Appendix A

NMR Glossary

Alignment medium: cf. **Residual dipolar couplings**.

Alignment tensor: cf. **Residual dipolar couplings**.

Assignment (**resonance assignment**): the process by which the signals in a spectrum are correlated to the **NMR-active nuclei** that give rise to them. A particular nucleus (e.g. Hα of Ala^{281} of the protein pleckstrin) is said to be ***assigned*** if its resonance frequency (e.g. 4.327ppm) is known. Equally, a resonance **signal** or **peak** is said to be ***assigned*** if it is known which nucleus or nuclei give rise to it. Hence, ***NOE assignment*** is the process of identifying which nuclei give rise to **peaks** in **NOESY** spectra.
For proteins, the first step of resonance assignment is to assign the backbone resonances H^N, N Cα, Cβ and C' of every residue (***backbone assignment***) using so-called triple-resonance experiments (e.g. HNCA). Protein sidechain resonance assignment (***sidechain assignment***) is carried out after backbone assignment. Usually, **TOCSY**-type experiments are used to connect all atoms in the sidechain (the entire **spin-system**) to already assigned "anchor" points in the backbone, e.g. the H^N/N and Hα/Cα groups.

Atom nomenclature: In the description of NMR experiments and other features, certain abbreviations or unusual naming conventions are used. Here is a list of the most important NMR specific atom/nucleus names:
CA: Cα
CB: Cβ
CO: carbonyl carbon, C' (IUPAC: C)
Carbon: by convention often refers to ^{13}C

D: deuteron, ^{2}H

D_2O: $^{2}H_2O$, heavy water

HA: Hα

H^N: amide proton (IUPAC: H)

Nitrogen: by convention often refers to ^{15}N

Proton: ^{1}H (hydrogen) nucleus.

Chemical shift (δ): the resonance frequency (the characteristic frequency of a nucleus) of NMR signals is most commonly given as ***chemical shift*** in units of **ppm**.

Correlation time (τ_c): a time constant that is a measure of how fast a molecule tumbles in solution. It depends on the size and the shape of a molecule (and also on many other parameters such as temperature, viscosity …). Small domains (5-10kDa) have ***correlation times*** in the range of 4-8ns.

COSY: **CO**rrelation **S**pectroscop**Y**. The ***COSY*** experiment correlates two **spins** that are connected via a single **J-coupling**. **HSQC**, **HMQC** are **heteronuclear *COSY*** experiments.

Diamagnetic: a substance with only paired electrons.

Dynamics (dynamical processes, backbone, slow, fast): ***dynamics*** are movements of parts of a molecule. Typically, backbone ***dynamics*** are measured in **^{15}N relaxation** experiments. They can be classified into fast (i.e. faster than the **correlation time**, nanosecond and sub-nanosecond time-scale) and slow (i.e. slower than the **correlation time**, micro to millisecond time-scale). **Exchange** is also a ***dynamical process***.

Ensemble: a family of structures that are calculated in parallel in a **NMR structure calculation**.

Exchange (intermediate, fast, slow, chemical and conformational): ***Exchange*** is a kinetic process between two (or more) states that is characterised by a rate constant. In NMR spectroscopy, ***exchange*** is classified into fast, intermediate and slow depending on the size of the rate constant with respect to **chemical shift** (which is measured in the same units (Hz) as the rate constant). In the ***fast exchange*** regime, the rate is much larger than the difference in **chemical shift** between the two states and the NMR signal will be at the weighted average position of the two states. In ***slow exchange***, signals for both states are observable and the intensities are proportional to the populations of the two states. In ***intermediate exchange***, the transition between the signals seen for slow and fast exchange takes place, resulting in very broad and often non-observable signals.
C***onformational exchange*** occurs when a structural element has more than one possible conformation. ***Chemical exchange*** is given when a chemical reaction is taking place, e.g. a ligand binds to the molecule that is measured or protons ***exchange*** with the solvent.

Exchangeable proton: A ^{1}H atom of a macromolecule that under mild conditions (neutral pH, ambient temperature and pressure) is replaced by a ^{1}H atom from the solvent (water) after a certain time. The time constant is important, since it is used to classify protons into: a) ***non-exchangeable***: all carbon-bound protons, that do not exchange at all with the solvent even over very long periods (years); b) ***exchangeabl***e: all OH, SH and NH protons that exchange within seconds or minutes; c) ***slowly exchanging***: protons that are in principle exchangeable, but have much slower rate (hours, days, weeks) than the other protons of the same type. ***Slowly-exchanging*** protons are in some way shielded from the solvent, for example H^N in hydrogen bonds in secondary structure elements; d) ***fast-exchanging***: OH and SH which exchange so fast with water that their signal is normally not observable.

Fourier Transform (**FT**): a mathematical operation that transforms time-domain data (intensities measured at discrete time intervals) into a frequency-domain spectrum and vice versa.

Heteronuclear: between nuclei that are not of the same isotope.

Heteronuclear NOE ({^{1}H}-^{15}N-heteronuclear NOE, hetNOE): a special case of the ***NOE*** experiment where magnetisation is transferred from ^{1}H to a **heteronucleus**. The ***NOE*** is actually a cross-relaxation rate, in that non-equilibrium states of one nucleus affects other nuclei. Since the ***NOE*** transfer rate depends on dynamics, the ***{^{1}H}-^{15}N-heteronuclear NOE*** is a very convenient way to identify flexible residues which have low ***hetNOE*** values.

Heteronucleus: a nucleus that is not of the same isotope as another. Very often, it means "other than ^{1}H", i.e. ^{15}N or ^{13}C.

HMQC: heteronuclear multiple-quantum correlation. This experiment is very similar to the **HSQC**. It also correlates ^{1}H to a **heteronucleus**. It is mostly used for ^{1}H,^{13}C correlations.

Homonuclear: between nuclei of the same isotope, e.g. ^{1}H-^{1}H or ^{13}C-^{13}C. Very often, it refers to experiments with unlabelled proteins that only deal with ^{1}H.

HSQC: heteronuclear single-quantum correlation. This type of experiment correlates ^{1}H to a **heteronucleus**, for example to ^{15}N. In fact, the 2D ^{1}H,^{15}N-HSQC is the most popular spectrum in biomolecular NMR because it yields one peak per ^{1}H/^{15}N group, i.e. almost exactly one peak per residue of a protein. Therefore the 2D ^{1}H,^{15}N-HSQC is a "fingerprint" experiment, in which changes in the environment of every residue can be easily followed. This is exploited in binding site mapping experiments (also known as NMR titrations).

Isotopic labelling (isotopic enrichment): enrichment of the abundance of a particular isotope in a macromolecule by (bio)synthesis from isotopically enriched precursors, e.g. $^{15}NH_4Cl$, $^{13}C_6$-glucose, heavy water ("D_2O").

J-coupling (**scalar coupling**): a through-bond interaction between nuclear spins. One manifestation of the ***J-coupling*** is the splitting of resonance lines. ***J-couplings*** can also be used to transfer magnetisation between two coupled spins. In proteins, mostly **heteronuclear *J-couplings*** are important because a) they are large and b) they can be used to transfer magnetisation from ^{1}H (the most sensitive nucleus) to ^{15}N and ^{13}C (which are quite insensitive).

J-couplings in proteins are measured either as line-splitting or as peak intensity ratios which depend on the size of the coupling (quantitative J experiment). ^{3}J-couplings (J-couplings over 3 bonds) contain torsion angle information. For example, the size of the $^{3}J_{HNHA}$ coupling varies between 4 and 12Hz depending on the backbone conformation (helical or extended).

Linebroadening: the linewidth of a NMR signal (**resonance line**) is dependent on R2, the transverse relaxation rate. If R2 is increased, the signals are broader. Since the peak volume (the integral over all points of the signal) stays constant, a broader signal has a lower intensity. This means that very strongly broadened peaks have such a low intensity that they disappear in the noise of the baseline, i.e. they are no longer detectable.

Linewidth: width of a NMR **resonance line**, measured at half-height.

Lorentzian: a function that is obtained by **Fourier transformation** of an decaying exponential function. The formula of a ***Lorentzian line*** for a signal in the frequency domain at $\omega=\omega_0$ is given by:

$$I(\omega) = R2 / (R2^2 + 4\pi^2(\omega-\omega_0)^2)$$

where **R2** is the transverse relaxation rate. It can be easily shown that the maximum peak height (at $\omega=\omega_0$) is equal to 1/**R2**. The half-height width of the peak is equal to **R2**/π.

NMR-active nucleus: a nucleus with **spin** I > 0. The most conveniently studied isotopes are of **spin** I= ½, i.e. ^{1}H, ^{13}C, ^{15}N, ^{31}P.

NOESY: **N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**. In a ***NOESY*** experiment, nuclei are correlated that are close in space (<5Å). Therefore, peaks in a ***NOESY*** contain distance information that can be used to determine the structure of a molecule. NMR jargon often refers to a ***NOESY*** peak as a "**NOE**"; a **NOE** between two nuclei therefore corresponds to a short distance.

Paramagnetic: a substance with one or more unpaired electrons.

Paramagnetic relaxation enhancement (PRE): the effect of a **paramagnetic** group (e.g. **spin-label**) on nuclear **spins**. The electron spin induces very strong relaxation, so that the signals of nearby nuclear spins are broadened (cf. **linebroadening**). PRE is distance dependent; therefore it can be used as a **restraint** in a **NMR structure calculation**.

Peak: NMR signal. A signal in a 1D NMR spectrum is typically a **Lorentzian** line. 2D signals are the convolution of two of these lines. Since 2D (and also 3D) NMR experiments are represented as contour plots in a similar manner as topographic maps, a 2D signal has the appearance of a peak.

ppm: parts per million, units of **chemical shift**.

R1: cf. **relaxation rates**

R2: cf. **relaxation rates**

Relaxation: a process by which a system returns to thermal equilibrium.

Relaxation rates (**R1**, **R2, ^{15}N relaxation**): relaxation of magnetisation is governed by exponential decay rates. There are two important relaxation rate constants: ***R1,*** the longitudinal relaxation and ***R2,*** the transverse relaxation rate. R1 and R2 depend on the

correlation time and hence on the size of the molecule, e.g. larger proteins have higher R2 rates. Both R1 and R2 also depend on local dynamics, therefore **^{15}N relaxation** experiments can be used to analyse backbone dynamics.

Residual dipolar couplings (RDCs): dipolar couplings are a through-space interaction between NMR active nuclei. In solution NMR, these couplings are averaged to zero due to the fast rotation of the molecule. It is however possible to re-introduce dipolar couplings by putting the molecule into a liquid medium that has some degree of order (**alignment medium**). For example, lipid bicelles orient their long axis along the static magnetic field of a spectrometer. Macromolecules collide with the oriented phase and become partially aligned themselves. ***RDCs*** provide information on orientation of the bond vectors with respect to an external reference frame (the "**alignment tensor**") and thus on the orientation between structural elements in the molecule.

Resonance frequency: the ***resonance frequency*** of a **NMR-active nucleus** is the frequency at which its signal appears in the spectrum.

Resonance line: NMR signal of a 1D (or a 1D trace in a 2D or 3D). A ***resonance line*** is characterised by its position in the spectrum (**resonance frequency**), its height (signal intensity) and its width (**linewidth**). Cf. **Lorentzian**.

Restraint (**structural R.**): structural information of NMR spectra is stored in a format called ***restraint***. A ***restraint*** defines a geometric value (a distance between two atoms, an angle between two bonds) with appropriate upper and lower bounds. In a **structure calculation**, the molecule is forced to fulfil the ***restraint***, i.e. the value of the distance or the angle is restrained to stay within the upper and lower bounds of the restraint.

Spin: quantum-mechanical property of a nucleus. In NMR, only nuclei with ***spin*** I>0 are observable. In conventional NMR jargon "***spin***" often means "**NMR active nucleus**".

Spin-label: a **paramagnetic** group that is attached to a macromolecule

Spin-system: all nuclei of the same isotope type that are connected via **J-couplings**. For example, all protons in an aromatic ring belong to a spin system. More importantly, the amino acid residue constitutes a spin-system for both carbon and proton, because there are no **homonuclear** J-couplings across the peptide bond.

Structure calculation: a NMR ***structure calculation*** combines **structural restraints** with known geometric information (bond lengths, atom radii, etc.). It is a molecular dynamics simulated annealing calculation that enforces the **structural restraints** to drive an initial random structure towards a final structure. Usually, a large number of structures (**an ensemble**) are calculated from different random seeds in parallel, so as to judge how well the restraints define the structure of a molecule.

Time-scale: order of magnitude of time (cf. **dynamics**).

Triple resonance experiments: 3D experiments that connect the resonances of three different isotopes (^{1}H, ^{15}N, ^{13}C). They are primarily used for protein **backbone assignment**. For example, the HNCO experiment connects the resonances of an amide group (H^N, N, C'). Similarly, a HNCA experiment connects amide proton and nitrogen to the Cα of its own residue, but also to the Cα of the previous residue. All ***triple resonance experiments*** make use of the large **heteronuclear J-couplings** in the protein backbone by which magnetisation is transferred from one nucleus to the other. The name of an experiment is a shorthand notation of the pathway the magnetisation takes: in the HN(CO)CA experiment, the magnetisation is transferred from H^N to N and *via* C' to Cα. The nuclei that are in brackets indicate the path the magnetisation takes; their **chemical shift** is not recorded. A **peak** in a 3D-HN(CO)CA is at the position of the frequencies of H^N, N of a given residue and CA of the preceding residue.

TOCSY: **TO**tal **C**orrelation **S**pectroscop**Y**. The ***TOCSY*** experiment transfers magnetisation via **J-couplings.** All nuclei in a **spin-system** are correlated (hence "total correlation"). For proteins, this means that all nuclei of the same isotope of a residue can

be assigned conveniently with ***TOCSY*** experiments. TOCSY experiments are used for protein sidechain assignment.

TROSY: **T**ransverse **R**elaxation **O**ptimised **S**pectroscop**Y**. The ***TROSY*** is an experiment that yields very similar spectra to the **HSQC**, only that it is optimised for large and deuterated proteins.