Full Protocol 2
DNA Biosensor

Overview

Estimated Experiment time: 4.5 hours
Level: Undergraduate
Expected outcome: Quantify DNA, observe the presence of DNA mutations and measure the kinetics of monolayer formation.
Similar molecular systems: Any nucleic acid assay.

Background

DNA is the genetic code of all living organisms of sizes ranging from a few microns, such as bacteria, to meters-long mammals, such as blue whales. The DNA sequence is unique to each organism, including humans. It consists of a string of nucleic acids that is translated into proteins, each having a specific function. Mutations – or alterations – in the DNA sequence can be caused in any organism by environmental factors (exposure to UV light, radiation, pollution, etc.). The probability of mutations occurring increases with time. In humans, mutations are the underlying cause of simple differences such as color-blindness, as well as serious diseases such as cystic fibrosis, sickle cell anemia, Tay-Sachs disease, phenylketonuria and many others.

The double-stranded DNA molecule, often described as a double helix, is constituted of four bases that form A-T and G-C base pairs. Base-pairing thus allows the assembly of two single-stranded DNA molecules, in a process named hybridization. The hybridization of complementary single-stranded DNA molecules is specific. In the context of biosensing, this allows the detection of specific DNA markers in human body fluids (e.g., blood, serum, saliva). In this experiment, a chip-based biosensor will be used to capture a DNA marker of interest. A single-stranded DNA probe that is complementary to the target DNA sequence is first immobilized on the sensor's surface. Then, the probe DNA-functionalized chip is exposed to the sample containing the target DNA. The immobilized probe DNA will capture and hybridize with the target DNA to create a change of state on the surface of the chip which is monitored and measured.

Material

- PBS (phosphate buffered saline) pH 7 with EDTA and urea :
  - 20 mM Phosphate (monobasic and dibasic)
  - 300 mM NaCl
  - 1 mM EDTA
  - 100 mM Urea
- Probe DNA:
  - 0.6 nmole of 16-mer — 5'-HS-$(CH_2)_6$-GGAA TGA AGT CGA TGG-3'

Probe DNA

- Target DNA:
  - 0.3 nmole of 16-mer — 5'-CCA TCG ACT TCA TTCC-3'
  - 1 nmole of 25-mer — 5'-TCT GTT ACC CCA TCG ACT TCA TTCC-3'
- Mutant DNA:
  - 0.3 nmole of 25-mer — 5'-TCT GTT ACC CAC TCG ATC TTA CTCC-3'
- 8 M Urea
- MCH: 2 mM 6-mercapto-1-hexanol in H_2O
- SPR prism
- SPR fluidic cell

Protocol

DNA Titration

1. Prepare 6 solutions of the following concentrations of 25-mer target DNA each in a total volume of 500 µL of PBS: 150, 200, 250, 300, 350 and 400 nM.
2. Prepare a 500 µL solution of 1,200 nM probe DNA in PBS.
3. Prepare a 500 µL solution of 400 nM 16-mer target DNA in PBS.
4. Prepare a 500 µL solution of 400 nM 25-mer mutant DNA in PBS.

SPR Setup

5. Place a SPR prism and the SPR fluidic cell in the P4SPR.
6. Turn on the computer, open the P4SPR software, proceed with the start-up routine, and begin recording your experiment (see appendix for detailed procedure).

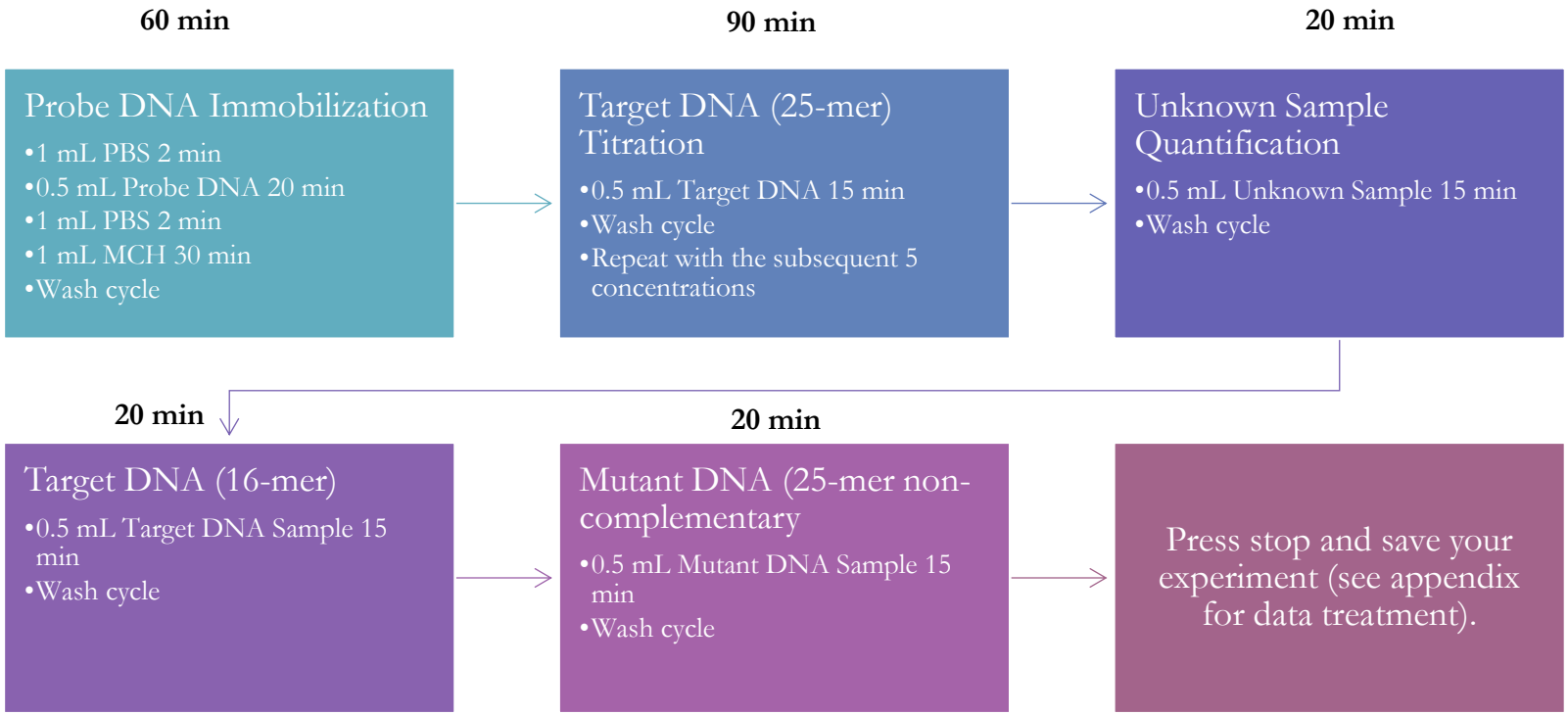
General considerations

- *Avoid injecting air bubbles into the P4SPR channels.*
- *Be aware that air bubbles can be introduced every time a syringe is inserted into or removed from a port.*
- *After each injection, visually inspect to see if air bubbles are present on the chip's surface. If so, push the bubbles away from the chip surface by doing pulsed injections manually.*
- *Carefully remove syringe from port to avoid backflow.*
- *When necessary, use the Y connector for simultaneous injections in the reference and sample channels.*

Biosensor Experiment

The following steps are time-sensitive as measures are taken in continuous time. Please ensure you have your material ready to inject ahead of time.

For the assay, we are defining the **wash cycle** as the following three steps: 1 mL PBS injected over 1 min, 1 mL Urea over 0.5 min, 3 mL PBS over 3 min.

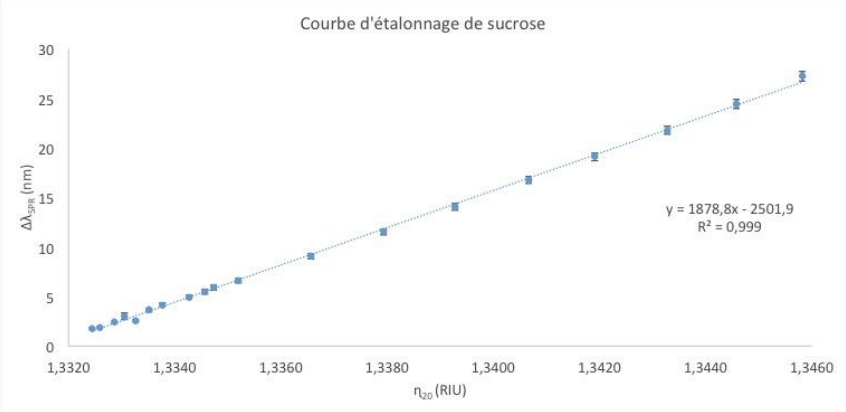


Questions for lab report

- What is the unknown sample's concentration?
- What is the kinetic rate of the probe DNA immobilization to the surface within the first 5 minutes of the reaction? To determine this value, you need the equation below and the sensitivity of the chip (see figure below).

$$\Gamma = \rho\left(\frac{-l_d}{2}\right) ln\left(1 - \frac{\Delta\lambda_{SPR}}{m(\eta_{adsorbed} - \eta_{solution})}\right)$$

Where Γ is the concentration at the sensor's surface in ng/cm², ρ is the density of DNA (1.7 g/mL), l_d is the penetration depth of SPR (230 nm), m is the SPR film sensitivity, and refractive indexes of adsorbed molecules ($\eta_{adsorbed}$ = 1.54 for DNA) and the bulk solution ($\eta_{solution}$ = 1.33 for aqueous solution).



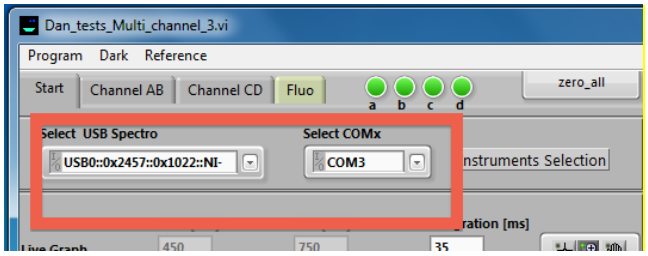
Titration curve of SPR sensor measured with sucrose solutions of various refractive indexes. $\Delta\lambda_{SPR}$ = 1878.8 η_{20} – 2501.9, whereas η_{20} is the refractive index at 20°C.

- Why is 6-mercaptohexanol important in this experiment?
- How is target DNA (e.g., 25-mer complementary) washed away from the surface using urea?

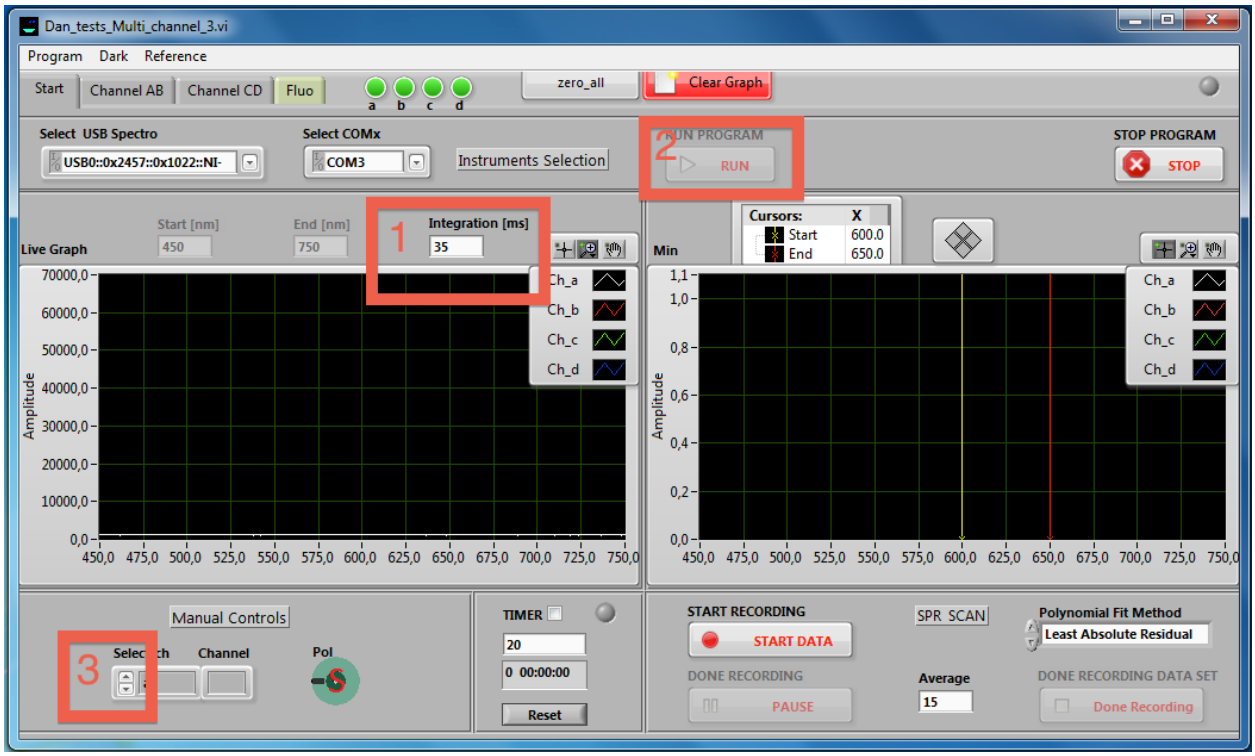
Appendix

P4SPR initiation routine

i. **Select the USB spectro** and **select COMx** using the drop down menu as shown below.



ii. Set the **integration** time 20 ms, click **Run**, and manually select each channel (**Select_ch**) using the arrows to see a signal in the left window.
Note: if the signal is too weak, adjust the integration time (20 ms to 50 ms).



iii. Ensure the polarizer (**Pol**) is in “S” position; if “P”, click on the letter to change to “S”. In the program menu, click on **Dark** and select **Start Dark**.

iv. Create a folder for your experiment and save the dark files in this folder by click on select this folder (DO NOT click on SAVE).

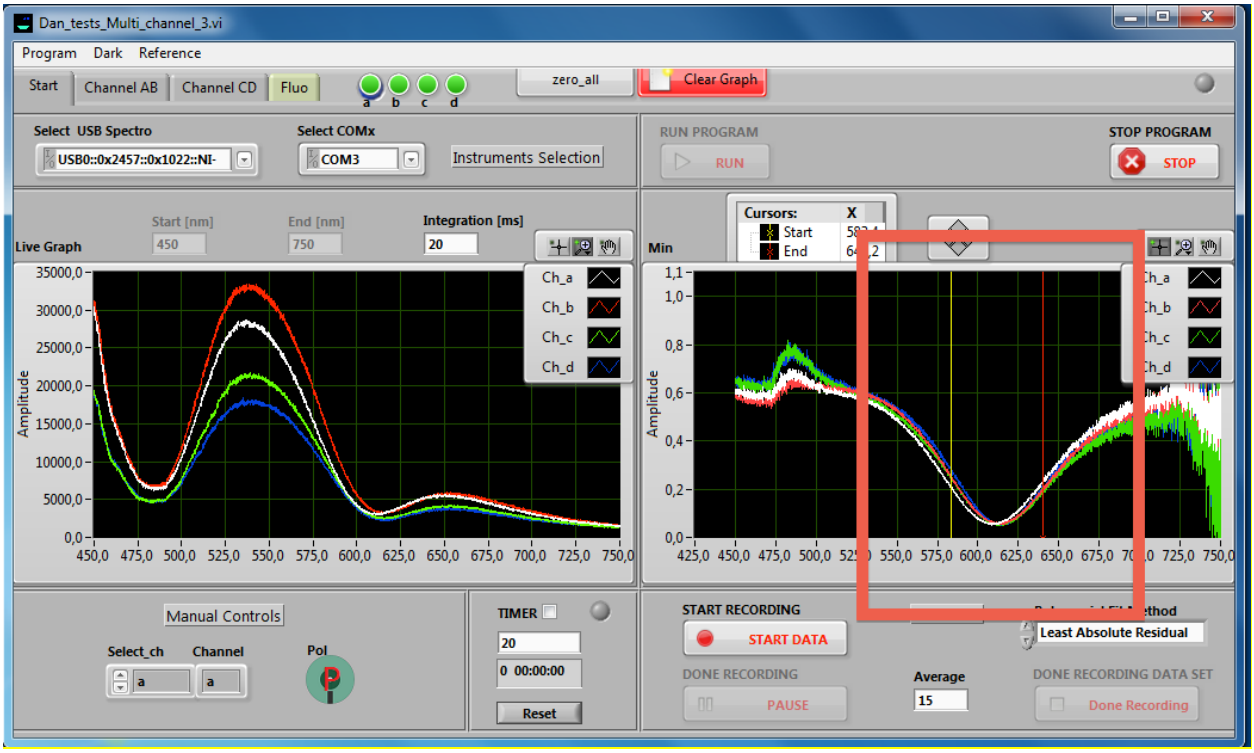
v. Once dark measurements are done, a popup window will appear. Close it.

vi. In the program menu, click on **Reference** and select **Start Reference**.

vii. Save the “Reference” files in the same folder as the “Dark” by click on select this folder (DO NOT click on SAVE).

viii. Once reference measurements are done, a popup window will appear. Close it.
Note: the polarizer should automatically switch to “P” from “S” once the reference measurements are done.

ix. The characteristic SPR absorbance spectrum for each channel (a to d) should appear overlapping in the right side window. On that graph, set the higher and lower tracking boundary (vertical yellow and red lines) about 50 nm apart and ensure the absorption band is in the middle.
Proceed to step x and beyond only if your material is all set for the kinetic experiment.



x. Press on **Start Data** located below the right window. Select the folder in which you saved the "Dark" and "Reference" files and save the kinetic data.

xi. Start an external timer to monitor your experiment.

xii. You can monitor the SPR shift in each individual channel by selecting tabs **Channel AB** and **Channel CD**.

xiii. You can set all channels to zero at all time by clicking on **zero_all**. This adjustment is recorded in your kinetic data file.

xiv. You can also clear the kinetic data from the channel windows by clicking on **Clear Graph**. This adjustment is NOT recorded in your kinetic data file and will erase previous data.

xv. Once the experiment done, press **Done Recording** and stop your timer.

Retrieve collected data

xvi. Go in the folder where you saved the data and look for four files named **Graph_a, Graph_b, Graph_c,** and **Graph_d** containing the kinetic data for each channel. Import these data into your data processing software of choice (e.g., TraceDrawer, excel, origin, matlab, etc.).

xvii. The time will be recorded in the first column, while the SPR wavelength can be found in the second column.

Acknowledgement: Chemistry Department at Université de Montréal.