

Supporting Information for:

Mode Recognition in UV Resonance Raman Spectra of Imidazole: Histidine Monitoring in Proteins

Gurusamy Balakrishnan, Andrzej A. Jarzecki, Qiang Wu, Pawel M. Kozlowski, Daojing Wang, and Thomas G. Spiro*

¹Department of Chemistry, Princeton University, Princeton, Princeton, NJ 08544, USA.

Pre-resonant Raman Experiment at 488 nm:

Imidazole (ImH) and ¹⁵N labeled (at both nitrogens) ImH were obtained from Aldrich and used as received. ImH with deuterium labeled at C₂ was prepared¹ by refluxing ImH in D₂O for 12 hrs at 60 °C. Then purified by repeated re-crystallization. Raman spectrum did not show bands that corresponding to native ImH and thus confirms that isotope exchange was complete. ImH (0.5 M) in 3M aqueous HCl solution were excited with 488 nm (CW Ar⁺ ion laser, 40 mW) to obtain pre-resonance Raman spectra of Imidazolium cation (ImH²⁺). Similarly, 0.5 M solutions of ImH in pH 7.4 phosphate buffer for neutral and 3 M aqueous NaOH for imidazolate (Im⁻) anion were used. Appropriate solvent backgrounds were subtracted for all spectra. In 3M NaOH, some neutral ImH remains, and the relevant bands were subtracted out using the ImH spectrum. Sample solutions were spun in a NMR tube during the Raman measurements. Back scattering (135 °C) geometry was used to collect the Raman spectra and dispersed in a triplemate (SPEX 1877) equipped with 1200 grooves/mm grating at the spectrometer stage. Raman signals were collected using intensified photodiode array (IRY1024, PI) as the multichannel detector. Two spectral windows were spliced to get the full spectrum, using a band with strong intensity that appears in both windows to normalize the spectrum. Raman bands from cyclohexane were used as the standard for wavenumber calibration.

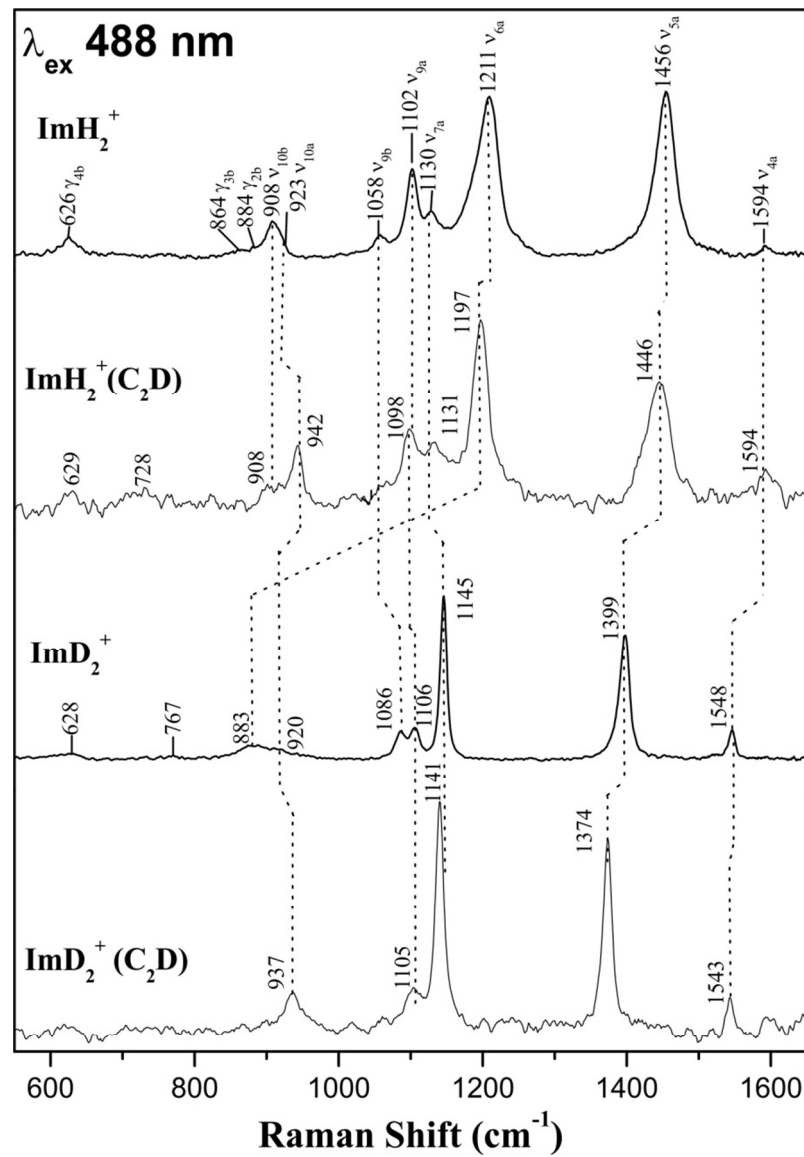


Figure S1. Pre-resonance Raman spectra excited at 488 nm for the indicated species in aqueous solution. Mode assignments and experimental frequencies are labeled.

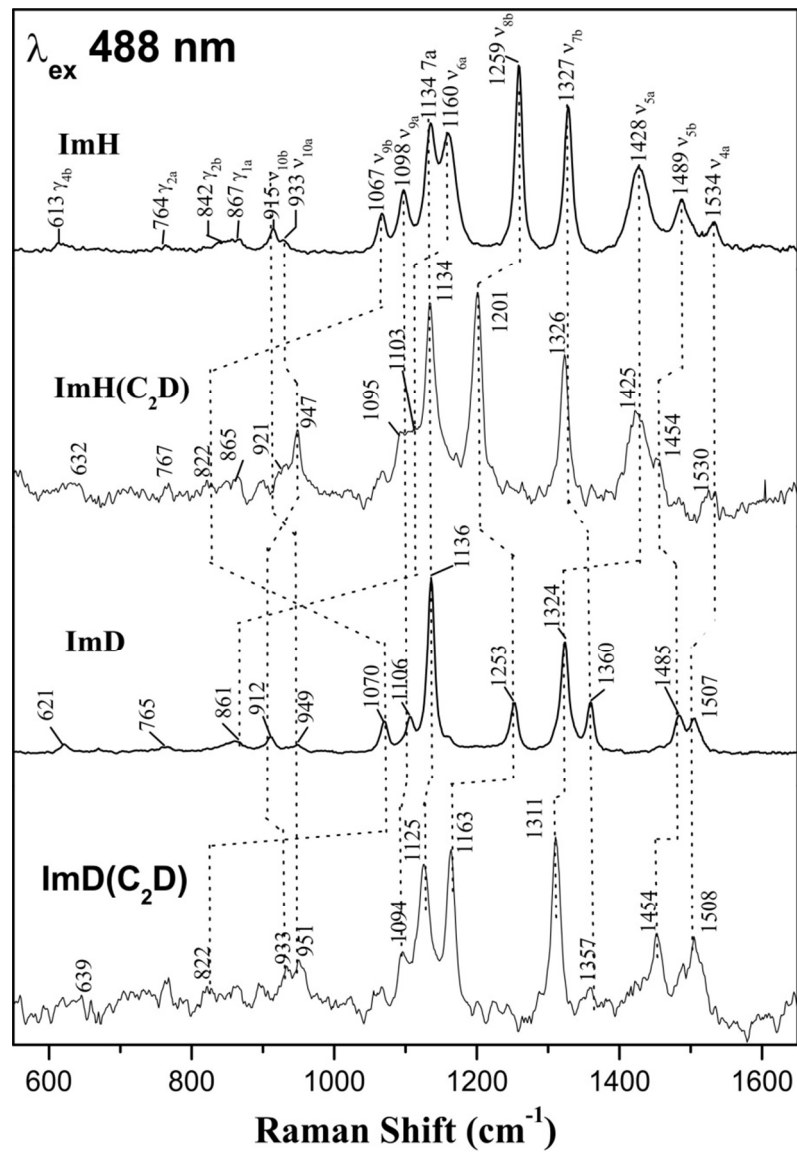


Figure S2. Pre-resonance Raman spectra excited at 488 nm for the indicated species in aqueous solution. Mode assignments and experimental frequencies are labeled.

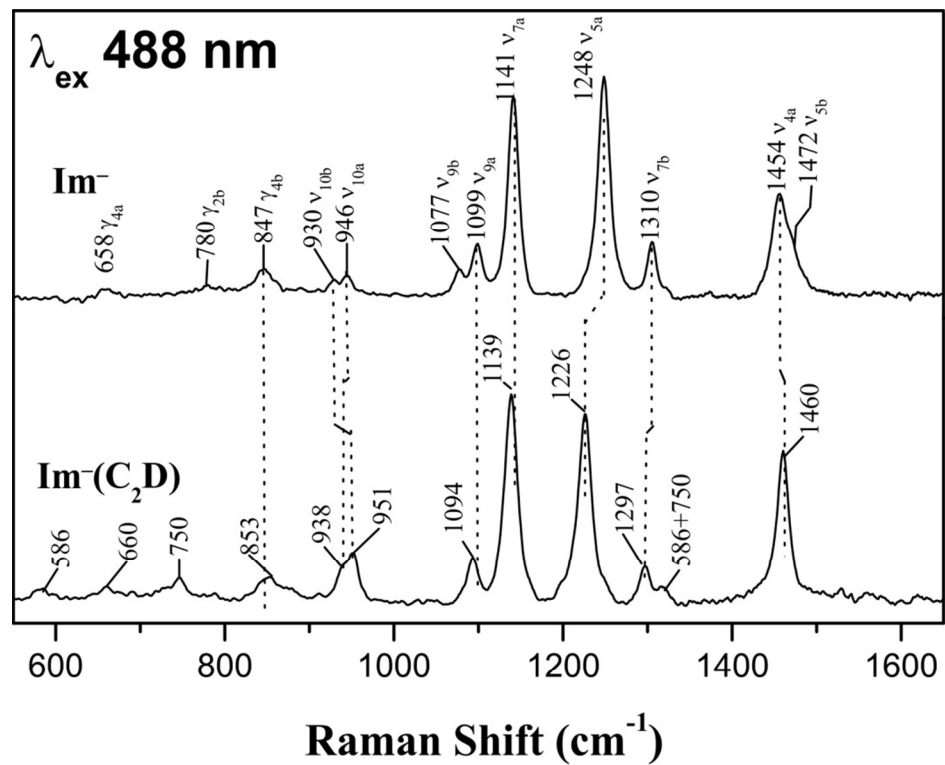


Figure S3. Pre-resonance Raman spectra excited at 488 nm for the indicated species in aqueous solution. Mode assignments and experimental frequencies are labeled.

Table S1. Optimized bond lengths (Å) of the indicated molecules using the B3LYP hybrid functional with a 6-31 G* basis set. Available experimental data are shown for comparison.

Parameter	Protonated				Neutral					Deprotonated		
	ImH ₂ ⁺	ImH ₂ ⁺ (H ₂ O) ₂	MeImH ₂ ⁺ (H ₂ O) ₂	Expl. ^a	ImH	ImH (H ₂ O) ₂	MeImH ^τ (H ₂ O) ₂	MeImH ^π (H ₂ O) ₂	Expl. ^b	Im ⁻	ImH ₂ ⁻ (H ₂ O) ₂	MeIm (H ₂ O) ₂ ⁻
r(N ₁ C ₂)	1.336	1.335	1.333	1.326	1.367	1.359	1.357	1.319	1.347	1.351	1.346	1.345
r(C ₂ N ₃)	1.336	1.335	1.336	1.328	1.315	1.321	1.319	1.361	1.323	1.351	1.346	1.346
r(N ₃ C ₄)	1.384	1.380	1.388	1.383	1.379	1.377	1.384	1.384	1.375	1.373	1.374	1.377
r(C ₅ N ₁)	1.384	1.380	1.382	1.376	1.381	1.379	1.380	1.378	1.369	1.373	1.374	1.375
r(C ₄ C ₅)	1.363	1.366	1.370	1.368	1.372	1.373	1.376	1.375	1.368	1.391	1.384	1.386
r(N ₁ H)	1.015	1.040	1.038	1.070	1.010	1.022	1.022	--	1.045			--
r(C ₂ H)	1.080	1.079	1.079	1.089	1.082	1.081	1.082	1.081	1.077	1.092	1.088	1.087
r(N ₃ H)	1.015	1.040	1.037	1.026			--	1.021				--
r(C ₄ H/C ₄ C ₆)	1.079	1.079	1.493	1.497	1.082	1.081	1.499	1.494	1.077	1.090	1.086	1.503
r(C ₅ H)	1.079	1.079	1.082	1.076	1.080	1.080	1.080	1.082	1.077	1.090	1.086	1.086
r(CH), Me			1.092				1.095	1.094				1.026
N ₁ (H)...H(O)H		1.716	1.726			1.892	1.902	1.911			1.827	1.826
N ₃ (H)...H(O)H		1.716	1.736			1.950	1.934	1.938			1.827	1.811

^a Neutron diffraction on HisH₂⁺Cl⁻ Ref. [2]. ^b Neutron diffraction on ImH at -170 °C Ref. [3].

Table.S2 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and isotopic shifts³ (Δ , cm⁻¹) of aqueous ImH₂⁺ and normal mode assignments.

v _i	ImH ₂ (H ₂ O) ₂ ⁺								ImD ₂ (D ₂ O) ₂ ⁺							
	⁴ PED(%)	Frequencies		Δ C ₂ D		Δ (¹⁵ NH) ₂		⁴ PED(%)	Frequencies		Δ (ND) ₂ -C ₂ D		Δ (¹⁵ ND) ₂			
	In-plane modes	⁴ Exp.	Calc.	⁴ Exp.	Calc.	⁵ Exp.	Calc.		⁴ Exp.	Calc.	⁴ Exp.	Calc.	⁵ Exp.	Calc.		
4a	v _{C2N} 16, v _{C2C} 34, δ_{NH} 28	1594	1579	0	-8	-8	-10	v _{C2C} 59, δ_{CH} 22	1548	1531	-5	-3	-3	-5		
5a	v _{NC} 17, v _{C2C} 34, δ_{NH} 16	1456	1447	-10	-9	-12	-8	v _{C2N} 40, v _{NC} 21, v _{C2C} 12	1399	1379	-25	-24	-21	-21		
6a	v _{C2N} 65, δ_{NH} 15	1211	1205	-14	-16	-7	-13	v _{NC} 42, v _{C2H} 14, δ_{CH} 23	883	880	--	-10	--	-7		
7a	v _{NC} 84	1130	1121	+1	-1	-12	-13	v _{NC} 61, v _{C2N} 14	1145	1135	-4	-6	-10	-11		
9a	v _{C2C} 23, δ_{CH} 64	1102	1093	-4	-9	0	0	v _{C2C} 20, δ_{CH} 64	1106	1095	-1	-7	0	-1		
10a	$\delta_{ring\ def.}$ 77, δ_{CH} 13	923	929	+19	+16	--	-16	$\delta_{rd.}$ 41, v _{C2N} 16, δ_{ND} 17	920	927	+17	+18	-8	-10		
5b	v _{C2N} 59, δ_{C2H} 26	1538	1525	--	-31	-10	-11	v _{C2N} 59, δ_{C2H} 25	1527	1509	--	-32	-8	-9		
6b	v _{C2N} 12, v _{NC} 23, δ_{NH} 48	--	1452	--	-7	--	-15	v _{NC} 31, δ_{ND} 45	924	941	--	-9	--	-4		
7b	v _{NC} 21, δ_{CH} 51	--	1315	--	-4	--	-1	v _{NC} 44, v _{C2N} 19, δ_{CH} 20	1379	1356	--	-4	--	-15		
8b	v _{C2N} 18, δ_{CH} 11, δ_{C2H} 50	--	1178	--	-358	--	-7	δ_{CH} 50, δ_{C2H} 37	1253	1252	--	-448	--	-8		
9b	v _{NC} 42, v _{C2H} 14, δ_{CH} 23	1058	1045	--	+52	-3	-3	v _{C2N} 23, δ_{C2H} 31	1086	1072	--	+104	-1	-1		
10b	$\delta_{ring\ def.}$ 85	908	918	0	0	--	-12	$\delta_{ring\ def.}$ 77, δ_{ND} 12	--	896	--	+39	--	-9		
	Out-of-plane modes															
1a	γ_{NH} 46	--	1049	--	-4	--	-1	γ_{ND} 33, γ_{CH} 30	--	734	--	+44	--	-1		
2a	γ_{CH} 98	--	747	--	+19	--	0	γ_{CH} 83	767	754	--	-1	--	-1		
4a	$\gamma_{ring\ def.}$ 83	--	625	--	-91	--	-10	$\gamma_{ring\ def.}$ 86	--	622	--	-88	--	-9		
1b	γ_{NH} 47	--	1005	--	0	--	-2	γ_{ND} 40	--	728	--	+2	--	-2		
2b	γ_{CH} 84	884	857	--	0	--	0	γ_{CH} 81	--	864	--	0	--	0		
3b	γ_{CH} 69	864	823	-136	-123	--	-1	γ_{CH} 60	--	842	--	-160	--	-2		
4b	$\gamma_{ring\ def.}$ 86	626	615	+3	0	--	-6	$\gamma_{ring\ def.}$ 88	628	611	--	0	--	-5		

¹Values given in bold are taken from resonance Raman spectra excited at 229 nm and others are from non-resonant Raman spectra excited at 488 nm.; ²Using B3LYP hybrid functional with 6-31 G* basis set followed by the SQM procedure[4-8].; ³Isotopic shifts (Δ ¹⁵N(data not shown) and Δ C₂D) are obtained from Raman spectra excited at 229 nm or 488 nm.; ⁴% distribution from major internal coordinate contributions are given.

Table.S3 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and isotopic shifts³ (Δ , cm⁻¹) of aqueous ImH and the normal mode assignments.

v _i	ImH(H ₂ O) ₂								ImD(D ₂ O) ₂							
	4,5PED(%)		Frequencies		Δ C ₂ D		Δ ¹⁵ N- ¹⁵ NH		4PED(%)		Frequencies		Δ ND-C ₂ D		Δ ¹⁵ N- ¹⁵ ND	
			Exp.	Calc.	Exp.	Calc.	Exp.	Calc.			Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
	In-plane modes								In-plane modes							
4a	v _{CC} 34, v _{C2N} 17, δ_{NH} 19, δ_{CH} 13		1534	1526	-4	-8	-6	-8	v _{CC} 38, v _{C2N} 24, δ_{CH} 17		1507	1504	+1	-7	+1	-7
5a	v _{CC} 18, v _{C2N} 15, v _{NC} 18, δ_{NH} 25		1428	1429	-3	-5	-13	-13	v _{C2N} 40, v _{NC} 20, δ_{CH} 12		1324	1328	-13	-20	-10	-17
6a	v _{C2N} 41, δ_{CH} 12, δ_{NH} 12, δ_{C2H} 15		1160	1149	-57	-47	-5	-10	$\delta_{ring\ def.}$ 32, δ_{ND} 43		861	871	--	-59	--	-4
7a	v _{NC} 86		1134	1138	0	-2	-14	-13	v _{NC} 80		1136	1134	-11	-6	-13	-13
9a	v _{CC} 23, δ_{CH} 43		1098	1091	-3	-11	0	-2	v _{CC} 24, δ_{CH} 53		1106	1104	-12	-21	-1	-2
10a	$\delta_{ring\ def.}$ 79		933	936	+14	+16	-13	-16	$\delta_{ring\ def.}$ 76		912	918	+21	+12	-14	-16
5b	v _{CC} 10, v _{C2N} 24, v _{NC} 19, δ_{C2H} 22,		1489	1479	-35	-28	-5	-8	v _{CC} 21, v _{C2N} 33, δ_{C2H} 18, δ_{C2H} 18		1485	1471	-31	-26	-4	-6
7b	v _{C2N} 28, v _{NC} 29, δ_{CH} 30		1329	1338	-3	-7	-4	-10	v _{C2N} 32, v _{NC} 26		1360	1351	-3	-2	-18	-16
8b	v _{C2N} 24, δ_{CH} 18, δ_{C2H} 40		1259	1253	-58	-61	-4	-3	δ_{CH} 30, δ_{C2H} 50		1253	1241	-90	-87	-10	-9
9b	v _{NC} 27, v _{C2N} 10, δ_{CH} 27, δ_{C2H} 12		1067	1056	-245	-445	-10	-10	v _{NC} 18, v _{C2N} 19, δ_{CH} 27, δ_{C2H} 13		1070	1057	-248	-247	-4	-2
10b	$\delta_{ring\ def.}$ 82		915	920	+6	+2	--	-15	$\delta_{ring\ def.}$ 51, v _{C2N} 10, δ_{ND} 11		949	941	+2	+9	-15	-13
	Out-of-plane modes								Out-of-plane modes							
1a	γ_{CH} 31, γ_{NH} 31 (891)		867	869	-2	-2	--	0	γ_{CH} 31, γ_{NH} 31		621	599	--	+17	--	-7
2a	γ_{CH} 82 (736)		764	722	+3	+12	--	0	γ_{CH} 82		765	722	--	+13	--	0
4a	$\gamma_{ring\ def.}$ 89 (658)		663	657	-107	-106	--	-9	$\gamma_{ring\ def.}$ 89		-	660	--	-110	--	-9
2b	γ_{CH} 74 (839)		842	829	--	-1	--	0	γ_{CH} 74		--	842	--	-2	--	0
3b	γ_{CH} 85 (827)		--	820	--	-125	--	0	γ_{CH} 85		--	820	--	-123	--	0
4b	$\gamma_{ring\ def.}$ 82 (618)		613	606	+19	+18	0	-7	$\gamma_{ring\ def.}$ 82		--	622	--	+4	+3	-1

¹⁻⁴ as in Table S2.

⁵Frequencies given in parenthesis are taken from reported IR spectra [9].

Table.S4 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and isotopic shifts (Δ , cm⁻¹)³ of aqueous Im⁻

ν_I	⁴ PED(%)	Frequencies		Δ_{C_2D}		$\Delta(^{15}N)_2$	
		Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
	In-plane modes						
4a	ν_{CC} 55, δ_{CH} 30	1454	1454	+3	0	-2	-3
5a	ν_{NC} 18, ν_{C_2N} 63	1248	1251	-22	-22	-17	-21
7a	ν_{NC} 88	1141	1157	-2	-7	-16	-15
9a	ν_{CC} 37, δ_{CH} 54	1099	1085	-5	-7	-2	-3
10a	$\delta_{ring\ def.}$ 70, δ_{CH} 10	946	950	-8	-7	-21	-19
5b	ν_{NC} 10, ν_{C_2N} 40, δ_{CH} 16, δ_{C_2H} 31	1472	1470	--	-38	-3	-9
7b	ν_{NC} 26, ν_{C_2N} 25, δ_{CH} 32	1310	1310	-13	-8	--	-6
8b	ν_{NC} 11, ν_{C_2N} 32, δ_{CH} 13, δ_{C_2H} 40	--	1220	--	-358	-4	-5
9b	ν_{NC} 42, ν_{C_2N} 11, δ_{CH} 26, δ_{C_2H} 12	1077	1080	--	+52	--	-14
10b	$\delta_{ring\ def.}$ 88	930	938	+21	+21	--	-13
	Out-of-plane modes						
2a	γ_{CH} 90	--	676	--	+3	--	0
4a	$\gamma_{ring\ def.}$ 85	658	643	+2	0	--	-7
2b	γ_{CH} 90	780	767	--	0	--	0
3b	γ_{C_2H} 80	--	738	--	-233	--	0
4b	$\gamma_{ring\ def.}$ 92	847	798	--	+33	--	-11

¹⁻⁴ as in Table S2.

Table.S5 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and their normal mode assignments of the indicated molecules. Experimental frequencies of HisH₂⁺ and HisD₂⁺ are shown for comparison. Calculated ¹⁵N isotopic shifts(cm⁻¹)³ also listed.

v _i	4-MeImH ₂ (H ₂ O) ₂ ⁺			Δ ¹⁵ N	HisH ₂ ⁺	4-MeImD ₂ (D ₂ O) ₂ ⁺			Δ ¹⁵ N	HisD ₂ ⁺
	⁴ PED(%)	Frequencies			Freq.	⁴ PED(%)	Frequencies			Freq.
		Exp.	Calc.		Calc.		Exp.	Exp.		Calc.
4a	v _{C4C5} (40), v _{C4-C6} (10), δ _{N3,1-H} (10,4)	1634	1614	-5	1634	v _{C4C5} (40), v _{C4-C6} (10)	1610	1589	-3	1610
5a	v _{N1-C2,5} (15,12), v _{C4C5} (8), δ _{N1-H} (34)	1490	1484	-8	1493	v _{N1-C2,5} (14,16), δ _{CH3} (44)	1412	1386	-17	1412
6a	v _{N1-C2,5} (39,10), δ _{N1,C2-H} (12,18)	1205	1186	-8	1200	v _{N3-C2} (10), δ _{ring} (24), δ _{C2H} (11), δ _{N3-D} (18)	985	983	-12	992
7a	v _{N3-C2,4} (25,5), δ _{N3,C2-H} (12,33), δ _{C5-H} (16)	1184	1166	-5	1186	v _{N1-C5} (23), v _{C4-C6} (16), δ _{C5-H} (40)	1260	1245	-4	1260
9a	v _{N1-C2,5} (5,50), δ _{C2,5-H} (8,18)	1089	1082	-5	1097	v _{N1-C2,5} (22,30), δ _{C2-H} (18)	1110	1097	-4	1114
10a	δ _{ring def.} (64), v _{N3C4} (16)	929	929	-15	928	δ _{ring def.} (36), v _{N3C4} (26)	918	910	-11	921
5b	v _{C2-N1,3} (33,29), δ _{C2H} (25)	1535	1526	-10	1535	v _{C2-N1,3} (33,28), δ _{C2H} (25)	--	1512	-10	1523
6b	v _{C4C5} (14), δ _{N3-H} (24), δ _{CH3} (24)	1441	1425	-7	--	δ _{ring} (37), δ _{N3,1-D} (14,23)	--	879	-5	--
7b	v _{N1,3-C5,4} (22,12) v _{N3-C2} 15 δ _{C5,4-H,C6} (18,20)	1270	1256	-9	--	v _{C4,C5} (12), v _{N3-C2,C4} (17,33), δ _{ring} (12)	1372	1350	-15	--
8b	v _{N1,3-C5,4} (5,23), δ _{CCH} (18), δ _{C5H} (19)	1296	1287	-1	1270	δ _{C5H} (16), δ _{C2H} (35)	--	1226	-7	--
9b	v _{C4C5} (18), δ _{N1-C2-N3} (22), δ _{CH3} (36)	1008	1003	-2	998	v _{C4C5} (16), δ _{C5H} (12), δ _{CCH} (44)	1023	1012	-1	1007
10b	v _{N3-C4} (16), δ _{ring} (38), δ _{CH3} (30)	978	973	-15	--	δ _{N1,3-D} (23,14), δ _{ring} (24)	--	942	-6	--
Methyl/Histidyl group modes										
	v _{N3-C2,4} (9,7), δ _{N3H} (12), δ _{CH3} (52)	--	1464	-6	1467	δ _{CH3} (76)	--	1454	-2	--
	δ _{CH3} (91)	--	1445	0	1443	δ _{CH3} (91)	--	1445	0	--
	δ _{CH3} (89)	1398	1391	0	--	δ _{CH3} (44), v _{N1-C2} (14), v _{C4-C6} (10)	1395	1396	-3	1396
	δ _{CCH} (72)	--	1056	0	--	δ _{CCH} (76)	--	1055	-1	--

¹Values are taken from resonance Raman spectra excited at 229 nm .; ²⁻⁴ as in Table S2.

Table.S6 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and normal mode assignments for the τ (N₁H) tautomer of 4-MeImH. Experimental frequencies of HisH are included for comparison. Calculated ¹⁵N isotopic shifts(cm⁻¹)³ are also listed.

v _i	τ -MeImH(H ₂ O) ₂ [N ₁ H]				HisH	τ -MeImD(D ₂ O) ₂ [N ₁ D]				HisD
	⁴ PED(%)	Frequencies		$\Delta^{15}\text{N}$	Freq.	³ PED(%)	Frequencies		$\Delta^{15}\text{N}$	Freq.
		⁵ Exp.	Calc.	Calc.	Exp.		⁵ Exp.	Calc.	Calc.	Exp.
4a	ν_{C4C5} (52), $\nu_{\text{C4-C6}}$ (13), $\delta_{\text{C5-H}}$ (10)	1577	1559	-3	1575	ν_{C4C5} (54), $\nu_{\text{C4-C6}}$ (15), δ_{ring} (10)	1572	1553	-3	1561
5a	$\nu_{\text{N1-C5,2}}$ (5,11), $\delta_{\text{N1-H}}$ (16), δ_{CH3} (51)	1454	1453	-14	1453	$\nu_{\text{N3-C4,2}}$ (25,29), δ_{ring} (10)	1308	1311	-21	1322
6a	$\nu_{\text{N1-C2,5}}$ (47,9), $\delta_{\text{N1-H}}$ (19), δ_{C2H} (11)	1158	1149	-9	1164	$\delta_{\text{N1-D}}$ (49), δ_{ring} (16)	--	876	-3	--
7a	$\nu_{\text{N1,3-C5,4}}$ (12,28), $\nu_{\text{C4-C6}}$ (16)	1260	1258	-7	1286	$\delta_{\text{C5,2-H}}$ (39,35)	1227	1216	-4	1234
9a	$\nu_{\text{N1-C5,2}}$ (43,11), $\delta_{\text{C5-H}}$ (21)	1087	1075	-5	1090	δ_{CCH} (25), ν_{C4C5} (22), δ_{ring} (18), δ_{ring} (10)	1019	1014	-5	1023
10a	$\delta_{\text{ring def.}}$ (66), ν_{N3C4} (14)	936	940	-15	940	$\delta_{\text{ring def.}}$ (61), ν_{N3C4} (14)	926	928	-14	926
5b	$\nu_{\text{C2-N3,N1}}$ (33,6), δ_{C2H} (24)	1494	1497	-7	1498	$\nu_{\text{C2-N3,N1}}$ (12,34), δ_{C2H} (24)	1489	1490	-7	1486
7b	$\nu_{\text{N3-C4,2}}$ (25,28), $\nu_{\text{C5-N1,C4}}$ (7,10)	1305	1314	-12	1324	$\nu_{\text{N1-C5,2}}$ (26,37), δ_{ring} (13), $\delta_{\text{N1-D}}$ (14)	1375	1357	-14	1376
8b	$\nu_{\text{N1,3-C5,2}}$ (12,6), $\delta_{\text{C5,2-H}}$ (32,28)	1231	1222	-5	1239	$\nu_{\text{N1,3-C5,4}}$ (20,25), $\nu_{\text{C4-C6}}$ (16)	1260	1250	-8	1274
9b	δ_{CCH} (48), ν_{C4C5} (20), δ_{ring} (18)	997	999	0	993	$\nu_{\text{N1-C5,2}}$ (27,29), $\delta_{\text{C5-H}}$ (12)	1100	1080	0	1099
10b	$\delta_{\text{ring def.}}$ (38), ν_{N3C4} (22)	977	975	-20	--	$\delta_{\text{ring def.}}$ (15), ν_{N3C4} (22), δ_{CCH} (39)	--	980	-16	991
	Methyl/Histidyl group modes					Methyl/Histidyl group modes				
	$\nu_{\text{C2-N1,3}}$ (19,8), $\delta_{\text{N1-H}}$ (12), δ_{CH3} (31)	--	1455	-1	1453	δ_{CH3} (75)	--	1467	-1	--
	δ_{CH3} (78)	--	1470	-2	1463	δ_{CH3} (82)	1456	1454	-3	--
	δ_{CH3} (88)	1390	1388	0	--	δ_{CH3} (87)	1390	1389	-1	1389
	δ_{CCH} (77)	--	1056	0	--	δ_{CCH} (78)	--	1057	0	--

¹⁻⁴ as in Table S5.

⁵ Values given in bold numbers are from overlapping bands assigned to both τ -MeIm and π -MeIm.

Table.S7 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and normal mode assignments for the π (N₃H) tautomer of 4-MeImH. Experimental frequencies of HisH are included for comparison. Calculated ¹⁵N isotopic shifts(cm⁻¹)³ are also listed.

v _i	π -MeImH(H ₂ O) ₂ [N ₃ H]			HisH	π -MeImD(D ₂ O) ₂ [N ₃ D]			HisD		
	⁴ PED(%)	Frequencies		$\Delta^{15}\text{N}$	Freq.	⁴ PED(%)	Frequencies		$\Delta^{15}\text{N}$	Freq.
		⁵ Exp.	Calc.	Calc.			Exp.	⁵ Exp.	Calc.	
4a	v _{C4C5} (42), v _{C4-C6} (14), $\delta_{\text{N3-H}}$ (13)	1596	1582	-4	1593	v _{C4C5} (50), v _{C4-C6} (16), δ_{C5H} (12)	1579	1563	-3	1570
5a	v _{C4C5} (18), v _{N3-C2,4} (11,8), $\delta_{\text{N3-H}}$ (28)	1426	1417	-9	1435	v _{N3-C4,2} (22,27), $\delta_{\text{N1-C5}}$ (14)	1326	1322	-20	1336
6a	v _{N3-C2} (43), $\delta_{\text{N3-H}}$ (16), δ_{C2H} (17)	1158	1128	-7	1164	$\delta_{\text{N1-D}}$ (54)	--	898	-1	--
7a	v _{N3-C2} (12), $\delta_{\text{C2,C5-H}}$ (19,48)	1260	1251	-3	1269	v _{N3-C2} (13), v _{N1-C5} (16), $\delta_{\text{C5-H}}$ (35)	--	1210	-10	1197
9a	v _{N1-C5} (53), $\delta_{\text{C5-H}}$ (17)	1103	1115	-7	--	δ_{CCH} (37), v _{C4C5} (20), δ_{ring} (15)	1019	1002	-3	1007
10a	$\delta_{\text{ring def.}}$ (52), v _{N3C4} (10)	--	936	-17	--	$\delta_{\text{ring def.}}$ (51), v _{N3C4} (14)	945	936	-18	947
4b	v _{C2-N1,3} (26,9), δ_{C2H} (18), δ_{CH3} (24)	1494	1487	-6	1498	v _{C2-N3,N1} (9,28), δ_{C2H} (17), δ_{CH3} (26)	1489	1483	-5	1486
7b	v _{N1,3-C5,4} (12,22), v _{C2-N1} (23)	1344	1346	-11	1360	v _{N3-C4} (40), δ_{ring} (13)	1366	1354	-8	1359
8b	v _{N1-C5,2} (26,11), v _{C4-N3,C6} (13,12), δ_{C2H} (16)	1231	1231	-11	1239	v _{N1-C5} (13), δ_{CH3} (13), δ_{C5H} (51)	--	1247	-3	--
9b	δ_{CCH} (14), v _{C4C5} (19), δ_{ring} (35)	1015	1010	-8	1011	v _{N1-C5} (54), $\delta_{\text{C5-H}}$ (15)	1100	1115	0	1100
10b	$\delta_{\text{ring def.}}$ (38), v _{N3C4} (12)	977	974	-12	969	$\delta_{\text{ring def.}}$ (29), v _{N3C4,C2} (11,12), δ_{CH3} (23)	982	969	-15	991
	Methyl/Histidyl group modes					Methyl/Histidyl group modes				
	v _{C2-N1,3} (14,17), δ_{CH3} (41)	--	1457	-6	1458	δ_{CH3} (42), v _{N1-C2} (18)	1456	1453	-4	--
	δ_{CH3} (89)	--	1448	0	--	δ_{CH3} (82)	--	1448	-1	--
	δ_{CH3} (80)	1390	1388	-1	--	δ_{CH3} (89)	1390	1389	0	--
	δ_{CCH} (79)	--	1052	0	--	δ_{CCH} (79)	--	1052	0	--

¹⁻⁵ as in Table S6.

Table.S8 Experimental¹ and calculated² vibrational frequencies (cm⁻¹) and normal mode assignments for 4-MeIm(H₂O)₂⁻. Experimental frequencies of His⁻ are included for comparison. Calculated ¹⁵N isotopic shifts(cm⁻¹)³ are also listed.

v _i	4-MeIm(H ₂ O) ₂ ⁻			His ⁻	
	⁴ PED(%)	Frequencies		Δ ¹⁵ N	Freq.
		⁵ Exp.	Calc.	Calc.	Exp.
4a	v _{C4C5} (46), v _{C4-C6} (14), δ _{C5-H} (15)	1536	1514	-1	1531
5a	v _{C2-N1,3} (25,42), δ _{C5-H} (12)	1260	1256	-12	1259
7a	v _{N1-C2} (13), v _{N1,3-C5,4} (39,13), δ _{C5-H} (15)	1233	1242	-17	1235
9a	δ _{ring def.} (50), v _{C4C5} (18)	1010	1008	-11	1009
10a	δ _{ring def.} (60), v _{N3-C4} (16)	951	958	-16	953
5b	v _{C2-N1,3} (15,18), δ _{C2-H} (24), δ _{CH3} (23)	1441	1444	-6	1438
7b	v _{C2-N1,3} (12,6), v _{N1,3-C5,4} (3,35)	1297	1312	-8	1315
8b	v _{C2-N1,3} (7,23), δ _{C5-H} (12), δ _{C2-H} (46)	--	1210	-10	--
9b	v _{N1-C2,5} (11,42), δ _{C2-H} (24)	1100	1110	-4	1104
10b	δ _{CCH} (52), δ _{ring} (16)	--	983	-10	--
Methyl/Histidyl group modes					
	v _{C2-H} (10), δ _{CH3} (57)	--	1481	-2	--
	δ _{CH3} (88)	--	1456	0	--
	δ _{CH3} (87)	--	1376	0	--
	δ _{CCH} (82)	--	1044	0	--

¹⁻⁴ as in Table S5.

Table S9. Internal coordinate coefficients^a in the computed eigenvectors and vibrational frequencies $\text{Im}(\text{H}_2\text{O})_2^-$.

v_i	Mode	$\text{Im}(\text{H}_2\text{O})_2^-$			
		$v(\text{C}_4\text{-C}_5)$	$v^+(\text{N-C}_2)$	$v^+(\text{N-C}_{4,5})$	Freq.
4a	$v(\text{C}_4\text{-C}_5)$	-0.344	0.068	0.120	1454
5a	$v^+(\text{N-C}_2)$	-0.077	-0.308	0.175	1251
7a	$v^+(\text{N-C}_{4,5})$	-0.051	-0.091	-0.327	1157
9a	$\delta^+(\text{C}_{4,5}\text{-H})$	0.179	-0.021	-0.025	1085
10a	δ^+ ring	0.013	-0.076	0.009	950
			$v^-(\text{N-C}_2)$	$v^-(\text{N-C}_{4,5})$	
5b	$v^-(\text{N-C}_2)$		-0.295	-0.160	1470
7b	$v^-(\text{N-C}_{4,5})$		0.229	-0.250	1310
8b	$\delta(\text{C}_2\text{-H})$		0.203	-0.131	1220
9b	$\delta^-(\text{C}_{4,5}\text{-H})$		-0.101	-0.206	1080
10b	δ^- ring		-0.006	0.070	938

^a Symmetry coordinate coefficients are given for pairs of bonds related by 2-fold symmetry:
 $v^\pm(\text{N-C}_2) = (\text{N}_1\text{-C}_2 \pm \text{N}_3\text{-C}_2)$; $v^\pm(\text{N-C}_{4,5}) = (\text{N}_1\text{-C}_5 \pm \text{N}_3\text{-C}_4)$; $\delta^\pm(\text{C}_{4,5}\text{-H}) = (\text{C}_4\text{-H} \pm \text{C}_5\text{-H})$;

Table S10. Internal coordinate coefficients^a in the computed eigenvectors and vibrational frequencies of ImH(H₂O)₂ and ImD(D₂O)₂.

v _i	Mode	ImH(H ₂ O) ₂						ImD(D ₂ O) ₂					
		v(N ₁ -C ₂)	v(C ₂ -N ₃)	v(N ₁ -C ₅)	v(N ₃ -C ₄)	v(C ₄ -C ₅)	Freq.	v(N ₁ -C ₂)	□(C ₂ -N ₃)	v(N ₁ -C ₅)	v(N ₃ -C ₄)	v(C ₄ -C ₅)	Freq.
4a	v(C ₄ -C ₅)	-0.035	-0.174	0.048	-0.065	0.254	1526	0.051	-0.215	0.002	-0.082	0.277	1504
5a	v ⁺ (N-C ₂)	-0.184	0.042	0.211	0.036	-0.194	1429	0.149	0.209	-0.038	-0.205	0.097	1328
6a	δ ⁺ (N-H/D)	0.209	0.023	-0.013	0.098	0.073	1149	-0.040	-0.012	0.050	0.012	0.027	871
7a	v ⁺ (N-C _{4,5})	-0.020	0.026	0.205	0.256	0.021	1138	0.095	0.038	0.158	0.270	0.053	1134
9a	δ ⁺ (C _{4,5} -H)	0.072	0.038	-0.091	0.084	-0.144	1091	-0.039	0.028	-0.031	0.098	-0.144	1104
10a	δ ⁺ ring	-0.040	-0.030	-0.013	0.042	0.005	936	0.040	0.024	0.036	-0.044	-0.002	918
5b	v ⁻ (N-C ₂)	0.208	-0.215	0.100	-0.041	-0.142	1479	-0.174	0.187	-0.113	0.024	0.207	1471
7b	v ⁻ (N-C _{4,5})	-0.024	0.221	0.132	-0.224	0.075	1338	-0.238	0.096	0.274	-0.106	-0.026	1351
8b	δ(C ₂ -H)	0.105	0.137	-0.012	-0.051	0.045	1253	-0.003	-0.109	-0.059	0.039	-0.028	1241
9b	δ ⁻ (C _{4,5} -H)	0.092	-0.052	0.156	-0.083	-0.049	1056	0.123	-0.043	0.130	-0.069	-0.063	1057
10b	δ ⁻ ring	0.022	0.014	-0.031	0.042	0.014	920	0.077	0.036	-0.088	0.037	-0.009	941

^a Internal coordinate coefficients are given for pairs of bonds related by pseudo 2-fold symmetry analogous to ImH₂⁺:

v[±](N-C₂) = (N₁-C₂ +/- N₃-C₂); v[±](N-C_{4,5}) = (N₁-C₅ +/- N₃-C₄); δ[±](C_{4,5}-H) = (C₄-H +/- C₅-H); δ[±](N-H/D) = (C₁-H/D +/- N₃-H/D).

Table S11. Experimental and computed vibrational frequencies(cm^{-1}) for the in-plane imidazole modes of the indicated molecules

ν_i	[tren]-Cu-Im-Zn-[tren]		[tren]-Cu-ImH		[tren]-Zn-ImH	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
4a	1493	1497		1568	1615	1567
5a	1280	1267	1449	1452	1444	1450
6a			1190	1186	1189	1185
7a	1185	1112		1133	1139	1128
9a	1107	1160		1104	1107	1103
10a	879	979		961	938	956
5b		1494		1517	1547	1516
6b						
7b	1337	1328	1336	1338	1334	1336
8b		1247	1270	1269	1267	1268
9b	1023	1081		1071	1078	1070
10b	906	953		928	954	928

Calculated frequencies of M-N stretching modes:

[tren]-Cu-Im-Zn-[tren]: Cu-N at 265 cm^{-1} and Zn-N at 227 cm^{-1} .

[tren]-Cu-ImH: Cu-N at 262 cm^{-1} .

[tren]-Zn-ImH: Zn-N at 221 cm^{-1} .

References

1. Vaughan, J. D.; Mughrabi, Z.; Wu, E. C. Kinetics of deuteration of imidazole, *Journal of Organic Chemistry* **1970**, *35*, 1141-1145.
2. Fuess, H.; Hohlwein, D.; Mason, S. A. Neutron-diffraction study of L-histidine hydrochloride monohydrate, *Acta Crystallographica Section B-Structural Science* **1977**, *33*, 654-659.
3. McMullan, R. K.; Epstein, J.; Ruble, J. R.; Craven, B. M. Crystal-structure of imidazole at 103-K by neutron-diffraction, *Acta Crystallographica Section B-Structural Science* **1979**, *35*, 688-691.
4. Pulay, P.; Fogarasi, G.; Pang, F.; Boggs, J. E. Systematic abinitio gradient calculation of molecular geometries, force constants, and dipole-moment derivatives, *Journal of the American Chemical Society* **1979**, *101*, 2550-2560.
5. Pulay, P.; Fogarasi, G.; Pongor, G.; Boggs, J. E.; Vargha, A. Combination of theoretical abinitio and experimental information to obtain reliable harmonic force-constants - scaled quantum-mechanical (SQM) force-fields for glyoxal, acrolein, butadiene, formaldehyde, and ethylene, *Journal of the American Chemical Society* **1983**, *105*, 7037-7047.
6. Rauhut, G.; Pulay, P. Transferable scaling factors for density-functional derived vibrational force-fields, *Journal of Physical Chemistry* **1995**, *99*, 3093-3100.
7. Rauhut, G.; Pulay, P. Transferable scaling factors for density-functional derived vibrational force-fields (VOL 99, Pg 3096, 1995), *Journal of Physical Chemistry* **1995**, *99*, 14572-14572.
8. Baker, J.; Jarzecki, A. A.; Pulay, P. Direct scaling of primitive valence force constants: An alternative approach to scaled quantum mechanical force fields, *Journal of Physical Chemistry A* **1998**, *102*, 1412-1424.
9. VanBael, M. K.; Smets, J.; Schone, K.; Houben, L.; McCarthy, W.; Adamowicz, L.; Nowak, M. J.; Maes, G. Matrix-isolation FTIR studies and theoretical calculations of hydrogen-bonded complexes of imidazole. A comparison between experimental results and different calculation methods, *Journal of Physical Chemistry A* **1997**, *101*, 2397-2413.