

#### **Text S4. The verification of the “r-translocation” simulation**

In the “r-translocation”, the movement of the anticodons of tRNAs was induced to pass through the free-energy minima in the classical, semi-hybrid and hybrid tRNA states during the EM-fitting simulation using the path-search algorithm [1]. Figs. S3(a) and S4(d) show that the path-search algorithm successfully induced the movement of the P-tRNA from the E-site and P-sites on the small subunit. Fig. S3(a) shows that at  $R_I > 37 \text{ \AA}$ , the positions of the anticodon of the P-tRNA in the PRE state in the “classical tRNA” simulation and the anticodon of the E-tRNA in the PRE state in the “r-translocation” simulation were close to each other (also see Fig. S3(b)). This indicates the movement of the tRNAs with respect to the small subunit from the classical P/P and E/E states into the hybrid A/P and P/E states was properly directed.

1. Ishida, H., *Branch Migration of Holliday Junction in RuvA Tetramer Complex Studied by Umbrella Sampling Simulation Using a Path-Search Algorithm*. *J. Comput. Chem.*, 2010. **31**: p. 2317-2329.