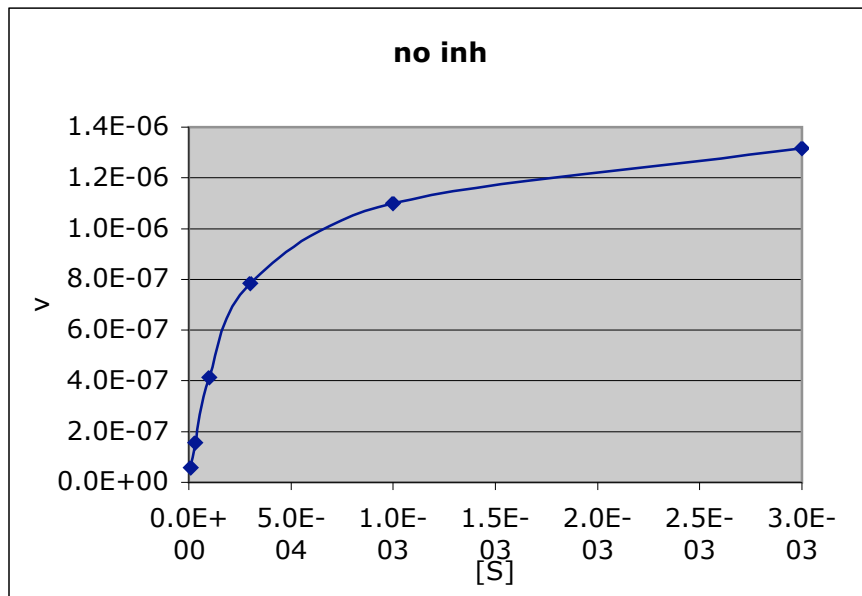


Problem 1

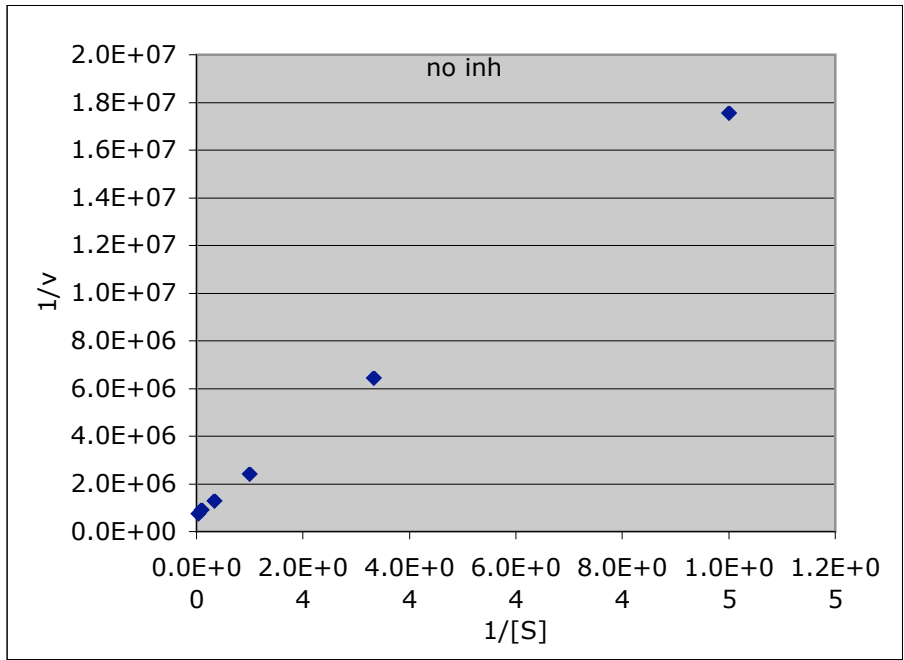
[I] (M) =	0	1E-08	3E-08	0.0000001	0.0000003	0.000001
[S] (μ M)	P (pmol)	P (pmol)	P (pmol)	P (pmol)	P (pmol)	P (pmol)
10	171	145	111	63	28	9.6
30	466	400	319	180	84	28
100	1240	1090	915	562	256	93
300	2350	2110	1880	1300	681	260
1000	3300	3190	3020	2580	1710	785
3000	3950	3840	3630	3410	2790	1690

[I] (nM) =	0	10	30	100	300	1000
[S] (μ M)	P (M)	P (M)	P (M)	P (M)	P (M)	P (M)
10	1.71E-06	1.45E-06	1.11E-06	6.30E-07	2.80E-07	9.60E-08
30	4.66E-06	4.00E-06	3.19E-06	1.80E-06	8.40E-07	2.80E-07
100	1.24E-05	1.09E-05	9.15E-06	5.62E-06	2.56E-06	9.30E-07
300	2.35E-05	2.11E-05	1.88E-05	1.30E-05	6.81E-06	2.60E-06
1000	3.30E-05	3.19E-05	3.02E-05	2.58E-05	1.71E-05	7.85E-06
3000	3.95E-05	3.84E-05	3.63E-05	3.41E-05	2.79E-05	1.69E-05

[I] (M) =	0.E+00	1.E-08	3.E-08	1.E-07	3.E-07	1.E-06
[S] (M)	v (M/s)	v (M/s)	v (M/s)	v (M/s)	v (M/s)	v (M/s)
0.00001	5.70E-08	4.83E-08	3.70E-08	2.10E-08	9.33E-09	3.20E-09
0.00003	1.55E-07	1.33E-07	1.06E-07	6.00E-08	2.80E-08	9.33E-09
0.0001	4.13E-07	3.63E-07	3.05E-07	1.87E-07	8.53E-08	3.10E-08
0.0003	7.83E-07	7.03E-07	6.27E-07	4.33E-07	2.27E-07	8.67E-08
0.001	1.10E-06	1.06E-06	1.01E-06	8.60E-07	5.70E-07	2.62E-07
0.003	1.32E-06	1.28E-06	1.21E-06	1.14E-06	9.30E-07	5.63E-07



[I] (M) =	0.E+00	1.E-08	3.E-08	1.E-07	3.E-07	1.E-06
1/[S]	1/v	1/v	1/v	1/v	1/v	1/v
100000	1.75E+07	20689655	27027027	47619048	107142857	312500000
33333.333	6437768.2	7500000	9404388.7	16666667	35714286	107142857
10000	2419354.8	2752293.6	3278688.5	5338078.3	11718750	32258065
3333.3333	1276595.7	1421800.9	1595744.7	2307692.3	4405286.3	11538462
1000	909090.91	940438.87	993377.48	1162790.7	1754386	3821656.1
333.33333	759493.67	781250	826446.28	879765.4	1075268.8	1775147.9



b	738853.43	1/Vmax			
m	168.33137	Km/Vmax			
Vmax	1.353E-06	M/s			
Km	0.0002278	M	228	μM	

[E]	4E-11	M
kcat	3.38E+04	s-1
kcat/Km	1.49E+08	M-1 s-1

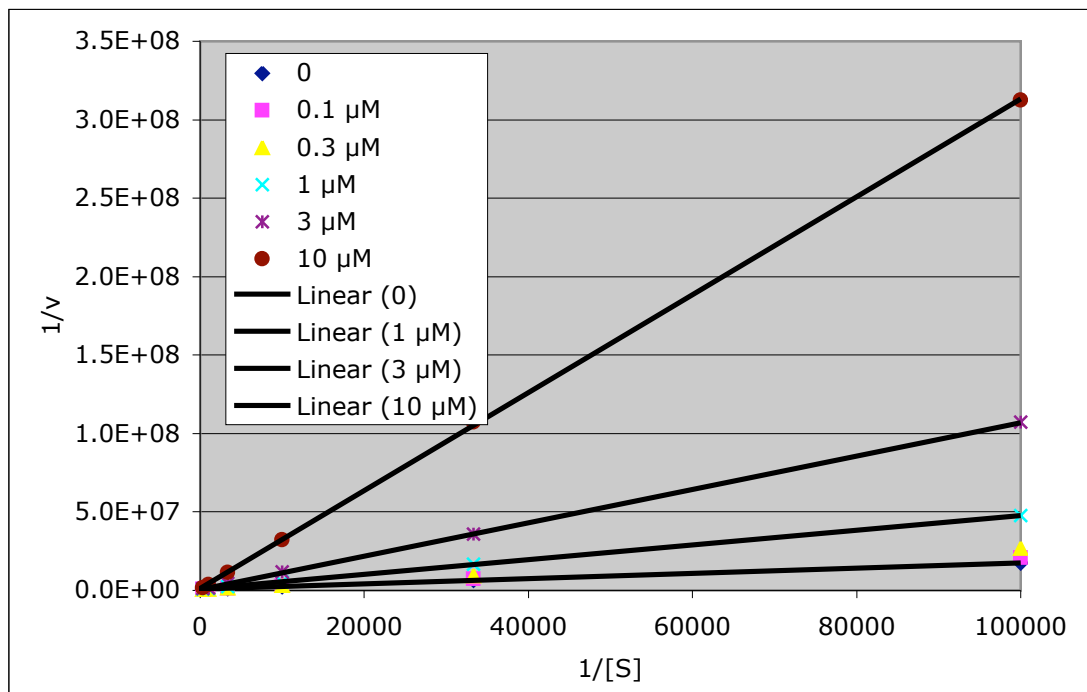
full analysis

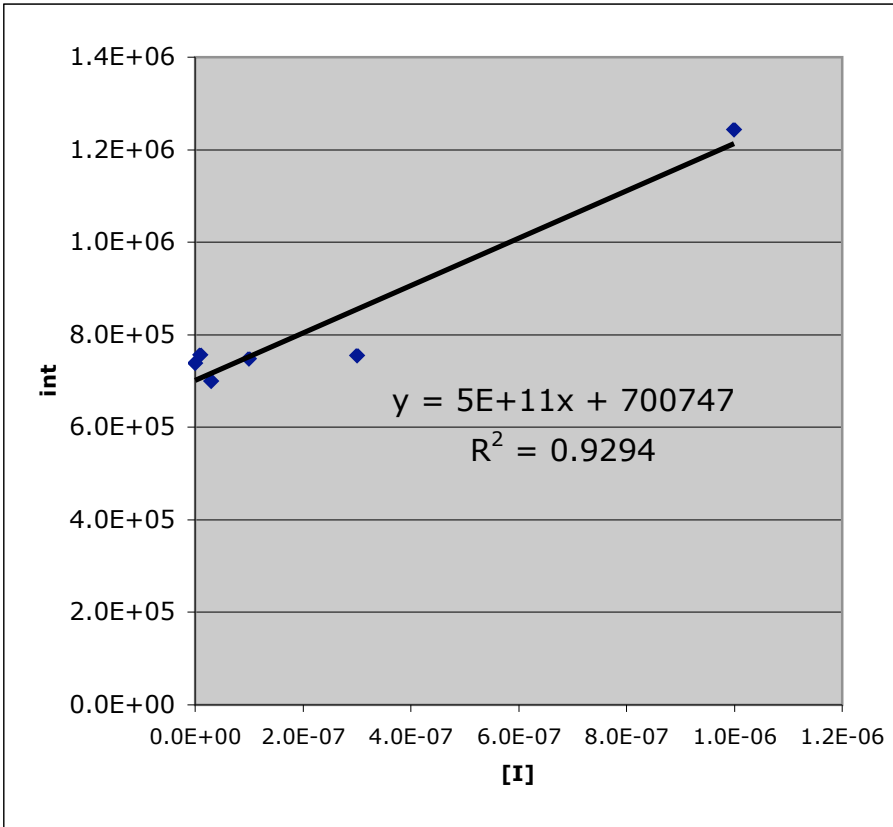
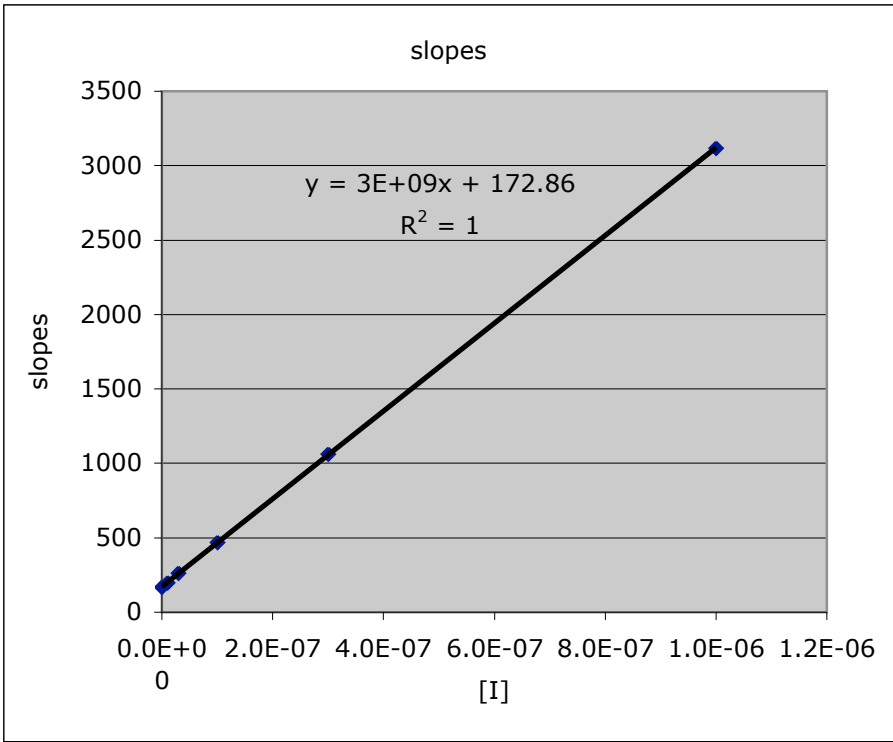
[I] (M) =	0.E+00	1.E-08	3.E-08	1.E-07	3.E-07	1.E-06
b	738853.43	756950.42	699694.77	748236.66	755401.98	1243301
m	168.33137	199.61984	263.02368	469.49068	1062.692	3118.7593
R ²	0.9999519	0.9999641	0.9999809	0.9999443	0.999958	0.9999356

			w/o last line		
int of b	7.01E+05	1/Vmax	7.33E+05	1/Vmax	
slope of b	5.12E+11	1/(Vmax KI')	7.19E+10	1/(Vmax KI')	
R2	9.29E-01		1.45E-01		
int of m	1.73E+02	Km/Vmax	1.71E+02	Km/Vmax	
slope of m	2.95E+09	Km/(Vm KI)	2.98E+09	Km/(Vm KI)	
R2	1.00E+00		1.00E+00		

Vmax	1.43E-06				
KI'	1.37E-06	M	1.3673887	μM	does not look dep on [I]
KI'	1.02E-05	M	10.195708	μM	(w/o last point)
Km	2.47E-04	M	0.2466733	mM	
KI	5.86E-08	M	58.639486	nM	
KI	5.74E-08	M	57.420225	nM	(w/o last point)

competitive inhibitor w/Ki = 59 nM





Problem 2

	[RR] (μM) =	v (nM/s)			
		3	10	30	100
[ATP] (mM)	0.1	5.9	6.9	7.0	7.6
	0.3	11.4	17.3	19.6	20.0
	1	18.3	32.9	44.5	47.9
	3	20.0	45.0	67.6	85.0
	10	20.9	51.6	89.0	122.0

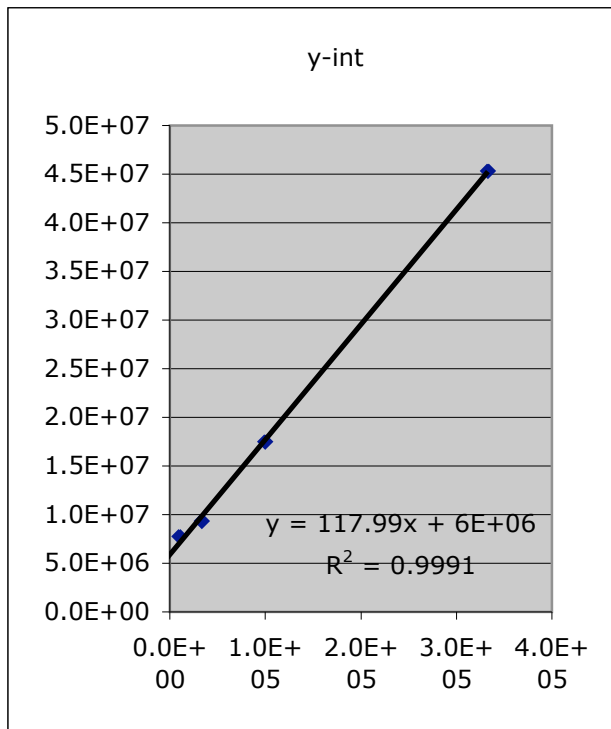
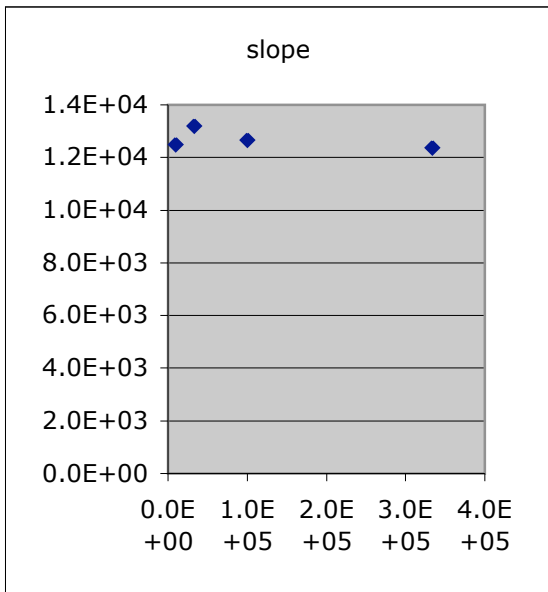
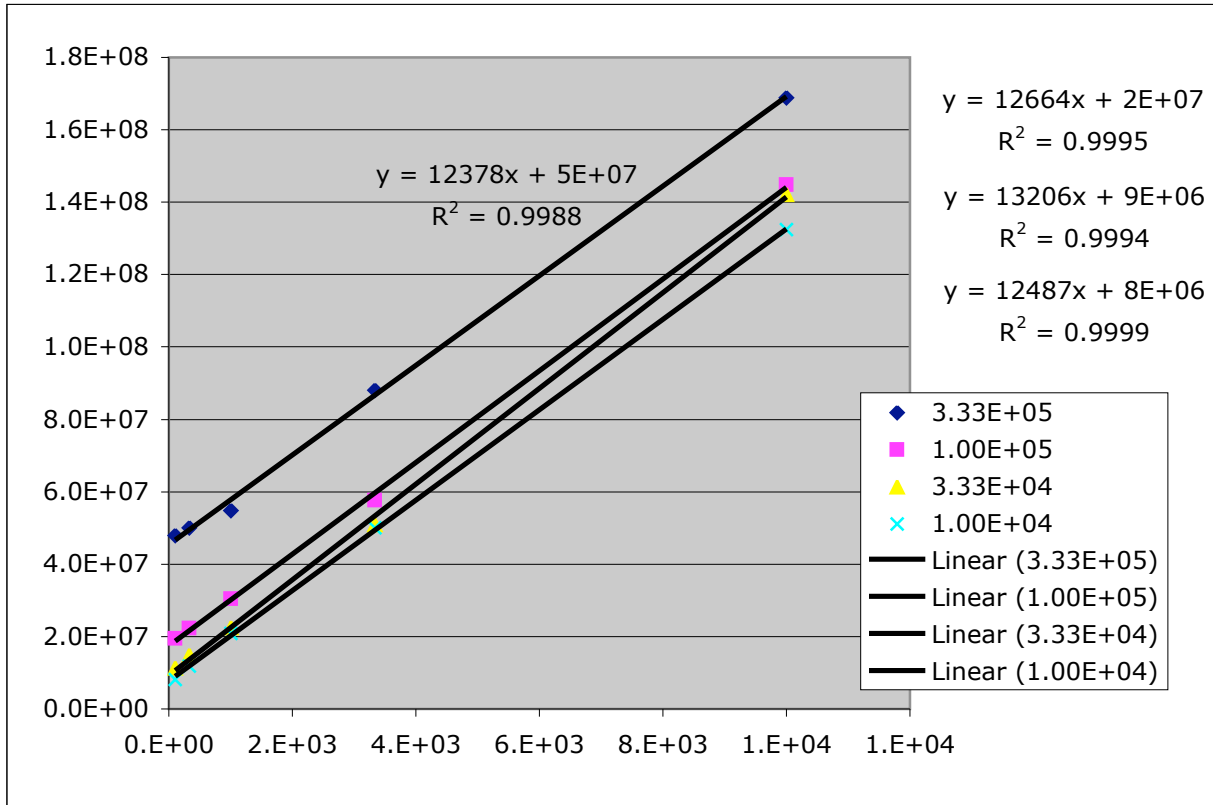
	1/v (s/M)	1/[S2]			
1/[S] (1/M)	3.33E+05	1.00E+05	3.33E+04	1.00E+04	
	1.00E+04	1.69E+08	1.45E+08	1.42E+08	1.32E+08
	3.33E+03	8.80E+07	5.77E+07	5.10E+07	5.00E+07
	1.00E+03	5.48E+07	3.04E+07	2.25E+07	2.09E+07
	3.33E+02	5.00E+07	2.22E+07	1.48E+07	1.18E+07
	1.00E+02	4.79E+07	1.94E+07	1.12E+07	8.20E+06
1/[S2]	3.33E+05	1.00E+05	3.33E+04	1.00E+04	
slope	1.24E+04	1.27E+04	1.32E+04	1.25E+04	
1/[S2]	3.33E+05	1.00E+05	3.33E+04	1.00E+04	
y-int	4.54E+07	1.75E+07	9.32E+06	7.77E+06	
R ²	9.99E-01	1.00E+00	9.99E-01	1.00E+00	

calculate values

1/V _{max} =	5.93E+06 s/M	int of ints
K _{m2} /V _{max} =	1.18E+02 s	slope of ints
V _{max} =	1.69E-07 M/s	
K _{m2} =	1.99E-05 M	19.9 μM
K _{m1} /V _{max} =	1.27E+04 s	avg of slopes
K _{m1} =	2.14E-03 M	2.1 mM
[E] _t =	1.00E-09 M	1.0 nM
[E] _t =	1.00E-09 M	
k _{cat} =	1.69E+02 s ⁻¹	168.7 s ⁻¹

specificity const

S1	7.88E+04 M ⁻¹ s ⁻¹
S2	8.48E+06 M ⁻¹ s ⁻¹



4. This problem is about the relationship between the structure and function of phosphofructokinase (PFK), which catalyzes the reaction:



This is a key reaction in glycolysis, and is highly regulated. PFK is a tetramer. Examine the crystal structures of the T & R forms of PFK. These are in the PDB files 1PFK (R state) and 6PFK (T state), and which can be downloaded from the website. Note that 6 PFK is the full tetramer bound to an effector, while 1 PFK shows a dimer bound to the products with ADP in the effector site. (The tetramer is essentially a dimer of dimers.)

- a) **Briefly describe the overall tertiary and quaternary structure of PFK. What kind of domains does it have (e.g. what class)?**

PFK is a homotetramer with D₂ dihedral symmetry. Each subunit has 2 α/β domains, with the active site in the cleft between. Each domain has a central twisted β saddle flanked by α -helices. There are many β - α - β motifs to make these domains, so the β sheets are parallel. Actually, the larger (N-terminal) domain has 7 strands, and the 5 central strands are parallel, but the outer ones are anti-parallel. It also has 4 long and 2 short α -helices. The smaller (C-terminal) domain is smaller with a 4-strand parallel β sheet and 5 α -helices. If you follow the chain, you will see that larger domain is interrupted after it has made 5 strands and 4 helices, it goes and makes and makes 4 helices and 4 strands (α - β - α - β - α - β - α - β), then the chain goes back and finishes the larger domain (α - β - β - α), and *then* it goes back to the last helix of the small domain.

- b) **Briefly describe the dimerization interface seen in the R state dimer.**

It is mainly an interaction between alpha-helices (no beta-strands involved). The helices in the small domain of one subunit interact with helices in the large domain of the other subunit.

- c) Examine the active site and effector site in the R state structure. Which amino acids are part of the sites binding the substrates and the effectors? Fill out the tables on the next pages for the most important residues you identify for PFK's function in terms of binding the 2 substrates (products) and effector. For the column in which you report distances, indicate which atoms you used to measure the distance (of the amino acid and the substrate or effector).

(1) Residues important for binding Fructose-6-P_i (Frc-1,6-bisP_i)

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Arg171	Loop/turn	Charge-charge interaction with P _i at C-1	3.8 Å (Arg-N to Pi-O)
Arg72	Loop/turn	Charge-charge interaction with P _i at C-6	2.7 Å (Arg-N to Pi-O)
Arg252	α-helix	Charge-charge interaction with P _i at C-6	2.7 Å (Arg-N to Pi-O)
His249	Loop/turn	H-bond/Charge-charge interaction with P _i at C-6	3.3 Å (His-N to Pi-O)
Asp127	Loop/turn	H-bond with 3-OH	2.7 Å (Asp-O to O)
Glu222	Loop/turn	H-bond with 4-OH	2.8 Å (Glu-O to O)
Thr125	Loop/turn (betw α & β)	H-bond with P _i at C-6	2.6 Å (Thr-O to Pi-O)
Asp103	α-helix	Coordinates Mg ²⁺ that binds P _i at C-6	1.9 Å (Asp-O to Mg)
Met169	Loop/turn	Ribose niche (no H-bonds)	

(2) Residues important for binding ATP (ADP) as substrate (product)

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Phe73	Loop/turn	C=O accepts H-bond from 2'-OH of ribose N-H H-bonds with 3-OH	2.5 Å O to O
Arg72	Loop/turn	Charge-charge interaction with P _i s	3 Å (Arg-N to P _i -O)
Gly102	Loop/turn (betw α & β)	N-H H-bonds with β-P _i	3 Å (N to P _i -O)
Arg77	α-helix	Forms niche for adenine	
Tyr41	α-helix	Forms niche for adenine	
Phe76	α-helix	Forms niche for adenine	
Gly104	α-helix	Forms niche for adenine	
Ser105	α-helix	Forms niche for adenine Donates H-bond to β-P _i	2.5 Å (O to Pi-O)
Met107	α-helix	Forms niche for adenine	
Gly108	α-helix	Forms niche for adenine	
Asp103	α-helix	Coordinates Mg ²⁺ that binds β-P _i	1.9 Å (Asp-O to Mg)
Asp129	Loop/turn	Charge interaction with Mg ²⁺ that binds 1-P _i & β-P _i	4 Å (Asp-O to Mg)
Gly11	Loop/turn	N-H H-bonds with β-P _i	2.8 Å (N to P _i -O)
Gly10	Loop/turn	VdW with phosphates	3-4 Å
Phe76	α-helix	Forms niche for adenine	3-4 Å

(3) Residues important for binding ADP as effector

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Tyr55	α -helix	Aromatic stacking with adenine	~ 3.7 Å
Arg54	Loop/turn	Charge interaction with β -P _i	2.8 Å (Arg-N to P _i -O)
Arg25	α -helix	Charge interaction with β -P _i	3 Å (Arg-N to P _i -O)
Arg21	α -helix	Charge interaction with β -P _i	3 Å (Arg-N to P _i -O)
Arg154	α -helix	Charge interaction with α -P _i	2.9 Å (Arg-N to P _i -O)
Lys213	Loop/turn	Charge interaction with α -P _i	3.3 Å (Lys-N to P _i -O)
Glu187	α -helix	-CO ₂ ⁻ coordinates Mg ²⁺ that binds α -P _i & β -P _i	2.1 Å (Asp-O to Mg)
Gly185	Loop/turn	C=O coordinates Mg ²⁺ that binds α -P _i & β -P _i	2.3 Å (Asp-O to Mg)
Lys214	Loop/turn	Backbone N-H donates H- bond to O-4 of ribose	3 Å
His215	Loop/turn	VdW with ribose	
Gly212	α -helix	VdW with adenine	
Ser58	Loop/turn	Possible H-b to α -P _i	2.9 Å
Asp59	Loop/turn	Possible H-b to 3'-OH	2.8 Å
Tyr319	α -helix	C-terminus interacts with Mg ²⁺ (ionic)	4.3 Å

- d) You have created a mutant in which Glu241 has been converted to Gln. Sketch below a graph of reaction velocity vs. [Frc-6-P_i], comparing mutant to normal PFK in these 2 conditions:
- in the presence of ADP
 - in the presence of high ATP (1mM, and no ADP)

It might be best to make 2 sketches – one in the first condition, and one in the second condition. These do not need to be quantitative. I just want to know in what way you would expect the mutant enzyme to be different from the normal enzyme.

The point is that Glu241 stabilizes the T state via an ionic interaction. Thus converting it to (uncharged) Gln would *destabilize the T state*. With ADP, which locks PFK into the R state, you would not see much effect. But in the presence of ATP, which stabilizes the T state, you would see some effect. The effect of the mutation would be to shift the curve to the left – to have more R state at lower [Frc-6-P_i]s.