MONOLITH

PRODUCT MANUAL

What is included in this product manual

MONOLITH

User Manual

Table of contents

1. About this user manual

This manual is a guide for using the Monolith system. It covers system specifications, safety considerations and installation as well as why and how to run experiments with Monolith. Please read this manual carefully before starting and make sure the contents are fully understood. Keep this manual available near the system for future reference. In case of loss, please contact NanoTemper Technologies customer support (**https://nanotempertech.com/support**) for a replacement copy of this manual.

1.1. Directions for more detailed information

There are two sources for further, more detailed information on scientific principles and recommendations for assay development as well as software usage. One is the **NanoPedia** knowledge base that is integrated into the MO.Control. It contains a variety of articles about the biochemistry of interaction analysis, MST/TRIC as a method and the operational functions of Monolith. A pdf version of the **NanoPedia** can be accessed through the NanoTemper Technologies **Explorer Community**.

The NanoTemper Technologies **Explorer Community** is an online community to obtain resources for NanoTemper products such as application notes, tech notes or protocols. It is also a place to exchange best practices directly with other Monolith users. You can also pose questions and have them answered by NanoTemper support guides. Follow **https://nanotempertech.com/be-an-explorer/** for access to the Explorer Community.

2. Safety information

To ensure operation safety, this system must be operated correctly. Carefully read this chapter to fully understand all necessary safety precautions before operating the system.

2.1. Symbols and descriptions

This section describes the safety symbols and descriptions used in this manual, as well as the labels on the system.

Please take a moment to understand what the signal words **WARNING! CAUTION** and **NOTE** mean in this manual.

WARNING! A **WARNING!** indicates a potentially hazardous situation which, if not avoided, could result in serious injury or even death.

CAUTION A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. **CAUTION** may also be used to alert against damaging the equipment or the system.

Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken the appropriate steps.

NOTE A **NOTE** provides additional information to help the operator achieve optimal system and assay performance.

Read manual label. This label indicates that you must read the manual before using the system. This label is positioned on the back of the instrument.

Warning symbol. This symbol, when used on its own or in conjunction with any of the following icons indicates the need to consult the provided manual, because a potential risk exists if the operating instructions are not followed. This label is positioned on the back of the instrument.

Warning symbol. This symbol indicates a possible risk for hand injuries by crushing and sharp edges. This warning label is positioned on the sample tray.

2.2. Use and misuse

Use the Monolith system only after having read and fully understood this user manual. Use the system only in perfect condition. If the system shows any signs of damage, stop operation and contact NanoTemper Technologies customer support.

Do not modify the system in any way. Do not use it for anything other than its intended purpose.

2.3. Safety instructions

WARNING! The drawer of the system can pinch or injure your hands or fingers. Keep fingers safe while opening and closing the drawer. Do not touch the instrument while parts are moving. Do not reach into the opening when the door is open.

WARNING! Connect the Monolith to the AC power supply using the supplied power cable. Since the instrument is assembled in line with the specifications for safety class IEC 61010-1:2010, it must only be connected to an outlet that has a ground contact.

WARNING! Do not attempt to change the Fuses. Consult NanoTemper Support (**https://nanotempertech.com/support**) if you think the fuse is broken.

WARNING! Danger of electric shock, fire and skin burns. Do not open the system (other than operating the drawer/loading hatch via the software). Do not reach into the hatch opening.

WARNING! Using hazardous or infectious substances in the system may pose a risk of explosion, implosion, release of gases or infection. Use only non-hazardous, non-infectious, aqueous samples. Dispose of used capillaries according to the substances contained in them and according to locally applicable regulations concerning chemical waste.

CAUTION The instrument contains an IR-laser module (invisible laser radiation class 3B according to IEC 60825-1: 2014). Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness. Do not attempt to open the instrument as this poses a risk of personal injury or damage to the instrument. When the instrument is used as intended it emits laser radiation of LASER CLASS 1.

CAUTION The system must be installed in a way that does not hinder access to the power switch and power plug.

CAUTION Broken glass can cut skin. Do not use if the front glass is broken.

CAUTION The weight of the Monolith instrument is approximately 27 kg, do not move the instrument alone (two persons required for transport). If you move the instrument alone, you risk personal injury or damage to the instrument.

CAUTION Only NanoTemper Technologies staff may service and open the instrument. Turn off the power switch and unplug the power cord before servicing the instrument, unless otherwise noted. Connect the equipment only to the delivered power source. Do not use extension cords. Have an electrician immediately replace any damaged cords, plugs, or cables. Not doing so poses a risk of personal injury or damage to the instrument.

NOTE Insufficient air supply can cause overheating of the system. Assure enough air supply by not covering the back of the system. Leave at least 15 cm of space between system and any wall or other obstruction.

CAUTION The instrument contains a temperature regulator to control the sample temperatures. Some accessible parts of the instrument can reach temperatures up to 50°C. Avoid touching the temperature-controlled parts of the instrument for a longer time when you have set the temperature controller to high temperatures.

CAUTION Only open the sample loading site when moving parts inside the instrument are at rest. Do not insert or remove a sample while linear actuator, laser or LED is at work. Moving parts in the instrument can be harmful.

CAUTION Do not use the instrument in a cold room.

CAUTION Turn off the main circuit breaker on the right side of the chassis, when the instrument is not in use.

CAUTION Only use water or 70 % ethanol to clean the instrument.

CAUTION Use the instrument only for biomolecule analytics with aqueous solutions and do not open the instrument on another site than for sample loading.

CAUTION Only use aqueous sample for analysis in the instrument.

CAUTION Do not use the instrument with hazardous substances or substances/ materials which pose a risk of infections.

3. The Monolith system

3.1. General

3.1.1. Intended use

The Monolith system provides fast and highly sensitive detection and quantification of molecular interactions in glass capillaries. The system is intended for research purposes only. It is not to be used for diagnostic purposes.

3.1.2. Conformity

The following safety and electromagnetic standards were considered:

- IEC 61010-1:2010/AMD1:2016 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements
- IEC 61010-2-010:2019 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials.
- IEC 60825-1:2014 Safety of laser products.
- Complies with 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3., as described in Laser Notice No. 56, dated May 8, 2019
- IEC 61326-1:2006 EMC, Electrical equipment for measurement, control and laboratory use – EMC requirements.
- IEC 61000-3-2:2006 EMC, Limits for harmonic current emissions (equipment input current up to and including 16A per phase).
- IEC 61000-3-3:2008 EMC, Limits

3.1.3. Identification

The identification labels (Figure 1) are positioned at the rear panel of the instrument. They include manufacturer information, system model name and serial number (SN), electrical requirements, and the CE conformity symbol.

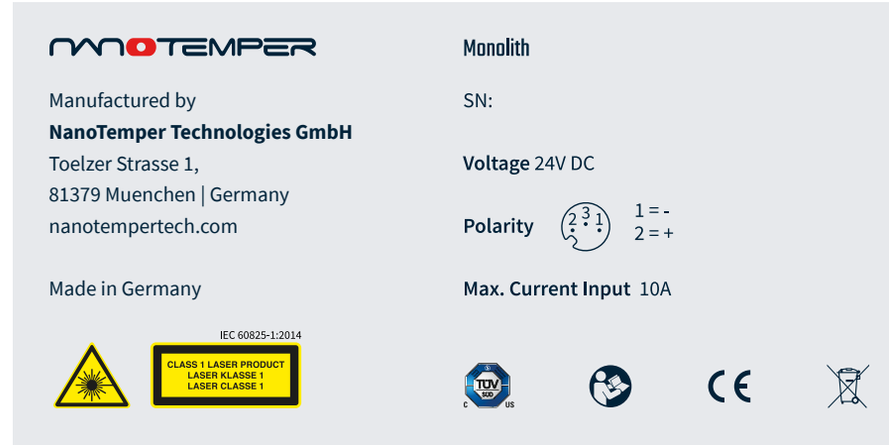

Figure 1: Identification labels for Monolith.

3.2. Technical information

3.2.1. Technical specifications

Electricity

Input Voltage	24 VDC (+10,-10%)
Overvoltage category	CAT I
Input current AC	10 A
Pollution degree	2

Environmental

Operating temperature	20 – 30 °C (indoor use only)
Storage temperature	-20 – 30 °C
Operating humidity	Dewpoint below 18 °C
Storage humidity	non-condensing
Operating altitude	Max. 2000 m

Monolith dimensions

Width	36 cm (14.17”)
Height	39,7 cm (15.63”)
Depth	57,9 cm (22.80”), with open drawer: 70,9 cm (27.91’’)
Weight	26.8 kg (59 lbs) net

IR Laser

Wavelength	1475 nm ± 15 nm
Power	120 mW max.
Monolith Laser classification	The device is LASER PRODUCT CLASS 1

Temperature control

Temperature control range	20 °C – 40 °C
Precision of temperature control	± 0.5 °C

Noise level

Noise level of Monolith	64 dB

3.2.2. Connections for input and output

Type	Function	Position
Ethernet	To connect the system to the Control PC/LAN via Ethernet cable.	Rear panel
AC Power	To connect the system to electrical power.	Rear panel

All ingoing and outgoing connections can be found on the rear panel of the instrument.

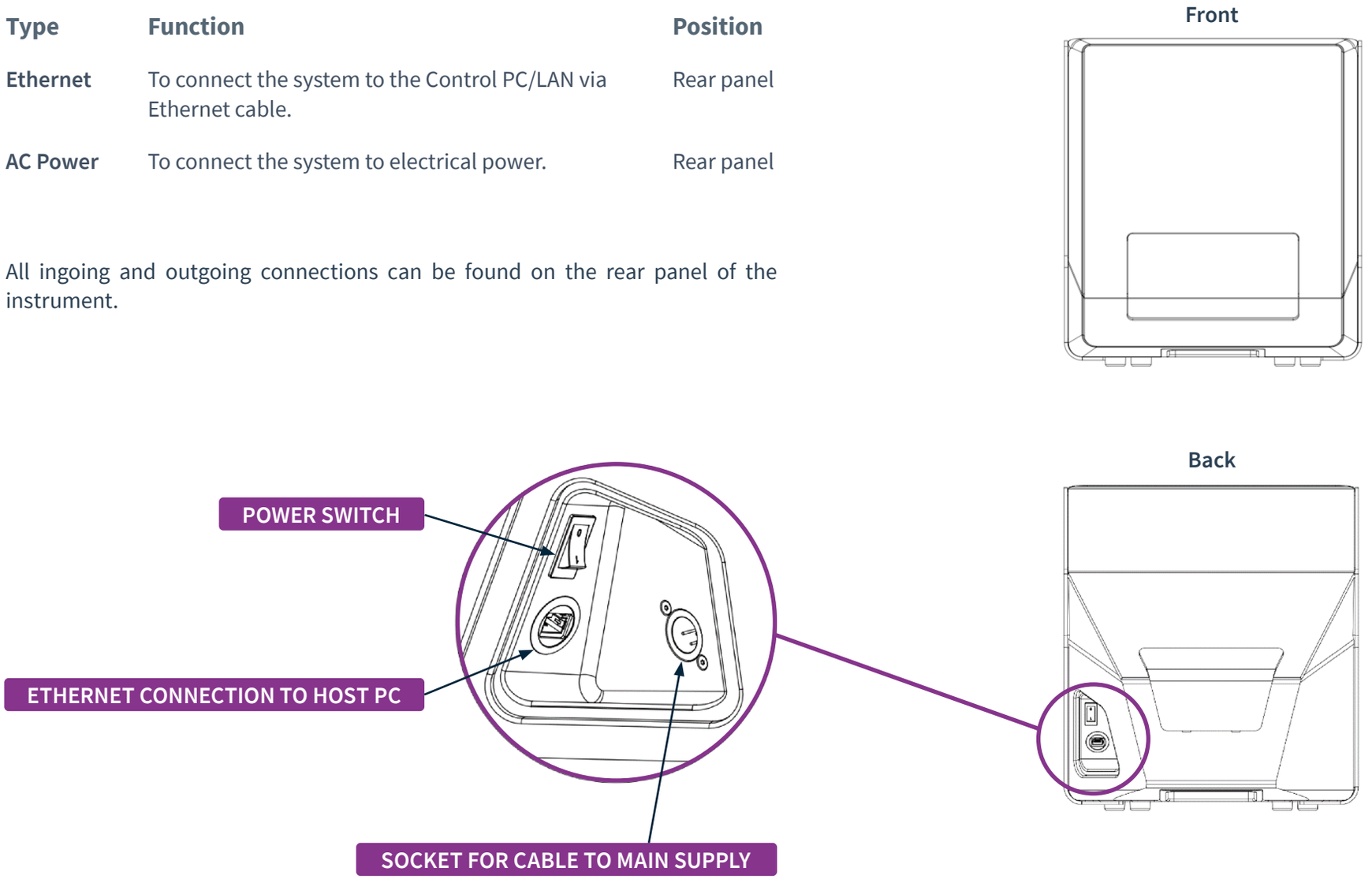

Figure 2: Connections on the Monolith device.

3.3. Legal

1. NanoTemper Technologies shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions on the use of NanoTemper Technologies software:
   - Copying software for purposes other than backup
   - Transfer or licensing of the right to use software to a third party
   - Disclosure of confidential information regarding software
   - Modification of software
   - Use of software on multiple workstations, network terminals, or by other methods
3. The content of this manual is subject to change without notice for product improvement.
4. This manual is considered complete and accurate at publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If a NanoTemper Technologies software program has failed, causing an error or improper operation, this may be caused by a conflict from another program operating on the controlling PC. In this case, take corrective action by uninstalling the conflicting product(s).
7. NanoTemper and Monolith are registered trademarks of NanoTemper Technologies GmbH.
8. Unauthorized resale is not permitted.

3.4. Limited warranty

Products sold by NanoTemper Technologies, unless otherwise specified, are warrantied to be free of defects in materials and workmanship for a period of one year from the date of shipment. If any defects in the product are identified during this warranty period, NanoTemper Technologies will repair or replace the defective part(s) or product free of charge.

This warranty does not apply to defects resulting from the following:

1. Improper or inadequate installation.
2. Improper or inadequate transport.
3. Improper or inadequate operation, maintenance, adjustment or calibration.
4. Unauthorized modification or misuse.
5. Use of unauthorized microwell plates and accessories.
6. Use of consumables, disposables and parts not supplied by an authorized NanoTemper Technologies distributor.
7. Corrosion due to the use of improper solvents, samples, or due to surrounding gases.
8. Accidents beyond NanoTemper Technologies' control, including natural disasters.

This warranty does not cover consumables like capillaries, reagents, labeling kits and the like. It also does not cover normal wear-and-tear.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact NanoTemper Technologies after confirming the model name and serial number of your NanoTemper Technologies system (see 3.1.3).

4. Monolith setup

The Monolith should be installed by NanoTemper Technologies personnel to ensure safety measures are taken and to confirm proper functionality of the instrument.

4.1. Scope of delivery

Upon receiving the system, please check package contents for completeness. The Monolith system package contains the following items:

Item	Description
Monolith system	-
User manual	This user manual
Cables	Power cord for power supply, network cable for connection to control notebook
Control notebook	Control notebook for Monolith system

4.2. Unpacking

The Monolith system should only be unpacked and installed by trained NanoTemper Technologies personnel to ensure proper functionality of the instrument upon delivery.

4.3. Startup

Connect the Monolith system to power by plugging in the power supply cable. Connect the Monolith system to the control laptop using the ethernet connection at the right side of the instrument. The system starts upon switching the power switch.

4.4. Cleaning

The Monolith system does not need any regular maintenance.

To clean the outside surface of the system, unplug the power supply at the right side of the instrument. Wipe the surface, including the front display, with a cloth slightly dampened with water or 70 % ethanol.

4.5. Software updates

Software updates of the embedded system can only be performed by instructed NanoTemper Technologies personnel and is part of regular maintenance visits. Software updates of MO.Control and MO.Affinity Analysis can be performed by the users within the respective software solution.

4.6. Installation requirements

To ensure operation safety, observe the following conditions:

- Only operate the Monolith instrument with the supplied external power supply (SPU63-105, Sinpro Electronic Co Ltd or Ael60US12, XP Power LLC or GS90A12-P1M, MeanWell or GS60A12-P1J, MeanWell).
- Only connect the external power supply of the Monolith to an electrical socket containing a protective conductor terminal.
- Ensure that the power plug of the external power supply is easily accessible. The Monolith instrument has to be installed in a way that it does not hinder the access to the external power supply and its power plug.
- Only operate the instrument with the delivered notebook/PC.
- Only operate the instrument with original Monolith capillary trays.

- The maximum noise level of the instrument is 64 dB(A). Only operate the instrument in an environment where this noise level is appropriate.
- Operate the instrument in a temperature range of 20 – 30 °C.
- Operate the instrument under non-condensing conditions and a dewpoint below 18 °C.
- Operate the instrument in an atmospheric pressure range of 800 – 1060 hPa.
- Do not operate the Monolith under conditions which pose a risk of explosion, implosion or the release of gases.
- Avoid strong magnetic fields and sources of high frequency. The instrument may not function properly when near a strong magnetic field or high frequency source.
- Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment and machine tools.
- Avoid dust and corrosive gas. Do not install the instrument where it may by exposed to dust, especially in locations exposed to outside air or ventilation outlets.
- To clean the instrument, only use water or 70 % ethanol.
- Do not install the instrument in a location where it may be exposed to direct sunlight.
- Install the instrument in a horizontal and stable position. (This includes a table, bench or desk upon which the instrument is installed).
- Ensure that no air conditioner blows air directly onto the instrument. This may prevent stable measurements.
- Install the instrument in a location that allows easy access for maintenance.

NOTE: *The above conditions do not guarantee optimal performance of this instrument.*

4.7. Installation and connecting cables

4.7.1. Preparation

Prepare a table which can bear a weight of about 50 kg (110 lbs) and has a free area of 80 cm (width, 31.5”) x 50 cm (depth, 20”). Put the Monolith instrument and the control notebook/PC on this free area.

CAUTION The weight of the Monolith instrument is approximately 27 kg (60 lbs), do not move the instrument alone (two persons required for transport/movement). If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

4.7.2. Connecting the power supply and the Monolith

Confirm that the power switch of the Monolith instrument is off (power switch is at the right).

WARNING! Only operate the Monolith instrument with the external power supply provided (SPU63-105, Sinpro Electronic Co Ltd or Ael60US12, XP Power LLC or GS90A12-P1M, MeanWell or GS60A12-P1J, MeanWell). Only use the supplied cables and plugs. If not doing so, you risk electric shock and fire.

Connect the external power supply to the electrical socket. Then connect the power supply to the Monolith instrument.

CAUTION Ensure that the power plug of the external power supply is well accessible. The Monolith instrument must be installed in a way that it does not hinder the access to the external power supply and its power plug.

Connect the Monolith instrument to the control notebook/PC by using the supplied network cable.

Switch on the Monolith and the notebook.

5. Maintenance and operation

Pay attention to the instrument operating environment and always keep it clean so that the instrument can be used in a stable condition over a long period. Do not place anything on top of the instrument.

5.1. Cleaning the Monolith

Switch off the instrument and remove the power plug of the external power supply from the electrical socket. Only use a dry cloth or a cloth dampened with water or 70 % ethanol for cleaning the instrument.

CAUTION Only use water or 70 % ethanol to clean the instrument.

5.2. Repackaging the Monolith for transport

The Monolith instrument should be repacked only by trained NanoTemper Technologies personnel to ensure safety and stability during transport. Please store the instrument box for that purpose. If the instrument box was discarded NanoTemper Technologies can provide replacement at the cost of packing and shipment.

5.3. Transporting the Monolith

Switch off the instrument and remove the power plug of the external power supply from the electrical socket. Do not carry the instrument alone, two people are needed for transportation.

CAUTION The weight of the Monolith instrument is approximately 27 kg (60 lbs), do not move the instrument alone, two persons required for transport/movement. If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

5.4. Malfunction

In case of a malfunction switch off the Monolith instrument and wait for five minutes, then switch on the instrument again. If the malfunction persists switch off the device, unplug the power cable and contact the NanoTemper Technologies customer support.

5.5. Repairing the Monolith

Do not try to repair the instrument. Contact the NanoTemper Technologies customer support for instrument repairs.

CAUTION The manual opening of the instrument is not permitted. Manual opening poses a risk of personal injury or damage to the instrument. Contact NanoTemper Technologies customer support if you need to open the instrument.

5.6. Waste disposal

Please dispose of used glass capillaries according to the substances contained in them and according to locally applicable regulations concerning chemical and glass waste.

5.7. System disposal

The system may need to be decontaminated before disposal. Please contact NanoTemper Technologies for more information.

This symbol indicates that this system may not be disposed of as unsorted municipal waste and must be collected separately. It must be disposed of according to locally applicable regulations regarding electrical and electronic equipment. The symbol is positioned at the back of the instrument.

6. Patents and intellectual property

Monolith, MST and TRIC technology are patent protected, especially by the following patents, US8431903B2, US8853650B2, US10345312B2, US8741570B2 including their application and registration in different other countries.

Contact

NanoTemper Technologies GmbH

Global headquarters:
Toelzer Strasse 1,
81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0
Fax: +49 (0)89 4522895 60

info@nanotempertech.com
nanotempertech.com

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MO.CONTROL 2

Software Manual

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1. General Information

The MO.Control 2 software is dedicated to running and analyzing MicroScale Thermophoresis (MST) experiments. It guides the user step-by-step through planning, setup and execution of experiments and helps to evaluate and analyze measured data. It also recommends assay improvement strategies and quantifies binding parameters such as dissociation constants (K_d) or EC_{50} values.

This manual explains the main functions integrated in the MO.Control 2 software. Additional and more detailed information can be found within the software itself. Due to the intuitive and self-explanatory design, the user is able to set up and perform MST experiments using MO.Control 2 without previous training.

2. System Requirements

If the necessary licenses have been purchased, MO.Control 2 software can be installed on additional computers for convenient data analysis and experimental planning. Computers must meet the following requirements:

Operating system	Windows 7/10 (x86 or x64)
CPU	Intel Core i5, 6th generation or higher
RAM	8 GB or higher
Hard disk	20 GB available space, SSD recommended
Display resolution	Full HD @ 125%
Software	Microsoft .NET Framework 4.5.1 or higher (included in installer of MO.Control 2 software)
Operating system language	English or German

An external computer mouse is necessary to access all software features.

3. Installation, License Activation, and Software Updates

The software can be installed on any computer and will automatically start in trial mode, when opened for the first time. The trial version offers full functionality and has to be activated with a license key purchased from NanoTemper Technologies after 30 days.

To activate the license, navigate to the main menu and select *Licensing Status*. Please follow the instructions provided. One software license is valid for one account per computer and licenses can be transferred between computers. To transfer a license, please deactivate it first via the *Licensing Status* dialogue and reactivate it on the new computer.

When a software update is available, a green button will appear next to 

button at the top right corner of the software window. This feature is accessible only if your device is connected to a network.

4. General Layout and Features

After starting MO.Control 2, the user is prompted to either “start a new session” or to “browse previous sessions”. A new session will create a .moc2 file in the specified location. Each .moc2 file can contain multiple experiments. Existing .moc2 files can be opened using “browse previous sessions”, and further experiments can be added.

Next, the user is asked to choose the excitation color filter for the upcoming experiment. This will trigger the adjustment of some experimental parameters and recommendations at later stages. The excitation color will be set automatically if the connected Monolith instrument contains only one excitation color filter. If the user chooses to browse a previous session, the session’s excitation color setting will automatically be applied. The excitation color can be changed at the start of each new experiment (see red highlight in **Figure 1**).

Protein labeling assistant, designed to help find best labeling strategy and labeling kit for the target protein, can be accessed by scanning the QR code (**Figure 1**).

4.1. Experimental Modes

In this screen, the user is also asked to choose the mode for the upcoming experiment. The options are (see **Figure 1**):

- **Pretest:** The *Pretest* routine guides you through basic tests that are recommended before conducting a binding experiment. This includes e.g. checking fluorescence after labeling and in label-free experiments. The *Pretest* is recommended when starting on a new or newly labeled target and allows to compare up to six different buffer conditions in a single experiment.
- **Binding Check:** The *Binding Check* routine guides you step-by-step through a quick and easy MST assay development set-up and analyzes whether a binding event can be detected (yes/no answer). The *Binding Check* allows to compare up to six different buffer conditions in a single experiment.
- **Binding Affinity:** The *Binding Affinity* routine provides detailed instructions for performing full, quantitative affinity measurements (K_d answer).

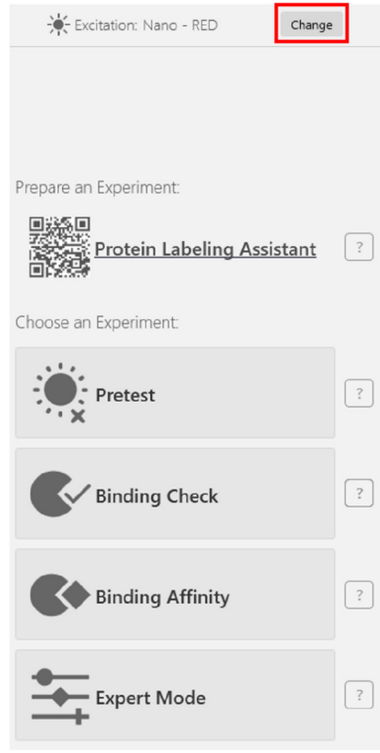


Figure 1: Initial setup of an experiment.

- **Expert Mode:** The *Expert Mode* gives you the freedom to use unconventional experimental setups, but does not offer features for instruction or automatic evaluation. Recommended only for advanced users.

4.2. Session Overview and Guidance Panels

Throughout the software, the left and right panels will always remain visible. The *Session Overview* panel on the left lists all experiments in the .moc2 file and indicates their respective experimental modes. These experiments can be selected, renamed (by clicking the *pen* button) and removed (by clicking the *X* button). Use the Save button to save a file at any time. An asterisk in the software title bar indicates unsaved changes in the file; the user will be prompted to save changes when closing the software. New experiments with blank settings can be started from the *Session Overview*.

The right panel is called the *Guidance*. It is context-sensitive and always shows information that is relevant to the task performed in the main window. Clicking on the *question mark* buttons ? will trigger item-specific information to be shown in the *Guidance*. Use this function if a term or a concept is unclear, or if you would simply like more information on a specific topic.

4.3. NanoPedia

Clicking the button NanoPedia in the top right corner of the software will open a separate window containing *NanoPedia*, an extensive knowledge base with background information concerning MicroScale Thermophoresis and further related topics (see **Figure 2**).

By default, the *NanoPedia* window is pinned, meaning it will always stay on top of the MO.Control 2 software window. Unpin it to trigger normal window behavior.

NanoPedia articles are sorted alphabetically. Use the magnifying glass button to search *NanoPedia* for a specific keyword.

Clicking on blue hyperlinks in the Guidance will open the corresponding *NanoPedia* entry.

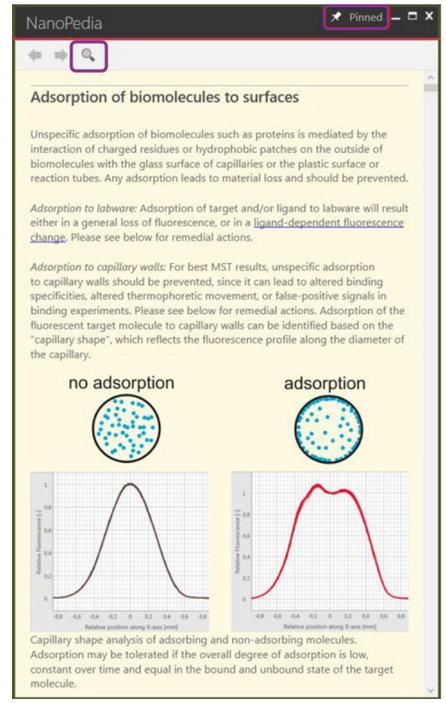


Figure 2: NanoPedia knowledge base. See highlights for pinning the NanoPedia window or searching for specific key words.

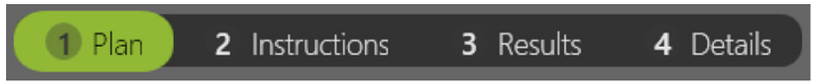
4.4. Starting a New Experiment

There are several ways to start a new experiment. The first one is to start a new session (see section 4). The second one is to use the *New Experiment* button in the *Session Overview* panel (see section 4.2). In both cases a blank experiment not containing any user-supplied information will be started. The third option is available after an experiment is finished. On the bottom right of the *Results* overview page (see section 5.3), the software will suggest a suitable follow-up experiment (see **Figure 3**). Use the arrow button to expand and access different experimental mode options. An experiment started from here will always contain previous user-supplied information and settings.

Figure 3: After an experiment finishes, start a new one directly from the Results page.

5. Running MST Experiments

5.1. Planning



Setting up a new experiment starts with the *Plan* page. While the exact setup of the *Plan* page is specific to the experimental mode, the general purpose is the same: to plan the current experiment. Information on interacting molecules, experimental conditions, affinities and concentrations can be entered.

Green fields are mandatory, since their information is used to calculate pipetting steps on the *Instructions* page. Other information is conveniently stored with the experiment, making it easy to keep track of assay optimization steps and experimental conditions (see **Figure 4**). Some fields already contain recommendations. For example, depending on the type of detector used, a typical target concentration is suggested.

Click the question mark buttons to show explanatory information on individual items in the *Guidance* panel.

Figure 4: Information stored with a typical experiment. The information ribbon at the top of the window shows measurement meta-data and information on experimental conditions. From top left to right: experiment number, experimental mode, target concentration, highest ligand concentration in the assay, buffer, capillary type, detector type and excitation power, MST power, comments, type of instrument, experimental status and date.

The information on the *Plan* page can be altered even after an experiment is finished. To do this, click the *Alter Data* button. Concentration and K_d calculations on later pages will be updated accordingly.

Temperature Control

This top ribbon also contains the temperature controls if supported by the Monolith instrument (see **Figure 5**). By default, measurements on Monolith instruments are carried out at 25 °C. If a different temperature is needed, click the thermometer icon to open the temperature control, then choose the desired temperature using the arrow buttons. Click *Set* to start the temperature control.

Figure 5: Temperature controls. Left panel: click the thermometer icon to open the temperature control. Right panel: choose the desired temperature using the arrow buttons, then click Set to start the temperature control.

5.2. Pipetting

The *Instructions* page uses the information previously entered on the *Plan* page to give specific, step-by-step pipetting instructions using experiment-specific molecule names and concentrations. Of course, the instructions are adapted to the chosen experimental mode. After preparation is complete and samples are loaded into the instrument, start the measurement.

5.3. Results and Details

Data acquisition can be followed on the *Results* page while experiments are running. After the measurement is finished, the *Results* section summarizes the findings of an experiment. The measurement is assessed, and the data and conclusion are

displayed here, along with recommendations for improving the assay. Depending on the experimental mode, the three panels on the *Results* page show different parameters. By clicking the *Review* buttons of the individual parameter panels, details concerning the respective parameter can be accessed (see sections 5.3.2 through 5.3.5).

A conclusion (e.g. a K_d or a definitive qualitative answer on binding) is given in the green box on the bottom left, if available. Recommendations for improving the assay are given on the bottom right. These recommendations are prioritized by relevance and should be considered one at a time. Use the *Guidance* panel and *NanoPedia* to get more information on assay optimization strategies by clicking question mark buttons and hyperlinks.

At the bottom of the *Results* page, click *Create Report* to create a PDF document containing all relevant information on the measurement for easy filing. The green button on the bottom right allows to start a new experiment. The software recommends an experimental mode; for example, if a *Binding Affinity* experiment was measured and needs improving, another *Binding Affinity* experiment will be suggested. By clicking on the button, a new experiment will be created and settings from the current experiment will be transferred. This is different from the *New Experiment* button in the Session Overview, which will open a new, blank experiment (see 4.2).

5.3.1. Quality Checks

The conclusion and consequential recommendations on the *Results* page are based on quality checks. These are computed automatically but can be overridden. Quality checks are used throughout the *Results* pages to visualize the results of checks performed on the measured data. Next to all quality checks is a question mark button providing additional information on the assessed parameter. Quality checks can have three different outcomes: positive, negative and inconclusive (see **Figure 6**). A negative quality check can be overridden if you disagree or if you think the detected issue is negligible.

Override by clicking on the quality check button. A positive quality check can of course also be overridden. Note the small arrow indicating a user-made decision to override a result. This information will be stored in the file. An inconclusive quality check means that user input is needed because additional data is required. In these cases, the software will provide information on the necessary input and how to get it (see section 6).

Figure 6: Quality Checks. Left panel: possible results. Center panel: overriding a negative quality check. Right panel: Overriding a positive quality check. Note the small arrow indicating an overridden quality check.

A conclusion (e.g. a K_d or a definitive qualitative answer on binding) will only be given if all quality checks are positive. The final decision on all quality checks is left up to the user.

5.3.2. Capillary Scans

The *Capillary Scans* panel shows the fluorescence peaks recorded for all individual capillaries. The peak shape indicates whether the fluorescent molecule is adsorbing to the inside capillary wall, while the peak height indicates fluorescence intensity. Both parameters are important for a successful MST experiment and are assessed by the software automatically.

Capillary scans are recorded before and after the MST measurement and can be displayed either together or separately. Capillary peak overlays enable easy visual assessment of adsorption, for example of target alone compared to target in complex. In *Pretest* and *Binding Check* experiments, this overlay is done automatically.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a capillary peak to display its number.

In this section, the software automatically assesses the following parameters and display the results as quality check symbols (see 5.3.1):

- Initial fluorescence intensity of each capillary (peak height)
- Fluorescence homogeneity (whether capillaries containing identical target concentration show identical fluorescence intensities)
- Adsorption (peak shape)
- Changes in initial fluorescence induced by the addition of ligand

5.3.3. MST Traces

The *MST Traces* panel shows all traces recorded for the individual capillaries. The shape of the MST traces gives information on homogeneity and photostability of the sample. Both parameters are important for a successful MST experiment and are assessed by the software automatically.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 5.3.1):

- Aggregation in the sample (shape of MST trace)
- Photobleaching behavior of the sample and whether it is changed by the addition of ligand (slope of the first seconds of the MST trace)

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over an MST trace to display its capillary number.

5.3.4. Signal-to-Noise Ratio (Binding Check)

For a qualitative answer on whether an interaction is taking place in the investigated sample, the MST signal of target is compared to the signal of complex (target + ligand). An interaction can only be assumed if the difference in the respective MST signals is large enough. The signal-to-noise ratio is used to evaluate the quality of the binding data. It is defined as the response amplitude divided by the noise of the measurement. A signal-to-noise ratio of more than 5 is desirable, while more than 12 reflects an excellent assay. The noise is defined as the average standard deviation of the mean of all points of target and complex, respectively, while the amplitude is the difference between the average complex and target signals.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a point to display its capillary number.

By default, the software will evaluate the measurement automatically. This means that the evaluation mode is chosen based on experimental parameters and to yield the best signal-to-noise ratio. To change the evaluation mode, deselect *Automatic Mode*, then choose an evaluation mode from the drop-down menu.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 5.3.1):

- Response amplitude
- Signal-to-noise ratio

5.3.5. Dose Response (Binding Affinity)

The *Dose Response* analysis displays concentration dependent changes in the measurement parameter (usually the MST signal), which are used to calculate K_d or EC_{50} values. The signal of each capillary is plotted against the ligand concentration in the capillary.

In this section, the software automatically assesses the following parameters and displays the results as quality check symbols (see 5.3.1):

- Response amplitude
- Signal-to-noise ratio

The signal-to-noise ratio is used to evaluate the quality of the binding data. It is defined as the response amplitude divided by the noise of the measurement. A signal-to-noise ratio of more than 5 is desirable, while more than 12 reflects an excellent assay. The noise is defined as the average standard deviation of all points from the fitted curve, while the amplitude is the difference between the unbound state and the bound state, or the two plateaus of the fitted curve. Only if both parameters can be determined, and if the signal-to-noise ratio is deemed sufficient, a binding curve will be fitted and a K_d or EC_{50} will be derived.

Individual capillaries can be selected as outliers by clicking on the respectively numbered box below the image. Quality checks and conclusions will be updated accordingly. Mouse over a point to display its capillary number.

By default, the software will evaluate the measurement automatically. This means that the evaluation mode is chosen based on experimental parameters and to yield the best signal-to-noise ratio. To change the evaluation mode, deselect *Automatic Mode*, then choose an evaluation mode from the drop-down menu. The default fit model is the K_d model, which can also be changed here.

6. Specificity Tests

When a ligand-induced fluorescence change is detected in an experiment, an additional specificity test is required to enable unambiguous interpretation of MST data and to distinguish between specific and non-specific effects. For samples labeled via the His-tag and RED-tris-NTA, it is recommended to perform an ECP-Test (EDTA/Control Peptide Test). For other samples, please perform an SD-Test (SDS denaturation test).

The automatic assessment performed by the MO.Control 2 software will identify these cases. It will suggest the appropriate specificity test and provide step-by-step instructions.

7. Data Visualization and Export

At the bottom of the *Results* page, click *Create Report* to create a PDF document containing all relevant information on the measurement for easy filing. This PDF report contains experimental settings, concentrations, user-supplied information and comments, and experimental results (capillary scans, MST traces and dose response/signal-to-noise ratio plots).

On each *Results Details* page, the image can be exported as a .png or .svg file by clicking on the *Export* button above the image.

To access the raw measurement data, open the measurement file in MO.Affinity Analysis 3 and export from there. Please refer to the MO.Affinity Analysis 3 user manual for more information.

All graphs can be zoomed and adjusted for optimal visualization. Check the *Auto Zoom* checkbox to adjust all data in the chart to the chart size. Zooming in-and-out of the chart is performed by scrolling the mouse wheel. Horizontal or vertical zooming can be performed by pressing shift or control on the keyboard while scrolling, respectively. Click and hold the mouse wheel and move the mouse to drag the chart (see **Figure 7**).

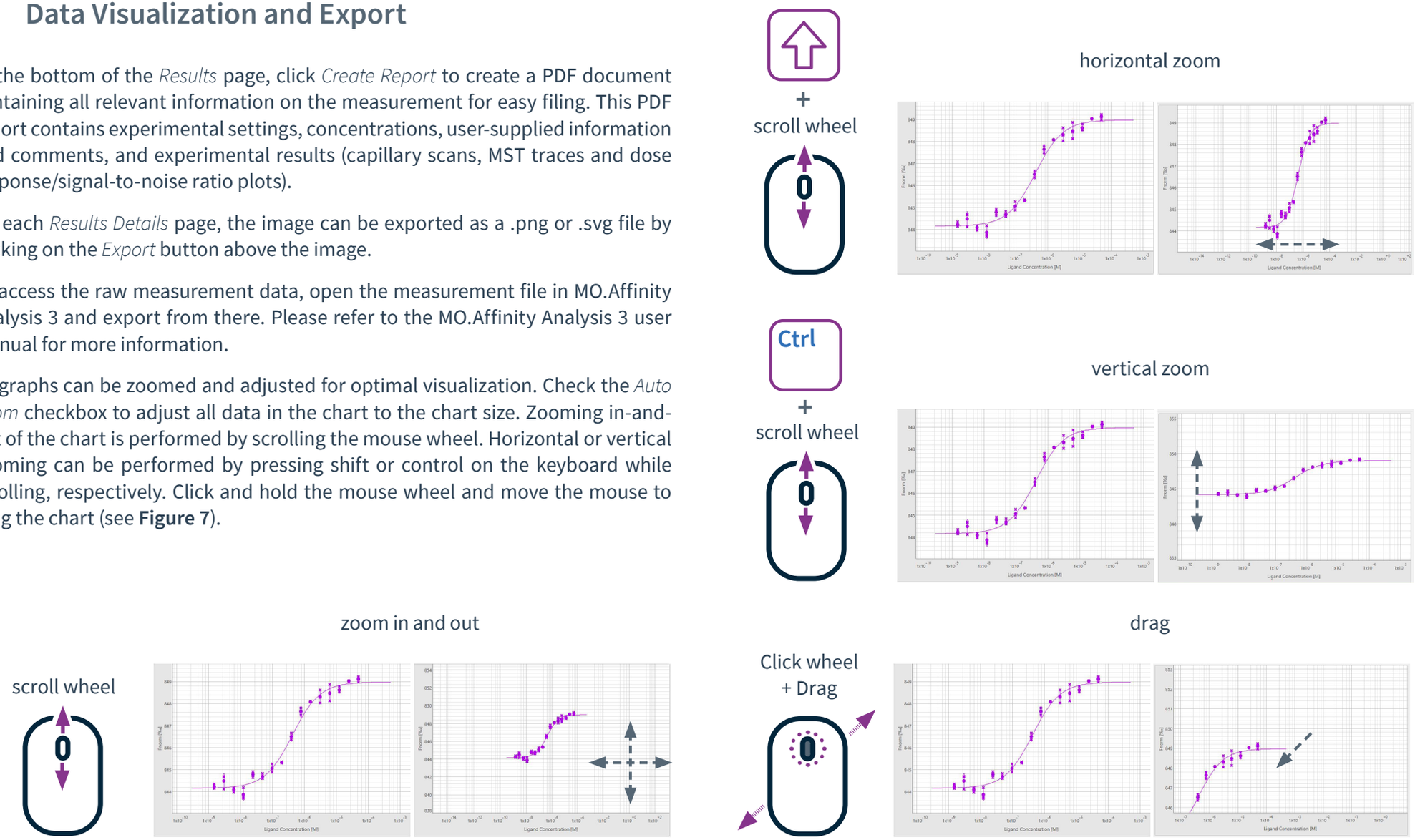


Figure7: Mouse control of chart visualization.

8. Patents and intellectual property

Monolith, MST and TRIC technology are patent protected, especially by the following patents, US8431903B2, US8853650B2, US10345312B2, US8741570B2 including their application and registration in different other countries.

Contact

NanoTemper Technologies GmbH

Global headquarters:
Toelzer Strasse 1,
81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0
Fax: +49 (0)89 4522895 60

info@nanotempertech.com
nanotempertech.com

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MO.AFFINITY ANALYSIS 3

Software Manual

Table of contents

1. General Information

The MO.Affinity Analysis 3 software allows straightforward analysis and evaluation of MicroScale Thermophoresis data. It allows quantification of binding parameters such as dissociation constants (K_d) or EC_{50} values and easy comparison of results e.g. for one target protein binding to different compounds.

The MO.Affinity Analysis 3 software guides the user through all important steps from data selection to evaluation by using a clearly organized submenu layout in the task bar. Creating an analysis file will retain the chosen settings for data analysis. Additionally, the software allows inspection and exporting of both raw and processed data at any step during data analysis.

This manual explains the main functions integrated in the MO.Affinity Analysis 3 software.

2. System Requirements

If the necessary licenses have been purchased, MO.Affinity Analysis 3 software can be installed on computers meeting the following requirements:

Operating system	Windows 7/10 (x86 or x64)
CPU	Intel Core i5, 6th generation or higher
RAM	8 GB or higher
Hard disk	20 GB available space, SSD recommended
Display resolution	Full HD @ 125%
Software	Microsoft .NET Framework 4.5.1 or higher (included in installer of MO.Affinity Analysis 3 software)
Operating system language	English or German

An external computer mouse is necessary to access all software features.

3. Installation, License Activation, and Software Updates

The software can be installed on any computer and will automatically start in trial mode, when opened for the first time. The trial version offers full functionality and has to be activated with a license key purchased from NanoTemper Technologies after 30 days.

To activate the license, navigate to the main menu and select *Licensing Status*. Please follow the instructions provided. One software license is valid for one account per computer and licenses can be transferred between computers. To transfer a license, please deactivate it first via the *Licensing Status* dialogue and reactivate it on the new computer.

When a software update is available, a green button will appear next to 

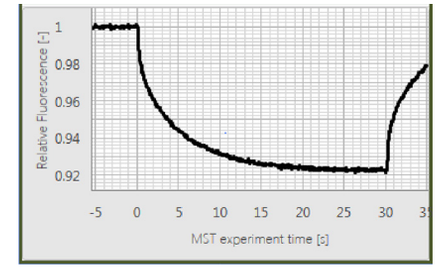
button at the top right corner of the software window. This feature is accessible only if your device is connected to a network.

4. Term Definitions

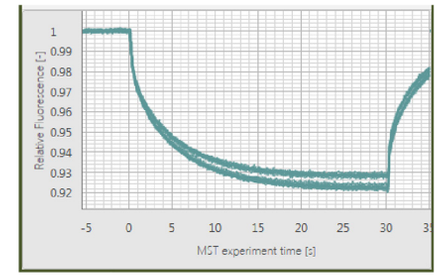
Target: The fluorescent molecule. The concentration of the target molecule is constant throughout a dilution series.

Ligand: Non-fluorescent binding partner. The ligand concentration is varied by serial dilution.

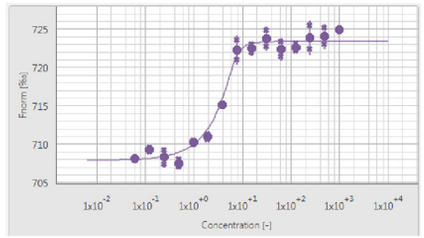
MST Trace: MST fluorescence signal over time. A typical MST trace contains an initial detection of the sample fluorescence (by default recorded for 3-5 seconds), followed by activation of the MST power to induce the temperature gradient and subsequent detection of thermophoretic changes in fluorescence (by default recorded for 20-30 seconds). Finally, MST power is deactivated and fluorescent recovery is monitored (recorded for a short period only).



MST Run: A run includes a series of MST traces, typically of a fluorescent target molecule versus a serial dilution of a ligand.



Merge Set: A series of replicates of MST runs, with identical MST power, LED/ excitation power as well as target concentrations. Data within one Merge Set will be averaged and error bars will be calculated and displayed. Note that the ligand concentrations do not necessarily have to be identical and can vary between merged MST runs.



Analysis Set: A complete dataset consisting of a number of Merge Sets or single MST runs, for parallel analysis and direct comparison. Dose response curves of runs and Merge Sets in Analysis Sets can be compared in the same charts with the MO.Affinity Analysis 3 software. All runs contained in one Analysis Set are analyzed with the same evaluation parameters.

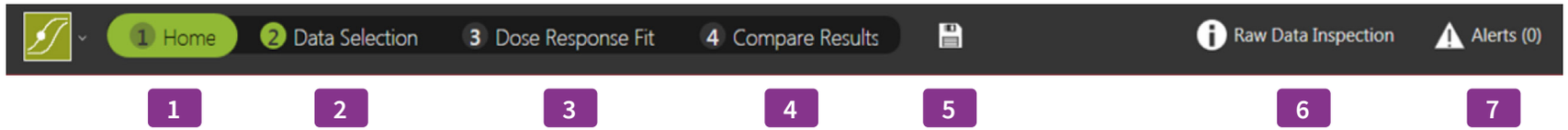
Analysis (file): A single Analysis Set, or a collection of Analysis Sets. The analysis can be saved at any time. An analysis file can be used to integrate a larger number of MST experiments for a comprehensive and systematic data analysis.

Raw Data: All fluorescence data recorded by the Monolith instrument: MST traces, capillary scans and shapes, initial fluorescence values and bleaching rates. All raw data can be viewed in the Raw Data Inspection tool (see **Figure 1** and section 5, point 6). Please note that the capillary scan displayed is the scan recorded before the MST measurement, not afterwards (applicable to MST measurements performed with MO.Control 2 software).

Figure 1: *MST Raw Data Inspection:* (A) Properties, (B) MST Traces, (C) Capillary Scan, (D) Capillary Shape, (E) Initial Fluorescence, (F) Bleaching Rate. Please note that the capillary scan displayed is the scan recorded before the MST measurement, not afterwards (applicable to MST measurements performed with MO.Control 2 software).

5. General Layout

All major functions of the MO.Affinity Analysis 3 software are organized in the task bar:



Four tabs guide the user through the process of MST data analysis:

1. **Home**
2. **Data Selection**
3. **Dose Response Fit**
4. **Compare Results**

It is recommended to complete all four steps in this order to ensure proper documentation and analysis of MST experiments. Movement between tabs during the analysis process is possible, e.g. to add additional files, edit names of Analysis sets, etc.

Additional buttons in the task bar are:

5. **Quick saving** of the analysis file.

6. **Raw Data Inspection** is available at any time during the analysis process by selecting the *Raw Data Inspection* button on the top right of the window. This will open a separate window which displays all experiment-associated settings and meta-data, as well as detailed charts of raw MST traces, capillary scans, overlays of capillary shapes, initial fluorescence values and bleaching rates. Selected runs and traces will be highlighted in both, the MO.Affinity Analysis 3 main window as well as in the *Raw Data Inspection* window. For detailed views of *Raw Data Inspection* options, see **Figure 1**.

7. **Alerts** will be displayed on the top right of the main window. Alerts include experimental inconsistencies as well as warnings about potential inconsistencies during data processing and fitting.

Context-related supporting information, such as term definitions and equations, can be found when clicking the buttons located on each page.

Anything you do in the software can be undone by pressing Ctrl + Z.

6. Saving and Exporting Data

The MO.Affinity Analysis 3 software allows for saving the current analysis at any time, using the drop-down menu in the top left (click ), the quick save button in the task bar  or navigation back to the *Home* tab.

Moreover, chart and tabular data can be exported where indicated using the export buttons (click ), which are located in the *Dose Response Fit* and *Compare Results* submenus as well as in the *Raw Data Inspection* section. Available image formats for export are .svg, .pdf and .png. Note that .svg and .pdf contain vector graphics which can be processed by graphic editing software. Tabular data are saved in .xlsx or .csv format. Results can also be saved as a condensed report in .pdf format on the *Dose Response Fit* and *Compare Results* screens.

7. Analysis Setup in the Home Screen

In the *Home* screen, create a new MST analysis or load a preexisting analysis file. Analysis files are saved in the .nta3 format. Changes in an analysis can be saved at any time.

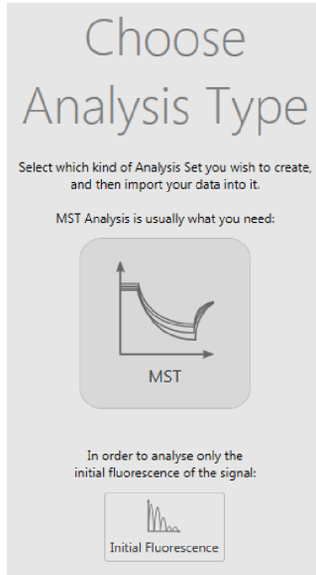
When creating a new analysis, enter an analysis name and optionally add comments, e.g. purpose of the analysis, assay conditions etc. Recently opened analysis files are listed chronologically. Start adding raw data to your analysis here, or in the next tab *Data Selection*.

8. Data Selection

See **Figure 2** for a summary of the options you have for *Data Selection*.

To add MST runs to the analysis, use the drag-and-drop function for .moc2 files, or use the load function to browse folders and select single or multiple files. MST runs of the selected file will appear in the *Data Selection* window as thumbnails of normalized *MST Traces* with a description of name, experiment settings and date. By changing the *View* option in the top panel, you can alter the presentation of the MST data thumbnails to *Dose Response*, *Capillary Shape* or *Initial Fluorescence*.

Before data analysis is performed, choose the type of analysis. To analyze binding by MST, click on the *MST* button in the *Choose Analysis Type* panel. In cases of ligand-induced fluorescence changes where the fluorescence values of each capillary are used to determine binding constants, click the *Initial Fluorescence* button.



Note: *Use the* Initial Fluorescence *analysis if there is a ligand-concentration dependent change in sample fluorescence >±20 %. Refer to the User Starting Guide or the MO.Control 2 software for more information.*

Note: *The data points presented in the "Dose Response" thumbnail view correspond to the ΔF_{norm} values determined after the MST power dependent time intervals (see section 9).*

For further analysis and determination of binding parameters, MST runs are combined into *Analysis Sets*. Clicking the *"Auto-Append"* button will create a single *Analysis Set* which contains all loaded MST runs as independent single runs. Alternatively, MST runs can be added by clicking the + symbol on the bottom right of each MST run thumbnail. This automatically creates a new *Merge Set.*

To add replicate runs to a *Merge Set,* drag-and-drop them there directly. Merged runs will be displayed with average values and error bars in the *Dose Response Fit* screen. The software allows the merging of runs if the runs were collected using the same

- LED/excitation power
- MST power
- Capillary type
- Acquisition mode (fluorescence channel) and optics module

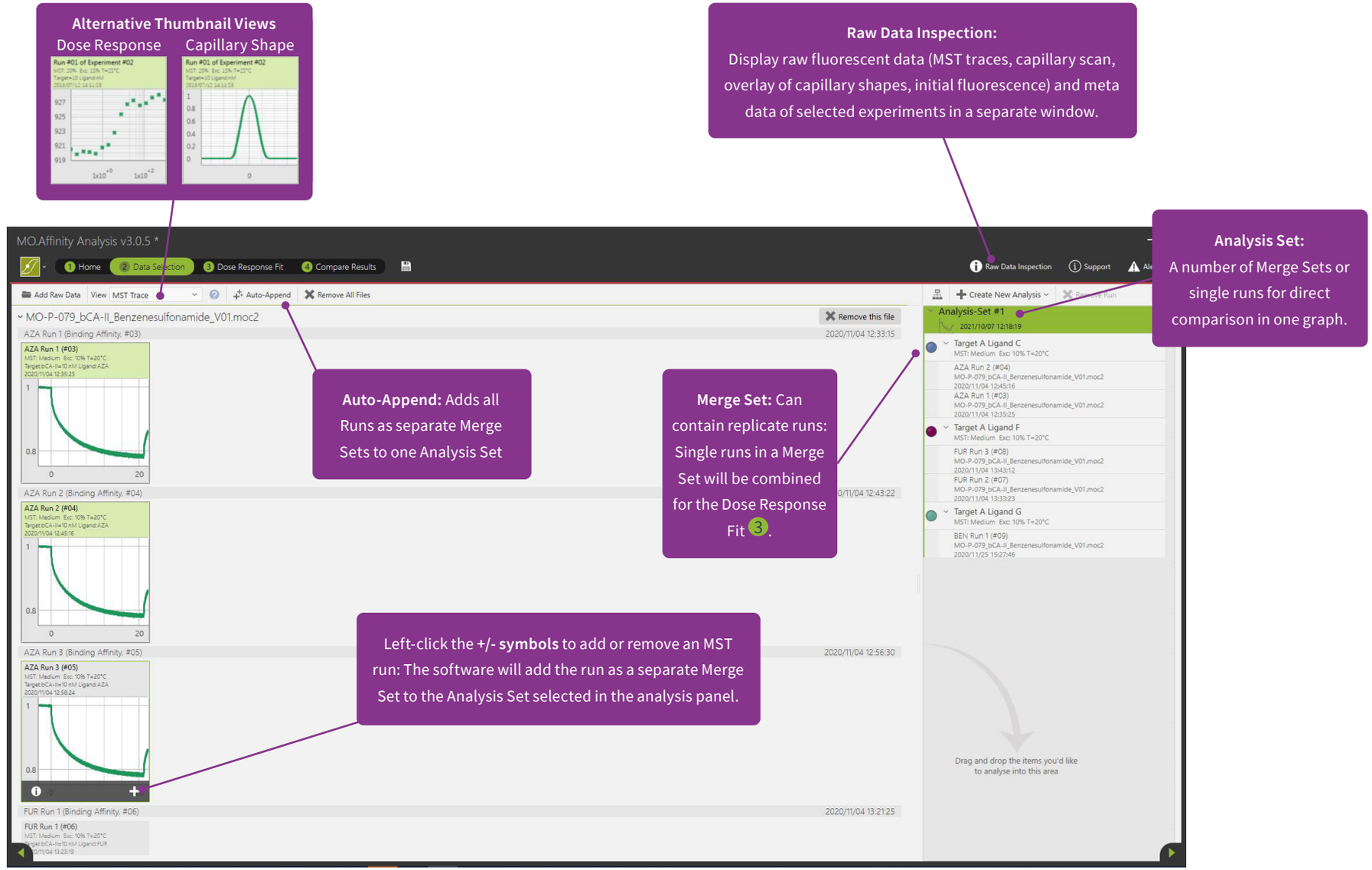
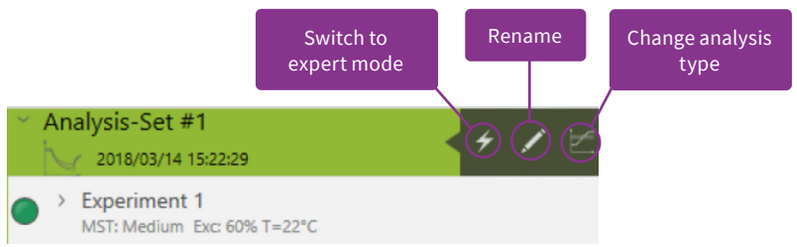


Figure 2: Create *Analysis* and *Merge Sets* from MST runs in the *Data Selection* menu.

Depending on the software used to perform the measurement, not all of these criteria may be saved in the file. As a result, it may not be possible to merge data collected with different software. When the user tries to add an incompatible run to a *Merge Set*, the software will reject the run and display an incompatibility message. To create a custom number of different *Merge* and *Analysis Sets*, drag-and-drop the MST run thumbnails.

Hint: Analysis Sets and Merge Sets can also be rearranged by simple dragging and dropping.

Please note that names of *Merge* and *Analysis Set* can be edited for better description and documentation by clicking the pen symbol on the respective flyout. The flyout appears upon mouse-over in the respective *Analysis Set* field (see screenshot below). Also, after an *Analysis Set* was created the analysis mode can still be switched between MST- and initial fluorescence analysis by selecting the respective button. Another button allows to switch to the expert mode for analysis (see next section for more details). Once *Analysis Sets* are created, binding data can be quantified.



9. Dose Response Fit

The *Dose Response* fit window allows for fitting MST data to obtain either dissociation constants (K_ds, using the law of mass action) or EC_{50} values (using the Hill equation). In the window, normalized MST traces as well as corresponding dose response plots of the selected MST data are shown.

Figure 3 summarizes the data analysis and fitting workflow.

By selecting either an *Analysis Set* or a *Merge Set* on the left, the respective MST traces and their dose response plots are displayed. By default, F_{norm}-values in the dose response plot are calculated from the ratio of normalized fluorescence F_0/F_1, where F_0 corresponds to the normalized fluorescence prior to MST activation. F_1 is by default determined after an optimal MST power-dependent time interval which yields the best signal-to-noise ratio.

Use the mouse or the arrow keys to navigate through the analysis tree in the left panel. The right arrow key expands an *Analysis Set* or *Merge Set*, while the left arrow key collapses it.

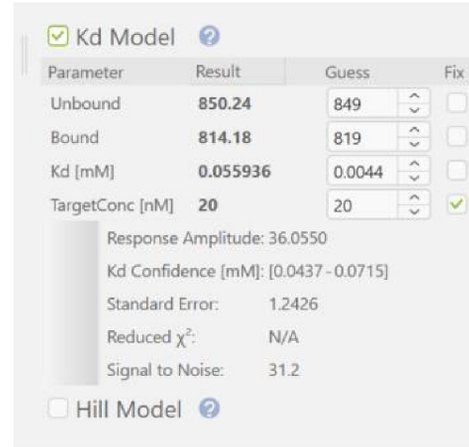
Data fitting is performed instantly after selecting the respective fit routine (K_d Model or Hill Model). Fitting requires initial values, which are determined automatically by the software (shown as *Guess* values in the fit model). Known parameters, such as target concentration, need to be fixed by checking the *Fix* checkbox. In some cases, it may be required to guide the fitting algorithm by manually entering initial *Guess* values.

Note: *The Hill fit should only be used if the interaction involves a cooperative binding mode. A 1:1 interaction should always be fitted using the K_d Model.*

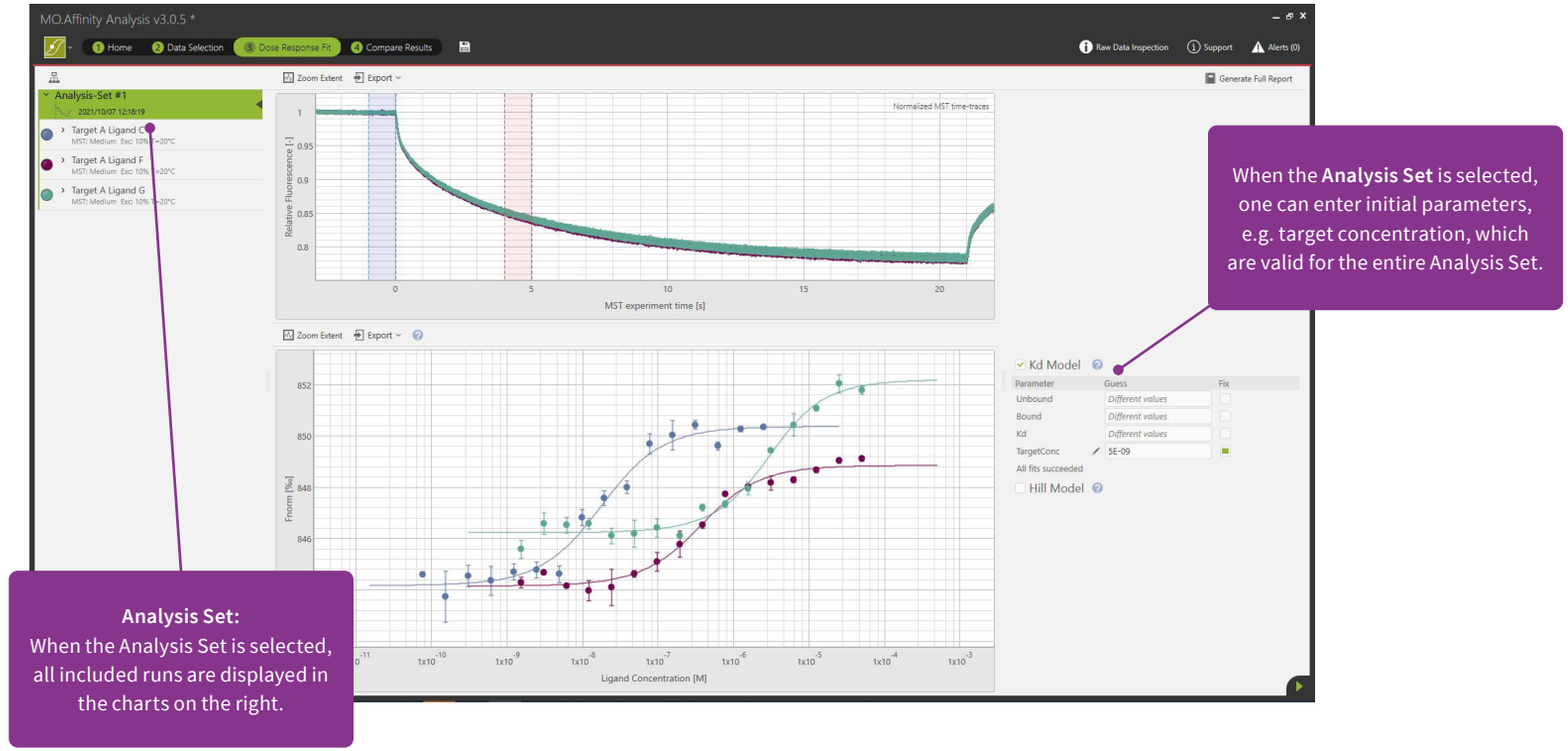
After a fit is performed, a range of statistical parameters is automatically calculated and displayed. For definitions, fit equations and more information, click the button.

Replicates within one *Merge Set* are displayed as average values and error bars representing the standard deviation. Fits are applied to the average values. To get an error estimation on the resulting K_d, fit the replicates individually and use this data to perform statistics.

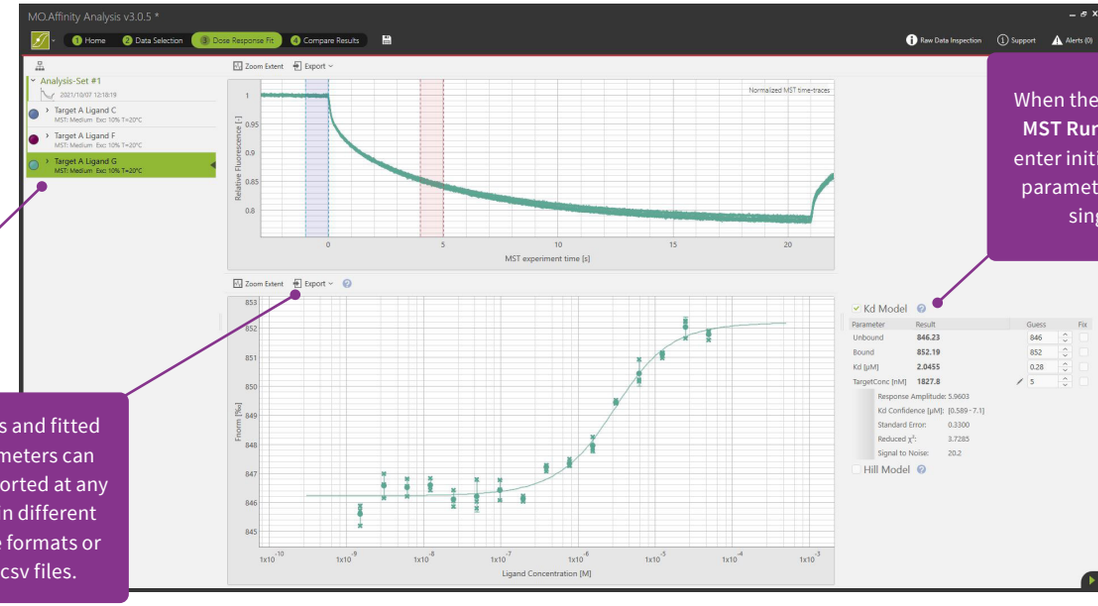
For in-depth data evaluation and fit refinement, single runs and MST traces can be highlighted either by selection on the left, or by clicking on the respective MST trace or data point in the graphs. After highlighting, outliers can be excluded from the fit (either greyed out or invisible).



Analysis Set selected (Overview + set global parameters)



Merge Set selected (Obtain binding affinities)

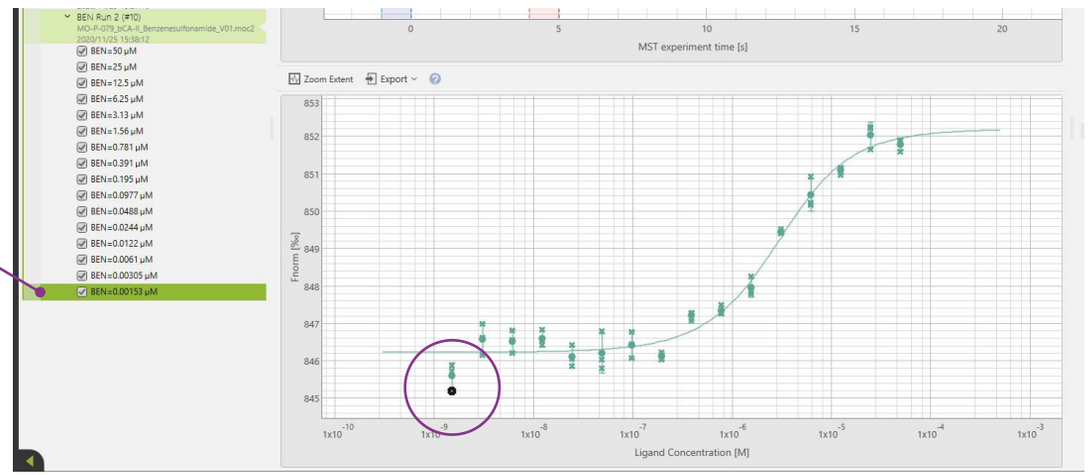


Merge Set:
When it is selected, all included runs are analyzed.

When the **Merge Set** or a single **MST Run** is selected, one can enter initial parameters and fix parameters for the particular single experiment.

Charts and fitted parameters can be exported at any time in different image formats or as .csv files.

Single Run selected (in-depth evaluation + removal of outliers)



Outliers can be marked with a left-click in the dose-response plot and either excluded from the analysis (, will be greyed-out), or additionally hidden from the chart .

Figure 3: Analysis of binding constants ans EC_{50}-values.

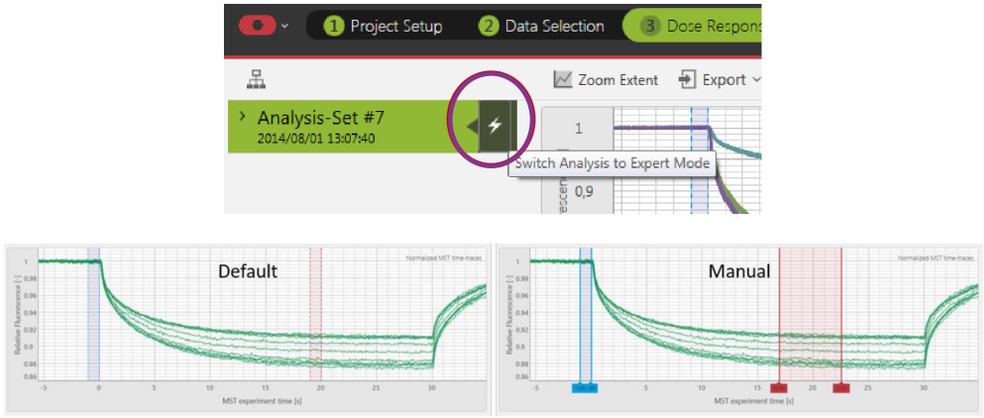
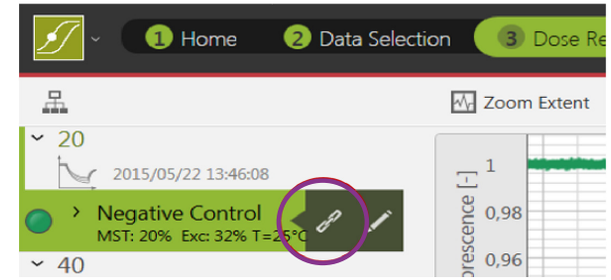


Figure 4: Activation of the Expert Mode and visualization of different cursor settings.

For a more in-depth evaluation of MST data, activate the *Raw Data Inspection* Raw Data Inspection. Here, effects such as sample aggregation, adsorption to capillary walls or fluorescence intensity variations in the titration series can be easily identified. Please see the MO.Control 2 software for more information on sample quality and assay optimization.

When preparing *Merge Sets* for presentation that contain non-binding negative control interactions, move the mouse cursor over the name of the *Merge Set*. A flyout will appear with a chain link symbol. Clicking this symbol initializes all enabled fit parameters as nonbinder, which means a horizontal line will be drawn through the points at their average value. This allows the comparison of nonbinders with binders in the *Compare Results* view. To revoke non-binder status, navigate to the data fitting section of the respective run and untick all unneeded checkboxes.



As an alternative to using the default analysis settings, the positions of F_1 and F_0 can be manually adjusted after enabling the *Expert Mode* for the *Analysis Set* (see **Figure 4**). Using this mode, the F_1 and F_0 cursors can be placed anywhere along the MST time traces. The *Expert Mode* should only be used if the default analysis procedure did not yield satisfying results.

Similarly, when working with an *Initial Fluorescence Analysis Set*, the Expert Mode can be enabled to analyze ligand-dependent photobleaching effects (bleaching rate). Please contact your NanoTemper Technologies Support for more information.

Chart visuals: Chart colors can be changed in the *Data Selection, Dose Response Fit* and *Compare Results* sections. All charts in the *Dose Response Fit* and *Compare Results* sections can be zoomed and adjusted for optimal visualization. Use the *Zoom Extent* button to adjust all data in the chart to the chart size. Zooming in-and-out of the chart is performed by scrolling the mouse wheel. Horizontal or vertical zooming can be performed by pressing shift or control on the keyboard while scrolling, respectively. Click and hold the mouse wheel and move the mouse to drag the chart (see **Figure 5**).

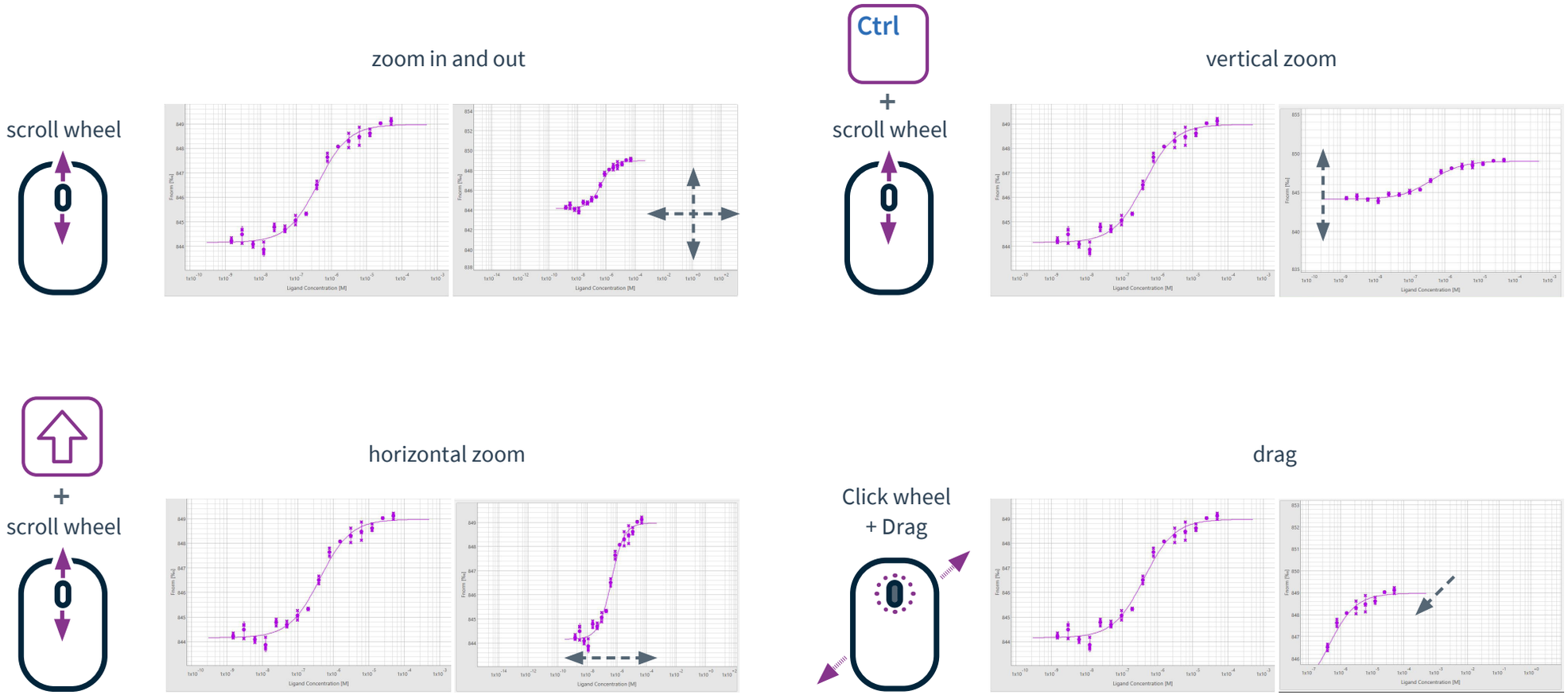
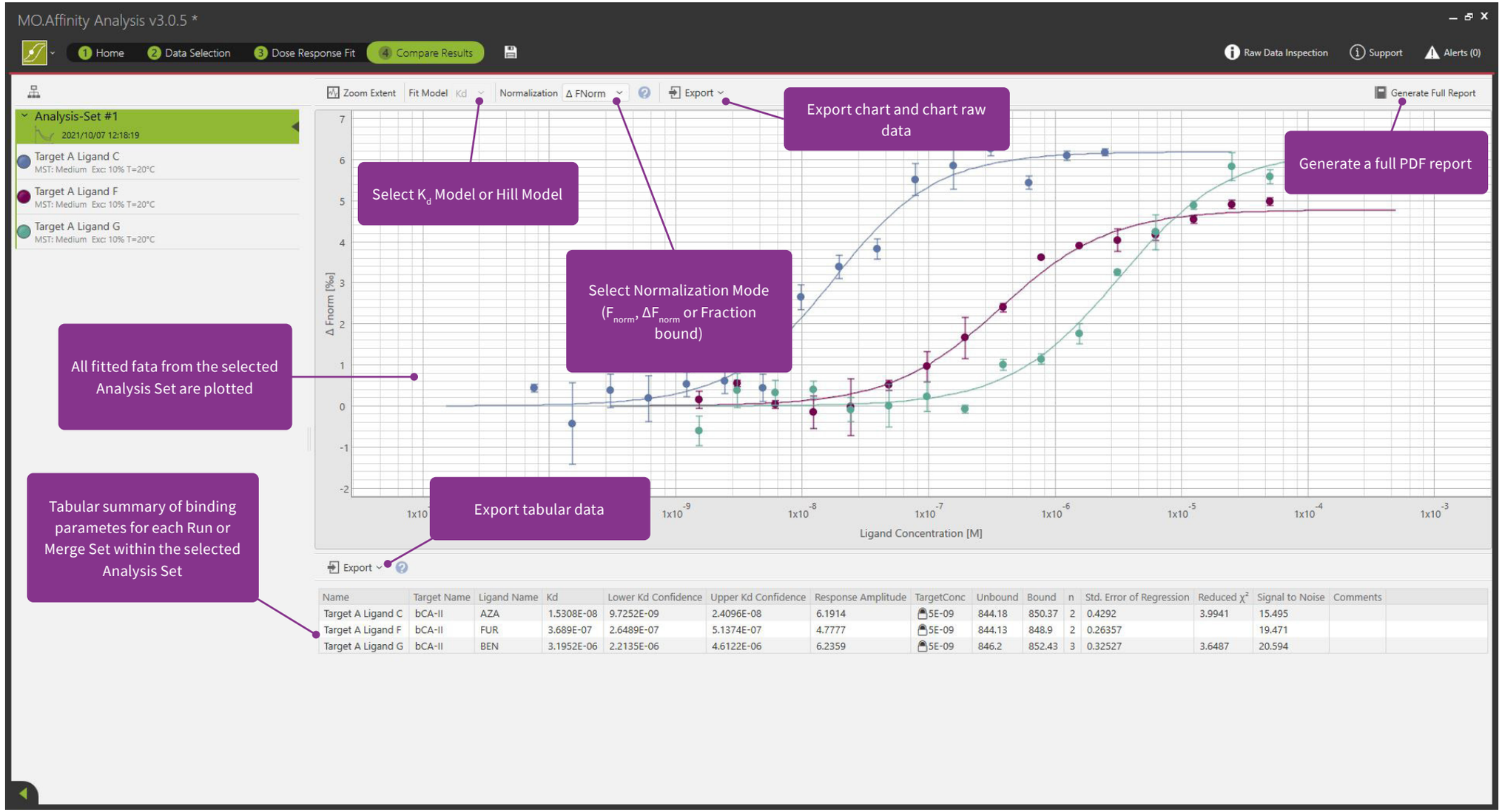


Figure 5: Mouse control of chart visualization.


MO.Affinity Analysis v3.0.5 *
1 Home
2 Data Selection
3 Dose Response Fit
4 Compare Results
Raw Data Inspection
Support
Alerts (0)
Zoom Extent
Fit Model
Kd
Normalization
Δ FNorm
Export
Generate Full Report
Analysis-Set #1
2021/10/07 12:18:19
Target A Ligand C
MST: Medium Exc: 10% T=20°C
Target A Ligand F
MST: Medium Exc: 10% T=20°C
Target A Ligand G
MST: Medium Exc: 10% T=20°C
Δ Fnorm [‰]
Ligand Concentration [M]
Export chart and chart raw data
Generate a full PDF report
Select Kd Model or Hill Model
Select Normalization Mode (Fnorm, ΔFnorm or Fraction bound)
All fitted fata from the selected Analysis Set are plotted
Tabular summary of binding parametes for each Run or Merge Set within the selected Analysis Set
Export tabular data
Export
Name | Target Name | Ligand Name | Kd | Lower Kd Confidence | Upper Kd Confidence | Response Amplitude | TargetConc | Unbound | Bound | n | Std. Error of Regression | Reduced χ² | Signal to Noise | Comments
Target A Ligand C | bCA-II | AZA | 1.5308E-08 | 9.7252E-09 | 2.4096E-08 | 6.1914 | 5E-09 | 844.18 | 850.37 | 2 | 0.4292 | 3.9941 | 15.495
Target A Ligand F | bCA-II | FUR | 3.689E-07 | 2.6489E-07 | 5.1374E-07 | 4.7777 | 5E-09 | 844.13 | 848.9 | 2 | 0.26357 | | 19.471
Target A Ligand G | bCA-II | BEN | 3.1952E-06 | 2.2135E-06 | 4.6122E-06 | 6.2359 | 5E-09 | 846.2 | 852.43 | 3 | 0.32527 | 3.6487 | 20.594

10. Compare Results

The *Compare Results* tab allows for a side-by-side comparison of MST runs and *Merge Sets* within an *Analysis Set.* In this tab, data and fitting results can also be exported in tabular and graphic format, including all binding data and the algorithms used. By selecting an *Analysis Set* on the left, all included data are plotted in the same chart. Selection of a *Merge Set* or a single MST run will highlight the selected experiments and grey-out the remaining experiments in the *Analysis Set.*

Dropdown menus to change the *Fit Model* (K_d or *Hill*) and display type (F_{norm}, ΔF_{norm}, *Fraction Bound*) are located on the top of the dose response chart. While the *Fraction Bound* normalization is best suited for a direct comparison of binding affinities, the ΔF_{norm} normalization provides additional information about amplitude size and direction (please contact your NanoTemper Technologies Customer Support for more information). Both charts and chart raw data can be exported as an image file (.svg, .png or .pdf) or text file (.csv or .xlsx) for further use and external analysis.

In addition to the visualization options, the *Compare Results* menu also includes a table summarizing number of averaged experiments (n), fit parameters, affinities and fit quality.

Finally, the *Generate Full Report* button summarizes all charts and tables into one single PDF. Click the *Generate Full Report* button in the *Dose Response Fit* view to obtain a report even with unfitted data.

11. Patents and intellectual property

Monolith, MST and TRIC technology are patent protected, especially by the following patents, US8431903B2, US8853650B2, US10345312B2, US8741570B2 including their application and registration in different other countries.

Contact

NanoTemper Technologies GmbH

Global headquarters:
Toelzer Strasse 1,
81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0
Fax: +49 (0)89 4522895 60

info@nanotempertech.com
nanotempertech.com

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MONOLITH CAPILLARIES

Accessory Manual

Table of contents

1. Monolith Capillary Chips

1.1. General Information

Specially-designed and precisely engineered, Monolith Capillary Chips are developed exclusively for use with the Monolith system. These single-use capillary chips enable researchers to analyze samples using small, microliter volumes. Manufactured in a state-of-the-art facility following stringent protocols and rigorous testing, Monolith Capillary Chips ensure optimal performance, maximum reproducibility and the highest quality results.

1.2. Content

Name	Store at
Monolith Premium Capillary Chips (16 chips with 24 capillaries each)	Store dry and in a dark place at room temperature

1.3. Capillary Chip Handling and Safety Information

Keep chip container closed to ensure capillaries are clean and dust-free. Hold individual capillary chips on both ends, not in the center. Use gloves. Dispose of capillary chips according to applicable regulations and according to the sample contained in them.

WARNING Broken glass can cut skin. Handle capillary chips carefully to avoid breaking. Do not touch broken capillary chips.

1.4. Packaging disposal

Dispose of the plastic capillary chip container according to applicable regulations. The cardboard can be recycled with paper products.

2. Monolith Capillary Kit

2.1. General Information

Specially-designed and precisely engineered, Monolith Capillaries are developed exclusively for use with the Monolith or Monolith Pico system. These single-use capillaries enable researchers to analyze samples using small, microliter volumes. Manufactured in a state-of-the-art facility following stringent protocols and rigorous testing, Monolith Capillaries ensure optimal performance, maximum reproducibility and the highest quality results.

2.2. Content

Name	Store at
Monolith Capillaries	Store dry and in a dark place at room temperature

2.3. Capillary handling and safety information

Keep vial closed to ensure capillaries are clean and dust-free. Hold individual capillaries at one end, not in the center. Use gloves. Dispose of capillaries according to applicable regulations and according to the sample contained in them.

WARNING Broken glass can cut skin. Handle capillary chips carefully to avoid breaking. Do not touch broken capillary chips.

2.4. Packaging disposal

All packaging, including the cardboard insert with foil, can be recycled with paper products.

PROTEIN LABELING

Accessory Manual

Protein Labeling Assistant

Assay development for MST and TRIC often begins with protein labeling. When you choose NanoTemper labeling kits — available in different chemistries and with optimized fluorophores — you get high-quality data from your assays. This interactive protein labeling assistant will help you find the best labeling strategy and labeling kit for your target protein.

The NanoTemper Technologies **Protein Labeling Assistan**t is a beta tool. We're test driving this to help make your assays more successful.

Follow **https://nanotempertech.com/labeling-bot/** for access to the **Protein Labeling Assistant**.

Contact

NanoTemper Technologies GmbH

Global headquarters:
Toelzer Strasse 1,
81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0
Fax: +49 (0)89 4522895 60

info@nanotempertech.com
nanotempertech.com

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NanoTemper cares for the future of our planet, therefore we want to do our best to protect it and reduce the environmental footprint of our products.

*This product manual is printed in Germany, using **EU Ecolabel approved paper from certified FSC® responsible forestry.***

NanoTemper Technologies GmbH

Global headquarters:

Toelzer Strasse 1,
81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0

Fax: +49 (0)89 4522895 60

info@nanotempertech.com

nanotempertech.com