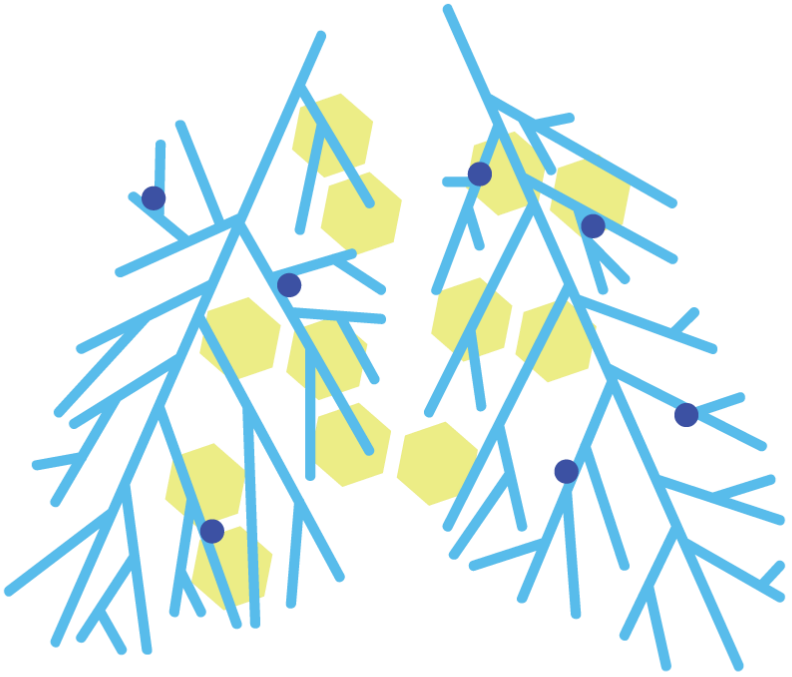


PROLUNG

DEGRADATION

EXPRESSION

LAB BOOK 10

iGEM Stockholm

Sialidase expression and purification for assay testing

Aim

- Express a large amount of active sialidase that will be further tested in an assay.
- Background information
    - To confirm that the expressed sialidase (from biobrick) was active, a larger amount had to be expressed and purified for testing. The same procedure that had been followed in earlier experiments, and showed highest enzyme yield, was repeated.
    - The Sialidase was expressed in E. coli BL21 cells and expression was induced using IPTG.
    - The Sialidase is a His-tagged 54 kDa protein and can therefore be purified using IMAC and afterwards be concentrated using the right filter.

Procedure:

- Cultivation and induction
    - *E. coli* BL21 cells containing the biobrick were cultivated in two 50 ml of LB medium with 20 mg/ml Chloramphenicol. When the culture reached an OD of 0.4 one of the cultures was induced with 0.5 mM IPTG. The other culture was used as a control. After induction the cultures were left overnight in room temperature.
    - iGEM 2016 protocol
- Sonication
    - Sonication was performed according to protocol. With the exception of DTT not being used in the lysis buffer.
    - iGEM 2016 protocol
- IMAC purification
    - IMAC performed according to protocol.
    - iGEM 2016 protocol
- SDS-PAGE

    - SDS-PAGE performed according to protocol. The purified samples were separated into 5 eluted fractions. With 2 samples there were 10 fractions in total. Five of them belonging to the uninduced control.
    - iGEM 2016 protocol with some modifications.

1. Protein samples and loading buffer were mixed at a ratio of 24 µL of protein sample and 6 µL of loading buffer in a fume hood. Loading buffer was prepared by staff at floor 2.
2. Samples were centrifuged at 13000 rpm for 60 seconds.
3. Samples were incubated for 5 minutes in a dry heating block at 95 °C.
4. Samples were centrifuged at 13000 rpm for 60 seconds.
5. Gels (Mini-Protean TGX from BIORAD) were mounted into a tank and the combs and strip at the bottom of the gel cast were removed.
6. The inner chamber was filled with 1x running buffer and the outer chamber was filled with running buffer up to the marking line on the tank. Running buffer was prepared by staff on floor 2.
7. The ladder was loaded into each gel with 2 uL of the ladder solution (product name?).
8. The rest of the wells were loaded with 10 uL of sample.
9. The gels were run at 150 V for 30 minutes.

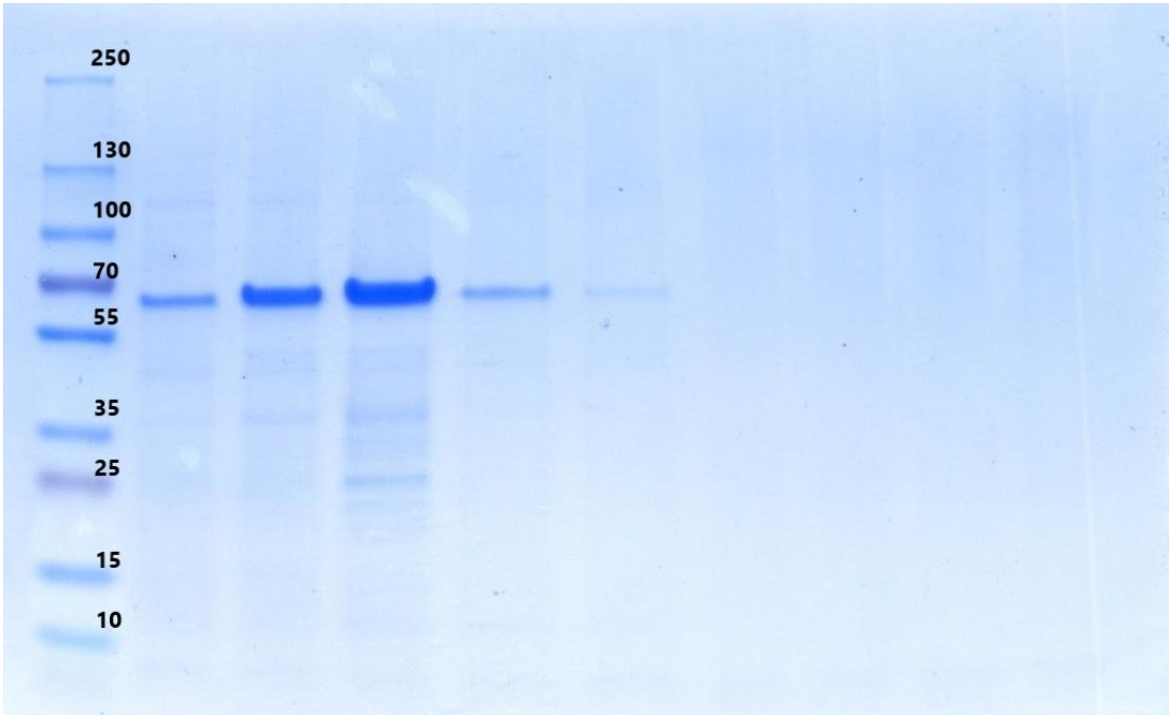
For staining of the proteins the protocol for SimplyBlue SafeStain was used with no modifications.

- Centrifugal filtration
    - *Amicon® Ultra-15 Centrifugal Filter Units* were used with a pore size of 10 kDa. The purpose of the filtration was to remove all imidazole, which is used for elution in IMAC.
    - The centrifugal filters usage guide was used as a protocol with some modifications.
        - The ultrafiltration membranes in *Amicon® Ultra-15* contain trace amounts of glycerine. A substance that may affect the the enzyme assay. Therefore, the filter was rinsed first with Tris-buffer, then with 0.1 M NaOH and lastly again with Tris-buffer.

        - The sample is added onto the filter, and is spun down for 10 minutes at 4000 x g (NOTE: The solution is never allowed to reach beneath the 500 ul mark above the filter). Afterwards Tris-buffer is added and the the sample is spun down again for 10 minutes at 4000 x g (this step is repeated twice). Lastly, you pipette up and down inside to column to remove residual protein from the filter. Afterwards extract your sample 500 ul at a time.
- Spectroscopic concentration measurement
    - Lastly, using absorbance measurement the concentration of the filtered enzyme was measured.

Results:

- SDS-PAGE



Wells	1	2	3	4	5	6	7	8	9	10
Gel	L	S1	S2	S3	S4	S5	C1	C2	C3	C4

L = Ladder

S = Sialidase, induced sample

C = Control, uninduced sample

- Spectroscopic concentration measurement

Blank = Tris-buffer

A_{280} = 0.497 ε = 65320 M^{-1} cm^{-1} L = 1 cm M = 54 *10^3 g/mol

c_{mol} = A / ε*L = 7.61 µmol/dm^3

c_g = M * c_{mol} = 0.41 µg/µl

Discussions:

The SDS-PAGE shows no sign of protein in the uninduced samples. The protein that gives the strongest band seems to be around the same size as it should be. With the goal of achieving a sample with high concentration enzyme, fractions 2 and 3 of the induced samples were pooled together in the filtration step. The spectroscopic concentration measurement shows the results after the filtration.

It is to be noted that fraction 3 does not seem to be completely purified. It has some faint bands beneath it. It was decided that the relation between the concentration of the desired protein and the faint bands was large and therefore it will hopefully not affect future experiments.

Conclusions:

- The experiment was a success and a sample with high concentration sialidase was produced.
- The next step is to use the enzyme in the enzyme assay and prove that it is active. It would be for the best if this step is done as early as possible. So that the enzyme will remain active.