

**Nanoscale coupled protein domain  
motion revealed by neutron spin echo  
spectroscopy**

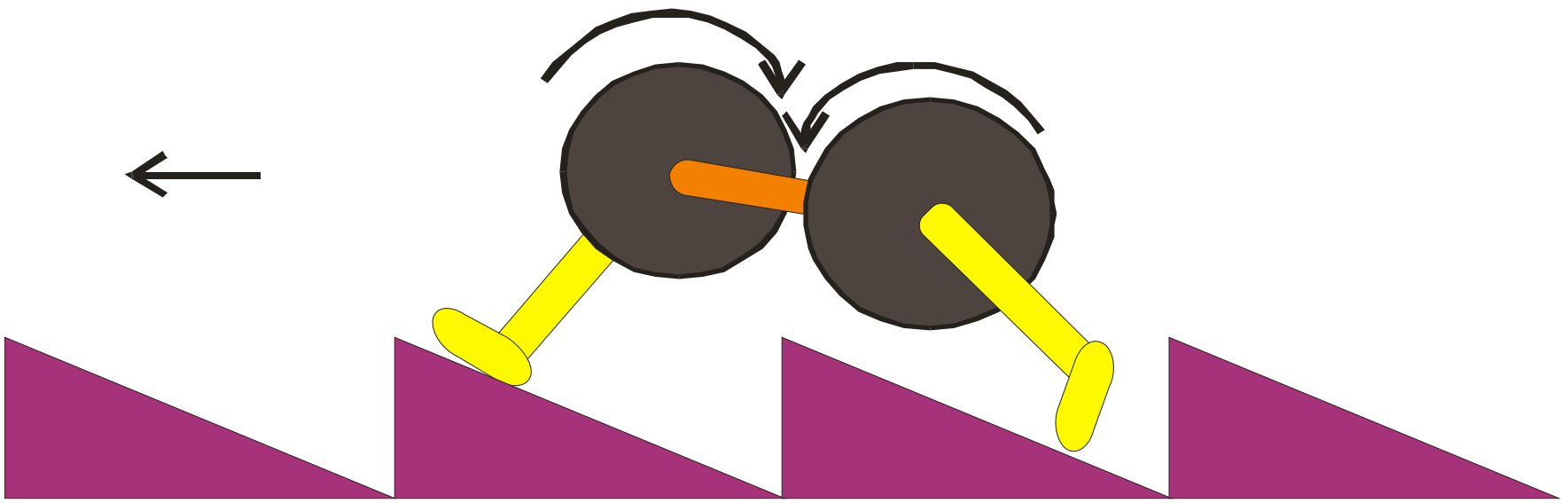
**David J E Callaway**

**New York University School of Medicine**

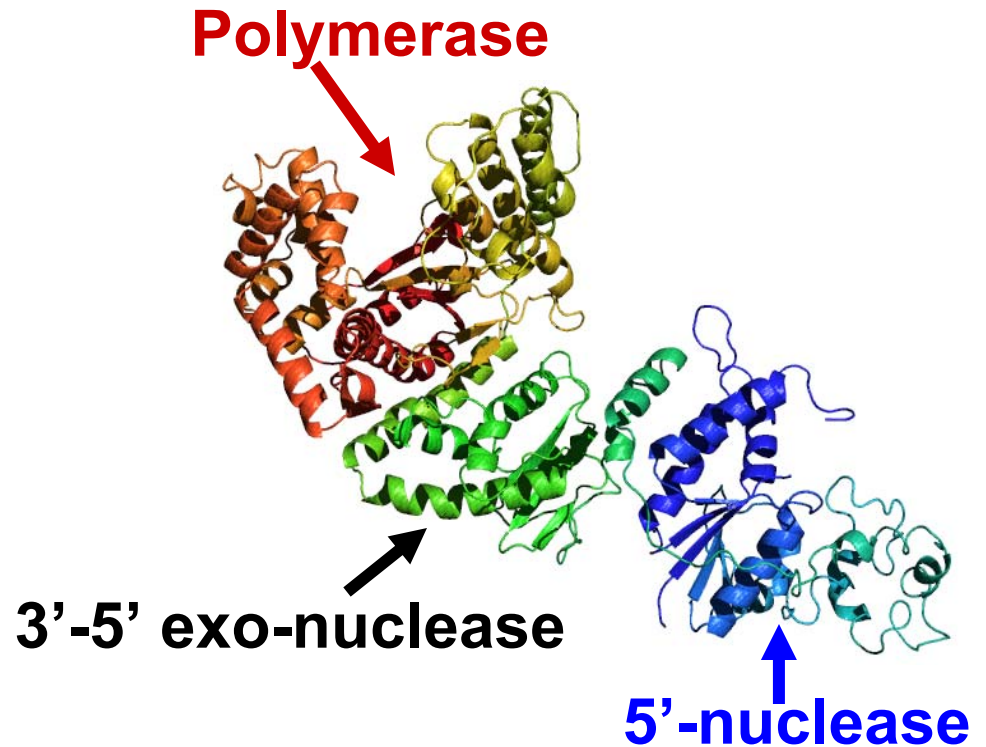
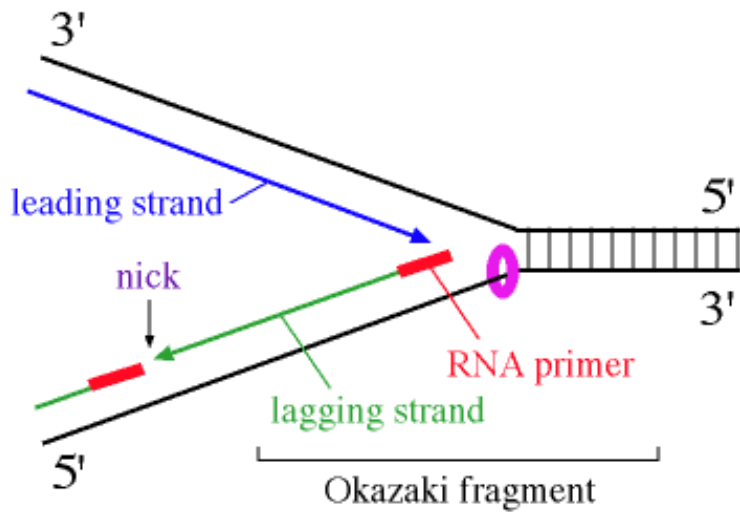
**David.Callaway@nyu.edu**

# Proteins are molecular machines

- Function is determined by both structure and **dynamics**
- Essential to probe **nanosecond dynamics**
- Engineer new **nanomachines!**

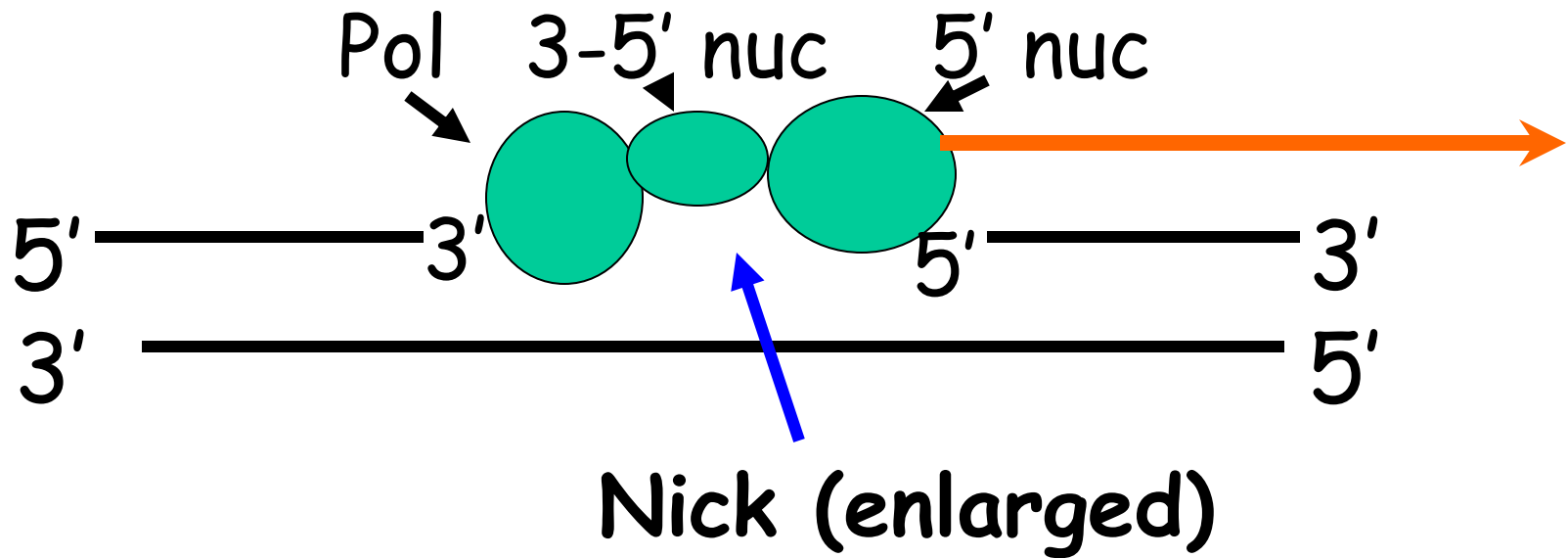


# DNA polymerase I: three enzymes in one protein



- Lagging strand DNA synthesis
- Strand replacement

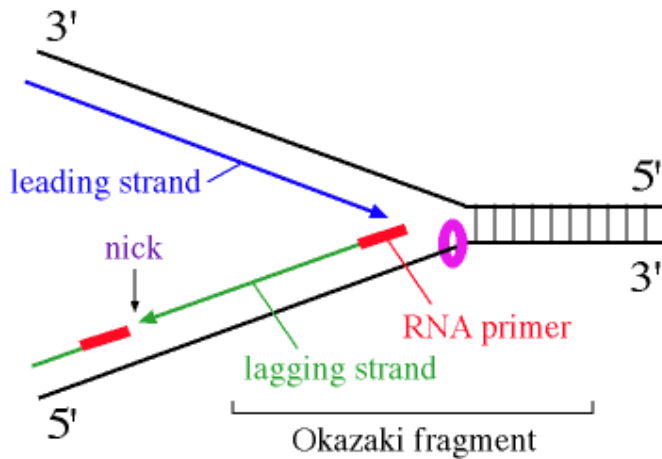
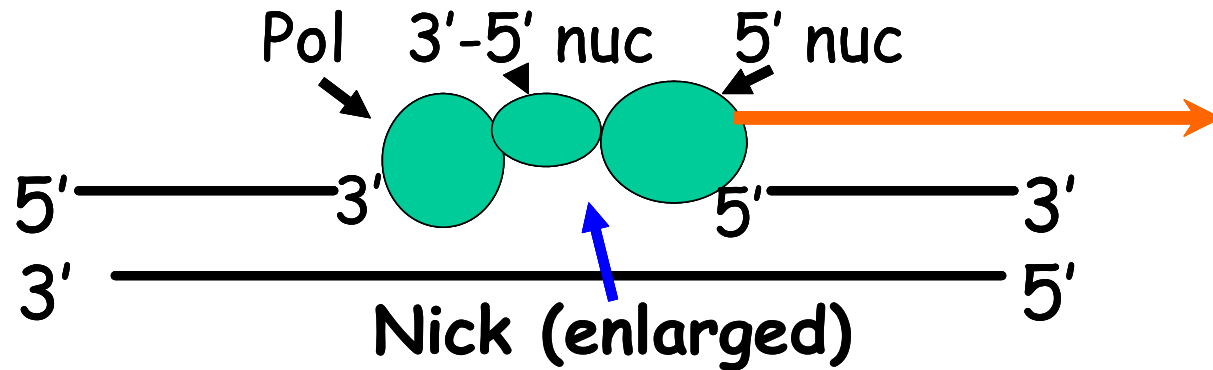
# Coordinated domain motion over 70 Å



In lagging strand synthesis pol and 5' nuc coordinate to leave only a nick

Nuclease is 10 times faster if polymerization occurs (Kornberg, Reich).

# Highly coordinated activities of different domains.



**The original hypothesis:**  
**Pol and 5' nuc are brought to juxtaposition**

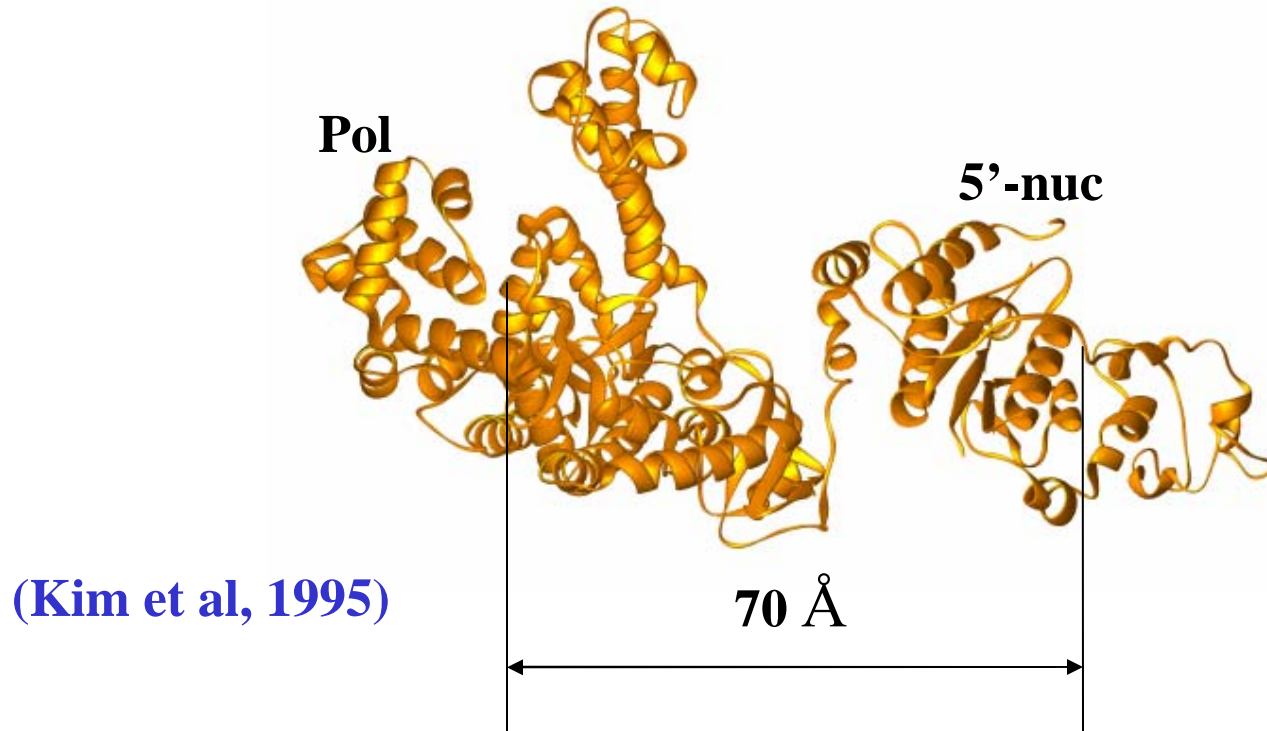
DNA replication  
2<sup>nd</sup> edition

A. Kornberg and T. Baker

# Taq polymerase $\approx$ Pol I

## Coupled domain function over 70 Å

---



Pol and 5'-nuc stimulate each other.  
(Kornberg, Reich, Ma...).

Active sites of the two domains are 70 Å apart

# Proteins **mix and match** modular domains (design our own **nanomachinery!**)

Lander, et al., (2001).  
Nature 409, 860-921

Initial sequencing and analysis of the human genome.

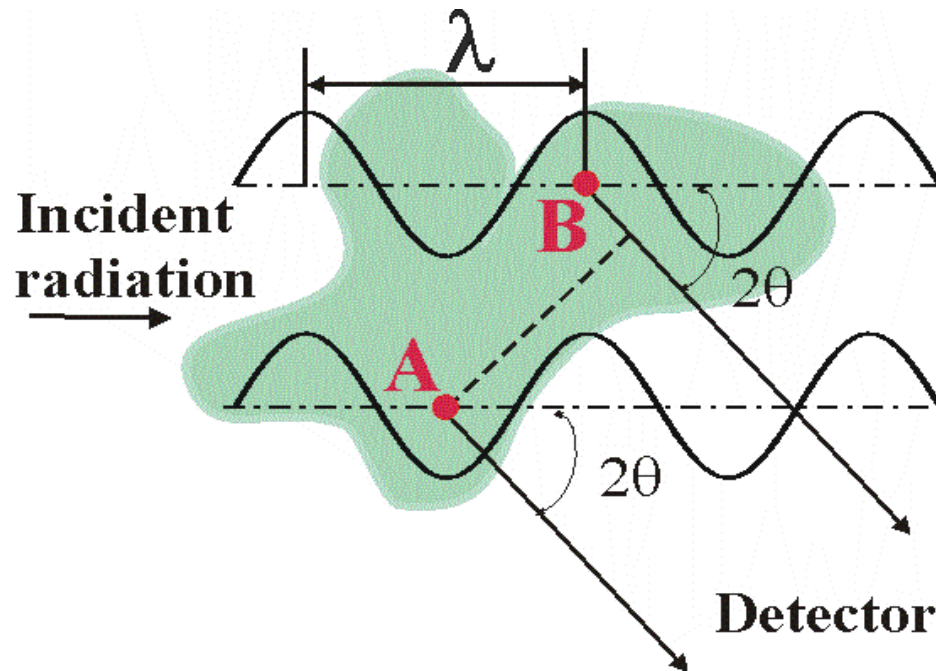
Venter, et al. (2001).  
Science 291, 1304.

The sequence of the human genome

Evolution → We **hypothesize** dynamic modular control

**Modular domains**  $\xrightarrow{\text{Different combinations}}$  **New proteins**  
**New functions**

# Small angle X-ray (SAXS) and neutron scattering



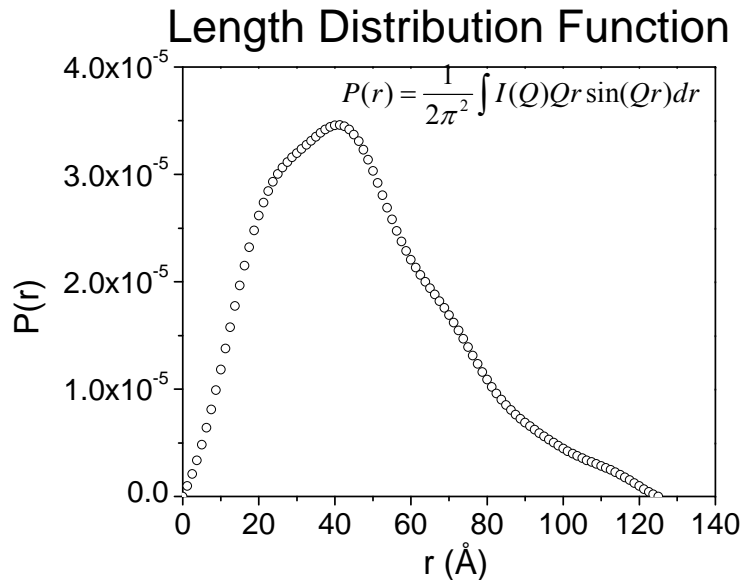
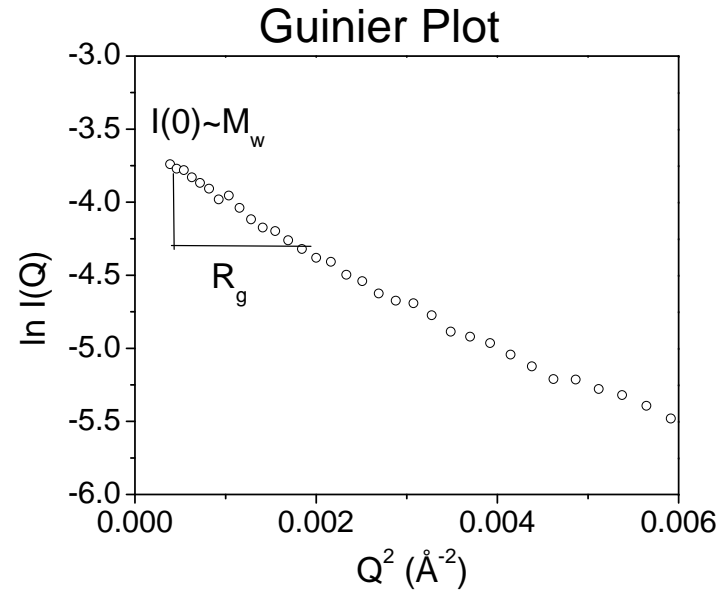
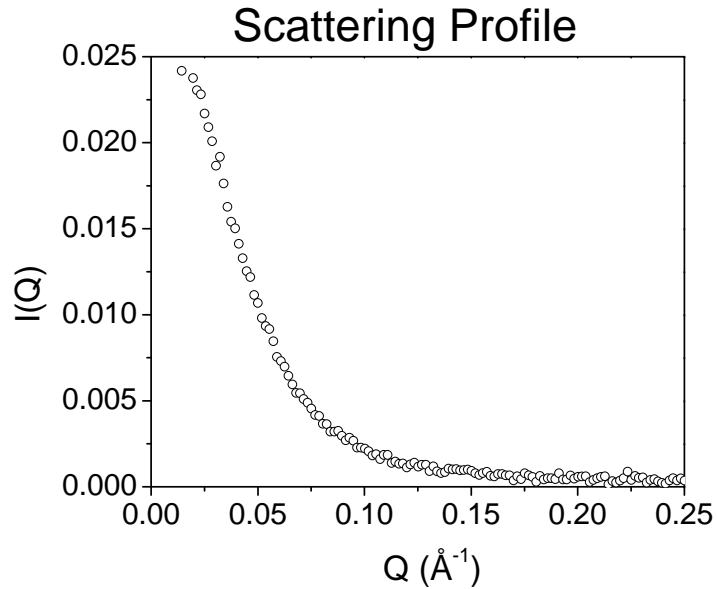
**Static structure factor:**

$$I(Q) = \langle A(\vec{Q}) \cdot A(\vec{Q})^* \rangle = b^2 \sum_i^N \sum_i^N \langle \exp(-iQr_{ij}) \rangle$$

**Momentum transfer  
or scattering vector:**

$$Q = \frac{4\pi \sin(\theta/2)}{\lambda}$$

# Solution small angle X-ray and neutron scattering



**Length scales measured: 5-500 Å**

**Size, shape, oligomer states,  
stoichiometry,  
and molecular envelope of protein  
complexes**

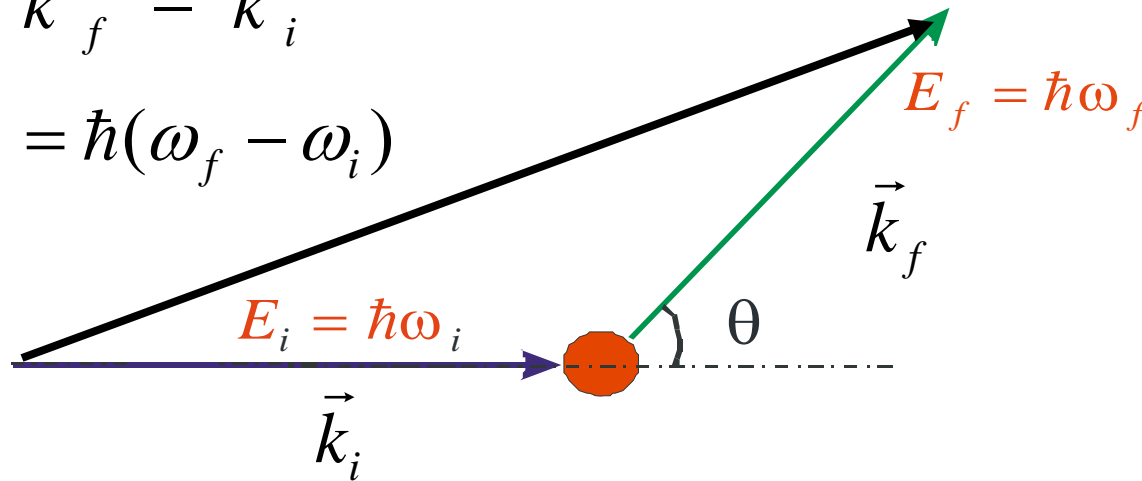
# Neutron scattering ideal for studying nanoscale dynamics of a protein

---

$$\vec{Q} = \vec{k}_f - \vec{k}_i$$

$$\Delta E = E_f - E_i = \hbar(\omega_f - \omega_i)$$

$$\omega \sim 1/\lambda^2$$

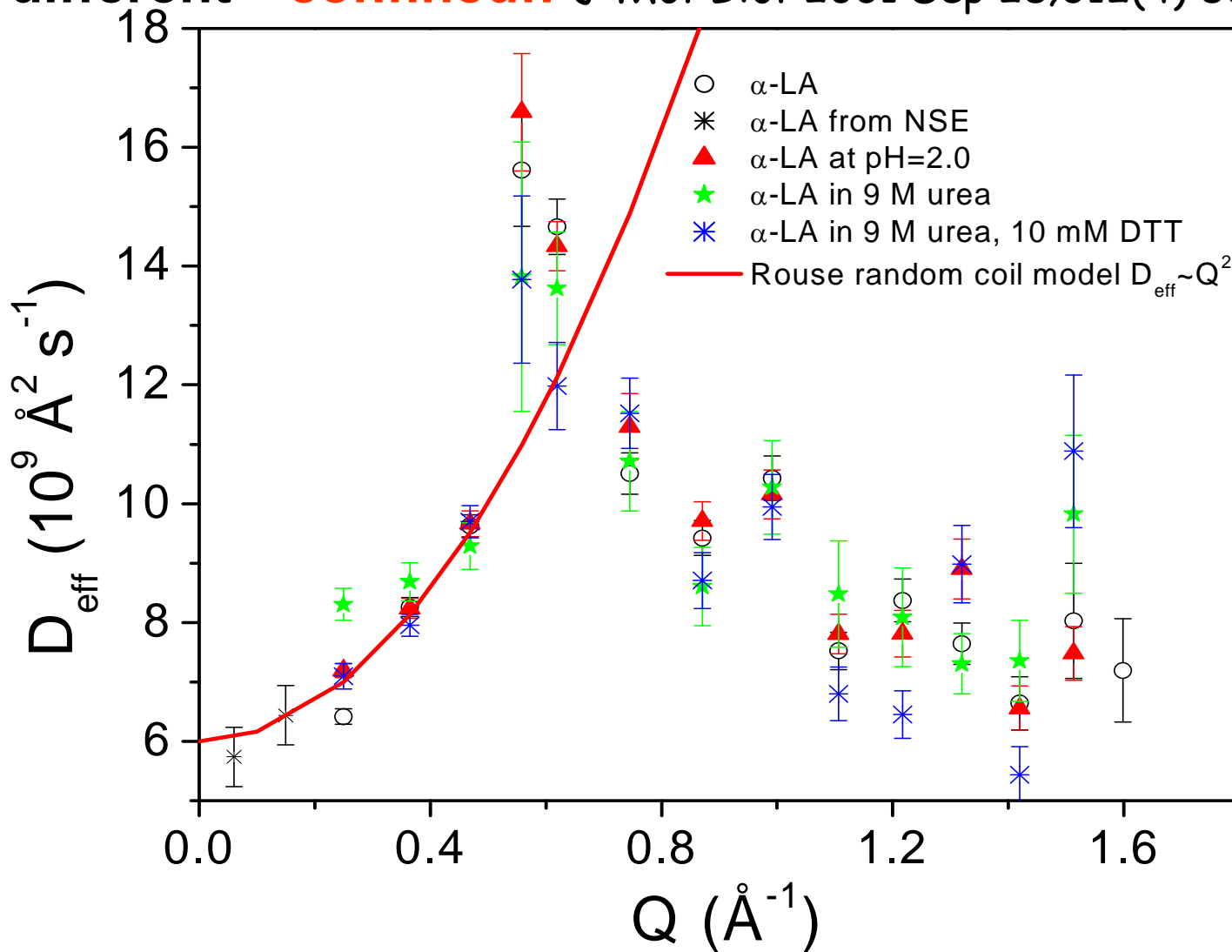


Neutrons change both **momentum** and **energy**

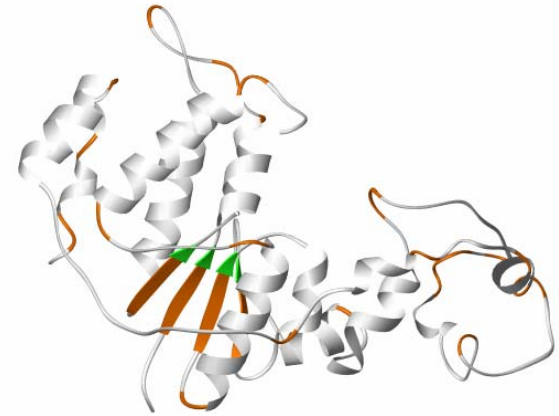
Neutron Spin Echo yields  $\Delta E/E \sim 10^{-5}$

# Nanosecond dynamics of **proteins** and **polymers** very

different—**confined!!** J Mol Biol 2001 Sep 28;312(4):865-73.



# Structural sensing by 5'-nuclease domain



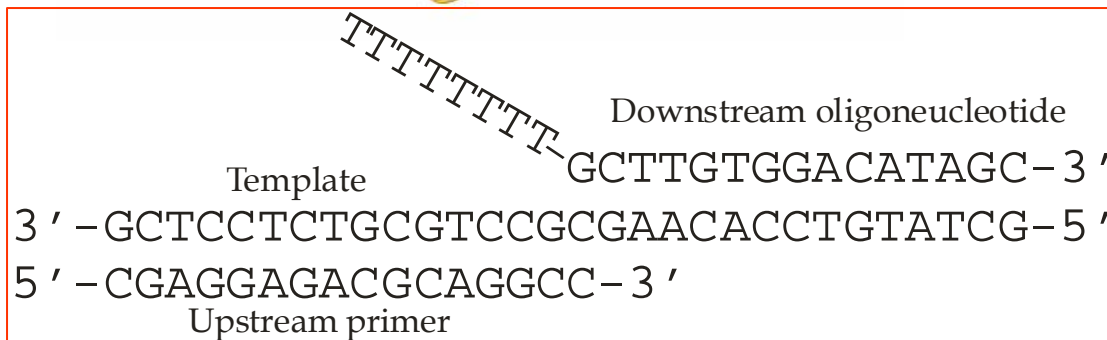
5' nuclease of Taq polymerase

**“Overlap flap” structure:**



Lyamichev, 1999

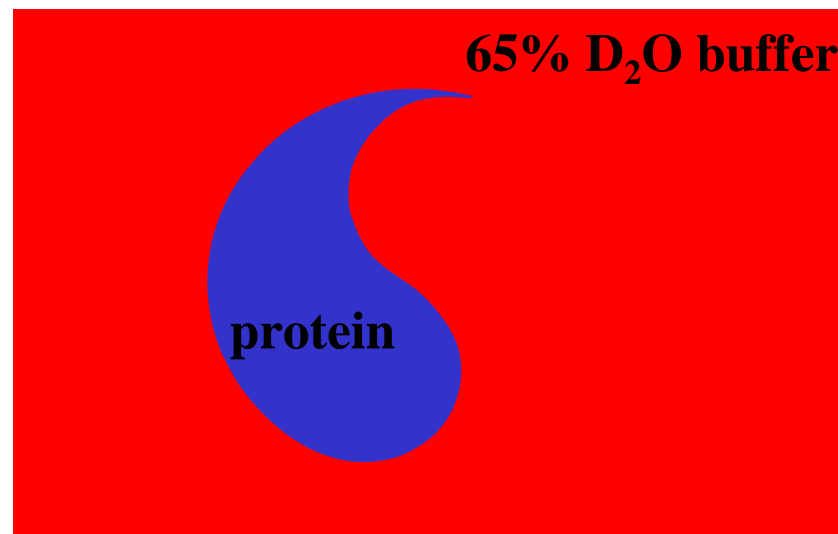
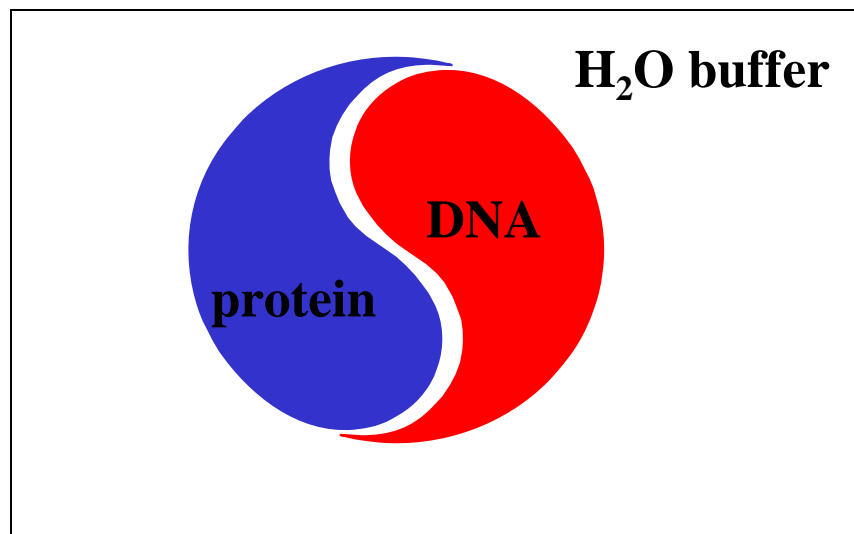
# Structural sensing by 5'-nuclease domain and domain-domain communication



**Will the “overlap flap DNA” substrate induce a conformation change in Taq polymerase?**

# Small angle neutron scattering study of Taq polymerase-DNA substrate complex

Scattering from multi-component system:  
contrast matching one component!!

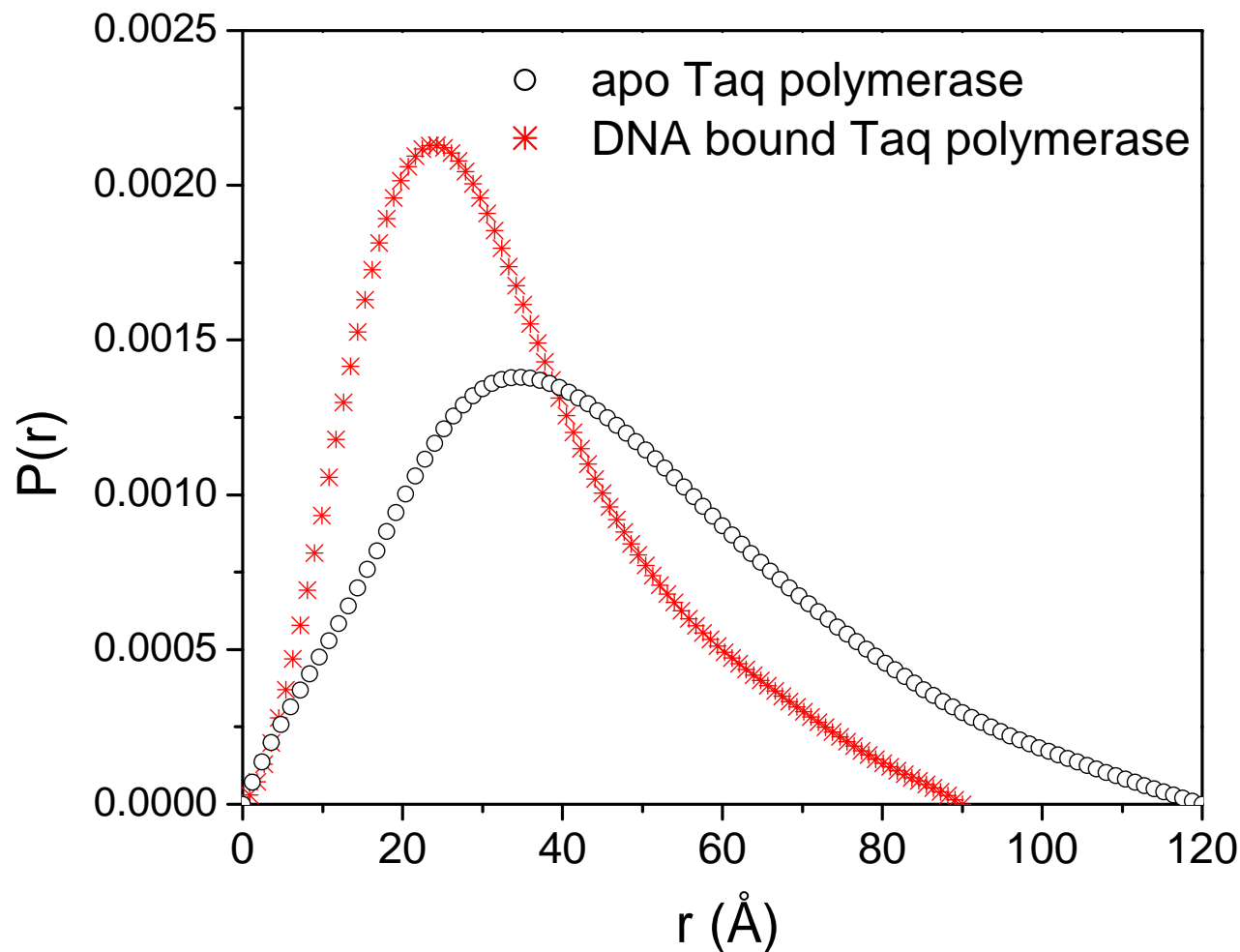


$$I(Q) = (\rho_p - \rho_s)^2 I'_p(Q) + (\rho_p - \rho_s)(\rho_L - \rho_s) I'_{pL}(Q) + (\rho_L - \rho_s)^2 I'_L(Q)$$

In 65% D<sub>2</sub>O buffer, the scattering length density of the buffer matches that of the DNA. DNA is “invisible” to neutrons.

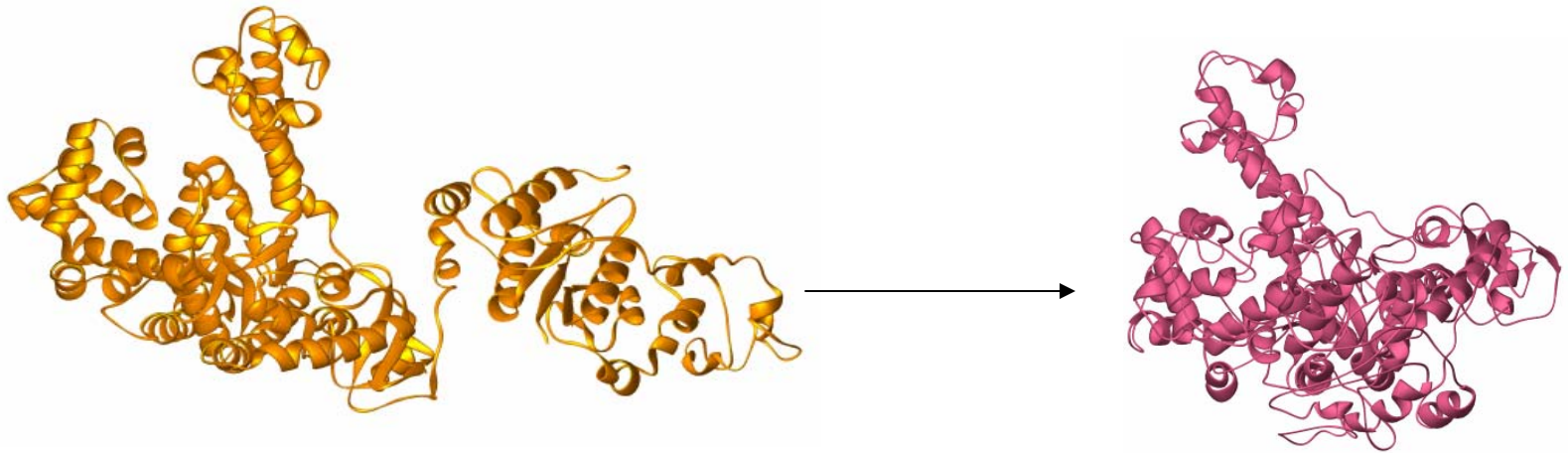
# A structure-specific DNA substrate induced conformation change in Taq polymerase

---



# The structure-specific DNA substrate brings the active sites into close proximity

---

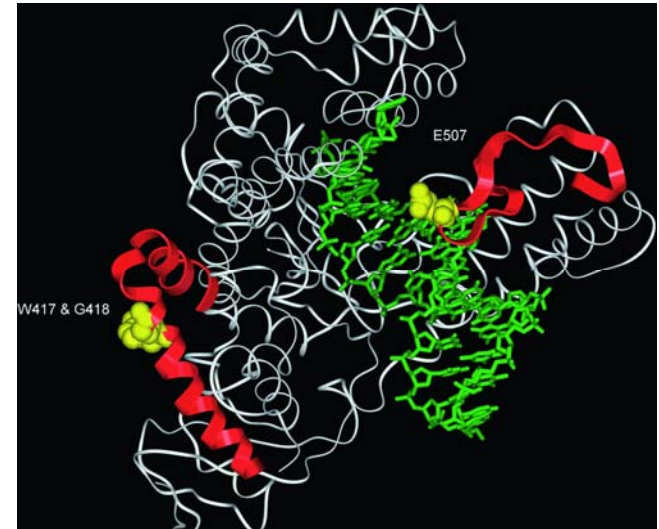
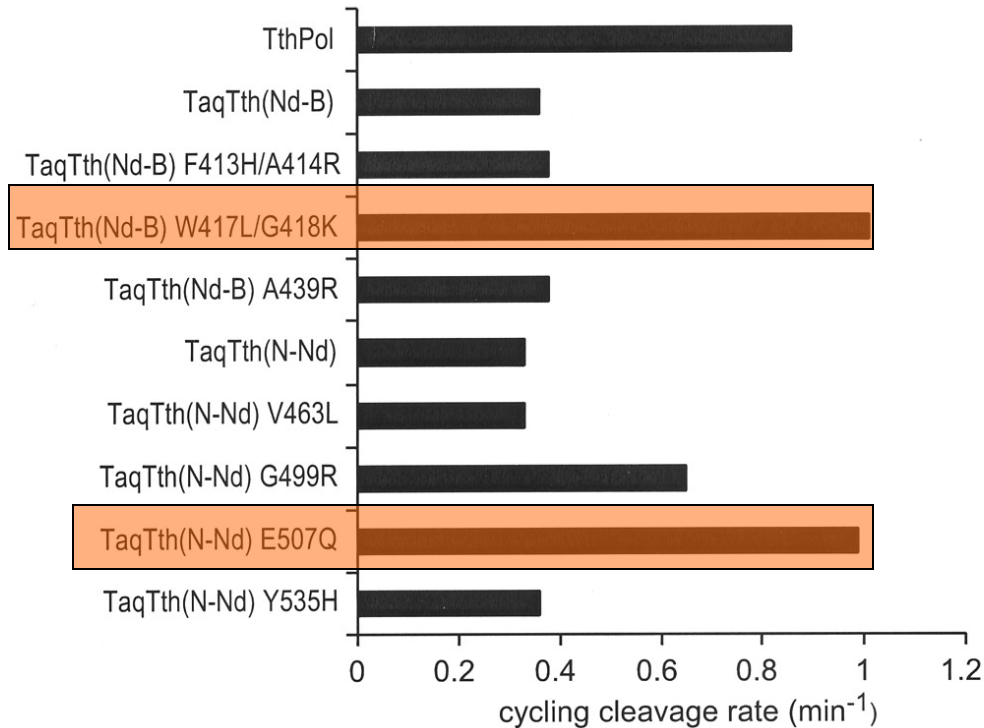


**Apo form**

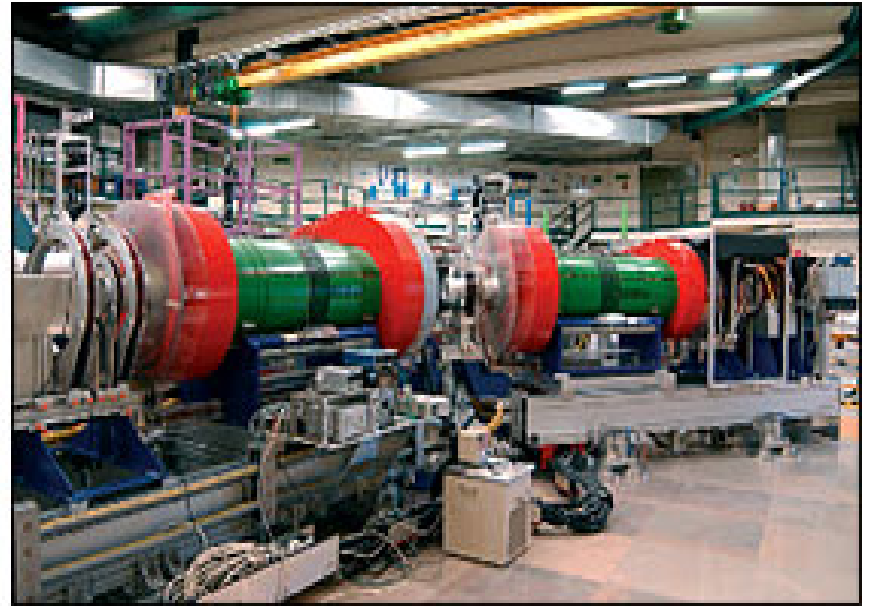
**DNA substrate  
bound form:  
two active sites  
are close**

J Biol Chem. 2004 Sep 10;279(37):39146-54.

# Domain-domain communication: mutations in the polymerase domain region affect the 5' nuclease activity



# Nanosecond Protein Domain Dynamics



Initiating events for global conformational changes (Taq) as they unfold in time (enzymatic transition)

**Neutron spin echo** is a promising and novel technique

**Larmor precession** to obtain unprecedented accuracy

# Essential point of NSE

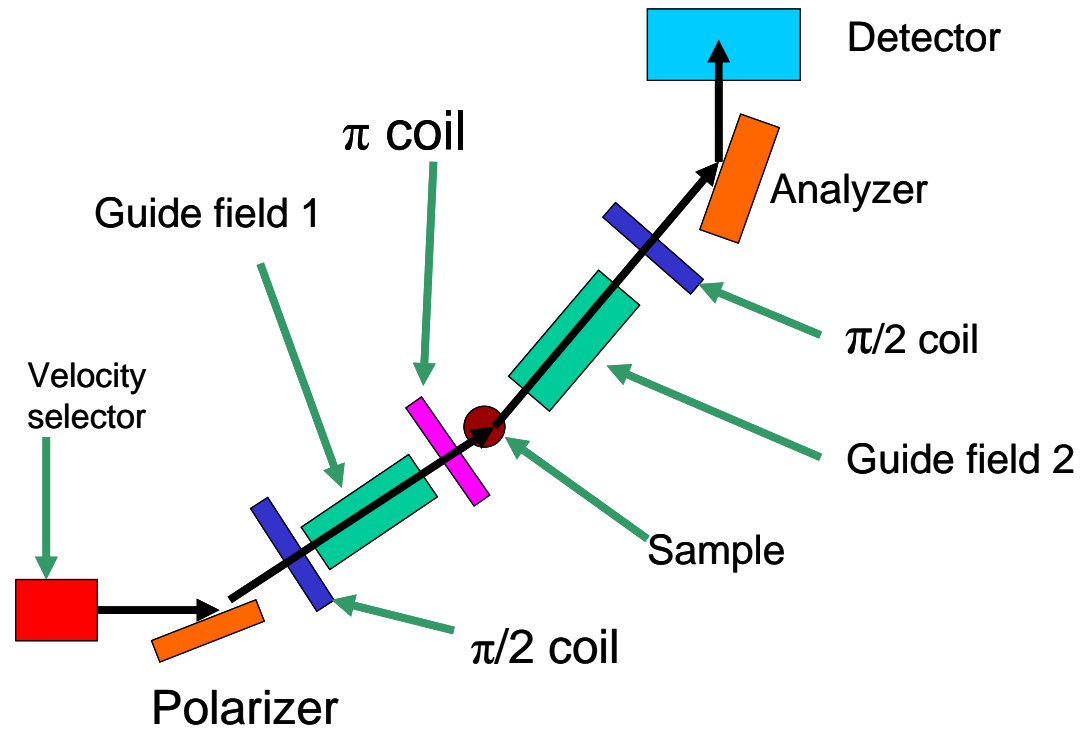
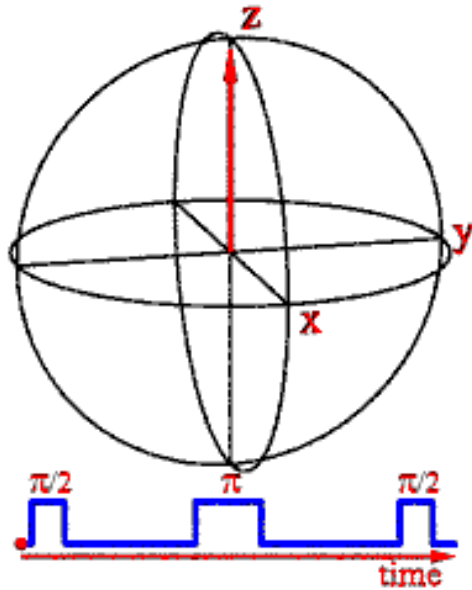
Other QENS methods find  $\Delta E$  from initial **and** final  $\lambda$  (double error!)

NSE encodes  $\Delta E$  in polarization, giving unprecedented (1 part in  $10^5$ ) accuracy in determination of energy

Allows wider spread of original  $\lambda$  (essentially **measure only  $\delta\lambda$** )

# Larmor precession of neutron spins in magnetic field

Ferenc Mezei



Measuring phase difference due to protein motion:

$$\phi_1 - \phi_2 \propto \left( \frac{1}{v_1} - \frac{1}{v_2} \right) \propto \frac{1}{v^2} \delta v \propto \frac{\hbar \omega}{v^3}$$

**Neutron Spin Echo yields  $\Delta E/E \sim 10^{-5}$**

# NSE does Fourier transform!

Spin rotates through angle

$$\phi = \omega_0 T = (\gamma B) (L/v) \sim B\lambda$$

( $\phi/\lambda$  independent of  $\lambda$ )

$$\cos(\delta\phi) \sim \cos[\delta\lambda (\phi/\lambda)]$$

$$\omega \sim 1/\lambda^2 \quad \text{so} \quad \delta\omega/\omega = -2 \delta\lambda/\lambda$$

$$\text{Polarization} = \int S(Q, \delta\omega) \cos(\delta\omega t) d(\delta\omega)$$

$$= S(Q, t), \quad \text{with "t"} \equiv \phi/(2\omega) \sim B$$

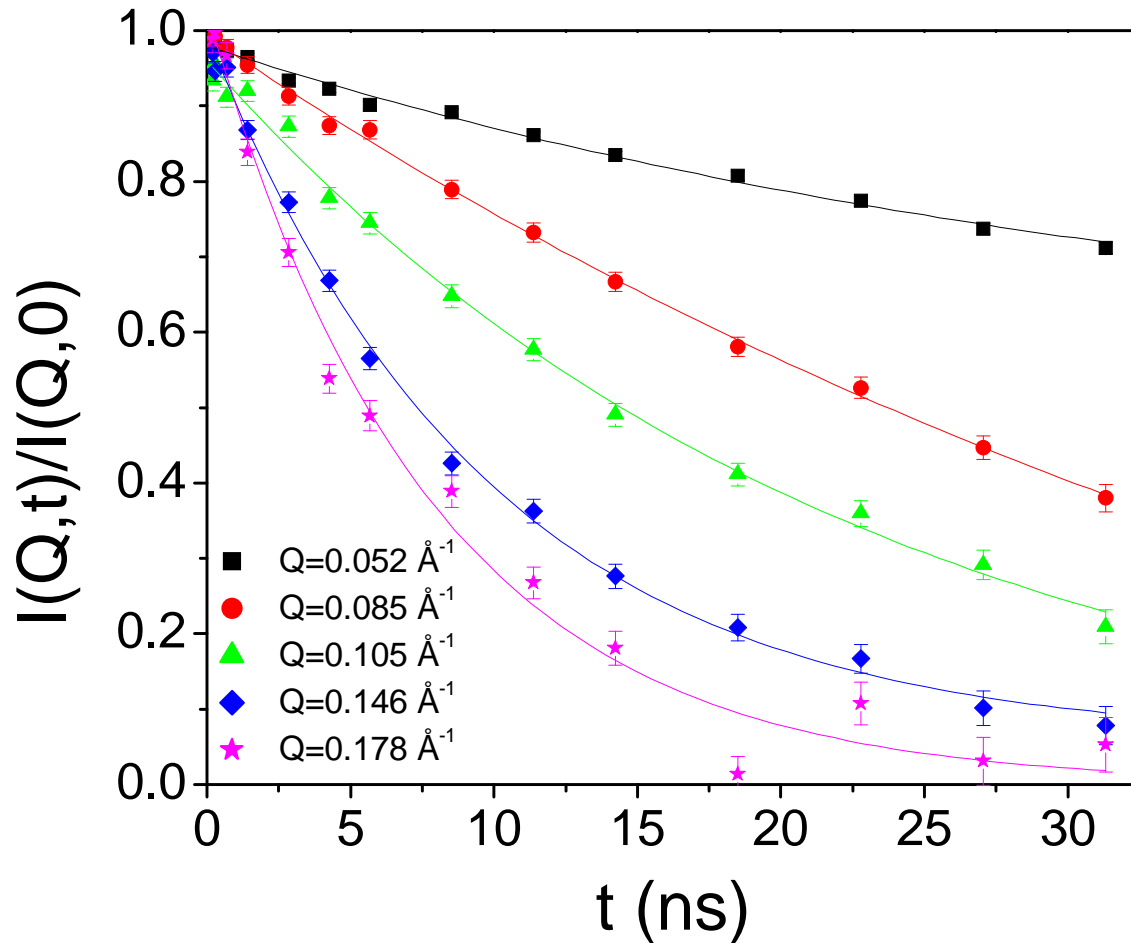
# Time and length scales probed by quasielastic neutron scattering

Conventional:  
ps to sub-ns, 1-10 Å

Current NSE:  
1ps-500 ns, 1-1000 Å

Near Future NSE:  
1ps-microsecond

# Neutron spin echo Spectra of Taq polymerase



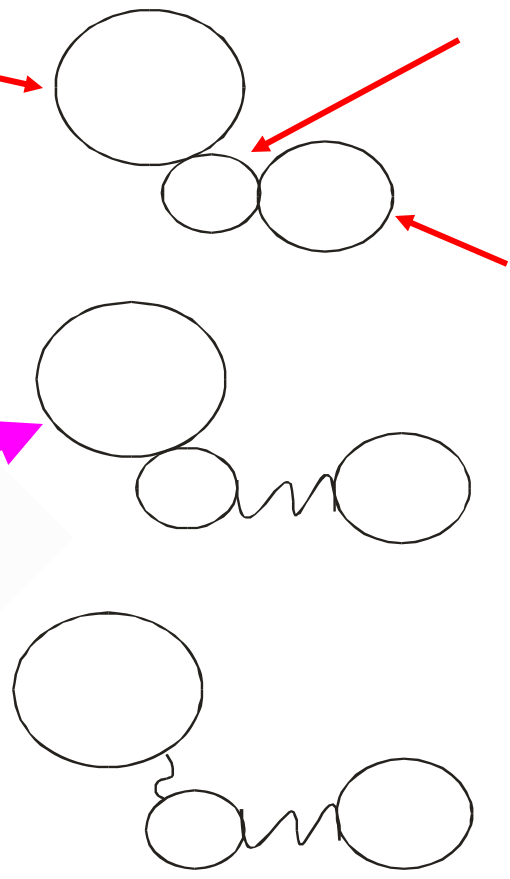
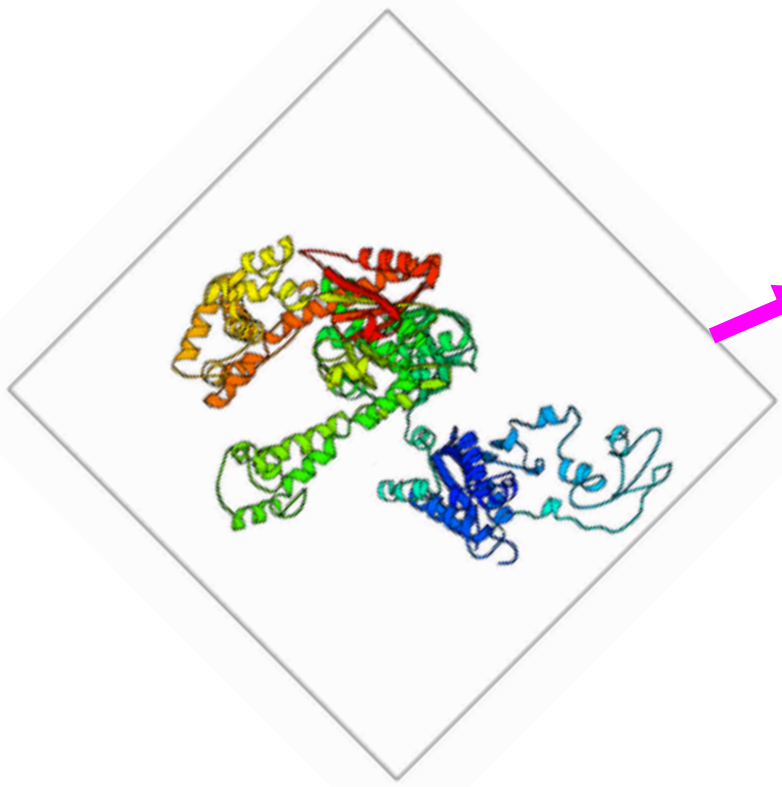
**Time-spatial correlation function  
on various length-scales up to 1000  $\text{\AA}$  !**

# Normal modes of Taq: rigid, one spring, two springs

Polymerase domain

3'-5' nuclease domain

5' nuclease domain



# Life at low $Re$

**Re**ynolds number large (ocean liner):

**Inertial**  $r = vt$  so  $\langle r^2 \rangle = v^2 t^2$

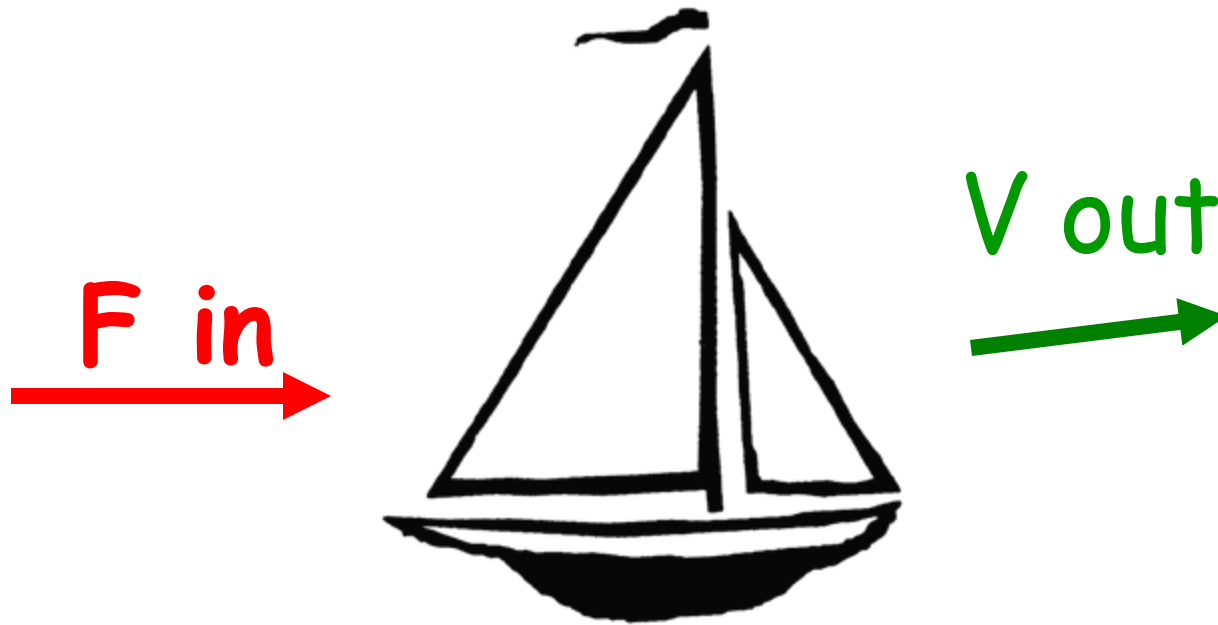
**Re**ynolds number small (**proteins**):

**Diffusive** (Brownian)  $\langle r^2 \rangle \sim D t$

**D** = diffusion const

(ping pong at bottom of molasses pool)

Mobility tensor  $\vec{v} = \overset{\leftrightarrow}{H} \vec{F}$



H is the **mobility tensor**, which yields the velocity of an object given the force applied on it

# Langevin description of coordinate evolution

Motion = Dynamics (**H**) x Static forces (**F**)

$$\frac{d}{dt} \vec{r}_j = \sum_k \vec{H}_{jk} \bullet \left[ -\frac{\partial U\{\mathbf{r}\}}{\partial \vec{r}_k} + \vec{f}_k(t) \right]$$

**H** is the **mobility tensor**, which yields the velocity of particle  $j$  given the force on particle  $k$

# First cumulant of dynamic structure factor $S(Q,t)$

$$\bar{\Gamma}(Q) = -\lim_{t \rightarrow 0} \frac{\partial}{\partial t} \ln[S(Q,t)]$$

yields **effective diffusion constant**

$$D_{\text{eff}}(Q) = \frac{\bar{\Gamma}(Q)}{Q^2}$$

$$S(Q, t) = S_1(Q)e^{-\Gamma_1 t} + S_2(Q)e^{-\Gamma_2 t} + \dots$$

## Cumulant expression

$$\bar{\Gamma}(Q) = -\lim_{t \rightarrow 0} \frac{\partial}{\partial t} \ln[S(Q, t)] = \frac{S_1 \Gamma_1 + S_2 \Gamma_2 + \dots}{S_1 + S_2 + \dots}$$

Effective diffusion constant  $D$   
 gives mobility tensor  $H$

operator

$$S(Q,t) \sim \langle \exp(-DQ^2t) \rangle \text{ so}$$

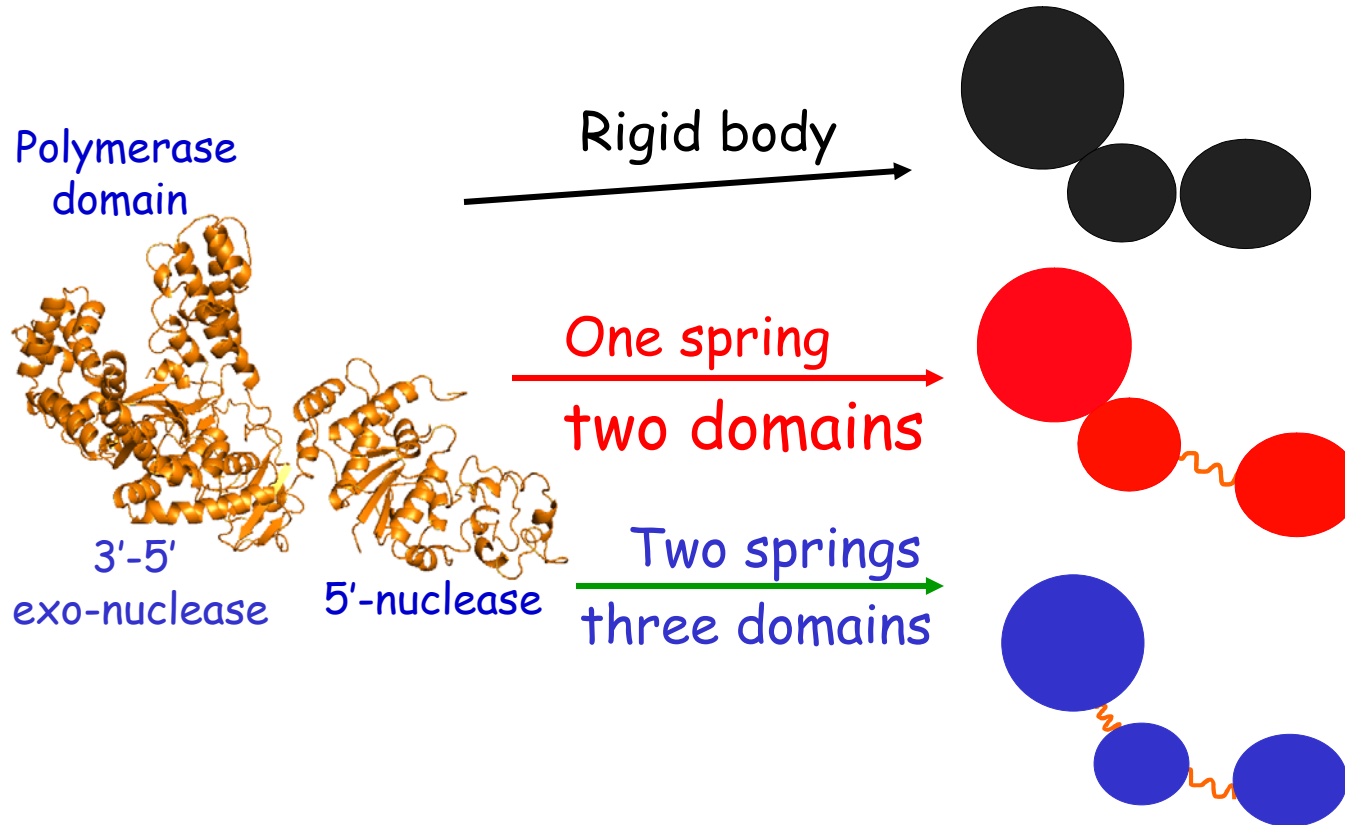
$$\Gamma(t=0) = -S'(0)/S(0) = \langle D \rangle Q^2 \text{ or}$$

$$D_{eff} = \frac{k_B T}{Q^2} \frac{\sum_{jl} \langle b_j b_l (Q \cdot H^T \cdot Q + L(j) \cdot H^R \cdot L(l)) e^{iQ \cdot (r_j - r_l)} \rangle}{\sum_{jl} \langle b_j b_l e^{iQ \cdot (r_j - r_l)} \rangle}$$

$$(L = Q \times r)$$

(calculate  $D_{eff}$  from crystal structure and  $H$ )

# Normal mode analysis of *Taq* polymerase



# Internal motion - normal modes

Simplest domain mobility tensor:

$$\overset{\leftrightarrow}{\mathbb{H}}_{j,k} = \zeta_j^{-1} \delta_{j,k} \overset{\leftrightarrow}{\mathbb{I}}$$

Domain friction constant

$(D = k_B T / \zeta)$   
First normal mode (2 domains + 1 spring):

$$D_{eff}(Q) = \frac{D_1 S_1(Q) + D_2 S_2(Q)}{S_{TaQ}(Q)}$$

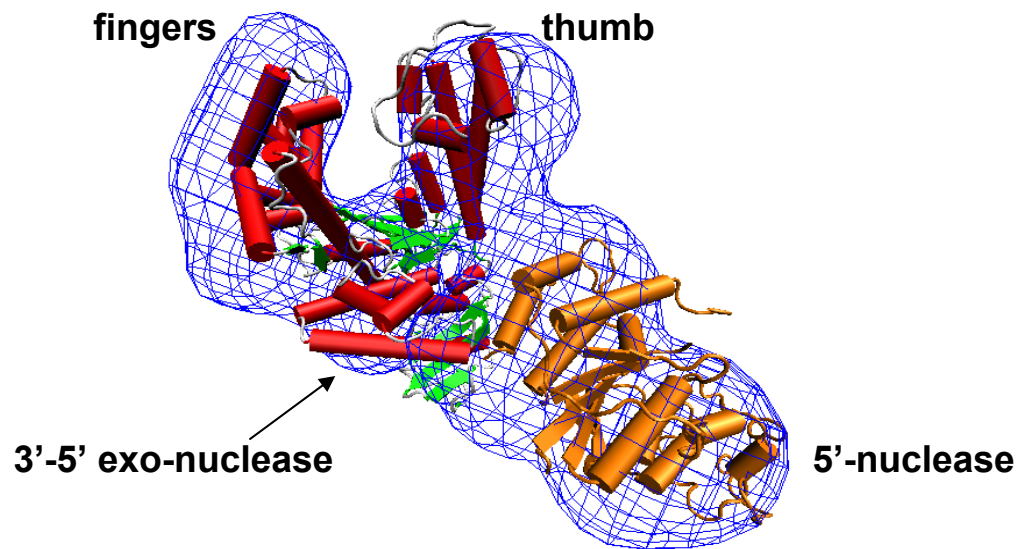
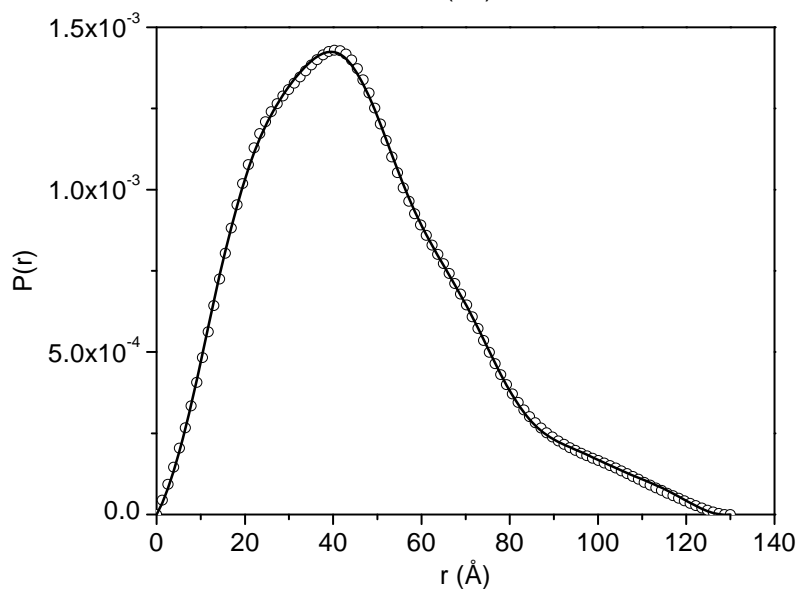
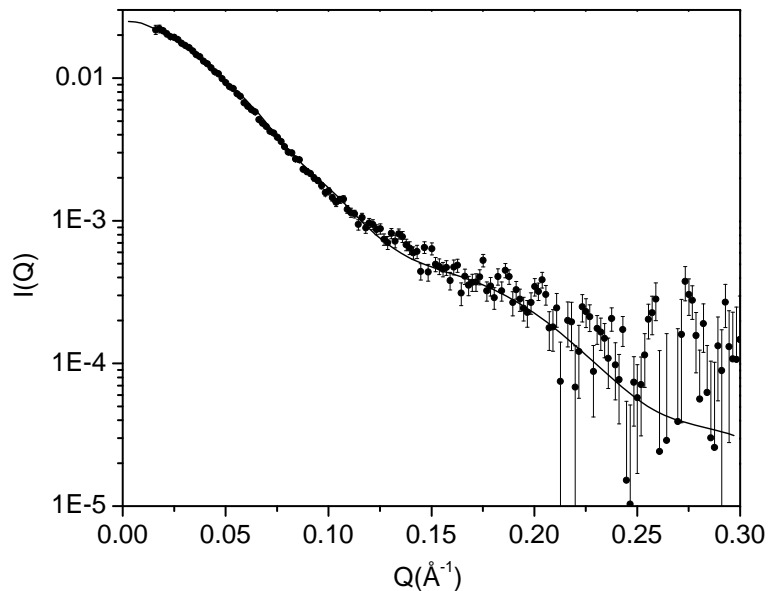
# Domain form factors

$$S_j(Q) = \sum_{m,n \in j} b_m b_n \frac{\sin [Q|r_m - r_n|]}{Q|r_m - r_n|}$$

First + Second normal modes  
(3 domains + 2 springs):

$$D_{eff}(Q) = \frac{D_{5'-nuc} S_{5'-nuc}(Q) + D_{3'-5'nuc} S_{3'-5'nuc}(Q) + D_{pol} S_{pol}(Q)}{S_{Taq}(Q)}$$

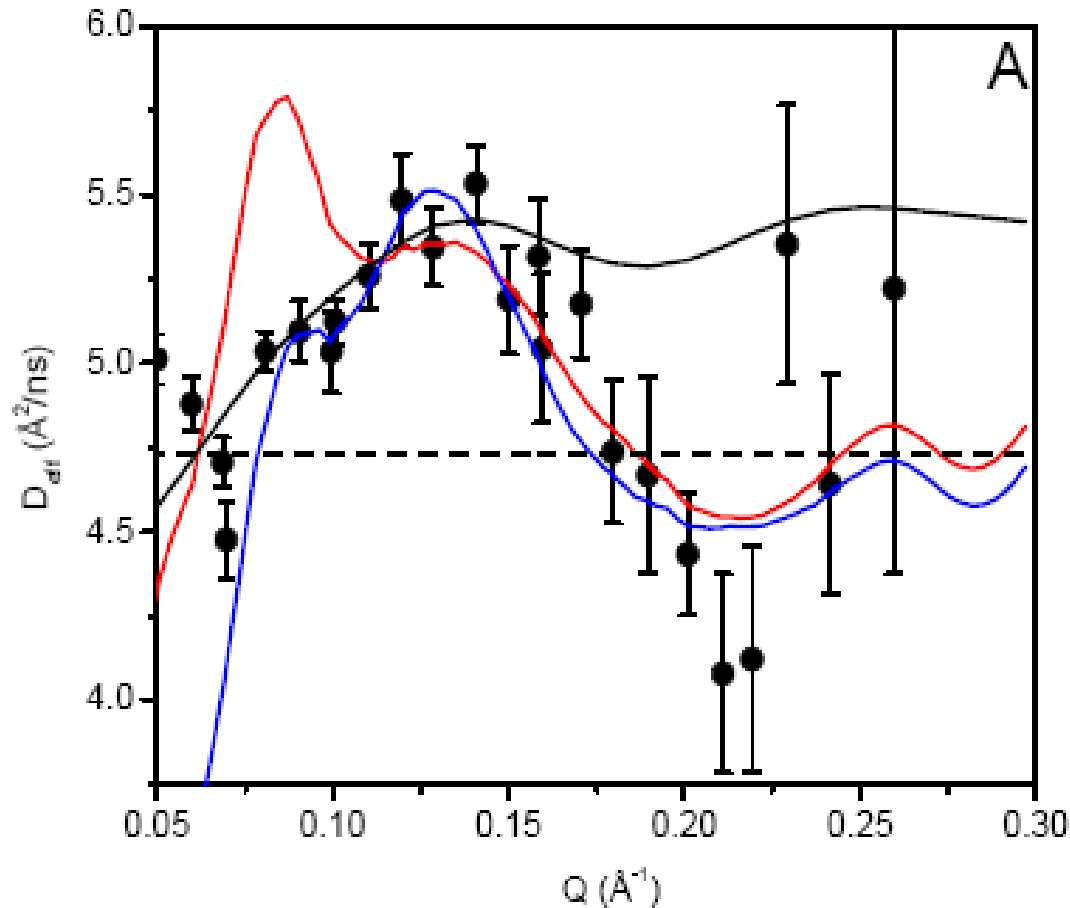
# The global conformation of TAQ from SAXS



**SAXS=crystal**  
**(not multiple structures)**

# NSE data (PNAS)

Rigid body **First mode** First+Second mode



First mode

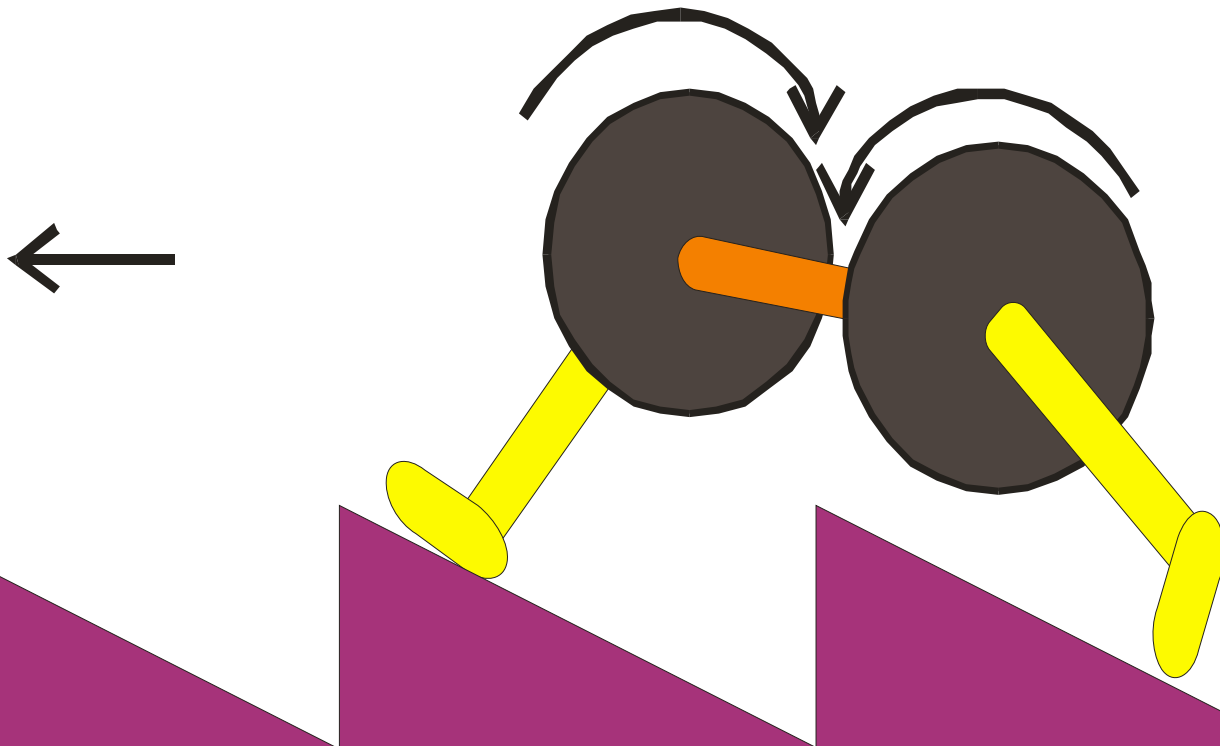
Estimate amplitude 10 Å

Equipartition theorem

$$\langle x^2 \rangle = \frac{k_B T}{k} = \left( \frac{k_B T}{\zeta} \right) \left( \frac{\zeta}{k} \right) = D\tau$$

# Molecular nanomachinery from proteins

## “Walking”



**Neutron spin echo** allows one to see  
coupled correlated domain motion

**Protein dynamics** significant in biology

Understand and harness protein  
**nanomachines**

Determine protein **mobility tensor**,  
defining internal domain motion

# Acknowledgements

Fox Chase Cancer Center

Zimei Bu

IFF Juelich

Dieter Richter

Michael Monkenbusch

Ralf Biehl

**IBM SUR Grant**