Using Lipid Nanodiscs for the *in vitro* Glycosylation of Membrane Proteins

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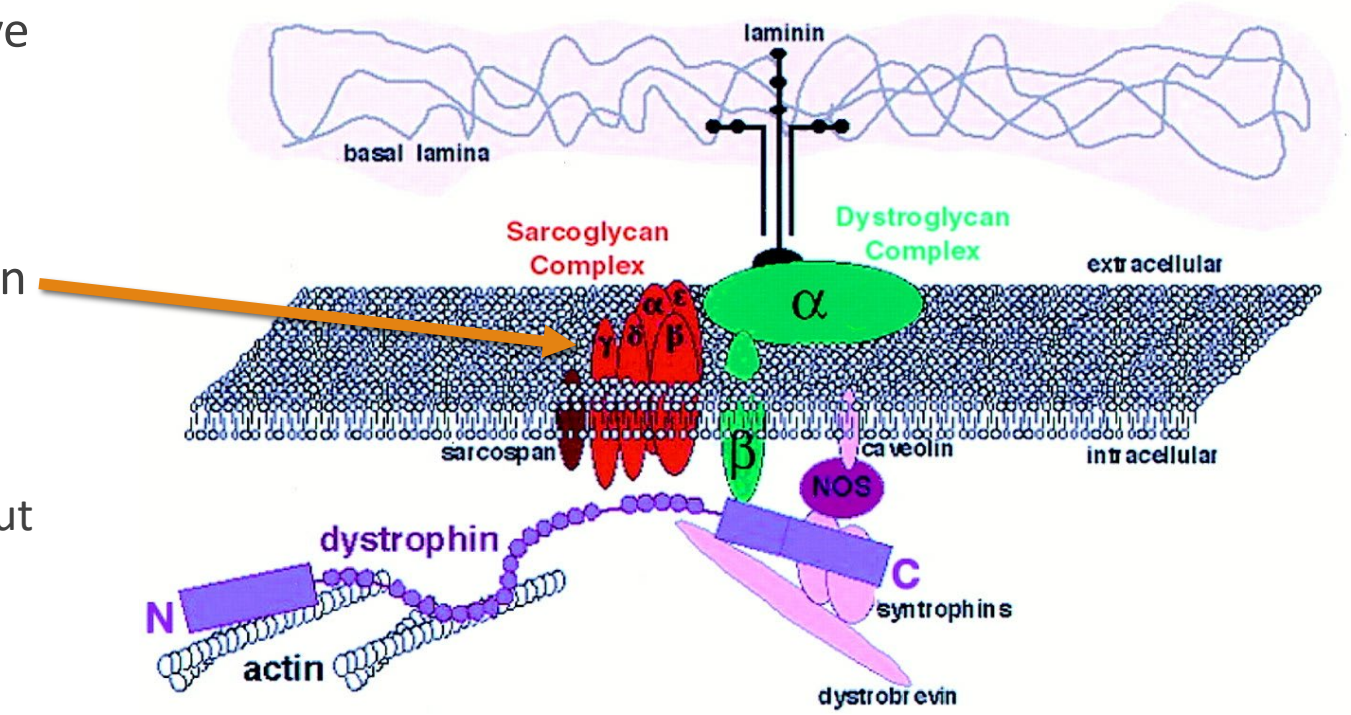
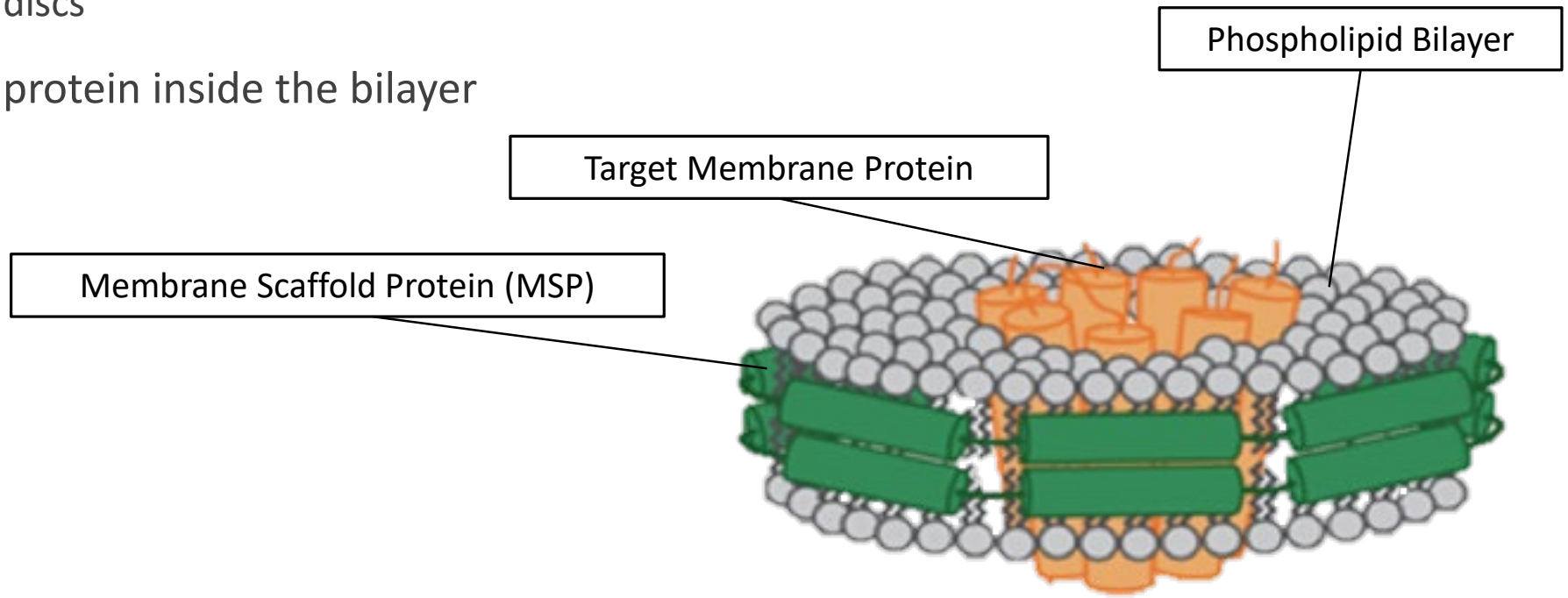
Muscular Dystrophy

- A genetic disease that causes progressive weakness and a loss of muscle mass
- Life expectancy: age 16 – early 20's
- Current protein of interest: γ-sarcoglycan
  - Glycosylation is important for function, however unsure if it causes changes in structure, function, or both
  - Issue is that the protein breaks up when put in NMR solution



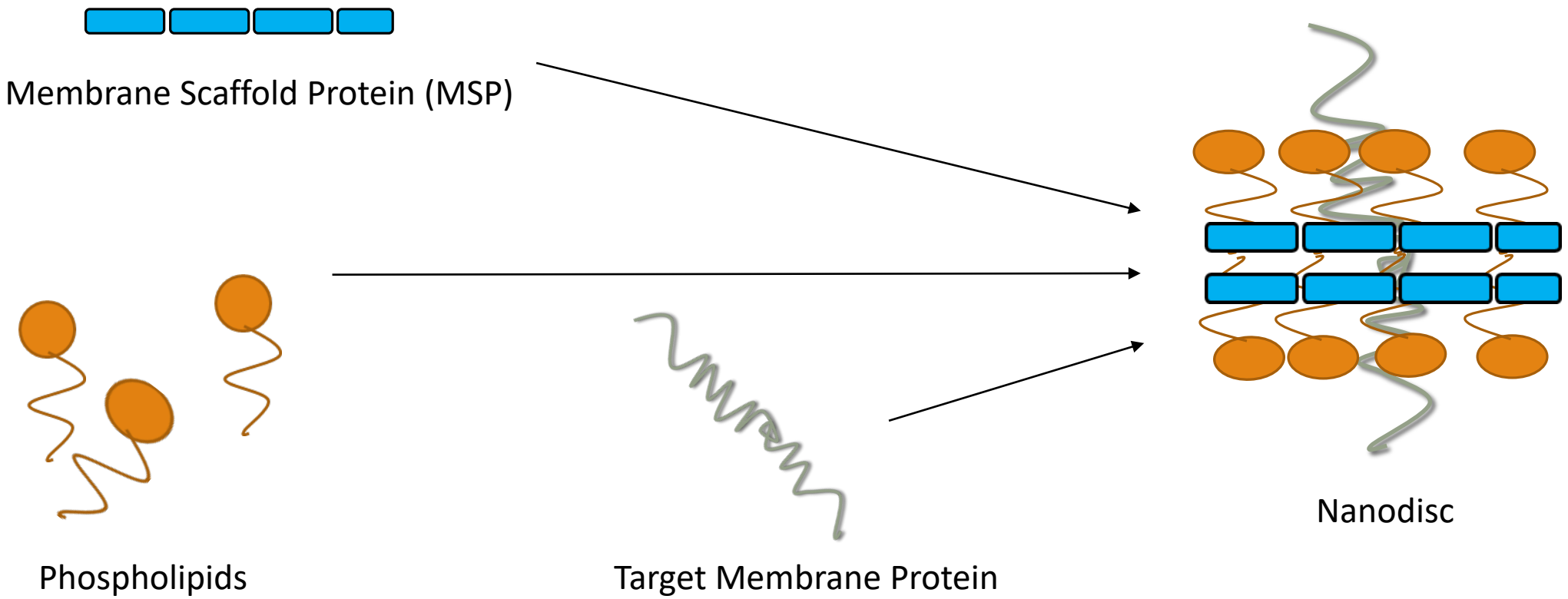
Nanodiscs

- Patch of a phospholipid bilayer
- Encircled by protein belt, membrane scaffold protein (MSP)
  - Controls diameter of discs
- Targeted membrane protein inside the bilayer



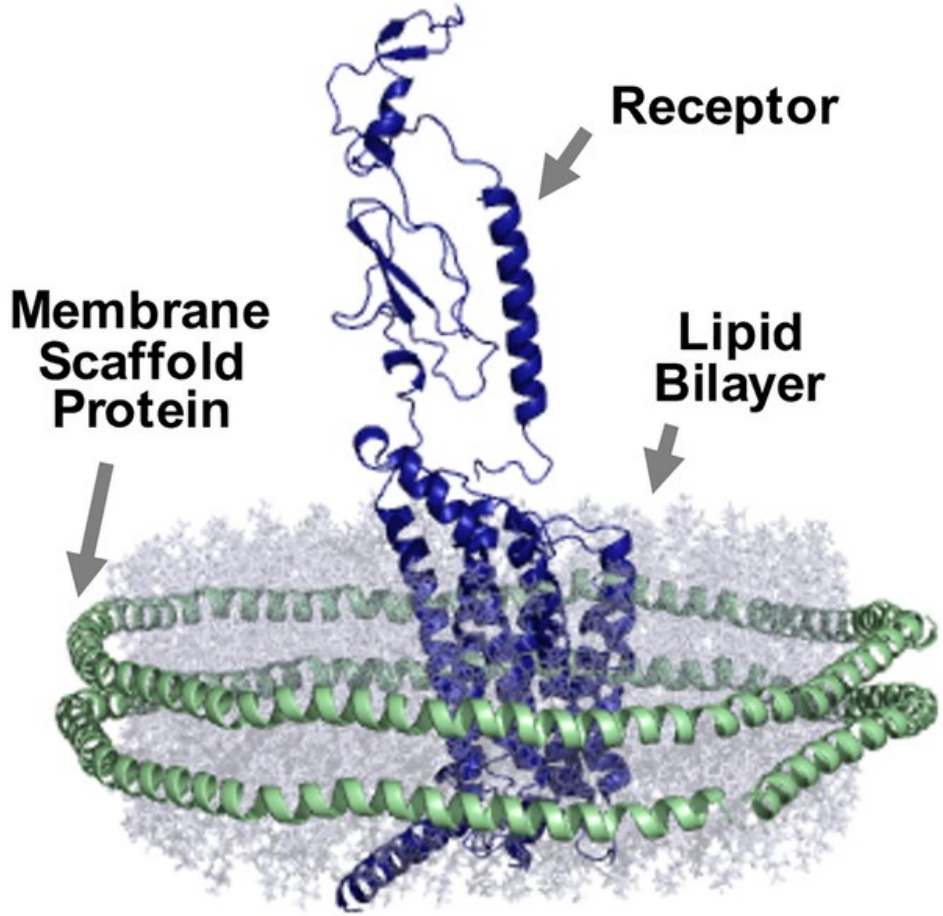
Bizcomjapan. What is a Nanodisc [Digital image].

Formation of Nanodiscs



Membrane Scaffold Protein (MSP)

- Amphipathic helical protein
- Encircles phospholipid bilayer
- Length of belt controls the size of the nanodisc
- Can be histidine-tagged or be non-tagged



PLOS. (2017, June 13). The purification method of GLP1R using nanodiscs. [Digital image].

Expression

<u>950mL flask:</u>

- 95 µL kanamycin
- 50 mL:
  - 200 µL cell starter:
    - 5 mL LB
    - 5 µL kanamycin
    - 10 µL cell stock
  - 50 mL broth (from 950 mL flask)
  - 95 µL kanamycin

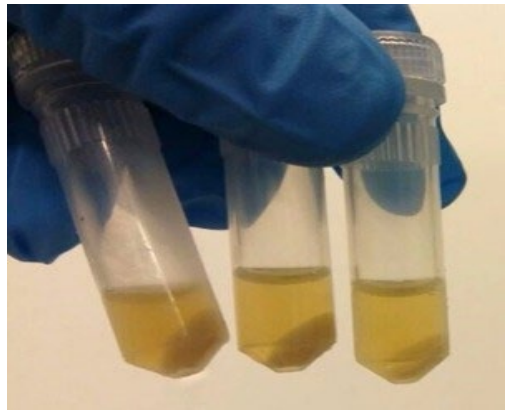
<u>Tested at 600 nm</u>:
1mL cell solution from each 950 mL flask

<u>Centrifuged for 2 min.:</u>
An amount of cell solution from Flask 1

<u>Centrifuged at 6500 rpm for 25 min. at 4 ℃:</u>
Cell growth from 4 flasks

- Supernatant removed

Purification

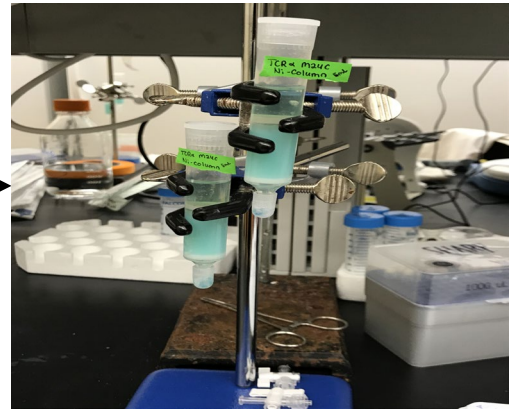


- Cell pellet
- 30 mL 20 mM phosphate buffer

Sonicator:

- Cells are lysed by 4 minutes of sonification for 2 pulses every 8 seconds at Ampl. 60 %

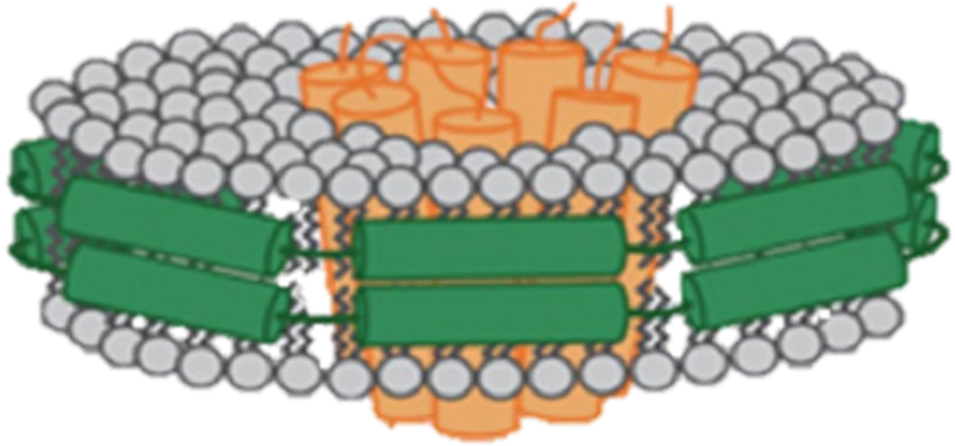
Centrifuged at 6500 rpm for 25 min. at 4 °C



- 25 mL Column Equilibrant
- ~ 35 mL sample
- 25 mL Column Equilibrant
- 25 mL (each) Buffer “A”, “B”, “C”, & “D”

Purpose of Nanodiscs

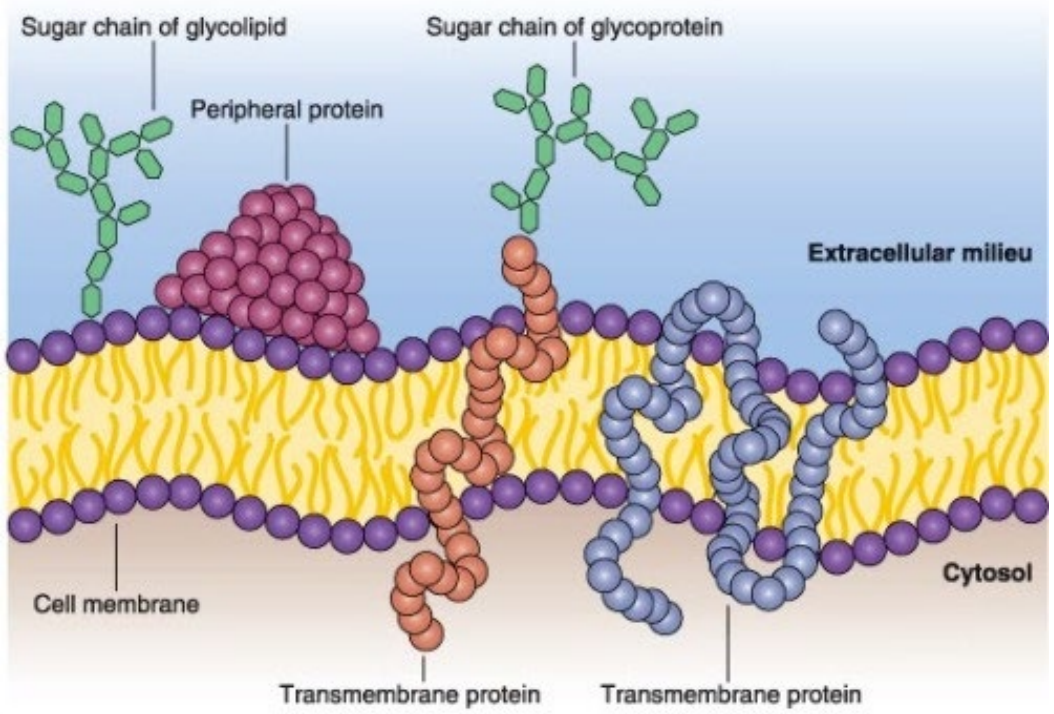
- Prevents the distortion/denaturation of native protein structure
- Keeps membrane protein in familiar environment
  - Phospholipid bilayer
- Membrane protein is soluble in solution while in nanodisc
- For glycosylation: allows access to both sides of the protein of interest



Inagaki, S., & Ghirlando, R. (2016, December 17). Nanodisc characterization by analytical ultracentrifugation [Digital image].

Glycosylation of Membrane Proteins *in vitro*

- Normally, membrane proteins and sugars are viewed independently to look at structure and function in the human body
- Glycosylation allows for more accurate study of function in the body
  - Sugars are attached to membrane proteins in the human body
- Known that glycosylation affects proteins
  - However, unsure if it changes its structure, dynamics, or both

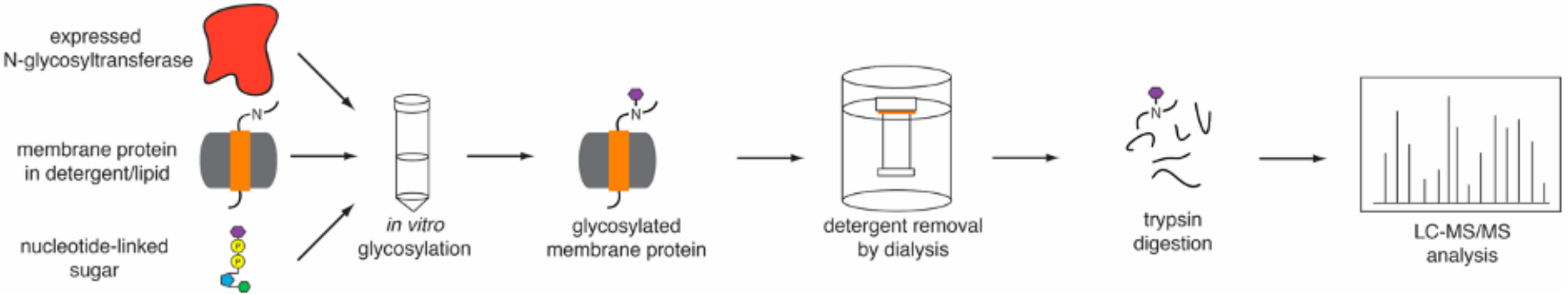


Other Proteins used in Glycosylation

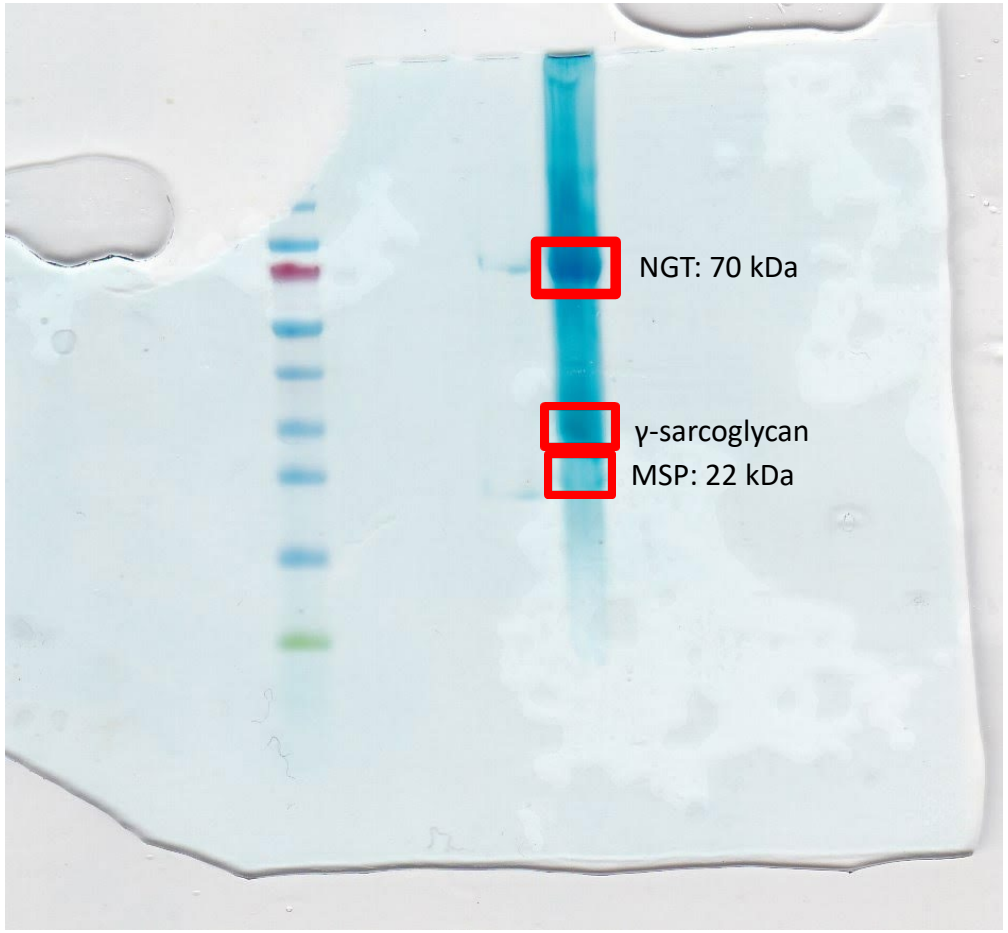
- Tobbacco etch virus (TEV) – used to cleave off histidine tag from MSP
- Dithiothreitol (DTT) – break any unwanted disulfide bonds
- N-Glycosyltransferase (NGT) – used to glycosylate *in vitro*

Methods of Glycosylation

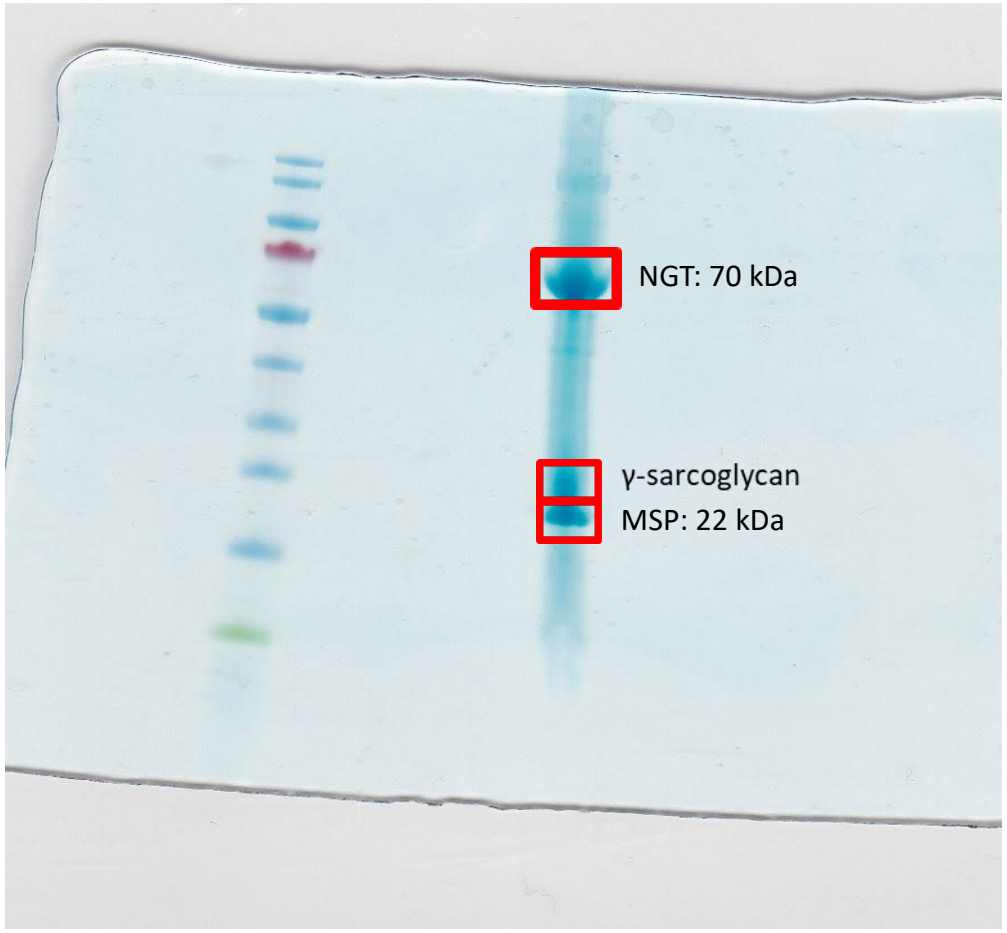
1. Add TEV and DTT to purified MSP sample and let dialyze in cold room overnight
2. Run Nickel column using 40mM Tris, 300 mM NaCl and 50 mM Imidazole followed by MSP sample
3. Dialyze MSP sample overnight, switching the bath once
4. Prep nanodisc with γ-sarcoglycan
5. Dialyze nanodisc sample in 20mM phosphate buffer
6. Concentrate sample
7. Separate samples into two separate tubes, adding NGT to both and UDP-glucose to the reaction tube, leaving on rotator for 24 hours
8. Dialyze both mixtures in DI water separately, freeze tubes, then put on lyophilizer to dry
9. Run gel of samples
10. Visualize and quantify data using mass spectrometry


expressed
N-glycosyltransferase
membrane protein
in detergent/lipid
nucleotide-linked
sugar
N
P
P
in vitro
glycosylation
N
glycosylated
membrane protein
detergent removal
by dialysis
N
trypsin
digestion
LC-MS/MS
analysis

Control

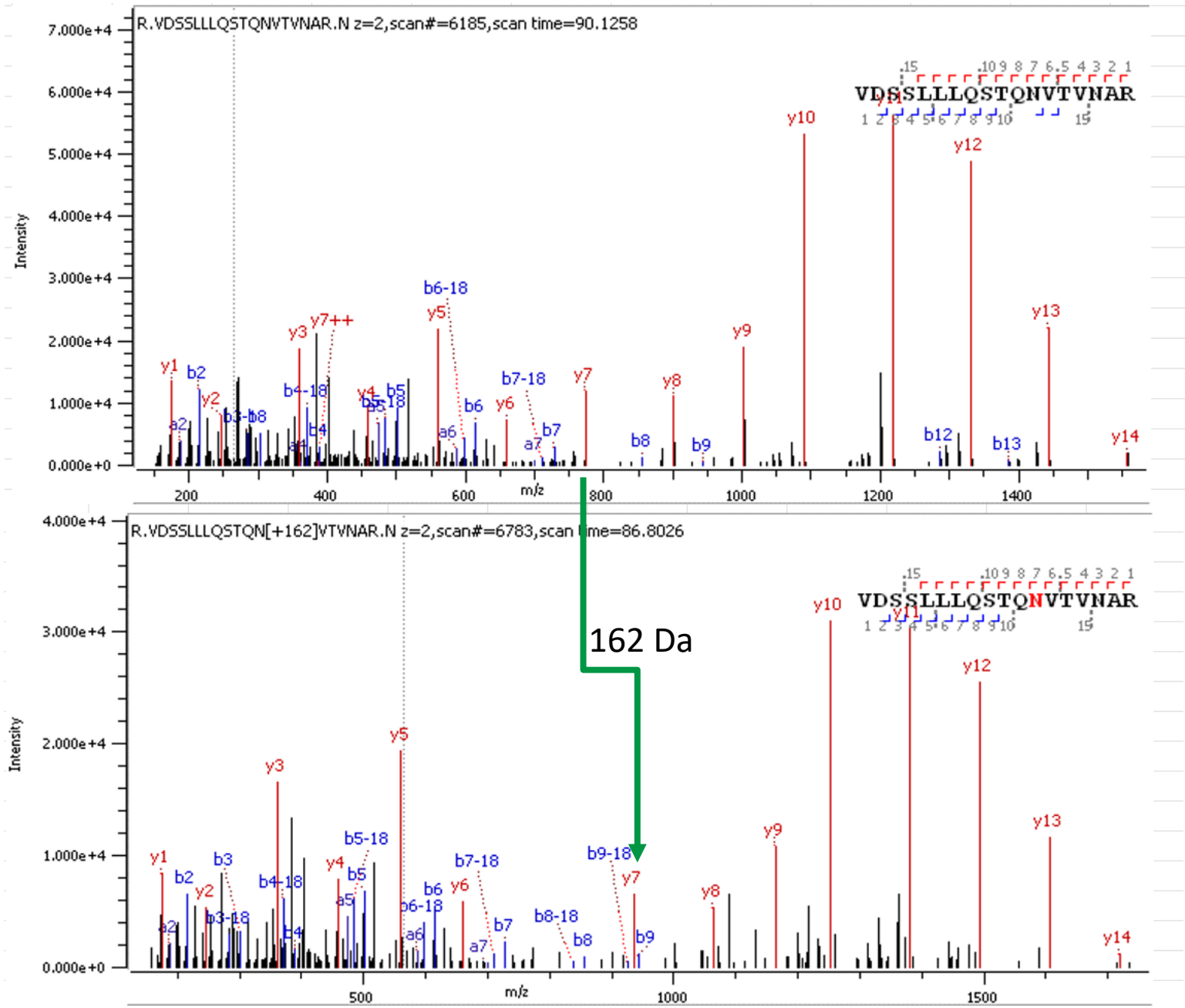


Reaction (Glycosylation)



Control

Reaction



Future Directions

- Glycosylate membrane proteins *in vitro* with larger sugars
- Look at structure of proteins in solution nuclear magnetic resonance (NMR)
- Determine how different membrane proteins function and interact in the body, such as in muscular dystrophy
  - How glycosylation affects protein structure or dynamics

References

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Images

Latter, W. Microbiological Methods [Digital image].