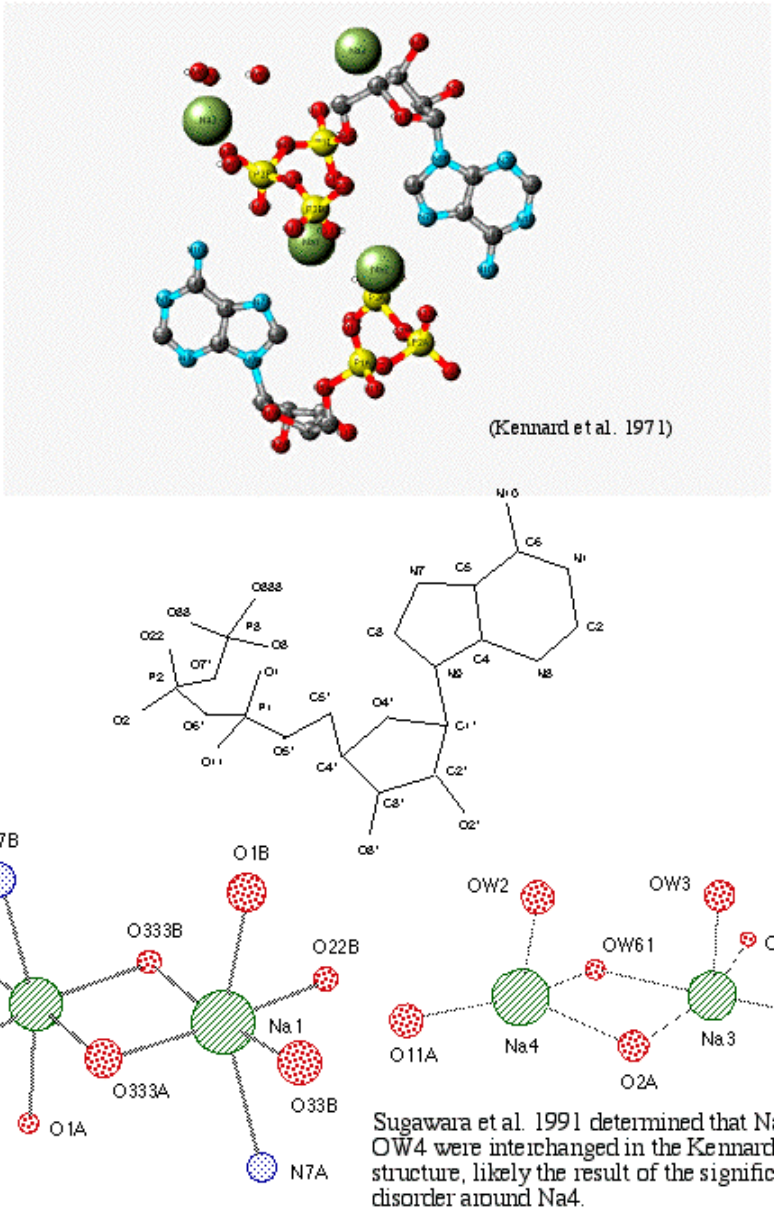
Investigating the Interaction of Metal Ions with Nucleotides and Macromolecular RNA Using Solid-State ^{23}Na, ^{25}Mg and ^{59}Co NMR for Direct Observation of the Metals

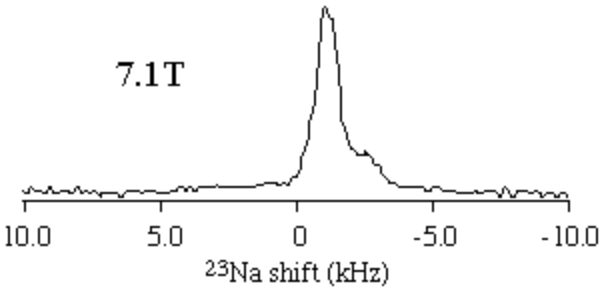
1. Sodium-Nucleotide Complexes

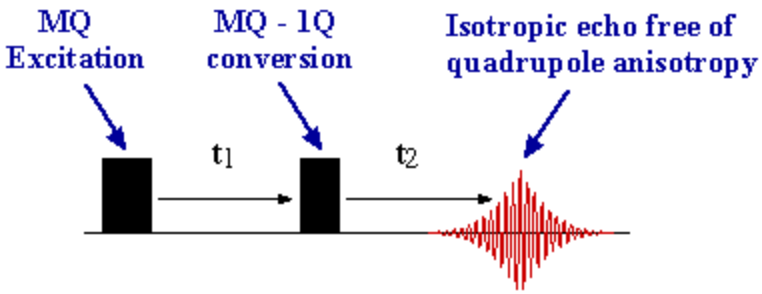
Metals ions are important to the structure of nucleic acids, which are poly-anionic in typical biological environments. For Example, we have studied Na_2ATP in the trihydrate form as a crystalline solid.

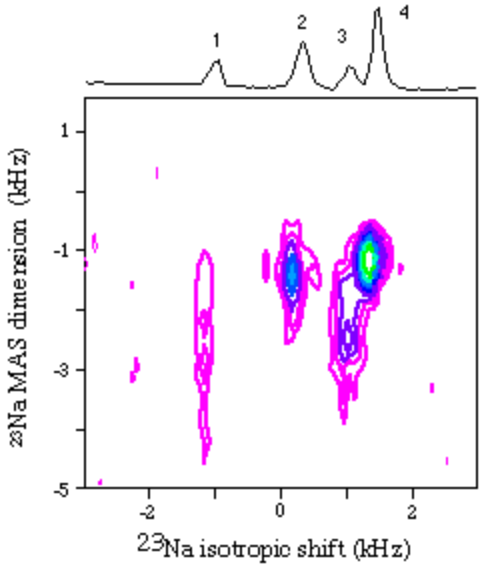


Sugawara et al. 1991 determined that Na4 and OW4 were interchanged in the Kennard structure, likely the result of the significant disorder around Na4.

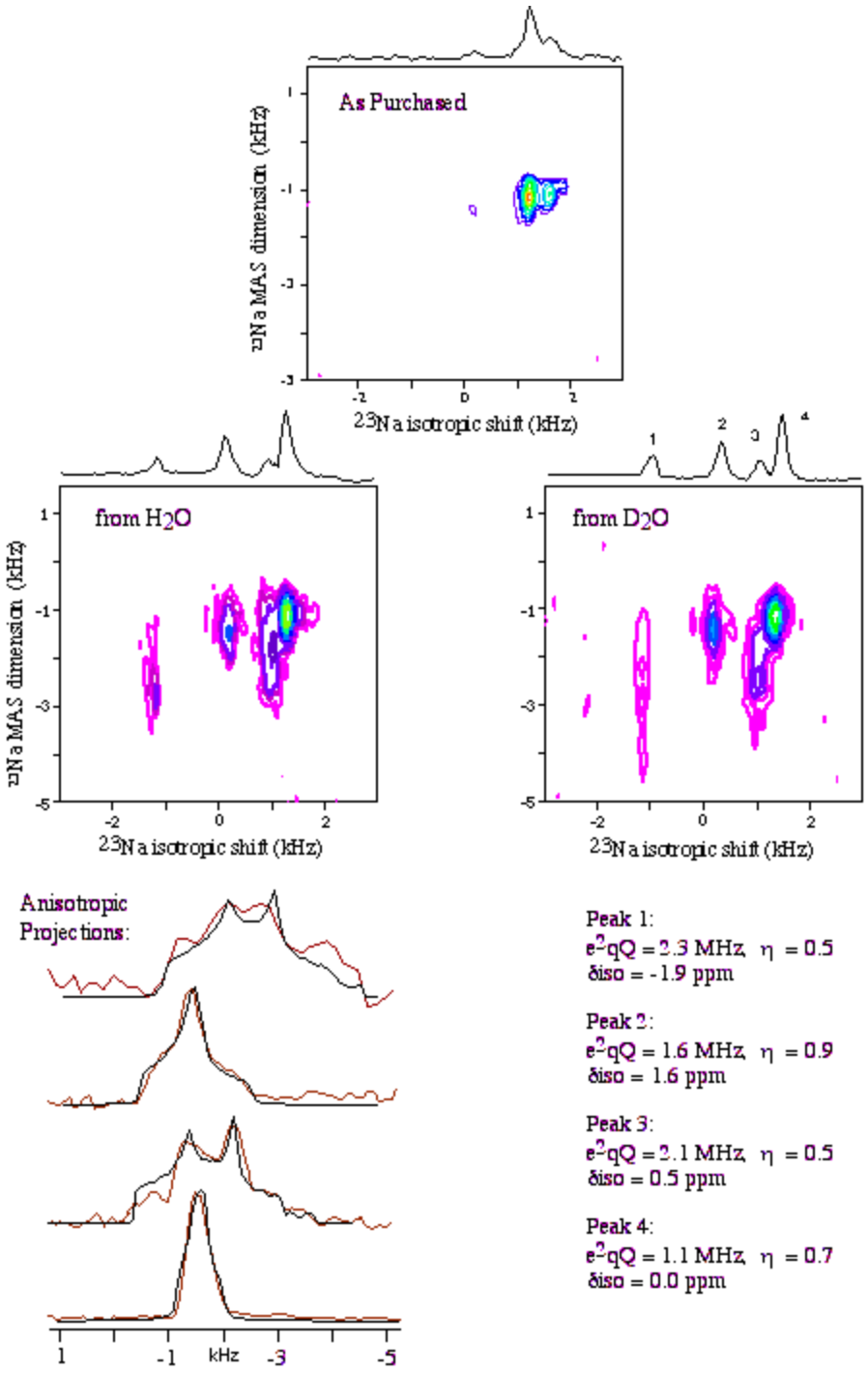
The MQMAS NMR experiment is used to resolve the four chemically inequivalent sites of the complex following recrystallization. MAS NMR alone fails to allow for the resolution of the sites.



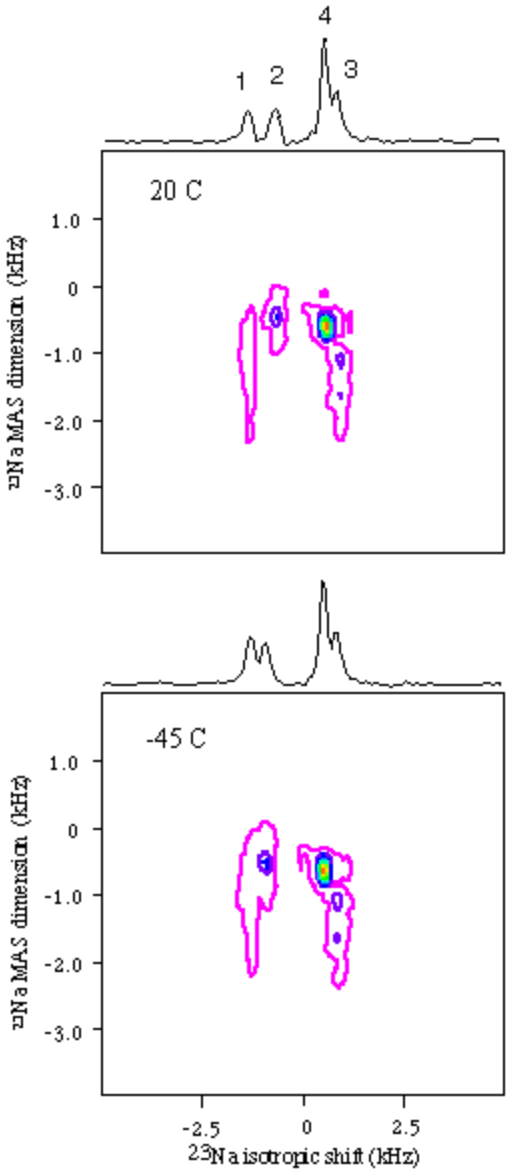




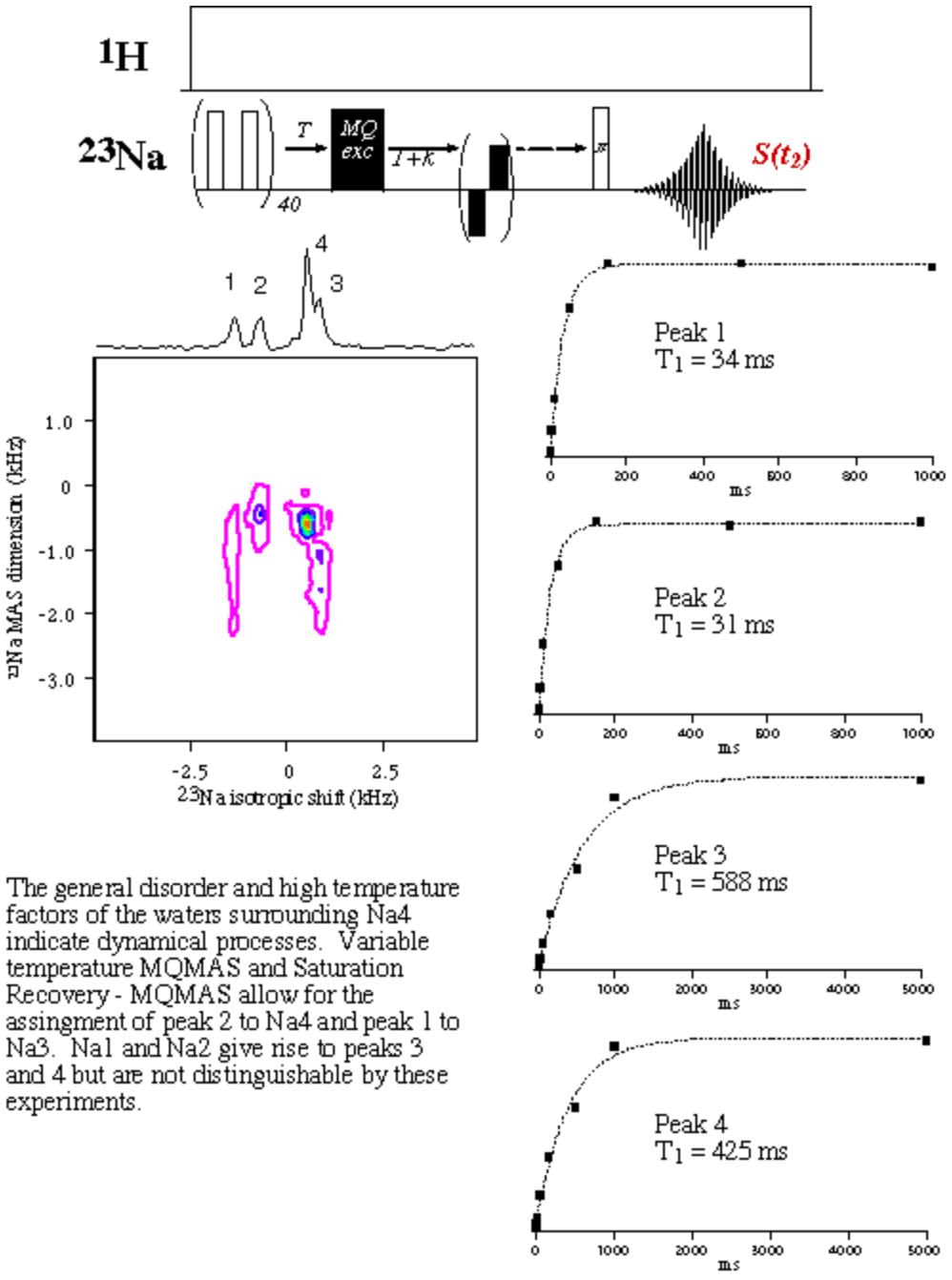
The MQMAS experiment shown above allows for the evaluation of the nuclear quadrupole coupling and chemical shift parameters of the resolved sodium sites under a variety of crystallization conditions.



With the site resolution that the MQMAS experiment provides, we can proceed to investigate the properties of the individual sodium resonances. These site-wise studies can take the form of recoupling experiments or, as in the following example, a variable temperature measurement. Note the distinct shift in peak 2.



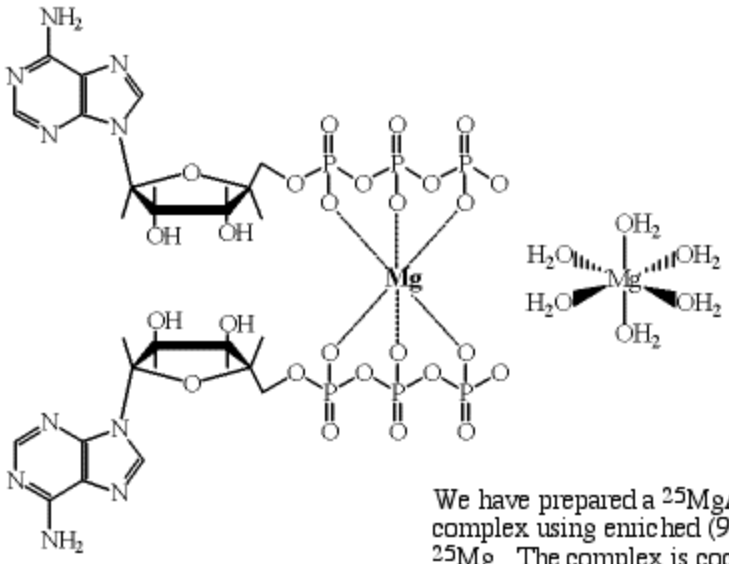
Furthermore, the relaxation properties of the individual sites can be measured. this is accomplished by the marriage of the saturation recovery experiment with the MQMAS experiment as follows.



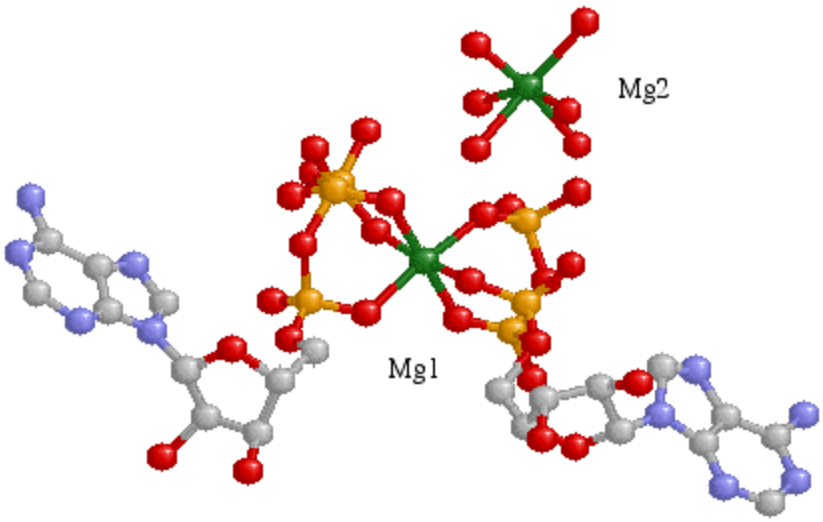
The general disorder and high temperature factors of the waters surrounding Na4 indicate dynamical processes. Variable temperature MQMAS and Saturation Recovery - MQMAS allow for the assingment of peak 2 to Na4 and peak 1 to Na3. Na1 and Na2 give rise to peaks 3 and 4 but are not distinguishable by these experiments.

2. ^{25}Mg NMR

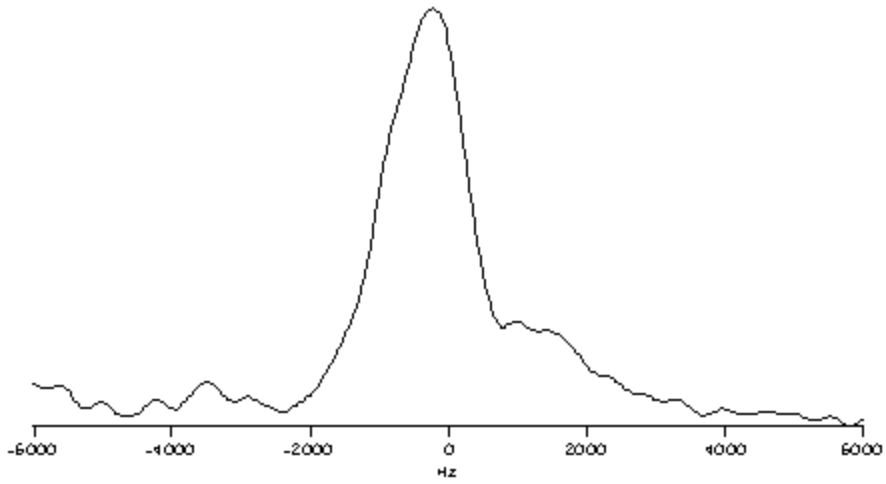
Arguably, the most important biological metal is magnesium. However, few techniques are available for the spectroscopic investigation of this closed shell, diamagnetic ion. Here we illustrate the use of solid-state NMR to investigate the NMR properties of magnesium(II) in complex with adenosine 5'-triphosphate and bis(2-pyridyl)amine (BPA). This complex has been previously studied crystallographically and the BPA allows for high quality crystals.

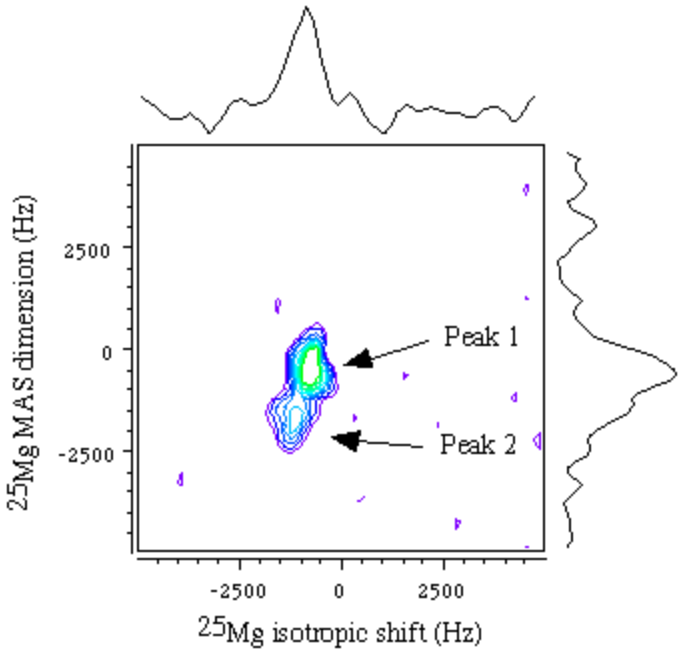


We have prepared a ^{25}MgATP complex using enriched (90%) ^{25}Mg. The complex is cocrystalized with bis(2-pyridyl)amine (BPA) and has been studied crystallographically by Cini et al. 1984.



As above, MQMAS provides site resolution and MAS NMR fails to provide site-wise information The experimental and simulated MQMAS data for MgATP is displayed below. These data were acquired at 11.7 T (30.6 MHz) with 10 kHz magic-angle-spinning

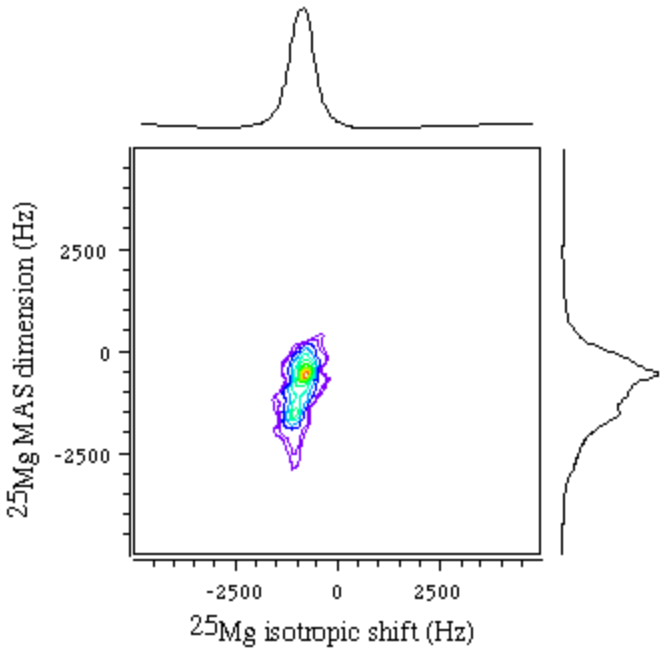




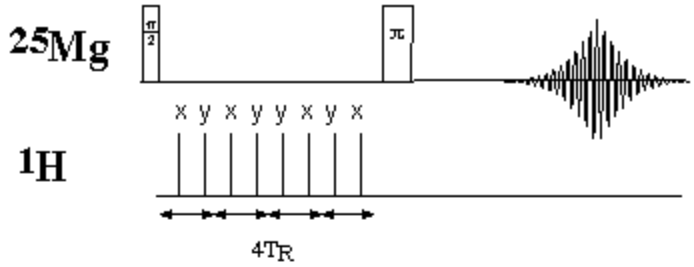
Simulation:

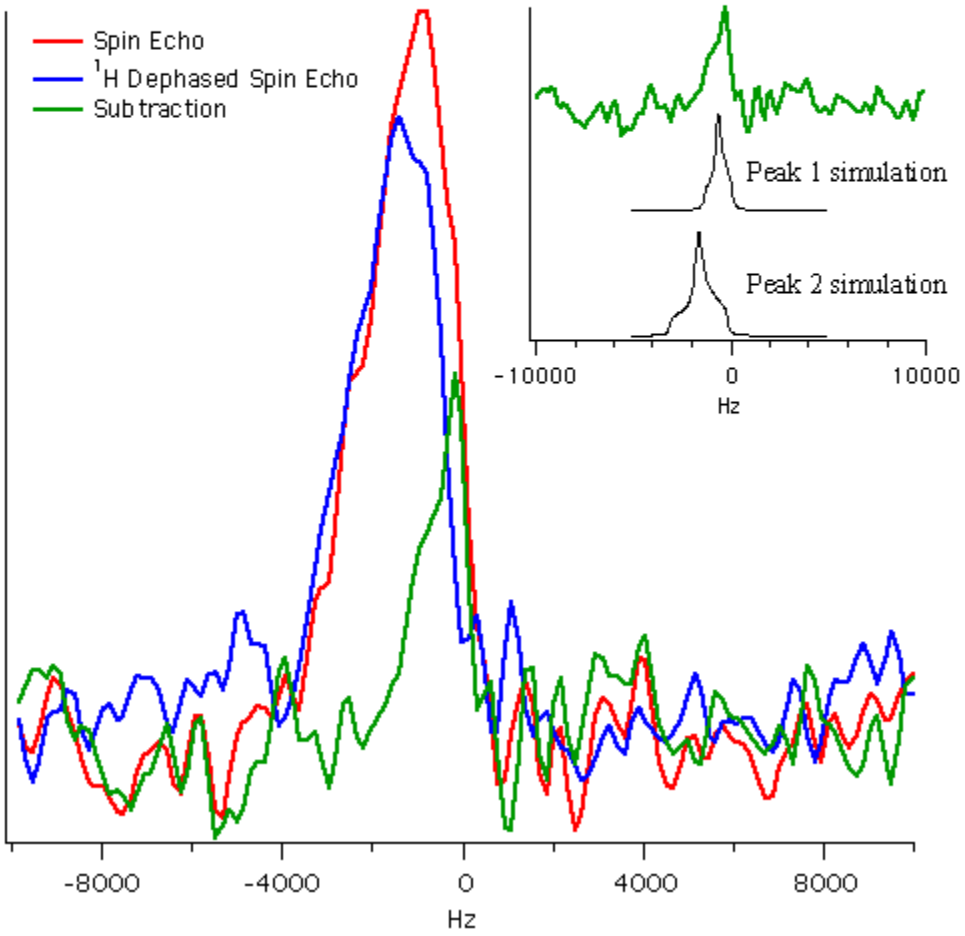
Peak 1:
$e^2qQ = 1.6$ MHz
$\eta = 1.0$
$\delta iso = -2.0$ ppm

Peak 2:
$e^2qQ = 2.3$ MHz
$\eta = 1.0$
$\delta iso = -9.0$ ppm



Two sites are clearly resolved, but sill we are unable to assign the two peaks of the spectrum to the pair of magnesium sites identified crystallographically. To assign the sites, we have developed a strategy based on the selective reintroduction of heteronuclear dipolar couplings using REDOR methodology. In this particular case, we will take advantage of the disparate amounts of strongly dipolar coupled protons in close contact with Mg2 vs. Mg1.

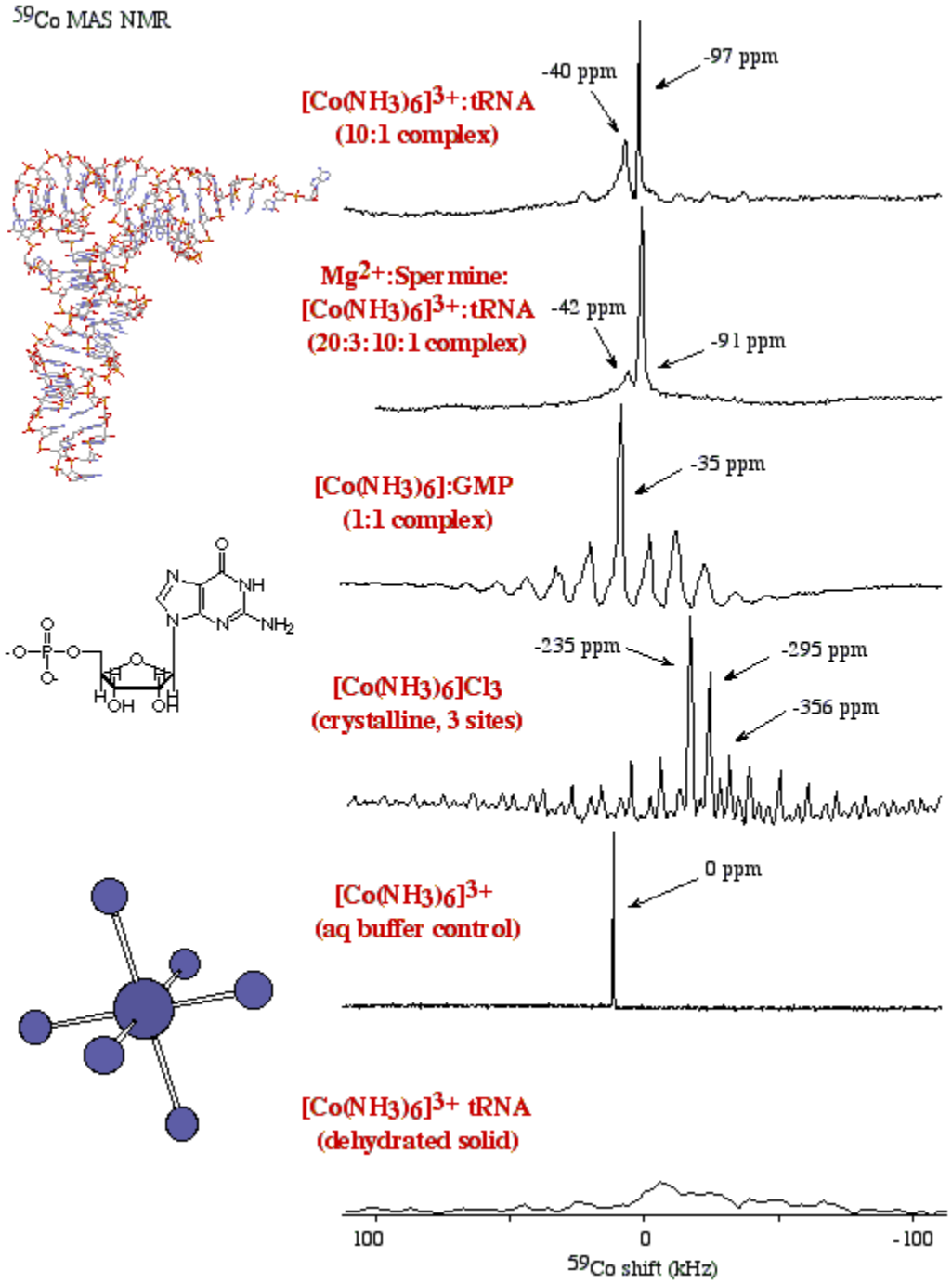




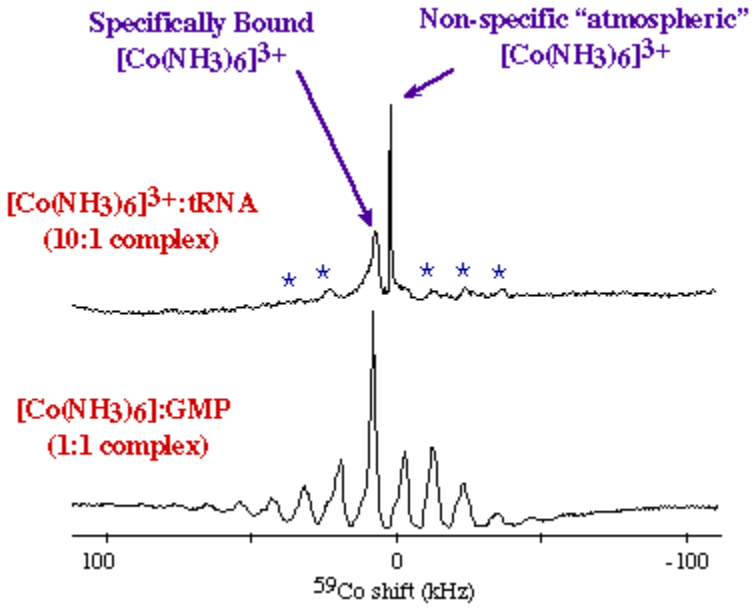
A reproducible change in the line shape of the magnesium resonance is observed upon the reintroduction of proton dipolar couplings that have been averaged away by magic-angle-spinning. By subtraction, it is clear that the signal being attenuated by the reintroduced dipolar couplings matches both the line shape and position of only one of the sites parameterized in the above MQMAS experiment. Thus, peak 1 can be assigned to Mg2 and peak 2 to Mg1.

3. Hexammine Cobalt(III) as a Solid-State NMR Spin Probe

The hexammine Cobalt(III) ion is a well established probe for solution NMR studies of metal binding sites in nucleic acids that have a preference for hydrated divalent metal ions. Solution NMR studies exploit NOE interactions between the exchange inert ammine protons of the hexammine cobalt(III) ion and the protons of the nucleic acid. In this work, we take advantage of the sensitivity of the 59-Co nucleus and the well established biochemistry of hexammine cobalt(III) ion binding to specific sites within RNA. This work has been carried out on tRNA samples which have been dialyzed against buffers containing hexammine cobalt(III) and assorted other monovalent and divalent ions. Samples are then precipitated with alcohol and analyzed as a hydrated solid. Furthermore, hexammine cobalt(III) - nucleotide complexes as crystalline solids are used as models for the sites formed in the tRNA samples.



Two peaks are clearly resolved in all spectra containing tRNA and hexammine cobalt(III). Furthermore, the peak -40 ppm is clearly diminished when competing cations are added to the sample. It is also clear that this same peak is very similar in position to the peak identified for the hexammine cobalt(III)-GMP nucleotide complex. As a result we interpret the data as follows:



Two classes of 59-Co resonances have been identified. the first of which are those assigned to specifically bound hexammine cobalt(III) ions that give rise to a peak flanked by spinning side bands (stars), a signal characteristic of hexammine cobalt(III) in a highly ordered solid. Furthermore, this peak shares many of the same properties as those observed for the crystalline hexammine cobalt(III)-GMP complex. The second class of resonance is devoid of spinning sidebands and is assigned to the hexammine cobalt(III) ion bound non-specifically to the tRNA. The rapid reorientation of these non-specifically bound ions gives rise to extensive averaging of the nuclear quadrupole interaction. These experiments demonstrate that this technique may be generalized to macromolecular RNA molecules, and provide a spectroscopic handle for studying the structure and dynamical properties of RNA at specific metal binding sites.