

Supplementary Materials

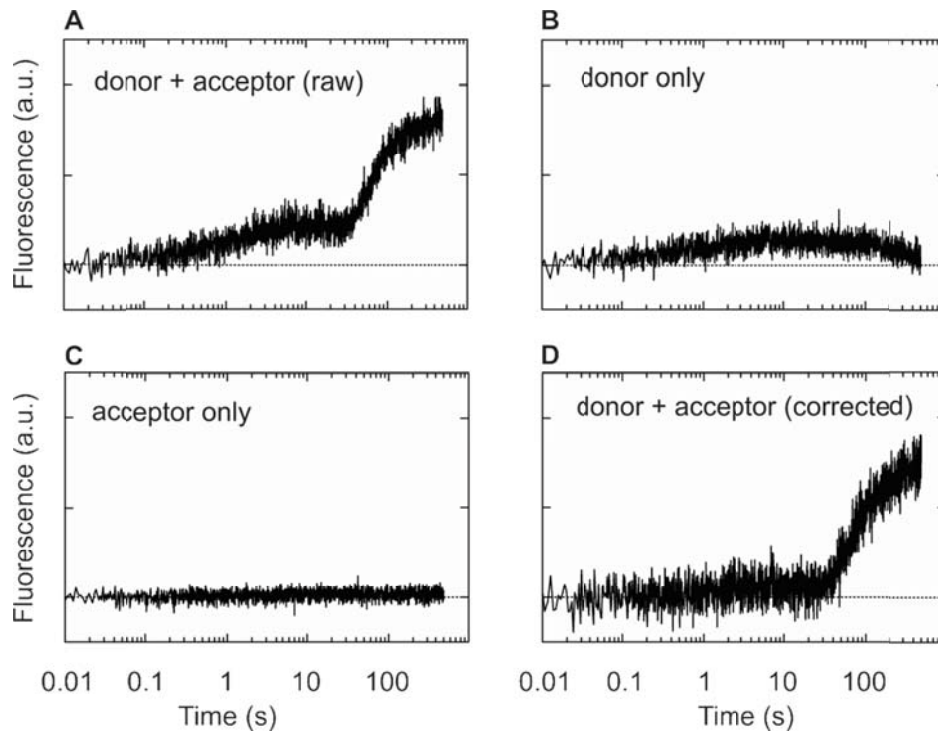
Signal recognition particle binds to translating ribosomes before emergence of a signal anchor sequence

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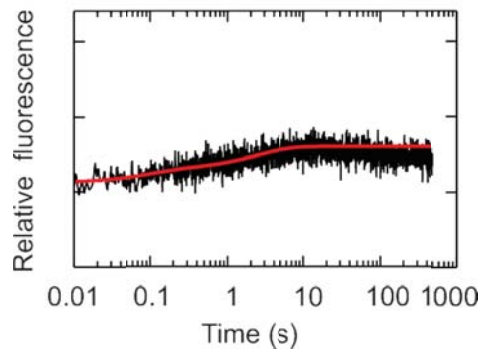
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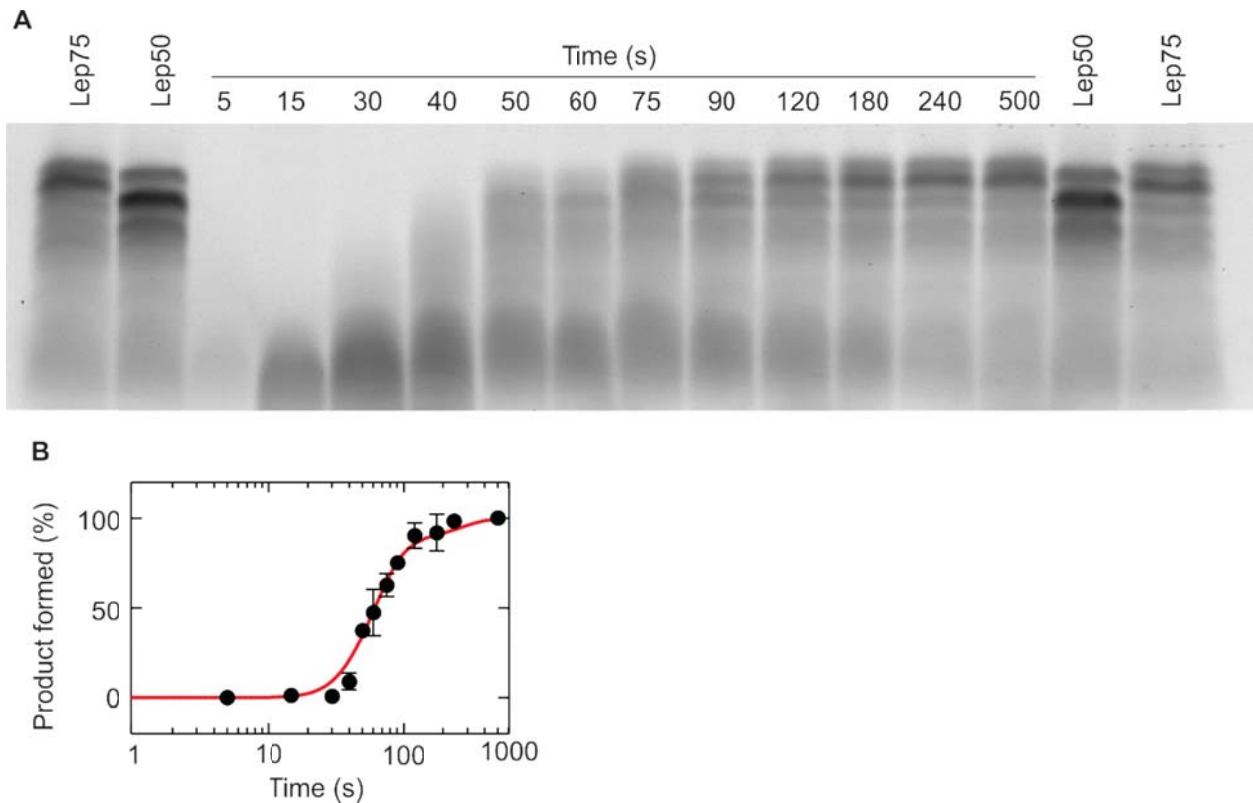
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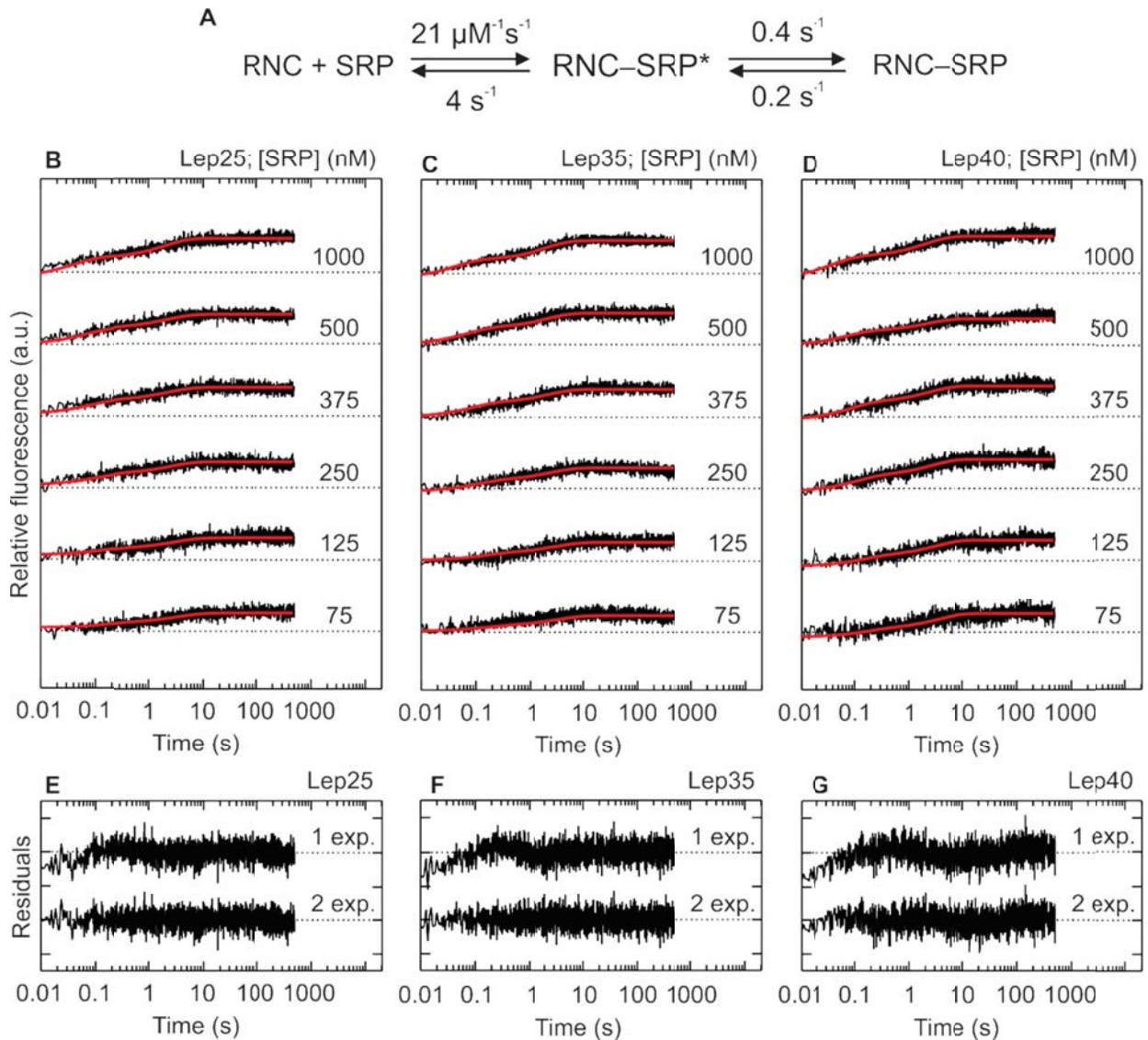
Supplementary Figure S1. Fluorescence controls for co-translational recruitment of acceptor-labelled Bpy-SRP to donor-labelled MDCC-RNCs. Translation of Lep75 mRNA was carried out in the presence of 250 nM SRP at 37°C. For all experiments, fluorescence excitation was at 410 nm and a 530 nm cut-off filter was used to filter fluorescence emission. **(A)** In the presence of both donor and acceptor fluorophores, rapid initial SRP recruitment and a slow conformational change are evident. **(B)** Initial SRP binding causes an increase in donor fluorescence followed by photobleaching at long times. Direct excitation of acceptor **(C)** does not contribute to the fluorescence signal. Corrected fluorescence for donor + acceptor **(D)** was calculated by subtracting fluorescence due to direct excitation of donor and acceptor fluorophores as well as the background signal due to buffer ($F = AD(A) - D(A) - A(A) - \text{buffer}(A)$). The corrected donor + acceptor trace shows that initial binding of SRP is detected predominantly by a change in donor fluorescence and the slow fluorescence increase starting at about 40 s is due to a change of FRET.



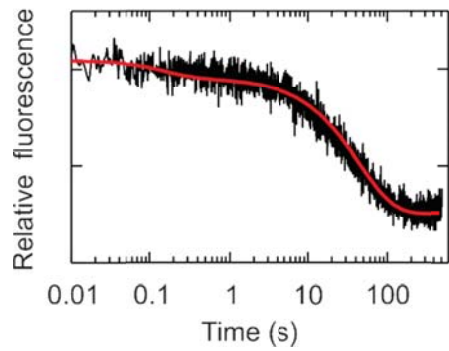
Supplementary Figure S2. Co-translational binding of SRP to the non-SRP substrate HemK75. Bpy-SRP (125 nM concentration after mixing) was rapidly mixed with MDCC-labelled HemK75-RNC (25 nM concentration after mixing). Experimental conditions, including translation factor concentrations, were identical to those used for other stopped-flow experiments involving Lep-RNCs (Methods). The red line is the fit obtained for co-translational binding of SRP to Lep25-RNC via global fitting, which closely resembles the data obtained with HemK75-RNC.



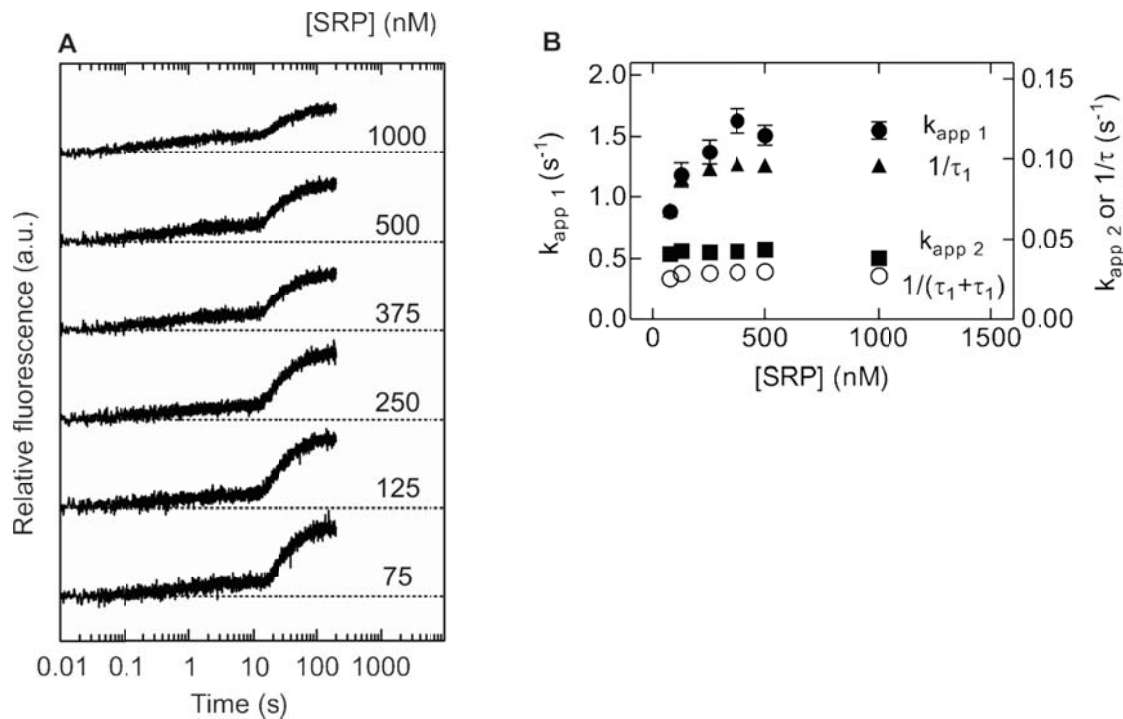
Supplementary Figure S3. Time course of Lep75 translation. **(A)** Translation of Lep75 mRNA was carried out using Bpy-labelled Met-tRNA^{fMet} at the same concentrations and conditions as used in the stopped-flow experiments (Methods). Aliquots taken at the indicated times were quenched and translation products separated on Tris-tricine SDS-PAGE and visualized in a fluorescence scanner. **(B)** Translation products 50 or more amino acids in length were quantified relative to the 500 s time point, yielding a transit time for Lep50 product formation of 72 ± 4 s. Average and standard deviation was computed for each time point based on four independent experiments. The double band observed for the longer-chain products, which has been reported before (1), is attributed to premature stalling of the RNC.



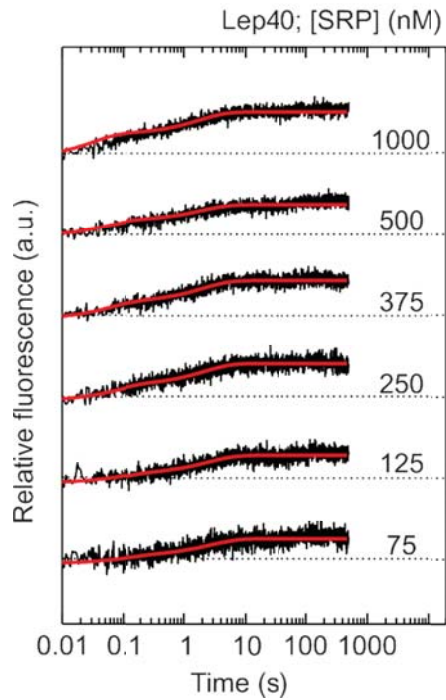
Supplementary Figure S4. Analysis of SRP binding to short-chain RNCs. **(A)** Two-step model used for global fitting. The rate constants obtained are indicated for each step. **(B, C, D)** Bpy-labeled SRP binding to MDCC-labeled Lep-RNCs of different chain lengths was measured during translation (Methods). The 18 traces used for the global fit are presented along with the results of the fit (red lines). **(E, F, G)** Comparison of residuals of one-step and two-step models of SRP binding, which demonstrates that one-exponential fits show a systematic deviation of predicted values from experimental data. Residuals were calculated from one-exponential and two-exponential fits to stopped-flow traces of co-translational Bpy-SRP (500 nM) binding to MDCC-labeled Lep-RNCs.



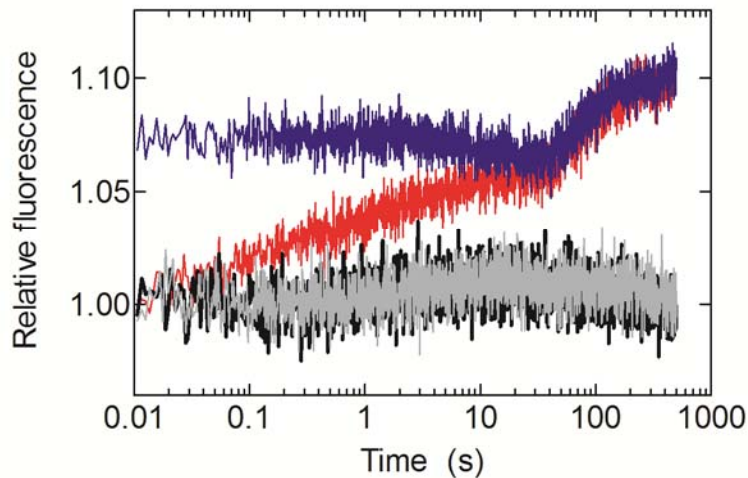
Supplementary Figure S5. Dissociation of the SRP–Lep75-RNC complex. Preparation of Bpy-SRP–MDCC-Lep-RNCs was carried out by translation of Lep75 mRNA by MDCC-labelled ribosomes (25 nM) in the presence of Bpy-SRP (75 nM) for 10 minutes at 37°C with the same translation components as in co-translational experiments. Dissociation of the complex was initiated by rapid mixing with unlabeled SRP (750 nM) at 37°C (Methods). The red line represents the result of global fitting.



Supplementary Figure S6. Co-translational SRP recruitment to RNCs in HiFi buffer containing polyamines. **(A)** Translation of Lep75 mRNA at increasing concentration of SRP. Translation of Lep75 mRNA was carried out in a stopped-flow apparatus in the presence of various concentrations of SRP (1 μ M – 75 nM, final concentrations top to bottom) at 37°C in HiFi buffer. FRET between MDCC-labelled ribosomes (25 nM final concentration) and Bpy-labelled SRP was monitored. The fluorescence traces are offset for clarity and a dotted line indicates the respective initial fluorescence. The rearrangement of the RNC–SRP complex starts at about 10 s in HiFi buffer, rather than at 40 s as in buffer A, consistent with a higher rate of translation in HiFi buffer, compared to buffer A. **(B)** Kinetic parameters of SRP–Lep75-RNC complex formation in HiFi buffer. Traces in panel a were fitted with a piecewise function describing an exponential increase starting at $t = 0$ and, following a delay defined by fitting parameter τ_1 , a second exponential increase was initiated. The apparent rate constant of the rapid phase (completed after 10 seconds) is k_{app1} , and the apparent rate constant of the slow phase (starting at τ_1) is $k_{app 2} = 1/\tau_2$.



Supplementary Figure S7. SRP recruitment to ribosomes translating Lep40 mRNA. Translation of Lep40 mRNA was carried out in the stopped-flow apparatus in the presence of Bpy-labelled SRP at various concentrations (75 nM–1 μ M, final concentrations), and FRET between MDCC-labelled ribosomes (25 nM final concentration) and Bpy-labelled SRP was monitored. Fluorescence traces are offset for clarity and dotted lines indicate the respective starting fluorescence. Red lines are obtained from global fitting of the associated data sets to the model in Figure 3.



Supplementary Figure S8. Rapid fluorescence change and SRP binding. Translation of Lep75 mRNA by MDCC-labeled ribosomes was initiated in the stopped-flow apparatus upon addition of translation components in the absence of SRP (black trace), in the presence of Bpy-labeled SRP (500 nM; red trace), or with initiation complex pre-equilibrated with Bpy-labeled SRP (500 nM) and addition of Bpy-labeled SRP (500 nM) together with translation components (blue trace). When Bpy-labeled SRP is present at the onset of translation, a rapid phase is observed. When Bpy-labeled SRP is pre-equilibrated with initiation complexes such that SRP is bound to ribosomes prior to mixing, the rapid phase is absent. Rapid mixing of MDCC-labeled initiation complex with a mixture of elongation factors EF-Tu (15 μ M), EF-Ts (0.1 μ M), and EF-G (2 μ M) reveals no fluorescence change (grey trace) indicating that the rapid fluorescence change is not due to non-specific protein interactions.

Reference

1. Ge Y, Draycheva A, Bornemann T, Rodnina MV, Wintermeyer W. (2014) Lateral opening of the bacterial translocon on ribosome binding and signal peptide insertion. *Nat Commun* 5, 5263.