NMR Handout

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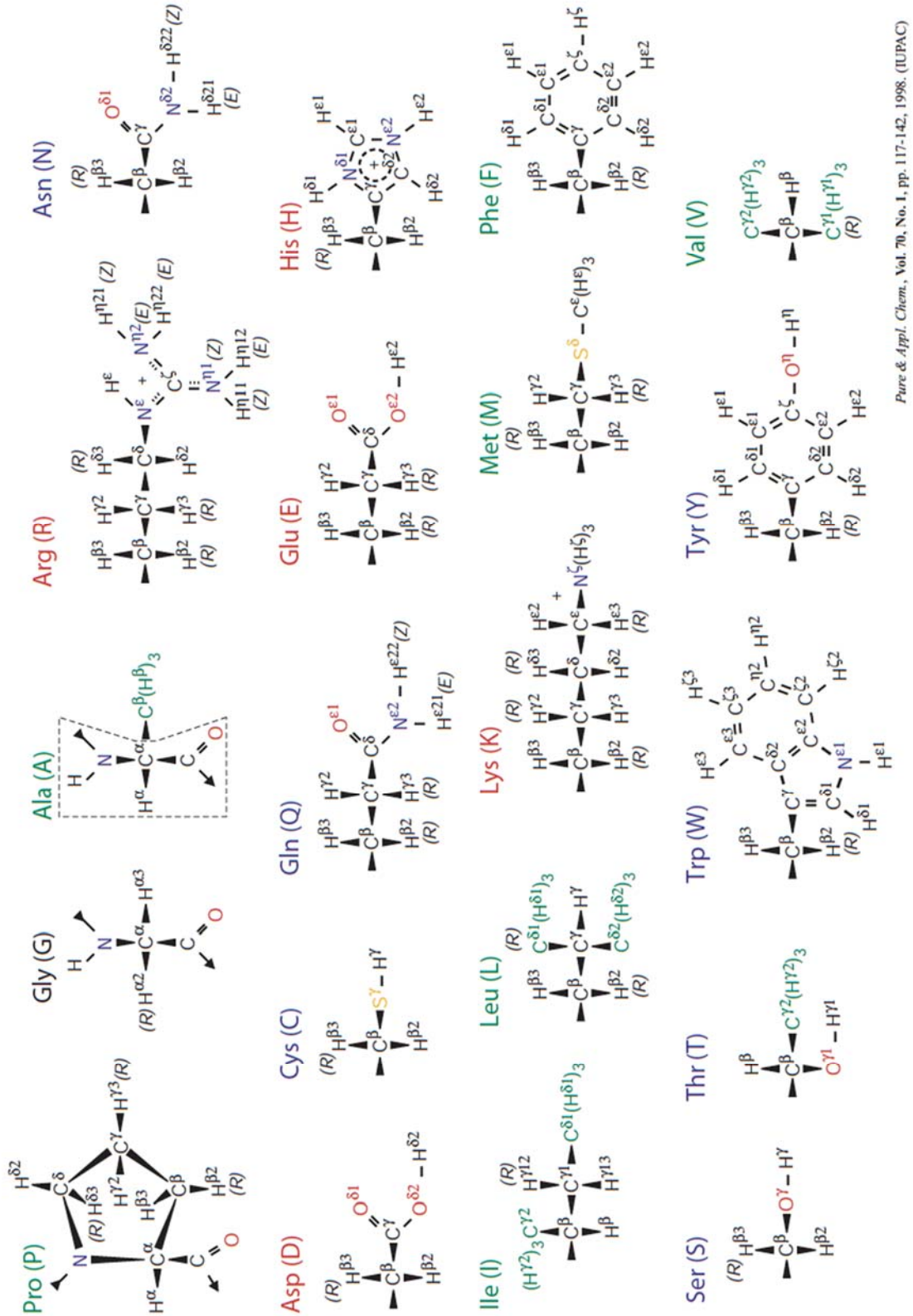
Annex 1. Structure of the 20 amino acids



Annex 2. Averaged aliphatic carbon chemical shifts

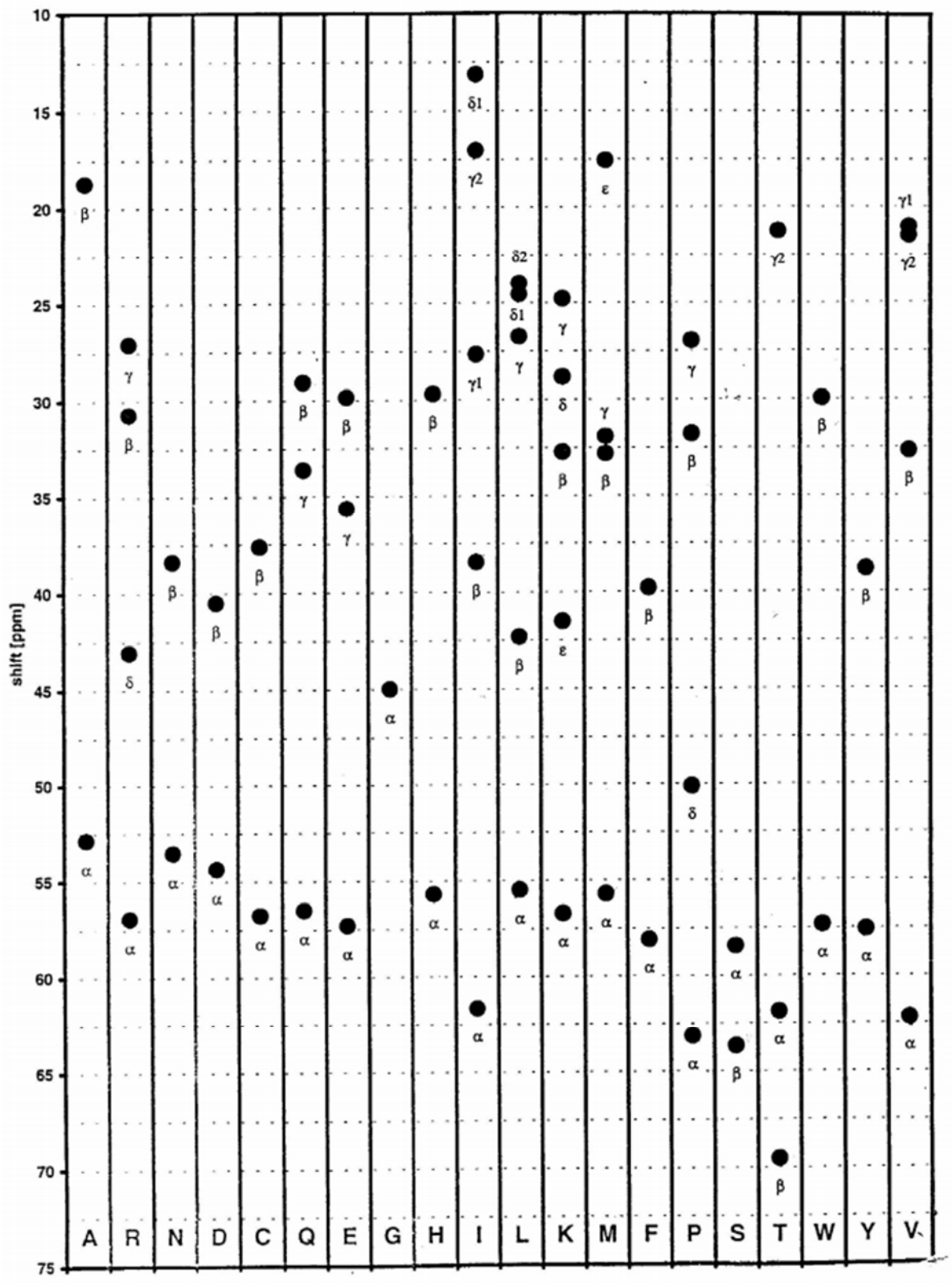


Figure was taken from http://www.protein-nmr.org.uk/wp-content/uploads/2012/10/ccpnmr_analysis_sh3_tutorial_figures.pdf

Annex 3. Average amino acid chemical shifts (in ppm) and standard deviations (in parentheses) categorized according to secondary structure type

Amino acid	$^{13}C^{\alpha}$			$^{13}C^{\beta}$			$^{13}C'$		
	β-strand	Random coil	α-helix	β-strand	Random coil	α-helix	β-strand	Random coil	α-helix
Ala	50.86 (1.28)	52.67 (1.76)	54.86 (0.94)	**21.72** (1.77)	19.03 (1.27)	18.27 (1.08)	175.3 (1.61)	177.39 (1.45)	179.58 (1.39)
Arg	54.63 (1.50)	55.96 (1.94)	59.05 (1.21)	32.36 (1.82)	30.53 (1.77)	30.00 (0.83)	175.04 (1.18)	175.91 (1.27)	**178.11** (1.70)
Asn	52.48 (1.18)	52.94 (1.43)	55.67 (0.99)	40.43 (1.89)	**38.22** (1.47)	38.28 (1.12)	174.55 (1.28)	174.98 (1.38)	176.74 (1.66)
Asp	53.41 (1.15)	54.09 (1.59)	57.04 (1.00)	42.78 (1.75)	40.76 (1.34)	40.50 (1.12)	175.15 (1.54)	176.01 (1.45)	**178.07** (1.80)
Cys	57.64 (1.94)	**58.8** (2.06)	62.86 (1.85)	29.48 (1.97)	29.75 (1.86)[b]	26.99 (0.84)[b]	**173.86** (1.83)[a]	174.77 (1.38)[b]	177.42 (1.35)[b]
	54.19 (1.64)[a]	57.68 (1.43)[a,b]	58.57 (1.59)[a,b]	43.79 (4.04)[a]	38.38 (1.39)[a,b]	40.02 (1.78)[a,b]	172.73 (1.05)[a]	175.85 (1.58)[a,b]	176.84 (0.47)[a,b]
Gln	54.33 (1.39)	55.94 (1.83)	58.61 (1.04)	31.92 (1.74)	**28.67** (1.73)	28.33 (0.79)	174.58 (0.94)	175.88 (1.53)	178.35 (1.15)
Glu	**55.55** (1.45)	56.39 (1.84)	59.30 (1.05)	32.45 (1.96)	30.02 (1.62)	29.20 (0.77)	175.01 (1.24)	176.11 (1.47)	178.46 (1.34)
Gly	45.08 (1.20)	45.34 (1.17)	47.02 (0.90)				**173.01** (2.59)	174.30 (1.80)	**176.31** (1.50)
His	54.8 (1.75)	55.78 (2.02)	59.62 (1.57)	32.2 (2.52)	29.62 (1.99)	29.91 (1.67)	**173.80** (2.24)	174.88 (1.68)	176.83 (1.16)
Ile	60.00 (1.51)	60.64 (2.08)	64.68 (1.66)	40.09 (1.85)	38.26 (2.06)	37.59 (1.08)	174.79 (1.41)	175.46 (1.65)	**177.49** (1.62)
Leu	53.94 (1.19)	54.85 (1.79)	57.54 (0.98)	44.02 (1.99)	**41.87** (1.70)	41.40 (1.11)	**175.16** (1.31)	**176.61** (1.77)	178.42 (1.70)
Lys	55.01 (1.00)	56.40 (1.80)	59.11 (1.19)	34.86 (1.79)	32.57 (1.30)	32.31 (1.08)	174.93 (1.25)	176.15 (1.40)	177.79 (2.22)
Met	**54.10** (1.46)	**55.12** (1.79)	58.45 (1.66)	**34.34** (2.44)	32.93 (3.05)	**31.70** (1.72)	**174.64** (1.47)	175.93 (1.54)	177.76 (1.77)
Phe	56.33 (1.31)	**56.94** (1.98)	60.74 (1.63)	41.64 (1.65)	39.43 (1.93)	38.91 (1.49)	174.15 (1.93)	175.28 (1.88)	**176.42** (1.74)
Pro	62.79 (1.22)	63.53 (1.26)	65.52 (1.01)	32.45 (0.93)	31.87 (0.96)	31.08 (0.84)	**176.41** (1.50)	176.91 (1.72)	178.34 (1.53)
Ser	57.14 (1.11)	58.35 (1.78)	60.86 (1.27)	65.39 (1.48)	63.88 (1.24)	62.81 (0.58)	**173.52** (1.55)	174.33 (1.22)	**176.51** (1.40)
Thr	61.10 (1.71)	61.59 (2.04)	65.89 (1.55)	70.82 (2.11)	69.75 (1.29)	68.64 (0.98)	173.47 (1.39)	174.62 (1.45)	176.62 (1.24)
Trp	56.28 (1.52)	57.62 (1.25)	60.03 (1.94)	**31.78** (1.76)	29.27 (1.10)	28.74 (1.15)	175.10 (1.80)	175.91 (1.32)	**177.81** (1.62)
Tyr	56.56 (1.59)	57.72 (2.14)	61.07 (1.72)	40.79 (1.77)	38.71 (2.00)	38.38 (0.89)	174.65 (1.64)	175.32 (1.54)	177.05 (1.51)
Val	60.72 (1.59)	61.80 (2.25)	65.96 (1.39)	33.81 (1.79)	32.68 (1.76)	31.41 (0.74)	174.66 (1.36)	175.76 (1.63)	177.75 (1.49)

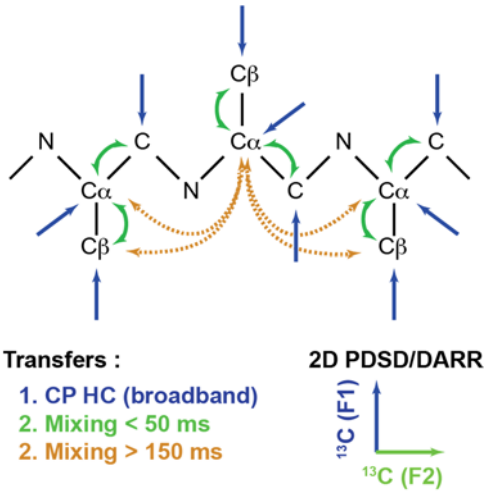
Amino acid	$^{1}H^{N}$			$^{1}C^{\alpha}$			^{15}N		
	β-strand	Random coil	α-helix	β-strand	Random coil	α-helix	β-strand	Random coil	α-helix
Ala	**8.59** (0.76)	8.11 (0.68)	7.99 (0.57)	**4.87** (0.46)	4.25 (0.35)	4.03 (0.31)	125.57 (4.80)	**132.52** (3.51)	121.65 (2.52)
Arg	8.57 (0.69)	8.17 (0.77)	8.03 (0.56)	**4.85** (0.47)	4.33 (0.37)	4.00 (0.33)	**122.60** (4.74)	**120.59** (4.42)	118.99 (2.56)
Asn	8.70 (0.55)	8.33 (0.72)	8.20 (0.66)	**5.26** (0.41)	4.60 (0.38)	4.45 (0.20)	**122.70** (4.18)	118.48 (4.58)	**117.60** (2.37)
Asp	8.56 (0.62)	8.39 (0.66)	8.05 (0.55)	5.01 (0.36)	4.64 (0.29)	4.44 (0.22)	123.82 (4.70)	120.69 (4.45)	119.90 (2.03)
Cys	9.00 (0.45)	7.81 (0.62)	8.22 (0.53)	5.18 (0.57)	4.63 (0.37)[b]	4.16 (0.25)[b]	123.27 (5.69)	117.01 (2.50)[b]	117.47 (3.04)[b]
	8.68 (0.98)[a]	8.53 (0.59)[a,b]	8.58 (0.48)[a,b]	5.21 (0.49)[a]	4.44 (0.29)[a,b]	4.53 (0.18)[a,b]	121.81 (4.34)[a]	118.62 (4.25)[a,b]	119.51 (2.44)[a,b]
Gln	8.51 (0.83)	8.25 (0.75)	8.11 (0.52)	4.97 (0.43)	4.26 (0.39)	4.03 (0.23)	123.14 (4.89)	119.73 (3.85)	118.59 (2.59)
Glu	8.66 (0.60)	8.29 (0.53)	8.32 (0.63)	4.76 (0.44)	4.28 (0.30)	3.99 (0.21)	123.52 (4.29)	120.87 (3.94)	119.89 (2.85)
Gly	8.27 (1.06)	8.34 (0.83)	8.23 (0.78)	4.09[c] (0.46)	3.95[c] (0.40)	3.84 (0.43)[c]	110.19 (4.20)	109.94 (4.09)	**107.34** (2.82)
His	**8.76** (0.79)	8.09 (0.83)	8.03 (0.68)	5.07 (0.50)	4.50 (0.51)	4.06 (0.54)	**121.65** (5.16)	**118.87** (4.98)	118.09 (3.17)
Ile	8.74 (0.66)	7.94 (0.66)	8.06 (0.56)	4.72 (0.42)	4.13 (0.36)	3.66 (0.30)	124.12 (4.93)	121.07 (5.17)	120.22 (2.75)
Leu	8.63 (0.67)	8.12 (0.72)	8.02 (0.56)	4.85 (0.43)	4.35 (0.36)	4.00 (0.27)	125.69 (4.14)	121.53 (4.30)	120.18 (2.46)
Lys	8.54 (0.63)	8.13 (0.66)	8.04 (0.61)	4.96 (0.46)	4.28 (0.31)	3.98 (0.26)	123.29 (4.76)	121.44 (4.19)	119.90 (2.93)
Met	8.43 (0.65)	8.37 (0.51)	8.05 (0.48)	4.94 (0.48)	4.55 (0.38)	4.03 (0.35)	121.67 (4.12)	120.19 (3.46)	118.69 (2.36)
Phe	8.80 (0.70)	7.95 (0.90)	8.21 (0.66)	5.17 (0.47)	4.62 (0.42)	4.11 (0.40)	121.95 (4.38)	119.41 (4.75)	119.12 (4.05)
Pro				4.72 (0.45)	4.41 (0.30)	4.13 (0.39)			
Ser	8.57 (0.65)	8.26 (0.74)	8.11 (0.50)	5.08 (0.48)	4.48 (0.35)	4.20 (0.19)	117.44 (4.19)	115.94 (4.13)	**114.78** (2.39)
Thr	8.50 (0.58)	8.22 (0.74)	8.10 (0.55)	4.81 (0.46)	4.33 (0.38)	4.02 (0.27)	**118.09** (4.86)	114.41 (5.70)	115.30 (3.72)
Trp	8.83 (0.73)	**7.59** (0.84)	8.24 (0.82)	**5.24** (0.41)	4.54 (0.24)	4.35 (0.40)	124.04 (5.43)	**120.57** (3.58)	120.48 (2.89)
Tyr	8.69 (0.73)	**7.90** (0.79)	8.10 (0.70)	5.00 (0.51)	4.55 (0.45)	4.14 (0.36)	122.55 (4.70)	120.05 (4.23)	119.67 (3.19)
Val	8.73 (0.61)	7.88 (0.75)	7.99 (0.63)	4.66 (0.42)	4.14 (0.40)	3.57 (0.34)	123.27 (5.05)	**119.66** (5.62)	119.53 (3.19)

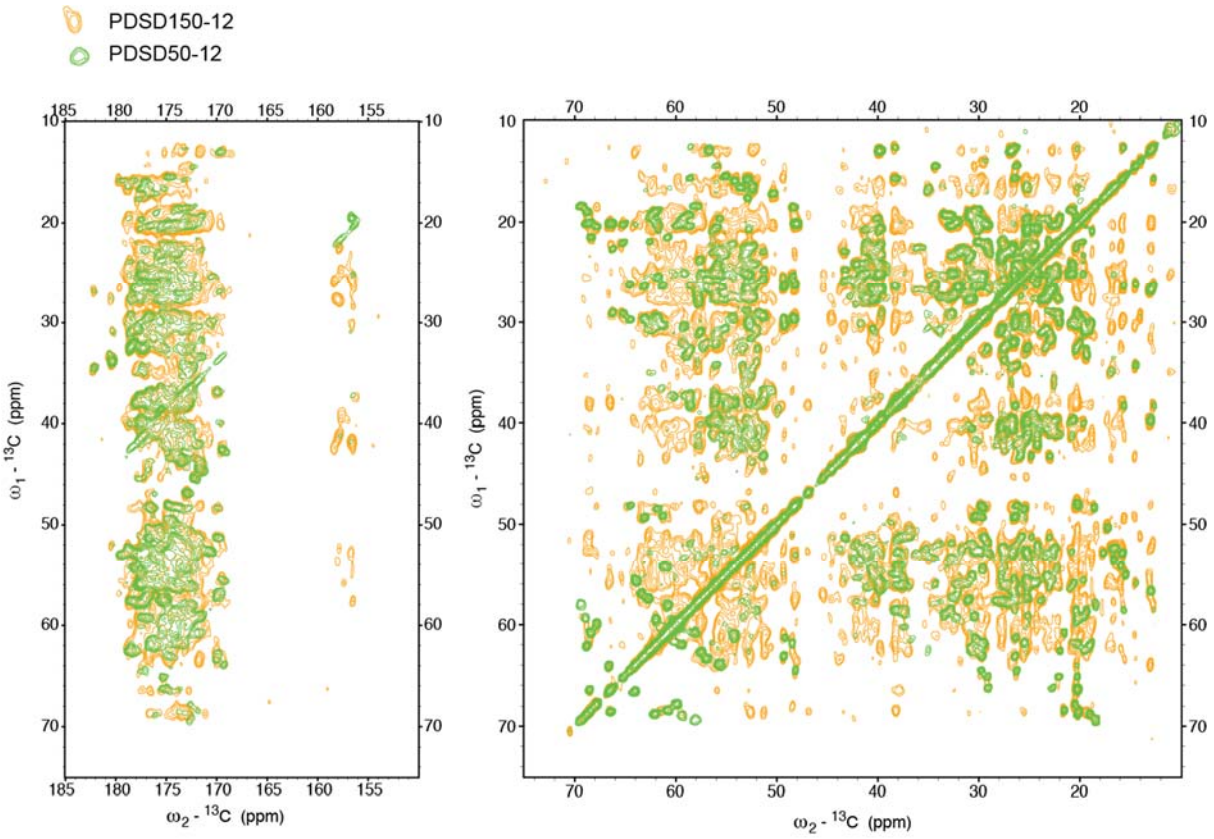
[a] Cys in the oxidized form.
[b] Number of the chemical shifts used in the statistical analysis is less than 10.
[c] Averaged value fro Gly.

Table extracted from reference : Wang Y. and Jardetzky O, Protein Sci. 2002, 11, 852-61)

Annex 4. 2D and 3D ssNMR experiments

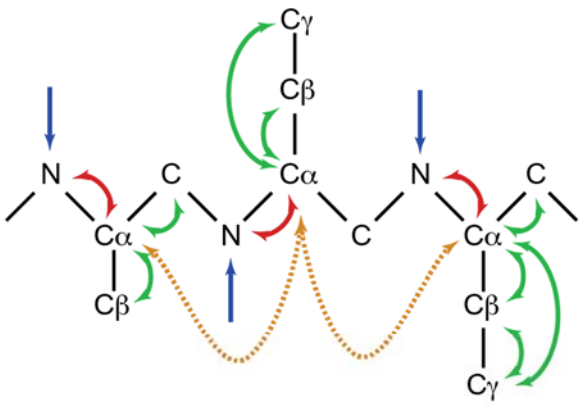
PDSD/DARR





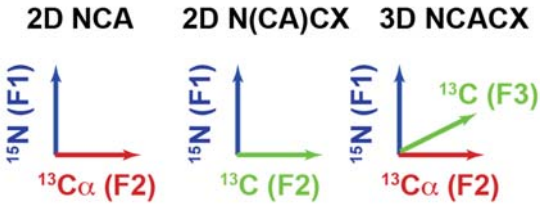
Annex 4. 2D and 3D ssNMR experiments

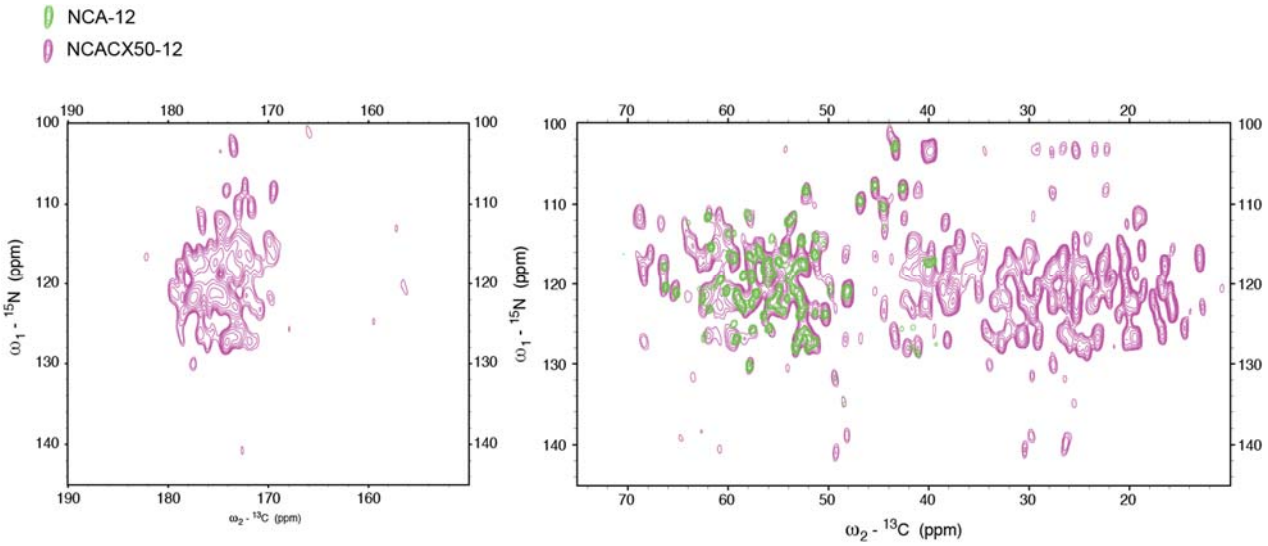
NCA/NCACX



Transfers :

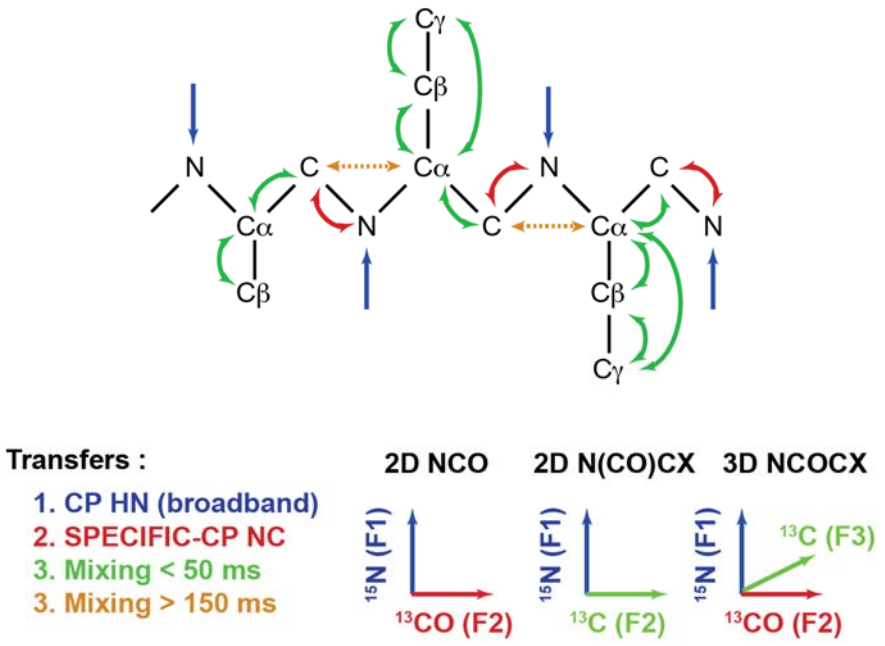
1. CP HN (broadband)
2. SPECIFIC-CP NC
3. Mixing < 50 ms
3. Mixing > 150 ms

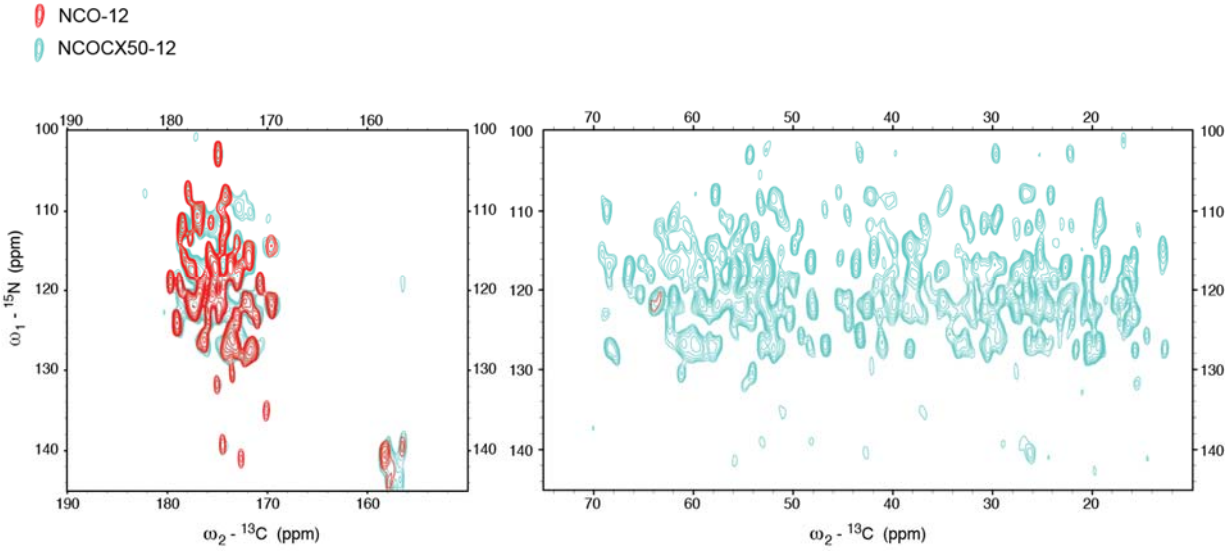




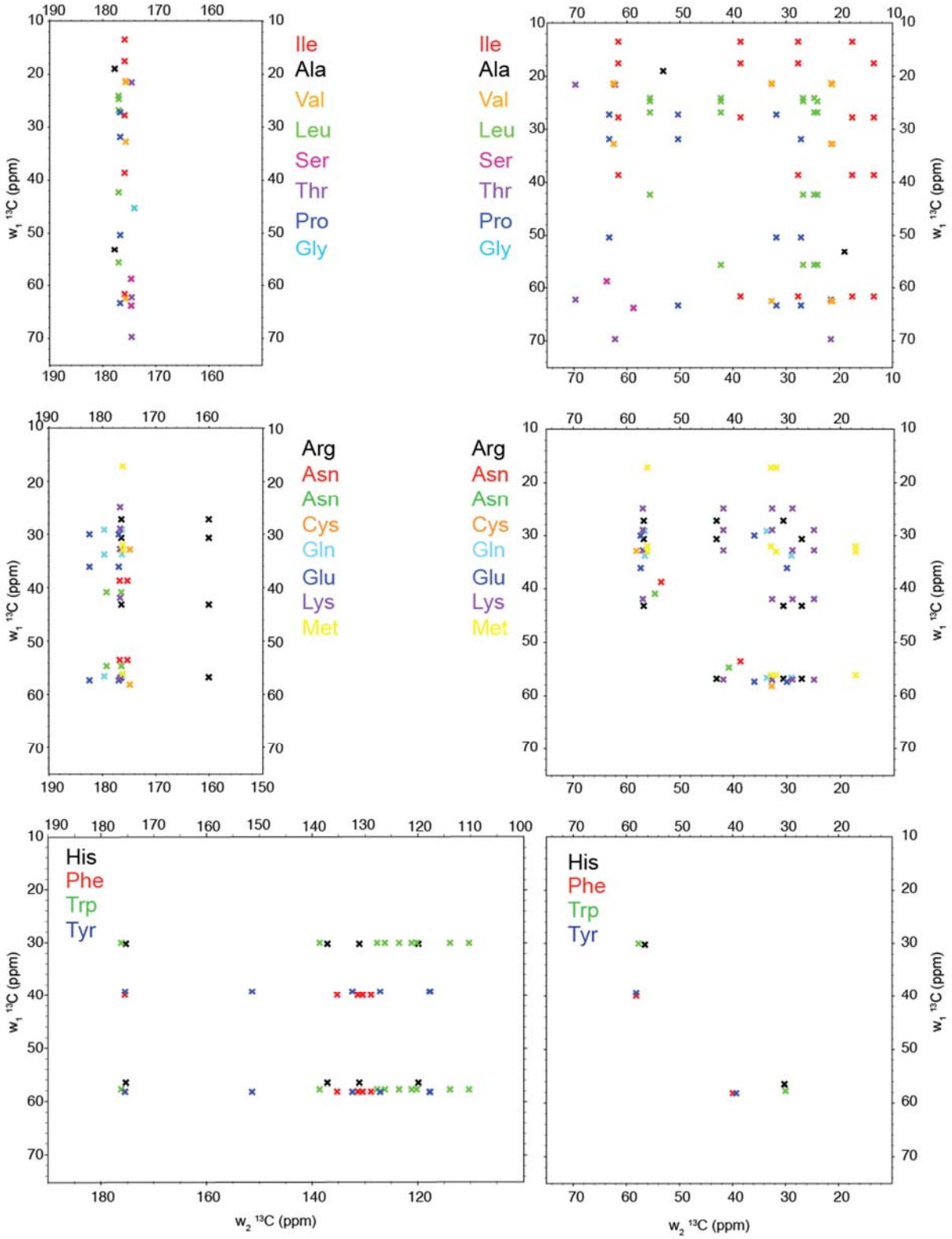
Annex 4. 2D and 3D ssNMR experiments

NCO/NCOCX



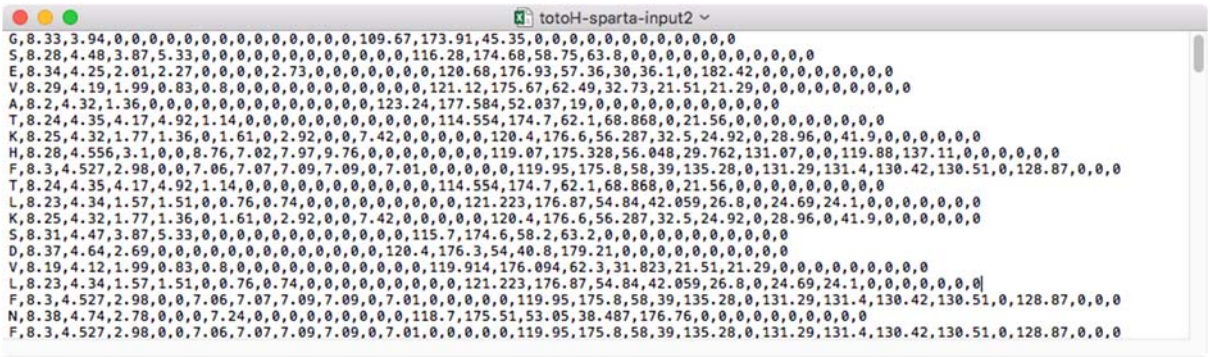


Annex 5. Characteristic intraresidue CC correlation patterns for the 20 amino acids

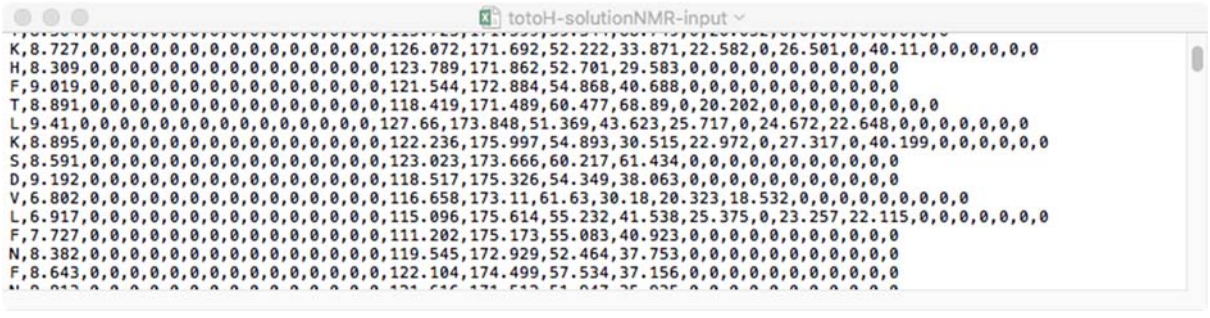


Annex 6. Procedure used for predicting backbone chemical shift from a crystal structure

1. Download the PDB file : toto.pdb
2. Create ^{1}H atoms (using the software Reduce)
   > in a terminal window, type : reduce –BUILD toto.pdb > totoH.pdb
3. Open the pdb file totoH.pdb and check that H atoms are present
4. Connect to the server of Sparta + (http://spin.niddk.nih.gov/bax/nmrserver/sparta/)
5. Follow the instructions :
   a. Select PDB Input file: totoH.pdb
   b. Chemical Shift Data for Comparison (optional) > NO [Optional input table of chemical shifts and sequence information. If this table is given, the output produced by SPARTA+ will compare the shifts in this input table and the corresponding predicted chemical shifts.
   c. Apply offset correction > NO
   d. Generate PDF graphic >NO
   e. Fill in the contact information (email)
   Submit
6. Check your email inbox, open the message from the Sparta+ server and save the 3 files : pred.tab ; struct.tab ; sparta.pdf
7. Generate a shift list (here, the FANDAS format <name>.txt was used) from the predicted chemical shifts indicated in the pred.tab file



8. Generate a shift list (here, the FANDAS format <name>.txt was used) from the solution NMR assignment table. (missing or unassigned resonances are labeled with a 0)



Annex 7. Procedure used to plot NC and CC correlation patterns from predicted or experimental chemical shifts

1. Download the shift list generated from the predicted chemical shifts (totoH-sparta-input)
2. Connect to the server FANDAS (http://tintin.science.uu.nl/services/FANDAS/html/main.php)
3. Follow the instructions for the Spectrum prediction
    a. Predict intra-residue ^{13}C-^{13}Cspectrum > PDSD50 & DARR50
        Save the peaklist with annotations as a <name>.peaks file
    b. Predict sequential ^{13}C-^{13}C spectrum > PDSD150
    c. Predict 2D ^{15}N-^{13}CA spectrum > NCA
    d. Predict 2D ^{15}N-^{13}CA-^{13}CX (intra) spectrum > NCACX50
    e. Predict 2D ^{15}N-^{13}CO spectrum > NCO
    f. Predict 2D ^{15}N-^{13}CO-^{13}CX (intra) spectrum > NCOCX50
4. Now, we have generated 6 peaklists in a Sparky format <name>.peaks from the backbone chemical shifts predicted from the crystal structure by using sequentially Sparta + and FANDAS servers.
5. Download the shift list generated from the solution NMR assignment table (totoH-solutionNMR-input)
6. Repeat the same procedure
7. Now, we have generated 6 peaklists in a Sparky format <name>.peaks from the solution NMR backbone and sidechain chemical shifts obtained on the soluble (U-^{13}C,^{15}N)-labeled protein.

Annex 8. Deducing K_d values from chemical shift perturbation along titration in the case of a 1:1 protein-ligand complex

$$P + L = PL$$

$K_d = (P)(L)/(PL)$

$P_0 = (P) + (PL)$

$L_0 = (L) + (PL)$

Injecting the later two equations in the K_d equation:

$K_d = [P_0-(PL)][L_0-(PL)]/(PL)$

A development of this equation leads to a quadratic equation:

$(PL)^2 - [P_0 + L_0 + K_d](PL) + P_0L_0 = 0$

A solution to this equation is: $\frac{(PL)}{P_0} = \frac{1}{2}\left(1 + \frac{K_d}{P_0} + \frac{L_0}{P_0} - \sqrt{\left(1 + \frac{K_d}{P_0} + \frac{L_0}{P_0}\right)^2 - 4\frac{L_0}{P_0}}\right)$

In the case of fast exchange, the observed chemical shift is the weighted average of the chemical shift of each form the protein (the free form and the bound form).

In other words: $\delta = \delta_{free}\left(1 - \frac{(PL)}{P_0}\right) + \delta_{bound}\frac{(PL)}{P_0}$

Thus $\delta - \delta_{free} = \left(\delta_{bound} - \delta_{free}\right)\frac{(PL)}{P_0}$

Thus $CSP_{obs} = \delta - \delta_{free} = \frac{(\delta_{bound} - \delta_{free})}{2}\left(1 + \frac{K_d}{P_0} + \frac{L_0}{P_0} - \sqrt{\left(1 + \frac{K_d}{P_0} + \frac{L_0}{P_0}\right)^2 - 4\frac{L_0}{P_0}}\right)$