Introduction to Nuclear Magnetic Resonance (NMR) Spectroscopy

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Phcg631 – Analysis of Natural Product Drugs I

(NMR Structure Elucidation)

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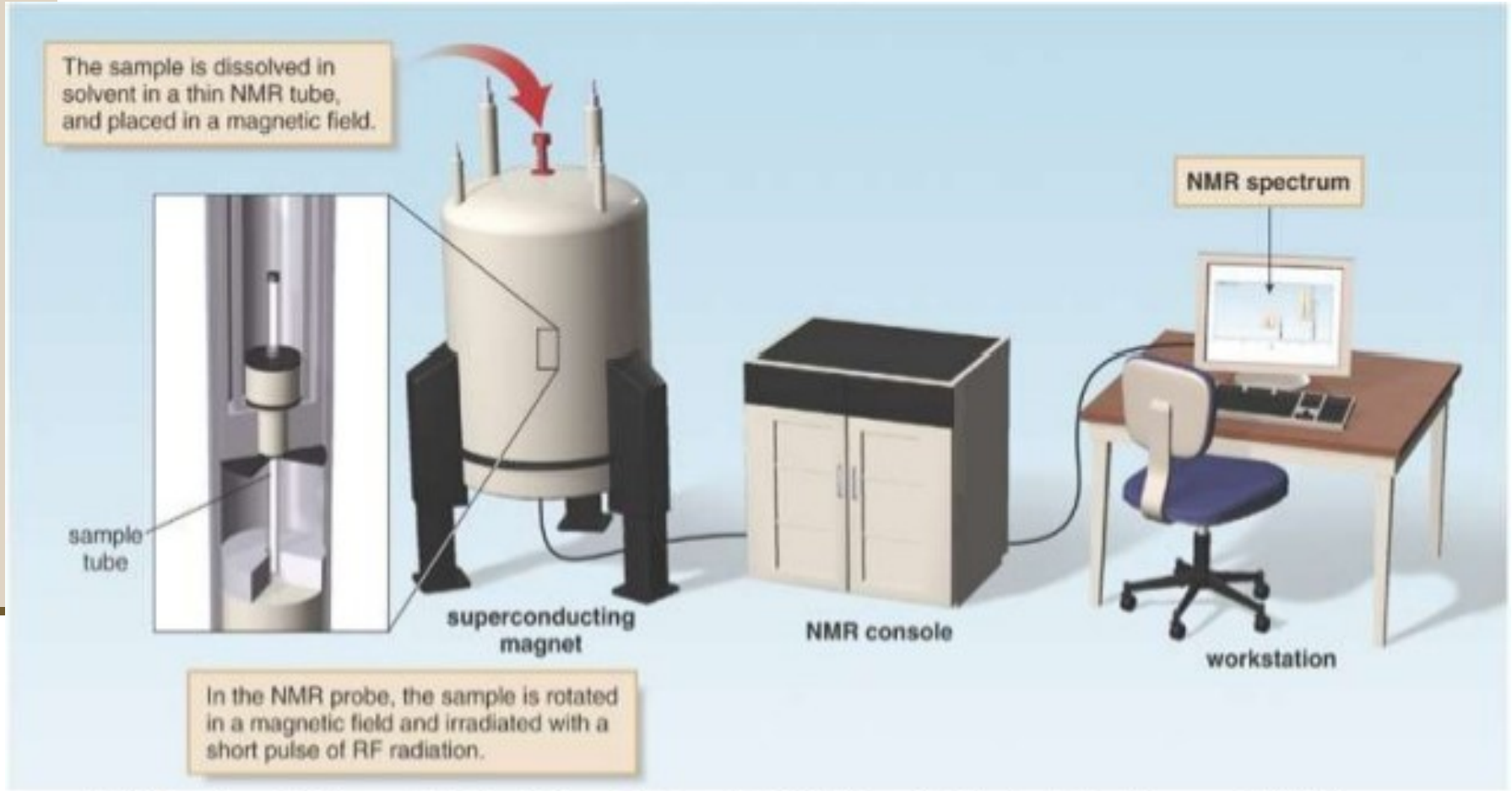
The NMR instrument!



An NMR spectrometer. The sample is dissolved in a solvent, usually $CDCl_3$ (deuterochloroform), and placed in a magnetic field. A radiofrequency generator then irradiates the sample with a short pulse of radiation, causing resonance. When the nuclei fall back to their lower energy state, the detector measures the energy released, and a spectrum is recorded. The superconducting magnets in modern NMR spectrometers have coils that are cooled in liquid helium and conduct electricity with essentially no resistance.

The NMR instrument!

The magnetic field!

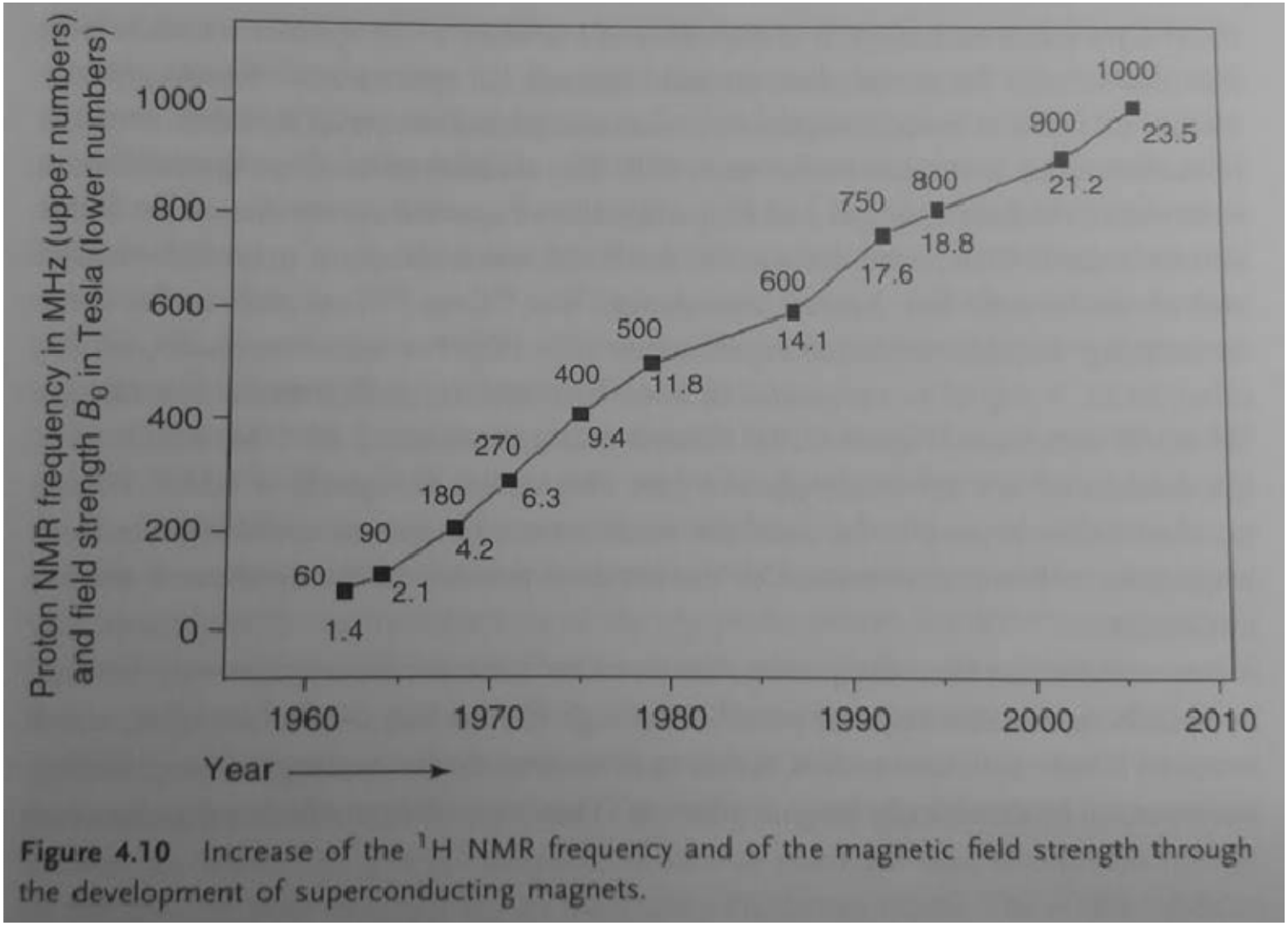
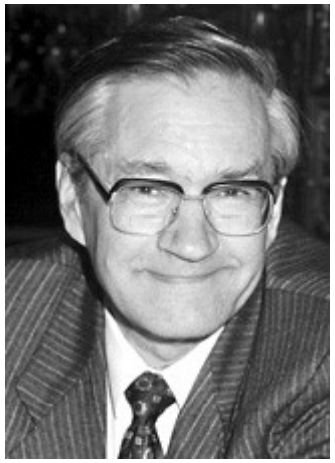


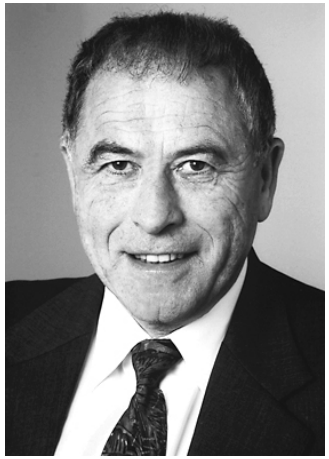
Figure 4.10 Increase of the ^{1}H NMR frequency and of the magnetic field strength through the development of superconducting magnets.

Nobel Prize in Chemistry - NMR

Richard R. Ernst 1991
"for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy".



- **Kurt Wüthrich 2002**
- *"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"*



Why NMR is so important?

- Solving structures of compounds like synthetics, impurities, natural products
- Identifying metabolites (NMR metabolomics, sTOCSY)
- Stereochemistry determination
- Follow reaction progress (hydrolysis, product formation, substrate consumption)
- Kinetics (dissociation constant, K_d of complexes, on-off rates in receptor ligand)
- Biomolecular nmr (protein, nucleic acids and carbohydrates)
- Molecular interactions (ligand binding sites, ligand binding poses, conformational changes upon interaction)
- Atomic distribution, quantities, bonds between atoms and elements
- Solid-state vs Liquid-state
- Imaging (MRI) for medicine diagnostic

Pros!

- Nondestructive analytical method.
- Multiple applications: structure elucidation, dynamics in solution, conformation, molecular complexes, reaction and somewhat function.

Cons!

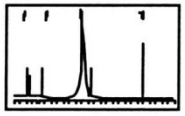
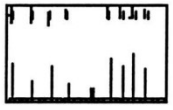
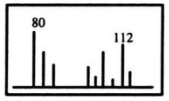
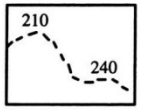
- NMR is one of the least sensitive analytical methods.
- Time-consuming.
- Sometimes the amount of information is big. Can be demanding on interpretation skills.
- Relatively expensive compared with other analytical methods (use of deuterated solvents, NMR cooling gases, sample tubes).
- As with other methods, NMR has “blind spots” and, depending on the goal, cannot serve as the only analytical technique.

NMR as compared to other spectroscopic methods

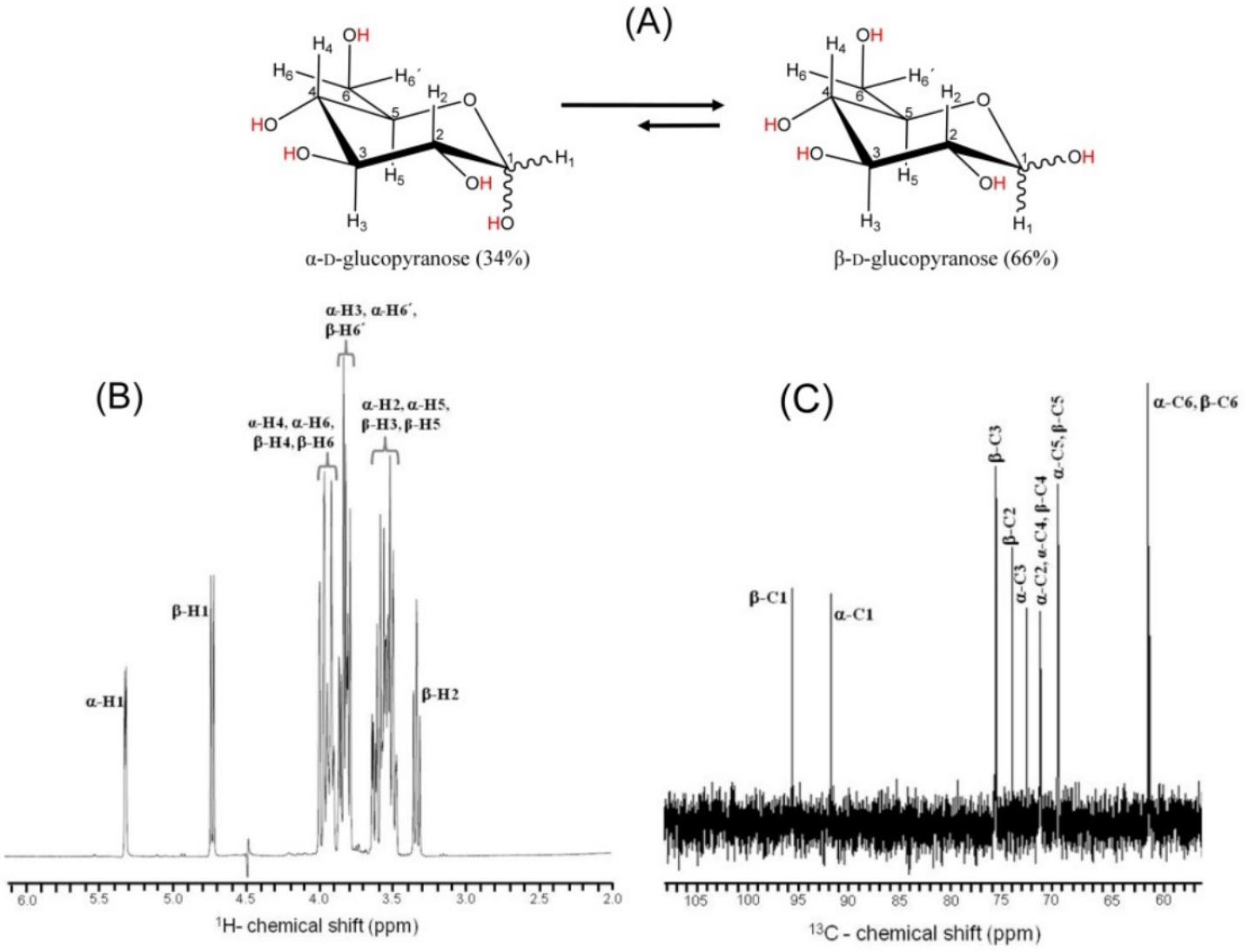
Modern techniques for structure determination of organic compounds include:

- **Mass spectrometry**
  - MW and chemical formula of the compounds
  - Structural information (fragmentation patterns)
- Infrared spectroscopy
  - Presence or absence of functional groups in compounds
- Ultraviolet-Visible spectroscopy
  - Absorption and reflectance in electron energy is directly related to atomic/molecular composition of compounds
- Nuclear magnetic resonance spectroscopy
  - Atomic (^{1}H, ^{13}C, ^{15}N, ^{19}F) framework of the compounds
  - Stereochemical view (conformation via NOE)
  - Dynamics and intermolecular interactions

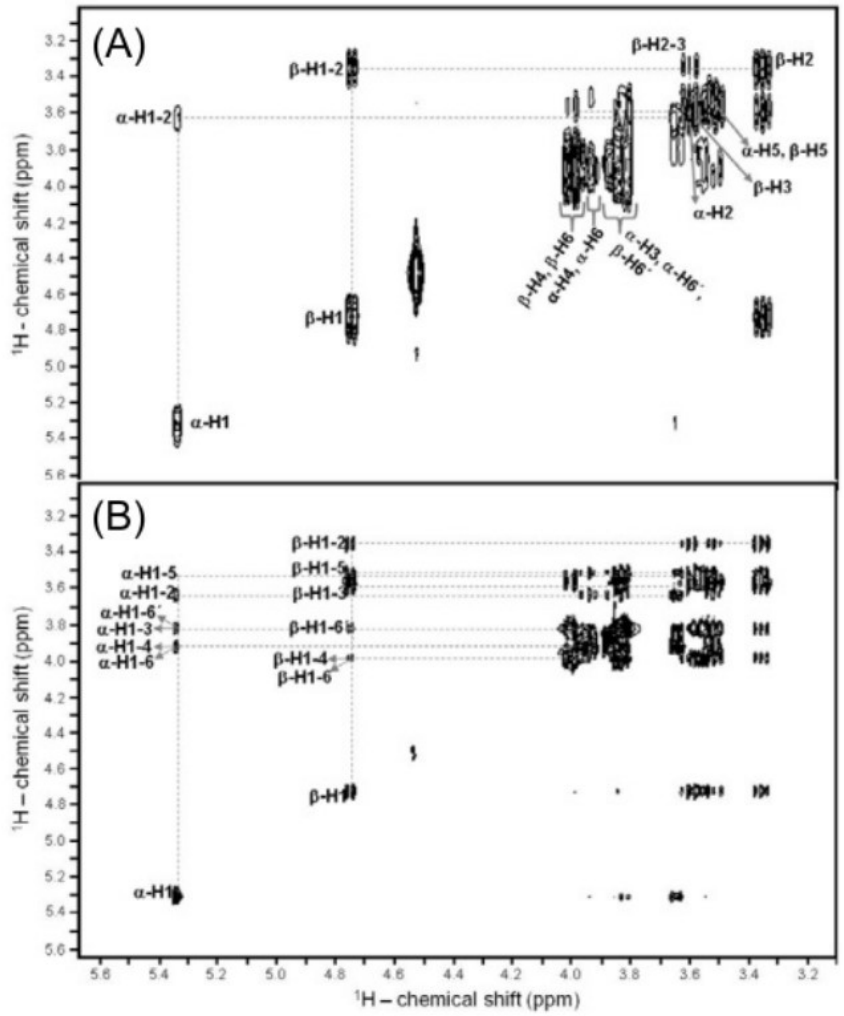
Characteristics of Important Spectroscopic Methods

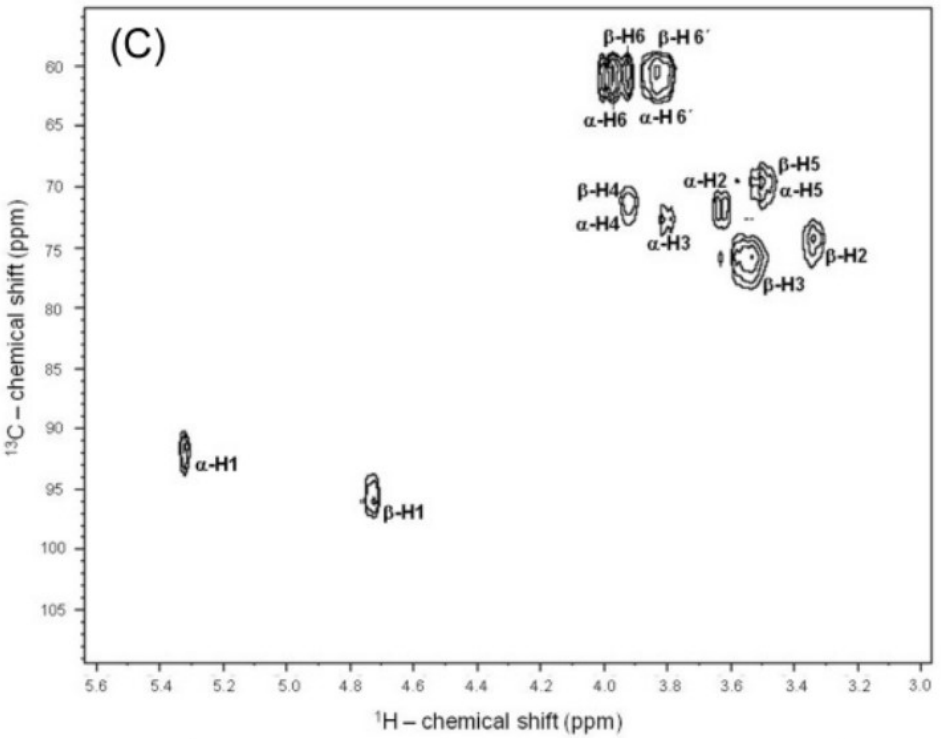
	H-1	*C-13*	*MS*	*IR/RAMAN*	*UV-VIS*	*ORD/CD*	*X-RAY*
•*Radiation type*	RF	RF	Not relevant	IR	UV to visible	UV to visible	X-ray
•*Spectral scale*	0-15 ppm	0-220 ppm	50-4000 amu	400-4000 cm^{-1}	200-800 nm	185-600 nm	Not relevant
•*Average sample*	≅ 1 mg	≅ 5 mg	< 1 mg	< 1 mg	< 1 mg	< 1 mg	Single crystal
•*Molecular formula*	Partial	Partial	Yes	No	No	No	Yes
•*Functional groups*	Yes	Yes	Limited	Yes	Very limited	Very limited	Yes
•*Substructures*	Yes	Limited	Yes	Limited	Limited	No	Yes
•*Carbon connectivity*	Yes	Yes	No	No	No	No	Yes
•*Substituent regiochemistry*	Yes	Yes	No	Limited	No	No	Yes
•*Substituent stereochemistry*	Yes	Yes	No	Limited	No	No	Yes
•*Analysis of isomer mixtures*	Yes	Yes	Yes (by GC/MS LC/MS)	Yes (by GC/IR)	No	No	Yes (if separate)
•*Purity information*	Yes	Yes	Yes	Yes	Limited	Limited	Limited
•*What is measured*	Peak areas Chemical shifts Coupling relaxation	Chemical shifts Coupling relaxation	Singly or multiple charged ions	Vibrational transitions	Electronic transitions	[α]	Relative atom positions *R/S* absolute stereochemistry
•*Typical units*	δ (ppm)	δ (ppm)	*m/z*	cm^{-1}	nm	nm	-
•*Typical representations*							 ORTEP

Structure elucidation (1D spectra)



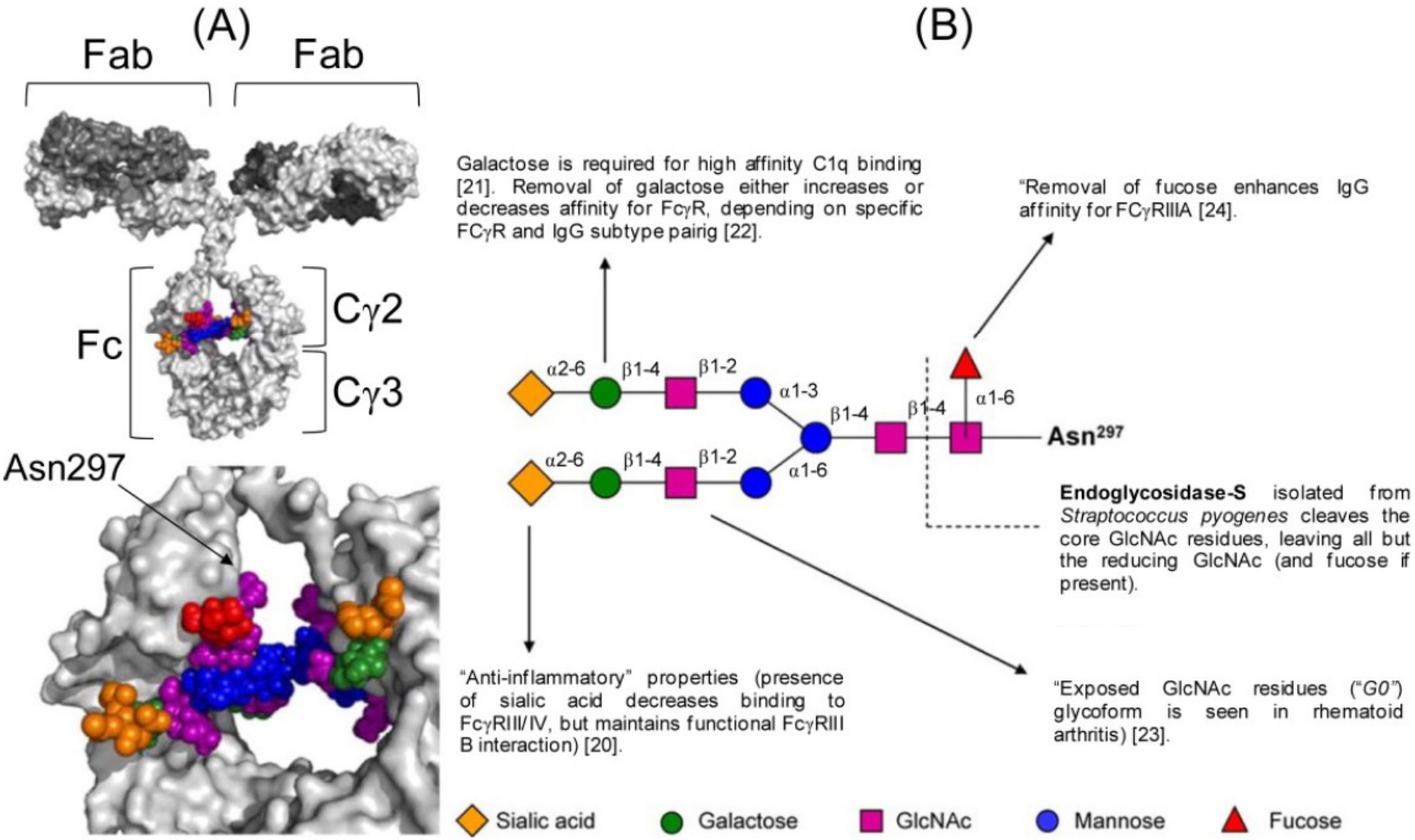
Structure elucidation (2D spectra)



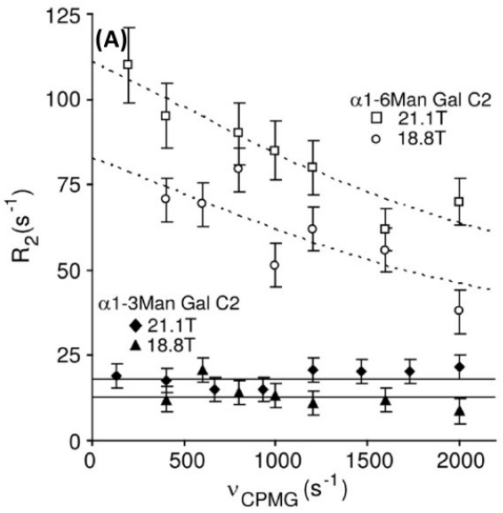


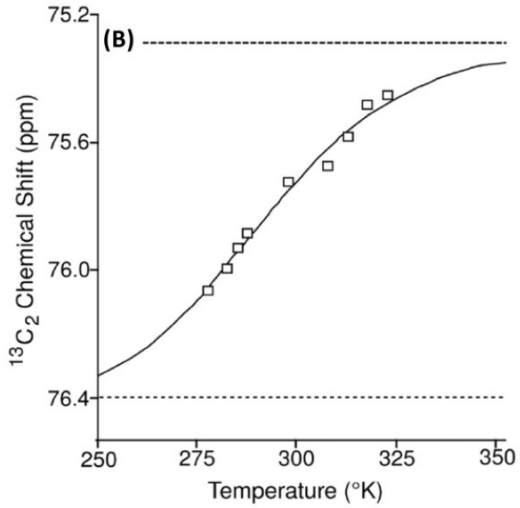
α-D-Glc*p*	β-D-Glc*p*
α-H1 – 5.32	β-H1 – 4.74
α-H2 – 3.63	β-H2 – 3.37
α-H3 – 3.83	β-H3 – 3.60
α-H4 – 3.92	β-H4 – 3.92
α-H5 – 3.50	β-H5 – 3.5
H6/6´ - 3.91/3.82	H6/6´ - 3.91/3.82
α-C1 – 91.4	β-C1 – 95.9
α-C2 – 71.8	β-C2 – 74.1
α-C3 – 72.4	β-C3 – 75.8
α-C4 – 71.2	β-C4 – 71.2
α-C5 – 69.8	β-C5 – 69.8
α-C6 – 60.3	β-C6 – 60.3

Molecular dynamics (spin-relaxation)

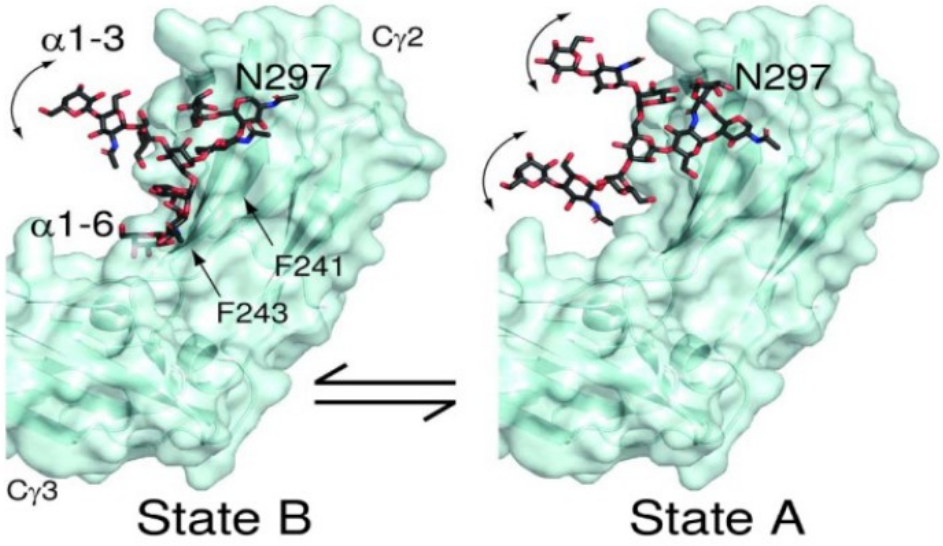


Molecular dynamics (spin-relaxation)

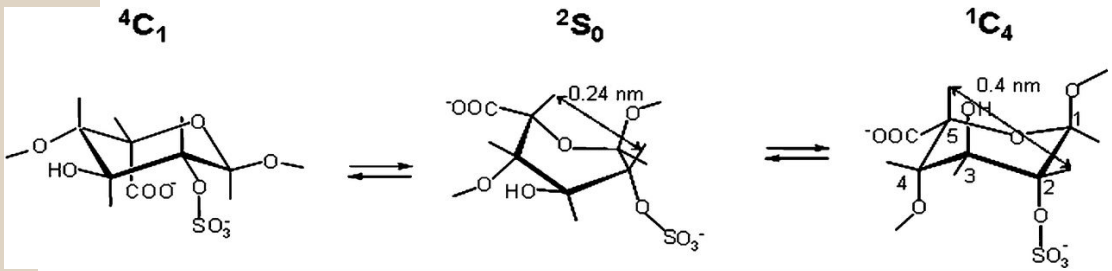




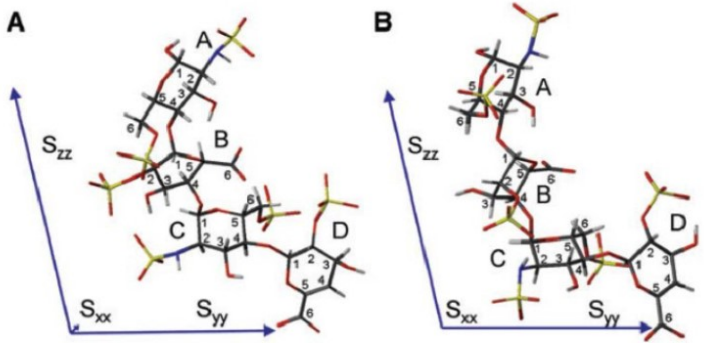
	α1-3Man-linked	α1-6Man-linked
	Relaxation rates	Relaxation rates
900 MHz	**Galactose ^{13}C6**	**Galactose ^{13}C6**
R_1	2.8 ±0.1 s^{-1}	3.1 ±0.4 s^{-1}
R_2	48.4 ±5.5 s^{-1}	59.7 ±9.2 s^{-1}
900 MHz	**Galactose ^{13}C2**	**Galactose ^{13}C2**
R_1	1.2 ±0.1 s^{-1}	1.2 ±0.2 s^{-1}
$R_{1\rho}$	20.1 ±1.0 s^{-1}	56.1 ±10 s^{-1}
$R_{2\ linewidths}$	40 s^{-1}	150 s^{-1}
600 MHz		
$R_{1\rho}$	14.7 ±1.1 s^{-1}	48.0 ±5.4 s^{-1}
$R_{2\ linewidths}$	28 s^{-1}	107 s^{-1}
R_2	29 s^{-1}	ND

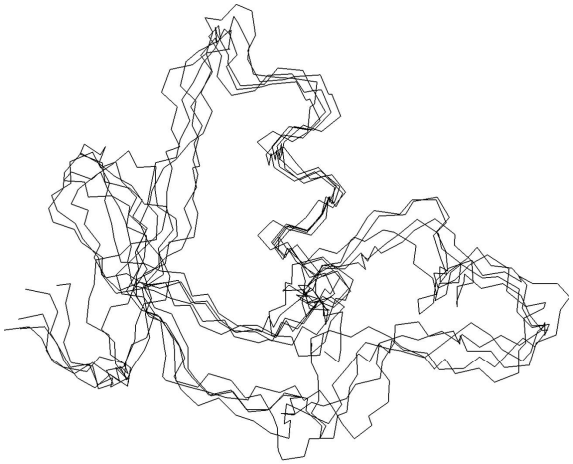


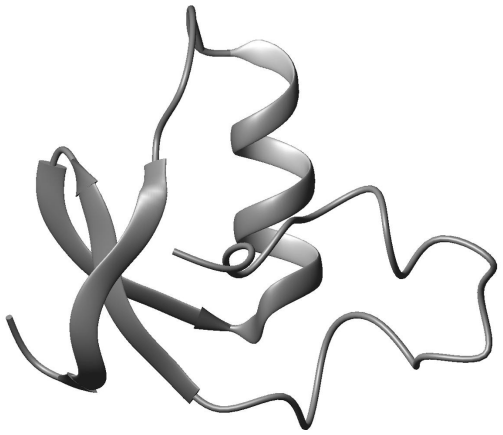
3D Structure (*J*-coupling, NOE, RDC)



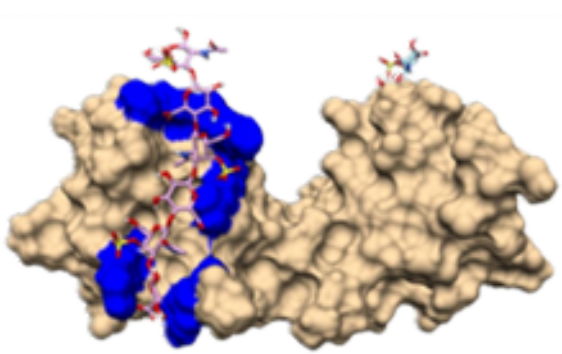
Compound number	$^3J_{H1\text{-}H2}$	$^3J_{H2\text{-}H3}$	$^3J_{H3\text{-}H4}$	$^3J_{H4\text{-}H5}$	$^1C_4$	$^4C_1$	$^2S_0$
1	4.0	6.6	5.2	3.7	45%	29%	26%
2	1.9	3.7	3.7	2.2	87%	13%	-
3	1.8	3.3	3.4	2.2	90%	-	10%
4	4.9	6.9	6.4	4.2	38%	45%	17%
5	2.5	4.5	2.8	2.2	75%	-	25%
6	2.5	4.6	3.1	2.3	75%	-	25%
7	4.0	7.5	3.6	3.1	35%	-	65%
8	5.2	9.8	4.1	4.0	10%	-	90%
9	2.6	5.9	3.4	3.1	60%	-	40%



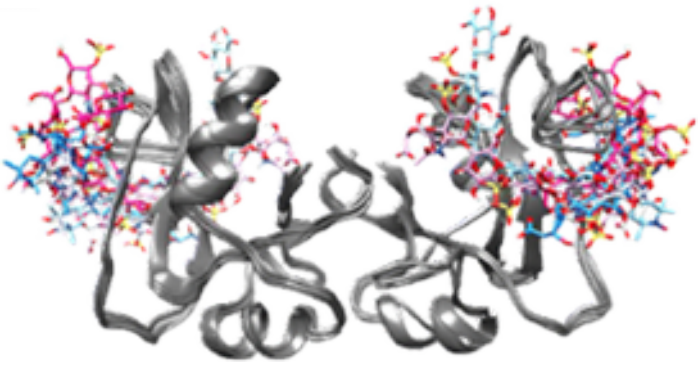




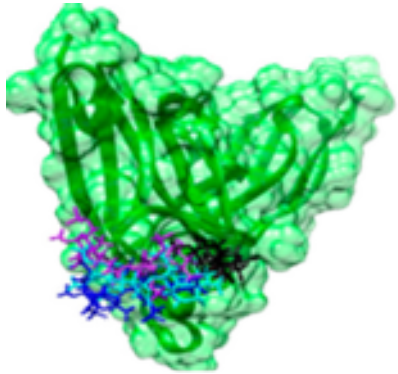
Intermolecular complexes



CCL5 dimer-chondroitin sulfate hexasaccharide



TSG-6 dimer-chondroitin sulfate hexasaccharide



LAR monomer-heparin pentasaccharide

CHEMICAL SHIFT PERTURBATION

SATURATION TRANSFER DIFFERENCE

TRANSFERRED NOE

PARAMAGNETIC RELAXATION ENHANCEMENT

WATERLOGSY

SOLID-STATE NMR

HADDOCK

Intermolecular complexes

Chemical shift perturbation of fondaparinux on ^{15}N-labeled hBD6