Part5: Algorithm to computationally estimate the effect of Pi

In order to estimate whether Pi has an activatory or an inhibitory effect on the enzymes, the computed probe binding energies were compared with those for the LDH from *L. plantarum* whose activity is known to be unaffected by Pi.

The binding energies of the Pi probe in the allosteric binding site (AS) and the COO probe in the catalytic binding site (CS) of LDH from *L. plantarum* were defined as $E_{AS,threshold}$ and $E_{CS,threshold}$, respectively. For the other LDH enzymes, the activity was considered to be enhanced by Pi, if the binding energy of the Pi probe in the allosteric binding site, $E_{Pi} \leq E_{AS,threshold}$. When the binding energy of Pi in the catalytic site $E_{Pi} \geq E_{CS,threshold}$, no inhibition by Pi was anticipated. The magnitude of activation and inhibition by Pi was assessed by computing and analyzing the following energy differences: $\Delta E_{AS} = E_{Pi} - E_{AS,threshold}$; $\Delta E_{CS} = E_{Pi} - E_{CS,threshold}$; $\Delta E_{CS}^{pr} = E_{Pi} - E_{COO}$. A strong enhancement of enzyme activity (designated by letter *A*) was assigned, if $\Delta E_{AS} \leq -2$ kcal/mol. The letter *a* designates weak activation, when $-2 \leq \Delta E_{AS} \leq 0$ kcal/mol. Strong inhibition (*I*) was assigned when both $\Delta E_{CS} < 0$ and $\Delta E_{CS}^{pr} \leq -2$ kcal/mol. Weak inhibition (*i*) was assumed when both $\Delta E_{CS} < 0$ and $-2 \leq \Delta E_{CS}^{pr} \leq 0$ kcal/mol. For $\Delta E_{CS} \geq 0$, no competition between Pi and COO in the catalytic site, and thus no inhibition, was expected (¬*I*).

The following relations were used to deduce the overall effect of the presence of Pi considering the possibility of Pi binding at the allosteric site and the catalytic site: $A \wedge \neg I \to A$; $a \wedge \neg I \to a$; $\square \wedge \neg I \to \neg E$ (no effect); $A \wedge i \to a \vee \neg E$; $\square \wedge i \to i$; $A \wedge I \to \neg E \vee i$; $\square \wedge I \to I$. Here, we use the common logical connectives (30) ∧ for "AND", ∨ for "OR", ¬ for negation "NOT" and □ for the cases when the allosteric binding site is occupied by FBP and thus not accessible for Pi.