**Supplement to:** I. S. Filimonov, A. P. Berzova, V. I. Barkhatov, A. V. Krivoshey, N. A. Trushkin, and P. V. Vrzheshch, Negative Cooperativity in the Interaction of Prostaglandin H Synthase-1 with the Competitive Inhibitor Naproxen Can Be Described as the Interaction of a Non-competitive Inhibitor with Heterogeneous Enzyme Preparation (ISSN 0006-2979, *Biochemistry (Moscow)*, 2018, Vol. 83, No. 2, pp. 119-128)

If we assume that the concentration of the inhibitor considerably exceeds the concentration of the enzyme (i.e., $I$ = const), it is easy to obtain the corresponding analytical expressions for the kinetics of inhibition.

The normalized rate for the cooperative model (9):

$$V_{norm}(I,t) = A_1 \cdot \exp(-\lambda_1 t) + A_2 \cdot \exp(-\lambda_2 t) + \\ + \frac{K_2 \cdot (K_1 + I \cdot \gamma)}{I^2 + 2 \cdot K_2 \cdot I + K_1 \cdot K_2}, \quad \text{(S1)}$$

$$\lambda_{1,2} = \frac{1}{2}\left(k_{-1} + 2 \cdot k_1 \cdot I + k_2 \cdot I + 2 \cdot k_{-2} \mp D\right), \quad \text{(S2)}$$

$$D = \sqrt{\left(k_{-1} + 2 \cdot k_1 \cdot I - k_2 \cdot I - 2 \cdot k_{-2}\right)^2 + 4 \cdot k_{-1} \cdot k_2 \cdot I}, \quad \text{(S3)}$$

where $k_1$, $k_2$ are elementary rate constants of the enzyme–inhibitor association; $k_{-1}$, $k_{-2}$ are rate constants of the enzyme–inhibitor dissociation ($K_1 = k_{-1}/k_1$, $K_2 = k_{-2}/k_2$), $A_1$ and $A_2$ are time-independent coefficients.

The normalized rate for the heterogeneous model (14):

$$V_{norm}(I,t) = A_\alpha \cdot \exp(-\lambda_\alpha t) + A_\beta \cdot \exp(-\lambda_\beta t) + \\ + \frac{1}{(1+\chi)} \cdot \frac{K_\alpha}{K_\alpha + I} + \frac{\chi}{(1+\chi)} \cdot \frac{K_\beta}{K_\beta + I}, \quad \text{(S4)}$$

$$\lambda_\alpha = k_{-\alpha} + k_\alpha \cdot I, \quad \text{(S5)}$$

$$\lambda_\beta = k_{-\beta} + k_\beta \cdot I, \quad \text{(S6)}$$

where $k_\alpha$, $k_\beta$ are elementary rate constants of the enzyme–inhibitor association; $k_{-\alpha}$, $k_{-\beta}$ are rate constants of the enzyme–inhibitor dissociation ($K_\alpha = k_{-\alpha}/k_\alpha$, $K_\beta = k_{-\beta}/k_\beta$); $A_\alpha$ and $A_\beta$ are time-independent coefficients.

If $K_2 >> K_1$ and taking into account that $0 \le \gamma < 2$ (see above), the free member of Eq. (S1) (the right side of Eq. (13)) becomes equal to the right side of Eq. (19). At the same time, the difference between the dimensionless $V_{norm}$ values in Eqs. (13) and (19) is comparable to the value of $K_1/K_2 << 1$.

Indeed, within the intervals $I << K_1$ and $I \sim K_1$, the $I$ value can be neglected in comparison to $K_2$; consequently, Eq. (19) is true. In the interval $K_1 << 1 << K_2$, let us neglect the $I$ value in relation to $K_2$ and $K_1$ in relation to $I$. If $\gamma \sim 1$ (comparison of two models is meaningful if $0 \le \gamma < 2$), Eq. (19) is true. The same is valid for the $\gamma$ intervals: $\gamma << 1$, but $\gamma \cdot I \sim K_1$; $\gamma << 1$, but $\gamma \cdot I << K_1$. If we neglect $K_1$ in comparison with $I$ within the interval $I \sim K_2$, then Eq. (19) is true if $\gamma \sim 1$. If $\gamma << 1$, right members of Eqs. (13) and (19) are comparable with the $K_1/K_2 << 1$ value, and in this case, the two models produce same results. If we neglect $K_1$ and $K_2$ in comparison with $I$ in the interval $I >> K_2$, then Eq. (19) is true if $\gamma \sim 1$. If $\gamma << 1$, the right members of Eqs. (13) and (19) are comparable with or significantly less than $K_1/K_2 << 1$, and both models produce equal results as well. Therefore, free members in Eqs. (S1) and (S4) are the same, if conditions (20)-(22) are fulfilled.

Moreover, if the following relations are fulfilled:

$$k_\alpha = 2 \cdot k_1, \quad \text{(S7)}$$

$$k_{-\alpha} = k_{-1}, \quad \text{(S8)}$$

$$k_\beta = k_2, \quad \text{(S9)}$$

$$k_{-\beta} = 2 \cdot k_{-2}, \quad \text{(S10)}$$

then $A_1 = A_\alpha$, $A_2 = A_\beta$, $\lambda_1 = \lambda_\alpha$, $\lambda_2 = \lambda_\beta$, i.e., Eqs. (S1) and (S4) completely coincide, and the heterogeneous model (14) describes kinetics of cooperative interaction of the inhibitor with the dimeric enzyme (9).

Comparison of the above chosen group of constants is, of course, completely illustrative.