Supplementary Data

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pH-Dependent random coil ^{1}H, ^{13}C, and ^{15}N chemical shifts of the ionizable amino acids: a guide for protein pK_a measurements

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Table S1 pH-dependent chemical shifts (ppm) of the ionizable amino acids in blocked Ac-Gly-X-Gly-NH_2 tripeptides [a]

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
N-terminal amine:		**Alanine-amide**	(pK_a 8.23)			
Ala	^{1}H	H (amine)	8.04			
		Hα	4.10	3.51	- 0.59	4.07
		Hβ (methyl)	1.54	1.27	- 0.27	1.52
	^{13}C	Cα	51.7	52.7	1.0	51.8
		Cβ (methyl)	19.3	22.9	3.6	19.5
		CO	176.0	184.6	8.5	176.5
	^{15}N	N (amine)	40.4	33.8	- 6.6	40.0
amide	^{1}H	H_2N (Z)	7.24			
		H_2N (E)	7.82			
	^{15}N	N	106.1			
C-terminal carboxylic acid:		**N-acetyl alanine**	(pK_a 3.55)			
acetyl	^{1}H	CH_3 (methyl)	2.02	2.00	- 0.02	2.00
	^{13}C	CH_3 (methyl)	24.3	24.7	0.4	24.7
		CO	176.8	176.1	- 0.7	176.1
Ala	^{1}H	HN	8.35	7.94	- 0.41	7.94
		Hα	4.33	4.12	- 0.21	4.12
		Hβ (methyl)	1.41	1.32	- 0.09	1.32
	^{13}C	Cα	51.4	53.7	2.3	53.7
		Cβ (methyl)	18.8	20.1	1.3	20.1
		CO	179.6	183.0	3.4	183.0
	^{15}N	N	110.5	115.7	5.2	115.7

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Aspartic acid: **Ac-Gly-Asp-Gly-NH_2** (pK_a 3.86)				
acetyl	^{1}H	CH_3 (methyl)	2.05	2.06	0.01	2.06
	^{13}C	CH_3 (methyl)	24.4	24.5	0.1	24.5
		CO	177.8	177.8	0.0	177.8
Gly(- 1)	^{1}H	HN	8.31	8.33	0.02	8.33
		Hα (avg.)	3.94	3.95	0.01	3.95
	^{13}C	Cα	45.4	45.4	0.0	45.4
		CO	174.8	174.6	- 0.2	174.6
	^{15}N	N	114.3	114.6	0.3	114.6
Asp	^{1}H	HN	8.55	8.38	- 0.17	8.38
		Hα	4.78	4.61	- 0.17	4.61
		Hβ (avg.)	2.93	2.70	- 0.23	2.70
		Hδ2 (carboxyl)	> 10			
	^{13}C	Cα	52.9	54.3	1.4	54.3
		Cβ	38.0	41.1	3.0	41.1
		Cγ (carboxyl)	177.1	180.3	3.2	180.3
		CO	175.8	176.9	1.1	176.9
	^{15}N	N	118.7	120.2	1.5	120.2
Gly(+1)	^{1}H	HN	8.50	8.47	- 0.03	8.47
		Hα (avg.)	3.91	3.90	- 0.01	3.90
	^{13}C	Cα	45.1	45.2	0.1	45.2
		CO	176.9	177.3	0.4	177.3
	^{15}N	N	110.6	110.8	0.1	110.8
amide	^{1}H	H_2N (Z)	7.10	7.10	0.00	7.10
		H_2N (E)	7.42	7.53	0.11	7.53
	^{15}N	N	107.2	107.6	0.4	107.6

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Glutamic acid: **Ac-Gly-Glu-Gly-NH_2** (pK_a 4.34)				
acetyl	^{1}H	CH_3 (methyl)	2.05	2.06	0.01	2.06
	^{13}C	CH_3 (methyl)	24.4	24.4	0.0	24.4
		CO	177.6	177.6	0.0	177.6
Gly(- 1)	^{1}H	HN	8.31	8.31	0.00	8.31
		Hα (avg.)	3.94	3.95	0.01	3.95
	^{13}C	Cα	45.3	45.3	0.0	45.3
		CO	174.6	174.7	0.1	174.7
	^{15}N	N	114.3	114.4	0.2	114.4
Glu	^{1}H	HN	8.45	8.57	0.12	8.57
		Hα	4.39	4.29	- 0.10	4.29
		Hβ (avg.)	2.08	2.02	- 0.06	2.02
		Hγ	2.49	2.27	- 0.22	2.27
		Hε2 (carboxyl)	> 10			
	^{13}C	Cα	56.0	56.9	1.0	56.9
		Cβ	28.5	30.0	1.5	30.0
		Cγ	32.7	36.1	3.5	36.1
		Cδ (carboxyl)	179.7	183.8	4.1	183.8
		CO	176.5	177.0	0.6	177.0
	^{15}N	N	119.9	120.9	1.0	120.9
Gly(+1)	^{1}H	HN	8.53	8.55	0.02	8.55
		Hα (avg.)	3.86	3.86	0.00	3.86
	^{13}C	Cα	44.9			
		CO	176.7	176.8	0.1	176.8
	^{15}N	N	111.0	111.1	0.1	111.1
amide	^{1}H	H_2N (Z)	7.08	7.09	0.01	7.09
		H_2N (E)	7.44	7.43	- 0.01	7.43
	^{15}N	N	107.2	107.3	0.0	107.3

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Histidine: Ac-Gly-His-Gly-NH_2 (pK_a 6.45) [c]				
acetyl	^{1}H	CH_3 (methyl)	2.03	2.03	0.00	2.03
	^{13}C	CH_3 (methyl)	24.5	24.5	0.0	24.5
		CO	177.4	177.4	0.0	177.4
Gly(- 1)	^{1}H	HN	8.29	8.28	- 0.01	8.28
		Hα (avg.)	3.88	3.87	- 0.01	3.87
	^{13}C	Cα	45.4	45.3	- 0.1	45.3
		CO	174.3	174.3	0.0	174.3
	^{15}N	N	113.9	114.2 [b]	0.3	114.2
His [c]	^{1}H	HN	8.55	~8.35 [d]	~ - 0.2	
		Hα	~ 4.75 [d]	4.59	~ - 0.2	
		Hβ (avg.)	3.25	3.08	- 0.17	3.12
		Hδ2	7.30	6.97	- 0.33	7.04
		Hε1	8.60	7.68	- 0.92	7.88
		Hδ1	>10			
		Hε2	>10			
	^{13}C	Cα	55.1	56.7	1.6	56.3
		Cβ	28.9	31.3	2.4	30.7
		Cγ	131.0	135.3	4.2	134.3
		Cδ2	120.3	120.0	- 0.3	120.0
		Cε1	136.6	139.2	2.6	138.6
		CO	174.8	176.2	1.5	175.9
	^{15}N	N	117.9	119.7 [b]	1.8	119.3
		Nδ1	175.8	231.3 [b]	56	219.1
		Nε2	173.1	181.1 [b]	8	179.3
Gly(+1)	^{1}H	HN	8.55			
		Hα (avg.)	3.94	3.88	- 0.06	3.89
	^{13}C	Cα	45.0	45.0	0.1	45.0
		CO	176.3	176.9	0.6	176.8
	^{15}N	N	111.0	111.5 [b]	0.5	111.4
amide	^{1}H	H_2N (Z)	7.09	7.09	0.00	7.09
		H_2N (E)	7.53	7.42	- 0.11	7.44
	^{15}N	N	107.4			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Cysteine: **Ac-Gly-Cys-Gly-NH_2** (pK_a 8.49)				
acetyl	^{1}H	CH_3 (methyl)	2.06	2.07	0.01	2.06
	^{13}C	CH_3 (methyl)	24.4	24.5	0.1	24.4
	^{13}C	CO	177.5	177.6	0.1	177.5
Gly(- 1)	^{1}H	HN	8.32			
		Hα (avg.)	3.98	3.97	- 0.01	3.98
	^{13}C	Cα	45.4	45.4	0.0	45.4
		CO	174.6	174.2	- 0.4	174.6
	^{15}N	N	114.3	114.7 [b]	0.4	114.4
Cys	^{1}H	HN	8.48			
		Hα	4.56	4.28	- 0.28	4.55
		Hβ (avg.)	2.97	2.88	- 0.09	2.97
		Hγ (thiol)	~ 2.0 [e]			
	^{13}C	Cα	58.5	60.6	2.1	58.5
		Cβ	28.0	29.7	1.7	28.0
		CO	175.0	176.9	1.9	175.1
	^{15}N	N	118.7	122.2 [b]	3.6	118.8
Gly(+1)	^{1}H	HN	8.58			
		Hα (avg.)	3.93	3.92	- 0.01	3.93
	^{13}C	Cα	45.0	45.1	0.1	45.0
		CO	176.6	177.1	0.5	176.7
	^{15}N	N	112.4	113.0 [b]	0.6	112.4
amide	^{1}H	H_2N (Z)	7.09	7.09	0.00	7.09
		H_2N (E)	7.46	7.5	0.04	7.46
	^{15}N	N	107.3			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Tyrosine: **Ac-Gly-Tyr-Gly-NH_2** (pK_a 9.76)				
acetyl	^{1}H	CH_3 (methyl)	2.01	2.03	0.02	2.01
	^{13}C	CH_3 (methyl)	24.4	24.5	0.1	24.4
		CO	177.3	177.4	0.1	177.3
Gly(- 1)	^{1}H	HN	8.21			
		Hα (avg.)	3.85	3.87	0.02	3.85
	^{13}C	Cα	45.2	45.3	0.0	45.2
		CO	174.2	174.2	0.0	174.2
	^{15}N	N	114.1	114.3 [b]	0.2	114.1
Tyr	^{1}H	HN	8.16			
		Hα	4.55	4.49	- 0.06	4.55
		Hβ (avg.)	3.02	2.94	- 0.08	3.02
		Hδ	7.14	6.97	- 0.17	7.14
		Hε	6.85	6.57	- 0.28	6.85
		Hη (phenol)	~ 9.3 [e]			
	^{13}C	Cα	58.0	58.2	0.3	58.0
		Cβ	38.6	38.7	0.1	38.6
		Cγ	130.5	123.8	- 6.7	130.5
		Cδ	133.3	133.2	- 0.1	133.3
		Cε	118.4	121.7	3.3	118.4
		Cζ	157.0	167.4	10.4	157.0
		CO	176.3	176.7	0.4	176.3
	^{15}N	N	120.1	120.7 [b]	0.6	120.1
Gly(+1)	^{1}H	HN	8.44			
		Hα (avg.)	3.82	3.83	0.01	3.82
	^{13}C	Cα	45.0	45.0	0.1	45.0
		CO				
	^{15}N	N	112.4			
amide	^{1}H	H_2N (Z)	7.07			
		H_2N (E)	7.21			
	^{15}N	N	107.1			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Lysine: Ac-Gly-Lys-Gly-NH_2 (pK_a 10.34)				
acetyl	^{1}H	CH_3 (methyl)	2.05	2.05	0.00	2.05
	^{13}C	CH_3 (methyl)	24.4	24.4	0.0	24.4
		CO	177.4	177.4	0.0	177.4
Gly(- 1)	^{1}H	HN	8.31			
		Hα (avg.)	3.94	3.94	0.00	3.94
	^{13}C	Cα	45.4	45.3	- 0.1	45.4
		CO	174.5	174.6	0.1	174.5
	^{15}N	N	114.4	114.5 [b]	0.1	114.4
Lys	^{1}H	HN	8.40			
		Hα	4.34	4.30	- 0.04	4.34
		Hβ (avg.)	1.82	1.78	- 0.04	1.82
		Hγ	1.44	1.36	- 0.08	1.44
		Hδ	1.68	1.44	- 0.24	1.68
		Hε	3.00	2.60	- 0.40	3.00
		Hζ (amine)	7.52	~ 1 - 2 [f]	~ - 6 [f]	7.52
	^{13}C	Cα	56.4	56.9	0.4	56.5
		Cβ	32.8	33.2	0.3	32.9
		Cγ	24.7	25.0	0.4	24.7
		Cδ	28.9	33.9	5.0	28.9
		Cε	42.1	43.1	1.0	42.1
		CO	177.0	177.5	0.5	177.0
	^{15}N	N	121.0	121.7 [b]	0.7	121.0
		Nζ (amine)	32.7	~ 25.2 [g]	~ - 7.5 [g]	32.7
Gly(- 1)	^{1}H	HN	8.50			
		Hα (avg.)	3.91	3.90	- 0.01	3.91
	^{13}C	Cα	44.9	44.9	0.0	44.9
		CO	176.8	176.8	0.0	176.8
	^{15}N	N	111.0	111.1 [b]	0.1	111.0
amide	^{1}H	H_2N (Z)	7.07			
		H_2N (E)	7.45			
	^{15}N	N	107.2			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	Δδ (A - HA)	calc. δ (pH 7)
		Arginine: Ac-Gly-Arg-Gly-NH_2	(pK_a ~ 13.9)			
acetyl	^{1}H	CH_3 (methyl)	2.05			2.05
	^{13}C	CH_3 (methyl)	24.4			24.4
		CO	177.5			177.5
Gly(- 1)	^{1}H	HN	8.31			8.31
		Hα (avg.)	3.94			3.94
	^{13}C	Cα	45.4			45.4
		CO	174.6			174.6
	^{15}N	N	114.4			114.4
Arg	^{1}H	HN	8.43			8.43
		Hα	4.36			4.36
		Hβ (avg.)	1.84			1.84
		Hγ	1.65			1.65
		Hδ	3.21	~ 3.04	~ - 0.17	3.21
		Hε (guan.)	7.19			7.19
		Hη (guan.)	6.64			6.64
	^{13}C	Cα	56.3			56.3
		Cβ	30.6			30.6
		Cγ	27.0			27.0
		Cδ	43.3			43.3
		Cζ (guan.)	159.5	~ 163.0	~ 3.5	159.5
		CO	176.7			176.7
	^{15}N	N	120.7			120.7
		Nε (guan.)	84.8			84.8
		Nη (guan.)	~ 71 [h]			~ 71 [h]
Gly(- 1)	^{1}H	HN	8.51			8.51
		Hα (avg.)	3.91			3.91
	^{13}C	Cα	44.9			44.9
		CO	176.5			176.5
	^{15}N	N	111.0			111.0
amide	^{1}H	H_2N (Z)	7.07			7.07
		H_2N (E)	7.46			7.46
	^{15}N	N	107.3			107.3

Table S1 - con't

[a] Recorded at 25 ^{o}C with 50 mM NaCl and 5% D_2O, unless indicated. Reported are the fit pK_a values and end point chemical shifts δ (ppm) of the of the acid (HA) and conjugate base (A) forms, along with the chemical shift change upon deprotonation (Δδ; negative is upfield) and the predicted shift at pH 7. The estimated errors are ± 0.05 for pK_a values (± 0.1 for arginine), ± 0.02 ppm for ^{1}H nuclei, ± 0.08 ppm for ^{13}C, and ± 0.06 ppm for ^{15}N. Blank values indicate not determined. Prochiral proton shifts are averaged. The C-terminal amide protons are assigned assuming Z/E as upfield/downfield.

[b] Recorded in 99% D_2O and corrected for the deuterium isotope shift.

[c] Data for neutral histidine is an average of ~80% $N^{\varepsilon 2}H$ and ~20% $N^{\delta 1}H$ tautomers.

[d] Estimated from (Kjaergaard et al. 2011).

[e] From the BioMagResBank (Ulrich et al. 2008).

[f] From (Takayama et al. 2008).

[g] From (Andre et al. 2007).

[h] From $^{13}C_6/^{15}N_4$-L-arginine (Table S2).

Table S2 pH-dependent chemical shifts (ppm) of $^{13}C_6/^{15}N_4$-L-arginine [a,b]

nucleus type	nucleus	δ (HAH) pH ~ 7	δ (HA) pH ~ 11.5	δ (A) pH > 15	Δδ due to amine (HA - HAH)	Δδ due to guan. (A - HA)
		α-COO- α-NH_3+ guan+	α-COO- α-NH_2 guan+	α-COO- α-NH_2 guan		
^{1}H	HN (amine)	7.81				
	Hα	3.77	3.26	3.19	- 0.51	- 0.07
	Hβ (avg.)	1.89	1.60		- 0.29	< |- 0.1|
	Hγ	1.67	1.60		- 0.07	< |- 0.1|
	Hδ	3.24	3.19	3.00	- 0.06	- 0.19
	Hε	7.22				
	Hη	6.67				
^{13}C	Cα	57.2	58.4	58.6	1.2	0.2
	Cβ	30.3	34.4	35.2	4.1	0.9
	Cγ	26.6	27.2	28.1	0.6	1.0
	Cδ	43.3	43.8	44.3	0.5	0.5
	Cζ	159.6	159.6	163.5	0.0	4.0
	CO	177.1	185.8	186.1	8.7	0.2
^{15}N	N (amine)	40.6	33.2	33.6	- 7.5	0.4
	Nε	84.4	85.6	91.5	1.2	5.9
	Nη	71.5	71.2	93.2	- 0.3	22

[a] Recorded at 25 °C for 100 mM $^{13}C_6/^{15}N_4$-L-arginine with 5% D_2O, 1 mM DSS, and initially 50 mM NaCl (final KOH > 10 M). Blank values indicate not determined due to rapid HX or spectral overlap. The $^1H^{\beta}$ shifts are averaged. Due to bond rotations, the two $^{15}N^{\eta}$ and four $^1H^{\eta}$ yield broad signals. The data for neutral arginine are tautomer averaged.

[b] Tabulated are the fit chemical shifts for the two-step sequential titration from pH 7 to 15.25 of the α-aminium (pK_a 9.15 ± 0.05) and then the guanidinium (pK_a 13.9 ± 0.1) moieties in the context of the α-carboxylate anion. The estimated fitting errors are ± 0.05 ppm for ^{1}H nuclei, ± 0.1 ppm for ^{13}C, ± 0.15 ppm for ^{15}N amine and $^{15}N^{\varepsilon}$, and ± 0.3 ppm for $^{15}N^{\eta}$.

Supplemental References

Andre I, Linse S, Mulder FAA (2007) Residue-specific pK(a) determination of lysine and arginine side chains by indirect ^{15}N and ^{13}C NMR spectroscopy: Application to apo calmodulin. J Am Chem Soc 129:15805-15813

Kjaergaard M, Brander S, Poulsen FM (2011) Random coil chemical shift for intrinsically disordered proteins: effects of temperature and pH. J Biomol NMR 49:139-149

Takayama Y, Castaneda CA, Chimenti M, Garcia-Moreno B, Iwahara J (2008) Direct evidence for deprotonation of a lysine side chain buried in the hydrophobic core of a protein. J Am Chem Soc 130:6714-6715

Ulrich EL, Akutsu H, Doreleijers JF, Harano Y, Ioannidis YE, Lin J, Livny M. Mading S, Maziuk D, Miller Z, Nakatani E, Schulte CF. Tolmie DE, Wenger RK, Yao HY, Markley JL (2008) BioMagResBank. Nucleic Acids Res 36:D402-D408