Chapter 8, Q 1,3,4,5,7,8
(Next week Q10-14)

2D NMR part II

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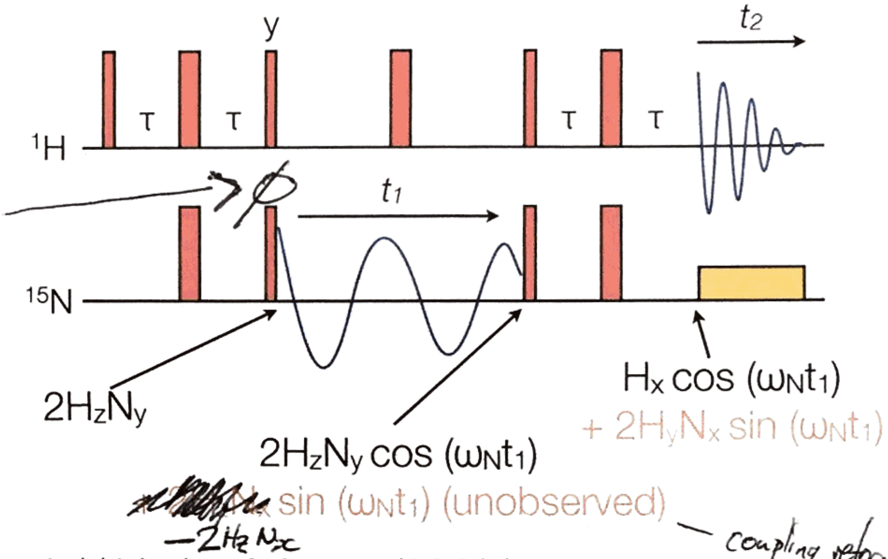
The HSQC experiment

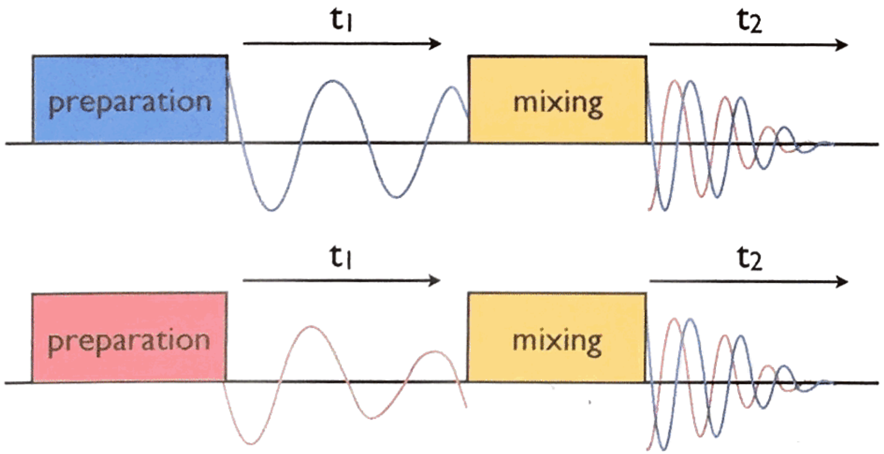
x, y
⇒ cos, sin modulation for phase sensitive detection



$\tau = 1 / (4J_{HN}) = 2.25$ ms (90 Hz)

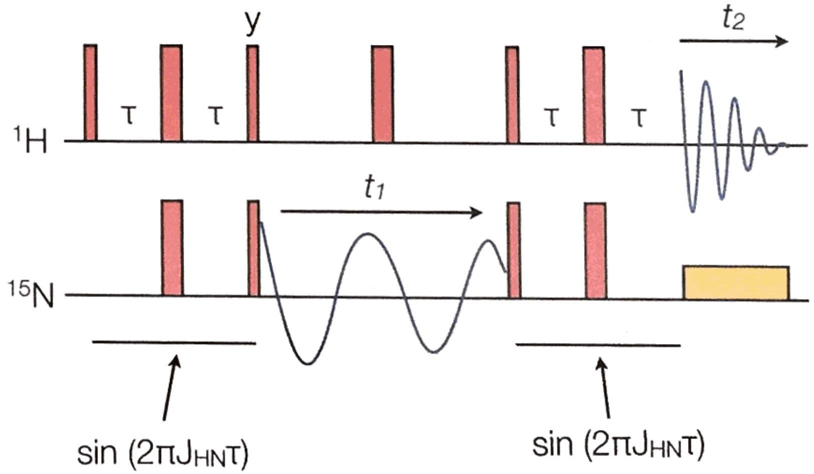
coupling refocussed
offset evolves

Quadrature detection in 2D



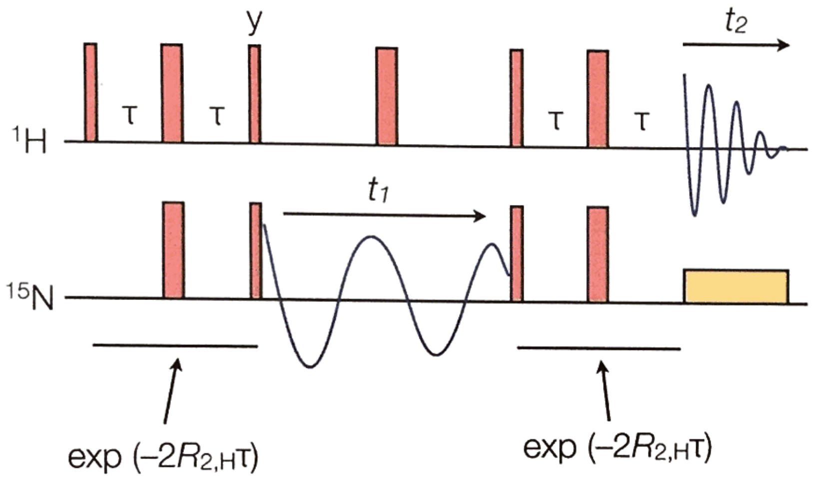
hypercomplex data

HSQC sensitivity



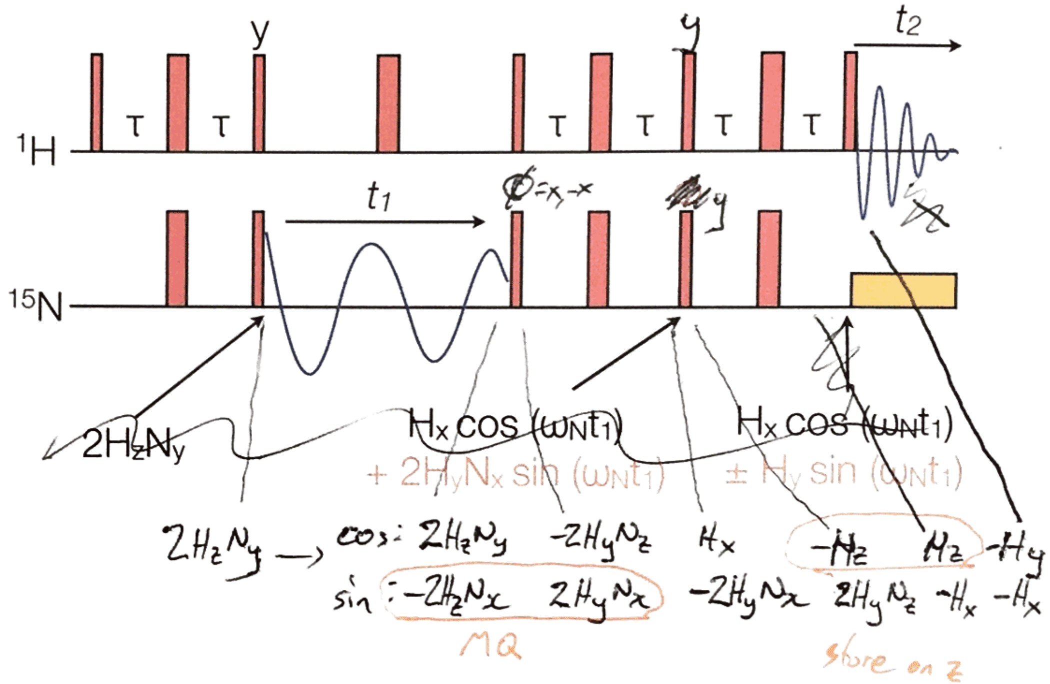
INEPT transfer efficiencies

HSQC sensitivity



Relaxation losses

The sensitivity-improved HSQC



Processing / sensitivity

Observed magnetisation =

$$H_x \cos(\omega_N t_1) \pm H_y \sin(\omega_N t_1)$$

$$\text{Sum} = 2\cos(\omega_N t_1)$$

$$\text{Difference} = 2i \sin(\omega_N t_1)$$

- 2x increase in signal by adding/subtracting adjacent FIDs
- Noise is also added
- Net gain of √2 in SNR
- Less in practice due to relaxation in longer sequence

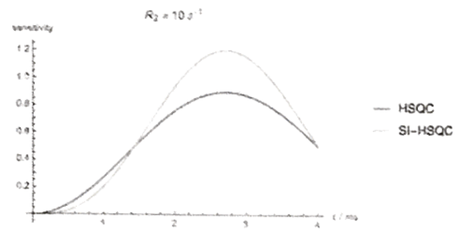
Comparison of HSQC and SI-HSQC sensitivity

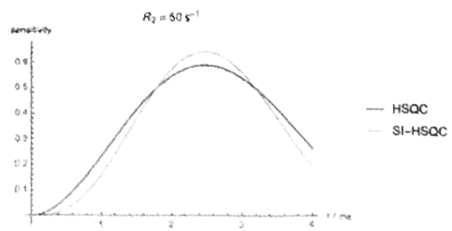
HSQC sensitivity:

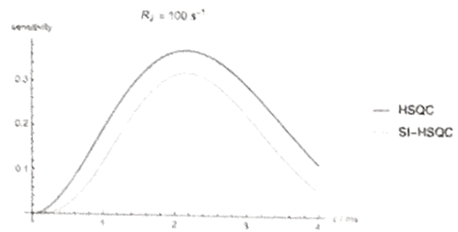
$$\sin^2(2\pi J_{HN}\tau)\exp(-4R_{2,H}\tau)$$

SI-HSQC sensitivity:

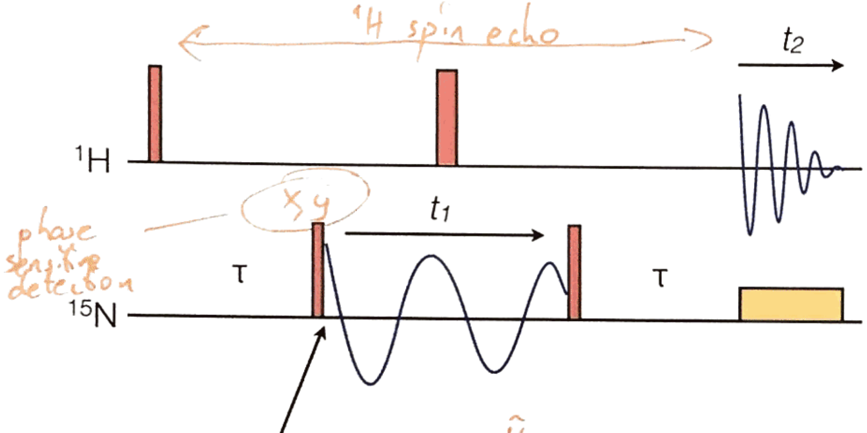
$$\sqrt{2}\sin^3(2\pi J_{HN}\tau)\exp(-6R_{2,H}\tau)$$







The HMQC experiment



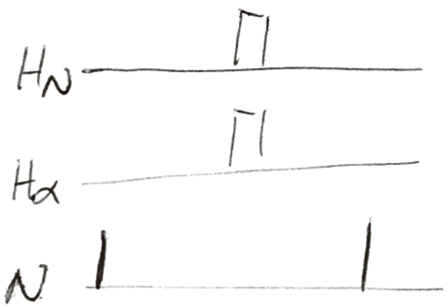
$2H_xN_y$ = ZQ + DQ $\xrightarrow{\pi \hat{H}_x}$ DQ + ZQ

$\tau = 1 / (2J_{HN}) = 4.5$ ms (90 Hz)

HMQC vs HSQC

- HMQC is simpler pulse sequence – less scope for calibration errors, and pulse imperfections (especially 180° pulses) don't matter so much
- Product operators during t_1:
  - HSQC: single quantum in-phase and anti-phase $2H_zN_y \Leftrightarrow N_x$
  - HMQC: multiple (zero + double) quantum
- Relative relaxation rates:
  - $R_2(N_x) < R_2(2H_zN_y) < R_2(2H_xN_y)$
- IMPORTANT EXCEPTION: methyl-TROSY HMQC!

Evolution of passive couplings during t_1



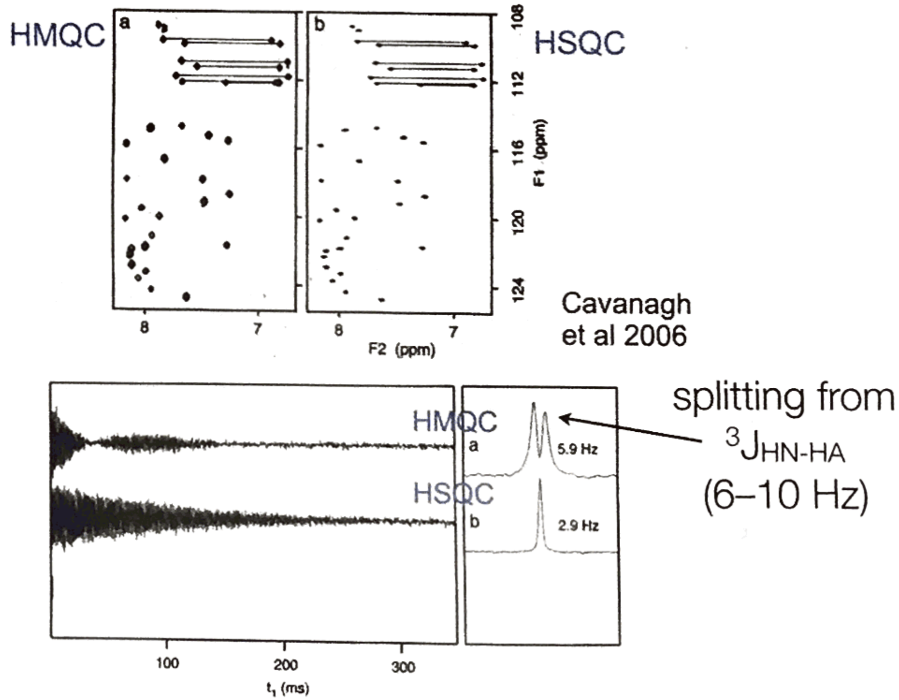
$(H_N)_x N_y \longrightarrow ?$

N – H_N coupling refocussed

N – H_α coupling refocussed

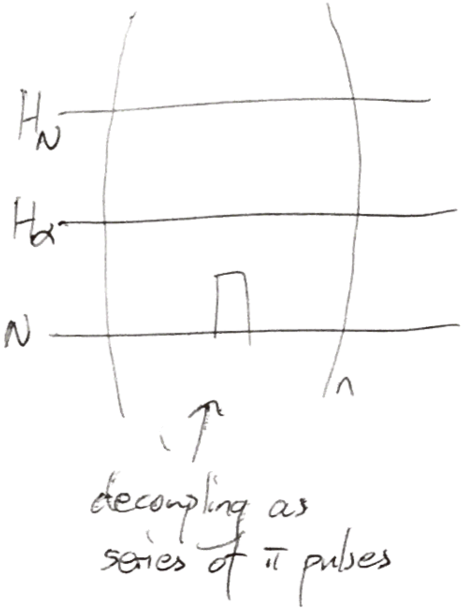
H_N – N coupling refocussed

H_N – H_α coupling <u>evolves</u>



Cavanagh et al 2006

Evolution of passive couplings during t_2

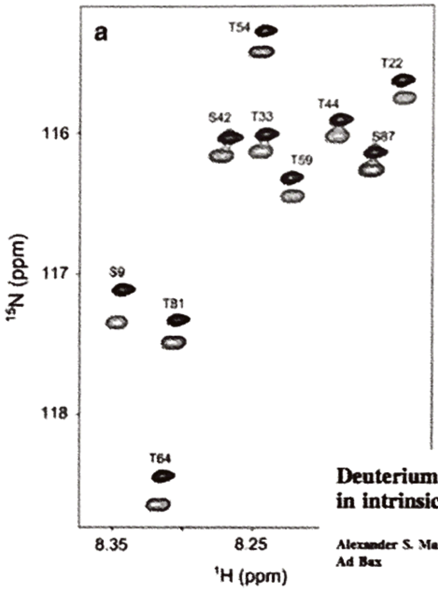


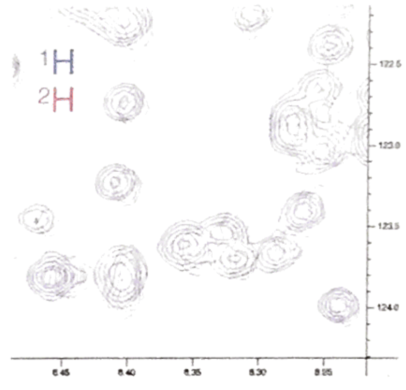
$(H_N)_x \longrightarrow ?$

H_N–N coupling refocussed

H_N–H_α coupling evolves

Effect of perdeuteration

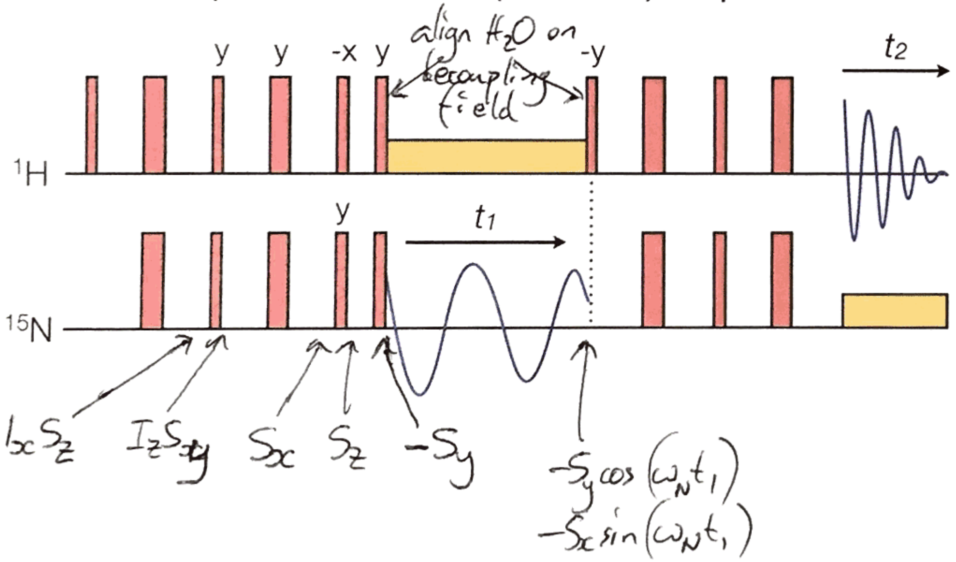




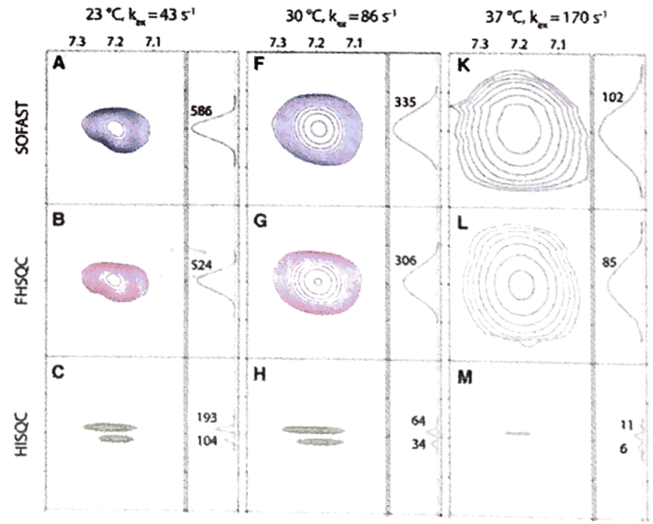
Deuterium isotope shifts for backbone ^{1}H, ^{15}N and ^{13}C nuclei in intrinsically disordered protein α-synuclein

Alexander S. Maltsev · Jinfa Ying · Ad Bax

The in-phase HSQC (HISQC) experiment



Comparison of HMQC, HSQC, HISQC



Yuwen, T. & Skrynnikov, N. R. *J. Biomol. NMR* **58**, 175–192 (2014).

Amide exchange

$$N_x H_z = N_x(H_\alpha - H_\beta)$$

quantum superposition involving both N and H spin states

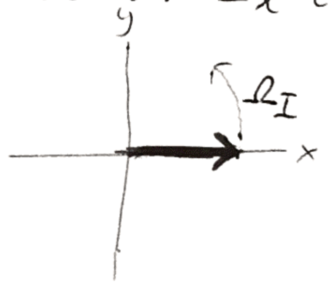
Chemical exchange of amide proton

=> coherence destroyed

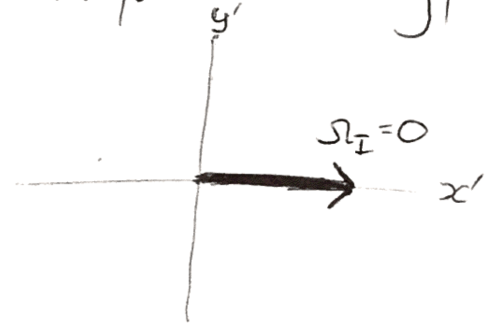
Vector model description of J-coupling

IS system (Ionel Soloman)

Consider I_x evolution:



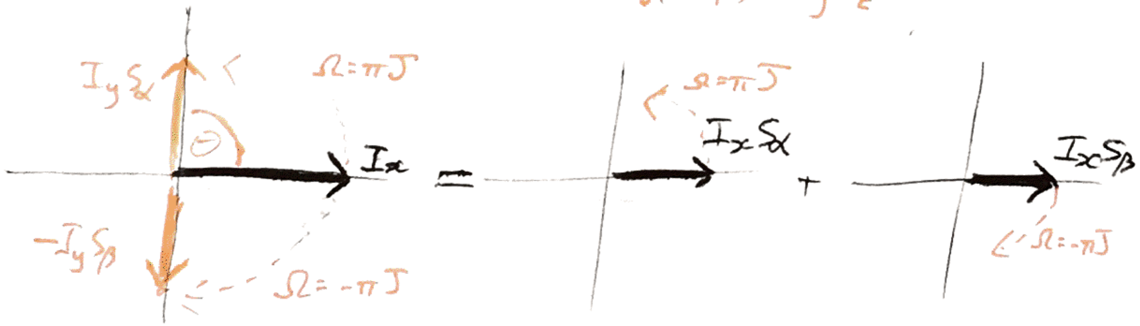
Transform into rotating frame:



Vector model description of J-coupling

I_x is really a mixture of S_α and S_β spin states:

$$I_x = I_x S_\alpha + I_x S_\beta \longrightarrow I_y(S_\alpha - S_\beta) = I_y S_z$$



$\Theta = \pi J \tau = \pi/2$ for complete conversion to antiphase

$$\Rightarrow \tau = \frac{1}{2J}$$

Decoupling (on-resonance)

- Coupling = splitting of resonances by frequency J
- Therefore, to observe (resolve) coupling, need to observe for time $\tau \geq 1 / J$
    - i.e. lifetime of coupled state must be $\geq 1 / J$
- Converse: reduce the lifetime, and coupling won't be observed
- Basic idea: exchange S_α <—> S_β with π pulse to refocus coupling evolution