Conformational Changes in Guanylate Kinase Studied by Osmotic Stress and SANS

Christopher Stanley[1,2], Susan Krueger[1], V. Adrian Parsegian[2], and Donald C. Rau[2]

[1]NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD; [2]Laboratory of Physical and Structural Biology, NICHD, National Institutes of Health, Bethesda, MD

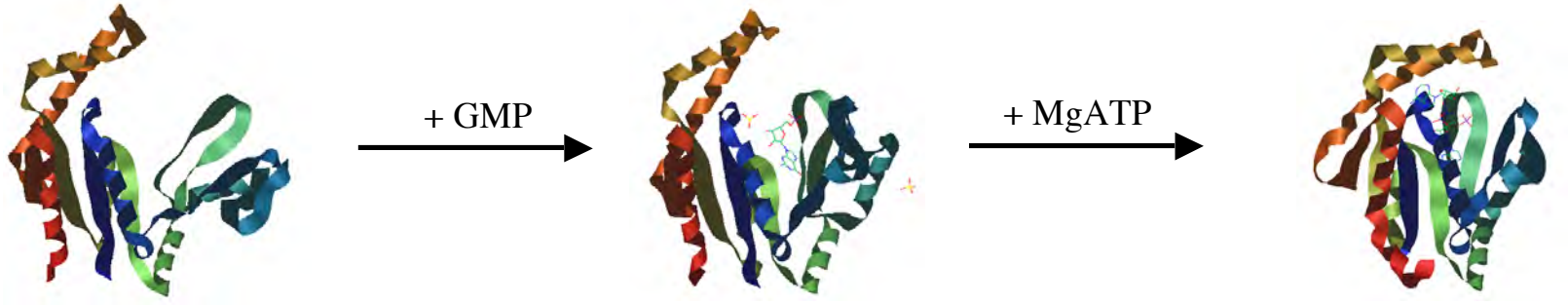
NIST

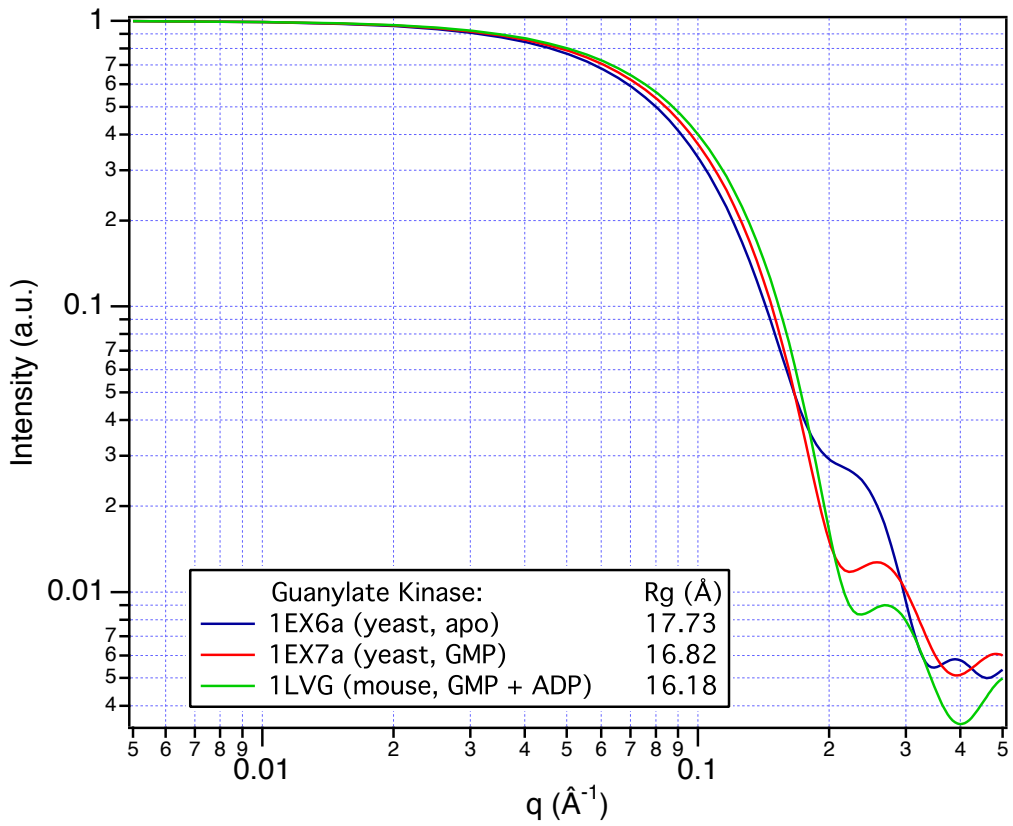
NATIONAL INSTITUTES OF HEALTH

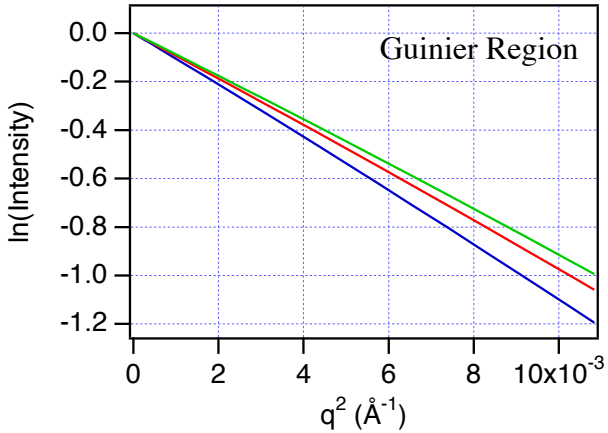
Abstract

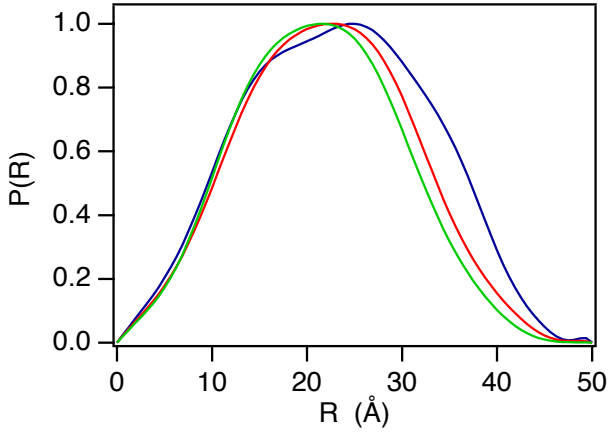
Protein conformational changes induced by ligand binding are accompanied by a change in the number of water molecules sequestered in pockets, cavities, and grooves. The significance of hydration to protein-ligand interactions has been illustrated using the osmotic stress technique. We are using small-angle neutron scattering (SANS) coupled with the osmotic stress technique to directly probe the connection between protein structural change and thermodynamics for guanylate kinase. We chose this enzyme because it is known to undergo a large conformational change upon binding the ligand GMP. We are able to follow this conformational change using SANS to determine the radius of gyration, R_g, and the pair-distribution function, $P(R)$, and now we are investigating protein mechanics by using osmotic stress to induce the conformational change in the absence of ligand. This should offer new opportunities for protein structure research by allowing the energetics of the conformational change to be measured apart from the ligand binding energy.

Simulated SANS from Crystal Structures

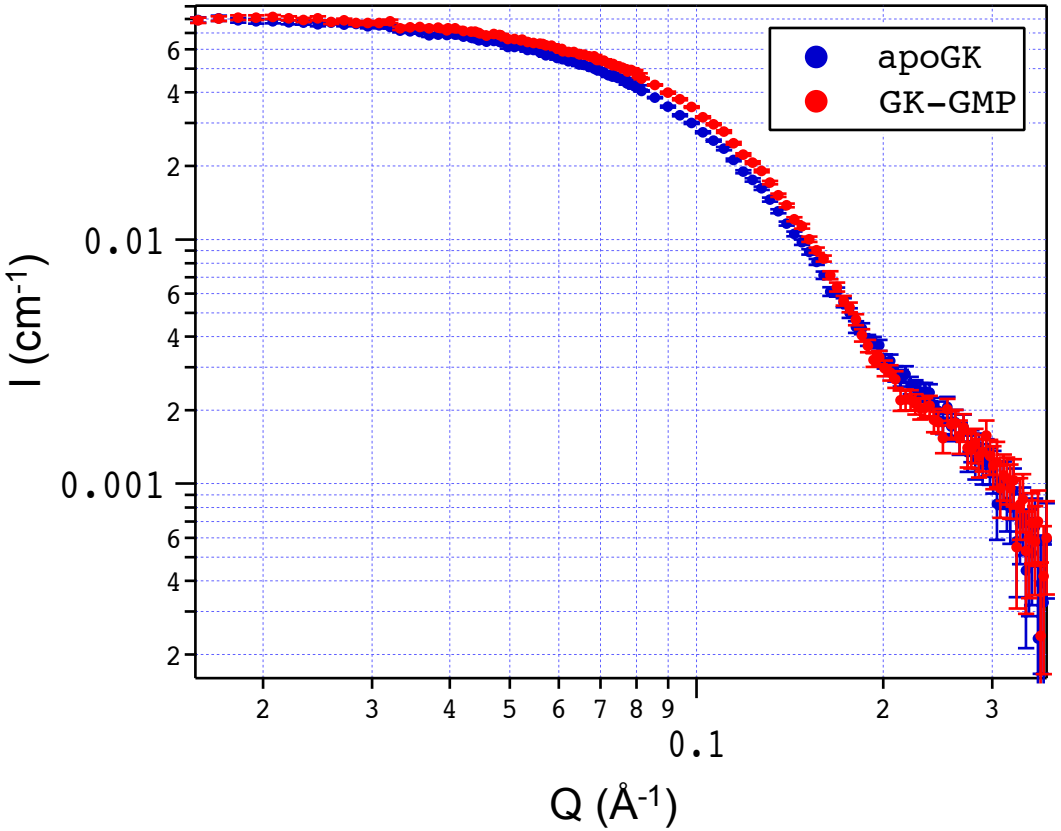








SANS from Guanylate Kinase



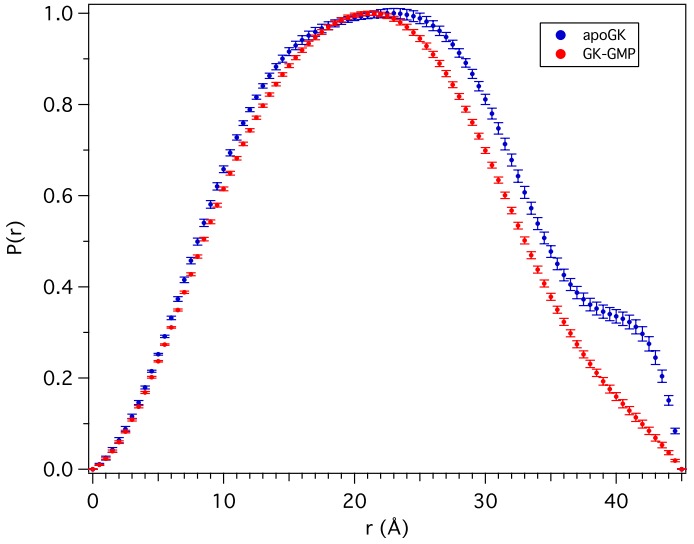
Guinier Fits:

apoGK	$R_g = 17.7 \pm 0.1$ Å
GK-GMP	$R_g = 16.4 \pm 0.1$ Å

in H_2O:

apoGK	$R_g = 18.1 \pm 1.0$ Å
GK-GMP	$R_g = 16.6 \pm 1.1$ Å

Pair-Distribution Function

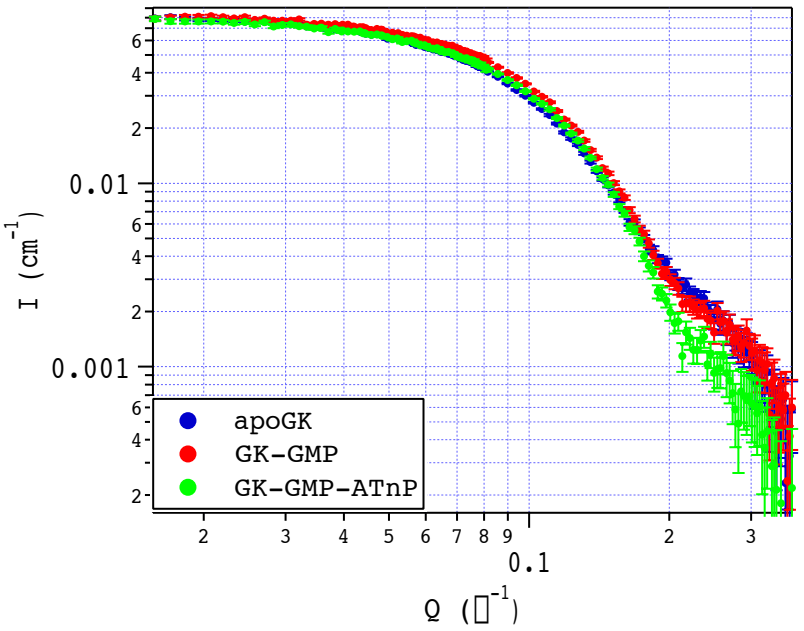


5 mg/mL guanylate kinase in 20 mM Tris-Cl (pH_m 7.7), 0.1 M KCl, D_2O
5 mM GMP added to observe the ligand-bound state

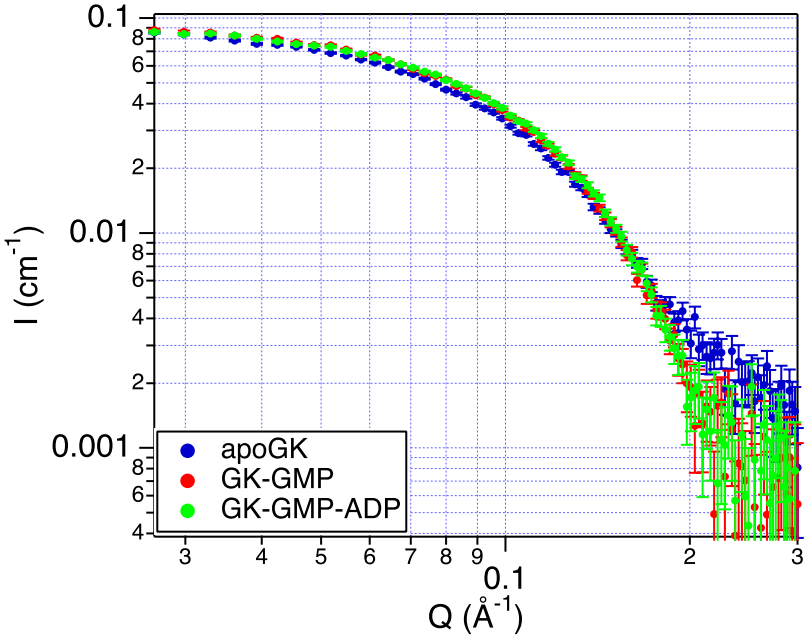
Data collected on NG7-SANS (NIST)

Investigating the Two-Ligand Bound State

ATnP (non-hydrolyzable ATP analog)



ADP



Guinier Fits:

GK-GMP-ATnP	$R_g = 16.9 \pm 0.1$ Å

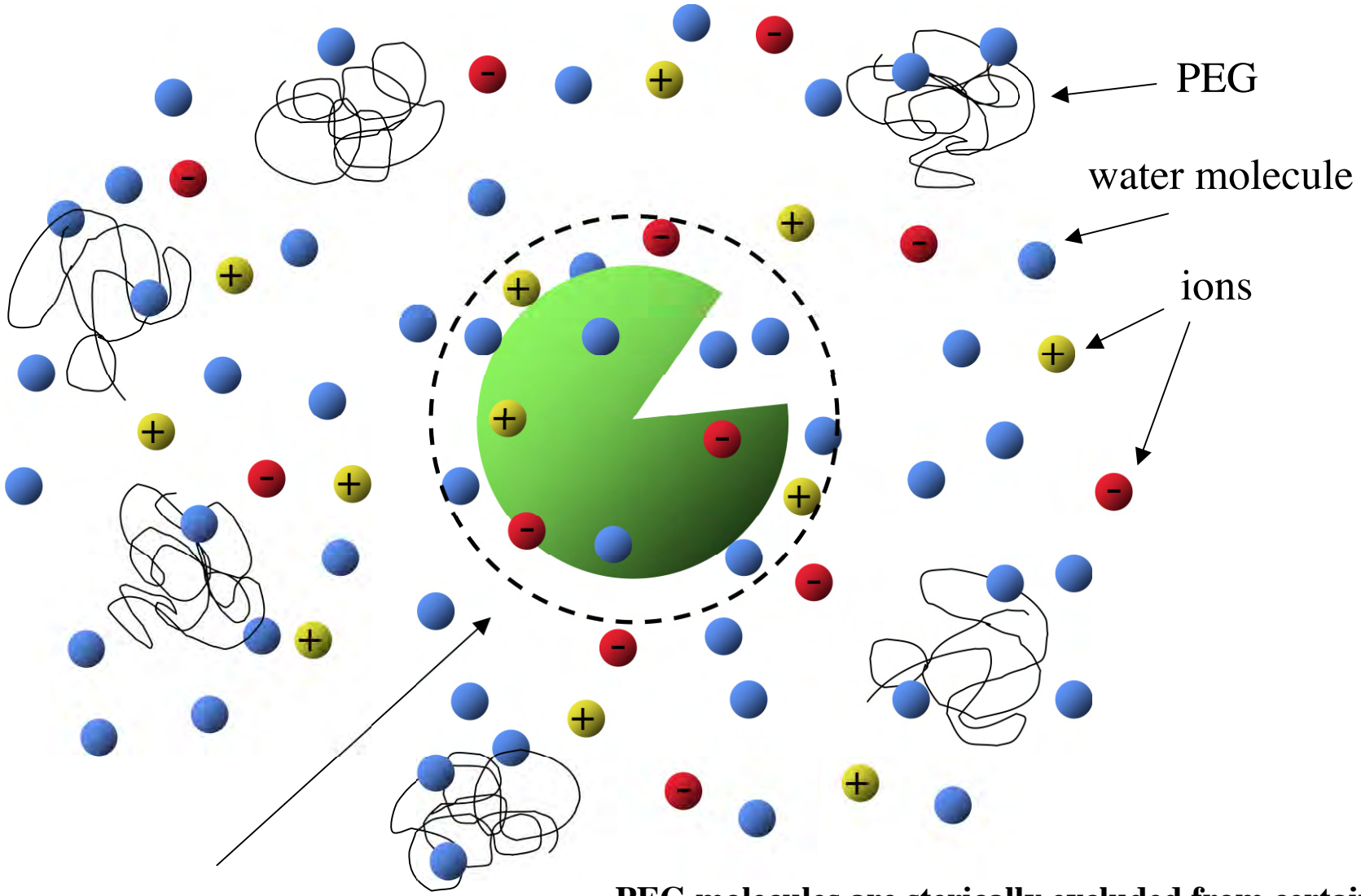
pre-phosphorylation conformation is probed
(crystals cannot be grown using this ligand*)

GK-GMP-ADP	$R_g = 16.2 \pm 0.1$ Å

post-phosphorylation conformation is probed

*N. Sekulic *et al. J. Biol. Chem.* **277,** 30236 (2002).

Osmotic Stress Technique

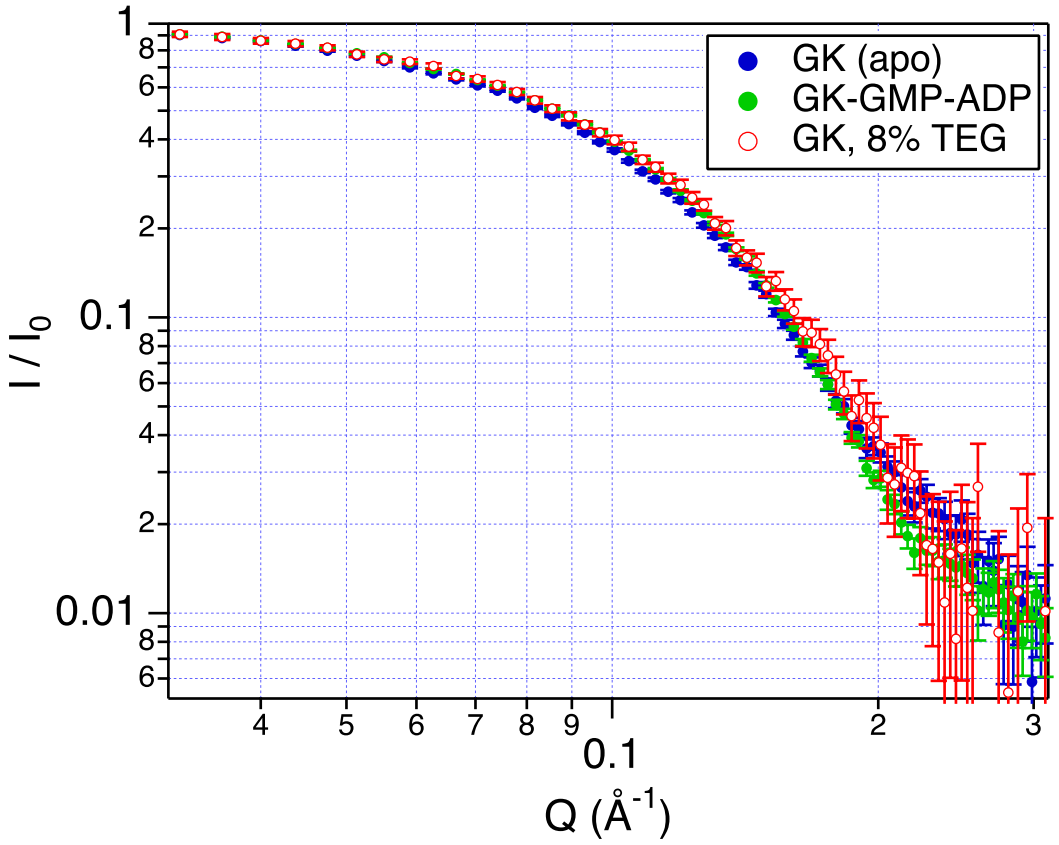


"Effective Semipermeable Membrane"

PEG molecules are sterically excluded from certain regions of the protein (e.g. binding site).

SANS from Guanylate Kinase + Solute

Triethylene glycol (TEG)



Guinier Fit:

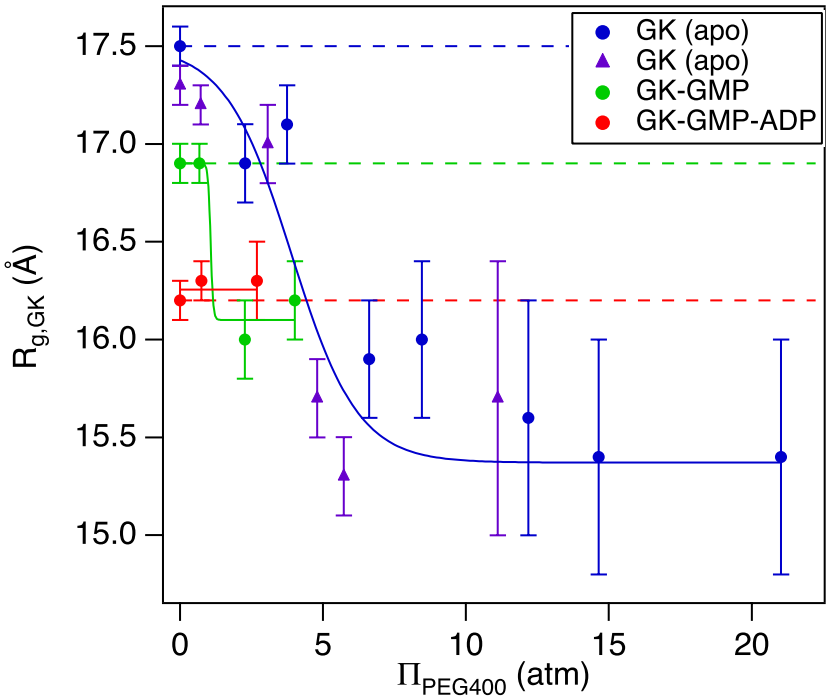
GK, 8% TEG $R_g = 16.5 \pm 0.2$ Å

In the absence of ligand, it appears that the enzyme undergoes a shape and size change due to the presence of TEG.

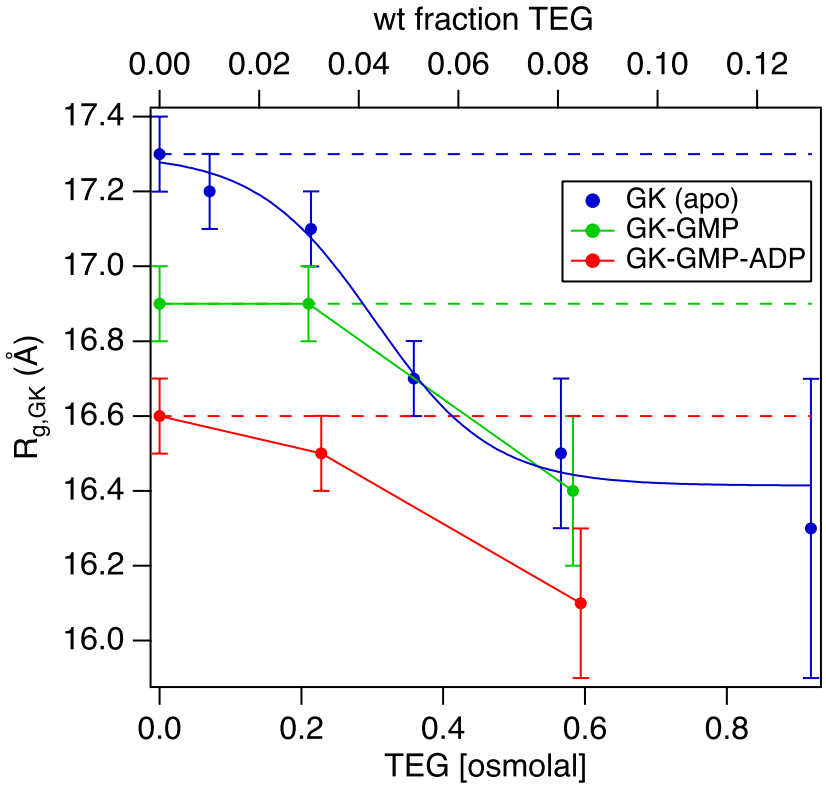
Data collected on NG3-SANS (NIST)

Osmotically Induced Conformational Change

PEG 400



TEG

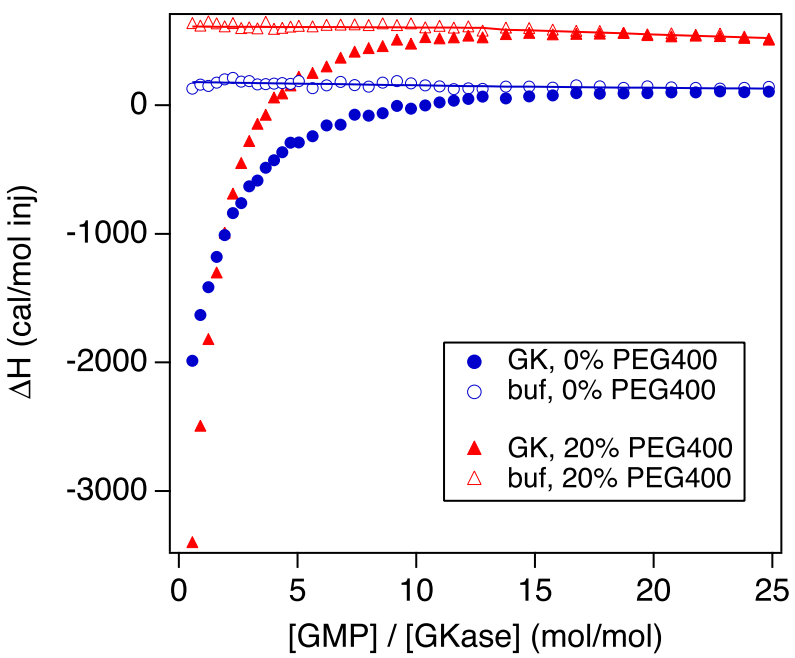


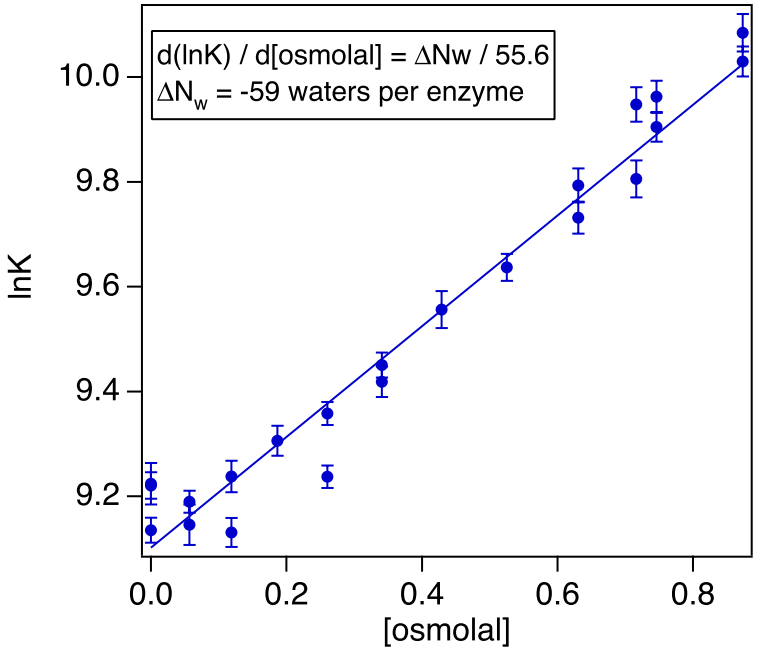
Radius of Gyration (R_g) values determined from Guinier fits to the SANS data (NG3 and NG7, NIST).

Isothermal Titration Calorimetry (ITC)

GMP Binding to GK

$$GK + GMP \rightleftharpoons GK \cdot GMP + nH_2O$$





$$dQ = Q_i - Q_0 + \frac{dV(Q_i - Q_0)}{V_0} + dQ_{GMP} f_{[GMP]}$$

Solvent Accessible Surface Area Calculations:

ΔN_w = -74 waters

T = 30 °C, 20 mM HEPES (pH 7), 100 mM KCl

Summary

- GK conformational change can be followed using SANS.
- Pre- and post-phosphorylation conformational states probed with ATnP and ADP.
- Osmotic stress can close the active site of GK in the absence of ligand.
- ITC measurements show $\Delta N_w \sim 60$ waters per enzyme associated with GMP binding.

Future Work

- SANS on GK with deuterated PEG to follow the conformational change unambiguously.
- ITC measurements with other solutes (e.g. TEG) and ATP analog binding.

Acknowledgement

Honggao Yan (Michigan State Univ.) for providing us with the plasmid for yeast guanylate kinase.