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## NMR spectroscopy, how can we be of assistance?

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## Properties of NMR spectroscopy

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NMR is very insensitive

NMR signal intensity is proportional to concentration

NMR is selective

NMR is non-invasive and non-destructive

NMR is not expensive

NMR can obtain detailed atomic information on the samples

NMR can analyze only one sample at a time

NMR can be performed on all states of the matter

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# NMR: the Universal Detector

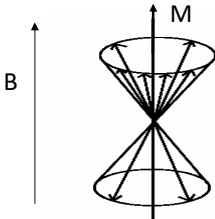
The periodic table shows NMR-active nuclei highlighted in red and green. Red highlights include <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N, <sup>19</sup>F, <sup>23</sup>Na, <sup>25</sup>Mg, <sup>29</sup>Si, <sup>31</sup>P, <sup>33</sup>S, <sup>35</sup>Cl, <sup>39</sup>K, <sup>41</sup>Ca, <sup>43</sup>K, <sup>45</sup>Ca, <sup>47</sup>Ca, <sup>49</sup>Ca, <sup>51</sup>V, <sup>53</sup>Cr, <sup>55</sup>Mn, <sup>57</sup>Fe, <sup>59</sup>Fe, <sup>61</sup>Fe, <sup>63</sup>Fe, <sup>65</sup>Fe, <sup>67</sup>Fe, <sup>69</sup>Fe, <sup>71</sup>Fe, <sup>73</sup>Fe, <sup>75</sup>Fe, <sup>77</sup>Fe, <sup>79</sup>Fe, <sup>81</sup>Fe, <sup>83</sup>Fe, <sup>85</sup>Fe, <sup>87</sup>Fe, <sup>89</sup>Fe, <sup>91</sup>Fe, <sup>93</sup>Fe, <sup>95</sup>Fe, <sup>97</sup>Fe, <sup>99</sup>Fe, <sup>101</sup>Fe, <sup>103</sup>Fe, <sup>105</sup>Fe, <sup>107</sup>Fe, <sup>109</sup>Fe, <sup>111</sup>Fe, <sup>113</sup>Fe, <sup>115</sup>Fe, <sup>117</sup>Fe, <sup>119</sup>Fe, <sup>121</sup>Fe, <sup>123</sup>Fe, <sup>125</sup>Fe, <sup>127</sup>Fe, <sup>129</sup>Fe, <sup>131</sup>Fe, <sup>133</sup>Fe, <sup>135</sup>Fe, <sup>137</sup>Fe, <sup>139</sup>Fe, <sup>141</sup>Fe, <sup>143</sup>Fe, <sup>145</sup>Fe, <sup>147</sup>Fe, <sup>149</sup>Fe, <sup>151</sup>Fe, <sup>153</sup>Fe, <sup>155</sup>Fe, <sup>157</sup>Fe, <sup>159</sup>Fe, <sup>161</sup>Fe, <sup>163</sup>Fe, <sup>165</sup>Fe, <sup>167</sup>Fe, <sup>169</sup>Fe, <sup>171</sup>Fe, <sup>173</sup>Fe, <sup>175</sup>Fe, <sup>177</sup>Fe, <sup>179</sup>Fe, <sup>181</sup>Fe, <sup>183</sup>Fe, <sup>185</sup>Fe, <sup>187</sup>Fe, <sup>189</sup>Fe, <sup>191</sup>Fe, <sup>193</sup>Fe, <sup>195</sup>Fe, <sup>197</sup>Fe, <sup>199</sup>Fe, <sup>201</sup>Fe, <sup>203</sup>Fe, <sup>205</sup>Fe, <sup>207</sup>Fe, <sup>209</sup>Fe, <sup>211</sup>Fe, <sup>213</sup>Fe, <sup>215</sup>Fe, <sup>217</sup>Fe, <sup>219</sup>Fe, <sup>221</sup>Fe, <sup>223</sup>Fe, <sup>225</sup>Fe, <sup>227</sup>Fe, <sup>229</sup>Fe, <sup>231</sup>Fe, <sup>233</sup>Fe, <sup>235</sup>Fe, <sup>237</sup>Fe, <sup>239</sup>Fe, <sup>241</sup>Fe, 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<sup>793</sup>Fe, <sup>795</sup>Fe, <sup>797</sup>Fe, <sup>799</sup>Fe, <sup>801</sup>Fe, <sup>803</sup>Fe, <sup>805</sup>Fe, <sup>807</sup>Fe, <sup>809</sup>Fe, <sup>811</sup>Fe, <sup>813</sup>Fe, <sup>815</sup>Fe, <sup>817</sup>Fe, <sup>819</sup>Fe, <sup>821</sup>Fe, <sup>823</sup>Fe, <sup>825</sup>Fe, <sup>827</sup>Fe, <sup>829</sup>Fe, <sup>831</sup>Fe, <sup>833</sup>Fe, <sup>835</sup>Fe, <sup>837</sup>Fe, <sup>839</sup>Fe, <sup>841</sup>Fe, <sup>843</sup>Fe, <sup>845</sup>Fe, <sup>847</sup>Fe, <sup>849</sup>Fe, <sup>851</sup>Fe, <sup>853</sup>Fe, <sup>855</sup>Fe, <sup>857</sup>Fe, <sup>859</sup>Fe, <sup>861</sup>Fe, <sup>863</sup>Fe, <sup>865</sup>Fe, <sup>867</sup>Fe, <sup>869</sup>Fe, <sup>871</sup>Fe, <sup>873</sup>Fe, <sup>875</sup>Fe, <sup>877</sup>Fe, <sup>879</sup>Fe, <sup>881</sup>Fe, <sup>883</sup>Fe, <sup>885</sup>Fe, <sup>887</sup>Fe, <sup>889</sup>Fe, <sup>891</sup>Fe, <sup>893</sup>Fe, <sup>895</sup>Fe, <sup>897</sup>Fe, <sup>899</sup>Fe, <sup>901</sup>Fe, <sup>903</sup>Fe, <sup>905</sup>Fe, <sup>907</sup>Fe, <sup>909</sup>Fe, <sup>911</sup>Fe, <sup>913</sup>Fe, <sup>915</sup>Fe, <sup>917</sup>Fe, <sup>919</sup>Fe, <sup>921</sup>Fe, <sup>923</sup>Fe, <sup>925</sup>Fe, <sup>927</sup>Fe, <sup>929</sup>Fe, <sup>931</sup>Fe, <sup>933</sup>Fe, <sup>935</sup>Fe, <sup>937</sup>Fe, <sup>939</sup>Fe, <sup>941</sup>Fe, <sup>943</sup>Fe, <sup>945</sup>Fe, <sup>947</sup>Fe, <sup>949</sup>Fe, <sup>951</sup>Fe, <sup>953</sup>Fe, <sup>955</sup>Fe, <sup>957</sup>Fe, <sup>959</sup>Fe, <sup>961</sup>Fe, <sup>963</sup>Fe, <sup>965</sup>Fe, <sup>967</sup>Fe, <sup>969</sup>Fe, <sup>971</sup>Fe, <sup>973</sup>Fe, <sup>975</sup>Fe, <sup>977</sup>Fe, <sup>979</sup>Fe, <sup>981</sup>Fe, <sup>983</sup>Fe, <sup>985</sup>Fe, <sup>987</sup>Fe, <sup>989</sup>Fe, <sup>991</sup>Fe, <sup>993</sup>Fe, <sup>995</sup>Fe, <sup>997</sup>Fe, <sup>999</sup>Fe, <sup>1001</sup>Fe, <sup>1003</sup>Fe, <sup>1005</sup>Fe, <sup>1007</sup>Fe, <sup>1009</sup>Fe, <sup>1011</sup>Fe, 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<sup>1637</sup>Fe, <sup>1639</sup>Fe, <sup>1641</sup>Fe, <sup>1643</sup>Fe, <sup>1645</sup>Fe, <sup>1647</sup>Fe, <sup>1649</sup>Fe, <sup>1651</sup>Fe, <sup>1653</sup>Fe, <sup>1655</sup>Fe, <sup>1657</sup>Fe, <sup>1659</sup>Fe, <sup>1661</sup>Fe, <sup>1663</sup>Fe, <sup>1665</sup>Fe, <sup>1667</sup>Fe, <sup>1669</sup>Fe, <sup>1671</sup>Fe, <sup>1673</sup>Fe, <sup>1675</sup>Fe, <sup>1677</sup>Fe, <sup>1679</sup>Fe, <sup>1681</sup>Fe, <sup>1683</sup>Fe, <sup>1685</sup>Fe, <sup>1687</sup>Fe, <sup>1689</sup>Fe, <sup>1691</sup>Fe, <sup>1693</sup>Fe, <sup>1695</sup>Fe, <sup>1697</sup>Fe, <sup>1699</sup>Fe, <sup>1701</sup>Fe, <sup>1703</sup>Fe, <sup>1705</sup>Fe, <sup>1707</sup>Fe, <sup>1709</sup>Fe, <sup>1711</sup>Fe, <sup>1713</sup>Fe, <sup>1715</sup>Fe, <sup>1717</sup>Fe, <sup>1719</sup>Fe, <sup>1721</sup>Fe, <sup>1723</sup>Fe, <sup>1725</sup>Fe, <sup>1727</sup>Fe, <sup>1729</sup>Fe, <sup>1731</sup>Fe, <sup>1733</sup>Fe, <sup>1735</sup>Fe, <sup>1737</sup>Fe, <sup>1739</sup>Fe, <sup>1741</sup>Fe, <sup>1743</sup>Fe, <sup>1745</sup>Fe, <sup>1747</sup>Fe, <sup>1749</sup>Fe, <sup>1751</sup>Fe, <sup>1753</sup>Fe, <sup>1755</sup>Fe, <sup>1757</sup>Fe, <sup>1759</sup>Fe, <sup>1761</sup>Fe, <sup>1763</sup>Fe, <sup>1765</sup>Fe, <sup>1767</sup>Fe, <sup>1769</sup>Fe, <sup>1771</sup>Fe, <sup>1773</sup>Fe, <sup>1775</sup>Fe, <sup>1777</sup>Fe, <sup>1779</sup>Fe, <sup>1781</sup>Fe, <sup>1783</sup>Fe, <sup>1785</sup>Fe, <sup>1787</sup>Fe, <sup>1789</sup>Fe, <sup>1791</sup>Fe, <sup>1793</sup>Fe, <sup>1795</sup>Fe, <sup>1797</sup>Fe, <sup>1799</sup>Fe, <sup>1801</sup>Fe, <sup>1803</sup>Fe, <sup>1805</sup>Fe, <sup>1807</sup>Fe, <sup>1809</sup>Fe, <sup>1811</sup>Fe, <sup>1813</sup>Fe, <sup>1815</sup>Fe, <sup>1817</sup>Fe, <sup>1819</sup>Fe, <sup>1821</sup>Fe, <sup>1823</sup>Fe, <sup>1825</sup>Fe, <sup>1827</sup>Fe, <sup>1829</sup>Fe, <sup>1831</sup>Fe, <sup>1833</sup>Fe, <sup>1835</sup>Fe, <sup>1837</sup>Fe, <sup>1839</sup>Fe, <sup>1841</sup>Fe, <sup>1843</sup>Fe, 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<sup>1949</sup>Fe, <sup>1951</sup>Fe, <sup>1953</sup>Fe, <sup>1955</sup>Fe, <sup>1957</sup>Fe, <sup>1959</sup>Fe, <sup>1961</sup>Fe, <sup>1963</sup>Fe, <sup>1965</sup>Fe, <sup>1967</sup>Fe, <sup>1969</sup>Fe, <sup>1971</sup>Fe, <sup>1973</sup>Fe, <sup>1975</sup>Fe, <sup>1977</sup>Fe, <sup>1979</sup>Fe, <sup>1981</sup>Fe, <sup>1983</sup>Fe, <sup>1985</sup>Fe, <sup>1987</sup>Fe, <sup>1989</sup>Fe, <sup>1991</sup>Fe, <sup>1993</sup>Fe, <sup>1995</sup>Fe, <sup>1997</sup>Fe, <sup>1999</sup>Fe, <sup>2001</sup>Fe, <sup>2003</sup>Fe, <sup>2005</sup>Fe, <sup>2007</sup>Fe, <sup>2009</sup>Fe, <sup>2011</sup>Fe, <sup>2013</sup>Fe, <sup>2015</sup>Fe, <sup>2017</sup>Fe, <sup>2019</sup>Fe, <sup>2021</sup>Fe, <sup>2023</sup>Fe, <sup>2025</sup>Fe, <sup>2027</sup>Fe, <sup>2029</sup>Fe, <sup>2031</sup>Fe, <sup>2033</sup>Fe, <sup>2035</sup>Fe, <sup>2037</sup>Fe, <sup>2039</sup>Fe, <sup>2041</sup>Fe, <sup>2043</sup>Fe, <sup>2045</sup>Fe, <sup>2047</sup>Fe, <sup>2049</sup>Fe, <sup>2051</sup>Fe, <sup>2053</sup>Fe, <sup>2055</sup>Fe, <sup>2057</sup>Fe, <sup>2059</sup>Fe, <sup>2061</sup>Fe, <sup>2063</sup>Fe, <sup>2065</sup>Fe, <sup>2067</sup>Fe, <sup>2069</sup>Fe, <sup>2071</sup>Fe, <sup>2073</sup>Fe, <sup>2075</sup>Fe, <sup>2077</sup>Fe, <sup>2079</sup>Fe, <sup>2081</sup>Fe, <sup>2083</sup>Fe, <sup>2085</sup>Fe, <sup>2087</sup>Fe, <sup>2089</sup>Fe, <sup>2091</sup>Fe, <sup>2093</sup>Fe, <sup>2095</sup>Fe, <sup>2097</sup>Fe, <sup>2099</sup>Fe, <sup>2101</sup>Fe, <sup>2103</sup>Fe, <sup>2105</sup>Fe, <sup>2107</sup>Fe, <sup>2109</sup>Fe, <sup>2111</sup>Fe, <sup>2113</sup>Fe, <sup>2115</sup>Fe, <sup>2117</sup>Fe, <sup>2119</sup>Fe, <sup>2121</sup>Fe, <sup>2123</sup>Fe, <sup>2125</sup>Fe, <sup>2127</sup>Fe, <sup>2129</sup>Fe, <sup>2131</sup>Fe, <sup>2133</sup>Fe, <sup>2135</sup>Fe, <sup>2137</sup>Fe, <sup>2139</sup>Fe, <sup>2141</sup>Fe, <sup>2143</sup>Fe, <sup>2145</sup>

## Macroscopic magnetization

A real sample has many spins which in a magnetic field are more likely to be found precessing in the state of lower energy. How much more likely is determined by the Boltzmann distribution:

$$\frac{N_{\beta \text{ spins}}}{N_{\alpha \text{ spins}}} = e^{-\Delta E/kT}$$

At the current operating field strength of the NMR equipment, the ratio is of the order of 0.999999. The tiny difference is the origin of the low sensitivity of NMR and therefore, the need for large amounts of material.

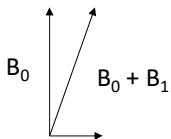


Nonetheless, this small difference generates a macroscopic magnetization **M** aligned with the field **B**.

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## Acting on the macroscopic magnetization

In order to perturb the nuclear spins it is necessary to apply a magnetic field  $B_1$  that is perpendicular to the static field  $B_0$

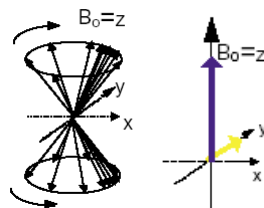


The field  $B_1$  is applied for a very short time and is called a pulse.

During its application the spins will precess about the axis of the resulting field, and they are no longer uniformly distributed - they develop coherence.

This causes the macroscopic magnetization to flip away from the direction of the  $B_0$  field.

How much it flips depends on the strength of the  $B_1$  field length of application of the pulse.

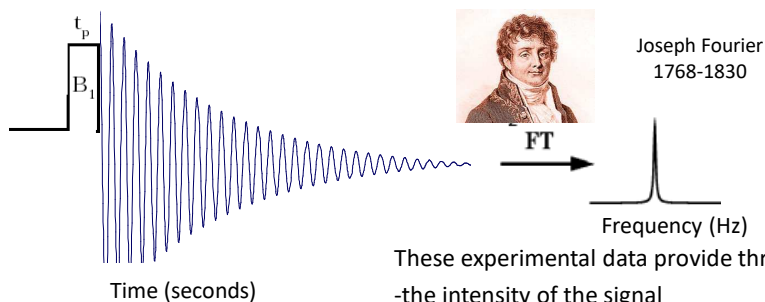


$$\theta = \gamma B_1 t$$

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## Performing an experiment

An NMR experiment is composed by two sections: the preparation, which can be as simple as the  $90^\circ B_1$  pulse, and the detection. The detector observes the decay of transverse magnetization.



These experimental data provide three pieces of information:

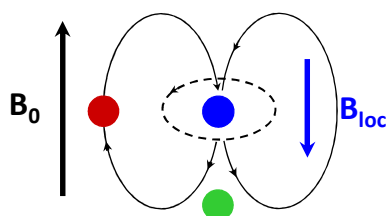
- the intensity of the signal
- the frequency of oscillation (position in the spectrum)
- the rate of transverse relaxation (width of the signal)

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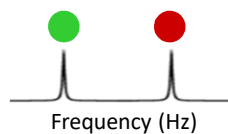
## Nuclear shielding

Even nuclei of the same nature show signals in different positions because the effective magnetic field is different from the static magnetic field  $B_0$  due to the influence of the surrounding environment - the *nuclear shielding*.

The nuclear shielding arises from the interaction (**coupling**) between the magnetic nuclei and the surrounding electrons which are also spins and therefore affected by the presence of the static magnetic field.



The situation depicted of the two nuclei would give rise to a spectrum that would look like this:



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## The chemical shift

The chemical shift is defined as the ratio between the nuclear shielding and the static magnetic field and is usually reported in ppm relative to a reference. This ratio is independent of the strength of the static field.

$$\delta = \frac{\nu_{\text{sample}} - \nu_{\text{reference}}}{\text{central frequency(Hz)}} \times 10^6 \text{ ppm}$$

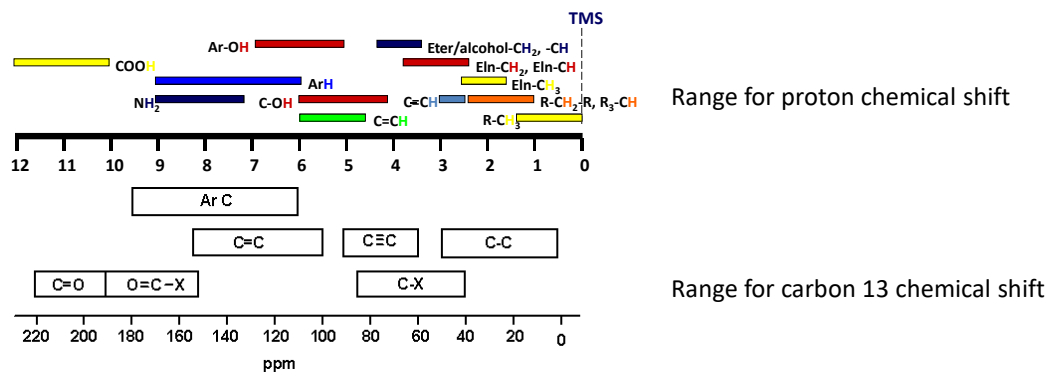
Data measured in different spectrometers can be compared.

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## The chemical shift

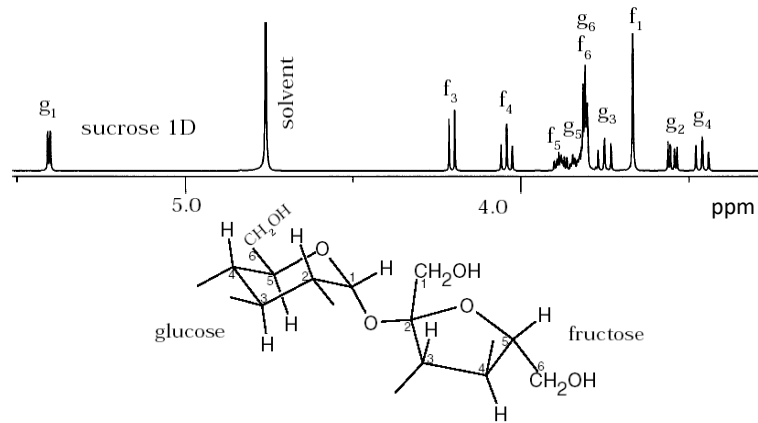
In molecules without unpaired electrons, different functional groups display characteristic chemical shifts.

As a rule of thumb the more electronegative the environment, the higher the chemical shift.



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## Spectrum of a simple biological molecule

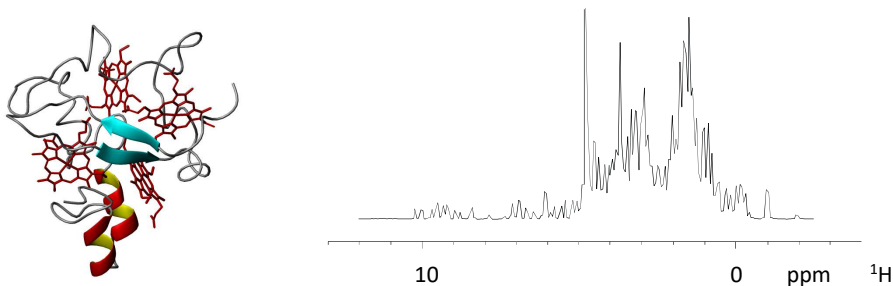


Clearly, even for a simple molecule like this, this spectrum is insufficient to perform the assignment.

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## A protein spectrum

Structure of cytochrome *c*<sub>3</sub> - a protein with 107 amino-acids and 4 hemes (13 kDa).



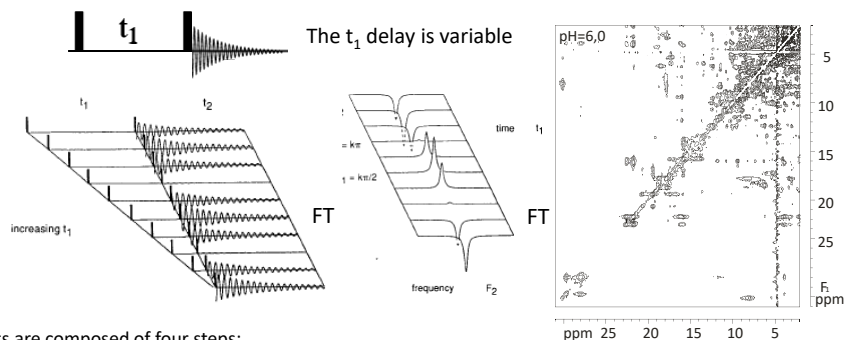
Thousands of proton signals had to be identified and their correlations measured

The resolution is so poor that

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## Multidimensional NMR spectroscopy

The development of multidimensional NMR methods allowed the characterization of larger and more complex molecules such as proteins and nucleic acids



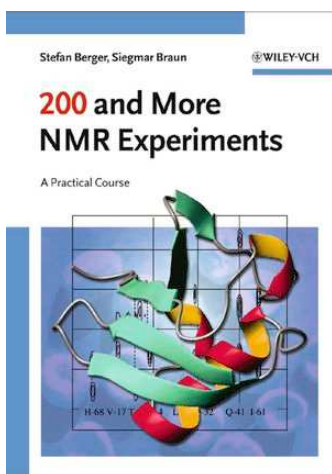
2D experiments are composed of four steps:

i) preparation; ii) evolution; iii) mixing; and iv) detection

Depending on what is actually done, different kinds of multidimensional experiments are obtained.

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## A zoo of NMR experiments



Surprisingly the zoo is just based on:

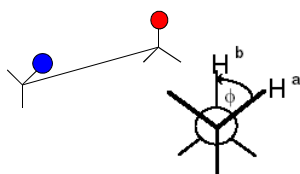
- two phenomena of coupling between magnetic spins
  - \*Scalar coupling, which is propagated through chemical bonds
  - \*Dipolar coupling, which is propagated through space
- relaxation
  - \*Spin-lattice relaxation T1
  - \*Spin-spin relaxation T2

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## Scalar couplings

Scalar couplings are designated by  ${}^nJ$ , where  $n$  indicates the number of bonds separating the nuclei.

**Geminal couplings  ${}^2J$**  - these couplings depend on the hybridization of the connecting atom. In order for these couplings to be observed the nuclei must have different chemical shifts.



**Vicinal couplings  ${}^3J$**  - these couplings display a well defined dependence on the dihedral angle between the nuclei. The relationship is generally known as *Karplus equation*.

There are different equations for different coupled nuclei, that provide information on **molecular geometry**.

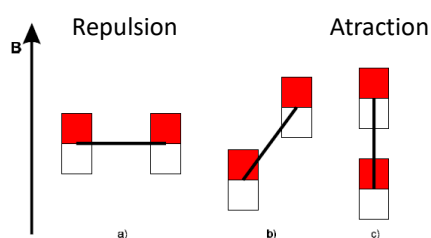


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## Dipolar coupling

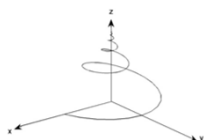
**Dipolar couplings** depend on the orientation of the vector connecting the interacting nuclei relative to the orientation of the  $B_0$  field. They usually are **not observed** in liquid samples, due to fast molecular reorientation (but see later for residual dipolar couplings). They are important in solids and liquid crystal samples.



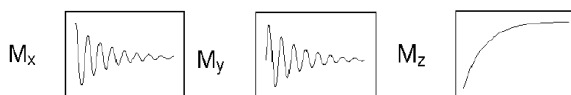
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## Relaxation: The return of the magnetization

When the  $B_1$  pulse is switched off, the spins gradually lose their coherence and the macroscopic magnetization returns to the direction of the  $B_0$  field. This is called **relaxation**, and follows an exponential decay: The free induction decay **FID**.



This process is described mathematically by the Bloch equations.



$$M_x(t) = [M_x(0)\cos\omega t - M_y(0)\sin\omega t]e^{-t/T_2}$$

$$M_y(t) = [M_x(0)\sin\omega t + M_y(0)\cos\omega t]e^{-t/T_2}$$

$$M_z(t) = M_{eq} + [M_z(0) - M_{eq}]e^{-t/T_1}$$

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## More on Relaxation

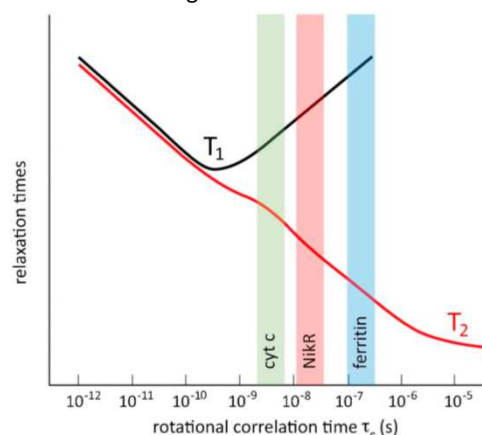
There are several mechanisms that give rise to local fluctuations of the magnetic field:

- dipolar interactions with other nuclei
- paramagnetic interactions with unpaired electrons
- quadrupolar interactions (for nuclei with  $I > \frac{1}{2}$ )

These mechanisms depend on molecular motions.

$T_1$  is shorter when the molecular motions are close to the Larmor frequency. Determines how fast an experiment can be repeated.

$T_2$  depends on motions of frequency close to the Larmor frequency and close to zero and therefore will continue to decrease as  $\tau_c$  increases. Relates inversely with linewidth.

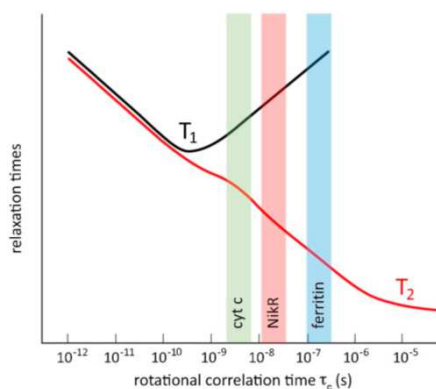


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## The issue of size

Size is challenging in three ways:



- 1 more signals
- 2 broader signals due to shorter T2
- 3 slower repetition due to longer T1

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## Protein NMR: Issues to consider

Sample must be soluble and remain monomeric to high concentration due to the low intrinsic sensitivity of the NMR phenomenon.

Low salt concentration is advantageous for improved experimental performance. At high salt heating during the experiment is a concern and tuning and matching is less efficient.

Sample must be stable over periods that can extend for more than a week at the experimental temperature to collect all the experiments necessary for structure determination.

You get your protein back.

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## Protein NMR

For all but the smallest proteins, isotopic labeling is necessary.

<i>Mol. Weight</i>	<i>Technique</i>	<i>Observed Spins</i>	<i>Dimensionality</i>
<10 kDa	Homonuclear	$^1\text{H}$	2D
10-15 kDa	$^{15}\text{N}$ -homonuclear <sup>†</sup>	$^1\text{H}, ^{15}\text{N}$	3D, 4D
15-30 kDa	Triple Resonance <sup>‡</sup>	$^1\text{H}, ^{15}\text{N}, ^{13}\text{C}$	3D, 4D
30-60 kDa	Triple Resonance/deuterated <sup>  </sup>	$^1\text{H}, ^{15}\text{N}, ^{13}\text{C}$	3D, 4D
60-100 kDa	Triple Resonance/deuterated/TROSY <sup>  </sup>	$^1\text{H}, ^{15}\text{N}, ^{13}\text{C}$	3D, 4D

<sup>†</sup>Requires uniform labeling of protein with  $^{15}\text{N}$ .

<sup>‡</sup>Requires uniform labeling with  $^{15}\text{N}$  and  $^{13}\text{C}$ .

<sup>||</sup>Requires uniform labeling with  $^{15}\text{N}$ ,  $^{13}\text{C}$  and replacement of CH groups with CD.

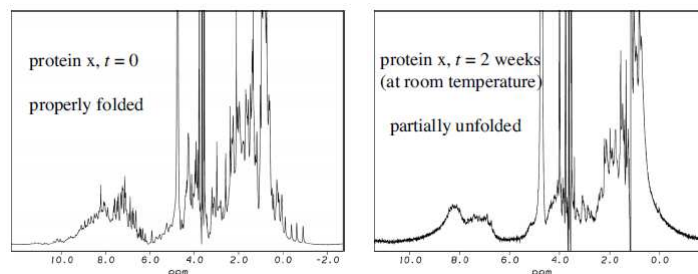
$^{15}\text{N}$  is cheap (~30€/l),  $^{13}\text{C}$  not cheap (~300€/l),  $^2\text{H}$  expensive (~1000€/l), specific side chain labeling (metyls) very expensive.

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## Obtaining a spectrum

Assessment of protein stability is mandatory before a structural determination is attempted.

Data collection takes more than one week and stability is crucial.



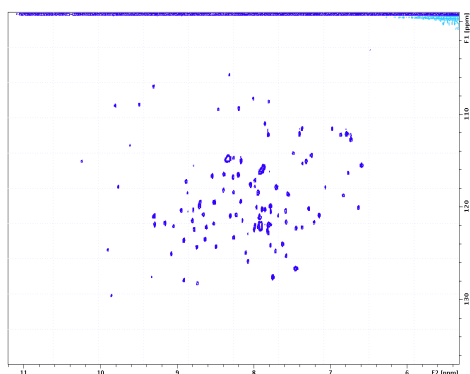
These spectra take less than 5 minutes to collect.

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## Looking at the spectrum

If your 1D data show that the protein is stable then you have to assess if the structure can be solved.

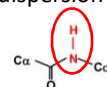
$^1\text{H}$ - $^{15}\text{N}$  HSQC



A spectrum looking like this tells the observer that you can produce your protein in adequate amounts

That it is pure (it should have ~1 peak per aminoacid)

That it is folded (good dispersion of peaks and no 'smears')



The assignment can be done setting the stage for all subsequent analysis

Protein structures- standard methods for up to 30kDa

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## How far can we go with solution NMR? With respect to structure

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2VDA

Solution structure of the SecA-signal peptide complex

PDB DOI: 10.2210/pdb2VDA/pdb

Classification: PROTEIN TRANSPORT

Organism(s): Escherichia coli

Expression System: Escherichia coli BL21(DE3)

Mutation(s): No

Deposited: 2007-10-01 Released: 2007-11-27

Deposition Author(s): Gellis, I., Bonvin, A.M.J.J., Keramisanou, D., Koukaki, M., Goundis, G., Karamanou, S., Economou, A., Kalodimos, C.G.

Experimental Data Snapshot

Method: SOLUTION NMR

Conformers Calculated: 200

Conformers Submitted: 10

Selection Criteria: LOWEST ENERGY

wwPDB Validation

Metric	Percentile Ranks	Value
Clashscore	2	2
Ramachandran outliers	0.8%	0.8%
Sidechain outliers	7.8%	7.8%

This is version 1.3 of the entry. See complete history.

Literature

Download Primary Citation

Structural Basis for Signal-Sequence Recognition by the Translocase Motor SecA as Determined by NMR

Gellis, I., Bonvin, A.M.J.J., Keramisanou, D., Koukaki, M., Goundis, G., Karamanou, S., Economou, A., Kalodimos, C.G. (2007) Cell 131: 756

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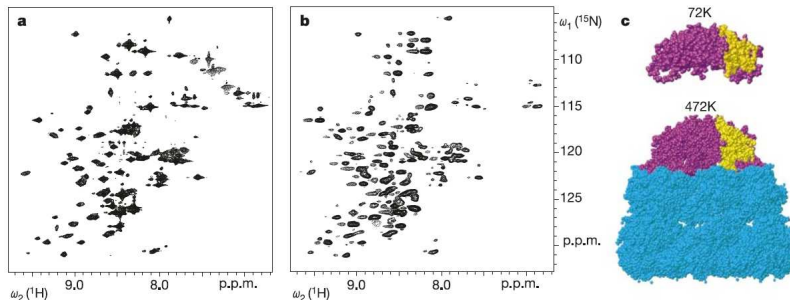
## How far can we go with solution NMR? With respect to function

### letters to nature

#### NMR analysis of a 900K GroEL–GroES complex

Jocelyne Fiaux<sup>1</sup>, Eric B. Bertelsen<sup>1</sup>, Arthur L. Horwich<sup>1</sup> & Kurt Wüthrich<sup>1\*</sup>

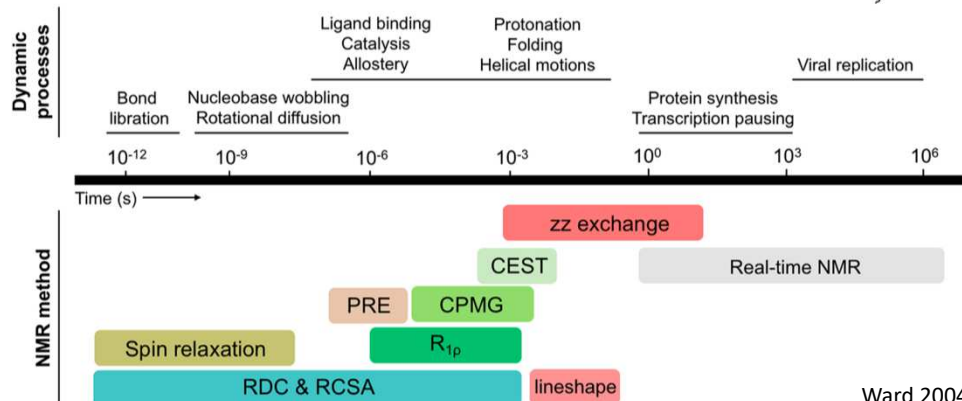
the same resonances whether free in solution or in complex with chaperonin; however, residues 17–32 show large chemical shift changes on binding. These amino acids belong to a mobile loop region of GroES that forms contacts with GroEL<sup>6–10</sup>. This establishes the utility of these techniques for solution NMR studies that should permit the exploration of structure, dynamics and interactions in large macromolecular complexes.



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## The biological playing field of NMR: Intrinsically Disordered Proteins

IDPs constitute ~33% of the proteome in eukaryotes, and are important in numerous diseases. About 70% of cancer related proteins are predicted to have long unstructured regions.



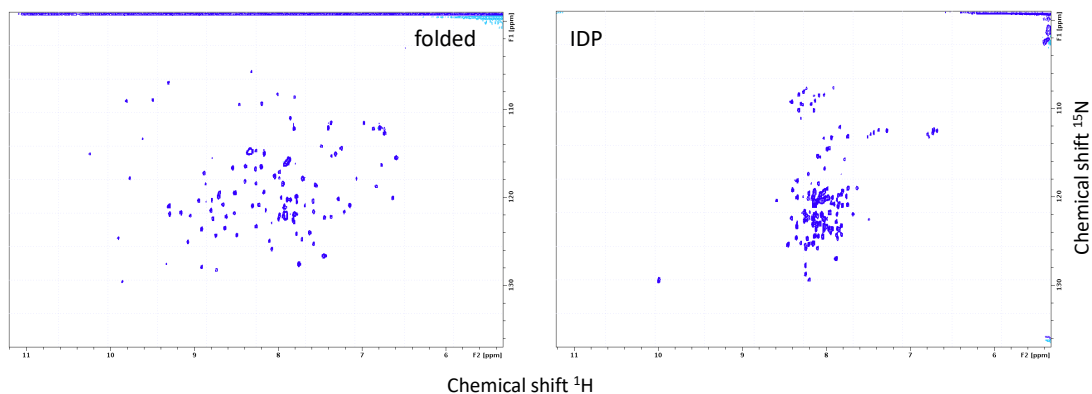
Ward 2004 J Mol Biol  
Daye 2022 Chem Rev

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## The biological playing field of NMR: IDPs

Analysis represents a challenge because of reduced spectral dispersion.

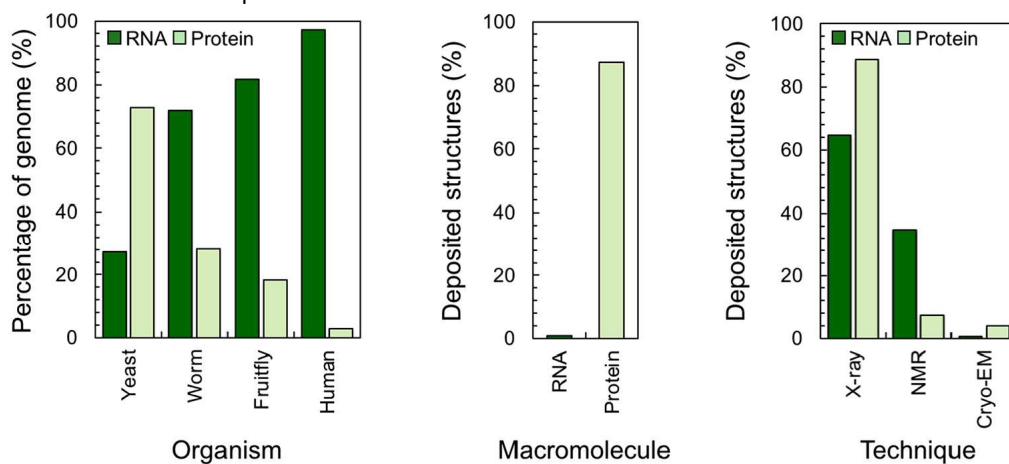
Protonless detection is essential due to additional spectral dispersion, but requires so-called cryoprobes with a sensitivity on up to 4X relative to standard probes.



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## The biological playing field of NMR: The "RNA world"

NMR contributes ~10% of protein structures in the PDB but ~35% of the RNA structures

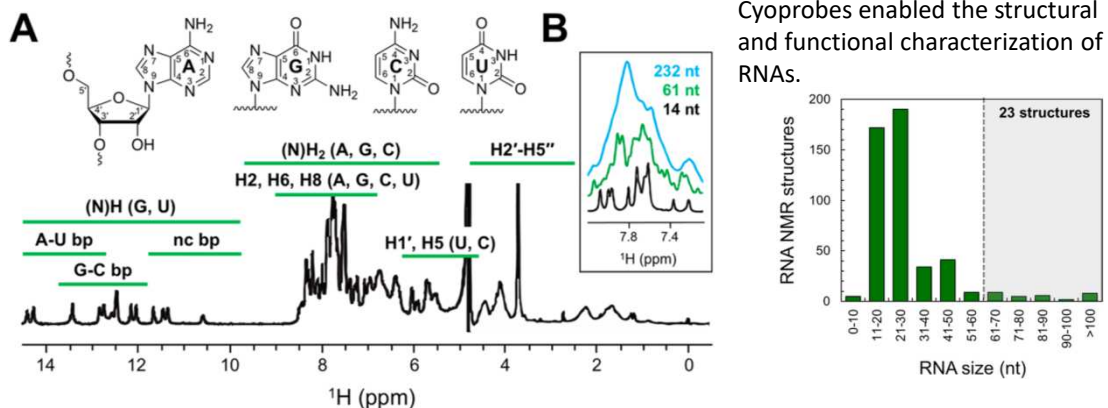


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## The biological playing field of NMR: The "RNA world"

Only 4 building blocks lead to poor spectral dispersion. A similar problem to that of IDPs.



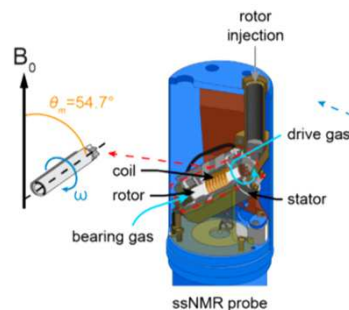
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## How does NMR handle solids?

The chemical shift and the spin-spin interactions depend on the orientation vs the magnetic field. Magic angle spinning allows averaging of the effect.

$$E = -\frac{\mu_{Iz}\mu_{Sz}}{r^3} (3\cos^2\theta - 1)$$



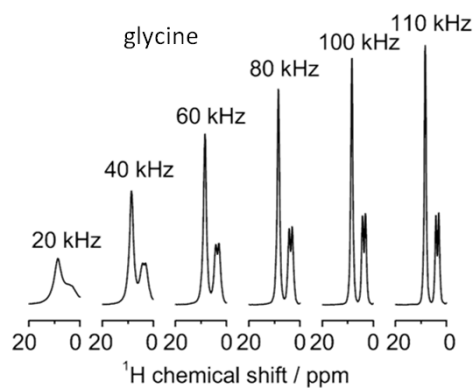
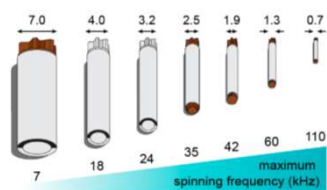
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## How does NMR handle solids?

Effect can be massive. Dipolar interaction depends on the gyromagnetic ratio of the nuclei involved and on the distance

Example: for two protons at 10 Å  $R^{DD} \sim 120$  kHz



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## In the solid state there is no size limit!

Angewandte  
Communications

Protein NMR Spectroscopy

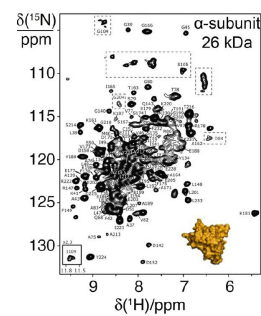
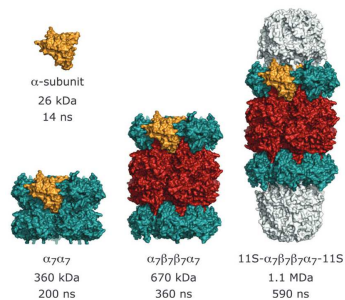
DOI: 10.1002/anie.201301215

### NMR Spectroscopy of Soluble Protein Complexes at One Mega-Dalton and Beyond\*\*

Andi Mainz, Tomasz L. Religa, Remco Sprangers, Rasmus Linser, Lewis E. Kay, and Bernd Reif\*

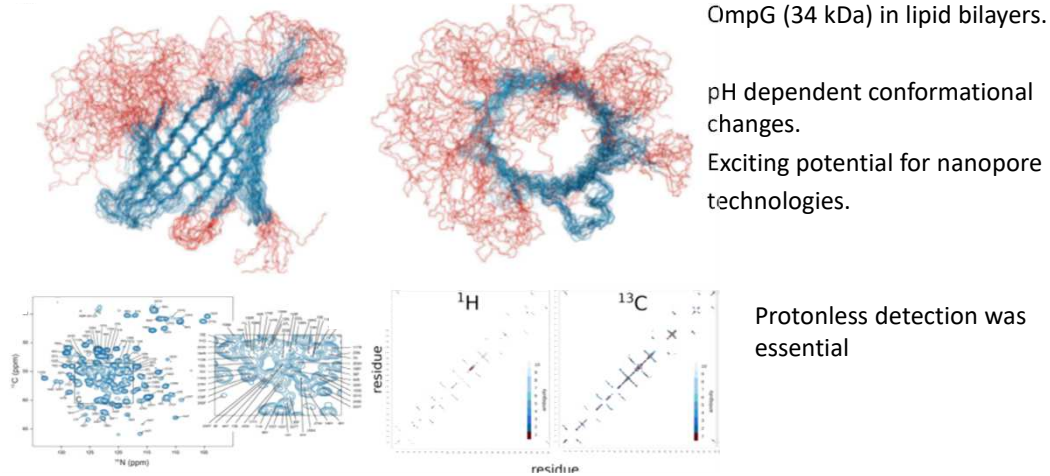
In Magic Angle Spinning SS NMR  
the linewidth is independent of  
the mass

The proteasome



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## The biological playing field of NMR: Membrane proteins



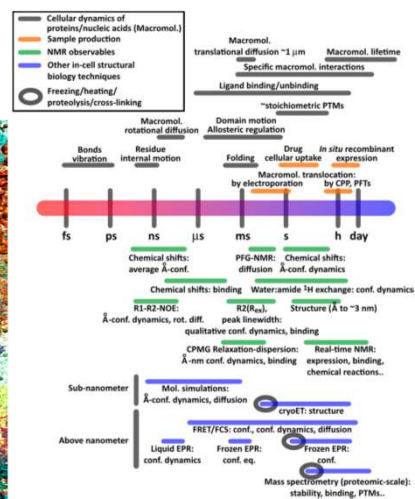
Ahlawat 2022 Chem Rev

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## NMR The biological playing field of NMR: In-cell NMR

The "real" physiological condition:

- 300 mM inorganic ions
- 200-200 mM metabolites
- 200-300g/l protein
- 20-100 g/l RNA

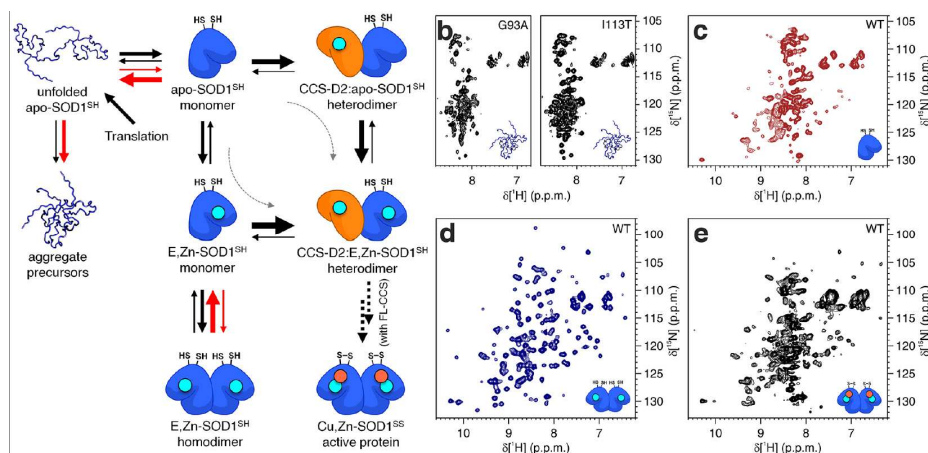


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## NMR The biological playing field of NMR: in-cell NMR

About 40% of the proteome contains metallic co-factors. How does the assembly take place?

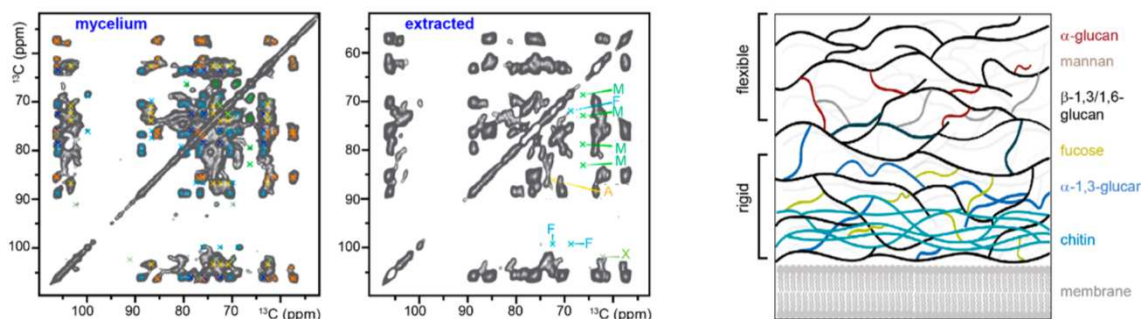


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## The biological playing field of NMR: fibers and polymers

The mycelium of a fungus can be analysed and a model for its structure developed

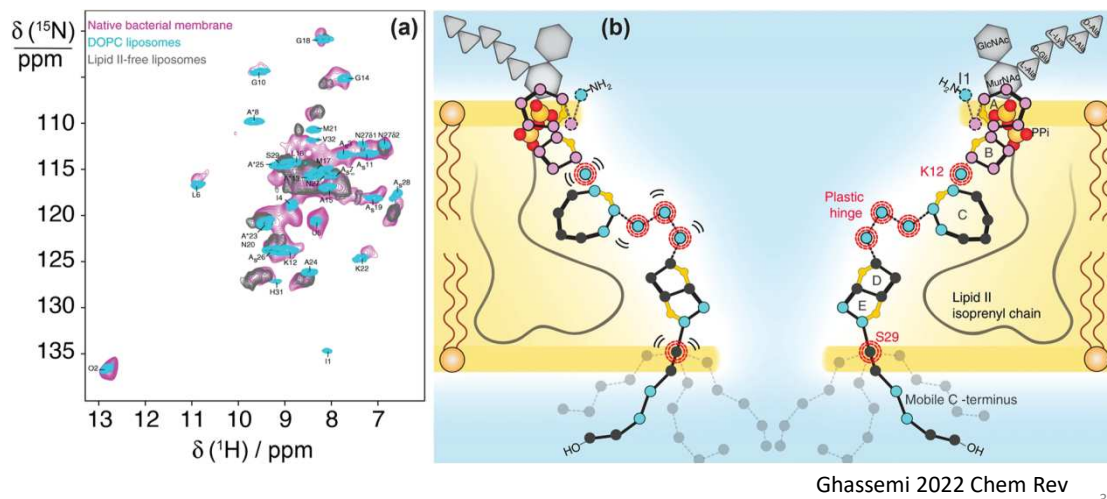


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## The biological playing field of NMR: fibres and polymers

The mode of action of lantibiotics on bacterial membranes can be investigated.



## Take home message

NMR spectroscopy can see “all”.

NMR spectroscopy can be performed in all states of the matter relevant to chemistry and biology and in conditions that can mimic those of the target systems.

NMR spectroscopy can investigate biological phenomena at diverse spatial and temporal scales with atomic resolution.

You get your sample back!