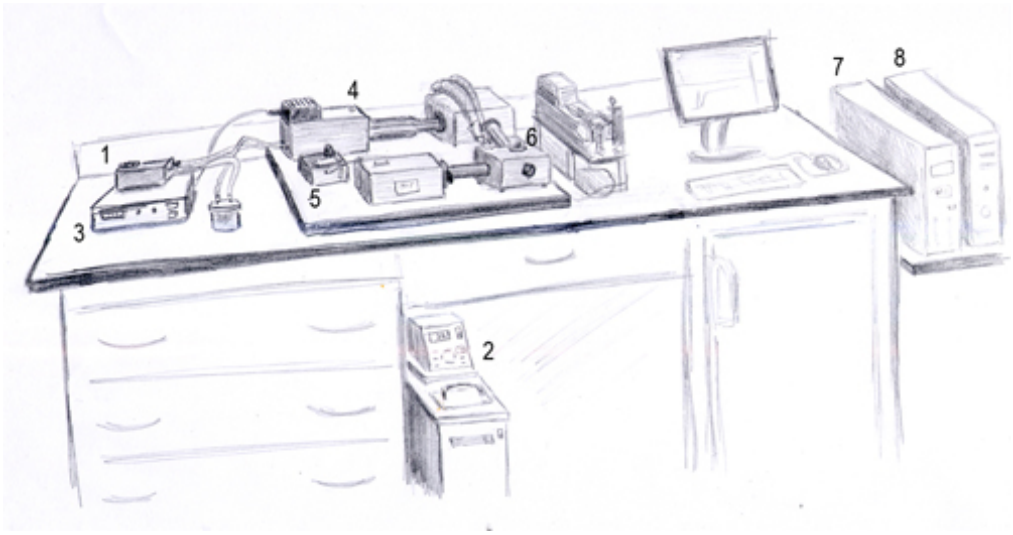
Using the Spectrofluorimeter

POWERING ON AND SETTING UP:

1. Turn on cooling box (1)
   Let run for 5-10 minutes
2. Turn on incubator (2) switches in the following order:
   Lowest switch on bath; highest switch on box; on/off button on box
   Let incubator run for ~5 minutes
3. Turn on computer (8)
   *Do not open Olis Globalworks program until AFTER photon counter and control box are on
4. Turn on "Power" to control box (3)
5. Push and hold "Ignite" on control box; hold until green light (4) comes on
   You want the control box to display 147-148 watts steadily
6. Turn on photon counter (5)
   *** THE COUNTER SHOULD NOT BE EXPOSED TO AMBIENT LIGHT
   Make sure all four manual slits, ESPECIALLY the one near the photon counter, are pushed in so no ambient light can enter and damage it.
7. Turn on Olis computer (7)
8. Place the cuvette holder in the well (8)



1. Cooling box 2. Water bath 3. Control box 4. Green light	5. Photon counter 6. Well 7. Olis computer 8. Computer computer

Using the Spectrofluorimeter

DATA COLLECTION SETUP

Program: Olis Globalworks
Click Data Collection tab
Open "DM45"
If: "The masterboard has timed out," then: "Retry" until functional.

Menus:

1) Parameters
   Where you change hardware settings
   Two PMTs and the photon counter (PC)
   "Hardware reset and calibration" if stuff isn't working
1. Operational Modes
   Where you can tell it about the assay
   Data Reduction Mode: Always "Photon Counter"
   Data Collection Mode: "Assay" (time-based)
2. Live Display
   Set "emission" = "excitation" = 310 nm
   Set duration of assay to 300s for FtsZ
   Set number of data points and frequency of data point collection, usually every 0.25 s
   *make sure (# data points)(frequency data taken) = (duration of assay)

Saving Files:
At the end of a reading, the Globalworks should automatically prompt the user to post data.
If it does not, click "Post these data" in the top right of the program window
Select the trace by clicking on it
Right-click the desired trace and save as an ASCII file **Using the Spectrofluorimeter**

DATA COLLECTION

Loading and Reading

1. Pre-chill all buffers
2. Using a gel loading tip, wash out the cuvette well with 2-3mL of water before initial use; take care to fill the cuvette fully and not to

overflow it. Hold the cuvette by the top and bottom, or by the corners.

3. Wash out the cuvette with buffer using gel loading tips 2X between runs.
4. Load all buffers, load protein, pipet well to mix.
5. Load any other proteins, pipet well to mix.
6. Final volume must not exceed 200 mL; this includes later additives like GTP
7. Wipe off cuvette with lens paper
8. Load into spectrofluorimeter, with "Q" on cuvette facing the left (for consistency)
9. On the computer, click "Collect Data," then press the spacebar
10. Let the first 30-60s pass to get a baseline reading; in the meantime, empty the old cuvette
11. After 30-60s, add 2mL GTP directly to the cuvette in the spec and mix well
    Brad uses a gel loading tip; Dan and Amy switch to a larger tip to mix
    Do not put the tip too far into the cuvette as this will affect the reading
12. Wash the old cuvette during the remainder of the assay