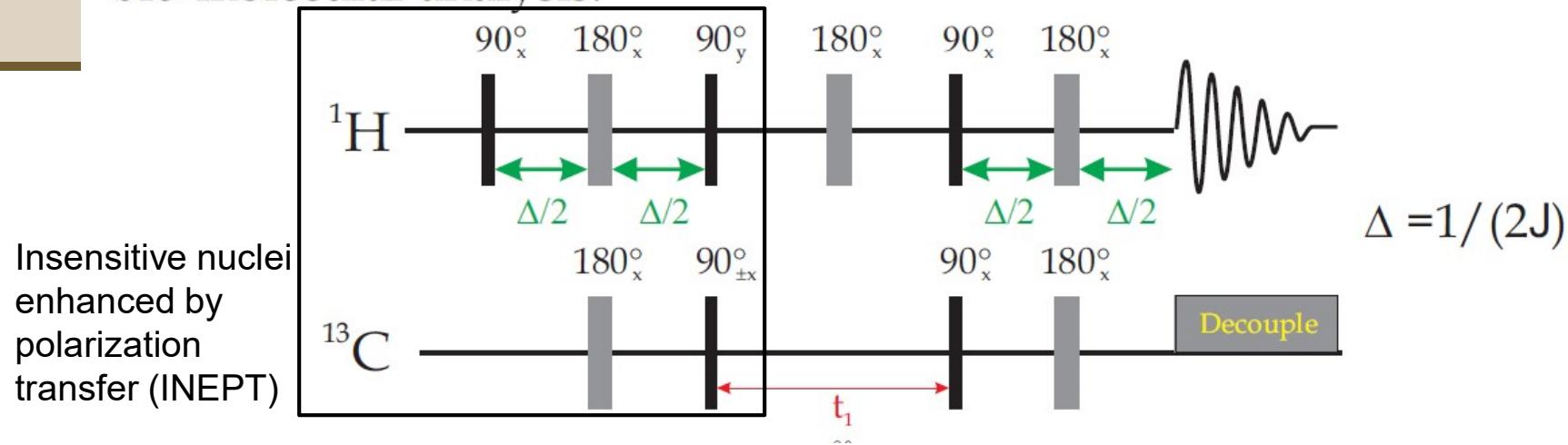


Heteronuclear Single Quantum Coherence/Correlation (HSQC)

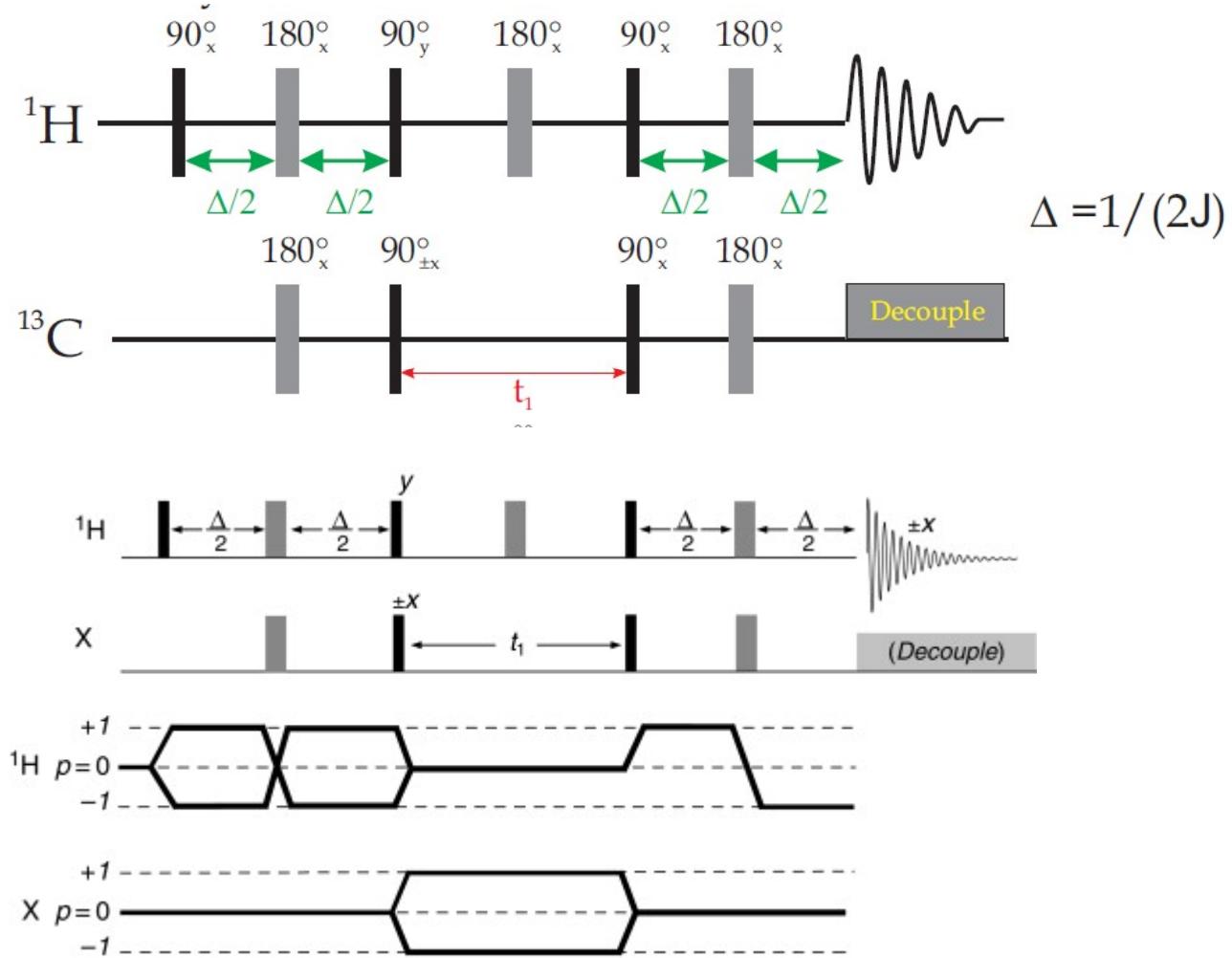
One of the more common ID experiments that yields *single bond correlations* (J-coupling). The sequence has a gradient coherence selection version (not shown) that greatly improves the selectivity and reduces artifacts.

Polarization of the ^1H is passed to ^{13}C , (via an INEPT sequence) where it is allowed to evolve (chemical shift). After the evolution period (t_1), it is passed back to the ^1H (reverse-INEPT) for detection.

The HSQC is the basis for many sequences that were developed for bio-molecular analysis.



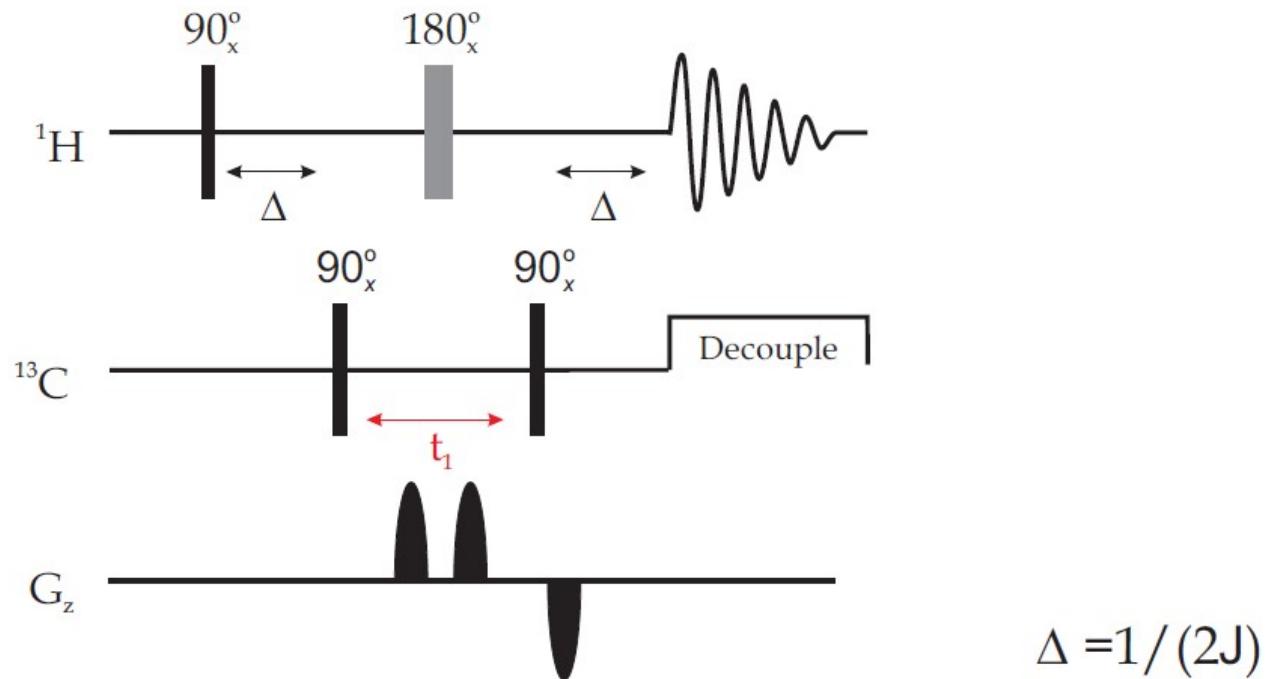
HSQC coherence transfer pathway



Heteronuclear Multiple Quantum Coherence/Correlation (HMQC)

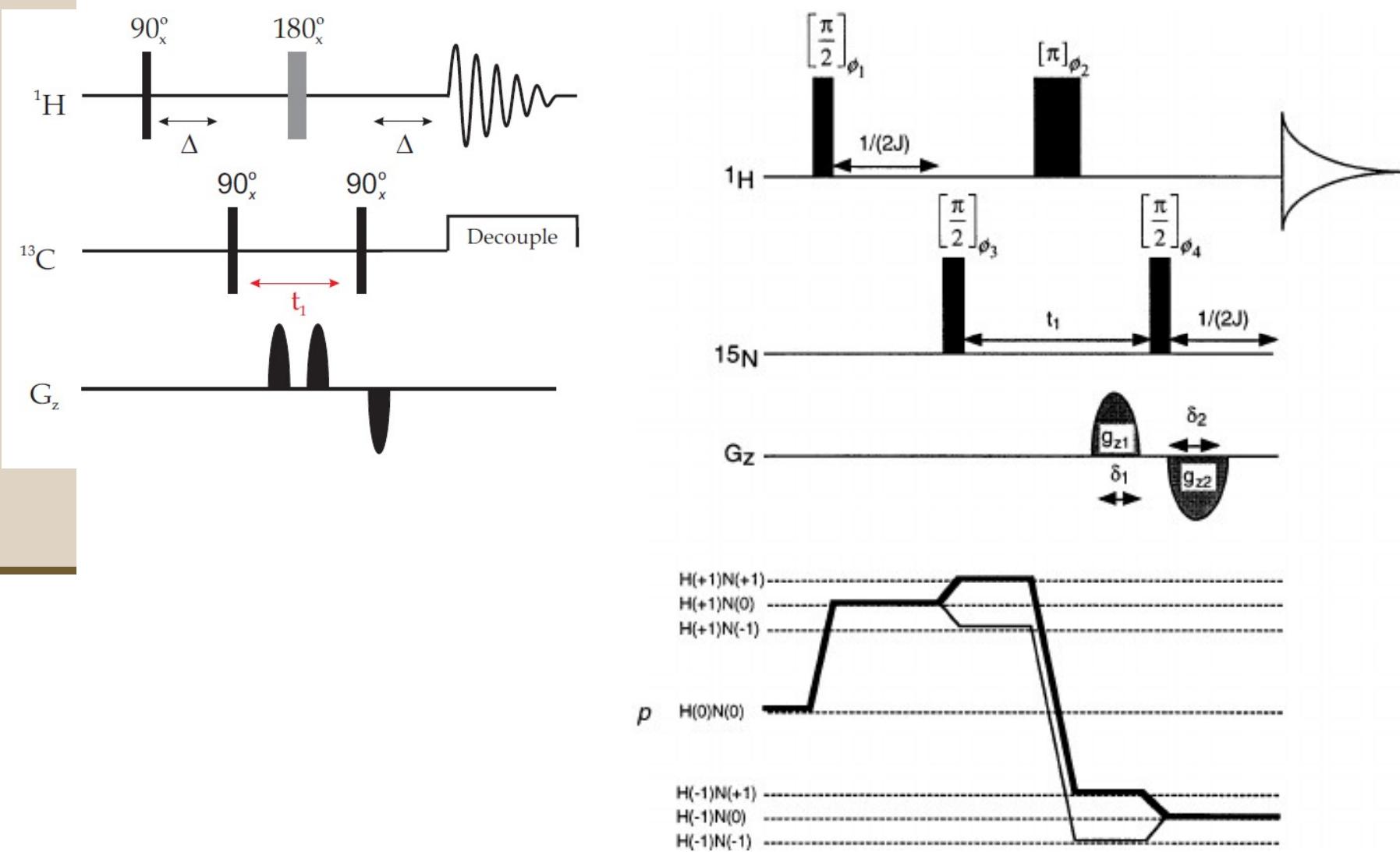
Another common ID experiment that yields *single bond correlations*. The main benefit of HMQC over HSQC is that HMQC is more robust against mis-calibrations in pulse widths and delay times. Also, HMQC has a simple modification that allows for long range correlations. There are both gradient and non-gradient versions of the sequence.

gHMQC:



HMQC coherence transfer pathway

Necessity of gradient pulses to simplify spectral complexity



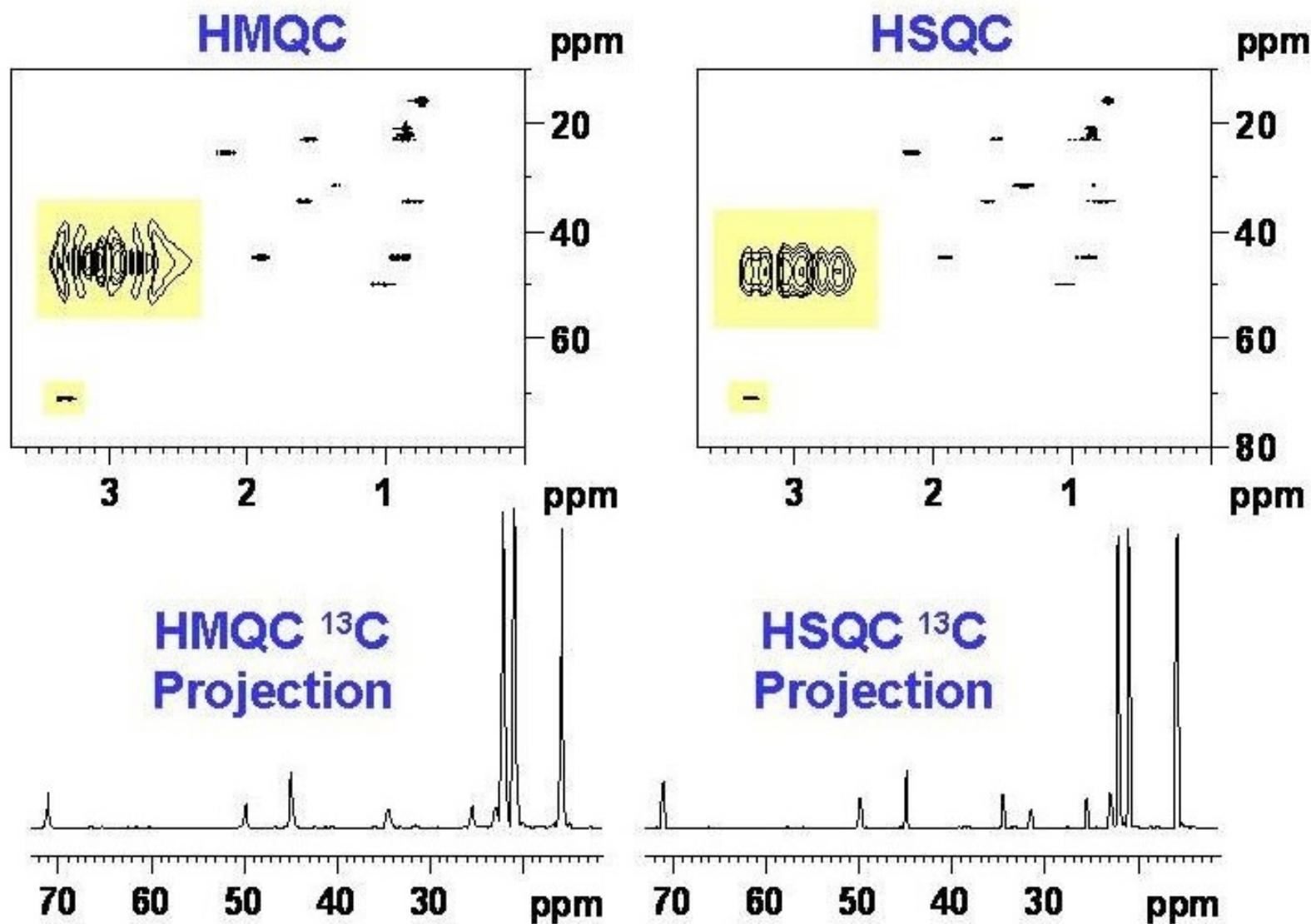
Why HMQC? Why HSQC?

- information content basically the same
- there are differences based on the relaxation of multiple quantum magnetization as opposed to single quantum
- there are differences based on the dipolar broadening of multiple quantum coherence as opposed to single quantum
- there are differences based on unresolved couplings that broaden signals in the directly detected dimension
- multiple quantum magnetization does not evolve with scalar coupling (can be an advantage)
- these can be different for ^1H - ^{15}N versus ^1H - ^{13}C , and size of the molecule
- these can be subtle, and depend on the application

HSQC vs HMQC analysis for proteins: Bax and coworkers, *J. Magn. Reson.* **86**, 304-318 (1990)

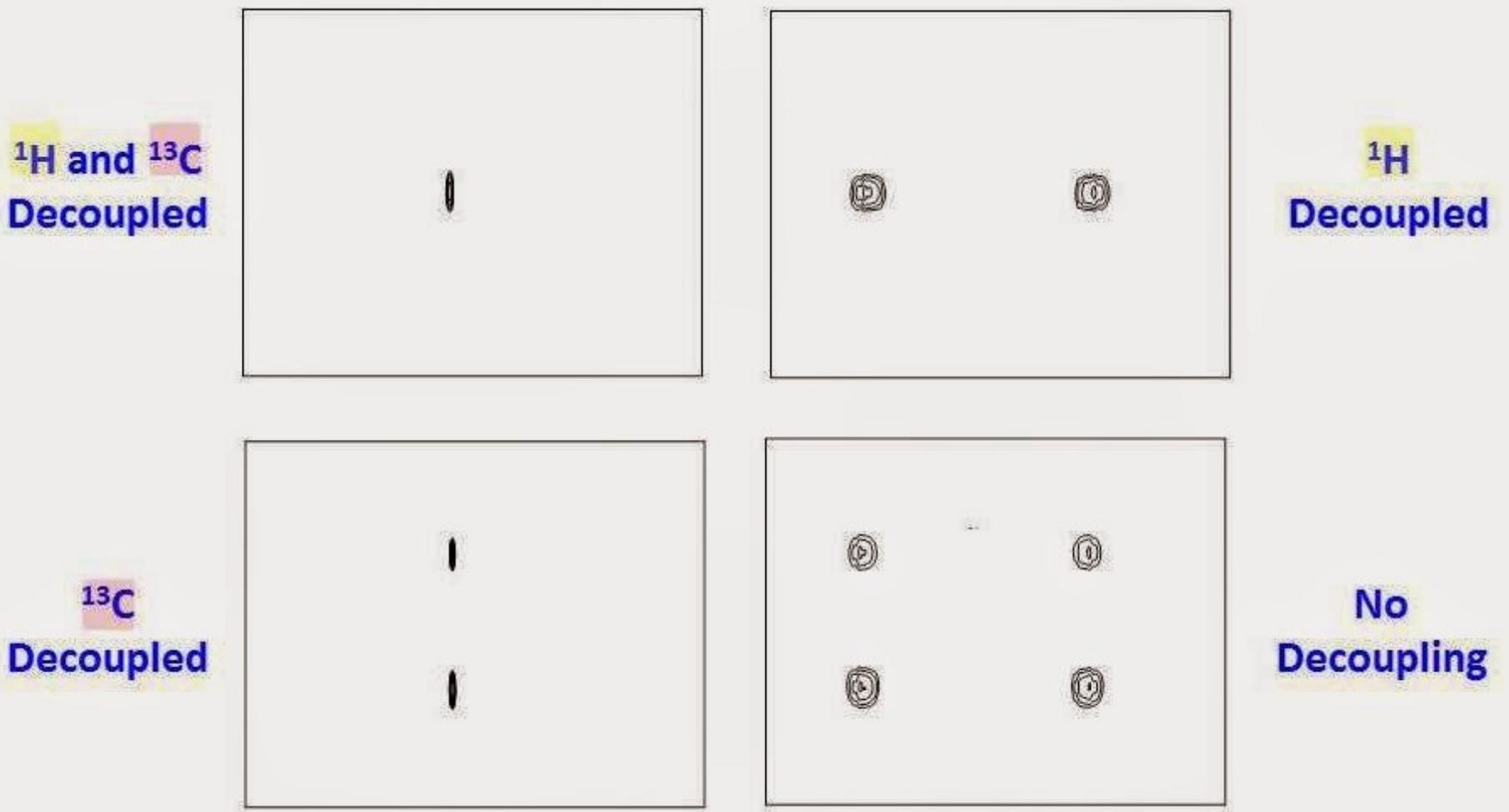
HMQC vs HSQC 2D NMR

^1H - ^{13}C HMQC / HSQC spectra of menthol at 300 MHz

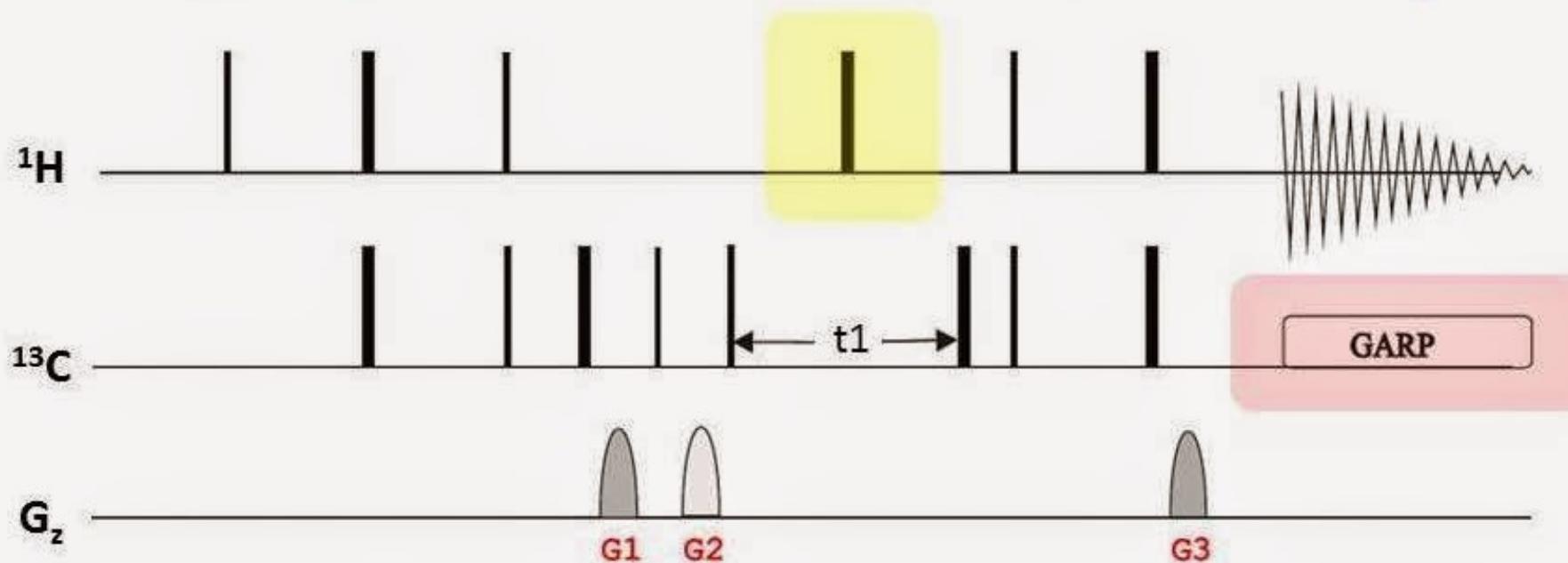


The role of decoupling in HSQC spectra

^1H - ^{13}C HSQC spectra of benzene



Decoupling elements in gHSQC pulse sequence



¹H Decoupling element for F1 domain

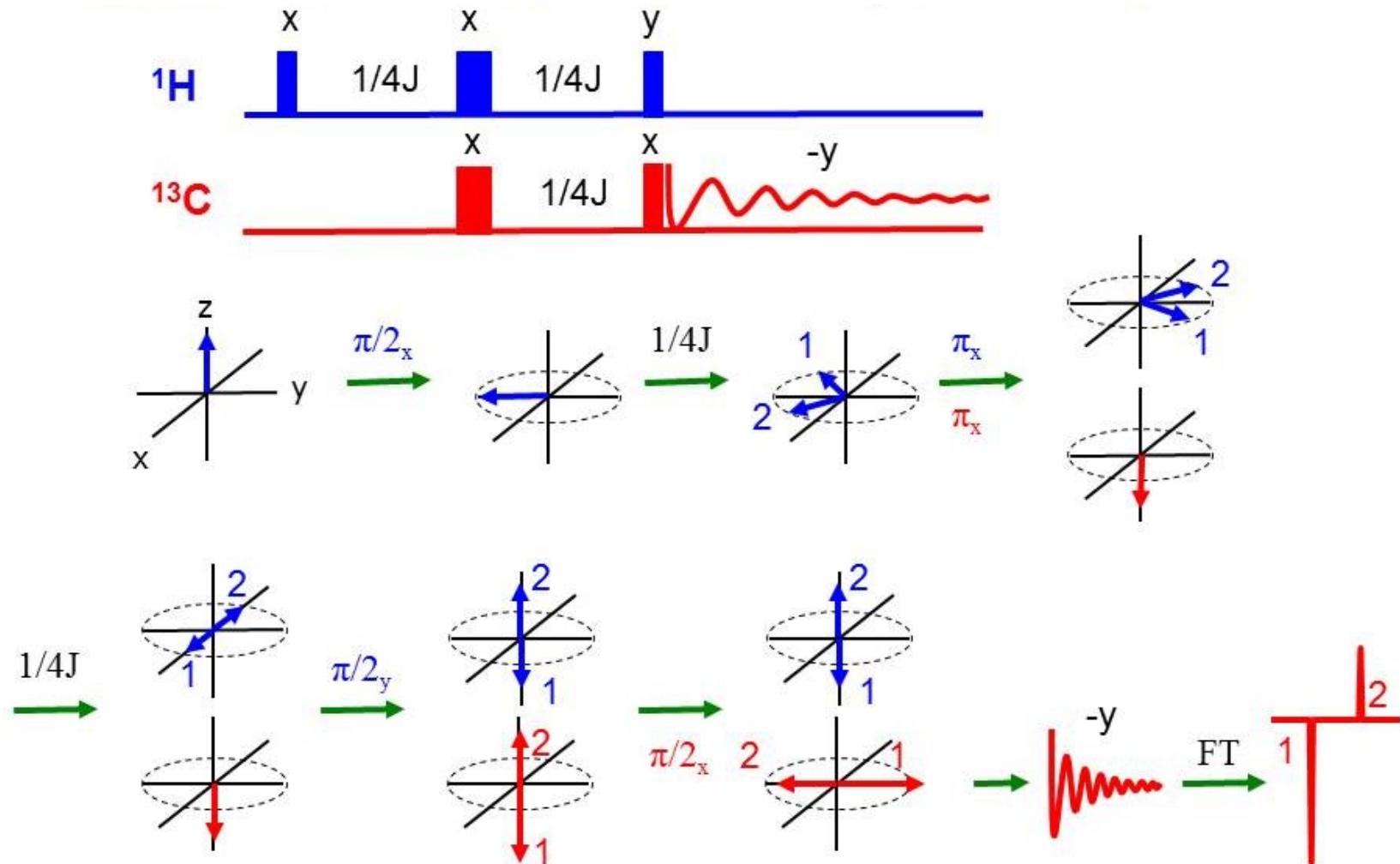
¹³C Decoupling element for F2 domain

GARP = Globally-optimized Alternating-phase Rectangular Pulses

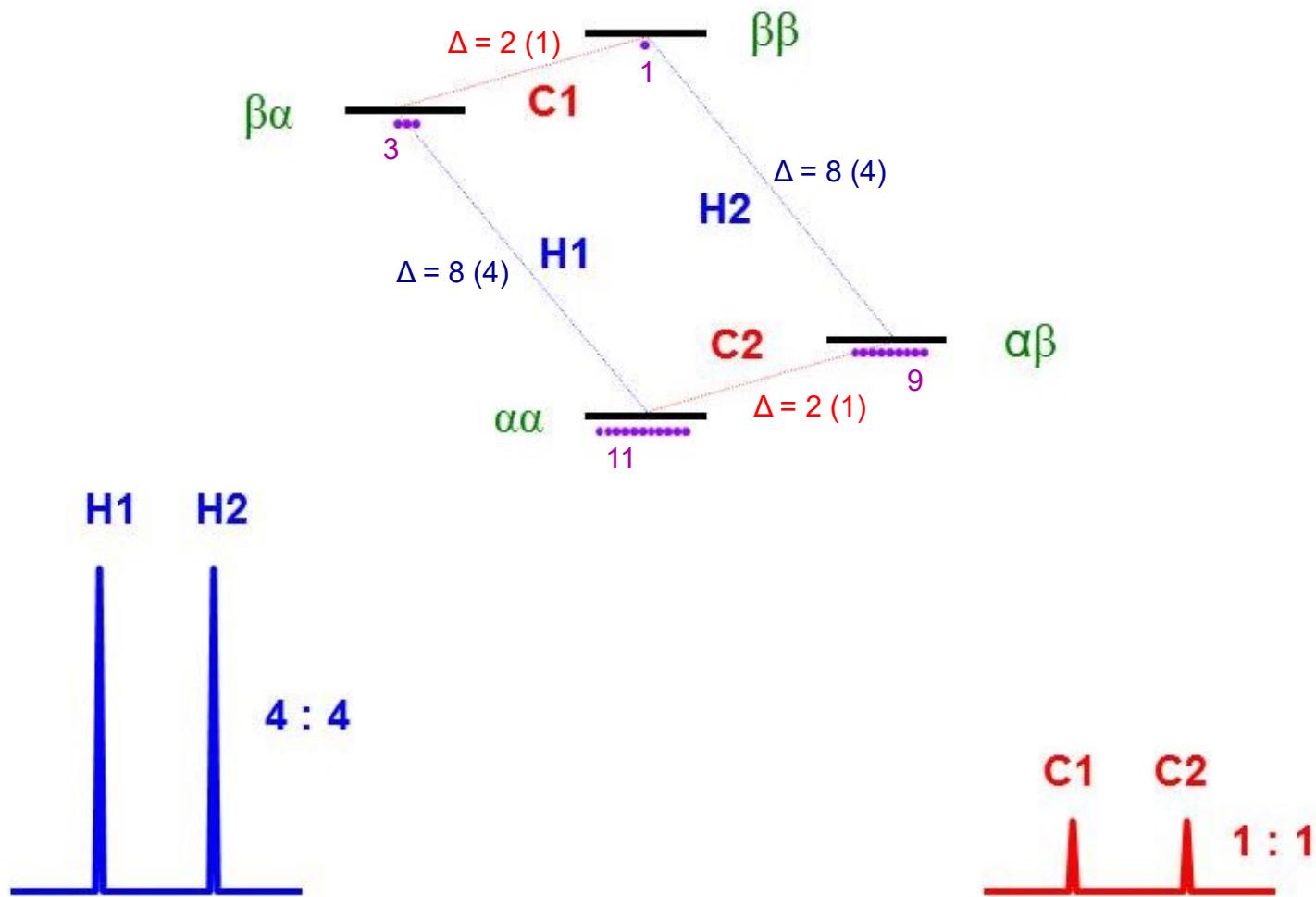
Polarization Transfer and Signal Enhancement

How Its Done – The "INEPT" Experiment

INEPT Insensitive Nuclei Enhanced by Polarization Transfer

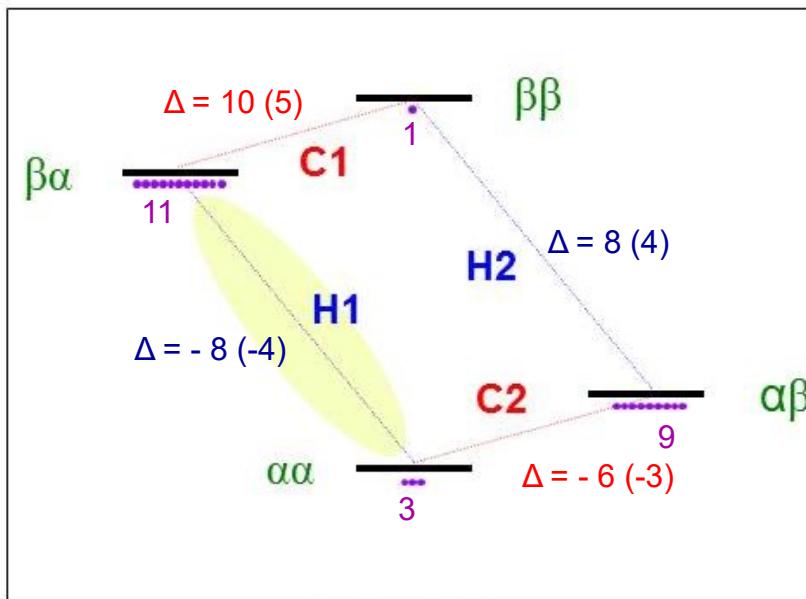


Equilibrium Energy Level Diagram for a ^{13}C – ^1H Spin Pair

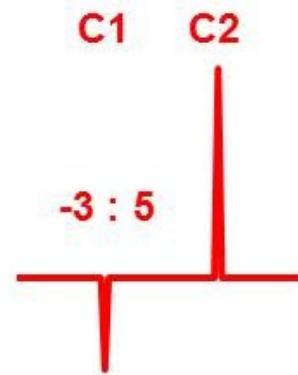
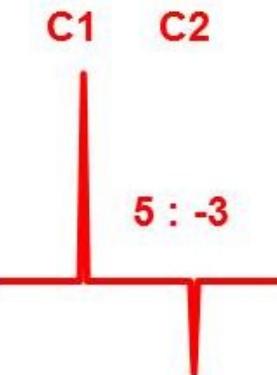
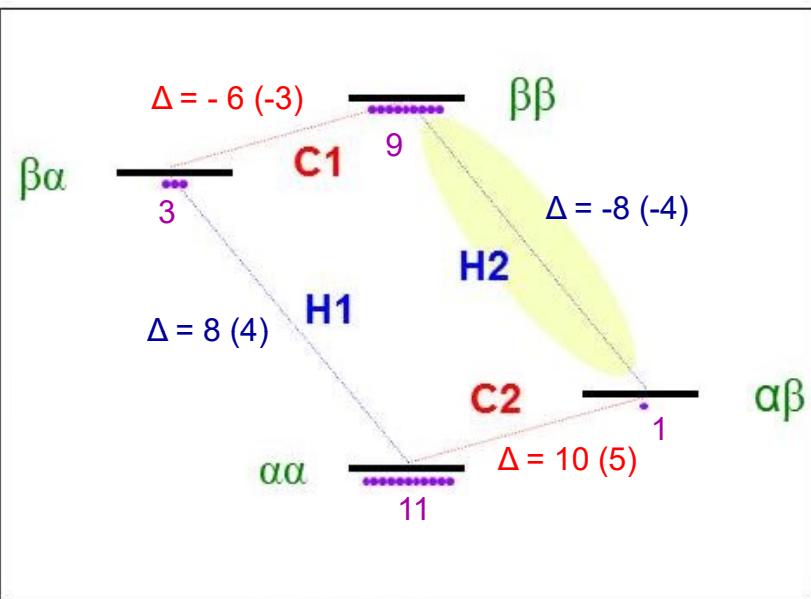


Polarization Transfer and Signal Enhancement

H1 Transition Inverted

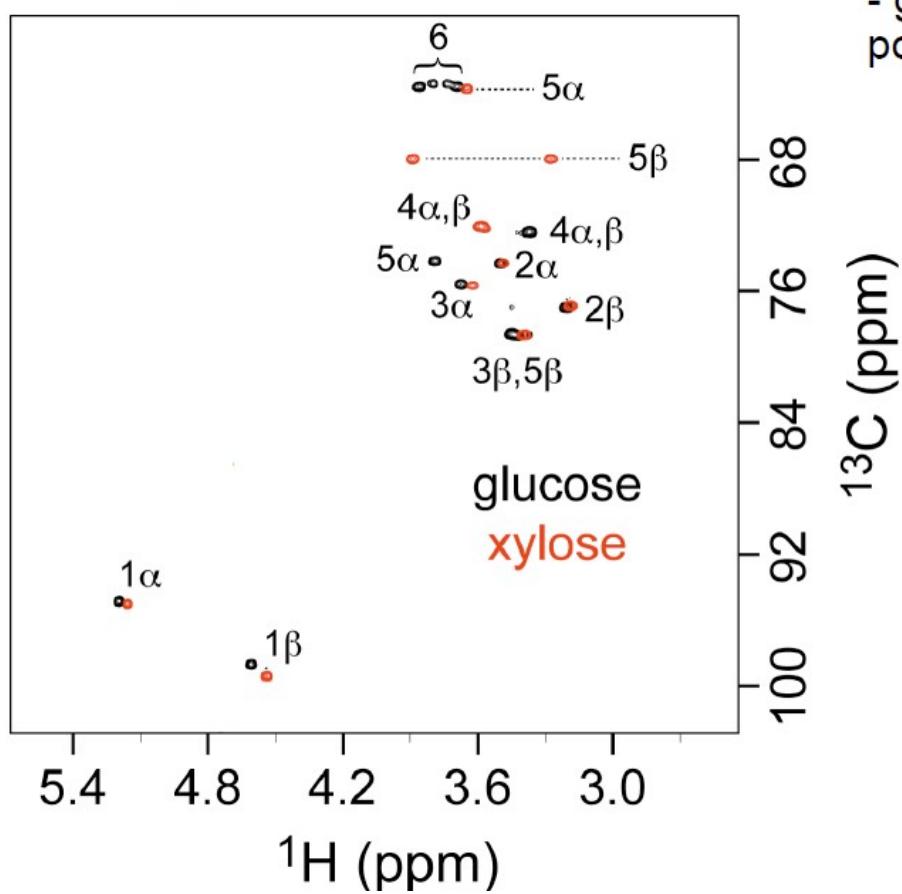


H2 Transition Inverted

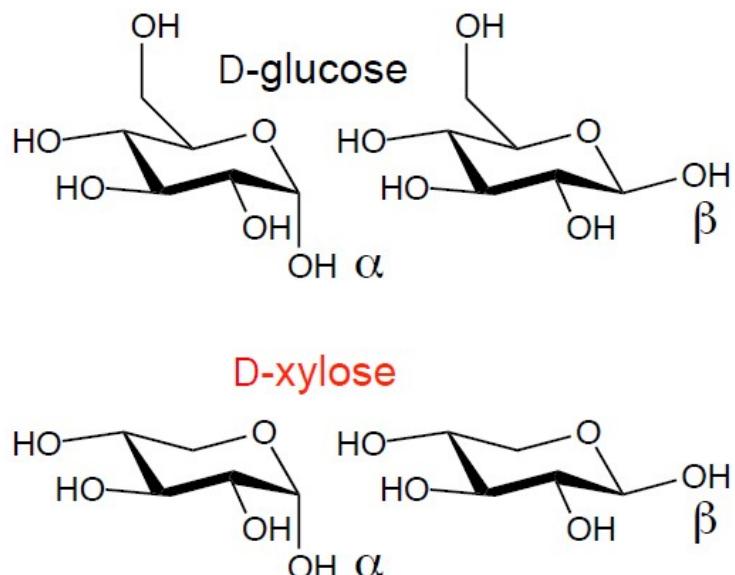


HSQC method in biomolecular NMR and small organic molecules

- for small molecules, with high concentrations, acquisition at natural isotopic abundance (~1.1%) is routine
- example: mixture of D-glucose and D-xylose (5 mM each, 40 minute total acquisition time)

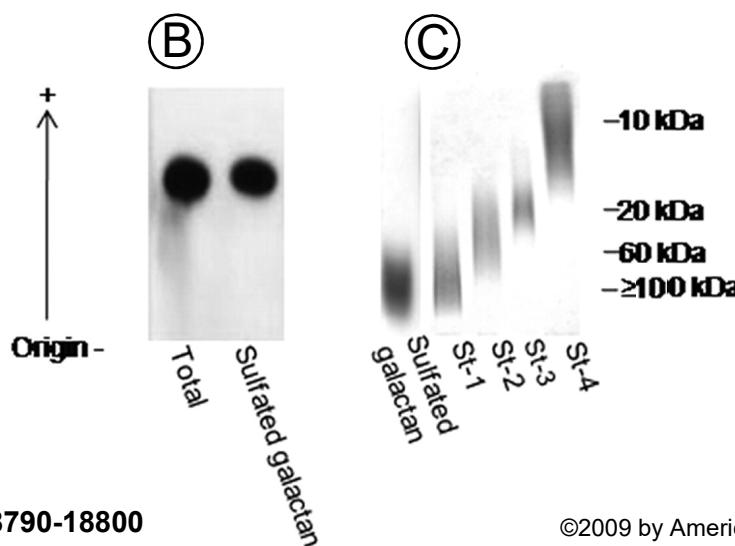
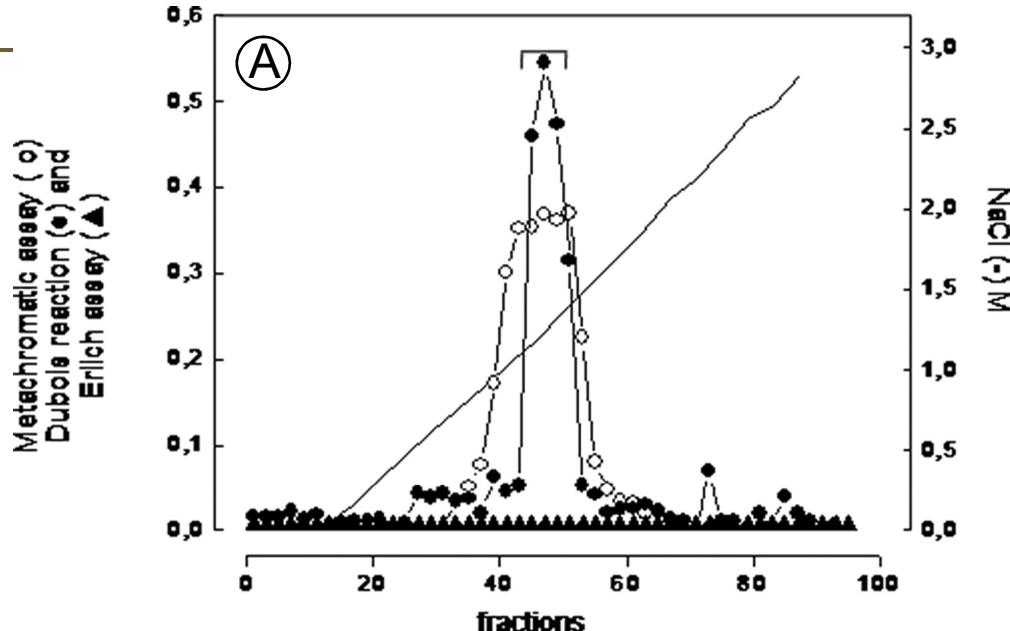


- gain $\gamma^1\text{H}/\gamma^{13}\text{C}$ factor in sensitivity (~4) for polarization transfer



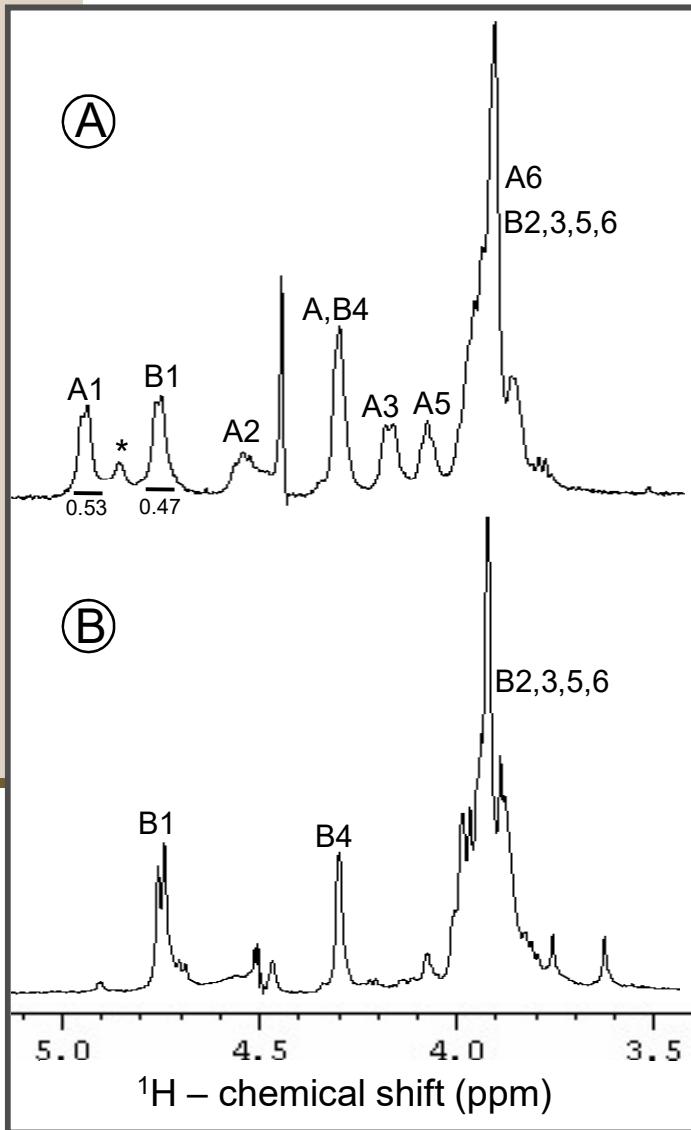
NMR STRUCTURAL DETERMINATION OF A SIMPLE SG

(A) Purification and (B and C) electrophoretic mobility of the SG from the egg jelly of sea urchin *Glyptocidaris crenularis*.



NMR STRUCTURAL DETERMINATION OF A SIMPLE SG

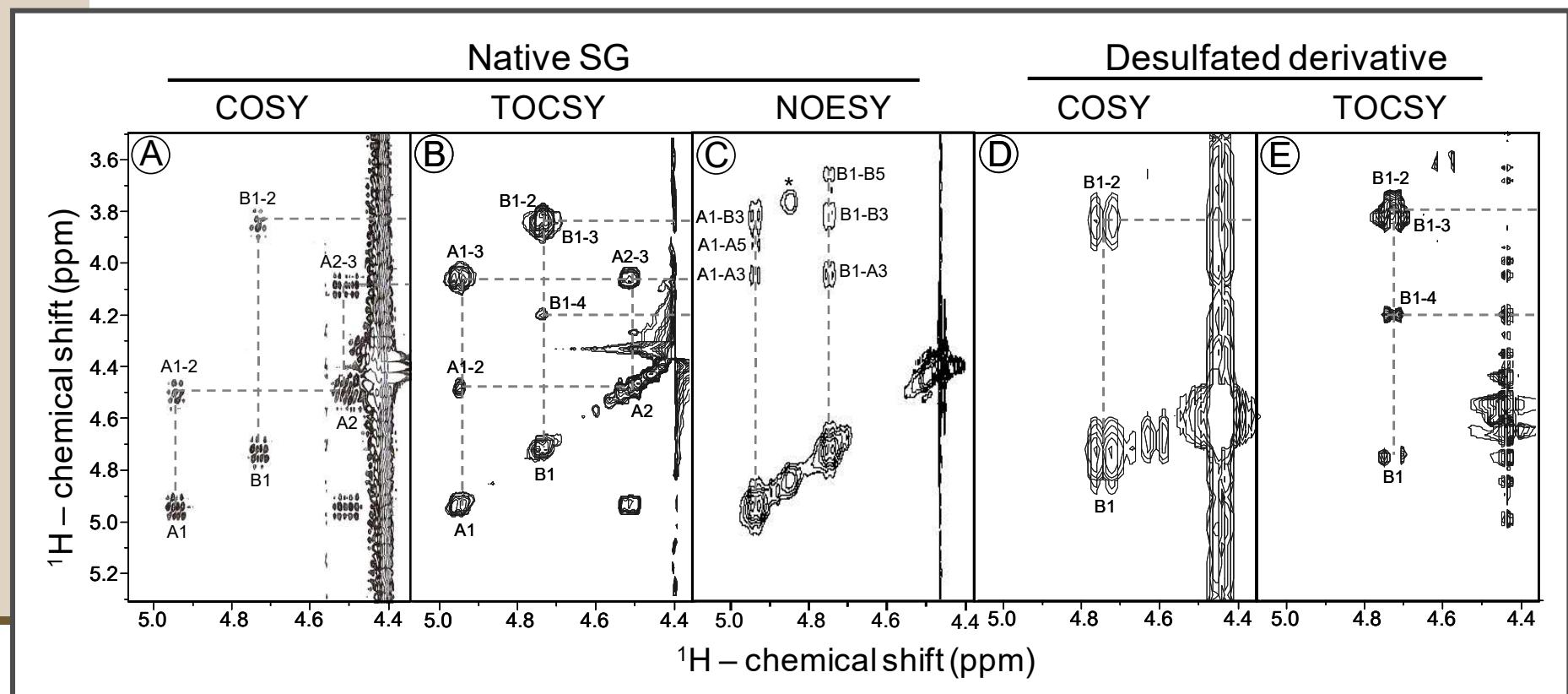
1D ^1H NMR spectra at 400 MHz of the (A) native β -SG from *G. crenularis* and (B) its desulfated derivative.



- two anomeric signals (A e B) with 1:1 ratio belong to the β -configuration (δ below 5.0 ppm) in the native compound
- just a single anomeric signal (B) at the desulfated derivative
- (A)-sulfated galactose
- (B)-non-sulfated galactose

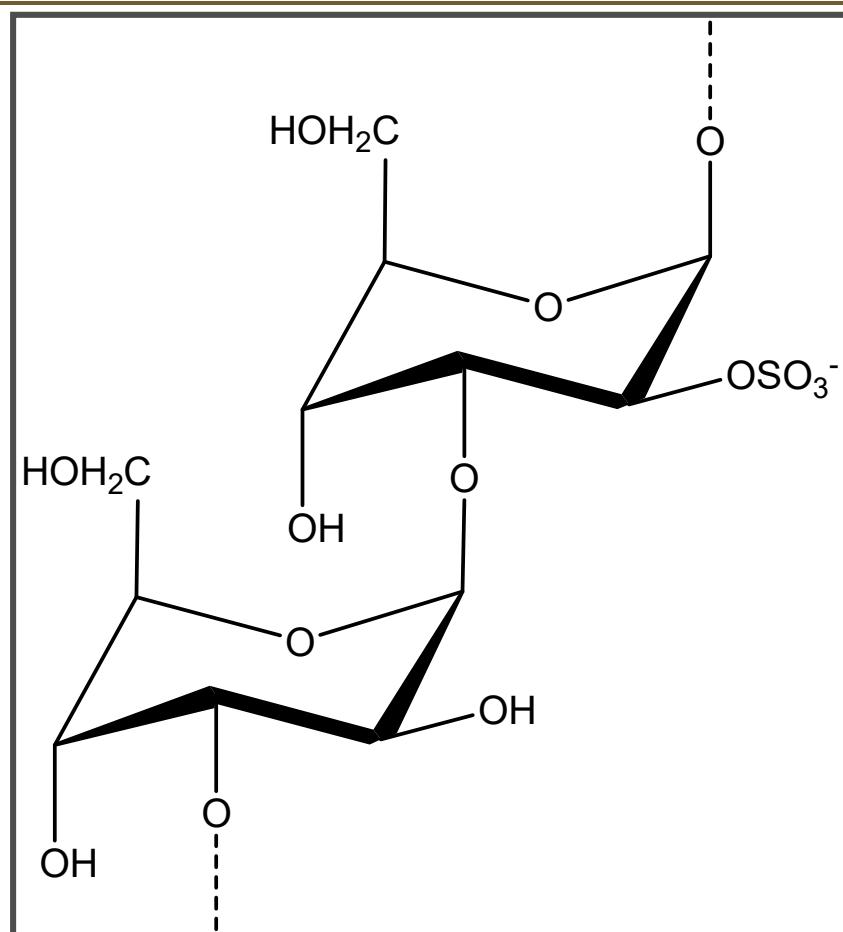
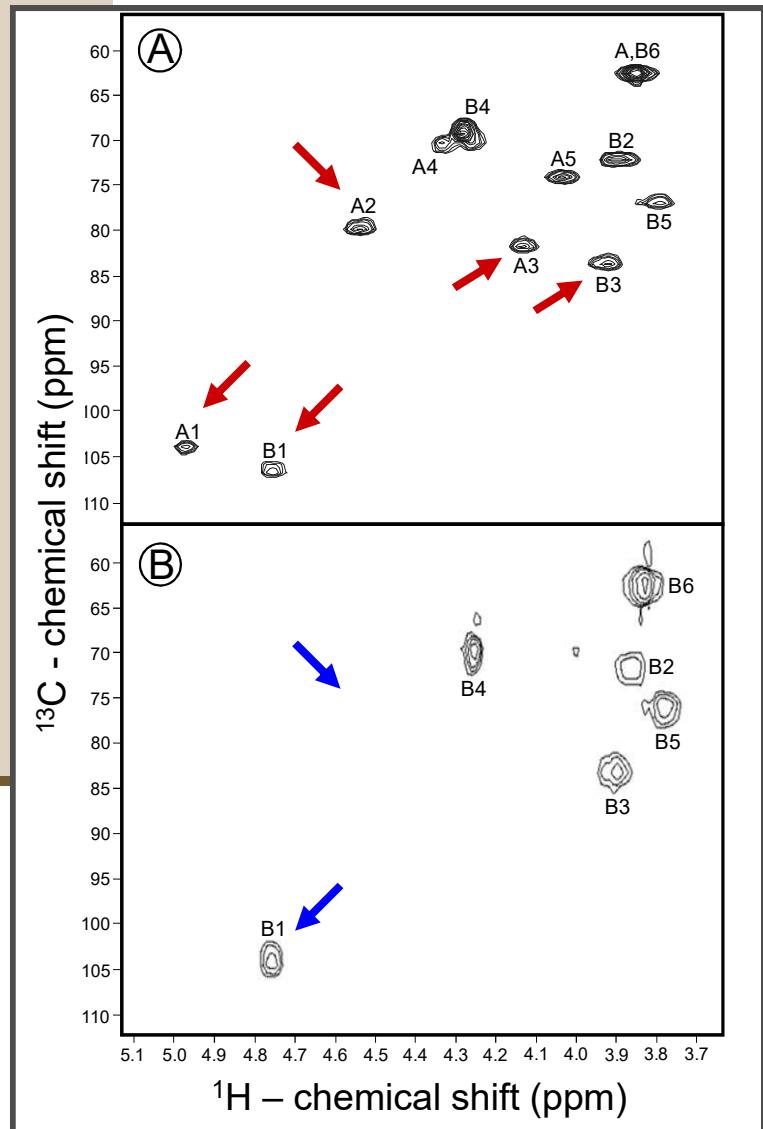
NMR STRUCTURAL DETERMINATION OF A SIMPLE SG

2D homonuclear ^1H - ^1H NMR spectra at 400 MHz (anomeric region) of the (A-C) native β -SG from *G. crenularis* and (D and F) its desulfated derivative.



NMR STRUCTURAL DETERMINATION OF A SIMPLE SG

2D heteronuclear ^1H - ^{13}C HSQC spectra at 400 MHz of the (A) native β -SG from *G. crenularis* and (B) its desulfated derivative.



¹⁵N-NMR CHARACTERIZATION OF GLYCOSAMINOGLYCANs (GAGs)

NMR characterization of GAGs is largely based on ¹H- and/or ¹³C-resonances and analysis by ¹⁵N is quite rare.

DISADVANTAGES OF ¹⁵N-NMR (poorly sensitive):

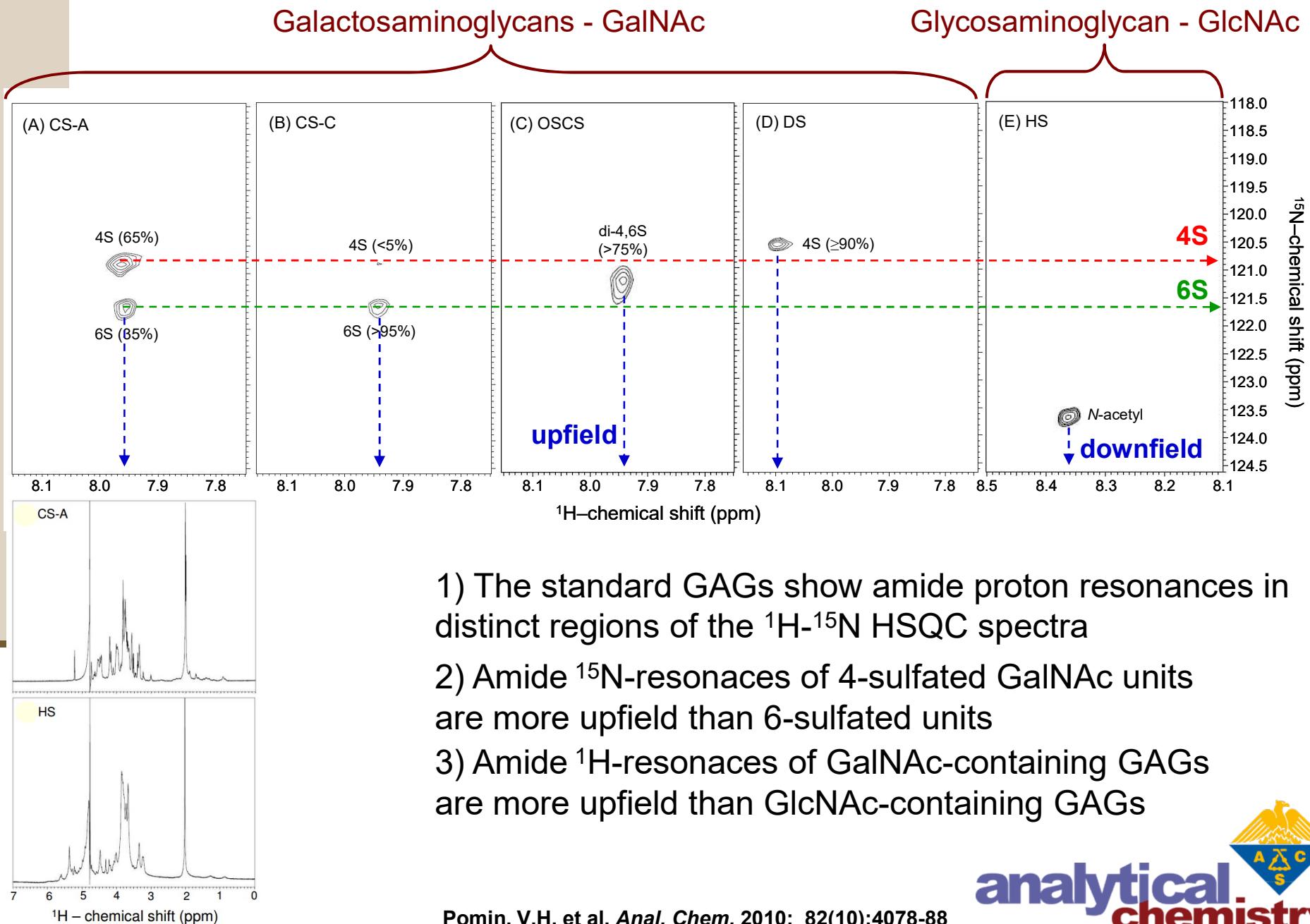
- very low natural abundance (0.37%)
- very low magnetic susceptibility ($\Delta E = \gamma \hbar B_0$ modulated by the low negative gyromagnetic ratio value of ¹⁵N)

Hence, ¹⁵N-isotope labeling techniques are considerably required, although some analyses (industrial sources) still work at natural abundance!

Table of some nuclei properties important for NMR detection.

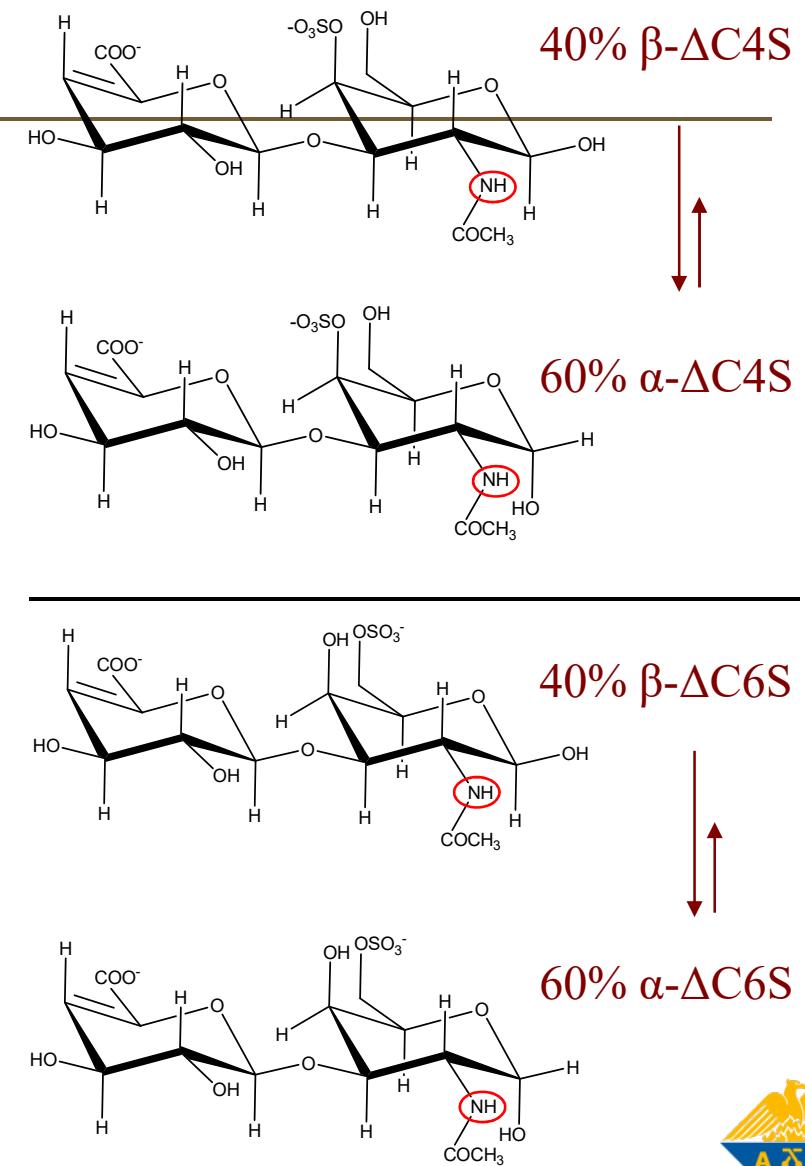
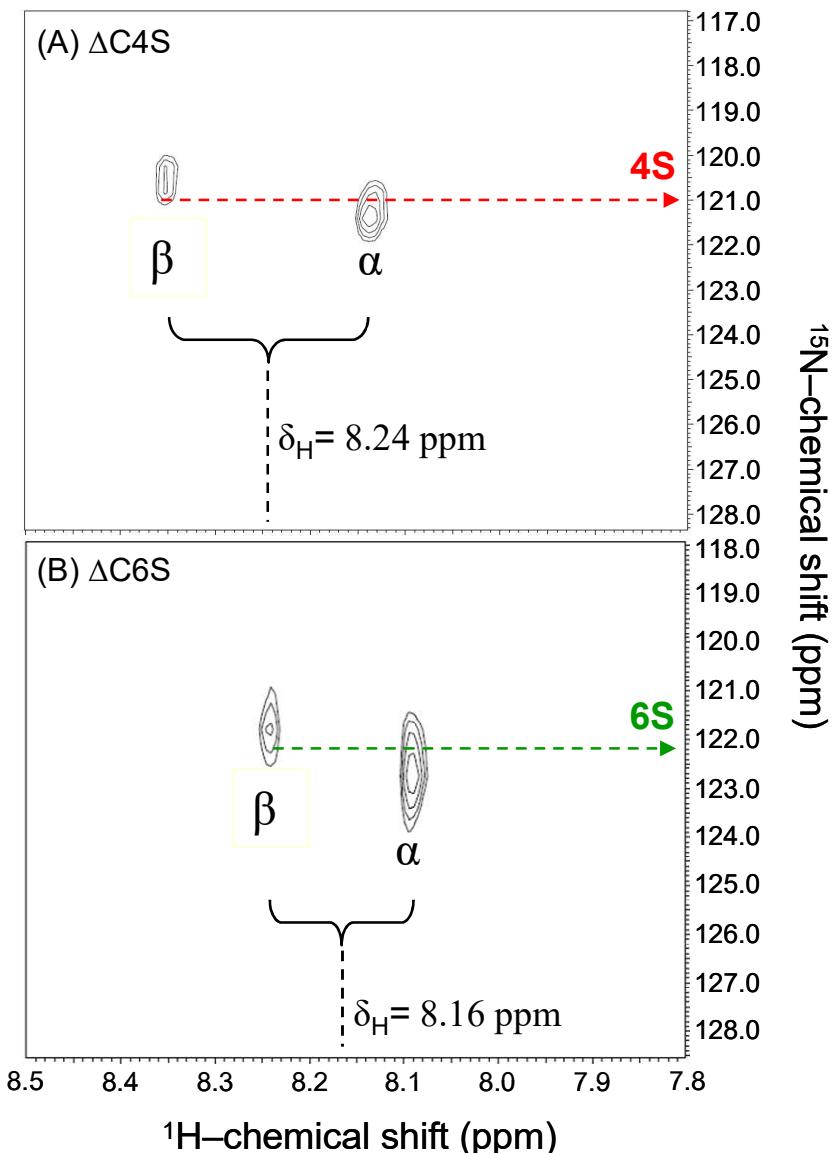
Nuclide	Spin	Natural abundance	Gyromagnetic ratio γ [10^7 rad T$^{-1}$ s$^{-1}$]	NMR Frequency (at 18.8 Tesla)
Proton (¹ H)	$\frac{1}{2}$	99.985	26.7522	799.734 (1)
Carbon-12 (¹² C)	0	98.9	-	-
Carbon-13 (¹³ C)	$\frac{1}{2}$	1.108	6.7283	201.133 (1/3.976)
Nitrogen-14 (¹⁴ N)	1	99.63	1.9338	57.820 (1/13.831)
Nitrogen-15 (¹⁵ N)	$\frac{1}{2}$	0.37	-2.7126	81.093 (1/9.861)

¹⁵N-HSQC spectra of native GAGs – at natural abundance!

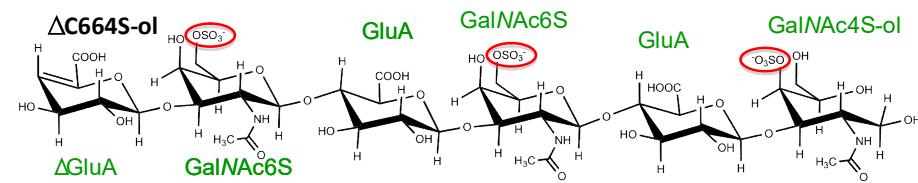
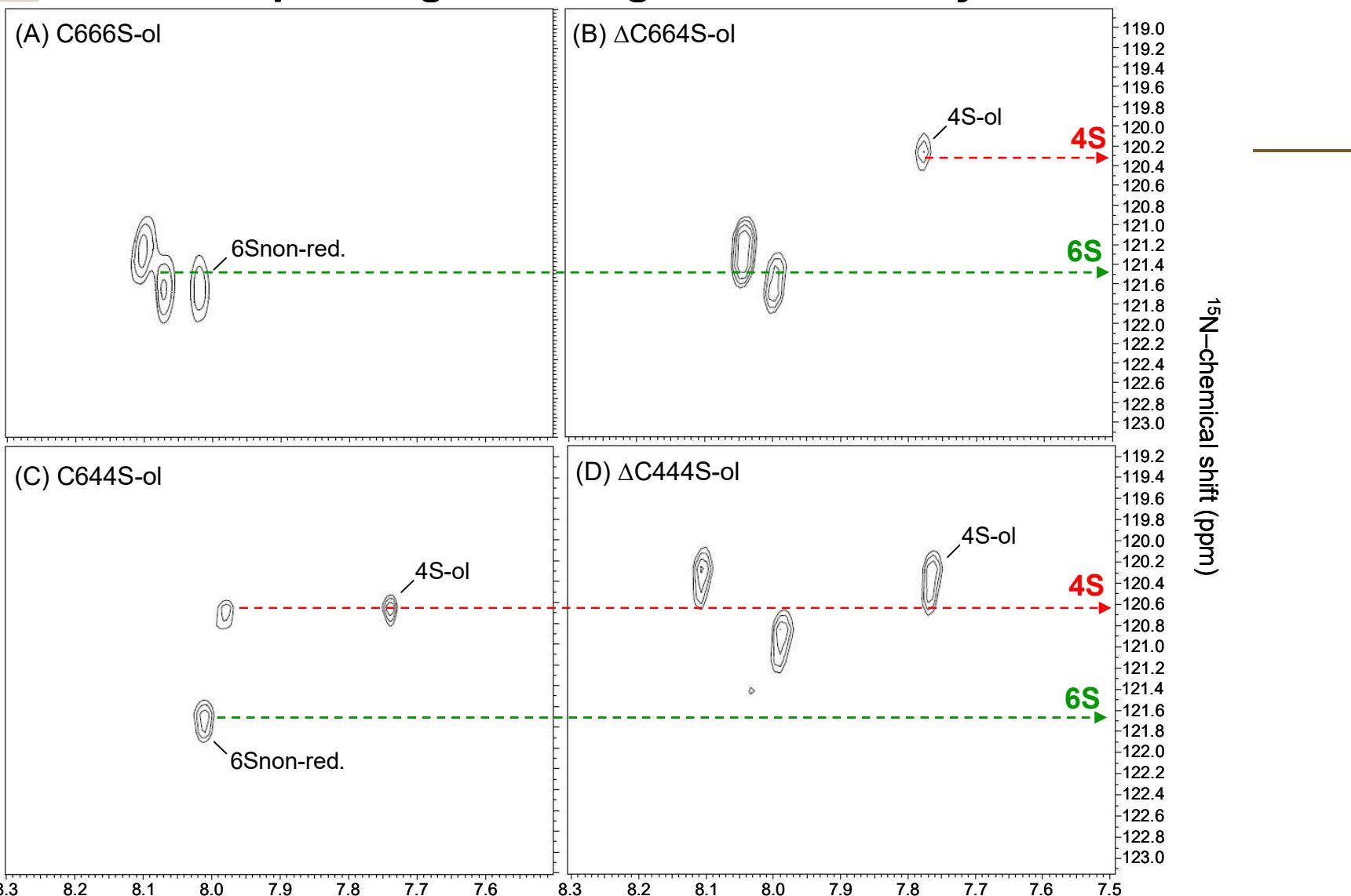


Pomin, V.H. et al. *Anal. Chem.* 2010; 82(10):4078-88

¹⁵N-HSQC spectra of CS disaccharides



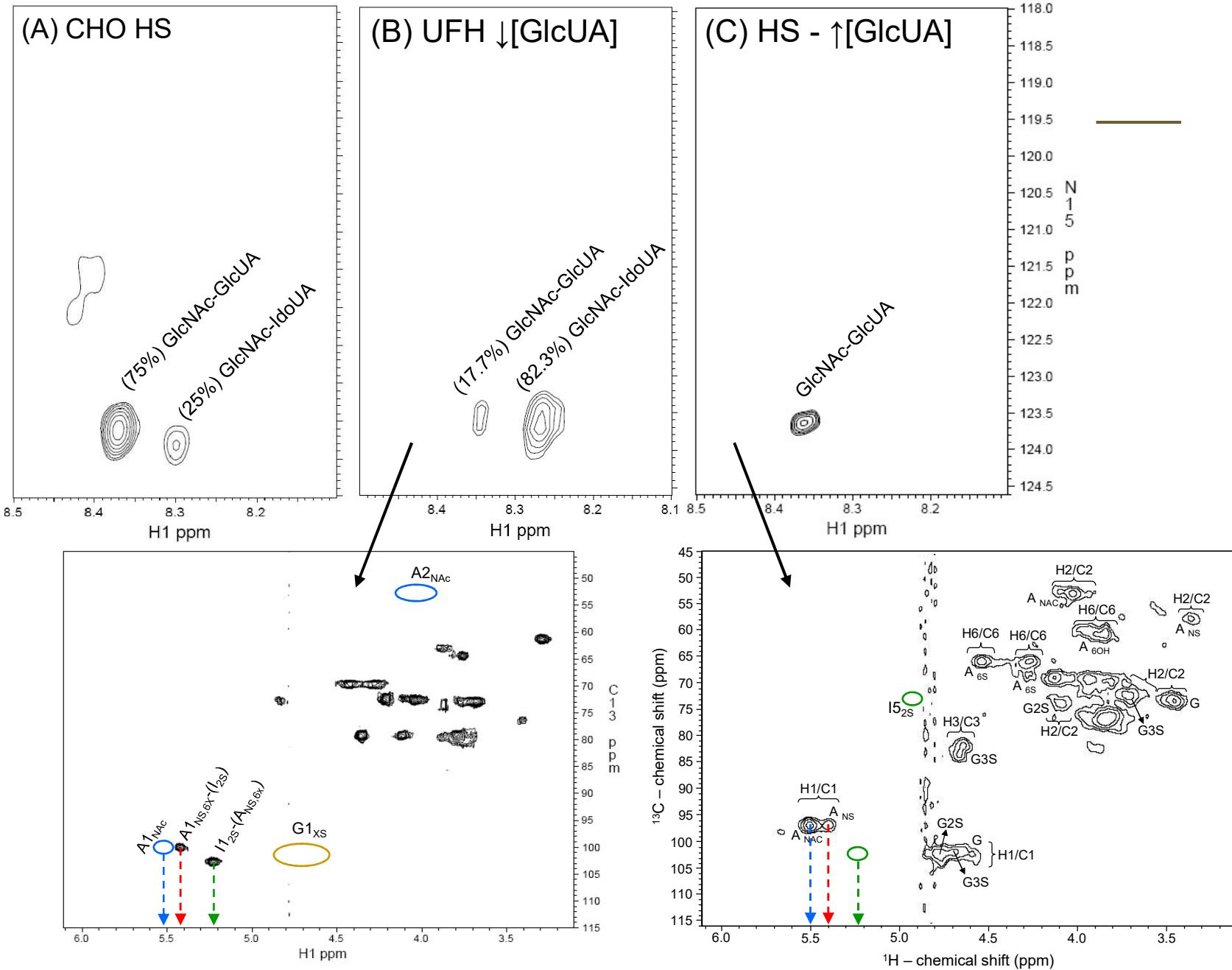
Sequencing of CS oligosaccharides by ^{15}N -HSQC



¹⁵N-HSQC

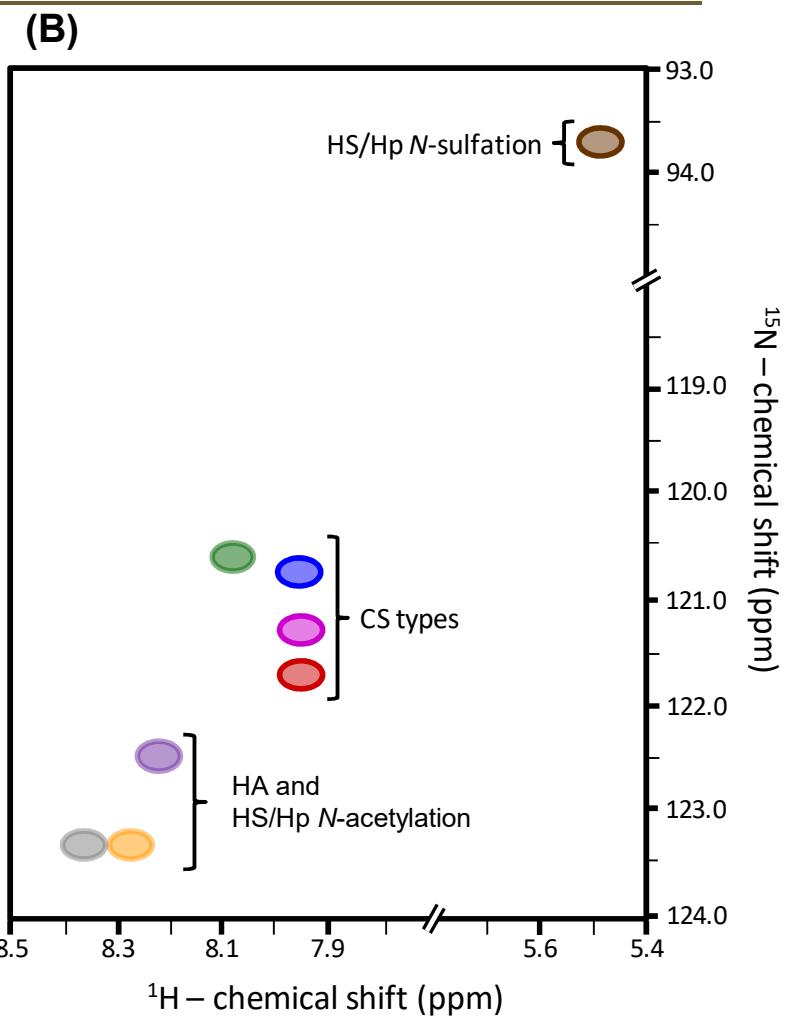
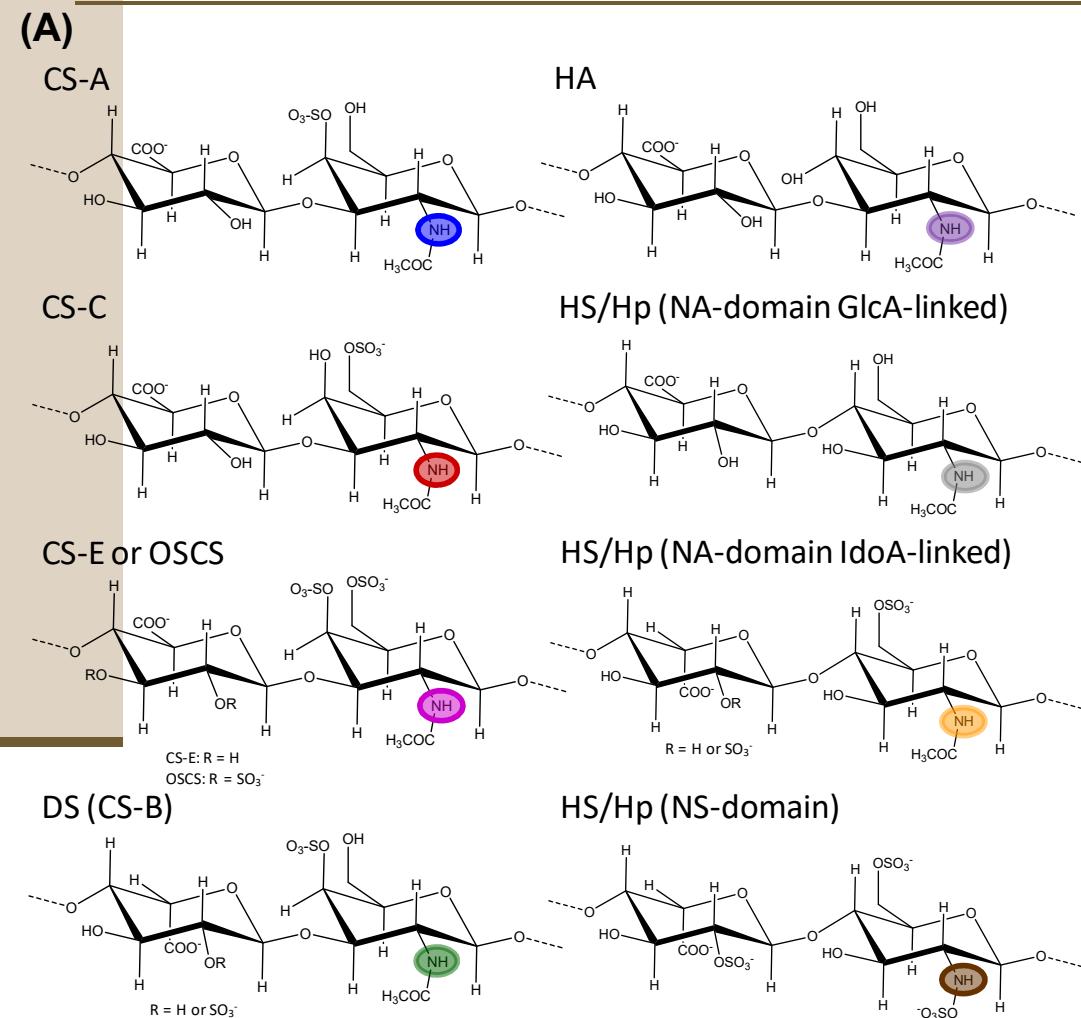
¹³C-HSQC

¹⁵N-¹H assignment of N-acetylated glucosamines in Hep/HS



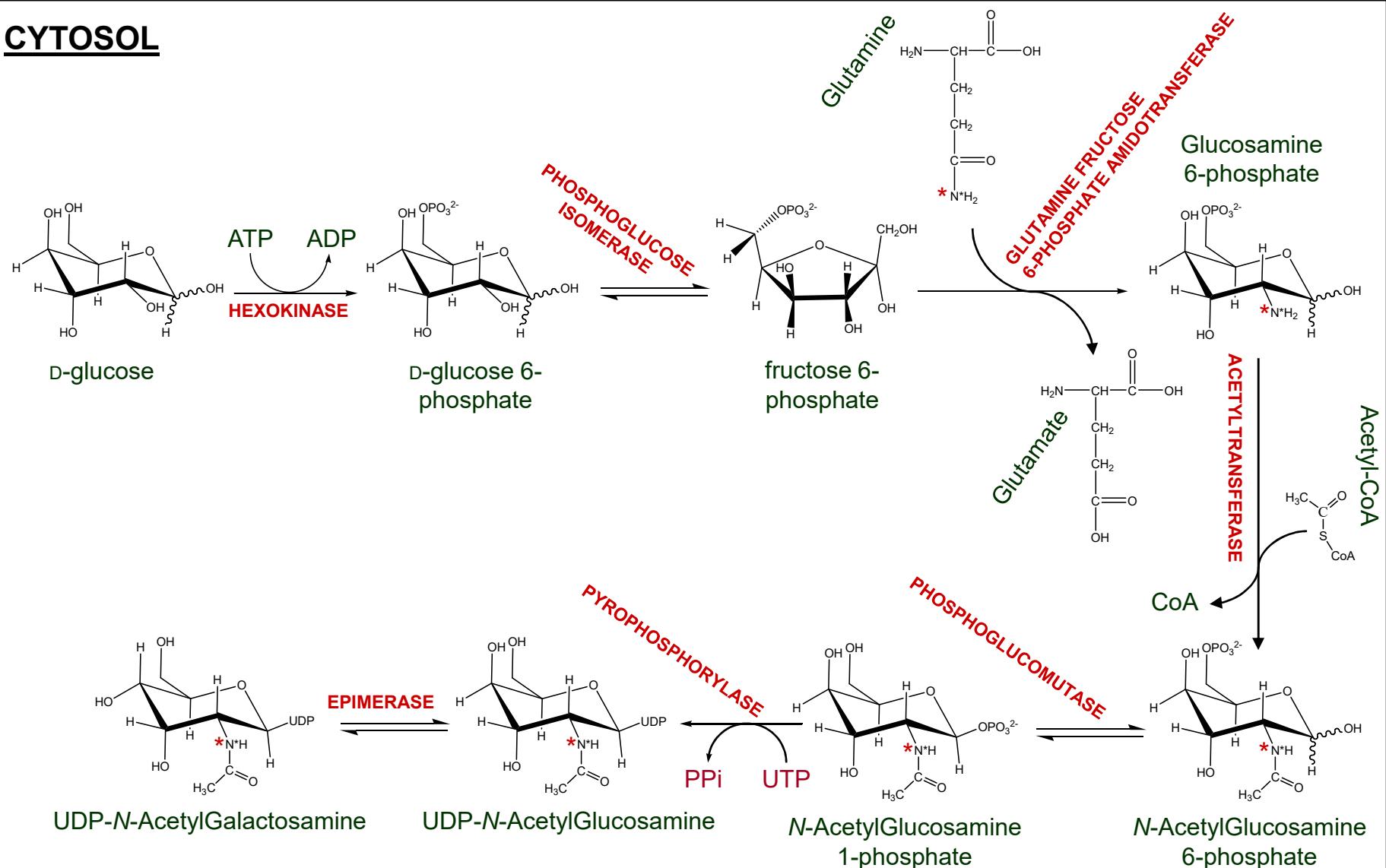
¹⁵N-HSQC spectra of GAGs

(A) Structural representation of classical GAGs and (B) hypothetical ¹H-¹⁵N HSQC spectrum showing their respective amide ¹H-¹⁵N cross-peaks.

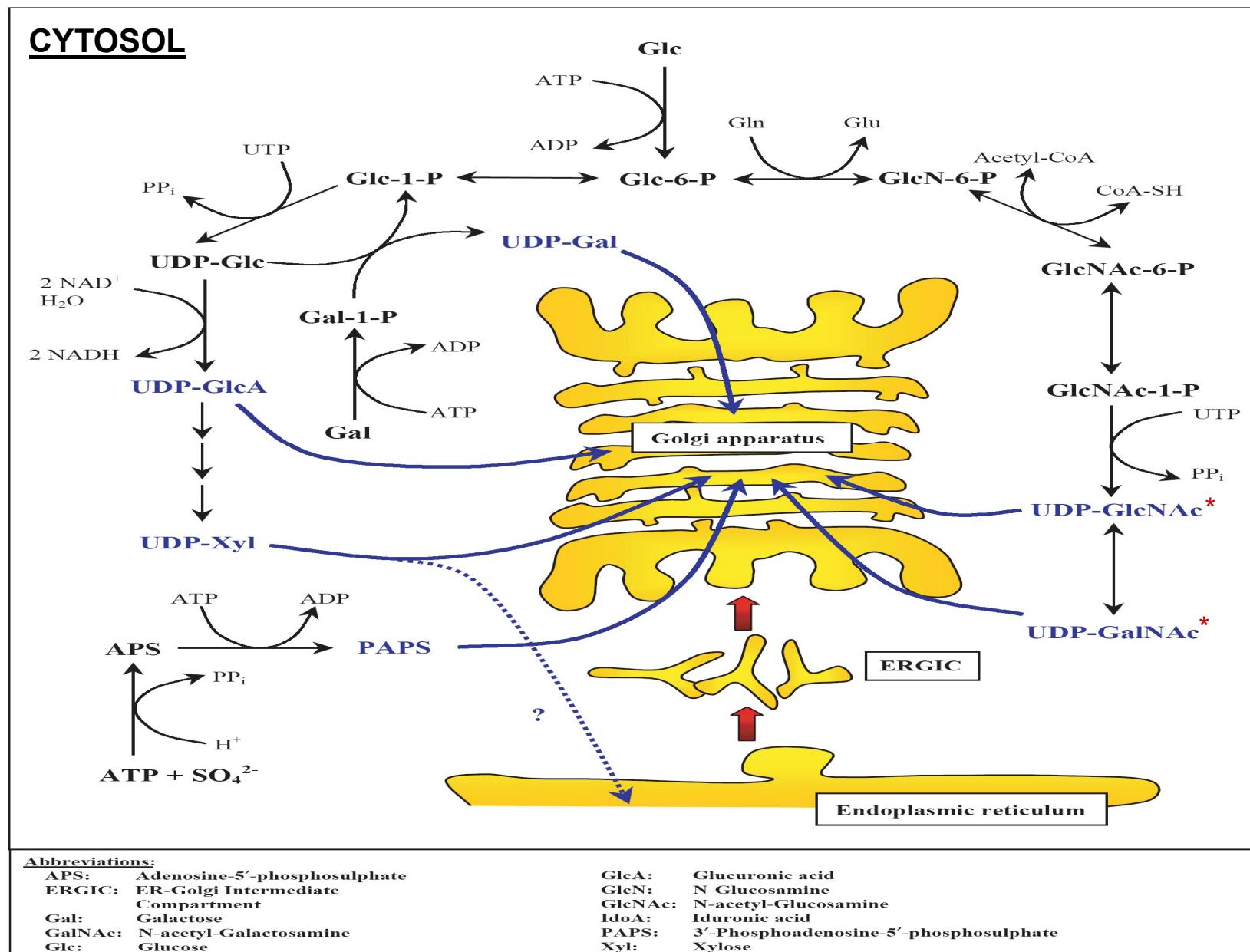


Biosynthetic pathway for ^{15}N -labeled N-acetyl-hexosamines

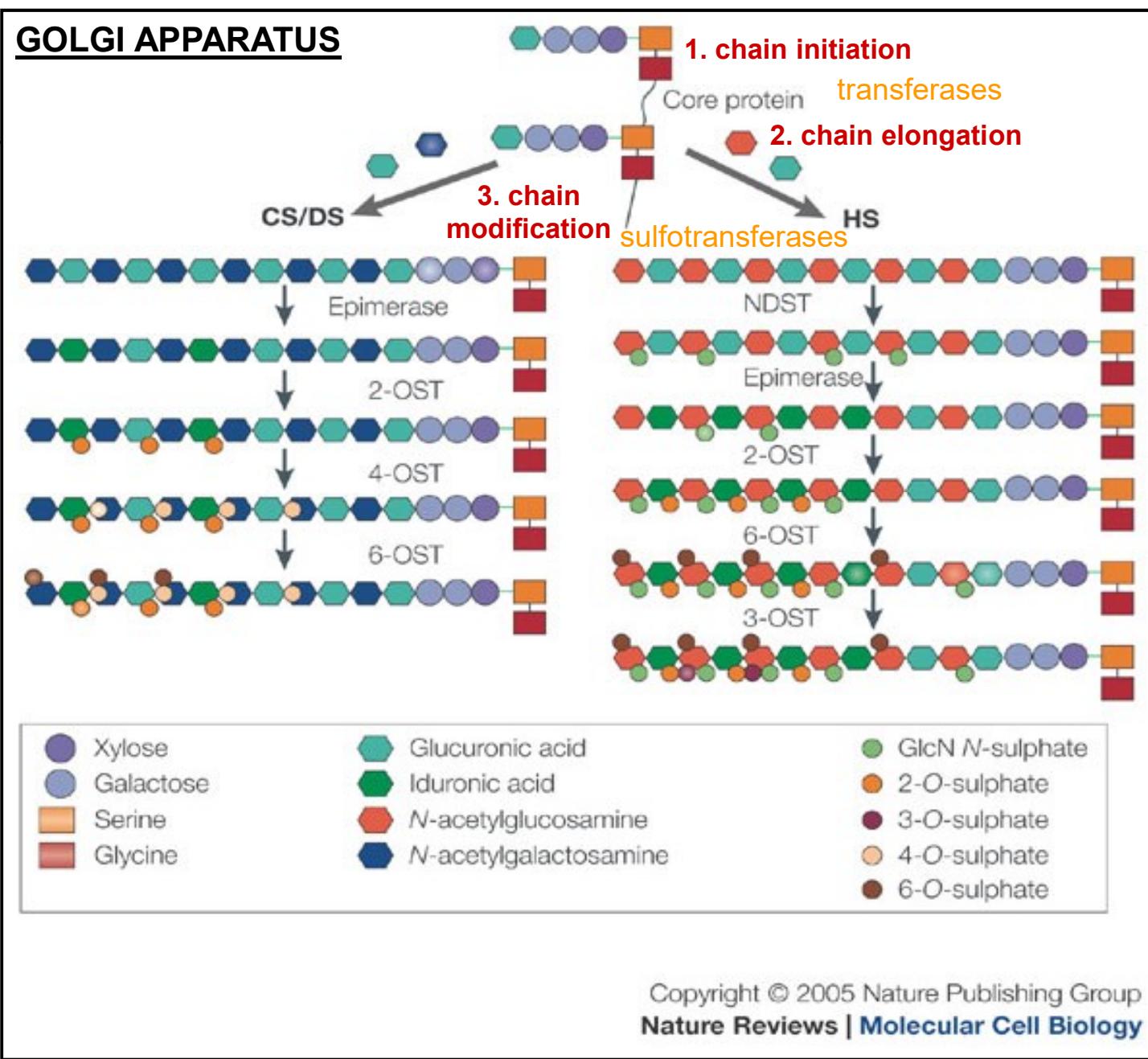
CYTOSOL



Trafficking of UDP-hexoses into GOLGI for GAG biosynthesis

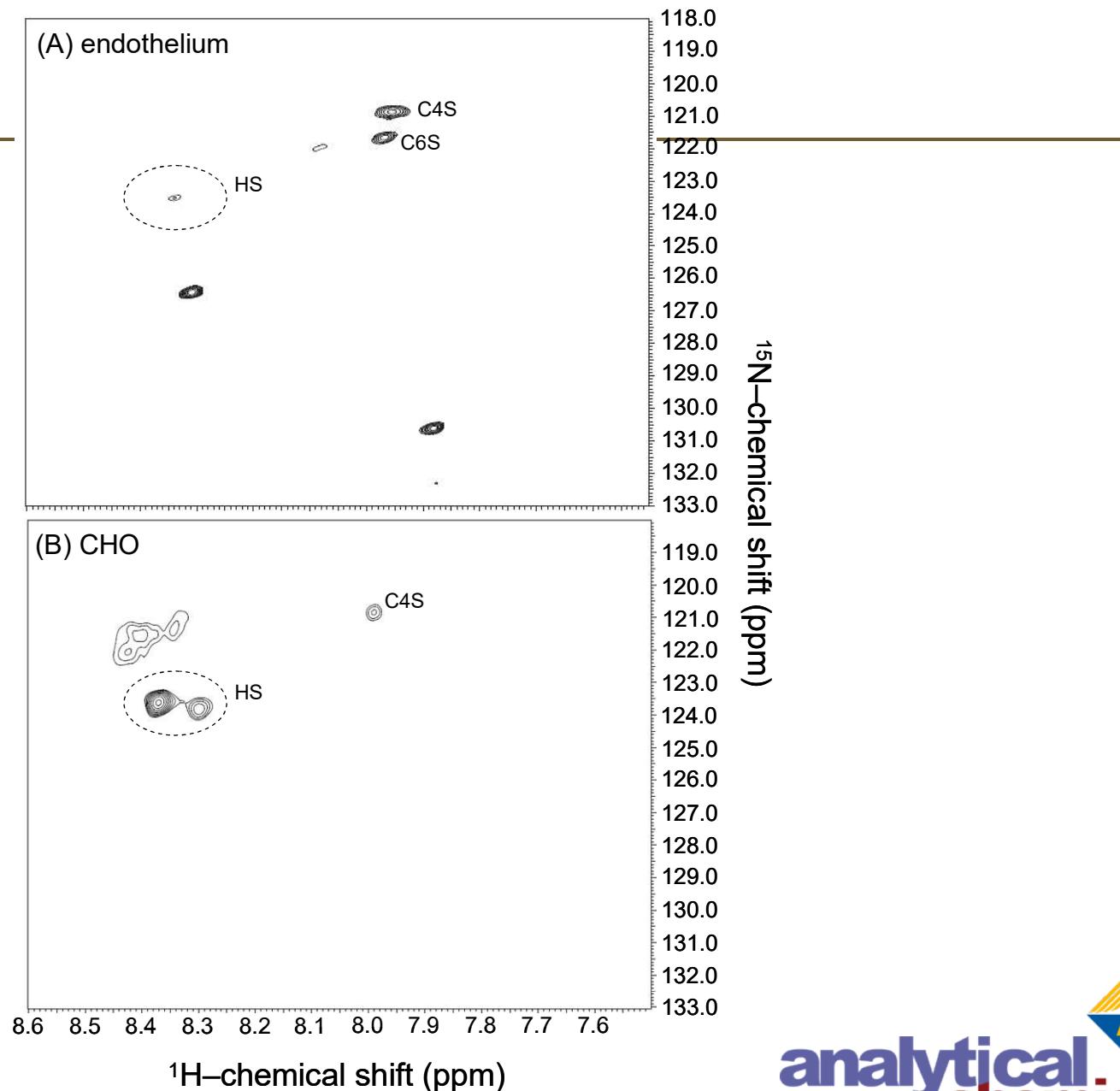


GAG biosynthesis in GOLGI apparatus



^{15}N -gHSQC spectra of cellular ^{15}N -labeled GAGs incubated 24 h with ^{15}N -Gln

Yield of $\sim 600 \mu\text{g}$



Isotopomeric MS analysis of the endothelial pure Δ C4S dimers incubated 24h with ^{15}N -Gln

