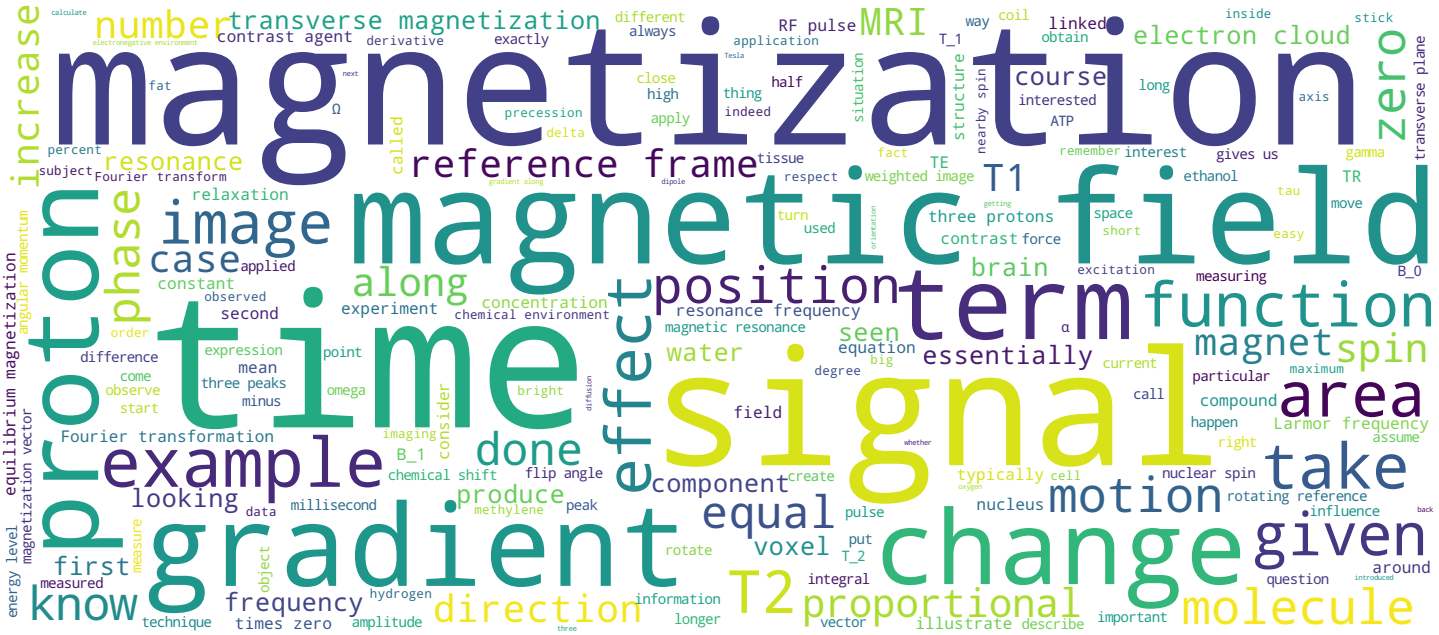
10.4 Measurement of chemical shift

Fundamentals of Biomedical Imaging

Prof. Rolf Gruetter



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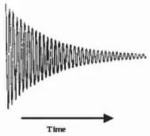
10-4. How can we measure chemical shift ?

MR spectroscopy

Free induction decay (FID) signal:

$$S(t) \propto M_{\perp}(0) e^{-i\omega t} e^{-t/T2}$$



distinguish resonance frequency

→ Fourier transformation (real part only):

$$G(\omega) \propto M_{\perp}(0) \frac{1}{(1+x^2)}$$

So how can we-- and this brings us to the question on how can we measure chemical shift? And this is done by looking at the free induction decay signal. So we have our signal as a function of time, a is the transverse magnetization times zero times *e ^ - i omega (Ω)t* times *e ^ -t / T2* We've seen that previously that expression, that's our time signal. Now let's say you have a signal that is separated by 1 ppm. So that is one millionth change in the resonance frequency. Would we be able from such a signal to be able to see a change in one millionth of the resonance frequency? Even if we place ourselves into the rotating reference frame where it might be easier to see that there are multiple frequencies present, we're not very good at detecting in time domain signals the change in frequency. And so here we are going to use, what is used here is the Fourier transformation, and I'll show you here the Fourier transformation of this function but only the real part, and we'll not take the full expression here. We're just interested in the fact that the Fourier transformation is proportional to the transverse magnetization of times zero times this function here which is the Fourier transformation of this term here, and *x* here is given by the frequency-- that's the argument here-- minus the chemical shift.

Notes

Summary

0m 04s

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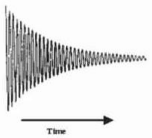

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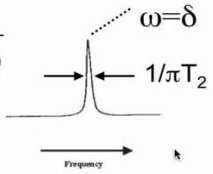
$$x \equiv (\omega - \delta) 2\pi T_2$$



Area of resonance ∝ M(0) ∝ number of nuclei ∝ concentration (if relaxation can be neglected)

$$S(0) \propto \int_{-\infty}^{\infty} G(\omega) e^{i\omega t} d\omega \Big|_{t=0} = \int_{-\infty}^{\infty} G(\omega) d\omega \propto M_0$$

That's the exact resonance frequency of our compound times *2π* times *T2*. So what does this look like? We'll take this signal here, Fourier transform it, and we're getting this line shape here. This function here is a Lorentzian function. It has its maximum when Ω is equal to delta (Δ), then *x* gets zero. This is maximally and the full width at half maximum here is given by *1 / π T2* That's the full width of half maximum of the function of Fourier transformed, which is also called the peak. Now the area of the resonance is proportional to the magnetization at times zero, this here. This is proportional to the number of nuclei and therefore proportional to the concentration if one can neglect the effect of relaxation in particular here T1 relaxation. So in this case, we are obtaining a signal, the area of this resonance here is proportional to the concentration. So to verify this, we have the signal at times zero is equal to the inverse Fourier transform of *G(Ω)* which is *e ^ i Ω t (d Ω)* but now we'll evaluate this at times zero to obtain the signal at times zero. So that is the integral of this function *G(Ω)*. So that's the integral of this.

Notes

Summary

1m 33s

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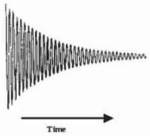

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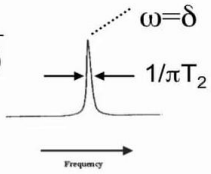

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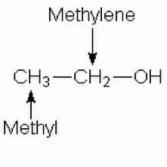
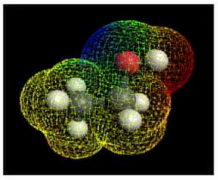
$$S(0) \propto \int_{-\infty}^{\infty} G(\omega) e^{i\omega t} d\omega \Big|_{t=0} = \int_{-\infty}^{\infty} G(\omega) d\omega \propto M_0$$

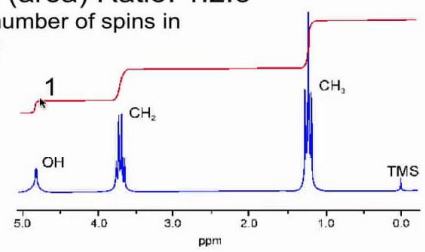
Example: Ethanol





Integral (area) Ratio: 1:2:3

(relative number of spins in molecule)



10-10

That's nothing but the area of the function, and we can see that this is now-- the area is proportional to this term here so it's proportional to *M zero* if we neglect the effects of relaxation. Now let me illustrate this with a molecule that most of us have been exposed to in their life and that's ethanol. So ethanol's found in, among others, in nice wines. Here's its structure. It's got a CH3 group, the methylene group and an OH group, the alcohol here. Here's the 3D rendering. Here's the oxygen. Here are the carbons and in white are the hydrogens which we are interested in. And if you now take a milliliter of wine or ethanol and you put it into your NMR spectrometer, then you observe three peaks, similar to here. Three peaks here, this is the reference peak of the reference compound TMS, that sets us the zero ppm scale. What we are observing is three peaks, if we know there's only one compound in there, then we know we have three different types of protons in that molecule that are subject to three different chemical environments. But we actually know more because we can also calculate the area under the curve, that is this function here. And we'll see here this is the red curve.

Notes

Summary

3m 04s

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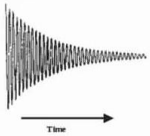

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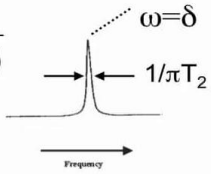

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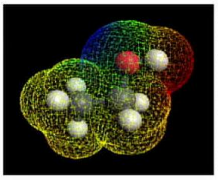
$$S(0) \propto \int_{-\infty}^{\infty} G(\omega) e^{i\omega t} d\omega \Big|_{t=0} = \int_{-\infty}^{\infty} G(\omega) d\omega \propto M_0$$

Example: Ethanol

Methylene

$CH_3—CH_2—OH$

Methyl



Integral (area) Ratio: 1:2:3

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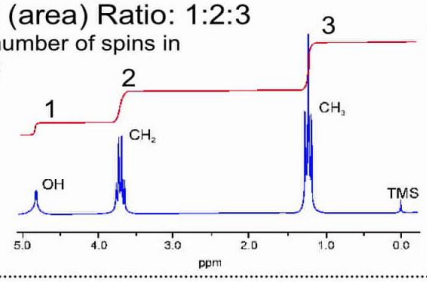


10-10

That's just integrating from infinity to whatever frequency is plotted here. We'll see an increase here, that's the area of this peak here. Then this area here is twice as high as this one, and this area is three times as high here. So if we know that there's only one compound in our sample, aside from TMS, and we know that the ratio is 1: 2: 3, then we know that there are three protons that have essentially the same chemical environment. They are to the right so they have a lower ppm so they are in a less electronegative environment. We have two protons in a more electronegative environment and one proton in the most electronegative environment. This is actually where we expect the OH groups to be, and so it's easy to deduce from this data that this is the most probable molecule that we are observing. So we'll stick with the example of ethanol for what is to come.

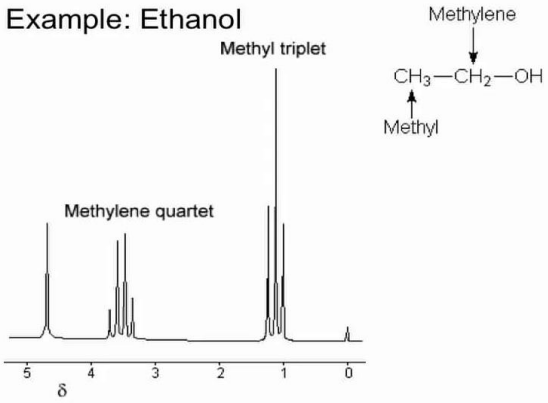
Notes

Summary

4m 32s

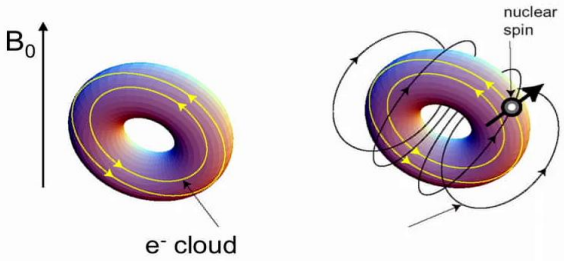
Ex. illustration of chemical proximity
(triplet & quartet)

Example: Ethanol



Hyperfine splitting

nucleus ⇒ tiny magnetic field linked to its dipole:



10-11

There's an additional effect of chemical proximity that I want to illustrate, and we'll stick here with the example of ethanol. So we have here, again, here's TMS, here's the methyl signal, the methylene and here's the OH signal. Here's the chemical shift scale here in ppm. So on the horizontal axis and as a a reminder, here is the molecule that we are discussing. Now if you look at the methyl signal, we actually see this is not one resonance but there's three signals that we see. And for methylene, we see four signals. And what I want to explain here in the next few minutes is the origin of these extra resonances that are observed. And essentially what they are dealing with here is a hyperfine splitting of the resonance frequency. The main mechanism that we have to consider here is that the nucleus which has a nucleomagnetic moment produces a very, very small magnetic field linked to its dipole. So we have *B zero*, we have the electron cloud. And now if we have the same electron cloud but we have a nearby nuclear spin, then this nuclear spin will produce an extra magentic field, this magnetic moment that adds to the magnetic field of *B zero*, and therefore, has an effect on the electron cloud.

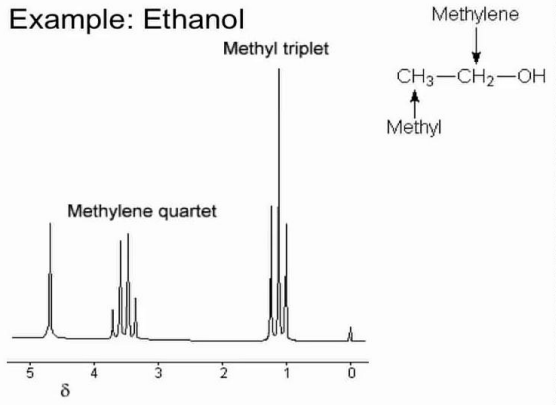
Notes

Summary

5m 34s

Ex. illustration of chemical proximity
(triplet & quartet)

Example: Ethanol



Nearby spin-1/2: ^1H resonance will split into two of equal magnitude (doublet)

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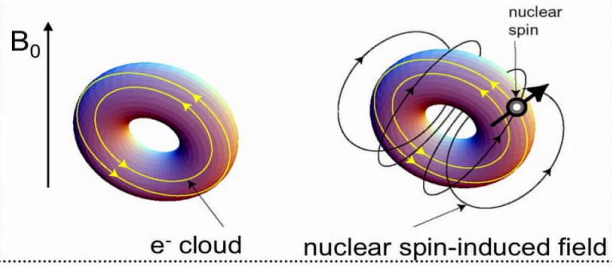
changes polarity if spin is “up” or “down”

⇒ affects the e cloud in the molecule⟶ alters the magnetic field at a nearby nucleus:



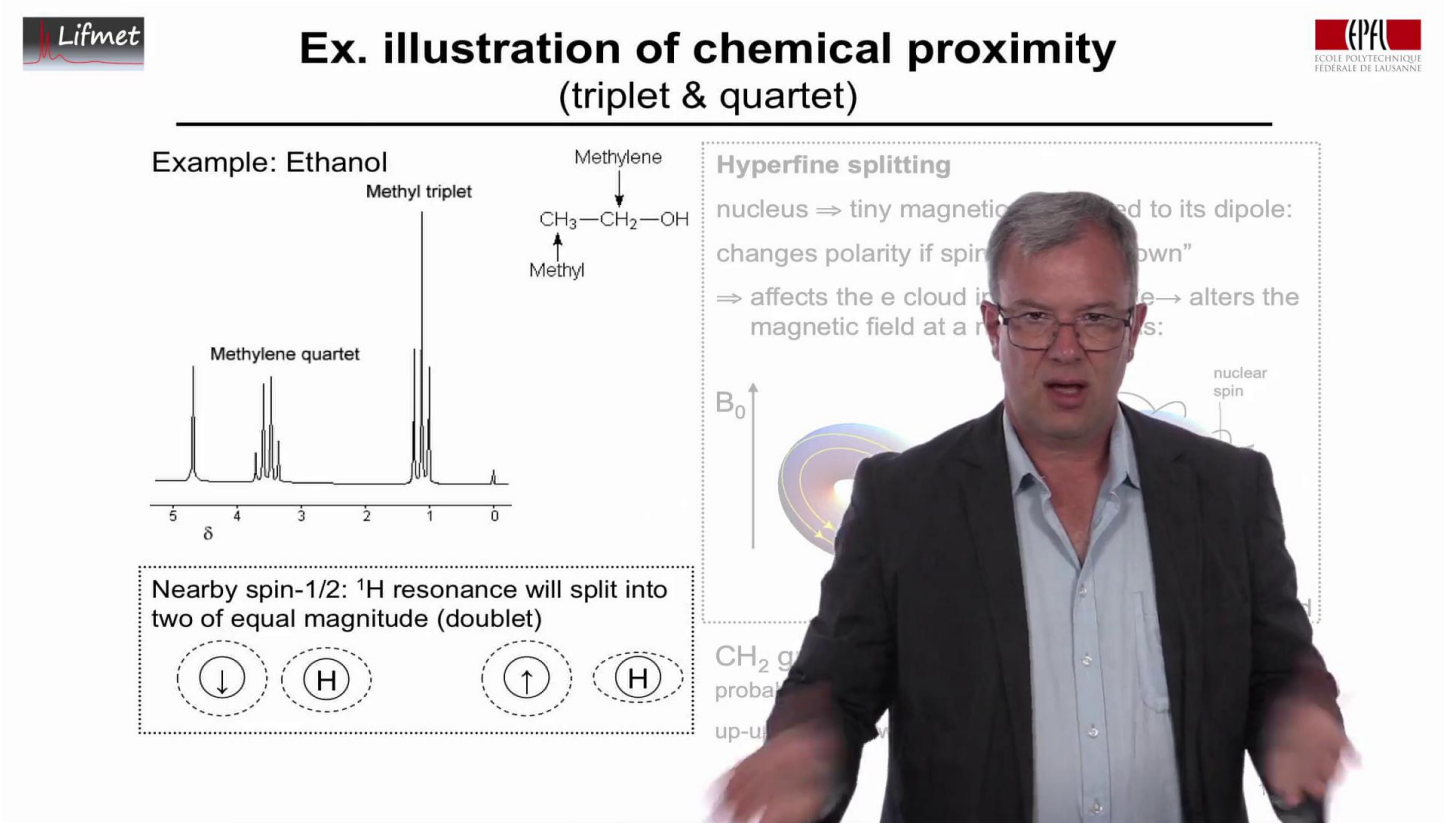
10-11

So this additional tiny magnetic field that's linked to the dipole of the nucleus this additional magnetic field for a spin one half nucleus like hydrogen, like the proton is either oriented up or oriented down. So this changes polarity, and this will affect the electron cloud in the molecule and therefore alter the magnetic field at a nearby nucleus. So if I have a nuclear spin here, this nuclear spin points either in this direction or in that direction. This will create an additional magnetic field. This creates an additional reorientation of the electron cloud and at the site of the nucleus that we're measuring, it will then, depending on its orientation, create a small change in the resonance frequency or the Larmor frequency. So let's say we have a nearby spin one half, and we'll look here at the proton resonance. So we are here interested in this proton here, and we have now a nearby spin one half nucleus. Here it's pointing down, and here it's pointing up. And so therefore it produces a-- in our nucleus hydrogen of interest which is this guy, the electron cloud will change its configuration ever so slightly depending on whether that nearby spin one half is oriented upwards or downwards with respect to the external magnetic field, and this results in a split of the resonance frequency and the intensity of the resulting two peaks is exactly the same.

Notes

Summary

7m 04s



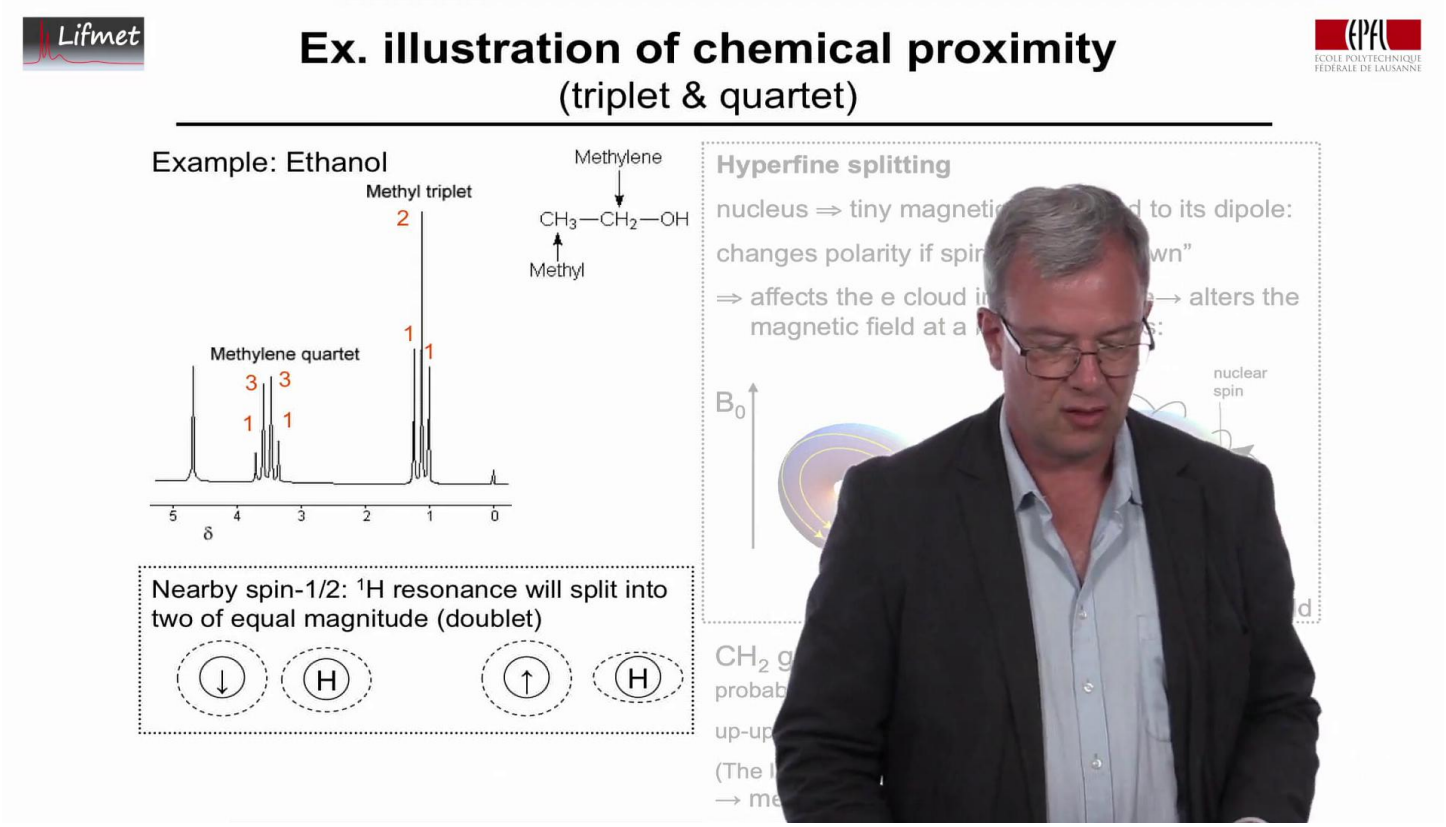
Now why is it exactly the same? Now remember we had dealt with the Boltzmann distribution. So we had said the probability whether it's up or down, that probability is essentially 50 percent. Well, one is 50.00001 percent and the other one is 49.99999 percent but in terms of intensity we don't see these small differences, so it is essentially the 50 percent up or down and so therefore the number of protons that see the spin up or the number of protons that see a spin one half down is the same and therefore we're seeing this signal split into two peaks of equal magnitude, and that's called a doublet. Now for a CH2 group, so if we're now here on the methyl, we have nearby the methylene, that's two protons. That's these guys, and these two protons, we'll assume here that they are chemically indistinguishable, and so we have four probabilities: up up, down down, up down and down up. So four probabilities. They are of equal possibilities, four configurations of equal probability: up up, down down. That's easy. Up down and down up, we cannot distinguish them because the two dipole fields of the two protons in the CH2 group cancel each other so up down or down up will produce the same change on the electron cloud.

Notes

Summary

8m 44s

Ex. illustration of chemical proximity
(triplet & quartet)



And so we have a triplet peak with a relative intensity ratio of 1: 2: 1. We're here observing three protons, but the next molecule that this interacts with has two protons, so we have four possibilities: one, one and two, that's the up down, down up. Now at the CH2 position, if I look at the protons that are here, they are seeing three protons from the methyl, three protons. So three protons, and they are-- because the methyl typically rotates very fast-- the three protons, they are having a number of combinations, and actually we have eight combinations. We have up up up, up up down, up down up, down up up, down down up, down up down, up down down and down down down so if you count this, this was eight possibilities, and so we have a quartet. Up up down, up down up, and so on are indistinguishable, same thing for the other cases. They cannot be distinguished. So this gives us a quartet, a resonance which is split into 1: 3: 3: 1 intensity structure.

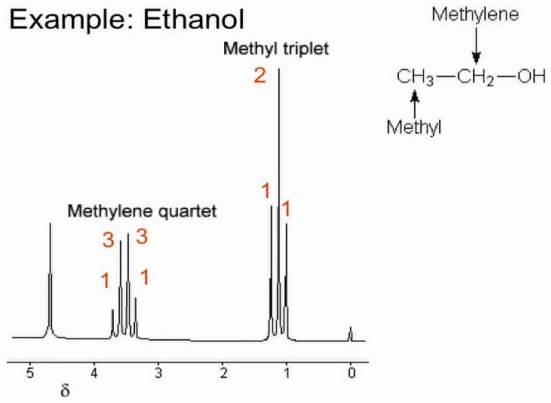
Notes

Summary

10m 27s

Ex. illustration of chemical proximity
(triplet & quartet)

Example: Ethanol



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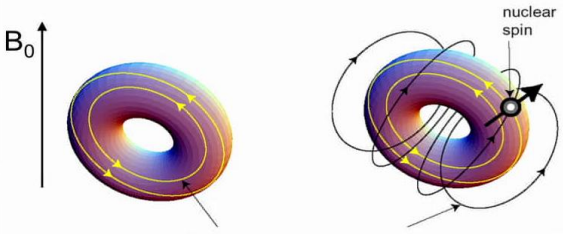


CH_2 group ⟶ four combinations (with equal probability):

up-up, down-down, up-down, down-up

(The latter two produce the same magnetic field)
⟶ methyl **triplet** (relative intensity ratio **1:2:1**)[10-11]

So to conclude here, in the case of ethanol we see three peaks, we have already a good idea that there most likely are three protons in the same chemical environment, but what this kind of splitting tells us indeed this whatever is in here is close to a CH2, and whatever is in here is close to a CH3, and from the relative intensity 2: 3 of the entire area, two to three, it is easy to deduce that the molecule that one observes here is indeed ethanol.

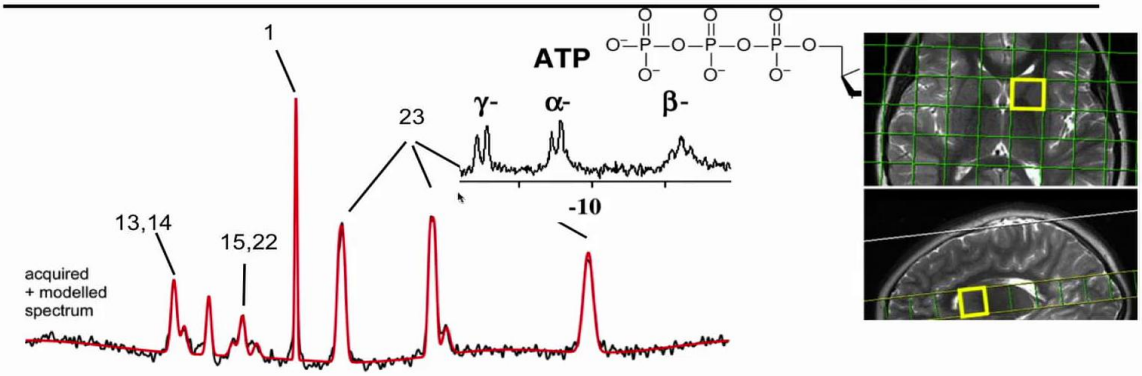
Notes

Summary

11m 48s

Ex. ^{31}P NMR spectroscopy

Phosphate metabolism is at the heart of cellular energetics



1: phosphocreatine (PCr)
13: phospho-ethanolamine (PE)
14: phosphocholine (PC)
15: glycerophosphocholine (GPC)
22: glycerophosphoethanolamie(GPE)
23: ATP

10-12

So I want to give here an example and that is phosphorus spectroscopy. Well, phosphorus spectroscopy was actually the first application of strong magnetic fields to living humans. There was an interest in looking at phosphorus metabolism, phosphate metabolism in the human brain as well as in muscle, so these were the first in vivo applications, and I want to illustrate here the type of information that one can get. So this is a phosphorus spectrum, and this is taken from the brain region here indicated in yellow. One observes the signal due to phosphocreatine. We have phosphomonoesters such as phospho-ethanolamine and phosphocholine. These are membrane anabolic compounds. Then one has glycerophosphocholine and glycerophosphoethanolamine, breakdown products of lipid metabolism. And then we have adenosine triphosphate. I presume you're all familiar with ATP, its structure, so we have adenosine triphosphate, so we know there's three phosphates in it, and indeed we have three resonances. The alpha (***α***) is the one that is closest to the ribose. This is the ***α***, this is the beta (β), this is the gamma (γ), and we can observe three peaks related to ATP. And what we can also see here is the splitting here.

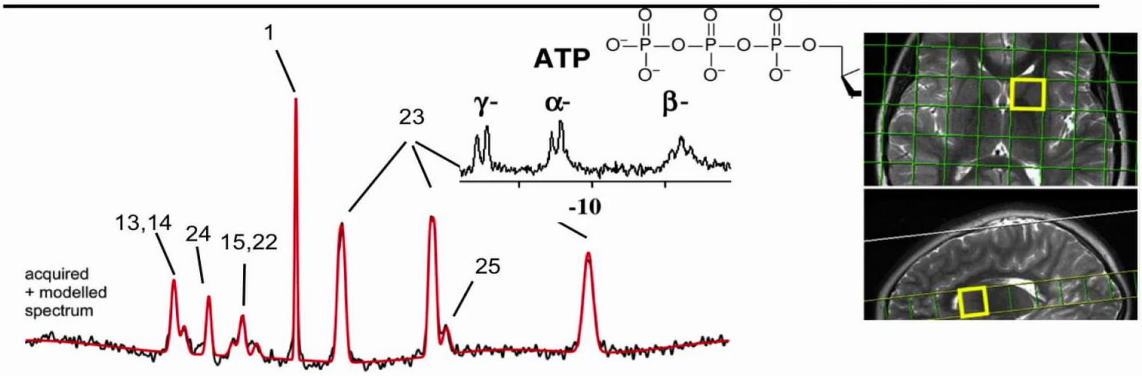
Notes

Summary

12m 25s

Ex. ^{31}P NMR spectroscopy

Phosphate metabolism is at the heart of cellular energetics



1: phosphocreatine (PCr)
13: phospho-ethanolamine (PE)
14: phosphocholine (PC)
15: glycerophosphocholine (GPC)
22: glycerophosphoethanolamie(GPE)
23: ATP
24: inorganic phosphate (Pi)
25:dinucleotides (NAD(P)[H])

Also measured:
Intracellular pH
Creatine kinase activity
ATPase activity

10-12

This tell us that there's a nearby phosphorous relative to this. Relative to this peak, there's a nearby phosphorous, and if we can see here, there's a 1: 2: 1 structure. It's not a very good signal-to-noise ratio here, but it indicates itself, and so we see that this must be linked to two phosphates nearby, and indeed this is the β, the middle one which sees this one and this one as well. We also have inorganic phosphate, which is resonance 24, which is this peak here, and dinucleotides NAD or NADP. Whether it's with the H or without the H, the dinucleotides contribute to this signal here. So these are the compounds that can be observed. They are linked to a large extent to cellular energetics. What is also measured is intercellular pH from the position of this peak creating kinase activity by measuring the conversion between phosphocreatine and ATP and ATPAs by measuring their conversion of inorganic phosphate and ATP. These are reactions that can also be measured. So this is an example of what the information that is obtainable and can be done. Here's the example of phosphorous. Like I mentioned, phosphorous, for historical reasons, was the first nucleus to be studied in vivo.

Notes

Summary

13m 52s

Credits

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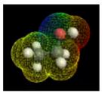

Ethanol production
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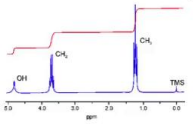

NMR
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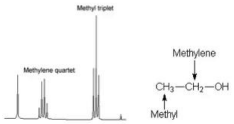
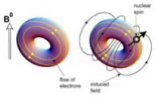
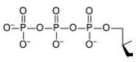


NMR
http://faculty.sdmiramar.edu/faculty/sdccd/fgarces/zCourse/All_Year/Ch251/a_LecLab/02_Lecture/01_LecNotes/20b_NMRSpectroscopy/NMRSpectroscopy.pdf



Nuclear spin
http://slideplayer.com/slide/781998/



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Notes

Summary

15m 15s