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Structural basis for an inositol pyrophosphate kinase surmounting phosphate crowding

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Abstract

Inositol pyrophosphates (such as IP7 and IP8) are multifunctional signaling molecules that regulate diverse cellular activities. Inositol pyrophosphates have ‘high-energy’ phosphoanhydride bonds, so their enzymatic synthesis requires that a substantial energy barrier to the transition state be overcome. Additionally, inositol pyrophosphate kinases can show stringent ligand specificity, despite the need to accommodate the steric bulk and intense electronegativity of nature’s most concentrated three-dimensional array of phosphate groups. Here we examine how these catalytic challenges are met by describing the structure and reaction cycle of an inositol pyrophosphate kinase at the atomic level. We obtained crystal structures of the kinase domain of human PPIP5K2 complexed with nucleotide cofactors and either substrates, product or a MgF_3^- transition-state mimic. We describe the enzyme’s conformational dynamics, its unprecedented topological presentation of nucleotide and inositol phosphate, and the charge balance that facilitates partly associative in-line phosphoryl transfer.

The inositol phosphates initially came to prominence as second messengers following the discovery that inositol 1,4,5-trisphosphate (IP3) releases Ca^{2+} from the endoplasmic reticulum¹. Since then, it has become widely recognized that IP3 is metabolized to form a large number of additional inositol phosphates that also function as cellular signals². More recently, the size of this signaling family was further expanded with the discovery that some of its members contain diphosphate groups^{3,4}. These so-called inositol pyrophosphates include diphosphoinositol penta-kisphosphate (IP7) and bisdiphosphoinositol tetrakisphosphate (IP8). There is now evidence that IP7 and IP8 regulate many cellular processes in eukaryotes, including apoptosis, vesicle trafficking, cytoskeletal dynamics,

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Author contributions

H.W., T.M.T.H. and S.B.S. designed experiments and wrote the manuscript. H.W. and S.B.S. performed the experiments. J.R.F. synthesized substrate for hPIP5K2.

Competing financial interests

The authors declare no competing financial interests.

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exocytosis, insulin signaling and neutrophil activation (reviewed in refs. 5–7 and also refs. 8,9).

The presence of diphosphate groups is a key, functionally important feature of the inositol pyrophosphates. It is the diphosphates that facilitate the ability of these molecules to compete with phosphatidylinositol-3,4,5-trisphosphate for binding to pleckstrin homology domains^{8,9}. This phenomenon seems to explain how IP7 can antagonize signaling by insulin⁹ and also regulate neutrophil activity⁸. There has also been one description of a more classical IP7 ‘receptor’: the Pho80–Pho85–Pho81 cyclin-dependent kinase complex¹⁰. IP7 augments the ability of Pho81 to inhibit kinase activity, and the ligand’s diphosphate group contributes to the specificity of the interaction¹⁰. The diphosphate groups can also be used for the nonenzymatic phosphorylation of a range of different proteins¹¹. This particular mechanism may help explain the multifunctionality of inositol pyrophosphate signaling.

The enzymatic synthesis of inositol pyrophosphates requires the formation of high-energy phosphoanhydride bonds¹², even though they are in a class of molecules that is unrivaled throughout nature in its degree of phosphate congestion. Thus, the active site of an inositol pyrophosphate kinase must accommodate considerable steric bulk and intense electronegativity yet retain selective substrate specificity^{13,14}. Moreover, this group of kinases must also overcome a substantial energy barrier to the transition state. There are two groups of enzymes that synthesize inositol pyrophosphates: inositol hexakisphosphate kinases 1, 2 and 3 (IP6K1, IP6K2 and IP6K3; ref. 15) and diphosphoinositol pentakisphosphate kinases 1 and 2 (PPIP5K1 and PPIP5K2; refs. 13,16); in yeasts, these groups of proteins are named Kcs1 (ref. 17) and Vip1 (ref. 18), respectively. These two groups of enzymes collaborate in the synthesis of IP8 through two complementary metabolic pathways. In one, IP6 is phosphorylated by IP6K, forming an isomer of IP7 with a diphosphate group at the 5 position (that is, 5-IP7)¹⁹. Next, 5-IP7 is further phosphorylated by PPIP5K to form IP8 (ref. 20). In the alternate pathway, IP6K adds a 5-phosphate to a second isomer of IP7, which is formed from IP6 by PPIP5K¹⁹. Previous analysis of inositol pyrophosphate structures¹⁹ have determined that this alternative IP7 isomer has a diphosphate at either the 1 or 3 position; the ambiguity reflects difficulties in discriminating between alternative products that are enantiomers. As it has not hitherto been demonstrated whether PPIP5Ks are 1- or 3-kinases, the exact structure of IP8 has also not previously been established.

The PPIP5K enzymes are of particular interest for exercising stimulus-dependent spatiotemporal control over inositol pyrophosphate synthesis. The activities of the N-terminal kinase domains of these enzymes (PPIP5K1^{KD} and PPIP5K2^{KD}) are modulated in response to certain cellular stresses^{7,10}, whereas separate inositol-lipid binding domains (PPIP5K1^{PBD} and PPIP5K2^{PBD}) mediate the receptor-regulated modulation of the intracellular compartmentalization of these enzymes¹⁴.

In this study, we use a structural and biochemical approach to determine how the catalytic challenges of pyrophosphorylation are met. To this end, we enlisted human PPIP5K2 as a representative member of the PPIP5K family. We report nine crystal structures and mutagenesis data on PPIP5K2 that define the basis for substrate recognition and selectivity, establish the reaction performed by the PPIP5Ks and the identity of the product, and demonstrate the reaction coordinate for creation of a high-energy pyrophosphate bond in IP7 and IP8.

RESULTS

Overall structure of hPPIP5K2^{KD}, an ATP-grasp kinase

We first determined crystal structures of hPPIP5K2^{KD}, including residues 37–366 or 41–366 (Supplementary Methods), which comprise the core catalytic domain of this particular protein; the length of the construct was varied to optimize crystallization (data not shown and ref. 16). These structures (Fig. 1a,b and Supplementary Results, Supplementary Table 1) reveal that the nucleotide nestles between two sets of antiparallel β -sheets, confirming the predictions^{13,18} of homology modeling servers that hPPIP5-K2^{KD} uses an ATP-grasp fold. The remainder of the hPPIP5K2^{KD} structure, mainly the N terminus, forms an $\alpha\beta\alpha$ domain (Fig. 1a and Supplementary Fig. 1a). The structure of hPPIP5K1^{KD} (84% sequence identity¹³) is most likely very similar to that of hPPIP5-K2^{KD}; dissimilar residues are confined to the surface distal to the active site (Supplementary Fig. 1b).

Detailed interactions between nucleotide and protein are shown in Supplementary Figure 2a. Nucleotide binding may stabilize protein conformation, as we could not crystallize the apoenzyme. Nevertheless, hPPIP5K2^{KD} hydrolyzed ATP at only a low rate (0.5 nmol mg protein⁻¹ min⁻¹) until a physiologically relevant substrate, 5-IP7, was also added; the rate of ATP hydrolysis then increased to 400 nmol mg protein⁻¹ min⁻¹. The Dali server²¹ identified hITPK1, another ATP-grasp inositol kinase (Fig. 1c,d), as the structure that is most homologous to hPPIP5K2^{KD}, although they share only 11% sequence identity. Furthermore, β 11 in the ATP-grasp region of hPPIP5K2^{KD} (Fig. 1a) does not occur in ITPK1 (Fig. 1c,d). Other regions of hPPIP5K2^{KD} also show considerable structural differences from ITPK1. For example, an insert unique to hPPIP5K2^{KD} forms an additional three-stranded β sheet (β 5, β 6 and β 7; Fig. 1). Similar insertions are also present in the sequences of hPPIP5K1 and the yeast homolog ScVip1.

Rationalization of substrate specificity of hPPIP5K2^{KD}

The ATP-grasp superfamily is noted for its considerable functional and structural divergence at the active site²². Indeed, the composition of the active site of ITPK1 (refs. 23,24) differs considerably from that of hPPIP5K2^{KD} (Fig. 2). For example, hPPIP5K2^{KD} uses only arginine and lysine residues to bind substrate, with the exception of Ser326 (Figs. 2a and 3a and Supplementary Fig. 3). The inositol phosphate binding pocket in EhITPK1 makes more general use of smaller and nonbasic residues such as glycine and glutamine²³ (Fig. 2b). The shapes of the two binding pockets are also substantially different (Fig. 2). EhITPK1 uses a relatively wide but flattened binding pocket that shows little substrate specificity²³. hITPK1, which phosphorylates a smaller range of substrates²⁴, has a binding pocket that is somewhat smaller but still loosely defined. In contrast, the highly specific hPPIP5K2^{KD} binds its substrates in two near-parallel grooves (marked with black arrows in Fig. 2c) that form a staggered 'H' shape. This binding pocket makes a perfectly tailored aperture for accommodating six phosphate or pyrophosphate groups attached to an inositol ring in the chair conformation. The biological and mechanistic consequences of the catalytic specialization of hPPIP5K2^{KD} are discussed below.

Substrate specificity for PPIP5Ks is biologically important in that it limits the variety of inositol pyrophosphate isomers that can be synthesized. As with hPPIP5K1 (refs. 13,14), we have found that the only important substrates for hPPIP5K2 are 5-IP7 and IP6 (the first-order rate constants are $346 \pm 15 \mu\text{g}^{-1} \text{min}^{-1}$ and $16 \pm 1 \mu\text{g}^{-1} \text{min}^{-1}$, respectively; $n = 3$, mean $\pm$ s.e.m.). There was negligible phosphorylation of 1-IP7 (0.09 ± 0.01), IP5 (0.028 ± 0.003) and PP-IP4 (0.75 ± 0.07) which, among the naturally occurring inositol phosphates, are the most similar to 5-IP7 and IP6. The stringent substrate specificity of hPPIP5K2 is achieved by the array of positively charged lysine and arginine residues lining the substrate

binding pocket, which makes polar contacts with every phosphate or pyrophosphate group in both IP6 and 5-IP7, and by the Mg^{2+} ions that bridge certain phosphate groups (Figs. 2c and 3a and Supplementary Fig. 3). Lys214 and the magnesium atom Mg5 interact specifically with the 5- β -phosphate of 5-IP7; the absence of the 5- β -phosphate in IP6 (Fig. 3a) may contribute to hPPIP5K2's preference for 5-IP7 as a substrate (see above). To further probe inositol phosphate recognition, we mutated basic residues lining the substrate binding pocket and assessed the effects of these mutations on catalytic activity against both IP6 and 5-IP7 (Fig. 3b). Most mutants showed sharply diminished activities (to less than 10% of wild-type enzyme activity). Less dramatic effects were observed for K53A and R273A mutations (25–50% of wild-type activity), as the original residues are more exposed to solvent. The K248A mutant showed the largest decrease in catalytic activity with either substrate (Fig. 3b). This lysine residue interacts with both the 1-phosphate of the substrates and the γ -phosphate of ATP throughout the catalytic cycle (Fig. 4; described further below).

Some inositol phosphate kinases (ITPK1 and IPMK) phosphorylate multiple positions around the inositol ring²⁵. The structural rationalization of this apparent promiscuity^{23,26} is that the loosely fitting active sites of these kinases allow several alternate substrates to bind in different orientations. In contrast, our structures reveal that IP6 and 5-IP7 fit into the active site of hPPIP5K2^{KD} in a near-superimposable orientation (Fig. 3a and Supplementary Fig. 4). Thus, for both substrates it is the 1-phosphate that is closest to the γ -phosphate of ATP (Fig. 4a,b and Supplementary Fig. 4). Moreover, when hPPIP5K2^{KD} crystals were soaked with ATP and 5-IP7 substrate, we obtained complexes that contained ADP and product (Fig. 4c), indicating that the crystallized enzyme retained its kinase activity. A difference Fourier map identified the product as 1,5-IP8 (Fig. 4c and Supplementary Fig. 5). Thus, PPIP5Ks are 1-kinases. Hitherto, it has not been determined whether PPIP5Ks phosphorylate the 1-position, the 3-position or both¹⁹. These two alternative reactions would yield products that are enantiomers, which have previously been difficult to distinguish between.

Induced-fit motion also contributes to specificity²⁷. A comparison of the AMP-PNP–enzyme crystal complexes with or without 5-IP7 indicates that substrate binding is accompanied by induced-fit motion in hPPIP5K2^{KD} of the side chains of three active-site residues: Arg262, Arg281 and Lys329 (Fig. 4d, Supplementary Fig. 6 and Supplementary Movies 1 and 2). The use of these flexible side chains enables the energy cost upon ligand binding to be lower than it would be if backbone rearrangement was necessary²⁷.

The reaction mechanism of hPPIP5K2^{KD}

To gain direct insight into the reaction mechanism, we obtained a crystal complex that contains 5-IP7, ADP and MgF_3^- (Fig. 4), as the latter matches the charge and geometry of the transition state of a phosphoryl transfer reaction²⁸. Indeed, in the active site, we detected MgF_3^- with near planar geometry (Fig. 4b and Supplementary Fig. 5d) that mimics a trigonal bipyramidal phosphoryl transition state²⁸. The magnesium atom was sandwiched 1.9 Å and 2.3 Å from the donor oxygen of ADP and the acceptor oxygen of the 1-phosphate of 5-IP7, respectively (Fig. 4b). For this total coordination distance of <4.9 Å, a new P-O bond can be formed by nucleophilic attack of the acceptor oxygen before the original P-O bond with the donor oxygen is broken, suggesting an in-line associative reaction mechanism²⁹. The three negative charges of the pentacoordinate (phosphorane) transition state seem to be balanced by electrostatic interactions with two Mg^{2+} ions, a water molecule, and the positively charged side chains of Lys248 and Arg213. This snapshot of charge neutralization in the transition state (Fig. 4b and Supplementary Fig. 7) helps explain how hPPIP5K2^{KD} overcomes the energy barrier to efficient catalysis.

Catalysis can also be enhanced when the geometry of the substrate is directed toward that of the transition state by conformational dynamics³⁰. A comparison between the substrate-bound ground and transition states of hPPIP5K2^{KD} (Fig. 4e and Supplementary Movies 1 and 2) revealed that Lys329 not only alters its conformation during this transition but also forms different contacts and gains two additional hydrogen bonds. Arg262 gains one hydrogen bond with 5-IP7 in the transition state. The entire inositol ring moves under the concerted actions of Lys54, Arg213, Lys214, Arg262 and Lys329 (Fig. 4e, Supplementary Fig. 6 and Supplementary Movies 1 and 2). Thus, these conformational changes close the gap between substrate and nucleotide. The position of C1 advances 0.7 Å closer to the γ -phosphate of ATP, and the entire inositol ring rotates 7° as it moves (Fig. 4e). Our crystal structures also show that hPPIP5K2^{KD} exploits a specific property of a phosphoester bond: its ability to rotate. The 1- α -phosphate group rotates 17° between ground and transition states (Fig. 4f) and then a further 19° after the phosphoanhydride bond is formed (Fig. 4f). These transitions are not available for inositol phosphate kinases that phosphorylate the hydroxyl groups of the inositol ring, which lack conformational flexibility. Furthermore, in hPPIP5K2^{KD} the inositol ring of IP6 and 5-IP7 is presented perpendicular to the plane of the nucleotide's β - and γ -phosphates, which avoids steric and electrostatic clashing between the nucleotide and the non-reacting oxygens on the 1-phosphate of the substrate (Fig. 5 and Supplementary Fig. 8). This topology distinguishes hPPIP5K2^{KD} from all of the other inositol phosphate kinases that phosphorylate hydroxyl groups (Fig. 5). Our data also indicate that the newly formed 1-pyrophosphate group is under considerable steric strain (Supplementary Fig. 9), which could favor product release.

A final and unexpected outcome of our studies was the identification of a basic three-dimensional blueprint within the active site that is shared by some other phosphoryl transferases (Fig. 6 and Supplementary Fig. 10). Both Lys248 and the metal-coordinating residue Asp321 are spatially and functionally conserved in ITPK1 and in several other kinases that do not use an ATP-grasp domain: namely, IP3K^{31,32}, AtIP5K³³ and protein kinase A³⁴.

DISCUSSION

Some of the signaling activities of inositol pyrophosphates rely on highly specific interactions with receptors¹⁰. Our stereoselective resolution of uncertainty concerning the structures of inositol pyrophosphates (Fig. 4) will now permit the chemical synthesis of physiologic isomers, the deciphering of their molecular interactions with receptors¹⁰ and the design of antagonists. The uniquely crowded three-dimensional phosphate topology of inositol pyrophosphates also endows them with high-energy properties¹²; exploitation of this free energy to nonenzymatically phosphorylate proteins may add to their multifunctionality^{6,11}. Nevertheless, that free energy also imposes a particular challenge upon a kinase; it must not only overcome a substantial energy barrier to synthesize the inositol pyrophosphates but also must do so at a high rate, as evidenced by the rapid turnover of these signaling molecules *in vivo*⁴. Our study shows how the active sites of PPIP5Ks have adapted to meet these demands: there is an extensive charge balance that facilitates a partly associative in-line phosphoryl transfer mechanism, a unique topological presentation of substrate to nucleotide and the exploitation of the conformational dynamics of both enzyme and substrate. Clearly, hPPIP5K2^{KD} has a highly specialized catalytic environment. Overall, the structural information that we provide here offers directions for studying the evolution of the active sites of diverse phosphoryl transferases and also offers templates for an underdeveloped area of research: the rational design of inhibitors that specifically target a particular inositol phosphate kinase.

METHODS

Protein expression and purification

The hPPIP5K2^{KD} constructs (residues 1–366, 37–366 and 41–366 and the single-site mutants) were each subcloned into a vector containing N-terminal tandem His₆ and maltose binding protein tags and a TEV protease cleavage site. ArcticExpress (DE3) competent cells (Stratagene) were transformed with the resultant plasmids. Recombinant proteins were purified using Ni-NTA agarose (Qiagen) and HiTrap Heparin HP columns (GE Healthcare), subjected to TEV cleavage, and further purified using Ni-NTA agarose and HiTrap Q Fast Flow and Superdex 200 columns (GE Healthcare). Further details are in Supplementary Methods.

Crystallization and structure determination

hPPIP5K2^{KD} (residues 37–366 or 41–366) was crystallized by vapor diffusion at 4 °C against a well buffer of 12% (w/v) PEG 3350, 20 mM MgCl₂, 0.1 M HEPES pH 7.0, 1 mM ATP or AMP-PNP, with or without 2 mM CdCl₂ (Supplementary Table 1). For ternary complexes, the crystals were soaked in 22% (w/v) PEG 3350, 10 mM MgCl₂ at pH 5.2 for 0–3 d with either 10 mM IP6 (Calbiochem; >99% pure) or 2 mM 5-IP7 (ref. 35), which was >80% pure³⁶. To generate the transition mimic, 10 mM NaF was also added to the soaking buffer. Diffraction data were collected with an in-house facility or with APS beamline 22-ID/22-BM and were processed with the HKL2000 program³⁷. Our initial structure of hPPIP5K2 (residues 37–366) was solved and auto-built with the PHENIX program³⁸ using single-wavelength anomalous dispersion techniques. Further manual rebuilding used COOT³⁹, and the structure was refined using REFMAC⁴⁰. Other structures were solved by rigid-body and direct Fourier synthesis or by molecular replacement approaches. Further details are in Supplementary Methods.

Enzyme assays and substrate preparation

Catalytic activities were determined by HPLC using radiolabeled substrates^{13,14}. Additional information is in Supplementary Methods. [³H]IP6 was purchased from PerkinElmer and repurified by HPLC¹⁴ (99% pure). The following [³H]-labeled inositol phosphates were prepared as previously described¹⁴: IP5 (>99% pure), PP-IP4 (93% pure), 1-IP7 (95% pure), 5-IP7 (97% pure).

Accession codes

Protein Data Bank: the previously determined crystal structures for EhITPK1, hIP3K, AtIP5K, hITPK1 and PKA are deposited under accession codes 1Z2P, 1W2C, 2XAN, 2Q7D and 1L3R, respectively. Newly acquired structures are deposited under accession codes 3T54, 3T99, 3T7A, 3T9A, 3T9B, 3T9C, 3T9D, 3T9E and 3T9F.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Overall structure of hPPIP5K2^{KD}, a member of the ATP-grasp fold superfamily (a) Ribbon diagram of hPPIP5K2^{KD}. The $\alpha\beta\alpha$ domain (residues 42–124 and 330–366) is shown in yellow. The two antiparallel β -sheet clusters of the ATP-grasp domain are colored green (residues 125–148 and 244–329) and blue (residues 149–243). Lighter shading of each color highlights structural elements that are present in hPPIP5K2^{KD} but not in ITPK1 (see later panels). For example, the three-stranded β -sheet that is unique to hPPIP5K2^{KD} is colored cyan. Shown in stick models are 5-IP7 and AMP-PNP; the latter is deeply buried (9% solvent accessible). (b) Electrostatic surface plot colored in blue and red, which respectively indicate positive and negative electrostatic potentials at physiological pH. (c) Ribbon diagram of EhITPK1 shown with bound 1,3,4-IP3 as a gray stick model with orange and red phosphate groups. (d) Ribbon diagram of hITPK1 (crystal structure with an inositol phosphate has not yet been determined).

Figure 2. Comparison of substrate binding pockets for hPPIP5K2^{KD} and ITPK1
 (a,b) Substrates for hPPIP5K2^{KD} (5-IP7) (a) and EhITPK1 (1,3,4-IP3) (b) are shown in gray with orange and red phosphate groups. Residues that contact the substrate are also shown (gray spheres, carbon; red spheres, oxygen; blue spheres, nitrogen). ATP analogs are depicted as brown stick models. (c–e) Electrostatic surface representations of inositol phosphate substrate binding pockets of hPPIP5K2^{KD} (c), EhITPK1 (d) and hITPK1 (e). The overall sequence identity of hPPIP5K2^{KD} with EhITPK1 and hITPK1 is 8% and 11%, respectively.

Figure 3. Structural and mutagenic analysis of residues in hPPIP5K2^{KD} that participate in substrate binding

(a) Active site of hPPIP5K2^{KD}. Shown in green are residues that make polar contacts with substrates. 5-IP7 is shown with gray carbon and orange and red phosphate groups, and IP6 is shown in light blue. Carbon atoms on the inositol ring are numbered. The interactions of Lys214 with 5-IP7 are highlighted by dashed yellow lines. Mg4 and Mg5 (sky blue spheres) are two magnesium atoms that are only present in crystal complexes that also contain substrate (Mg5 was present only in the hPPIP5K2^{KD}–5-IP7 complex). (b) Relative catalytic activities of wild-type and single-site mutants of hPPIP5K2^{KD}. First-order rate constants are shown relative to wild-type enzyme. The horizontal broken line emphasizes a 90% decrease in catalytic activity. Rates for wild-type enzyme: 5-IP7 = $346 \pm 15 \mu\text{g}^{-1} \text{min}^{-1}$, IP6 = $16 \pm 1 \mu\text{g}^{-1} \text{min}^{-1}$, $n = 3$, mean $\pm$ s.e.m. The data for the various mutants are mean values from two determinations, and s.d. were less than 10% of the mean values.

Figure 4. Snapshots of the reaction mechanism

(a–c) Ground (a), transition (b) and product (c) states of hPPIP5K2^{KD}. Key catalytic residues are shown as green and blue sticks. Inositol pyrophosphates and the nucleotide cofactors are shown (white, carbon; red, oxygen; orange, phosphorus; blue, nitrogen), as are catalytic magnesium atoms (magenta spheres) and a water molecule (WAT; blue sphere). The 1-carbon atom in the inositol ring is labeled. Bond distances (dotted lines) are denoted in angstroms (Å). The MgF₃⁻ transition state analog is depicted in purple (for Mg) and cyan (for F⁻). The simulated-annealing OMIT difference map for MgF₃⁻ is contoured at 5.0 σ. (d) Induced-fit motions of hPPIP5K2 upon 5-IP7 binding. The hPPIP5K2^{KD}-AMP-PNP structure (orange) is superimposed with the structure of hPPIP5K2^{KD}-AMP-PNP-5-IP7 (green). Metals in the catalytic center are colored magenta. Movements of side chains are highlighted with black arrows. (e) View highlighting some differences in the interactions of substrates with amino acids in the ground (green) and transition (purple) states. (f) Superimposition of inositol pyrophosphate bound in the ground (green), transition (purple) and product states (brown). Leaving and entering oxygen atoms are colored red. Movements are highlighted by solid arrows (detailed further in text). The dashed line shows the direction of nucleophilic attack. During catalysis, the relative positions of the three oxygen atoms of the active phosphoryl group are preserved, but the phosphorus atom moves about 1.4 Å and its configuration is inverted.

Figure 5. The relative topologies of nucleotide and substrate in inositol phosphate kinases

Comparison of inositol phosphate substrate orientation relative to the nucleotide cofactor by several inositol phosphate kinases (Protein Data Bank codes: hPPIP5K2^{KD}, 3T9D; EhITPK1 (ref. 24), 1Z2P; hIP3K³², 1W2C; AtIP5K³³, 2XAN) is shown. We have highlighted the angle between the plane of the nucleotide's β - and γ -phosphates and the plane of the inositol ring substrate. Numbers indicate phosphorylation positions on the inositol rings.

Figure 6. A conserved catalytic blueprint in hPPIP5K2^{KD}

This figure shows a spatially conserved catalytic blueprint in hPPIP5K2^{KD} (PDB code 3T9D), several other inositol phosphate kinases (PDB codes: hITPK1 (ref. 24), 2Q7D; hIP3K³², 1W2C; AtIP5K³³, 2XAN), and PKA³⁴ (PDB code 1L3R). The structures were aligned by superimposing the bound nucleotides; the color key denotes the origins of the lysine and aspartic acid side chains and the donor (leaving) and the acceptor (entering) groups.