



Supplementary Figure S2 Puromycin (Pmn) reaction.

(A) Puromycin reactivity of Bpy-MetPhe-tRNA^{Phe} bound to the ribosomes programed with mRNA-Alx in the absence (○) and presence (●) of EF-G (37°C, 1 mM Pmn). Given is the % of PRE complexes reacting with Pmn.

(B) Time courses of Pmn reaction with PRE and POST complexes double-labeled with Bpy and Alx. PRE complexes (0.2 μM after mixing) were mixed with Pmn (10 mM) in the

presence of HygB and EF-G (■) ($k_{\text{PRE}} = 0.16 \pm 0.01 \text{ s}^{-1}$). POST complexes were prepared by incubating PRE complexes with EF-G for 10 min at 4°C before mixing with Pmn (◆) ($k_{\text{POST}} = 45 \pm 6 \text{ s}^{-1}$).

(C) Same as in (B) with EF-G(H91A) (▼) ($k_{\text{PRE}} = 1.5 \pm 0.1 \text{ s}^{-1}$) or EF-G(H583K) (□) ($k_{\text{PRE}} = 1.6 \pm 0.1 \text{ s}^{-1}$) (4 μM EF-G); the rate of $k_{\text{POST}} = 48 \pm 7 \text{ s}^{-1}$ (●). The intrinsic rate of translocation into the Pmn-reactive POST intermediate ($k_{\text{TL Pmn}}$; **Table 1**) was calculated from k_{PRE} and k_{POST} as $1/k_{\text{TI Pmn}} = 1/k_{\text{PRE}} - 1/k_{\text{POST}}$. Shown is the % of POST complexes reacting with Pmn at a given time.