WEB308 - SOMT ammonsulfate precipitation

Benjamin Weigel

23rd September, 2014

Introduction

- refolded 2.5 mL 1 mg/mL SOMT in solubilization buffer in 50 mL Buffer 12 by fast dilution (in 50 mL tube)
- incubation over night at 4°C
- centrifuged at 16.000 x g / 4°C to remove precipitate
- took 5 mL aliquots and performed ammonium sulfate precipitation test (see Figure 1)

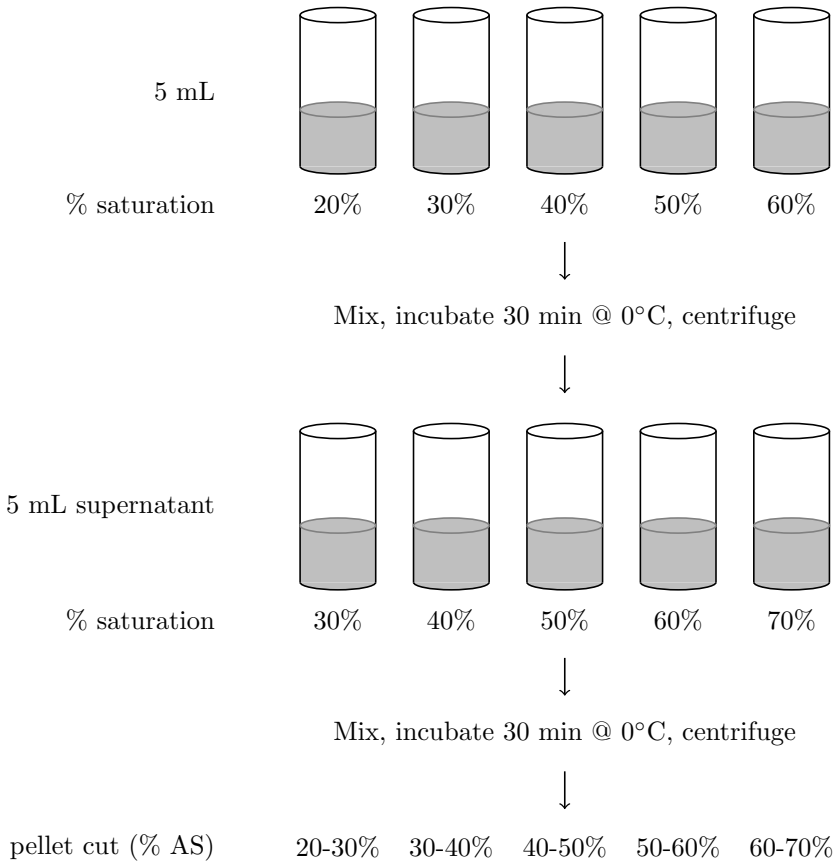


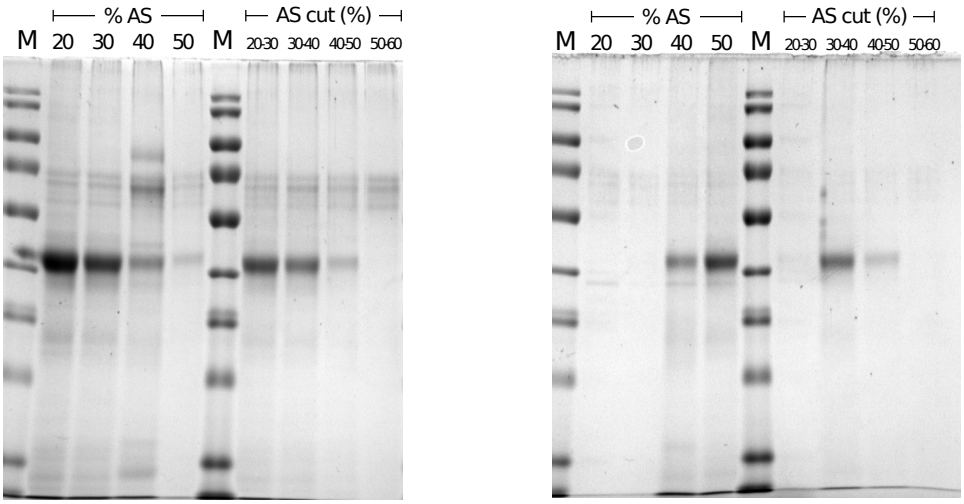
Figure 1: Experimental setup for the ammonium sulfate precipitation.

Table 1: Amount of Ammonium sulfate to add to 5 mL solution at each step.

AS cut	20-30%	30-40%	40-50%	50-60%	60-70%
AS (1^{st} step)	0.53 g	0.82 g	1.13 g	1.455 g	1.8 g
AS (2^{nd} step)	0.275 g	0.28 g	0.29 g	0.3 g	0.31 g

1 SDS-PAGE

- ca. 50 μg/mL protein in refolding reaction
- precipitation via ammonsulfate
- SDS-PAGE analysis of pellet & supernatant of first step
- SDS-PAGE analysis of pellet & supernatant of second step → 16 samples
- 200 uL = 10 ug protein → take 200 uL from precipitation in eppi & centrifuge
- resuspend AS pellet (1) in 50 uL PBS → 5 uL for Gel
- TCA precipitation with supernatant, take up pellet from TCA-precipitation in 10 uL PBS and analyze via SDS-PAGE
- resuspend protein pellet from AS-precipitation (2) in 0.25 mL PBS → 10 uL (ca 10 ug) on gel



(a) Supernatant

(b) Ammonium sulfate pellet

Figure 2: Ammonium sulfate precipitation test straight from refolding SOMT in refolding buffer 12. The majority of SOMT protein is precipitated in the 30–40% cut of ammonium sulfate saturation (Fig. 2b). However, there is a some SOMT still in solution even at a 50% AS saturation (Fig. 2a).

Table 2: 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
pellets	M	1^{st} precipitation				M	2^{nd} precipitation				M					
		20%	30%	40%	50%		30%	40%	50%	60%						
supernatant	M	1^{st} precipitation				M	2^{nd} precipitation				M	MTAN				
		20%	30%	40%	50%		30%	40%	50%	60%						