Sample Submission Guideline for NanoTemper Tycho NT.6
Differential Scanning Fluorimeter Detecting 350nm and 330nm

I. Sample Submission

1. Apply an account on the BCF reservation system using your official email address, which will be used to identify your affiliations. BCF will not accept service requests from public e-mail domains, such as gmail, outlook or yahoo, etc.
2. File sample submission form on-line under the instrument, “Tycho NT.6”.
3. After confirming the charges and experiment time through reservation system, samples and buffers may be submitted to Miss Jin-Hsuan Yu (Tel: 27855696 x4024, e-mail: bcf@gate.sinica.edu.tw). If BCF does not receive your samples before the scheduled time listed in the service request application form, the facility will add an extra charge for the delay, unless early notification is sent by emails more than two days in advance
4. BCF will not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). All experimental results are for research only. Without written permission from Academia Sinica, the user shall not claim, announce, or mislead the public into interpreting that the results of this testing is in any way related to the commercial development of the user. In addition, the user shall not in any form (including but not restricted to commercial marketing, for example advertisements, either online or offline, product packaging, catalogs, investment information etc,) use the title, logo, name, trademark or symbols that are that of Academia Sinica or similar to that of the facility, that gives the false impression of a commercial collaboration
5. Samples submitted to BCF should be non-hazardous, non-toxic and nonpathogenic. No radioactive or microbial samples are allowed.

II. Sample preparation

1. Samples submitted to BCF are non-hazardous, non-toxic and non-pathogenic.
   No radioactive or microbial samples are allowed.
2. Please spin your samples at 12,000 g for 5 minutes to remove possible precipitations.
3. 15 µl of sample is required for one experiment, which may be applied for buffer screening, QC (similarity), quick binding test, storage test or thermal shift (Ti).
4. Tycho NT.6 detects the fluorescence intensity at 350nm and 330nm, the emission wavelengths of the tryptophan (Trp) and Tyrosine (Tyr) residues. **0.1-0.5mg/mL** of concentration is preferable. However, if this concentration cannot be reached, please follow the simple guideline below for the least sample concentration:
   Sample concentration x number of tryptophan residue >>5µM
5. If possible, avoid substances that have fluorescence overlapping with that of proteins in your buffer, for example, Triton X-100. High concentration of Triton X-100 masks the unfold profiles.

III. During Experiments

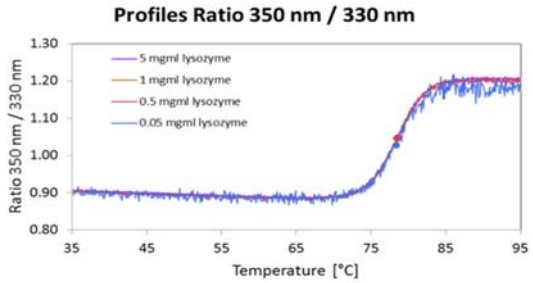
1. Samples are loaded to the capillaries (TY-C001) after spun on a benchtop centrifuge for 1 min.
2. Start measurements and monitor the ratio of fluorescence at 350 nm to that of 330 nm (ratio 350 / 330 nm) while the sample is heated from 35 to 95°C with 30 °C / min scanning rate.
3. Once the first measurement is finished, in the same capillaries, we will perform an additional measurement.
4. We will not recover your samples.

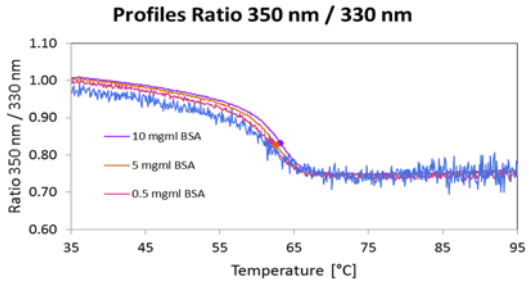
IV. After Experiments

1. Raw data in Excel spreadsheet formats can be downloaded via the link sent by the reservation system. A
2. Please note that data analysis is the responsibility of users.

3. Figures below show profiles of 2 proteins plotting ratio 350 nm / 330 nm vs. temperature and the derived Inflection temperatures.

#	Capillary label	Ti#1	Ti#2	Ti#3	Initial Ratio	Δ Ratio	Sample Brightness		Measured at	Keywords	Device
1	5 mgml lysozyme	78.6			0.9063	0.2991	1031.5		2019-02-19 11:47	screening ; reference	T6-146
2	1 mgml lysozyme	78.4			0.9030	0.2974	446.9		2019-02-19 11:47	screening ; reference	T6-146
3	0.5 mgml lysozyme	78.6			0.9027	0.2963	258.6		2019-02-19 11:47	screening ; reference	T6-146
1	10 mgml BSA	63.1			1.0079	-0.2451	2750.6		2019-02-19 12:33	screening ; reference	T6-146
2	5 mgml BSA	62.5			1.0035	-0.2430	1781.6		2019-02-19 12:33	screening ; reference	T6-146
3	0.5 mgml BSA	61.9			0.9951	-0.2433	226.7		2019-02-19 12:33	screening ; reference	T6-146





V. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example, “We acknowledge Tycho NT.6 DSF data collected by [operator] in the Biophysics Core Facility, funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201).”