中性子散乱による酵素ドメイン運動の観察

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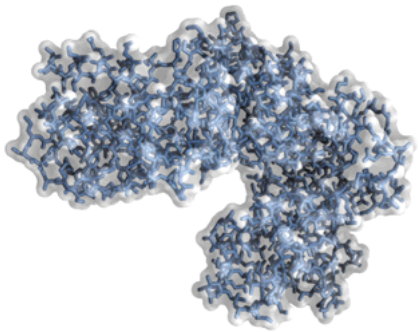
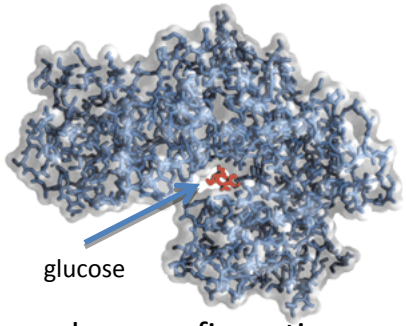
Introduction

Functionality of enzyme → large-scale dynamical displacements by binding substrates

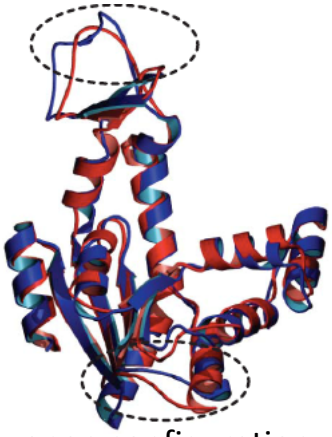


open configuration

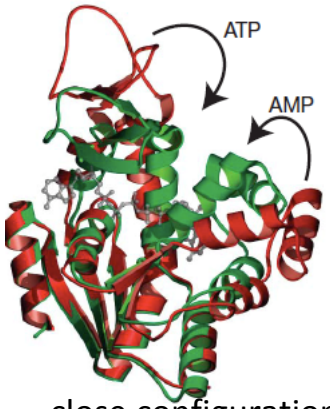


close configuration

locking of domains in hexokinase

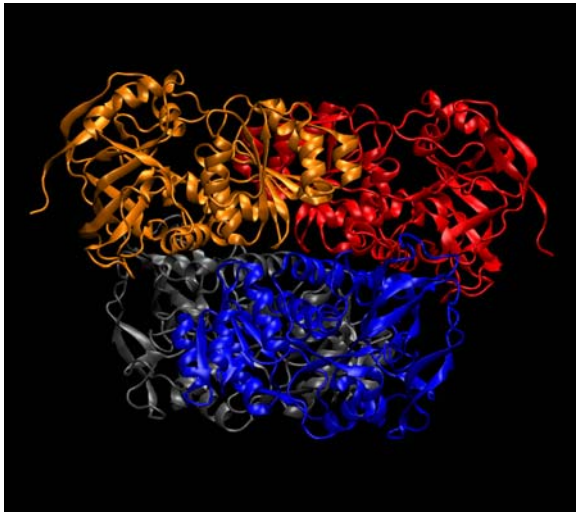


open configuration



close configuration

locking of domains in adenylate kinase



Cleft opening/closing motion of ADH

Thanks to ADH, we can drink beer!!

Benefit ?

1. increasing the specificity of transfer reactions.

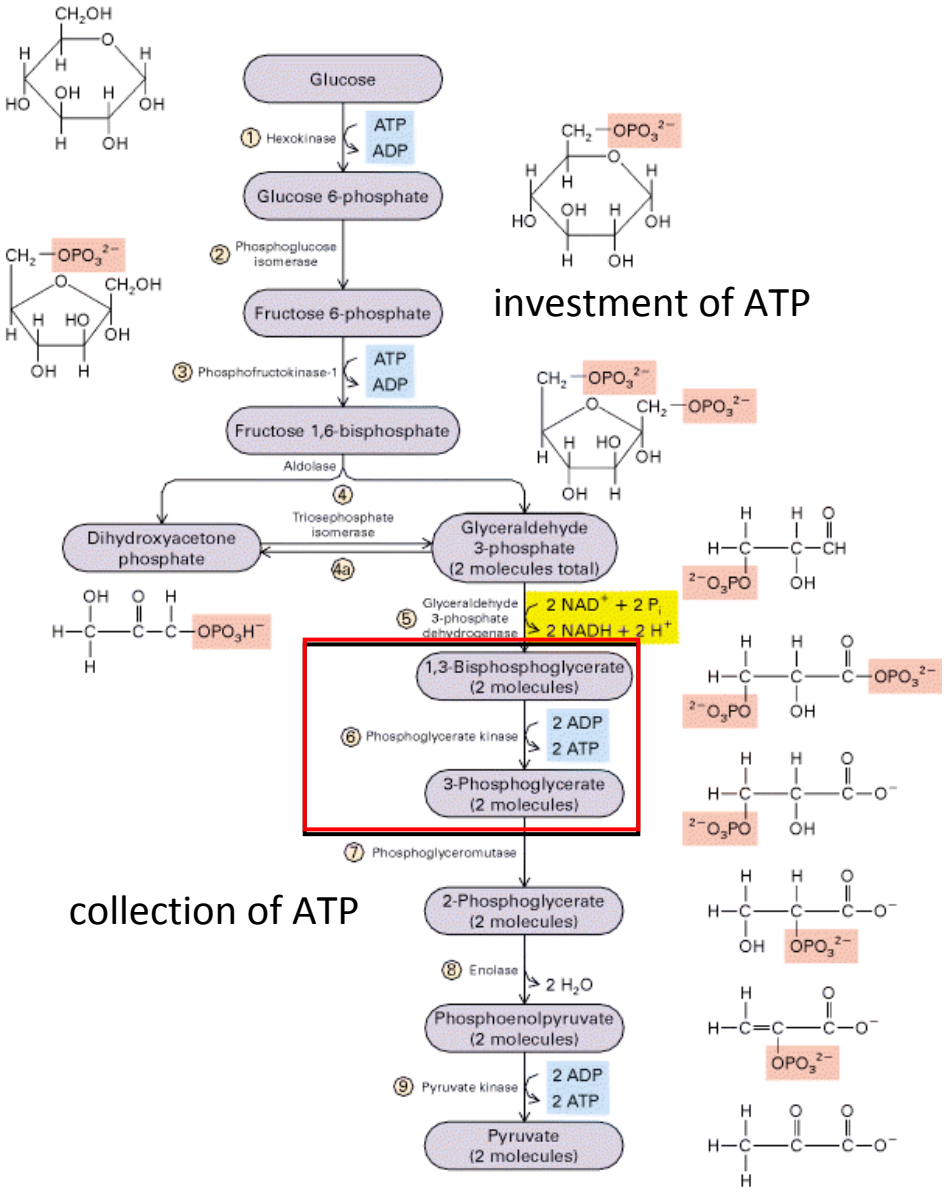
2. facilitating the transfer of atomic or functionality group.

3. shielding active center away from water.

Configuration change of enzyme is quite universal!!

phosphoglycerate kinase (PGK)

PGK is involved in the glycolytic process.

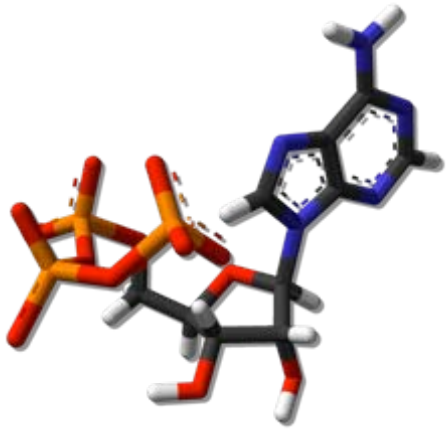


investment of ATP

collection of ATP

What is ATP?

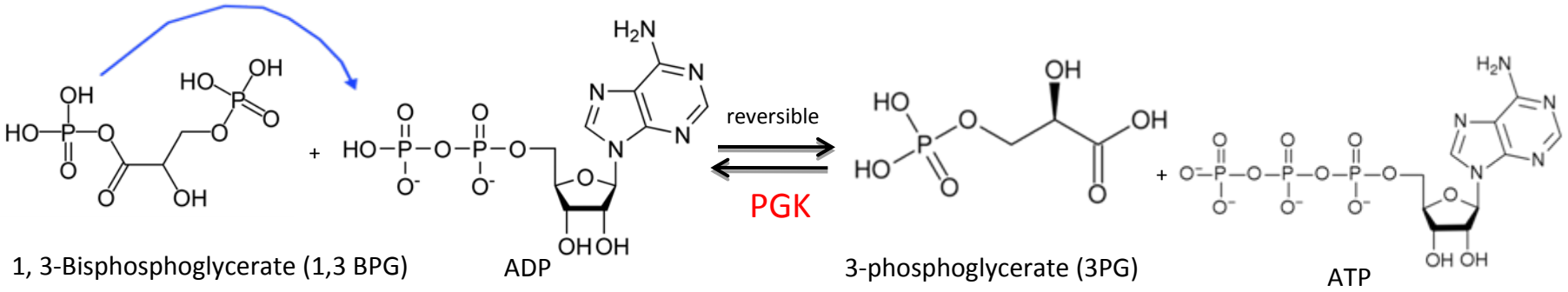
adenosine-5’-triphosphate (ATP)



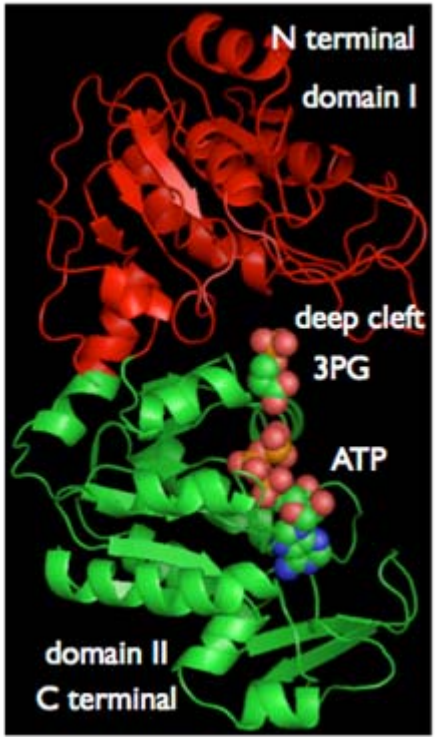
ATP is multifunctional nucleotide, and is most important in cell biology as a coenzyme that is the "molecular unit of currency" of intracellular energy transfer and normal concentration of ATP inside cell is 1~10mM.

What is PGK?

first step for the production of ATP in the glycolytic pathway



1, 3-Bisphosphoglycerate (1,3 BPG) ADP 3-phosphoglycerate (3PG) ATP

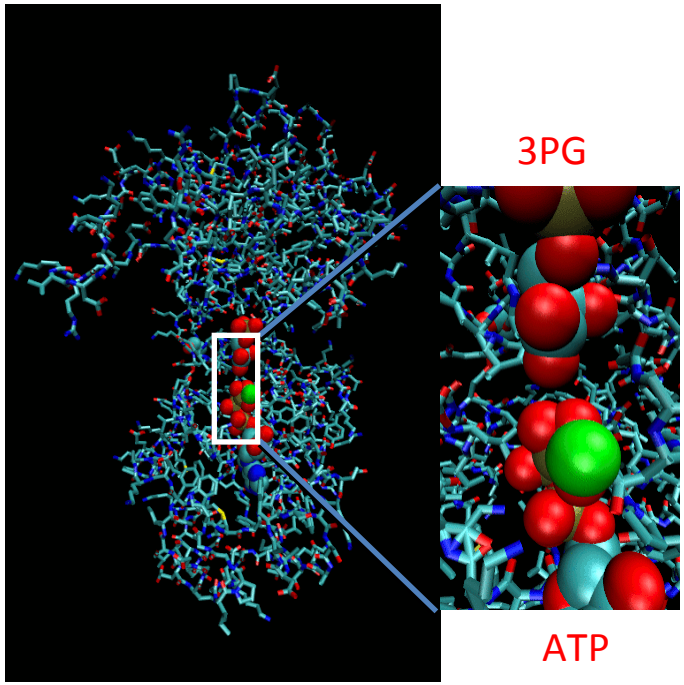


Crystalline structure of PGK

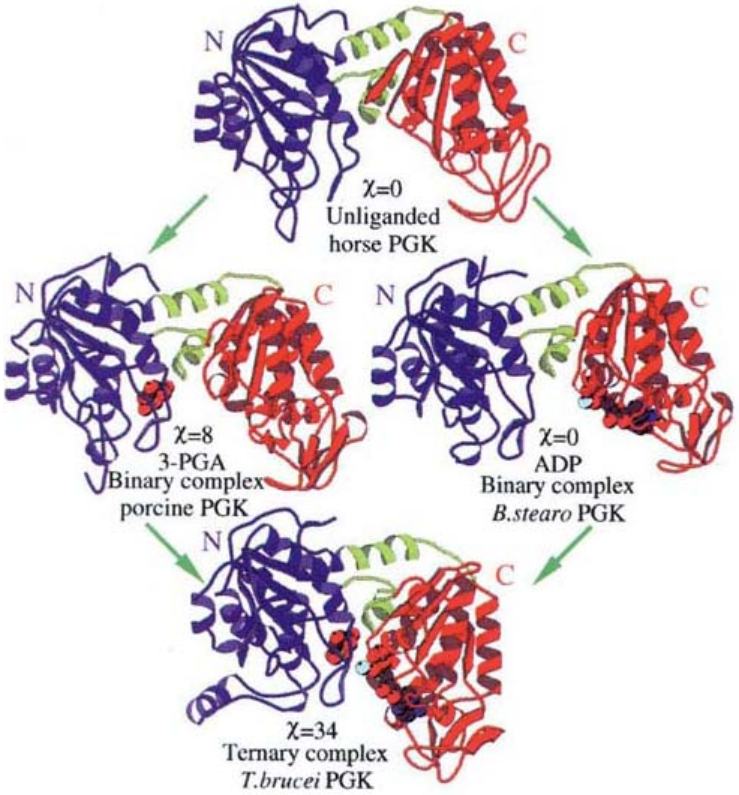
In open configuration, active sites for both substrates are separated about 12Å.

too far away from each other and not favorable for reaction

the idea of hinge-bending motion by Banks et al. (1986)



Experimental evidence of Hinge-bending motion of PGK



hinge closure increased by binding substrates

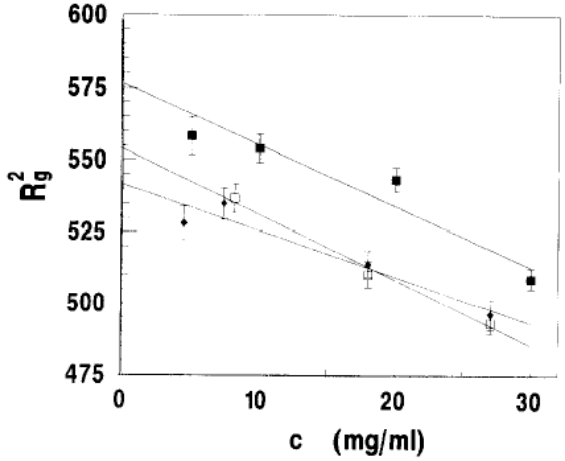
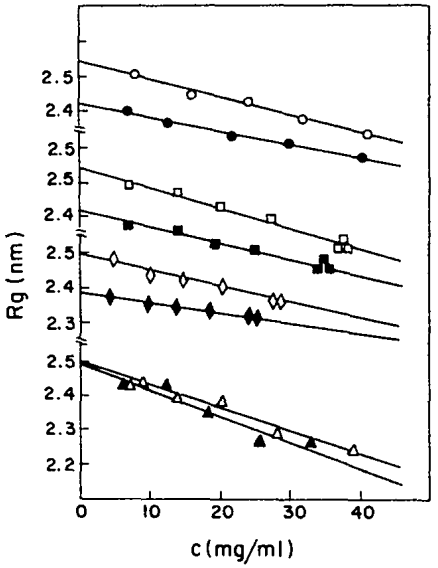
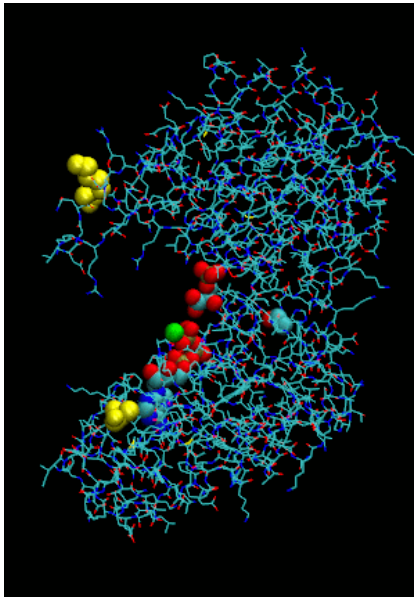


Fig. 3. Plot of R_g^2 against c for the 3 sulphate-free cases: (■) nativ PGK; (□) PGK + CrATP; (♦) PGK + PGA + CrATP.



decrease of R_g *by binding substrates*



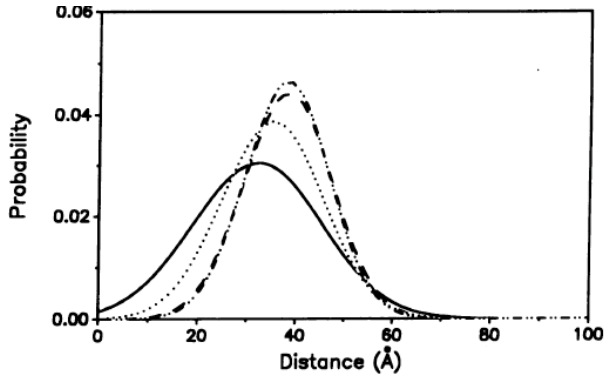
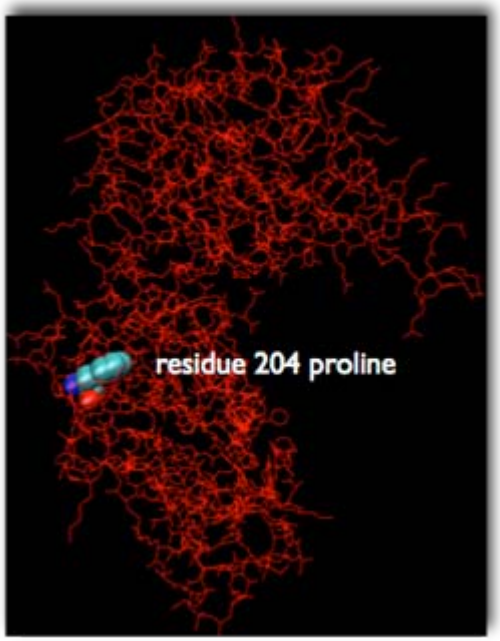


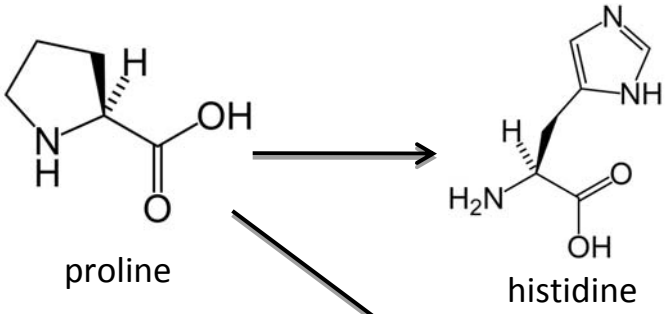
FIG. 4. Interprobe equilibrium distance distribution function without substrates (——), in the presence of ATP (– ·· –), in the presence of 3-PG (· · ·), and in the presence of ATP and 3-PG (– – –).

change of distribution function

Is Hinge-bending motion important for PGK?



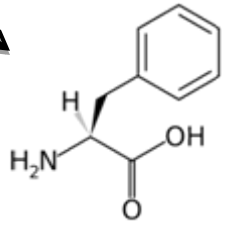
residue 204 proline belongs to Helix 7 (hinge region).



proline

histidine

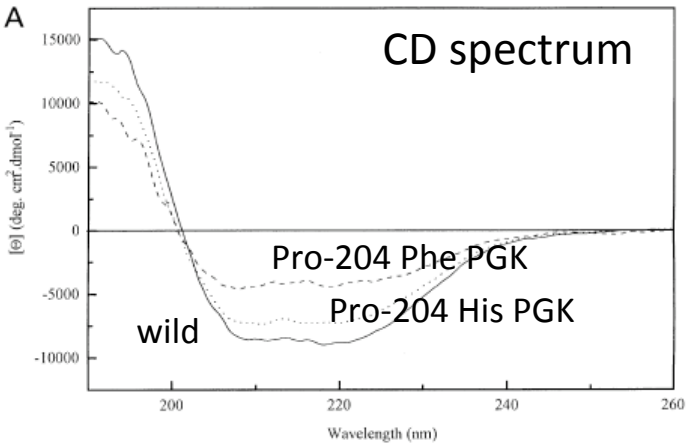
Pro-204 His PGK



phenylalanine

Pro-204 Phe PGK

What happened to PGK?



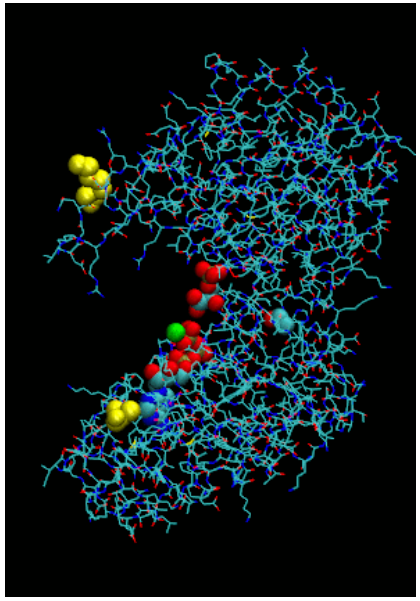
change of secondary structure

	specific acitvity (EU mg^{-1})	catalytic efficiency
wild PGK	468	460000
Pro-204 His PGK	4.5	1360
Pro-204 Phe PGK	1.4	-

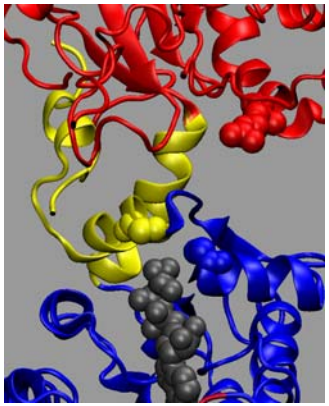
hinge-bending motion is strongly related to PGK's activity.

Purpose of this work

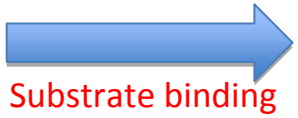
1. Direct observation of hinge-bending motion of PGK.

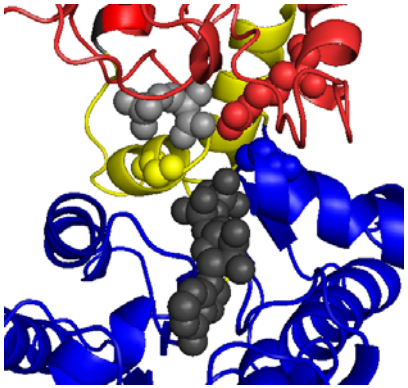


2. substrate induced configuration change of PGK. (more than change of Rg)



open configuration





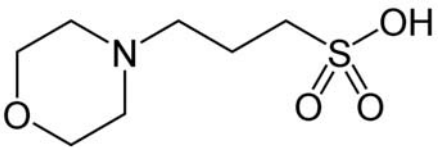
close configuration

Experimental

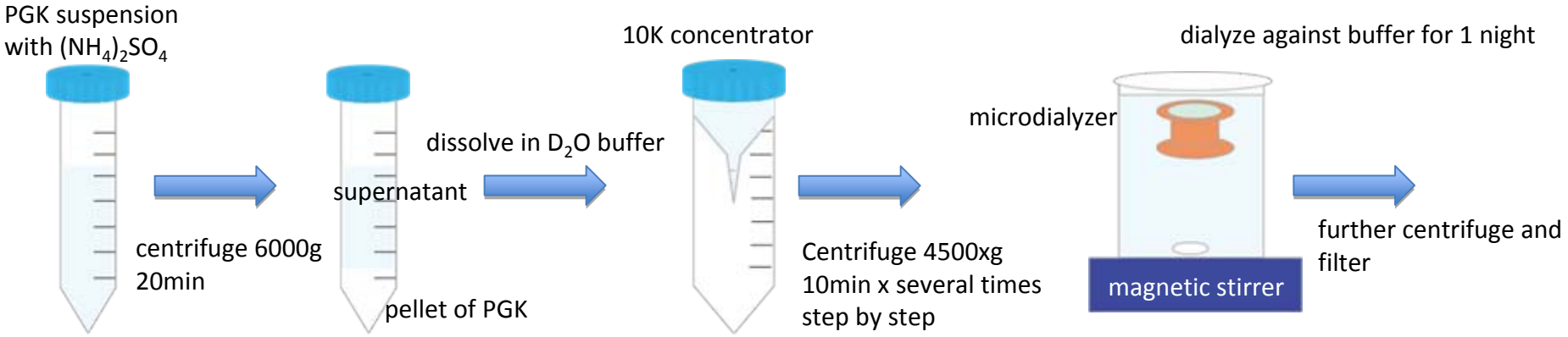
protein
phosphoglycerate kinase (PGK) (from Baker's yeast)
molecular weight M=44607 Da, R_g~23.9Å (from crystalline structure)
$C_{2009}H_{3232}N_{536}O_{599}S_4$

substrates
MgATP (complexed with Mg), M=507.181,$C_{10}H_{16}N_5O_{13}P_3$
3-phospho-D-glycerate, M=186.058, $C_3H_7O_7P$

buffer condition (pD=7.4)
D_2O
50mM NaCl
20mM MOPS
2mM EDTA



sample preparation



Instrumental

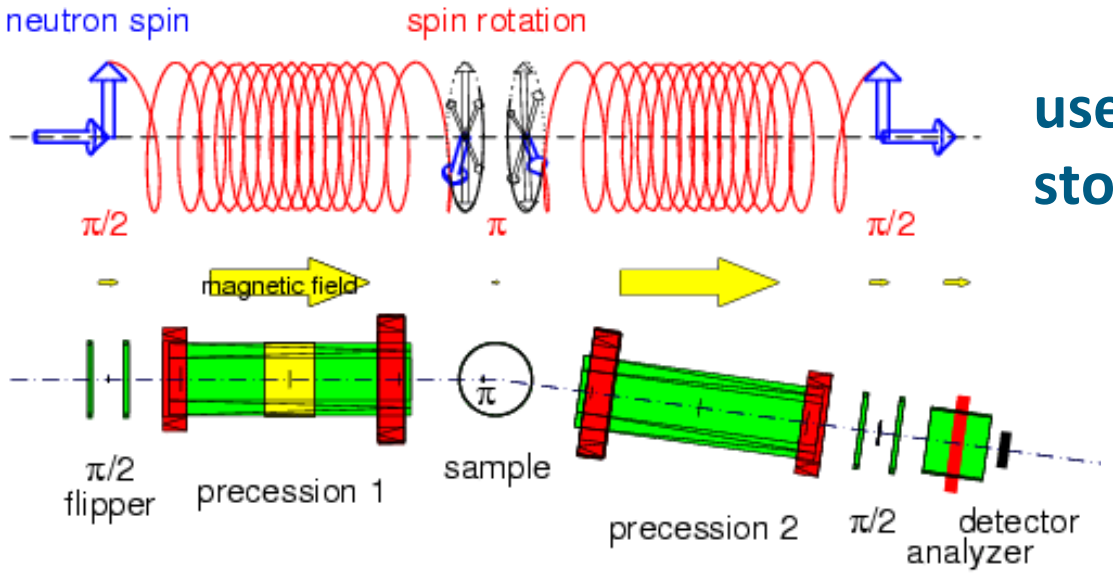
circular dichroism (CD): spectroscopy: Jasco J-810

Fluorescence spectroscopy: RF-1501 fluorospectrometer and LS55 luminescence spectrometer

dynamic light scattering (DLS): ALV-5000 at T=10°C

small angle neutron scattering (SANS): KWS 1, at FRM II λ =4.5Å at T=10°C

neutron spin echo (NSE): IN15, at ILL λ =6.3Å, 8.0Å, 10Å, 12Å and 15.9Å at T=10°C

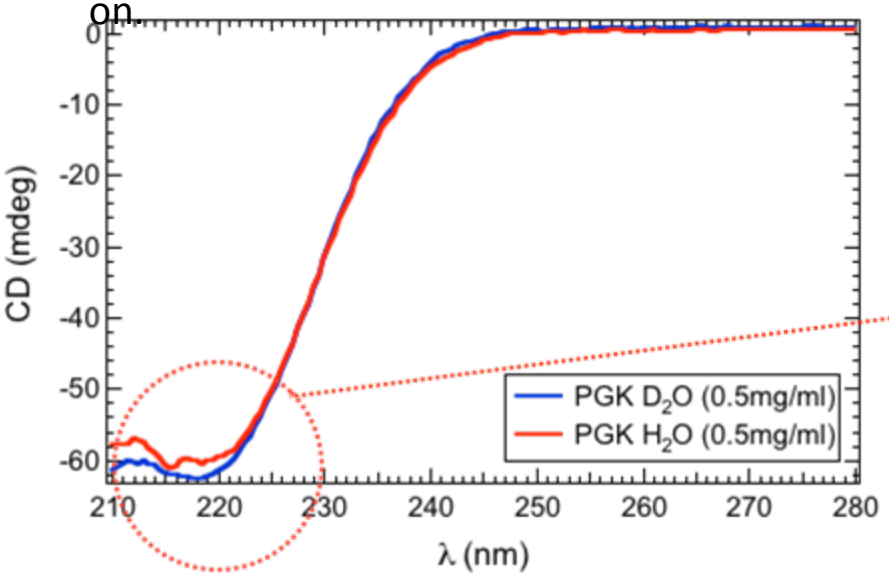


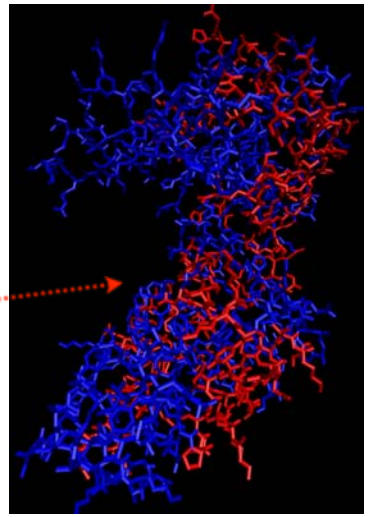
use the neutron spin as stop watch ...

...to detect tiny velocity changes

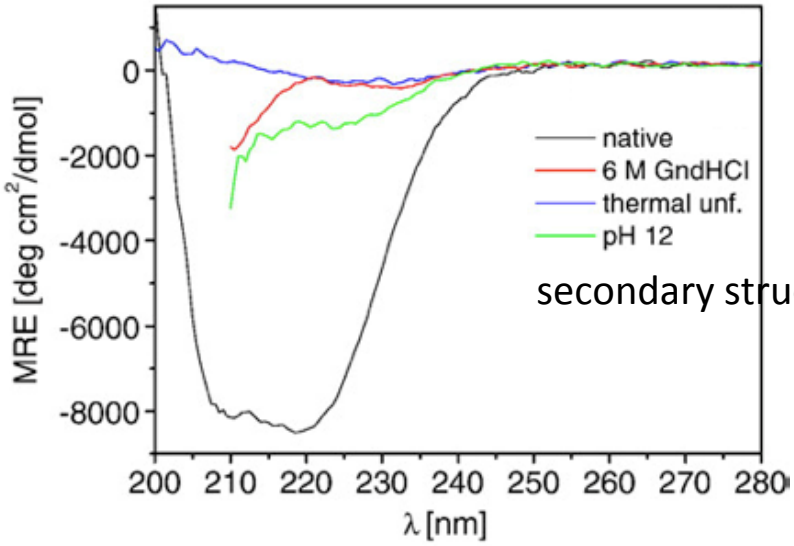
CD spectroscopy (secondary structure) D_2O effect?

secondary structure: α helix, β sheet, strand, helix and so on.





red part is α helix of PGK.

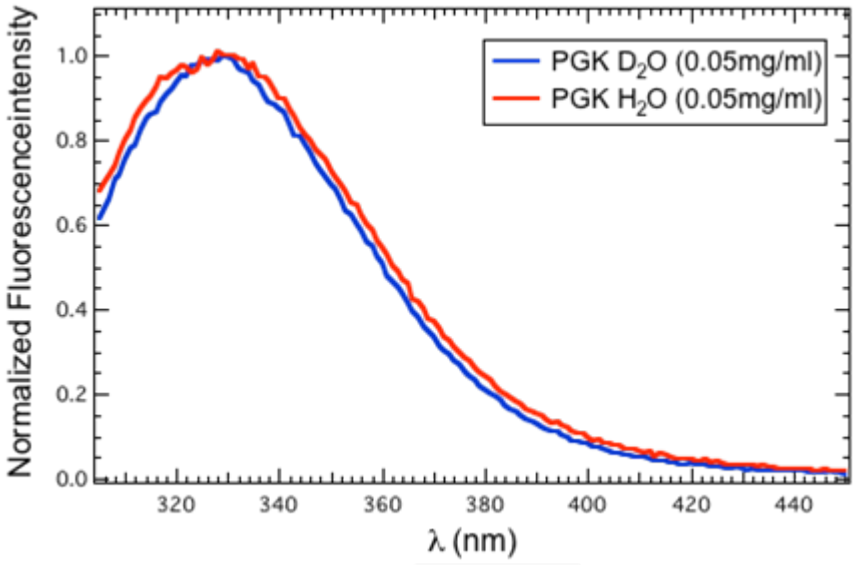


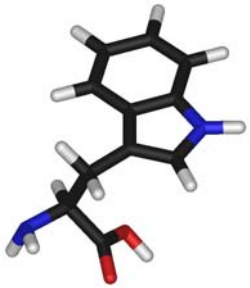
secondary structure becomes obscure by unfolding.

PGK is still native in D_2O buffer like in H_2O buffer.

Fluorescence spectroscopy

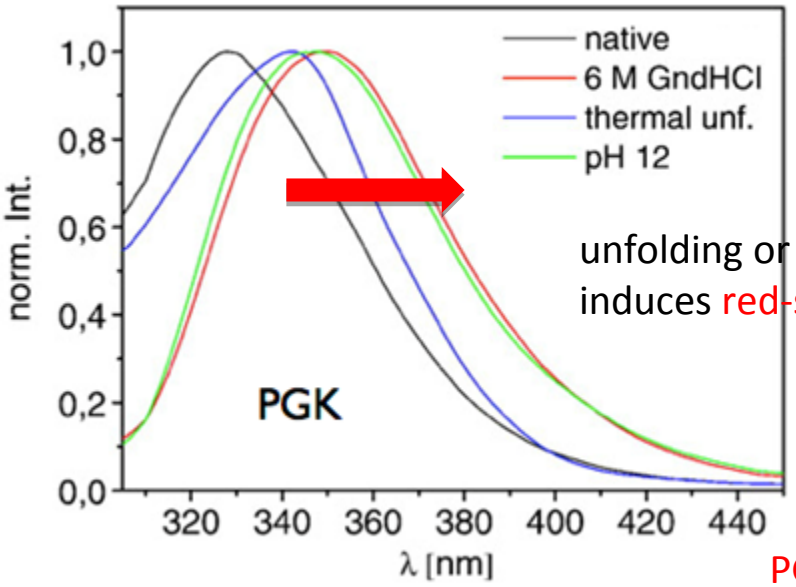
information from hydrophobic amino-acids tryptophan





tryptophan

sensitive to environmental polarity



unfolding or aggregation induces red-shift.

PGK is still native in D_2O buffer like in H_2O buffer.

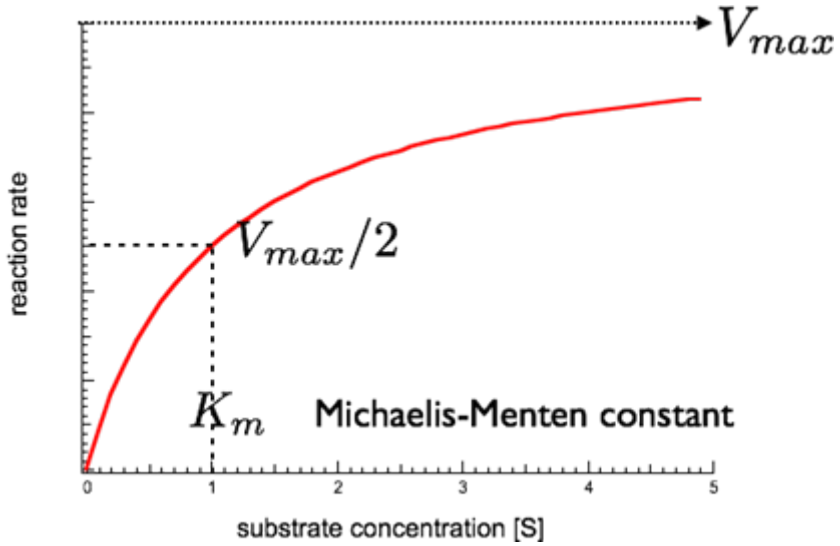
determination of substrates concentrations I

E (enzyme)+S (substrates) $\rightleftharpoons$ ES (complex with enzyme and substrates)

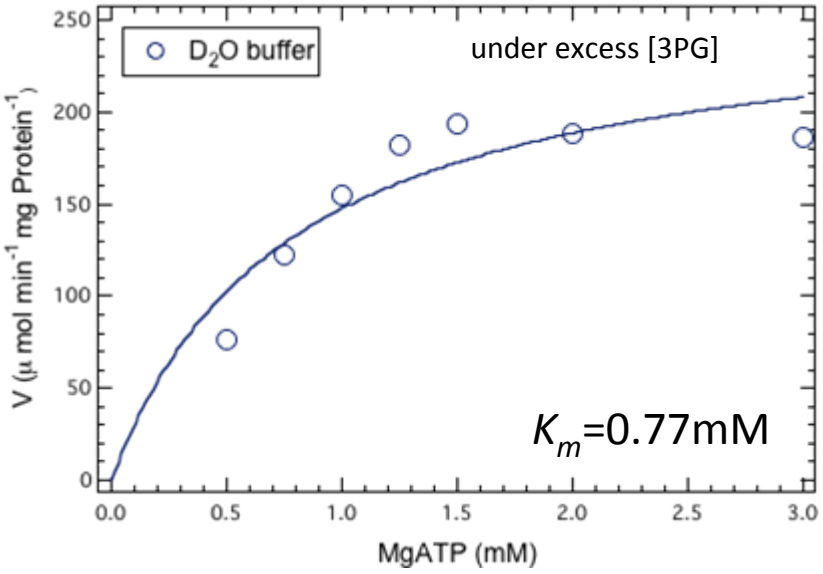
ES $\xrightarrow{k_{cat}}$ E+P (products)

reaction rate $v = k_{cat}[ES]$

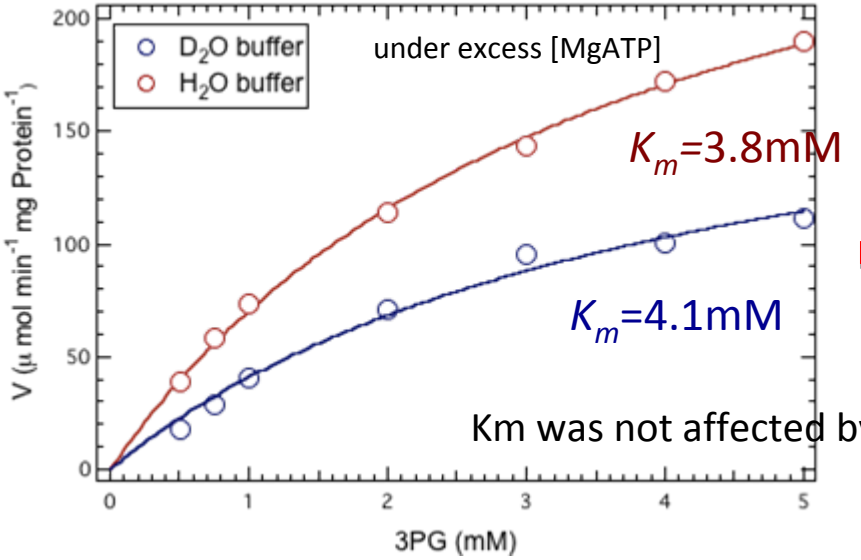
$$v = \frac{k_{cat}[E][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$



determination of substrates concentrations II



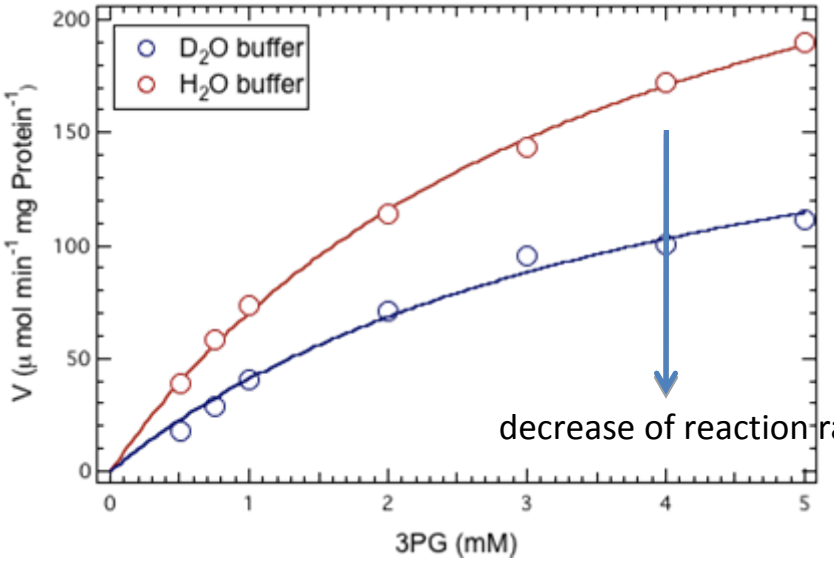
→ final concentration [MgATP]=8.0mM



→ final concentration [3PG]=41mM

Km was not affected by D2O.

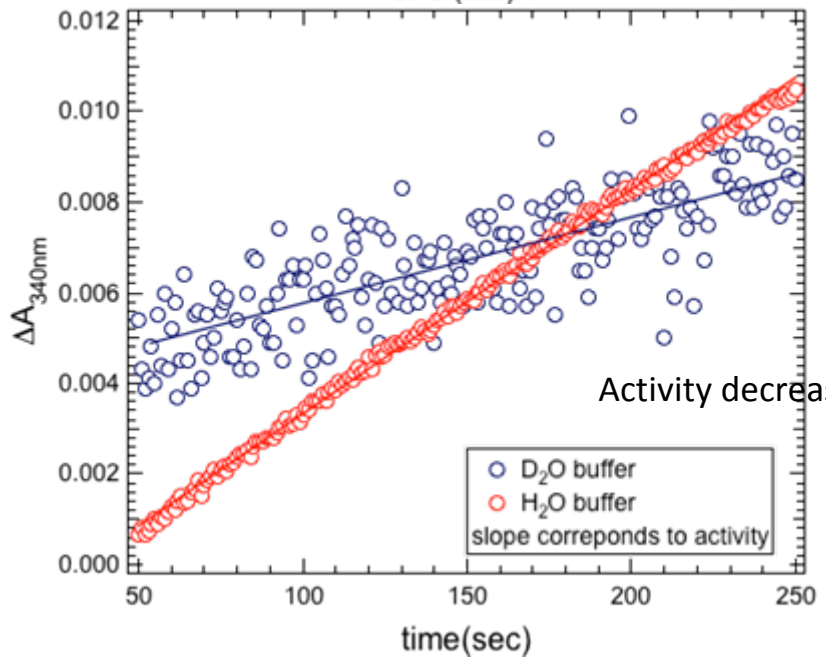
D_2O effect?



decrease of reaction rate for D_2O buffer.

GAP+β-NAD+P_i $\xrightarrow{\text{GAPDH}}$ 1,3-Diphosphoglycerate+β-NADH

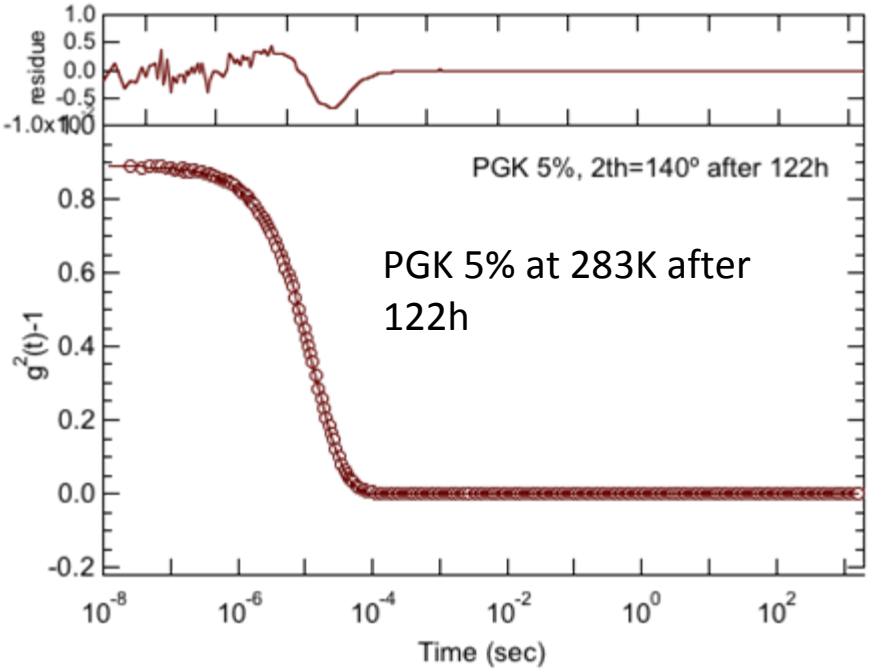
1,3-Diphosphoglycerate+ADP $\xrightarrow{\text{PGK}}$ 3PG+ATP

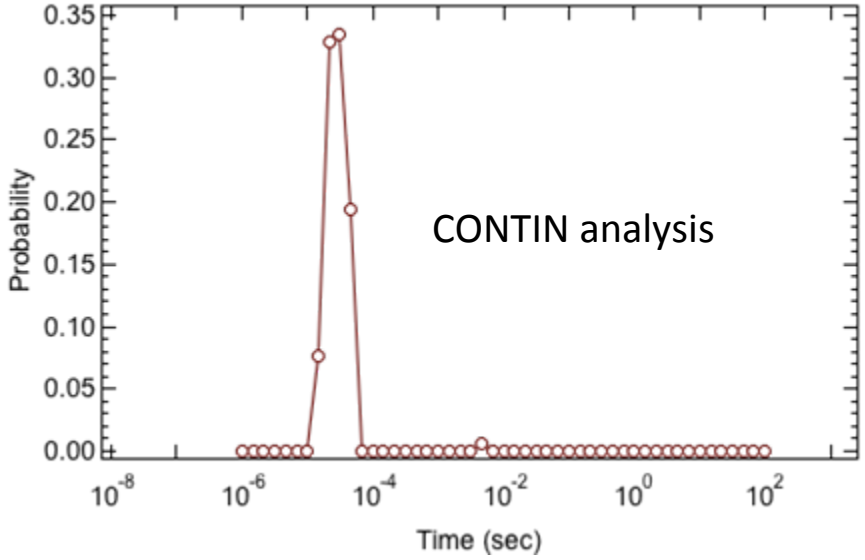


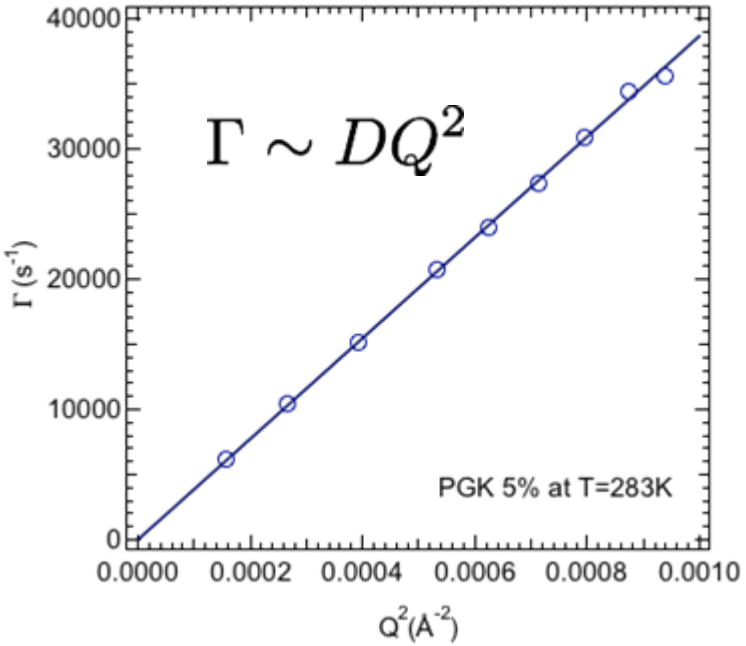
Activity decreased for D_2O buffer.

Viscosity? or other factor?

Sample stability?





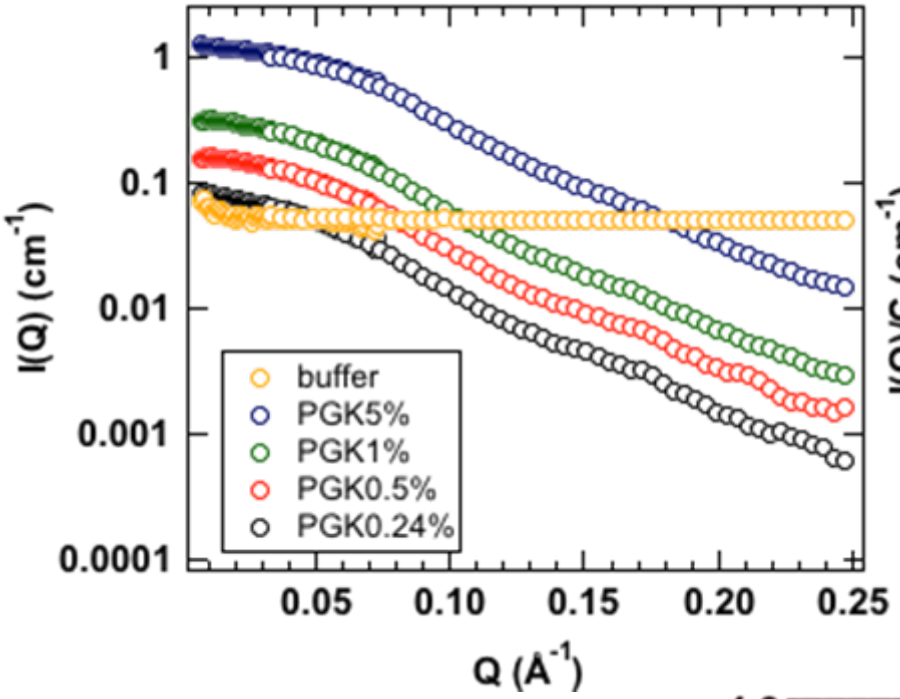


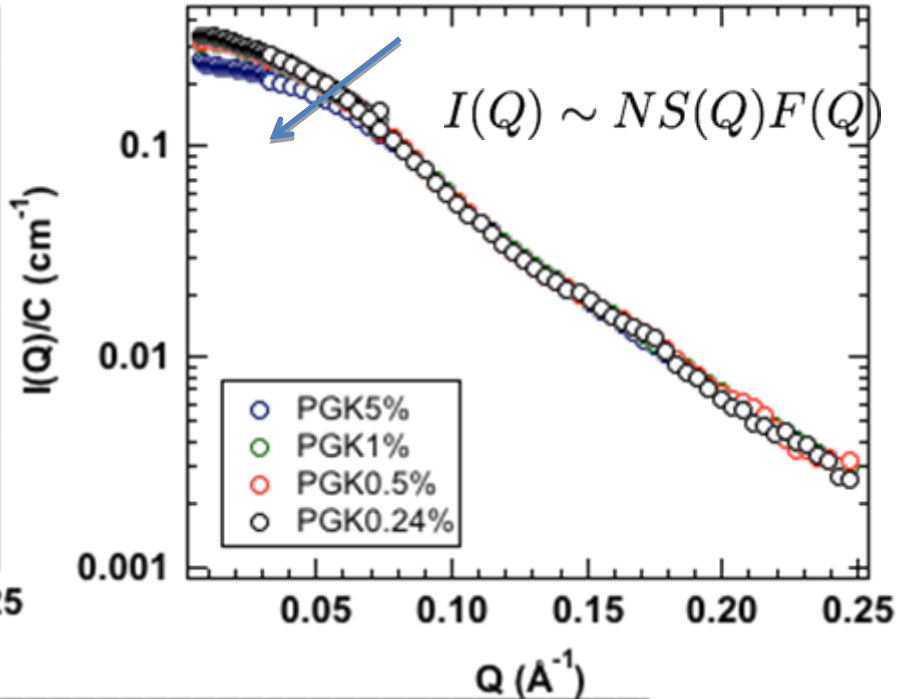
quite mono-disperse even at highest concentration at 283K

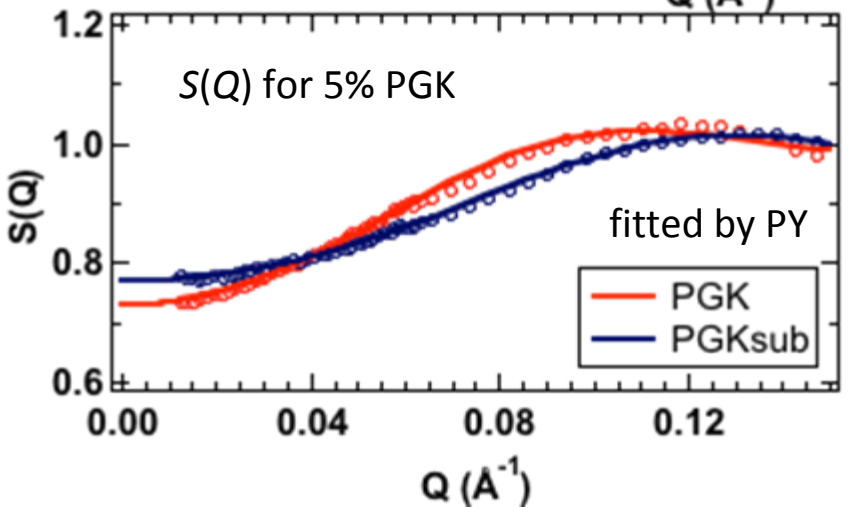
Possible for NSE experiments

Static : SANS

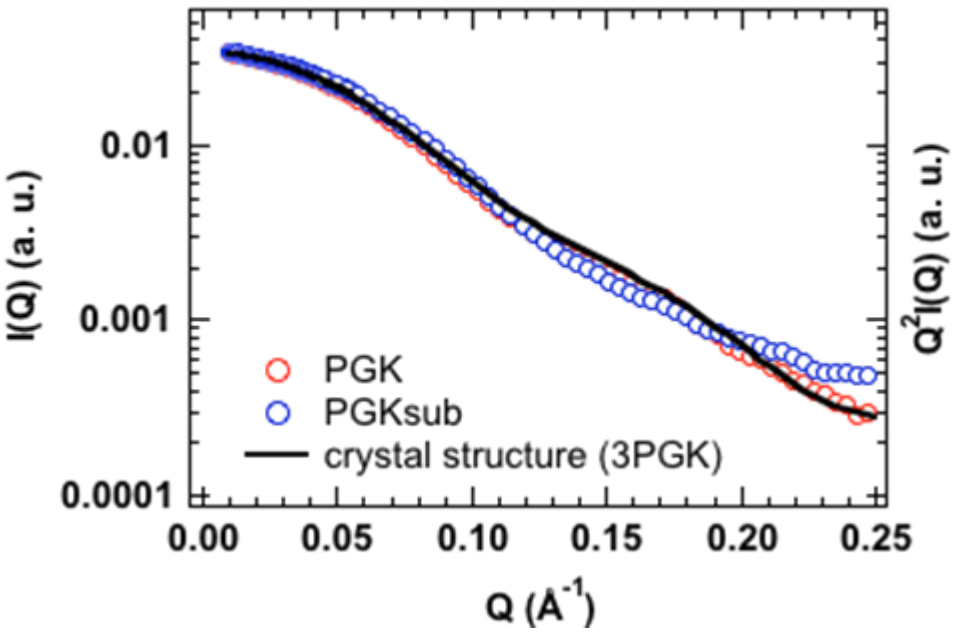
SANS profile

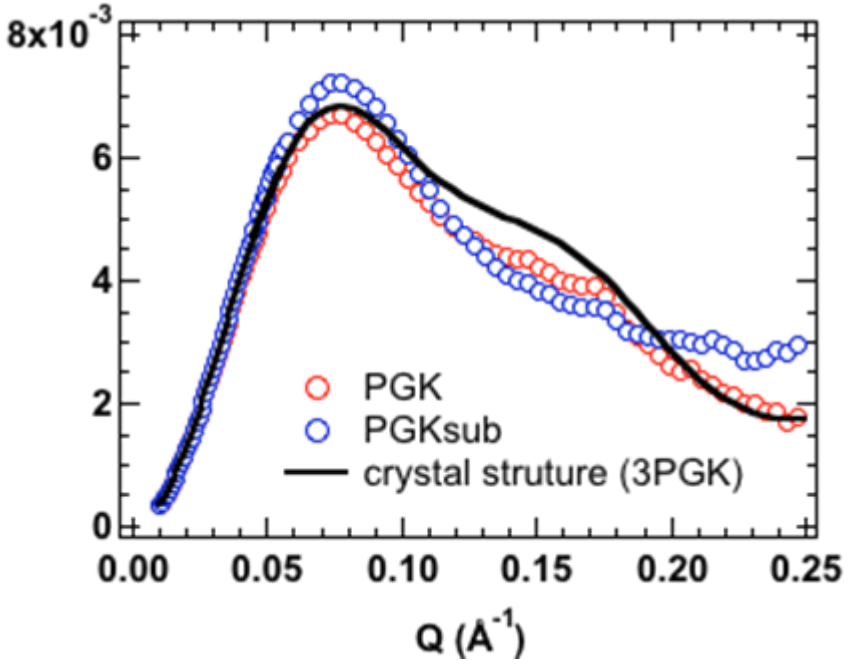




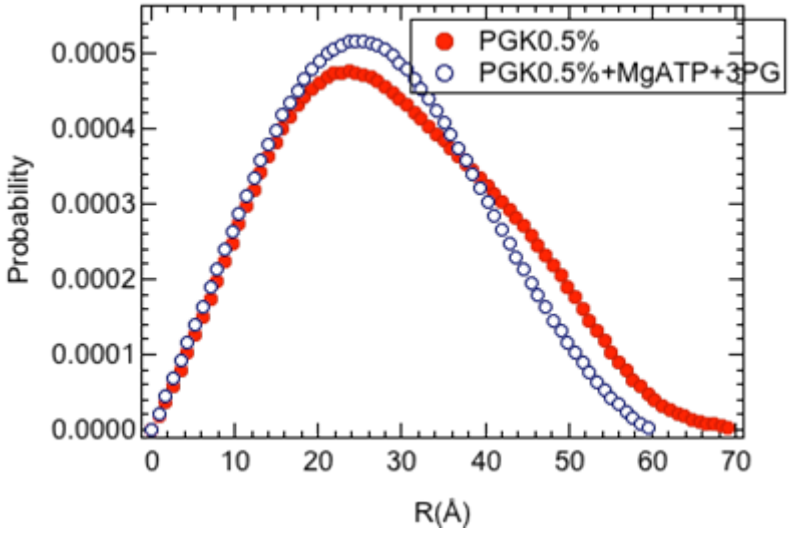


SANS profile form factor for PGK and PGKsub





	Rg (Å)
PGK (crystal)	23.9
PGK	23.7
PGKsub	22.5



1. PGK in solution has a more compact structure.

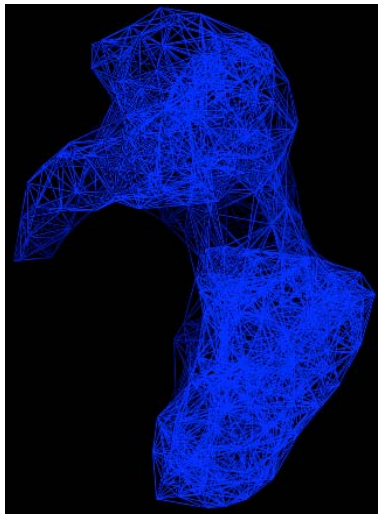
2. Substrate induced configuration change.

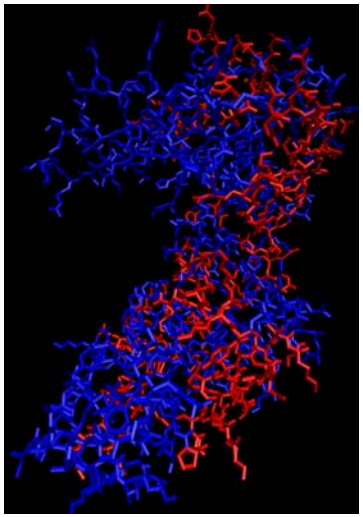
Different approach is needed!

Elastic normal mode

From web service calculation elNémo

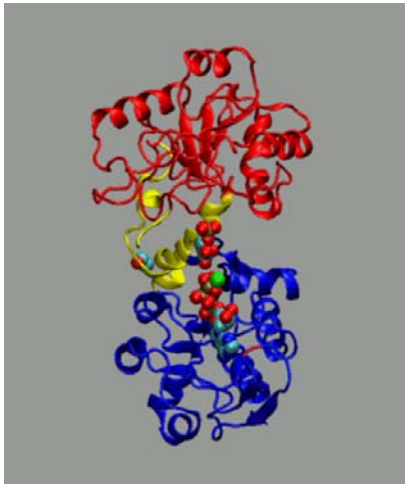
single parameter harmonic potential with asimplified protein model having only one point mass per residue is enough for low frequency NM.





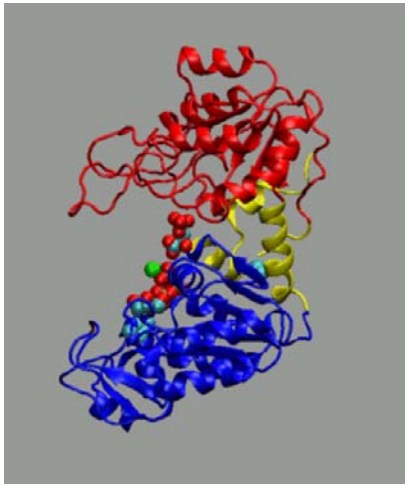
Elastic normal mode + original crystal structure → PGK in solution

NM 7



twisting

NM 8

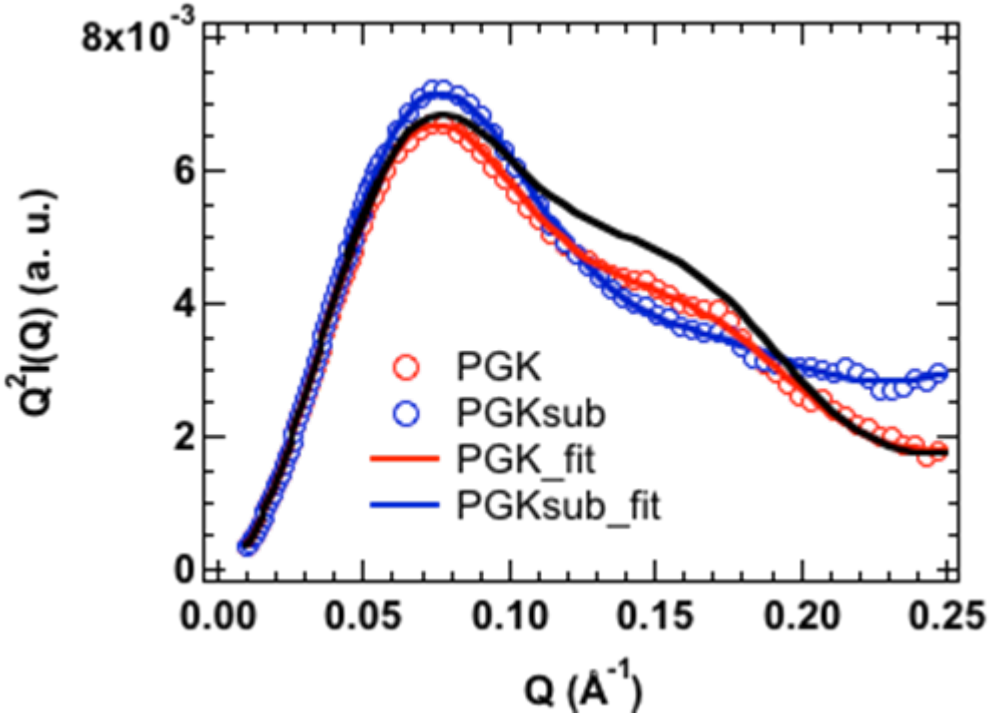


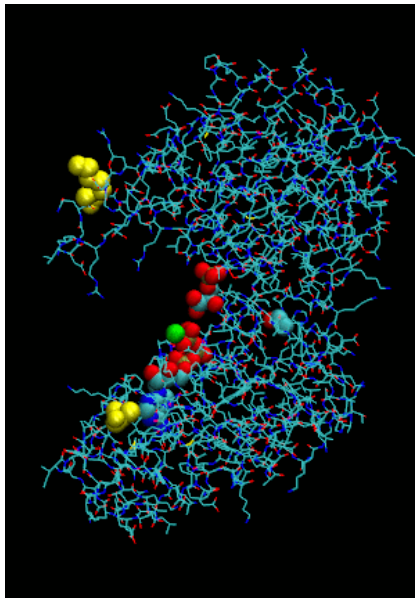
rocking

NM 9

rocking

Elastic normal mode + original crystal structure



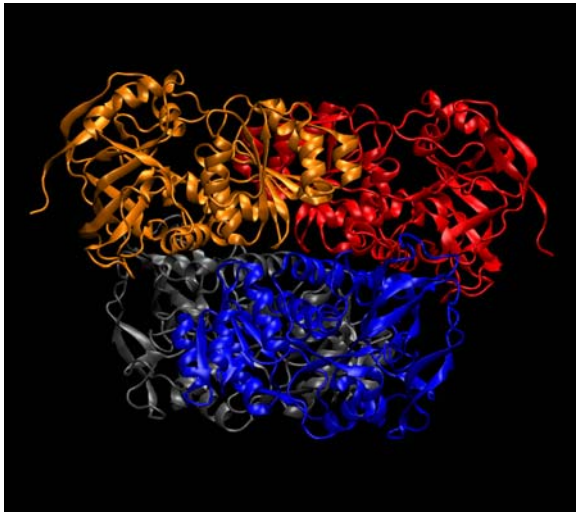


Near the active center

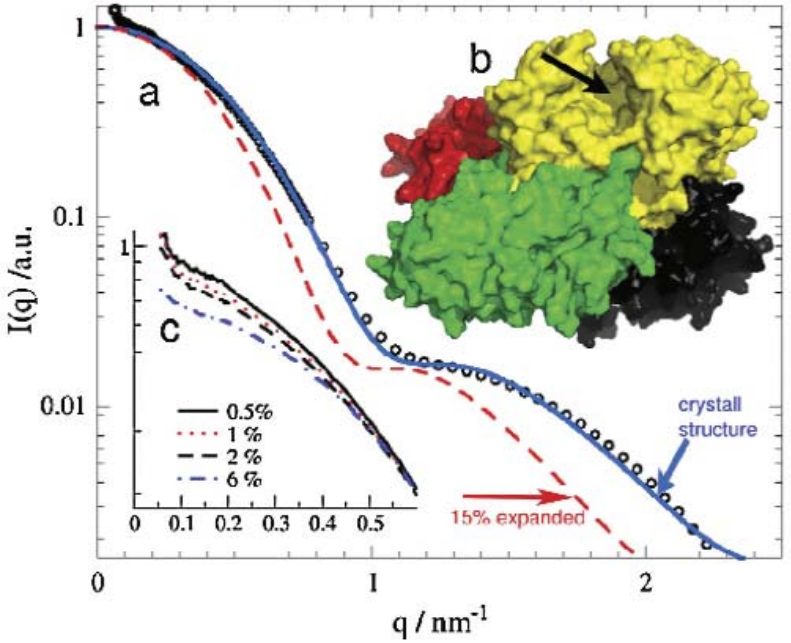
Mean atomic displacement mode [7, 8, 9]	Rg (Å)	Distance$_{38\text{-}371}$ (Å)	
[-1.4, 0.3, 2.2]	23.5	11.4	
[-0.6, -3.4, 0.3]	22.9	8.2	More compact strcuture

Low frequency NMs help to describe the PGK structure in solution.

The crystal structure cannot describe the protein structure in solution?



tetramer of ADH



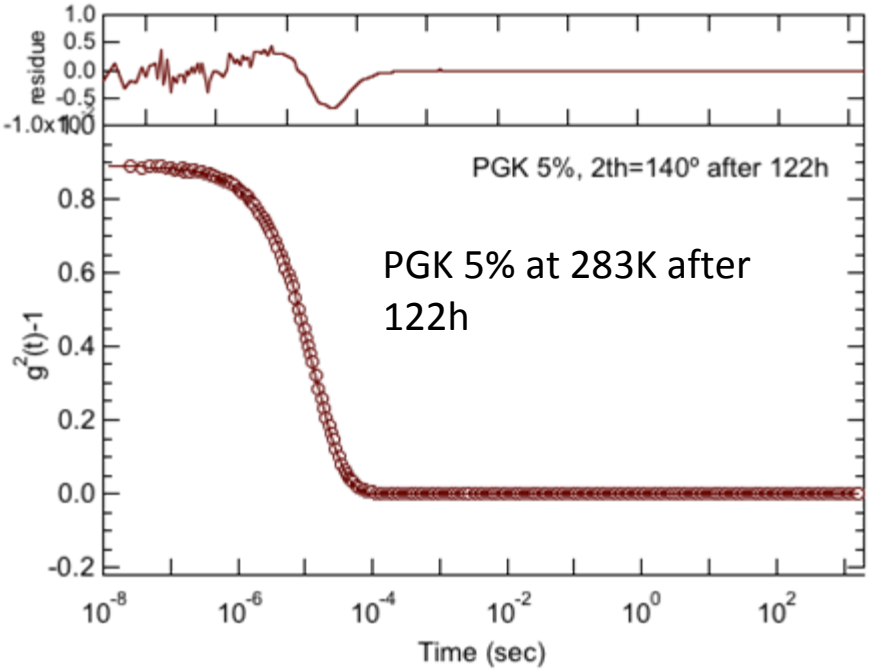
Crystal structure can describe the structure in solution for ADH.

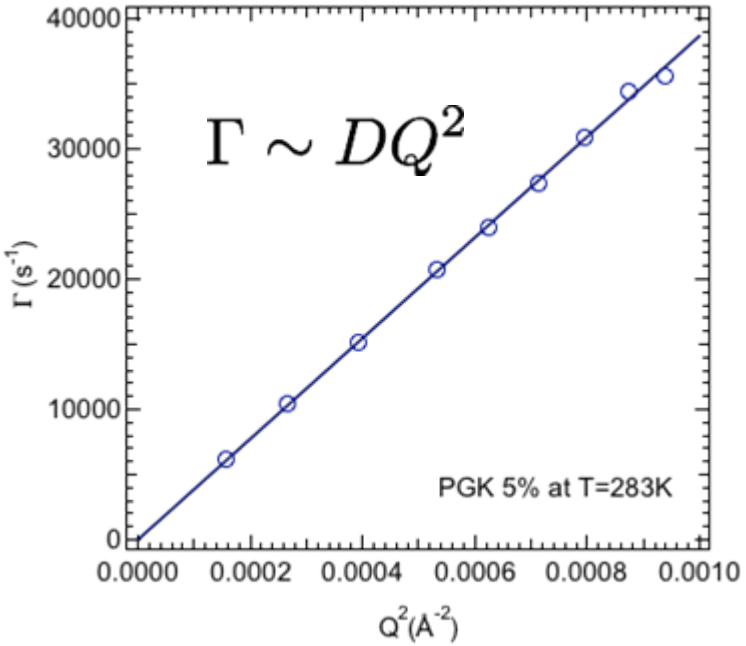
→ For rigid protein, the crystal structure might be used for the protein in solution.

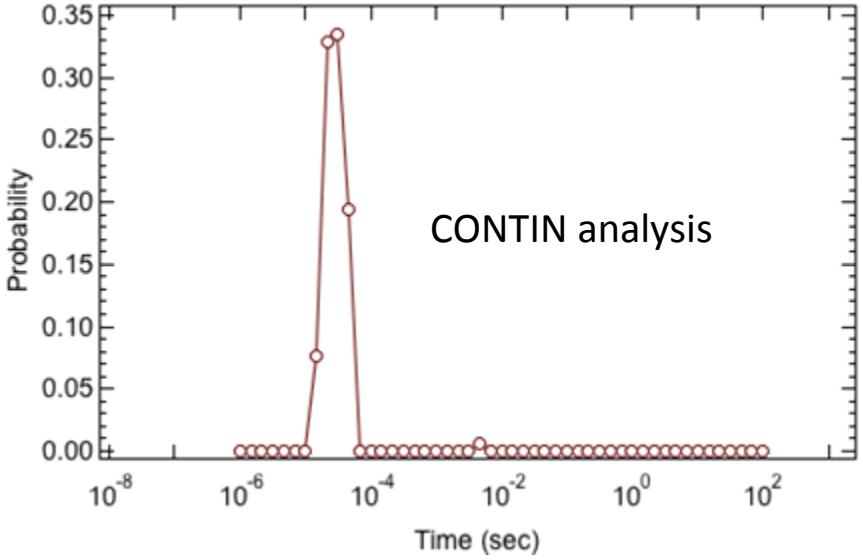
PGK is a quite flexible protein.

Dynamics: DLS and NSE

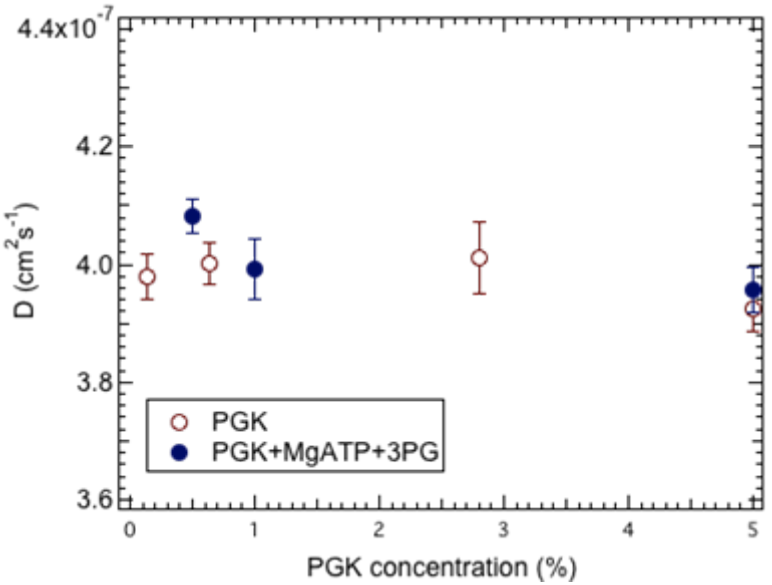
DLS


residue
PGK 5%, 2th=140° after 122h
PGK 5% at 283K after 122h
$g^2(t)-1$
Time (sec)


$\Gamma \sim DQ^2$
PGK 5% at T=283K
$\Gamma\ (s^{-1})$
$Q^2(Å^{-2})$


CONTIN analysis
Probability
Time (sec)

DLS for PGK and PGKsub

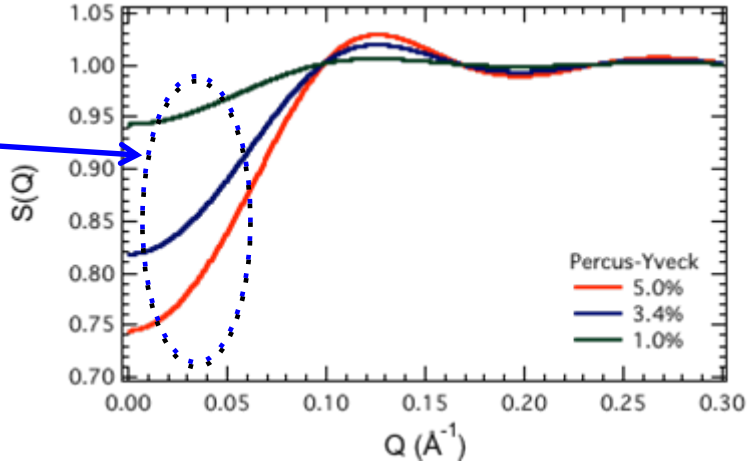


1. no difference between with and without substrates .

2. no concentration dependence of *D* in spite of large concentration dependence of *S(Q).*

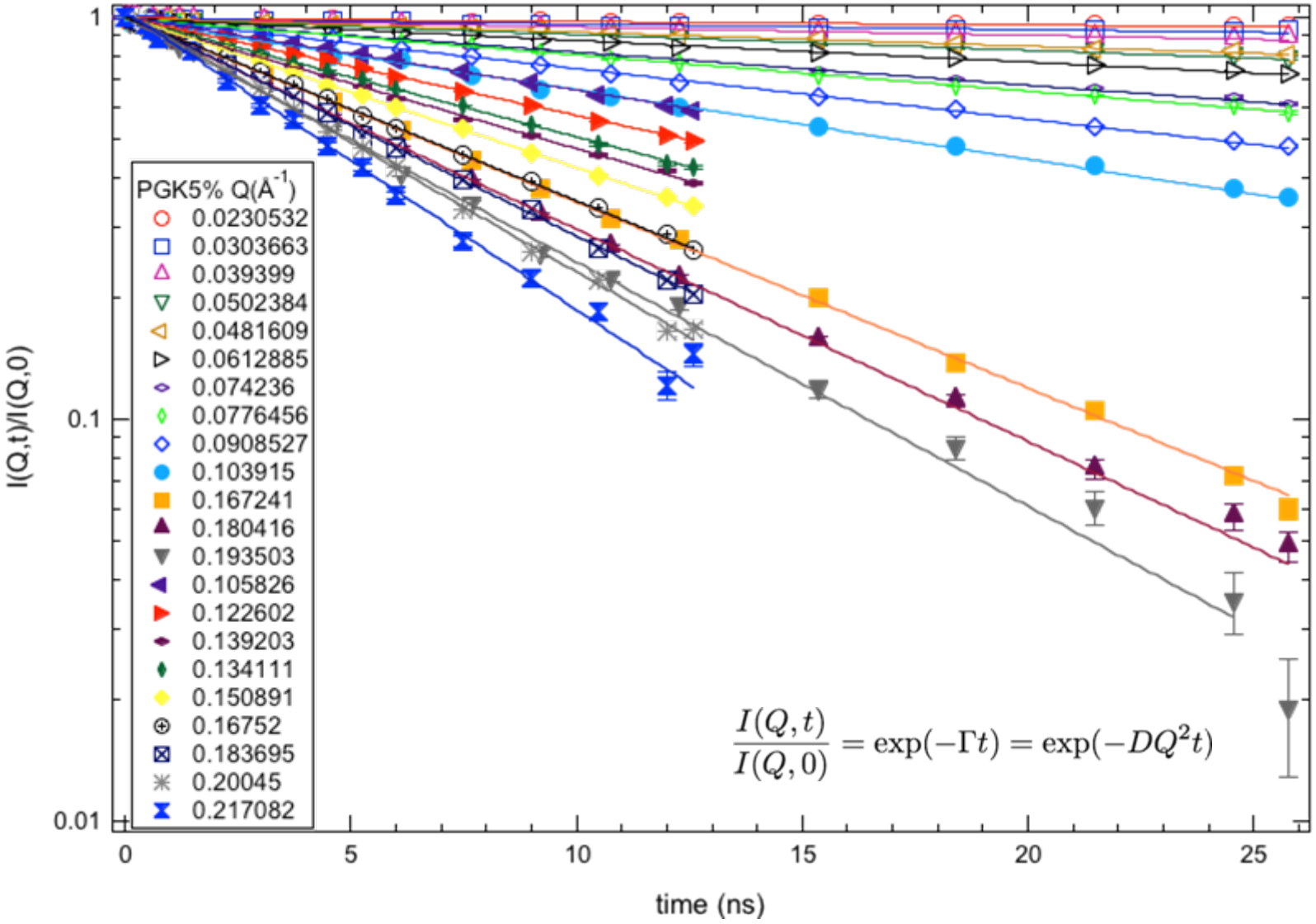
$$D(Q) = D_0 \frac{H(Q)}{S(Q)}$$

H(*Q*): hydrodynamic interparticle interactions

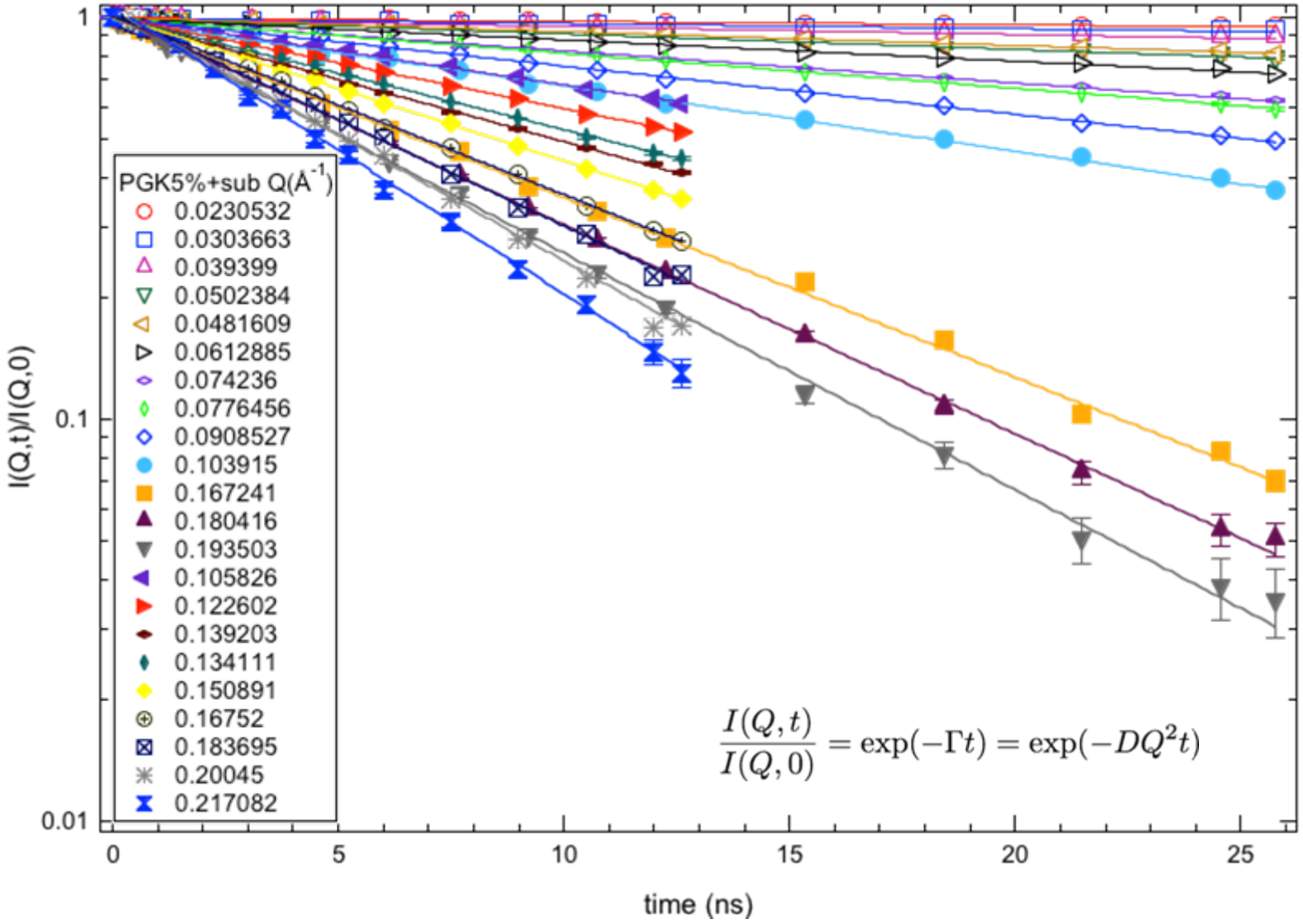


H(Q) cancel out the S(*Q*) effect at low *Q* region !!

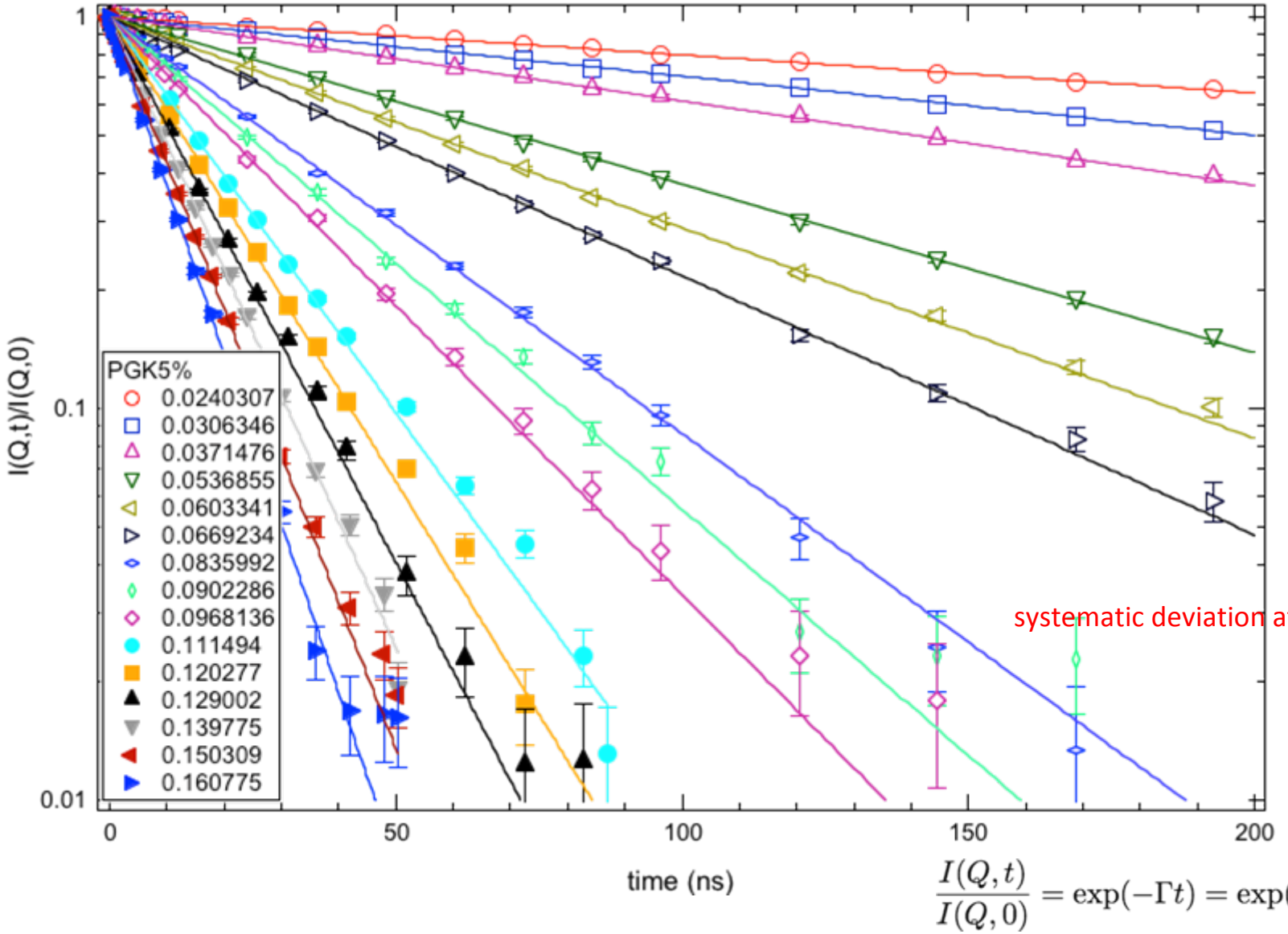
NSE: intermediate scattering function (short wavelengths)



NSE: intermediate scattering function (short wavelengths)

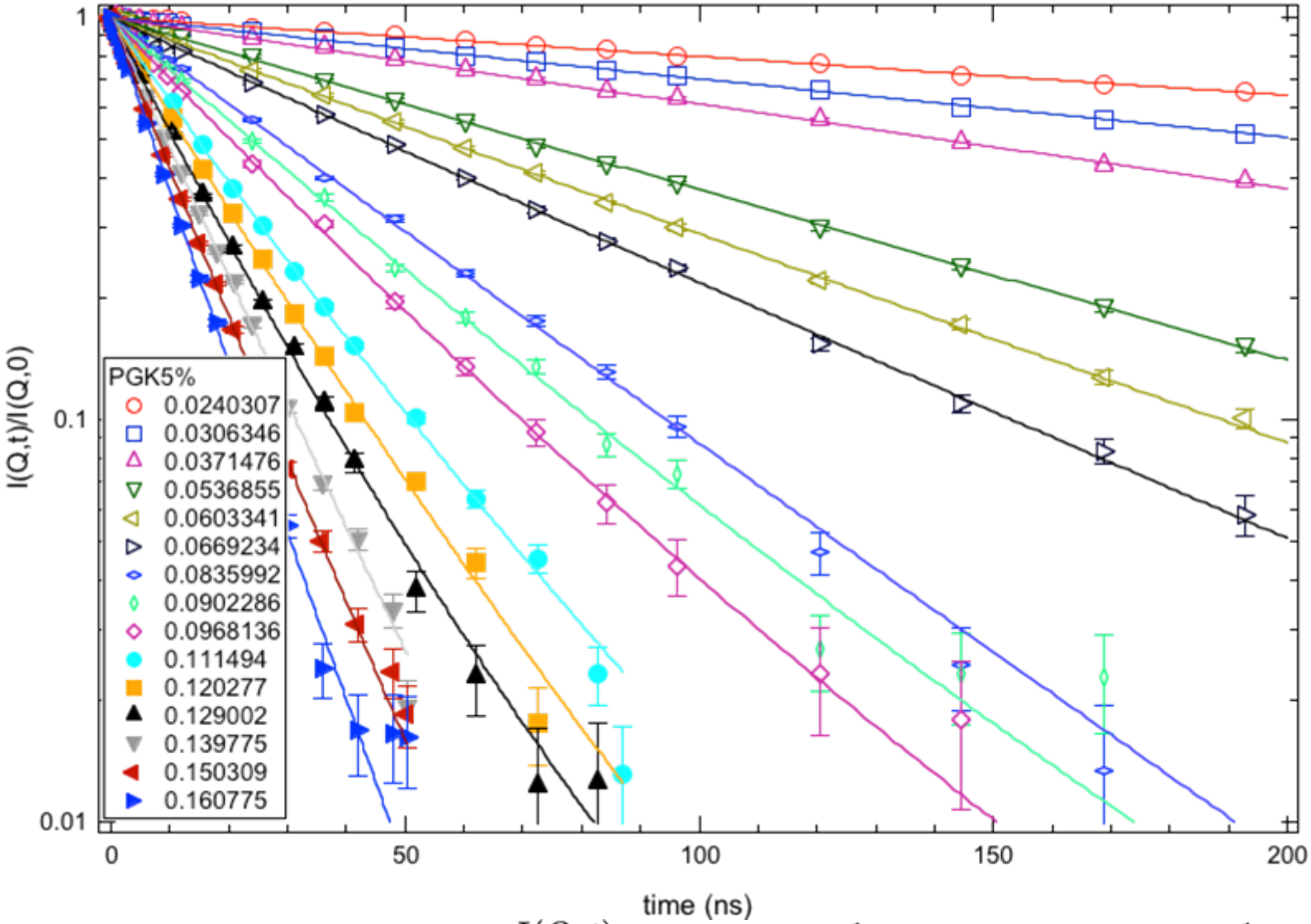


NSE: intermediate scattering function (long wavelengths)



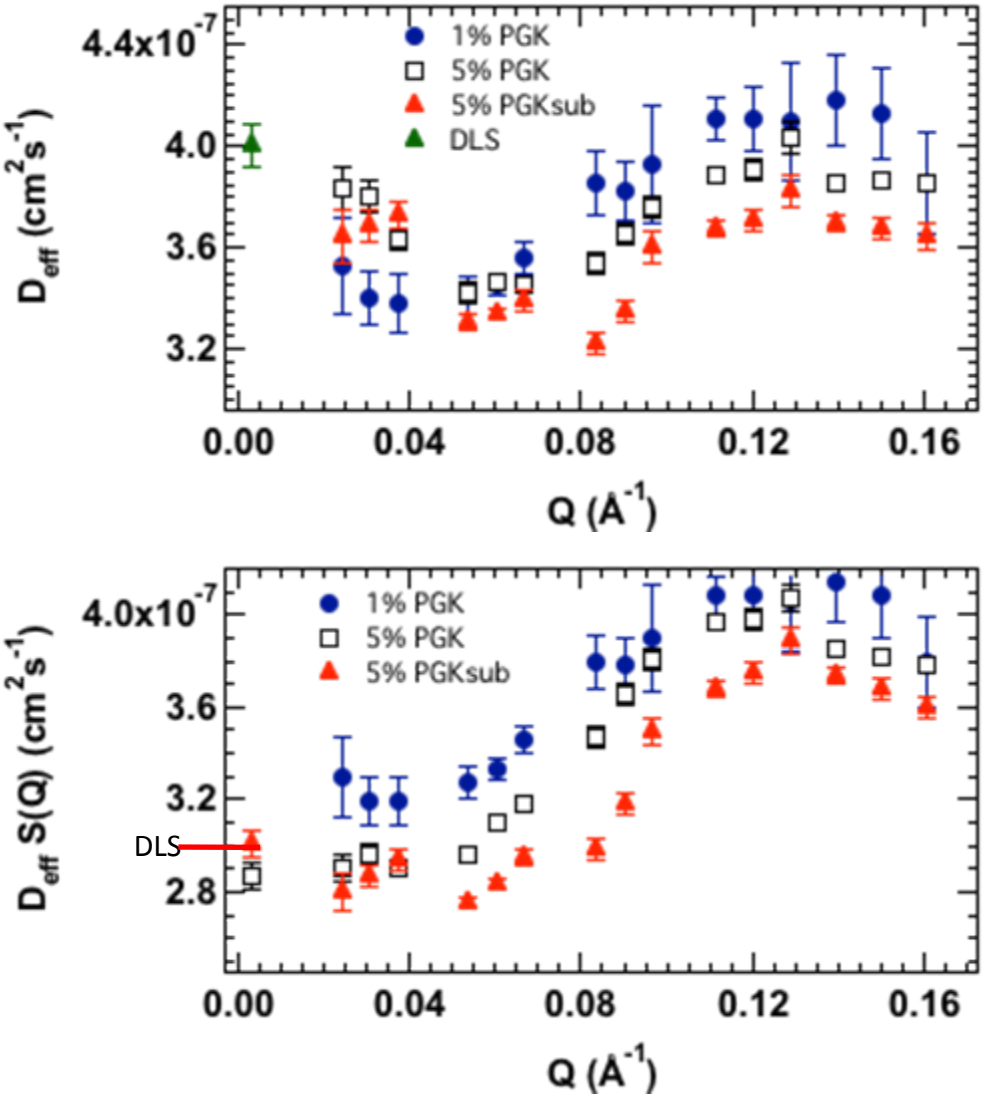
$$\frac{I(Q,t)}{I(Q,0)} = \exp(-\Gamma t) = \exp(-DQ^2 t)$$

NSE: intermediate scattering function (long wavelengths)



$$\frac{I(Q,t)}{I(Q,0)} = \exp(-\Gamma t + \frac{1}{2}K_2 t^2) = \exp(-DQ^2 t + \frac{1}{2}K_2 t^2)$$

NSE results



Deff*S(Q) was quite small compared to D from DLS!!

$$D_{eff}(Q) = D_0 \frac{H(Q)}{S(Q)}$$

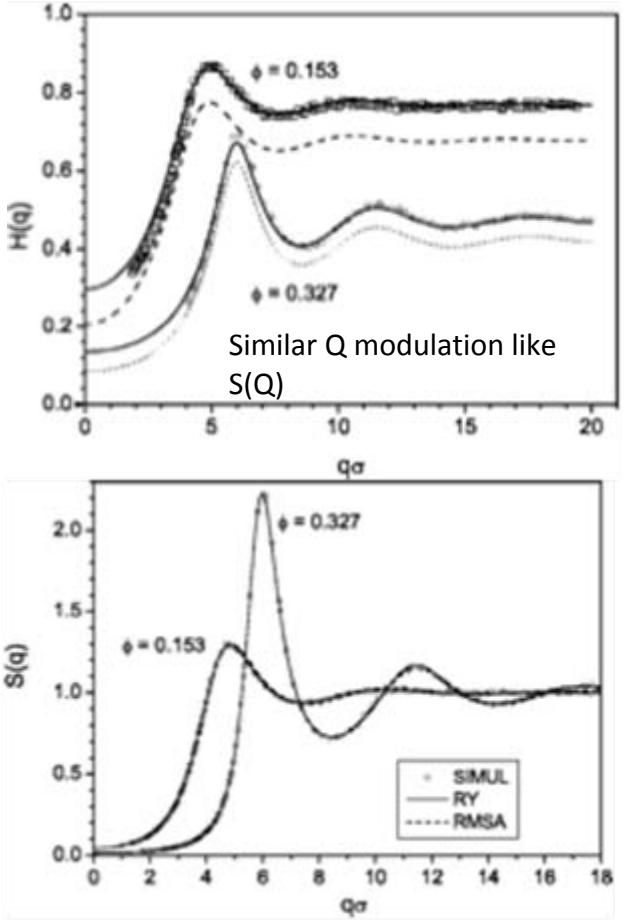
NSE results correction for H(Q)

Translational part

$$D_T(Q) = D_{T0}\frac{H_T(Q)}{S(Q)}$$

rotaitonal part

$$D_R = D_{R0}H_R$$



For hard sphere, high Q limit of H_T is given by

$$H_T = 1 - 1.831\Phi + ... < 1$$

For our volume fraction, $H_T(Q)$ was flat above Q=0.07Å^{-1}

$$H_T(Q) = H_{T,s} + H_{Td}(Q)$$

self distinct

Assumption

1. HT(Q) is constant due to the asymmetry shape of protein. (dominated by self part)

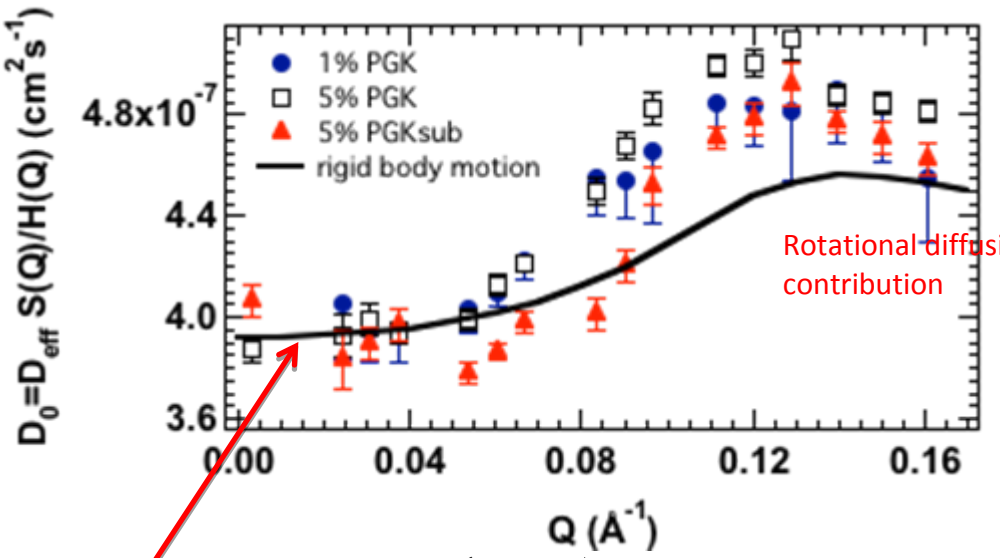
2. H_R=1 is concentration independent because of weaker coupling of HD to RD.

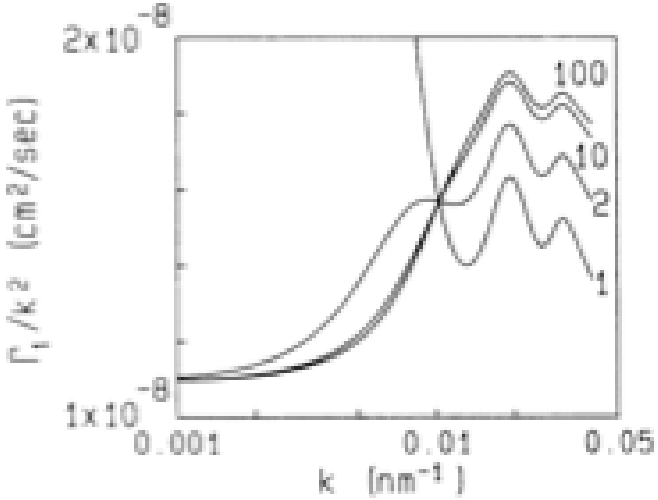
$$H_{T,5\%} = 0.74 \quad H_{T,1\%} = 0.81$$

$$H_R = 1.0$$

Detailed calculation is still missing!!

NSE results D_0 vs rigid body motion





Klein et al.

$$D_0(q) = \frac{1}{q^2}\frac{\sum_{j,k}\left\langle b_j e^{-i\mathbf{q}\mathbf{r}_j}\begin{pmatrix}\mathbf{q}\\ \mathbf{q}\times\mathbf{r}_j\end{pmatrix}\mathbf{D}\begin{pmatrix}\mathbf{q}\\ \mathbf{q}\times\mathbf{r}_k\end{pmatrix} b_k e^{i\mathbf{q}\mathbf{r}_k}\right\rangle}{\sum_{j,k}\left\langle b_j e^{-i\mathbf{q}\mathbf{r}_j} b_k e^{i\mathbf{q}\mathbf{r}_k}\right\rangle} = F(Q)$$

diffusion tensor

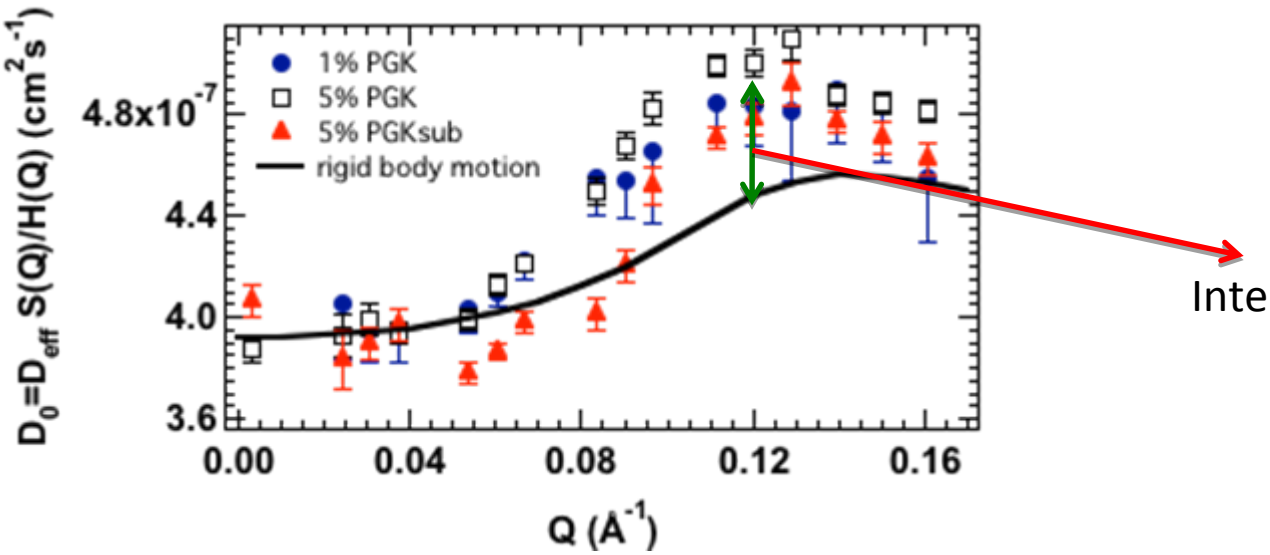
$$D_T = 3.94\times10^{-7} cm^2 s^{-1}$$
$$D_R = 3\times10^{6} s^{-1}$$

$$\boldsymbol{D} = \begin{pmatrix} \boldsymbol{D_T} & \boldsymbol{D_{TR}} \\ \boldsymbol{D_{RT}} & \boldsymbol{D_R} \end{pmatrix}$$

6X6 tensor

HYDROPRO :PDB file is used for calculation

NSE results Internal dynamics



How to describe the Internal dynamics?

full calculation

Relaxation time of internal dynamics

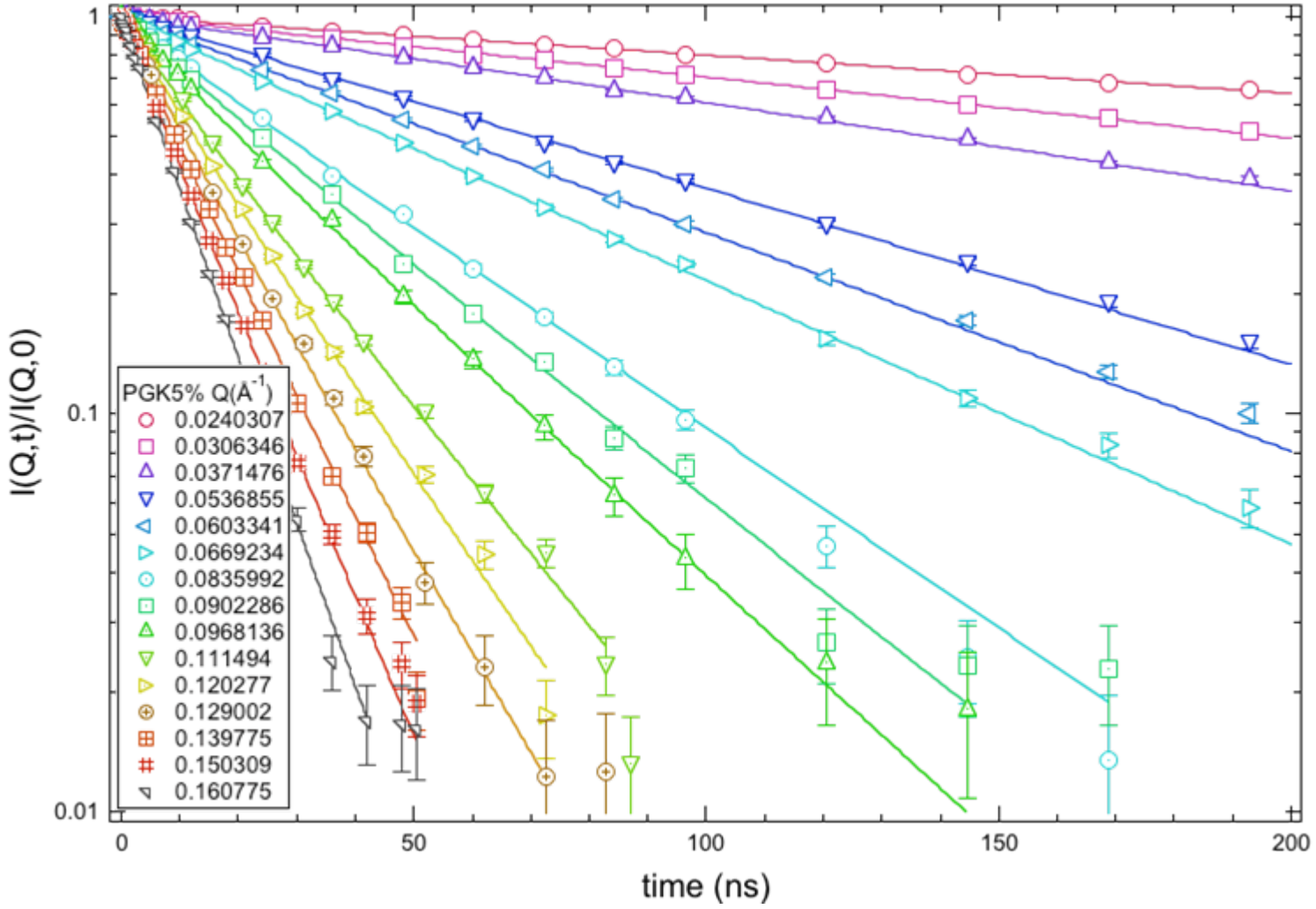
refer to Klein et al.

$$\frac{I(Q,t)}{I(Q,t=0)} = [(1-A(Q)) + A(Q)\exp(-\Gamma t)] \times \exp(-Q^2 D_t \frac{H_T}{S(Q)} t) \left(\sum_{l=0}^{\infty} S_l(Q) \exp(-l(l+1) D_r H_R t) \right) / \sum_{l=0}^{\infty} S_l(Q)$$

Amplitude of internal dynamics

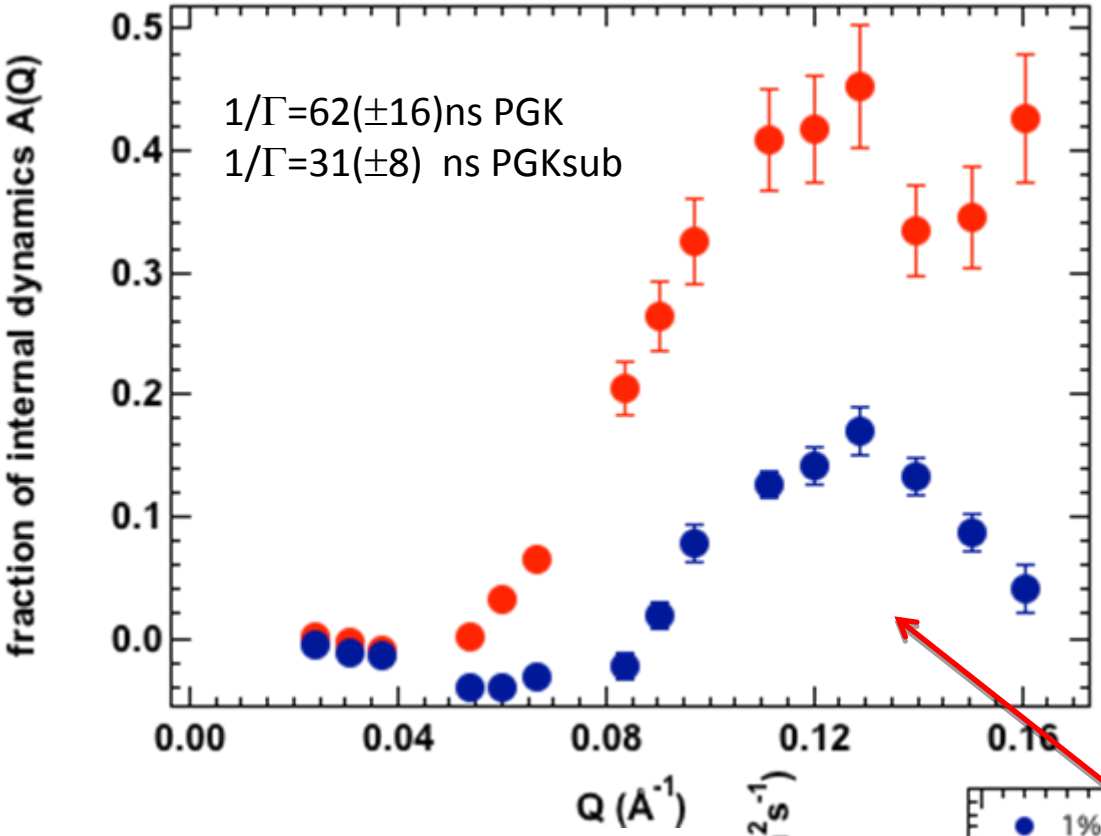
$$\text{with } S_l(Q) = \sum_m \left| \sum_i b_i j_l(Qr_i) Y_{l,m}(\Omega_i) \right|^2$$

NSE results fit by full calculation

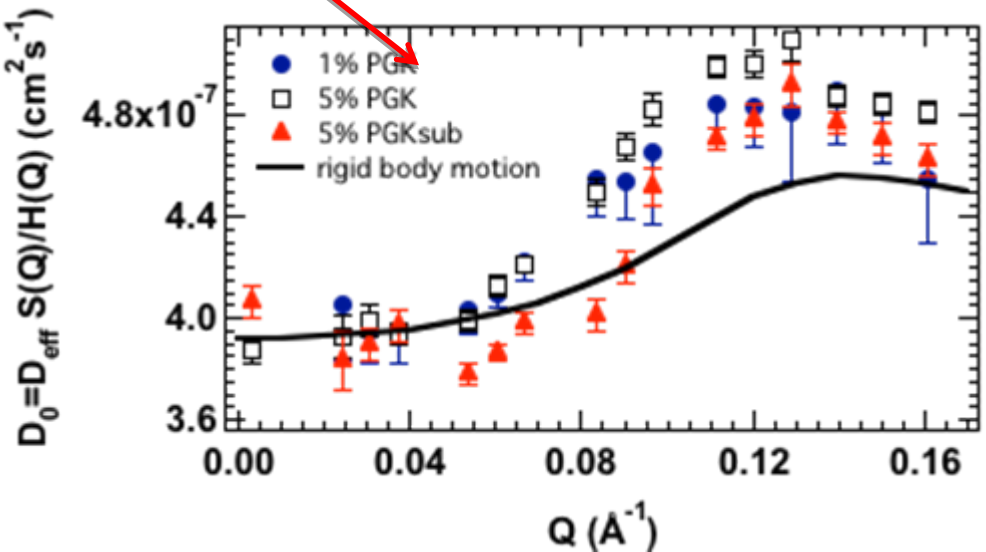


Well fitted by full calculation!!

Nature of internal dynamics



Same tendency



Nature of internal dynamics NM approaches

First-order approximation for small displacements along NM

$$I(Q,t) \propto I(Q) + \sum_{\alpha} a_{\alpha} e^{-\lambda_{\alpha} t} P_{\alpha}(Q)$$

Lambda is RR of the overdamped mode

Eigenvector of mode a

$$P_{\alpha}(Q) = \left\langle \sum_{k,l}^{N} b_k b_l \exp(i\boldsymbol{Q}(\boldsymbol{r_k} - \boldsymbol{r_l})) \cdot (\boldsymbol{Q} \cdot \boldsymbol{e_k^a})(\boldsymbol{Q} \cdot \boldsymbol{e_l^a}) \right\rangle$$

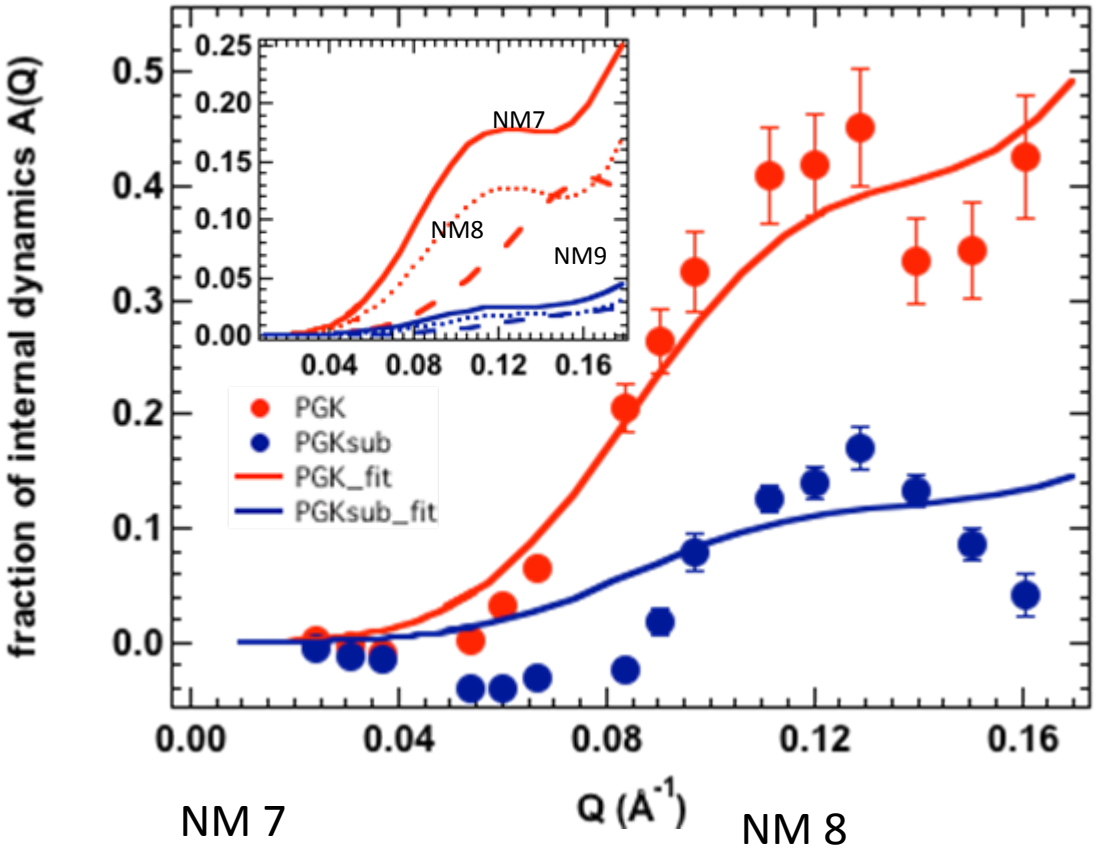
Intermediate scattering function $\frac{I(Q,t)}{I(Q,t=0)}$

$$a\hat{P}_{\alpha}(Q)e^{-\lambda_{\alpha} t} = \frac{\sum_{\alpha} a_{\alpha} P_{\alpha}(Q) e^{-\lambda_{\alpha} t}}{I(Q) + \sum_{\alpha} a_{\alpha} P_{\alpha}(Q)}$$

Contribution of NM

$$\hat{P}_{\alpha}(Q) = \frac{\sum_{\alpha} a_{\alpha} P_{\alpha}(Q)}{I(Q) + \sum_{\alpha} a_{\alpha} P_{\alpha}(Q)}$$

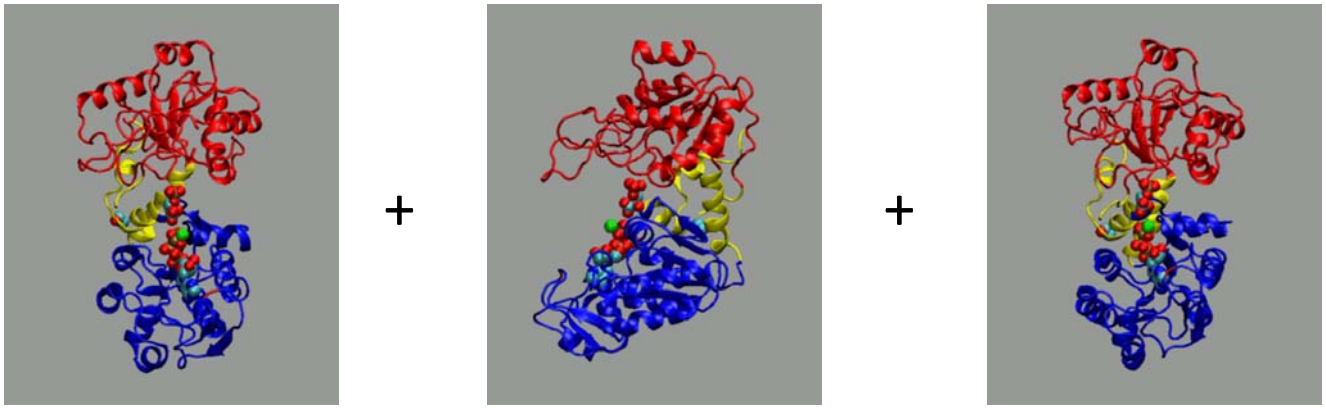
Nature of internal dynamics NM approaches



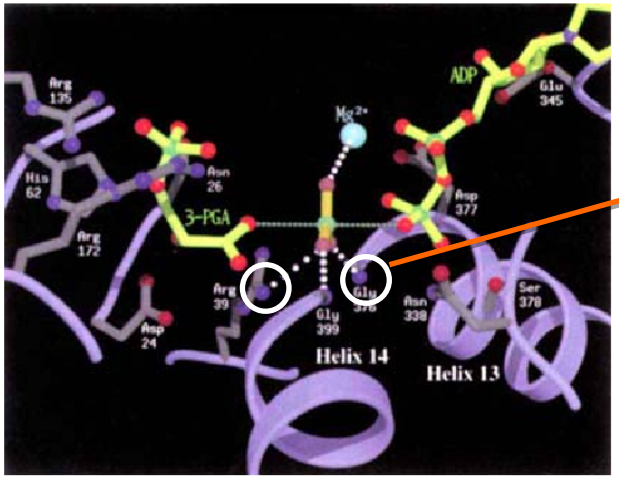
Mean atomic displacement

PGK=9.7±1Å,
PGKsub=4.5±0.9Å

NM 7 + NM 8 + NM 9



Relation to dynamics



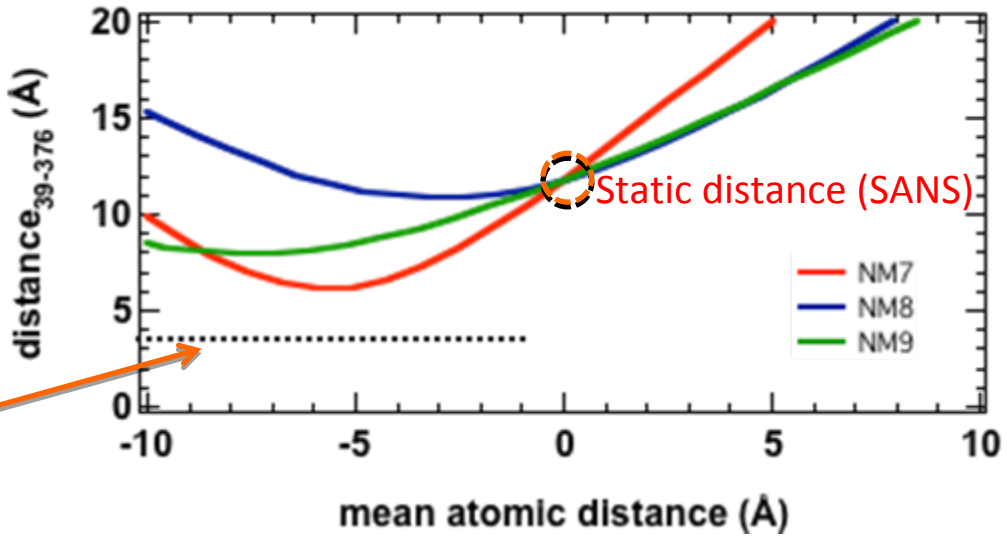


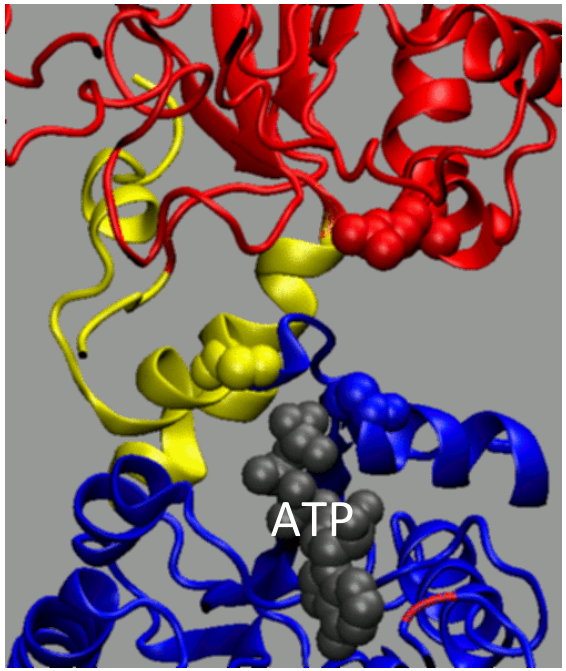
Figure 5 The active site of *T. brucei* PGK with the transition state modelled as a

$distance_{38\text{-}371}$=3.5Å (±2.5Å resolution)

Static distance (SANS) is not enough
to reach catalytic configuration

→ ns dynamics is already ready for
commencement of reaction!!

ca. turnover=350s^{-1}



Arg 38

Gly 371

mode 7

Summary

Dynamics is necessary for understanding the structure and the functionality.