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Project: iGEM_Munich2019 Shared Project
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Johanna + Sarah

Purification

- samples:
    - new Coiled-Coil L7Ae
    - new Coiled-Coil MCP
    - old Coiled-Coil MCP
    - Gag-L7Ae Fusionconstruct (purification with 10 CV highest salt concentration at the end)
- **Preparation**
Prepare Stock Solutions, filter them!
Prepare Buffer A, Buffer B, Equilibration Buffer, Wash Buffer
Prepare Elution Buffer (20 x 10 mL -> label 20 x 15 mL Falcons)
Prepare XY x FT Eppis, 10 x Wash Eppis, 20 x Elution Eppis
Equilibrate the heparin resin with 5 CV **Equilibration Buffer**
Prepare the sample: harvest the supernatant form the 6-well plate. Centrifuge 10 minutes at 2000 g.
Filtration: Filter the harvested supernatant through a 0.8 μm syringe filter
Load the sample on the resin -> collect Flow-through = XY mL = XY fractions
Wash
Wash with 10 CV Was Buffer = 10 fractions
Elution
Elute with a salt gradient (120 - 20000 mM NaCl) with 20 CV = 20 fractions

Salt Gradient

	NaCl [mM]	Buffer A [µL]	Buffer B [µL]	Buffer A [mL]	Buffer B [mL]
1	150	925	75	9.25	0.75
2	200	900	100	9	1
3	300	850	150	8.5	1.5
4	400	800	200	8	2
5	500	750	250	7.5	2.5
6	600	700	300	7	3
7	700	650	350	6.5	3.5
8	800	600	400	6	4
9	900	550	450	5.5	4.5
10	1000	500	500	5	5
11	1100	450	550	4.5	5.5
12	1200	400	600	4	6
13	1300	350	650	3.5	6.5
14	1400	300	700	3	7
15	1500	250	750	2.5	7.5
16	1600	200	800	2	8
17	1700	150	850	1.5	8.5
18	1800	100	900	1	9
19	1900	50	950	0.5	9.5
20	2000	0	1000	0	10

10 CV at the end of the gradient with **100 % Buffer B**
Regeneration
10 CV 1 M NaOH
10 CV 30 % 2-Propanol

- total elution was about 50-60 % from total load
- after purification the samples were concentrated by using 30 kDa centricons -> the concentrated samples are used for Western Blot and DLS