TA12 - Protein NMR

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique used by scientists to help determine the structure of a molecule. NMR is a beneficial tool for biochemists because it helps reveal the structure and dynamics of proteins. This is particularly useful for the development of new drugs, since many proteins have a significant role within pharmaceuticals. For example, the use of insulin to treat diabetes.

Firstly, a scientist must retrieve a pure sample of the required protein, as any impurities would obscure results and lead to false positives. The source of this protein may be natural, such as those extracted from humans or bacteria, or, alternatively, a protein might be synthesised through a technique called protein production.

When prepared, the sample is often dissolved in a buffer solution and adjusted to the correct conditions. Scientists need to be careful when choosing a solvent, as some can interfere with the NMR spectrum, particularly solvents containing simple hydrogen atoms. The sample is then placed in a thin walled glass tube. Although most NMR is carried out on aqueous samples, NMR using sold samples is also possible.

Key word: Buffer Solution

A buffer solution is a solution in which the pH does not change significantly when a small amount of acid or base is added to it.

NMR BASICS

• The sample is placed in a strong magnetic field which causes some nuclei to turn to face the same direction as the magnetic field is pointing, and some to turn to face the opposite direction. Those facing in the opposite direction are less stable.

• The sample is then exposed to radio waves of different energies (frequencies). When they are exposed to exactly the right amount of energy, some nuclei facing the opposite direction will flip so that they are facing in the same direction as the magnetic field. This flipping of the nucleus from one magnetic alignment to the other by the radio waves is known as the resonance condition.

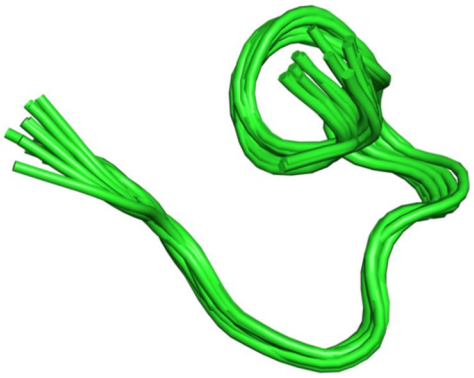
• It's possible to detect this interaction between the radio waves of just the right frequency and the nucleus as it flips from one orientation to the other and plot it as a peak on a graph.

• A graph produced from an NMR spectrometer shows a horizontal axis marked ‘chemical shift’ which is measured in parts per million (ppm). Each peak on the graph corresponds to the environment in which each nucleus is situated.

• The results can then be used to deduce a protein’s structure by combining results from several different tests. It is important to note that protein NMR is much more complex than NMR with simple molecules as proteins are generally large and intricate. Scientists use computer programmes, such as CYANA, to help them develop a 3D visualisation of their results.

Produced by Anna McKie at Newcastle University as part of a Nuffield Research Placement.

A typical full-size NMR machine can cost upwards of £500,000. However, a smaller, portable machine can be picked up for a few thousand pounds for use in classrooms and practical laboratories.



Protein NMR has many practical uses. For example, it was identified that the venom of the predatory snail *Conus geographus*, whilst being potent enough to kill humans, could be used as a strong painkiller by isolating some of the proteins in the toxin. The image on the right shows an isolated snail venom protein whose structure was determined using solution NMR.

PROBLEMS WITH PROTEIN NMR, AND EXPERIMENTAL RESULTS

One problem with generating models using techniques such as NMR is that the finished product is only a representation of experimental data. This is a crucial fact to acknowledge because the model may be a good or bad representation of the results.

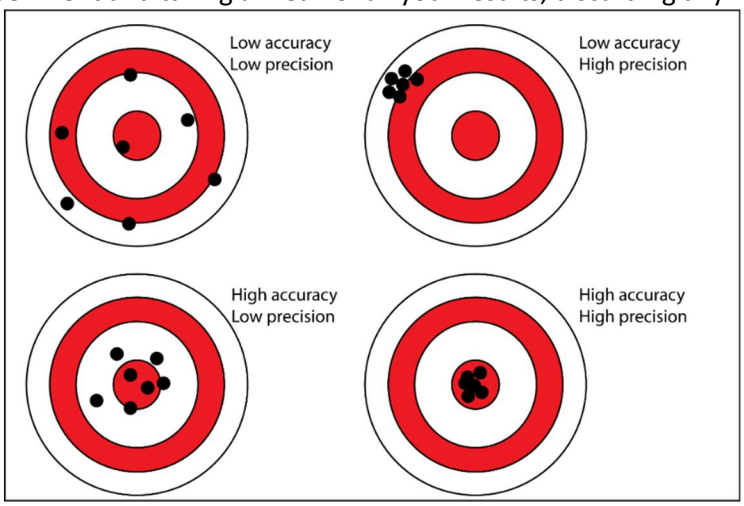
Every experiment has associated error. Error can be systematic or random:

SYSTEMATIC ERROR

Systematic errors have the same impact every time an experiment is repeated. They can be caused by the set-up of equipment, or the actual equipment itself. Systematic errors affect the accuracy of results. The accuracy refers to how closely the derived results match the 'true' value. The 'true' value is difficult to pinpoint as far as molecular modelling is concerned. In general, NMR accuracy is given by the degree of agreement between the model and a set of experimental data.

RANDOM ERROR

Random errors vary each time an experiment is repeated. They may be caused by human error, a faulty technique in taking the measurements, or by faulty equipment. Random errors cause readings to be spread out around the 'true' value, due to results varying in an unpredictable way from one result to another. The precision of the resulting structures is affected by random error. Precision refers to the degree of reproducibility and reliability of any measurements. Results can be made more precise by repeating an experiment and taking a mean of all your results, discarding any outliers.



A visual representation of accuracy and precision