

Expression, Purification and Basic Characterization of Target Protein Samples for Drug Binding Studies

Group 1 Laboratory Activities

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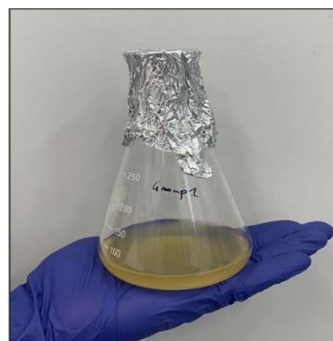
Day 1

Group 1

1) Transformation



2) Small-scale culture



After 8 h, $OD_{600} = 2.5$

Group 1

3) Large-scale culture, induction and cell harvesting

- Small scale (from Shapla ☺) → $OD_{600} = 8.9$ → 5.6 mL in 500 mL of LB
- After 40 min, $OD_{600} = 0.01$ → we added 10 mL ☺
- After 1.5 h, $OD_{600} = 0.43$
- Induction IPTG
- 20 min → centrifugation → pellet of 0.78 g

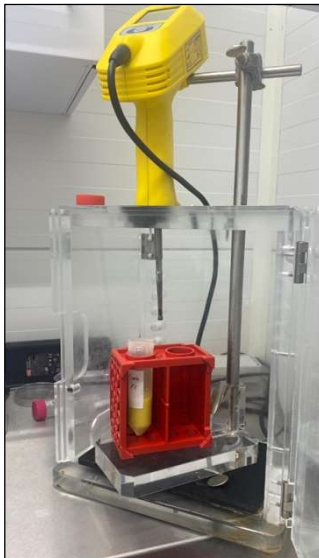


Day 2

Group 1

1) Sonication

6.28 g of pellet
(from Shapla ☺)



2) IMAC



Flow-through
Wash

E1 $OD_{600} = 4.067$ mg/mL ($A_{260/280} = 0.79$)
E2

Group 1

3) SEC



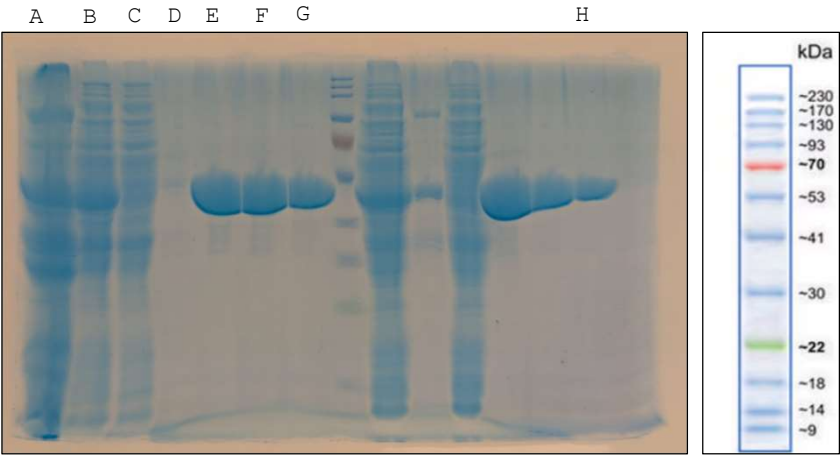
Fraction s	mg/mL	A _{260/280}
F3	0.957	0.67
F4	2.341	0.66
F5	2.426	0.67
F6	1.075	0.68
F7	0.257	0.80

4) IEX



5) SDS-PAGE

- A Insoluble
- B Soluble
- C Flow-through
- D Wash
- E IMAC E1
- F IMAC E2
- G SEC F4
- H IEX E

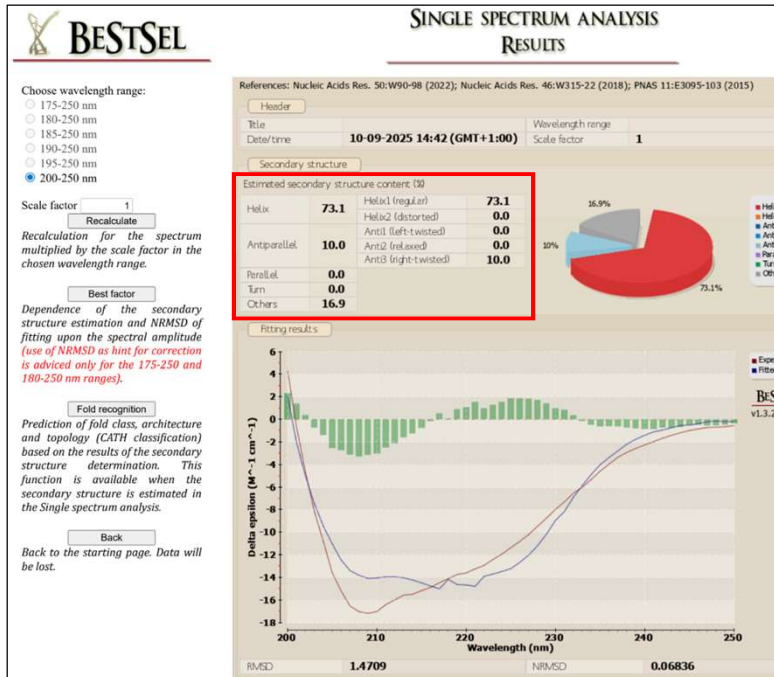


Group 1

Day 3

Group 1

1) CDsf F4



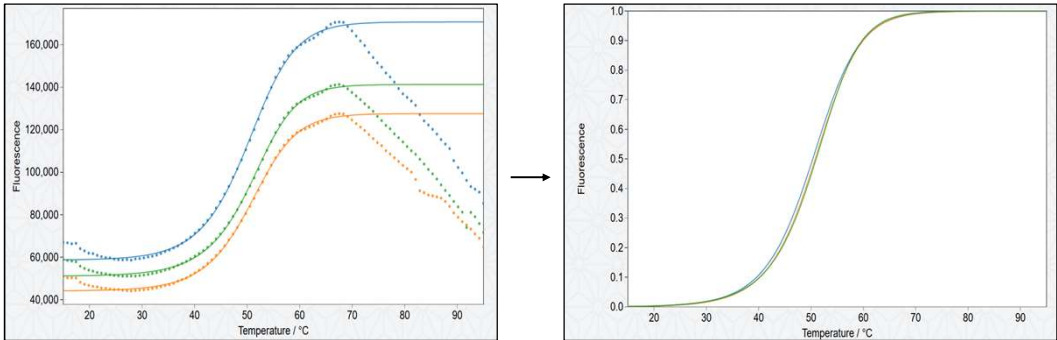
Day 3

Group 1



Group 1

2) DSFF F4

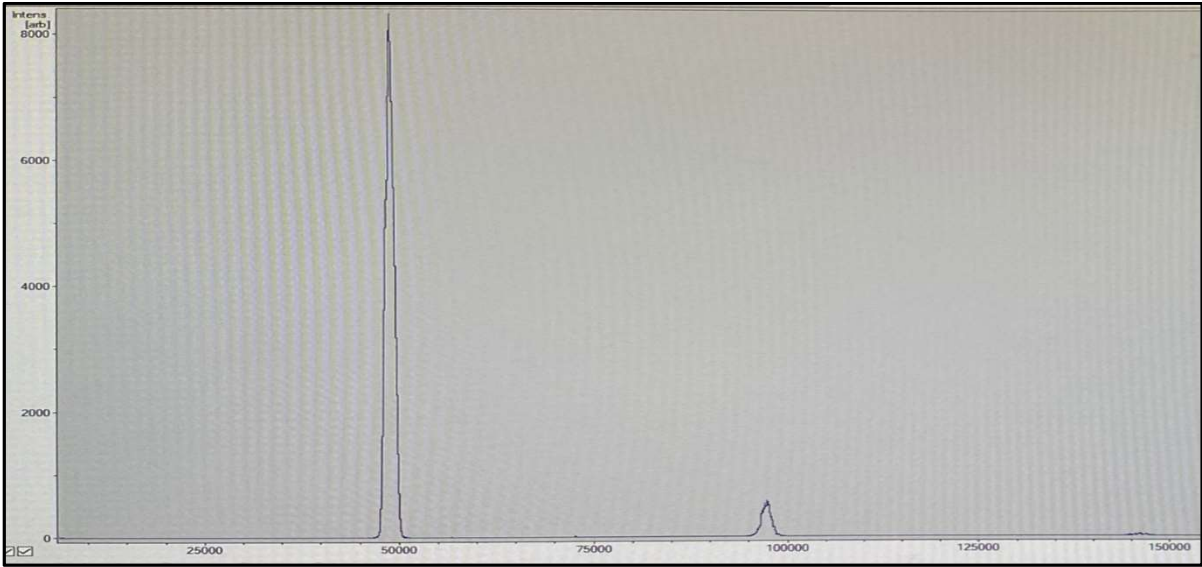


Name ^	Type	Tm	ΔTm	ΔTm / σ	R ²	Min T	Max T	Min F	Max F
A01	Assay	50.3			1.000	27.9	67.5	58,620	170,703
A02	Assay	50.8			1.000	27.9	67.5	44,122	127,550
A03	Assay	51.0			1.000	27.1	67.5	51,068	141,276

Group 1

3) MALDI-TOF F4

MW: 48.2 kDa



THANK YOU!

