Week 33 - Overview

Week 33 - Overview..... 1
Day 39: [August 10, 2026], Monday.....4
Day 40: [August 11, 2026], Tuesday.....7
Day 41: [August 12, 2026], Wednesday..... 15
Day 42: [August 13, 2026], Thursday.....18
Day 43: [August 14, 2026], Friday..... 21

Weekly summary:

During Week 33, we continued producing and purifying AKR1D1 fusion constructs, prepared ci2350-MBP mutant samples for sequencing, and initiated atcABC production. AKR1D1-SUMO and AKR1D1-Trx preparations were processed by affinity chromatography and analysed by SDS-PAGE. We also tested a plate-based assay with four enzyme preparations. The plate assay did not provide interpretable activity measurements, and the cuvette-assay entry remains incomplete. In this week we advanced with sample preparation and assay troubleshooting.

Main results of the week:

AKR1D1-SUMO material was lysed and processed by affinity chromatography on 11 August. The record states that 50 mL of clarified lysate was processed that day and that the remainder was stored at 4°C for later purification. AKR1D1-Trx material was lysed, clarified, filtered and affinity-purified on 12 August. Both preparations underwent concentration and buffer exchange. Final concentrations, recovery volumes and yields are not supplied.

The 13 August gel shows protein bands in the lanes assigned to AKR1D1-Trx and AKR1D1-SUMO eluates, including the concentrated preparations. Additional bands are visible. Plasmid preparations from double-mutant candidates were measured on 10 August, with reported concentrations ranging from 57.7 to 164 ng/µL. Samples were prepared for Economy and NightSeq sequencing, and submission identifiers were recorded for both single- and double-mutant candidates. These records document sample preparation and submission tracking; sequencing outcomes are not included.

Four atcABC cultures were monitored on 12 August. The recorded undiluted OD600 readings at 4.5 hours were 1.516, 1.531, 1.625 and 1.542. Inducer and cofactor-related additions are described, but their concentrations require reconciliation. On 13 August, the overview records that production was stopped and samples were stored at −80°C. Protein-expression and activity outcomes for these cultures are not documented.

On 14 August, a 96-well assay was set up using ci2350-MBP, AKR1D1-SUMO, AKR1D1-Trx and AKR1D1-His preparations at different sample volumes. Most conditions were prepared in triplicate, with recorded exceptions for AKR1D1-His. The notebook reports missing measurement signals and identifies use of cell-culture-grade plates as a suspected contributor. Without raw data and an instrument or plate check, the cause cannot be established. No enzyme-activity conclusion is supported by this run.

Day 39: [August 10, 2026], Monday

Weekly responsible: Eleonora
Lab participants: Cecilia, Hamsi, Hema, Ruiqi

Daily overview of experiments

We prepared pre-induction and post-induction AKR1D1-SUMO samples and a previously purified ci2350-MBP sample for SDS-PAGE. AKR1D1-Trx production and assay troubleshooting were listed among the activities, and culture OD600 values were recorded. Plasmids were extracted from double-mutant candidate cultures and measured by NanoDrop. The gel image and the outcome of the assay troubleshooting remain to be added.

Activity	***Protocol File***	***Comments***
SDS-PAGE of AKR-SUMO		
Preparation of TB Media		
Production of AKR1D1-TRX		
Enzyme Activity Assay Troubleshooting		
Double mutants plasmid extraction from pre culture inoculated at room temperature over the weekend		

Results and interpretation

SDS-PAGE of AKR-SUMO and ci-MBP

To evaluate protein expression prior to purification, SDS-PAGE analysis was performed on AKR1D1-SUMO samples. A total of nine samples were prepared: four pre-induction samples,

four post-induction samples, and one sample of eluted ci-MBP from a previous purification. (Note: The ci-MBP sample had undergone a second Vivaspin concentration step with an additional wash because the initial Vivaspin membrane failed to clear proteins below 30kDa).

For sample preparation, 30 µL mixtures were generated using 15 µL of cell sample (normalized to an OD600 of 20) and 15 µL of 2x SDS loading buffer. The loading buffer, originally prepared by Camille, was freshly supplemented with 10% beta-mercaptoethanol. The samples were heated at 95°C for 10 minutes and subsequently centrifuged for 30 seconds to collect any condensation.

Each well was loaded with 10 µL of the sample mixture, and 5 µL of the full-range rainbow molecular weight marker (Cytiva) was used as a reference. Electrophoresis was initiated at 80V for 15 minutes to allow the samples to stack, after which the voltage was increased to 160V for 1h. Following the run, the gel was rinsed in Milli-Q water, heated in a microwave for 30 seconds, and stained with Coomassie blue for 1 hour on an orbital shaker (100 rpm). Destaining was performed in three consecutive steps lasting 10 minutes, 30 minutes, and 1 hour, respectively, prior to gel imaging.

Insert SDS Gel:

The loading order is the following: AKR-SUMO before induction (4 wells), the ladder, AKR-SUMO after induction (4 wells), and ci_MBP eluate (after second vivaspin).

Production of AKR1D1-TRX :

AKR1D1 TRX OD values

Sample	Dilution Factor	OD at 600 nm	Actual OD
1	10	0.044	0.44
2	20	0.194	3.88

Nanodrop of double mutants

Sample	Concentration (ng/uL)	A 260/280	A 260/230
a	164	1.864	2.278
b	84	1.920	1.920

c	106	1.885	1.746
d	61.1	1.922	2.121
e	59.6	2.000	1.846
f	57.7	1.902	1.812
g	147	1.885	1.377
h	107	1.903	0.344

Day 40: [August 11, 2026], Tuesday

Weekly responsible: Eleonora
Lab participants: Cecilia, Hamsi, Hema, Ruiqi

Daily overview of experiments

We lysed AKR1D1-SUMO cells and processed part of the clarified lysate by affinity chromatography, followed by concentration and buffer exchange. AKR1D1-Trx culture densities and pellet weights were recorded. Single- and double-mutant candidates were prepared for sequencing, with sample identifiers recorded for Economy and NightSeq services. Two atcABC precultures were inoculated for overnight incubation

Activity	***Protocol File***	***Comments***
Full AKR1D1-trx SDS page, + old AKR1D1-sumo before induction		
Inoculation of atcABC		Two tubes of 5mL culture media is used for overnight incubation at 37°C with shaking of 170 rpm
Prep samples for sequencing		Samples were prepared for both single and double mutants and for Economy run and NightSeq.
Purification of AKR1D1-Sumo		

Results and interpretation

Sonication of AKR1D1-SUMO

Eight samples of AKR1D1-SUMO (cultivated on August 8) were thawed on ice, washed with 10 mL of cell wash buffer (500 mM NaCl, 20 mM sodium phosphate) per tube, and resuspended. The duplicate samples were then pooled into four distinct batches (A through D). The samples were centrifuged at 5000 x g for 10 minutes at 4°C. The resulting pellets were weighed, and

lysis buffer (500 mM NaCl, 20 mM sodium phosphate, 30 mM imidazole; same as binding buffer) was added at a ratio of 5 mL of buffer per 0.4 g of pellet weight.

Sample	Pellet Weight (g)	Lysis Buffer Added (mL)
A	2.897	36.2
B	3.670	45.88
C	3.373	42.16
D	1.971	24.16

Following resuspension, the batches were pooled and subjected to sonication on ice. The sonicator was operated at 30% amplitude for a total of 9 cycles, with each cycle consisting of 2 minutes of active time using a 3-second on/off pulse interval. No SDS-PAGE sample was collected following sonication. The lysate was subsequently divided into four 50 mL tubes and clarified via centrifugation at 20,000 x g for 1.5 hours at 4°C.

Purification of AKR1D1_SUMO via Column Binding

Immobilized metal affinity chromatography (IMAC) columns were equilibrated with binding buffer. The sample flow rate was maintained at 1 mL/min throughout the run. Due to time constraints, only 50 mL of the total 250 mL clarified lysate was processed through the column; the remaining 200 mL was stored at 4°C overnight for subsequent purification. The column was washed with 10 mL of binding buffer, and the target protein was eluted using 5 mL of elution buffer (500 mM NaCl, 20 mM sodium phosphate, 500 mM imidazole).

To exchange the buffer and concentrate the protein, a 2 mL aliquot of the eluate was loaded onto a Vivaspin centrifugal concentrator along with 5 mL of potassium phosphate buffer (pH 6.5). The mixture was centrifuged at 4000 x g for 30 minutes. An additional 5 mL of potassium phosphate buffer was then added, and the solution was pipetted up and down to prevent membrane fouling before a final centrifugation step.

AKR1D1 TRX - Overnight Cultures OD values

Sample	Dilution Factor	OD at 600 nm	Actual OD
A	20	0.377	7.54
B	20	0.503	10.6
C	20	0.426	8.52
D	20	0.384	7.68

Tube and pellet weight after Induction:

Empty tube weight - 12.5485

Sample	Tube weight (g)	Pellet weight (g)
A1	13.9516	1.4031
A2	13.9582	1.4097
B1	13.8668	1.3138
B2	13.9951	1.4466
C1	14.1248	1.5763
C2	14.0910	1.5425
D1	13.9812	1.4327
D2	13.9430	1.3945

Sample Prep for sequencing

Samples were prepared for Economy run and NighSeq to be sent to Microsynth

Sample	Concentration (ng/uL)	Samples for Economy run	Samples for NightSeq
a	164	4	0
b	84	1	3
c	106	2	2
d	61.1	1	3
e	59.6	1	3
f	57.7	1	3
g	147	3	1
h	107	2	2

- The concentration of the plasmid required for economy run = 40 to 100 ng/uL and volume required is 12 uL. We send the primers separately, 50uL of 10mM concentration primer.
- Inputs for NightSeq are → E.coli culture = 5uL, Plasmid = 1uL.

As the concentration of samples a, c, g and h are higher than the concentration range, it was diluted to 40 ng/uL when prepared for economy run. The other samples were not diluted and were used as such.

Sample	Concentration (ng/uL)	Required concentration (ng/uL)	Sample volume (uL)	Milli Q water volume (uL)	Final volume (uL)
a	164	40	2.92	9.08	12
c	106	40	4.52	7.48	12
g	147	40	3.26	8.74	12
h	107	40	4.48	7.52	12

Economy run sample code of double mutants:

Label Number	Sample Name	DNA Type	PCR Length (Bases)	PCR Purification	Primer Source	Primer Name
98913001	001	Plasmid	0		Enclosed	ci MBP rev
98913002	002	Plasmid	0		StandardPrimer	T7
98913003	003	Plasmid	0		StandardPrimer	T7term
98913004	004	Plasmid	0		Custom	EXTRA ci MBP
98913005	005	Plasmid	0		Enclosed	ci MBP rev
98913006	006	Plasmid	0		Enclosed	ci MBP rev
98913007	007	Plasmid	0		Custom	EXTRA ci MBP
98913008	008	Plasmid	0		Enclosed	ci MBP rev
98913009	009	Plasmid	0		Enclosed	ci MBP rev
98913010	010	Plasmid	0		Enclosed	ci MBP rev
98913011	011	Plasmid	0		Enclosed	ci MBP rev
98913012	012	Plasmid	0		Custom	EXTRA ci MBP
98913013	013	Plasmid	0		StandardPrimer	T7
98913014	014	Plasmid	0		Enclosed	ci MBP rev
98913015	015	Plasmid	0		Custom	EXTRA ci MBP

A1 - 98913**001 -**
A2 - 98913**002 -**
A3 - 98913**003 -**
A4 - 98913**004 -**
B1 - 98913**005 -**
C1 - 98913**006 -**
C2 - 98913**007 -**

D1 - 98913**008 -**
E1 - 98913**009 -**
F1 - 98913**010**
G1 - 98913**011**
G2 - 98913**012**
G3 - 98913**013**
H1 - 98913**014**
H2 - 98913**015**
P (primer) - 98913**016**

NightSeq sample code of double mutants - 5uL bacterial culture:

B2 - 11151**911**
B3 - 11151**912**
B4 - 11151**913**
C3 - 11151**914**
C4 - 11151**915**
D2 - 11151**916**
D3 - 11151**917**
D4 - 11151**918**
E2 - 11151**919**
E3 - 11151**920**
E4 - 11151**921**
F2 - 11151**922**
F3 - 11151**923**
F4 - 11151**924**
G4 - 11151**925**
H3 - 11151**926**
H4 - 11151**927**

NightSeq sample code of single mutants - 5uL bacterial culture:

V263I
E1 - 11151**928**
E2 - 11151**929**
E3 - 11151**930**
E4 - 11151**931**
F1 - 11151**932**
F2 - 11151**933**
F3 - 11151**934**
F4 - 11151**935**

R215A
A1 - 11151**936**

A2 - 11151**937**
A3 - 11151**938**
A4 - 11151**939**
D1 - 11151**940**
D2 - 11151**941**
D3 - 11151**942**
D4 - 11151**943**
E1 - 11151**944**
E2 - 11151**945**
E3 - 11151**946**
E4 - 11151**947**

Label Number	Sample Name	DNA Type	Primer Source	Primer Name
11151911	001	E.coli	StandardPrimer	T7
11151912	002	E.coli	StandardPrimer	T7term
11151913	003	E.coli	Custom	EXTRA ci MBP
11151914	004	E.coli	StandardPrimer	T7
11151915	005	E.coli	StandardPrimer	T7term
11151916	006	E.coli	StandardPrimer	T7
11151917	007	E.coli	StandardPrimer	T7term
11151918	008	E.coli	Custom	EXTRA ci MBP
11151919	009	E.coli	StandardPrimer	T7
11151920	010	E.coli	StandardPrimer	T7term
11151921	011	E.coli	Custom	EXTRA ci MBP
11151922	012	E.coli	StandardPrimer	T7
11151923	013	E.coli	StandardPrimer	T7term
11151924	014	E.coli	Custom	EXTRA ci MBP
11151925	015	E.coli	StandardPrimer	T7term
11151926	016	E.coli	StandardPrimer	T7
11151927	017	E.coli	StandardPrimer	T7term
11151928	018	E.coli	StandardPrimer	T7
11151929	019	E.coli	StandardPrimer	T7term
11151930	020	E.coli	Custom	EXTRA ci MBP
11151931	021	E.coli	Enclosed	ci MBP rev
11151932	022	E.coli	StandardPrimer	T7
11151933	023	E.coli	StandardPrimer	T7term

11151934	024	E.coli	Custom	EXTRA ci MBP
11151935	025	E.coli	Enclosed	ci MBP rev
11151936	026	E.coli	StandardPrimer	T7
11151937	027	E.coli	StandardPrimer	T7term
11151938	028	E.coli	Custom	EXTRA ci MBP
11151939	029	E.coli	Enclosed	ci MBP rev
11151940	030	E.coli	StandardPrimer	T7
11151941	031	E.coli	StandardPrimer	T7term
11151942	032	E.coli	Custom	EXTRA ci MBP
11151943	033	E.coli	Enclosed	ci MBP rev
11151944	034	E.coli	StandardPrimer	T7
11151945	035	E.coli	StandardPrimer	T7term
11151946	036	E.coli	Custom	EXTRA ci MBP
11151947	037	E.coli	Enclosed	ci MBP rev

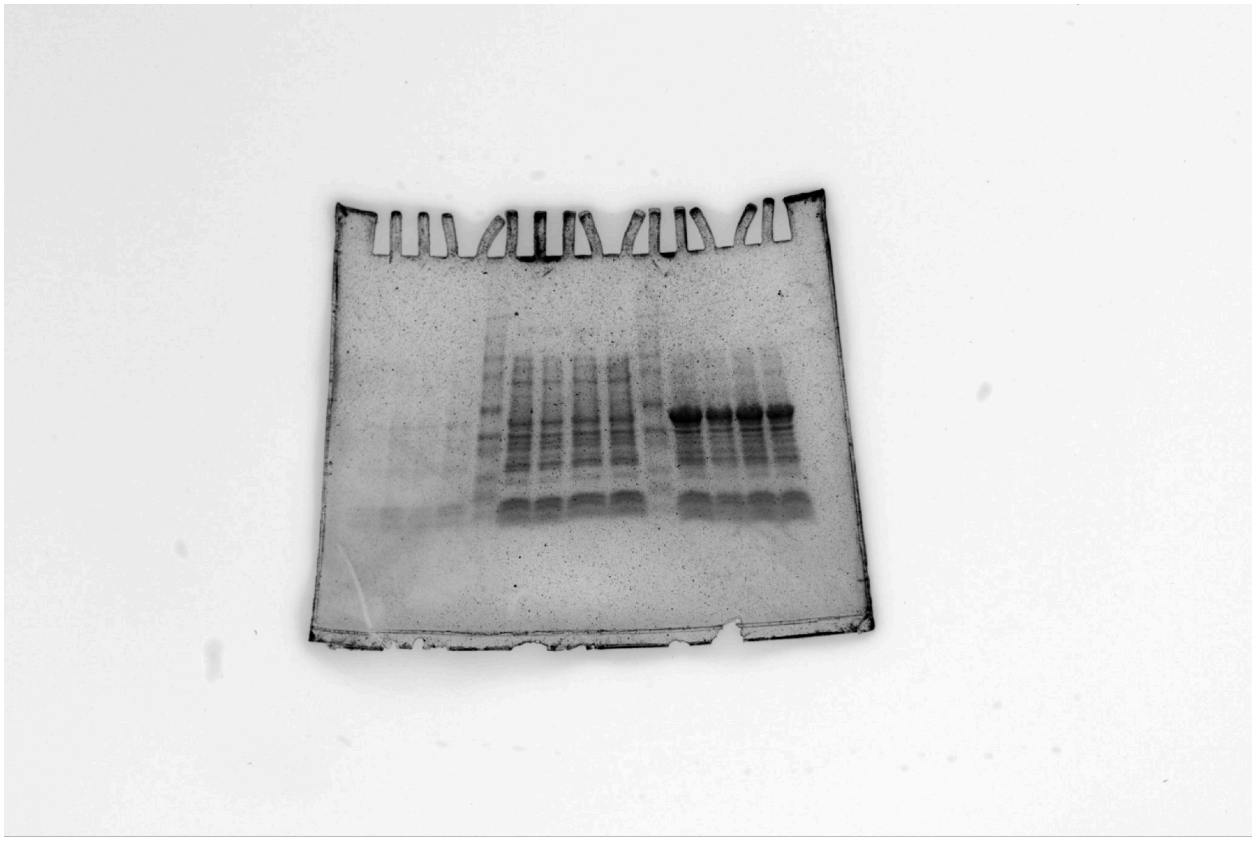
Mic

Inoculation and overnight incubation of atcABC

Two 5mL tubes were inoculated with atcABC, where a colony was picked and suspended in each of the tubes. The tubes were then placed in 37°C with shaking of 170 rpm overnight for production the next day

AKR1D1-Trx and AKR1D1-SUMO SDS

.



Day 41: [August 12, 2026], Wednesday

Weekly responsible: Eleonora
Lab participants: Cecilia, Hamsi, Hema, Ruiqi

Daily overview of experiments

We lysed, clarified, filtered and affinity-purified AKR1D1-Trx material, followed by concentration and buffer exchange. Four atcABC cultures were monitored before induction; iron citrate and sodium molybdate additions were recorded, although the stock and final concentrations require checking. Fluorescent reporter cultures and assay optimisation were listed, but the reporter identity and assay outcome remain incomplete.

Activity	***Protocol File***	***Comments***
Purification of AKR1D1-trx		
Production of atcABC		
Enzyme Activity Assay optimization		
Inoculation of fluorescence bacteria		5µL of amp in 5mL of LB-media; 37°C overnight cultivation

Results and interpretation

Production of atcABC

200mL broth is used to inoculate the overnight cultures. OD 600 for the pooled sample was measured to be 2.05 after 1:10 dilution. 1.46 mL of the overnight culture was inoculated in each of the culture flasks.
OD was recorded for every hour after the bacterial culture was inoculated. No dilution was done before induction of IPTG
The cultures were inoculated at 11:08 am and IPTG was induced at around 15:40 pm. 10 minutes before induction of 2mL of 100uM of IPTG , 2mL of 100uM Iron citrate and 2 mL of 1mM sodium molybdate were also added to the culture flask.

	1 hour	2 hours	3 hours	4 hours	4hour 30

					mins
1	0.029	0.113	0.587	1.194	1.516
2	0.031	0.099	0.622	1.203	1.531
3	0.027	0.087	0.532	1.138	1.625
4	0.028	0.080	0.503	1.030	1.542

Stock Solutions :

100mM Sodium Molybdate	10mL
Sodium Molybdate	0.242 g in 10mL water
10mM Iron Citrate	10mL
Iron Citrate	0.2499 g in 10mL water

Sonication of AKR1D1-trx

Four samples of AKR1D1-TRx (cultivated on August 8) were thawed on ice, washed with 10 mL of cell wash buffer (500 mM NaCl, 20 mM sodium phosphate) per tube, and resuspended. The samples were centrifuged at 5000 x g for 10 minutes at 4°C. The resulting pellets were weighed, and lysis buffer (500 mM NaCl, 20 mM sodium phosphate, 30 mM imidazole; same as binding buffer) was added at a ratio of 5 mL of buffer per 0.4 g of pellet weight.

Sample	Pellet Weight (g)	Lysis Buffer Added (mL)
1	1.09	13.6
2	1.06	13.3
3	0.95	11.9
4	1.08	13.6

Following resuspension, the batches were pooled and subjected to sonication on ice. The sonicator was operated at 30% amplitude for a total of 6 cycles, with each cycle consisting of 2

minutes of active time using a 3-second on/off pulse interval. The lysate was centrifuged at 20,000 x g for 1.5 hours at 4°C.
Afterwards, the lysate was filtered, first 1.2 µm, then 0.8µm, then 0.45 µm, and lastly 0.2 µm.

Purification of trx via Column Binding

Immobilized metal affinity chromatography (IMAC) columns were equilibrated with binding buffer. The sample flow rate was maintained at 1 mL/min throughout the run. After the flowthrough was collected the column was washed with 10 mL of binding buffer, and the target protein was eluted using 5 mL of elution buffer (500 mM NaCl, 20 mM sodium phosphate, 500 mM imidazole).

To exchange the buffer and concentrate the protein, a 4 mL aliquot of the eluate was loaded onto a Vivaspin centrifugal concentrator along with 2 mL of potassium phosphate buffer (pH 6.5). The mixture was centrifuged at 4000 x g for 30 minutes. An additional 3 mL of potassium phosphate buffer was then added, and the solution was pipetted up and down to prevent membrane fouling before a final centrifugation step (1mL of buffer added).

Day 42: [August 13, 2026], Thursday

Weekly responsible: Eleonora
Lab participants: Cecilia, Ruiqi

Daily overview of experiments

We lysed, clarified, filtered and affinity-purified AKR1D1-Trx material, followed by concentration and buffer exchange. Four atcABC cultures were monitored before induction; iron citrate and sodium molybdate additions were recorded, although the stock and final concentrations require checking. Fluorescent reporter cultures and assay optimisation were listed, but the reporter identity and assay outcome remain incomplete.

Activity	***Protocol File***	***Comments***
SDS-PAGE of AKR1D1 Sumo & Trx		
Stopped production of atcABC, sample stored at -80		

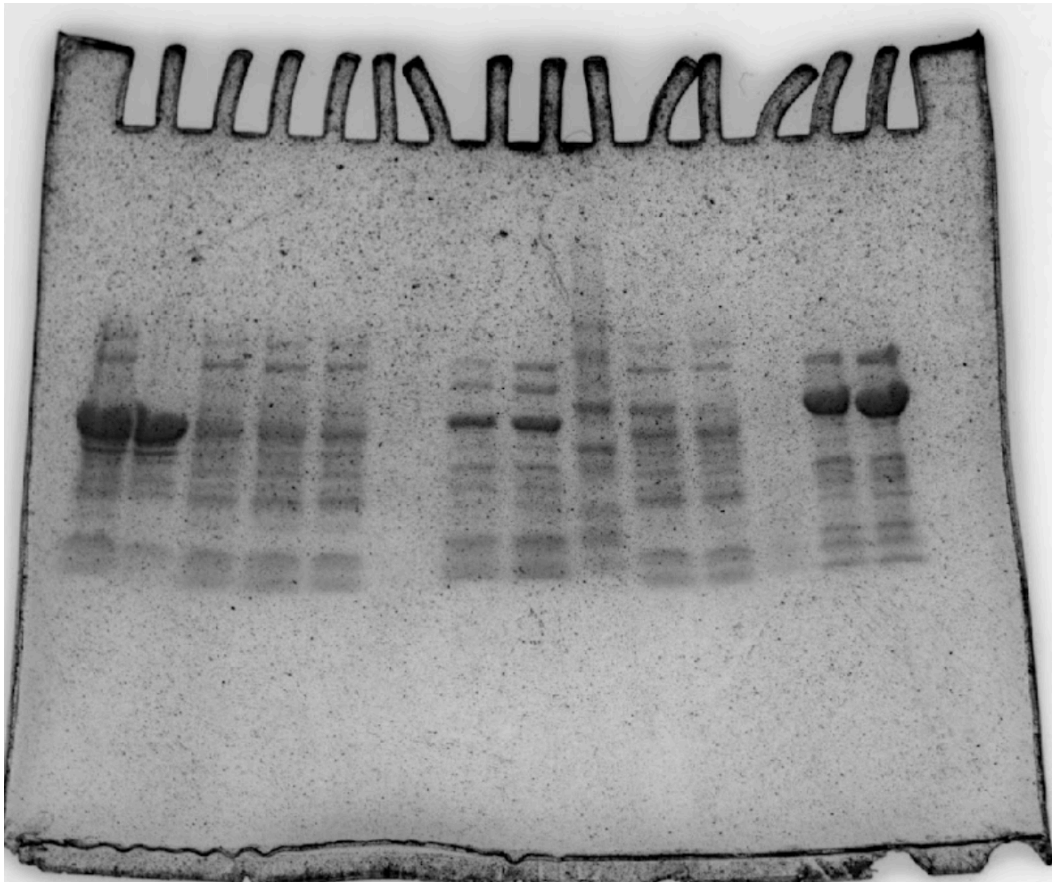
Results and interpretation

SDS-PAGE of AKR1D1_SUMO and AKR1D1_Trx

To evaluate protein purification, SDS-PAGE analysis was performed on AKR1D1-SUMO (purified on 11th of august) and AKR1D1-Trx (purified on 12th of august) samples. A total of thirteen samples were prepared: eight for AKR-Trx samples, five for AKR-Sumo.
For sample preparation, 30 µL mixtures were generated using 15 µL of cell samples and 15 µL of 2x SDS loading buffer. The loading buffer, originally prepared by Camille, was freshly supplemented with 10% beta-mercaptoethanol. The samples were heated at 95°C for 10 minutes and subsequently centrifuged for 30 seconds to collect any condensation.

For the pellet preparation, 15µL of lysate was centrifuged, supernatant discarded, and 15µL of 1% SDS added and resuspended. Then 15µL of the 2xSDS buffer was added as done for the other samples.

Each well was loaded with 10 µL of the sample mixture, and 5 µL of the full-range rainbow molecular weight marker (Cytiva) was used as a reference. Electrophoresis was initiated at 80V for 15 minutes to allow the samples to stack, after which the voltage was increased to 160V for 1h. Miliamper was set to 70 mA constantly. Following the run, the gel was rinsed in Milli-Q water, heated in a microwave for 30 seconds, and stained with Coomassie blue for 1 hour on an orbital shaker (100 rpm). Destaining was performed in three consecutive steps lasting 10 minutes, 30 minutes, and 1 hour, respectively, prior to gel imaging.



Well	Sample	Comment
1	AKR_Trx Lysate	
2	AKR_Trx Pellet of lysate	resuspended in 1% SDS
3	AKR_Trx Supernatant of lysate	
4	AKR_Trx Supernatant of Pellet Filtered	Filter used (µm): 1.2 -> 0.8 -> 0.45 -> 0.2

5	AKR_Trx Flowthrough	FT of sample through Ni Sepharose column
6	AKR_Trx Wash	Column wash with binding buffer
7	AKR_Trx Elutant	
8	AKR_Trx Elutant concentrated	Vivaspin, cutoff = 30kDa
9	Ladder	
10	AKR_SUMO Supernatant of lysate	
11	AKR_SUMO Flowthrough	FT of sample through Ni Sepharose column
12	AKR_SUMO Wash	Column wash with binding buffer
13	AKR_SUMO Elutant	
14	AKR_SUMO Elutant concentrated	Vivaspin, cutoff = 30kDa

Day 43: [August 14, 2026], Friday

Weekly responsible: Eleonora
Lab participants: Cecilia, Ruiqi and Hamsi

Daily overview of experiments

We set up a 96-well assay with four enzyme preparations and varied protein-sample volumes. Two deviations were recorded for AKR1D1-His: the nominal 25 µL sample condition without NADPH received no enzyme, and the 50 µL condition with NADPH was prepared in duplicate rather than triplicate. Missing measurement signals prevented interpretation. A cuvette assay and dilution series were also listed, but the reaction map and results remain incomplete.

Activity	***Protocol File***	***Comments***
Testosterone Assays Multiwell plate		Evaluate testosterone reduction across four enzyme purifications using a 96-well plate photometric assay.
Lab Cleanup and Autoclaving		All waste in the fume hood has been autoclaved
Testosterone Assay - cuvettes		

Results and interpretation

Testosterone Activity Assay (96-Well Plate)

This testosterone activity assay was performed in a 96-well plate to evaluate four enzyme purifications: ci_MBP (25°C cultivation, purified 29.07.), AKR1D1_SUMO (purified 11.08.), AKR1D1_Trx (purified 12.08.), and AKR1D1_His (purified 06.08.).

Each well contained a total reaction volume of 200 µL in potassium phosphate buffer, consisting of 50 µL sample master mix (0, 5, 15, 25, or 50 µL enzyme stock filled to volume with buffer), 140 µL testosterone master mix (15 µM target, 40 mM stock; 0.075 µL stock + 139.925 µL buffer per well), and 10 µL NADPH master mix (100 µM target, 20 mM stock; 1.0 µL stock + 9.0 µL

buffer per well). For control wells without NADPH, 10 µL potassium phosphate buffer was added instead. Master mixes were scaled up during preparation to account for pipetting losses.

Samples were tested in triplicate across seven conditions per enzyme construct (see table below): 0 µL sample (- NADPH), 25 µL sample (- NADPH), 0 µL sample (+ NADPH), 5 µL sample (+ NADPH), 15 µL sample (+ NADPH), 25 µL sample (+ NADPH), and 50 µL sample (+ NADPH).

	AKR1D1_His	AKR1D1_Trx	AKR1D1_SUMO	ci_MBP
Sample = 0µL No NADPH				
Sample = 25µL No NADPH				
Sample = 0µL NADPH				
Sample = 5µL NADPH				
Sample = 15µL NADPH				
Sample = 25µL NADPH				
Sample = 50µL NADPH				

Two experimental deviations occurred due to limited stock of AKR1D1_His: no enzyme was added to the 25 µL (- NADPH) control wells, and the 50 µL (+ NADPH) condition was prepared in duplicates rather than triplicates.

Additionally, cell-culture-grade plates were used instead of optical-grade absorbance plates, which likely caused the missing signals of the measurement.

Testosterone Assay was continued using Cuvettes following the conditions:

NADPH Solution 20mM	1mL
NADPH	16.67 mg
NADH Solution 20mM	1mL

NADH	13.31 mg

NADPH Solution 100 uM	
NADPH	120 uL of 20mM stock
Buffer	1080 uL
Testosterone 15 uM	
Testosterone	9 uL of 40mM stock
Buffer	16.67 mL

- Make a table for the amount of protein
- Series of dilution we did (1 , ½ , ¼ , ⅛ , 1/16)


IMPLEN
Tillhör
Tillämpad biokemi
Lunds universitet
Lunds Universitet
KC00907
Kinetics
0.90
0.85
0.80
0.75
0.70
0
5
10
15
20
25
A0 | An | dA | Slope | Result | R2
0.743 | 0.743 | 0.000 | --- | --- | 0.0000
Sample 1
Time 00:12:30
Abs 0.755
NANOPHOTOMETER
Responsible
Cedric Dicko, 073-649 42 22
- Ask responsible before using the equipment!
- Unsure of how to run the equipment? – ask responsible!
- If problem with the equipment – notify responsible!
- Good luck!