



Nano DSF

Initial Considerations:

- Generally, you want your protein of interest to be at a concentration 0.1-1.0 mg/mL. This isn't a hard rule, but most proteins will work above this threshold (see below for more information).
- Proteins with any Trp/Tyr are good to go. If you are lacking Trp, be sure to use the high-sensitivity quartz capillaries. If you don't have either Trp or Tyr in your target, consider using a different type of nDSF assay using SYPRO orange dye and a QPCR machine (The PANTA is not compatible with this form of nDSF. You can still use the DLS module though).
- If screening for (de)stabilizing ligands, a change in $T_m \geq 2^\circ$ is a typical cutoff for a significant result.
- Keep in mind that the DLS measurements are more accurate for larger proteins. Take the radii from these experiments with a grain of salt ($\pm 20\%$ accuracy)

General experimental workflow

1. Make a new experiment file and fill in the information for your samples. If you are measuring replicates, give the samples the same name in the spreadsheet (This way the analysis software can auto-merge the replicates). It's recommended to measure duplicates at a minimum due to issues such as air bubbles. Be sure to select the proper buffer.
2. Open the sample drawer, remove the magnetic clamp, and clean the reflective surface with 100% ethanol and lens paper. **Do not use a Kimwipe!**
3. Load your samples. It's best practice to tilt your tube of protein 90° so that it enters the capillary horizontally. This helps avoid air bubbles. As long as your capillary is 70% full, you're good to go.
4. Make sure the magnetic clamp is **fully** seated. If not, it will catch on to the instrument housing and the motor will seize up. This is easily fixable, see the note at the end of this document.
5. Run a discovery scan. Ideally you want to have a signal of 2000 or greater. Lower signal may work but may be less reproducible. If your samples have wildly different absorption coefficients, you may have to adjust the laser power to find the "sweet spot" of signal.
6. Run an isothermal DLS experiment. This will give you a sense for how monodisperse your sample is going in to the experiment.
7. Run a nDSF thermal gradient. We generally run from 25° to 90° at a step size of $1.5^\circ/\text{min}$. If the instrument warns that the data density will be insufficient, adjust to a $1.0^\circ/\text{min}$ step size.
8. Save your data, open the sample drawer, dispose of your capillaries into sharps waste, and clean the reflective surface with 100% ethanol and a kim wipe.

What to do in case the sample drawer catches the instrument housing:

1. Turn off the main power to the instrument.
2. Carefully re-seat the magnetic clamp

3. Hold the door flap open, and slowly push the sample drawer in by hand until it is flush with the rest of the instrument housing. Release the door flap, and power the instrument on again. Proceed to step 1 of the experimental workflow, skipping steps 2-4 above.

Data processing tips

- It's best to process the data in the analysis software, rather than exporting from the control software.
- Make sure to merge replicate datasets. This will give you nice statistical windows as shaded areas around the average value plotted in your data.
- Be sure to examine **all** the data traces to fully interpret the experiment. Sometimes the individual wavelength traces (or their first derivatives) are more informative than the ratio plots.
- Keep an eye on the turbidity and size traces to get a sense for the aggregation state of your protein. Sometimes you can see interesting trends such as multi-state unfolding or partial aggregation.
- In the export menu, you can export the formatted plots as .png or .pdf, as well as the raw data for processing in other software such as Prism.
- Nanotemper has a good library of advanced experimental techniques on their website.

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