Tycho NT.6 system

User manual

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1. About this user manual

This user manual gives guidance on the correct use of the Tycho NT.6 system. It covers system specifications, safety considerations and installation as well as why and how to run experiments with Tycho NT.6. 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 visit the NanoTemper Technologies Explorer Community at nanotempertech.com/explorer for a replacement copy of this manual.

2. The Tycho NT.6 system

2.1. General

2.1.1. Intended use

Tycho NT.6 performs fast, precise thermal stability measurements for checking the quality of proteins. The system is intended for research purposes only. It is not to be used for diagnostic purposes.

2.1.2. Conformity

The following safety and electromagnetic standards were considered:

- IEC 61010-1:2010 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements
- IEC 61010-2-010:2014 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 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

2.1.3. Identification

The identification label (Figure 1) is positioned at the rear panel of the device. It includes manufacturer information, system model name and serial number (SN), electrical requirements, and the CE conformity symbol.

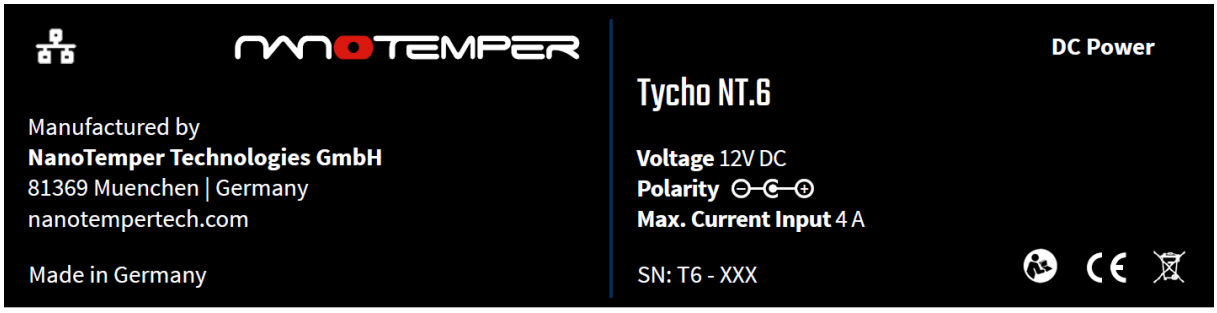


Figure 1: Identification label of Tycho NT.6.

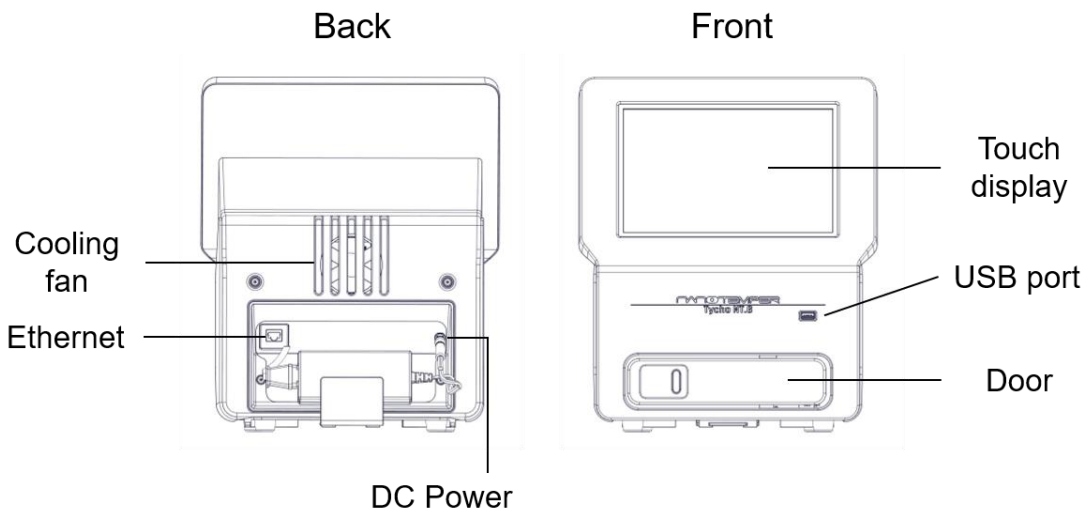
The serial number of the system, along with its versions of software and firmware, and its IP address (if connected to a LAN) can be found by tapping ⓘ on the *Start* screen.

2.2. Technical information

2.2.1. Technical specifications

Electricity		
Input external power supply	90–264 VAC ± 10%, 47–63 Hz, 230 VA max	
Output external power supply	12 VDC, 4.75 A max	
Electrical input Tycho NT.6 system	12 VDC, 4 A	
Environmental		
Operating temperature	15–30 °C (indoor use only)	
Storage temperature	-20–30 °C	
Humidity	0–80%, non-condensing	
Operating altitude	max 1000 m	
Tycho NT.6 dimensions	**System only**	**Including Capillary Station and power supply holder**
Width	31 cm (12.2”)	35.5 cm (14”)
Height	37 cm (14.5”)	37 cm (14.5”)
Depth	18 cm (7”)	21.5 cm (8.5”)
Weight	6.6 kg (14.6 lbs) net	7.4 kg (16.3 lbs) net
Required bench space	31 cm W x 27.5 cm D	35.5 cm W x 31 cm D
Power supply dimensions		
Width	10.1 cm (4”)	
Height	3.5 cm (1.4”)	
Depth	5 cm (2”)	
Weight	0.23 kg (0.51 lbs) net	
Temperature control		
Heating range	35°C–95°C	
Thermal ramp	30 °C/min	
Precision of thermal ramp slope	± 0.05 °C/min	

2.2.2. Connections for input and output



Type	Function	Position
Ethernet	To connect the system to the LAN via Ethernet cable	Back panel
DC Power	To connect the system to electrical power	Back panel
USB	To connect a USB memory stick for data export. Please only connect USB-compatible memory media.	Front panel

2.3. 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 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 contents of this manual are 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 laptop (PC). In this case, take corrective action by uninstalling the conflicting product(s).
7. NanoTemper is a registered trademark of NanoTemper Technologies GmbH in Germany and other countries.
8. Unauthorized resale is not permitted.

2.4. 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 found 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 operation, maintenance, adjustment or calibration.
3. Unauthorized modification or misuse.
4. Use of unauthorized capillaries and accessories.
5. Use of consumables, disposables and parts not supplied by an authorized NanoTemper Technologies distributor.
6. Corrosion due to the use of improper solvents, samples, or due to surrounding gases.
7. 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 2.1.3).

3. 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.

3.1. 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 have to read the manual before using the system. This label is positioned at the back of the device.

Warning symbol. This symbol indicates a surface that can heat up and cause burn injuries. This warning label is positioned on the sample tray.

3.2. Use and misuse

Use the Tycho NT.6 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.

3.3. Safety instructions

CAUTION The door of the system can pinch fingers. Keep fingers safe while opening and closing the door.

CAUTION Hot surfaces can cause skin burns. Don’t touch the thermal element (reflective silica surface of capillary tray) immediately after a measurement is finished. Wait until the system has cooled down. Tray temperature is indicated on the system display.

CAUTION The UV-LED contained in the system emits invisible ultraviolet radiation (UVB radiation) when in operation, which may be harmful to eyes and skin, even at brief periods of exposure. Do not look directly into the UV-LED during operation. Do not reach into the opening. If the system is used as intended, exposure to UV radiation cannot occur.

CAUTION Danger of electric shock, fire and skin burns. Do not open the system other than via the door. Do not reach into the door opening. Use only the provided power supply or a limited power supply according to system specifications (for example SPU63-105 by Sinpro Electronics Co., Ltd). Do not use extension cords. Replace damaged cables immediately.

CAUTION Mechanical moving parts within the system can injure hands or fingers. Do not reach into the opening.

CAUTION The system has to be installed in a way that does not hinder access to the external power supply and its power plug.

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

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 and glass waste.

NOTE Insufficient air supply can cause overheating of the system, which can lead to suboptimal performance. Assure sufficient air supply by not covering the cooling fan at the back of the system. Leave approx. 5 cm of space between system and wall.

4. Tycho NT.6 setup

4.1. Scope of delivery

Upon receiving the system, please check package contents for completeness. The Tycho NT.6 system package contains the following items:

Item	Description
Tycho NT.6 system	-
Tycho NT.6 Capillaries	High precision glass capillaries for Tycho NT.6 measurements Cat# TY-C001
Capillary station	Metal holder to be mounted at either side of the system. Includes positions for capillary vials and waste container
USB memory stick	For collecting data from the system
Quick start guide	Letter-sized sheet explaining Tycho NT.6 installation and setup.
Application guide	Letter-sized sheet explaining typical application examples and data interpretation.
User manual	This user manual
Power supply	-
Power supply holder	Metal holder to retain power supply in place at back of system

4.2. Unpacking

Open the box and remove the top layer of padding. Take out the smaller items (power supply, capillaries etc.) from their compartments and remove the second layer of padding. Insert hands into the cutouts on either side of the Tycho NT.6 system and lift it from the casing. pen the door of Tycho to remove the transportation lock band (red hook-and-loop fastener) (see Figure 2).

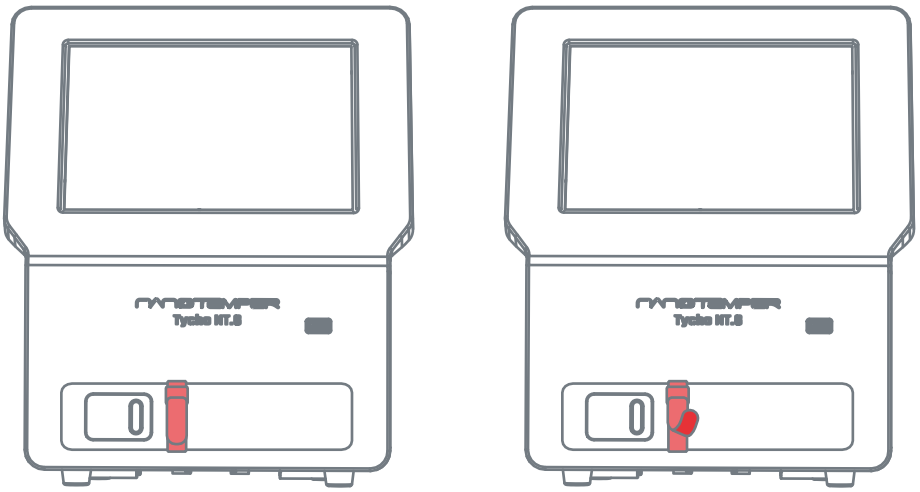


Figure 2: The transportation lock band (red hook-and-loop fastener) fixes the sample-holding arm to the door of Tycho. Lift the end of the band to remove. Open the door for better access.

It is recommended to keep all of the original packaging for possible future transport of the system.

4.3. Startup

Connect the Tycho NT.6 system to power by plugging in the power supply cable. The system starts up automatically. The touch display shows the *Start* screen when ready.

Connect the system to the local area network (LAN) via the Ethernet connection at the back if remote access is desired (see 4.8).

4.4. Time zone settings

When first using Tycho NT.6, the user is asked to set the time zone. Setting the right time zone will enable correct display of timestamps of all measured data.

It is possible to use Tycho NT.6 without correct time zone settings. In this case, all measurement dates and times are displayed as UTC (controlled universal time).

To modify the time zone setting, navigate to the *Start* screen. Tap the ⓘ icon. Tap the pen symbol, then select the correct time zone from the drop-down menu.

4.5. Capillary station

The Tycho NT.6 system comes with a convenient capillary station for convenient capillary handling (see Figure 3). Insert or remove the drawer by tilting it upwards (Figure 3B). Install the capillary station at the left or right side of Tycho NT.6 by sliding the supporting plate into the guiding rail until it clicks into place (Figure 3C).

Use the capillary station for storing capillary vials. Use it also to position an open capillary vial to facilitate capillary removal for sample loading (Figure 3D). Close the capillary vial when finished. The drawer of the capillary station can be used to collect used capillaries for disposal.

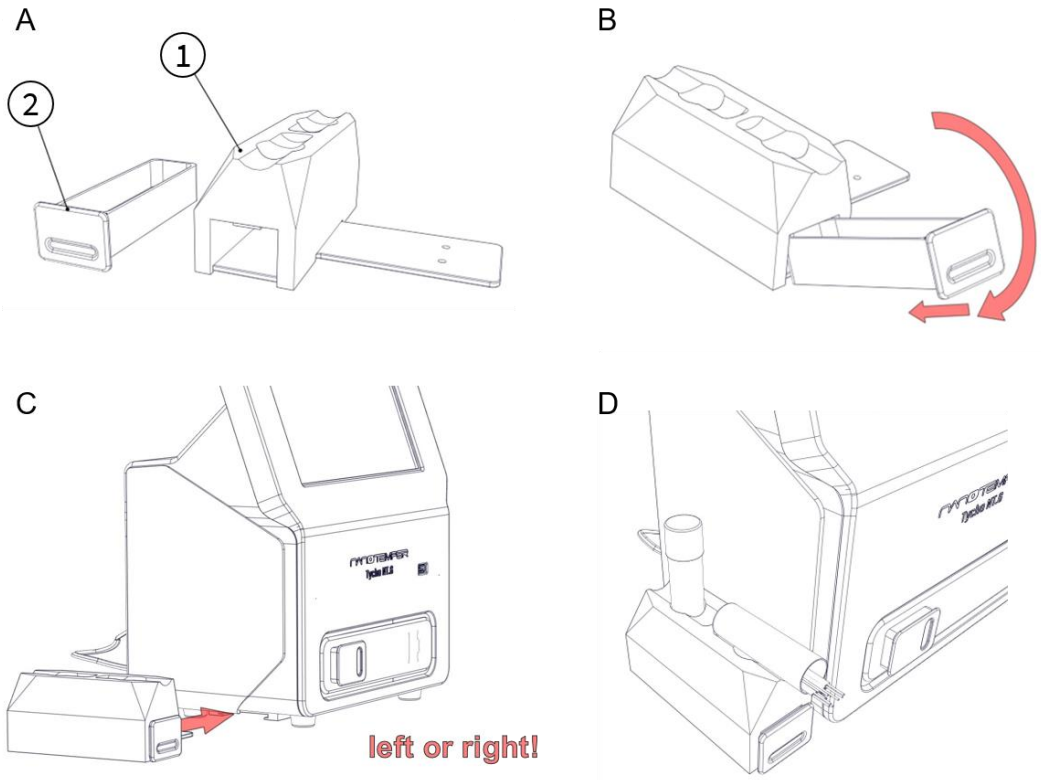


Figure 3: The Tycho NT.6 capillary station. (A) The capillary station base (1) and the drawer (2). (B) How to insert the drawer in to the base. Remove the drawer from the base by reversing the actions. (C) Install the capillary station at the left or right side of the Tycho NT.6 system by sliding the supporting plate into the guiding rail until it clicks into place. (D) When loading samples, positioning the open capillary vial on top of the capillary station facilitates the removal of individual capillaries.

4.6. Power supply holder

The power supply holder can be installed at the back of the Tycho NT.6 system (see Figure 4). Simply slide the holder plate into the guiding rail at the back until it clicks into place.

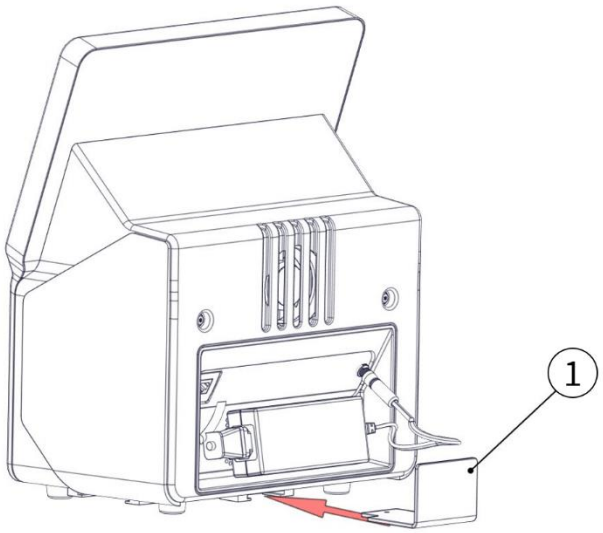


Figure 4: Installing the Tycho NT.6 power supply holder (1) at the back of the system.

4.7. Cleaning

The Tycho NT.6 system does not need any regular maintenance.

Before running an experiment, make sure that the capillary tray (reflective surface) is dirt- and dust-free. Clean with a scratch- and dust-free tissue and 99.8 % ethanol.

To clean the outside surface of the system, unplug the power supply at the back. Wipe the surface, including the touch display, with a cloth slightly dampened with water or ethanol.

4.8. Remote access

The Tycho NT.6 interface can be accessed remotely via browser from any computer in the same network. This conveniently allows data analysis and export from the user's desk.

To access, make sure that the Tycho NT.6 system is connected to the local area network (LAN) via the Ethernet connector at the back. Open a browser window and enter the Tycho system's IP address in the address bar. The address can be found by tapping ⓘ on the *Start* screen. Remote access functionalities are optimized for Google Chrome browser. Other browsers can be used as well.

Tycho is automatically assigned an IP address upon connection to the network. Please be aware that this works only for networks that use a DHCP server and assign IP addresses automatically to new devices. Depending on the exact network configuration, it may be necessary to manually "allow" new devices into the network by whitelisting their MAC addresses. The MAC address can also be found by tapping ⓘ on the *Start* screen.

Depending on the local network configuration, the IP address may change upon restart of the Tycho NT.6 system. In case the connection was bookmarked in the browser, the bookmark may need to be updated with the new IP address after restart.

Other types of networks, where IP addresses are fixed and/or assigned manually, are currently not supported.

4.9. Software updates

Connect a USB memory stick containing a software update file to the Tycho NT.6 system. The update will start automatically. Follow the instructions on the user interface to proceed.

Software update files are available from NanoTemper Technologies. Contact customer support for more information.

The software and firmware versions currently running on the system can be found by tapping ⓘ on the *Start* screen.

5. Using Tycho NT.6

5.1. Terms and definitions

This section explains the parameters measured with Tycho NT.6. For more details, see chapter 5.8.

Sample brightness	The relative, total fluorescence intensity of the sample is quantified as sample brightness. Tycho NT.6 systems are calibrated using a defined reference sample, which yields a sample brightness of 1. The sample brightness is independent of excitation power and therefore serves as a measure to quantitatively compare fluorescence intensities of different samples. If the samples contain identical proteins, e.g. when comparing different batches, the sample brightness can also be used to exactly determine the differences in protein concentration.
Ratio 350 nm / 330 nm	Over the course of the measurement, the fluorescence intensity of each sample is recorded at 330 nm and 350 nm. In the single wavelength detection, the change in fluorescence intensity (brightness) at a defined wavelength over temperature is monitored. The brightness ratio 350 nm / 330 nm however is a measure for a spectral shift in the emission profile of the Tryptophan (Trp) residues.
Initial ratio	Brightness ratio 350 nm / 330 nm at 35 °C.
Δ ratio	Difference of the ratio between 35 °C and 95 °C (start and end of measurement).
Similarity	Relative similarity of the unfolding profiles between measurement and reference capillaries. Profile similarity is determined by comparing the area under the profile curves.

5.2. Measuring principle and result output

The unfolding of a protein causes changes in the fluorescence emission properties of its fluorescent amino acid residues (tryptophan and tyrosine, mainly). Tycho NT.6 follows the unfolding process by recording sample fluorescence at 330 nm and 350 nm during thermal unfolding. A constant heating rate of 30 °C / min is applied to the sample, heating from 35 °C to 95 °C.

The recorded fluorescence signal is displayed as brightness and plotted against the temperature. The resulting curves are called unfolding profiles and are automatically analyzed for inflection points, also called inflection temperatures (T_i). The brightness ratio 350 nm / 330 nm is used to determine the initial ratio (at 35 °C) and Δ ratio (the difference between initial ratio at 35 °C and final ratio at 95 °C). All of these parameters are illustrated in Figure 5. They can be used to compare unfolding profiles to draw conclusions on the similarities between the analyzed samples.

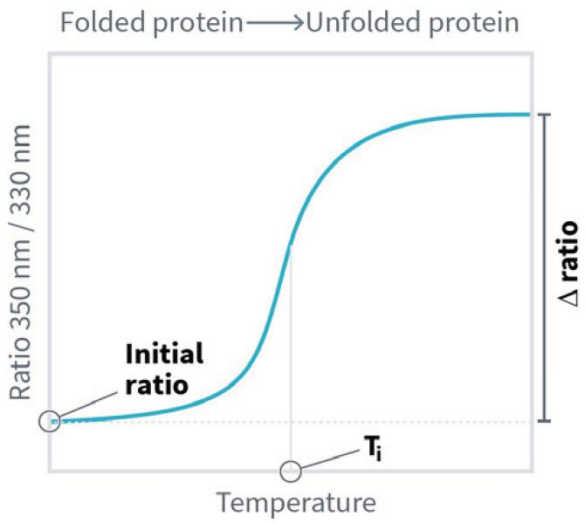


Figure 5: Typical unfolding profile illustrating (initial) ratio, Δ ratio and T_i.

5.3. General usage

Tycho NT.6 performs fast, precise thermal stability measurements for checking the quality of proteins. It records thermal unfolding profiles, automatically determines inflection temperatures (T_i) and quantifies sample brightness, initial ratio and Δ ratio. These parameters allow in-depth conclusions about a protein sample's stability and relative concentration. For application examples, see the section below.

The Tycho NT.6 system is controlled via its built-in screen. The straightforward, intuitive user interface enables users of all experience levels to run experiments. Integrated help features explain all functions and terminology. There are no levers, switches or movable parts besides the door. The system does not need to be switched off. Between measurements it will go into standby mode to save energy. Tap the screen to wake the system.

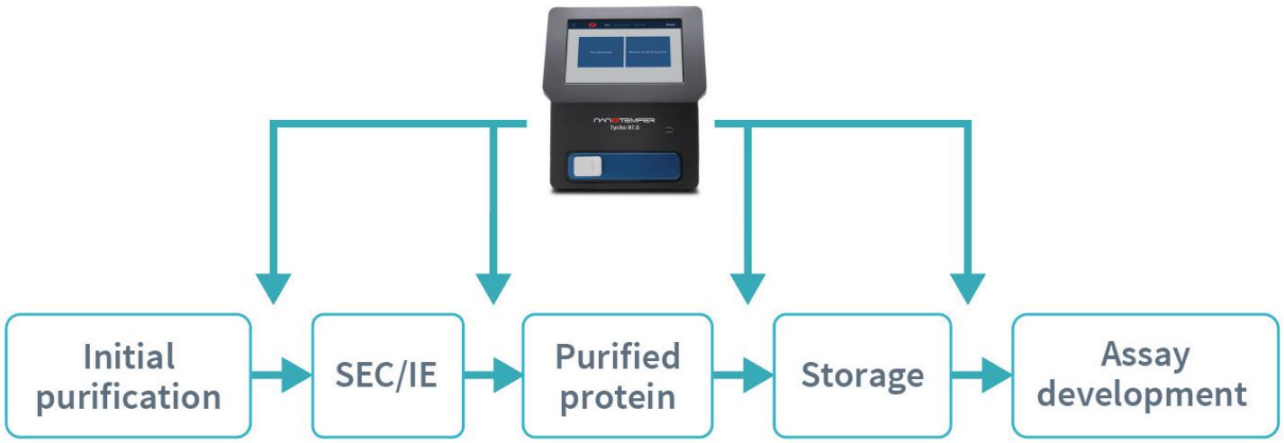


Figure 6: Examples of where Tycho NT.6 measurements fit into the protein purification and characterization workflow by performing 3-minute stability, functionality and similarity tests.

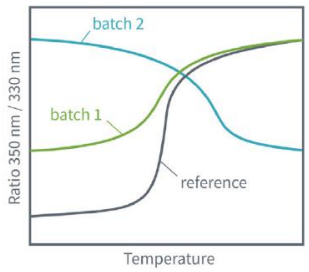
5.4. Applications

5.4.1. Similarity Tests

Similarity tests can yield helpful information at virtually any step of protein research. The sample brightness parameter quantified by Tycho NT.6 also allows concentration assessments. Similarity tests can therefore greatly help to ensure reproducibility and repeatability. Some examples are explained below and illustrated in Figure 7:

- Establish a reference of optimal integrity to compare against later stages, or future expression batches.
- Compare the sample at any time point to a previous expression step or previously expressed batch of the same protein to find out whether it is still happy (or whether it has degraded or unfolded).
- Compare different storage or stress conditions for each protein to optimize handling.

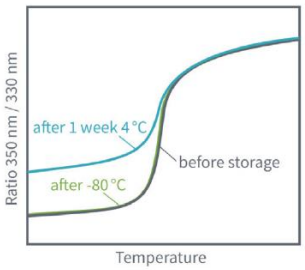
Batch-to-batch comparison



A decrease in Δ ratio compared to a reference sample indicates less folded protein (batch 1). A different unfolding profile indicates major differences between protein preparations (batch 2).

- Capture measurements during each step in protein purification preparations to ensure batch-to-batch similarity
- Identify and optimize critical steps in purification workflows
- Quickly identify major discrepancies between protein batches

Storage and stability



A decrease in Δ ratio indicates protein denaturation during storage.

- Record unfolding profiles before and after storage
- Quantify profile similarity using the comparison function to monitor relative quality and consistency of preparations
- Identical unfolding profiles before and after test conditions suggest minimal or no impact of storage effects on the protein quality

Figure 7: Examples of similarity test applications.

Please note that different buffers can affect the fluorescence properties of a protein sample. We recommend to analyze proteins for similarity only when they are in the same buffer.

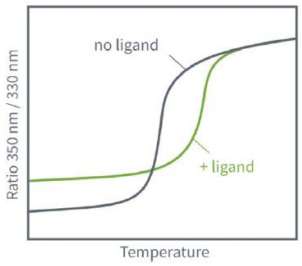
5.4.2. Functionality tests

Functionality tests can be performed when a ligand is available which is known to bind the protein of interest, for example a co-factor, co-enzyme, inhibitor, ATP, etc. When comparing the unfolding profile of the pure protein vs the protein mixed with the ligand, a change in the unfolding profile and its inflection temperatures (T_i) indicates that the two are binding. Typically, the initial ratio at the beginning of the experiment and/or the inflection temperature (T_i) of the protein are shifted upon interaction with a ligand, while the ratio value of the unfolded state is similar, unless the ligand itself fluoresces. In this case, it is recommended to use the single wavelength data to evaluate binding.

Thermal shift analysis allows conclusions about the functionality of the protein. Checking the functionality at intermediate steps of protein expression can help to identify critical steps and to make decisions about subsequent assays and their usefulness. Some examples of these intermediate steps are explained below and illustrated in Figure 8:

- After initial purification, knowing if the protein is expressed in sufficient quantities and behaving as expected helps to decide how to proceed.
- After chromatography (size exclusion, ion exchange, affinity…), different elution fractions can be assessed for best active protein content.
- Testing the final purified protein before storage allows to establish a reference of optimal integrity and functionality to compare against later stages, or future expression batches.
- Testing again after storage ensures that subsequent assays are only performed with active/functional protein.

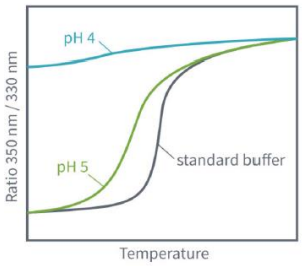
Folding and functionality



Loss of unfolding transition or loss of thermal shift during purification indicates protein denaturation.

- Perform during each step of purification processes to validate protein functionality
- Rapid yes/no answer on binding of ligands, substrates, ions or small molecules
- Shift in the initial ratio and/or shift in T_i (thermal shift) in presence of ligand indicates binding and functionality of the sample

Assay development



Optimize and determine buffers or formulations that better support protein stability. Loss or shift of T_i, or decrease in Δ ratio indicate destabilization and unfolding.

- Perform better assay development and optimization
- Rapidly screen buffers for assays or immobilization conditions, for example for biosensor experiments

Figure 8: Example of functionality test and assay development applications.

5.4.3. Assay development

Assay development can also benefit from Tycho NT.6 measurements. Find optimal experimental conditions and rule out those that destabilize the protein (Figure 8). For example, quickly test different immobilization and regeneration conditions before conducting an SPR experiment to save time and money. Another example is to test incubation conditions of a cell-based assay to ensure that the protein is functional throughout the assay.

5.5. Help features

The Tycho NT.6 user interface includes a help overlay feature which displays explanatory information for each screen. Tap the ? icon in the upper bar to display the overlay. Individual items on the screen are explained in brief (see Figure 9). Tap the ⊗ symbol (in the top left corner) or the bottom of the screen to close the overlay and return to the regular user interface.

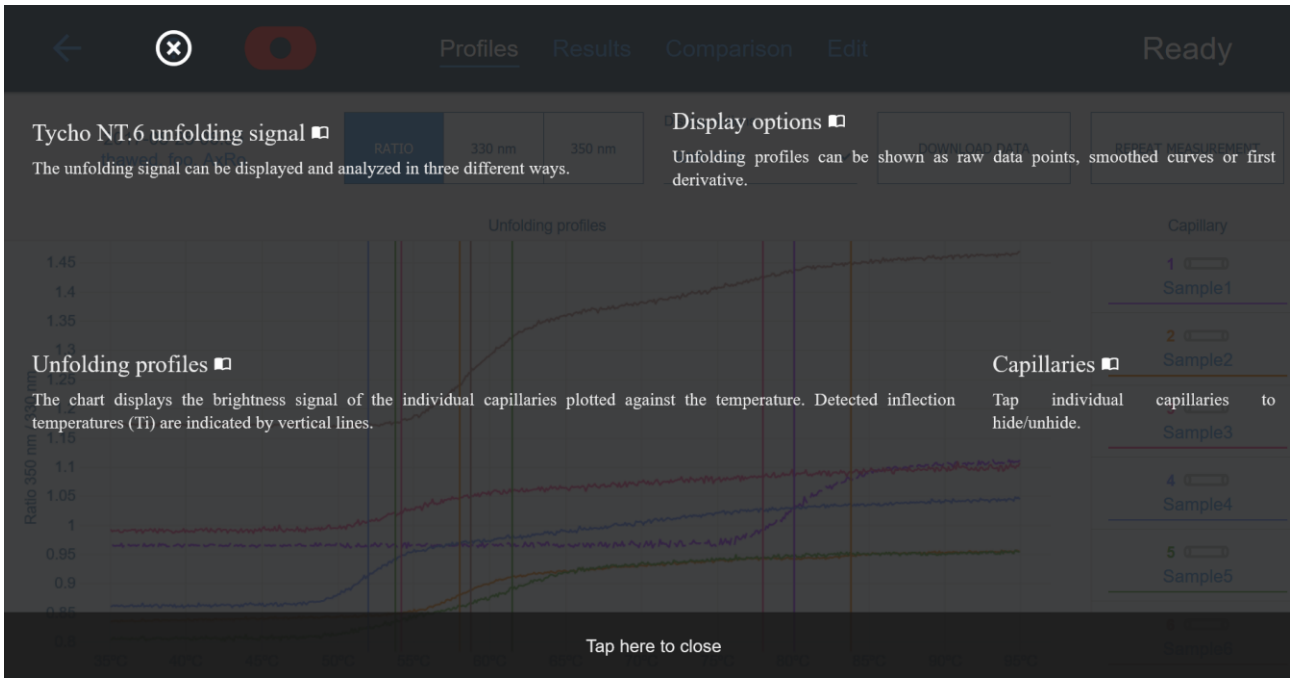


Figure 9: Overlay of the *Profiles* screen. Brief explanations are displayed for relevant topics. Each screen has its own, context-sensitive overlay. The book symbol indicates that more information on this topic is available in NanoPedia upon tapping.

For items labeled with a book symbol 🕮 more detailed information is available in the Tycho NT.6 NanoPedia knowledge base (see Figure 10). Tap such an item to open NanoPedia at the respective article.

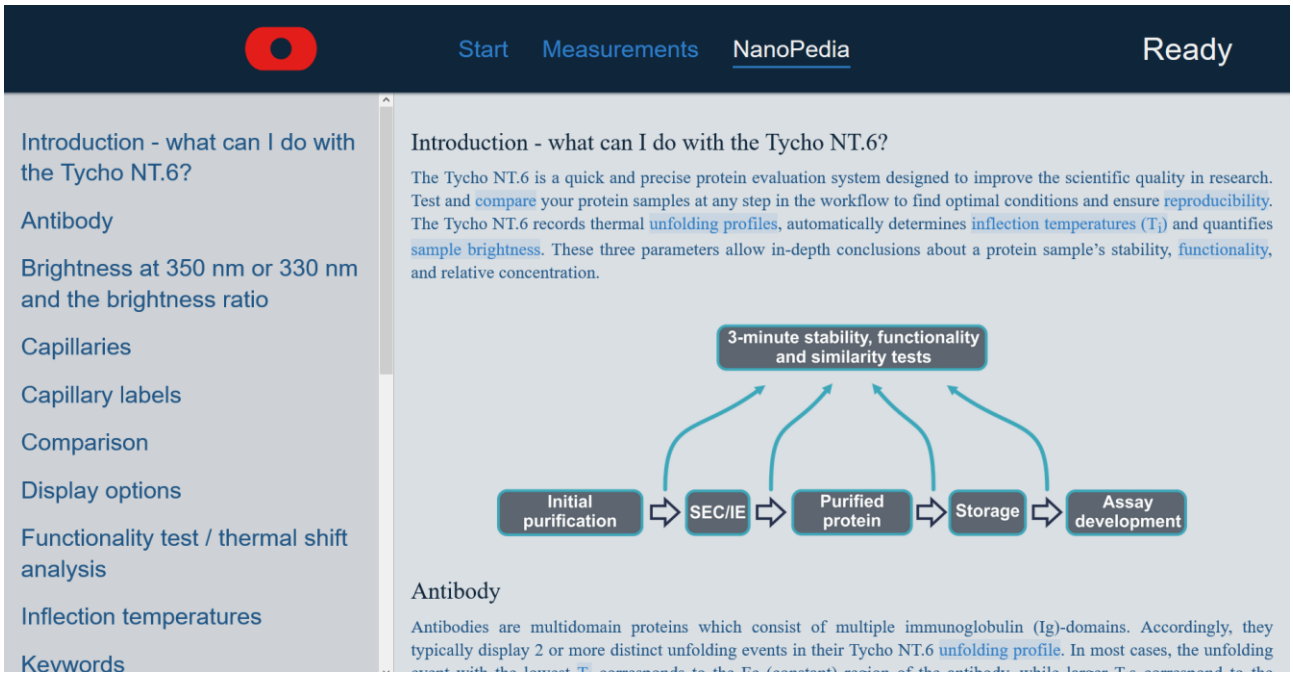


Figure 10: The Tycho NT.6 NanoPedia knowledge base. NanoPedia contains helpful background infromation on topics relevant to Tycho NT.6 measurements. Individual NanoPedia articles are sorted alphabetically. Words that link to other articles are highlighted blue. The navigation pane on the left side of the screen allows easy browsing.

NanoPedia contains more in-depth information on various topics relevant to Tycho NT.6 measurements. Individual NanoPedia articles are sorted alphabetically. Words that link to other articles are highlighted blue. The navigation pane on the left side of the screen allows easy browsing.

On the *Start* screen, the ⓘ icon is replaced by a ⓘ icon. Tap it to get information on NanoTemper Technologies customer support or on your Tycho NT.6 system (serial number, IP address etc.), or to change the time zone.

5.6. Running experiments

5.6.1. Tycho capillaries

Samples are loaded into single-use Tycho NT.6 capillaries. NanoTemper Technologies high-tech capillaries are designed for maximum ease-of-use and functionality. The high-purity glass and precise inner/outer diameters result in excellent sensitivity and data reproducibility. The capillaries are self-filling to reduce handling steps, and only require a few µL of sample. Up to 6 samples can be loaded per measurement.

5.6.2. Sample loading

Before running an experiment, make sure that the capillary tray (reflective surface) is dirt- and dust-free. Clean with a scratch- and dust-free tissue and 99.8 % ethanol.

Each sample is loaded into a capillary. Visual instructions on how to load samples can be found within the Tycho NT.6 user interface and in Figure 11. To access these instructions in the user interface, tap *New measurement* on the *Start* screen.

1. Open system door.
2. Open the magnetic lid of the capillary tray.
3. Clean the reflective surface of the capillary tray with a scratch- and dust-free tissue and 99.8 % ethanol prior to experiments. Keep the tray surface free from dust, dirt and scratches.

   CAUTION Hot surfaces can cause skin burns. Don't touch the thermal element (reflective silica surface of capillary tray) immediately after a measurement. Wait until the system has cooled down. Tray temperature is indicated on the display.
4. Load sample by dipping capillary into the liquid. Avoid liquid on the outside of the capillary. To do this, load from the surface of the sample instead of dipping the capillary far into the sample. Fill capillary completely. Don't touch the capillary in the center.
5. Place capillaries on capillary tray. Ensure that capillaries are inserted straight, not diagonally.
6. Close the magnetic lid.
7. Close the door. Tap *Start measurement*.

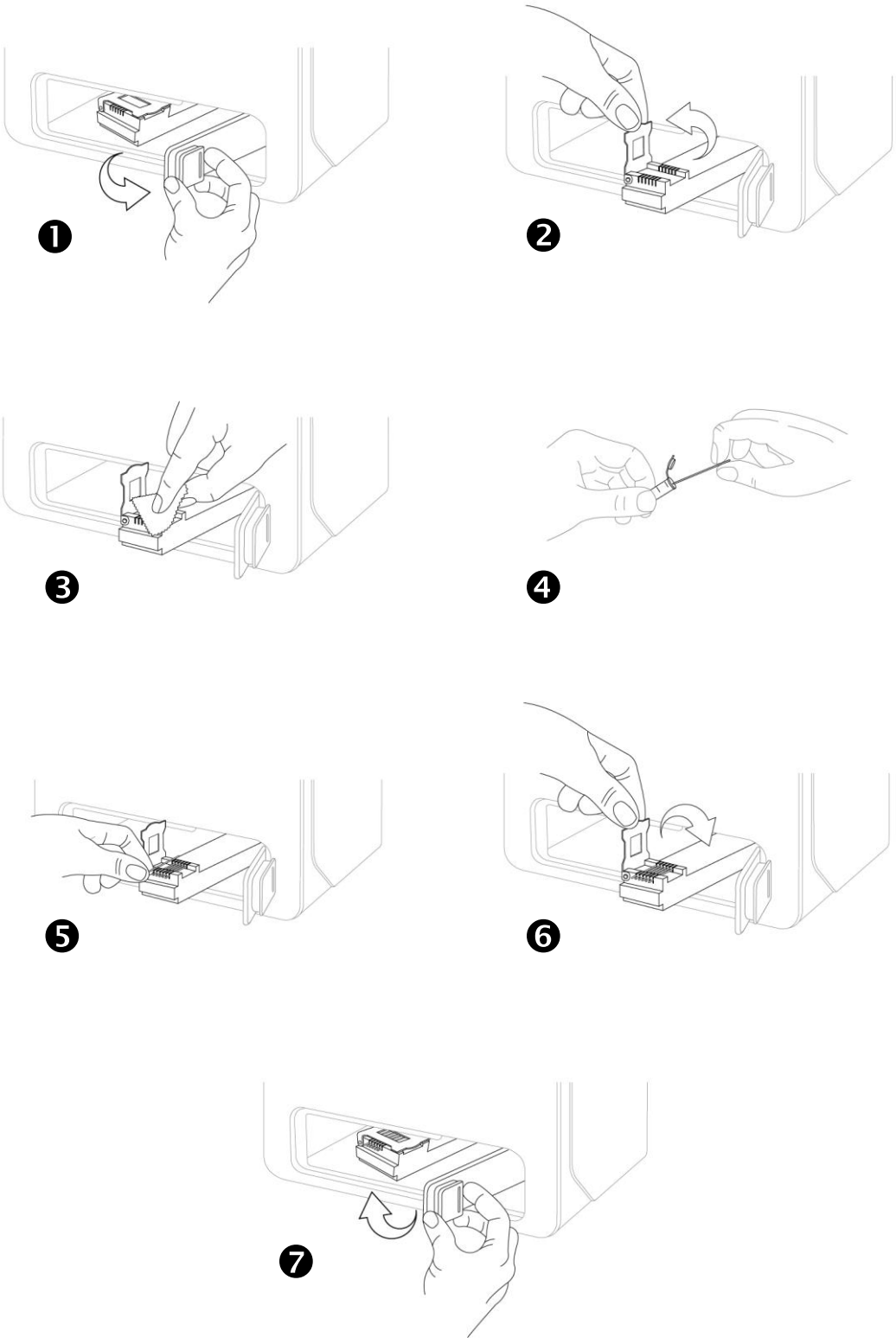


Figure 11: How to load samples into the Tycho NT.6 system. See text for a detailed description of the individual steps.

5.6.3. Measuring

Measurements can be started either from the *Start* view, or from inside a previous measurement by tapping *New measurement*. When starting a new measurement from inside a previous one, the user can decide to transfer annotations (capillary labels and keywords) and references to the new measurement. This is useful for replicate measurements, or other measurements with similar samples.

All measurements are run at a heating rate of 30 °C/min from 35 °C to 95 °C. While the measurement is running, data acquisition is displayed in real time. Annotation of samples or analysis of previously recorded data can be carried out in parallel.

To stop a measurement, open the door of the system.

CAUTION Hot surfaces can cause skin burns. Don't touch the thermal element (reflective silica surface of capillary tray) when hot. Wait until the system has cooled down. Tray temperature is indicated on the system display.

Tycho NT.6 automatically cools back down for the next measurement after a measurement is finished. Leave the door closed during the cooling period for fastest cooling. The display indicates the time remaining until the next measurement can be started. Cool-down takes approximately 3.5 min at an ambient temperature of 20-25 °C and with the door of the system closed.

Measurements can only be started if the temperature inside the system is below 34 °C. The door of the system can be opened at any time, but it is recommended to load new samples only after cool-down has completed to minimize sample warming.

5.7. Annotating measurements

For an instructional video on annotating measurements, see the USB memory stick delivered with your system.

A measurement can be annotated in two ways. Firstly, keywords can be added to the measurement itself. Suggestions for measurement keywords are: user initials, project abbreviation, protein name, measurement purposes like "batch comparison", "pH screen", "buffer test" etc. Each measurement can have an infinite number of keywords, each containing up to 100 characters. The date and time of the measurement are annotated automatically.

Secondly, each individual capillary measured can be labeled. A label can contain up to 40 characters. Typical examples of capillary labels are: protein name, concentration, batch number, buffer type, and specifics like "wild type", "untreated" etc.

Both keywords and labels are searchable. To search for a measurement, tap *Measurements* on the *Start* screen, or tap the magnifying glass symbol  when it appears in the top left corner of the screen.

Text can be copied and pasted when annotating keywords and capillary labels. Double-tap or long-tap a word to highlight it. Adjust the highlighted section as desired by moving the highlight handles. Tap *Copy* in the menu. To paste the copied text, long-tap where you would like to paste the text, then tap *Paste*.

5.8. Searching the measurement database

For an instructional video on searching the Tycho measurement database, see the USB memory stick delivered with your system.

The measurement database can be accessed by tapping *Measurements* or the magnifying glass symbol  at the top of the screen.

The database can be searched by (a combination of) the following:

- Measurement keywords or capillary labels (see 5.7): simply type in your search term. Only measurements containing the search term will be displayed in the results list.
- Time of measurement: tap *Select dates* in the top left corner, then use the sliders to narrow down the time frame to the desired time period/day. Only measurements from the selected time frame will be displayed in the results list.

Tap on any measurement in the results list to view or edit it. Use the magnifying glass symbol  again to return to the filtered results list.

To again display the complete list of measurements (without filters), delete any search terms and time frame selections.

The Tycho measurement database can hold approximately 30 000 measurements.

6. Results and data evaluation

6.1.1. Unfolding profiles and display options

The unfolding of a protein causes changes in the fluorescence emission properties of its fluorescent amino acid residues (tryptophan and tyrosine, mainly). Tycho NT.6 follows the unfolding process by monitoring sample fluorescence (brightness) at 330 nm and 350 nm during heating. The brightness ratio 350 nm / 330 nm is plotted against the temperature in the sample, and the resulting graph is called the unfolding profile (see Figure 12 for an illustration and details). Inflection temperatures (T_i) are calculated automatically. Unfolding profiles can be analyzed and displayed in different ways. The profiles can serve as a fingerprint of the protein, along with the brightness ratio, Δ ratio and inflection temperatures (T_i). Comparison of those parameters allows conclusions on the similarities between the analyzed samples. Please note that different buffers can affect the fluorescence properties of a protein sample. We recommend to analyze proteins for similarity only when they are in the same buffer.

In some cases, it can be preferable to display raw data points for seeing the most detail, or to display smoothed curves to get a better overview. The first derivative of the smoothed curve is an alternative display option for easier visualization of the data. In the first derivative, unfolding events are visible as peaks (maxima and minima) instead of inflections, which are easier to identify visually.

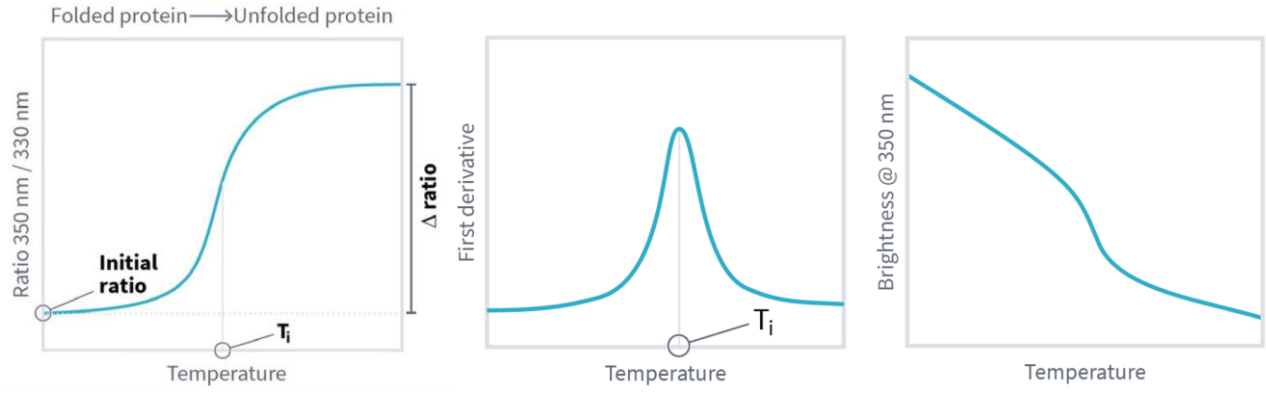


Figure 12: A typical unfolding profile displayed in ratio mode (left), first derivative of the ratio mode (center), and single wavelength detection mode at 350 nm (right). The inflection temperature T_i, (initial) ratio and Δ ratio are also indicated.

In addition to the ratio 350 nm / 330 nm, Tycho NT.6 also allows to inspect the single wavelength brightness signals. In the single wavelength detection (330 nm or 350 nm), we look at the change in brightness at a defined wavelength over temperature. The ratio 350 nm / 330 nm however is a measure for a spectral shift in the fluorescence emission profile of the Tryptophan (Trp) residues. This shift occurs in most unfolding events and is caused by the environmental change that the Trp residues undergo upon unfolding of the protein: In the folded state, Tryptophan residues (Trp) are often buried in the hydrophobic core of a protein and become surface-exposed during unfolding.

Since the ratio 350 nm / 330 nm typically obliterates effects of autofluorescent additives and general fluorescence decay with increasing temperature, this detection mode is typically more robust than single wavelength detection. Therefore, clear inflection temperatures (T_i) can be derived which might not be

visible in the single wavelength detection modes. Vice versa, it is also possible that unfolding events which do not trigger an emission peak shift are visible in the single wavelength data, but not in the ratio. It is therefore recommended to inspect all the obtained data, as important structural information might be obtained by comparing the unfolding profiles in the three analysis modes.

6.1.2. The ratio

The initial value of the ratio 350 nm / 330 nm (at 35 °C) contains valuable information. It mainly depends on the amount of tryptophan (Trp) residues and especially their location within the protein. Typical protein ratios range from 0.35 (all Trp residues buried) to 1.4 (all Trp residues solvent-exposed). A shift of the initial ratio e.g. after protein storage can therefore indicate a change in the amount of unfolded protein. A very low starting value (typically < 0.35) can be found for proteins without Trp residues.

Δ ratio is a valuable quality parameter for comparison with a reference. It describes the difference of the ratio at 35°C and 95°C. These values can also be used as convenient indicators of folded protein content: When comparing e.g. different batches of the same protein, a larger Δ ratio value indicates a higher folded protein content. Conversely, a small Δ ratio indicates a low folded protein content. Note that the absolute Δ ratio value is specific to each protein. It only allows to evaluate folded protein content relative to a reference sample of the same protein.

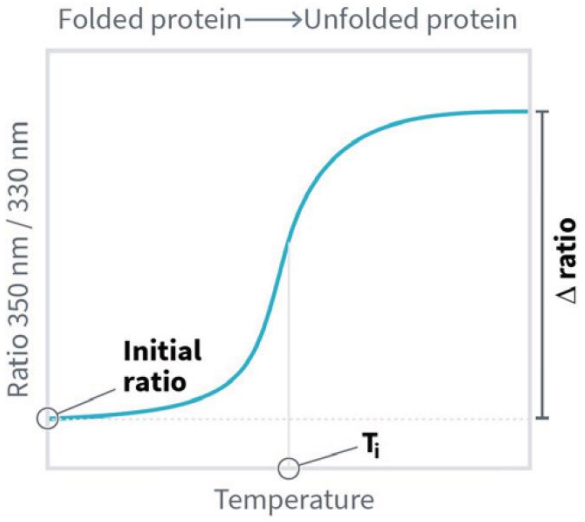


Figure 13: Typical unfolding profile illustrating ratio, Δ ratio and T_i.

6.1.3. Inflection temperatures (T_i)

Each inflection temperature (T_i) represents an unfolding event detected in the sample. Inflection temperatures are calculated automatically for the ratio signal and correspond to inflection points of the profile curve. Detected T_is are represented as vertical lines in unfolding profiles and results tables (see Figure 13). In results tables, the temperature at which the inflection occurs is also listed.

Tryptophan (and tyrosine) fluorescence is very sensitive to its environment, and this environment changes drastically upon unfolding. In a folded protein, the fluorescent residues are typically located in

its hydrophobic core. Upon unfolding, the residues come into contact with the (usually aqueous) buffer. This leads to a change in sample fluorescence regarding emission intensity and emission peak wavelength which appears in unfolding profiles as an inflection.

If more than one T_i is detected for a protein, this means that the protein has several domains that unfold more or less independently.

If no T_i is detected in a sample, this can have the following reasons:

- The protein in the sample did not unfold, for example because it was already unfolded.
- The unfolding event did not induce a detectable change in intrinsic fluorescence (rare).

6.1.4. Sample brightness

The relative, total fluorescence intensity of the sample is quantified as sample brightness. Tycho NT.6 systems are calibrated using a defined reference sample, which yields a sample brightness of 1. The sample brightness is independent of excitation power and therefore serves as a measure to quantitatively compare fluorescence intensities of different samples. If the samples contain identical proteins, e.g. when comparing different batches, the sample brightness can also be used to exactly determine the differences in protein concentration. Please note that different buffers can affect the fluorescence properties of a protein sample. We recommend analyzing proteins for similarity only when they are in the same buffer.

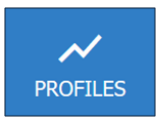
The sample brightness is the sum of brightness at 350 nm and brightness at 330 nm.

6.2. Comparing and referencing

Tycho NT.6 allows the comparison of unfolding profiles from different measurements. For an instructional video on comparing and referencing, see the USB memory stick delivered with your system.

Starting from the measurement containing the capillary you want to compare, tap *Comparison*. Select the capillary(s) to compare on the left, then select the reference to compare against on the right. Search for reference capillaries using measurement keywords or capillary labels. Search results can be sorted by newest first, popularity, or capillary label similarity. Repeat for all capillaries you want to compare. When finished, tap *Save and show*. The comparison as shown on the screen will be saved along with the measurement.

A comparison can be viewed from different angles: looking at the profiles, the inflection temperatures T_i, or the data (a summary of the samples' initial ratio, Δ ratio, sample brightness, and profile similarity).



The *Profiles* tab shows the unfolding profiles. Capillaries to be shown can be selected on the left. The colored profile belongs to the capillary(s) to be compared, while the gray profile belongs to the reference. Vertical lines indicate inflection temperatures (T_i).

The *T_i* tab shows the inflection temperatures (T_i) determined for all capillaries in the comparison, arranged by temperature. The colored numbers refer to the capillary(s) to be compared, while the gray numbers refer to the references.

The *Data* tab lists the the samples' initial ratio, Δ ratio, and sample brightness. For all parameters, the colored number refers to the capillary(s) to be compared, while the gray number refers to the reference. Finally, the unfolding profiles of the compared capillaries are analyzed for their similarity by calculating the difference in area between the profile curves. This results in a percentage value, with perfect similarity being 100 %.

Comparison data can be exported by tapping *Export to USB* (on the system) or clicking *Download data* (when accessing remotely). Comparison data is always exported along with the measurement containing the compared capillaries (not the measurement(s) containing the reference(s)).

A comparison will be saved automatically within a measurement file and is accessible upon re-opening of the measurement file. To overwrite a previously saved comparison, tap *Select references*. Next, simply select the capillary(s) to be compared, then the reference, as described above. It is not necessary to delete existing selections before overwriting.

6.3. Data export

Data can be exported directly at the system or remotely.
To export directly from the system, connect a USB memory stick at the front of the system and tap *Export to USB.* The USB memory stick needs to be a single FAT32 partition.
To export via remote access, click *Download data* and choose a location for saving the file.

All data is exported into a .zip file named after the timestamp of the measurement. The .zip file contains various formats sorted into subfolders, including raw data and profile images. More details are explained in the following sections.

If the exported measurement contains a comparison, all comparison data is exported as well. All files and Excel worksheets related to the comparison will feature the word "comparison" in the name.

6.3.1. Raw and processed data

The *raw and processed data* subfolder includes a Microsoft Excel spreadsheet file (.xlsx) that contains all of the measured data points, brightness, ratio and calculated T_i data along with the measurement keywords and capillary labels.

This Excel file comes with document properties that allow convenient handling of multiple files in Windows Explorer. To show these properties in Windows Explorer, navigate to the *View* tab and select the "Details" view. In the same tab, click on *Columns* and then on *Add Columns*. The following document properties are available:

Document property name	Information contained
Tags	All measurement keywords applied by the user (see 5.7)
Title	Serial number and software version of the Tycho system used to run the measurement
Authors	Serial number and software version of the Tycho system used to export the data

Of course these document properties can also be accessed by all available routes (left-clicking on a file in Windows Explorer and selecing *Properties*; from within Excel; etc.).

The .xlsx file is optimized for Microsoft Excel 2016. Other spreadsheet software can be used as well. Some of the functionalities described below may only work in Microsoft Excel 2016.

The information in the Excel file is arranged on worksheets. Pre-prepared charts are included in the spreadsheet. Modify the charts according to your needs by tweaking colors, fonts, sizes etc. Remove or add data rows by simply deleting or copy-pasting: selecting a chart will highlight the table cells containing the plotted data. Modify/drag the highlighted cells to add or remove data from the chart.

Ratio profile charts (raw, smoothed and first derivative) include the determined T_i as dots. To display the T_i temperature values in the chart, right-click on the T_i dots and enable data labels. Right-click again to format data labels to display x-values (temperatures) instead of y-values. Move labels by clicking to select an individual label, then dragging it to the desired position.

All worksheets have a pre-defined print layout to enable convenient one-page printing. If a chart is selected while printing, the chart will be printed in full-page format. Select the correct page orientation to match the chart's aspect ratio.

In addition to the spreadsheet, the raw and processed data folder also includes database files (.json format) for remote diagnostics by NanoTemper Technologies. They can also be used for automated data processing. If you'd like to learn more about this, please contact NanoTemper Technologies customer support.

6.3.2. Profile images

This folder contains all profile images of the measurement sorted into subfolders: raw and smoothed profiles and first derivative views of ratio, 330 nm and 350 nm. If the measurement contains comparisons, comparison profiles are also included in the respective subfolders.

Note that the exported image files (.svg and .png) will show exactly what is selected in the Tycho NT.6 user interface. Hidden capillaries will not be shown in the exported images, but will of course be included in the .xlsx files.

7. Troubleshooting

7.1. Broken capillaries

In case of broken capillaries on the capillary tray, use a paintbrush or tissue to carefully remove pieces of glass and to avoid scratching the tray.

CAUTION Broken glass can cut skin. Do not touch glass fragments. Dispose of capillaries according to the locally applicable regulations concerning glass waste.

7.2. Stopping measurements

To stop a running measurement, simply open the door of the system. Opening the door completely will immediately stop the measurement and start cool-down. All data collected before the interruption can be viewed as usual.

Interrupted measurements cannot be resumed. A new measurement can be started after cool-down.

Please be aware that the capillary tray may still be warm if a measurement or cool-down is interrupted. It is recommended to load samples only after cool-down is complete to avoid warming the samples.

CAUTION Hot surfaces can cause skin burns. Don't touch the thermal element (reflective silica surface of capillary tray) immediately after opening the system door. Wait until the system has cooled down. Tray temperature is indicated on the system display.

If the door is opened only partially, the measurement may continue, but any resulting data will be of poor quality due to incoming light. Please either open the door completely (to stop a measurement) or keep it closed (to measure).

7.3. Opening the door of the Tycho NT.6 system

The door of Tycho NT.6 can be opened at any time: in idle mode, during a measurement, or during cool-down. Opening the door is the way to stop a running measurement (see 7.2).

If the door is opened only partially, the measurement may continue, but any resulting data will be of poor quality due to incoming light. Please either open the door completely (to stop a measurement) or keep it closed (to measure).

Please be aware that the capillary tray may still be warm if a measurement or cool-down is interrupted. It is recommended to load samples only after cool-down is complete to avoid warming the samples.

7.4. Restarting Tycho NT.6

In case the system freezes, wait one minute. If it does not un-freeze, disconnect the power supply at the back of the system. Wait 30 seconds for complete shutdown, then reconnect the power supply. The system will start up again automatically.

7.5. Customer support

In case of any issues not described in this user manual, please visit the NanoTemper Technologies Explorer Community at nanotempertech.com/explorer. In the Explorer Community, you can find user manuals, FAQs, application notes and more supporting material, and you can get in touch with NanoTemper Technologies customer support staff.

8. Transport and disposal

8.1. Repackaging for transport

Remove all capillaries from the system before transport.

Always re-use the original packaging for transport. If the original packaging was discarded, please contact NanoTemper Technologies for a replacement.

Make sure to secure the sample-holding arm to the door with the provided transportation lock band (red hook-and-loop fastener) (see Figure 14).

Figure 14: Use the red hook-and-loop fastener to secure the sample-holding arm to the door.

8.2. Waste disposal

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

8.3. 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 has to be disposed of according to locally applicable regulations regarding electrical and electronic equipment. The symbol is positioned at the back of the device.

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