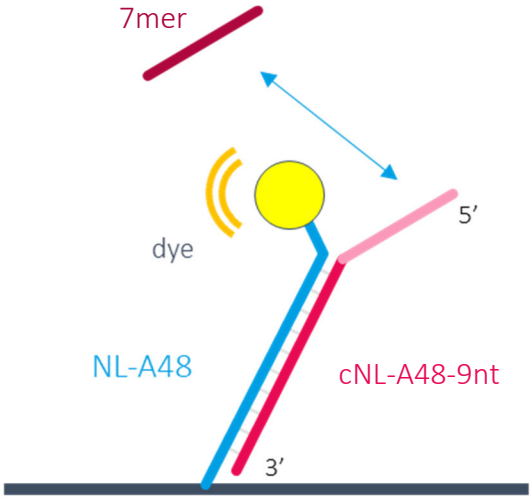
QC DRX kit: Fast kinetics
DNA hybridization kinetics using a 7mer oligo

Aims

- Quality control (QC) kit for checking functionality and performance of DRX or DRX2 devices.
- Determination of the kinetic values (k_{ON}, k_{OFF}, K_D) of a 7mer oligo hybridization to a single-stranded DNA nanolever overhang; values should lie in a ± 50% range to pass QC.



Workflow

Product description

Product Code DK-QC-A48-1

Measurement Time ~0 h 30 min

In this experiment, the fast DNA hybridization kinetics of a 7mer oligo binding to its complementary sequence on a 9nt DNA nanolever overhang is analyzed. The determined kinetic rates are compared to the "status quo" kinetic rates obtained from the multiple reproducibility studies using different DRX & DRX2 devices, different material batches and different operators on different days in the Dynamic Biosensors application labs.
We start by immobilizing a 9nt single stranded overhang sequence on the Biochip. Next, we flow in the lowest concentration of the 7mer oligo analyte for 10 seconds at fast flow rates (2000µL/min), directly followed by a 30 seconds buffer injection at the same flow rate (one-shot kinetics script). The 7mer oligo fully dissociates within 30 seconds. Hence, regeneration of the surface is not required and the four other concentrations of the 7mer analyte can be directly applied onto the surface in the order of increasing concentration.

TABLE 1 | Contents and storage information.

Material	Cap	Amount	Storage	Comments
cNL-A48-9nt / cNL-B48 (500 nM)	red	1x 100 µL	4°C	In PE40, good for 3 runs
7mer analyte (5 different concentrations prepared in 5 different 1.3mL autosampler vials with caps)	black	5x 1100 µL	4°C	Concentrations: 31.3, 62.5, 125, 250, 500 nM, good for 3 runs
PE40 / PE140	transparent	100 mL each	2-8°C	Attach PE40 to AUX inlet & PE140 to "Buffer" inlet
Passivation solution	silver	1x 10 mL	2-8°C	Ready to use; mix before run
Regeneration solution	black	1 x 300 µL	2-8°C	Ready to use
MPC-48-2-R1	In bag	1	2-8°C	Biochip

For *in vitro* use only.

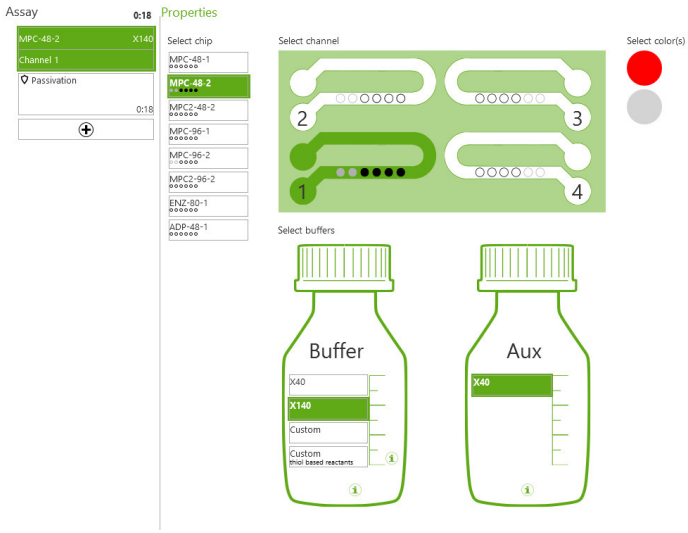
Planning the experiment

Before getting started, transfer all the required samples from the freezer to fridge to begin a slow thawing of the frozen samples.

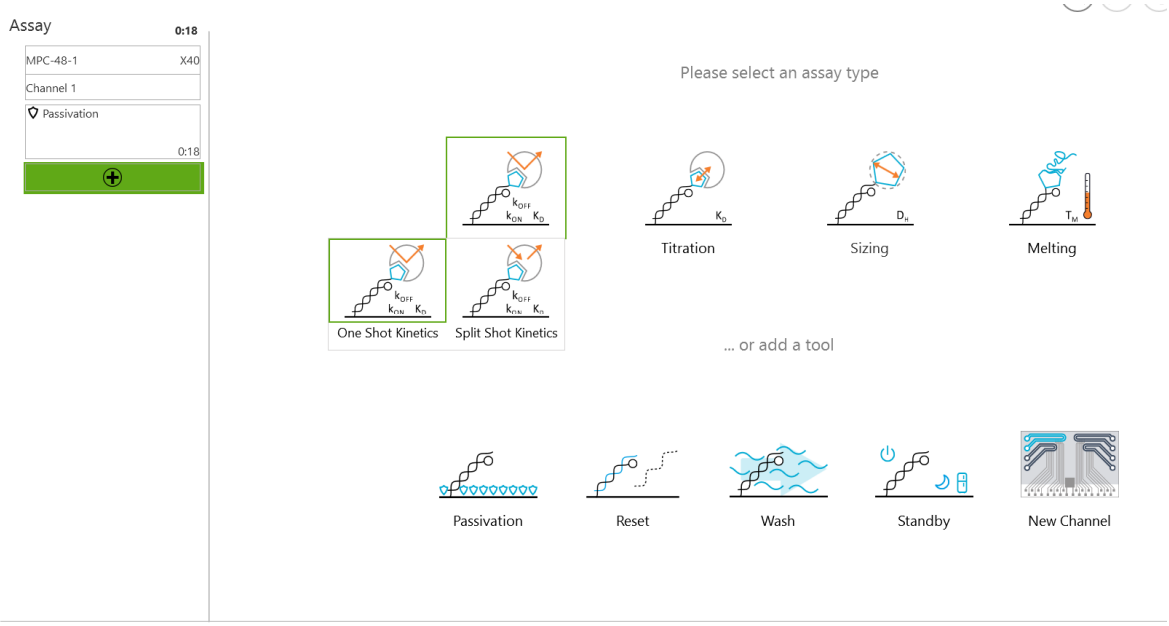
Your DBS application scientist will indicate the microfluidic channel and the spot on which measurements will be performed. With this information, it is time to begin your first switchSENSE® assay!

1. Start switchBUILD by clicking on the icon on the desktop followed by clicking icon to create a New new assay.

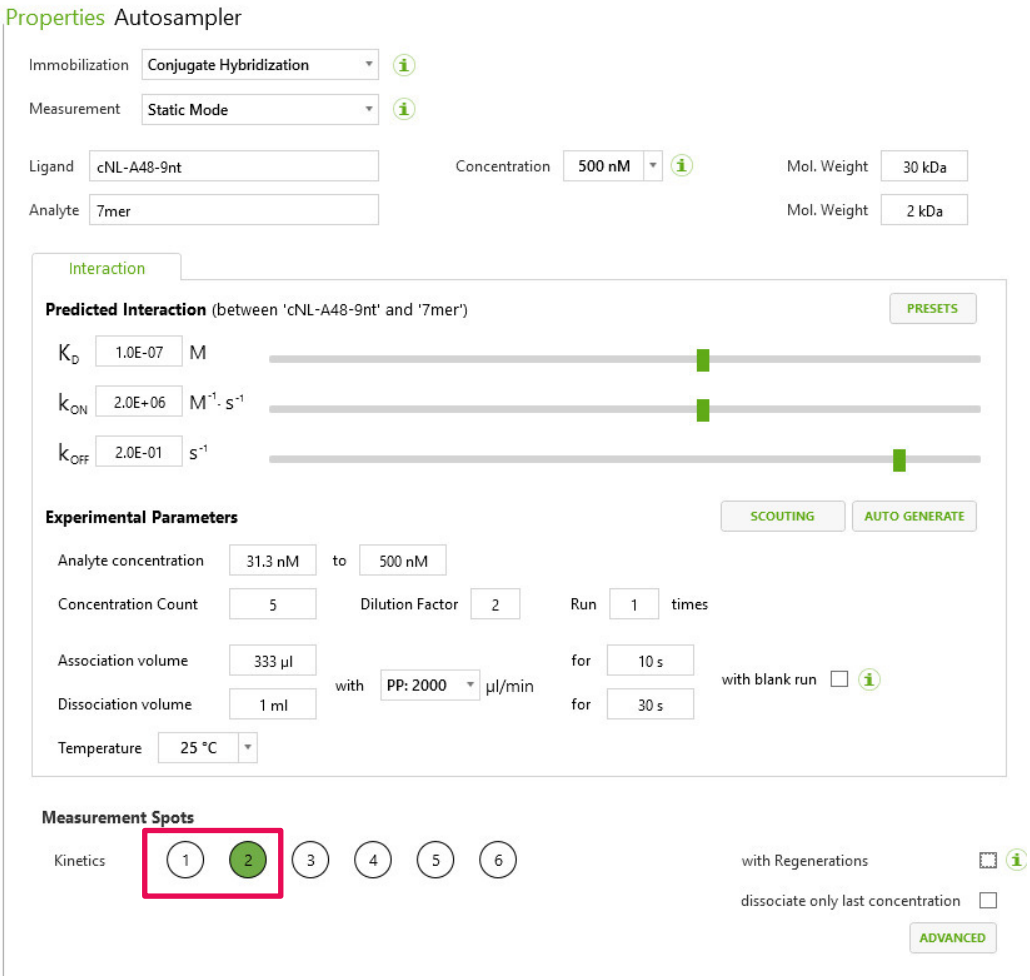
2. Next define the "Properties" of the assay. Select the flow channel in which you want to run the experiment by simply clicking on the channel. The selected channel is highlighted in green. To specify the type of Biochip used, select "MPC-48-2", which indicates that it is a Multi Purpose Chip with a functionalized DNA-length of 48 base pairs and two different DNA sequences. Furthermore, the running buffer "Buffer" (X140) and auxiliary buffer "Aux" (X40) can be specified. For this demo the default settings are appropriate. A "*Passivation*" assay element is inserted at the beginning of the "Assay" sequence by default in order to prevent unspecific binding on the biosensor surface.

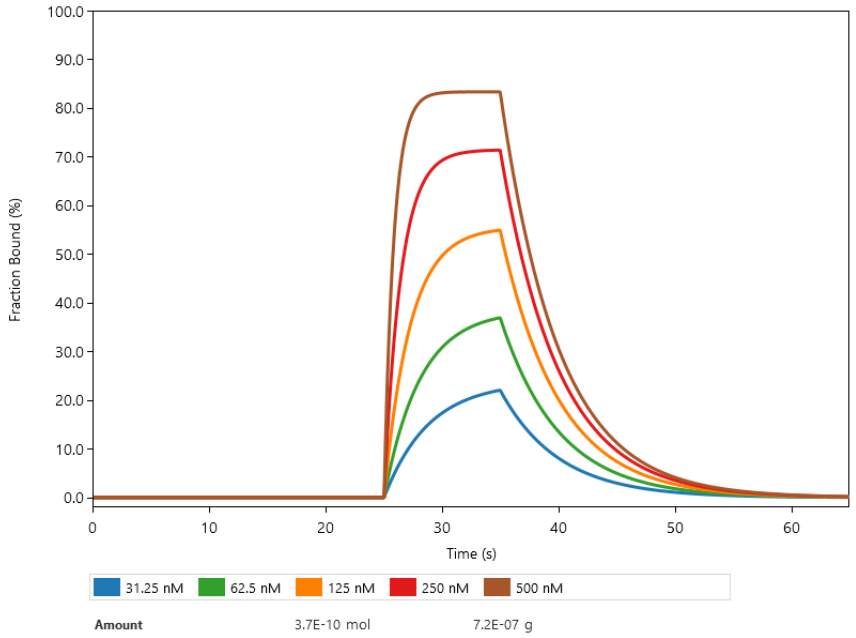


3. Click the icon below the "Passivation" box, move the mouse curser over the "Kinetics" assay element and select "One Shot Kinetics".

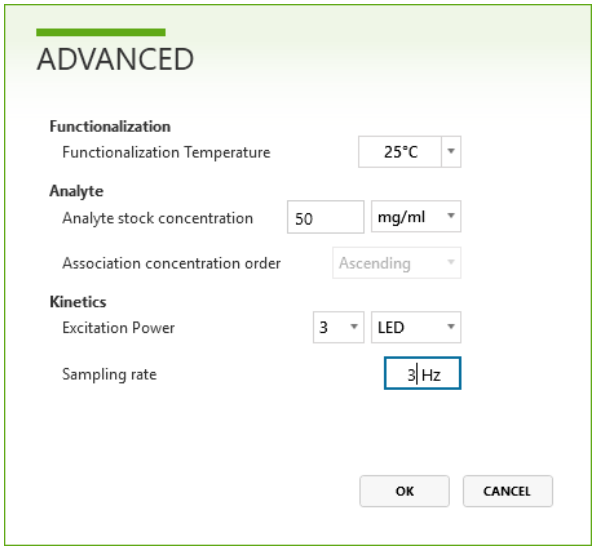


4. In the next interface, all measurement parameters and names can be set.
    - Leave Immobilization as “Conjugate Hybridization”.
    - Change Measurement to “Static Mode”. The 7mer oligo binding will affect the fluorescence signal upon binding in close proximity to the dye.
    - Name the ligand and the analyte as “cNL-A48-9nt” and “7mer” respectively.
    - Leave the ligand concentration at “500 nM”.
    - You can change the ligand molecular weight to 30 kDa and the analyte molecular weight to “2 kDa“. However, it is not mandatory to enter these values.
    - The predicted K_D, k_{ON}, and k_{OFF} values are around 1.0E-7 M, 2E6 M^{-1} s^{-1}, and 2E-01 s^{-1}, respectively. You can enter the exact or approximate K_D, k_{ON}, and k_{OFF} values by typing in or simply moving the sliders associated with the values.
    - For ”Experimental Parameters”, set the analyte concentration as 31.3 nM to 500 nM, concentration count as 5, dilution factor as 2, and (assay) run time as 1. Upon entering these values, the sensorgram on the right will automatically update to depict the predicted association and dissociation curves.
    - Set the Association/Dissociation flow rate to PP: 2000 µL/min if your DRX device is equipped with a peristaltic pump. If not, please select SP: 2000µL/min (SP stands for syringe pump). Set the 10 seconds association time and 30 seconds dissociation time. The Association & Dissociation volumes will update automatically. Due to the short measurement times, a blank run is not required, hence, leave the appropriate box unchecked.
    - Set the measurement temperature as 25°C.
    - **VERY IMPORTANT:** Select either electrode 1 or 2 as “Measurement Spots”!!! Only these two electrodes carry the correct NL-A sequence!
    - Uncheck the “with Regenerations” box as the analyte will fully dissociate within 30 seconds and this will tremendously lower the measurement time.
    - Uncheck the “dissociate only last concentration” to have a dissociation trace for all concentrations.





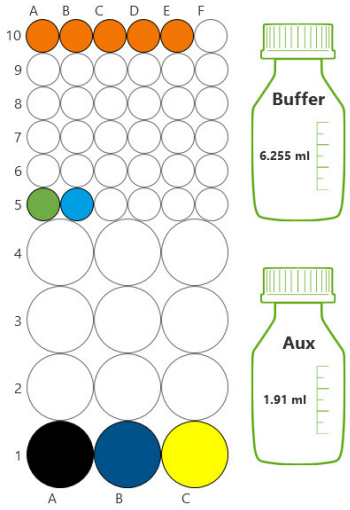
5. Next, click on ADVANCED and change the Excitation Power to LED "3" (to increase the signal-to-noise ratio) and the Sampling rate to "3 Hz" (to increase the recorded datapoints per second from 1 to 3 per second). Click on "OK".



6. After clicking on the *"Autosampler"* tab, you will see how the autosampler of the DRX should be loaded for the experiment. Here, you will find a list of all sample solutions and the required volumes and concentrations. To identify the sample position in the autosampler display on the right side of the window, select or click an item in the sample list, its respective position in the autosampler display is indicated with a green circle.

Properties Autosampler

	Sample	Volume (µl)	Conc (M)	Type	Vial
A1	Waste	-685		Waste	Large
B1	Water	filled		Water	Large
C1	Passivation solution	880		Solution	Large
A5	Regeneration solution	40		Solution	Small
B5	cNL-A48-9nt	40	5.00E-07	Ligand	Small
A10	7mer	348	3.13E-08	Analyte	Small
B10	7mer	348	6.25E-08	Analyte	Small
C10	7mer	348	1.25E-07	Analyte	Small
D10	7mer	348	2.50E-07	Analyte	Small
E10	7mer	348	5.00E-07	Analyte	Small



7. Save your switchBUILD script under a path and name that you can easily relocate again when uploading the script on switchCONTROL.

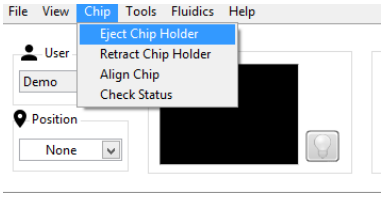
Congratulations, you just designed your own switchSENSE® experiment! We are happy to send a ready-to-use switchBUILD script. Please just drop us an email at support@dynamic-biosensors.com

Conducting the experiment

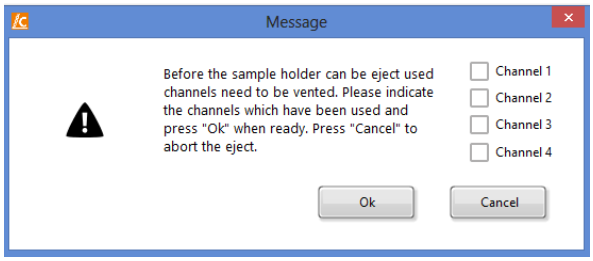
Your DBS application scientist will give you a short introduction to the materials to be used (samples, glass vials) and inform you if a biochip suitable for the measurements is already inserted (if so, skip steps 2 – 6), or if the chip needs to be exchanged (to insert the appropriate chip, follow steps 2 – 6).

1. Start switchCONTROL by clicking on the icon on the desktop and enter your name when prompted (the data will be saved under your folder name).

2. Click on **Chip → Eject Chip Holder** in the upper left corner of the software window to eject the chip holder.



3. The following message will pop up: measurement flow channels need to be vented before the chip can be ejected by the chip holder. Indicate which flow channels have been used by clicking in their respective checkboxes, and then click **OK**.

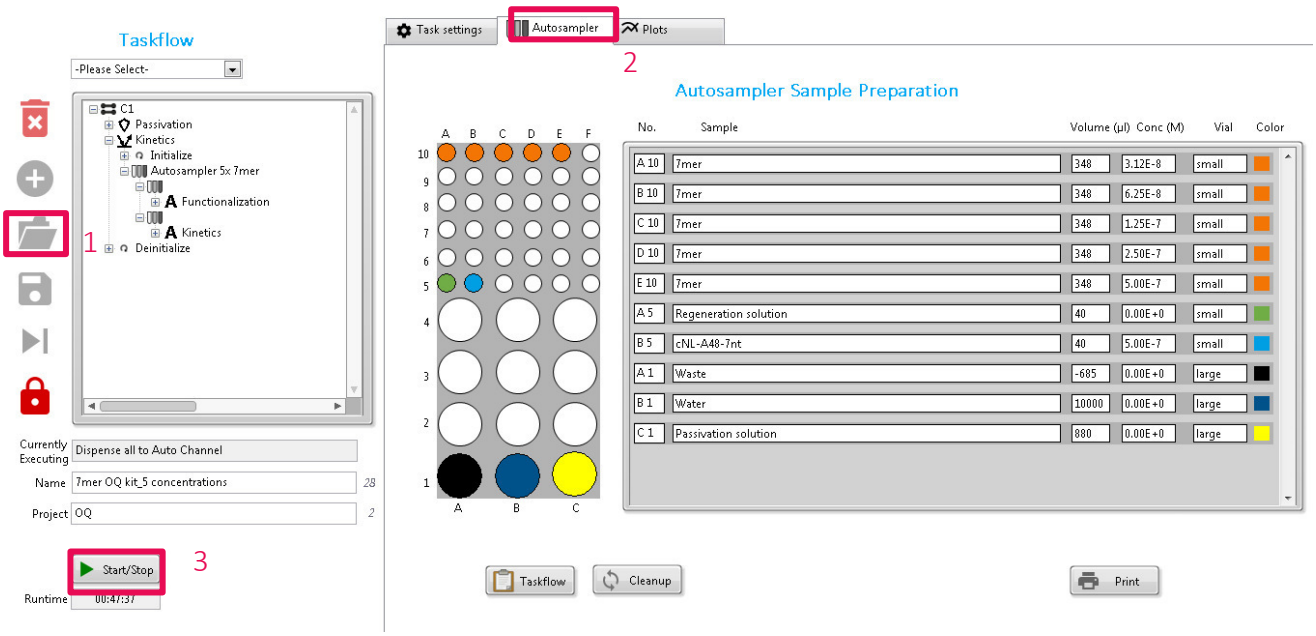


4. After venting, the chip holder will be ejected. Open the chipholder by pushing the white slider on the right to the back. Use the vacuum pen to remove the chip currently in place and insert your MPC-48-2-Y1 chip. Make sure to place it with the label facing upwards. Once the chip is in place, close the chipholder while pushing the white slider back.

White slider

5. Click on **Chip → Retract Chip Holder** to insert a new biochip and follow the instructions of the **Retract assistant**. First insert the new biochip, making sure to place it with the label facing upwards, and close the chip holder while pushing the white slider back. In the next step enter the Chip-ID, as shown on the packaging of your biochip. A message will pop up asking if the chip should be **aligned** – the alignment is necessary to precisely position the electrodes for measurement. An alignment should be performed for every newly inserted chip (except for a cleaning chip that is not used for measurements). The next message will ask you if you wish to **prime** the microfluidics system with the running buffer – the priming should be done if this is your first measurement of the day or if your running buffer is different from that of the prior measurement. Make sure there are two bottles of PE40 buffer connected to the "**Buffer**" and "**AUX**" reservoirs on the top left (exterior) of the instrument. Finally, you are asked if you want to **check the status** of the electrode and **regenerate** the channel before starting the experiment. These steps are not necessary for the purposes of this demo and the default settings can be maintained. The alignment and priming will take a few minutes. You can follow the alignment by watching the small camera window on the top left of the software window, where you will see the focusing on the four alignment crosses.

6. Load your assay into switchCONTROL by clicking on on the left of the software window {1}. Next, click on the "**Autosampler"** tab {2} to view the sample positions listed within the script, and load the sample solutions into the autosampler accordingly. Close the autosampler door, optionally change the run or project name and start the measurement by clicking on Start/Stop {3}.



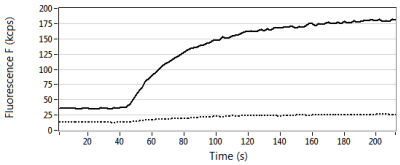
Measurement Steps

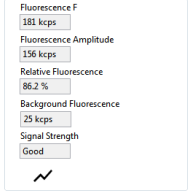
- Passivation

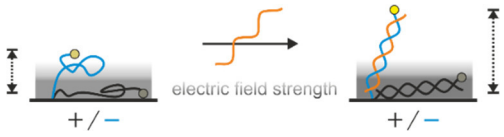
Before starting an experiment, the biosensor surface needs to be passivated to prevent unspecific surface binding, and thus enable maximal DNA-switchability. During the passivation step, the chip is incubated in passivation solution and the DNA levers are switched with a frequency of 0.2 kHz. A positive potential leads to a low fluorescence signal (as the fluorophore lies close to the surface), while a negative potential leads to a high fluorescence signal (as the fluorophore is positioned away from the surface). The passivation step takes 10 minutes and should be run completely if the channel had not already been passivated on the same day. If the channel has already been passivated, you can skip this step after a few minutes by clicking on . Therefore the taskflow needs to be unlocked first, by clicking on .

- Regeneration, Calibration and Dynamic Response

You will be able to follow the functionalization of the chip with the cNL-A48-9nt complementary DNA in real-time. At the start of the **regeneration**, the dsDNA is denatured by the injection of a regeneration solution and the measurement starts with the switching of ssDNA. After a short while, the fresh complementary strand is injected into the flow channel and the ssDNA is hybridized to dsDNA. As dsDNA is more rigid than ssDNA, the fluorophore is pushed further away from the surface during switching. Consequently, the switching amplitude increases during the hybridization. The regeneration takes ~ 10 – 15 minutes for completion.





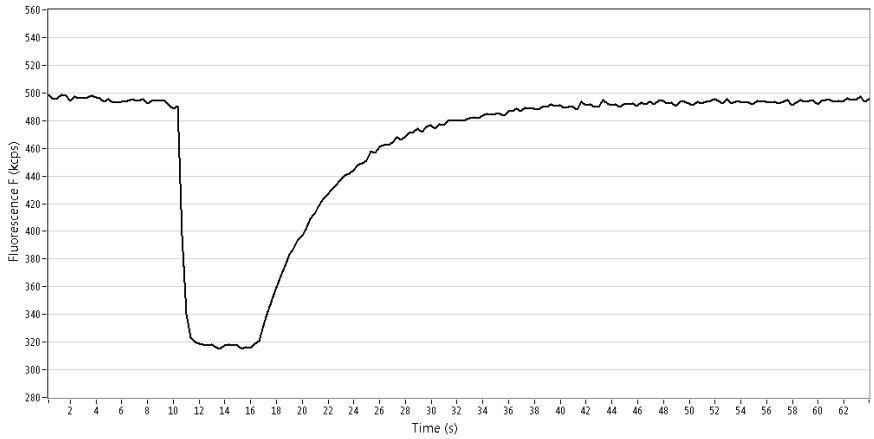


Association and Dissociation of the Analytes

After the functionalization of the chip with the 9nt overhang DNA is completed, the first concentration (31.3 nM) of the 7mer oligo is injected onto the electrodes for only 10 seconds. During the association and dissociation of the analytes, a fixed potential is applied to the electrodes, to keep the DNA nanolevers in an upright position.

You can now follow the binding to the 7mer oligo in real time: the fluorescence decreases over time, as the physico-chemical environment of the dye changes due the binding of the 7mer in close proximity to the dye. By clicking on “Color to display”, you can filter for “red only” so that the graph is exclusively auto scaled to the red signal response curve.

Directly after each association, running buffer is injected into the microfluidic channel (one-shot script). Again, you can follow the dissociation of the 7mer in real time, as the unbinding of the 7mer increases the fluorescence. You will see, that within 30 seconds of dissociation the initial fluorescence level is reached again. The 7mer fully dissociated from the 9nt overhang and a regeneration of the surface with cNL-A48-9nt is not required. Hence, the next highest concentration (62.5 nM) is picked up from the autosampler and directly injected onto the electrodes and dissociation is again monitored for 30 seconds to allow full dissociation. These steps are automatically repeated for the remaining 3 concentrations based on the generated switchBUILD script.

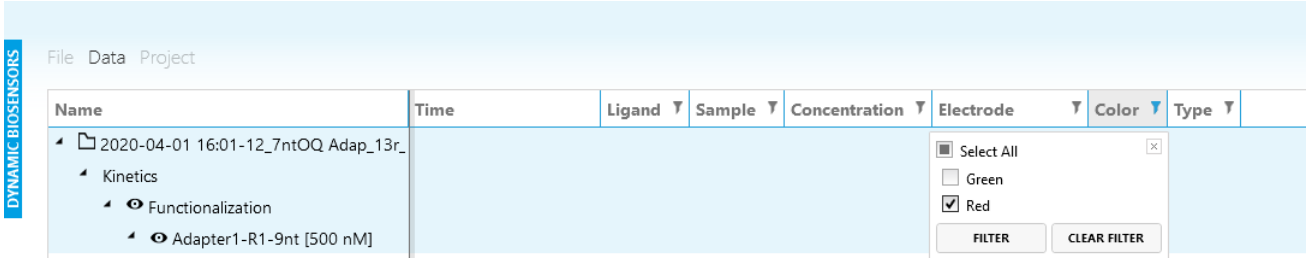


Exemplary real-time graph of 500nM 7mer binding to the overhang. Signal amplitude change for lower concentrations is smaller.

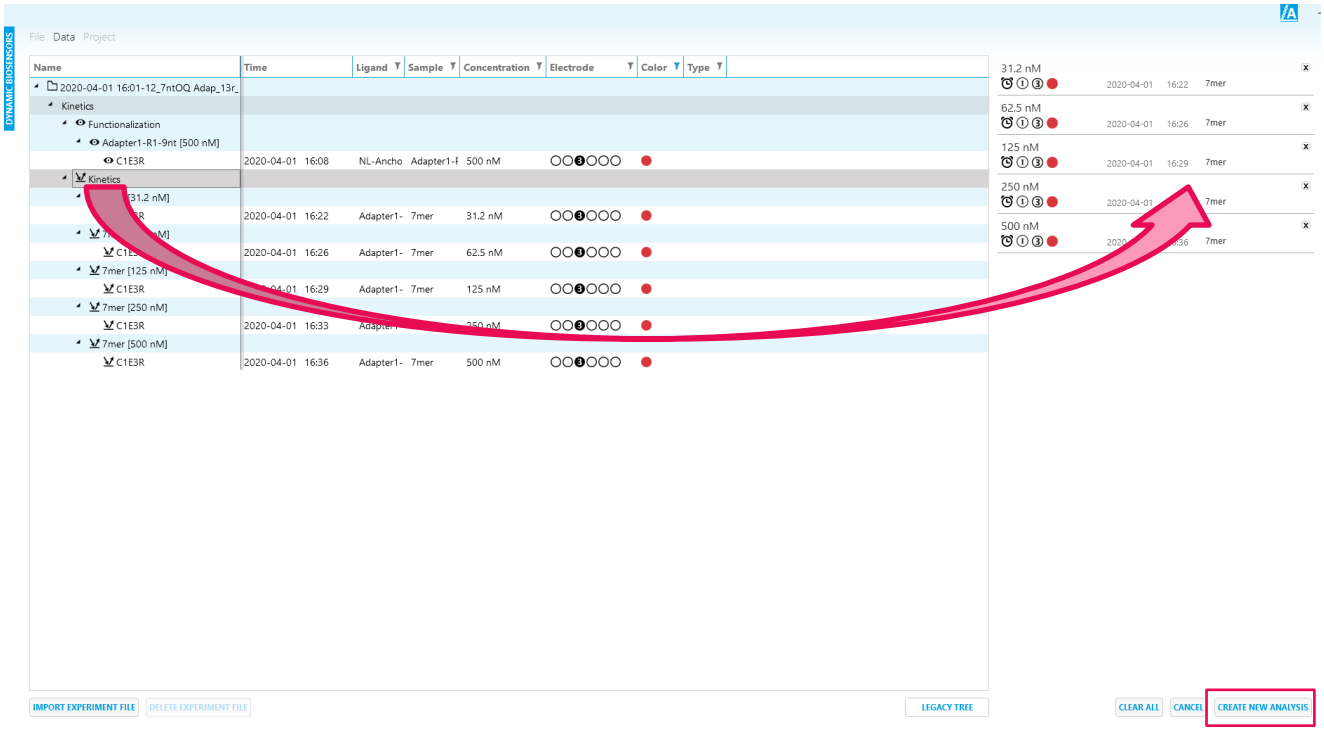
Data analysis with the switchANALYSIS software

1. Start switchANALYSIS by clicking on the icon on the desktop and click on + New to create a new analysis. Now, load your measurement file by clicking on "Choose measurement files or drag them here" and selecting your zipped measurement folder or, alternatively, by directly dragging & dropping the zipped folder into this area.

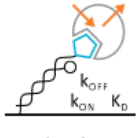
2. Now, all relevant measurement files are loaded. If you have a dual-color DRX2, first filter for the measurement color (red) by clicking on "Color" and checking "Red"



3. Next, drag & drop the entire Kinetics block (not the Functionalization) to the right side and click on "CREATE NEW ANALYSIS".

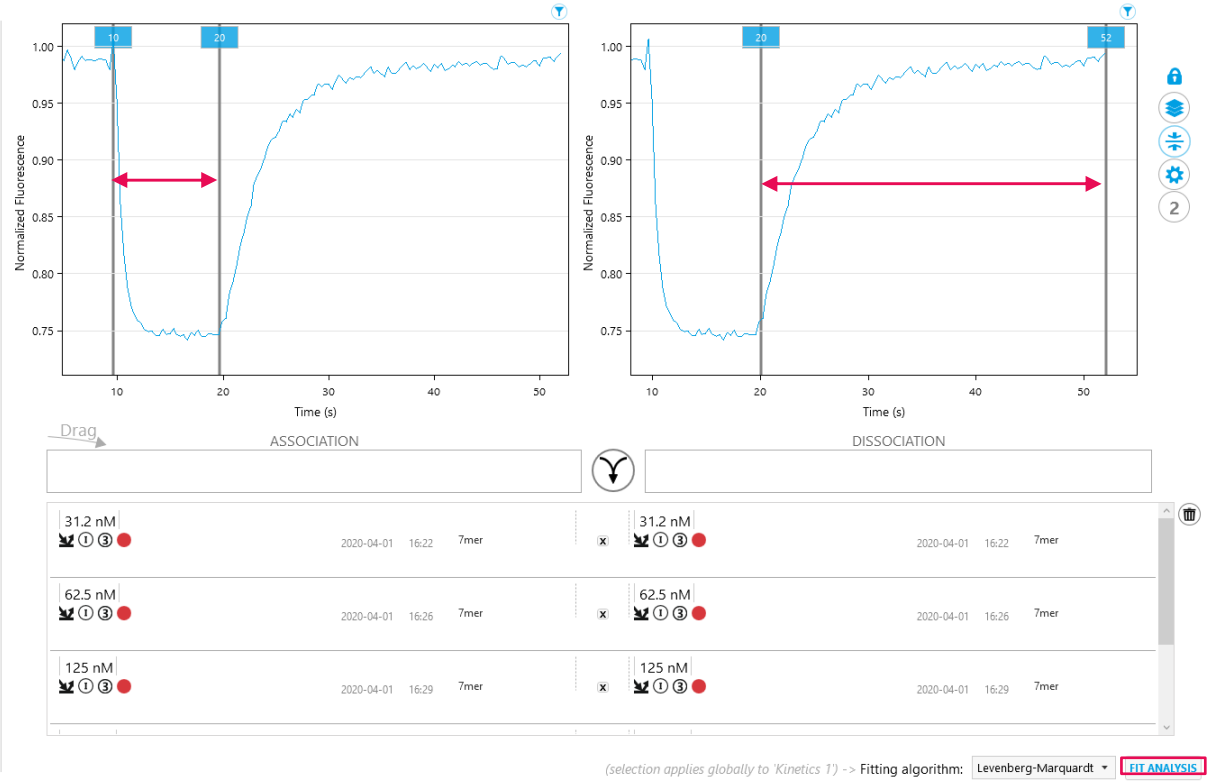


4. Now, you can select the analysis type. Please select "Kinetics".

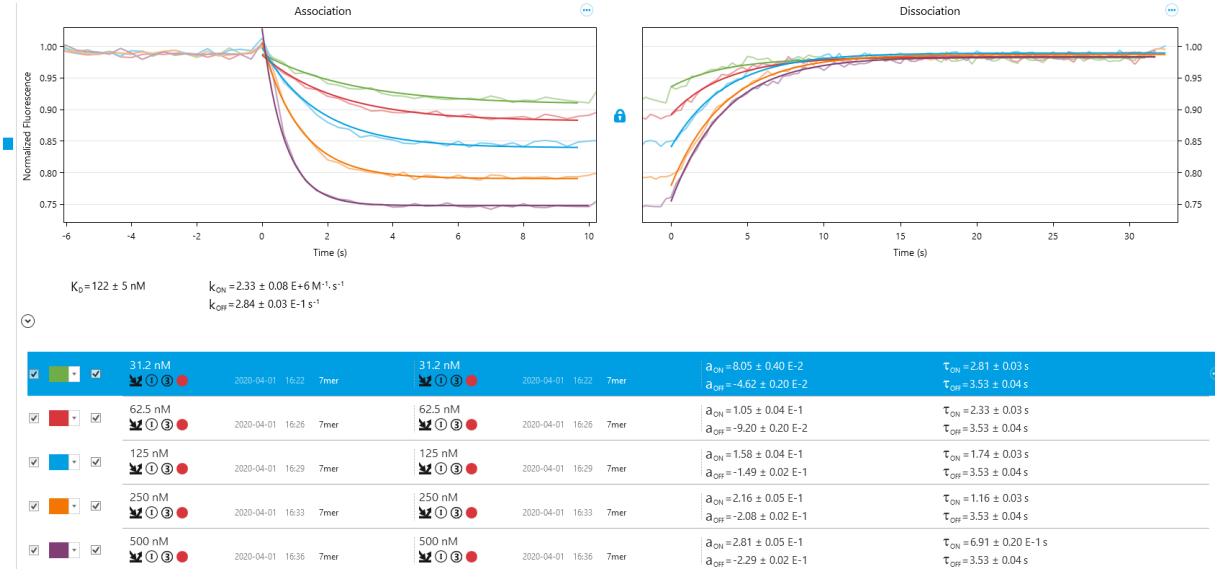


Kinetics

5. The one-shot association & dissociation curves will be automatically loaded into the appropriate “ASSOCIATION” or “DISSOCATION” columns. The fitting borders are already pre-set, however, please check and manually check these fitting borders to place them directly before the signal drop (starting fitting border) / before the signal increases again (ending association fitting border) and before the signal increases (starting dissociation fitting border) until the end of the graph (ending dissociation fitting border). Please check the fitting borders for all 5 concentration datasets. Next, please click on “FIT ANALYSIS”



6. You will see a mono-exponential fitting curve for the association and dissociation data. To incorporate all the data, the global mono-exponential fitting model is selected in the left column. One value for k_{ON}, k_{OFF} and K_D is calculated from all 5 concentration curves.
You can now compare these kinetic values to the stated ones. Please let us know in case you see any irregularities such as drifting baselines, misshaped curves, big airbubble spikes, or significantly deviating kinetic values.



Congratulations, you are a switchSENSE® expert user now!

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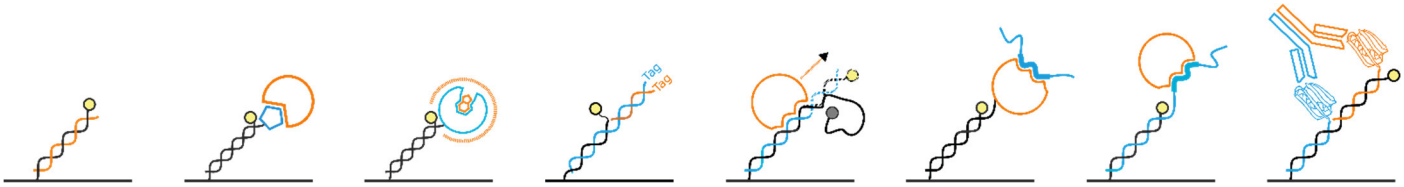

switchSENSE® is a proprietary measurement technology by Dynamic Biosensors GmbH. Instruments and biochips are engineered and manufactured in Germany.