Equilibrium Thermodynamic Effects of Sugar-based Polymers on Protein-complex Formation

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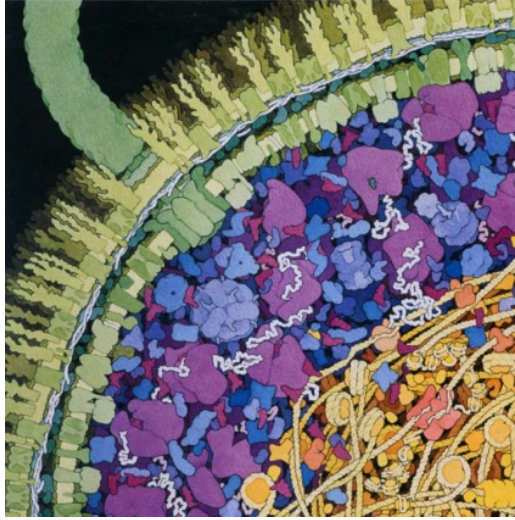
Research Question

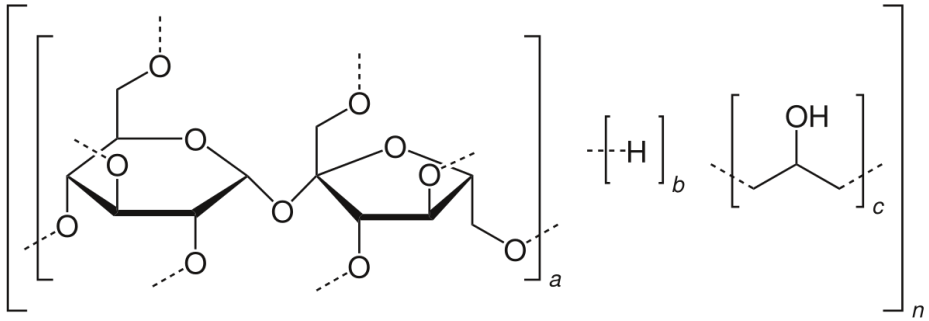


Above: Illustration showing crowded nature of cells

The cytoplasm is a crowded and complex environment. Macromolecular concentrations can exceed 300 g/L in cells, while dilute buffered solutions generally used for protein research have concentrations less than 10 g/L. Studies indicate that properties of proteins, such as stability and interactions, are altered based on the concentration of its surroundings. **Thus, the study of proteins in crowded solutions is imperative to our understanding of protein functions and interactions.** Synthetic crowders like polyethylene glycol (PEG), dextran, and Ficoll™ are often used as cosolutes to create crowded environments *in vitro* because they are relatively inert and commercially available in a range of molecular weights.

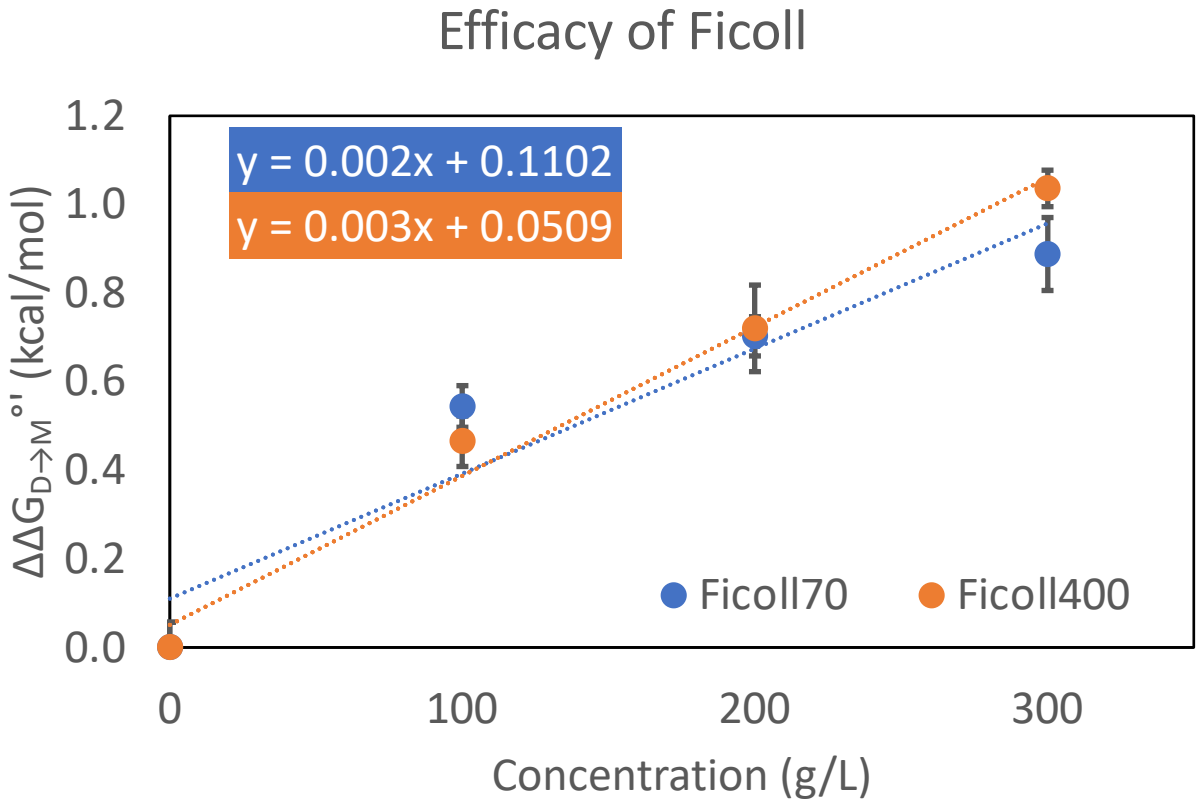
I investigated how crowding impacts the unfolding of a test protein.

I used a fluorine labeled protein, which can be observed with NMR. To model protein-complex formation, I used two homodimerizing variants of the 6-kDa immunoglobulin binding domain B1 of streptococcal protein G (GB1). ^{19}F NMR was utilized to study dimer formation because the two resonances represent the monomer and dimer.



Left: Chemical structure of Ficoll™

Results



Concentration	$\Delta\Delta G_{D\rightarrow M}^{\circ'}$ of Ficoll™ 70	$\Delta\Delta G_{D\rightarrow M}^{\circ'}$ of Ficoll™ 400
100 g/L	0.54 ± 0.05 kcal/mol	0.47 ± 0.06 kcal/mol
200 g/L	0.70 ± 0.04 kcal/mol	0.7 ± 0.1 kcal/mol
300 g/L	0.89 ± 0.08 kcal/mol	1.04 ± 0.04 kcal/mol

- Experiments were triplicated. Results are presented as averages. Uncertainties are presented as standard error of the mean. Both Ficoll™ 70 and Ficoll™ 400 stabilize GB1 Domain-Swap-Dimer (GB1 DSD) at all concentrations. Ficoll™ 70 has an m-value of $3 \pm 1 \frac{kcal \cdot mL}{mol \cdot g}$. Ficoll™ 400 has an m-value of $3 \pm 2 \frac{kcal \cdot mL}{mol \cdot g}$. At 100 g/L and 200 g/L the stabilizing effect is indiscernible.
- The data contribute to the developing theory of macromolecular crowding that sheds light on the significance of the physical characteristics of crowding agents. In future research, I will expand the range of concentrations to include 50 g/L. Additionally, exploring other crowding agents such as dextran polymers, glucose, sucrose, and maltose should be considered. Continued research of macromolecular crowding is critical for preserving chemically unstable pharmaceuticals.