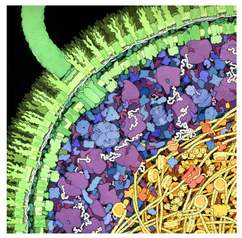
Investigation of the Effect of Crowding on Cooperativity in the Folding of Phosphoglycerate Kinase

Caleb M. Daugherty, *Andrei G. Gasic, Margaret S. Cheung*

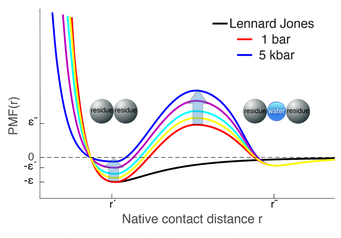
Department of Physics, University of Houston; Center for Theoretical Biological Physics, Rice University

Understanding the Folding Mechanism in a Cell Under Hydrostatic Pressure

- Experiments on the folding mechanics of Phosphoglycerate Kinase(PGK) confirm a two state thermal transition between the native and denatured states, and a multi-state transition under pressure denaturation.
- The introduction of crowding agents reduces the intermediate states under pressure denaturation.
- Classical folding theory is based on in vitro experimentation, but does not accurately predict folding mechanics in the crowded cellular environment.
- Understanding the in vivo folding process may lead to better treatments for disease associated with protein misfolding.

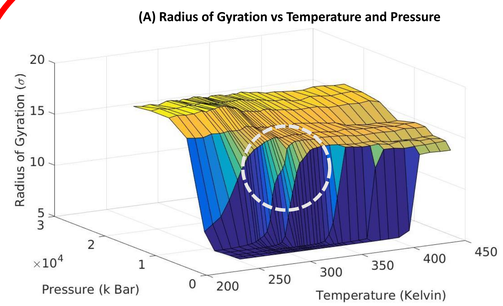


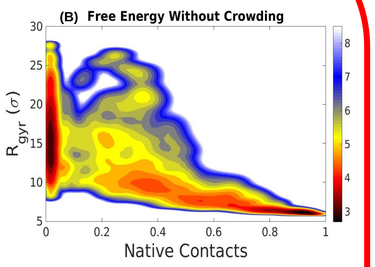
Crowded environment inside of an E. Coli bacterium



Overcoming the free energy barrier to alleviate water mediated contacts

Intermediate States Form Under High Pressure





- (A) Radius of gyration (R_g) plotted against temperature and pressure in the absence of crowding molecules.
- (A) The double sigmoidal shape (shown in circle) at 5 kbar and 293 Kelvin is indicative of an intermediate state in the absence of crowding.
- (B) Free energy profiles vs R_g and the fraction of native contacts (Q) with and without crowding agents.
- (B) The wide spread of basins at mid-range Qs confirms the presence of intermediate state.

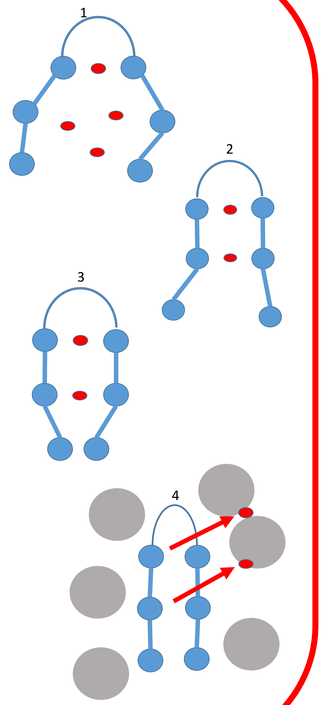
Cooperativity

1: As contacts begin to connect, water molecules must be forced out of the structure

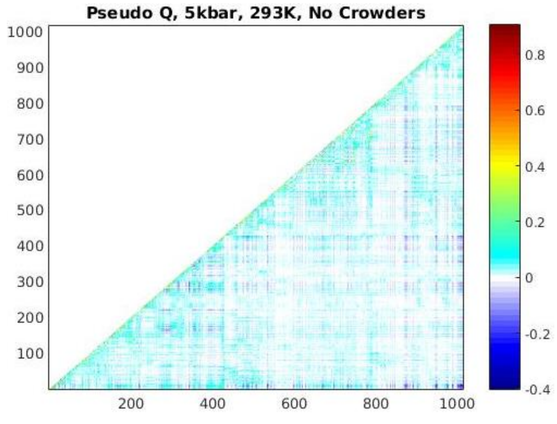
2: Individual contacts may become stuck in a water mediated state if the entropy change is not large enough to overcome the free energy barrier.

3: Water molecules may become trapped in the structure holding the protein in an intermediate state.

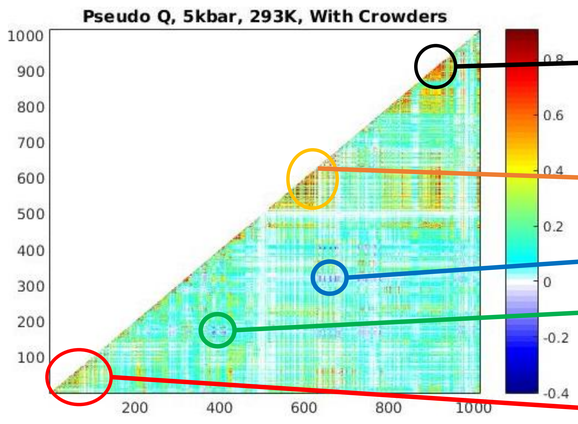
4: With crowding agents, excluded volume restricts protein conformations, and crowding agents compete for space with water molecules. The reduction of conformational entropy allows contacts to overcome the free energy barrier and desolvate.



Correlation of Water Mediated Contacts

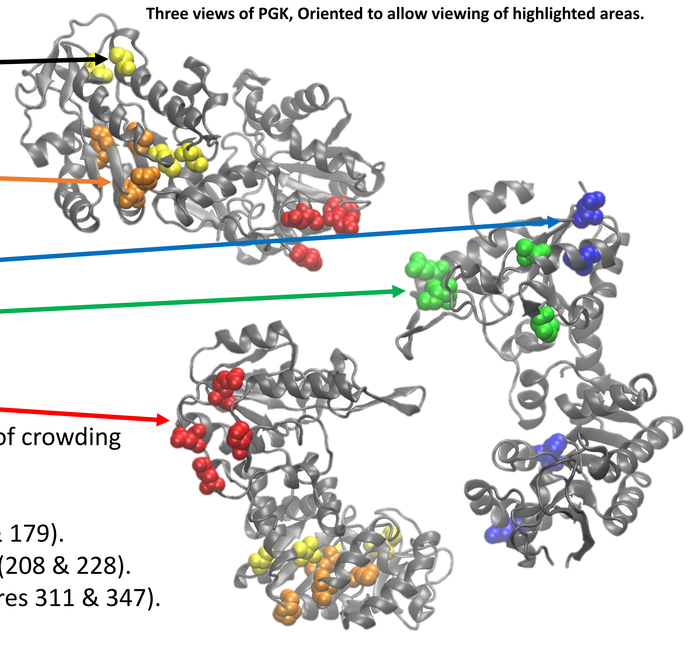


- Very little correlation is shown between water mediated contacts in the absence of crowding agents.



- Correlation increases substantially in the presence of crowding agents.
- High correlation is highlighted:
  - Red are contacts 62 (res 12 & 17), and 58 (res 10 & 179).
  - Orange are contacts 604 (res 208 & 233), and 568 (208 & 228).
  - Yellow are contacts 928 (res 332 & 365), and 889 (res 311 & 347).
- Anti-correlation is highlighted:
  - Blue are contacts 673 (res 224 & 268), and 319 (res 83 & 90).
  - Green are contacts 389 (res 124 & 143), and 172 (res 27 & 77).

Three views of PGK, Oriented to allow viewing of highlighted areas.



Continuation

- Investigate additional temperature and pressure simulations near the intermediate state with and without crowding.
- Gain a more quantitative understanding for cooperativity using statistical modeling similar to the Ising Model.

References

1. Ellis, R. John, and Allen P. Minton. "Cell biology: join the crowd." Nature 425.6953 (2003): 27-28.
2. Osváth, Szabolcs, Luu Manh Quynh, and László Smeller. "Thermodynamics and kinetics of the pressure unfolding of phosphoglycerate kinase." Biochemistry 48.42 (2009): 10146-10150.
3. N. Hillson, JN. Onuchic, and AE. García. "Pressure-induced protein-folding/unfolding kinetics." *Proc. Natl. Acad. Sci.* 96.26 (1999): 14848-14853.
4. MS. Cheung, AE. García, and JN. Onuchic. "Protein folding mediated by solvation: water expulsion and formation of the hydrophobic core occur after the structural collapse." *Proc. Natl. Acad. Sci.* 99.2 (2002): 685-690.
5. D. Apratim, et al. "Structure, function, and folding of phosphoglycerate kinase are strongly perturbed by macromolecular crowding." *Proc. Natl. Acad. Sci.* 107.41 (2010): 17586-17591.

Acknowledgments

MCB-1412532, PHY-1427654, & ACI-1531814

UNIVERSITY of HOUSTON
CENTER FOR ADVANCED COMPUTING & DATA SYSTEMS