Supplemental Information

A comparative study of ATP analogs for Kinase-catalyzed Photo-crosslinking

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Table of Contents:

III. Characterization of compounds

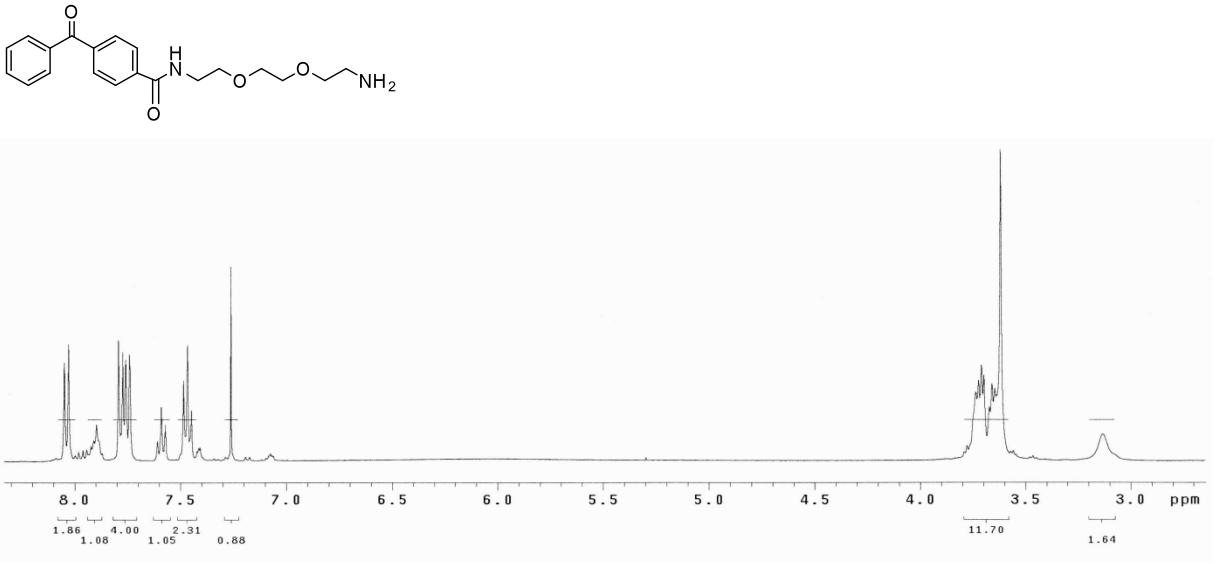


Figure S1: ^{1}H-NMR of compound **6** recorded in $CDCl_3$ solvent.

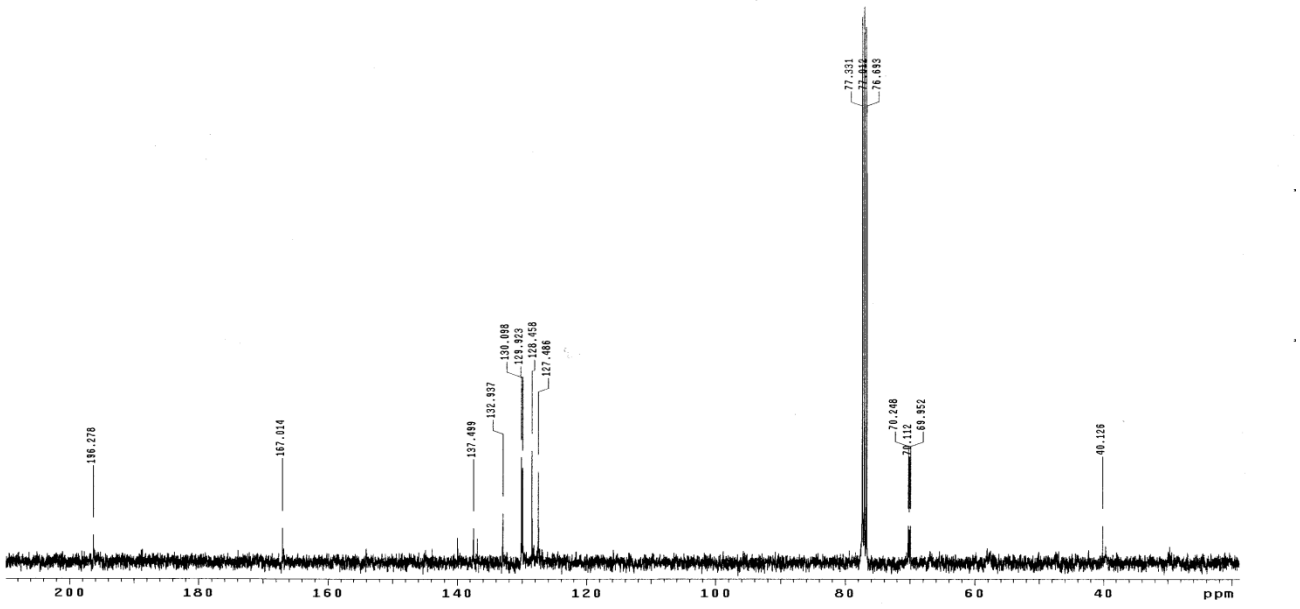
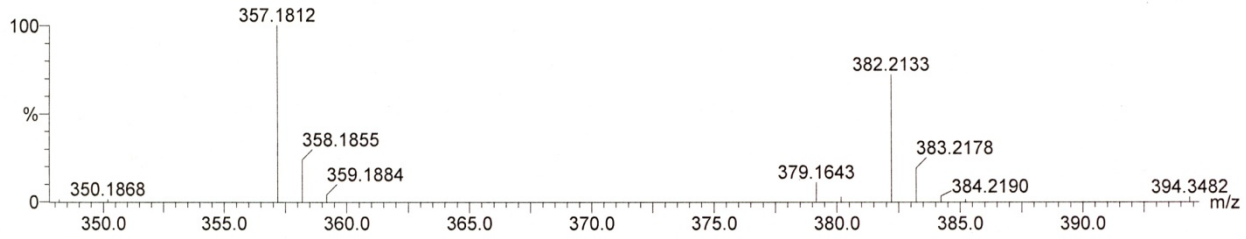


Figure S2: ^{13}C-NMR of compound **6** recorded in $CDCl_3$ solvent.



Minimum:				-1.5			
Maximum:		50.0	5.0	150.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
357.1812	357.1814	-0.2	-0.6	9.5	38.6	2.6	C20 H25 N2 O4
	357.1804	0.8	2.2	11.5	36.5	0.5	C19 H22 N6 23Na
	357.1822	-1.0	-2.8	-1.5	39.6	3.5	C7 H26 N8 O7 23Na
	357.1828	-1.6	-4.5	14.5	37.3	1.3	C21 H21 N6

Figure S3: HR-MS of compound **6** recorded with methanol solvent.

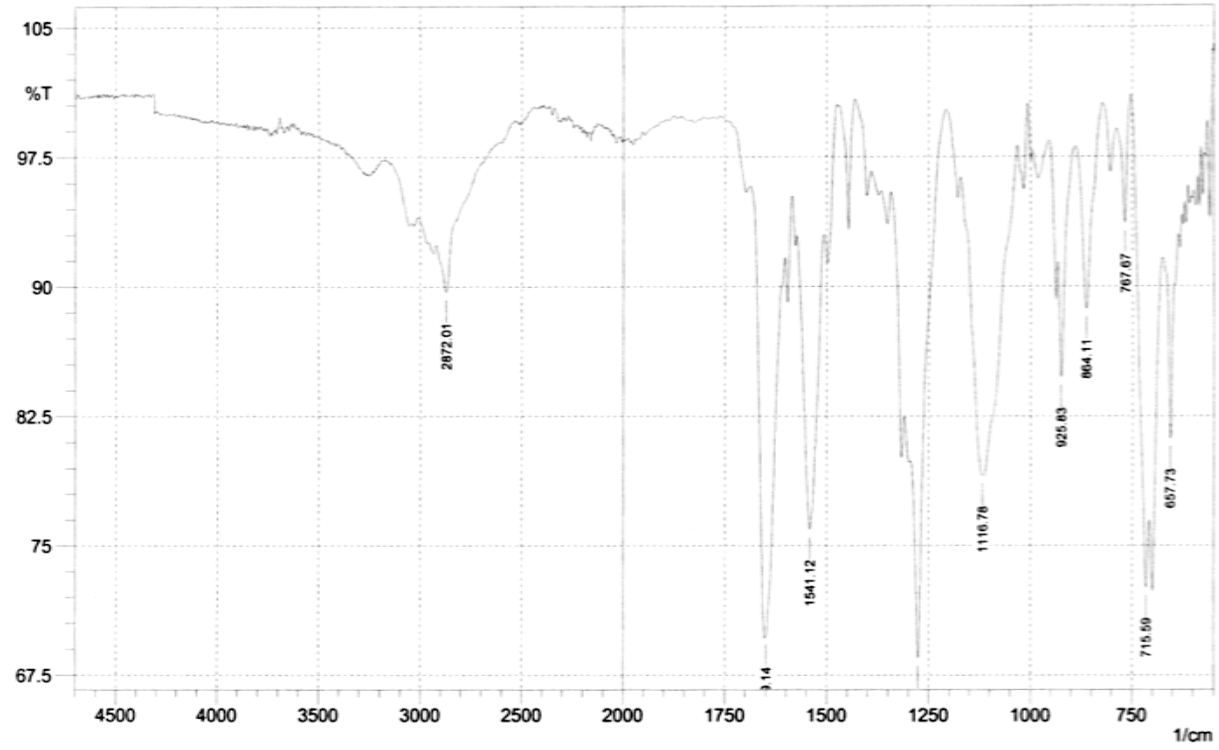
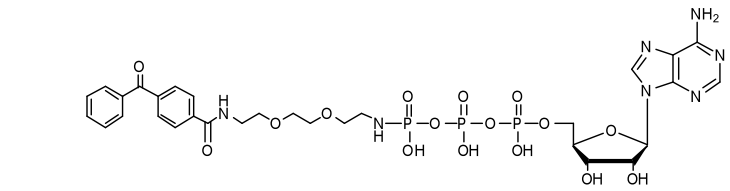


Figure S4: IR spectrum of compound **6**.



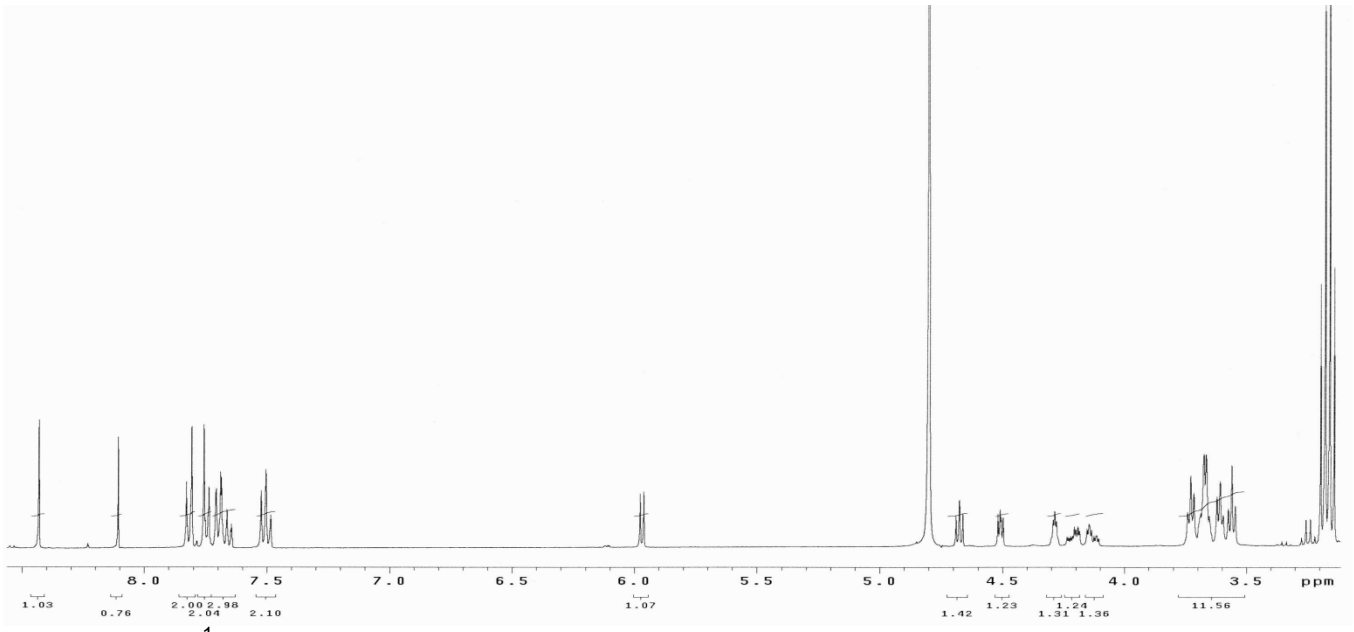


Figure S5: ^{1}H-NMR of ATP-BP **3** recorded in D_2O solvent.

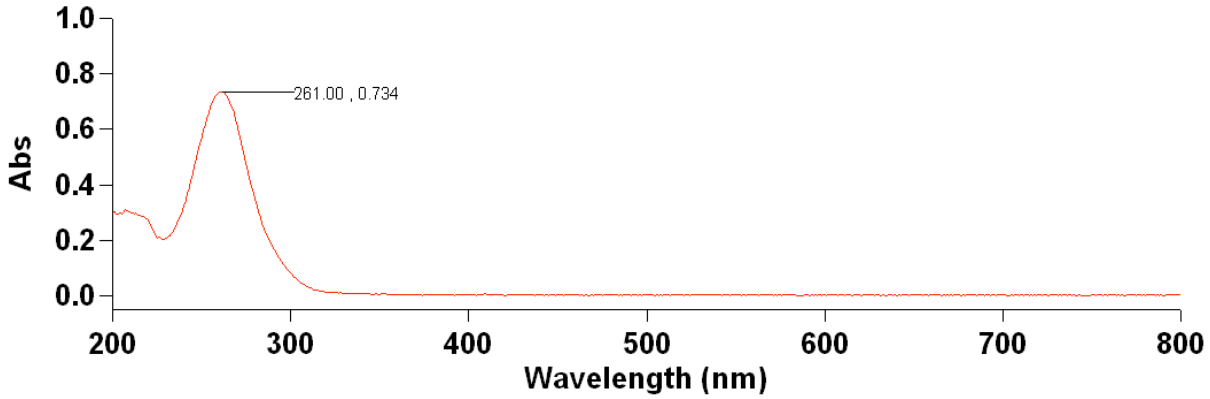


Figure S6: UV absorbance of ATP-BP **3**.

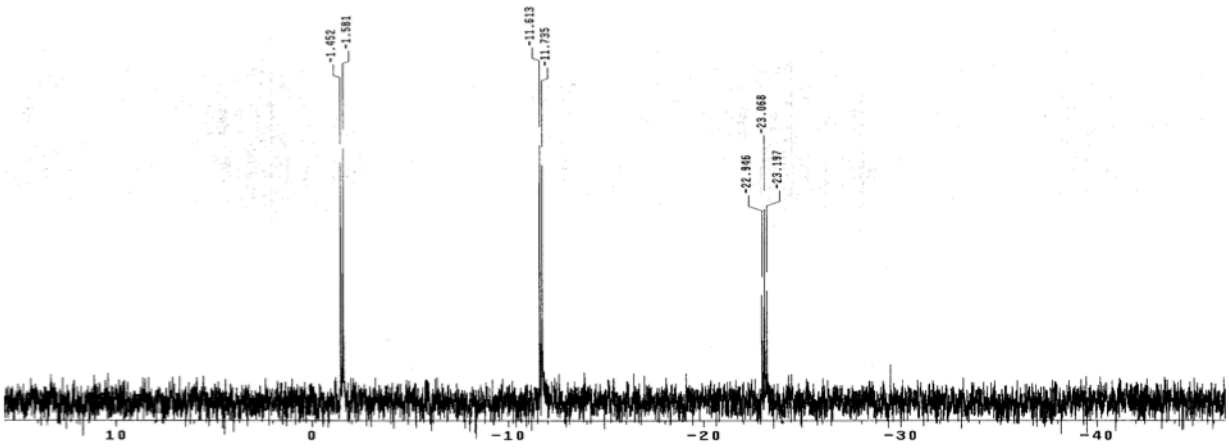
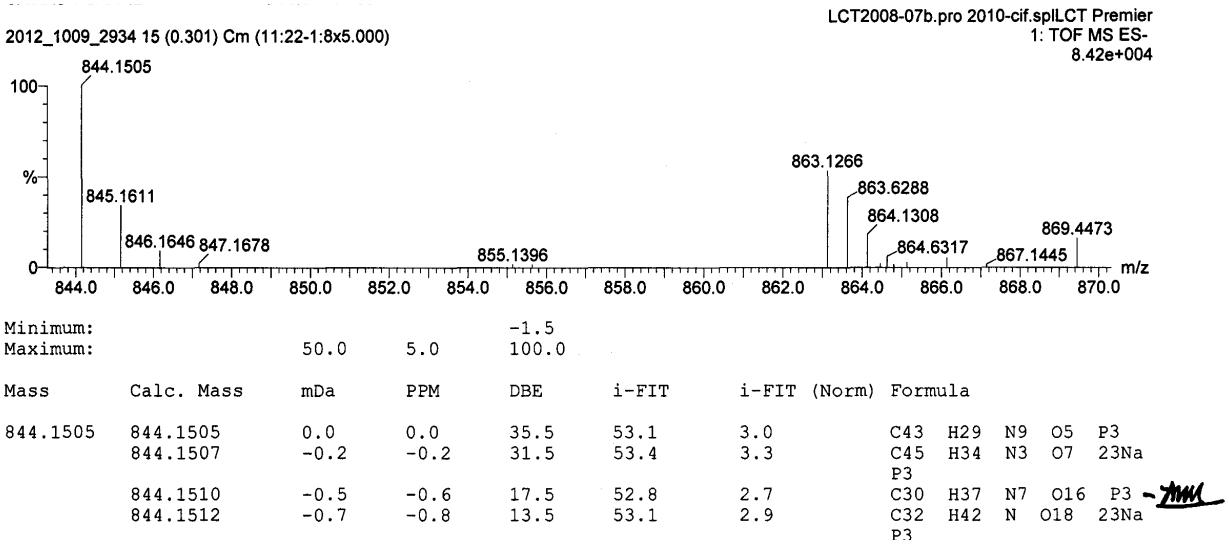


Figure S7: ^{31}P-NMR of ATP-BP **3** recorded in D_2O solvent.



Minimum:				-1.5								
Maximum:		50.0	5.0	100.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
844.1505	844.1505	0.0	0.0	35.5	53.1	3.0	C43	H29	N9	O5	P3	
	844.1507	-0.2	-0.2	31.5	53.4	3.3	C45 P3	H34	N3	O7	23Na	
	844.1510	-0.5	-0.6	17.5	52.8	2.7	C30	H37	N7	O16	P3	
	844.1512	-0.7	-0.8	13.5	53.1	2.9	C32 P3	H42	N	O18	23Na	

Figure S8: HR-MS of ATP-BP **3** recorded with methanol solvent.

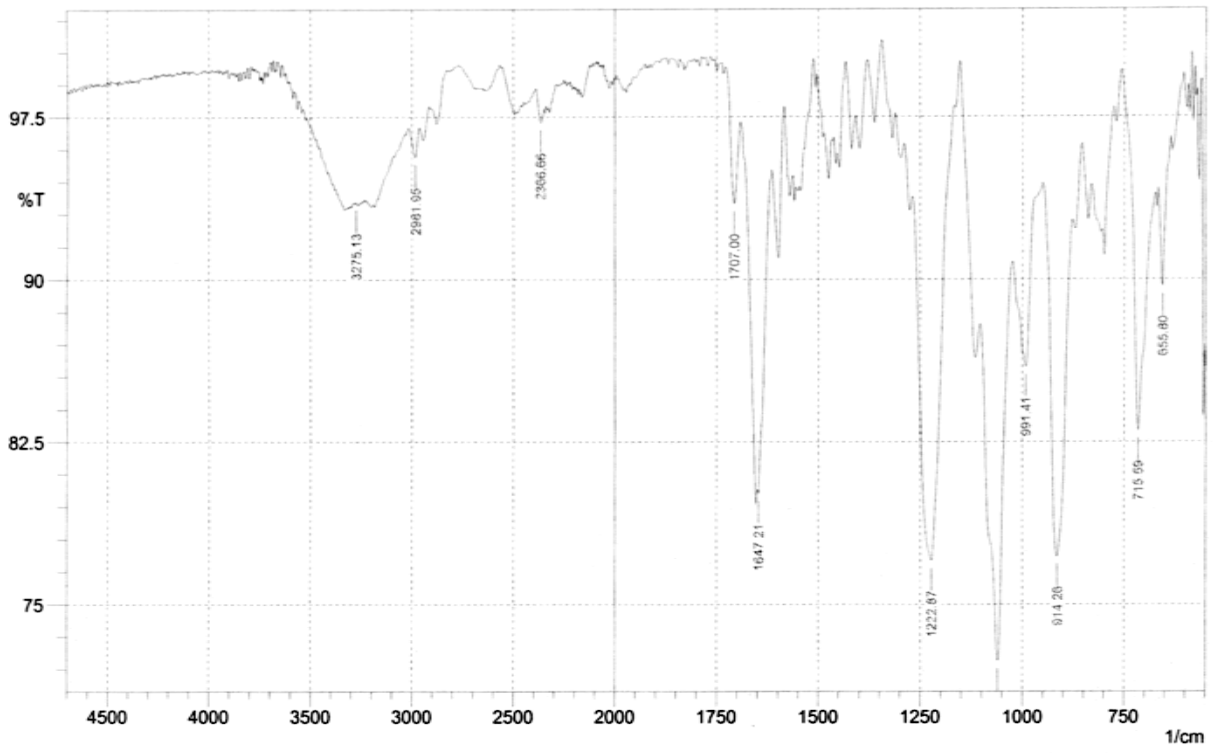
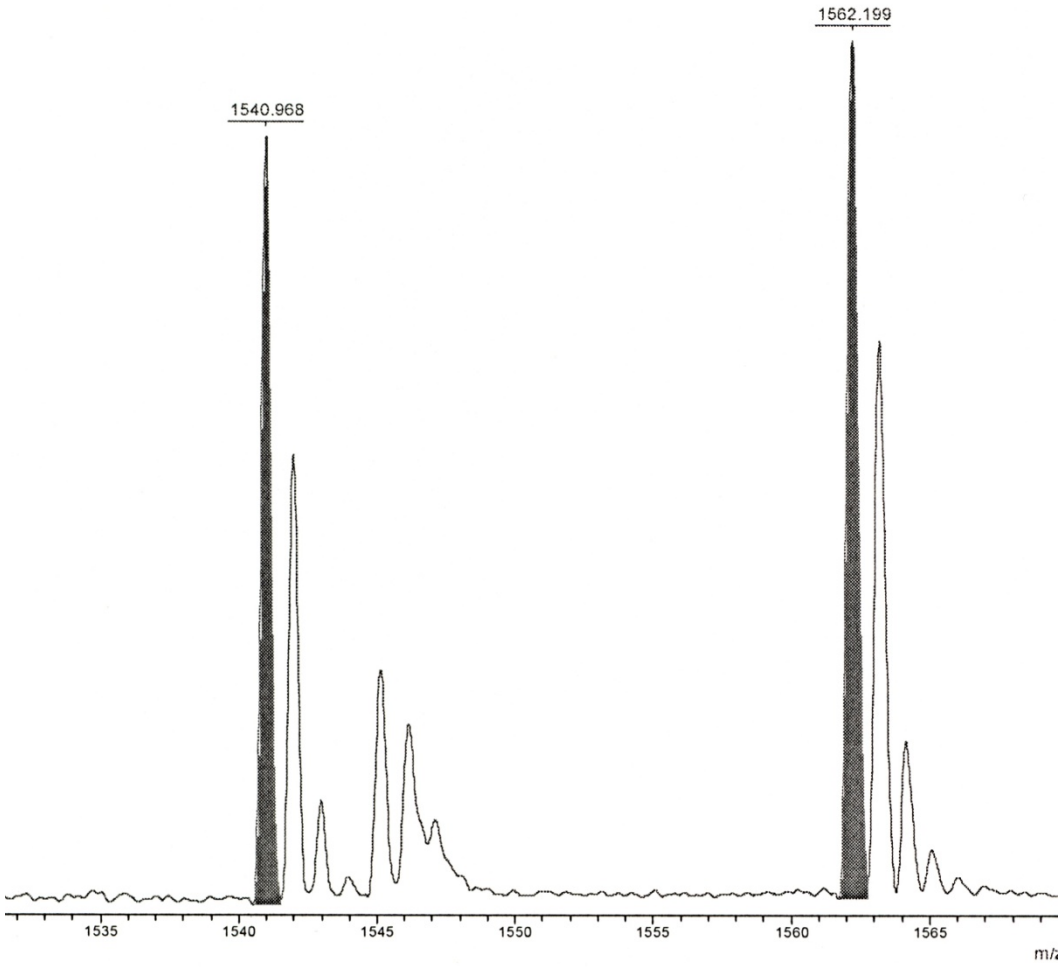


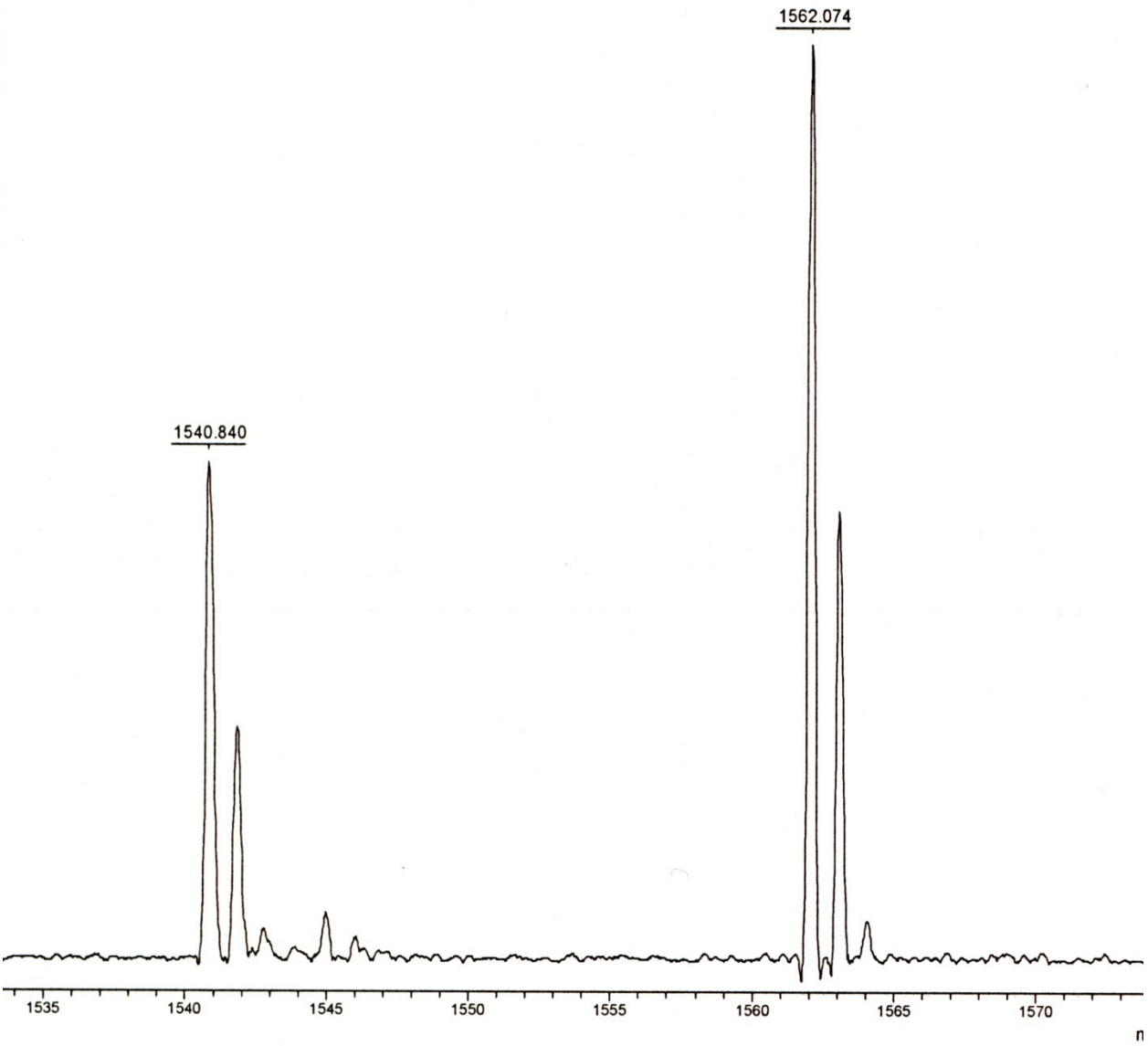
Figure S9: IR spectrum of ATP-BP **3**.

II. Quantitative mass spectrometric analysis:



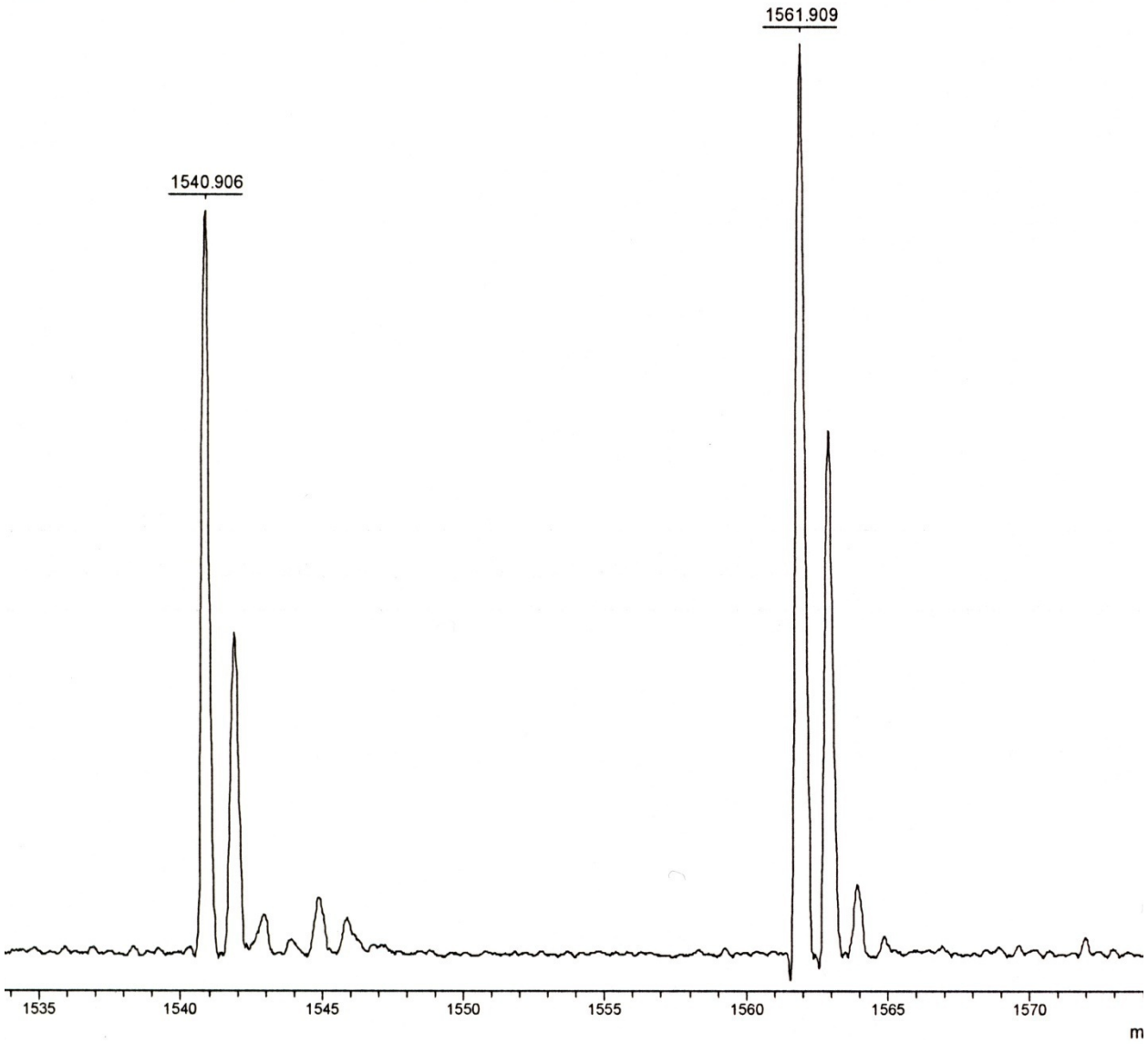
m/z	SN	Res.		Intens.	Area
1540.968	115.0	4094	813.42	317	
1562.199	129.6	3420	916.66	431	

Figure S10: (Trial 1) Quantitative MALDI-TOF MS of peptide substrate (RRREEE**T**EEE) with CKII kinase and either ATP **1** or ATP-benzophenone **3.** The peak at m/z ~1541 corresponds to heptamethylated phosphopeptide after reaction with ATP-benzophenone and acidic cleavage of phosphoramide bond, while the peak at m/z ~1562 corresponds to the deuterated heptamethylated phosphopeptide after phosphorylation with ATP. Percent conversion of this single trial was 74%.



m/z	SN	Res.	Intens.	Area
1540.840	21.2	5016	55.36	18
1562.074	39.0	5803	101.99	28

Figure S11: (Trial 2) Quantitative MALDI-TOF MS of peptide substrate (RRREEE**T**EEE) with CKII kinase and either ATP **1** or ATP-benzophenone **3.** Percent conversion of this trial is 65%. See Figure S1 for more details.



m/z	SN	Res.	Intens.	Area
1540.906	42.7	5160	161.56	50
1561.909	52.3	4335	197.63	72

Figure S12: (Trial 3) Quantitative MALDI-TOF MS of peptide substrate (RRREEE**T**EEE) with CKII kinase and either ATP **1** or ATP-benzophenone **3.** Percent conversion of this single trial is 74%. See Figure S1 for more details.

III. Autodock analysis:

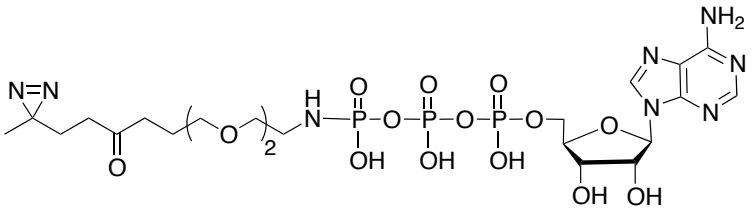


Figure S13. Structure of an ATP-diazirine (ATP-DAz) analog used in the docking studies in Figure 4 and 5 of the manuscript. This structure maintains the same polyethylene glycol linker as both the ATP-ArN_3 and ATP-BP analogs, but positions a dialkyl diazirine at the terminus.

A) CK2 kinase docking

The lowest energy binding mode that conforms to the kinase-catalyzed phosphorylation mechanism is in bold for each docking experiment. The grid box dimensions used in all the analysis was the same or similar.

Table S1. The grid dimensions and output file with all the different binding modes obtained from docking of ATP-ArN_3 (**2**).

center_x = 21.579 size_x = 22
center_y = 5.811 size_y = 30
center_z = 19.517 size_z = 18 exhaustiveness = 8.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	**-8.1**	**0.000**	**0.000**
2	-7.2	3.685	5.354
3	-7.2	8.785	10.991
4	-7.1	3.032	5.129
5	-7.1	4.889	7.208
6	-6.9	2.469	3.659
7	-6.9	3.559	5.621
8	-6.8	5.732	8.818
9	-6.7	10.940	13.509

Table S2. The grid dimensions and output file with all the different binding modes obtained from docking of ATP-BP (**3**).

center_x = 21.579 size_x = 22
center_y = 5.811 size_y = 30
center_z = 19.517 size_z = 18 exhaustiveness = 8

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	**-7.3**	**0.000**	**0.000**
2	-7.3	4.706	11.056
3	-6.7	1.462	2.275
4	-5.3	3.205	8.800
5	-5.2	4.051	8.731
6	-5.1	4.015	8.548
7	-5.0	4.001	8.547
8	-4.9	3.656	4.845
9	-4.8	3.301	9.279

Table S3. The grid dimensions and output file with all the different binding modes obtained from docking of an ATP-DAz analog, with the structure shown below (Figure S13).

center_x = 21.579 size_x = 22
center_y = 5.811 size_y = 30
center_z = 19.517 size_z = 18 exhaustiveness = 8

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	**-6.5**	**3.228**	**11.654**
3	-6.1	2.106	3.128
4	-5.8	4.891	12.286
5	-5.8	2.521	4.035
6	-5.7	3.274	11.896
7	-5.5	1.740	2.940
8	-5.4	2.944	5.893
9	-5.3	1.727	2.881

B) PKA kinase docking

The lowest energy binding mode that conforms to the kinase-catalyzed phosphorylation mechanism is in bold for each docking experiment. The grid box dimensions used in all the analysis was the same or similar.

Table S4. The grid dimensions and output file with all the different binding modes obtained from docking of ATP-ArN_3 (**2**).

center_x = 12.723 size_x = 28
center_y = 8.552 size_y = 20
center_z = 2.82 size_z = 16 exhaustiveness = 8

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.6	0.000	0.000
2	-7.6	2.552	4.617
3	**-7.2**	**3.196**	**15.716**
4	-7.2	2.061	3.817
5	-7.2	2.418	4.028
6	-7.2	2.202	14.849
7	-7.1	3.220	5.859
8	-7.0	2.486	4.993
9	-7.0	2.848	5.832

Table S5. The grid dimensions and output file with all the different binding modes obtained from docking of ATP-BP (**3**).

center_x = 12.886 size_x = 28
center_y = 8.549 size_y = 20
center_z = 2.924 size_z = 18 exhaustiveness = 8

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-10.2	0.000	0.000
2	-10.0	1.646	2.884
3	**-9.7**	**4.611**	**13.432**
4	-9.6	3.385	14.637
5	-9.6	4.480	14.024
6	-9.6	3.417	5.212
7	-9.6	3.992	6.090
8	-9.4	1.918	3.723
9	-9.2	2.214	4.154

Table S6. The grid dimensions and output file with all the different binding modes obtained from docking of ATP-DAz (Figure S13).

center_x = 12.723 size_x = 28
center_y = 8.552 size_y = 20
center_z = 2.82 size_z = 16 exhaustiveness = 8

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.5	0.000	0.000
2	-7.3	1.233	2.256
3	**-7.2**	**2.663**	**14.449**
4	-7.2	1.440	2.310
5	-7.1	2.304	14.531
6	-6.9	2.774	4.834
7	-6.8	4.594	6.423
8	-6.8	2.376	14.876
9	-6.8	2.321	3.800

IV. Figures from Docking Studies

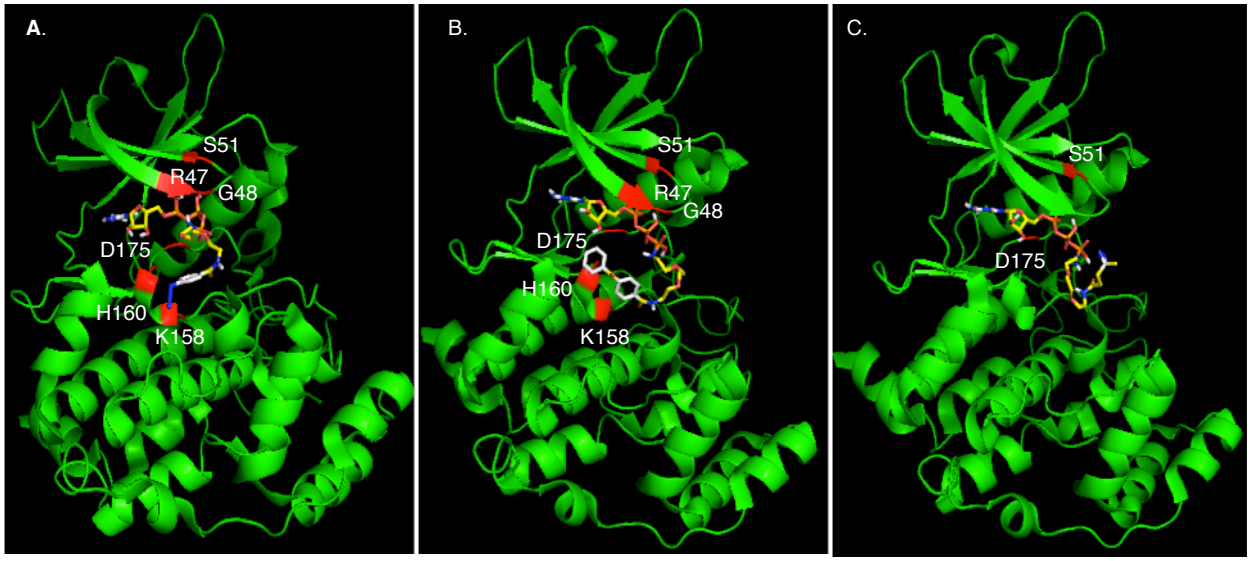


Figure S14. Docking of ATP-ArN_3 (A), ATP-BP (B), and ATP-DAz (C) (ball and stick structures) into the full CK2 kinase structure (green, pdb:1DAW). These structures are the full kinase images related to Figure 4 in the manuscript.

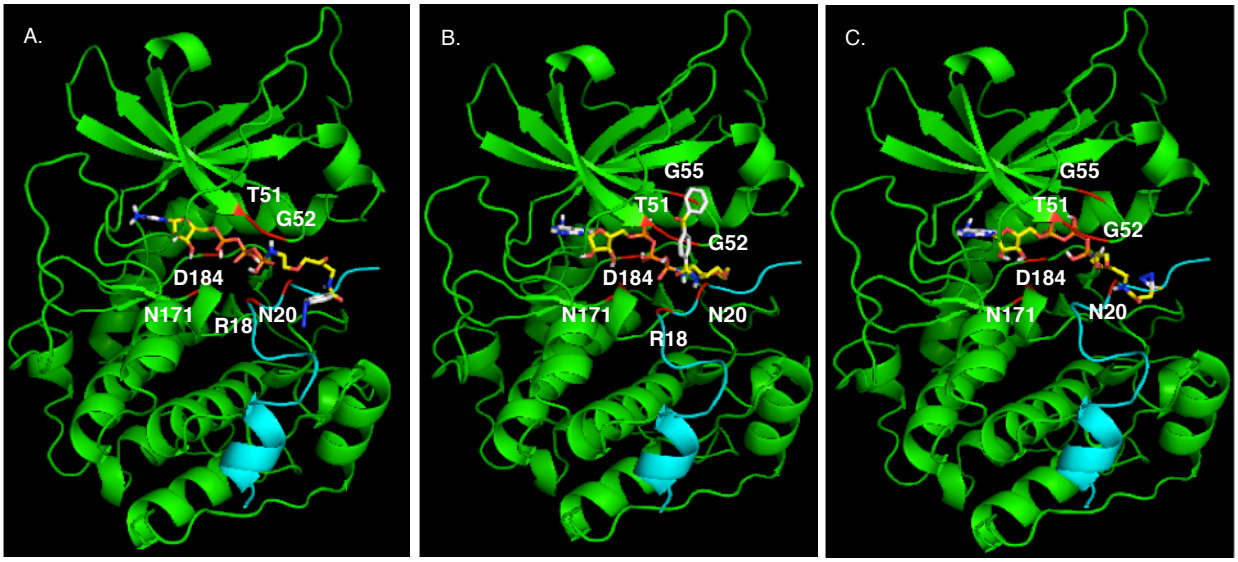


Figure S15. Docking of ATP-ArN_3 (A), ATP-BP (B), and ATP-DAz (C) (ball and stick structures) into the full PKA kinase structure (green) in complex with peptide substrate inhibitor (cyan). These structures are the full kinase images related to Figure 5 in the manuscript.

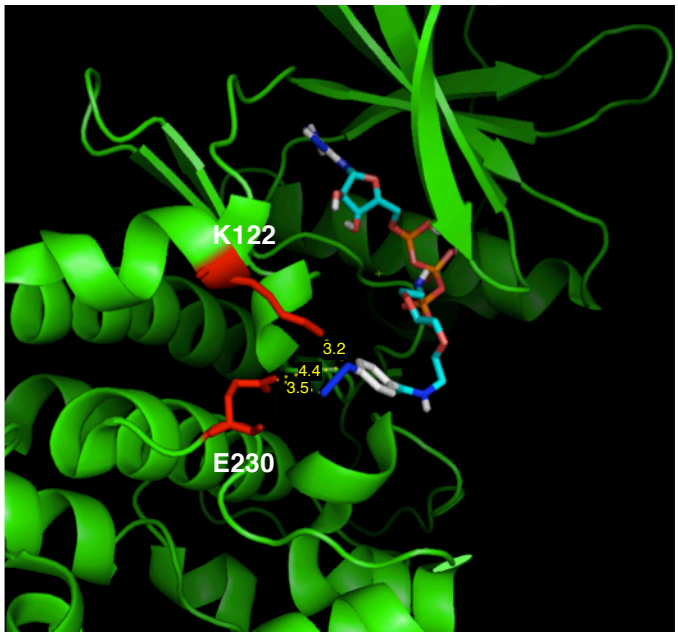


Figure S16: Distance measurements of amino acid residues of CK2 kinase residing near the arylazide group of ATP-ArN_3. The distances shown are from the azide nitrogen to the nearest atoms of K122 and E230. Images were created using Pymol after docking using Autodock.

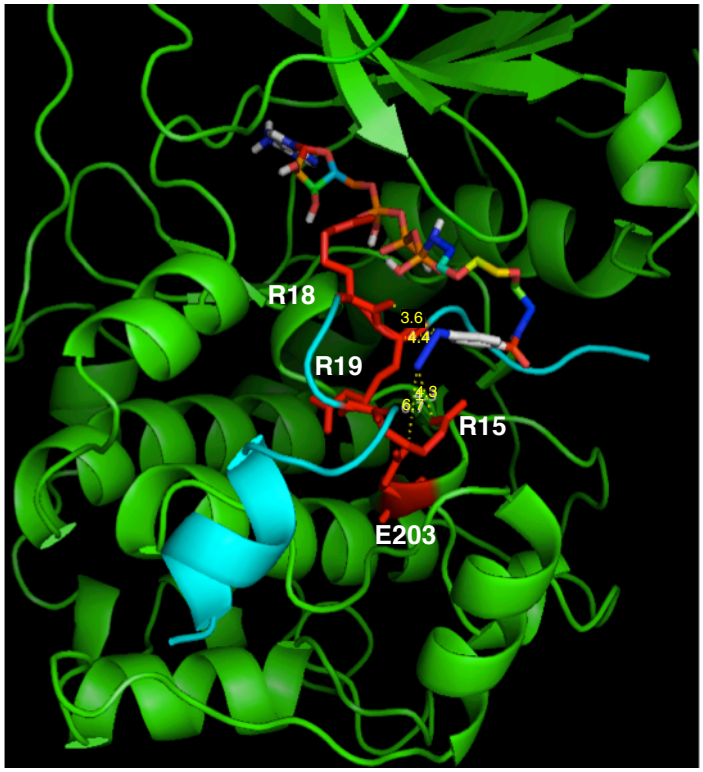


Figure S17: Distance measurements of amino acid residues of PKA kinase complex residing near the arylazide group of ATP-ArN_3. The distances shown are from the azide nitrogen to the nearest atoms of R15, R18, R19, and E203. Images were created using Pymol after docking using Autodock.

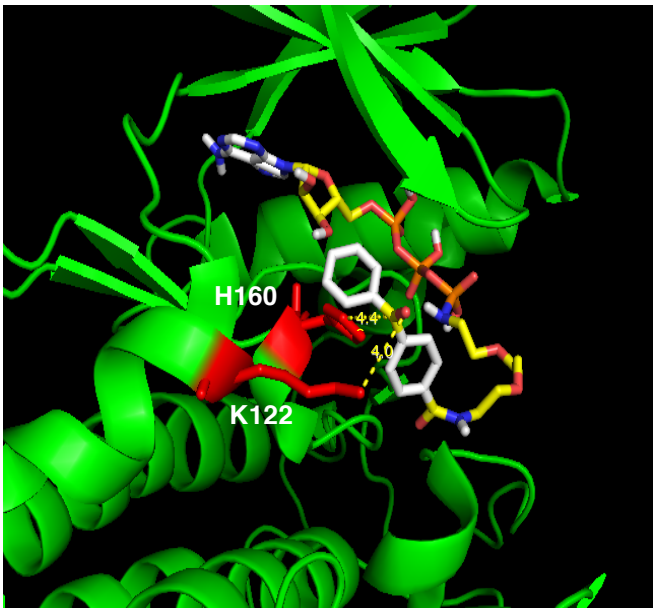


Figure S18: Distance measurements from amino acid residues of CK2 kinase to the benzophenone group of ATP-BP. Distances shown are from the benzophenone carbonyl to the nearest atoms of K122 and H160. Images were created using Pymol after docking using Autodock.

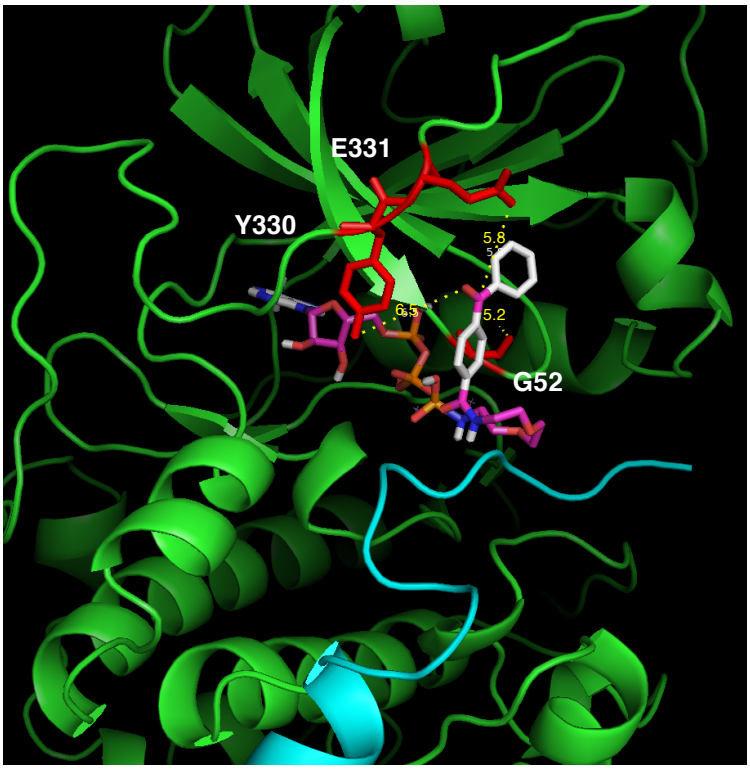


Figure S19: Distance measurements from amino acid residues of PKA kinase to the benzophenone group of ATP-BP. The distances shown are from the benzophenone carbonyl to the nearest atom of G52, Y330, and E331. Images were created using Pymol after docking using Autodock.