

1. As discussed several times in class, epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase which will phosphorylate tyrosines on selected substrates. EGFR (mol. wt ~180 kDa (180,000 g/mol)) is commercially available as a purified protein and is sold according to the number of units, where 1 unit is equal to the amount of enzyme necessary to catalyze the conversion of 1 pmol of phosphate from ATP to substrate tyrosine per minute at 37 C. V_{max} for this enzyme is 15,000 units per mg of enzyme.

a. Assume Michaelis-Menten kinetics and that the substrate concentration is much greater than K_M . How many moles of substrate are phosphorylated per second per mg of enzyme?

b. To test out the specificity of the enzyme, you set up 2 in vitro kinase reactions. For the first reaction, the solution contains 1 micromolar SHC, 100 mM ATP, and 10 mM $MgCl_2$. The second reaction contains 10 micromolar c-Cbl, 100 mM ATP, and 10 mM $MgCl_2$. K_M for EGFR-SHC is 1 μM , K_M for EGFR-c-Cbl is 2 μM . In both solutions, EGFR concentration is 1 μM .

For both in vitro kinase reactions, calculate how much of the enzyme is bound by substrate. State any assumptions/approximations you use in solving this problem. How does the fraction of enzyme bound affect the rate of reaction?

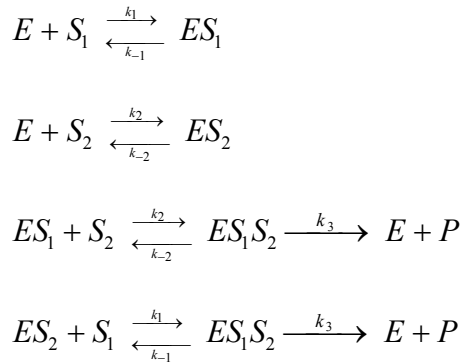
2. The Cheng-Prusoff Equation aims to relate the value of IC_{50} for a drug to the values of K_I , $[S]$, and K_M for the relevant enzyme [Cheng & Prusoff, *Biochemical Pharmacology* 22: 3099 {1973}]:

$$IC_{50} = K_I / \{ 1 + ([S] / K_M) \}$$

Determine how well this expression approximates the value of IC_{50} that could be calculated from our in-class treatment for competitive inhibitors of EGFR (which possesses $K_M = 17 \mu M$ for the relevant substrate) across a range of K_I from 10 nM to 10 μM , as a function of $[S]$ over the range 0.1 mM to 5 mM.

3. Many enzymes need to bind two different substrates in order to catalyze their reaction. For instance, kinases typically need to bind ATP along with a protein substrate in order to transfer a phosphate group from ATP to the protein substrate; the resulting product is then the phosphorylated protein substrate along with ADP. In order to analyze this situation, very common in cell signaling pathways, we need to extend the Michaelis-Menten treatment to obtain an analogous expression for the kinase reaction rate.

a. Let the protein substrate be denoted S_1 and ATP be denoted S_2 , and the phosphorylated protein substrate be denoted P . (We can neglect explicit consideration of the consequent ADP product here.) Under the assumption that S_1 and S_2 can bind to E independently, without any mutual interference and without any particular order being required, we can write the following reaction scheme:



Making assumptions similar to those for the single-substrate enzyme Michaelis-Menten treatment, develop an analogous expression for the enzymatic reaction rate:

$$d[P]/dt = k_3 [E_1S_1S_2]$$

in terms of the various individual-step rate constants and the total enzyme concentration, $[E]_0$.

b. Determine how a small-molecule kinase inhibitor drug, I , that is competitive with ATP for binding to the kinase, will affect the enzymatic reaction rate. Calculate the IC_{50} for this drug, for the the following parameter values: $K_I = 10 \text{ nM}$, $K_{M1} = 1 \text{ } \mu\text{M}$, $[S_1] = 1 \text{ } \mu\text{M}$, $K_{M2} = 100 \text{ } \mu\text{M}$, $[S_2] = 3 \text{ mM}$.