

AMR Placement Notes

23/08/24

Values I require

$R_0$ ,  $V_0$ ,  $T_0$ ,  $r$  from Vol to detector,  $I_0$ ,  $I_0$   
 $n$ ,  $\Delta n/dc$ ,  $c$ ,  $\text{MM}$ , flow Rate, ~~path length~~  
↓  
ref. index ↑ Molar Mass calculations

↓ Rh calculation

Flow Rate in mL/min

 $\epsilon$  = extinction coefficient in  $L/(mol \times cm)$ 

path Length in cm

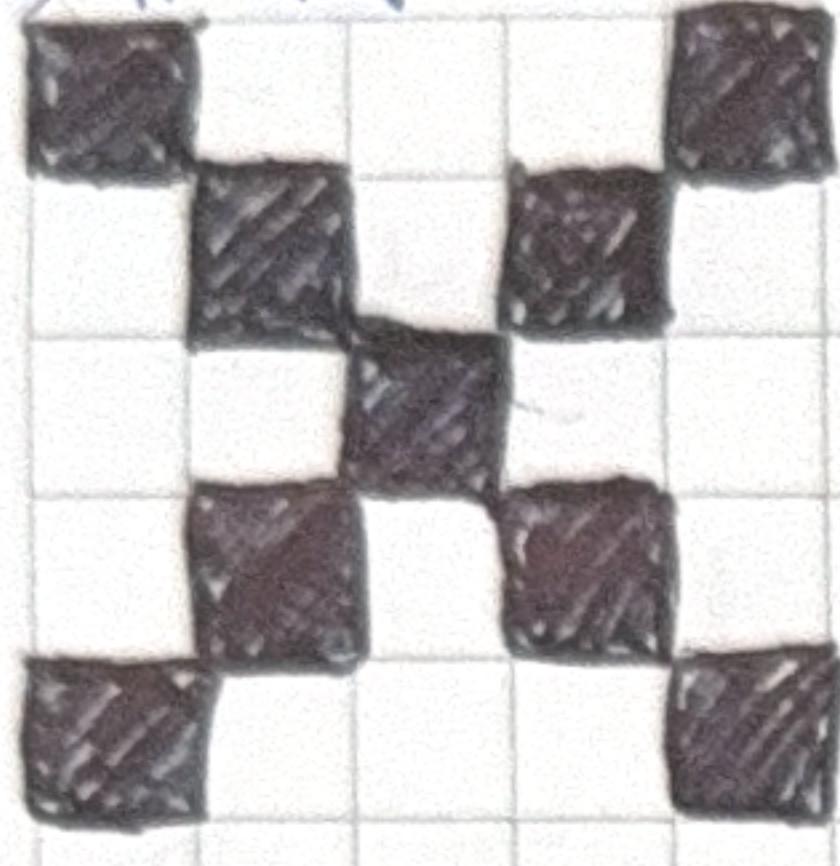
b a c b a

W3 - 1 - one to many 2 - min = 1 3 - '0' 4 - assoc. entity  
5 - binary XW5 1 - WID (q) 2 - Tab name 3 - try ms En  
4 - enID (pk), EnName, EnHealth, EnType  
5 - 5

Page I.

AMR 0.

Friday 30/08/21



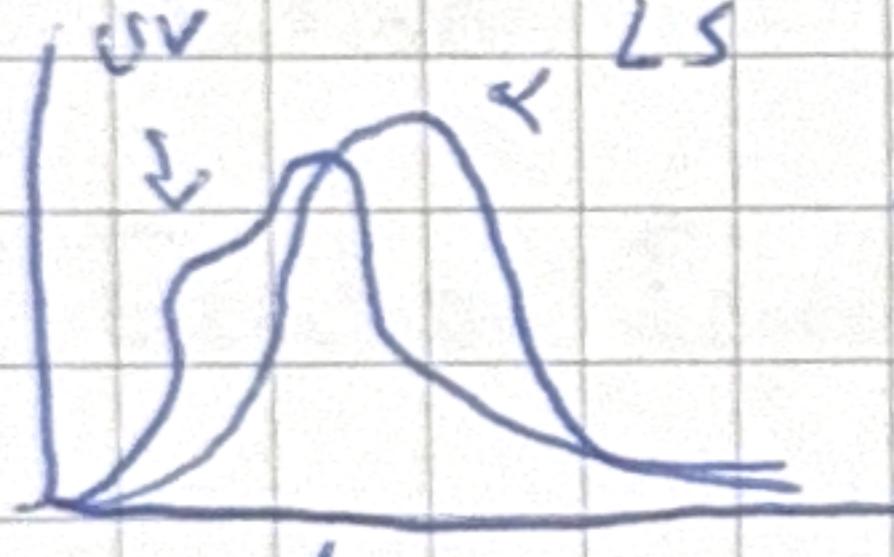
Started with checking the data from yesterday's overnight run AFY separation & 20nm polystyrene with SDS solvent.

Purpose is to compare data from AFY with the DLS machine.

Current issue is that Mar-Dean does not have a validated AFY method, so more in depth method development is required.

The data for the 100 nm particles is a lot nicer than the 20nm ones. 20nm have a lot of UV absorption issues - unknown why?

The 60 nm sample had great graphs - what's the issue?

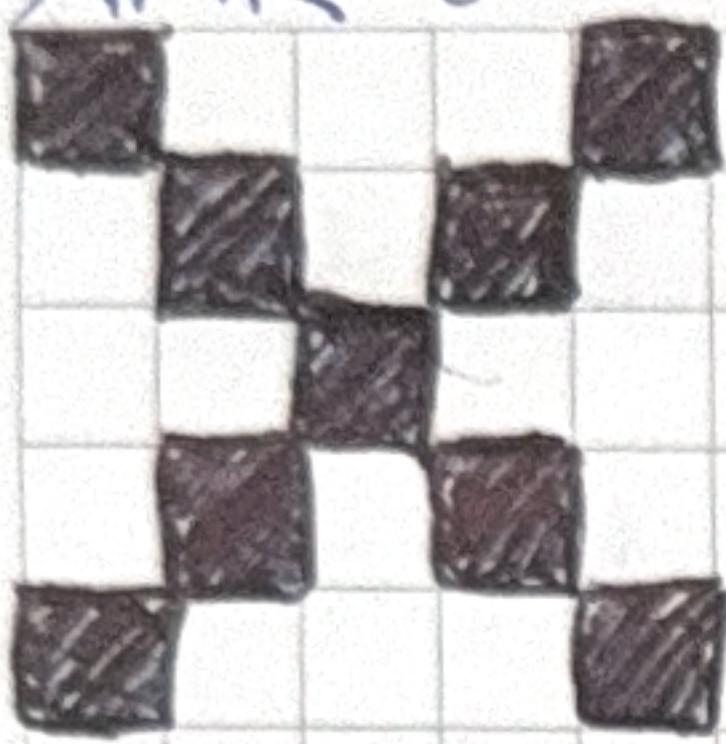


← issue is peaks follow diff patterns. UV is d-t

Mar-Dean will try to emulate tested BSA method & see if that helps. BSA is ~5nm, so that 'should' work on larger PSL molecules.

We found PSL method in the Wyatt handbook for 50nm. Will try, then compare to existing runs.

Friday 30/08/24



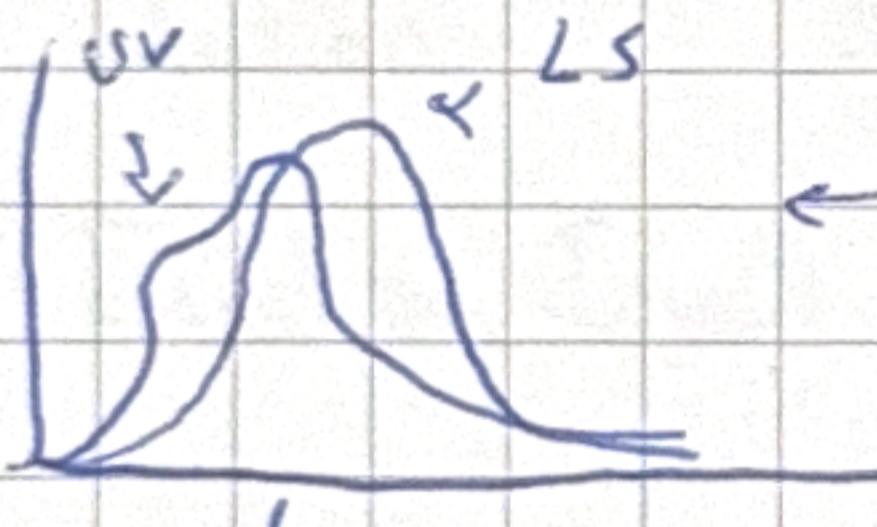
Started with checking the data from yesterday's overnight run <sup>AF4</sup> Separation & 20nm Polystyrene with SDS Solvent.

Purpose is to compare data from AF4 with the DLS machine.

Current issue is that Mar-Dean does not have a validated AF4 method, so more in depth method development is required.

The data for the 100nm particles is a lot nicer than the 20nm ones. 20nm have a lot of UV absorption issues - unknown why?

The 60 nm sample had great graphs - What's the issue?.



← issue is particles follow diff patterns. UV is diff

Mar-Dean will try to emulate tested BSA method & see if that helps. BSA is ~5nm, so that 'should' work on larger ~~PSL~~ PSL molecules.

We found PSL method in the Wyatt handbook for 50nm, will try, then compare to existing runs.

## The method -

Taken from Wyatt handbook - PSL long Channel Method.

Duration (min) X-flow starts (mL/min) X-flow end (mL/min)

1. Elution	1	0	1
2. Elution	2	1	1
3. Focus	3	1	1
4. Focus Inject	3	1	1
5. Focus	6	1	1
6. Elution	30	0.50	0.50
7. Elution	5	0.50	0.00
8. Elution Inject	10	0.00	0.00

Step 1 was added by ~~M-D~~, M-D, as the machine tends to produce better results when allowed to ramp up.

Step 6. n Time upped from 20 min  $\rightarrow$  30 min

Write notes on what each stage actually means, can use in my report (?).

Machines - UV Detector  
Agilent Infinity II - 1260  
Pump - G7111 A  
G7115 A - 1260 b.p.d WR

Friday 30/08 Riffle Coffee Samples

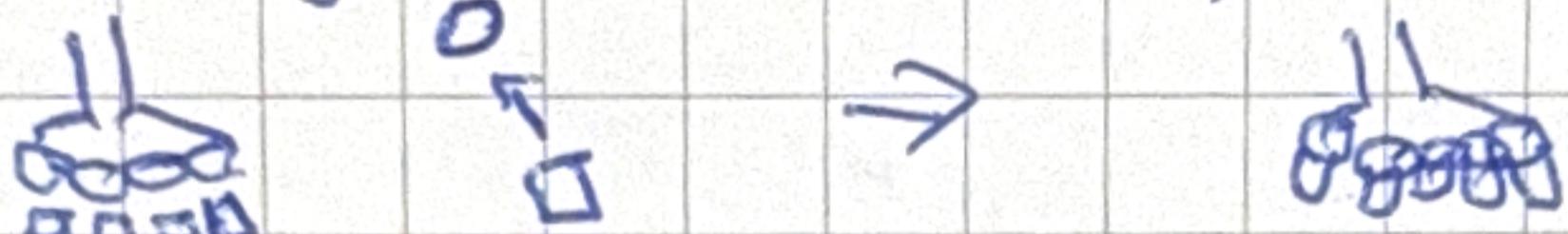
Why - Want to get a true representation of a bulk sample, removing bias that is added during things like transport.  
e.g. Truck driving = vibration = coffee gets displaced.

Steps 1. Label everything

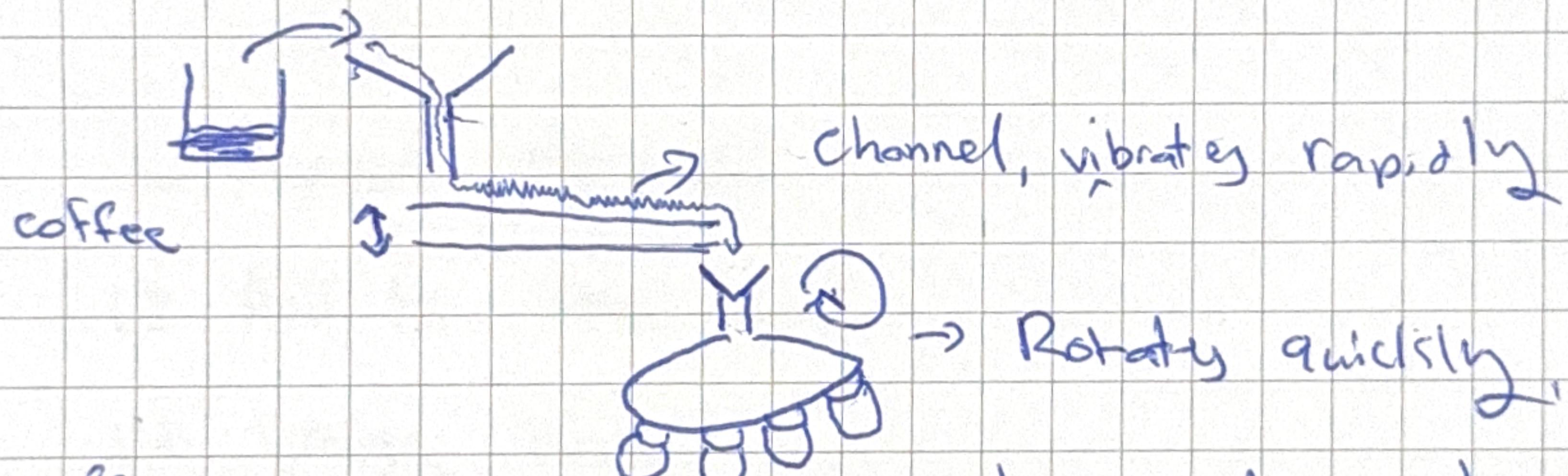
2. Weigh the coffee

3. Weigh the jars

4. Line up jars with riffler, attach all 8



5. Add Sample by the spoonful to ~~spoon~~ funnel



Coffee splits over channel due to vibrations, travels along channel then into spinning funnel separating to 1 of 8 jars.

Idea is by spinning, jars collect more even distribution of the coffee particles that are bounces along the channel.

Sometimes, coffee builds up after channel  $\xrightarrow{\text{if}}$

Possible reasons - coffee heats up (from vibration & channel getting hot)

$\Rightarrow$  Adding sample too fast.

30/08 Coffee Riffle Exp.

Step 6 brush any remain sample into channel

Step 7. Turn off the machine after all position sample & riffled.

8. Remove jars from rotator

9. Weigh full jars

10. Pour into plastic jars

11 choose 1 sample to split into a further 8 vials

12. Repeat Experiment

13. Add to small vials

14. Weigh Vials

Sep 6<sup>th</sup>, 2024 - Friday. 10:15 am

Started day in lab by placing AF4 machine in isopropanol for the weekend.

Aim: Gain a better understanding of the DLS machine & DLS techniques.

Assist in preparing 10mMol NaCl soln.

~~I~~ I prepared 10mMol NaCl, filtered with vacuum.

I then pipetted 20nm PSL into 4 cuvettes.

20nm PSL in NaCl

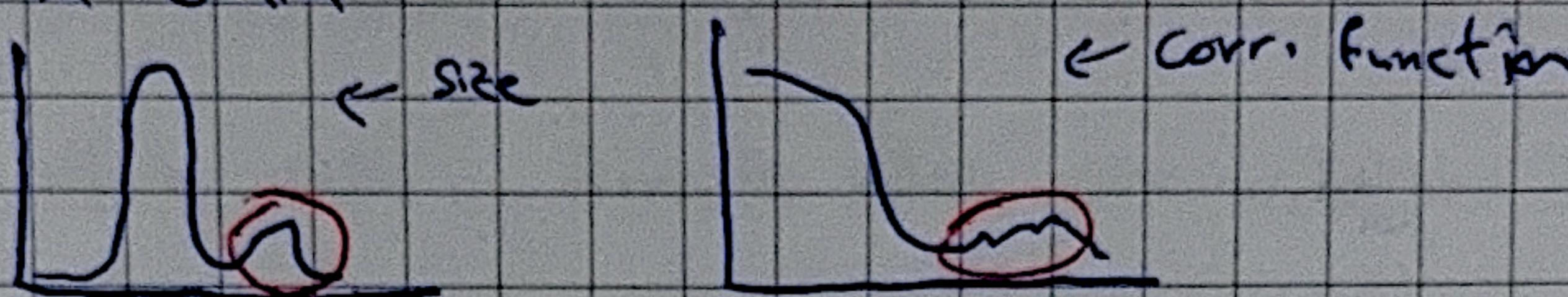
$\frac{1}{10}$ ,  ~~$\frac{1}{50}$~~ ,  $\frac{1}{100}$ ,  $\frac{1}{500}$

MD then explained the DLS machine & software:

Look into: Can you get concentration from FFF without an RI detector?

$2.17 \text{ g cm}^{-3}$

Our first Sample run of  $\frac{1}{10}$  concentration returned with  
issues in data



These indicated an issue with the sample, most likely  
due to dust / impurities.

We put through the F50 Sample, if issue persists,  
M-D to create new samples next week.

13 Sep 2024

- Start with running DLS analysis experiments on freshly prepared 80 nm PSL in NaCl Sample.

Issue with last week's sample was contamination (dust).

- There were issues with the FFF machine; pressure is higher than expected, though still below major concern levels. May flag if we cannot resolve. Potential causes:
  - air introduced while changing filter.
  - pipes compressed
  - contaminated solvent

Changed solvent from water to IPA (Iso-Propanol), as IPA can dissolve bubbles, will check outcome.

### DLS

M-D started filling out data tables to quantitatively compare the amount of data generated on the DLS machine.

So far, M-D has developed a method that is very good for monomodal 100 nm particle samples, and an 'OK' method for monomodal 20 nm particles.

She wants to develop a method that works well for both individually, with the assumption that that means it will work well for a bimodal 20/100 nm sample.

Once happy w/ DLS, move onto FFF.

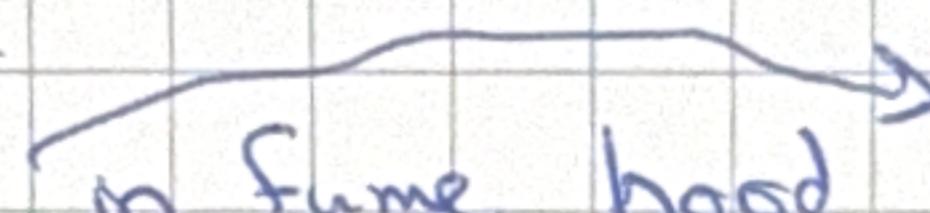
13.09

Watch Dark Waters - about PFAS

Read 'this is why you dream' ~, & 'Darwin's Doubts'

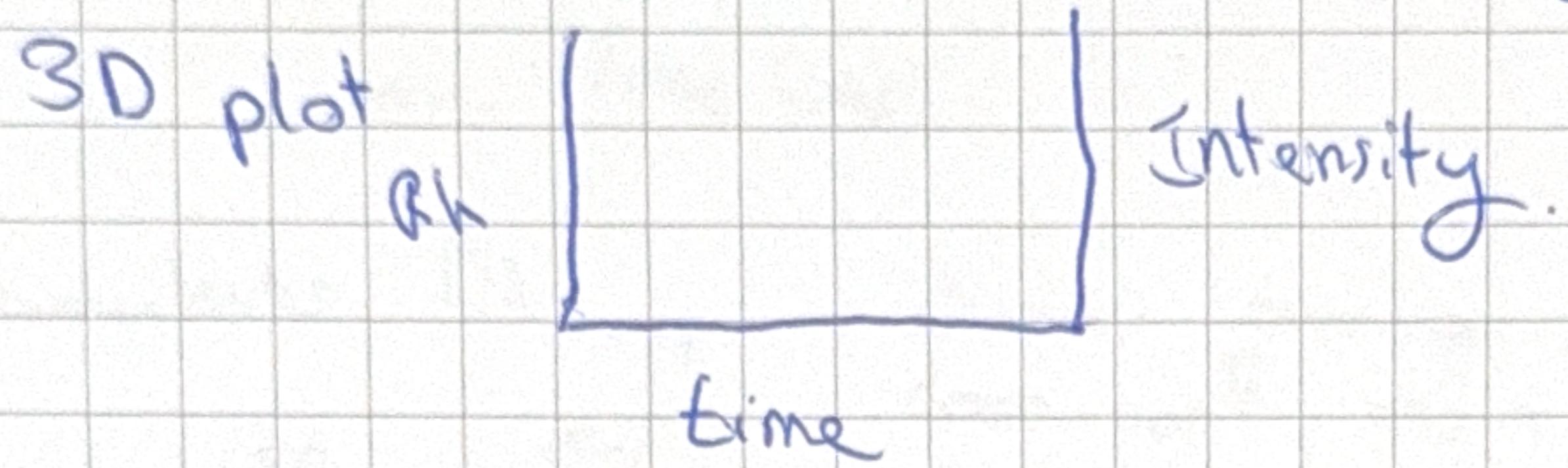
DLS Sample Issues + ~~\*~~

We re-prepared the ~~the~~ 20 nm sample, this time we did this differently:

- Compressed air blasted all vials
- Rinsed NaCl beaker
- Prepared all cuvettes in fume hood  9 vials
- Kept cuvette caps in sealed container.
- ~~Did~~ Did the cuvettes in order, but labelled after the particles were ~~the~~ pipetted inside, to minimise total time that cuvettes were uncovered -

13.09 ~~EE~~ Data Analysis Assistance

M-D requested help with some graphing / plotting:



$$k = 1.3808 \times 10^{-23} \text{ J K}^{-1}$$

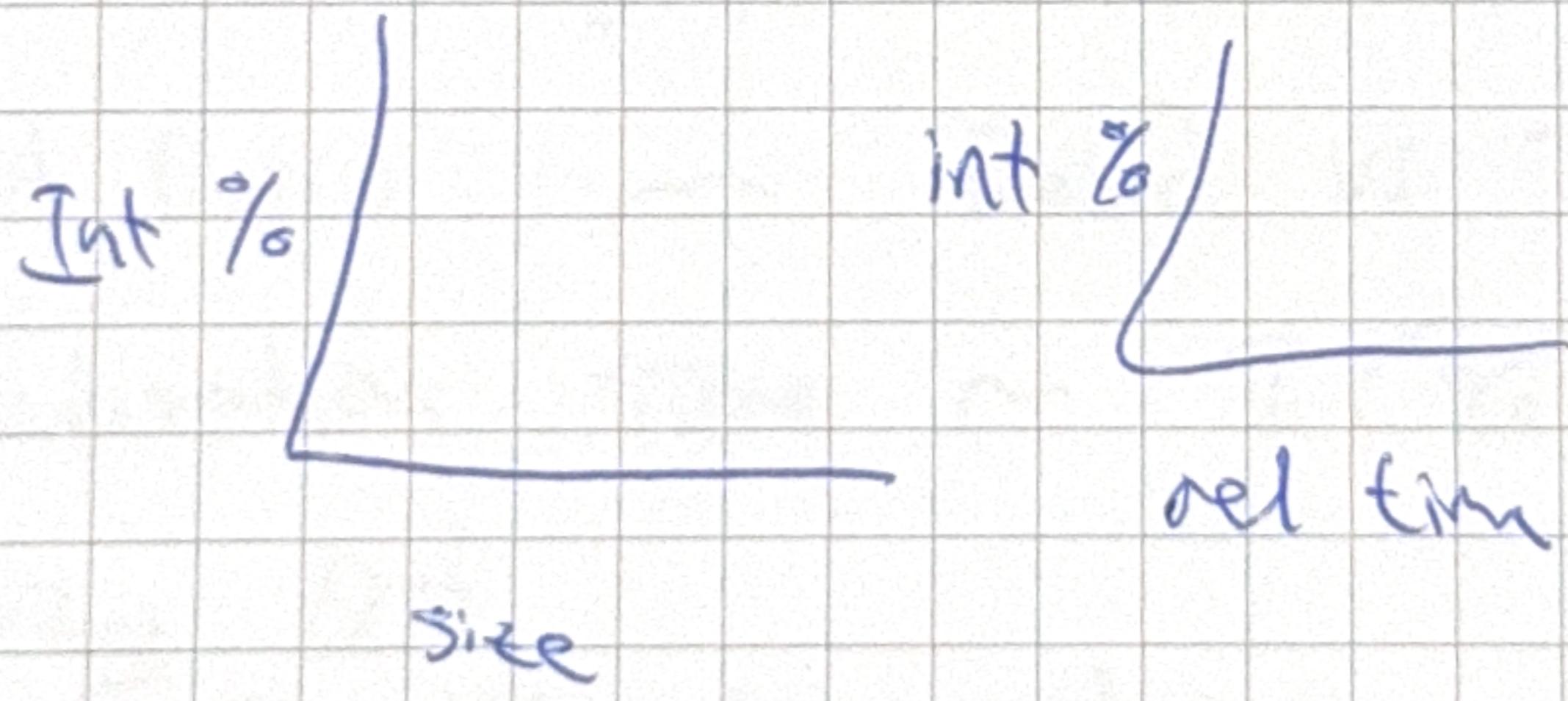
$$\alpha(H) = \frac{k_B T}{3\pi \eta D}$$

translational  
 $D = \text{diff. coeff.}$

$T = \text{abs. Temp}$

$\eta = \text{viscosity}$

- Row data table:
  - . quality indicator
  - . concentration
  - .  $k_B$ ,  $R$  & std. E.
  - . Diff. coeff. & std. E.



Intensity  $\rightarrow$  Size graph -  
 Area Under

Corr Coefficient  $g_{q-1}$

Correlogram

corr Time (ms)

Cumulants fit

corr coeff

$g_1$

cum. analysis fit of  $g_1$ ,  
corr coeff. as a function of corr  
time

corr time

same  $t$ .

Cumulant Resid.

Residuals vs corr time ms

Distr. fit. Intensity % vs D.L.F. ( $\text{nm}^2/\text{s}$ )

Distr. fit  $\sim g_1$ , corrcoeff vs time

Distr. Resid. Resid. vs Time

Relaxation Time  $\sim I(\zeta)$  vs Rel. Time  $\zeta$

size D.L.F.

$I\% \text{ vs size d.nm}$ ,

Number % vs size d.nm

Vol % vs d.nm

Try: Size dist plot  $y_1$   
Time from cornellogram / cumulants fit.

2. Diff. Dist  $\rightarrow$  relate to size using SE eqn

$$D = \frac{k_B T}{6\pi\eta n}$$

$\Rightarrow$

$$\Delta H = \frac{k_B T}{3\pi\eta D}$$

Size nm =  $y_1$  I % =  $y_2$ , time =  $\tau$

$$\eta = 1.011$$

$$T = 20 = 293.15 \text{ K}$$

$$k_B = 1.380649 \times 10^{-23}$$

$$44.16 - 147.7$$

Wed 8/18/09

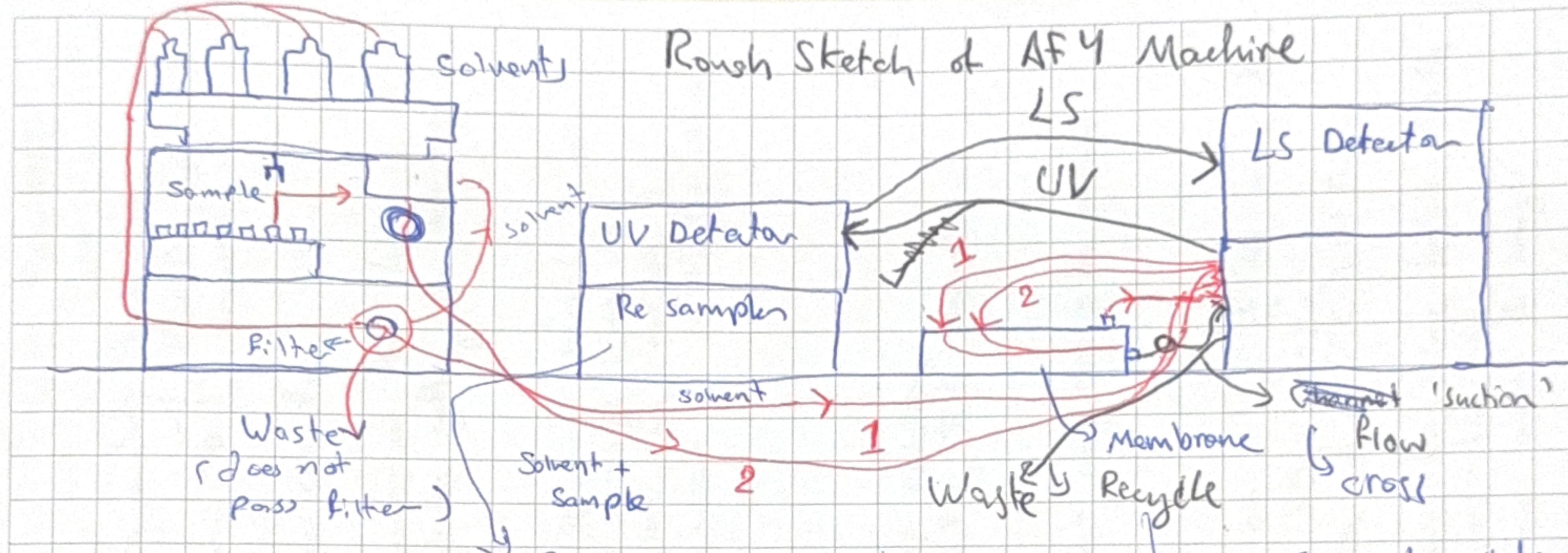
## Trouble Shooting Pressure Issues with FFF.

↳ Idea is that it's a filter problem.  
Why?

Issue is over past 2 weeks, pressure on FFF has steadily increased.

### Potential Reasons:

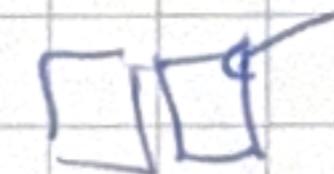
- Air introduced
  - Machine run through ~~isopropanol~~ iso-propanol, should have dissolved any bubbles.
- Filter scrunched up after being recently replaced
  - Have replaced filter.
- Physical contaminant
  - Maybe, though does not explain why P continuing going ~~down~~ up, # when in/out flows are at expected values;
- Issue with tubing:
  - We'll be disconnecting each channel separately to see what the issue is.



for Friday: ~~\*\*\*~~

VAMAS QCM & TM vials

Back Right Sample holder



- Add 200 μL to each & No -

Can separate peaks into their own sample vials.

Can recycle solvent

fr: 20/09

## Changing membrane (AF4)

### Method

- 10 kDa membrane - material: regenerated cellulose

Ref: ~~TM~~ ~~006~~

TN 6006

Handbook

Rev D, pg. 59

1. Soak in Milli Q water - leave for min ~~10~~ 1 hr onwards

2. Disconnect tubing from channel

3. Connect tubing to ~~to~~ 5-part union connector

4. Replace cross flow filter

↳ Used in case membrane has hole in it, to stop any particles getting into detectors / clogging tubing.

5. Take channel apart & rinse spacer, channel & frit

6. Align new membranes coated (shiny) side up

7. ~~&~~ Tighten screws according to instructions, ensuring that screws are tightened such that pressure is as evenly distributed as possible

### Why? / Aim:

- Further investigate pressure build up problem

- Learn more about membrane handling & preparation.

### Why Soak?

To remove any ~~stuck~~ fibers that may still ~~be~~ be present from production.

### What is a Dalton / kDa

(Unified atomic mass) unit (Da or u) =  $\frac{1}{12}$  the mass of an unbound neutral  $^{12}\text{C}$  atom.  $1\text{kDa} = 1000\text{ Da}$

The ~~real~~ atomic mass constant  $m_u = \frac{1}{12} m(^{12}\text{C}) = 1\text{ Da}$

### What does 10 kDa membrane mean?

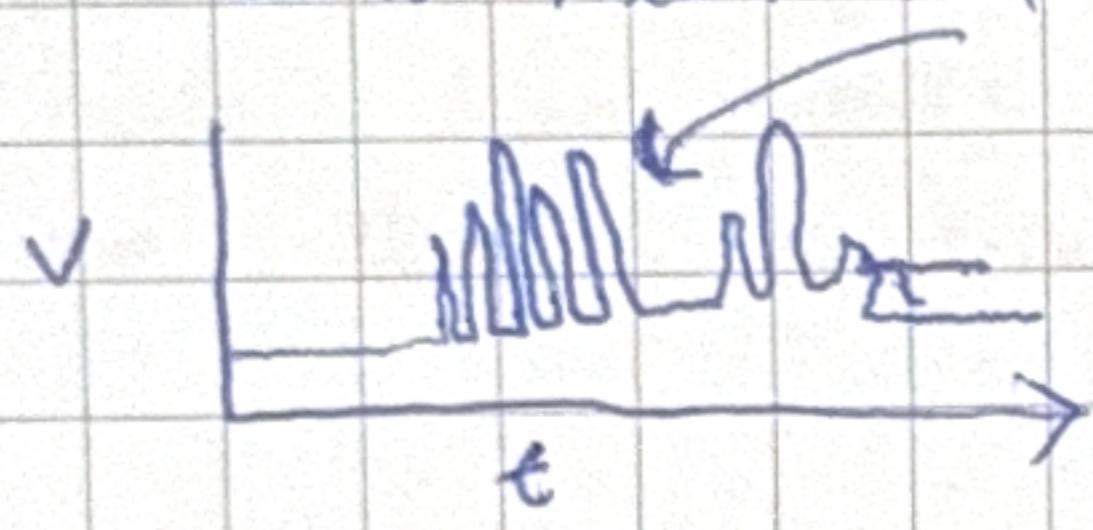
That is the 'cut-off' of the membrane. Anything below 10 kDa will pass through membrane, into cross-flow thin waste. Anything above will not. Should be a few orders of mag. Smaller than particles used in machine -

2019 Fri.

### Outcomes:

- Pressure continued to climb. 16:00 - 10:20.
- Good indicator that channel ~~was~~ was not the issue.

- 10:40 - Switched to water, pressure down <sup>as expected</sup>  
 - Although pressure is going down, a few spikes showed up on the detector, usually indicative of bubbles. This could mean that issue is resolved if pressure remains down.



Leak detected with machine ~ 12:05. Upon returning with newly replaced membrane channel, we found machine had turned off, with a pump leak ~~detected~~ error.

On closer inspection, ~~&~~ the recently replaced filter in the pump section ~~had~~ was identified as the source-

While unscrewing to replace, ~~M-D~~ observed that the screws were looser than expected - the most likely cause -

Filter replaced. Observed for a while, no ~~leak~~ further leakage observed.

25/09/24 SDS Sample Prep

1. Measure 0.5 g SDS  $\rightarrow$  in 1 L graded bottle  
(in fume hood)
2. Add water to beaker
3. Transfer water from beaker to bottle in fume hood
4. Continue until water ~~line~~ line hits 1 L mark
5. Add magnetic stirrer to bottle & place on stand
6. Assemble vacuum filter
7. Attach to vacuum bottle
8. Add small amount of ~~water~~ solution to it  
to check for leaks
9. if no leaks, continue till done

Th. 10/10/24

## Disc Centrifuge

Began the day w/ Asa showing me the principles of the centrifugal separation machine

Core concepts:

- Requires a gradient of Sucrose to 'control' the ~~fall~~ motion & the particles
- Density, not size based
  - Densest particles travel fastest / first
- Pros:
  - Quick (relatively)

~~Conns~~ Investigate Uses, pros/cons & Physics behind why it works (eqns - as well.)