An Introduction to Nuclear Magnetic Resonance Theory

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Black-and-white
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<Opening titles>

<Garlick to camera>

For a complete description of nuclear magnetic resonance, one requires a quantum mechanical approach. This, however, is often not helpful to the non-specialist. Using a classical approach, that is using classical physics, one can explain most of the theory of NMR although occasionally one must take quantum mechanic results on trust. This programme will take a classical approach wherever possible.

All nuclei have positive charge.

<Garlick narrates over animated diagrams>

Some also spin about their axes; that is they have angular momentum. Nuclei with spin of interest to biochemists are the proton, 13-carbon, and 31-phosphorus.

Possession of charge and spin gives a magnetic moment to these nuclei. This means they behave somewhat like tiny bar magnets. The magnetic moment, μ, has a direction, that is, it is a vector quantity and it lies along the axis of spinning. A collection of protons in water, say, would look like this.

If one now applies a large magnetic field, B_0, in the vertical direction, there are two consequences for the spinning nuclei. One, the magnetic moments will precess, that is, describe a cone about the B_0 field, rather like a gyroscope in the earth's gravitational field. And two, quantum mechanics dictates that there will only be certain orientations that will be allowed. For nuclei like protons and phosphorus, there are two options: with the field or against it. These two states have different energies. A lower energy is alignment with the field.

At thermal equilibrium there are more nuclei in the lower energy state, albeit an excess of only 1 in 10^4, and there is no preferred orientation around the cone.

<Garlick narrates over formula>

<formula>

$$\mu_0 = \gamma/2\pi \; B_0 (1- \sigma)$$

σ is always much less than 1

The frequency of precession, μ_0, that is the rate in cycles per second at which the magnetic moments are rotating, depends on three factors: one, which nucleus you're dealing with; two, what strength the magnetic field is; three, the electronic environment around the nucleus, that is whether, for example, the phosphorus nucleus is in a molecule of phosphocreatine or inorganic phosphate. Sigma is always much less than one.

<Garlick to camera>

So, this is why one has widely different precessional frequencies for different nuclei, but only slightly different frequencies for the same nuclei in different molecules.

<Garlick narrates over series of still and animated images, interspersed with talk to camera>

If one could think of an NMR motorway, where the positions of the towns indicate the precessional frequencies of the nuclei and the size of the towns indicates the total frequency range for the nucleus in question, such a motorway would be something like this.

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NMR1

Carbon	**45MH$_z$**
Phosphorus	**73MH$_z$**
Protons	**180MH$_z$**

Using a magnet of fuel strength 4.2 Tesla, if London were to represent 13-carbon at 45 megahertz, one would have to travel to Birmingham to reach the phosphorus frequency, and all the way up to Inverness to reach the frequency of protons. These precessional frequencies are what we want to measure. They tell us which groups of molecules are present in the sample.

<*To camera*> How do we measure them? <*Narration over images*> First, we set up the electronics so that we're dealing with the frequency range appropriate for the nucleus we wish to observe. What next? The old method of continuous wave NMR is very easy to understand. Going back to the motorway analogy, if one drives slowly through the different frequencies, energy is absorbed when the frequency of the applied radiation equals the precessional frequency.

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$h\mu = \Delta E$

This is, in fact, the point at which the energy of the applied radiation, hμ, equals the energy difference, ΔE, between the two allowed states. One therefore obtains an absorption spectrum, and in this the areas of the individual peaks correspond to the number of nuclei precessing at that particular frequency.

Let's look at an example. A sample contains two molecules, A and B, at high concentration with three times as much A as B. The precession frequency of A is μ_0 and of B is μ_1, slightly slower. After sweeping through the frequencies once, taking a minute or so, the spectrum looks like this. If the sample were much lower in concentration, one sweep through the frequencies would produce a spectrum where the peaks were virtually undetectable. Although one can repeat the sweeps many times and add the results together to achieve a reasonable spectrum, this is a very time consuming process.

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The method of continuous wave NMR was too slow, therefore, for biochemists with samples in the millimolar concentration range. They had to wait until the advent of Fourier transform NMR. This is a much faster method because all the frequencies are applied simultaneously with a very short pulse of radiation which lasts for tens of microseconds. Unfortunately, it's slightly more difficult to understand how one obtains a spectrum.

<*To camera*> In this case, one must not think of the individual spins but the resultant bulk magnetisation. <*Narration over images*> Let us consider just the A molecules in our original example. Where's the bulk magnetisation for these in a field, B_0? We've seen that the magnetic moments of each of the molecules are precessing around B_0 with frequency μ_0, but the resultant magnetisation, M_a, is static, and is in the B_0 direction because: A, there is a slight excess of moments with the field and, B, there is no preferred orientation of the moments around the cone. Let's put some axes on for working with.

Remember, we're still trying to find the frequency of precession and in our example this is μ_0. By convention, the signal is detected along the y-axis. There is no component of M_a in this direction though. To detect a signal, one must therefore tip M_a towards the y-axis. In classical terms, it's difficult to consider this situation because of the large B_0 field. One can, however, use an imaginary device to remove the B_0 field. It is called the rotating frame. If one imagines the set of axes to be rotating about z at a frequency μ_0, the individual magnetic moments will appear to freeze in their positions around the cone. This is exactly analogous to getting onto a revolving children's roundabout in a playground and finding that it now appears stationary.

Since the original precessional motion of the nuclear moments was due to the B_0 field, apparent cessation of that motion, due to the rotating frame, implies an effective magnetic field of zero. The co-ordinates for this rotating frame are given by x prime, y prime and z prime, the same as Z.

What happens if a static magnetic field, B_1, is now applied along the x prime axis in the rotating frame? It will cause the bulk magnetisation to precess in the z prime y prime plane. If the field is removed after a very short time, the magnetisation will still be in the z prime y prime quadrant. We have thus achieved what we wanted to, that is we have tipped the magnetisation towards the y prime axis. When we consider the nature of this B_1 field, we find that it is a pulse of radiofrequency radiation whose frequency is the same as that of the rotating frame, that is μ_0.

If the path lasts for a length of time such that it induces the magnetisation to precess until it lies along the y prime axis, it's called a 90 degree pulse. Now, when a 90 degree pulse is turned off, what happens? The bulk magnetisation returns to its thermal equilibrium position in a magnetic field by relaxation processes. <*To camera*> The process whereby magnetisation is re-established in the z direction is called longitudinal, or spin-lattice, relaxation. <*Narration over images*> The process responsible for the decay to zero of magnetisation in the x-y plane is called

transverse, or spin-spin, relaxation. Both processes are exponential and they are characterised by the time constants T_1 and T_2 respectively. So, how do we detect the signal and what does it look like? Well, we detect it in the rotating frame by subtracting a signal of frequency μ_0 from the signal obtained. Thus in our example, since the precession rate of A is μ_0, the detected signal for A will just be a decaying exponential. If, however, A were precessing at a frequency of μ_0 plus $\Delta\mu_0$, the signal would be a sinusoidal decaying exponential with a frequency $\Delta\mu_0$, that is the difference between its precessional frequency and that of the rotating frame. One could actually measure this frequency from the signal itself. This signal is called a free induction decay, or FID. The amplitude at the beginning of it depends on the number of sampled nuclei, that is, the concentration, while that at the end is just noise.

That was just a theoretical example. What happens in practice, say, with a biological sample like an isolated heart? They'll be two main differences. Firstly, the detected signal from one pulse will consist almost entirely of noise. This is because the concentrations of the molecules of interest will be very low, about 1 to 20 millimolar. One can overcome this problem, however, by adding together the signals from repeated pulses. One must not repeat the pulses too quickly after each other though or the size of the spectral peak will decrease. This is because the T_1 processes have not had time to re-establish the magnetisation in the z direction.

The second difference with biological samples is that the FIDs will look very complicated. <*To camera*> The information that we're looking for, that is the individual precessional frequencies, is still contained in them, but we need some help in unravelling it. The mathematical process of Fourier transformation is used for this and the required information is then displayed in the more conventional form of an absorption spectrum.

<*Narration over images*> If we now Fourier transform the FIDs from 1 pulse and 40 pulses, we can see the dramatic improvement in the visibility of the individual peaks. Looking at the 40 pulse spectrum, what have we achieved? Well, we've now

acquired the information that we set out to obtain in the beginning, that is the individual precessional frequencies for the phosphorus nuclei present in the sample. We can assign these frequencies to particular chemical groups or molecules.

<*To camera*> Having achieved this assignment, we can then proceed to the different experimental protocols, some of them simple, some of them highly sophisticated, which will enable us to investigate cardiac metabolism using this spectroscopic method.

<End credits>