SUPPLEMENTARY MATERIAL

An expression for the dependence of the observed rate constant \(k_{\text{obs}}\) on the concentrations of the effectors of the binary allosteric ribozyme (R), FMN (F) and theophylline (T), is derived as follows.

Equation a1 includes all forms of the uncleaved precursor RNA \(R_1\) at any instant, assuming that misfolded structures and the reverse reaction are negligible. Equation a2 includes all forms of the cleaved ribozyme \(R_1\) at any instant. Equation a3 reflects the total amount \(R_0\) of reacted and unreacted ribozyme.

\[
\begin{align*}
R_1 & = R + FR + TR + TFR \\
R_1' & = R' + FR' + TR' + TFR' \\
R_0 & = R_1 + R_1'
\end{align*}
\]

Equations b1–b4 represent the sums of the concentrations of the cleaved and uncleaved ribozyme in specific effector-bound states.

\[
\begin{align*}
R_S & = R + R' \\
FR_S & = FR + FR' \\
TR_S & = TR + TR' \\
TFR_S & = TFR + TFR'
\end{align*}
\]

During the ribozyme reaction, the fraction of RNA that remains unreacted at any instant \((Z)\) is represented by equation c.

\[Z = R/R_0\]

Since the complex \(TFR_S\) is expected to react with first order kinetics, the uncleaved TFR complex at any time \(t\) is represented by equation d, where \(k\) is the first order rate constant.

\[\text{[TFR]} = [TFR_S]e^{-kt}\]

By definition, \(k_{\text{obs}}\) for ribozyme cleavage is given by equation e at \(t = 0\).

\[k_{\text{obs}} = -d\ln(Z)/dt\]

The equilibrium constants for effector binding (Scheme 1) are given by equations f1–f4.

\[
\begin{align*}
K_R & = [FR_S]/([RS][F]) \\
K_T & = [TR_S]/([RS][T]) \\
K_{TF} & = [TFR_S]/([FR_S][T]) \\
K_{TF'} & = [TFR_S]/([TR_S][F])
\end{align*}
\]

This may be written as

\[k_{\text{obs}} = -d\ln((A + Be^{-kt})/C))/dt at t = 0\]

Upon differentiating equation i, where, \(A, B\) and \(C\) are constants, we get

\[k_{\text{obs}} = (-1) \times [1/(A + Be^{-kt})/C] \times (B(–k)e^{-kt})/C\] at \(t = 0\)

Substituting \(t = 0\),

\[k_{\text{obs}} = Bk/(A + B)\]

Then,\n
\[k_{\text{obs}} = k[FR_S]/[TFR_S] + [FR_S]/[TFR_S] + [TR_S]/[TFR_S] + 1\]

Multiplying equations f1 and f3,

\[K_R K_{TF} = [TFR_S]/([RS][F][T])\]

Rearranging,

\[R_S]/[TFR_S] = 1/(K_R K_{TF}[F][T])\]

Using equation g3 in the equation above gives

\[R_S]/[TFR_S] = 1/(\alpha K_R K_{TF}[F][T])\]

Similarly,

\[\text{[FR}_S]/[TFR_S] = 1/(\alpha K_{TF}[F])\]

Using equations m in k gives

\[
\begin{align*}
k_{\text{obs}} & = k[1/(1/\alpha K_{TF}[F][T]) + (1/\alpha K_T[T]) + (1/\alpha K_F[F])] + 1] \\
k_{\text{obs}} & = kaK_T K_F[F][T]/(1 + K_F[T] + K_T[T] + \alpha K_T[F][T]) \\
k_{\text{obs}} & = kaK_T K_F[F][T]/((1 + K_T[T]) + (K_F + \alpha K_T[F][T])[F])
\end{align*}
\]

Dividing the numerator and denominator by \((K_F + \alpha K_T[F][T])\) gives

\[
\begin{align*}
k_{\text{obs}} & = (\alpha k K_T K_F[F][T]/(K_F + \alpha K_T[F][T]))/((1 + K_T[T]/(K_F + \alpha K_T[F][T]) + [F])
\end{align*}
\]
The above equation can be rewritten as equation p, where apparent $V_{\text{max}} = (\alpha k K_F[T])/(1 + \alpha K_F[T])$ and apparent $K_m = (1 + K_F[T])/(K_F + \alpha K_F K_T[T])$.

$k_{\text{obs}} = (\text{apparent } V_{\text{max}} \times [F])/(\text{apparent } K_m + [F])$

(see equation 2 in text)