Kenyon et al. BMC Research Notes 2012, 5:131
http://www.biomedcentral.com/1756-0500/5/131

BMC Research Notes

RESEARCH ARTICLE Open Access

Conserved phosphoryl transfer mechanisms within kinase families and the role of the C8 proton of ATP in the activation of phosphoryl transfer

Colin P Kenyon*, Robyn L Roth, Chris W van der Westhuyzen and Christopher J Parkinson

Abstract

Background: The kinome is made up of a large number of functionally diverse enzymes, with the classification indicating very little about the extent of the conserved kinetic mechanisms associated with phosphoryl transfer. It has been demonstrated that C8-H of ATP plays a critical role in the activity of a range of kinase and synthetase enzymes.

Results: A number of conserved mechanisms within the prescribed kinase fold families have been identified directly utilizing the C8-H of ATP in the initiation of phosphoryl transfer. These mechanisms are based on structurally conserved amino acid residues that are within hydrogen bonding distance of a co-crystallized nucleotide. On the basis of these conserved mechanisms, the role of the nucleotide C8-H in initiating the formation of a pentavalent intermediate between the γ-phosphate of the ATP and the substrate nucleophile is defined. All reactions can be clustered into two mechanisms by which the C8-H is induced to be labile via the coordination of a backbone carbonyl to C6-NH_2 of the adenyl moiety, namely a "push" mechanism, and a "pull" mechanism, based on the protonation of N7. Associated with the "push" mechanism and "pull" mechanisms are a series of proton transfer cascades, initiated from C8-H, via the tri-phosphate backbone, culminating in the formation of the pentavalent transition state between the γ-phosphate of the ATP and the substrate nucleophile.

Conclusions: The "push" mechanism and a "pull" mechanism are responsible for inducing the C8-H of adenyl moiety to become more labile. These mechanisms and the associated proton transfer cascades achieve the proton transfer via different family-specific conserved sets of amino acids. Each of these mechanisms would allow for the regulation of the rate of formation of the pentavalent intermediate between the ATP and the substrate nucleophile. Phosphoryl transfer within kinases is therefore a specific event mediated and regulated via the coordination of the adenyl moiety of ATP and the C8-H of the adenyl moiety.

Background

The kinases are a large number of structurally diverse enzymes that play a critical role in numerous metabolic and signalling pathways and whose substrates may be a small molecule, lipid, or protein. The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC/IUB) commission on the classification and nomenclature of enzymes placed the enzymes that transfer high energy phosphate bonds from nucleotides into two divisions: the transferases (kinases) and the ligases (synthetases) [1]. The transferases have been placed in Division 2 and the ligases into Division 6 of the Enzyme Commission (EC) classification. The ligases catalyse the joining of two molecules with the concomitant hydrolysis of the pyrophosphate bond of ATP, while a kinase is defined as an enzyme which catalyses the transfer of the phosphate group from ATP (or GTP) to a substrate containing an alcohol, amino, carboxyl, or phosphate group as the phosphoryl acceptor [2,3]. The kinases have been classified into 25 families of homologous proteins, with the families assembled into 12 fold-groups based on the similarity of their structural folds [2,3]. This classification relays little information on the catalytic mechanisms employed in nucleotide binding and phosphoryl transfer. The critical question that needed to be answered was to

* Correspondence: ckenyon@csir.co.za
CSIR, Biosciences, Meiring-Naude Road, Pretoria, 0001 Gauteng, South Africa

what extent the functionality required for the catalysis of phosphoryl transfer is conserved within the 25 families or 12 fold-groups.

Furthermore, during research carried out on the effect of deuteration at the C8 position of ATP on the activity of a number of kinase and synthetase enzymes, it became evident that the C8 proton of ATP plays a direct mechanistic role in initiating phosphoryl transfer, and probably also in the regulation of catalysis via the regulation of the rate of reaction [4]. The Kinetic Isotope Effect (KIE) obtained in enzymes utilizing ATP deuterated at C8 was found to be significantly in excess of 2 and in most cases in excess of 10 at low ATP concentrations. *This is a primary effect and is therefore dependent on C8-H bond breaking*. To this end, the putative mechanistic role of the C8 proton in the catalysis of the 22 families within the 10-fold groups was also investigated in the context of the conserved catalytic residues of each group of kinases.

There are two accepted mechanisms by which catalysis might occur in nucleophilic substitution reactions: either by generation/formation of a leaving group or by the activation of the nucleophile, or both. Chemically phosphoryl transfer could occur by either by an S_N1 or S_N2 type reaction mechanism. However, within enzyme-catalysed systems it appears to occur predominantly via an in-line associative S_N2 type mechanism [5]. As the C8-H appears to play a direct role in initiating phosphoryl transfer [4], the main aim of this investigation was to establish mechanistically how this might occur within the 22 families or 12 fold-groups of the kinase enzymes, and also the extent to which these mechanisms might be conserved within the 22 families. One of the criteria required by the associative S_N2 type mechanism is the creation of a pentavalent intermediate between the γ-phosphate (γ-PO_4) of ATP and the substrate nucleophile. Accepted mechanisms leading to the creation of this pentavalent transition state and the activation of the γ-PO_4 as a leaving group, all require protonation of the α-phosphate (α-PO_4) or β-phosphate (β-PO_4) of ATP, either directly or via a carrier side-chain (Figure 1). Similarly, protonation or hydrogen-bonding of N7 of the ATP adenyl group with the protein would lead to the C8-H becoming more acidic. This, in turn, requires an acidic proton to protonate N7 and an electron-donating group to stabilise the developing positive charge on C8. This process would allow for the C8-H to protonate the α-phosphoryl group, initiating a tautomerization sequence of reactions involving the acidic proton on the α-phosphoryl group being transferred, via a coordinated basic amino acid side-chain such as lysine, to the β-phosphoryl group. This results in the concomitant activation of the γ-phosphoryl group to nucleophilic attack by the target residue or substrate through a pentavalent phosphorus intermediate. A general mechanism for this process is outlined in Figure 1. This general mechanism was used as the template to define and characterise the family specific mechanisms associated with 21 of the kinase families within 10 of the fold groups where sufficient structural information is available. To this end, the

PDB (the database) was searched for structures representing kinases within each family, based on the EC numbering. All kinase crystal structures containing any AMP, ADP, ATP or corresponding analogues were then visually examined for structurally conserved amino acid side-chains that were associated with the nucleotide or analogue. The inter-atomic distances between the identified amino acid side-chain residues and the nucleotide analogue were also noted. Having identified the conserved mechanisms associated with crystallized representatives of each family, sequence alignments were carried out with other members of each specific family where structural information is not available. This allowed the determination of the extent to which the conserved residues identified in the individual families of kinases by this mechanistic analysis as being responsible for catalysis within all these families.

Results

In this classification of the kinase enzymes based on their conserved phosphoryl transfer mechanisms, the framework for the overall organisation remains the same as that of Cheek *et al* [2,3]. However, individual kinases may have been transferred from one family to another based on the conservation of the mechanism. It has been demonstrated that C8-H plays a critical role in the activity of a range of kinase and synthetase enzymes [4]. In all cases, the mechanism associated with the activation and release of the C8-H is based on the proximity of the C8-H to the α- or β-PO_4, or to a proton carrier side-chain capable of transferring the proton from the C8-H to the α-PO_4. In most cases, the C8-H is rendered more acidic by hydrogen bonding networks mediating either a "push" mechanism via the coordination to the C6-NH_2 or a "pull" mechanism via the protonation of N7. In a significant number of cases a carbonyl group from the protein backbone is hydrogen bonded to the C6-NH_2 of the nucleotide (Figure 1). In cases where this does not occur, there is a residue with a labile proton hydrogen-bonded with N7. The tautomeric interconversion that gives rise to planar peptide bonds allows for the strong hydrogen bonding interaction and/or proton transfer between the backbone carbonyl and the C6-NH_2 [6,7]. Examples of the "push" and "pull" mechanisms for the kinase families are summarised Figures 2-3, while the detailed reaction mechanisms and the identified residue and interatomic distances outlined in Additional file 1: Table S3 and 4A. The Pfam superfamily and family classifications, as well

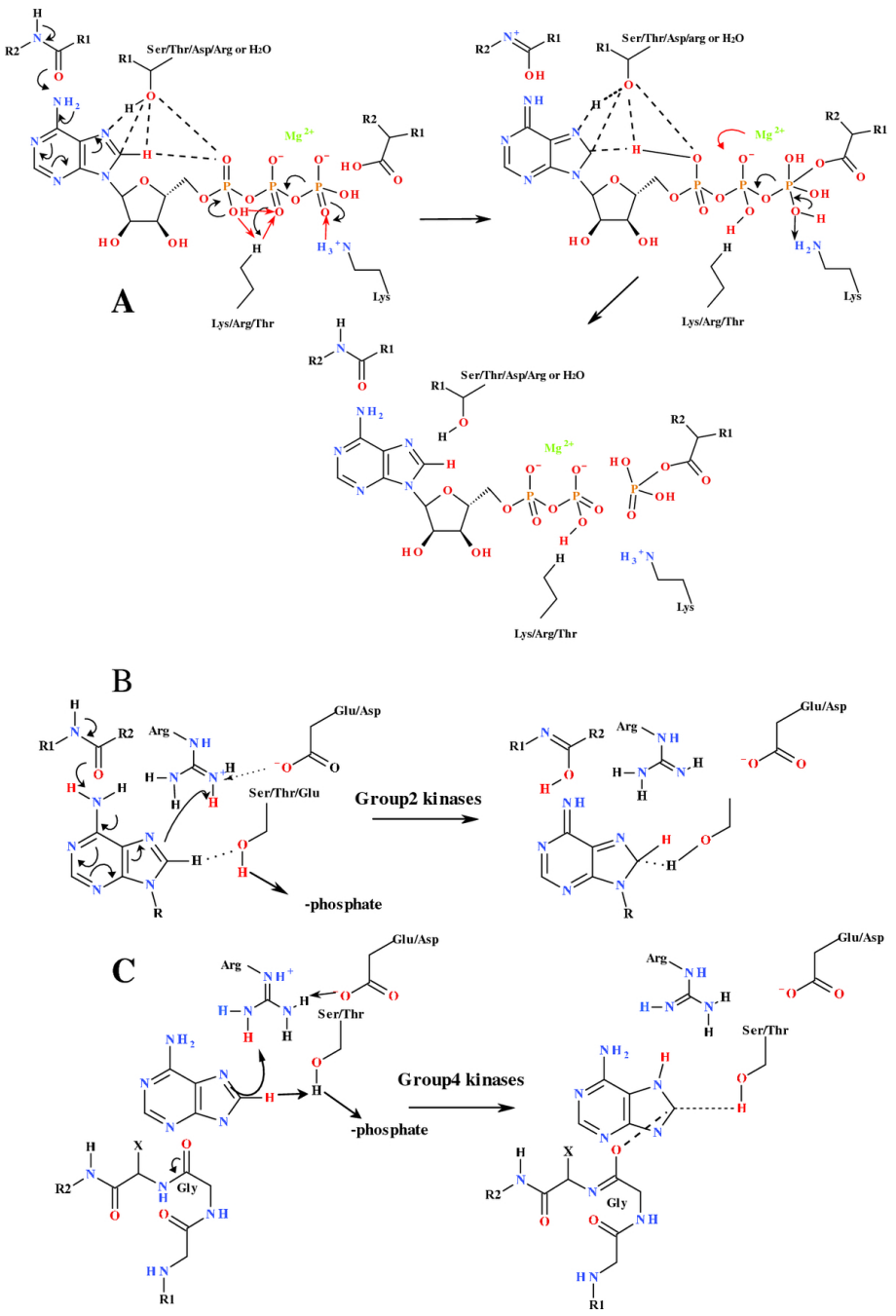


Figure 1 A. General mechanism for the induction of phosphoryl transfer from the C8H of the adenyl moiety. This is initiated via the coordination of the ATP adenyl moiety and effective proton transfer along the phosphate tail of ATP initiated from C8-H and mediated via range of conserved coordinating amino acid chains that are specific for each kinase group and family. As the KIE is a primary effect and is therefore dependent on C8-H bond breaking the C8-H is rendered more acidic by either of two mechanisms, a "push" mechanism mediated by the interaction of a backbone carbonyl at the C6-NH_2 or a "pull" mechanism mediated by the interaction via coordination of a proton donor at N7 or hydrogen bonding of C8-H to an adjacent carbonyl or alcohol (or acceptor group). The red arrows indicate direction of proton migration during the reaction. B. The "push" mechanism is initiated with the redistribution of electron density in the adenyl ring mediated by the coordination of the backbone carbonyl to C6-NH_2 via a high energy adenosine tautomer. C. The "pull" mechanism is mediated via the formation of a carbenoid species induced via the protonation of N7.

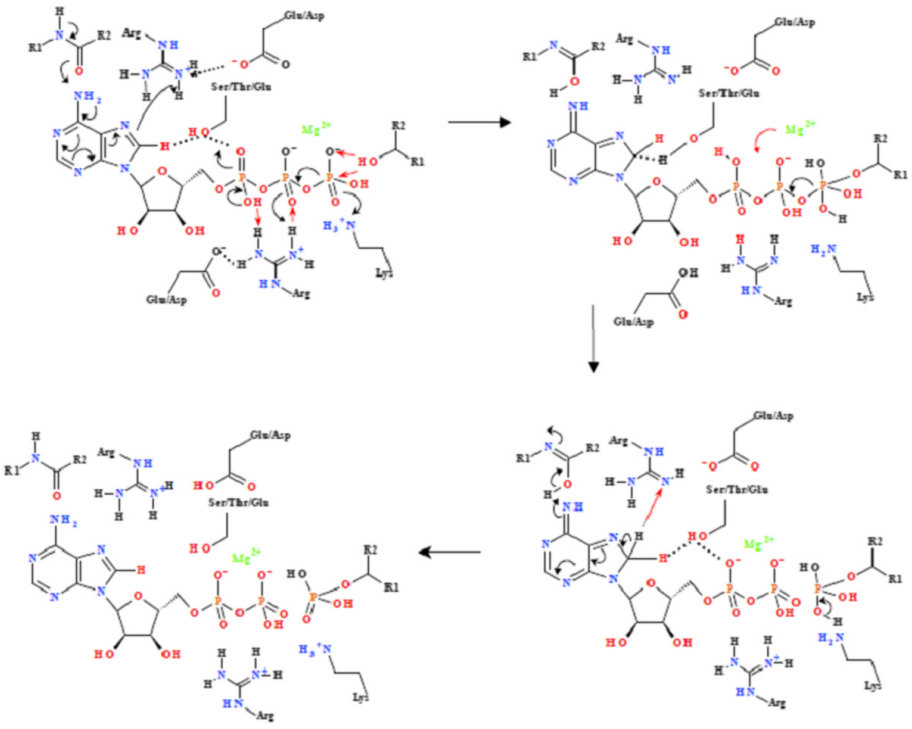


Figure 2 Phosphoryl transfer mechanism found in the Group 2 kinases (Rossmann-like fold and phosphoenolpyruvte carboxykinase-like sequences). The initiation of phosphoryl transfer occurs via the coordination of the ATP C6-NH_2 to a carbonyl arising from the protein backbone by the "push" mechanism resulting in the protonation of C8 via the coordination of a conserved Arg. This renders the C8-H more acidic, allowing for the protonation of the α-PO_4, via a conserved Ser/Thr carrier. There is a concomitant transfer of an H^+ from the α-PO_4 to β-PO_4 via a conserved Arg, thereby facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a simultaneous ATP-mediated deprotonation of the substrate -OH, allowing for the nucleophilic attack by the substrate to create the pentavalent intermediate and allow phosphoryl transfer. A protonated Lys then transfers the proton to the γ-PO_4, changing the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

as the sequence alignments showing the conserved residues within each family are shown in the supplementary information. Within each structurally conserved mechanism, the average interatomic distances between the conserved residues and nucleotide or nucleotide analogue are calculated and outlined in the tables. Figure 4 defines the spatial arrangement for the primary "structure" involved in the initiation of phosphoryl transfer for each of the remaining groups where structural information was available. The full mechanisms associated with these groups are shown in the supplementary information.

"Push" mechanism

The Group 2 kinases consisting of the Rossmann-like kinases and the phosphoenolpyruvate carboxykinase families are used as the example of the "push" mechanism (Figure 2, (Additional file 1: Table S3). These enzymes fall within the P-loop containing nucleoside triphosphate hydrolase superfamily (Additional file 1: Table S4, Figures S8 & S9). The mechanism relies on a side-chain carrier associated with the transfer of the C8-H to the α-PO_4, and then the activation of the nucleophile substrate occurs directly via the protonation of the γ-PO_4. The protonation of the α-PO_4 from C8-H in other mechanisms may occur directly (see below).

The initiation of phosphoryl transfer occurs via hydrogen bonding of the ATP C6-NH_2 to a carbonyl group arising from the protein backbone, with the average hydrogen-bond distance from the C6-NH hydrogen to the backbone carbonyl oxygen being 2.101 ± 0.699 Å. This reaction relies on the "push" mechanism which, by the hydrogen bonding of C6-NH_2 to the backbone carbonyl and the concomitant protonation of the developing anion at C8 by a conserved Arg residue acting as a Brønsted acid, produces a methylene group at C8 (Figure 2). On the opposite face of the adenyl moiety is a coordinated Thr/Ser which acts in the transfer of H^+ to the α-PO_4. The change in the N7-C8 hybridization facilitates the protonation of the α-PO_4 via the Thr/Ser, via the re-protonation of the Thr/Ser by C8-H. The effective protonation of the α-PO_4 therefore occurs via the coordinated Ser/Thr as the C8-H is not within direct hydrogen bonding distance of

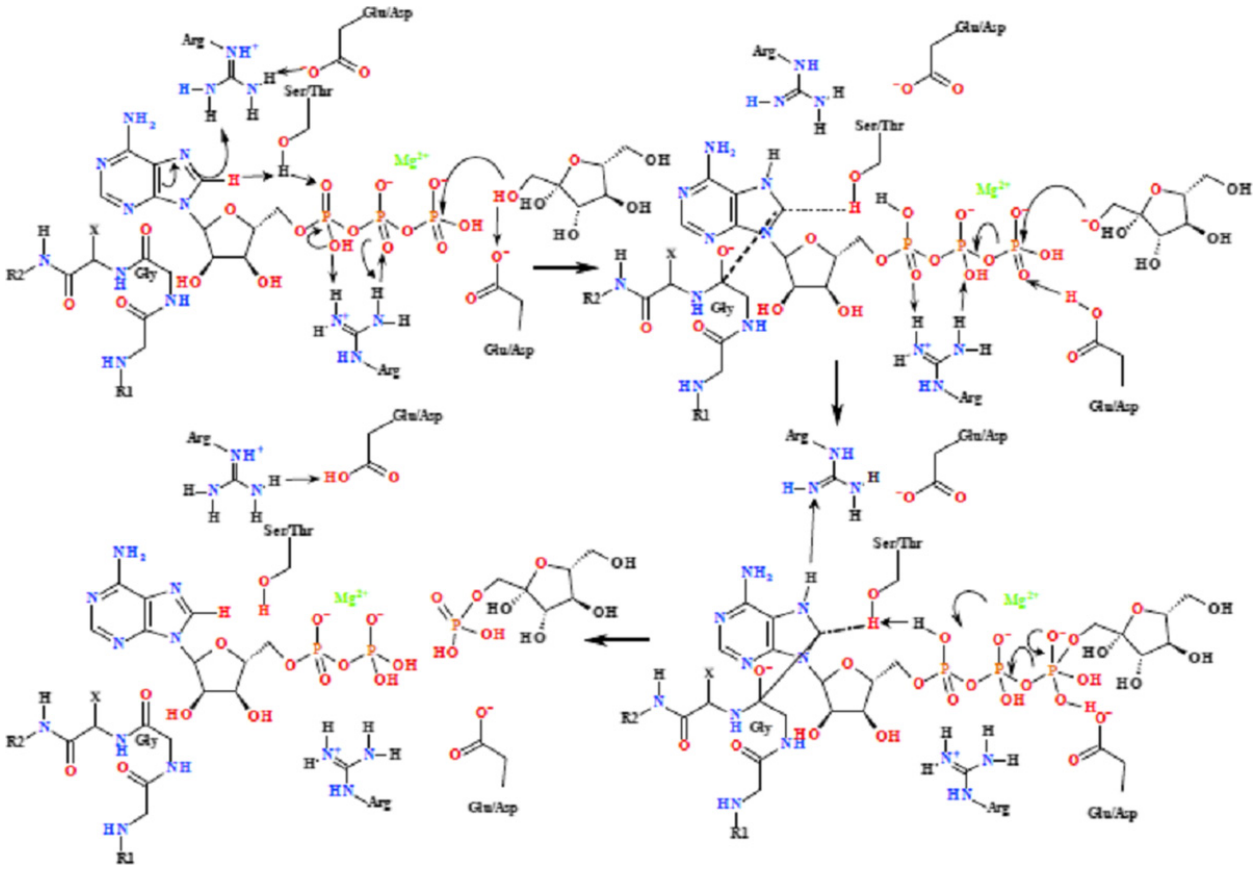


Figure 3 Phosphoryl transfer mechanism found in the Group 4 kinases (hexokinase family with polyol substrate). This occurs via coordination of an arginine residue to the N7/C8 of the imidazole moiety mediating the change in C8 hybridization from sp2 to sp3 hybridized, and altering the protonation of N7 and C8. Protonation of the N7 occurs via a conserved Arg residue with the NH_2 being coordinated directly to N7, with an interatomic distance of 3.065 ± 0.823 Å. The Arg residue in Group 4 kinases is always stabilized by an associated Asp/Glu residue. The reaction occurs via a carbene mechanism with the carbene being stabilized via the interaction of a conserved backbone Gly carbonyl that is within bonding distance of C8, causing C8-H to become more acidic, allowing for the protonation of the α-PO_4, via a conserved Ser/Thr. There is a concomitant transfer of an H^+ from the α-PO_4 to the β-PO_4 via a conserved Arg, thereby facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a concomitant Asp-mediated deprotonation of the substrate -OH, allowing for the nucleophilic attack by the substrate. This creates the pentavalent intermediate and allows phosphoryl transfer. The protonated Asp then transfers the proton to the γ-PO_4, changing the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

the α-PO_4, the interatomic distance being 4.322 ± 1.010 Å. The Thr/Ser to C8-H interatomic distance and the Thr/Ser to the α-PO_4 inter-atomic distance were found to be 3.797 ± 1.697 Å and 3.200 ± 0.558 Å, respectively. The effective transfer of an H^+ between the α- and β-PO_4 occurs via a co-ordinated conserved Arg residue for the Rossmann-like and phosphoenolpyruvate carboxykinase families. The co-ordinated Arg acts as a base by receiving a proton from the α-PO_4 and also acts as an acid by protonating the β-PO_4, with the conserved Arg to α-PO_4 and β-PO_4 interatomic distances being 2.953 ± 1.197 Å and 2.847 ± 1.143 Å, respectively. The substrate nucleophile is activated directly via the coordinated γ-PO_4 and this probably plays a role in the translocation of the Mg^{2+} from being β-PO_4/γ-PO_4 co-ordinated to being β-PO_4/α-PO_4 coordinated. A γ- PO_4-coordinated Lys also ensures the creation of the pentavalent intermediate after the nucleophilic attack by the substrate with the lysine δ-NH_3 to γ-PO_4 inter-atomic distance being 2.073 ± 0.480 Å. The migration of the Mg^{2+} to the β-PO_4/α-PO_4 co-ordination ensures that the proton arising from the C8 returns to the C8-H position. This occurs with the concomitant re-protonation of the co-ordinated Arg via the Ser/Thr/Asp and the subsequent return of the delocalization of the electrons of the adenine. Enzymes employing the "push" mechanism in the Group 2 in the P-loop containing nucleoside triphosphate hydrolase superfamily in which the C8-H dependent kinetic isotope effect has been

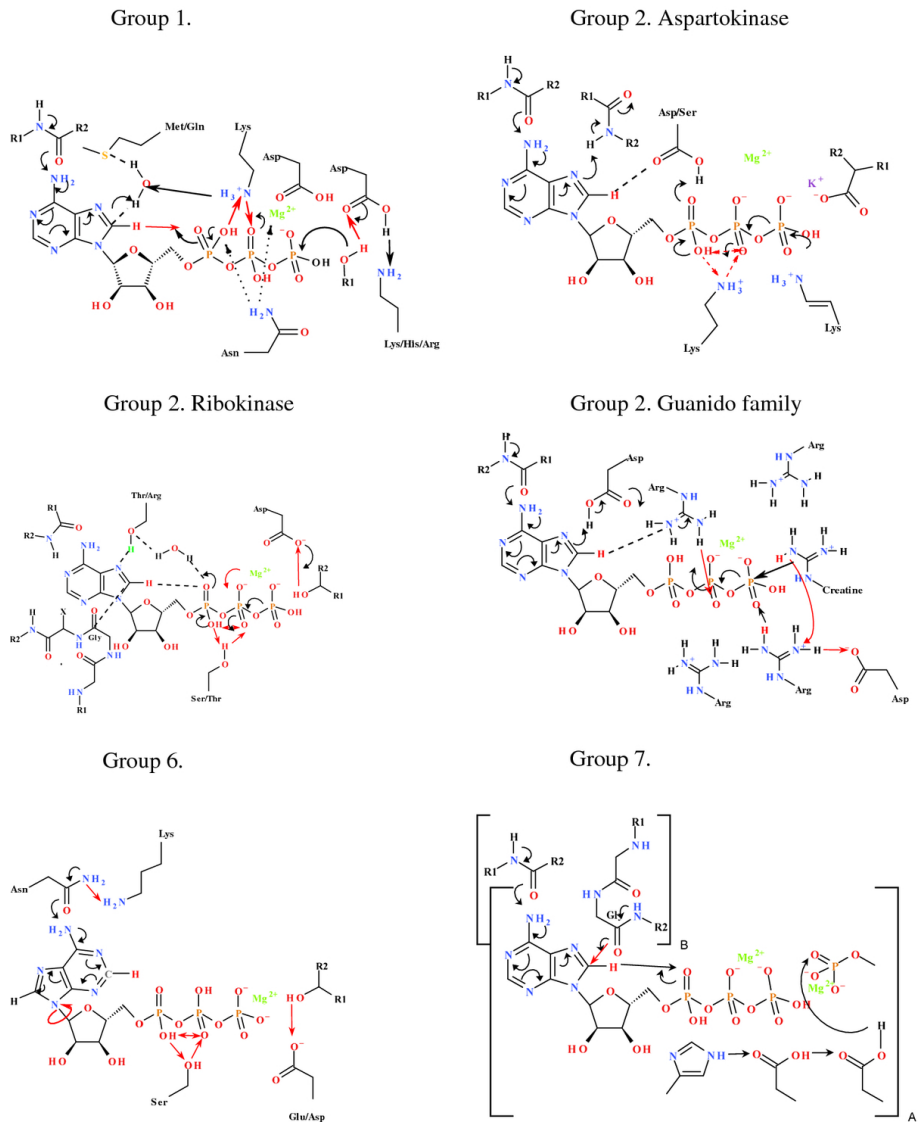


Figure 4 Spatial arrangements of residues implicated in the phosphoryl transfer mechanism found in the Group 1, Group 2 (aspartokinase family), Group 2 (ribokinase family), Group 3 (Guanido family), Group 6 and Group 7 kinases. The bracket indicates two distinct protein subunits.

demonstrated are shikimate kinase (4) and adenylate kinase (unpublished data).

"Pull" mechanism

The Group 4 kinases rely on a distinctive mechanism for the activation of the C8-H based on the protonation of N7 initiating the creation of a carbene at C8. This is referred to as the "pull" mechanism. The Group 4 kinases fall within the Actin-like ATPase and Ribokinase-like superfamilies (Additional file 1: Table S8). The C8-H to α- or β-PO_4 interatomic distance = 4.932 ± 0.876 Å (Figure 3, Additional file 1: Table S7). A significant difference between the Group 4 kinases and all other groups is the role of the adenyl ring associated Arg residue in the initiation of phosphoryl transfer. In this group there is no coordination of the ATP C6-NH_2 to a carbonyl arising from the protein backbone. Instead the Arg NH_2 is co-ordinated directly to N7, with the N7 inter-atomic distance being 3.155 ± 0.815 Å. It is proposed that it is this coordination within the Group 4 kinases that initiates the release of the C8-H with the concomitant initiation of phosphoryl transfer (Figure 3). Also making up the "pull mechanism", the Arg residue hydrogen bonded to the adenyl N7 in the Group 4 kinases is always stabilized by an associated Asp/Glu residue, assisting in the protonation of N7. Hydrogen

bonding to C8-H by the hydroxyl moiety of a Ser/Thr, is associated with the transfer of the C8-H to the α- or β-PO_4. The C8-H to α- or β-PO_4 inter-atomic distance is 4.932 ± 0.876 Å, while the C8-H to Ser/Thr hydroxyl oxygen and Ser/Thr hydroxyl hydrogen to α-PO_4 distances are 3.495 ± 0.446 Å and 2.672 ± 0.537 Å, respectively (Figure 3, Additional file 1: Table S7). The Arg and Ser residues are situated on opposite faces of the imidazole moiety of the adenyl group. The reaction occurs via a carbene mechanism with the carbene being stabilized via the interaction of a conserved backbone carbonyl that is within bonding distance of C8 at 2.781 ± 0.488 Å (Figure 3, Additional file 1: Table S7). The backbone carbonyl usually arises from a Gly residue. Once the hybridization of C8 from sp^2 to sp^3 has occurred, the protonation of the α-PO_4 by the Ser and the concomitant release of the C8-H occurs, affording a stable carbene. The transfer of the proton between the α- and β-PO_4 groups occurs via a coordinated Arg residue which is 2.756 ± 0.708 Å and 3.095 ± 0.908 Å, respectively, from the α- and β-PO_4 groups. The activation of the nucleophile substrate hydroxyl group occurs via deprotonation by a coordinating Asp, rendering it more nucleophilic (polyol-OH hydrogen to the Asp oxygen average inter-atomic distance = 2.533 ± 0.583 Å). As discussed previously, once the proton translocation has occurred, a pentavalent phosphorus(V) intermediate is formed between the substrate and the γ-PO_4, which is now in a position to act as a leaving group. The substrate is activated to nucleophilic attack on the γ-PO_4 by deprotonation of a hydroxyl group by a hydrogen bonded Asp (polyol -OH to Asp inter-atomic distance is 2.533 ± 0.583 Å). The substrates phosphorylated by the hexokinase, FGGY, and ROK kinases are polyols while short-chain carboxylic acids are the substrates for the acetokinase family. The Group 4 kinases fall within the actin-like ATPase and ribokinase-like superfamilies (Additional file 1: Table S8). The C8-H dependent KIE has been demonstrated in hexokinase a member of the Actin-like ATPase superfamily of Group 4 [4].

Group 1 kinases

The identified conserved amino acids in the Group 1 kinases, comprising the protein Ser/Thr-Tyr kinases, atypical protein kinases, lipid kinases and the ATP-grasp families are outlined in Supplementary Information (Additional file 1: Table S1). The identified residues associated with phosphoryl transfer distinguish the Group 1 kinases into 3 groups; (1) The protein kinase superfamily comprising families containing the protein kinase domain and protein tyrosine kinase domain, (2) the protein kinase superfamily comprising the choline kinase/ethanolamine kinase family, the phosphotransferase enzyme family and the amino glycoside/hydrourea antibiotic resistance kinase family, (3) and the lipid and ATP grasp kinases consisting of the phosphatidylinositol-4-phosphate 5-kinase and inositol polyphosphate kinase families (Additional file 1: Table S2, Figures S2, S3 & S4). The proposed reaction mechanism for the Protein kinase superfamily comprising families containing the protein kinase domain and protein tyrosine kinase domain relies on the "push" mechanism and the spatial arrangement for the initiation of the reaction is shown in Figure 4 (Additional file 1: Figure S1). The Protein kinase superfamily and protein kinase family are distinguished from all other superfamilies/families in that a conserved methionine residue is found within the active site in close proximity to C8. It is proposed that a water molecule is hydrogen bonded between the sulphur of the methionine and C8 of the adenyl ring creating the proton addition complex analogous to the Wheland intermediate implicated in electrophilic aromatic substitution. The C8-H is within H-bonding distance of the α-PO_4 (3.050 ± 0.271 Å) and the H^+ is transferred directly to the α-PO_4, allowing for the effective transfer of the H^+ to the β-PO_4 via a coordinating lysine or arginine residue. Other than the ATP-grasp kinases, the nucleophile in all cases is an -OH, which is deprotonated by a coordinating Asp and Asn/Lys/His/Arg combination, rendering it more nucleophilic. The rearrangement of the coordination of the Mg^{2+} probably contributes to the creation of the pentavalent intermediate and as a result of the protonation of the β-PO_4 by the coordinating Lys, ensures that the γ-PO_4 is a better leaving group. As the γ-PO_4-phosphorylated nucleophile leaves the pentavalent transition state, the H^+ coordinated to the Asp and Lys/His/Arg, which arises from the nucleophile, then "returns" to the γ-PO_4. This allows for the migration of the Mg^{2+} to be β-PO_4/α-PO_4 coordinated and the return of the H^+ at the β-PO_4 to the α-PO_4. Finally, C8 is re-protonated and subsequently the delocalization of the electrons of the adenine returns to its most stable tautomer. The detailed reaction mechanism is outlined in the Supplementary Information (Additional file 1: Table S1).

A major variation in the conserved catalytic mechanism within this group, that arises between the protein Ser/Thr-Tyr kinases and atypical protein kinases on one hand, and the lipid and the ATP-grasp kinases on the other, is the difference lies in the activation of the substrate for nucleophilic attack on the γ-PO_4. Generally the protein Ser/Thr-Tyr kinases and atypical protein kinases function via a conserved Asp/Asn residue that allows for the deprotonation of the substrate -OH while the lipid kinases appear to function using a conserved Asp/Lys residue. There are however examples of conserved amino acid functionality e.g. Asp to Glu. Furthermore, substrate activation by the ATP-grasp kinases, which utilize acid

substrates *e.g.* the acetate, butyrate and propionate, appears to make use of the mechanism found in the Group 4 kinases in conjunction with a number of His residues (see Group 4, Additional file 1: Table S7).

The differentiation of the Group 1 kinases can be extended to 3 distinct "sub-groups" based on the sequence alignments of the conserved functionality identified by the mechanism by which phophorylation is initiated (Additional file 1: Figures S2, S3 & S4). In each case, the residues involved in the initiation of phosphoryl transfer are conserved within the sub-group. Other than these conserved residues, there is very little sequence homology within the families. Based on the Pfam family classification the protein Ser/Thr-Tyr kinase family, α-PO_4 to β-PO_4 the proton transfer is mediated via a coordinated lysine while in the choline kinase family the α-PO_4 to β-PO_4 transfer is mediated via a conserved Arg residue (Additional file 1: Table S1, Figure S2 & S3). However, two structures which fall within the phosphotransferase family also utilise Lys for the α-PO_4 to β-PO_4 proton transfer. These sequences however, are not similar to the protein Tyr kinase sequences (Additional file 1: Figure S2 & S3). The conserved functionality within the lipid kinases depends on the complexities/order of domain the assembly. Taking the order of the functional domain assembly into account, the identified functional residues within the families of the lipid kinases remain conserved (Additional file 1: Figure S4).

Group 2 kinases

The Group 2 kinases consist of the Rossmann-like kinases, phosphoenolpyruvate carboxykinase, phosphoglycerate kinase, aspartokinase-like kinases, phosphofructokinase-like kinases, ribokinase-like kinases, thiamine pyrophosphokinase and glycerate kinase.

The mechanism associated with the Rossmann-like kinases and phosphoenolpyruvate carboxykinase has been outlined in the definition of the "push" mechanism. Structurally and mechanistically, the pantothenate and deoxyguanosine kinases differ from the other kinases located in the Rossmann-like and phosphoenolpyruvate carboxykinase families in that only one Arg is implicated in the phosphoryl transfer mechanism and the Arg is located on the opposite face of the ribose sugar (the "D-face") (Additional file 1: Table S3). This Arg plays a role in the protonation of the C8. However, this residue is also in close enough proximity to the α-PO_4 and the β-PO_4, to facilitate proton transfer. A primary sequence alignment of the structures within the Rossmann-like kinases (members of the P-loop containing nucleoside triphosphate hydrolase PFam superfamily) shows two distinct sub-families, grouped based on the conserved residues associated with reaction mechanisms (Additional file 1: Table S4, Figure S8 & S9). The conserved amino acid residues identified in Additional file 1: Table S3 are highlighted in Additional file 1: Figures S8 & S9. The two distinct modes of functionality identified are not necessarily conserved within a family (Additional file 1: Table S4 & Figures S8 & S9). The shikimate kinase, uridine kinase and adenylate kinase families all contain two conserved Arg residues in the active site linked to the proton translocation (Mechanism 2A) while the second family contains only one conserved Arg residue (Mechanism 2B).

The initiation of phosphoryl transfer with the aspartokinase family within Group 2 also relies on the "push" mechanism however, the spatial arrangement of the residues responsible for the reaction differ from the P-loop containing nucleoside triphosphate hydrolase superfamily (Figure 4, Additional file 1: Figure S6). Mechanistically, the aspartokinase family diverges from the Rossmann-like kinases and phosphoenolpyruvate carboxykinase in that there is no Arg associated with the adenyl ring to facilitate the protonation of C8. Within the aspartokinase family protonation of C8 is achieved via the coordination of a backbone amide. However, common to this family is the transfer of the C8-H to an intermediate Asp/Ser which leads to the protonation of the α-PO_4, as well as the presence of a co-ordinating Lys which is responsible for the inter-α-PO_4/β-PO_4 transfer (Figure 4; Additional file 1: Table S4, Figure S6, Figure S10). Within the aspartokinase family, the enzyme aspartokinase has an additional Arg residue between the Asp which facilitates the deprotonation of C8 and the protonation α-PO_4. A primary sequence alignment of the proteins making up the aspartokinase family identified all the functional residues in a region of relatively high sequence homology (Additional file 1: Table S4 & Figure S10).

The Group 2 ribokinase-like superfamily use the distinctive "pull" mechanism based on the protonation of N7 initiating the formation of a carbene at C8 as occurs in the Group 4 kinases. As occurs in the Group 4 kinases the carbene is stabilized via a reaction with a coordinated backbone carbonyl arising from a conserved Gly residue. The Group 2 ribokinase-like superfamily requires a conserved Thr/Arg for the protonation of N7 and subsequent protonation of the α-PO_4 from C8 via a conserved H_2O. The proton transfer between the α- and β-PO_4 is also mediated via a conserved Thr/Lys (Figure 4, Additional file 1: Table S4, Figure S7, Figure S11). A distinguishing feature of this group is the interaction of a peptide bond with the adenyl group, spanning C6-NH_2 and N1, possibly stabilising the adenyl ring. The protonation of C8 occurs via the coordination of a conserved Thr/Lys. Within the ribokinase-, PFK- and hexokinase-like superfamilies the "pull" mechanism is achieved by similar spatial arrangements achieved by 3 distinct groups as is evident from their sequence alignments

(Additional file 1: Figures S11). Also included in the Group 2 is phosphoglycerate kinase family [2,3]. This family, however, carries out the reverse reaction and therefore does not require the C8-H to facilitate phosphoryl transfer. Therefore, within the structure of phosphoglycerate kinase, no residues responsible for facilitating the formation of the pentavalent intermediate between the substrate and ATP are found. Within the phosphomethyl pyrimidine kinase and ADP-specific phosphofructokinase/glucokinase families of the ribokinase superfamily the reactions are either ADP dependent or transfer pyrophosphate and therefore do not have a residue responsible for proton transfer between the α- and β-phosphate.

Group 3 kinases

The reaction mechanism identified for the Group 3 kinases is limited to the structural information obtained from the guanido family comprising creatine and arginine kinase (Figure 4, Additional file 1: Table S5, Figure S14). There is, however, significant sequence and structural homology between these enzymes (Additional file 1: Table S5 & Figure S15). As with many kinase groups discussed, this reaction is initiated by the "push" mechanism via coordination of a carbonyl group arising from the protein backbone, to the ATP C6-NH_2 (spatial arrangement outlined in Figure 4). The acidification of the C8-H allows for the protonation of the coordinated Arg, allowing proton shuffling resulting in the required tautomeric forms of the α- and β-PO_4 residues. This results in the protonation of the β-PO_4 via the electron delocalization across the Arg guanidium group and proton transfer from the Arg NεH to β-PO_4. The creatine/arginine substrate guanidium group is activated by deprotonation via a coordinating Arg residue allowing for the formation of the pentavalent intermediate between the substrate and the γ-PO_4 as a leaving group (Figure 4, Additional file 1: Table S5). The inductive effect of the Mg^{2+} renders the renders the γ-PO_4 residue more electrophilic, promoting addition of the creatine nucleophile. The protonation state is restored by the interaction of proximal Asp and Arg residues, and the proton translocation is reversed after phosphoryl transfer. The conserved functionality within Group 3 is as outlined (Additional file 1: Figure S15). The Arg responsible for the substrate deprotonation is stabilized via a coordinated Asp residue which acts to deprotonate the Arg prior to the deprotonation of the substrate.

Group 4 kinases

The Group 4 kinases are outlined as the definition of the "pull" mechanism. Within the FGGY family of the Actin-like superfamily, two distinct sub-families may be defined in terms of the residues required for functionality (Additional file 1: Table S8 & Figures S17 & S18). Within the ROK family of the Actin-like ATPase superfamily, the amino acid residues involved in phosphoryl transfer cannot be identified as no crystal structure containing a nucleotide exists (Additional file 1: Table S8 & Figure S4). The functionality is well conserved within the acetokinase family (Additional file 1: Table S8 & Figure S20). The residues involved in phosphoryl transfer within this acetokinase family differ from those in the hexokinase, FGGY, and ROK kinases. This mechanism involves a number of conserved His residues and may not involve the C8-H. However, protonation of the phosphate backbone of ATP is still required for phosphoryl transfer. The proton may originate from the substrate and be transferred along to the α-PO_4 via a cascade of conserved His residues (Additional file 1: Figure S20). The hexokinase family appears to have a similar mechanism as occurs within all the Group 4 kinases; however, only a single structure containing a nucleotide ligand exists (Additional file 1: Table S8 & Figure S21).

Group 5 kinases

Group 5 (TIM β/α-barrel kinases) contains pyruvate kinase as the example which, as with phosphoglycerate kinase in Group 2, carries out the reverse reaction synthesizing ATP. Both examples contain no amino acid side-chains associated with the acidification of C8-H coordinated to the adenyl group of the nucleotide. Side-chains deemed to be necessary for the acidification of C8-H are the coordinating backbone carbonyl group capable of interacting with the N6-NH_2 and/or side-chains coordinated with either N7 or C8.

Group 6 kinases

The Group 6 kinases (Figure 4, Additional file 1: Table S9) comprises three known structures that have a conserved active site in which the adenyl ring is in a *syn* conformation relative to the ribose, allowing for the coordination of the C3-H to the α-PO_4. The critical question that arises as to the putative role C3-H may play in inducing phosphoryl transfer due to its proximity to the α-PO_4 and hence, by analogy to the situation in the case of the C8-H in other group, would be purely speculative. No KIE evidence was obtained in steady-state enzyme reactions to suggest that the C3-H of adenine plays a similar role in this group of kinases as the C8-H does within the other groups [4]. It is however conceivable that the ATP only binds in the *syn* conformation and is induced to rotate on its glycosidic bond to the *anti* conformation as part of the overall mechanism to binding specificity. Using the same theme of using conserved amino acid residues within a group of kinases to suggest mechanistic implications, it is proposed that this reaction is initiated by the coordination of the conserved Asn γC-carbonyl group to the ATP C6-NH_2

(Additional file 1: Figure S22). The coordination of a conserved lysine with the concomitant delocalization of the electrons of the adenyl group results in the re-hybridization of C8 from sp^2 to sp^3, that, along with the change of the conformation of the adenyl group from the *syn* to the *anti*-conformation there allows for the protonation of C8 by the lysine residue forming the carbene with the concomitant protonation of the α-PO_4 from C8. The proton translocation from the α-PO_4 creating the pentavalent intermediate then occurs. The lysine residue serves two roles, one being the deprotonation of the Asn amide in the activation of the amide to facilitate the deprotonation of the ATP C6-NH_2, and the second role is in the protonation of C8 in the stabilization of the carbene. A proton required for the creation of the pentatvalent intermediate also originates from the substrate via the Glu/Asp deprotonation of the substrate. The members of Group 6 are within the Ribosomal protein S5 s-like superfamily comprising the GHMP family (Additional file 1: Table S10 & Figure S23).

Group 7 kinases

The Group 7 kinases comprise thiamine monophosphate kinase and selenide water dikinase. The thiamine monophosphate kinase (EC 2.7.4.16) is from a prokaryotic system and uses ATP to phosphorylate thiamine monophosphate to thiamine pyrophosphate, while producing ADP. This thiamine monophosphate kinase differs from the thiamine pyrophosphate kinase (Group 2) and 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (Group 3) in that the latter two are pyrophosphotransferases transferring a pyrophosphate group from a nucleoside triphosphate, such as ATP to the hydroxyl of thiamine. All enzymes in Group 7 are homo-dimeric, with the active site comprising amino acid residues from both subunits (Figure 4, Additional file 1: Table S11, Figure S24). It is only on the formation of the dimer that the reaction can occur, since the carbonyl group coordinating ATP C6-NH_2 in the "pull" mechanism implied here, as well as a conserved backbone carbonyl which stabilizes the carbene formed at C8, both arise from residues in one subunit, with the remainder of the coordinating residues arising from the second subunit. The initiation of phosphoryl transfer therefore only occurs on dimerization, enabling adequate association of the various residues with the ATP molecule. The reaction mediated by the thiamine monophosphate kinase is initiated once proton transfer has occurred from C8-H to the α-PO_4. This is followed by protonation of the substrate via a cascade involving a conserved His residue and a series of Asp residues (Figure 4; Additional file 1: Figure AF 10). The interatomic distances are: α-PO_4 to His = 2.748 ± 1.25 Å, His to Asp = 4.020 ± 1.411 Å and Asp to Asp = 3.923 ± 1.357 Å. The protonation of the substrate allows the pentavalent intermediate to be formed, and the coordination of the Mg^{2+} to migrate from being the β-PO_4/γ-PO_4 coordinated to being α-PO_4/β-PO_4 coordinated, with the concomitant re-protonation of C8.

Groups 8

There is only one member in each of Group 8, Group 9, Group 10 and Group 11 kinases. Group 8 consists of riboflavin kinase from *Schizosaccharomyces pombe* and *Homo sapiens* (flavokinase family) (Additional file 1: Table S12). The postulated phosphoryl transfer mechanism is similar to all other groups utilizing the "push"mechanism (Additional file 1: Table S12, Figure S25 & Figure S26). The mechanism occurs via coordination of the adenyl C6-NH_2 and protonation of C8 via a coordinated Lys changing C8 from sp2 to sp3 hybridization, and alters the protonation of C8-H. The C8-H becomes more acidic, allowing for the protonation of the β-PO_4, via a conserved Asp to Arg proton transfer. The H^+ transfer is directly to the β-PO_4, facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a simultaneous Glu-mediated deprotonation of the substrate-OH that allows for the nucleophilic attack by the substrate, creating the pentavalent intermediate and allowing phosphoryl transfer. The protonated Glu then transfers the proton to the γ-PO_4 changing the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

Groups 9 to 12 kinases

Group 9 contains dihydroxacetone kinase from *Citrobacter freundii*, Group 10 contains glycerate kinase from *Neisseria meningitides* and Group 11 polyphosphate kinase from *Escherichia coli* (Additional file 1: Table S13). Not enough structural data was found for Groups 9, 10 and 12 to allow for the elucidation of the reaction mechanisms. The postulated phosphoryl transfer mechanism found in the Group 11 kinases which utilizes the "push"mechanism but also undergoes an autophosphorylation of a histidine residue (polyphosphate family) (Additional file 1: Table S14, Figure S27 & Figure S28) [8,9]. The mechanism occurs via coordination of the adenyl C6-NH_2 from the protein backbone to the ATP C6-NH_2 and an Asn carbonyl which acts to stabilize the carbene formed at C8. The C8-H becomes more acidic, allowing for the protonation of the α-PO_4, via a conserved Arg proton transfer. A second conserved Arg transfers the proton from the α-PO_4 to the γ-PO_4 facilitating the formation of the pentavalent intermediate to allow for phosphoryl transfer. There is a simultaneous deprotonation of a conserved His allowing for the nucleophilic attack of the His for the γ-PO_4. There is also a putative deprotonation of the substrate phosphate

by a second His allowing for the concomitant formation of the phosphate dimer (polymer). The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

Discussion

The role of C8 in electrophilic substitution reactions is not unprecedented in nucleotides under basic conditions, where the lone pairs on N3 and N7 are available to donate electron density through the conjugated system to facilitate the electrophilic attack at C8 [10-12]. A number of conserved mechanisms have been identified whereby the C8H of ATP plays a direct role in the initiation of phosphoryl transfer in kinase reactions. A required aspect of this is the locking of the adenyl moiety into position by interactions with C6-NH_2, impacting on the delocalization of electrons in the adenyl moiety as a result. Within the group classification as outlined by Cheek *et al*, this role of the C6-NH_2 is found in all groups other than Group 4. In these groups resonance interactions giving the backbone peptide bond the planar nature with double character and the concomitant impact on the C6-NH_2, facilitates this process via the charge distribution across the peptide bond and the charge to charge interaction with the N6-NH_2. In Group 4 the interaction of a conserved Arg residue interacting with the N7/C8 facilitates the change in hybridization of C8 back to sp^2 with the concomitant release of the C8H initiating phosphoryl transfer.

It is from this point that phosphoryl transfer is initiated via a number of family-specific proton transfer cascades. Two mechanisms therefore arise by which the adenyl moiety of ATP plays a direct role in the initiation of phosphoryl transfer; a "push" mechanism arising from the coordination of a conserved backbone moiety coordinated to the C6-NH_2 and a "pull" mechanism arising from the protonation of N7 to facilitate the creation of a carbene at C8. Each of these mechanisms would allow for the regulation of the rate of formation of the pentavalent intermediate between the ATP and the substrate nucleophile. Phosphoryl transfer within kinases is therefore a specific event mediated and regulated via the coordination of the adenyl moiety of ATP. Within families the alignment of sequences generally gave very low sequence identities. However, the residues associated with phosphoryl transfer are always conserved and, more importantly, the residues identified for their role in the general acid and base catalysis associated with the initiation of the reaction and the creation of the pentavalent transition are also always conserved within families. The secondary structure elements within families are conserved. The evolution of these kinase catalytic mechanisms appears to be convergent evolution as within groups and families both prokaryotic and eukaryotic organisms are found. This is especially born out in the Protein Ser/Thr-Tyr kinases and Atypical protein kinases as the enzymes arise from a large array of organisms utilizing varied substrates. In all cases phosphorylation is of the substrate hydroxyl group. The evolution of these kinase catalytic mechanisms appears to be based on controlling the rates two simultaneous mechanisms that occur during substrate phosphorylation. The first being the rate of deprotonation of the substrate nucleophile and the second being the rate of protonation fteγ-$PO_4$of the ATP to allow for the formation of the pentavalent intermediate. In thekinase enzymes therefore, within the catalytic site of the enzyme, the rate of reaction is dependent on two factors, the induced pK_a of the substrate and rate of induction of the C8H to become labile allowing for the effective transfer of a proton to the γ-PO_4 of the ATP to allow for the formation of the pentavalent intermediate. These mechanisms have therefore evolved independently but convergently to achieve specific rates of reactions. These reaction rates probably allow for the overall rate of reaction of each enzyme to be regulated within a range. The range is dictated by the mechanistic classification. Each mechanistic group has a different number of bonds through which the tautomerization and proton translocations need to occur.

A significant number of kinase enzymes also undergo large molecular dynamic motions during the course of each reaction. The molecular dynamics associated with the binding mechanisms and their role in the orientation of specific amino residues to ensure the release of the C8-H to allow for phosphoryl transfer is borne out in a number of systems. In the remodeling of the σ54* by the bacterial enhancer protein belonging to the NtrC subclass of the AAA + ATPases, the ATP binding site resides at the interface between two subunits [13-15]. Comparing the ATP-bound structure with the ADP-bound structure demonstrated large-scale conformational changes concomitant with ATP binding. Movement induced in the walker subunit in strand β7 and Linker 1 is associated with the partial unwinding and translocation of helix H8 and with the upward and outward role of the L1 loop and its σ54-binding GAFTA motif. Concomitant to the major conformational changes which occur in the walker subunit associated with σ54-binding is a major re-alignment of the residues associated with the "push" mechanism. The "push" mechanism utilized is similar to that used by the Group 3 kinases. A comparison of the inter-atomic distances in the ATP- and ADP-bound structures in the residues associated with the "push" mechanism demonstrated significant reduction in the inter-atomic distances (PDB: 3M0E and 1NY6[14,15]. The inter-atomic distances for the ADP- and ATP-bound structures between the

ATP/ADP C6-NH_2 to backbone carbonyl (Val140) inducing the tautomeric change in the adenyl ring, are 2.710 and 2.053 Å, respectively. The reaction occurs via a carbene mechanism with the carbene being stabilized via the interaction of a conserved backbone carbonyl (Gly170) that is within bonding distance of C8, causing C8-H to become more acidic, allowing for the direct protonation of the α-PO_4, with the inter-atomic distances for the ADP- and ATP-bound structures being 4.688 and 3.896 Å, respectively (See Group 7 kinases). The proton transfer creating the pentavalent intermediate occurs via Arg357 with the inter-atomic distances for the ADP- and ATP-bound structures being .5.807 and 2.221 Å to the α-PO_4, 8.673 and 4.046 Å to the β-PO_4 and 2.254 to the γ-PO_4, respectively. The conformational changes induced in the structure of the ATP are caused by the interactions of Arg299 on the Rfinger subunit interacting with the ATP in the active site of the Walker subunit, the inter-atomic distances for the ADP- and ATP-bound structures being between Arg299 and the β-PO_4 being 11.136 and 4.056 Å, respectively.

Cyclin dependent kinase-2 (CDK2) is a Ser/Thr protein kinase belonging to Group 1 which only shows catalytic activity when the subunit is bound by the allosteric Cyclin protein and the catalytic domain has been phosphorylated on Thr160 located within the kinase "activation loop" motif [16,17]. This enzyme also undergoes significant catalytic site closing motion on binding the peptide substrate and a second Mg^{2+} ion [18]. The high-resolution refinement of CDK2 reveals 12 ordered water molecules in the ATP binding pocket of the apoenzyme and five ordered waters in that of the ATP complex. Despite a large number of hydrogen bonds between ATP-phosphates and CDK2, binding studies of cyclic AMP-dependent protein kinase with ATP analogues show that the triphosphate moiety contributes little and the adenine ring is most important for binding affinity [19,20]. Critical to the Group 1 mechanism is the ordered water molecule that resides between the Glu131 αC-C = O and C8-H. The extent of the molecular dynamics associated with the binding of the substrate and the ATP is indicated in the change in the inter-atomic distances between the structure only containing an γ-S ATP and 1 Mn^{2+} and a structure containing ADP, MgF_3, 2 $Mg^{2+}$and the peptide substrate (Additional file 1: Table S1).

Structural evidence for the formation of the pentavalent intermediate in a bimolecular system as found in a kinase reaction has been shown in the phosphorylation of arginine by arginine kinase [21]. Using the transition state analogue complex, the nitrate mimics the planar γ-phosphoryl during associative in-line transfer between ATP and arginine and it is possible to measure how precisely the reactants are pre-aligned by the enzyme. The alignments were found to be exquisite. Substrates are positioned not only in close proximity but with orbitals aligned close to optimally. Consistent with in-line transfer, the donor and acceptor atoms (ADP β-O, and guanidinyl N_η) are positioned to form bonds within 0.2° orthogonal to the phosphoryl (nitrate) plane. With respect to the guanidinyl N_η, the optimal direction for nucleophilic attack by its lone pair is at an angle of approximately 110° to the N_η-C_γ bond in a plane perpendicular to the guanidinium plane. The nitrate nitrogen, mimicking the P_γ, is at 115°, close to ideal.

Conclusions

The kinase enzymes have been classified into 25 families of homologous proteins, with the families assembled into 12 fold-groups based on the similarity of their structural folds [2]. However, this classification relays little, if any, information on the catalytic and regulatory mechanisms employed in nucleotide binding and phosphoryl transfer. Within a single group, both prokaryotic and eukaryotic organisms are represented with kinase isoenzymes that appear to be kinetically and functionally distinct based on the rate of phosphoryl transfer and the regulation thereof. Clearly the regulation of enzyme activity in kinases is complex, which manifests in the apparent K_M of the kinases ranging from less than 0.4 μM to in excess of 1000 μM for ATP (Carna Biosciences, Inc., Kinase Profiling Book:http://www.carnabio.com, [22]). It is conceivable that the various conserved "push" and "pull" mechanisms associated with the release of C8-H, the proton transfer cascades and the resultant/concomitant creation of the pentavalent transition state are the mechanism by which the kinase enzymes achieve this 2500 fold variation in the K_M. Permutations and combinations of the various mechanisms identified are therefore employed by the kinase enzymes to achieve the variation in enzyme kinetics of this diverse group of proteins effectively carrying out the same phosphorylation reaction. The initiation and regulation of the rate of this reaction is achieved by the manner in which the C8-H is induced to be labile and the mechanism by which the resultant proton transfer cascade is achieved in the ultimate creation of the pentavalent transition state between the γ-phosphate of the ATP and the substrate nucleophile. Within the active site of the enzyme the rate of reaction is therefore governed firstly by the various mechanisms by which the C8-H is induced to be labile and secondly, by the mechanisms by which the resultant effective translocation of a proton from C8-H to the pentavalent transition state between the γ-phosphate of the ATP and the substrate nucleophile occur. In kinase enzyme steady state enzyme kinetics the overall perceived reaction rate therefore comprises the contribution of this catalytic effect as well as the contribution of the first order concentration effect of the ATP. The rate of

reaction in kinases is therefore dictated by the hydrogen bonding network that is set up between the amino acids on the surface of the protein binding site, the ATP molecule and any substrate molecule(s) that may be present. The key result of this interaction network is the change in the distribution of electron density in the heteroaromatic ring by two possible means, each facilitating the weakening of the C8-H bond and translocation of the proton to the triphosphate chain. This, in turn, ensures the formation of the pentavalent transition state through the hydrogen-bond mediated activation of the gamma phosphate group and subsequent nucleophilic attack thereof by the substrate. A number of conserved mechanisms have been identified by which the translocation of the C8 proton and subsequent activation of the gamma phosphate may occur. The contribution of these steps, both the electron redistribution in the heterocycle and subsequent activation of the triphosphate chain, to the overall reaction rate depends on the free energy contribution of each hydrogen bond used by the specific conserved mechanisms in question to the activation energy of the pentavalent transition state. The free energy contributions across the entire system, both small molecules and the protein, can probably be considered as a linear combination of the wave functions describing the electron distribution in the heterocycle, as well as the subsequent hydrogen bonding events.

Methods

Structural bioinformatics

Discovery Studio® (Accelrys Inc) was used for all molecular modelling protocols. Structures were identified within the wwPDB using the EC numbers in the families and groups outlined by Cheek *et al* [2,3]. Only structures containing AMP, ADP, ATP or their non-hydrolysable analogues were used. Interatomic and hydrogen bonding distances were measured between the nucleotide and the coordinating amino acid. where required the pyrimidine/β-D-sugar torsion angle of the nucleotide, Thr/Ser -OH torsion angle and the torsion angles within the coordinating arginine residues were rotated to optimize the interatomic distances obtained. Where required an energy-minimization was then carried out on the structure and a "bump" check was carried out on the structure to ensure that no inappropriate interactions occurred. Energy minimizations were carried out using the standard Accelrys Discovery Studio protocols using the 'Smart Minimizer' algorithm over a maximum of 10000 steps to a RMS equal to 0.1 and an energy change of 0.0. All the structural informatics accession numbers and the associated references are included in the supplementary information. All structural informatics references to be included in the additional information.

Sequence alignments and structural analysis

The protein sequence alignments were carried using the Accelrys Discovery Studio (Client 2.5) sequence alignment software, using the dynamic alignment with a gap penalty of 10, a multiple gap extension penalty of 0.05 and the BLOSUM scoring matrix. PFam searches were carried out to identify the kinase superfamilies and families. Sequence alignments were carried out within the superfamlies/families to identify the extent to which the amino acids associated with catalysis are conserved. The conserved secondary structure elements within families were also used to facilitate the sequence alignments. All sequence alignments are showing the conserved residues and the secondary structure elements are included in the supplementary information.

Additional material

Additional file 1: Supplementary information.

Acknowledgements

This work was supported by the Council for Scientific and Industrial Research (CSIR) parliamentary grant.

Authors' contributions

CPK formulated the concepts, carried out protein structural analysis, sequence alignments, defined reaction mechanisms and drafted and edited the manuscript, RLR carried out sequence alignments and edited the manuscript, CWvdW defined reaction mechanisms and edited the manuscript and CJP defined reaction mechanisms and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 3 January 2012 Accepted: 8 March 2012
Published: 8 March 2012

References

1. Florkin M, Stotz EH: **Enzyme Nomenclature.** *Comprehensive Biochemistry* 1973, **13.**
2. Cheek S, Zhang H, Grishin NV: **Sequence and structure classification of kinases.** *J Mol Biol* 2002, **320**:855-881.
3. Cheek S, Ginalski K, Zhang H, Grishin NV: **A comprehensive update of the sequence and structure classification of kinases.** *BMC Struc Biol* 2005, **5(6)**:1-19.
4. Kenyon CP, Steyn A, Roth RL, Steenkamp PA, Nkosi TC, Oldfield LC: **The role of the C8 proton of ATP in the regulation of phosphoryl transfer within kinases and synthetases.** *BMC Biochemistry* 2011, **12**:36-53.
5. Fersht A: *Structure and mechanism in protein science: guide to enzyme catalysis and protein folding* New York: W. H. Freeman & Co; 1999.
6. Pauling L, Corey RB, Branson HR: **The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain.** *Proc Natl Acad Sci USA* 1951, **37**:205-211.
7. Edison AS: **Linus: Pauling and the Planar peptide bond.** *Nature Struc Biol* 2001, **8**:201-202.
8. Ahn K, Kornberg A: **Polyphosphate kinase from Escherichia coli. Purification and demonstration of a phosphoenzyme intermediate.** *J Biol Chem* 1990, **265**:11734-11739.
9. Zhu Y, Huang H, Lee SSK, Xu W: **Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis.** *EMBO Reports* 2005, **6**:681-687.

10. Heller SR: **^{1}H NMR studies on deuterium-hydrogen exchange at C-5 in uridines.** *Biochem Biophys Res Commun* 1968, **32**:998-1001.
11. Bergstrom DE, Brattesani AJ: **Halogenation of tubercidin by N-halosuccinimides. A direct route to 5-bromotubercidin, a reversible inhibitor of RNA synthesis in eukaryotic cells.** *Nucl Acid Res* 1980, **8**:6213-6219.
12. Zhang B, Bailey VC, Potter BVL: **Chemoenzymatic synthesis of 7-Deaza cyclic adenosine 5'-diphosphate ribose analogues, membrane-permeant modulators of intracellular calcium release.** *J Org Chem* 2008, **73**:1693-1703.
13. Neuwald AF, Aravind L, Spounge JL, Koonin EV: **AAA+: A class of chaperonin-like ATPases associated with the assembly, operation, and disassembly of protein complexes.** *Genome Res* 1999, **9**:27-43.
14. Lee S-Y, DeLaTorre A, Yan D, Kustu S, Nixon BT, Wemmer DE: **Regulation of the transcriptional activator NtrC1: structural studies of the regulatory and AAA+ATPase domains.** *Genes Dev* 2003, **17**:2552-2563.
15. Chen B, Sysoeva TA, Chowdhury S, Guo L, De Carlo S, Hanson JA, Yang H, Nixon TB: **Engagement of arginine finger to ATP triggers large conformational changes in NtrC1 AAA+ATPase for remodelling bacterial RNA polymerase.** *Structure* 2010, **18**:1420-1430.
16. Radzio-Andzelm E, Lew J, Taylor S: **Bound to activate: conformational consequences of cyclin binding to CDK2.** *Structure* 1995, **3**:1135-1141.
17. Stevenson LM, Deal MS, Hagopian JC, Lew J: **Activation mechanism of CDK2: role of cyclin binding versus phosphorylation.** *Biochemistry* 2002, **41**:8528-8534.
18. Bao ZQ, Jacobsen DM, Young MA: **Briefly bound to activate: Transient binding of a second catalytic magnesium activates the structure and dynamics of CDK2 kinase for catalysis.** *Structure* 2011, **19**:675-690.
19. Schulze-Gahmen U, De Bondt HL, Kim S-H: **High-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: Bound waters and natural ligand as guides for inhibitor design.** *J Med Chem* 1996, **39**:4540-4546.
20. Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massagué J, Pavletich NPs: **Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex.** *Nature* 1995, **37**:313-20.
21. Zhou G, Somasundaram T, Blanc E, Parthasarathy G, Ellington RW, Chapman MS: **Transition state structure of arginine kinase: Implications for catalysis of bimolecular reactions.** *Proc Natl Acad Sci USA* 1998, **95**:8449-8454.
22. **Carna Biosciences, Inc., Kinase Profiling Book.** [http://www.carnabio.com].

doi:10.1186/1756-0500-5-131
Cite this article as: Kenyon *et al.*: Conserved phosphoryl transfer mechanisms within kinase families and the role of the C8 proton of ATP in the activation of phosphoryl transfer. *BMC Research Notes* 2012 **5**:131.

ADDITIONAL INFORMATION

Table AF 1A. Kinases representing **Group 1** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured interatomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**Met-S or Gln-C=O to Aden-NH_2[1]**	**C8-H to α-PO_4**	**Lys-NH_3 to α-PO_4[2]**	**Lys-NH_3 to β-PO_4[2]**	**Asp-COOH to β-PO_4[3]**	**Asp-COOH to Mg^{2+}[3]**	**Substrate H^+ removal[4,5,6]**
Protein Ser/Thr-Tyr kinases / Atypical protein kinases							
Choline kinase (Choline-OH) 3G15 (*Homo sapiens*)	Gln207Cα 2.004	2.809	Arg146 1.956	Arg146 3.798	Asp330 2.942	Asp330 2.242	Asp306 Asn311
Protein Ser/Thr kinase (Protein Ser/Thr-OH) 2BZK (*Homo sapiens*)	Pro123 Cα 3.322	4.226	Lys67 1.830	Lys67 3.025	Asp186 3.104	No Mg^{2+}	Asp167 Asn172
Phosphorylase kinase (Glycogen phosphorylase-Ser-OH) 2PHK (Rabbit)	Asp104 1.934 Met106 5.412	3.654	Lys48 2.489	Lys48 3.947	Asp167 3.204	Mn^{2+} coordinated	Asp149 Asn154 Arg7
Homoserine kinase P00547 (*Escherichia coli*)	NS						
1 Phosphotidylinositol 4-phosphate 5-kinase 2GK9 (*Homo sapiens*)	No ADP		Lys167	Lys167	Asp227	Asp227	Asp227 Lys171
Streptomycin 6-kinase P08077 (*Streptomyces griseus*)	NS		Arg64	Arg64	Asp219	Asp219	His199 Asp201
Ethanolamine kinase[7] Q9HBU6 (*Homo sapiens*)	NS		Arg167	Arg167	Asp329	Asp329	Asp310 Asn315
Streptomycin 3'-kinase P18150 (*Streptomyces griseus*)	NS		Arg60	Arg60	Asp210	Asp210	Asp190 Asn195
Kanamycin kinase (Aminoglycoside-OH) 1L8T (*Enterococcus faecalis*)	Ser91 2.294 Met90 4.618	3.225	Lys44 1.770	Lys44 2.071	Asp208 2.711	Asp208 2.159	Asp190 Asn195
Methylthioribose kinase (APS[9]-ribose-OH) 2OLC (*Bacillus subtilis*)	Glu1151.786 Met114 4.452	3.052	Lys61 1.750	Lys61 2.508	Asp250 3.243	Asp250 2.492	Asp233 His235 Open
Viomycin kinase[7] D2B3F1 (*Streptosporangium roseum*)	NS		Arg61	Arg61	Asp211	Asp211	His187 Asp189
[Hydroxymetylglutaryl-CoA reductase ($NADPH_2$)] kinase	NS						
Tyrosine kinase (Tyrosine-OH) 1U54 (*Homo sapiens*)	Gln206 2.113 Met203 8.518	3.068	Lys158 2.762	Lys158 1.944	Asp270 3.104	Asp270 2.421	Asn252 Asn257 Arg356
[Isocitrate dehydrogenase ($NADP^+$)] kinase A3RX03 (*Ralstonia solanacearum*) prokaryote	NS		Arg225	Arg225	Asp399	Asp399	His376 Asp377
[Myosin light-chain] kinase[7] Q9H8B3 (*Homo sapiens*)	NS				Asp286	Asp286	His271 Asp274
Hygromycin-B kinase[7] P09979 (*Streptomyces hygroscopicus*)	NS						
Calmodulin dependent protein kinase (Ser/Thr-	Glu139 1.924	3.818	Lys91	Lys91 7.441	Asp205	Asp205	Glu188

Kinase	**Met-S or Gln-C=O to Aden-NH_2[1]**	**C8-H to α-PO_4**	**Lys-NH_3 to α-PO_4[2]**	**Lys-NH_3 to β-PO_4[2]**	**Asp-COOH to β-PO_4[3]**	**Asp-COOH to Mg^{2+}[3]**	**Substrate H^+ removal[4,5,6]**
OH) 3LIJ	Met138 4.446		2.864		5.362	2.578	Asn189 Lys185
Rhodopsin kinase (Ser/Thr-OH) 3C4W (*Bos taurus*)	Thr265 1.711 Met264 4.297	2.788	Lys216 2.221	Lys216 2.270	Asp332 3.008	Asp332 2.074	Asp314 Asn319 Lys316
β-Adrenergic-receptor kinase 1YM7 (*Bos taurus*)	No ADP						
[Myosin heavy-chain] kinase[7] Q4CQ29 (*Trypanosoma cruzi*)	NS						
[Tau protein] kinase 0050321 (*Homo sapiens*)	NS						
Macrolide 2'-kinase[7] Q47396 (*Escherichia coli*)							
1 Phosphotidylinositol 3-kinase (Inositol-OH) 1E8X (*Sus scrofa*) wild boar	Gln288Cα 2.776	3.117	Lys833 2.457	Lys833 3.122	Asp964 3.423	Asp964 2.799	Asp950 Asn951 His948
[RNA-polymerase]-subunit kinase[7] Q4A3R1 (Potato virus S)	NS						
1 Phosphotidylinositol 4,5-bisphosphate 3-kinase 2B3R (*Mus musculus*)	No ADP						
1-Phosphotidylinositol-4-phosphate 5-kinase 1BO1 (*Homo sapiens*)	No ADP		Lys214	Lys214	Asp369		Asp278 Lys218
Lipid kinases							
1D-Myoinositol triphosphate kinase 1W2C (*Homo sapiens*)	Gln249 1.756	2.818	Lys209 1.855	Lys209 2.428	Asp416 3.527	Mn^{2+} coordinated	Glu262 Lys264
Inositol tetrakiphosphate 5-kinase	NS						
1-Phosphotidylinositol-5-phosphate 4-kinase[7] P78356 (*Homo sapiens*)	NS		Lys214	Lys214	Asp269	Asp269	Asp278 Lys218
1-Phosphotidylinositol-3-phosphate 5-kinase[7] Q9Y217 (*Homo sapiens*)	NS						
Inositol-polyphosphate multikinase 2IF8 (*Saccharomyces cerevisiae*)	Gln118Cα 1.636	2.847	Lys31 3.933	Lys31 1.861	Asp325 2.677	No Mg^{2+}	Glu37 Lys133 His328
1-Phosphotidylinositol 4-phosphate 5-kinase 2GK9 (*Homo sapiens*)	No ADP		Lys167	Lys167	Asp227	Asp227	Asp208 Lys171
ATP-grasp fold							
Inositol tetrakiphosphate 1-kinase 2QB5 (*Homo sapiens*)	Gln188Cα 1.879	2.853	Lys157 1.543	Distorted	Asp295 3.423	Mn^{2+} coordinated	Asn297 His162 His167 His233
Pyruvate phosphate dikinase 1DIK (*Clostridium symbiosum*)	No ADP						

Kinase	**Met-S or Gln-C=O to Aden-NH_2[1]**	**C8-H to α-PO_4**	**Lys-NH_3 to α-PO_4[2]**	**Lys-NH_3 to β-PO_4[2]**	**Asp-COOH to β-PO_4[3]**	**Asp-COOH to Mg^{2+}[3]**	**Substrate H^+ removal[4,5,6]**
Pyruvate, water dikinase[7] Q8TKJ7 (*Methanosarcina acetivorans*)	NS						

Kinase	**Met-S or Gln-C=O to Aden-NH_2[1]**	**C8-H to α-PO_4**	**Lys-NH_3 to α-PO_4[2]**	**Lys-NH_3 to β-PO_4[2]**	**Asp-COOH to β-PO_4[3]**	**Asp-COOH to Mg^{2+}[3]**	**Substrate H^+ removal[4,5,6]**
Phosphomevalonate kinase 3GON **(FROM GROUP 2)**	50 4.557	2.715	4.57	3.916	Distorted	Distorted	
Mean[10] **Standard Deviation** **% Standard Deviation**	**2.082** **0.328** **20.561**	**3.050** **0.271** **8.887**	**2.293** **0.667** **29.105**	**2.545** **0.605** **23.770**	**2.984** **0.490** **16.429**	**2.373** **0.242** **10.218**	
Cyclin dependent kinase-2 3JST (1Mn^{2+})	Glu81 1.568	2.581	Lys33 2.921	Lys33 1.757	Asp127 8.312	α-β-PO_4 Mn^{2+} 5.299	
Cyclin dependent kinase-2 3QHW (3Mg^{2+})	Glu81 1.739	2.937	Lys33 1.744	Lys33 2.388	Asp127 5.940	α-β-PO_4 Mn^{2+} 4.674	

1. Coordination of the carbonyl of the backbone carbon and the thiol ether of Met to the hydrogen of the N6-NH_2 and C8 respectively.
2. The coordinated Lys acts to transfer the H^+ from the α-PO_4 to the β-PO_4.
3. Asp to β-PO_4 coordinated and Asp is Mg^{2+}
4. Residues associated with the deprotonation of the substrate.
5. Residues associated with the deprotonation of the substrate.
6. Residues associated with the deprotonation of the substrate.
7. Residues identified by sequence alignment using UniProt sequence (Accession Number as indicated).
   NS = No structure in PDB.

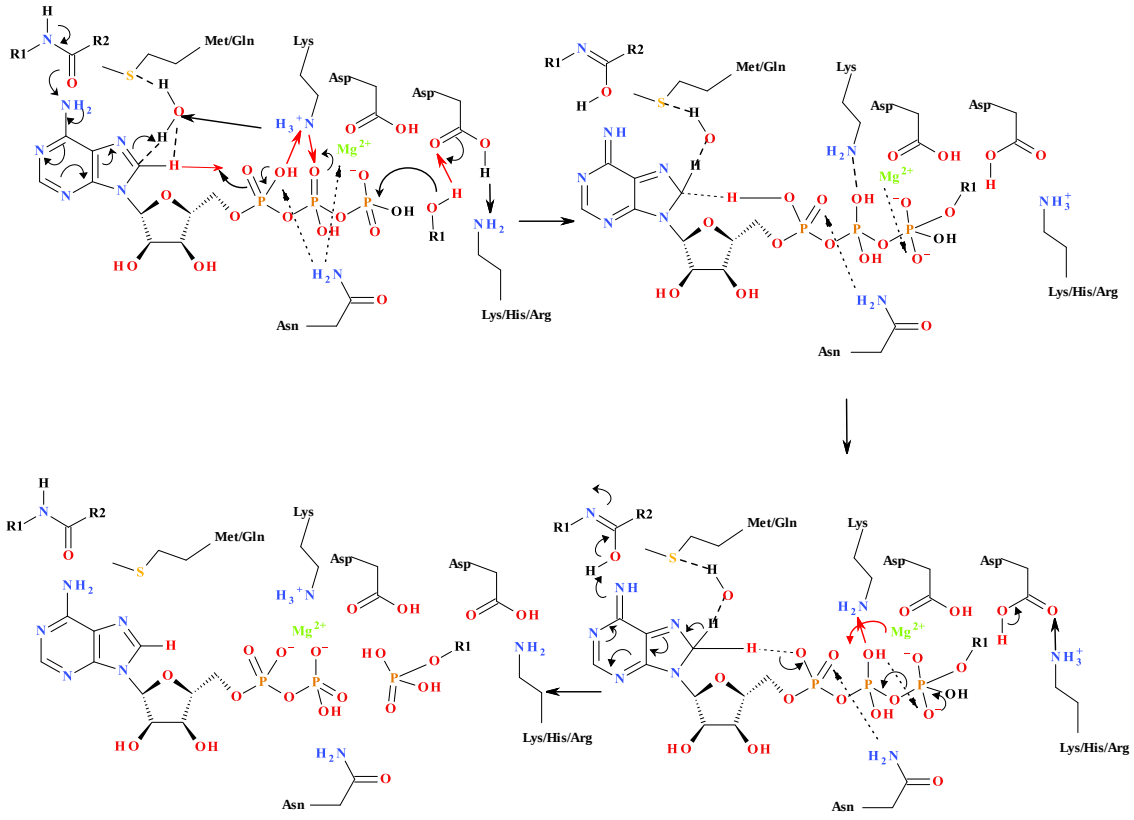


Figure MECH1. Phosphoryl transfer mechanism found in the Group 1 kinases. Within the Protein kinase superfamily and protein kinase family a conserved methionine residue is found within the active site. It is proposed that a water molecule is hydrogen bonded between the sulphur of the methionine and C8 of the adenyl ring creating the proton addition complex analogous to the Wheland intermediate implicated in electrophilic aromatic substitution. The coordination of the backbone carbonyl to C6-NH_2 creates a "push" mechanism that renders the C8-H more acidic, releasing the H^+ to the oxygen of α-PO_4. The "push" mechanism requires the protonation of C8 as part of rendering the C8-H more acidic. The C8-H is within H-bonding distance of the α-PO_4 (3.050 ± 0.271 Å) and the H^+ is transferred to the α-PO_4, allowing for the effective transfer of the H^+ to the β-PO_4 via a coordinating lysine or arginine residue. The mean interatomic distances between the Lys residue and the α- and β-PO_4 moieties are 2.293 ± 0.66 Å and 2.545 ± 0.605 Å, respectively. The protonation of the α-PO_4 and a concomitant transfer of an H^+ from the α-PO_4 to β-PO_4 via a conserved Lys facilitates the γ-PO_4 becoming activated for nucleophilic attack. There is a concomitant deprotonation of the substrate-OH via a conserved Asp. This allows for the nucleophilic attack by the substrate, creating the pentavalent intermediate, and the phosphoryl transfer. The protonated Asp then transfers the proton to the γ-PO_4. This changes the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

Table AF 1B. Group 1 kinases.

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
Group 1 Kinases: Ser/Thr-Tyr		
1. Protein Ser/Thr kinase (pdb|2BZK)	Protein kinase (CL0016)	Protein kinase domain (PF00069)
2. Phosphorylase kinase (pdb|2PHK)	Protein kinase (CL0016)	Protein kinase domain (PF00069)
3. Calmodulin-dependent protein kinase (pdb|3LIJ)	a. Protein kinase (CL0016) b. EF-hand like (CL0220)	a. Protein kinase domain (PF00069) b. EF hand (x3) (PF00036)
4. Rhodopsin kinase (pdb|3C4W)	a. Protein kinase (CL0016) b. RGS-like (CL0272)	a. Protein kinase domain (PF00069) b. Regulator of G protein signaling domain (PF00615)
5. β-Adrenergic-receptor kinase (pdb|1YM7)	a. Protein kinase (CL0016) b. RGS-like (CL0272) c. PH domain-like	a. Protein kinase domain (PF00069) b. Regulator of G protein signaling domain (PF00615) c. PH domain (PF00169)
6. Tyrosine kinase (pdb|1U54)	Protein kinase (CL0016)	Protein tyrosine kinase (PF00069)
7. Choline kinase (pdb|3GI5)	Protein kinase (CL0016)	Choline/ethanolamine kinase (PF01633)
8. Ethanolamine kinase (uniprot|Q9HBU6)	Protein kinase (CL0016)	Choline/ethanolamine kinase (PF01633)
9. Streptomycin 3-kinase (uniprot|P18150)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
10. Viomycin kinase (uniprot|D2B3F1)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
11. Hygromycin-B kinase (uniprot|P09979)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
12. Kanamycin kinase (pdb|1L8T)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
13. Methylthioribose kinase (pdb|2OLC)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
14. Macrolide 2'-kinase (uniprot|Q47396)	Protein kinase (CL0016)	Phosphotransferase enzyme family (PF01633)
15. Streptomycin 6-kinase (uniprot|P08077)	Protein kinase (CL0016)	Amino glycoside/hydrourea antibiotic resistance kinase (PF04655)
16. [Myosin heay-chain] kinase (uniprot|Q4CQ29)	nd[3]	Alpha kinase family (PF02816)
17. Homoserine kinase (uniprot|P00547)	Ribosomal protein S5domain 2-like superfamily (CL0329)	a. GHMP kinases N-terminal domain (PF00288) b. GHMP kinases C-terminal domain (PF08544)
18. [Myosin light-chain] kinase (uniprot|Q9H8B3)	Immunoglobulin superfamily (CL0011)	Immunoglobulin I-set domain (PF07679)
19. 1-Phosphatidylinositol 3-kinase (pdb|1E8X)	a. Ubiquitin (CL0072) b. C2 superfamily (CL0154) c. Tetratrico peptide repeat (CL0020) d. nd	a. PI3-kinase family, ras-binding domain (PF00794) b. Phosphoinositide 3-kinase C2 (PF00792) c. Phosphoinositide 3-kinase family, accessory domain (PIK domain) (PF00613) d. Phosphatidylinositol 3- and 4-kinase (PF00454)

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
20. 1-Phosphatidylinositol 4,5-bisphosphate 3-kinase (uniprot|O00443)	a. Ubiquitin (CL0072) b. C2 superfamily (CL0154) c. Tetratrico peptide repeat (CL0020) d. nd e. nd f. C2 superfamily (CL0154)	a. PI3-kinase family, ras-binding domain (PF00794) b. Phosphatide 3-kinase C2 family (PF00792) c. Phosphoinositide 3-kinase family, accessory domain (PIK domain) (PF00613) d. Phosphatidylinositol 3- and 4-kinase (PF00454) e. PX domain (PF00787) f. C2 domain (PF00168)
21. 1-Phosphatidylinositol 4-phosphate 5-kinase (pdb|2GK9)	nd	Phosphatidylinositol-4-phosphate 5-kinase (PF01504)
22. 1-Phosphatidylinositol 4-phosphate 5-kinase (pdb|1BO1)	nd	Phosphatidylinositol-4-phosphate 5-kinase (PF01504)
23. [Isocitrate dehydrogenase ($NADP^+$)] kinase (uniprot|A3RX03)	nd	Isocitrate dehydrogenase kinase/phosphatase (PF06315)
24. [RNA-polymerase]-subunit kinase (uniprot|QA3R1)		
25. [Hydroxynethylglutaryl-CoA reductase ($NADPH_2$)] kinase	No sequence	
26. [Tau protein] kinase	No sequence	
Group 1 Kinases: Lipid kinases		
1. 1D-Myoinositol triphosphate kinase (pdb|1W2C)	nd	Inositol polyphosphate kinase (PF03770)
2. Inositol-polyphosphate multikinase (pdb|2IF8)	nd	Inositol polyphosphate kinase (PF03770)
3. Inositol tetrakiphosphate 5-kinase (uniprot|Q9LY23)	nd	Inositol polyphosphate kinase (PF03770)
4. 1-Phosphatidylinositol-4-phosphate 5-kinase (pdb|1BO1)	nd	Phosphatidylinositol-4-phosphate 5-kinase (PF01504)
5. 1-Phosphatidylinositol-5-phosphate 4-kinase (uniprot|P78356)	nd	Phosphatidylinositol-4-phosphate 5-kinase (PF01504)
6. 1-Phosphatidylinositol-5-phosphate 5-kinase (uniprot|Q9Y217)	nd	FYVE zinc finger DEP domain TCP-1/cpn60 chaperonin family Phosphatidylinositol-4-phosphate 5-Kinase
Group 1 Kinases: ATP-grasp fold		
1. Inositol tetrakiphosphate 1-kinase (pdb|2QB5)	ATP-grasp (CL0179)	Inositol 1, 3, 4-trisphosphate 5/6-kinase (PF05770)
2. Pyruvate phosphate dikinase (pdb|1DIK)	a. nd b. nd c. Pyruvate kinase-like TIM barrel superfamily (CL0151)	a. Pyruvate phosphate dikinase, PEP/pyruvate binding domain (PF01326) b. PEP-utilising enzyme, mobile domain (PF00391) c. PEP-utilising enzyme, TIM barrel domain (PF02896)
3. Pyruvate water dikinase (uniprot|Q8TKJ7)	a. nd b. nd c. Pyruvate kinase-like TIM barrel superfamily (CL0151)	a. Pyruvate phosphate dikinase, PEP/pyruvate binding domain (PF01326) b. PEP-utilising enzyme, mobile domain (PF00391) c. PEP-utilising enzyme, TIM barrel domain (PF02896)

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
4. Phosphomevalonate kinase (pdb|3GON)	a. Ribosomal protein S5 domain 2-like (CL0329) b. nd	a. GHMP kinases N terminal domain(PF00288) b. GHMP kinases C terminal (PF08544)

[1] pfam clan classification in brackets
[2] pfam family/domain classification in brackets
[3] No Detectable (nd) similarity to conventional kinases
Where there are 2 or domains recognised, these are denoted by 'a', 'b', etc. One domain has been selected to position the protein within the table.

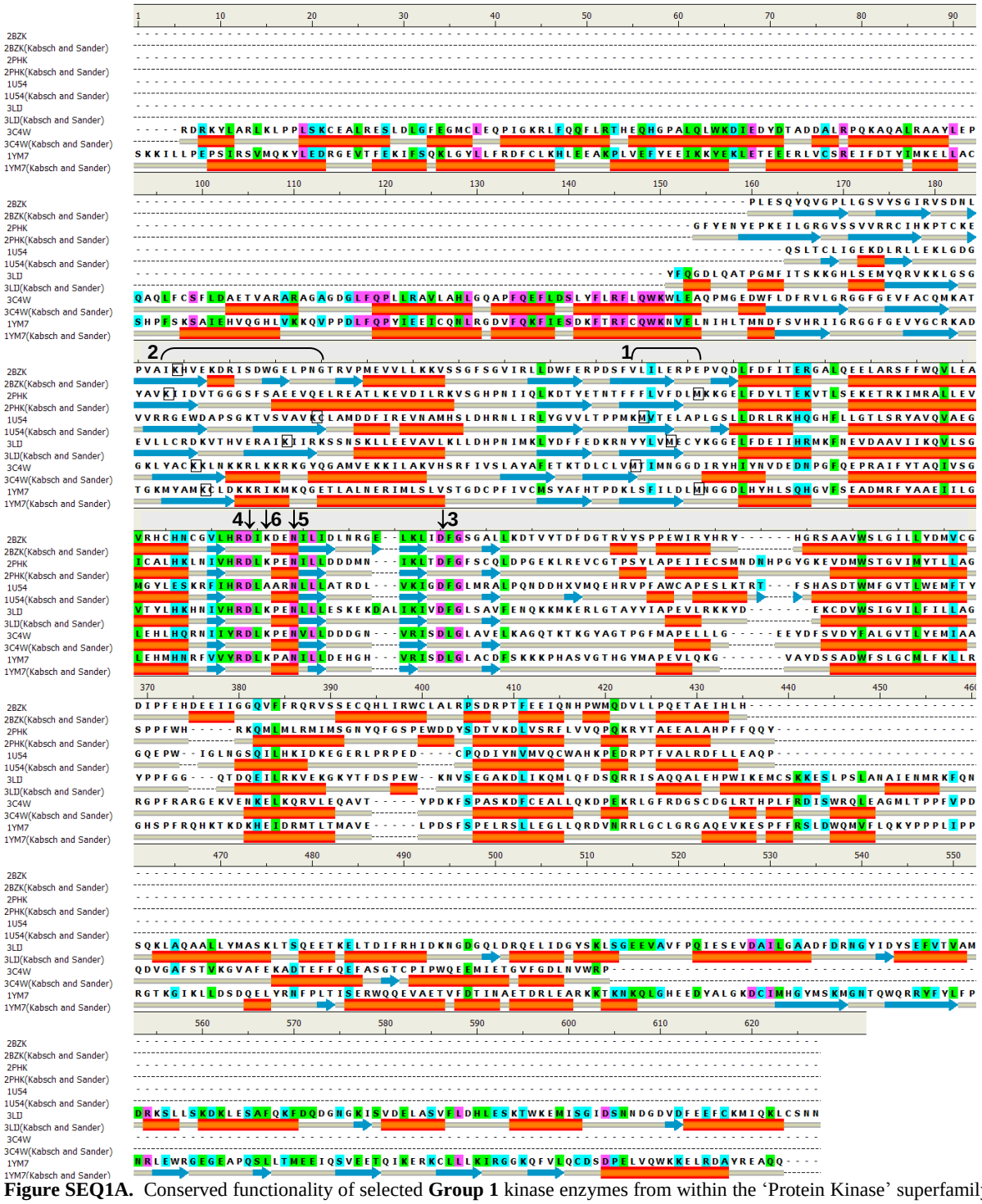


Figure SEQ1A. Conserved functionality of selected **Group 1** kinase enzymes from within the 'Protein Kinase' superfamily and 'protein kinase domain' family. 2BZK, Protein Ser/Thr kinase; 2PHK, phosphorylase kinase; 1U54, tyrosine kinase; 3LIJ, calmodulin dependent protin kinase; 3C4W, rhodopsin kinase; 1YM7, β-adrenergic-receptor kinase. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. The total percentage of identical residues in this alignment is 21.45%.

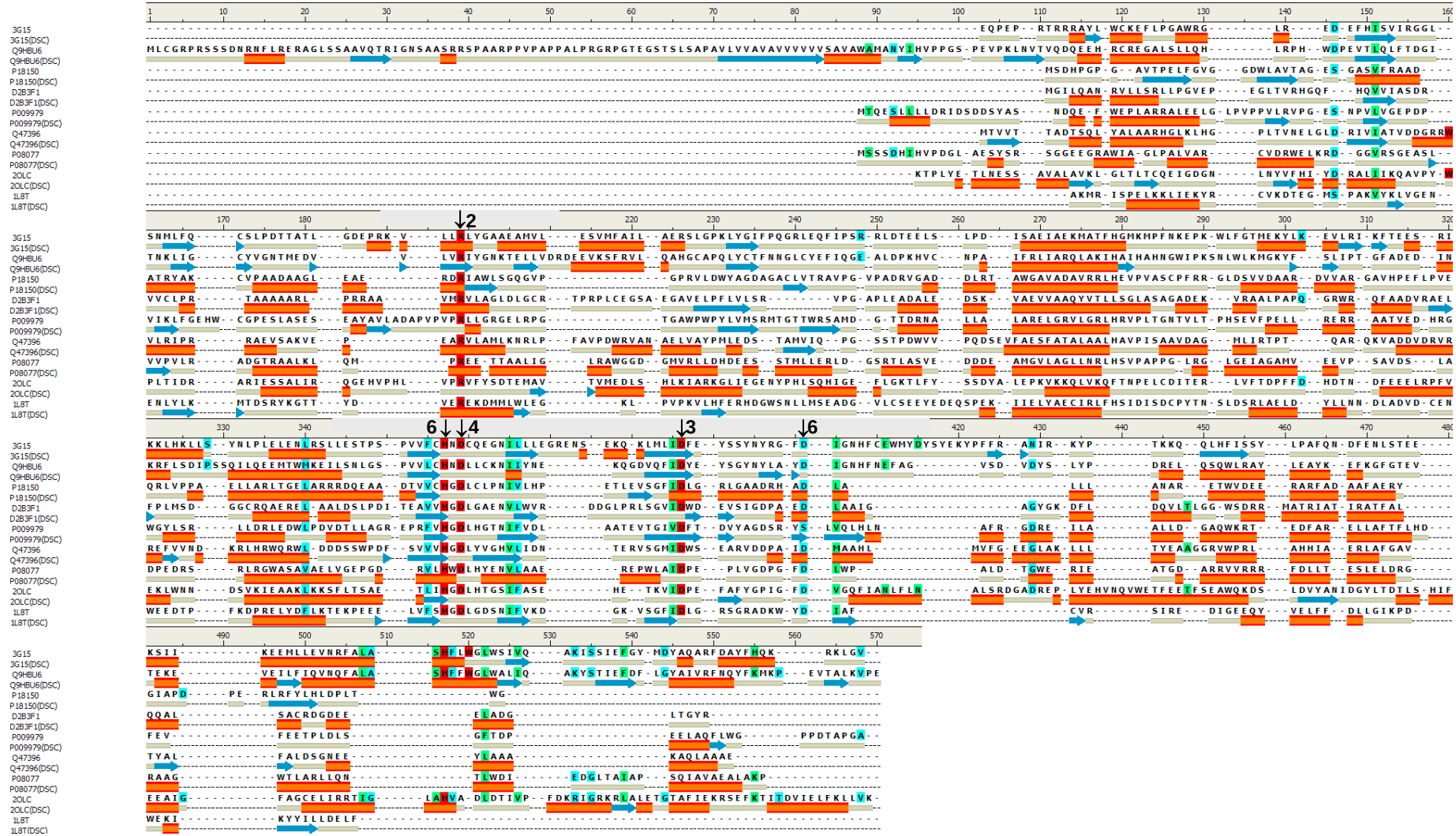


Figure SEQ1B. Conserved functionality of **Group 1** kinases that fall within the 'Protein Kinase' superfamily and 'choline/ ethanolamine kinase', 'amino glycoside/hydrourea antibiotic resistance kinase' and 'phosphotransferase' families. 3GI5, choline kinase; Q9HBU6, ethanolamine kinase; P18150, streptomycin 3'-kinase; D2B3F1, viomycin kinase; P09979, Hygromycin-B kinase; Q47396, macrolide 2'kinase; P08077, streptomycin 3'-kinase; 2OLC, methylthioribose kinase; 1L8T, kanamycin kinase. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A, except for 6 which indicates conserved His and Asp/Glu residues responsible for stabilization involved in Mg^{2+} binding and substrate deprotonation. The total percentage of identical residues in this alignment is 22.87%.

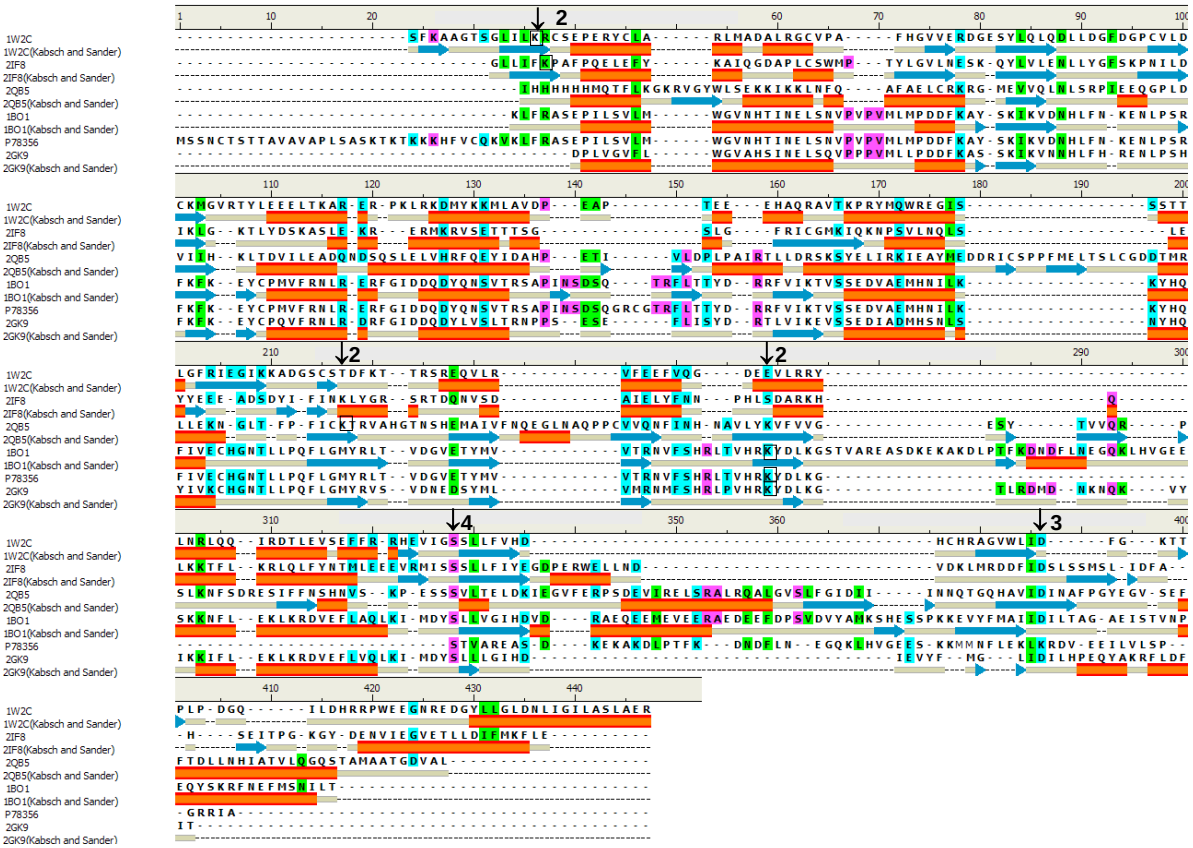


Figure SEQ1C. Conserved functionality within the 'phosphatidylinositol-4-phosphate 5-kinase' and the 'inositol polyphosphate kinase' families in **Group 1**. 1W2C, 1D-myoinositol triphosphate kinase; 2IF8, inositol-polyphosphate multikinase; 2QB5, inositol tetraphosphate 1-kinase; 1BO1, 1-phosphotidylinositol-4-phosphate 5-kinase; 2GK9, 1-phosphotidylinositol-4-phosphate 5-kinase; P78356, 1-phosphotidylinositol-5-phosphate 4-kinase. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. The total percentage of identical residues in this alignment is 22.87%.

Table AF 2A. Kinases representing **Group 2** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured interatomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**αC-C=O to Aden-NH_2**[14]	**N7-H to Arg**[1]	**C8-H to Arg**[1]	**C8-H to α-PO_4**	**Arg-NH_3 to α-PO_4**[2]	**Arg-NH_3 to β-PO_4**[2]	**Lys-NH_3 to γ-PO_4**[3]	**Thr to C8-H**[4]	**Thr to α -PO_4**[4]	**Arg to C8-H**[5]	**Thr to C8-H**[6]
Rossmann-like kinases											
Gluconate kinase[1L] 1KO5 (*Escherichia coli*)	Gln158 1.971	Arg120 3.273	Arg120 3.692	3.495 Direct	Arg124 2.940	Arg124 Distorted	Lys21 γ 1.734	Ser22 7.268	Ser22	5.24	4.21
Phosphoribulokinase 1A7J (*Rhodobacter sphaeroides*)	No ADP										
Thymidine kinase 2ORW (*Thermotoga maritima*)	Val139 1.778			4.738β	Lys16 1.678	Lys16 1.732					
Pantothenate kinase[1D] 2ZS9 (*Mycobacterium tuberculosis*)	NC	Arg238 4.556	Arg238 3.299	3.395	Arg238 1.640	Arg238 2.349	Lys103 β 2.023	Arg238		Arg238 3.299	Arg238
Uridine kinase[1L§] 2UVQ (*Homo sapiens*)	Asp215COOH 2.386	Arg168 4.116	Arg168 5.455	4.936	Arg172 3.178	Arg172 3.094	Lys36 β 2.744	Thr38 3.324	Thr38 2.591	Arg168 3.827	Thr38 3.526
Shikimate kinase[1L] 1L4U (*Mycobacterium tuberculosis*)	Arg153 1.959	Arg110 3.799	Arg110 4.012	3.697	Arg117 2.757	Arg117 1.783	Lys15 β-γ	Thr17 3.469	Thr17 2.767	Arg110 3.037	Glu31 3.113
6-Phosphofructo-2-kinase 2I1V (*Homo sapiens*)	Not Group 2			2.661	Lys168 4.237	Lys138 4.237	Lys47				
Deoxyguasine kinase[1D] 2JAS (*Mycoplasma mycoides subsp.mycoides*)	Asp83 1.611	Arg78 4.104	Arg78 2.492	5.310	Lys13 1.890	Lys13 1.820	Lys13 α-γ	Glu 31 2.592		Arg78 2.426	Glu31 3.113
Deoxyribonucleoside kinase 2JCS (*Drosophila melanogaster*)	No ADP	Arg94	Arg94		Lys22	Lys22	Lys22	Asp41			
Polyphosphate kinase 1XDP (*Escherichia coli*)	GROUP 3/11										
Phosphomevalonate kinase 3GON **(***Streptococcus pneumoniae* R6)	GROUP 1										
Adenylate kinase 2C95[1L] (*Homo sapiens*)	Gly177 1.906	Arg128 4.372	Arg128 4.789	4.042	Arg132 4.958	Arg132 2.136	Lys21 γ 1.865	Thr23 2.228	Thr23 3.621	Arg128 3.795	Arg23 2.249
Cytidylate kina+se 1QF9[1L] (*Dictyostelium discoideum*)	Arg176 1.812	Arg127 4.076	Arg127 4.734	3.338	Arg131 2.018	Arg131 3.720	Lys19 γ 2.139	Thr21 2.381	Thr21 2.739	Arg127 3.747	Arg21 2.486
Phosphoenolpyruvate carboxykinase											
Protein kinase (HPr kinase)[10,1D] 2GBL (*Synechococcus elongatus*)	SerA330 1.249	A326 3.618	A326 3.987	4.643	B459 4.055	B459 2.147	A294			A319 3.359	4.409α 3.253β
Protein kinase (HPr kinase)[10] 2GBL	SerA89 3.428	A88 away from site	A88 away from site	5.617	B226 3.799	B226 1.853	A52				
Phosphoenolpyruvate carboxykinase 1AQ2 (*Escherichia coli*)	Ile450 1.623	Arg449 4.541	Arg449 4.291	5.461	Arg333 4.491	Arg333 4.605	Lys254 β-γ	Thr256 3.512	Thr256 3.858		2.093β 2.130γ

Kinase	αC-C=O to Aden-NH_2[14]	N7-H to Arg[1]	C8-H to Arg[1]	C8-H to α-PO_4	Arg-NH_3 to α-PO_4^2	Arg-NH_3 to β-PO_4^2	Lys-NH_3 to γ-PO_4^3	Thr to C8-H[4]	Thr to α -PO_4^4	Arg to C8-H[5]	Thr to C8-H[6]
Glucokinase 3FGU FROM RIBOKINASE (*Homo sapiens*)	Leu415 3.861	Ser411 4.287	Ser411 4.409	5.583	Arg85 3.981	Arg85 4.382	Lys169	Ser411 4.395	Ser411 3.621		
Mean	**2.101**	**4.098**	**4.140**	**4.332**	**2.953**	**2.847**	**2.073**	**3.797**	**3.200**	**3.260**	**3.028**
Standard Deviation	**0.699**	**0.401**	**1.036**	**1.010**	**1.197**	**1.143**	**0.480**	**1.697**	**0.558**	**0.568**	**0.485**
% Standard Deviation	**33.28**	**9.77**	**25.02**	**23.32**	**40.52**	**40.15**	**23.16**	**44.70**	**17.45**	**17.415**	**16.022**
Phosphoglycerate kinase[8]	Reverse reaction										
Kinase	**Gln-αC-C=O to Aden-NH_2**	**N7 to backbone NH**	**Asp to N7**	**C8-H to α-PO_4**	**Lys-NH_3 to α-PO_4^2**	**Lys-NH_3 to β-PO_4^2**	**Lys-NH_3 to γ-PO_4^3**	**Thr to C8-H[4]**	**Thr to α-PO_4^4**		**Thr to C8-H[5a]**
Aspartokinase											
Carbamate kinase 1WE5 (*Escherichia coli*)	His262 1.812	Ala264 2.589	Tyr328 4.322	6.684	Lys271 4.012	Lys272 1.788	Lys209	Ser267 2.812	Ser267 5.622		Ser267 2.690
Aspartokinase 2J0W (*Escherichia coli*)	Tyr227 2.167	Lys257 3.619	Asp230 4.133	7.498	Lys257 2.243	Lys257 5.107		Asp230 2.509	Asp230 8.006		2.065
Acetylglutamate kinase 1OH9 (*Escherichia coli*)	Leu209 1.847	Thr211 2.220	Thr 211 2.950	6.674	Lys217 3.623	Lys217 2.355	Lys58	Asp212 1.917	Asp212 3.731		1.993
Uridylate Kinase 2BRI (*Pyrococcus furiosus*)	Tyr146 1.882	Asp149 2.894	Asp149 3.820	7.671	Arg49	Arg49	Arg49	Ser181	Ser181		
Mean	**1.927**	**2.831**	**3.806**	**7.271**	**3.159**	**2.758**		**2.677**	**5.032**		**2.554**
Standard Deviation	**0.163**	**0.594**	**0.607**	**0.435**	**0.805**	**1.589**		**0.646**	**2.311**		**0.685**
% Standard Deviation	**8.787**	**20.969**	**15.953**	**5.988**	**25.481**	**57.597**		**24.128**	**45.921**		**26.836**

Kinase	Gln- αC-C=O to Aden-NH_2	C8-H to Ser	Ser to α-PO_4	C8-H to α-PO_4	Thr-OH to α-PO_4^2	Thr-OH to β-PO_4^2	H-bonding to N7[1]	Asp to substrate-OH	Asp to γ-PO_4	C8 to Backbone C=O[13]	C8-H to α-PO_4^6
Phosphofructokinase											
6-Phosphofructokinase 4PFK (*Geobacillus stearothermophilus*)	Gln107 3.504	Direct	Direct	3.265	Arg72 1.793	Arg72 1.886	Lys77 3.619	No transfer group	No transfer group	Gly104 3.046	
6-Phosphofructokinase 3F5M (*Trypanosoma brucei*)	Arg203NH2 1.905	Direct	Direct	3.427	Arg173 1.922	Arg173 3.981	Arg203 4.257	No transfer group	No transfer group	Gly200 3.518	
1-Phosphofructokinase **2AJR**/2JG5[7] (*Thermotoga maritima*)	No ADP				Ser68	Ser68	Arg105			Gly102	
Diphosphate-fructose-6-phosphate 1-phosphotransferase **3K2Q**/3HN0/1KZH[7] (*Marinobacter aquaeolei* VT8)	No ADP				Arg78	Arg78	Arg81			Gly114	
Diacylglycerol kinase 2QV7[7]	Open site				Thr63	Thr63	Asn94			Gly91	

(*Staphylococcus aureus*)											
Kinase	**Gln- αC-C=O to Aden-NH_2**	**C8-H to Ser**	**Ser to α-PO_4**	**C8-H to α-PO_4**	**Thr-OH to α-PO_4[1]**	**Thr-OH to β-PO_4[1]**	**H-bonding to N7[2]**	**Asp to substrate-OH[3R]**	**Asp to γ-PO_4[14]**	**C8 to Backbone C=O[13]**	**C8-H to α-PO_4[6]**
Ribokinase											
Ketohexokinase 3HQQ (*Leishmania mexicana*)	No ADP				Thr253	Thr253		Asp258			
4-Fructokinase 3EPQ (*Bacillus subtilis*)	NC	Direct	Direct	3.041	Thr130 4.827	Thr130 1.757	Gln233 2.619	Asp103 NS	Asp103 3.617	Gly230 3.143	2.880
Ribokinase 1RKD (*Escherichia coli*)	NC	Direct	Direct	3.279	Thr223 2.276	Thr223 3.972	Thr250 3.437	Asp255 2.703	Asp255 3.244	Gly225 3.673	3.620
Adenosine kinase 2PKN (*Mycobacterium tuberculosis*)	NC	Direct	Direct	3.182	Thr223 3.036	Thr223 4.371	Thr253	Asp257 NS	Asp257 5.936	Gly225 3.385	3.705
Pyridoxal kinase 3IBQ (*Lactobacillus plantarum*)	Leu204 1.834	Direct	Direct	2.464	Thr211 2.292	Thr211 2.486	Arg203 6.737	Asp213 NS	Asp213 4.918		2.464
2-keto-3-deoxygluconate kinase 3KTN[7] (*Lactobacillus plantarum*)	No ADP				Thr239	Thr239		Asp278	Asp278		
Hydroxyethyl thiazole kinase 3HPD[7] (*Pyrococcus horikoshii*)	No ADP										
1-phosphofructokinase 2AJR[7] ((*Thermotoga maritima*)	No ADP										
Tagatose-6-phosphate kinase **2JG1**/2JGV (*Staphylococcus aureus subsp. aureus*)		Direct	Direct	2.805	Lys183 4.291	Lys183 2.213	Ser252 3.564	Asp254 NS	Asp254 3.696	Gly224 4.022	2.805
ADP-dependent phosphofructokinase 3DRW[12] (*Pyrococcus horikoshii*)	Val422 1.943	Direct	Direct	3.167	Asp287 3.079	ADP	Thr428 3.542	Asp433	Asp433		
ADP-dependent glucokinase[11] 1GC5 (*Thermococcus litoralis*)	Val440 2.106	Direct	Direct		Glu306 4.28	ADP	Thr446 3.621	Asp451	Glu451		
Phosphomethyl pyrimidine kinase 1UB0[7] /1JXH (*Thermus thermophilus*)	No ADP						Thr208	Cys210	Cys210		
Thiamin pyrophosphokinase											
Thiamin pyrophosphate kinase 2F17[12] (*Mus musculus*)	Gly129 3.241	Direct	Direct	2.331	Asp46 3.074	Pyr-PO_4	Arg131 4.983	Asp73	Asp73	Asn25 3.811	
Glycerate kinase											
Glycerate kinase 1TO6/1B8N/3CWC (*Neisseria meningitidis* serogroup A)	No ADP										
Mean	**2.865**			**3.200**	**3.163**	**3.086**	**4.042**		**4.429**	**3.390**	**3.337**
Standard Deviation	**1.812**			**0.799**	**0.979**	**0.961**	**1.366**		**1.235**	**0.398**	**0.398**
% Standard Deviation	**63.239**			**24.966**	**30.963**	**31.131**	**33.784**		**27.880**	**11.752**	**11.927**

1. Arg/Lys/Thr coordinated to C8-H and N7
2. Arg/Lys coordinated to the α- and β-PO_4

3. Lys responsible for the protonation of the γ-PO_4
4. Thr/Ser coordinated to the C8-H of the ATP residue responsible for the protonation of C8 during the rehybridization change from sp2 to sp3
5. Arg to C8-H taking the hybridization change from sp2 to sp3 and the concomitant conversion of C8-H to C8-H_2 into account.
6. Ser/Thr to C8-H taking the hybridization change from sp2 to sp3 and the concomitant conversion of C8-H to C8-H_2 into account.
7. Residues identified by sequence alignment using UniProt sequence (Accession Number as indicated)
8. Carries out reverse reaction.
9. Location of the arginine residue relative to the pyrimidine ring, D = on the D-orientation of the ribose, L = on the L-orientation of the ribose.
10. Active site comprises 2 subunits with amino acid numbering prefixed by A and B (see group 7).
11. ADP-dependent kinase requires β-PO_4 to be leaving group and therefore does not require proton transfer between α-PO_4 and β-PO_4.
12. Transfers pyrophosphate therefore requires β-PO_4 to be leaving group and therefore does not require proton transfer between α-PO_4 and β-PO_4.
13. C8-H induction mechanism, comprising the amino acid and the adenyl nitrogen to which it is coordinated and the creation of a carbene.
14. Substrate coordinated Asp/Glu.
15. Coordination of backbone carbonyl to the adenyl C6-NH_2.

§ adenyl group in crystal in *syn* conformation re-orientated to the *anti* conformation as found in the rest of the group.
NC = No coordinating residue.
IS = Incomplete structure
NS = No substrate.

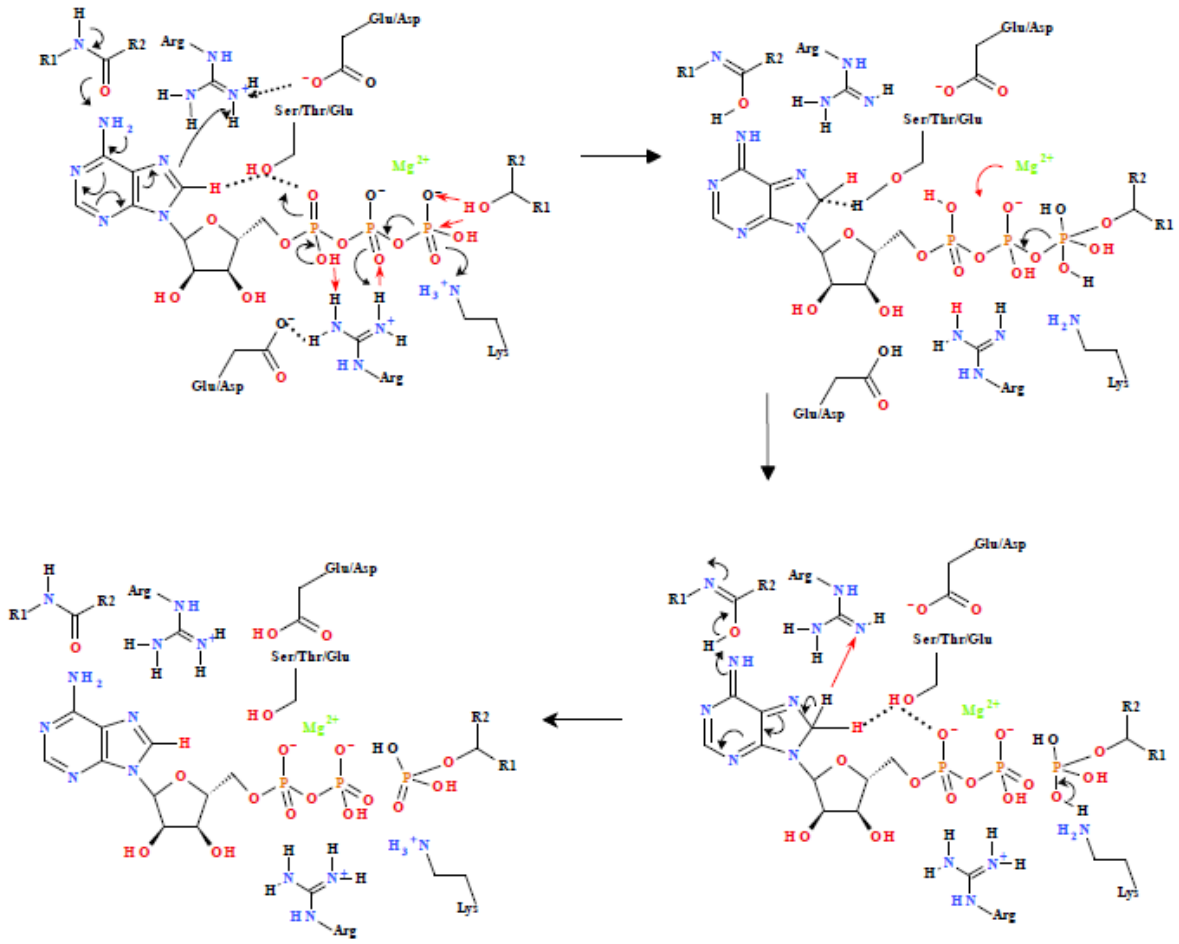


Figure MECH3. Phosphoryl transfer mechanism found in the Group 2 kinases (Rossmann-like fold and phosphoenolpyruvte carboxykinase-like sequences). The initiation of phosphoryl occurs via the coordination of the ATP C6-NH_2 to a carbonyl arising from the protein backbone by the "push" mechanism resulting in the protonation of C8 via the coordination of a conserved Arg. This renders the C8-H more acidic, allowing for the protonation of the α-PO_4, via a conserved Ser/Thr carrier. There is a concomitant transfer of an H^+ from the α-PO_4 to β-PO_4 via a conserved Arg, thereby facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a simultaneous ATP-mediated deprotonation of the substrate -OH, allowing for the nucleophilic attack by the substrate to create the pentavalent intermediate and allow phosphoryl transfer. A protonated Lys then transfers the proton to the γ-PO_4, changing the transfer of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

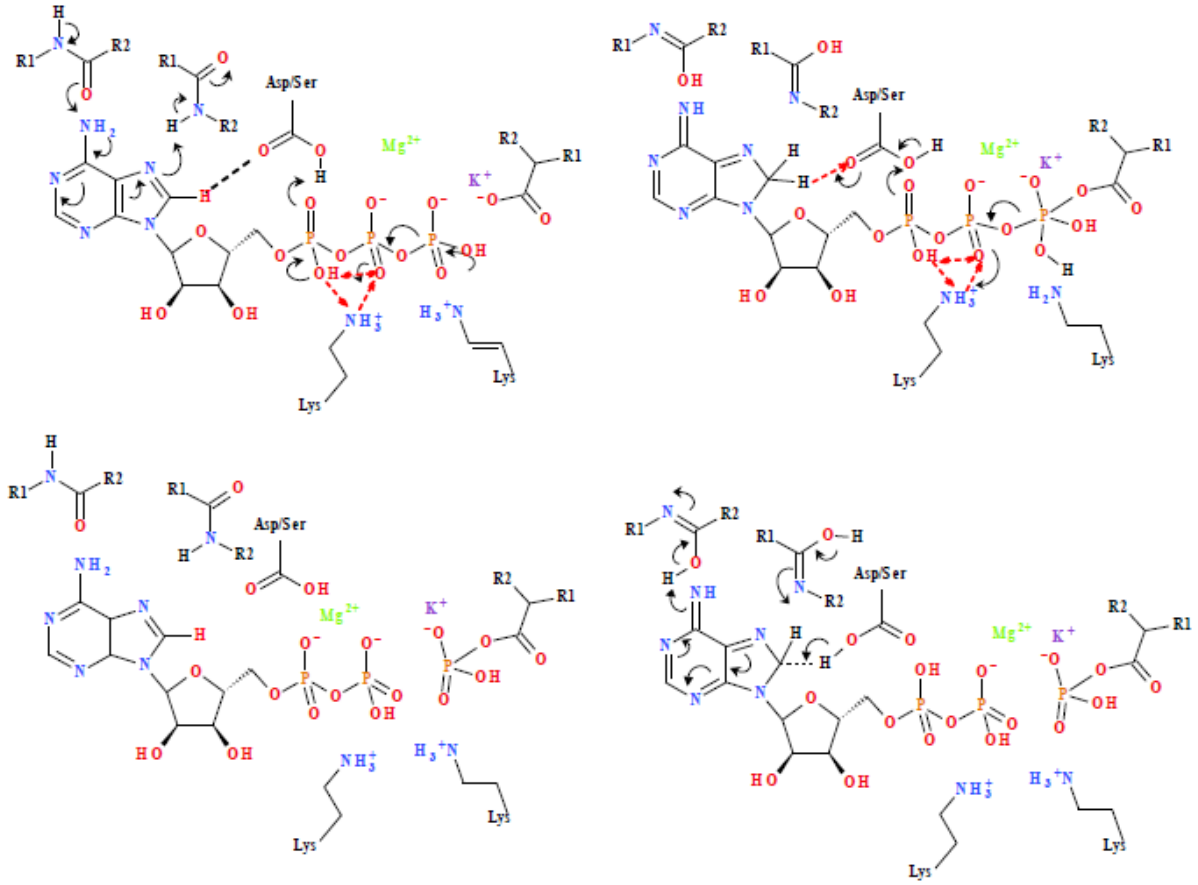


Figure MECH4. Phosphoryl transfer mechanism found in the Group 2 kinases (Aspartokinase-like sequences). Protonation of the C8 occurs via a backbone amide. The C8-H becomes more acidic, allowing for the protonation of the α-PO_4 via a Ser or Asp residue. There is a concomitant transfer of an H^+ from the α-PO_4 to β-PO_4 via a conserved Lys, facilitating the γ-PO_4 as a leaving group. The substrate moiety to be phosphorylated is a carboxylate group and therefore does not require deprotonation for the nucleophilic attack by the substrate. The protonated Lys then transfers a proton to the γ-PO_4, allowing the creation the pentavalent intermediate and subsequent phosphoryl transfer. A monovalent metal counter-ion such as K^+ facilitates the transfer of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the hybridization of the adenyl moiety to return to the "ground-state" tautomer.

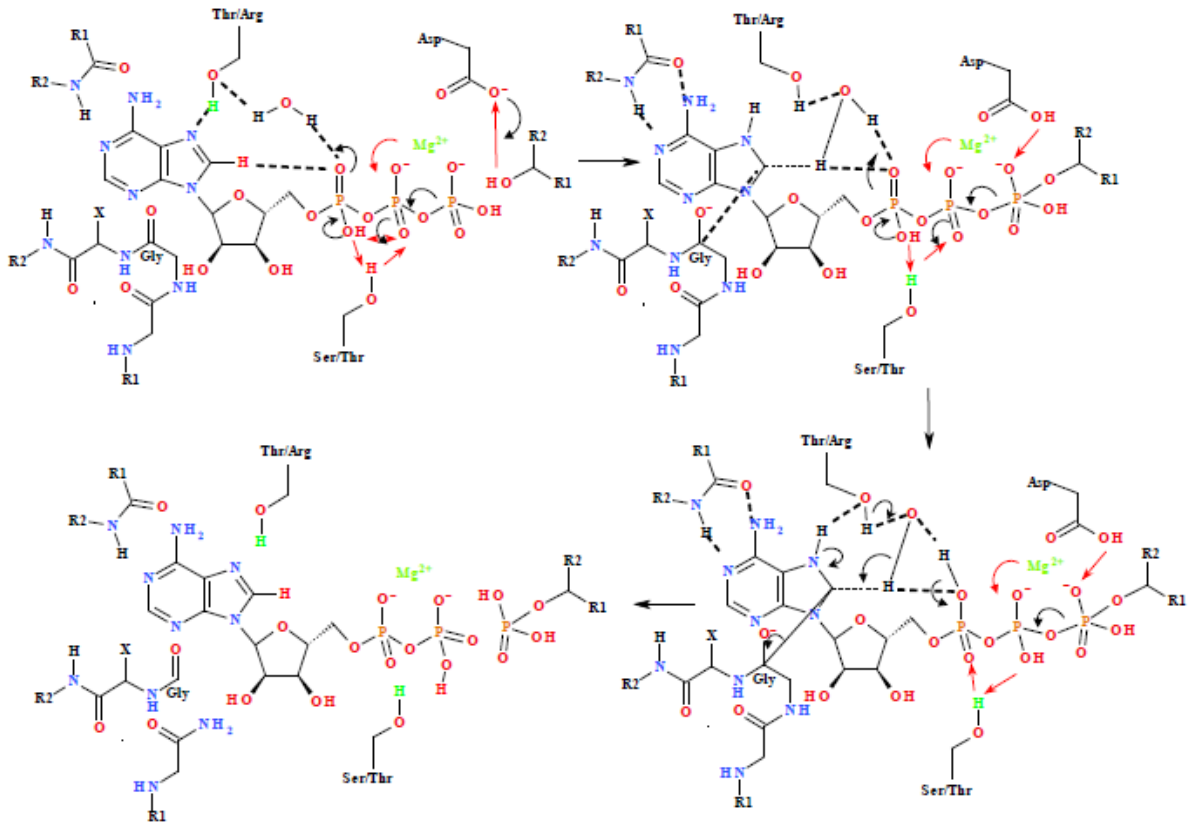


Figure MECH5. Phosphoryl transfer mechanism found in the Group 2 kinases (Ribokinase-like sequences). Generally within this group a backbone peptide bond is coordinated to the C6NH$_2$ and the N1. The protonation of N7 occurs via the coordination of a conserved Thr/Arg that renders the C8-H more acidic via the formation of a carbene, allowing for the direct protonation of the α-PO$_4$ with a concomitant transfer of an H$^+$ from the α-PO$_4$ to β-PO$_4$ via a conserved Thr/Ser/Lys. This facilitates the γ-PO$_4$ as a leaving group. There is a concomitant Asp-mediated deprotonation of the substrate –OH, allowing for the nucleophilic attack by the substrate. In all the structures available the only structure containing the non-ATP substrate is ribokinase where there is not sufficient order to identify the amino acid responsible for the transfer a proton to the γ-PO$_4$ creating the pentavalent intermediate and phosphoryl transfer. The proton arising from the substrate facilitates the transfer of the Mg^{2+} from being β-PO$_4$ to γ-PO$_4$ coordinated to being α-PO$_4$ to β-PO$_4$ coordinated. The H$^+$ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

Table AF 2B. Group 2 kinases

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]	Mechanism
Group 2 kinases (Rossmann-like sequences)			
1. Gluconate kinase (pdb|1KO5)	P-loop containing nucleoside triphosphate hydrolase	Shikimate kinase	2A
2. Phosphoribulokinase (pdb|1A7J)	P-loop containing nucleoside triphosphate hydrolase	Phosphoribulokinase / Uridine kinase family	2B
3. Thymidine kinase (pdb|2ORW)	P-loop containing nucleoside triphosphate hydrolase	Thymidine kinase	2B
4. Pantothenate kinase (pdb|2ZS9)	P-loop containing nucleoside triphosphate hydrolase	Phosphoribulokinase / Uridine kinase family	
5. Uridine kinase (pdb|2UVQ)	P-loop containing nucleoside triphosphate hydrolase	Phosphoribulokinase / Uridine kinase family	2A
6. Shikimate kinase (pdb|1L4U)	P-loop containing nucleoside triphosphate hydrolase	Shikimate kinase	2A
7. 6-Phosphofructo-2-kinase (pdb|2I1V)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	6-phosphofructo-2-kinase / Phosphoglycerate mutase family	
8. Deoxyguanisine kinase (pdb|2JAS)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	Deoxynucleoside kinase	2B
9. Deoxyribonuceoside kinase (pdb|2JCS)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	Deoxynucleoside kinase	2B
10. Polyphosphate kinase (pdb|1XDP)	n/a	Polyphosphate kinase	
11. Adenylate kinase (pdb|2C95)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	Adenylate kinase	2A
12. Cytidylate kinase (pdb|1QF9)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	Adenylate kinase	2A
13. HPr kinase (Protein kinase) (pdb|2GBL)	P-loop containing nucleoside triphosphate hydrolase / Phosphoglycerate mutase-like	KaiC	
14. Phosphoenolpyruvate carboxykinase (pdb|1AQ2)	PEP carboxykinase-like	Phosphoenolpyruvate carboxykinase	
15. Glucokinase (pdb|3FGU)	Actin-like ATPase	Hexokinase	2B
Group 2c (Aspartokinase)			
7. Carbamate kinase (pdb|1WE5)	nd[3]	Amino acid kinase	
8. Aspartokinase (pdb|2J0W)	a. nd b. ACT-like domain	a. Amino acid kinase family b. ACT domain	
9. Aspartokinase (pdb|2HMF)	a. nd b. ACT-like domain (x2)	a. Amino acid kinase family b. ACT domain (x2)	
10. Acetylglutamate kinase (pdb|1OH9)	a. nd b. ACT-like domain	a. Amino acid kinase family b. ACT domain	
11. Uridylate kinase (pdb|2BRI)	a. nd b. ACT-like domain	a. Amino acid kinase family b. ACT domain	
Group 2d (Ribokinase-like)			
1. Glucokinase: (pdb|3FGU)	Actin-like ATPase	Hexokinase	
2. Ketohexokinase: (pdb|3HQQ).	Ribokinase-like	PkfB carbohydrate kinase	

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]	Mechanism
3. 4-fructokinase: (pdb|3EPQ)	Actin-like ATPase	ROK (Repressor, ORF, Kinase)	
4. Ribokinase: (pdb|1RKD)	Ribokinase-like	PkfB carbohydrate kinase	
5. Adenosine kinase: (pdb|2PKN)	Ribokinase-like	PkfB carbohydrate kinase	
6. Pyridoxal kinase: (pdb|3IBQ)	Ribokinase-like	Phosphomethylpyrimidine kinase	
7. 2-keto-3-deoxygluconate kinase (pdb|3KTN)	Ribokinase-like	PkfB carbohydrate kinase	
8. Hydroxyethyl thiazole kinase (pdb|3HPD)	Ribokinase-like	hydroxyethyl thiazole kinase	
9. 1-phosphofructokinase (pdb|2AJR)	Ribokinase-like	PkfB carbohydrate kinase.	
10. Tagatose-6-phosphate kinase (pdb|2JG1)	Ribokinase-like	PkfB carbohydrate kinase.	
11. ADP-dependent phosphofructokinase (pdb|3DRW)	Ribokinase-like	ADP-specific Phosphofructokinase/Glucokinase .	
12. ADP-dependent glucokinase (pdb|1GC5)	Ribokinase-like	ADP-specific Phosphofructokinase/Glucokinase	
13. Phosphomethyl pyrimidine kinase (pdb|1UB0)	Ribokinase-like	ADP-specific Phosphofructokinase/Glucokinase	

[1] pfam clan classification in brackets
[2] pfam family/domain classification in brackets
[3] No Detectable similarity to conventional kinases
Where there are 2 or domains recognised, these are denoted by ‘a’, ‘b’, etc. One domain has been selected to position the protein within the table.

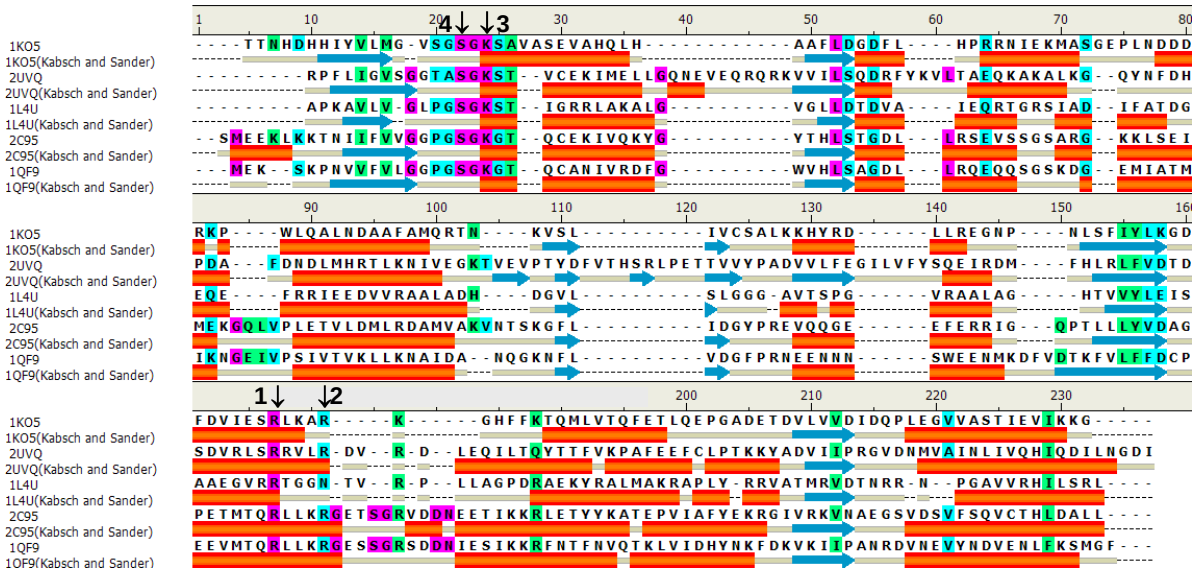


Figure SEQ2A. The **Group 2** kinases belonging to the 'P-loop containing nucleodside triphosphate hydrolase' superfamily comprising the 'shikimate kinase', 'phosphoribulokinase/uridine kinase' and 'adenylate kinase' families which contain two conserved Arg residues in the active site linked to the proton translocation (Mechanism 2A, Table 2B). Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. 1KO5, gluconate kinase; 2UVQ, uridine kinase; 1L4U, shikimate kinase, 2C95, adenylate kinase; 1QF9, cytidylate kinase.

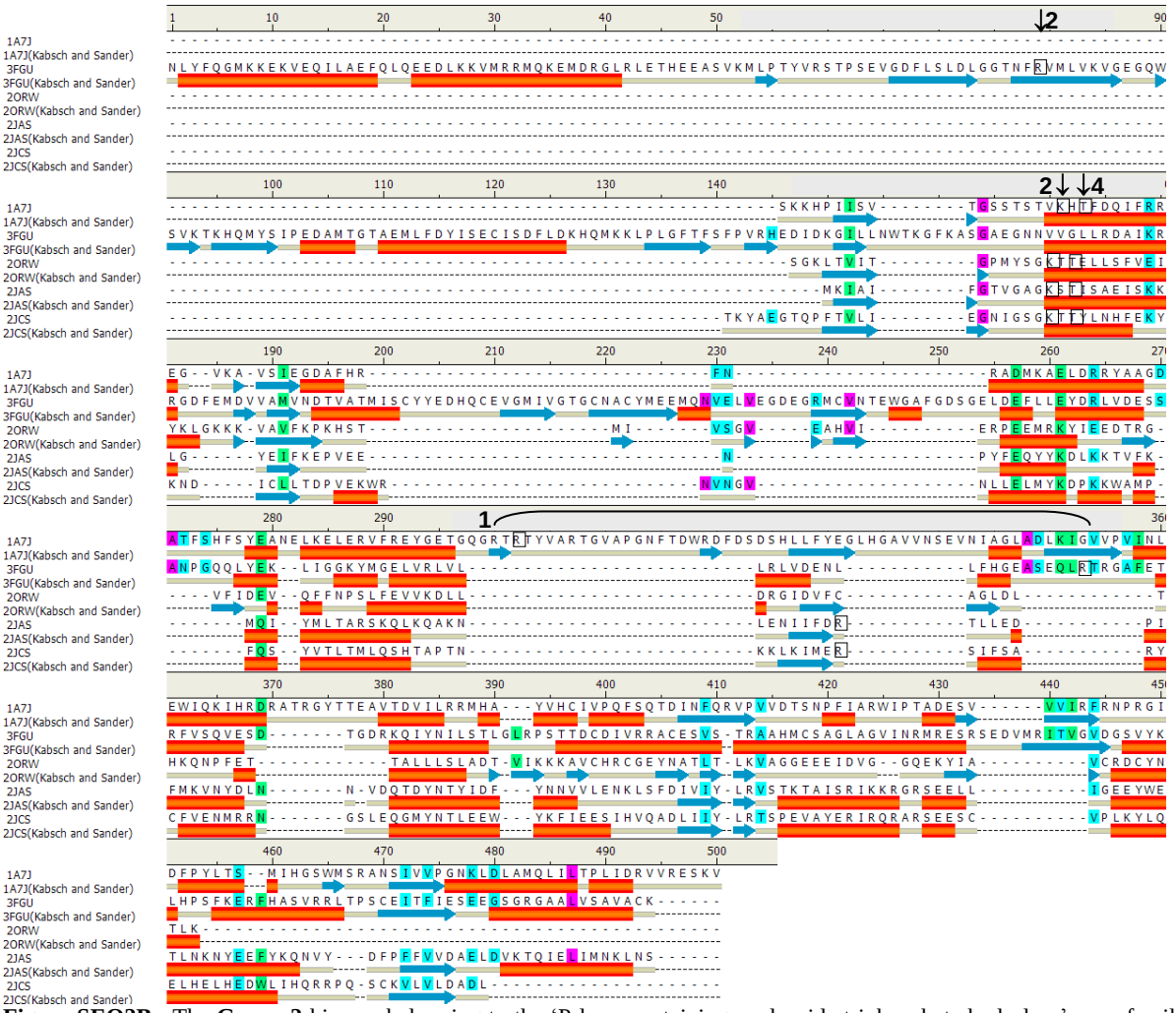


Figure SEQ2B. The **Group 2** kinases belonging to the 'P-loop containing nucleoside triphosphate hydrolase' superfamily comprising phosphoribulokinase /uridine kinase' and 'deoxynucleoside kinase' families which contain a single conserved Arg residue in the active site linked to the proton translocation (Mechanism 2B, Table 2B). The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 2A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. 1A7J; phosphoribulokinase; 2ORW, thymidine kinase; 2JAS, deoxyguanisine kinase; 2JCS, deoxyribonucleoside kinase; 3FGU, glucokinase.

2

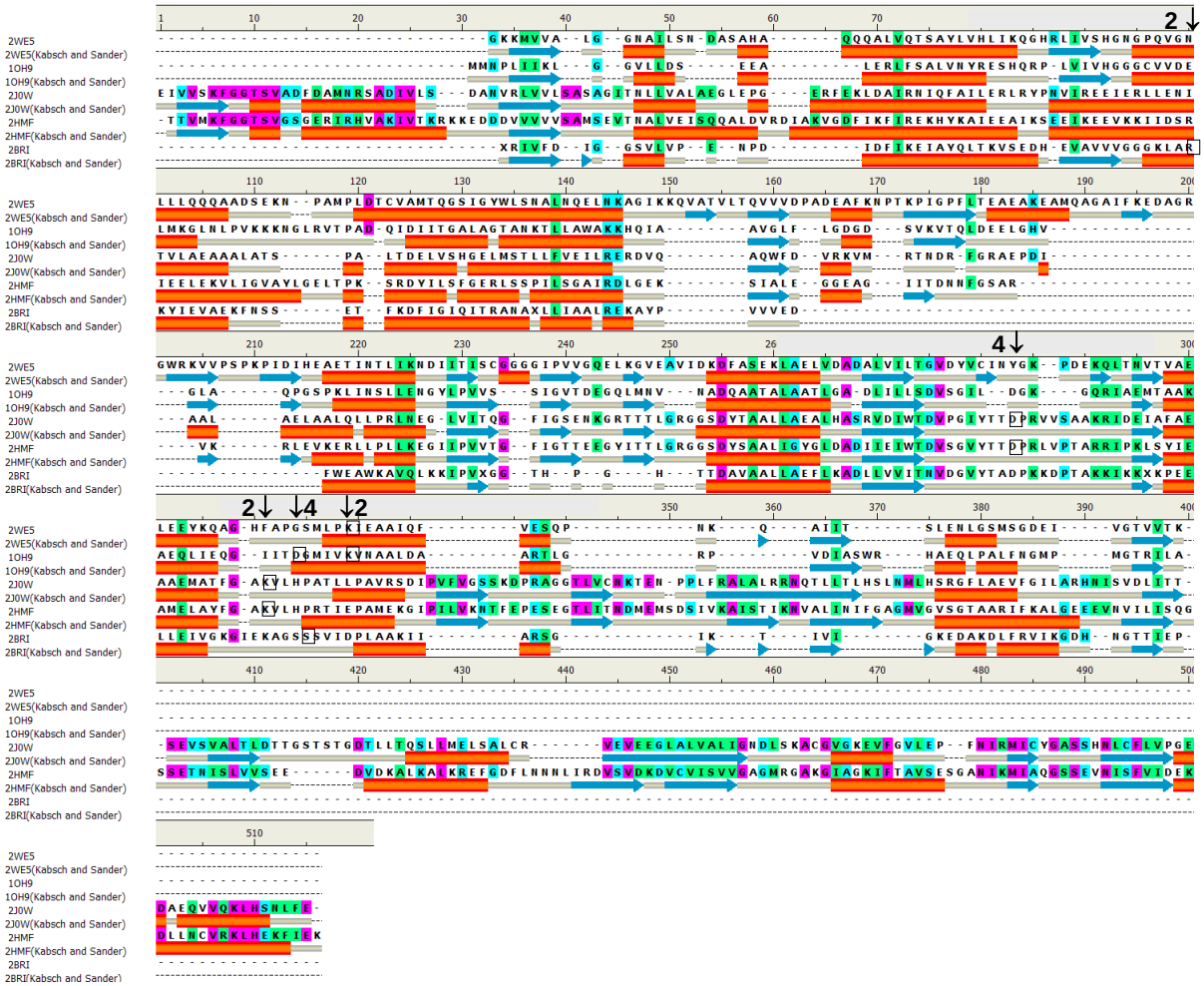
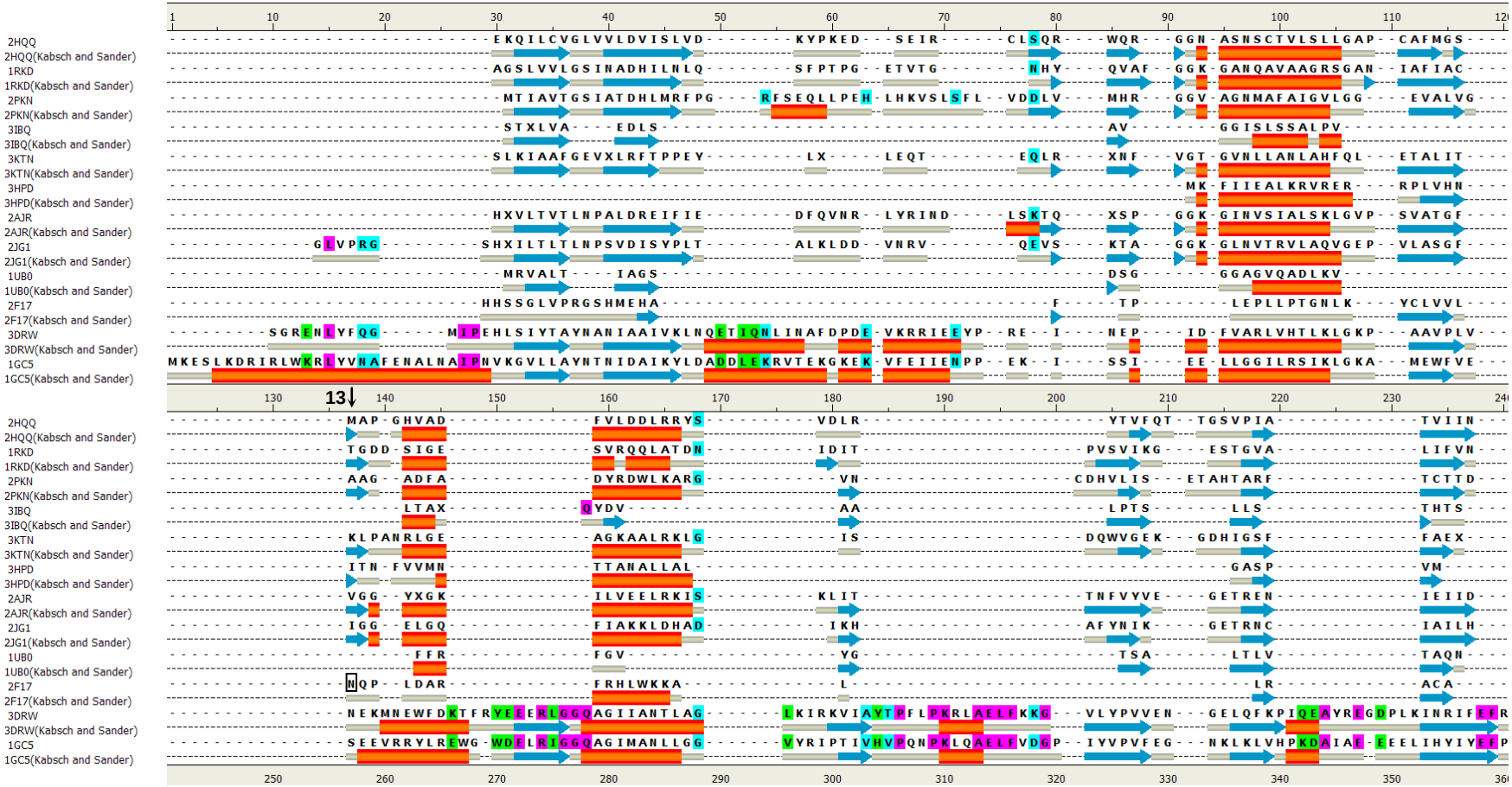
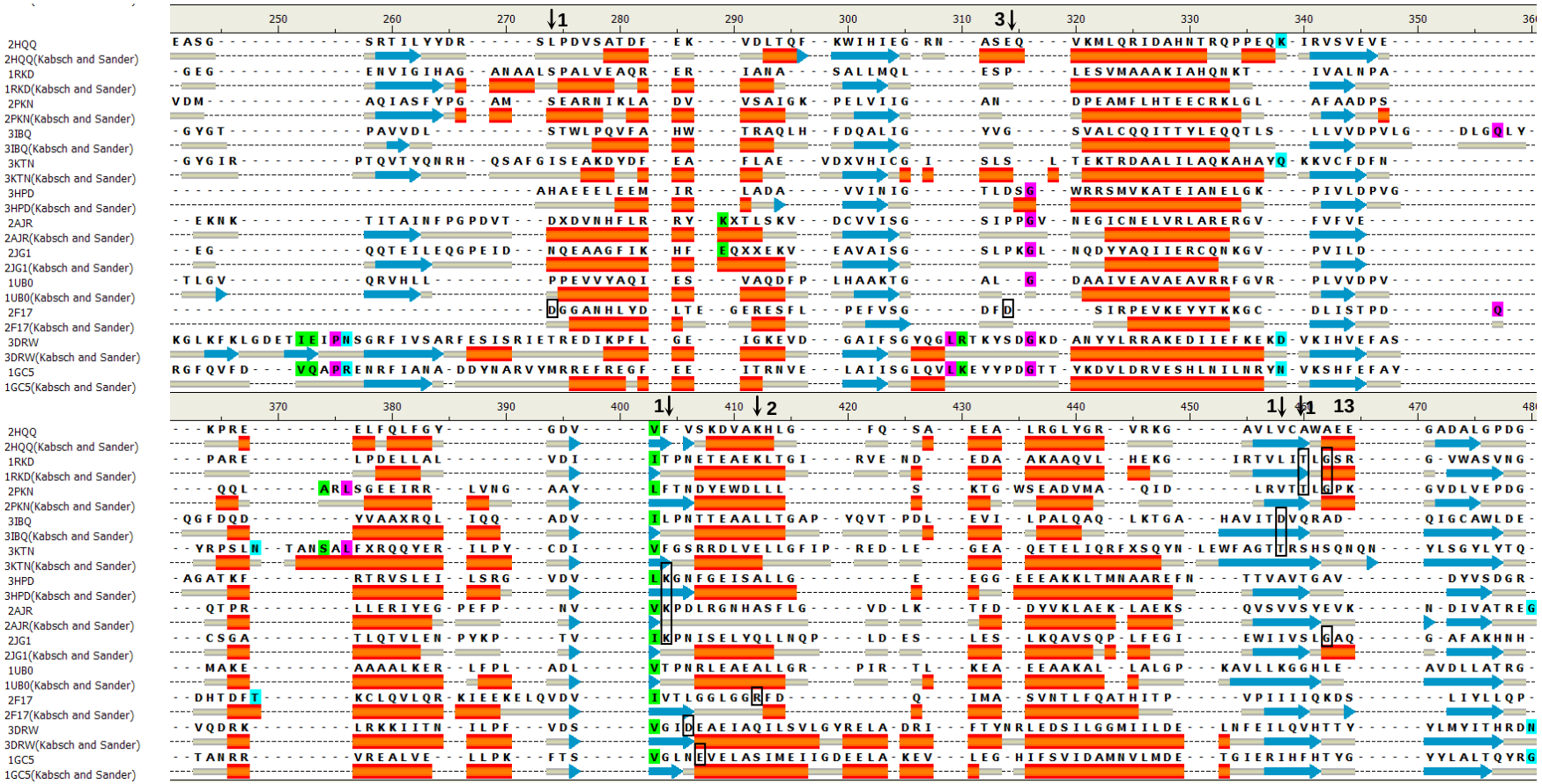


Figure SEQ2C. A sequence alignment of the proteins making up the Group 2 aspartokinase family. The aspartokinase enzymes and uridylate enzymes contain an additional Arg residue facilitating phosphoryl transfer. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 1A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 2A. 1WE5, carbamate kinase; 1OH9, acetylglutamate kinase; 2J0W, aspartokinase; 2BRI, uridylate kinase.


2HQQ
2HQQ(Kabsch and Sander)
1RKD
1RKD(Kabsch and Sander)
2PKN
2PKN(Kabsch and Sander)
3IBQ
3IBQ(Kabsch and Sander)
3KTN
3KTN(Kabsch and Sander)
3HPD
3HPD(Kabsch and Sander)
2AJR
2AJR(Kabsch and Sander)
2JG1
2JG1(Kabsch and Sander)
1UB0
1UB0(Kabsch and Sander)
2F17
2F17(Kabsch and Sander)
3DRW
3DRW(Kabsch and Sander)
1GC5
1GC5(Kabsch and Sander)
13


2HQQ
2HQQ(Kabsch and Sander)
1RKD
1RKD(Kabsch and Sander)
2PKN
2PKN(Kabsch and Sander)
3IBQ
3IBQ(Kabsch and Sander)
3KTN
3KTN(Kabsch and Sander)
3HPD
3HPD(Kabsch and Sander)
2AJR
2AJR(Kabsch and Sander)
2JG1
2JG1(Kabsch and Sander)
1UB0
1UB0(Kabsch and Sander)
2F17
2F17(Kabsch and Sander)
3DRW
3DRW(Kabsch and Sander)
1GC5
1GC5(Kabsch and Sander)

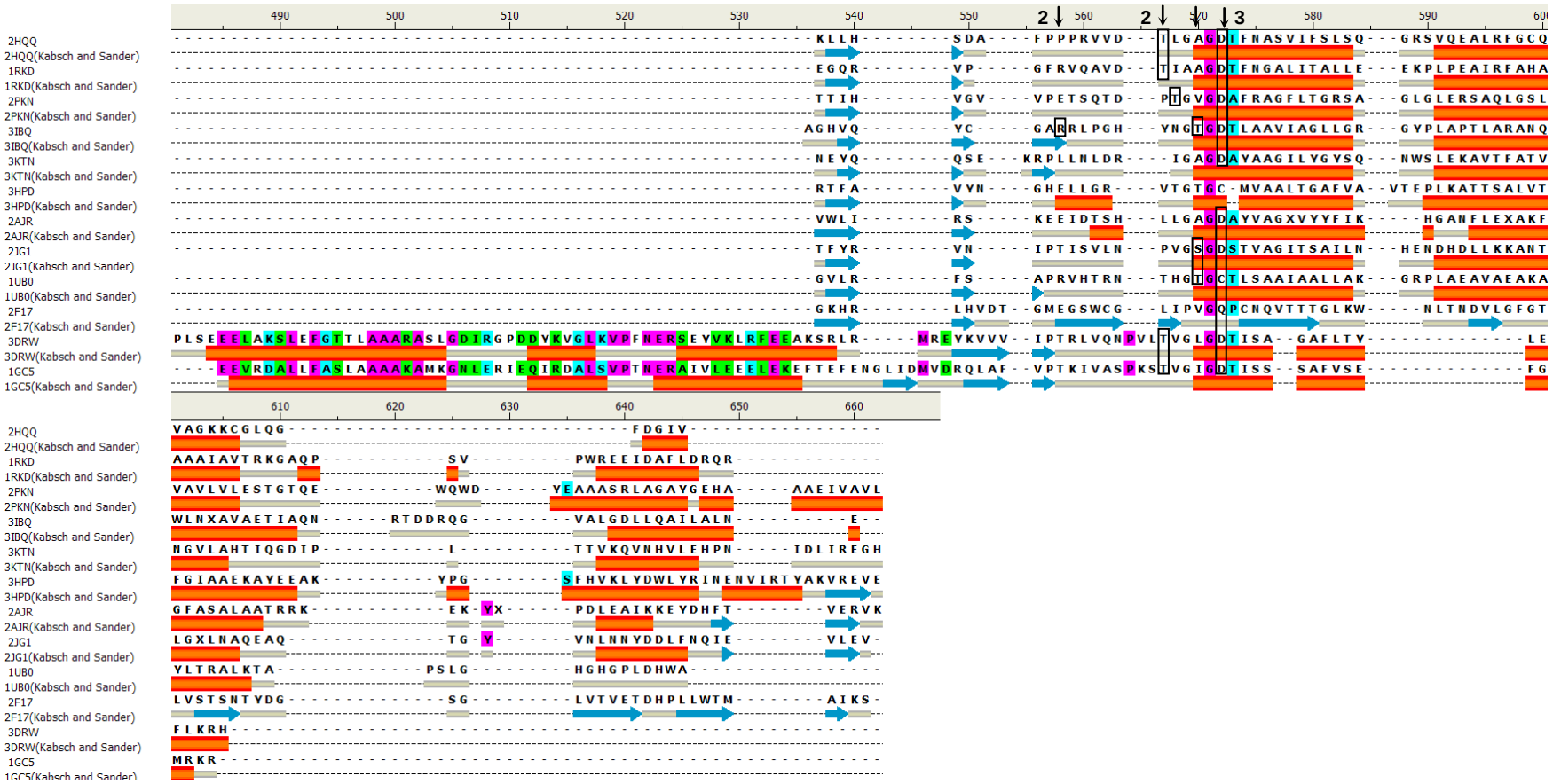


Figure SEQ2D. The conserved Thr and Asp residues responsible for the initiation of phosphoryl transfer within the ribokinase-like superfamily of the **Group 2** kinases. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 2A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 3HQQ, ketohexokinase; 1RKD, ribokinase; 2PKN, adenosine kinase; 3IBQ, pyridoxal kinase; 3KTN, 2-keto-3-deoxygluconate kinase; 3HPD, Hydroxyethyl thiazole kinase; 2AJR, 1-phosphofructokinase; 2JG1, tagatose-6-phosphate kinase; 3IBQ, Pyridoxal kinase; 3DRW, ADP-dependent phosphofructokinase; 1GC5, ADP-dependent glucokinase; 1UB0, phosphomethyl pyrimidine kinase; 2F17, Thiamin pyrophosphate kinase.

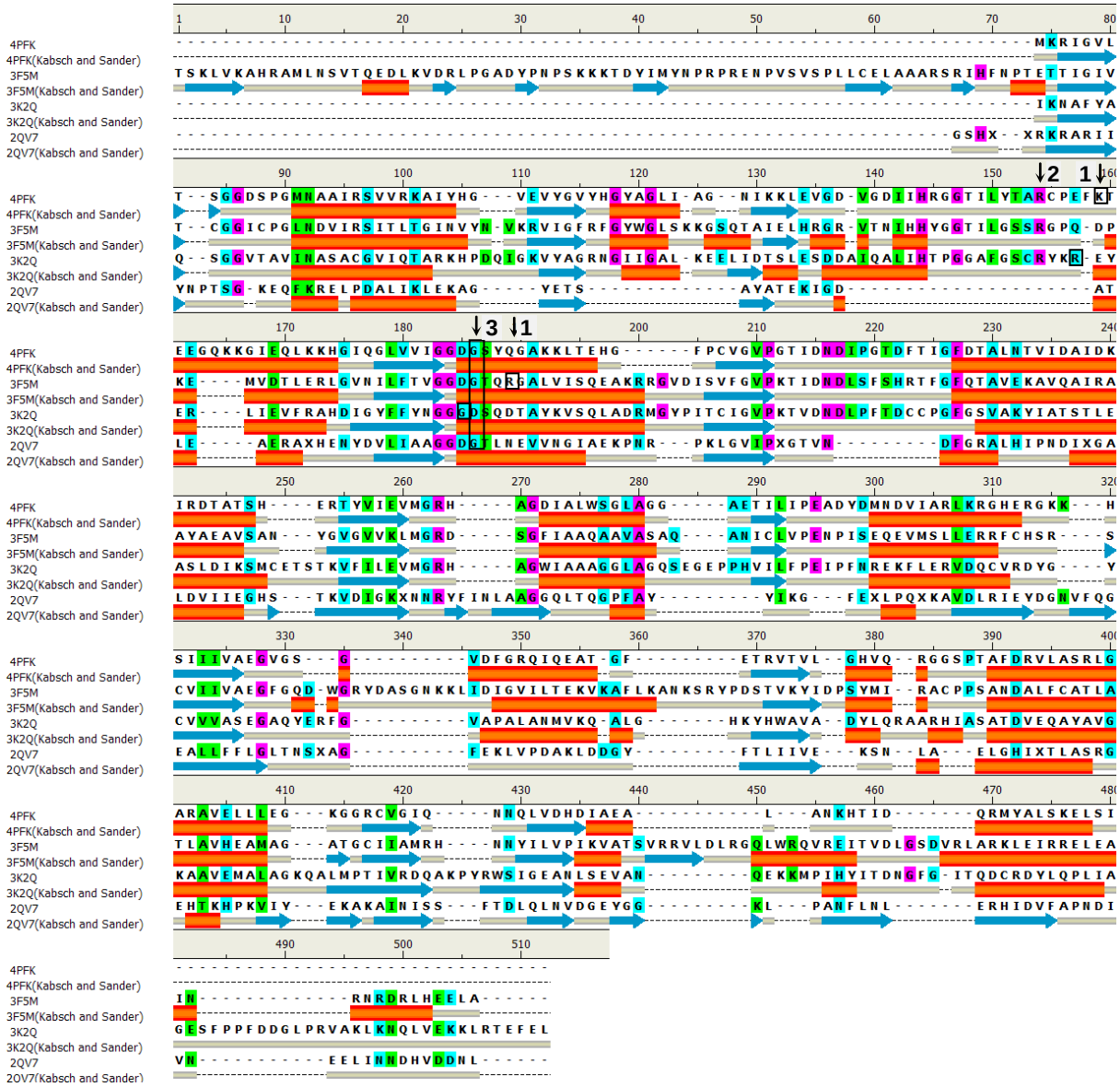


Figure SEQ2E. The conserved Arg and Lys residues responsible for the initiation of phosphoryl transfer within the PFK-like superfamily of the **Group 2** kinases. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 2A. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 4PFK, 6-Phosphofructokinase; 3F5M , 6-Phosphofructokinase; 2AJR, 1-Phosphofructokinase; 3K2Q , Diphosphate-fructose-6-phosphate 1-phosphotransferase; 2QV7, Diacylglycerol kinase.

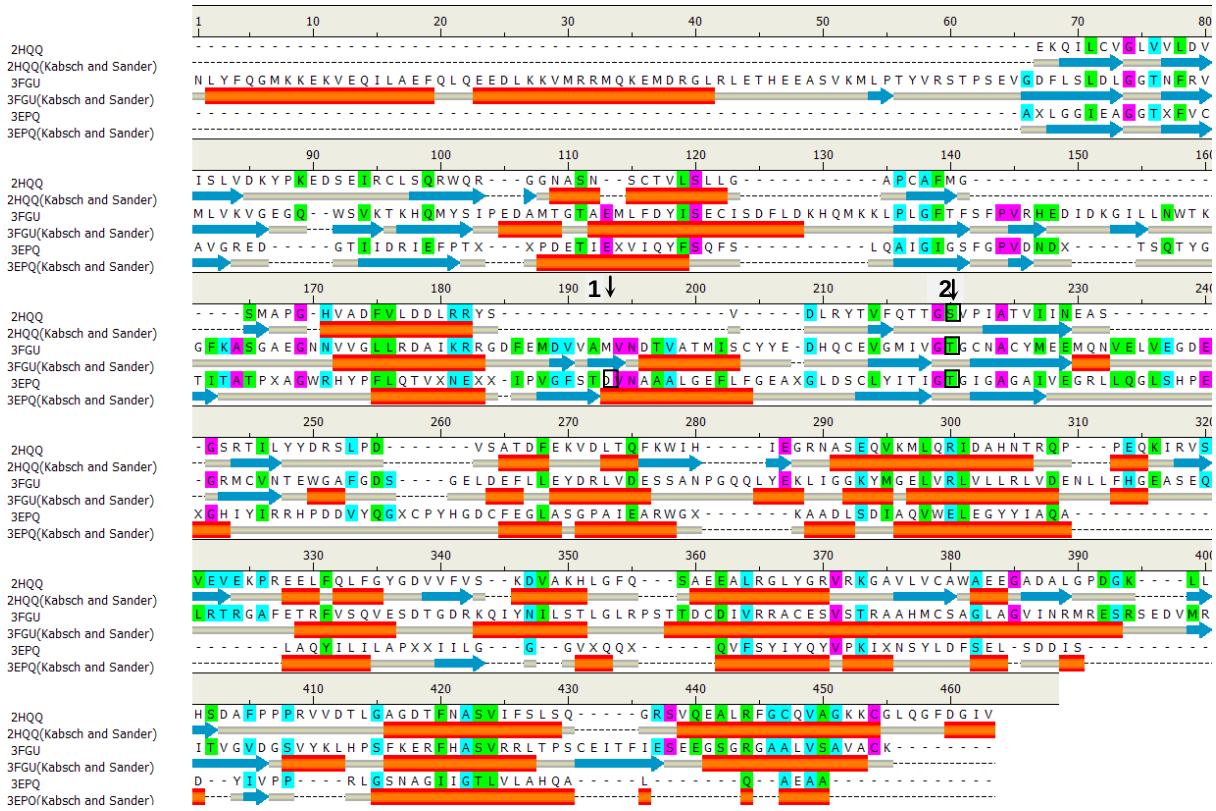


Figure SEQ2F. The conserved Thr and Asp residues responsible for the initiation of phosphoryl transfer within the Actin-like ATPase superfamily of the **Group 2** kinases. 3FGU, Hexokinase; 3HQQ, Ketohexokinase, 3EPQ, 4-Fructokinase.

Table AF 3A. Kinases representing **Group 3** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured interatomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**C-C=O to Adn-NH_2**	**N7 to Arg[1]**	**C8-H to Arg[1]**	**Arg[1] to α-PO_4**	**Arg[1] Nε to α-PO_4**	**C8-H to α-PO_4**	**C8-H to Asp[2]**	**Arg[3]-NH_3 to β-PO_4**	**C8-H to Arg[3]**	**Arg[3]-NH_3 to β -PO_4**	**Arg[4]-NH_3 to α-PO_4^-**	**Arg[5] PO_4^-**
Ferredoxin-like fold kinases												
Nucleoside bis-phosphate kinase 2BEF (*Dictyostelium discoideum*)	NC	Arg62 2.883	Arg62 2.725	NC	NC	3.249	His59 2.950	Thr98 3.853	Arg109	Arg109		
HPPK												
2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase 1EQ0 (*Escherichia coli*)												
Guanido kinases												
Guanidoacetate kinase[6] P51546 9 **(***Drosophila melanogaster*)			Arg287	Arg287	Arg287		Asp330	Arg125	Arg125	Arg127	Arg315	Arg231
Creatine kinase 2GL6 (*Homo sapiens*)	Gly328 3.068	Arg326 2.413	Arg326 2.413	Arg326 1.666	Arg326 2.338	3.689	Asp369 2.977	Arg164 1.847	Arg164 3.384	Arg166 1.635	Arg354 1.952	Arg270
Arginine kinase 1BG0 (*Limulus polyphemus*) Atlantic horseshoe crab	Ser122 2.233	Arg280 1.898	Arg280 1.898	Arg280 3.382	Arg280 1.935	4.574	Asp324 2.900	Arg124 1.867	Arg124 2.864	Arg126 1.812	Arg309 1.843	Arg229
Lombricine kinase[6] O15991 (*Eisenia foetida*) red worm			Arg281	Arg281	Arg281		Asp323	Arg118	Arg118	Arg120	Arg308	Arg324
Histidine kinase												
Histidine kinase												
[Pyruvate dehydrogenase (lipoamine)] kinase[6] Q9SBJ1(*Arabidopsis thaliana*)			Arg285	Arg285	Arg285							
[3-Methyl-2-oxobutanoate dehydrogenase (lipoamide)] kinase												
Polyphosphate kinase 1XDP From Group 9 (*Escherichia coli*)	Asp587 3.287	Arg564 3.213	Arg564 2.614	NC	NC	7.406	NC	Arg375 3.353		Arg405 2.994		Arg594 3.281
Mean	**2.651**	**2.156**	**2.156**	**2.524**	**2.137**	**4.132**	**2.939**	**1.857**	**3.124**	**1.724**	**1.898**	
Standard Deviation	**0.590**	**0.364**	**0.364**	**1.213**	**0.285**	**0.626**	**0.054**	**0.014**	**0.368**	**0.125**	**0.077**	
% Standard Deviation	**22.276**	**16.894**	**16.894**	**48.074**	**13.338**	**15.147**	**1.853**	**0.762**	**11.770**	**7.262**	**4.062**	

1. Arg coordinated to C8-H, α-PO_4, and the ArgNεH to β-PO_4.

2. Coordinated Asp residue responsible for the protonation C8 during the rehybridization change from sp2 to sp3.
3,4. ATP phosphate backbone stabilization.
5. Arg responsible for substrate deprotonation and γ=PO_4 protonation.
6. Residues identified by sequence alignment using UniProt sequence (Accession Number as indicated).
NC = No Coordinating residue
IS = Incomplete Structure.

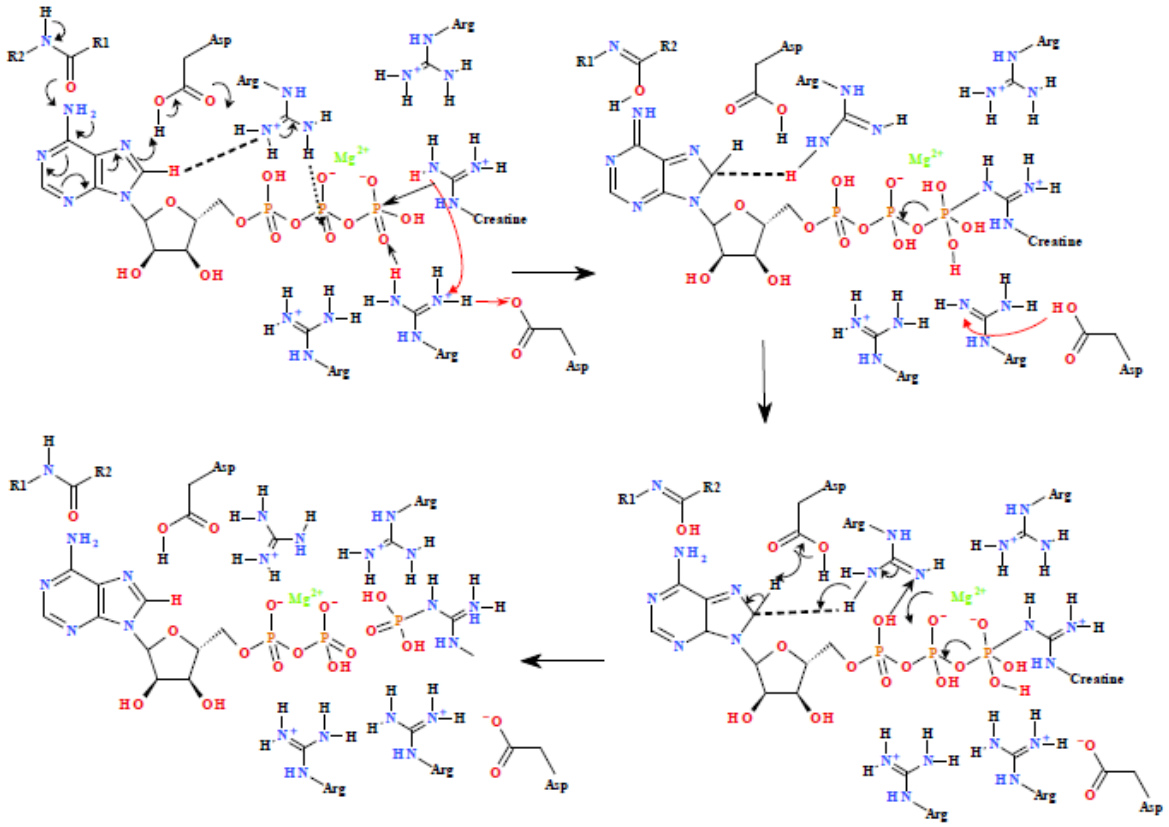
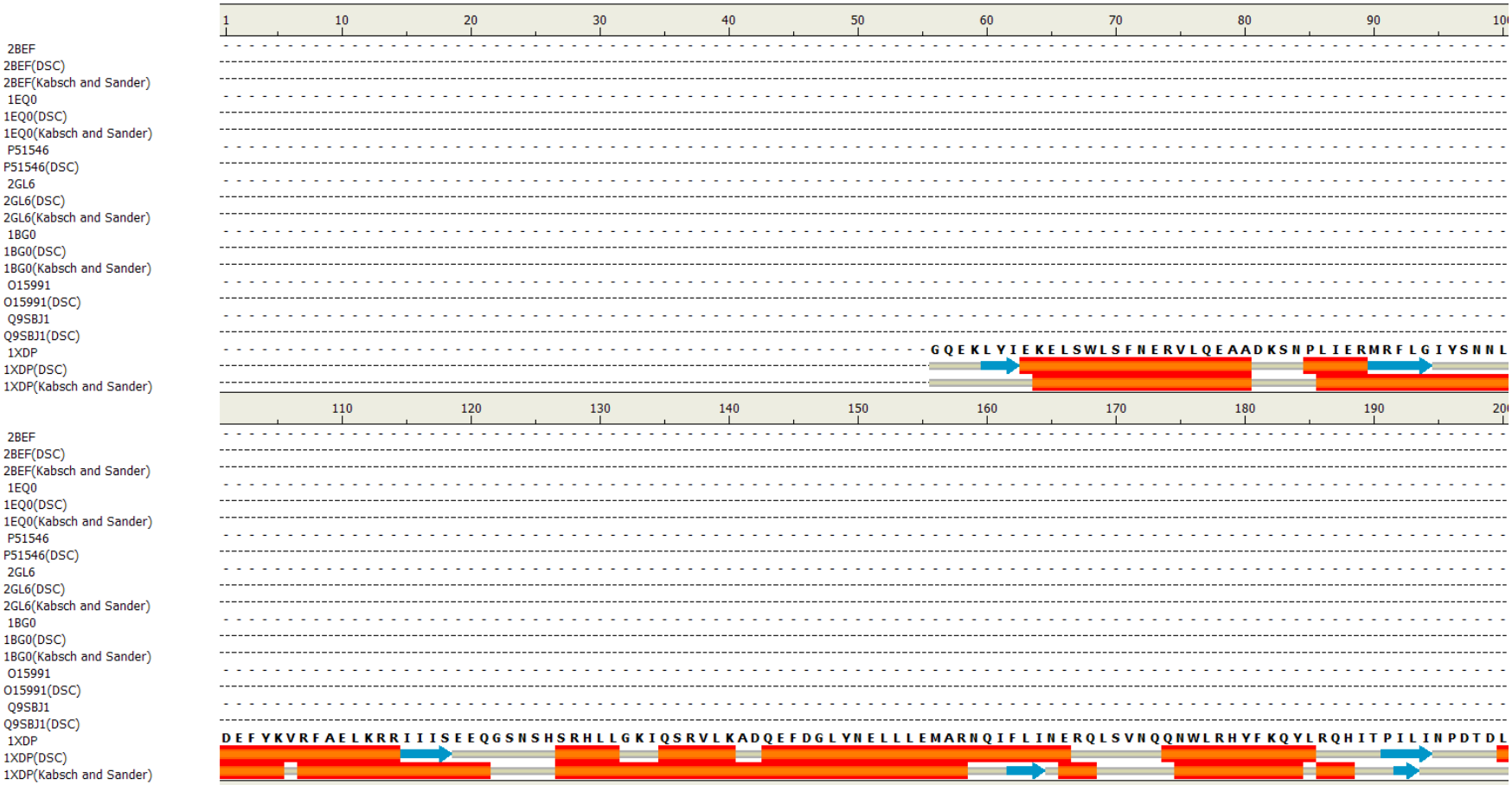


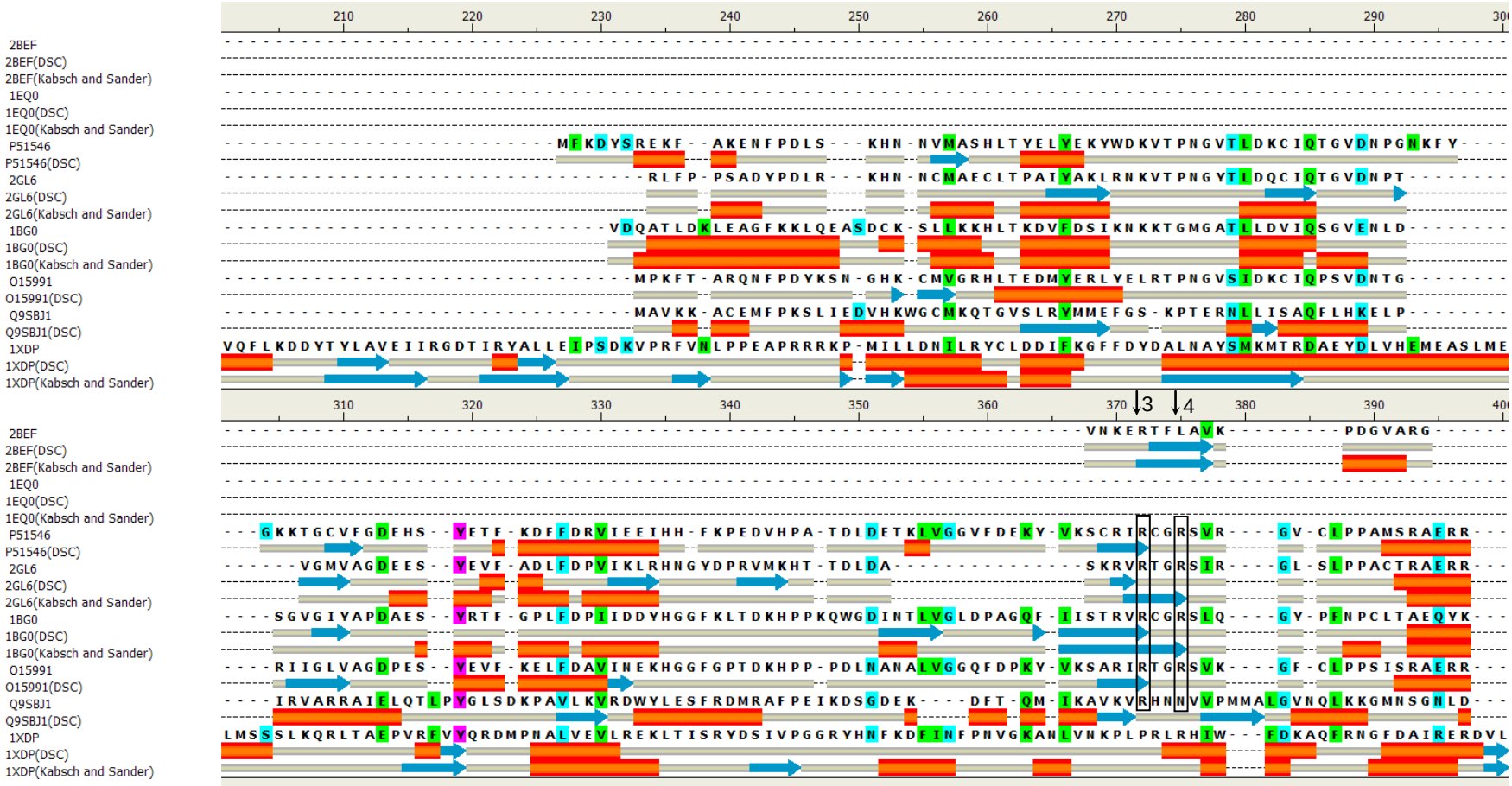
Figure MECH6. Phosphoryl transfer mechanism found in the Group 3 kinases (guanido family comprising creatine and arginine kinase). This occurs via coordination of the adenyl C6-NH_2 and protonation of C8 via a coordinated Asp changing C8 from sp2 to sp3 hybridization, and alters the protonation of C8-H. The C8-H becomes more acidic, allowing for the protonation of the β-PO_4, via a conserved Arg. There is a concomitant transfer of an H^+ to the β-PO_4, facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a simultaneous Arg-mediated deprotonation of the substrate-NH that allows for the nucleophilic attack by the substrate, creating the pentavalent intermediate and allowing phosphoryl transfer. The protonated Arg then transfers the proton to the γ-PO_4 changing the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

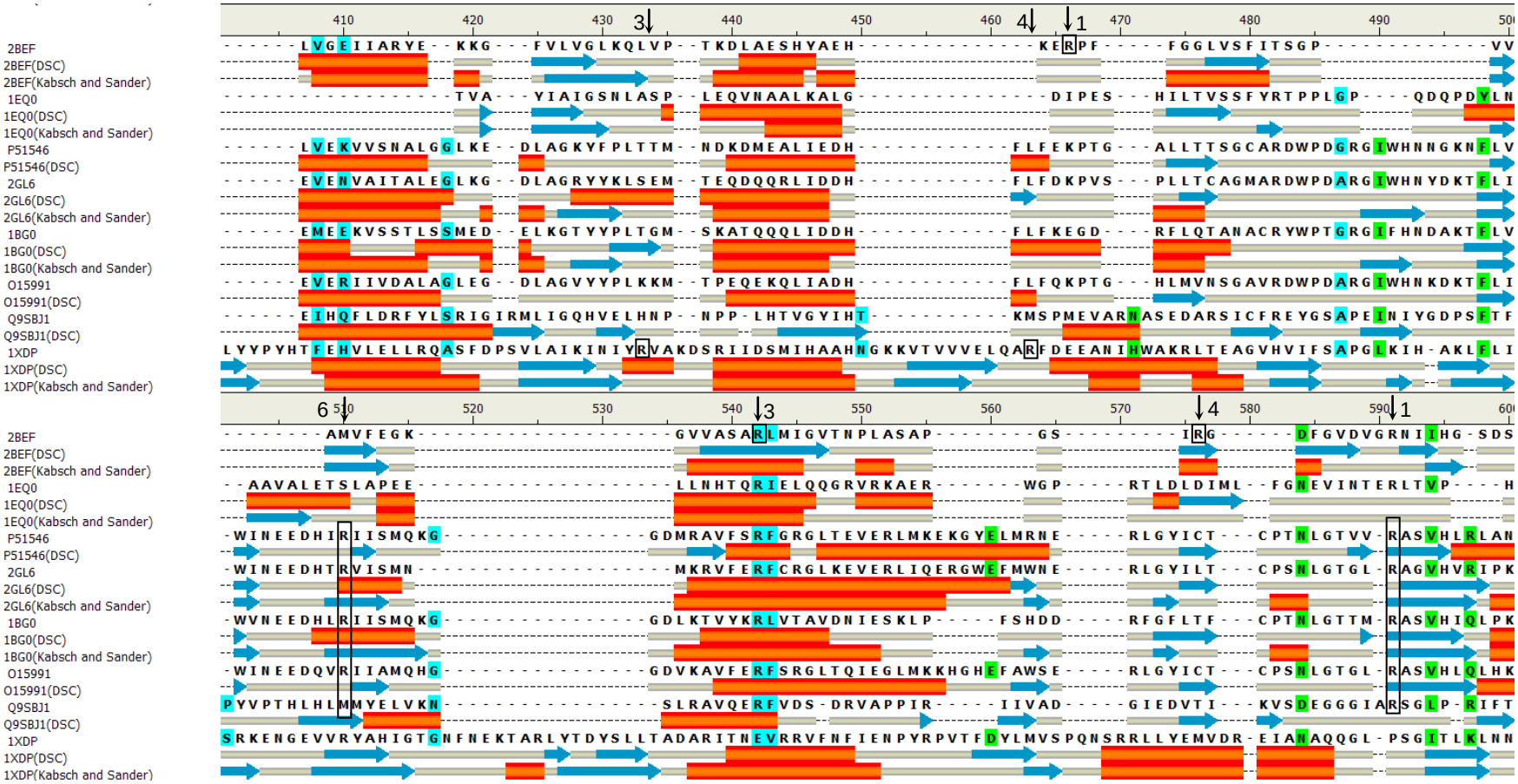
Table AF 3B. Group 3 kinases

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
Group 3 Kinases		
1. Nucleoside bis-phosphate kinase (pdb|2BEF)	nd[3]	Nucleoside diphosphate kinase (PF00334)
2. 2-amino-4-hydroxy-6-hydroxymethyldeihydopter-idine pyrophosphokinase (pdb|1EQ0)	nd	7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (PF01288)
3. Guanidoacetate kinase (uniprot|P51546)	a. nd b. gamma-glutamylcysteine synthetase/glutamine synthetase (CL0286)	a. ATP:guanido phosphotransferase, N-terminal domain (PF02807) b. ATP:guanido phosphotransferase, C-terminal catalytic domain (PF00217)
4. Creatine kinase (pdb|2GL6)	a. nd b. gamma-glutamylcysteine synthetase/glutamine synthetase (CL0286)	a. ATP:guanido phosphotransferase, N-terminal domain (PF02807) b. ATP:guanido phosphotransferase, C-terminal catalytic domain (PF00217)
5. Arginine kinase (pdb|1BG0)	a. nd b. gamma-glutamylcysteine synthetase/glutamine synthetase (CL0286)	a. ATP:guanido phosphotransferase, N-terminal domain (PF02807) b. ATP:guanido phosphotransferase, C-terminal catalytic domain (PF00217)
6. Lombricine kinase (uniprot|O15991)	a. nd b. gamma-glutamylcysteine synthetase/glutamine synthetase (CL0286)	a. ATP:guanido phosphotransferase, N-terminal domain (PF02807) b. ATP:guanido phosphotransferase, C-terminal catalytic domain (PF00217)
7. Pyruvate dehydrogenase kinase (uniprot|Q9SBJ1)	a. nd b. His Kinase A (phospho-acceptor) domain (CL0025)	a. Mitochondrial branched-chain alpha-ketoacid dehydrogenase kinase (PF10436) b. Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase (PF02518)

[1] pfam clan classification
[2] pfam family/domain classification
[3] No Detectable similarity to conventional kinases
Where there are 2 or domains recognised, these are denoted by 'a', 'b', etc. One domain has been selected to position the protein within the table.


1
10
20
30
40
50
60
70
80
90
2BEF
2BEF(DSC)
2BEF(Kabsch and Sander)
1EQ0
1EQ0(DSC)
1EQ0(Kabsch and Sander)
P51546
P51546(DSC)
2GL6
2GL6(DSC)
2GL6(Kabsch and Sander)
1BG0
1BG0(DSC)
1BG0(Kabsch and Sander)
O15991
O15991(DSC)
Q9SBJ1
Q9SBJ1(DSC)
1XDP
1XDP(DSC)
1XDP(Kabsch and Sander)
G Q E K L Y I E K E L S W L S F N E R V L Q E A A D K S N P L I E R M R F L G I Y S N N L
110
120
130
140
150
160
170
180
190
2BEF
2BEF(DSC)
2BEF(Kabsch and Sander)
1EQ0
1EQ0(DSC)
1EQ0(Kabsch and Sander)
P51546
P51546(DSC)
2GL6
2GL6(DSC)
2GL6(Kabsch and Sander)
1BG0
1BG0(DSC)
1BG0(Kabsch and Sander)
O15991
O15991(DSC)
Q9SBJ1
Q9SBJ1(DSC)
1XDP
1XDP(DSC)
1XDP(Kabsch and Sander)
D E F Y K V R F A E L K R R I I I S E E Q G S N S H S R H L L G K I Q S R V L K A D Q E F D G L Y N E L L L E M A R N Q I F L I N E R Q L S V N Q Q N W L R H Y F K Q Y L R Q H I T P I L I N P D T D L


2BEF
2BEF(DSC)
2BEF(Kabsch and Sander)
1EQ0
1EQ0(DSC)
1EQ0(Kabsch and Sander)
P51546
P51546(DSC)
2GL6
2GL6(DSC)
2GL6(Kabsch and Sander)
1BG0
1BG0(DSC)
1BG0(Kabsch and Sander)
O15991
O15991(DSC)
Q9SBJ1
Q9SBJ1(DSC)
1XDP
1XDP(DSC)
1XDP(Kabsch and Sander)
210
220
230
240
250
260
270
280
290
310
320
330
340
350
360
370
380
390
3
4


2BEF
2BEF(DSC)
2BEF(Kabsch and Sander)
1EQ0
1EQ0(DSC)
1EQ0(Kabsch and Sander)
P51546
P51546(DSC)
2GL6
2GL6(DSC)
2GL6(Kabsch and Sander)
1BG0
1BG0(DSC)
1BG0(Kabsch and Sander)
O15991
O15991(DSC)
Q9SBJ1
Q9SBJ1(DSC)
1XDP
1XDP(DSC)
1XDP(Kabsch and Sander)

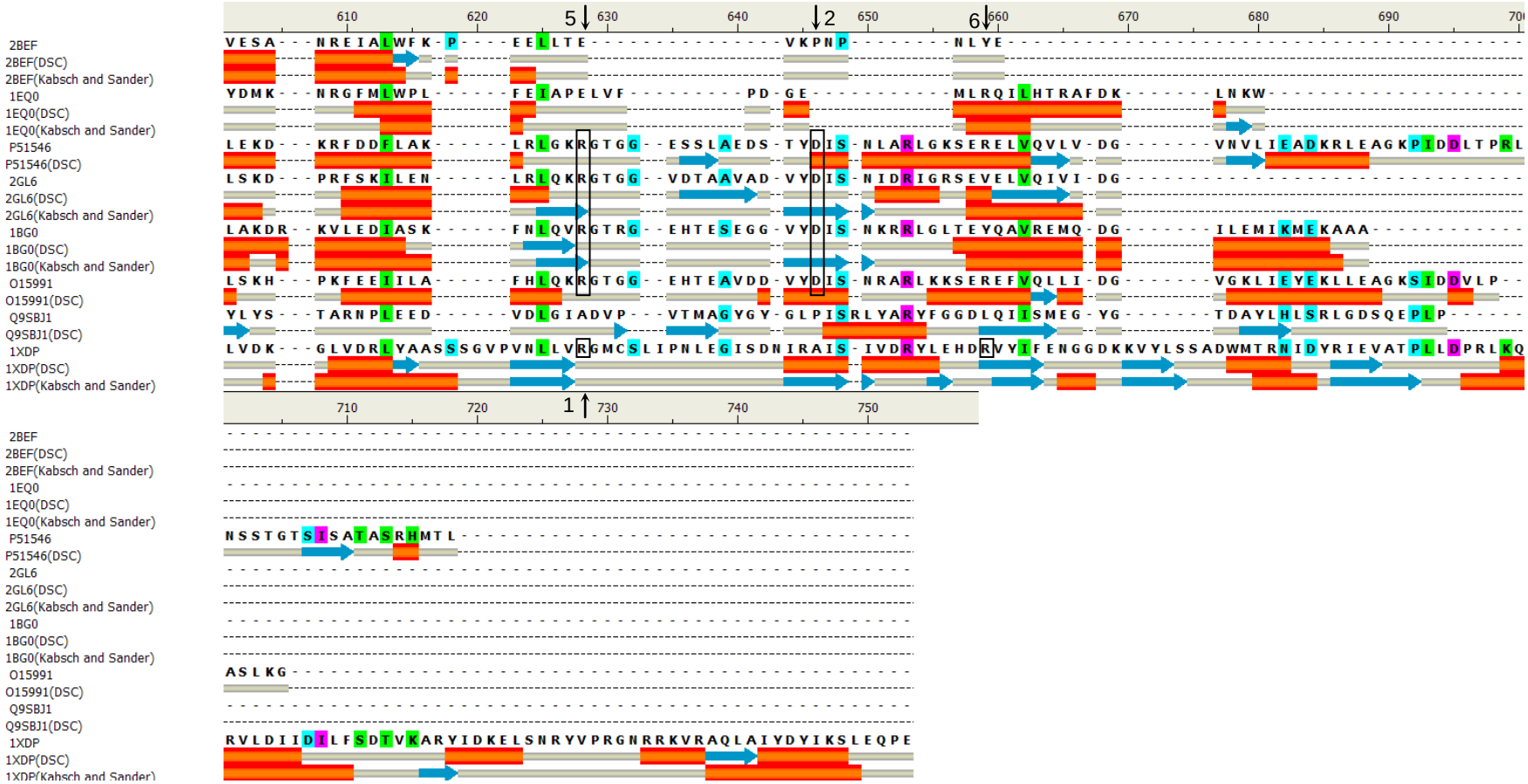


Figure SEQ3. A sequence alignment of the proteins making up the 'ATP guanido' kinase family within **Group 3** indicating the identified conserved amino acid residues responsible for initiating phosphoryl transfer. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 3A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. BEF 2, Nucleoside bis-phosphate kinase; 1EQ0, 2-amino-4-hydroxy-6-hydroxymethyldeihydopter-idine pyrophosphokinase; P51546, Guanidoacetate kinase; 2GL6, Creatine kinase; 1BG0, Arginine kinase; O15991, Lombricine kinase; Q9SBJ1, Pyruvate dehydrogenase (lopiamine) kinase; 1XDP, Polyphosphate kinase.

Table AF 4A. Kinases representing **Group 4** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured inter-atomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**C8-H to α-PO_4**	**Arg[1]**	**Glu[2]**	**Arg[3]**	**Arg[4]**	**Ser/Thr[5]**	**Ser/Thr[6]**	**Asp/Substrate[7]**	**Gly Loop[13]**
Ribonuclease H-like sequences									
Hexokinase[9,10] 1DGK (*Homo sapiens*)	7.097 5.825[9]	Lys785 αC=O 1.513	NCR	NCR	NCR	Thr863[11] 3.895	Thr863[11] 2.981	NSub	
Hexokinase[9,10] 2E2P (*Sulfolobus tokodaii*)	7.097 5.825[9]	Arg251 1.513	NCR	NCR	NCR	Arg251[12] 3.511	Arg251[11] 2.169	NSub	Leu245-Arg251 Gly248 3.550
Glucokinase[10] 3FGU (*Homo sapiens*)	4.650	Arg333 OA	Glu331 OA	Arg85[12] 2.987	Arg85[12] 3.197	Ser411[11] 3.487	Ser411[11] 3.313	Sugar/Asp205 2.041	O-P 2.681
Fructokinase 3LKI (*Xylella fastidiosa*)	2.878 Direct	Val266 αC=O 3.870		Lys203	Lys203 2.144		Thr238 3.268		Arg178 2.333
Rhamulokinase[10] 2CGJ **(***Escherichia coli*)	5.001	Arg37 6.090	Glu427 1.990	Arg17 2.152	Arg17 2.184	Thr259	Arg37[12] 3.849	Fructose/Asp237 1.813	Ile399-Gln405 Gly402 2.525
Mannokinase Q0BVN7 (*Granulibacter bethesdensis*)	NS								
Gluconokinase[10] 3LL3 (*Lactobacillus acidophilus*)	5.632	Arg305 3.595	Asp312 OAS	Lys15 OAS	Lys15 OAS	Thr259	Thr259 4.116	D-xylulose/Asp237 3.471	Asn396-Leu402 Gly400 2.245
L-ribulokinase 3JVP (*Bacillus halodurans*)	No ADP	Lys452	Asp455	Arg18	Arg18	Thr296	Thr296	Asp274	
Xylulokinase[10] 3HZ6 (*Chromobacterium violaceum*)	5.068	Arg412 4.004 N7	Asp433 OAS	Lys16 OAS	Lys16 OAS	Thr263 6.618	Thr263 3.450	D-xylulose/Asp241 3.019	Arg405-Ala411 Gly409 2.839
Erythritol kinase Q92NH0 (*Rhizobium meliloti*)	NS	Arg421	Glu441	Lys15	Lys15	Ser264	Ser264	Asp242	
Glycerol kinase[10] 1BWF **(***Escherichia coli*)	5.856	Arg436 3.951	Asp325 4.589	Arg17 2.229	Arg17 3.978	Arg436[12] 3.951	Arg436[12] 2.033	Glycerol/Asp245 2.755	Arg417-Ala414 Gly411 3.189
Pantothenate kinase[9,10] 3BF1 (*Thermotoga maritima*)	6.169 6.070[9]	His154 αC=O 3.116	NCR	NCR	NCR	Thr10 3.921	Thr10 2.507	Pantothenate/Asp10 5 2.414	
D-ribulokinase Q8YBC1 (*Brucella melitensis*)	NS	Arg351	Asp361	Arg20	Arg20	Thr286	Thr286	Asp258	
L-fuculokinase P11553 **(***Escherichia coli*)	NS	Arg322	Glu329	Lys27	Lys27	Thr273	Thr273		
L-xylulokinase B7MFE7 **(***Escherichia coli*)	NS	His405	Asp415	Lys16	Lys16	Thr265	Thr265	Asp243	
Allose kinase 3HTV **(***Escherichia coli*)	No ADP								

Kinase	**C8-H to α-PO_4**	**Arg[1]**	**Glu[2]**	**Arg[3]**	**Arg[4]**	**Ser/Thr[5]**	**Ser/Thr[6]**	**Asp/Substrate[7]**	**Gly Loop[13]**
2-dehydro -3-deoxygaltonokinase	NS								
Acetyl-glucosamine kinase 2CH6 (*Homo sapiens*)	4.382	Arg218 2.364	Glu222 OA	Arg14 3.654	Arg14 3.973	Ser27111 3.081	Ser27111 3.022	GlcNAc/Asp107 2.220	Leu267-Trp273 Ser271 3.554
Acetyl-mannosamine kinase 3EO3 (*Homo sapiens*)	No ADP								
Polyphosphate-glucose phosphotransferase 1WOQ (Arthrobacter sp.)								Polyphosphate substrate	
Beta-glucoside kinase Q926Y3 (*Listeria innocua*)	NS								
Acetate kinase9 1TUY (*Methanosarcina thermophila*)	4.487	Arg285 3.156	Asp286			Asn355 2.673		Glu384 Arg241 His208 His180	Ala330-Ser336 Gly331 2.161
Butyrate kinase βPO4 1SAZ (*Thermotoga maritima*)		Arg257 2.907				His307		Glu213 Arg214 His154 His182	Val300-Ala306 Gly304 2.946
Branched-chain-fatty-acid kinase Q3ETP1 (*Bacillus thuringiensis*)	NS								Ile322-Gly328 Gly326 2.566
Propionate kinase 1X3N (*Salmonella enterica* subsp. *enterica*)	4.512	Arg280 3.866				Asn330		Asp143 Arg236 His175 His203	
Mean	**4.932**	**3.155**		**2.756**	**3.095**	**3.495**	**2.672**	**2.533**	**2.781**
Standard Deviation	**0.876**	**0.815**		**0.708**	**0.908**	**0.446**	**0.537**	**0.583**	**0.488**
% Standard Deviation	**12.535**	**26.847**		**25.681**	**25.484**	**37.689**	**19.980**	**23.006**	**15.881**

1. N7 coordinated.
2. Stabilization of Arg[1] interatomic distance.
3. α-PO_4 coordinated Arg.
4. β-PO_4 coordinated Arg.
5. C8-H coordinated.
6. β-PO_4 coordinated.
7. Sugar-OH coordinated Asp.
8. γ-PO_4 coordinated.
9. C8-H β-PO_4 coordinated.
10. Optimization of the pyrimidine/β-D-sugar torsion angle.
11. Optimization of the Thr/Ser-OH torsion angle.
12. Optimization of the Arg torsion angles, not included in the interatomic distance calculation.
13. Loop containing conserved Gly responsible for the responsible for the stabilization of the carbene.

OAS = Active site partially open.
OA = orientated away from imidazole moiety of ATP.
NCR = no coordinating residue.
NSub = no substrate for phosphorylation in structure.

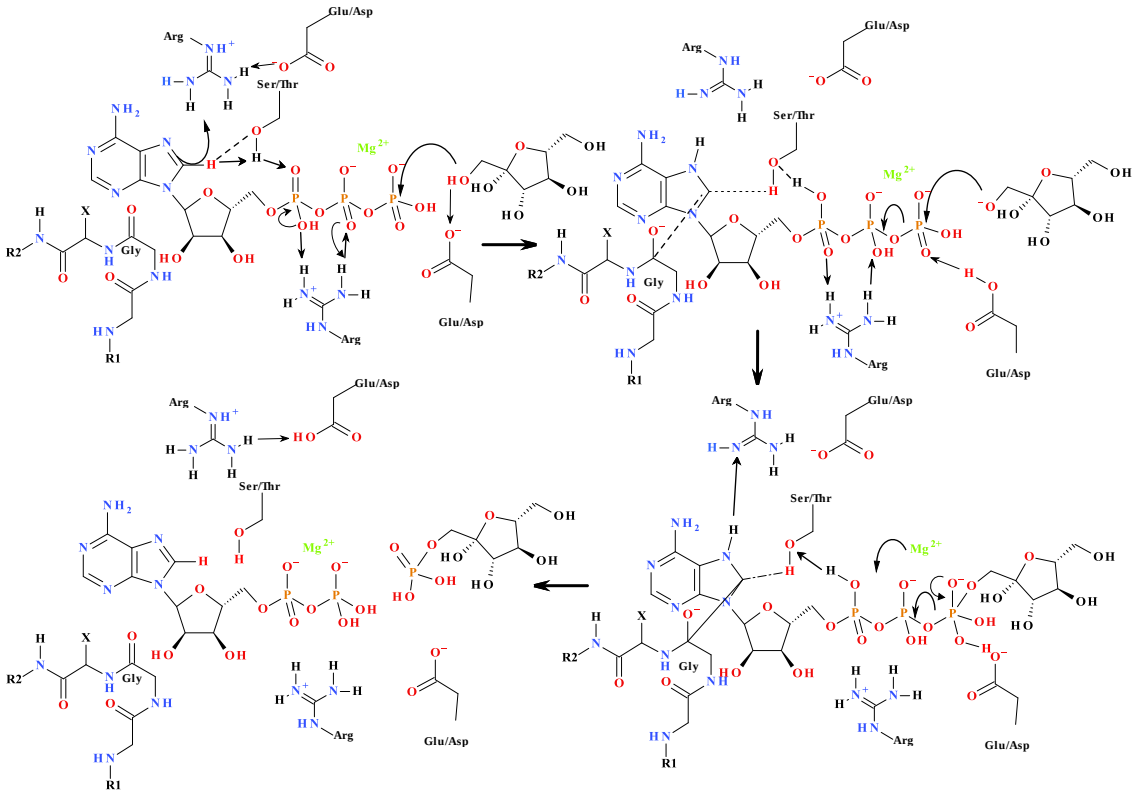


Figure MECH7. Phosphoryl transfer mechanism found in the Group 4 kinases (hexokinase family with polyol substrate). This occurs via coordination of an arginine residue to the N7/C8 of the imidazole moiety mediating the change in C8 hybridization from sp2 to sp3, and altering the protonation of N7 and C8. Protonation of the N7 occurs via a conserved the Arg residue with the NH_2 being coordinated directly to N7, with an interatomic distance of 3.065 ± 0.823 Å. The Arg residue in Group 4 kinases is always stabilized by an associated Asp/Glu residue. The reaction occurs via a carbene mechanism with the carbene being stabilized via the interaction of a conserved backbone carbonyl that is within bonding distance of C8, causing C8-H to become more acidic, allowing for the protonation of the α-PO_4, via a conserved Ser/Thr. There is a concomitant transfer of an H^+ from the α-PO_4 to the β-PO_4 via a conserved Arg, thereby facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a concomitant Asp-mediated deprotonation of the substrate –OH, allowing for the nucleophilic attack by the substrate. This creates the pentavalent intermediate and allows phosphoryl transfer. The protonated Asp then transfers the proton to the γ-PO_4, changing the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

Table AF 4B. Group 4 kinase

	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
Group 4 Kinases		
1. Hexokinase (pdb|1DGK)	Actin-like ATPase (CL0108)	Hexokinase 1 (PF00349) and Hexokinase 2 (PF03727)
2. Glucokinase (pdb|3FGU)	Actin-like ATPase (CL0108)	Hexokinase 1 (PF00349) and Hexokinase 2 (PF03727)
3. Fructokinase (pdb|3LKI)	Ribokinase-like (CL0118)	pfkB family carbohydrate kinase (PF00294)
4. Rhamulokinase (pdb|2CGL)	Ribokinase-like (CL0118)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
5. Mannokinase (uniprot|Q0BVN7)	Actin-like ATPase (CL0108)	ROK family (PF00480)
6. Glucokinase (pdb|3LL3)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
7. L-ribulokinase (pdb|3JVP)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
8. Xylulokinase (pdb|3HZ6)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
9. Erythritol kinase (uniprot|Q92NH0)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
10. Glycerol kinase (pdb|1BWF)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
11. Pantothenate kinase(pdb|3BF1)	Actin-like ATPase (CL0108)	Bordetella pertussis Bvg accessory factor family (PF03309)
12. D-ribulokinase (uniprot|Q8YBC1)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
13. L-fuculokinase (uniprot|P11553)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
14. L-xylulokinase (uniprot|B7MFE7)	Actin-like ATPase (CL0108)	FGGY family of carbohydrate kinases, N-terminal domain (PF00370) and C-terminal domain (PF02782)
15. Allose kinase (pdb|3HTV)	Actin-like ATPase (CL0108)	ROK family (PF00480)
16. 2-dehydro-3-deoxygalactonokinase (uniprot|P31459)	nd[3]	2-keto-3-deoxy-galactonokinase (PF05035)
17. Acetyl-glucosamine kinase (pdb|2CH6)	Actin-like ATPase (CL0108)	BadF/BadG/BcrA/BcrD ATPase family (PF01869)

18. Acetyl-mannosamine kinase (pdb|3EO3)	Actin-like ATPase (CL0108)	ROK family (PF00480)
19. Polyphosphate-glucoase phosphotransferase (pdb|1WOQ)	Actin-like ATPase (CL0108)	ROK family (PF00480)
20. Beta-glucoside kinase (uniprot|Q926Y3)	Actin-like ATPase (CL0108)	ROK family (PF00480)
21. Acetate kinase (pdb|1TUY)	Actin-like ATPase (CL0108)	Acetokinase family (PF00871)
22. Butyrate kinase (pdb|1SAZ)	Actin-like ATPase (CL0108)	Acetokinase family (PF00871)
23. Branched-chain fatty acid kinase (uniprot|Q3ETP1)	Actin-like ATPase (CL0108)	Acetokinase family (PF00871)
24. Propionate kinase (uniprot|P11868)	Actin-like ATPase (CL0108)	Acetokinase family (PF00871)

[1] pfam clan classification in brackets
[2] pfam family/domain classification in brackets
[3] No Detectable similarity to conventional kinases
Where there are 2 or domains recognised, these are denoted by ‘a’, ‘b’, etc. One domain has been selected to position the protein within the table.

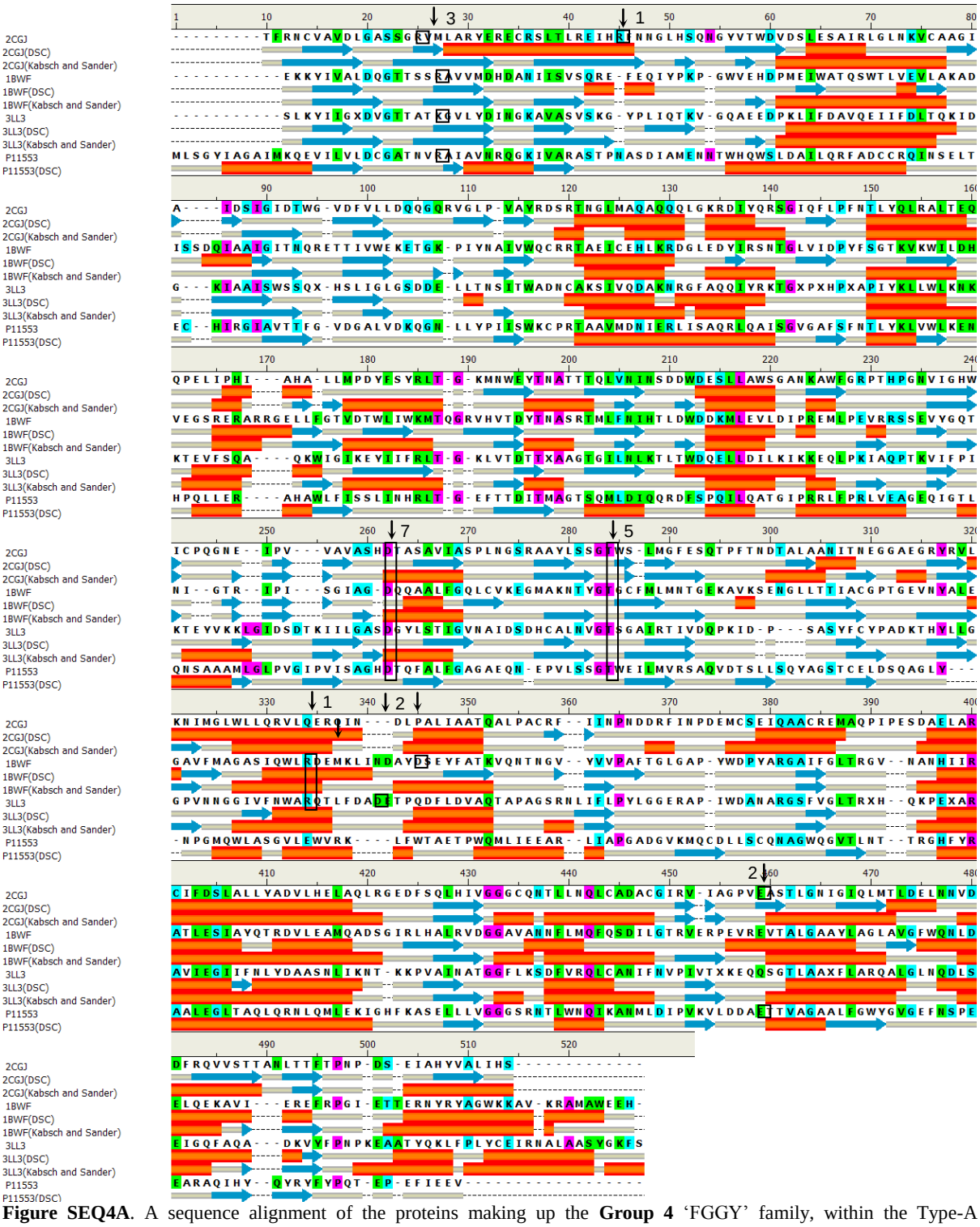


Figure SEQ4A. A sequence alignment of the proteins making up the **Group 4** 'FGGY' family, within the Type-A subfamily. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 2CGJ, Rhamulokinase; 3LL3, Gluconokinase; 1BWF, Glycerol kinase; P11553, L-Fuculokinase.

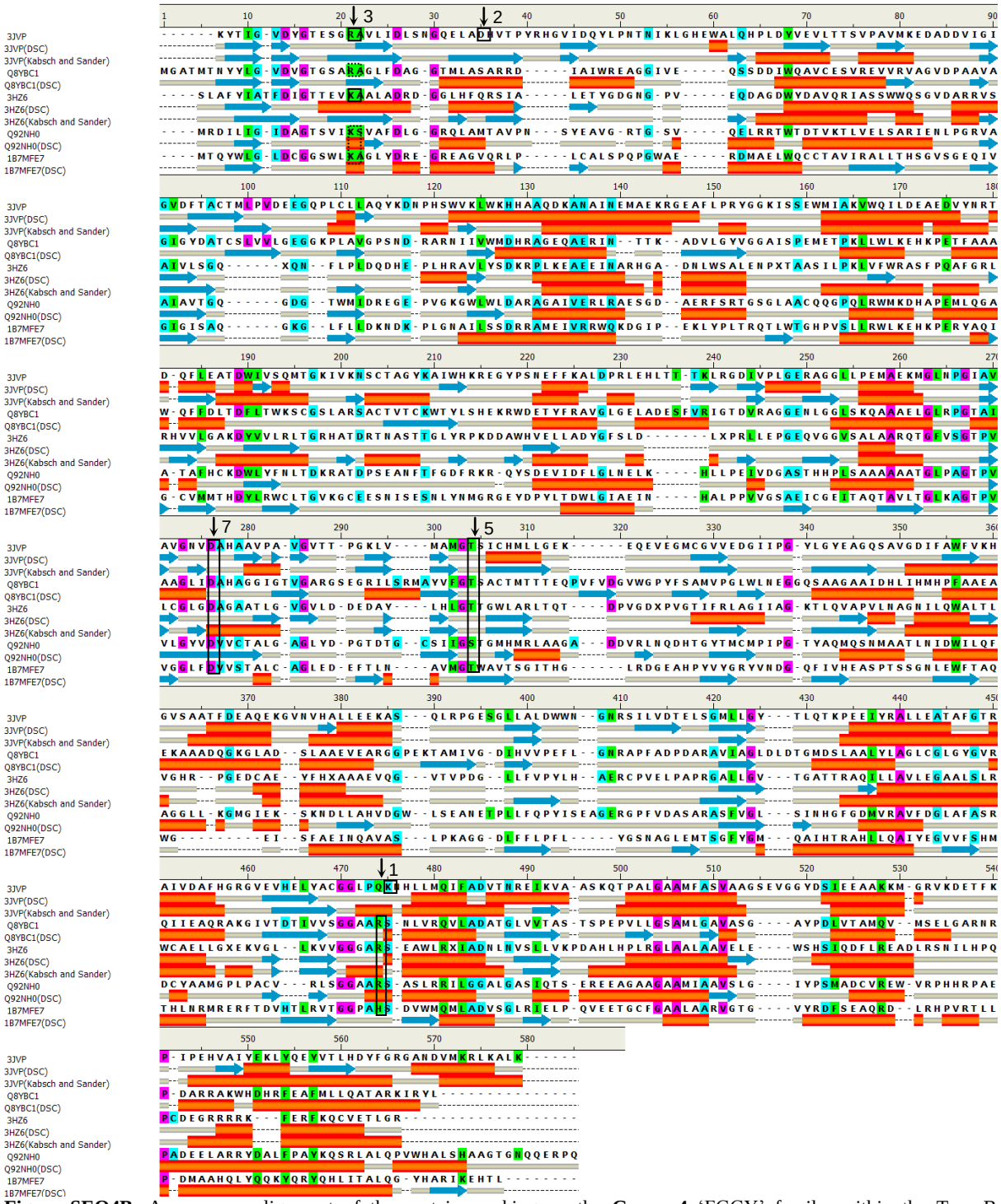


Figure SEQ4B. A sequence alignment of the proteins making up the **Group 4** 'FGGY' family, within the Type-B subfamily. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 3JVP, L-ribulokinase; Q8YBC1, L-ribulokinase; 3HZ6, D-Xylulokinase; Q92NH0, Erythretol kinase; 1B7MFE7, L-Xylulokinase.

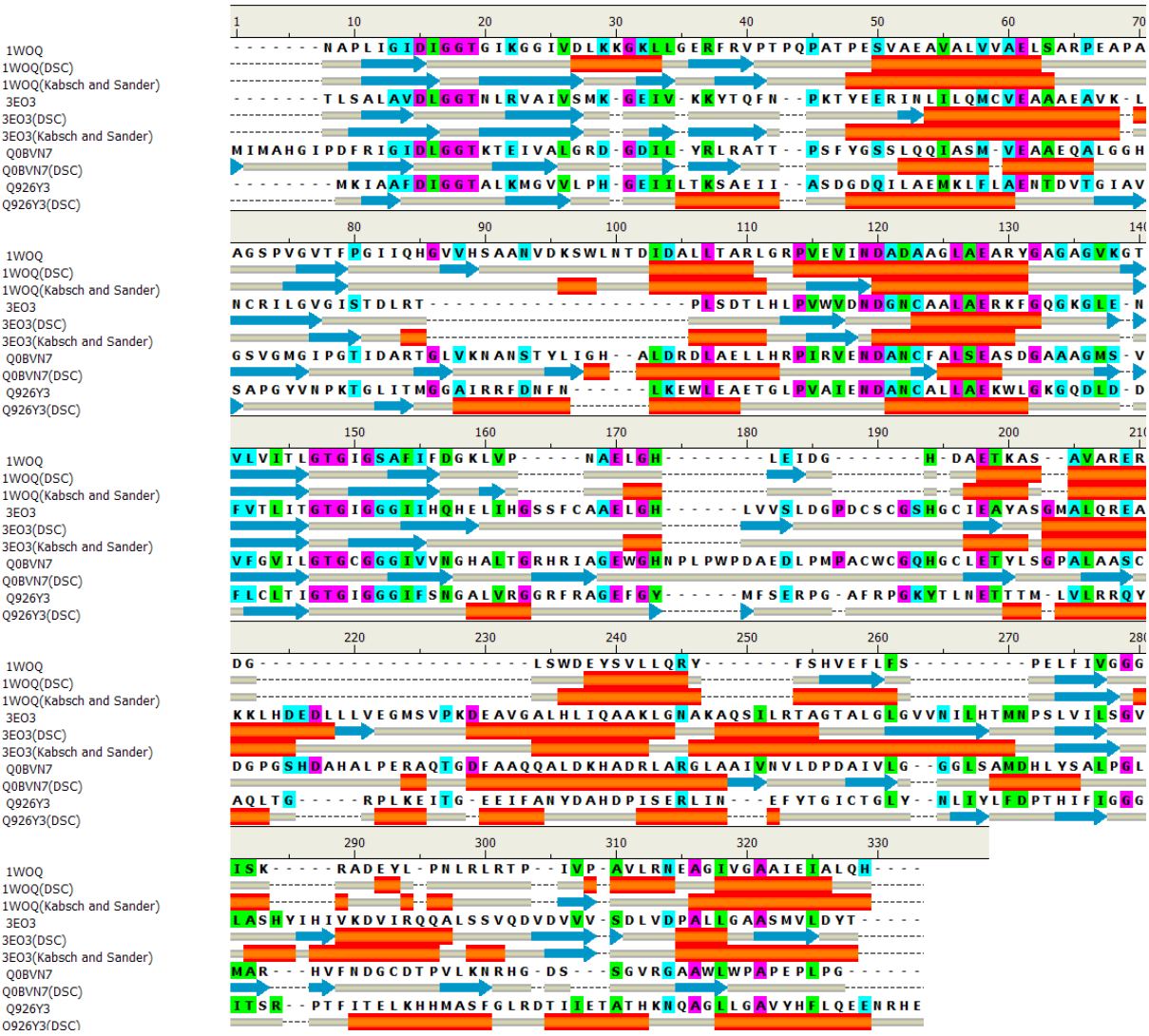


Figure SEQ4C. Conserved functional residues within the **Group 4** ROK family of kinases. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1WOQ, Polyphosphate-glucose phosphotransferase; 3EO3, Acetyl-mannoseamine kinase; Q0BVN7, Mannokinase; Q926Y3, β-Glucoside kinase.

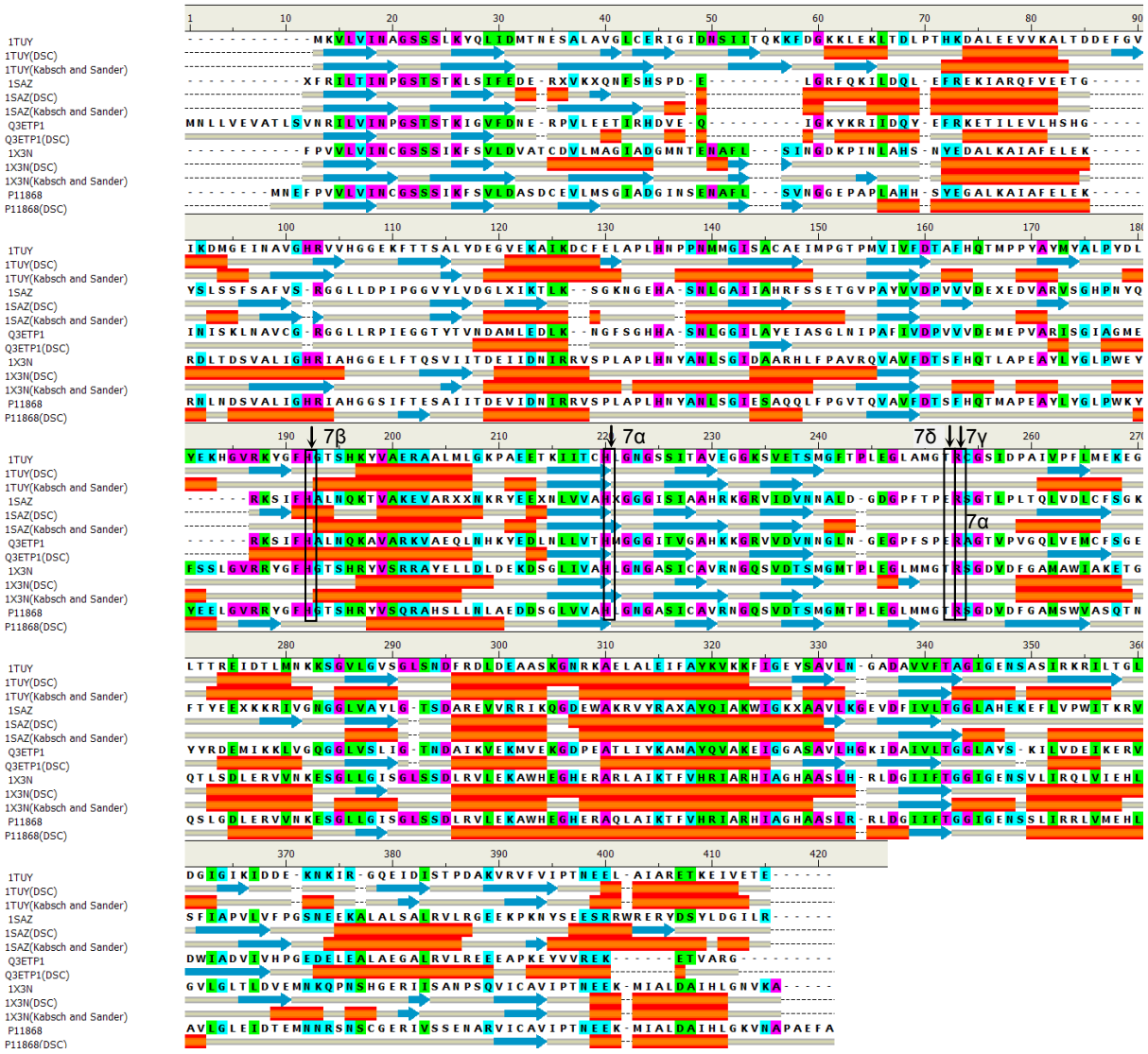


Figure SEQ4D. Conserved functional residues within the **Group 4** acetokinase family of kinases. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1TUY, Acetate kinase; 1SAZ, Butyrate kinase; Q3ETP1, Branched-chain-fatty-acid kinase; 1X3N/P11868, Propionate kinase.

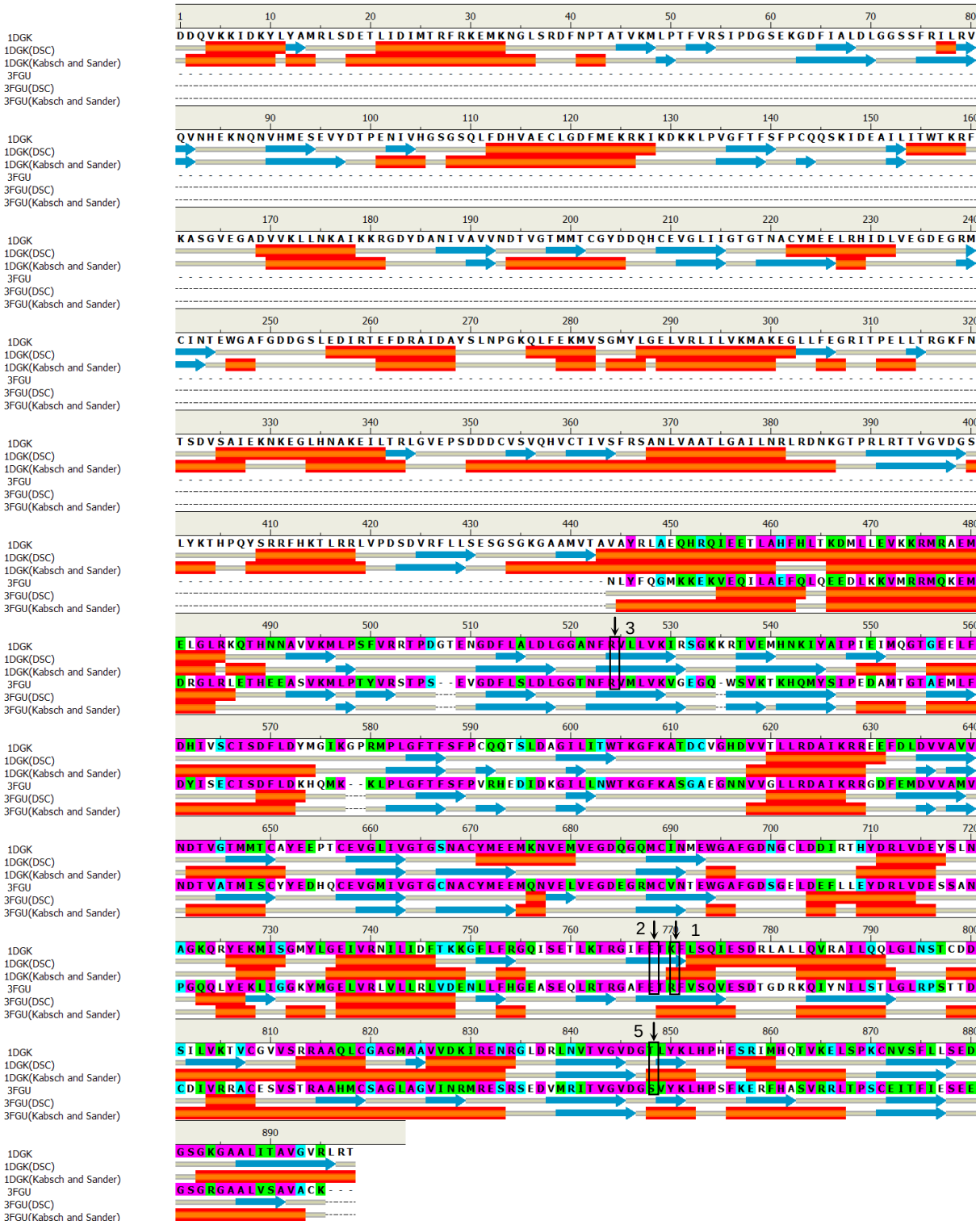


Figure SEQ4E. Conserved functional residues within the **Group 4** hexokinase family. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1DGK, Hexokinase; 3FGU, Glucokinase.

Table AF 5A. Kinases representing **Group 6** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured inter-atomic distances are shown. Also indicated are the residues associated with the rotation of the adenyl moiety from the *syn-* to the *anti-* conformation. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**Nucleo-tide Config.**[1]	**Asn-δO1 to Aden-NH_2**[2]	**Lys to Aden C6-NH_2**[3]	**Lys to Asn-NH_2**[3]	**Ser to C8-H**[4]	**Ser to α-PO_4**[4]	**Lys to C8-H**[5]	**Lys to α-PO_4**[5]	**C8-H to α-PO_4**[6]	**Lys/Ser to α-PO_4**[7]	**Lys to β-PO_4**[7]	**Glu-γC-C=O substrate**[8]
GHMP kinase												
Galactokinase 1S4E (*Pyrococcus furiosus*)	*Syn-*	His51 3.412	Tyr35 2.735	Tyr35 4.151	Ser106 7.155	Ser106 5.044						
	Anti-	His51 4.354	Tyr35 4.171	Tyr35 7.040	Ser106 2.860	Ser106 4.069				[9]	[9]	
Mevalonate kinase 2V8P (*Aquifex aeolicus*)	***Syn-***	Asn99 2.180	Lys 85 2.934	Lys85 3.465	Ser97 8.582	Ser97 3.574	-	-	-	-	-	
	Anti-	Asn99 4.782	Lys 85 6.068	Lys 85 5.056	Ser97 4.096	Ser97 2.990	Lys85 3.057	Lys85 3.326	3.030D	Ser97 2.990	Ser97 5.286	Asp130
Homoserine kinase 1FWK (*Methanocaldococcus jannaschii*)	***Syn-***	Asn62 1.971	Lys 87 3.046	Lys87 5.715	Ser98 6.375	Ser98 4.511	-	-	-			
	Anti-	Asn62 3.549	Lys 87 3.325	Lys 87 7.370	Ser98 2.791	Ser98 2.313	Lys87 4.797	Lys87 6.012	4.781[9]	Ser98 2.386	Ser98 3.153	Asp140
L-arabinokinase O23461 (*Arabidopsis thaliana*)		NS[7]										
Fucokinase Q8N0W3 (*Homo sapiens*)		NS[7]										
Shikimate kinase[11]		NS[7]										
ISPE[12] 2V2Z (*Aquifex aeolicus*)	***Syn-***	Asn99 1.919	Lys 85 3.324	Lys85 3.710	Ser97 9.325	Ser97 3.658	-	-	-			
	Anti-	Asn99 4.344	Lys 85 6.208	Lys85 4.377	Ser97 6.054	Ser97 4.431	Lys85 4.381	Lys85 4.278	4.672	Ser97	Ser97	
Phosphomevalonate kinase 1GON (*Streptomyces* sp.)	*Syn-*	Met35 3.066	Lys 94 2.545	Lys94 4.053	-	Ser106 5.698	-	-	-			
	Anti-	Met35 4.107	Lys 94 6.098	Lys94 9.114	Ser106 1.750	Ser106 1.675	Lys94 7.220	Lys94 7.495	2.716	Lys101 4.285	Lys101 2.810	Asp150
Mean		**4.2272**	**5.174**	**6.591**	**3.510**	**3.096**	**4.864**	**5.278**	**3.800**	**3.220**	**3.750**	
Standard Deviation		**0.451**	**1.337**	**1.899**	**1.647**	**1.159**	**1.737**	**1.850**	**1.079**	**0.970**	**1.341**	
% Standard Deviation		**10.658**	**25.835**	**28.817**	**46.925**	**37.438**	**35.718**	**35.047**	**28.388**	**30.128**	**35.777**	

1. Bold type indicates configuration of adenyl moiety relative to ribose sugar in the coordinated structure in the PDB.
2. Coordination of the carbonyl the γ-carbonyl of Asn to the hydrogen of the C6-NH_2 of the nucleotide with the Asn carbonyl acting as a general base catalyst.
3. Coordination of the Lys ε-NH_3 to the γ-carbonyl of Asn and the hydrogen of the C6-NH_2 of the nucleotide.

4. α- and β-PO_4 coordinated Ser/Thr.
5. Lysine coordination to C8-H and α-PO_4.
6. Direct transfer from C8-H to β-PO_4.
7. α- and β-PO_4 proton transfer.
8. Asp responsible for substrate deprotonation.
9. C8-H to β-PO_4 transfer therefore no residue required.
10. No structure in PDB.
11. archaeal shikimate kinase.
12. 4-(cytidine 5’-diphospho)-2-C-methyl-D-erythritol kinase.

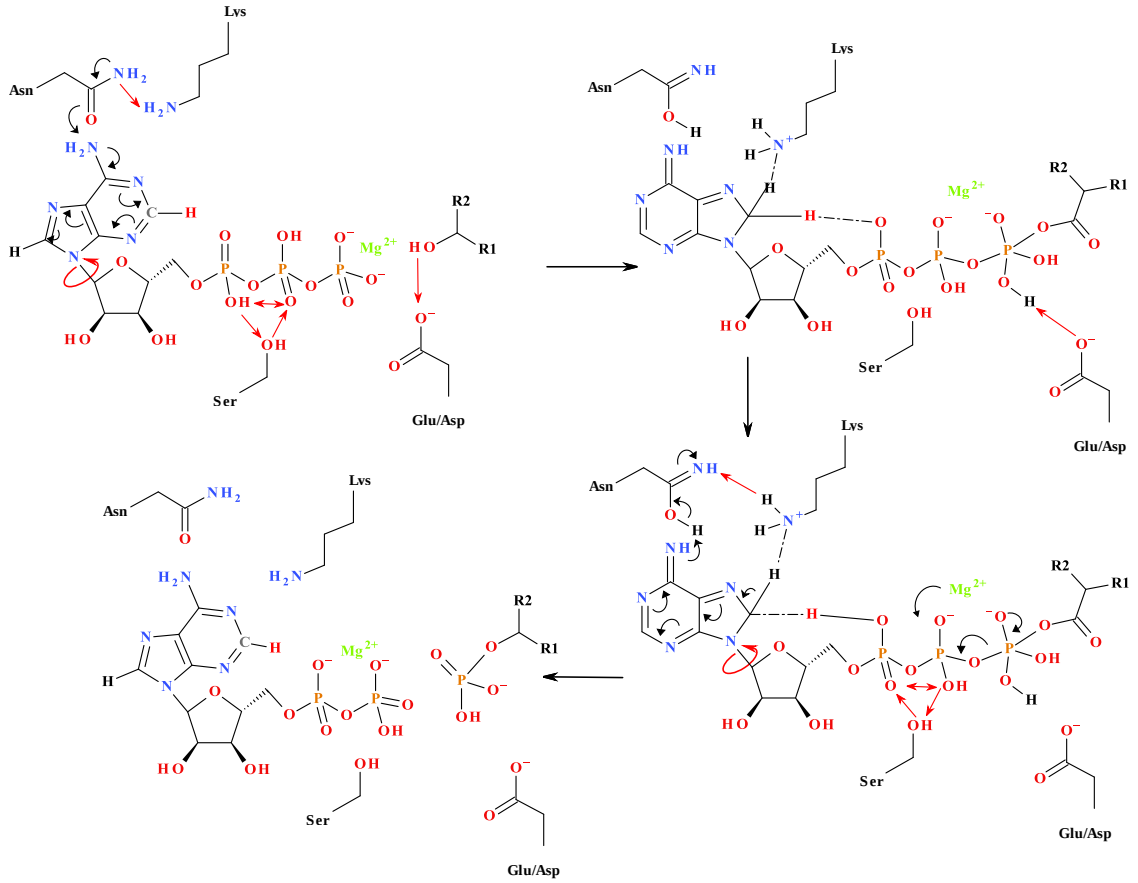


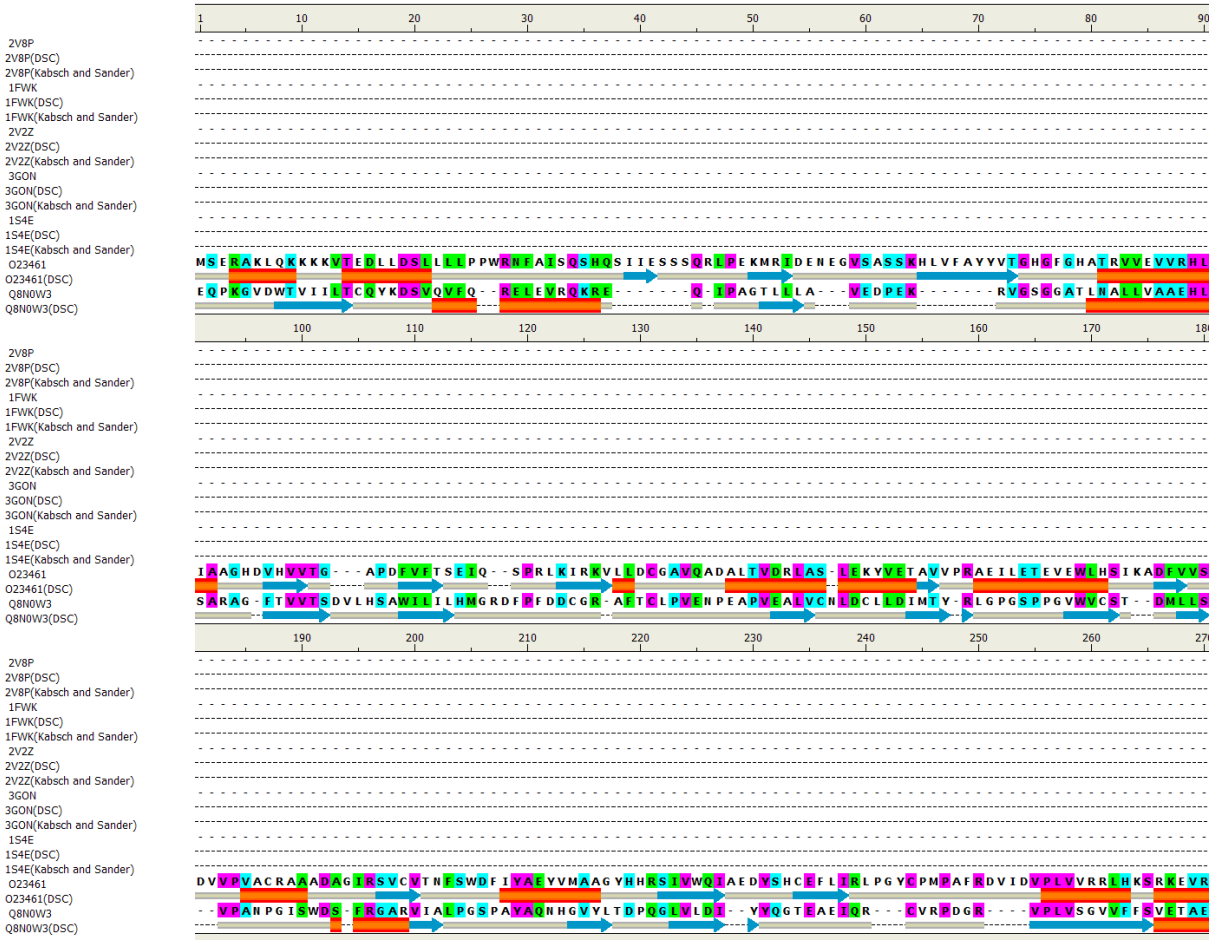
Figure MECH8. The postulated phosphoryl transfer mechanism found in the Group 6 kinases differs from all the other groups in that the adenyl group is in a *syn* conformation relative to the ribose, allowing for the coordination of the C8-H to the α-PO_4. The reaction is initiated by the coordination of the conserved Asn γC-carbonyl group to the ATP C6-NH_2. The coordination of a conserved lysine with the concomitant delocalization of the electrons of the adenyl group results in the re-hybridization of C8 from sp^2 to sp^3, that, along with the change of the conformation of the adenyl group from the *syn* to the *anti*-conformation there allows for the protonation of C8 by the lysine residue forming the carbene with the concomitant protonation of the α-PO_4 from C8. The proton translocation from the α-PO_4 creating the pentavalent intermediate then occurs. The proton required for the creation of the pentatvalent intermediate originates from the substrate via the Glu/Asp deprotonation of the substrate.

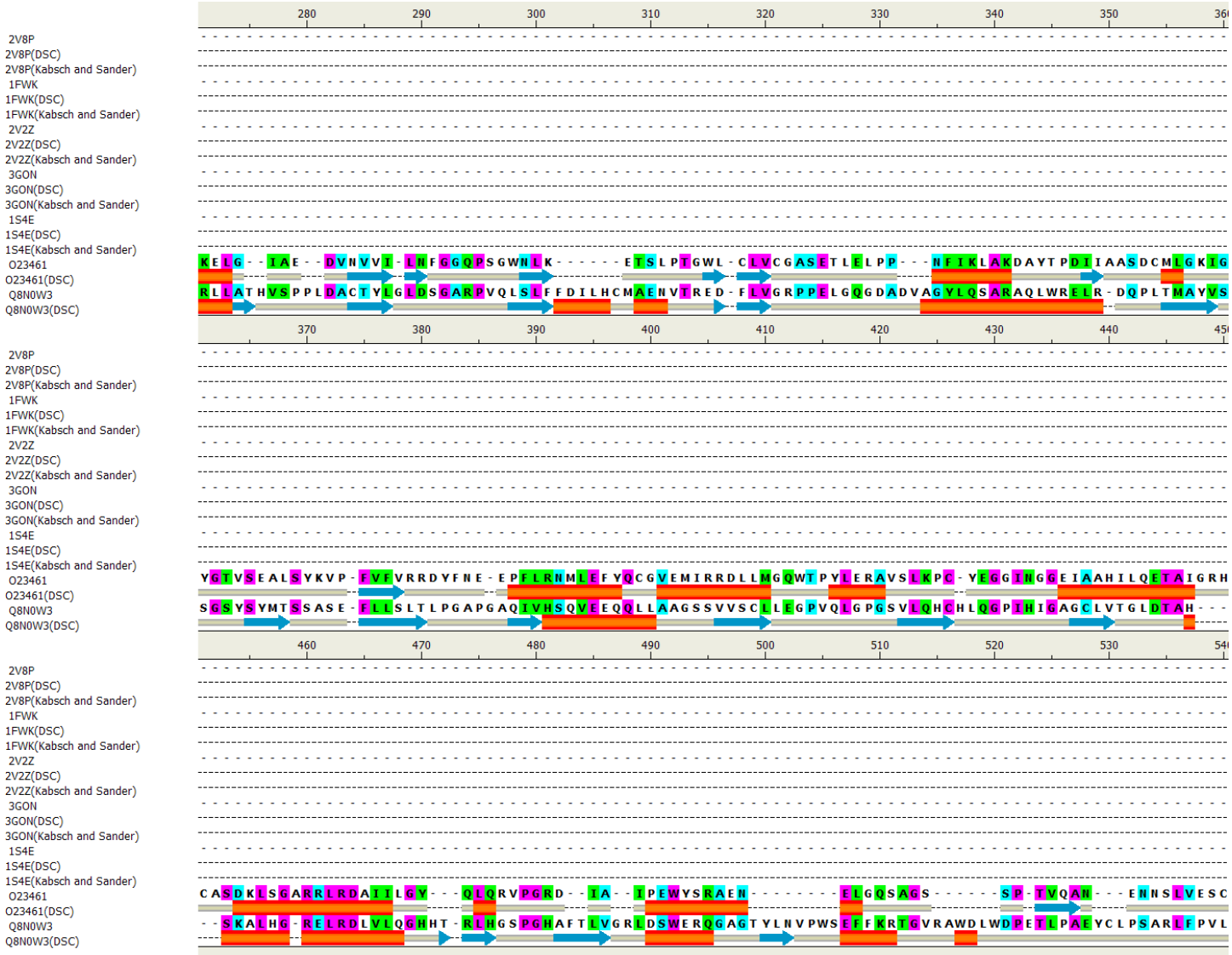
Table AF 5B. Group 6 kinases

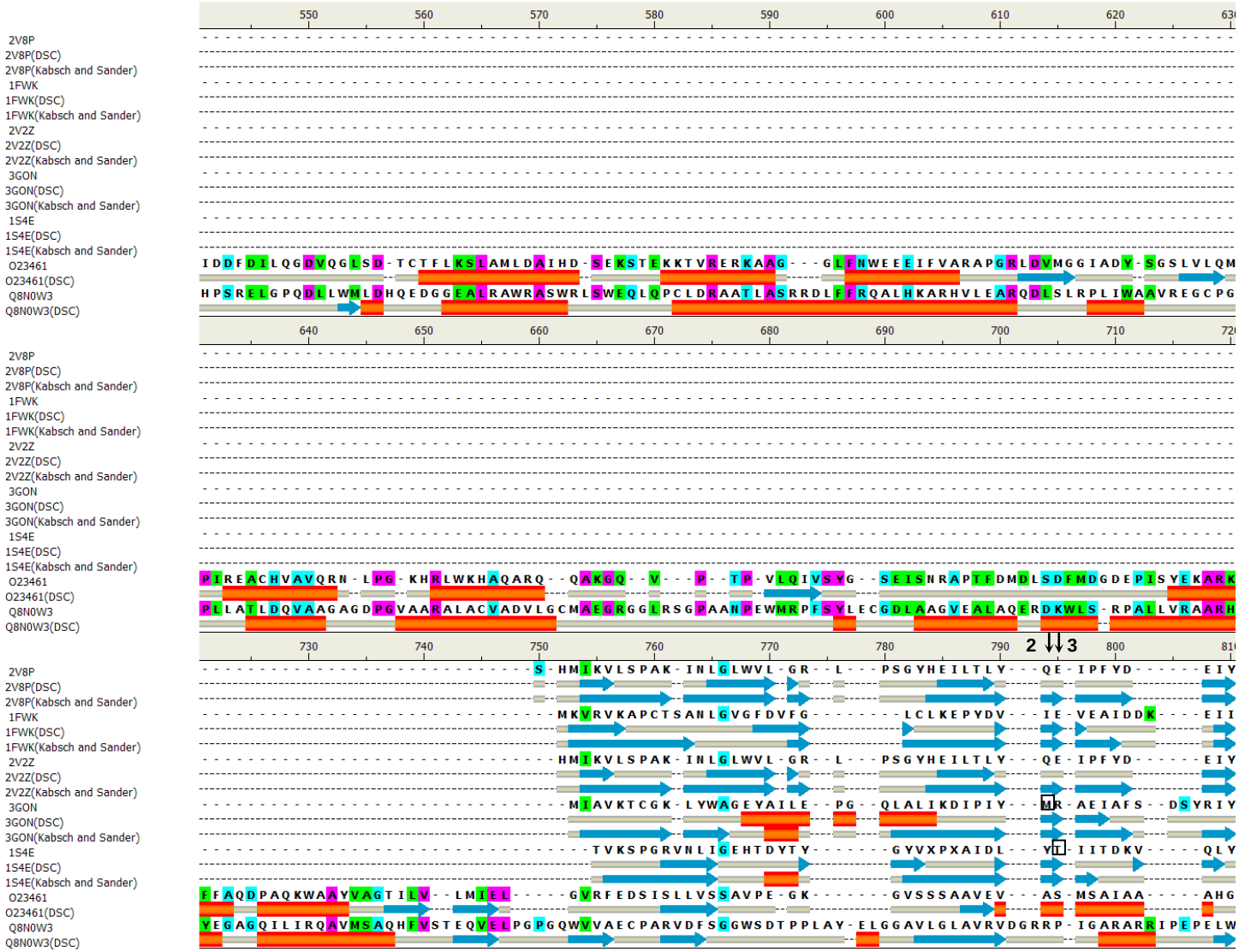
	SUPERFAMILY[1]	FAMILY / DOMAIN[2]
Group 6 Kinases		
1. Mevalonate kinase (pdb|2V8P)	Ribosomal protein S5 s-like (CL0329)	GHMP[4] kinase N terminal domain (PF00288) GHMP kinase C terminal domain (PF08544)
2. Homoserine kinase (pdb|1FWK)	Ribosomal protein S5 s-like (CL0329)	GHMP kinase N terminal domain (PF00288) GHMP kinase C terminal domain (PF08544)
3. L-arabinokinase (No sequence)	Ribosomal protein S5 s-like (CL0329)	GHMP kinase N terminal domain (PF00288) GHMP kinase C terminal domain (PF08544)
4. Fucokinase (uniprot|Q8N0W3)	Ribosomal protein S5 s-like (CL0329)	GHMP kinase N terminal domain (PF00288) GHMP kinase C terminal domain (PF08544)
5. Shikimate kinase (No sequence)	Ribosomal protein S5 s-like (CL0329)	GHMP kinase N terminal domain (PF00288) GHMP kinase C terminal domain (PF08544)

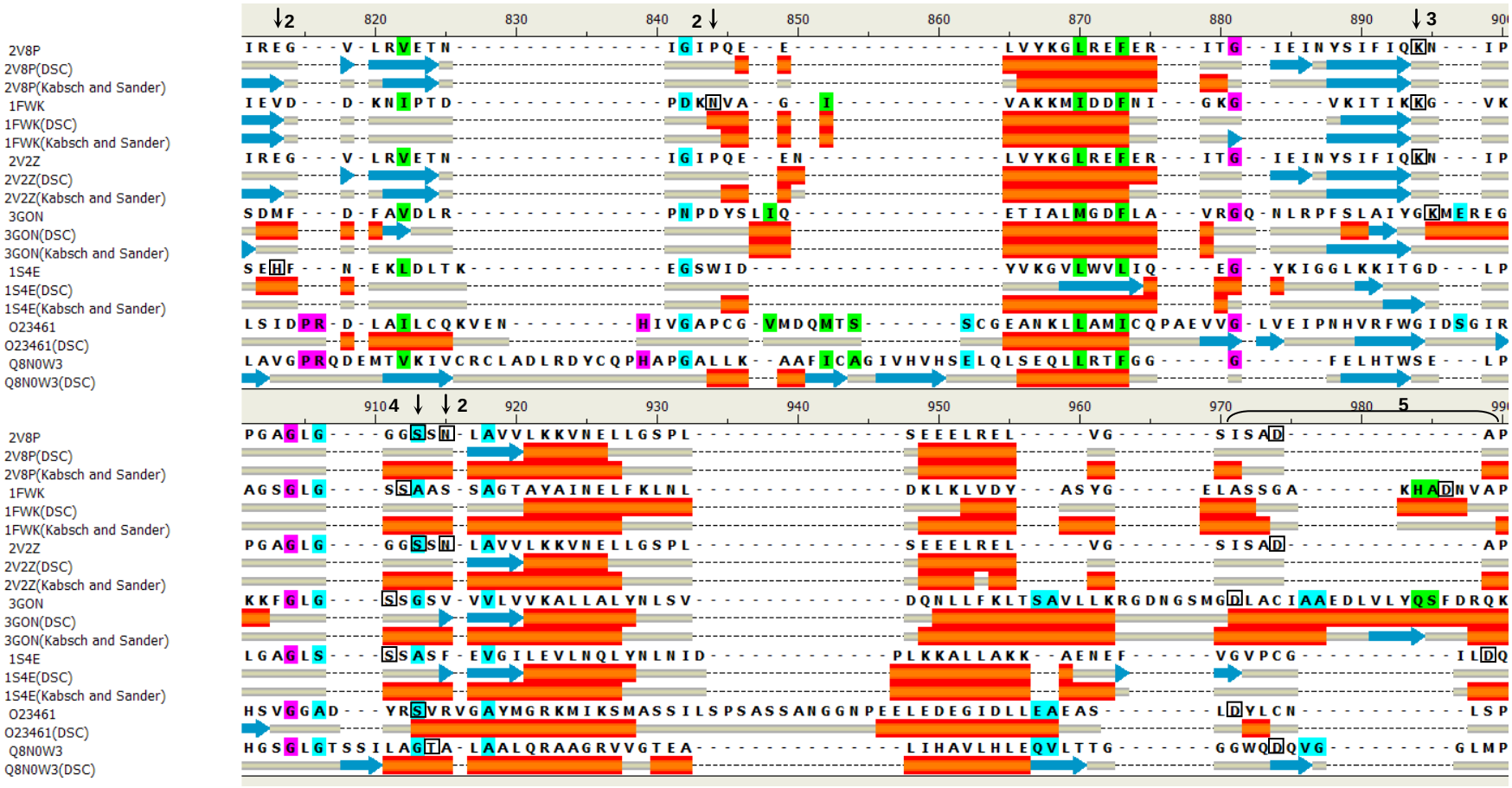
[1] pfam clan classification in brackets
[2] pfam family/domain classification in brackets
[3] No Detectable similarity to conventional kinases
[4] GHMP family includes galactokinases, homoserine kinases and mevalonate kinases

Where there are 2 or domains recognised, these are denoted by ‘a’, ‘b’, etc. One domain has been selected to position the protein within the table.


2V8P
2V8P(DSC)
2V8P(Kabsch and Sander)
1FWK
1FWK(DSC)
1FWK(Kabsch and Sander)
2V2Z
2V2Z(DSC)
2V2Z(Kabsch and Sander)
3GON
3GON(DSC)
3GON(Kabsch and Sander)
1S4E
1S4E(DSC)
1S4E(Kabsch and Sander)
O23461
O23461(DSC)
Q8N0W3
Q8N0W3(DSC)


2V8P
2V8P(DSC)
2V8P(Kabsch and Sander)
1FWK
1FWK(DSC)
1FWK(Kabsch and Sander)
2V2Z
2V2Z(DSC)
2V2Z(Kabsch and Sander)
3GON
3GON(DSC)
3GON(Kabsch and Sander)
1S4E
1S4E(DSC)
1S4E(Kabsch and Sander)
O23461
O23461(DSC)
Q8N0W3
Q8N0W3(DSC)
280 290 300 310 320 330 340 350
370 380 390 400 410 420 430 440
460 470 480 490 500 510 520 530


2V8P
2V8P(DSC)
2V8P(Kabsch and Sander)
1FWK
1FWK(DSC)
1FWK(Kabsch and Sander)
2V2Z
2V2Z(DSC)
2V2Z(Kabsch and Sander)
3GON
3GON(DSC)
3GON(Kabsch and Sander)
1S4E
1S4E(DSC)
1S4E(Kabsch and Sander)
O23461
O23461(DSC)
Q8N0W3
Q8N0W3(DSC)
550 560 570 580 590 600 610 620
640 650 660 670 680 690 700 710
730 740 750 760 770 780 790 800
2 3


2V8P
2V8P(DSC)
2V8P(Kabsch and Sander)
1FWK
1FWK(DSC)
1FWK(Kabsch and Sander)
2V2Z
2V2Z(DSC)
2V2Z(Kabsch and Sander)
3GON
3GON(DSC)
3GON(Kabsch and Sander)
1S4E
1S4E(DSC)
1S4E(Kabsch and Sander)
O23461
O23461(DSC)
Q8N0W3
Q8N0W3(DSC)

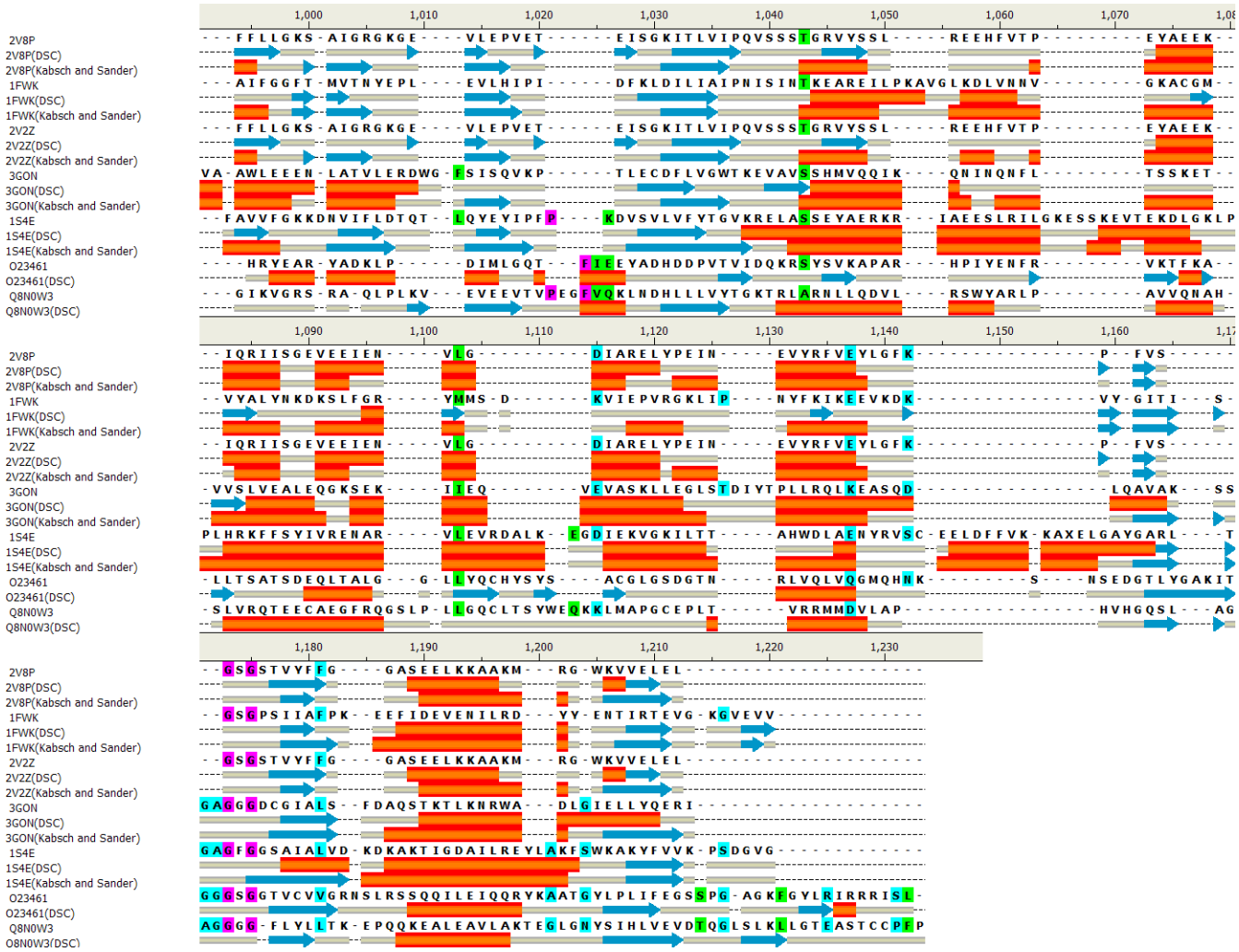


Figure SEQ9. Conserved functional residues within the **Group 6** kinases. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 4A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1SAE, Galactokinase; 2V8P, Mevalonate kinase; 1FWK, Homoserine kinase; 3GON, Phosphomevalonate kinase; O23461, L-arabinokinase; Q8N0W3, Fucokinase.

Table AF 6A. Kinases representing **Group 7** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured inter-atomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**Asn- □C-C=O to Aden-NH_2**[1]	**C8-H to α-PO_4**[2]	**α-PO_4 to β-PO_4**[3]	**His**[4]	**Asp**[5]	**Asp**[6]	**Asp**[7]	**Loop**
Group 7:AIR synthetase-like sequences								
Thiamine phosphate kinase 3C9S (*Aquiflex aeolicus*)	AsnB119 2.549	3.774	3.092	HisA219 4.132	Asp210 3.822	Asp43 3.490	Substrate 3.308	ValB115-SerB121 Gly118 2.299
Selenide, water dikinase 3FD6 (*Homo sapiens*)	MetA124 2.730	3.413	1.700	LysB32 1.700	AspB69 5.519	AspB110 5.443	AspB265 3.104	SerA158-ValA165 GlyA162 2.244
Selenide, water dikinase 2YYE (*Aquiflex aeolicus*)	HisB132 3.196	3.404	2.622	HisA132 2.413	AspA60 2.718	AspA83 2.835	AspA219 2.943	LeuB128-ThrB133 GlyB131 2.408
NAD^+ kinase 1Z0S **FROM GROUP 2 (***Archaeoglobus fulgidus*)	Ala180 2.746	Met127 2.973	Met127 3.487	β-PO_4 4.084				Direct transfer to β-PO_4
Mean	**2.825**	**3.883**	**2.471**	**2.748**	**4.020**	**3.923**	**3.118**	
Standard Deviation	**0.334**	**0.822**	**0.708**	**1.250**	**1.411**	**1.357**	**0.183**	
% Standard Deviation	**11.816**	**21.166**	**28.654**	**45.489**	**35.101**	**34.588**	**5.866**	

1. Coordination of the carbonyl of the backbone carbon of Asn to the hydrogen of the C6-NH_2 of the nucleotide with the Asn carbonyl acting as a general base catalyst.
2. Nucleotide adenyl C8-H coordination to α-PO_4.
3. Interatomic distance between α-PO_4 and β-PO_4 allowing for direct proton transfer.

4.-7. on substrate binding, the deprotonation of the substrate by a Asp residue leads to the inter-aspartate transfer of the proton to a coordinated His/Lys which acts to transfer the H^+ to the α-PO_4, the primary step in the creation of the pentavalent intermediate.

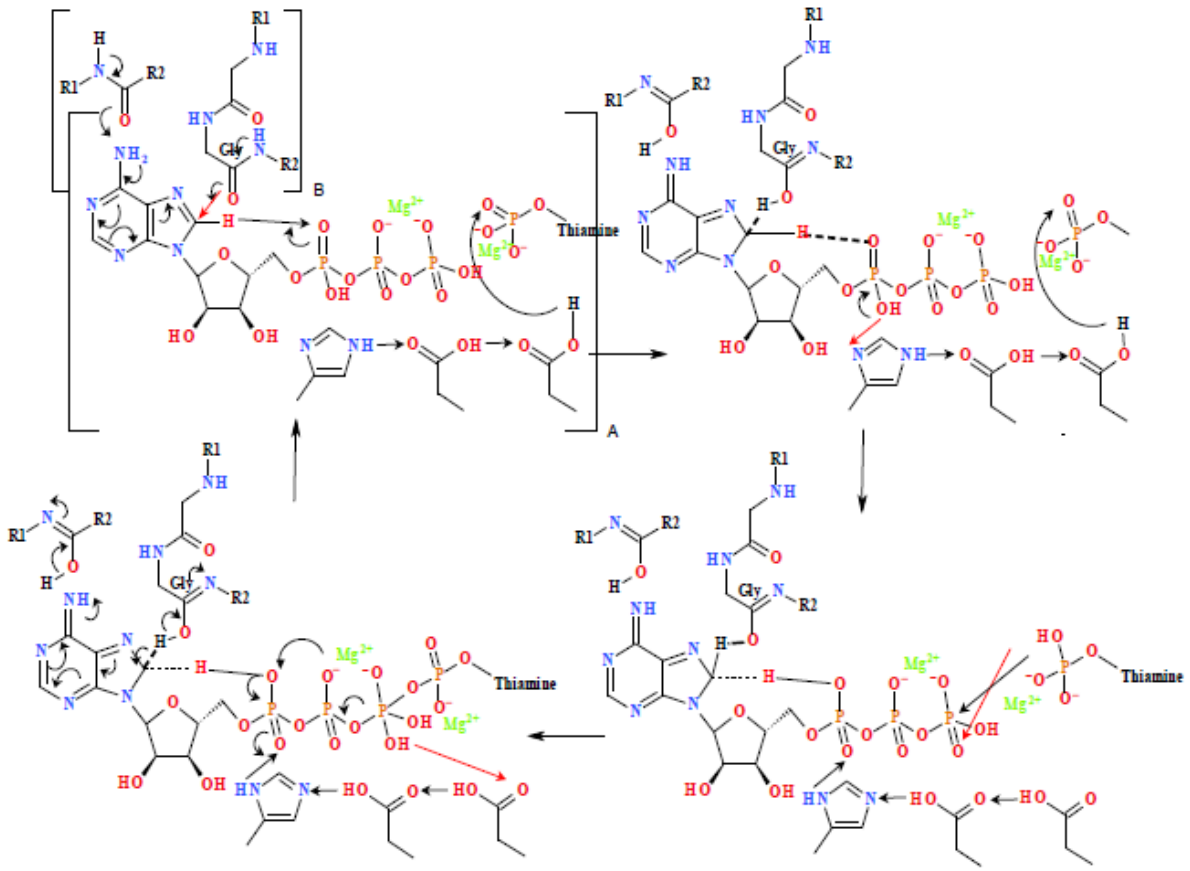


Figure MECH10. Phosphoryl transfer mechanism found in the Group 7 kinases differs from the other kinase groups in that the active site is dependent on the formation of the dimer between two subunits. This allows the reaction to be initiated via the coordination of a carbonyl arising from the protein backbone to the ATP C6-NH_2 on the one subunit (B) as does a conserved backbone carbonyl which acts to stabilize the carbene formed at C8, and the remainder of the coordinating residues arising from the second subunit (A). The reaction mediated by thiamine monophosphate kinase is initiated by the binding of the thiamine monophosphate. Once protonation has occurred from C8-H to the α-PO_4, the substrate is protonated via a cascade involving a conserved His residue and a series of Asp residues. The protonation of the substrate allows the formation of the pentavalent intermediate, the migration of the Mg^{2+} from being the β-PO_4/γ-PO_4 coordinated to being α-PO_4/β-PO_4 coordinated, and the concomitant re-protonation of C8.

Table AF 7A. Kinases representing **Group 8** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured inter-atomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**AA-αC-C=O to Aden-NH_2[1]**	**Asp to C8-H [1]**	**Asp/Arg[2]**	**Lys to C8-H[3]**	**Arg/Tyr to β-PO_4[4]**	**Glu[4]**
Group 8:Riboflavin kinase						
Riboflavin kinase[§#] 1Q9S (*Homo sapiens*)	His91 2.547	Asp96 2.955	Asp96/Arg21 5.201	His88 6.155	Arg21 3.642	Glu86
Riboflavin kinase[§#] 1N06 (*Schizosaccharomyces pombe*)	Leu99 1.723	Asp106 2.867	Asp106/Tyr108 4.642	Tyr108 3.799	Tyr108 1.990	Glu96
Riboflavin kinase[§#] 1N08 (*Schizosaccharomyces pombe*)	Leu99 1.747	Asp106 3.187	Asp106/Tyr108 4.491	Tyr108 3.524	Tyr108 3.732	Glu96
Riboflavin kinase[§#] 1NB0 (*Homo sapiens*)	His91 3.163	Asp96 3.404	Asp96/Arg21 4.123	Lys28 2.716	Arg21 4.324	Glu86
Mean	**2.211**	**3.153**	**6.250**	**3.346**	**4.3957**	
Standard Deviation	**0.825**	**0.270**	**1.870**	**0.562**	**1.276**	
% Standard Deviation	**37.293**	**8.569**	**29.918**	**16.823**	**29.028**	

§ inter-subunit in interaction.

1. Asp carboxyl group coordination to C8-H.
2. Asp to Arg coordination.
3. Arg to β-PO_4 coordination.
4. Lys to C8-H
5. deprotonation of the substrate by Glu.

\# rotation of histidine N-Cα-Cβ-Cγ torsion angle for optimum interatomic distance.

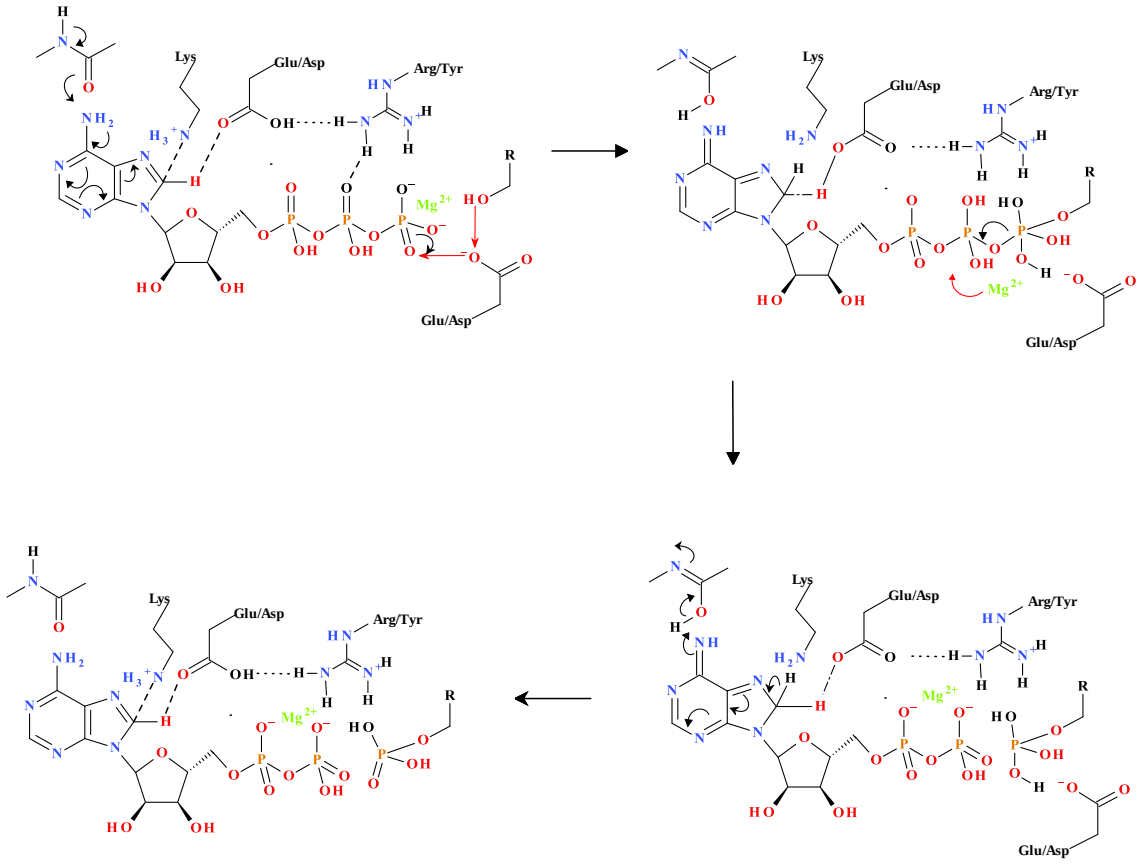


Figure AF 11. The postulated phosphoryl transfer mechanism found in the Group 8 kinases is similar to all other groups utilizing the "push"mechanism (flavokinase family). The mechanism occurs via coordination of the adenyl C6-NH_2 and protonation of C8 via a coordinated Lys changing C8 from sp2 to sp3 hybridization, and alters the protonation of C8-H. The C8-H becomes more acidic, allowing for the protonation of the β-PO_4, via a conserved Asp to Arg proton transfer. The H^+ transfer is directly to the β-PO_4, facilitating the formation of the pentavalent intermediate between the γ-PO_4 and the substrate nucleophile. There is a simultaneous Glu-mediated deprotonation of the substrate-OH that allows for the nucleophilic attack by the substrate, creating the pentavalent intermediate and allowing phosphoryl transfer. The protonated Glu then transfers the proton to the γ-PO_4 changing the coordination of the Mg^{2+} from being β-PO_4 to γ-PO_4 coordinated to being α-PO_4 to β-PO_4 coordinated. The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

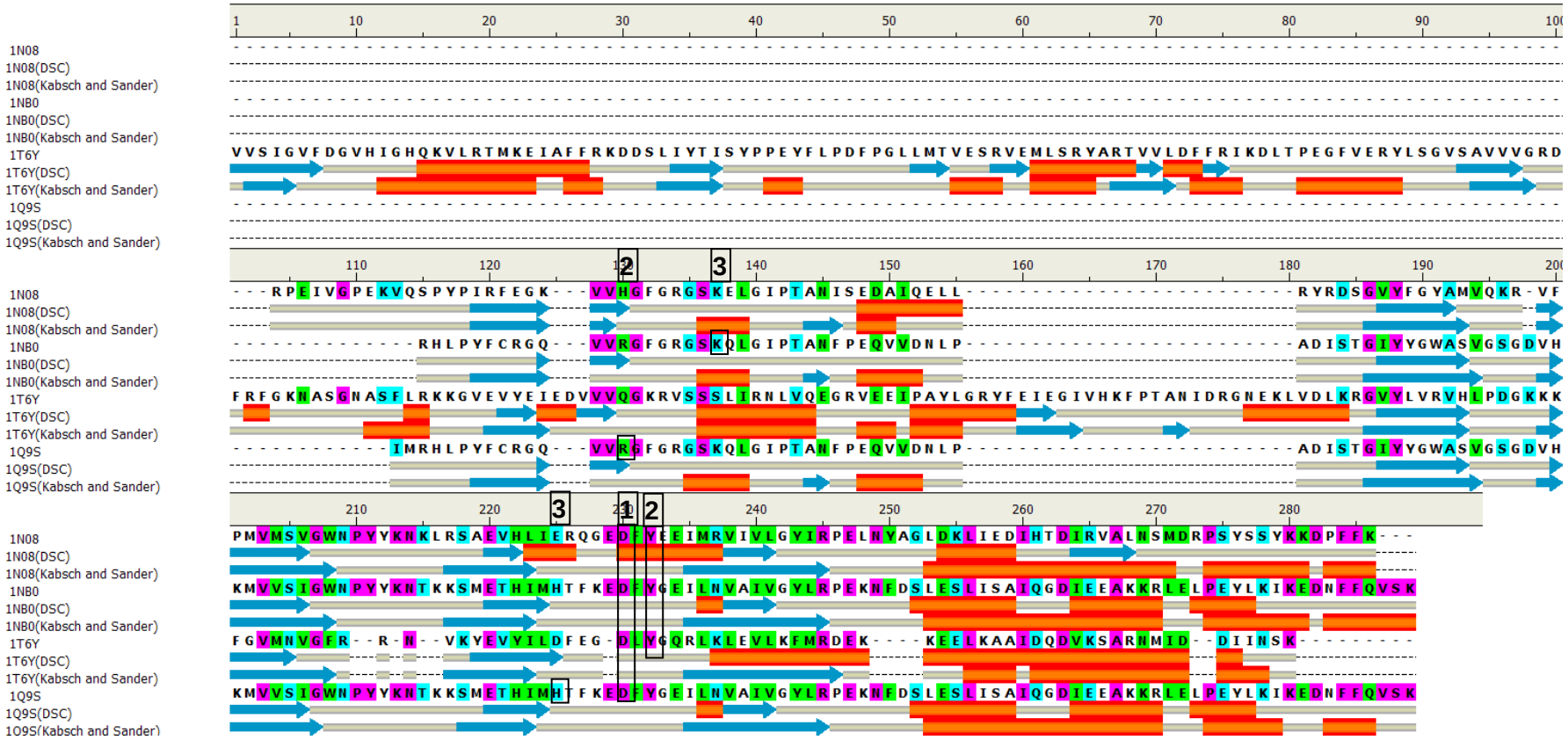


Figure SEQ12. Conserved functional residues within the **Group 8** kinases. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 7A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1N08; *Schizosaccharomyces pombe*, 1NB0; *Homo sapiens*, 1Q9S; *Homo sapiens*.

Table AF 8A. Kinases representing **Group 9 – 10** and the identified conserved amino acid residues associated with the catalysis of phosphoryl transfer and the measured inter-atomic distances are shown. The PDB or uniprot accession numbers are indicated. Conserved residues replaced by conserved functionality are indicated by 3-letter code.

Kinase	**EC**	**Met[1]**	**Thr[2]**	**His[3]**	**Thr[4]**								
GROUP 9: Dihydroxyacetone kinase													
Dihydroxyacetone kinase [§#] 2BTD **(***Escherichia coli*)	2.7.1.29	477	476	**Direct**	480								
GROUP 10: Glycerate kinase													
Glycerate kinase [4] (*Neisseria meningitidis*)	2.7.1.31	No ADP											
Kinase	**AA-αC-C=O to Aden-NH_2**	**N7 to Arg[1]**	**C8-H to Arg[1]**	**Arg[1] Nε to α-PO_4**	**Arg[1] Nε to α-PO_4**	**C8-H to α-PO_4**	**Arg[2]-NH_3 to α -PO_4**	**Arg[2]-NH_3 to β-PO_4**	**1st His[3] to γ-PO_4**	**Glu[4] to His**	**2nd His to γ-PO_4[5]**	**Glu to His[6]**	**Asn-γC-C=O to C8-H[7]**
GROUP 11: Polyphosphate kinase (legend as per Group 3 kinases)													
Polyphosphate kinase 1XDP From Group 9 (*Escherichia coli*)	Asp587 3.287	Arg564 3.213	Arg564 2.614	Arg564 3.003	Arg564 3.003	7.406	Arg405 2.994	Arg405 3.353	His435 2.901	Glu623 1.944	His592 2.901	Asp470 1.667	Asn45 2.906
Polyphosphate kinase 2O8R (*Porphyromonas gingivalis*)		Arg568	Arg568	Arg568	Arg568		Arg407	Arg407	His 437	Glu626	His595	Asp474	Asn46
GROUP 12: Integral membrane kinases													
No structures.													

§ two orientations of the ANP in the same molecule.

1. C8-H coordinated.
2. N7 coordinated.
3. C8-H to β-PO_4 coordinated.
4. No ATP in structure.

\# rotation of adenine around the adenine/ribose bond.

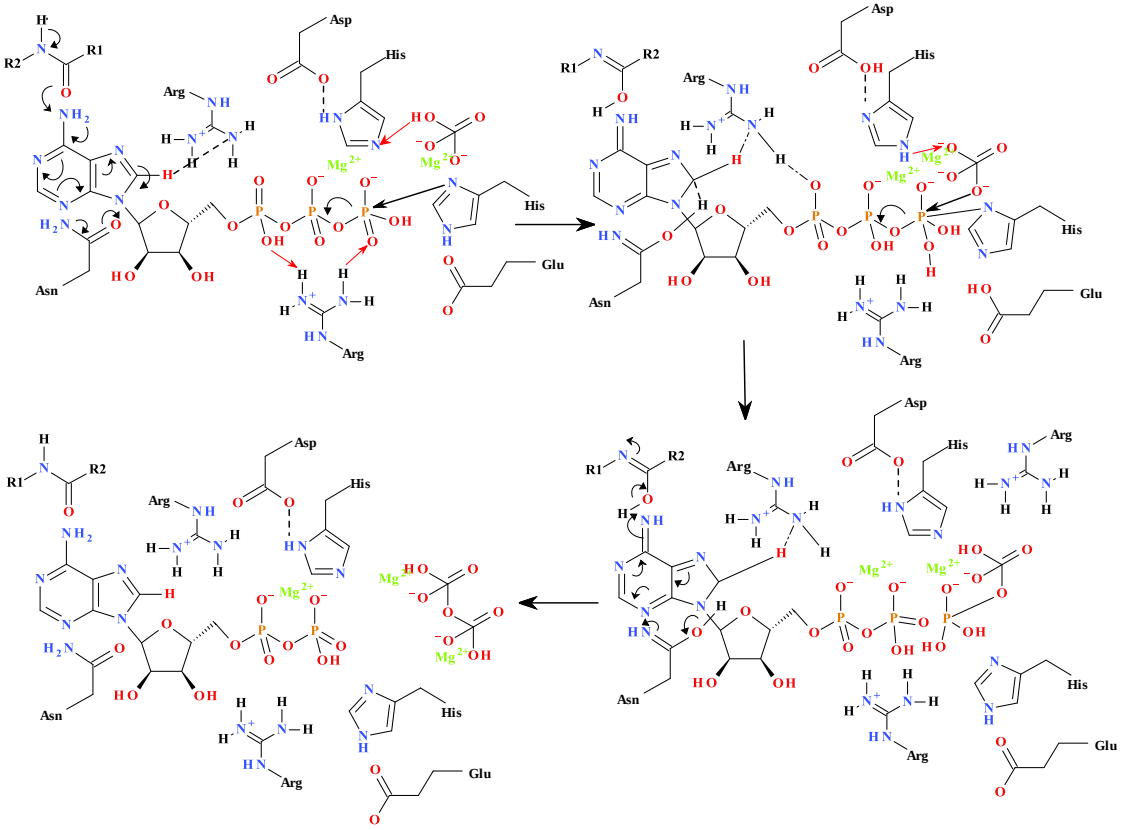


Figure AF 13. The postulated phosphoryl transfer mechanism found in the Group 11 kinases which utilizes the "push"mechanism but also undergoes an autophosphorylation of a histidine residue (polyphosphate family). The mechanism occurs via coordination of the adenyl C6-NH_2 from the protein backbone to the ATP C6-NH_2 and an Asn carbonyl which acts to stabilize the carbene formed at C8. The C8-H becomes more acidic, allowing for the protonation of the α-PO_4, via a conserved Arg proton transfer. A second conserved Arg transfers the proton from the α-PO_4 to the γ-PO_4 facilitating the formation of the pentavalent intermediate and to allow for phosphoryl transfer. There is a simultaneous deprotonation of a conserved His allowing for the nucleophilic attack of the His for the γ-PO_4. There is also a putative deprotonation of the substrate phosphate by a second His allowing for the concomitant formation of the phosphate dimer (polymer). The H^+ originally arising from the C8 is then transferred back to C8, allowing the electron density of the adenyl moiety to return to the "ground-state" distribution.

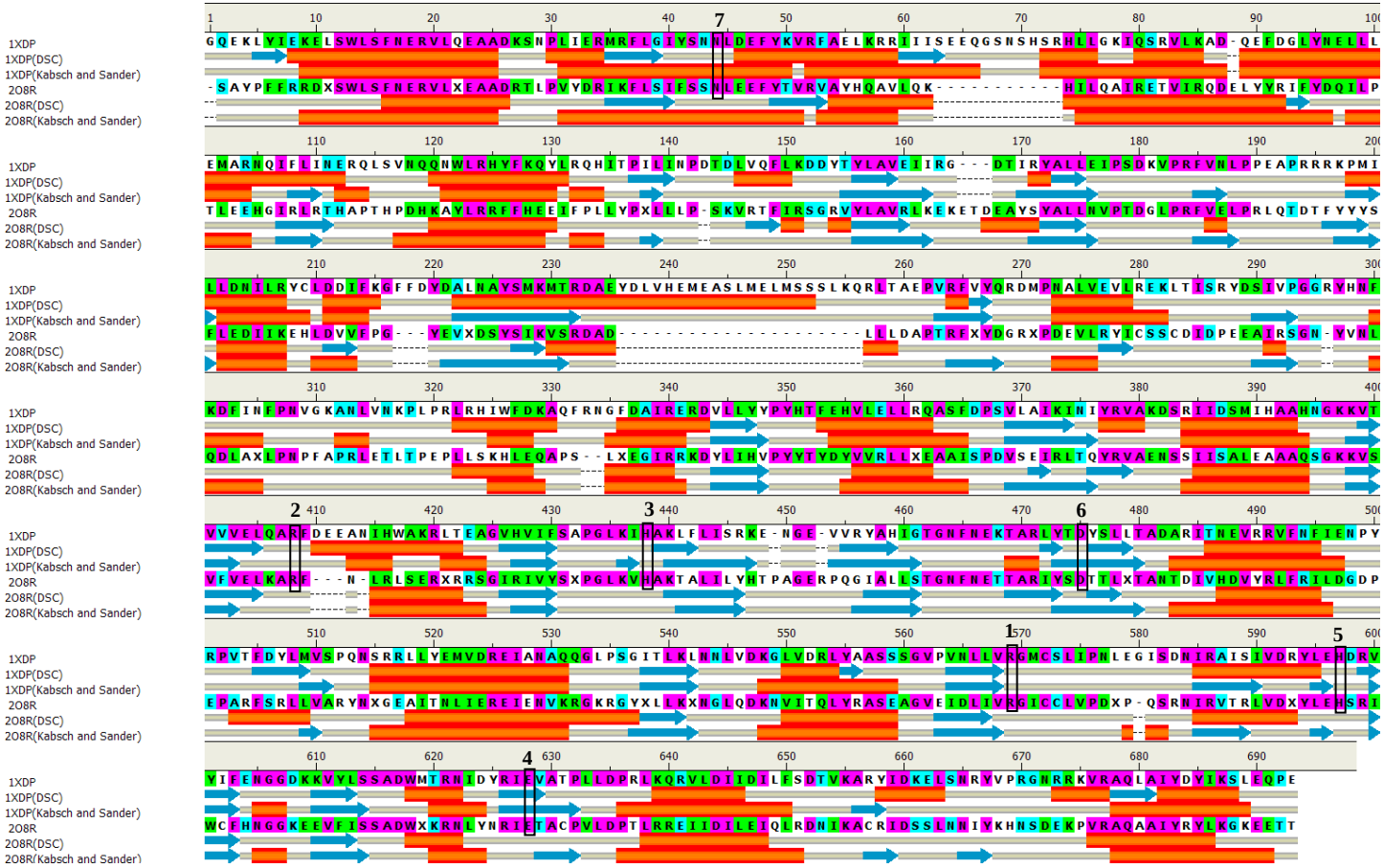


Figure SEQ14. Conserved functional residues within the **Group 11** kinase family. The indicated numbering is as per the identified residues as outlined in Sup Inf Table SI 8A. Pink = 100% identical, Green = >75% identical, turquoise = >50% identical. Secondary structure elements; Orange/Pink tube = Helix, Blue Arrow = Sheet, Grey = Coil. 1XDP; *Escherichia coli*, 2O8R; *Porphyromonas gingivalis*.

References (Additional file 1**)**

References outlined below refer to the crystal structures used for the analysis of all active sites in this investigation. Included are the RCSB protein databank accession codes for each protein used.

1. Hong, B. S., Allali_Hassani, A., Templ, W., Finerty Jr., P. J., Mackenzie, F., Domiv, S., Vedadi, M. and Park, H. W. (2010) Crystal structures of human choline kinase isoforms in complex with hemicholinium-3: single amino acid near the active site influences inhibitor sensitivity. *J. Biol. Chem. 285*, 16330-16340. (3G15.pdb)
2. Debreczeni, J.E., Bullock, A., Knapp, S., Von Delft, F., Sundstrom, M., Arrowsmith, C., Weigelt, J. and Edwards, A. (2010) PIM kinase. Structural Genomics Consortium, Oxford. Journal: To be Published. (2BZK.pdb)
3. Lowe, E.D., Noble, M.E., Skamnaki, V.T., Oikonomakos, N.G., Owen, D.J. and Johnson, L.N. (1997) The crystal structure of a phosphorylase kinase peptide substrate complex: kinase substrate recognition. *EMBO J. 16*, 6646-6658. (2PHK.pdb)
4. Thorsell, A.G., Uppenberg, J., Hogbom, M., Ogg, D., Arrowsmith, C., Berglund, H., Collins, R., Edwards, A., Ehn, M., Flodin, S., Flores, A., Graslund, S., Hammarstrom, M., Kotenyova, T., Nilsson-Ehle, P., Nordlund, P., Nyman, T., Sagemark, J., Stenmark, P., Sundstrom, M., Van Den Berg, S., Weigelt, J., Holmberg-Schiavone, L., Persson, C., Hallberg, B.M. (2010) Structure of Human Phosphatidylinositol-4-phosphate 5-kinase, type II, gamma. Journal**:** To be Published. (2GK9.pdb)
5. Fong, D.H. and Berghuis, A.M. (2002) Substrate promiscuity of an aminoglycoside antibiotic resistance enzyme via target mimicry. *EMBO J. 21,* 2323-2331. (1L8T.pdb)
6. Ku, S.Y., Smith, G.D. and Howell, P.L. (2007) ADP-2Ho as a phasing tool for nucleotide-containing proteins. *Acta Crystallogr.,Sect.D. 63*, 493-499. (2OLC.pdb)
7. Lougheed, J.C., Chen, R.H. Mak, P. and Stout, T.J. (2004) Crystal structures of the phosphorylated and unphosphorylated kinase domains of the Cdc42-associated tyrosine kinase ACK1. *J. Biol. Chem. 279***,** 44039-44045. (1U54.pdb)
8. Qiu, W., Hutchinson, A., Wernimont, A., Walker, J.R., Sullivan, H., Lin, Y.-H., Mackenzie, F., Kozieradzki, I., Cossar, D., Schapira, M., Senisterra, G., Vedadi, M., Arrowsmith, C.H., Bountra, C., Weigelt, J., Edwards, A.M., Bochkarev, A., Hui, R. and Amani, M. (2010) Crystal structure of full length CpCDPK3 (cgd5_820) in complex with Ca2+ and AMPPN. Journal: To be Published. (3LIJ.pdb)
9. Singh, P. and Tesmer, J.J.G. (2008) Crystal Structure of G protein coupled receptor kinase 1 bound to ATP and magnesium chloride at 2.7A. Journal: To be Published. (3C4W.pdb)
10. Lodowski, D.T., Barnhill, J.F., Pyskadlo, R.M., Ghirlando, R., Sterne-Marr, R. and Tesmer, J.J.G. (2005) The role of G beta gamma and domain interfaces in the activation of G protein-coupled receptor kinase 2. *Biochemistry 44*, 6958-6970. (1YM7.pdb)
11. Walker, E.H., Pacold, M.E., Perisic, O., Stephens, L., Hawkins, P.T., Whymann, M.P. and Williams, R.L. (2000) Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol.Cell 6,* 909-919. (1E8X.pdb)

12. Liu, L., Song, X., He, D., Komma, C., Kita, A., Virbasius, J.V., Huang, G., Bellamy, H.D., Miki, K., Czech, M.P. and Zhou, G.W. (2006) Crystal structure of the C2 domain of class II phosphatidylinositide 3-kinase C2alpha. *J. Biol. Chem. 281***,** 4254-4260. (2B3R.pdb)
13. Rao, V.D., Misra, S., Boronenkov, I.V., Anderson, R.A. and Hurley, J.H. (1998) Structure of type IIbeta phosphatidylinositol phosphate kinase: a protein kinase fold flattened for interfacial phosphorylation. *Cell 94*, 829-839. (1BO1.pdb)
14. Gonzalez, B., Schell, M.J., Letcher, A.J., Veprintsev, D.B., Irvine, R.F. and Williams, R.L. (2004) Structure of a human inositol 1,4,5-trisphosphate 3-kinase: substrate binding reveals why it is not a phosphoinositide 3-kinase. *Mol.Cell. 15***,** 689-701. (1W2C.pdb)
15. Holmes, W., and Jogl, G. (2006) Crystal structure of inositol phosphate multikinase 2 and implications for substrate specificity. *J. Biol. Chem. 281*, 38109-3811. (2IF8.pdb)
16. Structure of Human Phosphatidylinositol-4-phosphate 5-kinase, type II, gamma
17. Thorsell, A.G., Uppenberg, J., Hogbom, M., Ogg, D., Arrowsmith, C., Berglund, H., Collins, R., Edwards, A., Ehn, M., Flodin, S., Flores, A., Graslund, S., Hammarstrom, M., Kotenyova, T., Nilsson-Ehle, P., Nordlund, P., Nyman, T., Sagemark, J., Stenmark, P., Sundstrom, M., Van Den Berg, S., Weigelt, J., Holmberg-Schiavone, L., Persson, C. and Hallberg, B.M. (2006) Structural Genomics Consortium. Journal: To be Published. (2GK9.pdb)
18. Chamberlain, P.P., Qian, X., Stiles, A.R., Cho, J., Jones, D.H., Lesley, S.A., Grabau, E.A., Shears, S.B. and Spraggon, G. (2007) Integration of inositol phosphate signaling pathways via human ITPK1. *J. Biol. Chem. 282*, 28117-28125. (2QB5.pdb)
19. Herzberg, O., Chen, C.C., Kapadia, G., McGuire, M., Carroll, L.J., Noh, S.J. and Dunaway-Mariano, D. (1996) Swiveling-domain mechanism for enzymatic phosphotransfer between remote reaction sites. *Proc.Natl.Acad.Sci.USA 93*, 2652-2657. (1DIK.pdb)
20. Andreassi, J.L., Vetting, M.W., Bilder, P.W., Roderick, S.L. and Leyh, T.S. (2009) Structure of the ternary complex of phosphomevalonate kinase: the enzyme and its family. *Biochemistry 48***,** 6461-6468. (3GON.pdb)
21. Kraft, L., Sprenger, G.A. and Lindqvist, Y. Conformational changes during the catalytic cycle of gluconate kinase as revealed by X-ray crystallography. (2002) *J. Mol. Biol. 318***,** 1057-1069. (1KO5.pdb)
22. Harrison, D.H., Runquist, J.A., Holub, A. and Miziorko, H.M. (1998) The crystal structure of phosphoribulokinase from Rhodobacter sphaeroides reveals a fold similar to that of adenylate kinase. *Biochemistry 37*, 5074-5085. (1A7J.pdb)
23. Segura-Pena, D., Lutz, S., Monnerjahn, C., Konrad, M., and Lavie, A. (2007) Binding of ATP to TK1-like enzymes is associated with a conformational change in the quaternary structure. *J. Mol. Biol. 369***,** 129-141. (2ORW.pdb)
24. Chetnani, B., Das, S., Kumar, P., Surolia, A. and Vijayan, M. (2009) Mycobacterium tuberculosis pantothenate kinase: possible changes in location of ligands during enzyme action. *Acta Crystallogr.,Sect.D 65*, 312-325. (2ZS9.pdb)
25. Kosinska, U., Stenmark, P., Arrowsmith, C., Berglund, H., Busam, R., Collins, R., Edwards, A., Ericsson, U.B., Flodin, S., Flores, A., Graslund, S., Hammarstrom, M., Hallberg, B.M., Holmberg Schiavone, L., Hogbom, M., Johansson, I., Karlberg, T., Kotenyova, T., Moche, M., Nilsson, M.E.P., Nyman, T., Ogg, D., Persson, C., Sagemark, J., Sundstrom, M., Uppenberg, J., Uppsten, M., Thorsell, A.G., Van Den Berg, S., Weigelt, J., Welin, M and Nordlund, P. (2007) Structure of Human Uridine-Cytidine Kinase 1. Journal**:** To be Published. (2UVQ.pdb)

26. Gu, Y., Reshetnikova, L., Li, Y., Wu, Y., Yan, H., Singh, S., Ji, X. (2002) Crystal structure of shikimate kinase from Mycobacterium tuberculosis reveals the dynamic role of the LID domain in catalysis. *J. Mol. Biol. 319*, 779-789. (1L4U.pdb)
27. Kim, S.G., Cavalier, M., El-Maghrabi, M.R., Lee, Y.H. (2007) A direct substrate-substrate interaction found in the kinase domain of the bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. *J. Mol. Biol. 370*, 14-26. (2I1V.pdb)
28. Welin, M., Wang, L., Eriksson, S., Eklund, H. (2007) Structure-function analysis of a bacterial deoxyadenosine kinase reveals the basis for substrate specificity. *J. Mol. Biol. 366*, 1615-1623. (2JAS.pdb)
29. Egeblad-Welin, L., Sonntag, Y., Eklund, H. and Munch-Petersen, B. (2007) Functional studies of active-site mutants from Drosophila melanogaster deoxyribonucleoside kinase. Investigations of the putative catalytic glutamate-arginine pair and of residues responsible for substrate specificity. FEBS J. **274:** 1542-1551. (2JCS.pdb)
30. Zhu, Y., Huang, W., Lee, S.S., Xu, W. (2005) Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis. *Embo Rep. 6,* 681-687. (1XDP.pdb)
31. Bunkoczi, G., Filippakopoulos, P., Jansson, A., Longman, E., Von Delft, F., Edwards, A., Arrowsmith, C., Sundstrom, M., Weigelt, J. and Knapp, S. (2009) Structure of Adenylate Kinase 1 in Complex with P1, P4-Di(Adenosine)Tetraphosphate. Structural Genomics Consortium. Journal: To be Published. (2C95.pdb)
32. Schlichting, I. And Reinstein, J. (1999 pH influences fluoride coordination number of the AlFx phosphoryl transfer transition state analog. *Nat. Struct. Biol. 6*, 721-723. (1QF9.pdb)
33. Pattanayek, R., Williams, D.R., Pattanayek, S., Xu, Y., Mori, T., Johnson, C.H., Stewart, P.L. and Egli, M. (2006) Analysis of KaiA-KaiC protein interactions in the cyano-bacterial circadian clock using hybrid structural methods. *Embo J. 25*, 2017-2028. (2GBL.pdb)
34. Tari, L.W., Matte, A., Goldie, H. and Delbaere, L.T. (1997) Mg(2+)-Mn2+ clusters in enzyme-catalyzed phosphoryl-transfer reactions. *Nat. Struct. Biol. 4*, 990-994. (1AQ2.pdb)
35. Petit, P., Lagarde, A., Boutin, J.A., Ferry, G. and Vuillard, L. (2009) Effect of modulators on the structure and function of human glucokinase. Journal: To be Published. (3FGU.pdb)
36. Ose, T., Kitamura, M., Okuyama, M., Mori, H., Kimura, A., Watanabe, N., Yao, M. and Tanaka, I. (2009) Crystal Structure of Alpha-Xylosidase from Escherichia coli. Journal**:** To be Published. (1WE5.pdb)
37. Kotaka, M., Ren, J., Lockyer, M., Hawkins, A.R. and Stammers, D.K. (2006) Structures of R- and T-state Escherichia coli aspartokinase III. Mechanisms of the allosteric transition and inhibition by lysine. *J. Biol. Chem. 281*, 31544-3152. (2J0W.pdb)
38. Gil-Ortiz, F., Ramon-Maiques, S., Fita, I. and Rubio, V. (2003) The course of phosphorus in the reaction of N-acetyl-L-glutamate kinase, determined from the structures of crystalline complexes, including a complex with an AlF(4)(-) transition state mimic. J. Mol. Biol. **331:** 231-244. (1OH9.pdb)
39. Marco-Marin, C., Gil-Ortiz, F. and Rubio, V. (2005) The crystal structure of Pyrococcus furiosus UMP kinase provides insight into catalysis and regulation in microbial pyrimidine nucleotide biosynthesis. *J. Mol. Biol. 352*, 438-354. (2BRI.pdb)
40. Evans, P.R., Farrants, G.W. and Hudson, P.J. (1981) Phosphofructokinase: structure and control. *Philos. Trans. R. Soc. London, Ser.B 293*, 53-62. (4PFK.pdb)
41. McNae, I.W., Martinez-Oyanedel, J., Keillor, J.W., Michels, P.A., Fothergill-Gilmore, L.A. and Walkinshaw, M.D. (2009) The crystal structure of ATP-bound

phosphofructokinase from Trypanosoma brucei reveals conformational transitions different from those of other phosphofructokinases. *J. Mol. Biol. 385*, 1519-1533. (3F5M.pdb)
42. Liu, J., Lou, Y., Yokota, H., Adams, P.D., Kim, R. and Kim, S.H. (2005) Crystal structures of an NAD kinase from Archaeoglobus fulgidus in complex with ATP, NAD, or NADP. *J. Mol. Biol. 354*, 289-303. (1Z0S.pdb)
43. Crystal structure of Possible 1-phosphofructokinase (EC 2.7.1.56) (tm0828) from THERMOTOGA MARITIMA at 2.46 A resolution. Joint Center for Structural Genomics (JCSG). Journal: To be published. (2AJR.pdb)
44. Seetharaman, J., Lew, S., Wang, D., Neely, H., Janjua, K., Cunningham, K., Owens, L., Xiao, R., Liu, J., Baran, M.C., Acton, T.B., Rost, B., Montelione, G.T., Hunt, J.F. and Tong, L. (2009) Crystal structure of Pyrophosphate-dependent phosphofructokinase from Marinobacter aquaeolei, Northeast Structural Genomics Consortium Target MqR88. Journal: To be Published. (3K2Q.pdb)
45. Miller, D.J., Jerga, A., Rock, C.O. and White, S.W. (2008) Analysis of the Staphylococcus aureus DgkB structure reveals a common catalytic mechanism for the soluble diacylglycerol kinases. *Structure 16*, 1036-1046. (2QV7.pdb)
46. Morgan, H. P., McNae, I. W., Nowicki, M. W., Hannaert, V., Michels, P. A. M., Fothergill-Gilmore, L. A., and Walkinshaw, M. D. (2010) Allosteric mechanism of pyruvate kinase from *Leishmania mexicana* uses a rock and lock model. *J.Biol.Chem. 285*, 12892-12898. (3HQQ.pdb)
47. Nocek, B., Cuff, M., Volkart, L., and Joachimiak, A crystal structure of fructokinase from *Bacillus subtilis* complexed with ADP. To be Published. (3EPQ.pdb)
48. Sigrell, J. A., Cameron, A. D., Jones, T. A., and Mowbray, S. L. (1998) Structure of *Escherichia coli* ribokinase in complex with ribose and dinucleotide determined to 1.8 Å resolution: insights into a new family of kinase structures. *Structure 6*, 183-193. (1RKD.pdb)
49. Reddy, M. C. M., Palaninathan, S. K., Shetty, N. D., Owen, J. L., Watson, M. D., and Sacchettini, J. C. (2007) High resolution crystal structures of *Mycobacterium tuberculosis* adenosine kinase: insights into the mechanism and specificity of this novel prokaryotic enzyme. *J.Biol.Chem. 282*, 27334-27342. (2PKN.pdb)
50. Bagaria, A., Kumaran, D., Burley, S. K., and Swaminathan, S. Crystal structure of pyridoxal kinase from *Lactobacillus plantarum* in complex with ATP. Journal**:** To be Published. (3IBQ.pdb)
51. Satyanarayana, L., Burley, S. K., and Swaminathan, S. Crystal Structure of a putative 2-Keto-3-deoxygluconate kinase from *Enterococcus faecalis*. Journal**:** To be Published. (3KTN.pdb)
52. Jeyakanthan, J., Kuramitsu, S., and Yokoyama, S. Structure of hydroxyethylthiazole kinase protein from *Pyrococcus Horikoshii* Ot3. Journal**:** To be Published. (3HPD.pdb)
53. Joint Center for Structural Genomics (JCSG). Crystal structure of possible 1-phosphofructokinase (EC 2.7.1.56) (tm0828) from *Thermotoga maritina* at 2.46 Å resolution. Journal**:** To be Published. (2AJR.pdb)
54. Miallau, L., Hunter, W. N., Mcsweeney, S. M., and Leonard, G. A. (2007) Structures of *Staphylococcus aureus* D-tagatose-6-phosphate kinase implicate domain motions in specificity and mechanism. *J.Biol.Chem. 282*, 19948. (2JG1.pdb, 2JGV.pdb)
55. Kochinyan, S., Brown, G., Skarina, T., Singer, A. U., Wong, A. H. Y., Cuff, M. E., Guixe, V., Merino, F., Savchenko, A., Yakunin, A. F., and Jia, Z. Crystal structure of an ADP-dependent 6-phosphofructokinase from *Pyrococcus horikoshii* OT3. Journal**:** To be Published. (3DRW.pdb)

56. Ito, S., Fushinobu, S., Yoshioka, I., Koga, S., Matsuzawa, H., and Wakagi, T. (2001) Structural basis for the ADP-specificity of a novel glucokinase from a hyperthermophilic archaeon. *Structure 9*, 205-214. (1GC5.pdb)
57. Bagautdinov, B., Kuramitsu, S., Yokoyama, S., Miyano, M., and Tahirov, T. H. Crystal Structure analysis of phosphomethylpyrimidine Kinase (*ThiD*) from *Thermus Thermophilus* Hb8. To be Published. (1UB0.pdb)
58. Cheng, G., Bennett, E. M., Begley, T. P., and Ealick, S. E. (2002) Crystal structure of 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate kinase from *Salmonella typhimurium* at 2.3 Å resolution. *Structure 10*, 225-235. (1JXH.pdb)
59. Liu, J. Y., Timm, D. E., Hurley, T. D. (2006) Pyrithiamine as a substrate for thiamine pyrophosphokinase. *J.Biol.Chem. 281***,** 6601-6607. (2F17.pdb)
60. Rajashankar, K. R., Kniewel, R., Solorzano, V., and Lima, C.D. Glycerate kinase from *Neisseria meningitidis* (serogroup A). Journal**:** To be Published. (1TO6.pdb)
61. Schwarzenbacher, R., McMullan, D., Krishna, S. S., Xu, Q., Miller, M. D., Canaves, J. M., Elsliger, M. A., Floyd, R., Grzechnik, S. K., Jaroszewski, L., Klock, H. E., Koesema, E., Kovarik, J. S., Kreusch, A., Kuhn, P., McPhillips, T. M., Morse, A. T., Quijano, K., Spraggon, G., Stevens, R. C., van den Bedem, H., Wolf, G., Hodgson, K. O., Wooley, J., Deacon, A. M., Godzik, A., Lesley, S. A., and Wilson, I. A. (2006) Crystal structure of a glycerate kinase (TM1585) from *Thermotoga maritima* at 2.70 Å resolution reveals a new fold. *Proteins 65*, 243-248. (2B8N.pdb)
62. Osipiuk, J., Zhou, M., Holzle, D., Anderson, W., and Joachimiak, A. X-ray crystal structure of putative glycerate kinase 2 from *Salmonella typhimurium* LT2. Journal**:** To be Published. (3CWC.pdb)
63. Aleshin, A. E., Kirby, C., Liu, X., Bourenkov, G. P., Bartunik, H. D., Fromm, H. J., and Honzatko, R. B. (2000) Crystal structures of mutant monomeric hexokinase I reveal multiple ADP binding sites and conformational changes relevant to allosteric regulation, *J.Mol.Biol. 296***,** 1001-1015. (1DGK.pdb)
64. Petit, P., Lagarde, A., Boutin, J. A., Ferry, G., and Vuillard, L. Effect of modulators on the structure and function of human glucokinase. Journal**:** To be Published. (3FGU.pdb)
65. Satyanarayana, L., Burley, S. K., and Swaminathan, S. Crystal structure of fructokinase with bound ATP from *Xylella fastidiosa*. Journal**:** To be Published. (3KLI.pdb)
66. Grueninger, D., and Schulz, G. E. (2006) Structure and reaction mechanism of L-rhamnulose kinase from *Escherichia coli*. *J.Mol.Biol. 359***,** 787. (2CGJ.pdb)
67. Zhang, Z., Burley, S. K., and Swaminathan, S. The crystal structure of xylulose kinase from *Lactobacillus acidophilus*. Journal**:** To be Published. (3LL3.pdb)
68. Agarwal, R., Burley, S. K., and Swaminathan, S. Crystal structure of ribulokinase from *Bacillus halodurans*. Journal**:** To be Published. (3JVP.pdb)
69. Zhang, Z., Burley, S. K., and Swaminathan, S. Crystal structure of xylulokinase from *Chromobacterium violaceum*. Journal**:** To be Published. (3HZ6.pdb)
70. Bystrom, C. E., Pettigrew, D. W., Branchaud, B. P., O`Brien, P., and Remington, S. J. (1999) Crystal structures of *Escherichia coli* glycerol kinase variant S58-->W in complex with nonhydrolyzable ATP analogues reveal a putative active conformation of the enzyme as a result of domain motion. *Biochemistry 38*, 3508-3518. (1BWF.pdb)
71. Yang, K., Strauss, E., Huerta, C., and Zhang, H. (2008) Structural basis for substrate binding and the catalytic mechanism of type III pantothenate kinase. *Biochemistry 47*, 1369-1380. (3BF1.pdb)
72. Weihofen, W. A., Berger, M., Chen, H., Saenger, W., and Hinderlich, S. (2006) Structures of human *N*-acetylglucosamine kinase in two complexes with *N*-acetylglucosamine and with ADP/glucose: insights into substrate specificity and regulation. *J.Mol.Biol. 364*, 388. (2CH6.pdb)

73. Tong, Y., Tempel, W., Nedyalkova, L., Mackenzie, F., and Park, H. W. (2009) Crystal structure of the *N*-acetylmannosamine kinase domain of GNE. *Plos One 4*, e7165-e7165. (3EO3.pdb)
74. Patskovsky, Y., and Almo, S. C. Crystal Structure of *Escherichia coli* putative *N*-acetylmannosamine Kinase. Journal**:** To be Published. (2AA4.pdb)
75. Mukai, T., Kawai, S., Mori, S., Mikami, B., and Murata, K. (2004) Crystal structure of bacterial inorganic polyphosphate/ATP-glucomannokinase. Insights into kinase evolution. J.*Biol.Chem. 279*, 50591-50600. (1WOQ.pdb)
76. Gorrell, A., Lawrence, S. H., and Ferry, J. G. (2005) Structural and kinetic analyses of arginine residues in the active site of the acetate kinase from *Methanosarcina thermophila*. *J.Biol.Chem. 280***,** 10731-10742. (1TUY.pdb)
77. Diao, J., and Hasson, M.S. (2009) Crystal structure of butyrate kinase 2 from *Thermotoga maritima*, a member of the ASKHA superfamily of phosphotransferases. *J.Bacteriol. 191***,** 2521-2529. (1SAZ.pdb)
78. Hartley, A., Glynn, S. E., Barynin, V., Baker, P. J., Sedelnikova, S. E., Verhees, C., de Geus, D., van der Oost, J., Timson, D. J., Reece, R. J., and Rice, D. W. (2004) Substrate specificity and mechanism from the structure of *Pyrococcus furiosus* galactokinase. *J.Mol.Biol. 337***,** 387-398. (1S4E.pdb)
79. Sgraja, T., Alphey, M. S., Ghilagaber, S., Marquez, R., Robertson, M. N., Hemmings, J. L., Lauw, S., Rohdich, F., Bacher, A., Eisenreich, W., Illarionova, V., and Hunter, W. N. (2008) Characterization of *Aquifex aeolicus* 4-diphosphocytidyl-2C-methyl-d-erythritol kinase - ligand recognition in a template for antimicrobial drug discovery. *FEBS J. 275*, 2779. (2V8P.pdb, 2V2Z.pdb)
80. Zhou, T., Daugherty, M., Grishin, N. V., Osterman, A. L., and Zhang, H. (2000) Structure and mechanism of homoserine kinase: prototype for the GHMP kinase superfamily. *Structure Fold. Des. 8*, 1247-1257. (1FWK.pdb)
81. McCulloch, K. M., Kinsland, C., Begley, T. P., and Ealick, S. E. (2008) Structural studies of thiamin monophosphate kinase in complex with substrates and products. *Biochemistry 47*, 3810-3821. (3C9S.pdb)
82. Wang, K. T., Wang, J., Li, L. F., and Su, X. D. (2009) Crystal structures of catalytic intermediates of human selenophosphate synthetase 1. J.*Mol.Biol. 390*, 747-759. (3FD6.pdb)
83. Itoh, Y., Sekine, S. I., Matsumoto, E., Akasaka, R., Takemoto, C., Shirouzu, M., and Yokoyama, S. (2009) Structure of selenophosphate synthetase essential for selenium incorporation into proteins and RNAs. *J.Mol.Biol 385(5)*, 1456-69. (2YYE.pdb)
84. Karthikeyan, S., Zhou, Q., Osterman, A. L., and Zhang, H. (2003) Ligand binding-induced conformational changes in riboflavin kinase: structural basis for the ordered mechanism. *Biochemistry 42*, 12532-12538. (1Q9S.pdb)
85. Bauer, S., Kemter, K., Bacher, A., Huber, R., Fischer, M., and Steinbacher, S. (2003) Crystal structure of *Schizosaccharomyces pombe* riboflavin kinase reveals a novel ATP and riboflavin-binding fold. *J.Mol.Biol. 326*, 1463-1473. (1N06.pdb, 1N08.pdb)