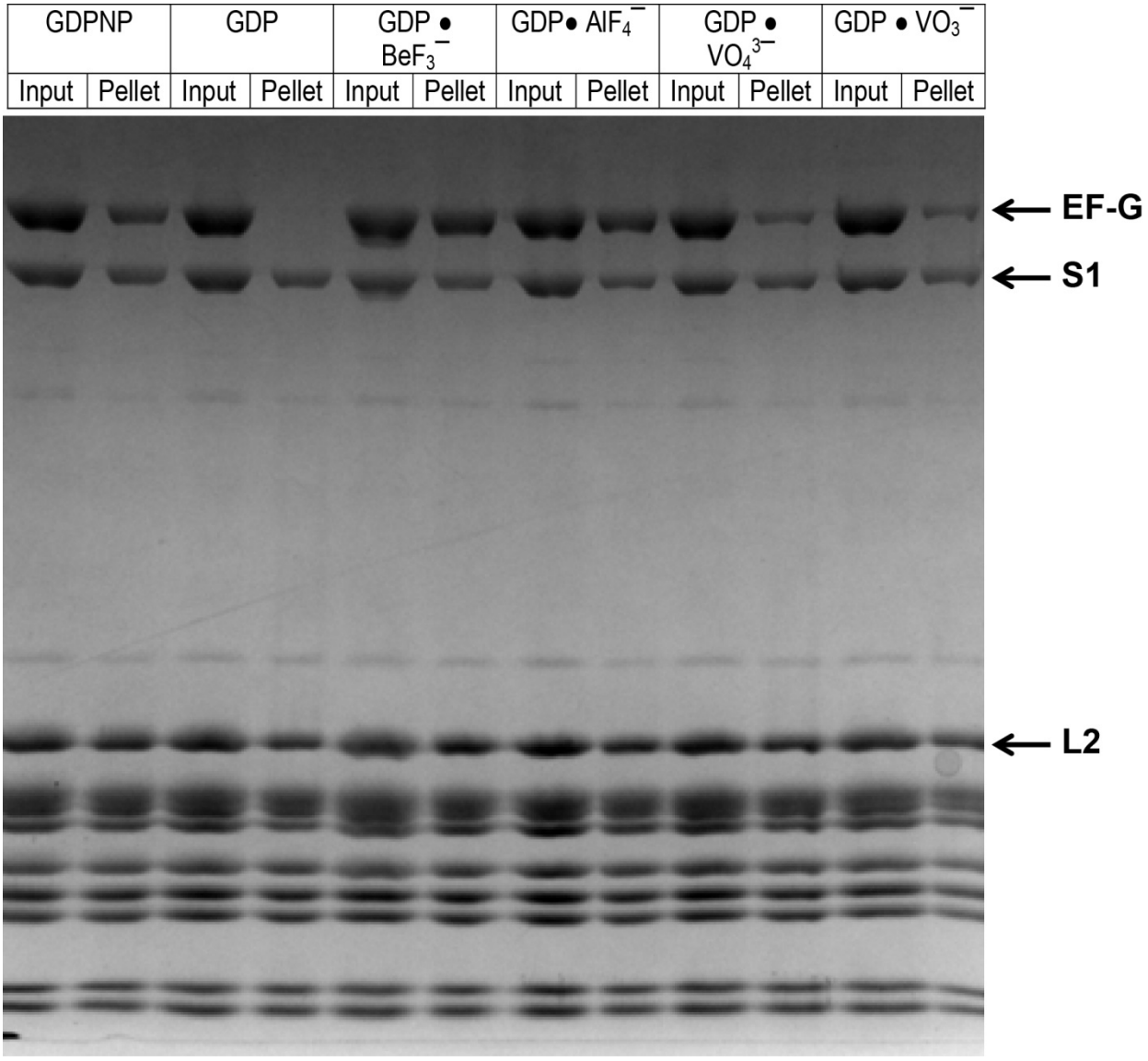
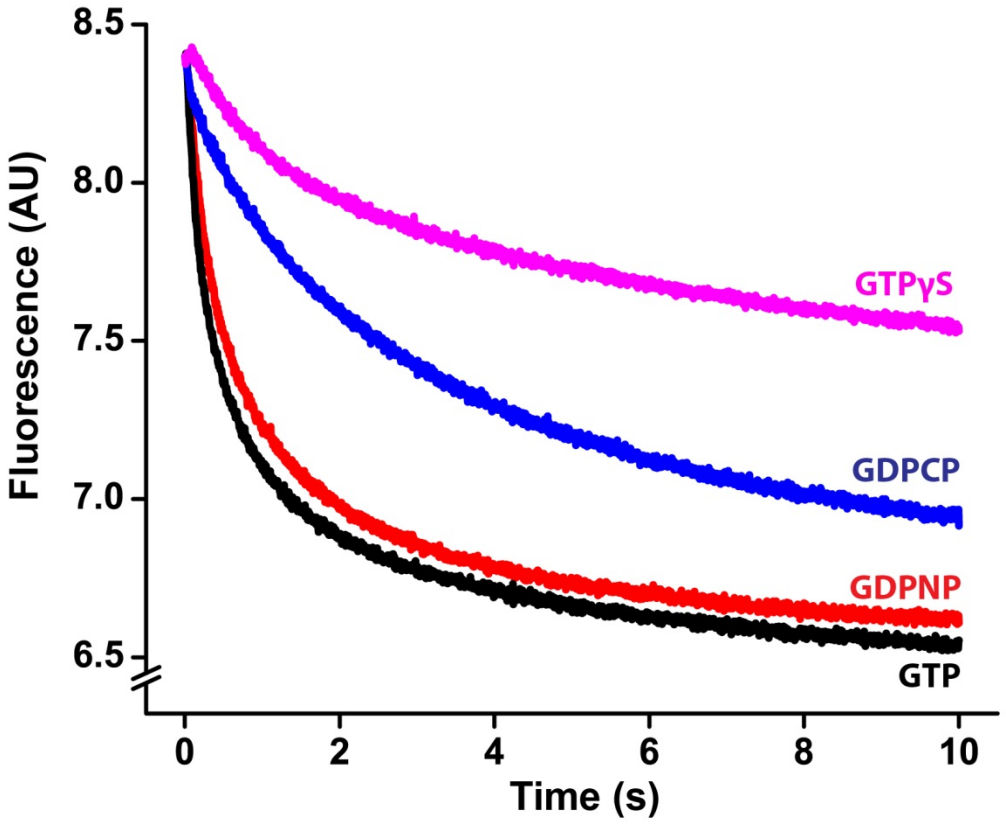
SUPPLEMENTARY MATERIALS



SUPPLEMENTARY FIGURE 1. Binding of EF-G to vacant ribosomes in the presence of various nucleotides and phosphate analogues (as indicated) measured by the pelleting assay. EF-G was incubated with vacant ribosomes. Half of each sample was pelleted through a sucrose cushion (pellet); the other half was used as a loading control (input). Protein content of ribosome pellets was analyzed using SDS-PAGE. The band corresponding to EF-G, the largest ribosomal proteins S1 and L2 are indicated by arrows. The cropped version of this gel is shown in Fig.1.



SUPPLEMENTARY FIGURE 2. Pre-steady-state kinetics of translocation in the presence of synthetic analogues of GTP. mRNA translocation was induced by mixing pretranslocation ribosomes (35 nM after mixing) with EF-G (1 μM after mixing) preincubated with 0.5 mM GTP (black), GDPNP (red), GDPCP (blue) or GTPγS (magenta). Experiments were performed in polyamine buffer at pH 7.5. mRNA translocation was detected by the quenching of fluorescein attached to the 3' end of mRNA using a stopped-flow apparatus.

SUPPLEMENTARY TABLE 1. Rates of mRNA translocation catalyzed by EF-G in the presence of GDPCP and GTPγS.

Nucleotide	k_1, s^{-1}	k_2, s^{-1}	$A_1/(A_1+A_2)$	k_{av}, s^{-1}
GDPCP	0.8±0.04	0.16±0.004	0.37±0.02	0.39±0.03
GTPγS	1.0±0.03	0.10±0.004	0.35±0.01	0.41±0.01

Rates of translocation induced by EF-G in the presence of GDPCP or GTPγS were measured in pre-steady-state stopped-flow kinetic experiments in polyamine buffer at pH 7.5. EF-G and ribosome concentrations after mixing were 1 μM and 35 nM, respectively. k_1 and k_2 are the rate constants of double-exponential fits of the mRNA translocation data; $A_1/(A_1+A_2)$ is the relative contribution of the faster phase to the total amplitude of fluorescein quenching. Weighted average values (k_{av}) for mRNA translocation rates were calculated by combining the rate constants derived from the two-exponential fits: $k_{av} = (k_1A_1 + k_2A_2)/(A_1 + A_2)$.

SUPPLEMENTARY TABLE 2. Rates of translocation induced by EF-G in the presence of GTP or GDP·BeF_3^- determined by fitting pre-steady-state stopped-flow kinetic data to stretched exponential function (Fig. 5).

Nucleotide	$k_{stretched}$, s^{-1}	β
GTP, pH 7.5	1.5±0.2	0.56±0.02
GDP•BeF_3^-, pH 7.5	1.2±0.5	0.60±0.06

Fluorescein quenching in kinetic translocation assay was fit to the stretched exponential function $y=y_0 +A*\exp(-k_{stretched}*t)^{\beta}$ where $k_{stretched}$ is stretched exponential rate constant; β is a stretched exponential numerical factor ($0<\beta\leq1$).