Estrogen binds to the estrogen receptor with $K_D^{est} = 0.1\ nM$ ($1 \times 10^{-10}\ M$). Testosterone binds with a much lower affinity $K_D^{tes} = 0.1\ mM$ ($1 \times 10^{-4}\ M$). One difference between estrogen and testosterone in the pocket is hydrogen bonding: estrogen has 0 unfulfilled bonds, while testosterone has 1 unfulfilled bond. If losing that hydrogen bond costs 20 $kJ \cdot mol$, is this enough to explain the difference in the affinity?

- Since K_D is an equilibrium constant, we can convert it to $\Delta G^{\circ\prime}$ by $\Delta G^{\circ\prime} = -RTln(K_D)$. So:

  $\Delta G^{\circ\prime}_{estrogen} = -RTln(K_D^{est}) = -0.0083{\times}300{\times}ln(10^{-10}) = 57.3\ kJ{\cdot}mol^{-1}$.

  This is the free energy to dissociate the estrogen from the protein at 1 M estrogen, $1M$ protein, pH 7.0.

- The loss of the hydrogen bond makes binding 20 $kJ \cdot mol^{-1}$ worse. This means our predicted dissociation free energy for testosterone is:

  $\Delta G^{\circ\prime}_{tes,predicted} = \Delta G^{\circ\prime}_{estrogen} - 20\ kJ{\cdot}mol^{-1} = 57.3 - 20 = 37.3\ kJ{\cdot}mol^{-1}$.

  (It is 20 $kJ \cdot mol^{-1}$ easier to pull apart testosterone:receptor than estrogen:receptor).

- Now, convert ΔG back to K_D.

$$\Delta G^{\circ\prime} = -RTln(K_D)$$

$$-\frac{\Delta G^{\circ\prime}}{RT} = ln(K_D)$$

$$e^{-\frac{\Delta G^{\circ\prime}}{RT}} = K_D = e^{-37.3/(0.0083\times300)} = 3 \times 10^{-7}\ M$$

- This K_D is still much lower (better binding) than the observed K_D^{tes} of $1 \times 10^{-4}\ M$, so some other interaction(s) must be involved in specificity.