

DNA Hybridization Reaction

Part 1 Analysis of the hybridization reaction between two DNA single strands

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Some notes on Thermodynamic Theory:

In chemistry, the Gibbs free energy represents an important parameter and is a state function (like enthalpy (H) and entropy (S)) that depends only on the initial and final states.

The difference in free energy of a reaction between the final state (free energy of the products) and the initial state (free energy of the reactants) defines the change in free energy.

$$\Delta G = G_{\text{final}} - G_{\text{initial}}$$

In chemistry it is an important parameter that describes the spontaneity of reactions. A chemical reaction proceeds from a higher free energy to a lower one:

If $\Delta G < 0$ the reaction is spontaneous towards the product

If $\Delta G > 0$ the reaction is spontaneous towards the reactants

If $\Delta G = 0$ the reaction is in equilibrium

If there is more than one reactant and/or product, the free energy is given by the sum of the individual free energies of each element in the reaction.

$$G = \sum G_i \quad \text{for each element 'i'}$$

$$\Delta G = \sum G_i(\text{product}) - \sum G_i(\text{reagent}) .$$

Free energy is a function that depends on three thermodynamic parameters: enthalpy (H), entropy (S), and temperature (T) and on the concentration of the element.

Usually reference is made to the free energy under standard conditions (components at 1 M concentration and determined in a solution with 1 M Na⁺)

$$G_0 = H - TS$$

The free energy associated to i-th component of chemical reactions, at standard condition and at the temperature T is:

$$G_{0i} = H_i - T S_i$$

Instead, the free energy (G_i) associated to i-th component of chemical reaction at not standard condition is:

$$G_i = G_{0i} + RT \ln(C_i)$$

C_i is the concentration of i-th component;

G_{0i} is free energy at standard condition of i-th component;

R is the gas constant (1.987 cal/Mol°K or 8.314 J/Mol°K);

T is the experimental temperature in °K.

For example in the hybridization reaction where two single strands (ssA and ssB) of DNA associate to form a duplex (dsD), we can represent the reaction as:



$$\Delta G = \Sigma G_i(\text{product}) - \Sigma G_i(\text{reagent}) .$$

If C_A , C_B , C_D are the instantaneous concentrations of the individual elements of the reaction, The instant free energy associated with this reaction is:

$$\begin{aligned} \Delta G &= G_D - (G_A + G_B) = G_{0D} + RT \ln(C_D) - (G_{0A} + RT \ln(C_A) + G_{0B} + RT \ln(C_B)) \\ &= \Delta G_0 + RT [\ln(C_D) - \ln(C_A) - \ln(C_B)] = \Delta G_0 + RT \ln\left(\frac{C_D}{C_A C_B}\right) \end{aligned}$$

and introducing $\Delta G_0 = \Delta H - T \Delta S$

$$\Delta G = \Delta H - T \Delta S + R T \ln\left(\frac{C_D}{C_A C_B}\right)$$

General Gibbs free energy (ΔG) equation

Remember that the concentrations are the instantaneous ones, not the initial ones

If ΔG is different from zero, then the instantaneous concentrations of the reactants and products change continuously so as to bring the reaction to equilibrium: $\Delta G=0$

If we know the concentrations of the two initial reagents (C_{Ai} and C_{Bi}), we can rewrite the energy equation:

Hybrid 'D' is formed by one strand of A and one of B. The concentration of D (C_D) is initially zero. The instantaneous concentrations (C_A and C_B) can be written as a function of the initial ones (C_{Ai} and C_{Bi}).

$$C_B = C_{Bi} - C_D$$

$$C_A = C_{Ai} - C_D$$

$$\Delta G = \Delta H - T \Delta S + R T \ln\left(\frac{C_D}{(C_{Ai} - C_D)(C_{Bi} - C_D)}\right)$$

General Gibbs equation also as a function of the initial concentrations

ΔG in J (or cal), ΔH in J/mol°K (or cal/mol°K), ΔS in J/mol (or cal/mol), concentrations in molar units, R is the gas constant 8.314 J/Mol°K (or 1.987 cal/Mol°K);

NOTE: by setting the free energy to zero, it is possible to determine the temperature as a function of the concentrations, or the concentrations as a function of the temperature.

Influence of the environment on the hybridization reaction

ΔG is influenced by the environment, in particular by the conditions of the solutions.

Generally ΔH and ΔS are measured in 1M Na⁺ solution. Therefore, in non-standard conditions of the solution, to the ΔG calculated with the previous equation it is necessary to introduce a variation of free energy due to the environment (ΔG_a).

$$\Delta G = \Delta H - T \Delta S + R T \ln \left(\frac{C_D}{(C_{Ai} - C_D)(C_{Bi} - C_D)} \right) + \Delta G_a$$

There are various methods to estimate the effect of the environment. Some act by modifying the entropy, others intervene only to modify the melting temperature (see the attachment).

Note: At constant temperature, the reaction tends to equilibrium, so the free energy tends to zero. In this hybridization equation, the only variables in time are the concentrations of the reactants/products, which therefore vary to make the free energy zero.

This concept is used to determine the melting temperature and the fraction of hybridized template (see next chapter)

To apply these equations it is necessary to estimate the thermodynamic parameters of enthalpy and entropy. In our works applied to the [DNA_Promix](#) platform, we use the Nearest Neighbor method which assigns to each internal nucleotide pair the experimentally determined thermodynamic values ΔS and ΔH . Furthermore, the initialization variations of ΔH and ΔS are considered

We will see in the next chapter in the [determination of the melting temperature](#).