The following questions pertain to Yuan, Y., Byrd, C., Shen, T., Simplaceanu, V., Tam, T., and Ho, C. (2013). Role of β/δ 101Gln in Regulating the Effect of Temperature and Allosteric Effectors on Oxygen Affinity in Woolly Mammoth Hemoglobin. *Biochemistry* **52**: 8888-8897.

1. The authors study Asian elephant, woolly mammoth, and mutant woolly mammoth hemoglobin. All three versions of hemoglobin have four binding sites for oxygen. Using the following data (available as a file on the desktop) and non-linear fitting in Excel, determine the P₅₀ and hill coefficient (h) for each version of hemoglobin. Error calculations are not needed.

	Theta		
pO2	Asian	Woolly	Mutant
(mmHg)	Elephant	Mammoth	Woolly Mammoth
	(E101)	(Q101)	(Q101 to K101)
0	0.00	0.00	0.00
1	0.00	0.02	0.13
2	0.03	0.11	0.40
3	0.08	0.24	0.62
4	0.15	0.37	0.75
5	0.25	0.50	0.83
6	0.34	0.60	0.88
7	0.44	0.68	0.91
8	0.52	0.75	0.93
9	0.60	0.79	0.95
10	0.66	0.83	0.96
11	0.72	0.86	0.97
12	0.76	0.88	0.97
13	0.80	0.90	0.98
14	0.83	0.91	0.98
15	0.85	0.93	0.98
16	0.87	0.94	0.98
17	0.89	0.94	0.99
18	0.90	0.95	0.99
19	0.91	0.96	0.99
20	0.92	0.96	0.99

	Asian Elephant (E101)	Woolly Mammoth (Q101)	Mutant Woolly Mammoth (Q101 to K101)
h			
P ₅₀			

2. Rank the three versions of hemoglobin from strongest to weakest affinity for oxygen.

Strongest	Weakest
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3. The authors suggest that the residue at position 101 of the β subunit is an important determinant of oxygen binding affinity. They argue that the chemical nature of the amino acid side chain at that position alters the ratio of the T and R states of hemoglobin at equilibrium without significantly affecting the intrinsic oxygen affinity of the T or R states. If the three hemoglobin versions were considered in the MWC model, rank the model fit parameters from smallest to largest value or indicate *no difference*.

K ^T _{site} Smallest	Largest
K_{site}^{R} Smallest	Largest
$K_0^{T \to R}$ Smallest	Largest

4. Draw a predicted hill plot showing the Asian elephant, woolly mammoth, and mutant woolly mammoth hemoglobins following the MWC model.

- 5. Asian elephant hemoglobin has glutamate at position 101. Woolly mammoth hemoglobin has glutamine at position 101. A mutant wooly mammoth hemoglobin studied by the authors has lysine at position 101.
 - a. Draw the chemical structure of glutamate, glutamine, and lysine side chains at pH 7.4.

- b. Make a list of all the types of interactions that each side chain could make with adjacent amino acid side chains within each hemoglobin structure beside your chemical structures in "a".
- c. Considering your rank ordering for the oxygen affinities of these three hemoglobin versions from #6, what chemical property at position 101 seems to be attributable to the change in the ratio of the T to the R state for hemoglobin?