Dynamics connect substrate recognition to catalysis in protein kinase A

Larry R Masterson1,2, Cecilia Cheng3, Tao Yu4, Marco Tonelli5, Alexandr Kornev3, Susan S Taylor3* & Gianluigi Veglia1,2*

Atomic resolution studies of protein kinases have traditionally been carried out in the inhibitory state, limiting our current knowledge on the mechanisms of substrate recognition and catalysis. Using NMR, X-ray crystallography and thermodynamic measurements, we analyzed the substrate recognition process of cAMP-dependent protein kinase (PKA), finding that entropy and protein dynamics play a prominent role. The nucleotide acts as a dynamic and allosteric activator by coupling the two lobes of apo PKA, enhancing the enzyme dynamics synchronously and priming it for catalysis. The formation of the ternary complex is entropically driven, and NMR spin relaxation data reveal that both substrate and PKA are dynamic in the closed state. Our results show that the enzyme toggles between open and closed states, which indicates that a conformational selection rather than an induced-fit mechanism governs substrate recognition.

RESULTS
Malleability of the X-ray ternary complex
Very few protein kinases have been crystallized with natural peptide substrates and even fewer with protein substrates5. Here we were able to crystallize PKA-C in complex with 5′-adenylyl-β,γ-imidodiphosphate (AMP-PNP) and a substrate peptide at 2.8-Å resolution (Fig. 1a,b and Supplementary Table 1). The peptide we used corresponded to residues 1–19 of PLN (PLN1–19)5, which composes the PKA-C recognition site (R_{13RAST17}). We found that nucleotide binding promotes synchronous motions among residues surrounding the active site that correlate with opening and closing of the enzyme's active site cleft. In the ternary complex, some of these dynamics are redistributed, but motions persist around the active site. Although these dynamics are not correlated to the chemical step (phosphoryl transfer), they occur on the same timescale as the rate-determining step of enzyme turnover5. Both NMR spectroscopy and X-ray crystallography indicated that the substrate, PLN, adopts an extended and dynamic conformation at the binding groove. The presence of dynamics in the substrate and at the active site of the enzyme cannot conform to an induced-fit recognition model but rather exemplify the conformational selection model of recognition5,19. Thermodynamic analysis confirmed that the binding events are entropically driven, providing further support for this mechanism. The coordinated motions that open and close the cleft of the enzyme underscore the role of conformational dynamics in the slow step of catalysis.

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P Hosphorylation via cAMP-dependent protein kinase A (PKA) is a ubiquitous signaling mechanism that regulates many cellular processes3. PKA targets a diverse array of substrates, including proteins localized in the cytoplasm, mitochondria, plasma membrane, sarcoplasmic reticulum membrane, nucleus, microtubules and actin filaments2. The architecture of this enzyme consists of a bean-shaped core that is conserved throughout the protein kinase family3. This core has two lobes that flank the active site: a small lobe formed by β-strands at the N terminus, which is primarily associated with binding and positioning ATP, and a large lobe that provides a docking surface for substrates or inhibitor proteins1. Most of the atomic resolution information on the catalytic subunit of PKA (PKA-C) is derived from X-ray crystallographic studies, which have mapped three major forms: apo (open state), nucleotide bound (closed state or binary complex) and nucleotide/inhibitor bound (closed state or ternary complex)4. The crystal structure of the closed state, which mimics the Michaelis complex has only been captured with kinase inhibitors (peptides or drugs) and in excess (inhibitory) concentrations of Mg2+ ions, precluding our understanding of the substrate recognition process. This problem is common to all protein kinases, whose crystal structures are trapped in inactive states with potent inhibitors. More importantly, information11,16 on protein kinase dynamics—the equilibrium fluctuations that enable the exploration of the free energy landscape—is scarce. These fluctuations influence enzymatic reactions because they are responsible for molecular recognition events involved in enzyme-ligand binding17,18 or can regulate the catalytic cycle by limiting access to certain conformations that allow turnover12,14,16.

Using a combination of NMR spectroscopy and X-ray crystallography, we provide a vivid picture of PKA-C substrate recognition along the reaction coordinates for the open, intermediate and closed conformational states, mapping out the dynamic landscape on fast (ps–ns) and slow (μs–ms) time scales. As a substrate, we used a peptide corresponding to the cytoplasmic domain of phospholamban (PLN), a single-pass membrane protein that inhibits the sarcoplasmic reticulum membrane calcium ATPase16. We found that nucleotide binding promotes synchronous motions among residues surrounding the active site that correlate with opening and closing of the enzyme's active site cleft. In the ternary complex, some of these dynamics are redistributed, but motions persist around the active site. Although these dynamics are not correlated to the chemical step (phosphoryl transfer), they occur on the same timescale as the rate-determining step of enzyme turnover16. Both NMR spectroscopy and X-ray crystallography indicated that the substrate, PLN, adopts an extended and dynamic conformation at the binding groove. The presence of dynamics in the substrate and at the active site of the enzyme cannot conform to an induced-fit recognition model but rather exemplify the conformational selection model of recognition15,19. Thermodynamic analysis confirmed that the binding events are entropically driven, providing further support for this mechanism. The coordinated motions that open and close the cleft of the enzyme underscore the role of conformational dynamics in the slow step of catalysis.
In the apo PKA-C molecule, the large lobe is packed against the active site face of the substrate-bound molecule, with PLN1–19 sandwiched between the two PKA-C molecules (Fig. 1a and Supplementary Fig. 2a,b). The apo conformation of PKA-C trapped in this complex is consistent with a previous apo structure1, where both lobes of the enzyme are disengaged from each other.

In the ternary complex, the PLN1–19 recognition sequence (residues 12–17) is clearly positioned in the active site groove between the lobes of PKA-C (Fig. 1b). As expected for a catalytically competent conformation, the P-site hydroxyl group of Ser16 in PLN1–19 is aligned to accept the γ-PO4 from AMP-PNP, similar to what is observed in the PKA-C crystal structure mimicking the transition state (Supplementary Fig. 2a). The electron densities of the PLN1–19 side chains were well defined at the active site, with a clear network of ionic interactions at the recognition site (Supplementary Fig. 2b). Specifically, the P – 2 and P – 3 arginine residues in PLN1–19 may form hydrogen bonds with Glu127 and Glu230 or Glu170 in PKA-C, respectively, in a manner similar to PKA–inhibitor structures5. In addition, two key backbone interactions between the glycine-rich loop and C terminus, which is likely because of conformational disorder.

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The crystal structure of the ternary complex indicated dynamic disorder at the small lobe when PKA-C is bound to the phosphorylatable substrate, PLN1–19. PKA-C was missing electron density for parts of the N (residues 1–16) and C termini (residues 339–342), as well as the conserved glycine-rich loop (residues 47 and 52–54) that positions the γ-phosphate. The unstructured substrate was also missing the first four residues at the N terminus and the last two residues at the C terminus. Strikingly, the B-factors of the small lobe in the ternary complex were significantly higher than in the apo form (Supplementary Fig. 3a,b). These data indicated higher malleability in the closed state relative to the open, unligated state of PKA-C.

### Supplementary Table 1 | Thermodynamic parameters for the binding of PKA-C to PLN1–19 in the presence or absence of nucleotide

<table>
<thead>
<tr>
<th>Configuration</th>
<th>ΔH (kcal mol⁻¹)</th>
<th>ΔS (kcal mol⁻¹ K)</th>
<th>−TΔS (kcal mol⁻¹)</th>
<th>Kd (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo form</td>
<td>−0.6 ± 0.1</td>
<td>0.017 ± 0.001</td>
<td>−5.1 ± 0.2</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>Binary complex</td>
<td>−1.2 ± 0.1</td>
<td>0.019 ± 0.001</td>
<td>−5.7 ± 0.1</td>
<td>10 ± 4</td>
</tr>
</tbody>
</table>

Data represent mean values ± one s.d. for triplicate measurements.
apo to the ternary complex (Supplementary Table 2), indicating that the entire enzyme is more compact upon ligand binding, in agreement with previous findings. However, changes in the H-X NOE and $R_e$ values in the binary and ternary complexes indicated an increase in backbone dynamics of the enzyme on fast and slow timescales, with a redistribution of motion throughout the backbone.

The apo form is monomeric and well folded, with a trimmed H-X NOE average value of 0.76 (Supplementary Table 2) and an effective rotational correlation time ($\tau_\text{r}$) of ~19 ns. Using H-X NOE as a proxy for the fast dynamics (ps–ns timescale), we found that the most relevant features are the marked dynamics (NOEs <0.6) in residues located between structural elements of the enzyme (Fig. 3a and Supplementary Fig. 5a). In particular, low NOE values were detected for all of the conserved loops, including the glycine-rich, catalytic, DFG, activation and P + 1 loops. Low H-X NOE values were also detected for residues within the acidic cluster (residues 328–334). In this region, Tyr330 forms electrostatic interactions with an essential arginine of the substrate recognition sequence (Fig. 3a and Supplementary Fig. 5a).

Surprisingly, very little conformational exchange is present in the apo form on the μs–ms timescale ($R_e$) (Fig. 3b and Supplementary Fig. 5a). The lack of these slow dynamics is also supported by analysis of the NMR inverse peak heights measured at various temperatures (Supplementary Fig. 6). Relatively small $R_e$ values were measured throughout the entire backbone, with the majority of values distributed around zero. Specifically, the small lobe does not show marked conformational exchange. Only a few residues interspersed throughout the large C-lobe show $R_e > 10$ Hz.

Saturation of PKA-C with the nucleotide (AMP-PNP) changed the backbone dynamics throughout the enzyme. Although a slight overall restriction of the protein backbone motion occurred (~0.03 increase in the average H-X NOE), increases in both fast and slow dynamics were detected for regions at the active site cleft (Fig. 3a,b and Supplementary Fig. 5b). This was apparent for the glycine-rich and the DFG loops, which are directly involved in nucleotide binding, as well as the activation loop, the P + 1 loop and the acidic cluster (Supplementary Fig. 5b). Other loops not directly involved in catalysis showed an increase in fast dynamics, such as the loops linking helices F and G. Other segments peripheral to the catalytic loops became more dynamically restricted.

Dynamics on a μs–ms timescale were pervasive in the presence of nucleotide, indicating conformational fluctuations in the binary complex (Fig. 3b and Supplementary Fig. 5b). Large changes in $R_e$ at the active site cleft were observed in the glycine-rich, DFG, activation and P + 1 loops, as well as in the C-helix. Notably, the C-helix residues lying on the face oriented toward the nucleotide-binding pocket showed higher $R_e$ values than those populating the opposite face (Supplementary Fig. 7). The conformational exchange rates detected for these residues likely reflect recruitment of the C-helix to the active site, which is a critical part of activation. Conformational exchange is also detected in regions far from the active site, such as the segments that flank the G-helix and the segment that links the conserved APE motif to the F-helix (Fig. 3b). The augmentation of dynamics was also propagated toward the C-terminal tail (residues 322–350), whose putative role is to position the small lobe for catalysis.

Upon saturation with the substrate, the ternary complex remained dynamic, with a redistribution of fast dynamics throughout the PKA-C backbone. For the glycine-rich loop, we observed a monotonic increase in fast dynamics, with the average NOE value dropping from ~0.8 in the apo, to ~0.7 in the binary complex, to ~0.5 for the ternary complex (Fig. 3a and Supplementary Fig. 5c). However, a significant reduction in fast dynamics was detected for residues at the active site located in the P + 1, DFG and activation loops. Subtle changes occurred throughout the enzyme, indicating that the entire backbone dynamics had reorganized after substrate binding. The reorganization of dynamics in the ternary complex was also apparent by the redistribution of μs–ms conformational exchange for many regions (Fig. 3b). For instance, the conformational exchange detected for the C-helix in the binary complex was quenched in the ternary complex, whereas the residues forming the base of the C-spine (Ile174, Met128 and Met231) became more dynamic. Large $R_e$ values were observed for residues throughout the small lobe in the ternary complex. Notably, $R_e$ values >25 Hz were observed in two regions intimately involved in the catalytic cycle, the glycine-rich and activation loops. The observed slow dynamics in these regions may reflect the conformational changes required for opening the active site for product release, the proposed rate-limiting step in the enzyme turnover.

Dynamics are synchronous with the slow step of catalysis
To determine whether the conformational exchange reports on opening and closing of the enzyme, we plotted $R_e$ versus the squared
difference of chemical shifts ($\Delta \omega$) between the apo and ternary complex (open and closed states) (Fig. 4a, b; see Supplementary Fig. 8 for full labeling of peaks)28. In the fast exchange limit, if a set of nuclear spins is affected by the same conformational exchange process (for instance, open-to-closed state transitions), a linear correlation is expected between $R_{ex}$ and $\Delta \omega^2$. Indeed, we found a linear correlation (correlation coefficient of 0.9) for many residues around the active site cleft upon addition of AMP-PNP (Fig. 4c). These residues were located in the conserved glycine-rich, DFG, activation and P + 1 loops, which line the entrance to the enzyme active site. Analogous to triosephosphate isomerase28, this linear correlation suggested that the motions of these residues report on the same exchange process ($k_{ex}$), which was the opening and closing of the enzyme active site. From the slope of the linear plot and the populations of free and bound states, it is possible to determine the exchange constant for the process, $k_{ex} = 203 \pm 18$ s$^{-1}$. This process was much slower than that of ligand (AMP-PNP) exchange between the free and bound states ($k_{on}[AMP-PNP] + k_{off}$), which we measured for phosphorylation of PLN$_1$–$20$ using a coupled enzyme assay ($\sim 23$ s$^{-1}$), indicating that the slow step of catalysis is dominated by the opening and closing of the enzyme18,29.

Allosteric network of PKA-c shows noncontiguous pathways

The dynamics induced by ligand binding proceeded through contiguous paths throughout the active site (Figs. 3b and 4c, residues colored in orange), where nearby residues are within van der Waals radii contact. However, noncontiguous effects occurred in remote regions of the enzyme (Fig. 4d, residues colored in red, Supplementary Fig. 8) that were not synchronous with the opening and closing motions. These dynamics are likely to report on conformational fluctuations other than the toggling between open and closed states and underscore the complexity of the allosteric network within the enzyme and its ability to funnel into dynamical different states. Other functional events such as binding to A-kinase anchoring proteins (AKAPs) and/or regulatory subunits may be correlated to these motions. In fact, a number of residues dynamically activated in the ternary complex are distal from the substrate-binding site and make close intermolecular contacts upon assembly with the type-I regulatory subunit30 (Fig. 4d). Therefore, the complexity of the dynamics we observed throughout
the backbone of PKA-C could be attributed to its ability to act as a scaffold for many other interactions within the cell.

**DISCUSSION**

The enzymatic mechanism of phosphoryl transfer for PKA-C has been extensively studied. Catalysis by PKA-C comprises three major steps: ligand (ATP and substrate) binding, chemical step (phosphoryl transfer) and product release. The chemical step is fast, whereas the product release constitutes the rate-determining step of the catalytic cycle. It has been hypothesized that the latter depends on the conformational transitions of the enzyme to eject both substrates in crystal structures with PKA-C. However, it should be noted that the recognition of PLN1–20 is entropically driven and governed by conformational selection rather than by an induced-fit mechanism.

The dynamic nature of the ternary complex is complemented by the X-ray crystal structure, in which electron density is absent or poor for several dynamic regions highlighted by NMR. These regions are located in the enzyme (particularly the glycine-rich loop at the active site) and substrate (terminal regions). This phenomenon may explain why, in general, it has been so difficult to trap peptide substrates in crystal structures with PKA-C. However, it should be aimed to understand the enzyme conformational transitions of the enzyme to eject both substrates in crystal structures with PKA-C. However, it should be noted that the recognition of PLN1–20 is entropically driven and governed by conformational selection rather than by an induced-fit mechanism.

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| Table 2 | Observed rate constants measured using the $R_{ex}/\Delta\delta^2$ correlation plot or from exchange of nucleotide involving NMR line shape analysis during ligand titration measurements |
|---|---|---|
| Observed kinetic constant | Ligand exchange* | Conformational exchange |
| $k_{on}$ (s$^{-1}$) | $3.37 \times 10^6 \pm 0.51 \times 10^6$ | $203 \pm 18$ |
| $k_{off}$ (s$^{-1}$) | $109 \pm 4$ | - |
| $k_{on}$ (s$^{-1}$)$^*$ | $3.36 \times 10^5 \pm 0.50 \times 10^5$ | - |
| $k_{conf}$ (s$^{-1}$)$^*$ | - | $10 \pm 2$ |
| $k_{conf}$ (s$^{-1}$)$^*$ | - | $193 \pm 17$ |

Values represent fitted values and associated errors from linear least-squares analysis (conformational rates, denoted 'conf') or mean ± one s.d. for six residues (ligand exchange rates, denoted 'ligand').

*The observation of a pseudo-first-order rate constant for $k_{on}$ is expected (for instance, $k_{on} = k_{off}$ (Ligand)).
Figure 5 | Model for the mechanism of the formation of a catalytically competent ternary complex. The apo form contains the C-spine residues (red), which are disengaged from the two lobes. Nucleotide binding completes the C-spine architecture and induces the conformational changes throughout the enzyme. The conformational fluctuations (opening and closing) present in the ternary complex limit the rate of catalysis.

noted that substrate recognition by this enzyme appears to be quite complex and is likely influenced by how the enzyme is funneled into a particular functional state. In fact, much stronger-binding peptides or drugs that inhibit PKA-C may invoke an induced-fit mechanism upon binding, when increased order and decreasing dynamics place the enzyme in an inhibitory state. This is likely the case with the inhibitor PKI5–24, which crystallized with PKA-C with a well-defined active site.

On the basis of the crystal structures and our current NMR analysis, it is possible to propose a general mechanism for events leading to catalysis (Fig. 5). We propose that formation of the Michaelis complex in PKA-C occurs mostly through a conformational selection mechanism, in which both open and closed states are populated by the enzyme, and ligand binding changes the population of these states. The apo enzyme is dynamically inert on the μs–ms timescale, with the two lobes of the enzyme disengaged. This is supported by the absence of conformational exchange detected by NMR and the relatively low B-factors of the apo enzyme crystal structure from this study. As we observe a linear behavior for \( k_{\text{obs}} \) and the \( ^{1}N \) chemical shift changes between the closed and open states, the apo enzyme likely contains very small populations of closed conformations, which were not detected because they may occur on a timescale that the NMR measurements were not able to probe. Nucleotide binding activates the enzyme both dynamically and allosterically. The nucleotide completes the C-spine architecture and engages the small and large lobes. This interaction functions as a pivot point for the two domains and induces the μs–ms conformational dynamics detected by NMR (Fig. 5). Subsequent changes in conformation and dynamics upon nucleotide binding prime the active site for catalysis. These changes are not likely to occur without the completion of the C-spine, as shown by a number of mutations, which disrupted this region and led to decreased catalytic activity. Crystallization of one of these mutated enzymes resulted in an open state under conditions in which a closed state is typically observed. In our work, we observe opening and closing of the enzyme in the presence of the nucleotide. This opening-closing equilibrium affords the enzyme more effective substrate binding (Fig. 5), which results in the cooperative enhancement in the binding affinity we measured.

An outstanding question that we addressed in this study is whether the backbone dynamics of the ternary complex correlate with any of the catalytic steps. To answer this, we analyzed all of the residues that experience conformational exchange in the ternary complex. We found that the highly conserved loops (glycine-rich, DFG, activation and P + 1 loops) as well as other catalytically important regions of the complex open and close in a synchronous manner. Moreover, the rate constant for opening the cleft (\( k_{\text{obs}} \)) measured in the ternary complex correlates well with the kobs measured for PLN1–19 phosphorylation. The presence of these dynamics in the ternary complex could constitute an entropic driving force for the enzyme to release the products once phosphorylation occurs, a process that has been identified as the slow step in catalysis. Therefore, the conformational fluctuations of the ternary complex probed by NMR correlate well with motions that limit enzyme turnover.

Although there is still an ongoing debate on the role of dynamics in the chemical step of enzyme catalysis, recent reports show a prominent role for conformational dynamics in substrate recognition and product release. Perhaps nowhere is this more apparent than in the highly regulated protein kinases. These conformational dynamics act locally and allosterically to tune the affinity and selectivity of enzymes, signaling proteins and receptors. In the case of PKA-C, the entire enzyme (catalytic loops, signal integration and core elements) is dynamic in all the major forms (apo, binary complex and ternary complex) that mimic the transitions through the catalytic cycle. These dynamics keep the enzyme from falling into inertia (dynamically uncommitted) states. The latter is reminiscent of the energetic counterweight hypothesis proposed for the adenylate kinase.

When bound, the substrate is also quite dynamic, possibly underlying a reciprocal dynamic adaptation between the two binding partners: although their conformations are held in one another, they remain dynamic before the catalytic event. This implies that the Michaelis complex trapped in this study is not a ‘tight’ structural complex (enthalpy driven) but remains ‘dynamically loose’ (entropically driven), as suggested earlier, and undergoes motions synchronous with the slow step of catalysis. This reciprocal dynamic adaptation of substrate and enzyme might be key for the ability of PKA-C to recognize a variety of substrates that typically exist as part of a macromolecular complex.

Recent papers highlight the importance of conformational entropy to the overall entropy of binding for both peptide substrates and drugs. For protein kinases, the role of conformational entropy in the context of drug design is relatively unexplored. The results presented here indicate that conformational entropy weighs significantly in the overall free energy of binding and must be taken into consideration for protein kinase drug design.

METHODS

Sample preparation. PKA-C was expressed and purified as described previously and PLN1–19 was expressed as in ref. 21. Peptide synthesis of PLN1–20 was performed using standard Fmoc chemistry on an Applied Biosystems Pioneer or CEM Liberty/Discover microwave synthesizer (for details see Supplementary Methods) and HPLC purified using a semipreparative C18 reversed-phase cartridge (Waters). Purities of pooled fractions were >95% as assessed by analytical HPLC (Vydac) and verified by ESI-MS (calculated 2,252.6 m/z, found 2,251.8 m/z). Activity of PKA-C was determined using steady-state enzymatic phosphorylation of Kemptide measured spectrophotometrically at 299 K (see details in the Supplementary Methods). Steady-state phosphorylation of full-length PLN and PLN1–19, was performed similarly and described in the Supplementary Methods.

X-ray crystallography. The PKA-C–AMP-PNP–PLN1–20 complex was obtained by combining a 1:10:10 molar ratio mixture of PKA-C (7 mg ml−1), PLN1–20, MgCl2 and AMP-PNP in 20 mM sodium acetate (pH 6.5), 180 mM KCl and 5 mM DTT. Crystals were harvested, transferred to mother liquor consisting of 15% glycerol, then flash frozen in liquid nitrogen. X-ray diffraction data was collected at the Advanced Light Source (Lawrence Berkeley National Laboratory) on beamline 2.2.1. Diffraction data were processed and scaled with HKL2000 (ref. 46) to 2.8 Å resolution.
The initial data clearly fit to a primitive hexagonal lattice, and the final data were integrated and scaled in the P6, space group. Initial phases were generated by molecular replacement using the coordinates of the PKA-C–ATP–PKI-5–24 complex without the PKI peptide (PDB: 1ATP) as a search model. The ternary complex crystallized with two molecules in the asymmetric unit (Z-scores of 26.5 and 38.7) corresponding to a solvent content of 52%. The first molecule corresponds to a ternary complex containing PKA-C, AMP-PNP-Mg2+ and PLN, and the second molecule corresponds to the apo form. The final R- and R-free values after data refinement (details in the Supplementary Methods) were 21.6% and 28.2%, respectively. The final model contained PKA-C residues 16–350 bound to AMP-PNP, two Mg2+ ions and PLN residues 5–15 in the first molecule and the apo form of PKA-C with residues 13–350 in the second molecule. Optimization of crystallization conditions and full details of data refinement are provided in the Supplementary Methods.

ITC measurements. ITC data were acquired on an MCT-ITC microcalorimeter (MicroCal Inc.). Stock solutions of PKA-C and PLN, were dissolved in 20 mM phosphate buffer solution (pH 6.5) containing 180 mM KCl and 4 mM MgCl2, and degassed. Titrations were conducted at 27 °C using 0.1 mM PKA-C in the absence or presence of 6 mM AMP-PNP and with a stock of synthetic PLN, (1.8 mM). The samples were stirred at 410 r.p.m. We used 20 injections separated by an equilibration period of 300 s (5 µl for the first, 10 µl for each of the remaining). A one-site binding model was assumed, the data were fit using MicroCal Origin software (version 5.0) and all data were repeated in triplicate.

NMR spectroscopy and data analysis. Typical NMR samples consisted of –500 µM PKA-C, 10 mM MgCl2, 90 mM KCl, 20 mM KH2PO4, 10 mM octanol-N-methylglucamide (MEGA-8), 20 mM DTT and 5% (v/v) 2H2O (uncorrected pH = 6.5). For the binary complex, PKA-C was saturated with 12 mM AMP-PNP, and the ternary complex was obtained by saturating the binary complex with 700 µM PLN, Solution NMR experiments were carried out on Varian instruments operating at 600.14 or 800.29 MHz. H’Larmor frequency, using an inverse-triple-axial gradient cryoprobe at 33 °C. The data were processed with NMRPIPE24 and all data were repeated in triplicate. The value <ωex> was measured using the method introduced by Palmer25 and references 26–28 and 32.

H-X NOE decay, and errors were taken from repeats of single data points. H-X NOE experiments were performed according to ref. 48, with TROSY detection49. Rρ and Rρβ were calculated according to fitting intensities to a single-exponential decay, and errors were taken from repeats of single data points. H-X NOE values were taken as the ratio of intensities from experiments performed with and without saturation. Errors in the H-X NOE were calculated via error propagation using root mean square noise of the spectra. Rρ was measured using the method introduced by Palmer25, with a Hahn echo period of 27T1/2 (10.8 ms). Peak intensities were used to determine Rρ according to refs. 24,25:

\[ R_{\rho} = \frac{C_{\rho}}{C_{\rho}^0} = \frac{\ln(\rho_\alpha) + \ln(\rho_b)}{\ln(\rho_\alpha) - \ln(\rho_b)} \]  

where Cρ = (ρα + ρβ) / (ρα − ρβ) and Cρ0 = (κ + 1) / (κ − 1). The intensities of the α, β and z spin states (Iα, Iβ and Iz) were obtained from two-dimensional experiments recorded in triplicate. The value <κ> was obtained from the trimmed mean of amides not showing chemical exchange. Errors in peak intensities were calculated by fitting intensities to a single-exponential decay and, if appropriate, were taken from repeats of single data points. H-X NOE values were calculated as the ratio of intensities from experiments performed with and without saturation. Errors in the H-X NOE were calculated via error propagation using root mean square noise of the spectra.

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Author contributions
L.R.M., C.C., T.Y., A.K., S.S.T. and G.V. designed experiments, analyzed data and wrote the paper, and L.R.M., C.C., T.Y. and M.T. performed experiments.

Competing financial interests
The authors declare no competing financial interests.

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