



UNIVERSITY OF GOTHENBURG

WORKSHOP ON CELL-FREE PROTEIN SYNTHESIS FOR NMR

May 28-30, 2013



May 28th

The program is deliberately loosely set to allow for improvisation when it comes to sample preparation.

10:00 Registration, overview of the program.

10:45 Setup of cell-free reactions.

Cell-free protein synthesis setup

1. Add the reagents to a 50 ml tube in the indicated order. Make sure to work on ice and use only filter tips. **See individual protocol setup paper!**
2. Place your reaction tube into one of the thermomixers at 30°C, 750 rpm
3. Add 1.15 ml creatine phosphate after 30 min of reaction.

12:30 Lunch at the NMR Centre

13:15 Seminar: “CFPS basics” Anders Pedersen

14:30 Purification of CFPS reactions

Ni-NTA purification for His-tagged constructs

Total and soluble samples will possibly be used for Western blots if stuff go wrong

We will try and use the AmiconPro spin-column format for purification:

1. Equilibrate 200 μ l NiNTA resin twice in AmiconPro tube (3 or 10 kDa MWCO as appropriate) with 2 ml buffer B, spinning at 600 x g for 1 min
2. Take out 6 μ l of the reaction as a total sample for SDS-PAGE
3. Take out 10 μ l of the reaction to a 1.5 ml tube and spin at maximum speed in a benchtop centrifuge. Spin the rest (the bulk) of the expression reactions at 4500 x g for 15 min.
4. Take out 6 μ l of the supernatant from the 1.5 ml tube as a soluble sample for SDS-PAGE
5. Add expression reaction supernatant and fill up with buffer A to the equilibrated NiNTA resin in the AmiconPro tube and incubate at room temp for one hour on roller table
6. Spin tube at 600 x g for 1 min, collect flow-through
7. Wash resin with 5 ml of buffer B, spin as above and collect wash fraction
8. Elute protein with 3x 500 μ l buffer C into a fresh 50 ml tube
9. Make SDS-PAGE samples from the eluate:
 - 13 μ l eluate
 - \pm 2 μ l DTT (0.5 M)
 - 5 μ l BOLT loading buffer

SDS-PAGE

1. Heat samples at 95°C on heating block for 5 min
2. Load samples on SDS-PAGE gels. Run SDS-PAGE for 30 min at a constant voltage of 200V
3. Stain, destain and scan gel(s).

Buffer change

1. Equilibrate PD-10 column with 25 ml of desired buffer (Tev: buffer D; for NMR buffer E)
2. Load pooled eluates and discard flowthrough
3. Elute with 3.5 ml buffer D
4. Estimate concentration optically if possible

Concentration of samples that does not require cleavage

1. Equilibrate Vivaspin ultrafiltration tubes of relevant cutoff with 3x NMR buffer
2. Concentrate sample until volume is ~280 µl
3. Add 20 µl D₂O and load 5 mm Shigemitube

16:30 Tag cleavage with Tev/FXa

1. Take out SDS-PAGE sample before adding Tev. Tev stock is 100 µM in 300 µl aliquots.
2. Add approximately 1:70 Tev:protein
3. Place on thermomixer at 24°C, 600 rpm for 6 hours, then 4°C ON

17:00 Participant presentations (4x)

18:00 *Pizza and suiting beverage at the NMR Centre*

May 29th

- 09:00** **Removal of Tev/FXa with affinity chromatography (NiNTA/FXa removal resin)**
- 11:00** **Seminar: “CFPS optimization” *Göran Karlsson***
- 12:00** ***Lunch at the NMR Centre***

Buffer change

1. Equilibrate PD-10 column with 25 ml of desired buffer (buffer E)
2. Load pooled eluates and discard flowthrough
3. Elute with 3.5 ml buffer D
4. Estimate concentration optically if possible

Concentration

1. Equilibrate Vivaspin ultrafiltration tubes of relevant cutoff with 3x NMR buffer
2. Concentrate sample until volume is ~280 μ l
3. Add 20 μ l D₂O and load 5 mm Shigemitube

15:00 **Seminar: “New tools for studying intrinsically disordered proteins” *Vladislav Orekhov***

16:00 **Participant presentations (4x)**

16:45 **Setup of NMR experiments on successful samples**

18:30 ***Dinner downtown***

May 30th

09:00 **Discussion of results, future directions**

11:00 **END**