Deducing the kinetics of protein synthesis *in vivo* from the transition rates measured *in vitro*

– Supporting Information –

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The supporting information consists of the four figures S1-S4:

Figure S1: Overall elongation rate as measured for a model protein *in vitro*.

Figure S2: *In-vitro* rates as measured for near-cognate accommodation and rejection after proofreading.

Figure S3: Codon-specific elongation rates *in vitro* and *in vivo*.

Figure S4: Incorporation of radioactively labeled amino acids for different dissociation rates.

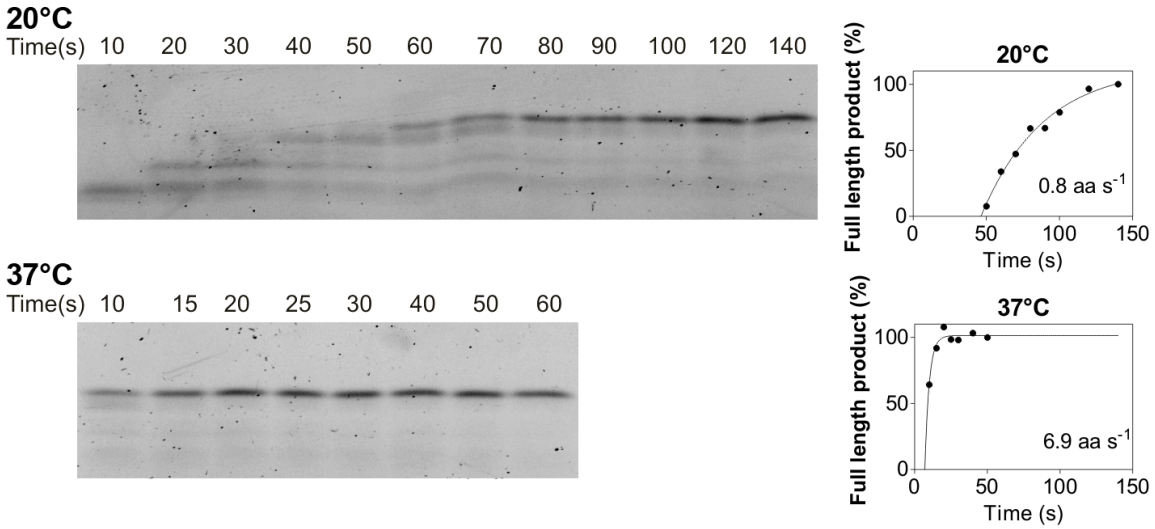

Figure S1. Kinetics of CspA translation *in vitro* at different temperatures. CspA mRNA, which codes for a 70 aa-long protein from *E. coli*, was prepared by T7 RNA-polymerase transcription. Ribosomes were synchronized by forming an initiation complex consisting of 70S ribosomes, CspA mRNA and a fluorescence derivative of initiator $tRNA^{fMet}$ carrying BodipyFL at the α-amino group of Met in the presence of initiation factors (IF1, IF2, and IF3) and GTP. Translation was carried out in a fully reconstituted translation system by adding initiation complexes (15 nM) to a mixture of EF-Tu–GTP–aminoacyl-tRNA (40 μM aminoacyl-tRNA, 100 μM EF-Tu in total), EF-G (3 μM), GTP (2 mM), phosphoenol pyruvate (6 mM), and pyruvate kinase (0.1 mg/ml) in HiFi buffer (50 mM Tris-HCl, pH 7.5, 30 mM KCl, 70 mM NH_4Cl, 3.5 mM free $MgCl_2$, 0.5 mM spermidine, and 8 mM putrescine) at the indicated temperatures [1]. In the absence of translation termination and ribosome recycling factors, translation was limited to a single round, i.e. at most one molecule of CspA was synthesized per ribosome. The reactions were stopped at the indicated time intervals and translation products separated on 16.5% Tris-Tricine-PAGE and visualized by the fluorescent reporter BODIPY-Fl at the N-terminus of the peptides [2] (left panels). The intensity of the full length product was quantified with ImageJ (right panel, circles). Average translation rates per codon, which depend on the elongation rates only, were determined by exponential fitting (fits in graphs of the right panel).

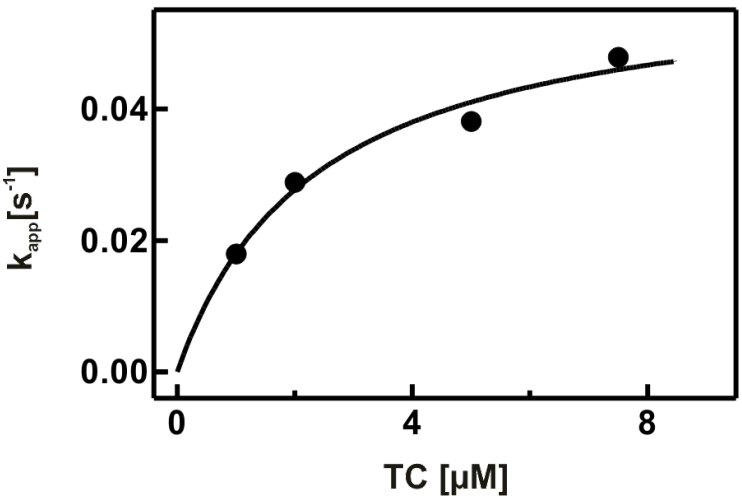

Figure S2. *In-vitro* values of the rates $\omega_{9,10} \equiv k_{5,\mathrm{nr}}$ and $\omega_{90} \equiv k_{7,\mathrm{nr}}$ for near-cognate accommodation and rejection after proofreading at 20 °C as determined by the experimental protocol described previously in Ref. [3]. The formation of f[^{3}H]Met[^{14}C]Phe was monitored under multiple-turnover conditions using initiation complexes 70S–mRNA(AUGCUC)–f[^{3}H]Met-tRNA$^{\mathrm{fMet}}$ (0.14 μM) and varying concentrations of the ternary complex EF-Tu–GTP–[^{14}C]Phe-tRNA$^{\mathrm{Phe}}$, which is near-cognate to the CUC codon. For each concentration of the ternary complex, the rates were determined from the linear slopes of the time courses. From the hyperbolic dependence of the concentration dependence of k_{app}, we calculated $\omega_{9,10} = 0.060 \pm 0.006/\mathrm{s}$ and $K_{\mathrm{M}} = 2.4\,\mu\mathrm{M}$. Using the previously measured efficiency $\omega_{9,10}/(\omega_{9,10} + \omega_{90}) = 1/15$ of the proofreading step [4], we then obtained the value $\omega_{90} = 0.84 \pm 0.08/\mathrm{s}$ for near-cognate rejection after proofreading.

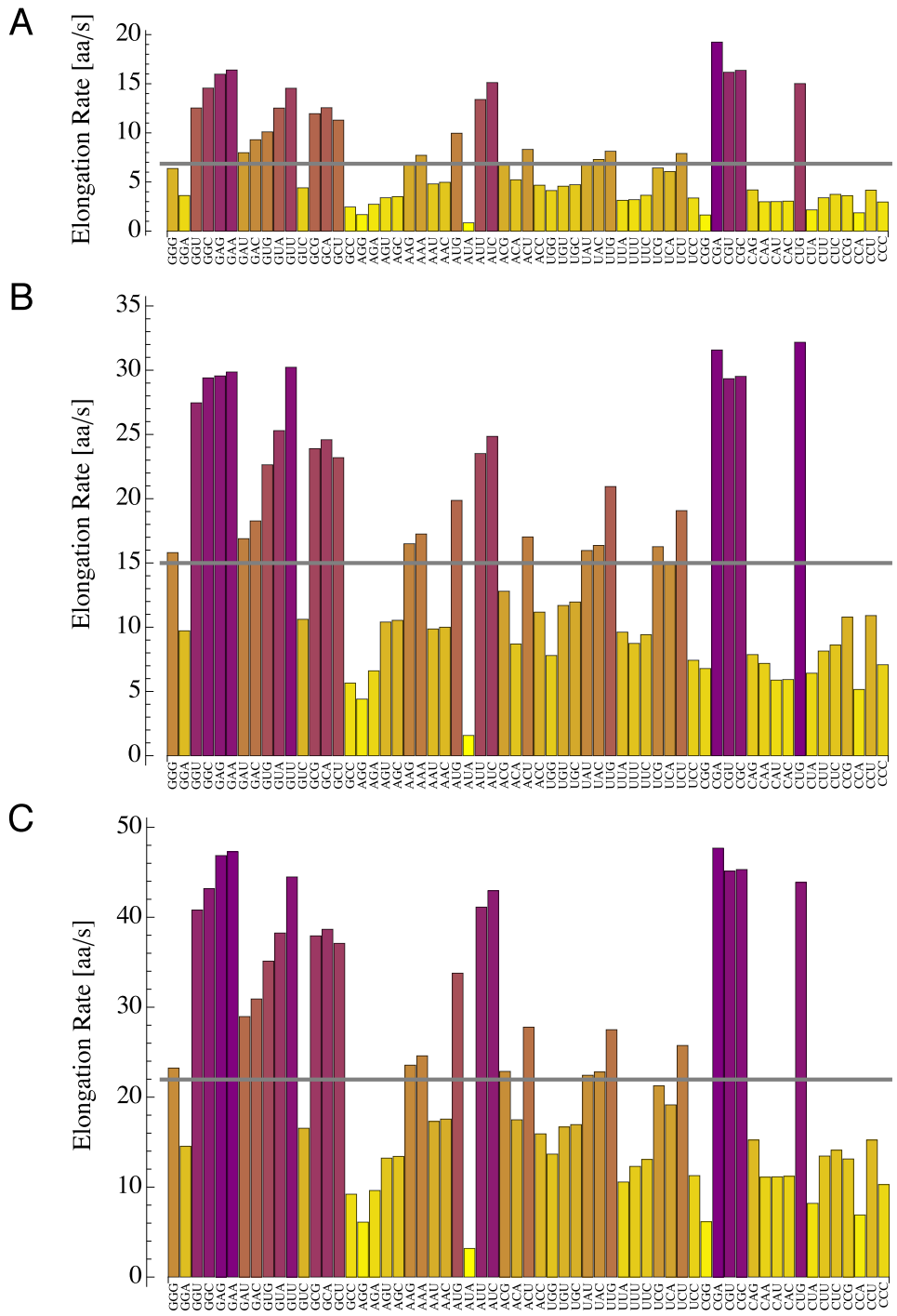

Figure S3. Codon-specific elongation rates $\omega_{c,\mathrm{elo}}$ in units of amino acids per second as calculated from Equation 17, see *Methods* section in the main text, using the decomposition of the codon-specific elongation times in Equation 7 and the complete sets of individual transition rates: (A) *In-vitro* values $\omega_{c,\mathrm{elo}}$ for the high-fidelity buffer at 37 °C, obtained from the individual rates in Table 1; (B, C) *In-vivo* values $\omega^{\star}_{c,\mathrm{elo}}$ for *E. coli* at growth conditions of (B) 0.7 dbl/h and (C) 2.5 dbl/h, calculated from the individual rates in Table 2.

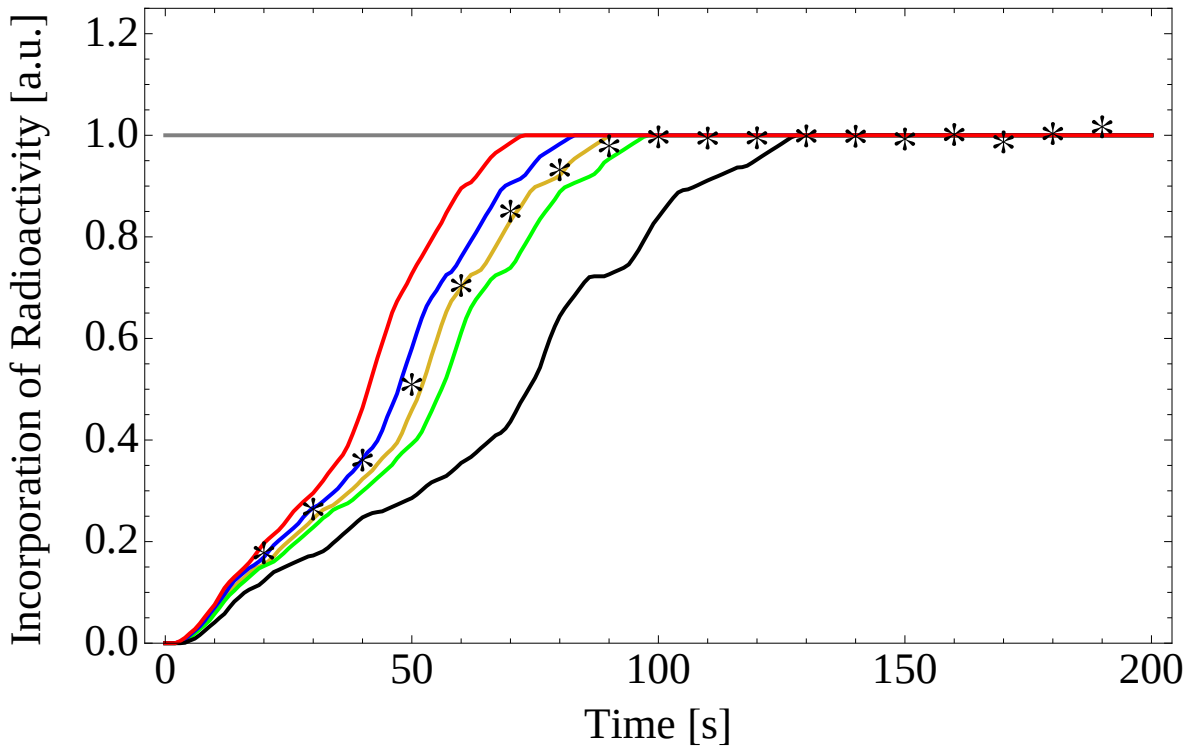

Figure S4. Experimental data (black stars) for the incorporation of radioactively labeled amino acids at a growth rate of 0.7 dbl/h [5] and simulation curves obtained for five different values of the initial dissociation rate ω_{off}. The orange simulation curve in the middle corresponds to $\omega_{\text{off}} = \omega^{\star}_{\text{off}} = 1400/\text{s}$, see Table 2. This value has been obtained from the minimization of the kinetic distance and provides an excellent fit to the data. The red, blue, green, and black curves have been obtained for simulations with $\omega_{\text{off}} = 2\,\omega^{\star}_{\text{off}}$, $1.2\,\omega^{\star}_{\text{off}}$, $0.8\,\omega^{\star}_{\text{off}}$, and $0.5\,\omega^{\star}_{\text{off}}$, respectively. Thus, changing the value of ω_{off} by 20% leads to a significant deviation of the simulation curve from the experimental data.

References

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