Diffusion

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Translational diffusion

- Global property – all residues in a protein have the same diffusion coefficient
- Stokes–Einstein relation to hydrodynamic radius:

$$D = \frac{kT}{6\pi\eta R}$$

Basic mathematics of diffusion

Fick's 1st law: $$J = D\frac{\partial c}{\partial z}$$

Continuity equation:
(conservation of mass) $$\frac{\partial c}{\partial t} = \frac{\partial J}{\partial z}$$

Fick's 2nd law:
('the diffusion equation') $$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial z^2}$$

Solution of the diffusion equation

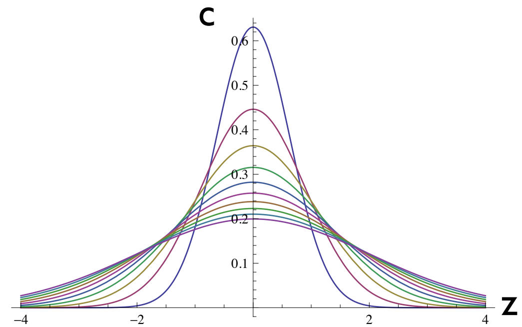
$\dfrac{\partial c}{\partial t} = D\dfrac{\partial^2 c}{\partial z^2}$ with initial condition $c(t=0) = \delta(z)$

has the solution: $c(x,t) = \dfrac{1}{\sqrt{4\pi D t}} \exp\left(-\dfrac{z^2}{4Dt}\right)$

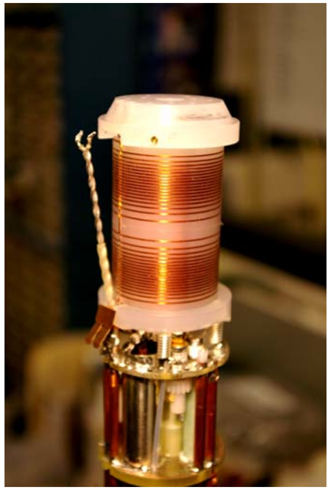
This is a Gaussian distribution with variance:

$$\langle z^2 \rangle = 2Dt$$

i.e. we now know the probability of moving a distance z in time t



NMR: pulsed-field gradients

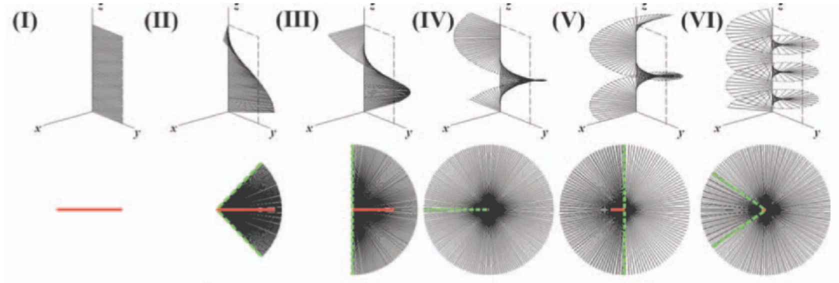


Disruption of field homogeneity

Magnetic field strength linearly proportional to position along z-axis:

$B = B_0 + G \cdot z$

Effect of gradients: the magnetisation helix



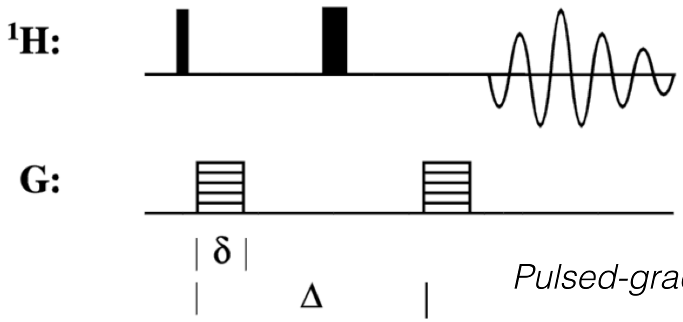
Phase from gradient applied for time δ: $\phi = \gamma G \delta z$

Helix pitch (phase = 2π): $z = \dfrac{2\pi}{\gamma G \delta}$

i.e. gradient strength defines a characteristic length scale

e.g. G = 0.55 T m^{-1}(100%), δ = 4ms => pitch ≈ 10 μm

NMR measurement of translational diffusion

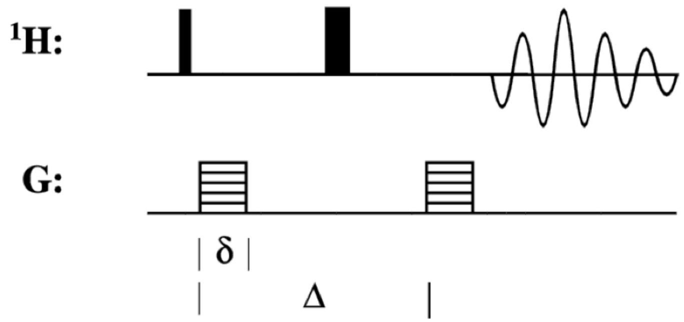


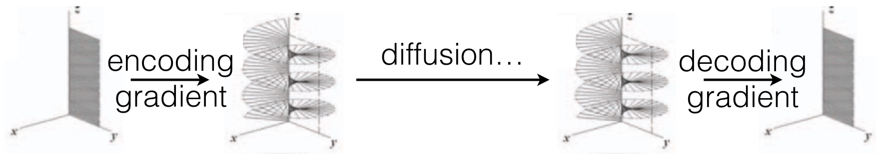
Pulsed-gradient spin-echo

Pairs of gradients encode & decode the z-position of spins.

Diffusion occurring during the delay Δ results in imperfect refocussing, and reduction in observed signal.

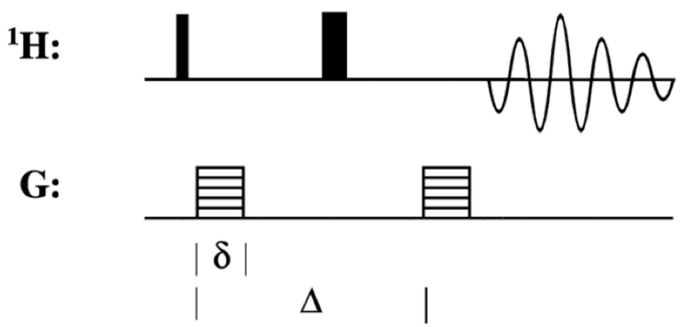
Qualitative description (the magnetisation helix)





Tighter helix => more sensitive to diffusion

The Stejskal-Tanner equation



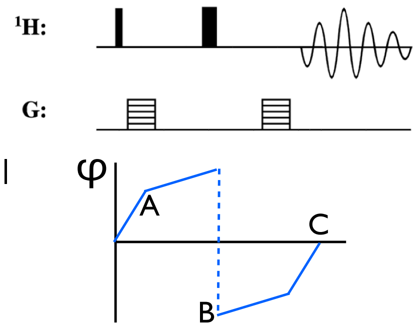
$$\frac{I}{I_0} = \exp\left[-(G\gamma\delta)^2(\Delta - \delta/3)D\right]$$

Derivation of the Stejskal-Tanner equation

phase $\phi = \omega t$

$\omega = \omega_0 + \gamma G z$

spin-echo: we can ignore ω_0 (chemical shift) as it will always be refocused



$\phi_A = \gamma G \delta z_1$

$\phi_B = -\gamma G \delta z_1$

$\phi_C = -\gamma G \delta z_1 + \gamma G \delta z_2 = \gamma G \delta (z_2 - z_1) = \gamma G \delta Z$

Derivation of the Stejskal-Tanner equation

Observed signal:

$$I(G) = \sum_{\text{spins}} (\cos\phi + i\sin\phi) = \sum_{\text{spins}} e^{i\phi} = \langle e^{i\phi}\rangle = \langle e^{i\gamma G\delta Z}\rangle$$

How to calculate average? Basic physics of diffusion!
We know the probability of displacement Z in time Δ has a Gaussian distribution: P(Z) ~ N(0, 2DΔ), so write average in terms of this:

$$I(G) \sim \int_{-\infty}^{\infty} e^{i\gamma G\delta Z} P(Z)dZ \;\sim \int_{-\infty}^{\infty} e^{i\gamma G\delta Z} e^{-Z^2/4D\Delta} dZ$$

Derivation of the Stejskal-Tanner equation

$$I(G) \sim \int_{-\infty}^{\infty} e^{i\gamma G\delta Z} P(Z)dZ \;\sim \int_{-\infty}^{\infty} e^{i\gamma G\delta Z} e^{-Z^2/4D\Delta} dZ$$

Looks formidable, but the integral is just a Fourier transform!

Conjugate variables are Z and γGδ

Fourier transform of a Gaussian with variance 2DΔ is a Gaussian with variance 1/2DΔ

$$I(G) \sim \exp\left[-(\gamma G\delta)^2 D\Delta\right] = I_0 \exp\left[-(\gamma G\delta)^2 D\Delta\right]$$

Optimisation of parameters

$$\frac{I}{I_0} = \exp\left[-(G\gamma\delta)^2(\Delta - \delta/3)D\right]$$

- I/I_0 – want to see substantial decay, ideally to 10%
- Gyromagnetic ratio – proton already most sensitive nucleus (apart from ^3H)
- Diffusion delta (Δ) – as small as possible (T_1 relaxation losses)
- Max. gradient strength – fixed limit, determined by gradient amplifier hardware
- Gradient length (δ) – limited by probe (<4 ms)

Convection

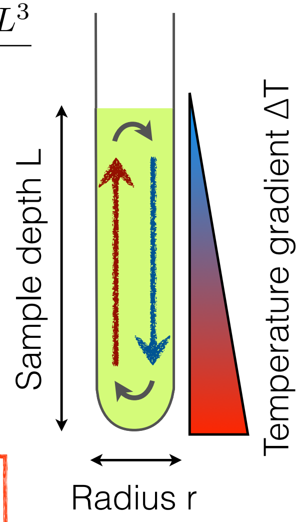
Rayleigh-Bénard number: $Ra = \dfrac{\alpha \Delta T g L^3}{\nu\kappa}$

Thermal expansion coefficient
$\alpha \approx 0.0002\ K^{-1}$

Kinematic viscosity
$\nu \approx 10^{-6}\ m^2\ s^{-1}$

Thermal diffusivity
$\kappa \approx 1.4 \times 10^{-7}\ m^2\ s^{-1}$

Acceleration due to gravity
$g = 9.8\ m^2\ s^{-1}$



Critical value for onset of convection: $Ra_{\text{crit}} \sim 200\dfrac{L^4}{r^4}$

Effect of convection on NMR

$$E(G) = \exp\left(-G^2\gamma^2\delta^2\Delta D\right)$$

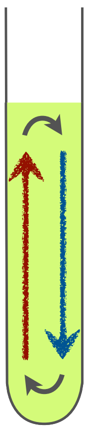
flow

$$E(G) = \exp\left(-G^2\gamma^2\delta^2\Delta D\right)\exp\left(iG\gamma\delta\Delta v\right)$$

$$E(G) = \exp\left(-G^2\gamma^2\delta^2\Delta D\right)\left[\exp\left(iG\gamma\delta\Delta v\right) + \exp\left(-iG\gamma\delta\Delta v\right)\right]/2$$

$$\tfrac{1}{2}\left(e^{ix} + e^{-ix}\right) = \cos x$$

$$E(G) = \exp\left[-G^2\gamma^2\delta^2\Delta D\right]\cos\left(G\gamma\delta\Delta v\right)$$



Effect of convection on NMR

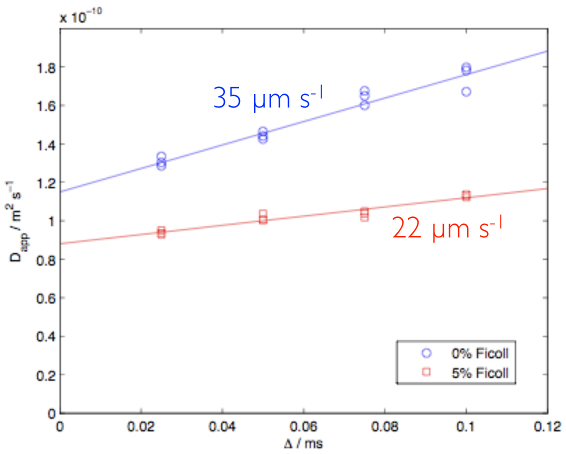
$$E(G) = \exp\left[-G^2\gamma^2\delta^2\Delta D\right]\cos\left(G\gamma\delta\Delta v\right)$$

$$\cos x \approx 1 - \frac{x^2}{2} + O(x^4)$$

$$\exp(-bG^2) \approx 1 - bG^2 + O(G^4)$$

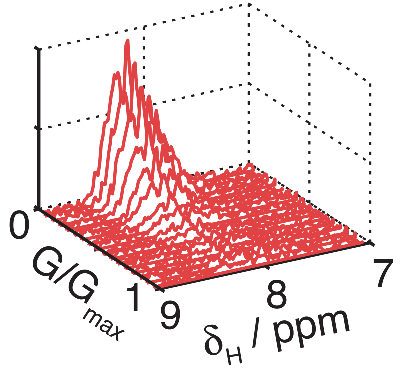
$$E(G) \approx \exp\left[-G^2\gamma^2\delta^2\Delta\left(D_0 + \frac{v^2}{2}\Delta\right)\right] \approx \exp\left[-G^2\gamma^2\delta^2\Delta D_{\text{app}}\right]$$

Convection compensation



$$D_{\mathrm{app}} = D_0 + \frac{v^2}{2}\Delta$$

Data analysis



$$\frac{I}{I_0} = \exp\left[-(G\gamma\delta)^2(\Delta - \delta/3)D\right]$$

$$D = \frac{kT}{6\pi\eta R}$$

Hydrodynamic Radii of Native and Denatured Proteins Measured by Pulse Field Gradient NMR Techniques†

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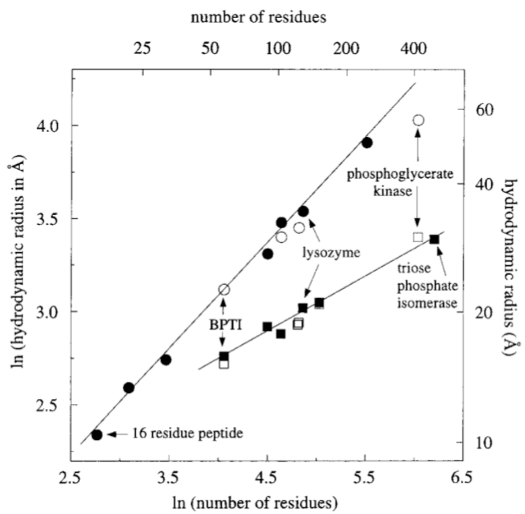
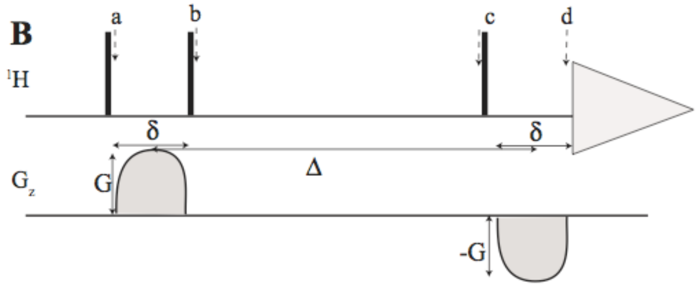
Received July 28, 1999; Revised Manuscript Received October 4, 1999



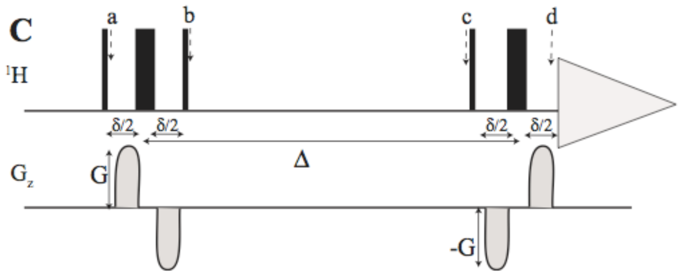
Refinement of pulse sequences

Pulsed-gradient stimulated-echo



Refinement of pulse sequences

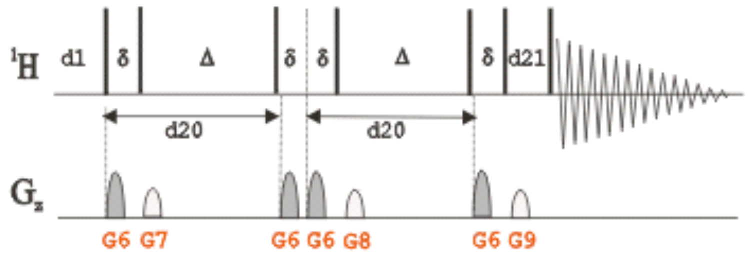
Stimulated echo with bipolar gradients



Refinement of pulse sequences

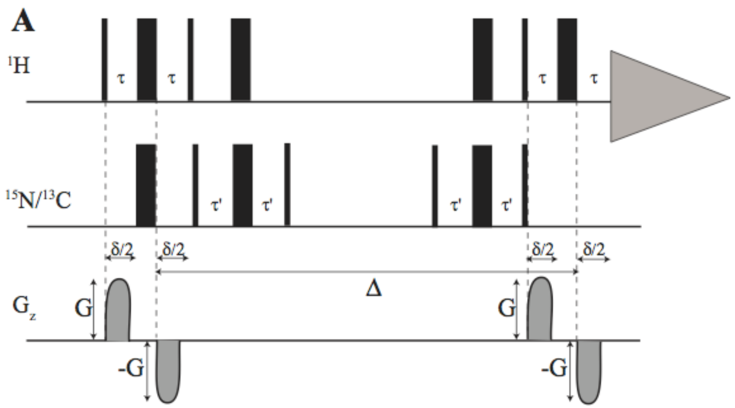
Double stimulated echo

dstegp3s



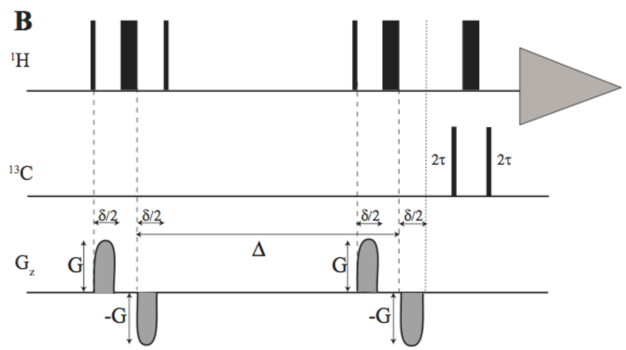
Refinement of pulse sequences

15N XSTE



Refinement of pulse sequences

Methyl-TROSY 1H STE-HMQC



2D diffusion experiments

^1H-^{15}N XSTE-HSQC