## 1. Consider the equilibrium

$$
M+X \leftrightarrow M X
$$

If the standard state free energy change is $\Delta \mathrm{G}^{\circ}=-14.2 \mathrm{kcal} / \mathrm{mol}$ for a 1 M standard state at $25^{\circ} \mathrm{C}$, for all components what is the standard state free energy change for a 1 mM standard state for all components?

## 2. Consider the protonation equilibrium

$$
\mathrm{M}+\mathrm{H} \leftrightarrow \mathrm{MH}
$$

If the standard state free energy change for this reaction is $-9.0 \mathrm{kcal} / \mathrm{mol}$ for a 1 M standard state at $25^{\circ} \mathrm{C}$ for all components, calculate the free energy change when both M and MH are 1 M , but a pH of 7 is maintained?
3. A protein, P , has 10 sites for a ligand, L .
a.) How many different configurations of the protein can occur in which 4 ligands are bound?
b.) If $L$ binds independently and identically to the 10 sites, with site binding constant $\kappa$, obtain an expression for the overall macroscopic binding constant, $\beta_{3}$ for the equilibrium

$$
3 \mathrm{~L}+\mathrm{P} \leftrightarrow \mathrm{PL}_{3}
$$

c.) Obtain an expression for the step-wise macroscopic binding constant, $\mathrm{K}_{6}$, for the equilibrium:

$$
\mathrm{L}+\mathrm{PL}_{5} \leftrightarrow \mathrm{PL}_{6}
$$

d.) For a protein with $\mathbf{n}$ independent and identical binding sites, what is the value of the ratio of the step-wise macroscopic binding constants, $\mathrm{K}_{1} / \mathrm{K}_{\mathrm{n}}$ ?
4. A homo-tetrameric protein (identical subunits) has a square structure with four binding sites for a ligand X , one site per subunit without cooperativity. X binds to each subunit with a different site binding constant, $\kappa_{\mathrm{i}}$. (i.e., $\kappa_{1}, \kappa_{2}, \kappa_{3}, \kappa_{4}$ ).

a.) Write expressions for the binding polynomial (molecular partition function) and $<\mathrm{X}>$ for this tetrahedral system in terms of:
i. the overall or stoichiometric binding constants, $\beta_{\mathrm{i}}$ (i.e., $\beta_{1}, \beta_{2}, \beta_{3}$ and $\beta_{4}$ ) and the free ligand concentration, $x$.
ii. the step-wise macroscopic binding constants, $\mathrm{K}_{\mathrm{i}}$ (i.e., $\mathrm{K}_{1}, \mathrm{~K}_{2}, \mathrm{~K}_{3}, \mathrm{~K}_{4}$ ) and $x$.
iii. the step-wise microscopic binding constant, $\mathrm{k}_{\mathrm{i}}$ (i.e., $\mathrm{k}_{1}, \mathrm{k}_{2}, \mathrm{k}_{3}, \mathrm{k}_{4}$ ) and $x$.
iv . the site binding constants, $\kappa_{\mathrm{i}}$ (i.e., $\kappa_{1}, \kappa_{2}, \kappa_{3}, \kappa_{4}$ ) and $x$.
v . the site binding constants, if all $\kappa_{\mathrm{i}}=\kappa$.
b.) Obtain expressions for the macroscopic constants, $\beta_{i}$ and $K_{i}$ and the step-wise microscopic constants $\mathrm{k}_{\mathrm{i}}$ in terms of $\kappa_{\mathrm{i}}$.

