User Manual
PR.ChemControl Software

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NanoTemper® Technologies' Prometheus™ instruments detect changes in the fluorescence emission of fluorescent amino acids upon unfolding using nanoDSF™, an advanced Differential Scanning Fluorimetry technology. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures. Furthermore, the instruments are equipped to investigate chemical unfolding and the free energy of unfolding ΔG in an extraordinarily straightforward and fast manner.

The PR.ChemControl software is dedicated to running and analyzing chemical unfolding (and optionally also refolding) experiments on Prometheus instruments.

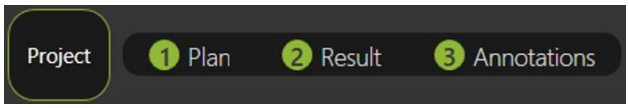
1. System Requirements

If the necessary licenses have been purchased, PR.ChemControl software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

Operating system:	Windows 7 64 Bit or higher
CPU:	Intel Core i5 or better
RAM:	8 GB or more
Hard disk:	20 GB or more free disk space available
Display resolution:	1600 x 900 or better
Software:	Microsoft .NET 4.6.2 framework (included in installer of PR.ChemControl software)
Operating system language:	English or German

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

2. Home Screen and General Usage



To perform a new measurement, start the PR.ChemControl software. Choose *Start New Session* or *Browse Previous Session*. If you start a new session, you will be prompted for a file name and location. The file will be saved in .prcc format. Alternatively, previous sessions can be loaded to analyze previous experiments or to add additional measurements. When you go back to the *Project* home screen later, you can enter more information on your project.

Next, select the size of the dilution series. Choose between 24 or 48 capillaries.

Use the *Save* button in the *Project Overview* panel on the left side at any time to save modifications of the file. An asterisk in the software title bar indicates unsaved changes in the open file. Closing the software will trigger a dialogue box asking whether you want to save the changes.

All experiments included in the current file are displayed in the *Project Overview* panel. Click on any of them to navigate to the corresponding experiment. Click the pen icon to change the displayed description.

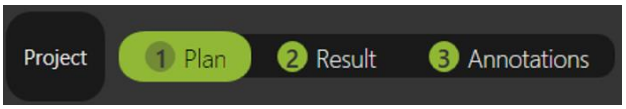
The keyboard shortcut ctrl + z will undo any action, while ctrl + y will redo.

Three tabs guide the user through running and analyzing chemical unfolding measurements:

1. Plan
2. Result
3. Annotations

Proceed from 1 to 3 to set up, run and analyze a chemical unfolding measurement. More details on each tab follow below.

3. Plan



In the *Plan* page, enter the experimental settings and conditions. When using a Prometheus NT.48, choose the number of samples in your assay (24 or 48). When using a Prometheus NT.Plex, an assay always consists of 24 samples.

Required fields are shaded green. Enter the highest concentration of denaturant (*Start Concentration*) in the experiment. Next, provide either a *Delta Dilution* (the increments of denaturant concentration steps in the experiment) or an *End Concentration*, which the software will use to calculate the increments. Load the samples, placing the capillary with the highest denaturant concentration in position 1.

For details on sample loading and capillary types, please refer to the Prometheus series instruments manual.

By default, chemical unfolding measurements are carried out at 20 °C. The target temperature can be changed either via the touch display of the Prometheus instrument or in the top left-hand corner of the software. The available temperature range is 15 °C – 60 °C.

Note: To run a measurement at a target temperature other than 20 °C, set the desired temperature, then wait until it is reached before you start the measurement.

Click *Start Measurement*. Suitable excitation power settings are automatically determined. The measurement and the analysis are subsequently performed automatically, and the software moves to the *Result* page.

Note: *It is recommended to check the fluorescence signal intensity of the sample before you prepare the assay. The fluorescence of any given protein depends on its number and position of fluorescent amino acids, and is therefore difficult to predict. Prepare one sample containing the protein at a concentration and in a buffer you are planning to use in the experiment and*

perform a fast thermal unfolding run using the PR.ThermControl software. This will give a preview of the fluorescence intensity of both the folded and the unfolded state. It is important to ensure that the fluorescence signal is within the range of the detector, and that the fluorescence ratio (350 nm/330 nm) yields a good unfolding signature. Please refer to the PR.ThermControl manual for details.

4. Result

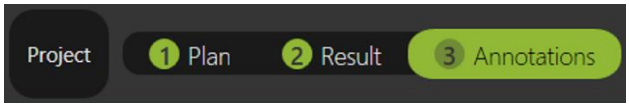
The fluorescence ratio (350 nm/330 nm) is plotted against the denaturant concentration for each capillary (blue dots). A fitting routine (green line) is automatically performed which returns two parameters displayed on the bottom left: ΔG for the unfolding reaction in pure buffer and c50 (also known as C_m), the concentration of denaturant at which 50 % of the protein is unfolded.

In addition to the fluorescence ratio, the values measured at the single wavelengths (330 nm and 350 nm) can be displayed by choosing the respective detectors. Using the fluorescence ratio signal is preferred because it is robust against pipetting errors or other irregularities. If the instrument is equipped with the Aggregation Optics, scattering data can also be accessed.

Choose the applicable fitting model depending on the investigated protein. The two-state model is selected by default. Use the two-state fit for proteins that exhibit one unfolding transition and the three-state fit for proteins that exhibit two transitions.

The graph can be exported by clicking the *Export* button. Options are to copy the graph to the clipboard, to save it as an image (.png or .svg file formats), or to export the raw data needed to recreate the graph in third-party software (.xlsx file format).

5. Annotations



Annotations for each capillary can be entered before or after the experiment. The annotations table by default contains a Capillary column and a Denaturant Concentration column, both of which are entered automatically. Columns can be added or removed by using the *Create New Column* and *Remove Column* buttons on the right side of the screen:

Annotations can either be entered by simple copy-and-paste from a spreadsheet software like Excel™ or manually. Annotations can be entered into multiple fields simultaneously after multi-selection, and subsequently sorted in ascending or descending manner by left-clicking on the column header:

Annotation tables can be exported in Excel™format (.xlsx).

6. Data Export

Each measurement and analysis performed can be exported to be used in third-party software. Throughout this software manual, different export options are mentioned. This section aims to give an overview regarding the file format and the content of each export.

6.1. Result

The Result tab contains three export options:

Clicking on *Export Raw Data* will create an Excel™ file (.xlsx) containing two sheets. The first sheet displays the results from the fitting procedure chosen, whereas the second sheet contains all the measured values and the annotations related to the sample.

The *Copy Chart to Clipboard* button will copy the graph displayed so you can paste it into another software.

The *Export Graph* button will create an image file (.png or .svg) of the graph.

6.2. Annotations

The export option is located at the bottom right of the screen. Clicking on it will save the annotation table as an Excel™ file at the location of your choice.

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