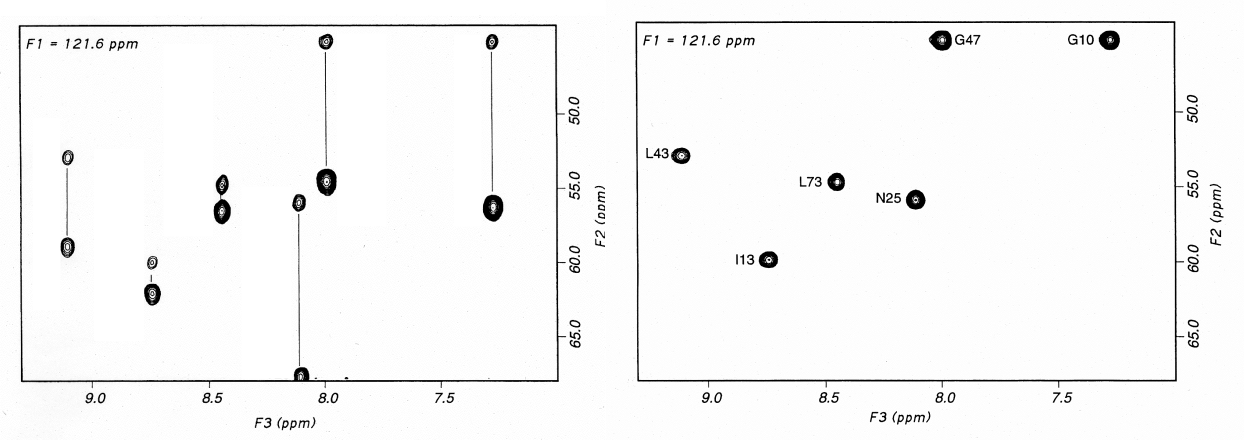
PROBLEM SET 6, BCMB/CHEM 8190

1) A NOESY experiment is conducted on a small protein using a relatively short mixing time (50 ms). For a particular glycine residue in the protein, the observed crosspeaks between ^{1}H nuclei are identified and the intensities determined. The observed intensities are shown below (arbitrary units). Using the distance between the two alpha protons in this amino acid (1.78 Å) as a distance standard, estimate the dihedral angle ϕ (phi) for this amino acid. Use other standard bond and angle geometries as necessary.

NOE intensities: $H\alpha_1$- $H\alpha_2$ = 28.0, $H\alpha_2$- H_N = 1.60, $H\alpha_1$- H_N = 0.5
Standard distances: $H\alpha_1$- $H\alpha_2$ = 1.78 Å, N-Cα = 1.46 Å, Hα-Cα= 1.1 Å, N-H_N = 1.08 Å
Standard dihedral angles: O-C-N-H_N = 180°

2) Below are planes from a 3D HNCA and a 3D HN(CO)CA spectrum of a particular protein. The planes are both at a ^{15}N chemical shift of 121.5 ppm. The signals in the HN(CO)CA spectrum are assigned.

a) Assign the individual signals in the HNCA spectrum (just the residue number).
b) In the HNCA spectrum, why are all of the upfield peaks weaker (lower intensity)?
c) In the HNCA spectrum, will upfield peaks always be weaker? Explain.



3) You've worked very hard, and almost have your protein backbone (and beta) resonances completely assigned. You have only a small stretch left, consisting of residues 124-130, shown below:

(123)	124	125	126	127	128	129	130	(131)
(LEU)	GLU	ILE	VAL	THR	ALA	ILE	PHE	(LYS)

Moreover, you have determined (correctly) that the data you have remaining in the spectra that you have collected are enough to complete the assignments.

You have collected high-resolution 2D ^{1}H, ^{15}N-HSQC and ^{1}H, ^{13}C-HSQC spectra. You can assume that the chemical shifts of the peaks in the HSQC spectra are 'perfect' in the sense that there are no errors associated with these values. You have also collected a variety of triple resonance assignment data as well as heteronuclear resolved NOESY data. Because these spectra are 3D, however, you could not collect these spectra with as much resolution. So, the chemical shifts obtained from the triple resonance and heteronuclear resolved NOESY spectra might differ from the 'correct' (HSQC) shifts by the following amounts:

^{15}N, $^{13}C\alpha$, $^{13}C\beta$: ± 0.2 ppm $^{1}H_N$, $^{1}H\alpha$, $^{1}H\beta$: ± 0.02 ppm

(you may assume that the ^{13}C=O chemical shifts that you have obtained from the HNCO experiment are perfectly correct, that is they have no errors associated with them)

It is also important to remember that certain triple resonance experiments are not all that sensitive, so that not all the peaks that might be observed actually are observed.

There are 7 remaining peaks in the ^{1}H, ^{15}N-HSQC spectrum, one corresponding to each of the 7 residues that remain unassigned:

	1	2	3	4	5	6	7
$^{1}H_N$ (ppm)	8.76	7.55	7.78	8.40	7.74	8.59	7.77
^{15}N (ppm)	119.1	112.9	124.0	121.8	120.6	116.5	123.8

There are 14 remaining peaks in the ^{1}H, ^{13}C-HSQC spectrum corresponding to alpha and beta resonances residues that remain unassigned:

	1	2	3	4	5	6	7	8	9	10
^{1}H (ppm)	2.30	4.08	3.85	3.73	3.77	1.84	1.90	4.02	2.30	4.42
^{13}C (ppm)	31.8	55.4	65.7	68.1	65.8	18.7	37.9	60.5	28.8	68.7

	1	2	3	4
^{1}H (ppm)	3.28	3.61	3.91	2.15
^{13}C (ppm)	40.1	63.9	60.5	38.8

Using the triple resonance and heteronuclear resolved NOESY data shown below, you are to assign the remaining resonances by placing the correct chemical shifts obtained from the HSQC spectra in the correct positions of the table (below, after the triple resonance data). The ^{13}C=O chemical shifts necessary to complete the table you will get from the HNCO experiment. There are 42 chemical shifts above from the HSQC data, and 7 carbonyl chemical shifts that you will get from the HNCO data, for a total of 49, the same number as the number of blanks in the table at the end. It is important to keep in mind that there is no ambiguity; there is only 1 way the data can fill out the table correctly. Also, all the chemical shifts are unique, and each is included in the table correctly

only once. So, there aren't any tricks ..... put each of the chemical shifts from the HSQC spectra in the table once, and each of the carbonyl chemical shifts from the HNCO data in the table once ..... and do it correctly using the triple resonance experimental data to tell you how. Also note that some of the chemical shifts for the flanking residues (123 and 131) are listed, whereas some cannot be determined.

Important crosspeaks in the 3D NOESY-^{1}H, ^{15}N-HSQC spectrum are also listed. You may find these helpful (but perhaps not necessary) in making the assignments above. Nevertheless, these data should of course be consistent with the assignments, and in addition should tell you of the nature of the secondary structure of this section of your protein. **So, state what the probable secondary structure of this section of your protein is and how this is indicated by the 3D NOESY-^{1}H, ^{15}N-HSQC crosspeak data shown.**

HNCA 13 peaks

	1	2	3	4	5	6	7	8	9	10	11	12	13
$^{1}H_N$	7.76	7.76	7.76	8.38	8.61	8.75	8.41	7.57	7.72	8.77	7.54	7.78	7.58
^{15}N	120.4	123.9	123.9	122.0	116.3	119.0	121.7	113.1	120.8	118.9	112.7	124.0	116.5
$^{13}C\alpha$	65.6	67.9	60.3	60.3	65.6[a]	68.2	64.0	55.6	60.7	65.9	63.8	55.3	60.4

[a] very large peak

HN(CO)CA 7 peaks

	1	2	3	4	5	6	7
$^{1}H_N$	8.39	8.60	7.72	8.78	7.53	7.62	7.75
^{15}N	121.9	116.6	120.7	119.2	112.9	116.1	123.7
$^{13}C\alpha$	63.7	65.6	60.6	66.0	55.5	60.6	68.0

HNCACB 23 peaks

	1	2	3	4	5	6	7	8	9	10	11
$^{1}H_N$	8.77	8.61	7.78	7.78	7.72	8.58	8.41	8.39	8.39	8.38	7.75
^{15}N	119.0	116.6	123.7	123.8	120.5	116.4	121.9	122.0	122.0	121.8	120.5
^{13}C	68.5[b]	39.0[b]	68.3	28.8[b]	65.8	65.8[a]	60.4	38.0[b]	39.9[b]	63.9	39.0[b]

	12	13	14	15	16	17	18	19	20	21	22	23
$^{1}H_N$	7.77	7.75	7.73	7.76	7.56	8.77	8.78	7.54	7.55	7.77	8.76	7.75
^{15}N	124.1	120.7	120.5	124.0	112.8	119.1	119.1	113.0	113.0	123.6	118.9	123.7
^{13}C	60.4	60.6	29.0[b]	68.8[b]	55.5	31.8[b]	68.2	37.9[b]	64.0	18.7[b]	66.0	55.3

[a] very large peak

[b] these peaks are 180 degrees out of phase with respect to the others

CBCA(CO)NH 13 peaks

	1	2	3	4	5	6	7	8	9	10	11	12	13
$^{1}H_N$	7.77	7.75	7.77	8.77	7.55	8.39	7.57	8.40	8.76	7.73	8.60	7.58	8.59
^{15}N	123.8	120.5	124.0	118.9	113.0	121.8	112.9	121.8	119.1	120.8	116.6	116.6	116.5
^{13}C	68.1	60.5	68.7	65.6	18.8	37.9	55.5	63.8	31.9	28.8	65.6	40.0	38.8

HN(CA)HA 13 peaks

	1	2	3	4	5	6	7	8	9	10	11	12	13
$^{1}H_N$	8.40	8.58	7.78	8.39	7.75	7.77	8.77	7.72	8.58	7.78	7.56	7.56	7.78
^{15}N	121.7	116.4	123.9	121.8	120.5	123.7	119.0	120.4	116.6	124.0	112.9	113.0	124.0
$^{1}H\alpha$	3.61	3.77	4.00	3.90	3.85	4.07	3.72	4.02	3.86	3.74	3.61	4.08	3.91

HBHA(CBCACO)NH 13 peaks

	1	2	3	4	5	6	7	8	9	10	11	12	13
$^{1}H_{N}$	7.77	7.75	8.60	8.60	8.40	7.55	8.41	8.76	7.74	8.77	7.77	7.54	7.77
^{15}N	124.0	120.5	116.4	116.4	121.9	113.0	121.8	119.0	120.6	119.1	123.8	113.0	123.9
^{1}H	4.40	4.01	2.14	3.85	3.60	1.85	1.91	2.29	2.30	3.77	3.74	4.10	3.89

HNCO 8 peaks

	1	2	3	4	5	6	7	8
$^{1}H_{N}$	7.58	7.77	8.60	8.41	8.76	7.54	7.75	7.77
^{15}N	116.5	124.0	116.6	121.8	119.0	113.0	120.5	124.0
$^{13}C=O$	177.6	174.4	177.2	179.1	178.5	178.2	178.8	179.2

3D NOESY-^{1}H, ^{15}N-HSQC important crosspeaks

	1	2	3	4	5	6	7	8	9	10	11	12
$^{1}H_{N}$	7.77	7.77	8.61	8.61	7.77	7.59	8.75	8.75	7.55	7.55	8.40	8.40
^{15}N	124.0	124.0	116.6	116.6	124.0	116.5	119.0	119.0	113.0	113.0	119.8	122.0
^{1}H	7.57	8.75	7.76	8.78	8.39	8.40	8.60	7.77	7.77	8 .39	7.77	7.60

	13	14	15	16	17	18	19	20	21
$^{1}H_{N}$	8.40	7.75	7.75	8.60	7.61	7.77	8.77	8.39	7.55
^{15}N	122.0	120.4	120.4	116.6	116.2	124.0	119.0	121.8	112.9
^{1}H	7.57	7.77	8.59	3.91	4.10	3.86	4.00	3.75	3.77

the intensities of NOEs 16-21 are “medium”, 1-15 are predominantly “strong”

Fill in all the blanks with the correct chemical shifts

	(LEU)	GLU	ILE	VAL	THR	ALA	ILE	PHE	(LYS)
	(123)	124	125	126	127	128	129	130	(131)
^{1}HN	8.41								7.60
^{15}N	119.6								116.3
$^{1}H\alpha$	3.91								3.99
$^{13}C\alpha$	ND*								57.8
$^{1}H\beta$	ND*								1.85
$^{13}C\beta$	ND*								31.9
$^{13}C=O$	179.2								176.6

*ND, could not be determined