BSc Pharmacology and Translational Medicine

Module: Principles of Pharmacodynamics and Pharmacokinetics
Lecture: Structural Analysis of Drugs and Their Targets
Date: Friday 21st October 2011

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Structural Analysis of Drugs and Their Targets

Lecture Outline

- Structural genomics

- Structural analysis
  - X-ray crystallography
  - NMR spectroscopy
    - Macromolecules
    - Small molecules
    - Interaction experiments
  - Mass Spectrometry
    - LC/MS

- Summary

Structural Analysis - Outline

- Knowledge of the chemical structure of biological molecules, and how these molecules are arranged in three dimensions can help us understand molecular interactions

- Understanding drug-target interactions are vital in pharmacology:
  - To explain the observed effects of different agents
  - In the development of novel therapies that act on a particular molecular target

- The majority of pharmacological interactions are either protein-protein or ligand-protein

- Spectroscopic tools to characterise proteins and small molecules are available and the most commonly used as they provide high-resolution, information rich data about molecular conformations and interactions.

Structural Genomics

Structural Analysis – Structural Genomics

- Structural genomics is the area of research concerned with solving the 3D structures of coding DNA

- Eventually will provide a library of structures that can be used in a complementary fashion, alongside genomics and other information

- Existing solved structures can help speed up future efforts due to the conserved nature of many macromolecular domains (e.g. binding sites in proteins). To date, >44000 macromolecular structures have been solved

- Several techniques are available for characterising the 3D conformation of macromolecules

- Two techniques can provide atomic resolution structures
  - X-Ray Crystallography
  - NMR Spectroscopy

- Other techniques (e.g. mass spectrometry) can assist in characterising a variety of molecules

X-Ray Crystallography

X-Ray Crystallography

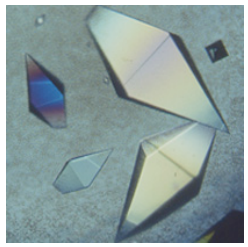
X-Ray Crystallography

- A method of determining the crystal structure of a compound

- The scattering/diffraction pattern observed when X-rays are applied to the compound can allow the **spatial location of the atom centres** in the molecule to be calculated

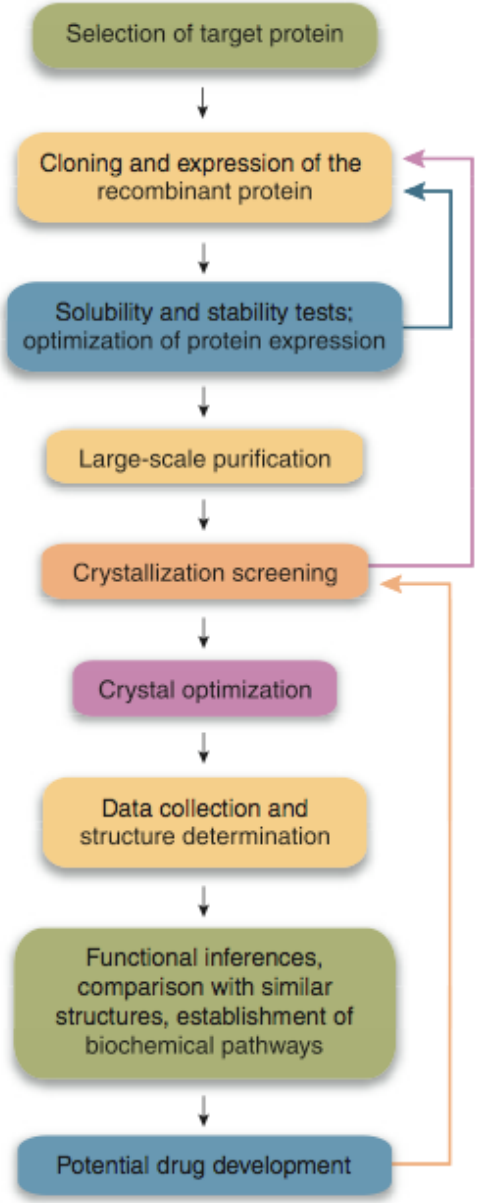
- Requires a considerable quantity compound as a crystal and success of the X-ray diffraction experiment is largely reliant on the **quality/purity** of the crystal

- Crystallisation conditions for a particular protein will vary and usually a large number of conditions must be tried before a pure crystal can be obtained



- A large number of different approaches including:
  - Vapor diffusion
  - Dialysis
  - Microfluidics

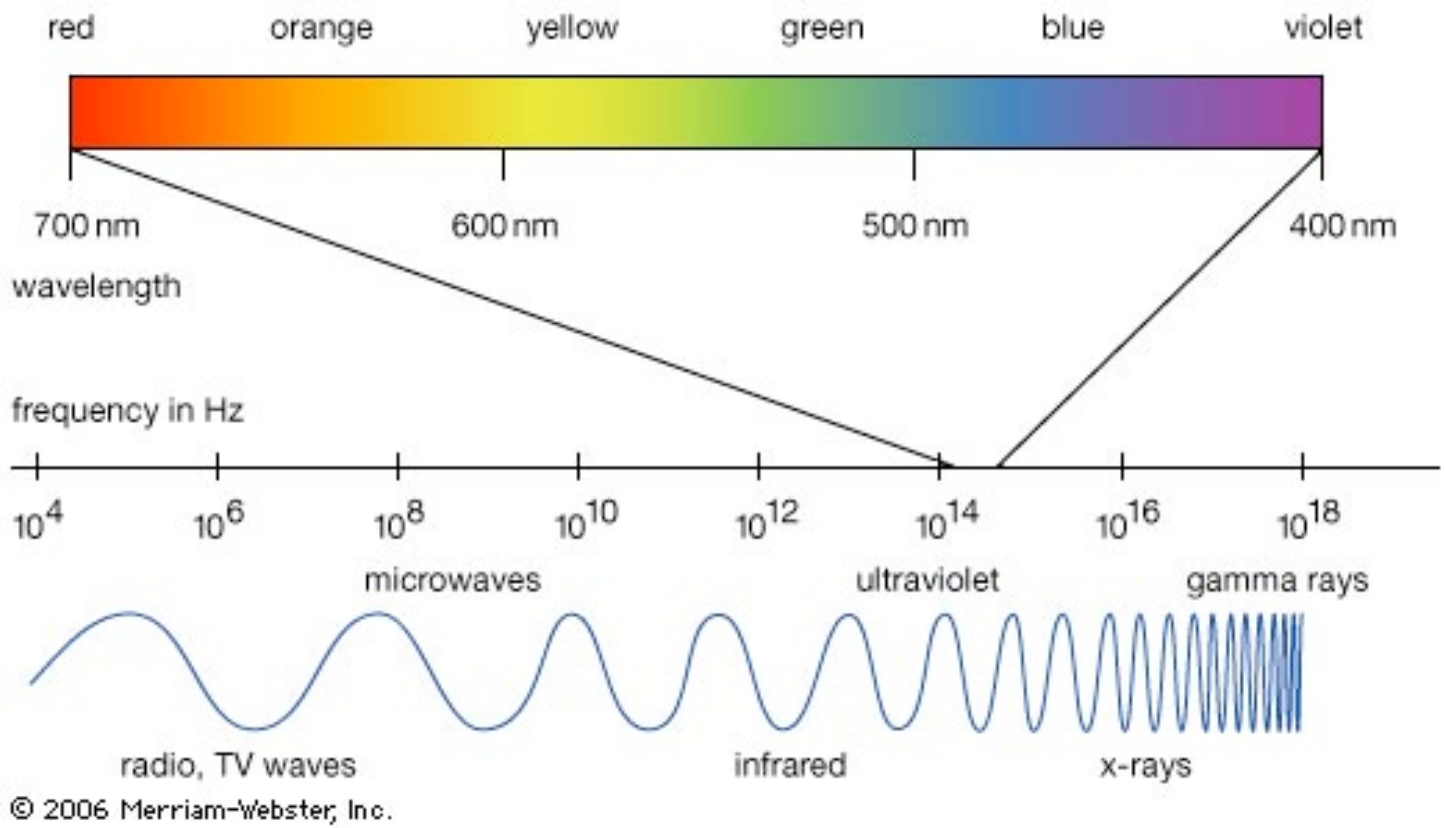
See Chayen E. and Saridakis *et al.* 2008



X-Ray Crystallography

X-Rays on the Electromagnetic Spectrum

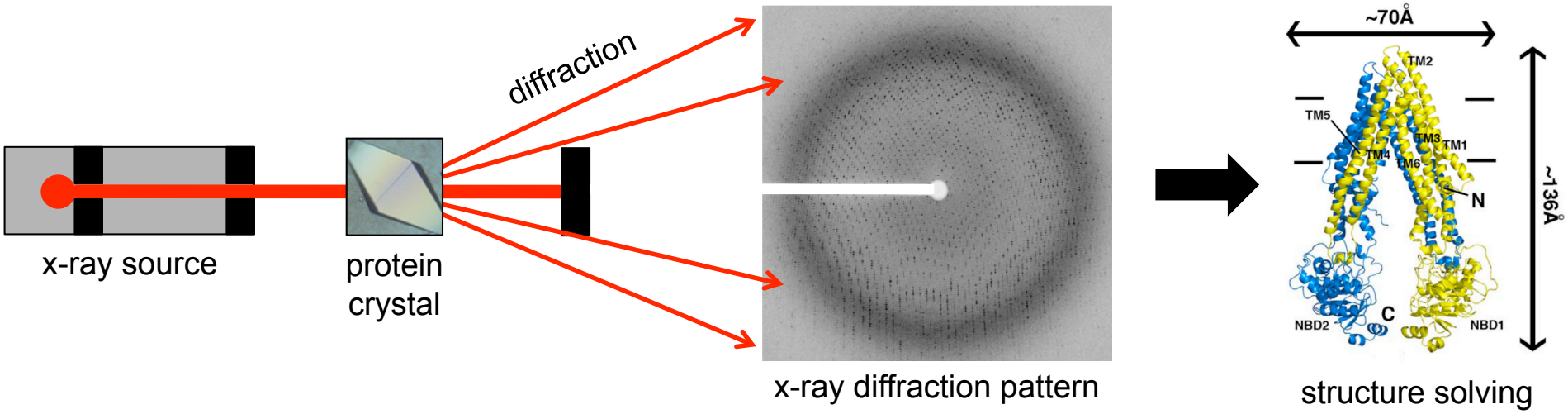
- Electronic transitions: X-rays / Ultra-violet (UV)
- Vibrational transitions: Infra-red (IR)
- Nuclear spin transitions: Radio waves (NMR)



X-Ray Crystallography

X-Ray Diffraction

- Protein crystals are bombarded with high energy X-rays
- A small proportion of the beam is refracted/scattered by the atoms in the crystal
- In a well-ordered (pure) crystal, a clear diffraction pattern can be observed using a detector
- A 3D electron density map can then be produced
- Knowledge of the amino acid sequence then allows the 3D structure to be calculated
- The resolution of the X-ray diffraction data determines how similar the model fit will be to reality



X-Ray Crystallography

- Benefits:
  - Good quality crystals can lead to high-resolution diffraction patterns
    - Very efficient structure solving using established algorithms
  - Robotics / high-throughput technologies can be employed
    - Rapid identification of optimal crystal growth conditions
    - Automation of main steps - efficient
    - Generation of large quantities of protein for analysis

X-Ray Crystallography

- Limitations:
  - The resolution of the diffraction patterns observed in X-ray crystallography experiments is partly determined by the size of the molecule in the crystal
  - Larger molecules cannot be very well resolved (e.g. large proteins)
  - Some proteins are difficult to crystallise
    - Affected by shocks and temperature fluctations
  - Some molecules crystallise in multiple orientations, distorting the resulting diffraction pattern
  - Exposure to X-rays may cause the protein to denature/degrade
    - A destructive process
  - Dynamics and *in situ* experiments are not possible
    - Experiments with ligand binding etc require separate crystals to be obtained

NMR Spectroscopy

NMR Spectroscopy – Outline

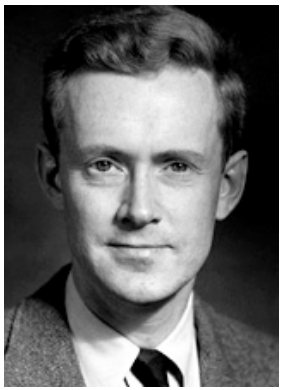
- Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful tools in modern science. Since its discovery >50 years ago, it has spread from physics to chemistry, biological science, materials science and medical diagnosis.

- Phenomenon of nuclear magnetic resonance (NMR) discovered in 1945
  - Bloch *et al.*
  - Purcell *et al.*

- Awarded Nobel Prize in Physics (1952) for the first experimental verifications of the phenomenon

- NMR is still the only method of determining the structure of a molecule in a liquid state (c.f. crystallography)

Bloch



Purcell

NMR Spectroscopy - Outline

- Nuclear Magnetic Resonance (NMR) Spectroscopy

- Generates spectra that describe the chemical environment of nuclei in biofluid molecules
  - Chemical structure
  - Interactions
  - Bulk properties can be studied
  - Diffusion
  - Compartmentation

- Typically concentrate on ^{1}H

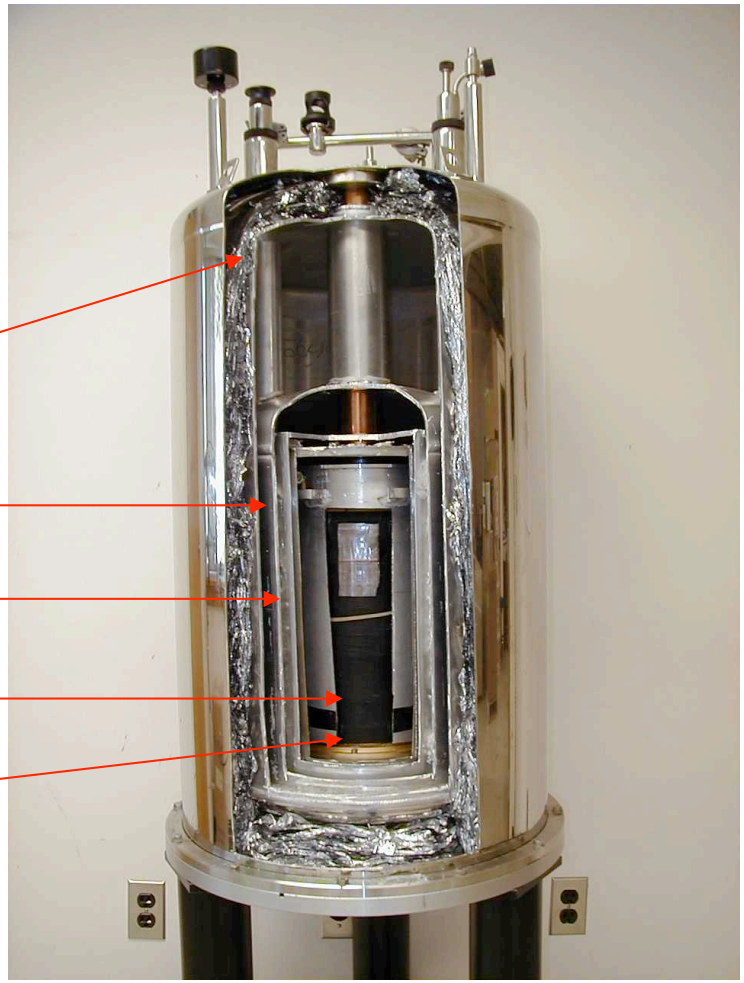
- Ubiquitous in biomolecules and drugs

- Good sensitivity

- LOD of ^{1}H NMR typically in micromolar range

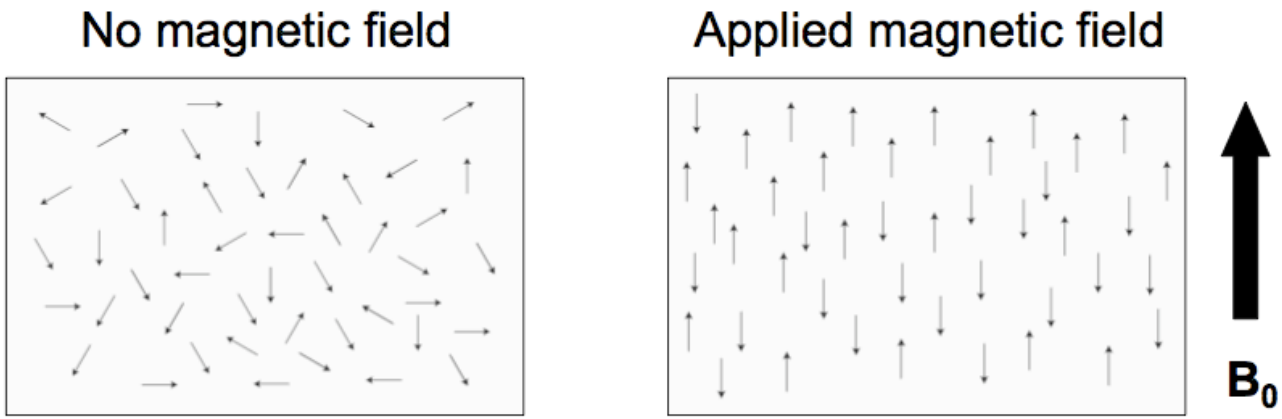
NMR Spectroscopy – Outline

- At thermal equilibrium in the Earth's magnetic field, the numbers of nuclear spins are almost equal between the upper and lower energy levels
- Increasing the field by millions of times gives workable energy differences



NMR Spectroscopy - Outline

- When placed in a magnetic field there will be a small net difference in the populations orient
  - -Boltzmann distribution
  - - Net magnetisation in direction of field



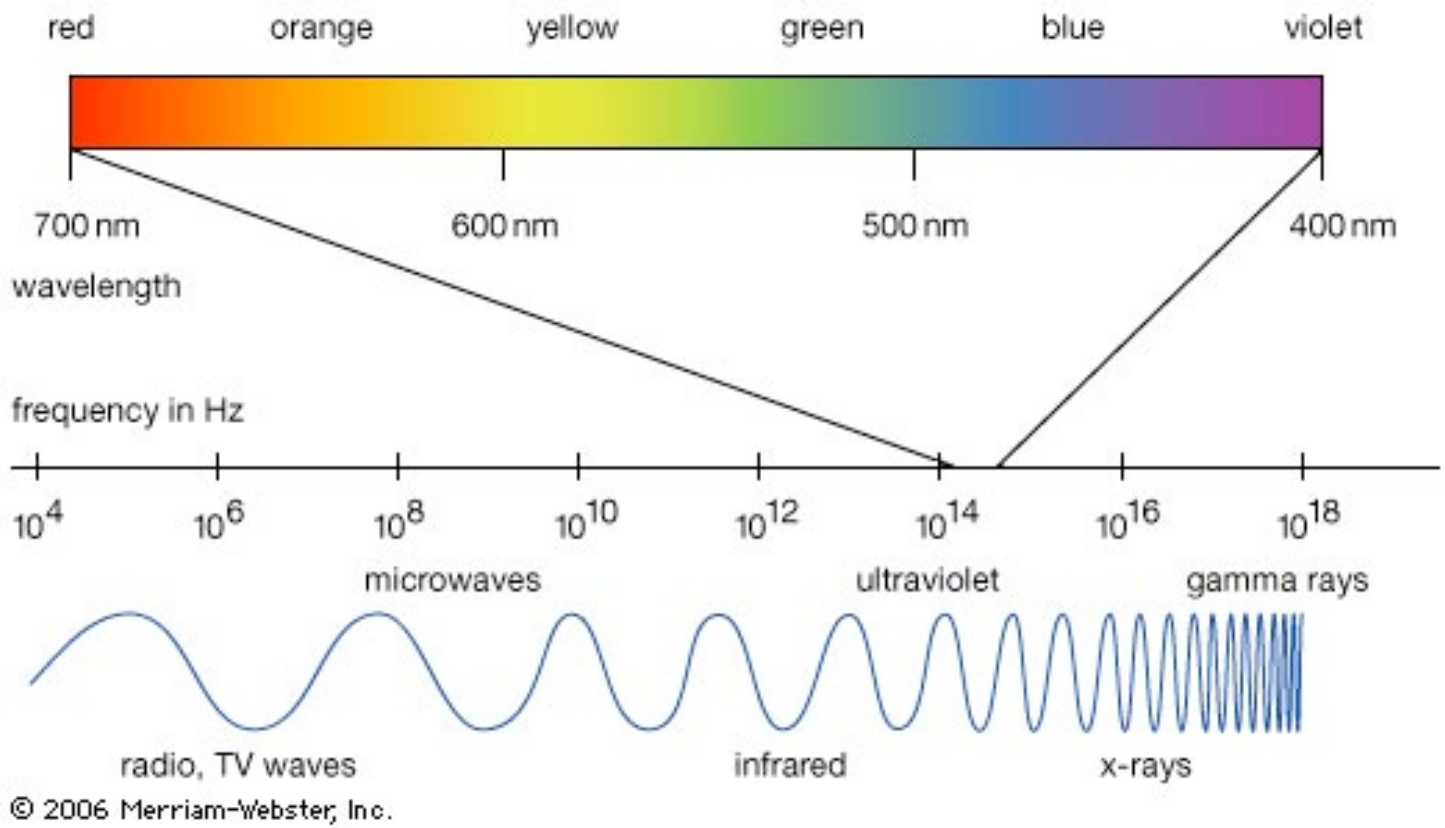
- By **disturbing the equilibrium condition** and observing the resulting **return over time**, we can determine the **resonance frequencies** of the nuclei in a sample

- Frequency is related to **chemical environment**

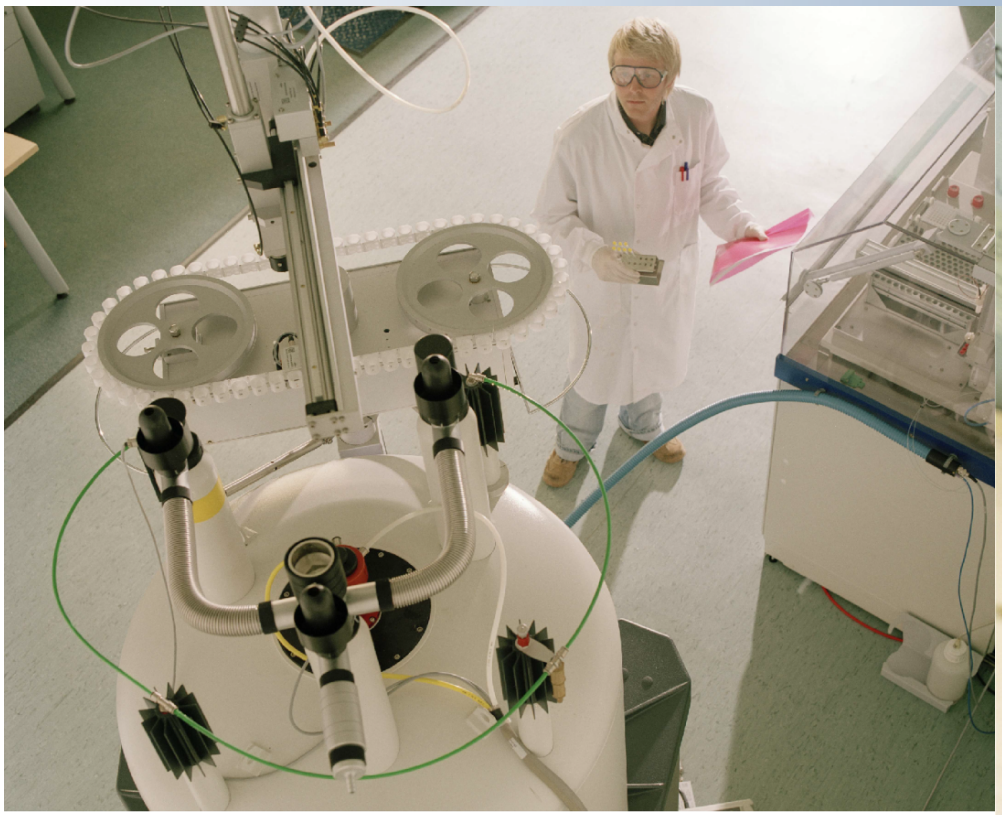
- When scaled to the magnet strength = **chemical shift**

NMR Spectroscopy on the Electromagnetic Spectrum

- Electronic transitions: X-rays / Ultra-violet (UV)
- Vibrational transitions: Infra-red (IR)
- Nuclear spin transitions: Radio waves (NMR)



NMR Spectroscopy



1. Place sample in a large magnet

2. Pulse with radiofrequency electromagnetic radiation

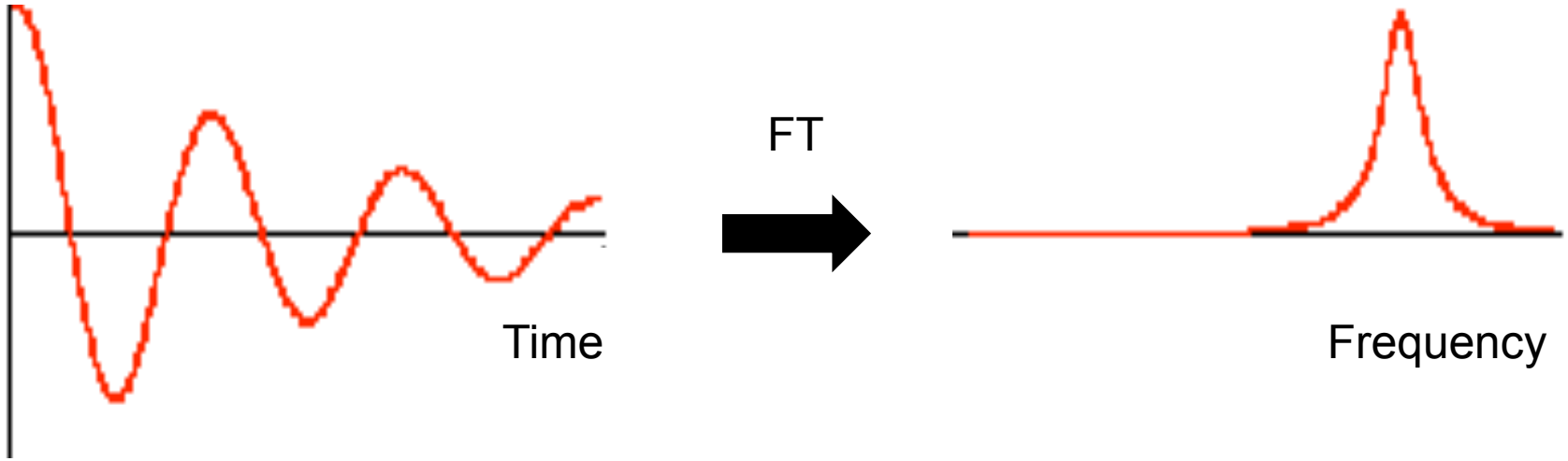
3. Detect resulting signal

Photos: © Metabometrix Ltd., London

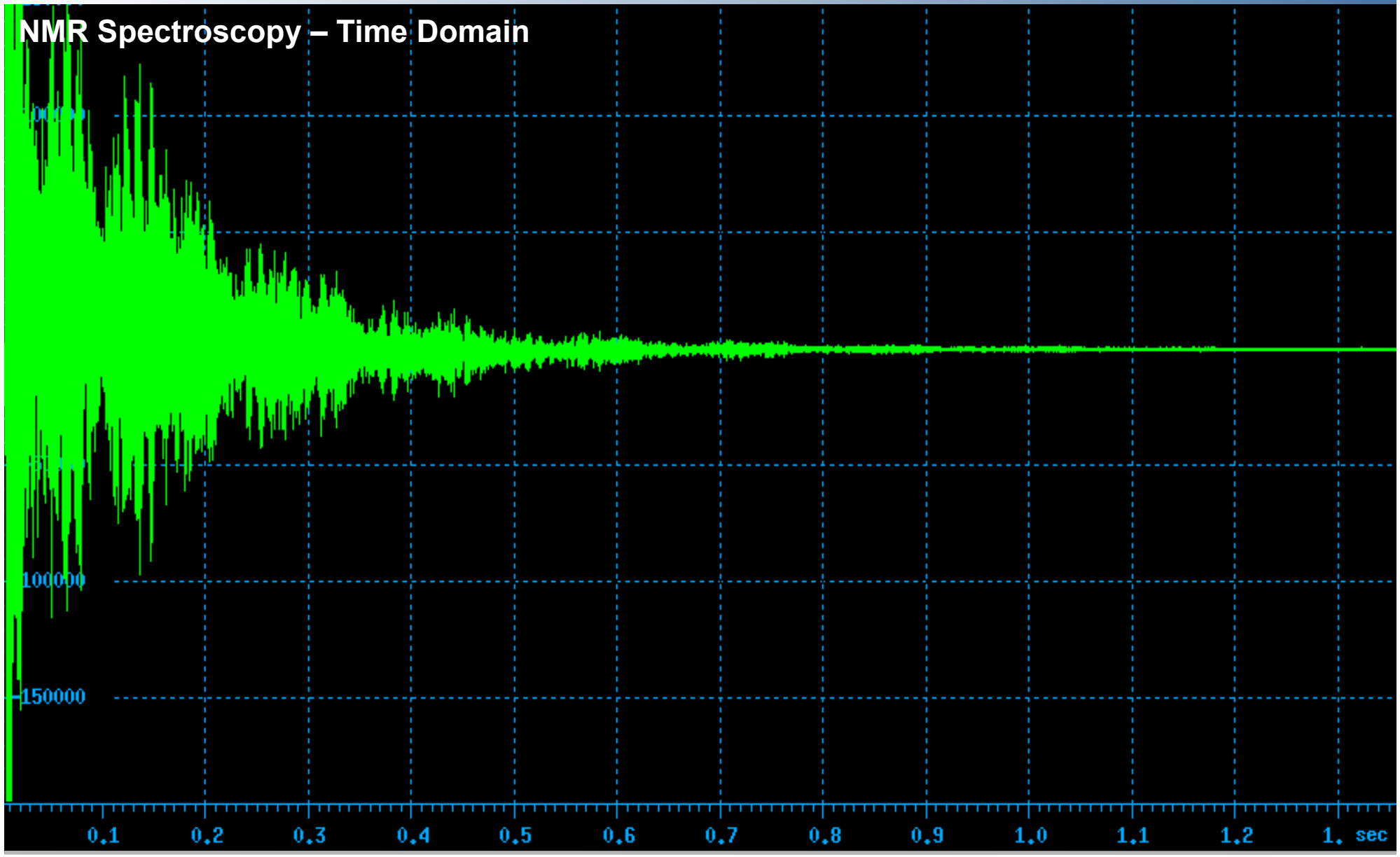
NMR Spectroscopy

- Nobel Prize in Chemistry (1991)
  - R.R. Ernst
  - Nobel Prize in development of NMR techniques
  - In particular Fourier Transform (FT)-NMR

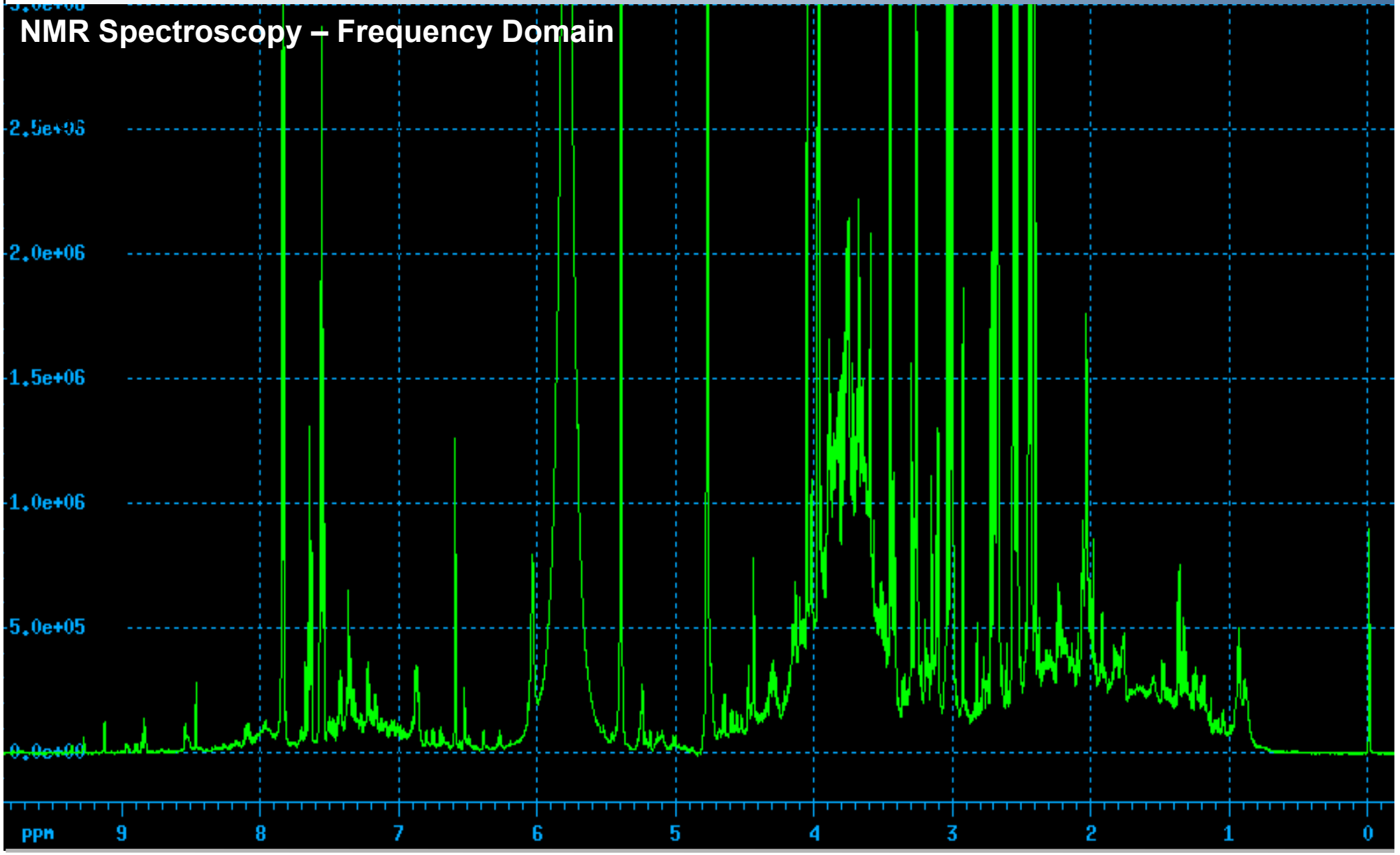
- Fourier transformation (FT)
  - Mathematical transformation of time dependent signal into frequency domain



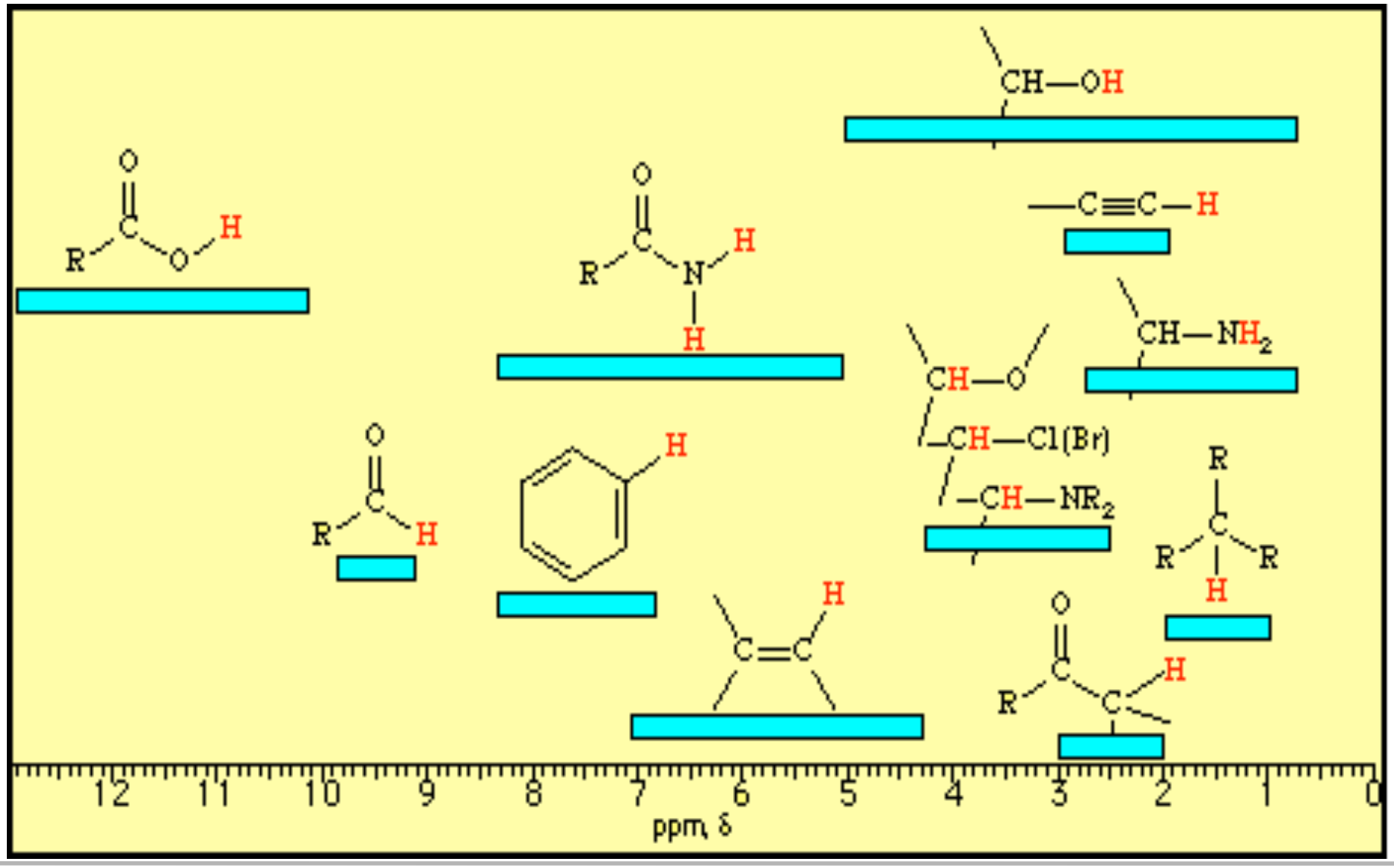
NMR Spectroscopy



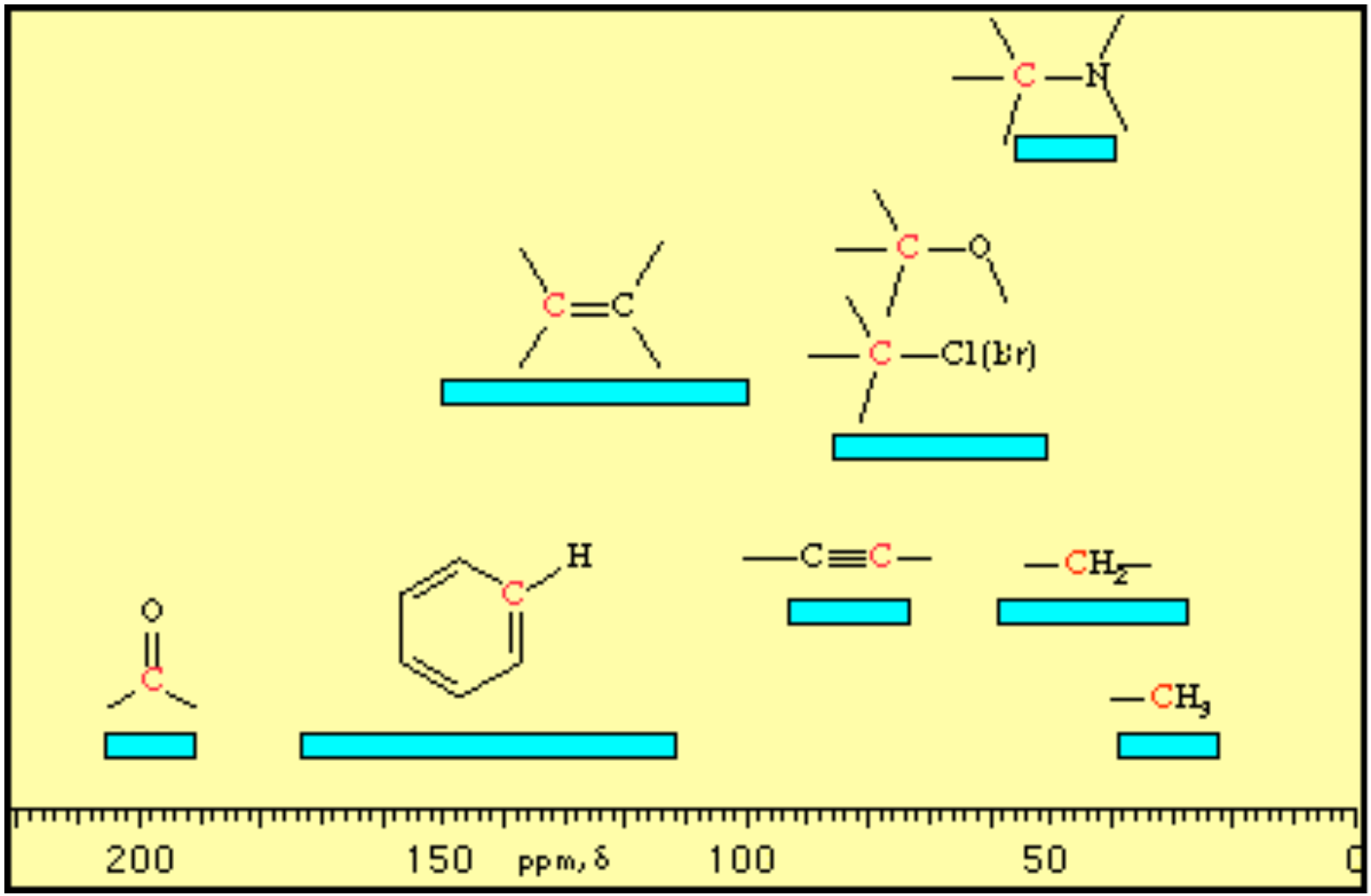
NMR Spectroscopy



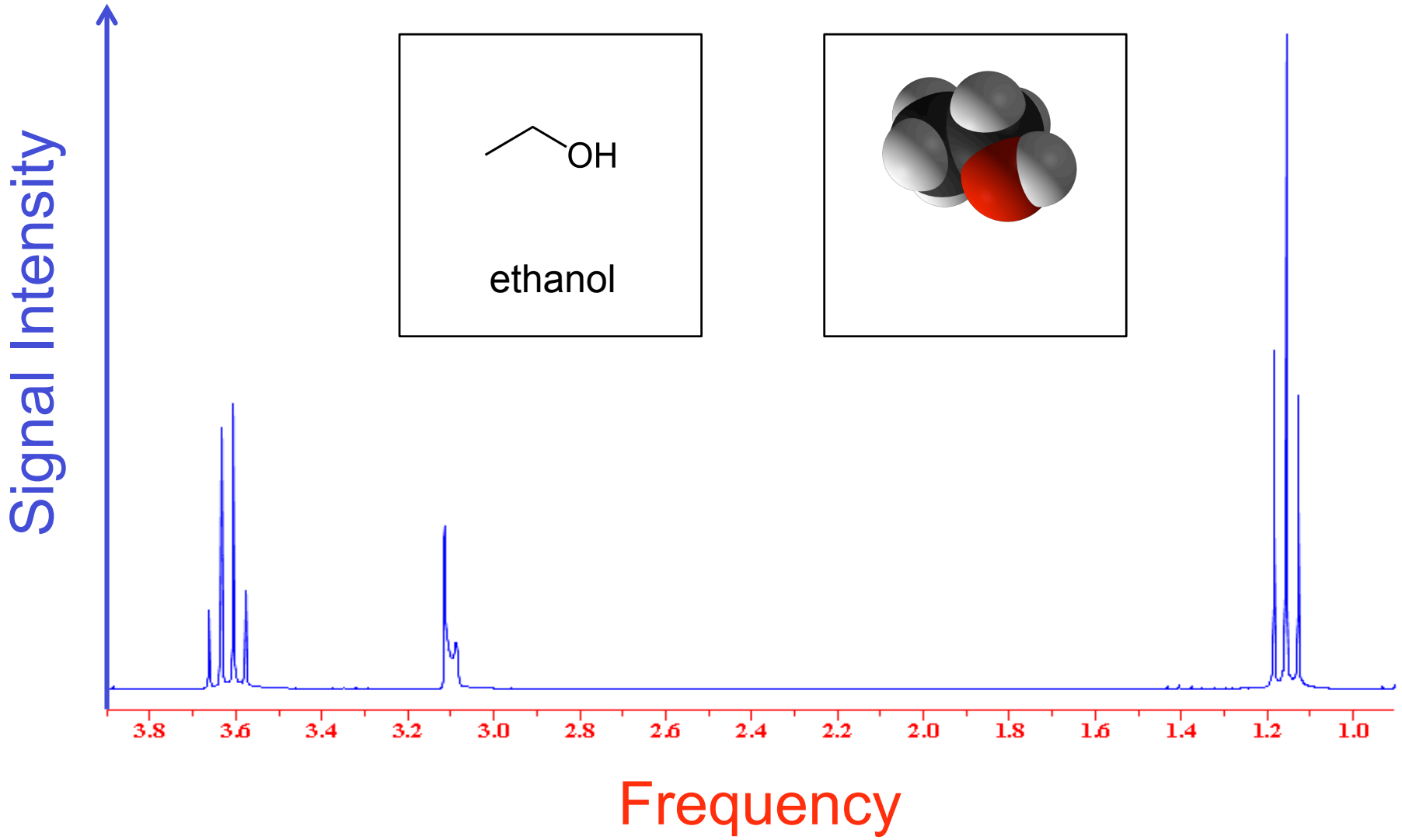
NMR Spectroscopy – ^{1}H NMR Chemical Shifts



NMR Spectroscopy – ^{13}C NMR Chemical Shifts

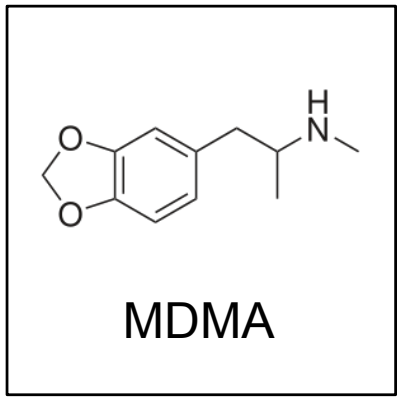


- **NMR Spectroscopy - ^{1}H NMR Spectrum of Ethanol**

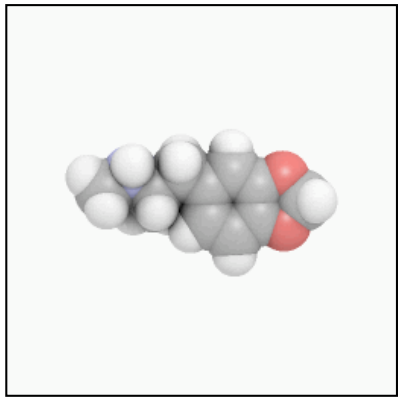


NMR Spectroscopy

- **NMR Spectroscopy - ^{1}H NMR of compound from suspected drugs haul**



MDMA

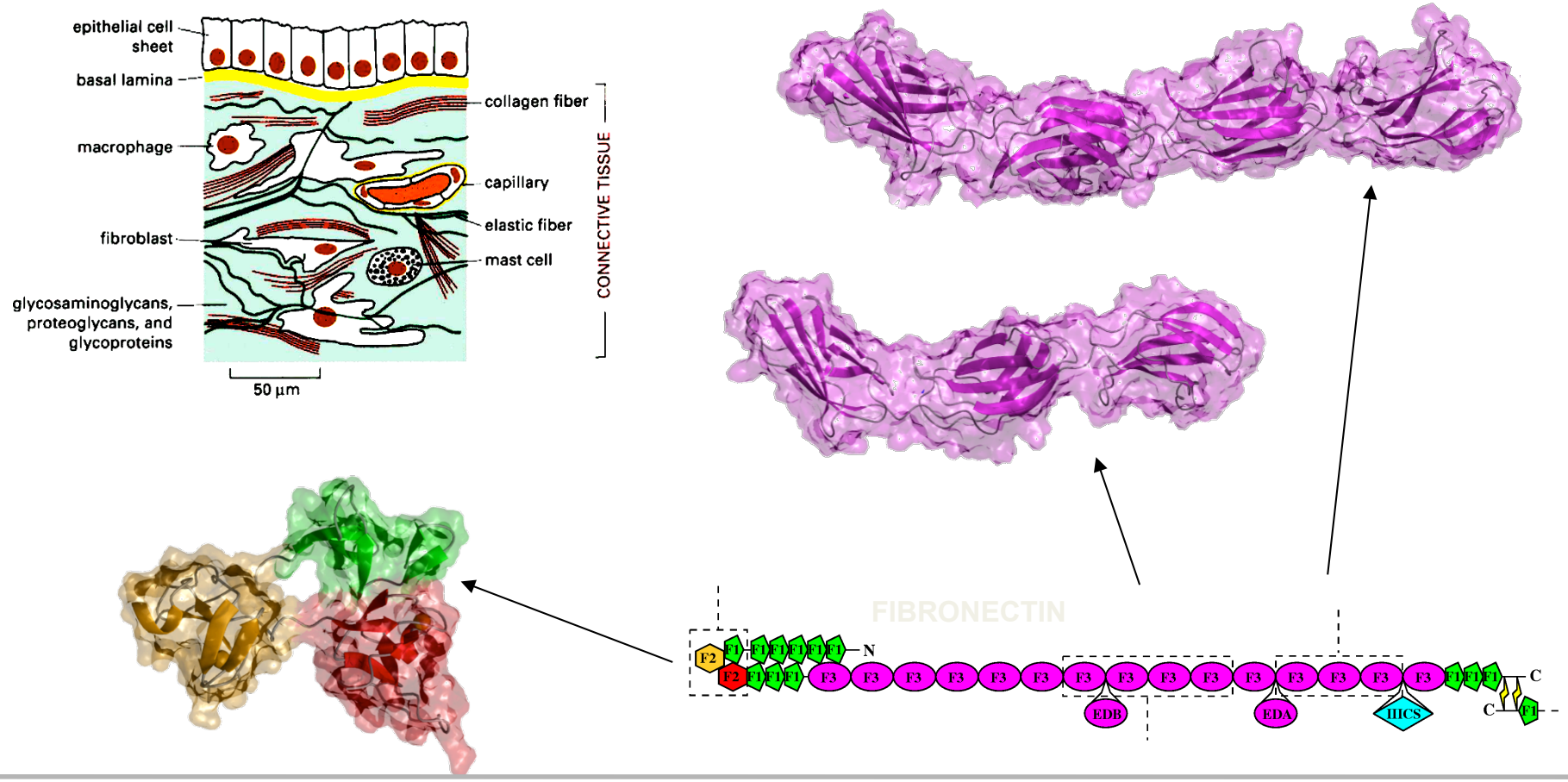


Frequency

Source: A. M Langford, Lothian and Borders Police Forensic Science Laboratory, Edinburgh

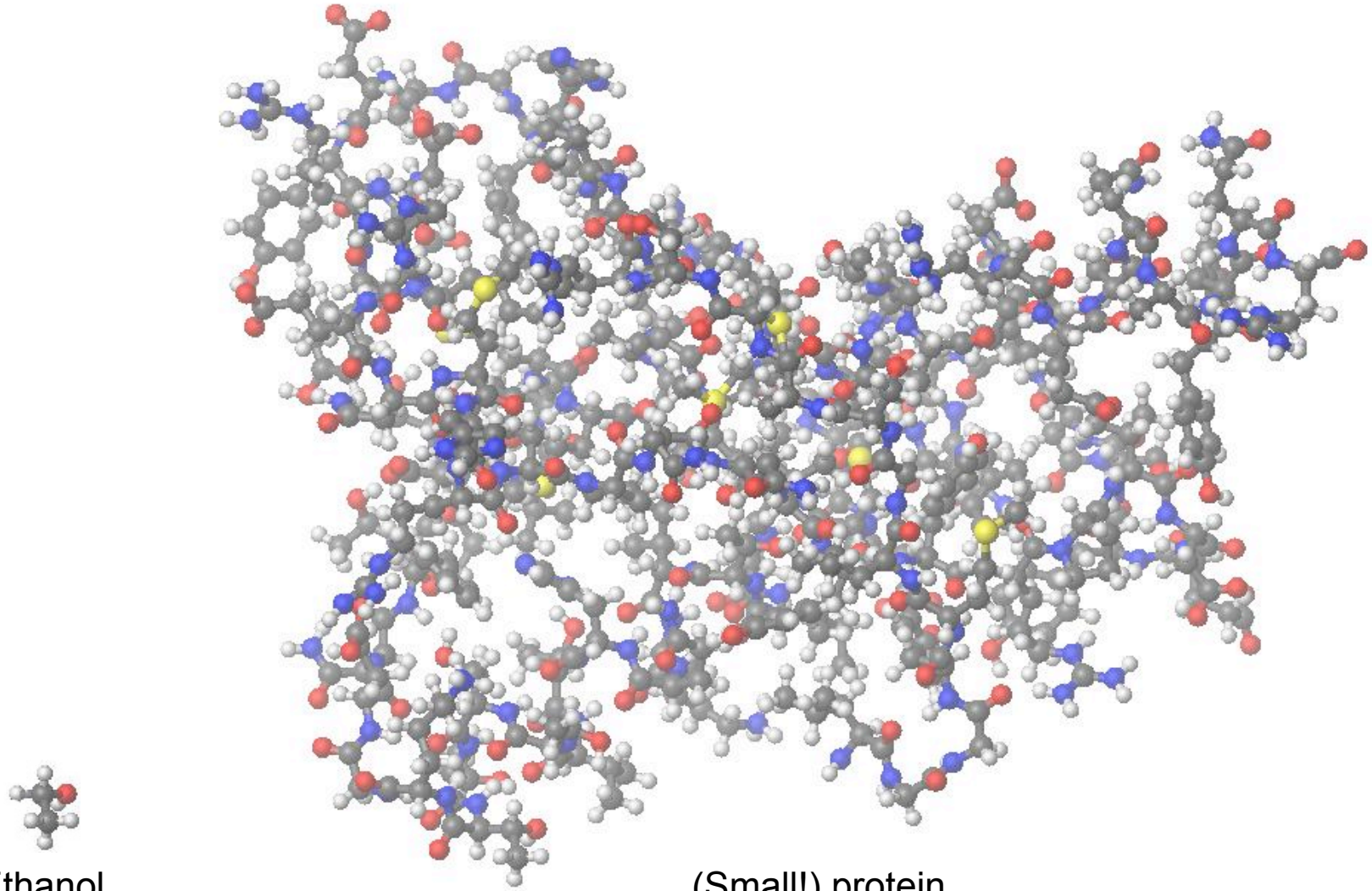
NMR Spectroscopy

▪K. Wüthrich shared the Nobel Prize in Chemistry (2002) for his work on the structure elucidation of large biomolecules such as proteins & nucleic acids



NMR Spectroscopy – Human Prion Protein

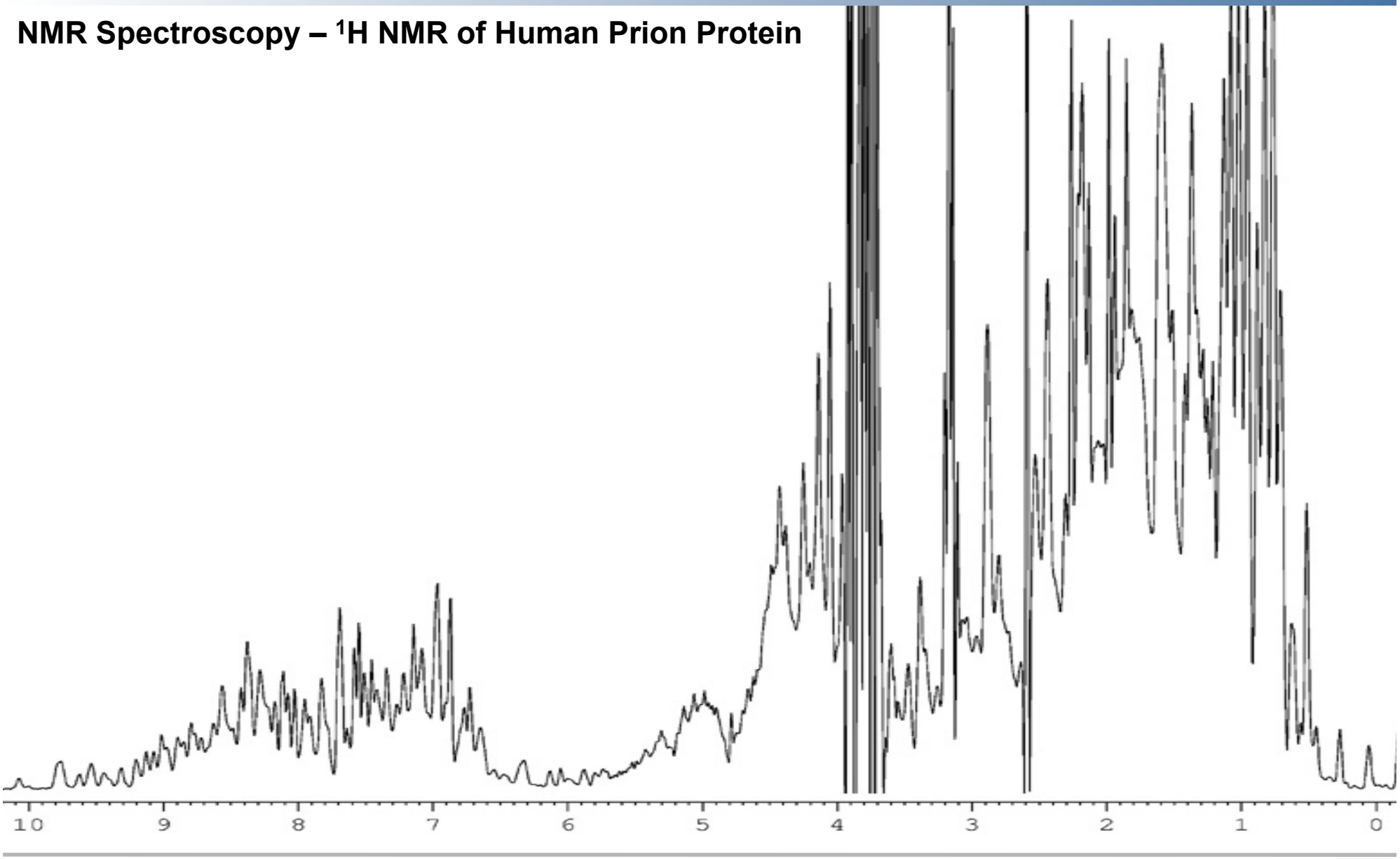
http://www.rcsb.org/pdb/explore/explore.do?structureId=1QM0



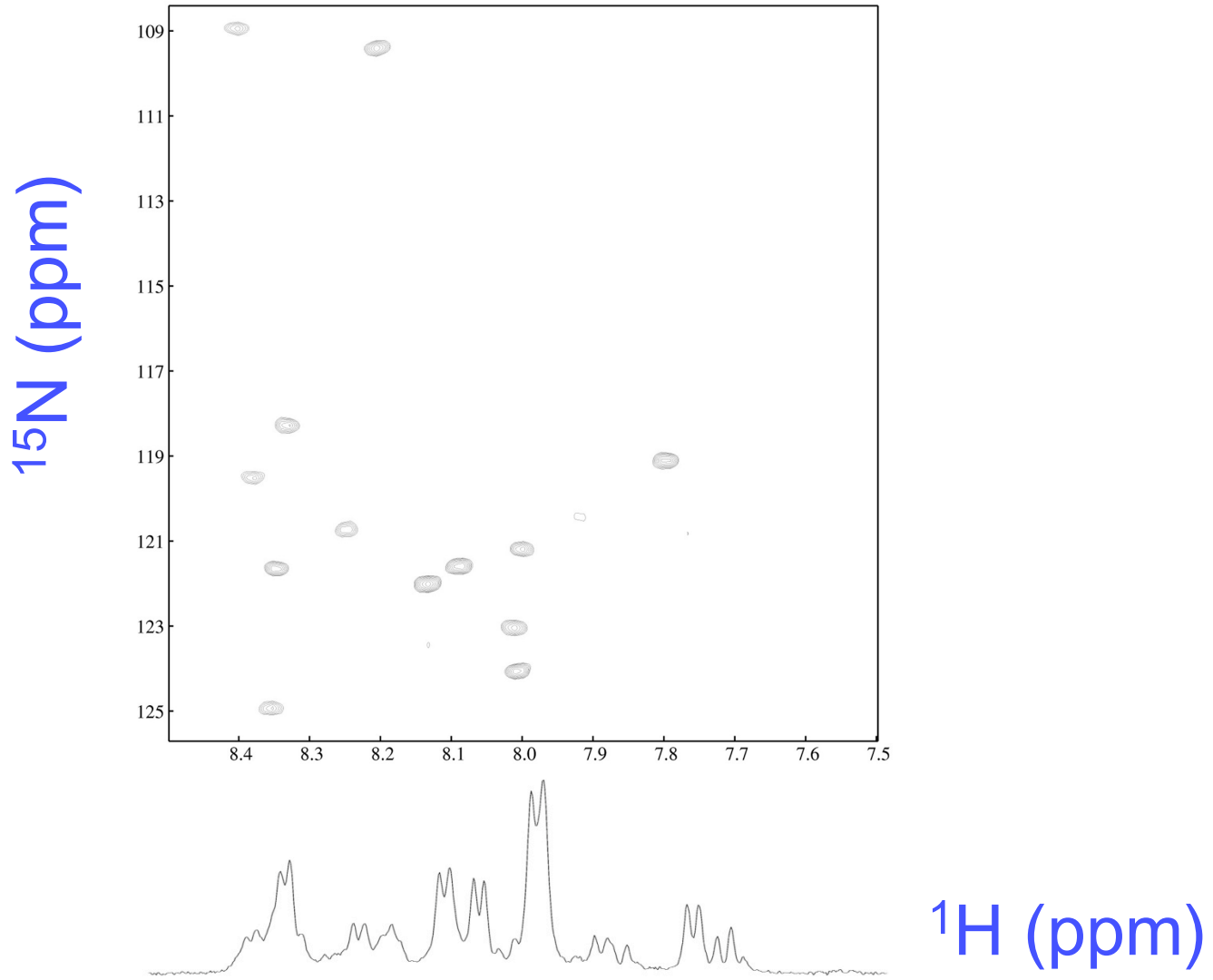
Ethanol

(Small!) protein

NMR Spectroscopy – ^{1}H NMR of Human Prion Protein



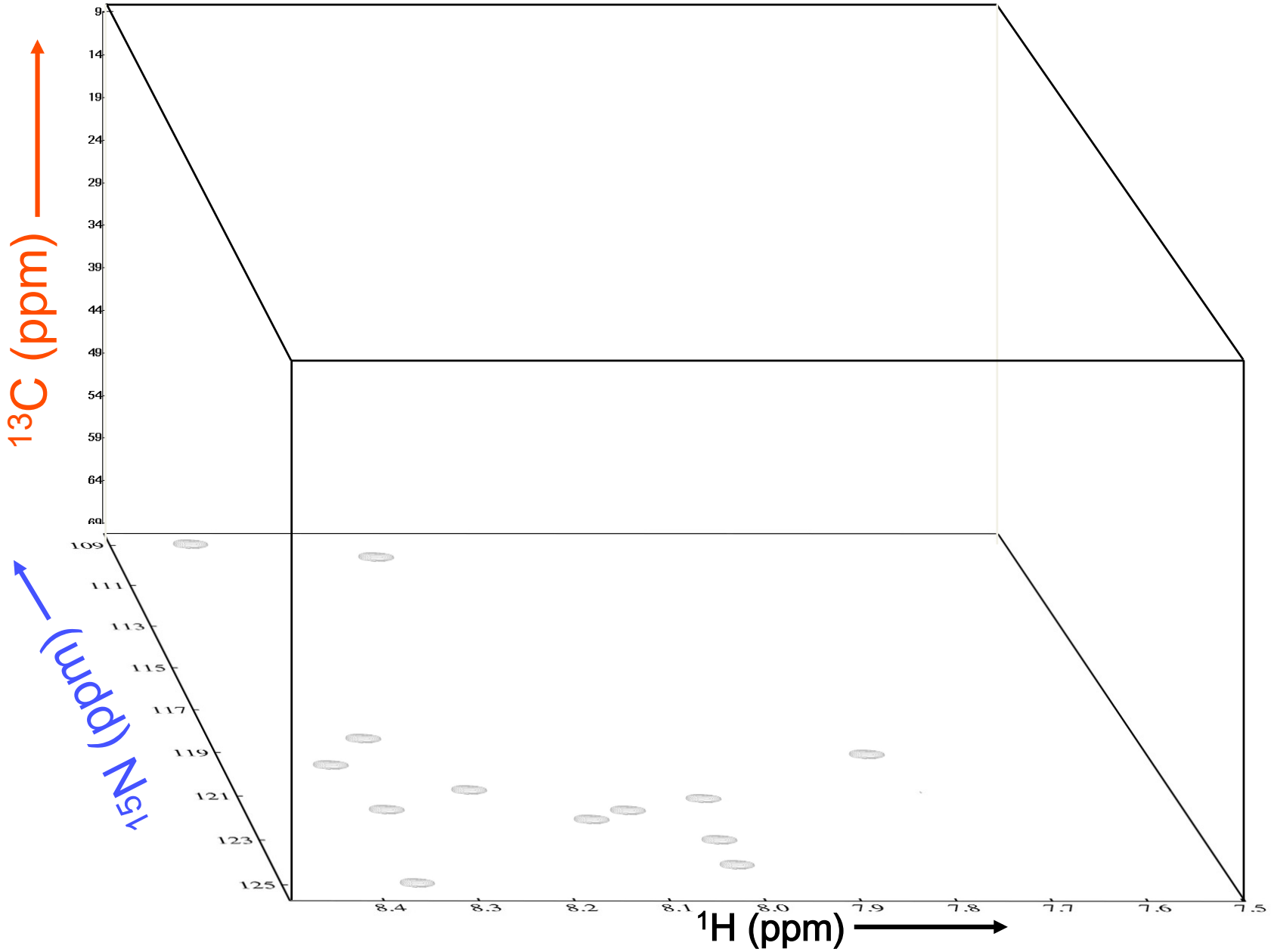
NMR Spectroscopy - Two dimensional simplification (HSQC spectrum)



NMR Spectroscopy

NMR Spectroscopy - Three dimensional spectrum…

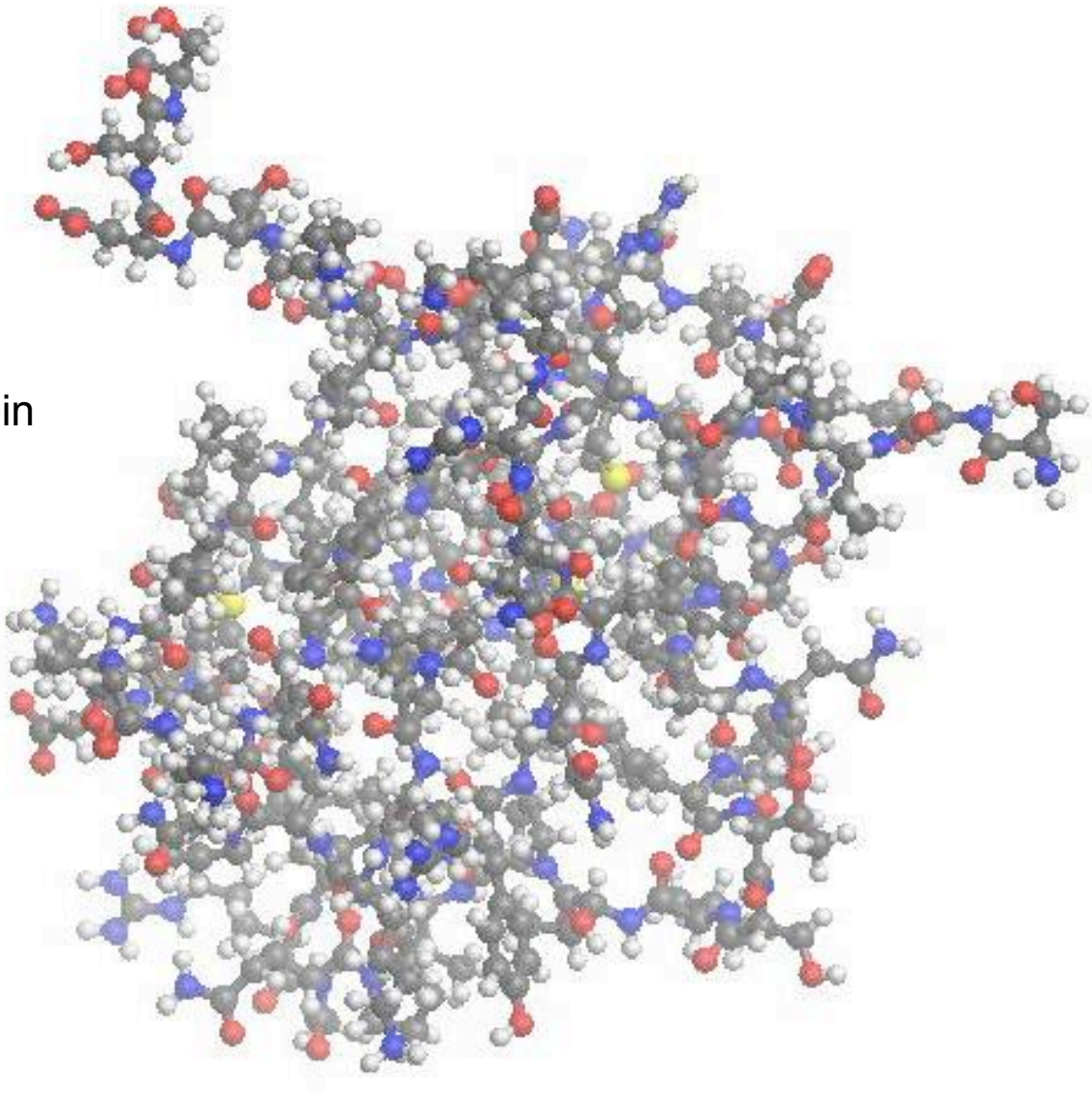
…up to 7D



NMR Spectroscopy - Example

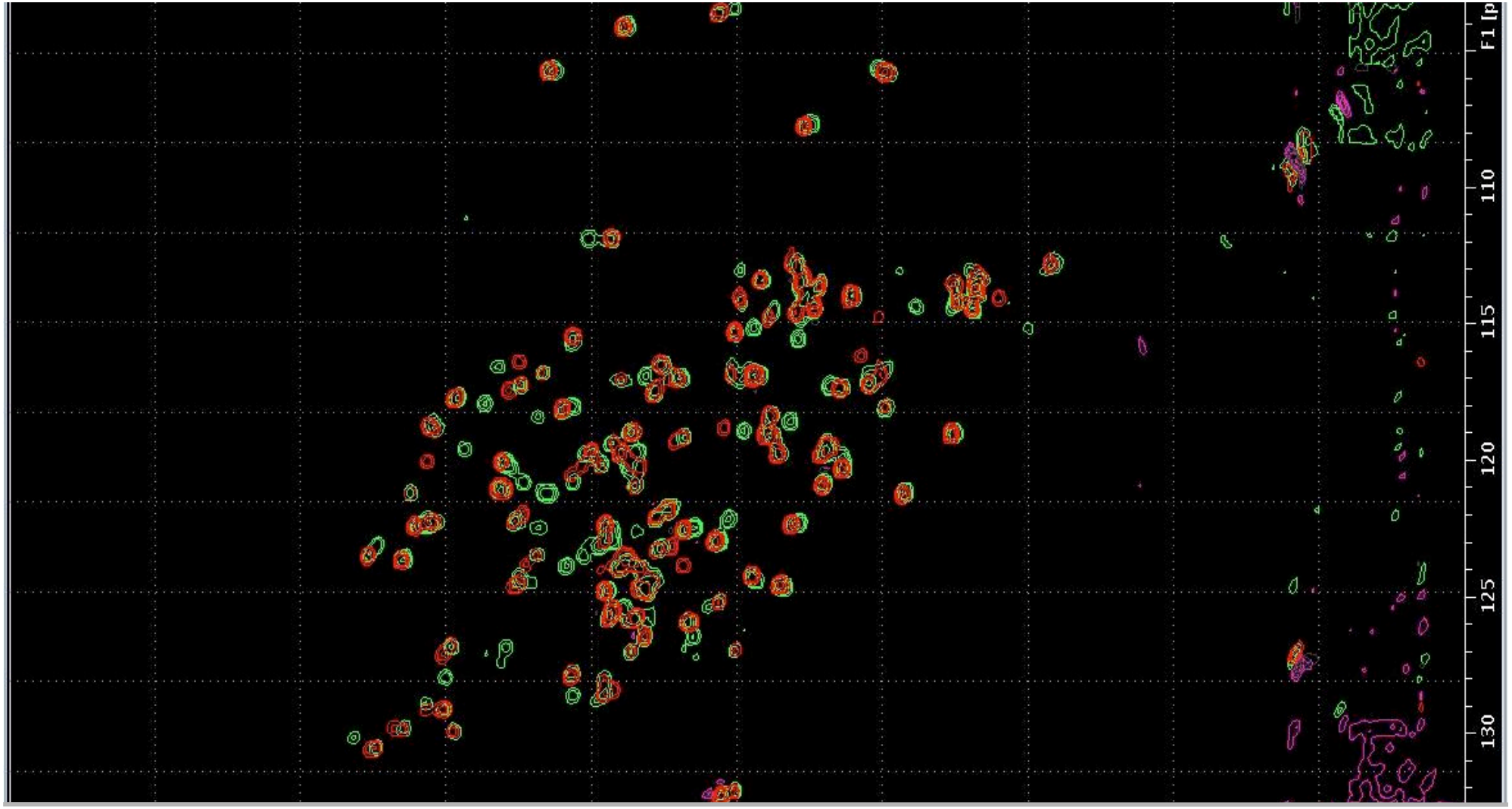
- Human PTB protein
  - Target of viral hijacking
  - Structure can be solved by NMR

- PTB = polypyrimidine tract binding protein



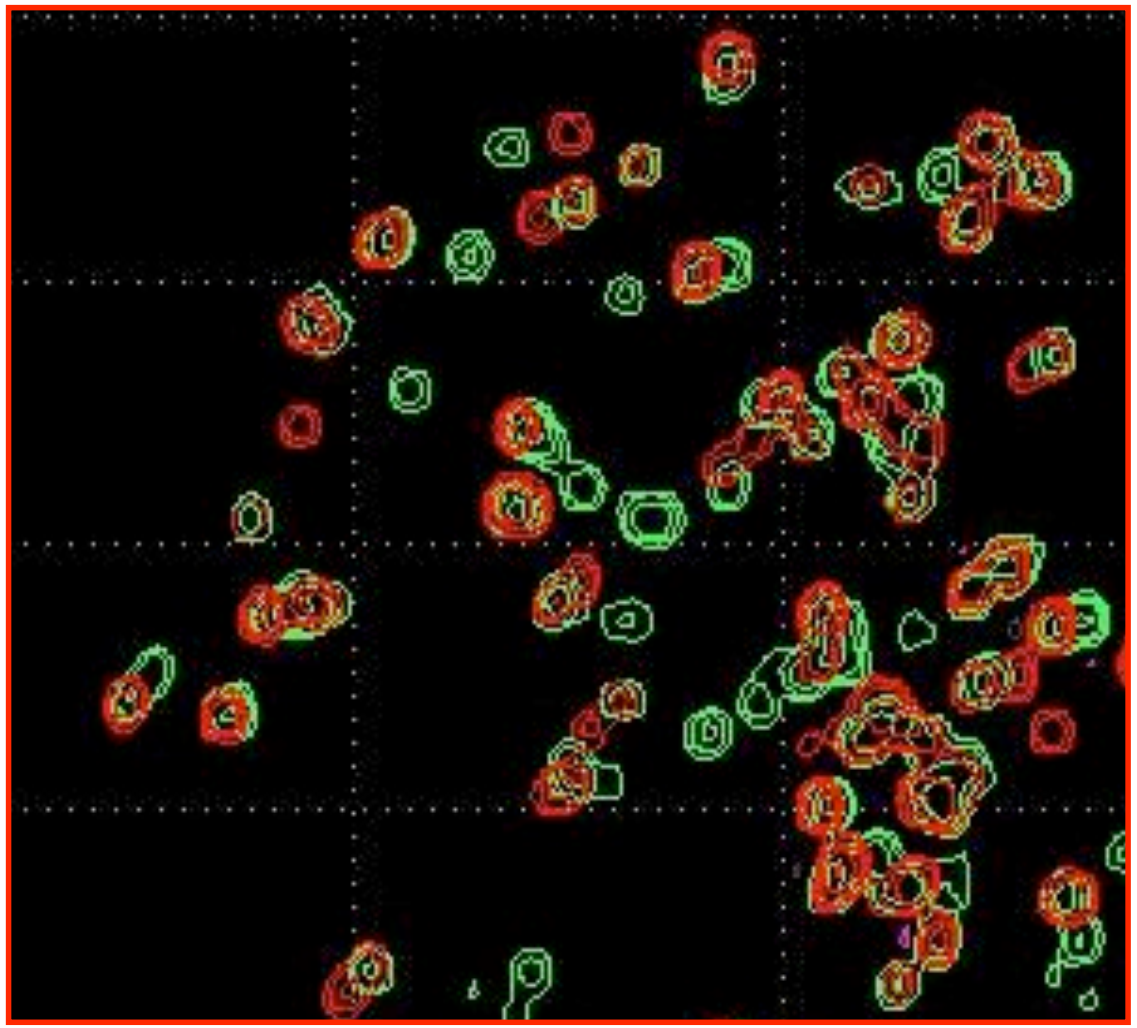
NMR Spectroscopy – Overlay of 2D Spectra

No RNA **RNA Added**



NMR Spectroscopy – Overlay of 2D Spectra (Zoomed)

No RNA **RNA Added**

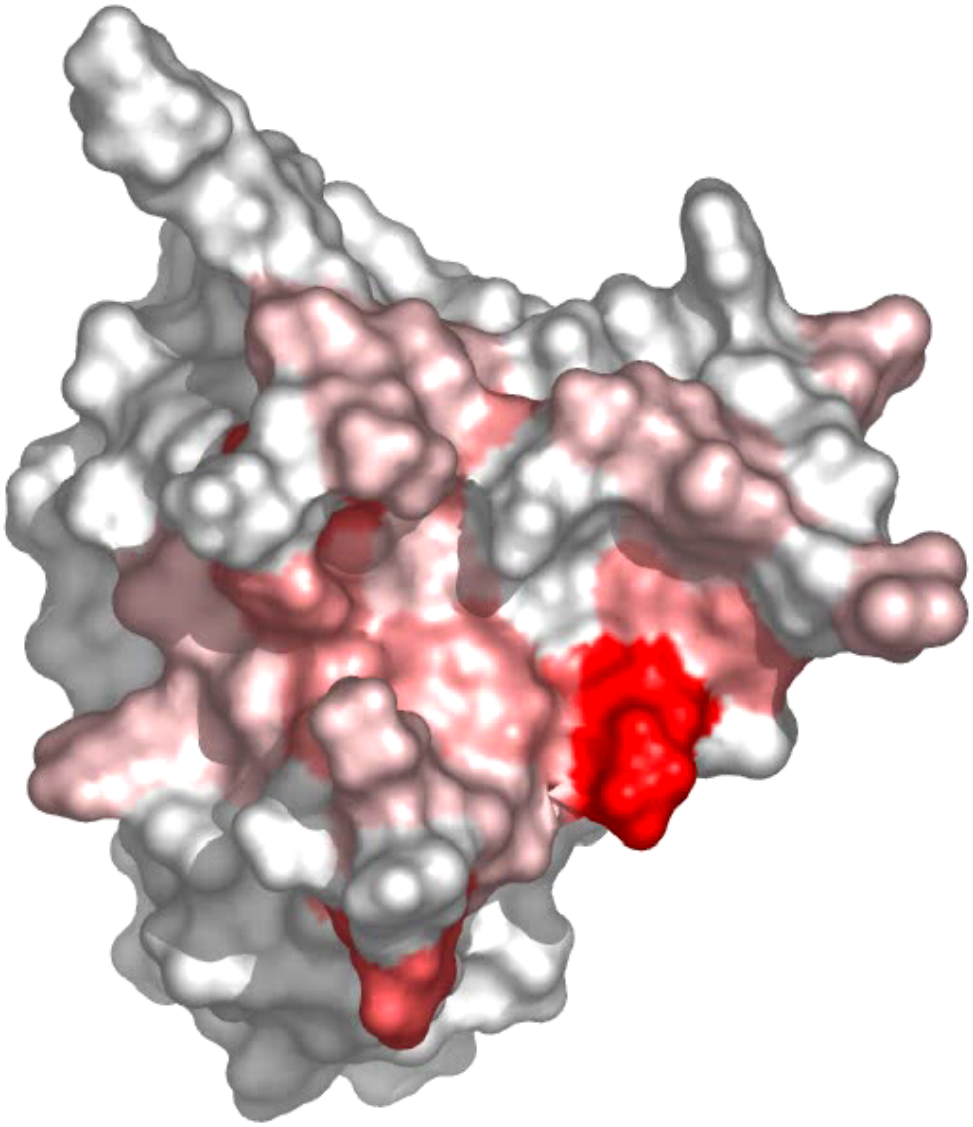


NMR Spectroscopy - Interactions

- Projection of frequency change onto the protein structure can reveal the site of interaction

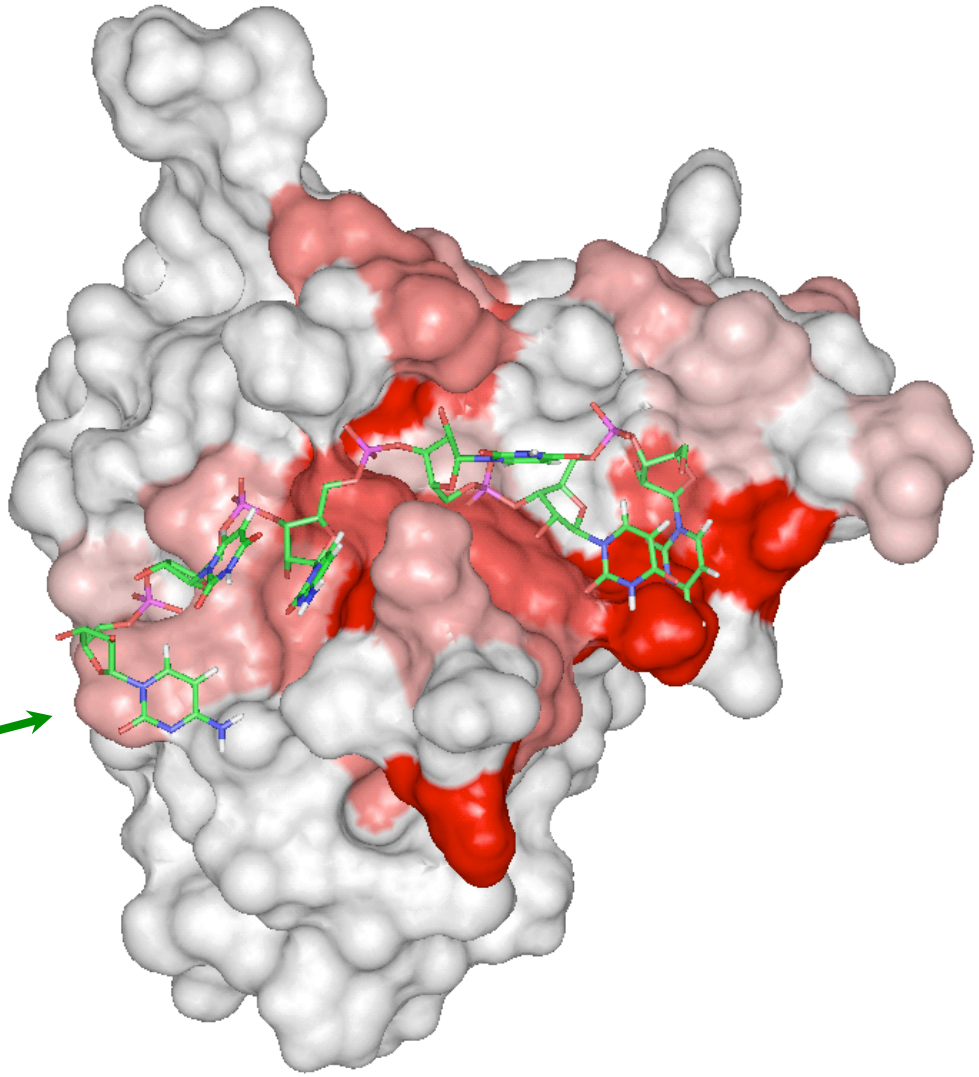
Large change in NMR frequency

No change in NMR frequency



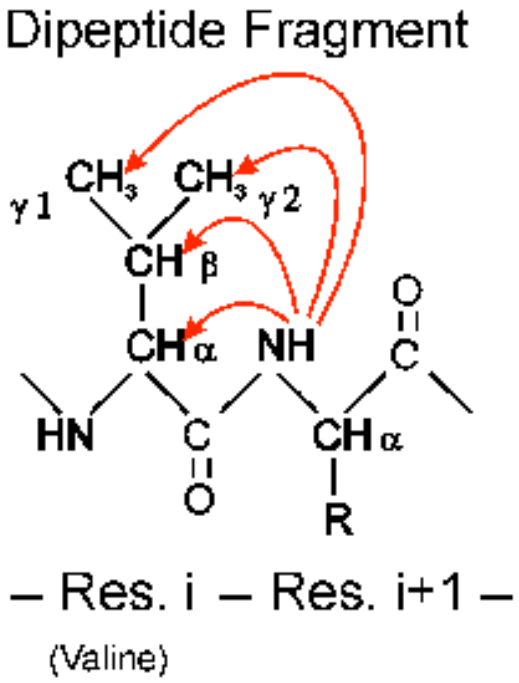
NMR Spectroscopy - Summary

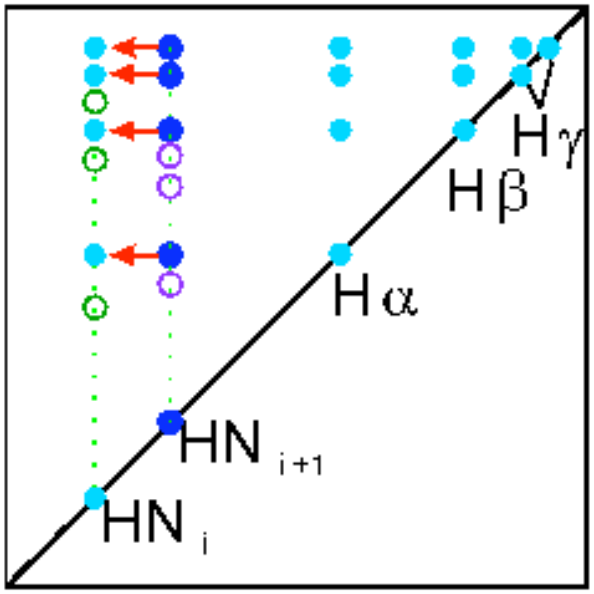
- Molecular modelling approaches can be used to visualise how molecules interact



NMR Spectroscopy – Information on Spatial Proximity

- 2D-Nuclear Overhauser Effect (NOE) experiments
- Couplings across space – stereo structure (peptides, proteins, etc.)





NMR Spectroscopy - Summary

- Some nuclei (e.g. ^{1}H, ^{13}C) are accessible to NMR spectroscopy
- NMR spectroscopy observes effect of disturbing spin populations using electromagnetic radiation
- Frequency, intensity and fine structure are the three principal features of an NMR spectrum
- Can be applied to structure elucidation, quantification, and medical diagnosis/imaging
- Powerful tool in metabonomics for profile generation
- Easily translatable results
- High resolution NMR – MAS of intact tissue – MRI in vivo

Mass Spectrometry

MS - Outline

- Characterises components by:
  - Mass-to-charge ratio (*m/z*)
  - Fragmentation pattern

- Used for structure elucidation
  - Masses of fragments give partial structural information
  - Tryptic digest can also be used – information on resulting peptides acquired

- A variety of spectrometers are available
  - Q-ToF and FTMS provide very high *m/z* accuracy/precision that can allow deduction of the empirical formula

- Note: other MS-based techniques are routinely used in structural analysis
  - MALDI/MS
  - GC/MS
  - CE/MS….. etc

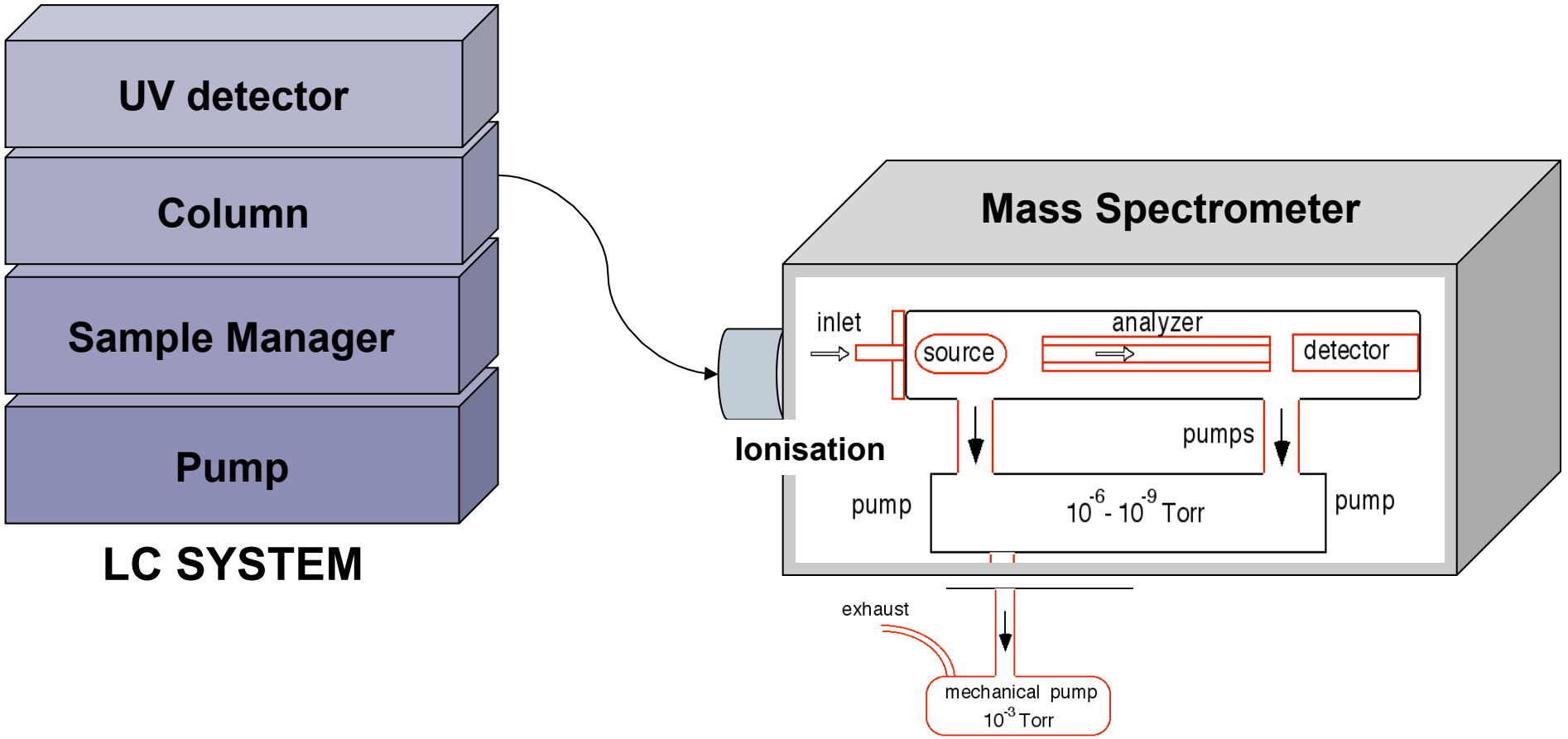
LC/MS - Summary

- MS can be used with chromatography
  - Separates components
  - Helps deconvolve complex mixtures
  - Retention times are usually very stable and provide an additional characteristic for i.d.

- Very sensitive (pmol) technique to detect minor components

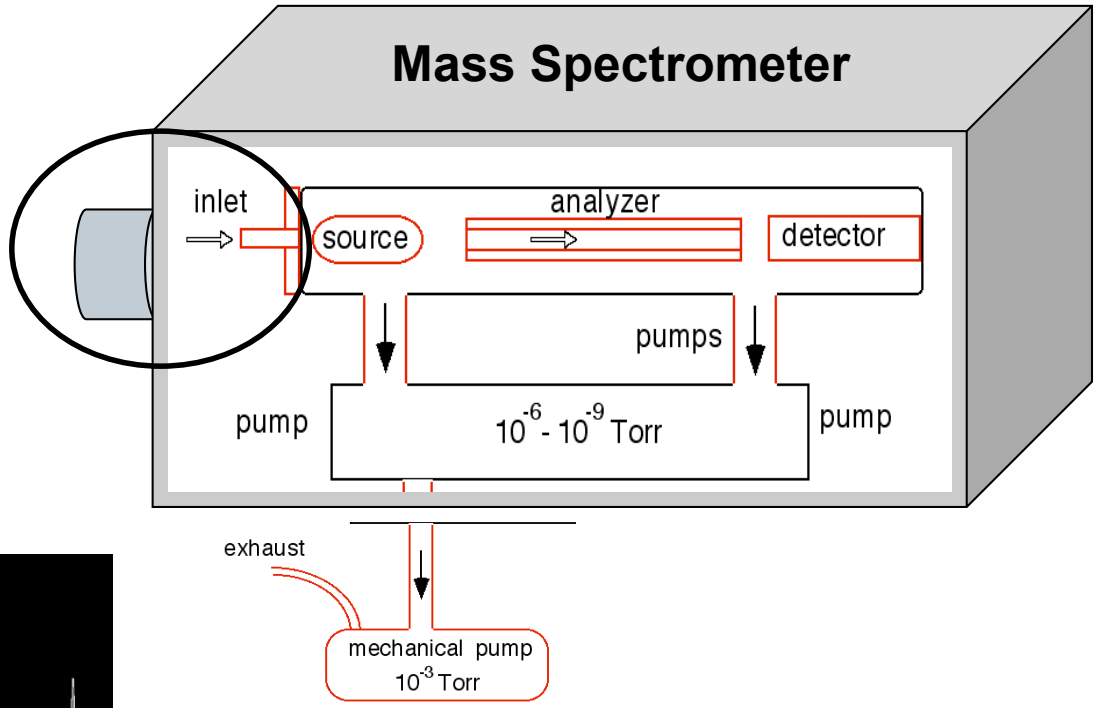
- Used extensively in combination with NMR for rapid structure elucidation of unknowns (e.g.)

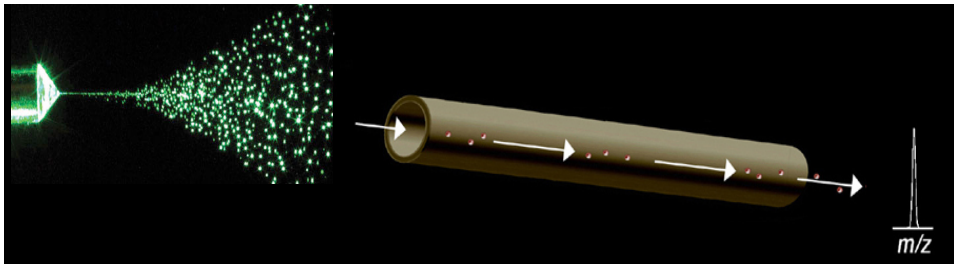
LC/MS - Setup


UV detector
Column
Sample Manager
Pump
LC SYSTEM
Mass Spectrometer
inlet
source
analyzer
detector
Ionisation
pumps
pump
10-6 - 10-9 Torr
pump
exhaust
mechanical pump
10-3 Torr

LC/MS – Electrospray Ionisation

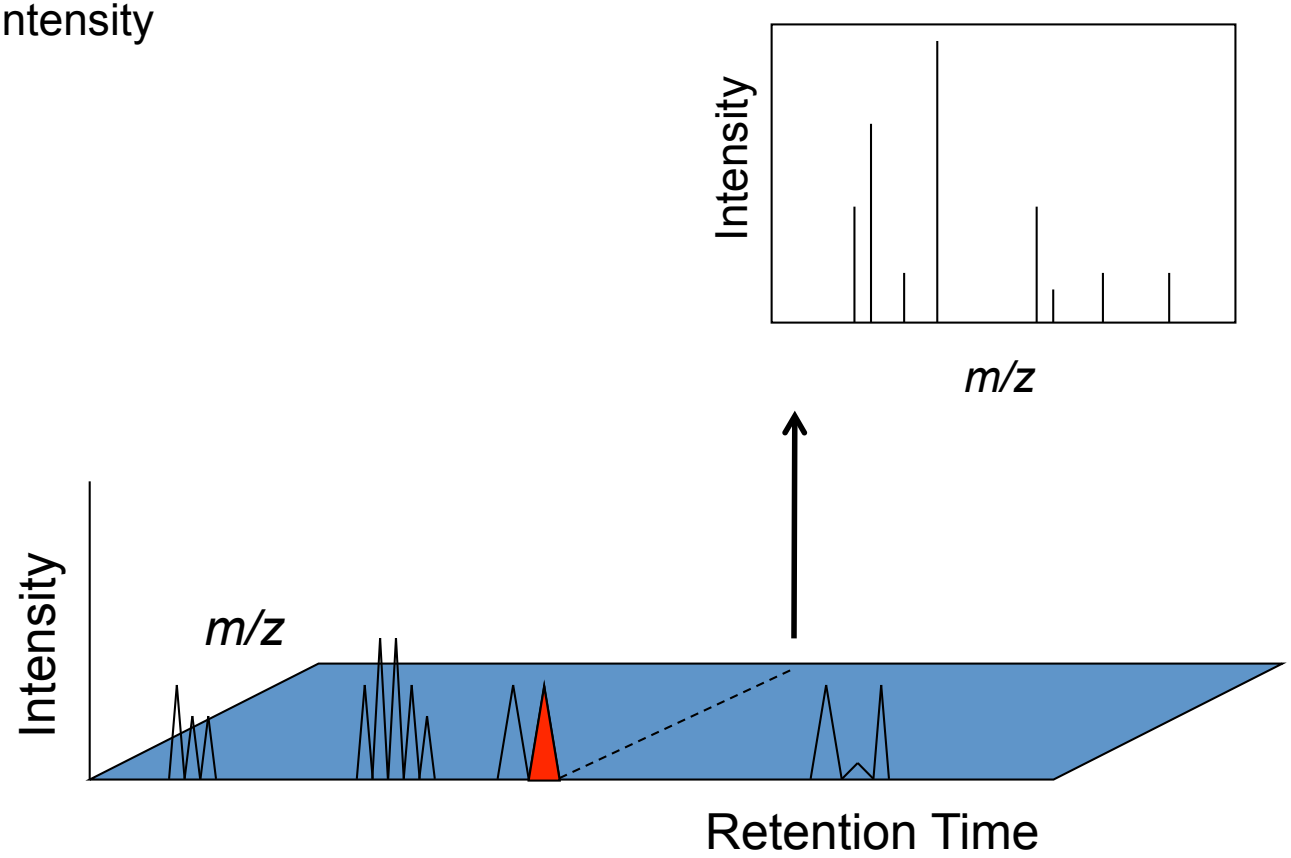
- Atmospheric pressure ionisation
- Soft ionisation technique
- Easily interfaced to LC
- Readily amenable to MS/MS analysis



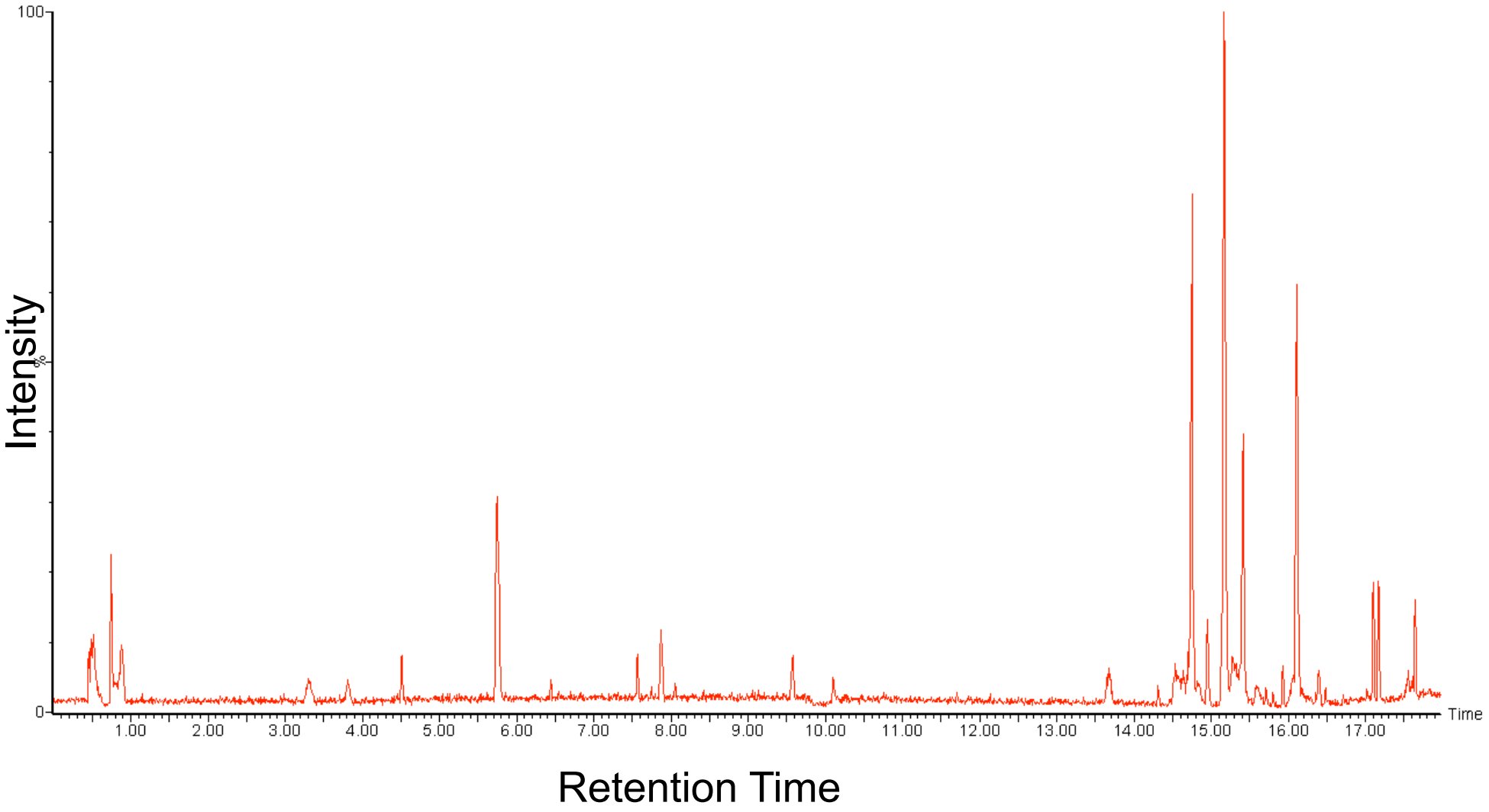


LC/MS – Separation and Detection

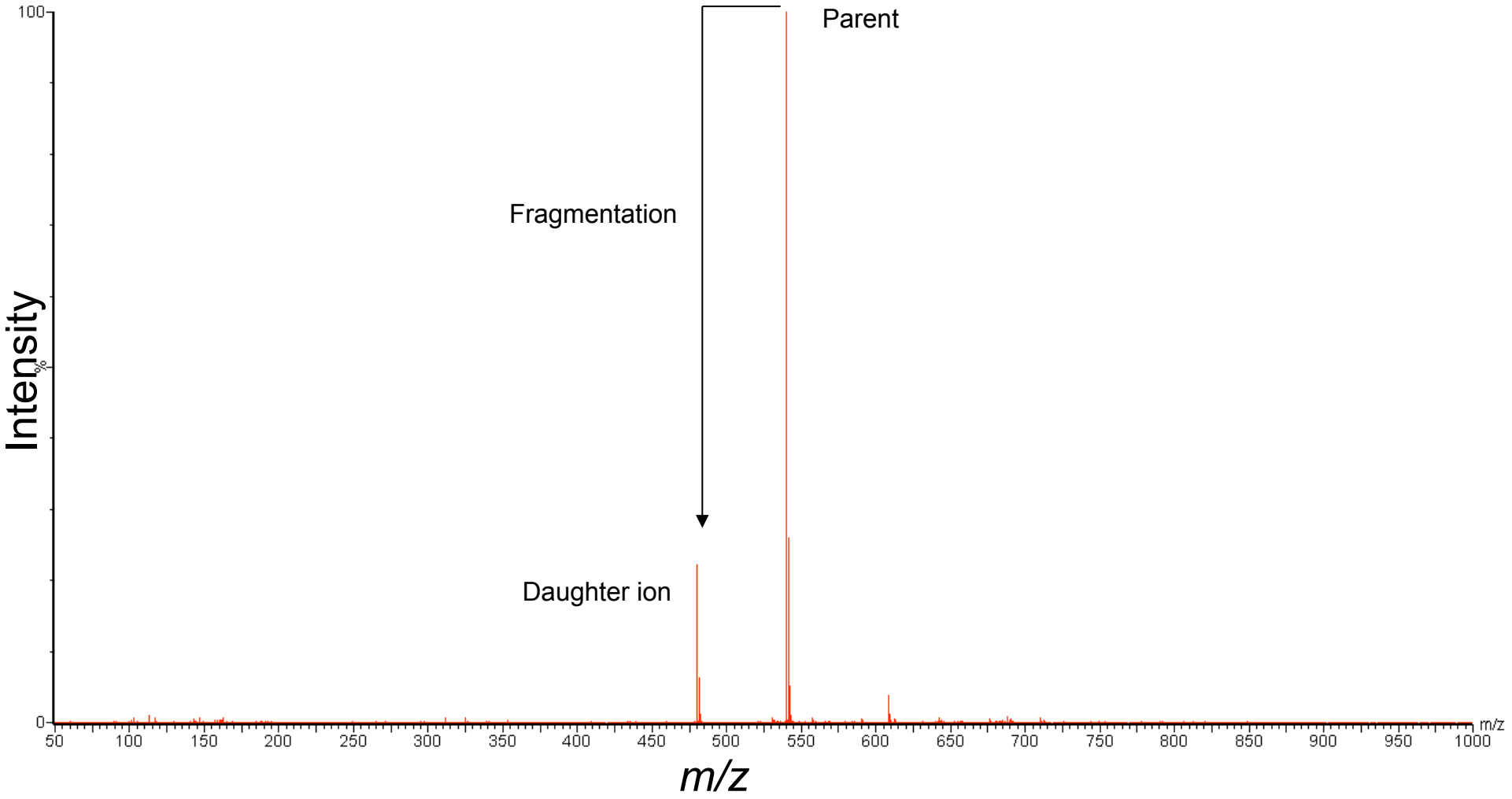
- 3D data
  - Mass-to-charge ratio (*m/z*)
  - Time
  - Intensity



LC/MS – Plasma Chromatogram



LC/MS – Spectrum



LC/MS

- Benefits:
- Rapid method for structural information
- Separates biofluid components and used in metabolism / PK studies
- Easily automated
- Information-rich datasets

LC/MS

- Limitations:

- Infusion MS generates very complex, overlapped spectra

- LC/MS is experimentally more complicated and generates (typically) very large datasets

- Mass spectra of trypic digests of proteins are very complex:
  - Require substantial expertise and computational power

- Suffers from ion suppression:
  - Can make observation/quantification of affected compounds difficult

Lecture Summary

Main Points

- Elucidating the structure of drugs and their targets is important in understanding the molecular mechanisms that they employ and can lead to the targeted development of new therapies

- The two main methods for characterising the 3D structure of large molecules such as proteins are:
  - X-ray crystallography (calculation of structure using diffraction pattern of a crystal)
  - NMR spectroscopy (calculation of structure using connectivity and distance measures)

- NMR is also used extensively to charaterise small molecules and metabolites, especially in complex mixtures

- Additional information can be obtained using complementary techniques such as mass spectrometry

- Characterising the interaction of drugs and their targets is possible using these and other techniques.