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Supplementary Materials for

Zooming in on protons: Neutron structure of protein kinase A trapped in a product complex

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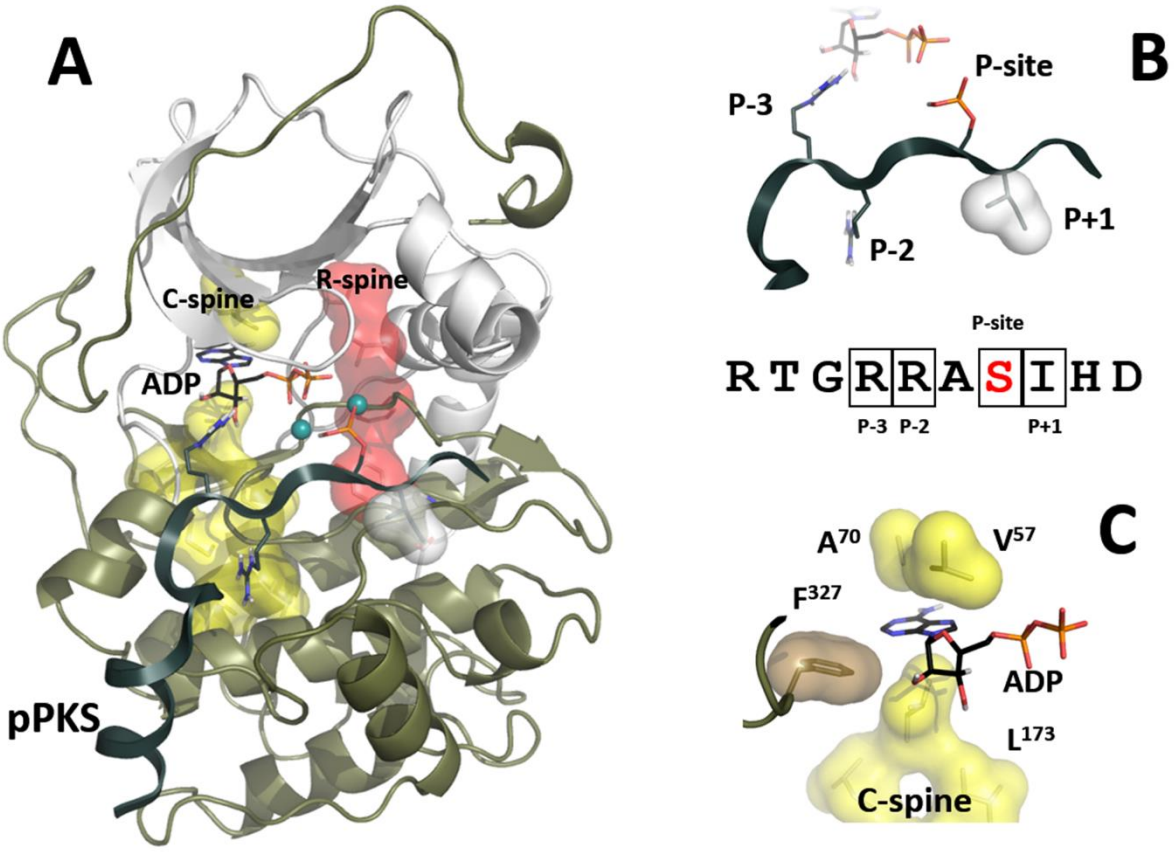

Fig. S1. Neutron structure describes the PKA-C product complex, PKA-C:Sr_2ADP:pPKS. (**A**) The overall structure showing PKA-C in cartoon representation with bound metals as teal-colored spheres; ADP in stick representation, colored with black carbon atoms; and pPKS as a dark gray ribbon. (**B**) Fragment of the phosphorylated substrate, pPKS, and its sequence highlighting the sites of the substrate's direct interactions with PKA-C. (**C**) Close-up view of the C-spine showing incorporation of the ADP's adenine ring highlighting features of the hydrophobic shell (Ref 21).

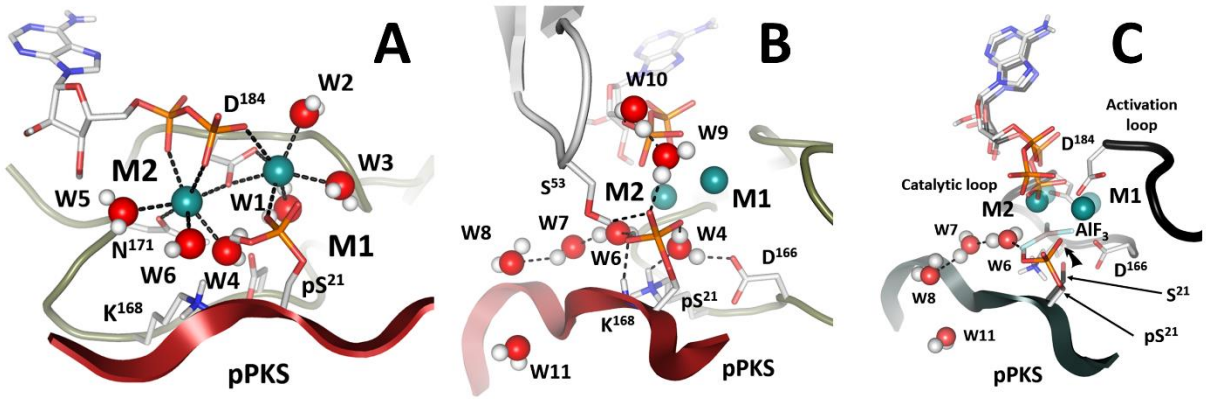

Fig. S2. The phosphorylation site. (**A**) Close-up view of the active site showing metal coordination for the product complex; Sr^{2+} ions are shown as teal-colored spheres and peptide is red ribbon. (**B**) Hydrogen bonding interactions of the pS21 phosphate group. The Sr^{2+} ions and peptide are shown with the same color as in panel A. (**C**) Superposition of the phosphorylation sites in the product complex PKA-C:Sr_2ADP:pPKS (carbon colored light gray) and transition state mimic PKA-C:Mg_2ADP:AlF_3:PKS (PDB code 1L3R, dark gray carbon atoms) showing rotation of the pS21 side chain in the product relatively to the position of S21 in the substrate. The dark gray ribbon is the peptide.

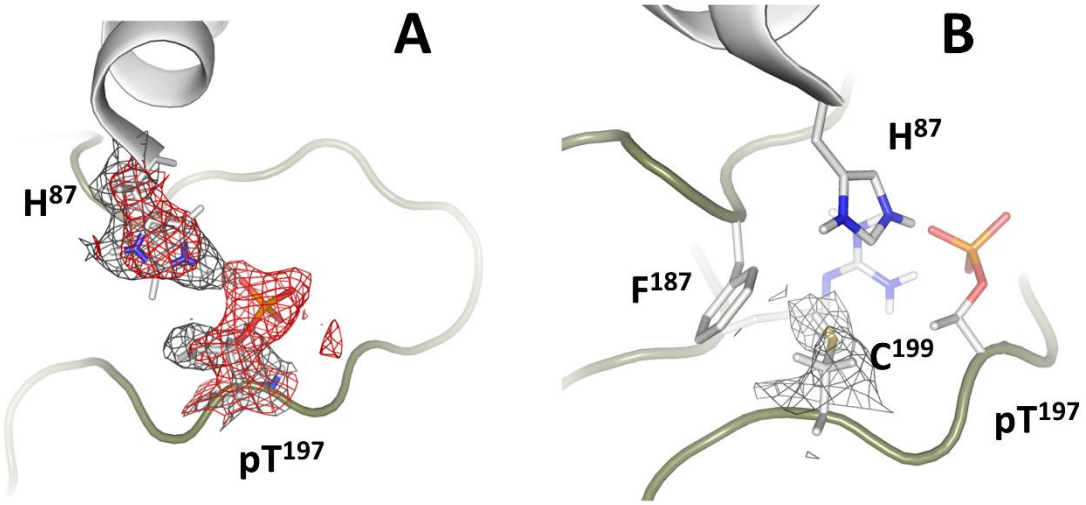

Fig. S3. Positive $2F_O - F_C$ electron (red) and nuclear (gray) density maps at 2.0σ demonstrate complementary features of X-ray and neutron diffraction techniques. (**A**) The pT197 phosphate is not visible in the nuclear, but excellently defined in the electron density map; electron density maps do not provide evidence for the protonation of His87, which is unambiguously demonstrated in the neutron structure. (**B**) The nuclear density map reveals that Cys199 is protonated in the product complex.

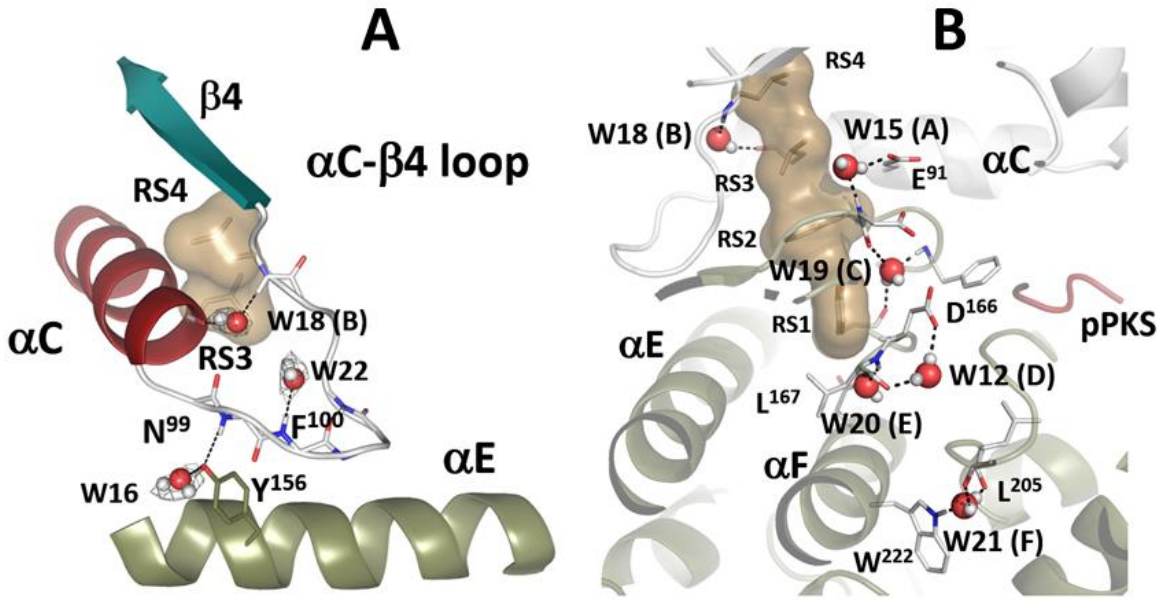

Fig. S4. Stable water molecules. (**A**) The αC-β4 Loop has one of the six conserved water molecules in its structure. (**B**) Entire collection of the six conserved water molecules in the product complex, initially identified by Knight *et al*. (A-F are the original water labeling) for the substrate complex of PKA C-subunit. Table S1 presents the potential roles for these waters and explains the labeling.

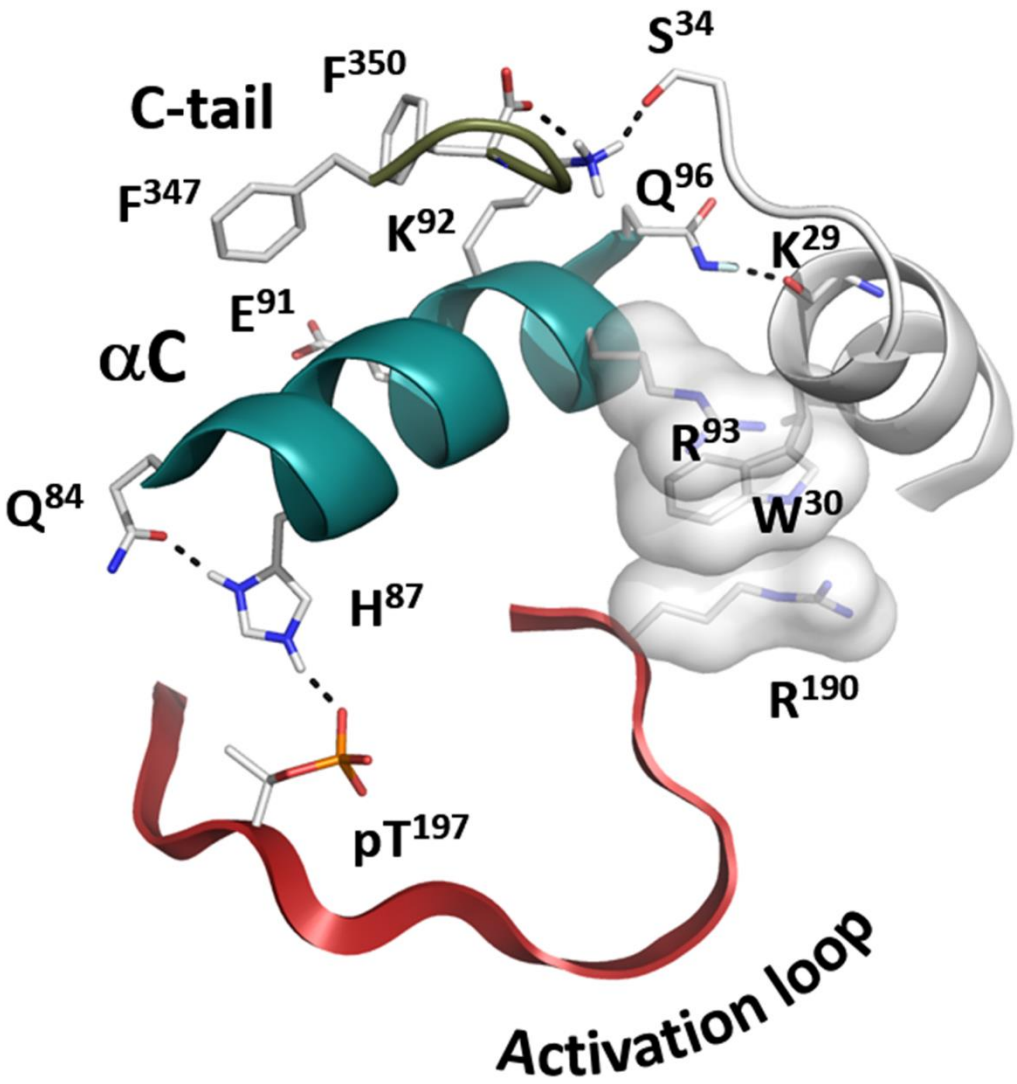

Fig. S5. The turn of the αC helix, Glu[91]-Lys[92]-Arg[93], is a signal integration motif. It anchors the N-lobe to the hydrophobic motif in the C-tail and to the activation loop through the αA helix.

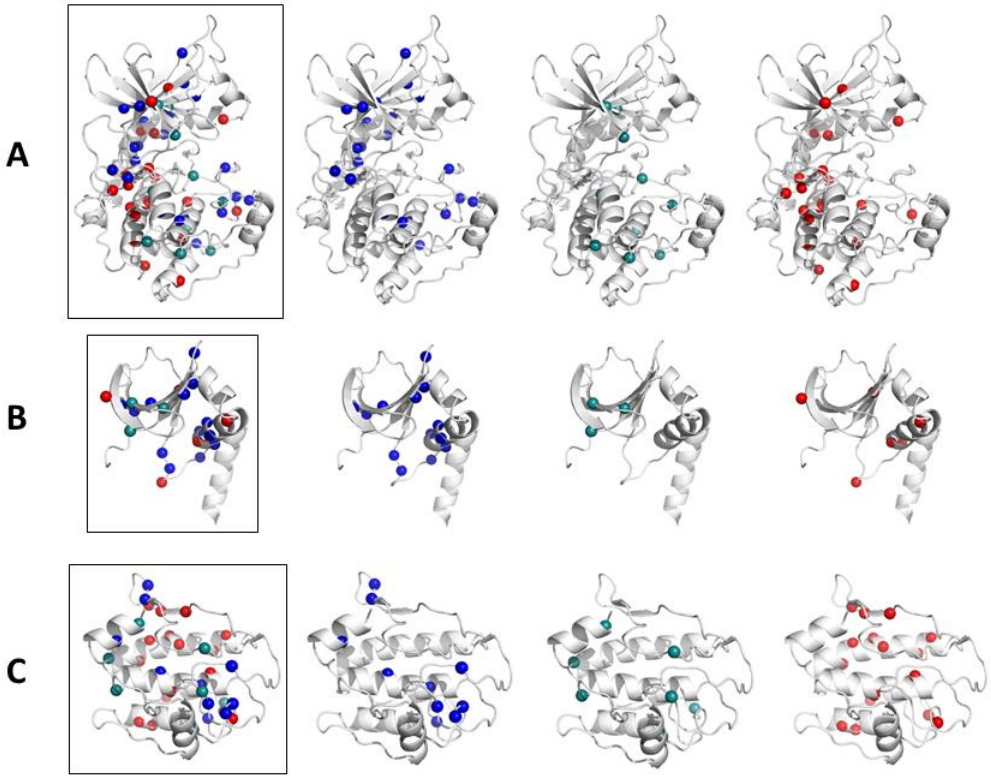

Fig. S6. Distribution of the identified backbone amides deconvoluted according to the occupancy of corresponding D atoms and structure. The amide groups are represented as spheres and color-coded according to the occupancy of the corresponding deuterium, D, atom: blue are amides with the D occupancy less than 0 and green with 0.01 to 0.5, both are classified as slow-exchanging and are the same in the NMR dataset; red amides have the occupancy of 0.51 to 1, classified as fully exchanged and are different compared to the NMR dataset. Panel (**A**) shows distribution of the backbone amides in the entire molecule, whereas panels (**B**) and (**C**) focus on the N-lobe and on the C-lobe, respectively.

Table S1. Stable water molecules. Comparison conserved water molecules identified in Knight, *et. al.* paper (*29*).

Water label		Corresponding water numbers in structures		Corresponding figure - contacts
Current structure	Ref. 29	Neutron Structure of the product (6E21)	X-ray structure, of the substrate (1ATP)	Possible Role
W15	A	1135	400	**Figure 2A and S4B** – anchors the side chain of E91 to F185 amide backbone, the RS2 of R-spine residue and contributes to the Lys72-Glu91 salt bridge that anchors the α and β phosphates to the αC helix
				Helps to define E91-K92-R93 – as a "Signal Integration Motif"
W18	B	1085	404	**Figure S4** - anchors the backbone of L106 and L95 residues (two R-spine residues)
				Contributes to R-spine assembly
W19	C	1023	403	**Figure S4B** – anchors the carbonyl backbone of Y164, the RS1 R-spine residues, and of D184 to the amide backbone of F187
				Helps to define HRD as a "Signal Integration Motif"
W12	D	1017	410	**Figure 1A and S4B** – Bridges the side chain of catalytic residue D166 and the backbone carbonyl of L167 anchored to αF helix. (molecule W12 in Fig. 1A)
				Stabilizes the catalytic loop
W20	E	1025	431	**Figure S4B** – links backbone amide of L167 to the side chain of D220
				Helps to anchor catalytic loop to the αF helix
W21	F	1001	568	**Figure 5C and D** – ties W222 to the main chain carbonyls of L205 and E203
				Anchors R280:E208 node to P+1 loop and defines D220-W222 as a "Signal Integration Motif"

Table S2. Backbone H/D exchange comparison. Residues with very slow-exchange patterns as identified by NMR in the PKA-C:Mg_2ADP:PKI complex are compared with the same set in the neutron diffraction structure categorized by the occupancy of the corresponding deuterium atom. (**A**) Residues with slow-exchange rates in the NMR structure. (**B**) Deuterium atom occupancies of these residues in the neutron diffraction structure of PKA-C:Sr_2ADP:p-PKS.

A. NMR: PKA-C:Mg_2ADP:PKI, 55 residues with $\varphi < 0.4$

47, 50, 58, 59, 69, 71, 72, 74, 84, 91, 92, 94, 95, 96, 97, 98, 102, 103, 104, 112, 116, 117, 128, 132, 138, 144, 146, 149, 150, 155, 157, 166, 174, 175, 179, 180, 182, 199, 205, 206, 208, 209, 210, 220, 221, 225, 227, 233, 266, 267, 274, 280, 283, 284, 294

B. Neutron: PKA-C:Sr_2ADP:pPKS, D atom occupancies for the same residues

33 identical residues with similarly low D atom occupancies		22 fully exchanged: occupancies > 0.5
23 with occupancies < 0	10 with occupancies < 0.5	
59, 69, 71, 74, 91, 92, 94, 96, 98, 103, 104,112, 116, 132, 174, 175, 199, 206, 209, 210, 221, 227, 280	50, 58, 72, 128, 138, 166, 205, 233, 274, 283	47*, **84**, **95**, **97**, **102**, **117**, 144#, 146*, **149**, **150**, **155**, 157*, **179**, **180**, 182#, **208**, **220**, **225**, 266*, **267**, **284**, **294**

Residues highlighted in bold are those for which the φ value increases in apo and/or binary compared to ternary

* For these residues φ value was determined in ternary but not in apo and binary states

For these residues φ value doesn't change between ternary and apo

Table S3. Room temperature crystallographic data collection and joint XN refinement statistics.

	PKAc-Sr$_2$ADP-pSP20 PDB ID 6E21	
Data collection:	**Neutron**	**X-ray**
Beamline/Facility	LADI-III/ILL	Rigaku HighFlux Home Lab
Space group	$P2_12_12_1$	
Cell dimensions:		
a, *b*, *c* (Å)	59.332, 79.898, 100.292	
α, β, γ (°)	90.00, 90.00, 90.00	
Resolution (Å)	10.00-2.50 (2.64-2.50) *	40.00-2.00 (2.07-2.00)
No. reflections measured	35466 (1965)	127950
No. reflections unique	10426 (1055)	30175 (2945)
R_{merge}	0.153 (0.181)	0.069 (0.542)
I/σ	7.7 (5.2)	14.3 (1.9)
Completeness (%)	62.0 (43.6)	91.7 (91.0)
Redundancy	3.4 (1.9)	4.2 (4.2)
Joint XN Refinement:		
Data rejection criteria	No observation & |F|=0	
Sigma cut-off	3.0	
No. reflections	10012	22849
R_{work}/R_{free}	0.226/0.275	0.187/0.217
No. atoms:		
Protein including H and D	5549	
Ligand, pSP20	307	
Ligand, ADP	39	
Metal	2	
Water	456	
B-factors:		
Protein	29.6	
Ligand, pSP20	26.7	
Ligand, ADP	22.7	
Metal	19.6	
Water	38.2	
R.m.s. deviations:		
Bond length (Å)	0.008	
Bond angles (°)	1.011	

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

Table S4. The software used in data collection and refinement.

The software and/or model	Reference
the Daresbury Laboratory *LAUE* suite program *LAUEGEN*	41, 42
LSCALE	43
SCALA	44
HKL3000	45
CCP4	46
starting model: PDB ID 4IAY	47
SHELX-97	48
nCNS	49
Coot	50