11-06-25

Yesterday each of us found a different Chl/Car, Chl a/Chl b and chlorofyl concentration:

	Mads	Koen	Eva	Jasmyn
Chl/Car	4.18	3.86	4.14	4.19
Chl a/Chl b	2.78	2.77	2.37	2.59
chl concentration ↳ mg/mL	0.63	1.59	0.86	1.24

ABSORPTION

- Today we started with preparing our samples for an absorption spectrum. We prepared tubes with 3mL B_1 and a bit (about half a milliliter) of our concentrated thylakoids
- We moved to the spectrometer in the dark room and installed an integration sphere. (For measuring the scattering as well)
- We put our samples into the spectrometer and retrieved graphs we will analyse later today. We saved our samples for fluorescence

FLUORESCENCE

- We determine fluorescence at 77 K (-196.15 °C). The trapping to the RC will be less efficient at this temperature, so we will measure more fluorescence.
- To do this we retrieved liquid nitrogen from the -1 floor. We carried it up the stairs because taking it into the elevator is a safety hazard.
- We set up the machine for low temperatures.
- We probe our samples from the absorption in a thin glass pipette (we put them carefully into the liquid nitrogen, otherwise the glass would burst) Then we quickly transported the pipette into the machine (into a vacuum tube filled with liquid nitrogen)
- We retrieved our graphs and could directly see the fluorescence (between 730 nm and 750 nm) Data-analysis we will do this afternoon.

Data analysis

- we began making 5 graphs of our own plant and 3 graphs of all of our plants. In this way we can interpreter our measurements. The graphs are normalized.

1. Emission plant vs absorption plant (room temp)
2. Absorption plant vs absorption AT (model plant)
3. Emission plant vs emission AT (room temp) + difference
4. Emission plant vs emission AT (low temp) + difference
5. Emission plant room temp vs low temp
6. Absorption all plants
7. Emission all plants (room temp.)
8. Emission all plants (low temp.)

The code in python is uploaded in Github.

We didn't finish all the graphs today, so we finished them at home.

1.24 mg/1mL, we need 0.5 mg

J $mL = \frac{0.5}{1.24} = 0.4$ mL

K $\frac{0.5}{1.59} = 0.314$ mL

M $\frac{0.5}{0.63} = 0.79$ mL

E $\frac{0.5}{0.86} = 0.58$ mL

- We put these quantities into tiny tubes. Pipet up and down to remix the thylakoids. Add 400 µL of Sol 1 ~~then~~

See protocol 'Sucrose gradient'

- 250 mL S3
- S2 to 0.5 mL (can be a bit more)

We ~~finished~~ finished the day with putting the tubes in the ultra-centrifuge at 40000 RPM (~205000 g) for 17h at 4°C. Tomorrow at 10:30 we'll continue with the gradient. One was a bit low concentrated and might fail. We discuss the graphs tomorrow morning.