**Dynamic Light Scattering (DLS) SOP**

NOTE: DLS and MALS run on the same computer and the both machines and software can collect data at the same time. Check to see if anyone is using it and, if so, make sure to speak to them about using it together.

NOTE: The cuvette you choose will depend on the sample volume you have. The glass cuvette holds ~10uL, whereas the plastic cuvettes hold ~80uL. The plastic cuvettes come with the benefit of not needing to be cleaned, but they are 3$ per cuvette and require a large sample volume.

NOTE: The MOST crucial thing is that your sample and buffer be dust free. You need to filter your buffers and keep the lids closed and you can either spin your sample for 10’ at 15k rpm and transfer to a new tube or use the Pall centrifuge filters.

1. Turn on the machine (Wyatt DynaPro NanoStar)
2. Turn on the laser by hitting tab until the “laser” button is highlighted, then hit enter. The button should go from yellow to green.
3. Select your cuvette
   * 1. NOTE: If using the glass cuvette, you will need to clean it before you use it to make sure that it did not collect dust as it sat. The glass cuvette is in the little door on the front of the DLS instrument.
4. Turn on the software (Dynamics V7)
5. Select “File -> New Experiment”
6. A pop up window saying it has selected default parameters will open, hit OK
7. Select “Parameters” and choose “Instrument”
8. You can change the acquisition time and the number of acquisitions on this page. I typically do the acquisition time of 5 seconds and 20 acquisitions.
   * 1. NOTE: The longer the acquisition time, the better your data will be and the less chance of dust ruining your read.
9. Choose “Sample” and select the type of protein, the buffer, and the cuvette you will be using
   * 1. Note: The glycerol content matters the most here. We typically use globular proteins for the fitting. Choose glass or plastic, depending on what you have chosen to use
10. Connect to the instrument by hitting “Connect”, the red circle should turn to green
11. Clean the glass cuvette by rinsing with filtered water 3 times, filtered ethanol 3 times, then drying it using a filtered air apparatus set up in MBB 4192, in the northwest corner of the lab.
    * 1. NOTE: It is critical that you remove all dust from the cuvette, as that will dominate your sample. Hold the cuvette at a downwards angle at all times and cover with the cap when not in use
12. Put a buffer sample in the cuvette, making sure to avoid getting any bubbles in the cuvette
    * 1. NOTE: It is very difficult to load the glass cuvette as it is such a small volume and the cuvette is difficult to look into. Looking through the side of the cuvette, you will be able to see where the sample goes and where your pipet tip is. Holding the cuvette against something white can help you see it. You need to be careful about bubbles, pipetting up and down to remove bubbles does not work and will likely damage your sample.
      2. **NOTE**: Practicing using buffer is a good idea so that you do not waste sample.
13. Open the door at the top of the instrument and insert the cuvette. It will only fit one way.
    * 1. NOTE: To prevent the sensors from getting fried, the laser turns off when you open the lid. Wait until the button goes back to green before running the experiment.
14. Hit run experiment and select the Analysis tab
15. As each acquisition runs, it will enter as one line in the table. You can monitor them as they come up.
16. The parameters to pay attention to are: radius, MW, %Pd (polydispersity), SOS, and baseline.
    * 1. NOTE: If the number appears in red, it means that the software has automatically “marked” it so it does not get included in the analysis.
      2. NOTE: radius is the number we care most about, the MW is fit as if the sample is a sphere. The WRC is not a sphere and so your MW will not fit well. The polydispersity tells you how diverse your sample is (i.e., how many species are being fit) – as such, lower is better. SOS is sum of squares and tells you how well your data fit the generated curve – the higher the SOS, the worse the fit. Anything higher than 15 I automatically mark and remove from consideration. The baseline is a measure of how low the autocorrelation function (the curve that the radius is extracted from) finally reaches – it should reach to 1. Anything below 0.995 or above 1.005 I discard and do not use, it means the curve is not good.
      3. **NOTE**: For buffer, you want an intensity of ~70000. You will likely get all marked samples as there is nothing to fit. If you do not get 70000 or it fits it to a very large size, it means there is dust and you need to clean the cuvettes and/or filter your buffer.
17. Remove the water and clean the cuvette
18. Put your sample in the cuvette, load it into the instrument, and hit run
19. Analyze your data
20. Mark anything with SOS>15, and anything outside of 0.995-1.005 for baseline. You can mark based on %Pd as well, anything that says polydisperse I remove
    * 1. NOTE: to mark something, right click on it and hit mark
      2. **NOTE**: You ran 20 acquisitions, if >10 are marked you need to re-do that sample.
21. Select the curve icon. This will bring you to the fitting itself, you can select regularization or cumulant fit
    * 1. NOTE: Regularization fits multiple species to your curve and will fit better. Cumulant fit treats it as one sample.
22. Select the histogram icon. This will bring you to a bar graph with the radius and MW, and the % of each species that fits to the value.
23. Export your data
    * 1. NOTE: You can export the table and the fit data by right clicking and hitting export (it will save as a csv file). You cannot export the histogram, but that is the number that we care about the most. Write that information down.
24. Save your file to the Chen Lab folder.