Supporting text for “Selective charging of tRNA isoacceptors explains patterns of codon usage” by Elf et al.

Quantitative description of the turnover of charged tRNAs
We define a parameter \( m_k \) as the average waiting time for a ribosome with an open A site programmed with a codon of type \( k \), according to

\[
m_k = 1/\sum_i t_i \alpha_i k_{ik}
\]  

[1]

The parameter \( k_{ik} \) is \( k_{cat}/K_m \) for the binding of a ternary complex containing EF-Tu, GTP and a tRNA isoacceptor \( i \) to a ribosome with a codon of type \( k \) in the A-site. The parameter \( k_{ik} \) is assumed to be zero for non-cognate interactions. \( t_i \) is the total concentration of isoacceptor \( i \) and \( \alpha_i \) is its fractional charging level \((0<\alpha_i<1)\). When cells are starved for one amino acid, \( \alpha_i=1 \), for all tRNAs cognate to the other nineteen amino acids. This follows from the fact that exactly balanced rates of supply of two, rate limiting, amino acids cannot be maintained in cells (J. Elf, manuscript). The total average time to translate a codon \( k \) is

\[
\tau_k = \tau_0 + m_k,
\]  

[2]

where \( \tau_0 \) is the time for peptidyl-transfer, translocation and other intra ribosomal steps (8).

The average protein elongation rate in the cell is given by

\[
v = 1/\sum_i f_i \tau_k
\]  

[3]

The total rate of consumption in protein synthesis per cell volume of a charged isoacceptor \( i \) is given by

\[
 j_i = rv \sum_k f_k t_i \alpha_i k_{ik} m_k
\]  

[4]

The parameter \( r \) is the total concentration of elongating ribosomes in the cell and the sum is taken over all cognate codons, \( k \). At steady state, the rate of deacylation of each isoacceptor \( i \) is equal to its rate of charging with amino acid, given by

\[
 j_i = s(1-\alpha_j) t_i c_i
\]  

[5]

The parameter \( s \) is the free concentration of the aminoacyl-tRNA synthetase specific for this amino acid and \( c_i \) is \( k_{cat}/K_m \) for the interaction between tRNA \( i \) and the synthetase. Equating Eqs [4] and [5] and forming ratios, lead to the following \( n-1 \) relations between the charging levels of the \( n \) isoaccepting tRNAs

\[
\frac{1-\alpha_i}{1-\alpha_j} t_i c_i = \frac{\sum_k f_k t_i \alpha_i k_{ik}/m_k}{\sum_k f_k t_i \alpha_j k_{ij}/m_j}
\]  

[6]

When there are two tRNA isoacceptors, each reading one synonymous codon, and \( c_0=0 \) for all \( i \), Eq [6] reduces to the equation in the main text. To obtain all \( \alpha_i \)-values, one more equation is required. This comes from the constraint that the total rate of supply of an amino acid must equal its consumption in protein synthesis:

\[
j_{\text{supply}} = rv \sum_i f_i ,
\]  

[7]

where the sum is taken over all codons cognate to the limiting amino acid. The maximal consumption of the amino acid in protein synthesis, \( J_{\text{max}} \), is given by Eq. 7 with all \( \alpha_i=1 \). Parameters used: \( \tau_0=0.05s \), \( k_{cat}=2 \times 10^{-5}M^{-1}s^{-1} \), cell volume \( 10^{-13} \) liters.

The sensitivity amplifications in Table 1 are calculated as

\[
100\frac{J_{\text{supply}}/J_{\text{max}} < 1 \text{ when } J_{\text{supply}}/J_{\text{max}} < 1 \text{ and 100 otherwise.}}{J_{\text{supply}}/J_{\text{max}} < 1 \text{ when } J_{\text{supply}}/J_{\text{max}} < 1 \text{ and 100 otherwise.}}
\]

Confidence intervals for codon usage
Assume that, for a particular amino acid, the total number of codons in a gene is \( n \). The probability that \( m \) of these codons have a particular identity was calculated by assuming a binomial distribution and estimating the mean value from the codon usage frequencies in table 1, normalized to make the sum of frequencies in a codon family equal to 1. A 95% confidence interval for the expected number of a specific codon was constructed symmetrically around the estimated mean (Table 2).

tRNA isoacceptor binding to synthetases and ribosomes

We designate the total concentration of an aminoacyl-tRNA synthetase by \( s_i \), the total concentration of an uncharged tRNA \( i \) in an isoacceptor family by \( t_i \), and the concentration of synthetase bound to the isoacceptor \( i \) by \( s_i \). With identical kinetics for the binding of different tRNAs in an isoacceptor family to the synthetase and pseudo-equilibrium between uncharged tRNA and the enzyme with dissociation constant \( K \), we get for all isoacceptors \( j \)

\[
\left(s_i - \sum_j s_j\right)\left(t_j - s_j\right) = K s_j
\]  

[8]

By forming ratios between pairs \((j, k)\) of isoacceptors, we get:

\[
\frac{t_j}{s_j} = \frac{t_k}{s_k}
\]  

[9]

That is, the enzyme bound concentration, ready for aminoacylation, is proportional to the total concentration of deacylated tRNAs, independently of synthetase concentration. Therefore, Eq. [6] is exact even if enzyme bound isoacceptors are neglected. Neglect of ribosome bound tRNAs also leads to exact ratios in Eq. [6], provided that there is no overlap in codon
reading among isoacceptors and provided that the
distribution of codons along mRNAs is unbiased.
In this case, the concentration of ribosome bound
isoacceptor \( i \) in a family is given by \( \gamma f_i \), where \( f_i \) is the
codon frequency and \( \gamma \) is a common constant of
proportionality. With this correction, Eq. [6] is recovered!

\[
\frac{(1-\alpha)\gamma f_j - \gamma f_i}{(1-\alpha)\gamma f_i - \gamma f_i} = \frac{f_j}{f_i} \iff \frac{(1-\alpha)\gamma f_i}{(1-\alpha)\gamma f_i - \gamma f_i} = \frac{f_i}{f_i} \tag{10}
\]

Cases, when codon reading among isoacceptors is
overlapping, have been analyzed numerically and no
significant deviations from Eq. [6] have been found. Due to
the special features of Eqs [8] and [10], the simplified Eq.
[6] is an excellent approximation.