Testing effects of glycocalyx inhibition by mannose on cell adhesion and mechanosensation of *Dictyostelium discoideum.*

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INTRODUCTION

Mechanosensation

- Cells can sense mechanical forces (e.g., shear flow) and react to these stimuli by moving directionally (away or towards the flow) through mechanosensation.

Dictyostelium discoideum

- Social amoeba *Dictyostelium discoideum* is a multicellular soil microorganism that reproduces as a unicellular amoeba under good conditions but produces aggregates and fruiting bodies when starved (Mohri et al., 2020).

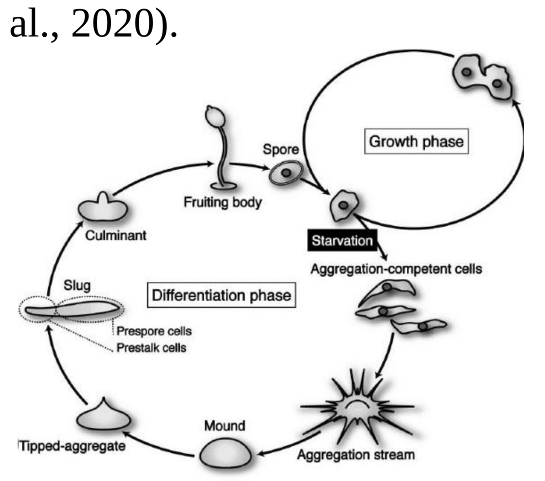


Figure 1. Life cycle of *Dictyostelium discoideum.*
https://www.mdpi.com/2218-273X/3/4/943

- *D. discoideum* is used in this study as a model organism to understand the mechanisms of directed motility in response to shear flow.
- Although proteins such as myosin II and cortexillin I have been implicated in mechanotransduction of *D. discoideum*, how these cells sense mechanical stimuli is not clear (Plaza-Rodríguez et al., 2022).

Cell Adhesion

- Cell adhesion is essential for cell migration.
- Cell adhesion can be mediated either by specific interactions with components of the extracellular matrix (ECM) or by non-specific interactions between the glycocalyx and the substrate (Delafrouz et al., 2020).

Glycocalyx

- The glycocalyx is the coating over the plasma membrane of all cells, mostly made up of sugars (Richter & Sanderson, 2023).

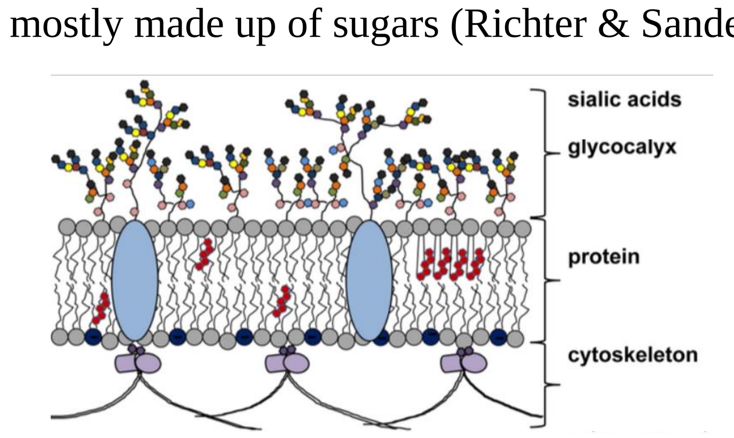


Figure 2. General structure of the plasma membrane surrounding mammalian cells. Image was modified from Atukorale et al., 2015.

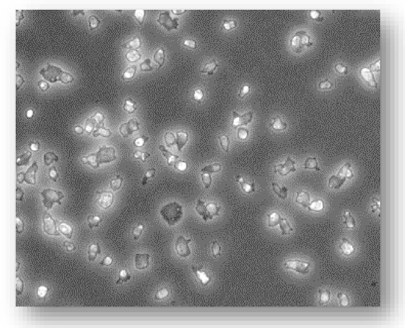
- Mannose is a key monosaccharide in the glycocalyx of *D. discoideum* (Sharkey & Kornfeld, 1989).

https://pubchem.ncbi.nlm.nih.gov/substance/2930.. https://pubchem.ncbi.nlm.nih.gov/compound/Mannitol.

Figure 3. Chemical structures of mannose and mannitol.

- **We hypothesized that inhibiting the glycocalyx by competition with mannose will decrease adhesion and mechanosensation in *D. discoideum* cells.**

APPROACH



D. discoideum cells

Grown on HL-5 Media (axenic cells)

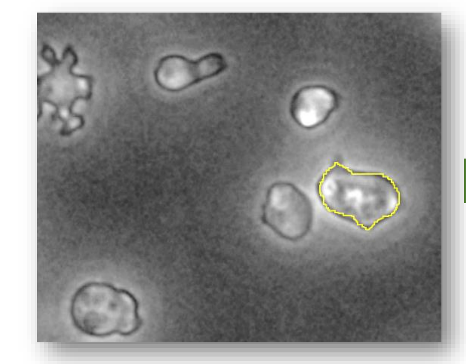
Grown on Bacterial Lawn (bacterial cells)

Inhibition of the Glycocalyx (+/- Mannose/ Mannitol)

Adhesion Assay

Mechanical Stimulation Assay

Morphology Assessment



Trace cell outlines

Measure Area and Circularity

RESULTS

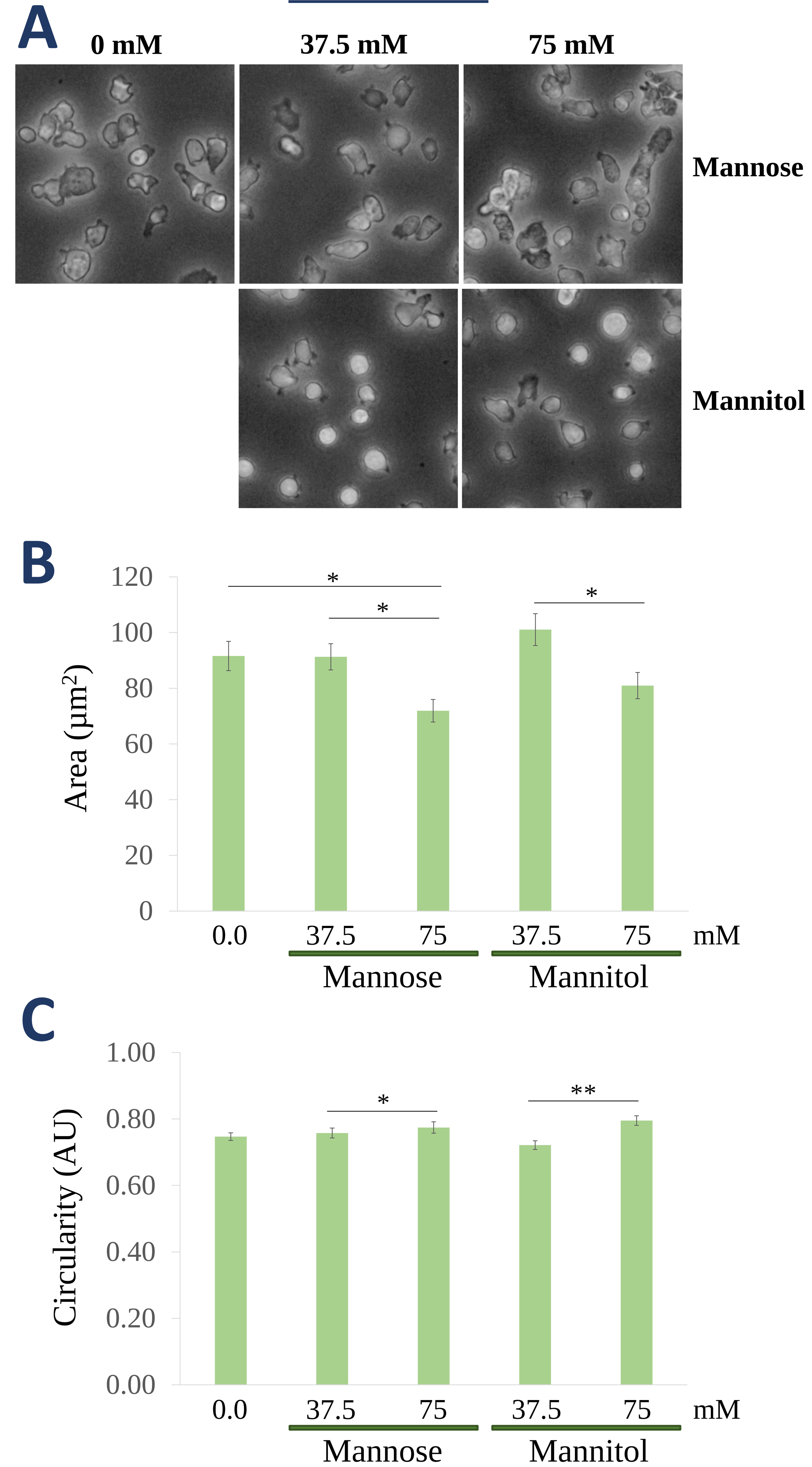


Figure 4. Morphology assessment of axenic *D. discoideum* cells following mannose and mannitol treatment. Cells were plated in buffer, allowed to attach for 30 minutes, and treated with mannose, mannitol or water for 20 minutes. **(A)** Images were acquired with phase contrast microscopy at 200X magnification. **(B, C)** Area (B) or circularity (C) was measured by manually tracing the cells in Fiji software. Data is shown as mean ± SE of 40 cells from 1 experiment. Differences were compared using ANOVA with a Student-Newman-Keuls post-test. *P<0.05, **P<0.001.

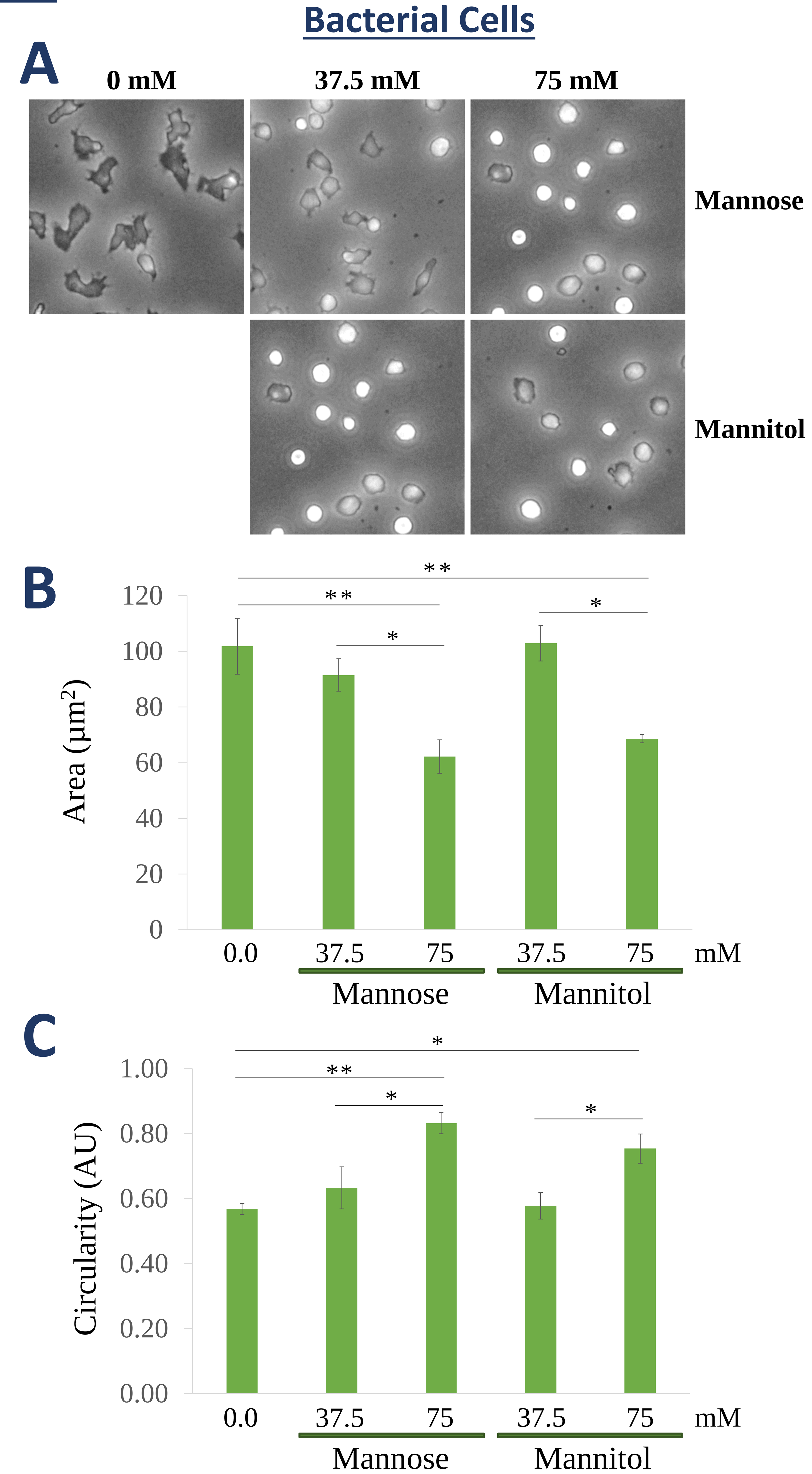


Figure 5. Morphology assessment of bacterially-grown *D. discoideum* cells following mannose and mannitol treatment. Cells were plated in buffer, allowed to attach for 30 minutes, and treated with mannose, mannitol or water for 20 minutes. **(A)** Images were acquired with phase contrast microscopy at 200X magnification. **(B, C)** Area (B) or circularity (C) was measured by manually tracing the cells in Fiji software. Data is shown as mean ± SE of 4 experiments. Differences were compared using ANOVA with a Student-Newman-Keuls post-test. *P<0.05, **P<0.001.

CONCLUSIONS

Area Assessment

- Axenic cells showed a significant decrease in area for high mannose concentration compared to control, whereas effects of mannitol were not statistically significant.
- Bacterially-grown cells showed a significant decrease in area with either mannose or mannitol treatment.

Circularity Assessment

- Axenic cells showed a slight increase in the circularity following mannose or mannitol treatment, although differences were not statistically significant compared to control.
- Bacterially-grown cells had a robust increase in circularity following treatment with mannose or mannitol.

Overall, it appears that mannose and mannitol have similar effects on cell morphology, with mannose being slightly more effective at inducing rounding in axenic cells compared to mannitol.

FUTURE DIRECTIONS

- Expand the data set for morphology assessment of axenic cells.
- Perform mechanical stimulation assays to test response to shear flow in bacterially-grown cells following mannose or mannitol treatments.
- Examine random and shear flow-directed migration of cells treated with mannose or mannitol.

REFERENCES

Atukorale, P. U., Yang, Y. S., Bekdemir, A., Carney, R. P., Silva, P. J., Watson, N., Stellacci, F., & Irvine, D. J. (2015). Influence of the glycocalyx and plasma membrane composition on amphiphilic gold nanoparticle association with erythrocytes. *Nanoscale*, 7(26), 11420–11432. https://doi.org/10.1039/c5nr01355k

Delafrouz, P., Zhao, J., Tian, W., & Liang, J. (2020). Effect of extracellular matrix stiffness gradient on durotaxis motion and cell migration of cells using dynamic cellular finite element model (DYCELFEM). *Biophysical Journal*, *118*(3). https://doi.org/10.1016/j.bpj.2019.11.1611

Mohri, K., Tanaka, R., & Nagano, S. (2020). Live cell imaging of cell movement and transdifferentiation during regeneration of an amputated multicellular body of the Social Amoeba Dictyostelium discoideum. *Developmental Biology*, *457*(1), 140–149. https://doi.org/10.1016/j.ydbio.2019.09.014

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 6251, Mannitol. Retrieved April 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Mannitol.

National Center for Biotechnology Information (2023). PubChem Substance Record for SID 2930, D-mannose, Source: BioCyc. Retrieved April 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/substance/2930.

Plaza-Rodríguez, A. I., Nguyen, L. T. S., Robinson, D. N., & Iglesias, P. A. (2022). Particle-based model of Mechanosensory Contractility Kit Assembly. *Biophysical Journal*, 121(23), 4600–4614. https://doi.org/10.1016/j.bpj.2022.10.031

Richter, J. R., & Sanderson, R. D. (2023). The glycocalyx: Pathobiology and repair. *Matrix Biology Plus*, *17*, 100128. https://doi.org/10.1016/j.mbplus.2023.100128

Sharkey, D. J., & Kornfeld, R. (1989). Identification of an N-acetylglucosaminyltransferase in Dictyostelium discoideum that transfers an "intersecting" N-acetylglucosamine residue to high mannose oligosaccharides. *The Journal of biological chemistry*, 264(18), 10411–10419.

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