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Supporting Information: Extended-sampling QM/MM simulation of biochemical reactions involving P–N bonds

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Table S1: Cartesian coordinates (Å) of the molecules P-N-product and P-N-intermediate, as used to generate the repulsive potential with *erepfit*. The structures were obtained from energy minimizations on the level B3LYP/aug-cc-pVTZ in vacuo.

PN-product

	x	y	z
C	−1.0760	1.2296	0.0
N	−0.2002	0.1559	0.0
C	−0.9982	−0.9869	0.0
C	−2.2823	−0.5390	0.0
N	−2.3187	0.8430	0.0
P	1.4965	0.2653	0.0
O	1.9723	−0.6759	−1.1922
O	1.9196	1.6662	0.0
O	1.9723	−0.6759	1.1922
H	−0.7121	2.2442	0.0
H	−0.5751	−1.9763	0.0
H	−3.1869	−1.1254	0.0
H	1.9944	−0.2124	2.0395
H	1.9944	−0.2124	−2.0395

PN-intermediate

	x	y	z
C	−2.7736	1.7845	0.0
C	−1.4192	1.5932	0.0
N	−1.2147	0.2152	0.0
C	−2.4710	−0.3281	0.0
N	−3.4219	0.5781	0.0
P	0.4567	−0.5869	0.0
O	−0.3169	−1.9890	0.0
O	0.8191	0.1611	1.3790
O	0.8191	0.1611	−1.3790
H	0.0896	0.6936	−1.7194
H	0.0896	0.6936	1.7194
H	−2.6281	−1.3922	0.0
H	−0.6037	2.2985	0.0
H	−3.3120	2.7190	0.0
O	1.8977	−1.3902	0.0
C	3.1649	−0.7384	0.0
H	3.9201	−1.5233	0.0
H	3.2862	−0.1220	−0.8925
H	3.2862	−0.1220	0.8925
H	0.3320	−2.7058	0.0

Table S2: The length of the P–N bond (in Å) in phosphohistidine resulting from geometry optimization performed on various DFT levels, with aqueous environment represented with the polarizable continuum model (PCM). The molecule of phosphohistidine is protonated at the histidine moiety, with a completely deprotonated phosphate group, which is the state appearing as P in the PMF in Fig. 4 (main text).

Basis set	B3LYP	PBE
aug-cc-pVTZ	1.91	1.93
SV	1.98	2.02
Huzinaga-MINI	2.13	2.23

Table S3: Cartesian coordinates (Å) of selected representative structures corresponding to the three significant states observed in the 2D QM/MM multiple walker metadynamics simulation of the analog of histidine phosphorylation performed with DFTB3/3OB-OPhyd-PNmod, leading to the PMF in Fig. 4C (main text).

Reactant (R)

	x	y	z
C	17.53	16.46	17.90
N	16.40	15.69	17.92
C	16.31	15.00	16.76
H	15.55	14.31	16.42
N	17.30	15.37	15.93
C	18.10	16.27	16.63
H	19.00	16.72	16.21
H	15.76	15.63	18.70
O	15.27	14.44	12.93
H	12.30	14.31	11.24
O	13.76	15.35	14.85
O	12.76	13.63	13.17
C	12.11	14.52	12.31
P	14.21	14.15	14.04
H	11.01	14.51	12.47
H	12.42	15.54	12.56
O	14.66	12.89	14.85
H	17.65	17.11	18.76

Product (P)

	x	y	z
C	18.46	18.02	15.89
N	18.42	17.46	17.18
C	17.57	16.39	17.13
H	17.32	15.74	17.91
N	17.11	16.31	15.86
C	17.63	17.27	15.07
H	17.42	17.32	14.04
H	19.01	17.77	17.88
O	14.81	15.52	16.27
H	11.78	11.29	12.09
O	16.60	13.75	15.59
O	12.80	11.96	13.84
C	11.72	11.74	13.09
P	15.96	15.10	15.32
H	11.00	11.04	13.50
H	11.11	12.65	12.83
O	15.70	15.55	13.87
H	18.96	18.97	15.61

Transition State (TS)

	x	y	z
C	17.27	18.25	15.05
N	17.64	17.29	15.98
C	16.70	16.32	15.87
H	16.67	15.39	16.50
N	15.72	16.58	15.05
C	16.09	17.78	14.44
H	15.45	18.25	13.66
H	18.36	17.42	16.64
O	13.17	15.74	15.90
H	11.65	10.98	14.16
O	14.94	13.90	15.44
O	12.01	13.01	14.57
C	12.21	11.72	14.82
P	14.07	15.02	14.88
H	13.33	11.46	14.97
H	11.69	11.44	15.84
O	13.91	15.26	13.36
H	17.93	19.02	14.82

Table S4: Cartesian coordinates (Å) of the states R, TS and P1 observed in the first step of the hydrolysis of TEPA, after energy minimization performed on the level B3LYP/aug-cc-pVTZ.

TEPA-R

	x	y	z
O	12.5020	14.5650	13.1404
P	13.3396	13.5874	13.8706
N	12.0808	12.4495	14.5743
C	12.3541	11.0976	15.1531
C	11.8735	12.1699	16.0351
N	14.1560	13.9844	15.2451
C	14.1294	15.3387	15.8480
C	15.3912	14.8053	15.2977
N	14.2848	12.4817	13.0882
C	14.1538	12.2379	11.6297
C	15.3809	12.8637	12.1561
H	11.6758	10.3249	14.8228
H	13.4044	10.8480	15.1559
H	10.8386	12.1916	16.3435
H	12.5854	12.6765	16.6672
H	13.9677	15.3395	16.9172
H	13.6247	16.1139	15.2876
H	16.1418	14.4063	15.9652
H	15.7403	15.2336	14.3688
H	13.4628	12.8662	11.0828
H	14.1583	11.1903	11.3634
H	15.5280	13.9191	11.9674
H	16.2717	12.2650	12.2838
O	14.7619	16.1636	12.3328
H	13.8007	16.0732	12.2822
H	14.9905	16.9588	11.8412
H	11.2177	12.5306	14.0442

TEPA-TS

	x	y	z
O	−1.3109	1.5157	−1.02508
P	0.0270	1.0092	−0.70064
N	−1.3326	−0.8311	0.50702
C	−1.4679	−2.2497	0.91936
C	−1.8623	−1.1782	1.85073
N	0.9050	1.4605	0.59287
C	0.4221	1.7370	1.95136
C	1.4107	0.6581	1.71502
N	0.8992	0.0011	−1.61651
C	1.2339	−1.4250	−1.55163
C	0.4444	−0.8883	−2.68820
H	−2.2380	−2.8344	0.43605
H	−0.5365	−2.7721	1.08983
H	−2.9141	−0.9959	2.02072
H	−1.2170	−0.9401	2.68401
H	−0.5982	1.4624	2.17630
H	0.7550	2.6849	2.35076
H	1.0693	−0.3650	1.77803
H	2.4547	0.8244	1.94165
H	0.7158	−2.0172	−0.81239
H	2.2857	−1.6367	−1.68582
H	−0.6131	−1.1130	−2.73035
H	0.9281	−0.7155	−3.64000
O	0.8609	2.6845	−1.88690
H	0.1545	3.3268	−2.03970
H	1.6298	3.1582	−1.54477
H	−2.1054	−0.5606	−0.09169

TEPA-P1

	x	y	z
O	12.2151	14.6929	13.3389
P	13.6354	14.3833	13.5473
N	12.1757	12.3358	15.1707
C	11.7900	10.9248	15.4099
C	11.7622	11.8905	16.5224
N	14.4724	14.8294	14.8711
C	13.9528	15.0282	16.2332
C	14.9900	14.0030	15.9763
N	14.5255	13.3051	12.7134
C	14.6107	11.8384	12.8682
C	14.0126	12.4218	11.6475
H	10.8505	10.5859	14.9958
H	12.5995	10.2096	15.3496
H	10.8021	12.2275	16.8882
H	12.5509	11.8701	17.2626
H	12.9445	14.6903	16.4226
H	14.2324	15.9737	16.6765
H	14.6782	12.9690	15.9911
H	16.0217	14.2045	16.2286
H	13.9487	11.3862	13.5913
H	15.6242	11.4633	12.8353
H	12.9386	12.3671	11.5278
H	14.5901	12.4617	10.7345
O	14.2862	15.8786	12.4214
H	13.5834	16.5387	12.3090
H	15.1039	16.3116	12.7109
H	11.4131	12.8332	14.7235

Table S5: Cartesian coordinates (Å) of the states R and P1 observed in the first step of the hydrolysis of TEPA, after energy minimization performed with DFTB3/3OB-OPhyd-PNmod. (Energy minimization of state TS was not performed.)

TEPA-R

	x	y	z
O	12.2937	14.5736	13.1267
P	13.2403	13.6703	13.8135
N	12.0424	12.3919	14.5990
C	12.4545	11.0978	15.1638
C	11.9051	12.1534	16.0444
N	14.0413	14.0871	15.2136
C	14.1782	15.4797	15.7055
C	15.3806	14.7214	15.2974
N	14.1926	12.5445	13.0238
C	14.1494	12.3475	11.5527
C	15.3736	12.8839	12.1847
H	11.8307	10.2529	14.8802
H	13.5283	10.9232	15.1489
H	10.8796	12.0791	16.3995
H	12.5881	12.7352	16.6609
H	13.9496	15.6020	16.7644
H	13.8017	16.2616	15.0473
H	16.0261	14.2838	16.0594
H	15.8626	14.9708	14.3533
H	13.5047	13.0231	10.9908
H	14.1137	11.3021	11.2468
H	15.6030	13.9435	12.0729
H	16.2258	12.2239	12.3464
O	14.6240	15.9701	12.5238
H	13.7045	16.1152	12.2695
H	15.1728	16.6822	12.1809
H	11.1498	12.3626	14.0943

TEPA-P1

	x	y	z
O	0.1440	1.6877	−1.3743
P	0.0917	0.4353	−0.6244
N	−1.1872	−0.5299	−0.5749
C	−2.6242	−0.3120	−0.3878
C	−1.8987	−1.3143	0.4378
N	1.3838	−0.2444	0.0437
C	2.0172	−1.5526	0.1912
C	2.8101	−0.3669	−0.2440
H	−3.2455	−0.6494	−1.2169
H	−2.9124	0.6395	0.0618
H	−1.9983	−2.3750	0.2056
H	−1.6650	−1.0798	1.4779
H	1.7395	−2.3112	−0.5405
H	2.1049	−1.9055	1.2205
H	3.1032	−0.2735	−1.2908
H	3.4665	0.1302	0.4718
O	−0.5541	1.6122	1.5631
H	−0.8366	2.5224	1.4189
H	−0.0877	1.5531	2.4038
H	2.0104	−2.5907	−3.8011
N	0.4001	−1.1881	−4.1958
C	1.7878	−1.6655	−4.3370
H	0.4312	−2.6337	−5.8218
H	0.3704	−0.1695	−4.2068
C	0.8699	−1.6918	−5.5064
H	2.5773	−0.9157	−4.3031
H	1.0180	−0.9604	−6.2955

Table S6: Cartesian coordinates (Å) of selected representative structures corresponding to the four significant states observed in the 2D QM/MM multiple walker metadynamics simulation of the two-step hydrolysis of TEPA performed with DFTB3/3OB-OPhyd-PNmod, leading to the PMF in Fig. 7 (main text).

TEPA-R

	x	y	z
O	14.27	15.91	11.67
P	14.97	15.81	13.03
N	15.07	17.55	13.58
C	16.25	18.45	13.40
C	14.99	18.63	12.65
N	14.00	15.23	14.32
C	13.98	13.96	15.07
C	12.85	14.40	14.24
N	16.58	15.35	13.09
C	17.07	14.04	13.35
C	17.17	14.59	12.01
H	17.08	17.92	12.92
H	16.54	18.94	14.26
H	14.95	18.34	11.62
H	14.29	19.43	12.99
H	14.00	14.01	16.20
H	14.61	13.22	14.74
H	12.06	14.88	14.75
H	12.57	14.05	13.26
H	16.33	13.23	13.53
H	17.85	13.90	14.08
H	16.46	14.04	11.29
H	18.08	14.99	11.54
O	14.66	12.25	12.47
H	14.38	11.91	11.61
H	14.85	11.63	13.20
H	14.52	17.66	14.51

TEPA-P1

	x	y	z
O	14.45	15.69	11.65
P	14.97	15.05	12.84
N	15.35	17.86	13.42
C	16.00	18.97	12.84
C	14.47	18.84	12.94
N	14.02	15.15	14.19
C	13.82	14.31	15.35
C	12.67	14.53	14.38
N	16.58	14.88	13.11
C	17.39	13.93	13.84
C	17.53	14.11	12.35
H	16.54	18.73	11.93
H	16.58	19.73	13.51
H	13.79	18.60	12.07
H	14.02	19.50	13.70
H	13.69	14.82	16.33
H	14.38	13.34	15.32
H	11.99	15.40	14.67
H	12.30	13.83	13.65
H	16.81	13.03	14.12
H	18.15	14.38	14.50
H	16.98	13.43	11.65
H	18.28	14.65	11.91
O	14.56	12.73	12.10
H	15.24	12.35	11.59
H	14.02	11.99	12.45
H	15.47	17.79	14.38

TEPA-I

	x	y	z
O	15.88	14.03	15.39
P	15.45	14.50	14.01
N	14.69	18.01	12.87
C	15.40	18.63	11.80
C	14.57	17.46	11.42
N	13.88	15.04	13.69
C	13.21	15.88	14.69
C	12.76	14.50	14.50
N	15.91	13.67	12.70
C	17.12	13.43	11.97
C	15.94	13.88	11.25
H	16.53	18.48	11.71
H	15.06	19.64	11.38
H	15.05	16.50	11.15
H	13.57	17.58	11.02
H	12.56	16.69	14.27
H	13.69	16.14	15.64
H	11.86	14.34	13.99
H	13.10	13.70	15.18
H	17.48	12.47	11.85
H	17.87	14.24	12.09
H	15.33	13.09	10.78
H	15.80	14.92	10.94
O	16.34	16.11	13.76
H	17.24	16.35	14.09
H	15.73	17.03	13.69
H	13.82	18.41	13.10

TEPA-P2

	x	y	z
O	16.23	15.83	14.61
P	15.67	14.62	13.93
N	15.64	18.25	11.66
C	15.65	19.46	12.54
C	15.11	18.17	13.02
N	14.19	14.21	14.68
C	12.90	15.01	14.65
C	12.99	13.62	14.16
N	15.28	14.93	12.39
C	16.10	14.38	11.09
C	14.79	13.79	11.54
H	14.96	20.20	12.08
H	16.65	19.75	12.79
H	14.11	17.82	13.28
H	15.79	17.51	13.58
H	12.87	15.88	14.02
H	12.44	15.21	15.62
H	12.94	13.32	13.06
H	12.67	12.80	14.77
H	17.09	14.00	11.33
H	16.10	15.14	10.33
H	14.73	12.74	12.01
H	13.85	14.12	11.15
O	16.48	13.26	14.03
H	16.65	12.58	14.73
H	15.09	18.36	11.14
H	16.51	17.74	11.46