

Thermal Cycling + Lumetics LINK™

INTRODUCTION

LINK is the software solution for automated analytical data import, analysis, and reporting. LINK scans network locations for new measurement files, extracts all useful data, and copies this data directly to a centralized database. The powerful analysis user-interface allows datasets to be aggregated, grouped, and visualized in the form of charts/tables/images. User-customizable analysis templates deliver rapid and error-free data visualization, with the ability to be replicated across many studies and shared within your organization.

All instrument settings and parameters calculated by the instrument software are copied to the LINK database, including full underlying raw data curves. Sample metadata may be easily assigned and incorporated within the analysis to integrate various product/process variables. LINK has been demonstrated to deliver time savings more than 90% and provide a highly affordable fully automated data management and analysis solution.

LINK currently supports multiple instruments specific to thermal cycling, including:

- Differential Scanning Calorimetry/Fluorimetry (DSC/DSF) – TA instruments, MicroCal (Origin & PEAQ), Uncle
- Nano Differential Scanning Fluorimetry (nDSF) – Prometheus
- qPCR – CFX96 Touch

THERMAL CYCLING DETAILS & APPLICATIONS

Thermal Cycling is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Conventionally Thermal Cycling can be used to induce protein denaturation, which then can be measured using qPCR. LINK may be utilized to assist directly in the following Thermal Cycling application areas:

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| <ul style="list-style-type: none"> • Characterizing protein unfolding • Thermal and chemical denaturation studies • Biological systems studies • Molecular interactions • Formulation, protein, viral vector profiling | <ul style="list-style-type: none"> • Discovery & development of small molecule drugs, biotherapeutics and vaccines • qPCR, RT-qPCR • Genotyping • GMO detection |
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INSTRUMENT SUPPORT DETAILS – DSC/DSF

TA DSC (Differential Scanning Calorimeters) measure temperatures and heat flows associated with thermal transitions in a material. Common usage includes investigation, selection, comparison, and end-use performance evaluation of materials in research, quality control and production applications. LINK supports multiple TA Instruments DSC systems. Imported measurements include output data for instrument/analysis settings, parameters, calculations, and raw data curves for temperature vs. Analysis Data, Baseline, Baseline Subtracted, Model, Power, Pressure, Residual, Time (seconds).

MicroCal DSC microcalorimeters enable characterization of the thermal stability of proteins and other biomolecules, primarily for biopharmaceutical development and manufacture. They are used for general stability studies, for biosimilarity and batch-to-batch comparability assessment, and for the optimization of purification and manufacturing conditions. LINK supports multiple MicroCal instruments, as well as the Origin software. Imported measurements include output data for instrument/analysis settings, parameters, calculations, and raw data curves for Cp and derivatives vs. Temperature. The full PDF from which the data was extracted is also imported as a series attachment within the LINK software.

Uncle from UnChained Labs is a one-stop protein stability platform that uses fluorescence, SLS and DLS detection to enable 12 different applications. Multiple measurements such as thermal melting, aggregation, and sizing can be performed with the

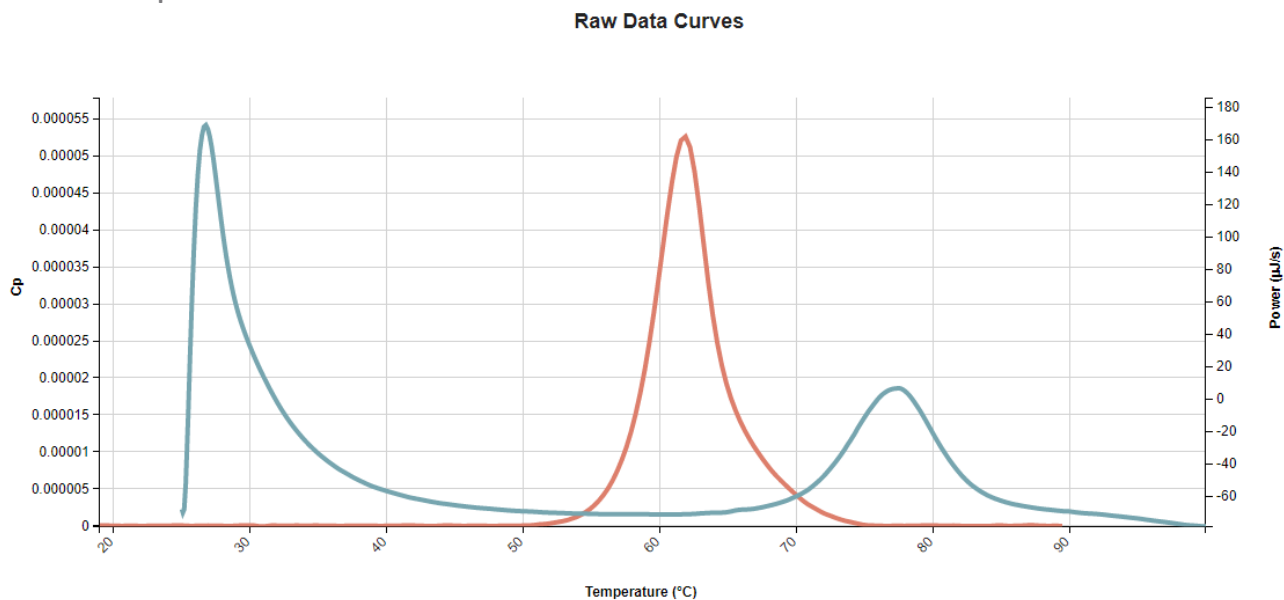


same samples in just one experiment. LINK supports Uncle output data for instrument/analysis settings, parameters, calculations, and raw data curves for multiple parameters including:

- Delay Time vs. Autocorrelation Function
- Particle Diameter vs. Intensity % & Mass %
- Temperature vs. BCM/nm, SLS 266nm/Count, and SLS 473nm/Count

The dynamic properties of UNCLE are supported through multiple visualization preferences and internal LINK calculations options.

Fig 1. Line Chart dashboard visualizing DSC/DSF measurement files plotting raw data curves for Cp and Power vs. Temperature.

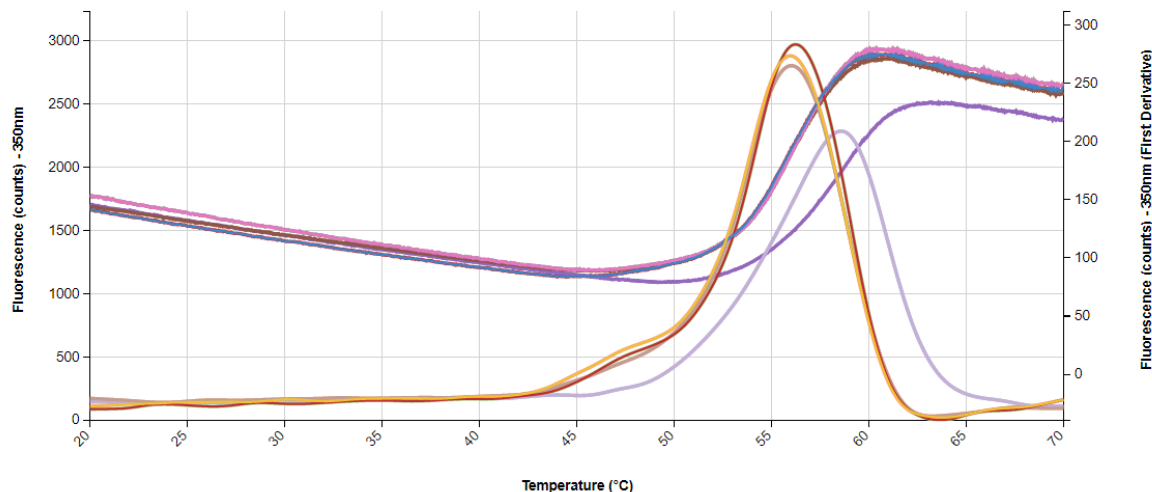


INSTRUMENT SUPPORT DETAILS – NANO DSF

Prometheus from Nanotemper characterizes protein unfolding by monitoring the intrinsic fluorescence signal of proteins as a measure of its folding state. A ratiometric measurement of the fluorescent signal is plotted against increasing temperature or concentration of a chemical denaturant to determine the T_m of a protein.

LINK supports multiple Prometheus instrument versions (NT.48, NT.Plex, NT.Plex plus NT.Robotic Autosampler) for instrument/analysis settings, parameters, calculations, and raw data curves for Time/Temperature vs Fluorescence 330 nm, Fluorescence 350 nm, Fluorescence Ratio, Scattering (mAU) as well as the first derivatives.

Fig 2. Line Chart dashboard visualizing Prometheus measurement files plotting raw data curves for Fluorescence at a Wavelength of 360 nm & First Derivative vs. Temperature.
Prometheus - Fluorescence at 350nm

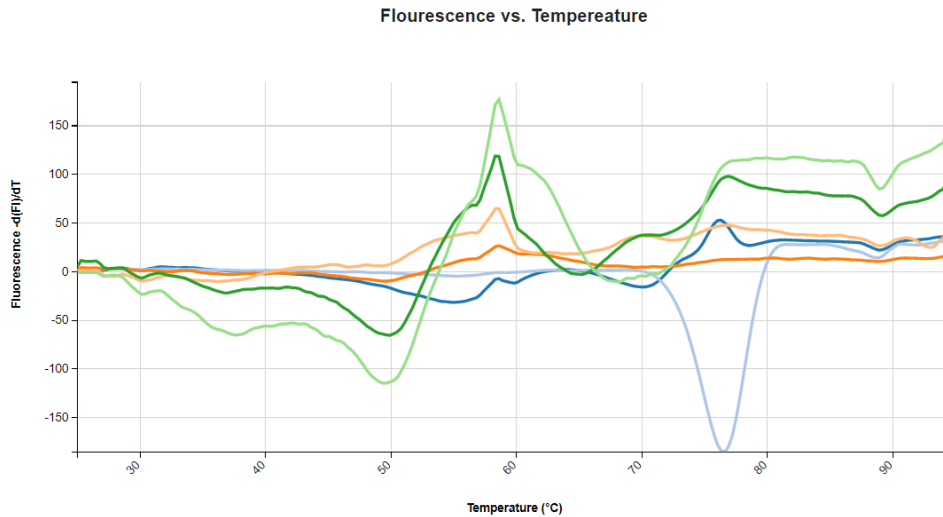


INSTRUMENT SUPPORT DETAILS –qPCR

CFX96 Touch from Bio-Rad is a six-channel real-time PCR instrument combines advanced optical technology with precise temperature control to deliver sensitive, reliable detection for single-plex or multiplex reactions.

LINK supports the CFX96 Touch multi-channel instrument for instrument/analysis settings, parameters, calculations, and raw data curves for Relative Fluorescence (RFU) and Fluorescence $-d(FI)/dT$ vs. Temperature. The dynamic properties of CFX96 Touch are supported through multiple visualization preferences and internal LINK calculations options.

Fig 3. Line Chart dashboard visualizing CFX96 Touch measurement files plotting raw data curves for Fluorescence vs. Temperature.



VISUALIZATIONS – LINK ANALYSIS

LINK's dynamic analytical suite will aggregate data and provide visualization tools to suit your specific needs. Imported Thermal Cycling measurement data can be analyzed using the LINK platform's customizable charts, tables, calculations, images etc. User-customized analysis templates deliver rapid and error-free data visualization with the ability to be exported as a word report to share across your organization. Measurement data can be visualized separately, as well as overlaid in the same chart and tables.

Fig 4. Raw Curve Data: Relative Fluorescence & 1st Derivative vs. Temperature

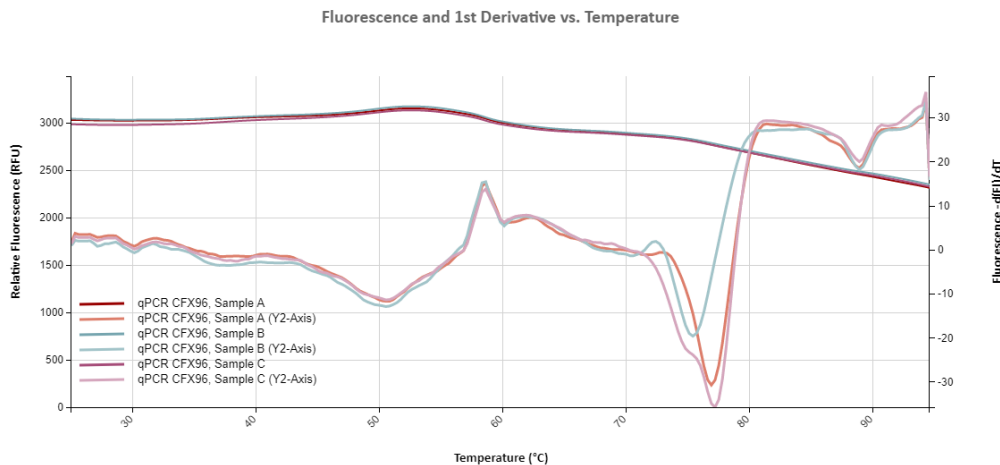


Fig 5. Raw Curve Data: Relative Fluorescence Ratio & 1st Derivative vs. Temperature

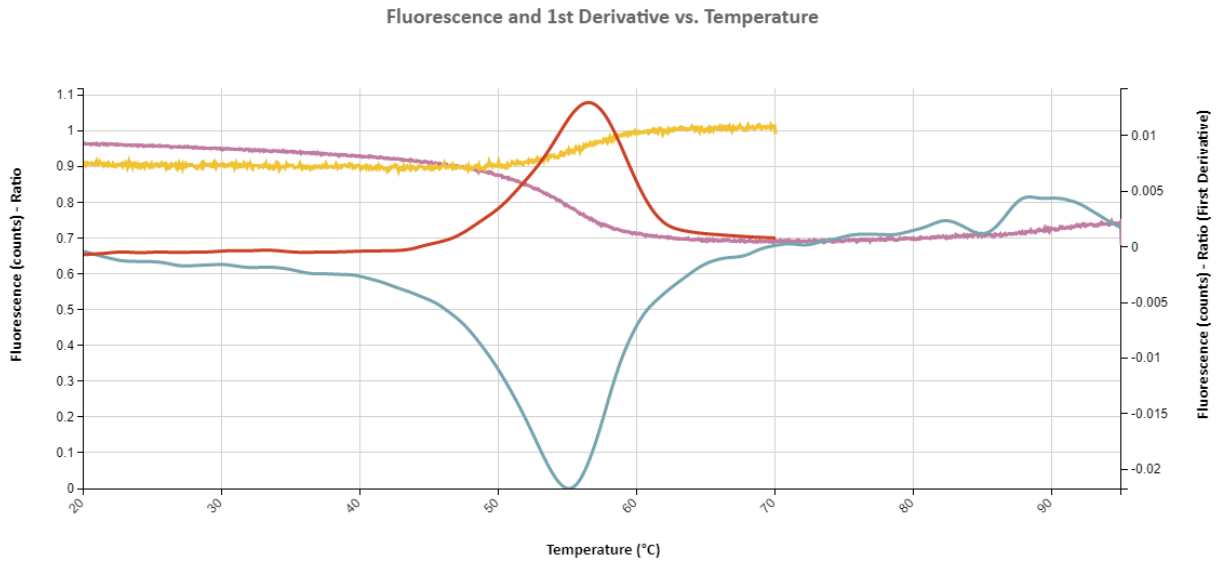


Fig 6. Raw Curve Data: Cp & 1st Derivative vs. Temperature

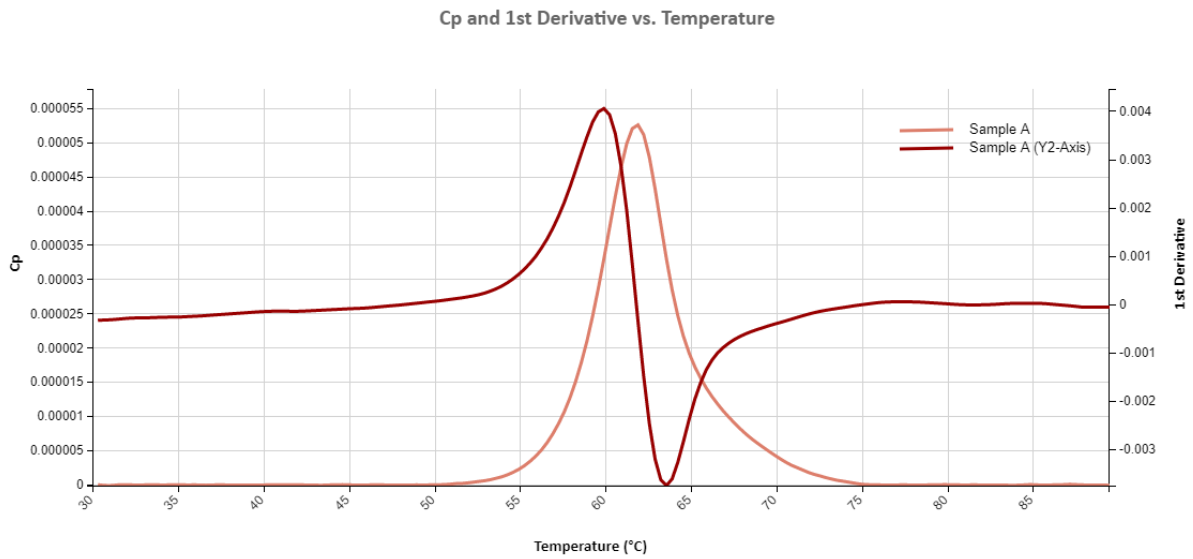


Fig 7. Raw Curve Data: Baseline & Baseline Subtracted vs. Temperature

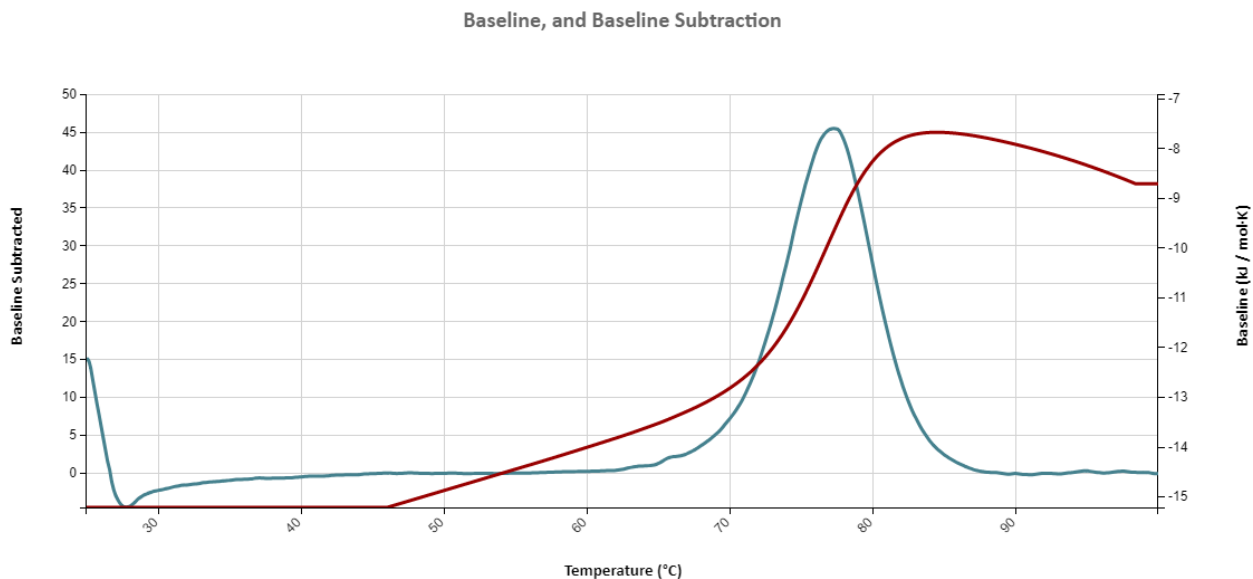


Fig 8. Tabular Summaries – Measurement Summary tables

Measurement Results

Sample Name	Nano DSC: Onset #1 (ratio)	Nano DSC: Inflection #1 (ratio)	Nano DSC: Inflection #2 (ratio)	Nano DSC: Inflection #3 (ratio)	Nano DSC: Inflection #4 (ratio)	Nano DSC: Inflection #5 (ratio)	TA DSC: Aw	TA DSC: delta-H (kJ/mol)	TA DSC: Tm (C)	MicroCal DSC: TOnset (C)	MicroCal DSC: Tm1 (C)	MicroCal DSC: Tm2 (C)	qPCR: Melt Temp
Sample A	41.37	54.83					0.75	496.20	77.01	60.49	66.53	74.62	58.50
Sample B	41.77	47.67	57.48	67.15	70.06	71					63.29	69.89	58.50
Sample C	40.99	53.14	52.43	56.30						59.04	69.87	79.46	58.50

DASHBOARD DOWNLOADS

These downloadable Analysis Dashboards are tailored to meet the most common needs of scientists using Thermal Cycling applications. If assistance is required, please contact LINK experts to assist in dashboard modifications or development of new dashboards of interest.

- Prometheus Dashboards: <http://lumetics.com/dashboards/Prometheus/Prometheus.zip>
- Uncle Dashboards: <http://lumetics.com/dashboards/Uncle/Uncle.zip>
- CFX96 Touch qPCR Dashboards: <http://lumetics.com/dashboards/CFX96/CFX96.zip>
- TA Instruments Dashboards: <http://lumetics.com/dashboards/TA/TA.zip>
- Microcal PEAQ Dashboards: http://lumetics.com/dashboards/MicroCal_PEAQ/MicroCal_PEAQ.zip

CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

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