

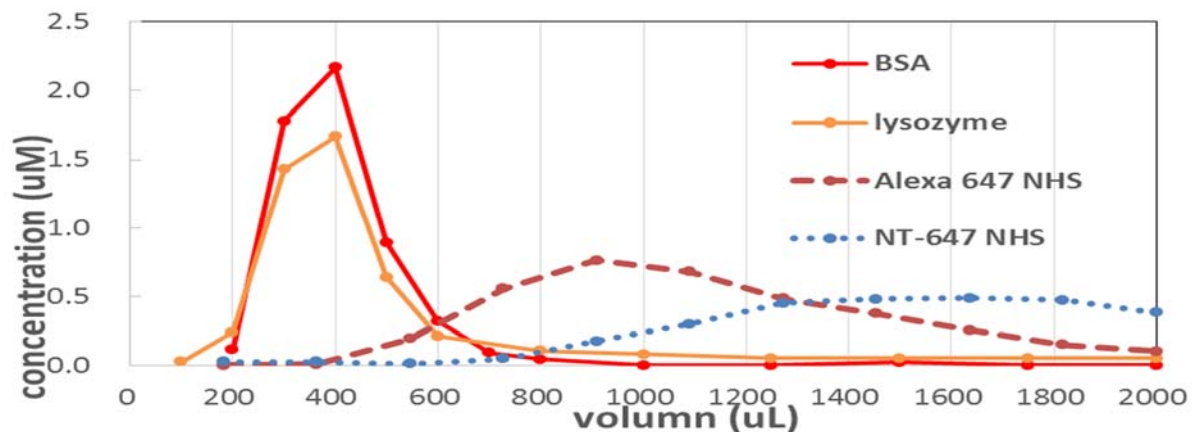
Monolith NT.115^{Pico} Simplified Procedures

STOP

This protocol is for qualified users operating Monolith NT.115^{Pico} in Biophysics Core Facility only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's User Guideline is highly recommended.

Labeling Dyes (Cy5/Alexa647/NT-647):

1. Dilute unlabeled protein in the labeling buffer (0.1-0.2M sodium bicarbonate, pH8.5) to make 20 μ M solution. Avoid primary amines and reducing reagents.
2. Add 30-50 μ L 20 μ M protein solution to an aliquot of 0.3 μ L, 10mM Alexa647NHS or NT-647NHS (Dye/Protein= \sim 3) and let the reaction proceed for 15-30 minutes in dark at room temperature. Since labeling dye is dissolved in DMSO, final DMSO concentration should be kept below 2% for most proteins. (To label on cysteine residues, add 50 μ L 20 μ M protein solution to 1 μ L, 10mM Alexa 647 C2 Maleimide and let the reaction proceed for 2 hours or 4 $^{\circ}$ C overnight, also in dark.)
3. Add 30-50 μ L buffer to the reaction to make 100 μ L and separate labeled protein from unreacted dye by desalting columns. (For example, from NanoTemper protein labeling manual: add 3x3mL buffer \rightarrow add 100 μ L sample + 400 μ L buffer \rightarrow add 600 μ L buffer and immediately collect the elution in the following amount: first tube 200 μ L, second 250 μ L, third 150 μ L \rightarrow save the labeled protein in the second tube.) Figure below shows the elution profile of the desalting column in BCF.



4. Measure the absorbance at A280 and A650 to calculate protein concentration and the degree of labeling (DOL), which is usually in the range of 0.8 to 1.2, depending on the protein sequence and labeling buffer.

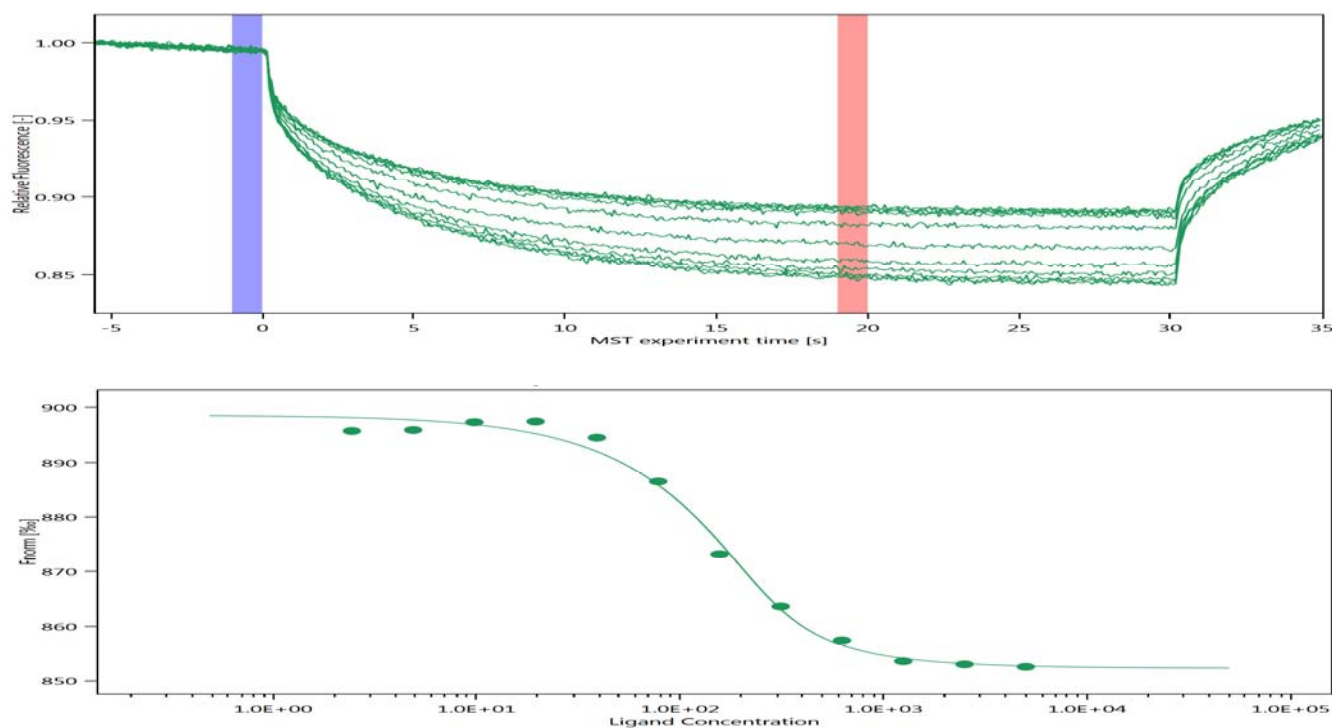
$$\text{protein_concentration} = (A280 - CF * A650) \div \epsilon_{\text{protein}}$$

$$DOL = (A650 / \epsilon_{\text{dye}}) / ((A280 - CF * A650) \div \epsilon_{\text{protein}})$$

5. Adjust the reaction time or the Dye/Protein ratio if DOL is not in the range.
6. 2-10 nM of fluorescently labeled protein is usually sufficient for the MST assay using Monolith NT.115^{pico}.

MO.Control and MO.Affinity.Analysis

- Turn on instrument and control PC.
- Create a folder in C:\Data\Institute\yymmdd to keep the results.
- Start MO.Control software.
- Select **+Start New Session** and create a new project (*filename.moc*).
- Choose an experiment from the list (Pretest, Binding Check, Binding Affinity and Expert Mode) and follow the procedures therein.
Pretest optimizes LED and MST power according to the intensity of the fluorescence; **Binding check** tests possible interactions;
Binding affinity measures K_D values using 16 capillaries;
Expert mode allows flexible experiment designs.
- Use MO.Affinity.Analysis software to analyze MST data for K_D values. Raw data and fitting results may be exported after analysis.



Shut Down

- Discard used capillaries in glass waste bin.
- Clean the sample tray.
- Transfer your results using DVD recorder.
- Shut down control PC and turn off NT.115^{Pico} instrument.

-----Old NTControl Software-----

NTControl - Check Fluorescence and Capillaries

1. Turn on instrument and control PC.
2. Create a folder in **C:\Data\Institute\yyymmdd** to keep the results.
3. Start NTControl software.
4. Choose **File: New Project** to create a new project (*filename.ntp*).
5. Perform a **Cap Scan** using about 5-20nM of labeled protein in the standard treated capillary to get an optimal fluorescent intensity to be around 10,000 counts (3,000 to 20,000) at LED power of 10-20%. Adjust LED power if necessary.
6. You may save a picture (*filename.png*) of the CapScan result by right-clicking inside the CapScan window and choosing **Save screenshot**. CapScan results are automatically saved in the project if MST experiments are executed.
7. Usually 0.005% to 0.05% detergent (Tween 20) is needed if standard treated capillaries are used. Other additives, such Pluronic F-127 or BSA, may be added.
8. Try premium coated capillaries if your protein non-specifically binds to the surface of standard treated capillaries.

NTControl - MST Experiments

9. Use Concentration Finder tool to simulate binding curves and adjust concentrations of the non-labeled binding partner.
10. Before serial dilution preparation, centrifuge labeled and non-labeled binding partners at highest speed (12,000 rpm) for 5 – 10 minutes.
11. Prepare 16 concentrations of non-labeled partners, 15 μ L each, by serial dilution in PCR tubes, including one sample of 0 concentration.
12. To each tube, add 15 μ L of the fluorescently labeled protein.
13. Fill out **Actual Experiment's Name**.
14. Perform **Cap Scan** at the level of LED power which was determined in step 9, starting from cap# 1 to cap#16. Save a Cap Scan screenshot.
15. Fill in concentrations for each capillary. For the 0 concentration, fill a very small number (for example, 1e-12) for later reference. Perform MST measurement at 20%, 40% and 60% MST power. Perform triplicates and examine the fluorescent consistency.

NTControl - Data Analysis and Export Report

16. Use NT Analysis software to analyze MST data.
17. Report is generated in html or pdf format for each set of experiment. Reports are automatically saved to Desktop\html. Please remember to move the files to your experimental folder.