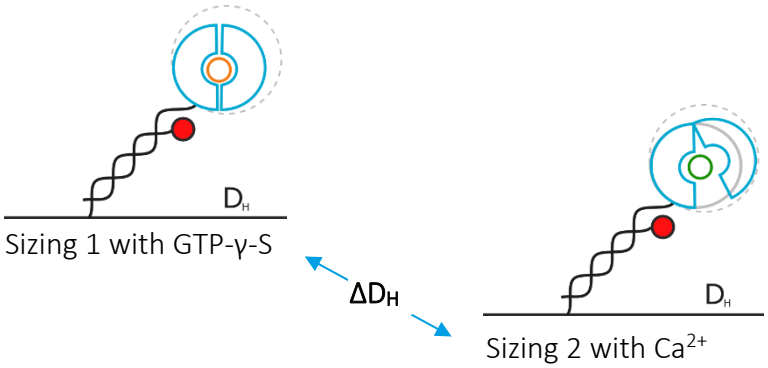
switchSENSE® Demo Kit

DK-Transglutaminase: Binding Induced Conformational Change (ΔD_H)

Sizing of Transglutaminase 2 with GTP-γ-S and Ca^{2+}

Aims

- Familiarization with the software tools for performing switchSENSE® experiments
- Determination of the hydrodynamic diameter (D_H) of transglutaminase 2 (TG2) bound to GTP-γ-S and Ca^{2+}



Workflow

Design your experiment	**switch**BUILD	Step-by-step guidance through the workflow
Perform your experiment	**switch**CONTROL	Finetuning of the experiment
Analyze your results	**switch**ANALYSIS	Simple drag´n´drop format for efficient data interpretation

Product description

Product Code DK-TG-1-B48

Measurement Time ~1 h 30 min

In this experiment, the conformational change of transglutaminase 2 (TG2) between the compact GTP-γ-S-bound and the extended Ca^{2+} bound state is analyzed. We start with immobilizing TG2 on the biochip and inject GTP-γ-S to force the protein into its compact conformation. During Stopped Flow we size the protein. After washing out the first analyte, we inject $CaCl_2$ and size again.

Before getting started, transfer one aliquot of each sample listed in Table 1 to the fridge to ensure slow thawing.

TABLE 1 | Contents and storage information

Material	Cap	Amount	Storage	Comments
cNL-A48 / cNL-B48 (500 nM each)	blue	3 x 65 µL	-20°C	in PE40
cNL-A48 / cNL-B48-TG2 (200 nM each)	green	3 x 40 µL	-20°C -80°C	in TE40
$CaCl_2$ (10 mM)	red	3 x 50 µL	-20°C	dilute in 950 µL PE40
GTP-γ-S (10 µM)	yellow	3 x 50 µL	-20°C	dilute in 950 µL PE40

TABLE 2 | Additional material required

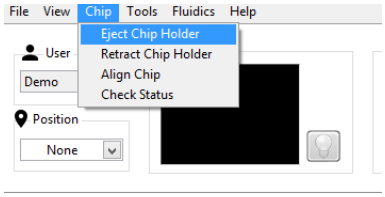
Material	Cap	Amount	Storage	Comments
MPC-48-2 biochip	-	1	2-8°C	use in DRX or DRX^2
PE40 buffer (1x): 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.4, 40 mM NaCl, 50 µM EDTA, 50 µM EGTA, 0.05 % Tween 20, sterile filtered	-	100 mL	2-8°C	Running Buffer Aux buffer
ddH_2O	-	1 x 12 mL	2-8°C	
Passivation solution (10x)	yellow	1 x 1 mL	2-8°C	dilute in 9 mL ddH2O
1x Regeneration solution	-	1 x 12 mL	2-8°C	

Large glass vials (10 mL), small glass vials (1.5 mL), caps required. For *in vitro* use only.

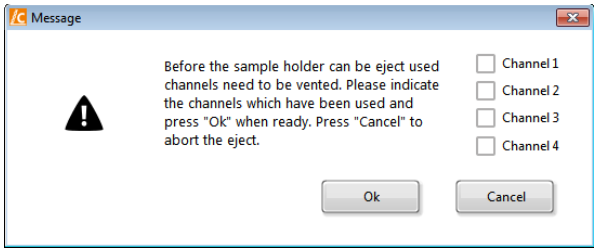
Conducting the experiment

1. **Start switchCONTROL** by clicking on the icon on the desktop and enter **your name** when prompted (the measured data will be automatically saved in a folder with your name).

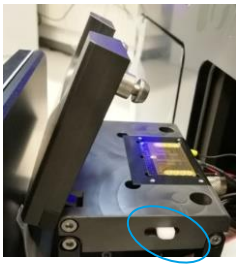
2. To insert a new chip, click on **Chip** → **Eject Chip Holder** in the upper left corner of the software window to eject the chip holder from the DRX.



3. The following message will pop up. Indicate which channels have been used on the inserted biochip by checking their respective checkboxes, and then click **Ok**.



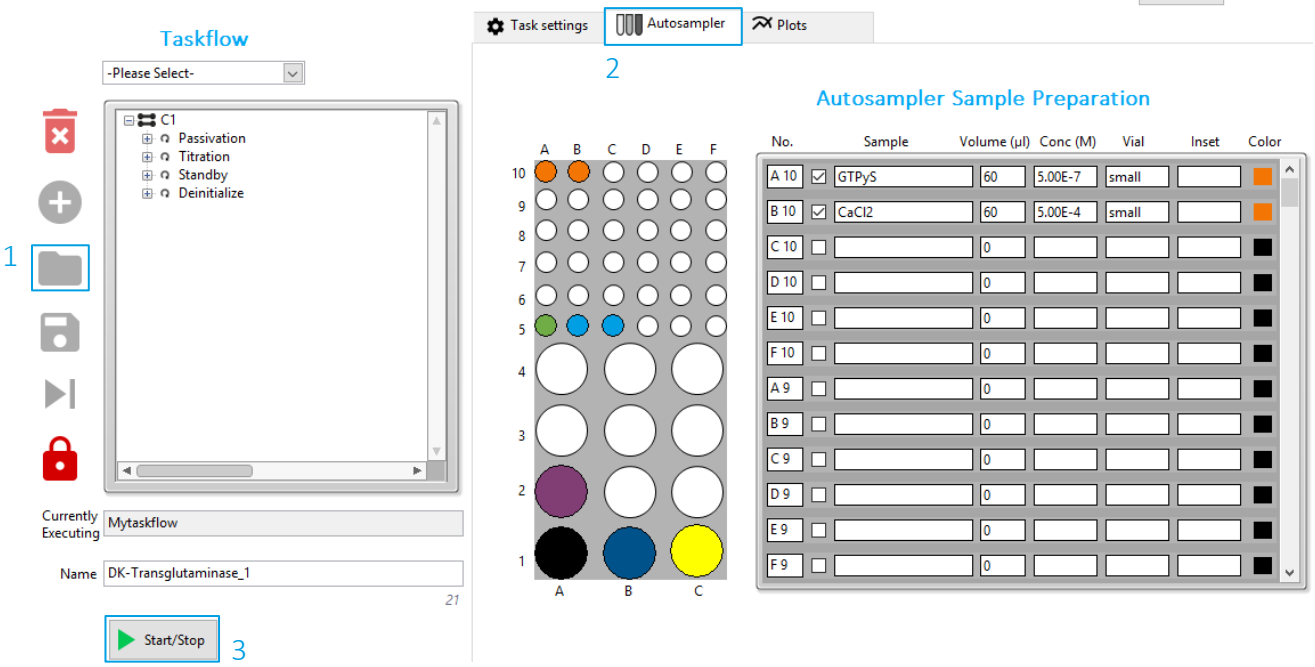
4. After venting, the chip holder will be ejected. Open the chip holder by pushing both white sliders on the right to the back. Use the vacuum pen to remove the chip currently in place and insert your **MPC-48-2** chip. Make sure to place it with the label facing upwards. Once the chip is in place, close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



5. Click on **Chip** → **Retract Chip Holder** to retract the new biochip into the DRX and follow the instructions of the **Retract assistant**. 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 every time a chip is placed into the DRX (except for a Cleaning chip which 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 that 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 electrodes 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. While the alignment and priming are taking place, please **prepare the samples**: Add 950 µL of PE40 buffer to 50 µL of each analyte sample (GTP-γ-S, $CaCl_2$).

7. **Load the taskflow** *DK-Transglutaminase_1* into switchCONTROL by clicking on  at 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 and start the measurement by clicking on  {3}.



Measurement Steps

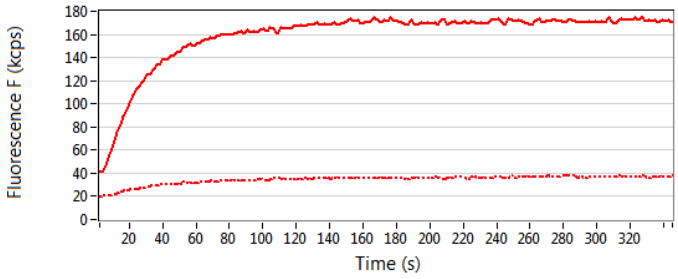
If working with a DRX² DualColor instrument, choose to display the **red dye only** by clicking on the color block next to *Display* above the measurement plots Display .

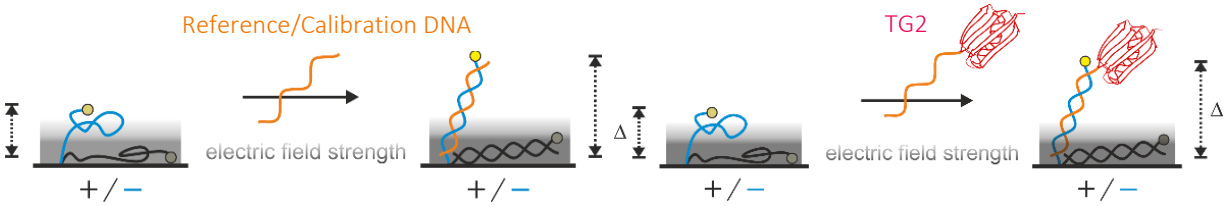
- Passivation

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. Positive potential leads to a low fluorescence signal (as the fluorophore lies close to the surface), while 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 . To do so, the taskflow needs to be unlocked first by clicking on .

- Functionalization

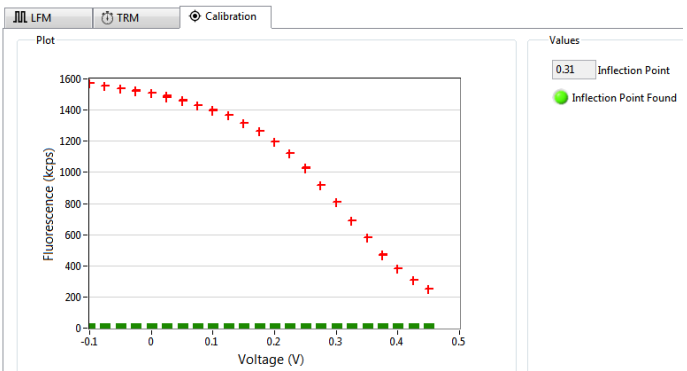
You will be able to follow the functionalization of the chip with first the reference complement DNA and later the TG2-conjugated complement DNA in real-time in the **red channel**. Before the *functionalization*, the dsDNA is denatured by the injection of a *regeneration* solution (high pH), therefore 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 form 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 functionalization takes about 15 minutes for completion.



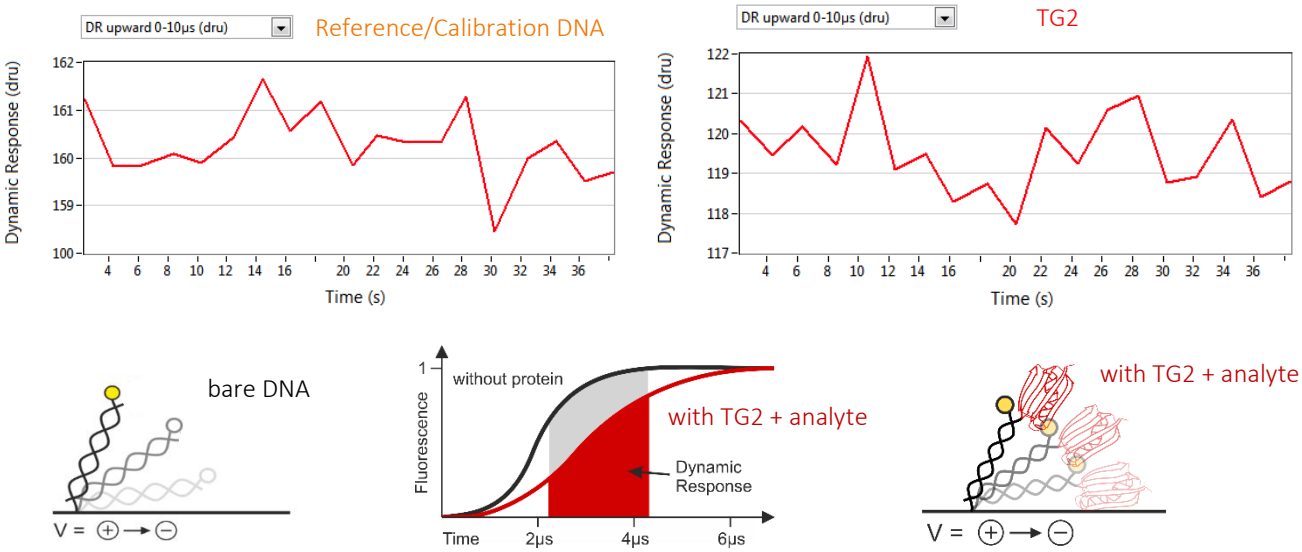


- Stopped Flow: Calibration and Dynamic Response

After hybridization of bare complementary DNA (for sizing calibration), and later of TG2-conjugated DNA with analytes, a *calibration* measurement determines the inflection point of the DNA on the selected electrode. This calibration measurement records the change of fluorescence intensity in response to the applied voltage. More positive voltages attract the negatively charged DNA and orient the DNA nanolevers closer to the quenching gold surface.



Subsequently, the standing-up curve is recorded during *Stopped Flow* with voltages according to the previously determined inflection point. It is used for calculating the absolute size of the conjugate, as well as the Dynamic Response. Therefore the "*DR upwards 0 - 10 µs*", plotted top left, is a measure of the switching speed of the nanolever and is derived from its time-resolved upward motion. The switching speed should be reduced on electrode spots 3 – 6 after the functionalization with TG2, compared to bare complementary DNA, as the bound protein increases the frictional drag of the DNA nanolever during switching.



- Titration of the analytes

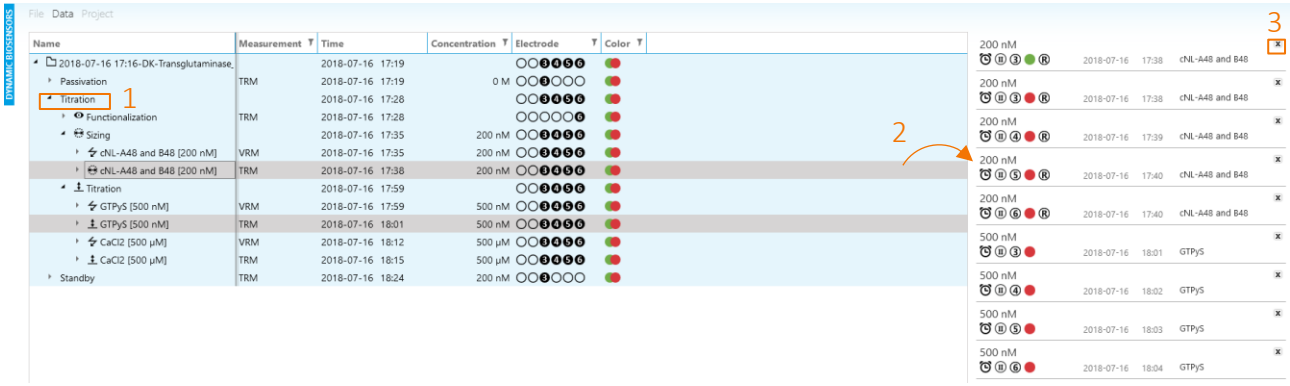
After functionalization of the electrode with TG2-conjugate, the first analyte GTP-γ-S is injected. During a waiting period of 60 seconds the fast kinetic is allowed to reach saturation. Subsequently, the TG2 + GTP-γ-S complex is sized during a Stopped Flow measurement.

GTP-γ-S is washed out with running buffer prior to the injection of the second analyte, $CaCl_2$. After an equilibration period the size of TG2 + Ca^{2+} is again determined in a Stopped Flow measurement.

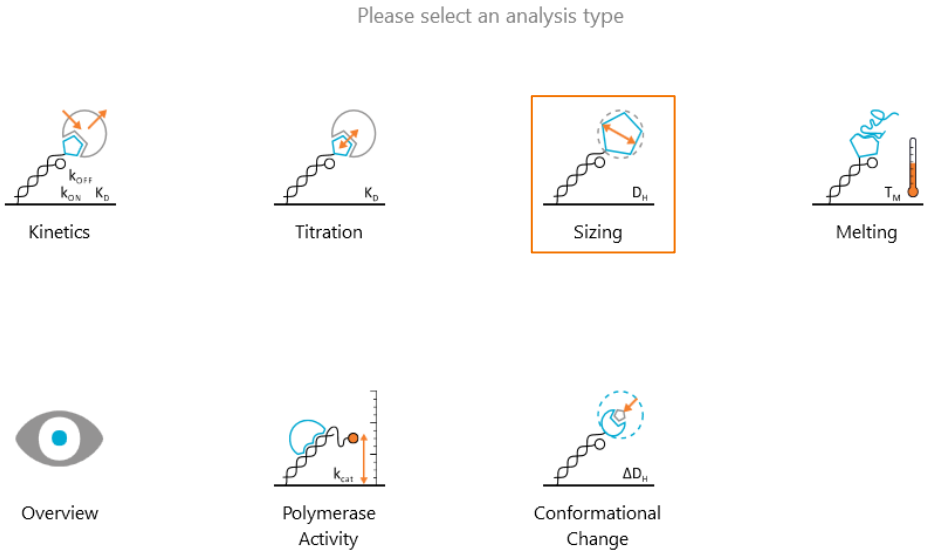
Data analysis with the switchANALYSIS software

1. Start switchANALYSIS by clicking on the icon on the desktop and click New on to create a new assay. Now you can load your data by clicking on **IMPORT EXPERIMENT FILE** in the lower left corner and selecting your zipped measurement data, which is saved in > folder with your username > subfolder with the date.

2. You will find the assay structure from the switchCONTROL taskflow (Passivation – Titration – Standby) when expanding the measurement file (indicated by the start time of your measurement) by **clicking on the triangles**. Further expansion of the *Titration* dataset {1} reveals data for the Functionalization, Sizing (of calibration DNA) and Titration of the analytes. To determine the size of TG2 in its closed state, click on the triangle in front of **Sizing and Titration**. This opens up all performed Calibrations and Sizings (and) from Stopped Flow measurements. Drag-and-drop the Sizings of calibration DNA (**cNL-A48 and B48**) and of TG2-conjugate with **GTP-γ-S** into the right column to select the data {2}. Both the red and green channel recorded data during the measurements, however, we did not use any green-labeled DNA, therefore **delete all green** marked data with {3}.

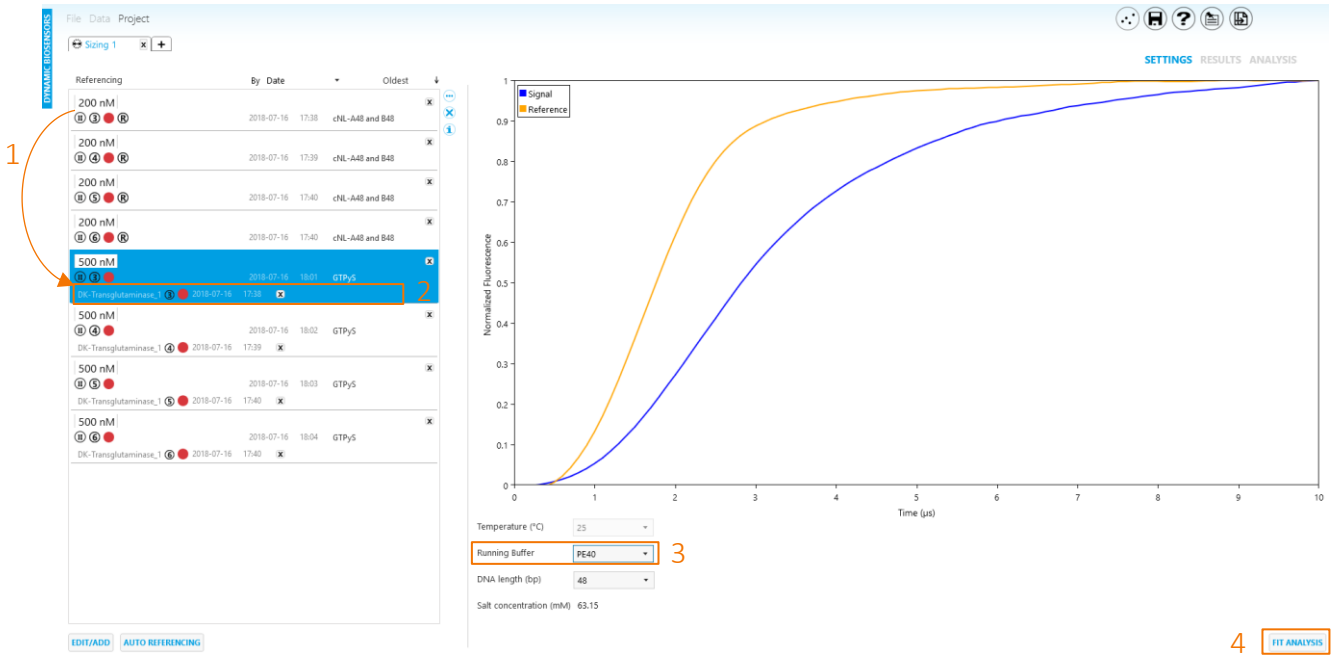


3. Click on **CREATE NEW ANALYSIS** in the lower right corner and select the **Sizing** analysis type to analyze the hydrodynamic diameter of TG2 in its closed state.



4. On the SETTINGS page you should first calibrate your data if not done automatically. To do so, drag-and-drop the **cNL-A48 and B48** measurement for each electrode on their respective **GTP-γ-S** measurement {1}. If a measurement is referenced or

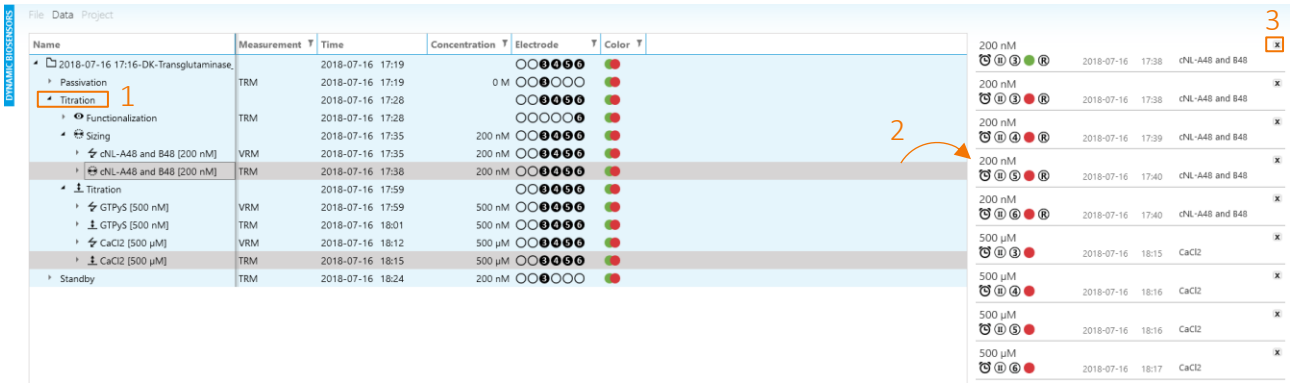
calibrated, it is indicated by the name and time of the reference measurement {2}. Set the *Running buffer* used (**PE40**) in the options below the graph {3}. Finally click on **FIT ANALYSIS** in the lower right corner {4}.



5. On the RESULTS page a fitted plot of the calibration DNA (Reference, orange) and the TG2-conjugate in its closed, compact state with bound GTP-γ-S (Signal, blue) sizing data is shown. The corresponding fit curves are indicated in grey and black, respectively. The left panel lists the calculated hydrodynamic diameter with the coefficient of determination (R^2) for each individual measurement (electrode 3-6). Below the graph an *average hydrodynamic diameter* D_H^{avg} of all selected ☑ sizing measurements is displayed. If more than one sizing measurement is selected, the standard error of the mean (SEM) is provided as well.

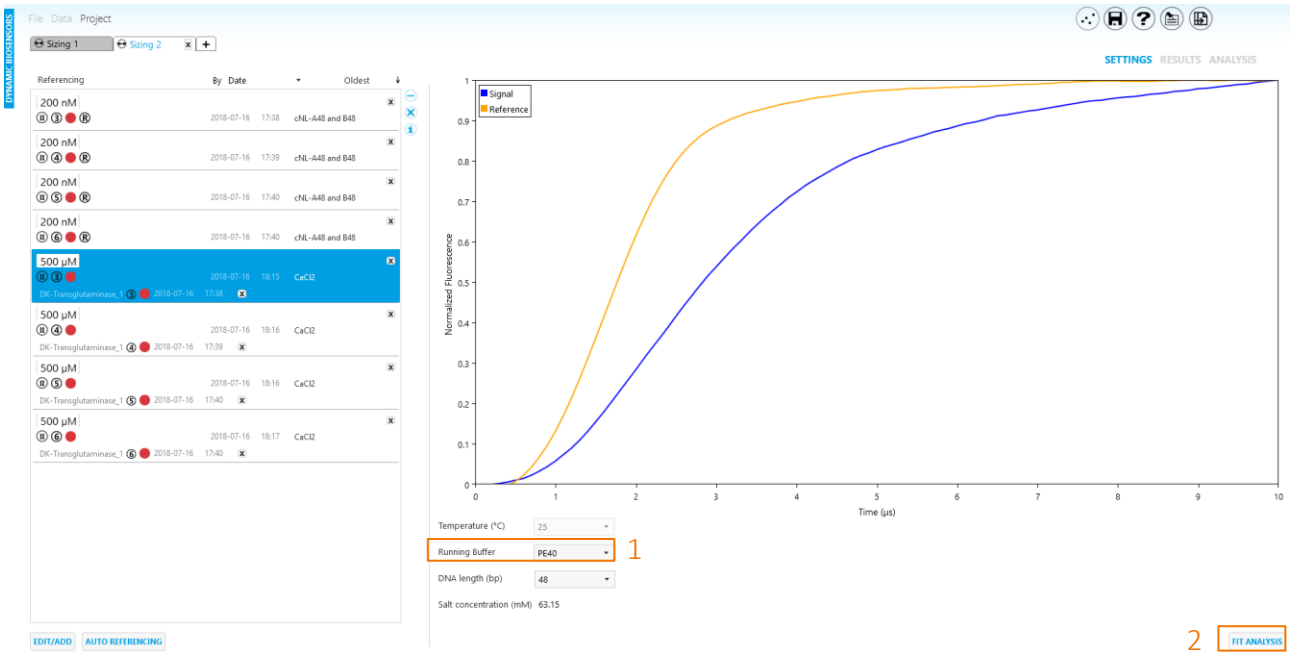
6. Next, click on the top next to "Sizing 1" on the **+ tab** to create a new sizing analysis.

To determine the size of TG2 in its open state, click again on the triangle in front of **Sizing and Titration**. This opens up all performed Calibrations and Sizings (and) from Stopped Flow measurements. Drag-and-drop the Sizings of calibration DNA (**cNL-A48 and B48**) and of TG2-conjugate with **$CaCl_2$** into the right column to select the data {2}. Both the red and green channel recorded data during the measurements, however, we did not use any green-labeled DNA, therefore **delete all green** marked data with ☒ {3}.



7. Click on **CREATE NEW ANALYSIS** in the lower right corner and again select the **Sizing** analysis type to analyze the hydrodynamic diameter of TG2 in its open state.

8. Calibrate the **CaCl2** size measurements again with the measurements of **cNL-A48 and B48** (see section 4) on the SETTINGS page. Set the *Running buffer* used (**PE40**) in the options below the graph {1}. Click on **FIT ANALYSIS** in the lower right corner to calculate the D_H of TG2 in its open, extended conformation {2}.



9. On the RESULTS page you will now find the *average hydrodynamic diameter* D_H^{avg} of all selected sizing measurements of TG2 + $CaCl_2$ below the graph. By subtracting the D_H of TG2 + GTP-γ-S from the D_H of TG2 + Ca^{2+} the **conformational change** between TG2 in its extended and compact state can be quantified.

10. Finally, you can save your analysis by clicking on .

Congratulations, you are a switchSENSE® expert user now!

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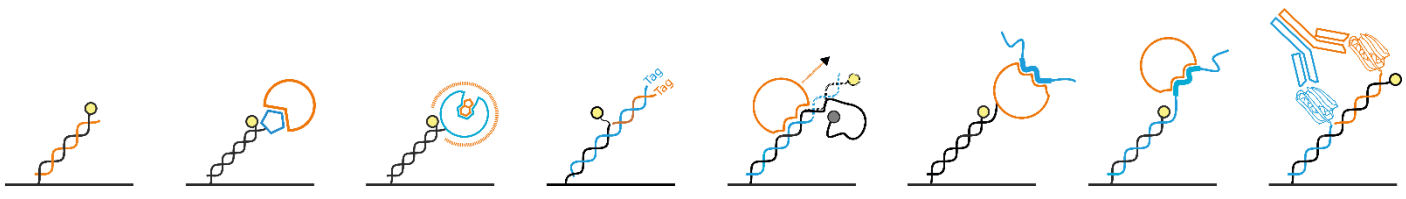

switchSENSE® is a proprietary measurement technology by Dynamic Biosensors GmbH.
Instruments and biochips are engineered and manufactured in Germany.