Identifying amino acids in protein NMR spectra:

1) Glycine (Gly, G)

Glycine is the only amino acid with 2 alpha protons (Hα1 and Hα2). Often the HN-Hα coupling is observed for both alpha protons, along the same amide H line of the COSY or TOCSY spectrum. The Hα1 to Hα2 coupling is usually quite strong, and can be seen in COSY or TOCSY spectrum. Sometimes the two alpha protons have equal or nearly equal chemical shifts, so the Hα1 to Hα2 coupling may not be observed.

Be careful not to confuse glycine with threonine: Note that Hα and Hβ of threonine have similar to Hα1 and Hα2 of glycine.

The ^{15}N amide nitrogen chemical shift is usually in the range of 104 to 115 ppm, slightly lower than the amide ^{15}N chemical shift of other amino acid types.

The ^{13}C alpha carbon chemical shift is usually in the range of 43 to 47 ppm, slightly lower than the ^{13}C alpha carbon chemical shift of other amino acid types.

2) Alanine (Ala, A)

Look for the strong methyl to Hα coupling in COSY or TOCSY.

Coupling from amide proton to methyl group is usually observed in TOCSY.

The ^{13}C alpha carbon chemical shift is usually in the range of 50 to 53 ppm, slightly lower than the ^{13}C alpha carbon chemical shift of other amino acid types (except glycine).

The ^{13}C beta carbon chemical shift is usually near 20 ppm, slightly lower ^{13}C beta carbon chemical shift of other amino acid types.

3) Valine (Val, V)

Look for 2 methyl groups coupled to the same beta proton, in COSY or TOCSY.

Coupling from Hα to both methyl groups is usually observed in TOCSY.

Coupling from amide proton to both methyl groups is sometimes observed in TOCSY.

4) Serine (Ser, S)

Chemical shifts of the two Hβ are distinctive, near 3.6 ppm (but don't confuse with Cys, which has two Hβ near 3.2 ppm).

5) Threonine (Thr, T)

Threonine is unique in that Hα and Hβ are both usually between 4 and 5 ppm. Sometimes the chemical shift of Hβ is greater than Hα.

Look for strong Hβ to Hγ peak in TOCSY and COSY (near alanine Ha to Hb). Unlike alanine, threonine usually has strong Hα to Hγ peak in TOCSY.

Hα to Hβ peak in COSY and TOCSY can usually be seen near the diagonal, between 4 and 5 ppm).

6) Cysteine (Cys, C)

Chemical shifts of the two Hβ are distinctive, near 3.2 ppm (but don't confuse with Cys, which has two Hβ near 3.6 ppm).

7,8) Aspartic acid (Asp, D) and Asparagine (Asn, N)

Chemical shifts of the two Hβ are distinctive, near 2.6 ppm (but similar to Hβ of Phe, His, Tyr, Trp).

In Asn and Gln, there is often a TOCSY peak between the two amine protons (near 6.9 to 7.6 ppm).

In Asn and Gln, there are often NOE peaks between the two amine protons (near 6.9 to 7.6 ppm) and the two Hβ near 2.6 ppm.

9-11) Glutamic acid (Glu,E), Glutamine (Gln,Q), Methionine (Met,M)

These three amino acid types are distinctive in that the two Hβ chemical shifts are greater than the two Hγ chemical shifts (Hβ near 2.2 ppm, Hγ near 2.6 ppm).

In Gln and Asn, there is often a TOCSY peak between the two amine protons (near 6.9 to 7.6 ppm).

In Gln and Asn, there are often NOE peaks between the two amine protons (near 6.9 to 7.6 ppm) and the two Hγ near 2.6 ppm.

The methionine methyl group is usually a sharp singlet line near 2 ppm, with no through bond coupling to any other protons.

12) Isoleucine (Ile, I)

The four-bond coupling between Hα and gamma methyl group is usually observed as a strong peak in TOCSY.

Coupling from amide proton to gamma methyl group is usually observed in TOCSY.

13) Leucine (Leu, L)

Look for 2 methyl groups coupled to the same Hγ, in COSY or TOCSY (be careful not to confuse with valine).

Coupling from Hα to both methyl groups is usually observed in TOCSY (be careful not to confuse with valine).

Coupling from amide proton to both methyl groups is sometimes observed in TOCSY (be careful not to confuse with valine).

14,15) Lysine (Lys, K) and Arginine (Arg, R)

Lys and Arg are difficult to distinguish since each has two Hβ near 1.7 ppm and two Hγ near 1.5 ppm.

In arginine, the side chain amide proton is often observed near 7.2 ppm, and coupling from side chain amide to Hε and Hδ is often observed.

The side chain amine of Lys is often not observed, or is often a broad peak.

Strong (often overlapping) peaks in COSY and TOCSY near 3.1 to 1.6 ppm are lysine Hδ to Hε.

Strong (often overlapping) peaks in COSY and TOCSY near 3.3 to 1.6 ppm are Arg Hδ to Hε.

16) Proline (Pro, P)

The Hγ to Hδ couplings appears in a relatively sparse region of the COSY and TOCSY spectrum, near 2.1 to 3.6 ppm).

NOE peaks from Hδ to amide HN of the next amino acid in the sequence are often observed.

17) Tryptophan (Trp, W)

The four protons on the ring farthest from the protein backbone (chemical shifts usually between 6.5 and 7.8 ppm) are coupled through TOCSY peaks (for 3, 4 and 5-bond couplings) and COSY peaks (for 3-bond couplings). In TOCSY, 3-bond coupling is usually stronger than 4-bond and 5-bond coupling, though all are usually observed.

The ring HN proton of tryptophan has a chemical shift near 10 ppm.

There is usually a strong NOE peak from the ring HN proton to the nearest C-H proton on the same 5-member ring.

There is usually a strong NOE peak from the ring HN proton to the nearest C-H proton on the 6-member ring.

There is sometimes a TOCSY peak from the ring HN proton to the nearest C-H proton on the same 5-member ring.

There is no TOCSY or COSY peak connecting Hβ to the ring protons.

There are usually strong NOE peaks from the two Hβ protons to the C-H proton on the 5-member ring.

18) Tyrosine (Tyr, Y)

There are usually 2 unique proton chemical shifts on the tyrosine ring (Hδ and Hε) near 7 ppm. Hδ1 and Hδ2 usually have equivalent chemical shifts, as do Hε1 and Hε2.

The two Hβ usually have strong NOE peaks to the ring proton nearest the Hβ.

There is no TOCSY or COSY peak connecting Hβ to the ring protons.

19) Phenylalanine (Phe, F)

There are usually 3 unique proton chemical shifts on the Phe ring (Hδ and Hε and Hζ) near 6.5 to 7.5 ppm. Hδ1 and Hδ2 usually have equivalent chemical shifts, as do Hε1 and Hε2.

The two Hβ usually have strong NOE peaks to the ring proton nearest the Hβ.

There is no TOCSY or COSY peak connecting Hβ to the ring protons.

20) Histidine (His, H)

The two C-H ring protons usually have chemical shifts between 6.5 and 8.5 ppm, with the ring proton nearest the Hβ having the lower chemical shift.

The two Hβ usually have strong NOE peaks to the ring proton nearest the Hβ.

Chemical shifts of histidine ring protons are usually quite pH dependent, due to the pKa of one of the ring N-H protons being near 6.5. Chemical shifts of the two ring C-H protons usually are higher at low pH. The histidine ring N-H protons are not usually observed.

The two histidine ring C-H protons are usually sharp lines (singlets). Sometimes a weak TOCSY or COSY peak is observed between the two C-H protons of the ring.

The two Hβ usually have strong NOE peaks to the ring C-H proton nearest the Hβ.