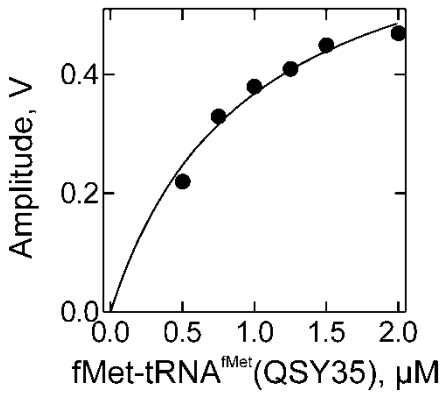
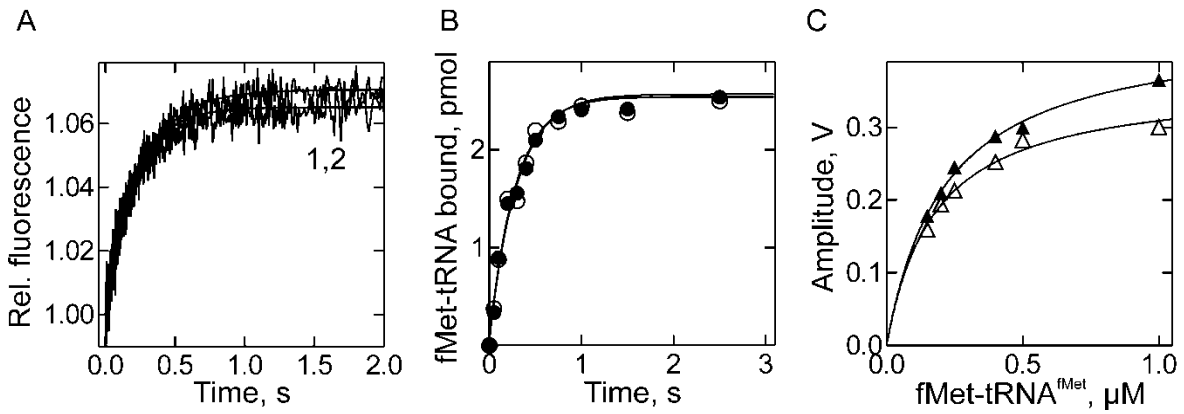


Suppl. Figure 1. Concentration dependence of the FRET amplitude during IF2(Atto)·GTP·fMet-tRNA^{fMet}(QSY) complex formation. Hyperbolic fitting yielded K_d = 1.0 ± 0.2 μM.



Suppl. Figure 2. Kinetics of IF2-dependent fMet-tRNA^{fMet} binding to the 30S subunit.

A. Stopped-flow. Time course of fMet-tRNA^{fMet}(Flu) (0.2 μM) binding to the 30S·IF1·IF2·IF3(Alx) complex (0.05 μM) monitored by FRET. Trace 1, 022mRNA; trace 2, 002mRNA.

B. Rapid filtration. Experiments were performed as in A, except that f[^{35}S]Met-tRNA^{fMet} was preincubated with IF2 (closed symbols) and 30S complex formation was determined by nitrocellulose filtration. Alternatively, f[^{35}S]Met-tRNA^{fMet} was added to the 30S*·IF2·GTP complex (open symbols).

C. Stopped-flow amplitudes. The amplitudes of the FRET changes (cf. panel A) were measured at increasing concentration of fMet-tRNA^{fMet}(Flu). Closed symbols, IF2 preincubated with fMet-tRNA^{fMet}(Flu); open symbols, IF2 preincubated with 30S* complex.