

As always, answer each question on a separate page and type narrative answers.

1. For a given protein-ligand complex, use the following data set to calculate the equilibrium dissociation constant (feel free to staple an Excel spreadsheet/graph as part of your answer):

[L] (M)	θ
0.002	0.17
0.004	0.29
0.008	0.44
0.012	0.55
0.014	0.58
0.016	0.62
0.018	0.64
0.020	0.67
0.022	0.69
0.026	0.72
0.028	0.74
0.030	0.75
0.032	0.76
0.034	0.77
0.036	0.78
0.038	0.79
0.040	0.80

2. With the use of site-directed mutagenesis, hemoglobin can be made in which the proximal His residues are replaced with Gly. If this modified Hb was assayed for O₂ binding in the presence of imidazole, would you expect this system to demonstrate cooperative O₂ binding? Explain.
3. Arthropods such as crabs and lobsters have O₂ transporting proteins that don't contain heme groups. Rather, the proteins use two copper I ions, each coordinated by three His imidazoles. Predict (draw) the structure of the complex involving these groups with a single O₂ molecule and speculate as to how O₂ association might lead to cooperative binding.
4. Using the Hill equation, plot an O₂ binding curve for a hypothetical 2-subunit Hb protein with $n=1.6$ and $P_{50}=12$ torr.