

SUPPLEMENTARY MATERIAL

An expression for the dependence of the observed rate constant (k_{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.

$R_1 = R + FR + TR + TFR$ **a1**

$R'_1 = R' + FR' + TR' + TFR'$ **a2**

$R_0 = R_1 + R'_1$ **a3**

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

$R_S = R + R'$ **b1**

$FR_S = FR + FR'$ **b2**

$TR_S = TR + TR'$ **b3**

$TFR_S = TFR + TFR'$ **b4**

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

$Z = R_1/R_0$ **c**

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

$[TFR] = [TFR_S]e^{-kt}$ **d**

By definition, k_{obs} for ribozyme cleavage is given by equation **e** at $t = 0$.

$k_{obs} = -d\{\ln(Z)\}/dt$ **e**

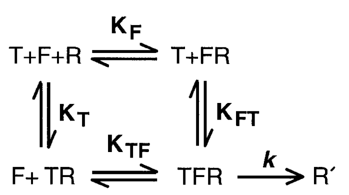
The equilibrium constants for effector binding (Scheme 1) are given by equations **f1**–**f4**.

$K_F = [FR_S]/([R_S][F])$ **f1**

$K_T = [TR_S]/([R_S][T])$ **f2**

$K_{FT} = [TFR_S]/([FR_S][T])$ **f3**

$K_{TF} = [TFR_S]/([TR_S][F])$ **f4**



Scheme 1. A kinetic framework for the activation of ribozyme function by two effectors. This kinetic model is based on three assumptions: (i) the ligand binding equilibrium is rapidly attained and thus has no influence on the rate of ribozyme cleavage; (ii) the ribozyme is active only when both effectors are bound; (iii) the ribozyme cleavage reaction does not affect the ligand binding equilibrium.

Further,

$K_{FT}/K_T = ([TFR_S][R_S])/([TR_S][FR_S])$ **g1**

$K_{TF}/K_F = ([TFR_S][R_S])/([TR_S][FR_S])$ **g2**

Therefore,

$\alpha = K_{FT}/K_T = K_{TF}/K_F$ (see equation **1** in text) **g3**

The constant α is the cooperativity coefficient as defined by Ehlert (1), which reflects the extent of binding cooperativity among effectors.

Since it is assumed that ribozyme cleavage does not affect equilibrium (Scheme 1), the concentrations of the ribozyme in its free state (R_S) and in its various complexed states (FR_S, TR_S and TFR_S) remain constant with time.

Hence, using equations **c** and **d** in **e** implies

$k_{obs} = -d\{\ln[([R_S] + [FR_S] + [TR_S] + [TFR_S]e^{-kt})/([R_S] + [FR_S] + [TR_S] + [TFR_S])]\}/dt$ at $t = 0$ **h**

where $R \approx R_S$, $TR \approx TR_S$ and $FR \approx FR_S$ during the initial phase of the cleavage reaction.

This may be written as

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

Upon differentiating equation **i**, where, A, B and C are constants, we get

$k_{obs} = (-1) \times [1/\{(A + Be^{-kt})/C\}] \times \{B(-k)e^{-kt})/C\}$ at $t = 0$. **j1**

Simplifying,

$k_{obs} = (Bke^{-kt})/(A + Be^{-kt})$ at $t = 0$. **j2**

Substituting $t = 0$,

$k_{obs} = Bk/(A + B)$ **j3**

Restoring the terms that make up A, B and C,

$k_{obs} = [TFR_S]k/([R_S] + [FR_S] + [TR_S] + [TFR_S])$ **j4**

Then,

$k_{obs} = k/([R_S]/[TFR_S] + [FR_S]/[TFR_S] + [TR_S]/[TFR_S] + 1)$ **k**

Multiplying equations **f1** and **f3**,

$K_F K_{FT} = [TFR_S]/([R_S][F][T])$ **l1**

$K_F K_{FT}[F][T] = [TFR_S]/[R_S]$ **l2**

Rearranging,

$[R_S]/[TFR_S] = 1/(K_F K_{FT}[F][T])$ **l3**

Using equation **g3** in the equation above gives

$[R_S]/[TFR_S] = 1/(\alpha K_F K_T[F][T])$ **m1**

Similarly,

$[FR_S]/[TFR_S] = 1/(\alpha K_T[T])$ **m2**

$[TR_S]/[TFR_S] = 1/(\alpha K_F[F])$ **m3**

Using equations **m** in **k** gives

$k_{obs} = k/\{(1/\alpha K_F K_T[F][T]) + (1/\alpha K_T[T]) + (1/\alpha K_F[F]) + 1\}$ **n1**

$k_{obs} = k\alpha K_F K_T[F][T]/(1 + K_F[F] + K_T[T] + \alpha K_F K_T[F][T])$ **n2**

$k_{obs} = k\alpha K_F K_T[F][T]/\{(1 + K_T[T]) + (K_F + \alpha K_F K_T[T])[F]\}$ **n3**

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

$k_{obs} = \{\alpha k K_F K_T[F][T]/(K_F + \alpha K_F K_T[T])\}/\{(1 + K_T[T])/(K_F + \alpha K_F K_T[T]) + [F]\}$ **o**

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

k_{obs} = (apparent $V_{max} \times$ [F])/(apparent K_m + [F]) (see equation **2** in text) **p**

REFERENCE

1. Ehlert,F.J. (1988) Estimation of the affinities of allosteric ligands using radiolabeled binding and pharmacological null methods. *Mol. Pharmacol.*, **33**, 187–194.