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Supplemental Information

Non-canonical Binding Site for Bacterial Initiation Factor 3 on the Large Ribosomal Subunit

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Supplemental Materials

Table S1. Summary of the apparent rate constants of subunit joining and FRET changes during 70S IC formation. Related to Figures 1 and S3.

FRET pair	Light scattering (SJ, GTP)[a] k_{app} (s^{-1})	FRET (SJ, GTP)[b] k_{app} (s^{-1})	FRET (SJ, GTPγS)[c] k_{app} (s^{-1})	FRET (chase, GTP)[d] k_{app} (s^{-1})
$IF1_4$(Atto540Q) - $IF3_{166}$(Alx488)	5.8 ± 0.4 (45 %) 0.8 ± 0.1 (55 %)	4.8 ± 0.3 (20 %) 1.0 ± 0.1 (70 %)	4.9 ± 0.3 (19 %) 1.1 ± 0.1 (65 %)	5.6 ± 0.5 (88 %)
$IF2_{757}$(Atto540Q) - $IF3_{166}$(Alx488)	5.9 ± 0.5 (48 %) 1.0 ± 0.1 (52 %)	4.2 ± 0.3 (32 %) 1.0 ± 0.1 (68 %)	3.7 ± 0.3 (38 %) 0.9 ± 0.1 (62 %)	6.0 ± 0.5 (80 %)
$30S_{S13}$(Alx488) - $IF3_{166}$(Atto540Q)	0.43 ± 0.05 (57 %) 0.012 ± 0.002 (43 %)	0.40 ± 0.05 (62%) 0.04 ± 0.01 (24 %)	0.36 ± 0.04 (58%) 0.03 ± 0.01 (26 %)	2.9 ± 0.3 (55%) 0.4 ± 0.1 (36 %)
$50S_{L33}$(Atto540Q) - $IF3_{166}$(Alx488)	1.9 ± 0.1 (34 %) 0.24 ± 0.04 (45 %)	1.2 ± 0.1 (83%)	1.3 ± 0.1 (100%)	n.d.

[a,b,c]0.05 μM 30S IC (formed using different fluorescence-labeled components) was mixed with 0.15 μM 50S subunits. SJ – subunit joining. In [b,c] FRET time courses were corrected by subtracting the donor fluorescence. All rates are derived from exponential fittings of time courses.

[d]0.05 μM 30S IC (formed using different fluorescence-labeled components) was mixed with 1.5 μM unlabeled IF3.

Rate values are represented as mean ± s.e.m; n.d. – not determined. Amplitude contributions of the dominant phases (comprising greater than 80 % amplitude change) are indicated in brackets.

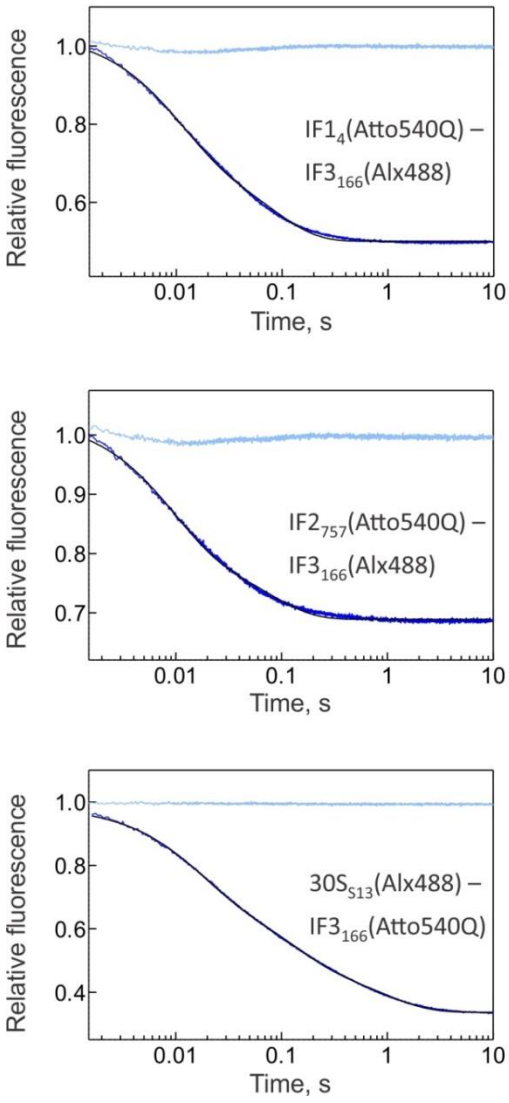

Figure S1. FRET changes upon IF3 binding to 30S PIC. Related to Figure 1.

Labeled 30S PIC (lacking IF3) (0.05 μM) were rapidly mixed with labeled IF3 (0.05 μM) and FRET changes between the indicated components were monitored with time (blue). Control experiments were performed in the absence of the acceptor dye (light blue). Black smooth lines represent exponential fitting of time courses. All reactions were performed in buffer TAKM$_7$ at 20°C.

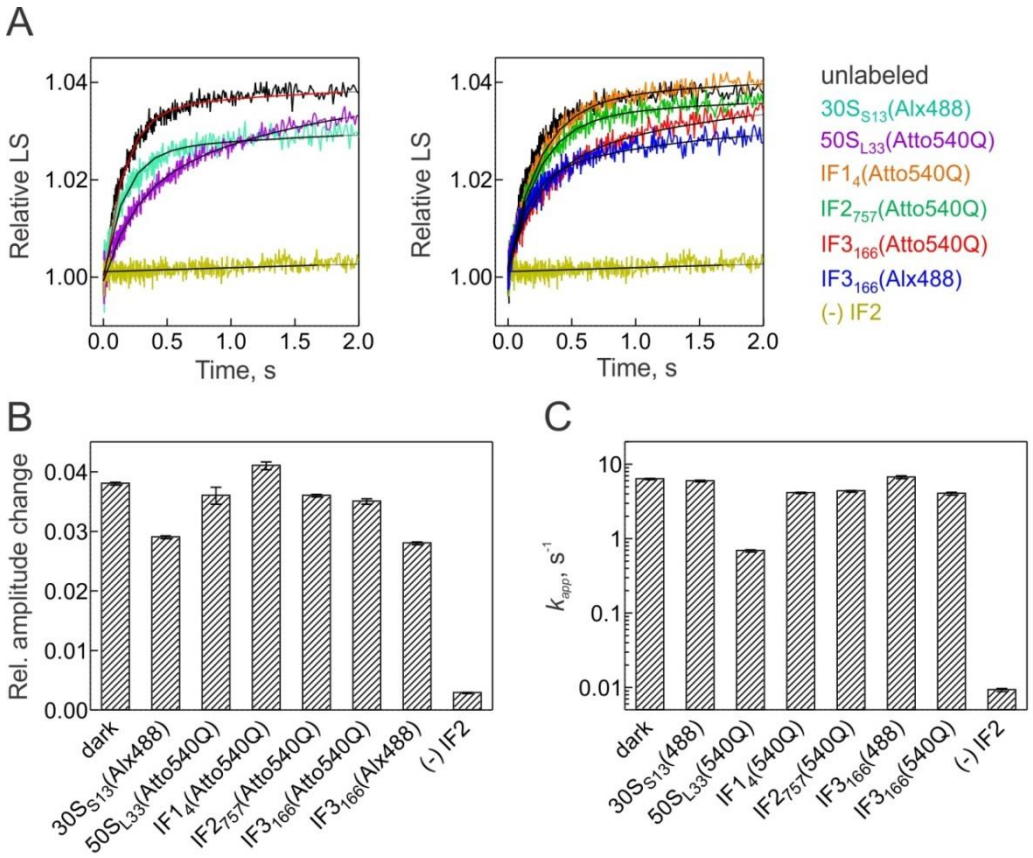

Figure S2. Activity of labeled ribosomal subunits and IFs. Related to Figure 1.

(A) 30S IC formed using indicated fluorescent components (0.05 μM) were rapidly mixed with 50S subunits (0.25 μM) and subunit association was monitored via changes in light scattering (LS). Positive and negative control measurements performed in the absence of any fluorescent label (black) or IF2 (gold), respectively, are depicted in both panels for comparison. Black smooth lines represent double-exponential fits of time courses.

(B-C) The relative change in amplitude at 2 s and the apparent rate of the predominant phase derived from double-exponential fitting of time courses in (A), respectively; error bars represent the standard error of the fit. All reactions were performed in buffer $TAKM_7$ at 20°C.

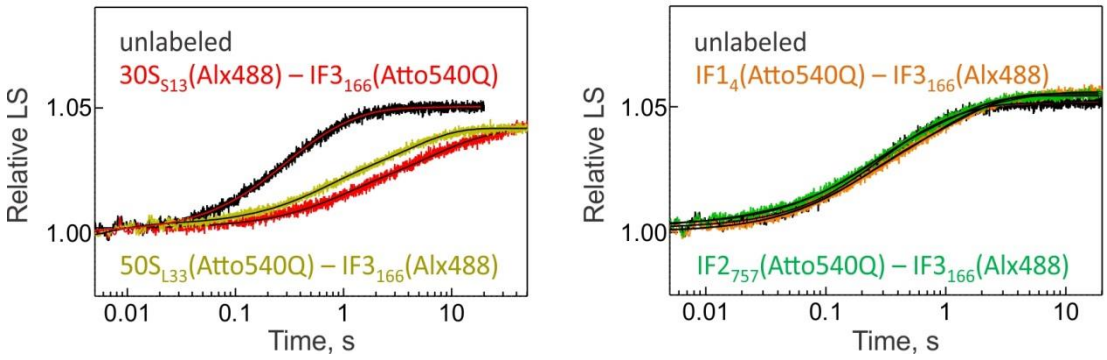

Figure S3. Kinetics of subunit joining monitored with different FRET pairs. Related to Figure 1.

30S IC formed in the presence of indicated components (0.05 μM) were rapidly mixed with 50S subunits (0.15 μM) and subunit joining was monitored with time. The same control measurement performed in the absence of any fluorescent label (black), is depicted in both panels for visual comparison. Fits derived from exponential fitting of time courses are shown as black smooth lines (see Table S1 for rates). All reactions were performed in buffer TAKM$_7$ at 20°C.