Protein Dimer Stability in Concentrated Solutions of Sugar Polymers

Owen M. Young[†], Thomas W. Redvanly[†], Claire J. Stewart[†], Gil I. Olgenblum[¥], Daniel Harries[¥], Gary J. Pielak[†,*,ʃ,§]

[†]Department of Chemistry, University of North Carolina at Chapel Hill (UNC-CH), [¥]Institute of Chemistry & the Fritz Haber Research Center, The Hebrew University, Jerusalem, [*]Department of Biochemistry & Biophysics, UNC-CH, [ʃ]Lineberger Cancer Center, UNC-CH, [§]Integrative Program for Biological and Genome Sciences, UNC-CH,

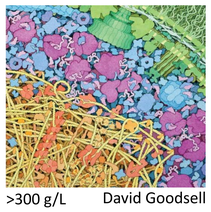
Claire Stewart's paper on a new protein crowding model

Shannon Speer's paper on macromolecular crowding

Abstract

Synthetic polymer solutions at concentrations of hundreds of grams per liter are used to stabilize proteins and mimic the crowded intracellular environment.[1] Several theories have been developed to explain the stabilizing effect of crowding, but almost all assume only contributions from hard (steric) contacts, ignoring soft (enthalpic) chemical interactions. We quantified the effects of the synthetic sugar-polymers, dextran and Ficoll™, as well as their monomers (glucose and sucrose, respectively), on two homodimer variants of the B1 binding domain of streptococcal protein G.[2,3] One dimer forms *via* domain swapping, while the other involves simple side-by-side dimerization. The dimers are fluorine labeled to facilitate detection using ^{19}F nuclear magnetic resonance spectroscopy. We measured the amounts of dimer and monomer as a function of temperature, cosolute size and cosolute concentration, allowing quantification of the stability (i.e., free energy), enthalpy, and entropy of dissociation. The cosolutes increase dimer stability and affect the enthalpy. We will analyze the data using a recently developed, more inclusive protein crowding model.[4]

Background



>300 g/L David Goodsell

...BUT

<10 g/L

- Classic crowding theory predicts only entropic contribution
- Two synthetic polymers:
  - dextran: polymer of glucose
  - Ficoll™: polymer of sucrose

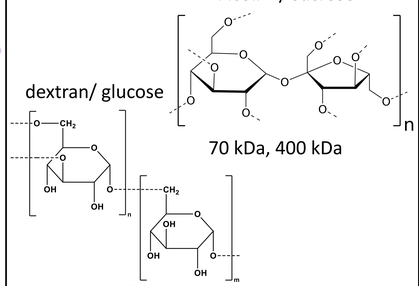
Classic Crowding Models: $\Delta G_{D\rightarrow M}^{\circ\prime} \approx -T\Delta S_{D\rightarrow M}^{\circ\prime}$ → Hardcore repulsions

New Model: $\Delta G_{D\rightarrow M}^{\circ\prime} = \Delta H_{D\rightarrow M}^{\circ\prime} - T\Delta S_{D\rightarrow M}^{\circ\prime}$ → Soft interactions; Hardcore repulsions, Soft interactions

Dimer ⇌ monomer equilibrium quantified by ^{19}F NMR:

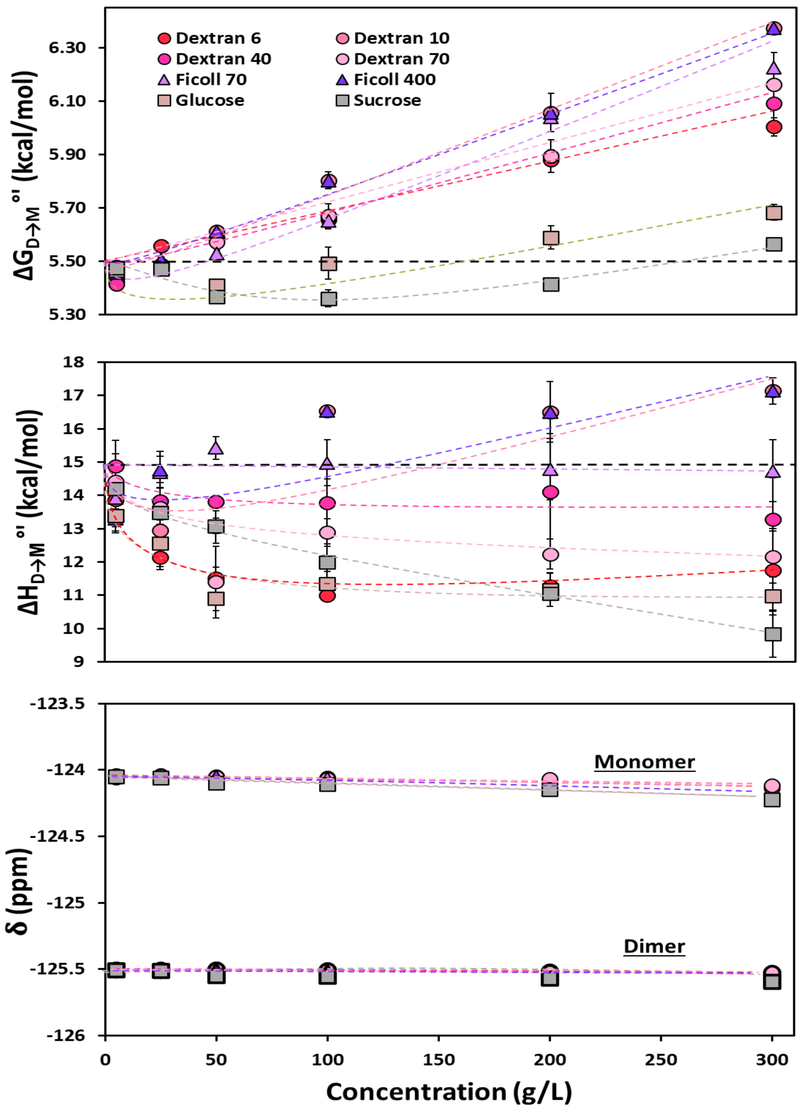
$$\Delta G_{D\rightarrow M}^{\circ\prime} = -RT\ln\left(\frac{Population\ Monomer}{Population\ Dimer}\right)$$

Ficoll™/ sucrose

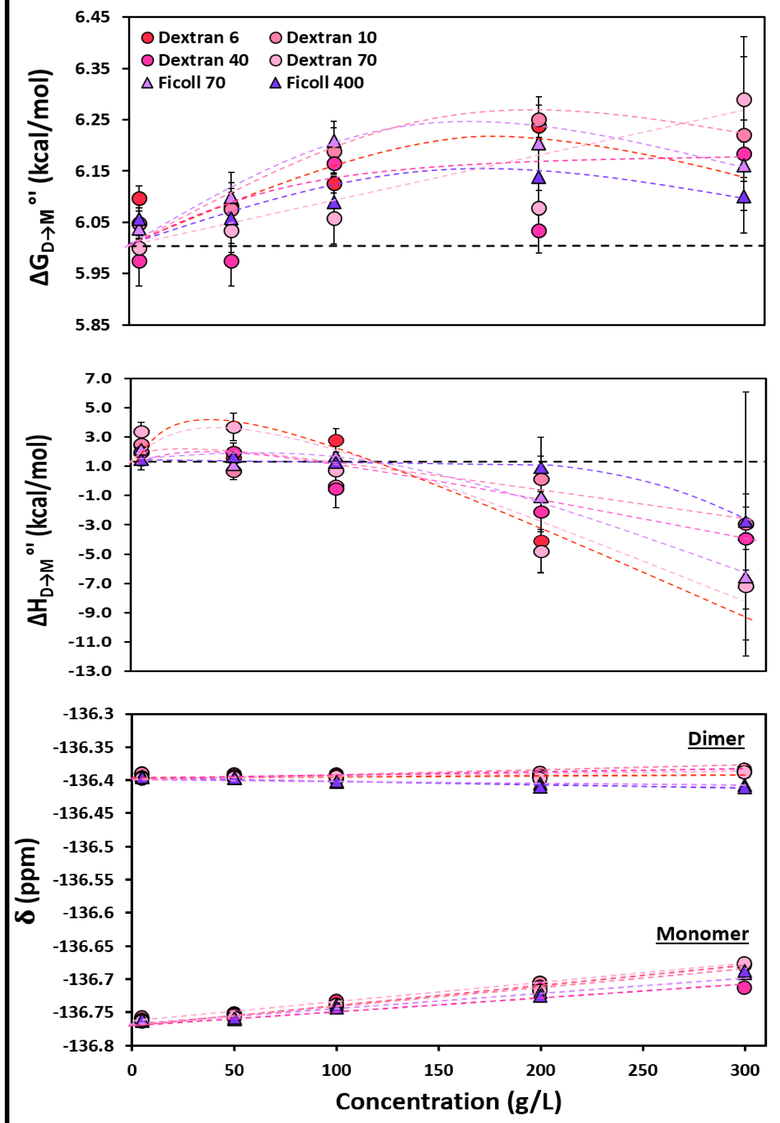


Results

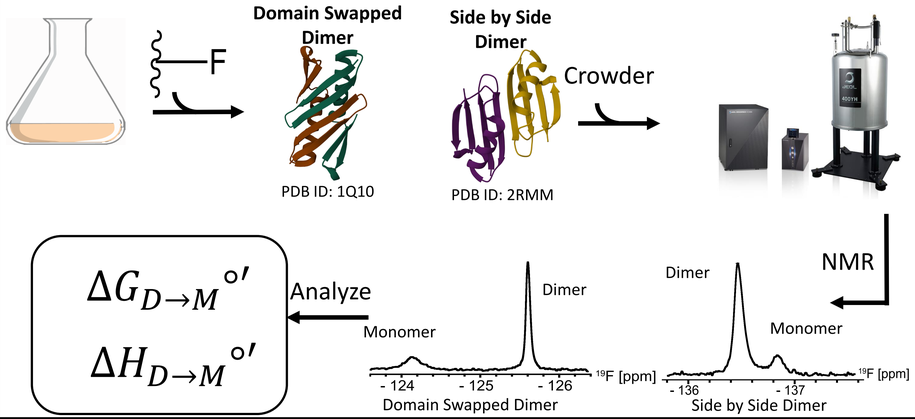
Domain Swapped Dimer



Side by Side Dimer



Methods



Above: Thermodynamic and chemical shift data for domain-swapped dimer and side-by-side dimer. Black dashed lines represent dimer data in buffer (20 mM NaP_i, 10% D_2O, 50 µM DSS). Colored dashed lines guide the eye and have no theoretical relevance.

Conclusions

- Crowding cosolutes stabilize dimer
- Non-negligible enthalpy component
- Cosolutes interact mostly with monomers
- New model needed to describe protein crowding

Acknowledgements and References

We thank Stu Parnham for spectrometer maintenance and advice, and the Pielak Lab for helpful discussions. Supported by the NSF, The University of North Carolina Office of Undergraduate Research, and a University of North Carolina's Department of Chemistry Chapel Family Undergraduate Research Award.

(1) Speer, S. L., Stewart, C., Sapir, L., Harries, D., Pielak, G. J. (2022). *Annu. Rev. Biophys. 51*, 267.
(2) Byeon, I.-J. L., Louis, J. M., Gronenborn, A. M. (2003). *J. Mol. Biol. 333*, 141.
(3) Guseman, A. J., Perez Goncalves, G. M., Speer, S. L., Young, G. B., Pielak, G. J. (2018). *Proc. Natl. Acad. Sci. USA 115*, 10965.
(4) Stewart, C. J., Olgenblum, G. I., Propst, A., Harries, D., Pielak, G. J. (2023). *Protein Sci. 32*, e4573.