



**Supplementary Figure S3** Kinetics of 50S and 30S translocation.

(A) Time courses of 50S (blue) and 30S (red) translocation at 23°C. The rates at 4  $\mu\text{M}$  EF-G were  $6 \pm 1$  (Bpy), and  $8 \pm 3$  (Alx), both results of single-exponential fitting.

(B) Time courses of Bpy-MetVal-tRNA<sup>Phe</sup> translocation on the 50S subunit (50S TL, blue trace) and mRNA(Alx) translocation on the 30S subunit (30S TL; red trace) (37°C). The rates obtained by two-exponential fitting were  $27 \pm 3$  (Bpy; 86% of the amplitude),  $28 \pm 3$  (Alx; 60% of the amplitude).

(C) Movements of the P-site deacylated tRNA monitored by fluorescence changes of tRNA<sup>fMet</sup>(Prf20) at 25°C (as in ref. (Pan et al, 2007)). Two-exponential fitting yielded  $15 \text{ s}^{-1}$  for the first and  $5 \text{ s}^{-1}$  for the second step. Inset, proflavin reporter in P-site tRNA<sup>fMet</sup>.

(D) Same as (C) at 37°C. Two-exponential fitting yielded  $30 \text{ s}^{-1}$  for the fast and  $13 \text{ s}^{-1}$  for the slow step.

## Supplementary References

Pan D, Kirillov SV, Cooperman BS (2007) Kinetically competent intermediates in the translocation step of protein synthesis. *Mol Cell* **25**: 519-529