Prometheus – NanoTemper

NT.48, NT.Plex, NT.Plex plus NT.Robotic Autosampler

INTRODUCTION

The Lumetics LINK™ software platform scans network locations for new measurement data files, copies data directly to a centralized database, and provides a powerful user interface for rapid multi-measurement multi-technique data aggregation, visualization, analysis, and reporting. LINK employs a client/server-based architecture where the LINK server hardware is provided by the end user and resides on the end user's network. The LINK client is a portable web-based application that may be placed on any computer with network connectivity to the LINK server. For successful import, the LINK webserver requires read access to the folders where user data resides.

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

DETAILS

LINK requires XLSX data file to import Prometheus data. The XLSX file must contain a sheet named "Overview" where A1 = "Capillary" and B1 = "Sample ID". Capillary must have values and all other metadata are in a row other than Capillary.

The following raw curve data may be imported, in addition to all available instrument/analysis settings and parameters calculated by the instrument software:

- Time/Temp. vs. Fluor. 330 nm
- Time/Temp. vs. Fluor. 330 nm (1st derivative)
- Time/Temp. vs. Fluor. 350 nm
- Time/Temp. vs. Fluor. 350 nm (1st derivative)
- Time/Temp. vs. Fluor. Ratio
- Time/Temp. vs. Fluor. Ratio (1st derivative)
- Time/Temp. vs. Scattering (mAU)
- Time/Temp. vs. Scattering (mAU) (1st derivative)

Helpful Notes:
- The LINK field 'Folding Type' is automatically created upon data import. This dynamically created LINKdb field will need to be added to the filter panel and a split applied to differentiate between refolding and unfolding.
- Criteria for valid Curve Data – must have one of the sheets below:
  - Ratio (Unfolding) **OR** Ratio (Refolding)
  - Ratio (First Derivative) (Unfolding) **OR** Ratio (First Derivative) (Refolding)
  - Ratio (1st deriv.) (Unfolding) **OR** Ratio (1st deriv.) (Refolding)
  - 330nm (Unfolding) **OR** 330nm (Refolding)
  - 330nm (First Derivative) (Unfolding) **OR** 330nm (First Derivative) (Refolding)
  - 330nm (1st deriv.) (Unfolding) **OR** 330nm (1st deriv.) (Refolding)
  - 350nm (Unfolding) **OR** 350nm (Refolding)
  - 350nm (First Derivative) (Unfolding) **OR** 350nm (First Derivative) (Refolding)
  - 350nm (1st deriv.) (Unfolding) **OR** 350nm (1st deriv.) (Refolding)
  - Scattering (Unfolding) **OR** Scattering (Refolding)
  - Scattering (First Derivative) (Unfolding) **OR** Scattering (First Derivative) (Refolding)
  - Scattering (1st deriv.) (Unfolding) **OR** Scattering (1st deriv.) (Refolding)

The Prometheus XLSX data file example is as follows:

|	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Capillary	Sample ID	Excitation Power	Start Temperature	End Temperature	Temperature Slope	Start Time	Duration	Onset #1 for Ratio	Onset #1 for Scattering	Inflection Point #1 for Ratio	Inflection Point #2 for Ratio	Inflection Point #3 for Ratio
2	1	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	46.8°C	44.3°C	56.9°C		
3	2	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	42.3°C	42.5°C	55.5°C		
4	3	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	29.1°C		46.2°C		
5	4	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	43.3°C	42.9°C	55.6°C		
6	5	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	42.5°C	42.6°C	55.4°C		
7	6	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	45.6°C	42.2°C	57.2°C		
8	7	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	46.3°C	42.6°C	57.9°C		
9	8	Sample A	3%	20°C	70°C	1.0°C/min	2016/12/21 09:57	0:50:00	44.2°C	40.3°C	57.0°C		

EXAMPLES

Included below are example dashboards from Prometheus measurement files:
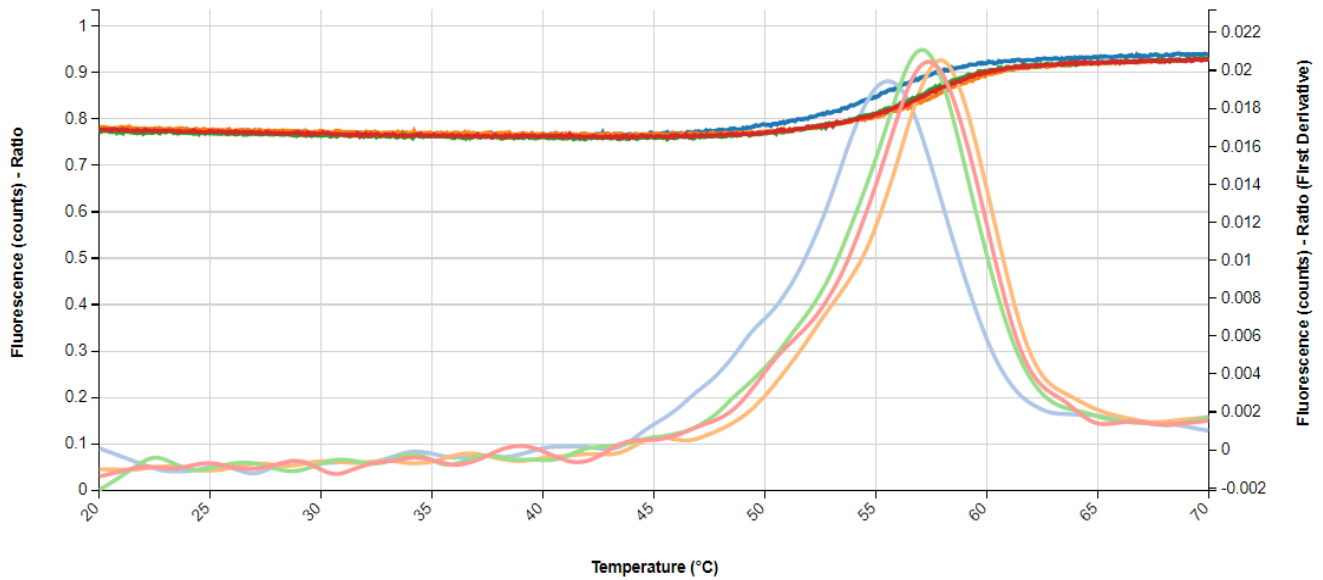
1. Line Chart raw data curves for Fluorescence Ratio vs. Temperature, split by Folding/Unfolding

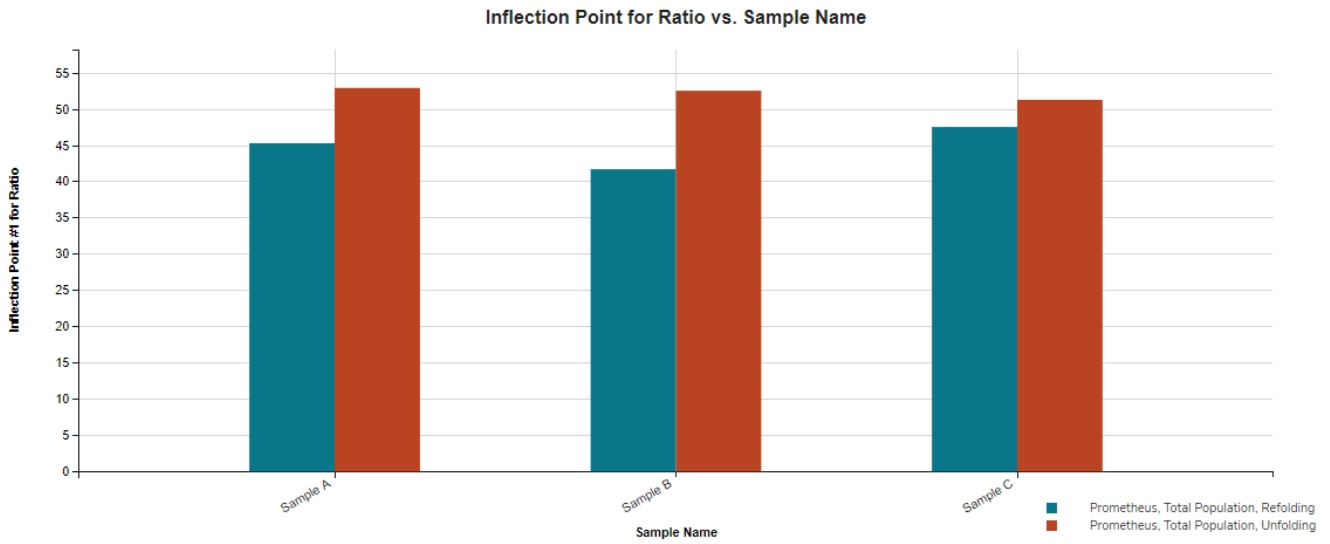### Prometheus - Fluorescence Ratio
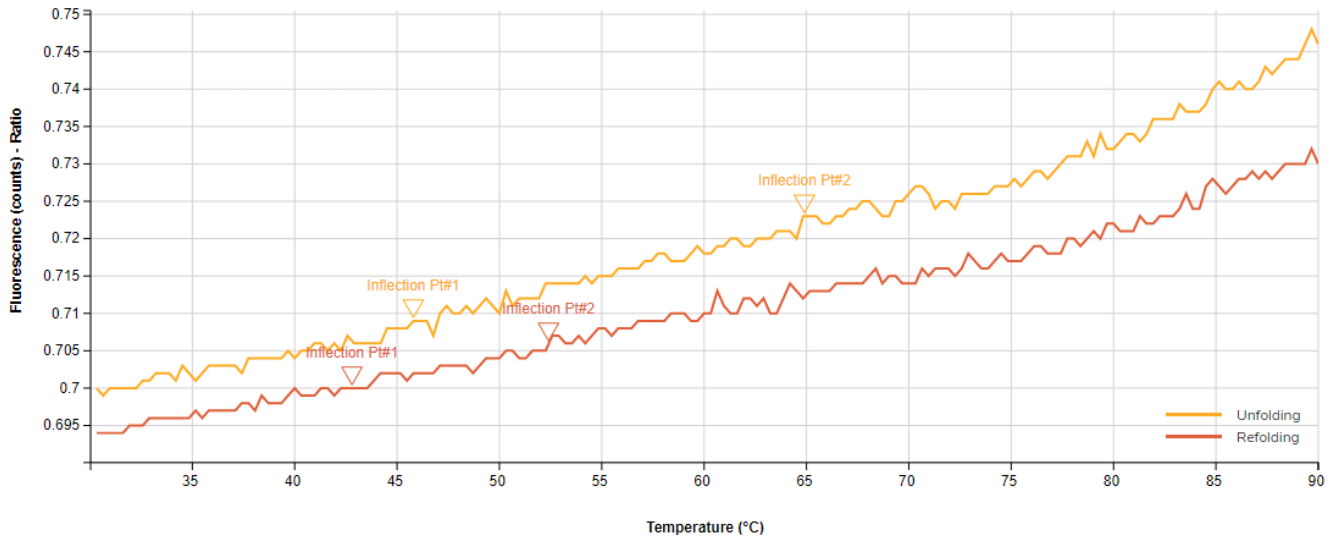
2. Line Chart plotting raw data curves for Fluorescence Ratio vs. Temperature

Fluorescence Ratio

3. Column Chart plotting raw data curves for Inflection Point vs. Sample, split by Folding/Unfolding

Inflection Point for Ratio vs. Sample Name

4. Tabular Summary examples

Measurement Summary Table – Instrument settings

Sample Name	Capillary	Folding Type	Analysis Date	Excitation Power (%)	Start Temperature (°C)	End Temperature (°C)	Temperature Slope (°C/min)	Duration
sample 1	1		2016-12-21 09:57:09	3	20	70	1	1899-12-31 00:50:00
sample 2	2		2016-12-21 09:57:09	3	20	70	1	1899-12-31 00:50:00
sample 4	4		2016-12-21 09:57:09	3	20	70	1	1899-12-31 00:50:00
sample 5	5		2016-12-21 09:57:09	3	20	70	1	1899-12-31 00:50:00

Measurement Summary Table – Measurmemnt Results

Sample Name	Capillary	Folding Type	Custom Marker #1 for Scattering - AVG	Custom Marker #1 for Ratio - AVG	Onset #1 for Ratio - AVG	Inflection Point #1 for Ratio - AVG
sample 1	1		5	3	46.80	56.90
sample 2	2		7	4	42.30	55.50
sample 4	4		8	5	43.30	55.60
sample 5	5		9	6	42.50	55.40

PROMETHEUS DASHBOARDS

LINK contains an extensive built-in dashboard library from LINK version 2.4.0.210401 and later. This function contains specific pre-created dashboards for all instruments and application groups.

CONTACT LUMETICS

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