WEB310 - SOMT Refolding & HIC

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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 NaOH

ATTENTION: Added 50 mL of an unknown solution (from DMEs bench) → to compensate 100 mL of 2 M $(NH_4)_2SO_4$ were added and then adjusted to pH 7

2 HIC

- equilibrated 4 mL phenyl sepharose matrix with 3 CV water & 3 CV 1 M $NH_4)_2SO_4$
- applied 200 mL of sample
- eluted stepwise from 1 M $(NH_4)_2SO_4$ to 20 % EtOH (see WEB309) 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 cleaning the HIC column with 4 CV of 1 M acetic acid and 4 CV of 0.1 M NaOH protein precipitated in the washed out solution → took a sample and centrifuged to collect pellet → large pellet of probably protein → do SDS-PAGE

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

lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Fraction	Flowthrough	M	1	2	3	4	5	6	7	8	9	10	11	12	13	14

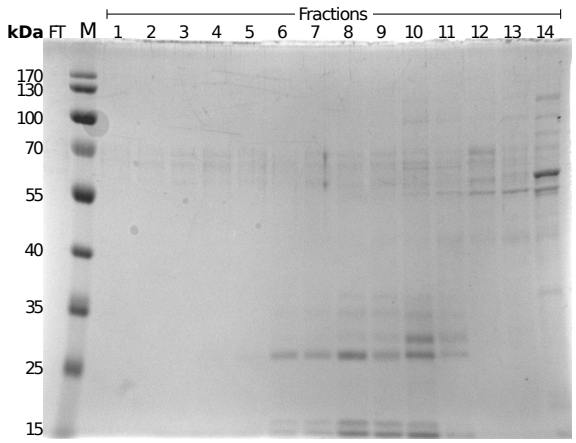

Figure 1: SDS-PAGE of fractions eluted from HIC.