Supporting Table 1. Data collection and refinement statistics.

	LT PKAc-Mg_2ADP-pSP20	RT PKAc-Mg_2ADP-pSP20	LT PKAc-Ca_2ADP-pSP20	LT PKAc-Sr_2ADP-pSP20	RT PKAc-Sr_2ADP-pSP20	LT PKAc-Ba_2ADP-pSP20
PDB ID	4IAD	4IAF				
Data collection						
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions						
a, *b*, *c* (Å)	57.522, 79.535, 97.914	59.213, 79.901, 99.637	57.808, 79.654, 98.356	56.537, 78.924, 97.331	59.484, 80.014, 100.177	58.288, 79.648, 98.709
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	40.00 (1.90) *	40.00 (2.20)	40.00 (1.55)	40.00 (1.60)	40.00 (2.00)	40.00 (1.85)
R_{sym} or $\boldsymbol{R_{merge}}$	0.038 (0.352)	0.086 (0.464)	0.027 (0.354)	0.029 (0.488)	0.072 (0.409)	0.064 (0.480)
$I / \sigma I$	54.0 (5.5)	14.9 (2.4)	48.0 (2.7)	53.3 (2.7)	15.2 (2.4)	16.2 (2.0)
Completeness (%)	96.0 (87.1)	97.0 (93.7)	97.3 (75.8)	99.6 (96.5)	95.3 (88.4)	97.4 (95.0)
Redundancy	7.9 (7.4)	4.0 (3.9)	5.5 (3.4)	5.7 (4.6)	3.9 (3.6)	3.7 (3.6)
Refinement						
Resolution (Å)	20.00-1.90	20.00-2.20	20.00-1.55	20.00-1.60	20.00-2.00	20.00-1.85
No. reflections	34466	18612	62900	55292	28271	34206
R_{work} / R_{free}	0.185 / 0.238	0.160 / 0.192	0.169 / 0.209	0.183 / 0.222	0.180 / 0.227	0.199 / 0.249
No. atoms						
Protein	2787	2781	2819	2844	2781	2793
Ligand/ion	29	29	29	29	29	29
Peptide	159	159	159	159	159	159
Water	288	260	395	284	196	255
B-factors						
Protein	25.9	30.4	22.1	25.0	34.4	25.6
Ligand/ion	18.3	21.0	14.1	17.0	23.5	21.9
Peptide	29.3	29.7	20.0	22.0	35.2	28.0
Water	32.4	42.3	32.0	32.1	37.0	31.6
R.m.s. deviations						
Bond lengths (Å)	0.012	0.004	0.010	0.008	0.015	0.022
Bond angles (°)	1.340	1.140	1.340	1.310	1.420	1.450

*For each structure one crystal was used to collect the dataset. *Values in parentheses are for highest-resolution shell.

Supporting Table 1. Data collection and refinement statistics. (contd.)

	LT PKAc-Mg_2AMPPCP-SP20
PDB ID	4IAC
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	56.809, 79.678, 97.954
α, β, γ (°)	90, 90, 90
Resolution (Å)	40.00 (2.15)
R_{sym} or R_{merge}	0.075 (0.441)
$I / \sigma I$	28.0 (4.2)
Completeness (%)	99.3 (98.3)
Redundancy	7.5 (6.9)
Refinement	
Resolution (Å)	20.00-2.15
No. reflections	21951
R_{work} / R_{free}	0.181 / 0.212
No. atoms	
Protein	2781
Ligand/ion	33
Peptide	155
Water	375
B-factors	
Protein	20.8
Ligand/ion	14.8
Peptide	18.1
Water	33.3
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.165

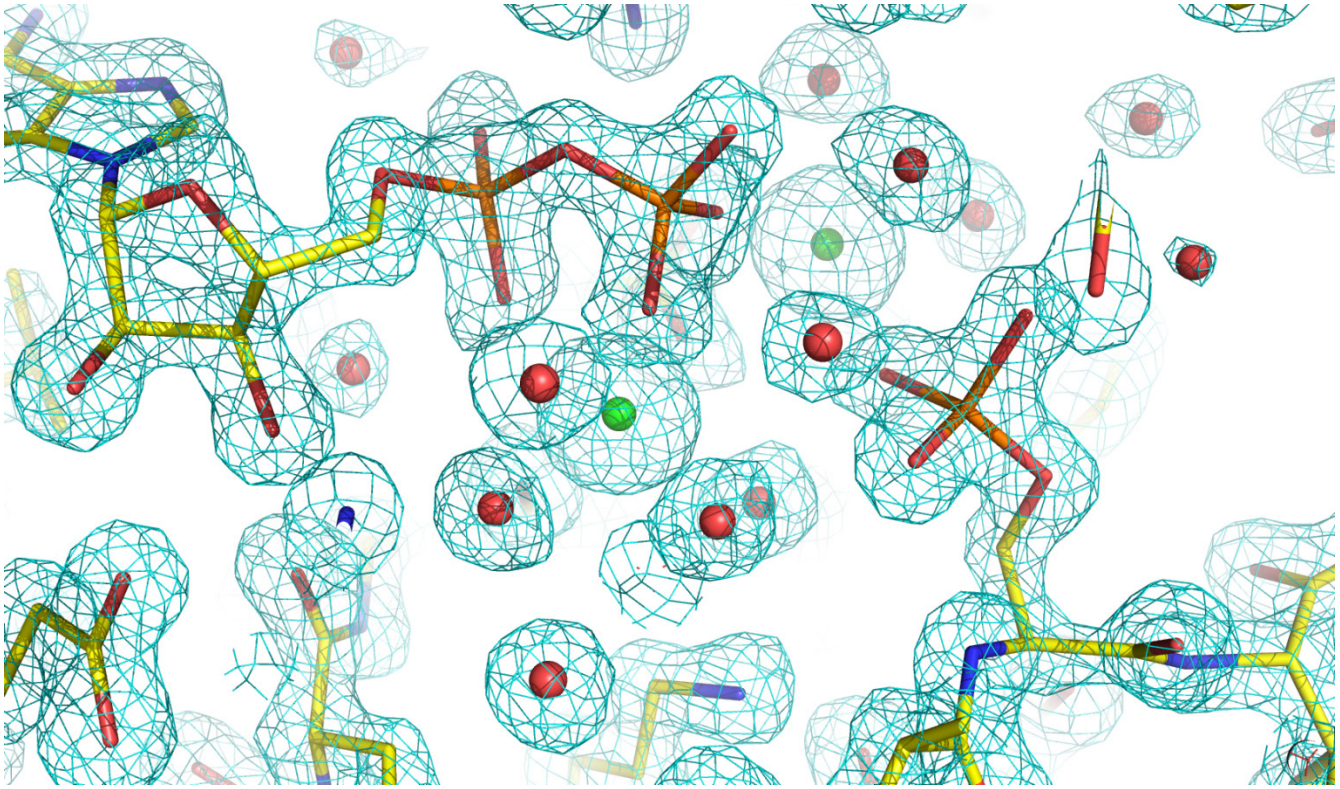
* For each structure one crystal was used to collect the dataset. *Values in parentheses are for highest-resolution shell.

Supporting Table 2. Coordination distances around M1 and M2 sites in complexes with excess Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}.

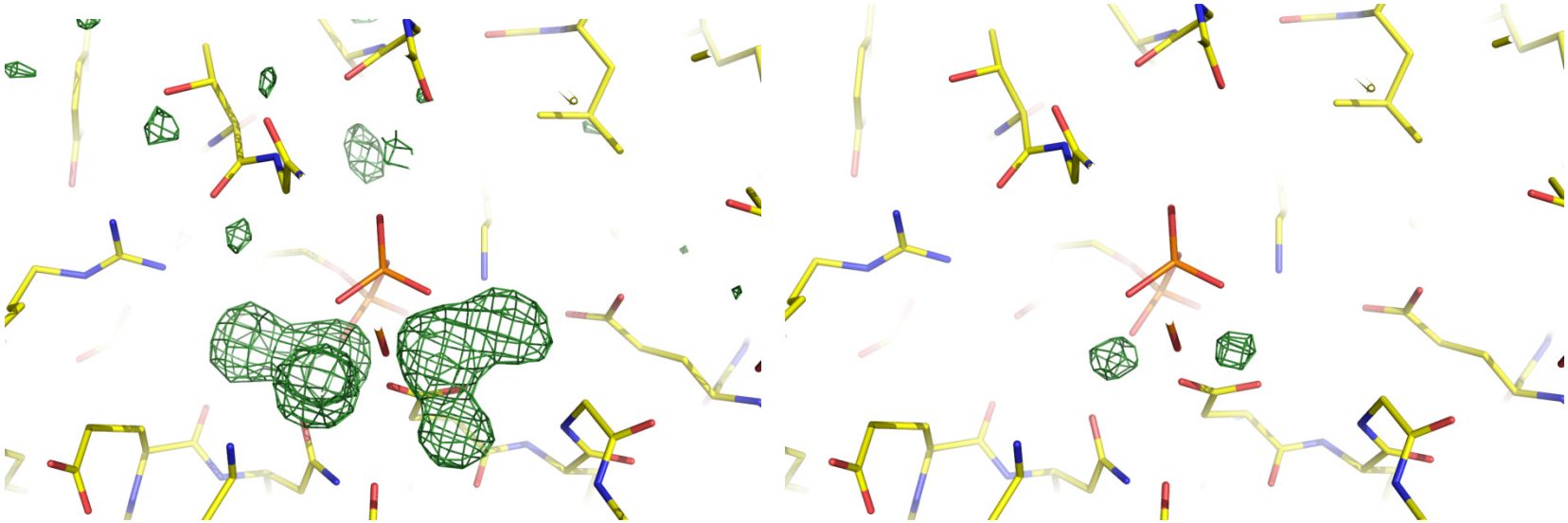
Distance values are given in Å; Ions are in order of increase of ionic radius**.**

M1 site	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	**M2 site**	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
ionic radii, Å*	0.72	1.06	1.26	1.42	ionic radii, Å*	0.72	1.06	1.26	1.42
$O_2(P_\alpha)$					$O_2(P_\alpha)$	2.0	2.3	2.4	2.5
$O_1(P_\beta)$	2.0	2.3	2.3	2.4	$O_1(P_\beta)$				
O_3 (P_β)					O_3 (P_β)	2.0	2.3	2.6	2.2
$O_{23}(pSer_{21})$	2.0	2.3	2.4	2.7	$O_{23}(pSer_{21})$				
O_1Asp_{184}	2.3	2.4	2.6	2.9	O_1Asp_{184}				
O_2Asp_{184}	2.3	2.5	2.6	2.7	O_2Asp_{184}	2.0	2.4	2.5	2.7
O_1Asn_{171}					O_1Asn_{171}	2.0	2.4	2.6	2.9
$H_2O(1)$	2.2	2.5	2.5	2.8	$H_2O(5)$	2.4	2.4	2.5	2.8
$H_2O(2)$	2.1	2.4	2.5	2.8	$H_2O(6)$		2.6	2.7	3.1
$H_2O(3)$		2.4	2.5	2.8	$H_2O(7)$	2.2	2.5	2.6	2.9
$H_2O(4)$			2.6	2.9					
Coordination number	6	7	8	8	Coordination number	6	7	7	7

*Metal ionic radius is dependent on the number of ligands the cation is coordinated to.

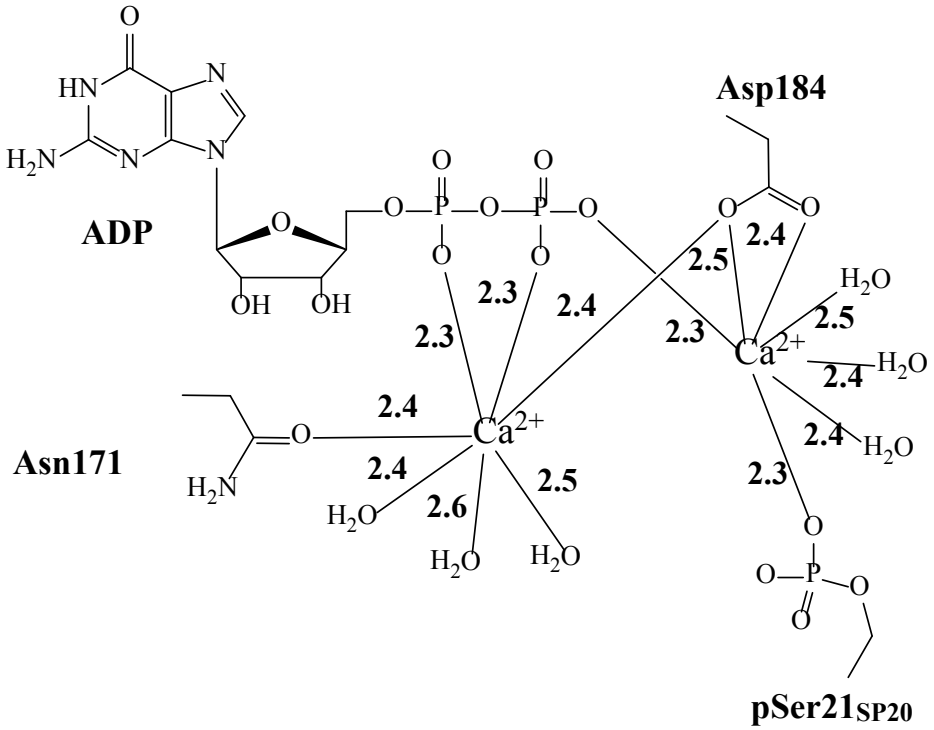


Supporting Figure 1a. The $2F_O$-F_C electron density map of the active site in PKAc-Sr_2ADP-pSP20 contoured at 2σ level.

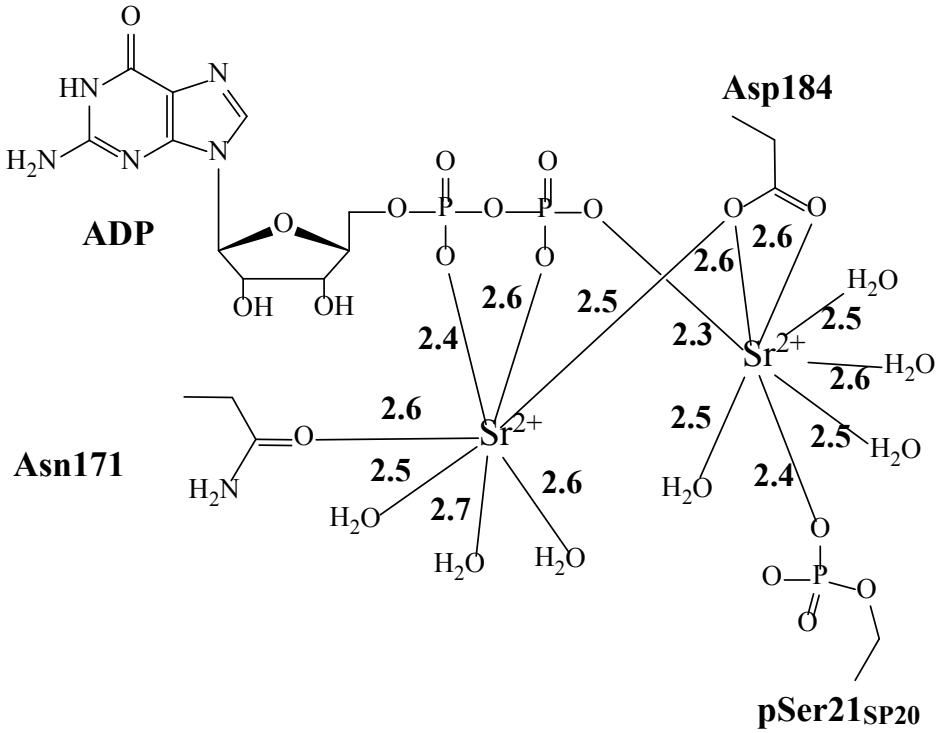


(a) **(b)**

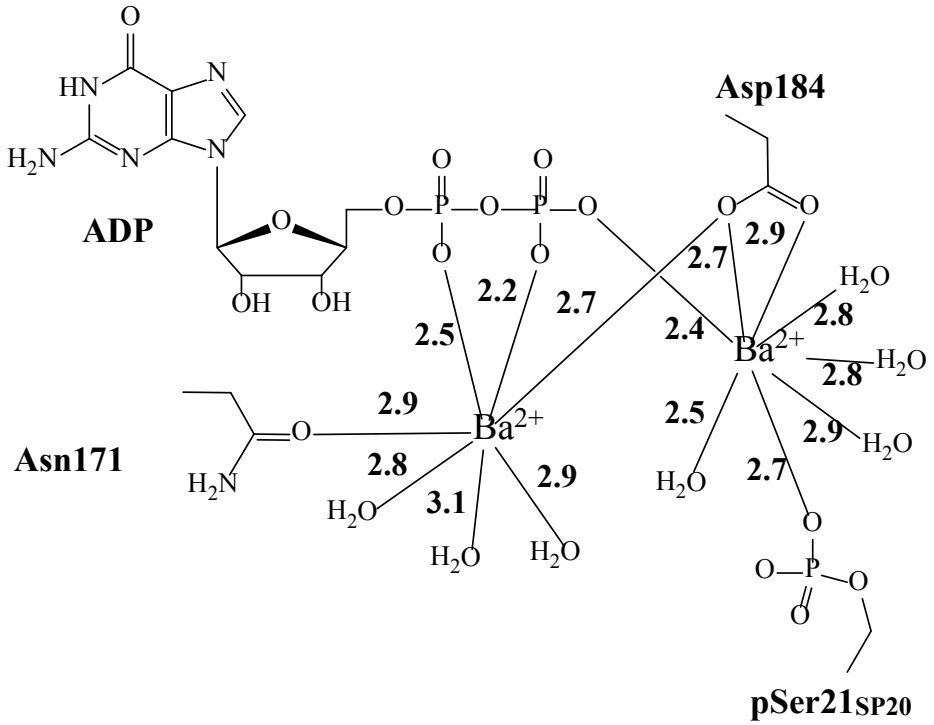
Supporting Figure 2. The omit F_O-F_C electron density map showing the locations of the metal ions and metal-bound water molecules. (a) in PKAc-Mg_2ADP-pSP20 at 3 σ level and (b) at 13 σ level.



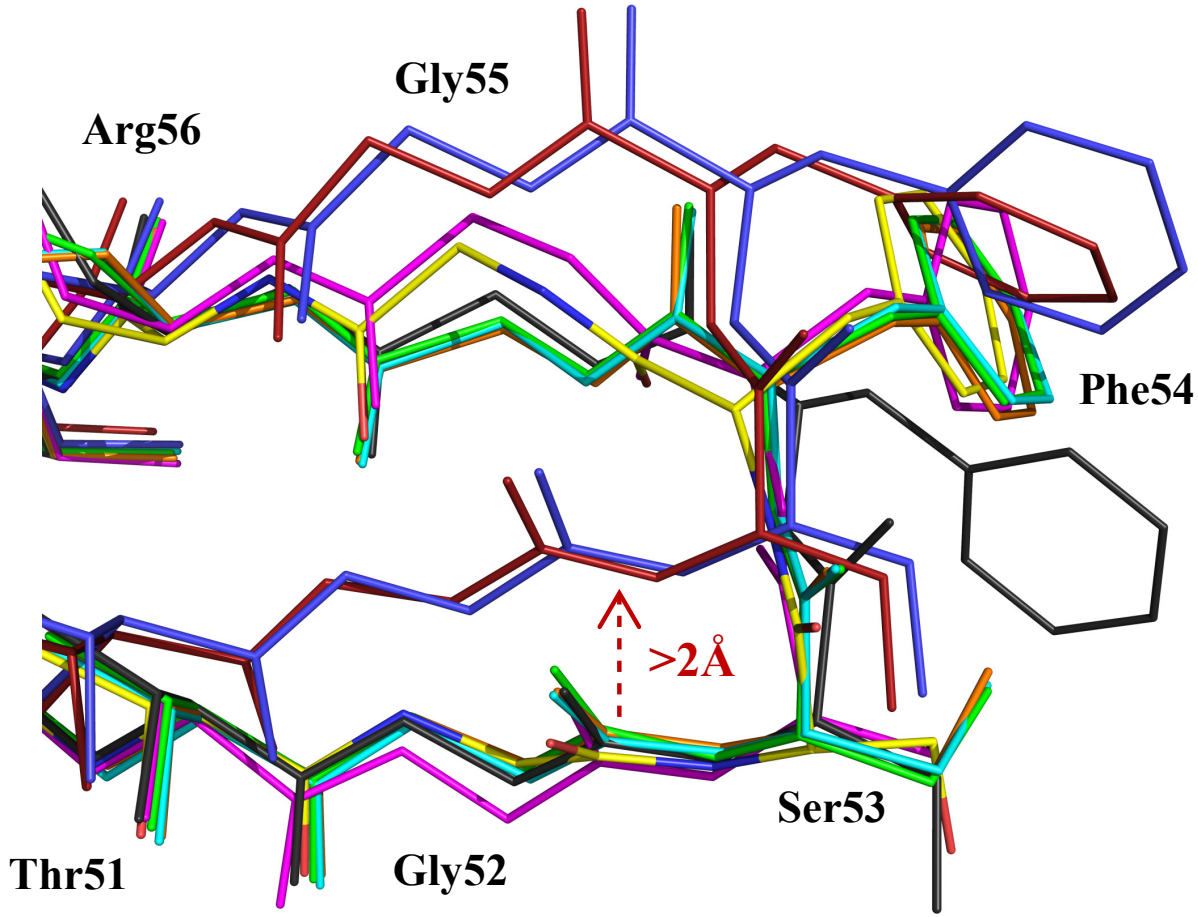
Supporting Figure 3a. Chemical diagram of the metal coordination in PKAc-Ca_2ADP-pSP20. Distances are in Å.



Supporting Figure 3b. Chemical diagram of the metal coordination in PKAc-Sr_2ADP-pSP20. Distances are in Å.



Supporting Figure 3c. Chemical diagram of the metal coordination in PKAc-Ba_2ADP-pSP20. Distances are in Å.



Supporting Figure 4. The superposition of glycine-rich loops in the low-temperature structures reported in this study.