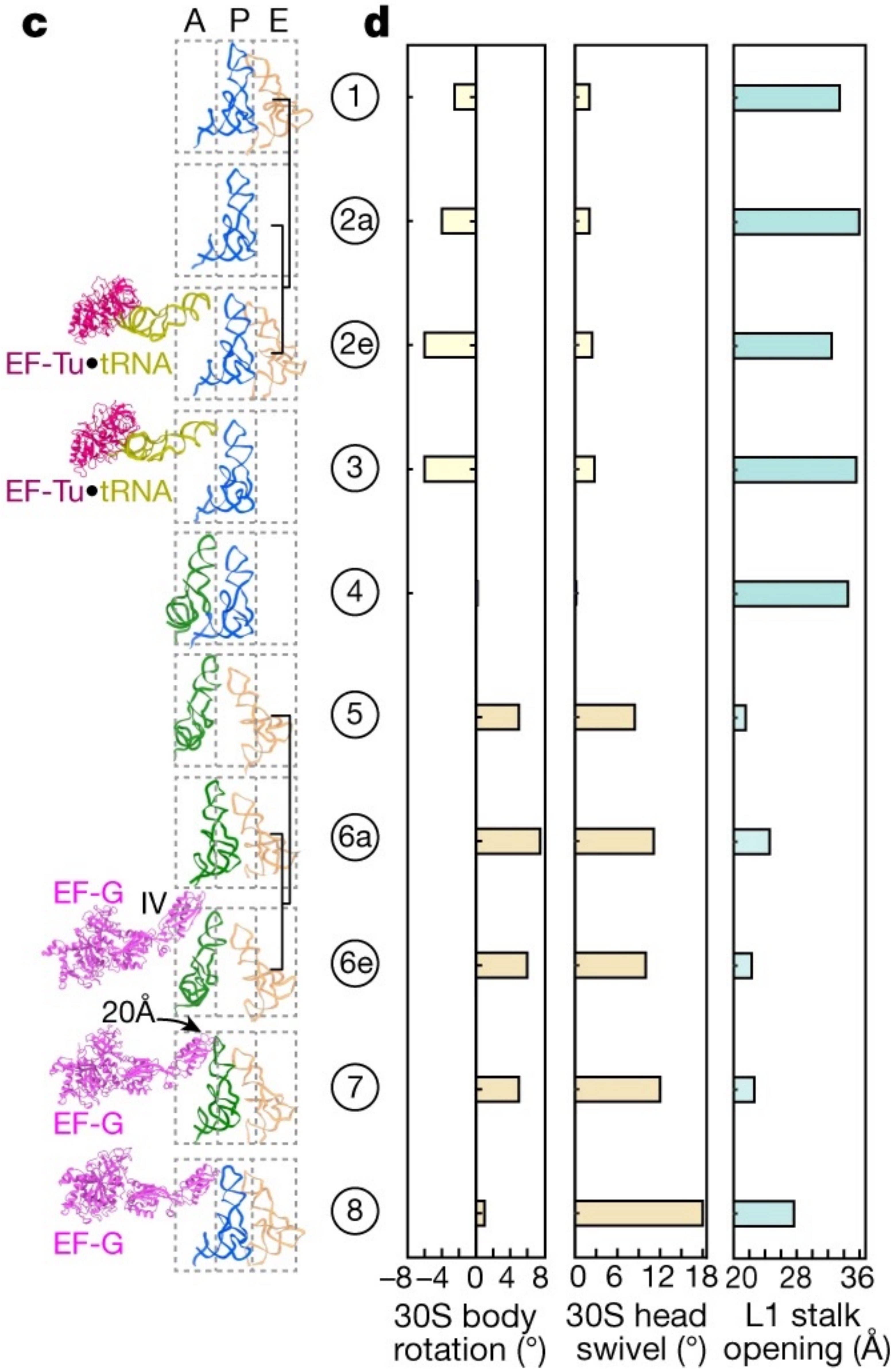


EF-Tu·tRNA binding and the disassociation of E-site tRNA are independent

2 Structure dynamics of translation in cells

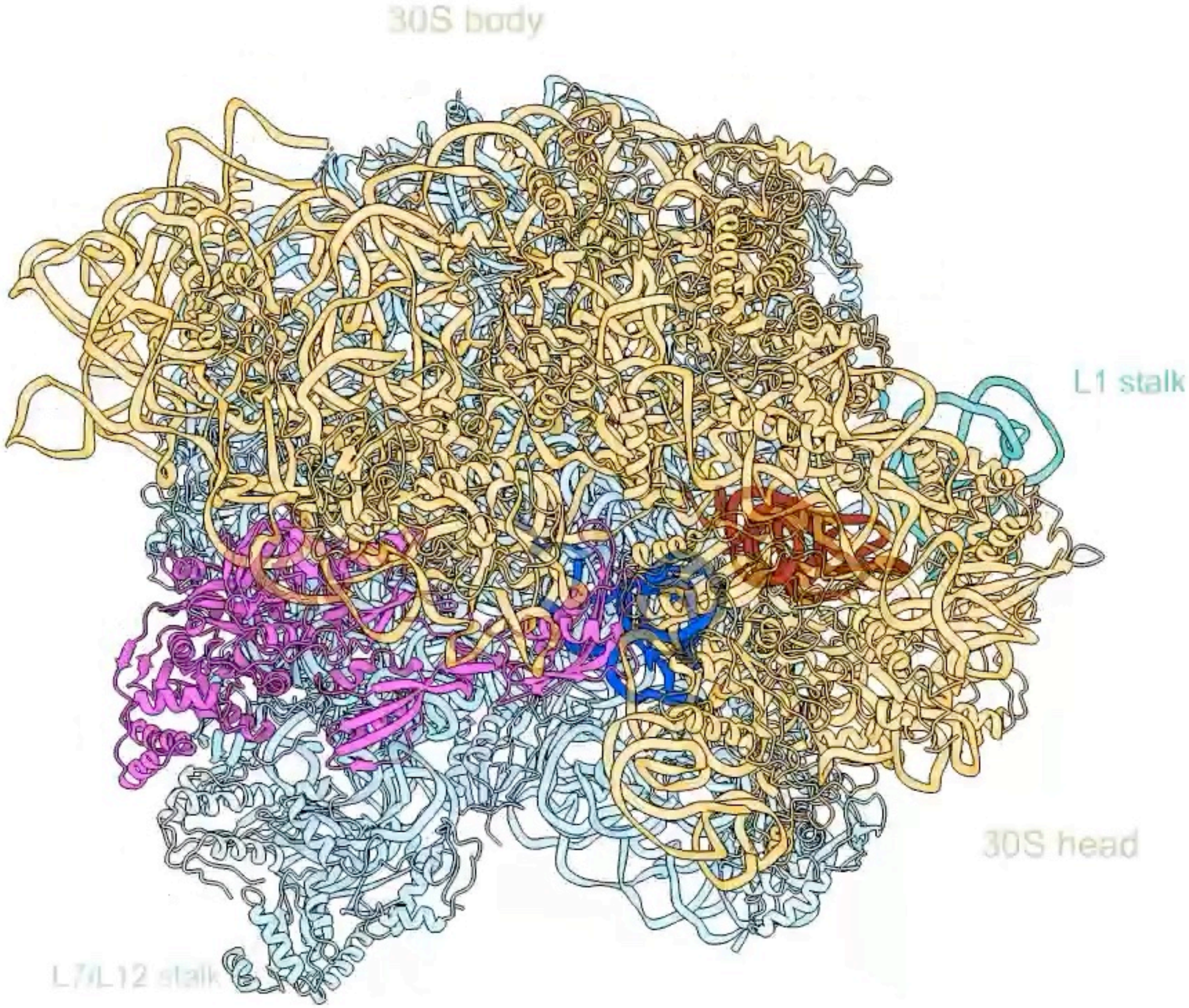
2.6 Inter-subunit rotation

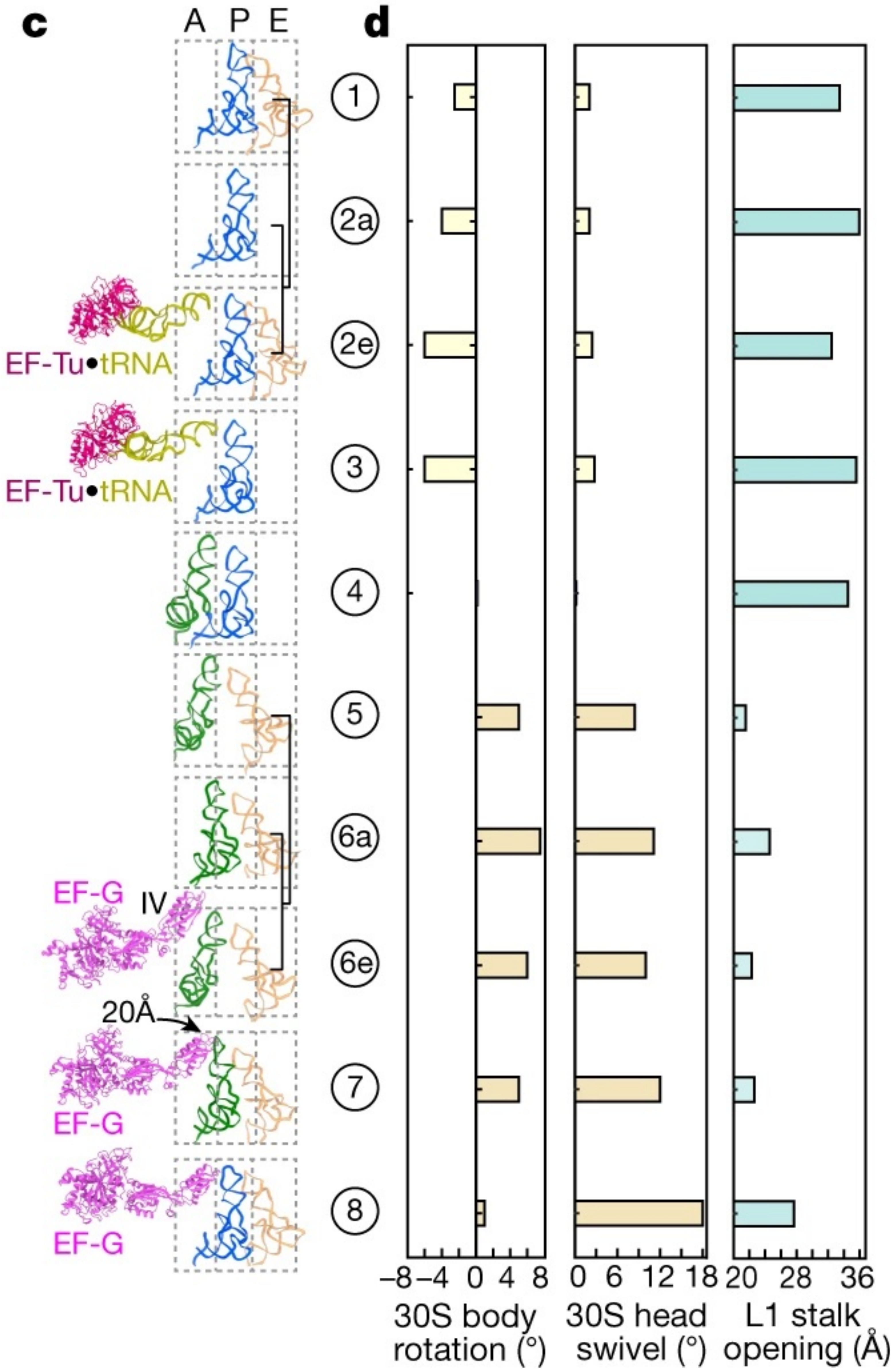




2 Structure dynamics of translation in cells

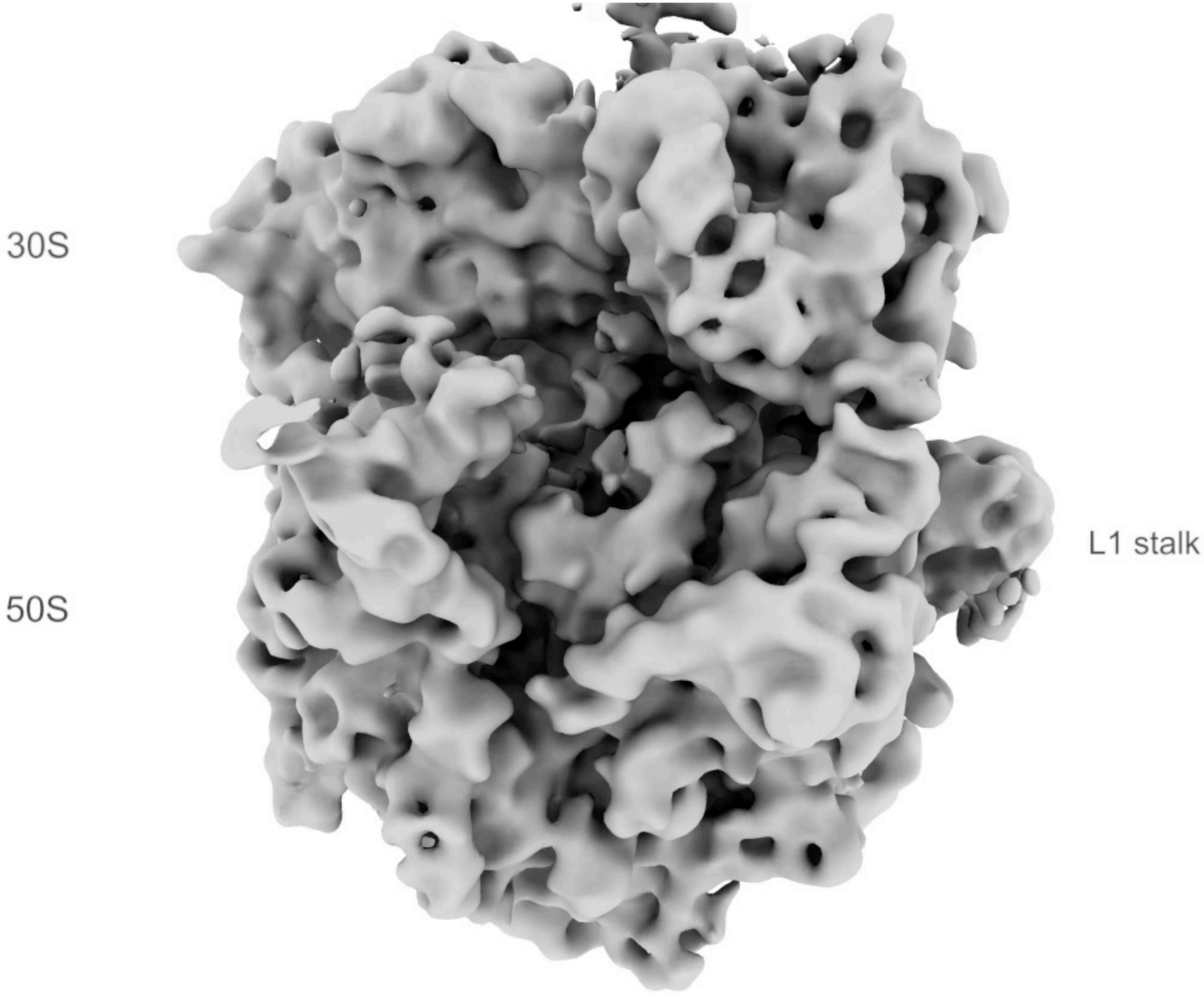
2.6 Inter-subunit rotation





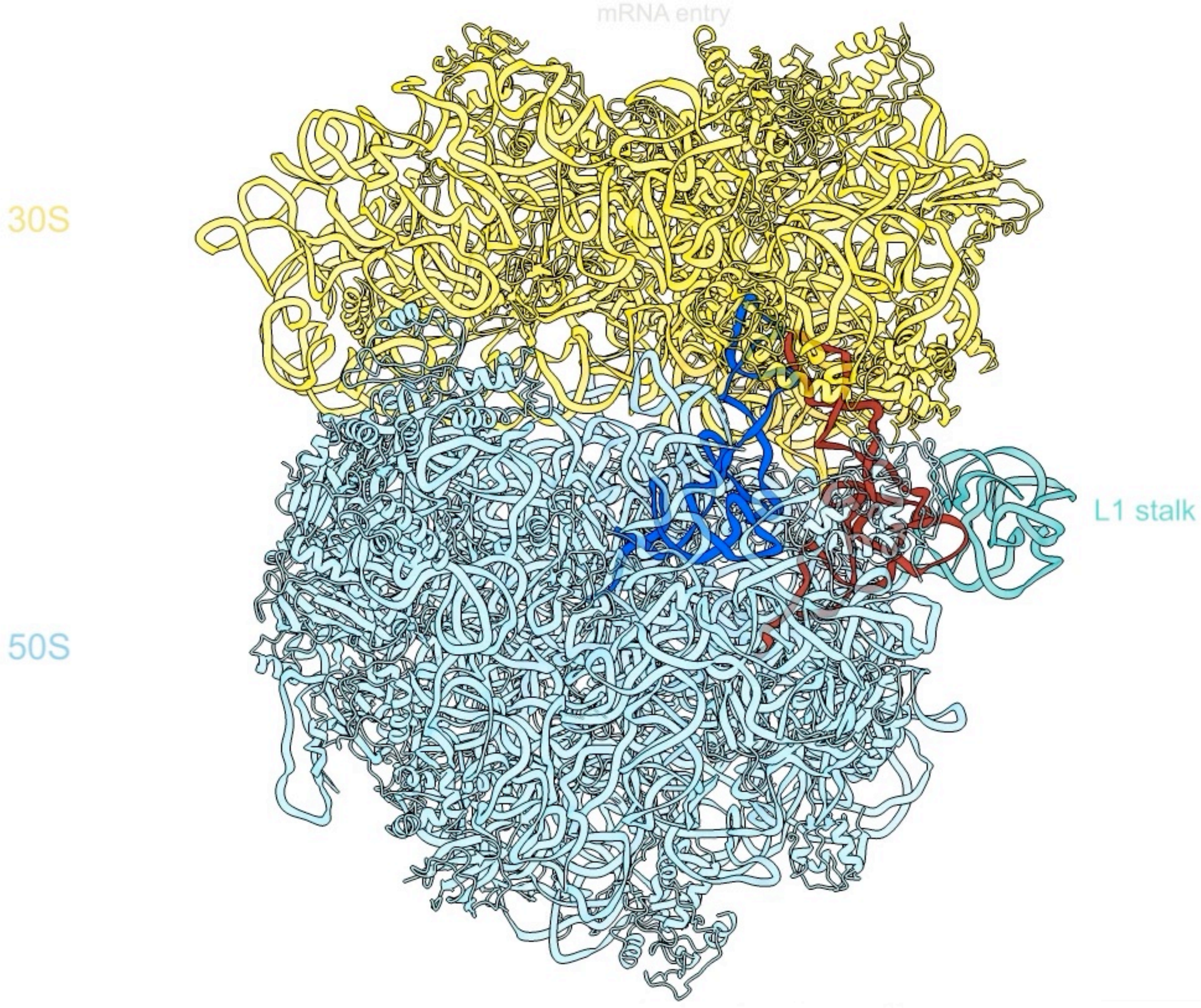
2 Structure dynamics of translation in cells

2.7 Translation elongation cycle



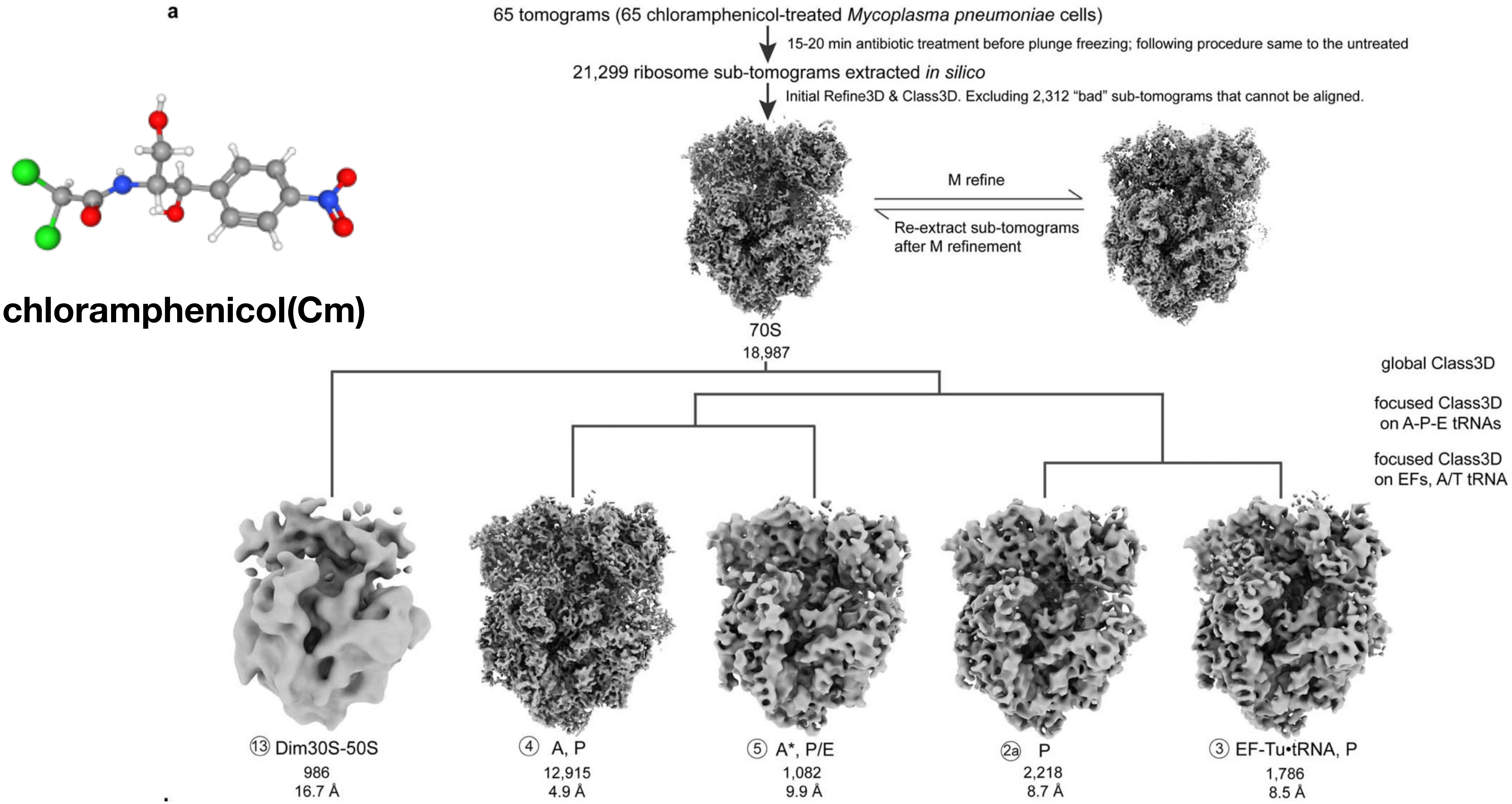
2 Structure dynamics of translation in cells

2.7 Translation elongation cycle



3 Antibiotics alter translation landscapes

3.1 In chloramphenicol(Cm)-treated cells



chloramphenicol(Cm)

65 tomograms (65 chloramphenicol-treated *Mycoplasma pneumoniae* cells)

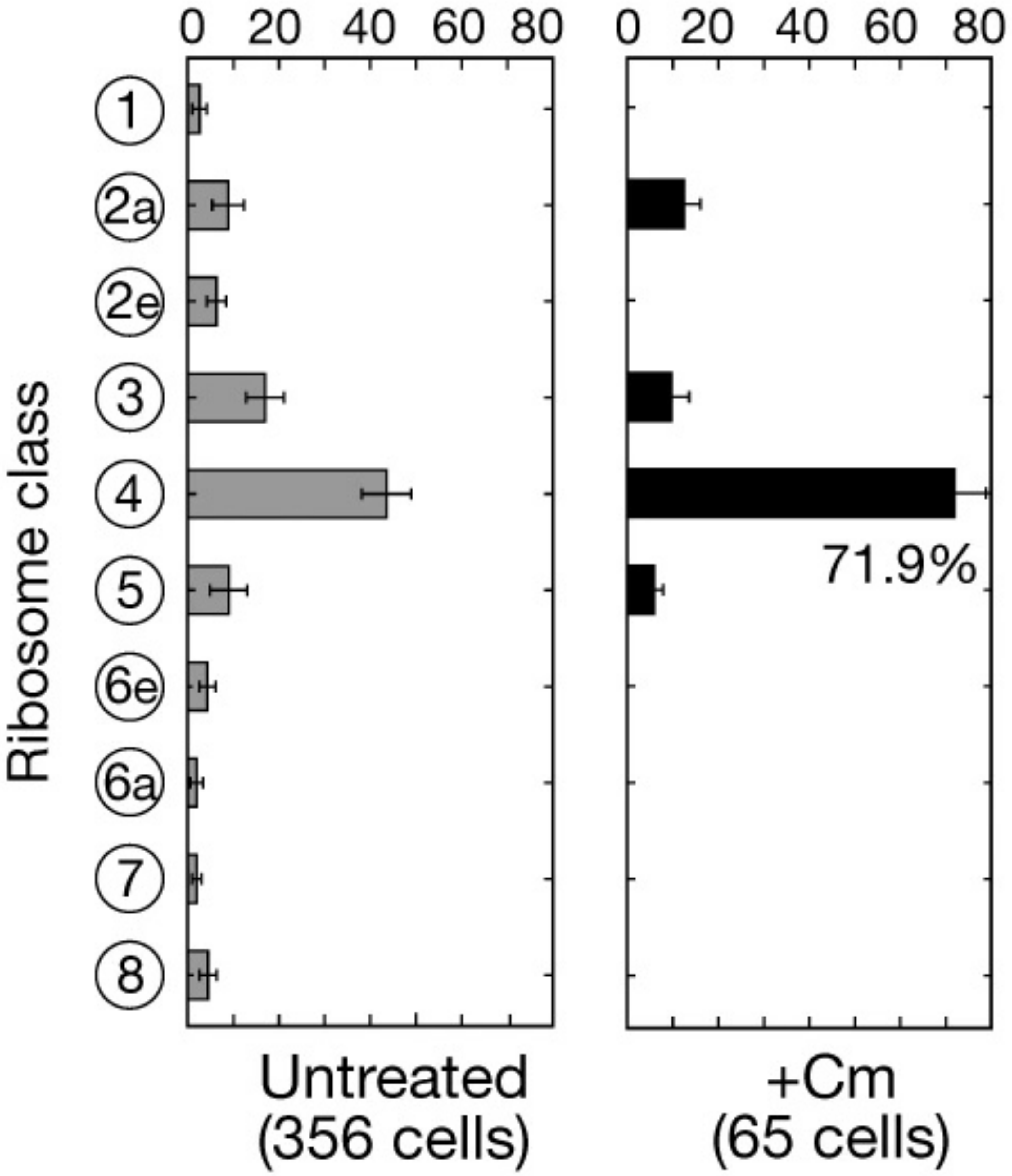
15-20 min antibiotic treatment before plunge freezing; following procedure same to the untreated

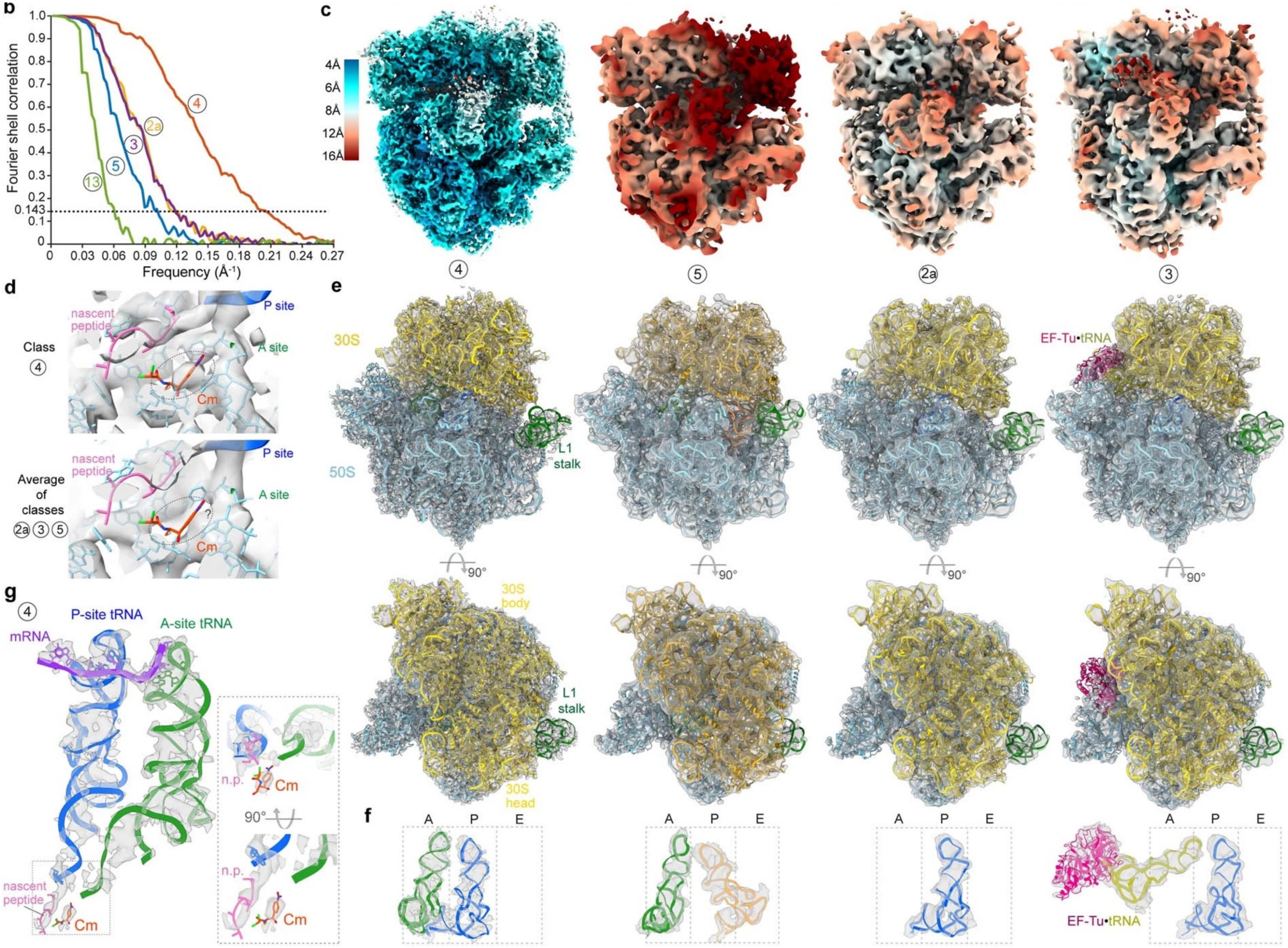
21,299 ribosome sub-tomograms extracted *in silico*

Initial Refine3D & Class3D. Excluding 2,312 "bad" sub-tomograms that cannot be aligned.

3 Antibiotics alter translation landscapes

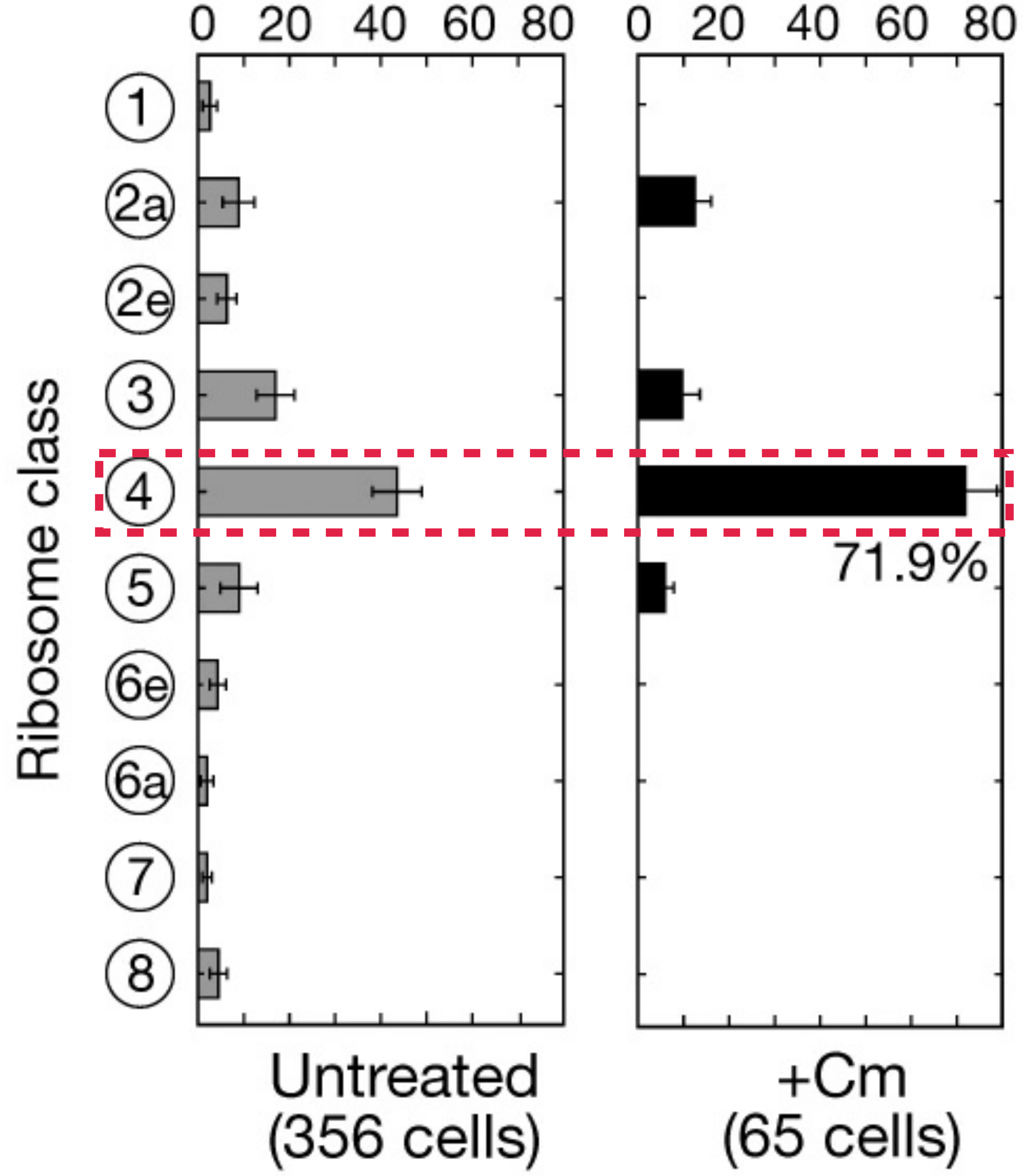
3.1 In chloramphenicol(Cm)-treated cells

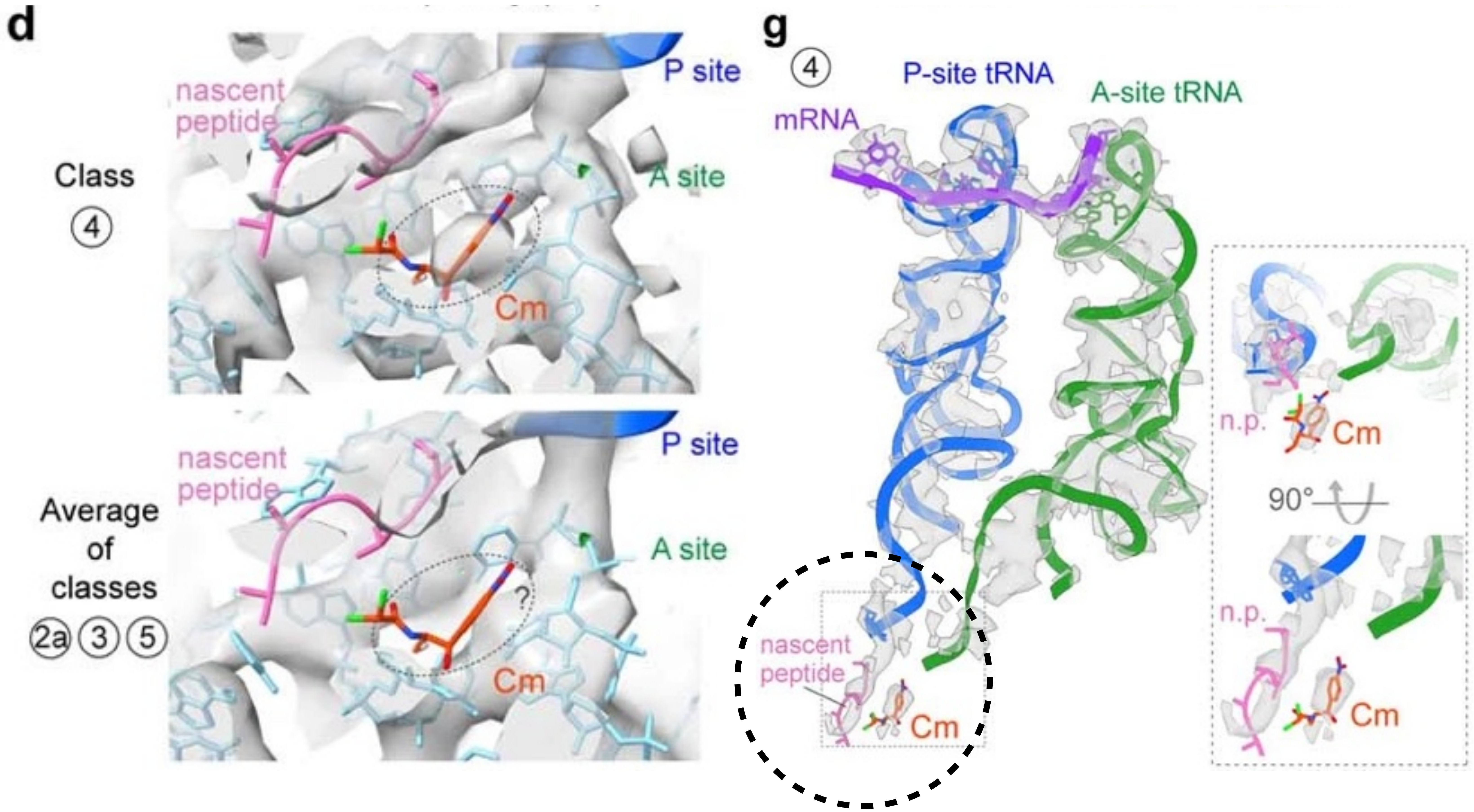




3 Antibiotics alter translation landscapes

3.1 In chloramphenicol(Cm)-treated cells



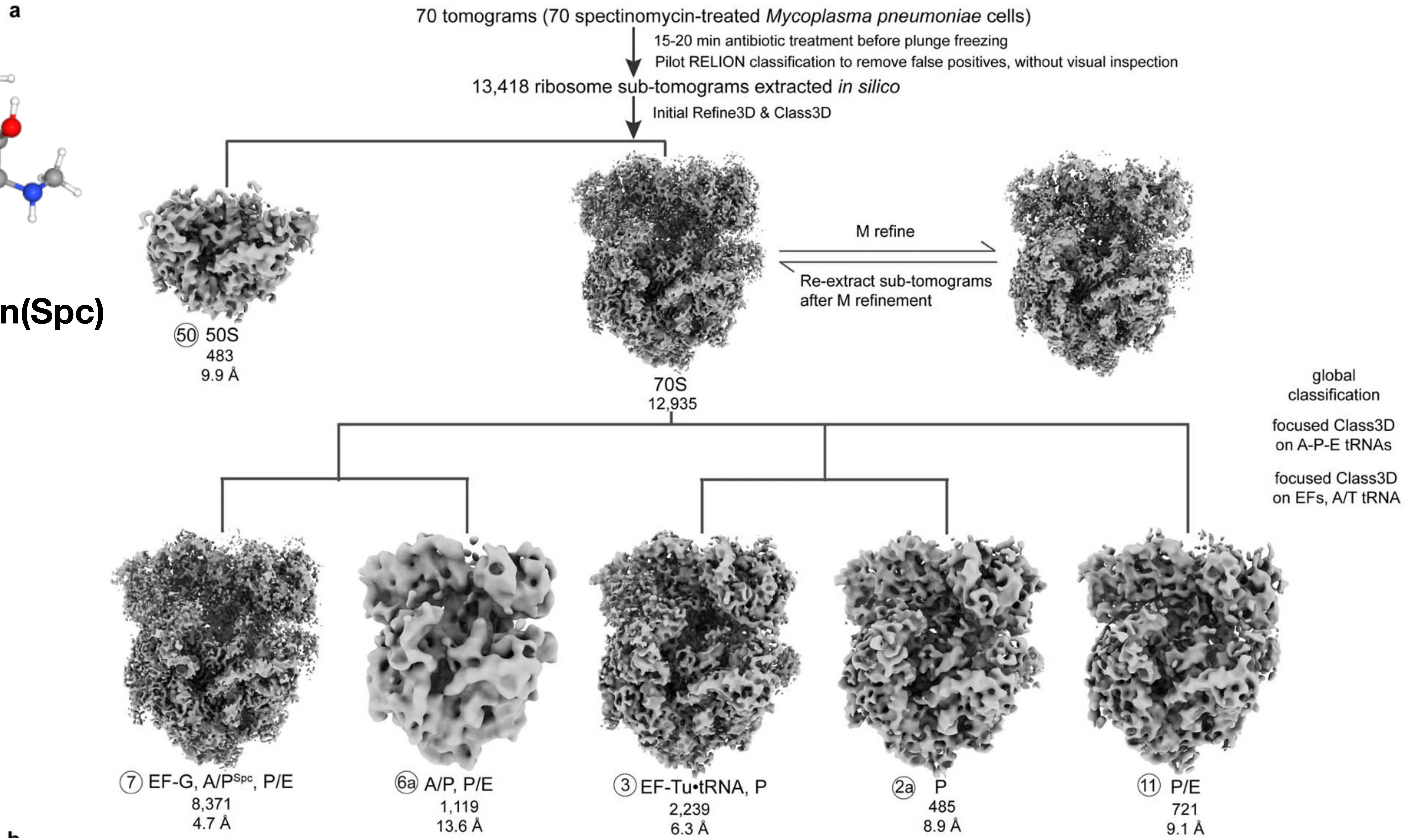


Cm inhibits peptide bond formation

3 Antibiotics alter translation landscapes

3.2 In spectinomycin(Spc)-treated cells

spectinomycin(Spc)



a

70 tomograms (70 spectinomycin-treated *Mycoplasma pneumoniae* cells)

15-20 min antibiotic treatment before plunge freezing

Pilot RELION classification to remove false positives, without visual inspection

13,418 ribosome sub-tomograms extracted *in silico*

Initial Refine3D & Class3D

㊿ 50S
483
9.9 Å

70S
12,935

M refine

Re-extract sub-tomograms after M refinement

global classification

focused Class3D on A-P-E tRNAs

focused Class3D on EFs, A/T tRNA

⑦ EF-G, A/P^{Spc}, P/E
8,371
4.7 Å

(6a) A/P, P/E
1,119
13.6 Å

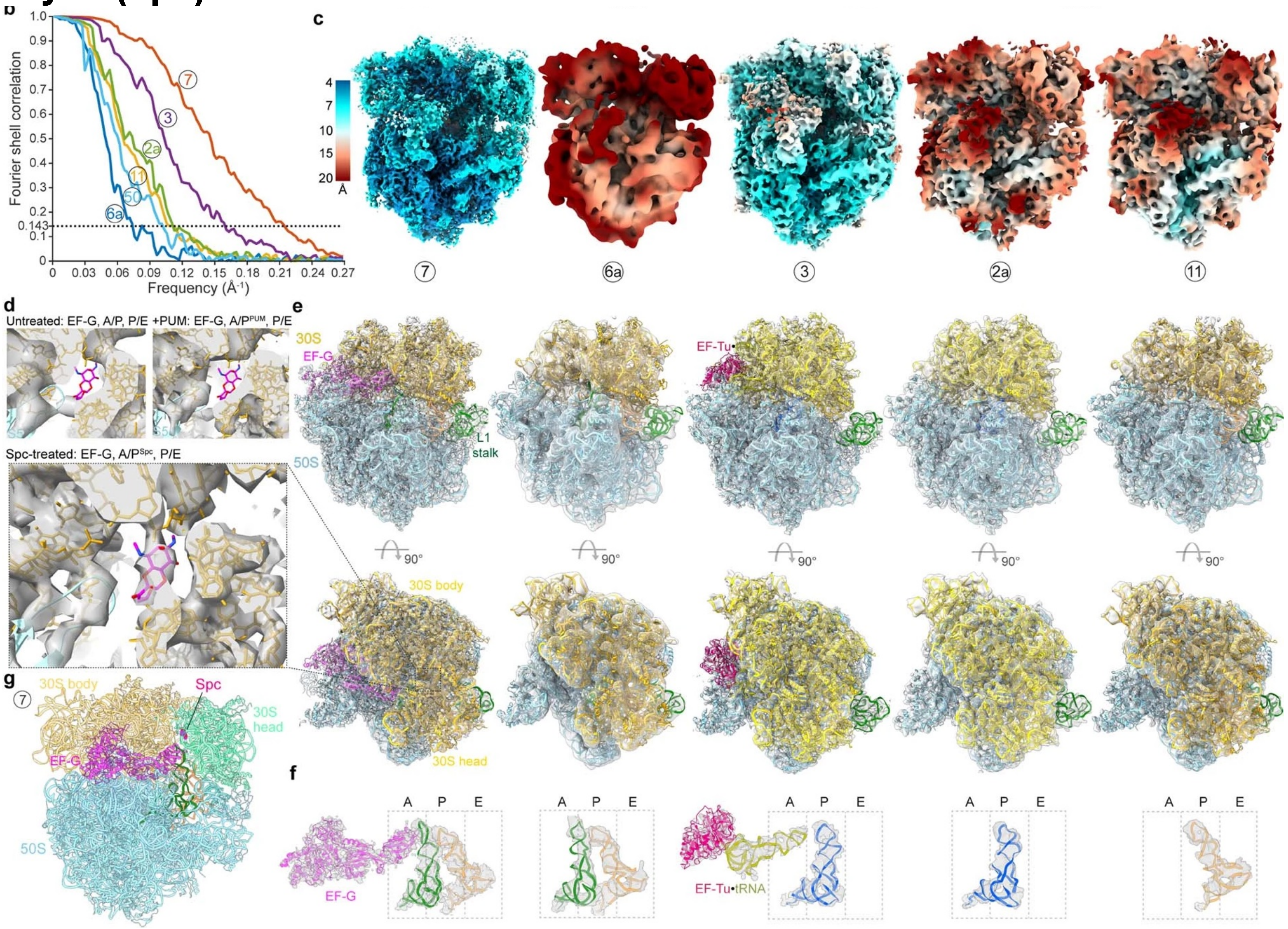
③ EF-Tu•tRNA, P
2,239
6.3 Å

(2a) P
485
8.9 Å

⑪ P/E
721
9.1 Å

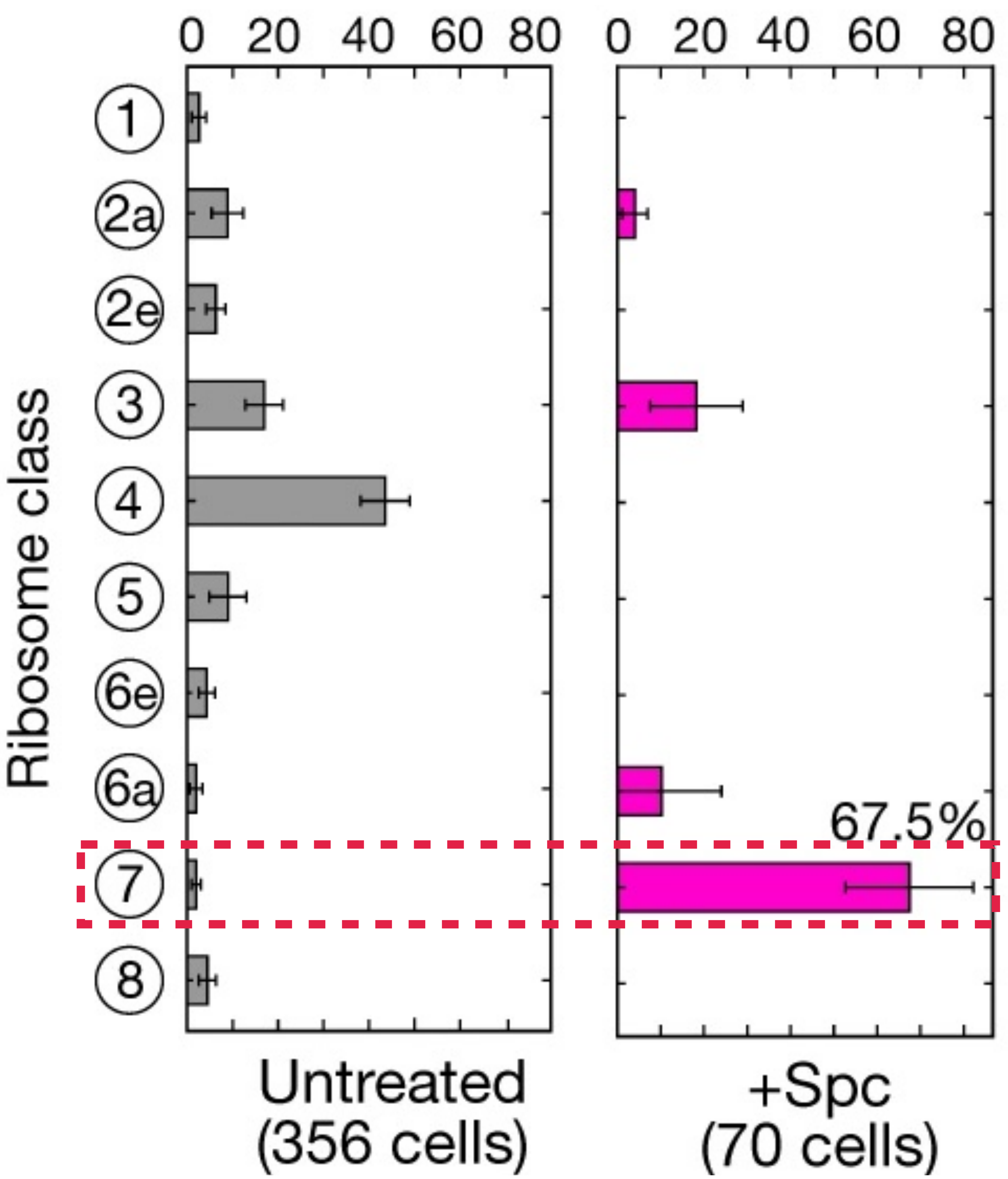
3 Antibiotics alter translation landscapes

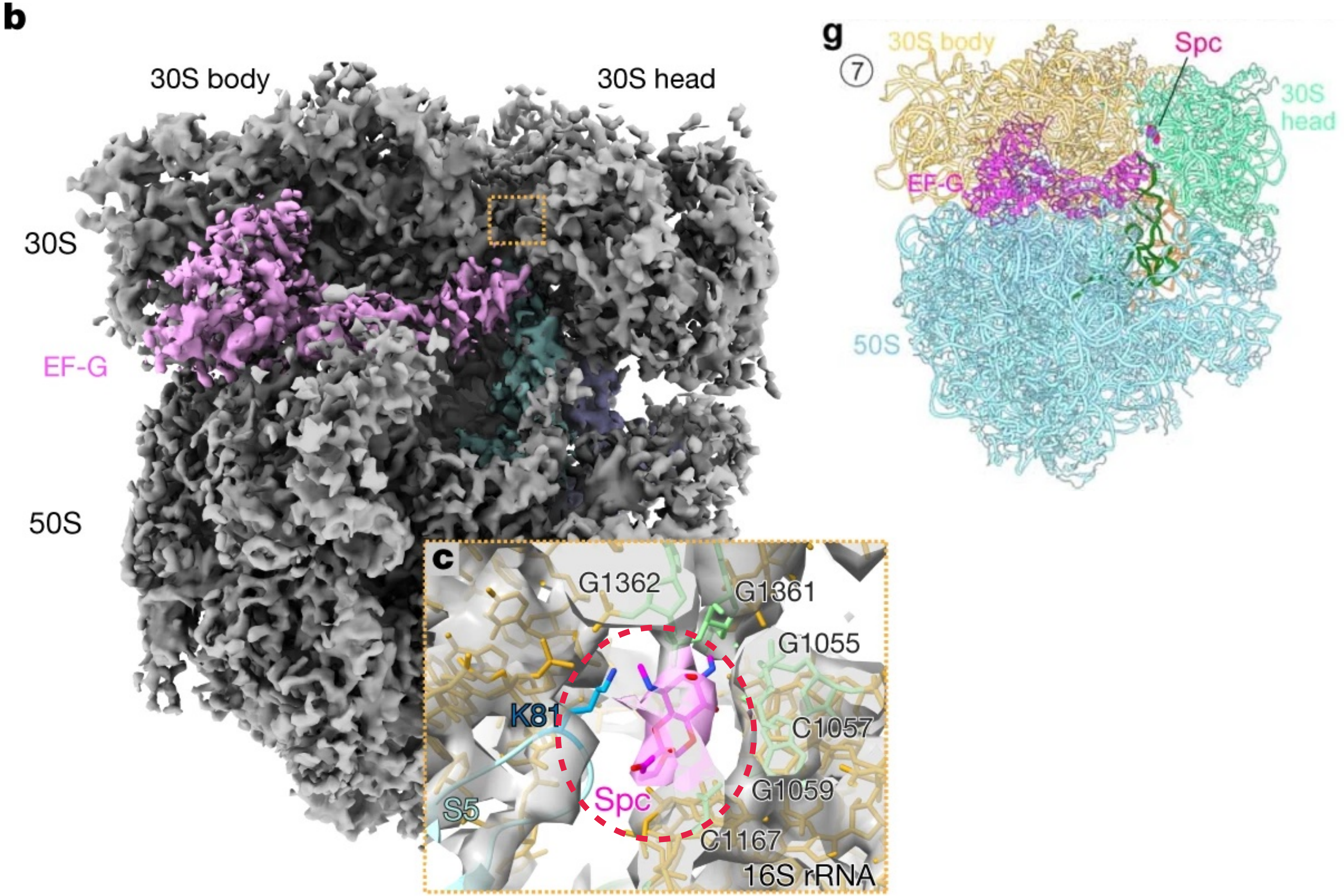
3.2 In spectinomycin(Spc)-treated cells



3 Antibiotics alter translation landscapes

3.2 In spectinomycin(Spc)-treated cells

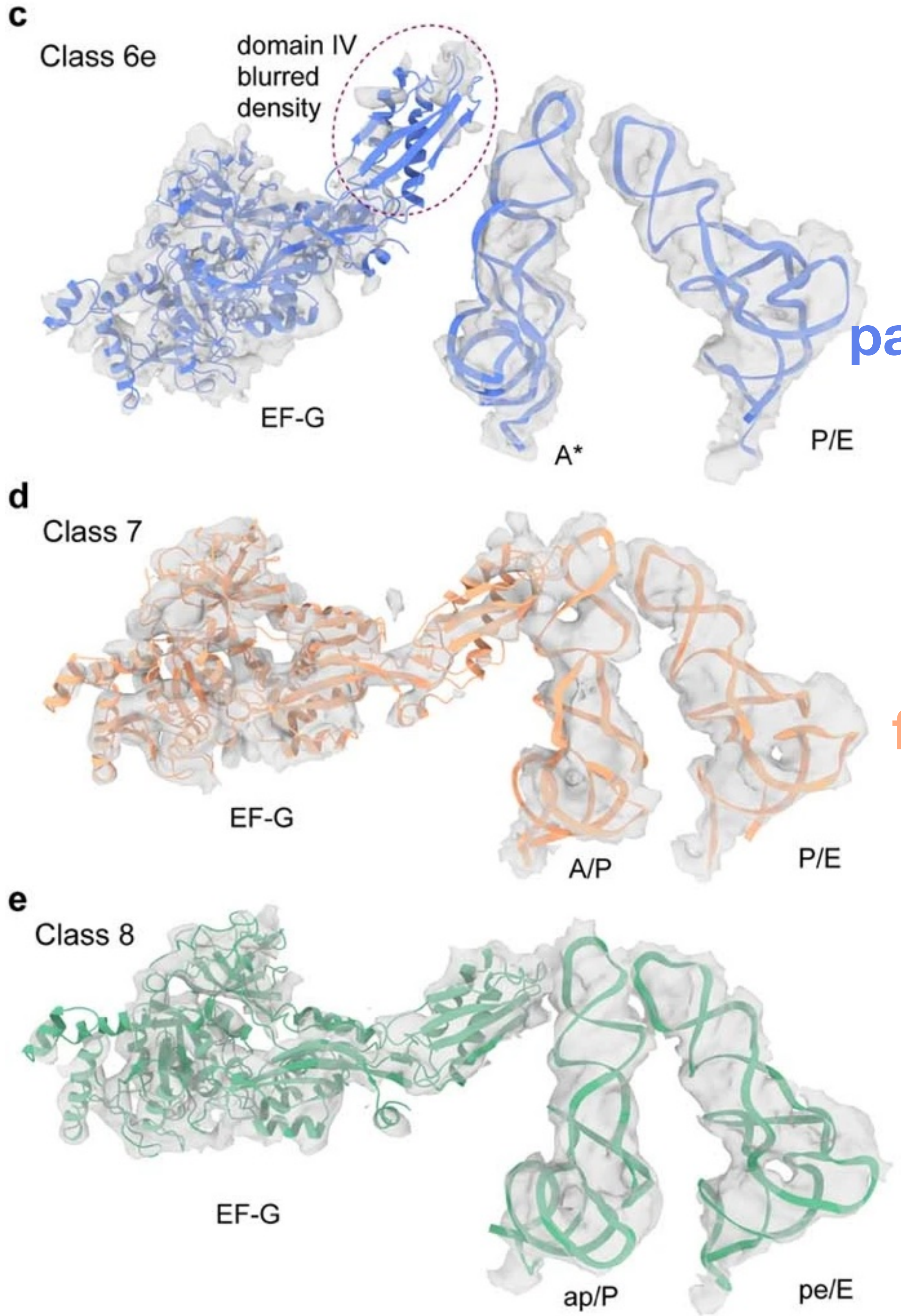




Spc inhibits translocation by blocking head swivel of the 30S

3 Antibiotics alter translation landscapes

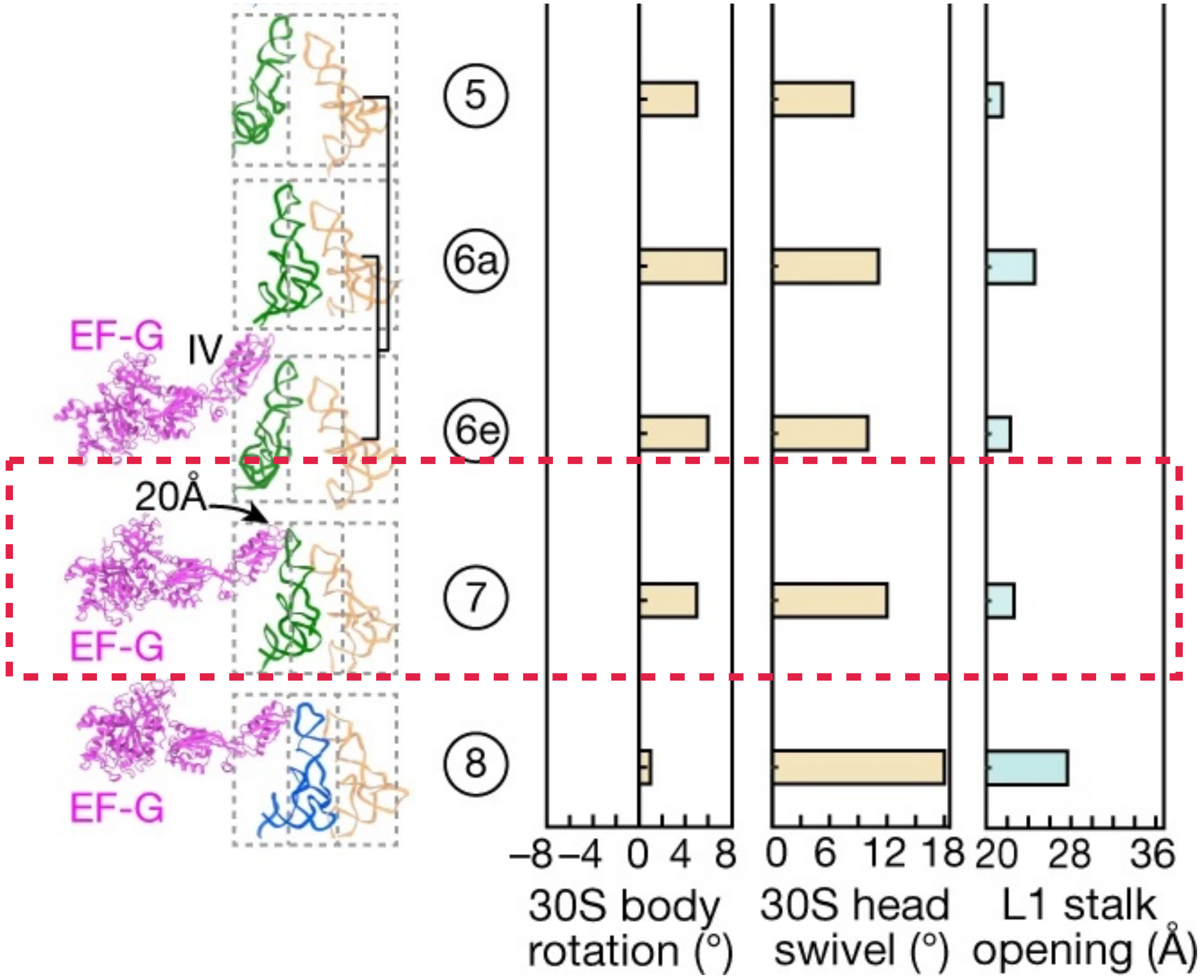
3.2 In spectinomycin(Spc)-treated cells



EF-G-bound partial hybrid state

EF-G-bound full hybrid state

The fully extended EF-G



Spc inhibits translocation by blocking head swivel of the 30S

3 Antibiotics alter translation landscapes

3.3 In pseudouridimycin(PUM)-treated cells

pseudouridimycin (PUM)

a

86 tomograms (86 pseudouridimycin-treated *Mycoplasma pneumoniae* cells)

15-20 min antibiotic treatment before plunge freezing; following procedure similar to the untreated

23,014 ribosome sub-tomograms extracted *in silico*

Initial Refine3D & Class3D. Excluding 2,029 "bad" sub-tomograms that cannot be aligned.

(50) 50S
5,671
8.7 Å

70S
15,314

M refine

Re-extract sub-tomograms after M refinement

global Class3D

focused Class3D on A-P-E tRNAs

focused Class3D on EFs, A/T tRNA

⑬ Dim30S-50S
898
20.7 Å

2a P
1,457
9.5 Å

③ EF-Tu•tRNA, P
1,128
9.3 Å

④ A, P
1,534
9.7 Å

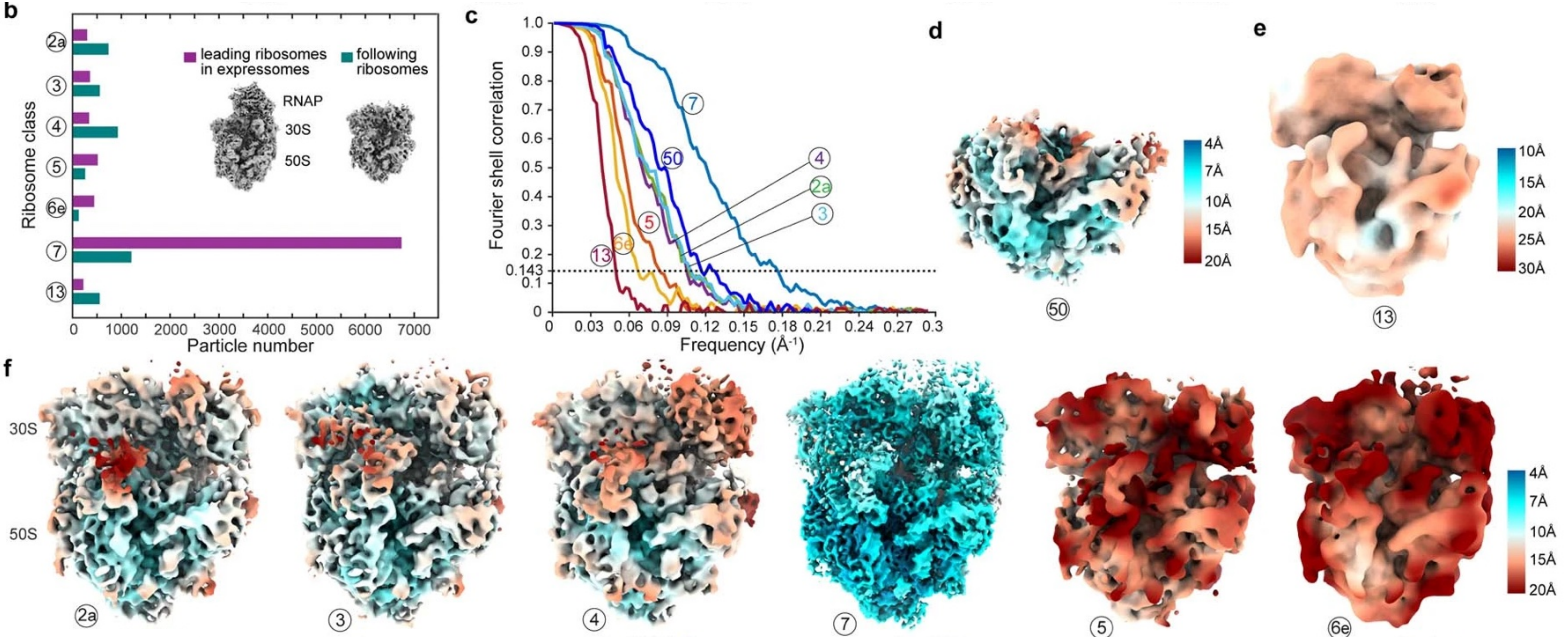
⑦ EF-G, A/P^{PUM}, P/E
8,730
5.7 Å

⑤ A*, P/E
940
12.1 Å

6e EF-G, A*, P/E
627
15 Å

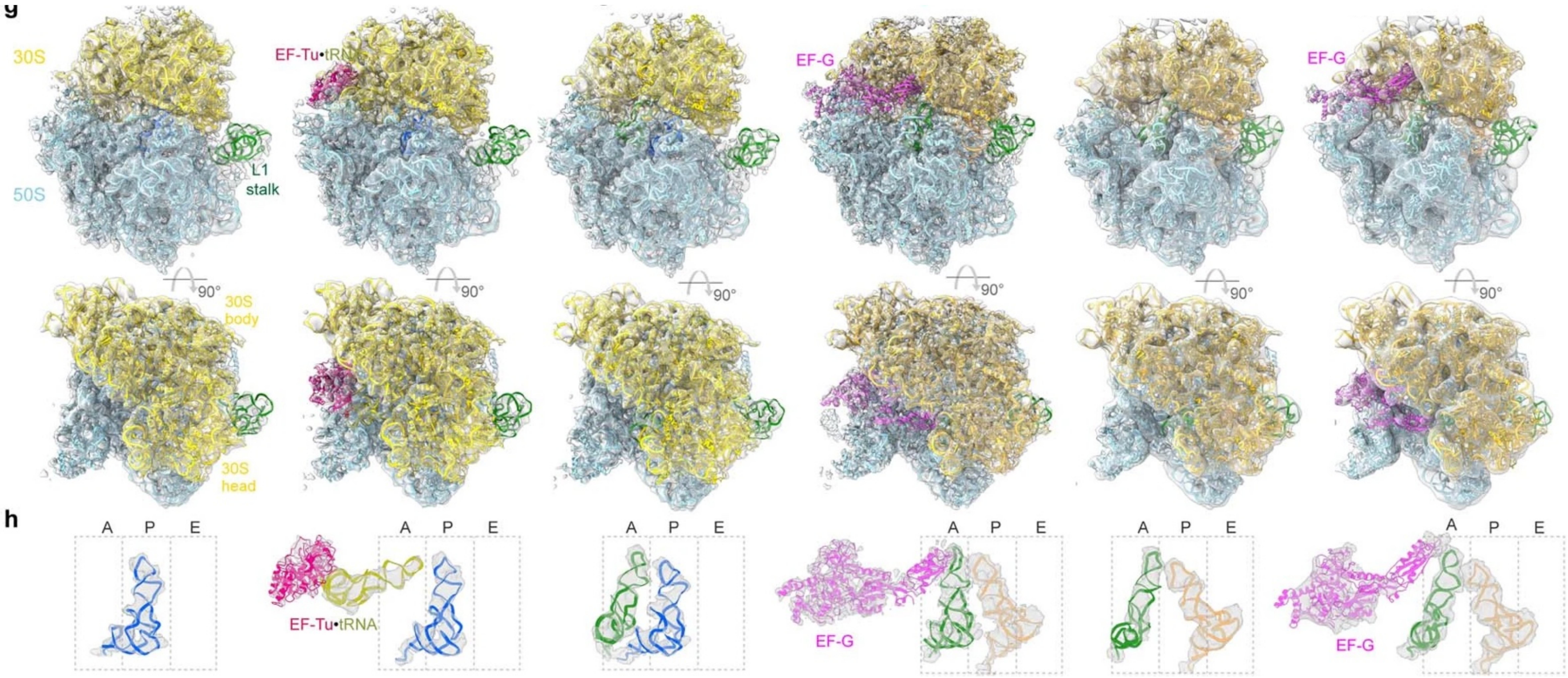
3 Antibiotics alter translation landscapes

3.3 In pseudouridimycin(PUM)-treated cells



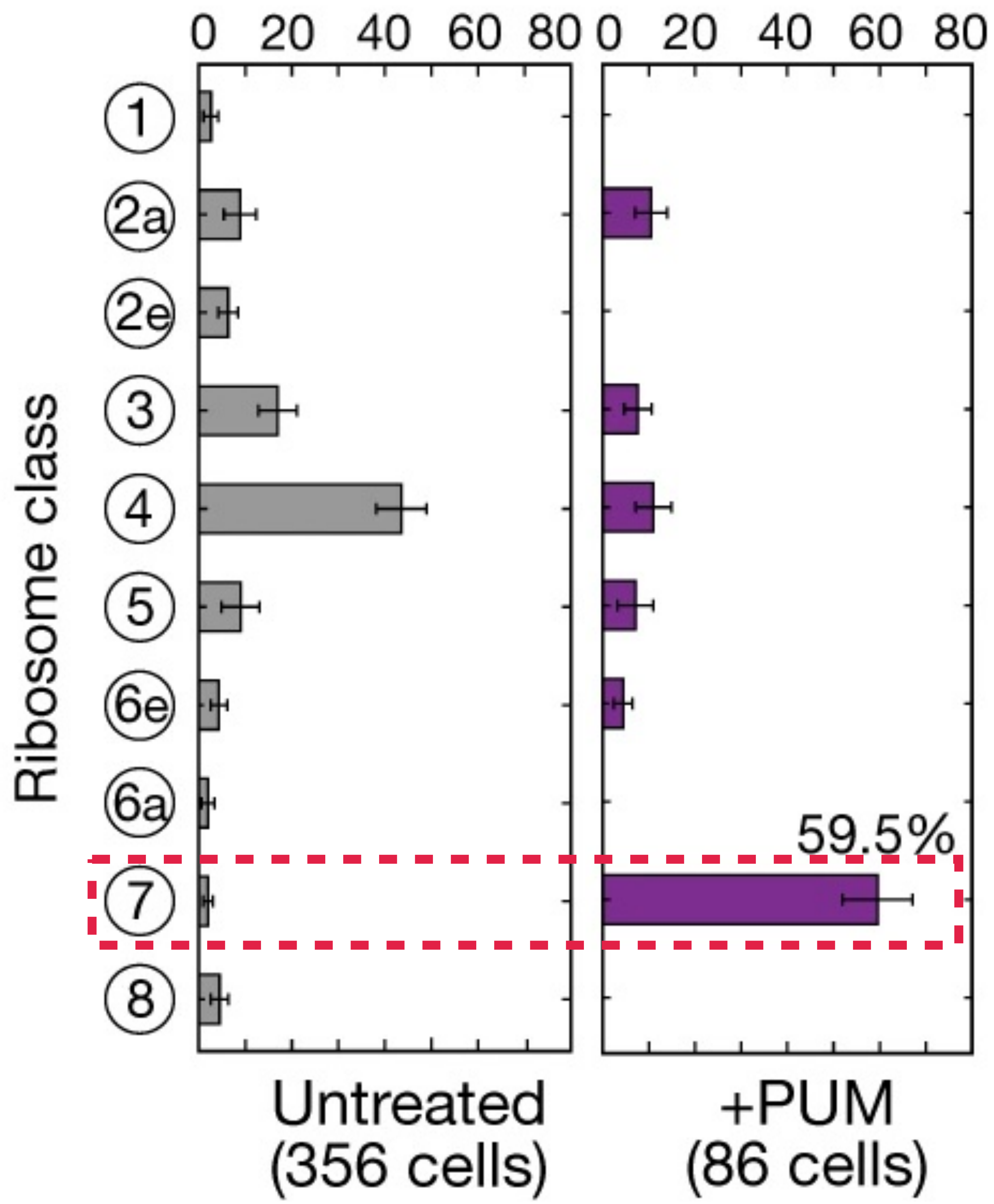
3 Antibiotics alter translation landscapes

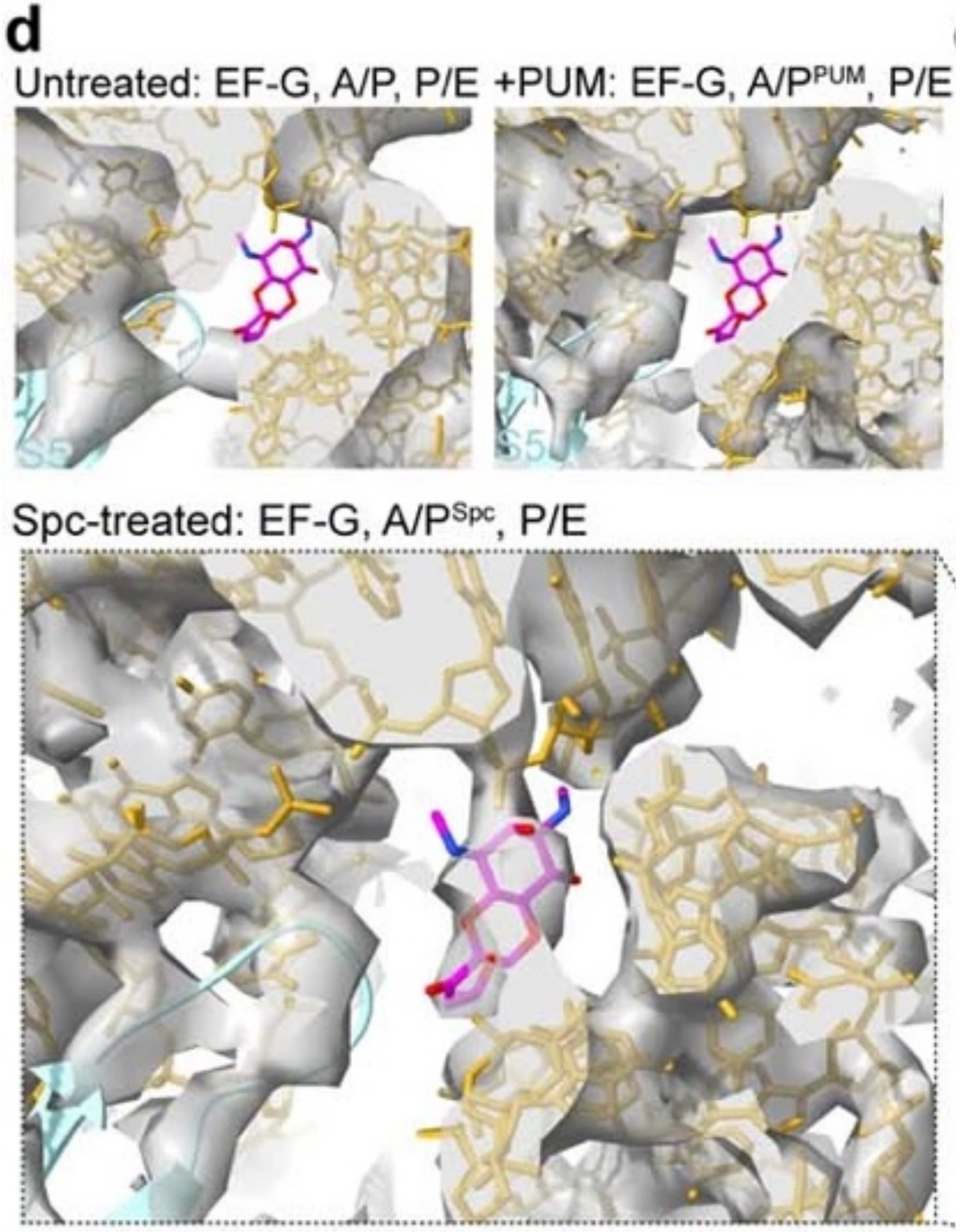
3.3 In pseudouridimycin(PUM)-treated cells

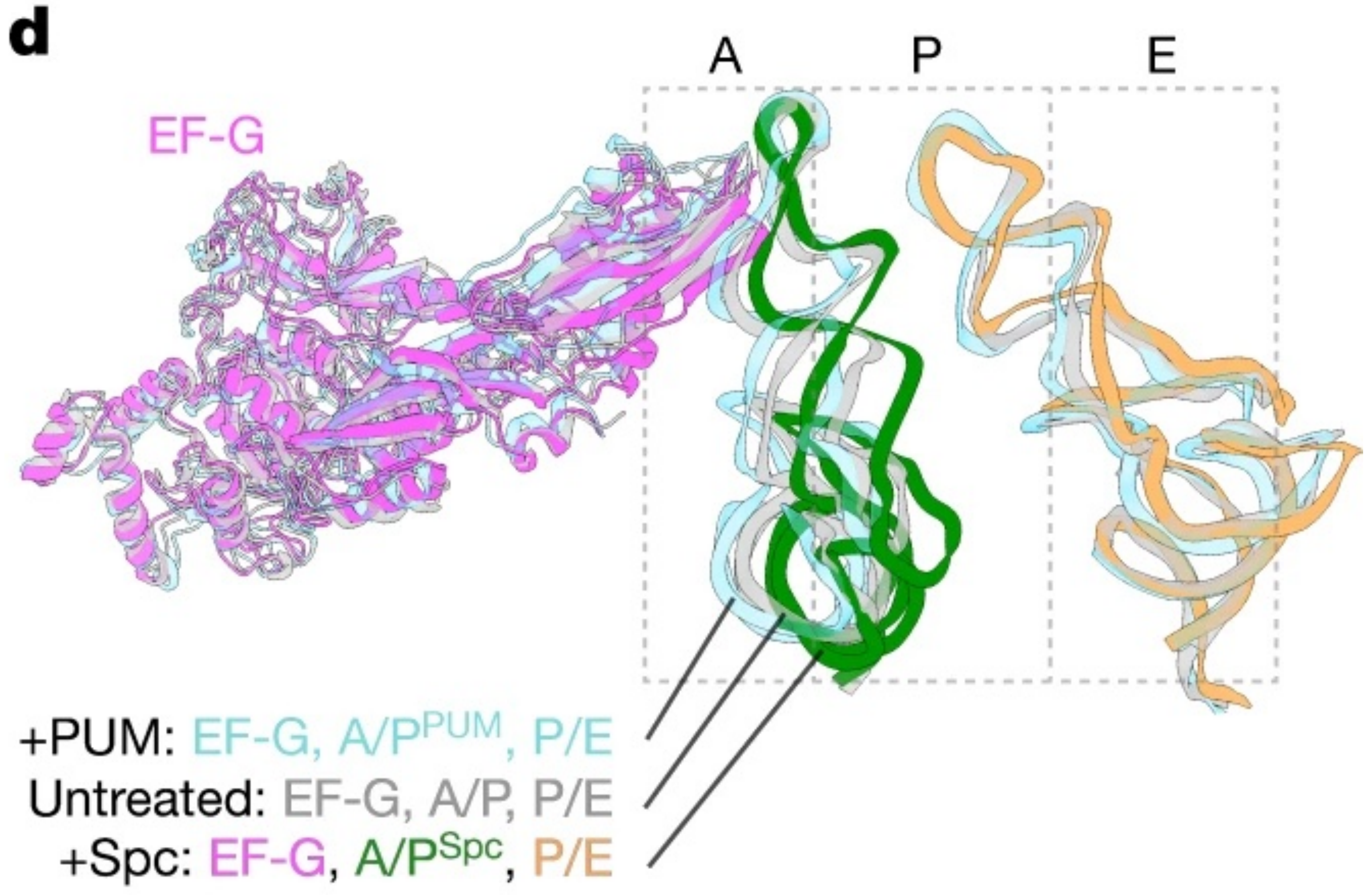


3 Antibiotics alter translation landscapes

3.3 In pseudouridimycin(PUM)-treated cells







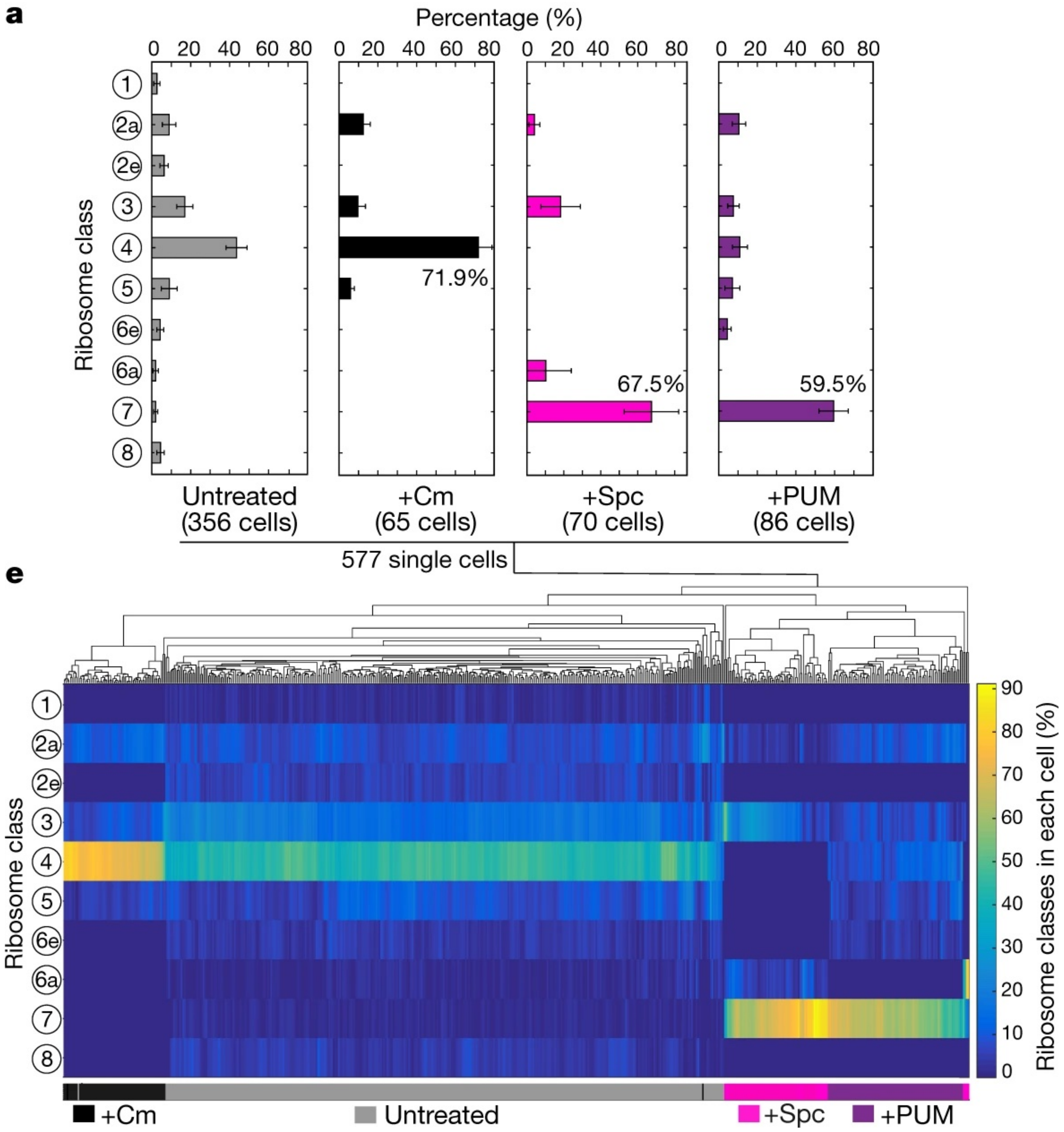
PUM blocks mRNA translocation

↓

mRNA translocation and 30S rotations are directly coupled

3 Antibiotics alter translation landscapes

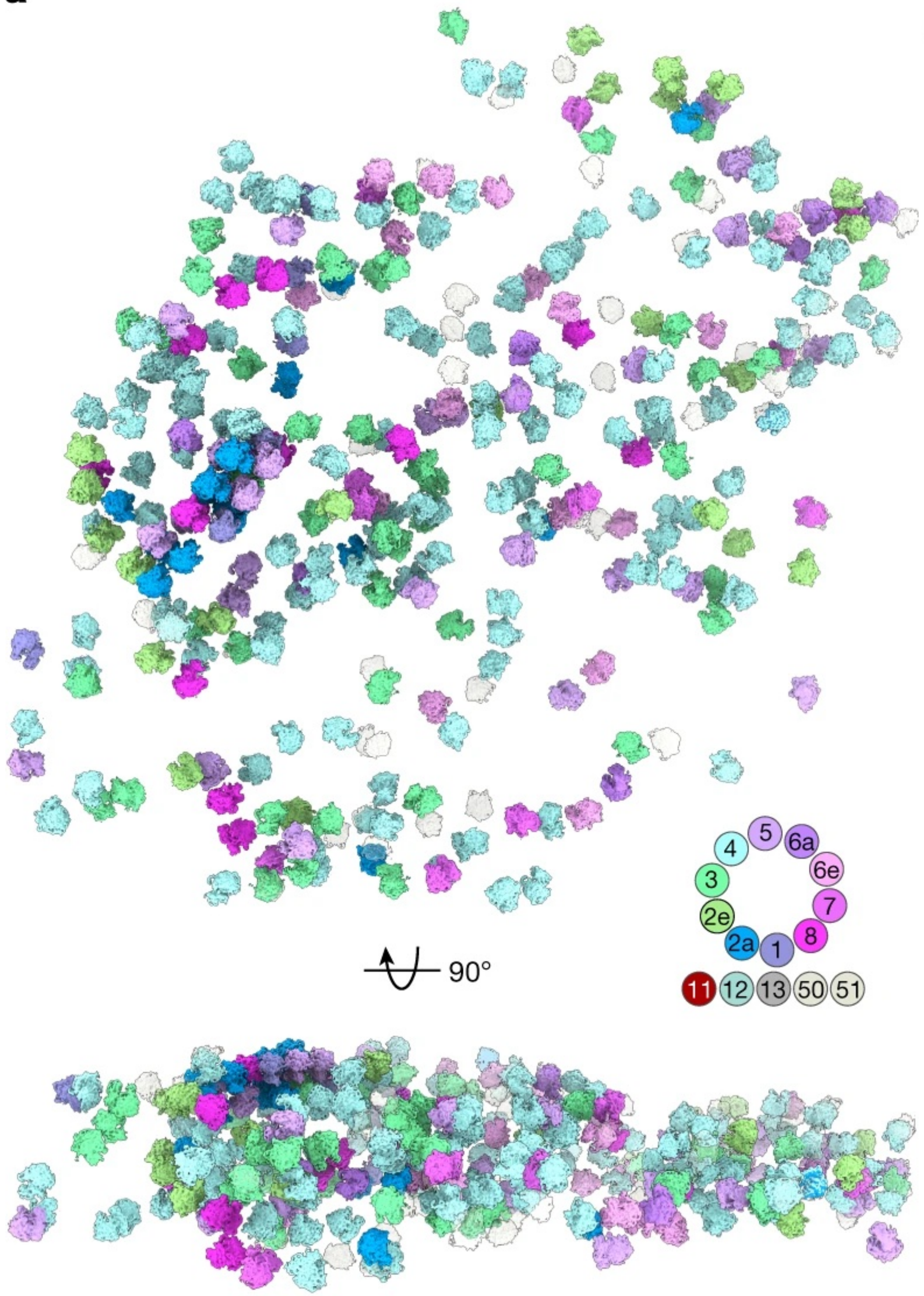
3.4 Translation landscapes are markedly reshaped by different antibiotics



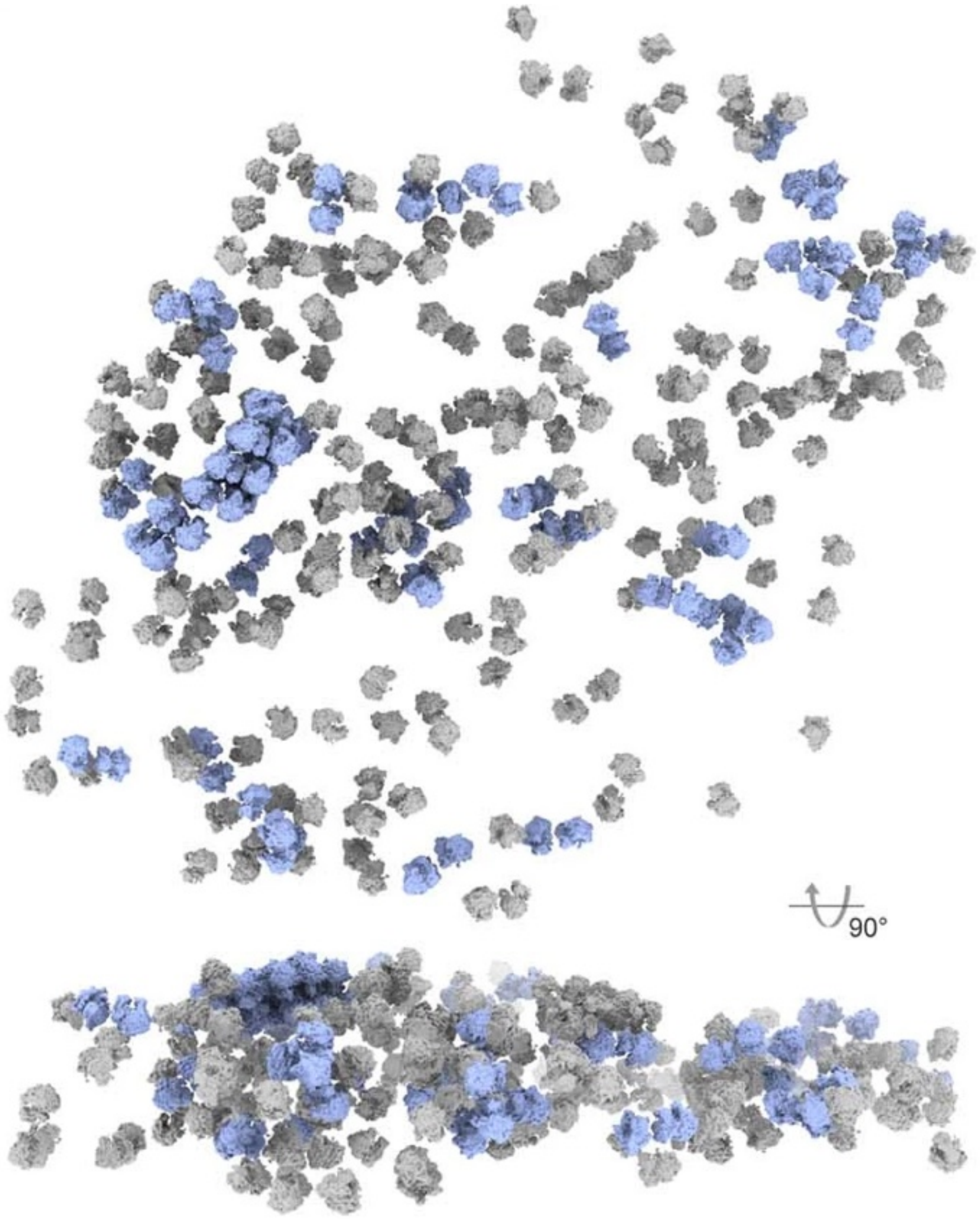
4 Spatial and functional organization of translation

4.1 Spatial analysis of ribosomes and polysomes in native untreated cells

a

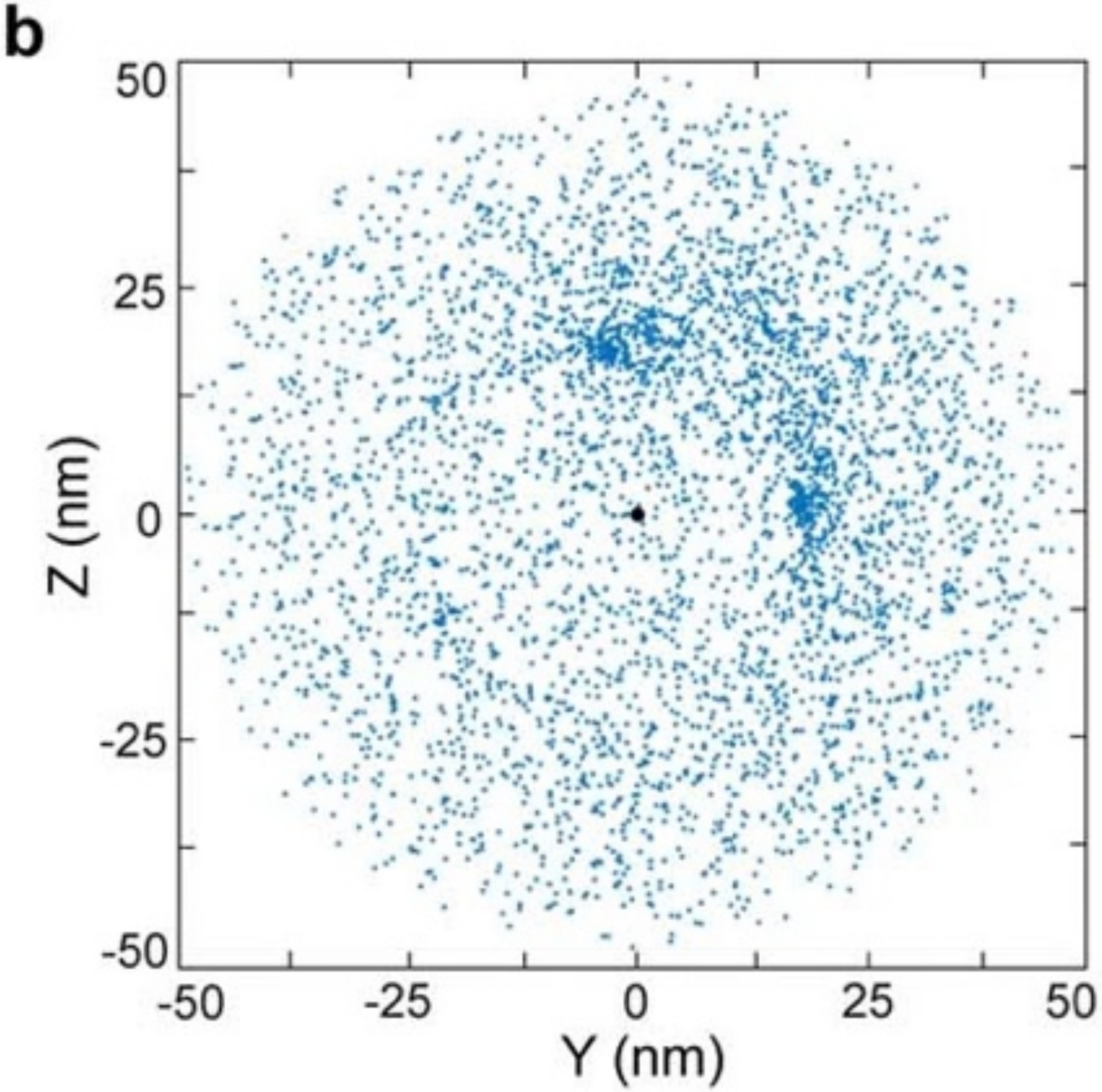


a



4 Spatial and functional organization of translation

4.1 Spatial analysis of ribosomes and polysomes in native untreated cells

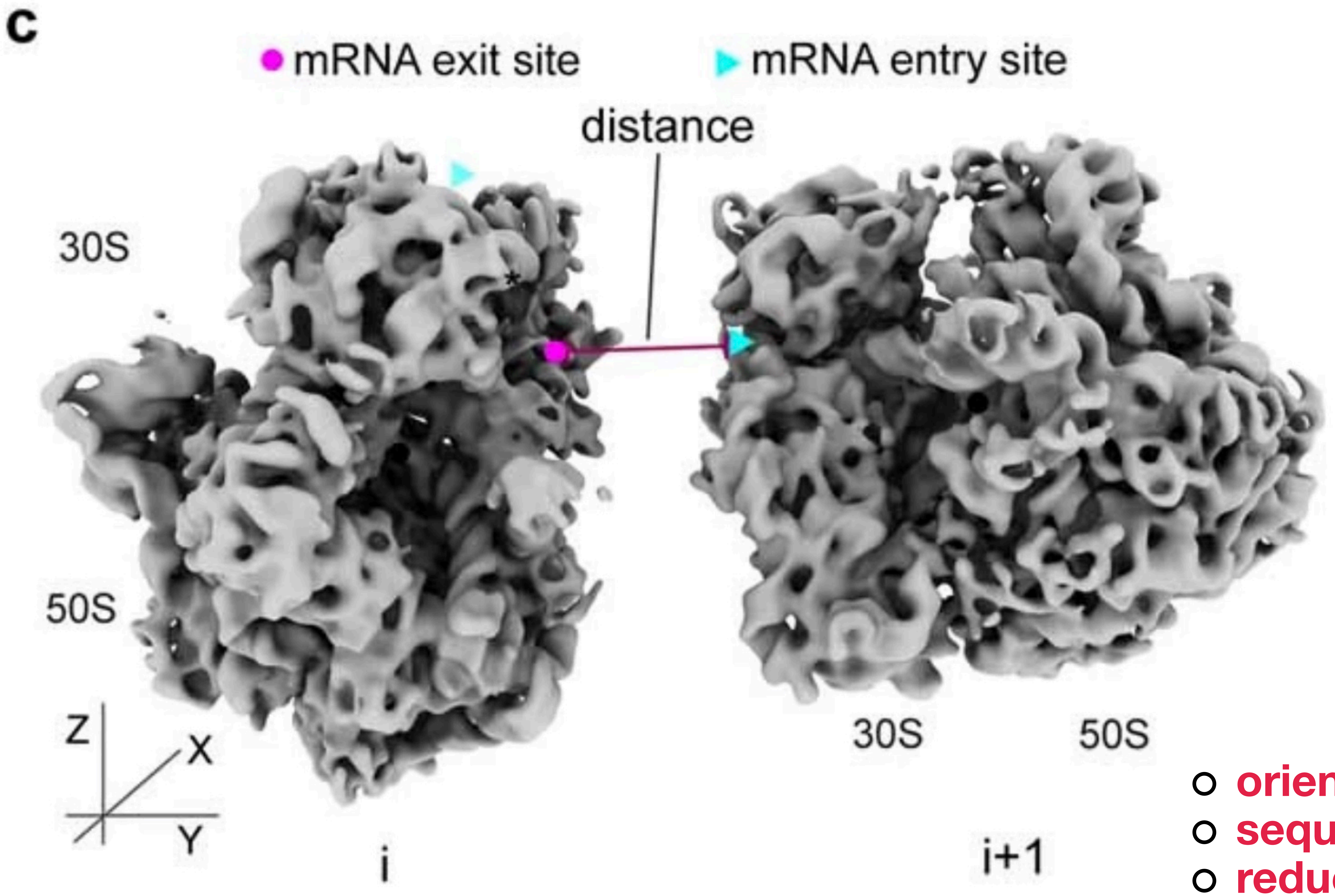


based on center-to-center distance

impossible to obtain the order of ribosomes within the polysome

4 Spatial and functional organization of translation

4.1 Spatial analysis of ribosomes and polysomes in native untreated cells

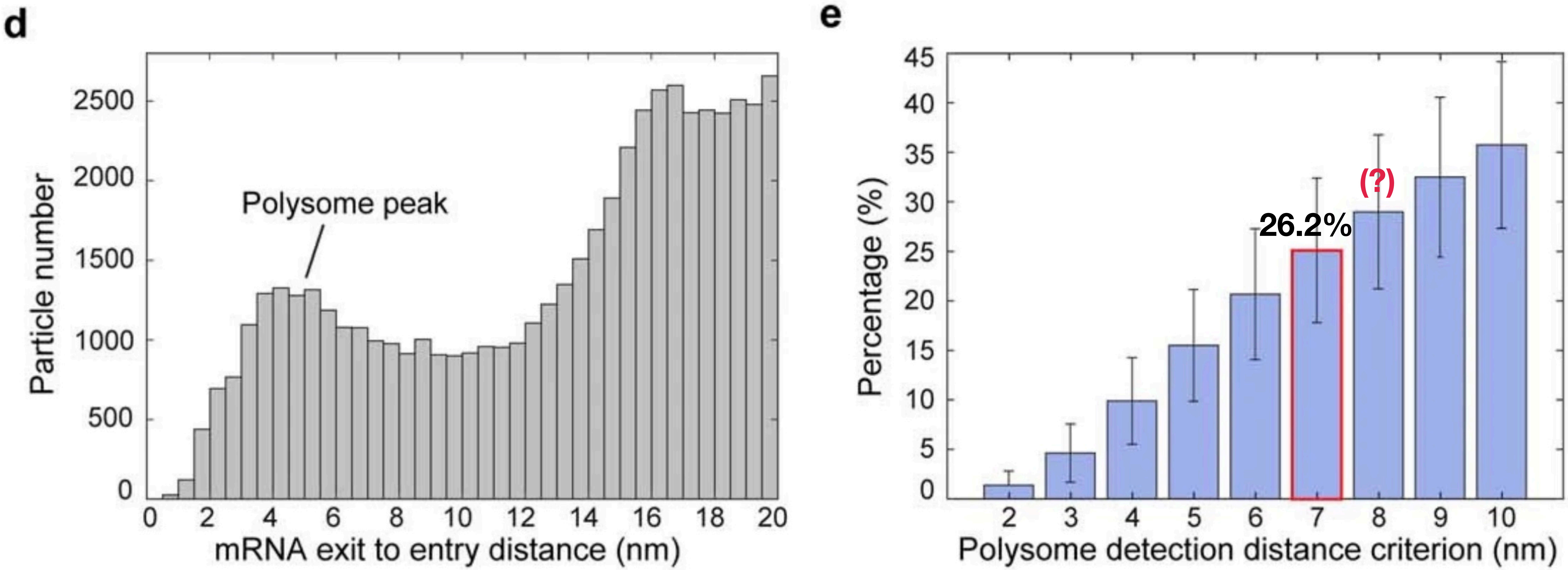


- ○ orientations
- ○ sequential information
- ○ reduce false annotation

based on the distance from mRNA exit of one ribosome to the mRNA entry of following ribosomes

4 Spatial and functional organization of translation

4.1 Spatial analysis of ribosomes and polysomes in native untreated cells



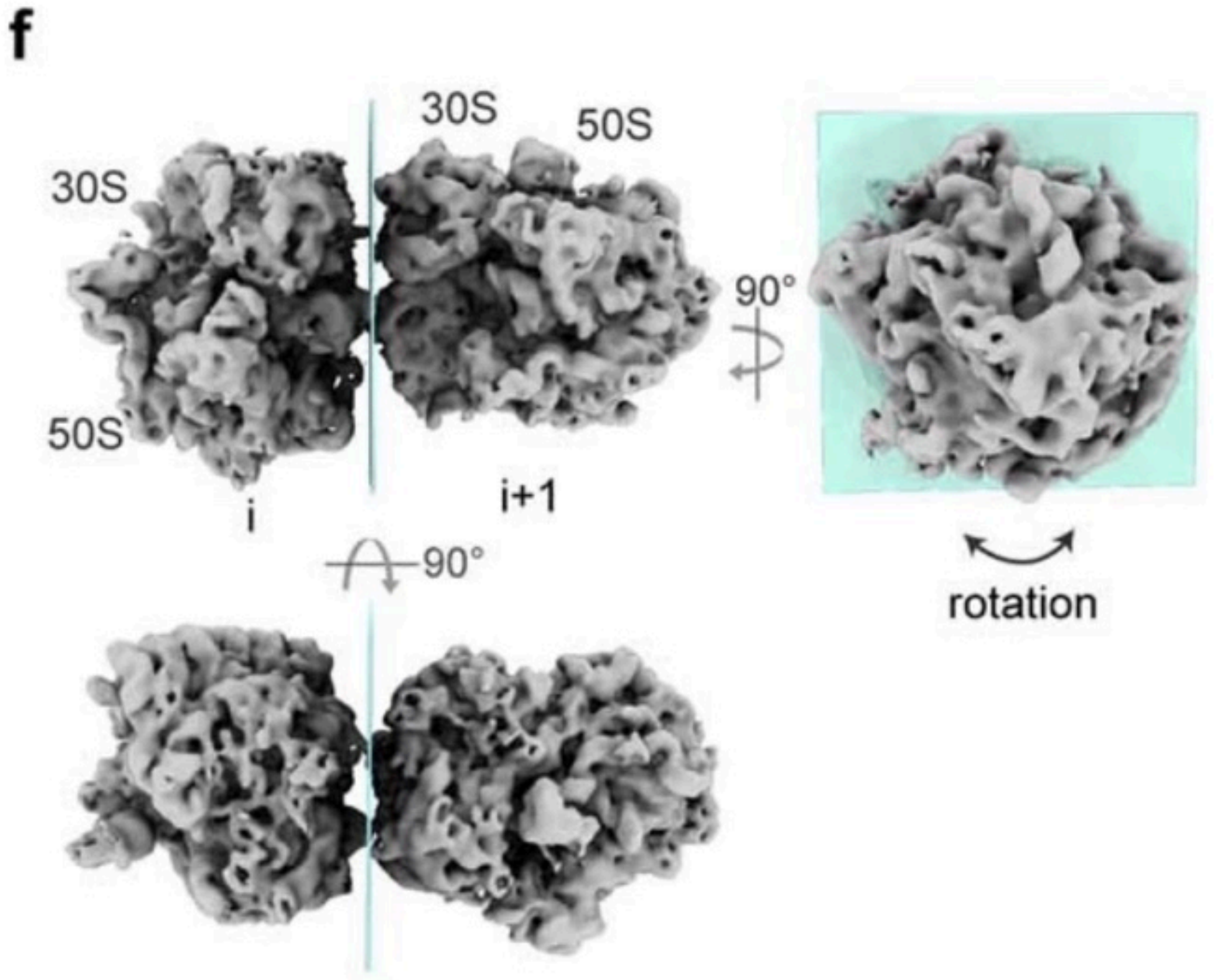
26.2%

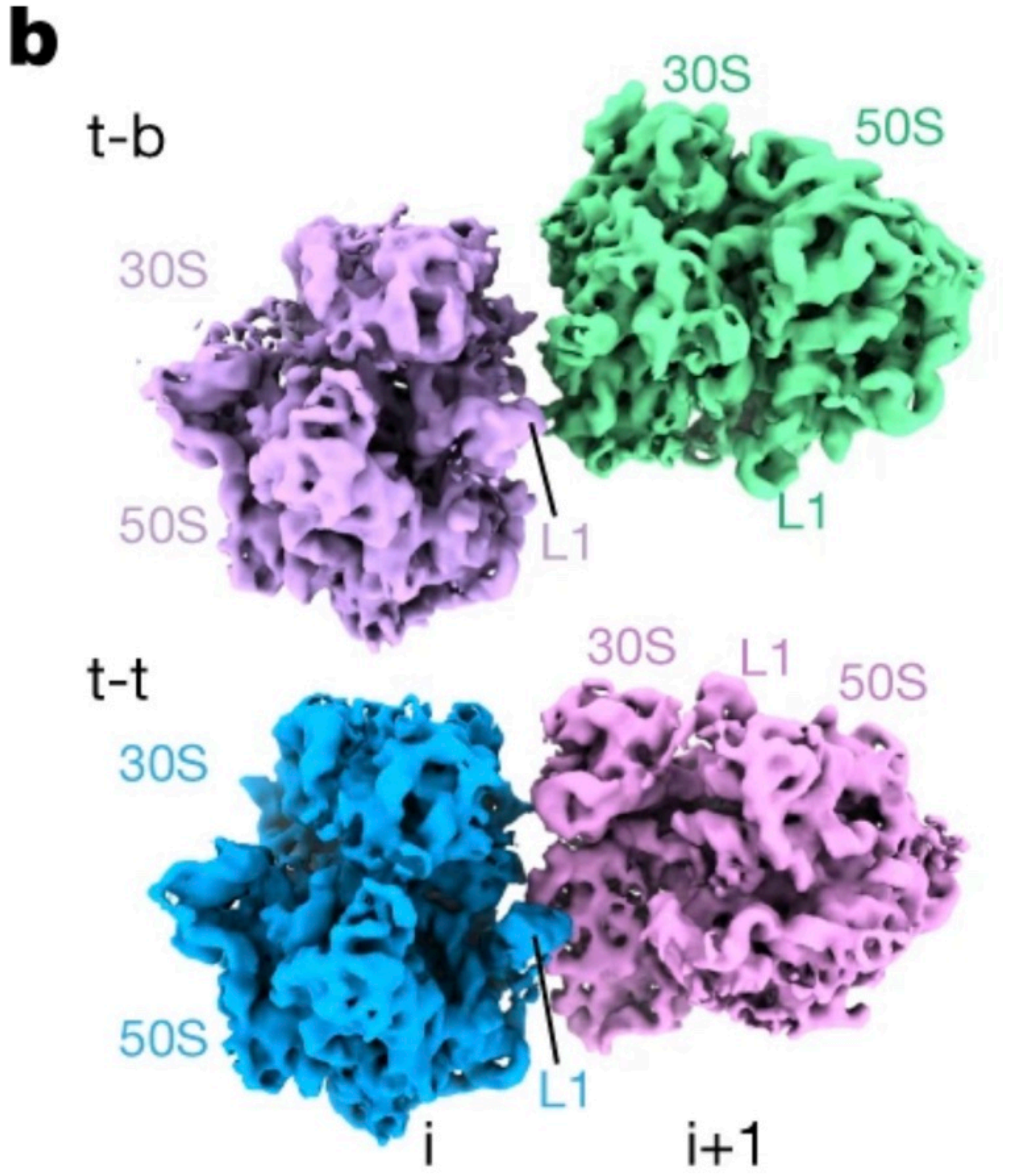
(?)

- **7 nm provided the best recovery rate and detection accuracy based on visual inspection**
- **26.2% is consistent with literature reporting that polysomes are often found at relatively low abundance (~30%) in bacteria**

4 Spatial and functional organization of translation

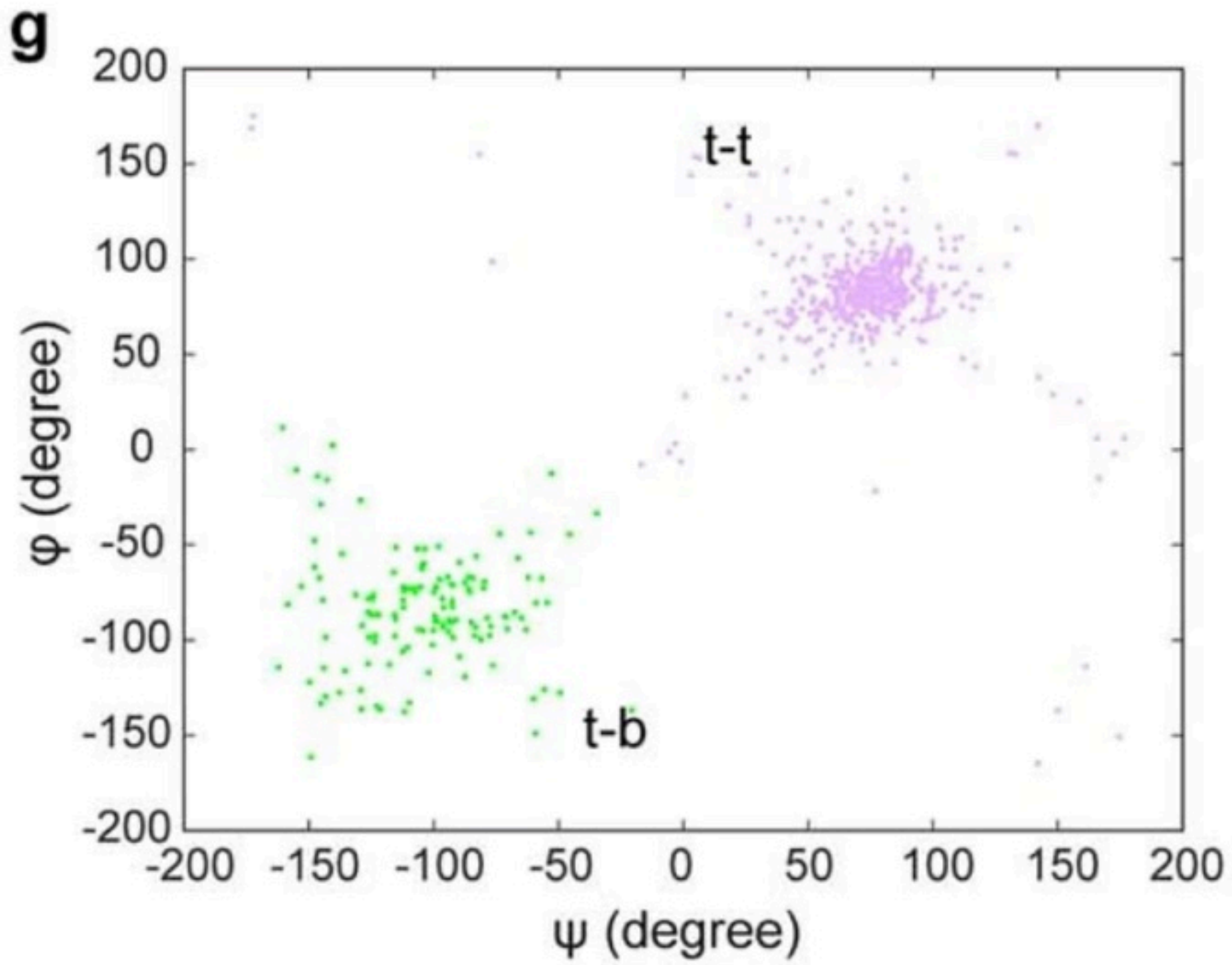
4.1 Spatial analysis of ribosomes and polysomes in native untreated cells

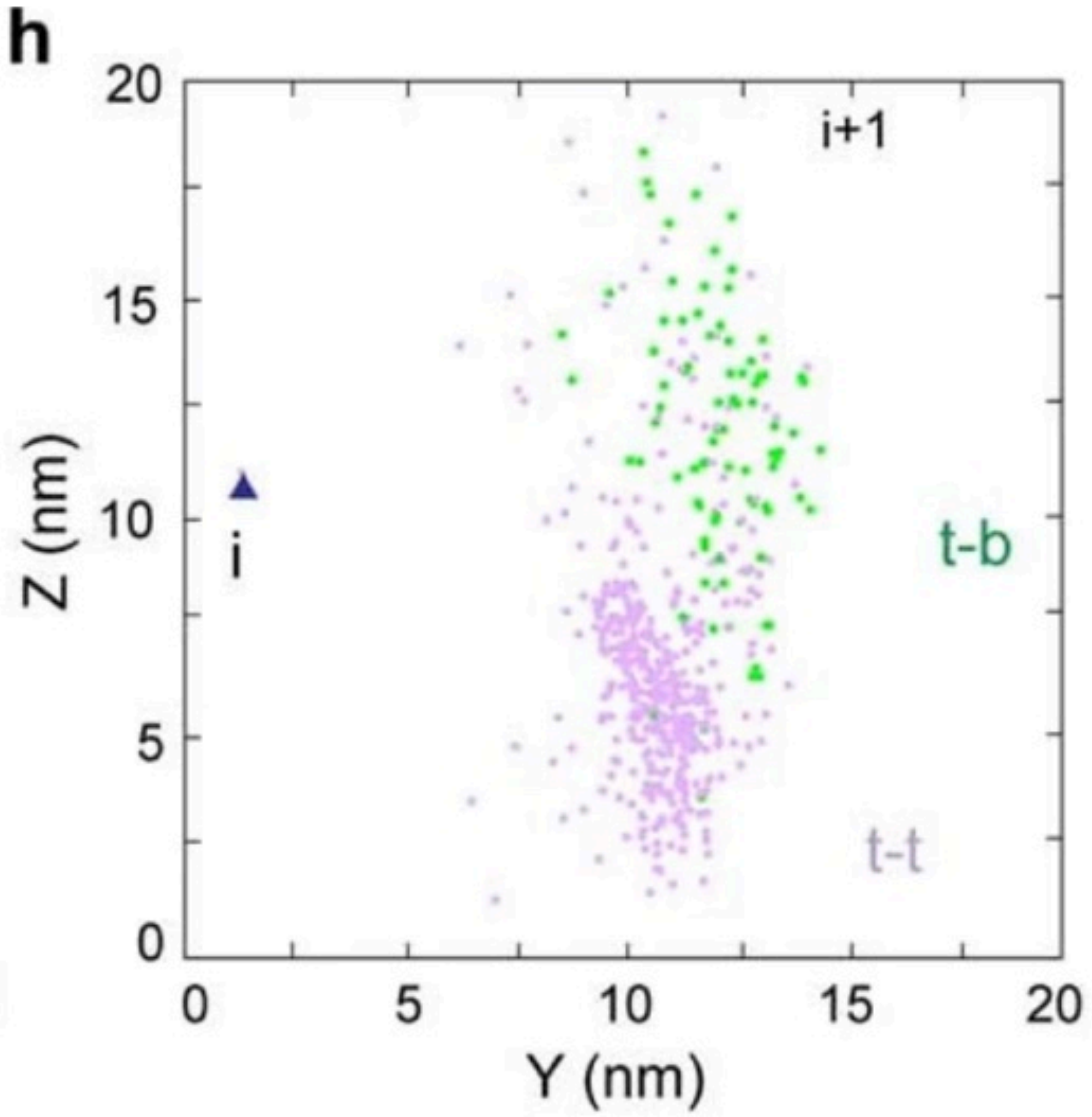


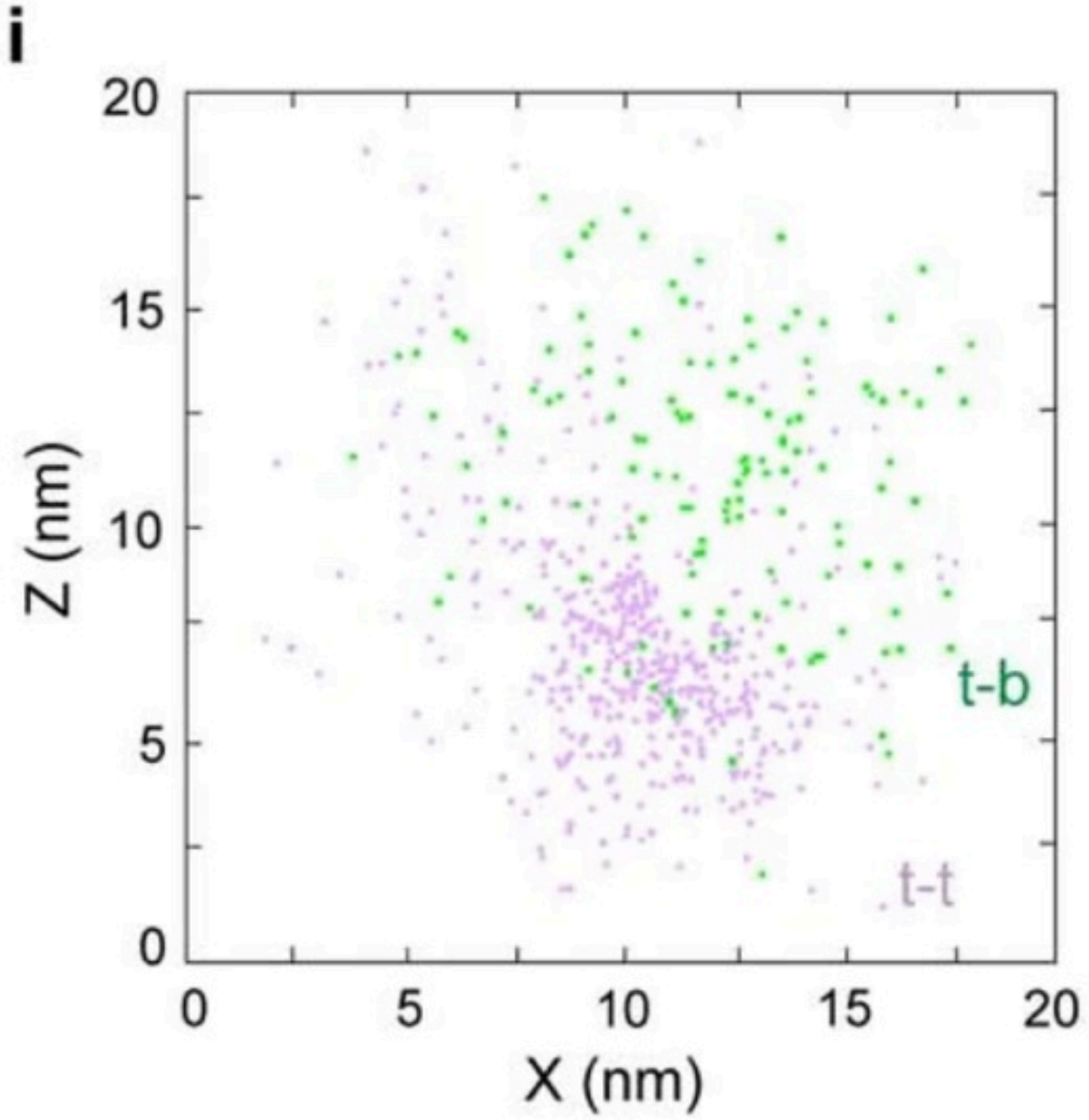


'Top-top' 78.5%
mRNA exit-to-entry distance 4.2±1.4nm

'Top-back' 21.5%
mRNA exit-to-entry distance 5.4±1.5nm

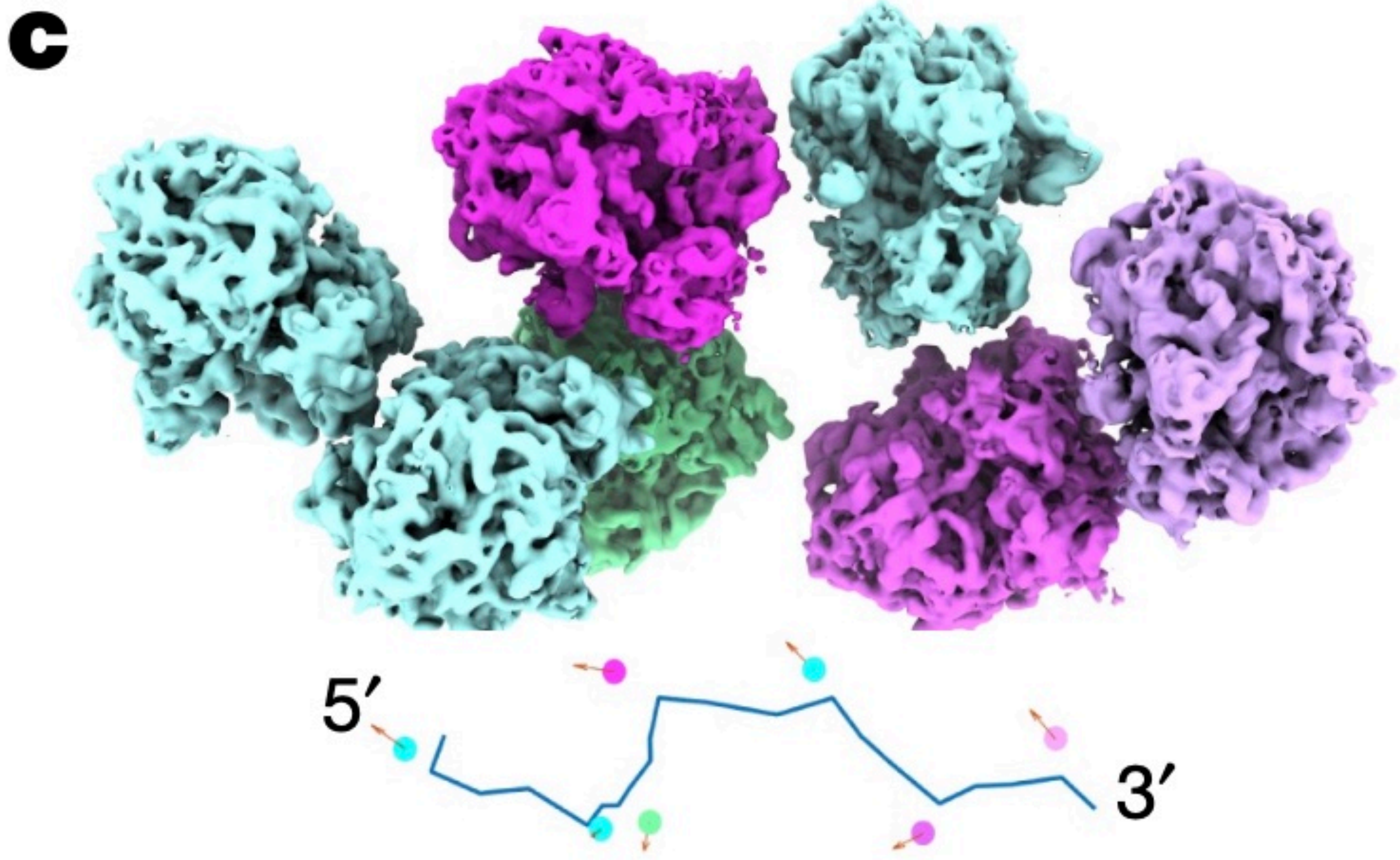




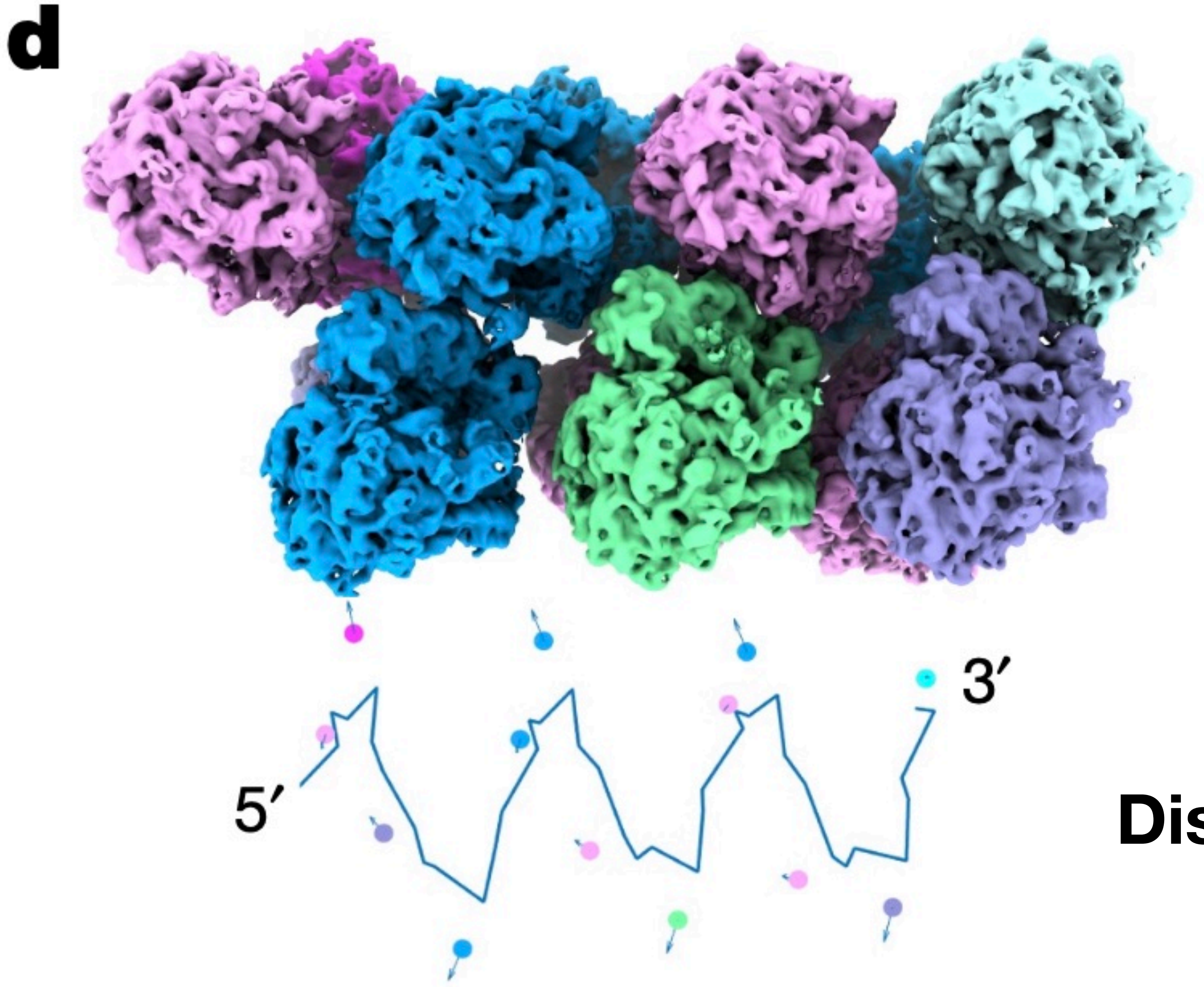


4 Spatial and functional organization of translation

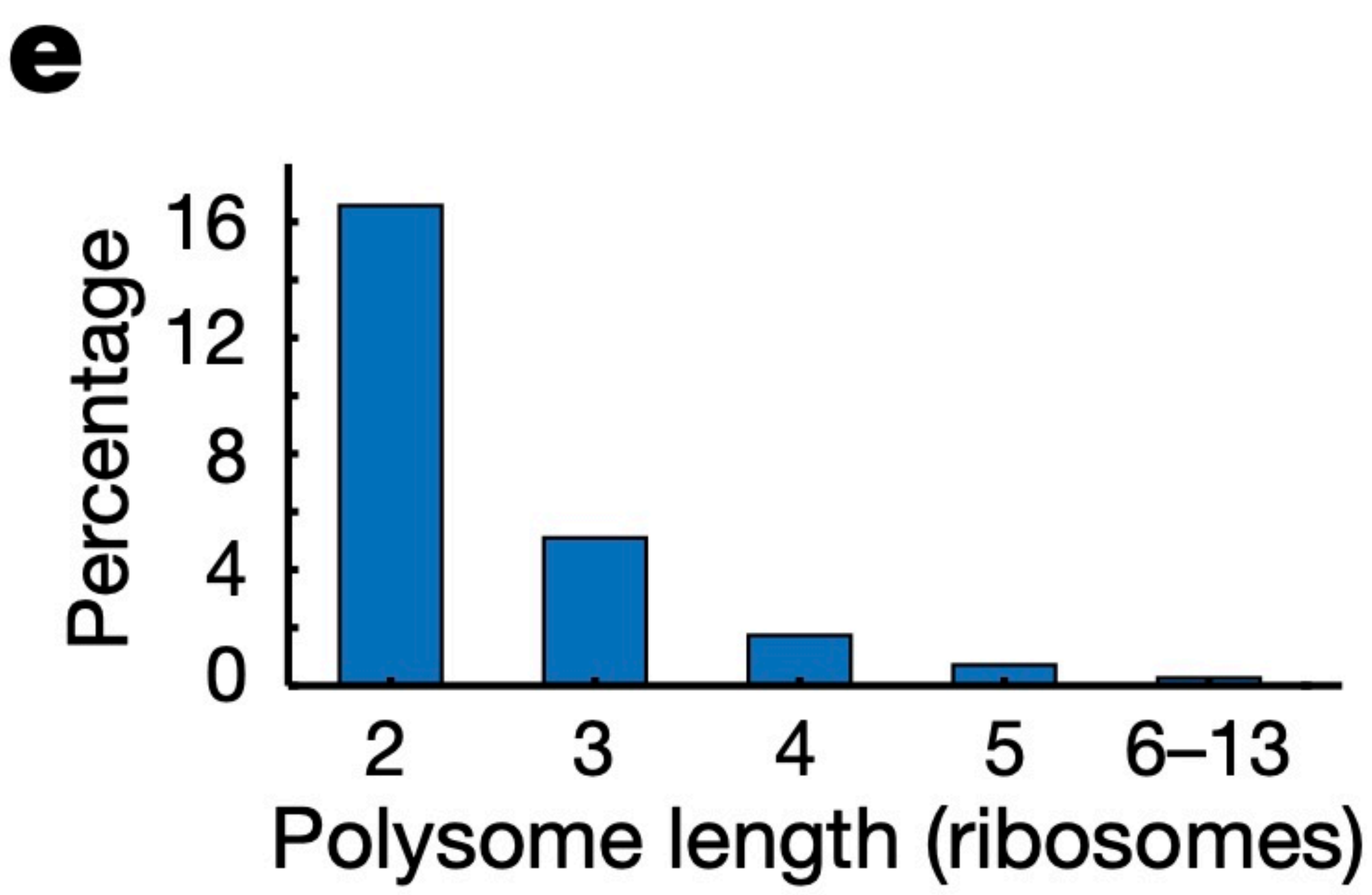
4.1 Spatial analysis of ribosomes and polysomes in native untreated cells



loose assembly



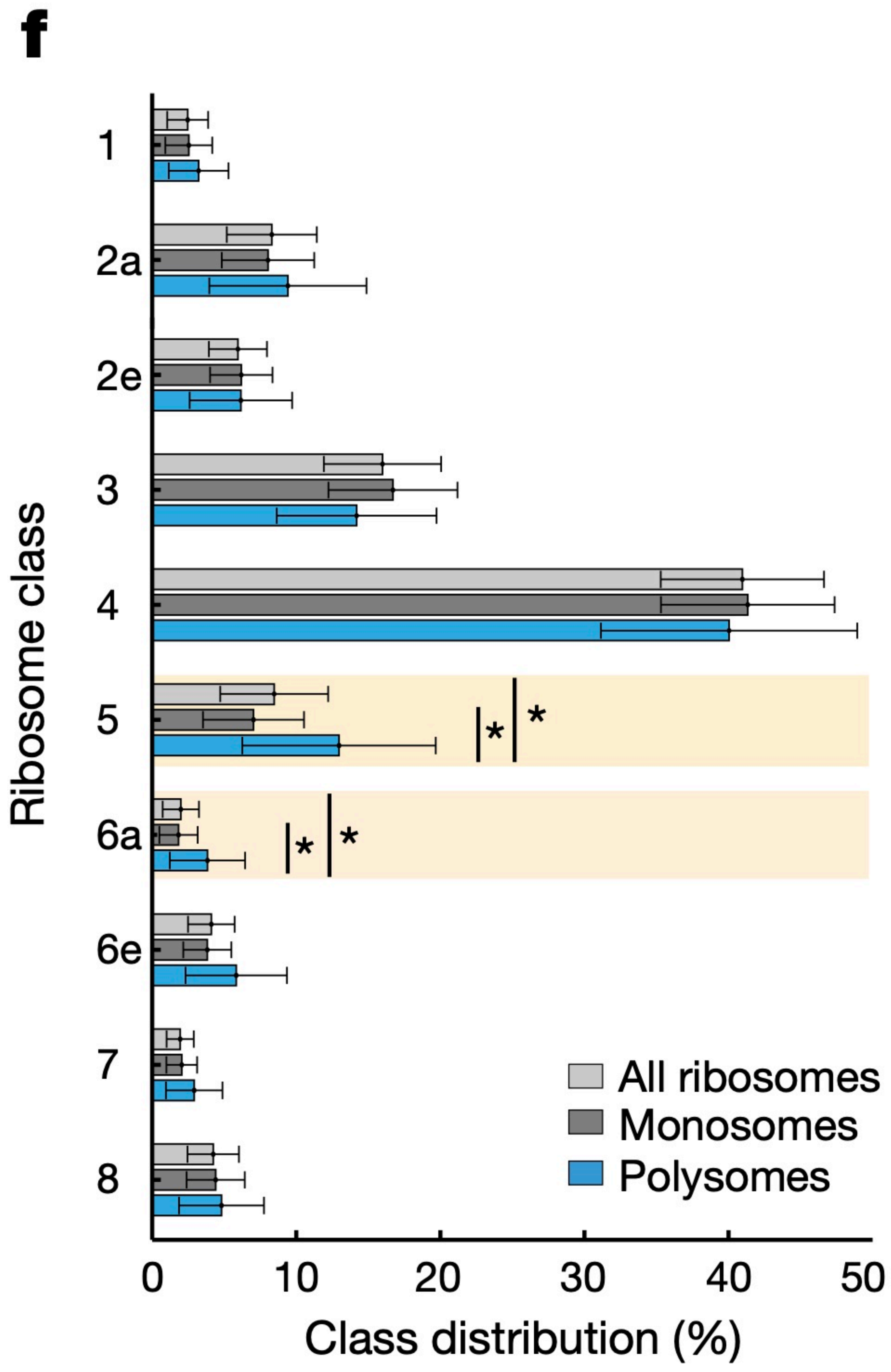
tight packing with helix-like configurations



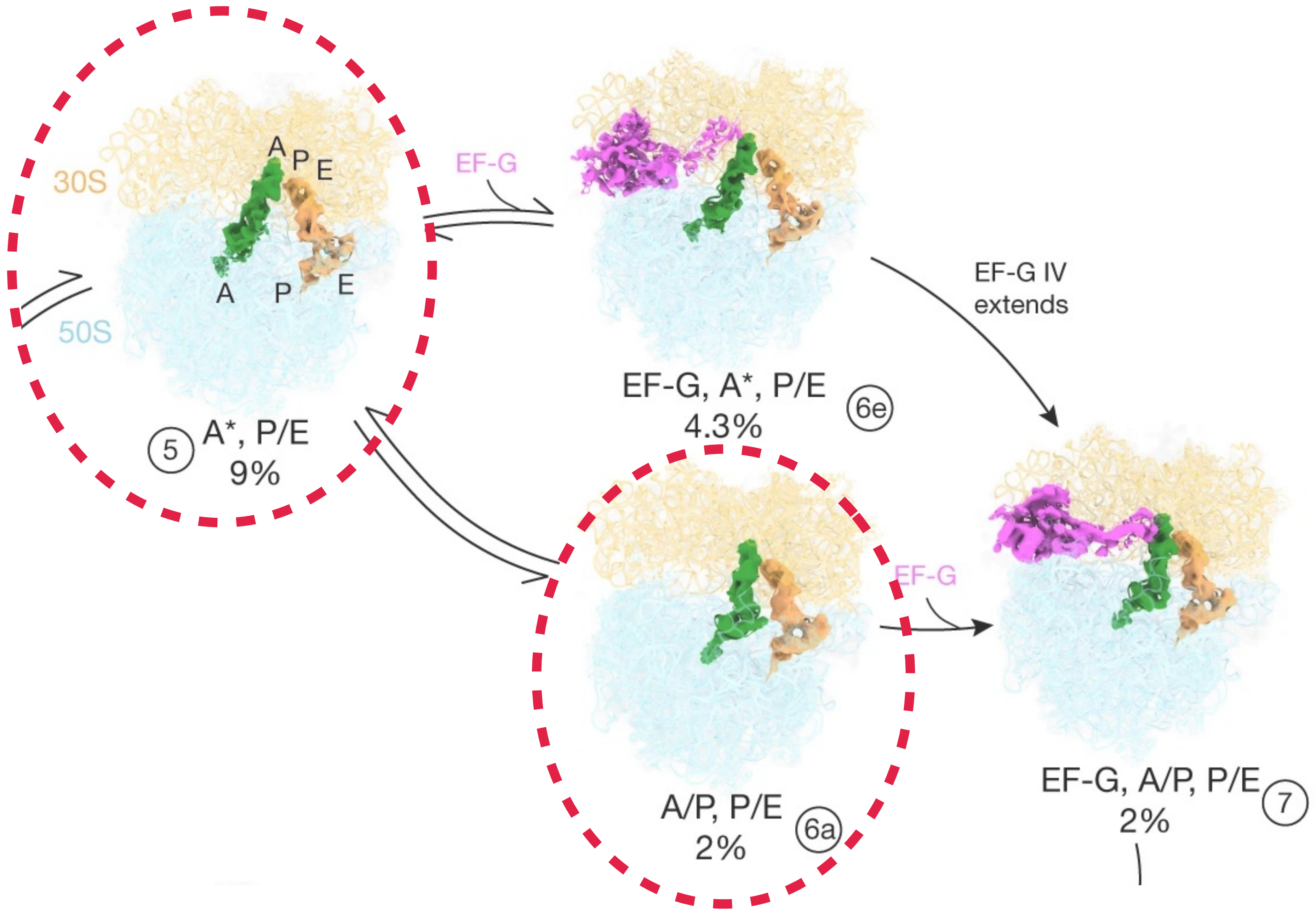
Distribution of polysome lengths

4 Spatial and functional organization of translation

4.2 Local coordination of translation elongation within the polysome

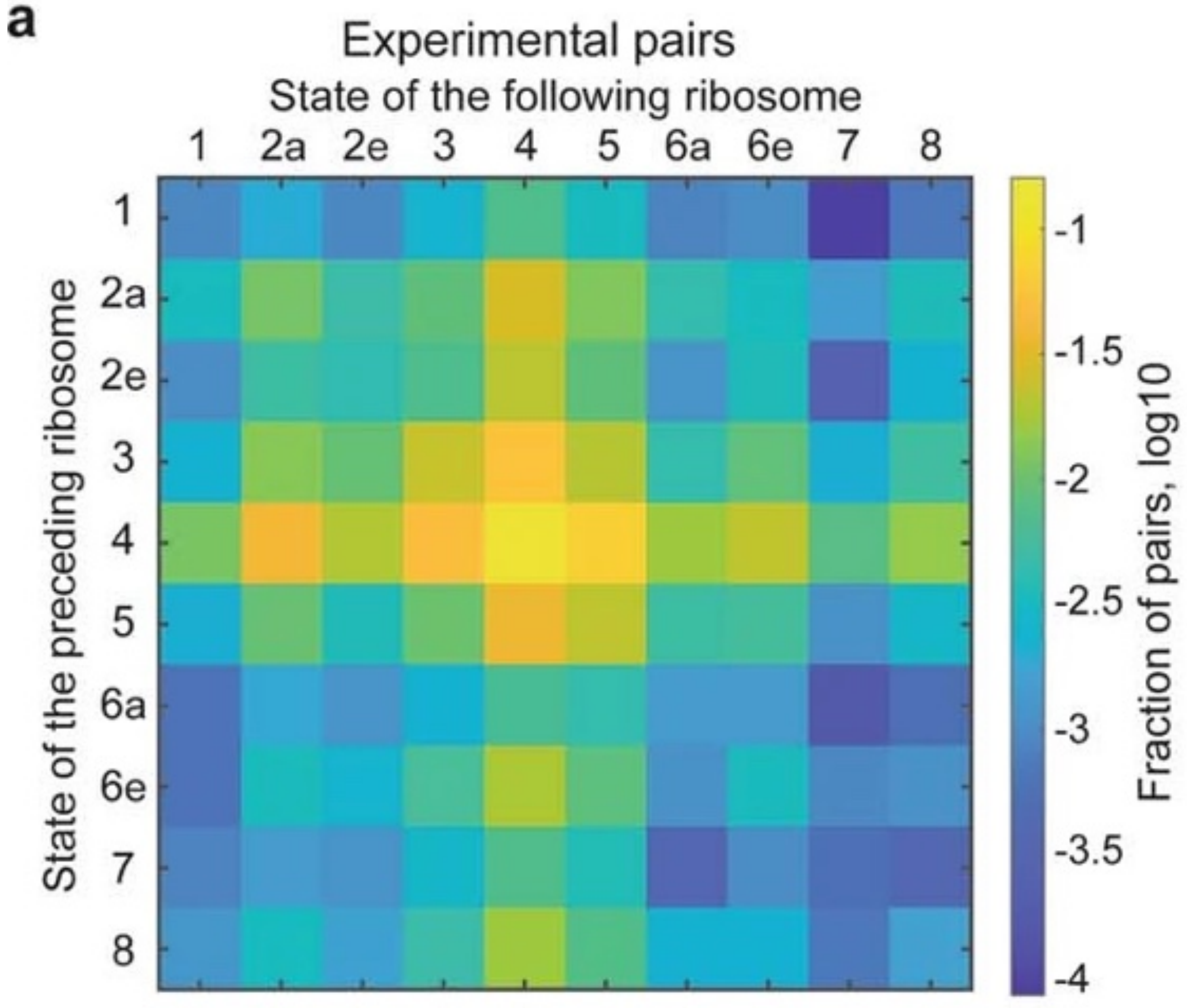


- Most states occurred equally frequently in both populations
- The fractions of two states before EF-G binding (class 5 and class 6a)are more frequent in polysomes

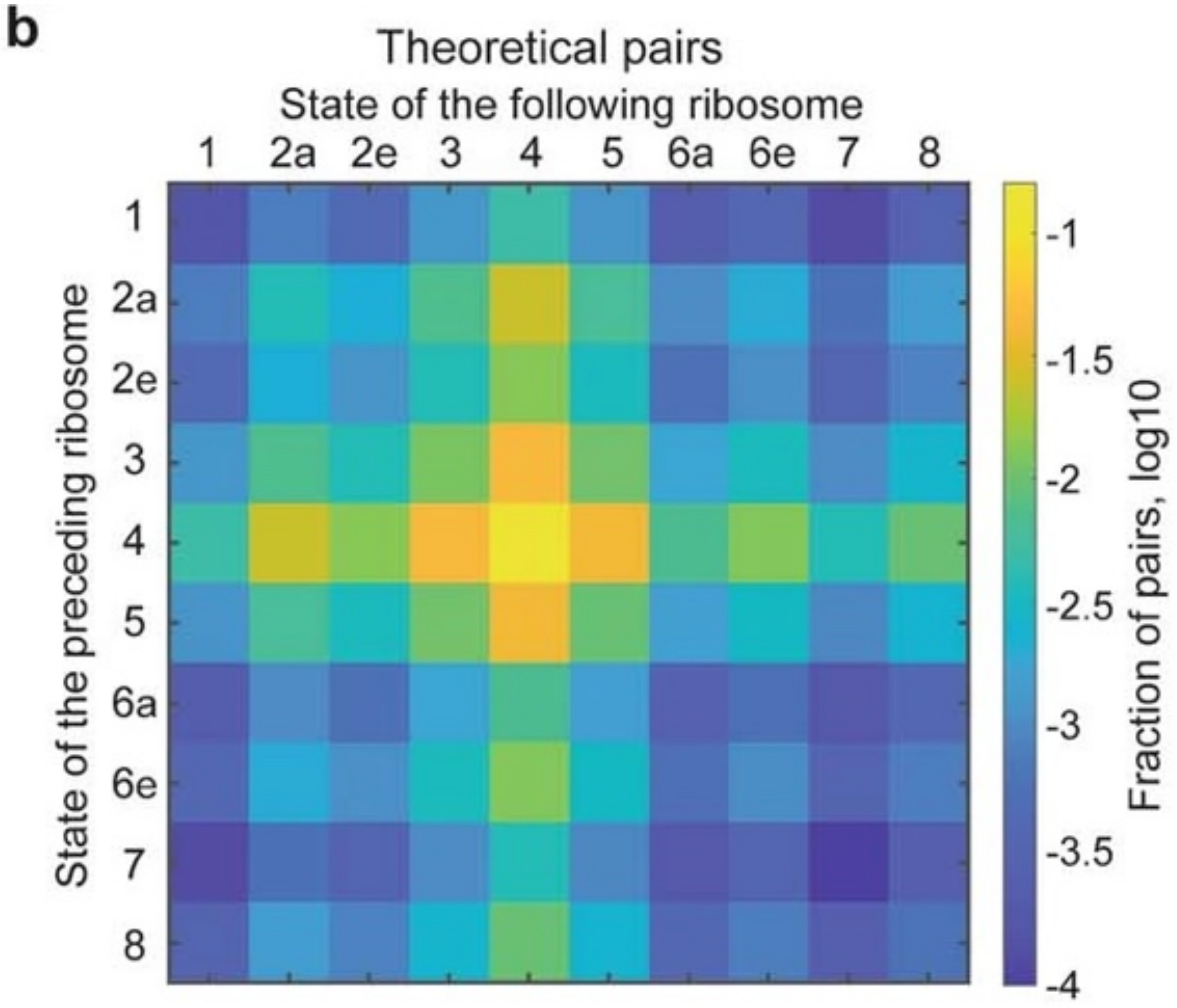


4 Spatial and functional organization of translation

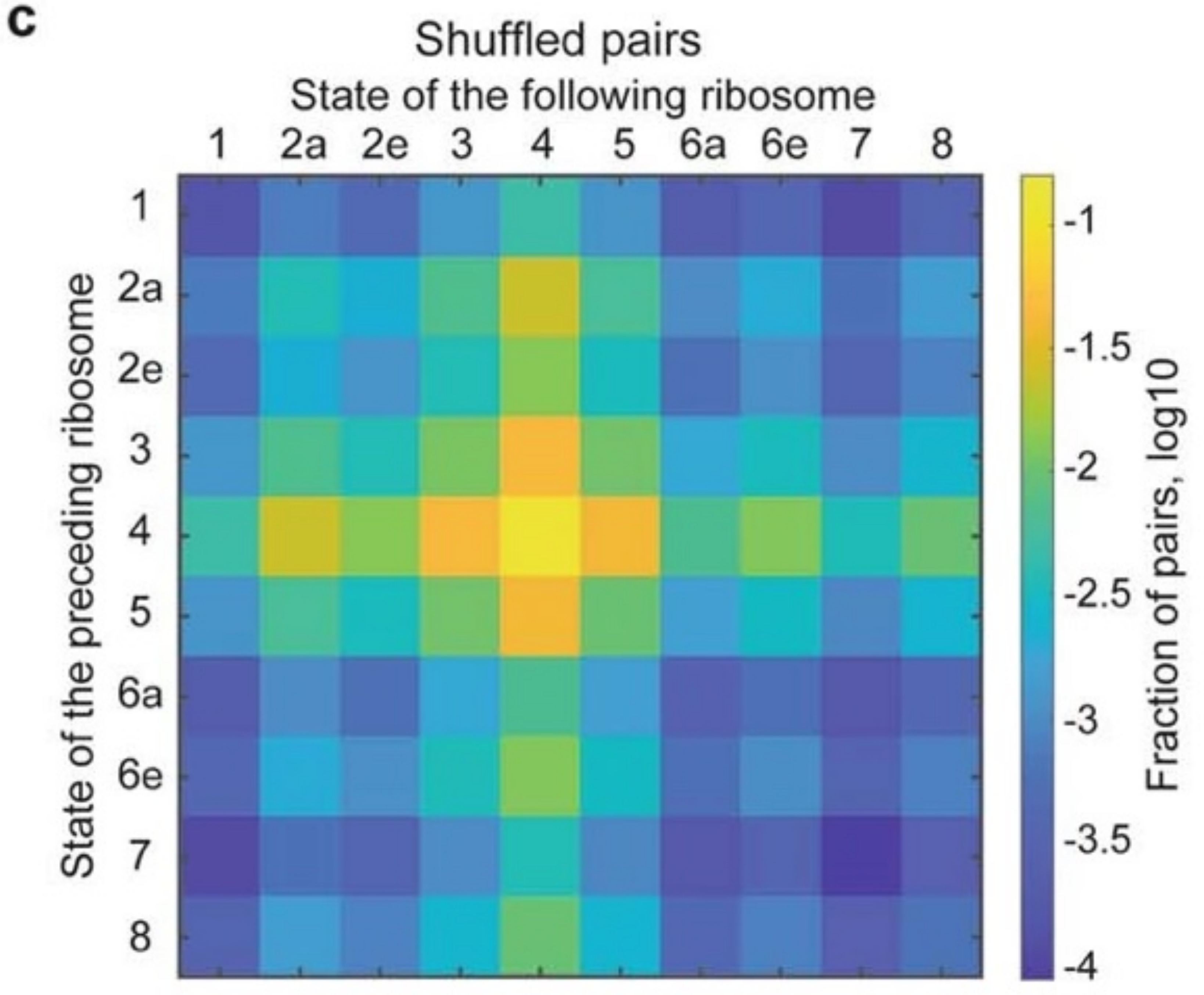
4.2 Local coordination of translation elongation within the polysome



From the experimental data

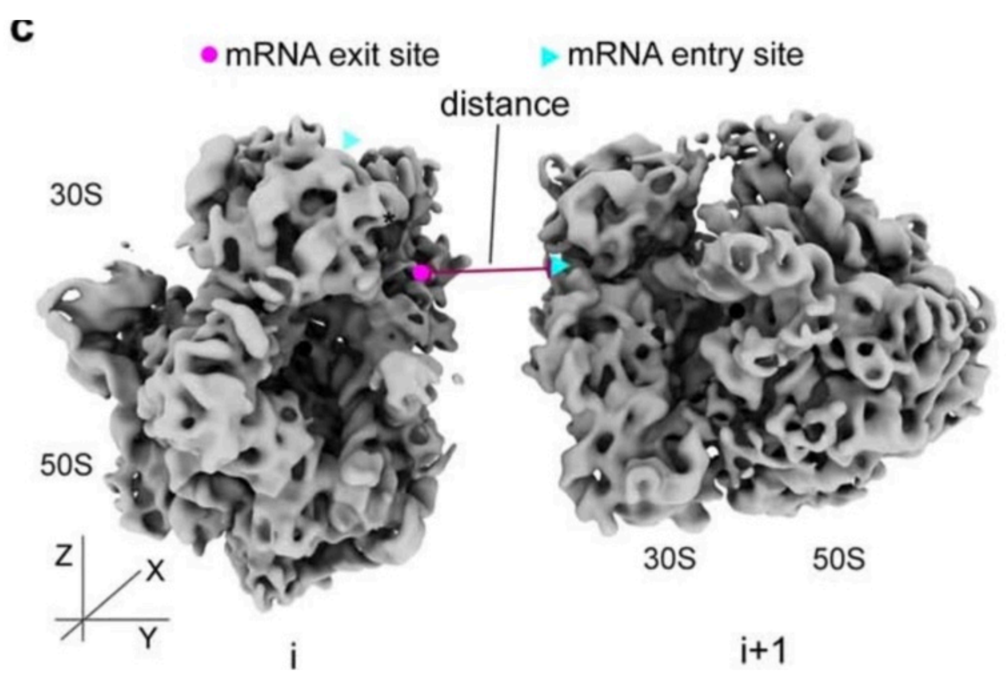


If no cross-influence within the polysome



Random shuffling of the experimental data

preceding ribosome i



following ribosome i+1

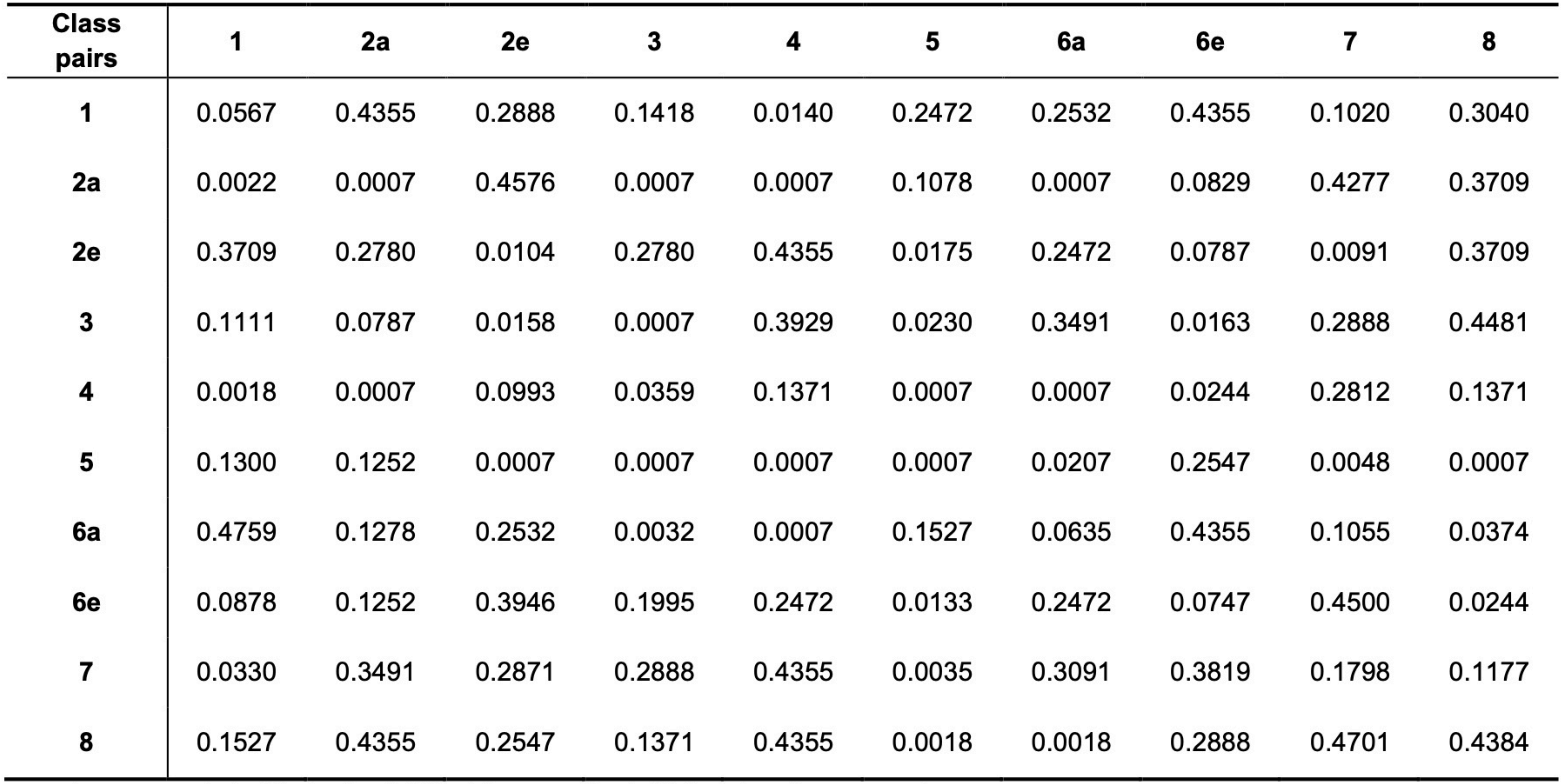
4 Spatial and functional organization of translation

4.2 Local coordination of translation elongation within the polysome

Polysome shuffling analysis using Permutation Test

4 Spatial and functional organization of translation

4.2 Local coordination of translation elongation within the polysome

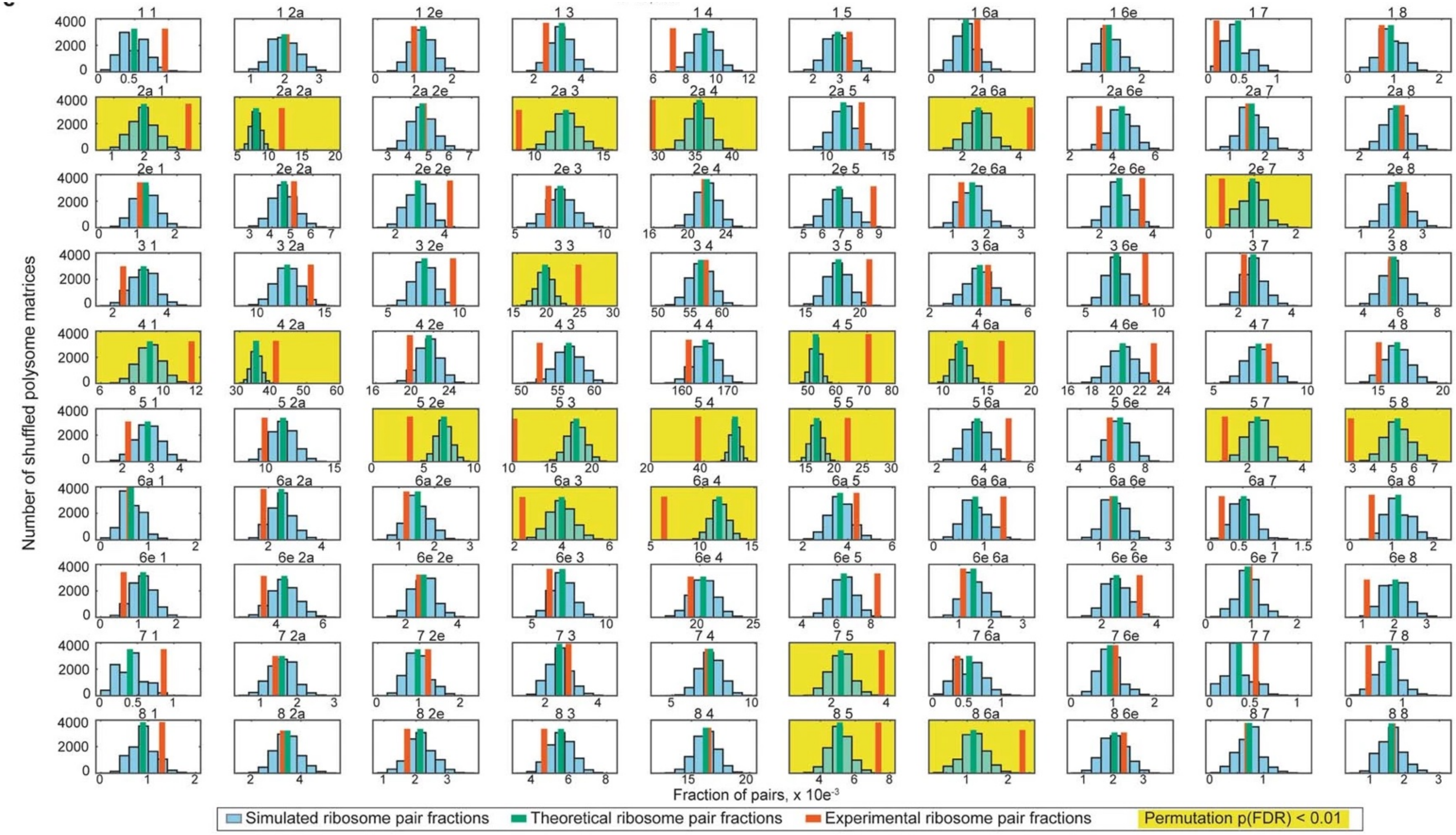


Class pairs	1	2a	2e	3	4	5	6a	6e	7	8
1	0.0567	0.4355	0.2888	0.1418	0.0140	0.2472	0.2532	0.4355	0.1020	0.3040
2a	0.0022	0.0007	0.4576	0.0007	0.0007	0.1078	0.0007	0.0829	0.4277	0.3709
2e	0.3709	0.2780	0.0104	0.2780	0.4355	0.0175	0.2472	0.0787	0.0091	0.3709
3	0.1111	0.0787	0.0158	0.0007	0.3929	0.0230	0.3491	0.0163	0.2888	0.4481
4	0.0018	0.0007	0.0993	0.0359	0.1371	0.0007	0.0007	0.0244	0.2812	0.1371
5	0.1300	0.1252	0.0007	0.0007	0.0007	0.0007	0.0207	0.2547	0.0048	0.0007
6a	0.4759	0.1278	0.2532	0.0032	0.0007	0.1527	0.0635	0.4355	0.1055	0.0374
6e	0.0878	0.1252	0.3946	0.1995	0.2472	0.0133	0.2472	0.0747	0.4500	0.0244
7	0.0330	0.3491	0.2871	0.2888	0.4355	0.0035	0.3091	0.3819	0.1798	0.1177
8	0.1527	0.4355	0.2547	0.1371	0.4355	0.0018	0.0018	0.2888	0.4701	0.4384

p-values corrected with Benjamini-Hochberg method. FDR, false discovery rate.

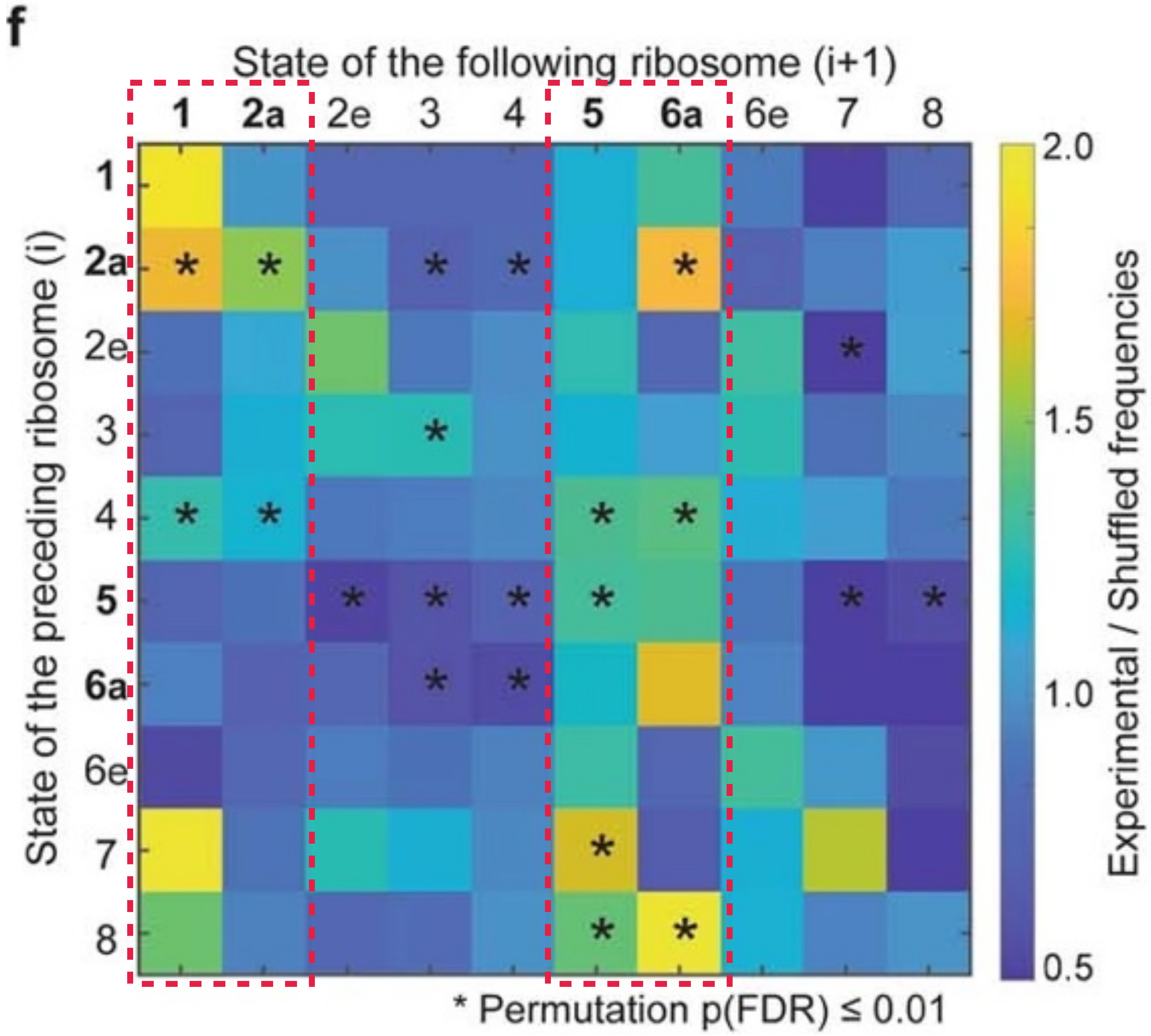
4 Spatial and functional organization of translation

4.2 Local coordination of translation elongation within the polysome



4 Spatial and functional organization of translation

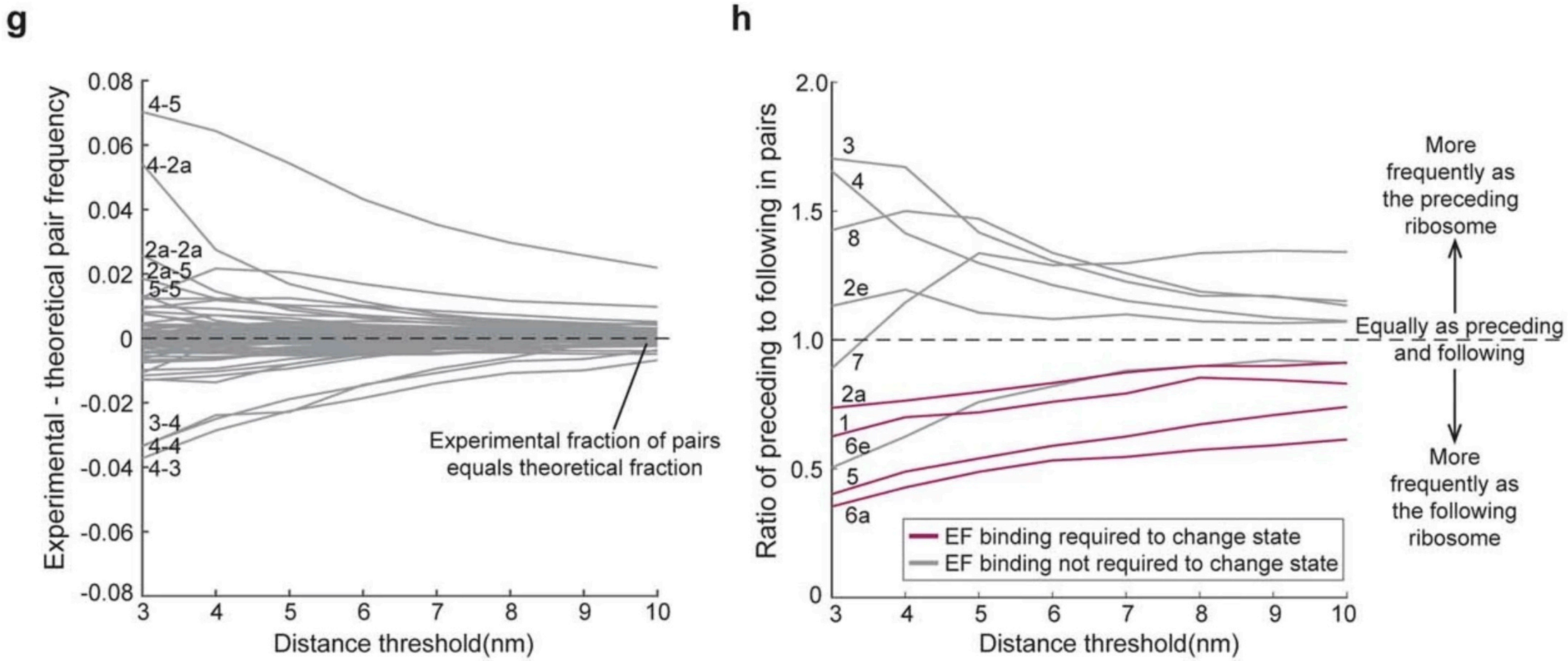
4.2 Local coordination of translation elongation within the polysome



Ribosomes of states that need elongation factor binding to proceed are more frequently engaged as the following ribosomes.

4 Spatial and functional organization of translation

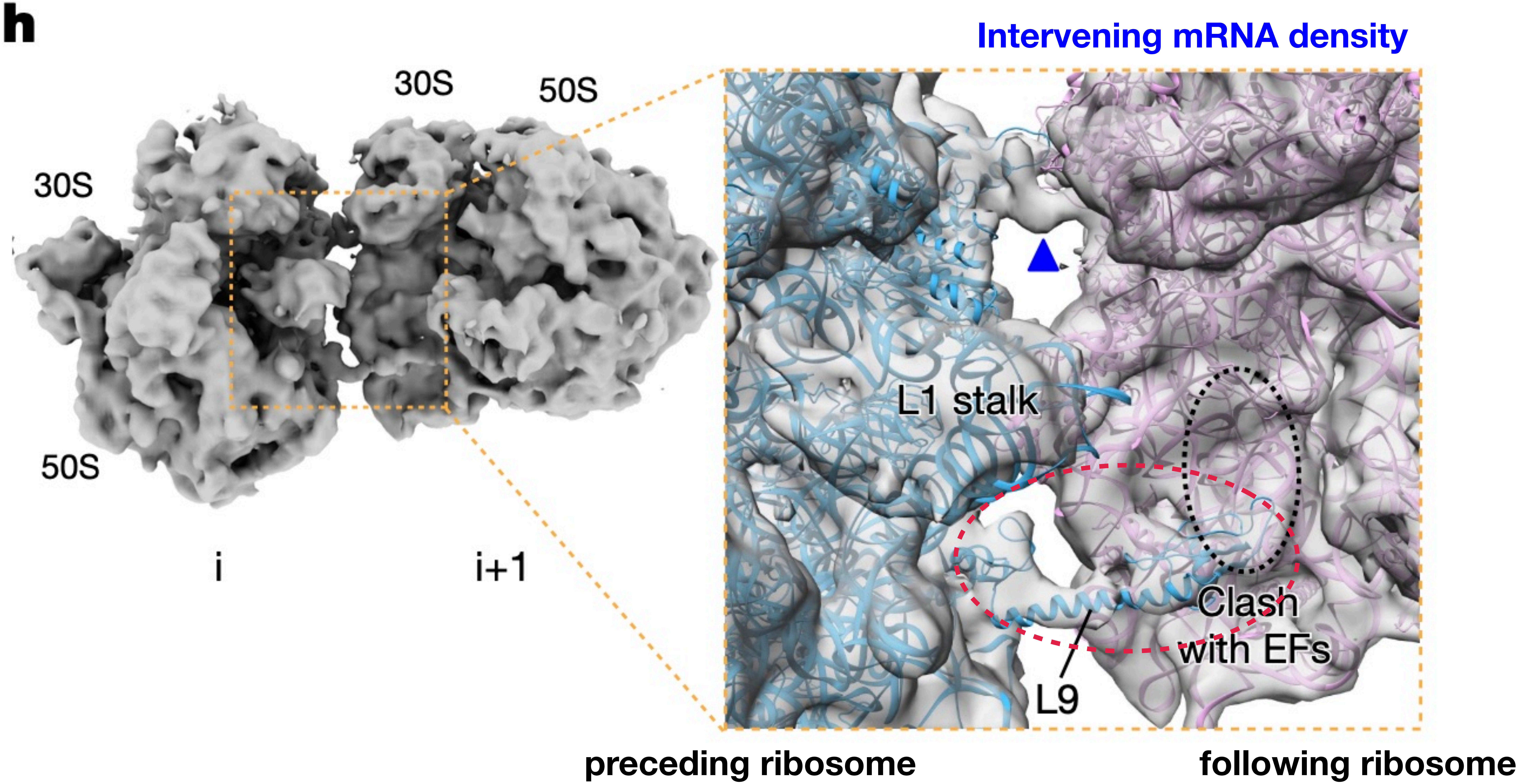
4.2 Local coordination of translation elongation within the polysome



Asymmetry increases as the threshold decreases

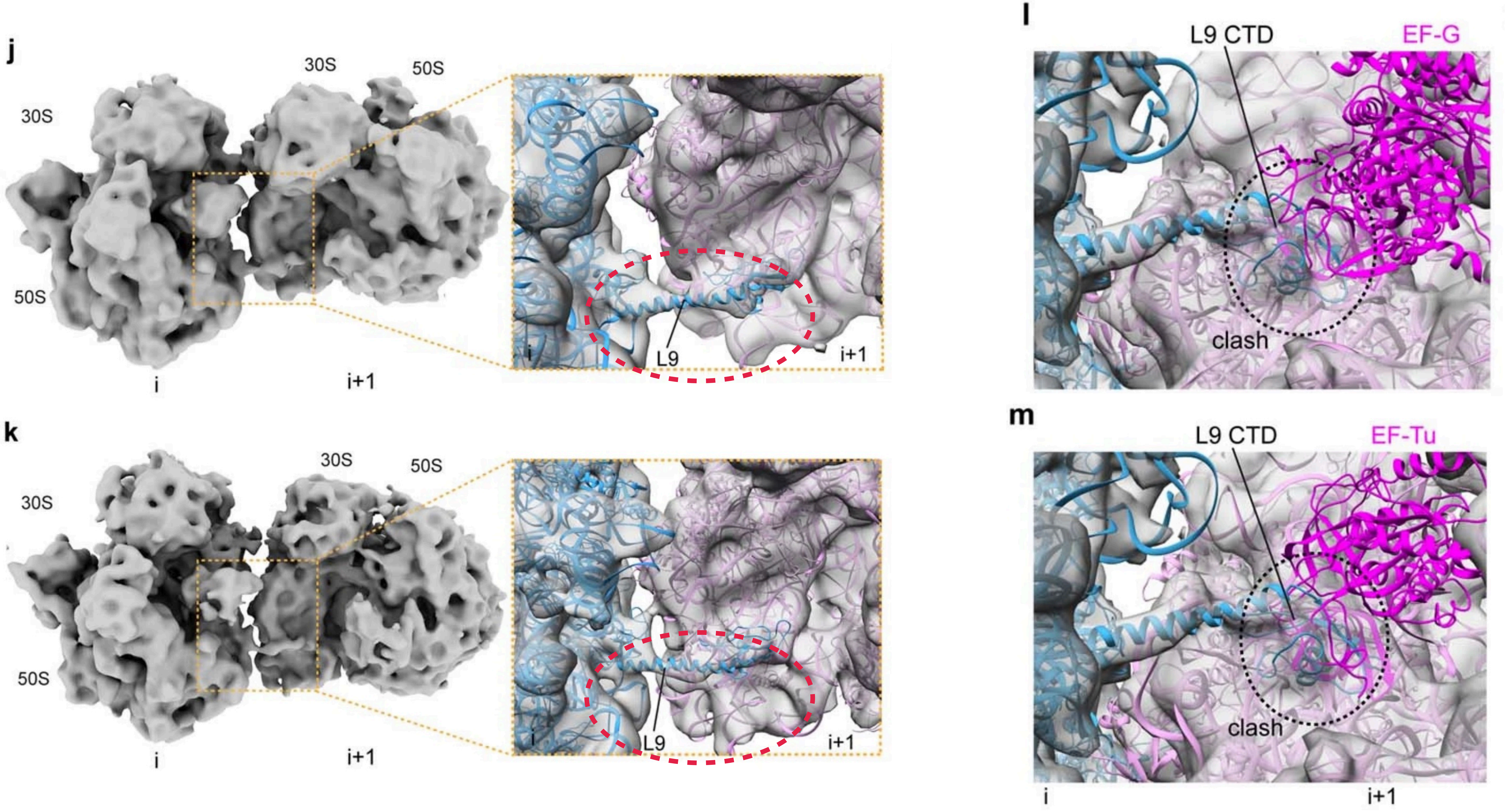
4 Spatial and functional organization of translation

4.3 An extended conformation of L9 sterically interferes with EF

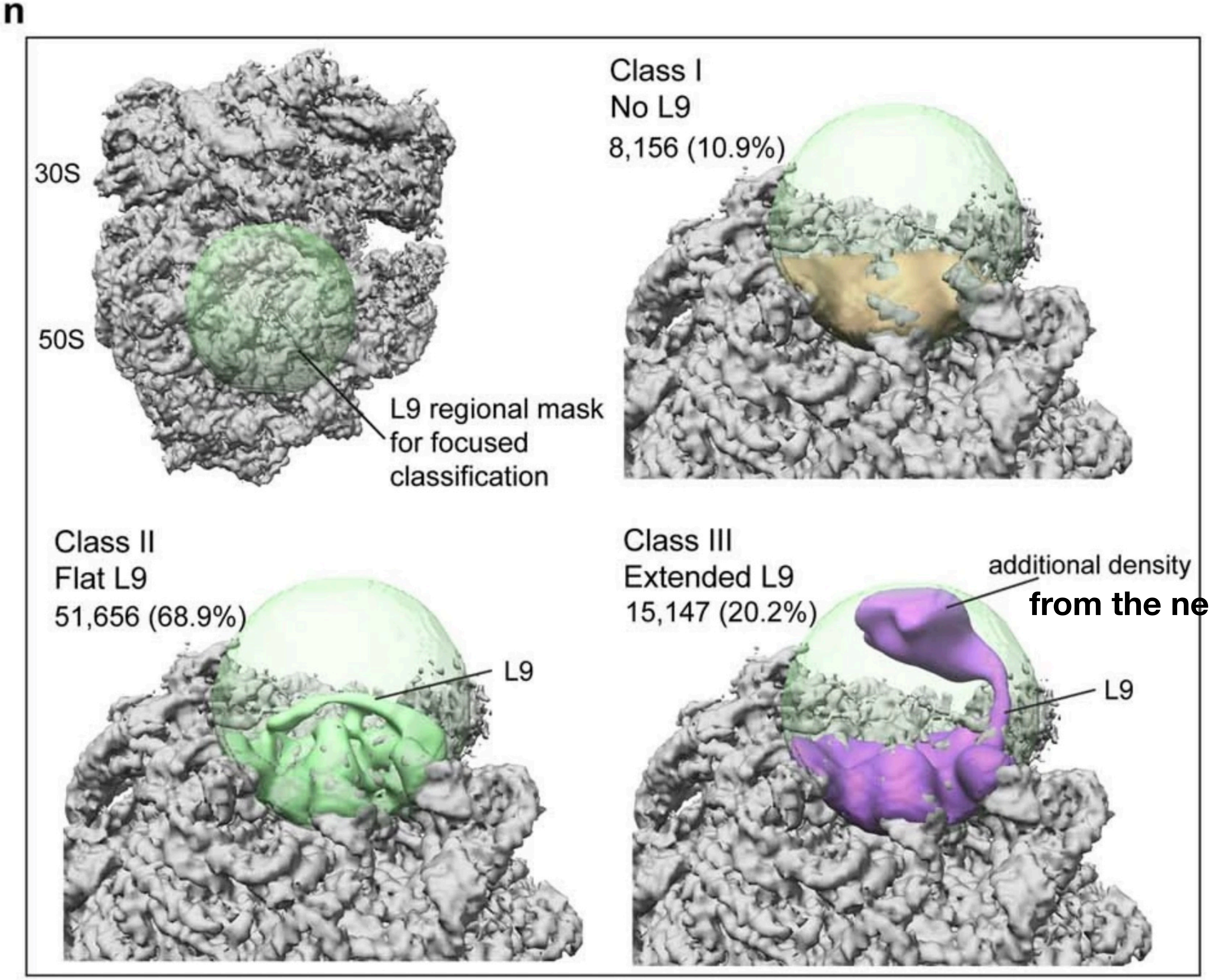


4 Spatial and functional organization of translation

4.3 An extended conformation of L9 sterically interferes with EF



4.3 An extended conformation of L9 sterically interferes with EF

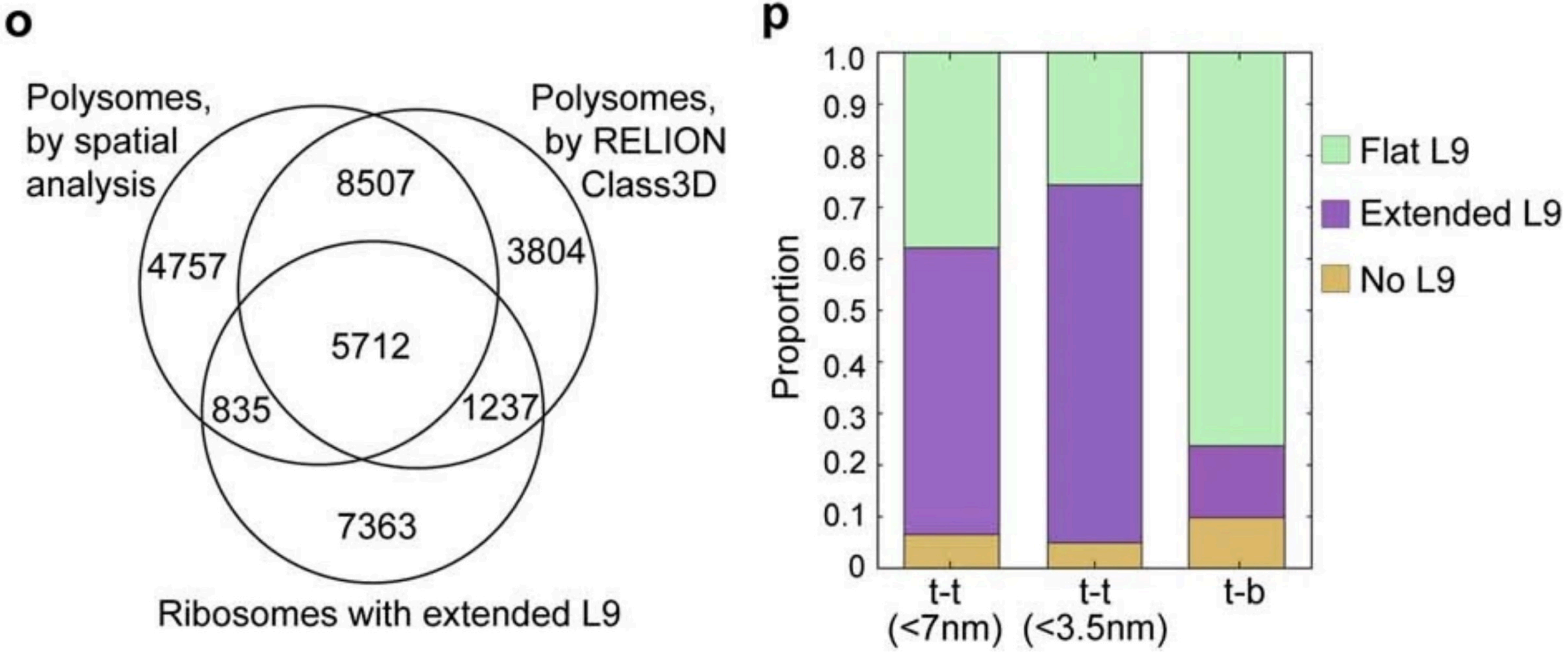


from the neighbouring ribosome

De novo focused classification on the L9 region

4 Spatial and functional organization of translation

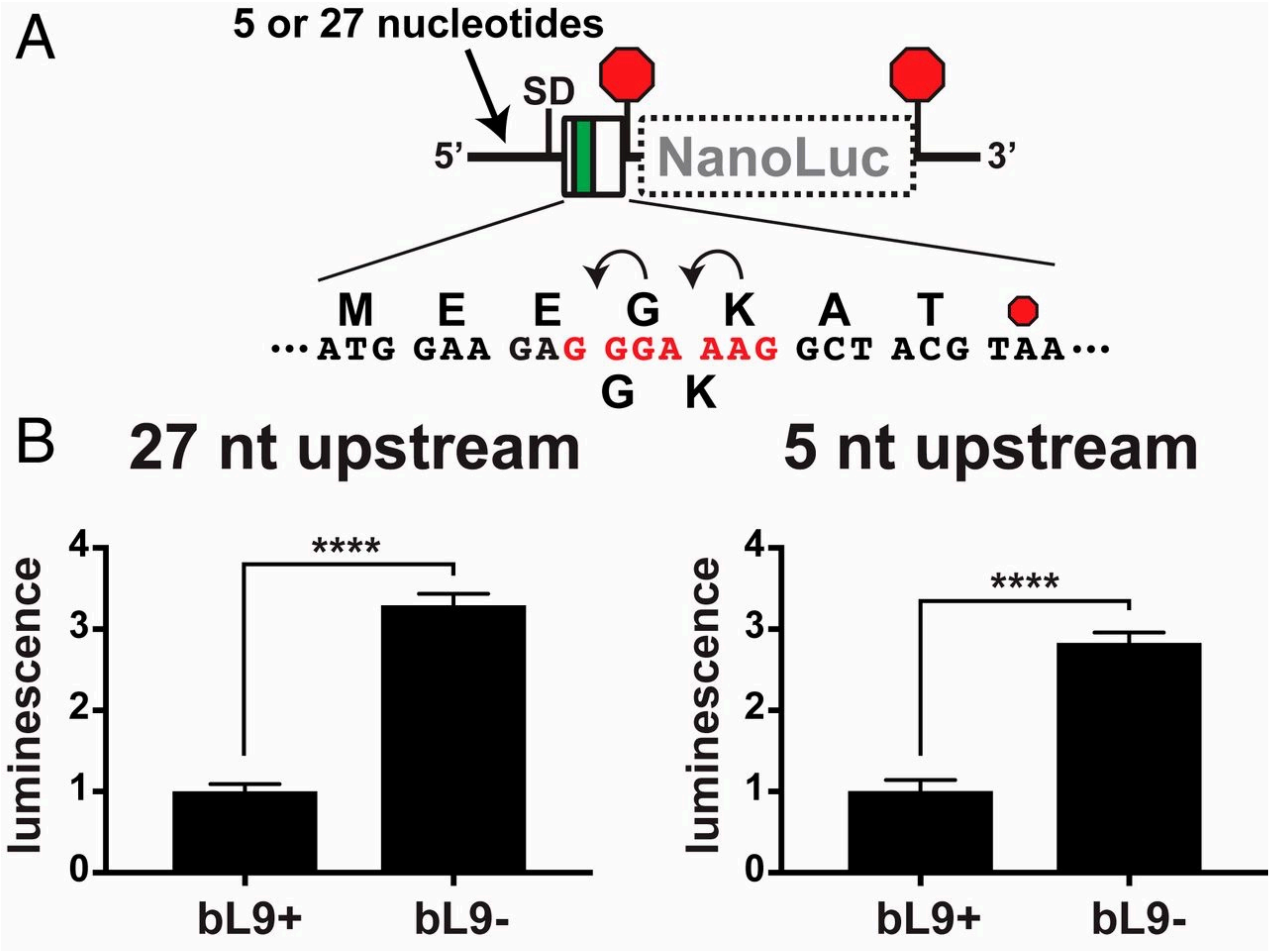
4.3 An extended conformation of L9 sterically interferes with EF



- L9 tends to have a flat conformation in single ribosomes
- L9 tends to adopt the extended conformation within tightly assembled polysomes

4 Spatial and functional organization of translation

4.3 An extended conformation of L9 sterically interferes with EF



L9's mutations cause increased frame shifting and ribosome compaction

(Smith et al., 2019)

Discussion

Summary

Visualize structure dynamics of translation inside *Mycoplasma pneumoniae* at atomic detail using cryo-ET and STA

Obtain a hi-res in-cell average map of all translating ribosomes

Build an atomic model and reveal distinct extensions

Classification resolves 13 major states and intermediates during translation

Animate translation elongation inside native cells

Show how antibiotics reshape the cellular translation landscapes

Map the organization of translating ribosomes and their association into polysomes

Extended ribosomal protein L9 facilitate translation fidelity