Seial Dilution Scheme

TL;DR

I want to dispense my compounds using serial dilutions because thy're easy. Here's how.

Background

- The last set of tests showed that plate type probably doesn't matter, they all looked similar
- The plastic of the plate might be absorbing some of the short wave light, I'll try to confirm that in this test
- In the 2018 tests, I had trouble mixing fatty acids dispensed by Echo with the assay mix, I think I'll have more luck with a serial dilution

Plan

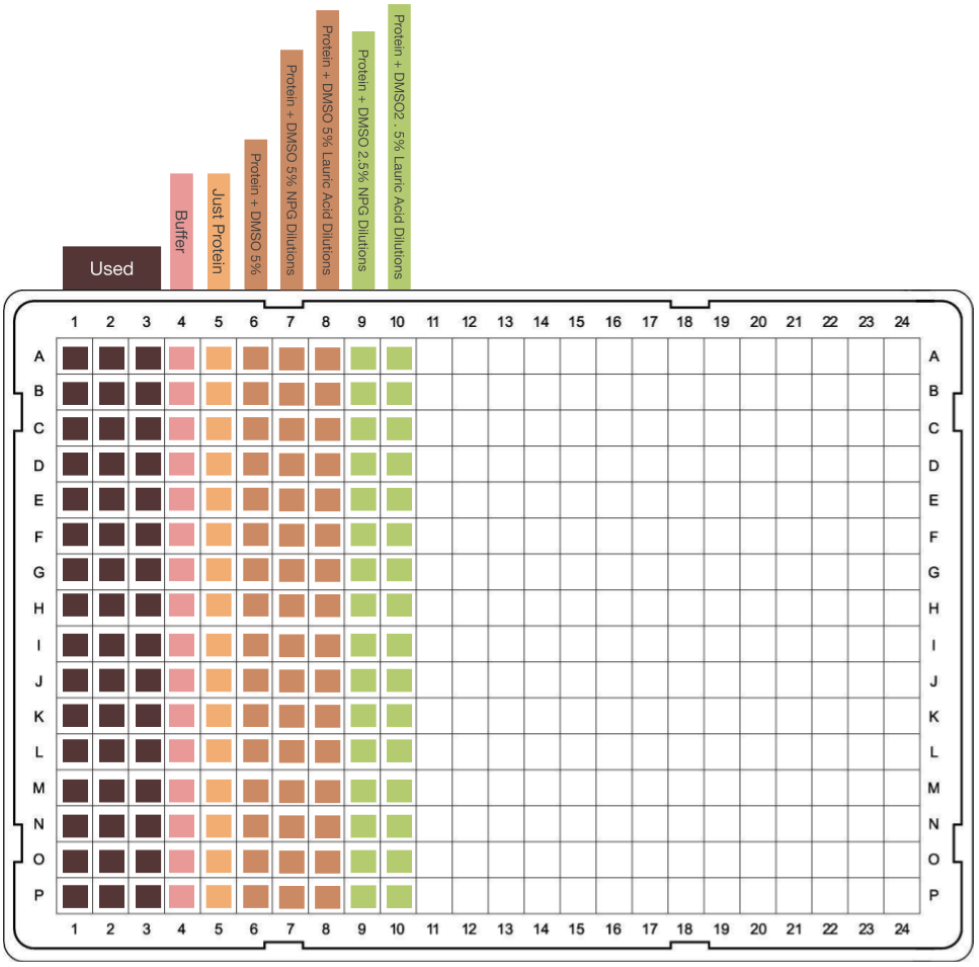
Here's what I want to keep fixed and why:

- `[Protein]` at 10 uM - Gives a good signal to noise ratio, not far off what we'd use for a classical titration
- `Conc of DMSO in each well` - DMSO can interact with P450 BM3, so I need to control this variable
- `Well Volume` 50 uM - suggested working volume for plates, seems like it gives a good signal
- `Number of substrate concentrations` 7 seems fine

Here's what I'll vary:

- `Substrate` - I'm trying Lauric Acid + N-Palmitoglycine
- `[Substrate]` - working range shouldn't exceed 10 mM in DMSO and 5% v/v DMSO in final mix (because vendors sell compounds in 10 mM DMSO and because 5% DMSO is about the limit of what BM3
- `Time Points` Just in case this thing is time sensitive, I really should know

Plate layout:



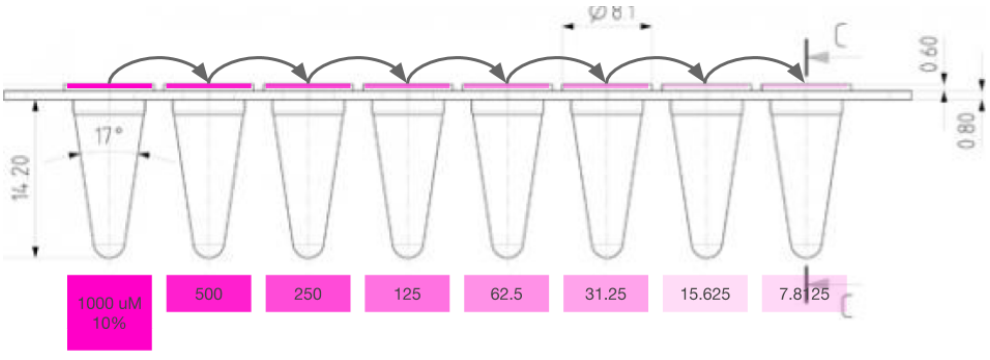
Looks cool. I think I'll do my serial Dilution in a PCR 8 strip and do repeats of each conc, which will fill a column.

Serial dilution scheme

For this test I want to keep my DMSO conc constant. For now I'll do the easy sounding option which is equal vols of double concentrate protein and substrate, each in the same buuffer. That means my starting protein stock has to be 20 uM.

I'm doing two stocks of each substrate - one where the final DMSO conc is 5%, and the other 2.5%. The DMSO stock of compound has to be 10 mM because that's what vendors sell the compounds as. So for each compound I'll do a 5 and 10% v/v 10 mM Compound in DMSO, in assay buffer. That means that the working range of compound conc is 0-500 uM for the 5%ers and 0-250 uM for the 2.5%.

Here's what the master stocks will look like for the 5% mixes:



These will get diluted by half again in the plate with the stock. The 2.5% ones are the same sort of thing, just half concs.

Lab Notes

6th June

Diluted the protein stock (BM3 WT heme, same as in plate selection from yesterday).

```
>>> conc = 983.4087522105264
>>> 6*16*25 # 6 rows times 16 cells per row times 25 uL of protein stock per well
2400
```

```
>>> 2400*1.5 # Times a safety margin
3600.0
>>> 3600.0*20 # 20 uM protein
72000.0
>>> 72000.0/conc
73.21472362144117
```

I measured the absorbance of this thing on a spec here. At some point I'll write a script to do concentration checks for me, but for now:

```
>>> a420 = 1.698913574
>>> extinction_coef = 95
>>> a420/extinction_coef
0.01788330077894737
>>> 0.01788330077894737 *1000 #mM to uM
17.88330077894737
```

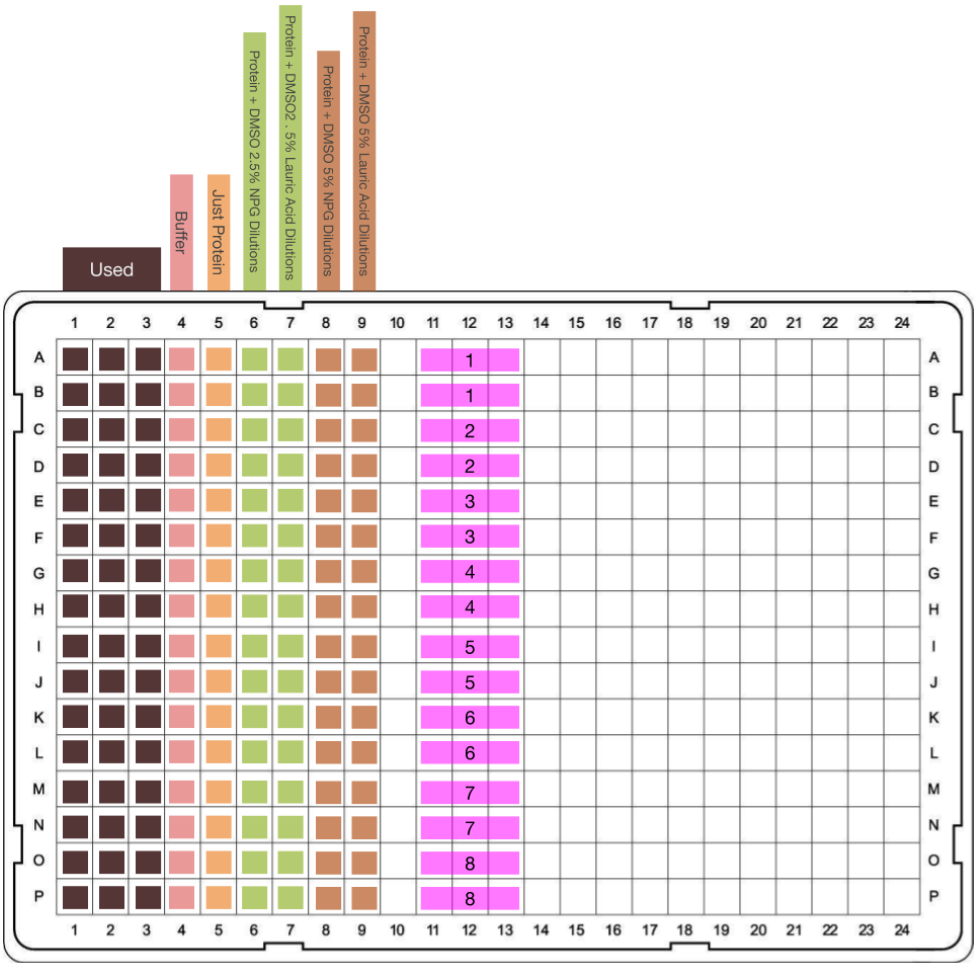
17 uM then, which will be diluted in half to a final concentraion of:

```
>>> 17.88330077894737/2
8.941650389473685
```

I diluted the compounds from old 10 mM stocks in DMSO. Might do it properly next time. Here's everything in mM

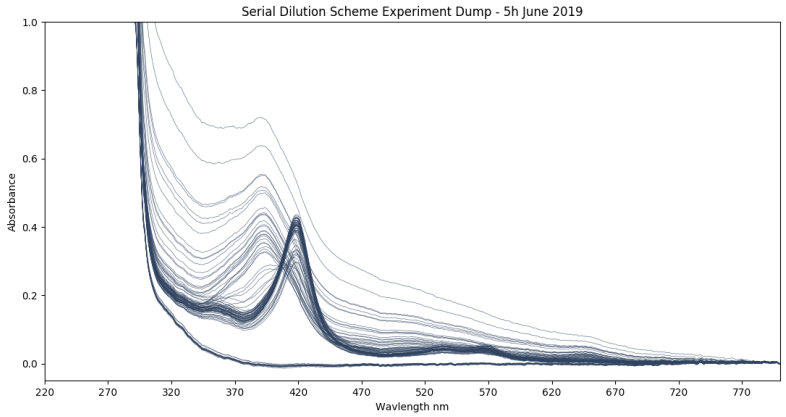
ID	1	2	3	4	5	6	7	8
NPG 5%	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125	0.00390625
Lauric 5%	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125	0.00390625
NPG 10%	1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125
Lauric 10%	1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125

I multichannel pipetted 25 uL of each of these into the 384 well plate (Corning 3640) annd made up the remaining 25 uL with my protein. I'll do a conc table later maybe. I span it all at 3700 rpm for 3 minutes then scanned it on the PheraSTAR fs on a 220-800 nm sweep of the wells. The layout looked like this in the end btw:

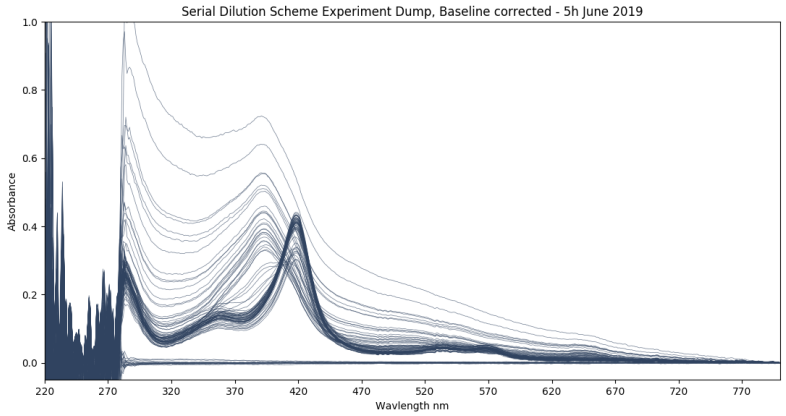


Here's the platereader data

And here's a dump of it:



Well it looks like there's some sort of shift there 👍👍. It also looks like the buffer only wells are also doing what I had previously though was scattering. I can subtract these control traces from the real ones, which looks like this:



It's starting to look like a normal titration! There's a sort of inflection point in there. I'll find out which traces are absorbing way more than the others

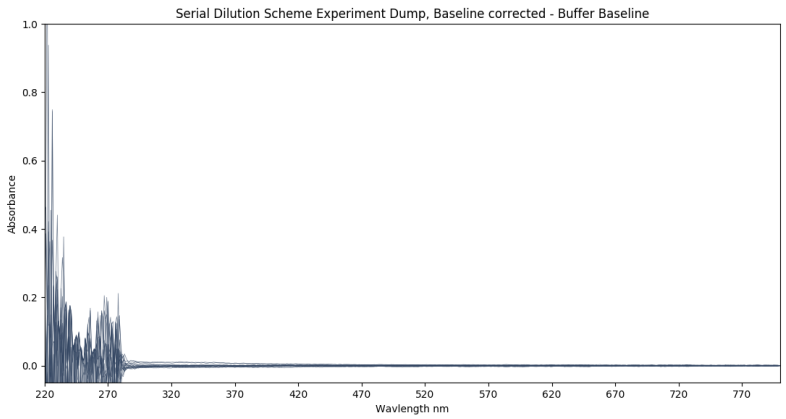
I sorted the traces by their A320 vales here because I think that part of the trace is supposed to be fairly constant. So far it looks like that's just something that's affecting rows 7 and 9, which both contained lauric acid. It's probably a compound specific thing, maybe it has its own absorbance. The worst affected wells are the ones with the highest lauric acid concentrations too.

Analysis

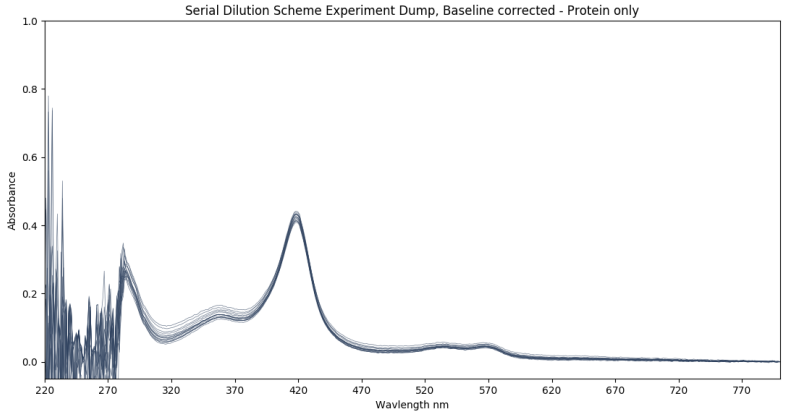
Aim - calculate Kd for the fatty acids from the data

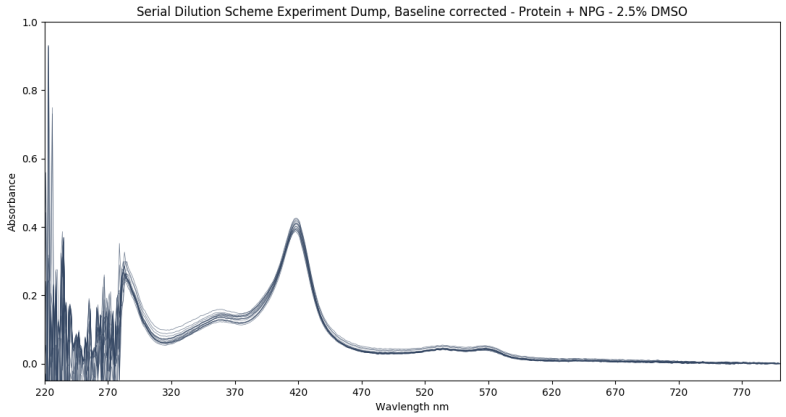
I plotted all the columns in the plate, some of them look ok!

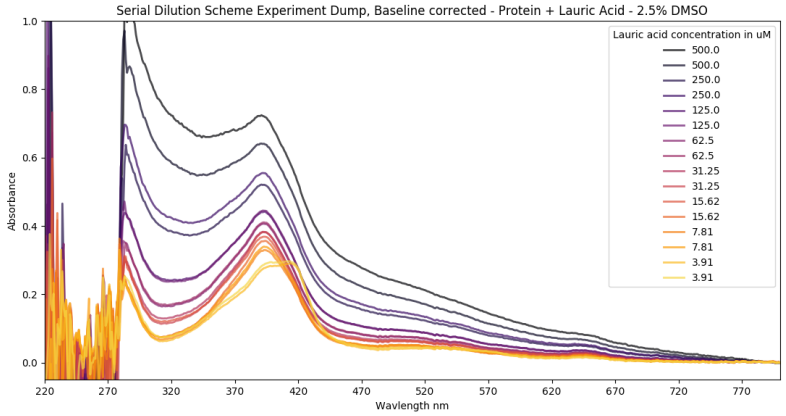
Here are my plots

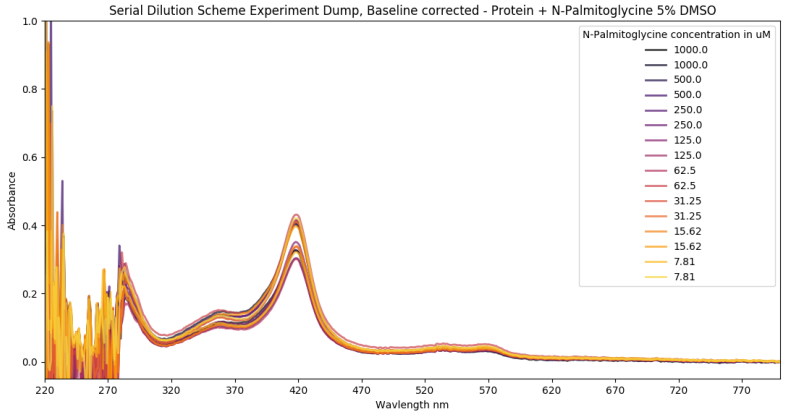


Here are my plots

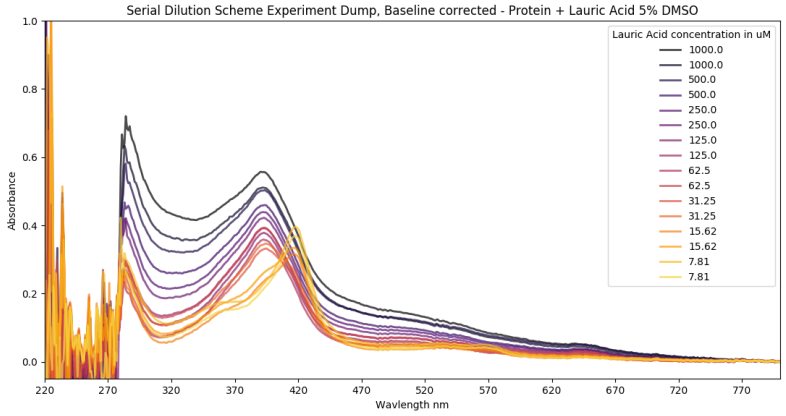




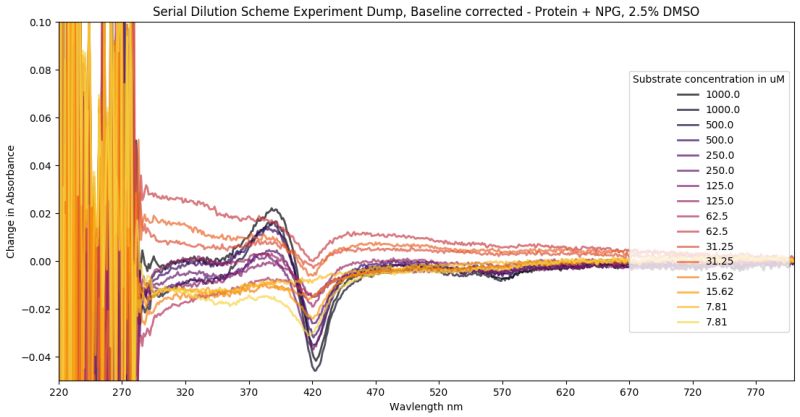


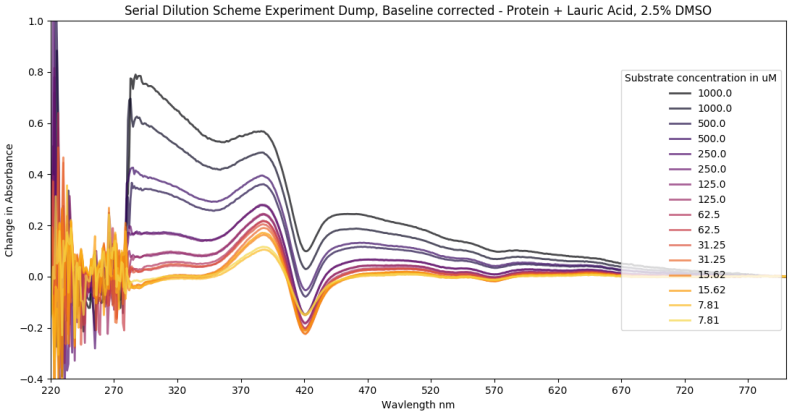


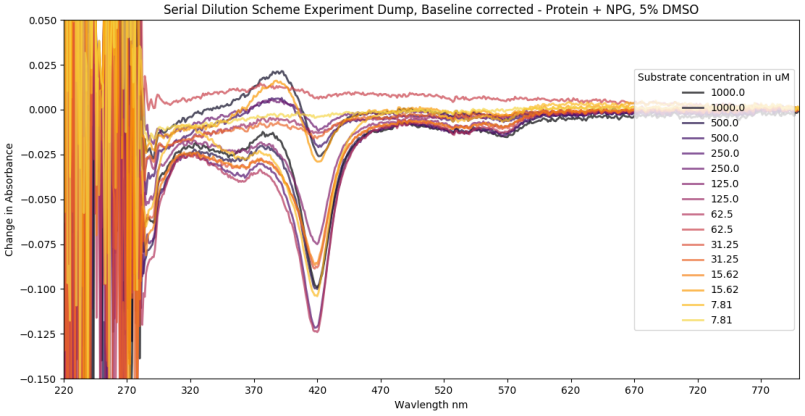
Here are my plots



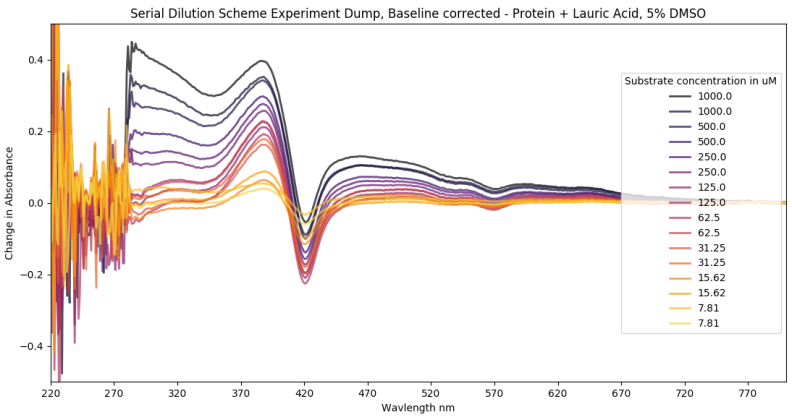
and some difference spectra



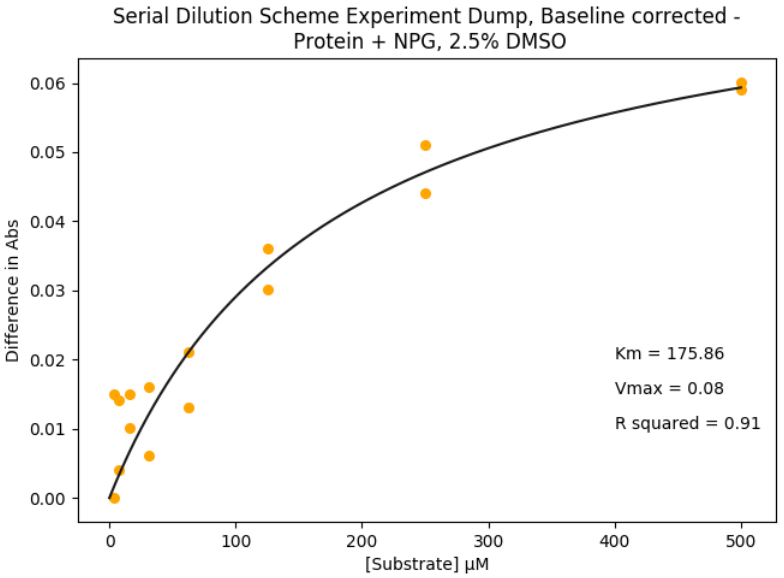


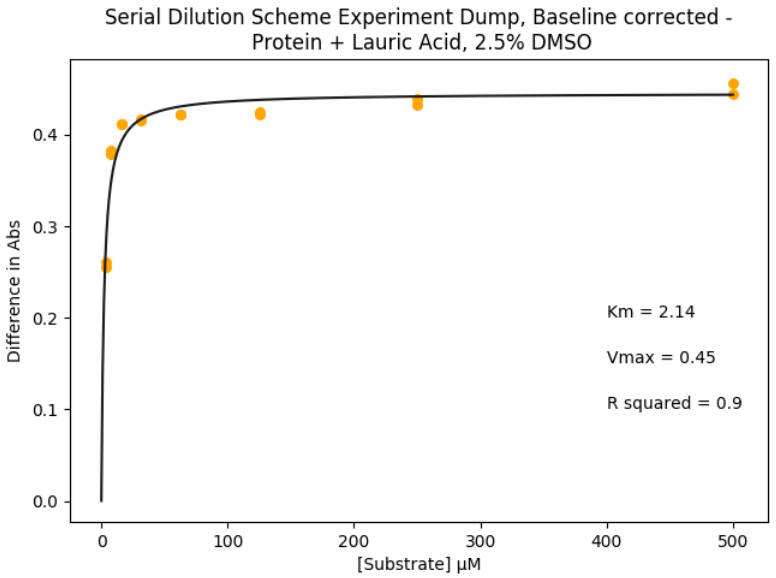


and some difference spectra

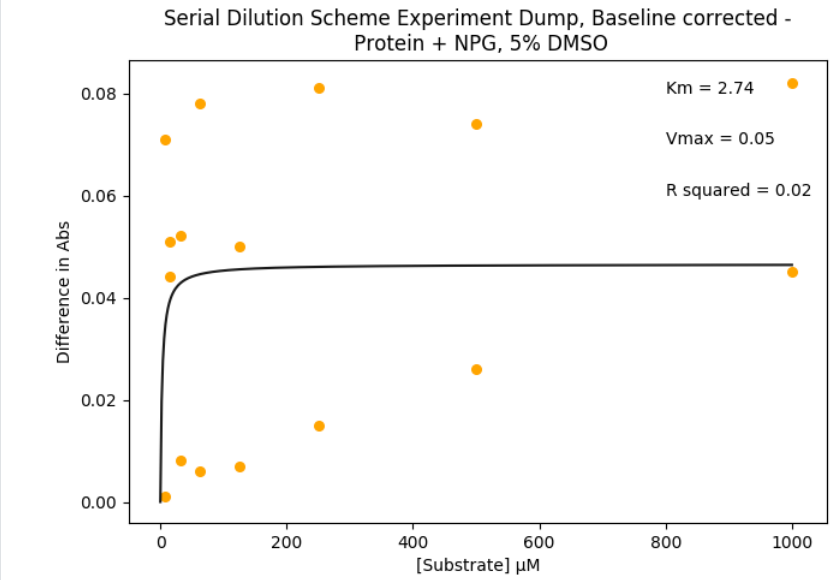


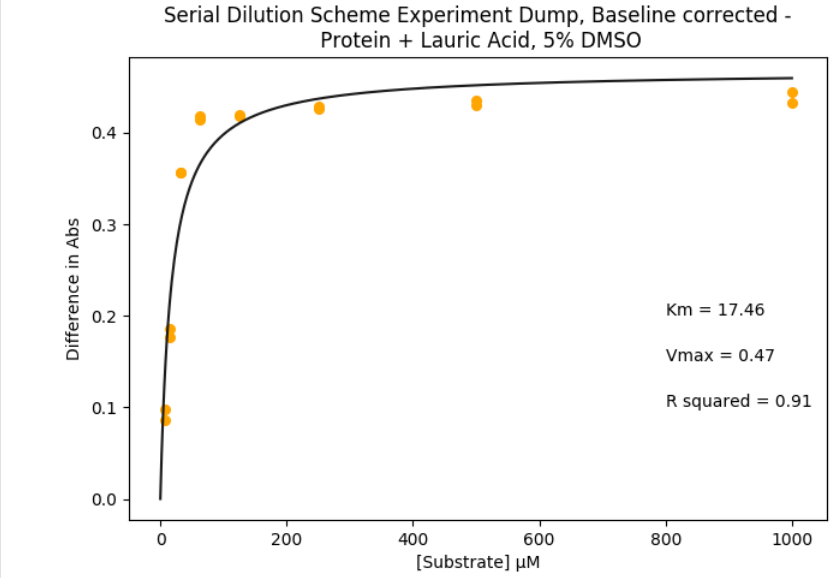
and some Michaelis Menten plots





and some Michaelis Menten plots





NPG is weird, will need to figure out what's going on there. Maybe in the next round of tests I should be making buffer blanks of each compound or something. Will need to do that at some point anyway.

Next Experiment

Here are some things that were lacking:

- The Protein + DMSO standard (because DMSO changes the spin of the BM3 heme sometimes, which may affect my measurements)
- Blanks for each compound (to correct for compound absorbance)
- Fresh compound stocks (I used old stocks because I was sad and tired)
- Repeating the measurements at different time points to see if this assay is at all time sensitive
- I also pipetted everything manually with a pipette that I haven't seen a calibration report for, which may affect things

So the next experiment will include:

- DMSO+Protein standard to zero my spectra against
- Compound blanks! It would be practical to replace on of the repeats with a blank, so each column is self contained
- Time point repeats - very easy to do. So far I don't suspect that time is a big factor over the time scales that I'm working on, so I might take a measurement every hour or something like that
- Fresh compound stocks, and maybe a larger array of BM3 substrates to get a feel for things?

I wonder how buffer conditions affect the measurements and reproducibility