

Methods and Material

Electron density map calculation:

The model 3DLS was modified by deleting the loop residues (W1040-T1050) and all hetero-atoms including water, ADP and Magnesium atoms. Random shifts were then applied to all atoms of the model (average shift 0.2 Angstroms) and the resulting model was refined with REFMAC5, using a maximum likelihood target function and Engh and Huber geometric restraints. The resulting electron density maps indicate clearly the positions of the missing atoms, including the loop residues (W1040-T1050) of chain A and the ADP ligand - see the attached electron density figures superimposed on the refined model 3DLS which includes these atoms.

Supplementary Figure Legends

Supplementary Figure 1: Structural comparison of PASK, PIM1 (PDB: 2BZK) and PKA (PDB: 1ATP) kinase domains. While the three structures show high similarity globally, PASK and PIM1 differ significantly from PKA in the N-terminal lobe (upper domain) due primarily to the additional β H1/ β H2 β -hairpin shown at the left near the top.

Supplementary Figure 2: Structure based sequence alignment of PASK.

Structure based sequence alignment of PASK with activation loop phosphorylation dependent (top) and activation loop phosphorylation independent (bottom) protein kinases. The PASK structure (PDB ID: 3DLS) was searched against DaliLite structure database. The final alignment was created using ESPript (1). The tryptophan residue in PASK and the three PIM isoforms (corresponding to Trp¹⁰⁴⁰ in PASK) is highlighted in green. The basic residue at the DFG+3 site in is highlighted in yellow. See text and Fig 4 for detail for explanation of the presence of basic residue in some activation loop phosphorylation independent kinases. The tyrosine residue involved in the active kinase conformation (corresponding to Tyr¹¹⁷⁹ in PASK) is highlighted in orange.

Supplementary Figure 3: Electron Density maps of PASK novel β -hairpin and ADP-Mg²⁺ complex.

[A]. Unbiased electron density map (2Fo-Fc) of β -hairpin contoured at σ 1.5. The position of β H1 and β H2 sheet is indicated.

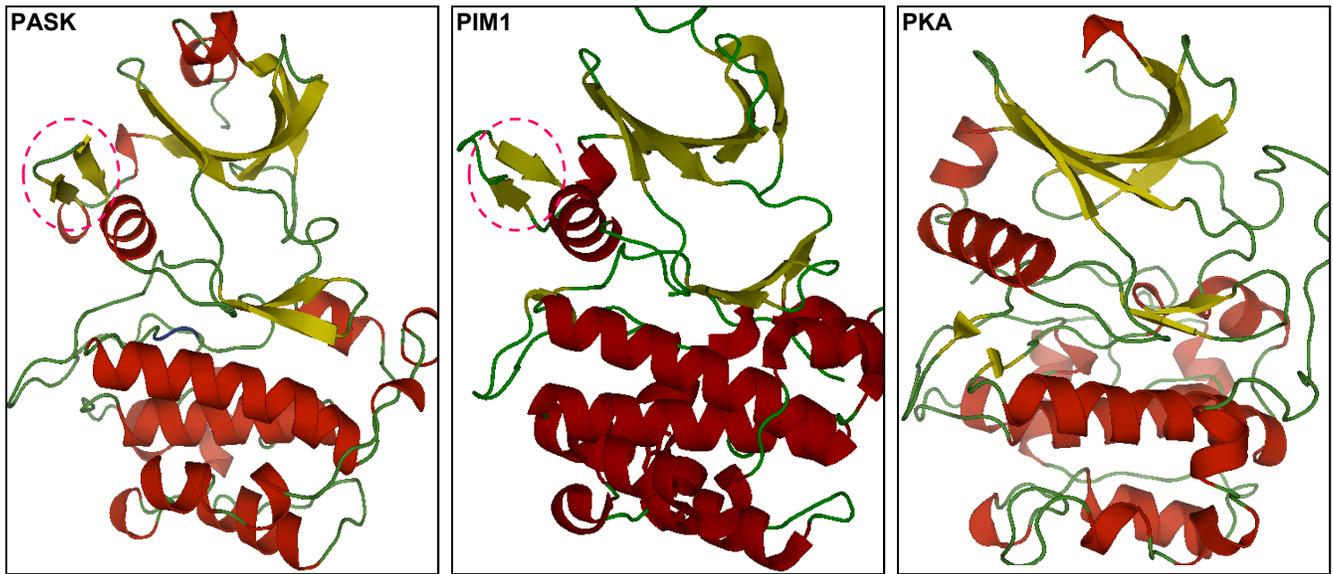
[B]. Unbiased electron density map (2Fo-Fc) of ADP and Mg²⁺ ions contoured at σ 1.5. See attached supplementary method for description of the procedure used to generate these maps.

Supplementary Figure 4: Stabilization of GSK3 β catalytic loop by phosphate from substrate.

A phosphate ion occupies and functions analogous to phosphorylated serine/threonine in activation loop dependent kinases in GSK3 β . Phosphate ion forms hydrogen bond with catalytic loop arginine (Arg¹⁸⁰) and DFG+3 lysine (Lys²⁰⁵) neutralizing positive charged catalytic core and aligning the catalytic residues for substrate catalysis.

Reference:

1. Gouet, P., Courcelle, E., Stuart, D. I. & Metoz, F. (1999). *Bioinformatics*. **15** 305-8



Supplementary Fig 1

| | | αA | | αB | | β1 | | β2 | | β3 | | βH1 | |
|---------------|--------|-----|---------------------|---------|--------|-------|-------------------|----------|--------|--------|-----------|--------------|--------------|
| P-dependent | PASK | 981 | KAVELEGLAACEGEYSQK | YSTMSPL | LGSGAF | FGFV | WTAVDKEK | ..NKEV | VVKF | IKKEK | VLEDC | WIEDPKL | |
| | AKT | 142 |KHRVTMNE | FEYLKLL | LGKGT | FGKV | ILVKEKA | ..TGRYY | AMK | ILKKE | EVIVA |KDEV | |
| | PKN | 603 | PGPALCSPLRKSPLTLED | FKFLAV | LGRGH | FGKVL | LSEFRP | ..SGELF | AIK | ALKK | GDIVA |RDEV | |
| | PDK1 | 64 | SLQHAQPPPPQPRKKRPED | FKFGKI | LGGFS | STV | VLARELAT | ..SREY | AIK | ILEKR | HIIKE |NKV | |
| | p70S6K | 82 |GPEKIRPEC | FELLRV | LGGY | YGV | FQVRKVTGANTGKIFAM | VV | LKKAM | IVRN |AKDT | | |
| | PKA | 39 |AHLDQ | FERIKT | LGTGS | FGRV | MLVKHKE | ..TGNHY | AMK | ILDKQ | KVVK |LKQI | |
| | p38A | 13 |LNKTIWEVPER | YQNLSP | VGSA | YGS | VCAAFDTKT | ..GLRV | AVK | KL | SRFPQSII |HA | |
| | ERK1 | 24 | VPGEVEMVKQPFDVGR | YTQLQY | IGEGAY | GMV | SSAYDHVR | ..KTRV | AIK | KIS | PPEHQT |YC | |
| | PASK | 981 | KAVELEGLAACEGEYSQK | YSTMSPL | LGSGAF | FGFV | WTAVDKEK | ..NKEV | VVKF | IKKEK | VLEDC | WIEDPKL | |
| | PIM1 | 119 |GKE | KEPLESQ | YQVGPL | LGGG | FGSV | YSGIRVSD | ..NLPV | AIK | IV | VEKDRISD |WGELPNG |
| P-independent | PIM2 | 21 |GGKDREAFAE | YRLGPL | LGGG | FGTV | FAGHRLTD | ..RLQV | AIK | IV | IPRNRVLG |WSPLSDS | |
| | PIM3 | 29 |AKADKESFEKA | YQVGAV | LGGG | FGTV | YAGSRIAD | ..GLPV | AVKH | VVKER | VTE |WGLSG | |
| | CDK6 | 1 |MEKDGLCRADQG | YECVAE | IGEGAY | GMV | FKAKDLKNG | ..GRFV | ALKR | VVR | VTGEEG |MP | |
| | CDK5 | 1 |MOK | YEKLEK | IGEGAY | GMV | FKAKDLKNG | ..HEIV | ALKR | VVR | VTGEEG |VP | |
| | GSK3B | 38 | TTVVATPGQGPDRPQEV | YTDTKV | IGNG | SG | FGV | VYQAKL | CDS | ..GELV | AIK | KV | LQDKRFK |
| | CHK1 | 1 |MAVPFVED | WDLVQT | LGGAY | GMV | QLAVNRVT | ..EEAV | AVK | IV | DMKRAVDCP | | |
| | PHKg1 | 9 |DSHSAQDFYEN | YEPKEI | LGRGV | SSV | VRR | CIHKPT | ..SQEY | AVK | IV | IVDTGGGSFSP | PEEVRELR |

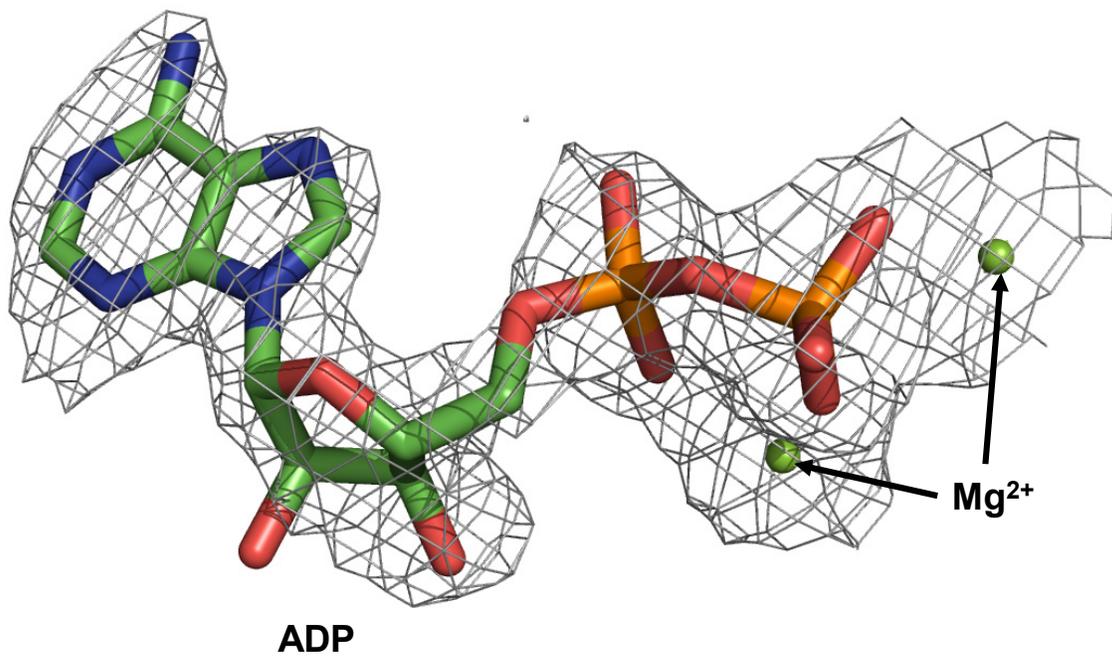
| | | βH2 | | αC | | β4 | | β5 | | αD | | |
|-------------|---------------|------|----------|-----------|----------|---------|---------|--------|-------------|--------------|------------|--------|
| P-dependent | PASK | 1047 | GKVTLE | IAILSR | VEHAN | ..IIK | VLDI | FE | ..NQG | FFQLVMEKHG | SGSLDL | FAFIDR |
| | AKT | 193 | AHTLTENR | VLQ | ..NSR | HPP | LTALKYS | FQ | ..THDR | LCFVMEYANGG | ELFP | HL |
| | PKN | 664 | ESLMCEKR | ILAAVT | ..SAG | HPP | LVNLF | FQ | ..TPEH | VCFVMEYSAGG | DLML | HI |
| | PDK1 | 125 | PYVTRERD | VMSRLD | ..HPP | FVNLYFT | FQ | ..DNEK | LYFGLSYAKNG | EL | LKY | IR |
| | p70S6K | 138 | AHTKARN | ILE | ..EVK | HPP | IVDLIYA | FQ | ..TGGK | LYLILEYLSGG | ELFM | QL |
| | PKA | 87 | EHTLNEKR | ILQ | ..AVN | FPFL | LVKLEFS | FK | ..DNSN | LFYVMEYVPGG | EMF | SH |
| | p38A | 66 | KRTYREL | LLKHMK | ..HEN | VIGLLD | V | TPARS | LEENF | PNVYLVTHL | MG | ADLN |
| | ERK1 | 83 | QRTLREIQ | ILLRFR | ..HEN | VIGIRDI | LR | ASTLE | AMRDVYIVQ | DLME | TDL | YK |
| | PASK | 1047 | GKVTLE | IAILSR | VEHAN | ..IIK | VLDI | FE | ..NQG | FFQLVMEKHG | SGSLDL | FAFIDR |
| | P-independent | PIM1 | 175 | TRVPEVV | LLKKVSSG | ..FSG | VIRLLD | WF | ..RPS | DFVLLERPEPVQ | DL | FD |
| PIM2 | | 78 | VTCPL | EVAL | LLWKV | GAGGG | HPG | VIRLLD | WF | ..TQEG | FMLVLERPL | PAQ |
| PIM3 | | 85 | ATVPL | EVV | LLRKV | GAGGG | ARG | VIRLLD | WF | ..RPG | DFLLVLERPE | PAQ |
| CDK6 | | 56 | LSTIREVA | VLRHLETPE | ..HPN | VVRLFDV | CTV | SRTD | RETKL | TLVFEH | VQ | DL |
| CDK5 | | 46 | SSALREIC | LLKELK | ..HKN | IVRL | LHDV | LHS | ..DKK | TLVFE | PCDQ | DL |
| GSK3B | | 95 | ..NR | ELQ | IMR | KLD | ..HCN | IVR | LRYF | FYSS | GEKK | DEYLVN |
| CHK1 | | 50 | ENIKKEIC | INKMLN | ..HEN | VVKFY | GHRR | ..EGNI | QYLF | LEYCSGG | EL | FD |
| PHKg1 | | 69 | EATLKEVD | ILRKVS | ..GHP | NI | IQ | LKDT | YET | ..NTFF | FLVFDL | MKRG |

| | | αE | | β6 | | β7 | | | | | | |
|-------------|---------------|------|--------|---------|---------|-------|-------|--------|--------|----------|--------|-----|
| P-dependent | PASK | 1105 | ASYIFR | QLVSAVG | YLR | ..LKD | IIHRD | IKD | ENIVIA | AEDFT | IKKI | DFG |
| | AKT | 250 | ARFYGA | EIVSALD | YHSEKN | VVYRD | LKLE | ENLMLD | ..KDG | H | IKI | DFG |
| | PKN | 723 | AIFYSA | CVVLGLQ | FLH | ..EHK | IVYRD | LKLE | DNLLD | ..TEG | VKI | DFG |
| | PDK1 | 182 | TRFYTA | EIVSALE | YH | ..GKG | IIHRD | LKLE | ENILLN | ..EDM | IQI | DFG |
| | p70S6K | 195 | ACFYLA | EISMALG | HLH | ..QKG | IIYRD | LKLE | ENIMLN | ..HQGH | VKL | DFG |
| | PKA | 144 | ARFYAA | QIVLTFE | YH | ..SLD | IIYRD | LKLE | ENLLD | ..QQGY | IQV | DFG |
| | p38A | 127 | VQFLIY | QILRGLK | YIH | ..SAD | IIHRD | LKLE | ENLAVN | ..EDCE | LKI | DFG |
| | ERK1 | 143 | ICYFLY | QILRGLK | YIH | ..SAN | VLHRD | LKLE | ENLLIN | ..TTCD | LKI | DFG |
| | PASK | 1105 | ASYIFR | QLVSAVG | YLR | ..LKD | IIHRD | IKD | ENIVIA | AEDFT | IKKI | DFG |
| | P-independent | PIM1 | 235 | ARSFFW | QVLEAVR | HCH | ..NRG | VVHRD | IKD | ENILID | LNRRGE | LKI |
| PIM2 | | 140 | SRCFFG | QVVAAIQ | HCH | ..SRG | VVHRD | IKD | ENILID | LRRC | AKL | DFG |
| PIM3 | | 147 | ARRFVA | QVLAAVR | HCH | ..SCG | VVHRD | IKD | ENILV | DLRSGE | LKI | DFG |
| CDK6 | | 122 | IKDMFF | QLLRGLD | FLH | ..SHR | VVHRD | LKLE | ENILV | ..SSG | IKL | DFG |
| CDK5 | | 103 | VKSFLP | QLLKGLG | FCH | ..SRN | VLHRD | LKLE | ENLLIN | ..NRGE | LKL | DFG |
| GSK3B | | 158 | VKLYMY | QLFRSLA | YIH | ..SFG | ICHRD | IKP | ENLLD | ..PDPTAV | LKL | DFG |
| CHK1 | | 107 | AQRFFH | QLMAGVV | YH | ..GIG | ITHRD | IKP | ENLLD | ..ERDN | LKI | DFG |
| PHKg1 | | 127 | TRKIMR | ALLEVIC | TLH | ..KLN | IVHRD | LKLE | ENILLD | ..DNMN | IKL | DFG |

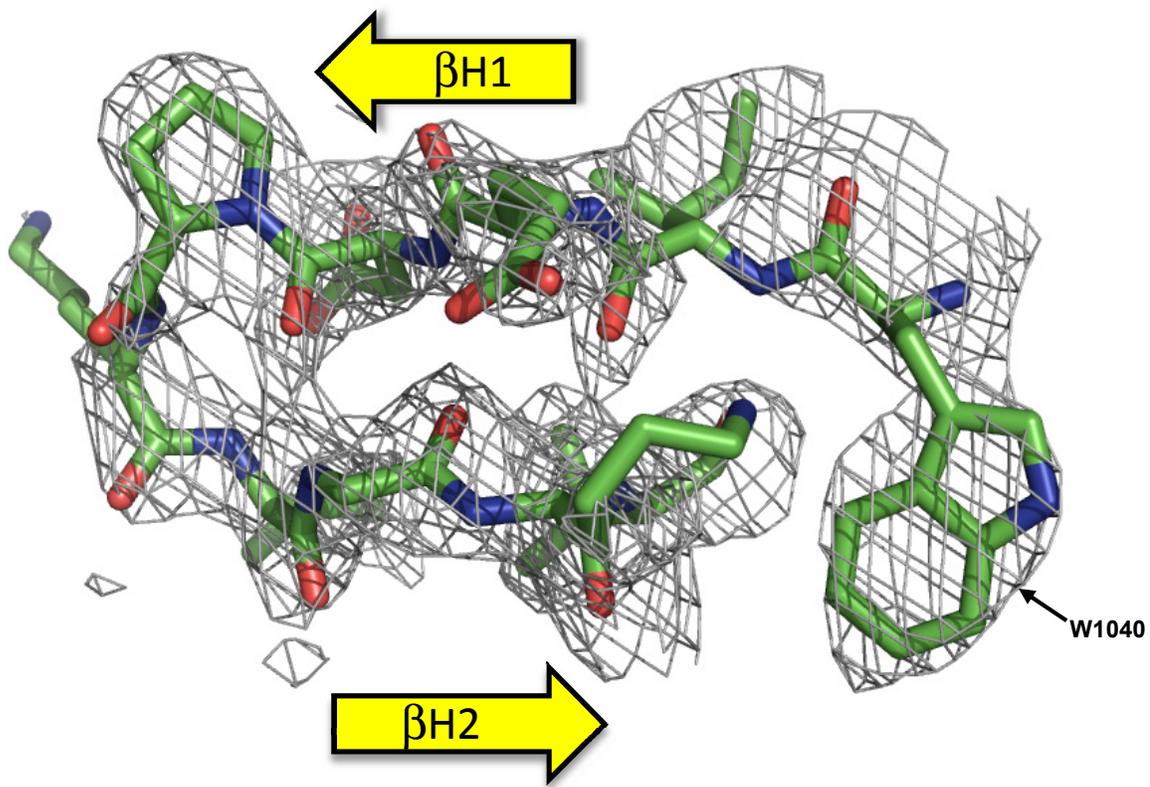
| | | αEF | | αF | | αG | |
|-------------|---------------|------|---------|----------|------------|----------|-------|
| P-dependent | PASK | 1169 | CAPEVLM |GNP | YRGP | PEL | EMWSL |
| | AKT | 316 | LAPVLE |DND | Y | GRAV | DWGL |
| | PKN | 788 | LAPVLT |DTS | Y | TRAV | DWGL |
| | PDK1 | 249 | VSP | PELLT |EKS | A | CKSS |
| | p70S6K | 260 | MAP | EILM |RSG | H | NRAV |
| | PKA | 206 | LAP | EIL |SKG | Y | NKAV |
| | p38A | 189 | RAP | EIML |NWMHY | Y | NQTV |
| | ERK1 | 211 | RAP | EIML |NSKG | Y | TKSI |
| | PASK | 1169 | CAPEVLM |GNP | YRGP | PEL | EMWSL |
| | P-independent | PIM1 | 299 | SP | PEWIR |YHR | YHGR |
| PIM2 | | 204 | SP | PEWIS |RHQ | YHAL | PA |
| PIM3 | | 211 | SP | PEWIR |YHR | YHGR | SA |
| CDK6 | | 186 | RAP | PEVLL |QSS | Y | ATPV |
| CDK5 | | 168 | RP | PDVLF |GAKLY | Y | STSI |
| GSK3B | | 223 | RAP | PELIF |GATDY | Y | TSSI |
| CHK1 | | 174 | VAP | PEL |RRFP | H | AEPV |
| PHKg1 | | 191 | LAP | EIK |ECSM | NEDHPGY | Y |

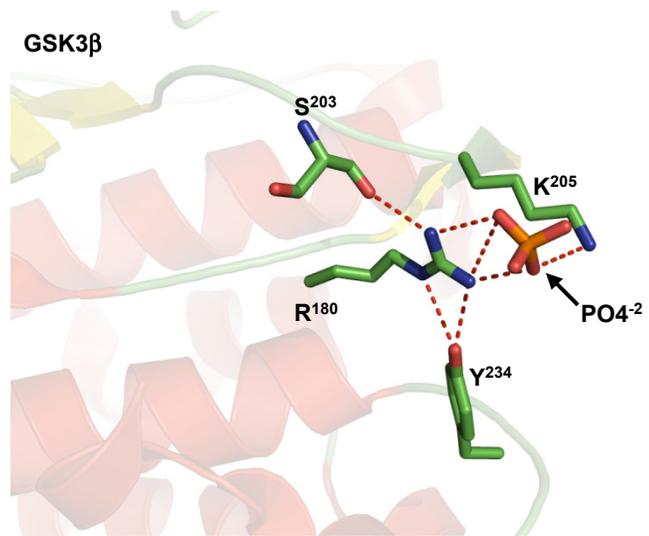
Supplementary Fig 2

[A]



[B]





Supplementary Fig 4