

This quiz is about protein-ligand binding. You study binding of ligand L to three proteins, A, B, and C at 25°C. You measured the concentration, [PL], of the ligand-bound form of each protein at various ligand concentrations. The total protein concentrations ( $[P_{\text{total}}]$ ) were A: 2  $\mu\text{M}$ , B: 3  $\mu\text{M}$ , C: 1  $\mu\text{M}$ . Your results are summarized in the table below. Using these data, answer the following questions. *Explain your assumptions.*

Protein	A	B	C
$[L_{\text{total}}], \mu\text{M}$	$[\text{PL}], \mu\text{M}$	$[\text{PL}], \mu\text{M}$	$[\text{PL}], \mu\text{M}$
10	0.549	0.480	0.038
20	0.867	0.831	0.138
40	1.216	1.309	0.390
60	1.402	1.616	0.590
80	1.517	1.830	0.719
100	1.595	1.987	0.800
200	1.776	2.394	0.941
500	1.904	2.726	0.990
1000	1.951	2.857	0.996

- (1) Is the ligand binding to each protein cooperative or non-cooperative? If the binding is cooperative, determine the Hill coefficient. Briefly (in 1-2 sentences) explain how you came to these conclusions.
- (2) Determine the  $K_d$  value for each protein. Briefly (in 1-2 sentences) explain how you did this.
- (3) Determine the  $\Delta G^0$  for each protein-ligand pair.
- (4) Which of the three proteins has the lowest affinity for the ligand? *Explain your reasoning.*