# Expression and Isolation of the Membrane Proteoglycan Syndecan-1 from *E. coli*

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Syndecan-1 is a membrane proteoglycan, which is a protein with glycosaminoglycans, or long carbohydrate chains, attached to its extracellular domain. The syndecan family is composed of various proteoglycans that each aid in cell-to-cell communication, and cell signaling pathways. My research involves the DNA alteration of a plasmid to incorporate the syndecan-1 protein along with its $His_6$-tagged TrpΔLE fusion partner. This fusion partner allows the normally membrane-integrated syndecan-1 protein to form inclusion bodies in the cytosol, allowing for overexpression of the protein. The syndecan-1 protein in its altered form weighs about 31.9kDa, and the TrpΔLE weighs approximately 14kDa.

**Cancer Significance:** The glycosaminoglycan chains that exist on the extracellular domain of syndecan-1 bind various stimulating agents such as growth factors or chemokines. This binding begins an intracellular enzyme cascade, which ultimately results in the shedding of the extracellular domain. This shed domain can still bind growth factors as it travels through the blood, and due to the association of growth related pathways, the syndecan-1 protein can propagate cancers through its shed glycosaminoglycan groups. Blood can be tested for the presence of shed syndecan-1, and large concentrations can indicate cancer.

**Research:** By overexpressing syndecan-1, we can characterize its structure, function, dynamics, and interactions in cell-like conditions. Once we can prove isolation of pure syndecan-1 protein, we can glycosylate (attach a glucose molecule) the protein to observe how its structure, and therefore function, are affected.

## Methods

**Expression**

Using Luria-Bertani medium and *E. coli* cells, grew 4 liters at a time in a 37 degree Celsius room. Induced cells with isopropyl β-D-1 thiogalactopyranoside (IPTG) to promote overexpression of protein. Cells are pelleted via centrifugation and kept in freezer.

**Lysis**

Cell pellets undergo sonication with Resuspension 1 and 2 buffers, followed by centrifugation and disposal of supernatant, respectively. The resulting supernatant is discarded and pellet dissolved in Binding buffer. The sample is subsequently sonicated, centrifuged, and dialyzed to remove possible lipids from sample.

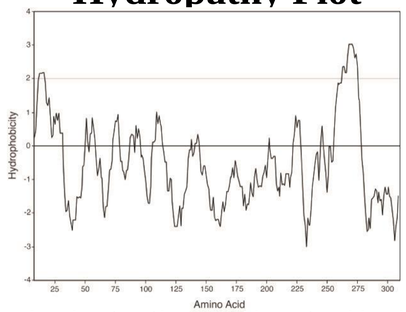
**Cleavage**

For every 10mg of protein, 30mg of cyanogen bromide, 80μL of DI water, and 320μL of formic acid is added to a tube containing the protein. The solution is placed on a rocker for at least three hours to ensure proper cleavage of the TrpΔLE fusion partner from the syndecan-1 protein.

**Size-Exclusion Chromatography**

The sample containing syndecan-1 and $His_6$-TrpΔLE is then run through a fast protein liquid chromatography (FPLC) protocol for the purpose of separating these two constituents. Five milliliter aliquots are collected in fraction tubes to isolate specific proteins and the resulting chromatogram indicates which fractions contain syndecan-1 protein.

## Hydropathy Plot



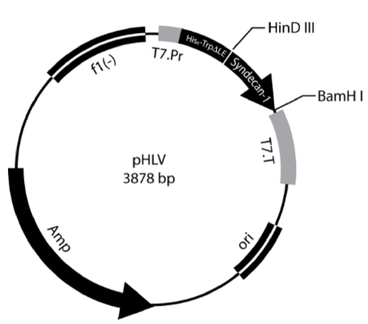

A hydropathy plot analyzes the amino acid sequence of the protein, and through the relative hydrophobicity of specific regions of the sequence, is able to provide an accurate estimate of the location of the transmembrane domain of the protein. In the above plot, the large sequence of residues from amino acid 254-274 exceeds two on the scale of hydrophobicity, indicating that this section is likely the transmembrane domain.

## Sequence

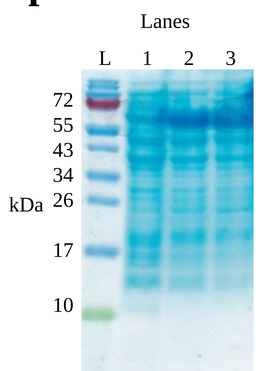
```
        10         20         30         40         50         60
MRRAALWLWL SALALSLQPA LPQIVATNLP PEDQDGSGDD SDNFSGSGAG ALQDITLSQQ
        70         80         90        100        110        120
TPSTWKDTQL LTAIPTSPEP TGLEATAAST STLPAGEGPK EGEAVVLPEV EPGLTAREQE
       130        140        150        160        170        180
ATPRPRETTQ LPTTHLASTT TATTAQEPAT SHPHRDSQPG HHETSTPAGP SQADLHTPHT
       190        200        210        220        230        240
EDGGPSATER AAEDGASSQL PAAEGSGEQD FTFETSGENT AVVAVEPDRR NQSPVDQGAT
       250        260        270        280        290        300
GASQGLLDRK EVLGGVIAGG LVGLIFAVSL VGFSLYRSKK KDEGSYSLEE PKQANGGAYQ
       310
KPTKQEEFYA
```

The above sequence is the altered amino acid sequence of syndecan-1. The alterations were replacement of cysteine residues with serine residues. The red-letter sequences are glycosylation sites, and the underlined portion indicates the transmembrane domain.

## Plasmid and Expression

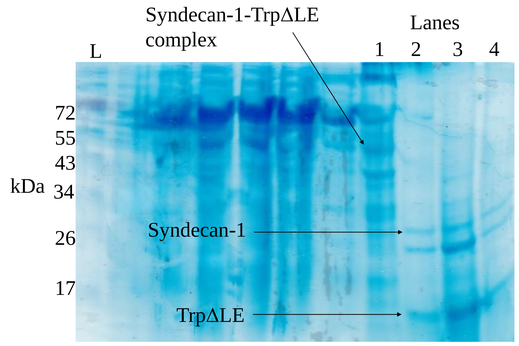



The pHLV plasmid was engineered to contain the human syndecan-1 protein, along with the TrpΔLE fusion partner, and the resulting plasmid was transformed into E. coli cells.

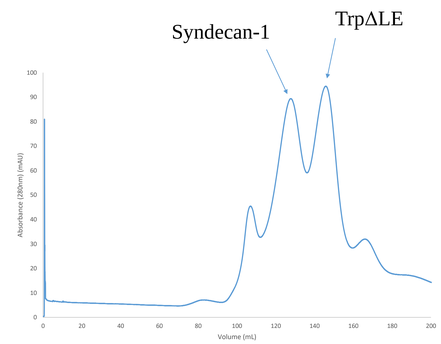



The lane denoted as "L" is the molecular weight ladder, with corresponding values shown next to the gel. The lanes 1, 2, and 3 are time of induction, 2 hours after induction, and 4 hours after induction, respectively.

## Results and Conclusions




Cleavage: The lane denoted as "L" is the molecular weight ladder, with corresponding values next to the gel. Lane 1 is uncleaved protein, and lanes 2-4 are cleaved protein.




Size-Exclusion Chromatography: A chromatogram from a Sephacryl S-200 HR column with FPLC SDS Buffer pH 8.2. The first and fourth peaks are assumed as endogenously expressed, while the second and third peaks are assumed to be syndecan-1 and TrpΔLE, respectively.

Mass Spectrometry: Spectra samples were dissolved in 98% tetrafluoroethylene (TFE), or 98% acetonitrile (ACN) and then diluted as necessary to produce readable spectra, with that dilution often being 1/250 at the greatest. To date, we have not had success in obtaining a spectra that apparently contains our syndecan-1 protein. We are continuing to test other ways in which we can have better success, such as MALDI-TOF and ElectroSpray ionization mass spectrometry.

**Conclusions:**

- Expression of the syndecan-1-TrpΔLE protein complex appears to be successful, however an endogenously expressed protein may be more highly expressed.
- The cleavage protocol is successful in separating the syndecan-1 protein from its fusion partner, and subsequent dialysis appears to further aid in purifying the sample.
- Size-exclusion FPLC provides adequate separation of proteins within the sample, but the lack of evidence for the syndecan-1 protein in the mass spectrometry chromatogram of its assumed peak leaves us unable to verify its isolation.

## Acknowledgements

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