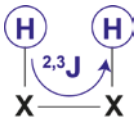
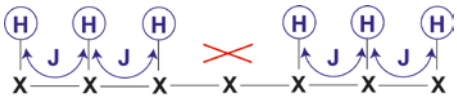
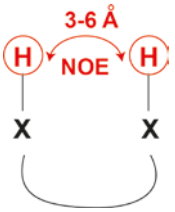
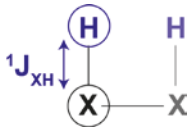
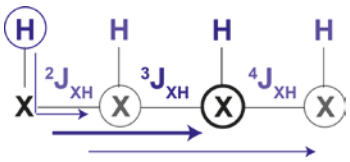
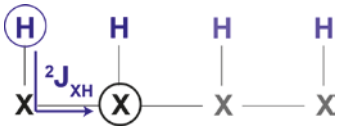
2D NMR

Type of technique and correlation spotted :

2D NMR detects couplings between different nuclei (either through bond or through space). Using 2D NMR allows to simplify overlapped spectra and to and to get valuable information for spectrum assignment or molecular structure determination.
The detected (direct) dimension is usually (but not always) ^{1}H. The second (indirect) dimension can be either 1H or another heteroatoms, typically (but not only) ^{13}C or ^{15}N.
Different 2D techniques allow to probe different bonding or geometric information.

	Through bond		Through space
Homonuclear (^{1}H-^{1}H)	COSY	TOCSY	NOESY
			
Heteronuclear (^{1}H-X)	1 bond	3 (+2,4) bonds	2 bonds
	HSQC	HMBC	H2BC
			

2D experiment setup :

* Start by acquiring a ^{1}H spectrum (useful 1) to check your samples 2) to have a high resolution spectrum)

- Command : **newnmr** (create a ^{1}H dataset)
- Command : **atma** (tuning) ; **rsh** (read standard shim file); **lock** (solvent); **topshim** (shim)
- Command : **zg** (start the experiment)

* Setup 2D experiment(s)

- Command : **edc** (create a new dataset); **rpar refe*** (read pre-set experiment) → choose **refe_#2D-exp-name** in user
- Command : **atma** (for heteronuclear experiment: tuning as it was not done before)
- Command : **zg** (start the experiment)

You can eventually repeat these steps to add more 2D experiments or add an heteronuclear 1D experiment
- Then use the command **multizg** (from the 1st experiment to start multiple experiments in a row)

You can eventually change some parameters:

- **ns** : change number of scans.
  Rem : The signal to noise (SNR) accumulate proportionally to the square root of the number of scans (ns)
  Ex: if 1024 scans are needed to have a SNR of 5, 4096 scans will be needed to have a SNR of 10
  → Consider using cryoprobes if your SNR is still too small within a decent acquisition time

- **O1P** and **SW** : the centre and the width (in ppm) of the spectral window
  (In F2 direct dimension and F1 indirect dimension)

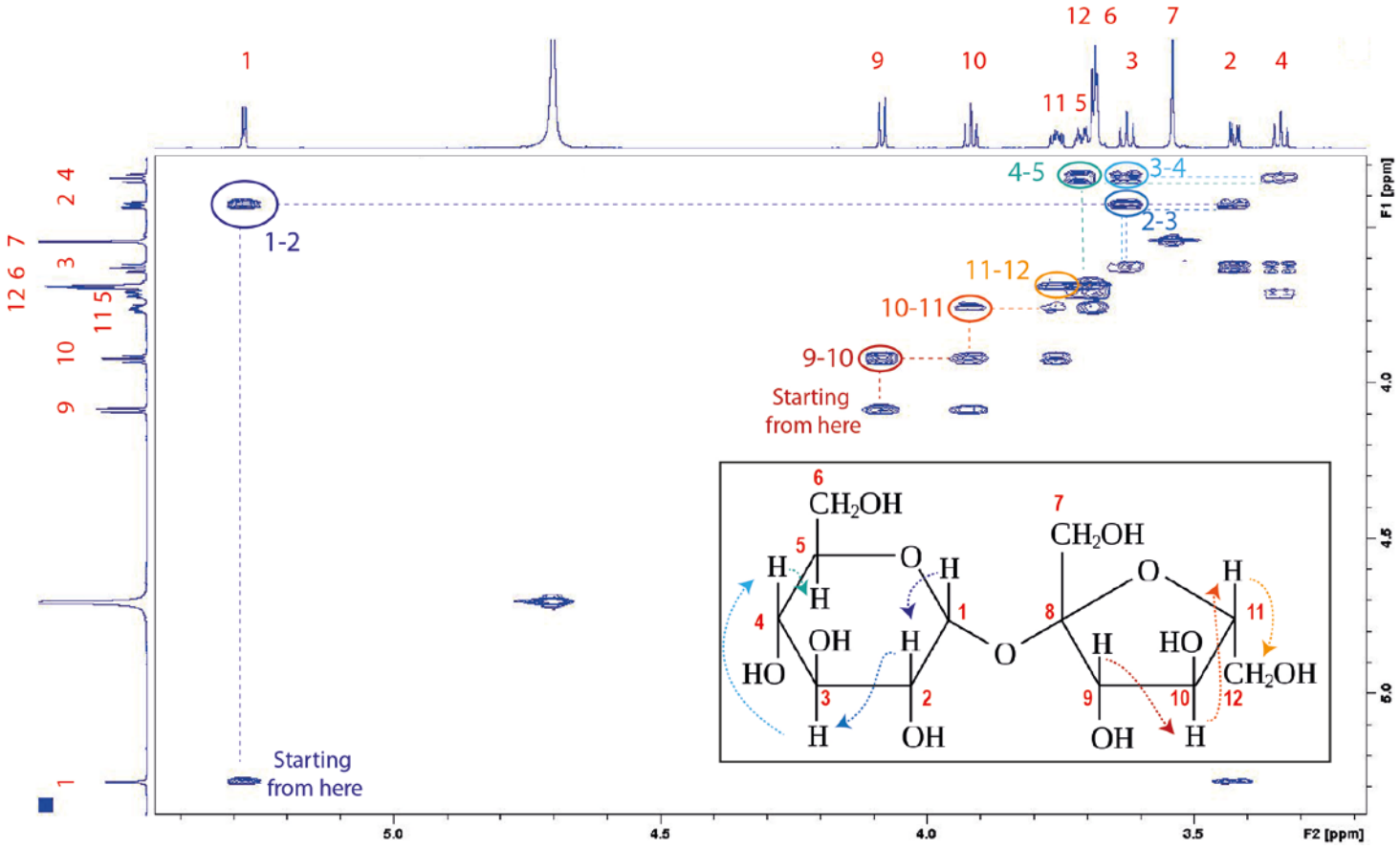
2D NMR : COSY

COSY: 1H-1H Correlation Spectroscopy

^{1}H-^{1}H Correlation Spectroscopy (COSY) shows the correlation between ^{1}H's which are coupled to each other. Mainly 2-bond and 3-bond ^{1}H-^{1}H coupling are visible by COSY.
The ^{1}H spectrum is plotted on both 2D axes. The cross-peaks (not on the diagonal) are symmetric to the diagonal and show the COSY correlations

Example : Saccharose

Starting from the anomeric proton (1) at 5.3 ppm one can assign one by one using COSY cross peaks almost all the ^{1}H of the 6 members ring except ^{1}H 6 (because of overlap). In a same way, starting form proton 9 one can assign the remaining ^{1}H on the 5 members ring.



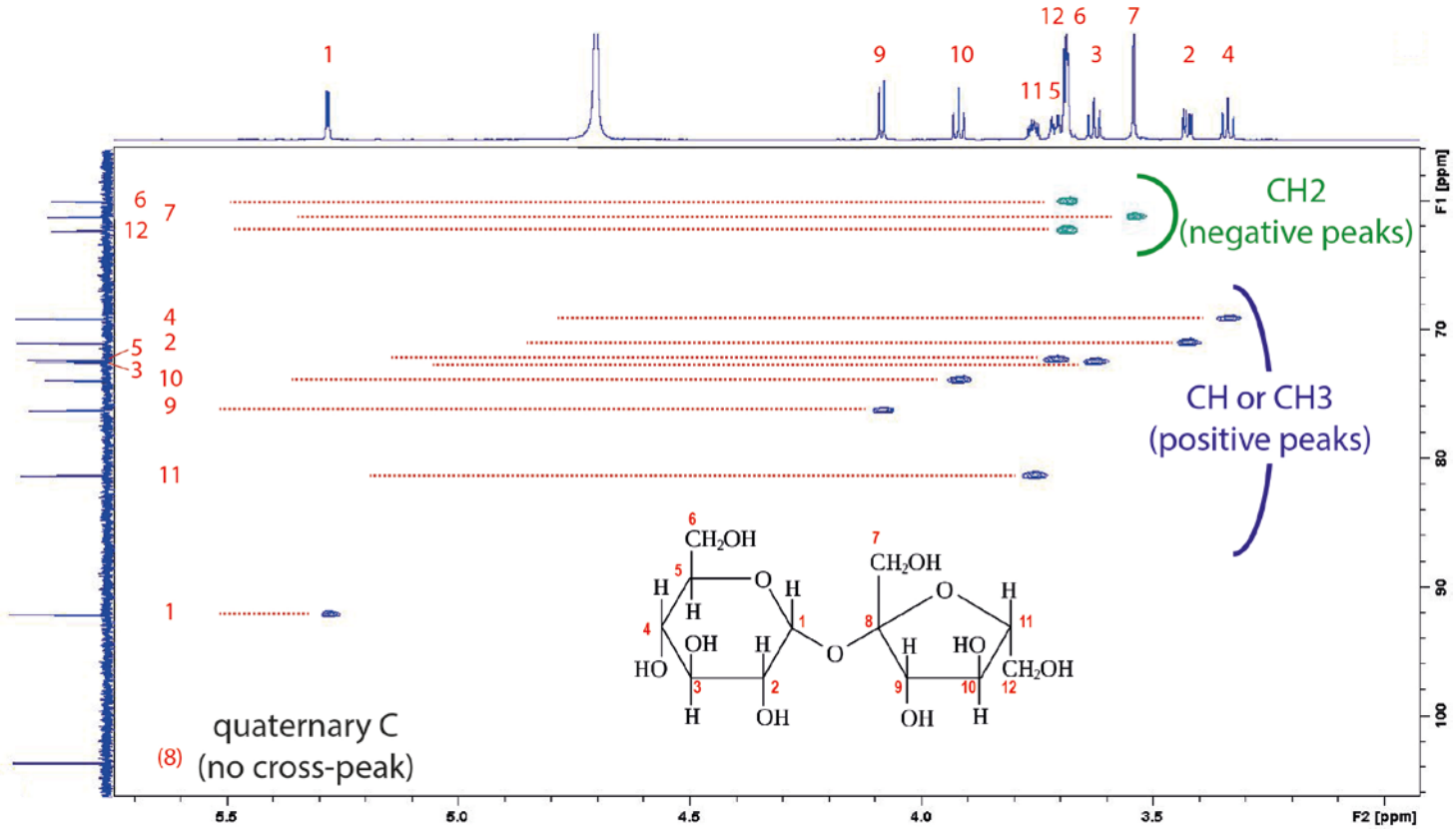
2D NMR : HSQC

1H-13C HSQC: Heteronuclear Single Quantum Coherence Spectroscopy

^{1}H-^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC) permits ^{13}C assignment as it shows which hydrogens are directly attached to which carbon atoms. The ^{1}H spectrum is shown on the horizontal axis and the ^{13}C spectrum is shown on the vertical axis.
Edited HSQC cross-peaks appear with positive intensity for CH and CH3 and negative for CH2 allowing a better spectral interpretation.

Example : Saccharose

Once or while the ^{1}H are assigned, using HSQC cross peaks the corresponding bonded ^{13}C resonances can be easily assigned.



2D NMR : HMBC

1H-13C HMBC: Heteronuclear Multiple Bond Correlation Spectroscopy

^{1}H-^{13}C Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC) show correlation between ^{1}H and ^{13}C at multiple bonds away from each other, usually 3 but as well 2 or 4. It permits the assignment of quaternary ^{13}C that have no hydrogens directly attached. The ^{1}H spectrum is shown on the horizontal axis and the ^{13}C spectrum is shown on the vertical axis.

HMBC is designed to see ^{1}H-^{13}C at 3 bond away, but depending on the angle between ^{1}H and ^{13}C (depending on their scalar coupling constant, see Karplus relation), especially when the angle is blocked, 3 bonds ^{1}H-^{13}C cross peak may disappear and 2 or 4 bonds ^{1}H-^{13}C cross peaks may appears. H2BC technique (2 bonds correlation only) may help to distinguish between 2 and 3 bonds correlations.

HMBC is as well possible between other pairs of nuclei, especially ^{1}H and ^{15}N.

Example : Saccharose

It is possible to assign the quaternary carbon using the correlation between proton (1) with carbon (8) through glycosidic bond.
The doubt on the assignment of carbons 12 and 6 (1H overlap) can be lifted using the correlation between proton (4) and carbon (6) and between proton (10) and carbon (12)