Interpretation of ¹H spectra
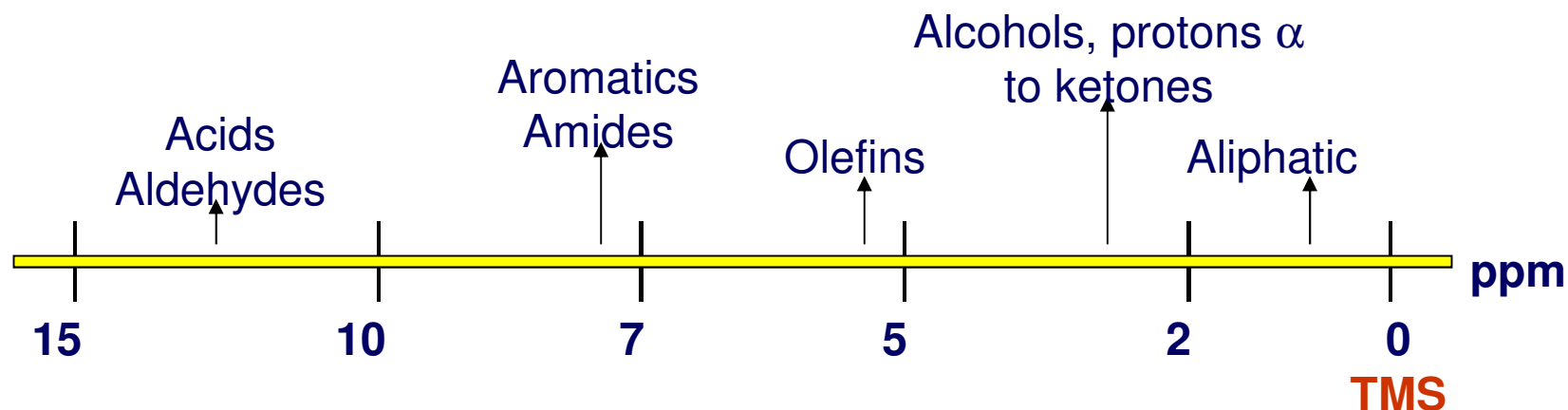
- The chemical shift range for ¹H is pretty small, from 15 to 0 ppm in most cases, although we can get peaks above 20 and below -5 ppm in some cases:

- The chemical shifts of different nuclei in a molecule arise due to differences in the local magnetic field or **shielding** (σ) felt by the nuclei in different spots of the molecule:

$$B_{eff} = B_o - B_{loc} \quad --- \quad B_{eff} = B_o(1 - \sigma)$$

Figure 8. Approximate proton chemical shifts.
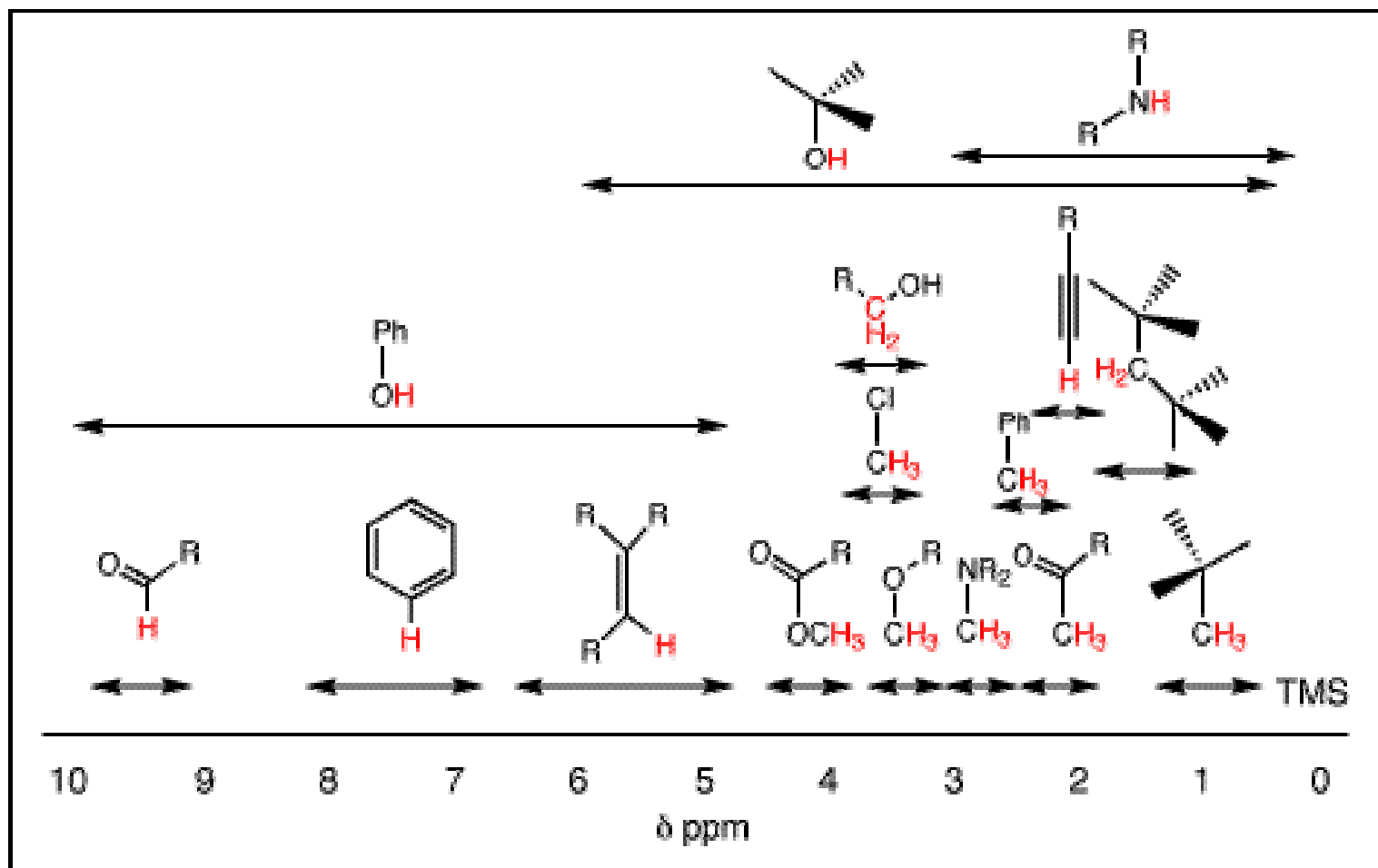
Origins of σ (B_{loc})

• The shielding of different nuclei depends on the electron density in its surroundings. We can dissect the contributions to the total shielding:

$$\sigma = \sigma^{dia} + \sigma^{para} + \sigma^{I}$$

• The term σ^{dia} is the diamagnetic contribution, which arises from the magnetic field opposing B_o from the electrons immediately surrounding the nucleus (**s** orbitals).

• σ^{para} is the paramagnetic term, and is generated by electrons in **p** orbitals (as well as bonds…). It is in favor of B_o.

• The third term, σ^{I}, is due to neighboring groups, and it can add or subtract from B_o, depending on the nature of the group and its spatial orientation. σ^{I} is the term that actually makes nuclei with similar characteristics in a molecule have different shieldings (and therefore chemical shifts).

• If we now consider our main players, ^1H and ^{13}C, we can see that since ^1H have only a **1s** orbital, σ^{dia} will dominate, while for ^{13}C (and other heavier atoms) σ^{para} will dominate because we have more **p** electron shells that can become occupied (lower energy than **p** orbitals in ^1H…)

Inductive contributions to σ^{dia}

• As we said, an isolated ¹H atom has a perfectly symmetrical distribution of its **1s** electrons around it.

H (1s)

• Now, when we add, say, a -CH_3 to it (and get methane), the electron cloud on the ¹H (on any of the 4) will become deformed, because the **electronegativity (E)** of the carbon will pull the **1s** electron of the ¹H towards it:

H (1s)   **C (sp³)**

• Since we lower the electron density around the nucleus, it will become **deshielded**, and therefore it will move towards lower fields (higher chemical shift).

• For example, if we consider hydrogen halides, we'll see that the more electronegative the halide is, the drop in shielding constants is inversely proportional to the **E** of the halide:

$$\sigma \text{ (HF)} < \sigma \text{ (HCl)} < \sigma \text{ (HBr)} < \sigma \text{ (HI)}$$

Mesomeric effects - EWGs and EDGs

• Now lets look at what happens when we have an olefinic or aromatic proton and we have a substituent that can have different *mesomeric effects* (**+M** or **-M**).

• For example, lets consider ethene and **EWGs** or **EDGs** as substituents. If we consider methylvinylketone, the chemical shifts of the olefinic protons will move downfield considerably, because since the ketone (an **EWG**) is taking electrons away from the double bond, the electron density around the ^1H will diminish:
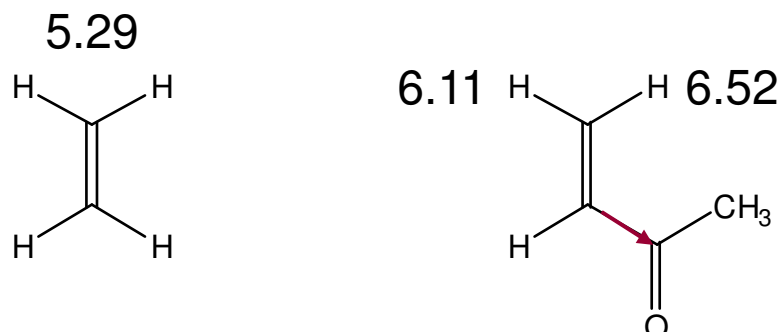

• If on the other hand we consider methylvinylether, the situation is reversed: The methoxy groups (an **EDG**), will donate electrons to the double bond, which will increase the electron density around the ^1H and therefore increase the shielding:
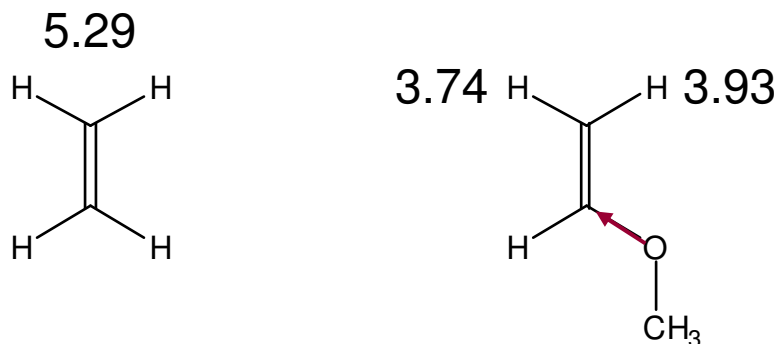
Mesomeric effects (continued)

• A similar reasoning can be used when we analyze the chemical shifts of ^{1}Hs on substituted aromatic systems.

• For example, in aniline we have an **EDG**, which has a **+M** effect. Since we'll have more electron density in the ring, all protons will be more shielded than the respective protons in benzene (7.24 ppm).

• Furthermore, if we draw resonant structures we can see that the **ortho** and **para** positions will have a larger electron density. Therefore, protons attached to the **ortho** or **para** carbons will be more shielded (lower chamical shift):
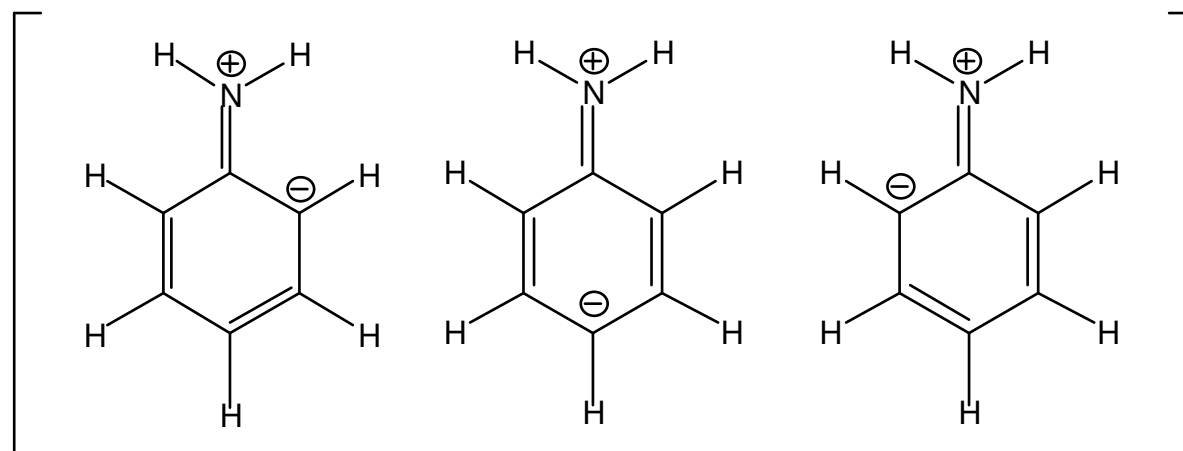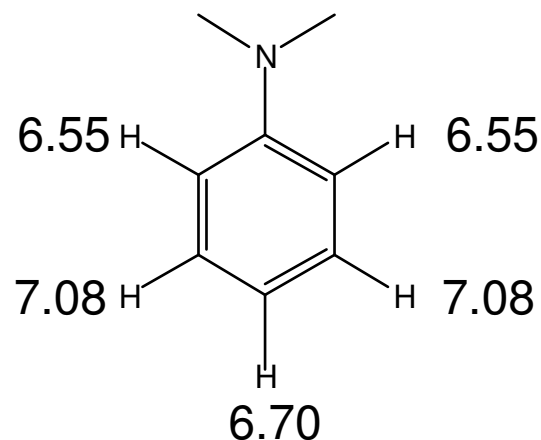
Anisotropic effects

• Any chemical bond is inherently anisotropic, i.e., it has a direction in space, and depending from which way we look at it, it will be different.

• When we subject the bonds (electron density) to an external magnetic field (B_o), there will be an induced magnetic moment which will also be anisotropic.

• Therefore, the magnetic environment of [1]Hs around these groups will be anisotropic. This means, depending were the [1]Hs are with respect to the group giving rise to the induced magnetic dipole, the effective magnetic field felt by the proton will vary.

• If we consider a single C-C bond, which has cylindrical symmetry, and think of the induced magnetic dipole to be centered right in the middle of the bond, it will look like this:
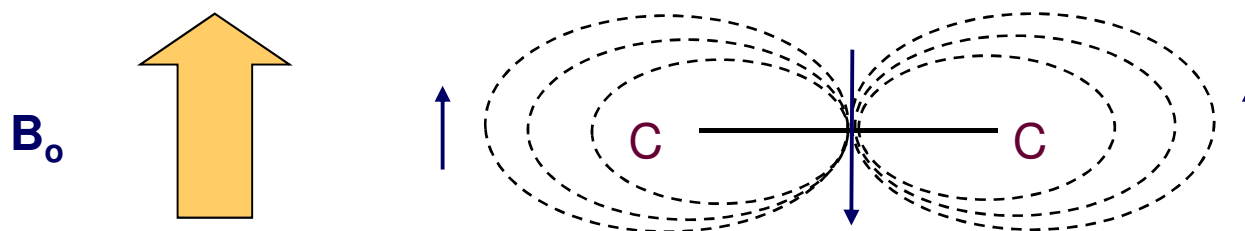

• What we see is that things on the ends of the bond will feel an increased magnetic field, while things sitting above or below will feel a slightly decreased effective field.

Anisotropic effects (…)
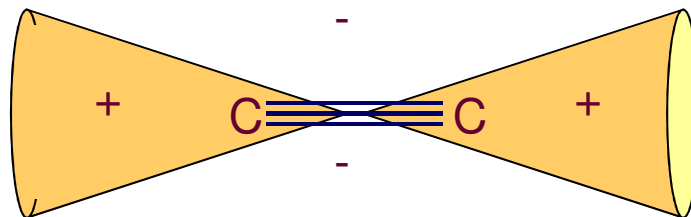
• We get two cones spanning from the center of the bond: Inside the cone, we will be deshielded, on the sides, we'll be shielded. At an angle of 54.7°, the effect is zero:
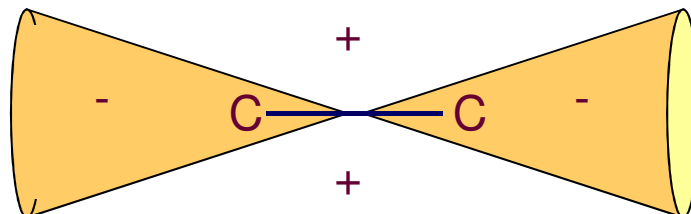

• For double bonds (C=O, C=C), the situation is similar:
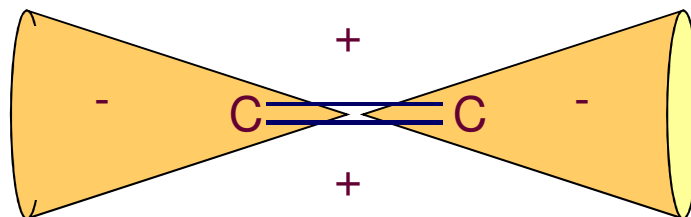
• For triple bonds, the induced magnetic dipole is such that the values of $\chi_{||}$ and $\chi_{\perp}$ are reversed:

Ring current effects

- One of the most pronounced effects arising from induced magnetic moments in a chemical group are due to aromatic rings.

- If we consider the ring current of the ring, it will generate a magnetic field perpendicular to the plane of the ring, that will be against the external magnetic field:
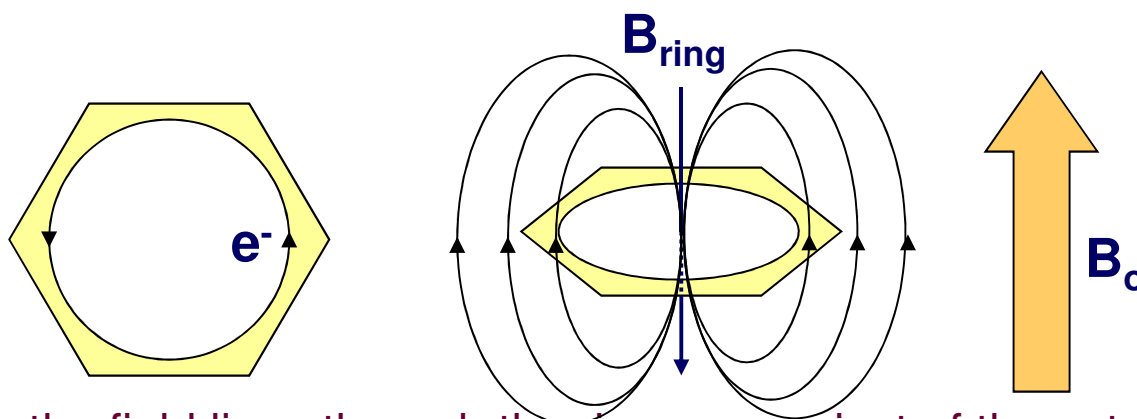

- As we see, the field lines through the ring are against of the external magnetic field (the induced magnetic moment will oppose the effect of B_o), but the 'return' lines, which go on the outside of the ring, are in favor of it.

- Therefore, we can safely assume that protons sitting on the plane of the ring and thereabouts will be deshielded, while those lying on top or below the ring will be shielded (i.e., higher fields and therefore lower chemical shifts.

Ring current effects (…)

• As was the case for single, double, and triple bonds, we can plot the value of the shielding as a function of the position in space of the ^1H under study. It will also be cone-shaped, with a shielding regions (-, lower chemical shift), and deshielding regions (+, higher chemical shift):
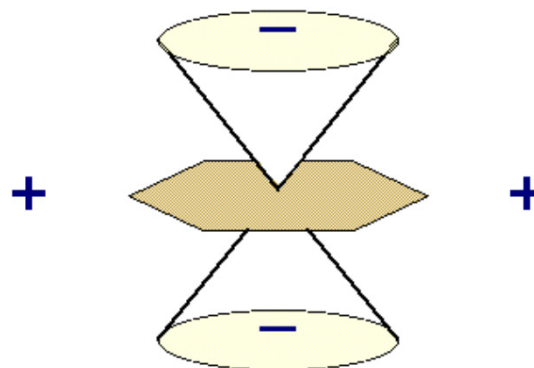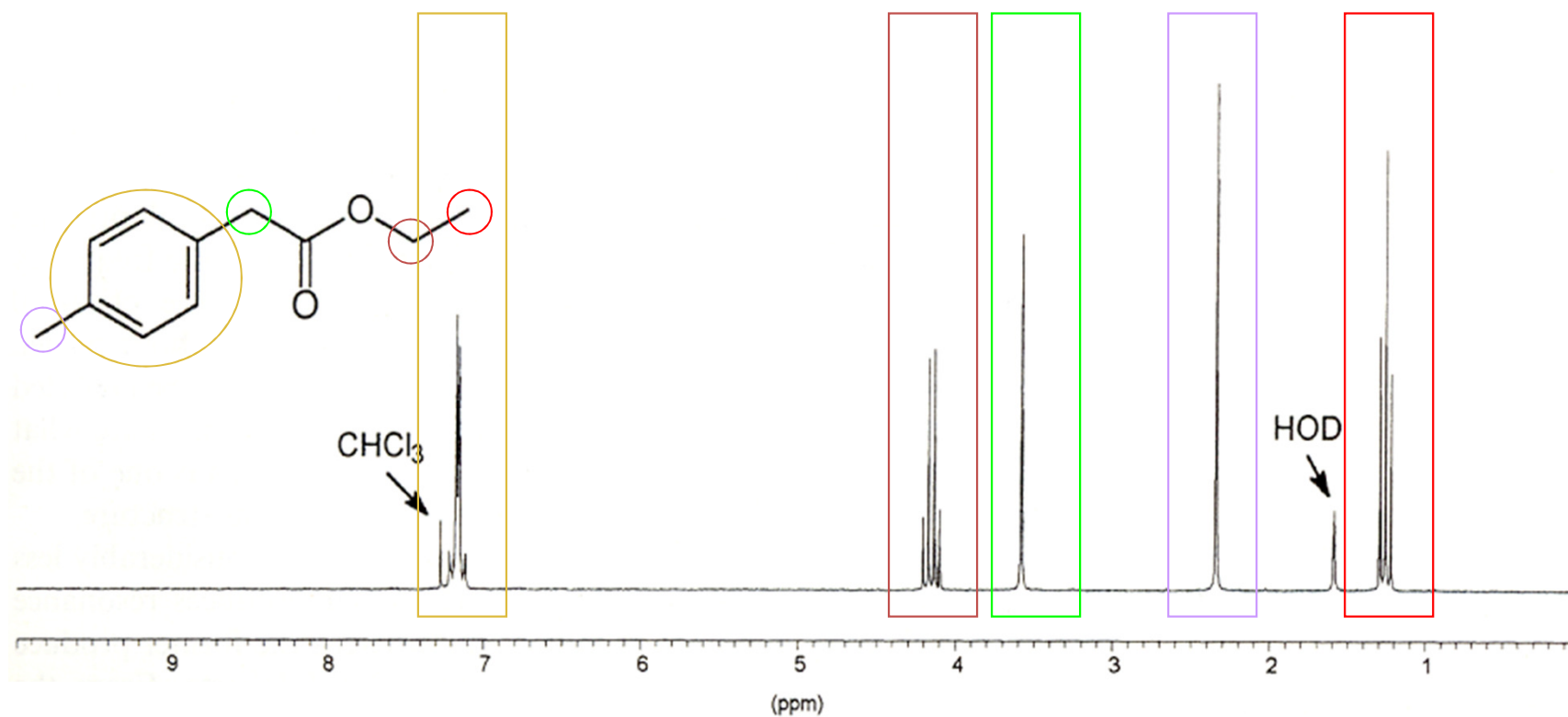

• Protons on the sides of the aromatic ring will feel a higher local magnetic field (higher ppm's), while those on top or bottom will feel a lower local magnetic field (lower ppm's).

• This is the reason why aromatic protons poking outwards from an aromatic ring have chemical shifts in the 6 to 9 ppm's:
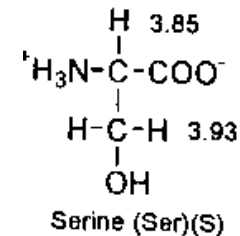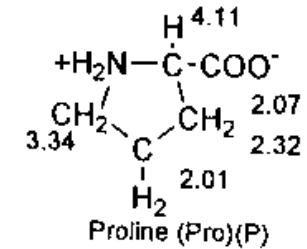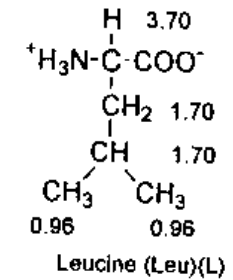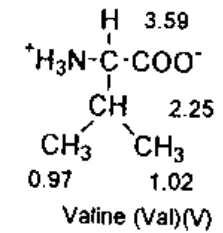

H 7.27

H 7.79

H 7.41
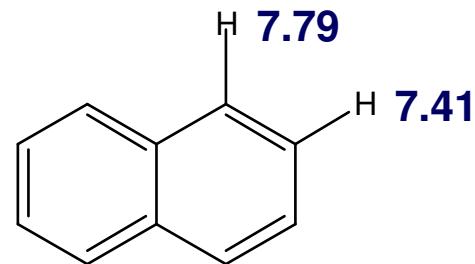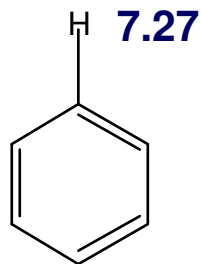
Type of Proton	Structure	Chemical Shift, ppm
Cyclopropane	C_3H_6	0.2
Primary	R-CH_3	0.9
Secondary	R_2-CH_2	1.3
Tertiary	R_3-C-H	1.5
Vinylic	C=C-H	4.6-5.9
Acetylenic	triple bond,CC-H	2-3
Aromatic	Ar-H	6-8.5
Benzylic	Ar-C-H	2.2-3
Allylic	C=C-CH_3	1.7
Fluorides	H-C-F	4-4.5
Chlorides	H-C-Cl	3-4
Bromides	H-C-Br	2.5-4
Iodides	H-C-I	2-4
Alcohols	H-C-OH	3.4-4
Ethers	H-C-OR	3.3-4
Esters	RCOO-C-H	3.7-4.1
Esters	H-C-COOR	2-2.2
Acids	H-C-COOH	2-2.6
Carbonyl Compounds	H-C-C=O	2-2.7
Aldehydic	R-(H-)C=O	9-10
Hydroxylic	R-C-OH	1-5.5
Phenolic	Ar-OH	4-12
Enolic	C=C-OH	15-17
Carboxylic	RCOOH	10.5-12
Amino	RNH_2	

This is an example of a NMR spectrum (^{1}H) for a drug like molecule.

CHCl$_3$

HOD

9   8   7   6   5   4   3   2   1

(ppm)

Amino acid structures and chemical shifts

H 3.73
^+H_3N-C-COO$^-$
CH$_2$ 1.87
CH$_2$ 1.70
CH$_2$ 1.46
CH$_2$ 3.02
NH$_3$$^+$
Lysine (Lys)(K)

H 3.51
^+H_3N-C-COO$^-$
4.22 H-C-OH
CH$_3$ 1.31
Threonine (Thr)(T)

H 3.72
^+H_3N-C-COO$^-$
CH$_2$ 2.06
CH$_2$ 2.33
C
O O$^-$
Glutamic Acid (Glu)(E)

H 3.98
^+H_3N-C-COO$^-$
CH$_2$ 3.11 3.27
7.40
Phenylalanine (Phe)(F)

H 3.98
^+H_3N-C-COO$^-$
3.18 H$_2$C C 7.08
NH
^+HN C 7.84
H
Histidine (His)(H)

H 3.59
^+H_3N-C-COO$^-$
CH 2.25
CH$_3$ CH$_3$
0.97 1.02
Valine (Val)(V)

H 3.74
^+H_3N-C-COO$^-$
CH$_2$ 1.87
CH$_2$ 1.67
CH$_2$ 3.22
N-H
C=NH$_2$$^+$
NH$_2$
Arginine (Arg)(R)

H 3.97
^+H_3N-C-COO$^-$
CH$_2$ 2.91
C
O NH$_2$
Asparagine (Asn)(N)

H 3.83
^+H_3N-C-COO$^-$
CH$_2$ 2.12
CH$_2$ 2.64
S
CH$_3$ 2.12
Methionine (Met)(M)

H 4.28
^+H_3N-C-COO$^-$
CH$_2$ 2.97 3.13
7.20
6.88
OH
Tyrosine (Tyr)(Y)

H 3.85
^+H_3N-C-COO$^-$
CH$_2$ 2.70
C
O O$^-$
Aspartic Acid (Asp)(D)

H 3.70
^+H_3N-C-COO$^-$
CH$_2$ 1.70
CH 1.70
CH$_3$ CH$_3$
0.96 0.96
Leucine (Leu)(L)

H 3.64
^+H_3N-C-COO$^-$
1.95 H-C-CH$_3$ 0.92
CH$_2$ 1.24, 1.45
CH$_3$ 0.99
Isoleucine (Ileu)(I)

H 3.73
^+H_3N-C-COO$^-$
CH$_2$ 2.10
CH$_2$ 2.43
C
O NH$_2$
Glutamine (Glu)(Q)

H 3.52
^+H_3N-C-COO$^-$
H
Glycine (Gly)(G)

H 4.24
^+H_3N-C-COO$^-$
7.54 CH$_2$ 3.38
7.20
CH 7.32
7.28
N
7.68
H
Tryptophan (Trp)(W)

H 4.11
^+H_2N-C-COO$^-$
CH$_2$ CH$_2$ 2.07 2.32
3.34 C
H$_2$ 2.01
Proline (Pro)(P)

H 3.95
^+H_3N-C-COO$^-$
CH$_2$ 3.05
SH
Cysteine (Cys)(C)

H 3.76
^+H_3N-C-COO$^-$
CH$_3$ 1.46
Alanine (Ala)(A)

H 3.85
^+H_3N-C-COO$^-$
H-C-H 3.93
OH
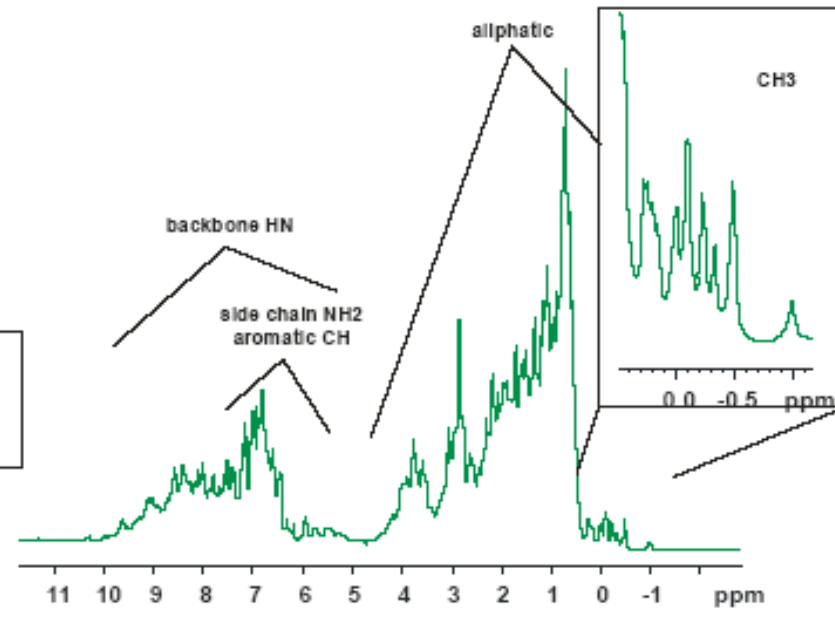Serine (Ser)(S)

Is my protein folded?

Unfolded 20 kDa protein
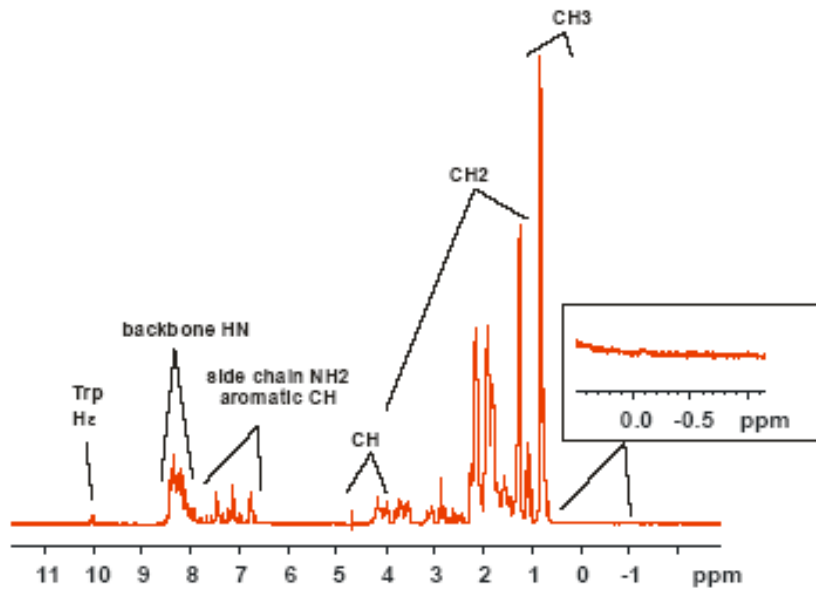
Folded 20 kDa protein

What specifically to look for in a nicely folded protein

notice aromatic/amide protons with shifts above 9 and below 7

notice alpha protons with shifts above 5

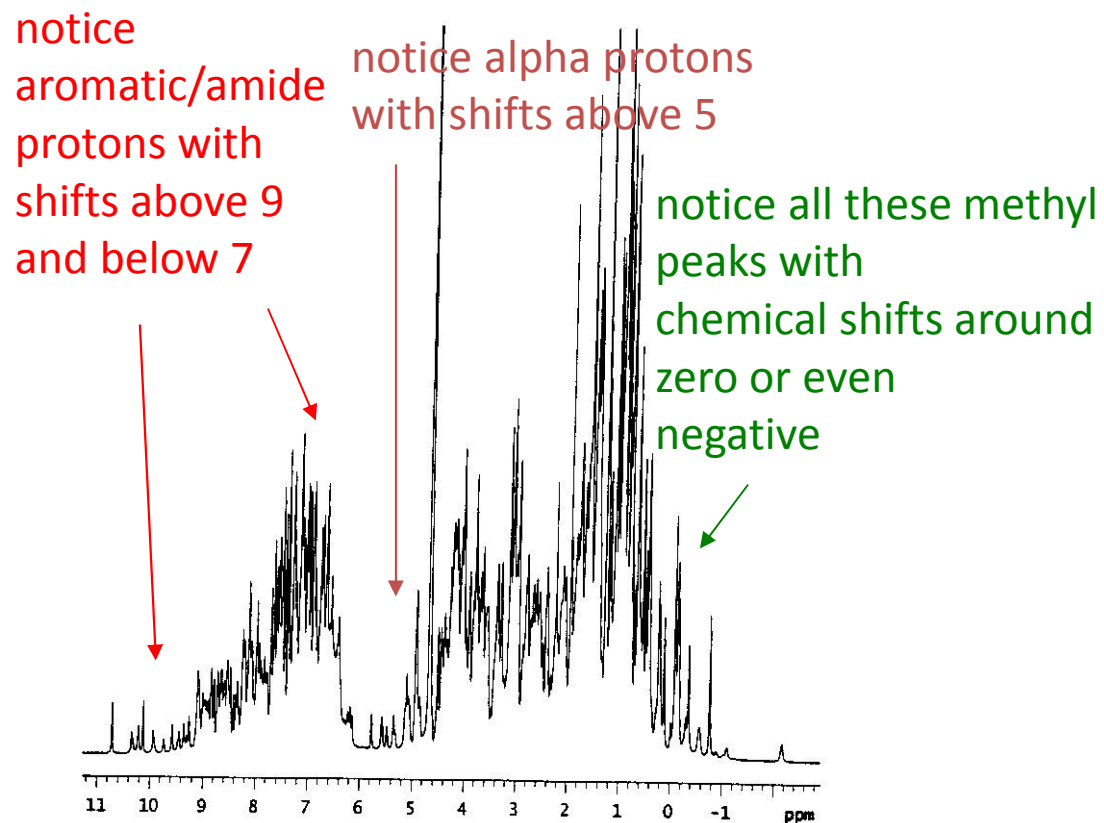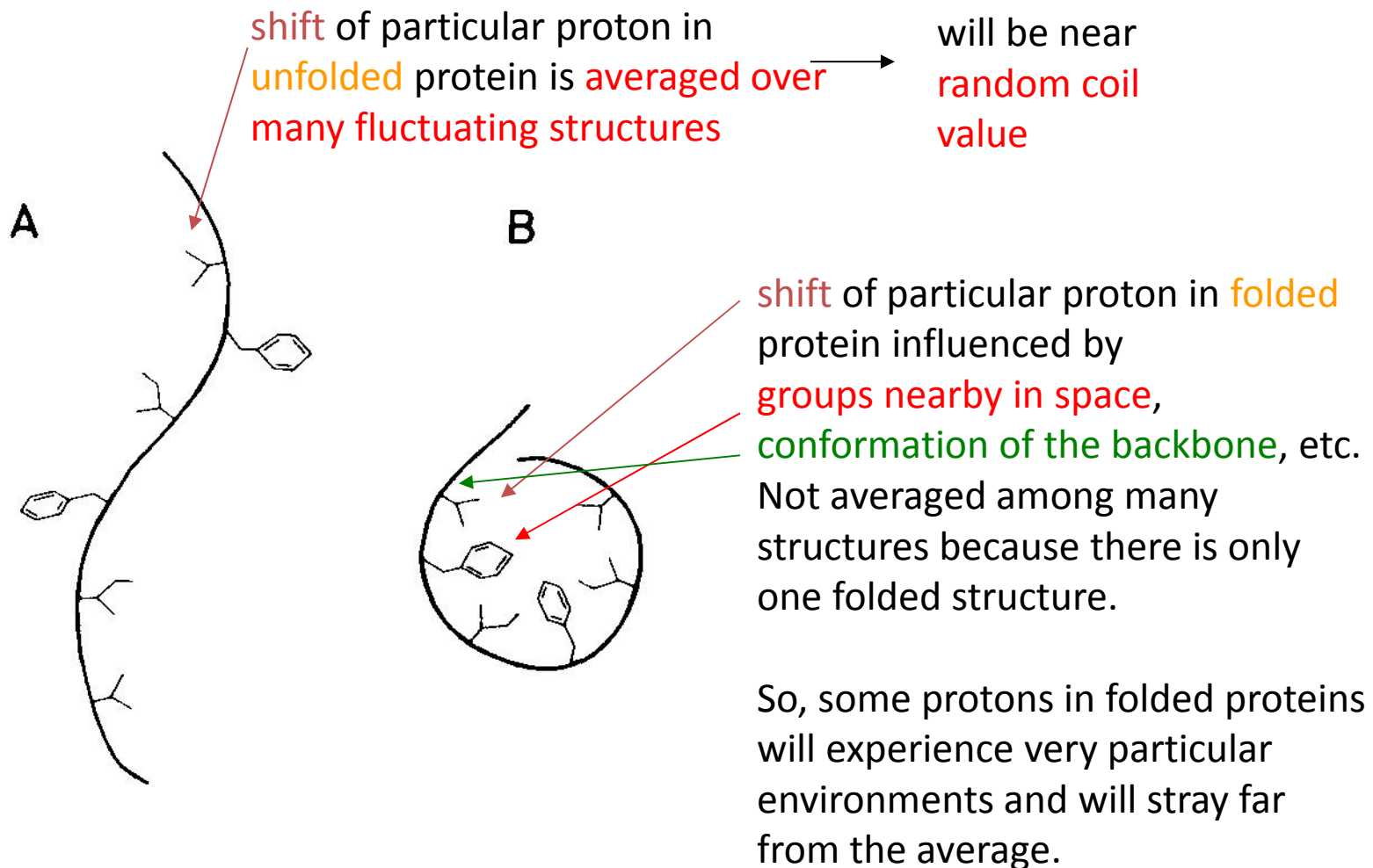notice all these methyl peaks with chemical shifts around zero or even negative

Fig. 1.2 The 750 MHz ^{1}H NMR spectrum of lysozyme.

A simple reason for the increased shift dispersion is that the environment experienced by ^{1}H nuclei in a folded protein (B) is not the same as in a unfolded, extended protein or "random coil" (A).
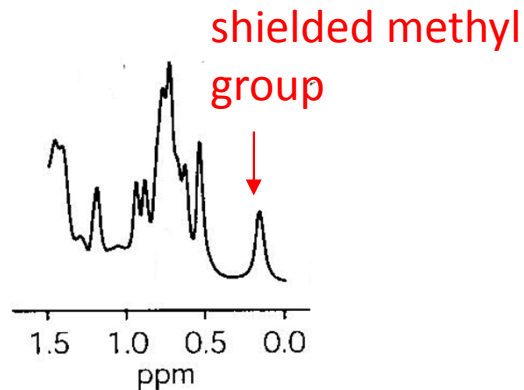
shift of particular proton in unfolded protein is averaged over many fluctuating structures → will be near random coil value

A

B

shift of particular proton in folded protein influenced by groups nearby in space, conformation of the backbone, etc. Not averaged among many structures because there is only one folded structure.

So, some protons in folded proteins will experience very particular environments and will stray far from the average.
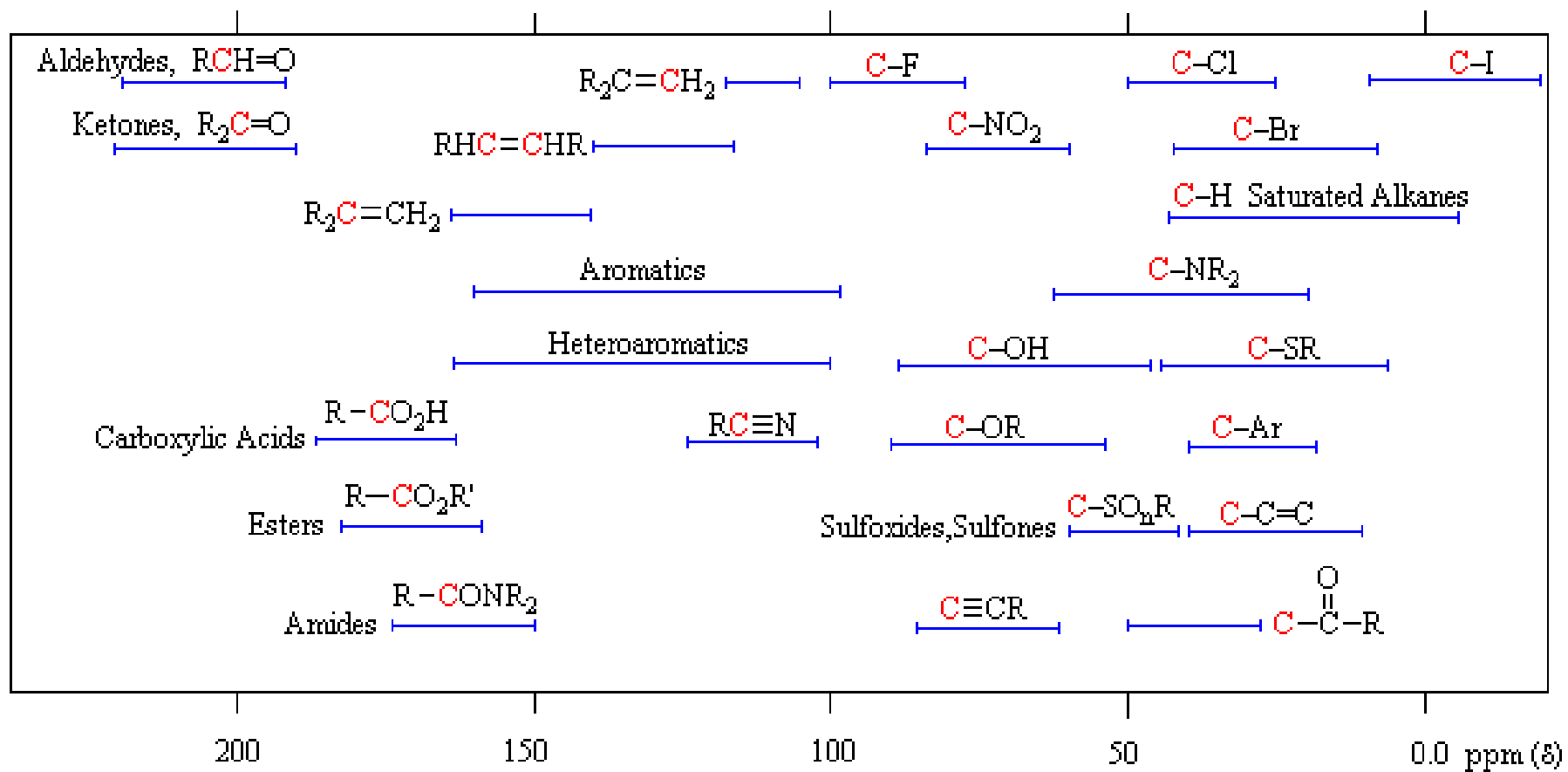
Example: shielding by aromatic side chains in folded proteins

Picture shows the side chain packing in the hydrophobic core of a protein--the side chains are packed in a very *specific* manner, somewhat like a jigsaw puzzle
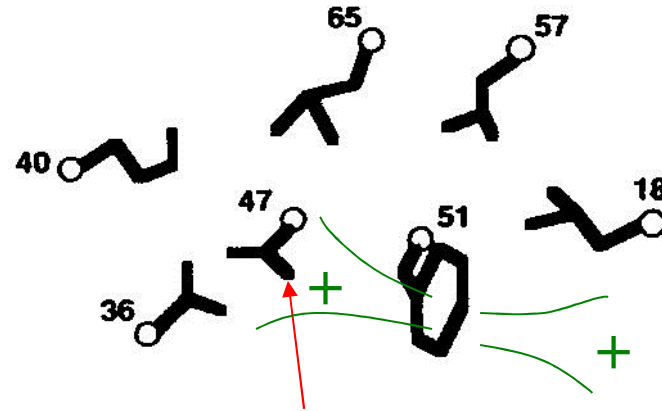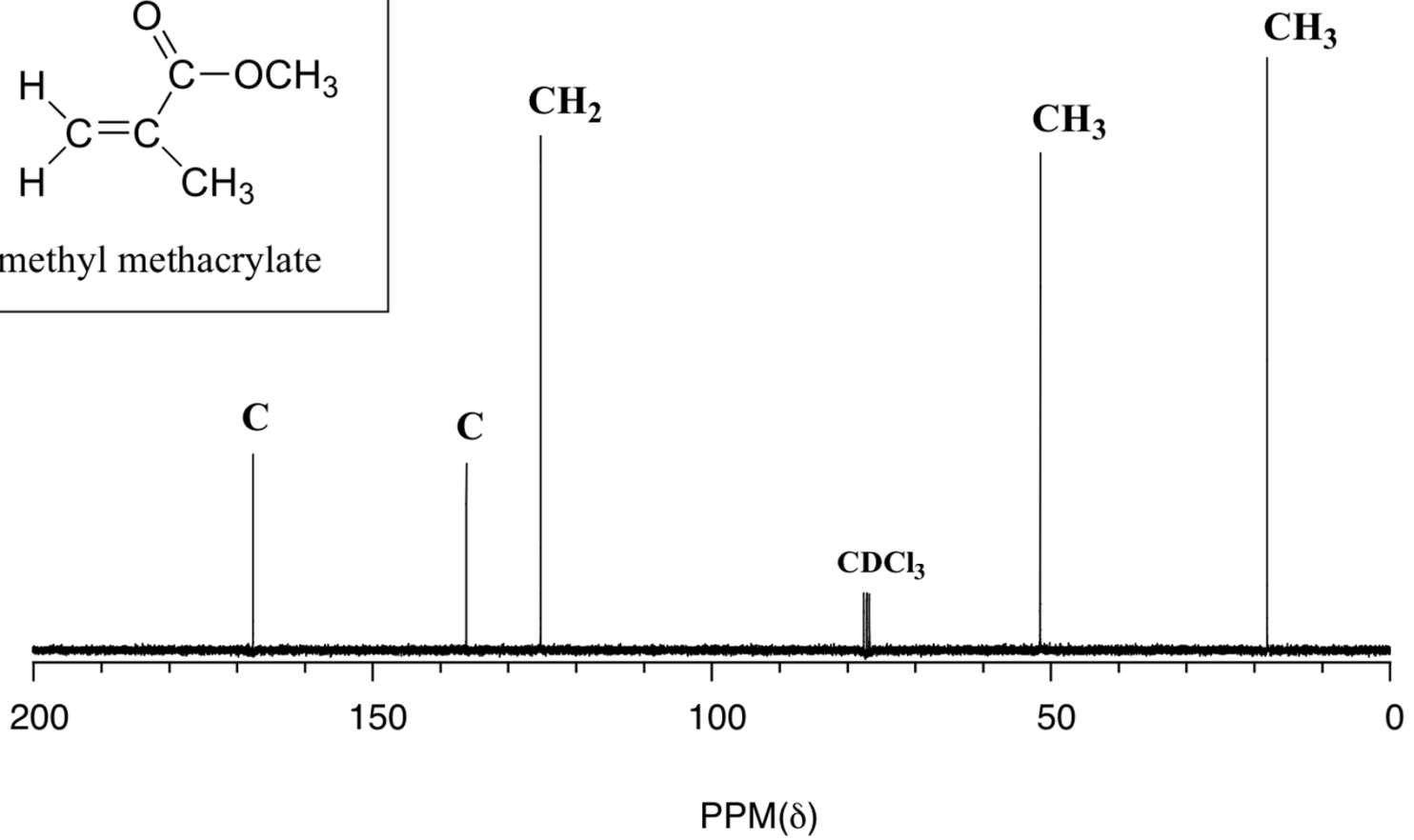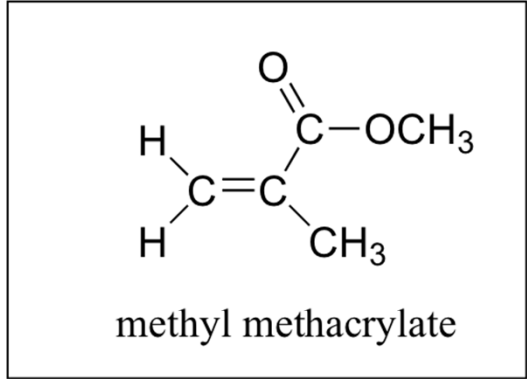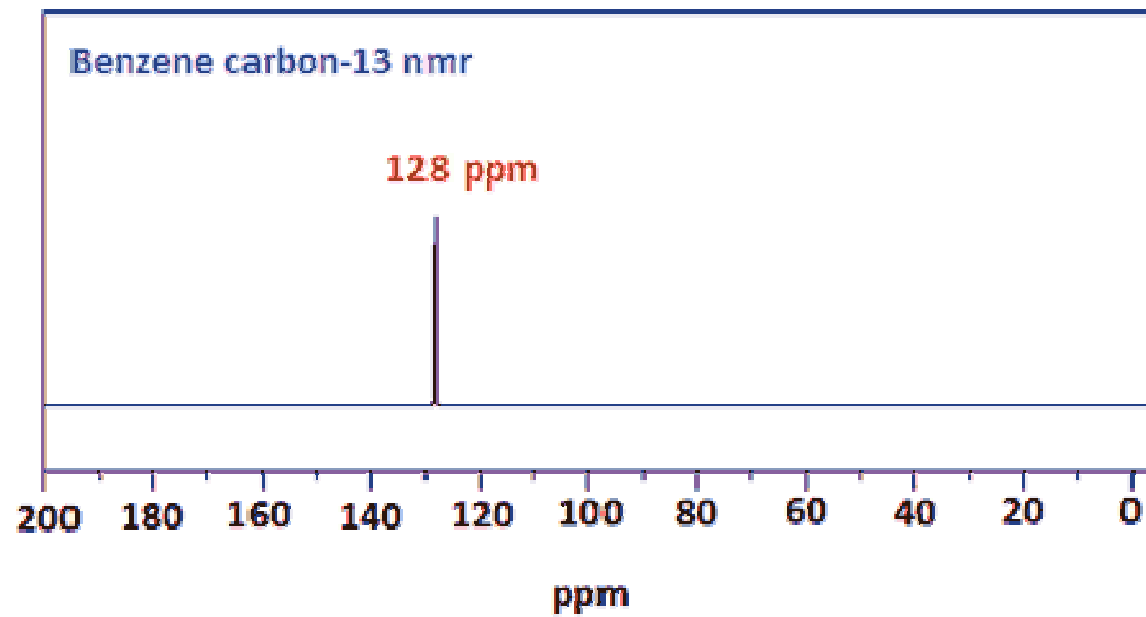

a consequence of this packing is that some protons may be positioned within the shielding cone of an aromatic ring such as Phe 51. Such protons will exhibit unusually low resonance frequencies (see picture at left). Note that such effects depend upon precise positioning of side chains within folded proteins
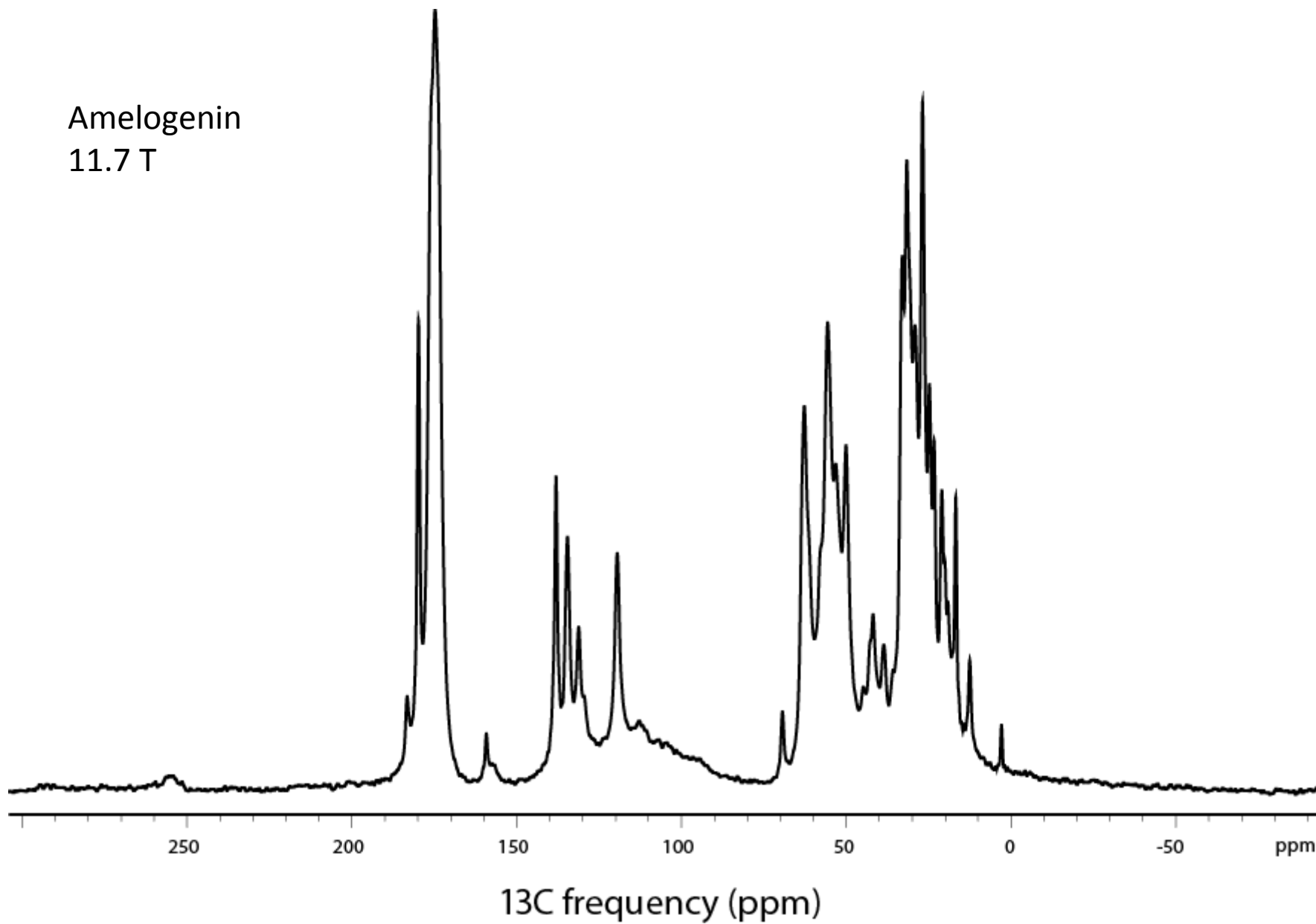

shielded methyl group

methyl region
of protein spectrum

Aldehydes, RCH=O

Ketones, R₂C=O

R₂C=CH₂

Carboxylic Acids R–CO₂H

Esters R—CO₂R'

Amides R–CONR₂

R₂C=CH₂

RHC=CHR

Aromatics

Heteroaromatics

RC≡N

C≡CR

C–F

C–NO₂

C–OH

C–OR

C–SO₂R

C–Cl

C–Br

C–H Saturated Alkanes

C–NR₂

C–SR

C–Ar

C–C=C

C–I

O
||
C–C–R

Sulfoxides,Sulfones

200   150   100   50   0.0 ppm (δ)

methyl methacrylate

Amelogenin
11.7 T

13C frequency (ppm)

SNIF-NMR®

Il contenuto isotopico di H_2O varia in funzione della distanza dalla costa marina, dall'altitudine, dalla latitudine e dalla condizione climitiche.

L'analisi della composizione isotopica dell'etanolo (CH_3CH_2OH) che si forma durante il processo di fermentazione consente di determinare l'origine e la genuinità del prodotto.
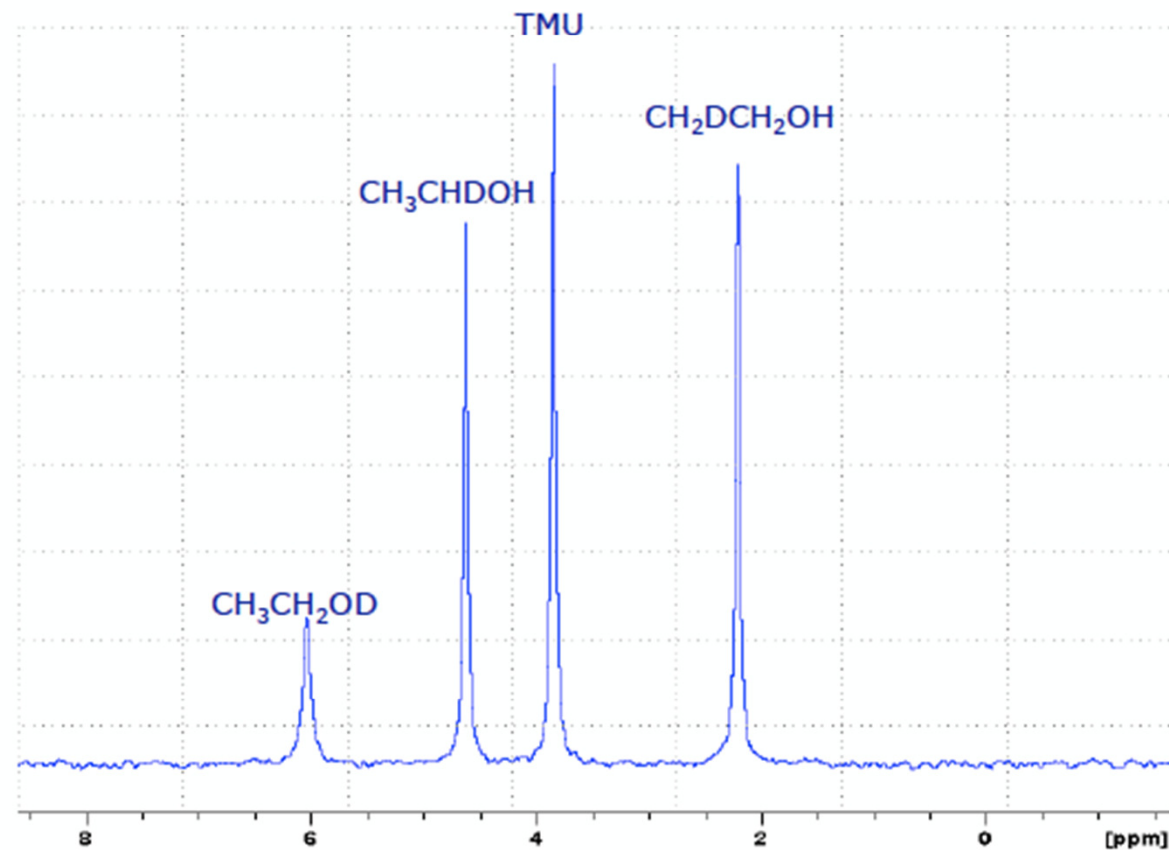
Infatti:

[CH_2DCH_2OH]: dipende dalla natura del vitigno

[CH_3CDHOH]: dipende dal clima e dall'eventuale arricchimento di mosto con zuccheri di altra origine.

SNIF-NMR®

Quindi è sufficiente acquisire uno spettro ^{2}H e analizzare [CH_2DCH_2OH]: [CH_3CDHOH]:

Questo metodo con decreto G.U. CEE del 03.10.1990 è riconosciuto come metodo per la determinazione del titolo alcoolimetrico naturale dei mosti di uve