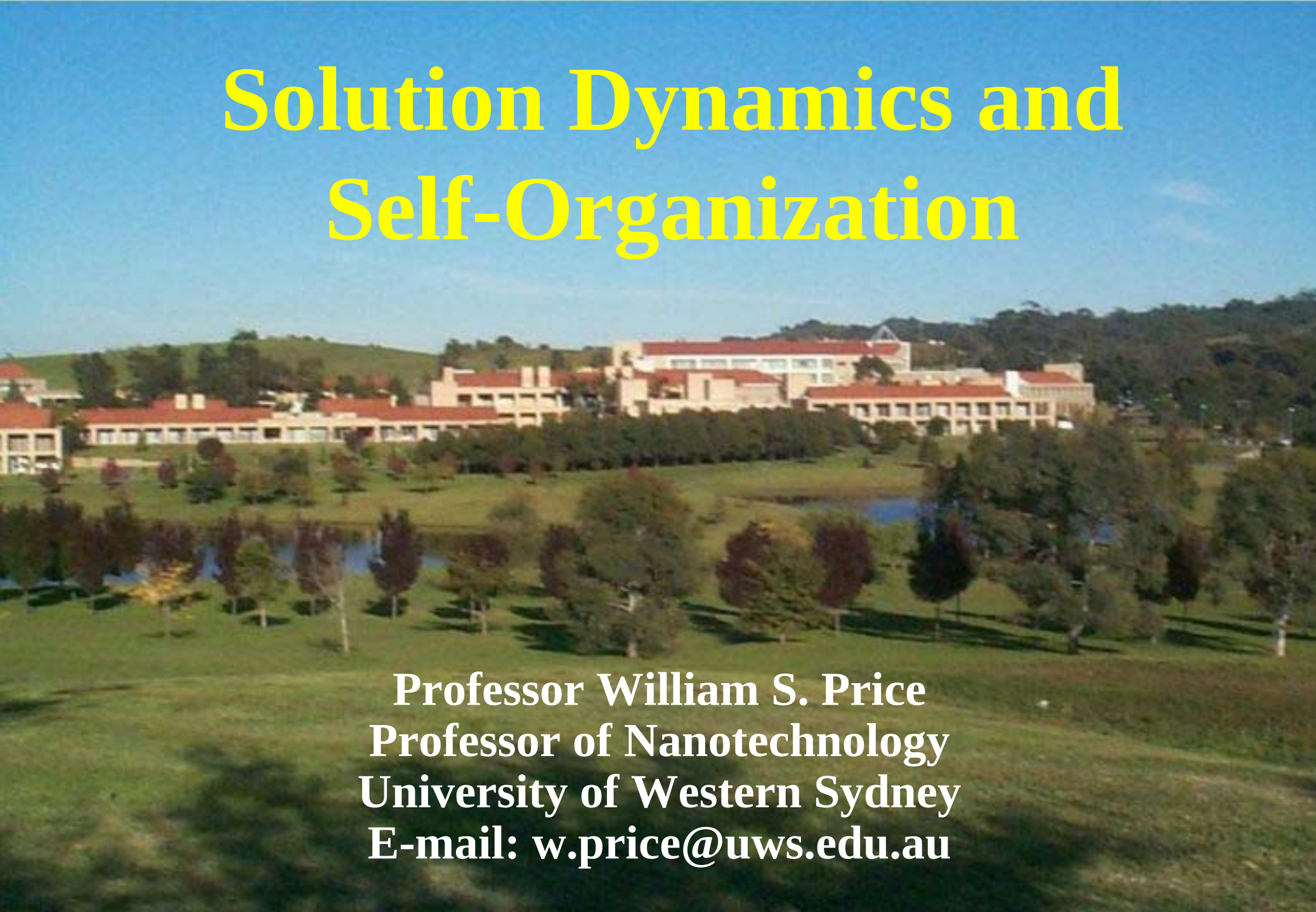
Solution Dynamics and Self-Organization



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Outline

A. Solution Dynamics and Self-Organization.

B. The Pulsed Gradient Spin Echo (PGSE) NMR method for measuring translational diffusion.

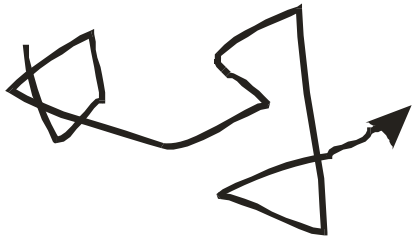
C. Some Examples
   1. Supercooled Water
   2. Isolated Water Molecules
   3. Alcohol Water Systems
   4. Drug Binding
   5. Aggregation and Crystallization of Lysozyme

Solution Dynamics

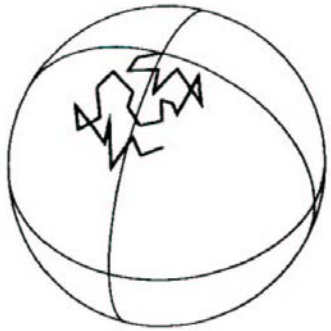
- **Includes:**
  - **Association → self organization and crystallisation**
  - **Binding (e.g., drug – protein or drug -DNA)**
  - **Phase changes**
  - Bio-thermodynamics (e.g., macromolecular crowding effects)
  - Exchange (e.g., transmembrane)
  - ….

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

Types of Motion



Translational (self-) Diffusion, D (m^2s^{-1})



Time taken to reorientate by ~ 1 radian

Reorientational Correlation Time, τ_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.

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Mw and Motion

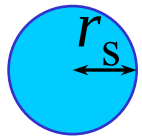
Translational diffusion

Stokes-Einstein equation
(only holds at infinite dilution)

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

← friction coefficient

sphere



$$f = n\pi\eta r_S$$

n = 4 (slip), 6 (stick)

Species	T (K)	D (m^2s^{-1}) × 10^9	M_W
H_2O	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

$$Mw \uparrow \quad \tau_c \uparrow \quad T_2 \downarrow$$

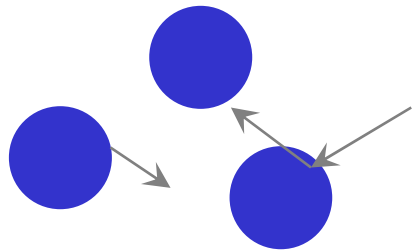
Diffusion as a Probe of Organization

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

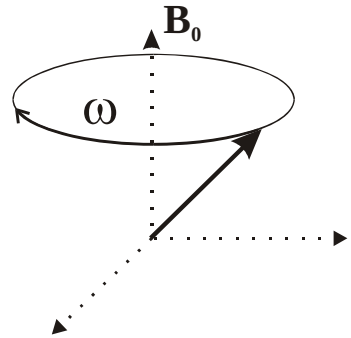
- Using spatially well-defined magnetic field gradients to spatially encode the translational motion of spins – this includes diffusion, flow, turbulence ….
- PGSE NMR measures self-diffusion not mutual diffusion.
- Also known as q-space imaging, PGSE (Pulsed Gradient Spin-Echo), DOSY (Diffusion Ordered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

The Mechanics of NMR Diffusion Measurements

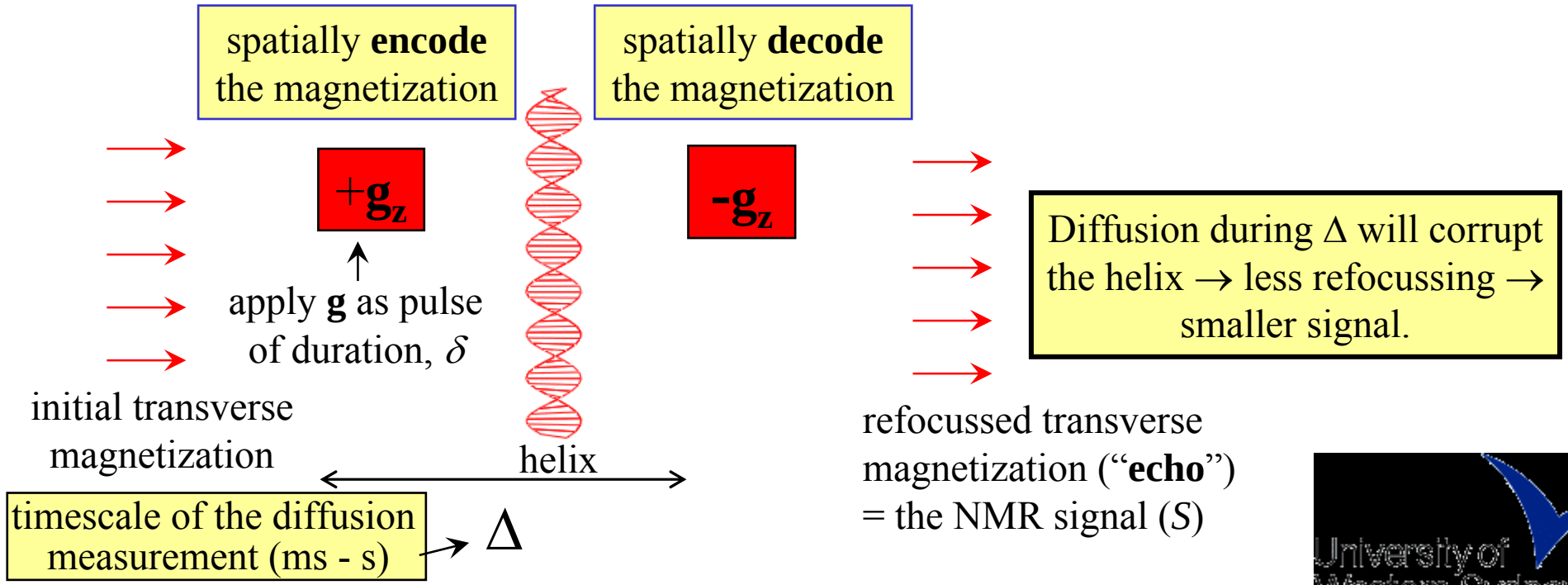
Larmor Equation

Resonance frequency (rad s^{-1}) ⟵ $\omega = -\gamma \mathbf{B}_0$ ⟵ Strength of the static magnetic field (T)

Gyromagnetic ratio (rad s^{-1} T^{-1})



- If the homogeneous field **(B_0)** is replaced by a gradient directed along the *z*-axis **(g_z)**, then ω will vary spatially along the *z*-axis: $\omega(z) = \gamma \mathbf{g}_z z$



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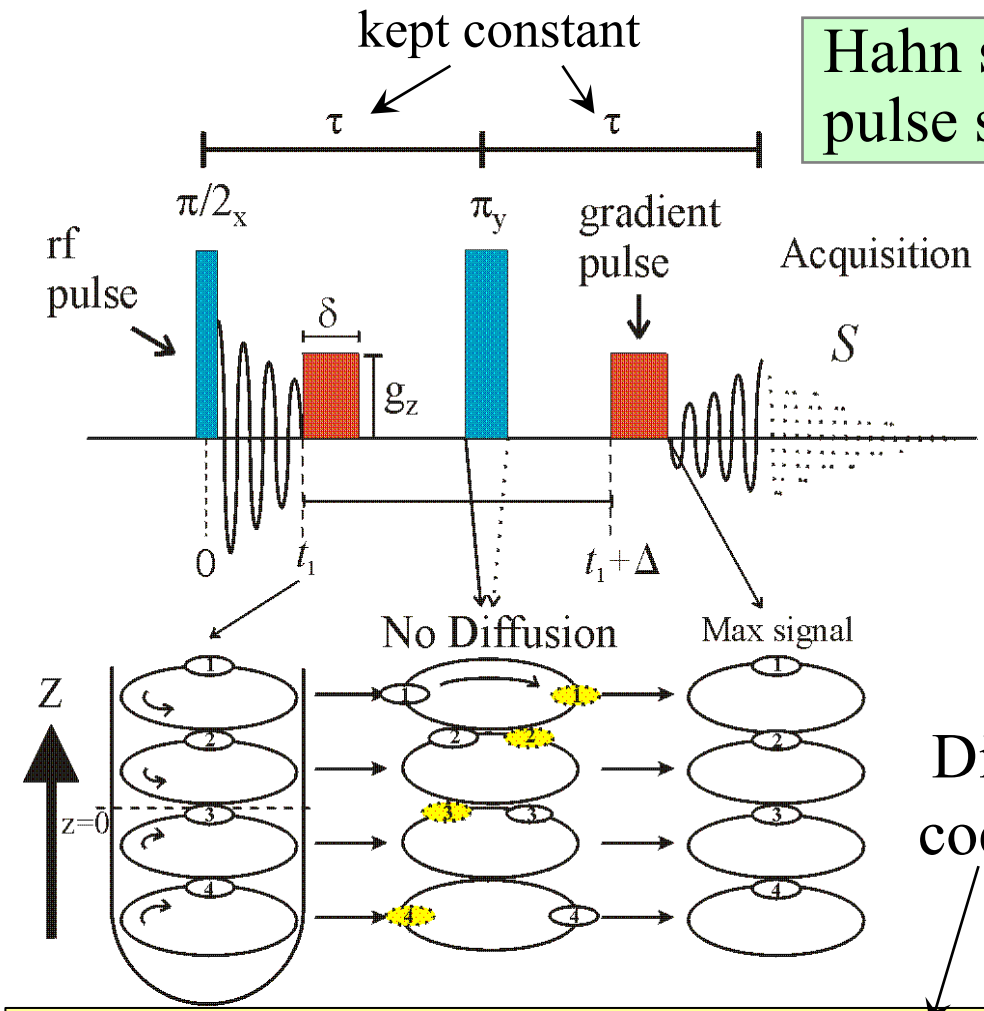
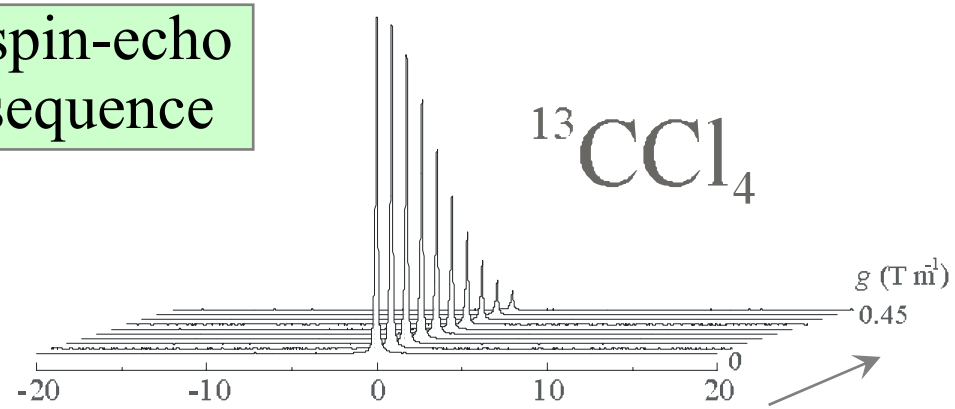
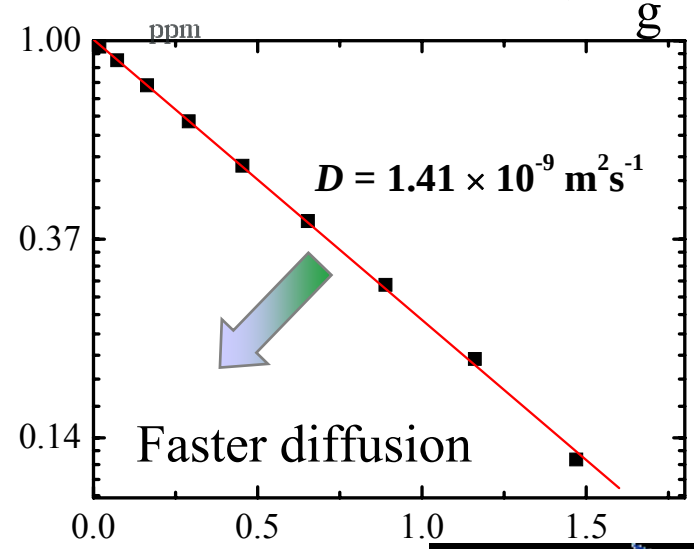
The PGSE NMR Experiment



Hahn spin-echo pulse sequence





Diffusion coefficient

Free Diffusion: $\ln(E) = \ln\left(S(g)/S(g=0)\right) = -D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)$

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What About Signal Attenuation due to Relaxation?

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

initial magnetization

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

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

Echo Signal Attenuation

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

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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} m^2s^{-1}.

  H_2O ↗ ↖ solid polymer

- If the mean square displacement during the experiment ($\Delta \sim 10$ ms – 1 s) is such that a sufficient population of spins make contact with the boundaries ⇒ <u>complicated</u> non-exponential attenuation profiles.

Experimentally this corresponds to barriers with characteristic distances, R □ 100 μm.

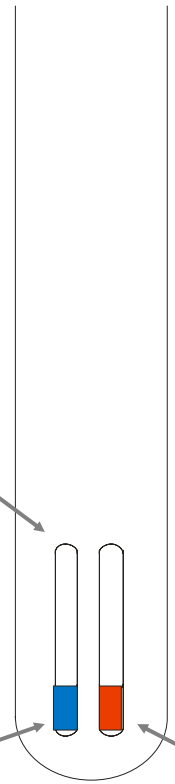
- At finite concentrations the diffusing species obstruct each other ⇒ $D \downarrow$
- Differential relaxation weighting is a problem in polydisperse systems.
- The **combination** of these effects can complicate data interpretation.

SUPERCOOLED WATER

- What is the nature of the water ↔ 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



5 mm NMR tube

0.13 mm i.d.
sealed capillaries

Water
(~ 0.5 µl, length ~ 8 mm)

Methanol
(for temperature calibration)

- Diffusion measured along the capillary.

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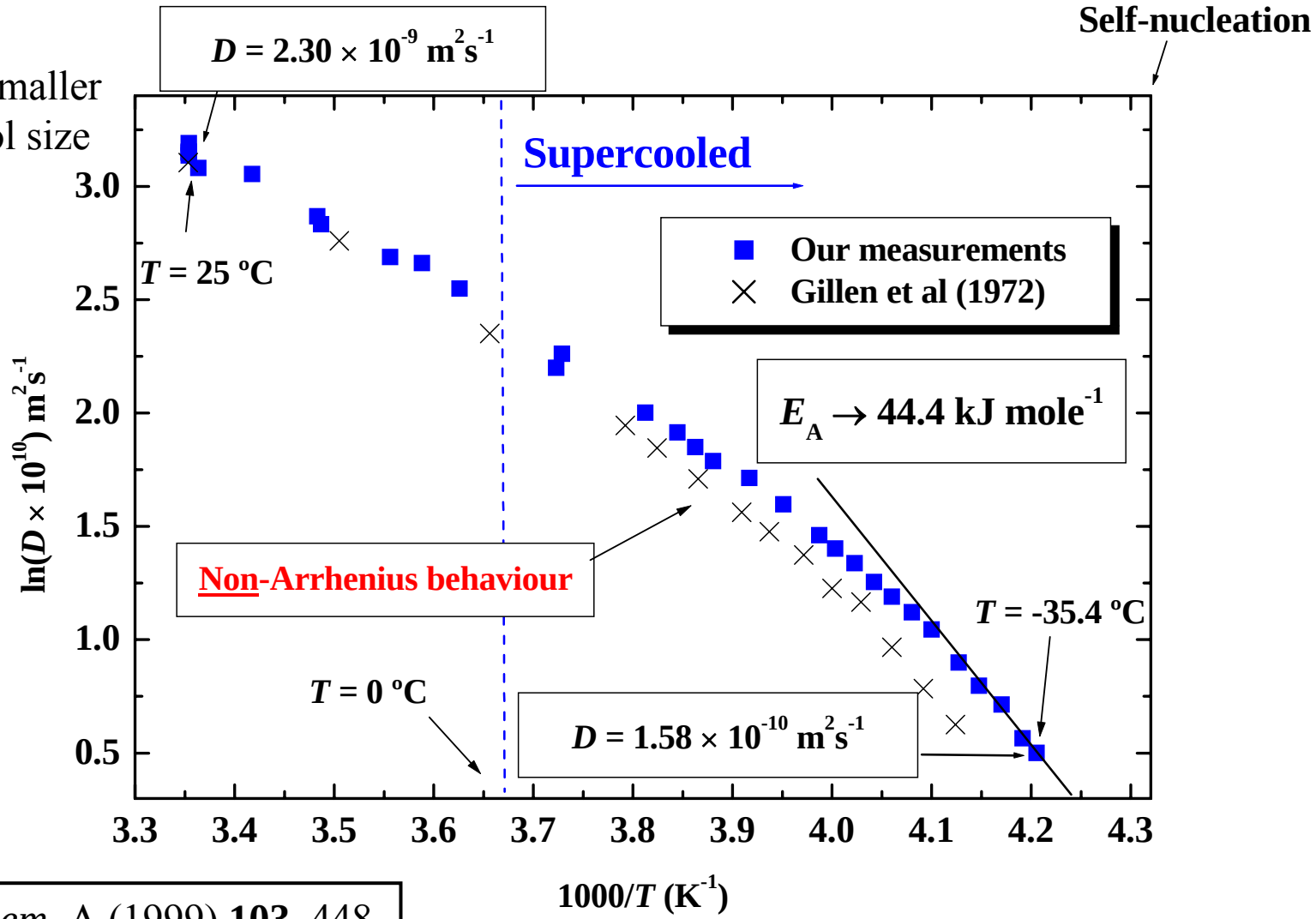
Supercooled ^1H_2O Diffusion

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)

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

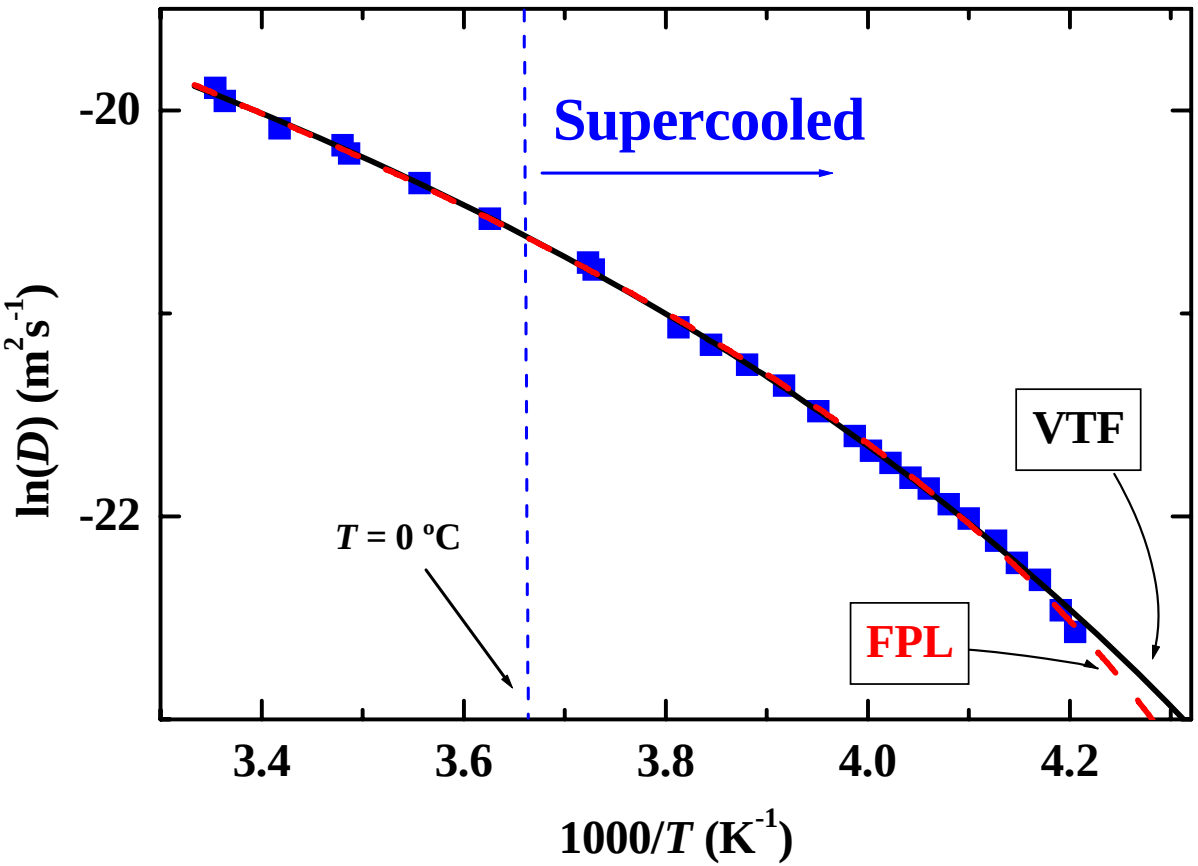
T_S: low temperature limit (singularity)
D_0, γ : fitting parameters

- VTF equation (smooth transition)

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

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

Modeling ^1H_2O Diffusion Using the FPL and VTF Relations



VTF

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

$B = 371 \pm 45$ K

$T_0 = 169.7 \pm 6.1$ K

FPL

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

$T_S = 219.2 \pm 2.6$ K

$\gamma = 1.74 \pm 0.10$

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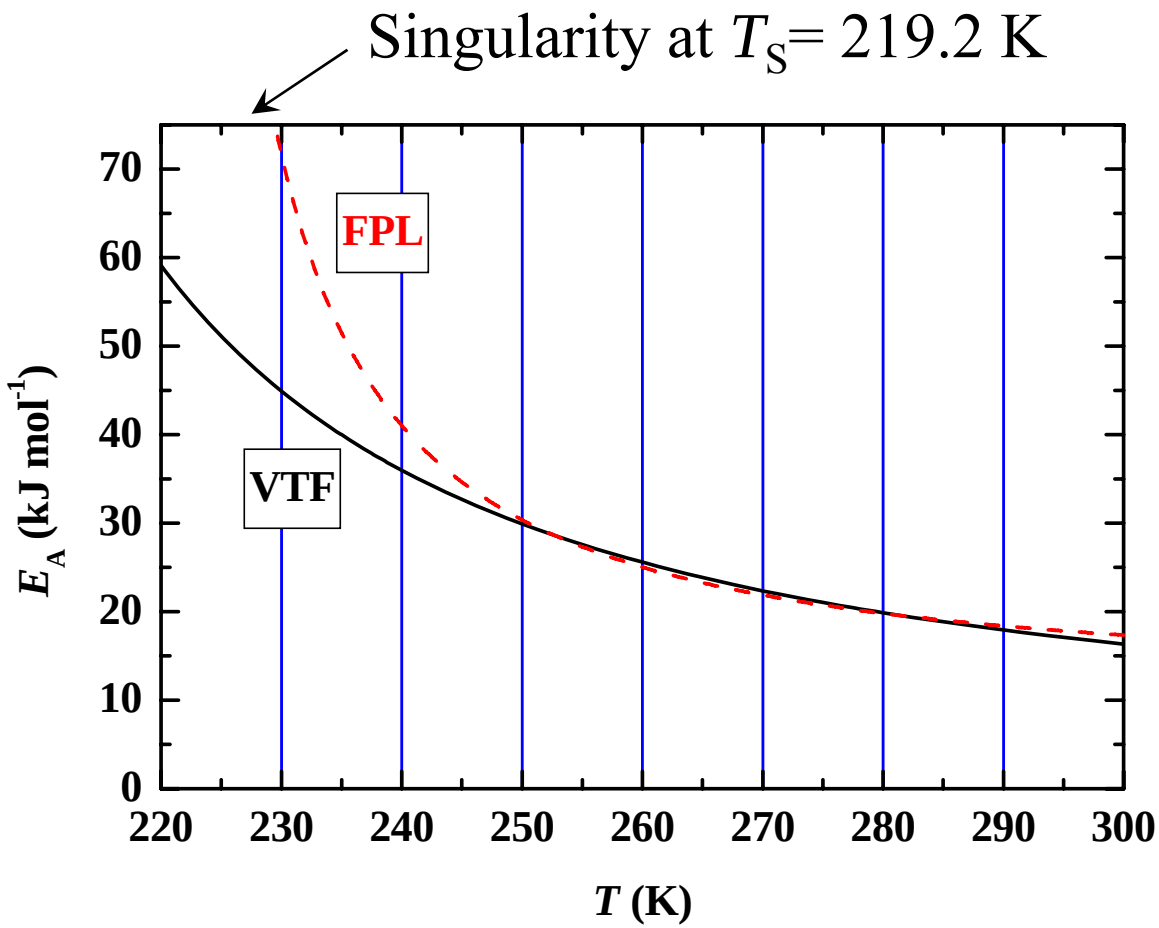
Apparent Activation Energy of ^1H_2O Diffusion



ISOLATED-WATER MOLECULES

- Anomalous behaviour of liquid water arises from hydrogen bond network.
- Water dissolved in a hydrophobic solvent

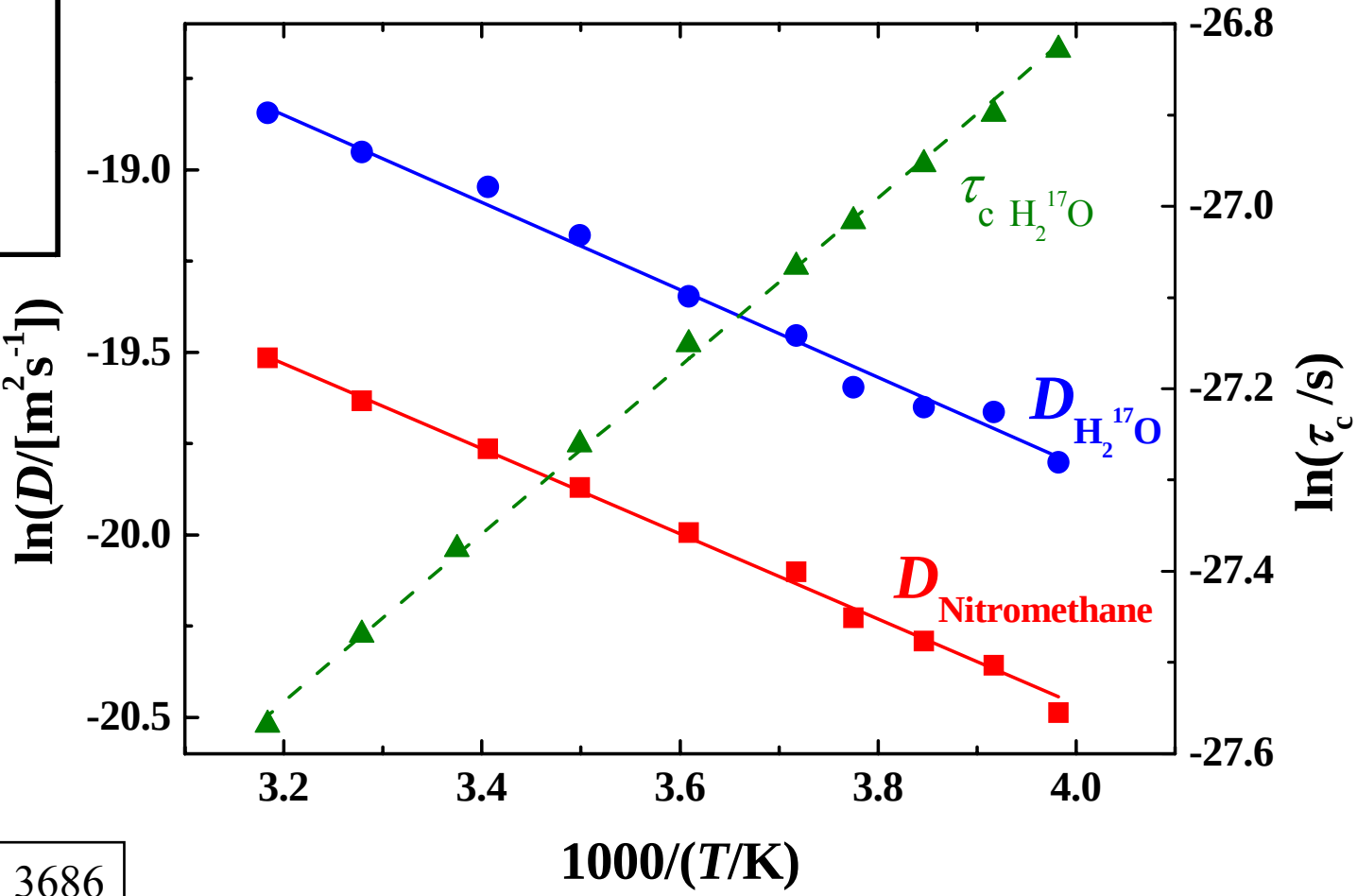
  ‘isolated’ water molecules.
- No hydrogen bonding between water molecules.
- Studied the diffusion (^{17}O PGSE) and ^{17}O longitudinal relaxation (T_1) of $H_2{}^{17}O$ dissolved in nitromethane.
- ‘Non-standard’, low γ nuclei are now becoming accessible to PGSE measurement with the increasingly larger applied gradients available.

^{17}O PGSE and Relaxation Measurements

	E_A (kJ mol^{-1})
$D_{H_2{}^{17}O}$	10.0 ± 0.3
$D_{Nitromethane}$	9.7 ± 0.2
$\tau_{c\,H_2{}^{17}O}$	7.7 ± 0.1

- D depends on inertial and solvent effects
- τ_c dominated by inertial effects

^{17}O $T_1 \rightarrow$ reorientational correlation time, τ_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)

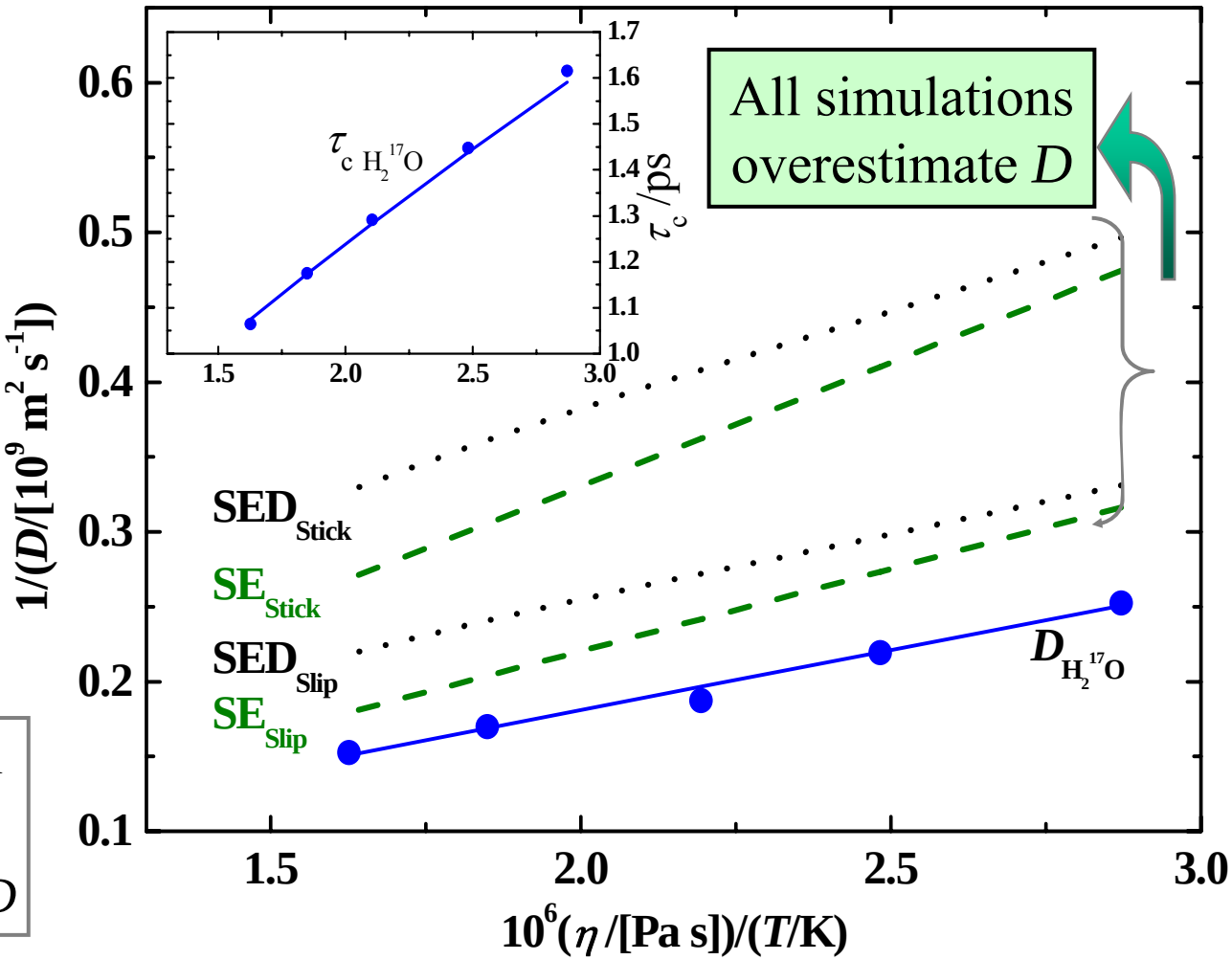
Stokes-Einstein-Debye (SED) Equation

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

H_2O Stokes radius, r_s = 1.21 Å

SE: lit. $\eta \rightarrow$ SE $\rightarrow D$

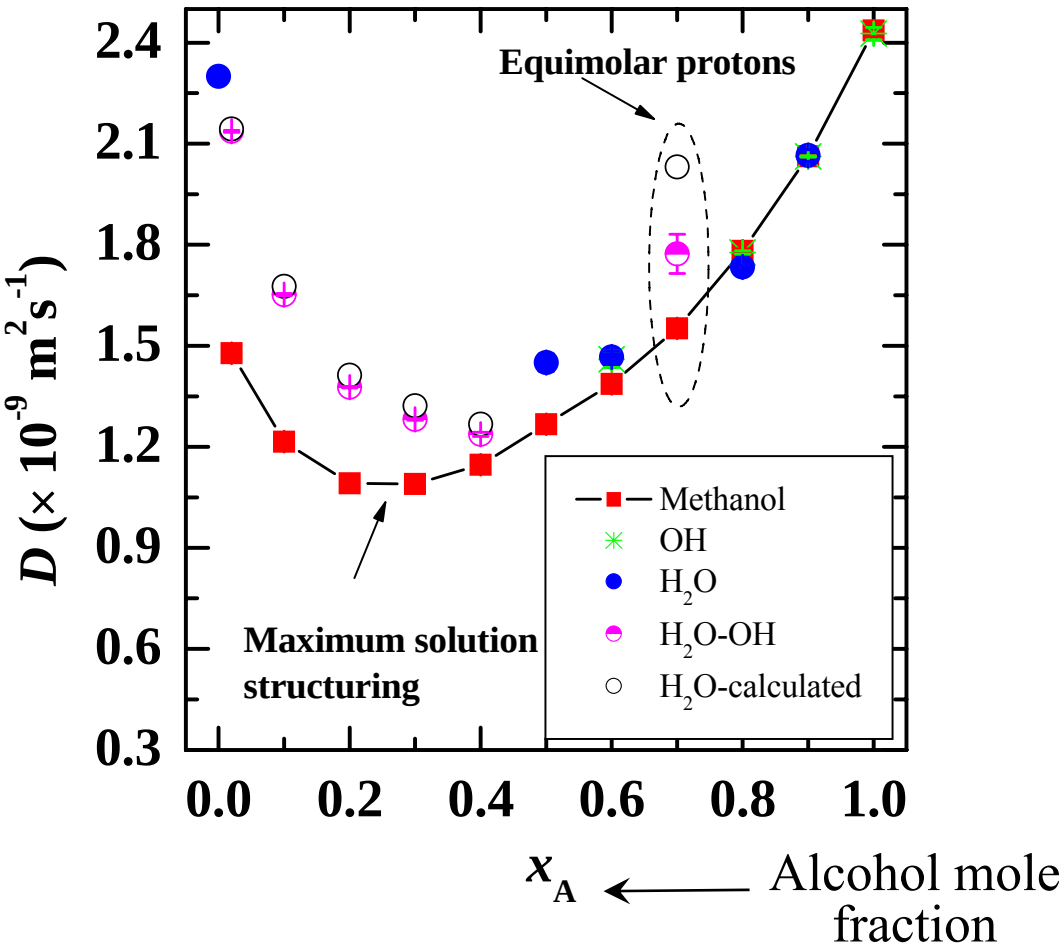
SED: $\tau_c \rightarrow$ SED $\rightarrow$ SE $\rightarrow D$



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



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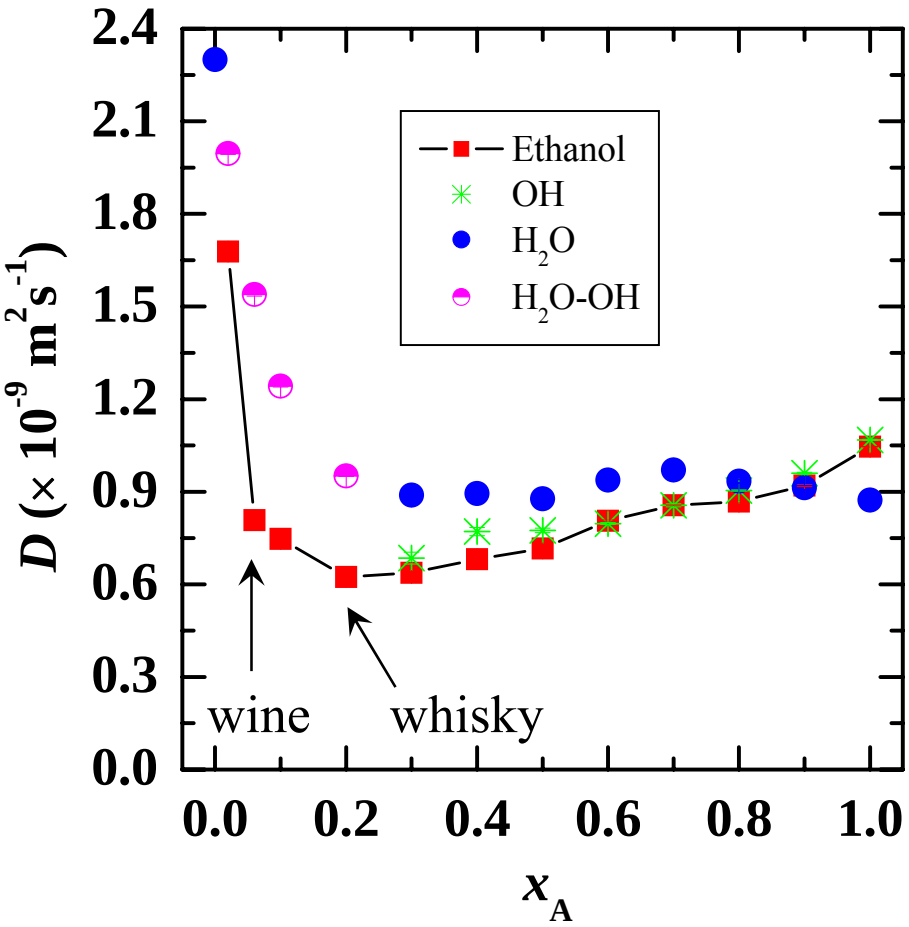
Diffusion in the Ethanol-Water System at 298 K



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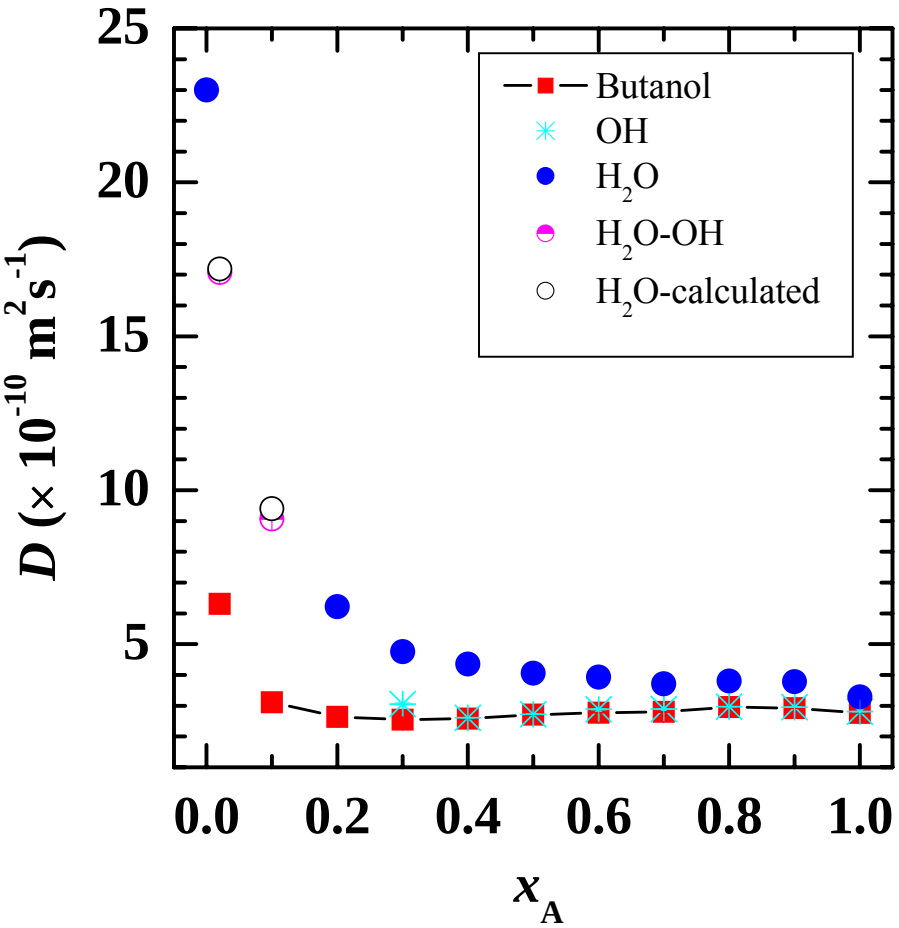
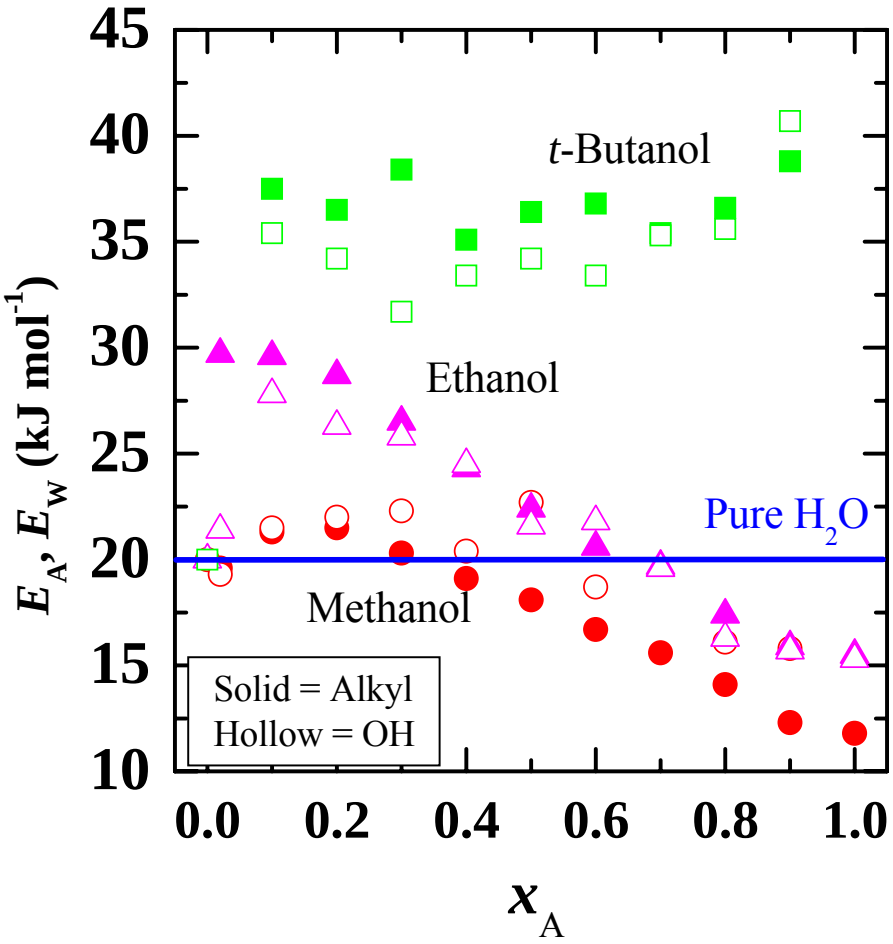
Diffusion in the *tert*-Butanol-Water System at 298 K



Arrhenius Activation Energy for Diffusion



Calculated from diffusion measured at 273, 285 and 298 K

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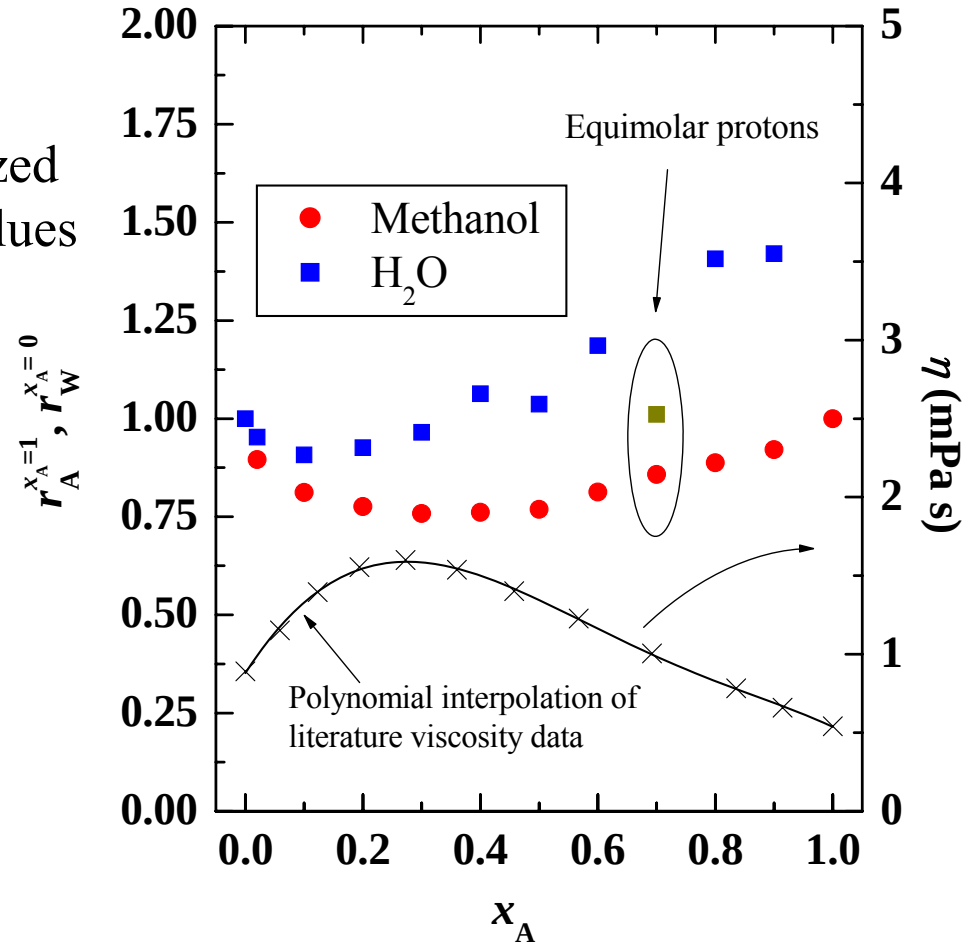
Stokes-Einstein Analysis of the Methanol System at 298 K

Stokes radii normalized to the pure solvent values



Bulk viscosity

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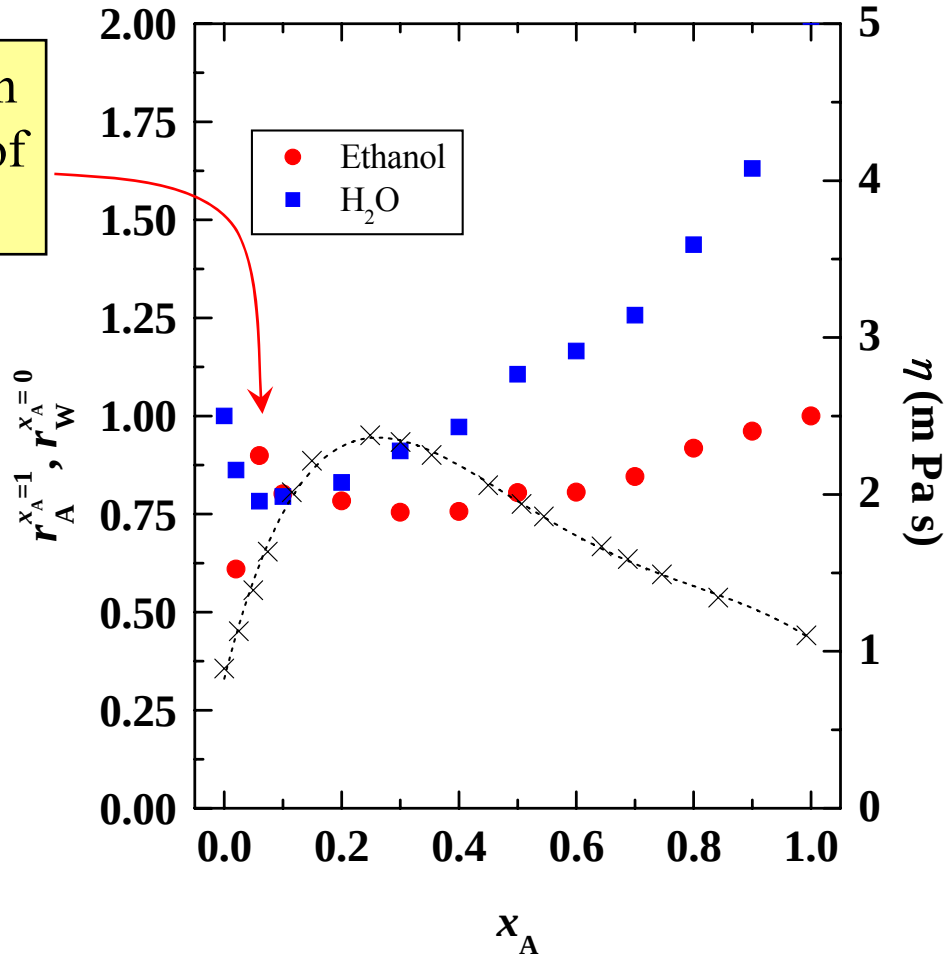
Stokes-Einstein Analysis of the Ethanol System at 298 K

Local maximum in the Stokes radius of the ethanol



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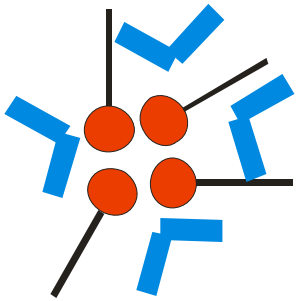
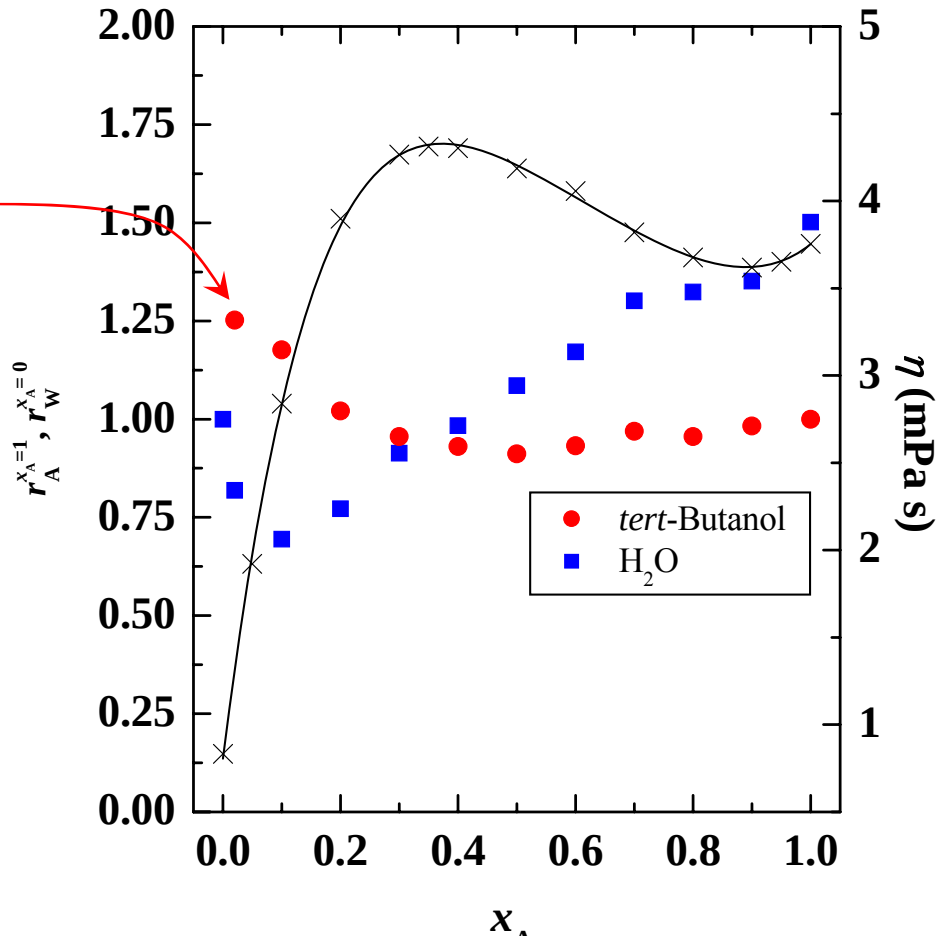
Stokes-Einstein Analysis of the *tert*-Butanol System at 298 K

butanol self-association





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

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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.

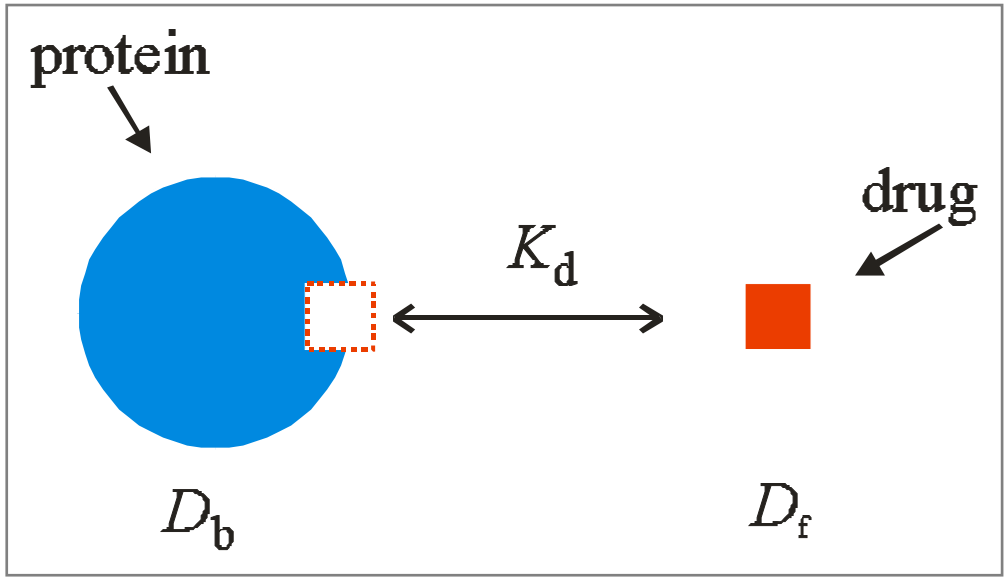
To do so it is necessary to:

i. Accurately measure *D* of the drug over large concentration ranges (esp. v. low concentrations) – ideally in non-deuterated samples (i.e., in $^{1}H_2O$ not $^{2}H_2O$).

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.

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Effects of Ligand Relaxation

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

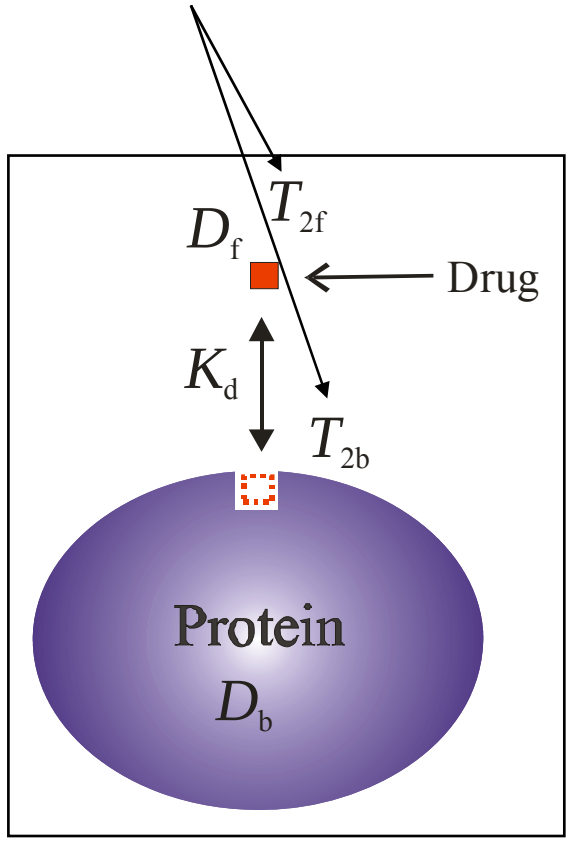
$$\frac{dS_f}{dt} = -(\gamma\delta g)^2 D_f S_f - \frac{S_f}{\tau_f} + \frac{S_b}{\tau_b} - \frac{S_f}{T_{2f}}$$

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

Ignoring relaxation effects

Population weighted average diffusion coefficient

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



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 answ
- Using it provides and additional source of information.

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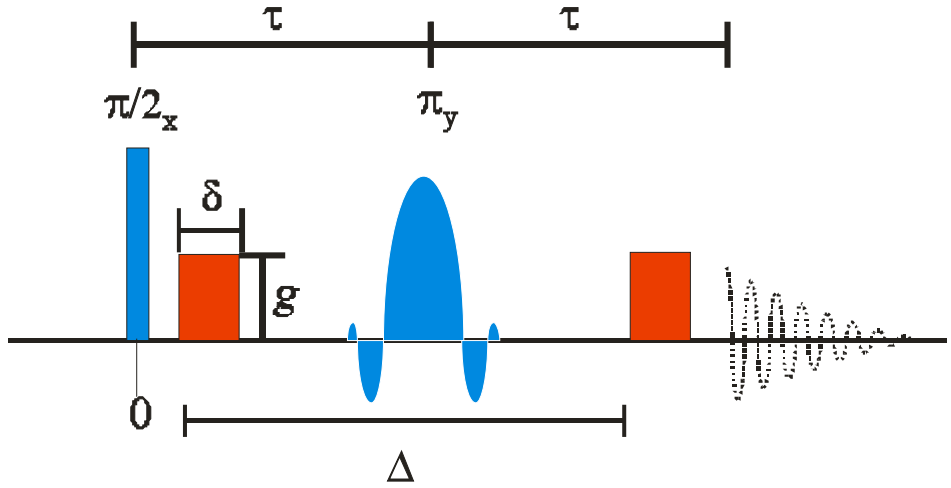
PGSE-WATERGATE



- 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.

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

initial magnetization

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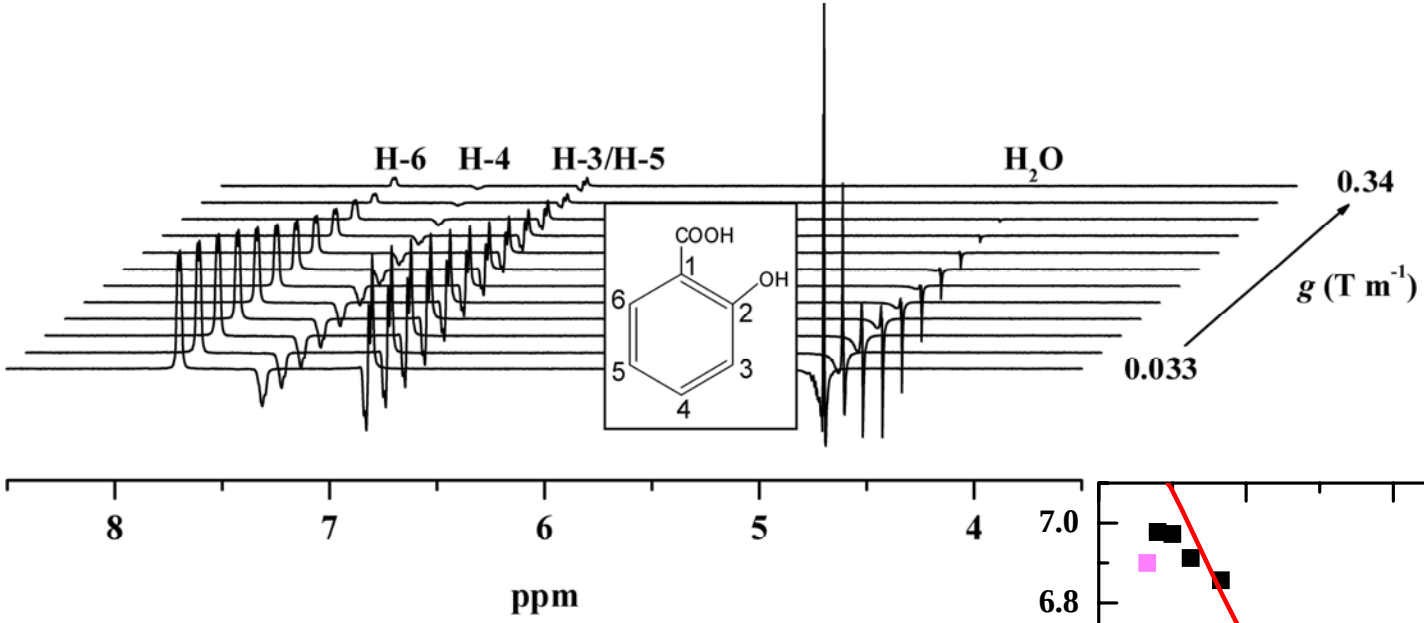
Salicylate binding to BSA



80 mM salicylate
0.5 mM BSA
90% ^1H_2O 298 K

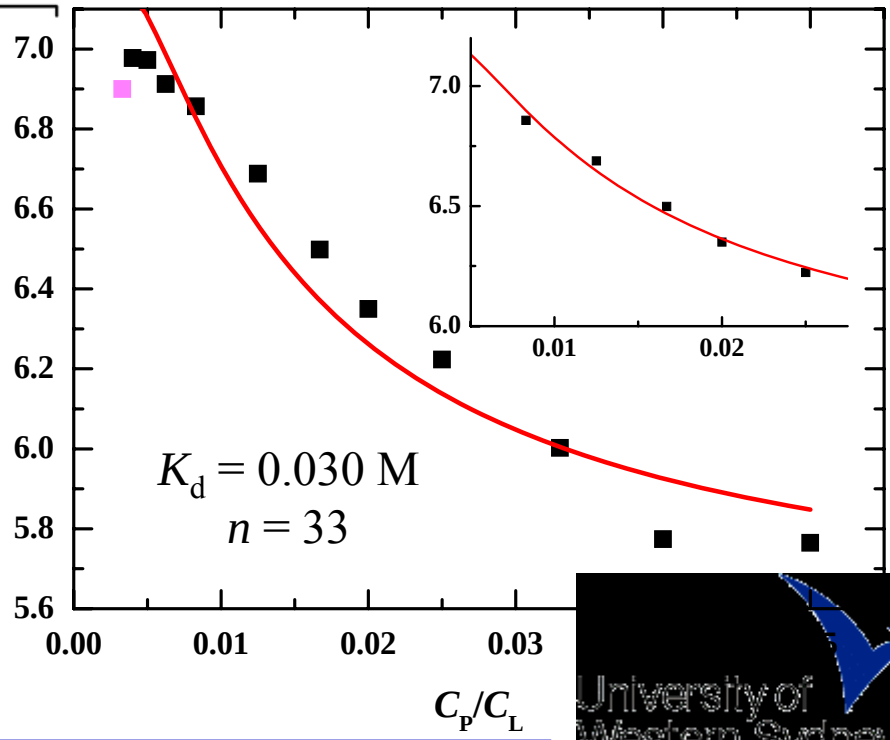
Two Site Model: $PL \overset{K_d}{\square} P + L$

$$D = (1 - P_b) D_f + P_b D_b$$

$$\alpha = \frac{(C_L + nC_P + K_d)}{2C_L}$$

$$P_b = \alpha - \sqrt{\alpha^2 - \beta}$$

$$\beta = \frac{nC_P}{C_L}$$



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

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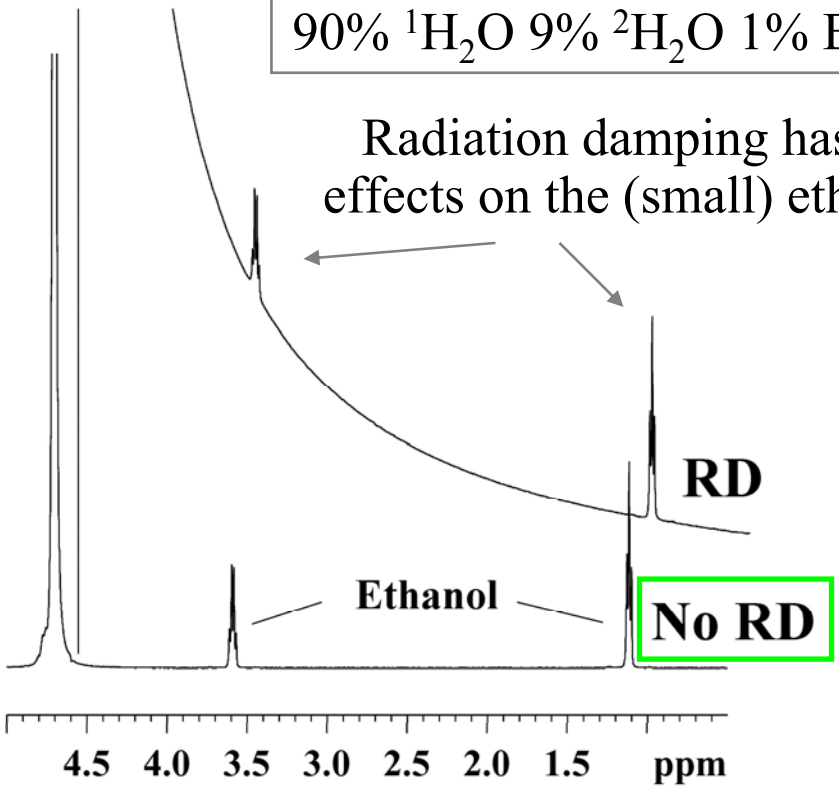
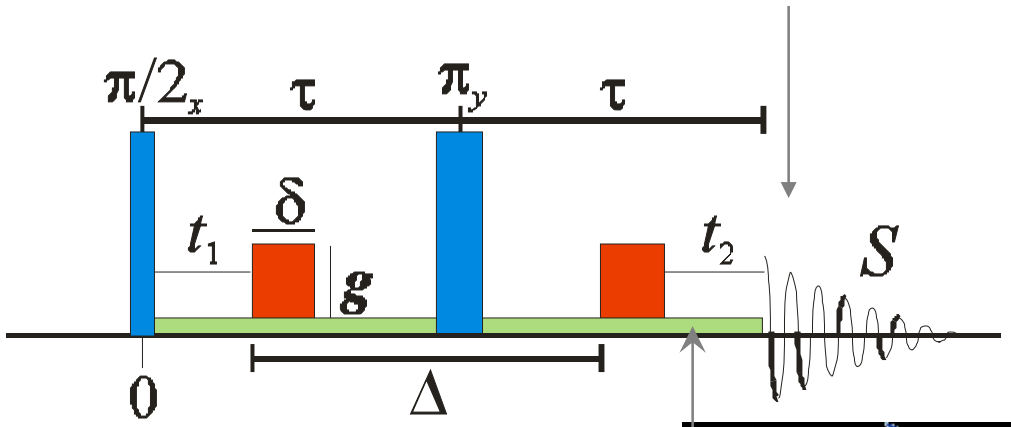
Using Q-Switching to counter Radiation Damping

- 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.

90% ^1H_2O 9% ^2H_2O 1% Ethanol at 500 MHz

Radiation damping has negligible effects on the (small) ethanol signals.





Q-swit

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

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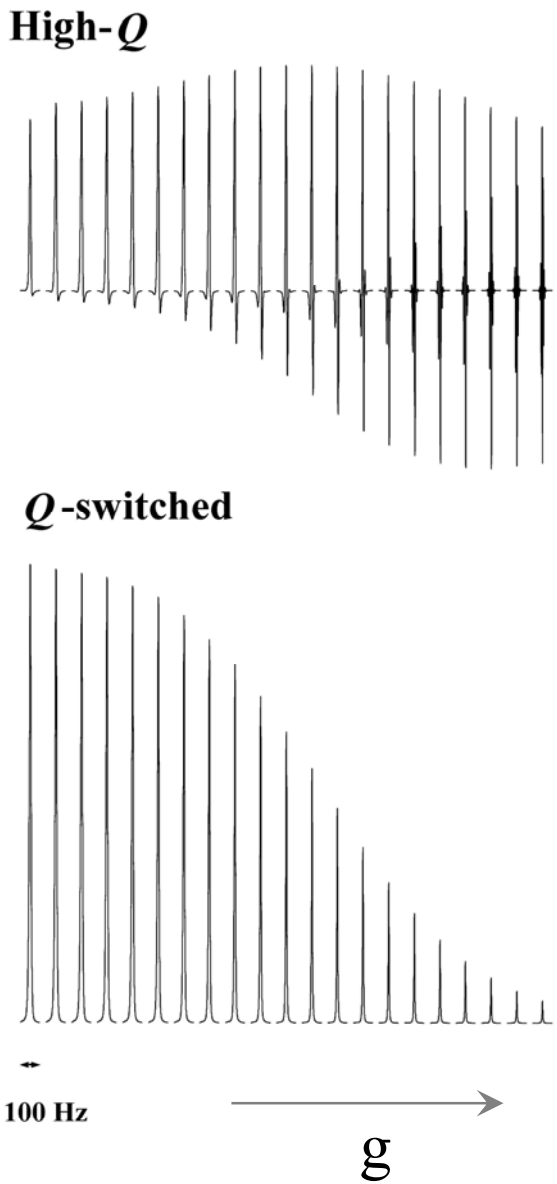
Using the PGSE-Q-Switch Sequence



90% ^1H_2O at 500 MHz
Δ = 30 ms, δ = 2 ms

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

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.

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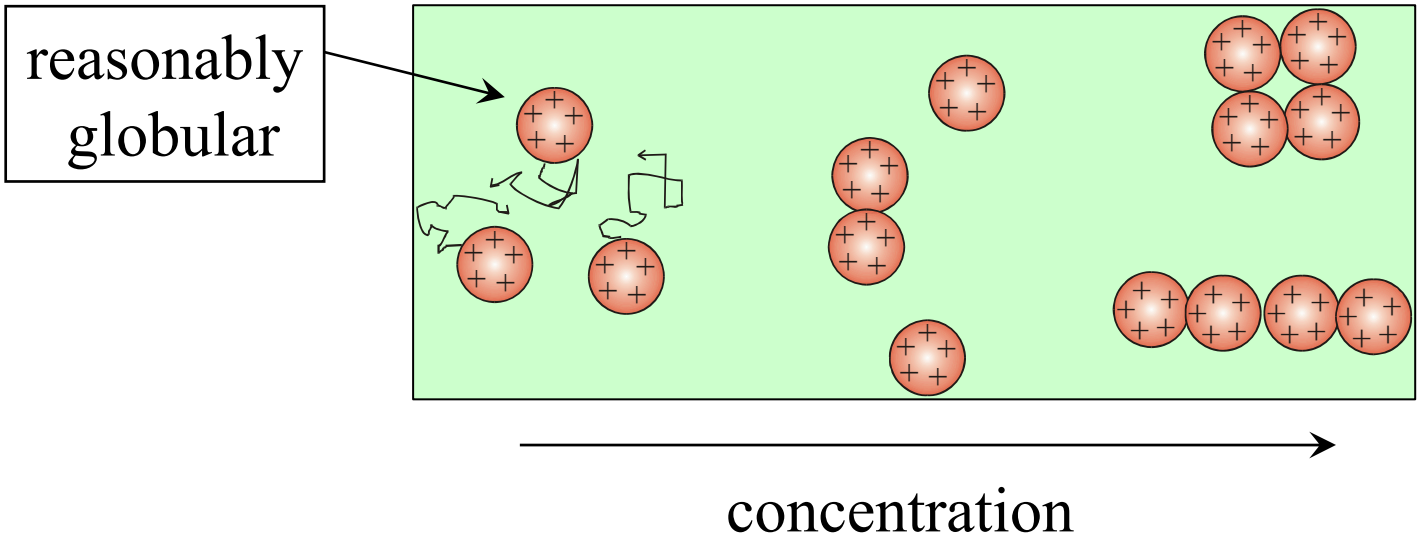
PROTEIN ASSOCIATION

- 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.
  $\Rightarrow$ need to consider size and electrostatic interactions

Aggregation of Lysozyme

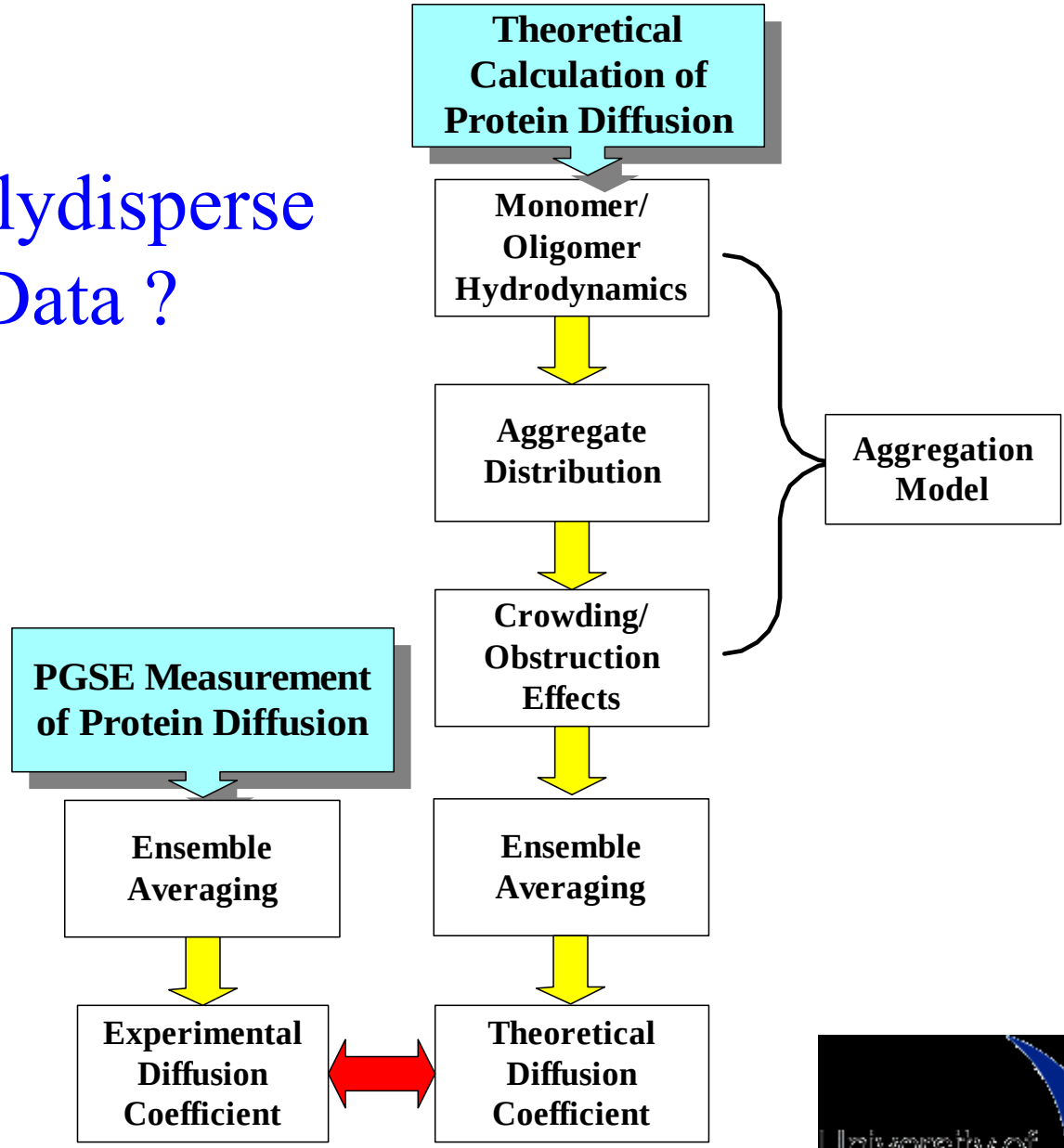
- Lysozyme - forms a complicated polydisperse system.



- 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. Δ.

Number concentration of the *i*th oligomer

Multiexponential Echo signal decay $\longrightarrow$ $$S(g) \propto \sum_i Mw_i n_i \exp\left(-bD_i\right) \exp\left(-2\tau/T_{2,i}\right)$$

Sum over the all oligomeric states; Magnetisation; Diffusion; Relaxation

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

1st Fudge: Neglect relaxation and normalise

$$E = \frac{S(g)}{S(0)} = \frac{\sum_i Mw_i n_i \exp\left(-bD_i\right)}{\sum_i Mw_i n_i}$$

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

Neglect higher terms

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

weight-averaged diffusion coefficient

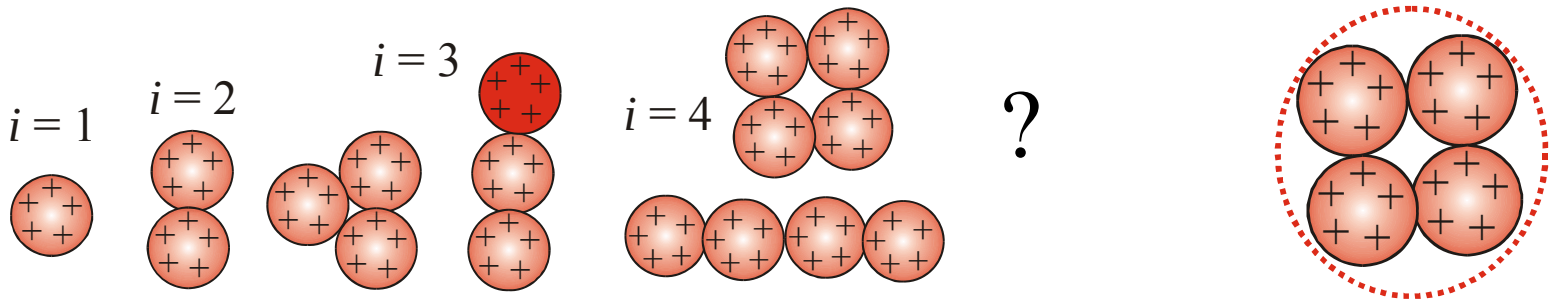
$$\langle D\rangle_W = \frac{\sum_i Mw_i n_i D_i}{\sum_i Mw_i n_i}$$

Effects of crowding are inherently included

- PGSE gives ~ $\langle D\rangle_W$ (actually $\langle D\rangle_W^C$).

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 ⇒ assume spherical shapes.



Infinite dilution

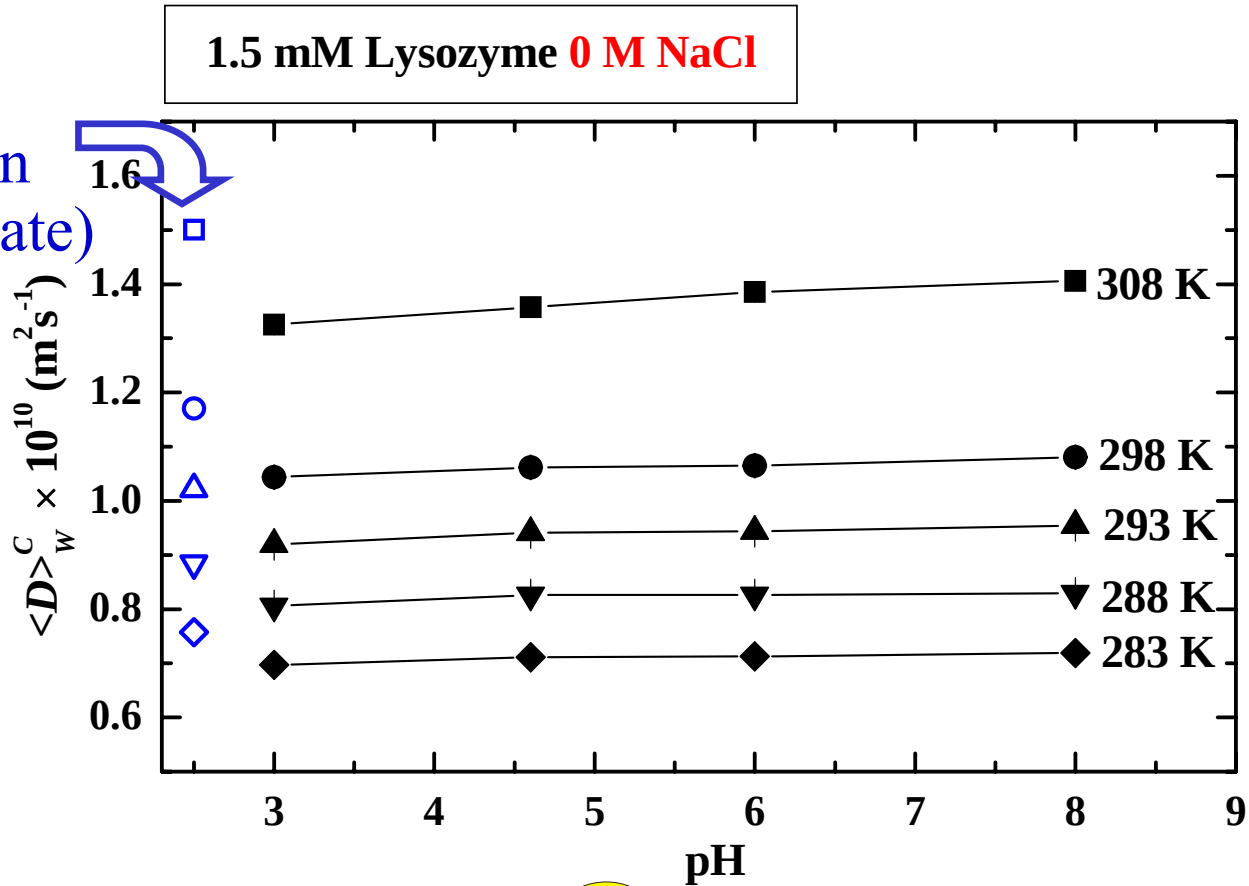
$$D^0 = \frac{kT}{6\pi\eta r_s} \quad \Rightarrow \quad D_i^0 = \frac{D_1^0}{\sqrt[3]{i}}$$

Diffusion coefficient of *i*-mer

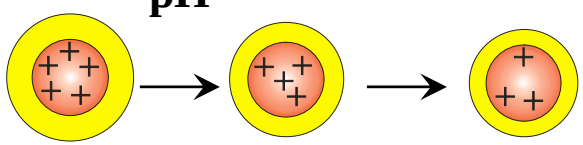
Importance of Charge Effects I

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

Bead model Approximation (theoretical estimate)



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



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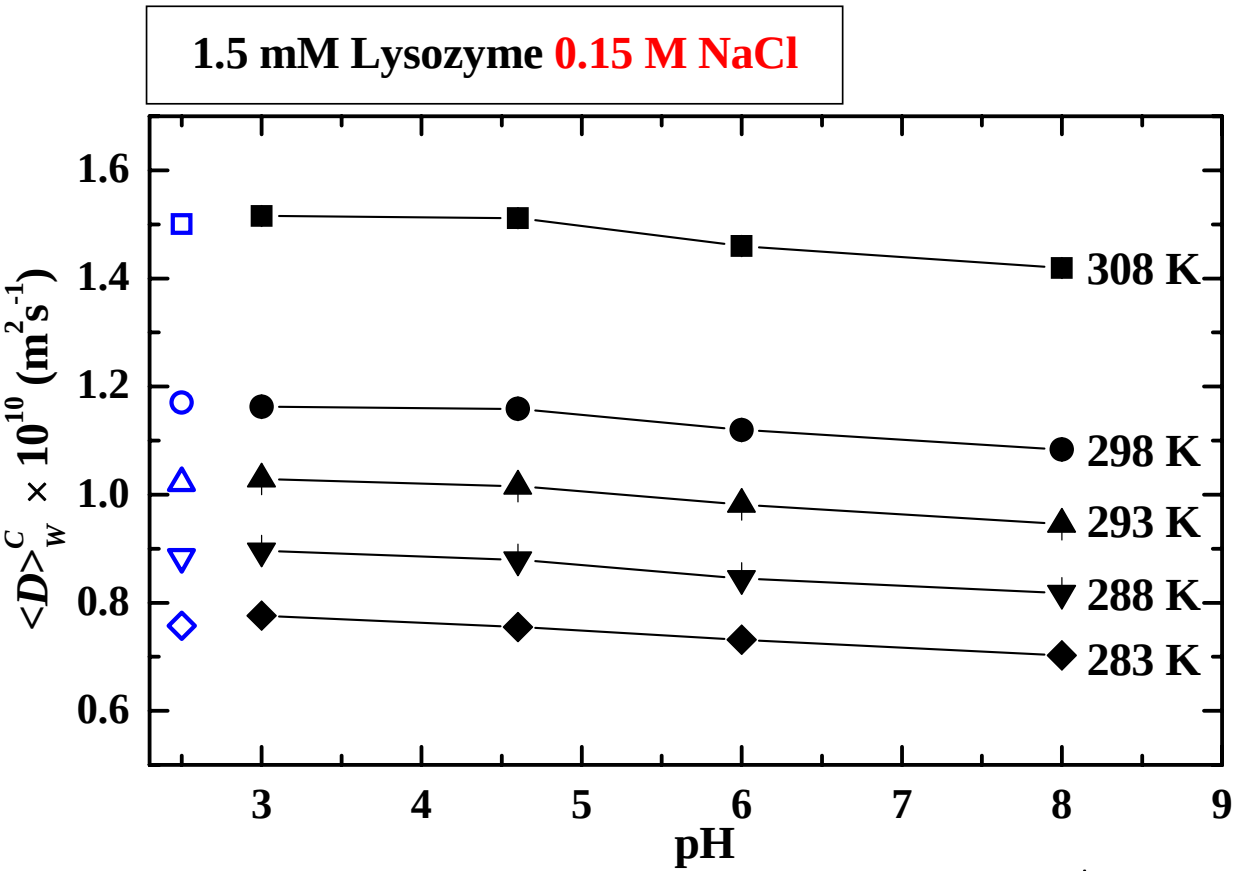
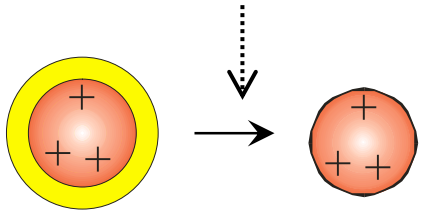
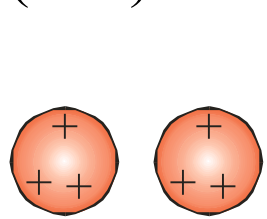
Importance of Charge Effects II



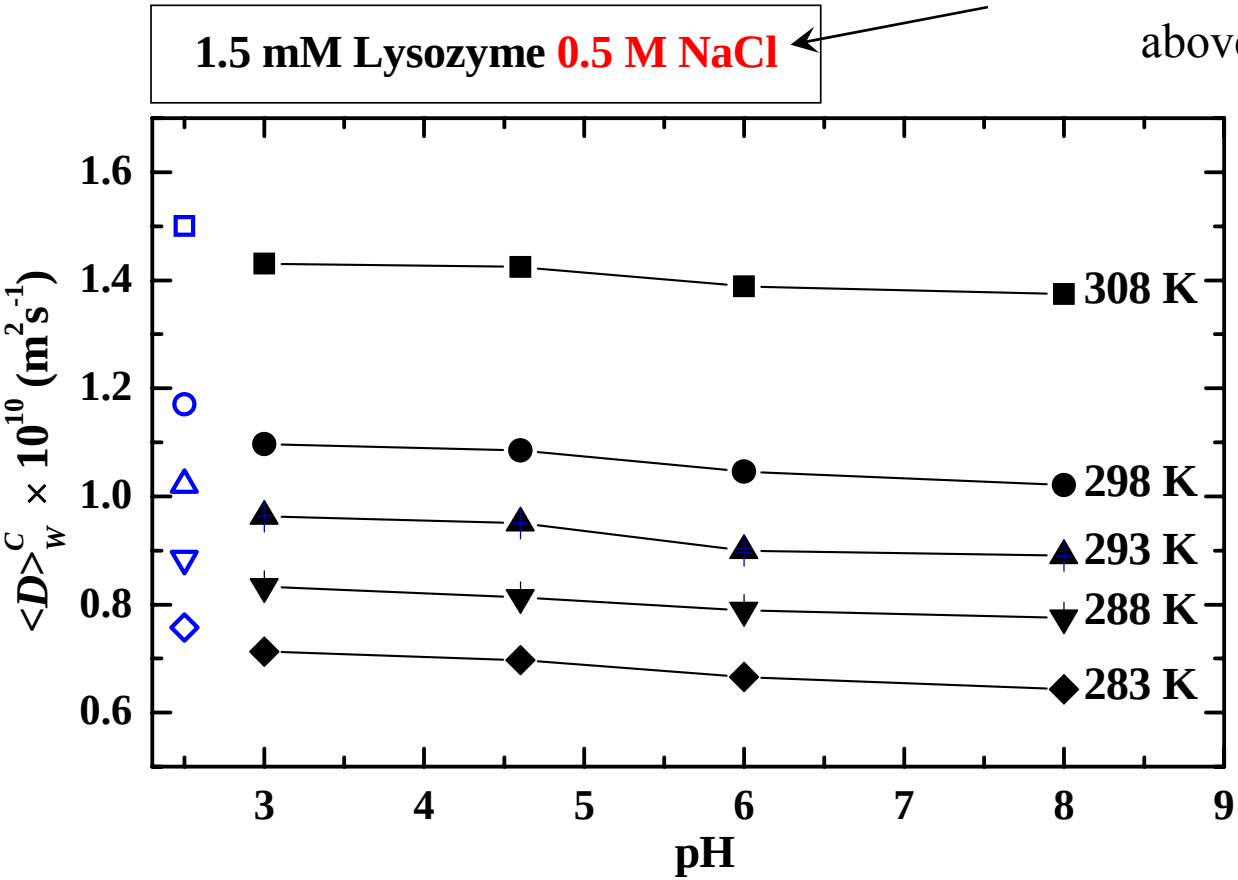
- Competition between electrostatic screening ($D \uparrow$) and aggregation ($D \downarrow$).





Importance of Charge Effects III

Electrostatic screening complete above 0.5 M NaCl



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

higher oligomers

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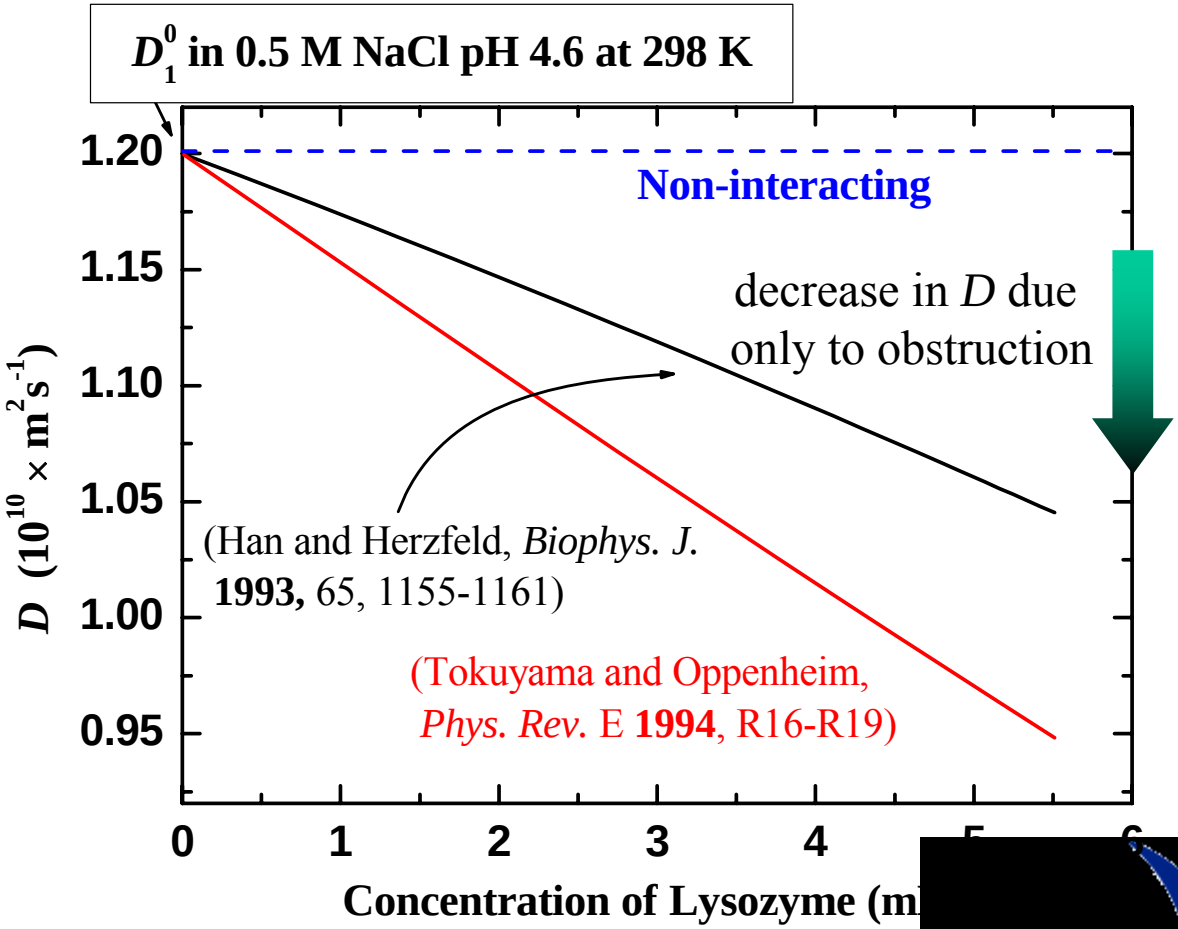
Two Models for the Effects of Obstruction on Diffusion in Lysozyme Solution

- 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)

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

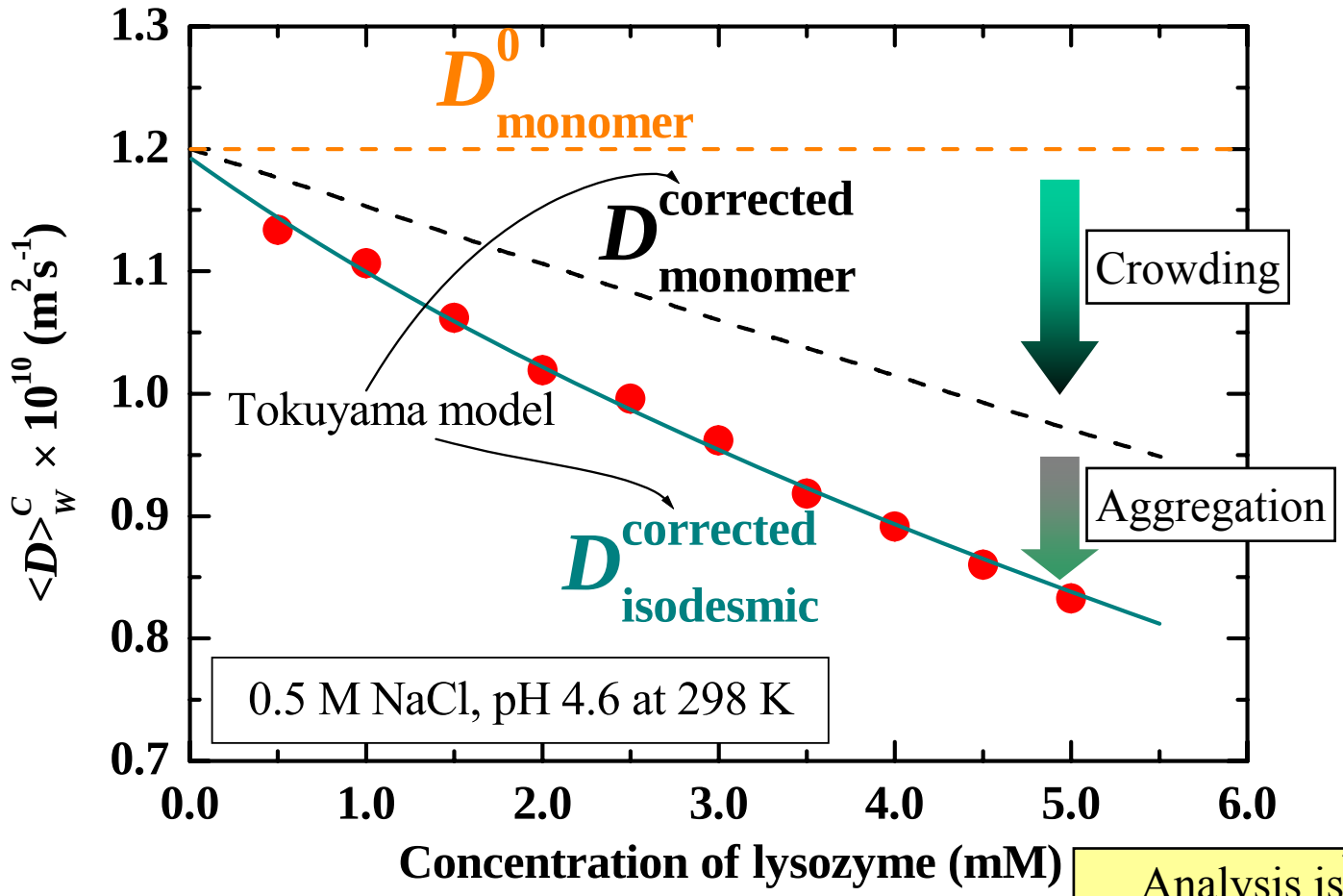
Sum over the different oligomeric states

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 averagin

Lysozyme Aggregation



Simulations:

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

Analysis is wildly wrong if obstruction is not accounted for.

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

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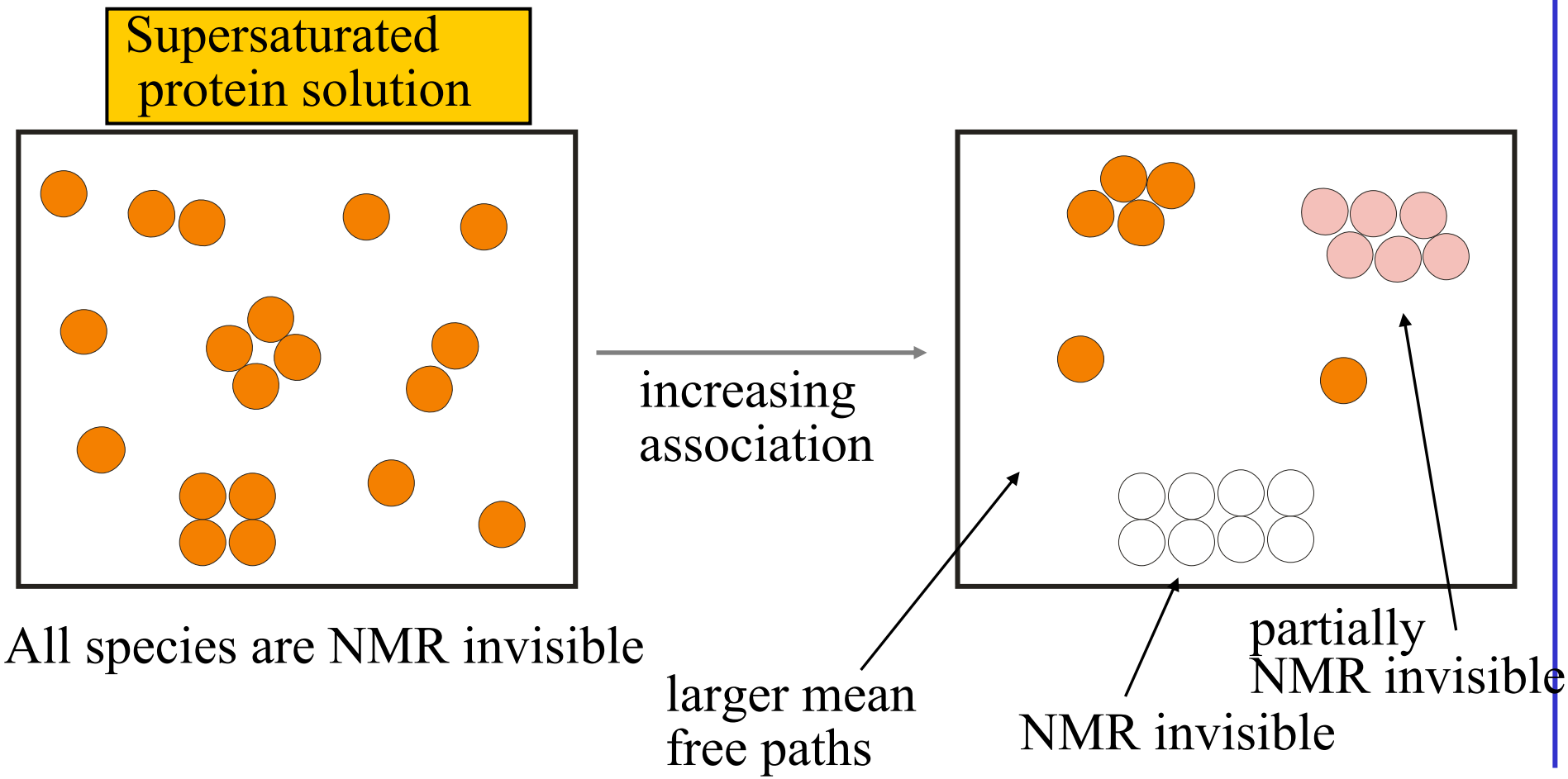
What Happens at Higher Protein Concentrations ?

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

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

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

What the PGSE experiment will 'see'



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

Probing the Time-Dependence of Aggregation

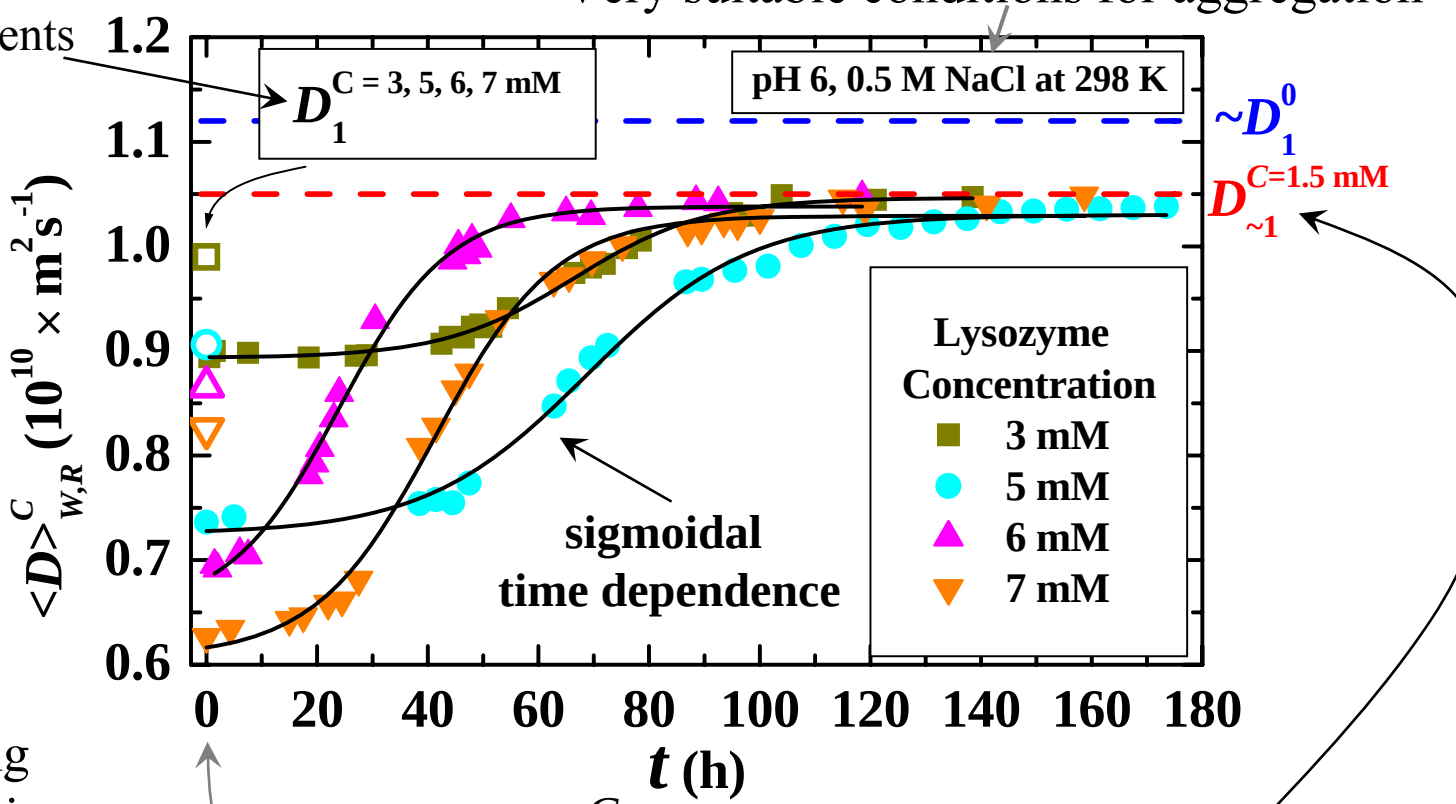
- As aggregation proceeds the larger aggregates (~ 'solid' phase) become 'NMR invisible' and PGSE measures only those still in solution.
- The protein remaining in solution diffuses faster due to less obstruction.
- Gradient strength permitting, the relaxation weighting can be 'tuned' to different M_w ranges.

⇒ **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

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

time scaling

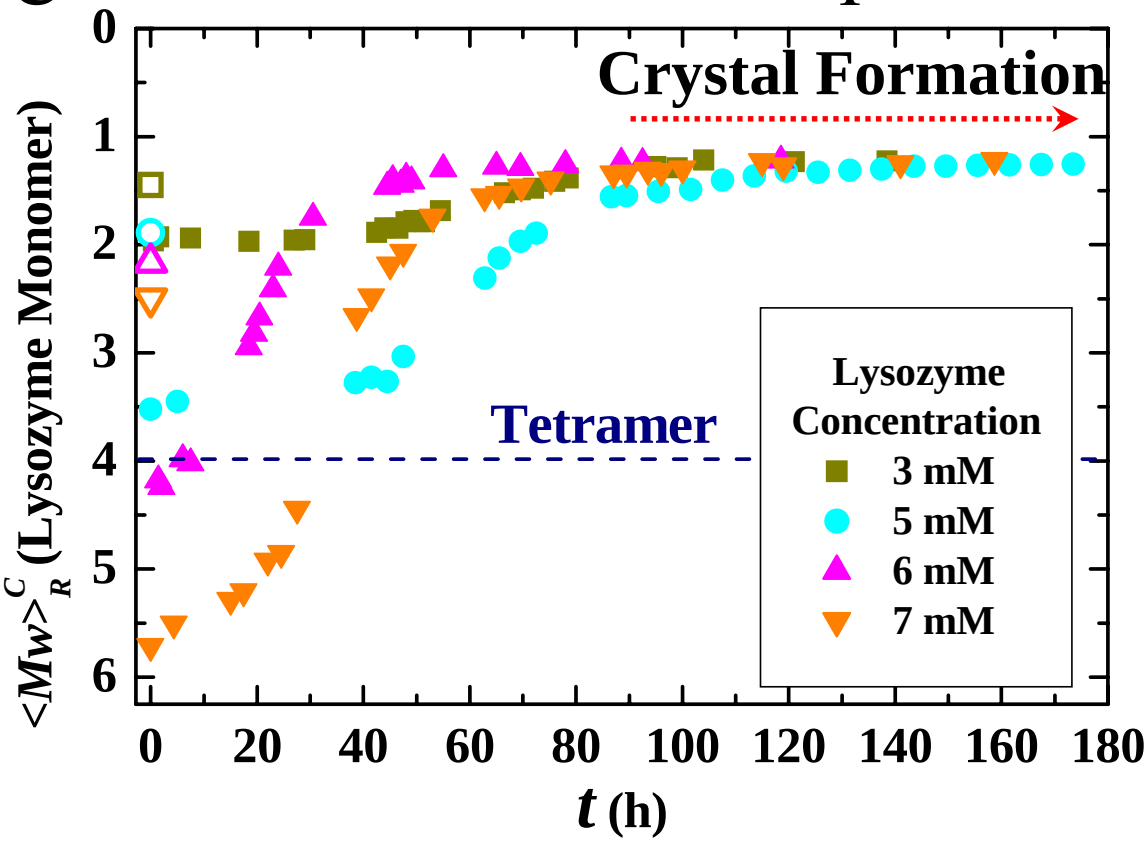
midpoint of inflection

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

Time-Dependence of Lysozyme Aggregation II

- Recast using the Stokes-Einstein Equation.

Of the aggregating species still in solution



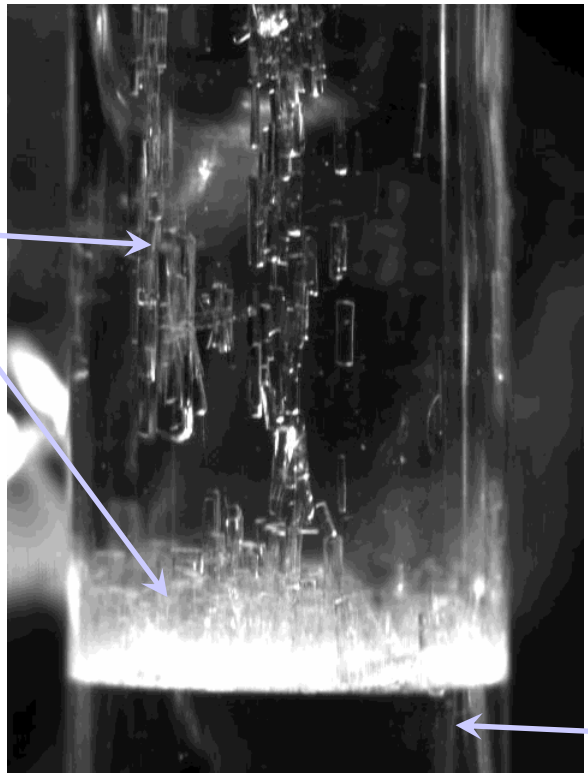
- It has been suggested that the critical nucleus is a tetramer and that the growth unit is an octamer.

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

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Using PGSE to Probe the Time-Dependence of Aggregation

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

- small no. of critical nuclei → long induction period and large crystals
- large no. of critical nuclei → 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 Thiacalix[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*".
- Schönfleder 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 Assymetric Polymer Membranes and Polyelectrolyte Multilayers*".

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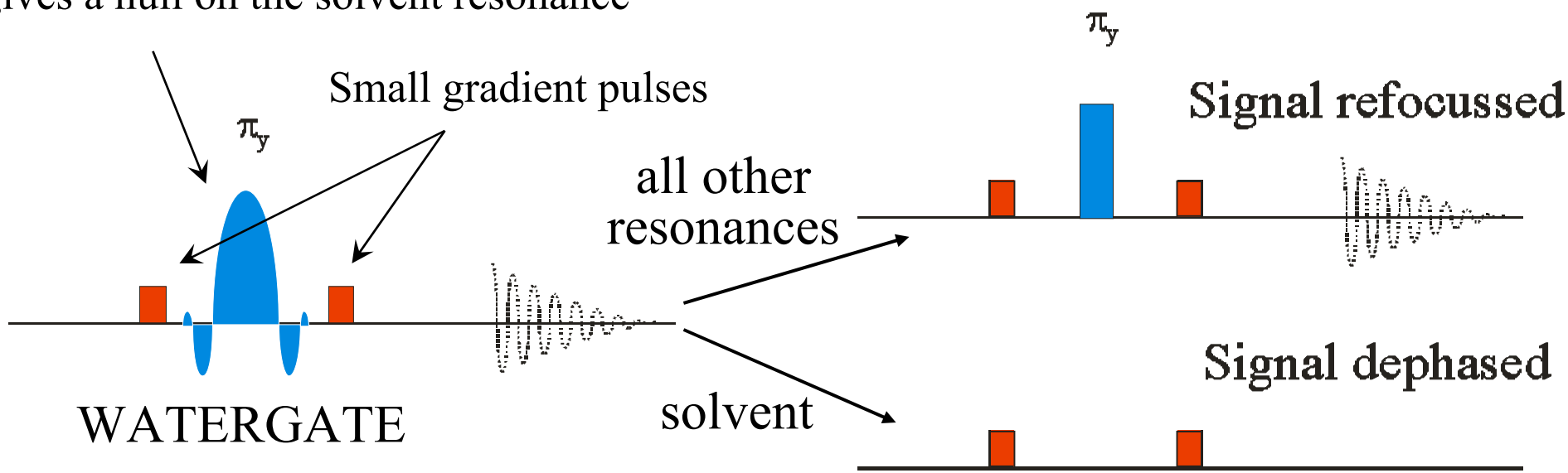
- **Hiroyuki Ide,** Ajinomoto, Japan.
- **Fumihiko Tsuchiya,** Applied Biosystems Japan.
- **Cécile Vigouroux,** Royal Institute of Technology, Sweden.
- **Markus Wälchli,** Bruker Biospin, Japan.

Water Suppression

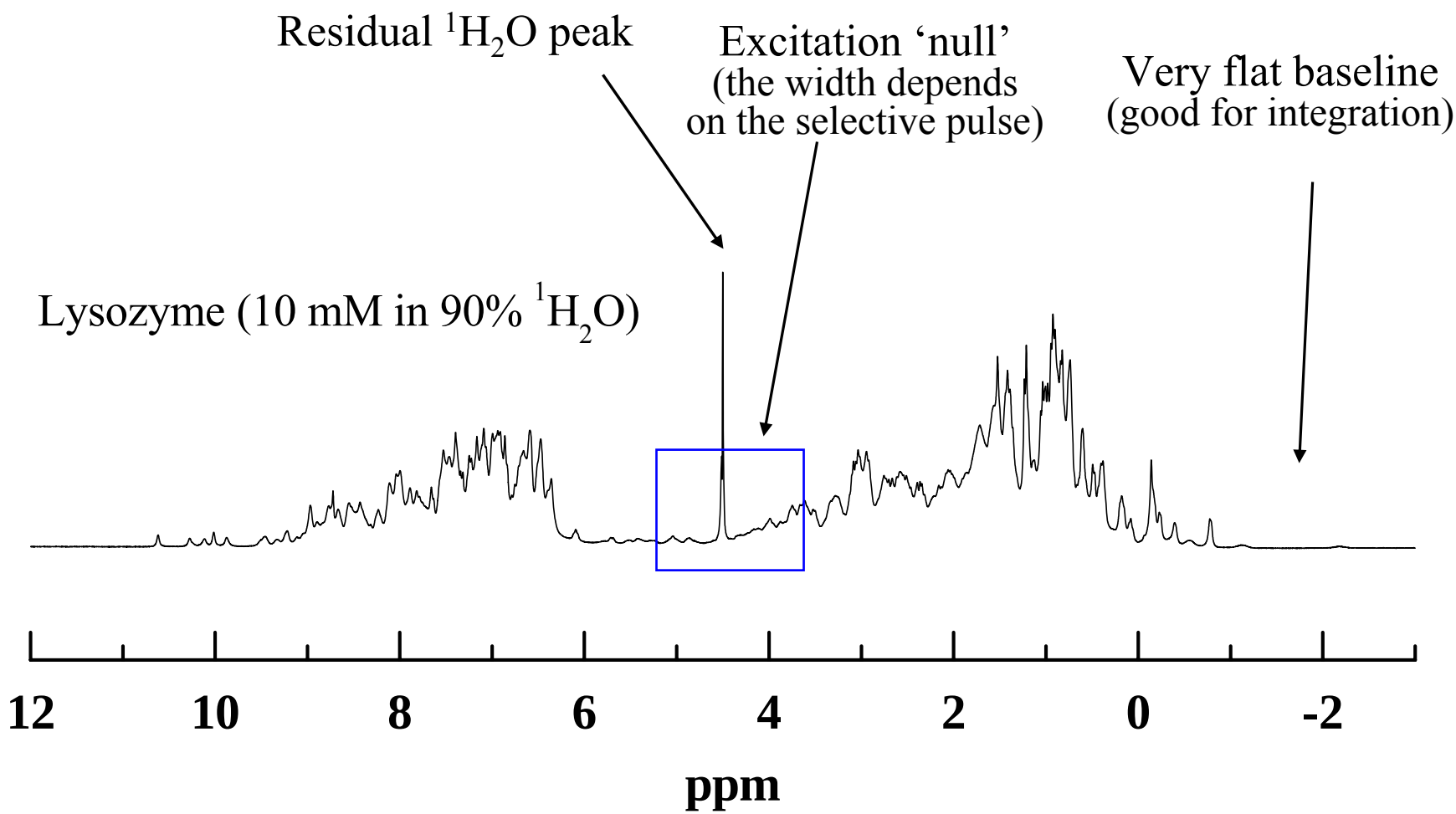
- One of the best suppression techniques is the WATERGATE sequence.

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



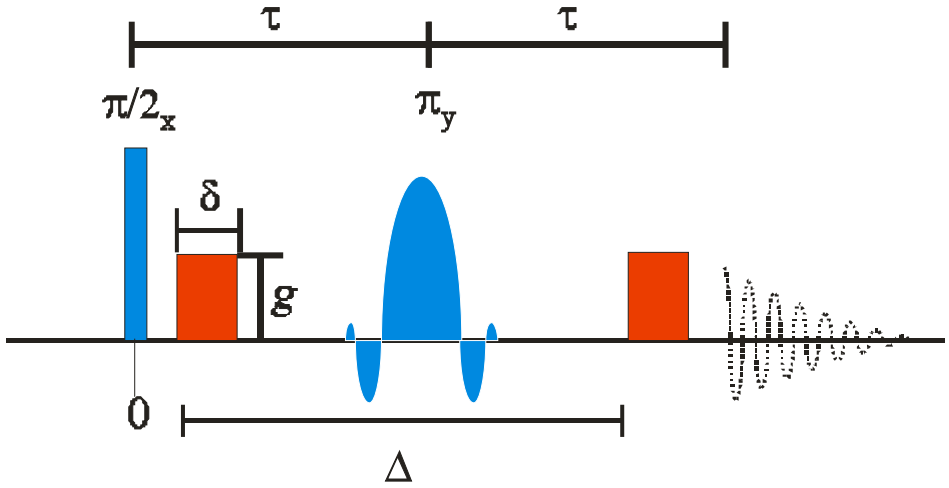
- The WATERGATE sequence resembles a PGSE sequence except diffusion effects (i.e., signal loss) are minimized.

WATERGATE



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

PGSE-WATERGATE



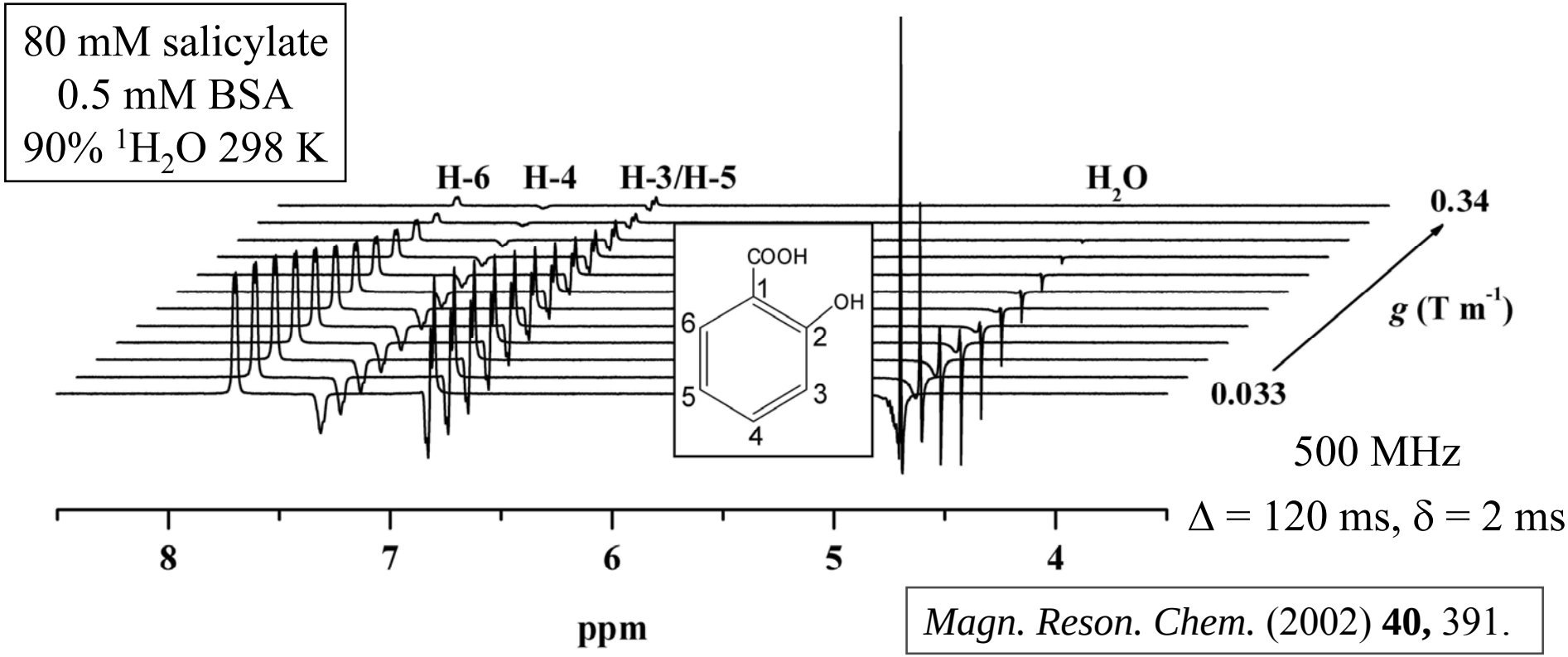
- A Hahn-based sequence is preferable to a Stimulated Echo-based PGSE sequence due to: (1) better removal of the protein resonances due to relaxation, (2) larger drug signal and (3) no complications from cross-relaxation effects.

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

M_0: initial magnetization

PGSE Spectra of Salicylate in BSA Solution

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



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

- 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.

Radiation damping rate constant

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

Static field strength

Filling factor

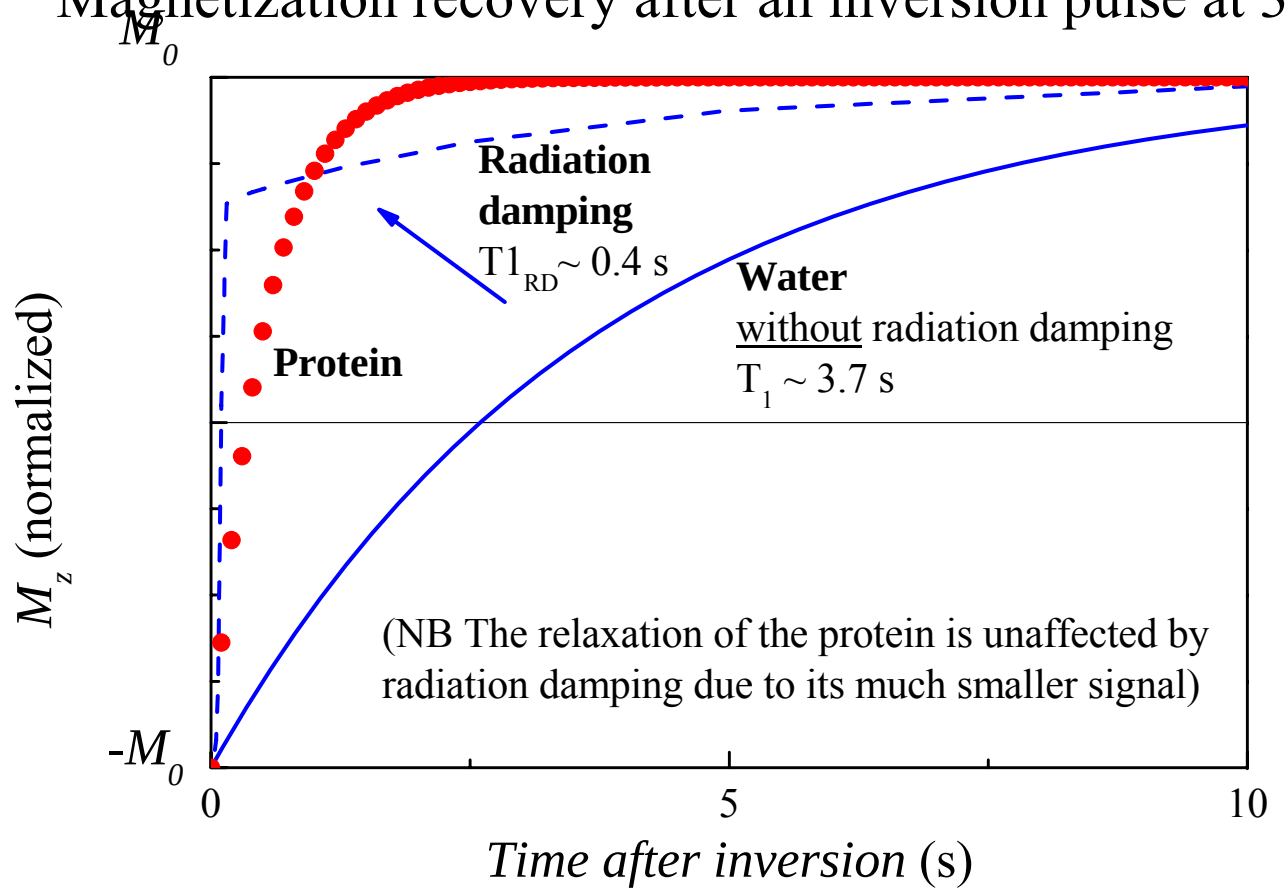
'Quality' factor of rf coil

Often: $T_{RD} << T_1$!

- 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, η or Q is not a solution when trying to detect small resonances.

Radiation Damping Effects on Relaxation

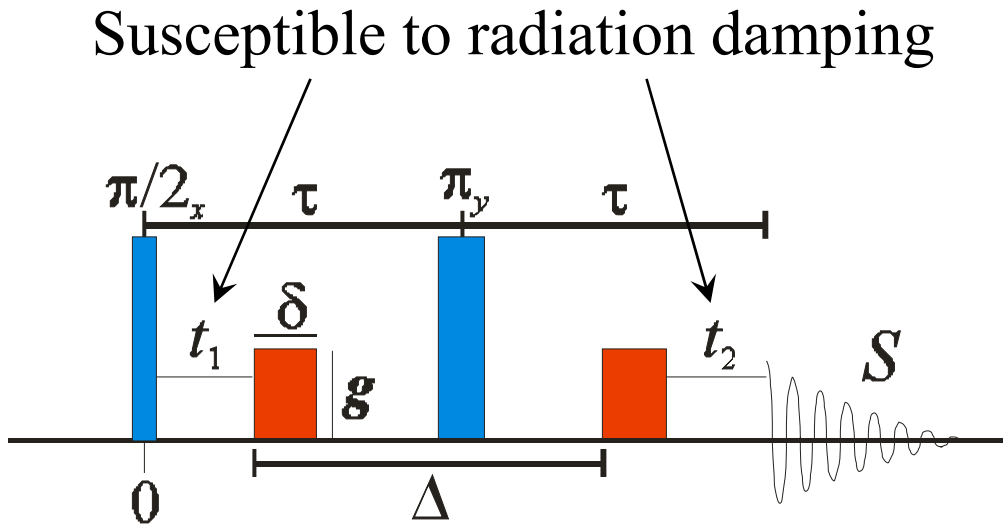
Magnetization recovery after an inversion pulse at 300 MHz



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

Radiation Damping and the PGSE Sequence

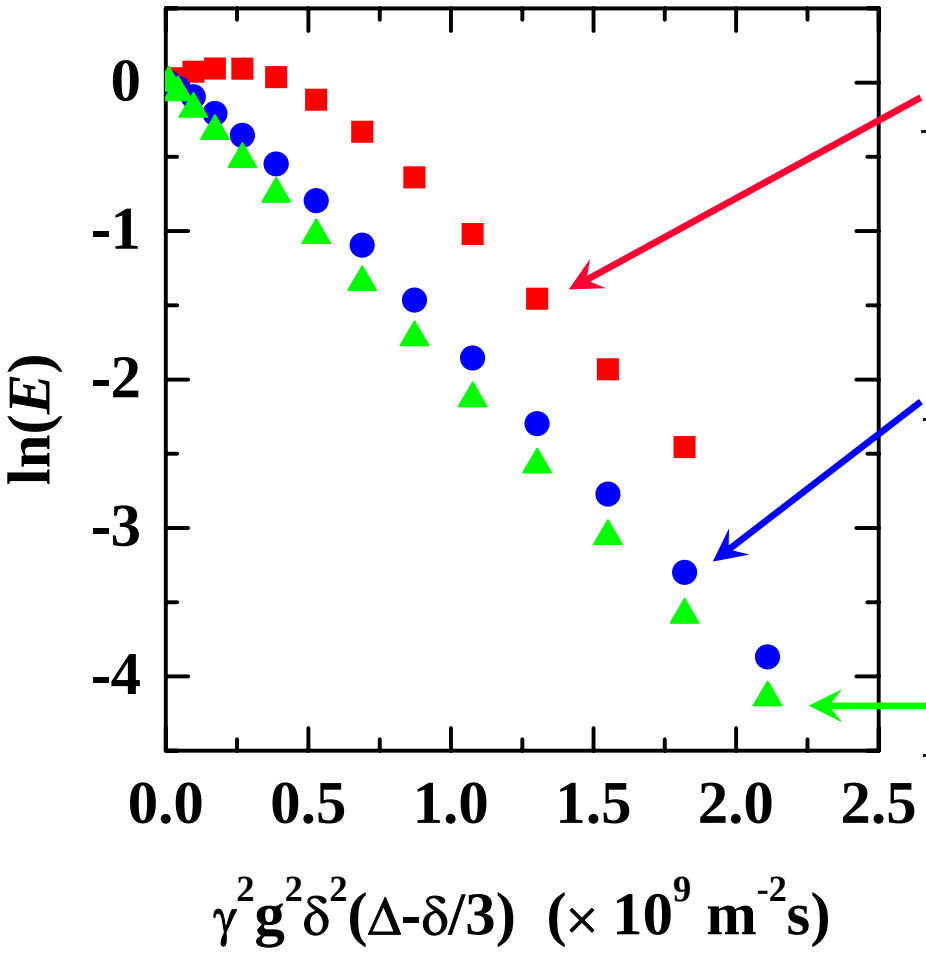
- Radiation damping effects are ~ negligible when the magnetization is gradient encoded (the vector sum of the net magnetization is zero).

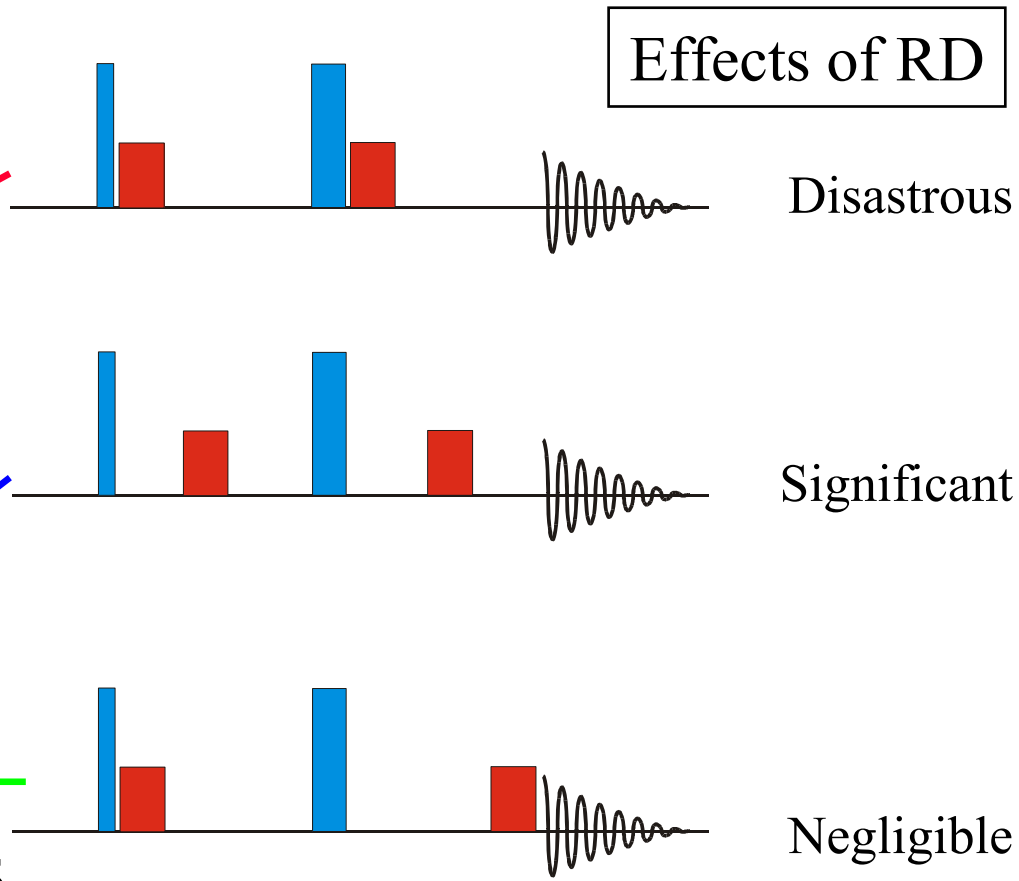


- The effects of radiation damping during t_1 are constant but change during t_2 in a complicated manner on the gradient parameters (δ, g, Δ).

Mild Radiation Damping Effects on PGSE

Small ^1H_2O sample at 300 MHz





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

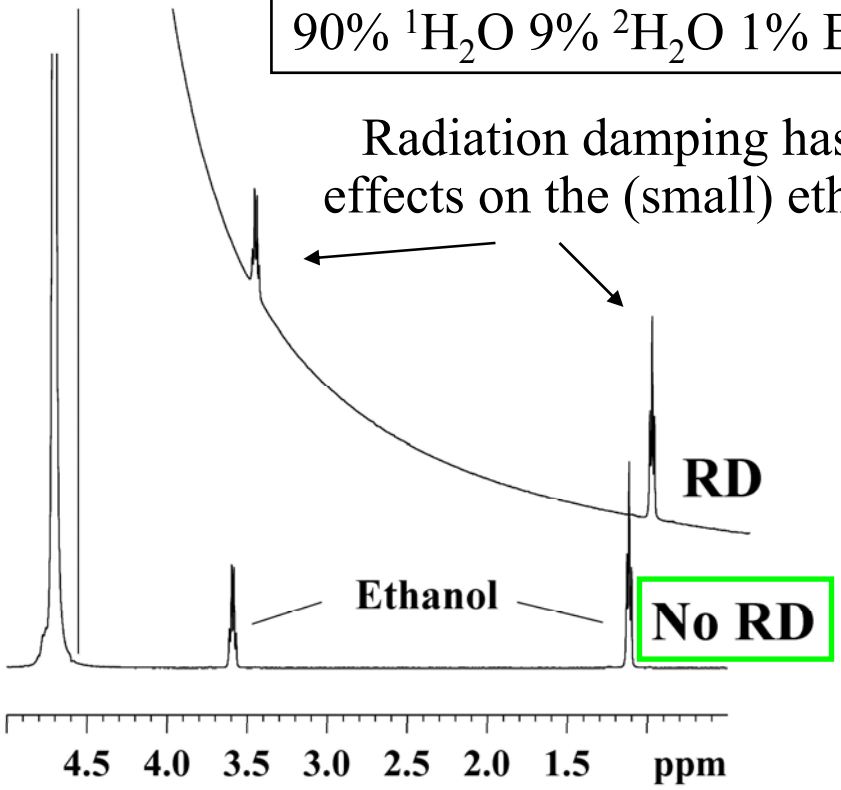
- Unfortunately, the best sequence is generally impracticable.

Using Q-Switching

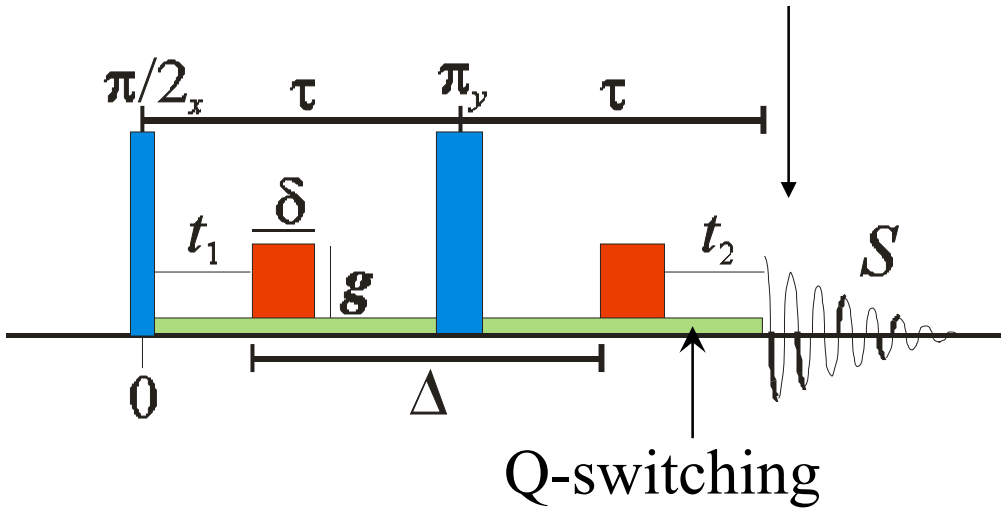
- The advantages of Q-switching (i.e., the rf circuitry is effectively disconnected) during acquisition are well-known.
- Less well-known are the advantages of switching during the sequence.

90% ^1H_2O 9% ^2H_2O 1% Ethanol at 500 MHz

Radiation damping has negligible effects on the (small) ethanol signals.

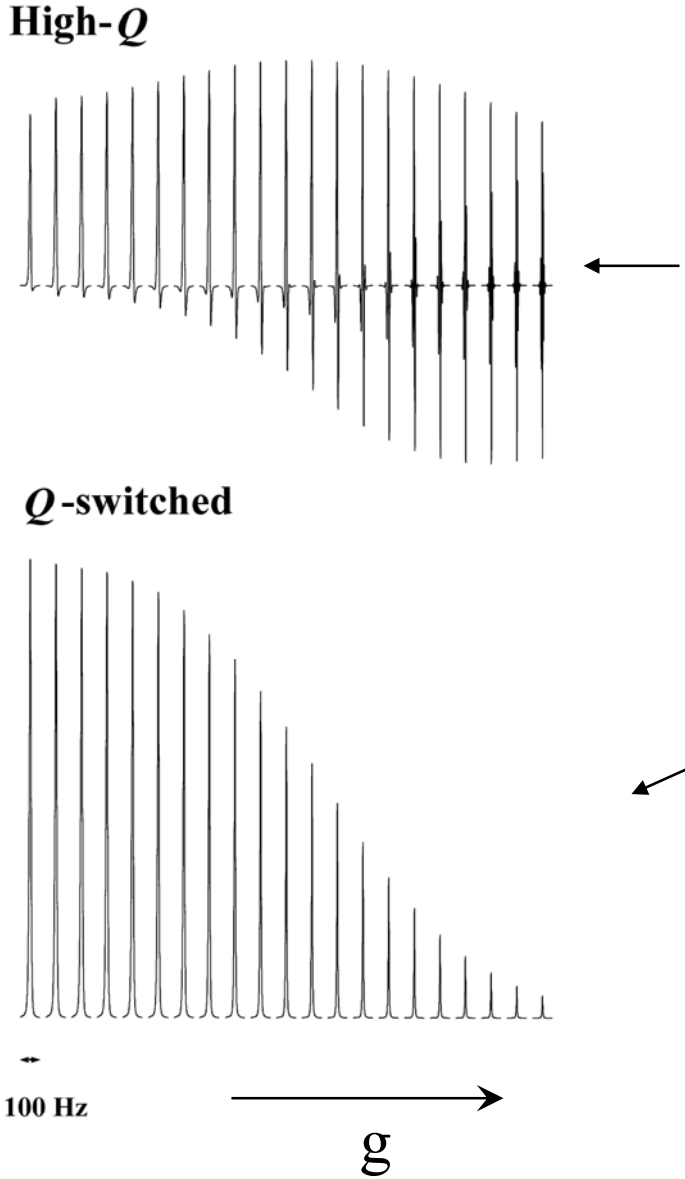


The initial part of the signal is free of radiation damping effects.



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

Using the PGSE-Q-Switch Sequence



90% ^1H_2O at 500 MHz
Δ = 30 ms, δ = 2 ms

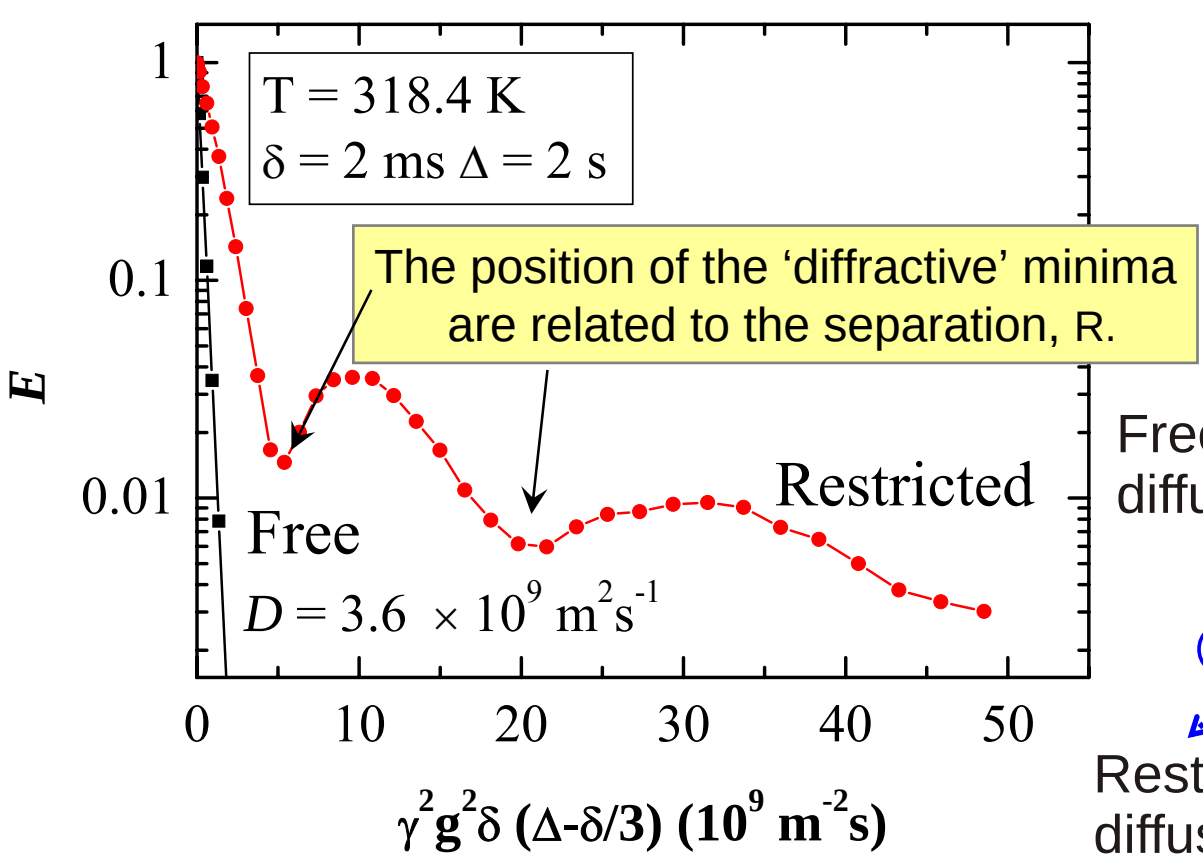
Normal PGSE spectra – very distorted
(High-Q is the normal spectrometer condition)

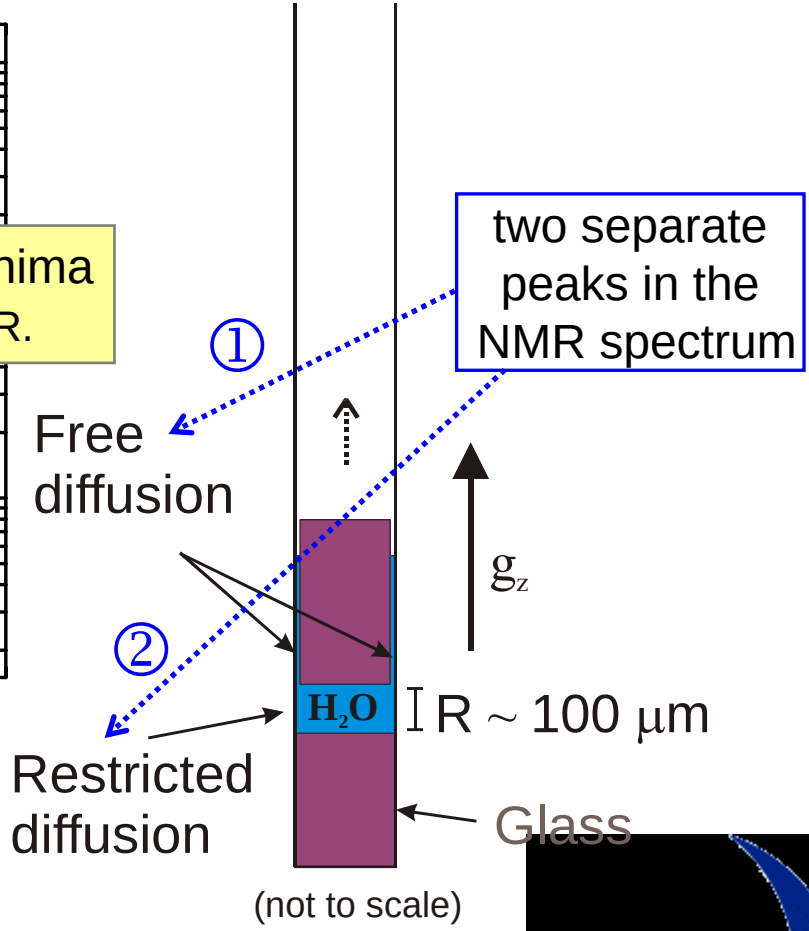
PGSE-Q-Switch spectra

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

Diffusive Diffraction

(between parallel PLANES)





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