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Journal club: Ramrath et al, 2013

C9940 3-Dimensional Transmission Electron Microscopy
S1007 Doing structural biology with the electron microscope

May 11, 2015

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EUROPEAN REGIONAL DEVELOPMENT FUND
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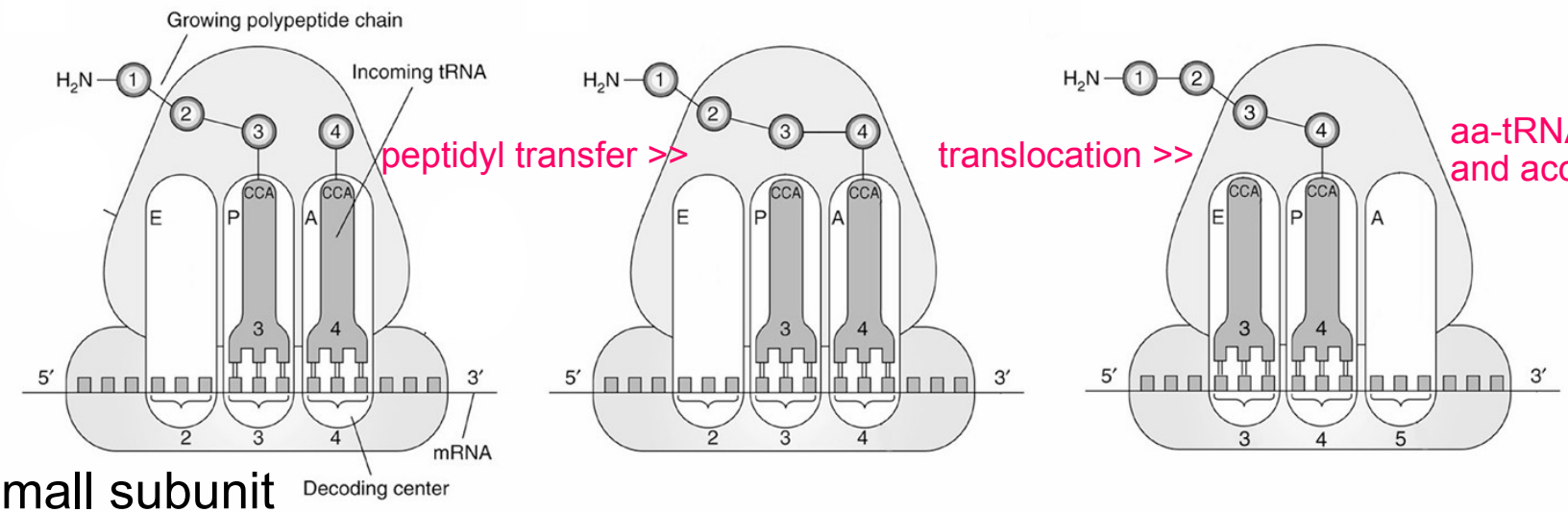
2007–13
OP Research and
Development for Innovation

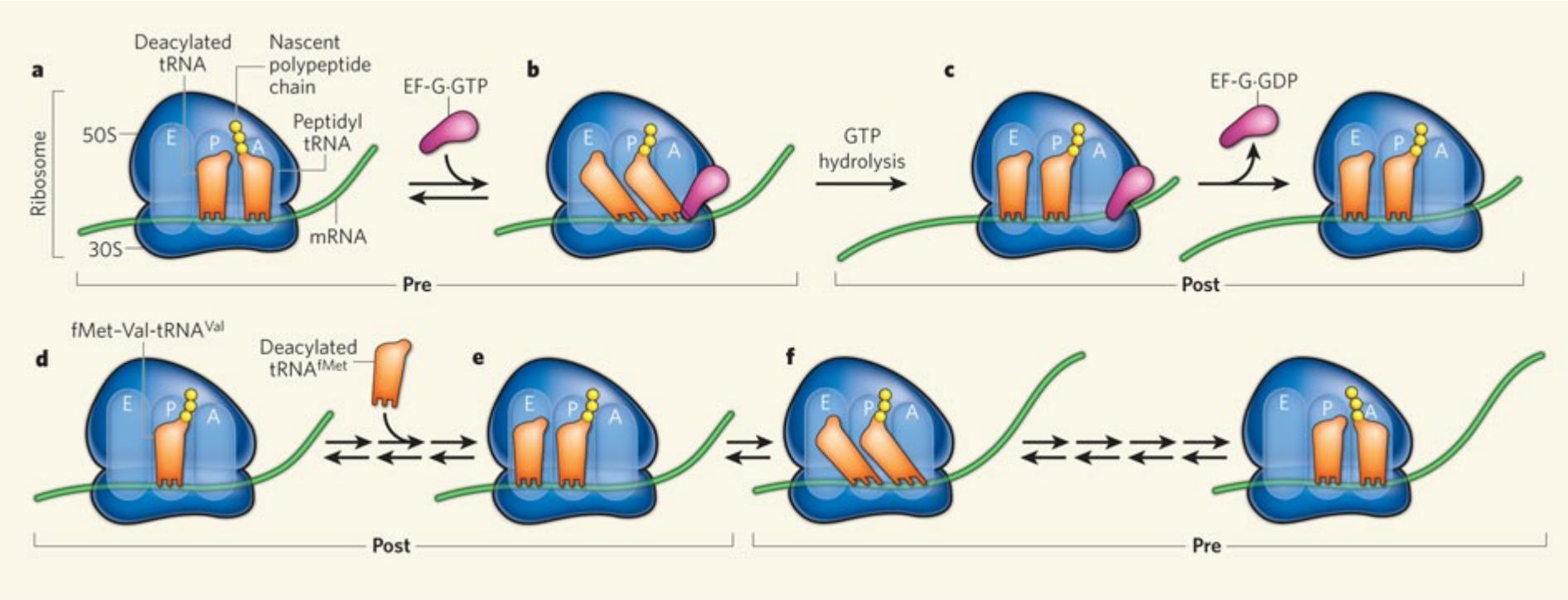
Elongation of the polypeptide chain by one amino acid

Large subunit

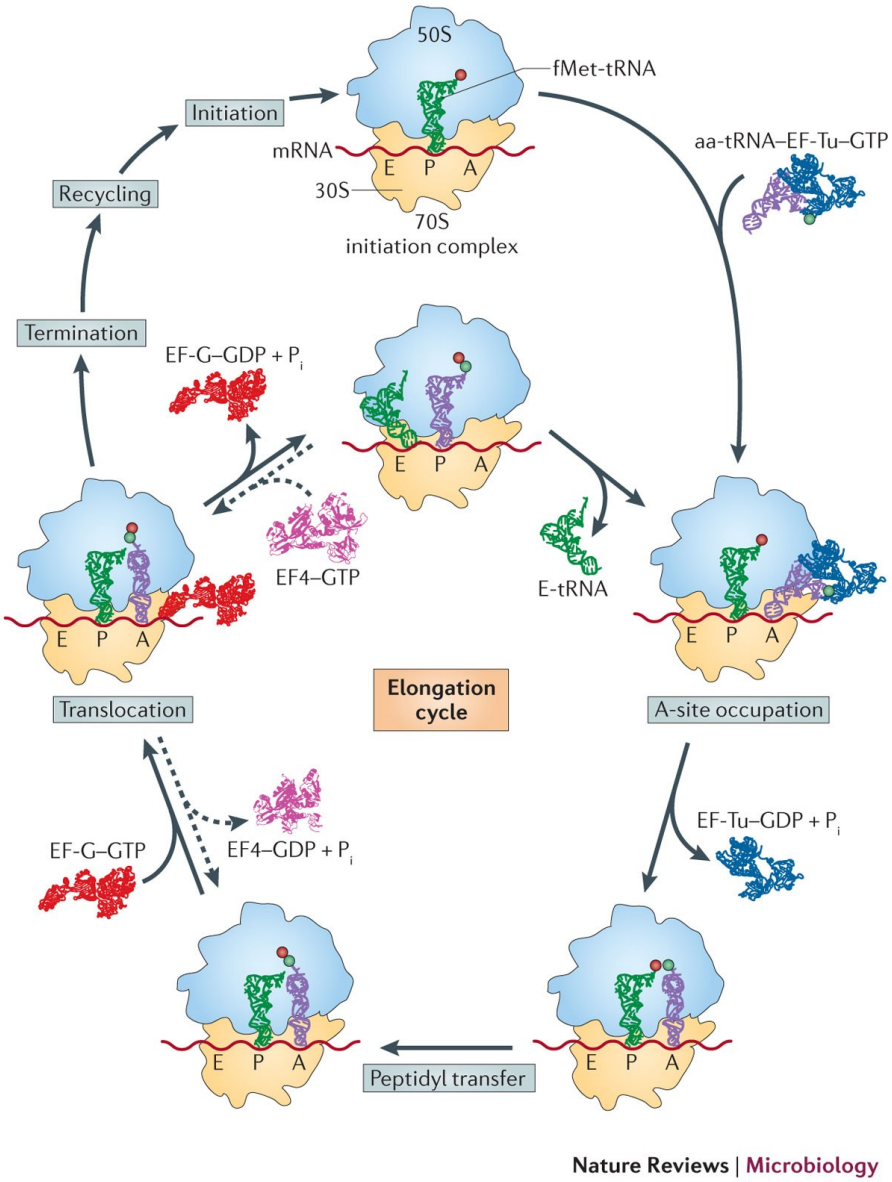


Small subunit

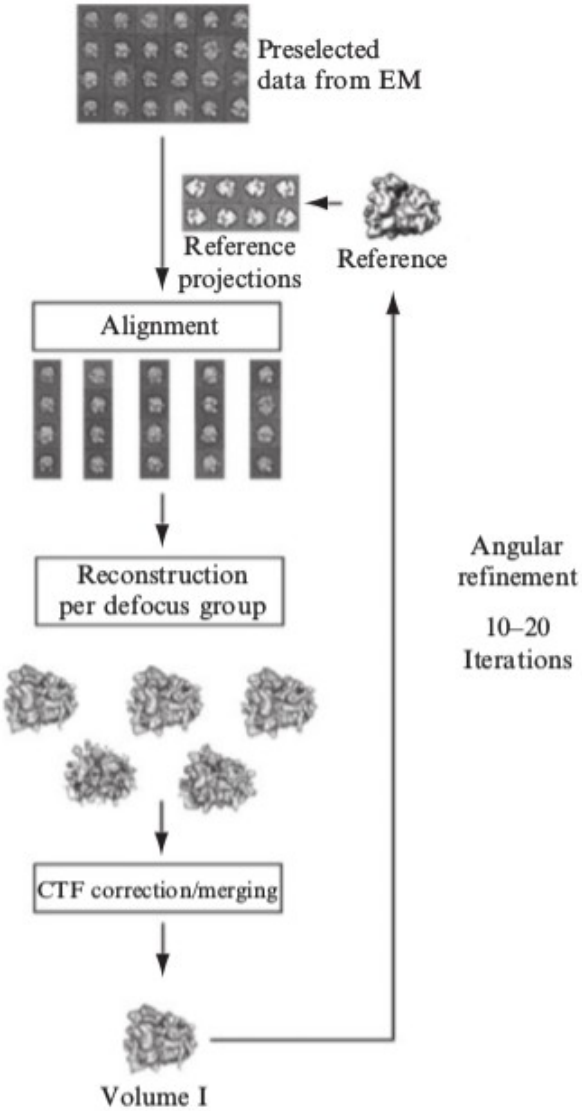
Adapted from Joachim Frank



Ehrenberg, Nature, Vol. 466
Figure 1



Yamamoto et al., (2014) Nature Microbiology, Vol12
Figure 2



"Normal" reconstruction
and refinement of orientations

Justus Loerke et al. (2010) Methods in Enzymology, Volume 483
Figure 2

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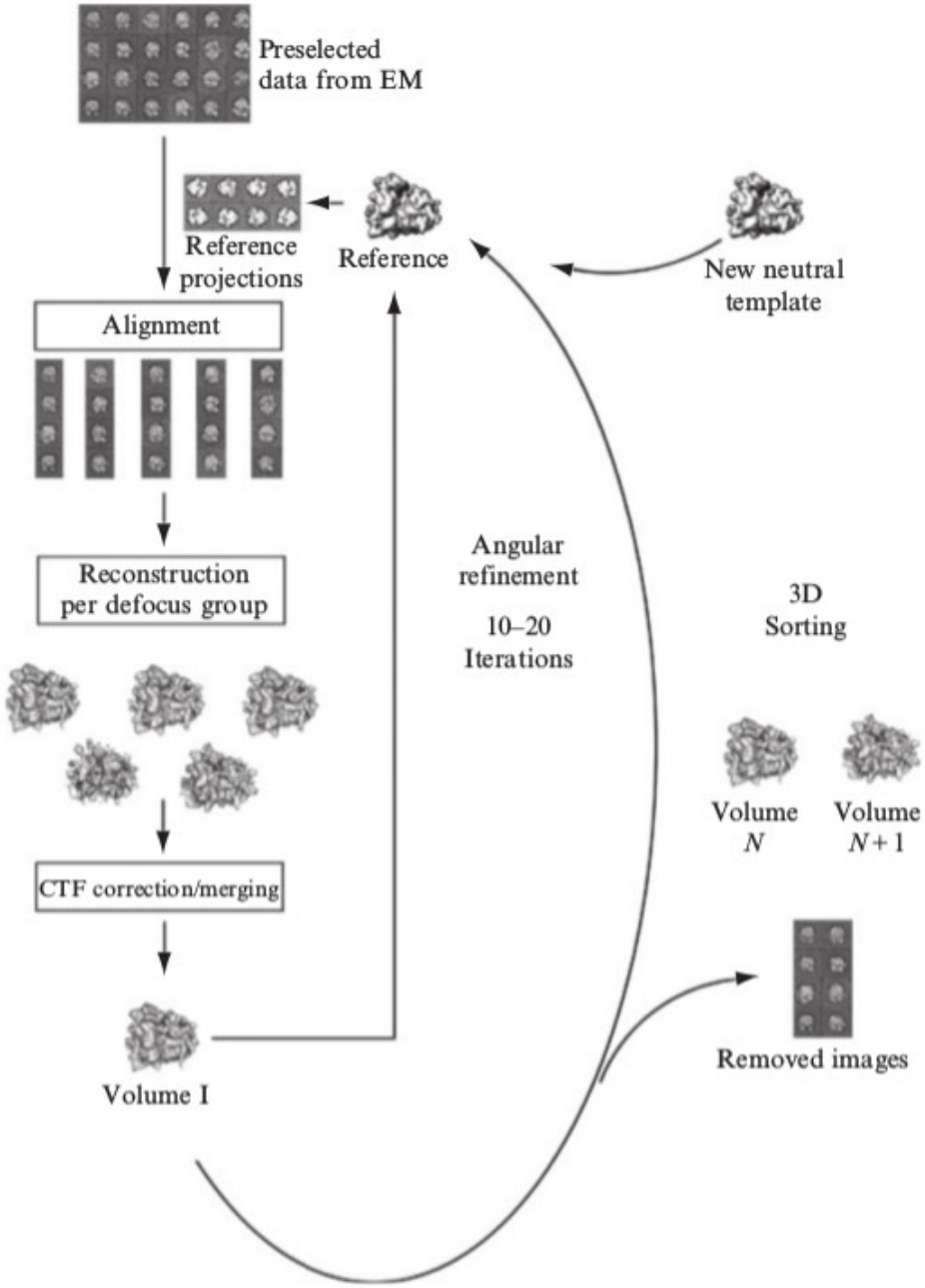

Justus Loerke et al. (2010) Methods in Enzymology, Volume 483
Figure 2

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Ramrath et al, 2013

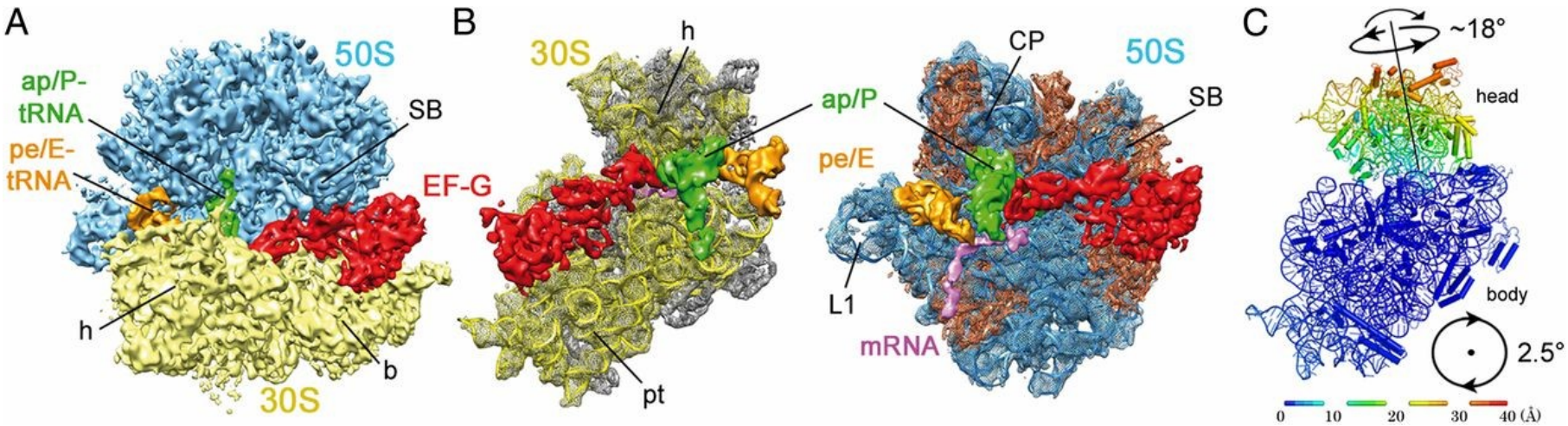
Question:

What's special about this complex?

Abbreviations used

- ASL: anticodon stem loop
- ASF: A-site finger (Helix H38)

Overview of the canonical TIPOSTcomplex.



David J. F. Ramrath et al. PNAS 2013;110:20964-20969

Fig. 1. Overview of the canonical TI POST complex. (A and B) Cryo-EM density of the ligands ap/P- and pe/E-tRNAs (green and orange), mRNA (magenta), and EF-G (red) in surface representation within the 70S complex [50S (blue) and 30S (yellow)] (A) or with a mesh representation of both subunits together with docked models of the 30S [16S (yellow) and S-proteins (gray)] and 50S [23S and 5S (blue) and L-proteins (orange)] (B). (C) View from the intersubunit space onto the 30S subunit in the presented authentic TI POST complex. Elements are colored according to their structural displacement compared with the classic conformation (30) upon a common 50S alignment.

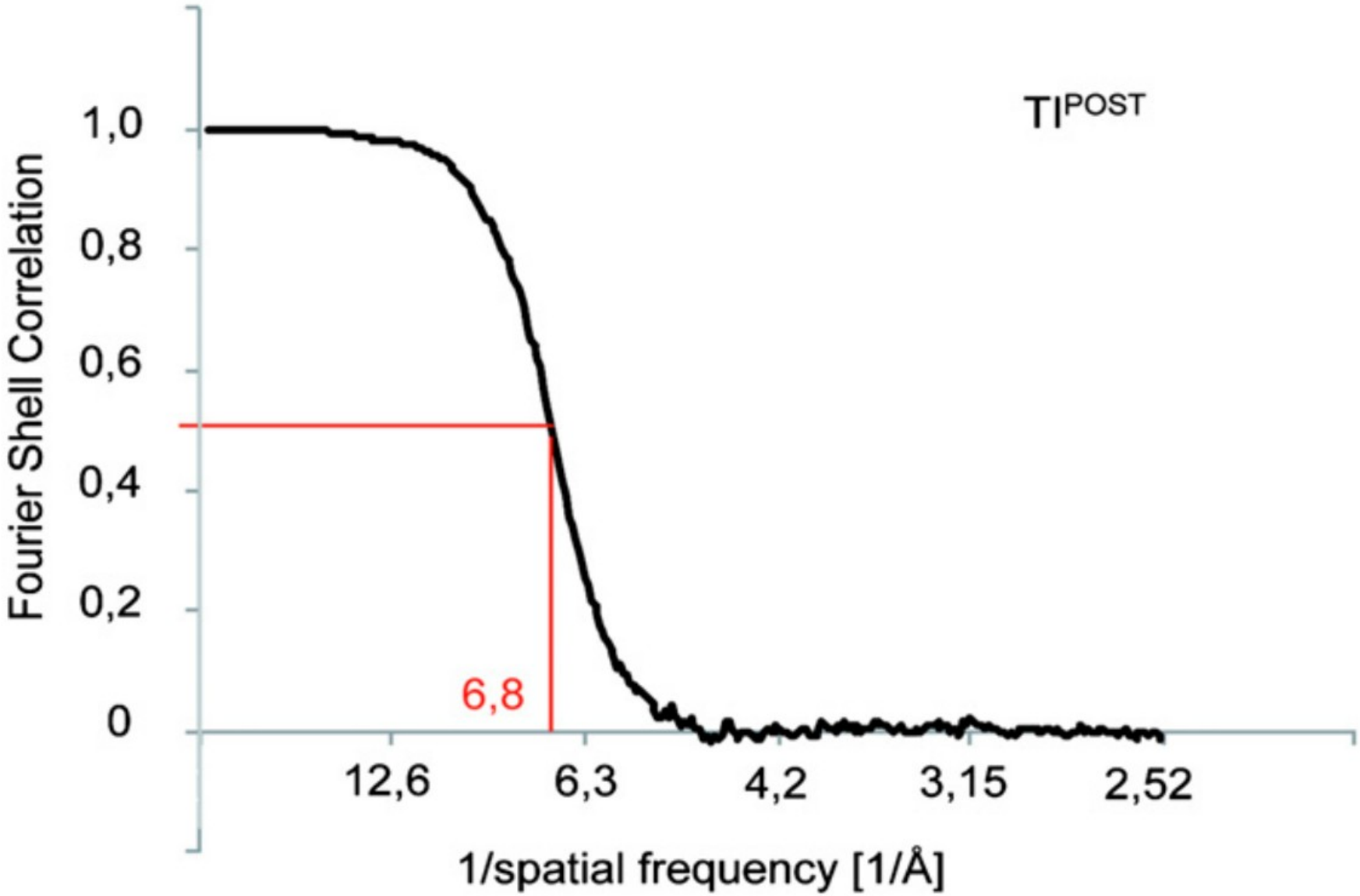


Fig. S1.
Resolution curves of the authentic TI POST . According to the 0.5 FSC cut-off, the resolution of the authentic TI POST complex is 6.8 Å.

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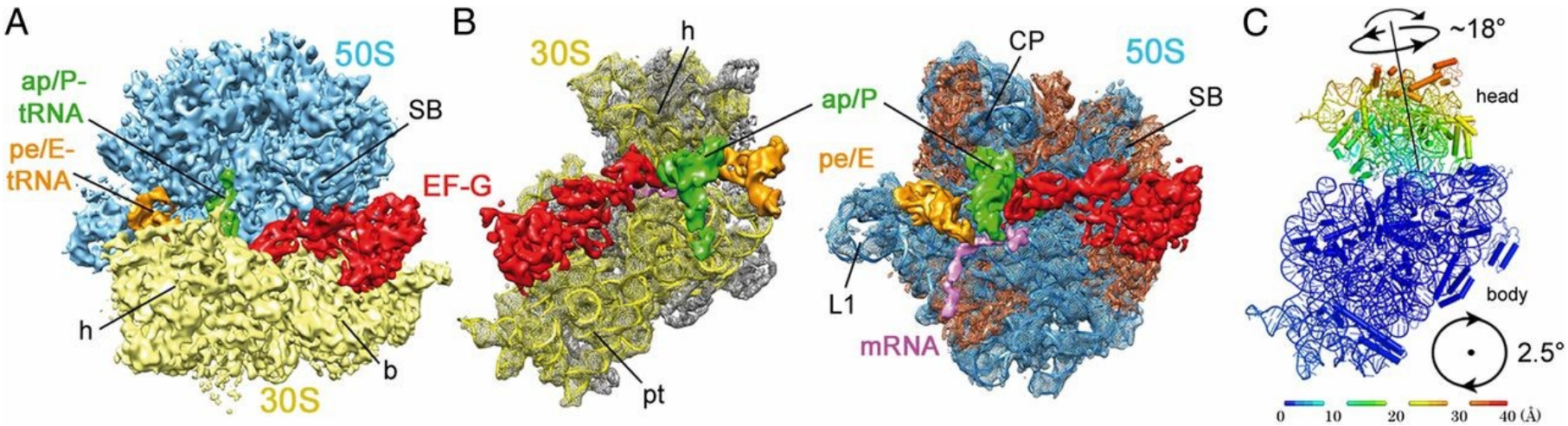
Overview of the canonical TIPOSTcomplex.



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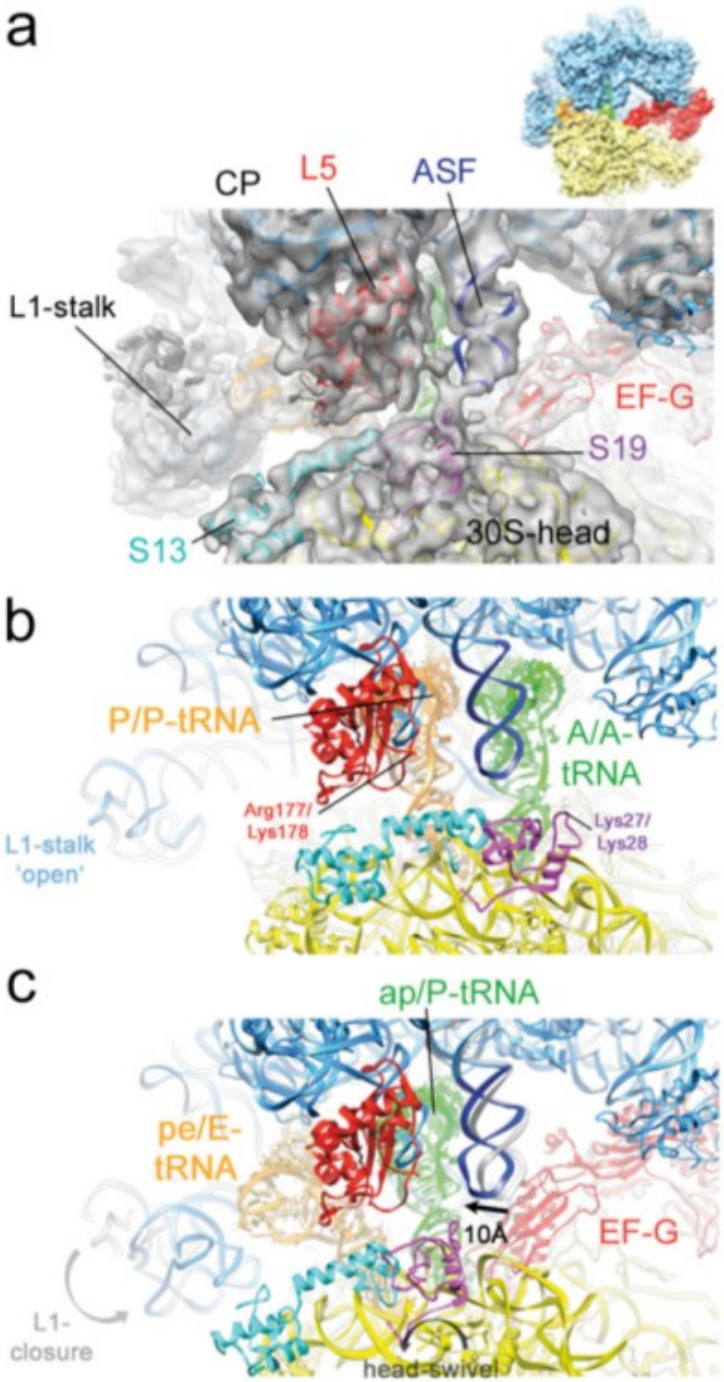

Fig. S2. Formation of a unique intersubunit bridge. (A) Cryo-EM density of the authentic TI POST shown as gray transparent surface with docked models of the ribosome [30S (yellow), 50S (blue), S13 (cyan), S19 (magenta)], EF-G (red), and tRNAs [ap/P-tRNA (green), pe/E-tRNA (orange)]. The map displays a continuous density between L5 (red), the ASF (dark blue), and S19 (magenta). (B) Cartoon representation of the ribosomal conformation in the classic unrotated classic pretranslocational state (15) with an opened L1-stalk indicating the contact between the ASF and S13. (C) Cartoon representation of the authentic TI POST indicating the structural changes of the 30S-head, ASF, and L1-stalk. The positions of L1-stalk and ASF as shown in B are displayed in transparent gray.

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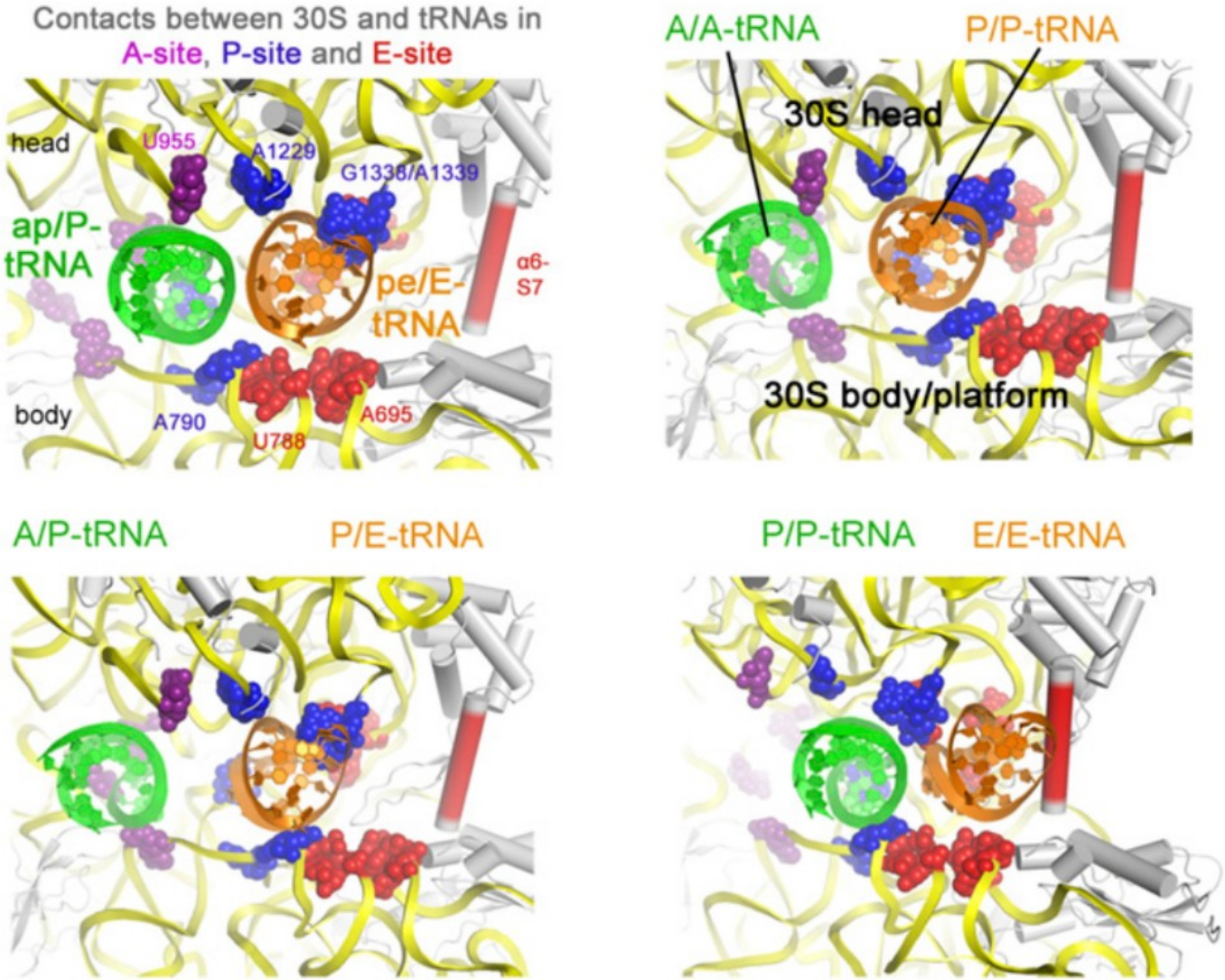

Fig. S3. Interactions of the 30S subunit and different bound tRNAs. Ribbon representation of the tRNA binding region on the 30S subunit. Residues that contact tRNAs in the A (magenta), P (blue), and E site (red) (25) are shown as spheres together with two tRNAs in chimeric intrasubunit (ap/P and pe/E), classic (A/A andP/P), intersubunit hybrid (A/P and P/E), or classic posttranslcoated (P/P and E/E) tRNA binding sites (14, 15).

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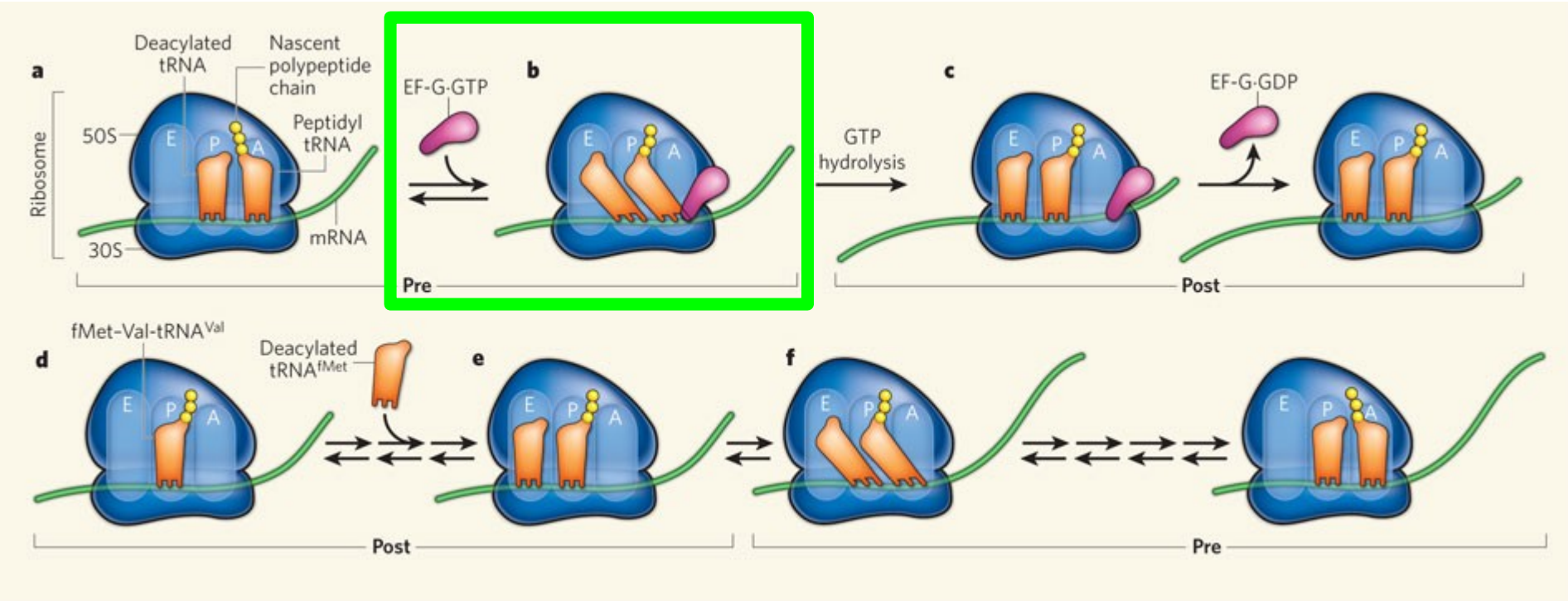
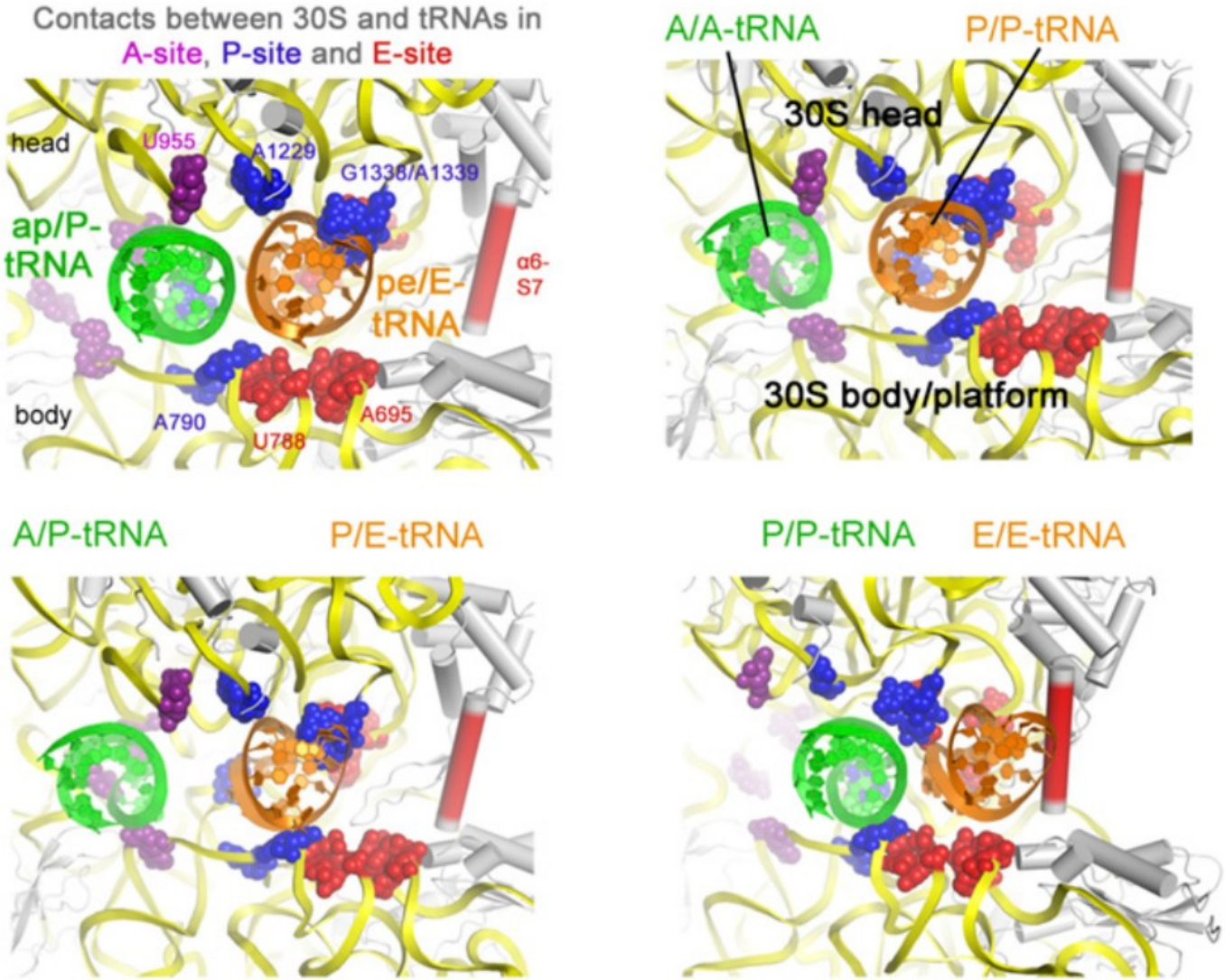


Ehrenberg, Nature, Vol. 466
Figure 1



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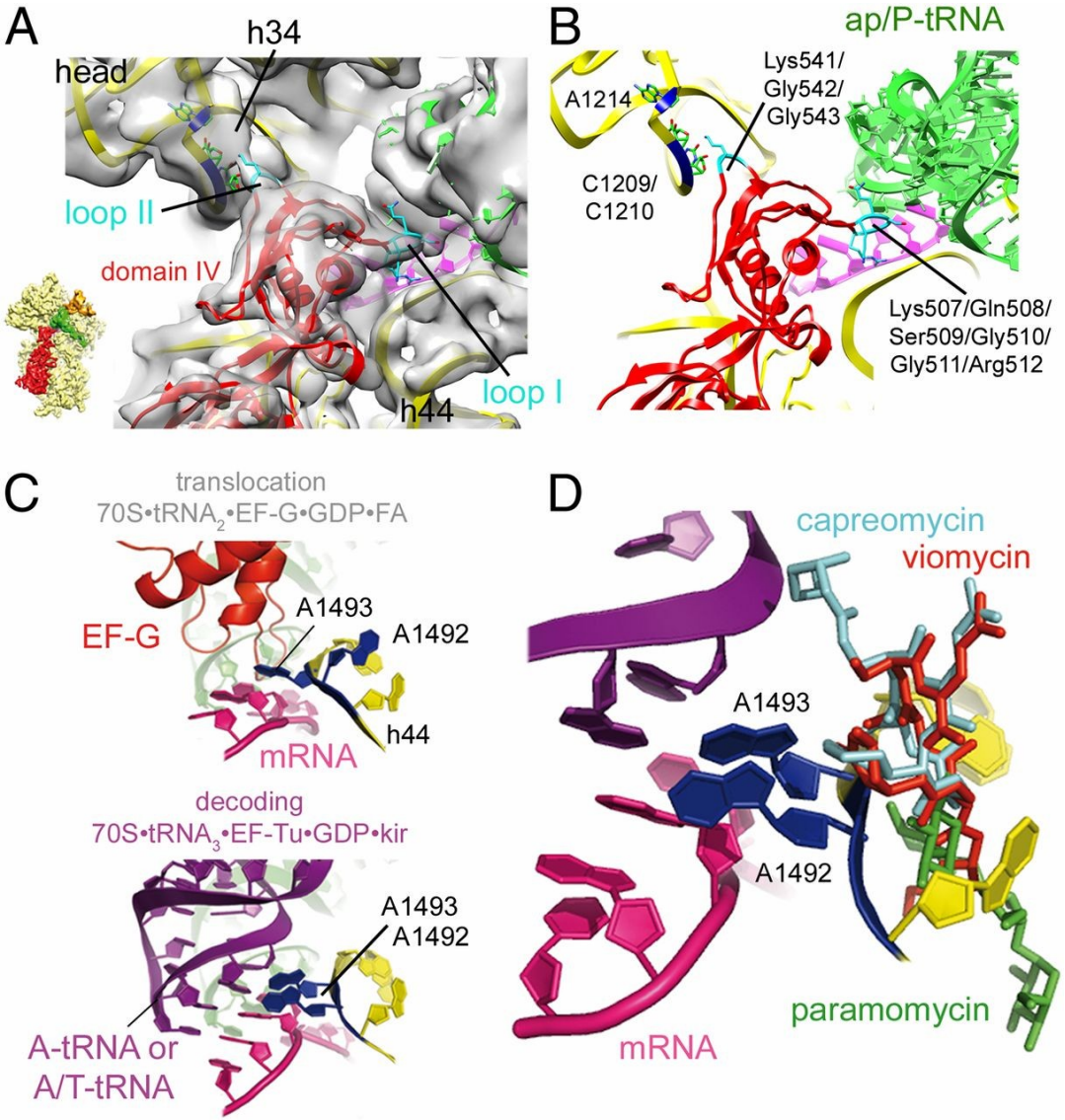
Contacts of EF-G domain IV. (A) Cryo-EM density of the presented TIPOST complex shown as transparent gray surface together with docked models of the 30S, mRNA, ap/P-tRNA, and EF-G (colored as in Fig. 1) in the indicated view (Left).



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Ligand positions within the authentic TIPOSTcomplex.

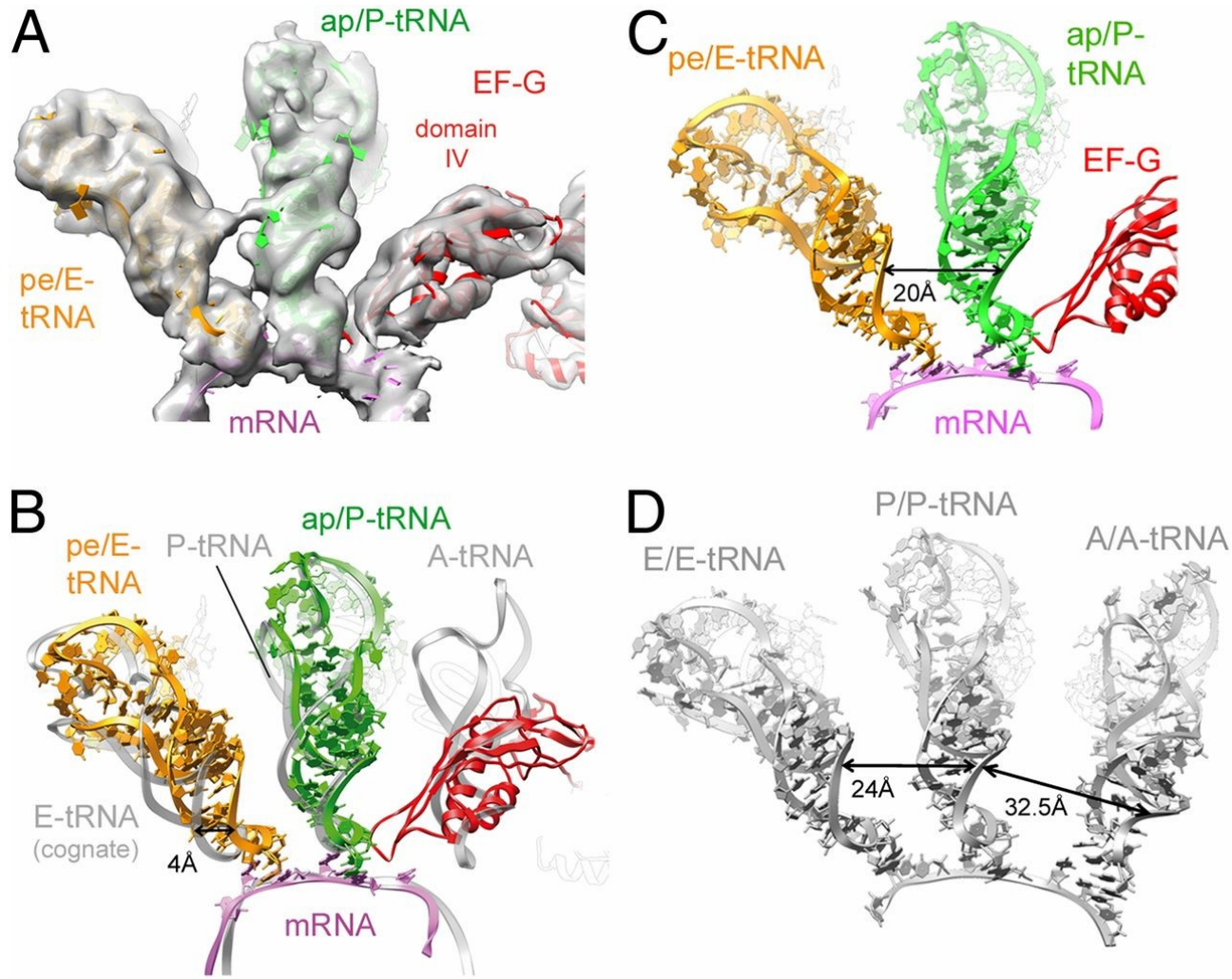

Fig. 3. Ligand positions within the authentic TI POST complex. (A) Cryo-EM density for the ligands within the presented authentic TI POST complex in a transparent surface representation together with docked models of EF-G (red), ap/P-tRNA (green), pe/E-tRNA (orange), and mRNA (magenta). (B) Superimposition of the ligands (as shown in A) together with tRNAs bound to classic A, P, and E sites (transparent gray) (23) upon a 50S alignment. (C and D) Ribbon representation of the ribosomal ligands within the present authentic TI POST complex (C), and an unrotated ribosomal complex with classic A, P, and E-tRNAs (23) (D). Highlighted are the distances between the last base pair within ASL of bound tRNAs.

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Ribosome–tRNA interactions.

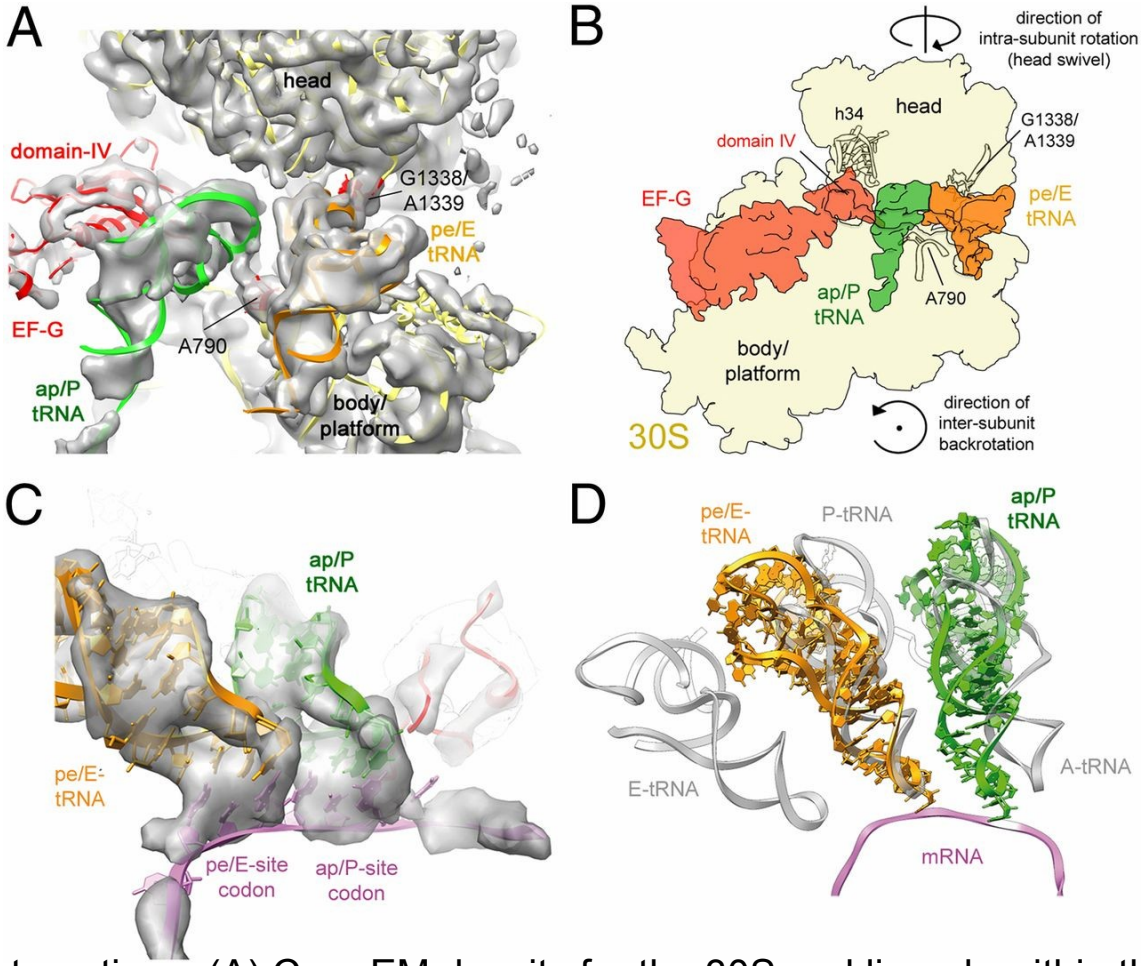

Fig. 4. Ribosome–tRNA interactions. (A) Cryo-EM density for the 30S and ligands within the presented authentic TI POST complex in a transparent gray surface representation together with docked models of the 30S (yellow), ap/P-tRNA (green), pe/E-tRNA (orange), and EF-G (red). (B) Schematic of the 30Ssubunit and bound ligands [EF-G (red), ap/P-tRNA (green) and pe/E-tRNA (orange)] from the presented authentic TI POST . (C) Cryo-EM density of the ligands within the presented authentic TI POST (transparent gray surface) together with docked models of EF-G, pe/E-tRNA, ap/P-tRNA, and mRNA (colored as in A). (D) Superimposition of the ligands from the present authentic TI POST complex with either tRNAs in classic A, P, and E-site (23) (transparent gray) upon a 30S head alignment.

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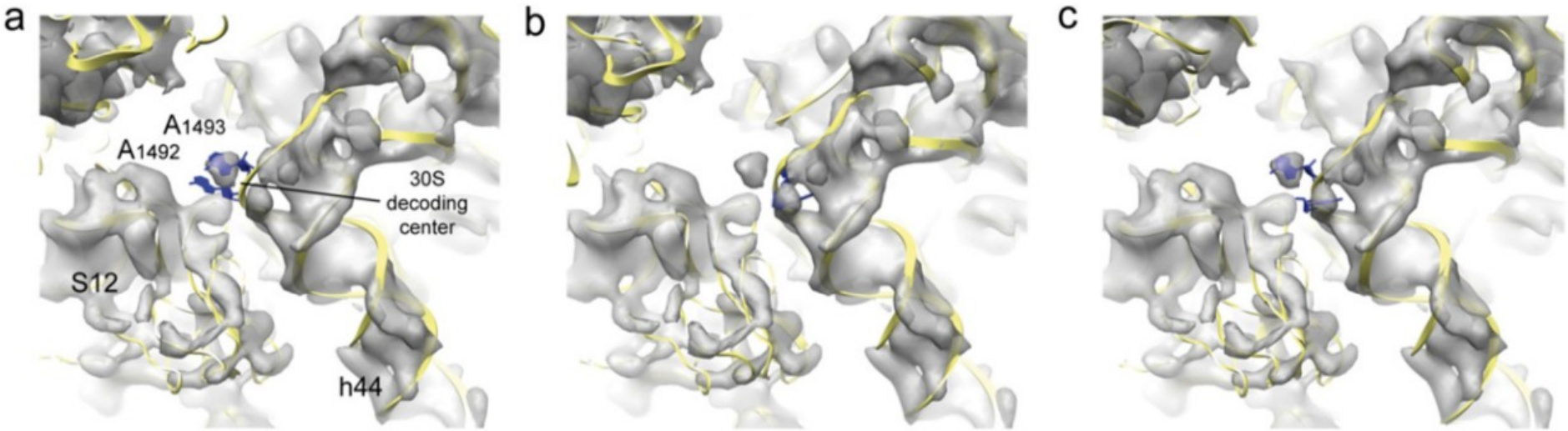

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Supplemental Figure 4

Thank you for your attention

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