Biophysics and
Biochemistry
Quantifier and qualifier

User manual

1 TABLE OF CONTENTS

2 INTRODUCTION

2.1 PURPOSE

This program was built for the Biophysics and Biochemistry Group of the Centre for Medicines Discovery (CMD), University of Oxford, formerly the Oxford branch of the Structural Genomics Consortium (SGC). As such, input and output are geared towards the equipment used and assays performed there, and the LIMS system used there.

The application is written in Python, packaged as a MS Windows executable with auto-py-to-gui and distributed as an installer compiled with Inno Setup Compiler

2.2 USER INTERFACE

The user interface is modelled after the design aesthetic/philosophy of current MS Office applications (on Windows 10).

Up to four assays can be pinned on the top half of home screen. To access the menu to pin assays, click on the pin symbol next to the “Pinned assays” heading.

Up to ten recent projects are shown on the bottom half of the home screen. As in MS Office applications, this list is automatically updated as projects are saved and opened.

Help button opens this user manual.

Version number, build date- and timestamp are displayed in the bottom left hand corner.

2.3 COLOUR SCHEME

For all plots, except heat maps, Dutch scientist Paul Tol’s muted colour scheme is used[click me].

	Colour	R	G	B	Hex

Indigo		51	34	136	#332288
Blue		68	119	170	#4477AA
Cyan		136	204	238	#88CCEE
Teal		68	180	153	#44B499
Green		17	119	51	#117733
Olive		153	153	51	#999933
Sand		201	204	119	#DDCC77
Rose		204	102	119	#CC6677
Wine		136	34	85	#882255
Purple		170	68	153	#AA4499
Pale grey		221	221	221	#DDDDDD

2.4 TYPES OF ASSAYS CURRENTLY SUPPORTED:

At present, all assays are tied to specific file formats, so do ensure that the files are formatted properly.

2.4.1 Endpoint assays (dose response/IC50 and single concentration/single shot)

Endpoint assays are non-continuous assays where an end state of an enzymatic reaction or an equilibrium state of a protein-protein, protein-peptide or protein-fluorescent tracer interaction (PPI) are measured.

Raw data files are saved by the BMG Labtech Pherastar plate reader's control software as plain text files, tab delimited, with the file extension set to ".xls". If you ever need to modify a raw data file, ensure you save it as "Text (tab delimited) (*.txt)" in Excel. After saving, change the file extension back to ".xls".

Plate formats: 96, 384, 1536

Assay types/formats/technologies:

- HTRF
- AlphaScreen (AS)
- Fluorescence Polarisation (FP)
- XMP/XDP Glo (Glo)

These are the types/formats/technologies available from the "Assay Type" selection list. For dose response/IC50 type assays, the data files need to be in a plate grid/array format. The program will parse the file and look for keywords, depending on the assay, before reading the raw data into memory. Single dose assays require a file in list format.

PPI assays (HTRF, AS and FP) are stored in the "AlphaScreen" table in the SGC/CMD SCARAB database. Glo assays are stored in the "Activity assay" table and thus the export tables differ.

2.4.2 DSF

Raw data files from the Roche Lightcycler480 are plain text files. When conducting an experiment, ensure that the sampling rate (number of data points) is not too great. Files sizes in excess of 10MB are easily achieved.

Files from the Agilent mx3005p are Excel files.

2.4.3 nanoDSF

Raw data files from the NanoTemper Prometheus are Excel files.

2.4.4 Enzymatic/Kinetic

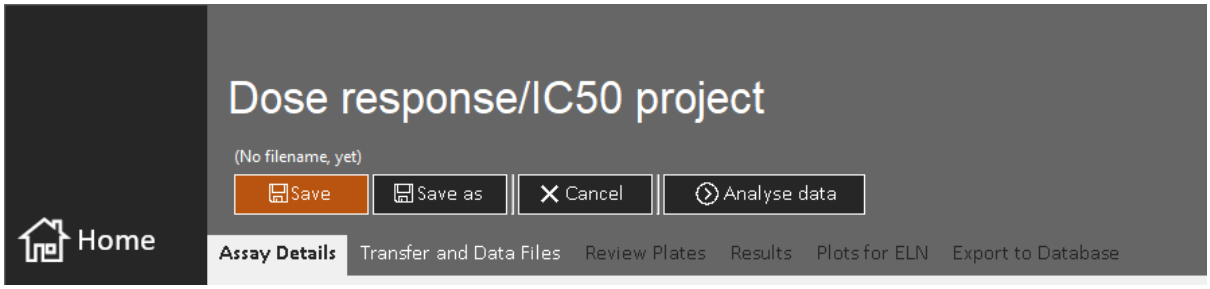
Kinetic assays work with the same file format as endpoint assays (assuming BMG Labtech Pherastar type plate reader). However, here we have one set of plate data per cycle/timepoint measured.

3 ASSAY ANALYSES

3.1 COMMON CONTROLS FOR ALL ASSAY ANALYSES:

To the top of the window you will see the type of analysis being carried out. If the file has been previously saved, the file name will be shown below.

Below the file name (or placeholder text) is a row of buttons. You can either “Save” or “Save as” as with any other standard application. “Cancel” will cancel the analysis. “Analyse Data” will perform the analysis, provided all necessary information and data have been entered. “Previous” and “Next” will switch between the tabs of each analysis.



3.2 ENDPOINT ASSAYS

For both dose response and single concentration assays, there are 6(5) tabs corresponding to each step of the analysis:

1. Assay Details
2. Transfer and Data Files
3. Review Plates
4. Results
5. Plots for ELN (N/A for single concentration)
6. Export Results to SCARAB

3.2.1 Assay Details

There are six sections to be filled out:

1. Assay Type:
   The selection of the assay type determines the keywords the program is looking for when parsing the raw data file, and the method of data normalization that is applied (e.g. whether loss or gain of signal corresponds to inhibition)
2. Protein/Enzyme
   CMD/SGC purification ID and concentration of the protein/enzyme being used
3. Peptide/Substrate:
   CMD/SGC compound ID of the peptide (protein-peptide interaction assays) or substrate (enzymatic assays) used
4. ELN Page:
   Electronic Laboratory Notebook page number of the experiment in the SCARAB LIMS.
5. Buffer:
   Composition of the assay buffer.
6. Solvent:
   Which solvent was used and at which concentration (per-cent, volume/volume). At present only one solvent can be accommodated per experiment.

3.2.2 Transfer and Data Files

In this tab, the user can select the transfer file from the Echo liquid handler and the corresponding raw data files:

1. To select a **Transfer File**, click on the 🗁 button in the “Select a transfer file” panel on the left-hand side of the tab. If the selected .csv file is a valid Echo transfer file, the destination plates produced in the Echo transfer run will be listed in the table below.
2. Depending on the assay type, either
   a. all files with the correct file extension (e.g., “.xls”) in the same directory as the transfer file will be displayed in the data file table, or
   b. you will have to open a data file via the […] button and the plate entries contained therein will be displayed in the data file table.
3. Once all files are loaded, you can assign raw data files to transfer file entries. You can either
   a. Drag files from the raw data file table to an entry in the transfer file entry table or
   b. select entries on the transfer file table, select corresponding entries in the raw data file table and assign via the [<<] button. You can select multiple entries in each table, and they will be assigned sequentially. If you select more entries on one side, they will not be assigned. Raw data files can be un-assigned from transfer file table using the [>>] button.

4. If it is necessary due to strong systematic effects on a plate (e.g., edge effects), you have the option to load a reference plate (e.g., an assay plate with no inhibitors and only DMSO additions) to normalize your sample plates against. This will simply divide the signal of the sample plates by the signal of the reference plate, well by well.

3.2.3 Review Plates

This tab contains a table with all sample plates and a heatmap plot representing the assay plate. Clicking on a plate in the table will update the heatmap plot. Also displayed are the average values (± SEM) for no addition wells (assay reagents only, 'Buffer'), DMSO backfill only wells ('DMSO') and control compound wells.

In case of single concentration screens, the user has the option to switch between the heatmap plot and a scatter plot showing the average per-cent inhibition value (with SEM over replicates shown as error bars). Samples with > 60 per-cent inhibition are highlighted. An option to change the threshold (e.g. set percentage value or within 3σ of control compounds will be added in later versions.

3.2.4 Results

3.2.4.1 Dose response

This tab contains a list of all samples with their IC_{50} values (± SEM of fit); a plot of and individual data set with the f curve and a list of the parameters of the curve fit (IC_{50}, Hill slope, top, bottom, R^2); or a summary plot with up to eight data sets.

For the individual plot, you can choose whether to show

i. raw data with an unconstrained sigmoidal curve fitted,
ii. normalised data with an unconstrained sigmoidal curve fitted, or
iii. normalised data with a constrained sigmoidal curve fitted (The following constraints apply: Top 100 ± 20 per-cent inhibition, bottom 0 ± 20 per-cent inhibition.);

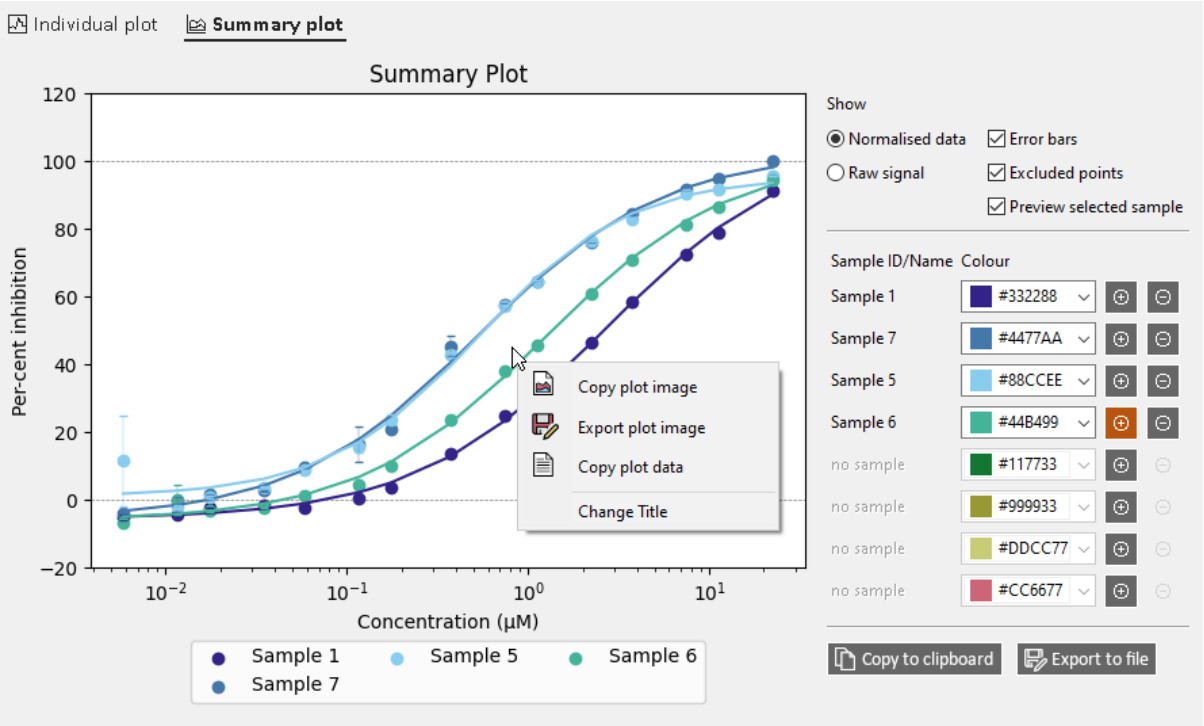
and whether excluded data points and error bars should be shown.

To exclude or re-include a data point, simply click on it. The curve fit will then automatically be updated. You can also choose whether to fit the data. This will normally be automatically determined based on a set of conditions for the normalised data set:

i. Maximum value >= 60 %
ii. Minimum value <= 40 %
iii. At least five data points with an SEM < 20%

The plot can be either copied to the clipboard, to be pasted into other applications, or saved as a PNG image file. There is also an option to export all plots as PNG images at once.

For the summary plot, you can add/remove data sets by selectin the sample in the sample list, and clicking on the ⊕ button on the right hand side of the plot's sample list. To remove a data set, click the ⊖ button. You can also choose between 9 different colour options.



The plots can be either copied to the clipboard to paste into other applications, saved as a PNG image file, or the data can be copied to clipboard. The title of the summary graph can be changed as well.

3.2.4.2 Single dose

For single dose experiments, this tab will contain a scatter plot with the per-cent inhibition values of each sample over all the plates analysed. See review plates section[click me].

3.2.5 Plots for ELN

Data series for each plate are plotted here. These can either be copied to the clipboard or saved as PNG image files.

N/A for single dose experiments.

3.2.6 Export Results to Database

Results table for the SGC/CMD's SCARAB database, which can be exported as a CSV file or copied to clipboard. Options to choose results tables for different databases might be added in later versions.

3.3 Continuous Assay: Differential Scanning Fluorimetry

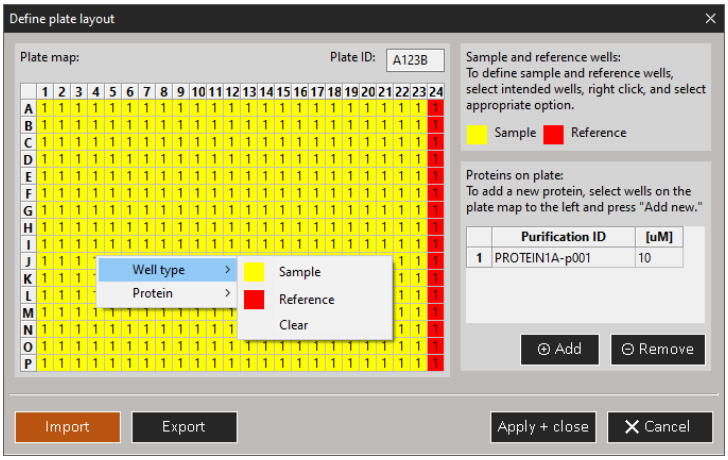
For DSF assays, there are 7 tabs corresponding to each step of the analysis:

1. Assay Details
2. Transfer and Data Files
3. Review Plates
4. Results
5. Plots for ELN
6. Export Results to Database
7. Plate map for Database

3.3.1 Assay Details

There are six sections to be filled out:

1. Device and plate format:
   This determines how the raw data files are parsed. Each manufacturer has their own file format, so this is critical for the analysis.
2. Obtain sample IDs:
   Sample IDs can be either obtained from an Echo transfer file or the data file (in the case of the Roche Lightcycler). Alternatively, the well designation can be used.
3. Plate layout(s):
   Since more than one protein can be used on a plate (e.g. a selectivity screen for one compound against a panel of proteins), a plate layout will have to be defined for each plate. You can either define a global layout or individual layouts for each plate. In the latter case, you first need to load a transfer file so that the number of plates analysed is known.
   Clicking on "Edit plate layout" will open the "Define plate layout" window. Here, you can select individual or groups of wells and assign them as either sample or reference wells and assign proteins to them. On the left hand side, you can add and remove proteins, and change the protein ID and concentration (in µM). If no reference wells have been specified, the analysis will be performed but a ΔT_m value will not be calculated.
   Plate layout files can be exported and imported for ease of use.



3.3.2 Transfer and Data Files

In this tab, the user can select the transfer file from the Echo liquid handler and the corresponding raw data files:

1. To select a **Transfer File**, click on the button in the "Select a transfer file" panel on the left-hand side of the tab. If the selected .csv file is a valid Echo transfer file, the destination plates produced in the Echo transfer run will be listed in the table below.

2. All files with the correct file extension (e.g., ".xls") in the same directory as the transfer file will be displayed in the data file table.
3. Once all files are loaded, you can assign raw data files to transfer file entries. You can either
    a. drag files from the raw data file table to an entry in the transfer file entry table or
    b. select entries on the transfer file table, select corresponding entries in the raw data file table and assign via the [>>] button. You can select multiple entries in each table, and they will be assigned sequentially. If you select more entries on one side, they will not be assigned. Raw data files can be un-assigned from transfer file table using the [<<] button.
4. If you chose individual plate layouts, click the edit button to launch the "Define plate layout" window. See Assay Details section for detailsclick me.

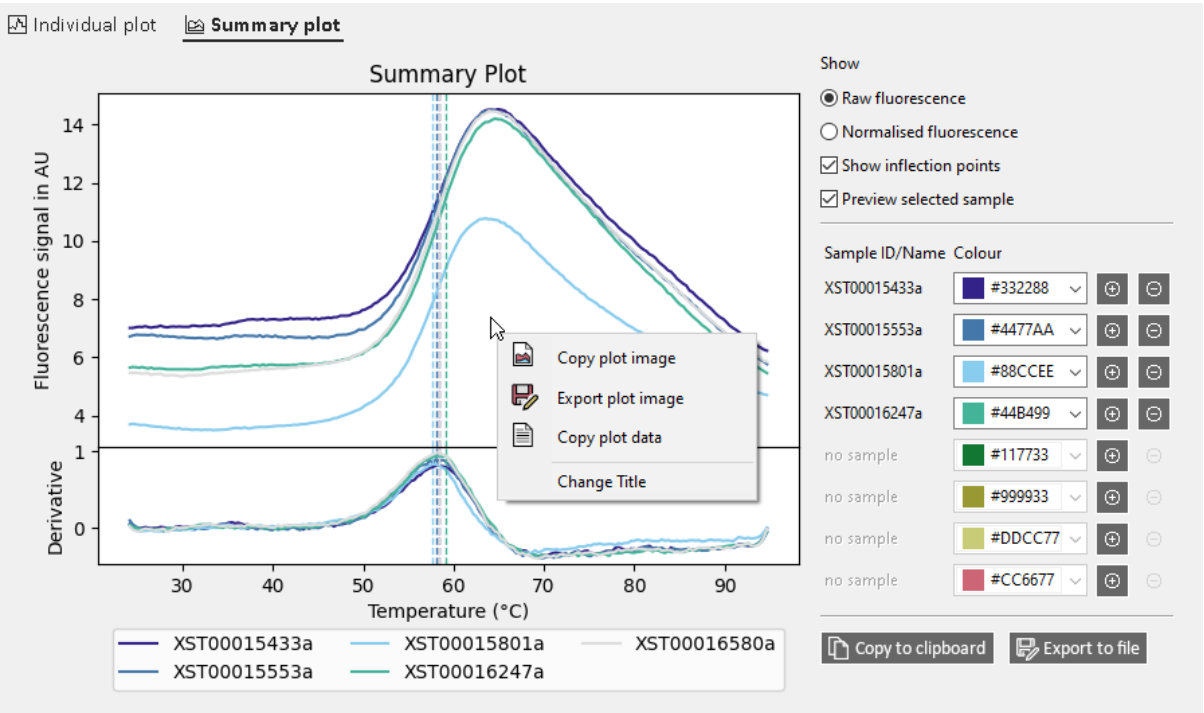
3.3.3 Review Plates

This tab contains a table with all sample plates, a heat map plot representing the assay plate (T_m values), a scatter plot showing the Tm values, and a preview plot of the melting curve and first derivative. Clicking on a plate in the table will update the plots. Clicking on a well on the heat map or data point on the scatter plot will update the preview plot. At this stage you can choose to exclude a well from being fitted.

3.3.4 Results

In this section, like with the other assays, you have a sample list and two plotting options: Individual plots and a summary plot, each with both the raw signals (either raw or normalised) and the first derivative on separate panels. The sample list contains all sample IDs and wells, their T_m and ΔT_m(if applicable). The plots can be either copied to the clipboard, to be pasted into other applications, or saved as a PNG image file. There is also an option to export all individual plots as PNG images at once.

On the summary plot, up to eight data sets can be displayed. You can add/remove data sets by selectin the sample in the sample list, and clicking on the ⊕ button on the right hand side of the plot's sample list. To remove a data set, click the ⊖ button. You can also choose between 9 different colour options.



3.3.5 Plots for ELN

Data series for each plate are plotted here. These can either be copied to the clipboard or saved as PNG files.

3.3.6 Export Results to Database

Results table for the SGC/CMD's SCARAB database, which can be exported as a CSV file or copied to clipboard. Options to choose results tables for different databases might be added in later versions.

3.3.7 Plate map for Database

Historically, DSF experiments were associated with a plate entry in the SCARAB database. This table provides the corresponding data.

3.4 CONTINUOUS ASSAY: NANODSF

For DSF assays, there are 6 tabs corresponding to each step of the analysis:

1. Assay Details
2. Results
3. Plots for ELN
4. Export Results to Database
5. Plate map for Database

3.4.1 Assay Details

There are four sections to be filled out:

1. Capillaries
   Click on the 🗁 button to load a Prometheus raw data file (.xlsx format). If the file is a valid Prometheus raw data file, a table will pop below the file path field. Fill out any empty fields and define whether the capillary is a sample or reference capillary (i.e. no sample, only protein, to calculate the ΔT_m value. It is possible to use multiple reference capillaries.
   It is also possible to leave this table blank, but ΔT_m values will not be calculated and the tables for the database will not be populated fully.
2. ELN Page
   Enter the number of your ELN page.
3. Compound Solvent
   Enter solvent and concentration in % (v/v) in the final experiment
4. Plate ID for database
   If desired, you can tick this box and enter a plate name. This will be used for the plate map for the database (see 3.4.4click me)

3.4.2 Results

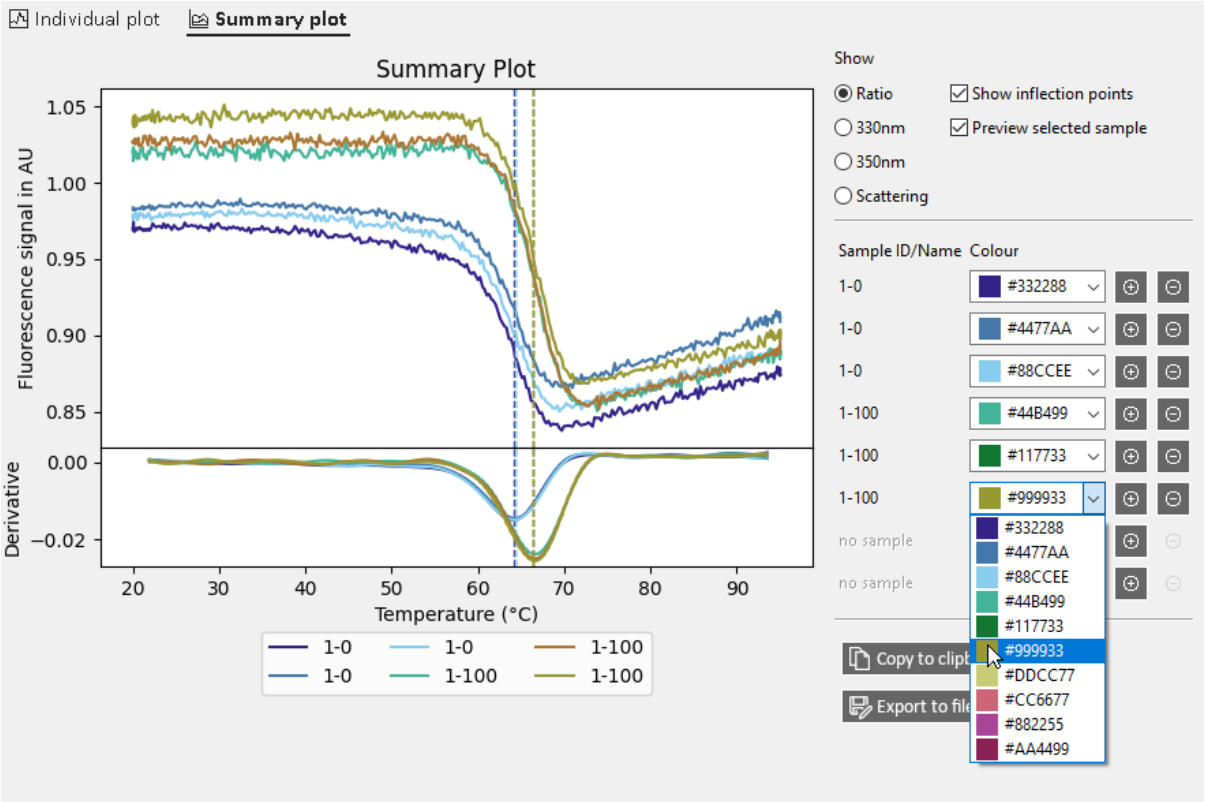
In this section, like with the other assays, you have a sample list and two plotting options: Individual plots and a summary plot, each with both the raw signals and the first derivative on separate panels.

For the individual plot, You can display the light scattering trace, and either the ratio of the fluorescence signals at 330 nm and 350 nm or one or both of the individual fluorescence traces (individual fluorescence traces and the ratio cannot be displayed at the same time). If scattering and fluorescence (either raw fluorescence or ratio) are both displayed, the light scattering signal will always be on the secondary (right) y-axis.

The plot can be either copied to the clipboard, to be pasted into other applications, or saved as a PNG image file. There is also an option to export all plots as PNG images at once.

For the summary plot, you can only choose one of the four options (fluorescence ratio, 330 nm or 350 nm fluorescence, or light scattering). Up to eight data sets can be displayed. You can add/remove data sets by selectin the sample in the sample list, and clicking on the ⊕ button on the right hand side of the plot's sample list. To remove a data set, click the ⊖ button. You can also choose between 9 different colour options.

The plots can be either copied to the clipboard to paste into other applications or saved as a PNG image file.



3.4.3 Export Results to Database

Results table for the SGC/CMD's SCARAB database, which can be exported as a CSV file or copied to clipboard. Options to choose results tables for different databases might be added in later versions.

3.4.4 Plate map for Database

Historically, DSF experiments were associated with a plate entry in the SCARAB database. This table provides the corresponding data.

4 TOOLS

4.1 CREATE ECHO TRANSFER FILE

4.2 PROCESS ECHO TRANSFER FILE

5 GLOSSARY

This glossary is by no means meant to apply universally. Entries will serve as a reference point for future revisions.

Assay plate	A microtiter plate containing the minimum components of an assay: i. the protein-peptide, protein-protein, protein-tracer or enzyme-substrate pair ("pair"), and ii. the detection reagents required to detect the interaction between the "pair".
BBQ	Biophysics
Capillary	Sample capillary for nanoDSF assays carried out with the Nanotemper Prometheus.
CSV (file)	Comma Separated Value. A plain text file in which values are separated by commas or tab stops. These are interpreted by MS Excel like a workbook with one single worksheet. Formatting and formulas cannot be saved in this format.
Data file	A file generated by a plate reader or other device collecting experimental data. Data files usually only contain the primary readout of the experiment.
ELN	Electronic lab notebook.
PNG (file)	Portable Network Graphics. An image file type offering lossless compression.
Raw data file	See data file.
Reference plate	An assay plate that does not contain any samples. It can, however, contain DMSO or other solvent addition to wells to capture the effect of solvent on the assay system.
Sample	A small molecule compound or biological molecule (peptide, protein fragment, protein) that is being tested in an assay.
Sample plate	An assay plate that also contains samples that are tested for their propensity to inhibit or improve the interaction between the "pair".
Transfer file	A log file showing every single transfer performed (or attempted) during the execution of a protocol on a liquid handler.
Z'	Measure for the quality of assay data. Values between 1 and 0.5 are typically considered "good". It is calculated as follows: $$Z' = 1 - \frac{3(\sigma_r + \sigma_c)}{\lvert\mu_r - \mu_c\rvert}$$ Where σ_r and σ_c are the standard deviation for the no sample **r**eference wells (no addition or solvent backfill only, corresponding to 0% inhibition) and the **c**ontrol compound wells (corresponding to 100% inhibition), respectively. μ_r and μ_c are the mean of the no sample reference wells and control compound wells, respectively.
Z' (robust)	Calculated as follows:

$$Z'_M = 1 - \frac{3(MAD_r + MAD_c)}{|M_r - M_c|}$$

Where MAD_r and MAD_c are the median absolute deviation for the no sample **r**eference wells (no addition or solvent backfill only, corresponding to 0% inhibition) and the **c**ontrol compound wells (corresponding to 100% inhibition), respectively. M_r and M_c are the median of the no sample reference wells and control compound wells, respectively.