Week 38 - Overview

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Weekly summary:

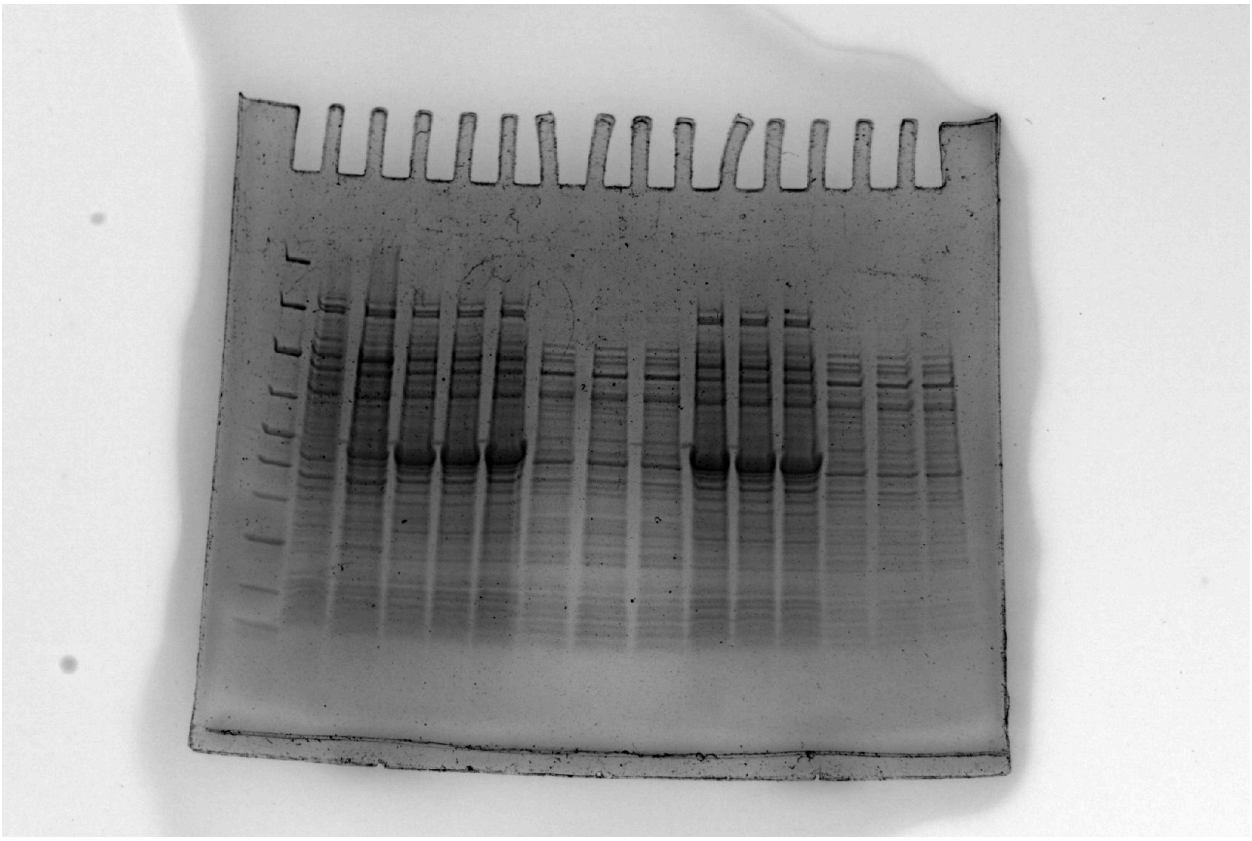
During Week 38, we focused on further optimizing AKR1D1 solubility by expressing AKR1D1-TrxA (the most soluble construct in Week 36), along with empty pET-11a as a negative control, at lower IPTG concentrations (0.2 and 0.4 mM instead of 1 mM), to test whether lowering the induction concentration would increase the soluble fraction of recombinant protein. The samples were analysed by SDS-PAGE. We also repeated testosterone reduction assays on ci2350-MBP.

Main results of the week:

After expressing AKR1D1-TrxA at 0.2 and 0.4 mM IPTG, we compared the band intensities of the whole-cell lysate and soluble fractions, to estimate solubility. Solubility was calculated as the AKr1D1.TrxA band intensity in the soluble fraction divided by its intensity in the whole.cell lysate fraction (x100), with all samples standardized by OD before loading. Expression at 0.2 mM yielded ~37.43% soluble AKR1D1-TrxA, compared with ~28.31% at 0.4 mM, indicating that lower IPTG concentrations increase the solubility fraction of AKR1D1-TrxA. This is relevant to our final design, since slower expression could lower the burden on the host bacteria. Further reductions in IPTG concentrations could be tested, and expression should be evaluated under the hypoxia-responsive promoter, as the expression may behave differently under hypoxic conditions.

Well Number	Sample
1	Ladder
2	pET-11a total fraction
3	AKR1D1-Trx uninduced total fraction
4	S1 0.2 IPTG - total fraction
5	S2 0.2 IPTG - total fraction

6	S3 0.2 IPTG - total fraction
7	S1 0.2 IPTG - soluble fraction
8	S2 0.2 IPTG - soluble fraction
9	S3 0.2 IPTG - soluble fraction
10	S1 0.4 IPTG - total fraction
11	S2 0.4 IPTG - total fraction
12	S3 0.4 IPTG - total fraction
13	S1 0.4 IPTG - soluble fraction
14	S2 0.4 IPTG - soluble fraction
15	S3 0.4 IPTG - soluble fraction



AKR1D1-TrxA bands were analysed, and their band intensities (raw volume) were divided to get the solubility percentage.

IPTG (mM)	Total protein Raw Volume	Soluble Protein Raw Volume	Solubility (%)
0.2	741	308	41.57
0.2	816	305	37.38
0.2	939	313	33.33
0.4	849	208	24.50
0.4	741	244	32.93
0.4	774	213	27.52

The testosterone reduction assays on ci2350-MBP and ci2350-MBP double mutant (R215A and V263I) were inconclusive, as no clear decrease in NADPH absorbance was observed during the run. This could mean either that the enzymes were inactive, possibly because they had been stored at 4ºC for almost two weeks, or that the assay conditions need to be changed. After discussing with our PI Johan, we concluded that the enzyme had probably degraded. We will therefore express and purify fresh protein, and repeat the assays. Results of the assays can be found in the notebook for Week 38.

Day 52: [September 15, 2026], Tuesday

Participants: Giulia, Patrik

Daily overview of experiments

Expression of AKR1D1-Trx was done, using different IPTG concentration to assess if lower induction concentrations would yield in relatively high solubility compared to high induction concentrations.

Activity	***Protocol File***	***Comments***
Expression of AKR1D1-TrxA	Protein expression lo… Protein expression (f…	Testing IPTG concentrations of 0.2 and 0.4 mM

Results and interpretation

Expression of AKR1D1-TrxA and pET-11a in E. coli Bl21(DE3)

In total 8 samples were put to cultivation. 1 sample of induced pET-11a (0.4 mM IPTG) to serve as expression control. 3 AKR1D1-TrxA with 0.2 mM IPTG, 3 AKR1D1-TrxA with 0.4 mM IPTG and one uninduced AKR1D1-TrxA control.

Samples were put to cultivation at 9:50, with 37ºC at 170 rpm. They were cultivated in 50 mL of TB Broth. They were induced at 13:57 (when OD was 1.5), and for 0.2 mM induction, 100 µL of IPTG stock was used, while for 0.4 mM induction, 200 µL of IPTG stock was added.

Samples were left to induce for 3 hours, until 17:00, when they were collected, centrifuged and stored as pellets. Additional samples for SDS and Bradford were taken, when cultivation was done.

SDS prep of samples for total protein determination

Prior to centrifuging, 1 ml aliquots were taken from each AKR1D1-TrxA culture. OD was measured and small aliquots were taken for SDS of total fraction. Specifically small aliquots were centrifuged, the supernatant was discarded and the pellet resuspended in MQ in suitable

volumes to reach OD20 (see calculations). Both the main samples and SDS aliquots were frozen until use the following day.

Construct	OD	Aliquote centrifuged (µL)	MQ H2O added to reach OD 20 (µL)
Pet11a	2.49	150	18.68
AkrTrx no induction	9.41	50	23.53
AkrTrx 1 (0.2 IPTG)	5.33	50	13.33
AkrTrx 2 (0.2 IPTG)	7.32	50	18.33
AkrTrx 3 (0.2 IPTG)	6.53	50	16.33
AkrTrx 1 (0.4 IPTG)	6.16	50	15.4
AkrTrx 2 (0.4 IPTG)	4.98	50	12.45
AkrTrx 3 (0.4 IPTG)	4.14	150	31.05

Day 53: [September 16, 2026], Wednesday

Participants: Giulia, Patrik

Daily overview of experiments

Lysis and SDS-PAGE of samples cultured yesterday, to assess AKR1D1-Trx tag effectiveness under low IPTG concentrations.

Activity	***Protocol File***	***Comments***
Sample prep for SDS-PAGE, preparing soluble and insoluble fractions for analysis by gel	Lysis by B-PER + Br… Cultures for tag asse…	To assess solubility of AKR1D1-Trx under different IPTG concentration (0.2 and 0.4 uM)
SDS-PAGE of AKR1D1-TrxA and pET-11a	SDS-PAGE	Note: the 50X SDS-running buffer is actually 5X, so dilute it 5X to make the 1x buffer

Results and interpretation

Sample prep for SDS-PAGE

Samples for soluble protein fraction were prepped according to the Lysis by B-PER protocol, using values and volumes from the excel spreadsheet. By accident, too much volume of cell wash buffer was added to pET11 and AKR1D1-Trx No Induction samples, but their soluble fractions were not analysed on the gel, so they were put aside (not thrown away).

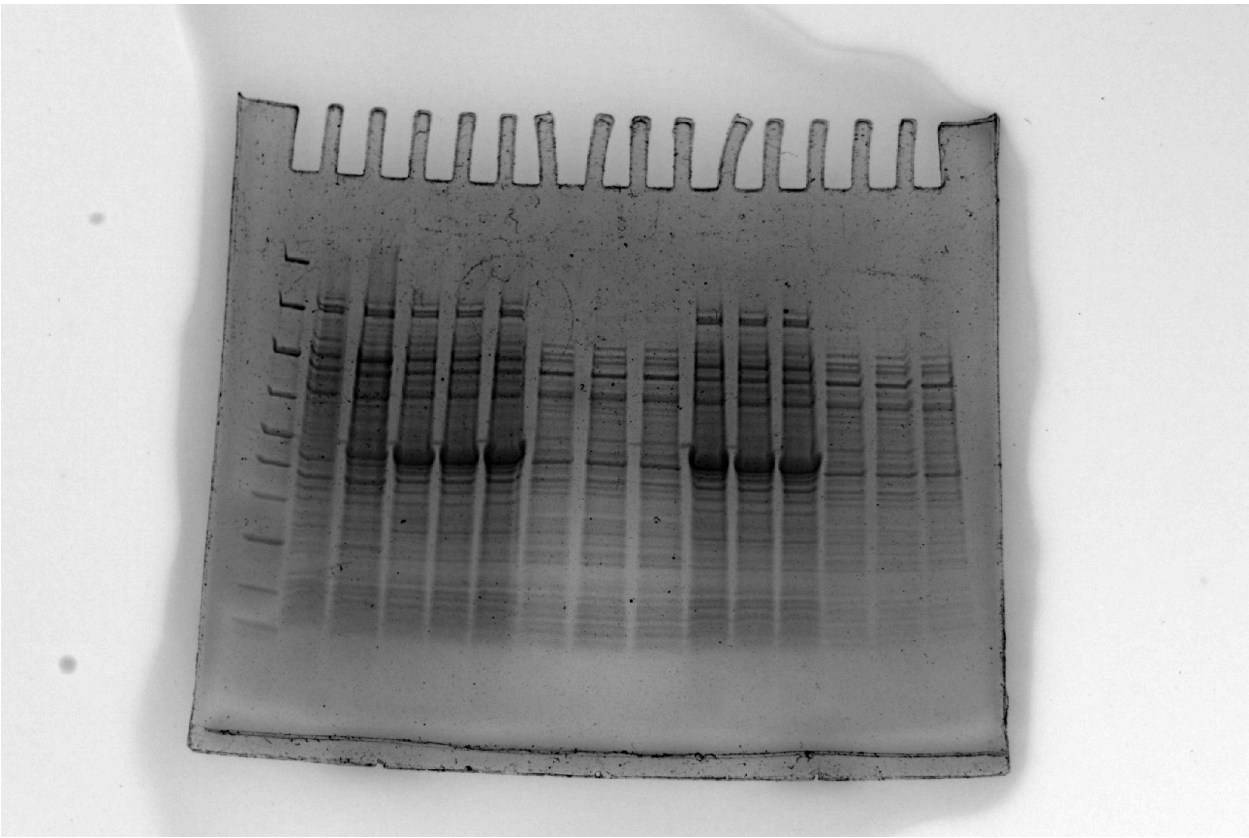
SDS-PAGE of AKR1D1-TrxA and pET-11a

Samples were loaded onto the gel, with 10 uL of sample and 5 uL of PageRuler Unstained Broad Ladder. The new 1X SDS Running buffer was made from the previous ‘50X’ stock, but it

was diluted only 5 times (not 50), due to previous gels having lower conductivity and frowning. The gel turned out good, with straight bands and no frowning of the gel. Following electrophoresis, it was stained and destained using standard protocol. The last destaining was done overnight.

Well Number	Sample
1	Ladder
2	pET-11a total fraction
3	AKR1D1-Trx uninduced total fraction
4	S1 0.2 IPTG - total fraction
5	S2 0.2 IPTG - total fraction
6	S3 0.2 IPTG - total fraction
7	S1 0.2 IPTG - soluble fraction
8	S2 0.2 IPTG - soluble fraction
9	S3 0.2 IPTG - soluble fraction
10	S1 0.4 IPTG - total fraction
11	S2 0.4 IPTG - total fraction
12	S3 0.4 IPTG - total fraction
13	S1 0.4 IPTG - soluble fraction
14	S2 0.4 IPTG - soluble fraction
15	S3 0.4 IPTG - soluble fraction

NOTE: in the future, dilute the stock SDS Running Buffer only 5 times



AKR1D1-TrxA bands were analysed, and their band intensities (raw volume) were divided to get the solubility percentage.

IPTG (mM)	Total protein Raw Volume	Soluble Protein Raw Volume	Solubility (%)
0.4	741	308	41.57
0.4	816	305	37.38
0.4	939	313	33.33
0.2	849	208	24.50
0.2	741	244	32.93
0.2	774	213	27.52

Day 54: [September 18th, 2026], Friday

Responsibles: TBD
Participants: Patrik, Ruiqi

Daily overview of experiments

Resuspending newly ordered plasmids, followed by transformation into E. coli BL21(DE3). Doing testosterone assays of ci2350_MBP, on a new spectrophotometric machine, allowing for multiple measurements at the same time.

Activity	***Protocol File***	***Comments***
Resuspending of new plasmids		
Transformation into E. coli BL21(DE3)		
Testosterone assays on ci2350_MBP		

Results and interpretation

Resuspending new plasmids

Newly received plasmid constructs ordered from TwistBioscience were resuspended in MilliQ H2O to the final concentration of 20 ng/µL. They were resuspended by slightly vortexing the tube (not heating).

Constructs resuspended and transformed:

- pET-BLANK-ci2350-His
- pET-BLANK-ci2350-MBP(no aaa)
- pET-BLANK-ci2350-MBP(double mutant)
- pFnrS-AKR1D1-SUMO
- pFnrS-ci2350-MBP

Transformation of resuspended plasmids into E. coli BL21(DE3)

After resuspension, the plasmids were transformed into E. coli BL21(DE3), using 2 µL of plasmid solution (40 ng of DNA in total) for each construct. Bacteria were transformed according to the standard protocol, and left overnight in the incubator at 37ºC.

Prep for testosterone assays

As needed, testosterone and NADPH stocks were freshly made. Testosterone stock (40 mM) was made by diluting 7.4 ng of testosterone into 724.46 µL of apsolut EtOH. NADPH stock was made by diluting 4.1 ng of NADPH in 984 µL of 100 mM potassium phosphate buffer (the same one we use for testosterone assays).

Testosterone assays

Considering a new spectrophotometer machine (can hold many cuvettes) was used for today's experiments, a dry run was made to get the feeling of how the machine is being used.

Dry run (30 mins reaction time):

Sample	Buffer (µL)	NADPH (µL)	ci2350-MBP (µL)	Testosterone
Blank	999.75	-	-	0.25 EtOH
Old NADPH	949.75	50	-	0.25 EtOH
Negative control	949.75	50	-	0.25 EtOH
Sample	899.75	50	50	0.25 T

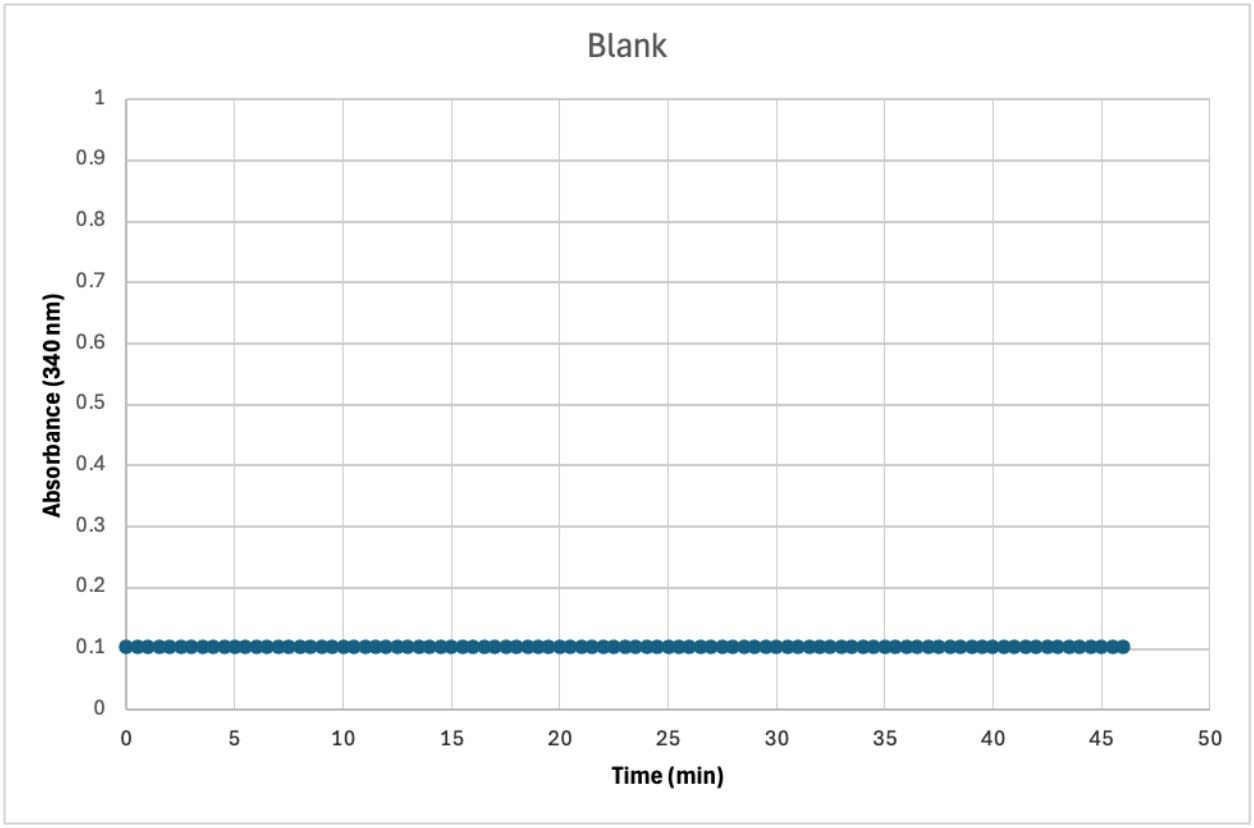
Old NADPH taken from the fridge was tested, to assess if its viable for assays (its absorbance was very low, meaning it degraded). In samples where EtOH is written, absolute ethanol was used, instead of testosterone solution.

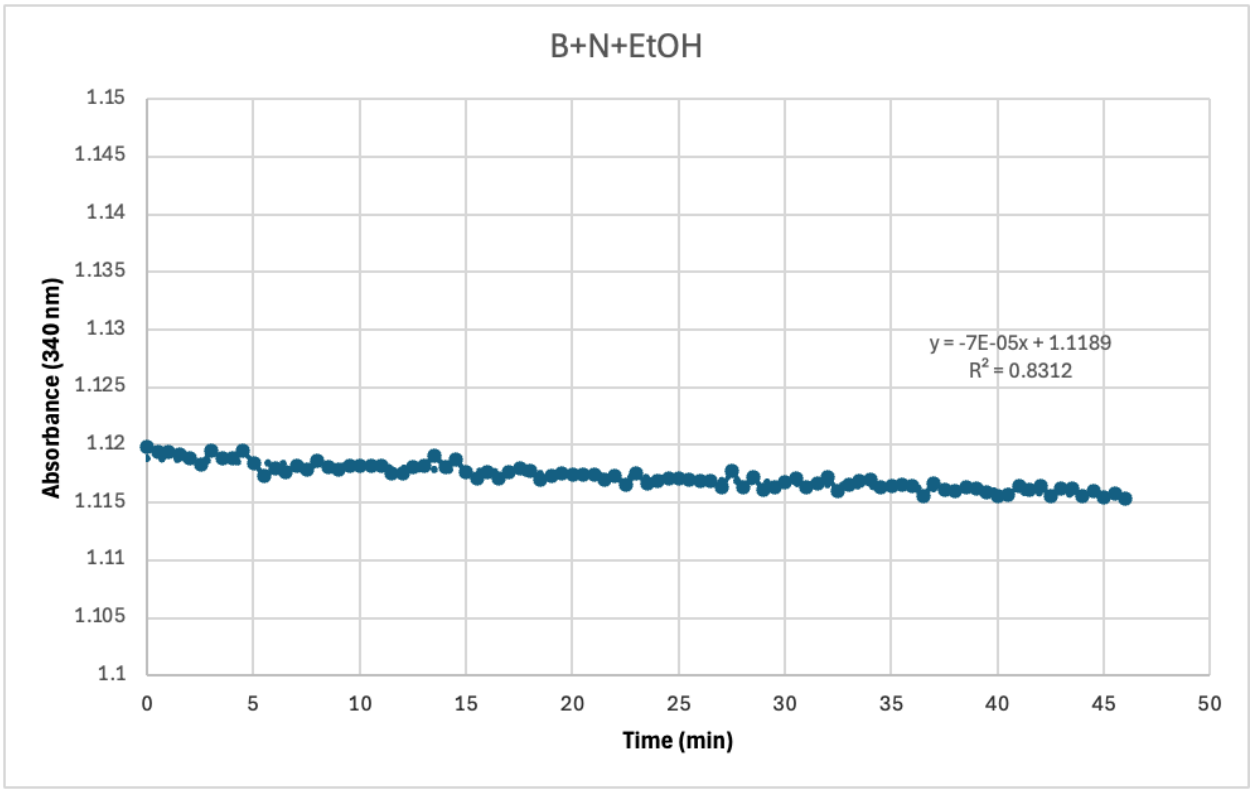
Actual run (~45 min reaction time):

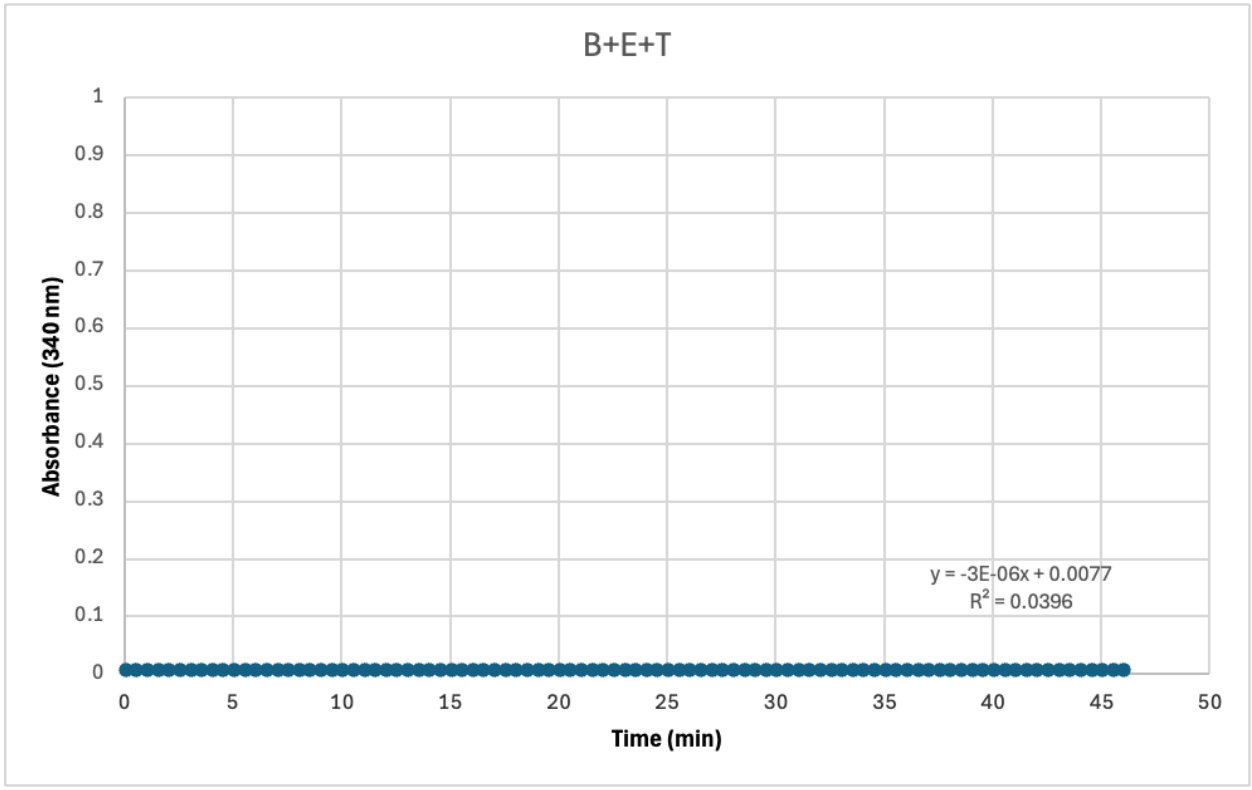
Sample	Buffer (µL)	NADPH (µL)	ci2350-MBP (µL)	Testosterone
Blank	999.75	-	-	0.25 EtOH

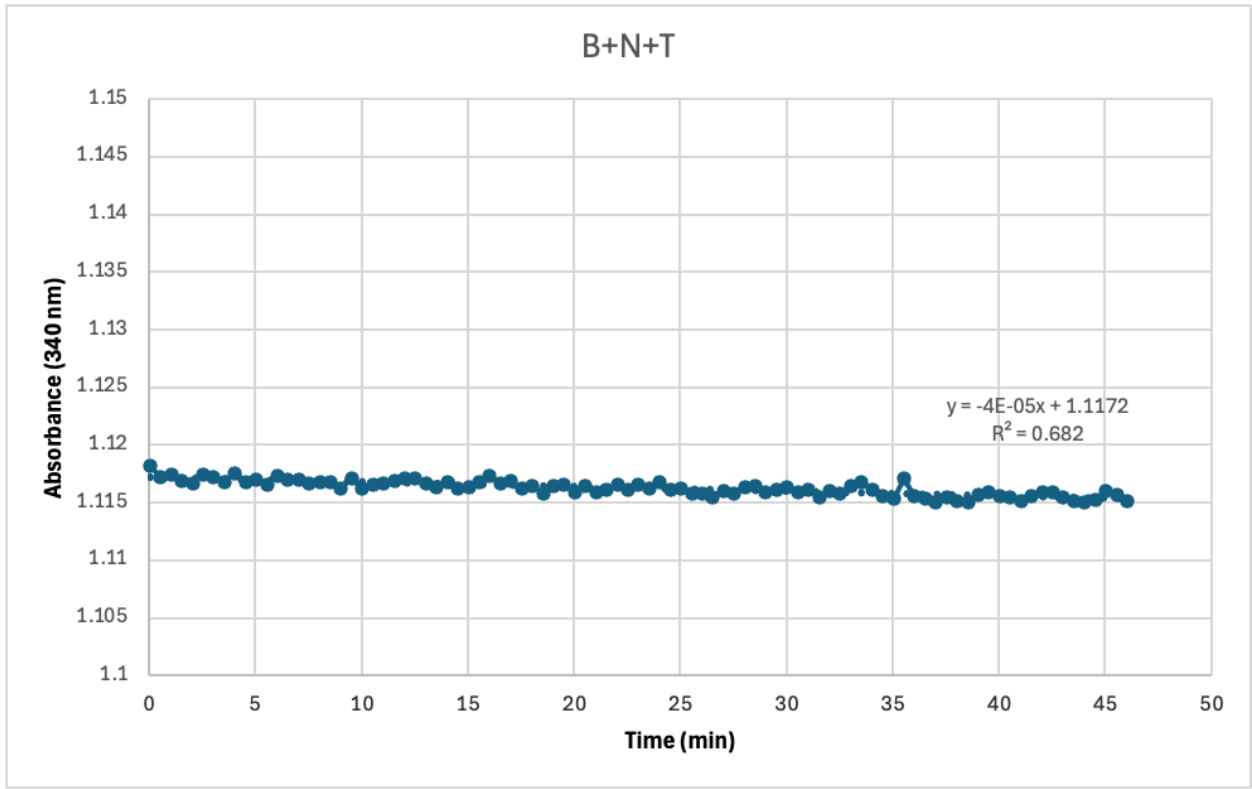
Negative control	949.75	50	-	0.25 EtOH
Negative control for testosterone background	949.75	50	-	0.25 T
Negative control for enzyme background	949.75	-	50	0.25 EtOH
Sample (triplicates)	899.75	50	50	0.25
Sample (double mutant, triplicates)	899.75	50	50	0.25

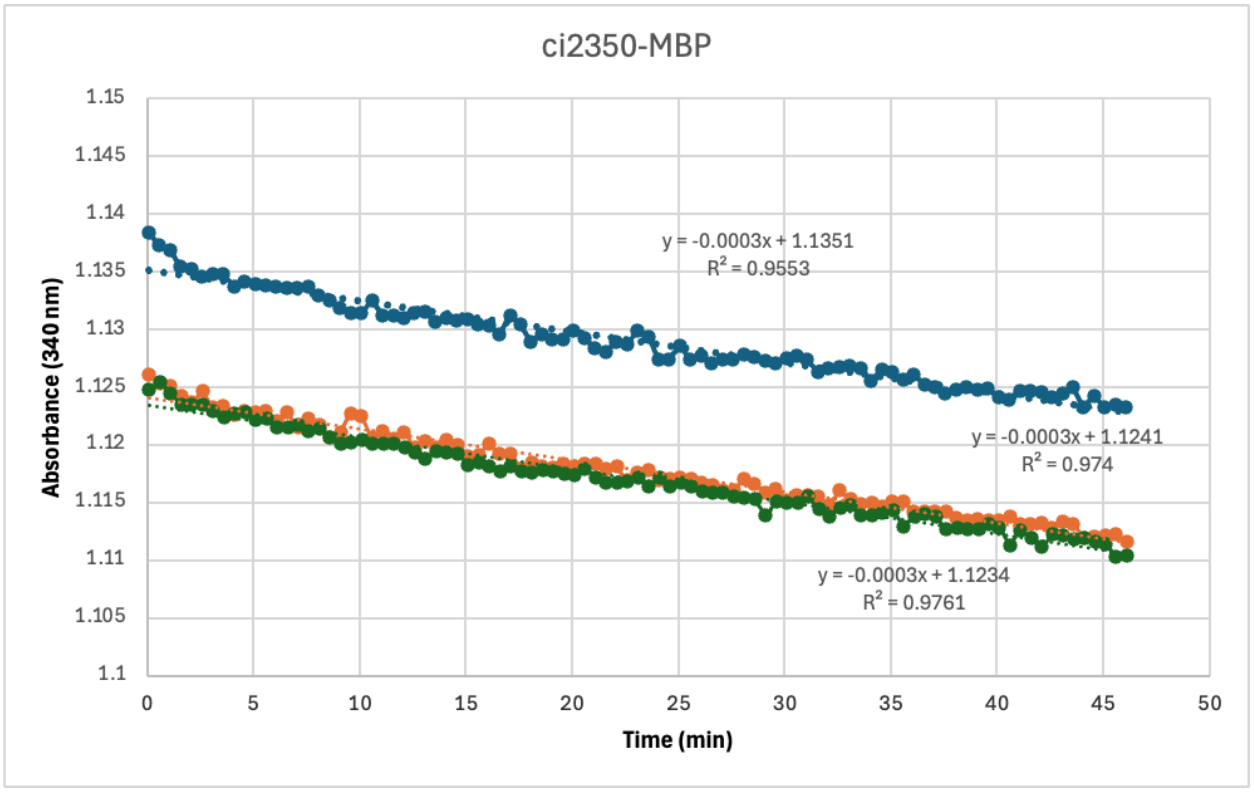
In all experiments there was almost no change in NADPH absorbance. After discussing with Johan, it was concluded the enzyme was inactive, due to it being stored in the fridge (not freezer) for two weeks. We have to repeat cultivation and purification before starting new testosterone assays.

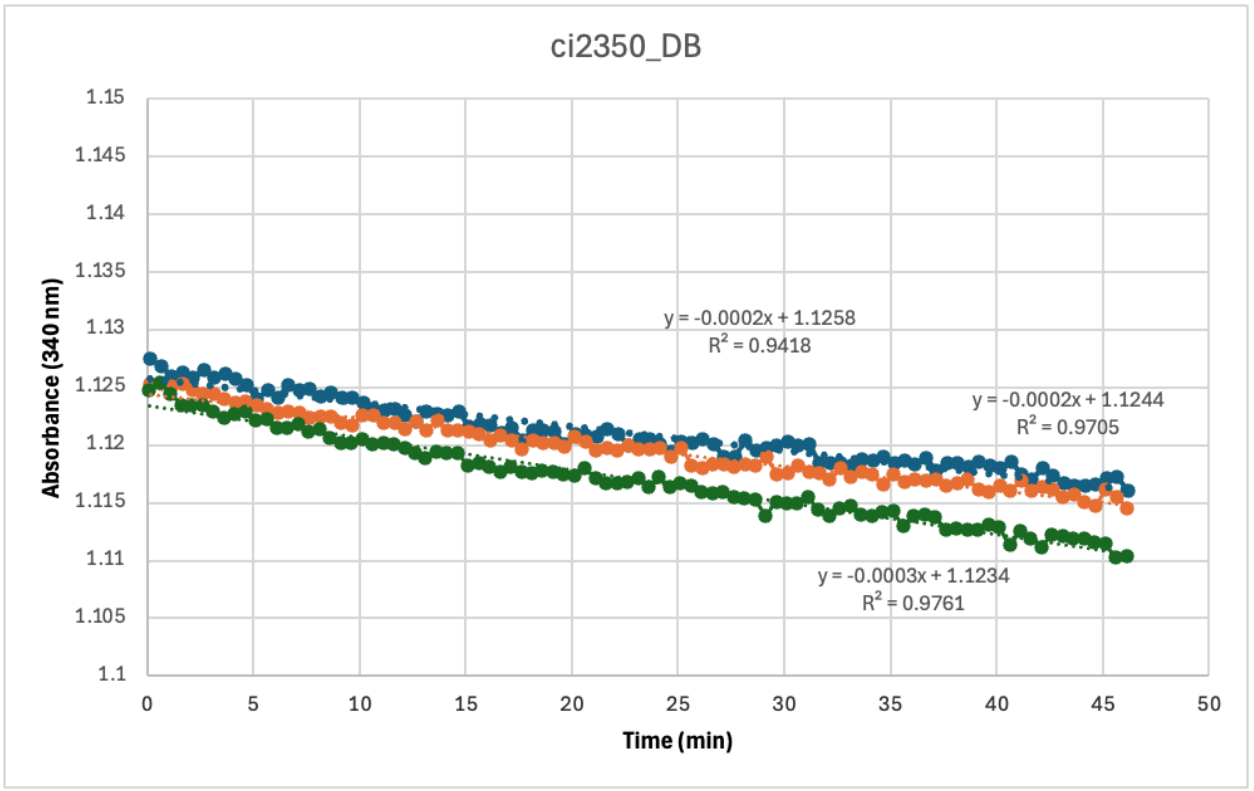

Blank
Absorbance (340 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
40
45
50
Time (min)


B+N+EtOH
Absorbance (340 nm)
1.15
1.145
1.14
1.135
1.13
1.125
1.12
1.115
1.11
1.105
1.1
y = -7E-05x + 1.1189
R² = 0.8312
0
5
10
15
20
25
30
35
40
45
50
Time (min)


B+E+T
Absorbance (340 nm)
Time (min)
y = -3E-06x + 0.0077
R² = 0.0396
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
40
45
50


B+N+T
Absorbance (340 nm)
Time (min)
y = -4E-05x + 1.1172
R² = 0.682
1.15
1.145
1.14
1.135
1.13
1.125
1.12
1.115
1.11
1.105
1.1
0
5
10
15
20
25
30
35
40
45
50


ci2350-MBP
Absorbance (340 nm)
Time (min)
y = -0.0003x + 1.1351
R² = 0.9553
y = -0.0003x + 1.1241
R² = 0.974
y = -0.0003x + 1.1234
R² = 0.9761


ci2350_DB
Absorbance (340 nm)
Time (min)
y = -0.0002x + 1.1258
R² = 0.9418
y = -0.0002x + 1.1244
R² = 0.9705
y = -0.0003x + 1.1234
R² = 0.9761