Supporting Information for :

Resolving uncertainties about phosphate hydrolysis pathways by careful free energy mapping

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I. Additional information about the reaction energetics

Table S1: The relative free energy (in kcal/mol) values for the hydrolysis of monomethyl pyrophosphate trianion in water. The data presented here have been utilized to generate the free energy surfaces presented of Figs. 7 and 8 of the main text. The surface was scanned in the gas phase (B3LYP/6-31+G*) as a function of R_1 and R_2 for different values of X, where the system was constrained at specific R_1, R_2, and X values and the energy is minimized with regard to all other coordinates. This is followed by a single point IEFPCM solvation calculation at the B3LYP/6-311++G** level, for each of the scanned points, to gauge the effect of solvent . The least energy paths start from the lower left corner of the lower panel and proceeds through the highlighted boxes . The intermediate panels correspond to the various proton transfer (PT) coordinates as depicted in Figs. 7 and 8 of the main text. The upper most panel corresponds to the product state. The minimum free energy paths corresponding to the associative, and associative/concerted mechanisms have been highlighted.

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.65 Å	47.50	40.85	35.18	31.62	19.27	8.15	-3.20	-6.14	-10.32	-13.42	-9.12	-8.65
1.8 Å	39.84	34.26	32.32	29.68	28.13	21.08	16.17	-2.25	-5.13	-8.03	-6.19	-5.08
1.9 Å	37.65	33.25	31.09	31.06	27.35	24.53	21.02	19.38	9.17	4.64	1.72	0.62
2.0 Å	37.08	33.91	32.01	33.17	28.19	29.46	27.12	20.12	11.32	9.52	6.17	1.37
2.1 Å	36.64	33.82	32.13	36.39	36.12	34.44	29.16	21.77	15.07	17.57	15.23	14.36
2.2 Å	34.45	32.37	36.39	35.39	37.01	39.95	39.01	38.17	34.32	32.39	20.12	27.12
2.4 Å	31.43	30.93	34.63	40.53	44.32	45.99	41.91	42.67	41.97	41.85	40.93	40.22
2.6 Å	27.32	26.93	33.79	38.03	45.23	46.68	47.21	48.12	43.17	44.17	46.71	50.07
2.8 Å	20.64	22.08	31.19	37.08	44.32	48.44	50.17	53.77	51.19	47.81	53.72	55.97
3.0 Å	15.37	18.82	29.23	34.88	42.60	47.12	49.74	52.77	53.17	55.22	55.37	55.91

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.8 Å	59.17	58.61	57.16	49.02	39.04	38.40	36.29	35.21	32.07	28.27	24.02	23.90
1.9 Å	51.23	50.99	50.73	45.20	40.92	39.29	38.92	38.04	37.91	34.32	30.07	25.27
2.0 Å	47.80	46.92	46.12	44.19	42.19	41.30	40.17	39.97	39.32	36.13	33.12	28.32
2.1 Å	45.92	44.23	40.28	43.91	44.70	44.03	41.71	42.91	41.12	40.16	37.12	29.17
2.2 Å	42.32	38.90	38.08	41.79	44.71	45.93	43.29	42.76	38.31	39.21	36.23	30.23

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.8 Å	64.12	59.30	52.32	50.92	49.72	47.31	47.99	48.91	46.31	45.13	41.92	36.64
1.9 Å	56.12	51.70	49.12	47.50	47.60	45.29	48.13	39.99	43.29	42.23	40.75	38.65
2.0 Å	50.29	46.74	44.61	46.17	46.44	45.03	43.29	46.16	44.71	43.76	41.58	40.85
2.1 Å	46.20	44.32	40.97	44.67	45.12	44.93	42.17	43.32	42.09	41.61	40.91	40.28
2.2 Å	41.23	38.10	37.33	41.12	43.92	44.17	42.92	41.67	40.13	39.92	39.01	38.27

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.8 Å	68.12	61.03	54.23	52.09	51.27	49.13	47.62	46.19	45.13	44.31	42.91	41.03
1.9 Å	56.22	51.07	50.17	48.50	48.06	45.99	48.31	40.49	44.92	43.23	41.28	38.02
2.0 Å	50.13	45.74	44.48	45.11	45.44	44.30	42.92	45.61	44.17	42.67	40.85	36.89
2.1 Å	44.80	41.16	39.46	42.60	43.73	44.11	41.72	44.80	43.54	42.61	40.91	35.51
2.2 Å	39.72	36.70	34.63	40.22	41.97	42.60	41.03	40.47	39.59	38.96	37.65	33.29

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.8 Å	67.03	59.53	53.12	51.92	49.23	48.01	46.37	45.17	44.02	41.73	39.13	39.65
1.9 Å	55.47	50.26	49.21	49.18	46.74	46.43	45.93	44.11	42.60	40.72	37.27	35.70
2.0 Å	47.81	43.32	43.10	44.41	44.97	45.01	43.48	42.41	40.78	37.23	32.17	33.32
2.1 Å	41.16	37.58	37.65	38.71	39.78	41.03	41.85	40.59	38.65	34.32	33.12	31.87
2.2 Å	35.14	32.0	32.63	35.2	37.08	39.34	39.97	33.53	37.46	33.23	32.87	30.50

R_2 \ R_1	1.68 Å	1.8 Å	2.0 Å	2.2 Å	2.4 Å	2.6 Å	2.8 Å	3.0 Å	3.2 Å	3.4 Å	3.6 Å	3.8 Å
1.65 Å	81.82	74.10	66.09	64.94	60.21	56.78	53.55	50.70	48.29	46.68	44.23	41.03
1.8 Å	62.05	55.53	50.92	49.07	41.72	43.17	40.56	37.71	35.67	33.19	31.07	29.86
1.9 Å	54.71	48.06	44.42	42.98	40.33	38.40	56.83	33.57	31.78	29.80	27.32	26.60
2.0 Å	43.27	43.39	39.28	39.43	41.72	36.27	35.19	32.71	29.92	28.32	26.21	25.16
2.1 Å	40.22	37.39	37.21	38.27	38.08	34.51	32.13	30.43	28.12	27.35	25.12	24.4
2.2 Å	30.92	31.07	32.06	33.18	34.07	33.21	31.23	30.0	29.07	27.51	25.73	25.29
2.4 Å	20.58	20.14	23.15	27.35	28.04	31.24	30.92	30.05	29.33	27.54	26.32	25.79
2.6 Å	8.61	5.31	15.02	17.29	23.27	25.62	27.12	28.32	27.12	27.32	26.07	25.46
2.8 Å	2.44	3.51	9.28	15.24	19.7	23.65	24.12	25.79	25.04	26.29	26.00	25.03
3.0 Å	-2.19	-0.62	5.77	11.85	16.18	21.27	22.2	23.78	24.0	24.53	24.17	23.53

Tables S2 – S3: The tables explores the sensitivity of the computed activation free energy barriers for the hydrolysis of phosphate monoesters along the associative and associative/concerted pathways towards different quantum potentials, and different solvation models are presented. All the gas phase, and the COSMO optimizations were carried out at the B3LYP/6-31+G* level. "gas opt" and "cosmo opt" designate , respectively, that the initial geometries used for the single point solvent calculations are gas phase and cosmo optimized.

Table S2: The energetics of the key points on the PES computed using the B3LYP and MP2 potentials at 6-311++G** level. The cases were presented where the influence of the solvent has been included by the IEFPCM and COSMO continuum models. Also, the performance of the COSMO model has been verified on both gas phase and cosmo optimized initial geometries at lower level (B3LYP/6-31+G*).

Method / **System**	IEFPCM		COSMO		COSMO	
	B3LYP/ 6-311++G** (gas opt)	MP2/ 6-311++G** (gas opt)	B3LYP/ 6-311++G** (gas opt)	MP2/ 6-311++G** (gas opt)	B3LYP/ 6-311++G** (cosmo opt)	MP2/ 6-311++G** (cosmo opt)
RS	0	0	0	0	0	0
Assoc. TS (1W, PT)	34.64	33.64	35.02	33.95	33.33	34.14
Assoc/con. TS (1W, PT)	38.62	40.62	40.75	42.57	35.10	39.56

Table S3: Comparing the activation free energy barriers between the direct proton transfer (1W) and water assisted proton transfer (2W) pathways at the TS corresponding to the Asso/conc mechanism. All the energies were computed using the B3LYP and MP2 potentials at the 6-311++G** level and the solvent effect has been included by the IEFPCM and COSMO models. The performance of the COSMO model on various initial starting geometries are also presented.

Method / **System**	IEFPCM		COSMO		COSMO	
	B3LYP/ 6-311++G** (gas opt)	MP2/ 6-311++G** (gas opt)	B3LYP/ 6-311++G** (gas opt)	MP2/ 6-311++G** (gas opt)	B3LYP/ 6-311++G** (casmo opt)	MP2/ 6-311++G** (casmo opt)
Assoc/con.TS (1W, PT)	38.62	40.62	40.75	42.57	35.10	39.56
Assoc/con.TS (2W, PT)	35.24	37.19	38.13	40.07	33.05	37.69

II. The coordinates that correspond to the key characteristic points on the potential energy surface.

Reactant State

P	105.433999	82.085999	96.531998
O	106.398490	82.725058	97.608786
O	105.430325	80.563675	96.709727
O	105.694241	82.816354	95.192413
O	103.959098	82.701066	97.050418
P	102.357432	82.676475	96.926952
O	101.838691	83.281695	95.629965
O	101.730152	81.304811	97.181133
O	101.906709	83.729890	98.203538
O	108.322090	80.830690	95.545526
H	107.425599	80.622400	95.891784
H	108.386715	81.810502	95.660569
C	101.519996	83.179001	99.440002
H	102.393529	82.966469	100.083218
H	100.882807	83.920217	99.957952
H	100.963019	82.240960	99.299140
O	108.209498	83.506163	95.890731
H	107.395267	83.458296	95.315574
H	107.672801	83.313663	96.745333

Associative TS (1W, PT):

P	105.166391	81.804983	97.015889
O	105.871398	82.525440	98.193575
O	104.777511	80.360291	97.174164
O	105.412679	82.669090	95.663048
O	103.392809	82.691568	97.277372
P	101.865170	82.521956	96.879753
O	101.429017	83.182764	95.569158
O	101.296581	81.105746	97.031820
O	101.151445	83.506973	98.125358
O	107.161866	81.307134	96.234721
H	107.600473	81.807403	96.941351
H	106.485694	82.138750	95.630192
C	101.519996	83.179001	99.440002

H	102.613536	83.176235	99.562735
H	101.078633	83.935700	100.120094
H	101.138946	82.182640	99.735912
O	106.499465	84.852565	96.821206
H	105.982340	84.368064	96.137291
H	106.355749	84.176533	97.539010

Associative/Concerted TS (1W, PT):

P	106.431517	81.377495	95.765314
O	107.356767	81.865412	96.893364
O	105.697777	80.086304	95.913243
O	106.055953	82.517111	94.753587
O	102.997968	82.568980	96.874867
P	101.471139	82.763555	96.767450
O	100.939308	83.696346	95.655001
O	100.608219	81.482613	96.941460
O	101.066242	83.732899	98.248304
O	107.716370	80.980384	94.568879
H	108.500211	81.449594	94.919329
H	107.183008	81.744871	94.055138
C	101.519520	83.178696	99.440233
H	102.590776	82.911214	99.389071
H	101.381151	83.916205	100.266287
H	100.962503	82.257205	99.719776
O	108.289197	83.917254	95.338579
H	107.380376	83.804118	94.968242
H	108.146986	83.373278	96.156120

Associative TS (2W, PT)

P	104.938465	82.097511	96.639277
O	105.475124	83.129318	97.729096
O	104.934893	80.647366	97.083771
O	104.874120	82.762479	95.239875
O	103.036207	82.529280	97.080852
P	101.525020	82.095478	96.999869
O	100.774681	82.463742	95.712934
O	101.213747	80.665403	97.470664
O	100.853058	83.176911	98.205051

O	107.071444	81.799476	96.190305
H	107.224221	81.399118	97.058642
H	107.280817	83.035858	96.182202
C	101.519996	83.179001	99.440002
H	102.604292	83.315485	99.309103
H	101.116327	84.012941	100.052668
H	101.357656	82.235044	99.998060
O	107.029506	84.106429	96.155368
H	106.285139	83.943942	95.507672
H	106.440813	83.967909	96.951767

Associative / Concerted TS (2W, PT)

P	106.016127	82.019156	96.388027
O	106.231602	83.081683	97.538219
O	105.669401	80.627586	96.843043
O	105.647136	82.715912	95.064966
O	102.294532	82.732482	96.672443
P	100.880730	82.219508	97.021725
O	99.712596	82.585791	96.066833
O	100.798519	80.735154	97.468294
O	100.475401	83.155876	98.508997
O	107.668499	81.843856	96.028947
H	108.060577	81.418978	96.811211
H	107.971808	83.119597	95.948615
C	101.519996	83.179001	99.440002
H	102.485761	83.376073	98.945738
H	101.320983	83.977420	100.193013
H	101.614527	82.217663	99.989613
O	107.676232	84.257055	95.958076
H	106.900317	84.075257	95.321439
H	107.122103	84.085679	96.772675