**Exercise 9: Spontaneity of chemical reactions**

The exercise is conducted using the Virtual Lab program and a spreadsheet. The online version of the application is available on the website:

<http://chemcollective.org/vlab/vlab.php>

The data obtained in the Virtual Lab program and the calculated values should be recorded in the appropriate tables under the 'ex9' tab in the spreadsheet "Physical Chemistry ".

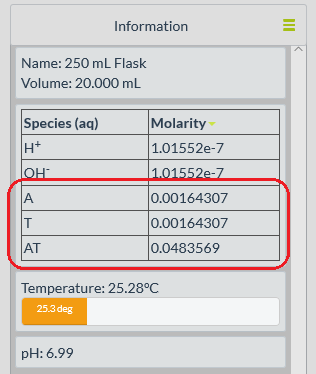
To launch all the tasks, select the following tabs:

***File → Load an Assignment → Chemical Equilibrium → DNA Binding Problem***

**I. Problem 1**

In the native structure of DNA, the following pairs of complementary bases have been identified: adenine – thymine and guanine – cytosine. Based on the reagents available in the Virtual Lab program, propose and conduct an experiment that demonstrates and explains the phenomenon of base complementarity in the DNA structure.

We need to conduct reactions between the bases found in DNA. We will need all possible combinations of nitrogenous base pairs: A-T, A-C, A-G, T-C, T-G, and C-G.  
We will use the chemical affinity values of the pure components, A, and we should observe a difference between the complementary pairs (A-T and C-G) and the others.

**1.** Conduct synthesis reactions using the available nitrogenous base solutions.  
Pour 10 cm³ of 0.1 M solutions of two selected bases into a 250 cm³ beaker, then repeat the procedure in separate beakers for the remaining solutions so that the obtained data allows the description of all possible reaction systems (a total of 6 reactions: A-T, A-C, A-G, T-C, T-G, and C-G).

**NOTE**: For each obtained system, set the temperature of the reaction vessel to 25°C. Next, record the equilibrium concentrations of the reagents in the spreadsheet. (Considering that each analyzed reaction occurs in a stoichiometric 1:1 system and uses starting solutions with equal volumes and concentrations, there is no need to record separate concentrations for individual substrates; it is sufficient to record only the concentrations CSUB and CPROD​, where CSUB is the concentration identical for both nitrogenous bases).

**2.** Using the obtained values, you need to determine the equilibrium constants for all the reactions considered at each temperature.

**Example reaction:** G + C = GC

To calculate the equilibrium constant of the reaction, we use the following formula:

KX = XGC/(XG∙XC)

KX – equilibrium constant determined based on mole fractions

XGC ; XG ; XC – mole fractions of individual reagents representing their content in the reaction mixture

To calculate the above constant, it is necessary to compute the mole fractions, according to the following example formulas:

XGC = NGC / (NGC+NG +NC)

XG = NG / (NGC+NG +NC)

XC = NC / (NGC+NG +NC)

NGC ; NG ; NC – the number of moles of individual reagents in the reaction mixture

To calculate the number of moles of individual reagents, the equilibrium concentration obtained from the Virtual Lab program should be multiplied by the volume of the solution. For example:

NC = NG = cSUB ∙ V

NGC = cPROD ∙ V

cSUB ; cPROD – equilibrium concentrations of reactants and products

V– the volume of the solution, i.e., 20 cm³ (remember to express the volume in dm³).

**3. Based on the obtained equilibrium constants, the chemical affinity of the pure components should be determined using the following formula:**

A=-ΔGr = R ∙ T ∙ ln(K)

R - gas constant: 8,3145 J∙K-1∙mol-1

T – temperature [K]

K – equilibrium constant

The calculations should be performed for each of the 6 nitrogenous base pairs.

**Report**  
Organise the collected data and calculated values in Table 1. Present the calculations and provide conclusions.

**II. Problem 2**

Among the reagents provided in the experiment is a substance labelled 'Unknown.' This is an unidentified nitrogenous base. Propose and carry out an experiment that will allow the identification of this compound.

We need to check all possible combinations again: G-X, C-X, A-X, and T-X.

We will calculate the chemical affinity values of the pure components A° for these combinations and determine which of the four bases has the greatest affinity for the unknown compound. The procedure is identical to Problem 1.

**Report**  
Organise the collected data and calculated values in Table 2. Present the calculations and provide conclusions.

**III. Problem 3**

How does the chemical affinity of pure components depend on the concentration of reagents and the temperature of the ongoing process?

**3a.** How does the chemical affinity of pure components depend on concentration?

Prepare the following solutions of **0.05 M, 0.03 M, 0.02 M, 0.01 M,** and **0.001 M dGMP** in **50 ml** quantities.

For example, for a **0.03 M** solution, using a **0.1 M** stock solution:

**0.1 M ∙ X ml = 0.03 M ∙ 50 ml**

**X = 15 ml of the 0.1 M solution**

**water = 50 ml – 15 ml = 35 ml**

We calculate the remaining solutions in the same way.

Pour **10 cm³** of **0.1 M dCMP** solution into a **250 cm³** beaker, and then add the same volume of **0.1 M dGMP** solution. After conducting the reaction, set the temperature in the vessel to 25°C and then record the equilibrium concentrations of the reagents.

Repeat the procedure for the subsequent concentrations, using **10 cm³** of the **0.1 M dCMP** solution and **10 cm³** of the **dGMP** solution with varying concentrations each time. For each system, calculate the mole fractions, equilibrium constant, and chemical affinity of the pure components as in Problem 1.

**NOTE: It is essential to record and include the concentrations of both substrates (non-stoichiometric ratio) as C1 and C2 in the calculations.**

**3b.** How does the chemical affinity of pure components depend on temperature?

Pour **10 cm³** of the **0.1 M** **dCMP** solution into a **250 cm³** beaker, and then add the same volume of the **0.1 M dGMP** solution. Set the temperature in the beaker to **36.6°C**, remembering to insulate it beforehand. Then, record the equilibrium concentrations of the reagents.

Next, gradually change the temperature of the beaker to **25, 15, 5,** and **0°C,** again recording the equilibrium concentrations of the reagents.

With the appropriate data, calculate the mole fractions, equilibrium constant, and chemical affinity of the pure components as in Problem 1.  
**NOTE: In this case, the concentrations of the substrates are equal (stoichiometric ratio) and can both be recorded as CSUB.**

**Report**  
Organize the collected data and calculated values in Tables 3 and 4, corresponding to subsections 3a and 3b. For both subsections, present the calculations and provide conclusions. Create a graph showing the dependence of the chemical affinity of pure components on temperature