## Supporting Information.

**Supporting Table 1.** X-ray crystallographic data collection and the refinement statistics.

| | PKAc-ADP-pSP20 | PKAc-ADP-psSP20 | PKAc-SP20 |
|---|---|---|---|
| PDB ID | 4IB3 | 4O21 | 4O22 |
| **Data collection** | | | |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Cell dimensions | | | |
| *a*, *b*, *c* (Å) | 57.54, 79.31, 98.42 | 56.96, 79.26, 97.89 | 56.63, 78.62, 97.86 |
| $\alpha, \beta, \gamma$ (°) | 90, 90, 90 | 90, 90, 90 | 90, 90, 90 |
| Wavelength (Å) | 1.5418 | 1.5418 | 0.9798 |
| Resolution (Å) | 40.00 (2.30-2.20)* | 40.0 - 1.95 (2.02-1.95) | 50.0 - 1.70 (1.74-1.70) |
| No. reflections unique | 23288 (2174) | 32471 (3119) | 48412 (3290) |
| $R_{merge}$ | 0.112 (0.569) | 0.062 (0.56) | 0.086 (0.455) |
| $I / \sigma(I)$ | 20.3 (2.8) | 21.5 (2.2) | 13.3 (1.7) |
| Completeness (%) | 99.3 (93.6) | 98.5 (96.8) | 99.2 (92.5) |
| Redundancy | 7.3 (5.5) | 3.9 (3.9) | 5.9 (2.6) |
| | | | |
| **Refinement** | | | |
| Software | CNS | *phenix.refine* | *phenix.refine* |
| Resolution (Å) | 20.00-2.20 | 37.12 - 1.95 | 45.95 - 1.70 |
| No. reflections | 20083 | 27971 | 48408 |
| $R_{work}$ / $R_{free}$ | 0.182 / 0.228 | 0.167 / 0.215 | 0.186 / 0.219 |
| No. atoms | | | |
| Protein | 2804 | 2800 | 2830 |
| Ligand | 27 | 27 | |
| Peptide | 159 | 166 | 155 |
| Water | 254 | 421 | 271 |
| *B*-factors | | | |
| Protein | 28.9 | 22.78 | 25.54 |
| Ligand/metal | 39.4 | 47.36 | |
| Peptide | 30.0 | 24.42 | 24.78 |
| Water | 37.0 | 29.27 | 36.22 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.005 | 0.005 | 0.006 |
| Bond angles | 1.191 ° | 0.941 ° | 1.057 ° |

* Values in parentheses are for the highest-resolution shell. Data were collected from 1 crystal for each structure.

**Supporting Table 1 (contd.).** X-ray crystallographic data collection and the refinement statistics.

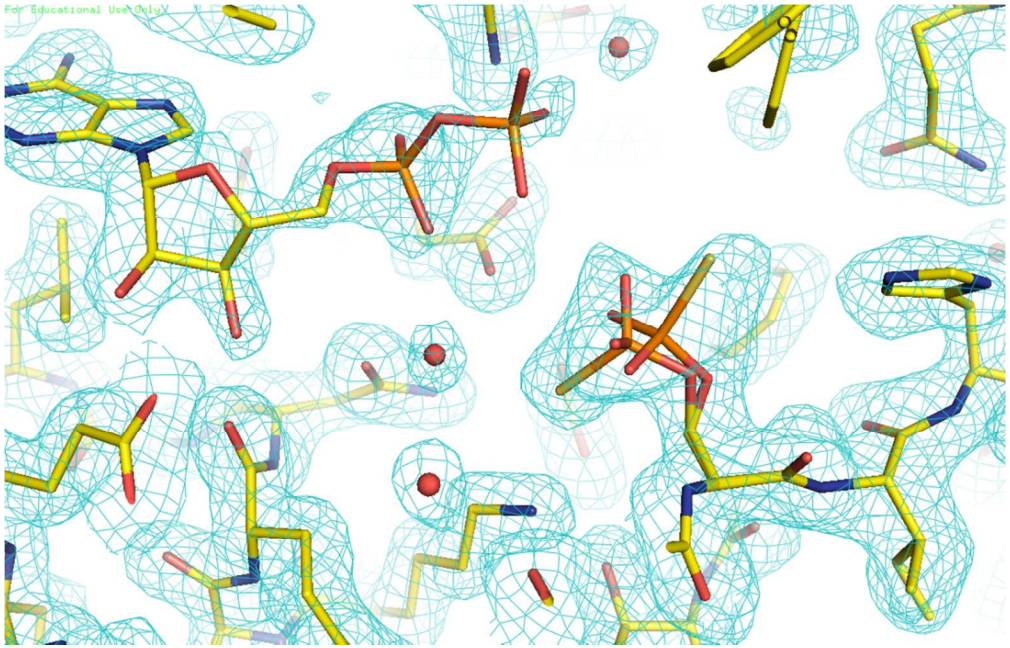
| | PKAc-$Na_2$ADP-pSP20 | PKAc-$K_2$ADP-pSP20 |
|---|---|---|
| PDB ID | 4IB0 | 4IB1 |
| **Data collection** | | |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 57.270, 79.202, 98.041 | 57.893, 79.795, 98.510 |
| $\alpha, \beta, \gamma$ (°) | 90, 90, 90 | 90, 90, 90 |
| Resolution (Å) | 40.00-1.87 (1.94-1.87) * | 40.00-1.63 (1.69-1.63) |
| No. reflections unique | 37374 (3447) | 57000 (5408) |
| $R_{merge}$ | 0.062 (0.542) | 0.047 (0.506) |
| $I / \sigma(I)$ | 30.6 (2.6) | 24.8 (2.1) |
| Completeness (%) | 99.3 (92.9) | 98.6 (95.1) |
| Redundancy | 6.8 (5.3) | 3.9 (3.6) |
| | | |
| **Refinement** | | |
| Software | SHELX-97 | SHELX-97 |
| Resolution (Å) | 20.00-1.87 | 20.00-1.63 |
| No. reflections | 37277 | 51855 |
| $R_{work}$ / $R_{free}$ | 0.216 / 0.287 | 0.195 / 0.223 |
| No. atoms | | |
| Protein | 2798 | 2821 |
| Metal | 2 | 2 |
| Ligand | 27 | 27 |
| Peptide | 159 | 159 |
| Water | 303 | 352 |
| *B*-factors | | |
| Protein | 30.1 | 20.8 |
| Ligand/metal | 27.2 | 16.3 |
| Peptide | 28.9 | 18.4 |
| Water | 38.4 | 28.2 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.022 | 0.022 |
| Bond angles | 0.036 Å | 0.037 Å |

**Supporting Table 2. Coordination distances around M1 and M2 sites in complexes with excess $Na^+$ and $K^+$, and comparison with $Mg^{2+}$ (4IAD)**

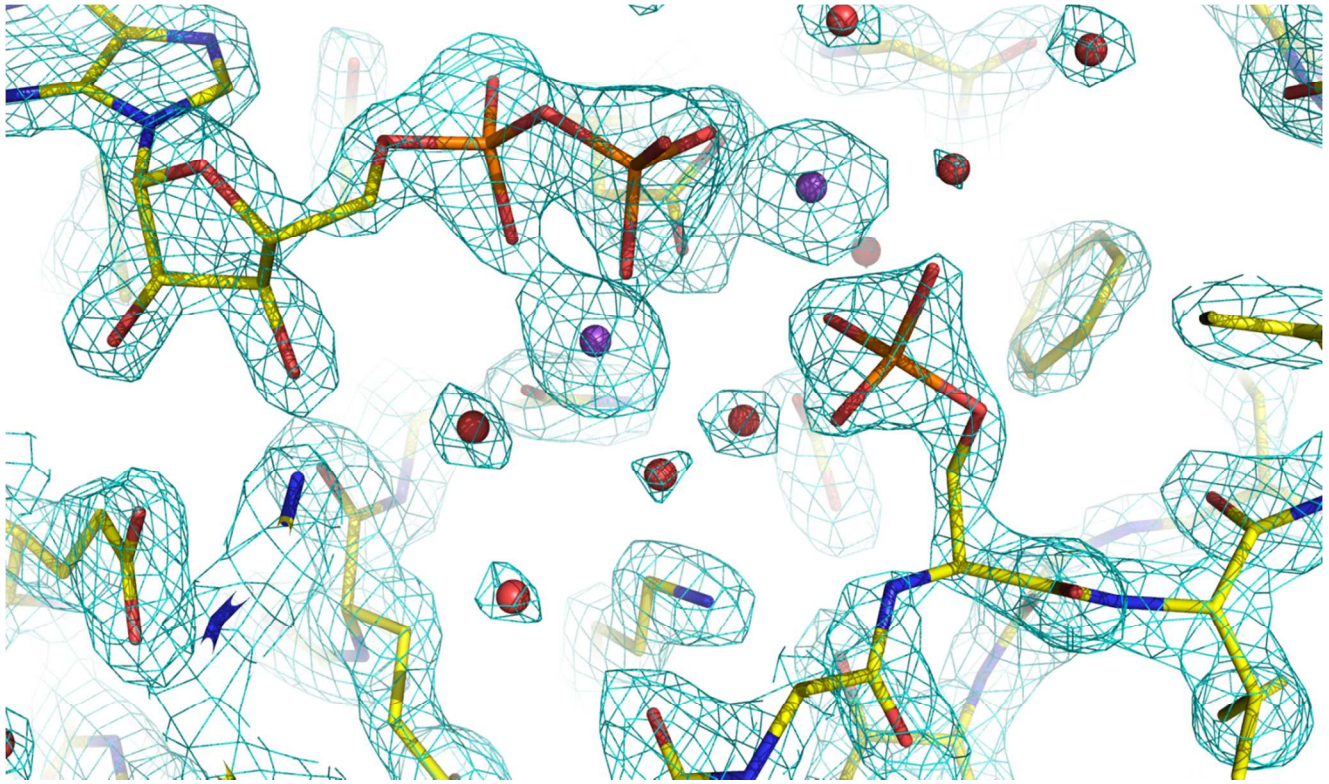
Distance values are given in Å; Ions are in order of increase of ionic radius**.**

| **M1 site** | $Mg^{2+}$ | $Na^+$ | $K^+$ | **M2 site** | $Mg^{2+}$ | $Na^+$ | $K^+$ |
|---|---|---|---|---|---|---|---|
| ionic radii, Å* | 0.72 | 1.12 | 1.46 | ionic radii, Å* | 0.72 | 1.12 | 1.46 |
| $O_2(P_\alpha)$ | | | | $O_2(P_\alpha)$ | 2.0 | 2.1 | 2.2 |
| $O_1(P_\beta)$ | 2.0 | 2.2 | 2.1 | $O_1(P_\beta)$ | | | |
| $O_3$ $(P_\beta)$ | | | | $O_3$ $(P_\beta)$ | 2.0 | 2.1 | 2.3 |
| $O_{23}(pSer_{21})$ | 2.0 | 2.3 | 2.3 | $O_{23}(pSer_{21})$ | | | |
| $O_1Asp_{184}$ | 2.3 | 2.6 | 2.5 | $O_1Asp_{184}$ | | | |
| $O_2Asp_{184}$ | 2.3 | 2.5 | 2.4 | $O_2Asp_{184}$ | 2.0 | 2.5 | 2.4 |
| $O_1Asn_{171}$ | | | | $O_1Asn_{171}$ | 2.0 | 2.6 | 2.5 |
| $H_2O(1)$ | 2.2 | 2.6 | 2.4 | $H_2O(5)$ | 2.4 | 2.5 | 2.4 |
| $H_2O(2)$ | 2.1 | 2.6 | 2.5 | $H_2O(6)$ | | 2.8 | 2.7 |
| $H_2O(3)$ | | 2.3 | 2.4 | $H_2O(7)$ | 2.2 | 2.7 | 2.5 |
| $H_2O(4)$ | | | | | | | |
| Coordination number | 6 | 7 | 7 | Coordination number | 6 | 7 | 7 |

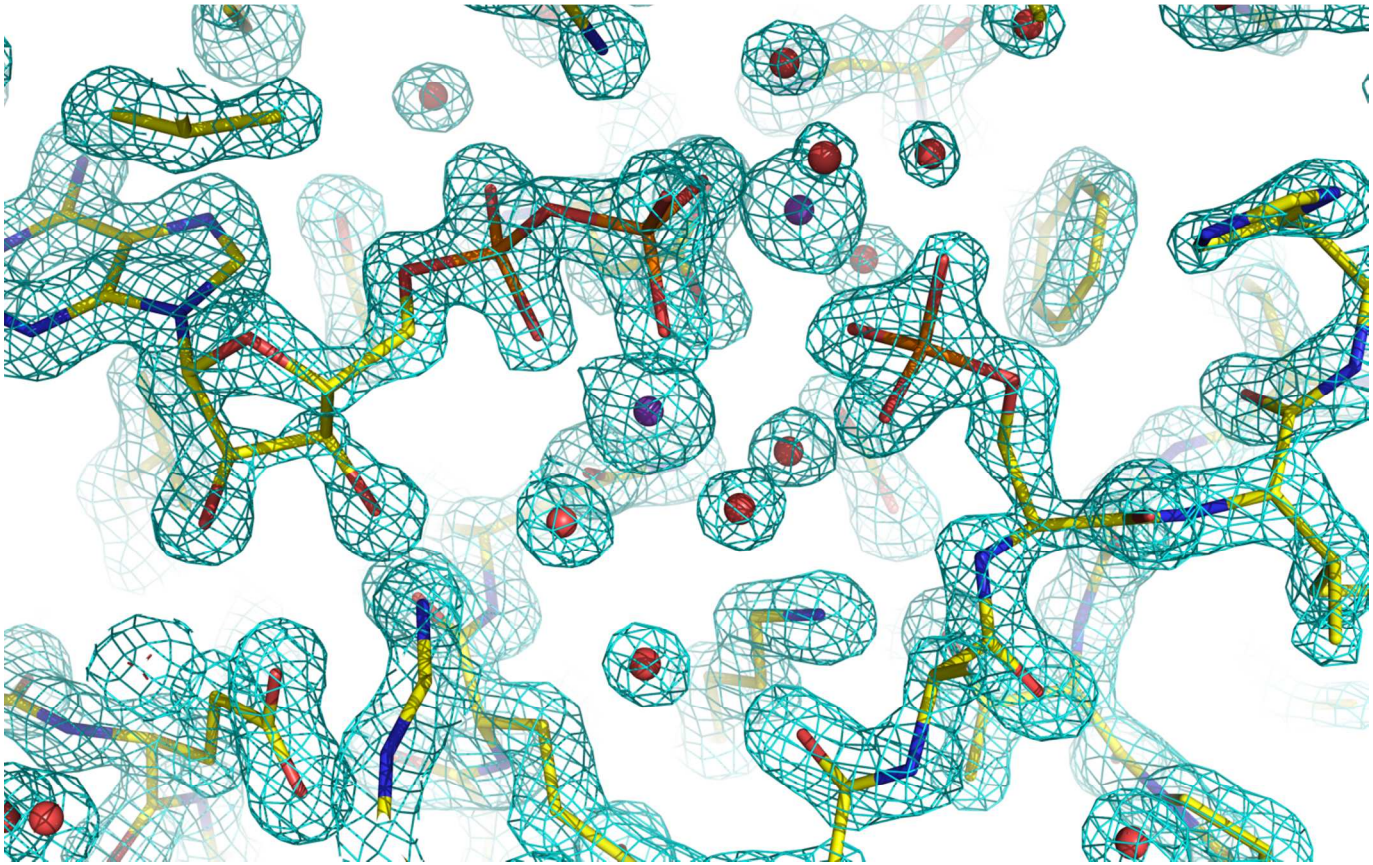
*Metal ionic radius is dependent on the number of ligands the cation is coordinated to.



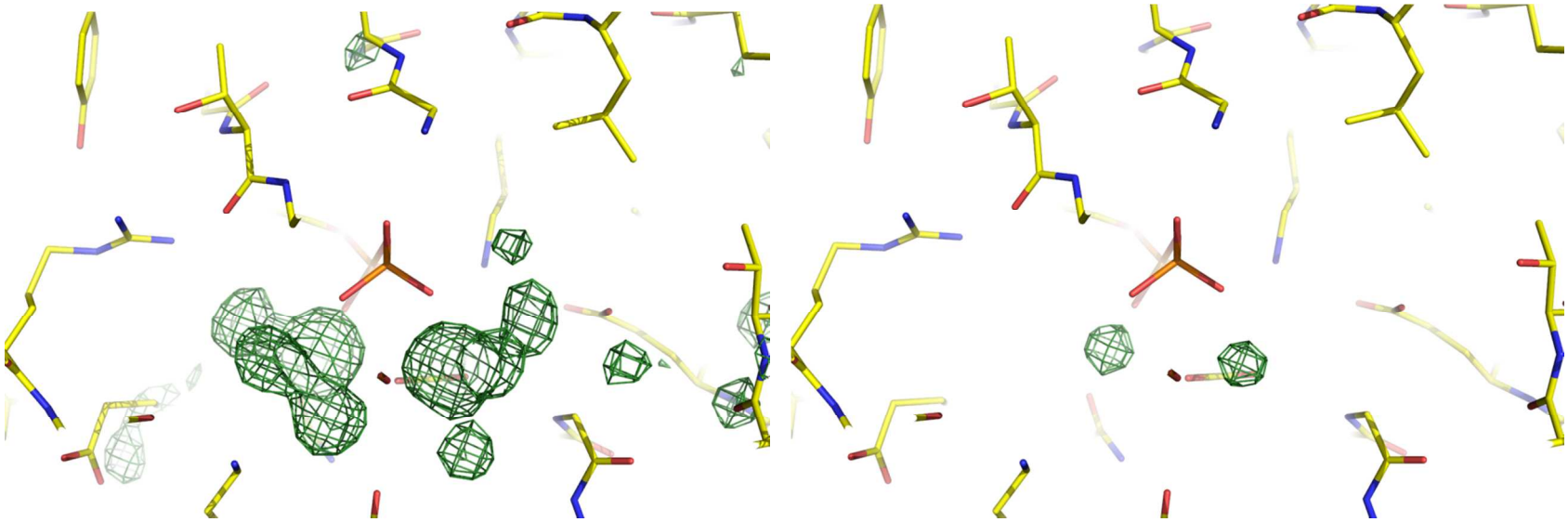

**Supporting Figure 1a.** The $2F_O$-$F_C$ electron density map of the active site in PKAc-ADP-psSP20 contoured at 2σ level.



**Supporting Figure 1b.** The $2F_O$-$F_C$ electron density map of the active site in PKAc-$Na_2$ADP-pSP20 contoured at $2\sigma$ level.

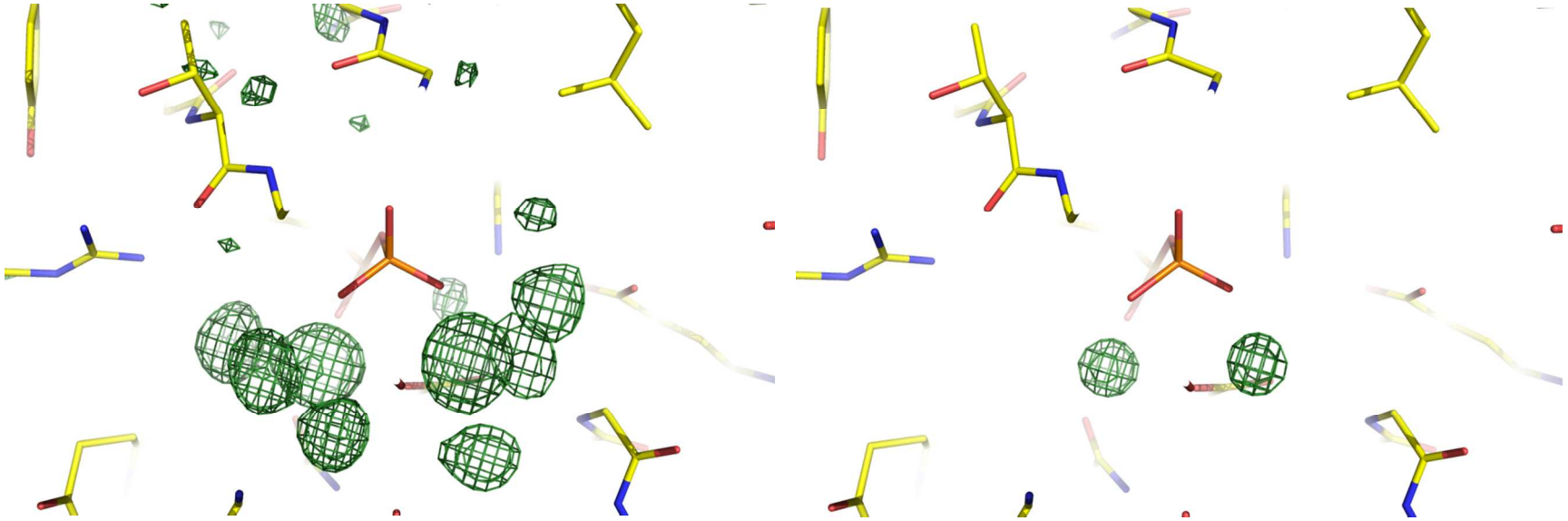


**Supporting Figure 1c.** The $2F_O$-$F_C$ electron density map of the active site in PKAc-$K_2$ADP-pSP20 contoured at 2σ level.



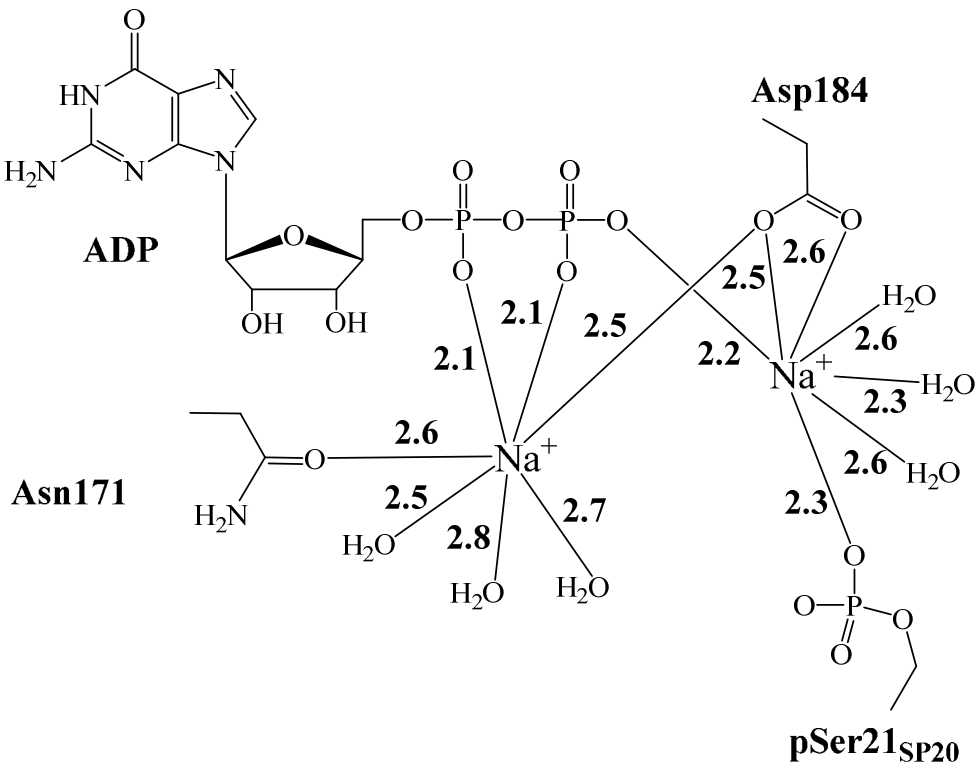
**(a)** **(b)**

**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-$Na_2$ADP-pSP20 at 3 $\sigma$ level and (b) at 13 $\sigma$ level.

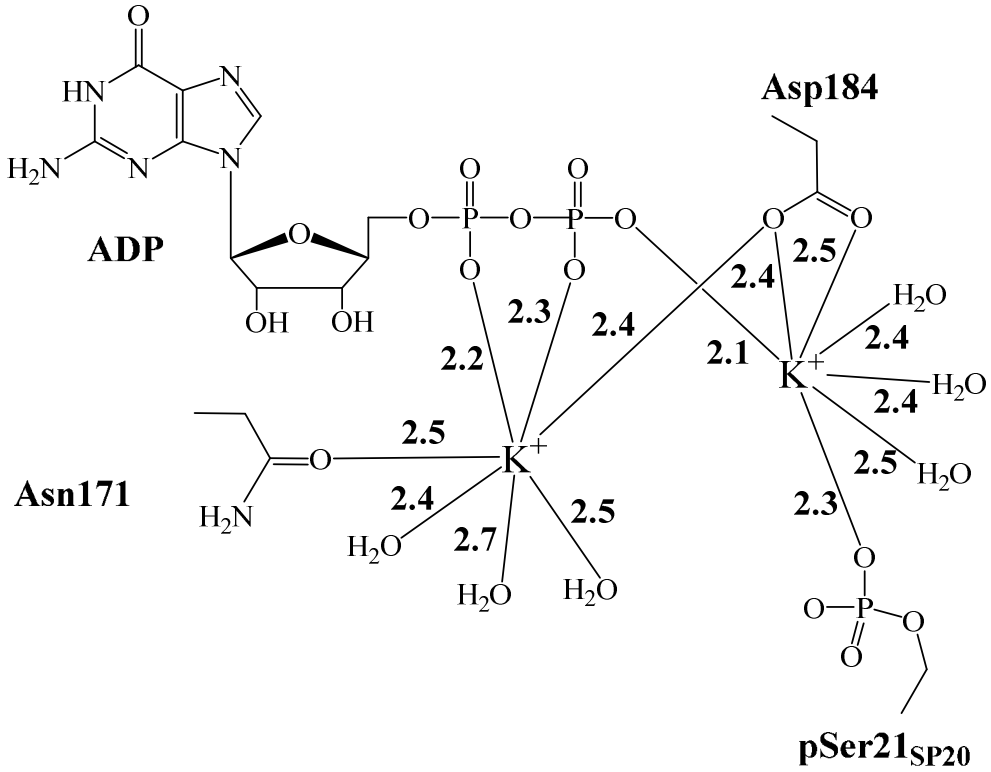


**(c)** **(d)**

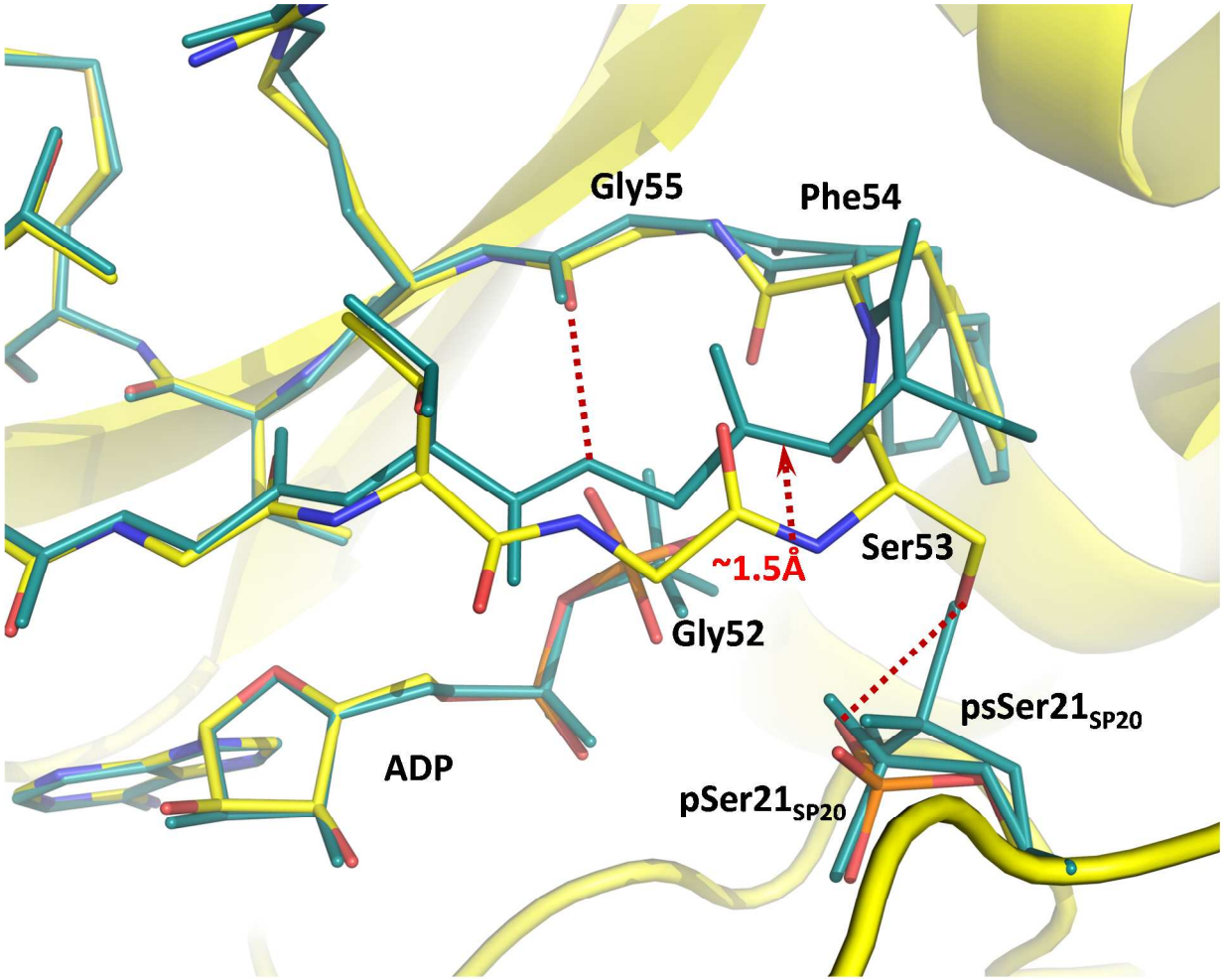
**Supporting Figure 2 (contd.).** The omit $F_O$-$F_C$ electron density map showing the locations of the metal ions and metal-bound water molecules. (c) in PKAc-$K_2$ADP-pSP20 at 3 $\sigma$ level and (d) at 13 $\sigma$ level.



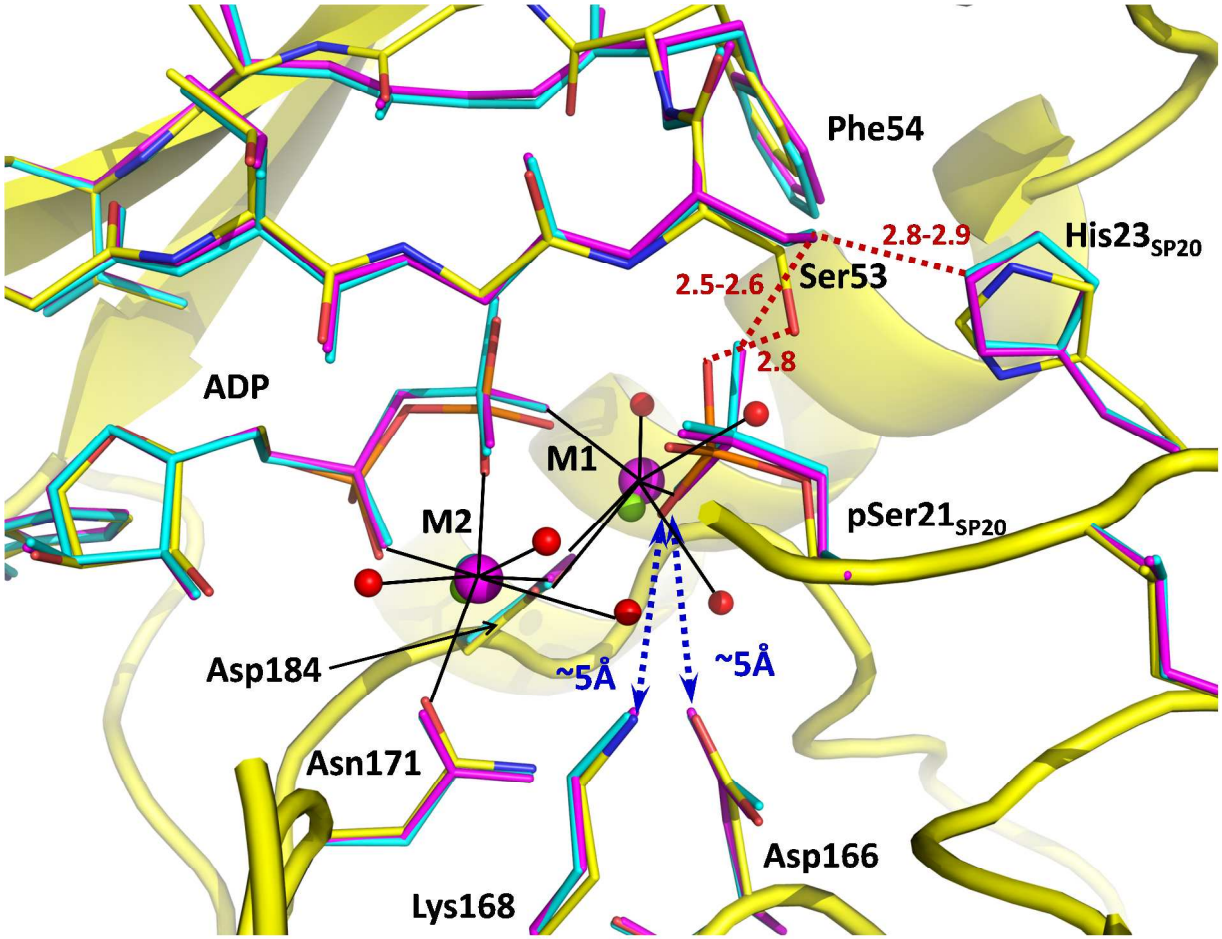

**Supporting Figure 3a.** Chemical diagram of the metal coordination in PKAc-$Na_2$ADP-pSP20. Distances are in Å.



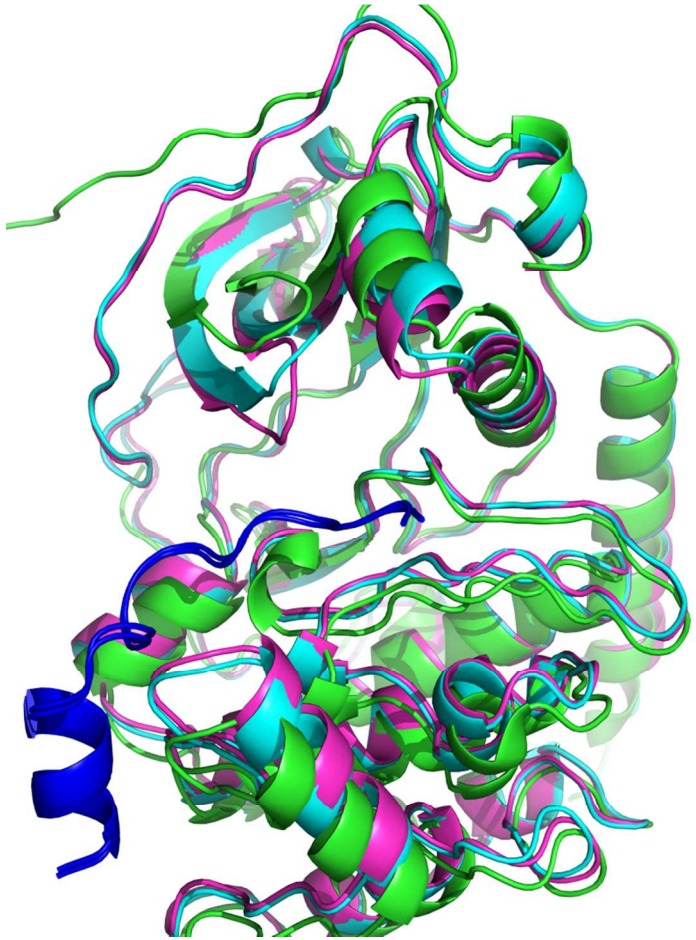

**Supporting Figure 3b.** Chemical diagram of the metal coordination in PKAc-$K_2$ADP-pSP20. Distances are in Å.




**Supporting Figure 4.** Superposition of active sites in PKAc-ADP-pSP20 (colored by atom type) and PKAc-ADP-psSP20 (cyan). Hydrogen bonds are depicted as red dashed lines.




**Supporting Figure 5.** Superposition of active site in PKAc-$Mg_2$ADP-pSP20 (PDB ID 4IAD; colored by atom type), PKAc-$Na_2$ADP-pSP20 (cyan), and PKAc-$K_2$ADP-pSP20 (magenta). Hydrogen bonds are depicted as red dashed lines. Metal coordination around $Na^+$ and $K^+$ is shown as black solid lines. Distances are in Å.



**Supporting Figure 6.** Superposition of apo-PKAc (PDB ID 1J3H; green),[1] PKAc-SP20 (cyan) and PKAc-Mg2ADP-pSP20 (PDB ID 4IAD)[2] in cartoon representation.

**References.**

(1). Akamine, P., Madhusudan, Wu, J., Xuong, N. H., Ten Eyck, L. F., and Taylor, S. S. (2003) Dynamic features of cAMP-dependent protein kinase revealed by apo-enzyme crystal structure. J. Mol. Biol. 327, 159-171.

(2). Gerlits, O., Waltman, M.J., Taylor, S., Langan, P., and Kovalevsky, A. (2013) Insights into the phosphoryl transfer catalyzed by cAMP-dependent protein kinase: An X-ray crystallographic study of complexes with various metals and peptide substrate SP20. *Biochemistry 52*, 3721-3727.