Supplementary Information to "pH-dependent absorption spectrum of oxyluciferin analogues in the active site of firefly luciferase"

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1 CpHMD oxyluciferin analogue structure and AMP parameters

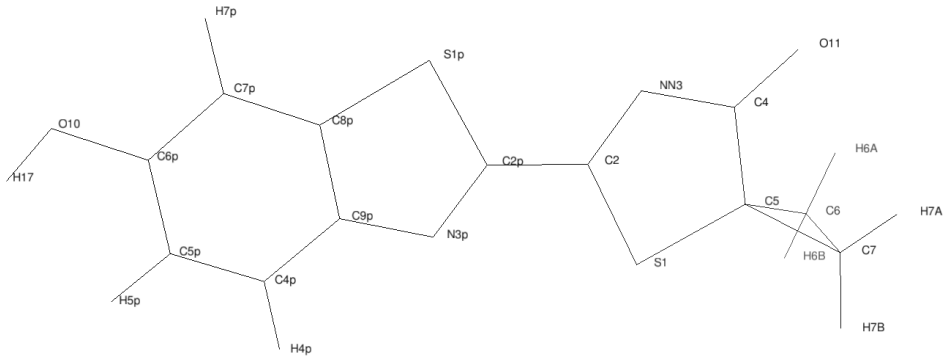

Figure S1: Structure and atom labels of the phenol-keto analogue

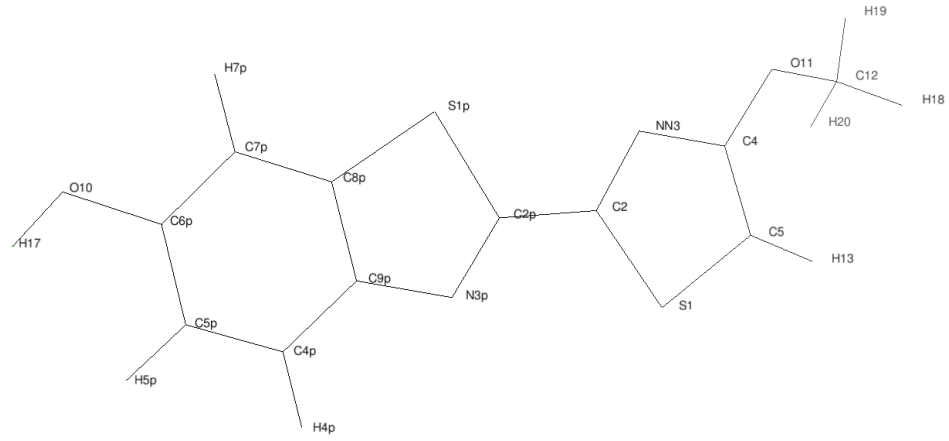

Figure S2: Structure and atom labels of the phenol-methoxy analogue

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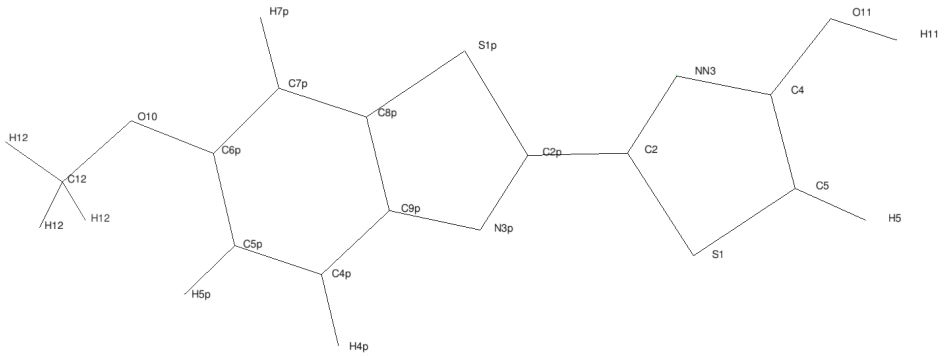

Figure S3: Structure and atom labels of the methoxy-enol analogue

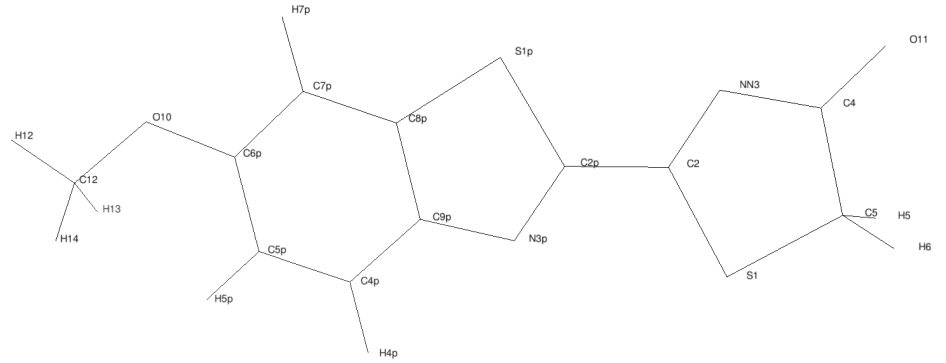

Figure S4: Structure and atom labels of the methoxy-keto analogue

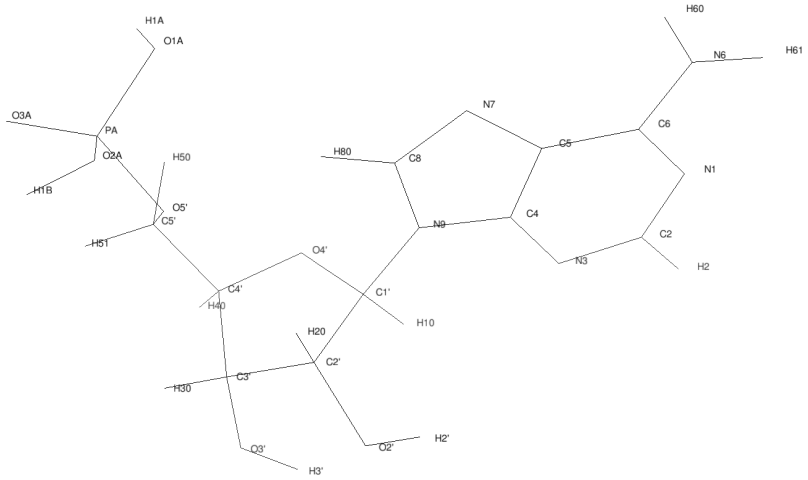

Figure S5: Structure and atom labels of the AMP1 model (first deprotonation of AMP)

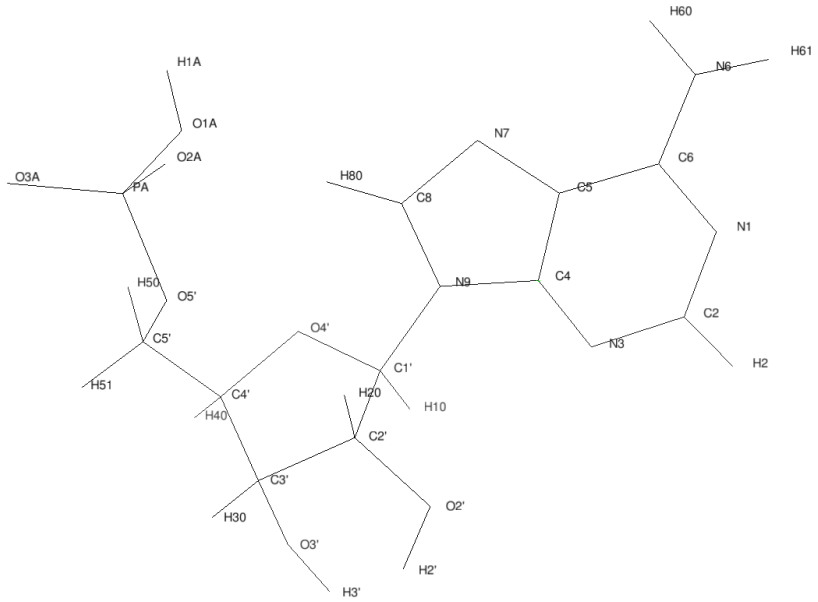

Figure S6: Structure and atom labels of the AMP2 model (second deprotonation of AMP)

Table S1: Atomic partial charges of the phenol-keto analogue

atom	protonated	deprotonated
NN3	-0.5932	-0.6446
C4	0.8344	0.7807
O11	-0.5635	-0.6072
C5	-0.0814	0.0448
C6	-0.2515	-0.3077
H6A	0.1577	0.1499
H6B	0.1577	0.1499
C7	-0.2515	-0.3077
H7A	0.1577	0.1499
H7B	0.1577	0.1499
S1	-0.1109	-0.2254
C2	0.2919	0.3447
C2p	0.3415	0.1142
N3p	-0.5195	-0.4196
C9p	0.3451	0.2867
C4p	-0.1860	-0.2457
H4p	0.1960	0.1709
C5p	-0.3819	-0.3617
H5p	0.1872	0.1489
C6p	0.4446	0.7181
O10	-0.6465	-0.7085
H17	0.4639	0.0000
C7p	-0.3674	-0.6176
H7p	0.2733	0.2418
C8p	-0.0056	0.1497
S1p	-0.0513	-0.1544

Table S2: Atomic partial charges of the phenol-methoxy analogue

atom	phenol	phenolate
NN3	-0.5250	-0.5855
C4	0.6718	0.6792
O11	-0.4096	-0.4232
C12	0.1013	0.1402
H18	0.0391	0.0174
H19	0.0391	0.0174
H20	0.0391	0.0174
C5	-0.5406	-0.5802
H13	0.2938	0.2781
S1	0.0203	-0.0756
C2	0.2730	0.3540
C2p	0.2869	0.1428
N3p	-0.5460	-0.4869
C9p	0.4151	0.2888
C4p	-0.2926	-0.2536
H4p	0.2057	0.1666
C5p	-0.2552	-0.3842
H5p	0.1984	0.1462
C6p	0.4033	0.6950
O10	-0.5944	-0.7375
H17	0.4237	0.0000
C7p	-0.4818	-0.6303
H7p	0.2684	-0.2411
C8p	0.0385	0.1528
S1p	-0.0742	-0.1801

Table S3: Atomic partial charges of the methoxy-enol analogue

atom	protonated	deprotonated
NN3	-0.3704	-0.4632
C4	0.5054	0.6869
O11	-0.5742	-0.7701
H11	0.4222	0.0000
C5	-0.5174	-0.6080
H5	0.2756	0.2309
S1	0.0433	-0.1096
C2	0.2372	0.2203
C2p	0.2545	0.3460
N3p	-0.5102	-0.6196
C9p	0.3294	0.4284
C4p	-0.2849	-0.3514
H4p	0.2075	0.2073
C5p	-0.2802	-0.2772
H5p	0.1712	0.1615
C6p	0.4582	0.4052
O10	-0.4119	-0.4200
C12	0.0809	0.1083
H12	0.0532	0.0356
H12	0.0532	0.0356
H12	0.0532	0.0356
C7p	-0.5258	-0.4990
H7p	0.2835	0.2652
C8p	0.1535	0.1125
S1p	-0.1070	-0.1614

Table S4: Atomic partial charges of the methoxy-keto analogue

atom	charge
NN3	-0.4334
C4	0.6102
O11	-0.4764
C5	-0.0544
H5	0.0838
H6	0.0838
S1	-0.1211
C2	0.2162
C2p	0.2002
N3p	-0.4278
C9p	0.3272
C4p	-0.2822
H4p	0.1873
C5p	-0.2043
H5p	0.1466
C6p	0.3657
O10	-0.3020
C12	0.0094
H12	0.0648
H13	0.0648
H14	0.0648
C7p	-0.4248
H7p	0.2370
C8p	0.0867
S1p	-0.0224

Table S5: Atomic partial charges of AMP1

atom	protonated	deprotonated
O1A	-0.6321	-0.7812
H1A	0.4654	0.4693
PA	1.1388	1.2882
O2A	-0.6321	-0.8252
H1B	0.4654	0.0000
O3A	-0.6934	-0.8252
O5'	-0.3102	-0.4252
C5'	-0.1428	-0.3227
H50	0.1280	0.1541
H51	0.1280	0.1541
C4'	0.2779	0.6098
H40	0.0810	0.0210
C3'	0.2923	-0.1131
H30	0.0223	0.1264
O3'	-0.7217	-0.6709
H3	0.4598	0.4301
C2'	0.3076	0.2388
H20	-0.0201	0.0693
O2'	-0.7209	-0.6803
H2'	0.4312	0.4220
O4'	-0.4885	-0.6094
C1'	0.1640	0.5023
H10	0.0932	0.0489
N9	-0.1443	-0.3618
C8	0.2919	0.3900
H80	0.1520	0.1573
N7	-0.6252	-0.6553
C5	-0.0045	-0.1813
C6	0.7920	0.9858
N6	-0.9613	-1.0895
H60	0.4303	0.4551
H61	0.4303	0.4551
N1	-0.7806	-0.9280
C2	0.5455	0.5836
H2	0.0791	0.0636
N3	-0.6936	-0.8000
C4	0.3954	0.6446

Table S6: Atomic partial charges of AMP2

atom	protonated	deprotonated
O1A	-0.7812	-0.9493
H1A	0.4693	0.0000
PA	1.2882	1.4113
O2A	-0.8252	-0.9493
O3A	-0.8252	-0.9493
O5'	-0.4252	-0.5525
C5'	-0.3227	-0.1520
H50	0.1541	0.1042
H51	0.1541	0.1042
C4'	0.6098	0.4956
H40	0.0210	0.0144
C3'	-0.1131	-0.0508
H30	0.1264	0.1054
O3'	-0.6709	-0.6897
H3	0.4301	0.4171
C2'	0.2388	0.1866
H20	0.0693	0.1045
O2'	-0.6803	-0.6946
H2'	0.4220	0.4135
O4'	-0.6094	-0.5789
C1'	0.5023	0.6100
H10	0.0489	0.0127
N9	-0.3618	-0.5616
C8	0.3900	0.6831
H80	0.1573	0.0205
N7	-0.6553	-0.6693
C5	-0.1813	-0.2804
C6	0.9858	1.0255
N6	-1.0895	-1.1068
H60	0.4551	0.4480
H61	0.4551	0.4480
N1	-0.9280	-0.9676
C2	0.5836	0.5659
H2	0.0636	0.0474
N3	-0.8000	-0.8124
C4	0.6446	0.7465

2 Selected titrating residues

Table S7: This table reports the type and numbering of all titrating residues selected using the iterative approach described in the main text. Of course, the analogue (544th residue) and AMP (545th residue) are included as well. In the first iteration of the selection, only HIS 245, GLU 344, LYS 439, AMP and analogue were considered.

AMP1+phenol-methoxy	AMP1+phenol-keto	AMP1+methoxy-enol	AMP1+methoxy-keto
GLU 83	GLU 83	GLU 83	GLU 83
ASP 107	CYS 108	ASP 107	ASP 107
CYS 108	LYS 206	CYS 108	CYS 108
LYS 206	HIS 244	LYS 206	LYS 206
HIS 244	HIS 245	HIS 244	HIS 244
HIS 245	GLU 344	HIS 245	HIS 245
GLU 311	LYS 354	GLU 311	GLU 311
GLU 344	ASP 356	GLU 344	GLU 344
LYS 354	ASP 357	LYS 354	LYS 354
ASP 357	HIS 431	ASP 357	ASP 357
LYS 439	LYS 439	LYS 439	LYS 439
LYS 443	LYS 443	LYS 443	LYS 443
CYS 447	CYS 447	CYS 447	CYS 447

AMP2+phenol-methoxy	AMP2+phenol-keto	AMP2+methoxy-enol
ASP 107	CYS 108	ASP 107
CYS 108	LYS 206	CYS 108
LYS 206	HIS 244	LYS 206
HIS 244	HIS 245	HIS 244
HIS 245	GLU 344	HIS 245
GLU 311	LYS 354	GLU 311
GLU 344	ASP 357	GLU 344
LYS 354	LYS 364	LYS 354
ASP 356	HIS 419	ASP 356
ASP 422	HIS 431	ASP 422
HIS 431	LYS 439	HIS 431
LYS 439	CYS 447	LYS 439
LYS 443		LYS 443
CYS 447		CYS 447

3 CpHMD-based titration curves

Below are reported all titration curves obtained using CpHMD. Some pK_a values are obviously not fully converged, especially when Hill factors n are significantly far from 1.0. Nevertheless, CpHMD trajectories were not meant to produce accurate pK_a values, but rather to sample the most probable protonation microstates, suitable for tens of thousands of QM/MM calculations.

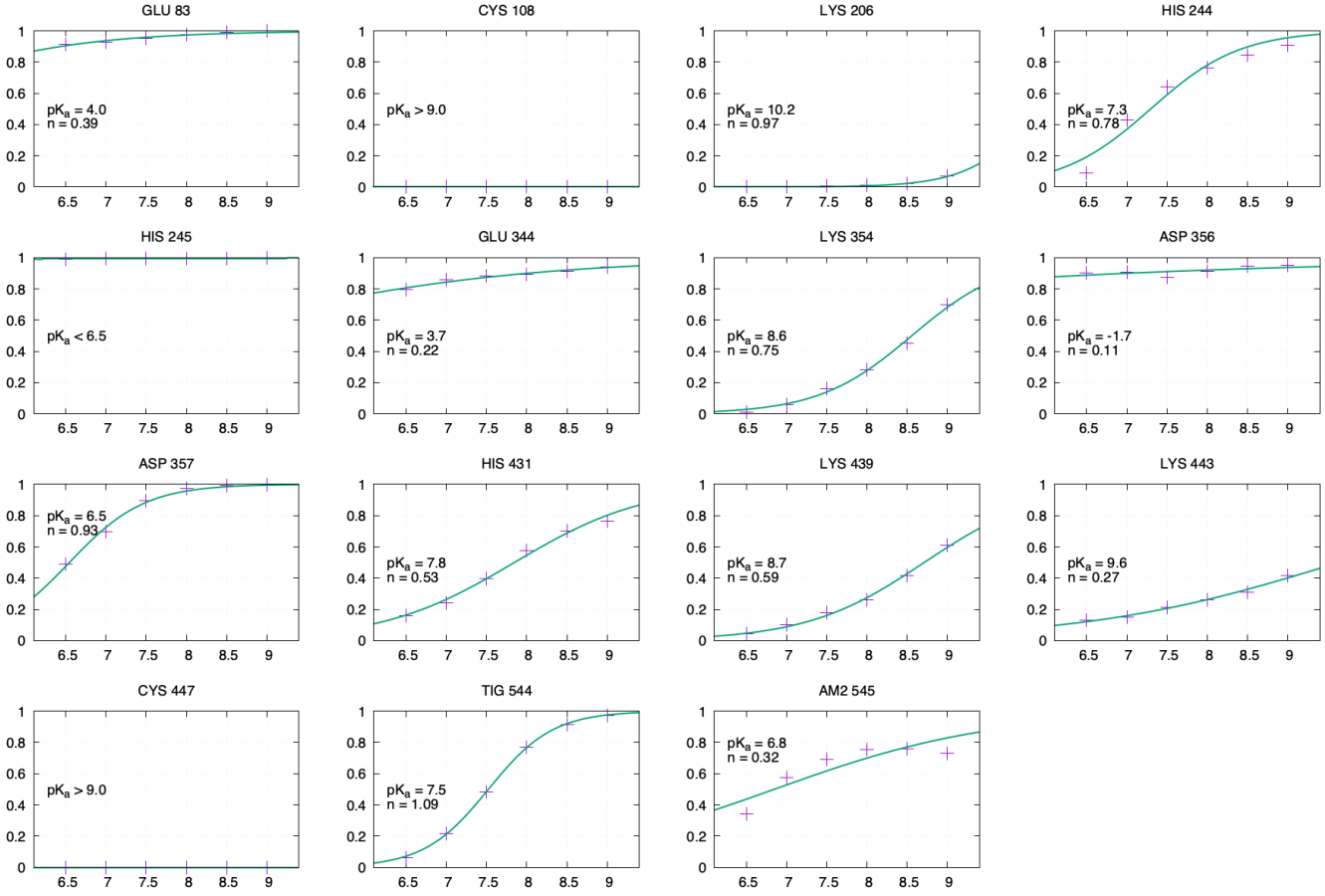

Figure S7: AMP1 (indicated as AM2) + phenol-keto (indicated as TIG 544)

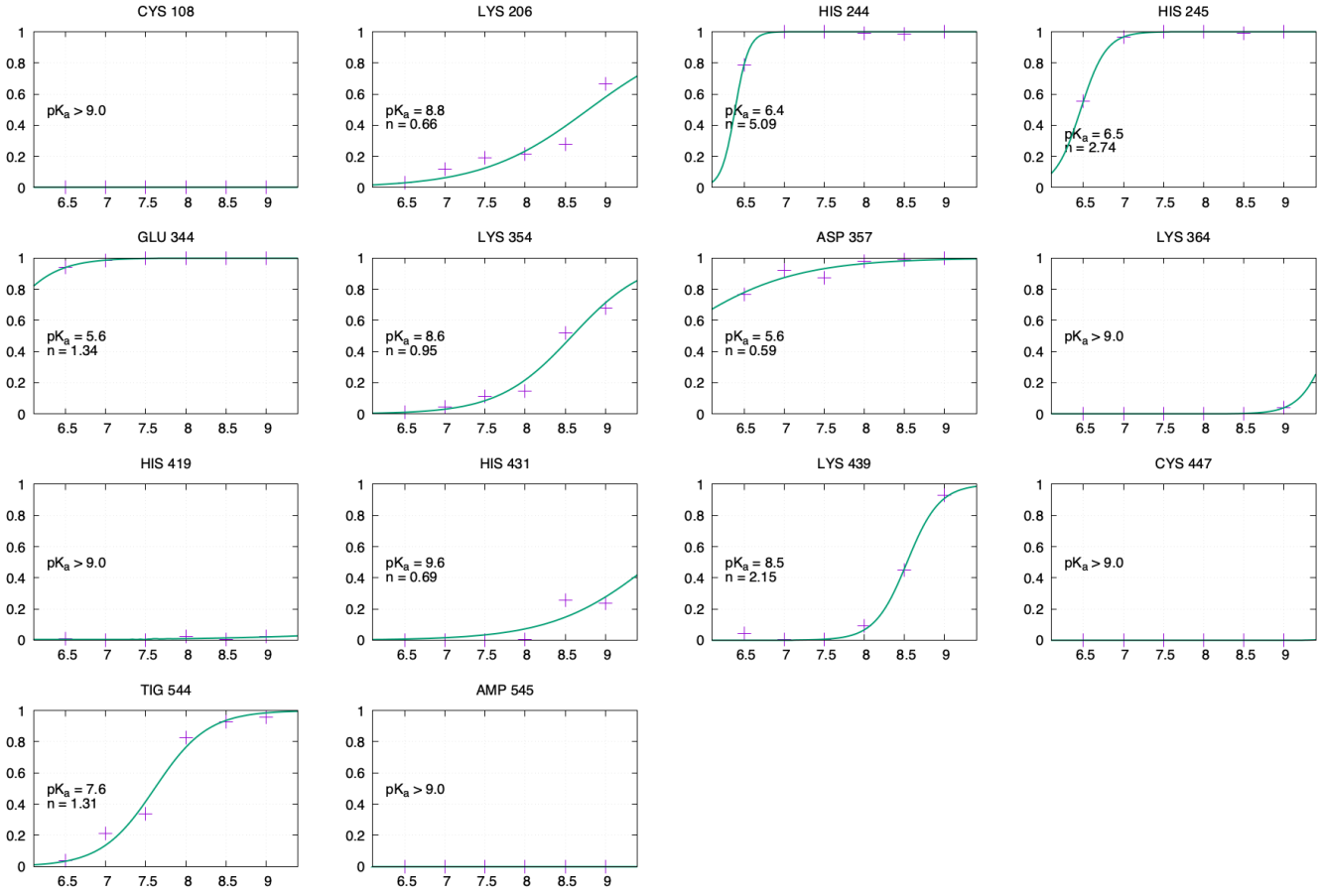

Figure S8: AMP2 (indicated as AMP) + phenol-keto (indicated as TIG 544)

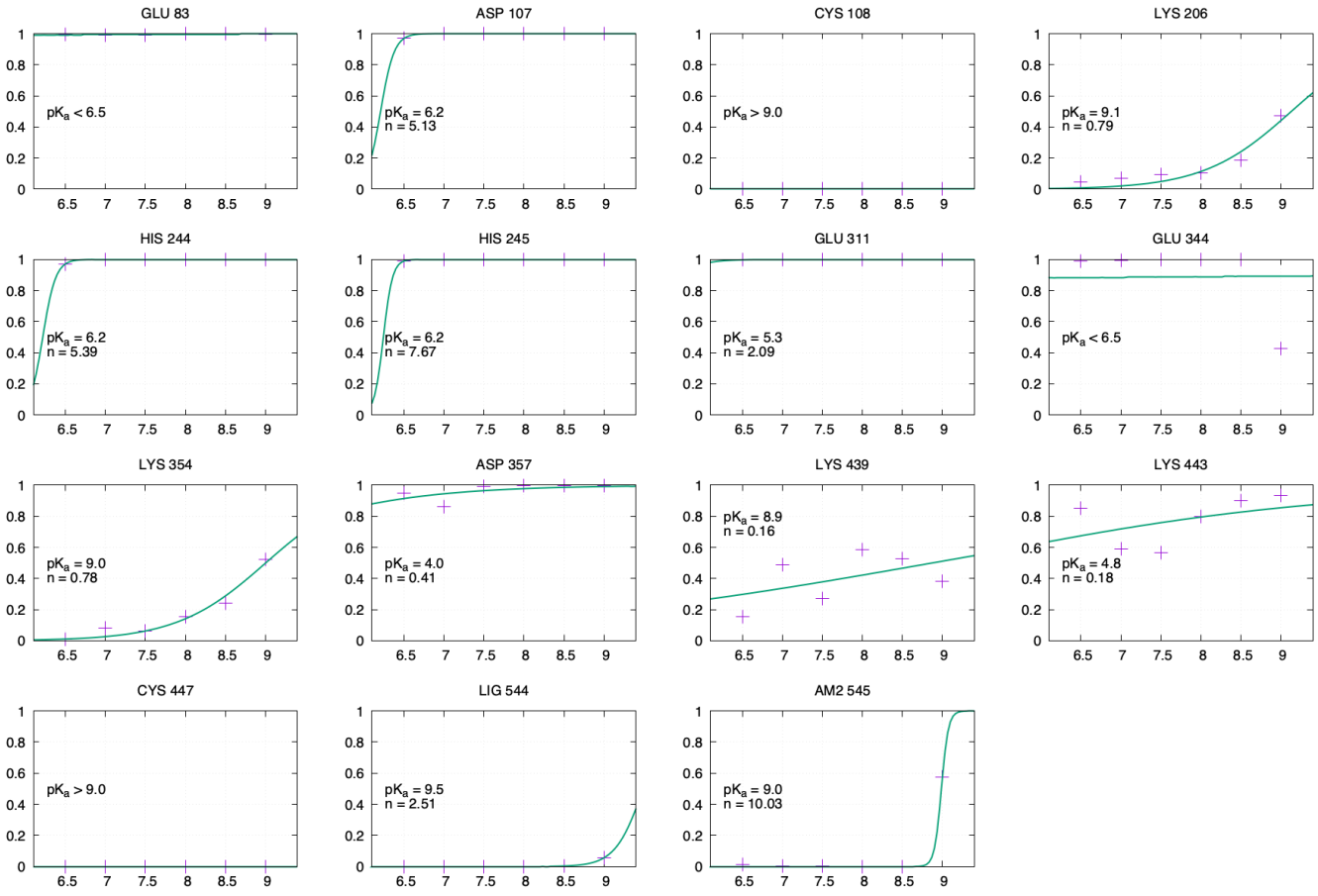

Figure S9: AMP1 (indicated as AM2) + phenol-methoxy (indicated as LIG 544)

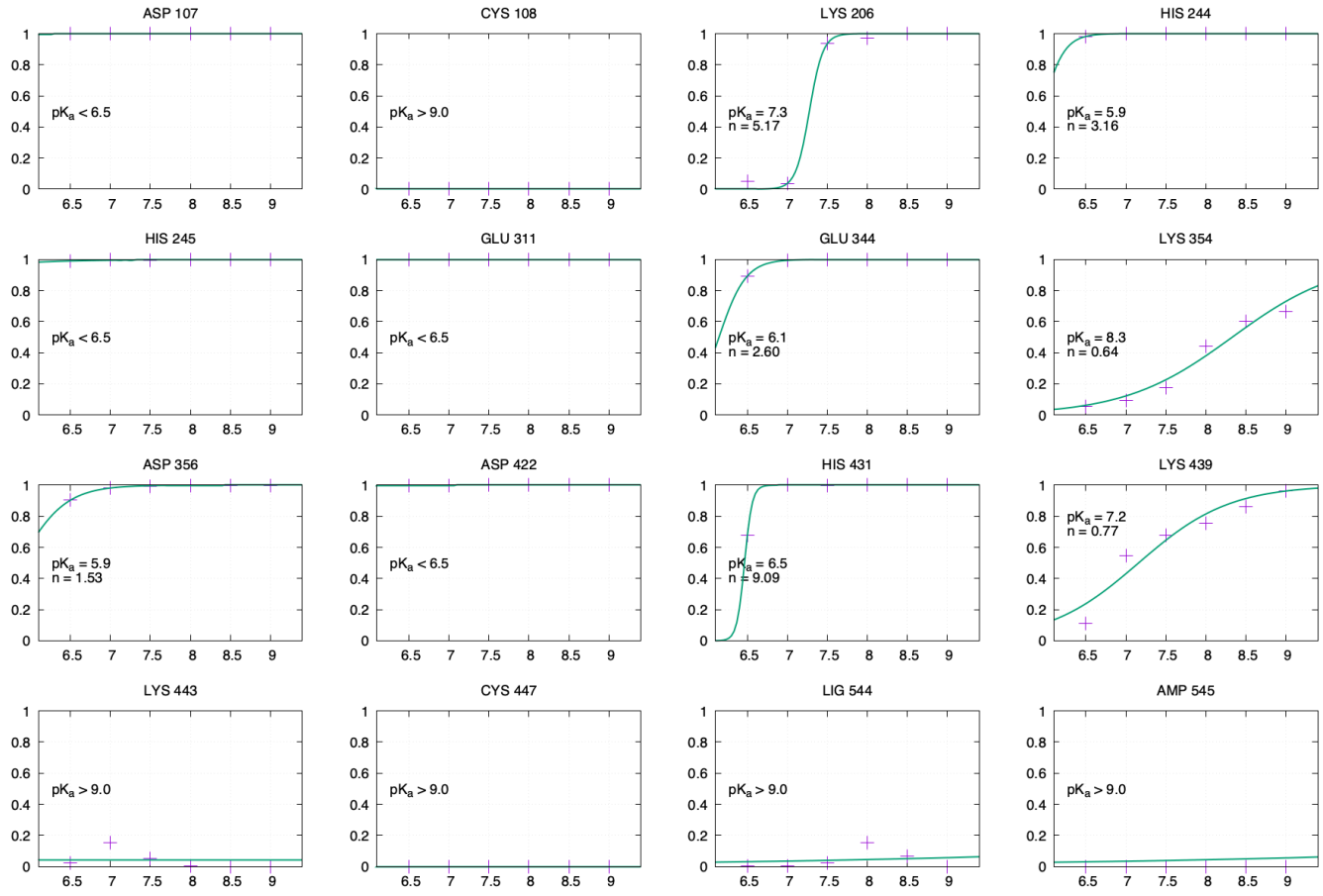

Figure S10: AMP2 (indicated as AMP) + phenol-methoxy (indicated as LIG 544)

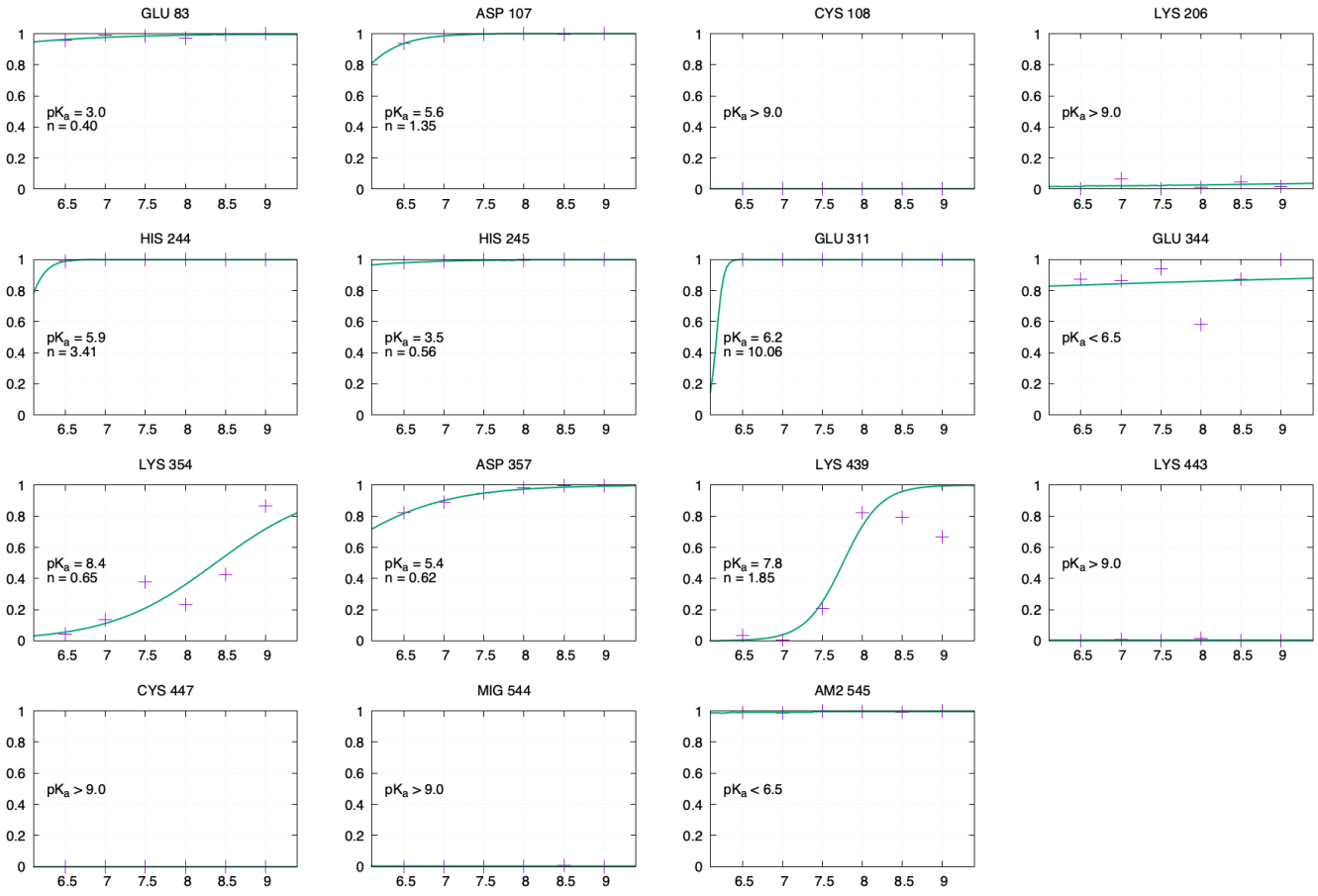

Figure S11: AMP1 (indicated as AM2) + methoxy-enol (indicated as MIG 544)

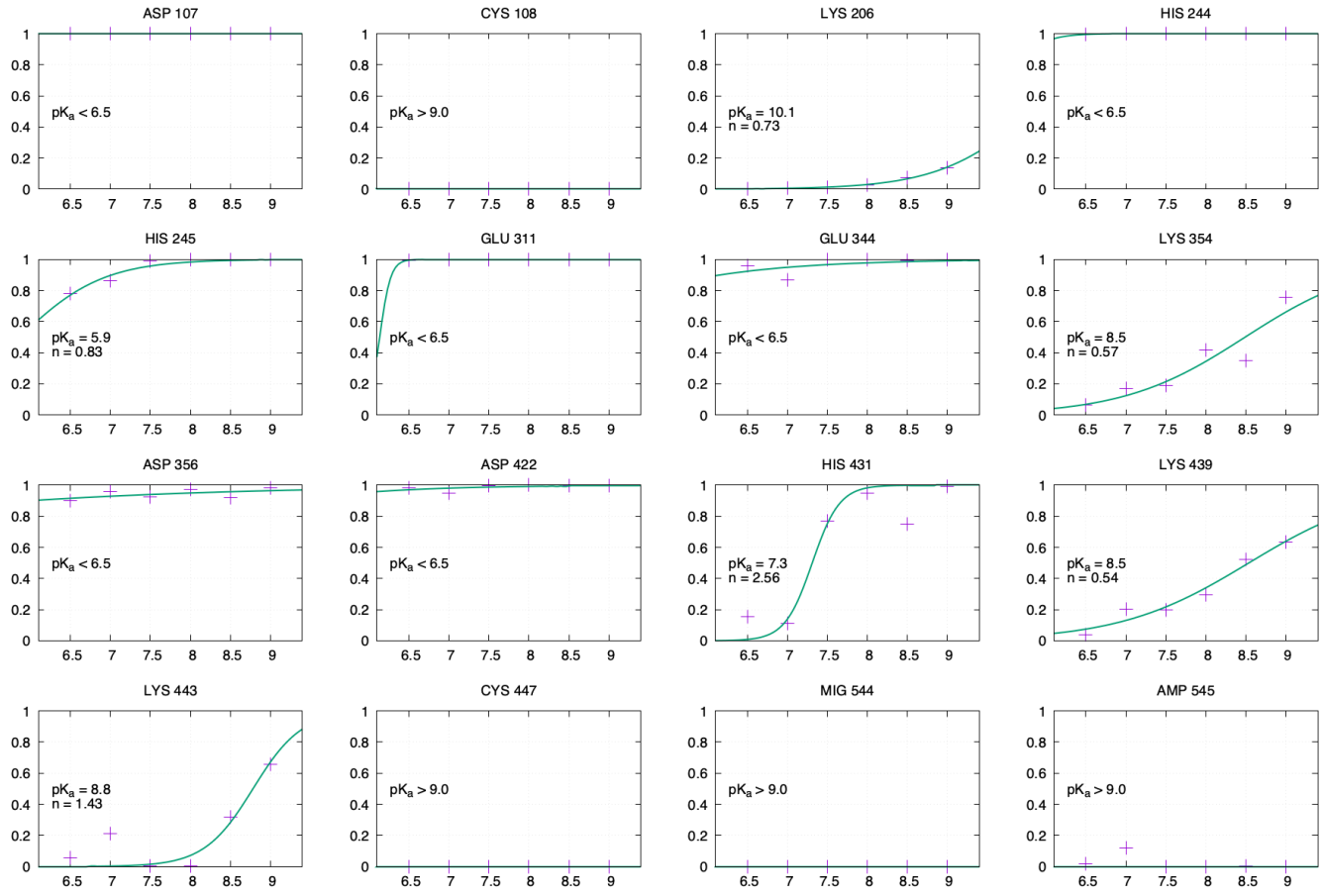

Figure S12: AMP2 (indicated as AMP) + methoxy-enol (indicated as MIG 544)

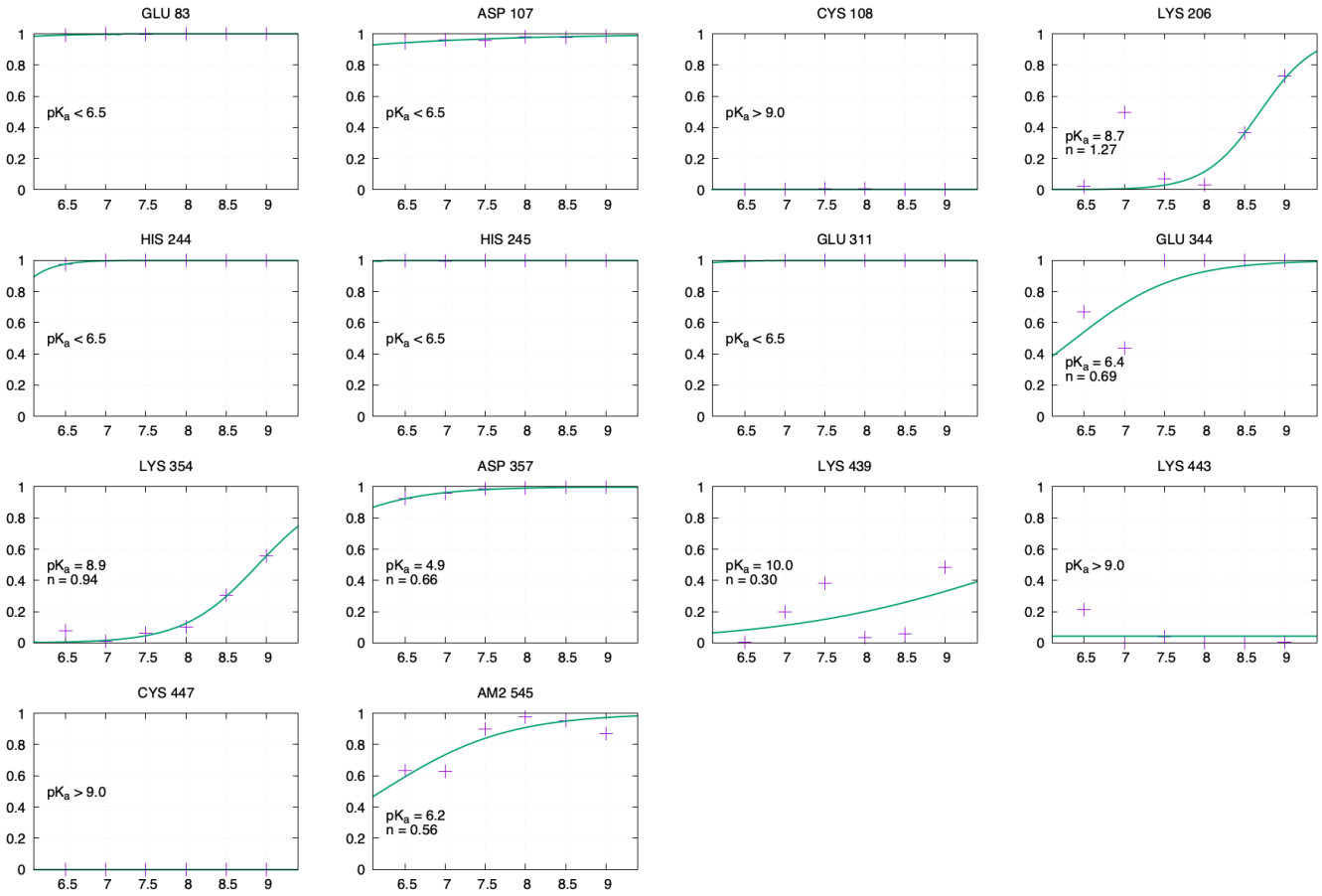

Figure S13: AMP1 (indicated as AM2) + methoxy-keto (not titrating)

4 CpHMD trajectory analysis: correlations between protonation microstates

Below are reported plots of individual protonation states of a subset of titrating residues, for each considered pH trajectory. Blue: positively charged; white: neutral; light red: negatively charged (-1); red: negatively charged (-2).

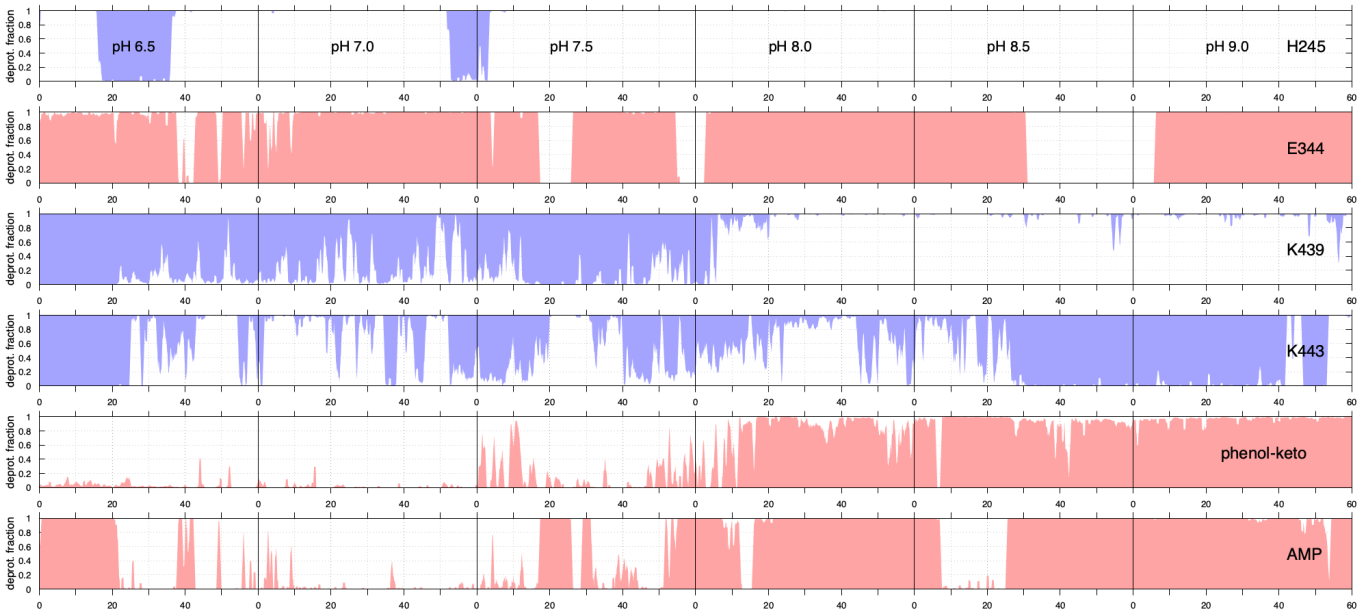

Figure S14: Phenol-Keto + AMP1

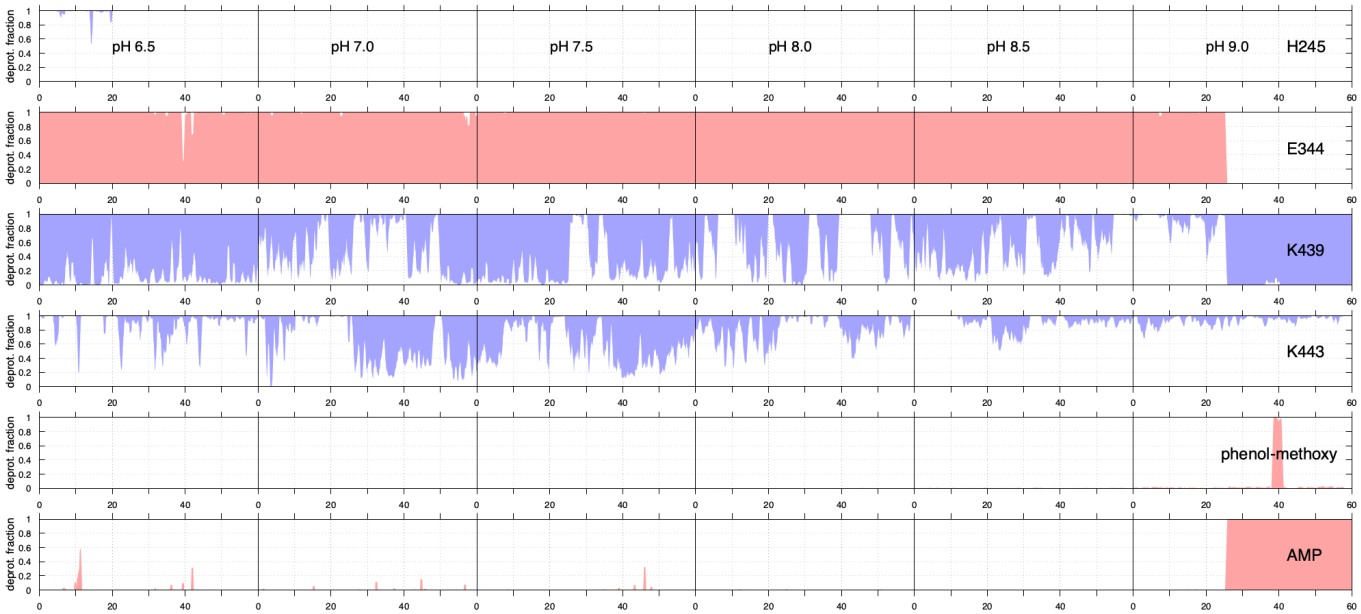

Figure S15: Phenol-methoxy + AMP1

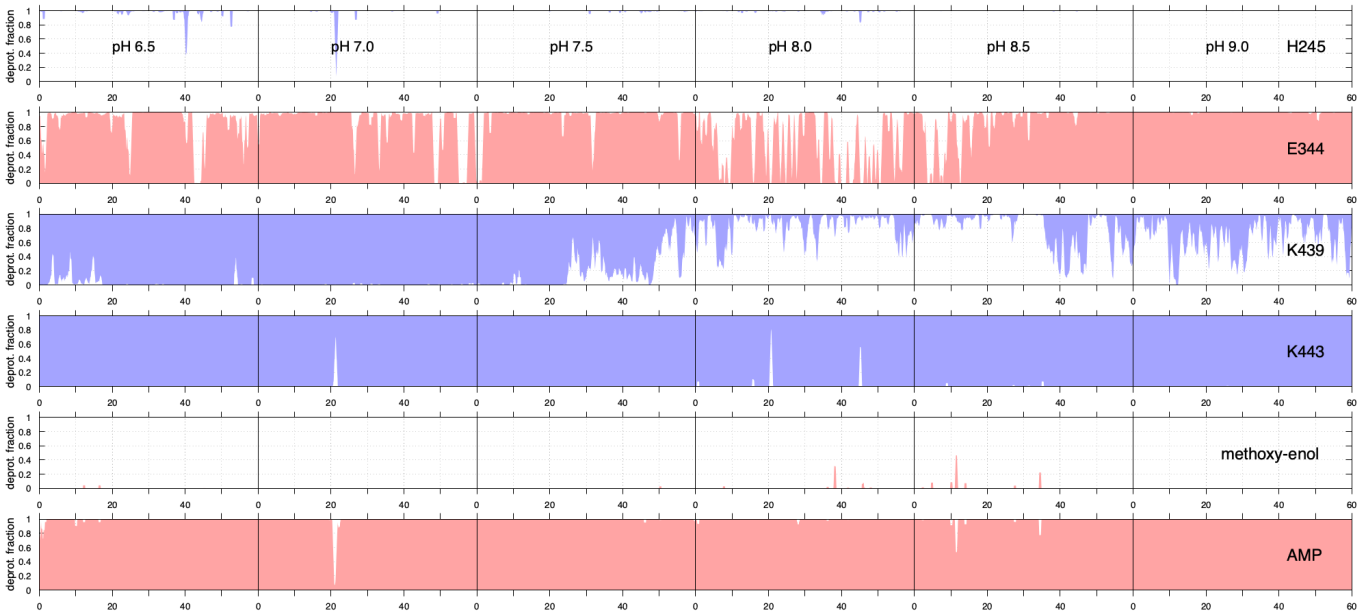

Figure S16: Methoxy-enol + AMP1

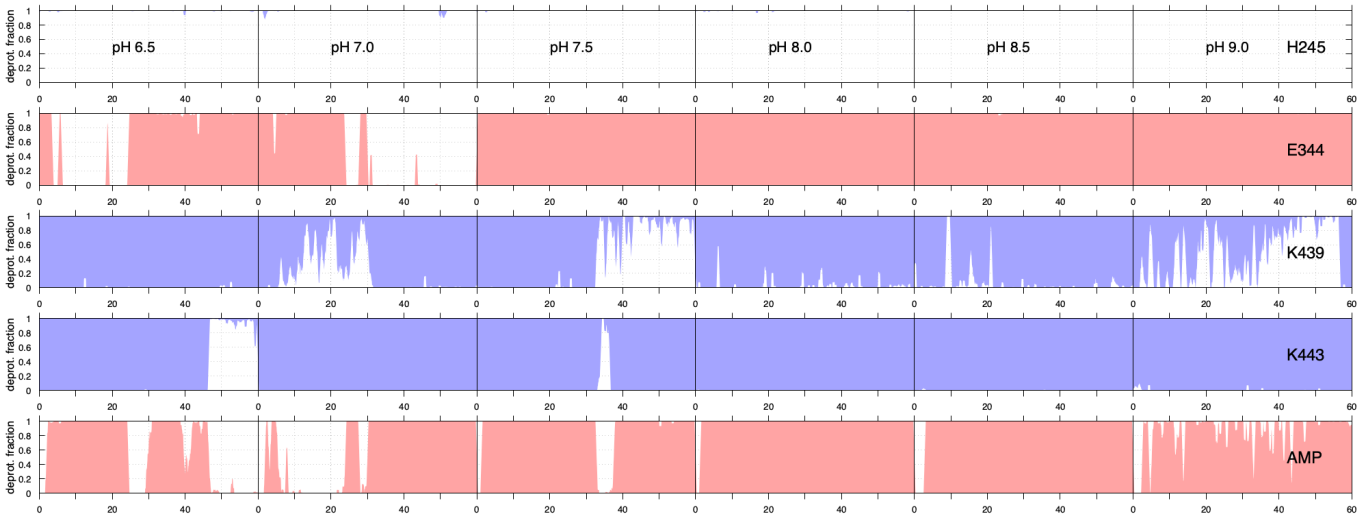

Figure S17: Methoxy-keto + AMP1

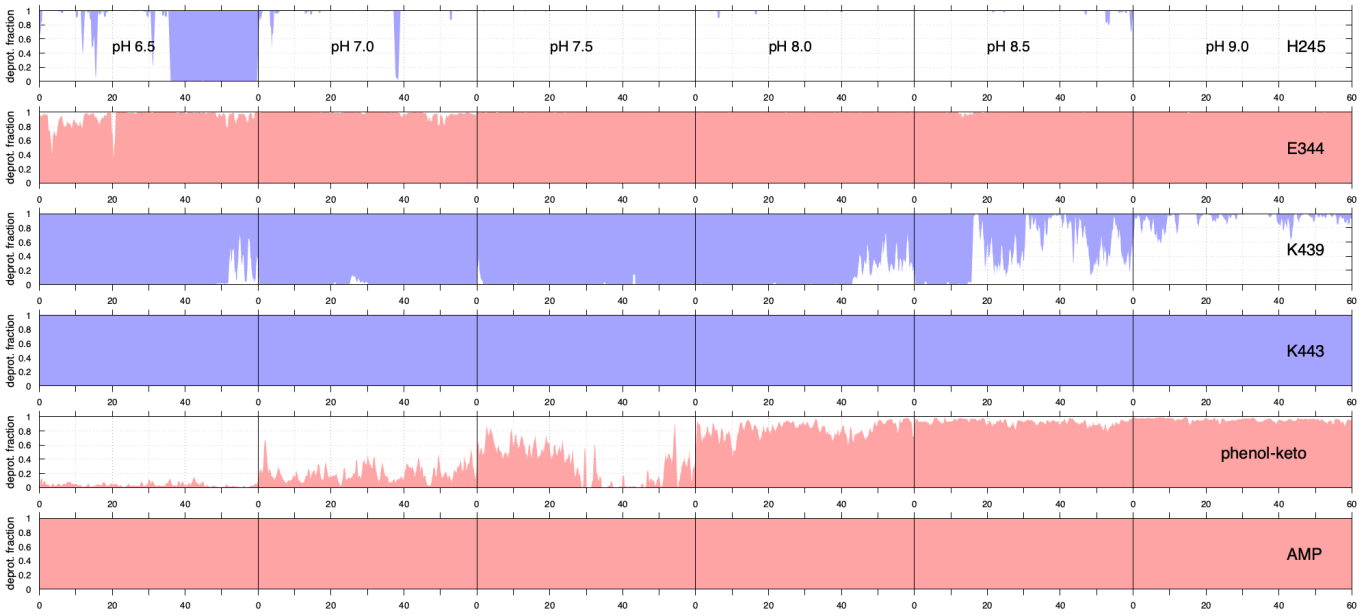

Figure S18: Phenol-keto + AMP2

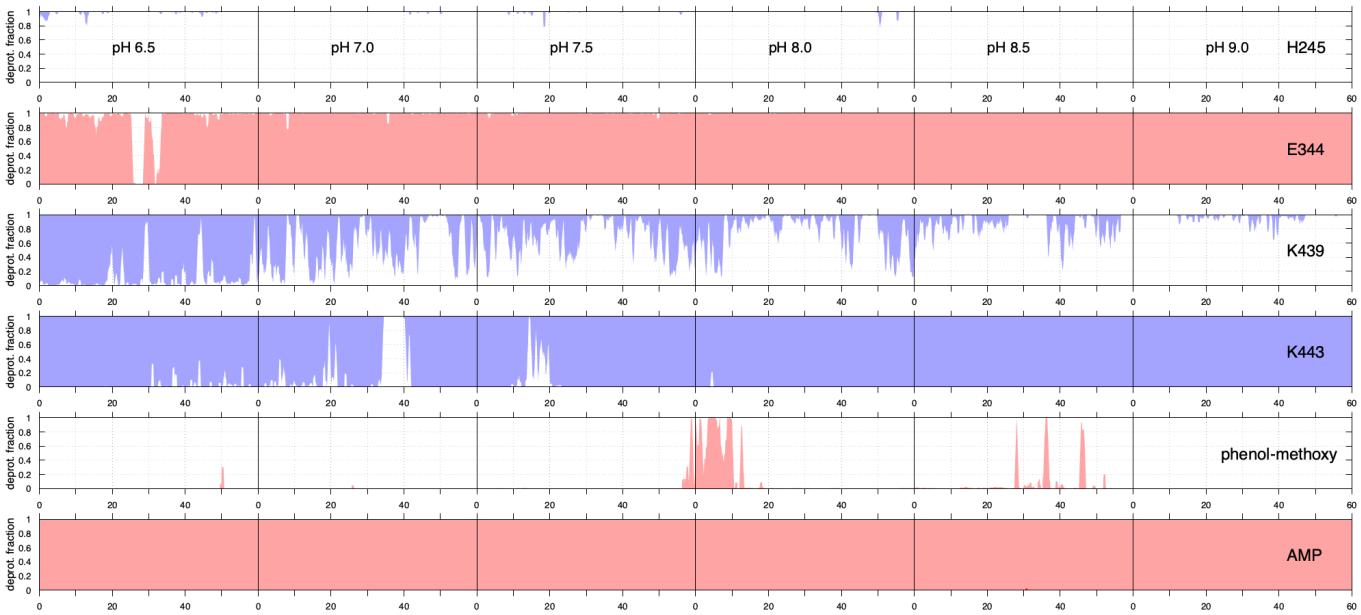

Figure S19: Phenol-methoxy + AMP2

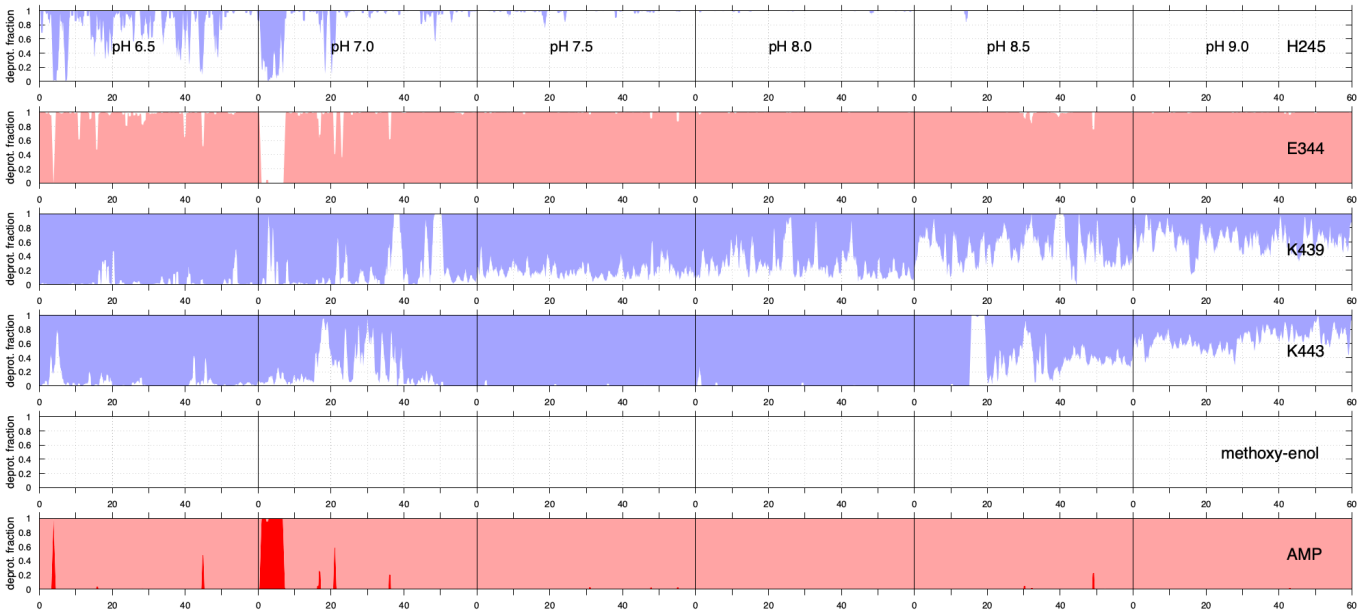

Figure S20: Methoxy-enol + AMP2

5 Analysis of the distance-based interactions between analogue, AMP and the most important residues.

From left to right: phenol-keto, phenol-methoxy, methoxy-enol, methoxy-keto. The radius of each circle is inversely proportional to the average distance between the selected amino-acid titratable atom and the considered AMP/analogue heavy atom. The color scale corresponds to the distance standard deviation (scale bar at the bottom of each Figure).

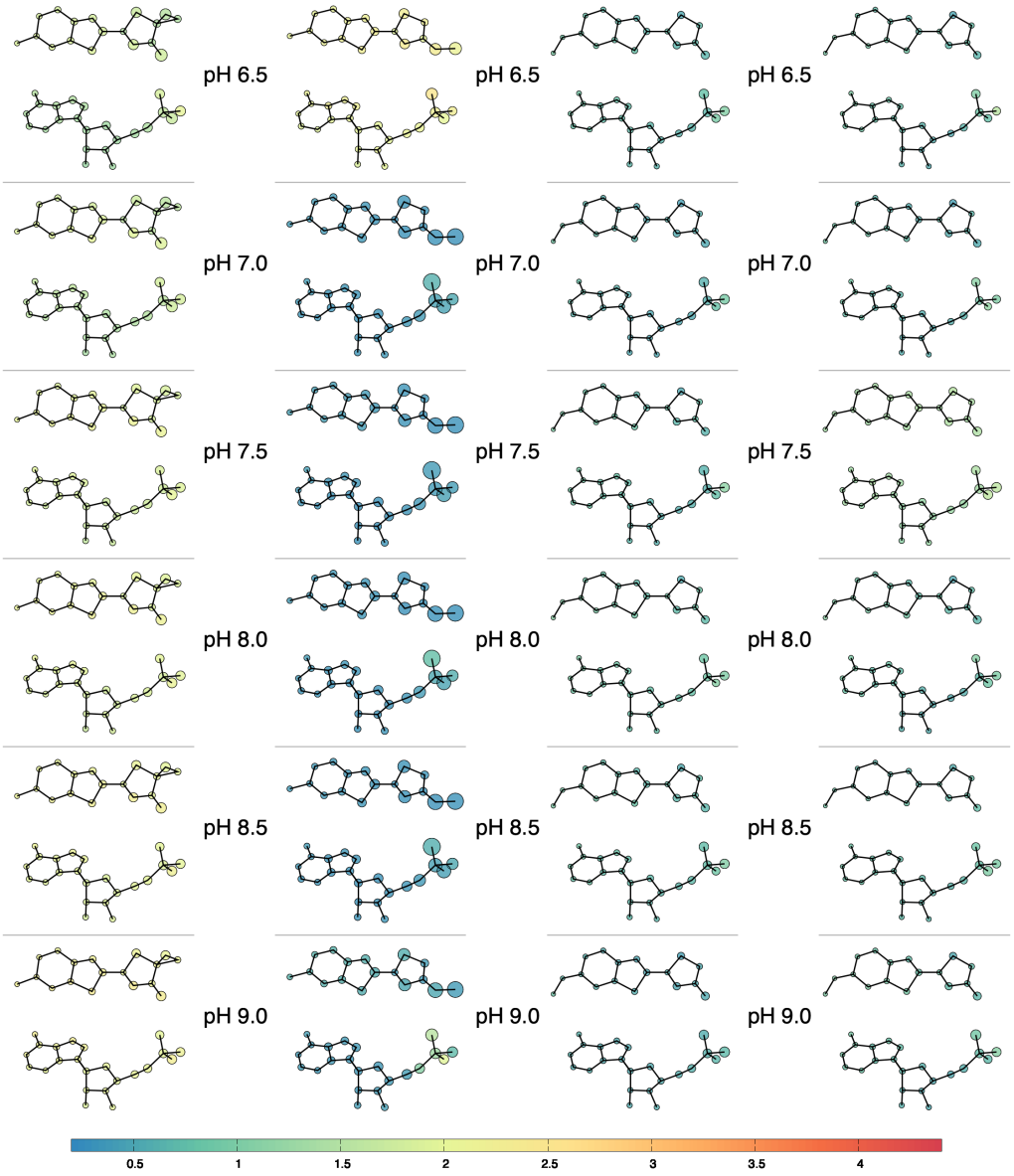

Figure S21: Distance distribution between analogue/AMP1 and residue H245

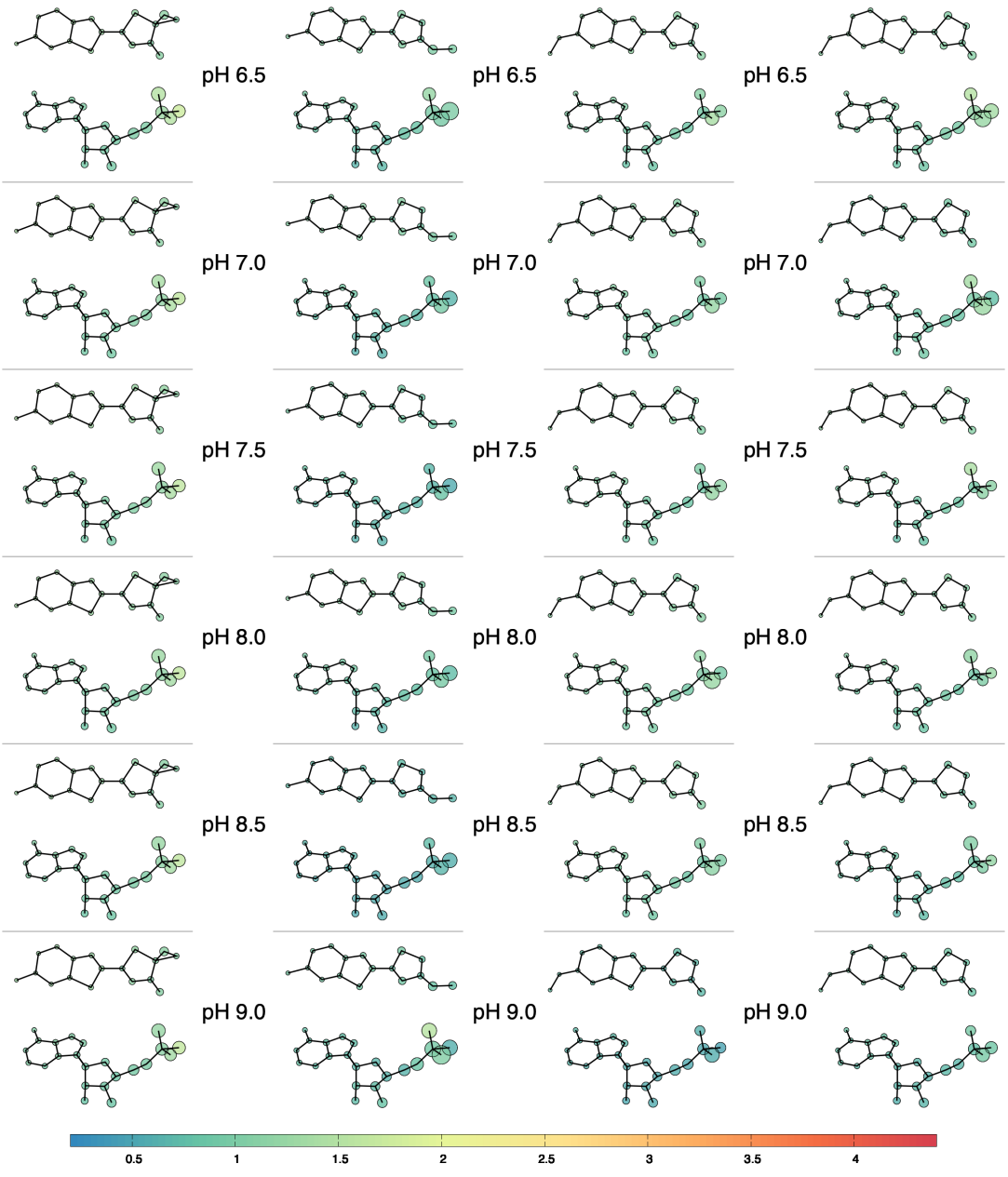

Figure S22: Distance distribution between analogue/AMP1 and residue E344

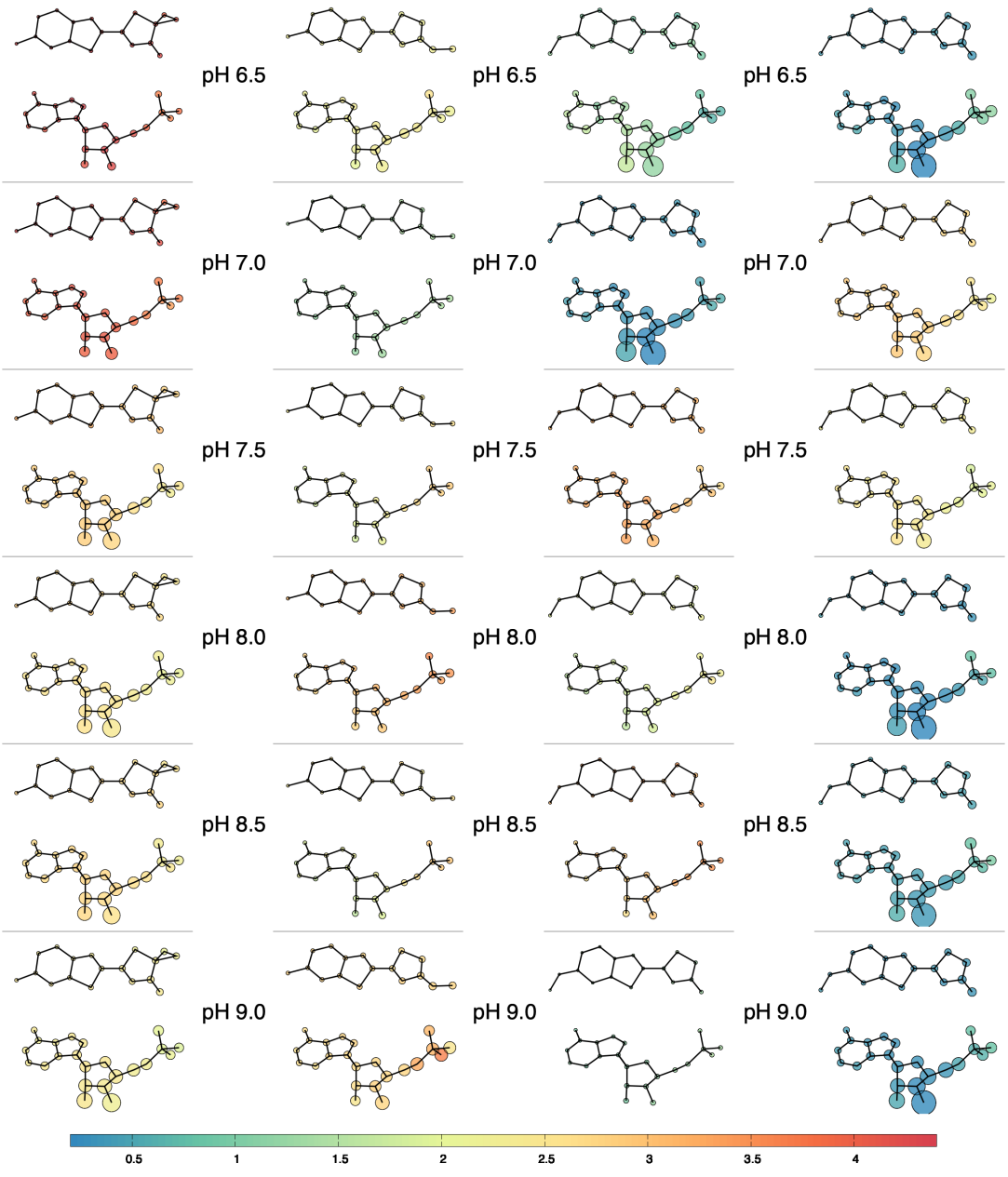

Figure S23: Distance distribution between analogue/AMP1 and residue K439

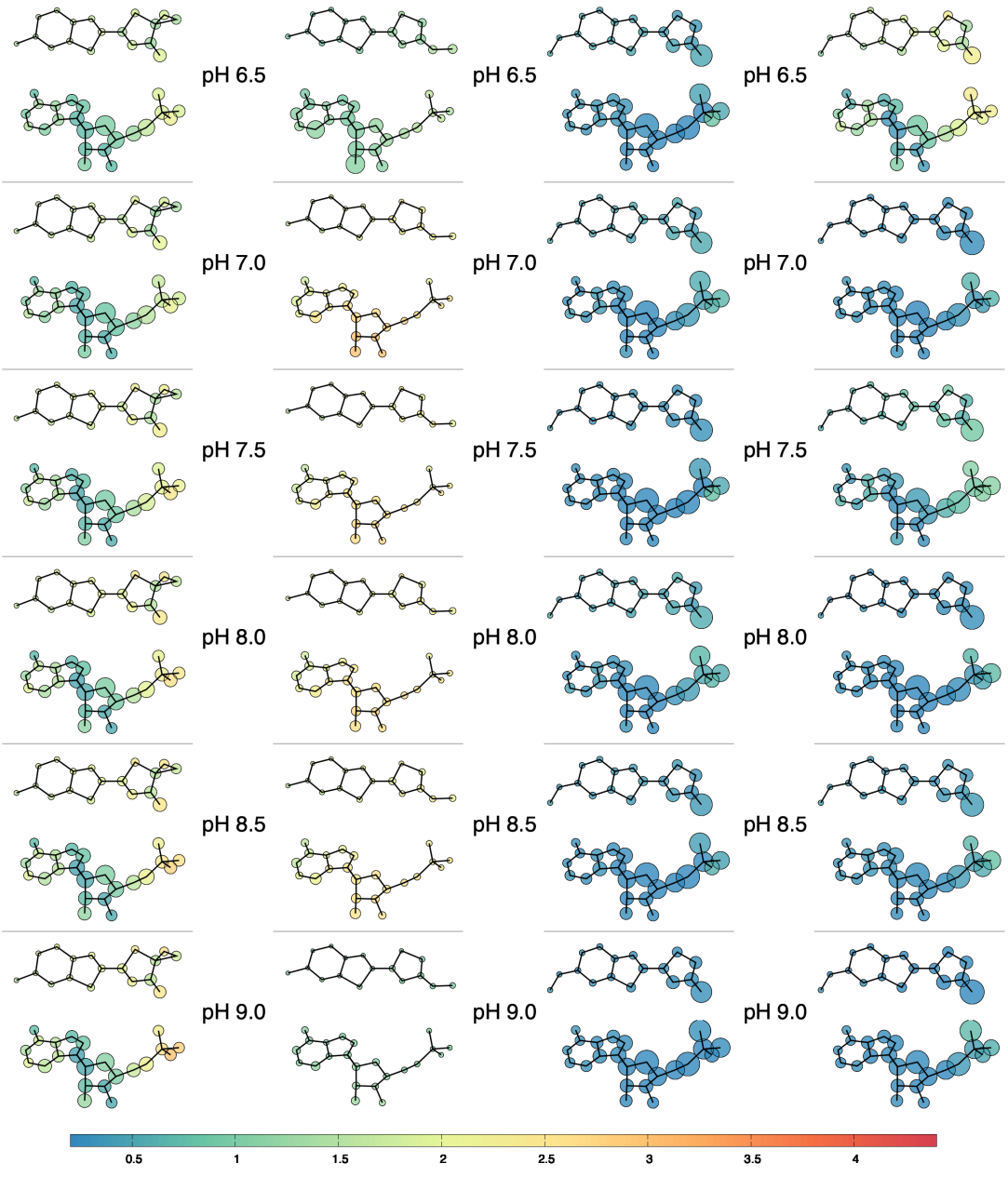

Figure S24: Distance distribution between analogue/AMP1 and residue K443

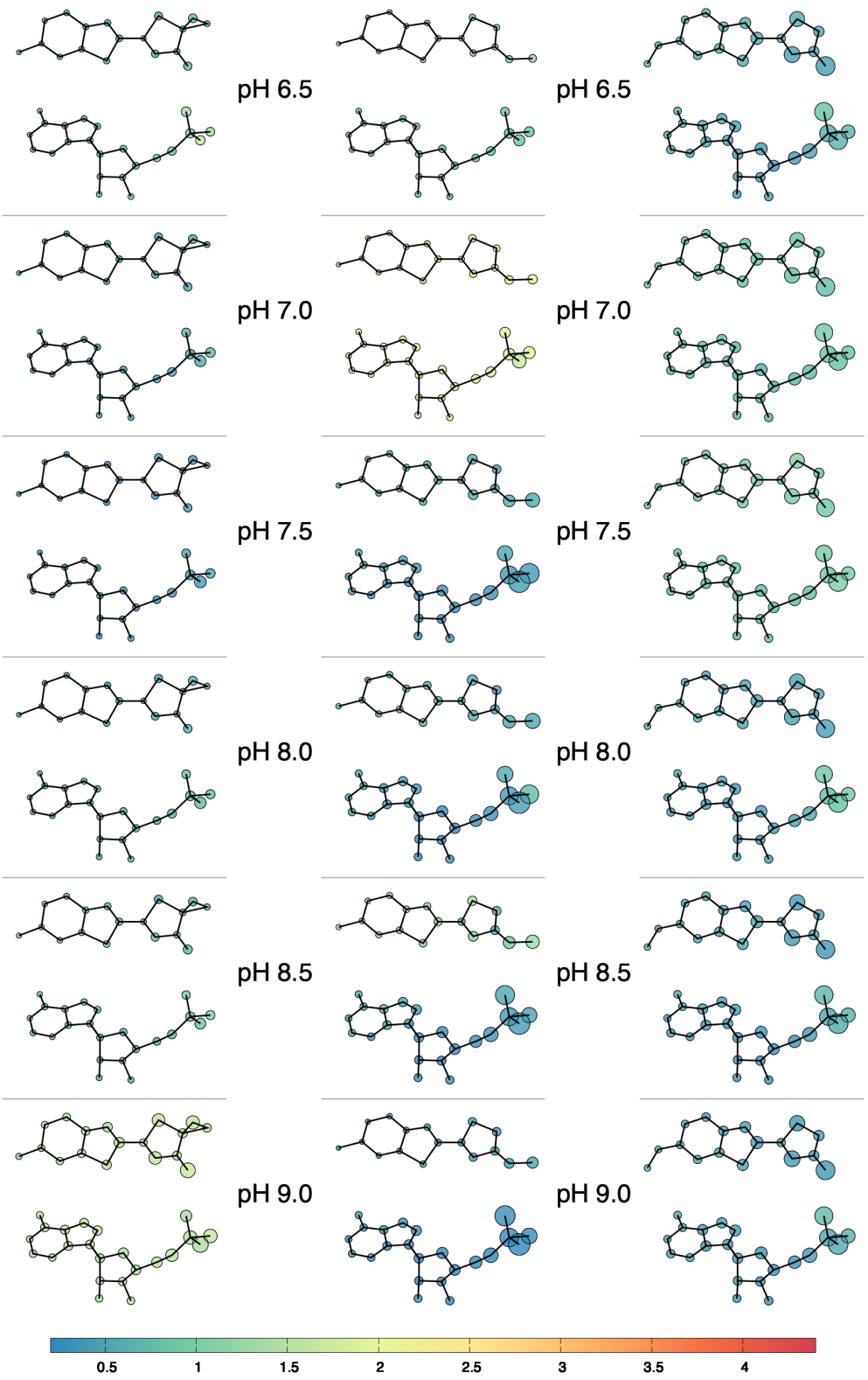

Figure S25: Distance distribution between analogue/AMP2 and residue H245

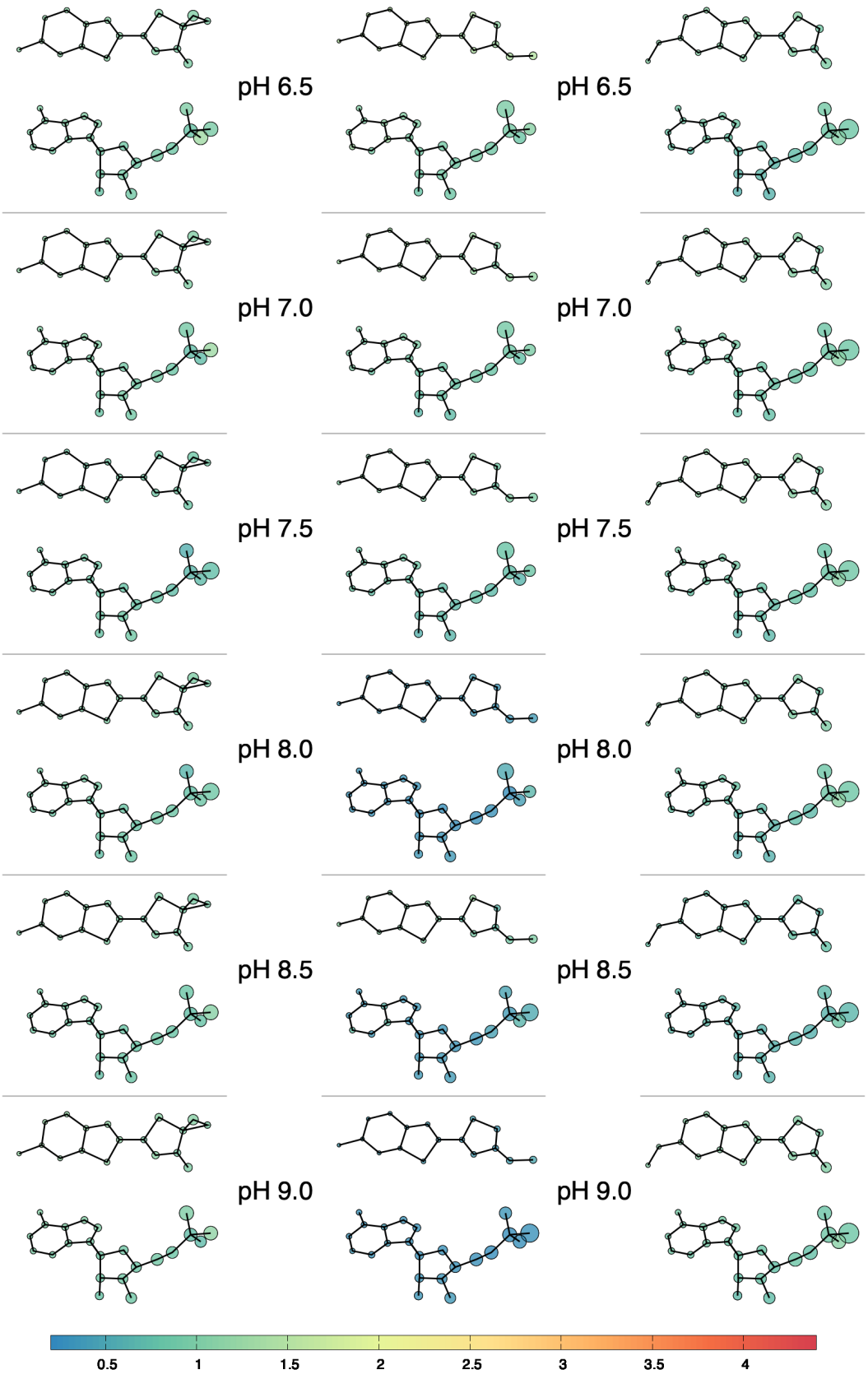

Figure S26: Distance distribution between analogue/AMP2 and residue E344

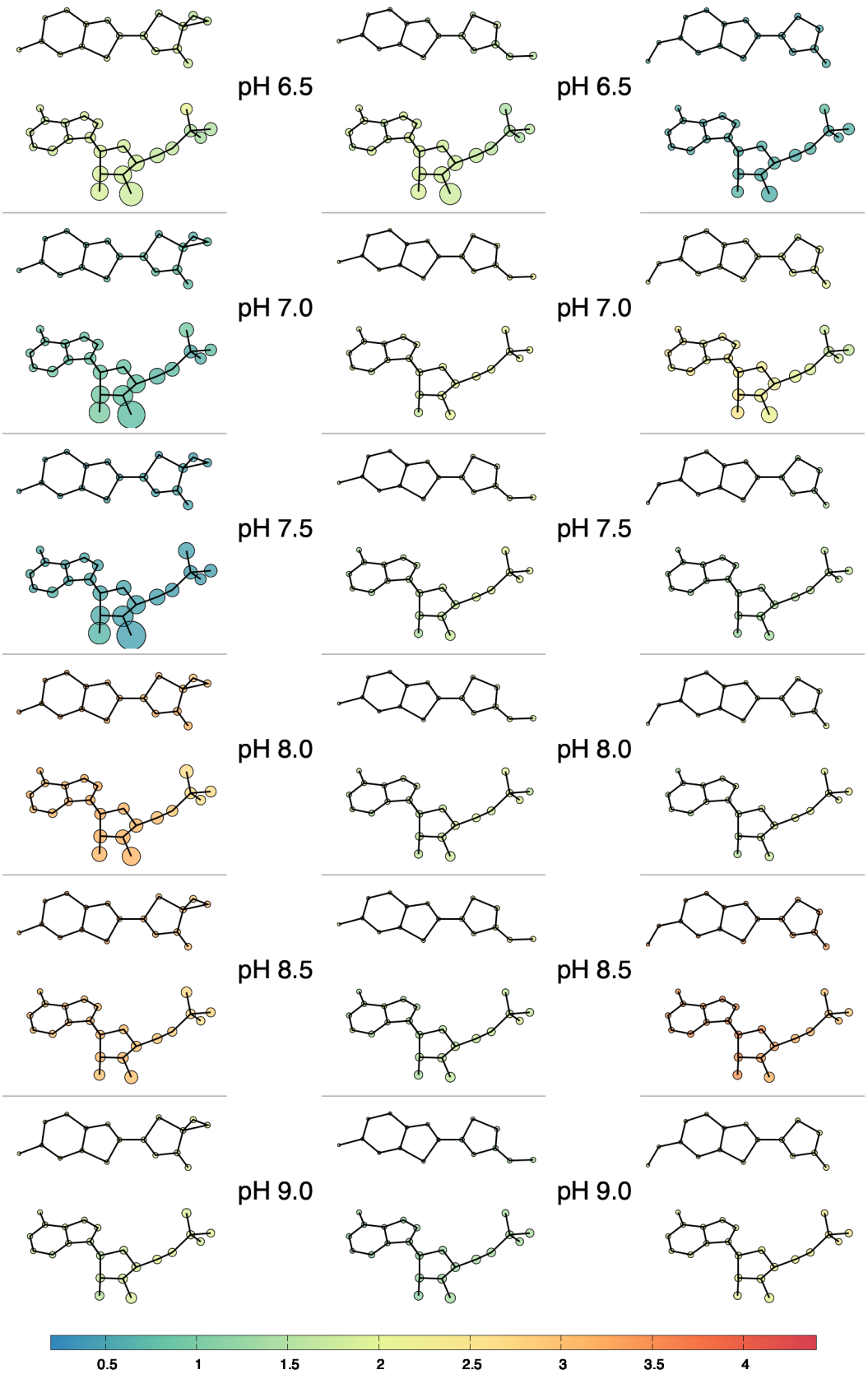

Figure S27: Distance distribution between analogue/AMP2 and residue K439

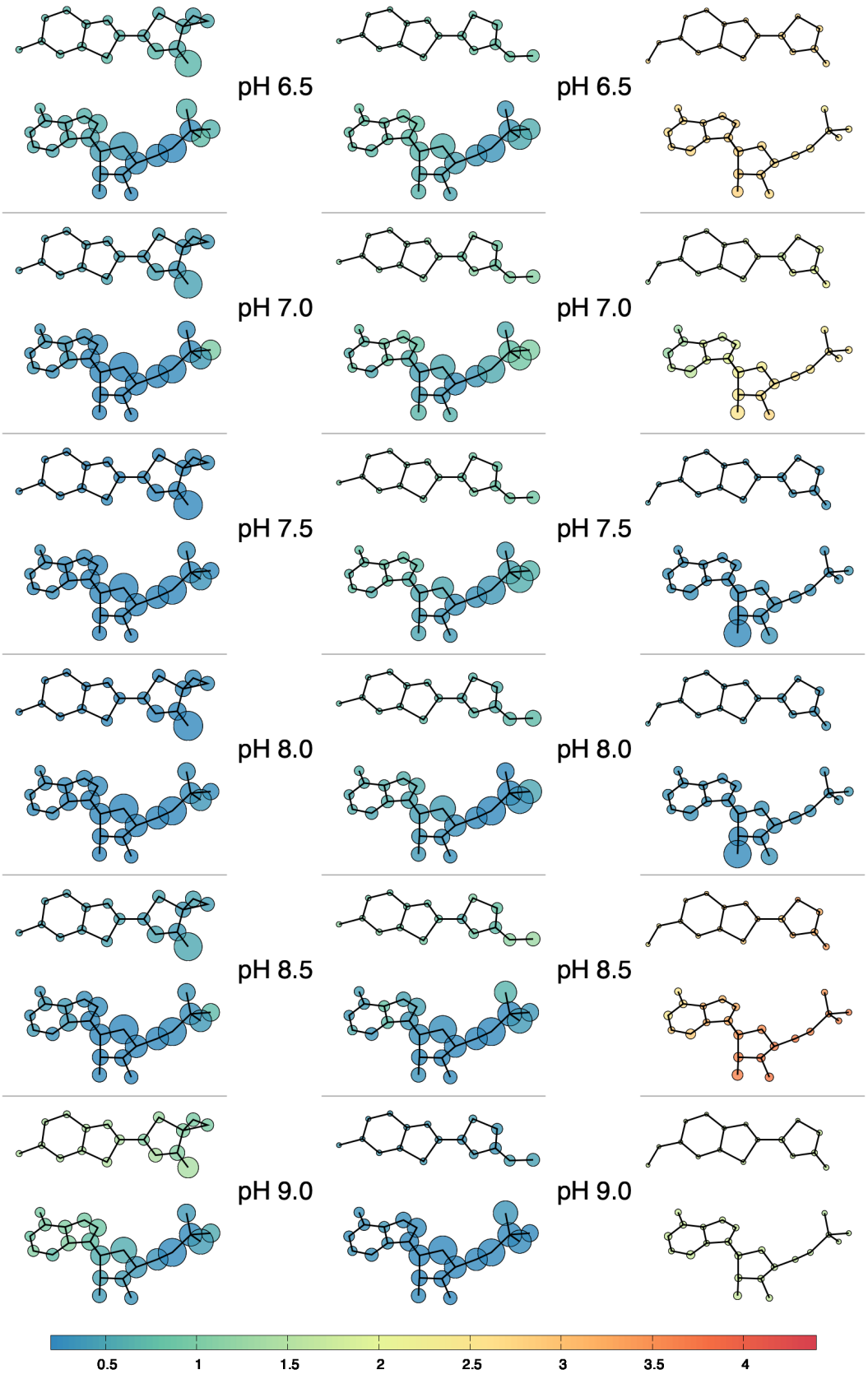

Figure S28: Distance distribution between analogue/AMP2 and residue K443

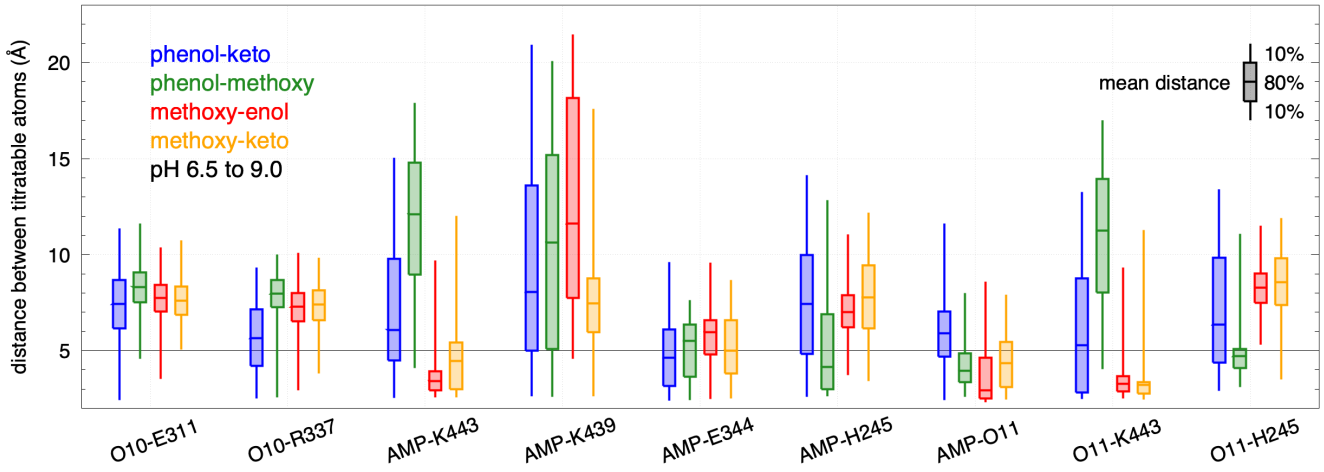

Figure S29: Candle plot of the distance distributions. Rectangles correspond to 80% of the distribution. Lines above and below show the tails of each distribution. Mean distances are also reported in each rectangle. A horizontal line at 5Å, corresponding to the cutoff value used in the main analysis, is included for a better comparison with the main text. For each analogue, O11 (resp. O10) indicate the oxygen atom close to (resp. far from) AMP.

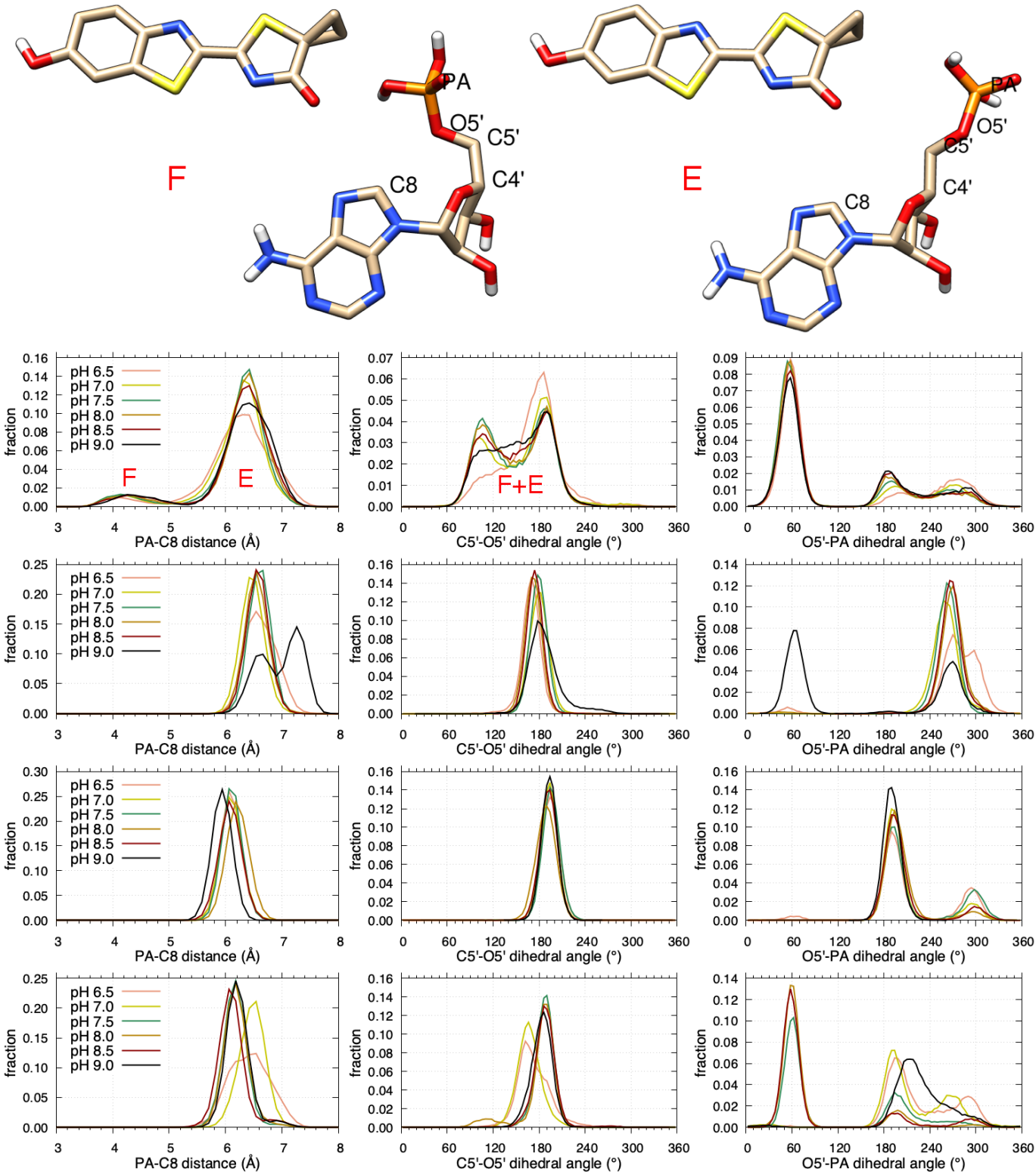

Figure S30: Normalized distribution of C8-PA AMP distances (in Å), C5'-O5' dihedral angle (in degree) and O5'-PA dihedral angle (in degree). From top to bottom: phenol-keto, phenol-methoxy, methoxy-enol, methoxy-keto. The phenol-keto distance distribution reveals two conformers: a folded one (F) and an extended one (E). The transition from one conformer to the other is driven by a rotation arround the C5'-O5' bond. The uni-modal to tri-modal distribution associated to the rotation around the O5'-PA bond reveals analogue-dependent and pH-dependent steric hindrance near the AMP phosphate group.

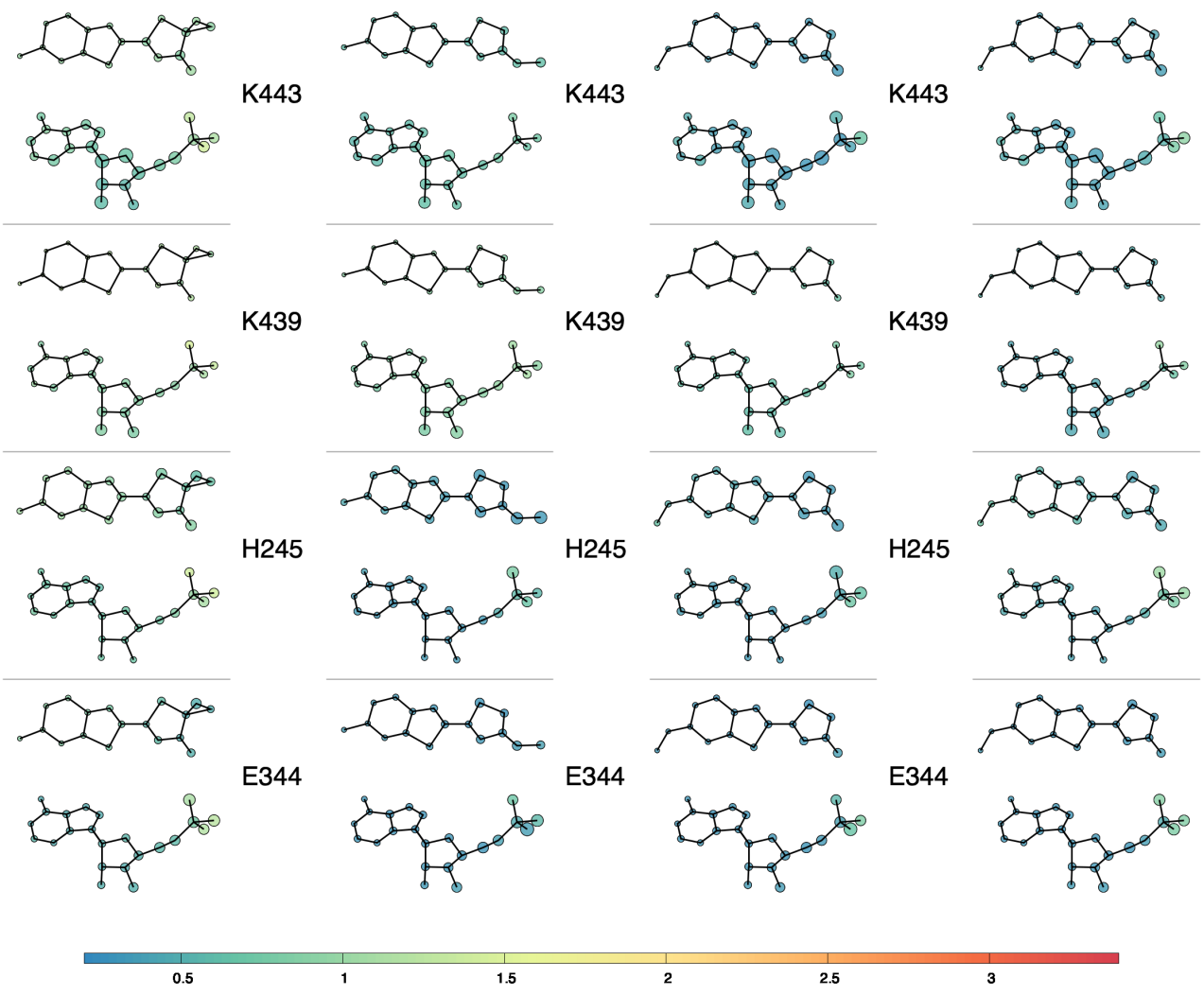

Figure S31: Analysis of the contacts between C_β of selected residues defining the luciferase cavity, and the analogue and AMP atoms.

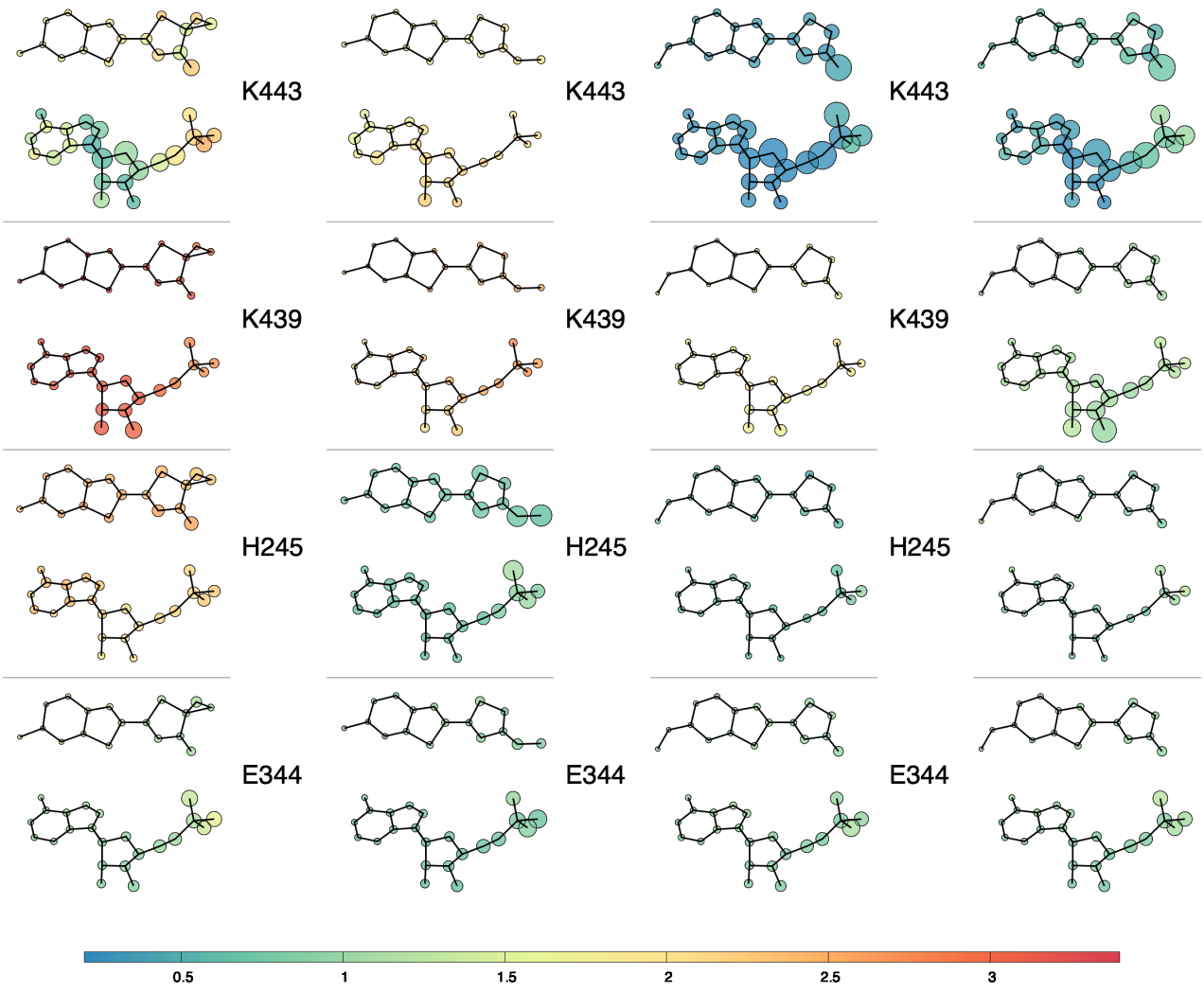

Figure S32: Analysis of the contacts between side-chain terminal atoms of selected residues defining the luciferase cavity, and the analogue and AMP atoms.

6 TDDFT spectrum for phenol-keto analogue in acidic conditions

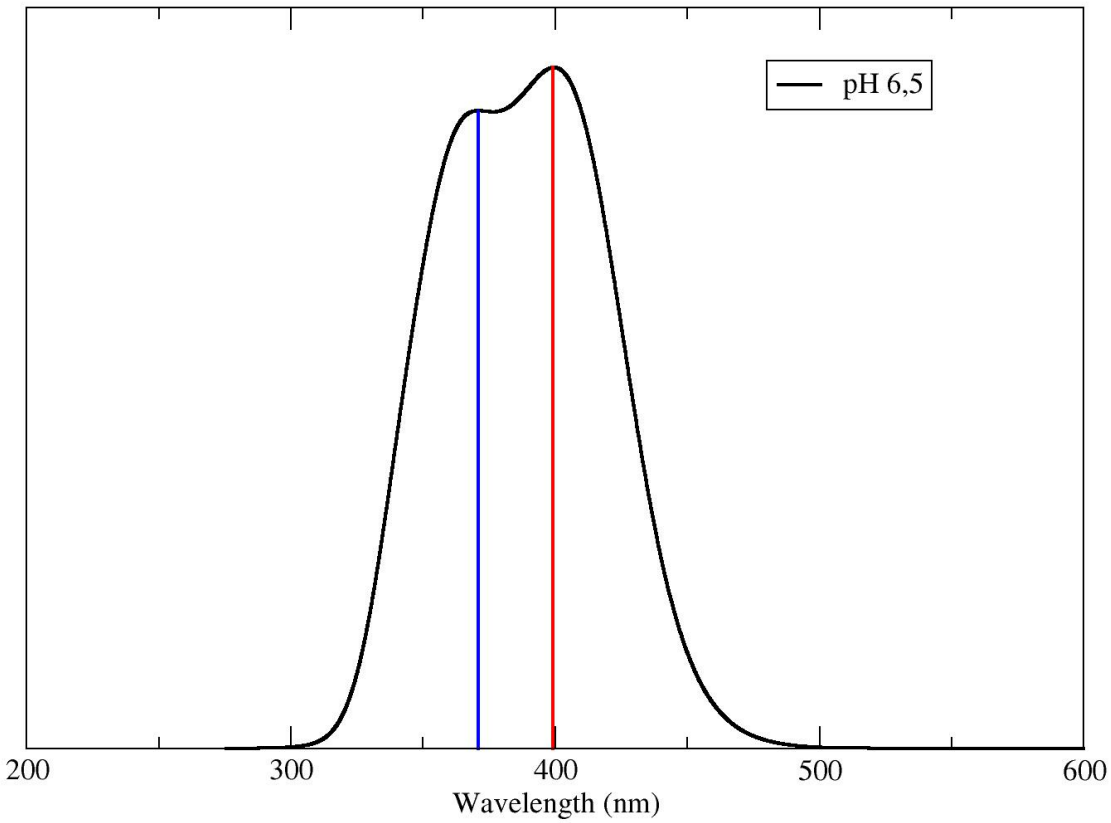

Figure S33: Absorption spectrum of the phenol-keto analogue in luciferase, at pH=6.5, using 1000 structures extracted from the corresponding CpHMD trajectory. Sticks indicate the average maximum absorption wavelengths.