

Determination of ^{15}N Chemical Shift Anisotropy from a Membrane-Bound Protein by NMR Spectroscopy

Supporting Information

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Table S1. Measured backbone amide- ^{15}N transverse cross-correlation rates (η_{xy}) for various residues of cytb₅.

Residue	$\eta_{xy}(\text{s}^{-1})$	Residue	$\eta_{xy}(\text{s}^{-1})$
K7	3.23±0.23	D58	16.39±0.93
D8	3.73±0.37	A59	14.75±1.17
V9	8.47±0.46	T60	16.16±0.91
K10	14.51±1.36	E61	18.72±2.20
Y11	14.32±0.89	N62	14.75±0.83
Y12	14.94±2.30	F63	16.41±0.48
T13	12.89±1.07	E64	15.73±0.89
L14	15.99±1.36	D65	14.38±0.63
E15	18.40±3.07	V66	16.35±1.11
E16	15.68±0.96	G67	12.43±0.50
I17	19.65±2.27	H68	9.62±2.29
K18	19.26±0.79	S69	10.35±0.26
K19	16.14±0.82	T70	15.70±1.44
H20	15.99±1.35	D71	16.82±0.84
N21	14.63±1.05	A72	15.78±1.23
H22	16.63±1.11	R73	15.24±0.71
K24	18.36±0.24	E74	16.64±0.87
T26	16.12±1.04	S76	13.38±0.51
W27	16.09±0.78	K77	16.46±0.22
L28	13.84±1.20	T78	13.67±0.34
I29	13.48±0.65	F79	13.86±0.71
H31	16.74±2.18	I80	14.51±0.27
V34	15.89±2.77	I81	17.28±1.51
Y35	17.69±0.89	G82	13.92±0.76
D36	15.51±0.74	E83	14.56±1.40
T38	18.62±1.87	L84	16.49±0.81
K39	14.56±1.34	H85	16.07±1.12
F40	10.06±0.73	D87	14.68±0.91
L41	17.21±0.23	D88	17.89±1.87
E42	13.60±0.26	R89	16.19±0.64
E43	15.11±0.51	L92	11.9±1.08
H44	11.37±0.62	S93	5.07±1.03
E48	17.21±1.79	K94	6.57±0.47
E49	13.60±0.25	E97	6.79±0.74
V50	16.05±1.12	T98	6.76±0.98
L51	16.40±1.30	L99	6.52±0.87
R52	17.01±0.53	T101	5.79±0.62
E53	16.52±0.97	T102	7.74±0.52
Q54	16.52±0.46	D104	7.88±1.43
A55	15.58±0.55	A132	11.77±1.39
G56	13.29±1.10	D133	7.66±0.24
G57	14.20±0.88	D134	2.64±0.07

Table S2. Calculated backbone amide-¹⁵N CSA values (in ppm) for various residues of cytb₅. Calculations were performed by taking effective internuclear distance between amide nitrogen and proton (r_{NH}) as 1.023 Å while angle β between NH dipolar vector with CSA tensor described in principal axis system was varied from 12° to 20°.

Residue	$\Delta\sigma_{12^\circ}$	$\Delta\sigma_{13^\circ}$	$\Delta\sigma_{14^\circ}$	$\Delta\sigma_{15^\circ}$	$\Delta\sigma_{16^\circ}$	$\Delta\sigma_{17^\circ}$	$\Delta\sigma_{18^\circ}$	$\Delta\sigma_{19^\circ}$	$\Delta\sigma_{20^\circ}$
K7	-63.1	-63.9	-64.8	-65.8	-66.9	-68.0	-69.3	-70.7	-72.2
D8	-67.0	-67.8	-68.8	-69.8	-71.0	-72.2	-73.6	-75.1	-76.7
V9	-129.3	-131.3	-133.5	-135.9	-138.7	-141.7	-145.0	-148.7	-152.9
K10	-180.4	-183.9	-187.9	-192.4	-197.6	-203.4	-210.2	-218.0	-227.2
Y11	-143.4	-145.8	-148.4	-151.3	-154.5	-158.1	-162.1	-166.6	-171.7
Y12	-159.9	-162.7	-165.8	-169.3	-173.3	-177.7	-182.8	-188.4	-194.9
T13	-107.7	-109.2	-110.9	-112.8	-114.9	-117.1	-119.7	-122.4	-125.5
L14	-141.1	-143.4	-145.9	-148.7	-151.8	-155.3	-159.2	-163.6	-168.5
E15	-164.8	-167.8	-171.1	-174.8	-179.0	-183.8	-189.2	-195.3	-202.3
E16	-137.0	-139.2	-141.6	-144.3	-147.3	-150.6	-154.3	-158.4	-163.0
I17	-186.8	-190.6	-194.9	-199.7	-205.3	-211.7	-219.2	-227.9	-238.4
K18	-159.1	-161.8	-164.9	-168.4	-172.3	-176.7	-181.3	-187.3	-193.7
K19	-159.9	-162.8	-165.9	-169.4	-173.4	-177.8	-182.9	-188.5	-195.0
H20	-140.9	-143.1	-145.6	-148.4	-151.6	-155.1	-159.0	-163.3	-168.2
N21	-132.3	-134.3	-136.6	-139.1	-142.0	-145.1	-148.6	-152.4	-156.7
H22	-132.3	-134.3	-136.6	-139.1	-141.9	-145.1	-148.5	-152.4	-156.7
K24	-160.5	-163.3	-166.5	-170.0	-174.0	-178.5	-183.5	-189.3	-195.8
T26	-131.4	-133.4	-135.7	-138.2	-141.0	-144.1	-147.5	-151.4	-155.6
W27	-176.7	-180.0	-183.9	-188.2	-193.1	-198.6	-205.0	-212.4	-220.9
L28	-143.8	-146.1	-148.7	-151.6	-154.9	-158.5	-162.5	-167.1	-172.1
I29	-123.2	-125.0	-127.1	-129.4	-131.9	-134.7	-137.8	-141.2	-145.0
H31	-160.3	-163.2	-166.3	-169.9	-173.8	-178.3	-183.4	-189.1	-195.6
V34	-135.1	-137.2	-139.6	-142.2	-145.1	-148.4	-152.0	-156.0	-160.5
Y35	-168.9	-171.9	-175.4	-179.4	-183.8	-188.8	-194.5	-201.0	-208.5
D36	-162.2	-165.1	-168.3	-171.9	-176.0	-180.6	-185.7	-191.6	-198.3
T38	-149.0	-151.4	-154.2	-157.3	-160.8	-164.6	-169.0	-173.8	-179.3
K39	-117.8	-119.5	-121.4	-123.5	-125.9	-128.5	-131.4	-134.5	-138.0
F40	-92.9	-94.1	-95.5	97.1	-98.8	-100.6	-102.7	-104.9	-107.4
L41	-181.8	-185.4	-189.4	-194.0	-199.2	-205.2	-212.1	-220.1	-229.6
E42	-170.6	-173.7	-177.2	-181.2	-185.8	-190.9	-196.7	-203.4	-211.1
E43	-197.6	-201.8	-206.7	-212.3	-218.8	-226.3	-235.2	-246.0	-259.3
H44	-128.5	-130.4	-132.6	-135.1	-137.7	-140.7	-144.0	-147.7	-151.8
E48	-155.0	-157.6	-160.6	-163.9	-167.6	-171.8	-176.5	-181.8	-187.8

E49	-123.6	-125.5	-127.5	-129.8	-132.3	-135.1	-138.2	-141.7	-145.5
V50	-171.2	-174.4	-178.0	-182.0	-186.6	-191.7	-197.6	-204.3	-212.1
L51	-176.9	-180.3	-184.1	-188.5	-193.4	-199.0	-205.3	-212.7	-221.4
R52	-201.7	-206.2	-211.3	-217.3	-224.1	-232.2	-241.8	-253.5	-268.4
E53	-184.1	-187.7	-191.9	-196.6	-202.0	-208.2	-215.3	-223.6	-233.5
Q54	-202.2	-206.7	-211.9	-217.9	-224.8	-232.9	-242.6	-254.5	-269.6
A55	-175.3	-178.6	-182.3	-186.6	-191.4	-196.8	-203.1	-210.3	-218.6
G56	-125.1	-127.0	-129.1	-131.4	-134.0	-136.8	-140.0	-143.5	-147.4
G57	-115.6	-117.3	-119.2	-121.3	-123.6	-126.1	-128.9	-132.0	-135.4
D58	-164.9	-167.9	-171.2	-174.9	-179.1	-183.9	-189.3	-195.4	-202.4
A59	-121.1	-122.9	-124.9	-127.1	-129.6	-132.3	-135.3	-138.6	-142.3
T60	-158.2	-161.0	-164.1	-167.5	-171.4	-175.8	-180.7	-186.2	-192.5
E61	-200.1	-204.5	-209.6	-215.4	-222.1	-229.9	-239.3	-250.6	-264.9
N62	-159.1	-161.8	-164.9	-168.4	-172.3	-176.7	-181.7	-187.3	-193.7
F63	-166.1	-169.1	-172.5	-176.3	-180.6	-185.4	-190.9	-197.1	-204.3
E64	-169.6	-172.7	-176.2	-180.2	-184.7	-189.7	-195.5	-202.1	-209.6
D65	-171.9	-175.1	-178.7	-182.8	-187.4	-192.6	-198.5	-205.3	-213.2
V66	215.5	-220.7	-226.8	-234.0	-242.4	-252.7	-265.4	-282.1	-306.7
G67	-141.1	-143.4	-145.9	-148.7	-151.8	-155.3	-159.2	-163.6	-168.5
H68	-204.1	-208.7	-213.9	-220.1	-227.2	-235.5	-245.6	-258.0	-273.9
S69	-110.4	-112.0	-113.7	-115.7	-117.8	-120.2	-122.8	-125.6	-128.8
T70	-126.0	-127.9	-130.0	-132.3	-135.0	-137.8	-141.0	-145.0	-148.5
D71	-167.6	-169.6	-172.9	-176.8	-181.1	-185.9	-191.4	-197.7	-204.9
A72	-156.6	-159.3	-162.3	-165.7	-169.5	-173.7	-178.5	-184.0	-190.1
R73	-161.5	-164.3	-167.5	-171.1	-175.2	-179.7	-184.8	-190.6	-197.3
E74	-197.2	-201.4	-206.3	-211.8	-218.3	-225.8	-234.6	-245.3	-258.4
S76	-150.2	-152.7	-155.5	-158.6	-162.1	-166.0	-170.4	-175.4	-181.0
K77	-161.1	-163.9	-167.1	-170.7	-174.7	-179.2	-184.3	-190.1	-196.7
T78	-116.3	-117.9	-119.8	-121.9	-124.2	-126.8	-129.6	-132.7	-136.2
F79	-134.1	-136.2	-138.5	-141.1	-144.0	-147.2	-150.7	-154.7	-159.1
I80	-132.5	-134.5	-136.8	-139.3	-142.2	-145.3	-148.8	-152.7	-157.0
I81	-166.7	-169.7	-173.1	-176.9	-181.2	-186.1	-191.6	-197.9	-205.2
G82	-125.4	-127.3	-129.4	-131.8	-134.4	-137.2	-140.4	-143.9	-147.9
E83	-139.0	-141.3	-143.7	-146.5	-149.5	-152.9	-156.7	-161.0	-165.7
L84	-154.5	-157.1	-160.1	-163.4	-167.1	-171.2	-175.9	-181.1	-187.1
H85	-157.3	-160.0	-163.1	-166.5	-170.3	-174.6	-179.5	-185.0	-191.2
D87	-93.6	-94.9	-96.3	-97.9	-99.6	-101.4	-103.5	-105.8	-108.3
D88	-133.4	-135.5	-137.8	-140.3	-143.2	-146.4	-149.9	-153.8	-158.2
R89	-139.2	-141.4	-143.9	-146.6	-149.7	-153.1	-156.9	-161.2	-165.9
L92	-112.6	-114.3	-116.1	-118.1	-120.3	-122.7	-125.4	-128.4	-131.6
S93	-55.2	-55.8	-56.6	-57.4	-58.4	-59.4	-60.5	-61.7	-63.0
K94	-87.0	-88.1	-89.4	-90.8	-92.4	-94.1	-96.0	-98.0	-100.3

E97	-91.6	-92.8	-94.2	-95.7	-97.4	-99.2	-101.2	-103.4	-105.8
T98	-86.8	-88.0	-89.3	-90.7	-92.2	-93.9	-95.8	-97.9	-100.1
L99	-83.2	-84.3	-85.6	-86.9	-88.4	-90.0	-91.8	-93.7	-95.9
T101	-52.1	-52.8	-53.5	-54.3	-55.1	-56.1	-57.1	-58.2	-59.5
T102	-97.5	-98.8	-100.3	-101.9	-103.7	-105.7	-107.9	-110.3	-112.9
D104	-90.0	-91.2	-92.5	-94.0	-95.7	-97.4	-99.4	-101.6	-103.9
A132	-120.1	-121.9	-123.9	-126.1	-128.5	-131.2	-134.2	-137.4	-141.1
D133	-100.9	-102.4	-103.9	-105.6	-107.5	-109.6	-111.9	-114.4	-117.2
D134	-101.2	-102.6	-104.1	-105.8	-107.7	-109.8	-112.1	-114.6	-117.4

Table S3. Ratio of transverse cross-correlation rate (η_{xy}) and transverse relaxation rate (R_2) for various residues of cytb₅. R_2 was determined using CPMG based HSQC pulse sequence.¹

Residue	η_{xy}/R_2	Residue	η_{xy}/R_2
K7	0.31	D58	0.69
D8	0.32	A59	0.55
V9	0.58	T60	0.67
K10	0.73	E61	0.78
Y11	0.62	N62	0.67
Y12	0.68	F63	0.69
T13	0.50	E64	0.70
L14	0.62	D65	0.67
E15	0.69	V66	0.81
E16	0.60	G67	0.62
I17	0.75	H68	0.79
K18	0.62	S69	0.51
K19	0.68	T70	0.57
H20	0.62	D71	0.69
N21	0.59	A72	0.67
H22	0.59	R73	0.68
K24	0.68	E74	0.77
T26	0.58	S76	0.65
W27	0.72	K77	0.68
L28	0.63	T78	0.53
I29	0.56	F79	0.59
H31	0.68	I80	0.59
V34	0.60	I81	0.69
Y35	0.70	G82	0.56
D36	0.68	E83	0.61
T38	0.64	L84	0.66
K39	0.54	H85	0.67
F40	0.44	D87	0.44
L41	0.73	D88	0.59
E42	0.71	R89	0.61
E43	0.77	L92	0.52
H44	0.57	S93	0.27
E48	0.66	K94	0.41
E49	0.56	E97	0.43
V50	0.71	T98	0.41
L51	0.72	L99	0.40
R52	0.78	T101	0.26
E53	0.74	T102	0.46
Q54	0.78	D104	0.42
A55	0.72	A132	0.54
G56	0.56	D133	0.47
G57	0.53	D134	0.47

Table S4. Backbone amide-¹⁵N CSA values determined from biomolecules using solution NMR approach.

System	CSA range	Average CSA	Reference
HIV-1 protease		170 ppm	(2)
Ubiquitin	-125 to -216 ppm	157±19 ppm	(3)
GATA1-DNA complex		168±20 ppm	(4)
Ribonuclease-H	-129 to -213 ppm	-172±13 ppm	(5)
GB3	-111 to -240 ppm Helix: -178 ppm Sheet: -181 ppm	-178 ppm	(6)
Ubiquitin	-138 to -207 ppm	-169 ppm	(7)
GB3 in liquid crystalline medium	-148 to -190 ppm Helix: -173±7 ppm Sheet: -162±6 ppm	-166±9 ppm	(8)
Lysozyme in bicelles		-174 ppm	(9)
Ubiquitin in liquid crystalline medium		162±4 ppm	(10)
C12A- <i>p8</i> ^{MTCPI}	-160 to -245 ppm	-180 ppm	(11)
Ubiquitin in liquid crystalline medium	Helix: -166±9 ppm Sheet: -161±6 ppm	-163±8 ppm	(12)

Table S5. Backbone amide-¹⁵N CSA values determined from peptides and proteins using solid-state NMR approach.

System	CSA [$\delta_{11} - 0.5 * (\delta_{22} + \delta_{33})$]	CSA span ($\delta_{11} - \delta_{33}$)	Reference
Ac[1- ¹³ C]Ala [¹⁵ N]AlaNH ₂	165 ppm	185 ppm	(13)
Gly[¹⁵ N]GlyH ₂ O	160 ppm	175 ppm	(14)
GB1	-109 ppm; [$\delta_{11} - \delta_{iso}$] Helix: -115 ppm Sheet: -106 ppm		(15)
NAG	161 ppm	183 ppm	(16)
Collagen powder	169 ppm	181 ppm	(16)
[1- ¹³ C] glycyl ₂ [¹⁵ N]alan yl ₃ -gramicidin A	156 ppm	169 ppm	(17)
N-AcVal Leu: Val	156 ppm	170 ppm	(18)
N-AcVal Leu: Leu	156 ppm	174 ppm	(18)
(Ala) _n	Helix: 158 ppm	175 ppm	(19)
(Ala) ₅	Sheet: 148 ppm	157 ppm	(19)
(Ala, Val) _n	Helix: 154 ppm	159 ppm	(20)
(Gly) _n	Sheet: 152 ppm	160 ppm	(21)
(Gly, Ala) _n	Helix: 162 ppm	168 ppm	(21)
Asp(OBzl) _n	Helix: 159 ppm Sheet: 154 ppm	Helix: 166 ppm Sheet: 162 ppm	(22)
Tripeptide AGG and GGV		157- 176 ppm	(23)
NAV	153-166 ppm [at different spinning rates]	169-179 ppm	(24)
NAVL Valine	145 to 158 ppm [at different spinning rates]	168-177 ppm	(24)
NAVL Leucine	150 to 159 ppm [at different spinning rates]	169-174 ppm	(24)

Table S6. Backbone amide-¹⁵N CSA values determined from peptides and proteins using computational approach.

System	CSA [$\delta_{11} - 0.5 * (\delta_{22} + \delta_{33})$]	Reference
Cytochrome c ₅₅₁	142 to 159 ppm	(25)
GB3	Helix: 159 ppm Sheet: 158 ppm	(26)
NMA, N-formyl-alanyl-X and GB3	-155 to -168 ppm Helix: -164 ppm Sheet: -160 ppm Mean: -160 ppm	(27)
Dipeptides	138 to 186 ppm Helix: 144 (Ala-X) and 155 ppm (X-Ala) Sheet: 169 (Ala-X) and 178 ppm (X-Ala)	(28)
GB1	$\delta = [\delta_{11} - \delta_{iso}]$ Helix: 108 ppm Sheet: 102 ppm	(29)
GB3	$\delta = [\delta_{11} - \delta_{iso}]$ Helix: 109 ppm Sheet: 104 ppm	(29)

Readers are referred to a recent review article by Saito et al. for a detailed study on various CSA tensors and values determined from different types of NMR techniques.³⁰

Figure S1. Residues of cytb₅ within 12.5Å distance from paramagnetic center Fe(III) of the heme unit.

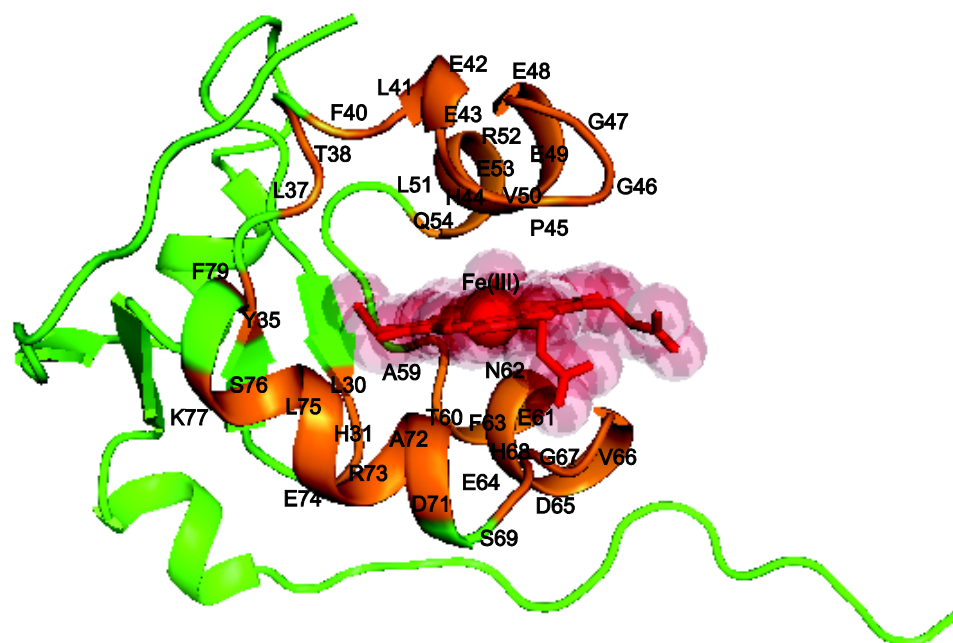
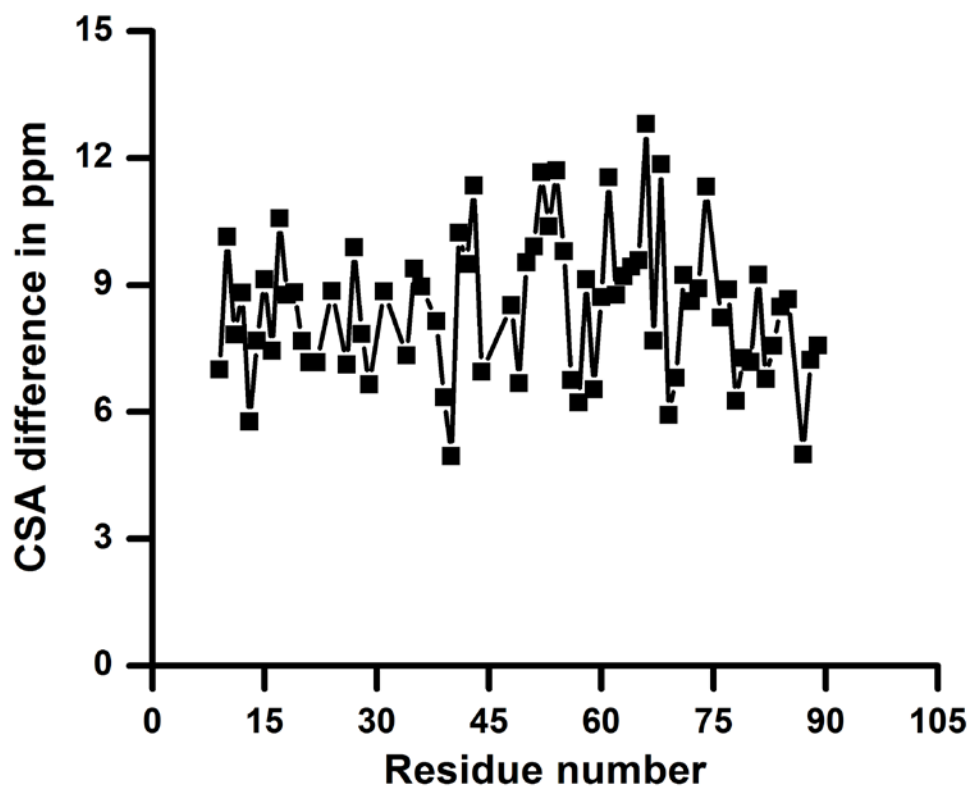


Figure S2. ^{15}N CSA difference calculated for internuclear distances ($r_{\text{N-H}}$) 1.023 and 1.04 Å.



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