UGSF - UMR8576
Institute for Structural and
Functional Glycobiology

Workshop on Glycobiology NMR

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- Short presentation
- Structure of carbohydrates
- Structural approaches
- Use of NMR for glycan structure
- Classical NMR experiments
- What use of NMR for glycobiology
  - *de novo* sequencing
  - Glycomics profiling
  - Surface analysis: HR-MAS NMR
  - DOSY NMR
  - Protein-carbohydrate interaction

Unité de Glycobiologie Structurale et Fonctionnelle
UGSF, UMR 8576, **LILLE**

Monothematic institute on glycobiology

- 12 groups
- 60 researchers (CNRS, University, INSERM, INRA)
- 30 masters et doctor students
- 20 technical staff

Understand the relationships between **structure** and **functions** of sugar complex molecules

Three main approaches:

- **Structural** Glycobiology and Modelisation
- Functions and Regulations of Glycosylation **Enzymes**
- Glycobiology and **Pathologies**

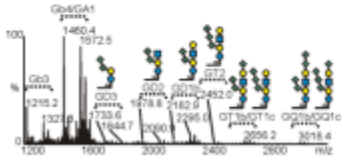
Structural Glycobiology of Host-Pathogens Interactions

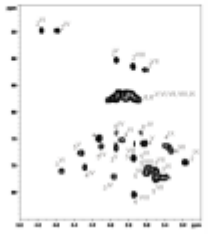
MS

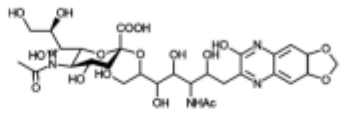
NMR

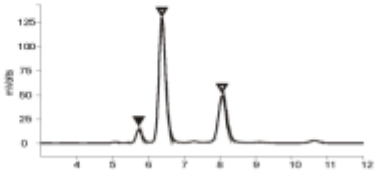
Sugar chemistry

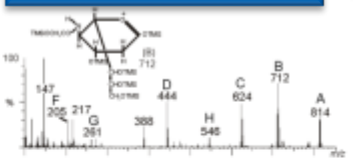
GC/MS



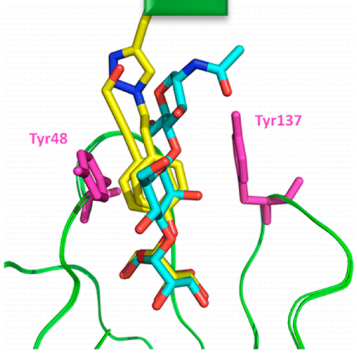


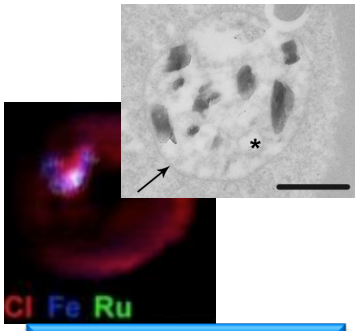


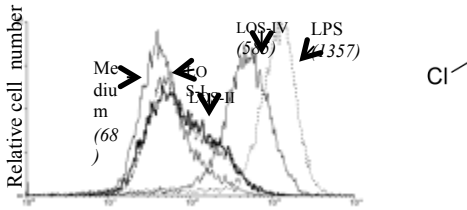


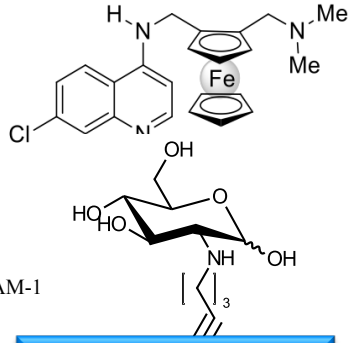


Glycoconjugates
Structures and functions









Protein structure

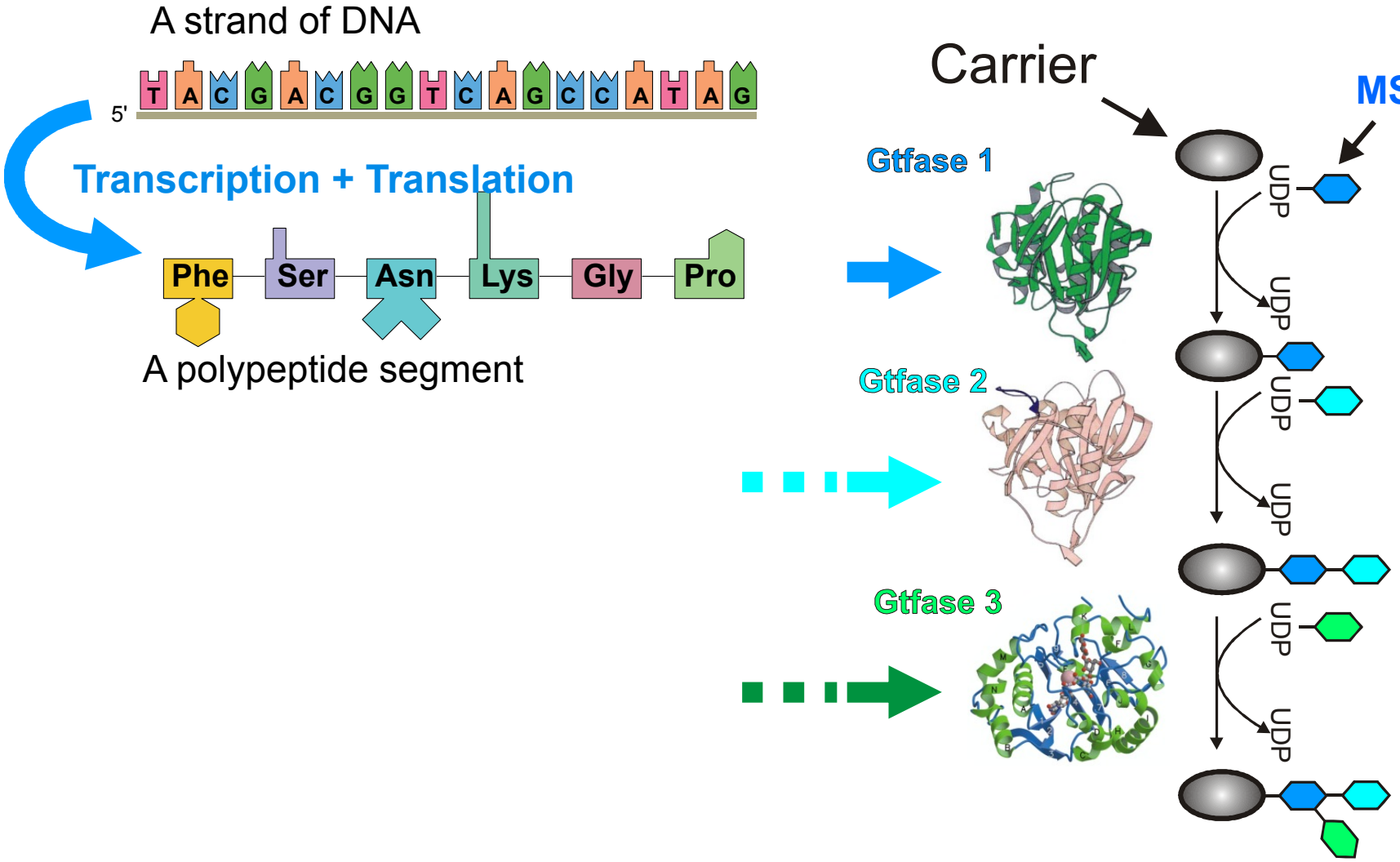
Imaging

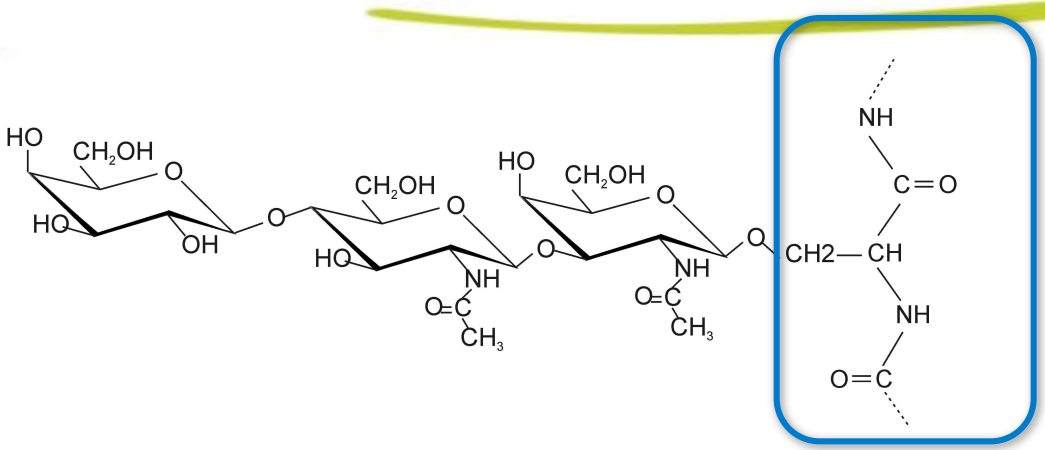
Cell biology

Synthesis

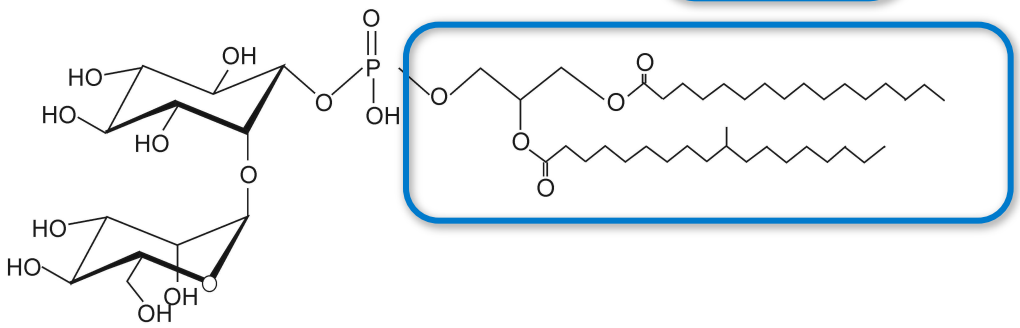
http://plateforme-pages.univ-lille1.fr

A post-translational modification

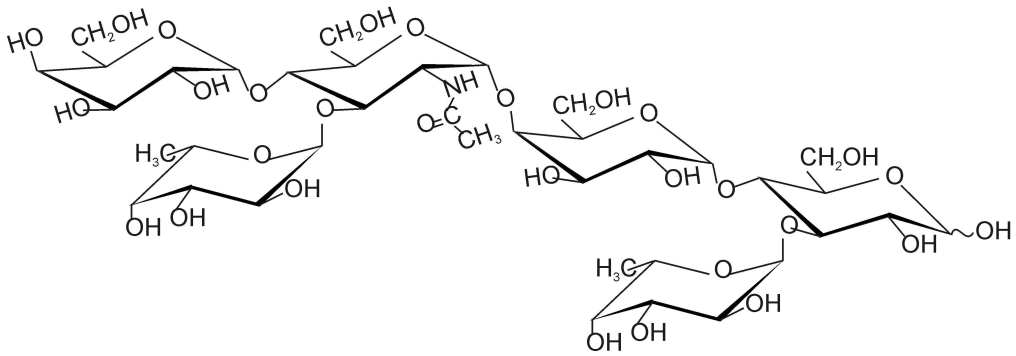




Glycoproteins



Glycolipids

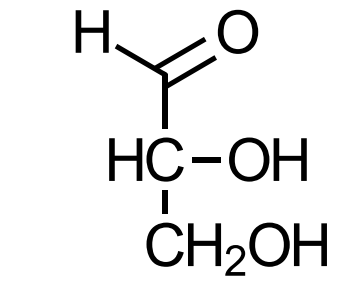


Free sugars and polysaccharides

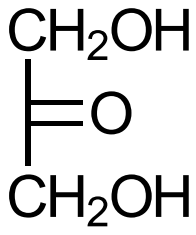
Structure of carbohydrates

Definitions

- Carbohydrates are made of monosaccharides
- Monosaccharides are poly-hydroxy-aldehydes ou poly-hydroxy-ketons
- All monosaccharides have an asymetric carbon, except for dihydroxyacétone



D-glyceraldehyde

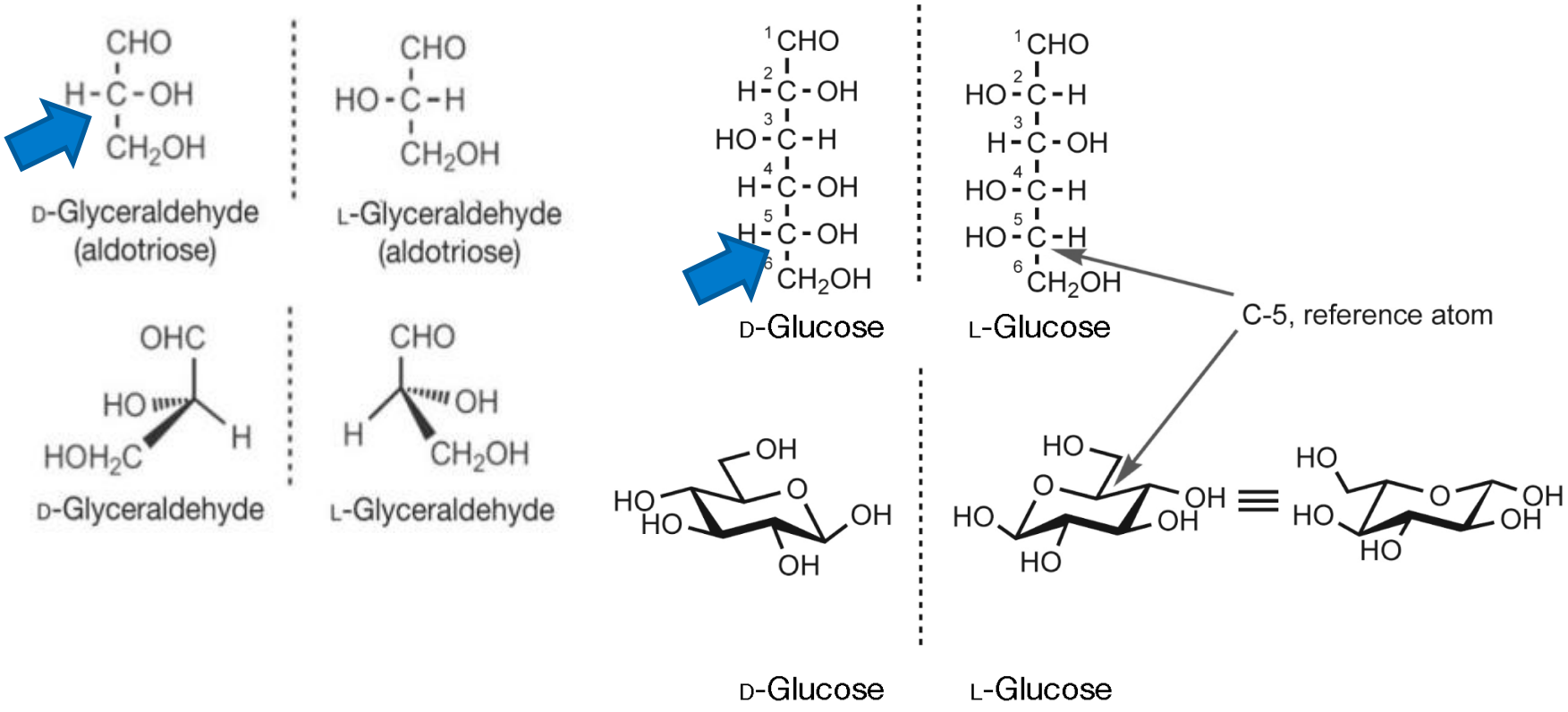


Dihydroxyacetone

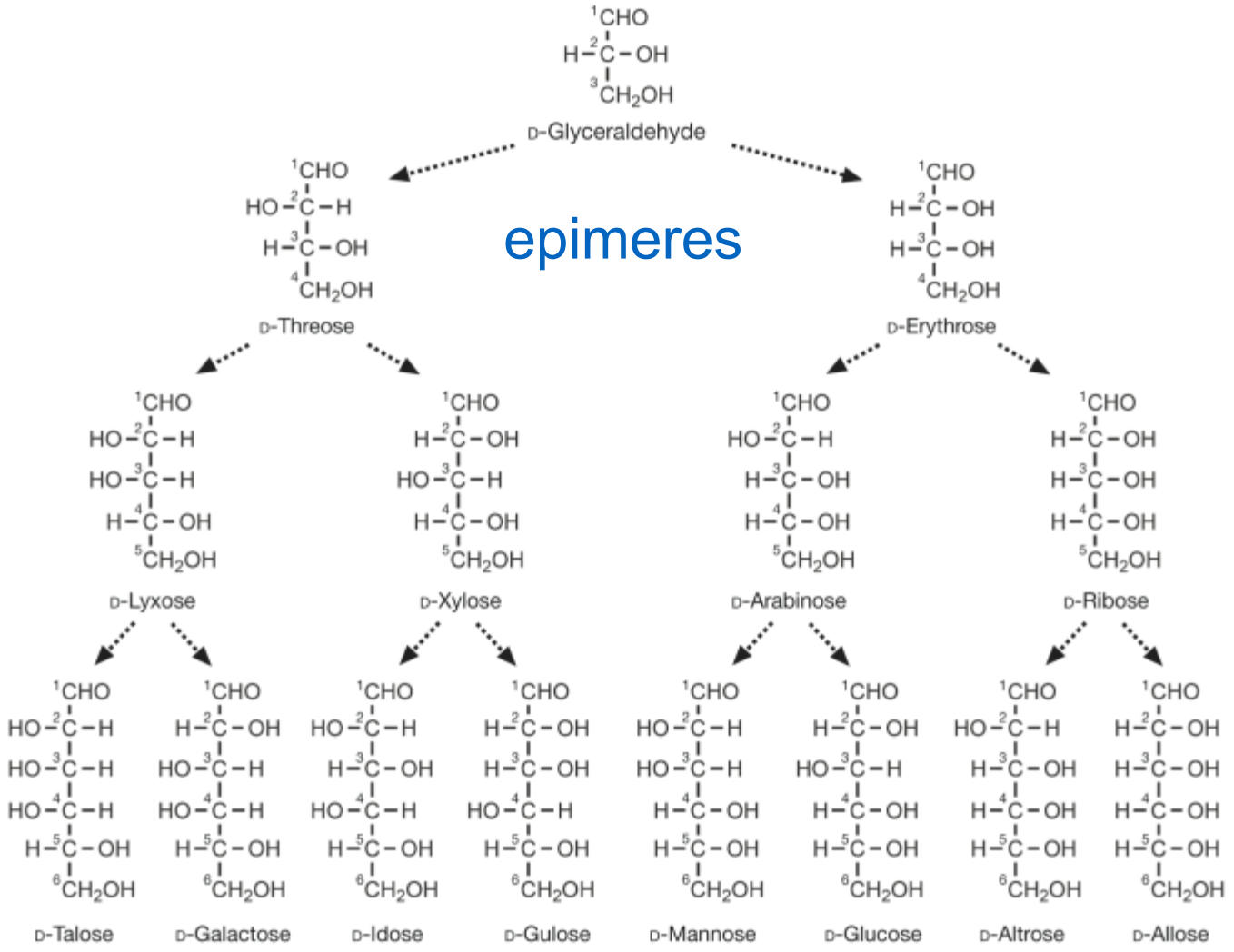
Aldoses

ketoses

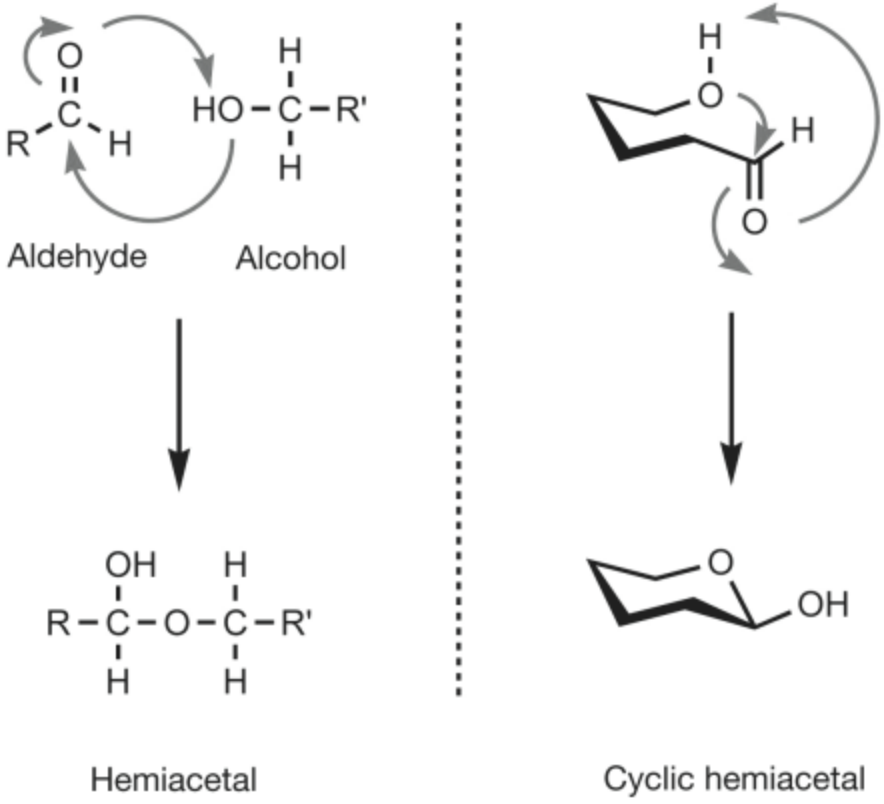
D/L Configuration



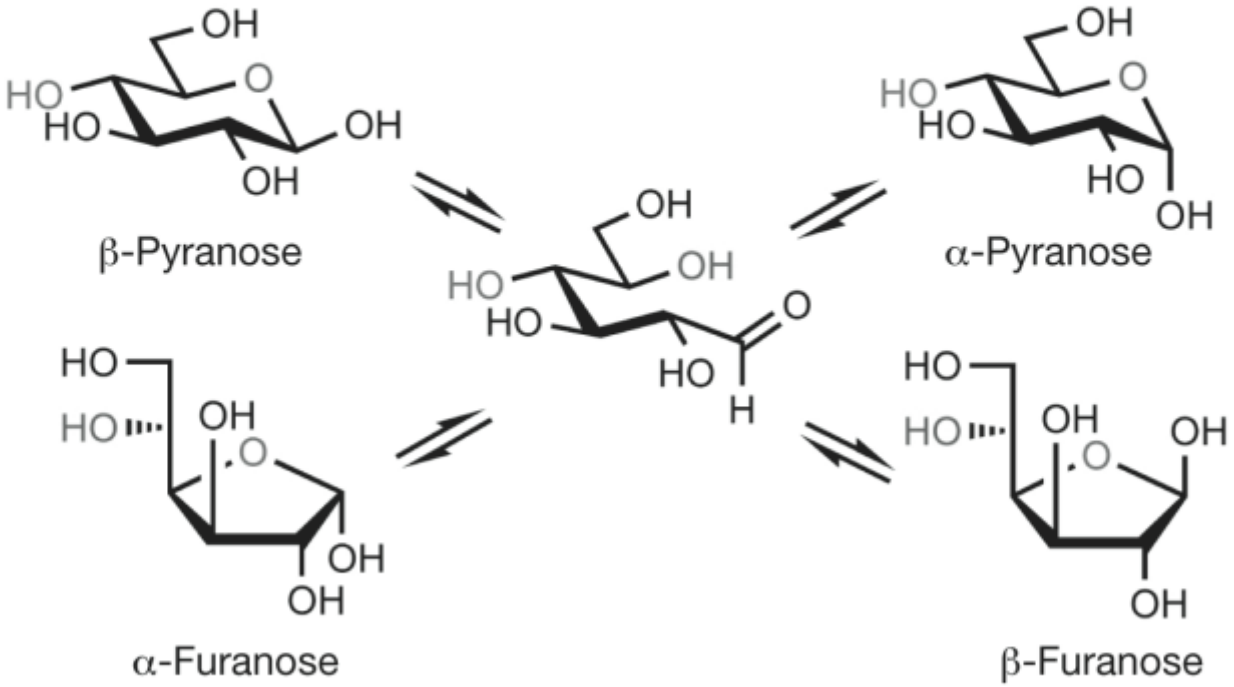
Aldoses D series



Formation of hemi-acetals

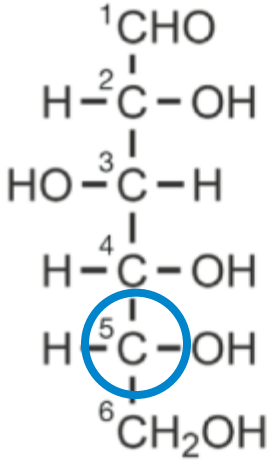


Equilibrium during cyclisation of D-glucose



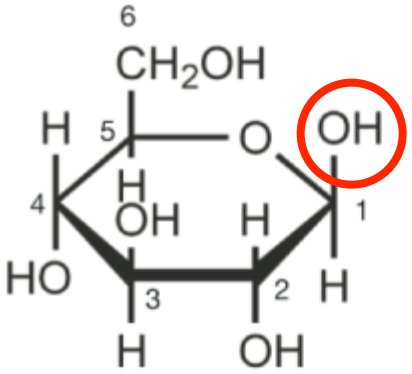
Fischer & Haworth projections

Fischer



D-Glucose

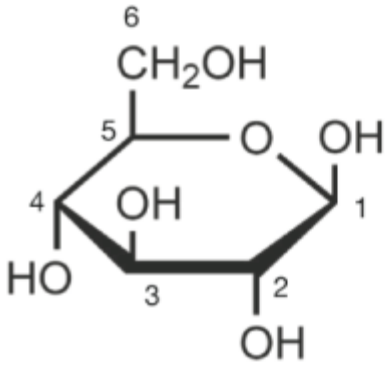
Haworth



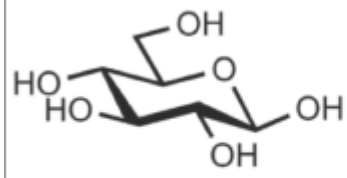
β-D-Glucopyranose

Abbreviated Haworth

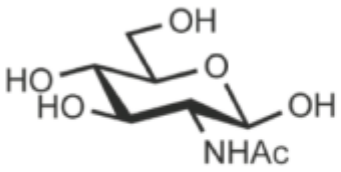
≡



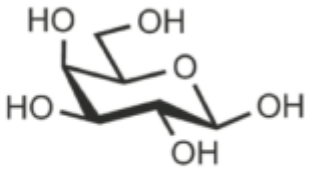
Common monosaccharides in vertebrates



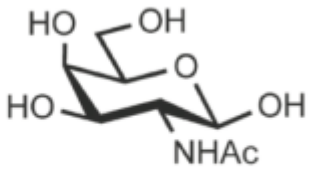
D-Glucose
(Glc)



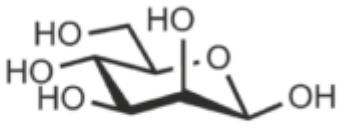
N-Acetyl-D-glucosamine
(GlcNAc)



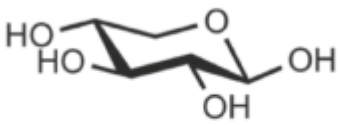
D-Galactose
(Gal)



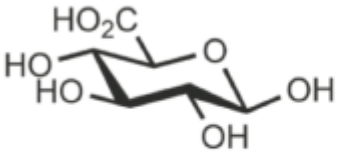
N-Acetyl-D-galactosamine
(GalNAc)



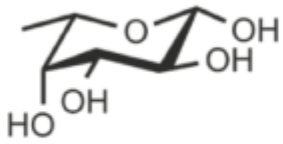
D-Mannose
(Man)



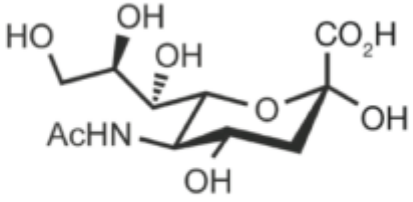
D-Xylose
(Xyl)



D-Glucuronic acid
(GlcA)



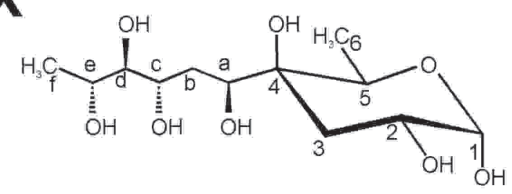
L-Fucose
(Fuc)



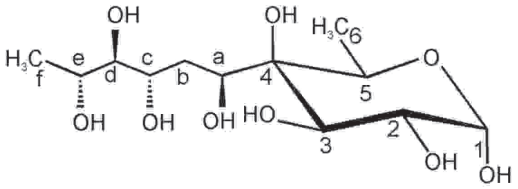
N-Acetylneuraminic acid
(NeuAc)

Many other monosaccharides
500 to 600 known

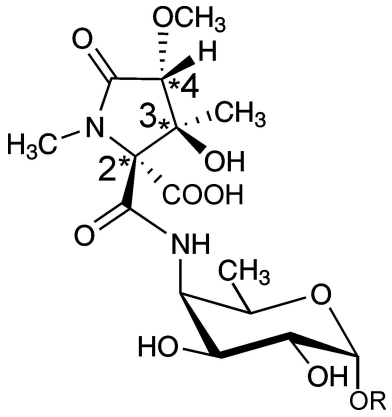
X

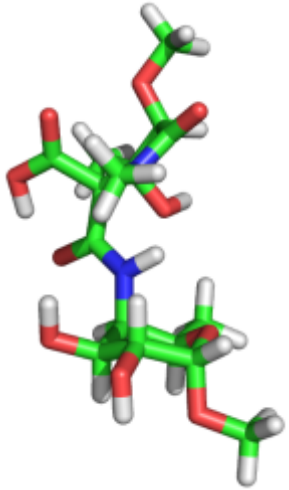


Caryophyllose



Caryophyllose
Hydroxylé

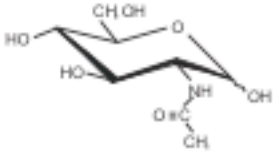




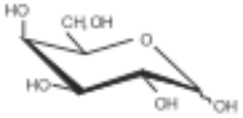
Structural Diversity

1:monosaccharides

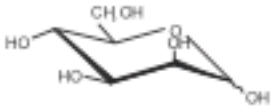
D-Glc*p*NAc



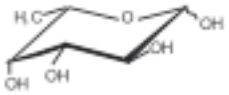
D-Gal*p*



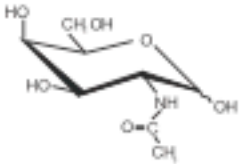
D-Man*p*



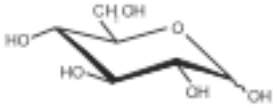
L-Fuc*p*



D-Gal*p*NAc

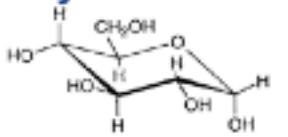


D-Glc*p*

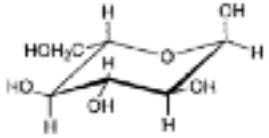


2: D/L isomery

α-D-Glc*p*

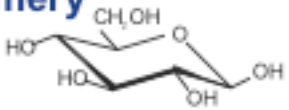


α-L-Glc*p*

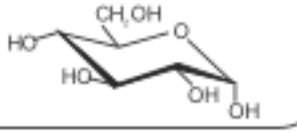


3: α/β anomery

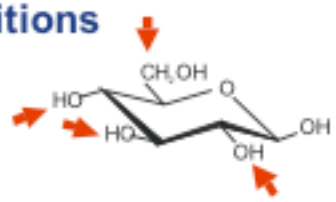
β-D-Glc*p*



α-D-Glc*p*

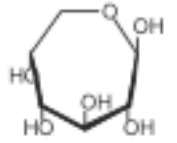


4:positions

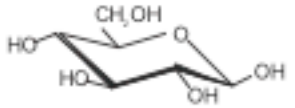


5: size of ring

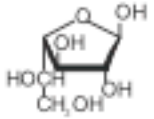
β-D-Glchepta



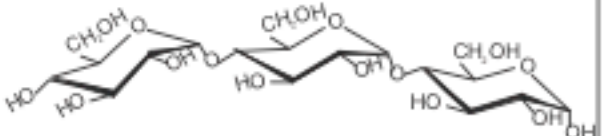
β-D-Glc*p*

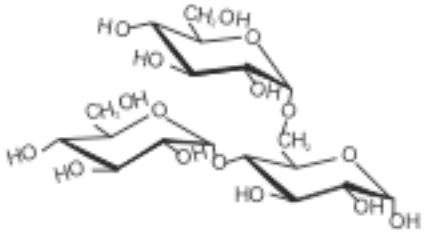


β-D-Glc*f*

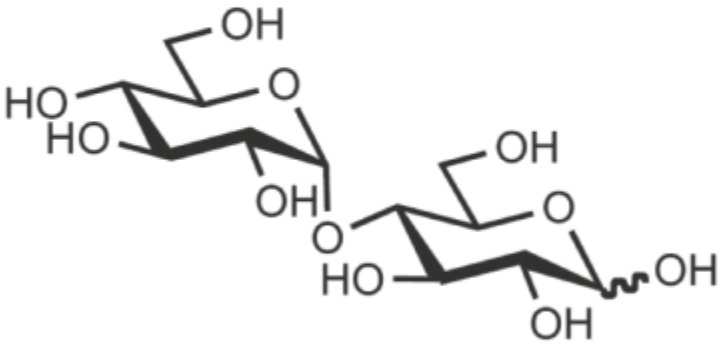


6: branching pattern

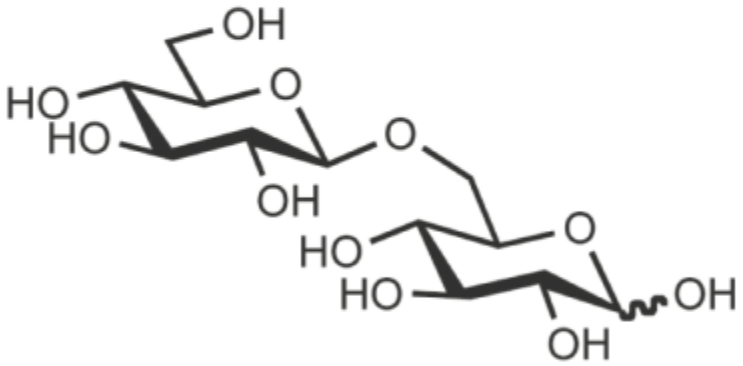




Two isomeres of disaccharides

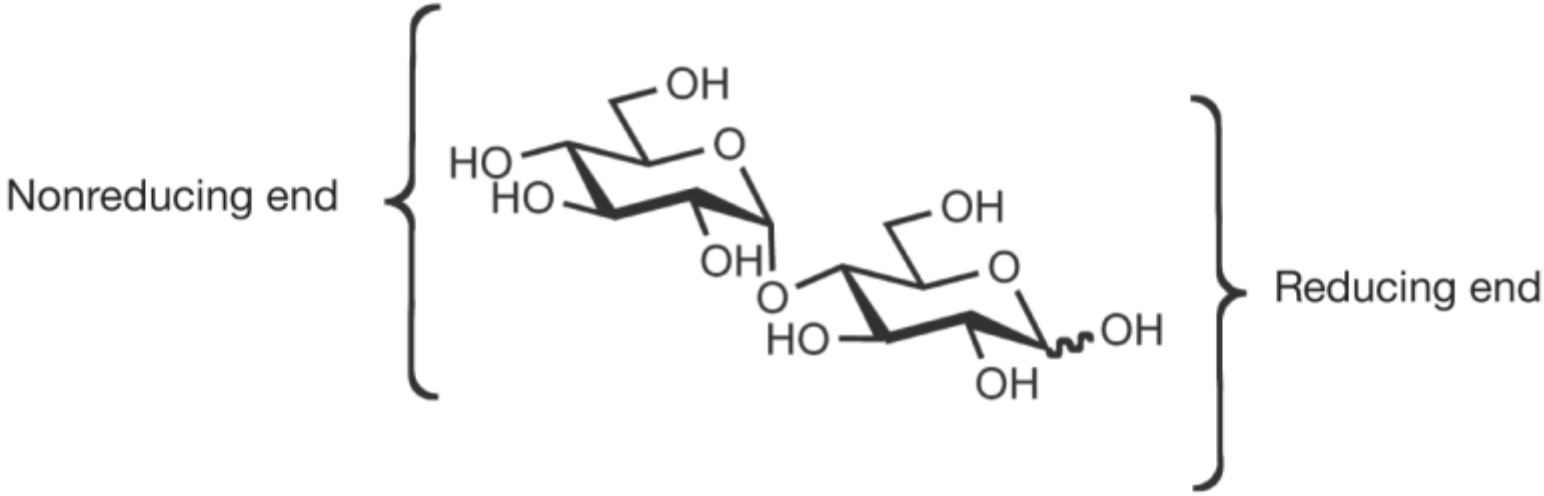


Glcα1-4Glc
(maltose)

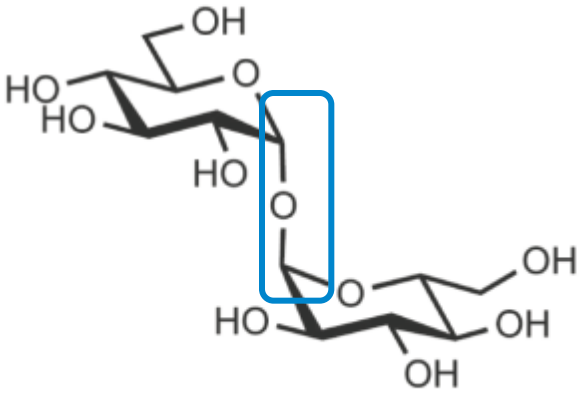


Glcβ1-6Glc
(gentiobiose)

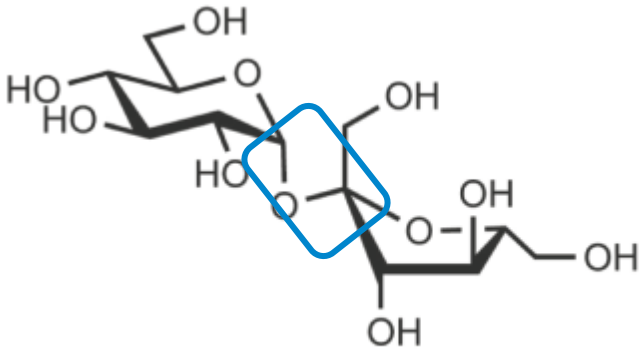
Reducing and non-reducing ends



Disaccharides WITHOUT reducing extremities



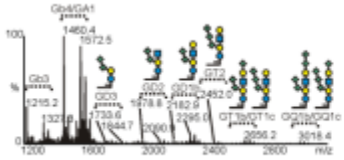
Glcα1Glcα1
(trehalose)



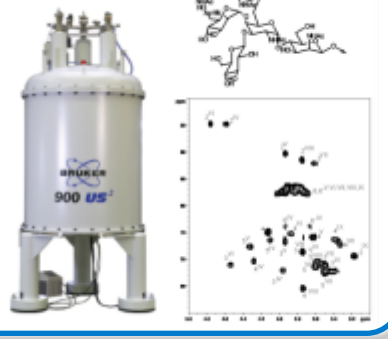
Glcα2Fruβ
(sucrose)

Structural Approaches

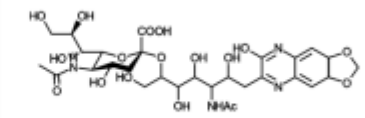
MS

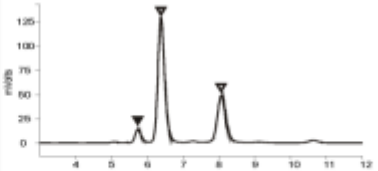


NMR



Sugar chemistry

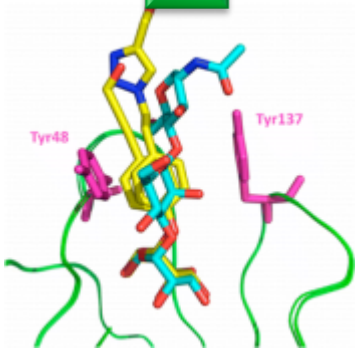




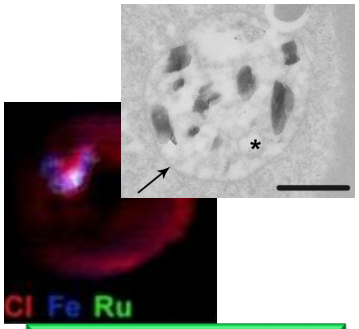
GC/MS

Structures of glycoconjugates

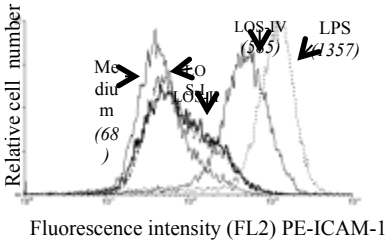
Functions of glycoconjugates



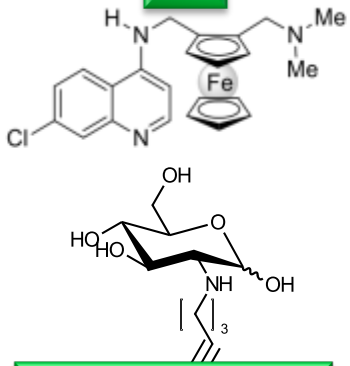
Protein structure



Imaging



Cell biology



Synthesis

Acronym	Technique	Description	Use
FACE	fluorophore-assisted carbohydrate electrophoresis	gel-electrophoresis-based chromatographic technique for separating samples derivatized with an anionic fluorophore	separation, identification, and quantification of labeled mono- and oligosaccharides
GLC or GC	gas-liquid chromatography or gas chromatography	gas-phase chromatographic technique for separating volatile derivatized samples	sugar composition and linkage analysis; usually interfaced with MS
HPAEC-PAD	high-pH anion-exchange chromatography–pulsed amperometric detection	ion-exchange liquid chromatographic separation technique carried out at high pH	separation, identification, and quantification of mono- and oligosaccharides without derivatization
HPCE	high-performance capillary electrophoresis	chromatographic technique for separating charged molecules	separation, identification, and quantification of charged glycans; sometimes inter faced with MS
HPLC	high-pressure liquid chromatography	chromatographic technique for analytical and preparative separations	separation of all classes of glycans and glycoconju gates; may be interfaced with MS
HPTLC	high-performance thin-layer chromatography	chromatographic technique for analytical separations	glycolipid characterization
SDS-PAGE	sodium dodecyl sulfate– polyacrylamide gel electrophoresis	gel electrophoresis technique for separation of proteins according to molecular weight	glycoprotein characterization
PAS	periodic acid–Schiff reaction	colorimetric determination of sugars	detection of glycans
NMR	nuclear magnetic resonance	1D NMR spectroscopy	number and anomeric configuration of monosaccharides in a glycan
COSY	correlation spectroscopy	2D NMR spectra; cross-peaks indicate protons joined by few bonds	identity and anomeric configuration of monosaccharides in a glycan
TOCSY	total correlation spectroscopy	2D NMR spectra; cross-peaks define whole spin system (e.g., one monosaccharide residue)	identity and anomeric configuration of monosaccharides n a glycan
NOESY	nuclear Overhauser effect spectroscopy	2D NMR spectra; cross-peaks indicate protons close in space	sequence analysis, conformational analysis
ROESY	rotating-frame NOESY	2D NMR spectra; cross-peaks indicate protons close in space; better than NOESY for oligosaccharides	sequence analysis, conformational analysis
HMBC	heteronuclear multiple bond spectroscopy	2D NMR spectra; cross-peaks indicate proton and C, N, or P atom linked by few bonds	assignment of NMR signals to atoms in structure; sequence and substitution analysis
HSQC	heteronuclear single- quantum coherence spectroscopy	2D NMR spectra; cross-peaks indicate proton and C, N, or P atom linked by one bond	assignment of NMR signals to atoms in structure
MS	mass spectrometry	technique for mass measurement of gas-phase ions	primary structure analysis of biopolymers
FAB	fast atom bombardment	MS ionization technique	mass mapping and sequence analysis of glycans and glycolipids
MALDI	matrix-assisted laser desorption ionization	MS ionization technique	mass mapping of glycans and glycoconjugates; important for glycomics
ESI	electrospray ionization	MS ionization technique	molecular weight and sequence analysis of glycans and glycoconjugates; glycoproteomics
CAD-MS/MS	collisionally activated decomposition mass spectrometry/mass spectrometry	tandem MS technique in which fragment ions are produced from a selected parent ion via collisions with an inert gas	sequence analysis of glycans and glycoconjugates

Analytical tools

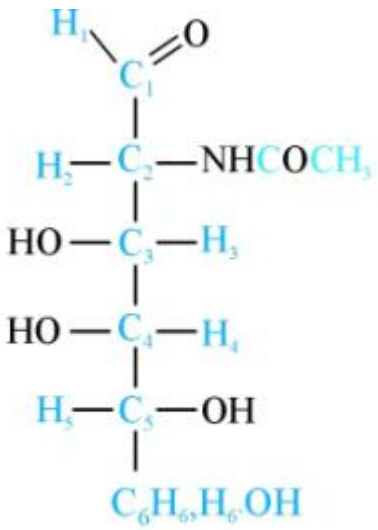
Technics	Information
Mass spectrometry (MS)	➢ Molecular weight ➢ Monosaccharide sequence ➢ Substitution (methyl, acetyl...)
Gas chromatography-mass spectrometry (GC-MS)	➢ Monosaccharidic composition ➢ Linkage ➢ Conformation D and L
NMR	➢ Carbon configurations ➢ Conformation ➢ Anomer (α or β) ➢ Linkage ➢ Substitution

Use of NMR for glycan structure

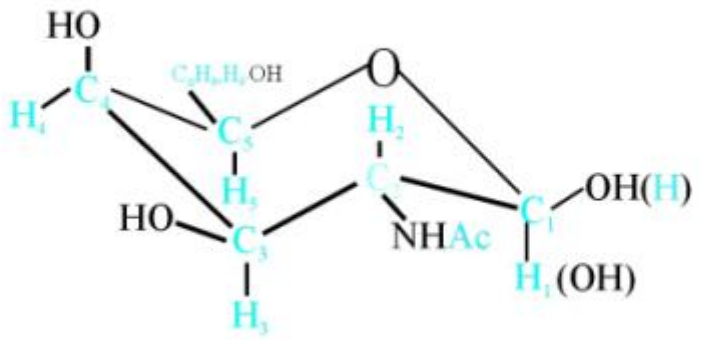
What does NMR provides?

- ✓ Chemical shifts of all individual carbons and protons — Linkage
- ✓ Spin systems of all monosaccharides
- ✓ Intra residues $^{3}J_{H,H}$ correlations
- ✓ ^{1}H-^{1}H coupling constants of vicinal protons — Composition
- ✓ ^{1}H-^{13}C coupling constant of anomeric carbons — α-β anomery
- ✓ Inter residues ^{1}H-^{1}H nOe correlations
- ✓ Inter residues $^{3}J_{H,C}$ correlations — Sequence

Which atoms do we observe?



N-acetyl-D-Galactosamine

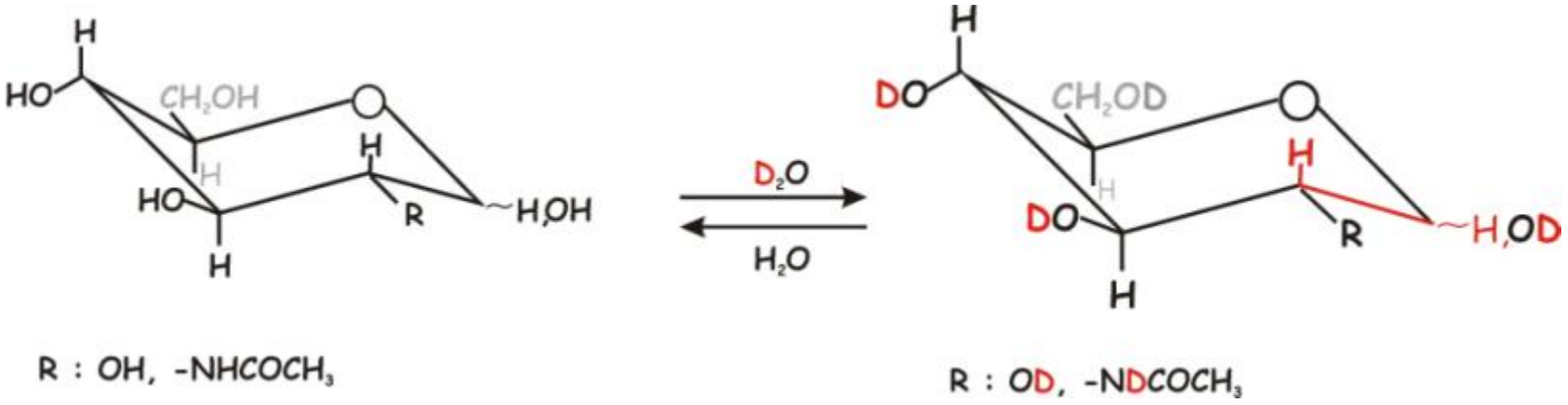


N-acetyl-(α)β-D-Galactopyranosamine

Mostly ^{1}H and ^{13}C, but also ^{31}P

Chemical exchange

Exchange all mobile/non informative protons (-NH, -OH) by deuterium

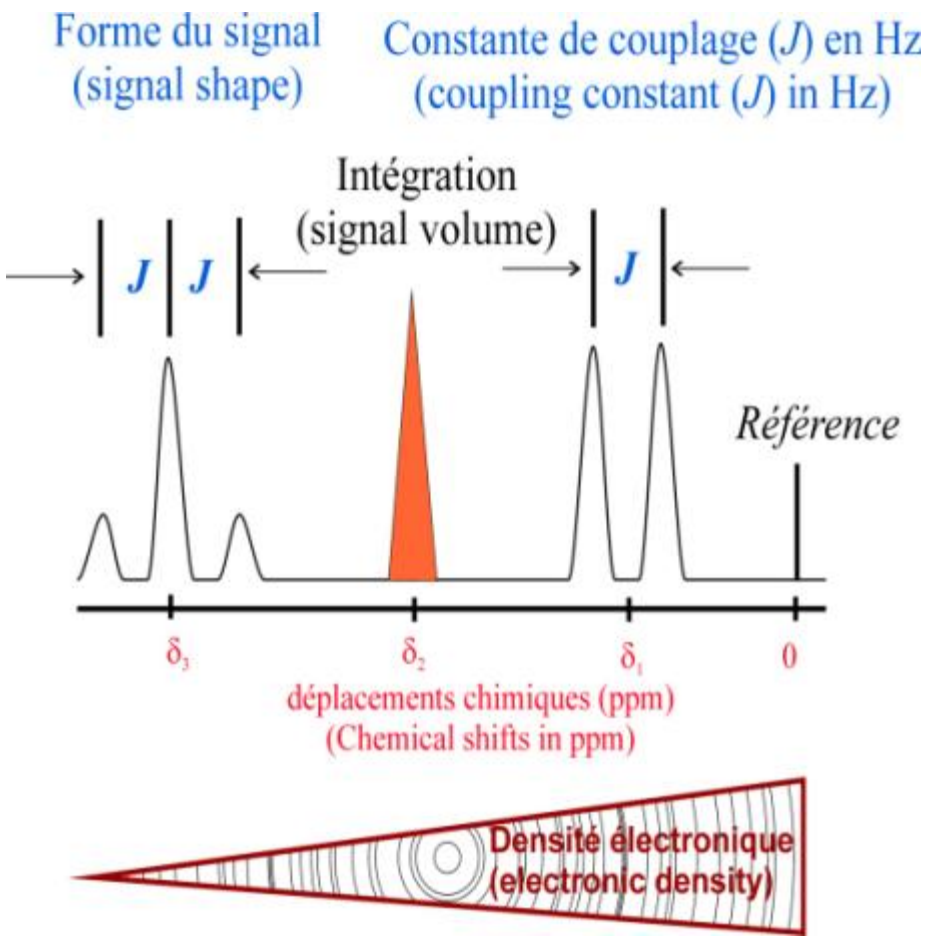


R : OH, -NHCOCH$_3$

R : OD, -NDCOCH$_3$

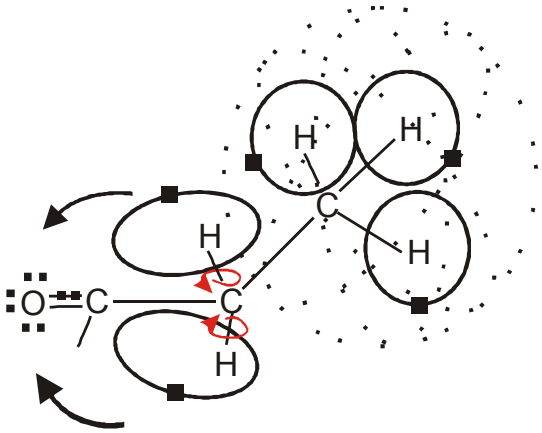
increase signal/noise ratio

Parameters to look for



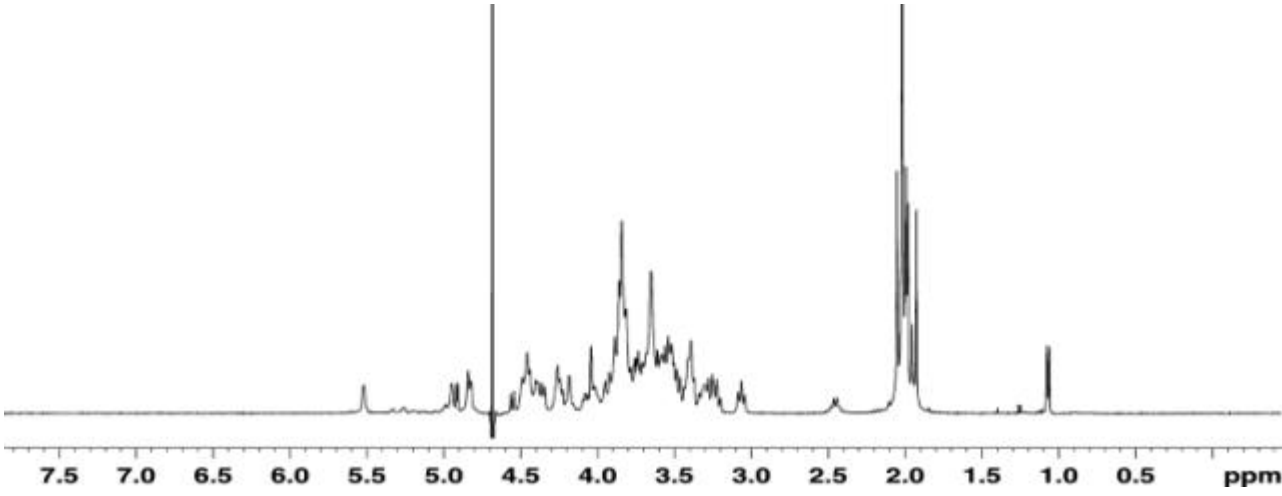
1. electronic density
2. chemical shifts (ppm)
3. signal integration
4. coupling constant (Hz)
5. nOe effect
6. relaxation time (T1 et T2) (s)

1 Influence of electronic density

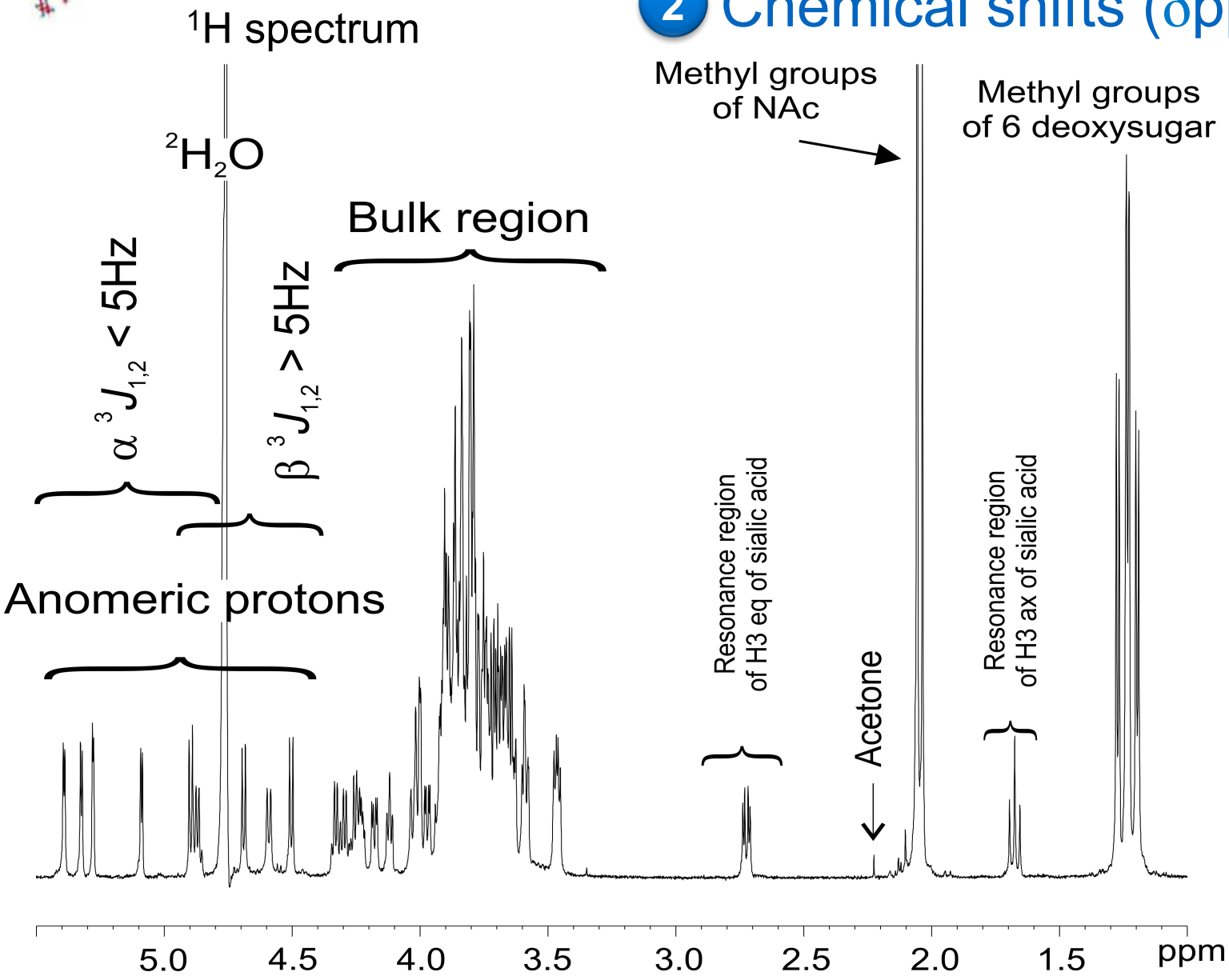


low electronic density

high electronic density

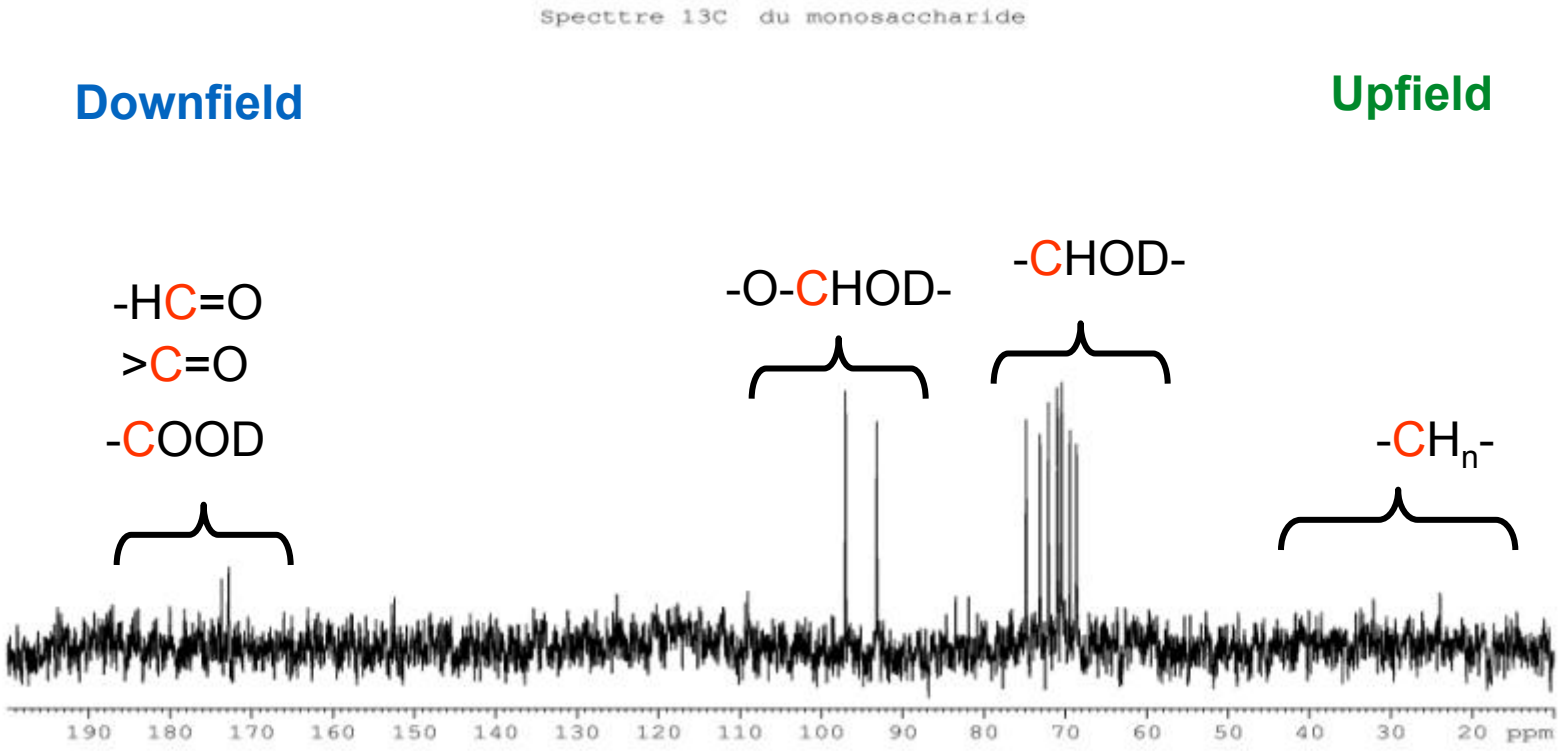


2 Chemical shifts (δppm)

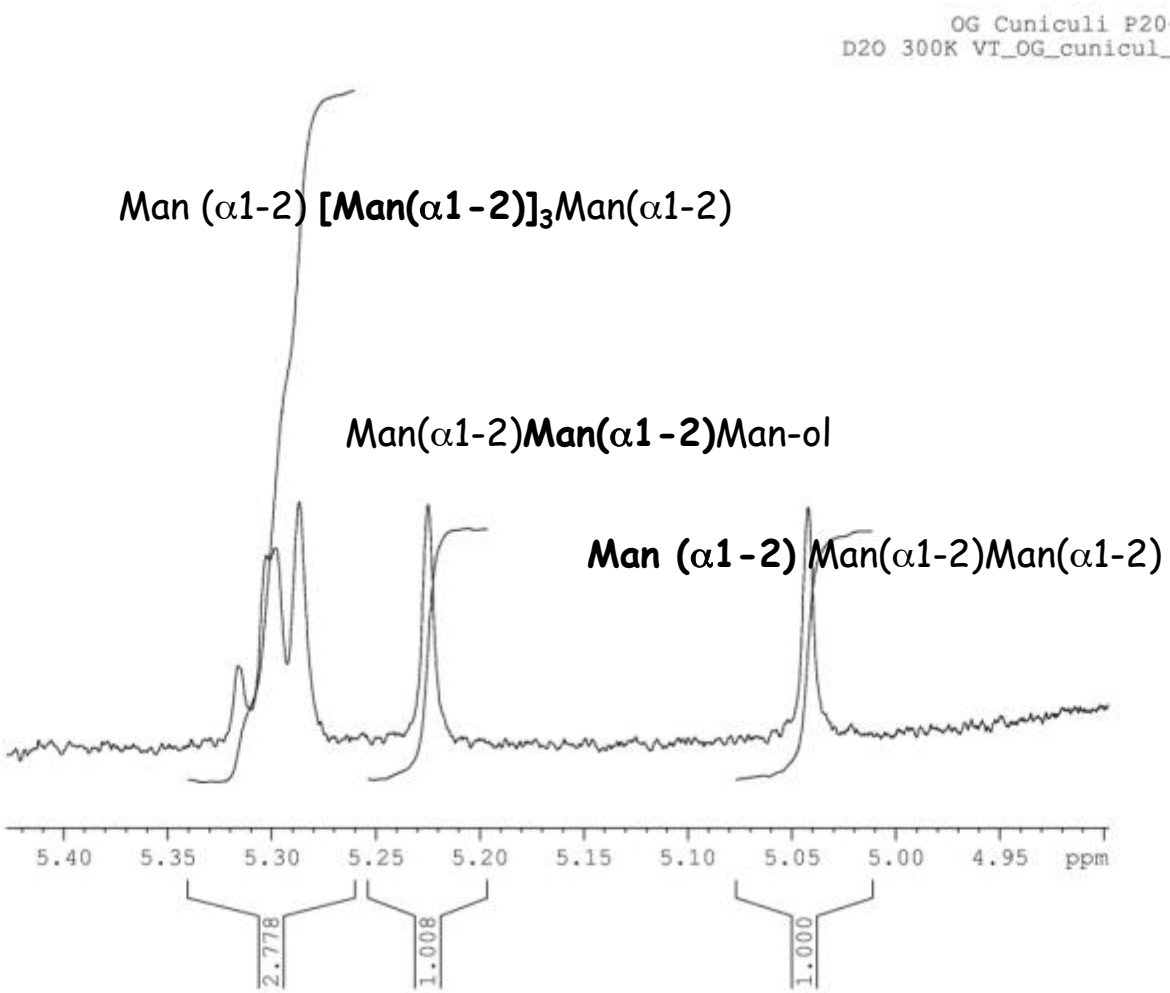


2 Chemical shifts (δppm)

^{13}C spectrum

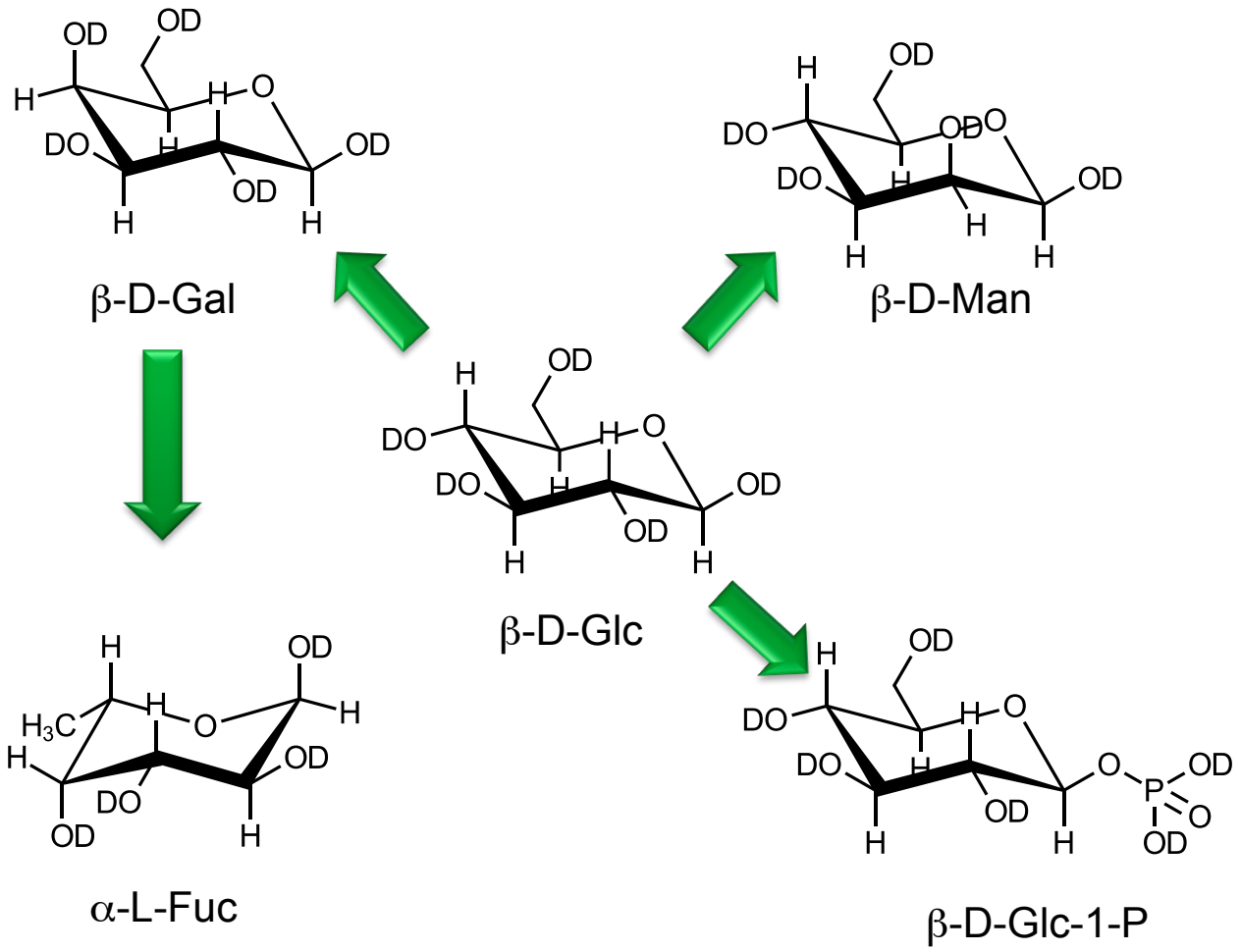


3 Signals intensities



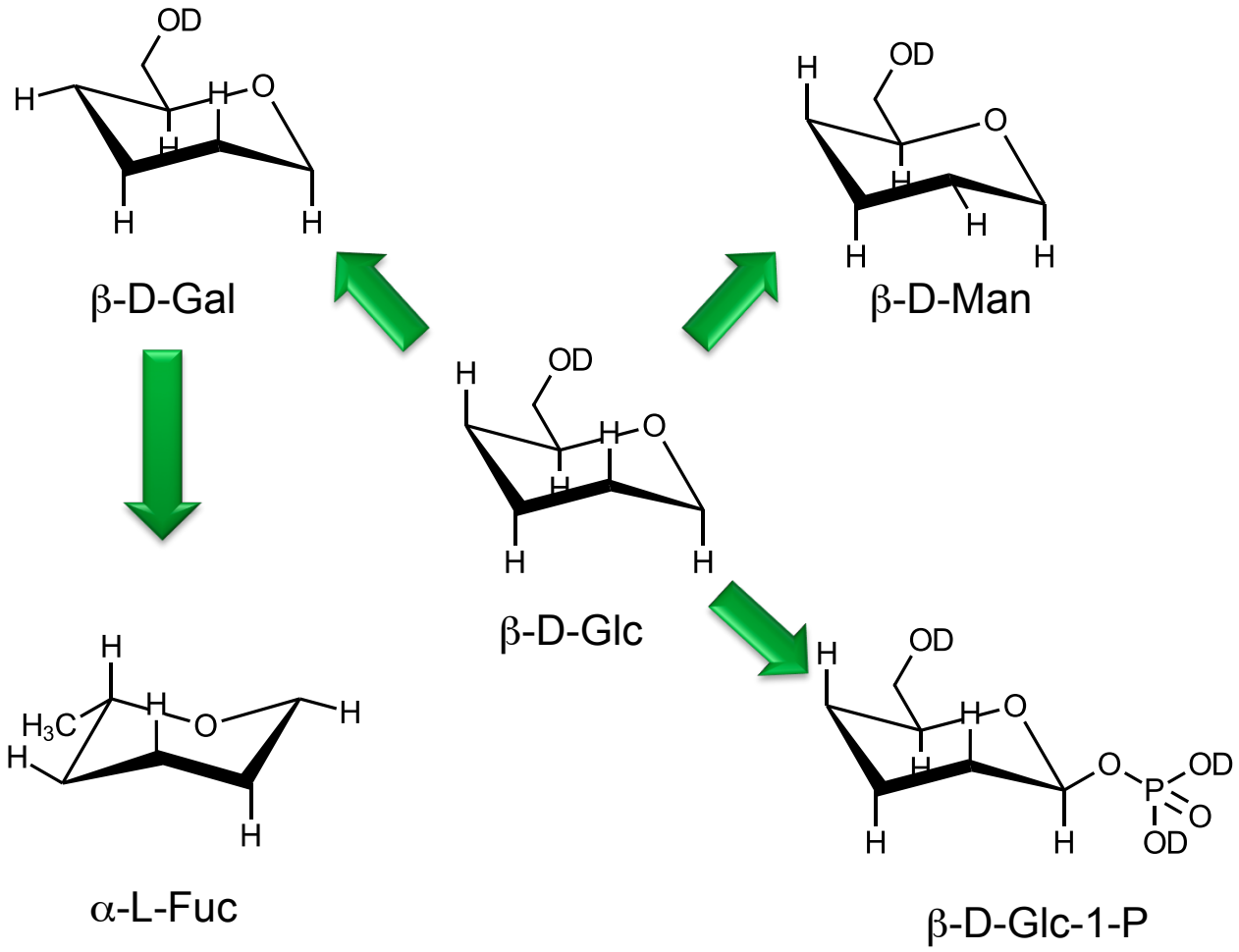
4 Coupling constant (Hz)

How to define the configuration of monosaccharides?



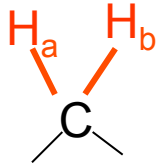
4 Coupling constant (Hz)

How to define the configuration of monosaccharides?

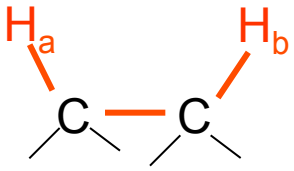


4 Coupling constant (Hz)
Values for scalar homonuclear H-H

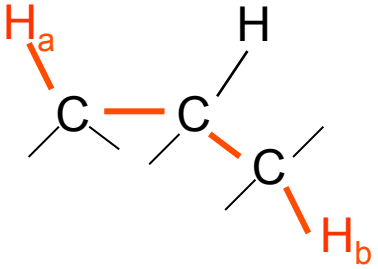
Number of covalent linkages → $^{X}J_{H,H}$ ← Atoms



$^{2}J_{Ha,Hb}$ Geminal (~ 10 à 15Hz)



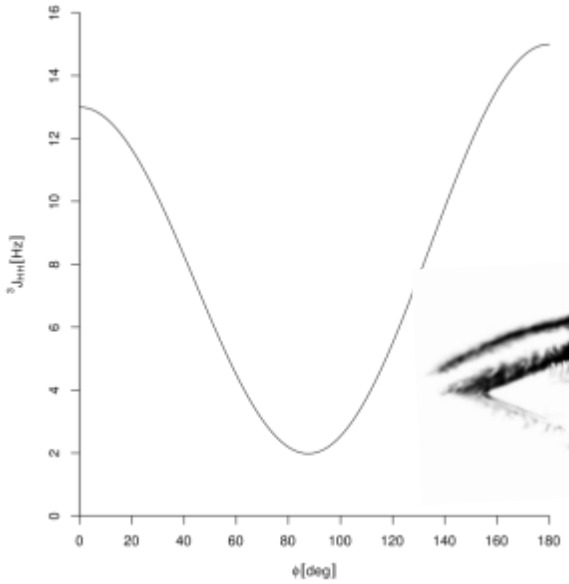
$^{3}J_{Ha,Hb}$ Vicinal (~ 1 à 12 Hz)

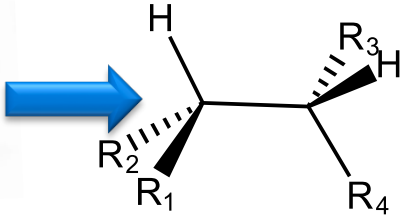


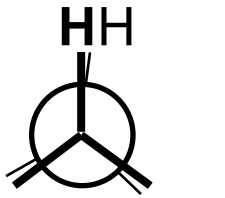
$^{4}J_{Ha,Hb}$ Long distance (< 2Hz)

4 Coupling constant (Hz) Values for scalar homonuclear H-H

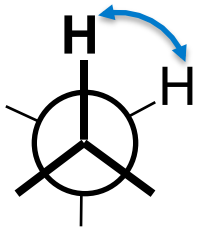
$^3J_{H,H}$ can be predicted from the dihedral angle between two protons



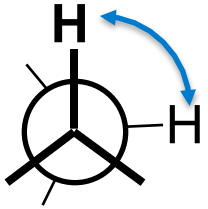




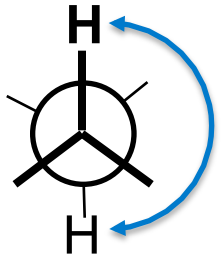
Φ=0
^3J=8-12 Hz



Φ=60
^3J=2-5 Hz



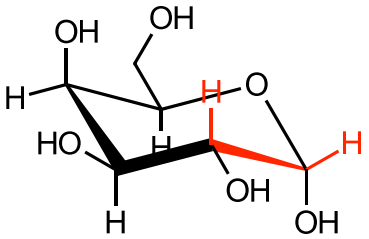
Φ=90
^3J=0-2 Hz

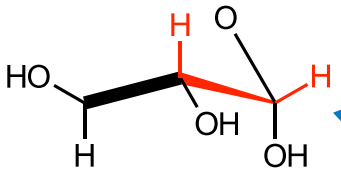


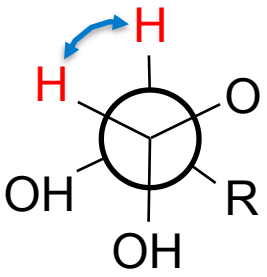
Φ=180
^3J=8-15 Hz

4 Coupling constant (Hz) Value estimation

α-D-galactose



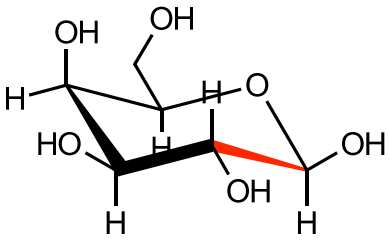


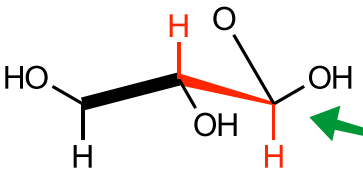


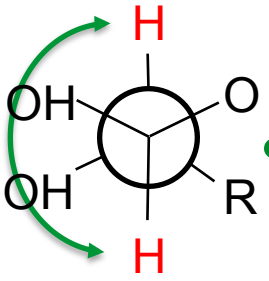
Φ ~ 60 °

$^{3}J_{H,H}$ < 5 Hz

β-D-galactose







Φ ~ 180 °

$^{3}J_{H,H}$ > 5 Hz

4 Coupling constant (Hz) Shape

The shape depends on the number of neighbouring atoms

Pascal Triangle		Number of signals	
1	0 neighbor	singlet	1
1 1	1 neighbor	doublet	2
1 2 1	2 neighbors	quadruplet	4
1 3 3 1	3 neighbors	octuplet	8
1 4 6 4 1	4 neighbors	hexadecatuplet	16

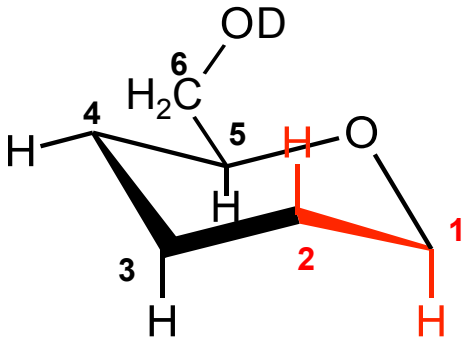
4 Coupling constant (Hz)

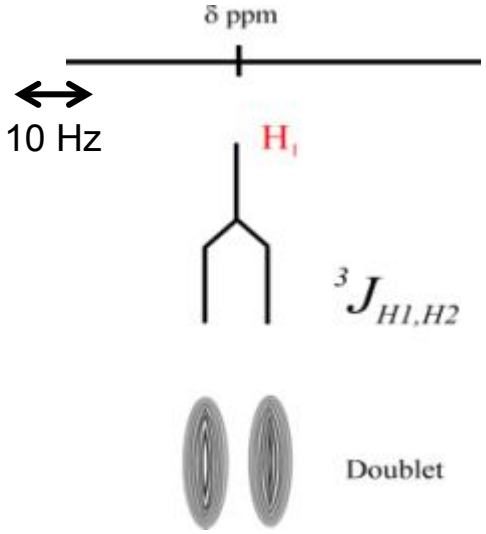
Determination of the spin system of β-D-Galactose in two dimensions

H1-H2

Φ = 180°

$^3J_{H1,H2}$ ~ 10Hz





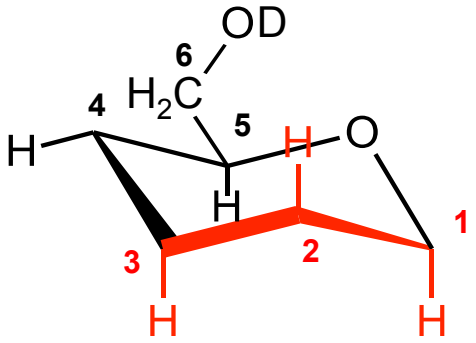
H2-H1 = H1-H2

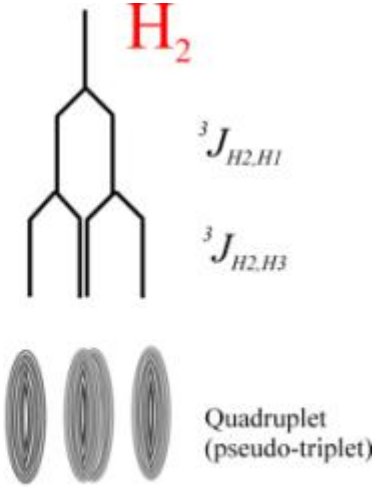
$^3J_{H2,H1}$ ~ 10Hz

H2-H3

Φ = 180°

$^3J_{H2,H3}$ ~ 10Hz





$^3J_{H2,H1}$ and $^3J_{H2,H3}$ almost similar

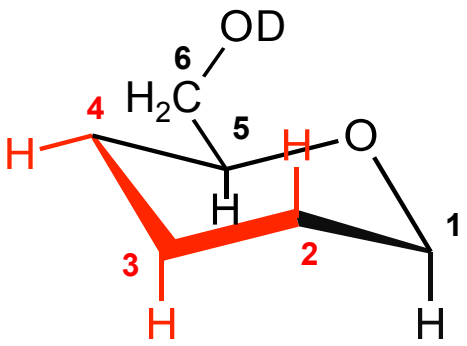
Shape on 1D spectrum

4 Coupling constant (Hz)

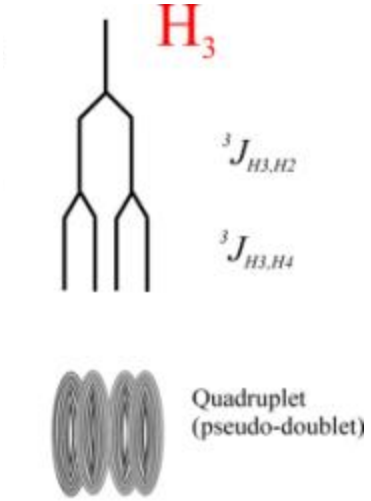
H3-H4

Φ = 60°

$^3J_{H3,H4}$ ~ 3Hz



10 Hz

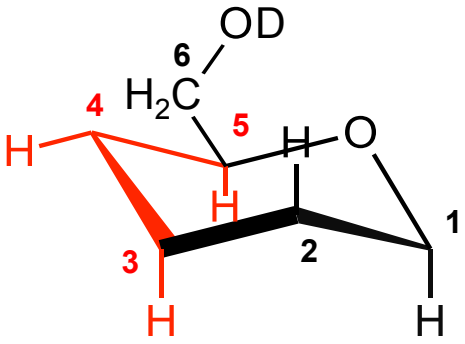


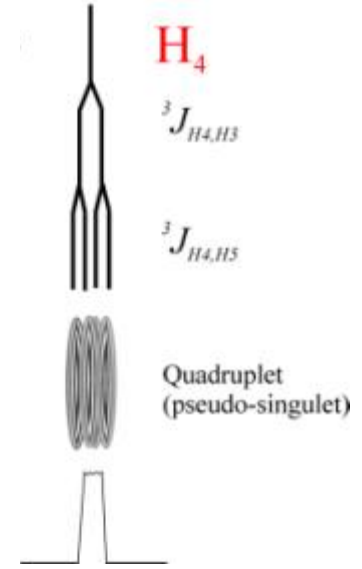
Shape on 1D spectrum

H4-H5

Φ = 60°

$^3J_{H4,H5}$ ~ 3Hz

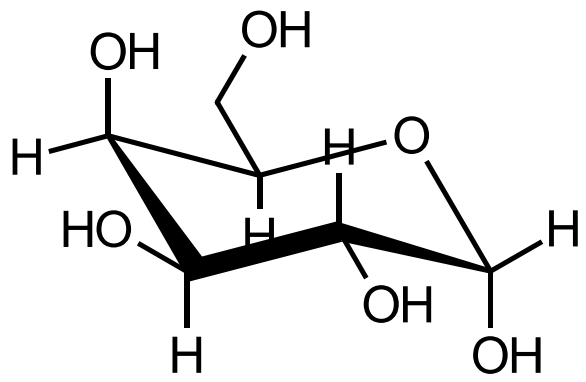




Shape on 1D spectrum

4 Coupling constant (Hz)

Summary for β-D-Gal configuration



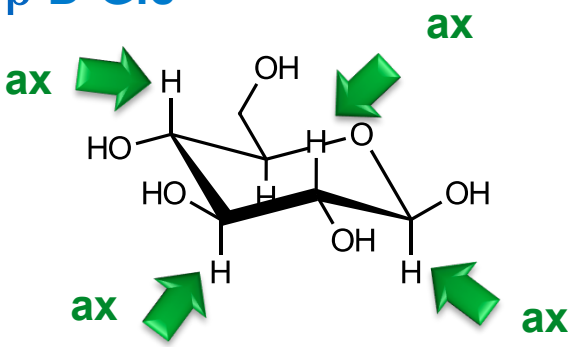
$^{3}J_{H1,H2}$ ~ 10Hz = Large
$^{3}J_{H2,H3}$ ~ 10Hz = Large
$^{3}J_{H3,H4}$ < 5Hz = Small
$^{3}J_{H4,H5}$ < 5Hz = Small

L, L, S, S

No need of $^{3}J_{H5,H6}$ and $^{3}J_{H5,H6'}$ to establish the configuration

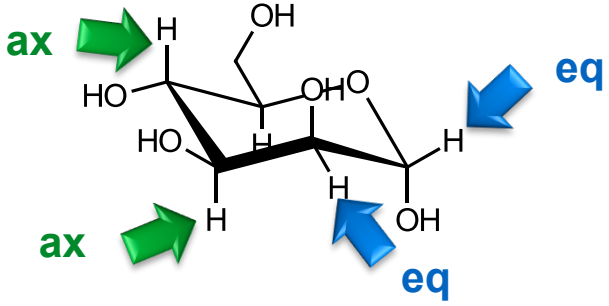
4 Coupling constant (Hz)

β-D-Glc



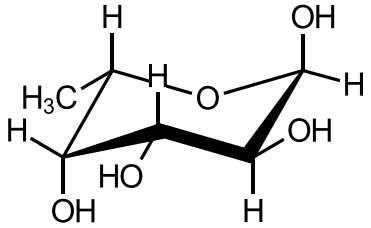
L, L, L, L

α-D-Man



S, S, L, L

α-L-Fuc



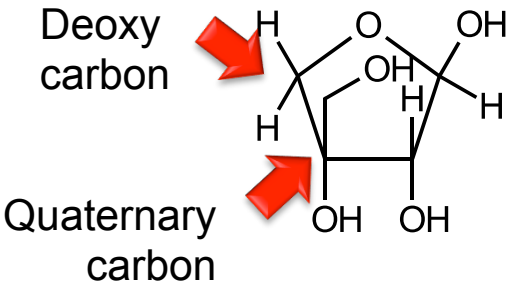
S, L, S, S

4 Coupling constant (Hz)

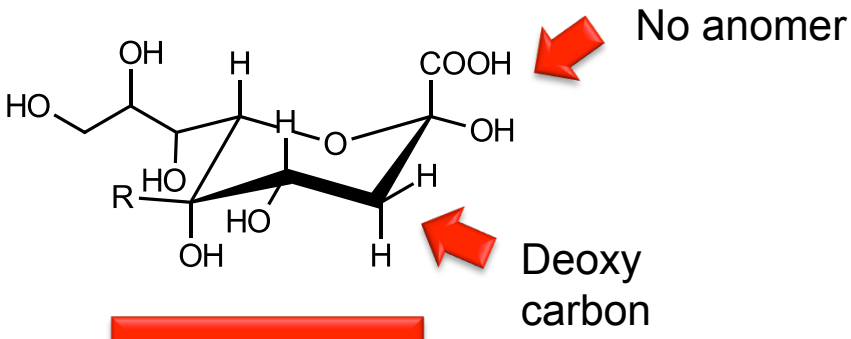
Koerner table

vicinal coupling constants				Stereochemistry of aldopyranosic residues		
$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	**Configuration**	**Conformation**	**Exemples**
L	**L**	**L**	**L**	**β-*gluco***	$^4C_1$	**β-D-Glc, β-D-GlcNAc, β-D-Qui**
L	L	L	S	α-*ido*	$^1C_4$	(α-D-Idose)
L	L	S	L	impossible		
L	**L**	**S**	**S**	**β-*galacto***	$^4C_1$	**β-D-Gal, β-D-GalNAc, β-L-Fuc, β-D-Fuc**
				α-*altro*	$^1C_4$	(α-D-Altrose)
L	S	L	L	impossible		
L	S	L	S	impossible		
L	S	S	L	β-*allo*	$^4C_1$	(β-D-Allose)
L	S	S	S	β-*gulo*	$^4C_1$	(β-D-Gulose)
S	**L**	**L**	**L**	**α-*gluco***	$^4C_1$	**α-D-Glc, α-D-GlcNAc, α-D-Qui**
S	L	L	S	β-*ido*	$^1C_4$	(β-D-Idose)
S	L	S	L	impossible		
S	**L**	**S**	**S**	**α-*galacto***	$^4C_1$	**α-D-Gal, α-D-GalNAc, α-L-Fuc, α-D-Fuc**
S	S	L	L	α-*manno*	$^4C_1$	α-D-Man, α-D-ManNAc, α-L-Rha
				β-*manno*	$^4C_1$	β-D-Man, β-D-ManNAc
S	S	L	S	α-*gulo*	$^1C_4$	(α-D-Gulose)
S	S	S	L	α et β-*altro*, α-*allo*	$^4C_1$	(α or β-D-Altrose, α-D-Allose)
S	S	S	S	α et β-*ido*, α-*gulo*	$^4C_1$	(α et β-D-Idose)
				α-*gulo*	$^4C_1$	(α-D-Gulose)
				α et β-*talo*	$^4C_1$	(α et β-D-Talose)

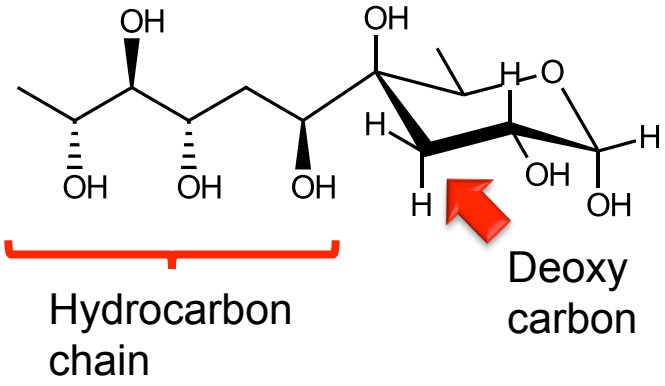
4 Coupling constant (Hz) Pitfalls



Apiose



Sialic acids



Caryophilose

5 nOe effects

Dipolar coupling when distance<5Å

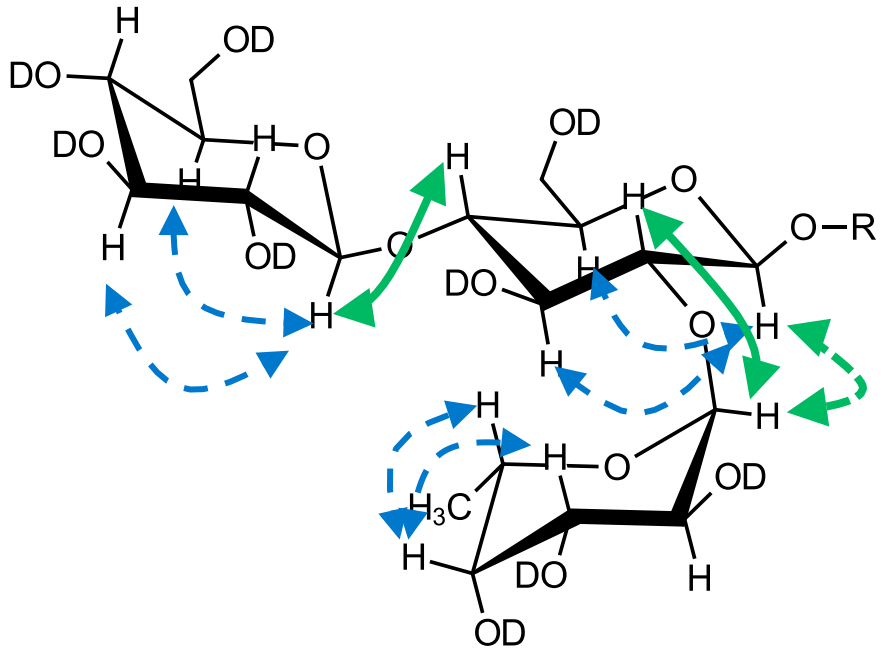
Inter-residues, linkage

Inter-residues, not linkage

Provide linkage/sequence informations

Intra-residues

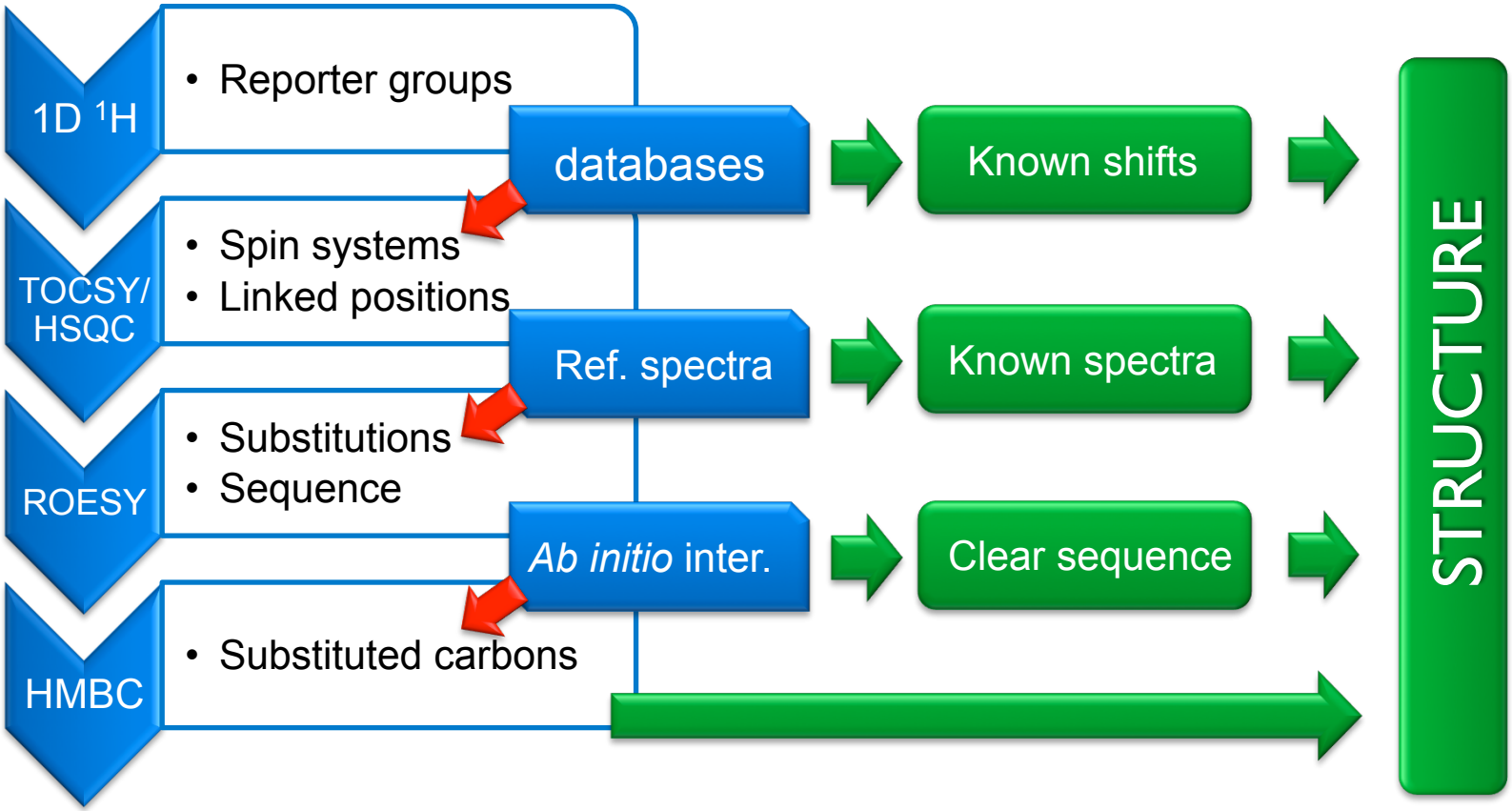
Help to determine the spin system



experiments : ROESY (MW<2500 Da), NOESY (MW>2500 Da)

Classical NMR experiments

Strategy


1D ^{1}H
• Reporter groups
databases
Known shifts
TOCSY/ HSQC
• Spin systems
• Linked positions
Ref. spectra
Known spectra
ROESY
• Substitutions
• Sequence
Ab initio inter.
Clear sequence
HMBC
• Substituted carbons
STRUCTURE

Useful databases

- **Sweet-DB**

http://www.glycosciences.de/sweetdb/

- **BCSDB (Polysaccharides)**

http://csdb.glycoscience.ru

- **BMRDB (Biological Magnetic Resonance Data Bank)**

http://www.bmrb.wisc.edu/metabolomics/query_metab.php

- **Glycobase**

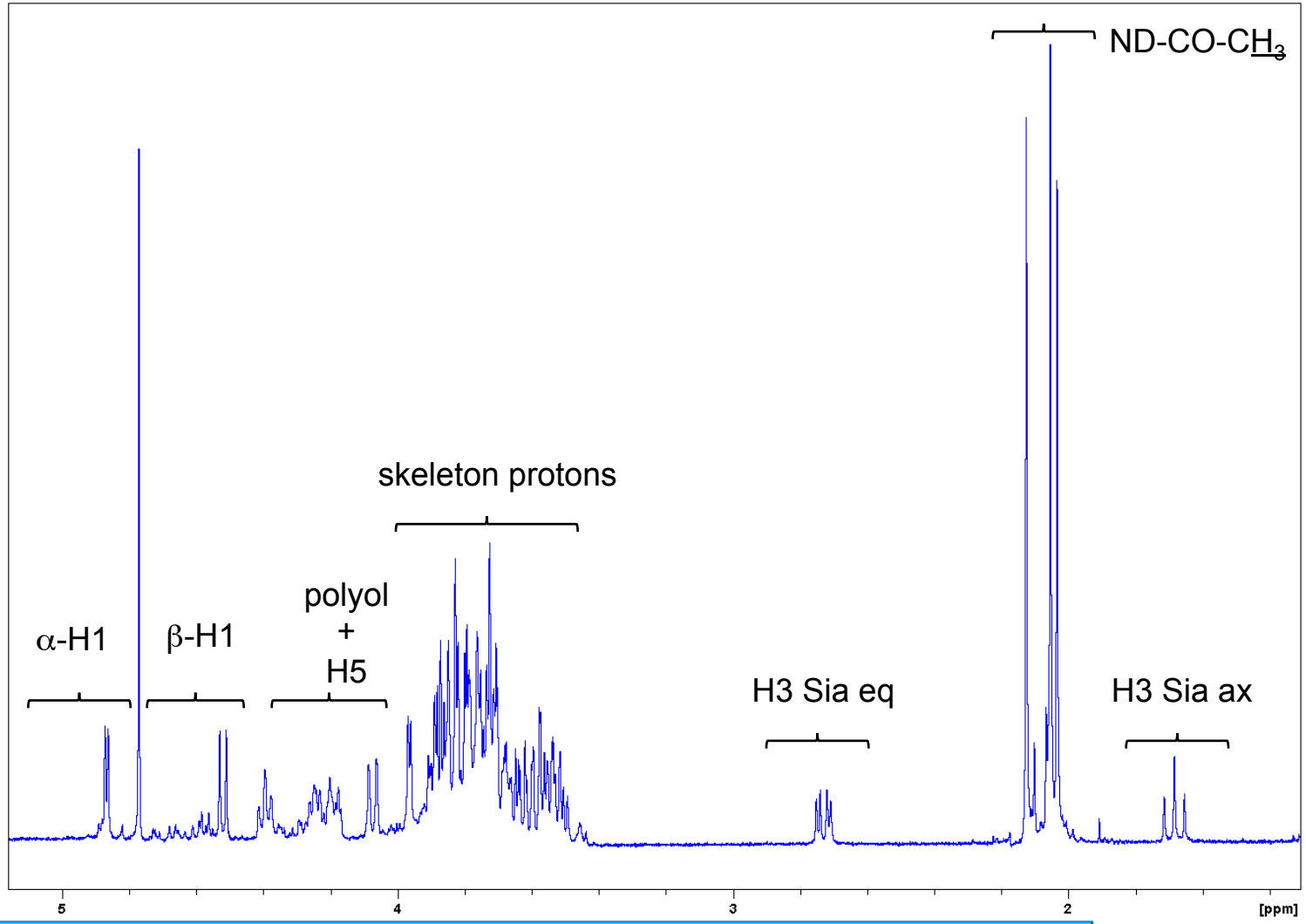
http://glycobase.univ-lille1.fr/base/

Proton 1D
Easy answers

Spectra usually too complex to be interpreted completely by a first order approach

- How many **monosaccharides**? → Anomeric signals
- How many **oligosaccharides**? → Relative intensities of anomeric signals
- Is there **sialic** acid? → H-3 ax and H-3 eq
- How many **aminated** sugar? → *N*-Acetamido group
- How many **deoxysugars**? → -CH_3 groups
- How **pure** is the sugar fraction? → Non sugar signals

Proton 1D



Number of sugar residues + nature of some monosaccharides

Proton 1D
Concept of structural reporter groups

1. A sugar is defined by the protons resonating at clearly distinguishable positions:
   - Anomeric protons
   - Mannose H-2 and H-3
   - Sialic acid H-3
   - Deoxyhexose H-5 and CH_3
   - Galactose H-3 and H-4
   - Amino sugars

2. Each signal is influenced by its environment in a specific manner that can be defined

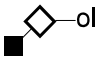
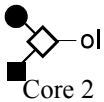
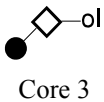
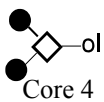
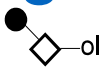
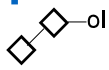
3. Influences on reporter were established owing to the availibility of sugars of increasing complexities

It is possible to predict the structure of a sugar from a **limited set** of signals

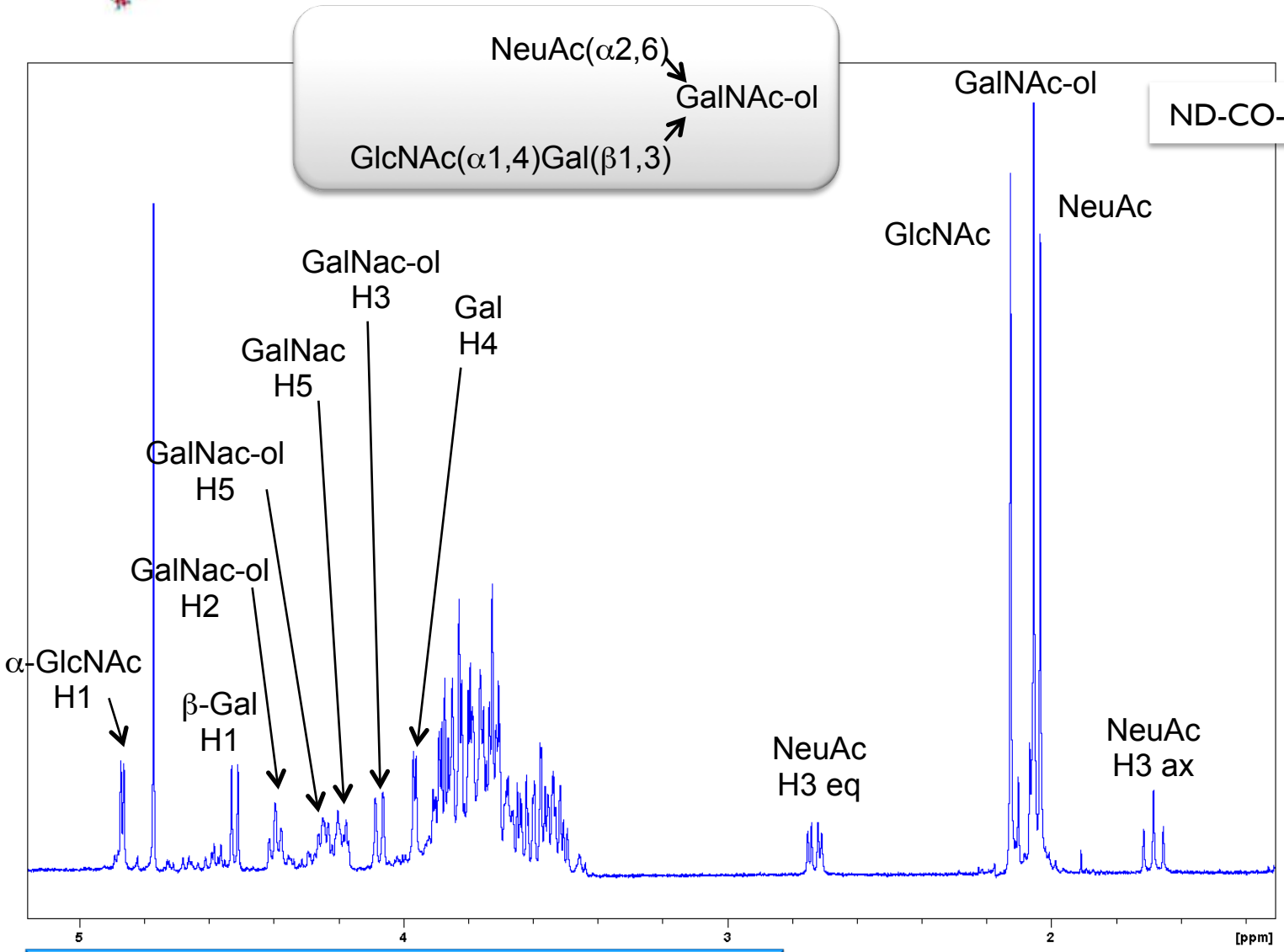
- Vliegenthart JFG *et al*. (1983) High-resolution, ^{1}H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins
- Kamerling et al. (1992) High-Resolution ^{1}H-Nuclear Magnetic Resonance Spectroscopy of Oligosaccharide-Alditols Released from Mucin-Type O-Glycoproteins

Proton 1D

Concept of structural reporter groups

			 Core 1	 Core 2		 Core 3	 Core 4		 Core 5	 Core 6
GalNAc-ol	*H-2*	4.252	4.395	4.395		4.287	4.280		4.395	4.242
	H-3	3.850	4.065	4.061		3.996	3.984		3.888	3.841
	H-4	3.390	3.507	3.468		3.546	3.519		3.680	3.379
	H-5	3.928	4.196	4.281		4.141	4.230		3.749	4.021
	H-6	3.668	3.69	3.931		3.65	3.905		3.647	3.933
	H-6'	3.647	3.628	n.d.		n.d.	n.d.		3.647	n.d.
	NAc	2.055	2.050	2.066		2.037	2.044		2.049	2.046
Other residues			**βGal**	**βGal**	**βGlcNAc (6)**	**βGlcNAc**	**βGlcNAc (3)**	**βGlcNAc (6)**	**βGalNAc**	**βGlcNAc**
	H-1	-	4.478	4.468	4.538	4.604	4.600	4.543	5.103	4.553
	H-2	-	3.564	3.542	n.d.	n.d.	n.d.	n.d.	4.235	n.d.
	H-3	-	3.671	n.d.	n.d.	3.584	n.d.	n.d.	3.921	n.d.
	H-4	-	3.901	3.901	n.d.	n.d.	n.d.	n.d.	4.043	n.d.
	H-5	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.073	n.d.
	H-6	-	n.d.	n.d.	3.932	3.950	3.949	3.931	n.d.	3.928
	H-6'	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	NAc	-	-	-	2.066	2.085	2.081	2.063	2.060	2.059

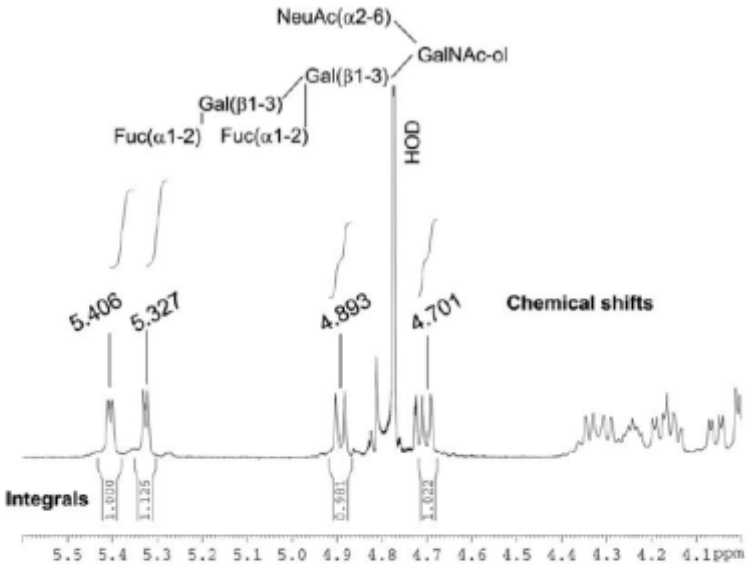
Proton 1D

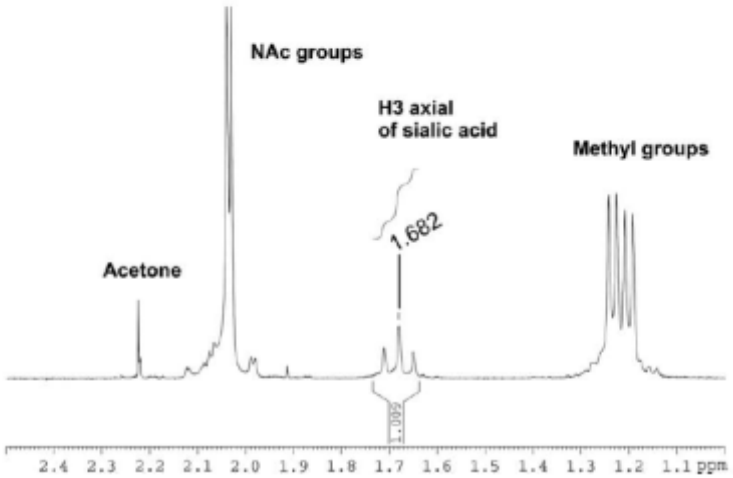


3 monosaccharides, 1 sialic acid, 2 HexNAc

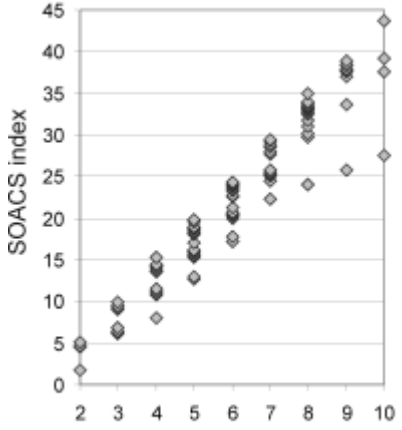
Proton 1D
SOACS index

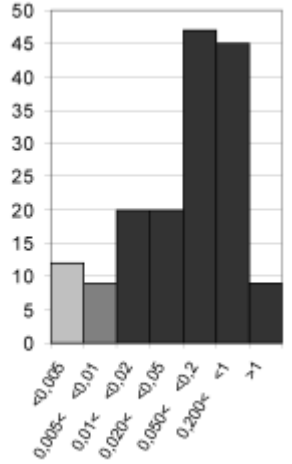
Sum of Anomeric Chemical Shifts





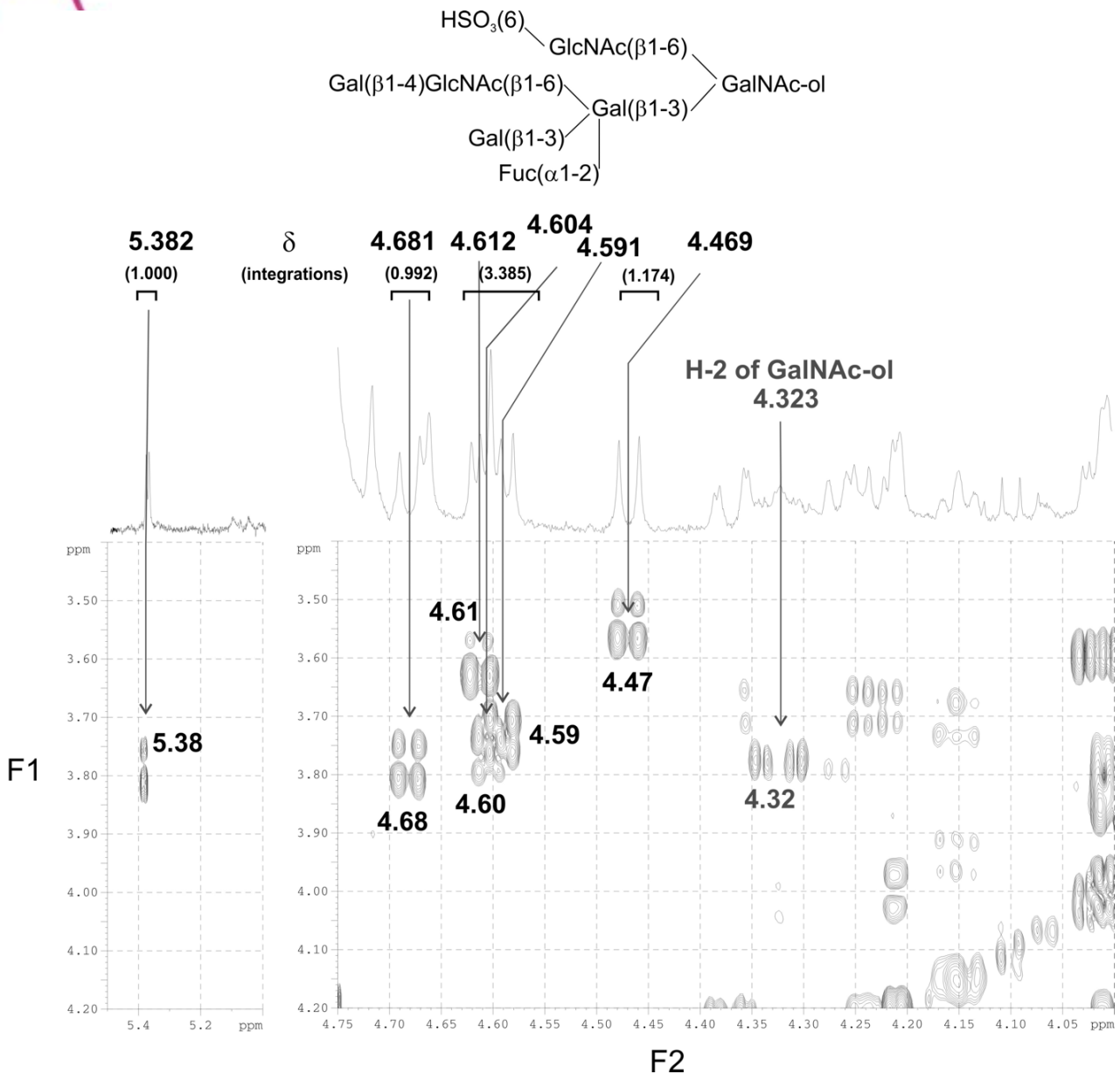
Reference number	Scheme	SOACS-ol Index	LINUCS ID	Reference number	Scheme	SOACS-ol Index	LINUCS ID
44		33,627	1667	65		41,915	12180
45		32,109	5009	66		19,592	639
48		23,272	8948	67		24,139	1674
49		23,018	4288	68		28,629	1675
50		28,308	8949	69		28,504	1673
53		33,102	8952	70		33,876	12558
54		32,777	8954	71		39,222	1678
55		38,142	8953	72		24,026	1676
56		38,135	12363	73		43,519	1679





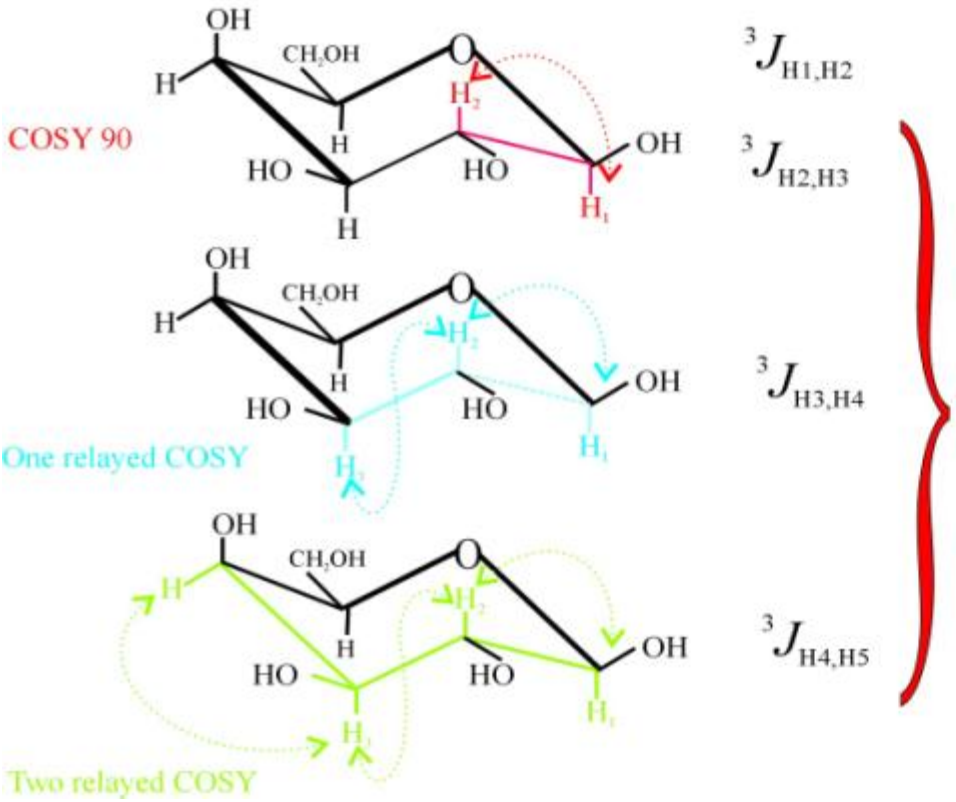
Maes *et al.*, (2009) Carbohydr Res. 344(3): 322-30.

Proton 2D
SOACS index

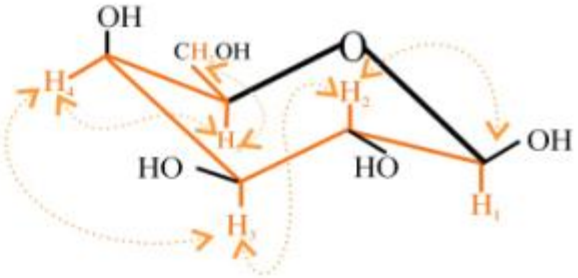


2D experiments

Relayed COSY



TOCSY
(with variable mixing time)



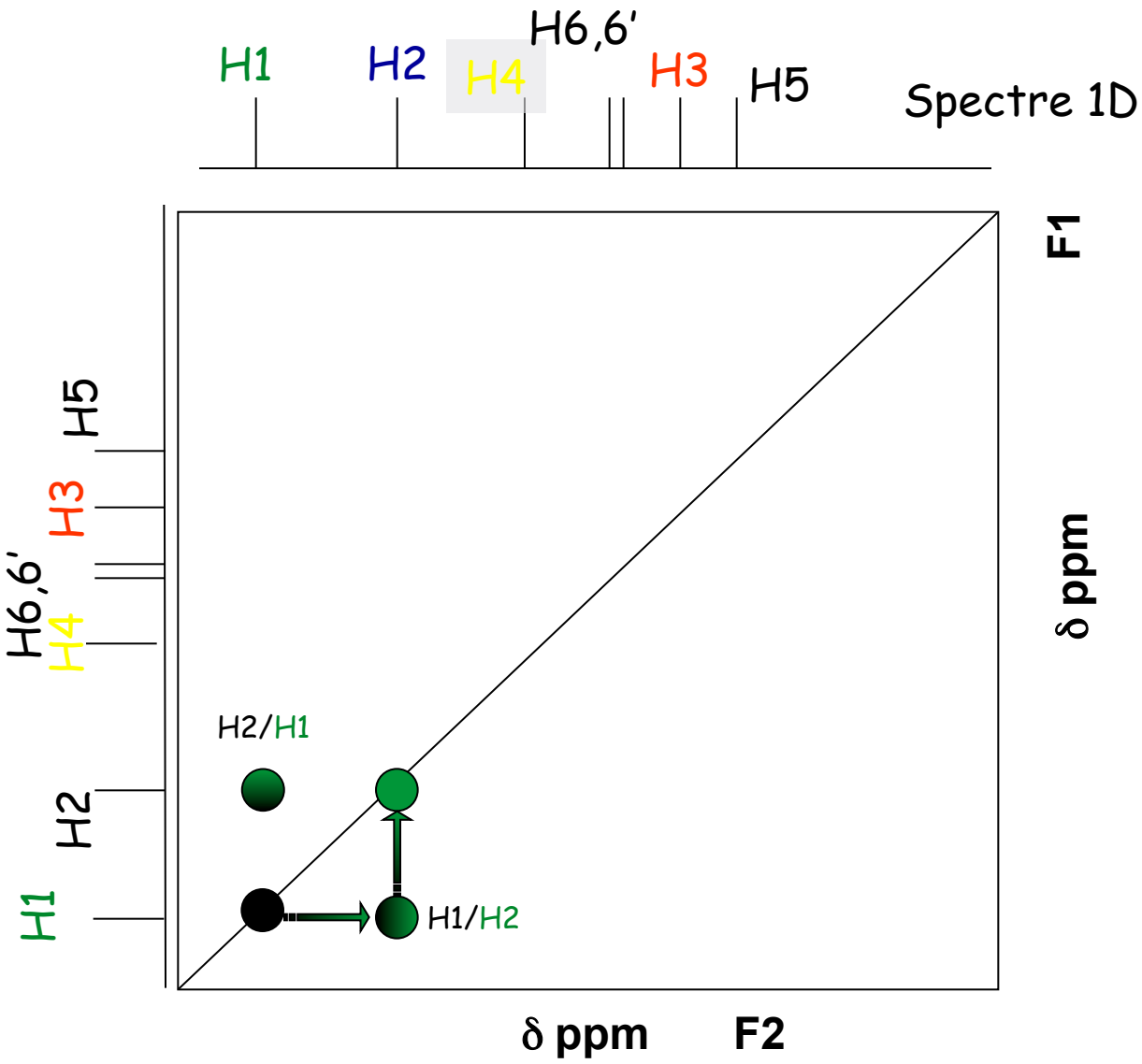
Total spin system

COSY : COrrelation SpectroscopY

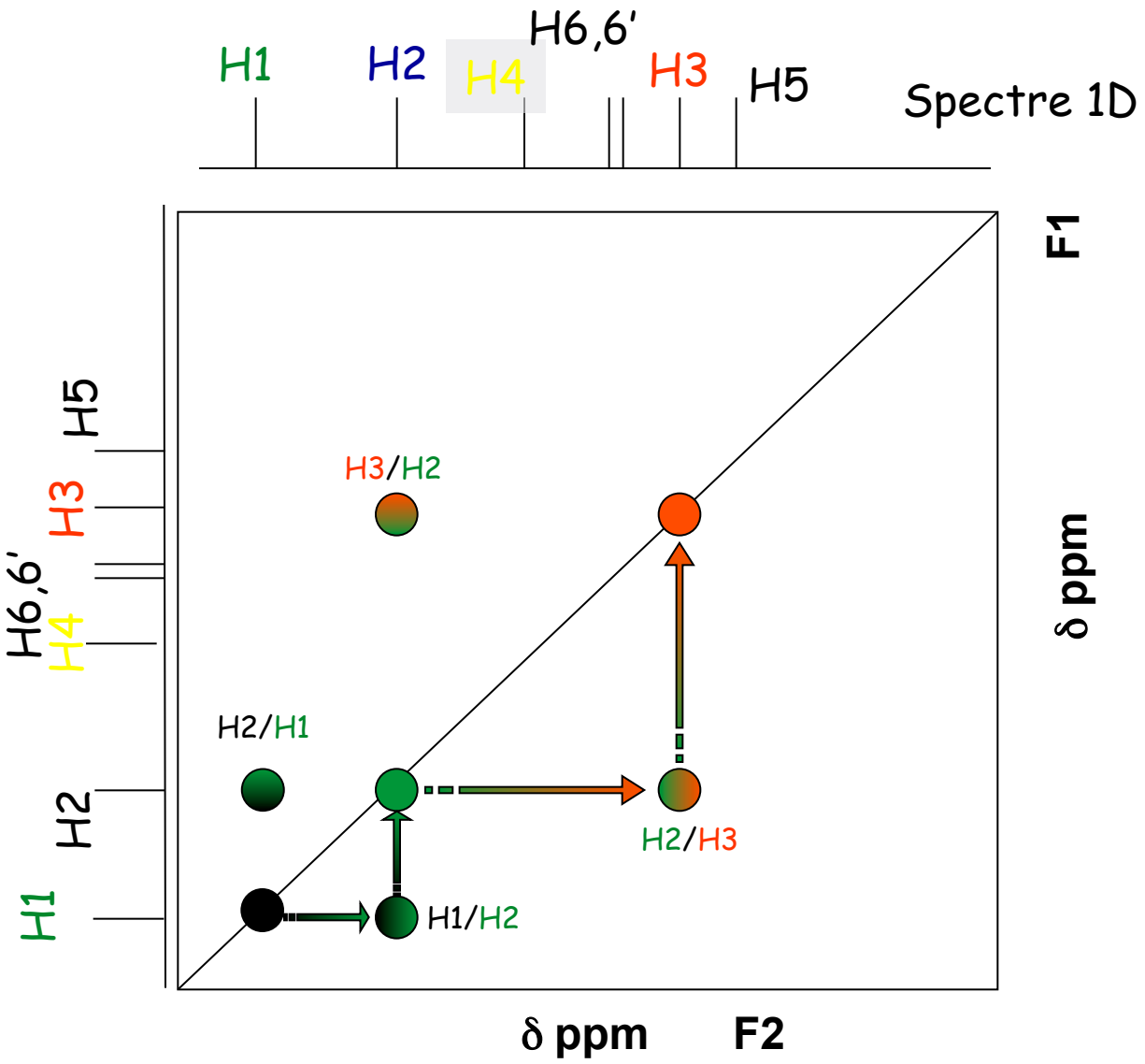
TOCSY : TOtal Correlation SpectroscopY
(temps de mélange variable)

- chemical shifts values
- coupling constant measurement
- sugar configuration

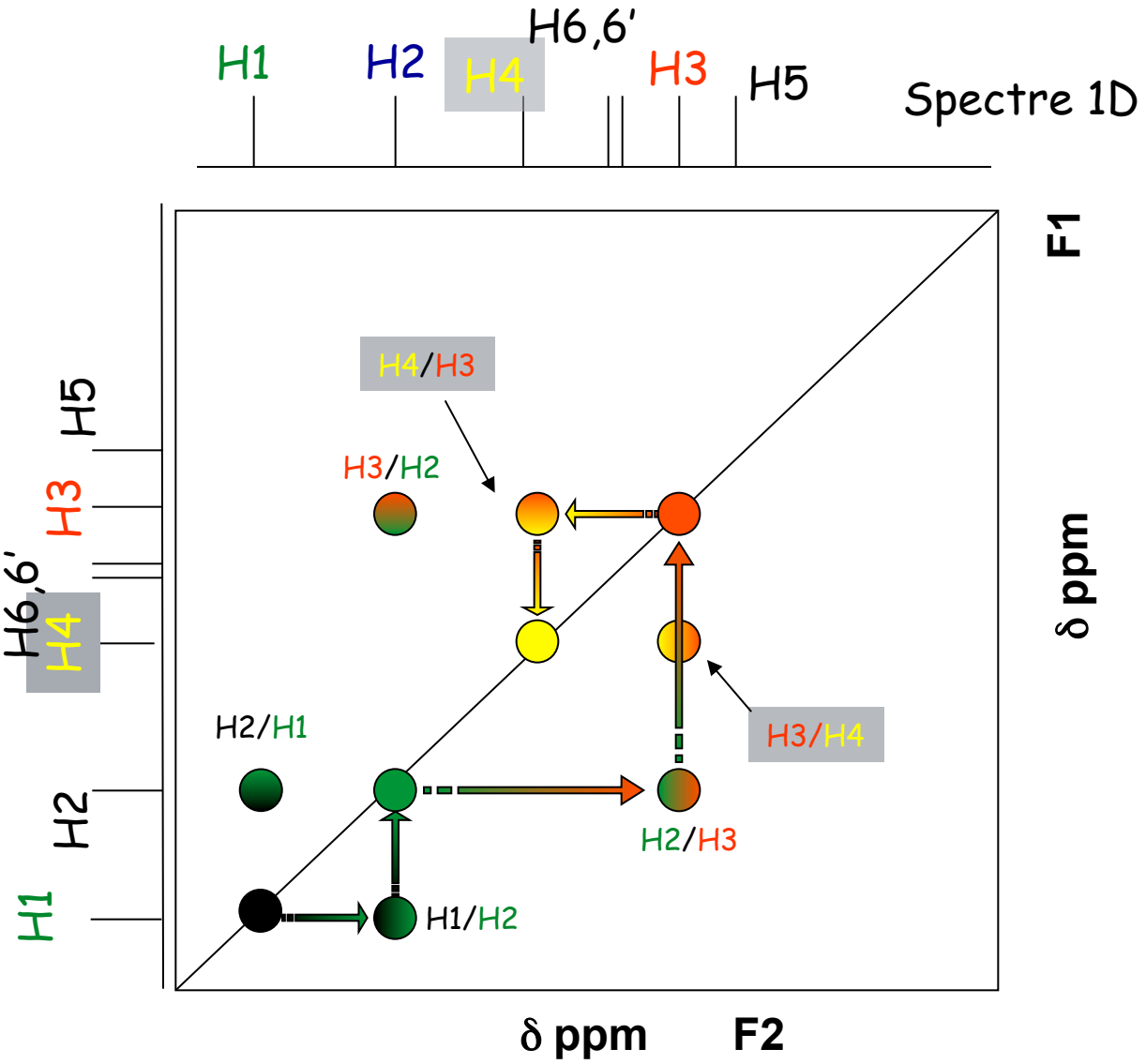
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H5
H3
H6,6'
H4
H2
H1
H2/H1
H1/H2
δ ppm
F2

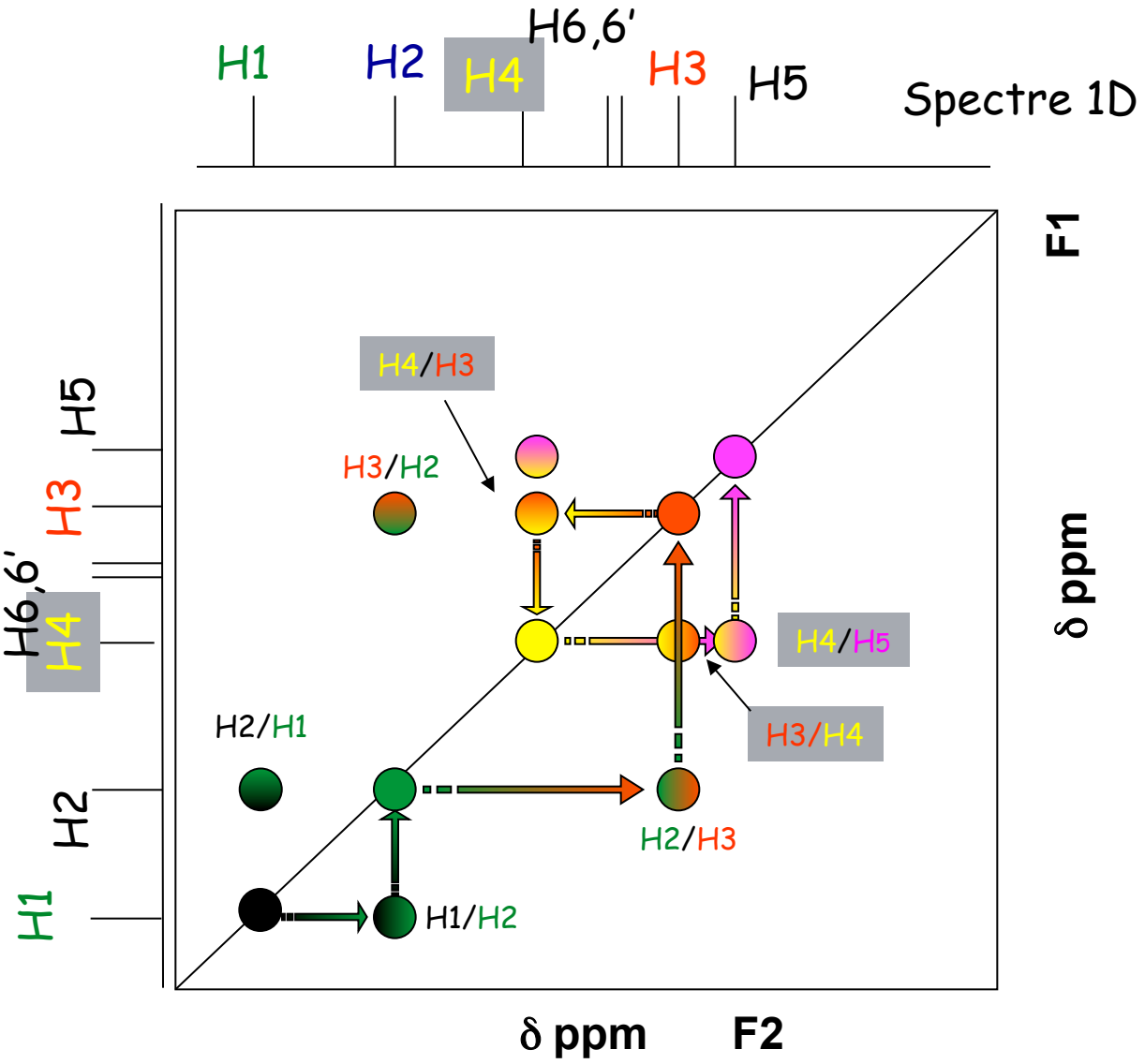
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H5
H3
H6,6'
H4
H2
H1
H3/H2
H2/H1
H2/H3
H1/H2
δ ppm
F2

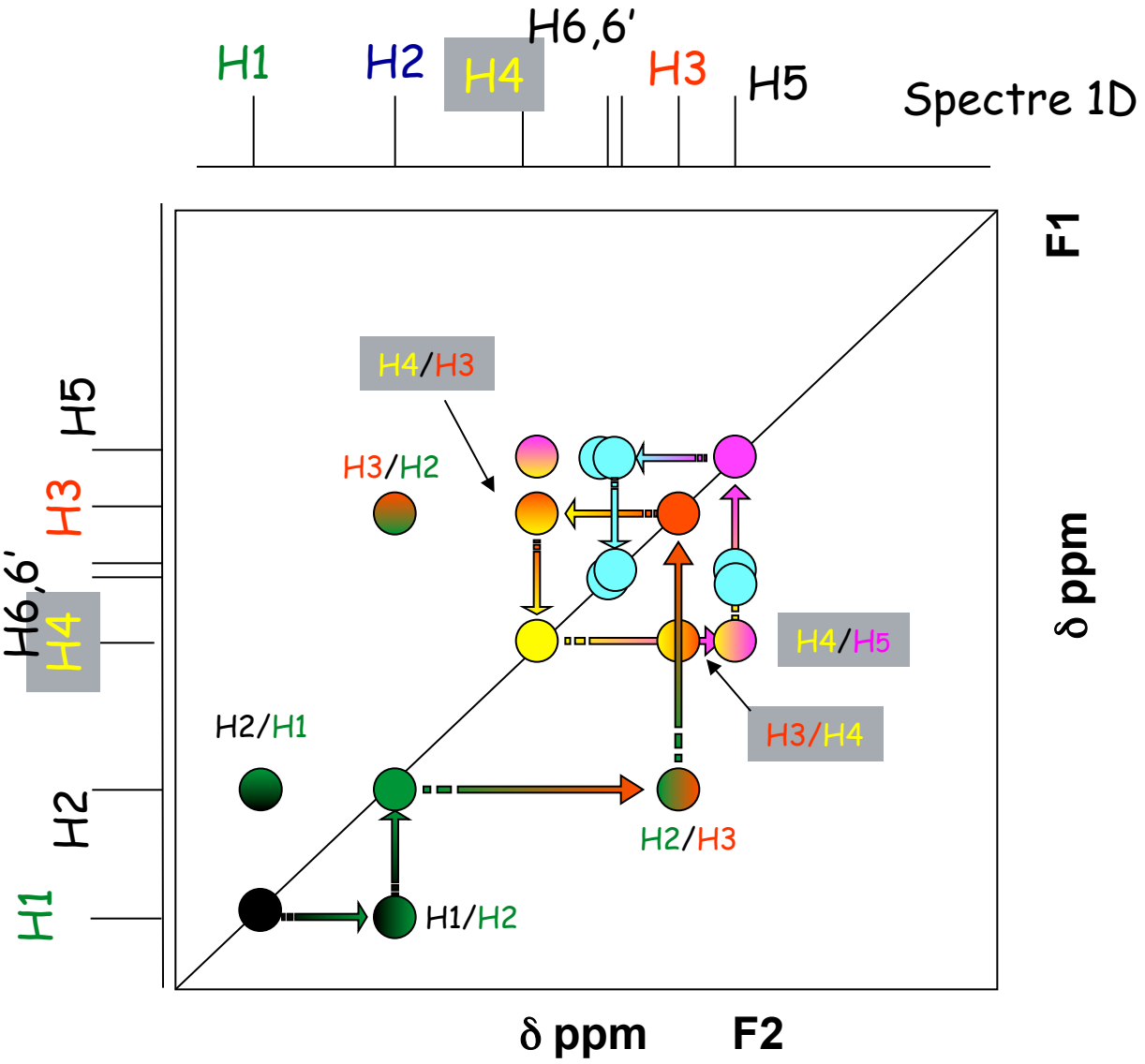
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H4/H3
H3/H2
H2/H1
H3/H4
H2/H3
H1/H2
δ ppm
F2

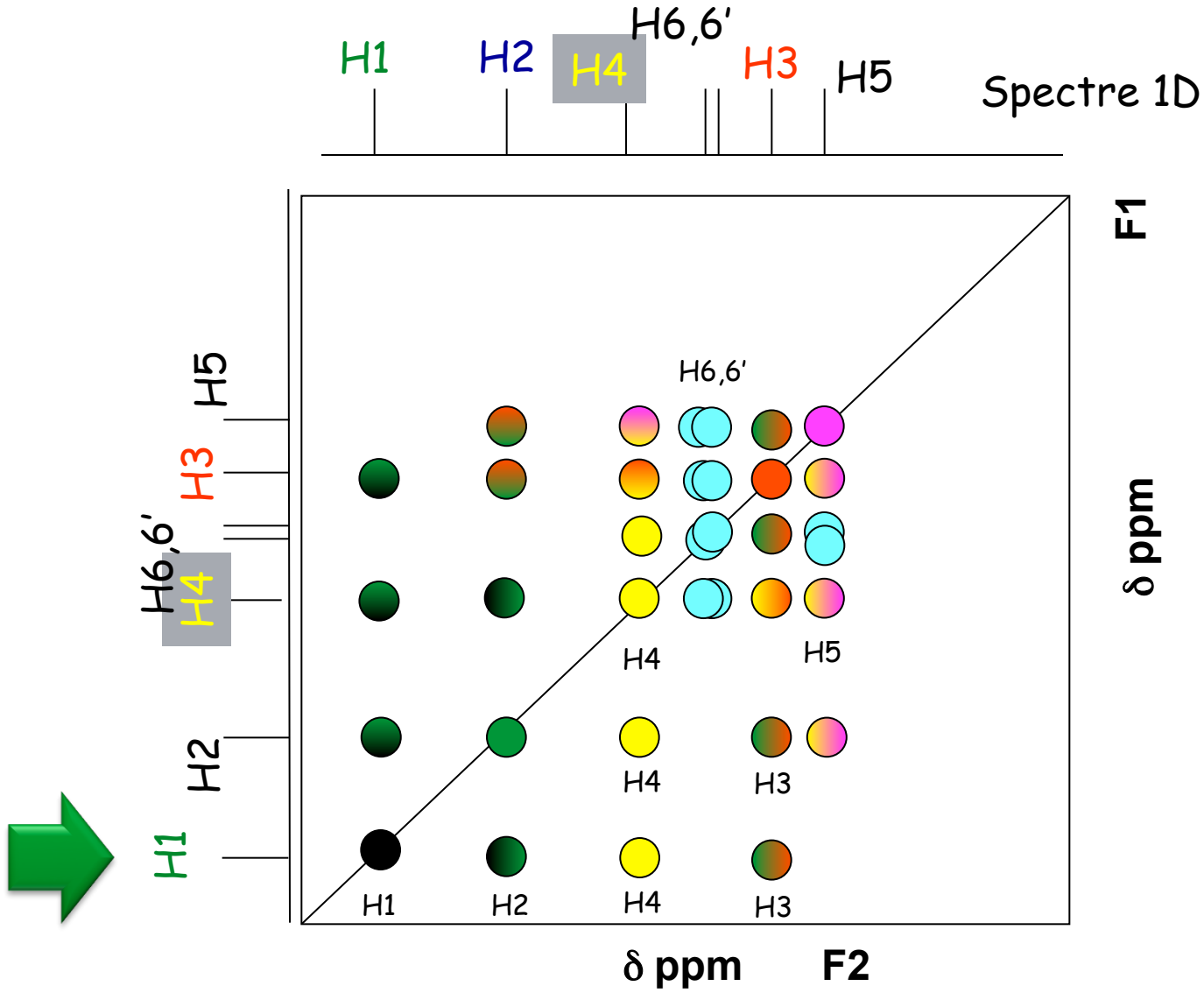
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H4/H3
H3/H2
H4/H5
H3/H4
H2/H1
H2/H3
H1/H2
H5
H3
H6,6'
H4
H2
H1
δ ppm
F2

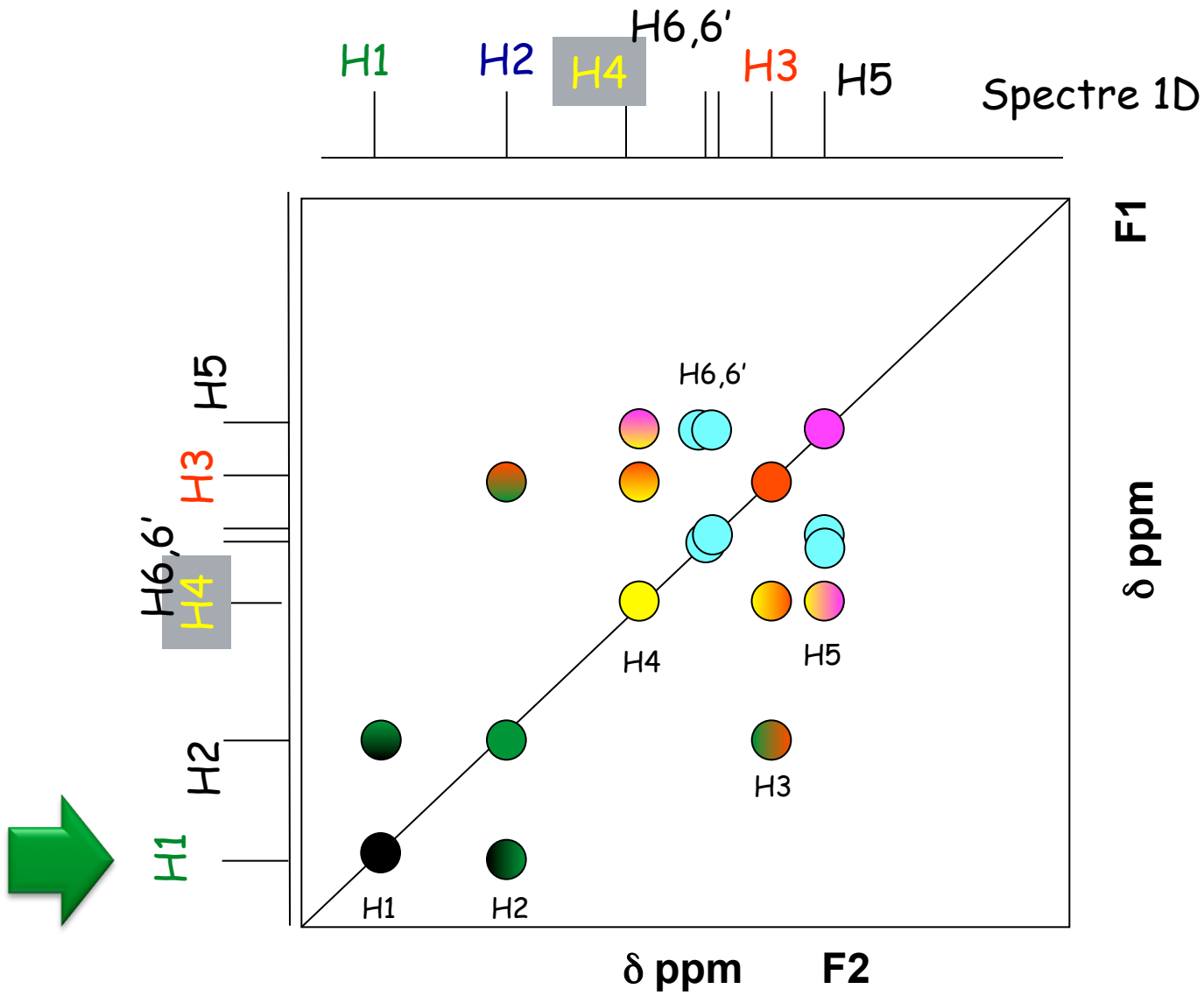
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H4/H3
H3/H2
H4/H5
H3/H4
H2/H1
H2/H3
H1/H2
δ ppm
F2

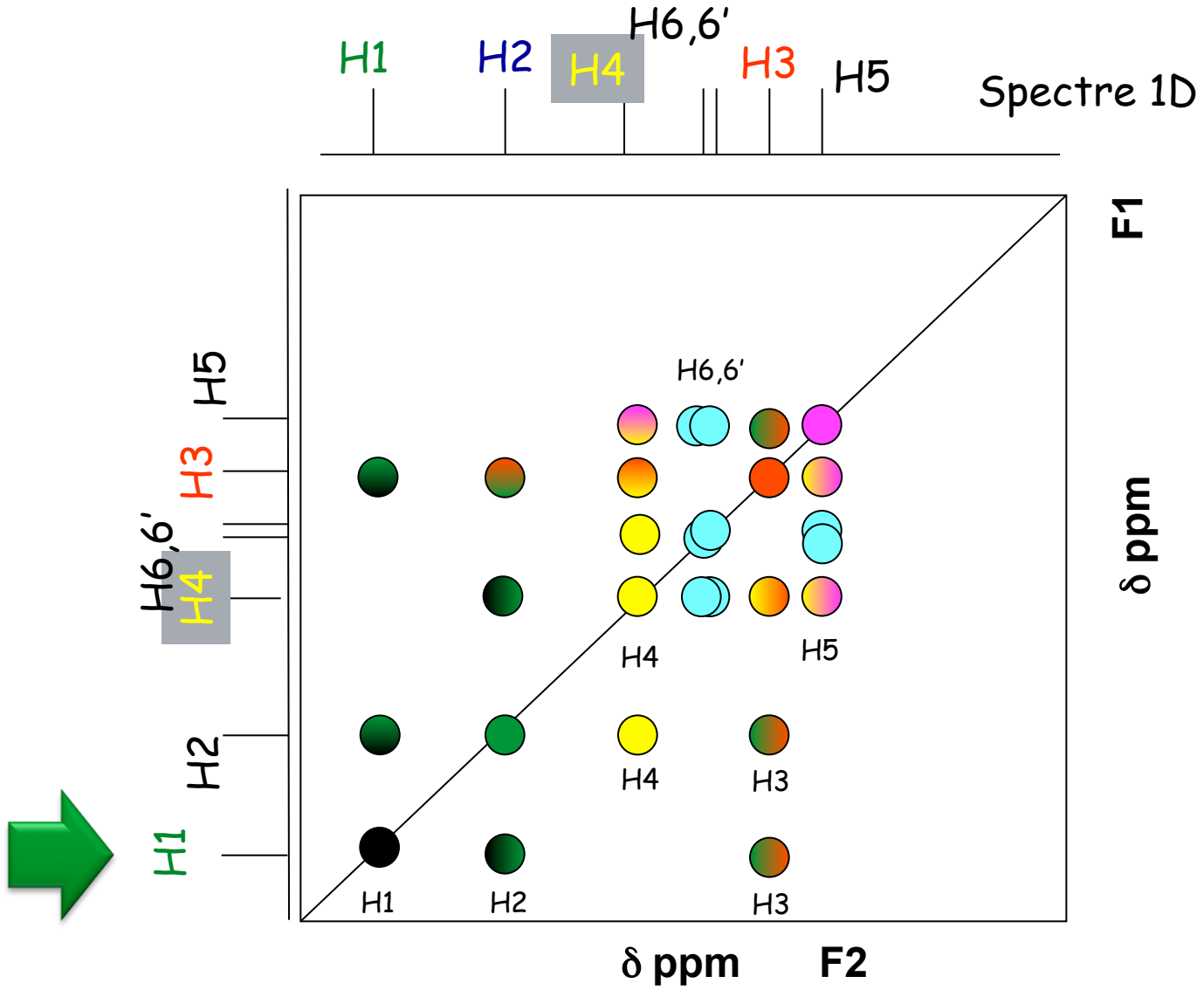
TOCSY


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H6,6'
H5
H3
H6,6'
H4
H4
H5
H4
H3
H2
H1
H1
H2
H4
H3
δ ppm
F2

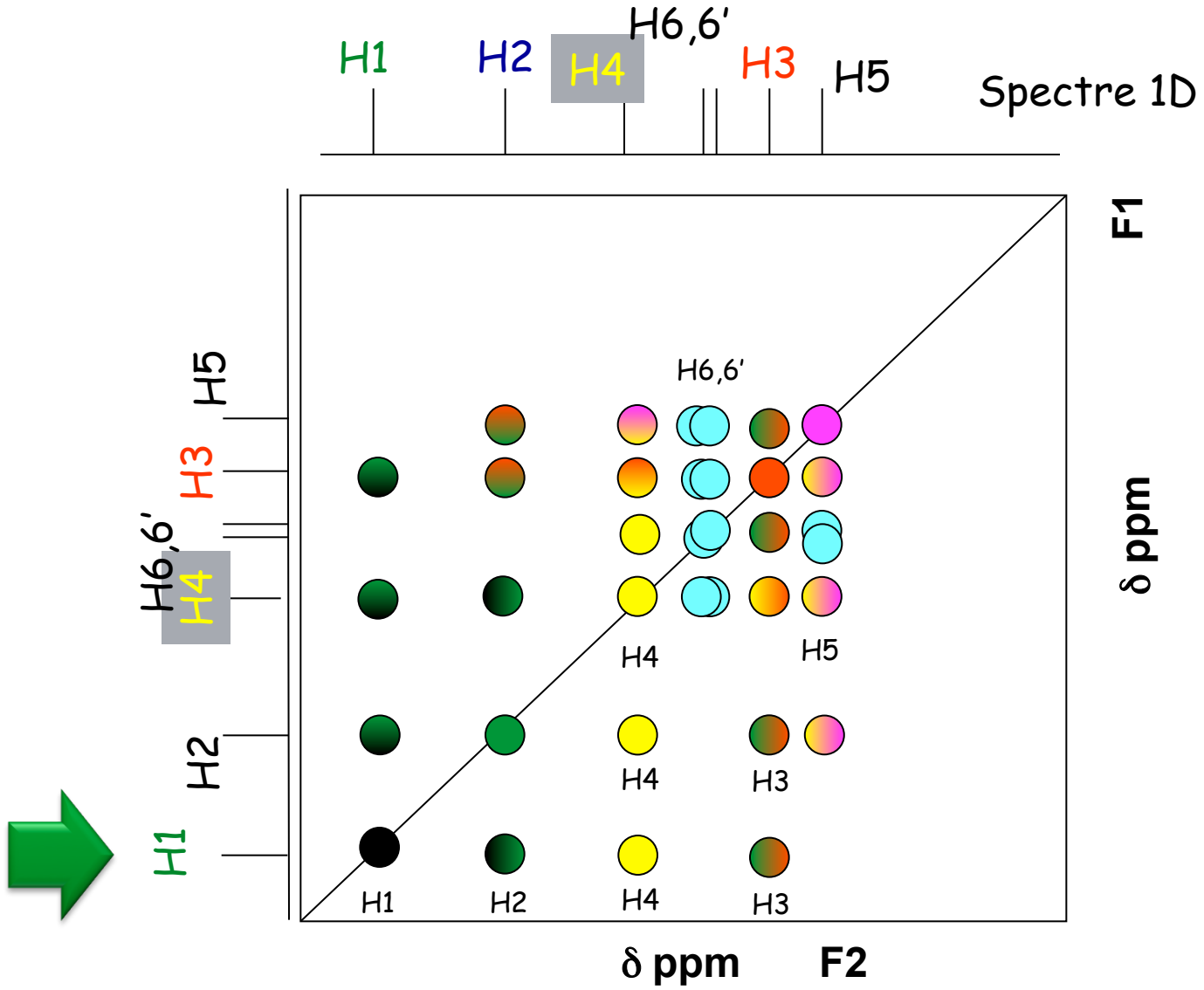
COSY90


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H6,6'
H5
H3
H6,6'
H4
H4
H5
H3
H2
H1
H1
H2
δ ppm
F2

COSYR1


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H6,6'
H5
H3
H6,6'
H4
H4
H5
H4
H3
H2
H1
H1
H2
H3
δ ppm
F2

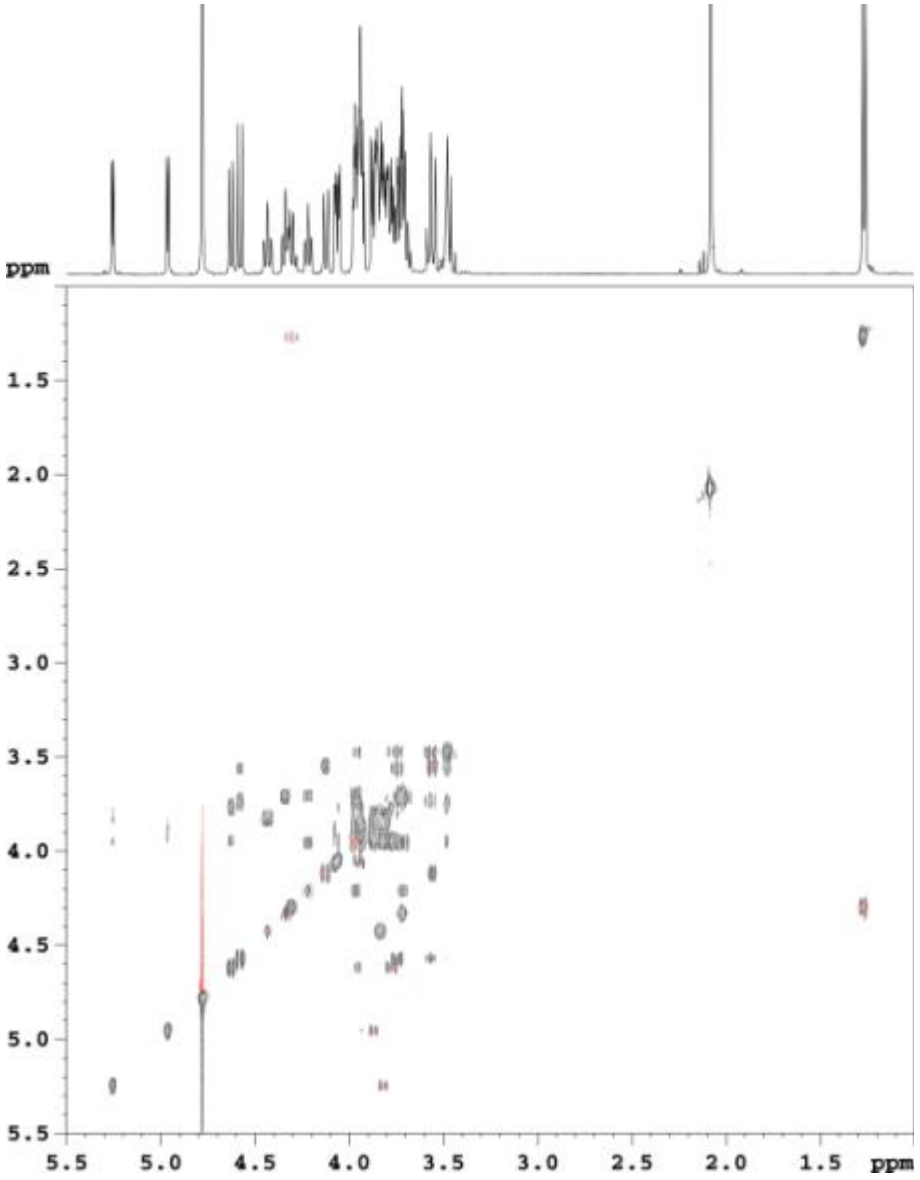
COSYR2


H1
H2
H4
H6,6'
H3
H5
Spectre 1D
F1
δ ppm
H6,6'
H5
H3
H6,6'
H4
H4
H5
H4
H3
H2
H1
H1
H2
H4
H3
δ ppm
F2

TOCSY

Mixing time

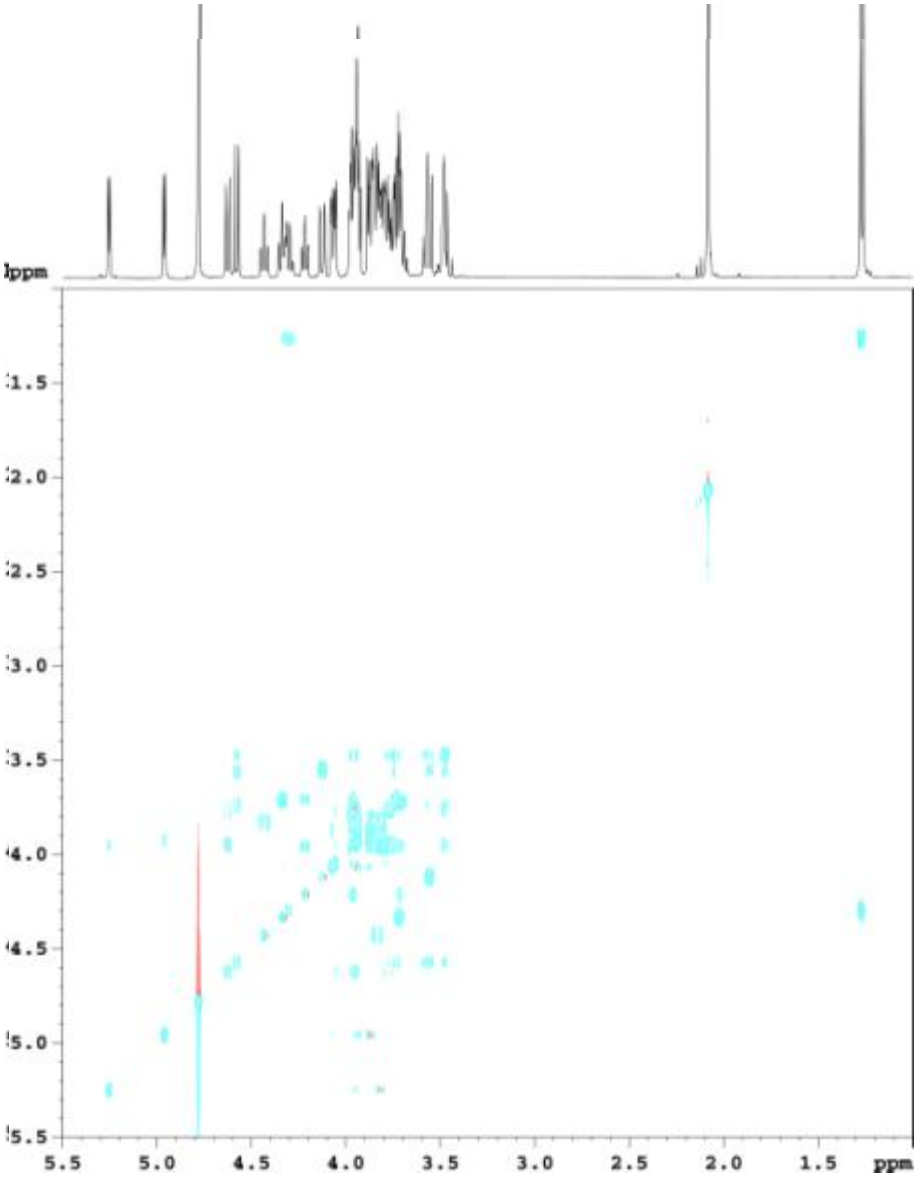
40 ms



TOCSY

Mixing time

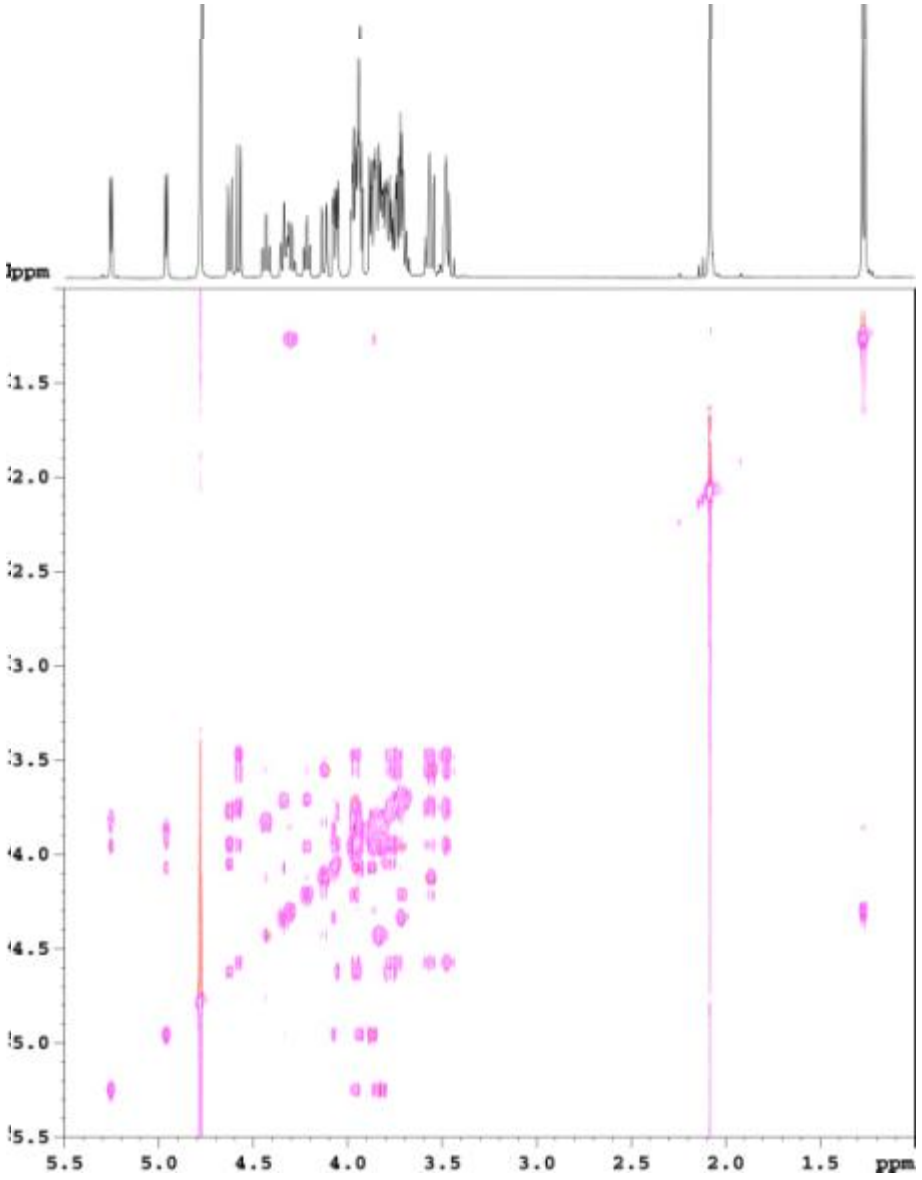
60 ms



TOCSY

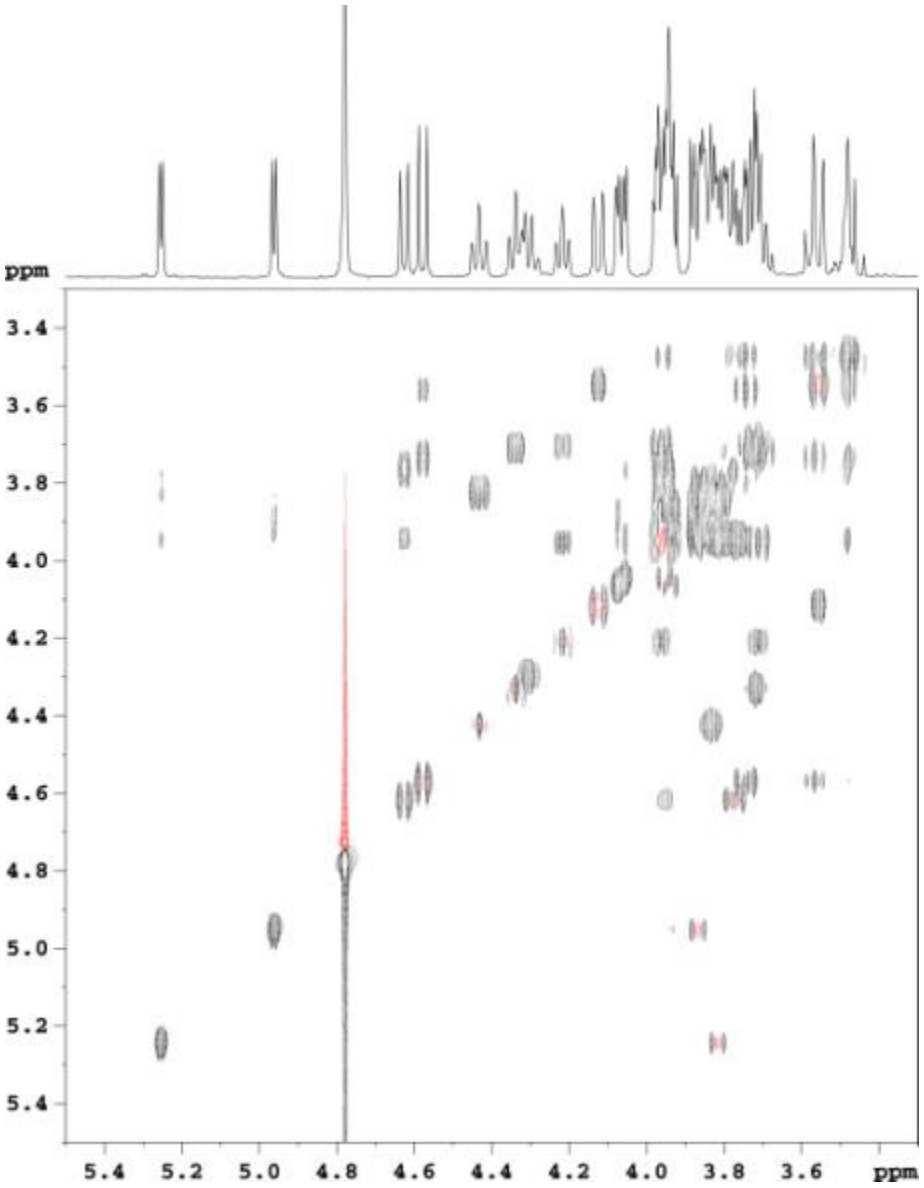
Mixing time

100 ms



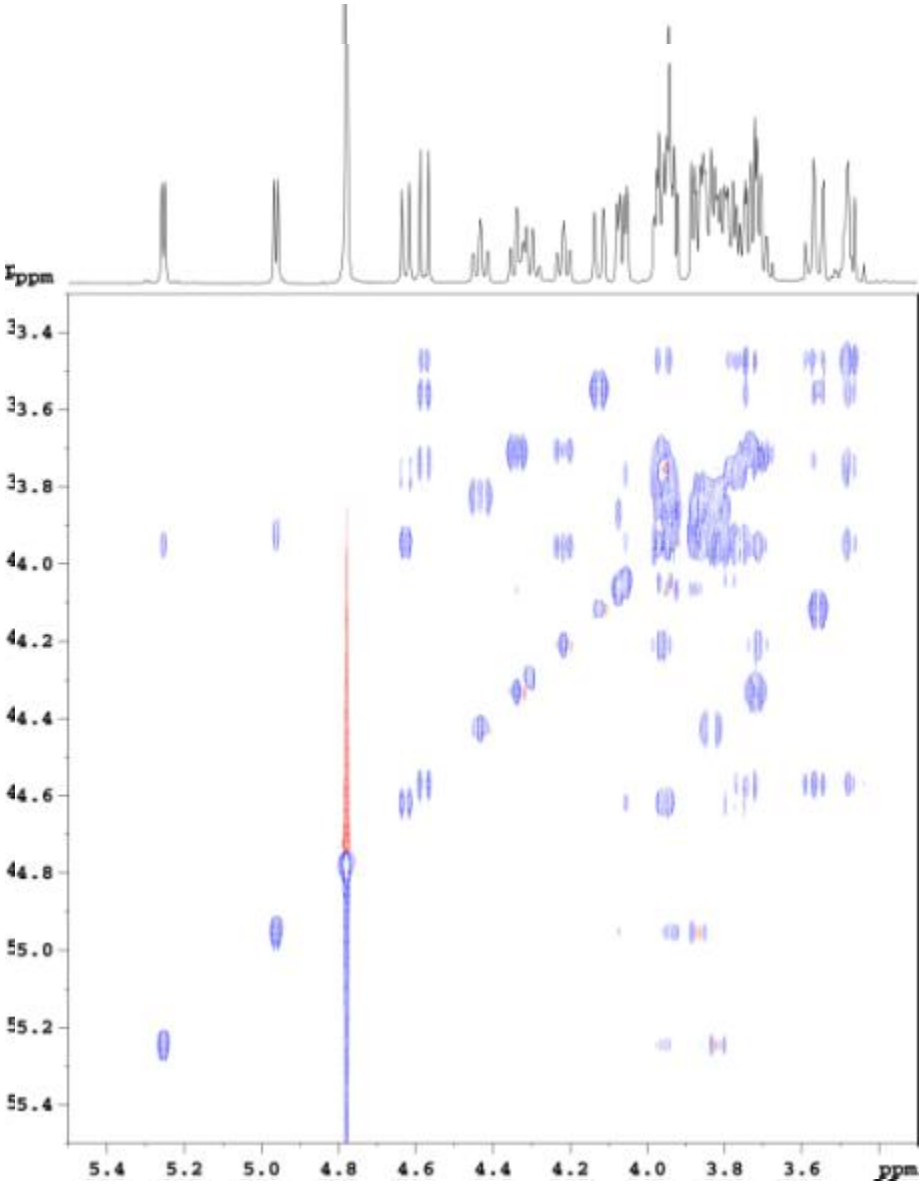
TOCSY

40 ms



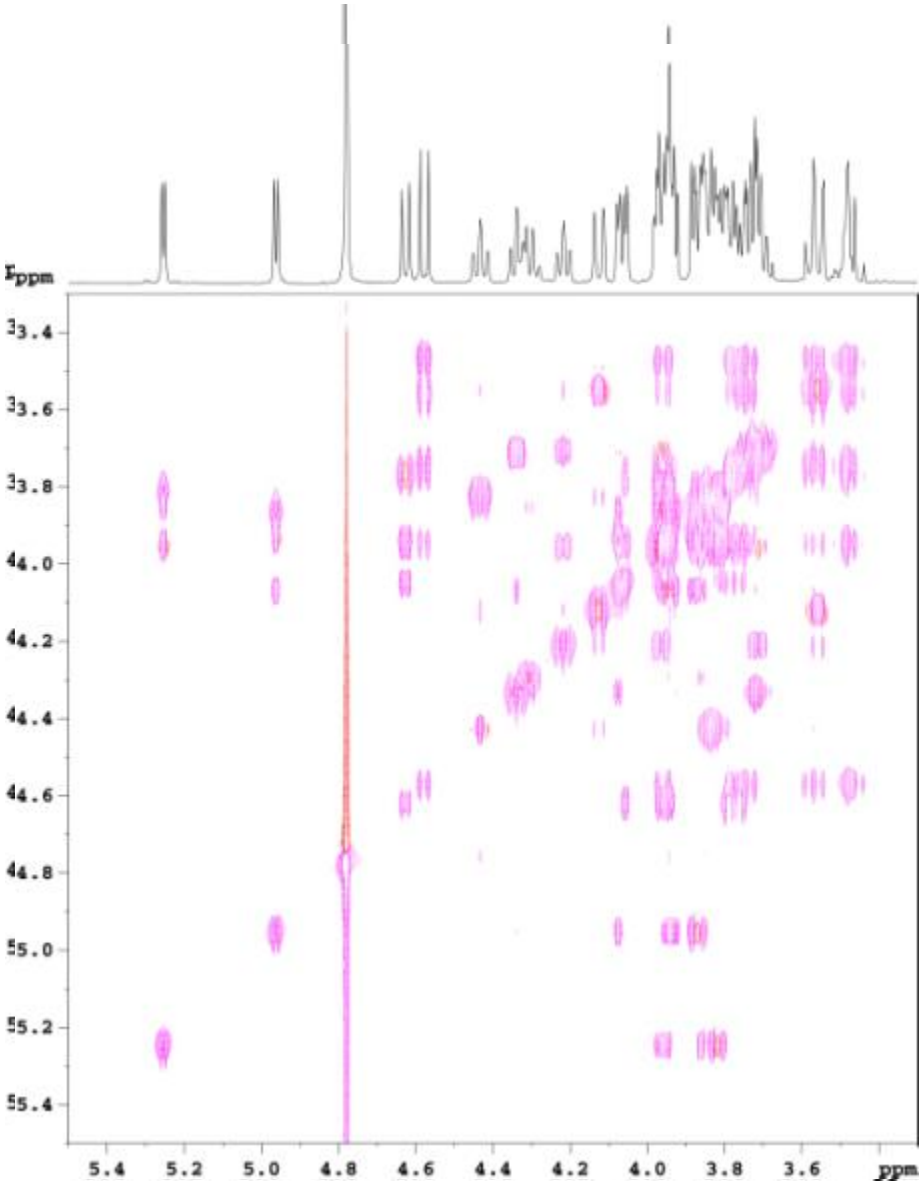
TOCSY

60 ms

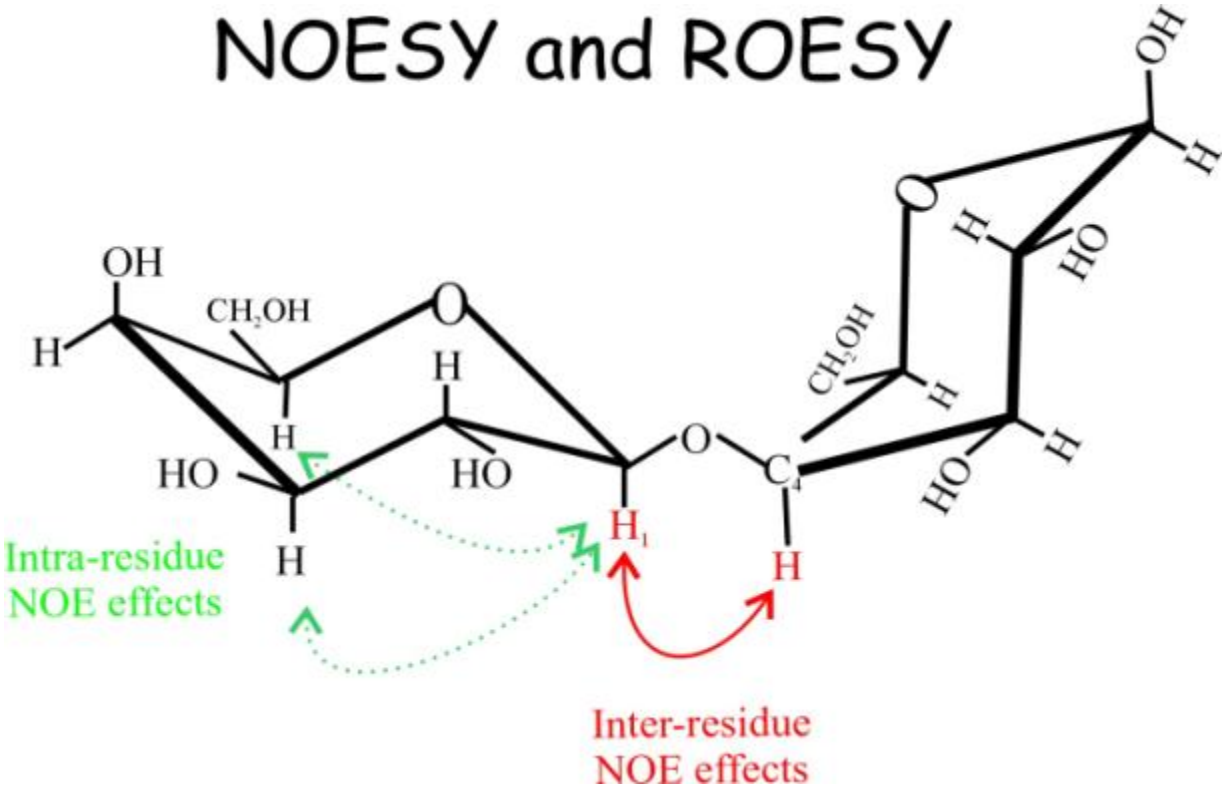


TOCSY

100 ms

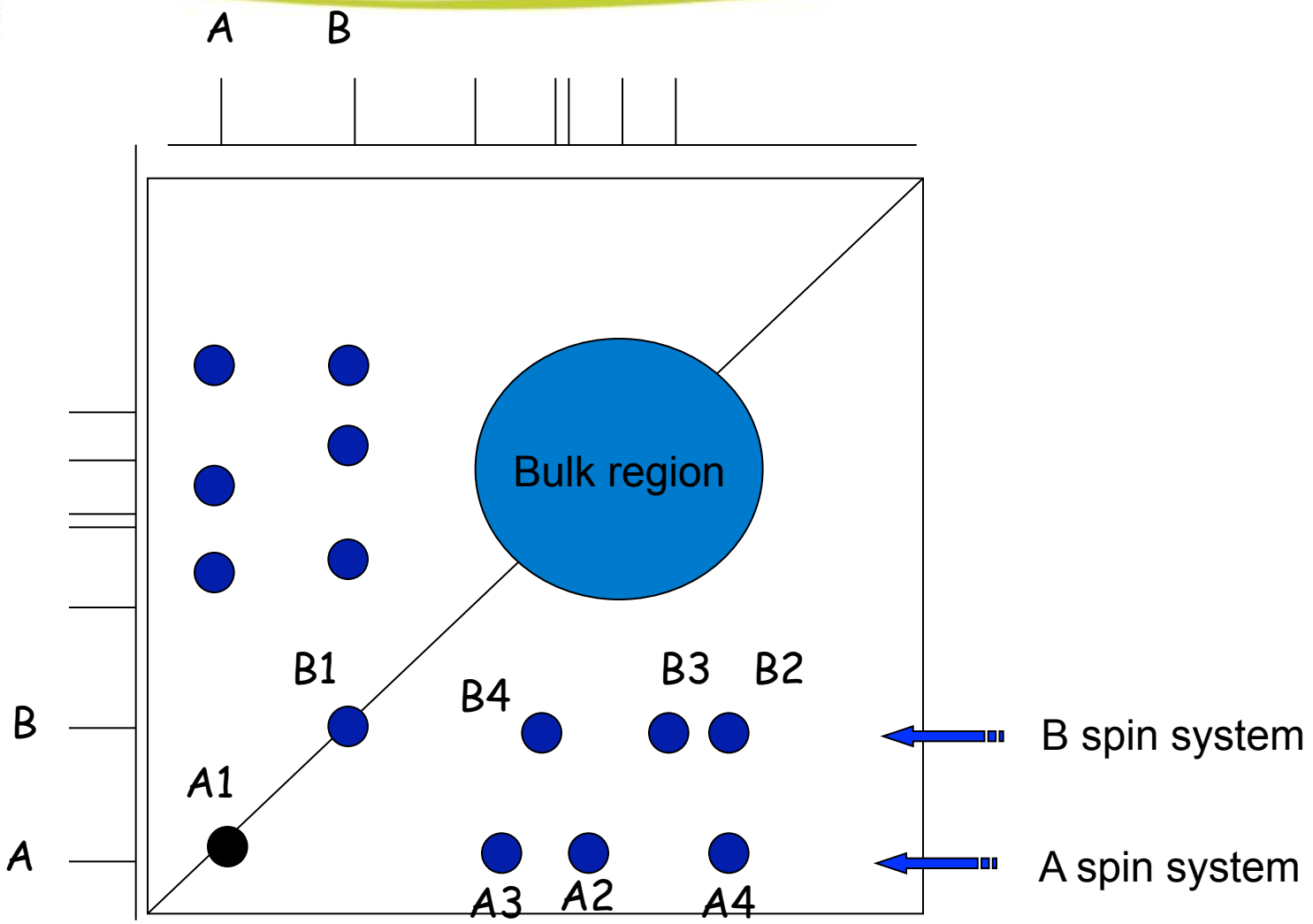


NOESY and ROESY



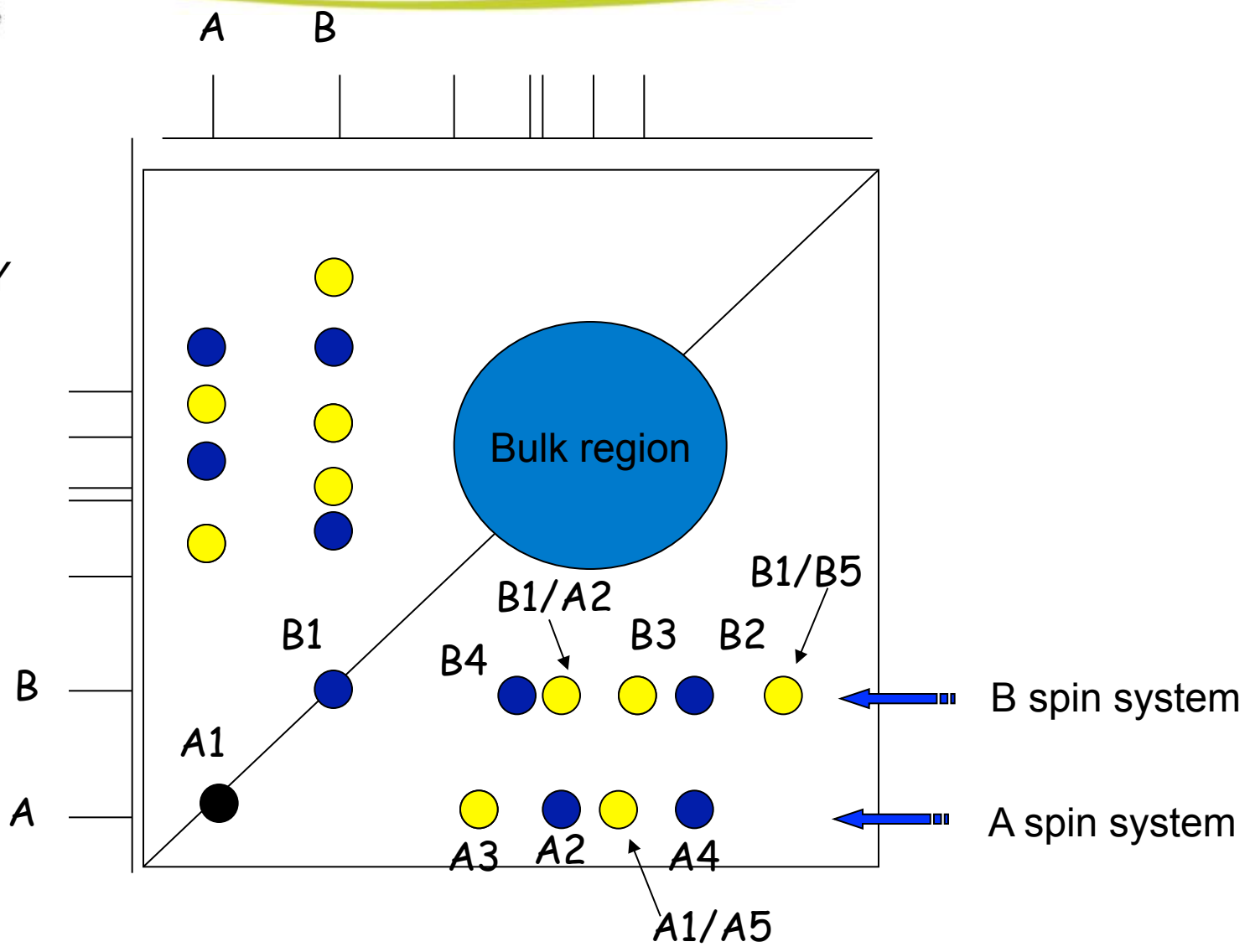
ROESY : Rotating Overhauser Effect SpectroscopY (MW<2kDa)
NOESY : Nuclear Overhauser Effect SpectroscopY (MW>2kDa)

Blue TOCSY


A
B
Bulk region
B1
B4
B3
B2
B
A1
A
A3
A2
A4
B spin system
A spin system

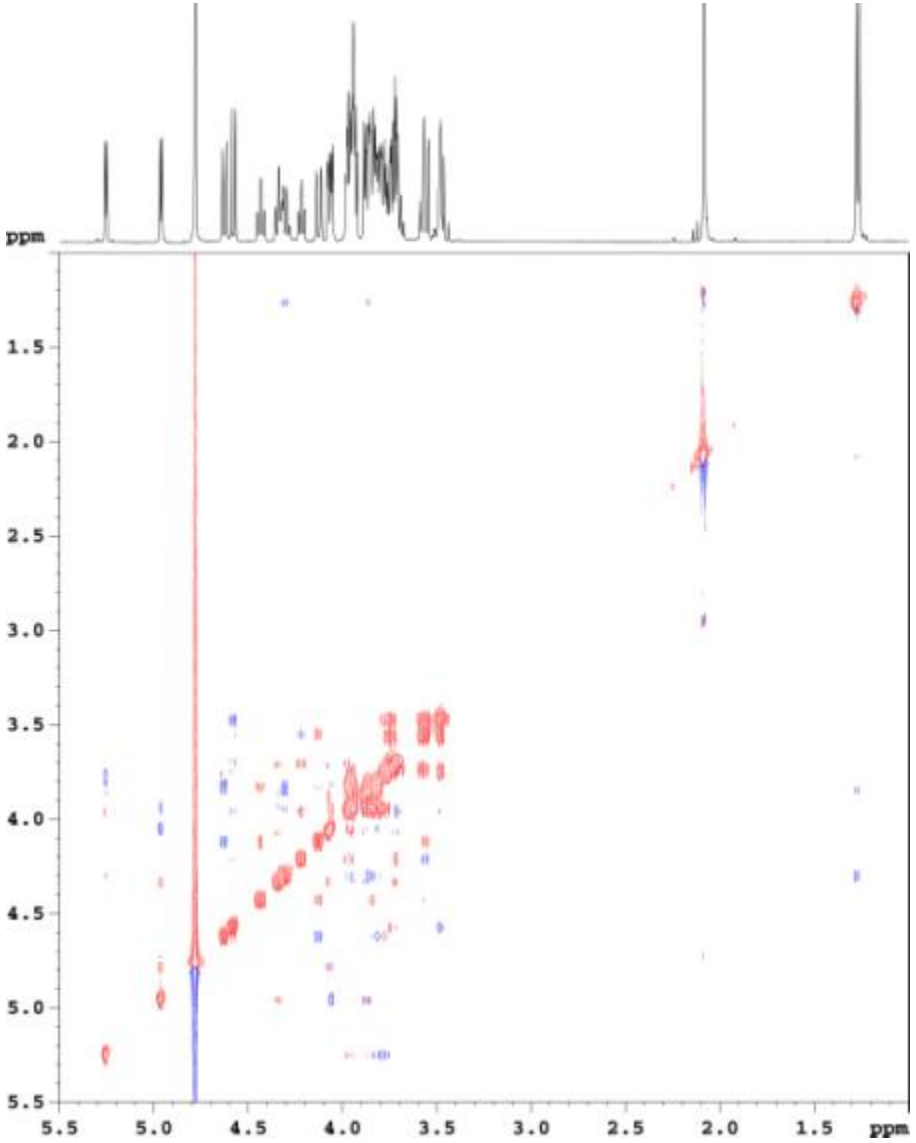
Blue TOCSY

Yellow ROESY

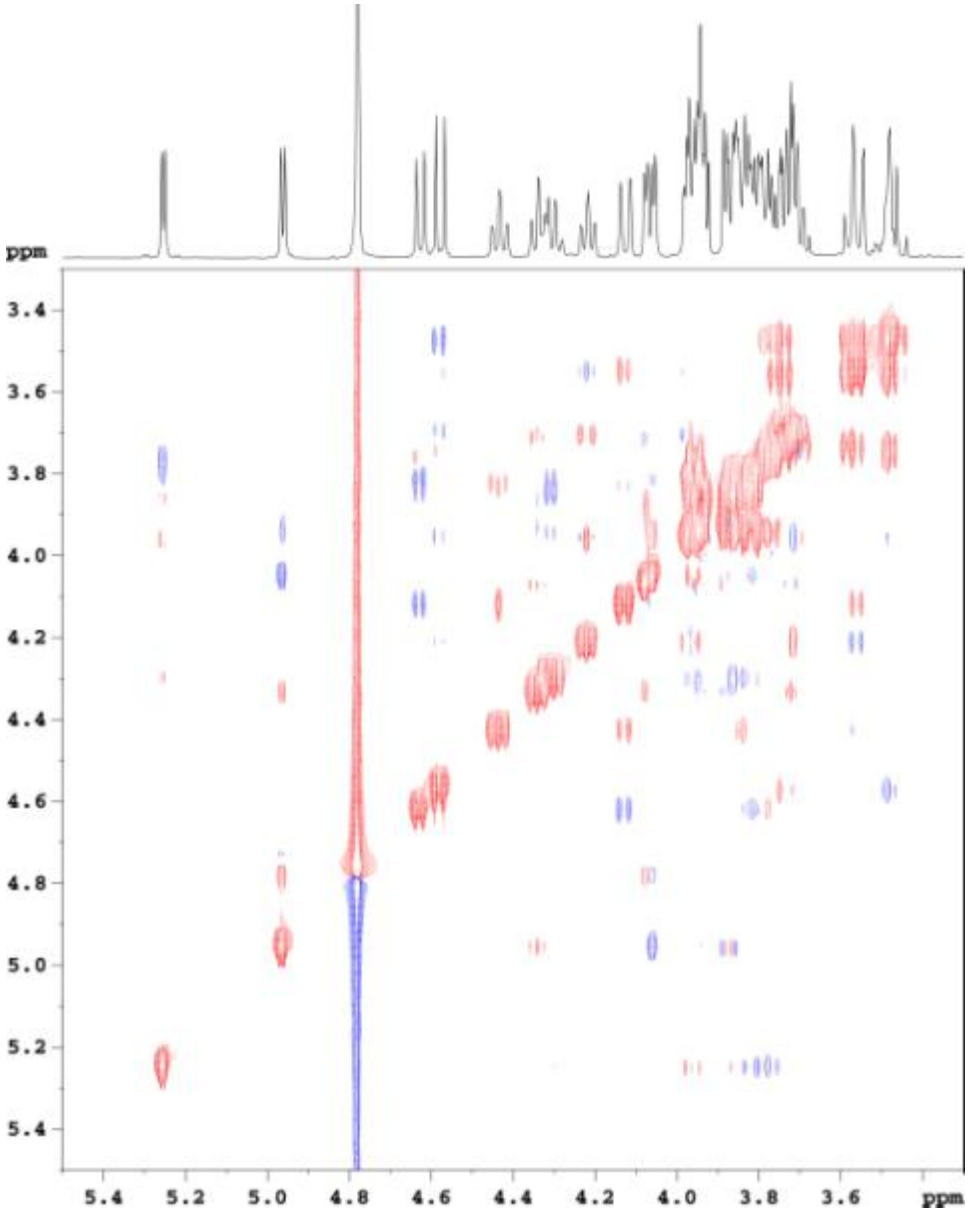


B linked to A in 2 position

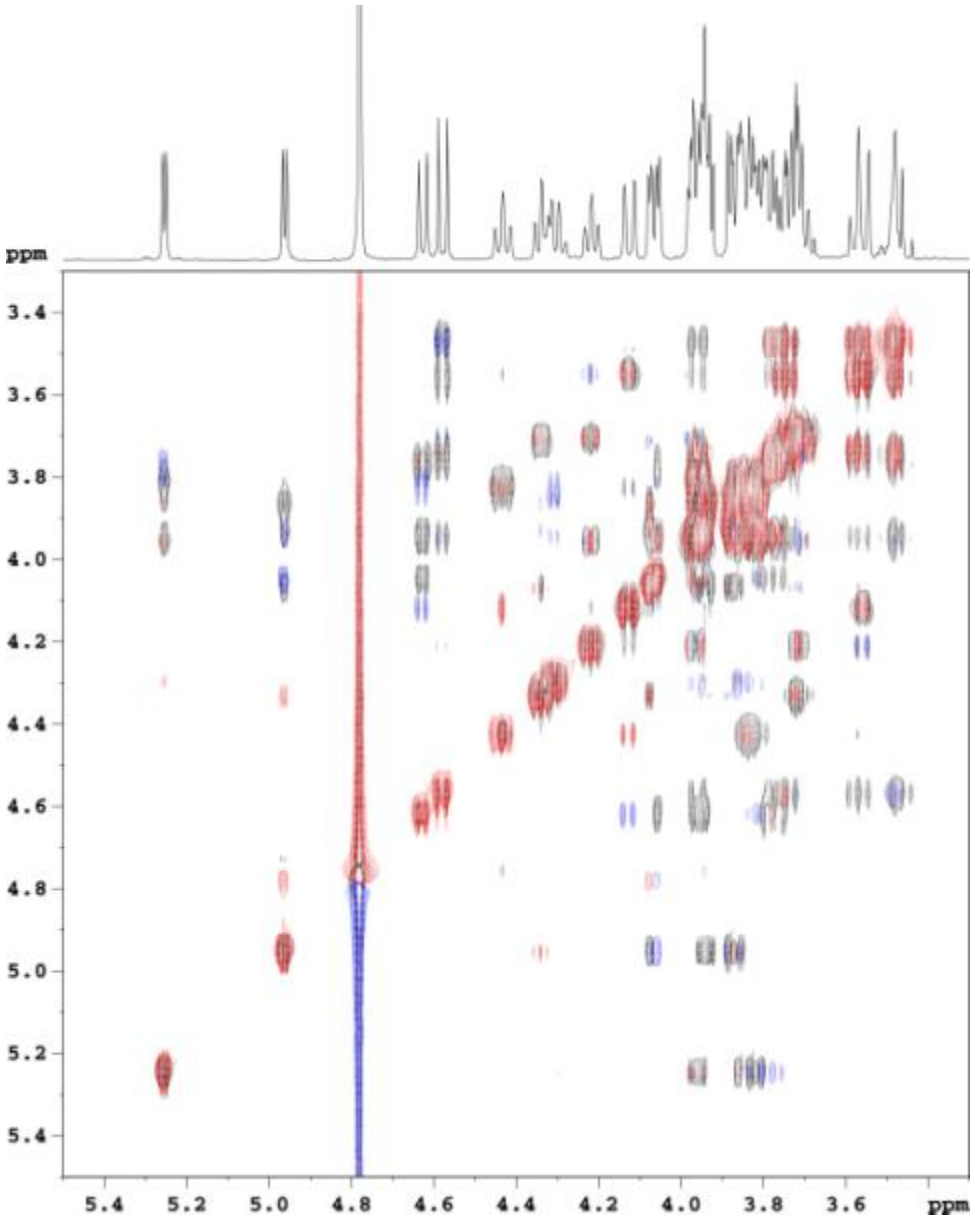
NOESY



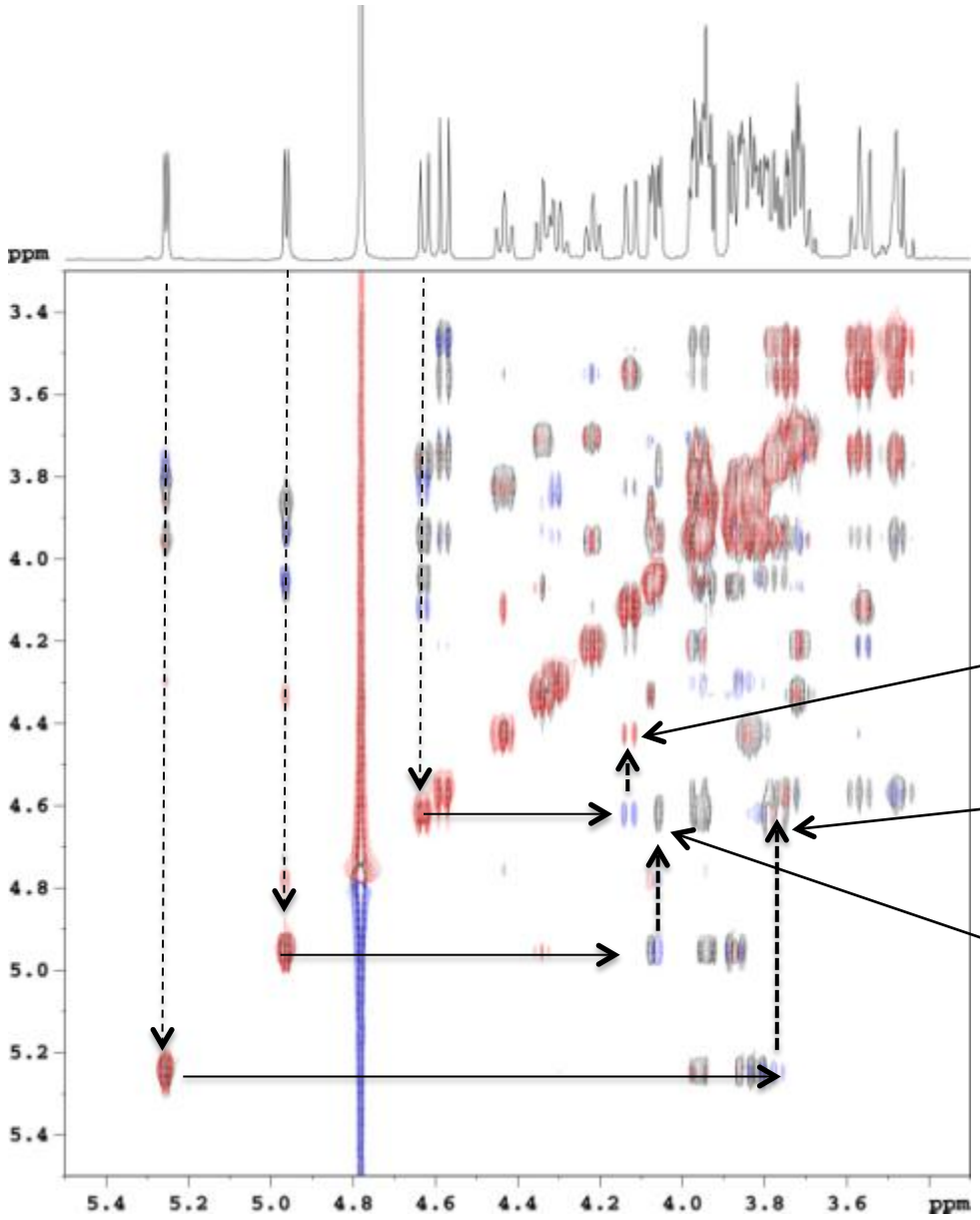
NOESY



NOESY + TOCSY



NOESY + TOCSY

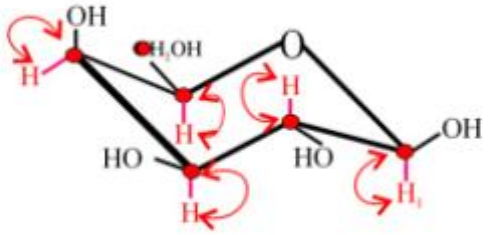


H3 of GalNAc-ol

H2 of β-Gal

H4 of β-Gal

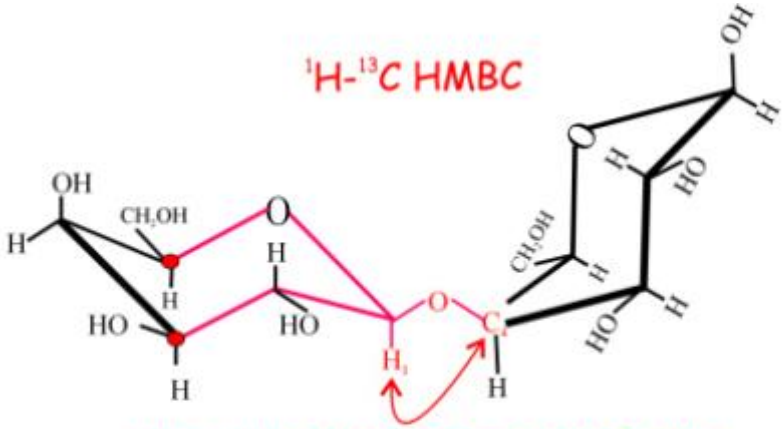
^{1}H-^{13}C HMQC



Direct heteronuclear correlation

$^{1}J_{H,C}$ ~ 145 Hz

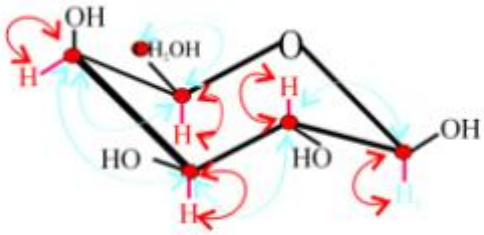
^{1}H-^{13}C HMBC



Vicinal heteronuclear correlation

$^{3}J_{H,C}$ ~ 7 Hz

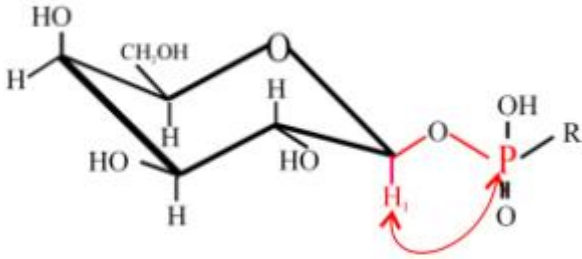
^{1}H-^{13}C HMQC-TOCSY



Direct heteronuclear correlation

correlated with associated spin systems (^{1}H-^{13}C and ^{13}C-^{1}H)

^{1}H-^{31}P HMQC



Direct heteronuclear correlation

$^{3}J_{H,P}$ ~ 7 Hz

HMQC : Heteronuclear Multi Quanta Coherence

HSQC : Heteronuclear Simple Quantum Coherence

HMQC-TOCSY : Heteronuclear Multi Quantum Coherence-TOtal Correlation spectroscopY

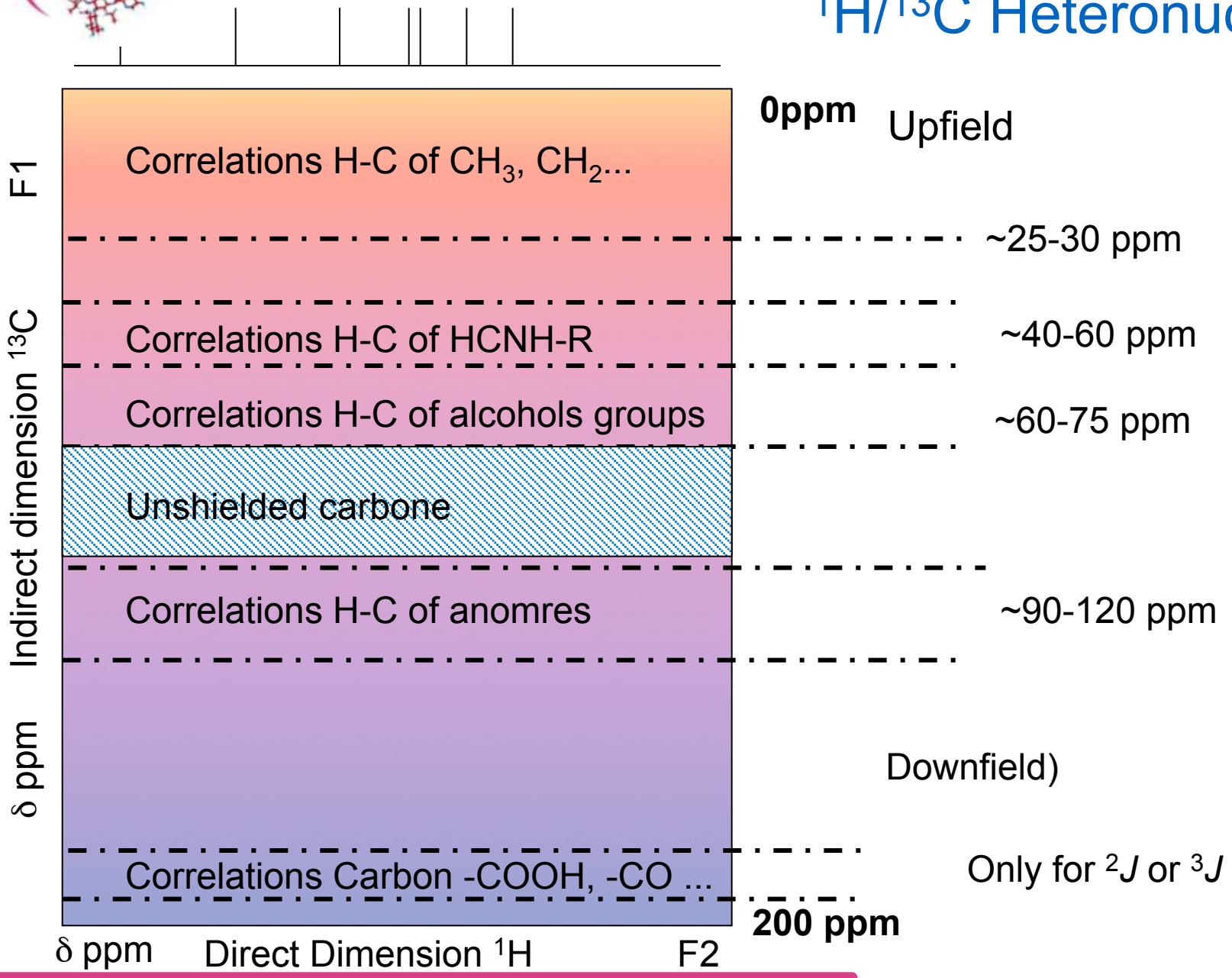
HMBC :Heteronuclear Multiple Bound Coherence

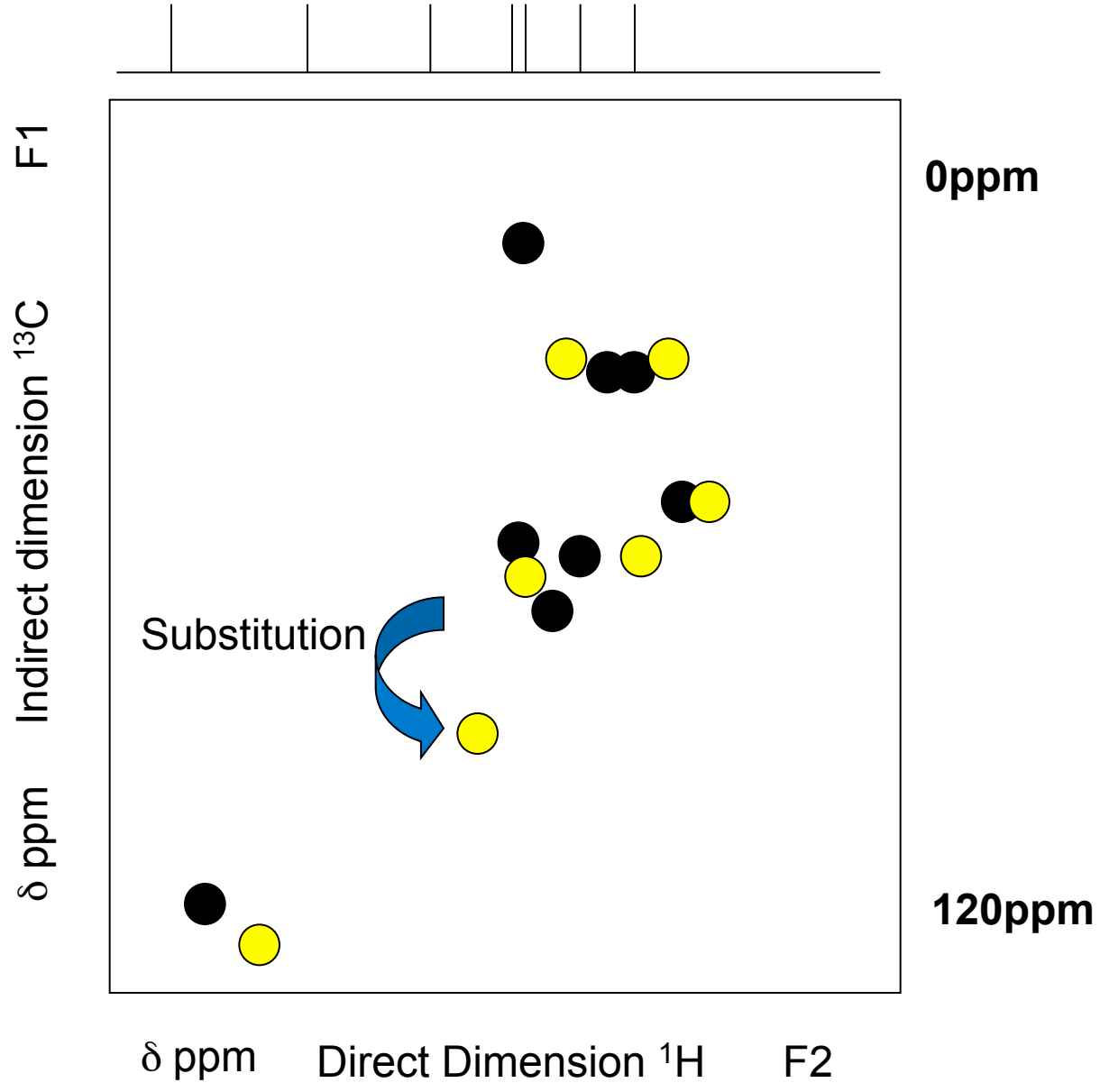
➡ Chemical shifts of heteroatoms

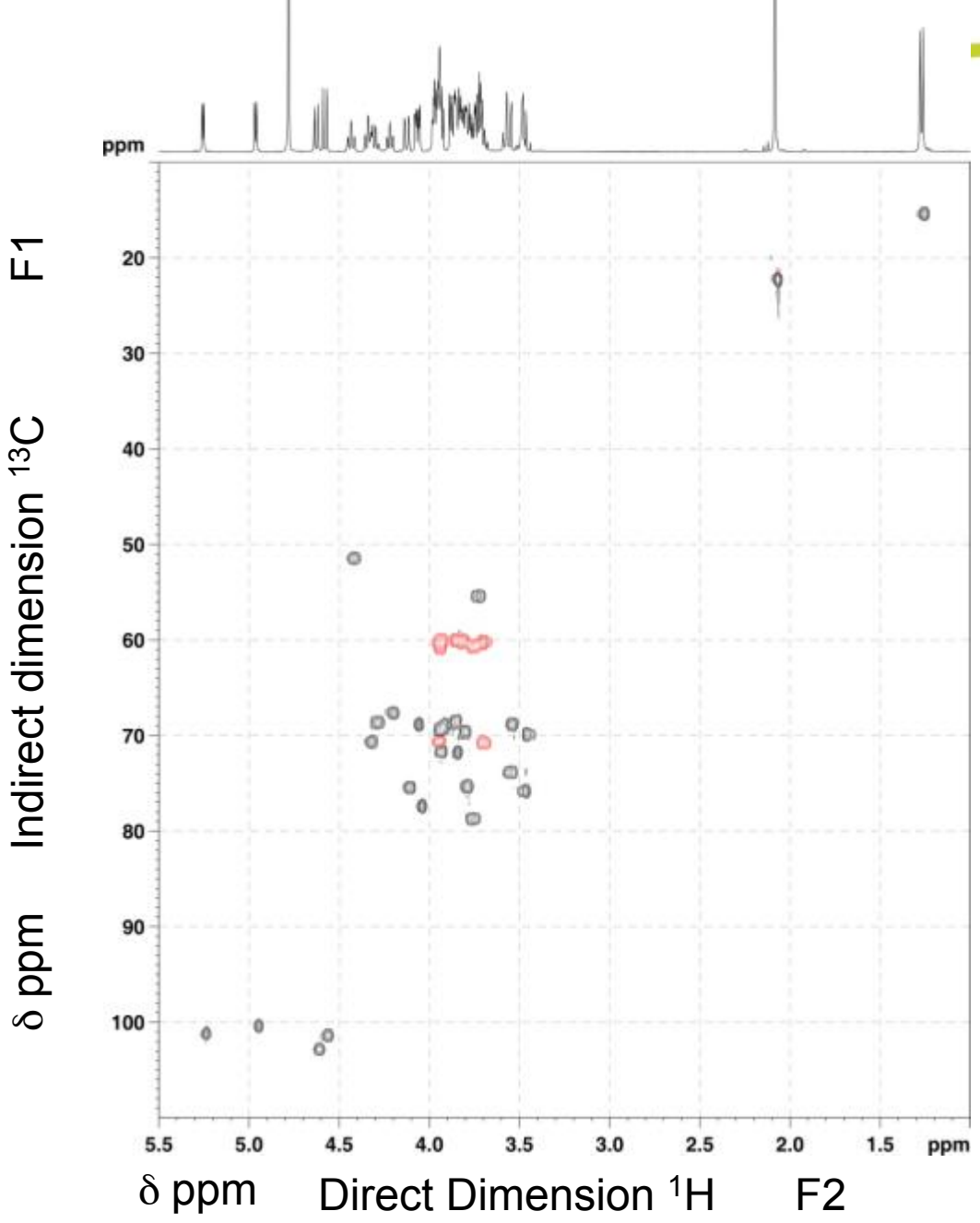
➡ Direct $^{1}J_{H,X}$ coupling constants

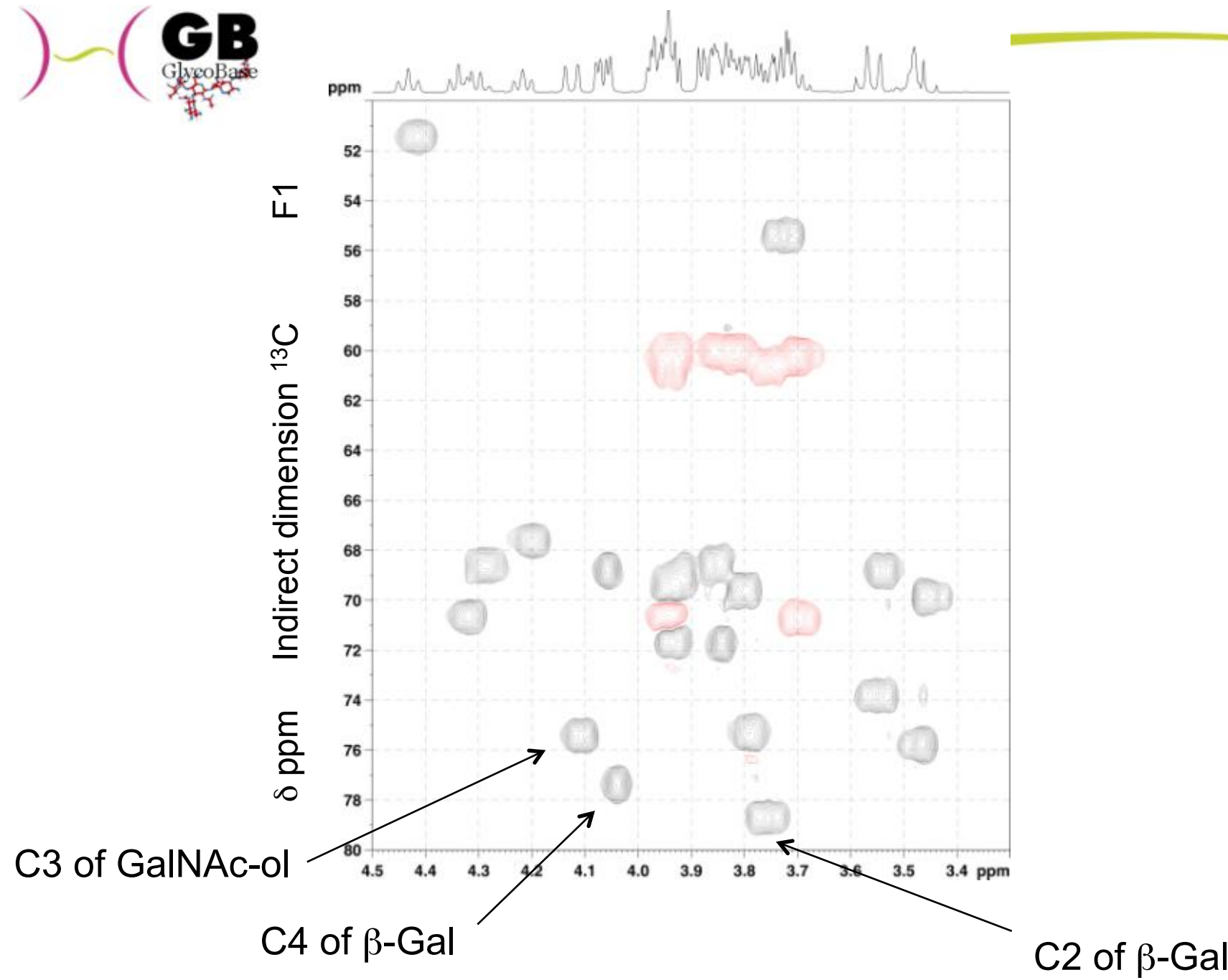
➡ Substitution and sequence

$^{1}H/^{13}C$ Heteronuclear




F1
Indirect dimension ^{13}C
δ ppm
0ppm
120ppm
Substitution
δ ppm
Direct Dimension ^{1}H
F2


ppm
20
30
40
50
60
70
80
90
100
δ ppm Indirect dimension ^{13}C F1
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
δ ppm Direct Dimension ^{1}H F2


ppm
52
54
56
58
60
62
64
66
68
70
72
74
76
78
80
F1
Indirect dimension ^{13}C
δ ppm
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4 ppm
C3 of GalNAc-ol
C4 of β-Gal
C2 of β-Gal

What use of NMR for glycobiology?

- *de novo* sequencing
- Glycomics profiling
- Surface analysis: HR-MAS NMR
- DOSY NMR
- Protein-carbohydrate interaction

What use of NMR for glycobiology?

- De novo sequencing

De novo sequencing

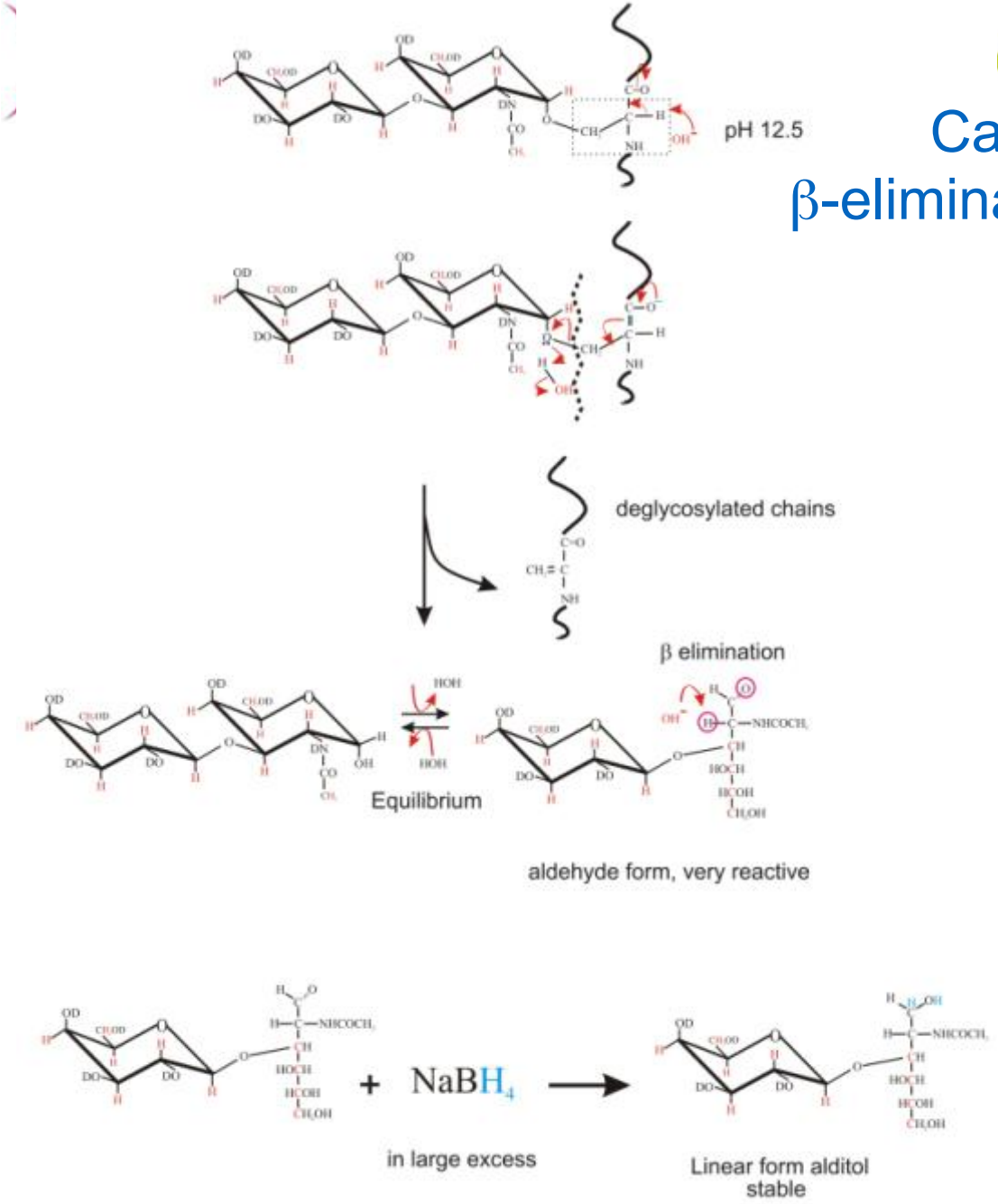
Establish the **exact sequence** of:

- A pure oligosaccharide (dp<15)
- A simple mixture of small oligosaccharides (dp<8)
- A pure polysaccharide with limited heterogeneity
- A simple mixture of homogeneous polysaccharides

Will provide **all possible structural parameters** of the molecules:

- Composition
- Sequence
- Anomery
- Substitutions
- …/…

Case study of O-glycan after β-elimination in reducing condition



SO_3-Hex-Hex-HexNAc-ol
|
deHex

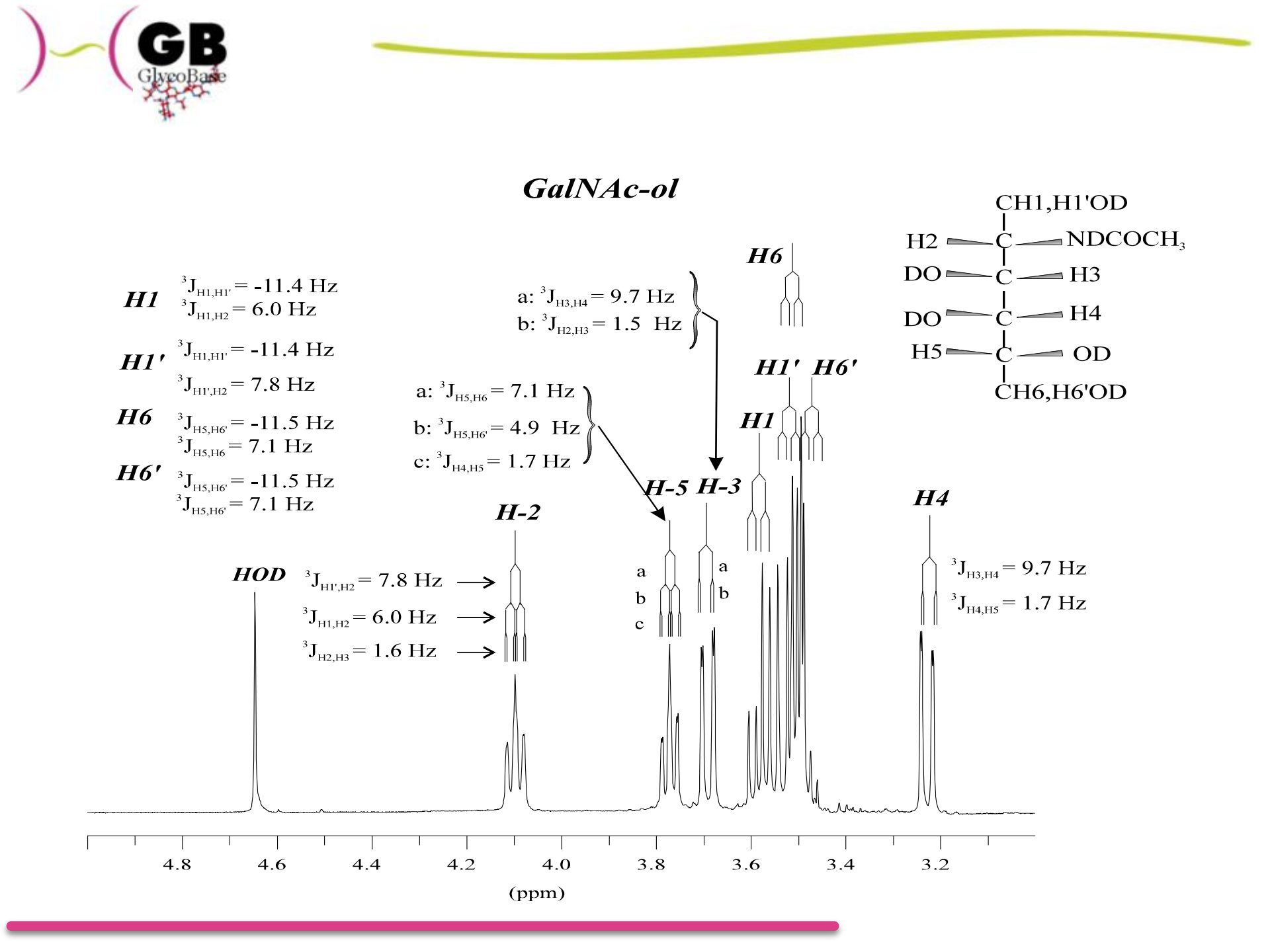
MS & MS/MS

↑

HPLC

↑

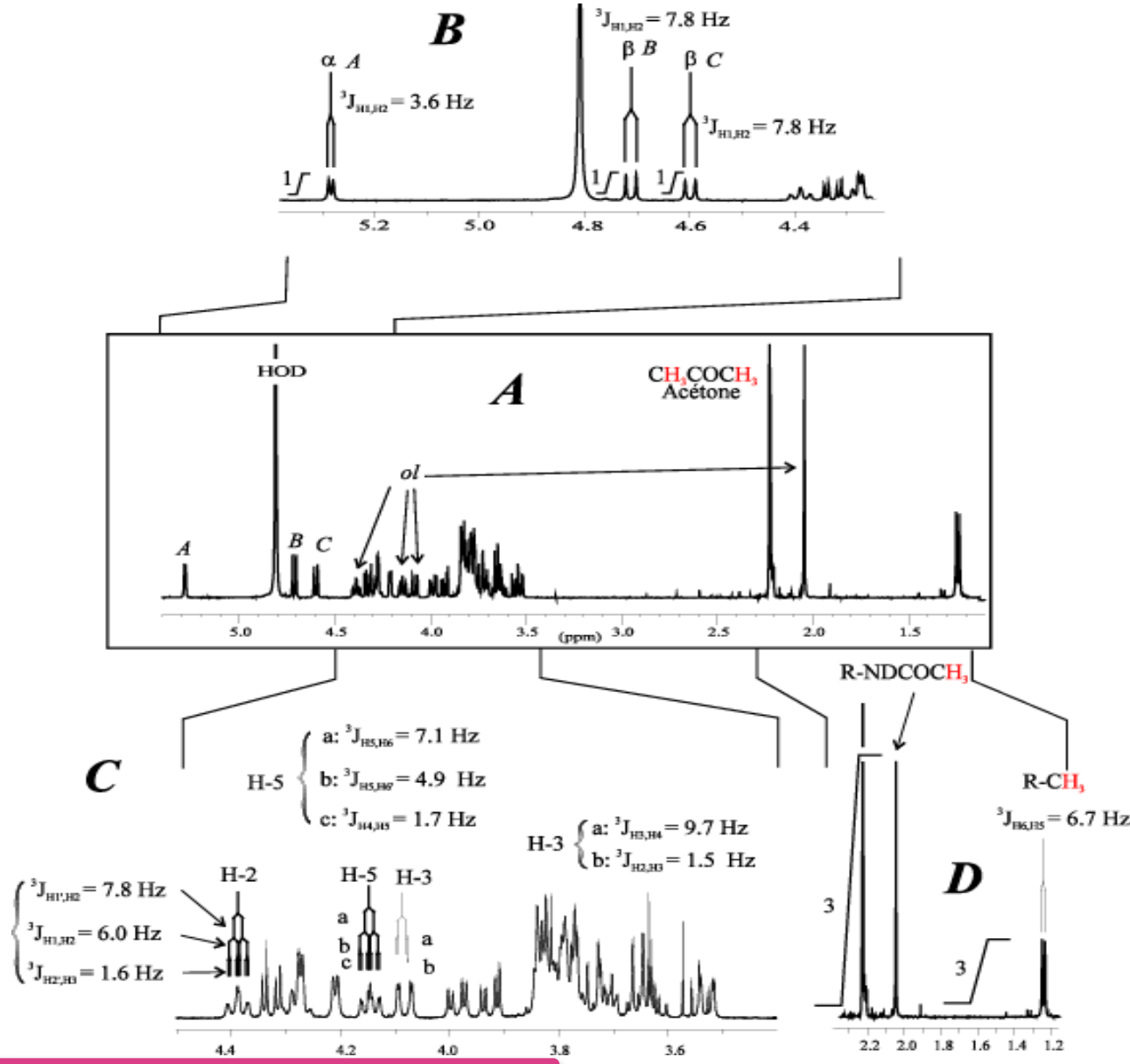
Oligosaccharide-alditol


GalNAc-ol
H1
$^3J_{H1,H1'}$ = -11.4 Hz
$^3J_{H1,H2}$ = 6.0 Hz
H1'
$^3J_{H1,H1'}$ = -11.4 Hz
$^3J_{H1',H2}$ = 7.8 Hz
H6
$^3J_{H5,H6'}$ = -11.5 Hz
$^3J_{H5,H6}$ = 7.1 Hz
H6'
$^3J_{H5,H6'}$ = -11.5 Hz
$^3J_{H5,H6'}$ = 7.1 Hz
a: $^3J_{H3,H4}$ = 9.7 Hz
b: $^3J_{H2,H3}$ = 1.5 Hz
a: $^3J_{H5,H6}$ = 7.1 Hz
b: $^3J_{H5,H6'}$ = 4.9 Hz
c: $^3J_{H4,H5}$ = 1.7 Hz
H6
H1' H6'
H1
H-5 H-3
H-2
H4
HOD
$^3J_{H1',H2}$ = 7.8 Hz
$^3J_{H1,H2}$ = 6.0 Hz
$^3J_{H2,H3}$ = 1.6 Hz
a
b
c
a
b
$^3J_{H3,H4}$ = 9.7 Hz
$^3J_{H4,H5}$ = 1.7 Hz
CH1,H1'OD
H2
C
NDCOCH3
DO
C
H3
DO
C
H4
H5
C
OD
CH6,H6'OD
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
(ppm)

1D NMR

Establishment of:

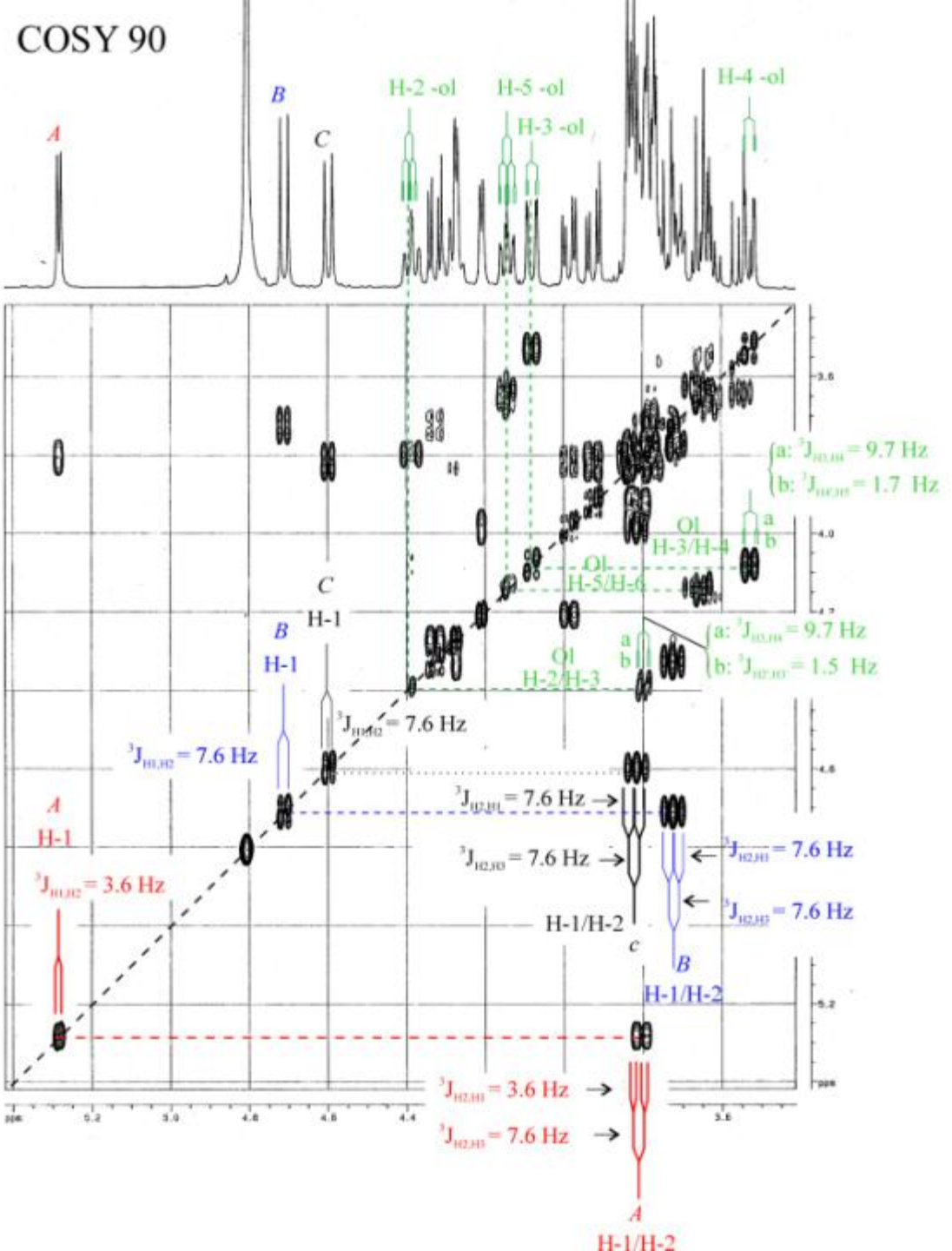
- ^{1}H δ of anomers and GalNAc-ol
- $^{3}J_{1H,H2}$
- ^{3}J of GalNAc-ol



COSY 90

Establishment of:

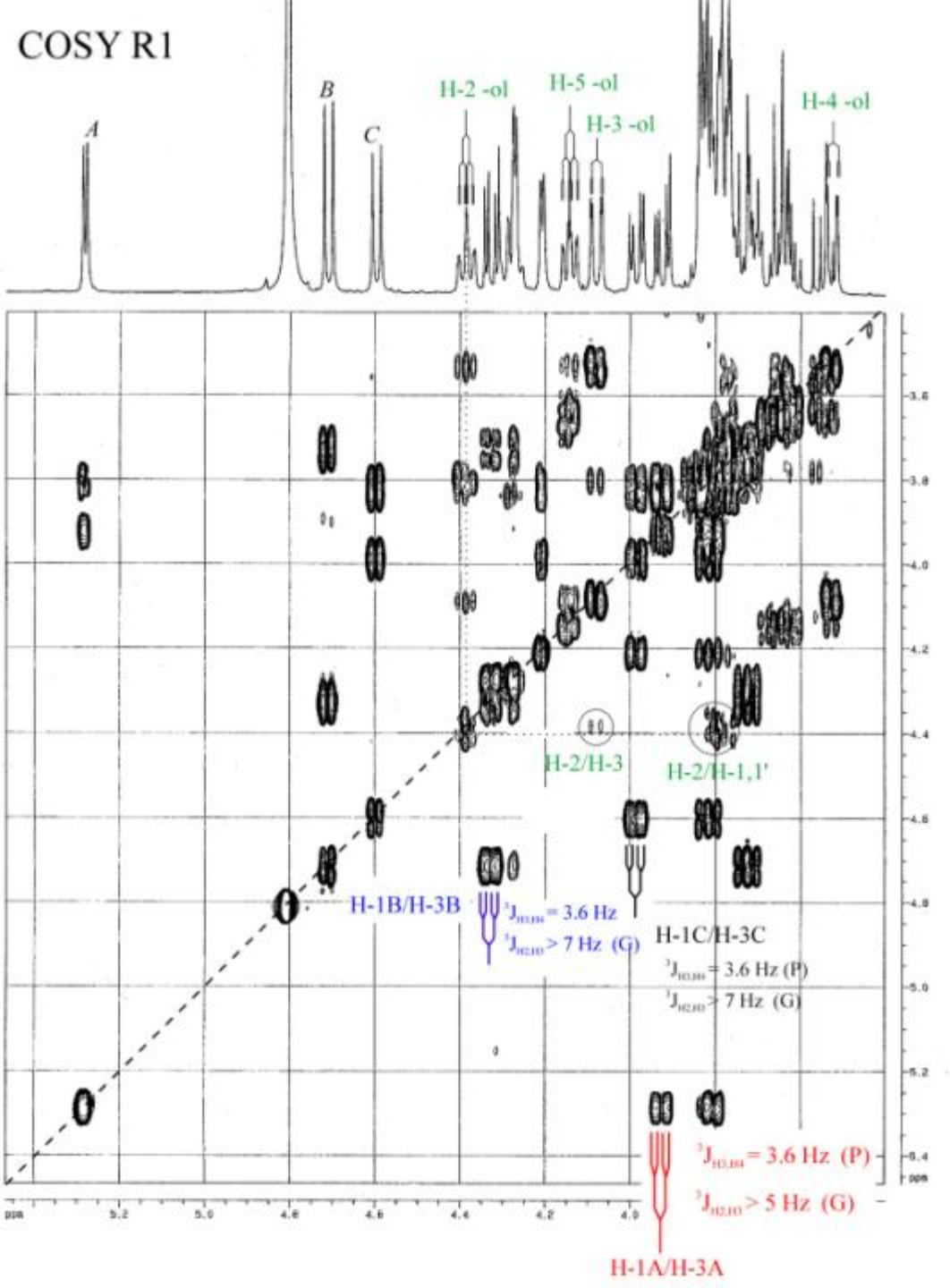
- H1-H2 correlations
- H2 δ
- $^{3}J_{H2,H3}$



COSY R1

Establishment of:

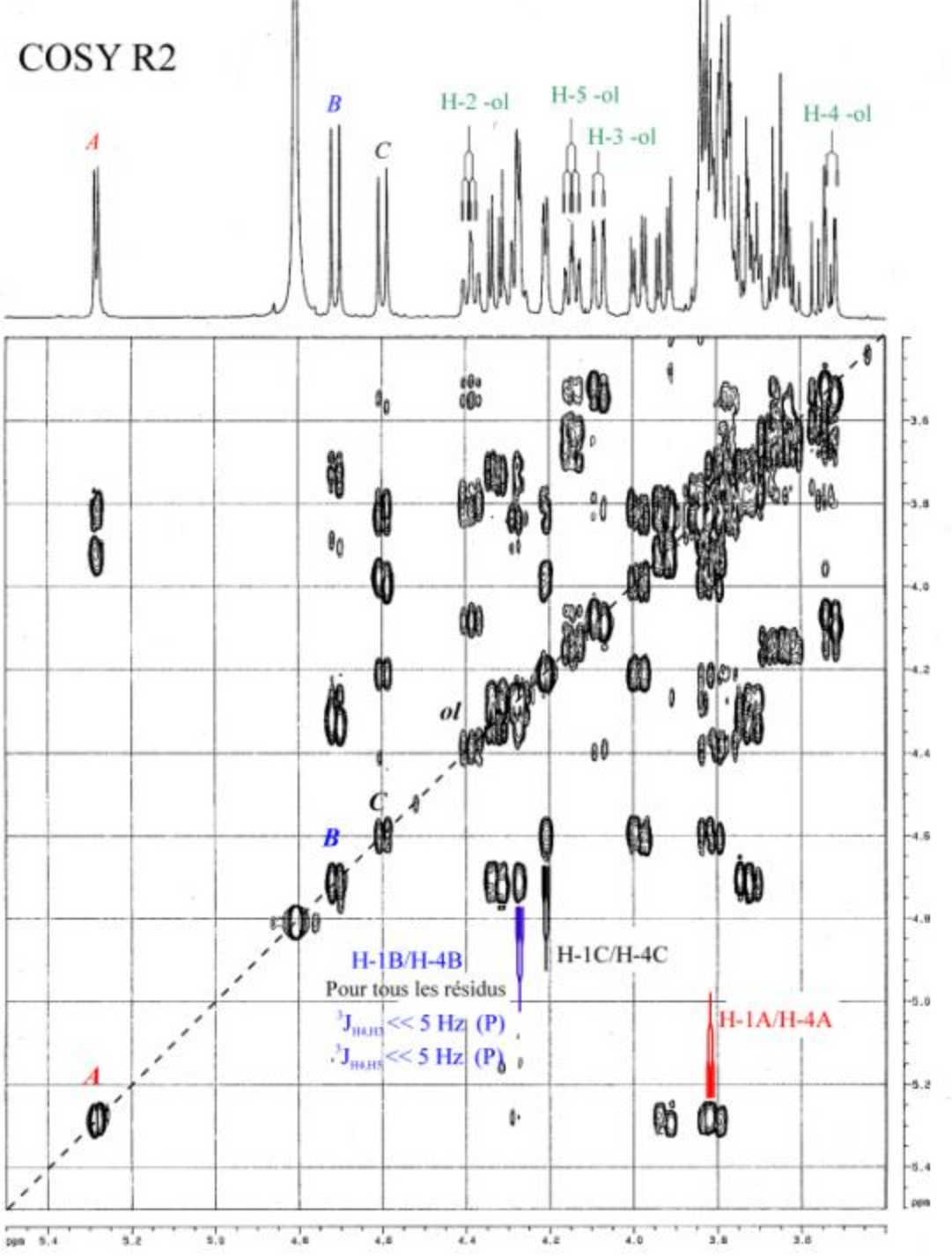
- H1-H3 correlations through H2
- H3 δ
- $^{3}J_{H3,H4}$



COSY R2

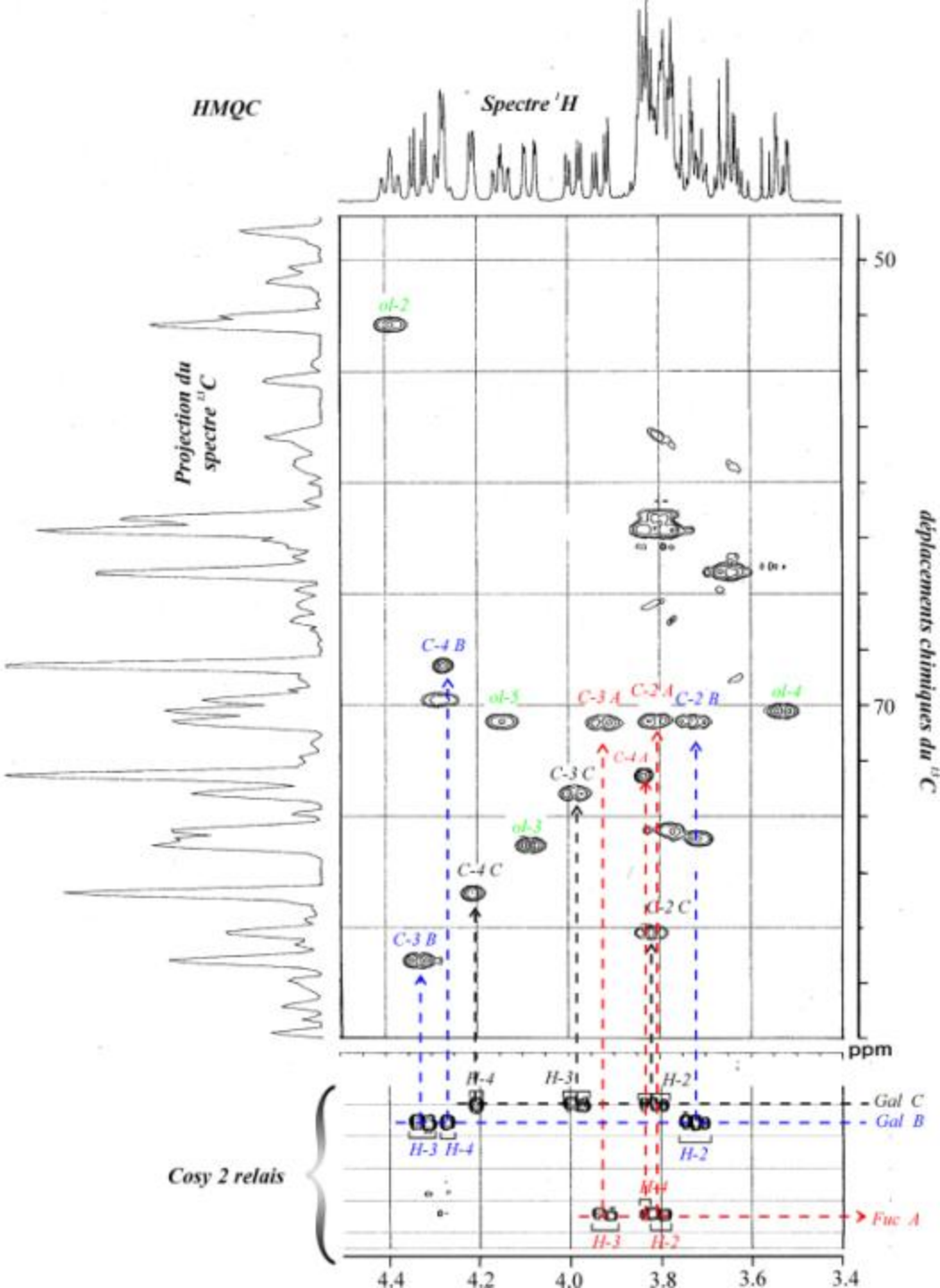
Establishment of:

- H1-H4 correlations through H2
- H4 δ
- $^{3}J_{H4,H5}$



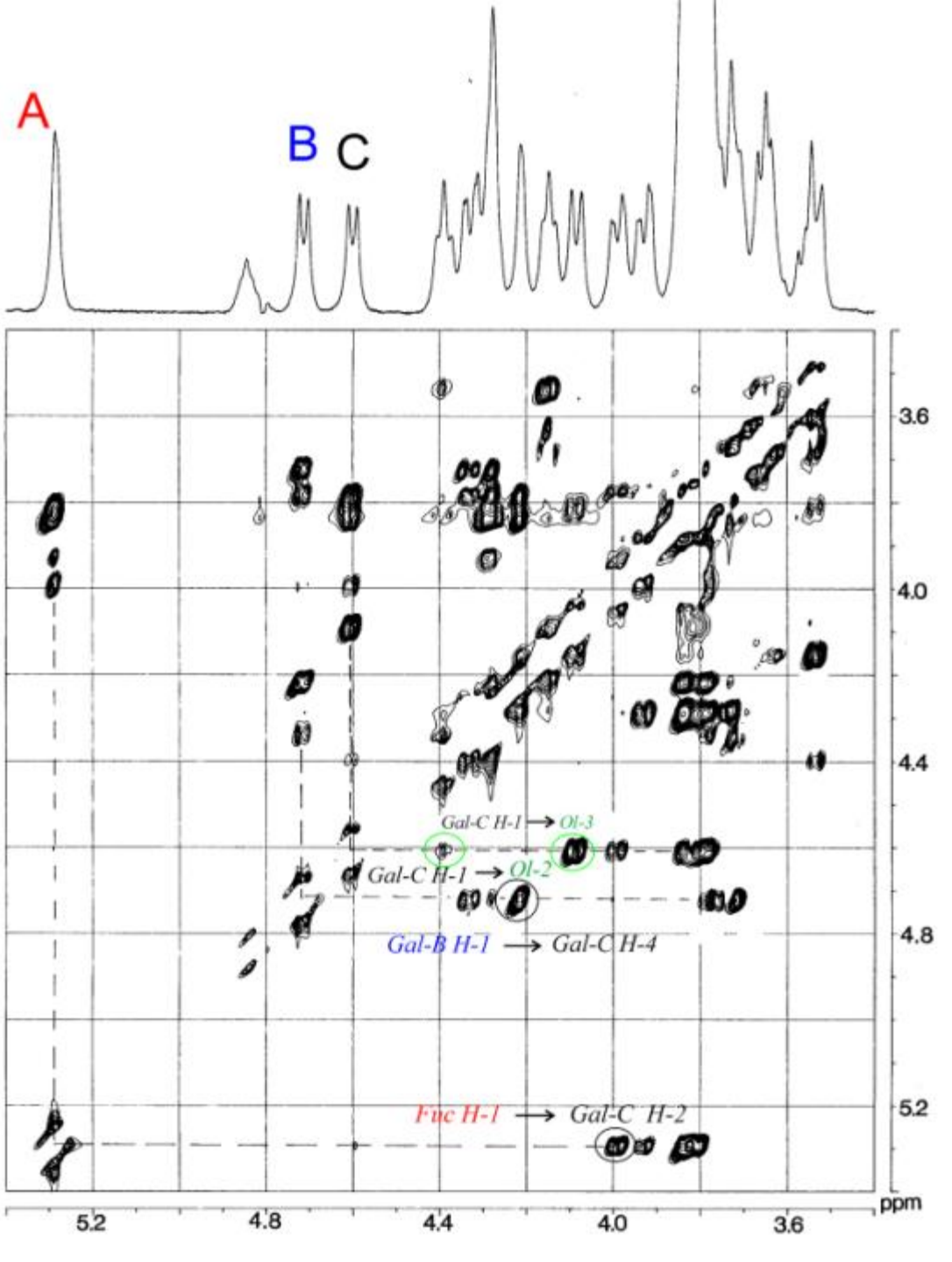
Establishment of:

- H-C correlations
- C δ
- Positions of substitutions ($\Delta\delta$ ^{13}C > + 4-5Hz)

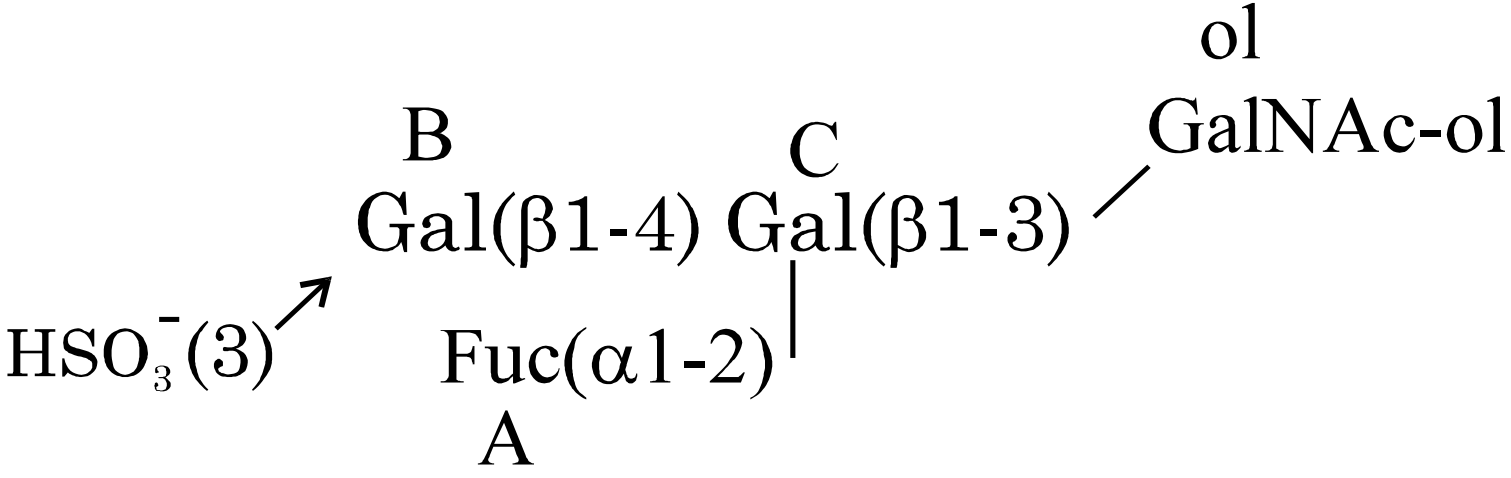


Establishment of:

- Dipolar couplings
- Sequence



Solution



What use of NMR for glycobiology?

- Glycomics profiling

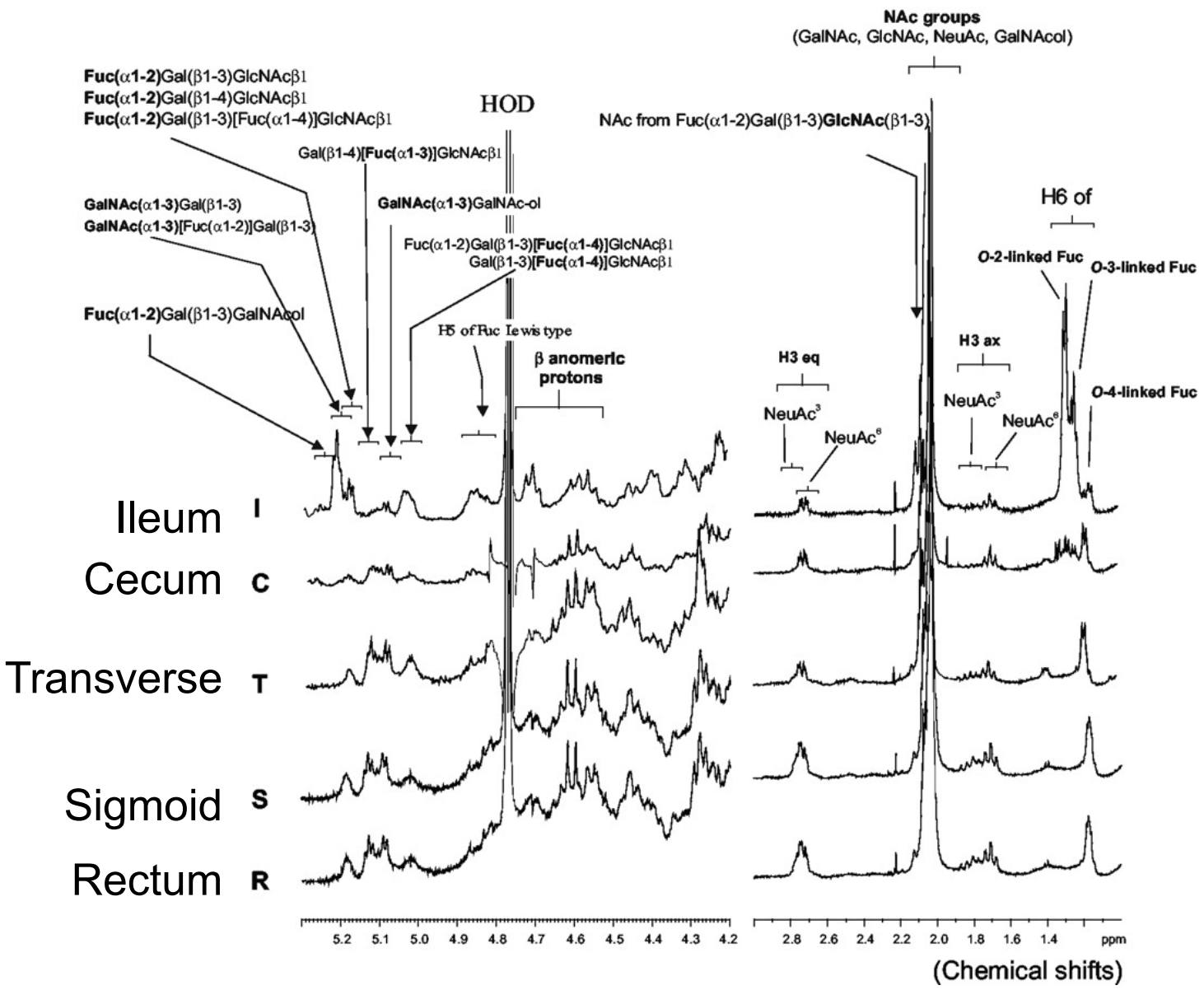
Glycomics analysis

Establish the **glycan profile** of:

- A complexe mixture of oligosaccharides
- A pure polysaccharide with high heterogeneity
- A complexe mixture of polysaccharides

Will provide **limited set** of relevant parameters

- Composition
- Partial sequence
- Anomeric ratios
- Presence of motifs


NAc groups
(GalNAc, GlcNAc, NeuAc, GalNAcol)
Fuc(α1-2)Gal(β1-3)GlcNAcβ1
Fuc(α1-2)Gal(β1-4)GlcNAcβ1
Fuc(α1-2)Gal(β1-3)[Fuc(α1-4)]GlcNAcβ1
HOD
NAc from Fuc(α1-2)Gal(β1-3)GlcNAc(β1-3)
Gal(β1-4)[Fuc(α1-3)]GlcNAcβ1
GalNAc(α1-3)Gal(β1-3)
GalNAc(α1-3)[Fuc(α1-2)]Gal(β1-3)
GalNAc(α1-3)GalNAc-ol
Fuc(α1-2)Gal(β1-3)[Fuc(α1-4)]GlcNAcβ1
Gal(β1-3)[Fuc(α1-4)]GlcNAcβ1
H6 of
O-2-linked Fuc
O-3-linked Fuc
Fuc(α1-2)Gal(β1-3)GalNAcol
H5 of Fuc Lewis type
β anomeric protons
H3 eq
H3 ax
O-4-linked Fuc
NeuAc3
NeuAc6
NeuAc3
NeuAc6
Ileum I
Cecum C
Transverse T
Sigmoid S
Rectum R
5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2
2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 ppm
(Chemical shifts)

E. Maes

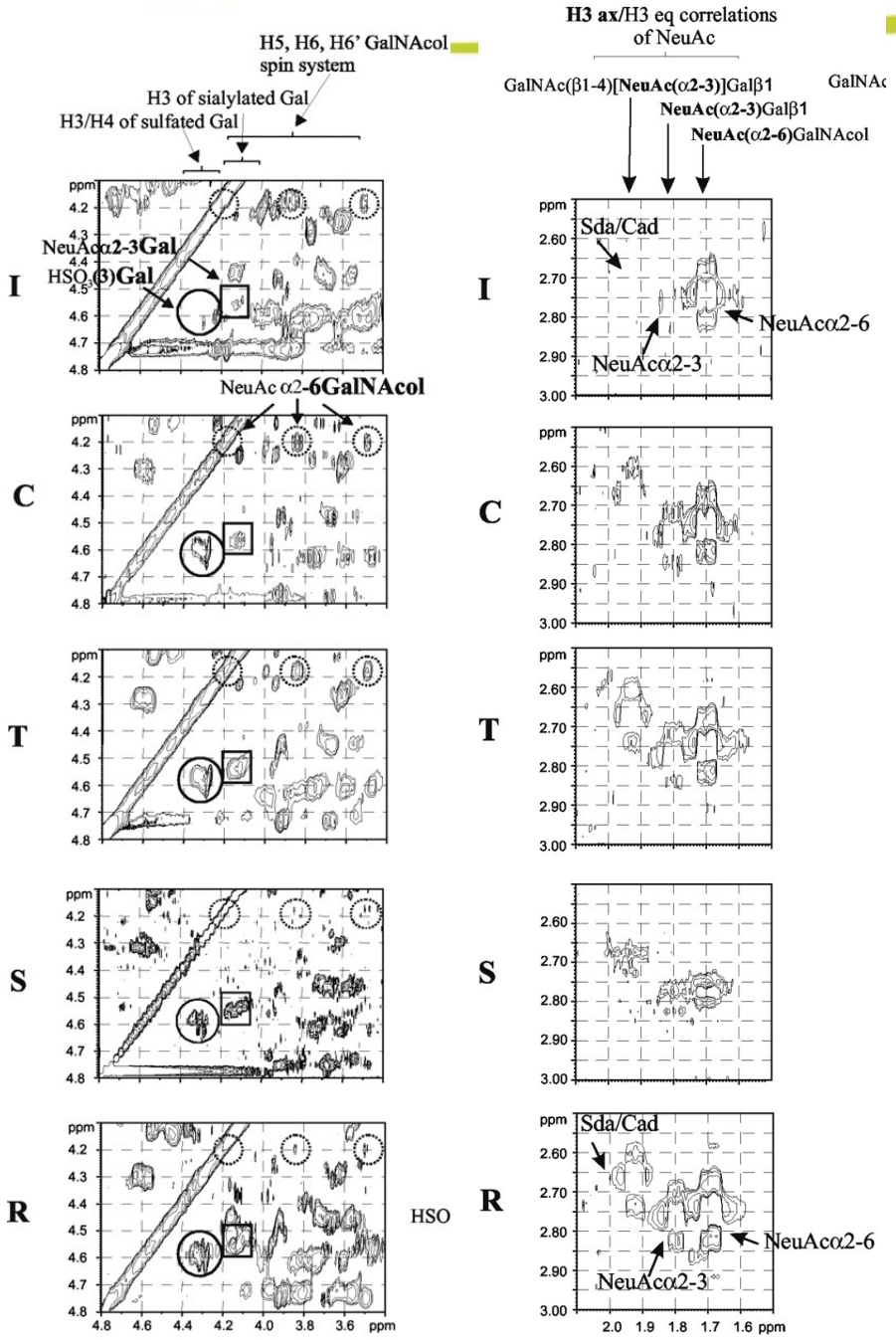
Ileum

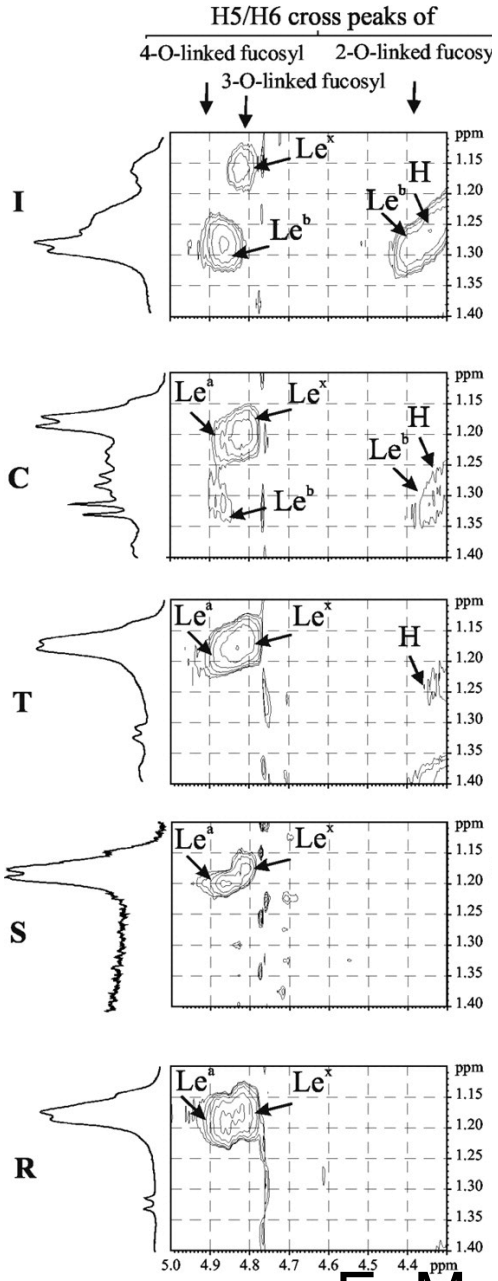
Cecum

Transverse

Sigmoid

Rectum





E. Maes

Glycomics profile

	Assignments of signals (ppm)[a]				Estimated abundance									
					Ileum		Cecum		Transverse		Sigmoid		Rectum	
	H3eq	H3ax			D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
α2-3 linked NeuAc	2.75-2.78	1.79-1.81			±	+	+	+	++	++	++	++	+++	++
α2-3 linked NeuAc in Sda/Cad	2.63-2.67	1.91-1.93			-	-	+	-	+	-	++	-	++	±
α2-6 linkedNeuAc	2.72-2.75	1.68-1.71			++	++	++	++	++	++	++	++	+++	+++
	H1	H2	H3	H4										
O-3 Sialylated Gal	4.53-4.59	3.5-3.6	4.08-4.13	3.92-3.95	±	-	+	-	++	+	++	+	+++	++
O-3 Sulfated Gal	4.56-4.61	3.7-3.8	4.31-4.33	4.26-4.28	-	-	+	+	++	++	++	++	+++	++
	H6	H6'												
O-6 Sulfated GlcNAc	4.4-4.3	4.3-4.2			-	-	-	-	-	±	-	+	±	+
	H1	H2	H5	H6										
α1-2 linked Fuc in H group[b]	5.15-5.35	3.76-3.84	4.26-4.32	1.18-1.29	++	++	+	+	±	-	-	-	-	-
α1-2 linked Fuc in Le^b group	5.27-5.29	3.72-3.82	4.34-4.39	1.28-1.29	++	++	+	+	±	-	-	-	-	-
α1-3 linked Fuc in Le^x group	5.11-5.15	3.69-3.71	4.81-4.82	1.13-1.19	+	+	++	+	++	++	+++	++	+++	++
α1-4 linked Fuc in Le^a group	5.01-5.06	3.80-3.81	4.82-4.86	1.15-1.20	-	-	+	+	++	++	+++	++	+++	++
α1-4 linked Fuc in Le^b group	5.02-5.04	3.81	4.86-4.87	1.27	++	++	+	+	-	-	-	-	-	-
	H1	H2	H3	H4										
α1-3 linked GalNAc core 5	5.06-5.07	4.22	3.92	4.04	+	+	+	+	+	+	+	+	+	+
GalNAc in A group	5.18-5.23	4.24	3.90-3.93	4,0	+	+	+	+	-	-	-	-	-	-
GalNAc in A Le^b group	5.23-5.27	4.17-4.18	3.97	3.97	+	+	±	-	-	-	-	-	-	-

E. Maes

What use of NMR for glycobiology?

- Surface analysis: HR-MAS NMR

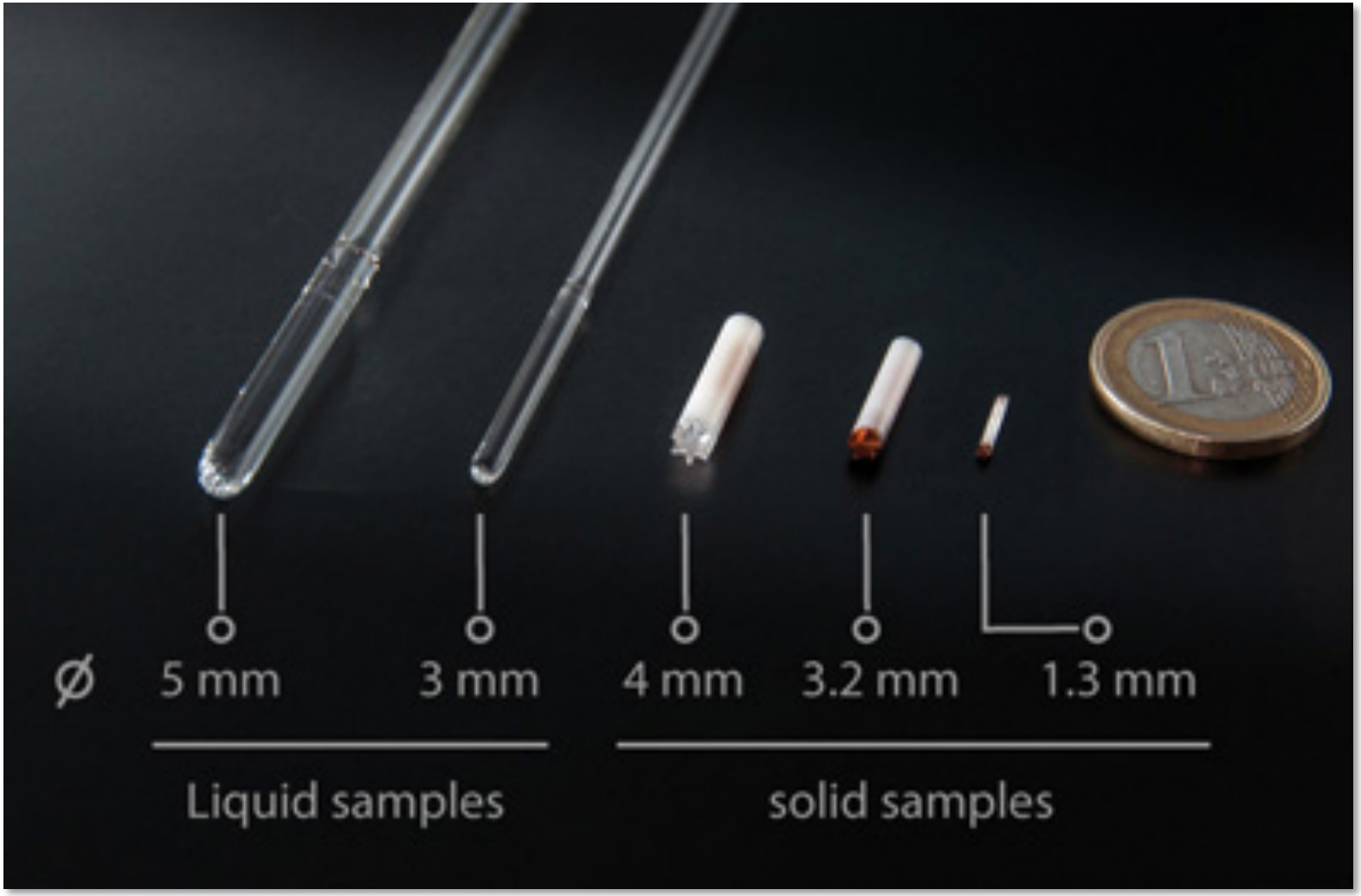
High-Resolution at Magic Angle NMR
What does it do?

Acquires NMR spectra of intact cells

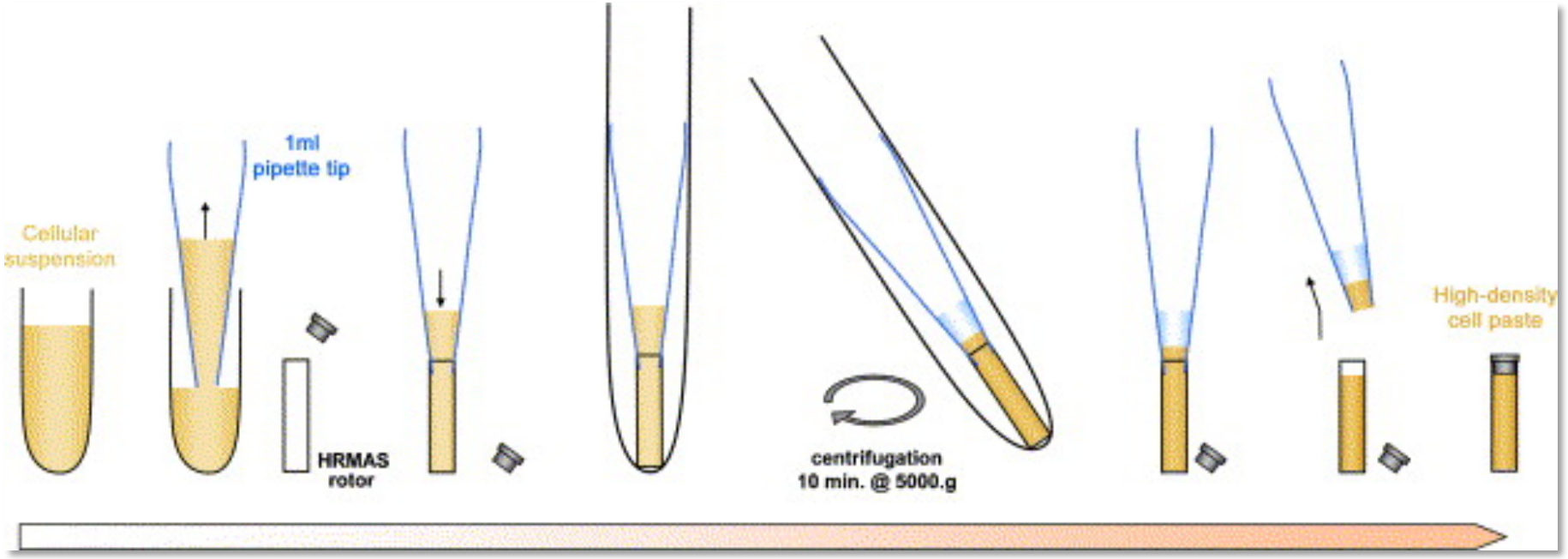
- No purification needed
- NMR signal is proportional to quantity AND mobility of molecules
- Holistic vision of cell surface
- Observe mobile components, potentially present at the surface of cells

Ideally suited for the study of surface glycocalix

How does it work?



How to fill the rotor?



You need 12 to 200 µL of cell paste

Hanoulle *et al.* BBRC 2005

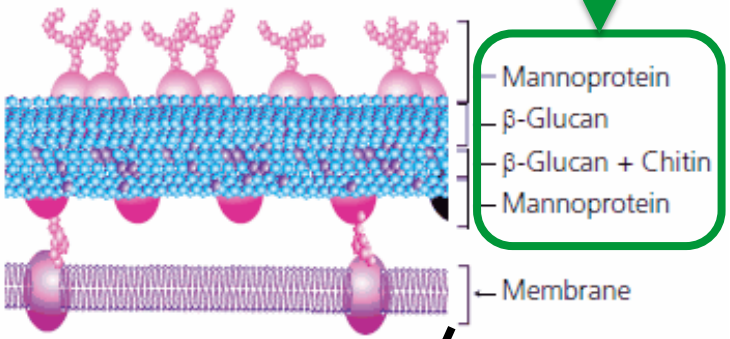
How to acquire the data?

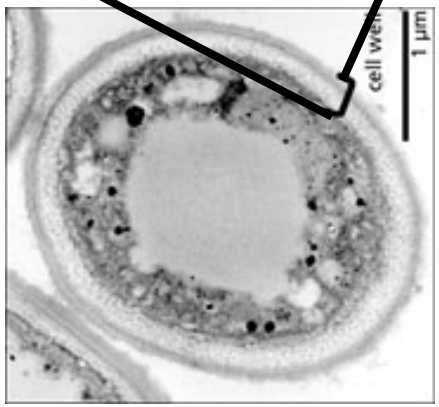
- Load into HR-MAS probe
- Tilt to 54,74° magic angle
- Rotate to 8000rd/s
- Acquire data with any NMR pulse

Cell walls of microbes

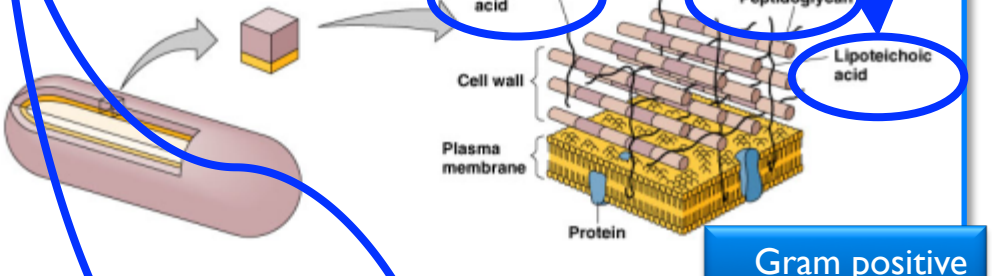
SUGARS

Fungus

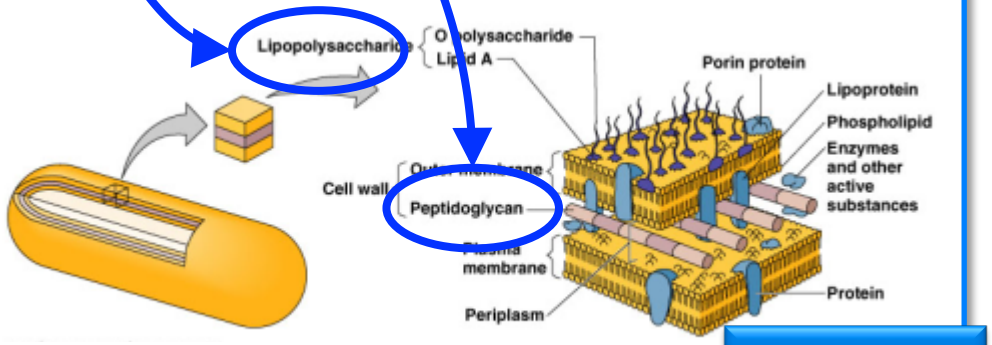




Bacteria

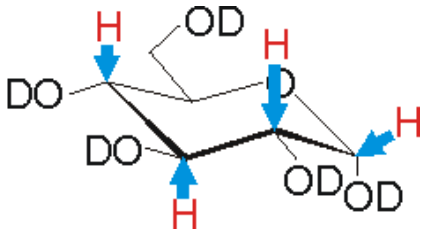


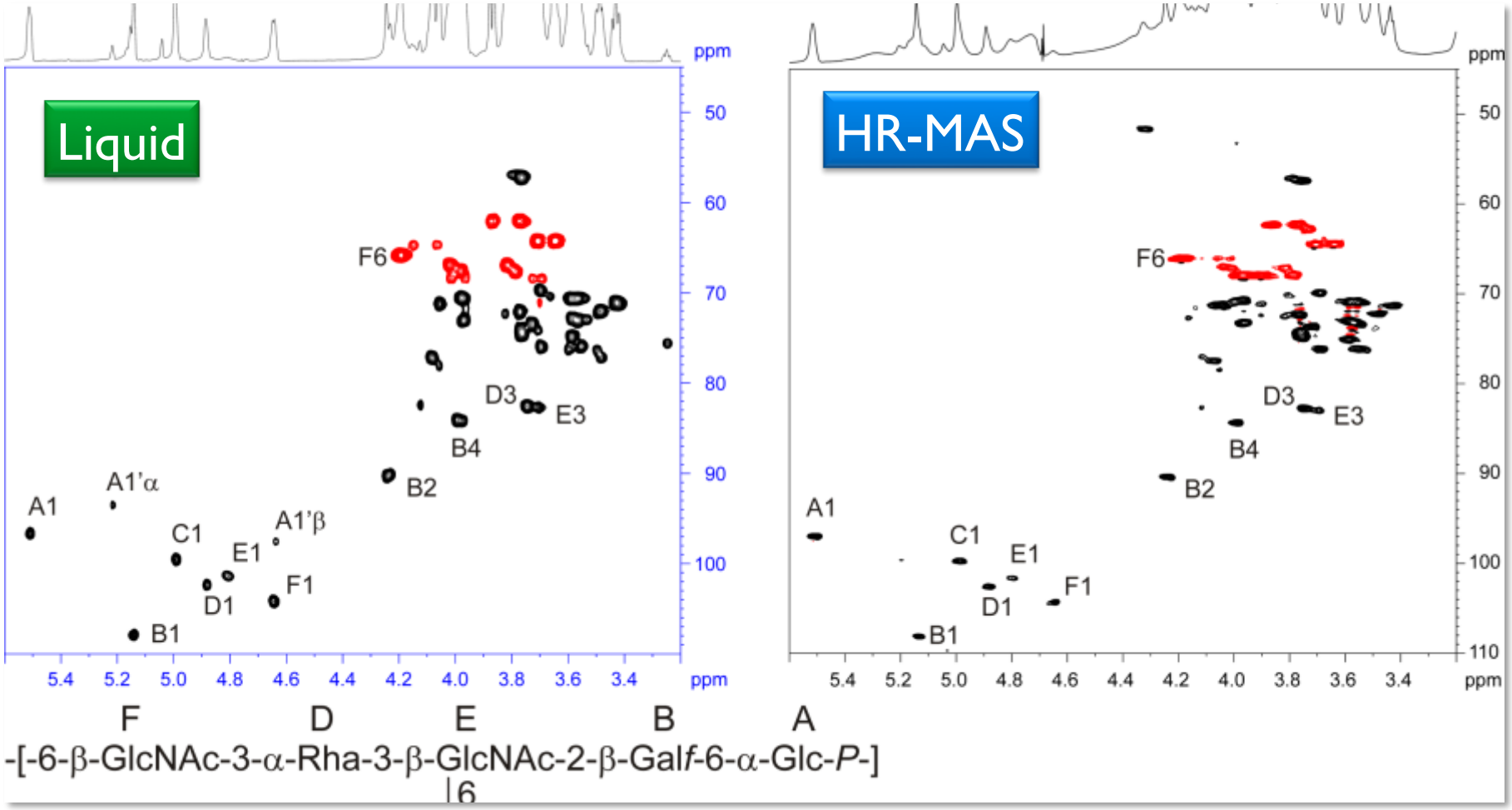
Gram positive



Gram negative

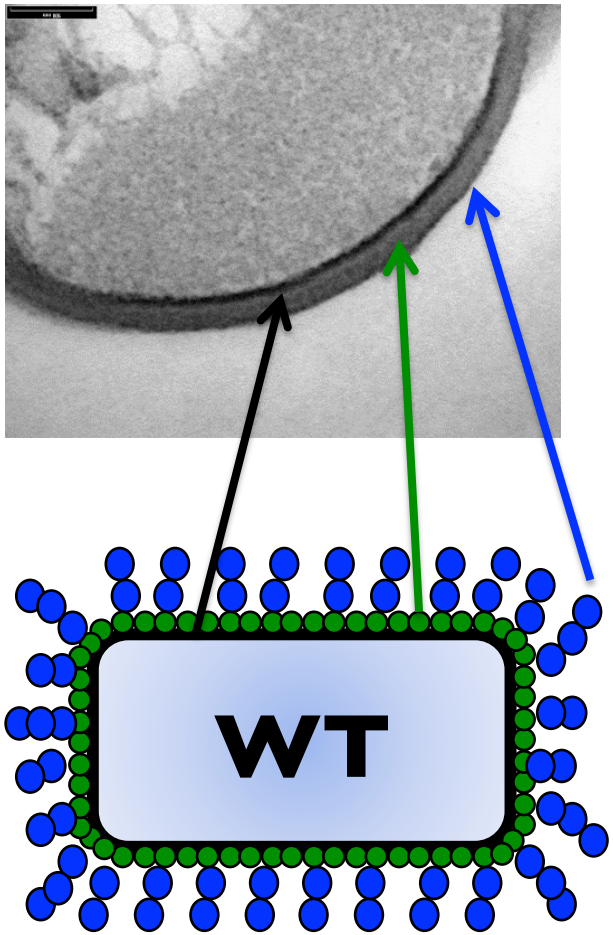
Liquid vs HR-MAS

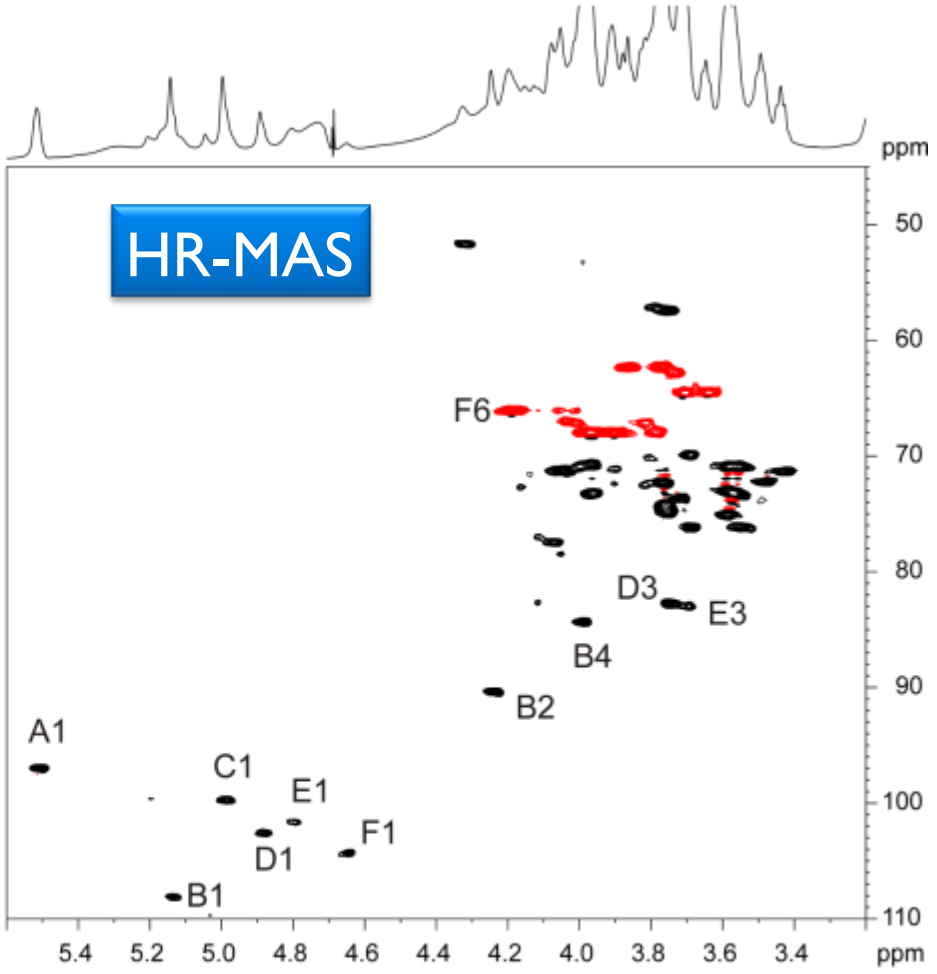




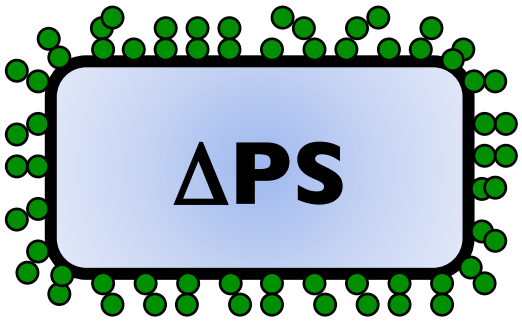
Ainsworth *et al*., mBio 2014

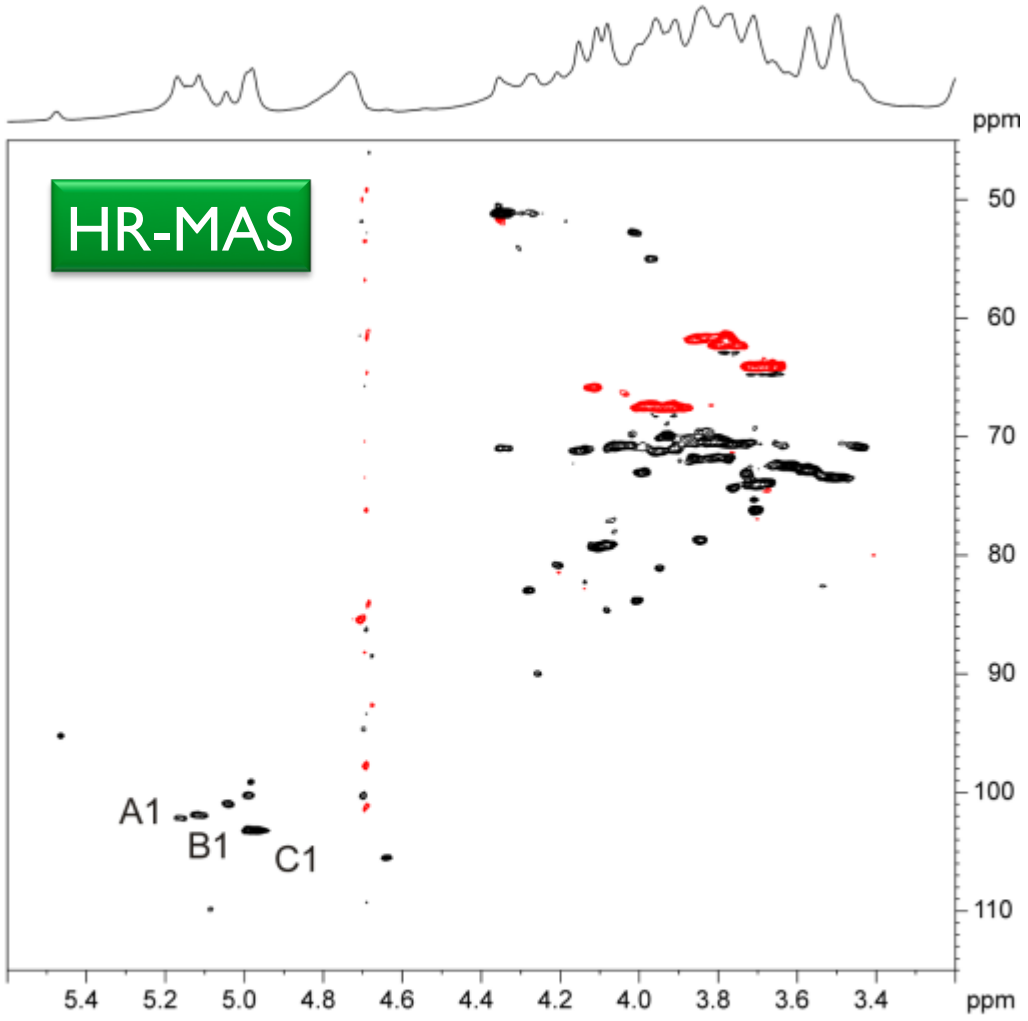
Identification of external free-moving layer



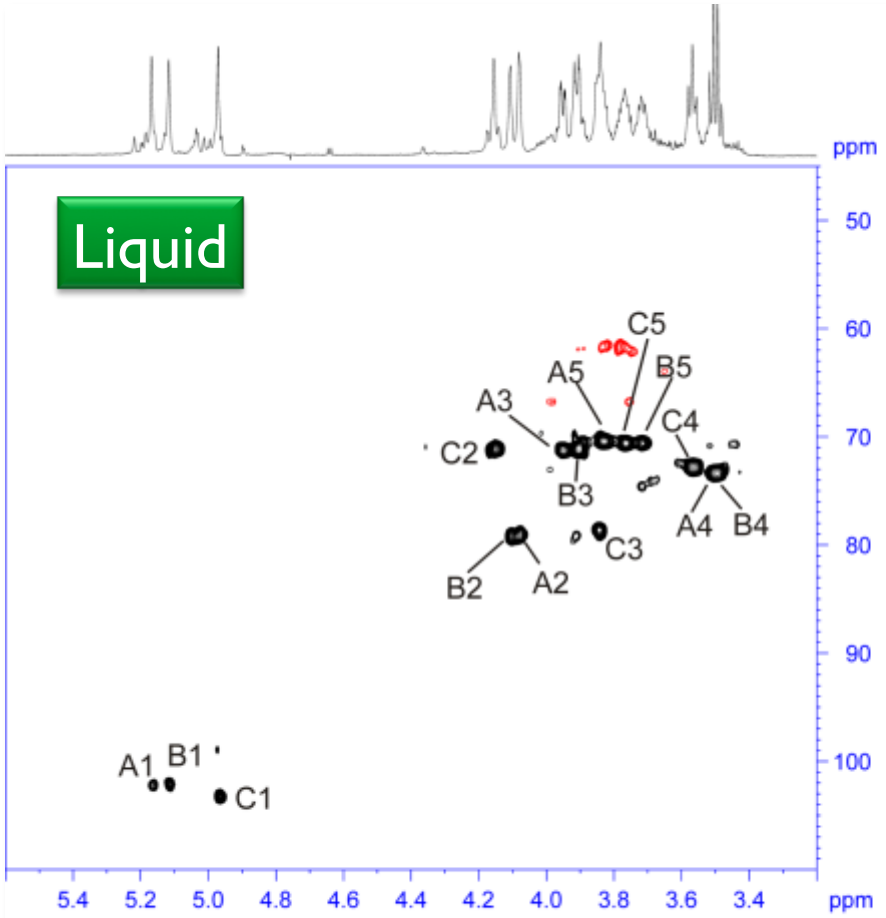


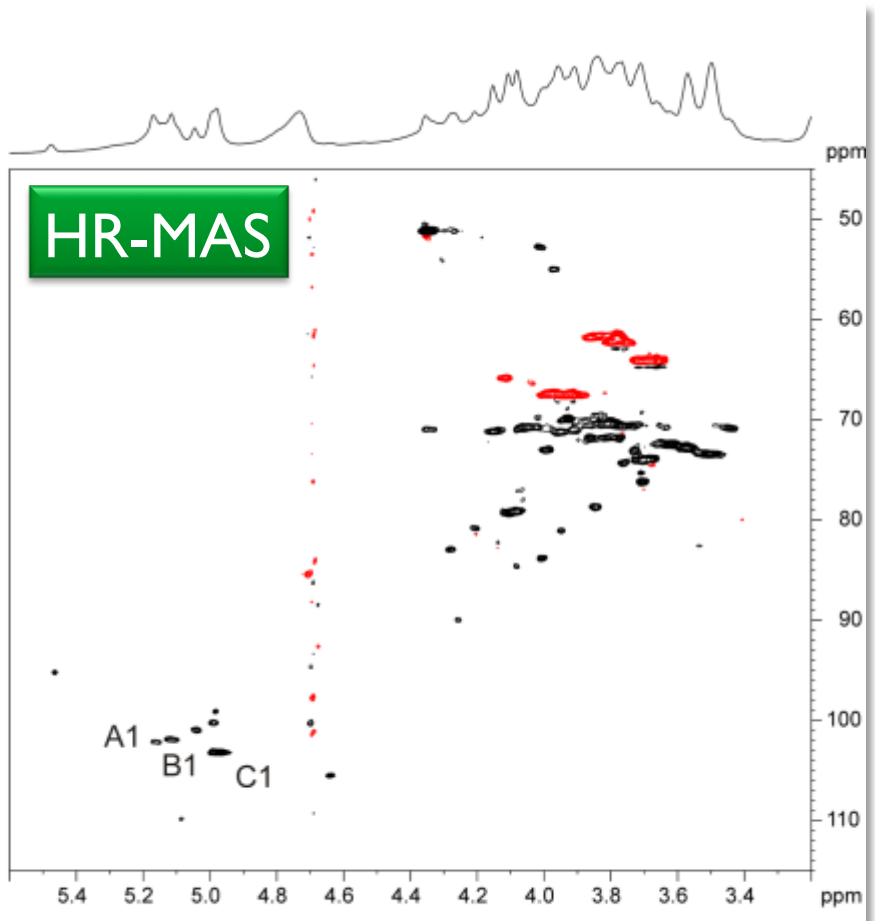
Identification of internal condensed layer





Structural analysis of internal condensed layer



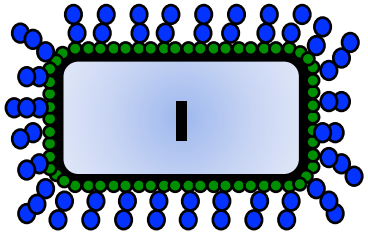


B A C

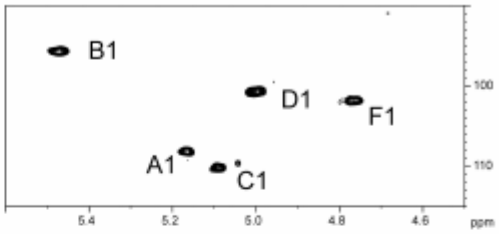
-2-α-L-Rha-2-α-L-Rha-3-α-L-Rha-

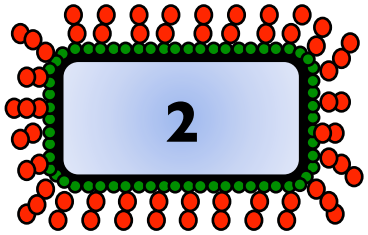
Polysaccharides shuffling

HR-MAS

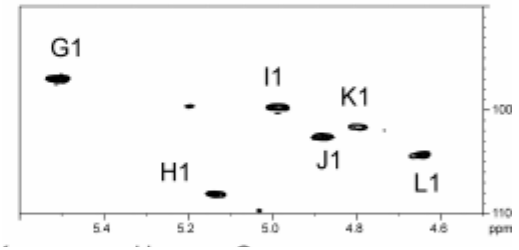


-(6-αGlc-3βGal-3βGlcNAc-2βGal-
-6αGlcNAc-P)



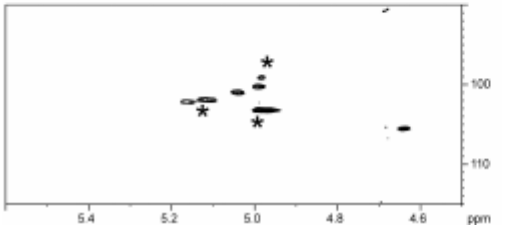


(6βGlcNAc-3αRha-3βGlcNAc-2β
Gal-6αGlc-P)
I
αGlc

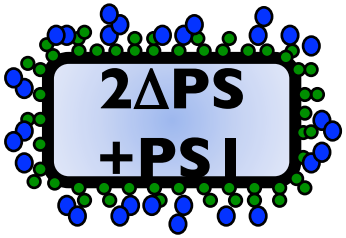


Minus PS2

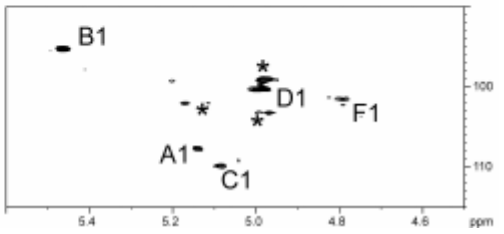
-2αLRha-2αLRha-3αLRha



Plus PS1



-2αLRha-2αLRha-3αLRha
+
(6βGlcNAc-3αRha-3βGlcNAc-2β
Gal-6αGlc-P)
I
αGlc



Applications

- Bacterial surface polysaccharides
- Bacterial glycolipids
- Bacterial lipids
- Bacterial metabolites
- Fungus polysaccharides and glycoproteins
- Many others to try and lot's of fun…/…

Usage

- Screen mutants/culture conditions
- Discover molecules
- Follow metabolism
- Check the *in vivo* structure

What use of NMR for glycobiology?

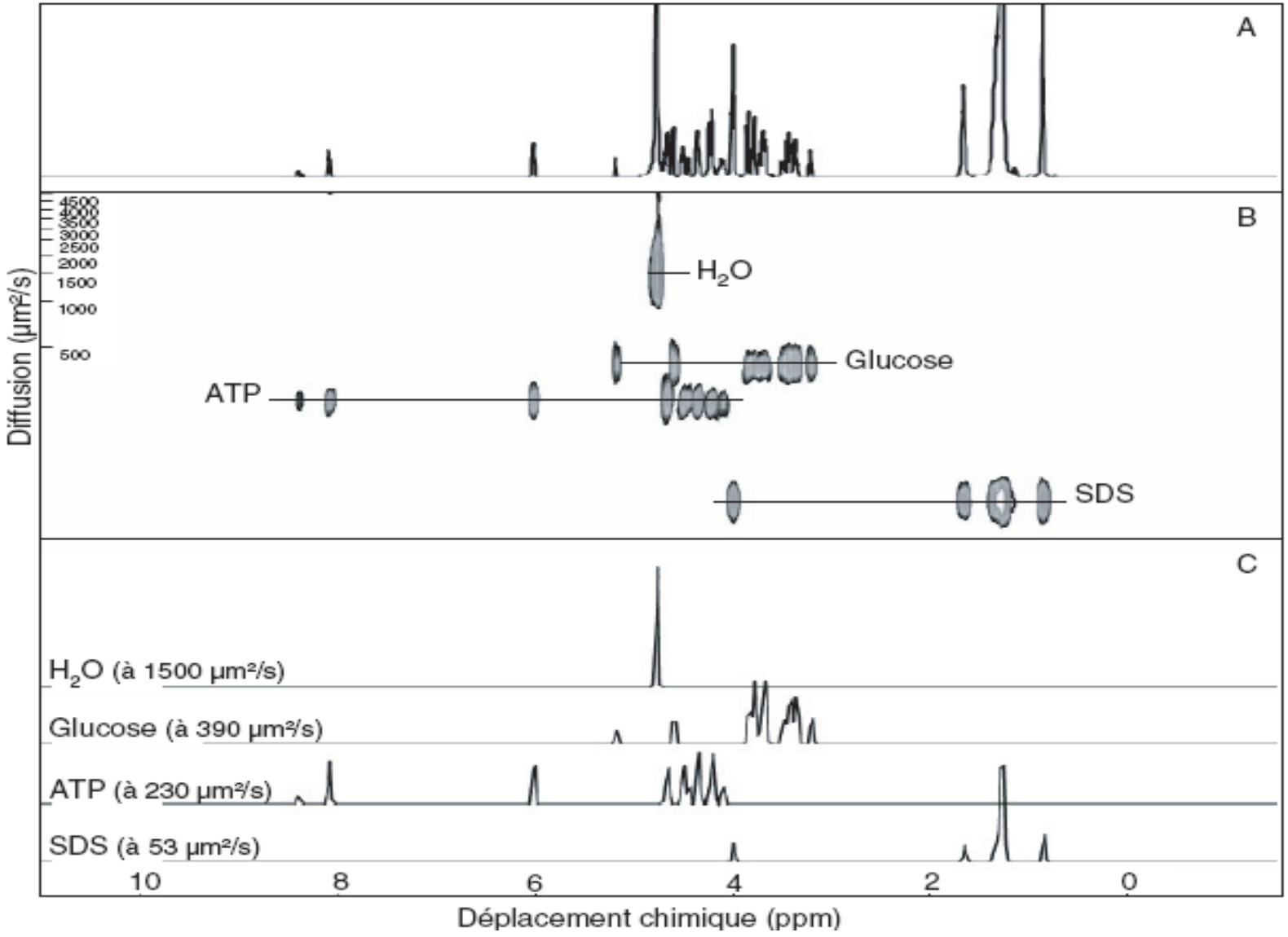
- DOSY NMR

DOSY

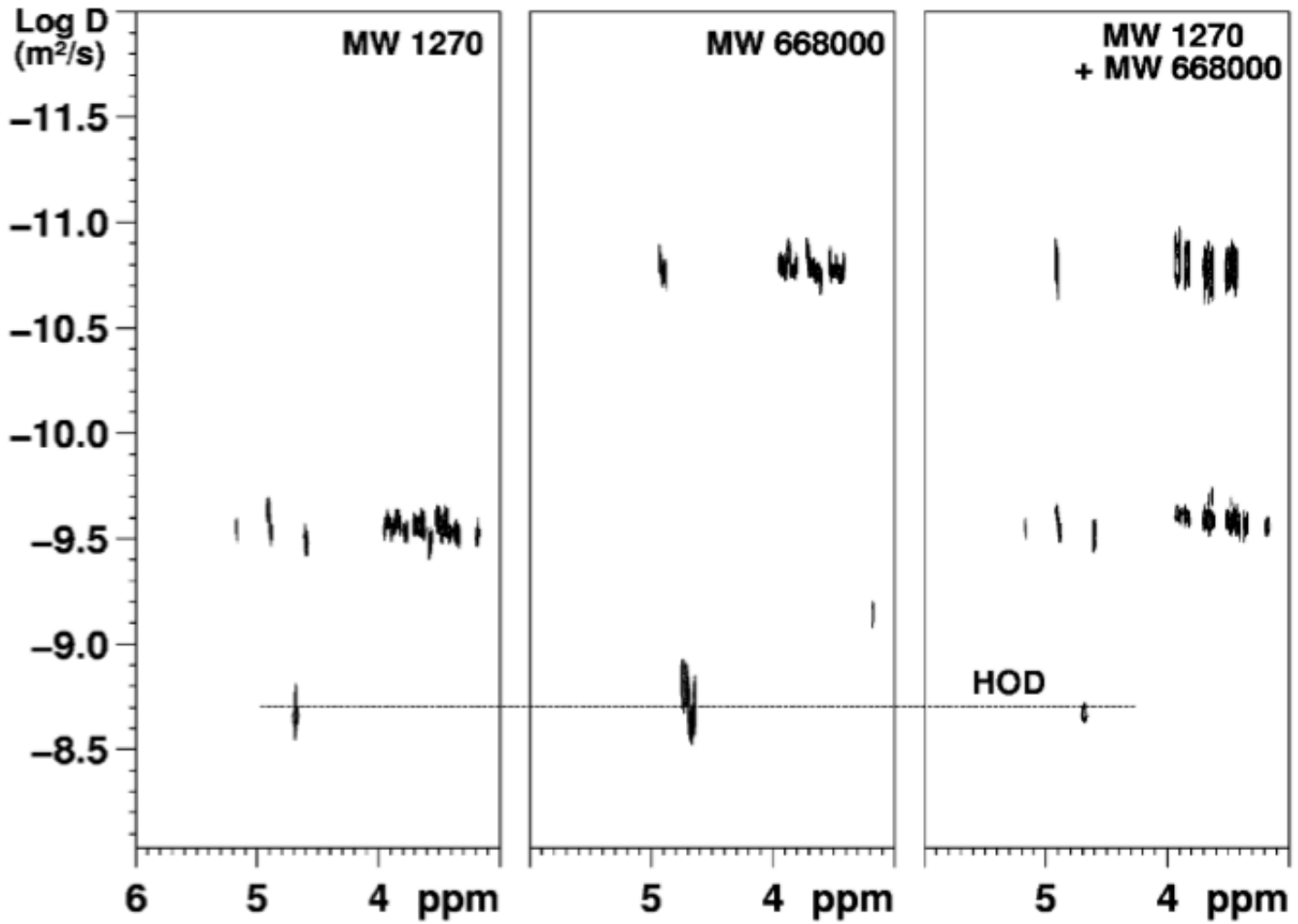
Diffusion-ordered spectroscopy (DOSY)

- separate the NMR signals of different species according to their diffusion coefficient.
- resolve mixtures of molecules with differing sizes
- referred as ‘NMR size exclusion chromatography’

DOSY

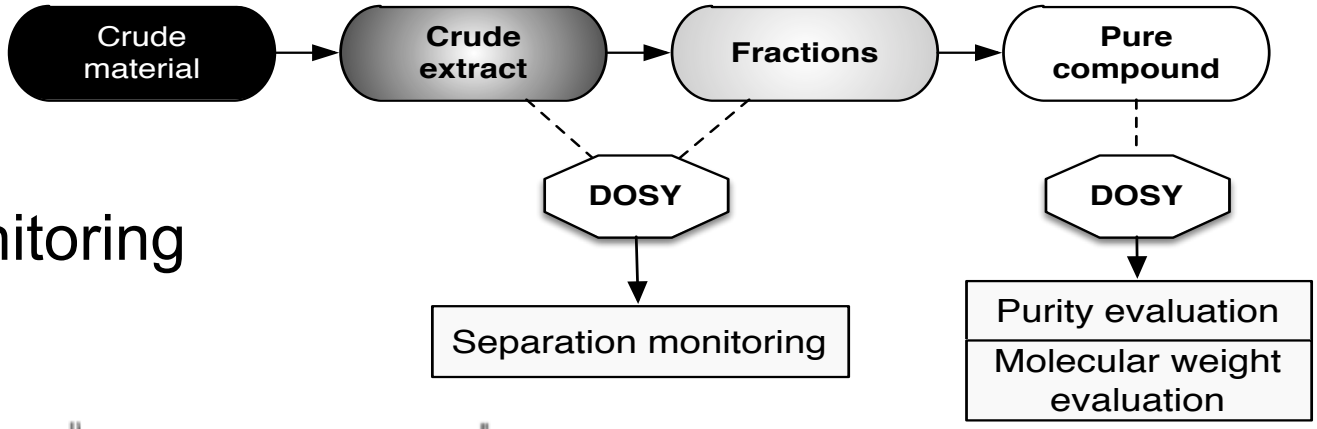

A
B
C
Diffusion (µm²/s)
4500
4000
3500
3000
2500
2000
1500
1000
500
H2O
Glucose
ATP
SDS
H2O (à 1500 µm²/s)
Glucose (à 390 µm²/s)
ATP (à 230 µm²/s)
SDS (à 53 µm²/s)
10
8
6
4
2
0
Déplacement chimique (ppm)

DOSY

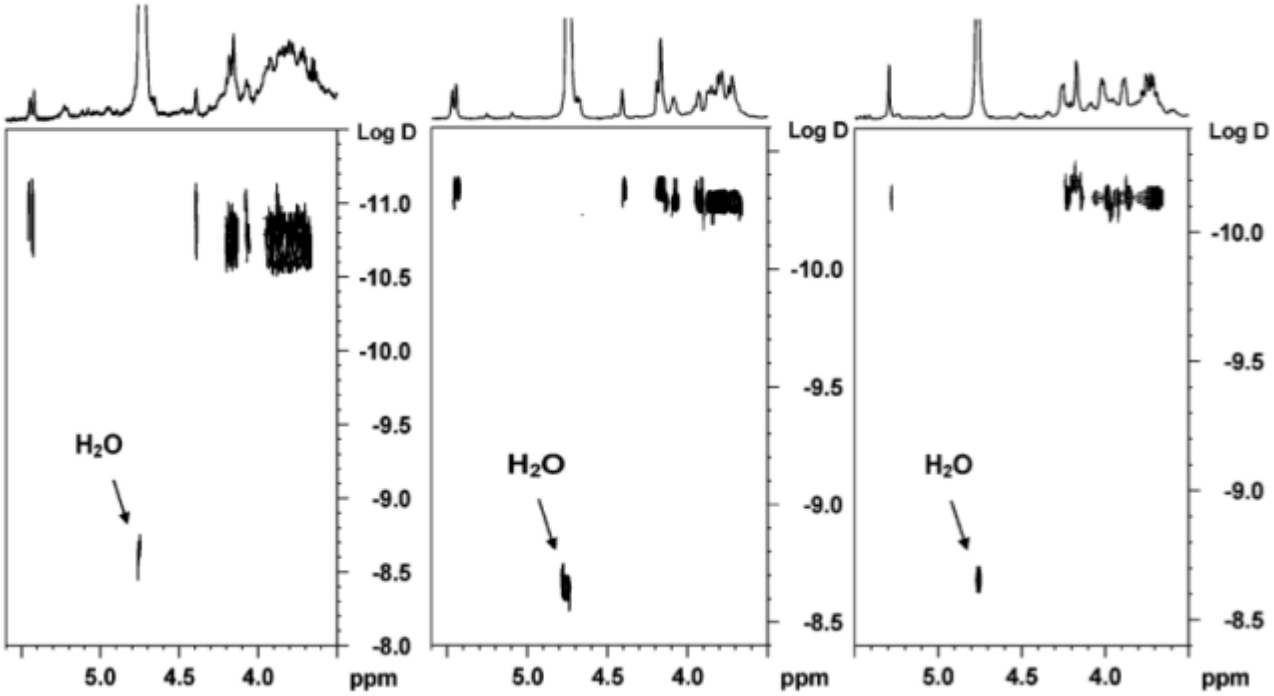


Viel *et al*. *Biomacromol.* 2013

DOSY exemples of applications



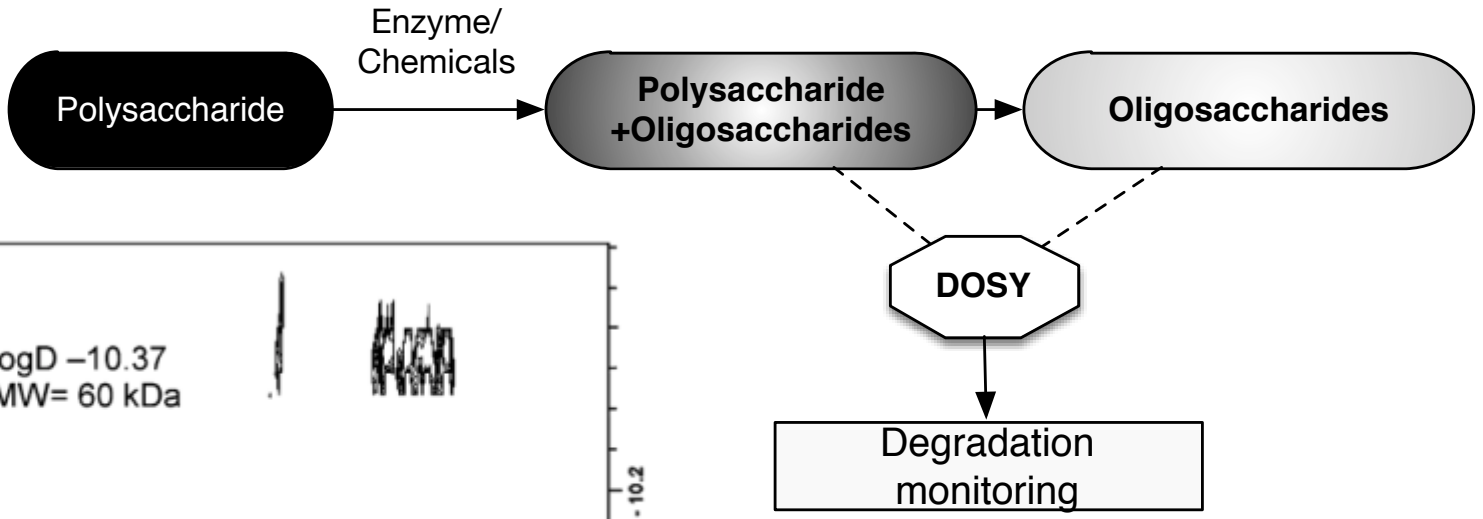
Purification monitoring

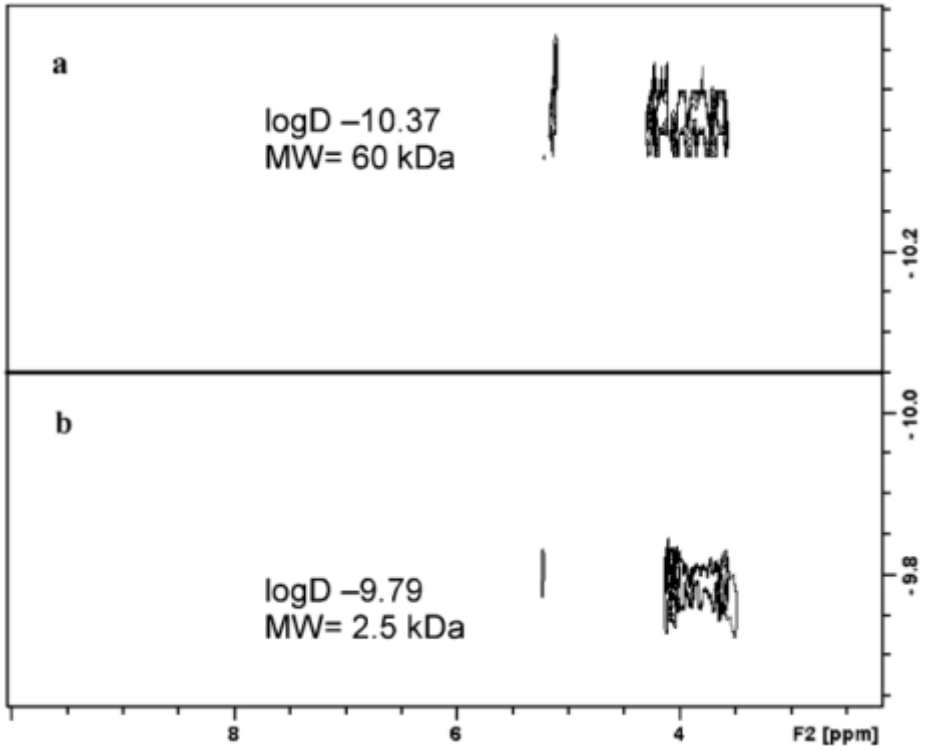


Suarez *et al*. *Biomacromol.* 2006

DOSY exemples of applications

Depolymerization monitoring



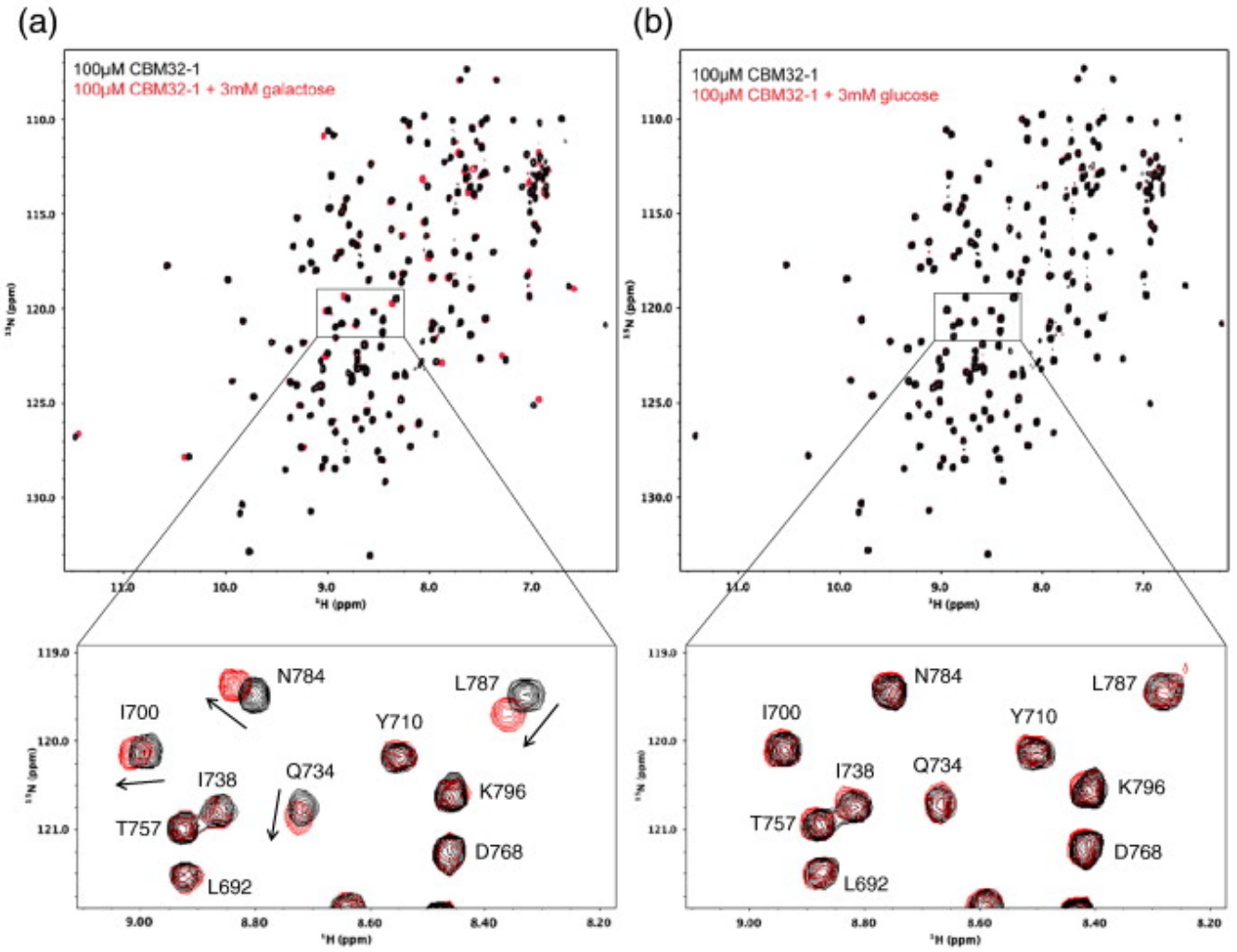


Pietro *et al*. *glycoconj.* 2007

What use of NMR for glycobiology?

- Protein-carbohydrate interaction

Protein interaction by 1H-15N NMR



Galactose

Glucose

Grondin *et al.* JMB 2014

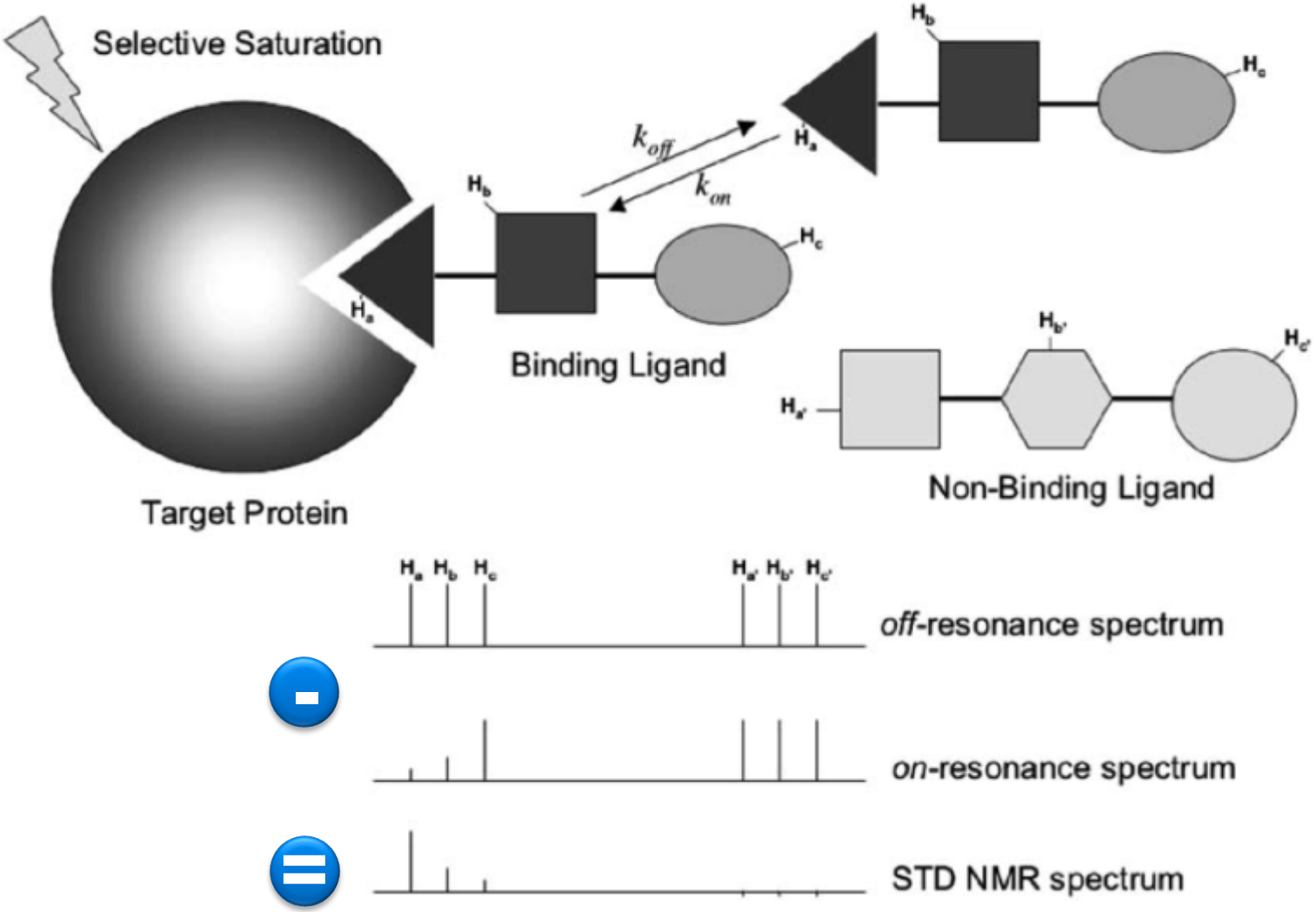
Saturation transfer difference (STD) NMR

Used for studying **protein–ligand** interactions in solution.

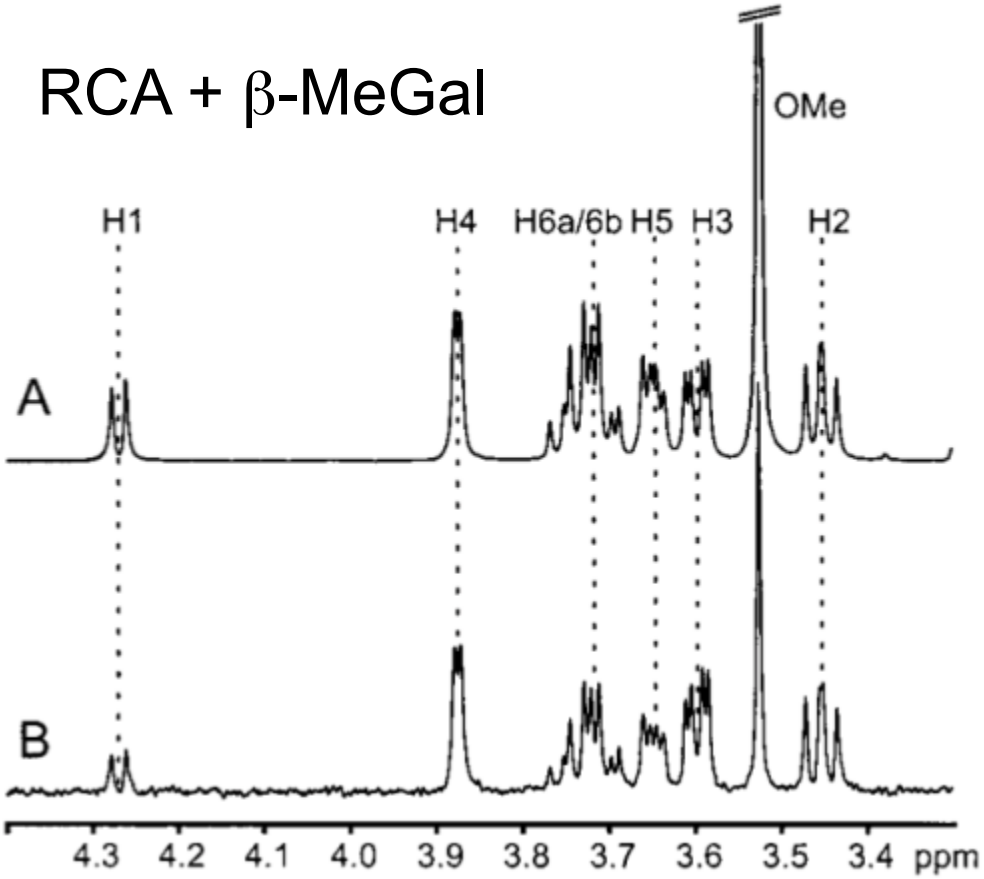
- Identify the binding glycan bound to its receptor protein
- Ligand protons in close contact receive higher saturation, which results in stronger STD NMR signals.
- The STD NMR is easy to implement
- Only small amounts of native protein are required.

Mayer, M., Meyer, B. (1999) Characterization of ligand binding by saturation transfer difference NMR spectroscopy. *Angewandte Chemie, International Edition*, 38, 1784–1788

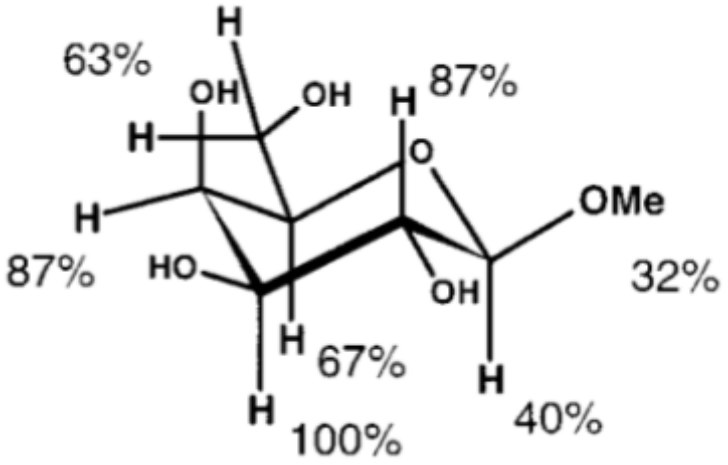
STD NMR


Selective Saturation
k_{off}
k_{on}
Binding Ligand
Target Protein
Non-Binding Ligand
off-resonance spectrum
on-resonance spectrum
STD NMR spectrum

RCA + β-MeGal

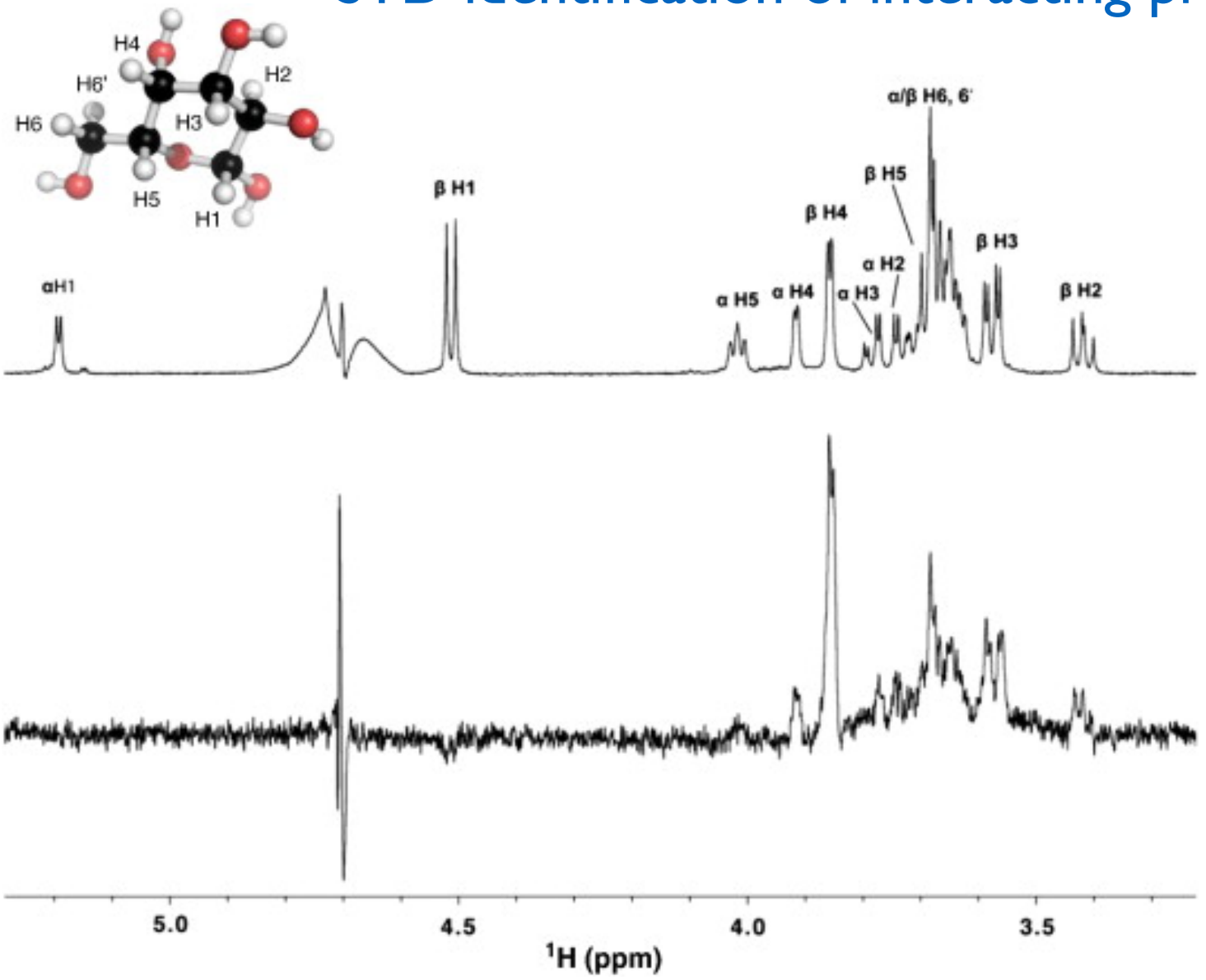


STD



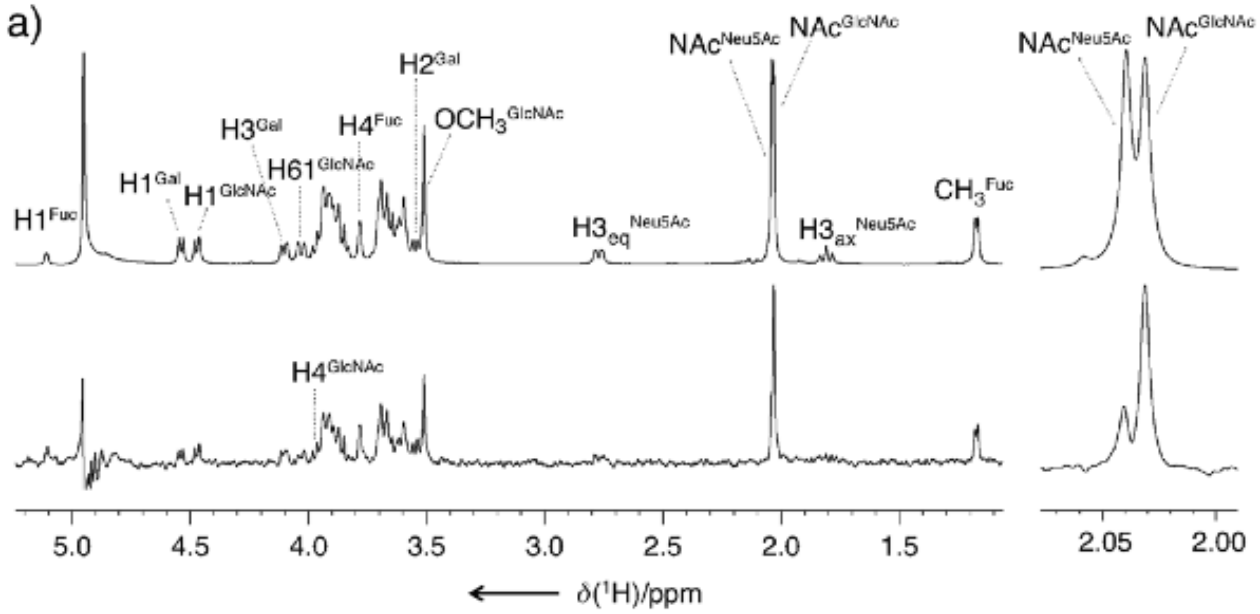
Mayer & Meyer *JACS* 2011

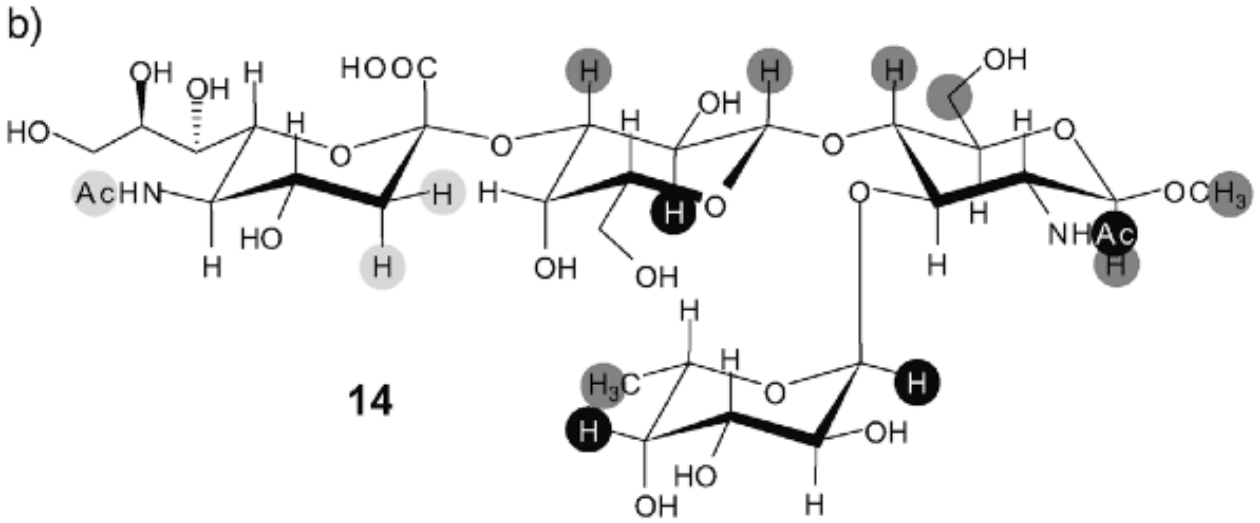
STD identification of interacting protons



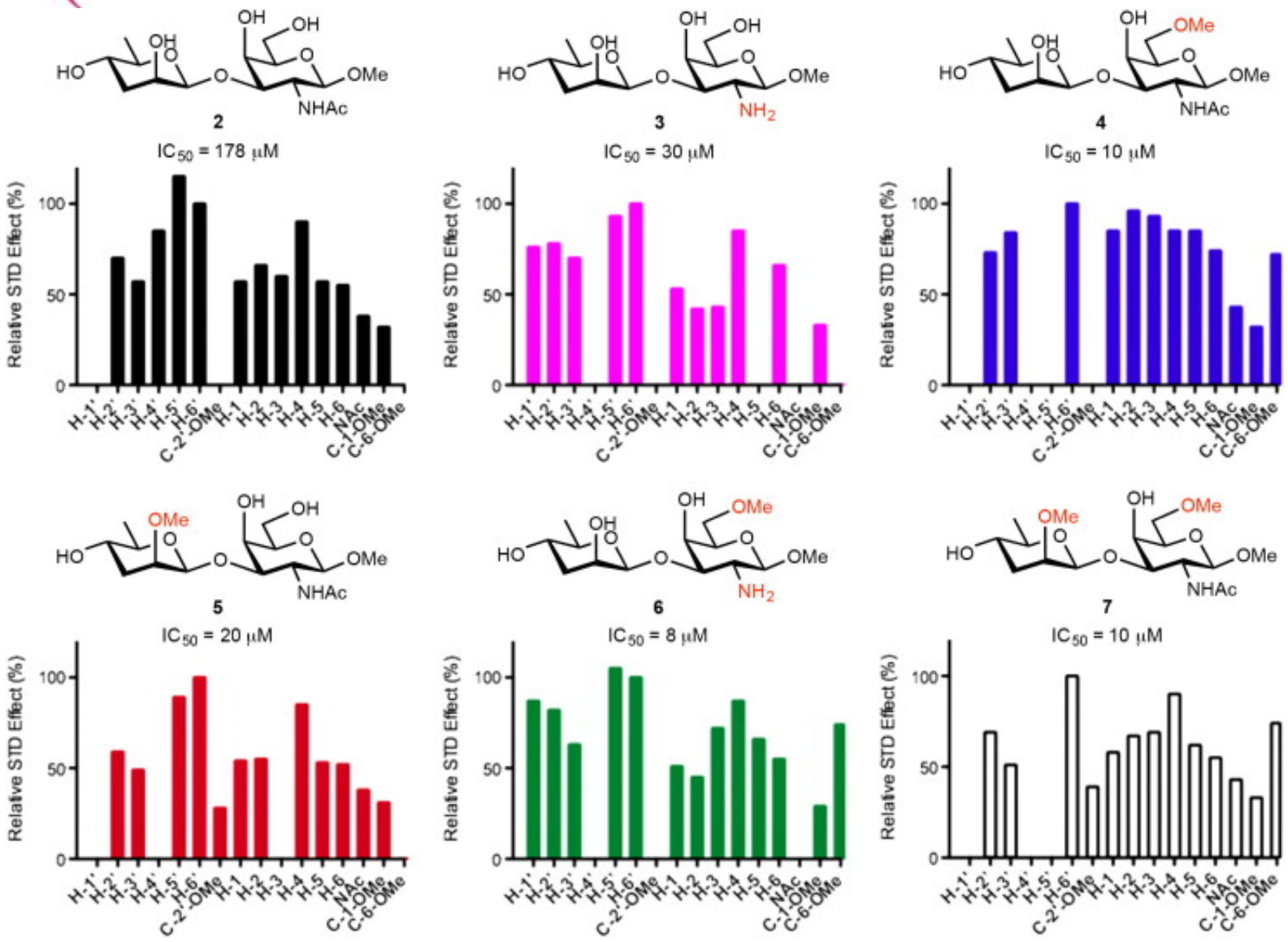
Grondin *et al.* JMB 2014

Interaction between Slex and virus particles





Fiege et al. *JACS* 2012



Cui *et al.*, CR 2014

Acknowledgement

E. Maes

NMR Expert
Carbohydrates

X. Trivelli

NMR Expert
Small molecules
Proteins

IR THC RMN