Mean time modeling of ribosome recycling in the presence of FA

The kinetics of the mechanism in Figure 1 can be described by a linear differential equation system consisting of the time-dependent probabilities of being in the different ribosomal states and of the rate constants connecting these states. Integration of the system, from zero to infinite time, results in an algebraic equation system (Eq. S1) from which the average time that the system spends in each ribosomal state can be derived (1).

\[
\begin{align*}
-p_0(0) &= -(k_{RERF}[RERF] + k_{G1}[G]) \tau_0 + q_{RERF}\tau_{RERF} + q_{G1[GTP]} \tau_{G1[GTP]} + q_{G1[\tau_{G1[GDP]}]} \\
-p_{G(GTP)}(0) &= k_{G1}[G] \tau_0 - (q_{G[GTP]} + k_{GTP1}) \tau_{G1[GTP]} \\
-p_{G(GDP)}(0) &= k_{GTP1} \tau_{G1[GTP]} - (q_{G} + k_{FA1}[FA]) \tau_{G1[GDP]} + q_{FA1} \tau_{G1[GDP]} \ FA \\
-p_{G(GDP)FA}(0) &= k_{FA1}[FA] \tau_{G1[GDP]} - q_{FA1} \tau_{G1[GDP]} \ FA \\
-p_{RERF}(0) &= k_{RERF}[RERF] \tau_0 - (q_{RERF} + k_{G2}[G]) \tau_{RERF} + q_{G2[GTP2]} \tau_{RERF} \ G1[GTP] + q_{G2} \tau_{RERF} \ G1[GDP] \\
-p_{RERF[G(GTP)]}(0) &= k_{G2}[G] \tau_{RERF} - (q_{G[GTP2]} + k_{GTP2}) \tau_{RERF \ G1[GTP]} \\
-p_{RERF[G(GDP)]}(0) &= k_{GTP2} \tau_{RERF \ G1[GTP]} - (q_{G2} + k_{split} + k_{FA2}[FA]) \tau_{RERF \ G1[GDP]} + q_{FA2} \tau_{RERF \ G1[GDP]} \ FA \\
-p_{RERF[G(GDP)]FA}(0) &= k_{FA2}[FA] \tau_{RERF \ G1[GDP]} - q_{FA2} \tau_{RERF \ G1[GDP]} \ FA
\end{align*}
\]

(S1)

Here \( p_{\text{comp}}(0) \) are the initial probabilities of being in the different ribosomal states, \( \text{comp} \), at incubation start. Solving the system of linear, algebraic equations with the initial condition \( p_{\text{comp}}(0) = 1 \) gives the average times that the ribosome spends in each of the states depicted in Figure 1 before it is split into subunits. The average times for the FA-free complexes, \( \tau_0 \), \( \tau_{G(GTP)} \), \( \tau_{G(GDP)} \), \( \tau_{RERF} \), \( \tau_{RERF[G(GTP)]} \), and \( \tau_{RERF[G(GDP)]} \), have been reported earlier (2). The sum of these six average times is the recycling time in the absence of FA, \( \tau_{\text{rec}} \), as specified in Eq. 1 of the main text in terms of A-parameters, defined in Table 1. In the presence of FA the total recycling time, \( \tau_{\text{recFA}} \), is the sum of \( \tau_{\text{rec}} \) and an additional delay time, \( \tau_{\text{recI}} \), which is the sum of the average times, \( \tau_{G(GDP)FA} \) and \( \tau_{RERF[G(GDP)]FA} \) that the ribosome spends in the two FA-bound states \( R_{G(GDP)FA} \) and \( R_{RERF[G(GDP)]FA} \), respectively (Figure 1):

\[
\tau_{\text{recFA}} = \tau_{\text{rec}} + \tau_{\text{recI}} = \tau_{\text{rec}} + \tau_{G(GDP)FA} + \tau_{RERF[G(GDP)]FA}
\]

(S2)

The average times of the inhibited states are obtained from Eq. S1 and given by

\[
\begin{align*}
\tau_{G(GDP)FA} &= \frac{k_{FA1}[FA]}{q_{FA1}} \left( \frac{k_{\text{cat}}}{k_{M1}} \right)_{G1} q_{RERF} \left( k_{\text{cat}} / k_{M2} \right)_{G2} \left( 1 + \frac{q_{G2}}{k_{split}} \right) + [G] \\
\tau_{RERF[G(GDP)]FA} &= \frac{k_{FA2}[FA]}{q_{FA2} k_{split}}
\end{align*}
\]

(S3)
The Michaelis-Menten parameters \((k_{\text{cat}}/K_M)_{G1}\) and \((k_{\text{cat}}/K_M)_{G2}\) in Eq. S3 are defined in Table 2. We defined two new A-parameters, \(A_6\) and \(A_7\) (Table 1), and expressed the total time of ribosome recycling in the presence of FA (Eq. S2) in terms of a total of seven A-parameters in Eqs. 1 and 2 of the main text.

**Biphasic recycling kinetics of the FA-inhibited ribosome: curve fitting**

FA is a slow inhibitor of translocation and ribosome recycling in the sense that a ribosome stays inhibited by the drug during long times compared to the time for the uninhibited reactions. In such cases the inhibition kinetics displays biphasic behavior, as in Figure 4A, with a slow phase of amplitude, \(f_S\), and average time, \(\tau_S\), as well as a fast phase amplitude, \(f_F\), and time, \(\tau_F\), where \(f_S + f_F = 1\). The slow phase amplitude, \(f_S\), approximates the fraction of ribosomes that become inhibited by FA, \(p\). In ribosome recycling, the average time of the fast phase, \(\tau_F\), decreased with decreasing amplitude, \(f_F\), and was therefore in the curve fitting approximated as \(f_F \cdot \tau_{\text{rec}}\), where \(\tau_{\text{rec}}\) is the average recycling time in the absence of FA as obtained in experiments parallel to those performed in the presence of the drug. We also defined a time \(\tau_I\) related to \(\tau_S\) through \(\tau_S = \tau_I + (1 + f_F) \cdot \tau_{\text{rec}}\), corresponding to the difference in reaction time between an inhibited and an uninhibited ribosome. Taking into account that there was a slow phase with average time \(\tau_X\) and small amplitude \(u_0\) present also in the absence of FA, we simultaneously fitted exponential functions to time traces obtained in the presence and absence of FA (see Figure 4A) according to:

\[
y_0(t) = \left( (1 - u_0) \left( f \left( t, \tau_{\text{rec}} \right) \right) + u_0 \left( f \left( t, \tau_{\text{rec}}, \tau_X \right) \right) \right) + bg_1
\]

\[
y_{FA}(t) = \left( f_F \left( (1 - u_0) \left( f \left( t, f_F \cdot \tau_{\text{rec}} \right) \right) + u_0 \left( f \left( t, f_F \cdot \tau_{\text{rec}}, \tau_X \right) \right) \right) + (1 - f_F) \left( (1 - u_0) \left( f \left( t, (1 + f_F) \cdot \tau_{\text{rec}}, \tau_I \right) \right) + u_0 \left( f \left( t, (1 + f_F) \cdot \tau_{\text{rec}}, \tau_X, \tau_I \right) \right) \right) \right) + bg_2
\]

(S4)

Here, \(y_0(t)\) refers to recycling in the absence of FA. A fraction 1-\(u_0\) of these reactions represents recycling of fully active ribosomes with average time \(\tau_{\text{rec}}\) and a fraction \(u_0\) represents recycling of partially active ribosomes with an additional slow step of average time \(\tau_X\). \(y_{FA}(t)\) refers to recycling in the presence of FA. A fraction \(f_F\) of the total amplitude belongs to the fast phase and a fraction \(f_S\) to the slow phase and of both these phases a fraction \(u_0\) has an additional slow step with average time \(\tau_X\).

This means that the functions in Eq. S4 describe processes that flow through one, two or three consecutive irreversible steps with rate constants as shown in Eq. S5:

\[
y_0 = \left\{ \begin{array}{c}
(1 - u_0) \quad \begin{array}{c}
\rightarrow \frac{1}{\tau_{\text{rec}}} \rightarrow \frac{1}{\tau_X}
\end{array}
\vspace{0.5em}
\end{array} \right\}
\]

\[
y_{FA} = f_F \left\{ \begin{array}{c}
(1 - u_0) \quad \begin{array}{c}
\rightarrow \frac{1}{(f_F \cdot \tau_{\text{rec}})} \rightarrow \frac{1}{\tau_X}
\end{array}
\vspace{0.5em}
\end{array} \right\} + \left\{ \begin{array}{c}
(1 - f_F) \quad \begin{array}{c}
\rightarrow \frac{1}{(1 + f_F) \cdot \tau_{\text{rec}}} \rightarrow \frac{1}{\tau_I} \rightarrow \frac{1}{\tau_X}
\end{array}
\vspace{0.5em}
\end{array} \right\}
\]

(S5)
Accordingly, the functions of the type $f(t, \tau_1)$, $f(t, \tau_1, \tau_2)$, and $f(t, \tau_1, \tau_2, \tau_3)$ in Eq. S4 correspond to the decrease of the difference between final and currently accumulated end products in processes with one, two or three consecutive steps. The total time for each time trace can then be calculated according to:

$$
\int_0^\infty f(t, \tau_1, \tau_2, \ldots, \tau_i) dt = \tau_1 + \tau_2 + \ldots + \tau_i
$$

(S6)

and consequently the total recycling times obtained from Eq. S4 are:

$$
\int_0^\infty y_0 dt = (1-u_0)\tau_{rec} + u_0(\tau_{rec} + \tau_x) = \tau_{rec} + u_0\tau_x
$$

(S7)

$$
\int_0^\infty y_{FA} dt = f_F((1-u_0)f_F\tau_{rec} + u_0(f_F\tau_{rec} + \tau_x)) + f_S((1-u_0)((1+f_F)\tau_{rec} + \tau_i)) + u_0((1+f_F)\tau_{rec} + \tau_x + \tau_i) = \tau_{rec} + f_S\tau_i + u_0\tau_x
$$

(S8)

The slow step with small amplitude, $u_0$, and average time $\tau_x$, was present in both the presence and absence of FA and was therefore treated as a background reaction of no functional significance. The fitting of Eq. S4 to the experimentally obtained time traces resulted in precise estimates of the average time in the absence of FA, $\tau_{rec}$, the average time that an inhibited ribosome spends in either of its inhibited states, $\tau_i$, and the fractional amplitude of the fast phase, $f_F$, for all combinations of FA, EF-G and RRF concentrations. From these parameters the fraction of the slow phase, $f_S$, and the slow phase average time $\tau_S$ could also be derived.

**Biphasic recycling kinetics of the FA-inhibited ribosome: model parameters**

Since FA-inhibited recycling kinetics are biphasic it is useful to define a per cycle probability, $p_i$, that a ribosome becomes drug inhibited. This probability can be derived from a modified version of the scheme in Figure 1 in which the drug dissociation rate constants $q_{FA1}$ and $q_{FA2}$ are set equal to zero, and we ask for the probability, $p_i$, that a ribosome starting in state $R_0$ is irreversibly inhibited and never splits into subunits. The inhibition probability is conveniently derived from the splitting probability $1-p_i$, computed by multiplying the average time, $\tau_{RRF(GDP)}$, spent in complex $R_{RRF(GDP)}$ with the rate constant $k_{split}$. By setting $q_{FA1}$ and $q_{FA2}$ equal to zero in Eq. S1 and solving the resulting set of algebraic equations, with the initial condition $p_0(0) = 1$, we find:

$$
1-p_i = k_{\text{split}}\tau_{RRF(GDP)} = \frac{K_{12}}{[FA]+K_{12}} \left( \frac{1}{w[FA]/([FA]+K_{12})+1} \right)
$$

(S9)

From this an expression for $p_i$, (Eq. 9 of the main text) can be obtained. The parameter $w$ is given by Eq. 10. Forming the ratio between $\tau_{recI}$ in Eqs. S2 and S3 and $p_i$ in Eq. 9 leads to the following expression for the time $\tau_i$ that an inhibited ribosome spends in either one of its inhibited complexes:
\[\tau_i = \frac{\tau_{\text{rec}}}{\rho_i}\]

\[= \frac{[FA] + K_{i2}}{[FA](1+w) + K_{i1} + wK_{i2}} \left( \frac{1}{q_{FA1}} \frac{(Q + [G])}{K_{i1}} + \frac{1}{q_{FA2}} \frac{1}{K_{i2}} \right) ([FA](1+w) + K_{i1}) \tag{S10}\]

We note that the first factor in Eq. S10 is close to \(1/(1+w)\) when the inhibitory constants are similar, \(K_{i1} \approx K_{i2}\). This similarity and the small variation in \(w\) with the FA concentration account for the linear dependence of \(\tau_i\) on [FA] (Figure 4C). The second factor in Eq. S10 predicts, furthermore, that the slope of the almost straight line increases with increasing ratio between the EF-G concentration, [G], and the RRF concentration, [RRF], as observed experimentally (Figure 4C).

Concerning the y-axis intercepts, \(\tau_i([FA]=0)\), of the straight lines in Figure 4C they are given by

\[\tau_i([FA]=0) = \frac{1}{q_{FA1}} \frac{K_{i2}w}{K_{i1} + wK_{i2}} + \frac{1}{q_{FA2}} \frac{K_{i1}}{K_{i1} + wK_{i2}}, \tag{S11}\]

where the \(w\)-value limits at the intercepts are given by

\[w([FA]=0) = \frac{(k_{\text{cat}} / K_M)_{G1}}{K_{\text{RRF}} [RRF]} (Q + [G]) \tag{S12}\]

**Growth inhibitory impact of FA inhibition of recycling and translocation**

The growth rate, \(\mu_e\), of a bacterial population in logarithmic phase is constant and given by (3,4):

\[\mu_e = \frac{[R_e]}{\tau_{\text{efA}} \rho_0} \tag{S13}\]

Here, \(\tau_{\text{efA}}\) is the average time for a peptide elongation cycle in the presence of FA, \([R_e]\) is the intracellular concentration of elongating ribosomes and \(\rho_0\) is the concentration of amino acid residues (or peptide bonds) in the whole proteome. Assuming that ribosomes are, during FA inhibition, mainly in elongation or recycling phase, the concentration of elongating ribosomes \([R_e]\) is related to the total ribosome concentration, \([R_T]\), through:

\[ [R_e] = f_e [R_T] = \frac{N_p \tau_{\text{efA}}}{N_p \tau_{\text{efA}} + \tau_{\text{recFA}}} [R_T] \tag{S14}\]

where \(\tau_{\text{recFA}}\) is the average ribosome recycling time in the presence of FA and \(N_p\) the average number of amino acid residues per protein. Under the assumption that \([R_T]\) does not vary with [FA] the generation time, \(\tau_G\), is given by:
\[
\tau_G = \frac{\ln 2}{\mu_e} = \tau_{eFA} \left(1 + \frac{\tau_{recFA}}{\tau_{eFA} N_p} \right) \ln 2 \cdot \rho_0 \left[\frac{R_f}{R_f} \right] = \tau_{eFA} \left(1 + r \right) \ln 2 \cdot \rho_0 \left[\frac{R_f}{R_f} \right] \tag{S15}
\]

Eq. S15 corresponds to Eq. 11 of the main text and the parameters \(\tau_{recFA}, \tau_{eFA}\) and \(r\) are given by Eqs. 2, 12 and 13, respectively. To account for ribosome-bound and free EF-G we define parameters \(\tau_{GeFA}\) and \(\tau_{GrecFA}\), which are the average times that EF-G remains ribosome-bound during an elongation cycle and during ribosome recycling, respectively. It follows from Eq. 12 that \(\tau_{GeFA}\) is given by:

\[
\tau_{GeFA} = \tau_{eFA} - \left(\tau_{Tu} + \left(\frac{K_M}{k_{cat}}\right)_{G} \frac{1}{[G]}\right) = \tau_{eminG} + \left(\frac{1}{q_{F2} [FA]} + \frac{1}{q_{F2} [RFA]} + \frac{1}{q_{F3} [RFA]} \right) [FA] \tag{S16}
\]

Subtracting the times spent in the EF-G-free ribosomal states (\(\tau_0\) and \(\tau_{RRF}\), see (2)) from the total recycling time in the presence of FA, given by Eqs. 1 and 2, gives an expression for \(\tau_{GrecFA}\):

\[
\tau_{GrecFA} = \tau_{recFA} - \left(\frac{1}{k_{RRF} [RRF]} + \frac{Q}{k_{RRF}} \left(\frac{1}{q_{RRF}} + \frac{1}{k_{RRF}} \right) \right) \tag{S17}
\]

Hence, in terms of A-parameters \(\tau_{GrecFA}\) is given by:

\[
\tau_{GrecFA} = \left(A_1 + \left(\frac{1}{k_{RRF}} \right) \frac{1}{[RRF]} \right) + \left[A_2 + \left(\frac{1}{k_{RRF}} \right) \frac{1}{[RRF]} \right] [FA] + \left(A_3 + \left(\frac{1}{k_{RRF}} \right) \frac{1}{[RRF]} \right) (1 + Q) + A_f [FA] \tag{S18}
\]

Assuming that the ribosome is either in elongation or recycling phase, the concentration of ribosome-bound EF-G, \([G_B]\), is linked to the total ribosome concentration, \([R_f]\), through:

\[
[G_B] = [R_f] \frac{N_p \tau_{GeFA} + \tau_{GrecFA}}{N_p \tau_{eFA} + \tau_{recFA}} = [R_f] r_g, \tag{S19}
\]

Here \(r_g\) is the fraction of ribosomes that are bound by EF-G. Neglecting the time for regeneration of EF-G from the GDP to the GTP form, off the ribosome, the free concentration of GTP-bound EF-G is given by:

\[
[G] = [G_f] - [R_f] r_g, \tag{S20}
\]

where \([G_f]\) is the total intracellular concentration of EF-G. An expression for the free EF-G concentration, \([G]\), can be derived by combining Eqs. S19 and S20 with Eqs. S16 and S17 for \(\tau_{GeFA}\) and \(\tau_{GrecFA}\) and Eqs. 12 and 2 from the main text for \(\tau_{eFA}\) and \(\tau_{recFA}\) and solving the resulting cubic
equation. Knowledge of \([G]\) can then be used in Eqs. S16 and S17 to find the fraction of EF-G-bound ribosomes, \(r_G\), in Eq. S19.

Under conditions of EF-G-deficiency in the living cell or in the test-tube an interesting situation arises (5). When, for instance, \([G_T] < 0.8[R_T]\), most EF-G will be ribosome-bound \(([G] \approx 0)\) even at low FA concentration so that \([G_B] \approx [G_T]\). Then, according to Eqs. S15 and S19, the total rate of protein synthesis in the cell and the generation time are determined by the total EF-G concentration and the average time per EF-G cycle:

\[
\tau_G = \frac{\tau_{GeFA}}{N_p} \left(1 + \frac{\tau_{GeFA}}{[G_T]} \right) \cdot \ln \frac{2 \cdot \rho_0}{[G_T]} = \frac{\tau_{GeFA}}{N_p} (1 + r) \cdot \ln \frac{2 \cdot \rho_0}{[G_T]} \tag{S21}
\]

where the impact ratio, \(r\), introduced in Eq. 11 of the main text, is now given by:

\[
r = \frac{\tau_{GeFA}}{\tau_{GeFA} N_p} \tag{S22}
\]

Note that the differences between the expressions for \(\tau_G\) in Eqs S15 and S21 are that \(N_p \tau_{eFA}\), \(\tau_{recFA}\) and \([R_T]\) in Eq. S15 are swapped for \(N_p \tau_{GeFA}\), \(\tau_{geFA}\) and \([G_T]\) in Eq. S21, respectively. These alterations reflect that when EF-G is in excess, the total rate of protein synthesis is determined by the total ribosome concentration and the inverse of its cycle time and that when the ribosome is in excess, the rate of protein synthesis is dominated by the total EF-G concentration and the inverse of its cycle time.

Under EF-G deficiency the impact ratio \(r\) can be written in terms of A-parameters as:

\[
r = \frac{1}{N_p \tau_{eminG}} \left(1 + \frac{1}{q_{F2}[FA] + \kappa_{i1}} + \frac{1}{q_{F2} \kappa_{i2}} + \frac{1}{q_{F3} \kappa_{i3}} \right) [FA] \tag{S23}
\]

When the [FA]-containing terms dominate, Eq. S23 simplifies to:

\[
r = \frac{1}{N_p} \left( \frac{A_b [FA]}{[RRF]} + A_r \right) \tag{S24}
\]

Eq. S24 is very similar to Eq. 14 of the main text, except that the [G]-containing term in the numerator of Eq. 14 is missing, as expected.
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