SEPTEMBER

WEEK 4

Ethanol Precipitation

- Simply add 9 volumes ice-cold ethanol (100%) to one volume of buffer containing the protein in 8M urea.
- Incubate at least 1h at -20°C and then spin down (7000rpm, 1h) the precipitate.
- Wash the pellet 4-5 times with 90% ice-cold ethanol, remove the supernatant as good as possible
- Resuspend the pellet in 1X PBS with 0.1% SDS.

Result

- There was very little precipitation.
- Nanodrop conc. : 0.729mg/ml
- We did CD of 0.6uM protein.
- It showed required negative peak for alpha and beta structure.
- It had more alpha structure than beta.
- There is not much difference in structure for different temperature.

Protein purification using new method

- Induction
- Lysis
- Urea treatment
- Ni NTA purification
- Ethanol precipitation
- Concentrating Protein

26/09/2021

Induction

- Primary inoculum (20 ml)
- Secondary inoculum (2l), induced with 1mM IPTG 35C for 5hr.
- Pellet down and stored in -20C.

27/09/2021

Lysis

- Lysed with 30 ml of lysis buffer and sonicated.

Urea Treatment

- Washed twice with 30ml buffer (50mM NaPB, 0.5M NaCl, 0.5% Triton X)
- Wash with 30ml 10mM CaCl2.
- Wash with 30 ml Extraction buffer (50mM NaPB, 8M urea, 0.5M NaCl).
- Collect the supernatant.

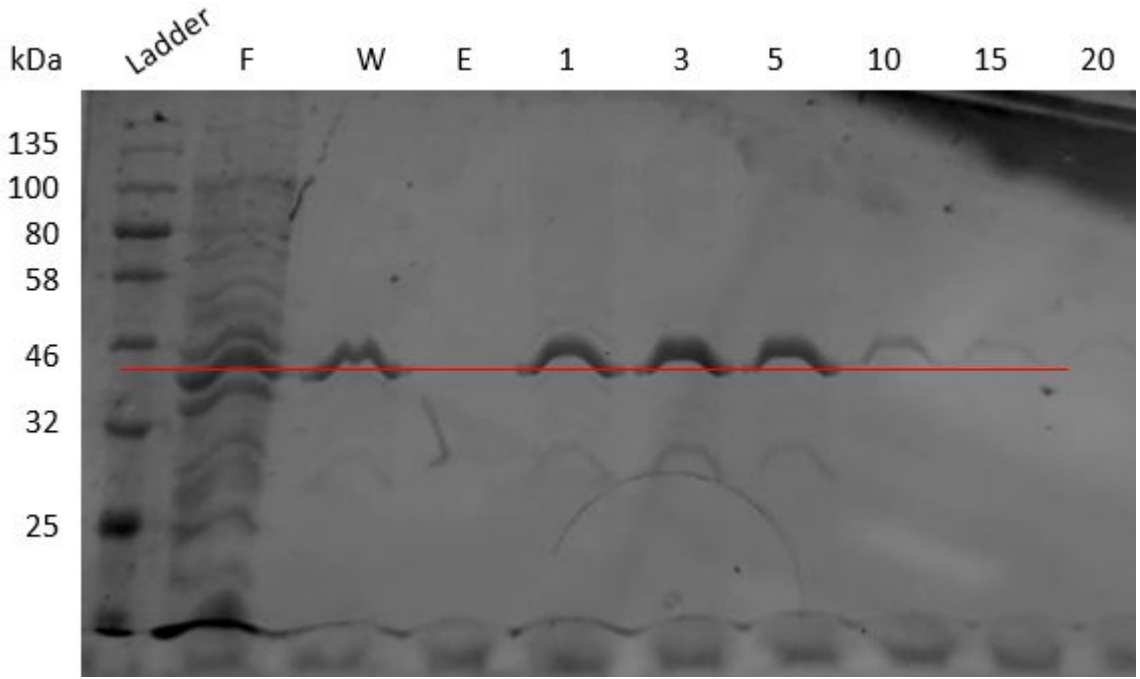
Ni NTA Purification

- Equilibrate with 30 ml equilibration buffer (50mM NaPB, 500mM NaCl, 8M urea)
- Incubate protein with beads for 1hr.
- Load the protein in column. Collect flow through.
- Wash the column with 50 ml wash buffer (50mM NaPB, 500mM NaCl, 20mM Imidazole, 8M urea).
- Add 50 ml Elution buffer (50mM NaPB, 100mM NaCl, 400mM Imidazole, 8M urea).
- Collect 31 fractions of elution.

27/09/2021

SDS PAGE

Annotations:
F: Flow Through
W: Wash Flow Through
E: Elution Flow Through
1-20: Elution Fractions



Ethanol precipitation

- Simply add 9 volumes ice-cold ethanol (100%) to one volume of buffer containing the protein in 8M urea.
- Incubate for 2hr at -20°C and then spin down (7000rpm, 1h) the precipitate.
- Wash the pellet 4-5 times with 90% ice-cold ethanol, remove the supernatant as good as possible
- Resuspend the pellet in 1X PBS with 0.1% SDS.

Note: If the protein is precipitating add more volume of buffer.

Concentrating Protein

- The protein is concentrated using amicon filter.
- During this experiment, the protein is precipitating.
- Thus, we changed the buffer composition.

Buffer	Stock solution	Working solution	Vol for 40 ml
NaPB	5M	200mM	1.6 ml
PBS	10X	1X	4 ml
Glycerol	50%	10%	8ml
SDS	10%	0.1%	0.4 ml

28/09/2021

Nanodrop concentration

Ɛ = 73.59 Mol. mass = 43.262 kDa

mg/ml	A280	A260/A280
1.000	1.70	0.69

Protein purification of wash flow through

- Did ethanol precipitation.
- Divided in 2 parts- one we suspended in buffer containing SDS and the other without buffer.
- We observed that there is more precipitation in buffer without SDS.
- Then we concentrated the protein using amicon filter

Nanodrop concentration

Ɛ = 73.59 Mol. mass = 43.262 kDa

#	mg/ml	A280	A260/A280
Protein with SDS	3.2	5.47	0.82
Protein without SDS	0.250	0.43	1.75