UNIVERSITÉ Grenoble Alpes

Laboratory Report

Molecular interactions: nanocalorimetry of DNA molecules

Hervé Guillou

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Instructions for the report

The report must have a title your name and the date of the practical, it should be written in french or english, be concise and if needed the references should be noted. It contains several sections as follows. This file and others are available for download at the following url: `https://cloud.neel.cnrs.fr/index.php/s/xbKxsNXfCss5eTf` In general a lot of informations are available there, in particular the datafiles. The problem that we propose to investigate here is the thermodynamics of hybridation of DNA by studying several assembly architectures differing from the canonical duplex. The first nanostructure we propose to investigate is made of four DNA strands forming a Holliday jucntion. Holliday junctions are occuring in vivo during particular processes associated with the chromosomal replication. The simplified structure is represented on the figure 1. The sequences that we will study have been optimized by N. Seeman [1] and the whole field of structural DNA nanotechnology started base on his seminal paper [2]
The second architecture involves only two strands forming a loop as pictured in the figure 2.
We base our analysis on the following set of sequences for the HJ:

ST1: CGCAATCCTGAGCACG

ST2: CGTGCTCACCGAATGC

ST3: GCATTCGGACTATGGC

ST4: GCCATAGTGGATTGCG

The Loop forming sequences are:

LOOPSTP1: GCTTCTATTGACATGCCACCGTGCTTAGAGTG

LOOPSTP2: CACTCTAAGCACGGTGGCATGTCAATAGAAGC

LOOPSTP2L4: CACTCTAAGCACGGTGTTTTGCATGTCAATAGAAGC

LOOPSTP2L8: CACTCTAAGCACGGTGTTTTTTTTGCATGTCAATAGAAGC

LOOPSTP2L16: CACTCTAAGCACGGTGTTTTTTTTTTTTTTTTGCATGTCAATAGAAGC

LOOPSTP2L32: CACTCTAAGCACGGTG$(T)_{32}$GCATGTCAATAGAAGC

LOOPSTP2L64: CACTCTAAGCACGGTG$(T)_{64}$GCATGTCAATAGAAGC

The datasheets are provided on the cloud.

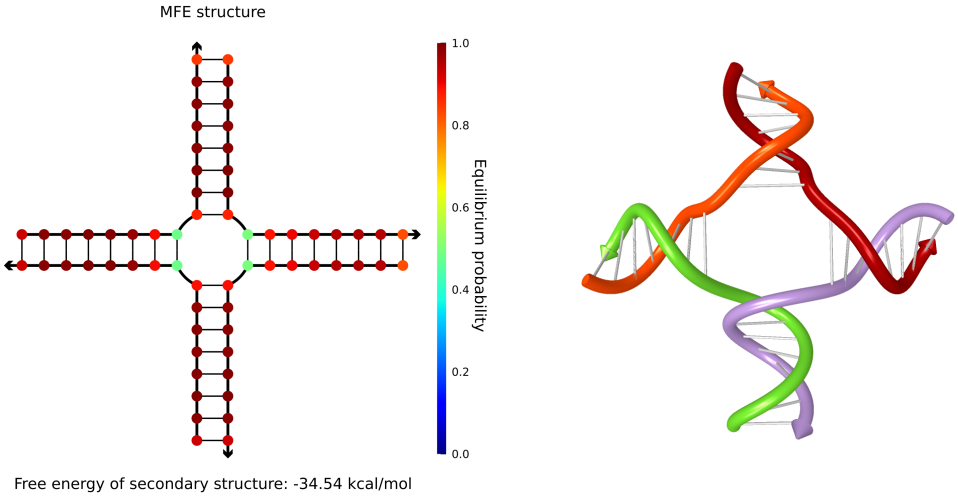

Figure 1: Holliday junction formed by four strands of DNA. On the left a simplified geometrical approach shows the specific hybridation of the strand. The right image is a cartoon of the helical structure. The exact structure of the Holliday junction has been solved by structural tools. Such molecular assembly are often the corner stone of larger DNA structures

Figure 2: Loop formed by two strands with partial complementarity. The complementary sequences are 16 bases long. We propose to study the effect of the length of the loop on the thermodynamic properties.

Introduction, evaluated on 4 points

Here you should present the position of the problem and its context several points should be discussed:

- Holliday junctions, what biological functions
- Express where they are used in DNA origami and where you kind find loop like structues, draw schematics and base your finding on reference [].
- precise by a color code, the the duplex forming sequences that your group investigated
- Predictions of thermodynamic models nupack[1]and mfold or dinamelt [2].

Experimental method, evaluated on 3 points

Describe in short the experimental methods that are used in the lab to evaluate the thermodynamic parameters of molecular interactions. Specify their advantages and limitations:

- preparation of the solutions (what sequences and what concentrations, some critical discussions are expected)
- DSC, differential scanning calorimetry, NanoDSCIII from CSC (now TAintruments)
- ITC, Isothermal scanning calorimetry, NanoITC from CSC

You can base your text on the introduction given in the file *DSC-Principle2.pdf* available on the Cloud.

Experimental analysis of real DSC data evaluated on 3 points

For the data that you have been assigned you should plot and comment:

1. raw data (P vs T)
2. the background and justify its choice
3. ΔC_p vs T in correct units
4. ΔH and ΔS

I strongly advise you to base your analysis on the file *Analysis-DSC-step-by-step-using-python.pdf* In order to save space you can combine graphs. Don't forget to givesinformations on the concentrations.

[1] `http://nupack.org/partition/new`
[2] `http://www.unafold.org/Dinamelt/applications/hybridization-of-two-different-strands-of-dna-or-rna.php`

comparison with models (2 points)

We ask you at least to compare your results for ΔH and ΔS with the current model available on web servers. The importance of salt conditions can be tested, in our experiments they are of two types dpenedning on the buffer generally:

- 50 mM Na^+ and 10 mM Mg^{++}
- 0.3 M Na^+

Bonus: if you are interested you can establish a model of chemical reaction occuring in our systems. To keep it simple and feasible we propose you to describe the system with the following reaction scheme: A + B $\rightleftarrows$ AB where the laws of mass action apply and the equilibrium constant fix the evolution of the concentrations of the species are fixed by the equilibrium constant:

$$K_{eq} = \frac{[AB]}{[A][B]} = \exp\left(-\frac{\Delta G}{RT}\right)$$

The enthalpy of the system is set with simplificative hypothesis to:

$$\Delta H(T) = \Delta H_{AB} \times \frac{\partial n_{AB}}{\partial T}$$

Can you develop a numerical code to fit $\Delta Cp(T)$ to the chemical model ?

analysis of titrations (5 points)

The ITC par is less stabilized and still the focus of understanding. The method suffers from instabilities and drifts that we do not completely understand. We propose several things:

- focus on the calibration of the instrument, this includes the measurement of the compensation power dissipated in the cell when a known energy is brought by Joule dissipation. The integration of the power with respect to time gives the energy. The ratio between the actual energy and the prescribed energy set in the experimental design should gives a multiplication factor close to 1.0 in principle. The analysis of the dispersion and the drift is a relevant information regarding the experimental design. The experimental design aims at giving the proper concentrations of DNA strands in the reaction vial and in the syringe so as the heat released by the hybridisation can be detected. A good ITC experiments aims also at determining the binding constant of the chemical reaction. In the case of DNA the conditions are difficult to achieve. You should discuss this fact.
- focus on the blank titration of buffer into buffer, this should gives you some criteria to define the minimum quantity of strands to inject.
- focus on strand titrations, the evaluation of the reaction enthalpy can usually be done, the evaluation of the binding constant is much more problematic. Discuss why this is the case and what kind of experiment you should perform. Is ti possible considering the characteristics of the instrument.

Some python scripts in the form of jupyter notebook are available in the ITC folder to guide you with these tasks.

global conclusion (3 points)

in particular comment on the coherence of the thermodynamic parameters from part 3 and 4 and on the total amount of mole of A and B to perform a titration experiment were A is initialy present in the volume of 1 mL and B in the 300 μL seringe. The titration should be complete (the initial number of mole of A is exhausted and B is in excess at the end of the 25 injections)

References

List all sources cited in the report.

References

[1] N C Seeman and N R Kallenbach. "DESIGN OF IMMOBILE NUCLEIC ACID JUNCTIONS". In: *Biophysical Journal* 44.2 (Nov. 1983), pp. 201–209. DOI: 10.1016/S0006-3495(83)84292-1. URL: http://dx.doi.org/10.1016/S0006-3495(83)84292-1.

[2] Nadrian C. Seeman. "Nucleic acid junctions and lattices". In: *Journal of Theoretical Biology* 99.2 (Nov. 1982), pp. 237–247. ISSN: 00225193. DOI: 10.1016/0022-5193(82)90002-9. URL: https://linkinghub.elsevier.com/retrieve/pii/0022519382900029 (visited on 07/04/2019).