

Structural Biology
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Lecture - 27
Basic Principles of NMR and Instrumentation

Hi everyone, welcome again to the course of structural biology, we are continuing with various structural biologic techniques or we have discussed about X ray crystallography. Now we are in the module where we are discussing about nuclear magnetic resonance. In the last class I have started introducing NMR I talked about basic application of NMR the very basic concept introductory concept of spectroscopy and few ideas regarding NMR.

Today, we will talk about magnetism, we will talk about nuclear spin, we will talk about different energy level and also we will talk about instrumentation in NMR. In addition to that, I would also let you know about some major contributors in the field of NMR spectroscopy. So, let us start with showing NMR, nuclei with an odd mass or odd atomic numbers have nuclear spin in a similar fashion, you see in a spin up electrons.

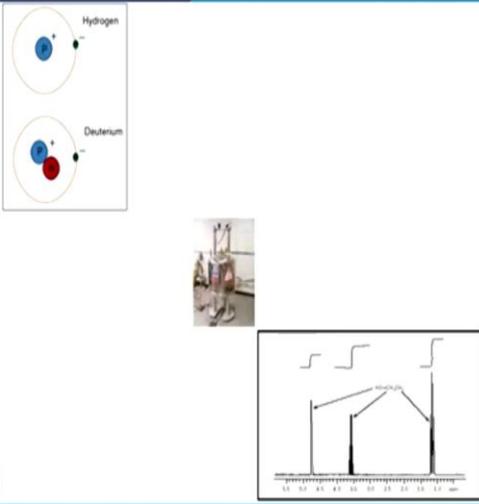
This includes ^1H proton and ^{13}C but not ^{12}C , the spins of the nuclei are sufficiently different that NMR experiments can be sensitive for only one particular isotope of one particular element, we talked about little bit in abundance, you know. The NMR behavior of ^1H the proton and ^{13}C nuclei has been exploited by organic chemists and structural biologists since they provide valuable information that can be used to deduce the structure of organic and bio compounds.

Our demonstration will be majorly based on these 2 elements, because if you look at in protein, they are the major network in any polymer they are the major network in any small molecule, they are the major components but we will also talk about others when needed.

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NMR Spectroscopy:

- NUCLEAR
- MAGNETIC
- RESONANCE



The slide includes diagrams of Hydrogen and Deuterium nuclei, a photograph of an NMR spectrometer, and an NMR spectrum plot showing peaks at different chemical shifts.

So, as I told NMR spectroscopy 3 component as the name suggests nuclear, magnetic, resonance. Yesterday in the last class, we talked about nucleus. Today we will talk about resonance and in between there is magnet and magnetic property.

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Concept of Magnetism:

Since a nucleus is a charged particle in motion, it will develop a magnetic field

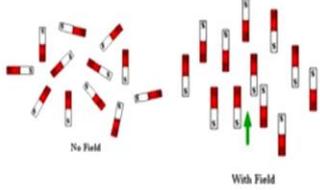
^1H and ^{13}C have nuclear spins of $1/2$ and so they behave in a similar fashion to a simple, tiny bar magnet

These nuclei together in a sample are oriented randomly in absence of a magnetic field

A combination of spin and a charge confers the nuclei with properties of a magnet

So a magnetic dipole moment is associated with every nucleus. As a result, every nucleus which has a charge and spin becomes now a magnet, a small magnet

In the absence of a magnetic field, these are randomly oriented but when a field is applied they line up parallel to the applied field, either spin aligned or spin opposed



The diagram illustrates the concept of magnetism for nuclei. On the left, labeled 'No Field', several red and white bar magnets representing nuclei are shown in random orientations. On the right, labeled 'With Field', the same bar magnets are shown aligned vertically, representing their orientation in an external magnetic field.

So, let us come to the concept of magnetism. Since a nucleus is a charged particle in motion, it will develop a magnetic field, ^1H and ^{13}C have nuclear spins of half and so they behave in a similar fashion to a simple tiny bar magnet. So, if you see when there will be no field they are disorganized in their orientation like this and when a field is applied, they would be properly oriented.

These nuclei together in a sample are oriented randomly in absence of magnetic field as I talked about. A combination of spin and a charge confers the nuclei with properties of a

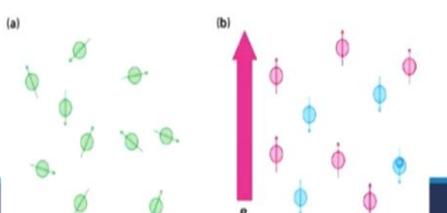
magnet. So, a magnetic dipole moment is associated with every nucleus. As a result, every nucleus which has a charge and spin becomes now a magnet, a tiny small magnet. In the absence of the magnetic field, these are randomly oriented but when a field is applied, they lineup parallel to the applied field, either spin aligned or spin opposed. So, see, there are 2 conditions, spin aligned and spin opposed, we will talk about them.

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Nuclear Spins:
Nuclear spins are oriented randomly in the absence of an magnetic field but have a specific orientation in the presence of an external magnetic field B_0

Some nuclear spins are aligned parallel to the external field
Lower energy orientation
More likely

Some nuclear spins are aligned antiparallel to the external field
Higher energy orientation
Less likely



The diagram consists of two parts, (a) and (b). Part (a) shows several green circles representing nuclear spins, each with a small arrow pointing in a different direction, indicating random orientation. Part (b) shows a red arrow pointing upwards, labeled B_0 , representing the external magnetic field. Below this arrow, there are two groups of spins: pink circles with arrows pointing up (parallel to B_0) and blue circles with arrows pointing down (antiparallel to B_0). The pink spins are more numerous than the blue spins.

Nuclear spins are oriented randomly in the absence of magnetic fields but have a specific orientation in the presence of an external magnetic field B_0 , some nuclear spins are aligned parallel to the external field as I told they have lower energy orientation and more likely. So, you if you see here they were randomly oriented and when applied field is there they are aligned and some are aligned on the direction where the applied magnetic field is given.

Whereas, others are opposite to that, so some nuclear spins are aligned anti parallel to the external field, if you see the pinks are aligned with the parallel to the external field, whereas the blue ones are anti parallel to the external field. And in this case there would be higher energy orientation and less likely, by more likely and less likely I mean, more populated and less populated. In the lower energy in the stable state there are more populated more nuclear with there in the higher energy which is coming through like opposite anti parallel alignment, it is less populated.

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Concept of two spin states:

In NMR, electromagnetic (EM) radiation is used to "flip" the alignment of nuclear spins from the low energy, spin aligned state to the higher energy spin opposed state

The energy required for this transition depends on the strength of the applied magnetic field

The energy difference between the spin states is quite small and corresponds to the radio frequency range of the EM spectrum

So, from there we are getting the concept of 2 spin states. In NMR electromagnetic radiation is used to flip the alignment of nuclear spins from the low energy spin aligned state to the higher energy spin opposed state. So, you see that there are 2 energies and there happened there is ground state and excited state and there happen spin flip. The energy required for this transition depends on the strength of the applied magnetic field.

So, here, the applied magnetic field, the external magnetic field have a very critical role, because the flipping depends on the amount of energy provided. The energy difference between the spin states is quite small and corresponds to the radio frequency range of the electromagnetic spectrum. We have talked about that NMR is used radio frequency and this is one of the reasons.

So, why radio frequency? Because it is good enough to spin flip which we want here and because it is low energy, this enables NMR to be non destructive, non invasive and when we talks about non destructive, non invasive there are many application but what comes first in our mind is MRI which is a really good technique by which we could now identify a lot of disease conditions.

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As this diagram shows, the energy required to cause the spin-flip, ΔE , depends on the magnetic field strength at the nucleus

With no applied field, there is no energy difference between the spin states

As the field increases, separation of energies of the spin states increases with the frequency required to cause the spin-flip.

$\Delta E = 0$

$x \rightarrow \Delta E$ $2x \rightarrow \Delta E'$

E_2 E_2'

E_1 E_1'

ΔE $\Delta E'$

Increasing magnetic field

$\Delta E' > \Delta E$ since $B_0' > B_0$

So, as this diagram shows here, the energy required to cause the spin flip ΔE , depends on the magnetic field strength at the nucleus. With no applied field, there is no energy difference between the spin states. So, there are 2 spin states we understand them but without any external applied magnetic field, there is no difference that ΔE is 0. But then you start increasing the application of external magnetic field there is distance.

So, when you apply, let us say x amount it is ΔE . When you apply $2x$ amount it is $\Delta E'$. And it is easy to understand that $\Delta E'$ is greater than ΔE , because the applied magnetic field B_0 is greater than, B_0' is greater than B_0 . As the field increases, the separation of energies of the spin state increases with a frequency required to cause the spin flip.

(Refer Slide Time: 09:10)

Magnetic Dipole Moment and Gyromagnetic Ratio:

The tiny magnets are randomly oriented in absence of external magnetic field and oriented when magnetic field is applied

Now, if we consider I being the net nuclear spin number then the nucleus could only stay in spin states $2I+1$, which means in spin state 2 for 1H or ^{13}C according to the rules of quantum mechanics $2 \times \frac{1}{2} + 1 \rightarrow 2$

If you look at the nucleus now, which has a magnetic dipole because of its charge and spin, the magnetic dipole moment (μ) is equal to $\gamma \cdot I$

The parameter, γ , is basically a constant for a particular type of nucleus. It is known as the 'gyromagnetic ratio'.

γ varies

Magnetic dipole moment and Gyromagnetic ratio, as I talked about the tiny magnets are randomly oriented in absence of external magnetic field and oriented when magnetic field is applied. Now if we consider I, being the net nuclear spin number then the nucleus could always stay in spin states $2I + 1$ which means in spin state 2 for ^1H proton or ^{13}C , according to the rules of quantum mechanics, there would be so it is $2I + 1$. So; if it is half spin, 2 into half plus 1 which is 2.

So, there are 2 spins tests. If you look at the nucleus now which has a magnetic dipole because of its charge and spin, so from where it is happening, we know the nucleus have charge because of the charged proton and it up spin, because all the nucleones both protons and neutrons all at spin. So, because of its charge and spin the magnetic dipole moment μ is equal to γ into I. The parameter γ is basically a constant for a particular type of nucleus, it is known as gyromagnetic ratio. So, what you have to remember here is particular type of nucleus. So, γ varies depending on particular type of nucleus.

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Gyromagnetic ratio is an important parameter which is useful for understanding NMR.

The **gyromagnetic ratio** of a particle or system is the ratio of its magnetic moment to its angular momentum, which is here called as nuclear spin angular momentum, denoted as I

SI unit is the radian per second per tesla ($\text{rad}\cdot\text{s}^{-1}\cdot\text{T}^{-1}$) or, equivalently, the coulomb per kilogram ($\text{C}\cdot\text{kg}^{-1}$).

(μ) is a vector quantity because it has a direction and similarly (I) is a vector quantity because it has a direction

So therefore I can split μ into three components, that is μ_x , μ_y and μ_z

The gyromagnetic ratio is an important parameter which is useful for understanding NMR. The gyromagnetic ratio of a particle or system is the ratio of its magnetic moment to its angular momentum which is here called as nuclear spin angular momentum and denoted as I. So, γ is the ratio of its magnetic moment with angular momentum. The SI unit is the radian per second per Tesla or equivalently the coulomb per kilogram.

μ is a vector quantity because it has a direction and similarly, I is a vector quantity because it has a direction. So, both μ and I both have direction. So, because μ have direction, you

know, it is a vector, it have a direction. So, what you could do? You could split it into different directions let us consider you split it into x, y and z, so there would be 3 components μ_x , μ_y and μ_z .

(Refer Slide Time: 12:32)

When we apply a magnetic field, this magnetic dipole interacts with the external magnetic field (B_0) and the energy of this interaction is given the formula: $E = -\mu \cdot B_0$

This is a dot product with the magnetic field

So as I said we can split μ as μ_x , μ_y and μ_z and the magnetic field, B_0 can also be split as be B_x , B_y , B_z , which are the components of the magnetic field along the three different directions

This dot product can therefore be written as: $E = \mu_x B_x + \mu_y B_y + \mu_z B_z$

Assuming that I apply the magnetic field in the z direction, what happens is the x and y components of the magnetic field no longer exist (they are zero) and therefore only the z component $\mu_z B_z$ remains

Handwritten notes on the slide: Red arrows point to the $\mu_x B_x$ and $\mu_y B_y$ terms in the equation. A red circle highlights the $\mu_z B_z$ term. To the right, the handwritten equation $E = \mu_z B_z$ is written.

When we apply a magnetic field, this magnetic dipole interacts with the external magnetic field and the energy of this interaction is given by the formula energy equal to minus mu into B_0 . B_0 is the external magnetic field. This is a dot product because of its vector property with the magnetic field. So, it is a multiplication but a dot product because of the vector property.

So, as I said we can split μ as μ_x , μ_y and μ_z and the magnetic field B_0 can also be split as B_x , B_y , B_z which are the components of the magnetic field along the 3 different directions. This dot product can therefore be written as $E = \mu_x B_x + \mu_y B_y + \mu_z B_z$. Assuming that we apply the magnetic field in the z direction, what happened is there will be no x and y component like because they are 0. So, because they are 0 the total component is 0. So, it would be this and therefore only z component is $\mu_z B_z$, this will remain. So, now, $E = \mu_z B_z$ and dot B_z .

(Refer Slide Time: 14:06)

μ_z is nothing but γI_z , where I_z is the z-component of the spin angular momentum. So this is our external magnetic field, B_0 which we have applied in the Z-direction and therefore the total final energy of interaction becomes equal to

$E = -B_0 \gamma I_z$

Now, this μ_z is nothing but γI_z , where I_z is the z component of the spin angular momentum because spin angular momentum is I , is also a vector could be dissected into 3 coordinates. So, we only get the z coordinate. So, this is our external magnetic field B_0 which we have applied in the Z direction therefore the total final energy of the interaction becomes equal to $E = -B_0 \gamma I_z$ that is the total energy.

(Refer Slide Time: 14:43)

Spin States:
 Now according to quantum mechanics if you have a spin, I , it has a possibility of $2I+1$ spin states

What is a spin state?
 Spin state is basically what we could refer to discrete energy levels

Similarly the energy levels corresponding to this angular momentum, which is denoted by I , is also quantized and it has $2I+1$ different levels

For example, when $I = 1/2$, which is a case for protons and Carbon 13, $2I+1=2$. So that means a hydrogen atom or hydrogen nucleus has now two spin that is denoted as $+1/2$ or $-1/2$

That means this I has two possible values: $I = \frac{1}{2} (h/2\pi)$ or $-1/2 (h/2\pi)$. Here, h is the Planck's constant

We are talking about spin states we see that there is possibility of 2 states developed. According to quantum mechanics, if you have a spin I , it has a possibility of $2I + 1$ spin states, we talked about this. So, what is a spin state? Spin state is basically what we could refer to discrete energy levels. Similarly the energy levels corresponding to this angular momentum which is denoted by I is also quantized and it has $2I + 1$ different level which I talked about just before.

So, for example; when I equal to half which is the case of protons and carbon 13, $2I + 1 = 2$. So that means, the hydrogen atomic or hydrogen nucleus has now 2 spin states that is denoted as plus half and minus half that means, this I_z has 2 possible values I_z equal to half $h / 2\pi$ or minus half $h / 2\pi$, where h is Planck's constant.

(Refer Slide Time: 15:55)

If we put this value of I_z in the equation for the energy of interaction, E , it results in: $E = B_0 \gamma (1/2) (h / 2 \pi)$ or $E = -B_0 \gamma (1/2) (h / 2 \pi)$ depending on the value of I_z we use

we can see that now this energy of interaction has two values depending on whether the spin is either in the $+1/2$ or $-1/2$ state

So therefore what has happened that we have created two energy levels because of the interaction of this nucleus with the magnetic field

One energy level corresponds to the lower energy value which is when the spin is in $+1/2$ state. That is the ground state energy level, also called as the alpha state

beta state
alpha state

Now if we put this value of I_z in the equation for the energy of interaction, E , it results in $E = B_0 \gamma (1/2) (h / 2 \pi)$ or $E = -B_0 \gamma (1/2) (h / 2 \pi)$, depending on the value of I_z we use, so 2 different state again energy state. We can see that now, this energy of interaction has 2 values depending on whether the spin is either in the plus half or minus half state. So, therefore what has happened that we have created 2 energy levels because of the interaction of this nucleus with our external magnetic field.

One energy level corresponds to the lower energy value which is when the spin is in the plus half state. That is the ground state energy level, also called as the alpha state. So, we talked about energy splitting, the lower state is alpha state and we will see the higher state is beta state.

(Refer Slide Time: 17:08)

When $I_z = -1/2$, the total energy is positive as shown here and that becomes the excited state energy level and is called as the beta-state

The difference between two energy levels becomes: $\Delta E = B_0 \gamma (1/2) (h/2\pi) - (-B_0 \gamma (1/2) (h/2\pi)) = B_0 \gamma (h/2\pi)$ The nuclei now get distributed between the two states

When we apply a magnetic field, which we call as B_0 , and put a sample in that magnetic field, all the nuclei in the sample are exposed to the field and get distributed into one of the two energy levels

With this, in the terminology of nuclear magnetic resonance, we have looked at the nucleus and the magnet and the interaction between the nucleus and the magnet.

When I_z equal to minus half, the total energy is positive as shown here and that becomes the excited state energy level and is called as the beta state. The difference between 2 energy levels becomes $\Delta E = B_0 \gamma \frac{h}{2\pi} - (-B_0 \gamma \frac{h}{2\pi}) = B_0 \gamma \frac{h}{\pi}$, the nuclei now get distributed between 2 states as I told, alpha state and beta state. When we apply a magnetic field which is we are calling as B_0 and put a sample in that magnetic field.

All the nuclei in the sample are exposed to the field and get distributed into one of the 2 energy levels. So, you have sample there the nucleus are randomly oriented, you apply external magnetic fields, you have 2 states, lower energy state which is alpha state and higher energy state which is beta state. With this in the terminology of nuclear magnetic resonance, we have looked at the nucleus and the magnet and the interaction between the nucleus and the magnet.

(Refer Slide Time: 18:27)

Resonance:

Now the next term is resonance

What happens next is essentially that we supply an external energy which will take the molecules from the ground state to the excited state

This energy has to match the gap between the two energy levels, denoted as ΔE

Only then the molecules will absorb the applied energy

The matching of the Gap is a critical concept and is also the general idea of spectroscopy



15

Now, the next term is resonance. So, we talked about nuclei, we talked about magnet and now it is resonance. What happens next is essentially that we supply an external energy which will take the molecules from the ground state to the excited state. So, we need this. So, we have to provide an external energy, this energy has to match the gap between the 2 energy levels denoted as ΔE and if it match only then the molecule will absorb the applied energy.

The matching of the gap is a critical concept and is also a generalized idea of spectroscopy. So, if you look at spectroscopy, for different types of spectroscopy, we target different things, we have different rays but there is a gap and there is a resonance to find the gap or matching the gap.

(Refer Slide Time: 19:37)

Resonance:

When we apply an energy which matches this energy level gap, it is called 'resonance'

In resonance, the molecules which are now in alpha, the ground level state, get excited and they absorb the energy and go to the beta state

And in this moment we justifies the term:
Nuclear Magnetic Resonance

↑
different nuclei

↑
spin magnetic charge positive spin nucleus

↑
matching the gap

→ Nuclear Magnetic Resonance



16

When we apply an energy which matches this energy level gap, it is called resonance. In resonance the molecules which are now in alpha, the ground state level, get excited and they absorb the energy and go to the beta state and in this moment, we justify the term nuclear magnetic resonance, because we talk about different nucleus, we talk about how these tiny magnets are formed because of charge coming from proton spin coming from nuclear.

And now we know the resonance the matching of the gap. So, these different nuclei, tiny magnetic with charge and spin matching the gap, develop the technique called nuclear magnetic resonance.

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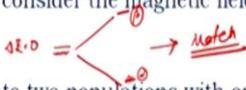
The overall idea of NMR now can be said as follows:

Take a sample which contains molecules in which the atoms have non-zero nuclear spin

Keep the sample in a magnetic field, consider the magnetic field is applied along Z-direction

The molecules now get distributed into two populations with one set of nuclei in the alpha state and other set in the beta because of their interaction with the magnetic field

Take the molecules which are in the ground state to the excited state by applying an energy which is equal to the difference in energy level that is ΔE .



17

So, let us come to the overall idea of NMR, you have to take a sample which contains molecule in which atoms have non zero nuclear spin, if you have zero nuclear spin, you will not get any effects. So, you have to be knowledgeable about the fact that the sample you are picking up should have atoms or at least an isotope of an atom which have non zero nuclear spin. Keep the sample in a magnetic field consider the magnetic field is applied along the z direction. So, you could put it in any direction but let us put it in z direction.

So that other 2 would be not present like nullified making your case simpler, the molecules now get distributed into 2 populations with one set of nuclei in the alpha state and other set in the beta because of their interactions with the magnetic field. Take the molecules which are in ground state to the excited state by applying an energy which is equal to the difference in energy level that is ΔE .

So, what happened, you have this 1 state and another state in same energy, where $\Delta E = 0$, now you apply energy, so they are going to 2 different places calling alpha and beta. And now we have to put energy to match. That is what more or less basic principles of NMR is.

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The overall idea of NMR now can be said as follows:

This is called stimulated excitation and stimulated emission

So therefore the total net energy absorbed will depend on the difference in the population of how many nuclei are in the ground state minus the nuclei in the excited state; if they are equal then there is no net excitation and the signal will be zero

That means no energy will be absorbed

But if the difference in the population between the ground state and the excited state is not zero and if the ground state is more populated than the excited state, there will be more number of nuclei absorbing than the nuclei coming down from the excited state to the ground state

This process is called stimulated excitation and stimulated emission. So, therefore, the total net energy absorbed will depend on the difference in the population of how many nuclei are in the ground state minus the nuclear in the excited state. In case if they are equal then there would be no net excitation and the signal would be 0, there would be no signal that means no energy would be absorbed.

But if the difference in the population between the ground state and the excited state is not 0 and if the ground state is more populated than the excited state, there will be more number of nuclei absorbing than the nuclei coming down from the excited state to the ground state which is the matching energy and that is where the signal comes.

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In summary, there will be a net positive absorption of the energy and that is what we rely on in NMR

That means all depends on the net difference, the word 'difference' is very important here, in the population between the two states

This concept of population difference is applicable in general across all spectroscopy; wherever we talk about energy quantization, we talk about population difference and distribution

That distribution comes from a famous law known as the Boltzmann law

Boltzmann law says that under equilibrium conditions, there is always more population in the ground state compared to the excited state

That means under standard conditions you will always have more nuclei which are sitting in the ground state than in the excited state.



19

In summary, there will be a net positive absorption of the energy and that is what we rely on in NMR. That means all depends on the net difference, the word difference is very important here in the population of the 2 states the alpha and the beta state. The concept of population difference as I talked earlier is applicable in general across all spectroscopy, wherever we talked about energy quantization, we talked about population difference and distribution and hence they are matching.

That distribution comes from a famous law which is known as Boltzmann law. Boltzmann law says that under equilibrium conditions there is always more population in the ground state compared to the excited state. That means under standard condition, you will always have more nuclei which are sitting in the ground state than the number of nuclei which is present in the excited state and the ratio of the population between the 2 states is given by the write up which we are putting on the Boltzmann equation.

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Strength of the Magnetic Field:

The strength of the magnetic field is given in different units: Tesla, Gauss or MHz, 1 Tesla = 10^4 Gauss

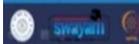
The most common way to indicate the magnetic field strength is to give in terms of Larmor precession frequency

The frequency of precession the frequency at which the magnetic the nucleus precesses around magnetic field is given by this frequency: $\nu = \gamma B_0 / 2\pi$

Now here B_0 is measured in units of Tesla or it can be units of Gauss (1T = 10^4 Gauss)

If we put in the values of γ and B_0 , which is typically used, we get ν in the Megahertz regime (denoted as MHz)

Magnetic Field Strength	
In MHz	In Tesla
300 MHz	7.05 T
500 MHz	11.75 T
700 MHz	16.45 T
900 MHz	21.15 T



20

Coming to the strength of the magnetic field, the strength of the magnetic field is given in different units Tesla, Gauss or Megahertz, one Tesla equal to 10 to the power 4 Gauss. The most common way to indicate the magnetic field strength is to give in terms of Larmor precession frequency, the frequency of precession the frequency at which the magnetic nucleus precesses around magnetic field is given by this frequency $\nu = \gamma B_0 / 2\pi$.

Now, here B_0 is measured in units of Tesla or it can be in units of Gauss if we put in the values of γ and B_0 which is typically used we get ν in the megahertz regime. So, if you see when we get 7.05 Tesla, this is actually megahertz 300 megahertz, 11.75 Tesla is 500 megahertz, 16.45 Tesla is 700 megahertz and 21.15 Tesla is 900 megahertz and that is why you see that we use megahertz to say the power of NMR instrument.

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The table clearly tells you the fact that why the NMR systems mentioned in terms of Megahertz

We say that we have a 300 or 400 Megahertz spectrometer etc. So essentially the magnetic field is what is determining the frequency

When somebody says that she has a 500 Megahertz NMR spectrometer in the laboratory, what she means to say is that she has a magnet which is 11.75 Tesla

So the point here is that the resonance frequency depends on the magnetic field directly. To put this in perspective the earth's magnetic field is very small, it is 0.5 Gauss

So the magnetic field we use in NMR is really orders of magnitude higher compared to what is earth's magnetic field. We use very strong magnetic fields and the associated precautions, which has to be taken, will be considered when we come to the discussion on hardware aspects

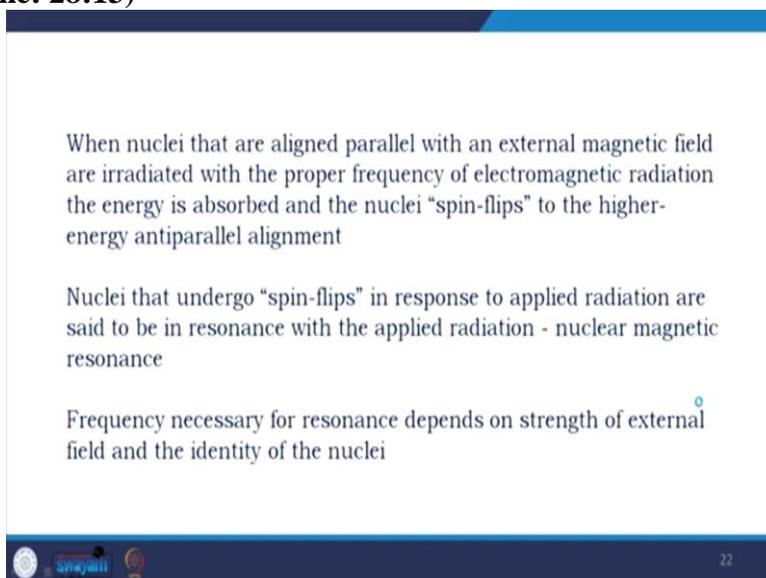


21

The table I showed you clearly tells you the fact that why NMR system mentioned in terms of megahertz. We say that we have a 300 or 400 megahertz spectrometer. So, essentially the magnetic field is what is determining the frequency. When somebody says that he or she has 500 megahertz NMR spectrometer in the laboratory, what he or she means to say is that a magnet which is 11.75 Tesla.

So, the point here is that the resonance frequency depends on the magnetic field directly to put this in perspective the Earth's magnetic field is very small which is 0.5 Gauss. So, the magnetic field we use in NMR is really orders of magnitude higher compared to what Earth's magnetic field is. We use very strong magnetic fields and the associated precaution which has to be taken will be considered when we discuss about the hardware aspect.

(Refer Slide Time: 28:13)



When nuclei that are aligned parallel with an external magnetic field are irradiated with the proper frequency of electromagnetic radiation the energy is absorbed and the nuclei "spin-flips" to the higher-energy antiparallel alignment

Nuclei that undergo "spin-flips" in response to applied radiation are said to be in resonance with the applied radiation - nuclear magnetic resonance

Frequency necessary for resonance depends on strength of external field and the identity of the nuclei

22

When nuclei that are aligned parallel with an external magnetic field are irradiated with proper frequency of electromagnetic radiation, the energy is absorbed and the nucleus spin flips to the higher energy anti parallel alignment. Nuclei that undergo spin flips in response to applied radiation are said to be in resonance with the applied radiation, nuclear magnetic resonance. Frequency necessary for resonance depends on strength of the external field and the identity of the nuclei.

So, I talked about the NMR spectroscopy, the nucleus we discussed, the resonance we discussed and the magnetic property, magnetism we discussed. Now we will talk about the hardware of this. So, we will discuss NMR hardware. But before going to NMR hardware, I would also like to give you a brief history of how NMR.

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History of NMR Development:

1938 - NMR in molecular beams Rabi (Columbia University)	Protein NMR: In 1985, Michael Williamson, Havel and Wüthrich reported the first solution-state protein structure — that of proteinase inhibitor IIA from bull seminal plasma
1946 - NMR of Liquids and Solids Purcell, Torrey, Pound (Harvard) Bloch, Hansen, Packard (CalTech)	The results were met with disbelief
1952 - First commercial NMR spectrometer	It was not until several structures solved initially using NMR were solved again using crystallography that the NMR technique was accepted
1962 - First Superconducting Magnet for NMR	In 2002, Wüthrich was rewarded with the Nobel Prize in Chemistry
1968 - First Pulse Fourier Transform NMR	
1969 - First Concept of MRI Scanners	
1971 - First 2D NMR Experiment - COSY (Jean Jeener)	
1985 - Protein Structures	
2009 - First Gigahertz NMR Spectrometer	

25

As a technique developed in 1938, NMR in molecular beams by Rabi from Columbia University, who first have taken the NMR setup and NMR signal in 1946 NMR of liquid and solids are performed by Purcell, Torrey, Pound from Harvard University, Bloch, Hansen and Packard, from Caltech University. In 1952, the first commercial NMR spectrometer is developed. In 1962, the first superconducting magnet for NMR is created or designed.

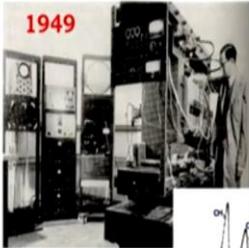
In 1968, first Pulsed Fourier Transform NMR which again revolutionize the process how NMR works. In 1969 the first concept of MRI scanner which is now playing a very important role in medical science, in 1971 first 2D NMR experiment, COSY by Jean Jeener. In 1985, the first protein structure was solved. In 1985 as I told, Michael Williamson, Havel and Wuthrich reported the first solution state protein structure which is a proteinase inhibitor 2A from bull seminal plasma.

Initially they were met with a lot of disbelief, people who are not agreed to what their finding was and it was not until several structures solved initially using NMR were actually solved again using crystallography. And when they found that the structure, the assault by NMR is having the similar information when solved by X ray crystallography then NMR was actually accepted. And in 2002 Wuthrich was rewarded with a Nobel Prize in Chemistry because of his contribution in solving protein structure. In 2009, the first gigahertz NMR spectrometer is developed.

(Refer Slide Time: 32:03)

Journey of NMR Spectrometer Instrument:

1949



The very first NMR hardware set up

This was happened in Noble Laureate Felix Bloch's lab in Stanford providing the first ever characteristic signal



One of the first commercial NMR spectrometer equipped by electromagnet

1964



Trub-Tauber KIS-1




26

So, coming to the journey of NMR spectrometer instrument, in 1949 the first machine was developed, the very first NMR hardware setup. This was happened in Nobel Laureate Felix Bloch's lab in Stanford providing the first ever characteristic signals. If you see they have taken ethanol and they get 3 signals, 3 peaks, we will talk about that later. In 1964, Trub Tauber KIS 1 the first commercial NMR spectrometer which was equipped by electromagnet was arrived and you see that how much change in the sophistication of the data collection.

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Journey of NMR Spectrometer Instrument:

1970

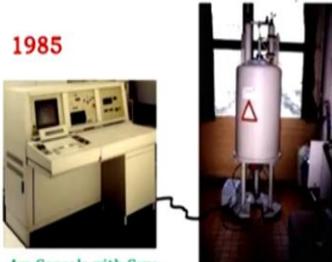
HFX-90



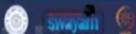
A bit better version of the first commercial NMR spectrometer equipped by electromagnet

NMR spectrometer with a cryo-magnet

1985



Am Console with Cryo-Magnet


27

Then in 1970 HFX 90 came. This is a bit better version of the first commercial NMR spectrometer equipped by electromagnet. So, then in 1980 Am Console with Cryo Magnet, the first Cryo Magnet developed, so this is a NMR spectrometer with the cryo magnet.

(Refer Slide Time: 33:33)

Journey of NMR Spectrometer Instrument:

2000



A current routine, everything is automated

Research laboratories are equipped by several huge spectrometers with very high intensity of magnetic fields

The present NMR Spectrometer

2002

AVANCE 750 WB

17.6 T;
Supercooled He - 1,0K
Boiling temperature 4,2K (Joule-Thompson)



28

In the year 2000 there was like fully commercialized and fully automated NMR instrument. So, you could only do a current routine everything is automated. And in 2002, you see the present NMR spectrometer which is currently used. The research laboratories are equipped by several huge spectrometers with very high intensity of magnetic fields.

(Refer Slide Time: 34:05)

Components of NMR spectrometer:

- Magnet:** Superconducting magnet, shim coils, liquid helium and nitrogen container, anti-vibration legs
- Probe:** RF coil to pulse and receive signals, gradients, temperature probes, lock coil, pre-amplifiers
- Console:** Electronics for generating RF pulses, power and gradient amplifiers, lock system, temperature control etc
- Computer:** Data storage, processing and analysis, communication with console

29

So, the components of NMR spectrometer, the first one which is very important is magnet superconducting magnets, shim coils, liquid helium and nitrogen container, anti vibration legs. Vibration is extremely critical. So, when the NMR instruments set they have anti vibration legs which absorb the vibration and not let the instrument getting the vibration. Probe, RF coil to pulse and receive signals, gradients, temperature probes, lock coil, pre amplifiers.

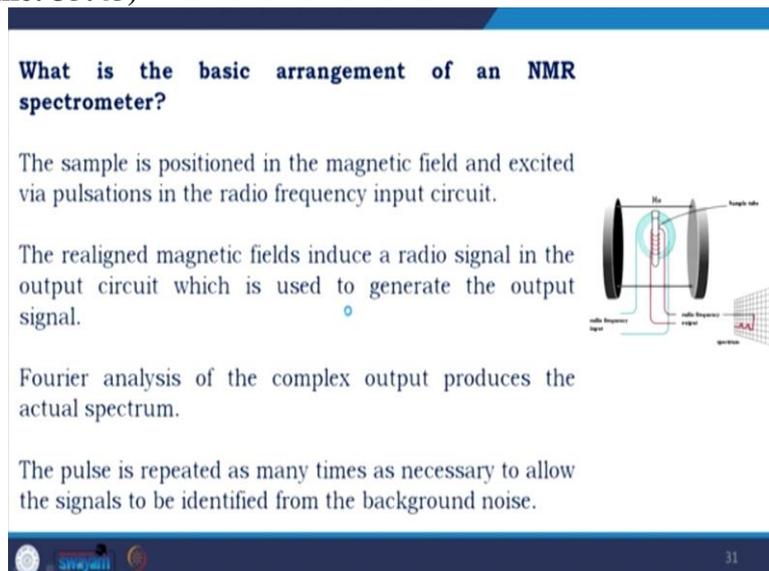
Console, the electronics for generating RF the radio frequency pulses, power and gradient amplifiers, lock system, temperature control etcetera in the console, computer for data storage, processing and analysis, communication with console. So, these are the,

(Refer Slide Time: 35:07)



Major components, now if you see, this is the pictorial demonstration of major components. This is the superconducting magnet generates a powerful magnetic field that is tens of thousands of times stronger than the Earth magnetic field as I told, samples are placed within this magnetic field and exposed to radio waves. Spectrometer transmits and receives the radio frequency waves used to make the NMR measurement. The computer is the instrument control and data processing.

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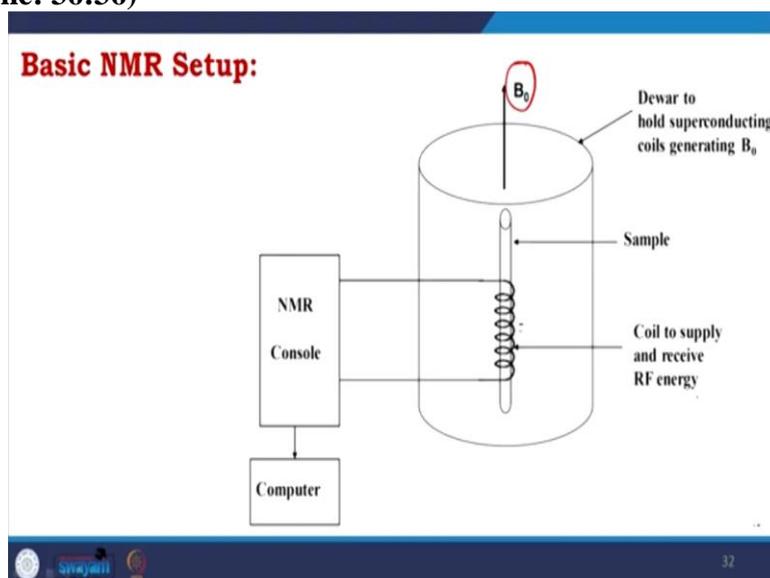


So, what is the basic arrangement of an NMR spectrometer? The sample is positioned in the magnetic field and excited via pulsations in the radio frequency input circuit. The realigned

magnetic fields induce a radio signal in the output circuit which is used to generate the output signal. Fourier analysis of the complex output produces the actual spectrum which we are going to talk about.

The pulse is repeated as many times as necessary to allow the signals to be identified from the background noise. You use more pulses and your signal would be enhanced and your signal by noise ratio would be improved that is good for getting better data.

(Refer Slide Time: 36:36)



So, this is again the basic setup this is the external magnetic field, this is a Dewar to hold the superconducting coils generating the external magnetic field, here the sample is put and here the coil to supply and receive the radio frequency energy, the radio frequency energy comes from the NMR console and the data acquired, it generate the radio frequency energy and it get the data and send it to the computer.

(Refer Slide Time: 37:11)

Superconducting Magnet:

A **superconducting magnet** is an electromagnet made from coils of superconducting wire

They must be cooled to cryogenic temperatures during operation

In its superconducting state the wire has no electrical resistance and therefore can conduct much larger electric currents than ordinary wire, creating intense magnetic fields

Superconducting magnets can produce greater magnetic fields than all but the strongest non-superconducting electromagnets and can be cheaper to operate because no energy is dissipated as heat in the windings.



So, we are talking about superconducting magnets. What is superconducting magnets? The superconducting magnets are electromagnets made from coils of superconducting wire. They must be cooled to cryogenic temperatures during the operation. In its superconducting states that wire has no electrical resistance and therefore can conduct much larger electric currents than ordinary wire creating intense magnetic fields.

Superconducting magnets can produce greater magnetic fields than all but the strongest non superconducting electromagnets and can be cheaper to operate because no energy is dissipated as heat in the windings. So, if you see this is the superconducting magnet, if you go inside you will see that there are superconducting wires and this is the super fine high resolution demonstration.

(Refer Slide Time: 38:20)

The NMR magnet:

The superconducting coils are made of niobium-titanium or niobium-tin alloy (for high field strengths)

These wires are wound miles long with uniform diameter throughout

The magnet is rested on vibration free legs to avoid vibrations from the ground to be transferred to the magnet

The higher the field strength, higher is the sensitivity of NMR

The higher the field strength, higher is the resolution (The resolution varies as B_0)

The magnetic field is not constant and slowly *drifts* with time. Typical drift rate is 8-10 Hz per hour

Protein NMR

The superconducting coils are made up neobium titanium or neobium titanium alloy for high field strength. These wires are wound miles long with uniform diameter throughout. The magnet is rested on vibration free legs, as I talked about to avoid vibration from the ground to be transferred to the magnet. The higher the field strength, higher is the sensitivity of the NMR. The higher the field strength, higher is the resolution.

So, when you increase the field strength, you increase the resolution, about these we will discuss specially, when we are going to discuss protein NMR. The magnetic field is not constant and slowly drifts with time. Typical drift rate is 8 to 10 hertz per hour. So, this you have to include in your experimental consideration.

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Console:

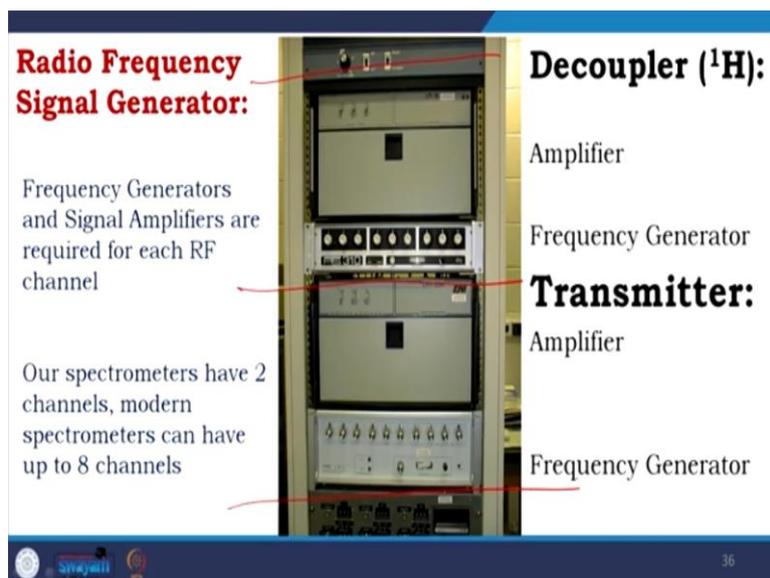
The purpose of the console is to house the various spectrometer units in a compact arrangement, as well as, providing shielding from interference such as electro-magnetic radiation from external sources



35

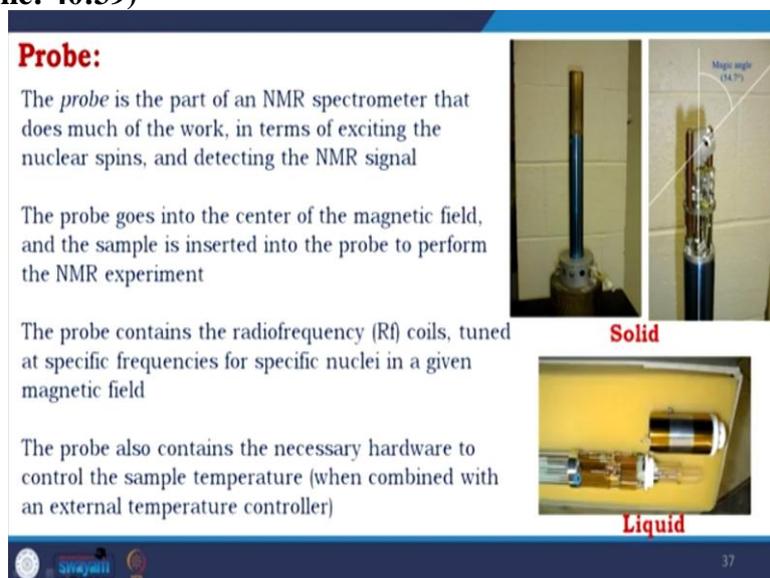
Console, so the purpose of the console is to house the various spectrometer units in a compact arrangement as well as providing shielding from interference such as electromagnetic radiation from external sources. This is a console with a computer.

(Refer Slide Time: 39:57)



Radio frequency signal generator, if you see, here you will see 2 parts decoupler and transmitter both of them have amplifier as well as frequency generator. So, frequency generators and signal amplifiers are required for each radio frequency channels. Here we have shown one where it have 2 channels, this is one channel and this is the other one. But the modern spectrometers can have up to eight channels.

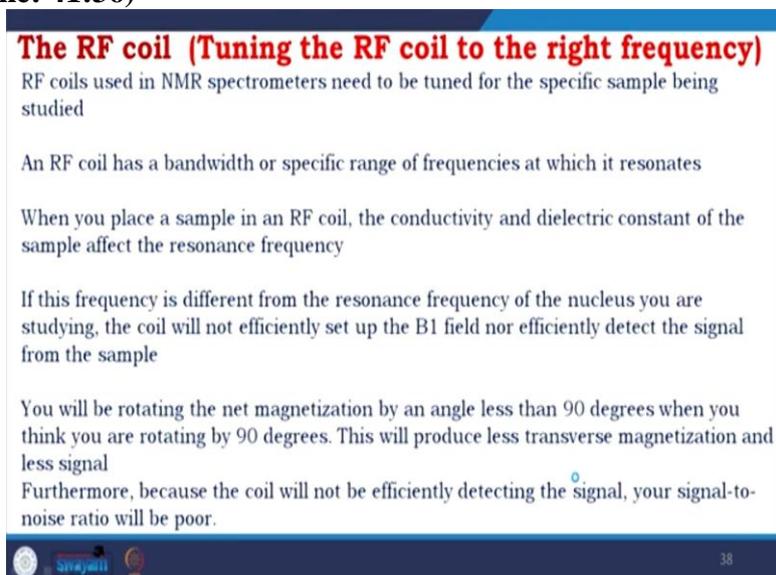
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Probe, the probe is the part of an NMR spectrometer that does much of the work in terms of exciting the nuclear spins and detecting the NMR signals. The probe goes into the center of the magnetic field and the sample is inserted into the probe to perform the NMR experiment. The probe contains the radio frequency coils tuned at specific frequencies for specific nuclei in a given magnetic field. The probe also contains the necessary hardware to control the

sample temperature when combined with an external temperature controller. So, these are probes which are used for solid state and these are probe which used for liquid.

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The RF coil (Tuning the RF coil to the right frequency)
RF coils used in NMR spectrometers need to be tuned for the specific sample being studied

An RF coil has a bandwidth or specific range of frequencies at which it resonates

When you place a sample in an RF coil, the conductivity and dielectric constant of the sample affect the resonance frequency

If this frequency is different from the resonance frequency of the nucleus you are studying, the coil will not efficiently set up the B1 field nor efficiently detect the signal from the sample

You will be rotating the net magnetization by an angle less than 90 degrees when you think you are rotating by 90 degrees. This will produce less transverse magnetization and less signal

Furthermore, because the coil will not be efficiently detecting the signal, your signal-to-noise ratio will be poor.

38

The RF coil which is necessary, tuning the RF coil to the right frequency. The RF or radio frequency coils used in NMR spectrometers need to be tuned for the specific sample being studied and RF coil has a bandwidth or specific range of frequencies at which it resonate where the resonance happened. When you place a sample in a radio frequency coil the conductivity and dielectric constant of the sample affect the resonance frequency.

If this frequency is different from resonance frequency of the nucleus you are studying, the coil will not efficiently set up the B 1 field nor efficiently detect the signal from the sample, so the matching is very important. You will be rotating the net magnetization by an angle less than 90 degrees, when you think you are rotating by 90 degrees. This will produce less transverse magnetization and definitely less signal.

Furthermore, because the coil will not be efficiently detecting the signal, your signal to noise ratio will be poor. So that is why it is very important to tuning the radio frequency coil to the right frequency.

(Refer Slide Time: 43:06)

Different NMR System:



This is a collection I have made to show you that there are different NMR systems, depending on the magnetic power which we are already talked about. All of them are Bruker system, where you see 300, 400, 500, 600, 800, 900, 1.2 gigahertz which is the most powerful one up to now is present, there are in between but these is how a system look like. So, I have talked about the basic concept of magnetism, the basic concept of resonance and the hardwares required for doing the NMR experiments.

I will end up this class by talking about some pupil with their landmark contribution to the field of NMR. I talked about history before. Here I take pupils who are contributing in NMR and because of their contributions they are awarded with different prizes. One the Nobel Prize winners, you all know about Nobel Prize so I am not going to define that.

(Refer Slide Time: 44:27)

People with landmark contribution to the field of NMR:

Noble Prize winners

The **Varian Young Investigator Award** at ENC is intended to recognize a single investigator, for his/her achievements in any area of magnetic resonance. The **award** is to be granted to people in the first 12 years after their Ph. D

The Günther **Laukien Prize** is a **prize** "to recognize recent cutting-edge experimental NMR research with a high probability of enabling beneficial new applications". The **prize** was established in 1999 in memoriam to Günther **Laukien**, who was a pioneer in NMR research

I will also take the names of Varian Young Investigator awardees who actually got this award for their achievement in any area of magnetic resonance, their contribution. The award is to be granted to pupil in the first 12 years after their PhD. So, they are mostly young investigators. And the Laukien prize on the name of Gunther Laukien which is to recognize recent cutting edge experimental NMR research with a high probability of enabling beneficial new application. This prize was established in 1999 in memoriam of Gunther Laukien, who was a pioneer in NMR research. I will talk about Nobel Prize winners.

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Noble Prize for contribution in in NMR Spectroscopy:

Otto Stern, USA (Year 1943): For the contribution to the development of molecular ray method and his discovery of the magnetic moment of the proton

Isidor I. Rabi, USA (Year 1944): For resonance method for recording the magnetic properties of atomic nuclei

Felix Bloch, USA and Edward M. Purcell, USA (Year 1952): For their discovery of new methods for nmr precision measurements and discoveries in connection therewith

Richard R. Ernst, Switzerland (Year 1991): For the contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy

41

First, so Otto Stern, from US in the year 1943 for the contribution to the development of molecular ray method and his discovery of the magnetic moment of the proton. Isidor I Rabi, I already talked about Rabi, because he got the first signal. So, he is from US, he got Nobel Prize in the year 1944 for resonance method for recording the magnetic properties of atomic nuclei, this is Isidor Rabi. Felix Bloch, we again talked about Felix Bloch in the history part and Edward Purcell.

They got Nobel Prize in the year 1952 for their discovery of new methods for NMR precision measurements and discoveries in connection therewith, so these are Bloch and Purcell, Richard R Ernst from Switzerland in the year 1991 for the contributions to the development of the methodology of high resolution nuclear magnetic resonance spectroscopy.

(Refer Slide Time: 46:56)

Noble Prize for contribution in in NMR Spectroscopy:

Kurt Wüthrich, Switzerland (Year 2002): For the development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution



Paul C. Lauterbur, USA and Peter Mansfield, United Kingdom (Year 2002): For their discoveries concerning magnetic resonance imaging



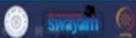

42

Kurt Wuthrich, from Switzerland, I already talked about his contribution for the development of nuclear magnetic resonance spectroscopy for determining the 3 dimensional structure of biological macromolecules in solution, as I talked earlier, he is the first one or in the group of pupils who have first solved the structure of protein using NMR technique, this is Kurt Wuthrich and Paul Lauterbur, from US and Peter Mansfield, from United Kingdom at the year 2002 for the discoveries concerning magnetic resonance imaging or MRI. So, these are the 2 pupils.

(Refer Slide Time: 47:48)

Varian Prize Winners:

2012 Ray Freeman and Weston A. Anderson	Nuclear Magnetic Double Resonance
2011 Gareth Alun Morris, The University of Manchester, UK	INEPT
2010 Martin Karplus, Harvard University, Cambridge, Massachusetts	Karplus equations
2009 Albert W. Overhauser, Purdue University, West Lafayette, IN	NOE & Dynamic Polarization
2008 Alexander Pines, UC Berkeley, and Lawrence Berkeley National Laboratory	Cross Polarization
2007 Alfred G. Redfield, Brandeis University, Waltham, Massachusetts	Spin Dynamics
2006 John S. Waugh, MIT, Cambridge, Massachusetts	Average Hamiltonian Theory (AHT)
2005 Nicolaas Bloembergen, University of Arizona, Tucson, Arizona	Nuclear Magnetic Relaxation
2004 Erwin L. Hahn, Professor Emeritus, University of California, Berkeley	Spin Echoes
2002 Jean Jeener, Universite Libre de Bruxelles, Belgium	Two-dimensional NMR



43

So, this is the list of Varian Prize winners, in 2002 Jean Jeener for 2 dimensional NMR, in 2004, Edwin L. Hahn, professor from university California, Berkeley for spin echoes, in 2005, Nikolas Bloomberg in University of Arizona for Nuclear Magnetic relaxation. In 2006 John Waugh from MIT for average Hamiltonian theory or AHT, 2007 Alfred Redfield from

Brandeis University for spin dynamics, 2008 Alexander Pines from UC Berkeley and Lawrence Berkeley National Laboratory for cross polarization.

Albert Overhauser from Purdue University for NOE and dynamic polarization, Martin Karplus from Harvard University for Karplus equation, Gareth Alun Morris, the University of Manchester for INEPT, Ray Freeman in 2012 and Weston Anderson for nuclear magnetic double resonance. So, for time constraints,

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Laukien Prize Winners:

- 1999 Konstantin Pervushin, Roland Riek, Gerhard Wider, and Kurt Wüthrich; TROSY
- 2000 Lucio Frydman; Quadrupolar MQMAS
- 2001 Peter Boesiger, Klaas Prüssmann, Markus Weiger; Sensitivity-encoded magnetic resonance imaging
- 2002 Ad Bax, Aksel Bothner-By and James Prestegard; Residual dipolar couplings of weakly aligned molecules in solution
- 2003 Jacob Schaefer; REDOR Technique for Solid State NMR
- 2004 Lewis E. Kay; NMR of Biological Macromolecules
- 2005 Stephan Grzesiek; J couplings across hydrogen bonds
- 2006 Thomas Szymanski, Eriks Kupce, Ray Freeman, and Rafael Bruschweiler; Acceleration of Multi-dimensional NMR by novel procedures for scanning data space and efficiently processing results to obtain a conventional spectral representation
- 2007 Robert G. Griffin; High-field dynamic nuclear polarization (DNP) for sensitivity enhancement in solid-state MAS NMR
- 2008 Malcom H. Levitt; Optimized pulses and pulse sequences to enhance the power of liquid & solid state NMR
- 2009 Daniel P. Weitekamp; PASADENA and BOOMERANG significantly improve NMR force detection by circumventing the problems of inhomogeneous magnetic fields
- 2010 Paul T. Callahan; Contributions to the study of polymeric and heterogeneous materials by advanced NMR exchange, diffusion and relaxation techniques, and for his innovative q-space-diffusion-related developments that were relevant in the context of the development of diffusion-tensor imaging
- 2011 Daniel Rugar, John Mamin, and John Sidles; Magnetic Resonance Force Microscopy (MRFM)
- 2012 Klaes Golman and Jan Henrik Ardenkjær-Larsen; Dissolution-DNP NMR

44

There is a huge list. I am not going to the Laukien Prize winners, I am keeping it for you guys to take a look.

(Refer Slide Time: 49:31)

Jokes from Experimental Nuclear Magnetic Resonance Conference:

Reproduced through the courtesy of Dr. Sue Hilder, Department of Chemistry, University of South, Canada.

NMR practical jokes.

"Why, 'spine'! You've inverted the wheel!"

Eric is alive and well, working as an NMR Spectroscopist in Cleveland.

And, through a bizarre set of circumstances involving the choice of location for IUC 1991, the city of St. Louis inadvertently became known for its tall food.

So, I am finishing this with a collection of cartoons from different NMR aspects which you could go through and enjoy. With that I am finishing this class. Thank you very much for listening.