WEB322 - COMT expression

Benjamin Weigel

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1 Day 1

- 500 mL over night culture (+ 200 $\frac{\mu g}{ml}$ Amp) of **ZYP-5052** (autoinduction medium) inoculated with toothpick of COMT (3) from Th. Vogt

2 Day 2

- measured $OD^{600} = 10.56 \rightarrow 500\ \mu l$ **SDS-PAGE** sample
- harvested cells by centrifugation (10.000 x g, 4°C , 5 min)
- inoculated 250 mL culture of **LB** (+ 200 $\frac{\mu g}{ml}$ Amp) with 1.8 mL of overnight AI culture
- started incubation at 10.50

time	sample	OD^{600}	comments
10.50	AI	10.56	
13.00	LB culture	0.852	
18.00	LB culture	3.6	SDS-PAGE sample NI

3 Purification from AI-medium expression

- resuspended pellet (∼3 g) in 60 mL **Lysis Buffer** (50 mM Tris/HCl, 0.5 M NaCl, 10% (v/v) glycerol, 10 mM Imidazole, 1% Tween 20)
- added a spatula tip of HEWL and incubated for 15 min at RT while inverting
- lysed cells by sonication (3 times for 30 s at 70% amplitude, 1s-1s on-off-cylce)
- added DNase buffer and 200μl DNase I and incubated on ice for 15 min
- removed debris by centrifugation (20 min, 10.000 x g, 4°C)

3.1 ÄKTA

- filtered through a 0.45 μm filter and injected onto 1 mL HiTrap Talon FF column on ÄKTA

! buffers, lysate and also the collection tubes were cooled

- column was equilibrated with **buffer A** (50 mM Tris/HCl, 500 mM NaCl, 10% (v/v) glycerol, 2.5 mM imidazole, pH 7.4)

- after sample injection the column was washed with 4 CV of buffer A and 4 CV of 5% buffer B

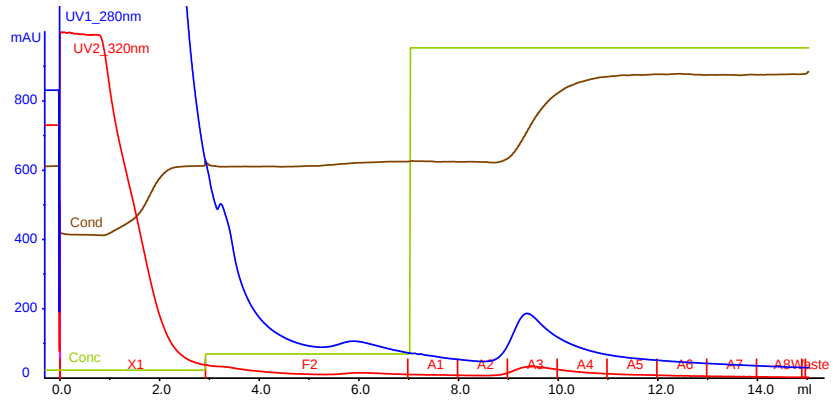
- eluted with 8 CV **buffer B** (50 mM Tris/HCl, 500 mM NaCl, 10% (v/v) glycerol, 300 mM imidazole, pH 7.4)

!!! only a very small protein peak elutes at 100% B (Fig. 1a) → SDS-Gel to clarify what went wrong in purification (Fig. 2a)

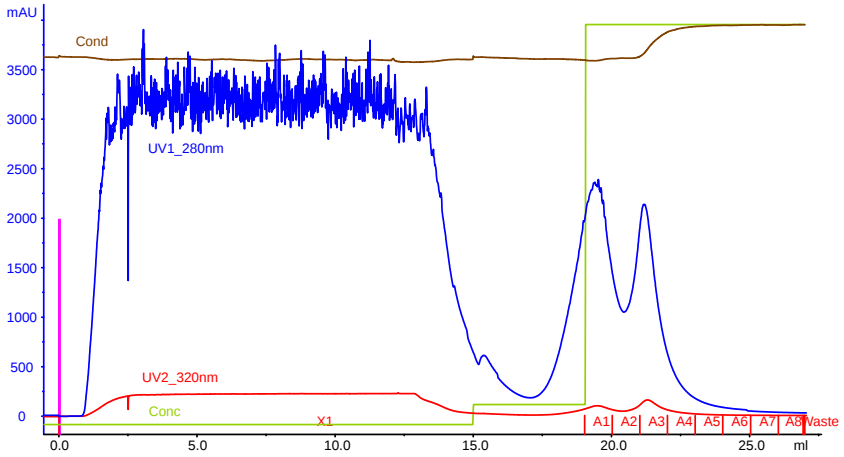
- precipitated 100μl of fractions X1 and A3 with TCA and used for SDS-PAGE

4 Purification from LB-medium expression

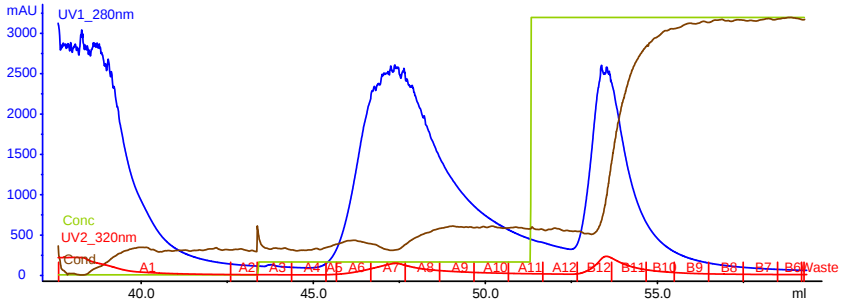
- harvested cells by centrifugation (10.000 x g, 4°C , 5 min) and stored at -20°C



(a) ÄKTA-Run 1. Co-NTA, application @ 10 mM imidazole

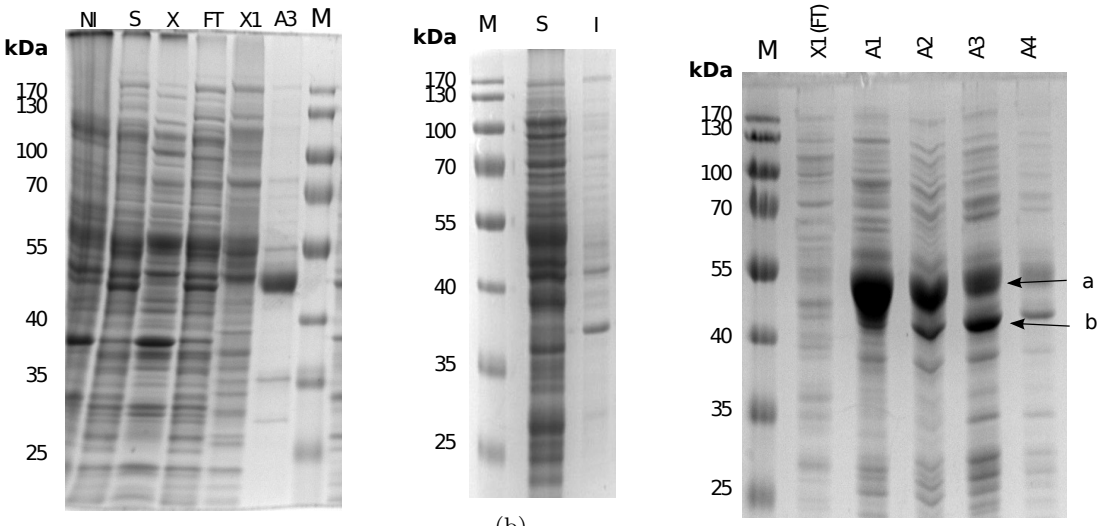


(b) ÄKTA-Run 2. Ni-NTA, application @ 2.5 mM imidazole



(c) ÄKTA-Run 3. Ni-NTA, application @ 2.5 mM imidazole, prolonged steps

Figure 1: ÄKTA Runs. **1a**: Raw lysate was injected at 2 mL/min on a HiTrap Talon FF 1mL (Co-NTA). **1b**: The flow-through of 1a was diluted with 3 volumes of buffer A.2 and 30 mL were injected at 0.5 mL/min on a HisTrap FF 1mL (Ni-NTA). **1c**: The same protocol as used before (Fig. 1b), however the wash and elution steps were prolonged.



(a)
10% Gel

(b)
12% Gel

(c)
12% Gel

Figure 2: COMT purification.
2a: COMT purification using 1 mL HiTrap Talon FF (left side). NI - after induction (total), S - after induction (soluble), X - not sure (discard), FT - ÄKTA flowthrough, X1 - fraction X1, A3 - elution fraction A3.
2b: Flowthrough, that was collected during COMT purification using 1 mL HiTrap Talon FF. The flowthrough stood at room temp for ∼3 hours. The turbid solution was centrifuged to separate soluble and aggregated protein. S - soluble, I - insoluble.
2c: Flowthrough, that was collected during first COMT purification trial (2a) was diluted with 3 volumes of **buffer A.2** (50 mM Tris/HCl, 500 mM NaCl, 10% (v/v) glycerol, pH 7.4). 30 mL of the diluted flowthrough (2.5 mM imidazol concentration) were applied to a HisTrap (Ni-NTA) 1 mL FF column at 0.5 mL/min flow. X1 - flowthrough, A1:4 - fractions A1 to A4.