

# **Solution Dynamics and Self-Organization**

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# Outline

- A. Solution Dynamics and Self-Organization.
- B. The Pulsed Gradient Spin Echo (PGSE) NMR method for measuring translational diffusion.
- C. Some Examples
  - 1. Supercooled Water
  - 2. Isolated Water Molecules
  - 3. Alcohol Water Systems
  - 4. Drug Binding
  - 5. Aggregation and Crystallization of Lysozyme

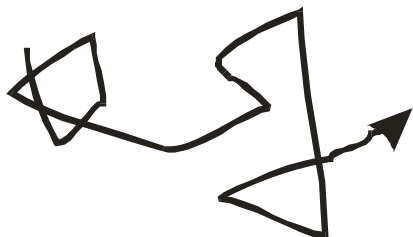
# Solution Dynamics

## ■ Includes:

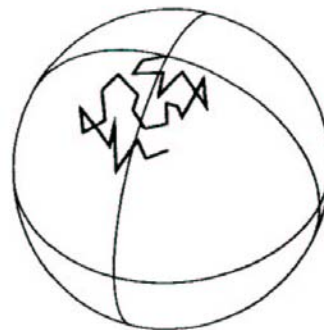
- **Association** → self organization and crystallisation
- **Binding** (e.g., drug – protein or drug -DNA)
- **Phase changes**
- **Bio-thermodynamics** (e.g., macromolecular crowding effects)
- **Exchange** (e.g., transmembrane)
- ....

➤ **How a molecule interacts with its neighbours and surroundings.**

# Types of Motion

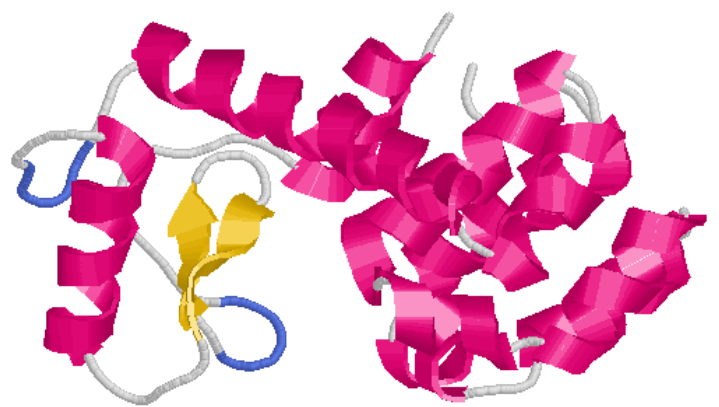


**Translational (self-)  
Diffusion,  $D$  ( $\text{m}^2\text{s}^{-1}$ )**



Time taken to reorientate  
by  $\sim 1$  radian

**Reorientational  
Correlation Time,  $\tau_c$  (s)**



**Lysozyme**

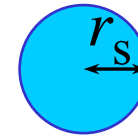
Different parts of molecule may have different reorientational motions, but a single diffusion coefficient characterises the whole molecule.

■ NMR can probe both types of motion.

# Mw and Motion

## ■ Translational diffusion

sphere



**Stokes-Einstein equation**  
(only holds at infinite dilution)

$$D = \frac{kT}{f}$$

← friction coefficient

$$f = n\pi\eta r_s$$

$n = 4$  (slip),  $6$  (stick)

Species	T (K)	$D$ (m <sup>2</sup> s <sup>-1</sup> ) × 10 <sup>9</sup>	$M_w$
H <sub>2</sub> O	298	2.26	18
Glycine	298	1.05	75
glucose	298	0.67	180
inorganic phosphate	298	0.61	95
creatine phosphate	298	0.52	211
sucrose	298	0.52	342
ATP	298	0.37	507
insulin	293	0.082	5700
lysozyme	298	0.108	14500
hemoglobin	293	0.063	64500
tobacco mosaic virus	293	0.005	40000000

$$D \propto \frac{1}{\sqrt[3]{M_w}}$$

## ■ Reorientational motion

$$M_w \uparrow \quad \tau_c \uparrow \quad T_2 \downarrow$$

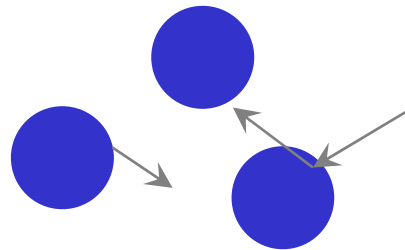
# Diffusion as a Probe of Organization

## Advantages:

- Organization and association generally involve changes in molecular weight and hydrodynamic properties → diffusion is a natural probe of such phenomena.
- Data analysis is facilitated by diffusion being a property of the **entire molecule** (excluding exchangeable groups)

## Complications:

- **Finite Solute Concentrations:** Inter-particle collisions and interactions (i.e., ‘obstruction effects’) also influence the measured diffusion coefficient. Nuisance OR source of information?



# PGSE NMR Diffusion Measurements

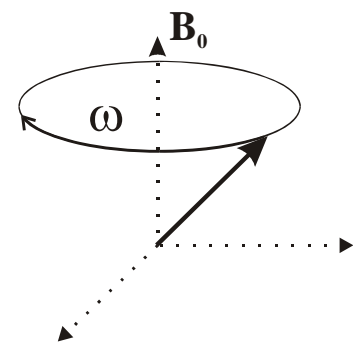
- Using spatially well-defined magnetic field gradients to spatially encode the translational motion of spins – this includes diffusion, flow, turbulence ....
- PGSE NMR measures self-diffusion not mutual diffusion.
- Also known as q-space imaging, PGSE (Pulsed Gradient Spin-Echo), DOSY (Diffusion Ordered Spectroscopy) or NMR diffusometry.

# The Mechanics of NMR Diffusion Measurements

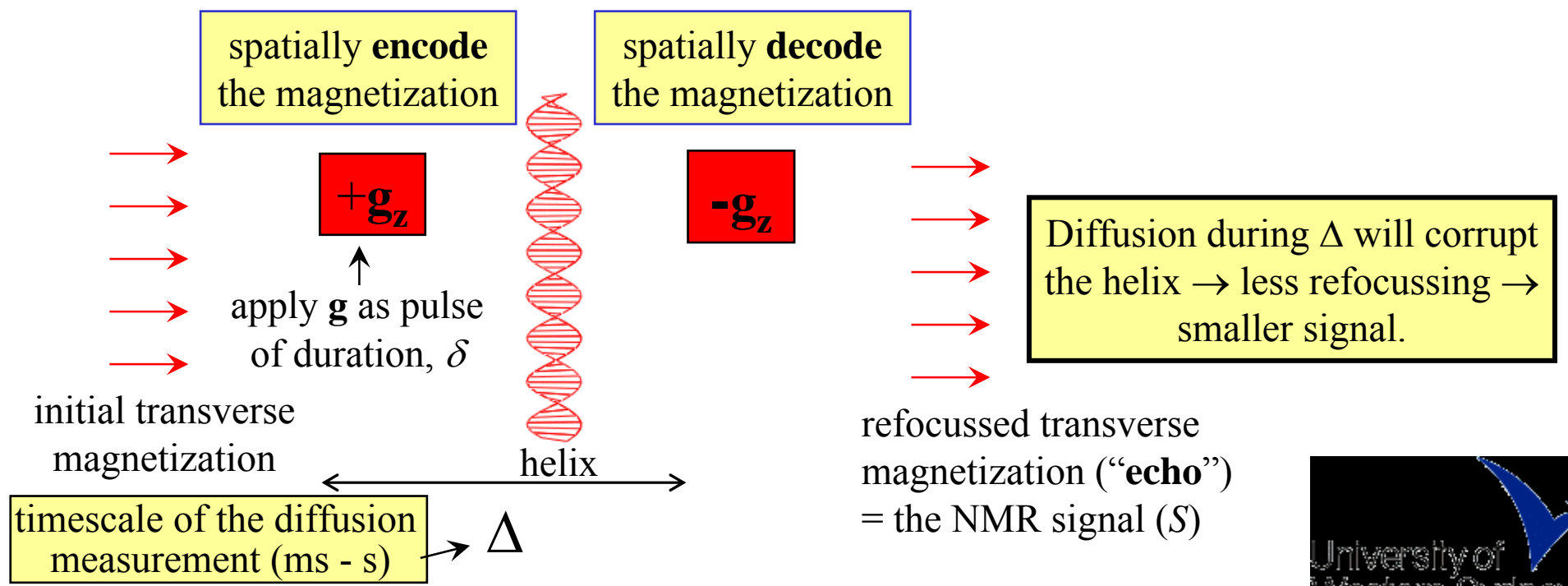
## Larmor Equation

Resonance frequency (rad s<sup>-1</sup>)  $\longrightarrow \omega = -\gamma \mathbf{B}_0$   $\longleftarrow$  Strength of the static magnetic field (T)

↑  
Gyromagnetic ratio (rad s<sup>-1</sup> T<sup>-1</sup>)



If the homogeneous field ( $\mathbf{B}_0$ ) is replaced by a gradient directed along the  $z$ -axis ( $\mathbf{g}_z$ ), then  $\omega$  will vary spatially along the  $z$ -axis:  $\omega(z) = \gamma \mathbf{g}_z z$





# What About Signal Attenuation due to Relaxation?

$$\text{Echo signal} \rightarrow S(g) = \underbrace{M_0 \exp\left(-\gamma^2 g^2 D(\Delta - \delta/3)\right)}_{\text{Attenuation due to Diffusion}} \underbrace{\exp\left(-2\tau/T_2\right)}_{\text{Attenuation due to Relaxation}}$$

↑
initial magnetization

- Normally, the attenuation due to relaxation is normalised out.

$$E = \frac{S(g)}{S(0)} = \frac{\exp\left(-\gamma^2 g^2 D(\Delta - \delta/3)\right) \exp\left(-2\tau/T_2\right)}{\exp\left(-2\tau/T_2\right)} = \exp\left(-\gamma^2 g^2 D(\Delta - \delta/3)\right)$$

Echo Signal Attenuation

- Unfortunately, this ‘elegant’ solution is not general - it only holds for a single component.

# A Summary of the Characteristics of PGSE

- “Rule of Thumb”  $M_w \uparrow D \downarrow$

PGSE can measure diffusion in the range of  $10^{-9} - 10^{-14} \text{ m}^2\text{s}^{-1}$ .

$\begin{matrix} \nearrow & & \nwarrow \\ \text{H}_2\text{O} & & \text{solid polymer} \end{matrix}$

- If the mean square displacement during the experiment ( $\Delta \sim 10 \text{ ms} - 1 \text{ s}$ ) is such that a sufficient population of spins make contact with the boundaries  $\Rightarrow$  complicated non-exponential attenuation profiles.

Experimentally this corresponds to barriers with characteristic distances,  $R \square 100 \mu\text{m}$ .

- At finite concentrations the diffusing species obstruct each other  $\Rightarrow D \downarrow$
- Differential relaxation weighting is a problem in polydisperse systems.
- The **combination** of these effects can complicate data interpretation

# SUPERCOOLED WATER

- What is the nature of the water  $\leftrightarrow$  ice transition?
- Self-nucleation temperature is about 231 K.
- Metastable 'Grey area' from 273 to 231 K.
- PGSE is one of the few applicable techniques.

# Sample for Supercooled Water Diffusion Measurements

Using a small sample volume makes it possible to approach the self-nucleation temperature

0.13 mm i.d.  
sealed capillaries

5 mm NMR tube

Water

(~ 0.5  $\mu\text{l}$ , length ~ 8 mm)

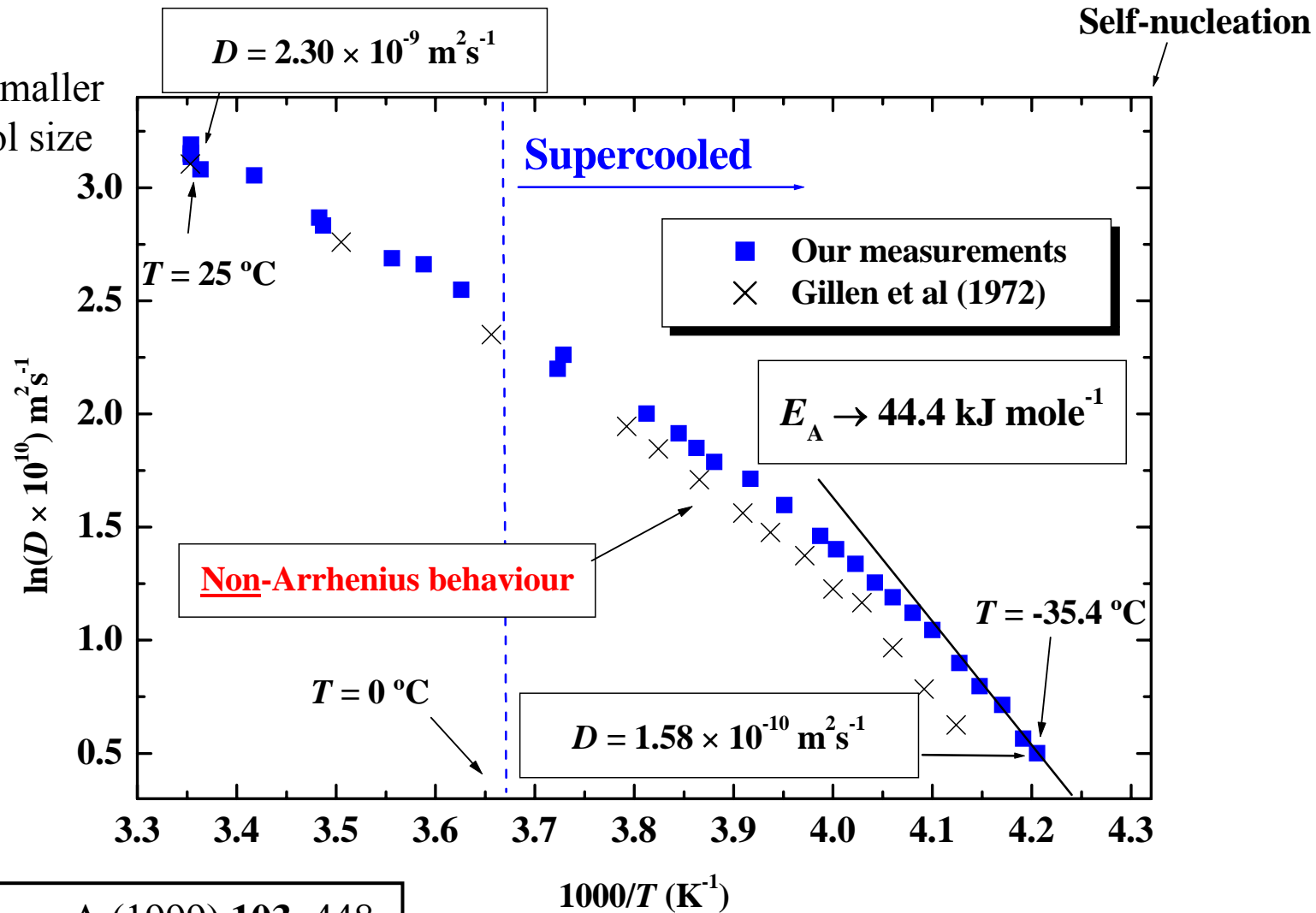
Methanol

(for temperature calibration)

- Diffusion measured along the capillary.

# Supercooled $^1\text{H}_2\text{O}$ Diffusion

errors in  $D$  are smaller than the symbol size



*J. Phys. Chem. A* (1999) **103**, 448

# Fractional Power Law (FPL) and Vogel-Tamman-Fulcher (VTF) Relations

- FPL equation (sharp transition from water to ice)

$$D = D_0 T^{1/2} \left( \frac{T}{T_S} - 1 \right)^\gamma$$

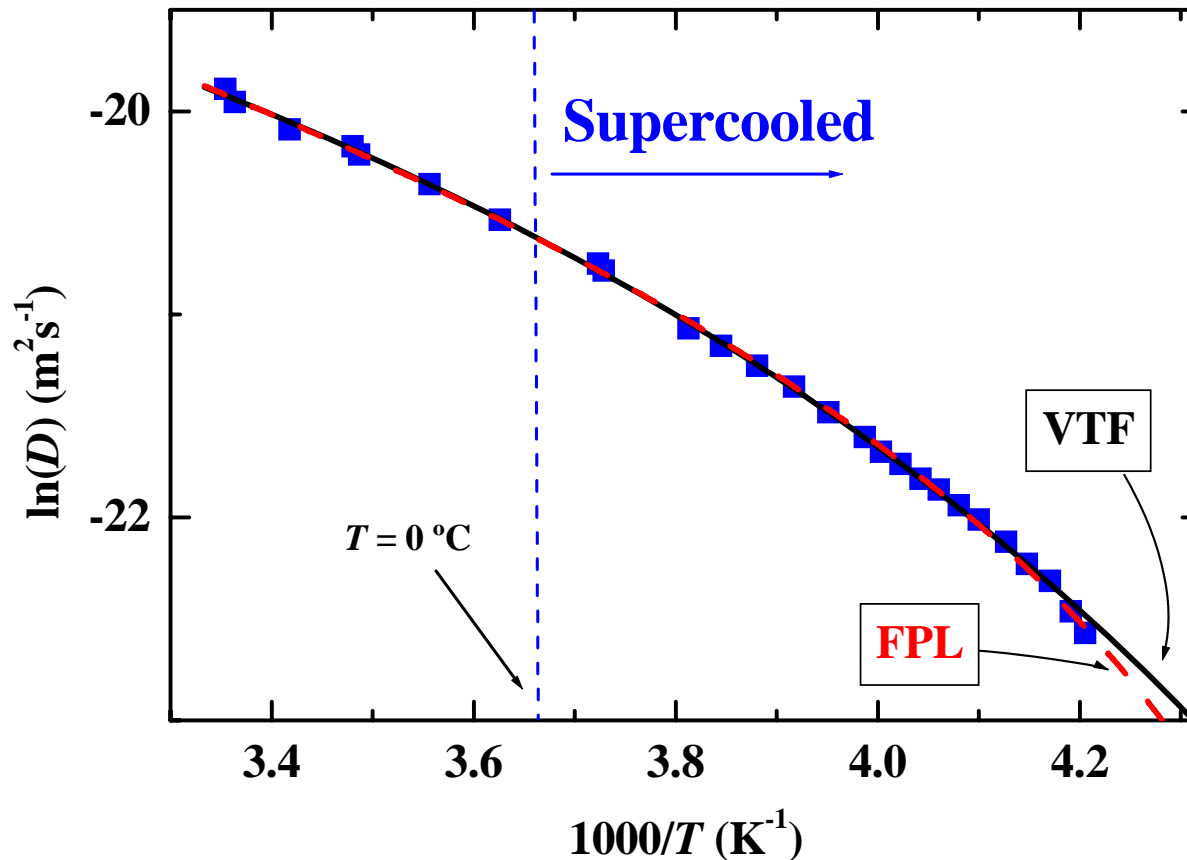
$T_S$ : low temperature limit  
(singularity)  
 $D_0, \gamma$ : fitting parameters

- VTF equation (smooth transition)

$$D = D_0 \exp\left\{-B/(T - T_0)\right\}$$

$T_0$ : related to the glass  
transition temperature  
 $D_0, B$ : fitting parameters

# Modeling $^1\text{H}_2\text{O}$ Diffusion Using the FPL and VTF Relations



VTF

$$D_0 = 4.00 \pm 0.87 \times 10^{-8} \text{ m}^2\text{s}^{-1}$$

$$B = 371 \pm 45 \text{ K}$$

$$T_0 = 169.7 \pm 6.1 \text{ K}$$

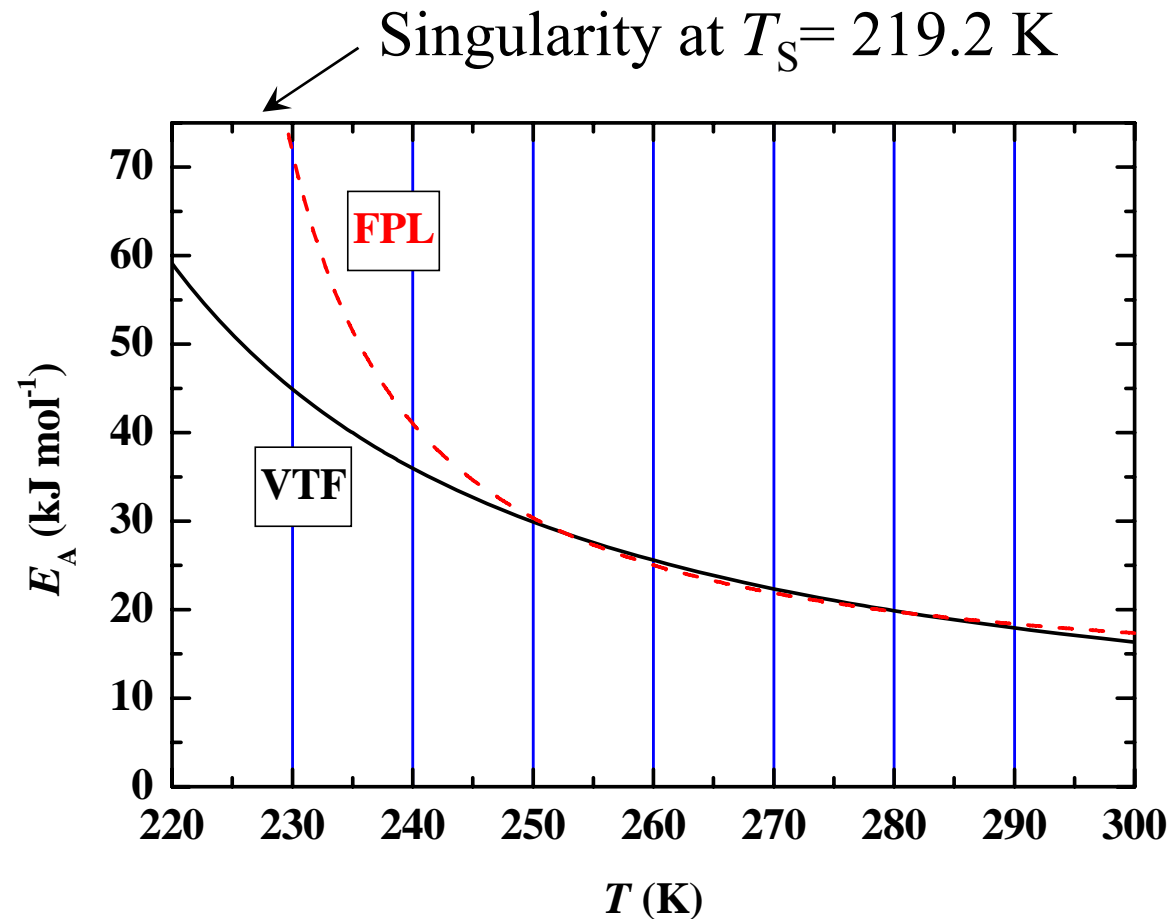
FPL

$$D_0 = 7.66 \pm 0.24 \times 10^{-10} \text{ m}^2\text{s}^{-1}$$

$$T_S = 219.2 \pm 2.6 \text{ K}$$

$$\gamma = 1.74 \pm 0.10$$

# Apparent Activation Energy of $^1\text{H}_2\text{O}$ Diffusion



# ISOLATED-WATER MOLECULES

- Anomalous behaviour of liquid water arises from hydrogen bond network.
- Water dissolved in a hydrophobic solvent  
‘isolated’ water molecules.
- No hydrogen bonding between water molecules.
- Studied the diffusion ( $^{17}\text{O}$  PGSE) and  $^{17}\text{O}$  longitudinal relaxation ( $T_1$ ) of  $\text{H}_2^{17}\text{O}$  dissolved in nitromethane.
- ‘Non-standard’, low  $\gamma$  nuclei are now becoming accessible to PGSE measurement with the increasingly larger applied gradients available.

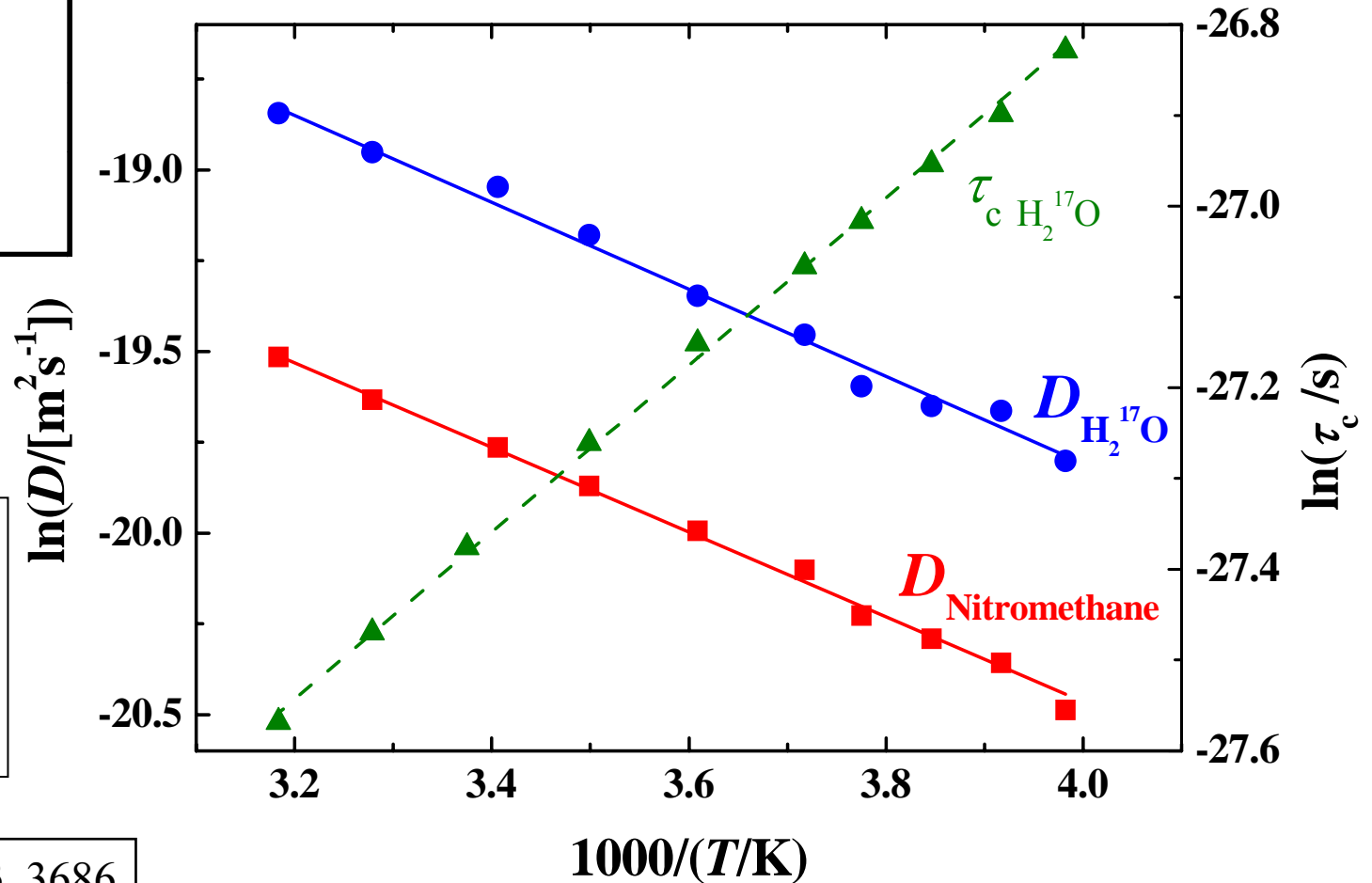
# $^{17}\text{O}$ PGSE and Relaxation Measurements

	$E_A$ (kJ mol $^{-1}$ )
$D_{\text{H}_2^{17}\text{O}}$	$10.0 \pm 0.3$
$D_{\text{Nitromethane}}$	$9.7 \pm 0.2$
$\tau_{c\text{H}_2^{17}\text{O}}$	$7.7 \pm 0.1$



- $D$  depends on inertial and solvent effects
- $\tau_c$  dominated by inertial effects

$^{17}\text{O}$   $T_1 \rightarrow$  reorientational correlation time,  $\tau_c$



*J. Chem. Phys.* (2000) **113**, 3686

# Correlation between Reorientational Correlation Time and Diffusion in the Hydrodynamic Continuum Model

Stokes-Einstein (SE) Equation

$$D = \frac{kT}{n\pi\eta r_s}$$

$n = 4$  (slip),  $6$  (stick)

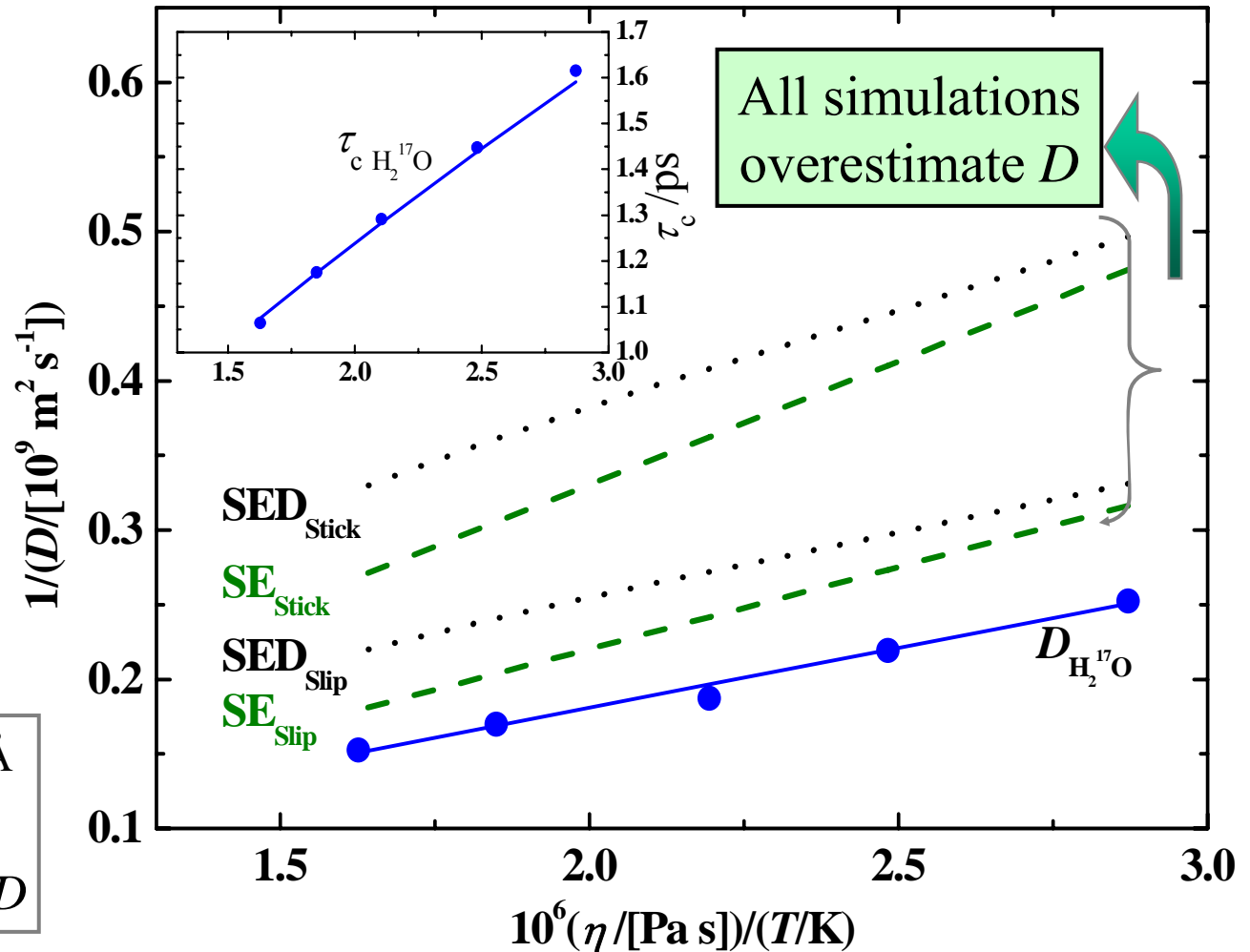
Stokes-Einstein-Debye (SED) Equation

$$\tau_c = \frac{4\pi r_s^3 \eta}{3kT}$$

H<sub>2</sub>O Stokes radius,  $r_s = 1.21 \text{ \AA}$

**SE:** lit.  $\eta \rightarrow \text{SE} \rightarrow D$

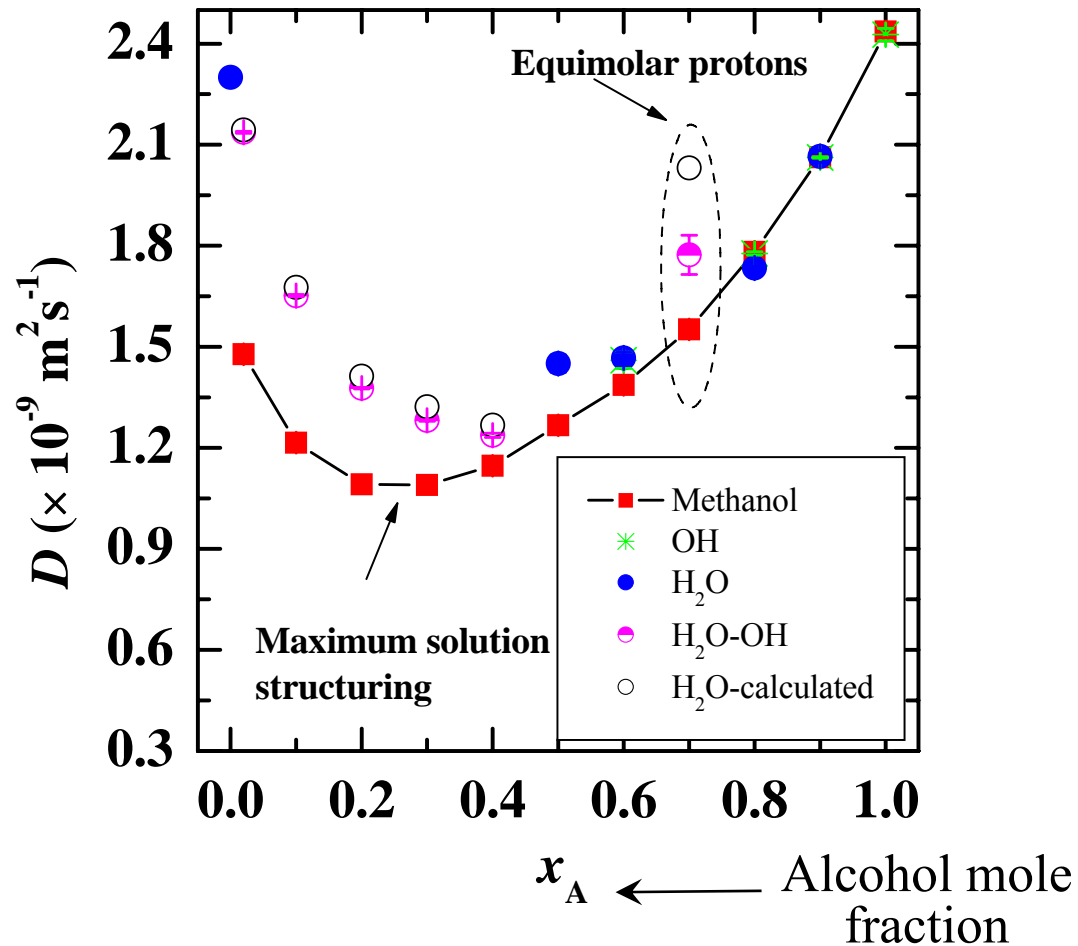
**SED:**  $\tau_c \rightarrow \text{SED} \rightarrow \text{SE} \rightarrow D$



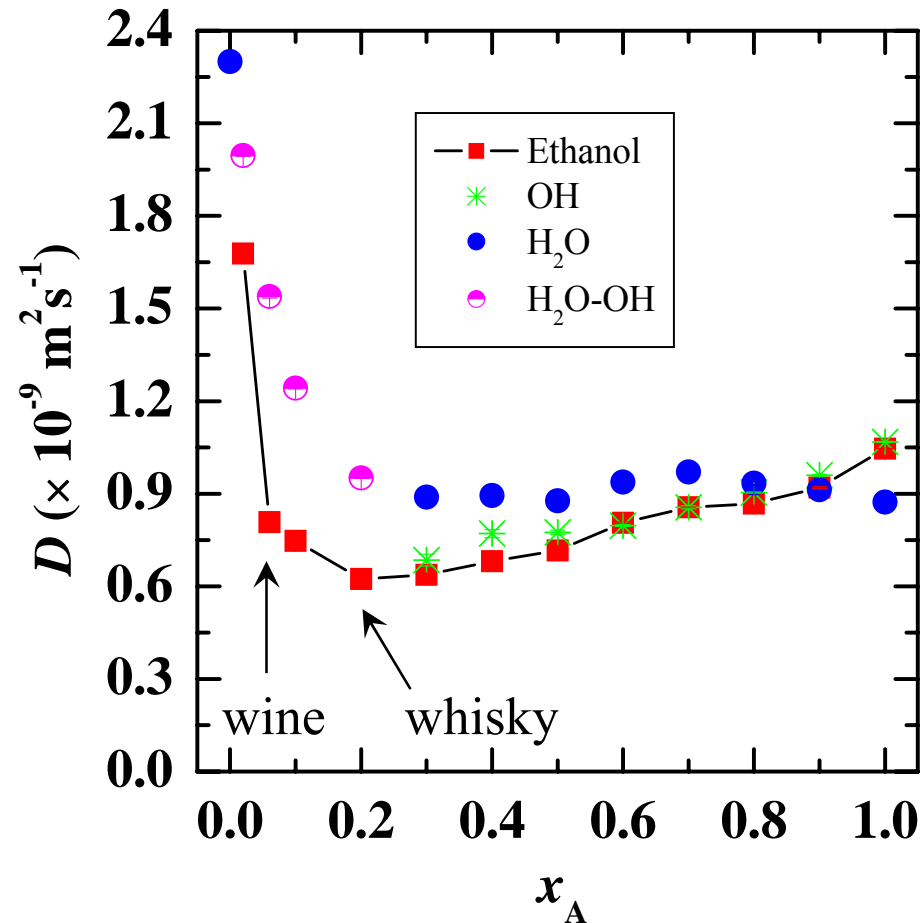
# ALCOHOL-WATER

- Alcohols are amphiphilic → complicated solution chemistry.
- Methanol, Ethanol and *tert*-Butanol differ only in the size of the alkyl group.
- “Primordial” lipids - models for micelle assembly.

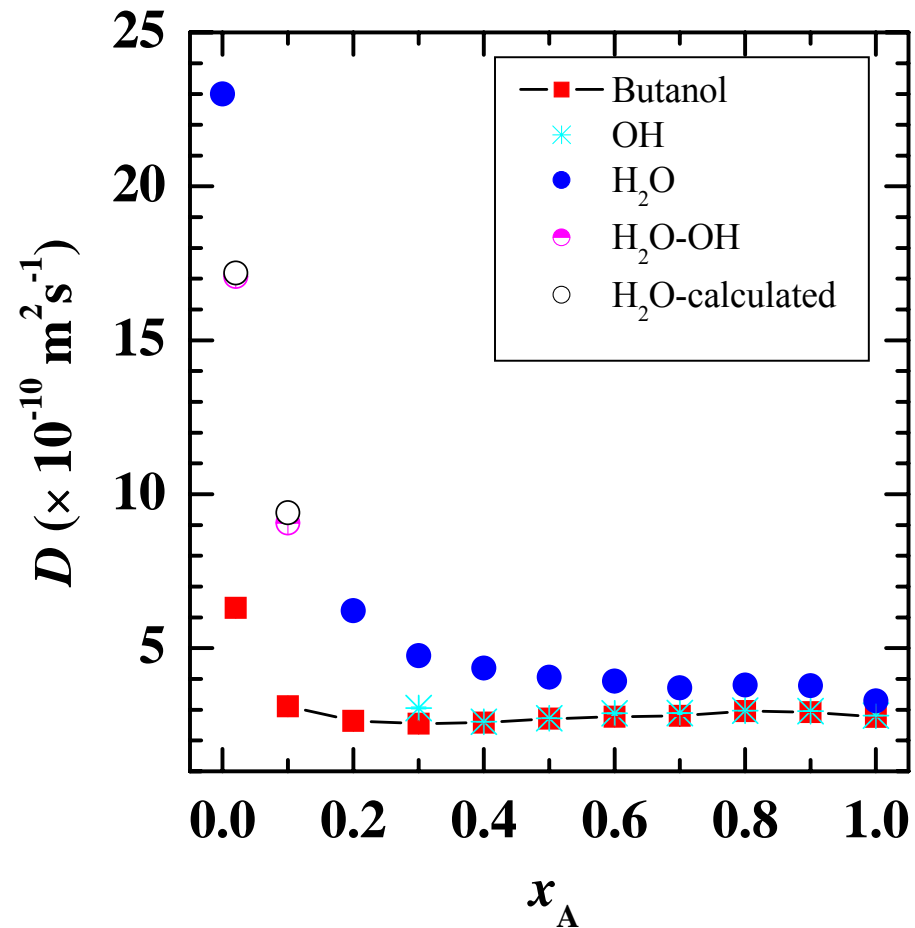
# Diffusion in the Methanol-Water System at 298 K



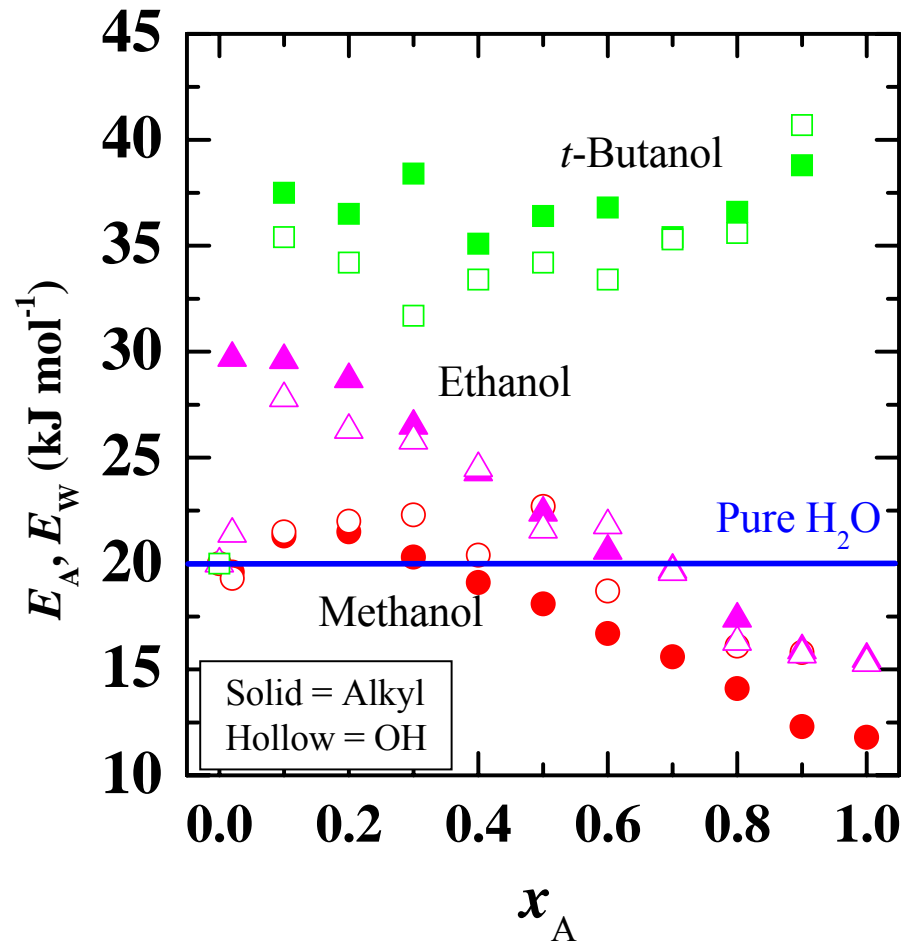
# Diffusion in the Ethanol-Water System at 298 K



# Diffusion in the *tert*-Butanol-Water System at 298 K



# Arrhenius Activation Energy for Diffusion

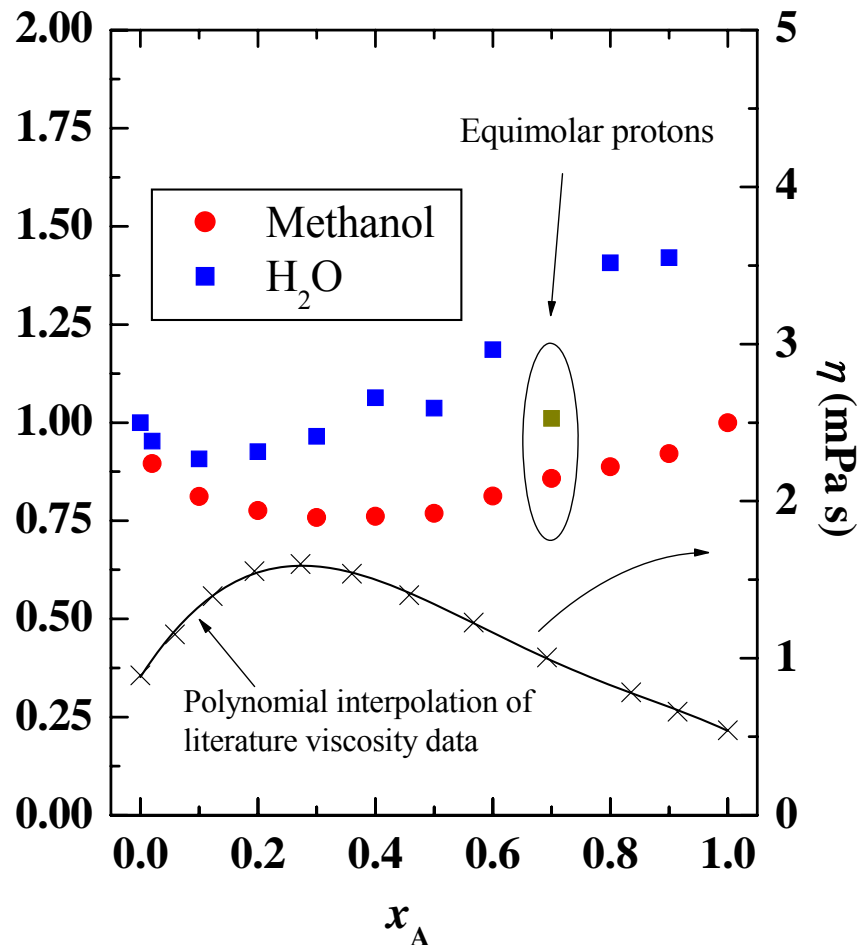


Calculated from diffusion measured at 273, 285 and 298 K

# Stokes-Einstein Analysis of the Methanol System at 298 K

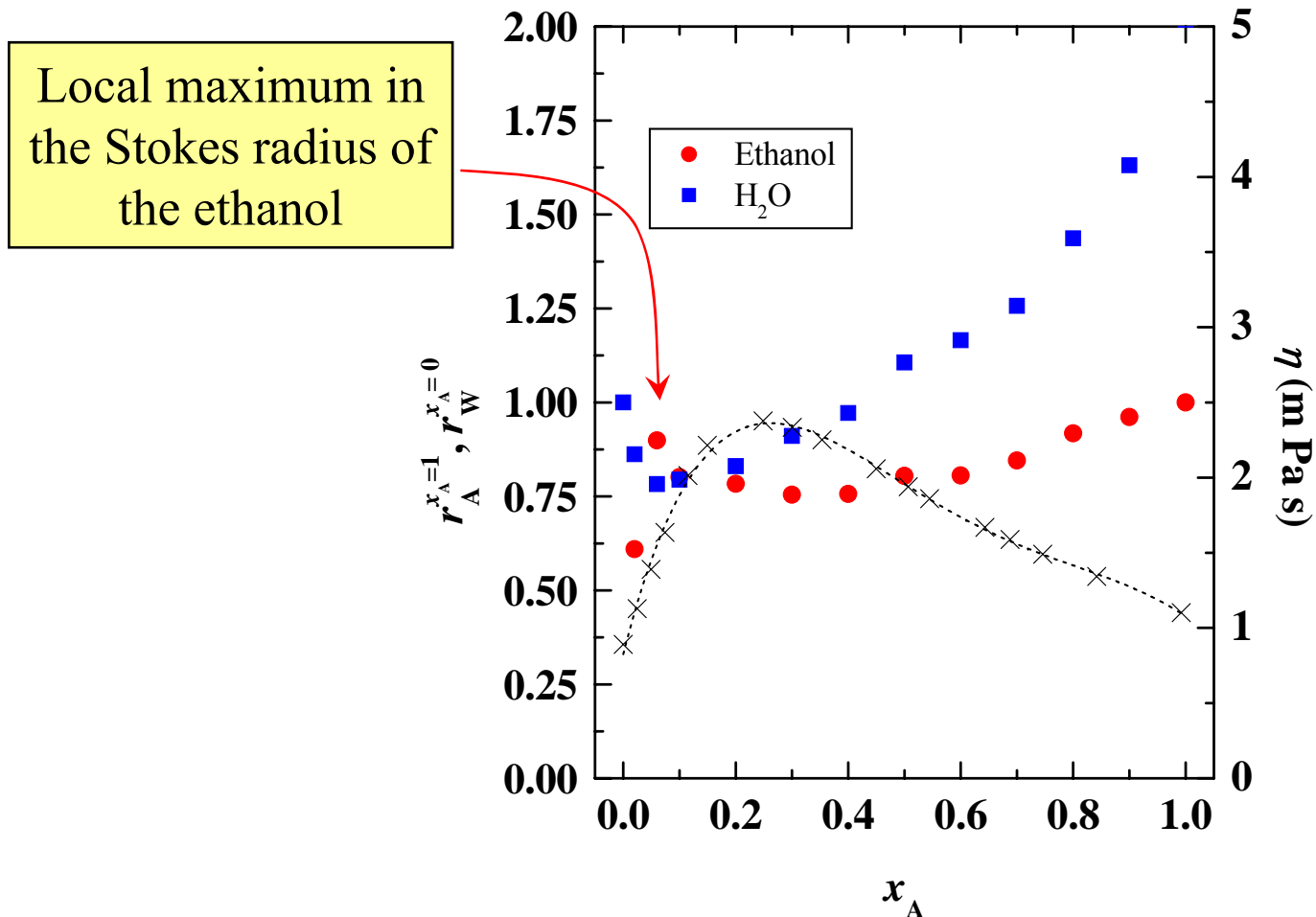
Stokes radii normalized to the pure solvent values

$$r_A^{x_A=1}, r_A^{x_A=0}$$



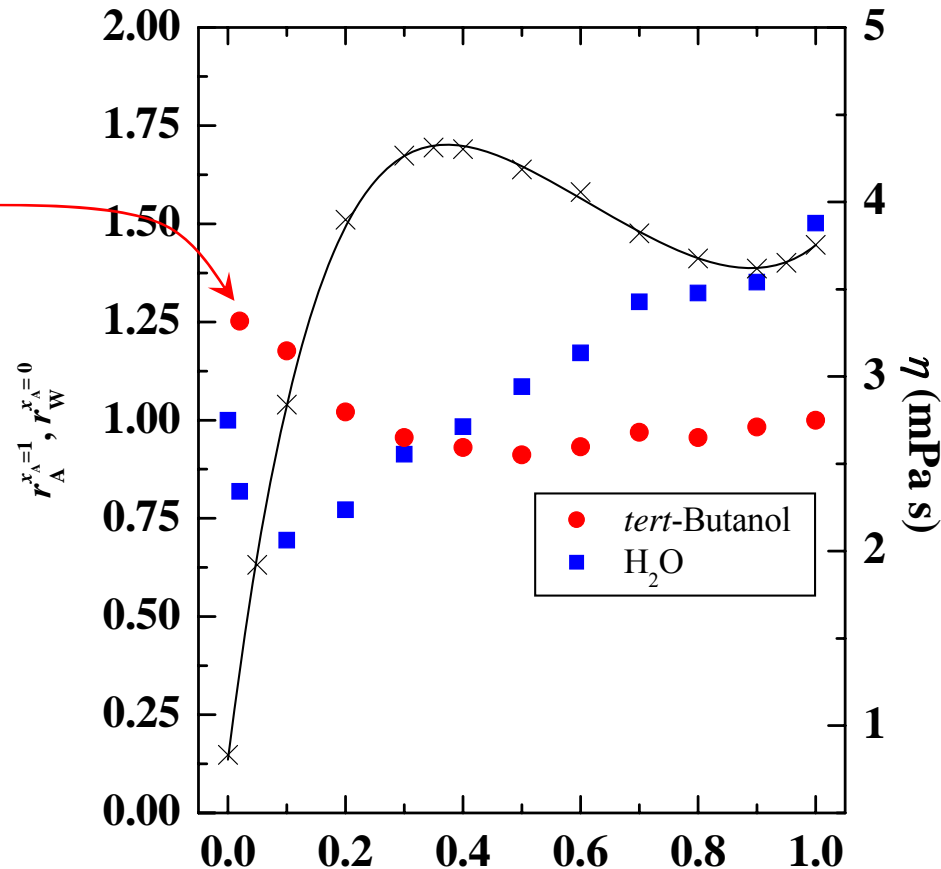
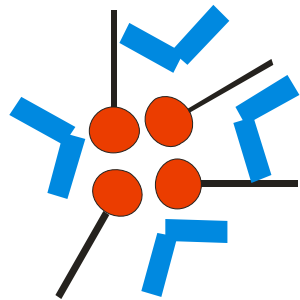
Bulk viscosity

# Stokes-Einstein Analysis of the Ethanol System at 298 K



# Stokes-Einstein Analysis of the *tert*-Butanol System at 298 K

butanol  
self-association



The data is consistent with small butanol clusters (e.g., tetramer, pentamer).

# Alcohol-Water Summary

- At low  $x_A$  the alcohol molecules associate due to hydrophobic hydration. The alcohol molecules sit at the centre of hydration shells.
- As  $x_A$  increases there comes a point where there are insufficient  $H_2O$  to form the shells.
- The three alcohols have quite different properties.
- *tert*-Butanol has the strongest hydrophobic hydration but the weakest H-bonding.
- Ideally, we would like to study the very low  $x_A$ .

# DRUG BINDING

- Diffusion is a very powerful method for screening drugs and characterizing their binding properties.

# The Practicalities of Diffusion-based Binding Assays

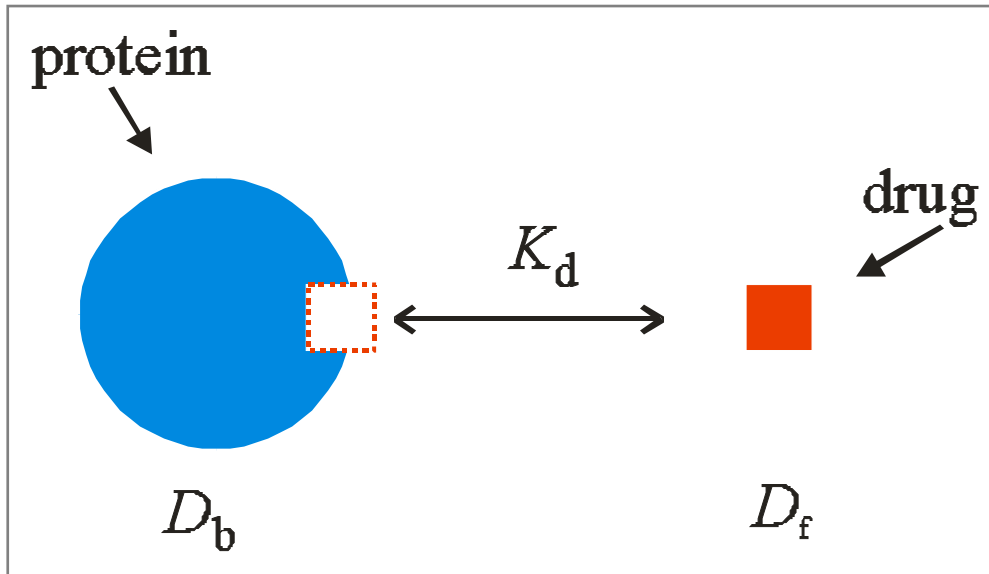
## Wish to determine:

- i. Is there any binding.
- ii. Dissociation constants.
- iii. Decide if there are one or more classes of binding constants.

## To do so it is necessary to:

- i. Accurately measure  $D$  of the drug over large concentration ranges (esp. v. low concentrations) – ideally in non-deuterated samples (i.e., in  $^1\text{H}_2\text{O}$  not  $^2\text{H}_2\text{O}$ ).
- ii. Remove the signals of the receptor.
- iii. Consider the effects of NMR relaxation on the measurement.

# The Basis of Diffusion-based Binding Assays



$D_b = D$  of protein (v. slow)  
 (~ unchanged by drug binding)

$D_f = D$  of drug (fast)

■ If the exchange is fast on the NMR timescale, the **observed** drug diffusion coefficient  $D$ , will be the population weighted average of the diffusion coefficients:  $D = P_b D_b + P_f D_f$

■ Can use a diffusion filter to detect binding or a more detailed analysis to determine the dissociation constant ( $K_d$ ).

■ The use of NMR diffusion measurements to study drug binding is sometimes referred to as “Affinity NMR”.

General ref.: *Encyclopedia of Nuclear Magnetic Resonance* (2002) **9**, 364-374.

# Effects of Ligand Relaxation

- The bound and free states of the drug have different relaxation rates.

$$\frac{dS_f}{dt} = -(\gamma\delta g)^2 D_f S_f - \frac{S_f}{\tau_f} + \frac{S_b}{\tau_b} - \frac{S_f}{T_{2f}}$$

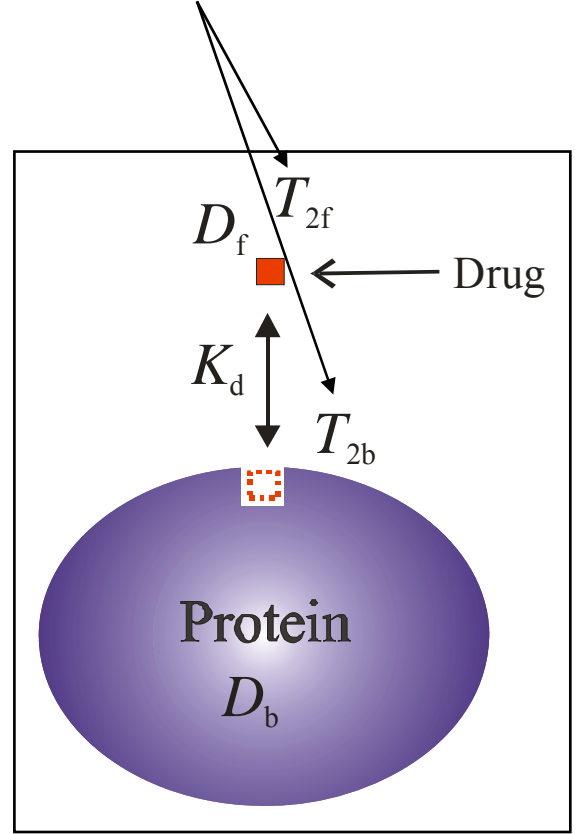
$$\frac{dS_b}{dt} = -(\gamma\delta g)^2 D_b S_b - \frac{S_b}{\tau_b} + \frac{S_f}{\tau_f} - \frac{S_b}{T_{2b}}$$

Ignoring relaxation effects

Population weighted average diffusion coefficient

$$S = \exp\left(-(\gamma\delta g)^2 [P_f D_f + P_b D_b] \Delta\right)$$

Kärger et al *Adv. Magn. Reson.* (1988) **12**, 1-89.

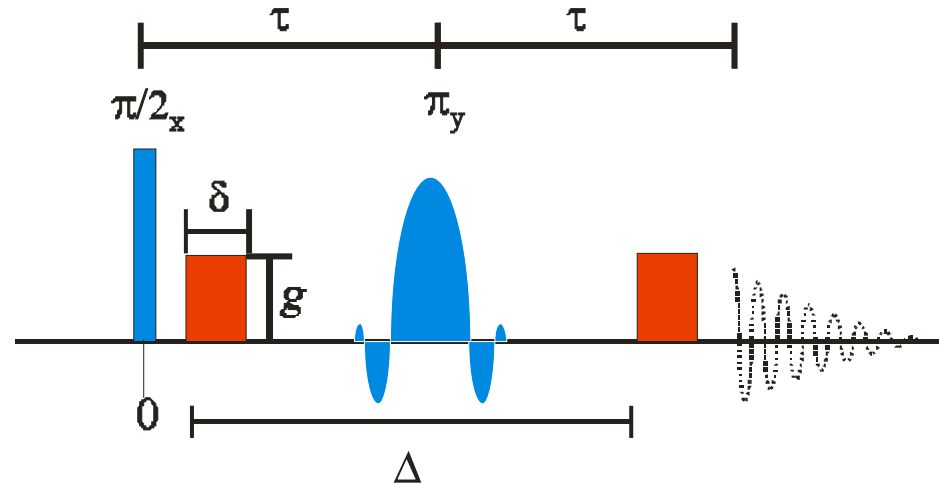


## Relaxation affects the population weighting:

- Ignoring it in the data analysis will result in incorrect answers
- Using it provides an additional source of information.



# PGSE-WATERGATE

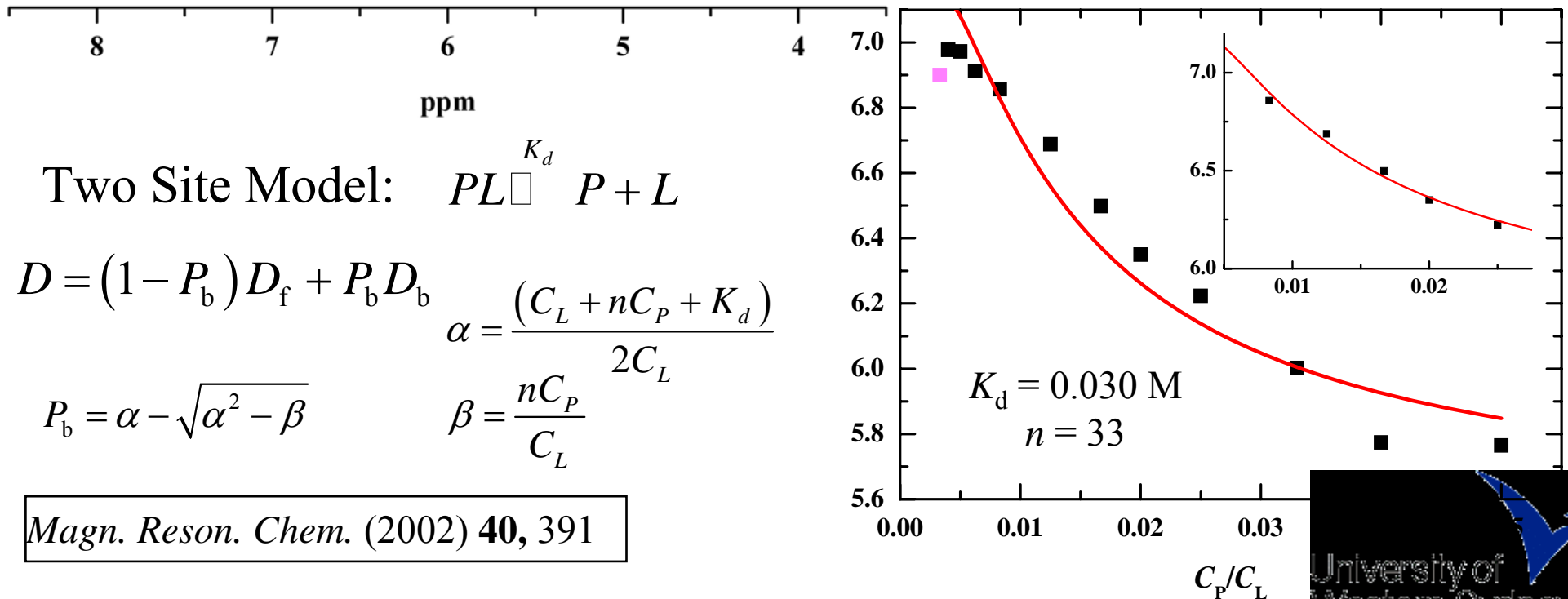
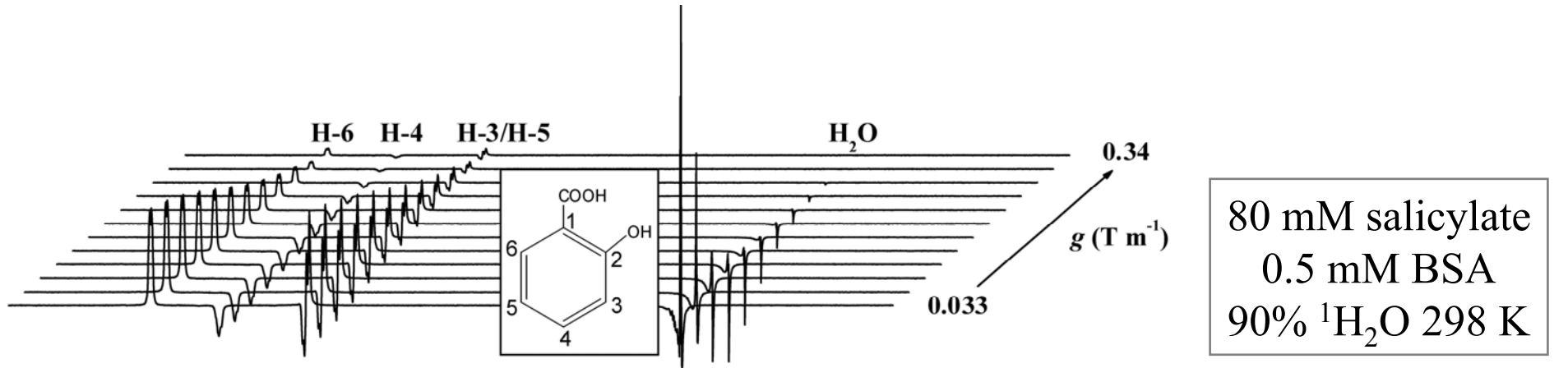


- A Hahn-based sequence is preferable to a Stimulated Echo-based PGSE sequence due to: (1) better removal of the protein resonances due to relaxation, (2) larger drug signal and (3) no complications from cross-relaxation effects.

$$\text{Echo signal} \rightarrow S(g) = \underbrace{M_0 \exp\left(-\gamma^2 g^2 D (\Delta - \delta/3)\right)}_{\text{Attenuation due to Diffusion}} \underbrace{\exp\left(-2\tau/T_2\right)}_{\text{Attenuation due to Relaxation}}$$

initial magnetization
Attenuation due to Diffusion
Attenuation due to Relaxation

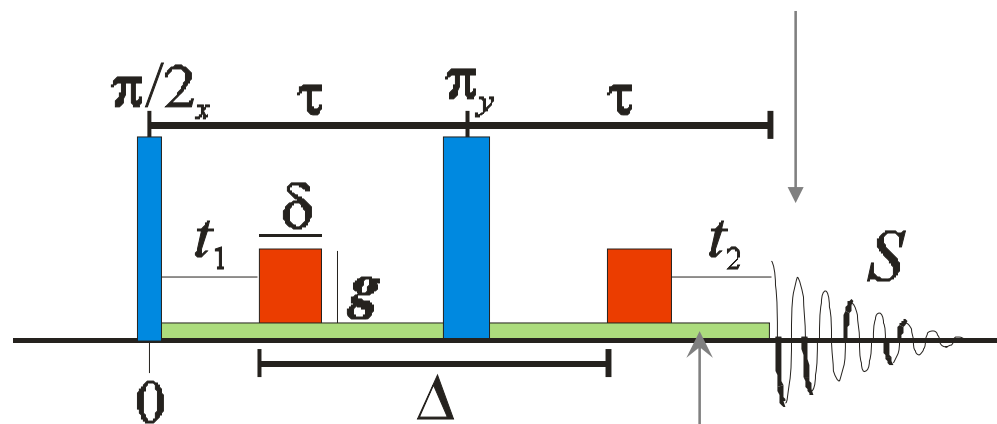
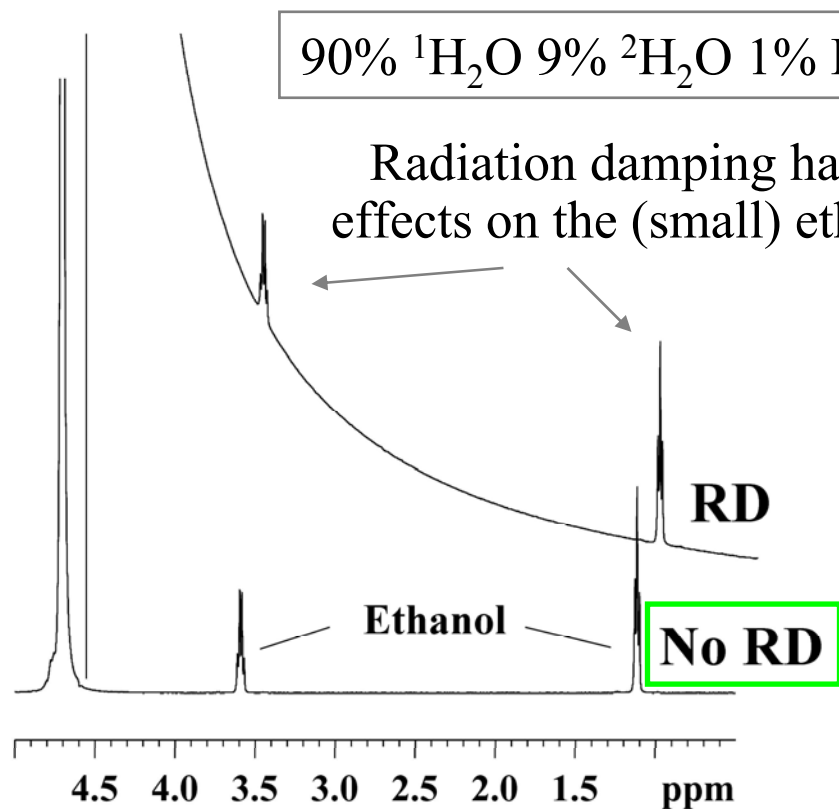
# Salicylate binding to BSA



*Magn. Reson. Chem.* (2002) **40**, 391

# Using Q-Switching to counter Radiation Damping

- The advantages of Q-switching (i.e., the rf circuitry is effectively disconnected) during acquisition are well-known.
- Less well-known are the advantages of switching during the sequence.

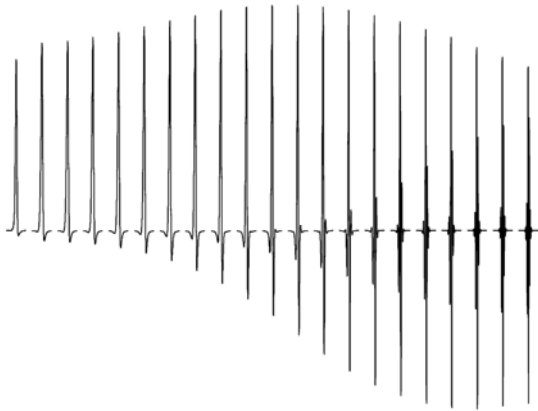


Q-switch

*Magn. Reson. Chem.* (2002) **40**, S128.

# Using the PGSE-Q-Switch Sequence

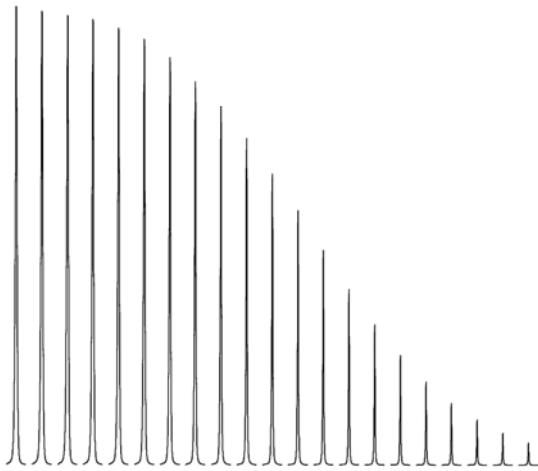
**High- $Q$**



90%  $^1\text{H}_2\text{O}$  at 500 MHz  
 $\Delta = 30$  ms,  $\delta = 2$  ms

Normal PGSE spectra – very distorted  
 (High- $Q$  is the normal spectrometer condition)

**$Q$ -switched**



PGSE-Q-Switch spectra

With  $Q$ -switching we have complete freedom to move the gradient pulses within the sequence and can work at any sample volume.

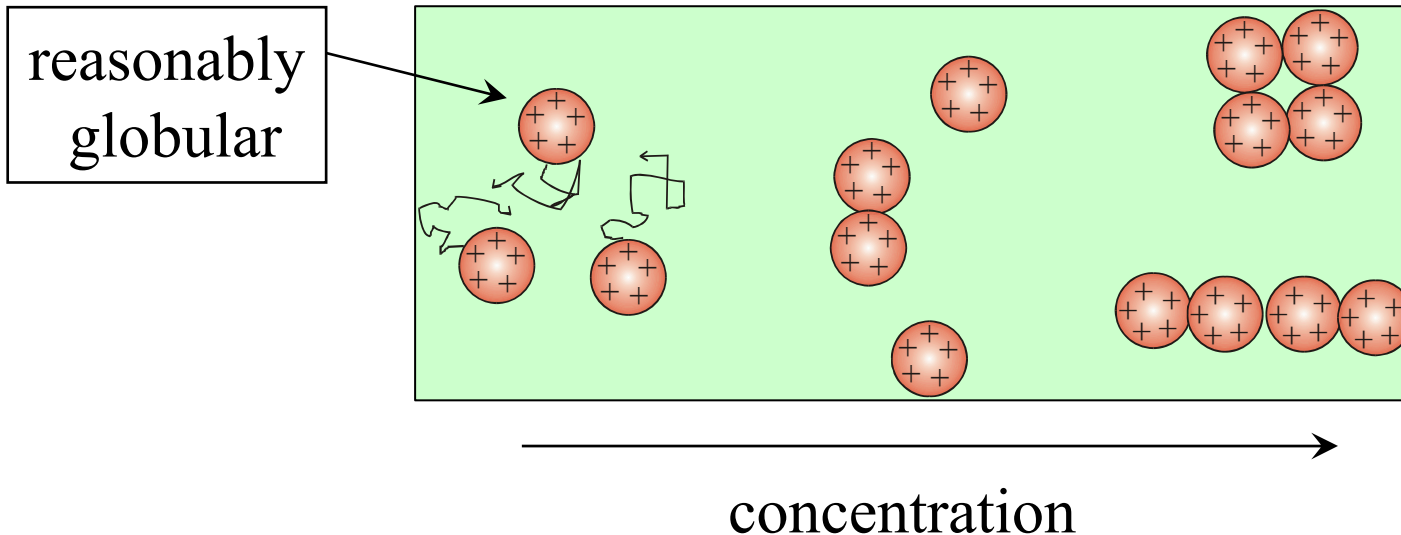
100 Hz  
 $\rightarrow$   
 $g$

# PROTEIN ASSOCIATION

- Involved in normal physiology and in disease (e.g., cataracts, Alzheimer's disease).
- Aggregation is the initial step in the crystallisation process.
- Proteins are both colloids and polymers.
  - ⇒ need to consider size and electrostatic interactions

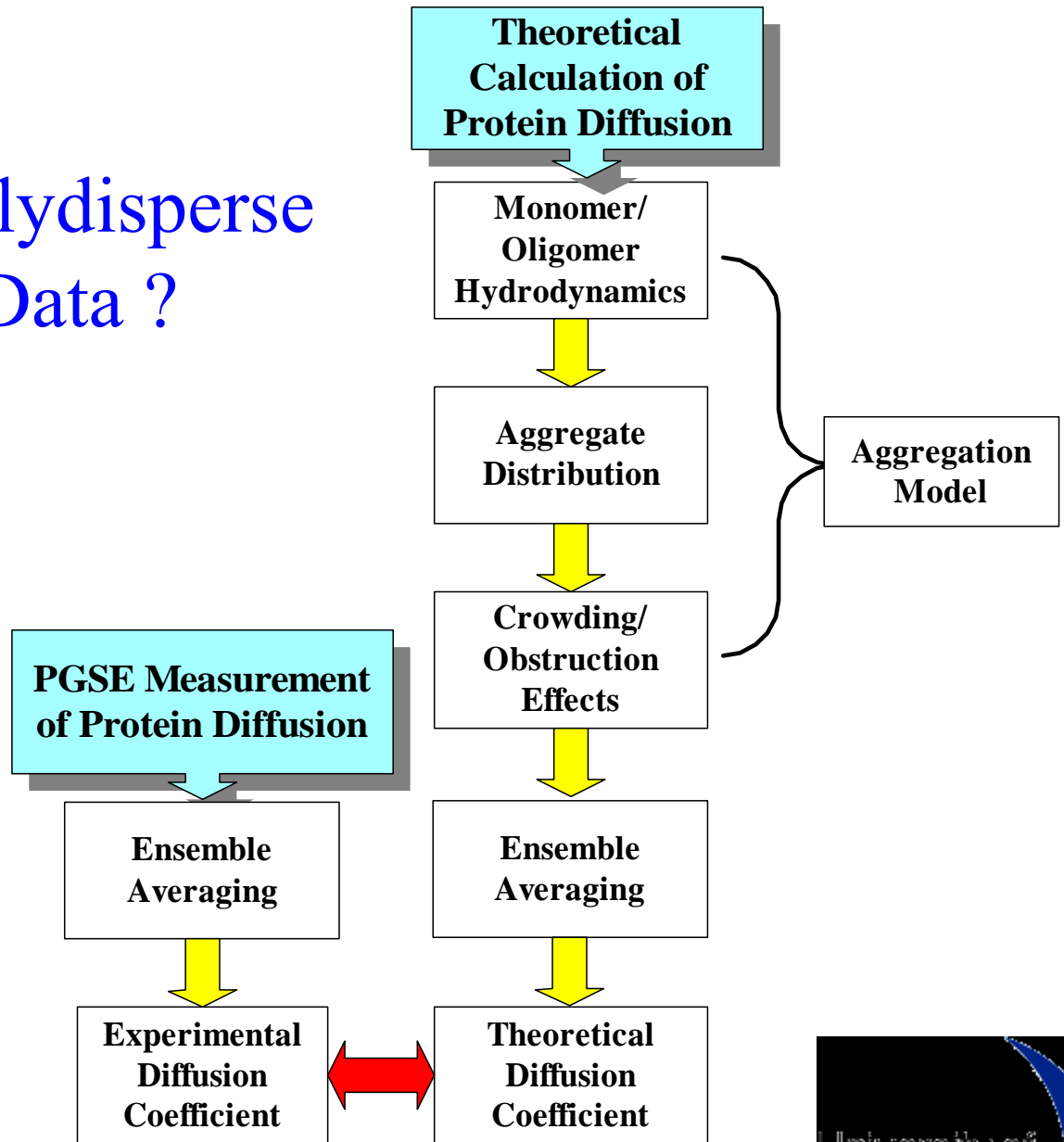
# Aggregation of Lysozyme

- Lysozyme - forms a complicated polydisperse system.



- Its aggregation state is sensitive to the solution environment (e.g., salt concentration, pH).
- The spectra of the different oligomeric states overlap.

# How to Analyse Polydisperse Protein PGSE Data ?



# PGSE of a Polydisperse System

- Assume slow exchange between the different oligomeric states w.r.t.  $\Delta$ .

Multiexponential Echo signal decay  $\rightarrow S(g) \propto \sum_i M w_i n_i \underbrace{\exp(-b D_i)}_{\text{Diffusion}} \underbrace{\exp(-2\tau/T_{2,i})}_{\text{Relaxation}}$

Sum over the all oligomeric states  $\rightarrow$  Magnetisation  $\rightarrow$  Diffusion  $\rightarrow$  Relaxation

Number concentration of the  $i$ th oligomer

$$b = \gamma^2 g^2 \delta^2 \left( \Delta - \delta/3 \right)$$

1st Fudge: Neglect relaxation and normalise

$$E = \frac{S(g)}{S(0)} = \frac{\sum_i M w_i n_i \exp(-b D_i)}{\sum_i M w_i n_i}$$

Probably OK for small oligomers as most of the signal is from the side-chains whose motion is reasonably independent of the overall reorientation rate.

But even highly aggregated protein solutions give single exponential echo decay.

- Some sort of microscopic population weighted **ensemble averaging** of the oligomeric diffusion coefficients to give a single average  $D$  (i.

# Ensemble Averaging of the Diffusion Coefficients

2nd Fudge: expand the exponential

$$\ln(E) = -b \langle D \rangle_W + \frac{b^2}{2} \left( \langle D \rangle_W^2 - \langle D^2 \rangle_W \right)$$

Neglect higher terms

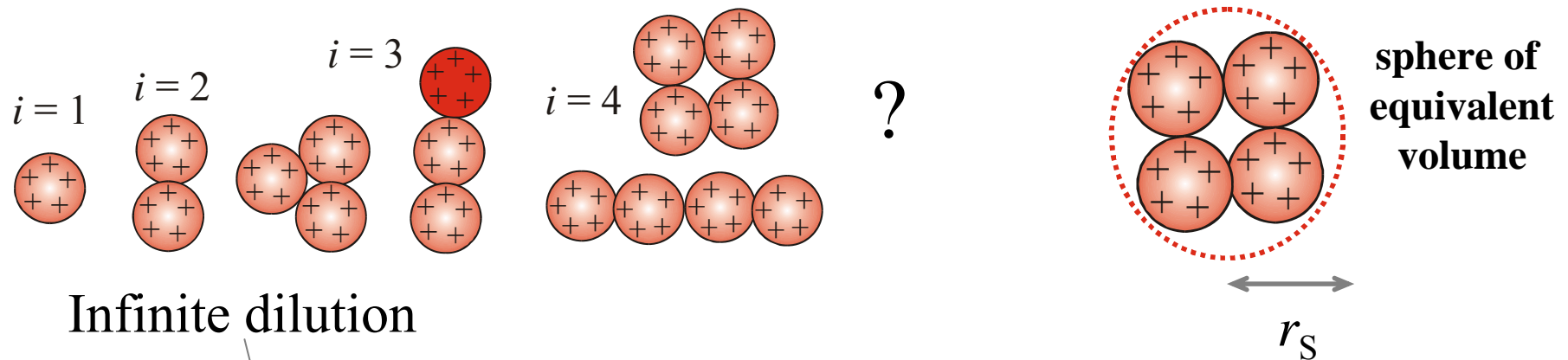
weight-averaged  
diffusion coefficient

$$\langle D \rangle_W = \frac{\sum_i M w_i n_i D_i}{\sum_i M w_i n_i}$$

■ PGSE gives  $\sim \langle D \rangle_W$  (actually  $\langle D \rangle_W^C$ ). Effects of crowding are inherently included.

# Modelling the Oligomer Hydrodynamics

- Monomers and higher oligomers have complex shapes.
- Models (numerical and analytical) do exist.
- However, given the quality of the diffusion data and lack of knowledge of the oligomeric shapes  $\Rightarrow$  assume spherical shapes.



Infinite dilution

$$D^0 = \frac{kT}{6\pi\eta r_s}$$

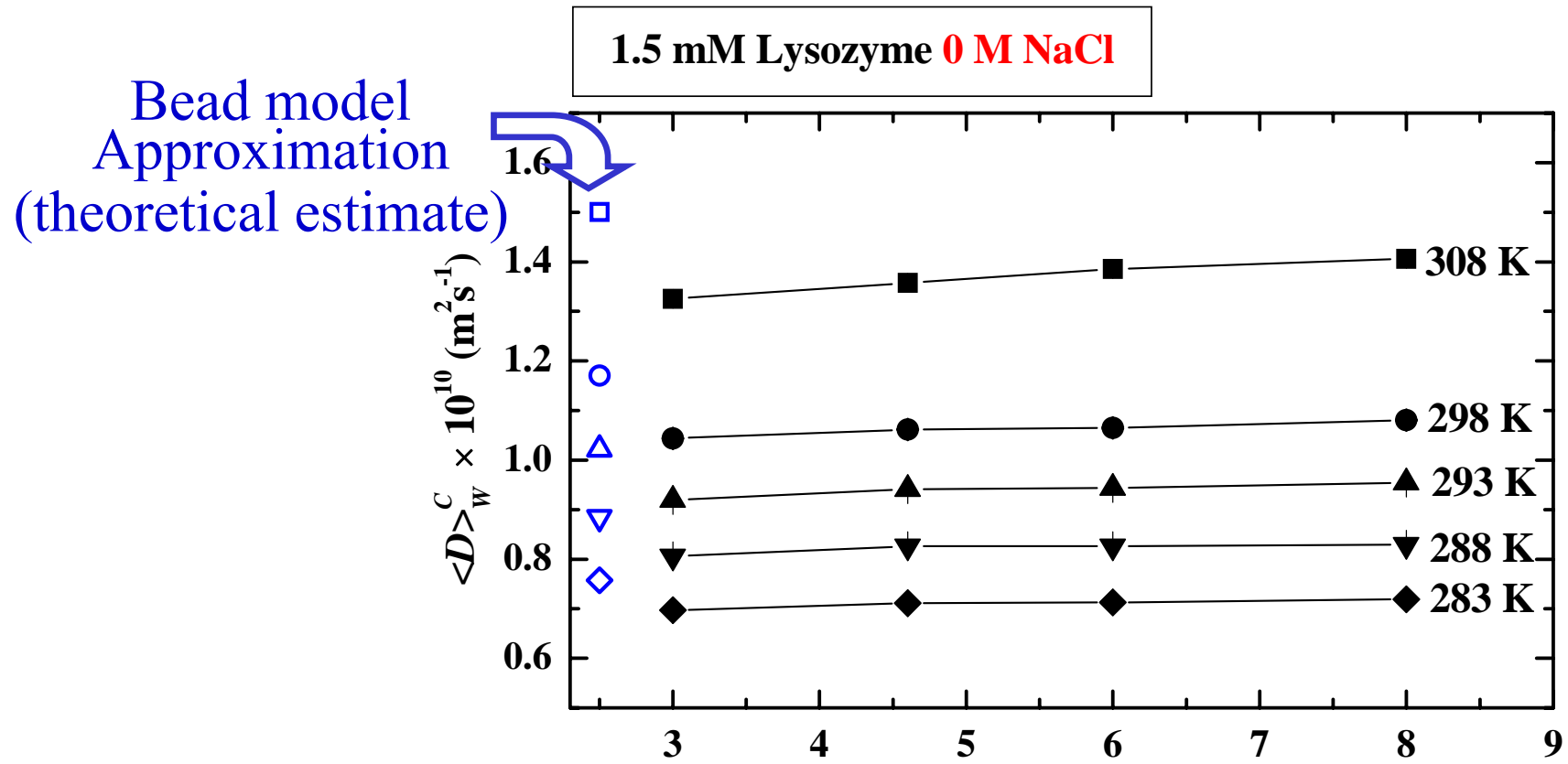


$$D_i^0 = \frac{D_1^0}{\sqrt[3]{i}}$$

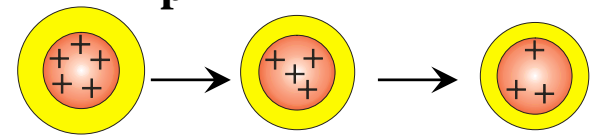
Diffusion coefficient of  $i$ -mer

# Importance of Charge Effects I

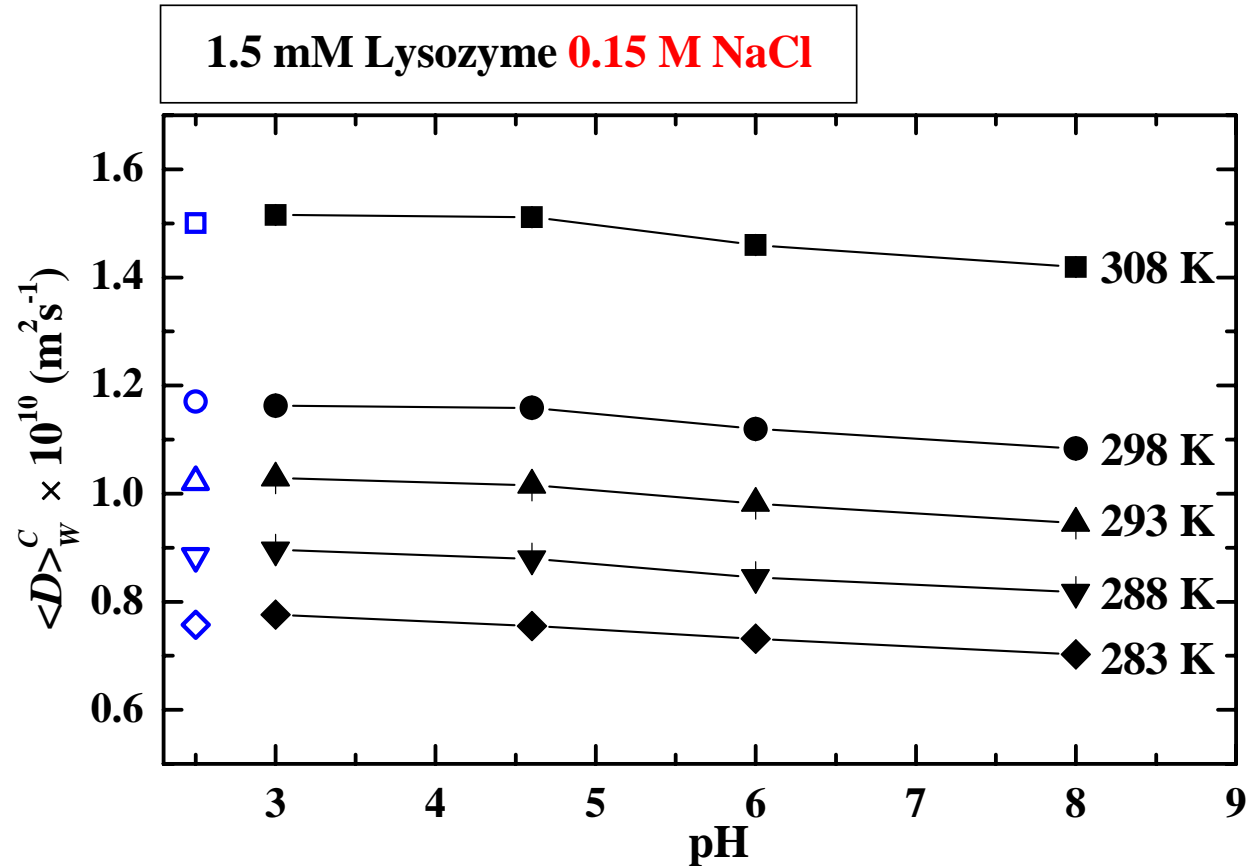
■ Isoelectric point ~ pH 11 → net positive charge at normal pH



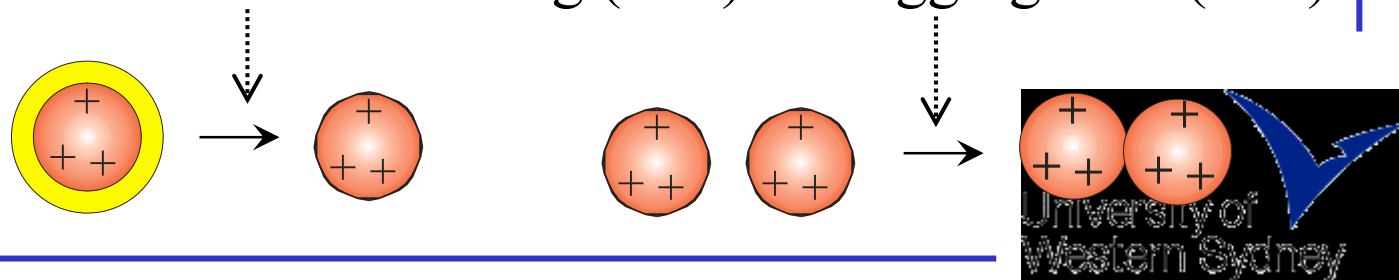
effective size of the lysozyme decreases as the pH increases → less obstruction



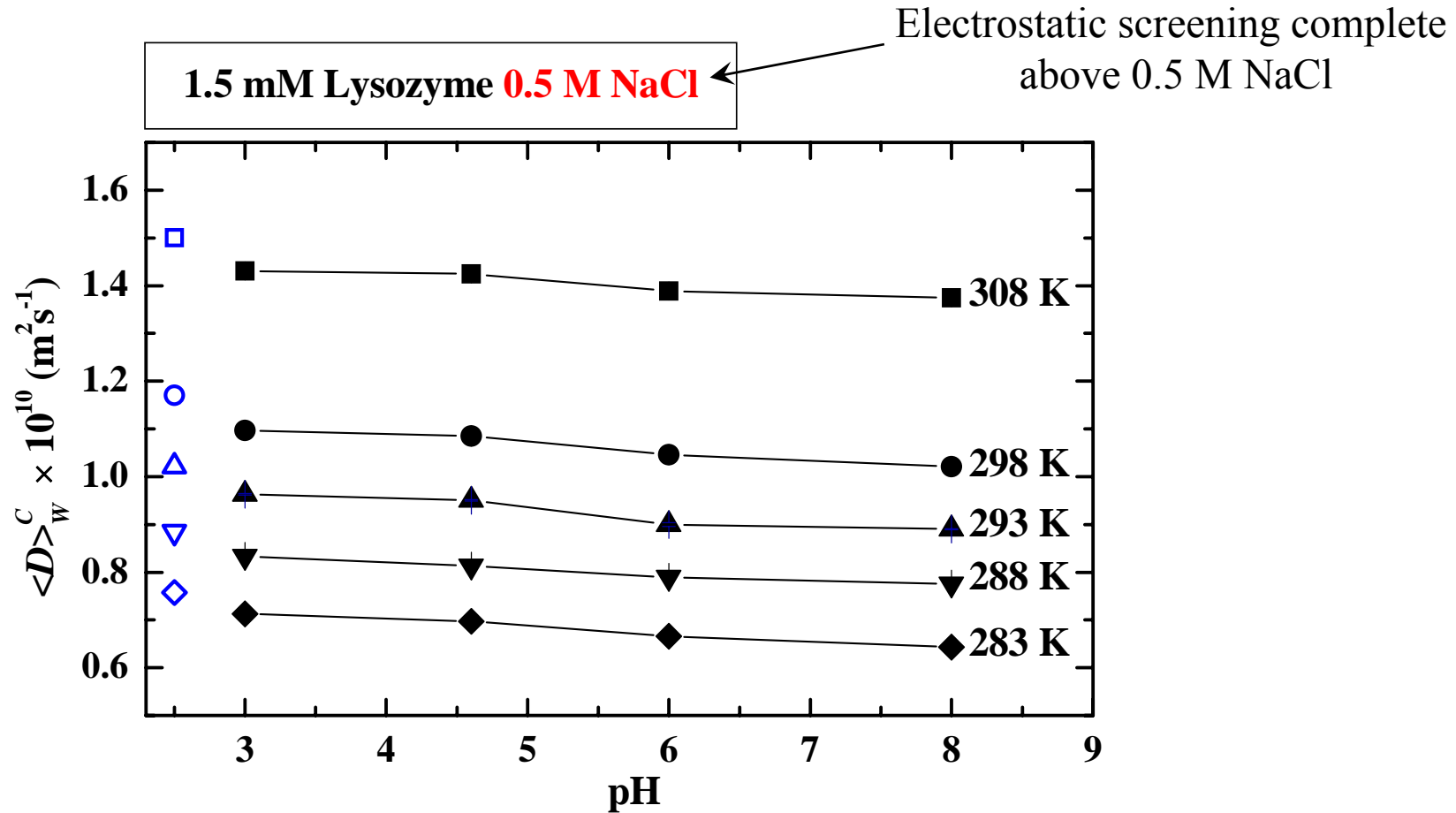
# Importance of Charge Effects II



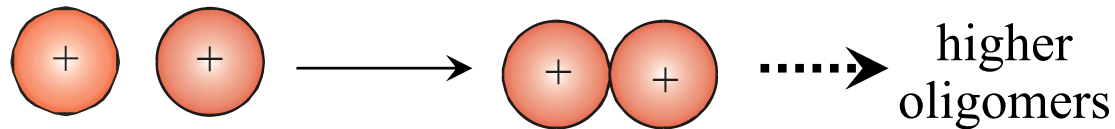
■ Competition between electrostatic screening ( $D \uparrow$ ) and aggregation ( $D \downarrow$ ).



# Importance of Charge Effects III



■ Conditions favourable for aggregation ( $D \downarrow$ ) – a DLVO problem.

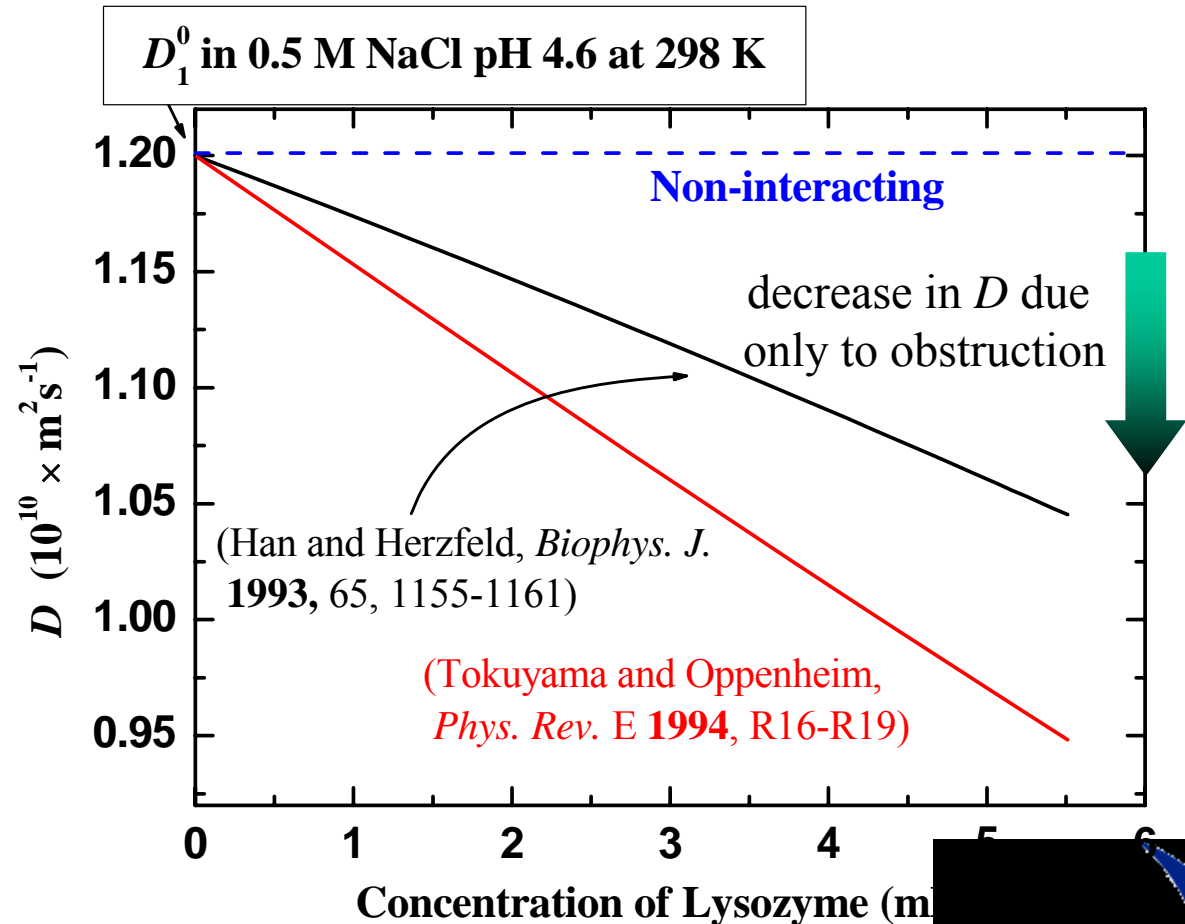


# Two Models for the Effects of Obstruction on Diffusion in Lysozyme Solution

■ Current models for obstruction

do not include:

1. Aggregation effects
2. Electrostatics



# The Theoretical Diffusion Coefficient

■ Combining all the steps and convenient fudges:

Includes concentration effects  
(i.e., obstruction)

$$\langle D \rangle_W^C = \sum_i \alpha_i D_1^0 i^{-\frac{1}{3}} f_C(C) = \langle D \rangle_W f_C(C)$$

Sum over the different oligomeric states

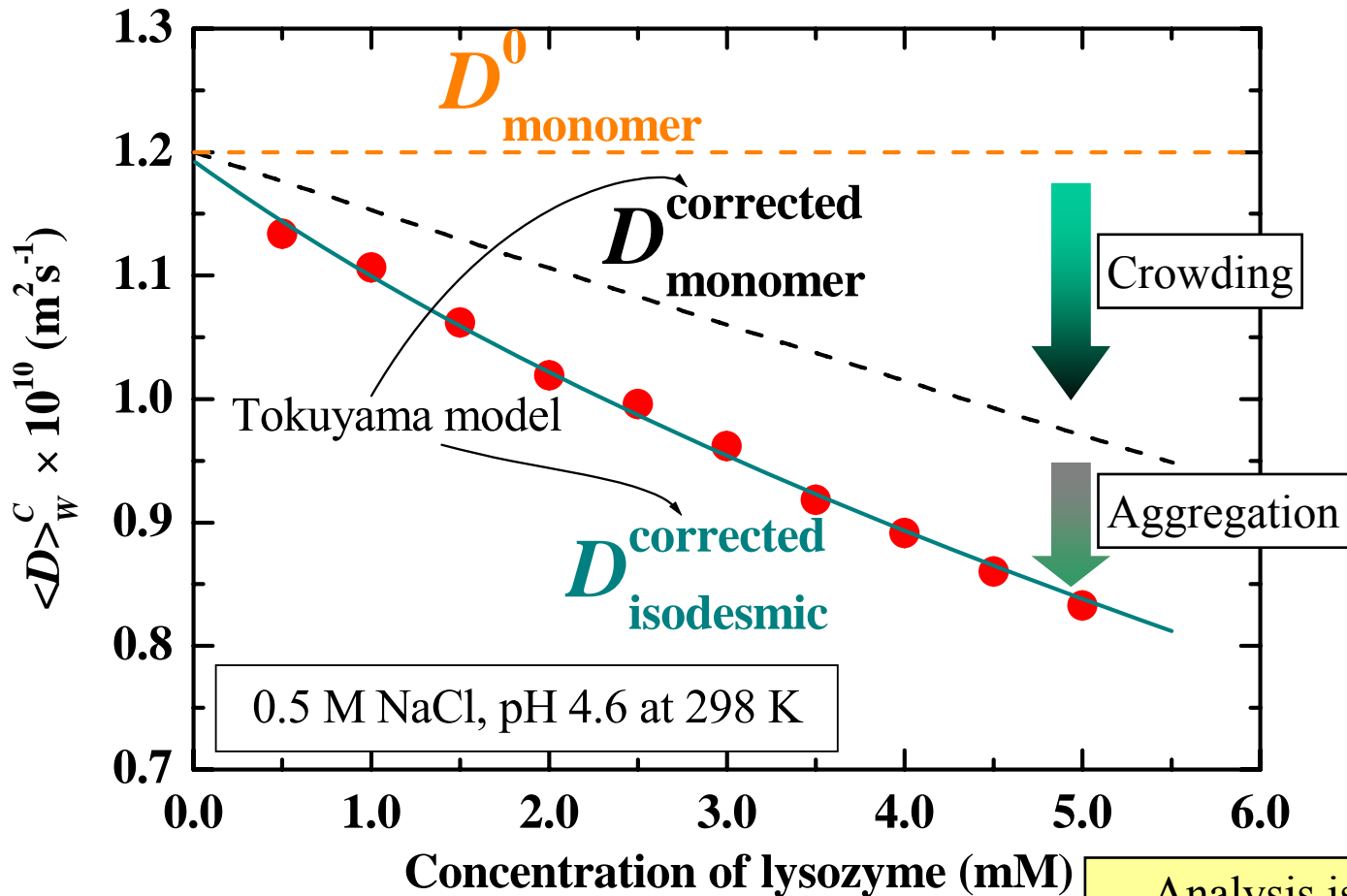
Mole fraction (from aggregation model)

Simplistic obstruction correction

■ The inclusion of ensemble averaging makes this equation identical to that obtained using the fast exchange but without ensemble averaging



# Lysozyme Aggregation



## Simulations:

1. Monomer-Dimer  
 $K_d = 313 \pm 555$   
(corrected)
2. Isodesmic  
 $K_e = 1056 \pm 173 \text{ M}^{-1}$   
(uncorrected)  
 $K_e = 118 \pm 12 \text{ M}^{-1}$   
(corrected)

Analysis is wildly wrong if obstruction is not accounted for.

*J. Am. Chem. Soc.* (1999) **121**, 11503

# What Happens at Higher Protein Concentrations ?

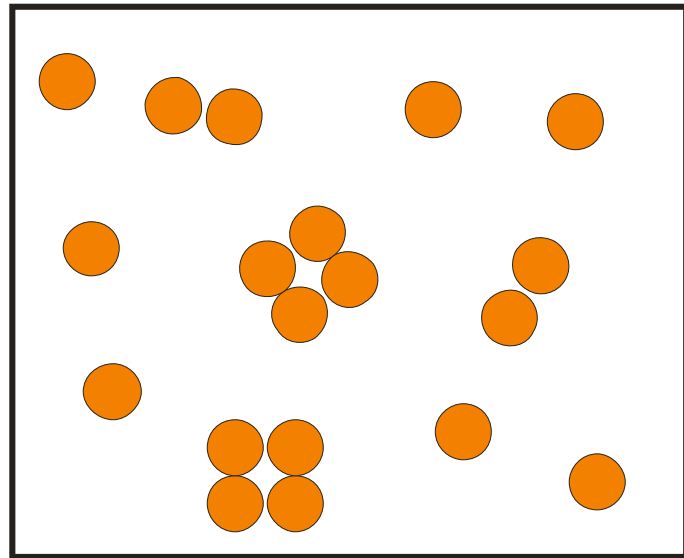
- With reasonably small aggregates neglecting relaxation weighting is ‘reasonable’.
- However, with more polydisperse systems **it must be considered.**

$$\langle D \rangle_{W,R}^C = \sum_i \alpha_i D_1^0 i^{-\frac{1}{3}} f_C(C) \underbrace{\exp(-2\tau/T_{2,i})}_{\text{Relaxation}} = \langle D \rangle_{W,R} f_C(C)$$

- It has the effect of a high molecular weight filter with a very broad cut-off.

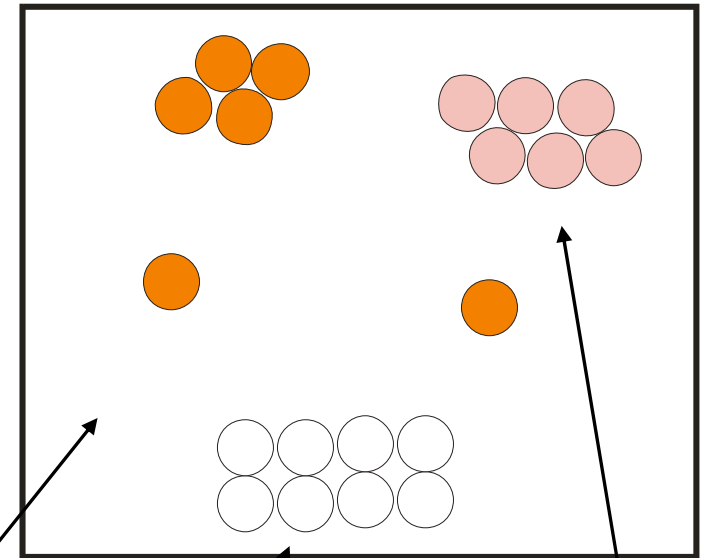
# What the PGSE experiment will 'see'

Supersaturated  
protein solution



All species are NMR invisible

increasing  
association



larger mean  
free paths

NMR invisible

partially  
NMR invisible

The PGSE experiment provides information  
on the smaller aggregates still in solution

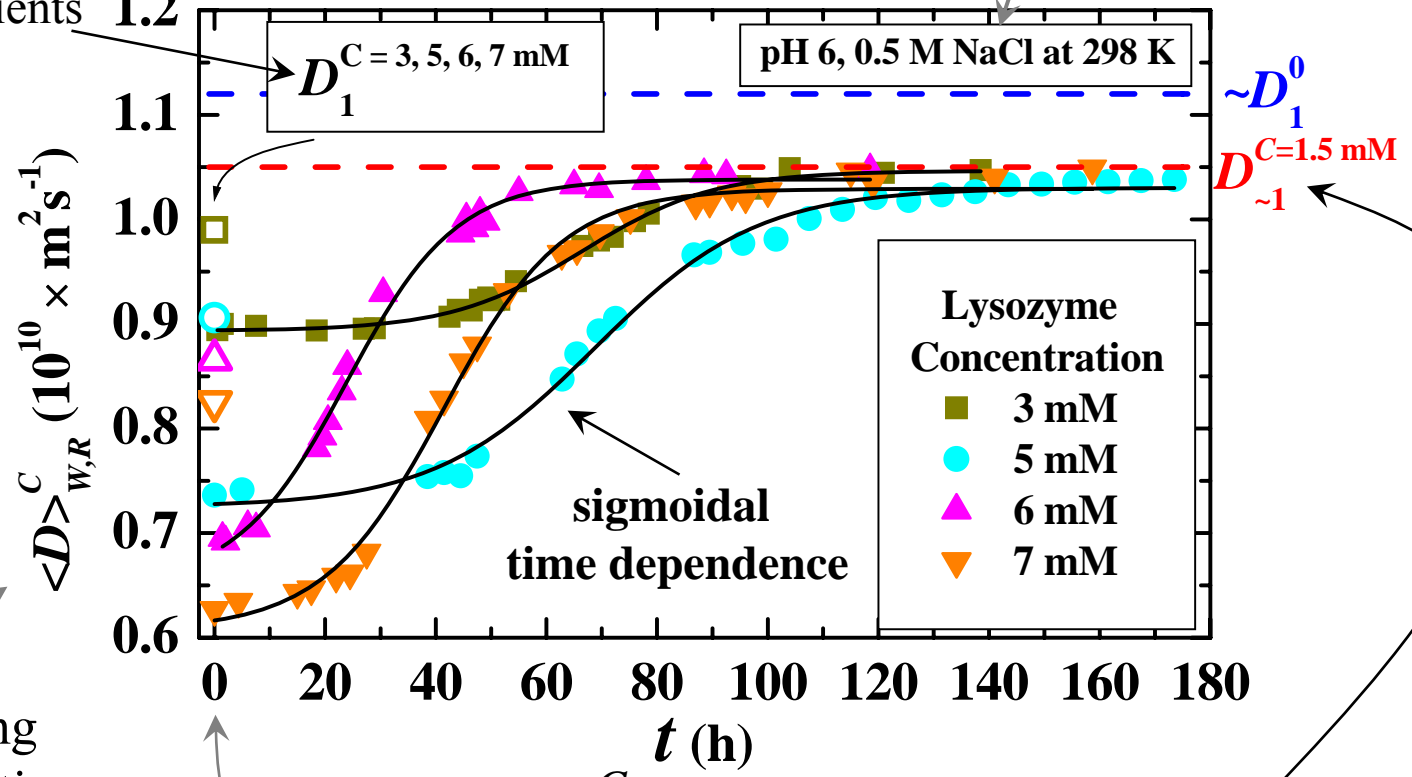
# Probing the Time-Dependence of Aggregation

- As aggregation proceeds the larger aggregates ( $\sim$  ‘solid’ phase) become ‘NMR invisible’ and PGSE measures only those still in solution.
  - The protein remaining in solution diffuses faster due to less obstruction.
  - Gradient strength permitting, the relaxation weighting can be ‘tuned’ to different  $M_w$  ranges.
- $\Rightarrow$  PGSE provides a means to studying the time-dependence of aggregation.**

# Time-Dependence of Lysozyme Aggregation I

obstruction corrected monomer diffusion coefficients

Very suitable conditions for aggregation



Of the aggregating species still in solution

$$\langle D \rangle_{W,R}^C = \frac{\langle D(t_0) \rangle_{W,R}^C - \langle D(t_\infty) \rangle_{W,R}^C}{1 + e^{(t-t_{\text{sigm}})/t_S}} + \langle D(t_\infty) \rangle_{W,R}^C$$

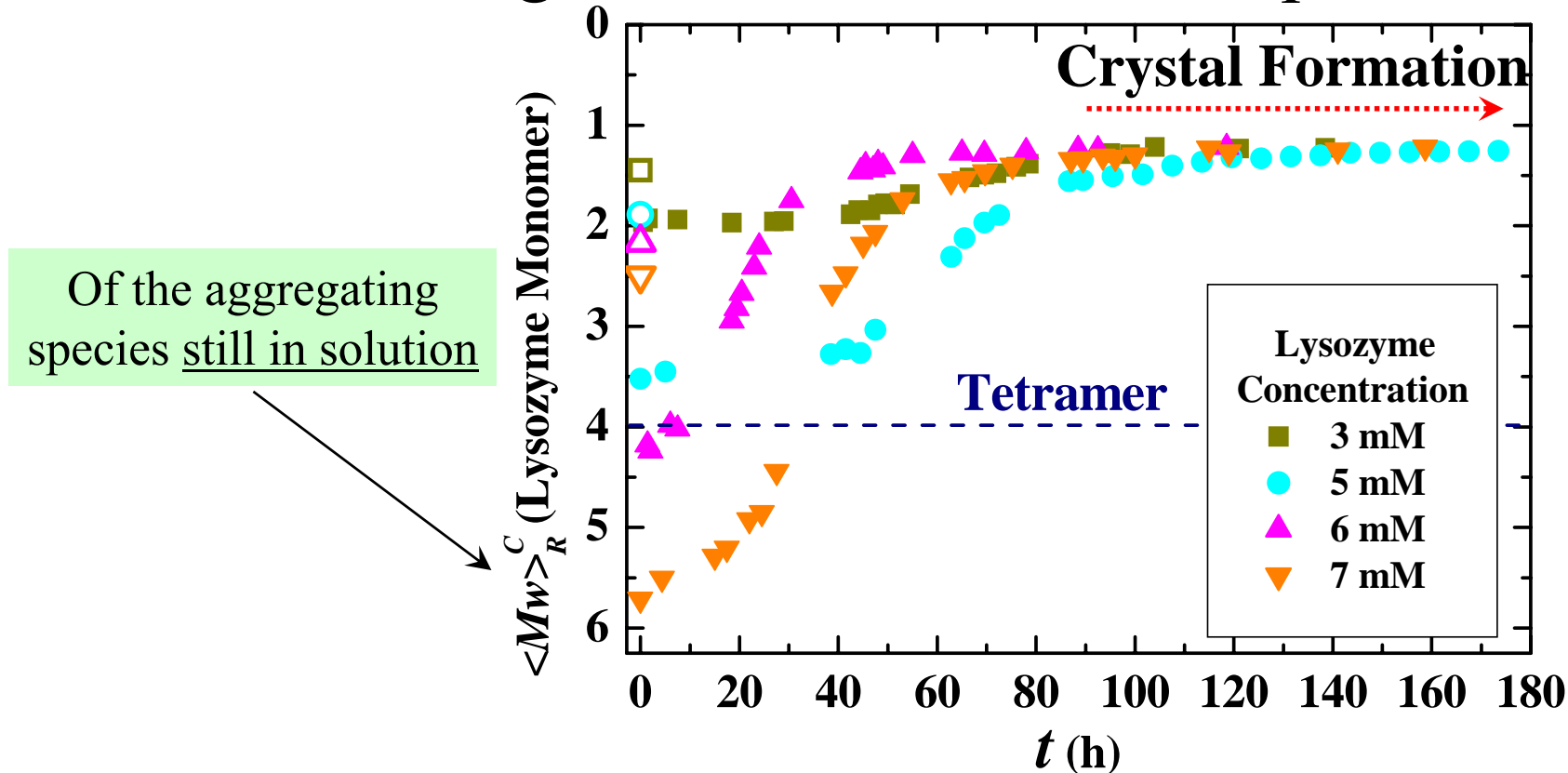
$t_{\text{sigm}}$  ← midpoint of inflection  
 $t_S$  ← time scaling

*Biophys. J.* (2001) **80**, 1585.



# Time-Dependence of Lysozyme Aggregation II

- Recast using the Stokes-Einstein Equation.



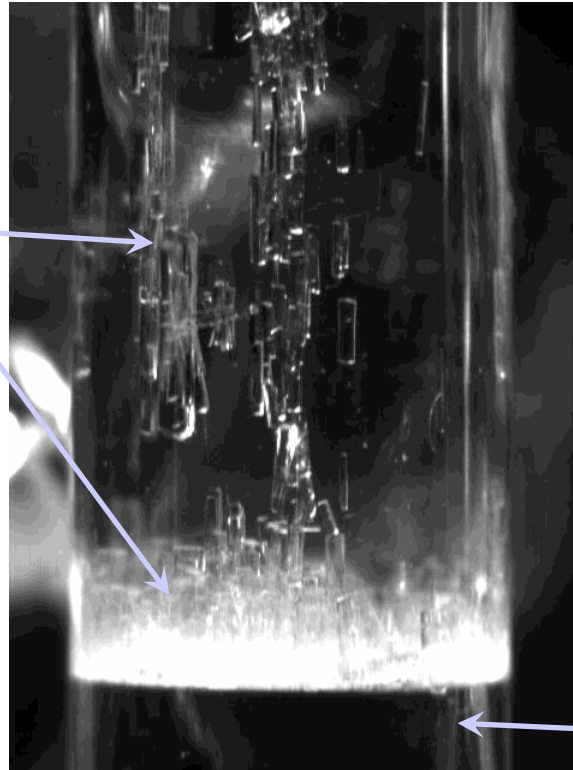
- It has been suggested that the critical nucleus is a tetramer and that the growth unit is an octamer.

# Time-Dependence of Lysozyme Aggregation III

- When the sample tube was removed at the end of the experiment lysozyme crystals were visible.

Initial solution = 5 mM Lysozyme

Lysozyme crystals are invisible to PGSE NMR



Shigemi  
NMR

# Using PGSE to Probe the Time-Dependence of Aggregation

Lysozyme Concentration	$\langle D(t_0) \rangle_w^c$ ( $\times 10^{-10} \text{ m}^2\text{s}^{-1}$ )	$\langle D(t_\infty) \rangle_w^c$ ( $\times 10^{-10} \text{ m}^2\text{s}^{-1}$ )	$t_{\text{sigm}}$ (h)	Slope at inflection ( $\times 10^{-17} \text{ m}^2\text{s}^{-2}$ )	Result
3 mM	$0.89 \pm 0.00$	$1.05 \pm 0.00$	$66.5 \pm 0.8$	9.4	Small no. large crystals
5 mM	$0.72 \pm 0.01$	$1.03 \pm 0.00$	$68.9 \pm 1.2$	14.8	Small no. large crystals
6 mM	$0.65 \pm 0.01$	$1.04 \pm 0.00$	$23.9 \pm 0.9$	27.4	Many small crystals
7 mM	$0.61 \pm 0.01$	$1.03 \pm 0.00$	$41.4 \pm 0.9$	27.0	Many small crystals

■ small no. of critical nuclei  $\longrightarrow$  long induction period and large crystals

■ large no. of critical nuclei  $\longrightarrow$  short induction and small crystals

# RELATED POSTERS

- ❑ Fröba et al (350) “*Mutual Diffusion Coefficients in Fluids by Dynamics Light Scattering*”.
- ❑ Gädke and Nestle (392) “*Apparent Longitudinal Relaxation of Mobile Spins in Thin, Periodically Excited States*”.
- ❑ Lang et al (356) “*Molecular Motions of Calix[4]Arene and Thiocalix[4]Arene in Solution Studied by NMR Relaxation*”.
- ❑ Marek et al (358) “*ESRI Study of Diffusion Processes in Poly(2-Hydroxyethyl Methacrylate) Gels and Concentrated Solutions*”.
- ❑ Fernandez et al (444) “*New Options for Measuring Diffusion in Zeolites by MAS PFG NMR*”.
- ❑ Schönfelder et al (470) “*NMR Studies of Diffusion and Pore Size Distribution on Water Containing Aquifer Rocks and Construction Materials*”.
- ❑ Grinberg (564) “*Ultraslow Molecular Dynamics of Organized Fluids: NMR Experiments and Monte-Carlo simulations*”.
- ❑ Roland et al (584) “*Influence of Phase Transitions on the Mobility of Organic Pollutants in Synthetic and Natural Polymers*”.
- ❑ Sagidullin et al (588) “*Water Diffusion through Assymmetric Polymer Membranes and Polyelectrolyte Multilayers*”.

# Acknowledgements

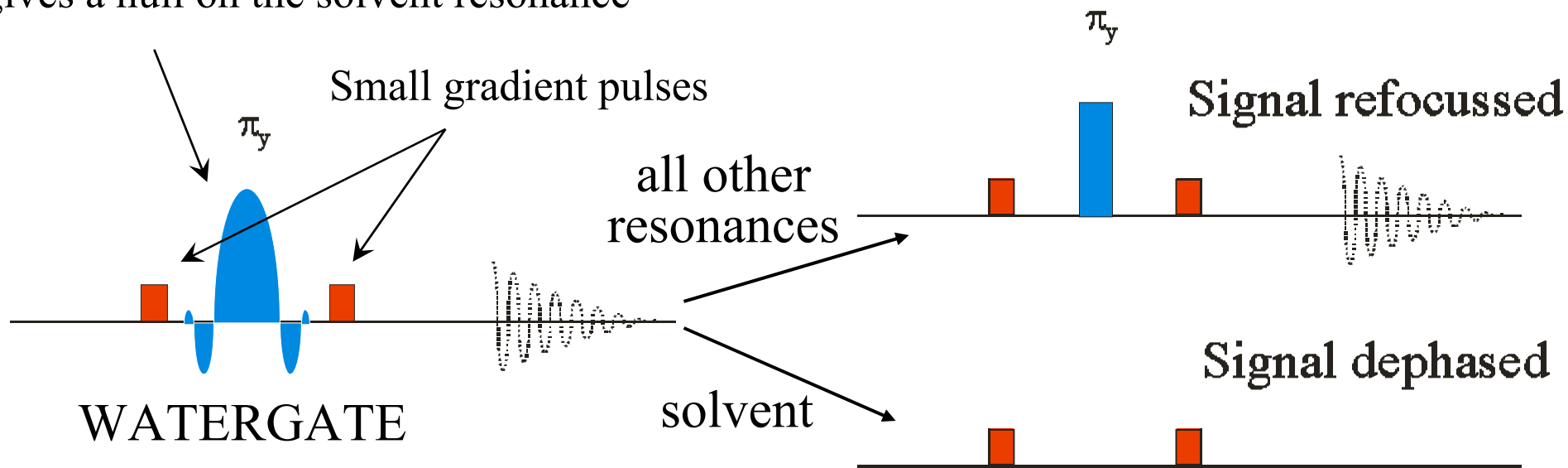
- **NSW State Government BioFirst Award.**
- **Peter Stilbs**, Royal Institute of Technology, Sweden.
- **Olle Söderman**, University of Lund, Sweden.
- **Fredrik Elwinger**, GE Healthcare, Sweden.
- **Yoji Arata**, Water Research Institute, Japan.
- **Hiroyuki Ide**, Ajinomoto, Japan.
- **Fumihiko Tsuchiya**, Applied Biosystems Japan.
- **Cécile Vigouroux**, Royal Institute of Technology, Sweden.
- **Markus Wälchli**, Bruker Biospin, Japan.



# Water Suppression

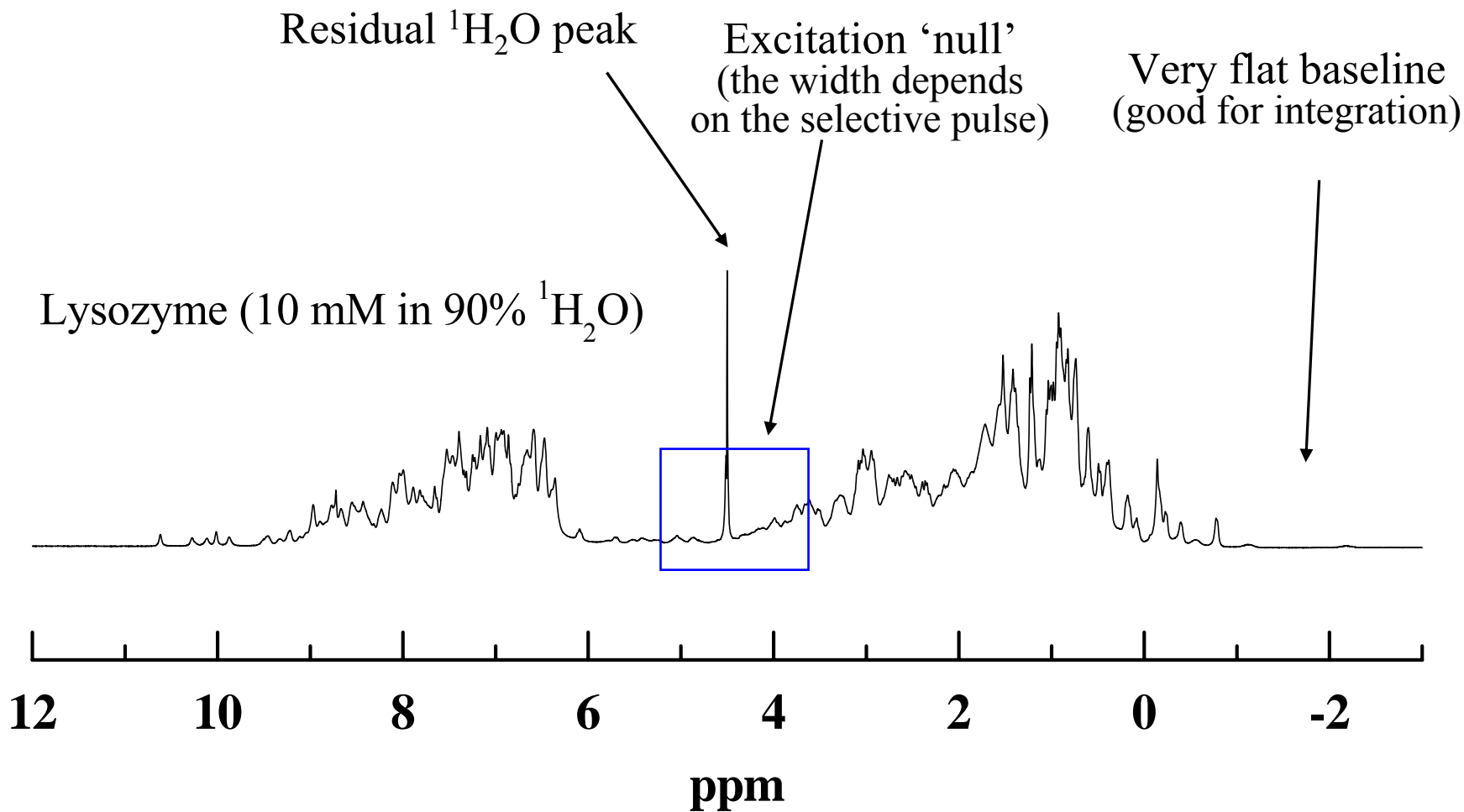
- One of the best suppression techniques is the WATERGATE sequence.

Selective  $\pi$  pulse (e.g., a binomial pulse) that gives a null on the solvent resonance



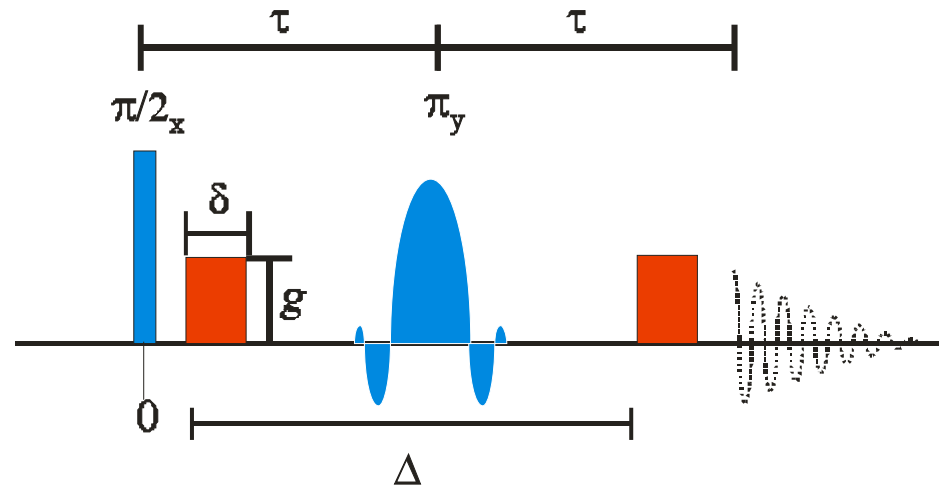
- The WATERGATE sequence resembles a PGSE sequence except diffusion effects (i.e., signal loss) are minimized.

# WATERGATE



■ Suppression of the water peak by a factor of at least **10000**.

# PGSE-WATERGATE



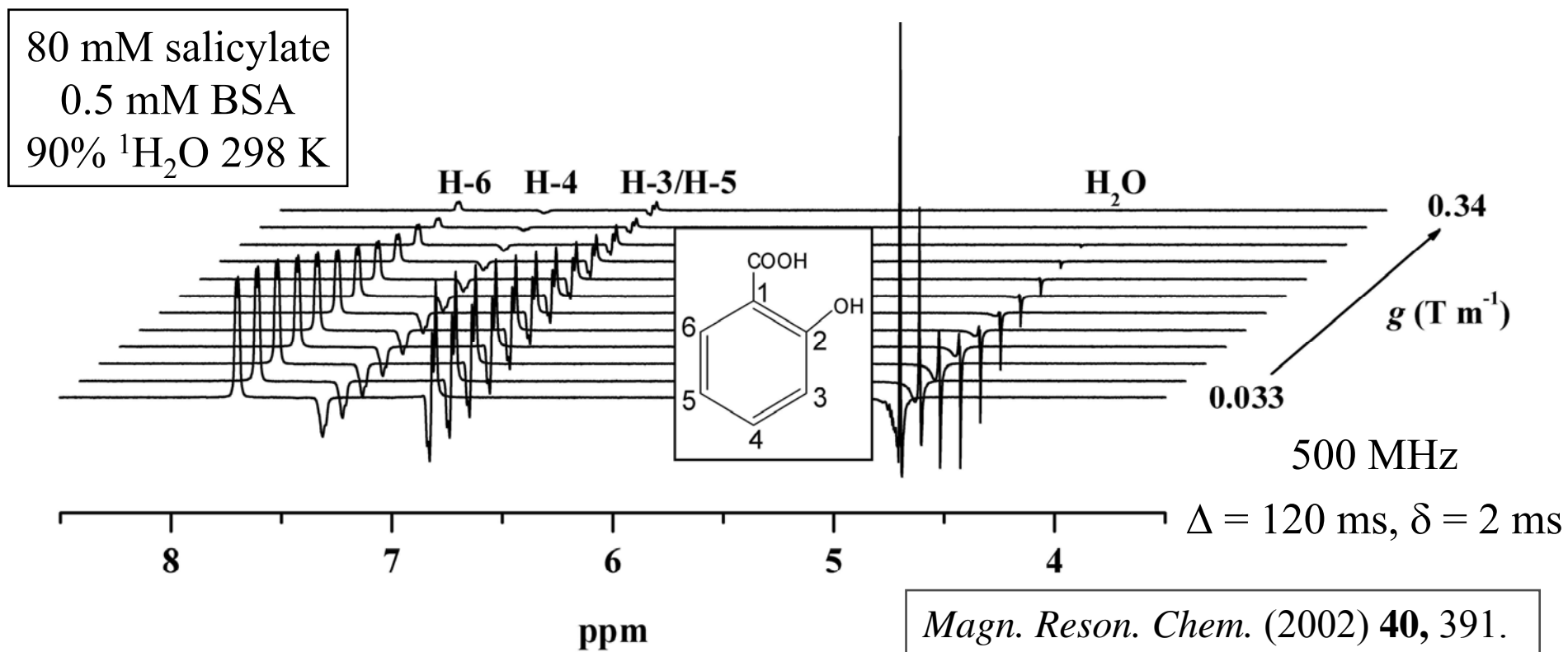
- A Hahn-based sequence is preferable to a Stimulated Echo-based PGSE sequence due to: (1) better removal of the protein resonances due to relaxation, (2) larger drug signal and (3) no complications from cross-relaxation effects.

$$\text{Echo signal} \rightarrow S(g) = \underbrace{M_0 \exp\left(-\gamma^2 g^2 D(\Delta - \delta/3)\right)}_{\text{Attenuation due to Diffusion}} \underbrace{\exp\left(-2\tau/T_2\right)}_{\text{Attenuation due to Relaxation}}$$

initial magnetization
Attenuation due to Diffusion
Attenuation due to Relaxation

# PGSE Spectra of Salicylate in BSA Solution

- The binding of salicylate to albumin governs its transport and tissue distribution.



- The protein background is negligible.
- Unless the solvent suppression is excellent, the three proton resonances appear to give different diffusion coefficients.

# Problems with NMR of Strong Signals

- ‘Strong’ samples present particular difficulties to NMR measurements due to the induction of radiation damping effects.
- Radiation damping is in effect a feedback loop between the sample magnetization and the rf coil/circuitry.

Static field strength

Radiation damping rate constant

$R_{\text{RD}} = \frac{1}{T_{\text{RD}}} = \left[ \frac{\mu_0 \gamma^3 \hbar^2 B_0 c_A}{8kT} \chi_{\text{water}} \right] \eta Q$

Filling factor

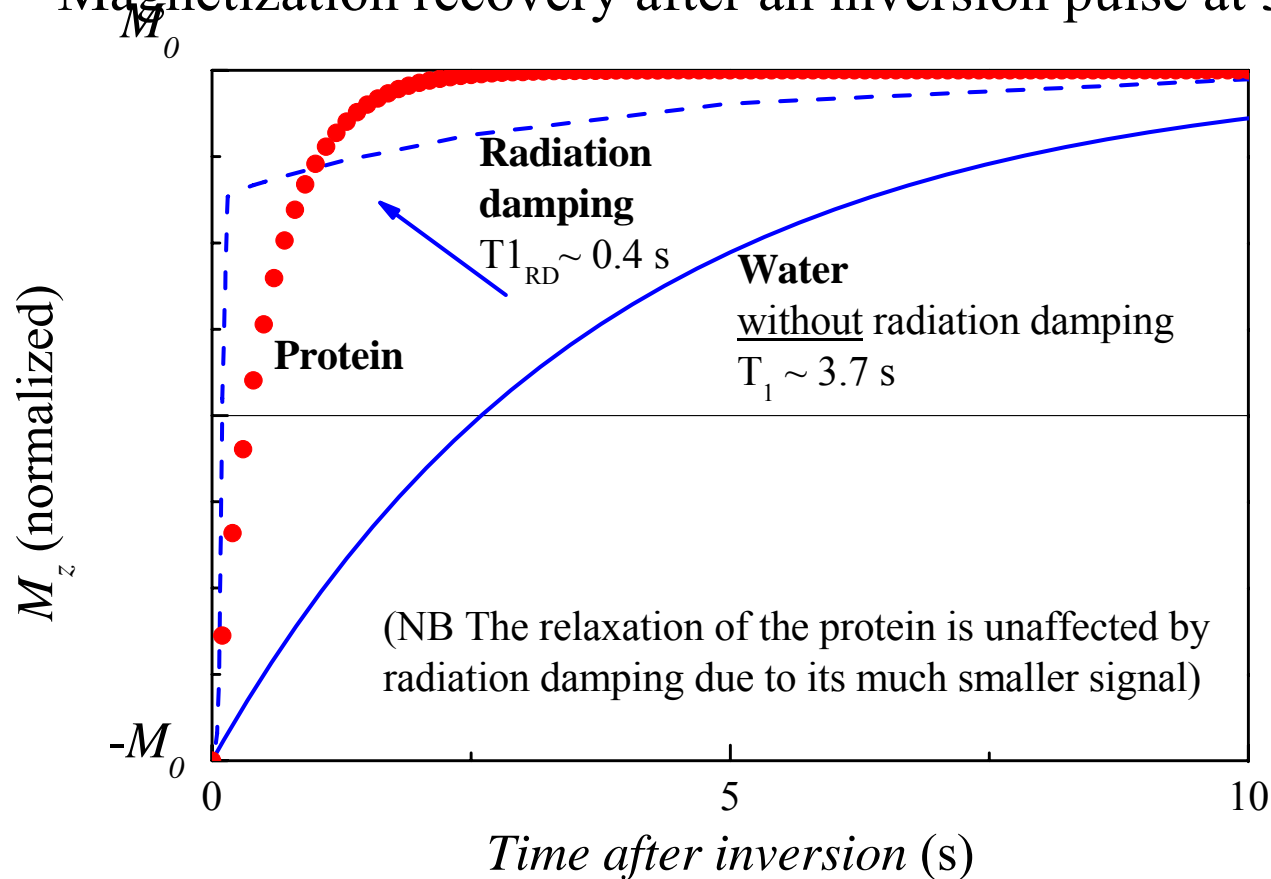
‘Quality’ factor of rf coil

Often:  $T_{\text{RD}} \ll T_1!$

- Gets worse in better spectrometers and more sensitive probes (e.g., cryoprobes). It affects both  $T_1$  and  $T_2$  (and therefore lineshape) and has very deleterious effects on pulse sequences – esp. PGSE sequences.
- Reducing  $B_0$ ,  $\eta$  or  $Q$  is not a solution when trying to detect small resonances.

# Radiation Damping Effects on Relaxation

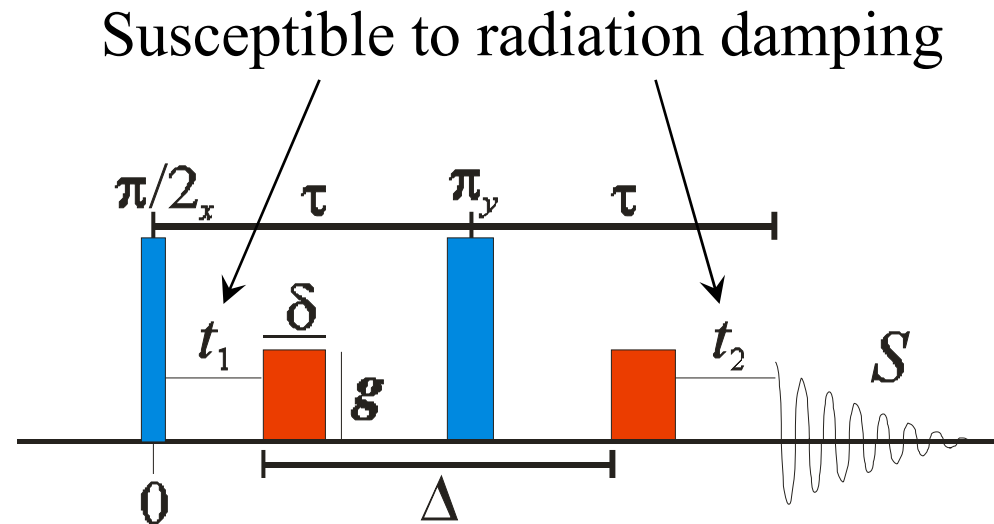
Magnetization recovery after an inversion pulse at 300 MHz



- The non-linear behaviour of the water makes pulse sequence design very difficult.

# Radiation Damping and the PGSE Sequence

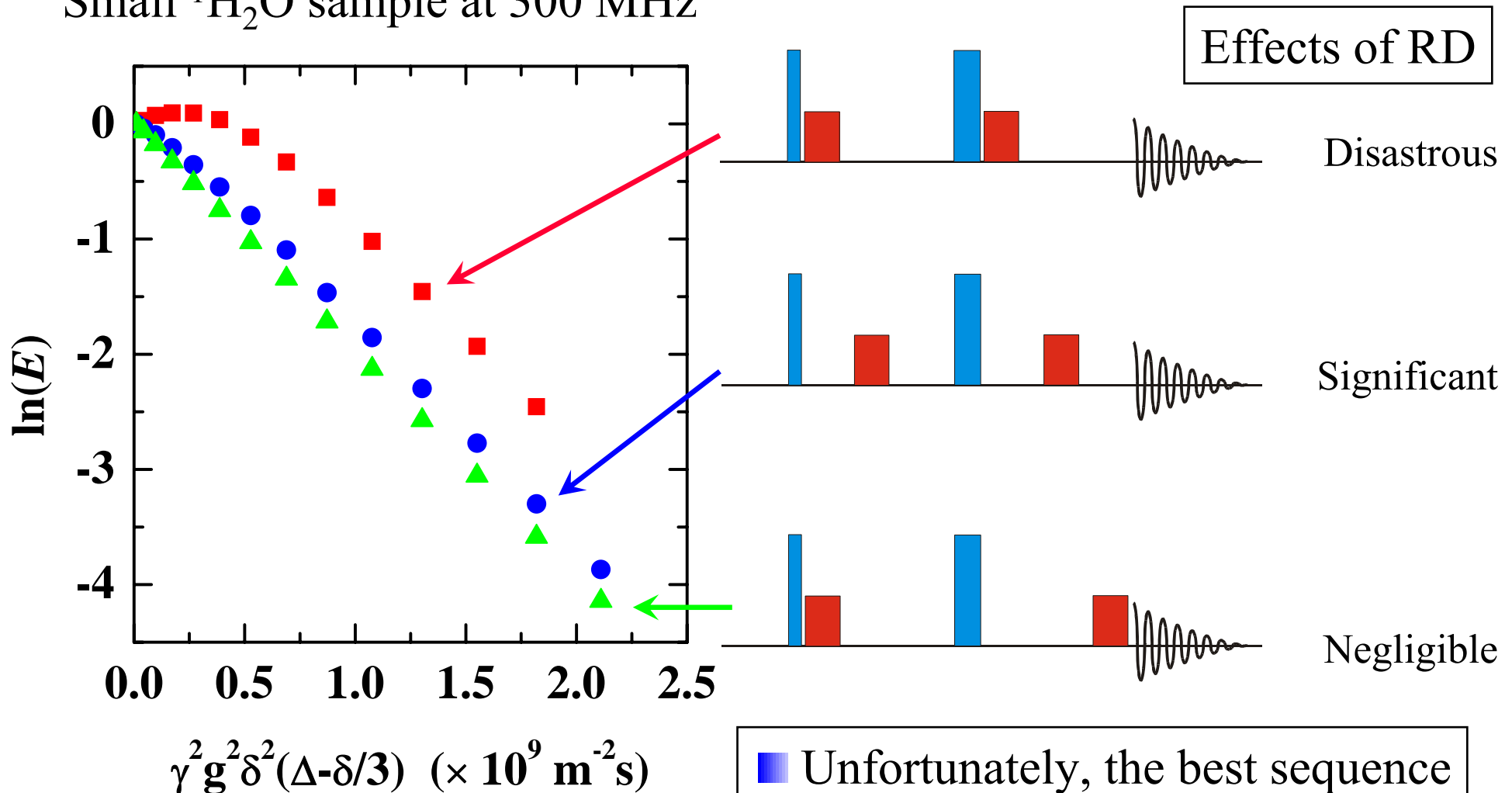
■ Radiation damping effects are  $\sim$  negligible when the magnetization is gradient encoded (the vector sum of the net magnetization is zero).



■ The effects of radiation damping during  $t_1$  are constant but change during  $t_2$  in a complicated manner on the gradient parameters ( $\delta$ ,  $g$ ,  $\Delta$ ).

# Mild Radiation Damping Effects on PGSE

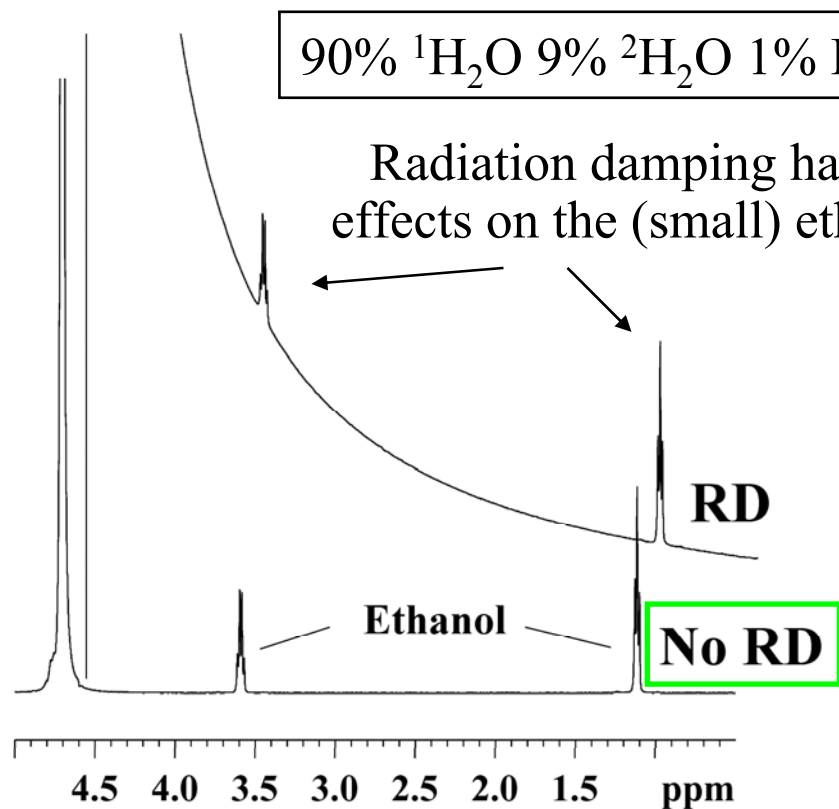
Small  $^1\text{H}_2\text{O}$  sample at 300 MHz



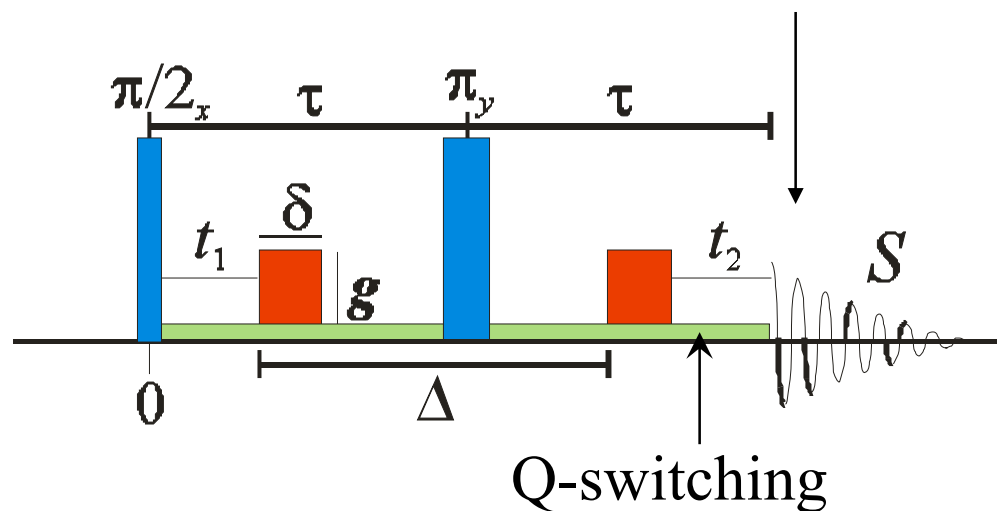
*J. Magn. Reson.* (2001) **150**, 49.

# Using Q-Switching

- The advantages of Q-switching (i.e., the rf circuitry is effectively disconnected) during acquisition are well-known.
- Less well-known are the advantages of switching during the sequence.



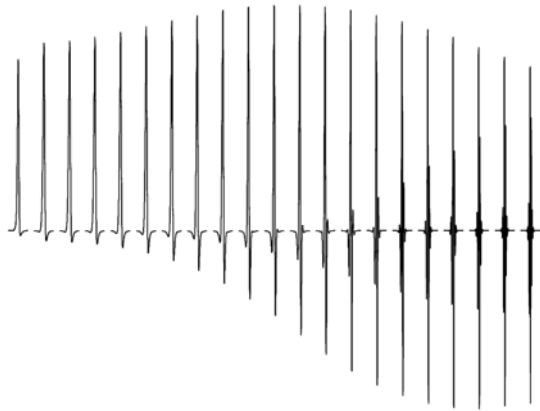
The initial part of the signal is free of radiation damping effects.



*Magn. Reson. Chem.* (2002) **40**, S128.

# Using the PGSE-Q-Switch Sequence

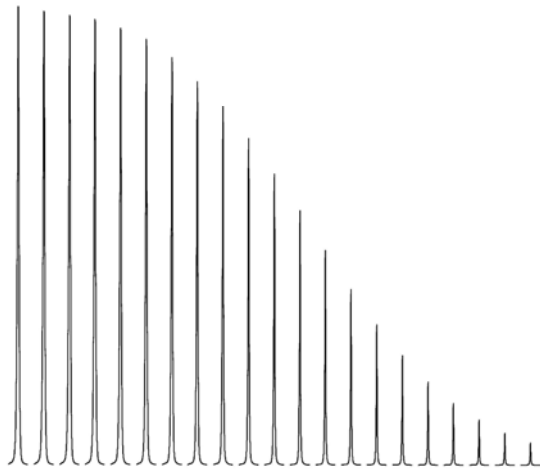
High- $Q$



90%  $^1\text{H}_2\text{O}$  at 500 MHz  
 $\Delta = 30$  ms,  $\delta = 2$  ms

← Normal PGSE spectra – very distorted  
 (High- $Q$  is the normal spectrometer condition)

$Q$ -switched



PGSE- $Q$ -Switch spectra

With  $Q$ -switching we have complete freedom to move the gradient pulses within the sequence and can work at any sample volume.

100 Hz  
 $\rightarrow$   
 $g$



# Diffusive Diffraction

(between parallel PLANES)

