

Identifying amino acids in protein NMR spectra:

### 1) Glycine (Gly, G)

Glycine is the only amino acid with 2 alpha protons (H 1 and 2). Often the HN-H coupling is observed for both alpha protons, along the same amide H line of the COSY or TOCSY spectrum. The H 1 to H 2 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 H 1 to H 2 coupling may not be observed.

Be careful not to confuse glycine with threonine: Note that H and H of threonine have similar to H 1 and H 2 of glycine.

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

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

### 2) Alanine (Ala, A)

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

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

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

The  $^{13}\text{C}$  beta carbon chemical shift is usually near 20 ppm, slightly lower  $^{13}\text{C}$  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 H 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 H are distinctive, near 3.6 ppm (but don't confuse with Cys, which has two H near 3.2 ppm).

#### **5) Threonine (Thr, T)**

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

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

H to H 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 H are distinctive, near 3.2 ppm (but don't confuse with Cys, which has two H near 3.6 ppm).

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

Chemical shifts of the two H are distinctive, near 2.6 ppm (but similar to H 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 H 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 H chemical shifts are greater than the two H chemical shifts (H near 2.2 ppm, H 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 H 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 H 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 H, in COSY or TOCSY (be careful not to confuse with valine).

Coupling from H 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 H near 1.7 ppm and two H 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 H and H 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 H to H.

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

## **16) Proline (Pro, P)**

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

NOE peaks from H 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 H to the ring protons.

There are usually strong NOE peaks from the two H 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 (H and H) near 7 ppm. H 1 and H 2 usually have equivalent chemical shifts, as do H 1 and H 2.

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

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

### **19) Phenylalanine (Phe, F)**

There are usually 3 unique proton chemical shifts on the Phe ring (H and H and H) near 6.5 to 7.5 ppm. H 1 and H 2 usually have equivalent chemical shifts, as do H 1 and H 2.

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

There is no TOCSY or COSY peak connecting H 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 H having the lower chemical shift.

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

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