LC Startup

- Change the in-line filter upon changing buffers or change after ~60 days of use
- Filter mobile phase using a 0.1 micron filter for aqueous buffers and a 0.02 micron filter for organic solvents
- Wyatt recommends using 200 ppm Sodium Azide (NaN3) for aqueous buffers
- Adjust the flow rate in 0.1 ml/min increments, allowing pressure to stabilize after each increase
- Upon storing LC for more than 2 days, change to filtered water containing ≥ 20 % EtOH
 Be careful to flush LC with miscible solvents ie. Toluene →100% EtOH → 100% H2O

DAWN HELEOS/TREOS

- Turn on Laser using the main LCD display
- Run the COMET (if applicable) while the instrument is warming up (~10 min)
- Upon starting, the 90° angle should be ~ 0.02 V or less (in aqueous systems)
- Turn off the COMET before starting run (COMET running time can be set in System window)

*Check the following instrument parameters in Astra Configuration:

- ✓ Make sure that your physical instrument is selected when starting a new method
- ✓ Normalize the detector for any new mobile phase (NIST BSA <u>https://srmors.nist.gov/view_detail.cfm?srm=927d</u>)
- ✓ Set calibration constant for MALS detector (calibrate detector with filtered Toluene about every 6 months)
- ✓ Set solvent refractive index (can be determined from the Optilab rEX)
- ✓ Set DNDC of sample (<u>www.ampolymer.com</u>) protein DNDC is ~0.185

Optilab rEX

(This instrument should not have backpressure so make sure outlet tubing is 0.030" ID or greater)

- Adjust temp to desired setting and allow instrument to warm up for ~30 min
- set the purge button to ON on the main LCD display during any change in buffer
- Adjust the LED intensity under the System Tab so that it is ~7.6 volts (adjust max power % manually)
- Turn off purge and zero instrument using the main LCD display
- Re-purge and re-zero the instrument until the drift is less than 5.0 E-8 RIU/min

*Check the following instrument parameters in Astra Configuration:

- ✓ If using the RI detector for your concentration, make sure that you select the RI detector in Astra 6
- ✓ Make sure that you select your physical instrument when starting a new method
- ✓ Set volume delays (alignment) for each new concentration detector

QELS (only for internal QELS)

- Calculate the solvent viscosity to the MALS temp on the front LCD display using SEDNTERP (<u>www.cauma.uthscsa.edu/software</u>)
- Turn on the QELS using the front LCD panel, under the QELS tab
- Make sure that the Dither is in the on position
- *Check the following instrument parameters in Astra Configuration
- ✓ Set the correct viscosity for your solvent (correct for the changes due to run temp using SEDNTERP)

UV Detector

- Turn on the lamps and allow them to warm up for ~30 minutes
- *Check the following instrument parameters in Astra Configuration
- ✓ If using the UV detector for your concentration, make sure that you select the UV detector in Astra 6
- ✓ Set the appropriate wavelength for your analysis
- ✓ Set the correct AU/Volt, Path Length and extinction coefficient (careful of the units, ASTRA 6 uses mL/(mg cm))
- ✓ Set volume delays (alignment) for each new AUX detector

Viscostar

- DO NOT START THE LC WITHOUT THE VISCOSTAR BEING POWERED UP
- When running the system, both the IP and DP purges must be off
- Increase the LC flow rate slowly so that the DP pressure remains on scale (± 0.73 psi)
- Set the flow rate to desired value, allow the instrument to stabilize, and zero the DP (Viscostar total volume is ~28mL)
- Each week, purge the IP and DP separately with ~10 mL of solvent and re-zero the DP
 - **When changing solvents, activate the DP purge from the main LCD display and flush instrument for 15 minutes at a flow rate of 0.2 mL/min. After 15 minutes, activate the IP purge and flush both for another 15 minutes. Set the flow rate to 0.1 ml/min and disable the IP and DP purge. Follow the startup procedure.