General Fluorescence Quenching (Tara)

1. **Cuvette**

- Store in MilliQ plus cuvette soap (use new soap every other day/every couple days)
- Before use put cuvette in a beaker with MilliQ then add soap (if preparing a fresh soap solution)
- Heat for at least 15 min in oven @ 40°C

2. **Sample Prep**

- While cuvette is heating prepare sample solution and metal spiking solution
    - NOTE: I used 25 mL for my sample
- Fluorescence standard (Southampton) if using to monitor intensity

3. **Prep for downstairs**

- Take cuvette out of oven, rinse with MilliQ and wrap in kim wipes
- Ensure you have everything you need to bring to the instrument room with you (if working in the 4th floor instrument room then disregard below list)
- This includes:
    - Kim wipes
    - MilliQ bottle
    - Cuvette
    - Pipettes (4 for pH adjustment, 1 for sample)
    - Pipette bulb(s)
    - Waste beaker
    - Micropipettes and corresponding tips
    - pH calibration buffers (4, 7 and 10)
    - HCl and NaOH for pH adjustment (only probably need the 0.1 M solutions)
    - Timer
    - Stir bar, beaker with sample
    - USB for saving data

4. **Running the experiment**

- Calibrate pH electrode (Follow manual directions)
- Ensure sample is proper pH and stirring
- Set up the Fluorescence spectrometer using the same settings as previously used (see **Section 5** for typical set-up)
    - If running for the first time, run your blank (see below) and then alter settings after you run your first sample in order to obtain a good intensity (Typically a higher intensity – aim for around 750-900 Intensity)
- Run a blank (just MilliQ) first to ensure no contamination
    - If you see a peak around 350ish or where they should not be then just put the cuvette back into the soap and MilliQ solution and into the oven for another 15 minutes and try again. If this doesn't work ask Holly to help you sonicate the cuvette. (Raleigh scattering will be seen typically at the higher emission wavelengths (above 500 nm) – this is NOT contamination)

- If blank looks good then tap cuvette dry
- Rinse cuvette three times with a little bit of your sample, returning liquid back to sample beaker after each rinse
- Run a scan of your sample (without any metal addition) and repeat the scan three times. Repeat for all other additions, waiting 15 minutes after each metal addition and ensuring pH is constant before each measurement.
- Titration is considered complete when there is no significant decrease in fluorescence intensity with increasing metal concentration
- NOTE: Aim for around 10 metal additions for a total titration

5. Fluorescence Spectrometer Set-up

a. On desktop double click **Scan**

b. **Setup**

Ensure **Emission** is clicked
Excitation: 270 or 275
Start: 300
Stop: 600

Slit width: 5nm (for both ex and em)*
PMT: High (or manual if you need to adjust signal)*

* Can adjust for better intensity signal

c. **Accessories (if an option)**

Multicell → # 2 (or whichever place you want to put your cuvette in)

d. **Autostore**

On: Prompt on start (ASCII csv)