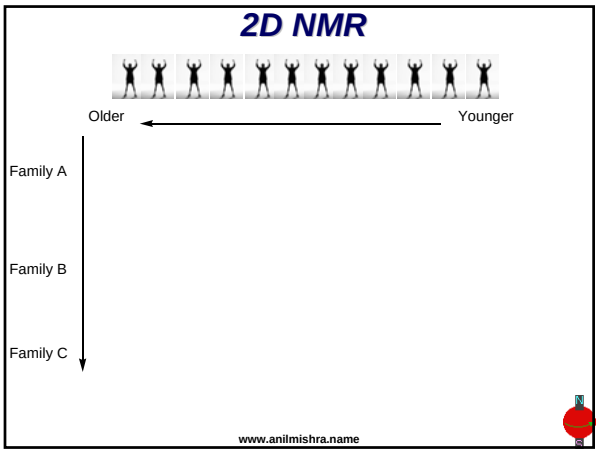
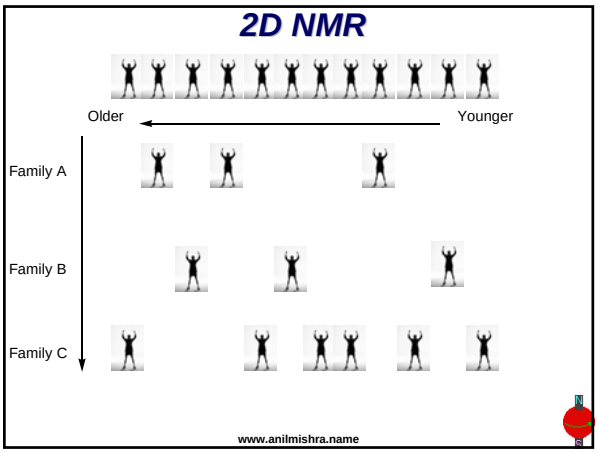
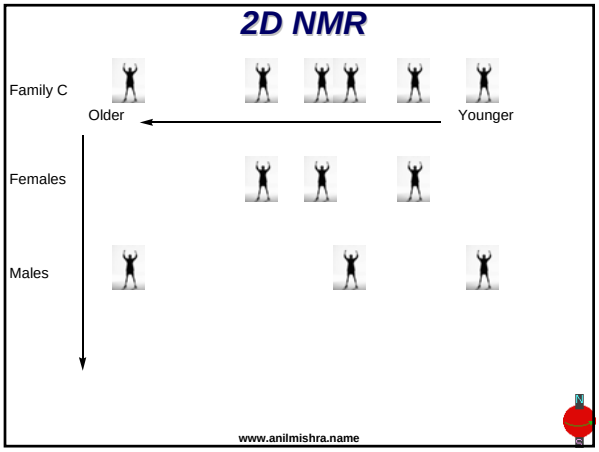
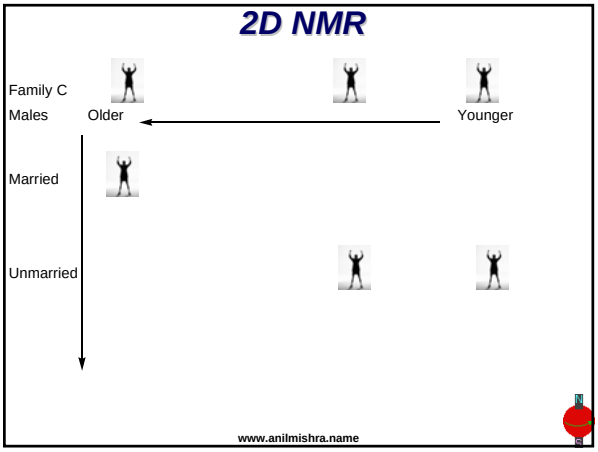
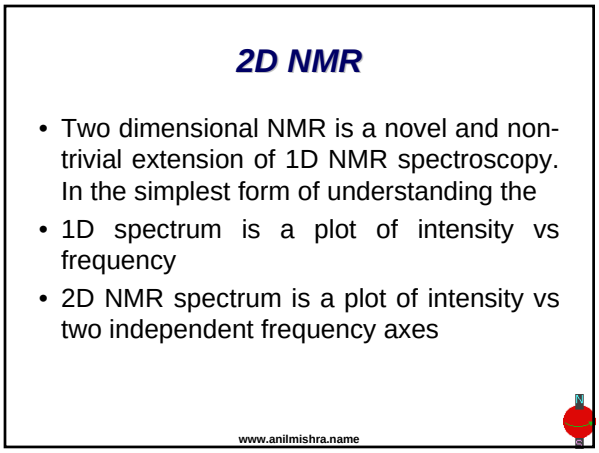
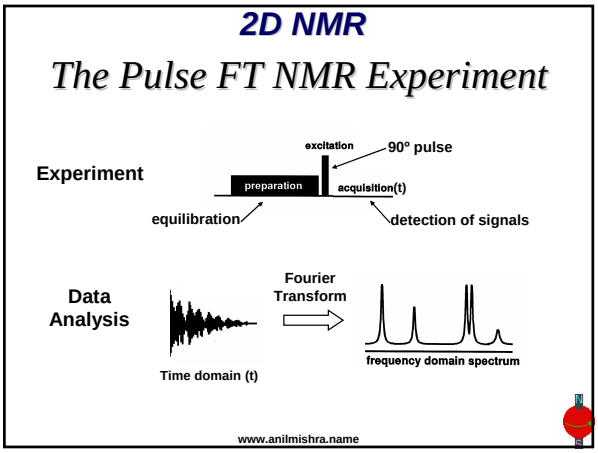

2D NMR
Older
Younger
Family A
Family B
Family C
www.anilmishra.name


2D NMR
Older
Younger
Family A
Family B
Family C
www.anilmishra.name

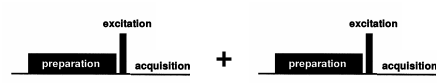

2D NMR
Family C
Older
Younger
Females
Males
www.anilmishra.name


2D NMR
Family C
Males
Older
Younger
Married
Unmarried
www.anilmishra.name

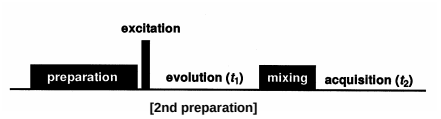

2D NMR
• Two dimensional NMR is a novel and non-trivial extension of 1D NMR spectroscopy. In the simplest form of understanding the
• 1D spectrum is a plot of intensity vs frequency
• 2D NMR spectrum is a plot of intensity vs two independent frequency axes
www.anilmishra.name


2D NMR
The Pulse FT NMR Experiment
Experiment
excitation
90° pulse
preparation
acquisition(t)
equilibration
detection of signals
Data Analysis
Fourier Transform
Time domain (t)
frequency domain spectrum
www.anilmishra.name

The 2D NMR Pulse Sequence



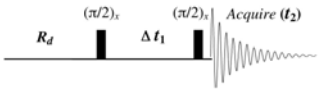
1D + 1D = 2D



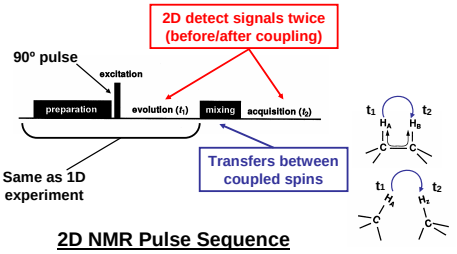
[2nd preparation]

2D NMR

- We know that NMR signals are detected as a function of time and 2D NMR thus implies we have NMR signal as a function of two independent time periods. Any 2D NMR scheme can be represented in general as below



2D NMR: Coupling is the Key

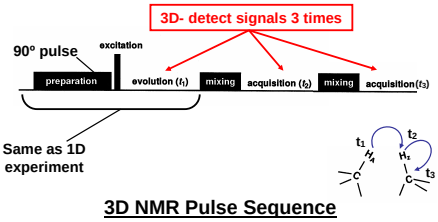


2D NMR Pulse Sequence

2D NMR

- In the preparation period equilibrium magnetization is built-up and transformed into coherences that evolve during the evolution (t_1) period.
- The evolution period is incremented systematically in successive experiments.
- During the mixing period a coherence /magnetization transfer is effected which then get detected during the detection period

Multi-Dimensional NMR:
Built on the 2D Principle

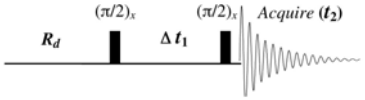


3D NMR Pulse Sequence

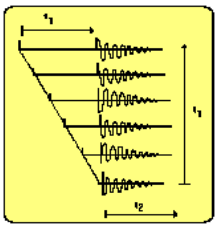
➢**Experiments are composites → acronyms are composites**

2D NMR Spectroscopy

- A two-dimensional NMR experiment involves a series of one-dimensional experiments.
- Each experiment consists of a sequence of radio frequency pulses with delay periods in between them.
- It is the timing, frequencies, and intensities of these pulses that distinguish different NMR experiments from one another.
- During some of the delays, the nuclear spins are allowed to freely precess (rotate) for a determined length of time known as the **evolution time**.
- The frequencies of the nuclei are detected after the final pulse.
- By incrementing the evolution time in successive experiments, a two-dimensional data set is generated from a series of one-dimensional experiments.



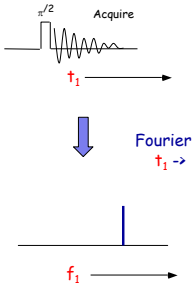
2D NMR



www.anilmishra.name

2D NMR

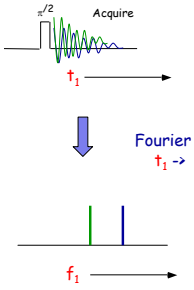
General One Dimensional Experiment



www.anilmishra.name

2D NMR

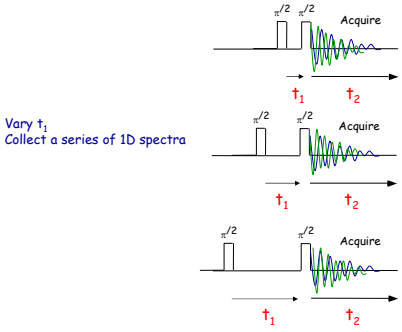
General One Dimensional Experiment



www.anilmishra.name

2D NMR

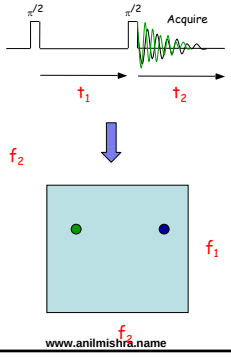
General Two Dimensional Experiment



www.anilmishra.name

2D NMR

General Two Dimensional Experiment

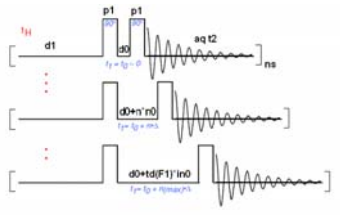


www.anilmishra.name

2D NMR

Creating Multiple Dimensions in NMR

- collect a series of FIDS incremented by a second time domain (t_1)
  - evolution of a second chemical shift or coupling constant occurs during this time period
- the normal acquisition time is t_2.
- Fourier transformation occurs for both t_1 and t_2, creating a two-dimensional (2D) NMR spectra

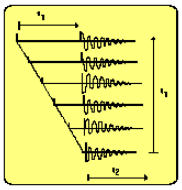


Relative appearance of each NMR spectra will be modulated by the t_1 delay

www.anilmishra.name

NMR Signal in Two-Time Periods

- The systematic incrementation of the t_1 interval and direct detection of NMR signal during t_2 gives two dimensional time domain data

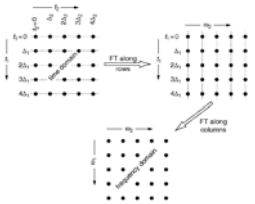


- All 2D experiments are a simple series of 1D experiments collected with different timing

www.anilmishra.name

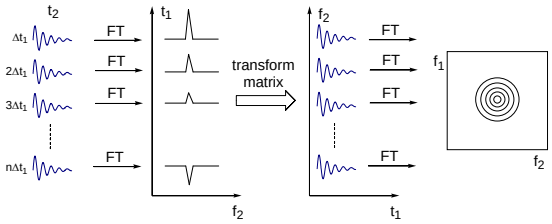
2D FT

- The time domain signal S(t1,t2) up on two dimensional Fourier transform yields two dimensional spectrum S(ω1, ω2).



www.anilmishra.name

PROCESSING 2D DATA



n is the number of increments

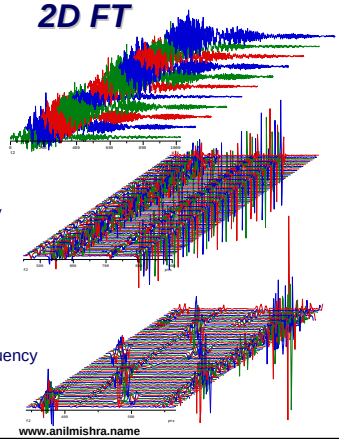
www.anilmishra.name

2D FT

time - time

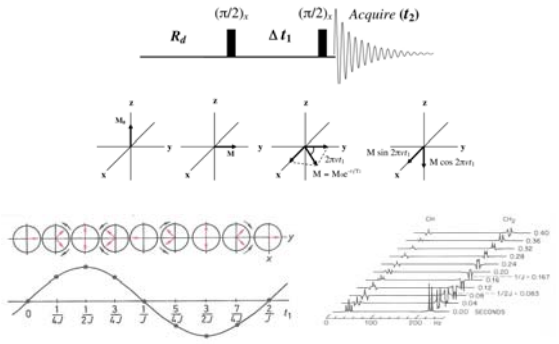
time - frequency

frequency - frequency



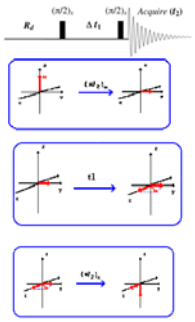
www.anilmishra.name

2D FT



www.anilmishra.name

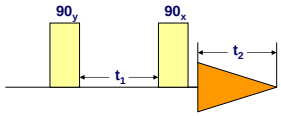
2D NMR



www.anilmishra.name

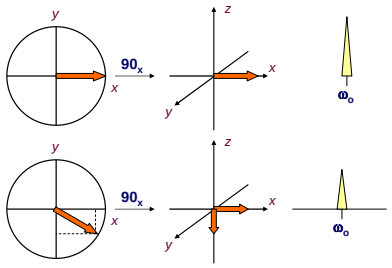
2D NMR

- IN the the ***COSY*** pulse sequence. Think of this pulses, were **t1** is the preparation time:



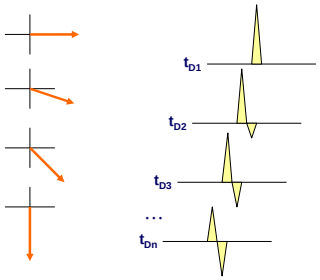
www.anilmishra.name

2D NMR



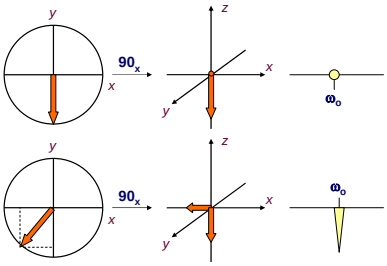
www.anilmishra.name

2D NMR



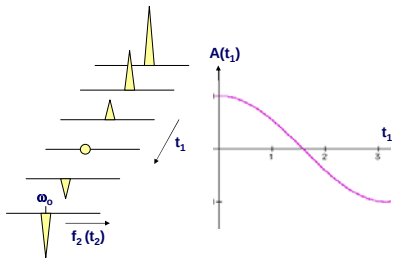
www.anilmishra.name

2D NMR



www.anilmishra.name

2D NMR



www.anilmishra.name

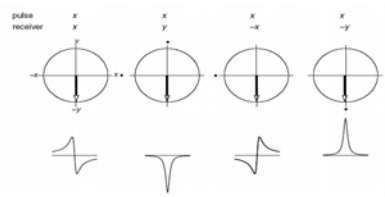
2D NMR

Processing 2D NMR Data

- How Is Phase Information Generated in a 2D Spectra?
  - *Need to collect both the Real (sin) and Imaginary (cos) component of the signal*

Re = A cos(φ) + D sin(φ)	φ = const.	for zero order correction
Im = A sin(φ) - D cos(φ)	φ = k Ωt	for linear (first order) correction

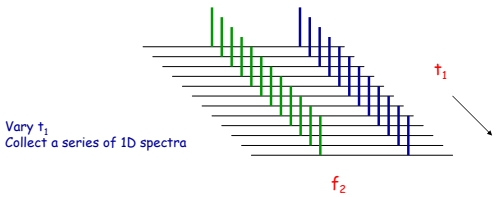
  - ***Need to collect sin and cos modulated data***
  - ***Phase of peak is determined by the relative phase of pulse and receiver***



www.anilmishra.name

2D NMR

General Two Dimensional Experiment

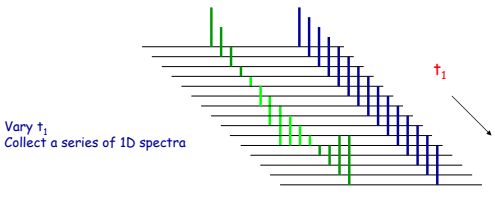


Here, the intensities of | and | do not change as a function of the t_1 evolution time

www.anilmishra.name

2D NMR

General Two Dimensional Experiment



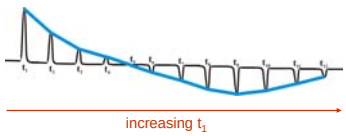
Whereas here, the intensity of | is modulated as a function of the t_1 evolution time

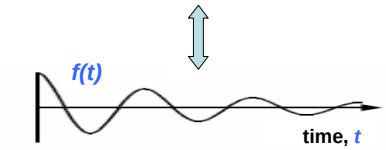
www.anilmishra.name

2D NMR

Creating Multiple Dimensions in NMR

- collect a series of peaks incremented by a second time domain (t_1)
  - Peak intensity (phase) will modulate at a frequency corresponding to the correlated chemical shift and/or coupling constant



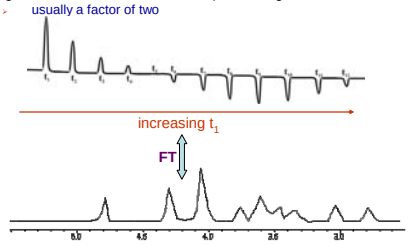


www.anilmishra.name

2D NMR

Creating Multiple Dimensions in NMR

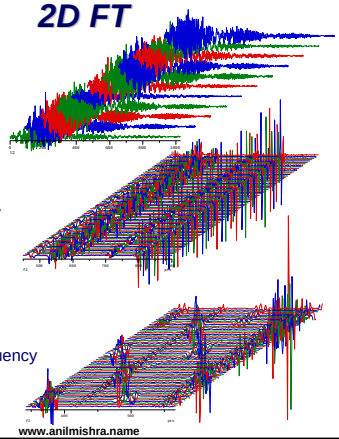
- Number of FIDs or Spectra collected as a function of $t_1$will determine:
  - number of data points in t_1
  - resolution of the t_1 dimension
  - experiment time
    - **Number of scans *x* acquisition time *x* number of t_1 points**
    - **1D NMR ~few mins. → 2D ~few hours → 3D ~ few days**
- t_1 increment will determine the sweep-width in t_1
  - usually a factor of two



typically significantly lower digital resolution in t_1

www.anilmishra.name

2D FT

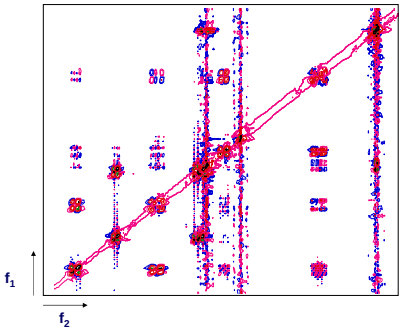


www.anilmishra.name

2D NMR

The same with some real data

Now the ***contour-plot*** showing all the ***cross-peaks***:

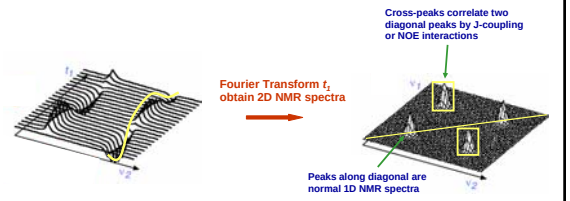


www.anilmishra.name

2D NMR

Creating Multiple Dimensions in NMR

- Spectra is now processed along t_1 dimension
  - Similar to t_2 processing
- Diagonal peaks are normal 1D spectrum
- Off-diagonal or cross-peaks indicate correlation between diagonal peaks
- Processed spectra is actually three-dimensional, where peaks now have volumes.

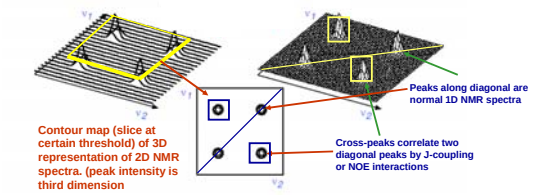


www.anilmishra.name

2D NMR

Displaying Multiple Dimensions in NMR

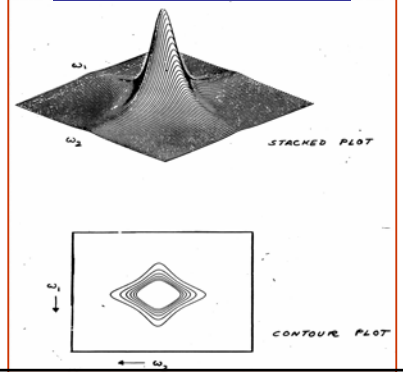
- Since spectra is 3D, data is typically displayed as contour plot
  - Single slice through the peak intensity above the noise
    - ***Referred to as a threshold***
  - Peaks represented by series of concentric ellipses (contours)
  - More contour lines → more intense the peak



www.anilmishra.name

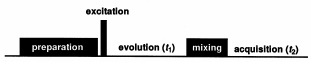
2D NMR

REPRESENTATION OF A 2D SPECTRUM

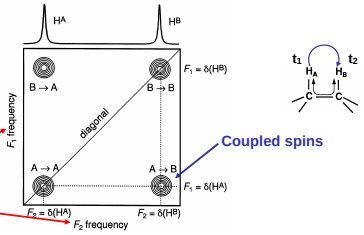


The 2D NMR Spectrum

Pulse Sequence

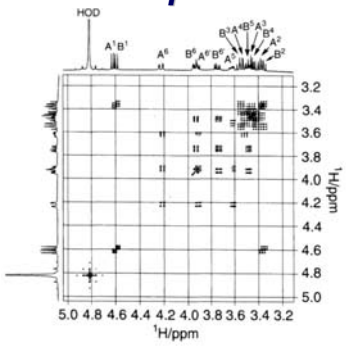


Spectrum



www.anilmishra.name

2D Spectra



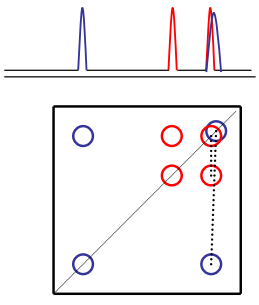
www.anilmishra.name

The Power of 2D NMR:

Resolving Overlapping Signals

1D — **2 signals overlapped**

2D — **2 cross peaks resolved**



www.anilmishra.name

2D NMR

TWO –DIMENSIONAL SPECTROSCOPY

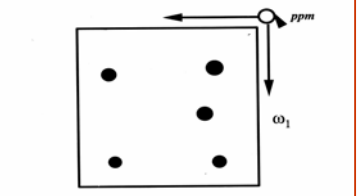
2D NMR ALLOWS YOU TO :

(a) SPREAD INFORMATION INTO TWO–DIMENSIONS

INCREASE RESOLUTION.

(b) EXPLOIT NMR PARAMETERS e.g. J COUPLINGS

d–d INTERACTIONS, TO GET *MORE INFORMATION*



How is the ω_1 axis is generated ?

www.anilmishra.name

2D NMR

J-Correlated Spectroscopy

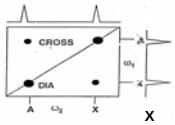
What Happens?

- *Information Transfer* between spins that are *J*-coupled.

A

What Do We See?

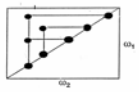
- *Cross Peaks* between *J*-coupled spins.



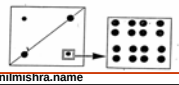
X

What do We Learn?

- How to identify spin systems and (sometimes), assign resonances.

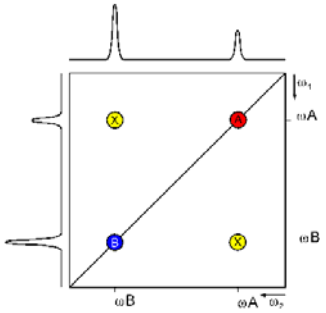


- obtain coupling constants from fine structures of cross peaks.



www.anilmishra.name

2D NMR



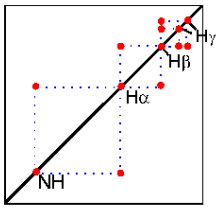
www.anilmishra.name

Homonuclear correlation - COSY

- ***COSY*** stands for ***COrrelation SpectroscopY***, and for this particular case in which we are dealing with homonuclear couplings, ***homonuclear correlation spectroscopy***.
- What COSY is good for is to tell which spin is connected to which other spin. The off-diagonal peaks are this, and they indicate that those two peaks in the diagonal are coupled.

www.anilmishra.name

Homonuclear correlation - COSY

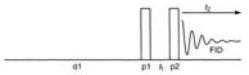


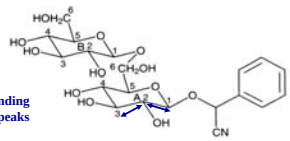
www.anilmishra.name

2D COSY Spectroscopy

COSY-90 2D NMR

- Correlates chemical shifts that are coupled
  - P1 is 90º pulse (many varieties)
  - Diagonal is the normal 1D spectrum
  - Off-diagonal peaks or cross-peaks identifies nuclei that are coupled





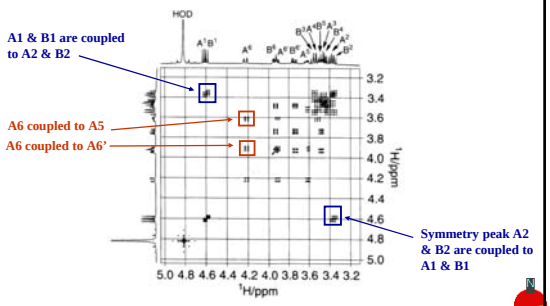
Example: diagonal corresponding to A2 should have two cross-peaks connecting to A1 and A3

www.anilmishra.name

2D COSY Spectroscopy

COSY-90 2D NMR

- Correlates chemical shifts that are coupled



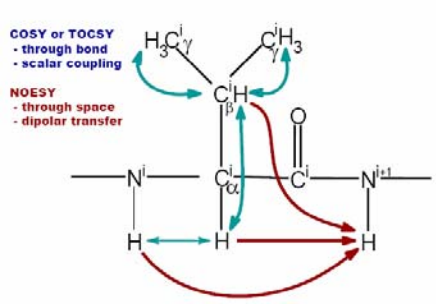
www.anilmishra.name

2D Experiments

- **COSY** [Correlated Spectroscopy] or H,H – COSY experiment
- **HETCOR** [HETeronuclear CORrelated] or C, H –COSY (Correlated spectroscopy) experiment
- **HSQC ; HMQC** [Reversed 2D Heteronuclear (H,C)- Correlated NMR Spectroscopy]
- The Gradient selected (gs) **HMBC** Experiment
- The **TOCSY** Experiment

www.anilmishra.name

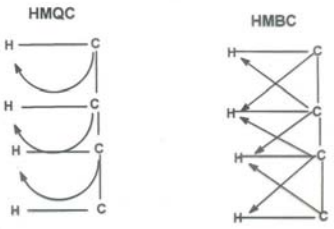
2D Experiments



www.anilmishra.name

2D Experiments

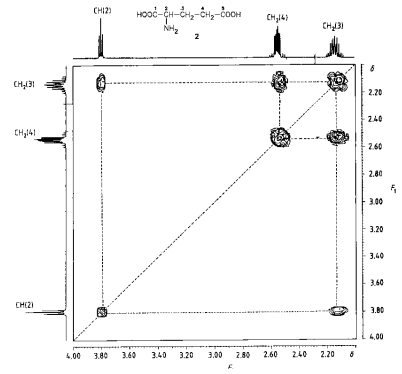
- HMQC – heteronuclear multiple quantum coherence
- HMBC – heteronuclear multiple bond coherence



www.anilmishra.name

2D NMR

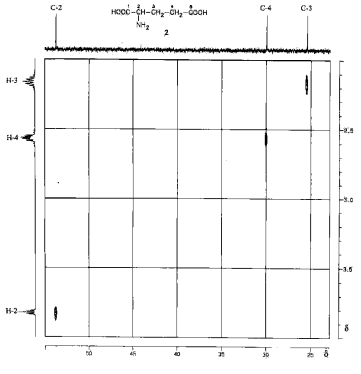
Glutamic Acid COSY



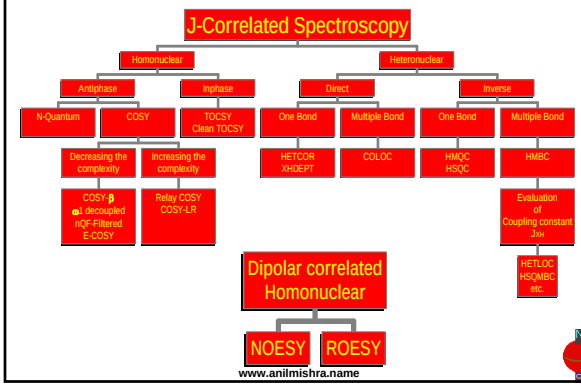
www.anilmishra.name

2D NMR

Glutamic Acid HetCOR



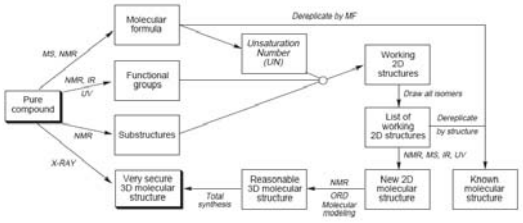
Experiments used for structure elucidation



www.anilmishra.name

Spectral Interpretation

General Process for Structure Elucidation of an Unknown



www.anilmishra.name

Spectral Interpretation

General Process for Structure Elucidation of an Unknown

- Normally the molecular formula is derived from a combination of ^{13}C NMR, DEPT and MS data.
- Using IR, UV and ^{13}C NMR the functional groups can be proposed
- ^{1}H NMR coupling data or 2D NMR correlations are used to assemble substructures
- The substructures are then combined into 'working structures' using all possible combinations of the substructures
- Check structures for consistency with the 2D-NMR data and MS fragmentations *etc.*
- ^{13}C chemical shifts of the surviving structure(s) are then compared with literature, database or predicted values to confirm the 2D structure of the molecule.
- To determine the relative stereochemistry of the molecule, ^{1}H coupling constant (*J*) and Nuclear Overhauser (NOE) data is used

Need to Verify as early as possible if the structure has already been identified

- Don't want to waste time and effort re-discovering a compound
- Done by using a combination of molecular formula, substructure and chemical structure databases

www.anilmishra.name

NMR Spectroscopy

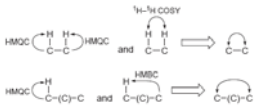
Possible Strategy using H-H and H-C correlations

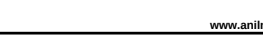
- COSY sees 2,3 & 4 bond correlation which leads to confusion

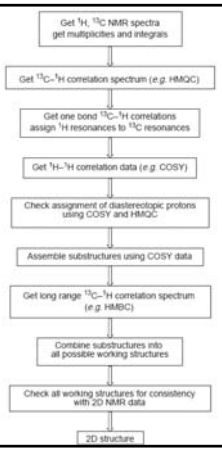
- Impossible to distinguish between 3 and 3-bond correlations in HMBC

- Angular dependence of $^{3}J_{HH}$ and $^{3}J_{CH}$ means that some expected cross peaks will not appear in the COSY and HMBC spectra

- Lower resolution in indirect ^{13}C dimension



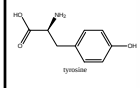


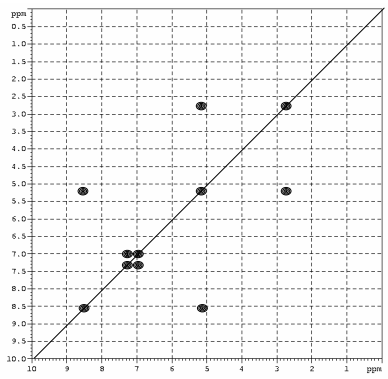


www.anilmishr

COSY

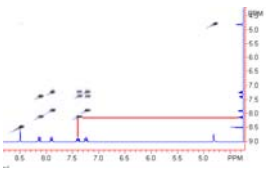
Tyr-COSY

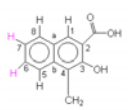




www.anilmishra.name

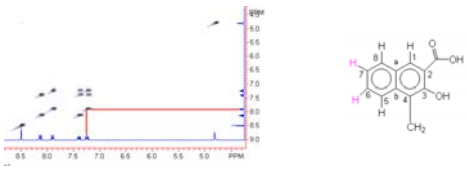
COSY of Pamoic Acid



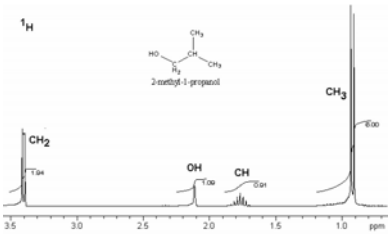


www.anilmishra.name

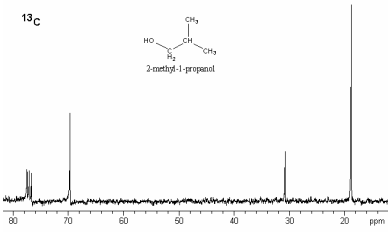
COSY of Pamoic Acid



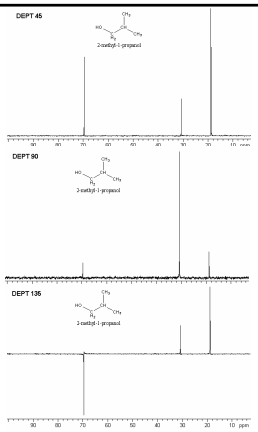
www.anilmishra.name



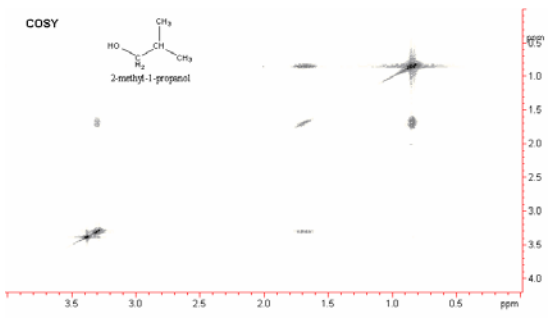
www.anilmishra.name



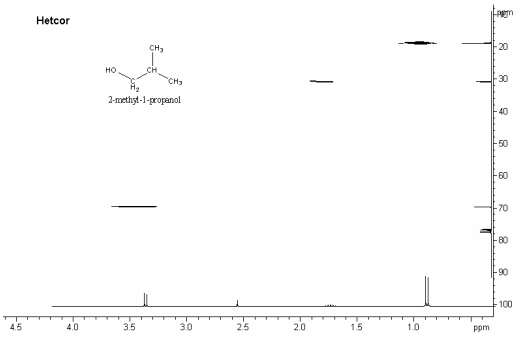
www.anilmishra.name



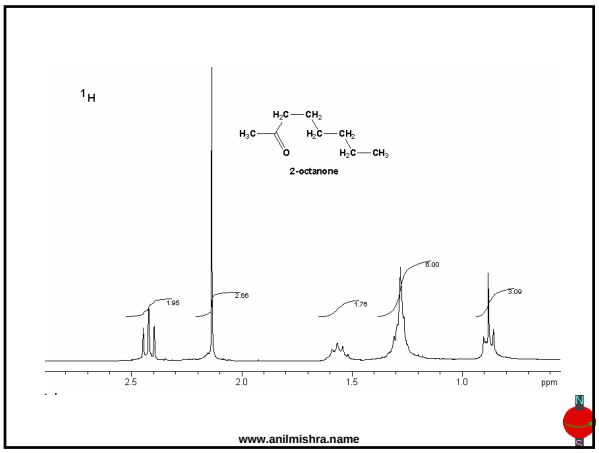
www.anilmishra.name

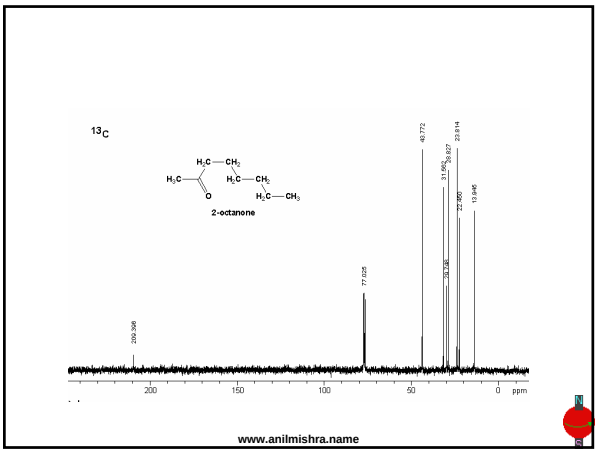


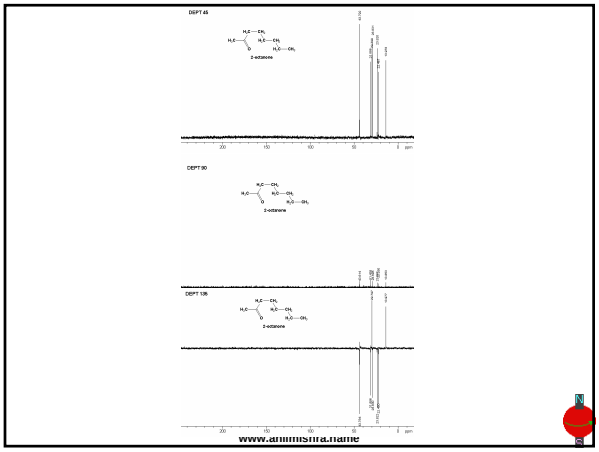
www.anilmishra.name

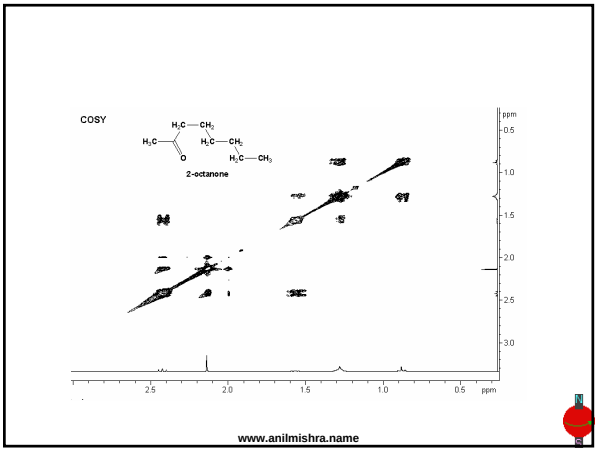


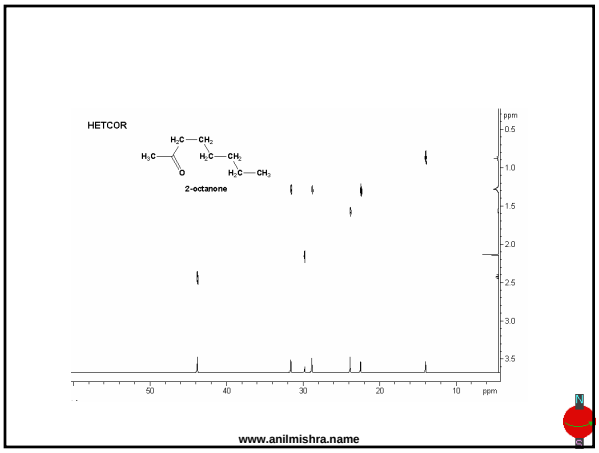
www.anilmishra.name











Online Resources

- Animations
- http://mutuslab.cs.uwindsor.ca/schurko/nmrcourse/animations.html

www.anilmishra.name