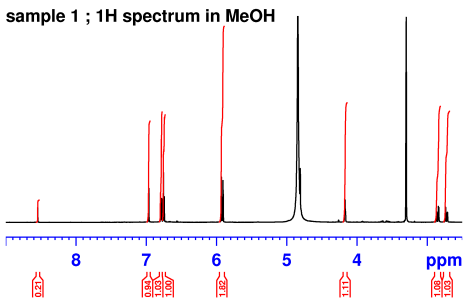
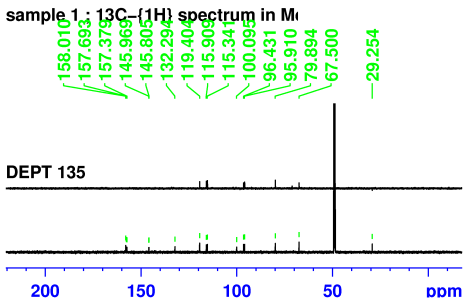
Applications of 600 MHz Nuclear Magnetic Resonance Spectroscopy.

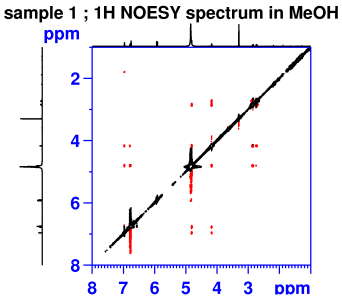
Part 1 : Small molecules.

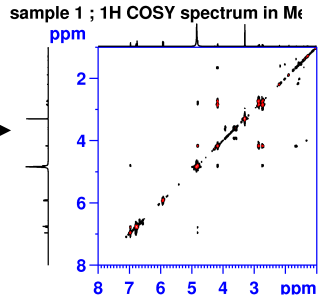
Harold Toms NMR Manager
SBCS

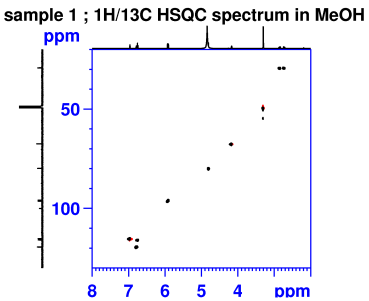
Funtumia elastica (Silkrubber) is a medicinal plant. It is a medium-sized African rubber tree with glossy leaves, milky sap, and long woody seedpods. The bark is the medicinal portion. Funtumia has important antioxidant, antifungal, anti-inflammatory, and antibiotic properties. It is traditionally used in its native environment, tropical Africa, to treat asthma, allergies, and other respiratory issues, as well as malaria.

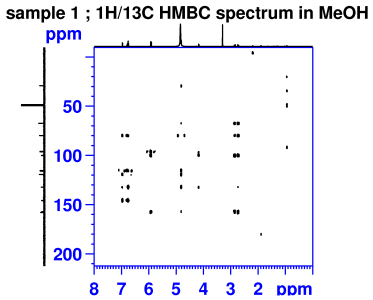

sample 1 ; 1H spectrum in MeOH
8
7
6
5
4
ppm


sample 1 ; 13C-{1H} spectrum in M
158.010
157.693
157.379
145.969
145.805
132.294
119.404
115.909
115.341
100.093
96.431
95.910
79.894
67.500
29.254
DEPT 135
200
150
100
50
ppm


sample 1 ; 1H NOESY spectrum in MeOH
ppm
2
4
6
8
8 7 6 5 4 3 ppm


sample 1 ; 1H COSY spectrum in M
ppm
2
4
6
8
8 7 6 5 4 3 ppm


sample 1 ; 1H/13C HSQC spectrum in MeOH
ppm
50
100
8 7 6 5 4 ppm


sample 1 ; 1H/13C HMBC spectrum in MeOH
ppm
50
100
150
200
8 7 6 5 4 3 2 ppm

SDBS Compounds and Spectral Search

Compound Name:

'%,*'for the wild card.
eg. %benzene » ethylbenzene...

Molecular Formula:

C, H, then the other elements are alphabetical order, '%,*' for the wild card

Molecular Weight:

to

Numbers between left and right columns
Up to the first place of a decimal point

CAS Registry No.:

'%,*' for the wild card.

SDBS No.:

'%,*' for the wild card.

Atoms:

C(Carbon)		to	
H(Hydrogen)		to	
N(Nitrogen)		to	
O(Oxygen)		to	
F(Fluorine)		to	
Cl(Chlorine)		to	
Br(Bromine)		to	
I(Iodine)		to	
S(Sulfur)		to	
P(Phosphorus)		to	
Si(Silicon)		to	

Numbers between left and right columns.

Spectrum:

Check the spectra of your interest.

☐ MS ☐ IR
☑ ^{13}C NMR ☐ Raman
☐ ^{1}H NMR ☐ ESR

IR Peaks(cm^{-1}): Allowance ±10

',' or space is the separator for multiple peaks.
Use '-', to set a range:. eg. 550-750,1650 3000-

Transmittance < 80 %

^{13}C NMR Shift(ppm): Allowance , 79.8945, 67.4997, 29.2541, ±3

',' is the separator for multiple shifts, eg. 129.3,18.4,...

No shift regions:

Range defined by two numbers separated by a space, eg. 110 78,...

^{1}H NMR Shift(ppm): Allowance ±0.2

No shift regions:

MS Peaks and intensities:

Mass and its intensity are a set of data separated by a space, eg. 110 22,..

Search Clear Hit: 20hit

SDBS Search Results: 1 - 1 out of 1 hits

SDBS No	Molecular Formula	Molecular Weight	MS	CNMR	HNMR	IR	Raman	ESR	Compound Name
22007	C15H14O6	290.3	Y	Y	Y	Y	N	N	(-)-epicatechin

Return to Search

SDBS Information

SDBS No.: 22007

Compound Name:
(-)-epicatechin

Molecular Formula: $C_{15}H_{14}O_6$

Molecular Weight: 290.3

CAS Registry No.:
490-46-0

Spectral Code:
Mass :
^{13}C NMR : in DMSO-d_6
^{1}H NMR : 400 MHz in DMSO-d_6
IR : nujol mull
IR : KBr disc

Chemical Information:

Return to Search:
Return to Result:

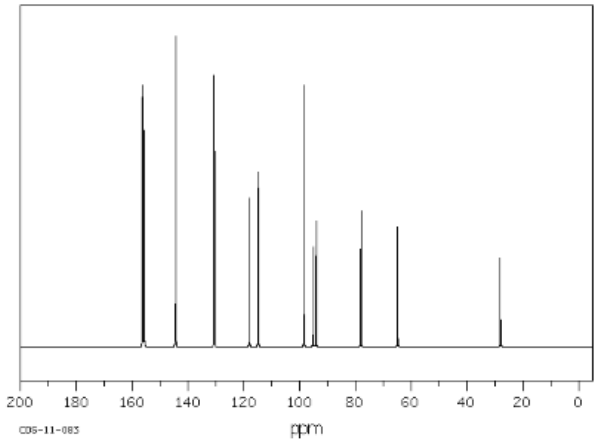
SDBS-^{13}C NMR SDBS No. 22007CDS-11-083

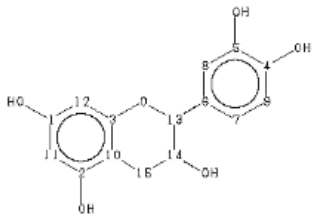
22.53 MHz

$C_{15}H_{14}O_6$

0.029 g : 0.5 ml DMSO-d_6

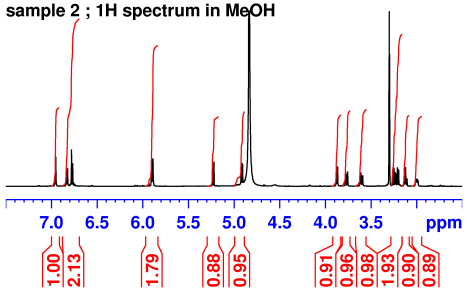
(-)-epicatechin



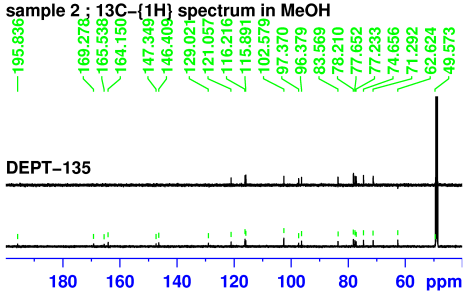


ppm	Int.	Assign.
156.41	859	1 *
156.16	901	2 *
155.68	750	3 *
144.42	1000	4 #
144.35	982	5 #
130.55	941	6
117.91	515	7 *1
114.85	591	8 *1
114.72	531	9 *1
98.47	909	10
95.12	344	11
94.09	437	12
78.01	472	13
64.89	416	14
28.07	309	15

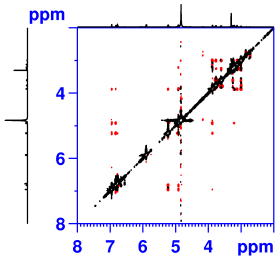
sample 2 ; 1H spectrum in MeOH



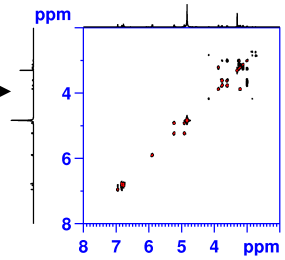
sample 2 ; 13C–{1H} spectrum in MeOH



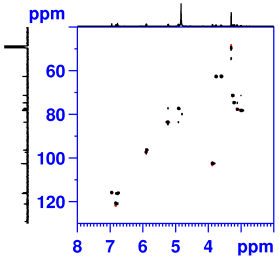
sample 2 ; 1H NOESY spectrum in MeOH



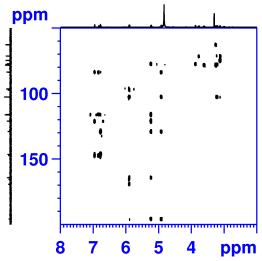
sample 2 ; 1H COSY spectrum in MeOH



sample 2 ; 1H/13C HSQC spectrum in MeOH



sample 2 ; 1H/13C HMBC spectrum in MeOH



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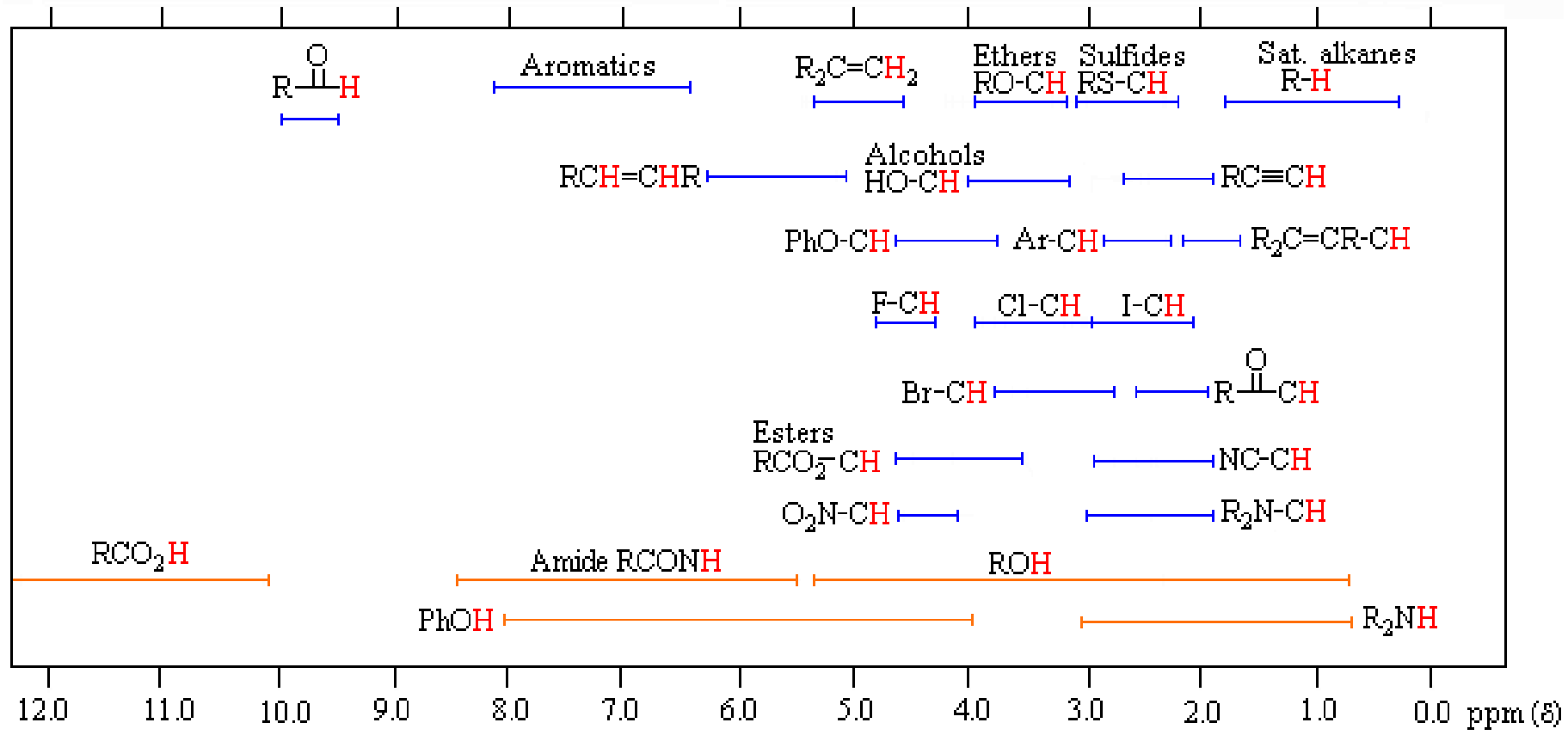
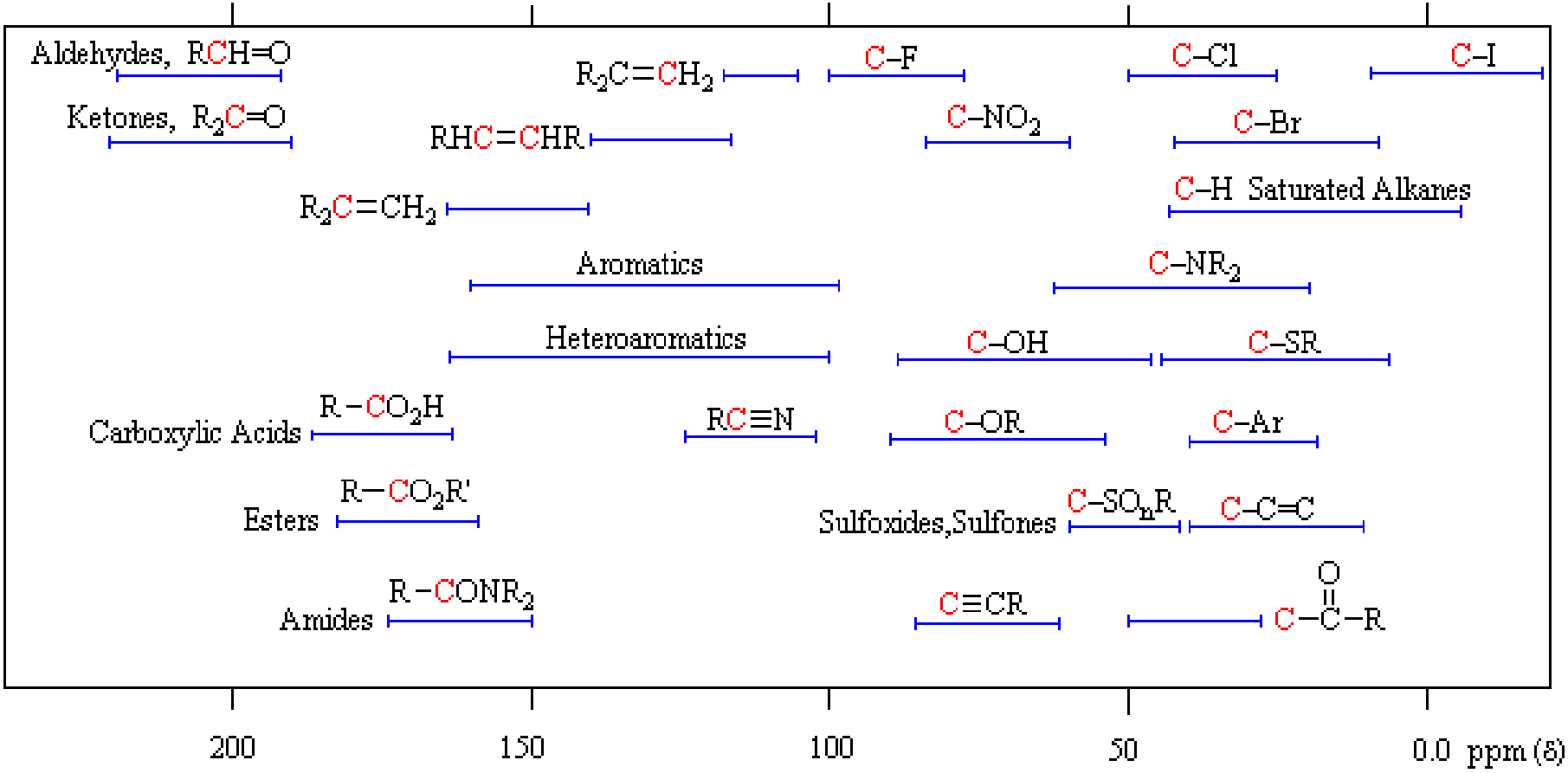
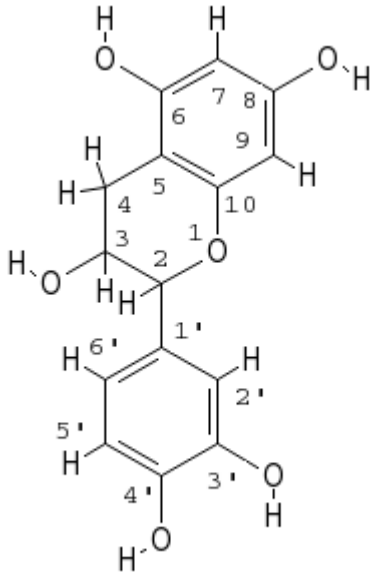
^{1}H chemical shift of some common groups in $CDCl_3$ solution

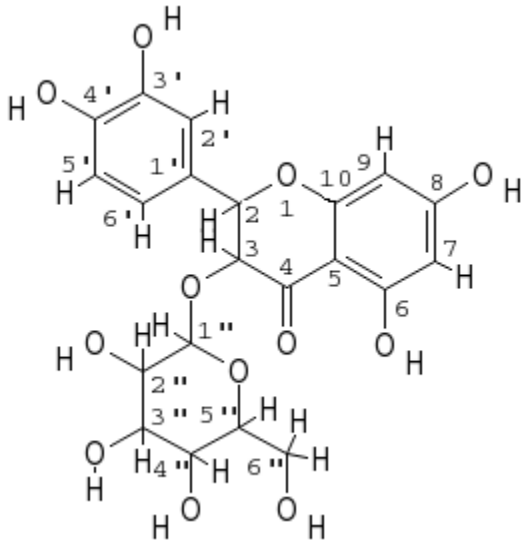


^{13}C chemical shift of some common groups in $CDCl_3$ solution





(-)-epicatechin



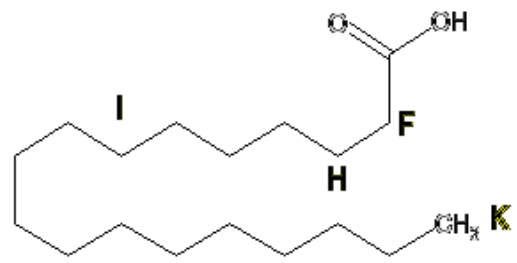
taxifolin-3-D-glucopyranoside

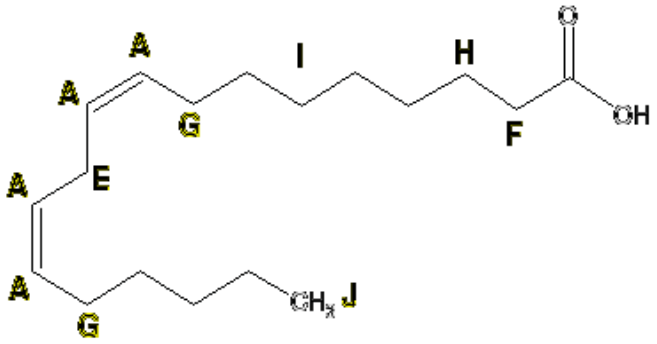
Applications of 600 MHz Nuclear Magnetic Resonance Spectroscopy.

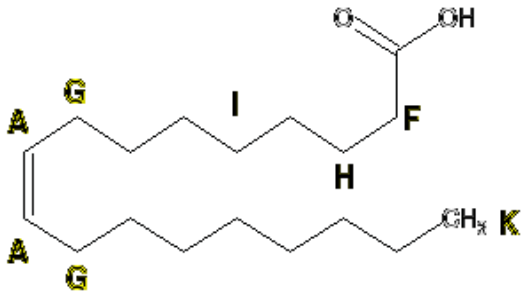
Part 2 : Mixtures.

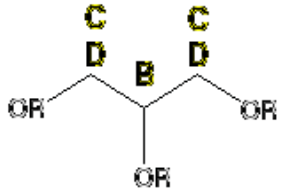
High resolution ^{1}H NMR spectroscopy can be used not only to determine the distribution of the various fatty acids constituting a vegetable oil sample but also to detect and quantify the levels of primary lipid oxidation products such as conjugated hydroperoxydienes and secondary products such as aldehydes

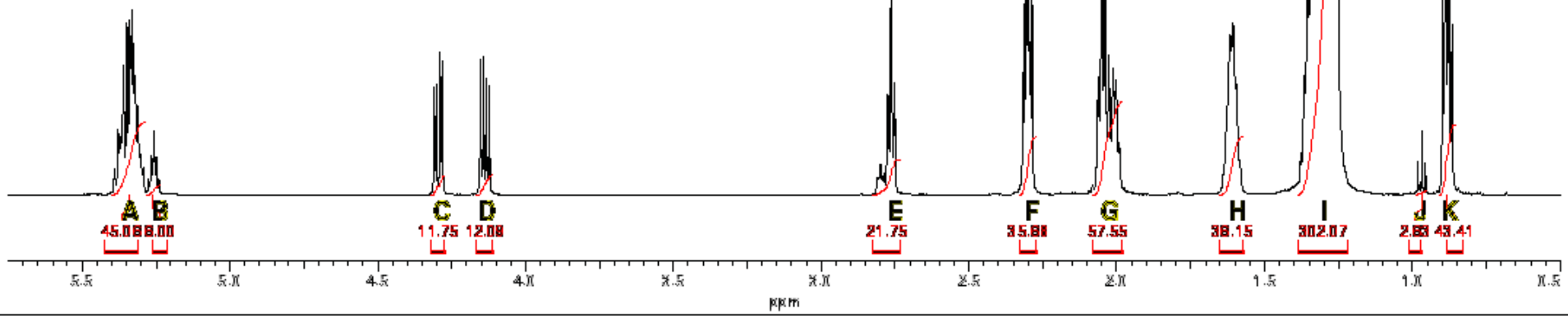
600.13 MHz ^{1}H NMR SPECTRUM OF A TYPICAL CULINARY OIL SAMPLE











ASSIGNMENTS

A acyl chain vinylic
B glycerol CH
C glycerol CH_2
D glycerol CH_2
E *bis*-allylic
F CH_2CO
G $CH_2C{=}C$
H $C\underline{H}_2CH_2CO$
I acyl chain $(CH_2)_n$
J w_3 acyl chain CH_3
K acyl chain CH_3

FATTY ACID DISTRIBUTION

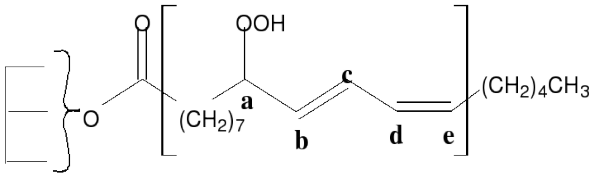
$$\%\text{saturated} = 100*(1 - (I_G/2*I_F)))$$

$$\%\text{monounsaturated} = 100*((I_G/(2*I_F)) - (I_E/I_F) + (I_J/(I_J + I_K)))$$

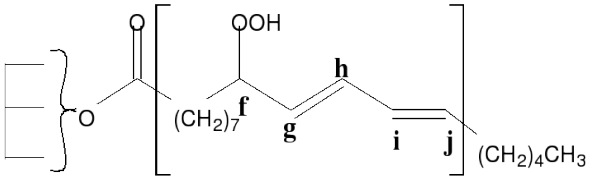
$$\%\text{diunsaturated} = 100*((I_E/I_F) - 2*(I_J/(I_J + I_K)))$$

$$\%\text{triunsaturated} = 100*(I_J/(I_J + I_K))$$

STRUCTURAL UNITS OF LIPID OXIDATION PRODUCTS (LOPS) DETECTABLE IN PEROXIDISED PUFAS BY HIGH RESOLUTION NMR SPECTROSCOPY



[I]



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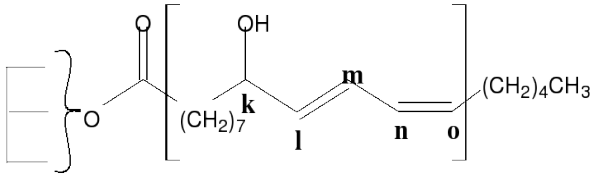

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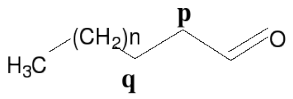

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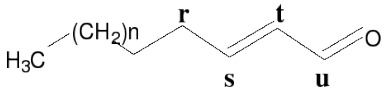
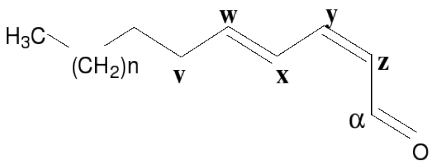
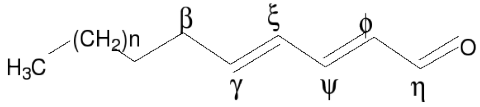


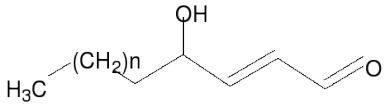
[V]



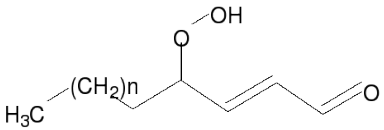
[VI]



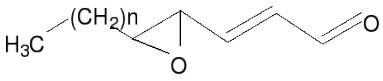
[VII]



[VIII]



[IX]

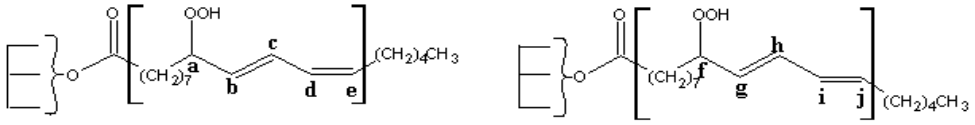


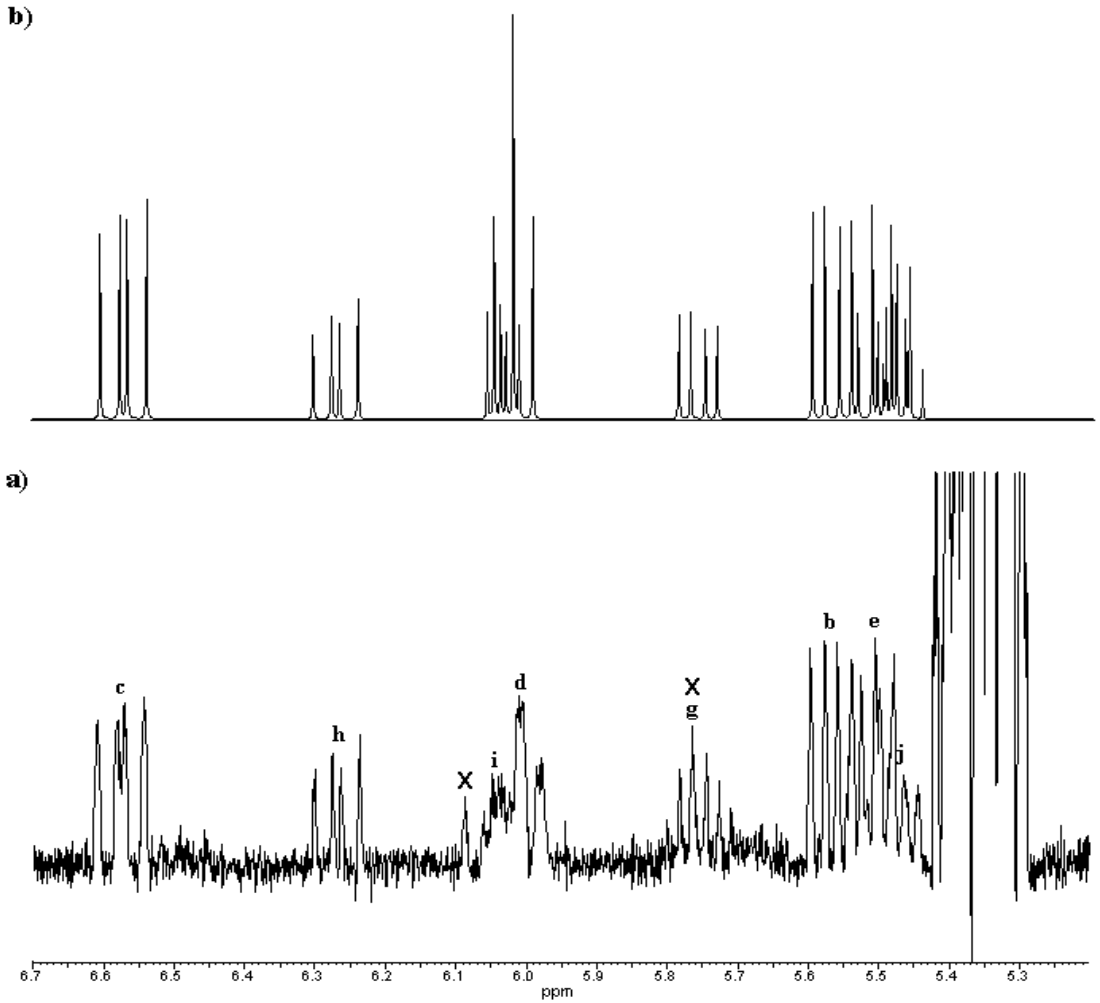
[X]

KEY:

[I] 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienylglycerol adduct
[II] 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienylglycerol adduct
[III] 9-hydroxy-*trans*-10,*cis*-12-octadecadienylglycerol adduct
[IV] *n*-alkanals
[V] *trans*-2-alkenals
[VI] *cis*,*trans*-alka-2,4-dienals
[VII] *trans*,*trans*-alka-2,4-dienals
[VIII] 4-hydroxy-*trans*-2-alkenals
[IX] 4-hydroperoxy-*trans*-2-alkenals
[X] 4,5-epoxy-*trans*-2-alkenals

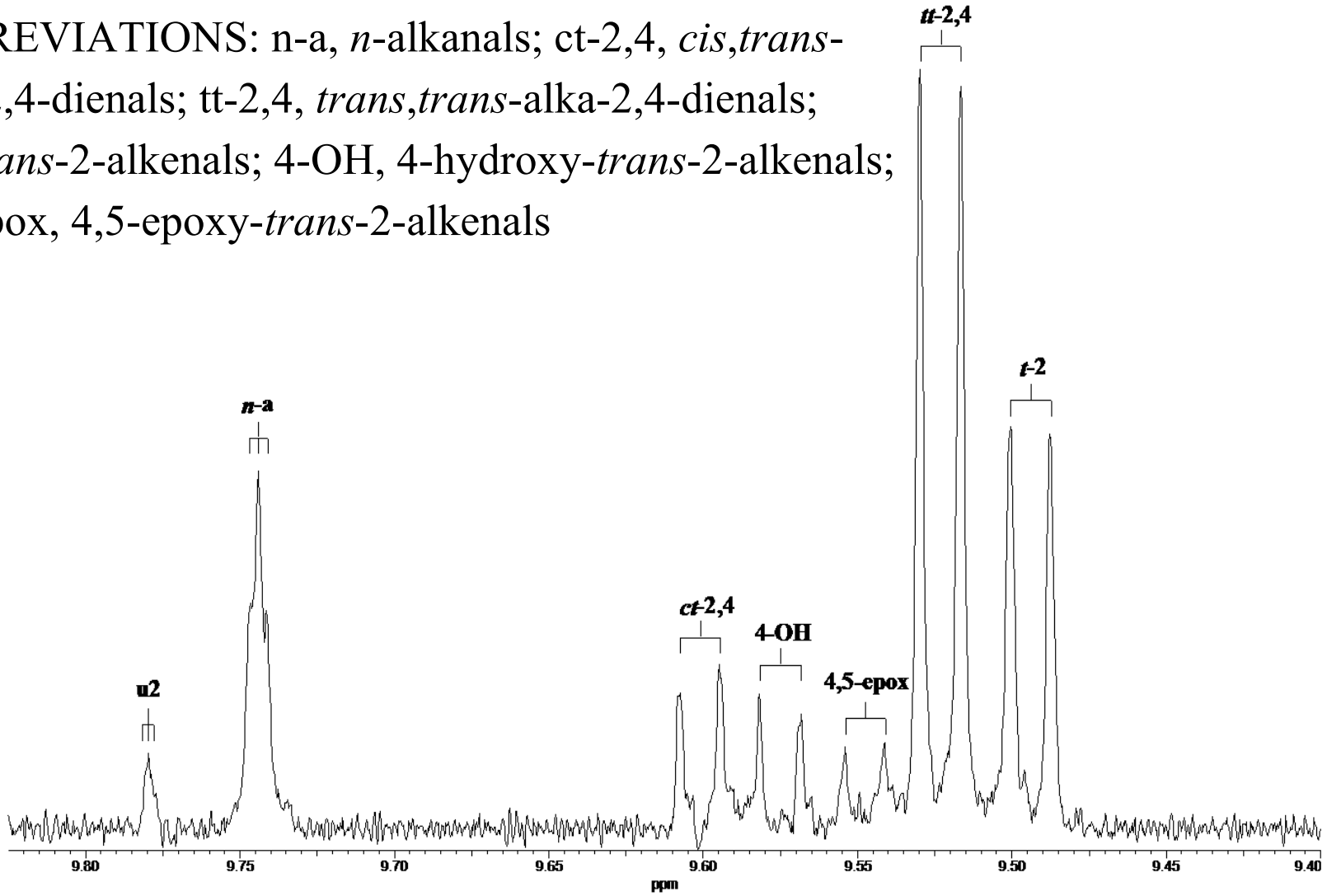
EXPANDED 5.20-6.70 PPM REGIONS OF (A) AN EXPERIMENTAL SPECTRUM OF AN AUTOXIDISED OIL SAMPLE AND (B) A CORRESPONDING COMPUTER-SIMULATED SPECTRUM GENERATED USING THE ACD/LABS NMR SOFTWARE SUITE





EXPANDED 9.400 - 9.825 PPM REGION OF THE 600.13 MHZ ^{1}H NMR SPECTRUM OF A HEATED CORN OIL SAMPLE HIGHLIGHTING THE FORMATION OF A VARIETY OF LOP SPECIES

ABBREVIATIONS: n-a, *n*-alkanals; ct-2,4, *cis*,*trans*-alka-2,4-dienals; tt-2,4, *trans*,*trans*-alka-2,4-dienals; t-2, *trans*-2-alkenals; 4-OH, 4-hydroxy-*trans*-2-alkenals; 4,5-epox, 4,5-epoxy-*trans*-2-alkenals



Applications of 600 MHz Nuclear Magnetic Resonance Spectroscopy.

Part 3 : Large molecules.

Disadvantages of using NMR for structure determination compared to X-ray methods:

- Size of the structures it can handle. Most NMR spectroscopists focus on molecules no larger than 60 kilodaltons (about 180 amino acids). X-ray crystallographers have solved structures up to 2,500 kilodaltons—40 times as large.

- Methods for determining structures by NMR spectroscopy are much younger than those that use X-ray crystallography.

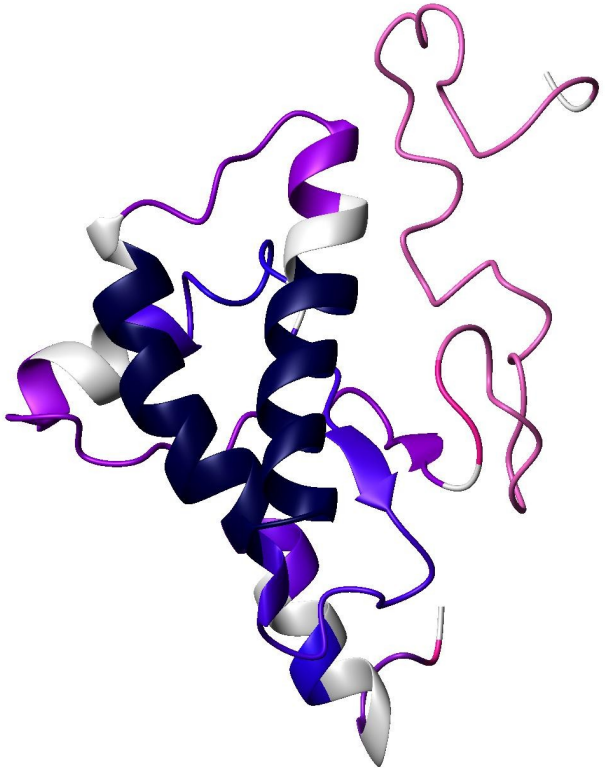
So why use NMR at all?

- It uses molecules in solution, so it is not limited to those that crystallize well. (Remember that crystallization can be an uncertain and time-consuming step in X-ray crystallography.)

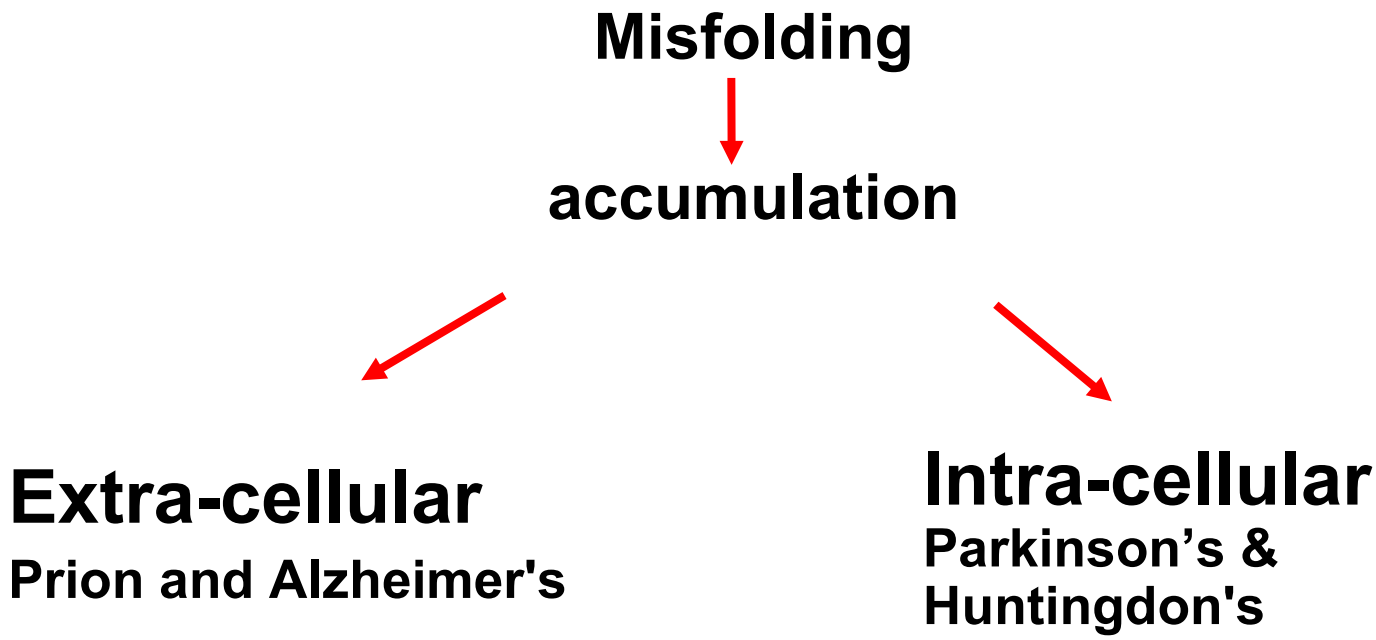
- NMR also makes it fairly easy to study properties of a molecule besides its structure—such as the flexibility of the molecule and how it interacts with other molecules. With crystallography, it is often either impossible to study these aspects or it requires an entirely new crystal.

Using NMR and crystallography together gives researchers a more complete picture of a molecule and its functioning than either tool alone.

Structural biology of the Prion Protein



Protein Misfolding Diseases



What Causes Prion Disease ?

1982: Stan Prusiner isolated the infectious agent- a single protein- The Prion protein

Prion:

Proteinaceous Infectious Particle

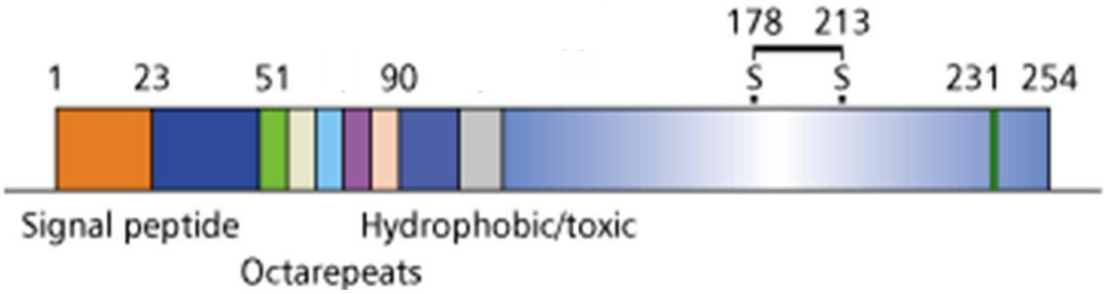
Controversial: (It was believed RNA or DNA was required for replication)

The Prion Protein

Single Polypeptide Chain

209 amino acids; residues 23-231

Glyco-protein, GPI anchored to cell surface



Two Prion Protein (PrP) isoforms

identical primary sequence

α

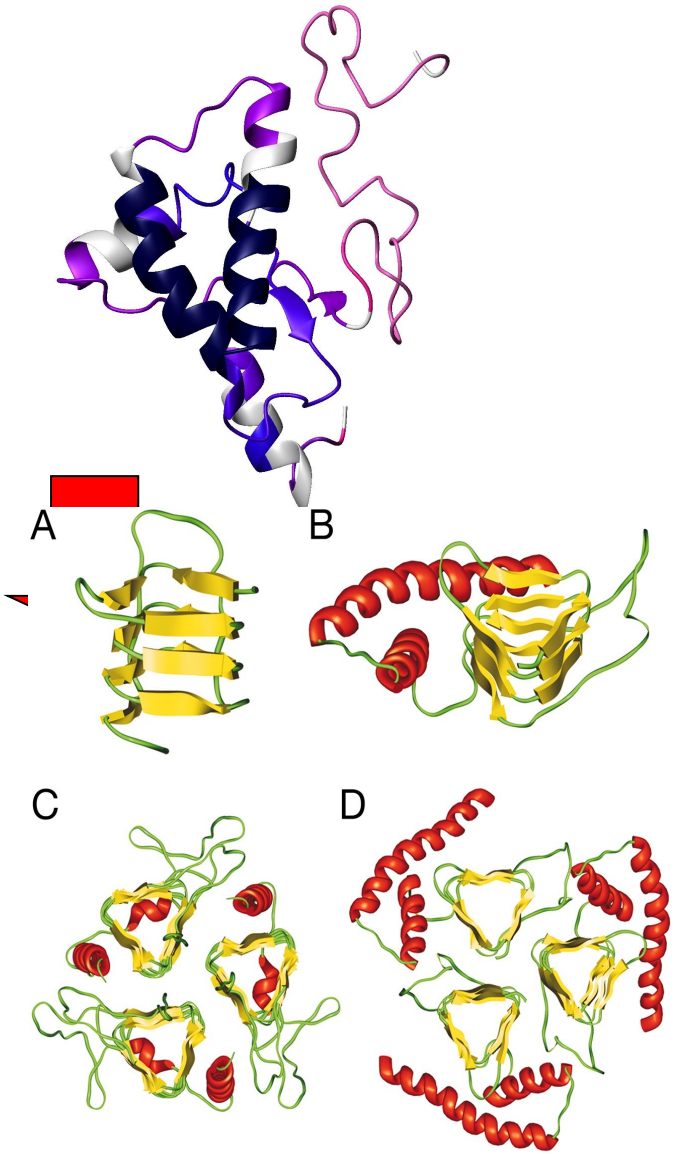
PrP-Cellular

Ubiquitous in normal neuronal cells
High Helical content
Soluble
Susceptible to proteolysis

β

PrP-Scrapie

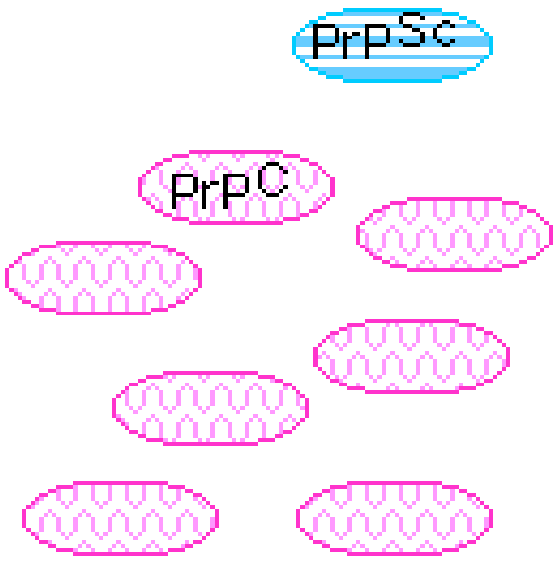
Present in infectious material
High β-sheet content
Insoluble
Resistant to proteolysis



Mechanism of Prion replication

<u>Infectious Diseases</u> $PrP^C \rightarrow PrP^{Sc}$

Template assisted conformational switch



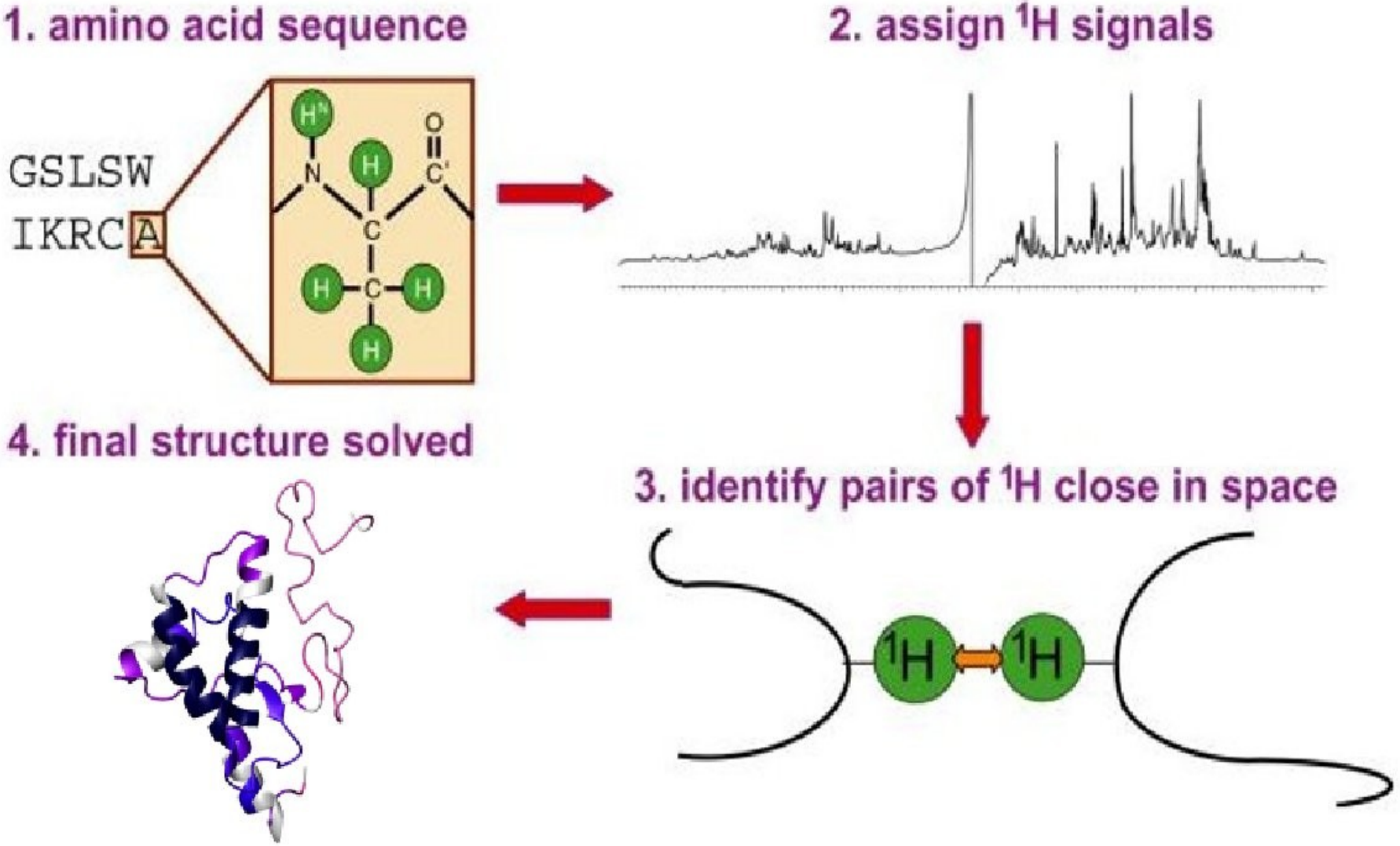
amyloids

Misfolded prion protein acts as a seed or template to nucleate further misfolding

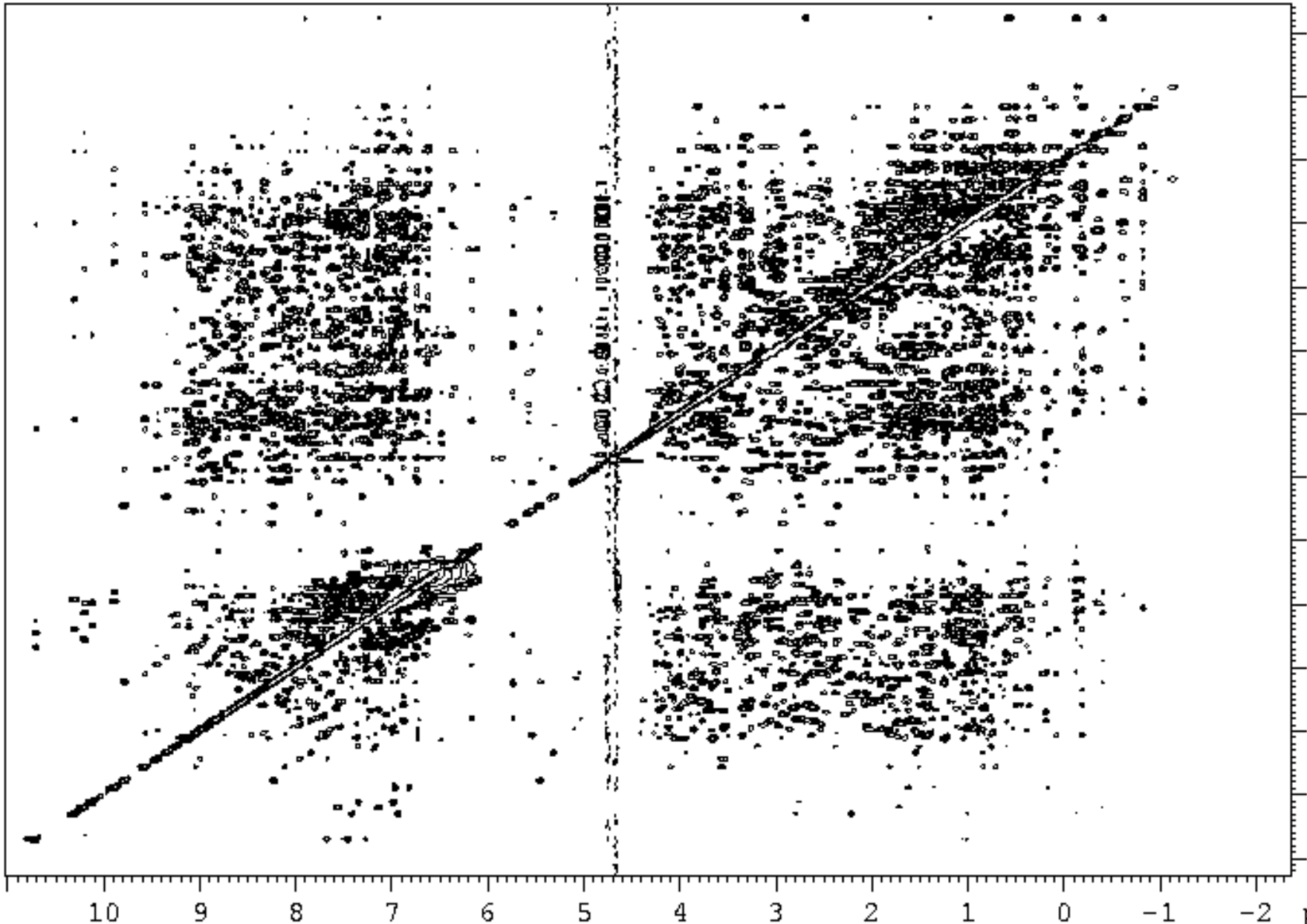
<u>Inherited Diseases:</u> Mutations in the primary sequence promote misfolding

Information obtained from chemical shift data

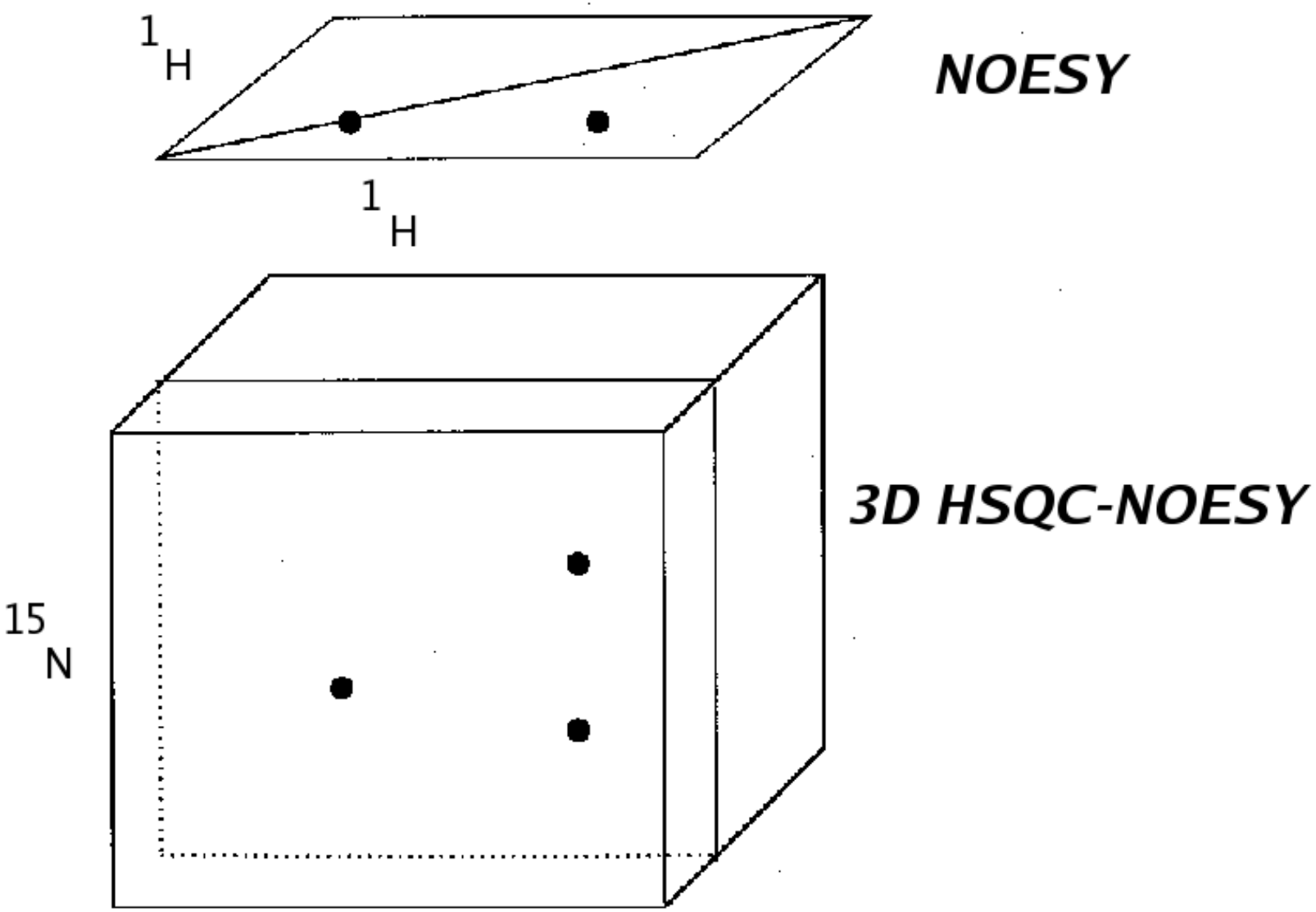
General procedure for obtaining structure by NMR



^{1}H NOESY spectrum of a protein



Schematic HSQC-NOESY showing how 1H-1H overlap is removed by adding a 15N dimension



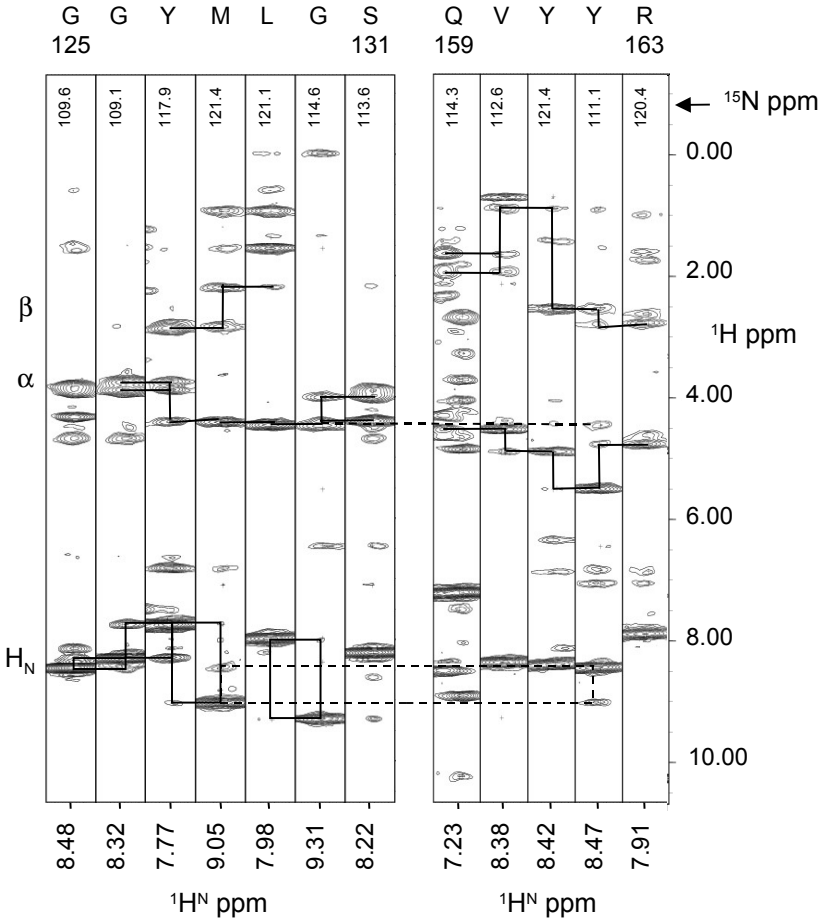
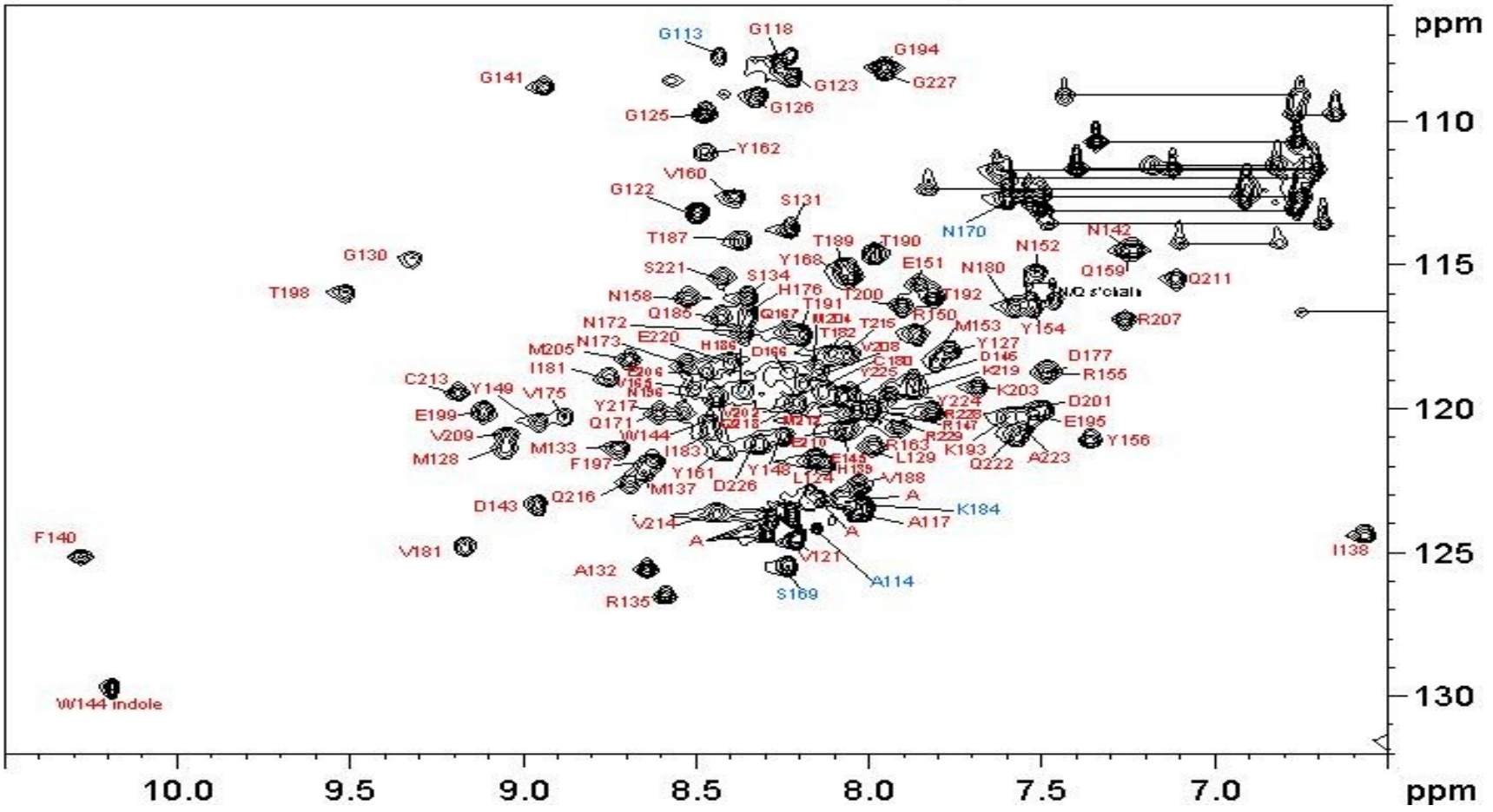
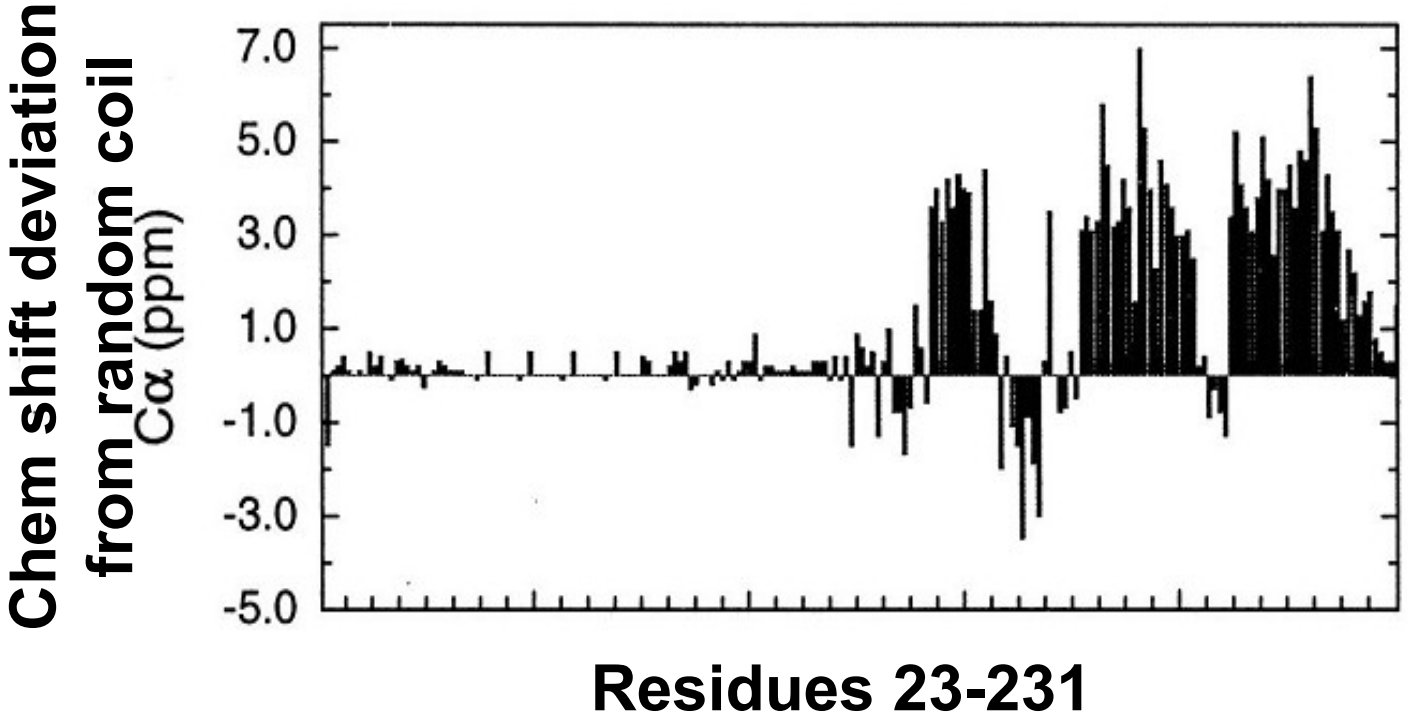


Figure 1: Series of strips from ^{1}H-$^{1}H_{N}$ slices of the 3D ^{15}N NOESY-HSQC collected at 600MHz (^{1}H) and 30°C. Each strip corresponds to a single residue in the two-stranded β-sheet, β1 (G125 - S131) and β2 (Q159 - R163). Solid lines connect sequential residues, dashed lines highlight connections occurring between the strands. The assignments are shown at the top of each strip .

15N HSQC assignments- NMR finger print



Structure of Prion Protein PrP^C



Metal ions and misfolding disease

Changes in metal homeostasis/ compartmentalisation

<u>Structural Role</u>

Triggering:

- Misfolding
- Oligomerization
  Aggregation
- Amyloid

Stabilization of fibrils

<u>Toxic-Oxidative Stress</u>

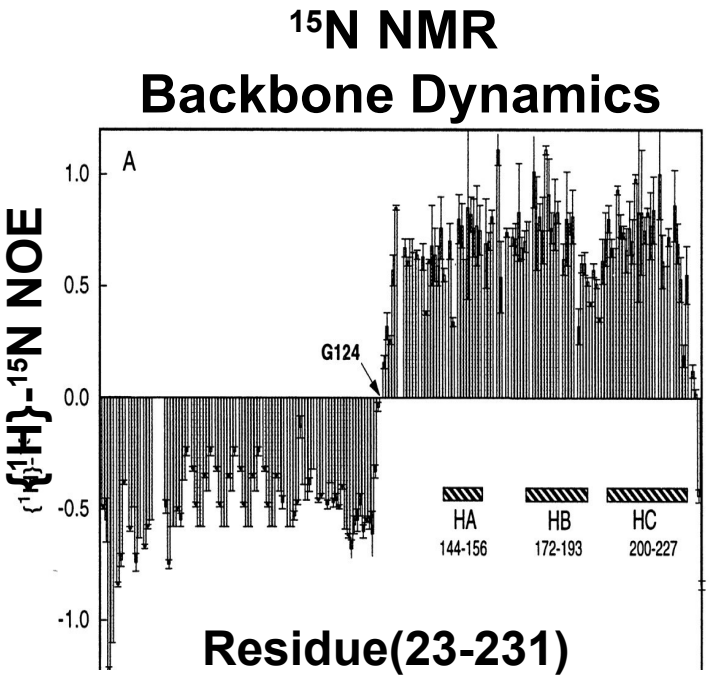
Cu(II) and Fe(III)

Fenton's cycling

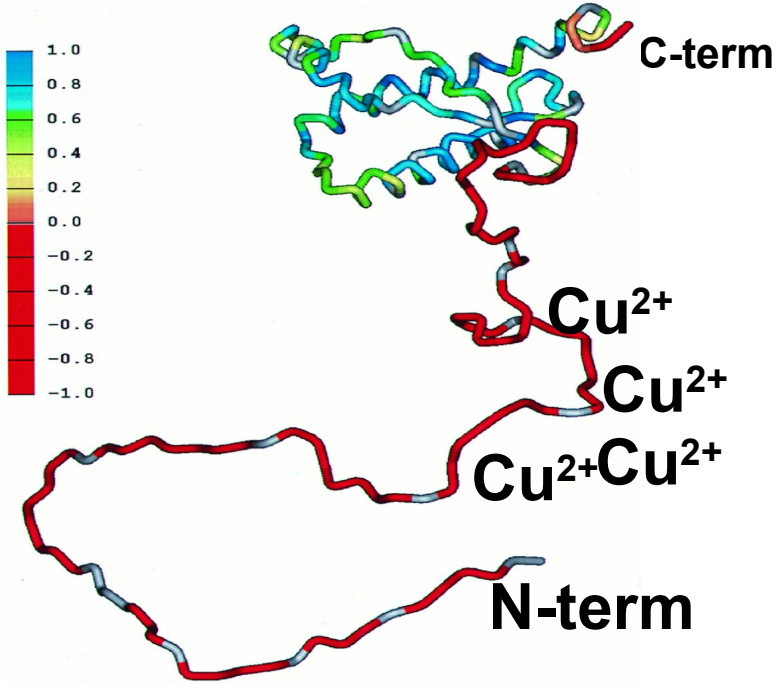
$OH^{\cdot}$ radicals

toxic to the cell

Highly flexible Cu^{2+} binding region

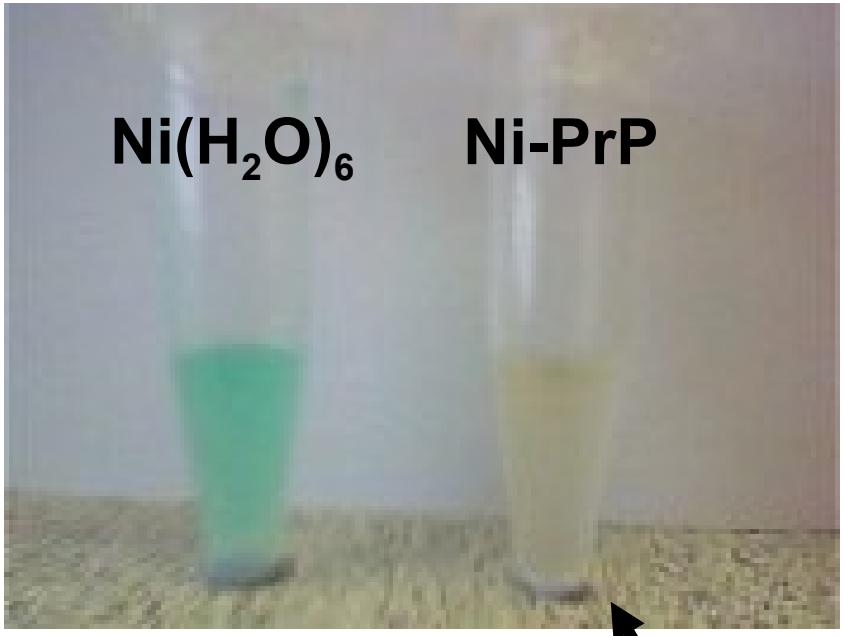


Viles *et al* Biochem 2001

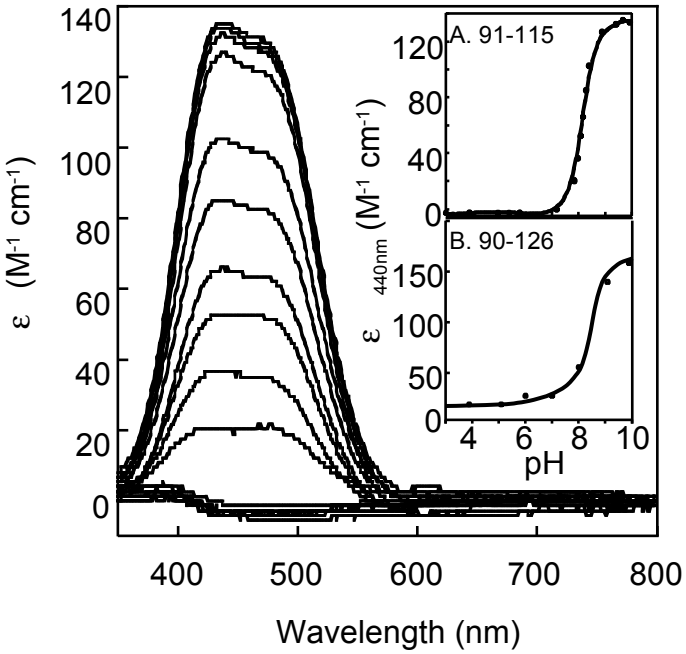


Octarepeats {GQPHGGGW}*
4 highly conserved

Ni^{2+} binding to PrP(90-126)

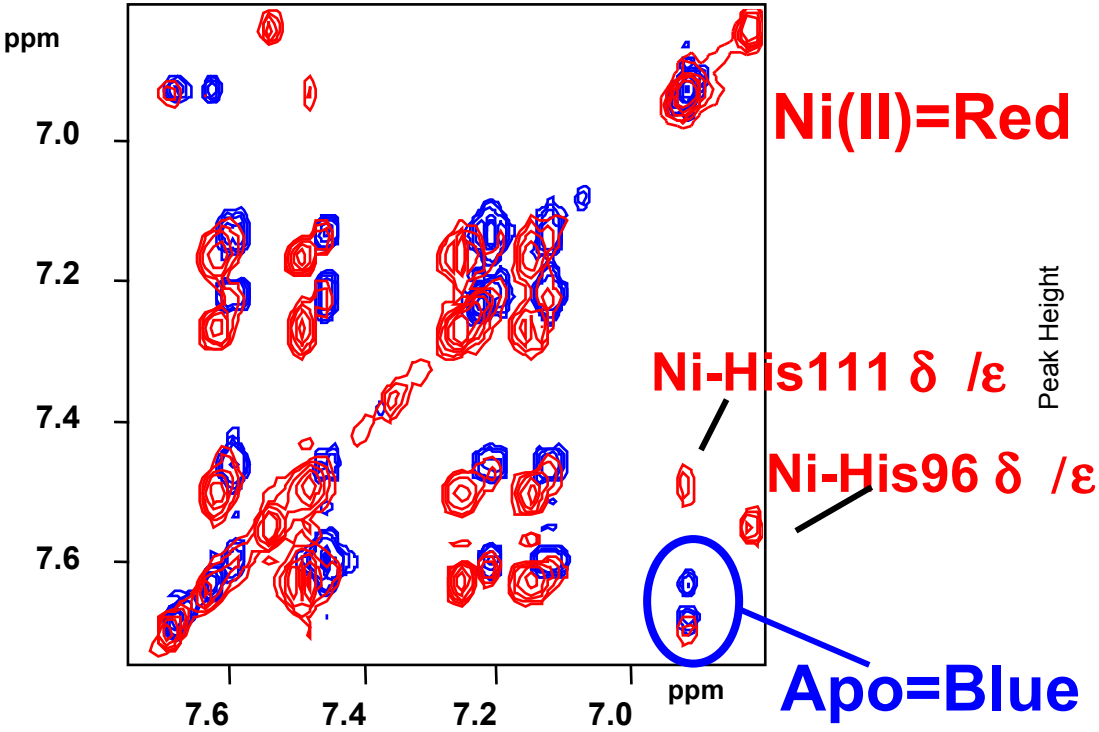


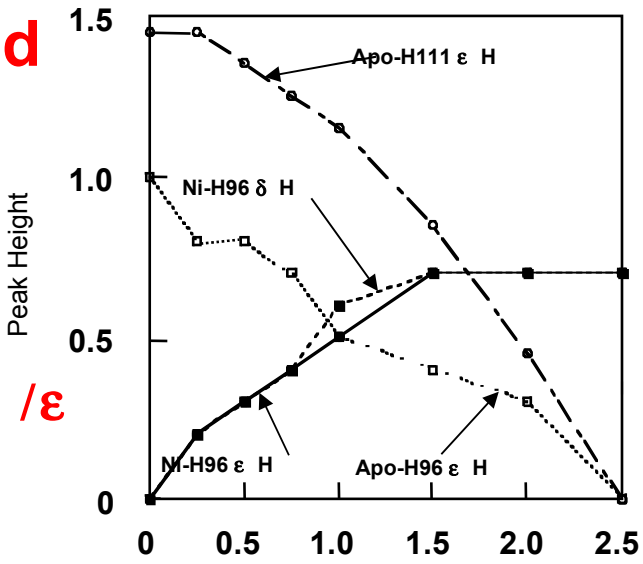
440 nm



^{1}H NMR Ni^{2+} binding is low-spin diamagnetic

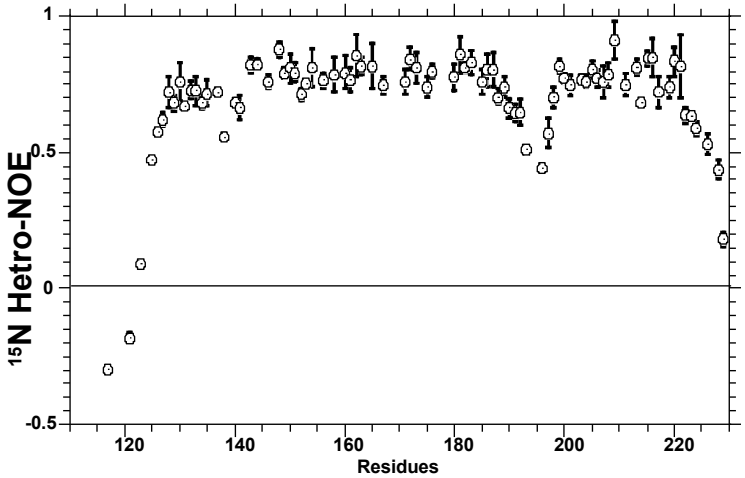
PrP(90-126)

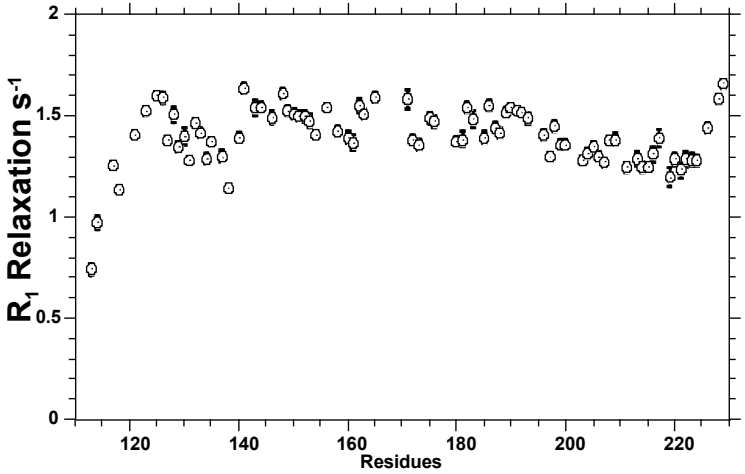


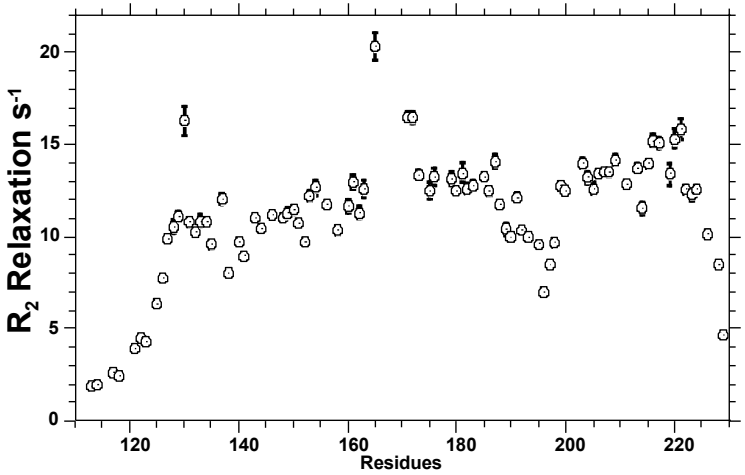


Jones,Viles et al (2005) JMolBiol

Information obtained from relaxation data





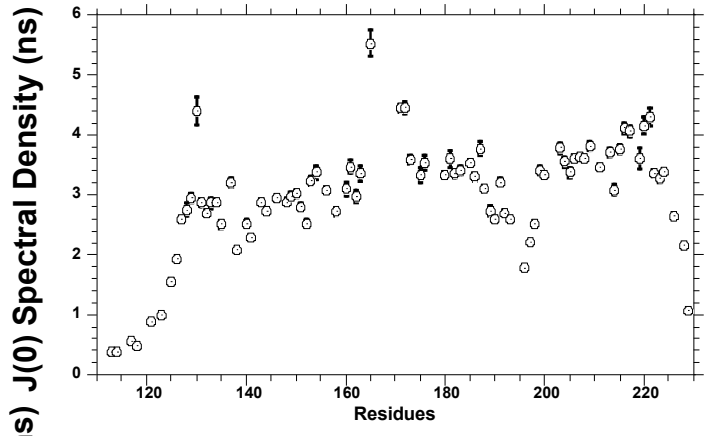


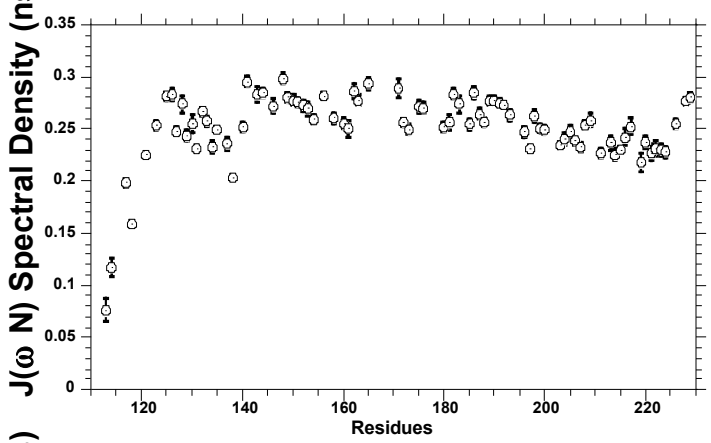
Relaxation data for mPrP (113-231).

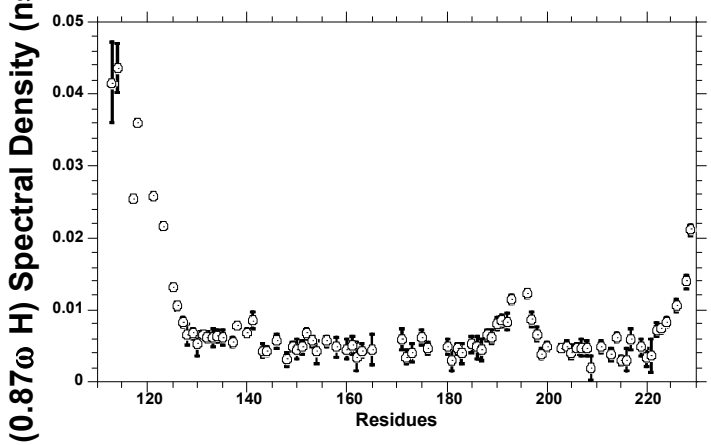
(a) heteronuclear NOE

and the relaxation rates,
(b) R_1 (= $1/T_1$) and
(c) R_2 (=$1/T_2$)

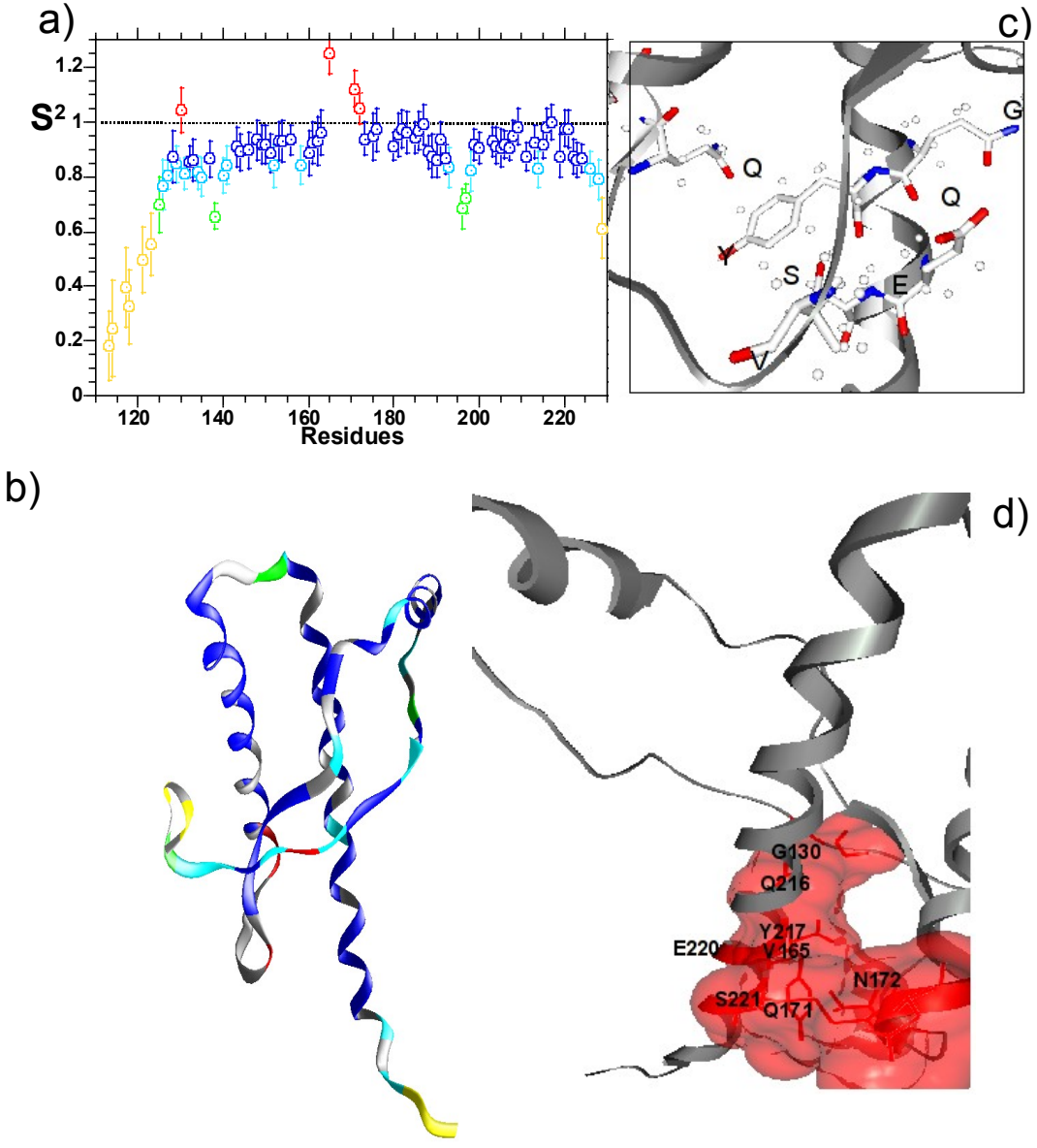
determined at 600 MHz. Sample conditions: 30ºC; pH 5.3; 20 mM acetate buffer.







Reduced spectral density functions: a) J(0), b) $J(\omega_N)$ and c) $J(0.87\omega_H)$ for mPrP(113-231) at 600 MHz. The high frequency spectral densities $J(0.87\omega_H)$ indicate fast pico-second motions typically observed in flexible regions of proteins. J(0) indicates sub-nanosecond flexibility of the NH bond vector, the smaller the value of J(0) the greater the flexibility. Uncharacteristically large J(0) (>4 ns) indicate residues with slow conformational fluctuations. Sample conditions: 30ºC; pH 5.3; 20 mM acetate buffer.



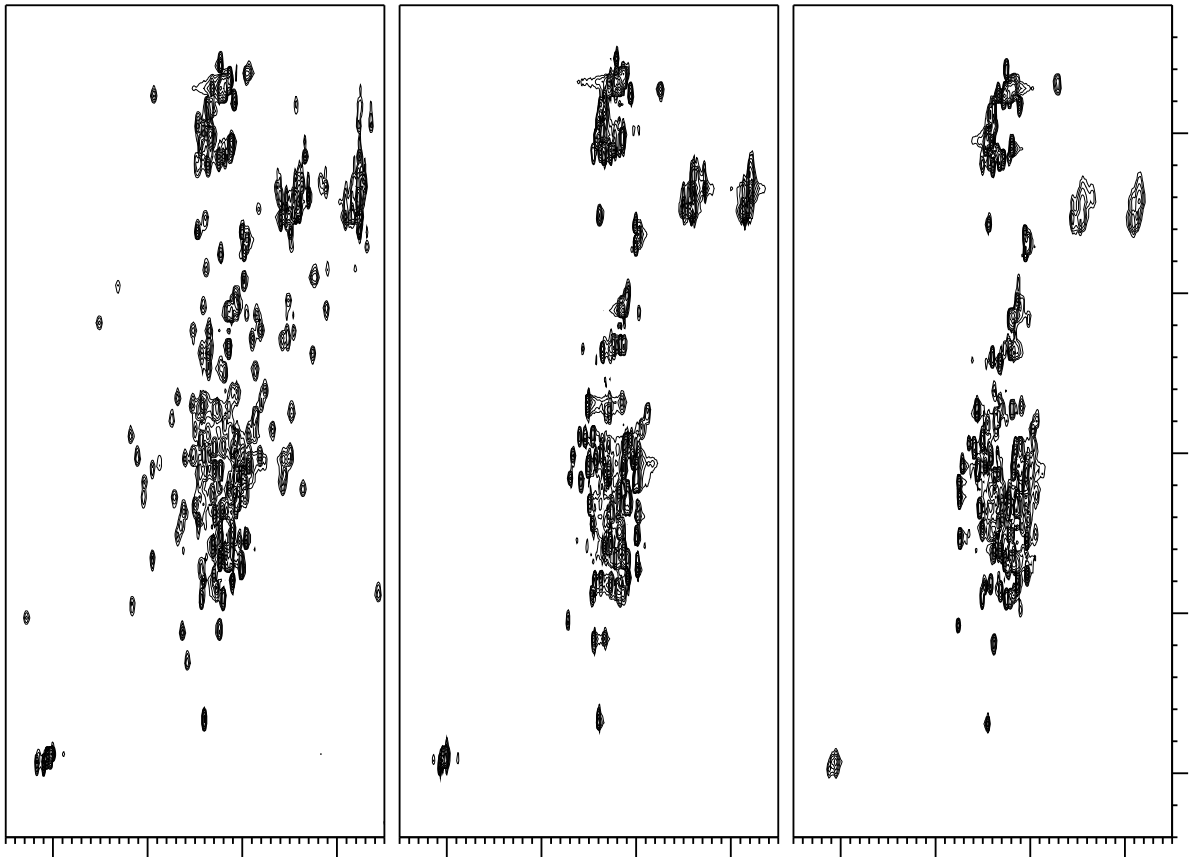
(a) Order parameters (S^2) for mPrP(113-231). Order parameters describe the amplitude range of the nanosecond time-scale motions between 0 flexible and 1.0 rigid. Residues that exhibit additional R_{ex} exchange motions are highlighted in red.

(b) Order parameter mapped onto the structure (1xyx) of $mPrP^C$: red $S^2>1.0$ (R_{ex} motions); blue $0.85<S^2<1.0$; cyan $0.75<S^2<0.85$; green $0.65<S^2<0.75$ and yellow $S^2<0.65$, residues in gray have no value calculated.

(c) Slow conformational fluctuations are localized to a distinct region within PrP^C, the orientation of side-chains that exhibit R_{ex} motions are highlighted, dots are proton positions.

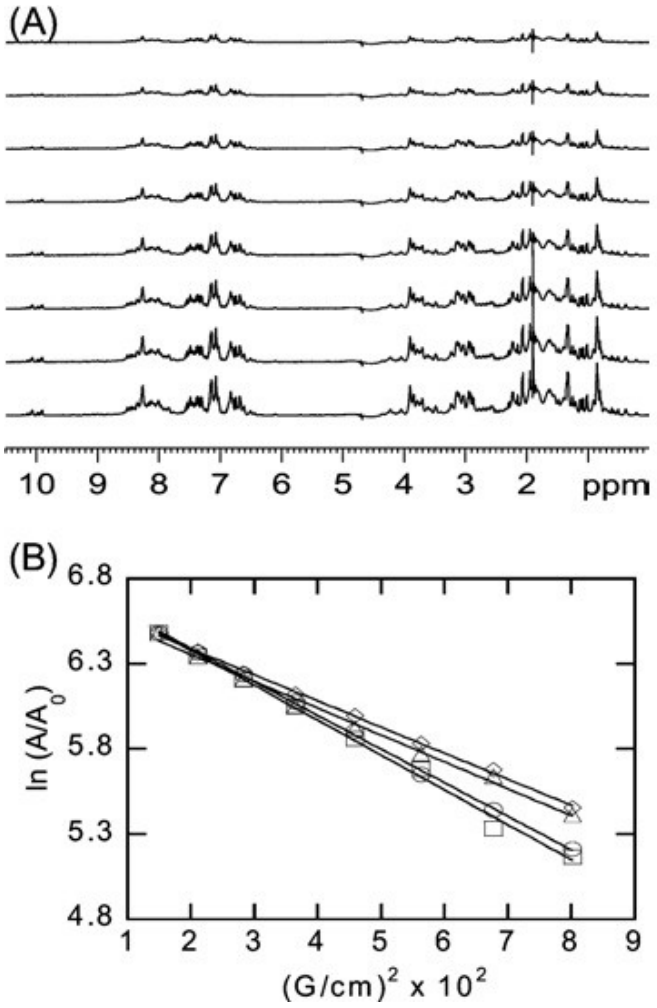
(d) The molecular surface, in red, generated by the residues that exhibit R_{ex} motions, within the context of the whole structured domain. This figure was generated using GRASP2.

Information obtained from diffusion data



Two-dimensional ^{15}N-HSQC spectra of mPrP-(23–231) (A) pH 5.21, 20 mM sodium acetate; (B) pH 4.11, 3.5 M urea, 150 mM NaCl and 20 mM sodium acetate; (C) pH 1.65, 3.5 M urea, 150 mM NaCl and 20 mM sodium acetate. Concentration of His-tagged PrP-(23–231), approx. 5 mg/ml; spectra were recorded at 30°C.

Translational diffusion measurements of mPr native, β-intermediate and acid denatured forms



(A) One-dimensional 1H NMR spectra of native mPrP-(23–231) (6 mg/ml) using STE with increasing field gradient strength between 28.3 and 12.2 G/cm.

(B) The natural logarithm of the peak intensity for the aromatic region of the spectra was plotted against the corresponding square of the gradient strengths. Least square straight line fit to: (i) native state pH 5.2 (circle) (ii) pH 4.40, 3.5 M urea and 150 mM NaCl (square), (iii) pH 1.64, 3.5 M urea and 150 mM NaCl (diamond) and (iv) pH 3.67 after incubation at 37◦C (triangle).

Acknowledgements:

Epicatechin/Taxifolin – Olivia Corcoron & Samuel Osei-Djarbeng (UEL)

Cooking Oils – Chris Silwood & Martin Grootveld (LSBU)

Prion Protein – John Viles & group (QMUL)
(especially Chris Jones and Dara O'Sullivan)