3D NMR

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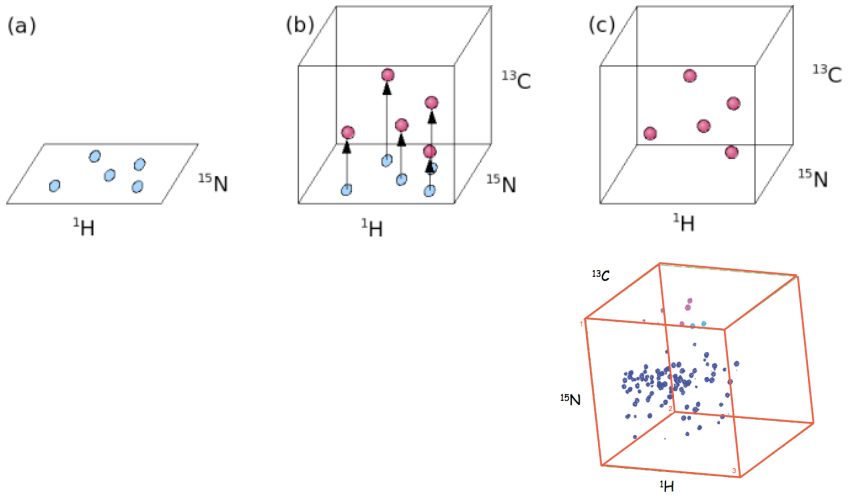
3D NMR



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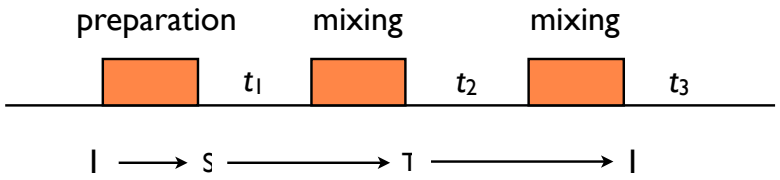
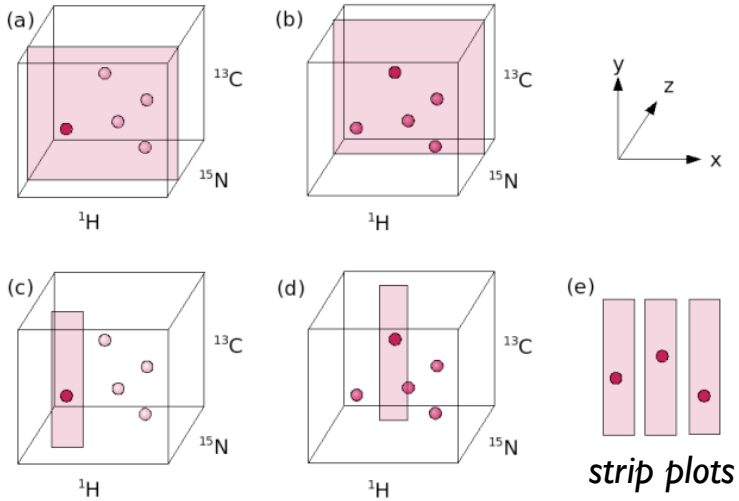
3D NMR



- 2 indirect dimensions, independently incremented evolution times
- Much longer acquisition than 2Ds (hours-days)
- SNR decreases by $\sqrt{2}$ for each extra dimension (quadrature detection, need to record separate sin and cos components)
- Longer sequences – lower sensitivity

Visualising 3D NMR spectra



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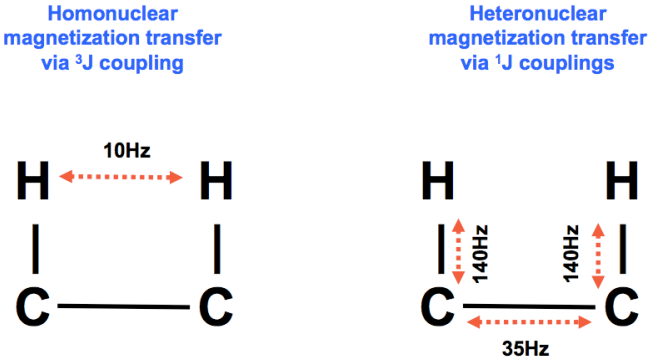
Effective long-range magnetisation transfer



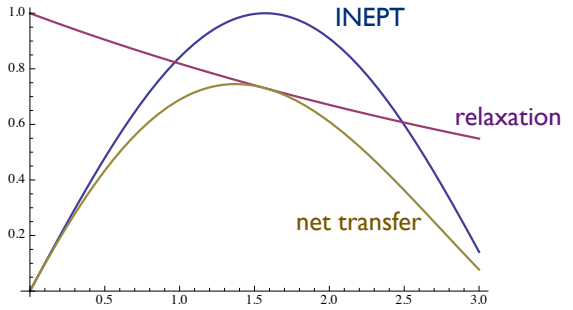
$T_{INEPT} \approx 1 / 2J$

$SNR \sim ns^{1/2} \cdot conc \cdot B_0^{3/2} \cdot \gamma_{ex} \cdot \gamma_{obs}^{3/2}$

Michael Sattler

Optimisation of transfer efficiencies

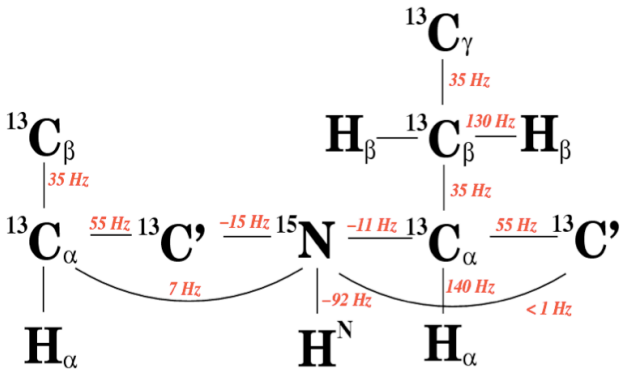
INEPT transfer vs relaxation



$$\text{transferred signal} \propto \sin(\pi J \tau) \exp(-R_2 \tau)$$

INEPT relaxation

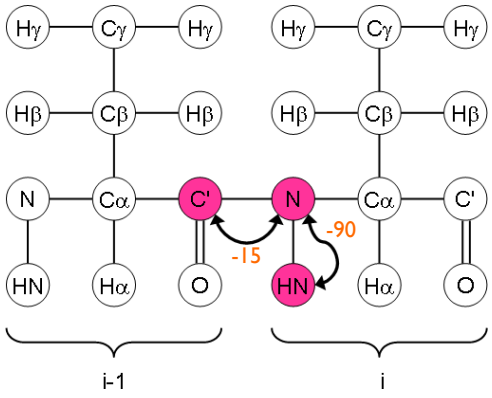
Backbone ^1J and ^2J couplings

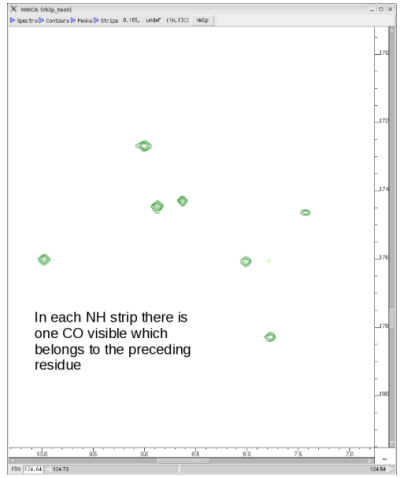


- *J couplings proportional to gyromagnetic ratio (N.B. negative couplings involving ^{15}N)*
- *^2J couplings very small!*

Michael Sattler

The HNCO experiment





CO chemical shifts very sequence dependent

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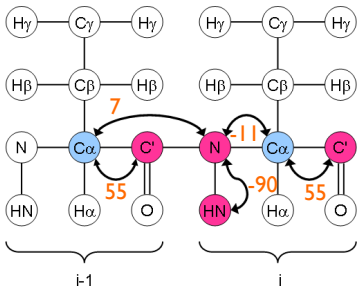
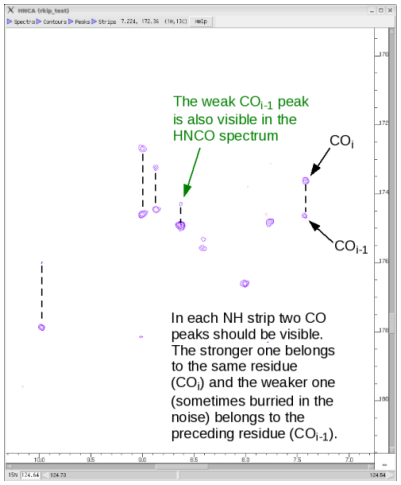
HN(CA)CO

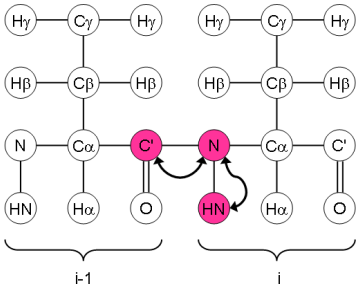


HN(CA)CO links
HN_i with C'_{i-1} and C'_i

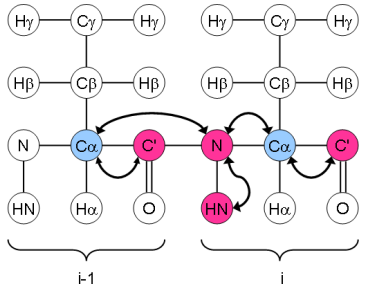
$^2J_{NC'}$ < 1 Hz, so transfer via CA is required



HNCO / HN(CA)CO

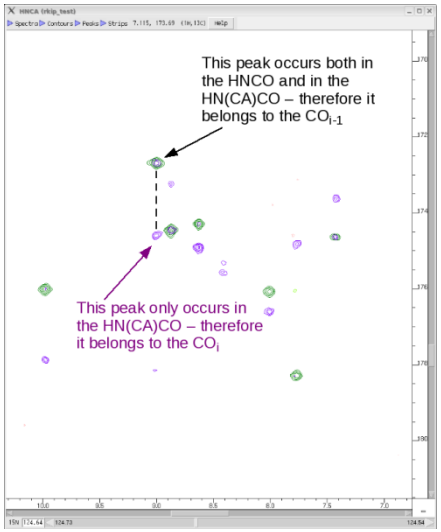


HNCO links
HN_i with C'_{i-1}

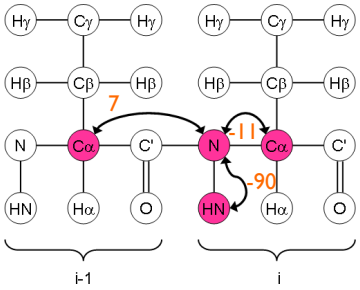


HN(CA)CO links
HN_i with C'_{i-1} and C'_i

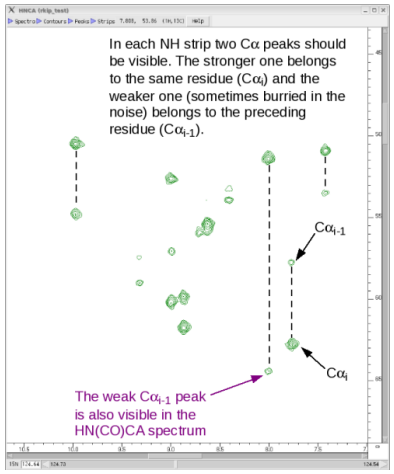
HNCO / HN(CA)CO



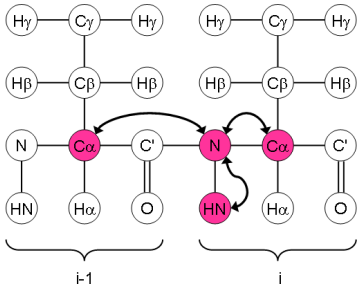
HNCA



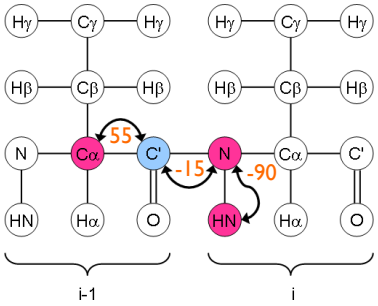
HNCA links
HN_i with CA_{i-1} (weak)
and CA_i (strong)



HNCA / HN(CO)CA



HNCA links
HN_i with CA_{i-1} (weak)
and CA_i (strong)



HN(CO)CA links
HN_i with CA_{i-1}

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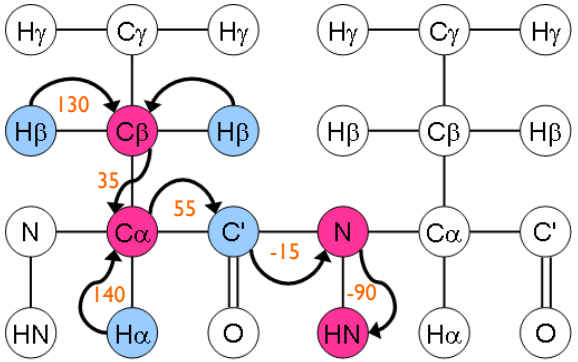
CBCA(CO)NH



HA/HB polarisation transferred to HN

With deuterated protein, 'out-and-back' HN(CO)CACB experiment must be used – longer, less sensitive

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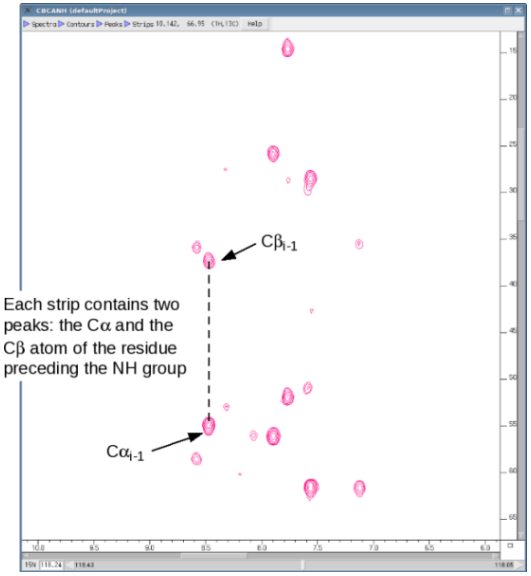
CBCA(CO)NH



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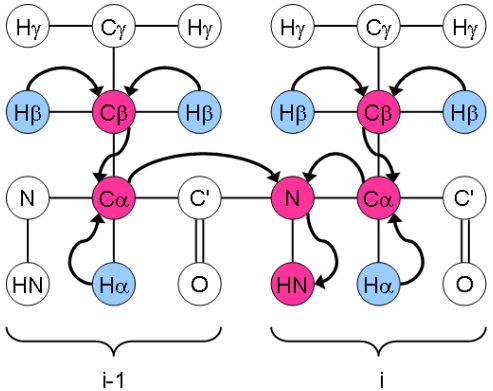
CBCANH



HA/HB polarisation transferred to HN

With deuterated protein, 'out-and-back' HNCACB experiment must be used – longer, less sensitive

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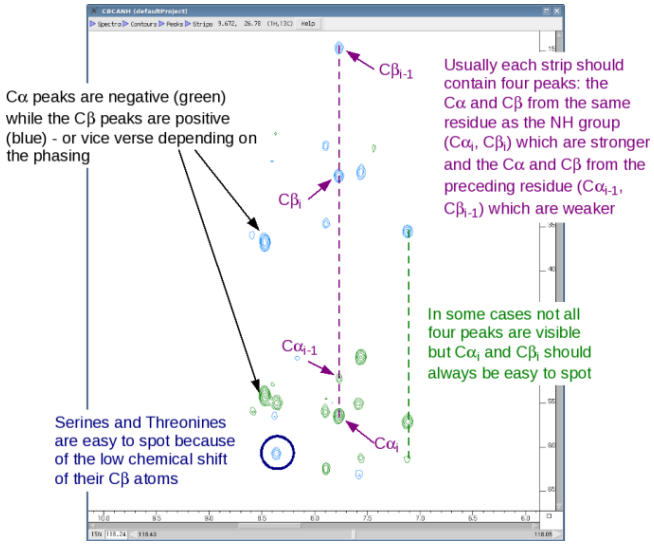
CBCANH



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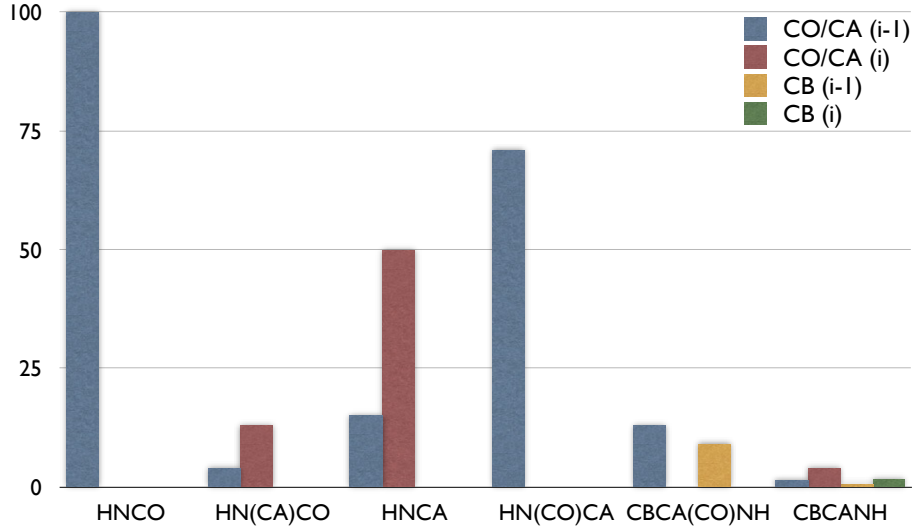
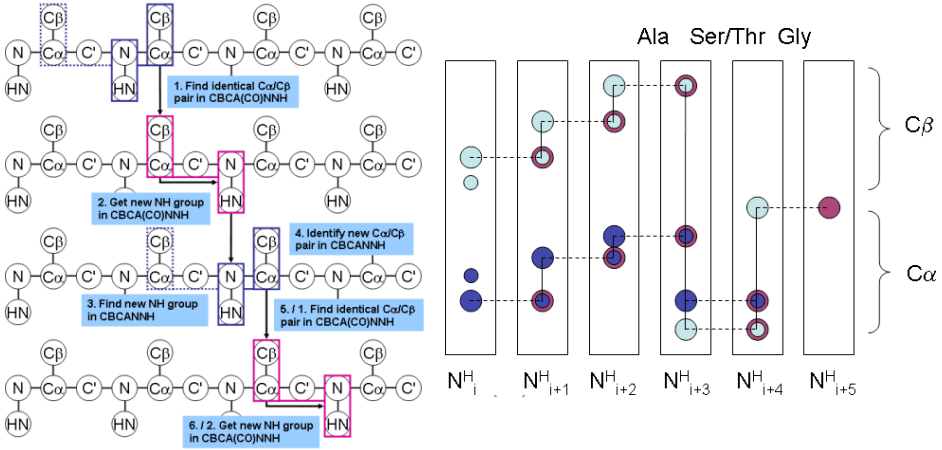
Relative sensitivity of 3D experiments



The assignment process



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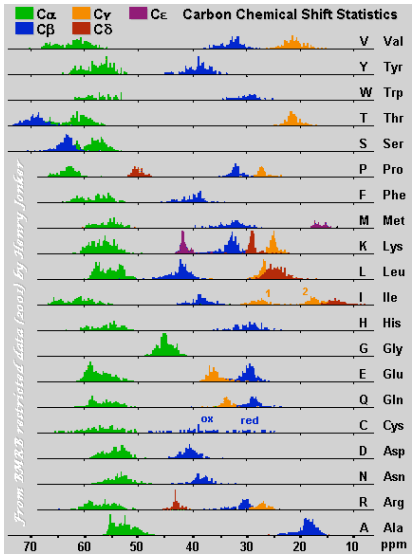
Spin system typing



- CA and CB shifts are extremely useful for determining residue type
- Extent of CA and CB chemical shift dispersion is dependent on secondary structure formation – bad for IDPs!
- Ser/Thr very easy to identify – characteristic CB shifts (bonded to electronegative oxygen)
- Ala – unique CB shift around 17 ppm
- Gly – unique CA shift around 45 ppm – and no CB resonances!
- Others are less clear, but usually can restrict to a few possibilities
- Prolines are only observed in *i*-1 experiments – e.g. CBCA(CO)NH

Sequence analysis – identifying assignment checkpoints

alpha-synucein

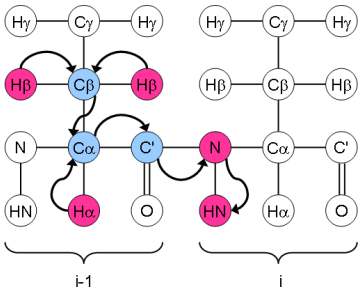
```
         10          20          30          40          50          60
MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKTK

         70          80          90          100         110         120
EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP

        130         140
DNEAYEMPSE EGYQDYEPEA
```

Sequence analysis – identifying assignment checkpoints

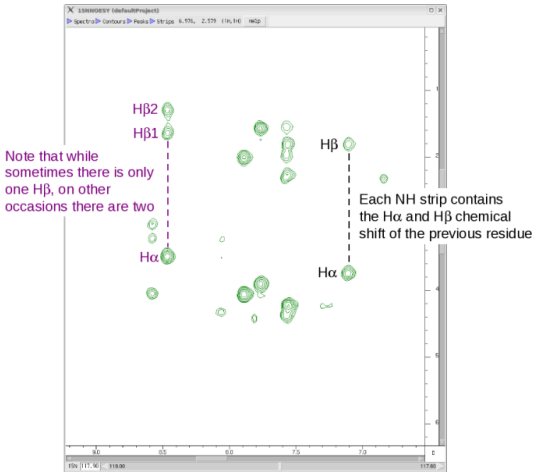
ddFln5

```
                                                            650         660
                                                           KPAPS AEHSYAEGEG

        670         680         690         700         710         720
LVKVFDNAPA EFTIFAVDTK GVARTDGGDP FEVAINGPDG LVVDAKVTDN NDGTYGVVYD

        730         740         750
APVEGNYNVN VTLRGNPIKN MPIDVKCIEG
```

Sidechain assignment: HBHA(CO)NH



Essentially identical pulse sequence to CBCA(CO)NH – chemical shift encoded on ^{1}H rather than ^{13}C



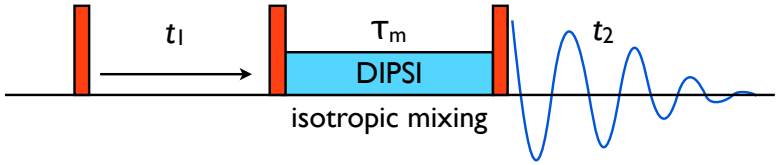
Sidechain protons and magnetic equivalence

Sidechain assignment: TOCSY

TOtal Correlation SpectroscopY

Extension of HBHA(CO)NH – wouldn't it be nice to get cross-peaks from ALL the spins in a sidechain?

2D TOCSY experiment



$$I_{1,z} \rightarrow -I_{1,y} \rightarrow -I_{1,y}\cos(\omega_1 t_1) + \text{other terms} \rightarrow I_{1,z}\cos(\omega_1 t_1)$$

eliminated by phase cycling — *at start of mixing period*

Isotropic mixing

$$\mathcal{H} = -\sum_i \omega_i I_{iz} + \sum_{i,j} 2\pi J_{ij} \mathbf{I}_i \cdot \mathbf{I}_j$$

$$= -\sum_i \omega_i I_{iz} + \sum_{i,j} 2\pi J_{ij} I_{iz} I_{jz}$$

when Δω >> J (weak coupling)

DISPI spin-lock manipulates the effective Hamiltonian to remove chemical shift differences ('isotropic mixing') so:

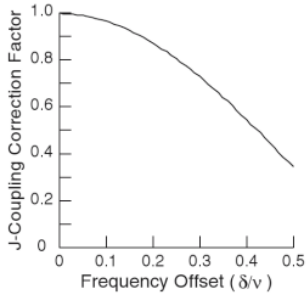
$$\mathcal{H}_{\text{isotropic mixing}} = \sum_{i,j} 2\pi J_{ij} \mathbf{I}_i \cdot \mathbf{I}_j$$

All spins are strongly coupled! Magnetisation will transfer between all spins in the spin system.

Isotropic mixing

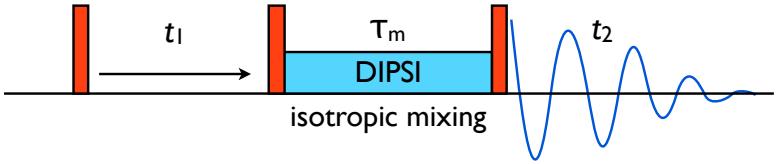
The spin-lock reduces the effective chemical shift difference between spins, increasing the efficiency of TOCSY transfer.

The magnitude of this effect depends on the strength of the spin-lock relative to the frequency difference:



Fundamentals of Protein NMR Spectroscopy, Rule & Hitchens

2D TOCSY experiment



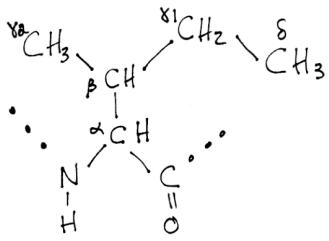
$$I_{1,z} \rightarrow -I_{1,y} \rightarrow -I_{1,y}\cos(\omega_1 t_1) + \text{other terms} \rightarrow I_{1,z}\cos(\omega_1 t_1)$$

eliminated by phase cycling — *at start of mixing period*

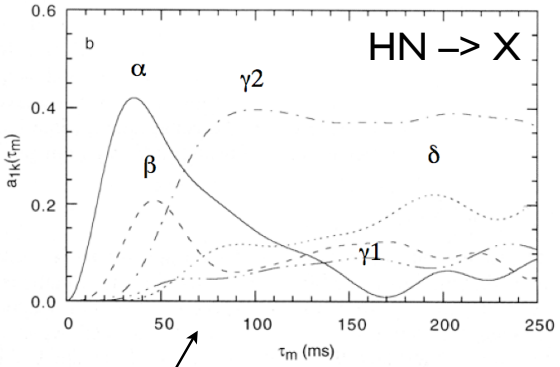
$$\cos(\omega_1 t_1) I_{1,z} \xrightarrow{\text{DIPSI}} \sum_j \sin^2(\pi J_{\text{eff}} \tau_m)\cos(\omega_1 t_1) I_{j,z}$$

TOCSY transfer coefficient

TOCSY transfer efficiency



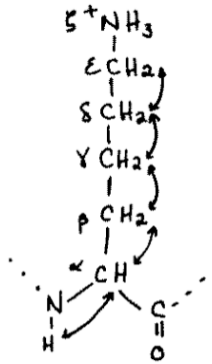
isoleucine spin system



Neglecting T_2 relaxation!

Cavanagh

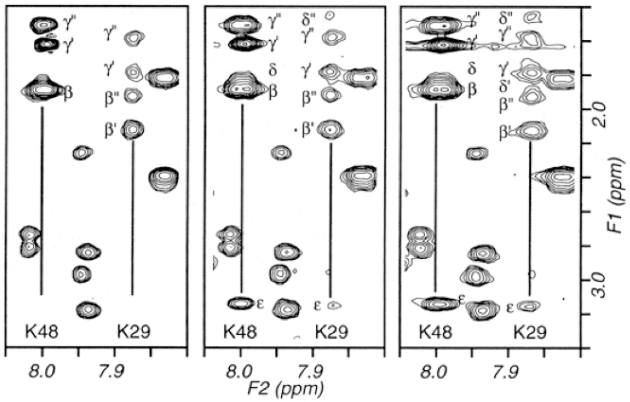
Optimising TOCSY mixing time



lysine spin system

Ubiquitin: 2D ^{1}H-^{1}H TOCSY

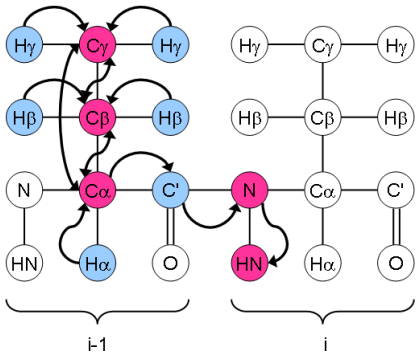
showing amide-aliphatic region



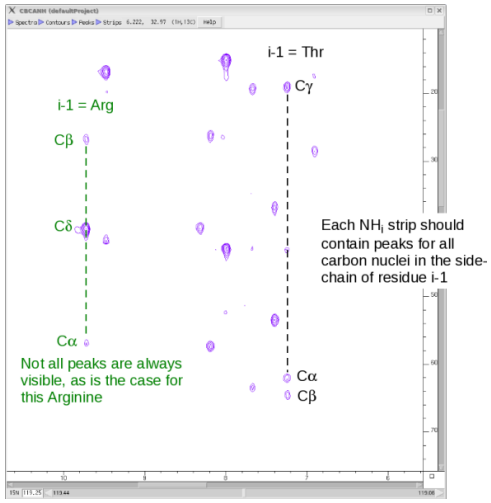
48 ms 83 ms 102 ms

Cavanagh

CC(CO)NH



TOCSY transfer between aliphatic carbons



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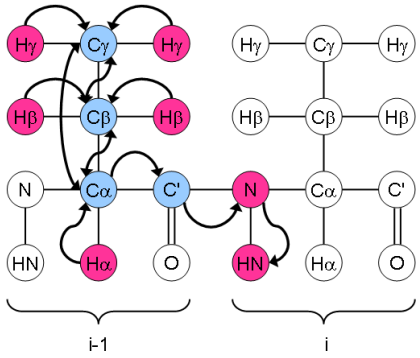
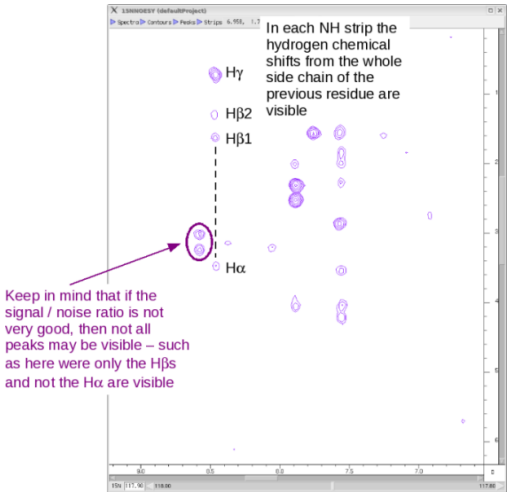
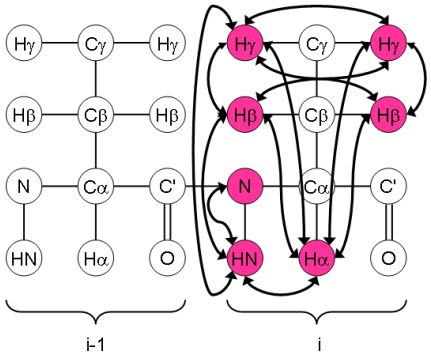
H(CCO)NH



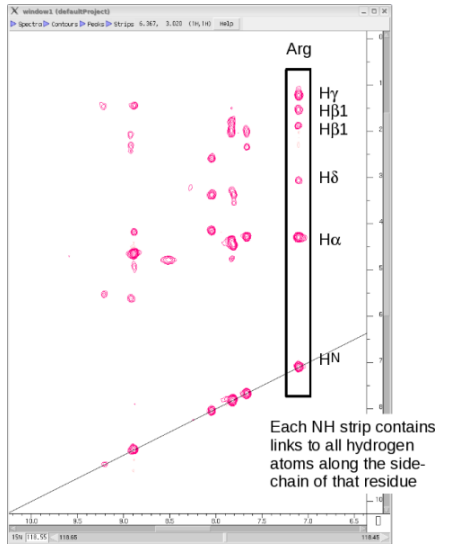
TOCSY transfer between aliphatic carbons



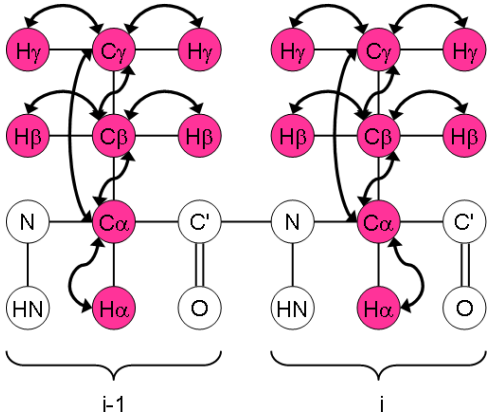
15N TOCSY-HSQC



TOCSY transfer between protons



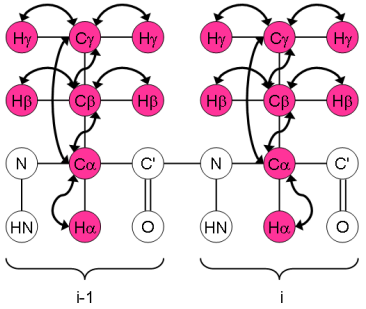
HCCH TOCSY



TOCSY transfer between carbons

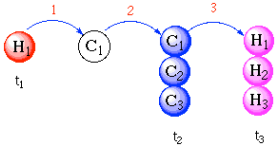
HCCH TOCSY

Which chemical shifts to encode for 3D experiment?



HcCH

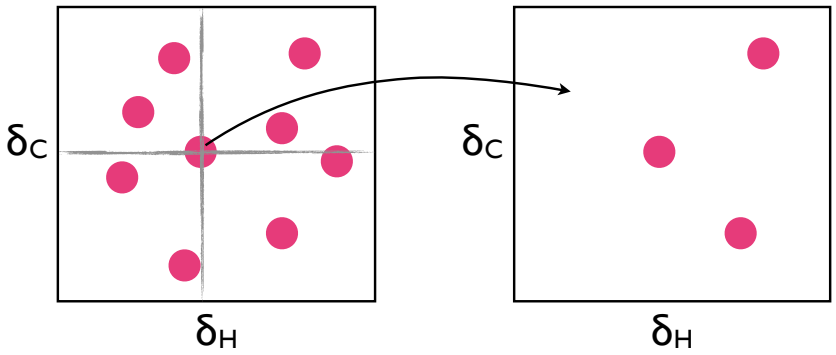
^{1}H correlations to CH group



hCCH

^{13}C correlations to CH group

4D HCCH TOCSY

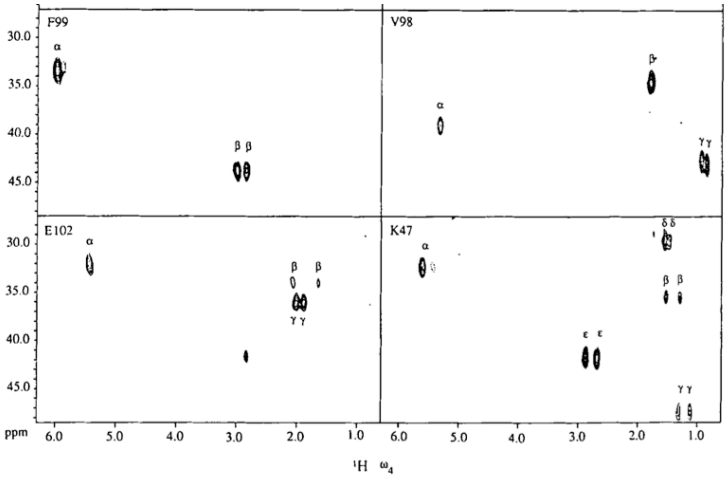


Each point in 2D $^{1}H,^{13}C$ spectrum is correlated with a second 2D $^{1}H,^{13}C$ spectrum showing coupled spins

Problem – huge acquisition time!
(can be addressed using non-uniform sampling…)

Olejniczak et al (1992) J Biomol NMR

4D HCCH TOCSY



40 x 9 x 10 x 1024 points – 6.4 days acquisition

Olejniczak et al (1992) J Biomol NMR