

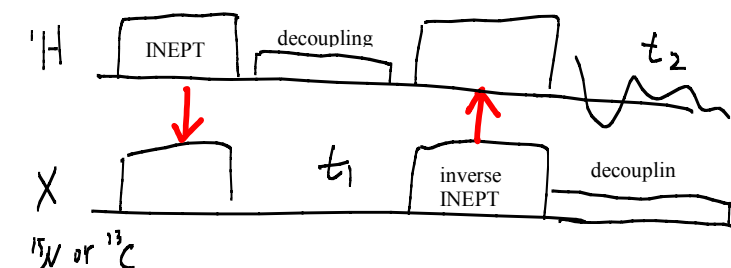
## Chem590N1 Feb. 1, 2008

Palmer's Chapter 7.1-7.3 HMQC, HSQC, TROSY, combination with NOESY & TOCSY

### 1. Heteronuclear experiments

Proton 2D and 3D homonuclear experiments become ineffective for proteins larger than 10 kDa. Spectral degeneracy, as a result of the increased linewidth and number of resonances make assignment difficult or impossible. In addition, fast spin-spin relaxational decay causes too much signal loss during the long mixing periods dictated by the small homonuclear scalar couplings ( $^3J < 10$  Hz).

Heteronuclear NMR experiments, which rely on the much larger one- and two-bond scalar couplings ( $^1J_{N-H} \sim 90$  Hz,  $^1J_{C-H} \sim 140$  Hz), circumvent those problems for proteins up to 30 kDa. The heteronuclears have much wider chemical shift ranges (amide  $^{15}N$  40 ppm, alpha  $^{13}C$  25 ppm, in contrast with 4 ppm for amide  $^1H$ ), very effective in reducing overlap and degeneracy. The TROSY technique further improves resolution greatly and proteins as large as several hundreds kDa have been investigated.  $^{15}N$  and  $^{13}C$  (as well as  $^2H$  for TROSY) isotope labeling is required.



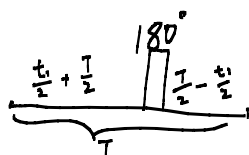
#### HSQC (heteronuclear single quantum correlation)

schematic draws of the pulse sequence

See example N-H hsqc spectra in Fig. 7.2b, c.

- \* one peak per amide group
- \* two peaks for  $NH_2$

\* peaks have no fine structures (such as seen in COSY): suppressed by (a) decoupling (b) choosing single quantum coherence during  $t_1$ .



*Constant time version*  
pro: same relaxation for all  $t_1$  data points, leading to sharp peaks.

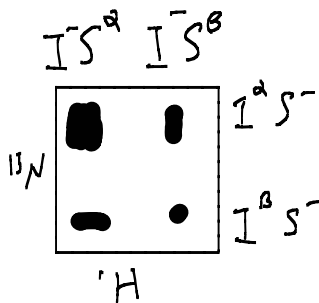
con: signal loss from relaxation.

#### HMQC (heteronuclear multiple quantum correlation)

Multiple quantum evolution during  $t_1$ . (a) H-X J-coupling is active, resulting doublets fine structure in  $F_1$ . (b) This is *heteronuclear* MQ. If only consider X, still single quantum and resonance frequency is simply  $\Omega_X$ . This is different from homonuclear 2Q,  $\Omega_X + \Omega_X$ .

## TROSY transverse relaxation optimized spectroscopy

Relaxation interference between dipolar interaction and CSA interaction (Section 5.4.5) results in very different relaxation rates (or linewidths) for different spin states (or different sub-peaks in the fine structure). The CSA cross relaxation term at high magnetic field becomes very close to the dipolar term; the narrowest peak relaxs very slowly. TROSY experiment selects only the narrowest peak, thus dramatically improves resolution. Remote  $^1\text{H}$ - $^1\text{H}$  dipolar interactions reduce the linewidth narrowing achievable by TROSY, and therefore, the optimal samples for TROSY need to have most proton replaced by  $^2\text{H}$ .

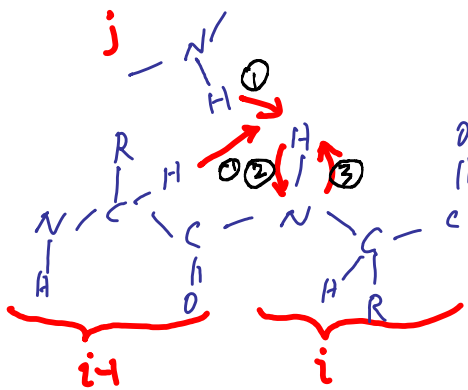


Linewidths variation for sub-peaks within fine structure

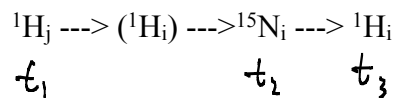
(notice in Fig. 7.10a contours are drawn, where the apparent linewidths could be misleading. But once you realize the broader the peak, the weaker the intensity, you will find Fig. 7.10a is consistent with the linewidth representation shown here.)

## 2. Heteronuclear-edited experiments

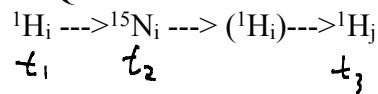
3D and 4D experiments combining homonuclear correlation (NOESY, TOCSY) with heteronuclear correlation (HSQC, HMQC, TROSY) to provide great resolution and resonance connectivities.



### NOSY-HSQC



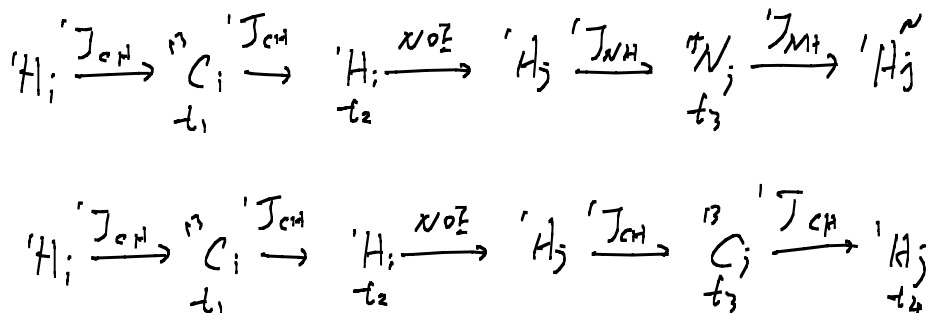
### HSQC-NOESY



$^1\text{H}_j$  can be  $\text{-CH}$ ,  
meaning large band  
width (sw) need.  
HSQC-NOESY make  
more efficient use of  
exp. time.

#### 4D $^{13}\text{C}/^{15}\text{N}$ (or $^{13}\text{C}/^{13}\text{C}$ ) HMQC-NOESY-HMQC

Better resolution from addition dimension; can now tolerate broader lw of HMQC in the sparse 4D spectra. HMQC consists of less number of pulses than HSQC, therefore it is easier to implement and optimize in the 4D.



#### 3. HCCH-TOCSY and HCCH-COSY

Use  $^{13}\text{C}$ - $^{13}\text{C}$  scalar coupling. For large proteins, the following three step transfer is more efficient than the one step transfer between protons.

