Supporting Information

Quantum Chemical Calculations of Amide-^{15}N Chemical Shift Anisotropy Tensors for a Membrane-Bound Cytochrome b_5

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Table S1. The variation of the principal components of backbone amide-^{15}N CSA tensors in ppm are tabulated with distance from backbone amide-^{15}N of interest for few residues of cytb$_5$. Calculated isotropic chemical shift (δ_{iso}) represents the average of three principal components [δ_{iso}= (δ_{11}+δ_{22}+δ_{33})/3]; CSA ($\Delta\delta$) is determined using the relation $\Delta\delta = \delta_{11} - 0.5 \times (\delta_{22}+\delta_{33})$.

Residue	Distance	δ_{11}	δ_{22}	δ_{33}	δ_{iso}	CSA ($\Delta\delta$)	δ_{11}- δ_{33}
K19	4Å	239.9	91.3	50.1	127.1	169.2	189.8
	5 Å	236.2	91.1	48.6	125.3	166.4	187.7
	6Å	234.2	91.9	46.9	124.3	164.8	187.2
T60	4Å	253.1	73.4	73.3	133.3	179.7	179.8
	5Å	251.2	73.3	72.9	132.5	178.1	178.3
	6Å	252.3	73.0	72.6	132.6	179.5	179.7
N62	4Å	229.4	97.4	57.2	128.0	152.2	172.2
	5Å	227.9	99.7	54.4	127.3	150.9	173.6
	6Å	228.9	96.6	60.0	128.5	150.6	168.9
	8Å	229.1	97.8	65.9	130.9	147.2	163.2
E64	4Å	232.8	74.4	57.1	121.5	167.1	175.7
	5Å	232.9	74.2	57.6	121.6	167.0	175.3
	6Å	233.5	73.5	58.1	121.7	167.7	175.3
D71	4Å	253.7	80.2	54.1	129.3	186.5	199.6
	5Å	245.2	82.3	49.3	125.6	179.4	195.9
	6Å	244.8	82.2	48.9	125.3	179.3	195.9
	8Å	246.7	80.7	53.9	127.1	179.4	192.8

Table S2. Backbone amide-^{15}N CSA principal components (δ_{11}, δ_{22} and δ_{33} in ppm) obtained from quantum chemical calculations for residues of cytb$_5$. The calculations were performed by taking residues within 5Å distance from the residue of interest. Asymmetry parameter (η) is given by the relation $\eta = (\delta_{22}-\delta_{33}) / (\delta_{11}-\delta_{iso})$.

Residue	δ_{11}	δ_{22}	δ_{33}	Calc δ_{iso}	Expt δ_{iso}	CSA $\Delta\delta$	span (Ω) $=\delta_{11}-\delta_{33}$	η
K10	232.4	99.5	49.3	127.1	127.3	158.0	183.1	0.48
Y11	217.9	111.2	37.7	122.3	121.9	143.5	180.2	0.77
Y12	229.7	91.5	58.7	126.6	118.3	154.6	171.0	0.32
T13	220.9	78.1	40.1	113.0	115.5	161.8	180.8	0.35
L14	248.2	104.1	50.5	134.3	123.4	170.9	197.7	0.47
E15	242.7	84.7	64.8	130.7	116.8	168.0	177.9	0.18
E16	230.5	76.0	45.2	117.2	118.9	169.9	185.3	0.27
I17	215.9	73.5	50.0	113.1	121.2	154.2	165.9	0.23
K18	236.7	77.1	51.3	121.7	116.5	172.5	185.3	0.23
K19	236.2	91.1	48.6	125.3	114.3	166.4	187.7	0.41
H20	230.7	77.4	58.6	122.3	121.8	162.7	172.1	0.17
N21	233.7	77.0	68.9	126.5	118.2	160.7	164.8	0.08
H22	203.5	100.1	58.8	120.8	117.5	124.1	144.7	0.50
K24	205.7	77.0	49.8	110.8	118.4	142.3	156.0	0.29
S25	226.4	81.0	47.6	118.3	115.5	162.1	178.9	0.31
T26	229.1	76.7	58.0	121.3	125.3	161.8	171.1	0.17
W27	242.3	97.1	59.4	132.9	127.1	164.0	182.8	0.34
L28	227.1	79.0	41.7	115.9	116.2	166.8	185.5	0.34

I29	253.7	78.5	58.8	130.3	120.8	185.1	194.9	0.16
L30	244.8	85.9	66.1	132.2	121.9	168.8	178.7	0.18
H31	223.3	82.9	65.8	124.0	125.8	149.0	157.5	0.17
K33	244.3	80.0	64.0	129.4	121.9	172.3	180.3	0.14
V34	234.8	85.4	54.7	125.0	120.8	164.7	180.1	0.28
Y35	235.5	91.5	48.0	125.0	126.5	165.7	187.4	0.39
D36	228.9	84.5	61.4	124.9	119.6	156.0	167.5	0.22
L37	231.5	99.6	67.1	132.7	122.9	148.1	164.4	0.33
T38	236.7	71.1	58.3	122.0	119.4	172.0	178.3	0.11
K39	233.5	74.0	52.5	120.0	117.5	170.3	181.0	0.19
F40	217.7	76.9	65.3	120.0	120.4	146.6	152.4	0.12
L41	225.0	87.6	44.2	118.9	119.8	159.1	180.8	0.41
E42	227.3	79.4	48.0	118.2	112.6	163.6	179.3	0.29
E43	216.2	85.0	55.6	118.9	118.3	145.9	160.6	0.30
H44	222.3	81.9	39.5	114.6	120.4	161.6	182.7	0.39
G46	198.2	78.7	31.2	102.7	110.3	143.2	167.0	0.50
E48	236.9	81.6	45.0	121.2	121.6	173.6	191.9	0.32
V50	240.9	81.7	54.2	125.6	112.7	173.0	186.7	0.24
L51	217.8	81.6	46.2	115.2	116.5	154.0	171.6	0.34
R52	230.8	84.7	55.6	123.7	119.1	160.7	175.2	0.27
E53	243.2	76.6	61.9	127.2	116.7	174.0	181.3	0.13
Q54	230.0	78.2	55.0	121.1	112.9	163.4	175.0	0.21
A55	243.3	83.8	72.8	133.3	121.3	165.0	170.6	0.10
G56	234.5	83.9	46.1	121.5	111.3	169.5	188.4	0.33
G57	207.4	76.4	25.9	103.3	105.3	156.2	181.5	0.48
D58	248.2	90.6	46.1	128.3	117.7	179.8	202.0	0.37
A59	217.0	99.0	48.7	121.6	129.1	143.1	168.3	0.53
T60	251.2	73.3	72.9	132.5	117.6	178.1	178.3	0.00
E61	232.1	74.8	40.7	115.9	119.6	174.4	191.4	0.29

N62	227.9	99.7	54.3	127.3	116.4	150.9	173.6	0.45
F63	215.9	85.9	50.0	117.2	118.3	147.9	165.9	0.36
E64	232.9	74.2	57.6	121.6	120.4	167.0	175.3	0.15
D65	233.7	91.0	45.9	123.5	121.7	165.2	187.8	0.41
H68	211.8	104.3	25.0	113.7	120.2	147.1	186.7	0.81
T70	218.4	65.9	41.9	108.7	117.0	164.5	176.5	0.22
D71	245.2	82.3	49.3	125.6	122.0	179.4	195.9	0.28
A72	221.4	83.2	50.2	118.3	124.9	154.7	171.2	0.32
R73	212.4	91.1	34.9	112.8	119.7	149.5	177.6	0.56
E74	253.8	72.8	59.5	128.7	123.6	187.7	194.3	0.11
S76	227.3	75.4	41.4	114.7	115.1	168.9	185.8	0.30
K77	231.9	85.9	46.8	121.5	120.4	165.5	185.1	0.35
T78	224.4	67.8	49.4	113.9	110.0	165.8	175.0	0.17
F79	238.2	72.4	62.8	124.5	118.4	170.6	175.5	0.09
I81	237.9	89.1	38.4	121.8	120.0	174.1	199.5	0.44
G82	215.0	70.0	30.1	105.1	111.0	164.9	184.9	0.36
E83	236.4	84.9	59.5	126.9	118.7	164.2	176.9	0.23
L84	232.5	94.8	50.7	126.0	123.3	159.8	181.8	0.41
H85	221.6	116.1	34.4	124.0	130.8	146.4	187.2	0.84
S93	214.9	81.7	39.8	112.1	115.9	154.1	175.1	0.41
K94	230.8	94.2	31.3	118.7	124.6	168.0	199.5	0.56
M96	219.2	87.9	45.4	117.5	121.7	152.6	173.9	0.42
E97	228.8	71.6	56.2	118.9	122.8	164.9	172.6	0.14
T98	226.6	78.6	57.2	120.8	116.6	158.6	169.3	0.20
I100	215.3	102.7	39.5	119.2	123.4	144.2	175.8	0.66
T101	226.6	93.7	32.1	117.5	119.1	163.7	194.5	0.56
T102	214.3	79.6	34.7	109.5	117.6	157.2	179.6	0.43

Table S3. Parallel ($\delta_{||}$, ppm) and perpendicular ($\delta_{\perp}$, ppm) components of experimentally measured amide-^{15}N CSA derived using the relations ($3\delta_{iso}$ + 2CSA)/3 and ($3\delta_{iso}$ - CSA)/3, respectively for alpha-helix and beta-sheet residues of cytb$_5$. The experimental CSA for backbone amide residues were obtained by assuming axially symmetric CSA tensors for an effective internuclear distance $r_{N\text{-}H}$ = 1.023 Å and a constant angle $\beta = 18°$ that least shielded component (δ_{11}) of CSA tensor makes with the N-H bond vector.[1]

Residue	$\delta_{\|\|}$	$\delta_{\perp}$	Residue	$\delta_{\|\|}$	$\delta_{\perp}$
K10	267.4	57.3	Q54	274.7	32.0
Y11	229.9	67.8	G56	204.6	64.7
Y12	240.1	57.4	G57	191.2	62.3
L14	229.5	70.3	D58	243.9	54.6
E15	242.9	53.7	T60	238.1	57.4
E16	221.8	67.5	E61	279.1	39.8
I17	267.3	48.1	N62	237.6	55.9
K18	237.6	55.9	F63	245.6	54.7
K19	236.2	53.3	E64	250.7	55.2
H20	227.8	68.8	D65	254.1	55.5
W27	263.8	58.8	T70	211.1	70.0
L28	224.6	62.0	D71	249.6	58.2
I29	212.7	74.9	A72	244.0	65.4
V34	222.2	70.2	R73	242.9	58.0
Y35	256.1	61.6	E74	280.0	45.4
L41	261.2	49.1	S76	228.7	58.3
E42	243.8	47.1	K77	243.2	58.9
E43	275.1	39.9	T78	196.4	66.8
E48	239.2	62.7	F79	218.8	68.1
V50	244.5	46.9	I81	247.7	56.1
L51	253.4	48.1	G82	204.6	64.2
R52	280.3	38.5	E83	223.2	66.5

E53	260.3	45.0	L84	240.5	64.6

REFRENCES

(1) Pandey, M. K.; Vivekanandan, S.; Ahuja, S.; Pichumani, K.; Im, S. C.; Waskell, L.; Ramamoorthy, A. *J. Phys. Chem. B* **2012**, *116*, 7181

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Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr ; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03 (Revision C.2). Wallingford CT: Gaussian, Inc.;* **2004**.