Electronic Supplementary Information: Planarity and out-of-plane vibrational modes of tryptophan and tyrosine in biomolecular modeling.

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Structures of tryptophan (Trp) and tyrosine (Tyr) side chains that are capped with neutral methylamide terminal groups are shown in Fig. S1.

Table S1 provides the torsion angle sets that C36 applies for atoms in the rings of Trp and Tyr.

Figs. S2a and S2b provide respectively the sum of angles around NE1 and the calculated $d_\perp$ for NE1 of Trp with methylamide terminal groups in solution during 200 ps of molecular dynamics (MD) simulation. These figures indicate that the sum of angles and $d_\perp$ of the side chain ring fluctuated in the same range for both methylamide-terminated Trp and the zwitterion (Figs. 4a and 4c in the main text). Fig. S2c shows the calculated time autocorrelation function of $d_\perp$, and Fig. S2d provides its Fourier transform. Figs. S4 provides analogous results obtained from NVE MD simulations for zwitterionic Trp. Comparing these figures with the first row of Fig. 10 indicates that out-of-plane vibrational frequencies are similar; consequently, these frequencies are independent of the terminal groups and the MD simulation ensemble (NVE or NPT). Analogous results for atom CG in Tyr with two methylamide terminal groups and in zwitterionic Tyr with NVE MD simulations are provided in Fig. S3 and Fig. S5, respectively. These results are similar to the results shown in Fig. 5 and the first row of Fig. 11 for the Tyr zwitterion.

The average sum of angles and average $d_\perp$ of all atoms in the rings of Trp or Tyr with methylamide terminal groups were calculated over 200 ps of MD simulation. In addition, these parameters were calculated for zwitterionic Trp or Tyr in NVE MD simulations. Fig. S6 and Fig. S7 provide these for Trp and Tyr, respectively, with results shown for zwitterion (NVE or NPT MD) or neutral methylamide terminal groups (NPT MD). Again, results are very similar.

These results confirm that employing charges on the terminal groups of Trp or Tyr zwitterions do not have notable effects on out-of-plane motions of atoms in the rings of these molecules. Furthermore, the similarity between results of NVE and NPT MD simulations prove that including temperature and pressure control algorithms in NPT MD simulations have not affected the out-of-plane motions of atoms.

The time autocorrelation function and its Fourier transform for fluctuations in out-of-plane deformation distances of atoms CE3 in Trp and CZ in Tyr are shown in Figs. S8 and S9, respectively. These figures are described in the main text.

Tables S2 and S3 provide all calculated wavenumbers obtained by applying all-atom normal mode analysis (NMA) on minimized zwitterionic Trp and Tyr and on Trp and Tyr with methylamide terminal groups (Fig. S1), on the basis of the C36 FF. These tables also provide averaged out-of-plane frequencies and their standard deviations for these amino acids in solution, which were obtained by applying instantaneous NMA. Dot products of eigenvectors for the minimized zwitterionic structure and minimized methylamide terminal group structure were calculated to find similar out-of-plane modes for the different architectures. These modes were chosen as the reference modes for averaging out-of-plane motions in the methylamide terminal group structure over 200 ps MD simulations by the dot product method, as explained in the methodology section of the main paper.

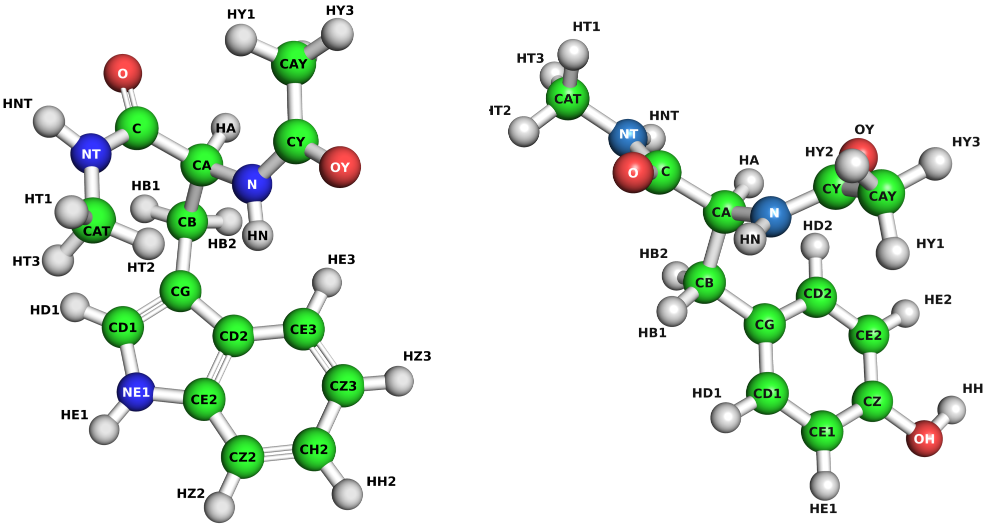
To confirm the visual identification of out-of-plane normal modes, changes in out-of-plane displacement ($\Delta d_{\perp}$) of atoms in the rings of minimized Trp and Tyr were calculated for a quarter time period of each vibrational mode. Figs. S10 and S11 provides these results for Trp and Tyr, respectively. Out-of-plane modes are labeled as listed in Tables S2 and S3. In most cases, out-of-plane modes are indicated by larger $\Delta d_{\perp}$. In some cases, out-of-plane motion was visually clear yet no one atom showed $\Delta d_{\perp} > 0.1$ Å within the normal mode normalization of 1 Å^2. In these modes, animations revealed that motions were largely planar or backbone/linker vibrations, accompanied by very small change for ring atoms. Modes H and V in Trp and modes J and K in Tyr are examples. In some cases with small $\Delta d_{\perp}$, most rings atoms made small out-of-plane motions simultaneously. Modes A and B for both amino acids are examples. Some modes such as 53 and 57 in Trp or modes 48 and 51 in Tyr had small $\Delta d_{\perp}$ yet were not named as out-of-plane modes. Instead, planar or backbone/linker vibrations slightly moves atoms that neighbor a ring, causing very small changes in $d_{\perp}$ of CG atom.

The frequencies and frequency distribution obtained for out-of-plane motions of methylamide terminal group amino acids using the C36 FF are compared with results for the amino acid zwitterions (NVE or NPT MD simulations) and with literature data in Fig. S12 and Fig. S13 for Trp and Tyr, respectively. The frequencies and frequency patterns of zwitterionic Trp or Tyr in NVE and NPT MD simulations are extremely similar, which confirms the independence of out-of-plane vibrations from the temperature and pressure control algorithms. The frequencies and frequency patterns for the zwitterion and methylamide terminal group structures are similar though not the same. Some modes (e.g., G, H of Tyr) split into multiple modes in the methylamide terminal group structures. Average frequencies for some modes became more separated on the frequency spectrum (e.g., A vs. B, P vs. R, and M vs. N of Tyr) while others showed more similar frequencies (e.g., J, K, L of Tyr). Changes were within the range of the standard deviations, though sometimes only at the extremes of that range. Differences within the MD results for each architecture were small compared to differences when compared with out-of-plane frequencies from prior Raman and infrared spectroscopy and quantum calculation results. However, some differences were observed such as mode D in Trp (Fig. S12). Mode D has frequencies of 566.5 ± 378.1 cm^{-1} and 266.9 ± 81.8 cm^{-1} for Trp as a zwitterion or with methylamide terminal groups, respectively (Table S2 or Fig. S12). Mode D of zwitterionic Trp has a big standard deviation. As explained in the main paper, we have applied the dot product only using eigenvector components for atoms in the rings because we were interested in the out-of-plane motions of these atoms. Since atoms such as CB are involved in the D mode vibration, and we have not included this atom in the dot product calculations, the method could not perfectly monitor similar modes during MD simulations.

Movies TRP01-TRP81.mpg are animations of vibrational modes for minimized Trp zwitterion on the basis of the C36 FF. These animations have been built by using VMD software.[1] Modes are listed in Table S2.

Movies TYR01-TYR72.mpg are animations of vibrational modes for minimized Tyr zwitterion on the basis of the C36 FF. These animations have been built by using VMD software.[1] Modes are listed in Table S3.

File par_all36_tor_FJ-LMM-MLG_PCCP.prm provides the changed torsion angle and added improper torsion angle parameters of set “15,-1” for Trp and set “10,-1” for Tyr as we incorporated them into a standard CHARMM 36 parameter file.



(a) (b)

Figure S1: (a) Tryptophan and (b) tyrosine with neutral methylamide terminal groups. Labels show atom ID assignments used in molecular simulations.

Table S1: CHARMM 36 (C36) torsion angle sets used for the rings of Trp and Tyr.[2]

Trp						**Tyr**					
Set	Atom Names[a]				K_{tor}(kcal/mol)[b]	Set	Atom Names[a]				K_{tor}(kcal/mol)[b]
1	CG	CD2	CE2	NE1	6.5	1	CB	CG	CD2	HD2	4.2
2	CD1	NE1	CE2	CD2	6.5	2	CB	CG	CD1	HD1	4.2
3	CG	CD1	NE1	CE2	6.0	3	CG	CD2	CE2	HE2	4.2
4	CD1	CG	CD2	CE2	5.0	4	CG	CD1	CE1	HE1	4.2
5	NE1	CD1	CG	CD2	5.0	5	CD1	CG	CD2	HD2	4.2
6	CE3	CZ3	CH2	HH2	4.2	6	HD1	CD1	CE1	CZ	4.2
7	HE3	CE3	CZ3	CH2	4.2	7	HD1	CD1	CG	CD2	4.2
8	CZ3	CH2	CZ2	HZ2	4.2	8	CE1	CZ	CE2	HE2	4.2
9	HZ3	CZ3	CH2	CZ2	4.2	9	HE1	CE1	CZ	CE2	4.2
10	CG	CD2	CE3	HE3	4.0	10	HE1	CE1	CZ	OH	4.2
11	CG	CD2	CE3	CZ3	4.0	11	CZ	CE2	CD2	HD2	4.2
12	CG	CD2	CE2	CZ2	4.0	12	OH	CZ	CE2	HE2	4.2
13	NE1	CE2	CD2	CE3	4.0	13	CB	CG	CD2	CE2	3.1
14	CE2	CZ2	CH2	CZ3	3.0	14	CB	CG	CD1	CE1	3.1
15	CE2	CZ2	CH2	HH2	3.0	15	CG	CD2	CE2	CZ	3.1
16	CE2	CD2	CE3	HE3	3.0	16	CG	CD1	CE1	CZ	3.1
17	CE2	CD2	CE3	CZ3	3.0	17	CD1	CE1	CZ	CE2	3.1
18	CD2	CE3	CZ3	CH2	3.0	18	CD1	CE1	CZ	OH	3.1
19	CD2	CE3	CZ3	HZ3	3.0	19	CD1	CG	CD2	CE2	3.1
20	CD2	CE2	CZ2	HZ2	3.0	20	CE1	CZ	CE2	CD2	3.1
21	CD2	CE2	CZ2	CH2	3.0	21	CE1	CD1	CG	CD2	3.1
22	CE3	CD2	CE2	CZ2	3.0	22	OH	CZ	CE2	CD2	3.1
23	NE1	CE2	CZ2	HZ2	3.0	23	HD1	CD1	CE1	HE1	2.4
24	NE1	CE2	CZ2	CH2	3.0	24	HD2	CD2	CE2	HE2	2.4
25	CD1	NE1	CE2	CZ2	3.0						
26	CD1	CG	CD2	CE3	3.0						
27	CB	CG	CD2	CE2	3.0						
28	CE3	CZ3	CH2	CZ2	3.1						
29	HD1	CD1	CG	CD2	2.8						
30	HD1	CD1	NE1	CE2	2.6						
31	CB	CG	CD2	CE3	2.5						
32	CB	CG	CD1	NE1	2.5						
33	HE3	CE3	CZ3	HZ3	2.4						
34	HZ3	CZ3	CH2	HH2	2.4						
35	HZ2	CZ2	CH2	HH2	2.4						
36	CB	CG	CD1	HD1	1.2						
37	HE1	NE1	CE2	CD2	0.8						
38	HD1	CD1	NE1	HE1	0.4						
39	HE1	NE1	CE2	CZ2	0.2						
40	CG	CD1	NE1	HE1	0.05						

[a] Atom names are taken from Fig. 1. These names differ from the parameter names used in a C36 parameter file.

[b] Numerical force constants are those implemented in NAMD[3] software. Parameters are $n = 2$ and $\chi = \pi$ for all of these torsion angles.

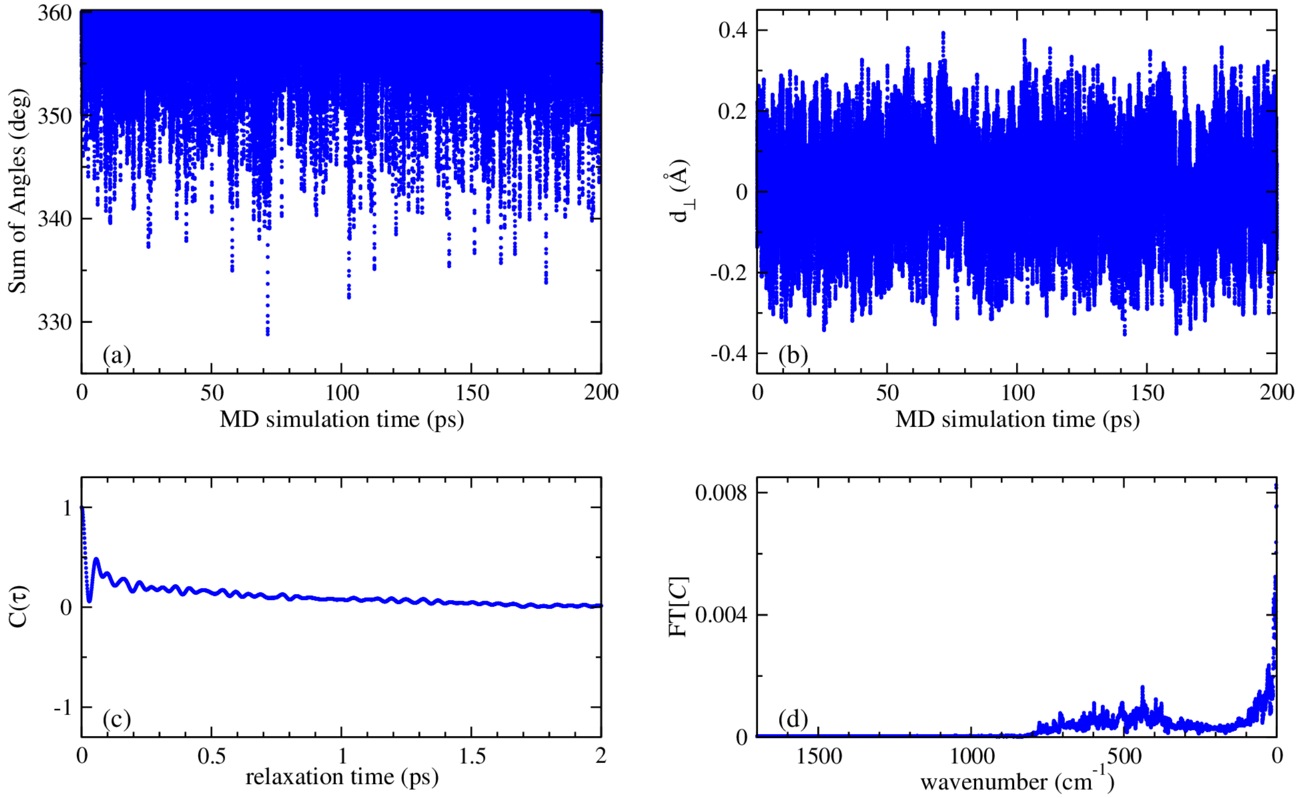


Figure S2: (a) The sum of angles around NE1 and (b) the calculated $d_\perp$ for NE1 in Trp with methylamide terminal groups as a function of simulation time by applying the original C36. (c) The time autocorrelation function of $d_\perp$ and (d) its Fourier transform for atoms NE1, CD1, HE1, and CE2 in Trp with methylamide terminal groups. A sum of 360° and $d_\perp = 0$ correspond to planarity.

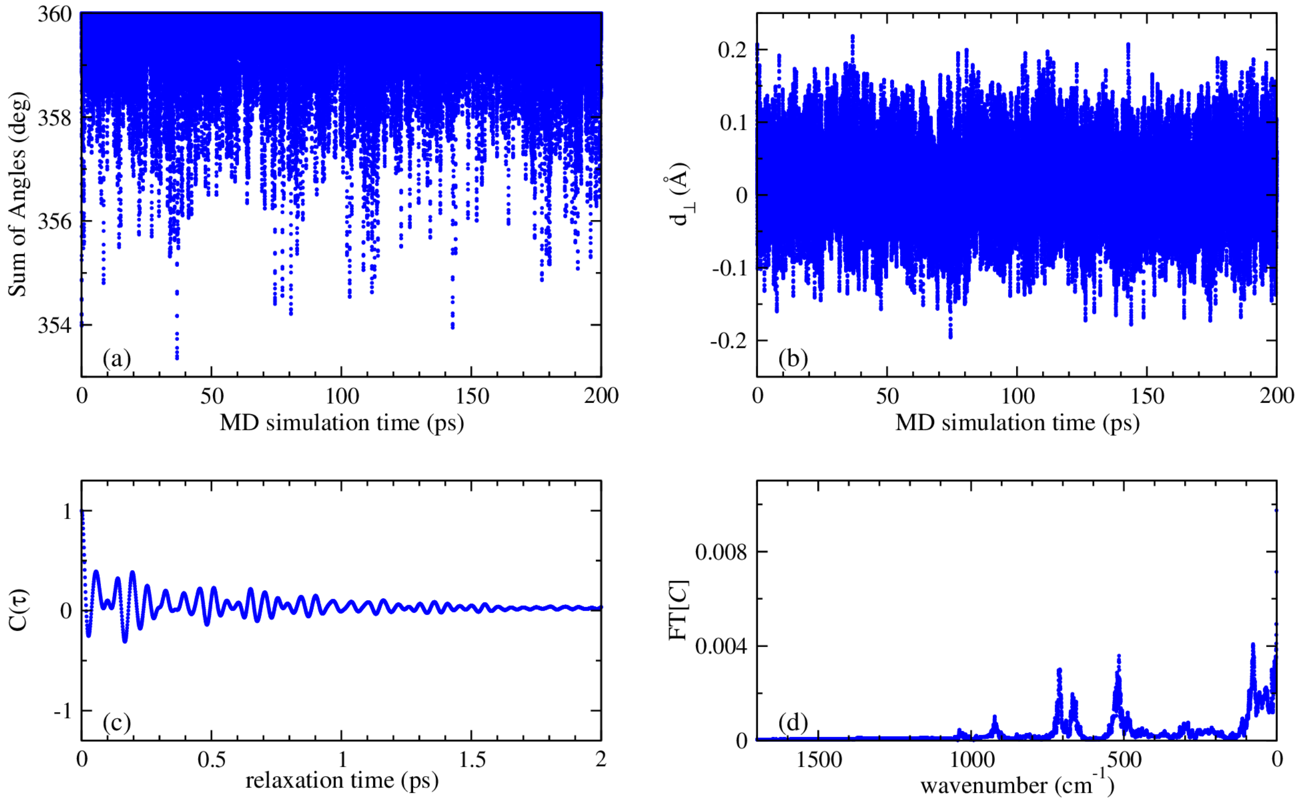


Figure S3: (a) The sum of angles around CG and (b) the calculated $d_\perp$ for CG in Tyr with methylamide terminal groups as a function of simulation time by applying the original C36. (c) The time autocorrelation function of $d_\perp$ and (d) its Fourier transform for atoms CG, CD1, CD2, and CB in Tyr with methylamide terminal groups. A sum of 360° and $d_\perp = 0$ correspond to planarity.

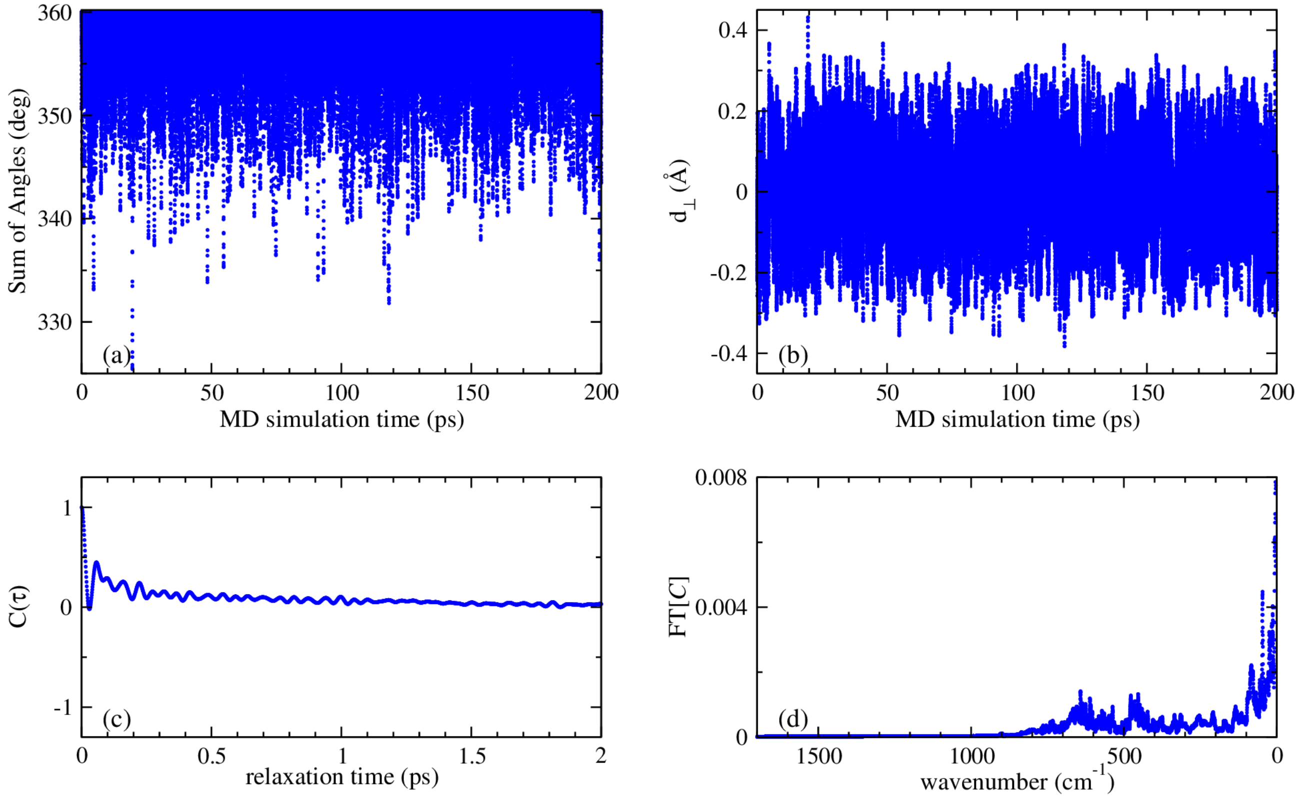


Figure S4: NVE MD simulation results. (a) The sum of angles around NE1 and (b) the calculated $d_\perp$ for NE1 in zwitterionic Trp as a function of simulation time by applying the original C36. (c) The time autocorrelation function of $d_\perp$ and (d) its Fourier transform for atoms NE1, CD1, HE1, and CE2 in zwitterionic Trp. A sum of 360° and $d_\perp = 0$ correspond to planarity.

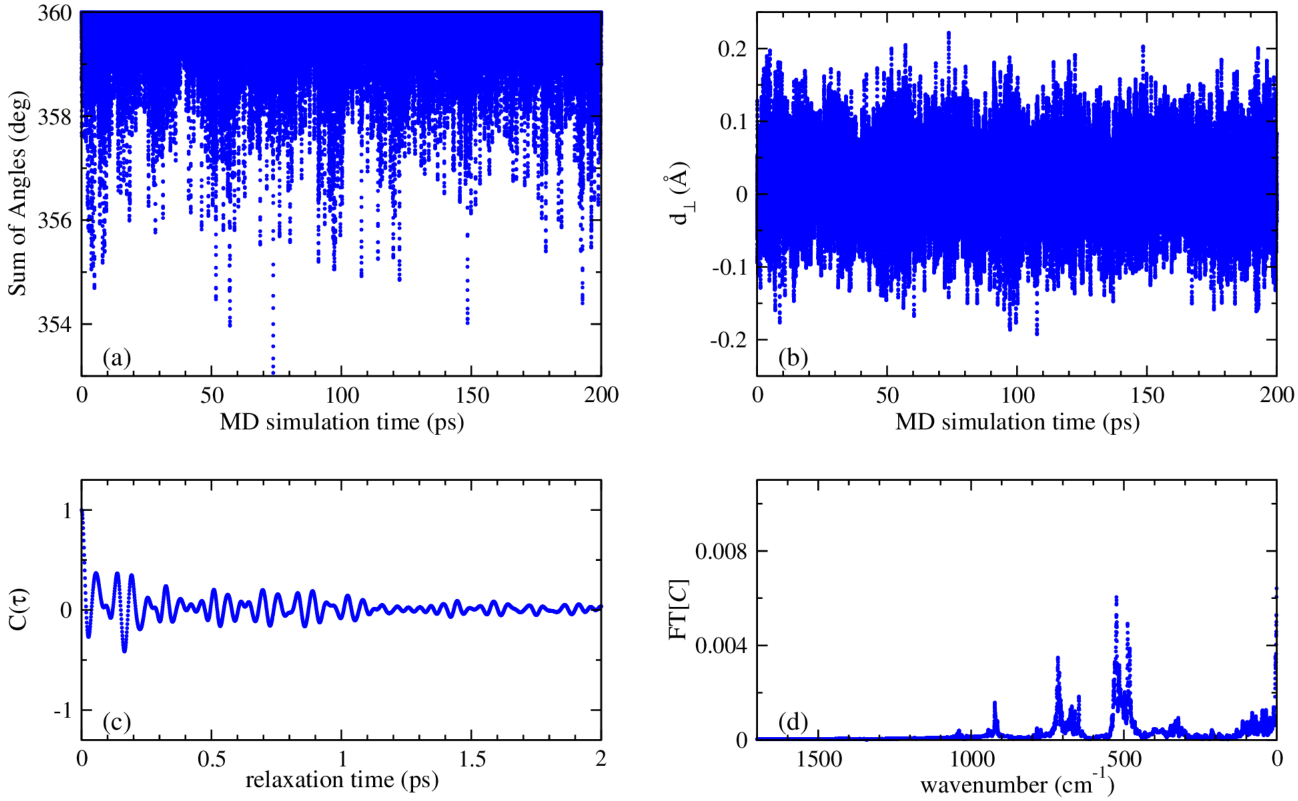


Figure S5: NVE MD simulation results. (a) The sum of angles around CG and (b) the calculated $d_\perp$ for CG in zwitterionic Tyr as a function of simulation time by applying the original C36. (c) The time autocorrelation function of $d_\perp$ and (d) its Fourier transform for atoms CG, CD1, CD2, and CB in zwitterionic Tyr. A sum of 360° and $d_\perp = 0$ correspond to planarity.

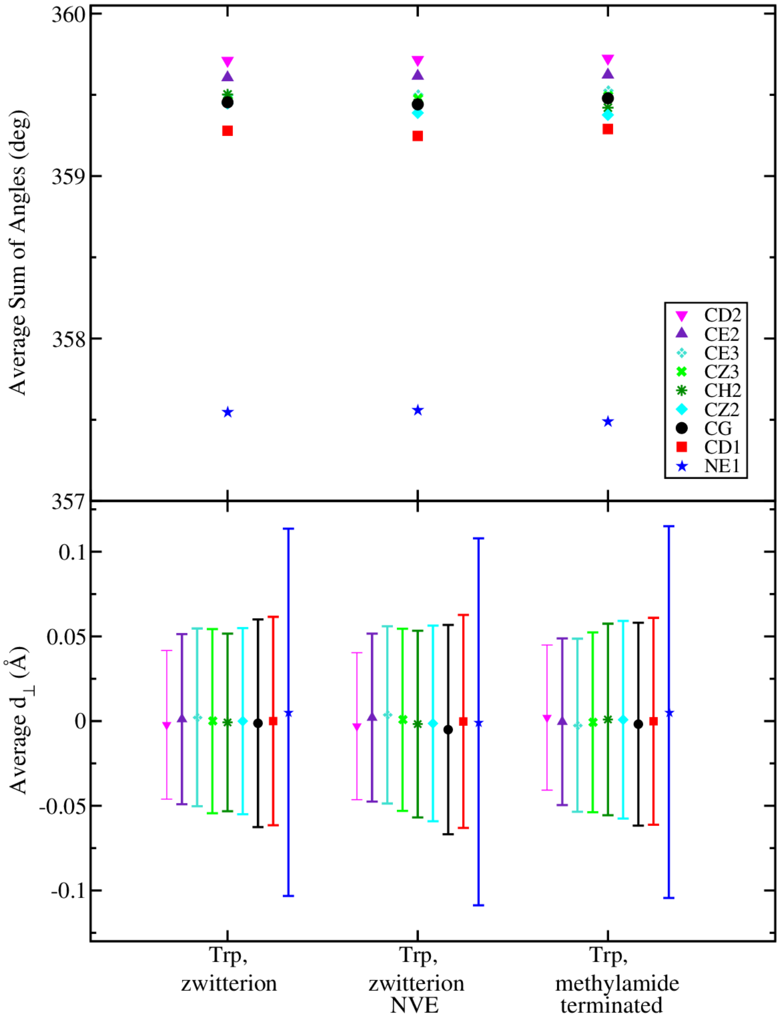


Figure S6: Variability in the average sum of bond angles and the average $d_\perp$ for atoms in the ring of Trp as a zwitterion (NPT and NVE MD simulations) and with neutral methylamide terminal groups averaged over 200 ps; atom labels in the legend are taken from Fig. S1.

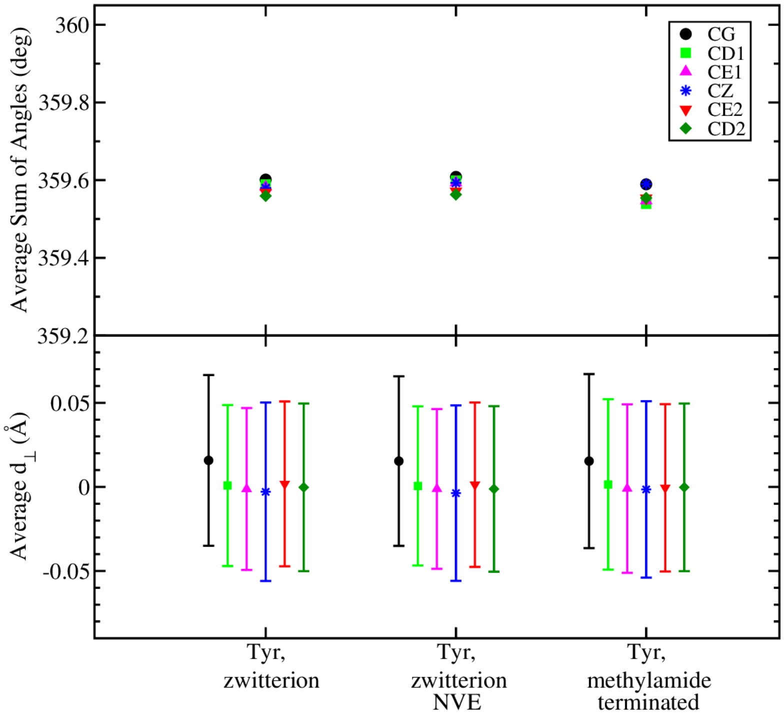


Figure S7: Variability in the average sum of bond angles and the average $d_\perp$ for atoms in the ring of Tyr as a zwitterion (NPT and NVE MD simulations) and with neutral methylamide terminal groups averaged over 200 ps; atom labels in the legend are taken from Fig. S1.

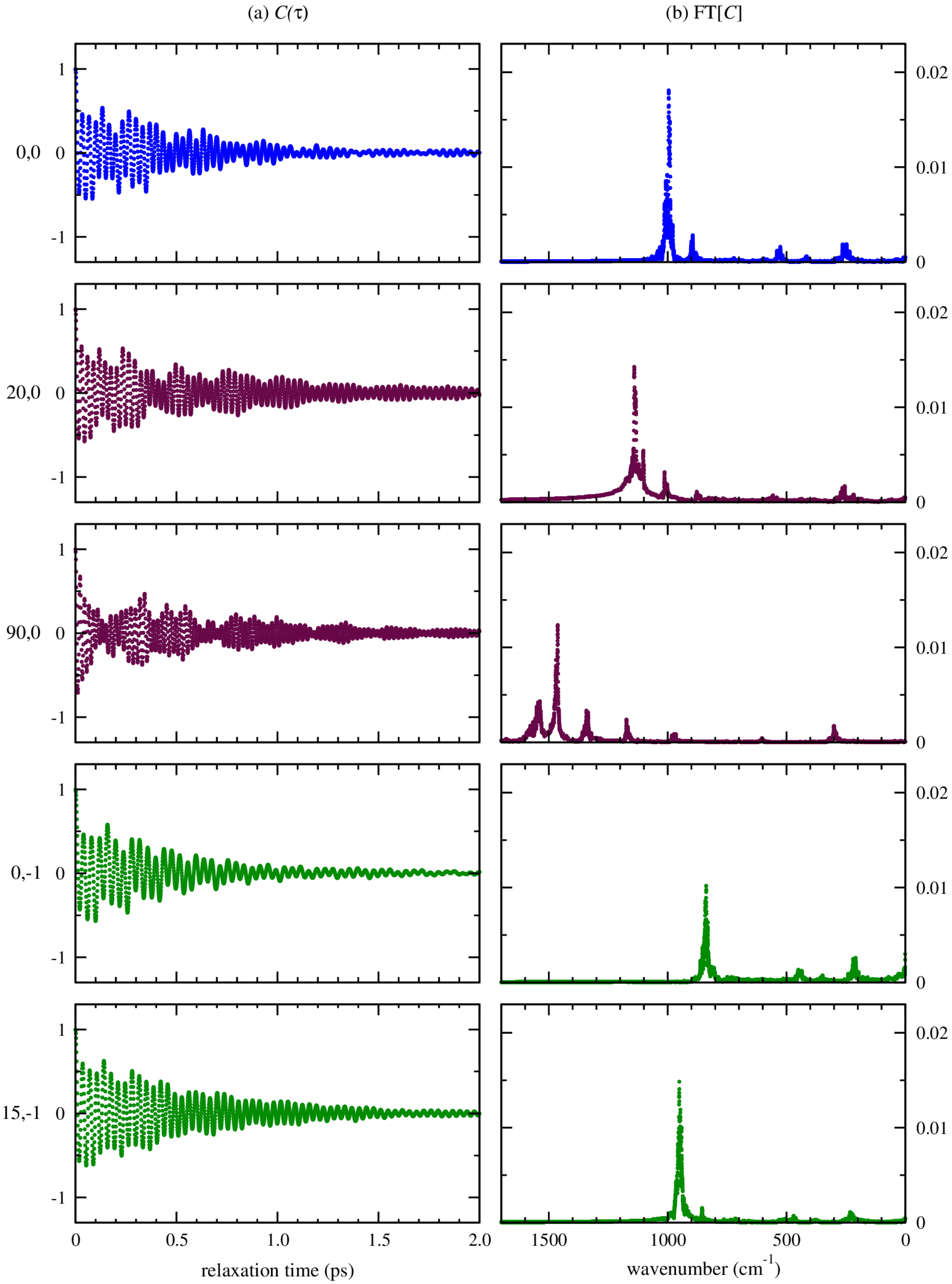


Figure S8: (a) The time autocorrelation function of $d_\perp$ and (b) its Fourier transform for atoms CE3, CD2, CZ3, and HE3 of Trp under some FF sets.

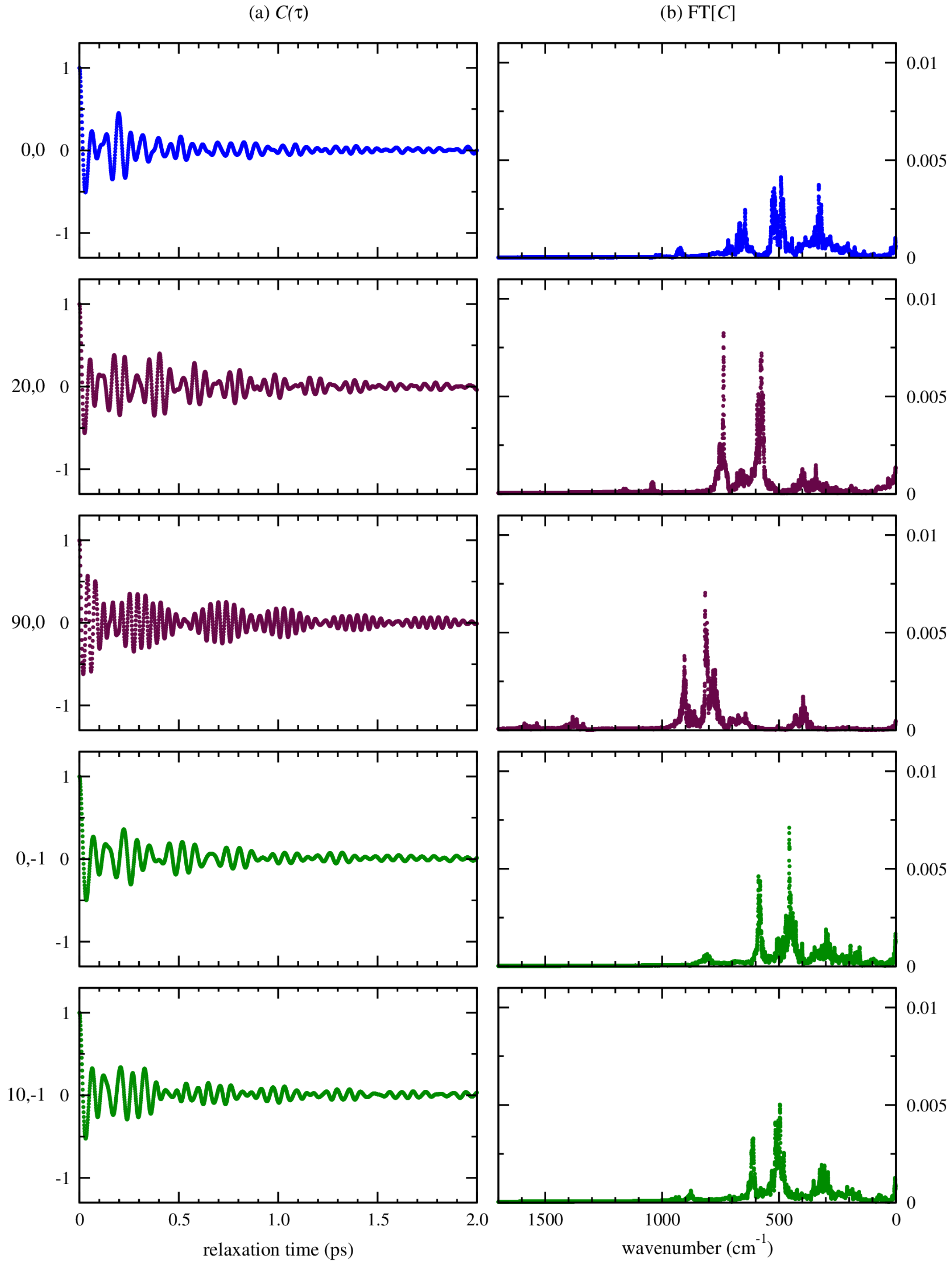


Figure S9: (a) The time autocorrelation function of $d_{\perp}$ and (b) its Fourier transform for atoms CZ, CE1, OH, and CE2 of Tyr under some FF sets.

Table S2: Calculated vibrational frequencies for minimized structures and averaged out-of-plane vibrational frequencies in aqueous solution for Trp as a zwitterion and with methylamide terminal groups on the basis of the C36 FF (set 0,0). Standard deviations are over the 2×10^5 steps of the MD trajectory. Mode names provide labels in Figure 15. Underlines indicate modes that combine out-of-plane and planar components.

Mode		Trp zwitterion Wavenumber (cm^{-1})			Trp with methylamide terminal groups Wavenumber (cm^{-1})		
No.	Name	Minimized	Avg.	Std.	Minimized	Avg.	Std.
1		0.00					
2		0.00					
3		0.00					
4		0.00					
5		0.00					
6		0.00					
7		46.12					
8		54.96					
9		70.74					
10		115.57					
11		166.91					
12		193.90					
13	A	197.48	183.0	22.3	209.53	183.0	37.8
14	B	235.83	219.3	40.2	199.82	215.3	40.7
					244.70	192.9	37.1
15		247.33					
16	C	318.52	322.4	73.3	296.52	308.0	42.9
					304.70	300.9	56.6
17	D	386.73	566.5	378.1	337.64	266.9	81.8
18	E	390.54	593.0	382.3	376.31	561.8	344.9
19	F	411.31	389.8	35.3	412.57	391.7	32.3
20	G	428.76	398.2	38.0	421.23	393.1	32.4
21	H	462.27	453.4	52.3	459.59	423.1	62.4
22		492.40					
23	I	529.94	514.9	18.7	527.76	515.5	21.4
24	$\underline{J}$	544.18	543.5	10.2	547.82	548.3	39.5
25		549.65					
26	K	596.39	585.9	79.1		568.2	69.3
						585.3	48.1
27		681.48					
28	L	686.29	678.5	56.7	664.03	677.1	32.9
					706.34	688.6	24.3
29	M	729.83	710.3	38.2		710.9	36.8
30	$\underline{N}$	743.49	726.3	147.1		720.6	147.8
31	O	748.42	756.3	15.6		754.9	22.3
32	P	766.23	727.6	90.2		753.5	35.4
33	$\underline{Q}$	772.75	757.1	54.1		753.8	59.4
34	R	782.89	773.3	23.3		790.4	221.5
35		820.74					
36		876.10					
37	S	894.85	884.8	70.9	896.1	882.3	58.7

Table S2 (continued)

		Trp zwitterion			Trp with methylamide terminal groups		
	Mode	Wavenumber (cm^{-1})			Wavenumber (cm^{-1})		
No.	Name	Minimized	Avg.	Std.	Minimized	Avg.	Std.
38	T	900.42	887.1	71.8	896.1	882.3	58.7
39		970.73					
40	U	972.93	984.2	81.5	969.91	977.0	72.0
41	V	997.93	1010.7	49.5	925.92	969.6	238.9
					969.91	996.1	46.3
					995.26	998.4	44.5
42	W	1009.25	1009.4	77.9	1017.56	1010.5	85.4
43	X	1020.12	1014.4	39.6	1017.56	1010.5	85.4
44	Y	1027.17	1022.6	19.9	1031.76	1030.0	17.5
45		1044.54					
46		1061.87					
47		1090.53					
48		1158.30					
49		1186.20					
50		1229.04					
51		1269.94					
52		1293.09					
53		1301.33					
54		1346.46					
55		1359.94					
56		1389.80					
57		1394.36					
58		1413.20					
59		1440.18					
60		1451.10					
61		1469.96					
62		1483.17					
63		1503.55					
64		1602.09					
65		1611.13					
66		1613.53					
67		1639.63					
68		1661.53					
69		1718.33					
70		2857.89					
71		2888.48					
72		2905.17					
73		3053.40					
74		3053.95					
75		3055.80					
76		3059.81					
77		3083.48					
78		3142.51					
79		3253.02					
80		3262.30					
81		3679.85					

Table S3: Calculated vibrational frequencies for minimized structures and averaged out-of-plane vibrational frequencies in aqueous solution for Tyr as a zwitterion and Tyr with methylamide terminal groups on the basis of the C36 FF (set 0,0). Standard deviations are over the 2×10^5 steps of the MD trajectory. Mode names provide labels in Figure 16. Underlines indicate modes that combine out-of-plane and planar components.

		Tyr zwitterion			Tyr with methylamide terminal groups		
Mode		Wavenumber (cm^{-1})			Wavenumber (cm^{-1})		
No.	Name	Minimized	Avg.	Std.	Minimized	Avg.	Std.
1		0.00					
2		0.00					
3		0.00					
4		0.00					
5		0.00					
6		0.00					
7		26.98					
8		52.78					
9	A	78.01	126.5	221.1	81.00	99.1	97.6
10		109.14					
11	B	178.76	122.8	54.9	180.28	177.6	32.6
12		199.03					
13		230.48					
14		272.44					
15		301.68					
16		316.39					
17	C	372.08	321.0	22.3	340.36	292.1	41.1
18	D	413.95	400.4	21.2	413.56	398.6	30.9
19	E	421.53	406.8	20.5	432.12	414.7	40.3
20		429.35					
21		447.55					
22	F	491.07	508.2	18.3	468.23	476.3	51.5
23	G	523.46	475.1	21.6	508.48	502.4	37.3
					583.55	549.3	46.2
24	H	643.43	664.7	28.7	628.00	623.8	29.0
					654.06	654.6	31.1
					673.00	675.8	25.4
25		666.93					
26		677.32					
27	I	697.17	650.0	32.7	686.40	686.8	28.7
28	J	770.36	724.8	56.5	800.00	750.2	81.8
29	K	824.10	768.3	110.3	800.00	750.2	81.8
30		844.04					
31	L	873.71	857.3	93.0	804.9	754.8	67.2
32	M	895.15	884.3	82.6	831.62	846.1	23.1
33	N	922.17	883.1	77.3	922.12	890.8	105.4
34	O	938.38	889.6	75.5	927.30	898.4	75.5

Table S3 (continued)

		Tyr zwitterion			Tyr with methylamide terminal groups		
	Mode	Wavenumber (cm^{-1})			Wavenumber (cm^{-1})		
No.	Name	Minimized	Avg.	Std.	Minimized	Avg.	Std.
35		953.49					
36		994.76					
37		1003.30					
38	P	1025.24	1038.4	68.2	1025.51	1006.1	61.2
39	R	1058.40	1052.1	60.5	1053.01	1067.6	59.5
40	Q	1070.42	1030.5	79.6	1073.93	1068.4	28.6
41		1087.97					
42		1127.83					
43		1174.46					
44		1206.30					
45		1219.00					
46		1281.50					
47		1307.19					
48		1338.84					
49		1370.13					
50		1374.02					
51		1406.68					
52		1424.63					
53		1434.64					
54		1450.45					
55		1462.67					
56		1492.96					
57		1511.30					
58		1545.30					
59		1607.53					
60		1640.16					
61		1666.59					
62		2854.48					
63		2890.98					
64		2904.99					
65		3053.53					
66		3054.83					
67		3057.29					
68		3059.85					
69		3143.81					
70		3250.37					
71		3264.52					
72		3682.69					

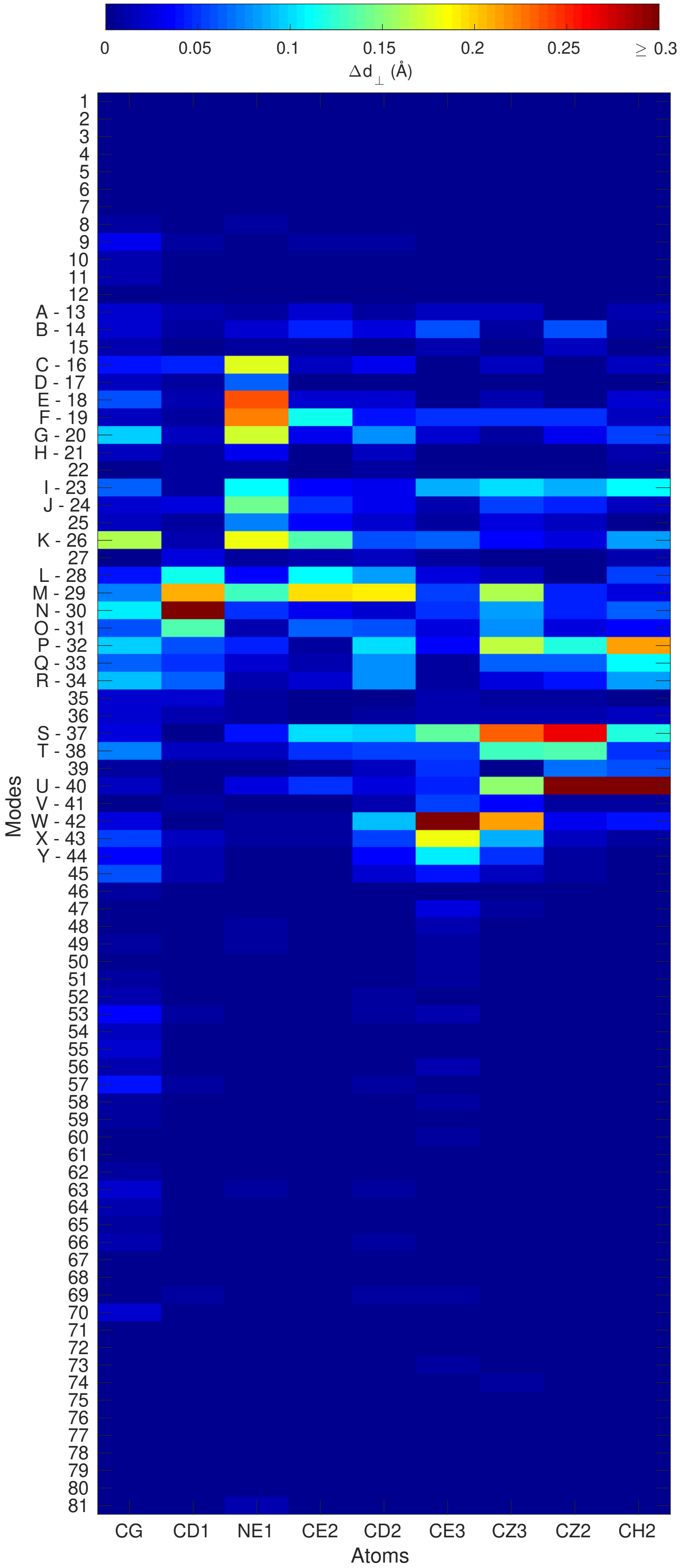


Figure S10: The maximum change in out-of-plane displacement ($\Delta d_{\perp}$) of atoms in the rings of minimized Trp in a quarter time period vibration of each mode.

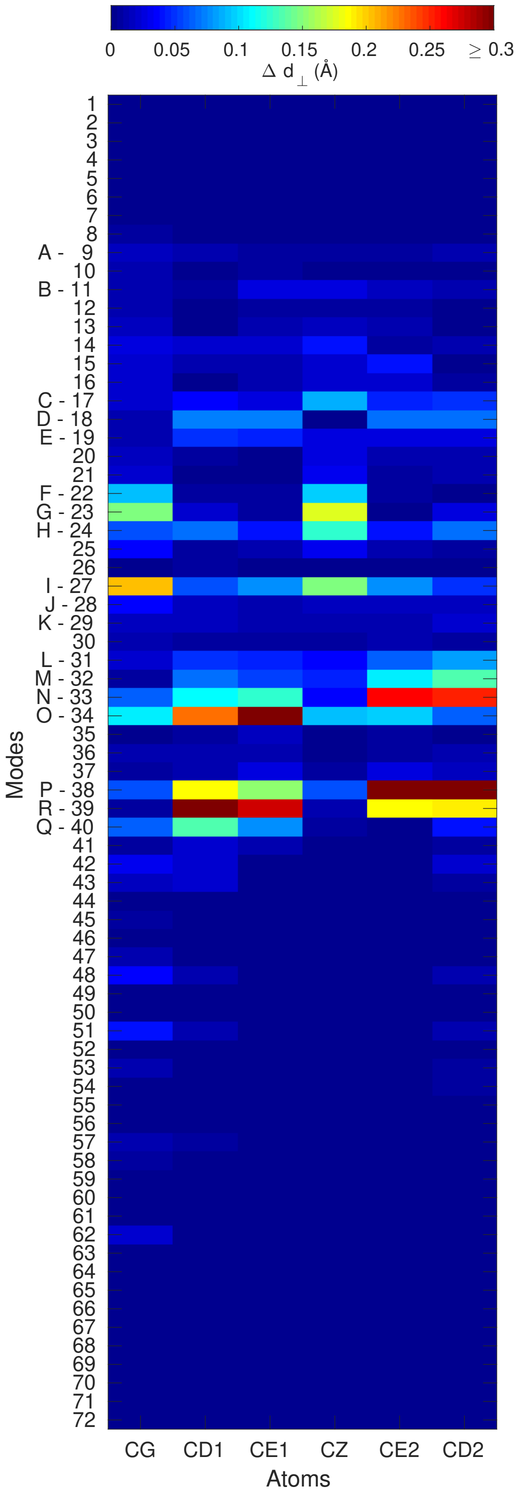


Figure S11: The maximum change in out-of-plane displacement ($\Delta d_{\perp}$) of atoms in the rings of minimized Tyr in a quarter time period vibration of each mode.

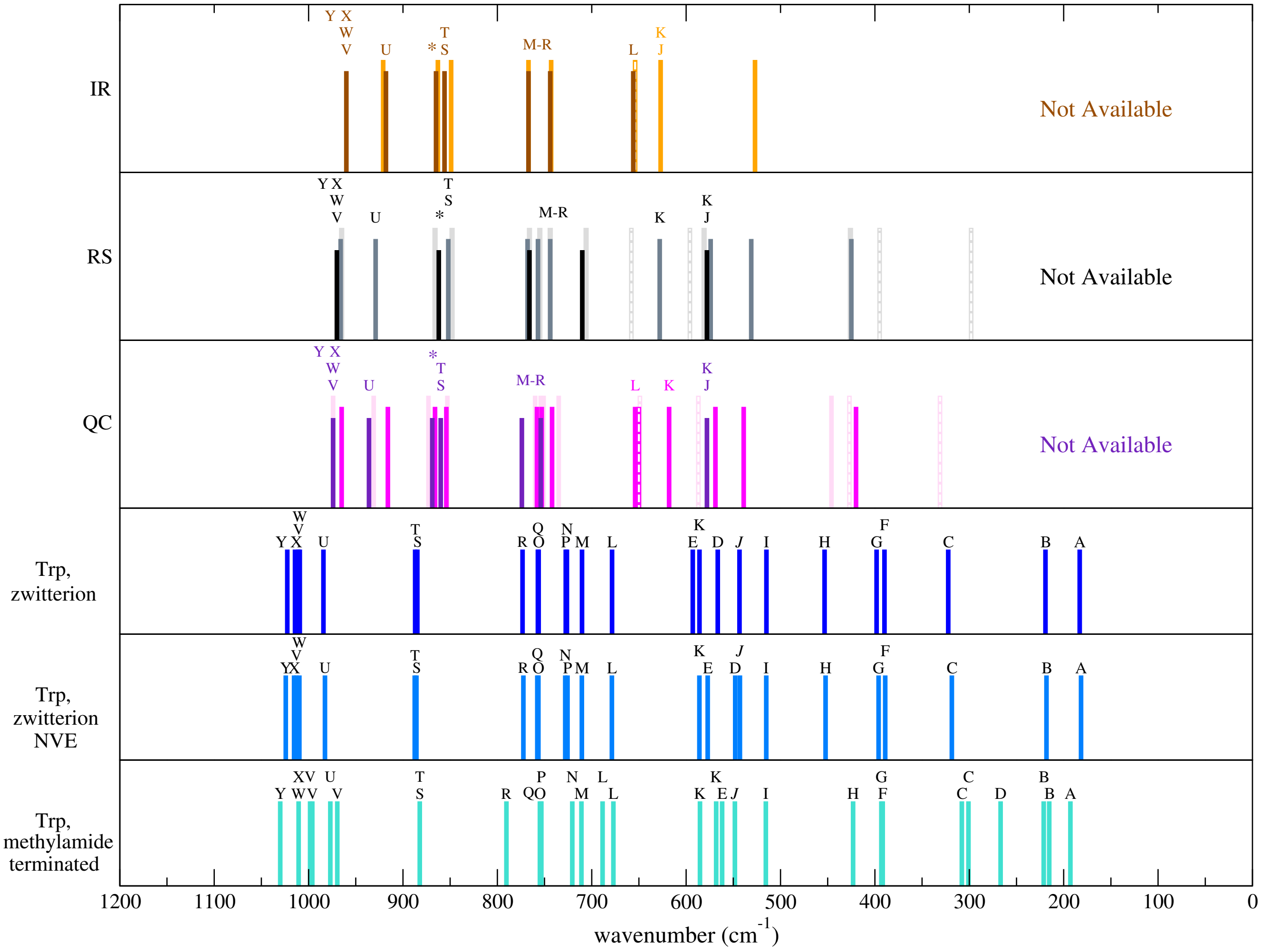


Figure S12: Normal mode frequencies for Trp. All peaks in all panels present frequencies of out-of-plane vibrations of Trp. The first panel presents prior infrared spectra (IR) in solution (brown) and solid phase (orange) of Trp.[4] The second panel presents prior Raman spectra (RS) of Trp in solution (black[5] and grey[4]) and solid phase (light grey).[6] The third panel presents prior quantum calculations (QC) of Trp + $7H_2O$ (indigo)[5], Trp in solution (magenta),[4] and solid phase (pink).[6] The fourth to sixth panels show out-of-plane vibrational modes for Trp as a zwitterion in NPT and NVE MD simulations and with methylamide terminal groups in NPT MD simulations, respectively. These modes were calculated by instantaneous NMA on the basis of C36, and averaged using MD of aqueous solution over 200 ps. Similar modes are shown with the same letter. Modes from IR, RS, and QC from different sources are shown with different heights; otherwise the y-axis has no units.

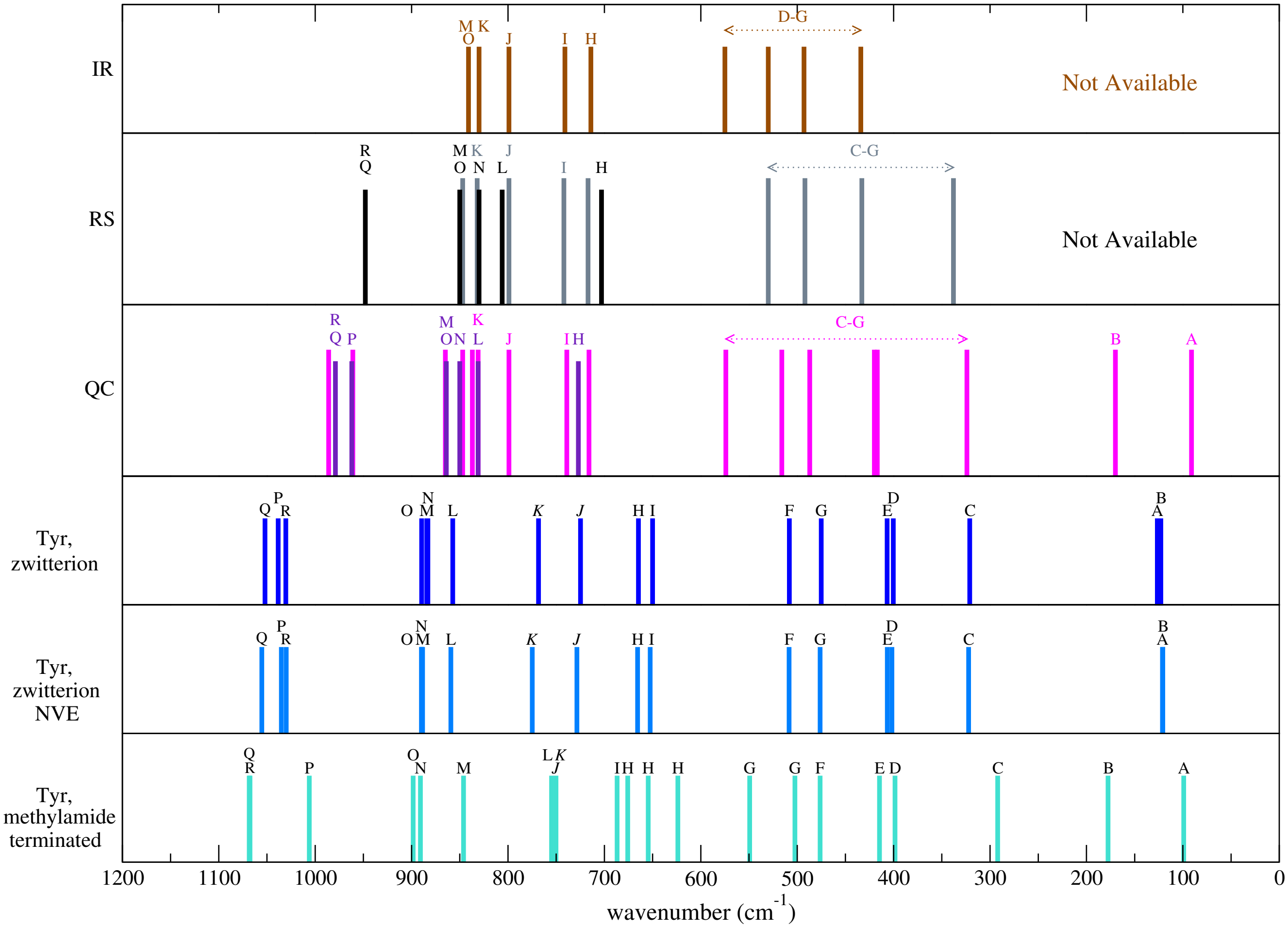


Figure S13: Normal mode frequencies for Tyr. All peaks in all panels present frequencies of out-of-plane vibrations of Tyr. The first panel presents prior solid phase infrared spectra (IR, brown) of Tyr.[7] The second panel presents prior Raman spectra (RS) of Tyr in solution (black[5] and grey[7]). The third panel presents prior quantum calculations (QC) of Tyr + $7H_2O$ (indigo)[5] and Tyr in solution (magenta).[7] The fourth to sixth panels show out-of-plane vibrational modes for Tyr as a zwitterion in NPT and NVE MD simulations and with methylamide terminal groups in NPT MD simulations, respectively. These modes were calculated by instantaneous NMA on the basis of C36, and averaged using MD of maqueous solution over 200 ps. Similar modes are shown with the same letter. Modes from RS and QC from different sources are shown with different heights; otherwise the y-axis has no units.

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