

Thrombin Analyzer Tutorial

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Abstract

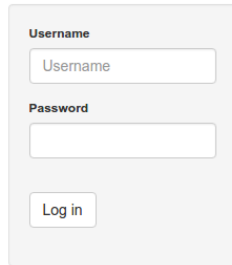
This tutorial guides the user through the [Thrombin Analyzer](#) application for data analysis in thrombin generation experiments.

Contents

1	Introduction	2
2	Login and logout	2
3	Instructions	4
4	Calibration	4
5	Thrombin generation	6
6	Parameters	7
7	Dataset	8
8	Tutorial	13
9	Important notes from Dmitriy	13

1 Introduction

Follow [this link](#) to open Thrombin Analyzer (further referred to just as “the app”) in Your browser. You should see the following result:

A login form with a light grey background. It contains a label "Username" above a text input field with the placeholder "Username". Below this is a label "Password" above another text input field. At the bottom is a "Log in" button.

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Figure 1. Thrombin Analyzer start page.

If You see Fig. 1 in Your browser, You are now ready to use Thrombin Analyzer. The following sections will show in details how to do that.

2 Login and logout

You see Fig. 1 in Your browser, as discussed in Sec. 1, and now You have to login. A combination of login and password should have been sent to You by me earlier. You type them in as in Fig. 2a and if both the username and the password are correct, You are logged in and You see the main page of the app as in Fig. 2b.

The grey sidebar of the app consists of four sections — **Dataset**, **Calibration**, **Thrombin generation**, and **Demo signals**. **Calibration** and **Thrombin generation** sections allow to work with individual signals, **Dataset** — with datasets consisting of many signals, and **Demo signals** allows to download some sample data in case there is no experimental data available on Your computer, but You are still eager to try out the app.

The white main panel consists of seven tabs — **Instructions**, **Dataset**, **Calibration signal**, **Thrombin generation signal**, **Parameters**, **Demo signals**, and **Tutorial** that visualize the results for corresponding sections of the grey sidebar. The sections below will discuss them all in details.

Pressing the **Log out** button will log You out of the main page of the app. Upon logout, Your session will be cleared, so when logging in again You will start from scratch.

a)

Username

dmmar

Password

Log in

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b)

Thrombin Analyzer

Hello dmmar!

Log out

Dataset

Load dataset

Choose File

No f...sen

Load parameters

Choose File

No f...sen

Load results

Choose File

No f...sen

Calibration

Load calibration data file

Choose File

No file chosen

Thrombin generation

Load thrombin generation data file

Choose File

No file chosen

Demo signals

Select a demo signal to try out models on data!

None

Instructions

Dataset

Calibration signal

Thrombin generation signal

Parameters

Demo signals

Tutorial

You are successfully logged in the Thrombin Analyzer application! Press [Log out](#) in the topright corner of the sidebar to log out of the application.

This application performs data analysis in thrombin generation experiments. Below is the step-by-step guide that explains how to use it.

Calibration

Thrombin generation

Parameters

Dataset

Demo signals

Calibration signal tab visualizes the results of analysis of a calibration signal. Use the [Load calibration data file](#) button and choose a suitable signal to load. Then, choose a model to fit the loaded signal using the [Select model to fit calibration signal](#). Parameters obtained from the model fitted to the signal will be shown in [Parameters](#) tab.

Thrombin generation signal tab visualizes the results of analysis of a thrombin generation signal. Use the [Load thrombin generation data file](#) button and choose a suitable signal to load. Then, choose a model to fit the loaded signal using the [Select model to fit thrombin generation signal](#). Use [Thrombogram](#) tab to visualize the thrombogram and the thrombin velocity of the signal. Parameters obtained from the model fitted to the signal will be shown in [Parameters](#) tab.

Analyze a calibration or a thrombin generation signal or both following the steps above and check the parameters with the [Parameters](#) tab.

Dataset tab visualizes the results of the analysis of a dataset from a thrombin generation experiment. Use the [Load dataset file](#) to load a dataset, and press [Analyze!](#) to analyze it. Overlay thrombograms and check the details of the performed analysis using the [Overlay](#) and [Overlay details](#) tabs.

If You have the results and the parameters available for the dataset, You can alternatively load them using the [Load parameters](#) and [Load results](#).

In case there are no data on Your computer, download some of the provided demo signals by choosing from options in [Select a demo signal to try out models on data!](#). The data You choose is visualized in [Demo signals](#) tab. Download data, and analyze it following the steps above.

Good luck!

Dmitriy Markovich

Figure 2. a) Login with the personal credentials. b) Main page of the app when logged in.

3

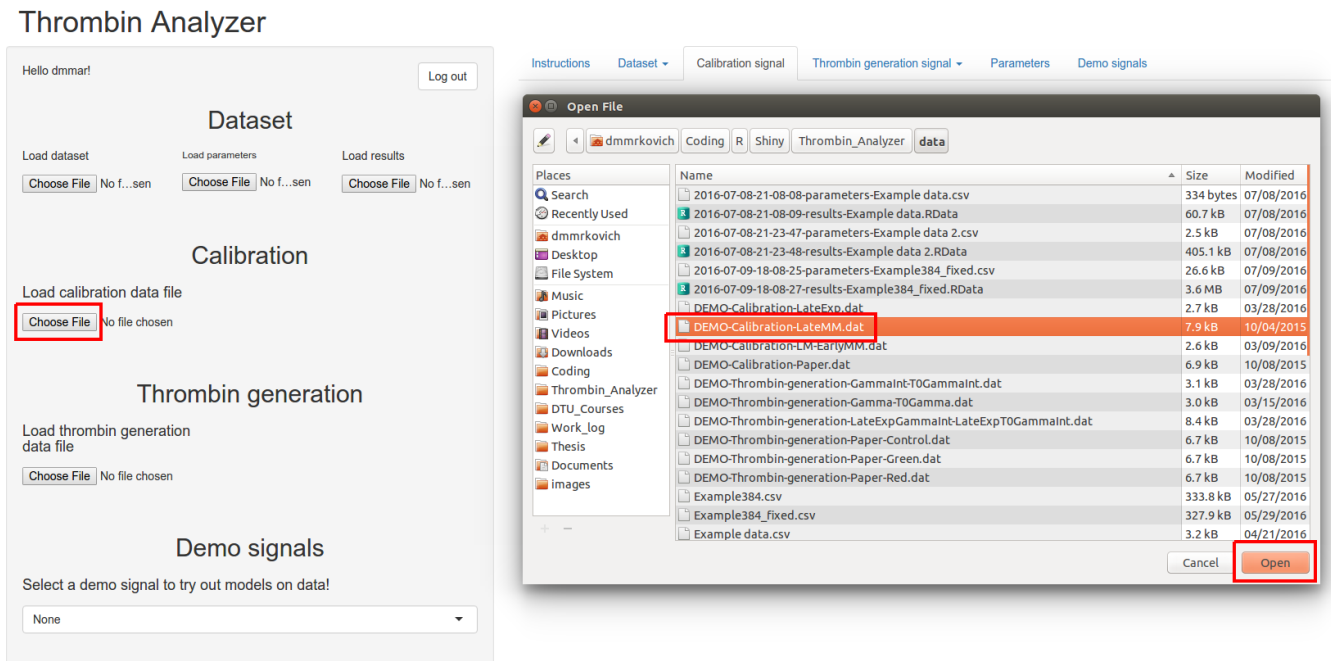
3 Instructions

The **Instructions** tab gives a brief reminder about how to use the app, see Fig. 2b.

4 Calibration

Calibration signal tab visualizes the results of analysis of a calibration signal. Use the **Load calibration data file** button and choose a suitable signal to load (see Fig. 3a). When loaded, the signal will be visualized as in Fig. 3b. You might have noted that two values are present at the top and the bottom of the plot. The top one shows the ratio of the maximum fluorescence in the experiment to the first value of fluorescence measured, and is the measure of how much the signal has increased compared to the initial value. The bottom number shows the ratio of the duration of the experiment to the time point where the first derivative of the signal had its maximum, and it is the measure of how much the duration of the experiment exceeds the peak time of the first derivative. These two measurements are used later to decide which model to fit to the signal.

a)



b)

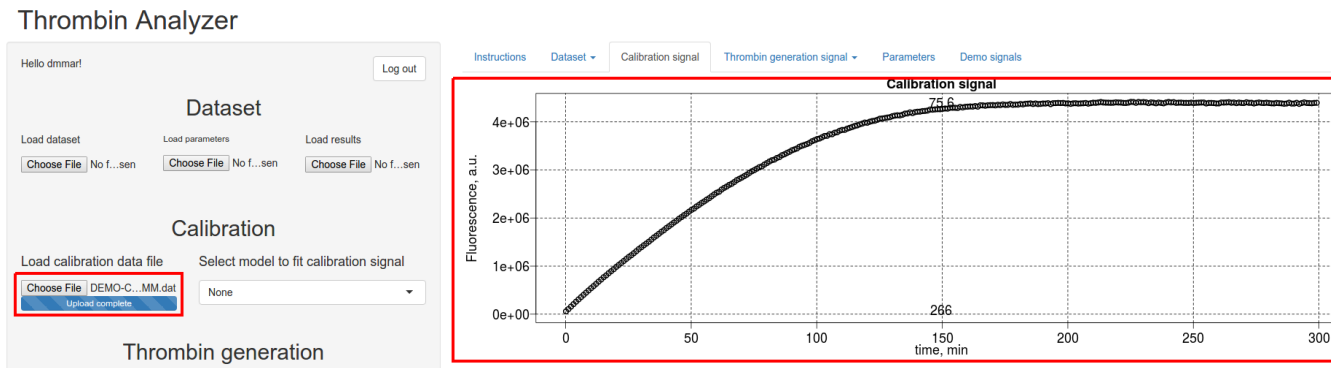
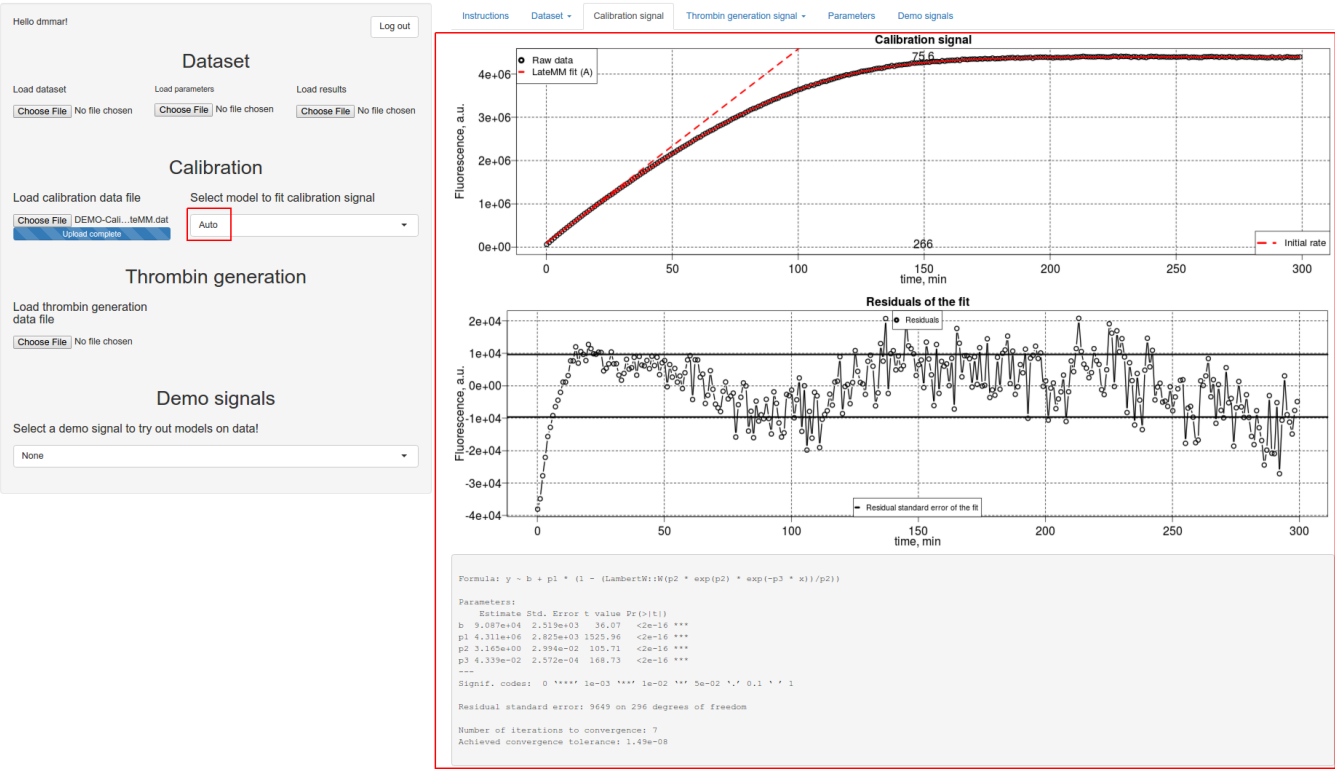


Figure 3. a) Loading a calibration signal. b) Visualization of the loaded calibration signal.

Note that on Fig. 3b an additional menu becomes available when a calibration signal is loaded — **Select model to fit calibration signal**, with a default value **None** chosen. It offers a collection of models to choose from to fit to the calibration signal. The simplest option for the user comes first — **Auto**, that uses

the two measurements discussed above to select a proper model and fit it to the calibration data. So, selecting **Auto** from the menu will produce the results depicted in Fig. 4a.

a)



b)

Thrombin Analyzer

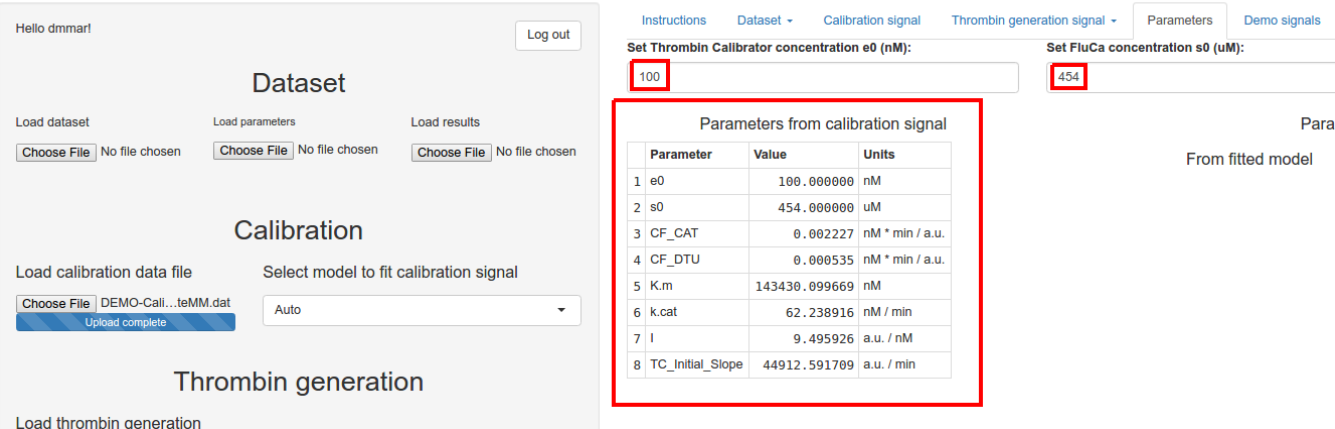


Figure 4. c) Auto model fitted to the loaded calibration signal. d) Parameters from the model fitted to the loaded calibration signal. Inputs define the initial Thrombin Calibrator concentration e_0 and the initial FluCa generation concentration s_0 .

As Fig. 4a shows, the **Auto** model chose the **LateMM** model for this signal and fitted it to the data. The solid red line represents the fit, and the dashed red line represents the initial rate of change of fluorescence of the signal according to the model. The residual differences of the data and the model are visualized in the plot below, where the two solid black lines represent the residual standard error of the fit. The summary of the fit is also visualized — it shows the formula that has been used to fit the data, the estimated values of parameters and their standard errors, the residual standard error of the fit and fit convergence information.

From the fitted calibration signal several parameters can be obtained, that are visualized in the **Parameters** tab, see Fig. 4b. The most important parameter is the calibration factor, and there are two values for it in the app — the standard **CF_CAT** value and the alternative more accurate **CF_DTU**.

5 Thrombin generation

Thrombin generation signal tab is similar to Calibration signal tab, but works with thrombin generation signals instead. The result of loading a signal and using the Auto option to process it gives the result visualized in Fig. 5.

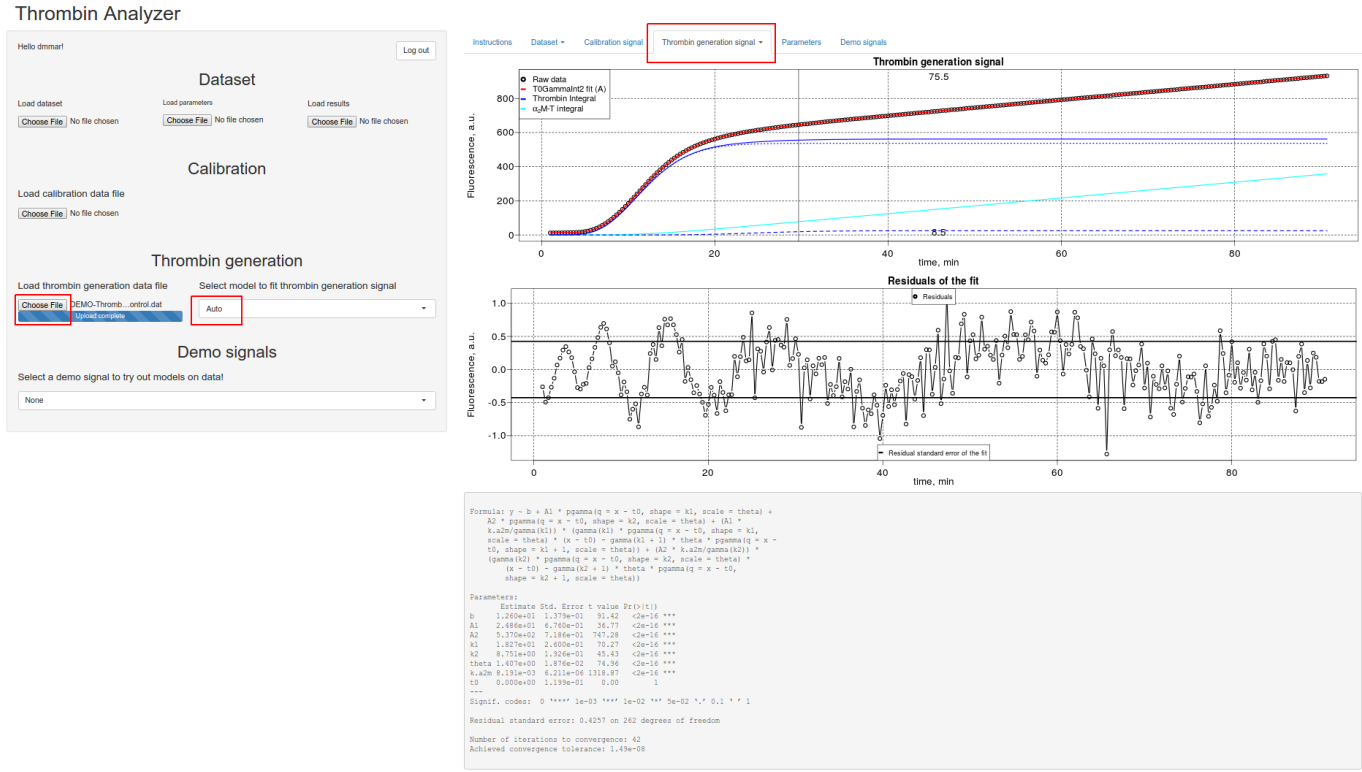


Figure 5. Loaded thrombin generation signal fitted with “Auto” option.

An additional feature for thrombin generation is to visualize the thrombogram and thrombin velocity of the signal. To do that, select the **Thrombogram** tab from the **Thrombin generation** tab, and the results should be like in Fig. 6.

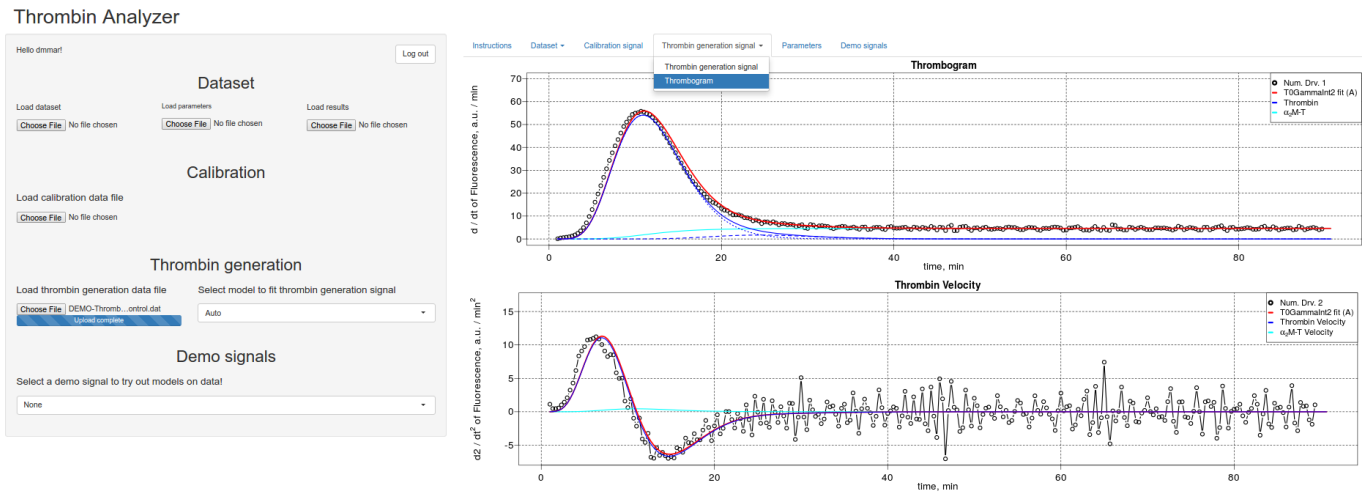


Figure 6. Thrombogram and thrombin velocity of a thrombin generation signal fitted with “Auto” option.

Finally, the CAT parameters for the thrombin generation signal are presented in the parameters tab, see Fig. 7. Parameters obtained from the model fitted to the data are shown to the left, and parameters estimated numerically — to the right.

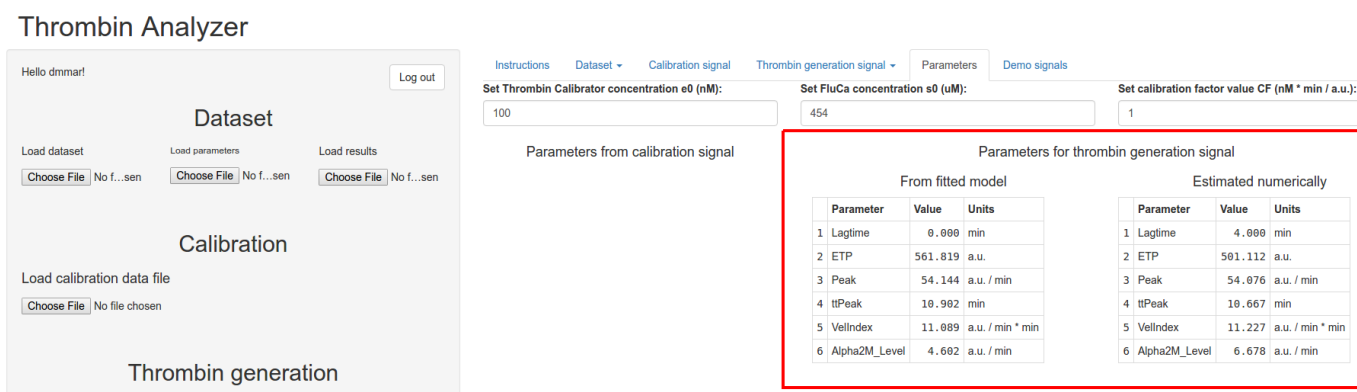


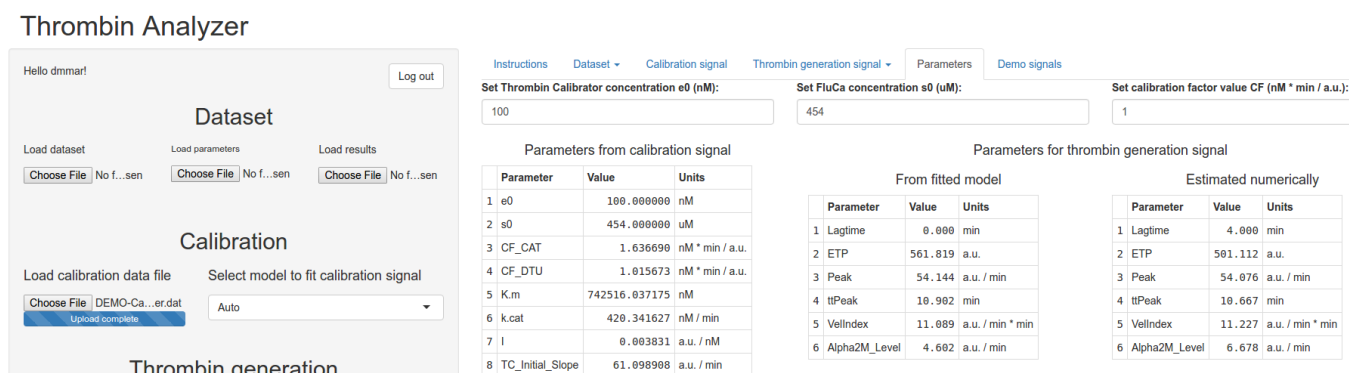
Figure 7. CAT parameters for the thrombin generation signal visualized in the Parameters tab.

6 Parameters

With both calibration signal and thrombin generation signal loaded into the app and processed, this tab shows the parameters one obtains in the standard CAT experiment, see Fig. 8a.

If the values of the initial concentrations e_0 of the Thrombin calibrator and s_0 of the substrate are different from 100 nM and 454 μ M correspondingly, You can enter the proper values and the parameters of the calibration signal will be automatically re-calculated. After that, if You change the value of the CF from 1 to one of the calibration factors provided by the calibration signal the parameters for thrombin generation signal from the fitted model will be re-calculated, see Fig. 8b.

a)



b)

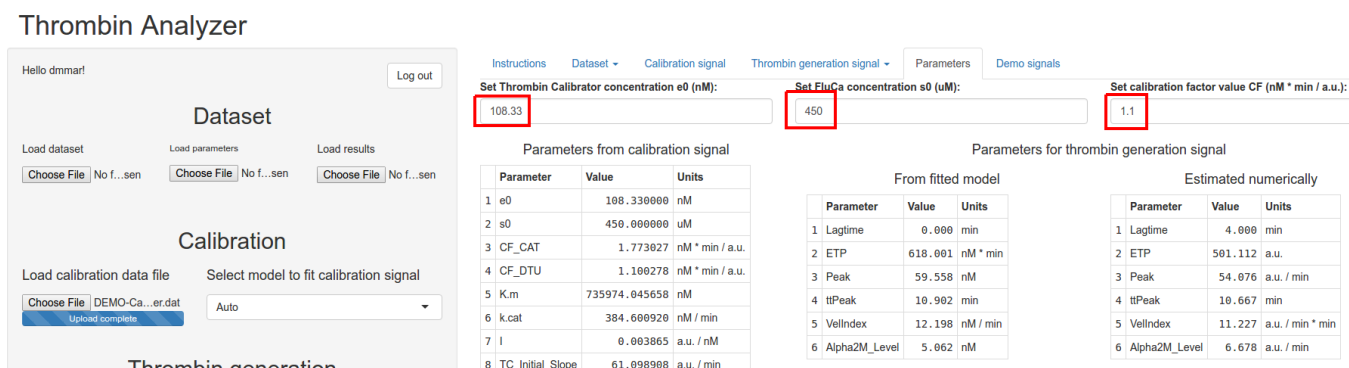


Figure 8. a) CAT parameters visualized in the Parameters tab. b) CAT parameters with changes visualized in the Parameters tab.

7 Dataset

Dataset tab allows to analyze datasets from thrombin generation experiments. When a dataset is loaded into the app, it is visualized and analyzed when the user presses the **Analyze!** button. After the analysis is done, the standard CAT parameters obtained from the fits to raw data are visualized. They can be downloaded in a separate file, as well as the information about the fitted models, and loaded into the application later.

Below is a step-by-step instruction that clarifies how to use the **Dataset** tab.

1. Navigate to the **Load dataset file** button and press it. Choose the dataset file to upload, and press **Open**:

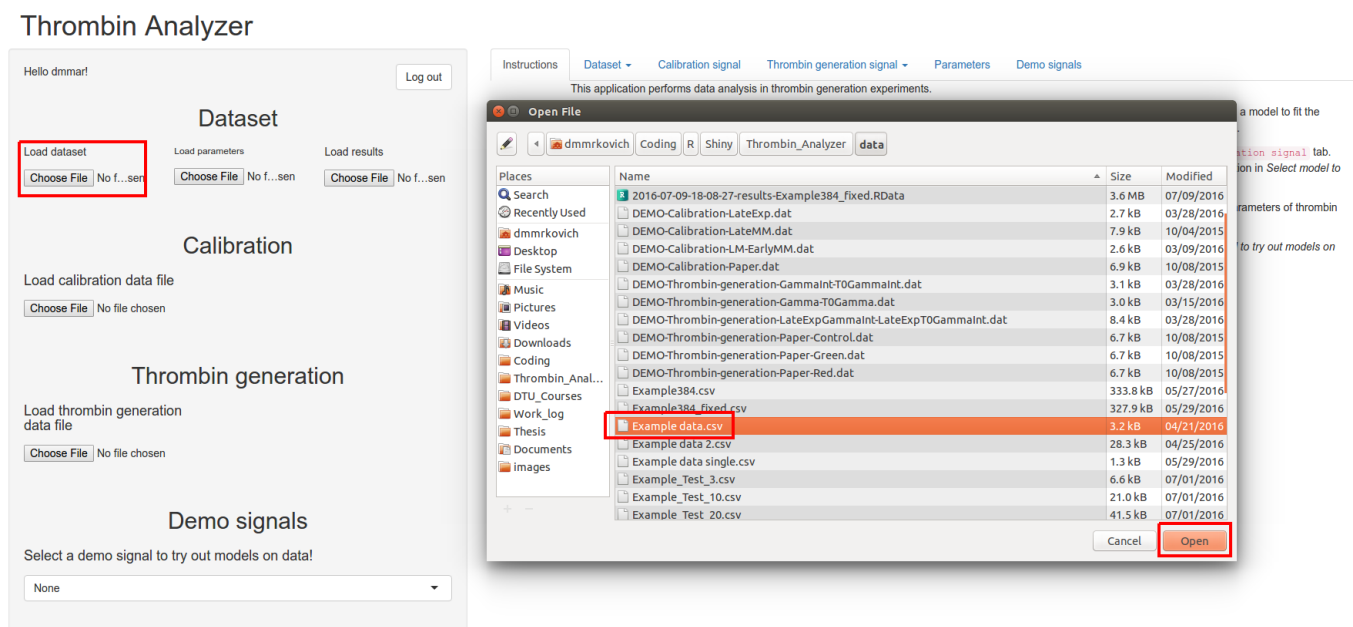


Figure 9. Load dataset file

Remember that the data in the .csv file is expected to be exactly in the following format:

```
Time [min];Compound 1;Compound 2;Compound 3
1;159552;149228;151170
2;153199;151531;150912
...
119;3689312;3441499;171452
120;3694577;3447236;172900
```

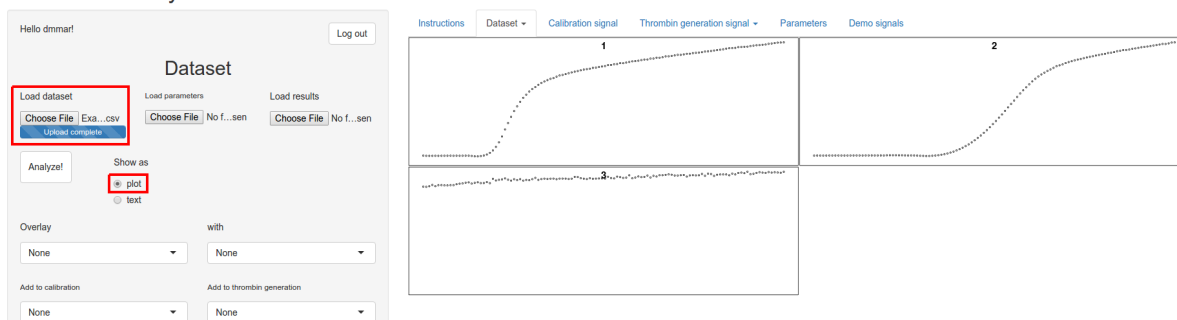
No additional information should be stored in the file, **only raw fluorescence traces!**

2. If the datafile is loaded correctly, the signals will be visualized, and the **Dataset** tab will look like in Fig. 10.

X-axis the time axis, and visualizes the data in the first column of the dataset file (**Time__min_**). The data in the second column (**Compound_1**) is considered as the first signal, and is visualized against time in the topleft plot with number 1. Number 2 corresponds to the signal in the third column (**Compound_2**), and so on. Y-axis is the fluorescence axis, and its limits are from zero to the maximum fluorescence of the visualized signal. This visualization is supposed to give the user an idea about how does the signal look like in general compared to zero fluorescence. For example, signals 1 and 2 are ok, whereas signal 3 shows no significant thrombin generation and therefore would not be analyzable.

a)

Thrombin Analyzer



b)

Thrombin Analyzer

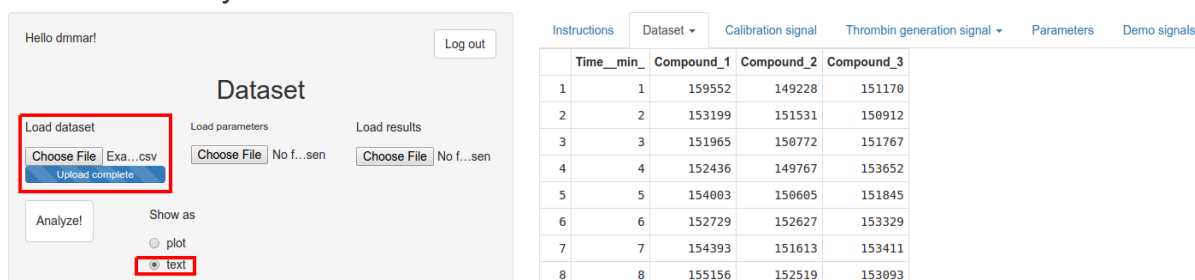
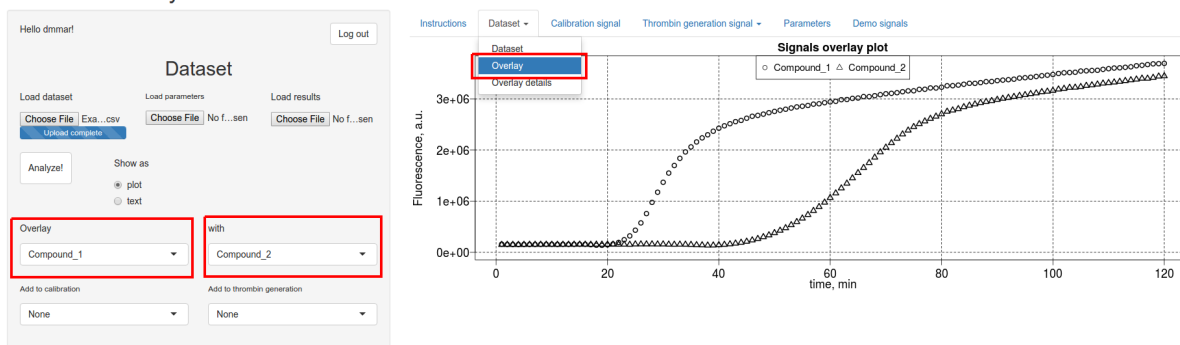


Figure 10. Loaded dataset visualization a) as plot and b) as text.

3. If You would like to compare the raw signals visually before doing analysis, You can select the **Overlay** tab with inputs that are now available in the **Dataset** section. Choose the column names of the signals You would like to overlay and switch to the **Overlay** tab to visualize them against each other:

a) Compound 1 vs Compound 2

Thrombin Analyzer



b) Compound 1 vs Compound 3

Thrombin Analyzer

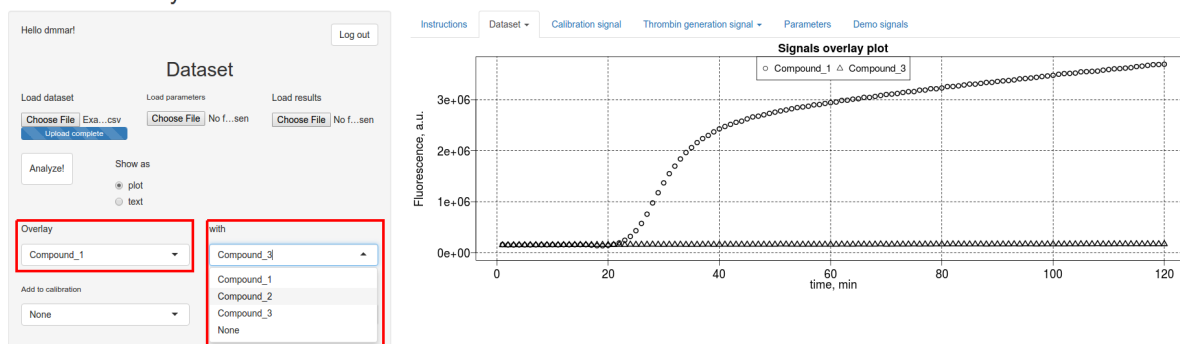


Figure 11. Overlay of raw signals

Choosing **None** as one of the names will disable the visualization.

- The fully automated analysis of the dataset is performed if You press the **Analyze!** button.

When the analysis is ready, the waitbar on top of the tabs disappears and the obtained parameters are visualized as in Fig. 12.

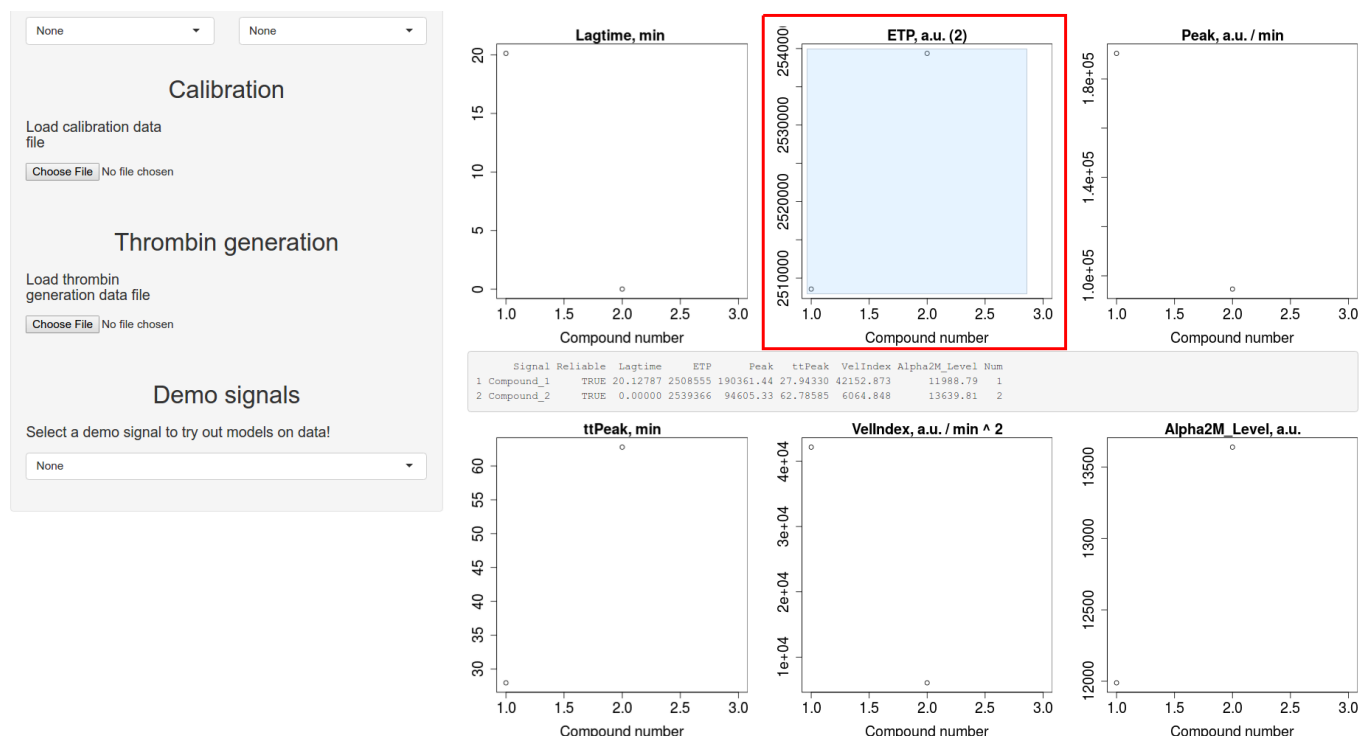


Figure 12. Analyzed dataset with CAT parameters visualized

Each plot shows values of a certain parameter (Lagtime, ETP, etc.) versus the compound number. Circles represent the reliable parameters, meaning that those parameters were obtained from the models that have both the contribution of thrombin and α_2 M-thrombin. If that was not the case, the obtained parameters are considered unreliable, and will be represented with crosses. If a signal was not fitted at all, its parameter values are NA, and are not shown on the plots. The range of y-axis of each of the plots is computed according to max and min of the reliable parameters, therefore some unreliable parameters may not be shown (typical situation for the ETP plot).

ETP is a special plot among all others. In the main title of this plot, the value in parentheses is the count of non-NA values of parameters in the dataset. Moreover, this plot is interactive. You can make a selection with the left mouse button, and all the non-NA parameters of the dataset that fall into selection will be displayed in the middle. This gives a convenient way to quickly compare some of the parameter values from different compounds between each other.

So, the plots represent the following parameters dataset:

```
Signal;Reliable;Lagtime;ETP;Peak;ttPeak;VelIndex;Alpha2M_Level
1;Compound_1;TRUE;20.1278671784601;2508555.56363607;190361.432073557;27.9433040504615;42152.8795676397;11988.7890445844
2;Compound_2;TRUE;0;2539331.17454722;94606.8473592663;62.7857999845869;6065.10099545375;13640.4392964936
3;Compound_3;FALSE;NA;NA;NA;NA;NA;NA;NA
```

Note that the parameters dataset from above can be downloaded by using the now available download link **Download parameters**.

- If You would like to check and compare the results of the performed analysis against the two signals, choose them and switch to **Overlay** again, see Fig. 14a.

a)

Thrombin Analyzer

Hello dnmr! Log out

Dataset

Load dataset: Choose File Exa...csv Upload complete

Load parameters: Choose File No f...sen

Load results: Choose File No f...sen

Analyze! Show as plot text

Download parameters Download results

Overlay: Compound_1 with: Compound_2

Add to calibration: None Add to thrombin generation: None

Calibration

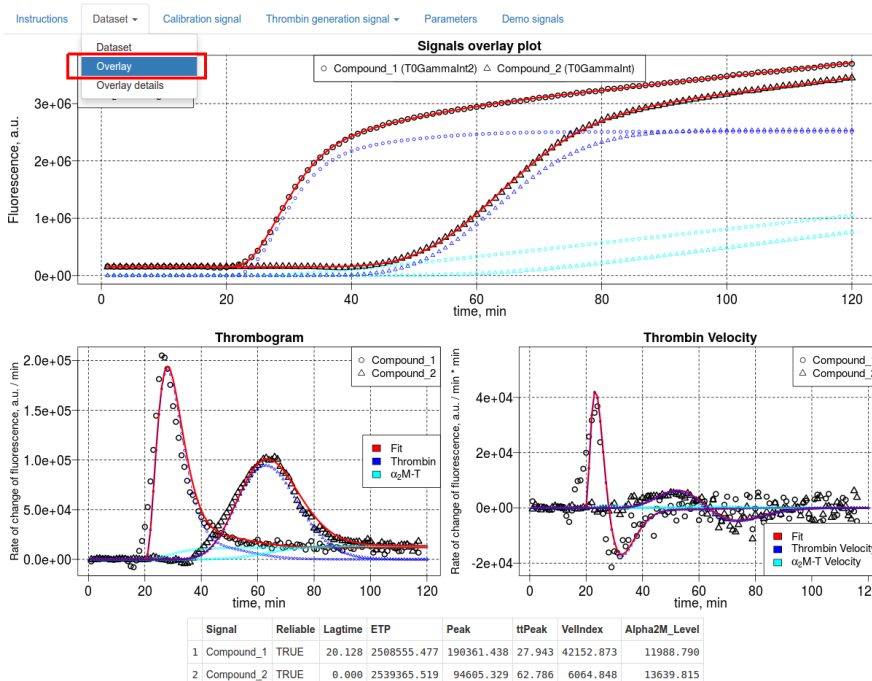
Load calibration data file: Choose File No file chosen

Thrombin generation

Load thrombin generation data file: Choose File No file chosen

Demo signals

Select a demo signal to try out models on data!



b)

Thrombin Analyzer

Hello dnmr! Log out

Dataset

Load dataset: Choose File Exa...csv Upload complete

Load parameters: Choose File No f...sen

Load results: Choose File No f...sen

Analyze! Show as plot text

Download parameters Download results

Overlay: Compound_1 with: Compound_2

Add to calibration: None Add to thrombin generation: None

Calibration

Load calibration data file: Choose File No file chosen

Thrombin generation

Load thrombin generation data file: Choose File No file chosen

Demo signals

Select a demo signal to try out models on data!

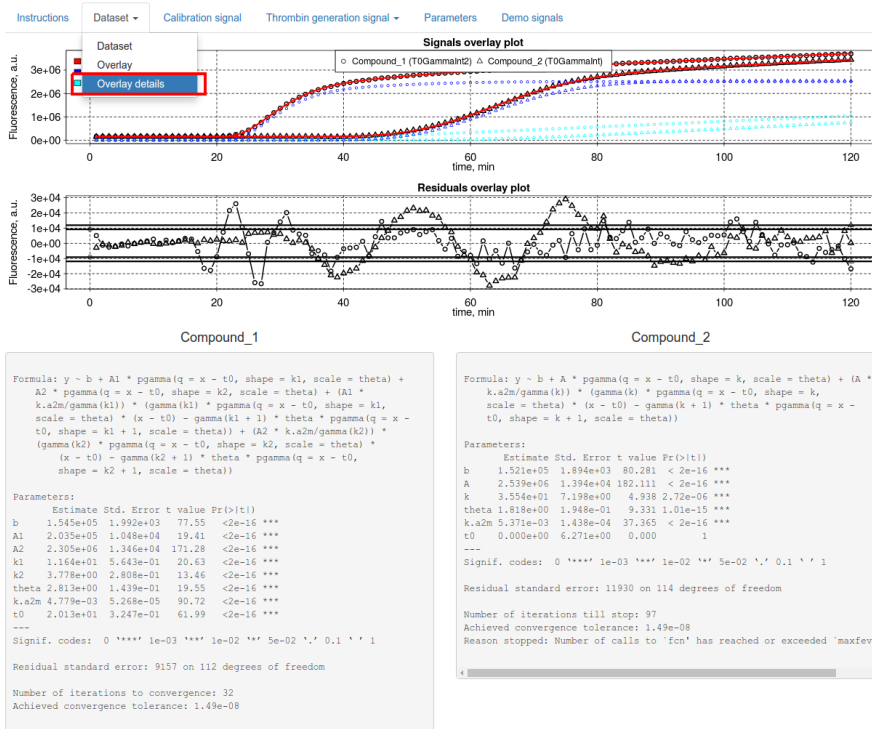


Figure 13. a) Overlay of analyzed signals. b) Detailed overlay of analyzed signals.

This time, it will show the raw data, the fits to raw data with the separate contributions of thrombin and α_2 M-T, and the same for the first derivative of the signals (Thrombogram) and the second derivative (Thrombin velocity). The table below the plots will show the values of the CAT parameters characterizing the two chosen signals.

The **Overlay details** tab will show the fits overlaid with data, the residuals of the fits, and fit summaries for overlaid signals, see Fig. 14b.

6. Analysis is done, so You can download the .csv file with parameters using the **Download parameters** button and download the information about the fitted models using the **Download results** button. The results will be downloaded in a .RData file, that You can later load into the app.
7. Suppose You did the analysis yesterday, and today You would like to check it again. Several options are available in this case.
 - (a) You are just interested in the visualization of parameters. Then, navigate to **Load parameters** and press it to load parameters. They will be visualized in the app when loaded.
 - (b) You want to perform a full check of the analysis You did earlier. You **load the dataset**, You see it visualized in the app and the next step is to load the saved parameters and results. You use the **Load parameters** and **Load results** buttons to load the parameters .csv file and the results .RData file into the app. When they are loaded, You see Fig. 12 and Fig. 14.
8. If You would like to investigate a single thrombin generation signal from the dataset on Your own, You can use the **Add to thrombin generation** input to add the signal to **Thrombin generation** tab of the app.

a)



b)

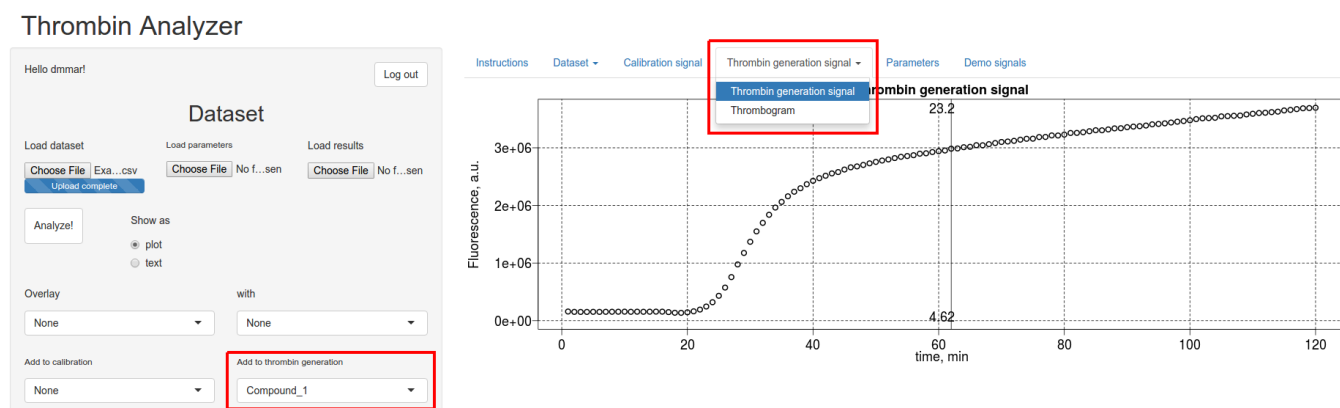


Figure 14. a) Adding a single thrombin generation signal from the dataset to **Thrombin generation** tab. b) A single signal from the dataset is added to the **Thrombin generation** tab.

Note that all information about the analysis of the signal will be erased — You will have to start the analysis from scratch. Check Sec. 5 for further details.

8 Tutorial

The **Tutorial** tab shows this tutorial directly in the app using the standard pdf-viewer of Your browser.

9 Important notes from Dmitriy

1. Please do not try to load files in the wrong format into the app — will cause malfunction, since there is no file format check guard yet.
2. the same for to load datasets with calibration signals and analyze them — there is no any type check of the signal yet.
3. In the same fashion, **Add to calibration** input is not working yet.
4. Remember that nonlinear fitting is not expected to give exactly the same results each time You run it — this is why it is always good to check the fit with Your own eyes.
5. If something is not ok, try to re-choose the input, switch back and forth between the tabs or etc — sometimes the synchronization lags. If nothing helps, **press F5 to reload the app** and start from scratch.
6. If there is a bug of the functionality that is supposed to work fine with the correct premises for using it, document the steps that produce the bug and write me an [e-mail](#), better with screenshots.