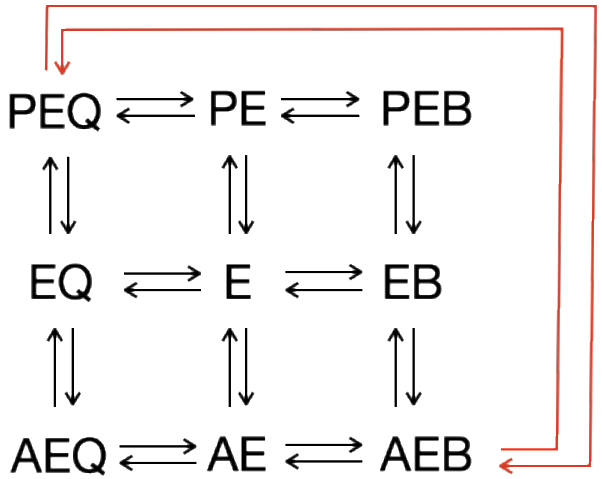
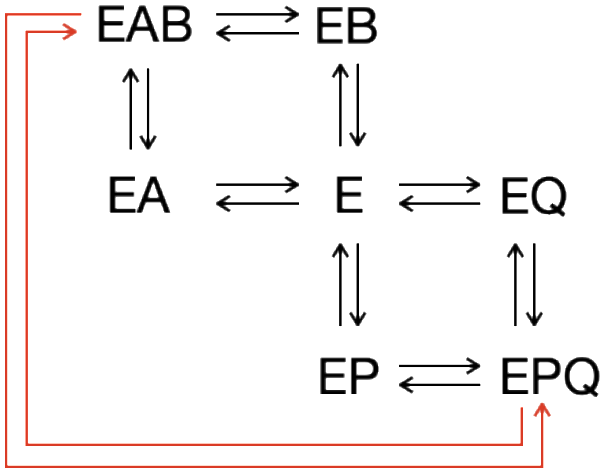
monomeric enzyme kinetics and effectors (activators and inhibitors)

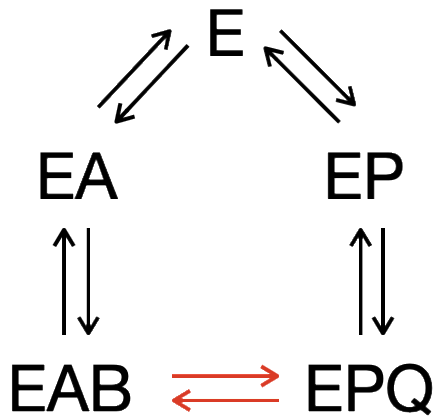
basic modelling of biological networks

derive enzyme kinetics for $A + B \rightleftharpoons P + Q$.

three different state-transition diagrams







The rate equations differ in their denominator and not in their numerator!

derive enzyme kinetics for $A + B \rightleftharpoons P + Q$.

three different state-transition diagrams, spot the logic!

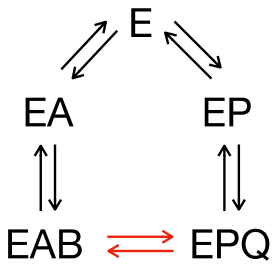
Reaction

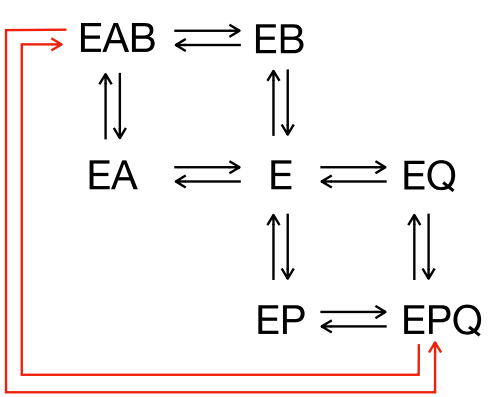
$$A + B \rightleftharpoons P + Q$$

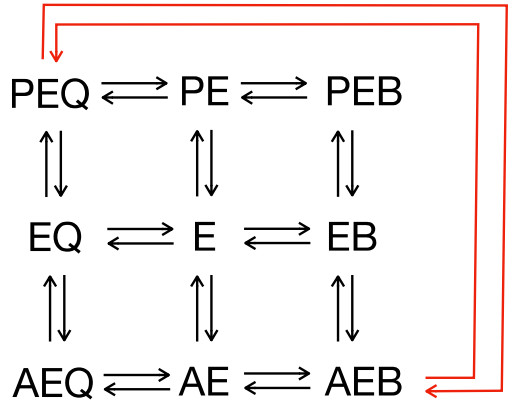
Rate equation

$$v = \frac{V_M^+ \prod_i \frac{s_i}{K_{S,i}} - V_M^- \prod_j \frac{p_j}{K_{P,j}}}{\mathcal{B}}, \quad \mathcal{B} = \mathcal{A} \times \mathcal{C}$$

Catalytic site mechanisms







Catalytic site binding polynomial $\mathcal{C}$

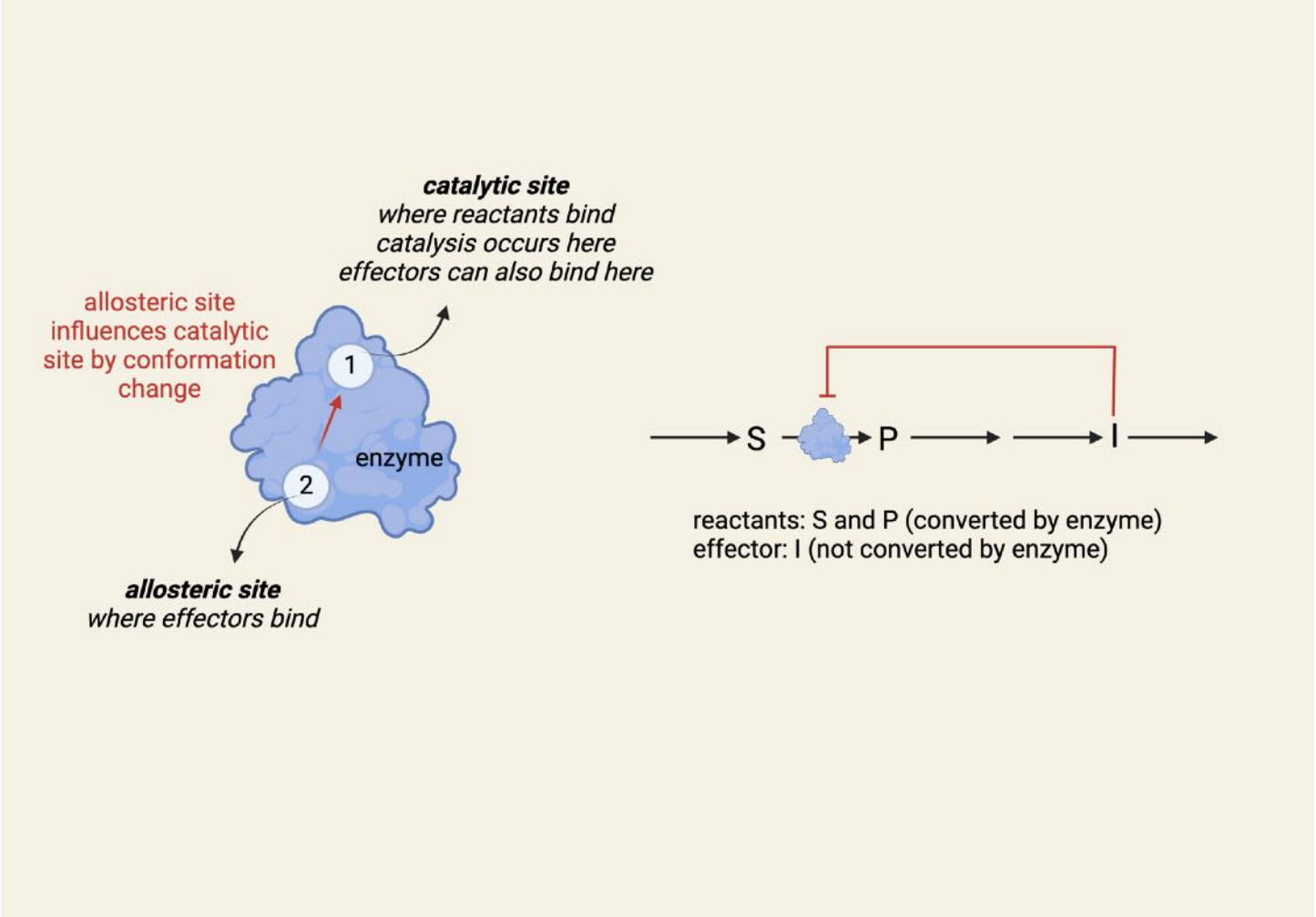
$$1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}$$

$$1 + \frac{a}{K_A} + \frac{b}{K_B} + \frac{ab}{K_A K_B} + \frac{p}{K_P} + \frac{q}{K_Q} + \frac{pq}{K_P K_Q}$$

$$\left(1 + \frac{a}{K_A} + \frac{p}{K_P}\right)\left(1 + \frac{b}{K_B} + \frac{q}{K_Q}\right)$$

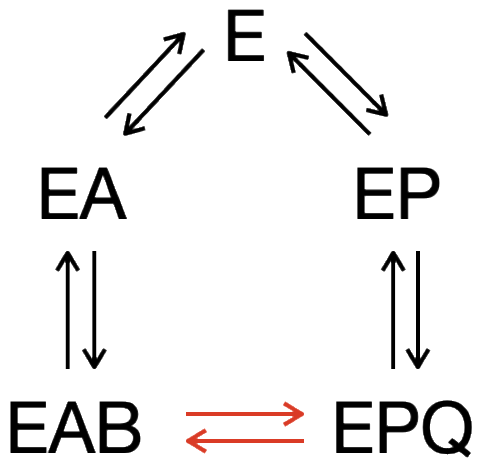
every state in the state-transition diagram has a term in the binding polynomial

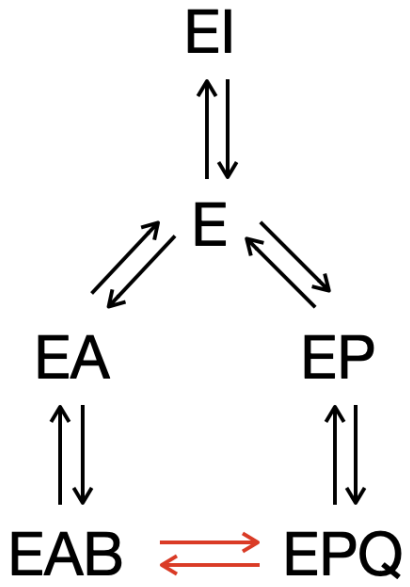
Catalytic and allosteric sites on enzymes



inhibiting effector binding in the catalytic site

Assume now that the inhibitor binds to the catalytic site to that no other reactant can bind to it.
How does this mechanism change?



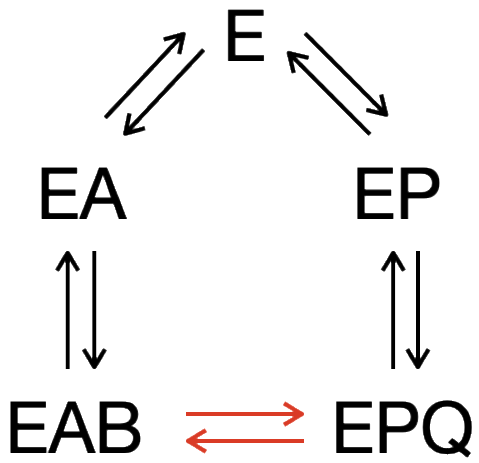


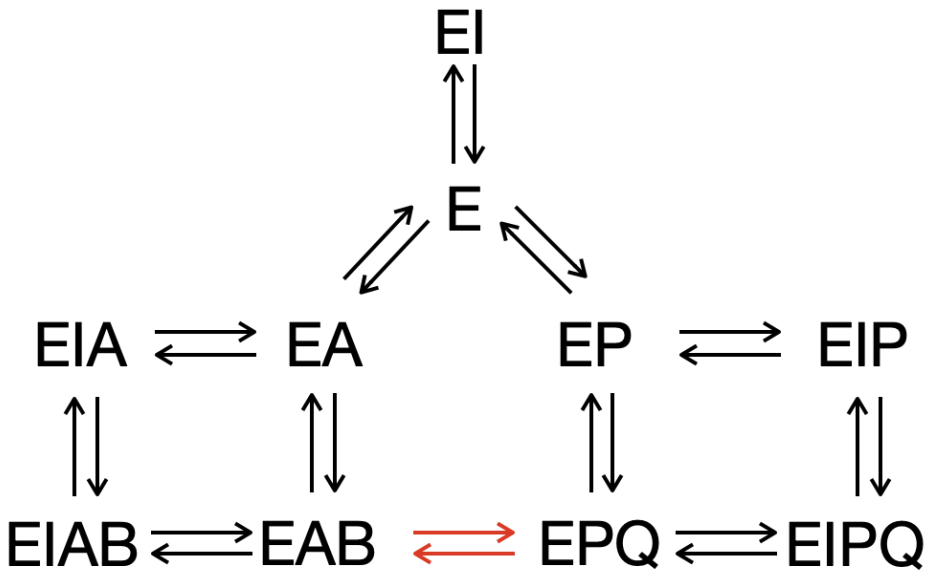
Derive the new binding polynomial

$$\mathcal{B} = 1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q} + \frac{i}{K_I}$$

inhibiting effector binding in an allosteric site

Assume now that the inhibitor binds to an allosteric site.
How does this mechanism change?



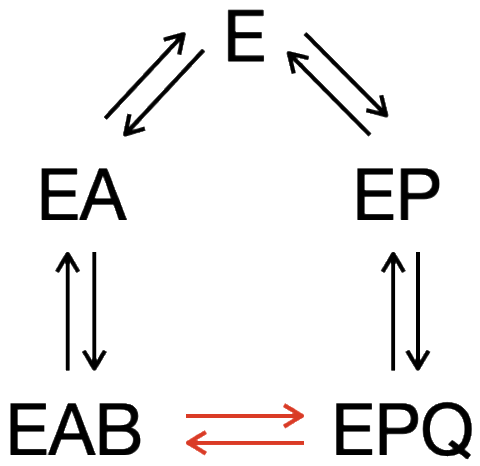


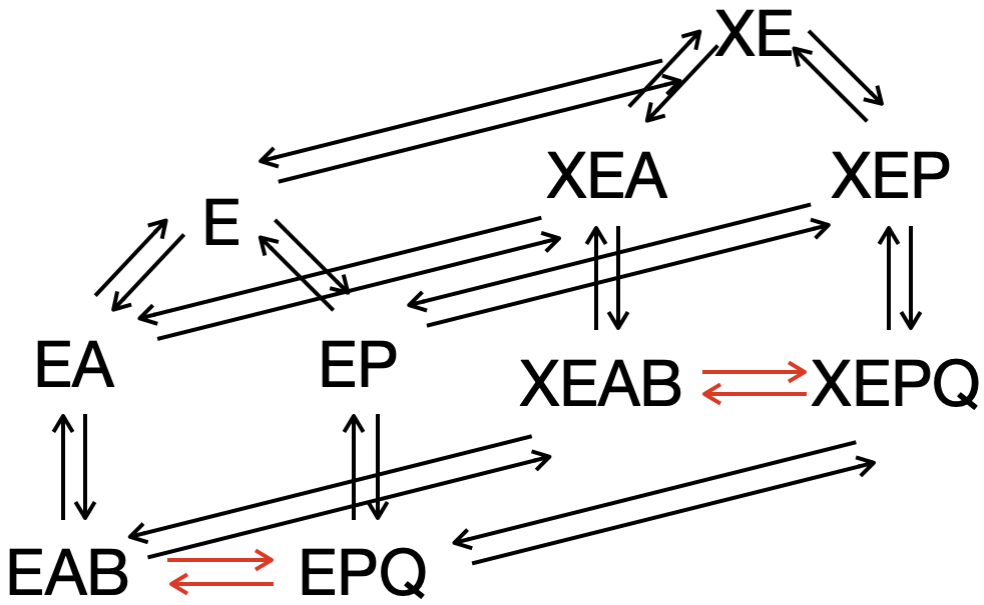
Derive the new binding polynomial.
Does it factorise?

$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a \cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p \cdot q}{K_P K_Q}\right)}_{\mathcal{C}} \underbrace{\left(1 + \frac{i}{K_I}\right)}_{\mathcal{A}}$$

activating effector binding to allosteric site

Assume now that an activator binds to an allosteric site.
How does this mechanism change?

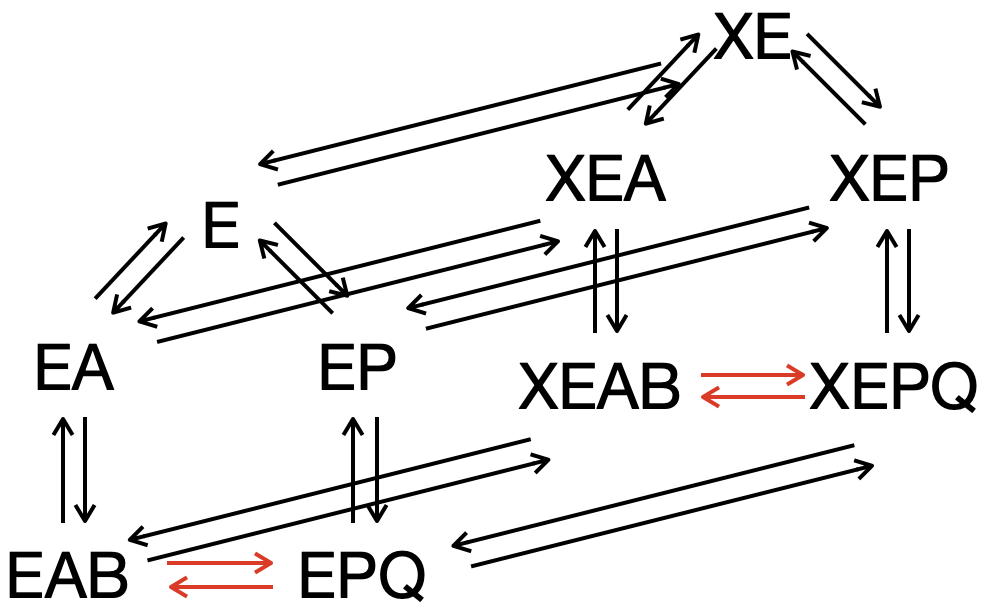




activating effector binding to allosteric site

now the denominator and numerator change of the rate equation

Derive the rate equation:



$$v = k^{+}eab + k'^{+}exab - k^{-}epq - k'^{-}expq$$

$$v = k^{+}\frac{e\cdot a\cdot b}{K_A K_B} + k'^{+}\frac{e\cdot a\cdot b\cdot x}{K_A K_B K_X} - k^{-}\frac{e\cdot p\cdot q}{K_P K_Q} - k'^{-}\frac{e\cdot p\cdot q\cdot x}{K_P K_Q K_X}$$

$$e_T = e + ea + eab + ep + epq + ex + eax + eabx + epx + epqx$$

$$= e\underbrace{\left(1 + \frac{a}{K_A} + \frac{a\cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p\cdot q}{K_P K_Q}\right)}_{\mathcal{C}}\underbrace{\left(1 + \frac{x}{K_X}\right)}_{\mathcal{A}}$$

$$= e\mathcal{C}\mathcal{A} \quad \Rightarrow \quad e = \frac{e_T}{\mathcal{C}\mathcal{A}} = \frac{e_T}{\mathcal{B}}$$

$$v = \frac{k^{+}e_T\frac{ab}{K_A K_B} + k'^{+}e_T\frac{abx}{K_A K_B K_X} - k^{-}e_T\frac{pq}{K_P K_Q} - k'^{-}e_T\frac{pqx}{K_P K_Q K_X}}{\mathcal{B}}$$

$$= \frac{k^{+}e_T\frac{ab}{K_A K_B}\left(1 + \alpha\frac{x}{K_x}\right) - k^{-}e_T\frac{pq}{K_P K_Q}\left(1 + \beta\frac{x}{K_x}\right)}{\mathcal{B}}$$

$$\mathcal{B} = \underbrace{\left(1 + \frac{a}{K_A} + \frac{a\cdot b}{K_A K_B} + \frac{p}{K_P} + \frac{p\cdot q}{K_P K_Q}\right)}_{\mathcal{C}}\underbrace{\left(1 + \frac{x}{K_X}\right)}_{\mathcal{A}}$$

Pyruvate kinase (PK) often mutated in cancer

activated by FBP, patients have different PK mutations

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Cancer-associated mutations in human pyruvate kinase M2 impair enzyme activity

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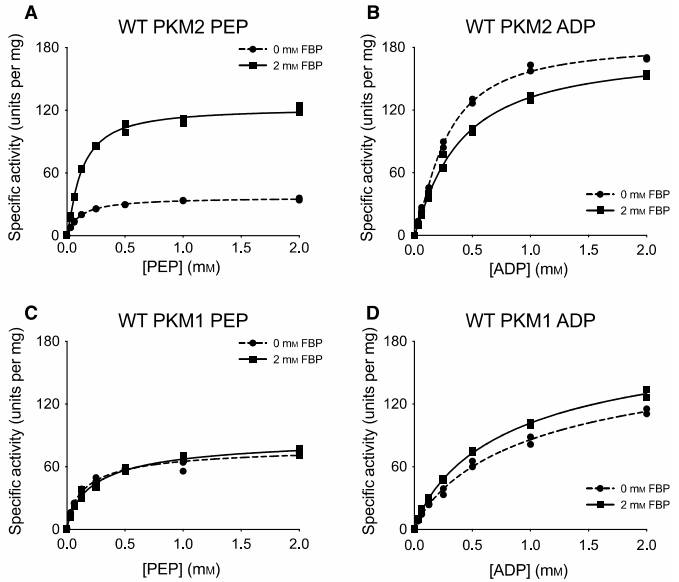
Mammalian pyruvate kinase catalyzes the final step of glycolysis, and its M2 isoform (PKM2) is widely expressed in proliferative tissues. Mutations in PKM2 are found in some human cancers; however, the effects of these mutations on enzyme activity and regulation are unknown. Here, we characterized five cancer-associated PKM2 mutations, occurring at various locations on the enzyme, with respect to substrate kinetics and activation by the allosteric activator fructose-1,6-bisphosphate (FBP). The mutants exhibit reduced maximal velocity, reduced substrate affinity, and/or altered activation by FBP. The kinetic parameters of five additional PKM2 mutants that have been used to study enzyme function or regulation also demonstrate the deleterious effects of mutations on PKM2 function. Our findings indicate that PKM2 is sensitive to many amino acid changes and support the hypothesis that decreased PKM2 activity is selected for in rapidly proliferating cells.

Keywords: allostery; cancer; enzymology; mutation



Pyruvate kinase (PK) often mutated in cancer

activated by FBP, patients have different PK mutations

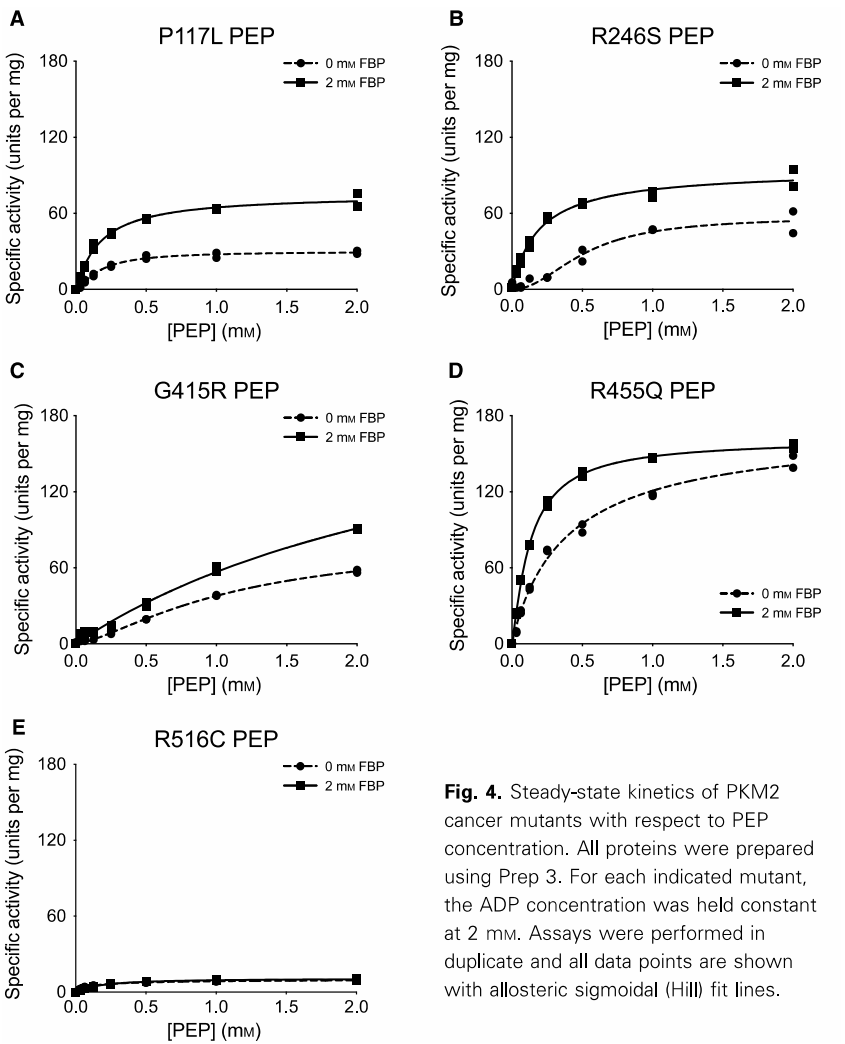


Fig. 4. Steady-state kinetics of PKM2 cancer mutants with respect to PEP concentration. All proteins were prepared using Prep 3. For each indicated mutant, the ADP concentration was held constant at 2 mM. Assays were performed in duplicate and all data points are shown with allosteric sigmoidal (Hill) fit lines.

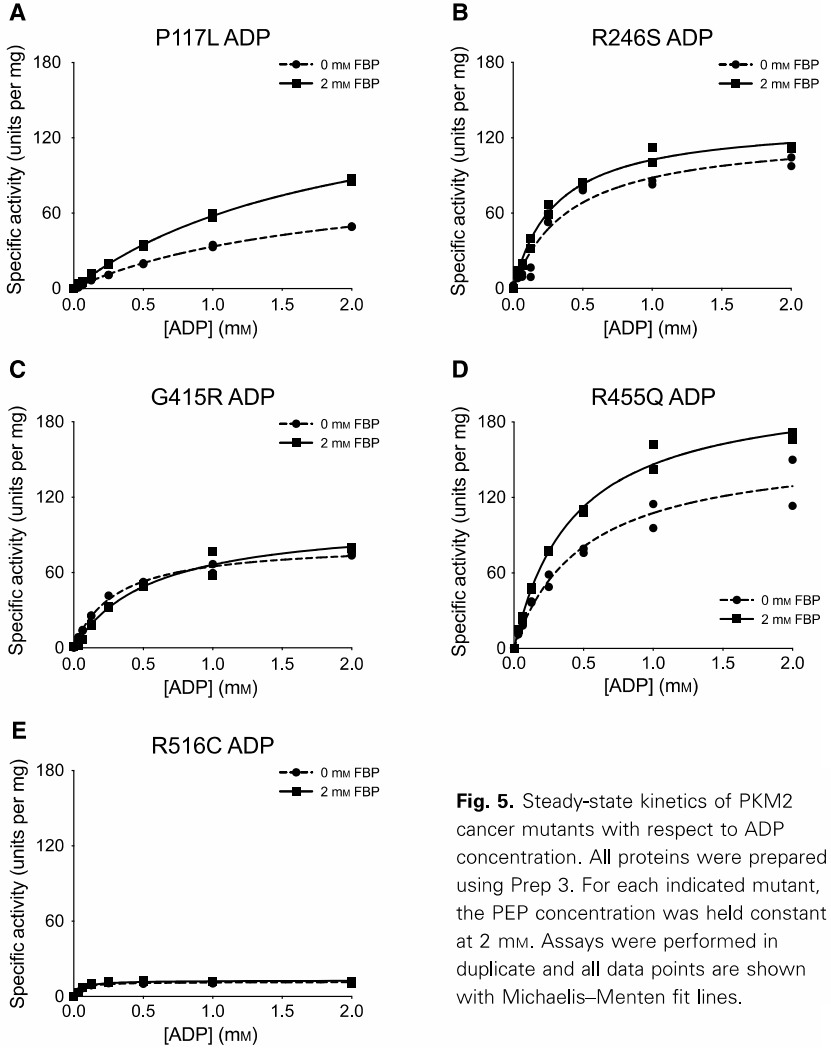


Fig. 5. Steady-state kinetics of PKM2 cancer mutants with respect to ADP concentration. All proteins were prepared using Prep 3. For each indicated mutant, the PEP concentration was held constant at 2 mM. Assays were performed in duplicate and all data points are shown with Michaelis–Menten fit lines.

Pyruvate kinase (PK) often mutated in cancer

activated by FBP, patients have different PK mutations

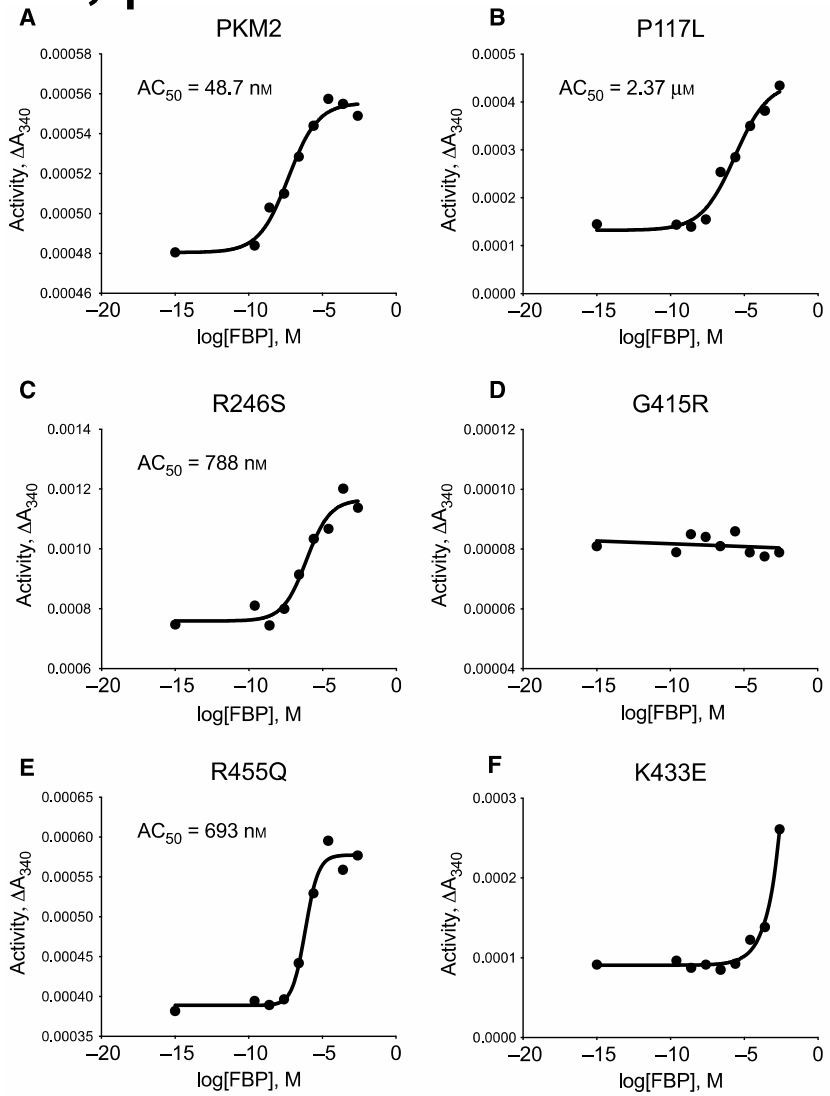


Fig. 6. Activation of PKM2 and PKM2 cancer mutants by FBP. All proteins were prepared using Prep 3. For each indicated mutant, activity was assessed with 2 mM ADP and 0.125 mM PEP and data points are means of duplicate measurements. Data were fit to a sigmoid dose–response curve with variable slope, except that G415R was fit with linear regression.