1. In most cases native proteins are in equilibrium with their denatured form:

\[ K_{eq} = \frac{\text{protein(native)}}{\text{protein(denatured)}} \]

For ribonuclease (a protein), the following equilibrium concentration data for these two forms were experimentally determined for a total protein concentration of \(1 \times 10^{-3}\) M:

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Native</th>
<th>Denatured</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>(9.97 \times 10^{-4}) M</td>
<td>(2.57 \times 10^{-6}) M</td>
</tr>
<tr>
<td>100</td>
<td>(8.6 \times 10^{-4}) M</td>
<td>(1.4 \times 10^{-4}) M</td>
</tr>
</tbody>
</table>

a) What is \(K_{eq}\) at 50 °C?

\[ K_{eq} = \frac{[\text{Protein(denatured)}]}{[\text{Protein(native)}]} = \frac{2.57 \times 10^{-6}}{9.97 \times 10^{-4}} = 2.58 \times 10^{-3} \]

b) What is \(\Delta H^o\) for the denaturation reaction, assuming \(\Delta H^o\) to be independent of temperature?

Use the integrated Van't Hoff equation:

\[ \ln \left( \frac{K_2}{K_1} \right) = \frac{-\Delta H^o}{RT} = \frac{-4.14}{8.314 \times 298} = -0.002 \]

\[ \Delta H^o = -4.14 \times 8.314 \times 298 = -83.9 \text{ kJ/mol} \]

(Note: Need to calculate \(K_{eq}\) at 100 °C as in part a)

c) What is \(\Delta G\) for this reaction at 100 °C when the native and denatured forms are at the concentration shown in the table?

\[ \Delta G = 0 \text{ at equilibrium} \]

d) What is \(\Delta G^o\) for the denaturation reaction (this is for the standard state temperature)?

\[ \Delta G^o = -RT \ln(K_{eq}) = -298 \times 2.58 \times 10^{-3} \]

\[ \ln K = \frac{1}{2.58 \times 10^{-3}} = \frac{8.314}{298} \times 373 = 8.59 \]

\[ \ln K = -8.59 \]

\[ \Delta G^o = -RT \ln K = -8.314 \times 298 \times (-8.59) = +21.2 \text{ kJ/mol} \]

(native protein is strongly favored)
2. Cystine (Cys-Cys) is the disulfide-linked dimer of cysteine (Cys). Cys will oxidize to Cys-Cys in the presence of O₂. T = 25°C.

<table>
<thead>
<tr>
<th>reaction</th>
<th>( E^\circ ) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{1}{2} \text{O}_2 + 2 \text{H}^+ + 2 e^- \rightarrow \text{H}_2\text{O} )</td>
<td>0.816</td>
</tr>
<tr>
<td>Cys-Cys + 2 H⁺ + 2 e⁻ \rightarrow 2 Cys</td>
<td>-0.340</td>
</tr>
</tbody>
</table>

a) What is the net chemical reaction for the oxidation of cysteine to cysteine?

\[
\text{2Cys} + \frac{1}{2}\text{O}_2 \rightarrow \text{Cys-Cys} + \text{H}_2\text{O}
\]

b) What is \( \Delta G^\circ \) for this reaction when all activities are at their equilibrium values?

To get the net reaction add top reaction to the reversed bottom reaction. Thus \( E^\circ_{\text{net}} = E^\circ_{\text{top}} - E^\circ_{\text{bott}} = 0.816 \text{V} - (-0.340 \text{V}) = 1.156 \text{V} \)

\[
\Delta G^\circ = -nF\Delta E^\circ = -(2)(96.5 \frac{\text{kJ}}{\text{mol} \cdot \text{V}})(1.156 \text{V}) = -223.1 \frac{\text{kJ}}{\text{mol}}
\]

c) What is the equilibrium constant for this reaction?

\[
\Delta G^\circ = -RT \ln K
\]

or \( K = e^{-\Delta G^\circ / RT} = e^{-\left(-223.1 \frac{\text{kJ}}{\text{mol}} / (8.314 \text{ J/K/mol}) \right)(298 \text{K})} = e^{90.05} = 1.28 \times 10^{39}
\]

d) What is the ratio [Cys-Cys]/[Cys] at 298 K for a 0.01 M solution of Cys in equilibrium with the O₂ in the air (P₀₂ = 0.2 atm)? You may assume the activity coefficients for all species = 1.

The reaction very strongly favors Cys-Cys. Thus the 0.01 M Cys converts almost completely to 0.005 M Cys-Cys.

We know this is an equilibrium problem, so

\[
K = \frac{[\text{Cys-Cys}]}{[\text{Cys}]^2 \sqrt{P_{O_2}}}
\]

or \( [\text{Cys}] = \sqrt{\frac{K \sqrt{P_{O_2}}}{[\text{Cys-Cys}]}} = \sqrt{\frac{(0.005)}{(1.28 \times 10^{39}) \sqrt{0.2}}}
\]

\[
= \sqrt{8.735 \times 10^{-42}} = 2.96 \times 10^{-21} \text{M}
\]

Thus \( \frac{[\text{Cys-Cys}]}{[\text{Cys}]} = \frac{0.005 \text{M}}{2.96 \times 10^{-21} \text{M}} = 1.69 \times 10^{18} \)