Supplementary Information: Decomposition of time-dependent fluorescence signals reveals codon-specific kinetics of protein synthesis

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Kinetic rates

Table S1: Kinetic rates as used in the Markov model description of the translation process [1].

Rates	k-notation	37°C	Units
κ_{on}	k_1	175 ± 25	$\mu M^{-1}\,s^{-1}$
ω_{off}	k_{-1}	700 ± 270	s^{-1}
ω_{rec}	k_2	1500 ± 450	s^{-1}
ω_{21}	$k_{-2,\text{co}}$	2 ± 0.6	s^{-1}
ω_{23}	$k_{3,\text{co}}$	1500 ± 450	s^{-1}
ω_{con}	k_4	450	s^{-1}
ω_{40}	$k_{7,\text{co}}$	1	s^{-1}

Table S2: Fitted *in-vitro* rates of ribosomal transitions. The standard deviation of the fitting parameters was calculated by analyzing the goodness-of-fit parameter χ^2.

Rates	37°C	Units
ω_{trans}	53 ± 4	s^{-1}
ω_{45_1}	109 ± 3	s^{-1}
ω_{45_2}	11 ± 0.5	s^{-1}
ω_{45_3}	43 ± 4	s^{-1}
ω_{45_4}	2 ± 0.1	s^{-1}
ω_{45_5}	20 ± 1	s^{-1}
ω_{end}	0.3	s^{-1}

Intrinsic Fluorescence Intensities

Table S3: IFIs obtained from fluorescence signatures of *phe1* to *phe5* mRNA translation

#	no EF-G	*phe1*	*phe2*	*phe3*	*phe4*	*phe5*
(IFI_1)	(1)	(1)	(1)	(1)	(1)	(1)
$IFI_1{}^{pep}$	1.124	1.146	1.081	1.168	1.183	1.157
$IFI_1{}^{trans}$		1.069				
IFI_2			1.019	1.015	0.977	0.980
$IFI_2{}^{pep}$			1.149			
IFI_3				1.320	1.390	1.383
$IFI_3{}^{pep}$				1.088		
IFI_4					1.068	(1.068)
$IFI_4{}^{pep}$					1.182	
IFI_5						0.988
$IFI_5{}^{pep}$						1.261

Table S4: IFIs obtained from fluorescence signatures of *phe4* mRNA translation under different ternary complex concentrations

#	0.15 μM	0.3 μM	2 μM	10 μM
(IFI_1)	(1)	(1)	(1)	(1)
$IFI_1{}^{pep}$	1.235	1.224	1.179	1.174
IFI_2	1.024	1.003	0.975	0.983
IFI_3	1.299	1.342	1.408	1.383
IFI_4	1.073	1.061	1.071	1.117
$IFI_4{}^{pep}$	1.172	1.176	1.186	1.189

Table S5: Averaged IFIs obtained from fluorescence signatures of *phe1* to *phe5* mRNA translation (± SD)

#	Fluorescence value	SD	SD [%]	Relative change [%]
(IFI_1)	(1)	-	-	-
IFI_1^{pep}	1.143	0.031	2.7	14.3
IFI_1^{trans}	1.069	-	-	6.9
IFI_2	0.998	0.017	1.7	0.2
IFI_2^{pep}	1.149	-	-	14.9
IFI_3	1.364	0.027	2.0	36.4
IFI_3^{pep}	1.088	-	-	8.8
IFI_4	1.068	-	-	6.8
IFI_4^{pep}	1.182	-	-	18.2
IFI_5	0.988	-	-	1.2
IFI_5^{pep}	1.261	-	-	26.1

Table S6: Averaged IFIs obtained from fluorescence signatures of *phe4* mRNA translation under different ternary complex concentrations (± SD)

#	Fluorescence value	SD	SD [%]	Relative change [%]
(IFI_1)	(1)	-	-	-
IFI_1^{pep}	1.203	0.028	2.2	20.3
IFI_2	0.996	0.019	1.9	0.4
IFI_3	1.358	0.041	3.1	35.8
IFI_4	1.080	0.022	2.0	8.0
IFI_4^{pep}	1.181	0.007	0.6	18.1

Additional figures: Markov Process Representations

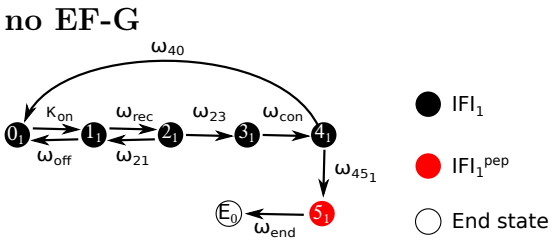

Figure S1: Representation of *phe* mRNA translation elongation as a Markov process without EF-G.

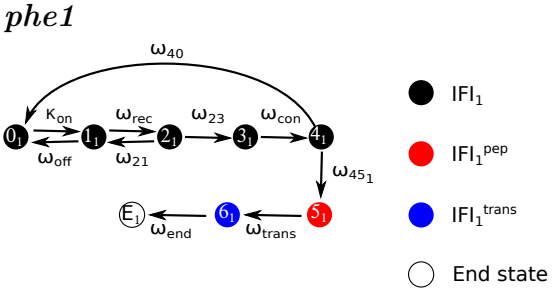

Figure S2: Representation of *phe1* mRNA translation elongation as a Markov process.

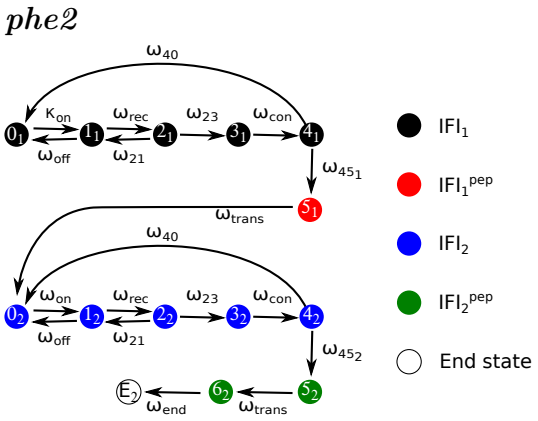

Figure S3: Representation of *phe2* mRNA translation elongation as a Markov process.

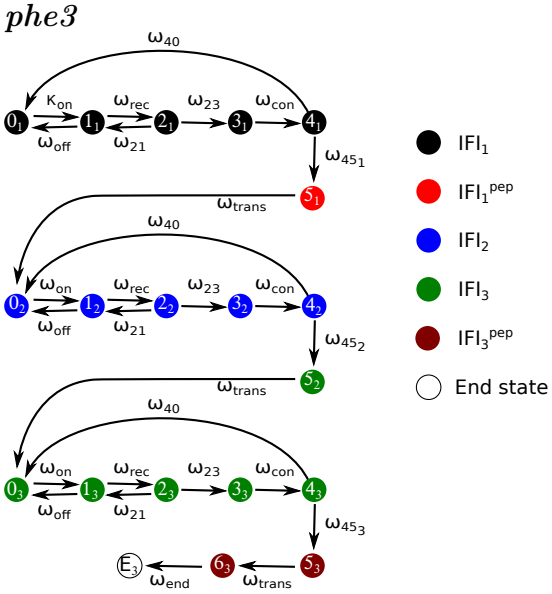

Figure S4: Representation of *phe3* mRNA translation elongation as a Markov process.

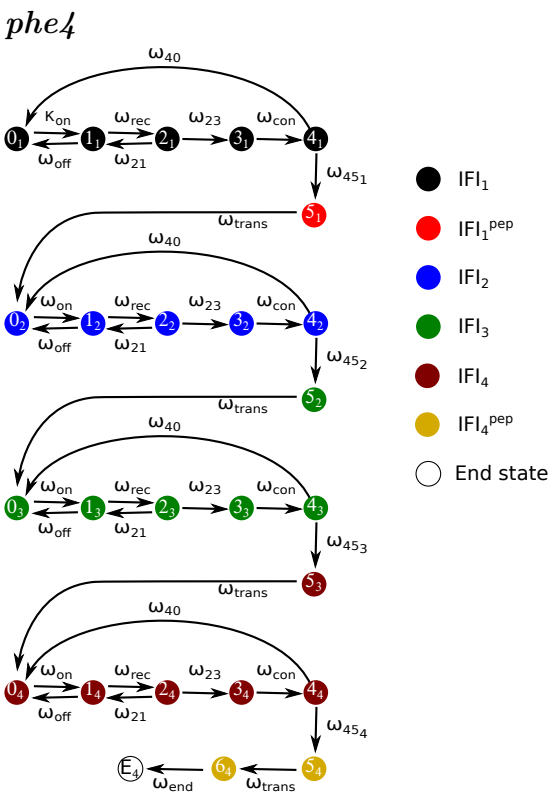

Figure S5: Representation of *phe4* mRNA translation elongation as a Markov process.

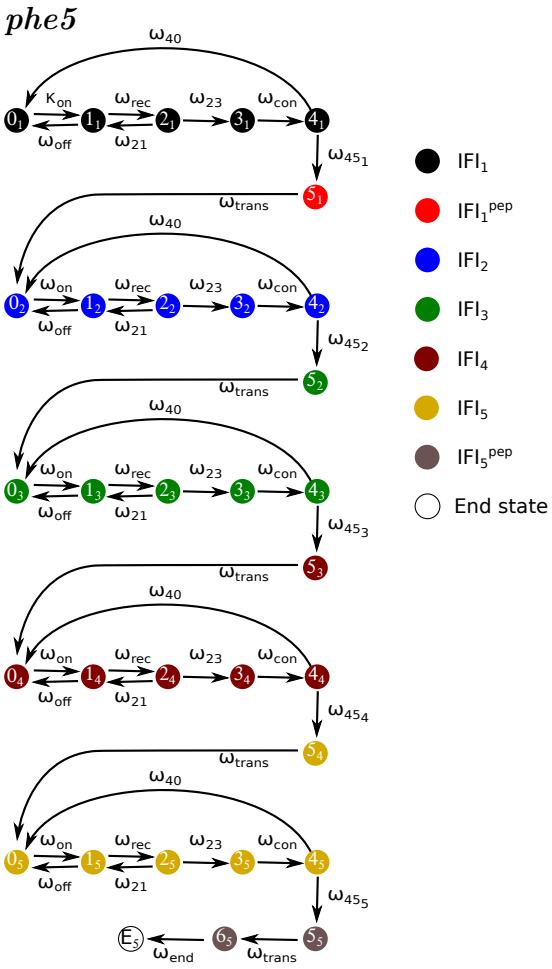

Figure S6: Representation of *phe5* mRNA translation elongation as a Markov process.

The figures S1-S6 represent Markov process descriptions of the *in-vitro* translation elongation cycle for truncated poly(U) mRNAs in the presence of only cognate ternary complexes. Each state of the Markov process corresponds to one sub-step of the elongation cycle. The fluorescent initiation complex consisting of a ribosome with BOF-Met-tRNA$^{\mathrm{fMet}}$ in the P site and the first UUU codon in the A site starts in state 0_1. Initial selection (0_1-4_1) is followed by A-site accommodation of the first Phe-tRNA$^{\mathrm{Phe}}$. After peptide bond formation (5_1), the ribosome translocates to the second Phe codon (state 0_2). The ribosomes repeat the elongation cycle until they reach the end of the truncated mRNA, thus ending up in an end state ($\mathrm{E_n}$) without mRNA in their A sites. Dots with the same color indicate states that are assigned the same intrinsic fluorescence intensity (IFI).

References

[1] S. Rudorf, M. Thommen, M.V. Rodnina, and R. Lipowsky. Deducing the kinetics of protein synthesis in vivo from the transition rates measured in vitro. *PLoS Computational Biology*, 10(10), 2014.