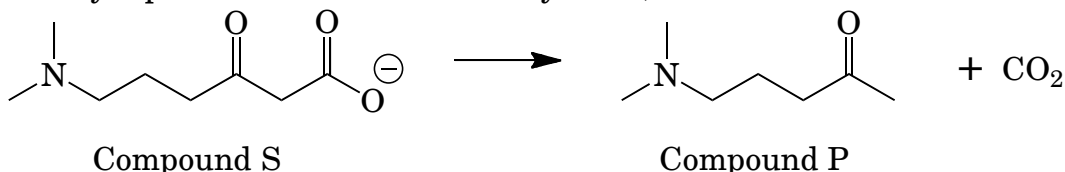
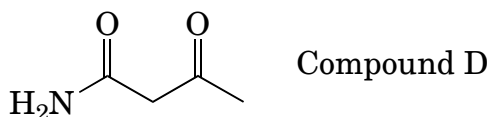


You have discovered a new enzyme, A, and you have begun to characterize it. As part of this process, you obtained structural information for enzyme A bound to compound D; a pdb file is on the course website. (Note: in the pdb file, compound D may show up in SwissPDBViewer as d247. Note also that the Enzyme A pdb file contains only a part of the structure of enzyme A.)



- You run a series of assays at 25°C on enzyme A. You discover that enzyme A is capable of catalyzing the reaction shown above. You measure the velocity for a range of S concentrations, and calculate the parameters shown in the table. What does this tell you about the enzyme?

	Enzyme A	Enzyme A + 10 μ M Compound D	Enzyme A + 1 μ M Compound D
K_m (mM)	5.7	5.7 (K_m app 62.65)	5.7 (K_m app 11.40)
k_{cat}	350	350	350
K_i		0.001	0.001
[I]		0.01	0.001



- You discover that compound D, in addition to assisting in obtaining the structural information, alters enzyme A activity. What is the effect of Compound D? Is this logical? Why? How is compound D associated with the enzyme?
- You mutate several different residues within the protein. Mutations are indicated by “Arg30Gln” (arginine at position 30 mutated to glutamine). Mutations at several different positions resulted in altered activity; data for five of these positions using compound S as substrate are shown below. Mutation of Lys127 to Ala, in addition to yielding the altered kinetics shown, resulted in an unstable protein. Why might this be true?

	Arg30Gln	Glu62Gln	Tyr75Phe	Glu77Gln	Lys127Ala
K_m (mM)	5.7	5.7	50	5.7	60
k_{cat}	5	40	100	5	30

- What is each mutant doing to the parameters for enzyme A? What do these parameter changes suggest about the possible role of these residues in the enzyme?
- How does enzyme A catalyze the reaction? (Please propose a possible reaction mechanism, preferably showing logical electron movement, and explain how it accounts for your observations above.)