Supplementary Material

Solid-state NMR spectroscopy of 10% 13C labeled ubiquitin: spectral simplification and stereospecific assignment of isopropyl groups; Mario Schubert, Theofanis Manolikas, Marco Rogowski and Beat H. Meier

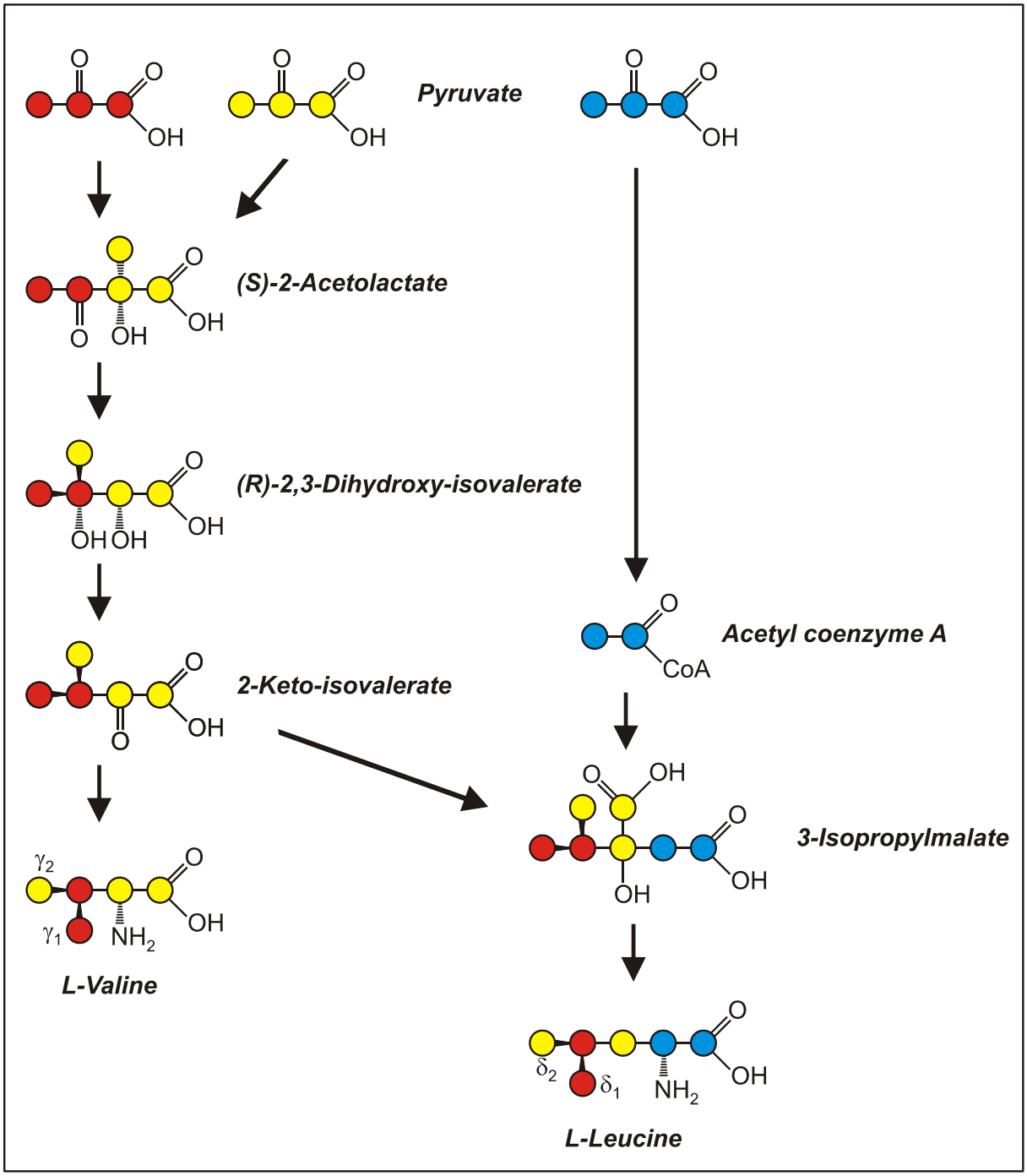

Figure S1: Schematic representation of the biosynthetic pathway for Val and Leu. Blocks of two or three carbons originating from the same glucose molecule, which is either 13C labeled or not, will stay together.

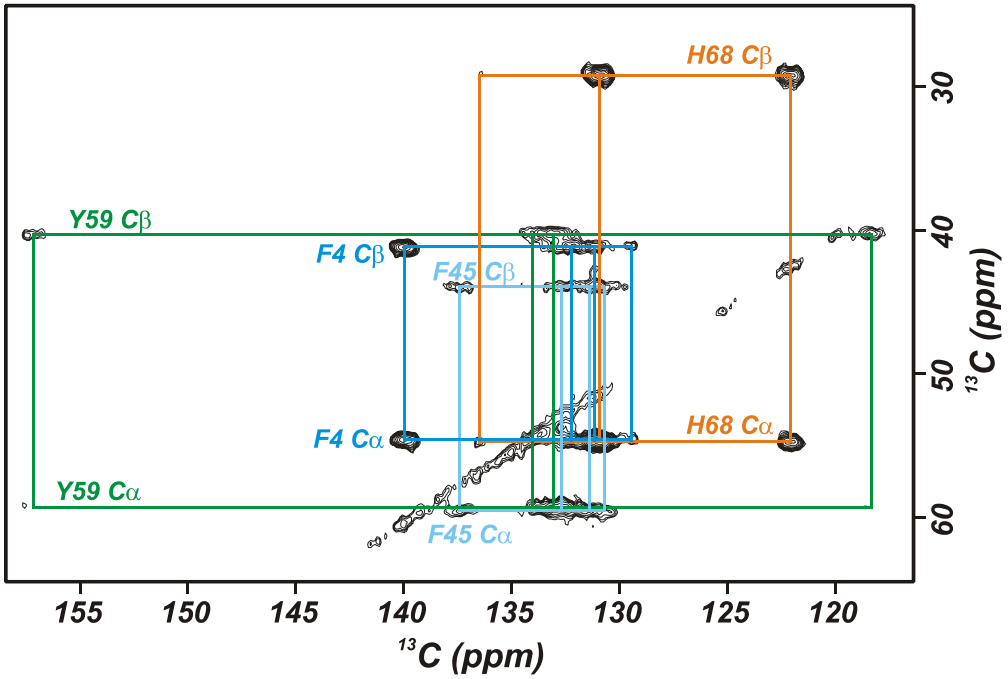

Figure S2: Cross-peaks between aromatic and backbone carbons in a PDSD spectrum of uniformly 13C/15N labeled ubiquitin.

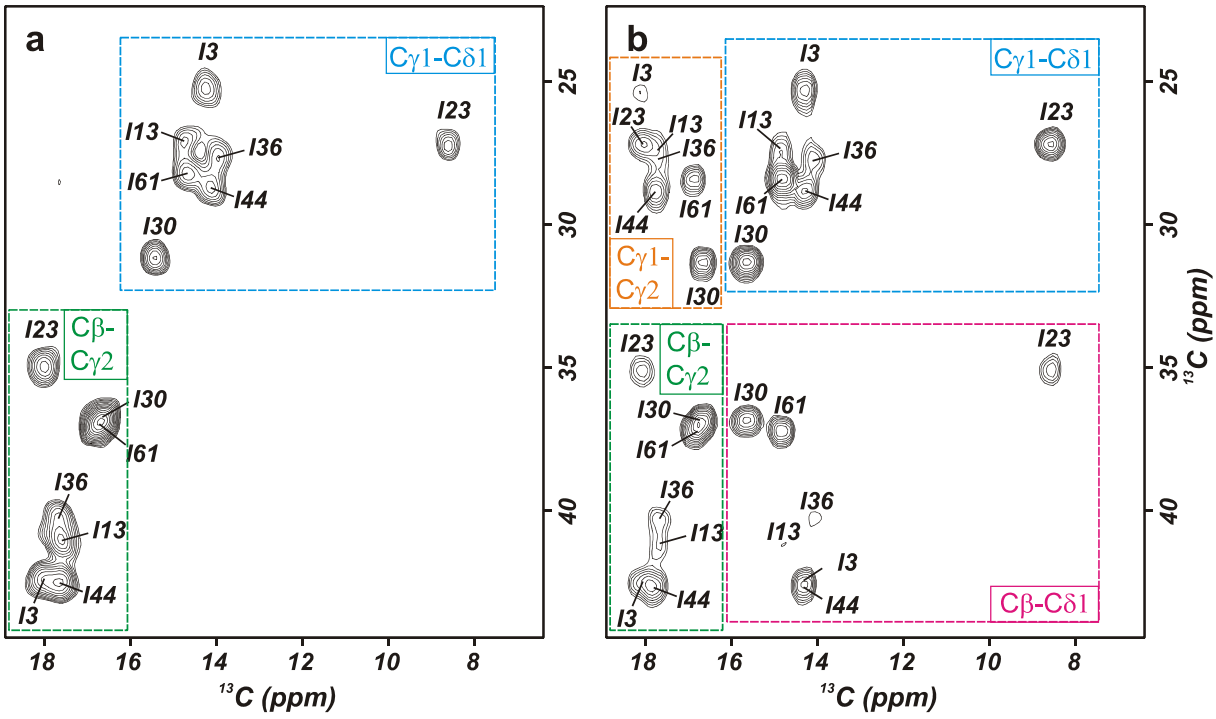

Figure S3. Region with side chain cross-peaks of Ile residues in PDSD spectra of 10% ^{13}C/100% ^{15}N labeled (a) and uniformly ^{13}C/^{15}N labeled ubiquitin (b). The labeling pattern in a 10% ^{13}C labeled sample leads to less side chain correlations of Ile. Cβ-Cδ1 and Cγ1-Cγ2 cross-peaks are very weak or absent, leaving only strong Cγ1-Cδ1 and Cβ-Cγ2 correlations in this region. A 10% ^{13}C labeled sample allows the straightforward distinction between Cγ2 and Cδ1 of isoleucines. Cα-Cβ cross-peaks are absent (Fig. 5), but Cα-Cγ1 correlations are present and since the spectrum is simplified in this region, those peaks are well suited to link side chain and backbone assignments.

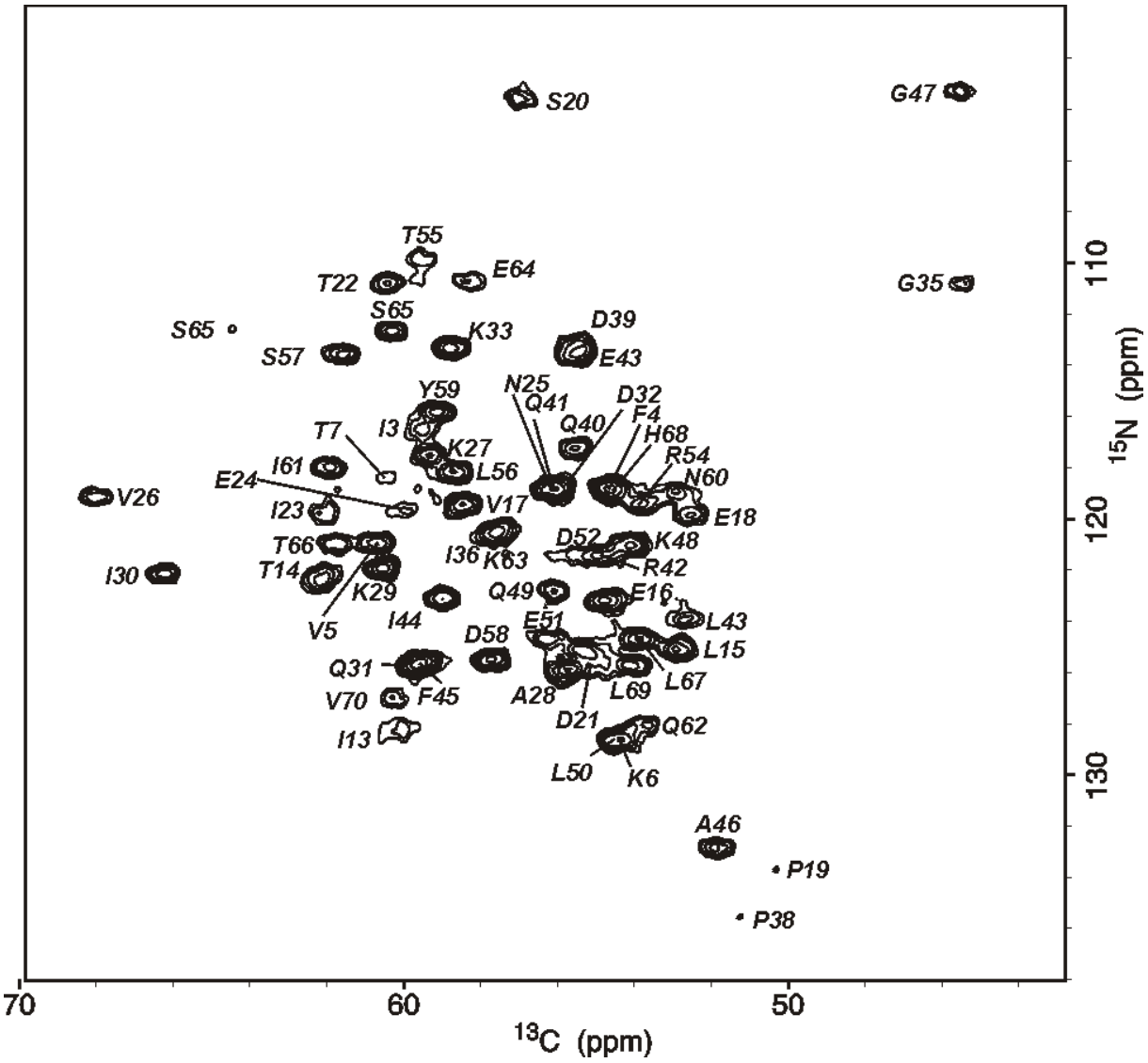

Figure S4. NCA correlation spectrum of uniformly $^{13}C/^{15}N$ labeled ubiquitin.

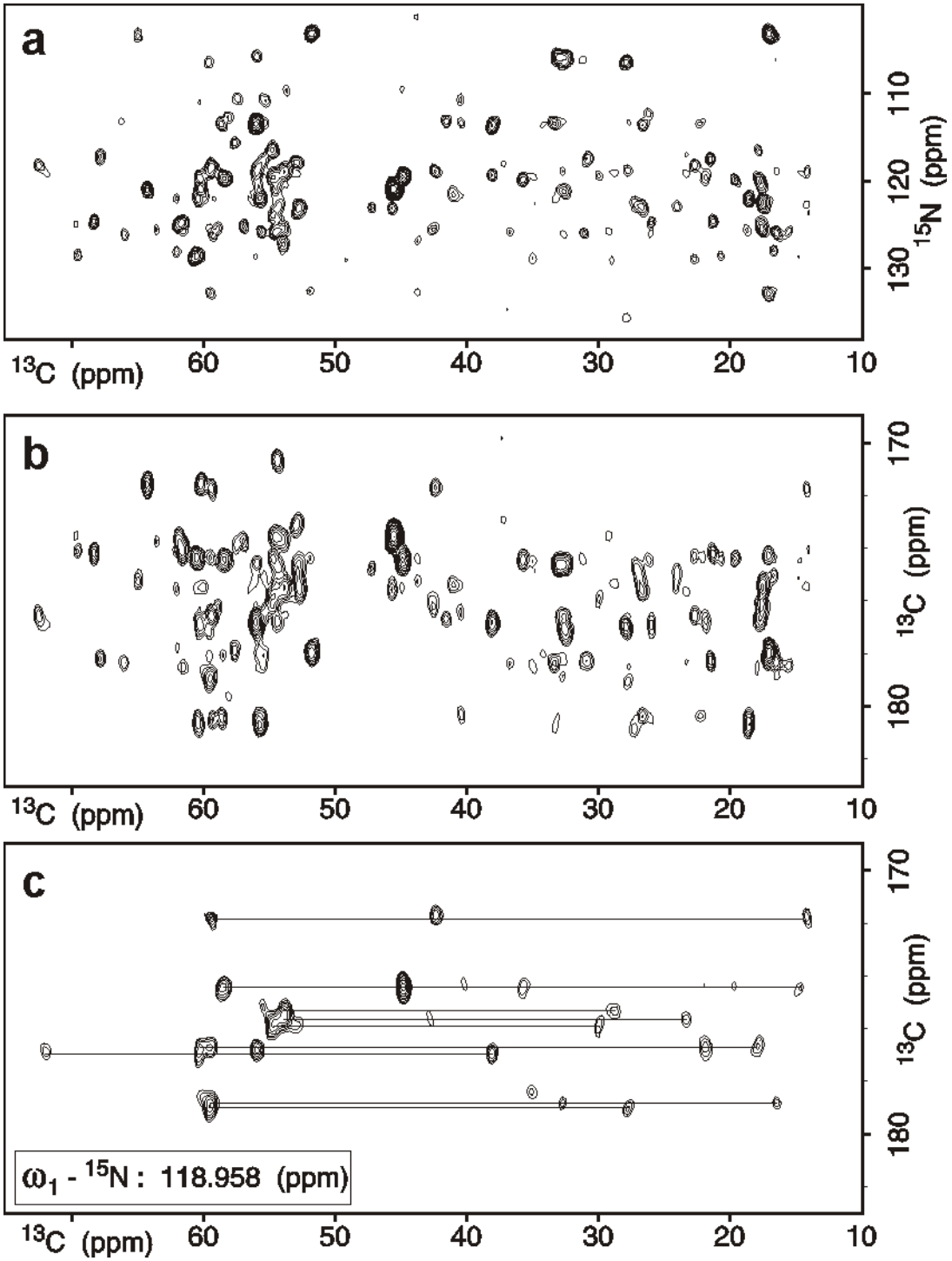

Figure S5. 3D NCOCX correlation spectrum of uniformly $^{13}C/^{15}N$ labeled ubiquitin. The spectrum was recorded using 64 scans, TPPI with 32 points and t_{1Max}= 5.44 ms in the ^{15}N dimension and 40 points and t_{2Max}= 6.8 ms in the $^{13}C'$ dimension resulting in a total measurement time of 48 h. The C'-CX transfer was achieved with proton driven spin diffusion period of 50 ms. A NC projection of the 3D is shown in (a), and a C'C projection in (b), respectively. A representative plane of the 3D is presented in (c).

Table S1: Stereospecific assigned chemical shifts for carbon resonances of Val and Leu in microcrystalline ubiquitin. Solution values in phosphate buffer pH 5.7 (Wand et al., 1996) are entered in parenthesis.

residue	$C\gamma_1$	$C\gamma_2$	$C\gamma$ [a]	$C\delta_1$ [a]	$C\delta_2$
V5	23.0 (22.7)	20.9 (21.1)			
V17	22.2 (22.5)	19.8 (19.8)			
V26	21.6 (21.8)	23.4 (23.9)			
V70	21.7 (21.6)	20.9 (20.9)			
L8			-	-	-
L15			27.3 (27.2)	27.0 (27.3)	24.1 (24.4)
L43			27.1 (26.9)	26.7 (26.5)	24.1 (24.4)
L50			~26.1 (26.2)[b]	~26.1 (26.1) [b]	19.5 (19.9)
L56			~27.1 (27.0)[b]	~27.1 (27.0) [b]	22.4 (23.4)
L67			28.6 (29.7)	25.3 (24.9)	23.6 (25.4)
L69			27.7 (27.7)	23.6 (24.1)	26.3 (26.4)
L71			-	-	-
L73			-	-	-

[a] The chemical shifts of $C\gamma$ and $C\delta_1$ could not be unambiguously distinguished with the presented data, values could be swapped.

[b] The chemical shifts of $C\gamma$ and $C\delta_1$ overlap.

Table S2: Observed chemical shifts of human ubiquitin microcrystals which amend the assignment list of Igumenova et al., 2004. Solution values in phosphate buffer pH 5.7 (Wand at al., 1996) are entered in parenthesis. “nr” stands for not reported.

residue	Cα	Cβ	Cγ	Cδ	Cε	Cζ
F4	54.6 (55.3[a])	41.2 (41.4[a])	140.0 (nr)	132.3 (132.2[b])	131.2 (131.1[b])	129.4 (129.6[b])
F45	59.5 (56.8[a])	43.9 (43.9[a])	137.3 (nr)	132.5 (132.4[b])	131.0 (132.4[b])	
Y59	59.0 (58.6[a])	40.3 (40.4[a])		133.0 (133.5[b])	118.4 (118.6[b])	
H68	54.7 (56.3[a])	29.3 (32.6[a])	130.9 (nr)	122.0 (120.4[b])	136.5 (137.4[b])	

[a] Values were measured in phosphate buffer pH 5.7 (Wand et al., 1996).

[b] Values were measured on encapsulated ubiquitin in low viscosity solvent (Flynn et al., 2002) at pH 5.0, available from the BioMagResBank database under accession number 5387.

References:

Flynn, P. F., Milton, M. J., Babu, C. R. and Wand, A. J. (2002) *J. Biomol. NMR*, **23**, 311-6.

Igumenova, T. I., McDermott, A. E., Zilm, K. W., Martin, R. W., Paulson, E. K. and Wand, A. J. (2004) *J. Am. Chem. Soc.*, **126**, 6720-7.

Wand, A. J., Urbauer, J. L., McEvoy, R. P. and Bieber, R. J. (1996) *Biochemistry*, **35**, 6116-25.