Seven-Fold Dynamical Symmetry in Solid-State Nuclear Magnetic Resonance

Malcolm H. Levitt

Physical Chemistry Division,

University of Stockholm, S-10691, Sweden

Phone: +46-8-16 23 73

Fax: +46-8-15 21 87

email: mhl@physc.su.se

Introduction

Nuclear magnetic resonance is a very useful physical effect discovered independently by two groups of American scientists in the 1940's. These researchers discovered that if ordinary matter is placed in a strong magnetic field, radio waves with well defined frequencies, like radio stations, are absorbed or emitted. One group first detected this effect in a large block of wax; another used ordinary tap water. The radio emissions are due to the atomic nuclei that sit at the heart of every atom. Some of these atomic nuclei are magnetic, and it is the rotatory motion ("precession") of these microscopic magnets that cause the detectable radio signals [1].

This effect was revolutionary because until then, heavy apparatus and high energies were thought to be necessary for investigating atomic nuclei. The NMR phenomenon showed that a fairly strong magnet and some army surplus radar components were sufficient for listening to the atomic nuclei in ordinary material such as water or wax. Purcell made the following beautiful remark in his 1952 Nobel lecture: "Commonplace as such experiments have become in our laboratories, I have not yet lost that sense of wonder, and of delight, that this delicate motion should reside in all ordinary things around us, revealing itself only to him who looks for it".

Since then, NMR has gone through an extraordinary expansion and is now the broadest and most versatile physical method for examining the structure and dynamics of matter. It is becoming familiar to non-scientists as the MRI (magnetic resonance imaging) method for revealing anatomic structure, where it is now used routinely in hospitals (medical practitioners have dropped the term "nuclear", which scares some patients). In scientific laboratories, it is used routinely by biologists, chemists and physicists for revealing the motions and three-dimensional structure of molecules, amongst many other applications. R. R. Ernst was awarded the Nobel prize in 1991 for some of the methodological developments that have turned NMR into such a powerful and ubiquitous experimental tool in contemporary science [2].

One remarkable feature of NMR is that even after 55 years of intense development, the methodology of the subject is still not stable. The field still contains a wealth of possibilities and displays a seemingly unlimited potential for new concepts and new sorts of experiments. In this article, I will sketch out one such new line of investigation, in which symmetry plays a prominent role.

Solid-state NMR and magic-angle spinning

Most nuclear magnetic resonance experiments are performed on liquid samples, or materials containing a lot of liquid, such as the human body. It turns out, for technical reasons that I will not go into, that the NMR signals from liquids tend to be strong and easy to detect.

Nevertheless, NMR is also possible in solid samples, although the experiments tend to be technically more difficult. The NMR signals of solids are usually weak and "broad", meaning that they cover a wide range of frequencies. The broadness of solid-state NMR spectra creates a number of technical problems, and makes it more difficult to interpret the signals that one receives, in terms of information about the molecular structure.

However, in 1959, a breakthrough was made by the group of R. Andrew at the University of Bangor in Wales. This group discovered that it is possible to make the NMR signals of solids more "narrow" by rotating the sample rapidly about a well-defined axis. The narrowing effect is dramatic when the sample is rotated about an axis that subtends an angle of around 54.7° with respect to the applied magnetic field [3]. This angle solves the equation $3\cos^2\theta - 1 = 0$ and is known in NMR as the *magic angle*. The magic angle may be visualized with the help of a piece of A4 paper (Fig.1a).

{Insert Fig.1 near here}

The *magic-angle spinning* method in solid-state NMR is illustrated in Fig.1b. The sample is packed into a small ceramic cylinder, called a rotor, which is span rapidly in a stream of air at around one million revolutions per minute about the magic angle axis. For reasons that I will not go into here, rapid magic-angle spinning (MAS) makes the NMR signals from solids both stronger and more informative. It is an ubiquitous method in modern solid-state NMR.

Recoupling

The NMR phenomenon relies on the fact that certain atomic nuclei behave as tiny magnets, which rotate in the applied magnetic field, and produce radio waves. In addition, these tiny magnets interact with each other, just like two compasses placed side by side. This effect is called the magnetic internuclear coupling.

The internuclear couplings are small but very informative. This is because the couplings depend in a very simple way on the distance between the atomic nuclei. An internuclear coupling is inversely proportional to the *cube* of the distance between the interacting nuclei. By measuring the small internuclear couplings, one may therefore measure the distances between atoms, and hence deduce a lot about the geometrical shape of a molecule. Scientists use this method to find out about the geometry of molecules, especially biological molecules such as proteins and nucleic acids [2].

This approach is now rather well-established for the NMR of *liquids*. Solid-state NMR, on the other hand, was long faced with serious obstacles in this regard. As mentioned above, it is often necessary NMR to rotate the sample rapidly at the magic angle, in order to get strong enough radio signals. Unfortunately, there is a catch. The magic angle rotation also has the effect of *decoupling* the nuclear spins. Effectively, the couplings between neighbouring nuclei get washed out by the sample rotation. Solid-state spectroscopists were therefore forced to choose between informative weak signals, and non-informative strong signals. This unfortunate situation ruled out many potential applications of solid-state NMR.

The situation changed at the end of the 1980's when several solid-state NMR groups discovered that the internuclear couplings could be reactivated by applying carefully chosen magnetic fields to the sample at the same time as the magic-angle rotation. This is called *recoupling* [4, 5, 6]. The physical idea is expressed in Fig.1b. Oscillating magnetic fields (actually, radio waves) are applied to the object at the same time as it is rotated. One can imagine that in some sense, the rotation of the applied fields cancels out the rotation of the sample.

Recoupling allows solid-state NMR spectroscopists to "turn on" the internuclear couplings at will. The magnetic recoupling fields may be turned off in order to detect strong NMR signals, and turned on again in order to access molecular-level information. This proves to be very useful, and recoupling is now used extensively in the application of solid-state NMR to biologically-important samples, and many other areas.

Recoupling contains two elements: Magic-angle spinning of the sample, and the application of additional magnetic fields. The question arises: How should one *synchronize* these two elements? The recoupling fields may be controlled with great precision and almost complete freedom. But how should this freedom be best exploited?

This is called the *recoupling design problem* in solid-state NMR.

Before examining how symmetry helps us address this problem, I would like to discuss two more general aspects of nuclear magnets in solids.

Symmetry and selection rules

Nuclear magnetic resonance is a form of *spectroscopy*, which is the branch of science concerned with the interaction of light particles (photons) and matter. In optical spectroscopy, the photons are visible; In NMR, on the other hand, the photons have a very low frequency and cannot be detected by the eye. The photons in NMR are detected instead by a radio receiver.

In university courses on spectroscopy, one learns about a fundamental link between the *symmetry* of the physical system and the spectroscopic *selection rules* [7]. One classic case is when an atom is embedded in a symmetrical environment, such as a crystal. Quantum mechanics predicts that the atom has a set of quantum mechanical *energy levels*. When light is absorbed, the atom makes a transition between these energy levels. In many cases, certain transitions are *forbidden* and do not occur, while certain other transitions are *allowed*. A very elegant body of theory predicts the *selection rules* for forbidden and allowed transitions on the basis of the symmetry of the atomic environment. These rules have been confirmed by innumerable experimental tests. Their prediction using symmetry arguments is one of the triumphs of quantum mechanics [7, 8].

One aspect of the spectroscopic selection rules is so self-evident that it is hardly ever stated explicitly: The symmetry of the problem is defined by the molecular environment, not by the incident light. This is because the interaction of one atom with its immediate neighbours is under ordinary circumstances far larger than the interaction of that atom with the incident light. The incident light probes the environment of the atom in a mild way, but does not change the essential symmetry of the system.

NMR is a very unusual form of spectroscopy. In particular, the magnetic interactions between the nuclei and the experimental apparatus are often much *larger* than the magnetic interactions of the nuclei with the molecular environment. As a result, the selection rules in NMR are often linked to the symmetry of the fields applied by the *apparatus* rather than to the symmetry of the immediate molecular environment. In other words, it is technically possible to generate selection rules in NMR, just by changing the symmetries of the magnetic fields applied to the sample, which are under complete experimental control.

This is a unique situation in NMR and creates a wealth of possibilities for experimental design.

Correlated nuclear magnets

{Insert Fig.2 near here}

As mentioned above, NMR is based on the fact that many atomic nuclei behave as tiny magnets. If the object is placed in a magnetic field, the nuclear magnets become slightly *polarized*. This means that the nuclear magnets tend to line up slightly along the external magnetic field, like compass needles. This is depicted in exaggerated form in Fig.2a. In reality, this "lining-up tendency" is almost completely overcome by the randomizing effect of heat, so that in reality there are only about 10 magnets out of one million that are aligned with the field, rather than being totally randomized.

Now consider Fig.2b. This shows, in exaggerated form, a different type of nuclear state. In this case there is no net tendency for any *individual* nuclear magnet to have any particular direction, but if one of the nuclear magnets in a molecule points in some direction, then its neighbour in the same molecule tends to point in the *same* direction. This is called a *correlated* state. Such states are very important in some of the modern developments of NMR.

One cannot create a correlated nuclear state by simply placing an object in a magnetic field. One must manipulate the nuclei from outside, and allow the nuclear magnets to interact with each other, in order to create a correlated nuclear state [2].

A pulse sequence with seven-fold symmetry

The C7 recoupling method [9] combines all of the elements sketched above: magic-angle spinning, recoupling, symmetry, selection rules, and correlated nuclear magnets.

The basic idea of C7 is to direct the motion of the nuclear spins in such a way that correlations between nuclear magnets develop naturally through their mutual couplings. The nuclear spins are encouraged to develop correlations by imposing carefully-chosen selection rules. These selection rules are generated by synchronizing the magic angle rotation of the sample with the applied recoupling fields in a seven-fold symmetrical way.

{Insert Fig.3 near here}

The dynamic symmetry of C7 is illustrated in Fig.3. The left part of Fig.3a depicts the magic-angle rotation of the sample in time, plotted as a helix. One may imagine this helix as the track traced out by a spot on the sample as it turns. The spot traces out a helix since the rotational motion goes on as a function of time, which is depicted in the figure as a linear motion in the third dimension.

The right part of Fig.3a depicts the dynamical symmetry of the recoupling fields that are applied by the experimental apparatus. These fields also trace out a helix, indicating that the direction of the recoupling fields (technically, their *phase*) also goes around a circular path as time proceeds. Fig.3a shows that these two helices have different pitch. The recoupling fields are timed so that they revolve twice for every complete rotation of the sample. The *winding numbers* of the two helices are therefore in a ratio of one to two.

Furthermore, the recoupling fields do not circulate continuously. They go round in jumps of 360°/7, so that they take seven complete steps in order to complete one whole revolution.

These are the basic elements of the C7 method: winding numbers for the sample rotation and the applied recoupling fields in a ratio of one to two, and seven steps for the revolution of the recoupling field.

Seven-fold symmetry and selection rules

The theory of C7 cannot be given here in any detail. However, I wish to give at least a flavour of how this method works.

The symmetry numbers, imposed by the apparatus, generate selection rules. These selection rules may be visualized in terms of a simple diagram [10]. One such diagram is shown in Fig.3b. One sees a set of levels, and lines leading between the levels. The levels may be thought of as possible states of the nuclear magnets, and the lines may be thought of as possible pathways which the nuclear states may follow.

The selection rule imposed by the experimental symmetry is represented by the wall with holes at regular intervals, on the right hand side of the diagram. Pathways which run into the wall are not permitted. Only the pathways that pass through holes in the wall contribute to the experimental outcome.

Fig.3b shows a symmetry diagram for C7. Note that the symmetry numbers 2, 1 and 7 are linked to the spacings between the levels on the diagram and the spacing between the holes in the barrier.

It may be seen that only one pathway passes through the holes in the barrier. This indicates that the evolution of the nuclear magnets is closely-controlled by the C7 experiment. Theory shows that this *symmetry-directed evolution* leads to a correlated nuclear magnetic state. The experimental seven-fold symmetry encourages the nuclear magnets to move from an uncorrelated state (Fig.2a) to a correlated state (Fig.2b).

These theoretical predictions have been verified in the laboratory [9,10,13,15,16-19].

Applications

What is all this good for?

One of the experiments under way in our laboratory in Stockholm is to do with the physical mechanism of *vision*, i.e. the fundamental physical processes that go on when light enters our eyes [11].

{Insert Fig.4 near here}

In the retina, at the back of each eye, are groups of specialized cells called *rods* and *cones* that have the function of catching light. We use the cones for colour vision in strong light and the rods for black-and-white vision in dim light. The rods and cones contain a protein called *rhodopsin* (Fig.4a). Each of these protein molecules is bound to a special molecule called *retinal*. This is the molecule that actually catches the light.

Retinal is a modified vitamin A molecule. The body makes this molecule from a number of sources, including a substance called carotene, which resembles two retinal molecules stuck together and which is responsible for the red colour of carrots. This is why eating carrots is good for our night vision.

Rhodopsin has been studied by hundreds of scientists for decades. It is known that the retinal molecule initially has a bent shape, but that when light hits the molecule, it straightens out [12]. The straightening-out of retinal acts like the release of a trigger, and causes the changes in the structure of the rhodopsin molecule. These changes initiate a complex sequence of events which end up in the activation of nerve cells in the retina at the back of the eye. The nerve impulse passes down the optic nerve into the brain, where the experience of seeing is eventually registered [11].

My group, in collaboration with others, is using solid-state NMR and the C7 experiment to investigate the first step in this process in more detail. We want to find out how the light energy is initially stored in the rhodopsin molecule, before the trigger is released and all the other things happen.

In order to do this, our collaborators in Holland help us obtain rhodopsin molecules in which two of the ordinary carbon nuclei in retinal have been replaced with magnetic carbon nuclei (these are called carbon-13 nuclei). We use the C7 method to construct a correlated magnetic state between these pairs of magnetic carbons. This correlated state allows us to detect the radio signals selectively from the retinal, at the heart of the large rhodopsin protein, without interference from the rest of the molecule.

Fig.4 shows some of our first experimental results in this area [13]. Fig.4b shows the ordinary carbon-13 NMR spectrum from the sample. The peaks in this spectrum come from the retinal, the protein, and some other material used to keep the protein stable and functional. There is not much to be got from this spectrum. Basically, it is too complicated to be useful.

Fig.4c shows the carbon-13 NMR spectrum from the same rhodopsin sample, but this time using C7 to generate the correlated nuclear state. By passing the NMR signals through this correlated state, we may select the NMR signals from the retinal alone. As may be seen, the spectrum now displays only two sharp peaks, which come from the two carbon-13 nuclei in each retinal molecule. It is possible to use this spectrum in order to understand what happens to the retinal when light is absorbed.

Using this method, we have started to build up a detailed geometrical picture of the retinal molecule before light is absorbed, and afterwards [15]. At the moment, there is no other physical method capable of this level of detail in a protein like rhodopsin.

Such studies are not of interest only to the science of vision. Rhodopsin is just one representative of a large and important family of proteins (called *G-protein coupled receptors*) that control a large number of essential processes in our body, including the sensory responses (taste, smell, etc.) and many neurological processes. By demonstrating the new method on rhodopsin, we make this experiment available to scientists who work on other members of this important family of proteins, which are already very important pharmacological targets. A future cure for serious medical conditions such as depression, schizophrenia, drug addiction and Parkinson's disease may one day evolve out of the molecular insights available from methods like the one sketched here.

Further Developments

The applications of symmetry to solid-state NMR have not stopped with C7. More recently, we have generalized the symmetry theory and discovered new classes of symmetries which cover a very wide range of applications [10, 16, 17]. Some of these new applications involve 9-fold, 10-fold and 11-fold symmetries [17]. Other research groups have also started to use symmetry arguments in their work [18, 19]. Symmetry and selection rules look poised to become an integral part of the thinking of solid-state NMR spectroscopists.

It is not clear whether this type of symmetry has any immediate applications outside NMR. As mentioned above, NMR is unique in that the interactions of the quantum-mechanical system with the experimental apparatus are usually stronger than the interactions of the nuclei with their immediate environment. There are few other technologies where this condition is satisfied, although laser spectroscopy and electron magnetic resonance may approach this situation in some circumstances.

Nevertheless, even if the applications of this work remains restricted to a rather narrow and technical field, the interesting dynamical aspects of the symmetries described here may serve as food for thought for a wider audience.

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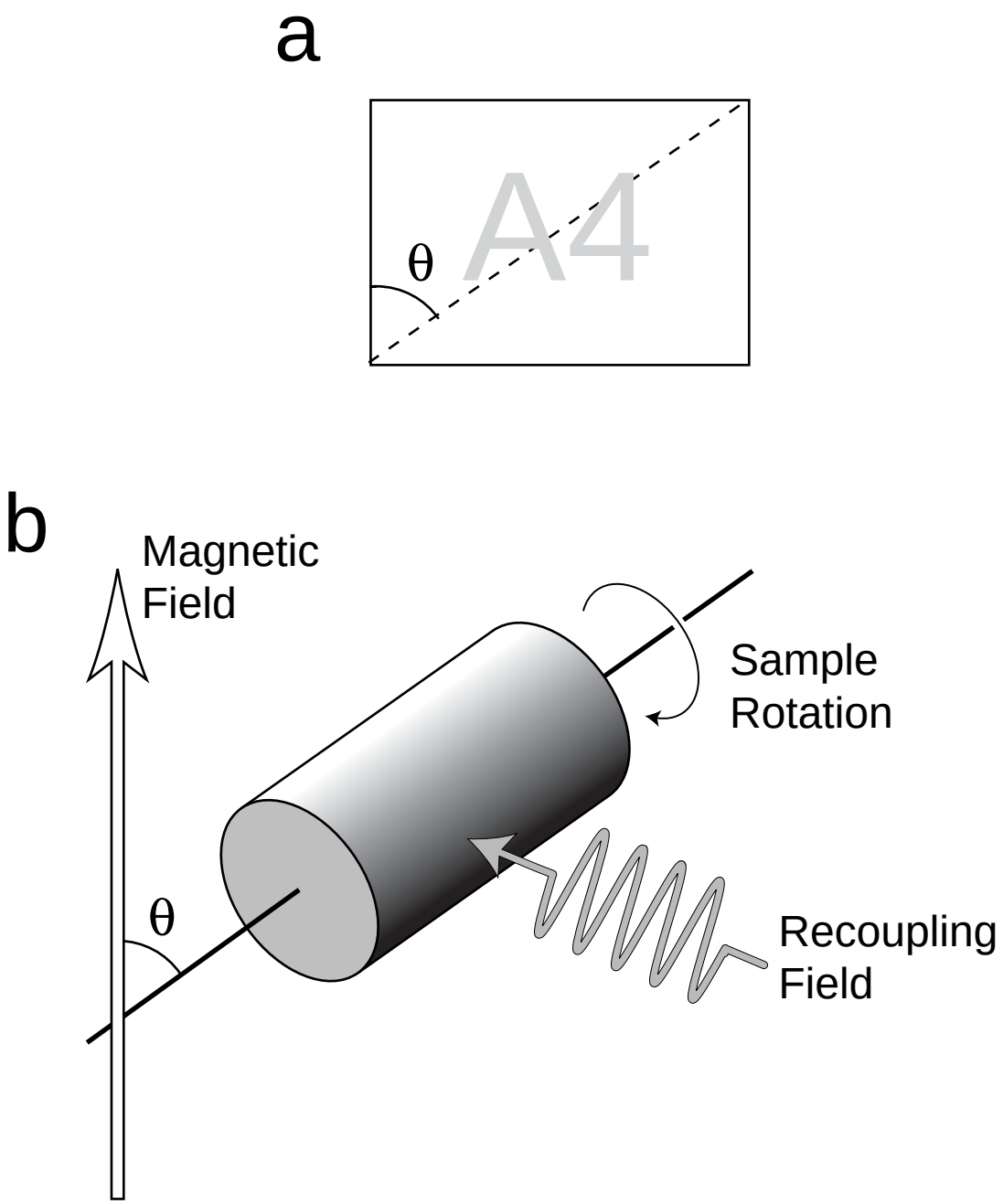
Figure Captions

Figure 1 (a) The magic angle θ is subtended by the diagonal and the short edge of a piece of A4 paper. (b) In the magic-angle-spinning NMR method, the sample is rotated rapidly about an axis that subtends the magic angle θ with respect to a large magnetic field. Recoupling is implemented by applying radio waves to the sample as it rotates.

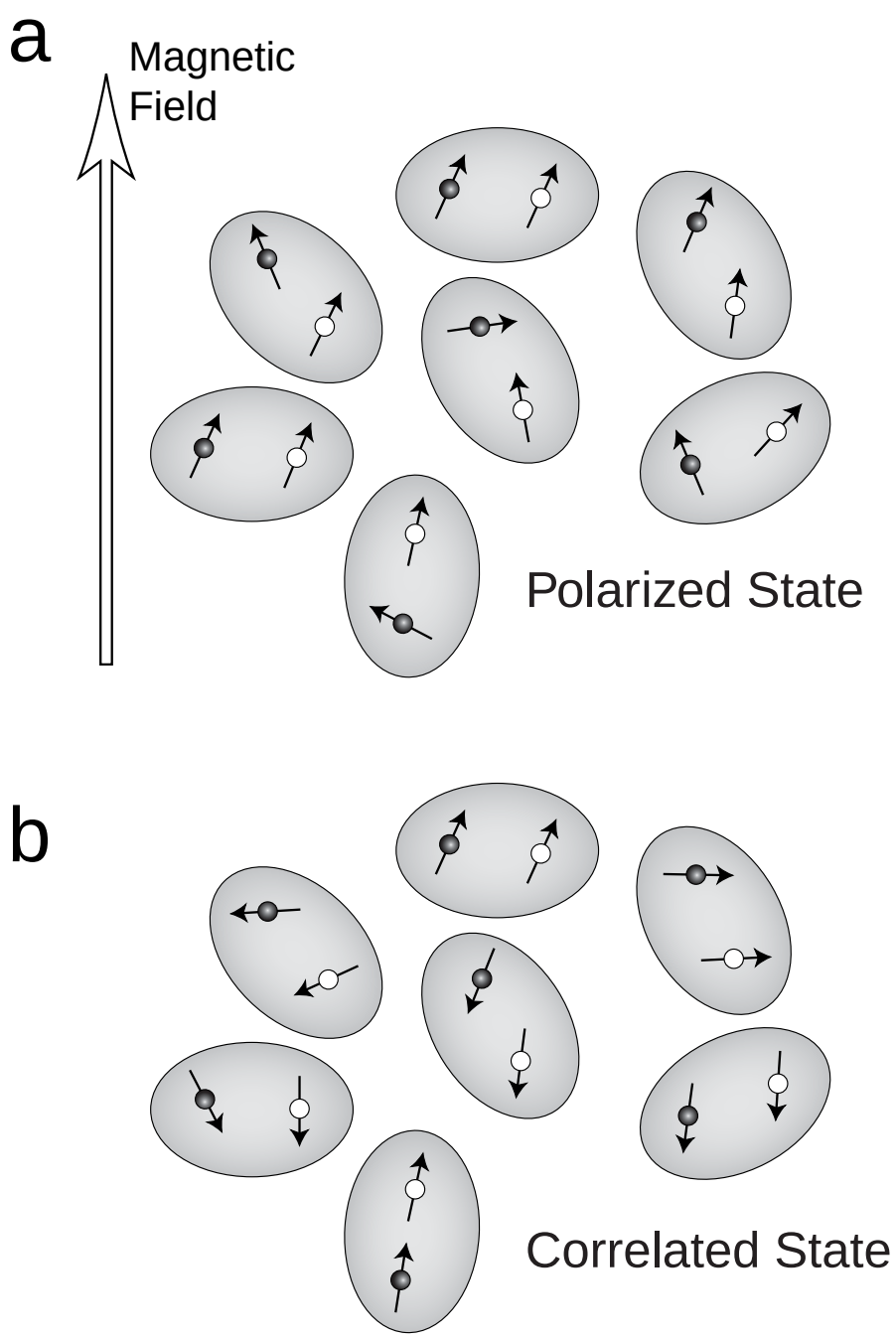
Figure 2 (a) Partially polarized nuclear magnets in a substance in which each molecule contains two magnetic nuclei. (b) A correlated nuclear magnetic state.

Figure 3 (a) The dynamic symmetry of the C7 recoupling method. The rotation of the sample is shown on the left; the rotation of the applied magnetic fields is shown on the right. Note that the magnetic fields advance in seven steps. (b) Selection rule diagram showing how the seven-fold symmetry restricts the evolution pathways for the nuclear magnets, leading to the development of a correlated state.

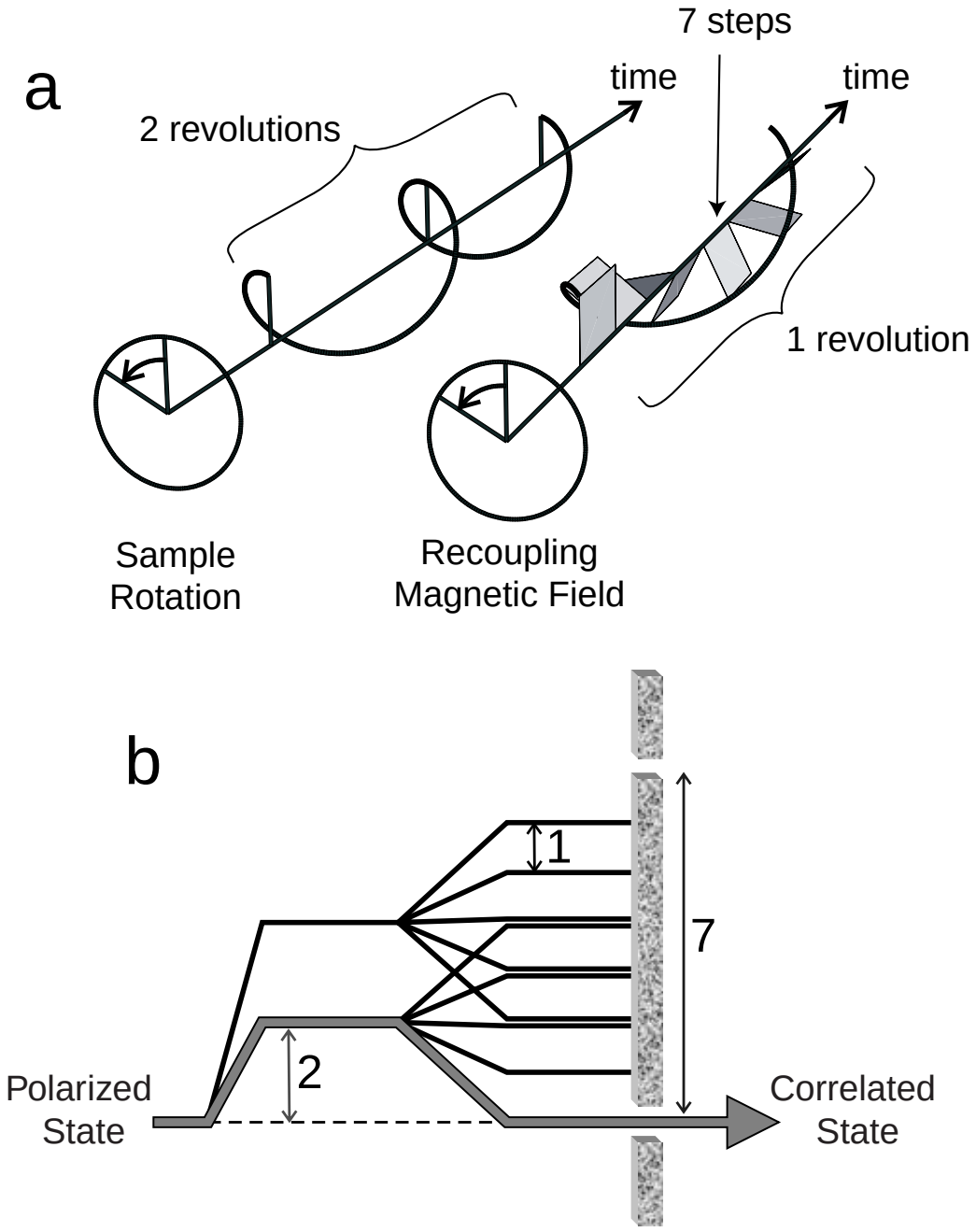
Figure 4 (a) The three-dimensional structure of the rhodopsin molecule. The retinal is shown as a space-filling model. (b) A carbon-13 NMR spectrum of rhodopsin, in which two of the carbon-12 nuclei in the retinal have been replaced by carbon-13. (c) By using C7, we pick these retinal carbon-13 signals out of the "background".



Levitt, Fig. 1



Levitt, Fig. 2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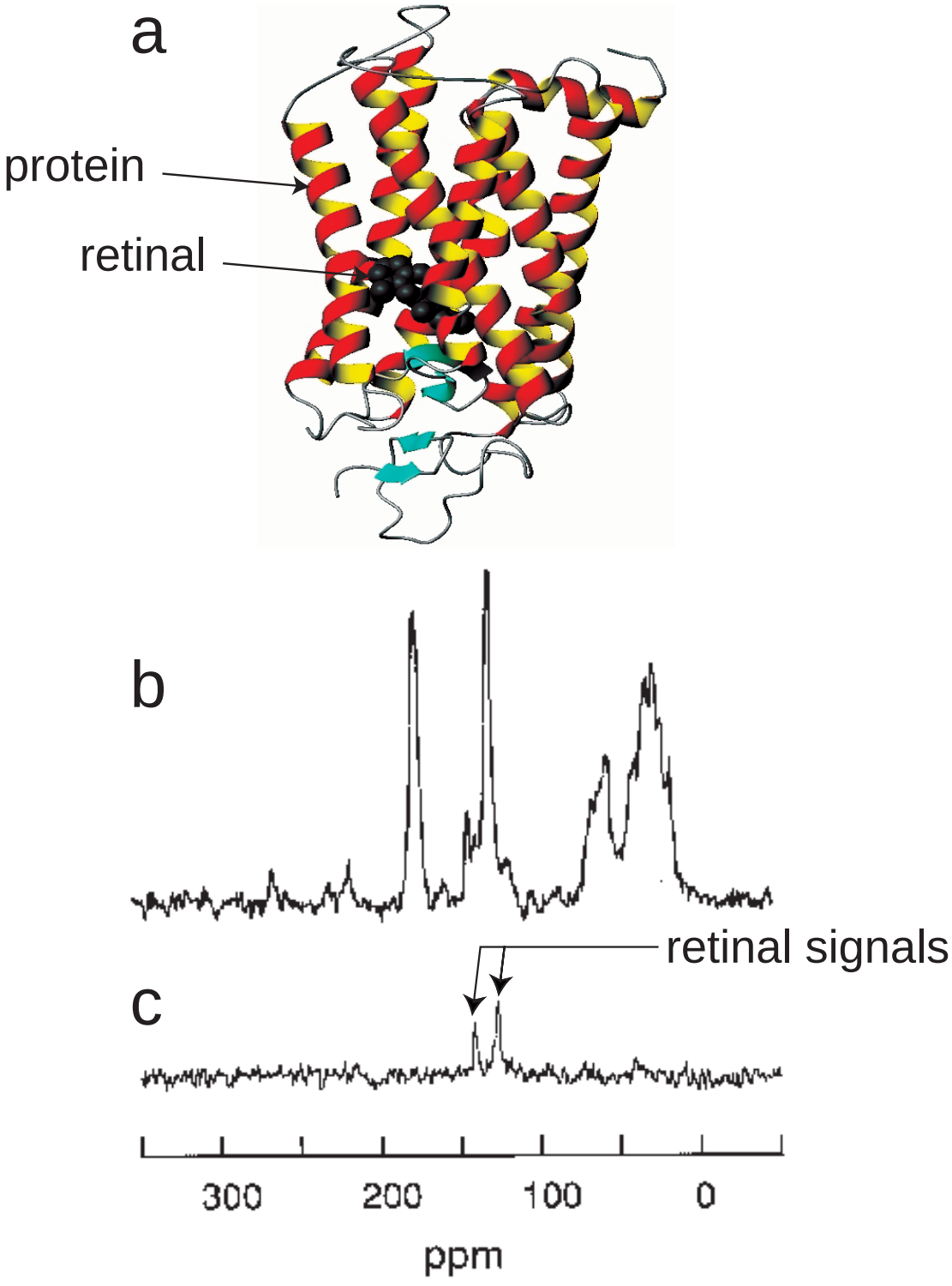
Levitt, Fig. 3



Levitt, Fig. 4