

The following questions pertain to Yuan, Y., Byrd, C., Shen, T., Simplaceanu, V., Tam, T., and Ho, C. (2013). Role of  $\beta/\delta 101\text{Gln}$  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  $P_{50}$  and hill coefficient ( $h$ ) for each version of hemoglobin. Error calculations are not needed.

pO <sub>2</sub> (mmHg)	Theta		
	Asian Elephant (E101)	Woolly Mammoth (Q101)	Mutant Woolly Mammoth (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)
<b>h</b>			
<b>P<sub>50</sub></b>			

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

Strongest---

---Weakest

3. The authors suggest that the residue at position 101 of the  $\beta$  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*.

$$K_{site}^T$$

Smallest---

---Largest

$$K_{site}^R$$

Smallest---

---Largest

$$K_0^{T \rightarrow R}$$

Smallest---

---Largest

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

