WEB311 - SOMT Refolding & HIC

Benjamin Weigel

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1 Refolding

- refolded 2.5 ml 1$\frac{mg}{mL}$ SOMT in 50 mL buffer 12 over night at 4°C
- added 1 Volume (50 mL) of 2 M $(NH_4)_2SO_4$
- adjusted pH to 7 using 5 M KOH → solution turned sligthly turbid
- centrifuged to remove precipitate (20.000 x g, 4°C , 30 min)

2 HIC

- equilibrated 4 mL phenyl sepharose matrix with 3 CV water & 3 CV 1 M $(NH_4)_2SO_4$ pH 7
- applied 100 mL of clarified sample
- eluted stepwise from 1 M $(NH_4)_2SO_4$ to 20 % EtOH (see WEB309), then 70 % EtOH, 0.1 M NaOH and 0.5 M NaOH, and collected 4 mL fractions
- for SDS-PAGE 1 mL of each fraction was precipitated using TCA and resuspended in 10 μl PBS and 2 μl SDS loading dye (heated to 95 °C and applied to gel)

ATTENTION: after increasing EtOH to 70% there is visible precipitate at the top of the column

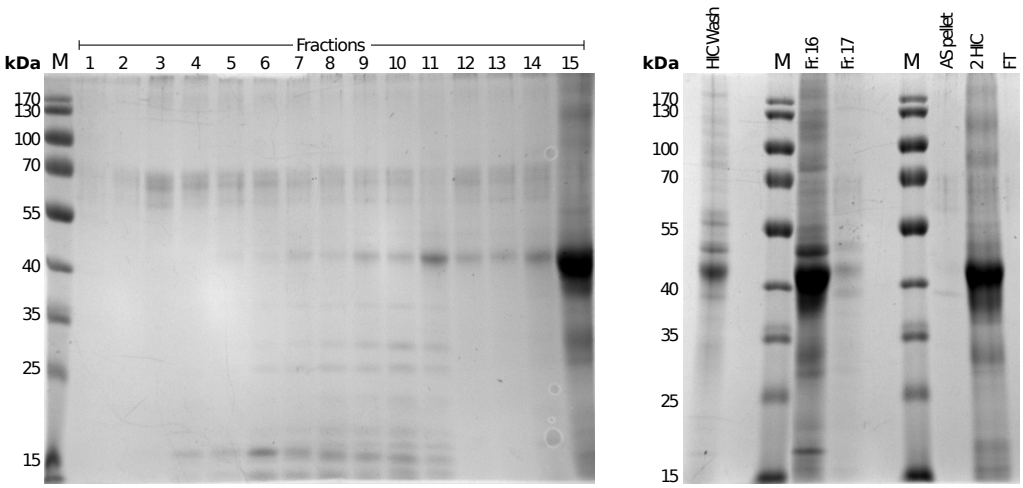

Figure 1: SDS-PAGE of fractions eluted from HIC.

Table 1: Setup of the gels for SDS-PAGE analysis

gel	lane	sample	comment
Gel 1 / Fractions	1	Marker	
	2	Fr. 1	1 M AS
	3	Fr. 2	1 M AS
	4	Fr. 3	0.8 M AS
	5	Fr. 4	0.8 M AS
	6	Fr. 5	0.6 M AS
	7	Fr. 6	0.6 M AS
	8	Fr. 7	0.4 M AS
	9	Fr. 8	0.4 M AS
	10	Fr. 9	0.2 M AS
	11	Fr. 10	0.2 M AS
	12	Fr. 11	0 M AS
	13	Fr. 12	0 M AS
	14	Fr. 13	20 % EtOH
	15	Fr. 14	20 % EtOH
	16	Fr. 15	70 % EtOH
Gel 2	1		
	2	HIC Wash	applied precipitate that appeared after stringent wash (0.1 M acetic acid [4 CV], 0.1 M NaOH [4 CV]) - WEB310
	3		
	4	Marker	
	5	Fr. 16	
	6	Fr. 17	
	7		
	8	Marker	
	9	AS pellet	precipitate from after AS addition
	10	2HIC	sample applied to HIC (after AS addition & centrifugation)
	11	FT	flowthrough from HIC column
	12		
	13		
	14		
	15		
	16		