



maudr

**Easily* generate data and model answers in enzyme kinetics
for undergraduate laboratory practicals**

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useR!, Salzburg, 9th July 2024

*if you know some r

Photo source: Wikipedia

Biochemistry 1 and 2 labs

practical write-up – summative in-class assessment

1. Tabulate the results of part 2 in the Table below (12 marks)

	[s] mM	0.167	0.330	0.660	1.33	2.7	5.4
Time (min)	6						
0.17		0.002	0.001	0.006	0.005	0.007	0.009
0.33		0.004	0.006	0.010	0.012	0.013	0.015
0.50		0.006	0.009	0.014	0.018	0.021	0.022
0.67		0.011	0.013	0.022	0.026	0.029	0.031
0.83		0.012	0.017	0.029	0.031	0.036	0.037
1.00		0.014	0.021	0.034	0.037	0.039	0.042

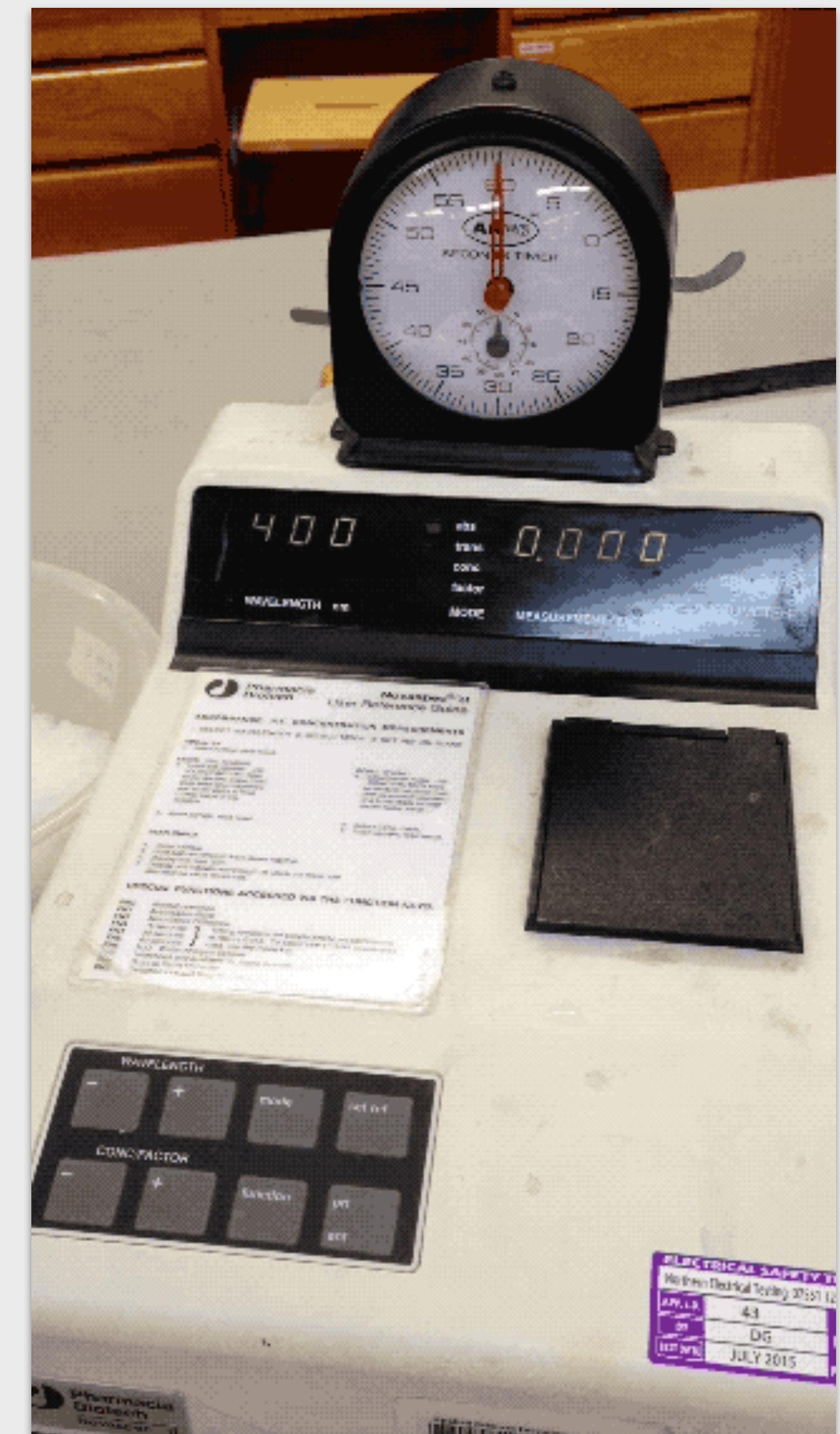
12

0ml of banana
0.001 0.002
0.006
0.015
0.021
0.026
0.034

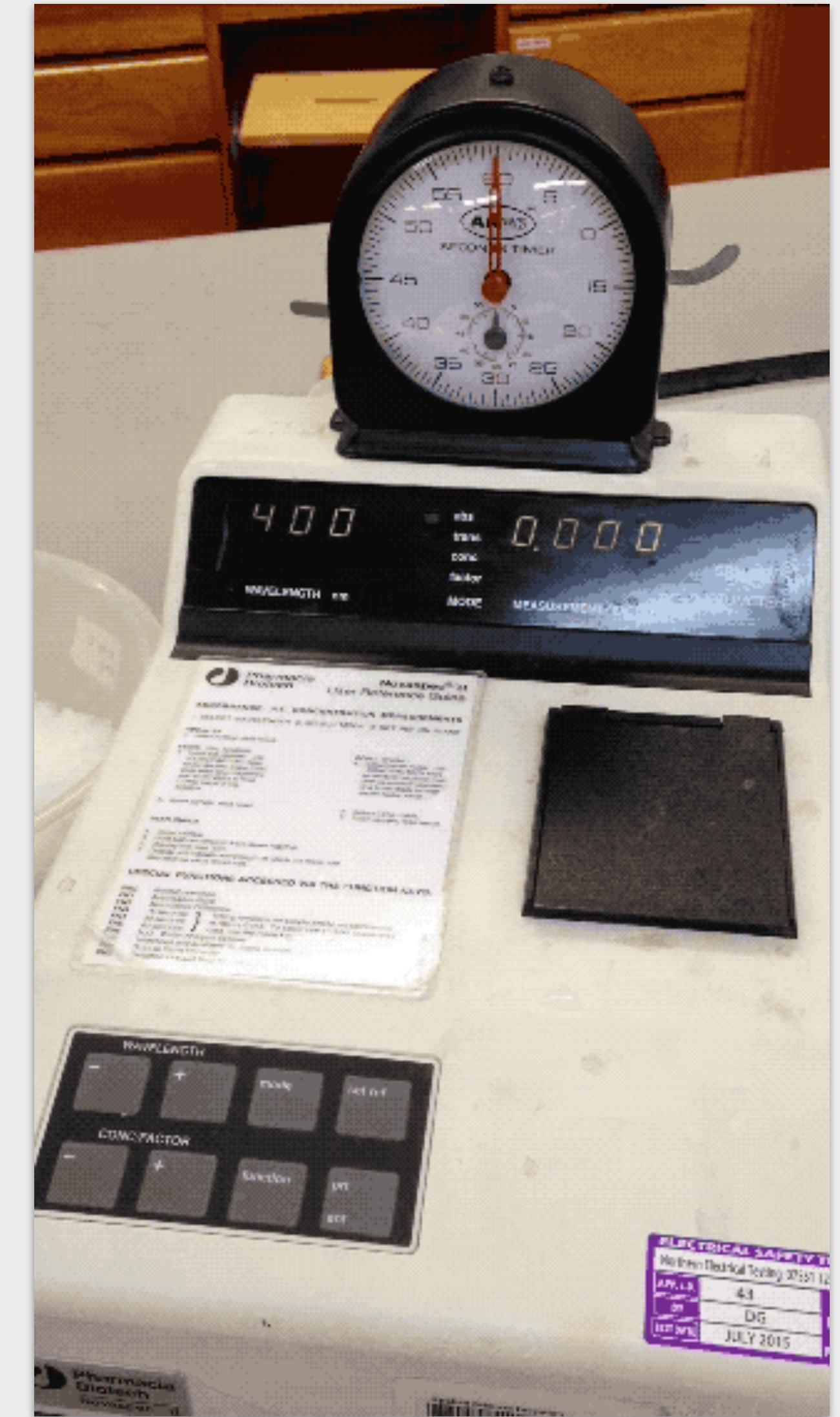
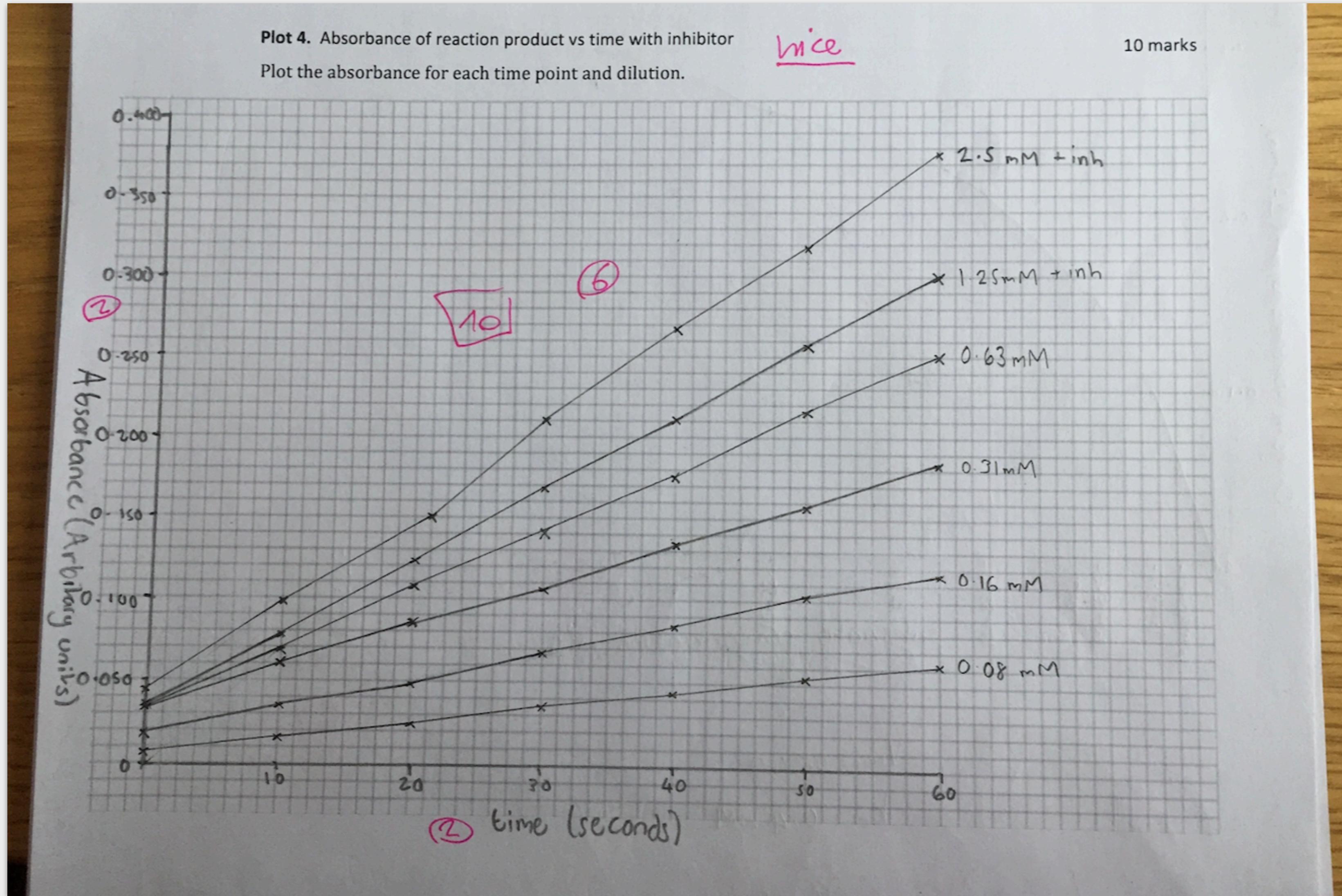
10ml of banana
10 0.010
20 0.083
30 0.087
40 0.091
50 0.094

40ml of banana
10 0.004
20 0.011
30 0.017
40 0.023
50 0.027

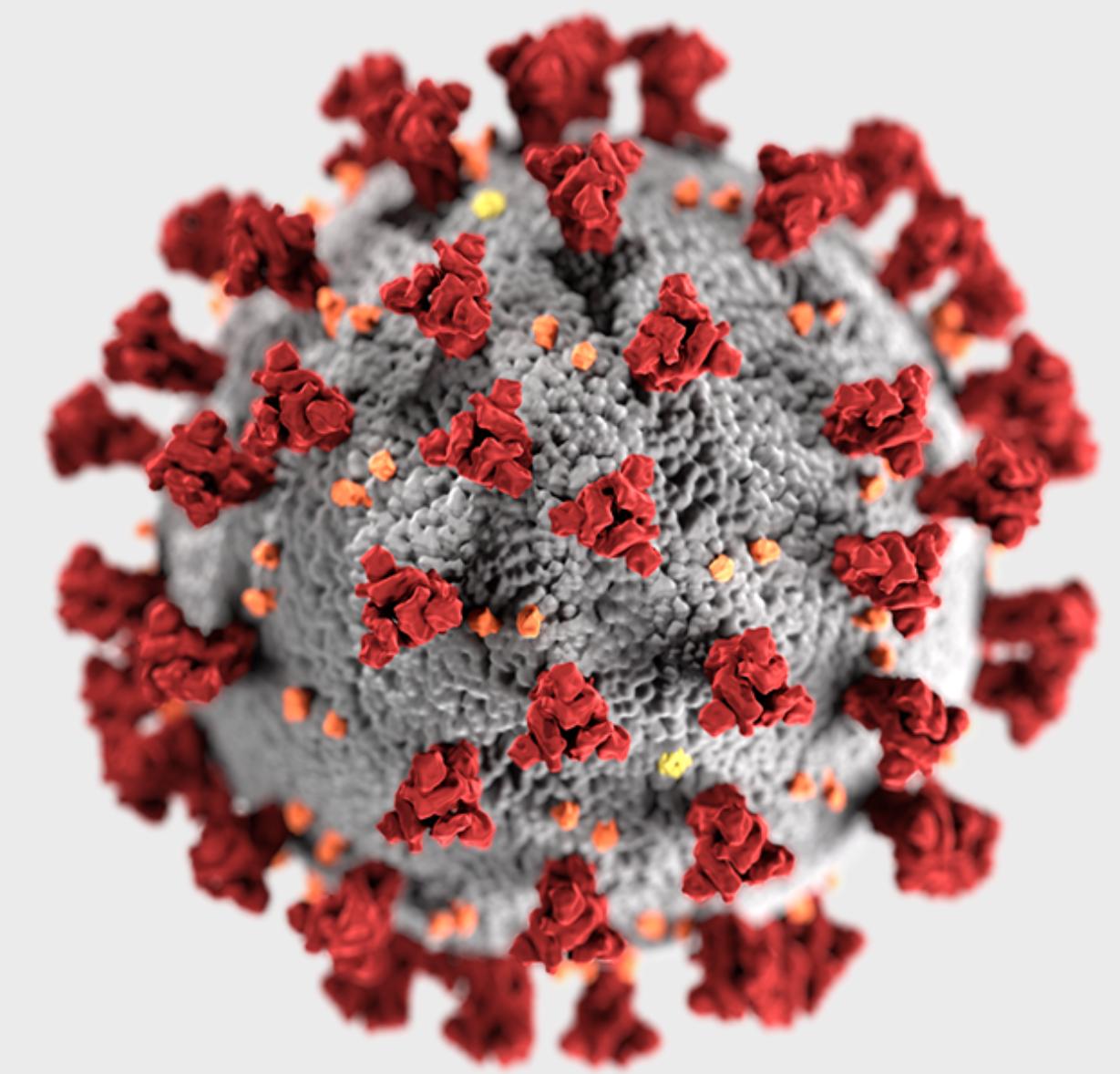
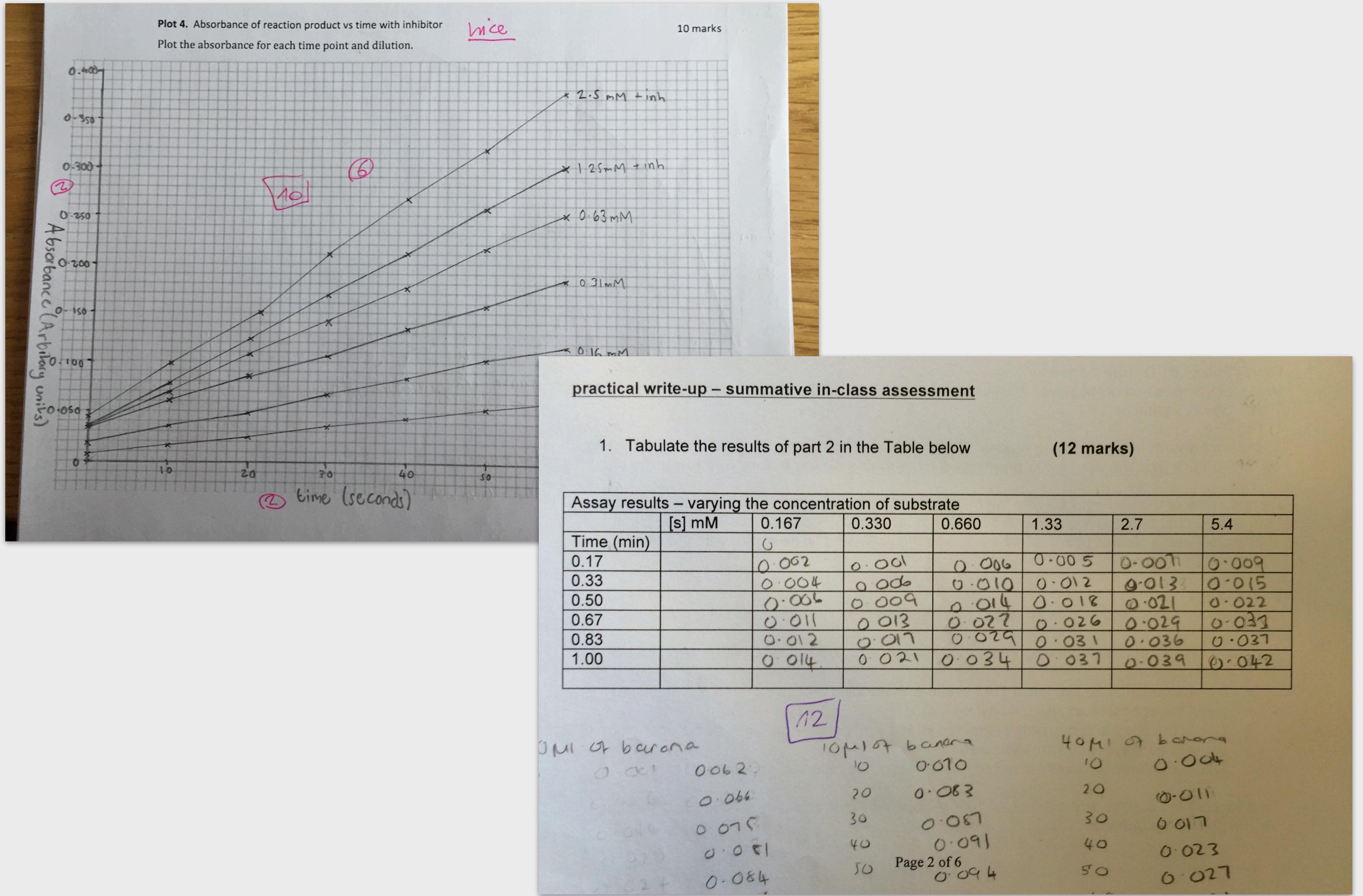
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Biochemistry 1 and 2 labs



Biochemistry 1 and 2 labs: *in silico*?



Starting from first principles,
it is possible to calculate performance
of an enzyme, including absorbance
of its product

🧐 Everybody who knows some biochemistry

First principles (1987)

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Kinetic Characterization of Yeast Alcohol Dehydrogenases

AMINO ACID RESIDUE 294 AND SUBSTRATE SPECIFICITY*

(Received for publication, October 28, 1986)

Axel J. Ganzhorn, David W. Green, Andrew D. Hershey, Robert M. Gould, and Bryce V. Plapp

From the Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52242

A three-dimensional model of yeast alcohol dehydrogenase, based on the homologous horse liver enzyme, was used to compare the substrate binding pockets of the three isozymes (I, II, and III) from *Saccharomyces cerevisiae* and the enzyme from *Schizosaccharomyces pombe*. Isozyme I and the *S. pombe* enzyme have methionine at position 294 (numbered as in the liver enzyme, corresponding to 270 in yeast), whereas isozymes II and III have leucine. Otherwise the active sites of the *S. cerevisiae* enzymes are the same. All four wild-type enzymes were produced from the cloned genes. In addition, oligonucleotide-directed mutagenesis was used to change Met-294 in alcohol dehydrogenase I to leucine. The mechanisms for all five enzymes were predominantly ordered with ethanol (but partially random with butanol) at pH 7.3 and 30 °C. The wild-type alcohol dehydrogenases and the leucine mutant had similar kinetic constants, except that isozyme II had 10–20-fold smaller Michaelis and inhibition constants for ethanol. Thus, residue 294 is not responsible for this difference. Apparently, substitu-

with the three-dimensional structure of horse liver alcohol dehydrogenase (Jörnvall *et al.*, 1978), and we have confirmed this model with a computer graphics system. A schematic view of the active site, as compared to the horse liver enzyme, has been presented (Plapp *et al.*, 1987). The only difference between the two isozymes within the alcohol binding pocket seems to be at position 294, where a methionine in alcohol dehydrogenase I is changed to a leucine in isozyme II. The extra methyl group could account for the difference in specificity for ethanol. Alcohol dehydrogenase III, the mitochondrial isozyme in *S. cerevisiae*, also has Leu-294 (Young and Pilgrim, 1985), whereas the enzyme from *Schizosaccharomyces pombe* has methionine (Russell and Hall, 1983). In order to evaluate the role of this amino acid residue, we used site-directed mutagenesis to change the ATG codon (for methionine) in the gene for alcohol dehydrogenase I to TTG (for leucine), purified the five enzymes, and compared their kinetic properties.

EXPERIMENTAL PROCEDURES

TABLE V

Kinetic constants for different alcohols

Substrates were varied over a 5-fold range up to 100 mM (ethanol, propanol), 50 mM (butanol), or 10 mM (pentanol, hexanol) with 2 mM NAD⁺. The enzyme concentrations were adjusted for each substrate so that reasonable velocities were obtained.

Isozyme	Substrate				
	Ethanol	Propanol	Butanol	Pentanol	Hexanol
<i>V, s⁻¹</i>					
I	340	120	51	29	16
I-leucine	500	120	79	72	57
II	130	89	98	88	71
III	490	88	92	47	31
<i>K_m, mM</i>					
I	17	27	55	37	9.5
I-leucine	19	20	11	8.8	3.3
II	0.8	2.6	2.9	3.8	1.4
III	12	11	7.7	5.2	1.9

Two input files is all you need

Enzyme parameters for different substrates + a list of students

reaction_parameters.xlsx

	A	B	C	D	E	F	G	H
1	rxn_substrate	Kcat	Km	Vmax	enzyme_conc	inhibition_actual		
2	ethanol	340	17	6.8	0.005	no_inhibition		
3	propanol	120	27	4.8	0.01	no_inhibition		
4	butanol	51	55	3.06	0.015	no_inhibition		
5	pentanol	29	37	2.32	0.02	no_inhibition		
6	hexanol	16	9.5	1.6	0.025	no_inhibition		
7	ethanol	340	25.33	6.8	0.005	competitive		
8	ethanol	340	10.2	4.08	0.005	uncompetitive		
9	ethanol	340	17	4.08	0.005	noncompetitive		
10	propanol	120	40.23	4.8	0.01	competitive		
11	propanol	120	16.2	2.88	0.01	uncompetitive		
12	propanol	120	37	2.88	0.01	noncompetitive		
13	butanol	51	81.95	3.06	0.015	competitive		
14	butanol	51	33	1.836	0.015	uncompetitive		
15	butanol	51	55	1.836	0.015	noncompetitive		
16	pentanol	29	55.13	2.32	0.02	competitive		
17	pentanol	29	22.2	1.392	0.02	uncompetitive		
18	pentanol	29	37	1.392	0.02	noncompetitive		
19	hexanol	16	14.155	1.6	0.025	competitive		
20	hexanol	16	5.7	0.96	0.025	uncompetitive		
21	hexanol	16	9.5	0.96	0.025	noncompetitive		
22								
23								

student_names.xlsx

	A	B	C	D	E	F
1	student_no	first_name	surname			
2	u123444	Maud	Menten			
3	u123333	Leonor	Michaelis			
4	u122222	Hans	Lineweaver			
5	u111111	Dean	Burk			
6						
7						
8						



enzyme_inhibition_useR - RStudio

enzyme_inhibition_useR — ~/Documents/work/huddersfield/research/collaboration/Richard

enzyme_kinetics_simulation.Rmd x

Source Visual

```
1 ---  
2 title: "Simulation of enzyme kinetics data"  
3 output: html_notebook  
4 ---  
5  
6 `r libraries`  
7  
8 ### Set the experimental parameters  
9  
10 All enzyme-related parameters are put into a single dataframe.  
11  
12 `r`  
13 # Import the parameters for the simulations  
14 rxn_params <- read_xlsx(here("data", "reaction_parameters.xlsx"))  
15  
16 # Reaction duration in minutes  
17 time <- c(.17, .33, .5, .66, .83, 1)  
18  
19 # Substrates  
20 rxn_substr <- unique(rxn_params$rxn_substrate)  
21  
22 # Type of inhibition  
23 inh_type <- unique(rxn_params$inhibition_actual)[-1]  
24  
25 # Substrate concentration in mM  
26 substr_conc <- c(0,10,20,40,80,160)  
27  
28 # Required params to calculate gradients  
29 cuv_vol <- 0.003 # volume of cuvette (l)  
30 eps <- 6220 # extinction coefficient  
31 enz_vol <- 0.1 # volume of enzyme added (ml)  
32  
33 `r`
```

16:4 (Top Level) ▾ R Markdown ▾

Environment History Connections Tutorial

155 MiB List C

Global Environment

Environment is empty

Files Plots Packages Help Viewer Pre... C

..

Name Size

Output files: experimental data for each student

U111111_DEAN_data_new.xlsx — Saved to my Mac

AutoSave Home Insert Draw Page Layout Formulas Review View Automate Acrobat

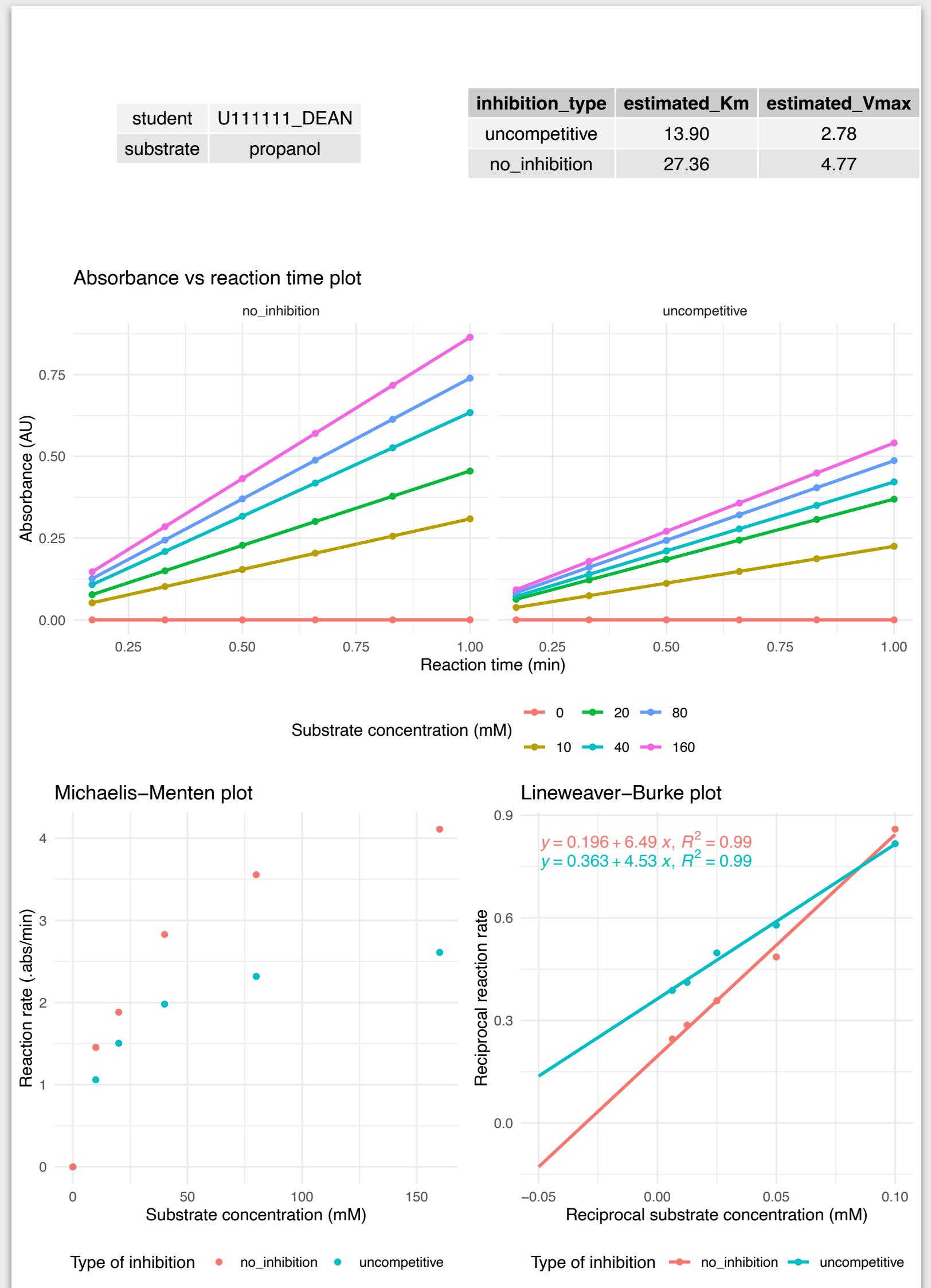
A17 fx |

	A	B	C	D	E	F	G	H	I	J
1	student_id	rxn_substrate	rxn_condition	rxn_time	0_mM	10_mM	20_mM	40_mM	80_mM	160_mM
2	U111111_DEAN	propanol	with_inhibitor	0.17	0	0.039	0.056	0.072	0.084	0.092
3	U111111_DEAN	propanol	with_inhibitor	0.33	0	0.075	0.109	0.14	0.164	0.179
4	U111111_DEAN	propanol	with_inhibitor	0.5	0	0.114	0.165	0.212	0.248	0.271
5	U111111_DEAN	propanol	with_inhibitor	0.66	0	0.15	0.218	0.28	0.328	0.358
6	U111111_DEAN	propanol	with_inhibitor	0.83	0	0.189	0.274	0.353	0.412	0.45
7	U111111_DEAN	propanol	with_inhibitor	1	0	0.228	0.33	0.425	0.497	0.542
8	U111111_DEAN	propanol	without_inhibitor	0.17	0	0.046	0.072	0.101	0.126	0.145
9	U111111_DEAN	propanol	without_inhibitor	0.33	0	0.089	0.14	0.196	0.246	0.281
10	U111111_DEAN	propanol	without_inhibitor	0.5	0	0.134	0.212	0.297	0.372	0.426
11	U111111_DEAN	propanol	without_inhibitor	0.66	0	0.178	0.28	0.392	0.491	0.562
12	U111111_DEAN	propanol	without_inhibitor	0.83	0	0.223	0.351	0.493	0.618	0.707
13	U111111_DEAN	propanol	without_inhibitor	1	0	0.269	0.423	0.594	0.744	0.852
14										
15										

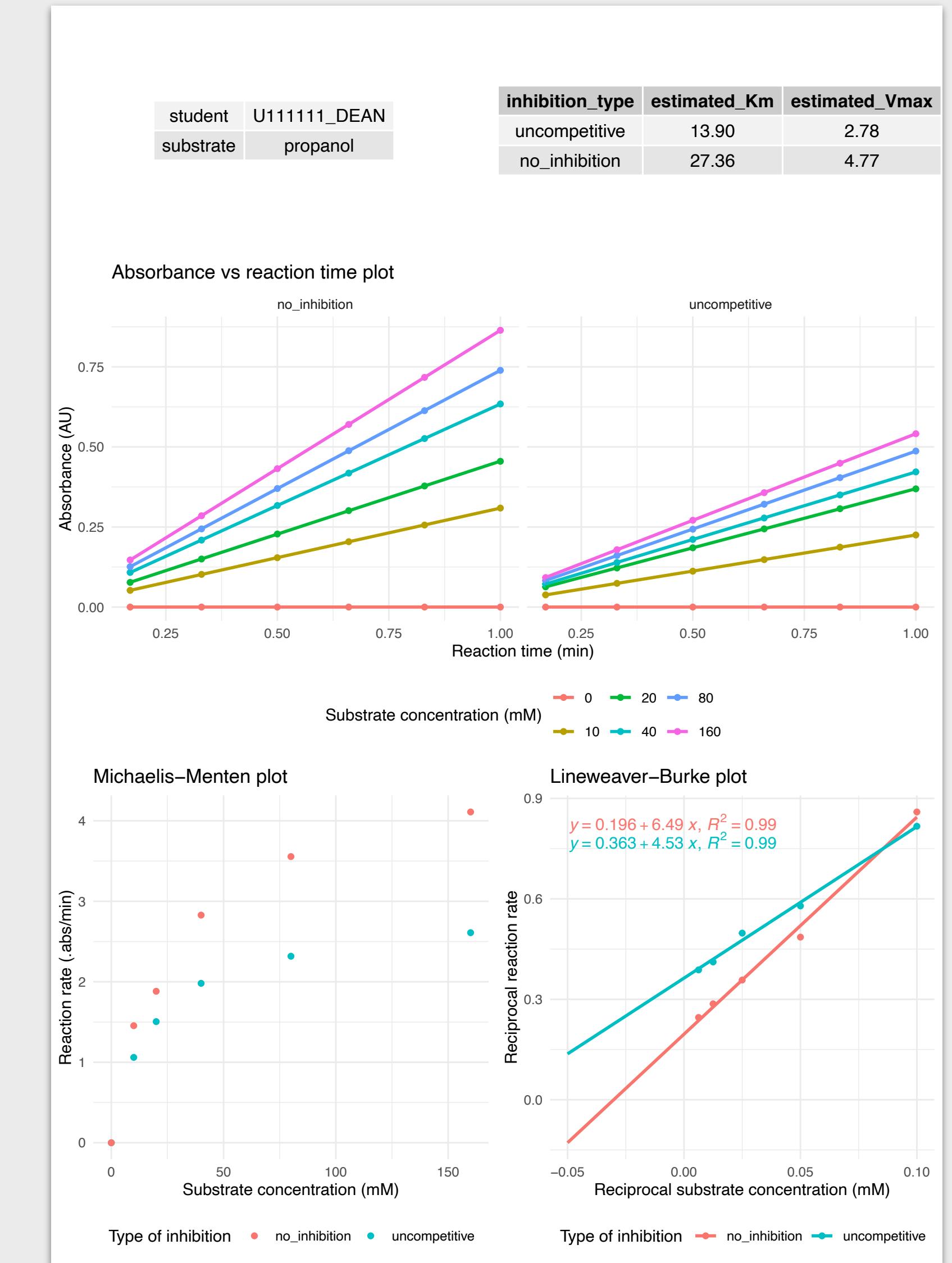
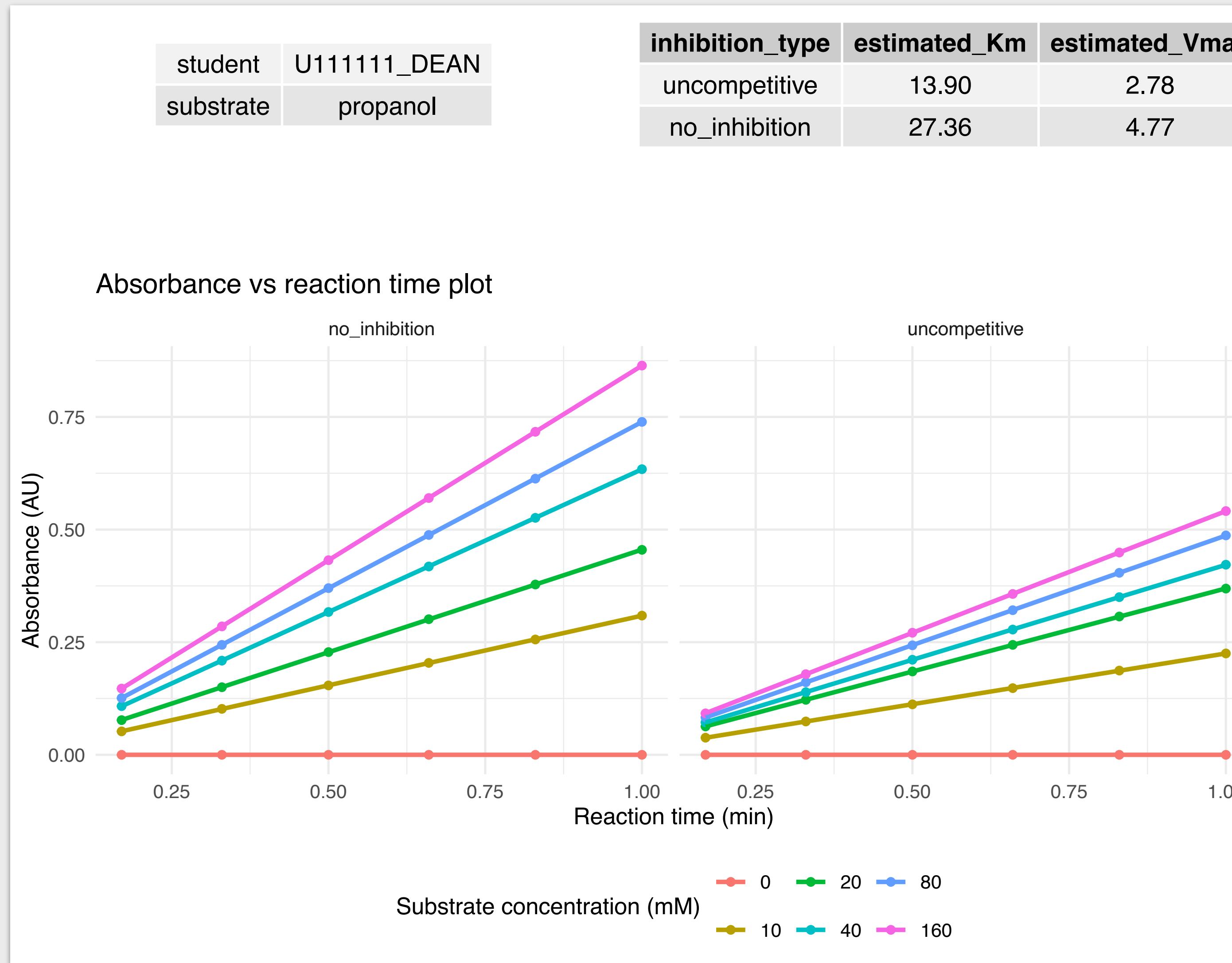
Sheet1 +

Ready Accessibility: Good to go

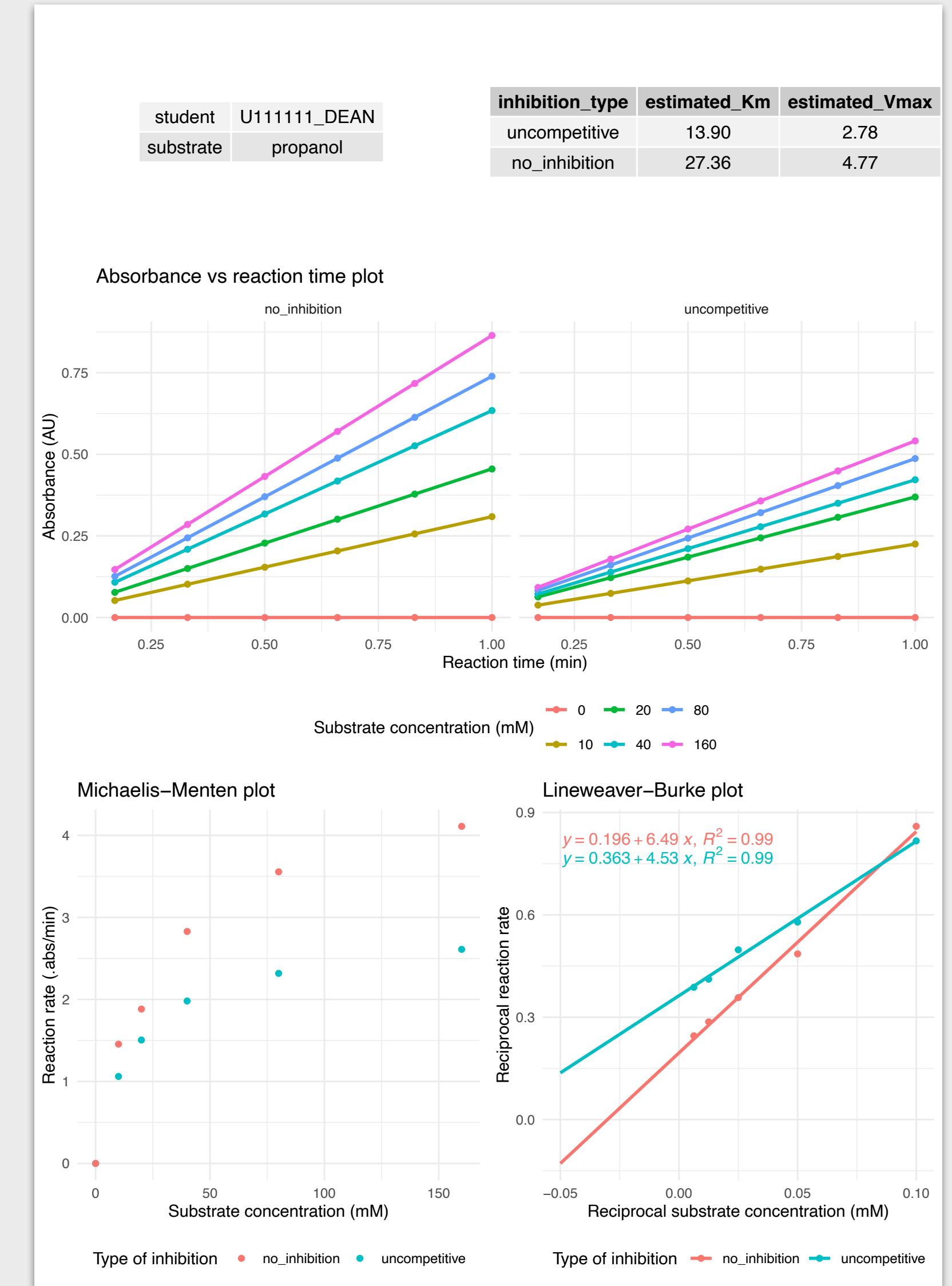
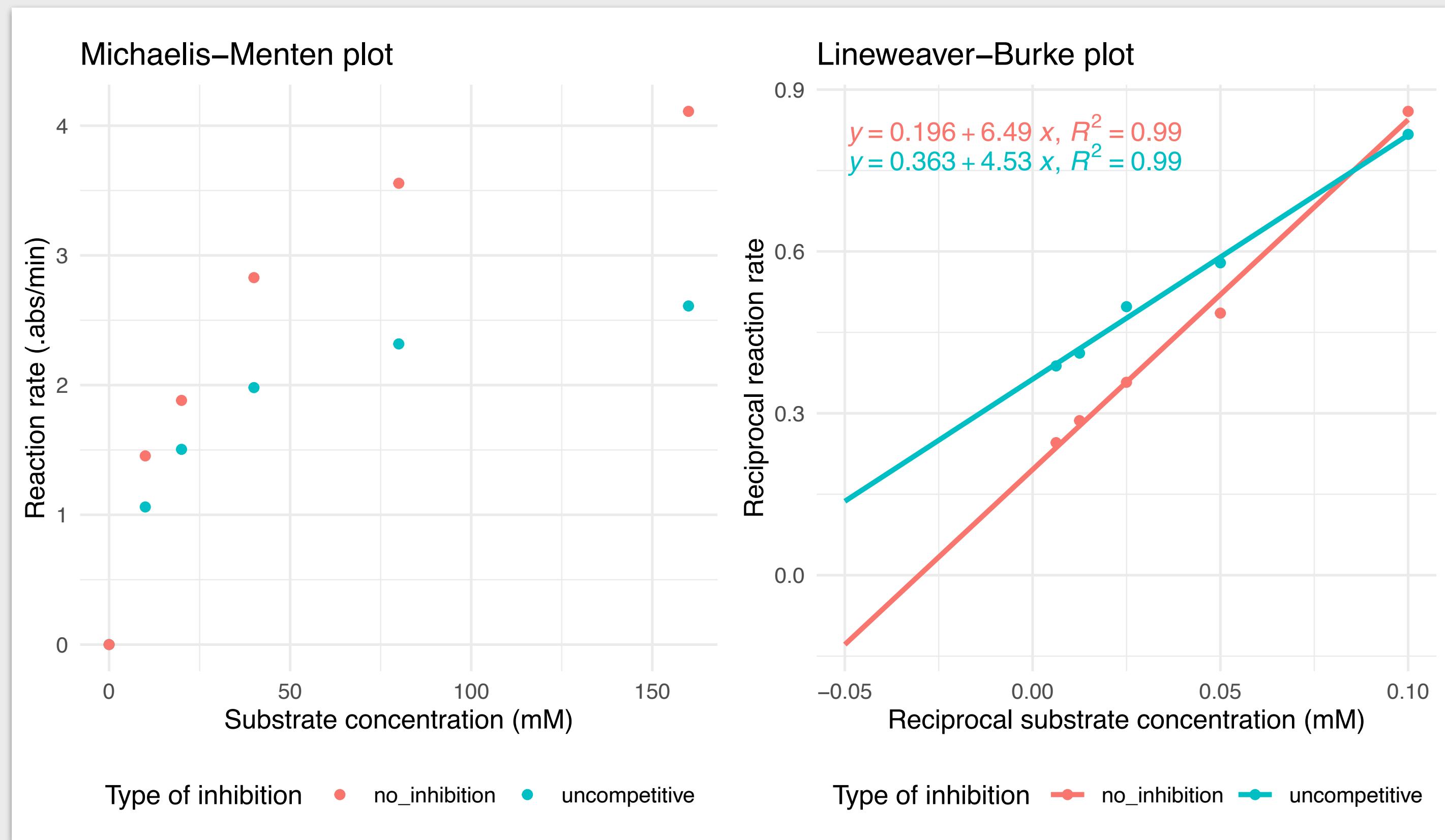
Output files: model answers for each student



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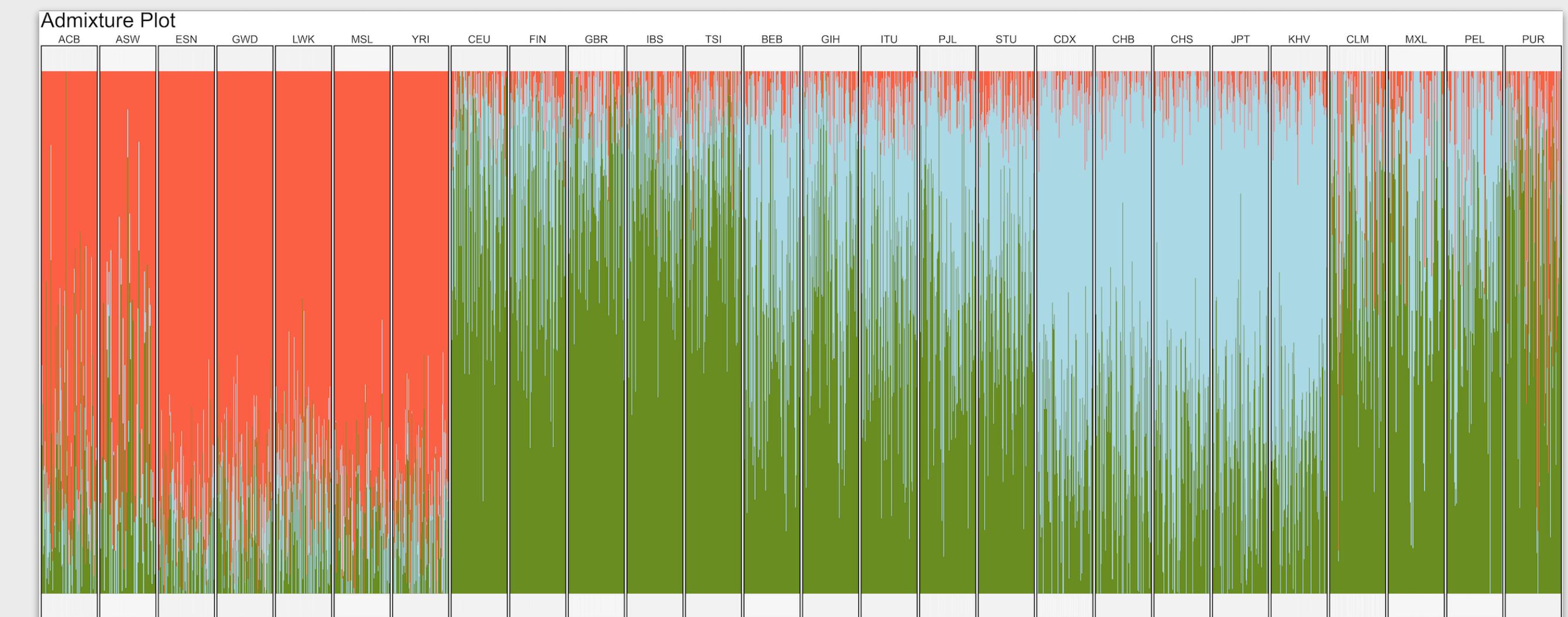
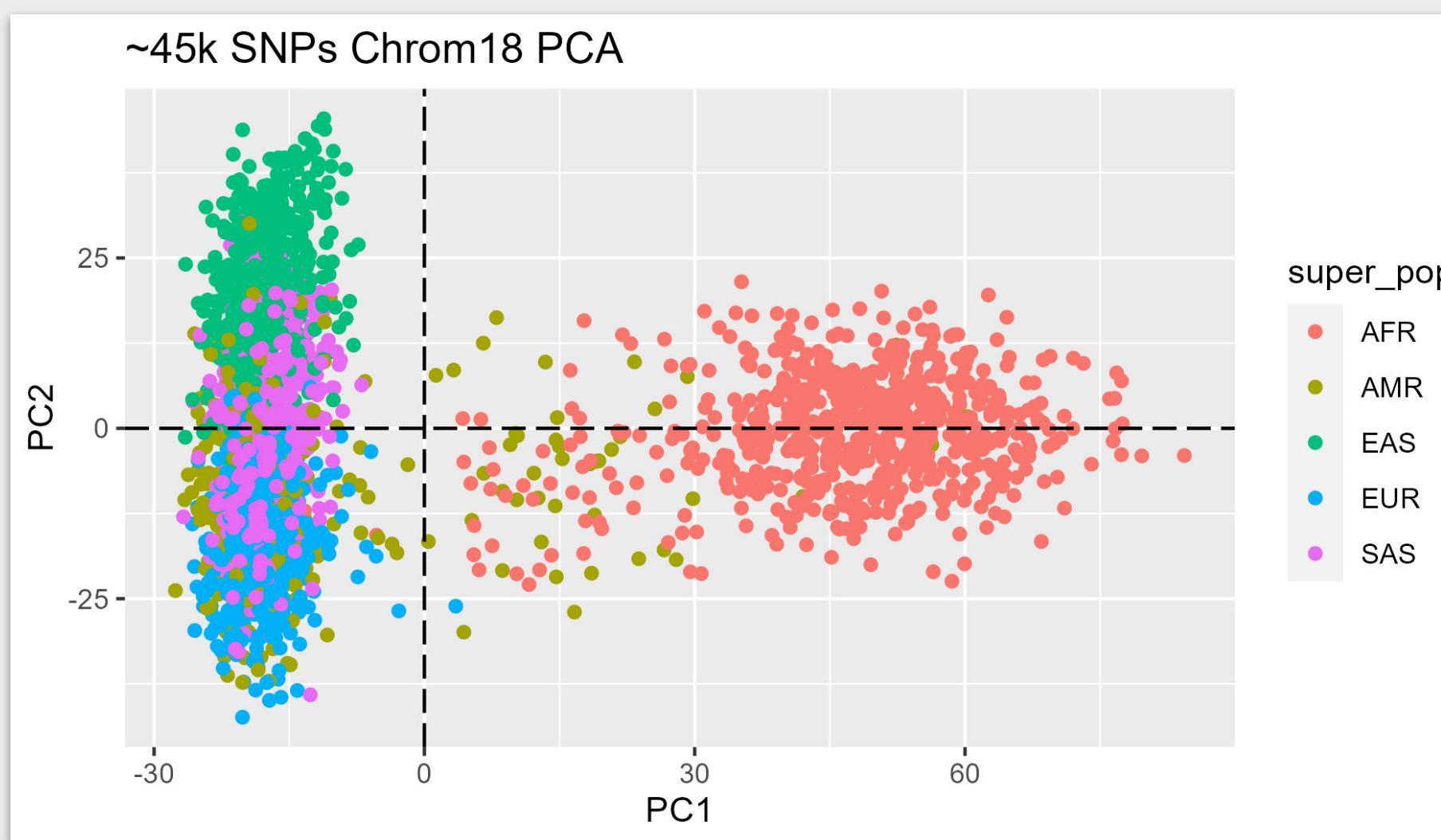


It works

- Can be applied to other enzymes, as long as their K_m , K_{Cat} and V are known
- Can be used *ad-hoc*
- Can be used as formative assignment, as model data for students who missed labs or to practice plotting and calculations
- This general approach can be applied to other topics

Other ideas (in development)

- Grab data from online repositories for selected genes/sequences/organisms (NCBI) to generate multiple sequence alignments with phylogenetic trees
- Download SNP data for a region of human genome (1000 Genomes Project) and generate PCA and ADMIXTURE plots for all combinations of selected super/populations



Thank you.

Watch this space: github.com/jarekbryk/maudr

- Dr Richard Bingham came up with the idea, found the source data and wrote the initial script
- Dr Shamus Burns found some bugs in my code
- Wayne Hon-Lau found some more bugs in it and also computed parameters and limits of reaction as his final year project