Supplemental Data

Visualization of the Hybrid State

of tRNA Binding Promoted

by Spontaneous Ratcheting of the Ribosome

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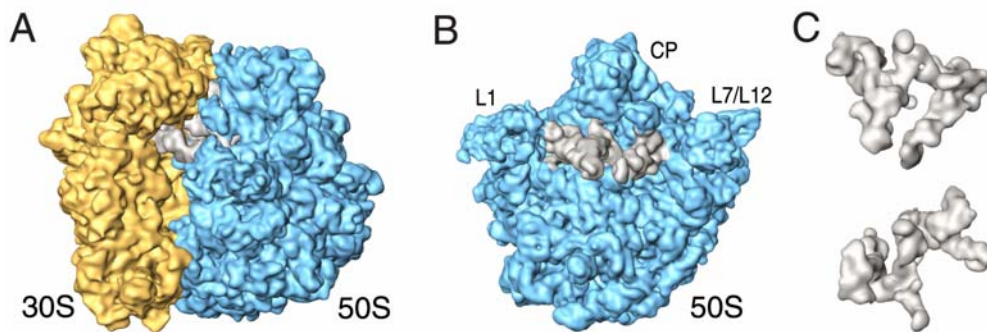Rachel Green, and Joachim Frank

Figure S1: *Initial reconstruction.* Cryo-EM reconstruction at 8.75 Å resolution obtained using the whole data set (216,517 particles). (A) 70S ribosome. Cryo-EM densities are shown for the small (yellow) and large (blue) subunits, and tRNAs (grey). (B) 50S subunit shown from the 30S subunit side. (C) Density corresponding to tRNAs. The viewpoints correspond to the top (upper panel) and back (lower panel) views of that shown in B.
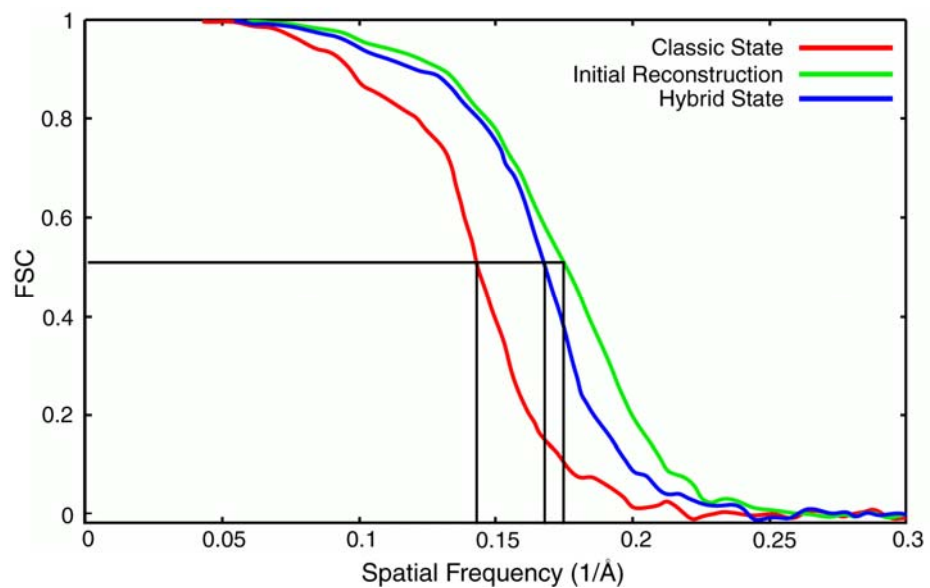
Figure S2: *Fourier Shell correlation (FSC) curves*. The resolution for the maps using a cutoff of 0.5 is 8.75 Å (initial reconstruction), 8.9 Å (hybrid state) and 10.5 Å (classic state).
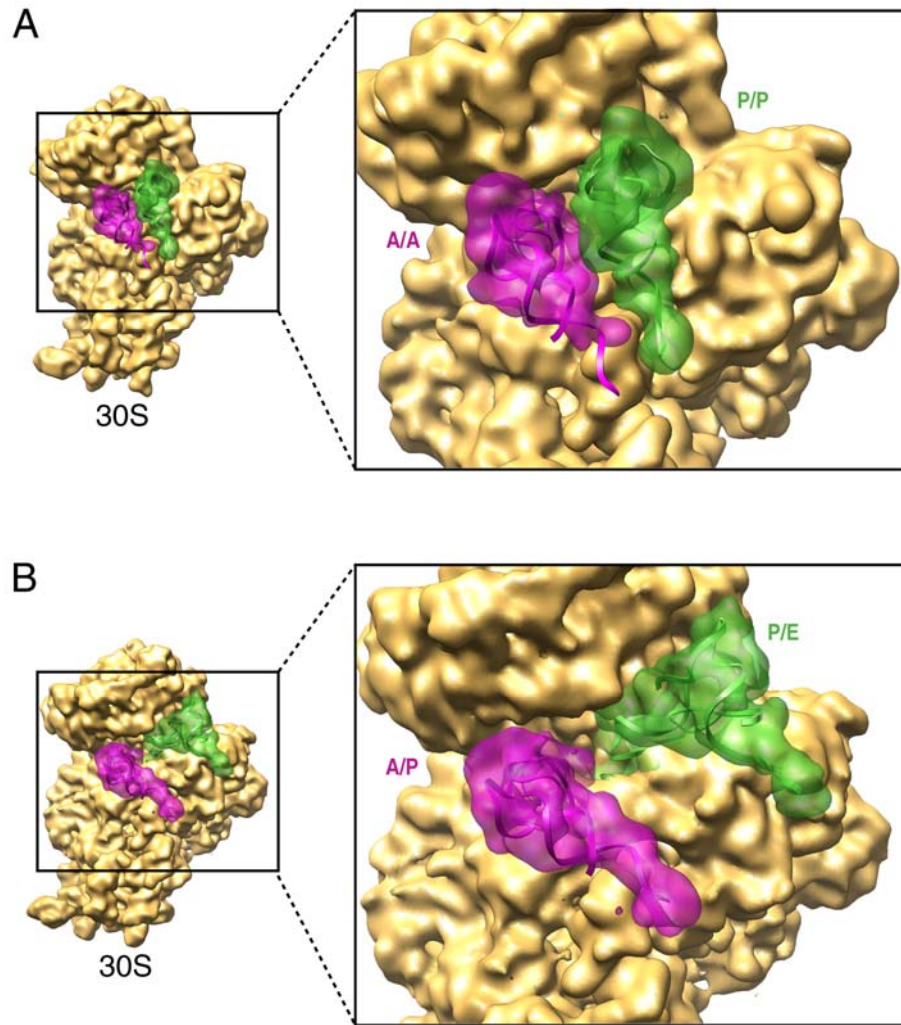
Figure S3: *Rigid-body fitting of tRNA atomic models in the experimental density maps.* (A) Close-up view of the fitted classic tRNAs. (B) Close-up view of the fitted hybrid tRNAs.
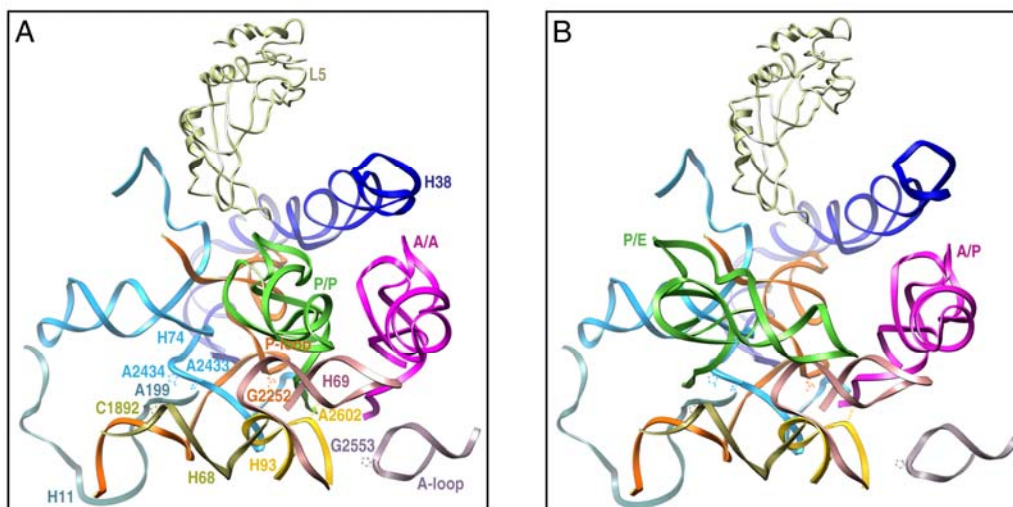
Figure S4: *Interaction of the tRNAs with the large subunit*. (A) Classic state A/A and P/P tRNAs in the 50S environment. (B) Hybrid state A/P and P/E tRNAs in the 50S environment. Protein L5, as well as 23rRNA fragments 197-228 (H11), 1189-1905 (H68), 2586-2608 (H93), 2547-2564 (A-loop), 1906-1934 (H69), 2227-2286 (P-loop) and 2372-2451 (H74) are shown. Residues C1892 (H68), A2602 (H93), G2252 (P-loop), A2433 and A2434 (H74) and G2553 (A-loop) are highlighted. The fitting has been done by real space refinement as described in the Methods section.
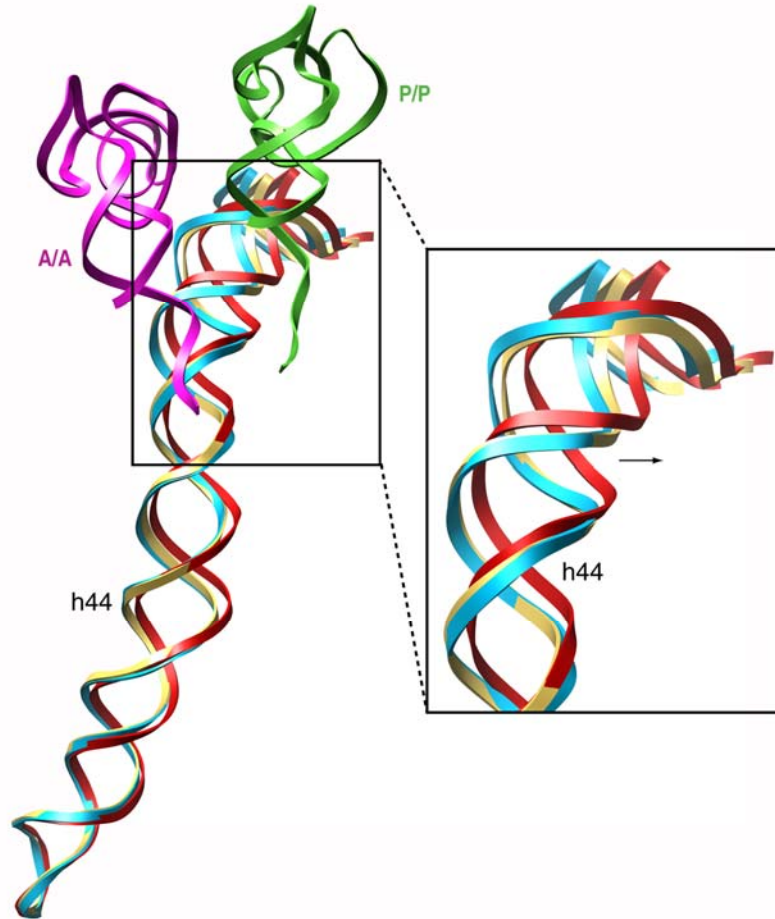
Figure S5: *Superimposed atomic models of 16S rRNA h44.* The classic state ribosome is shown in blue, the hybrid state ribosome in yellow and the EF-G bound ribosome in red. The lower portion of h44 has been kept fixed in the fitting to show the displacement of the decoding region (top of h44). The arrow points in the direction of the movement. A/A and P/P tRNAs are shown for clarity.