



The Analysis of Structure and Function on Mouse Protein Kinase D

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Group 9

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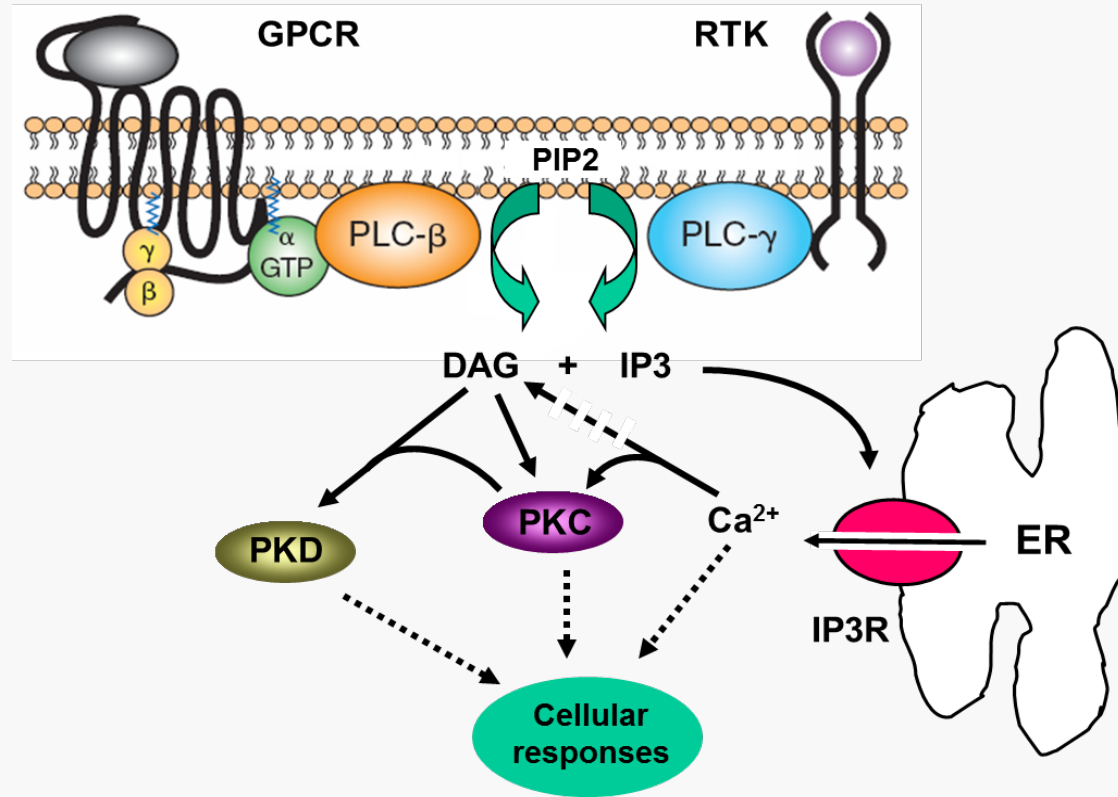
- Background of Protein Kinase D
- General gene and protein analysis on PKD1
- Structure prediction and analysis on PKD1
- Signaling pathways analysis on PKD
- Summary

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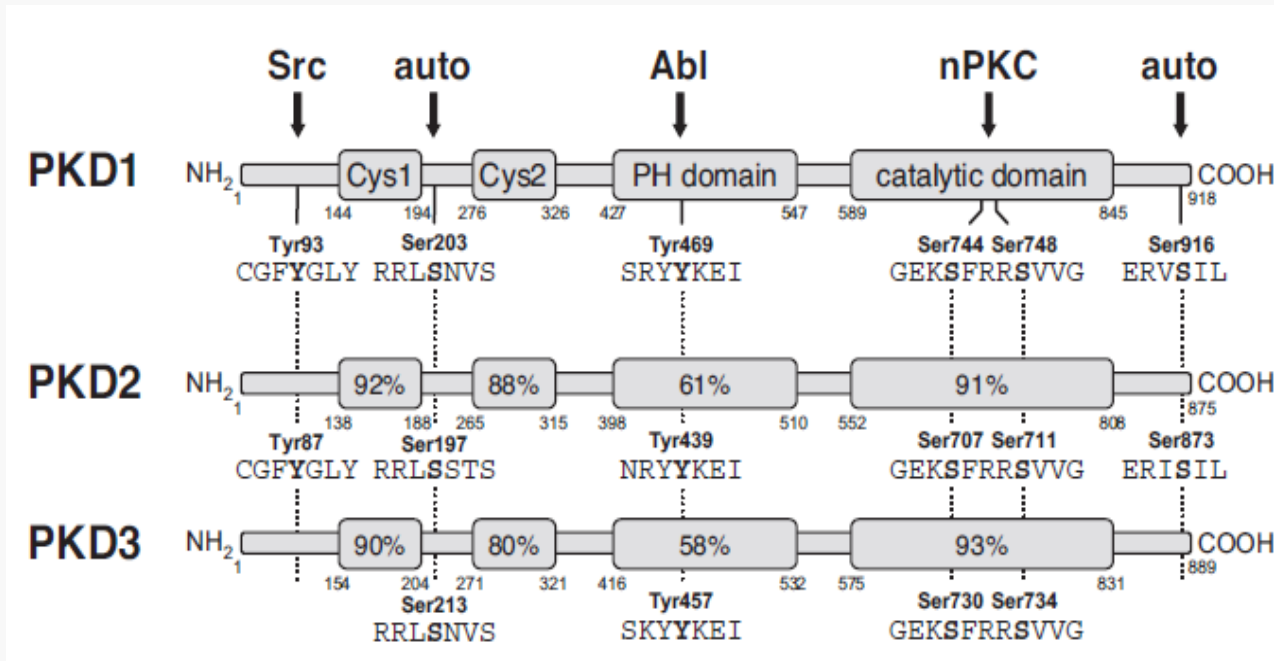
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Protein Kinase D (PKD) Introduction



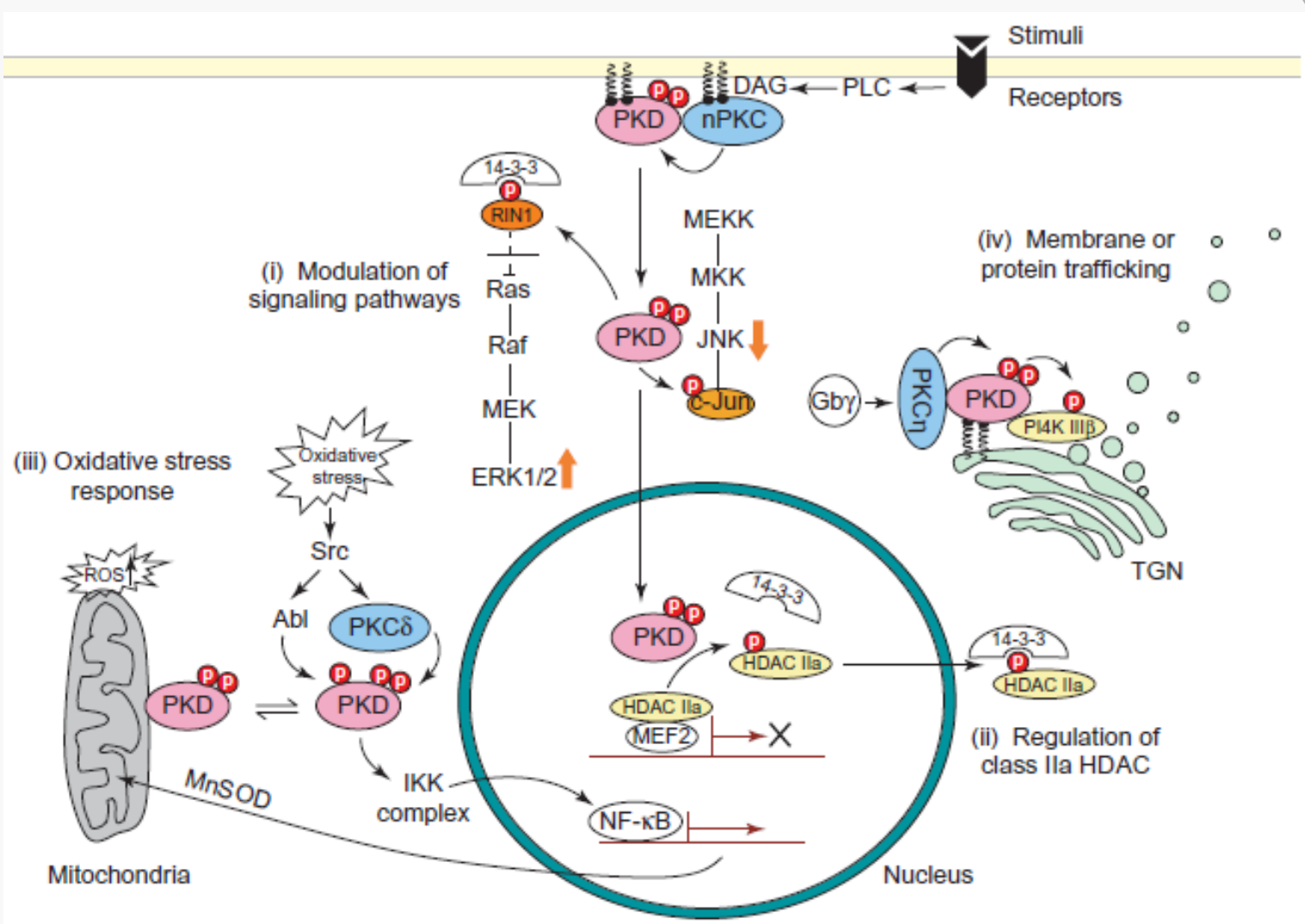
- PKD is a new family of second messenger stimulated kinases
- IP3R and PKD are downstream molecules of PLC pathway

Protein kinase D is a subfamily of Serine/threonine kinases



- PKDs have DAG binding domains
- PKDs are classified into CaMKs based on their catalytic structure and substrate specificity.
- Activities of PKDs are regulated by upstream kinases such as PKC, Src and Abl.

Major PKD signaling pathways and cellular responses

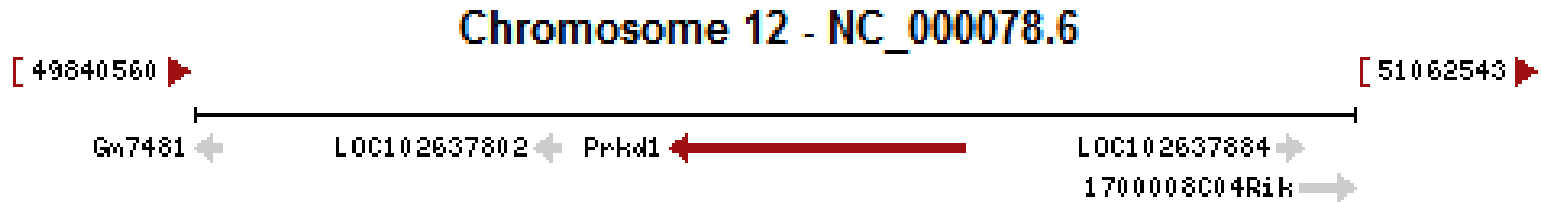


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PKD1 Gene Sequence

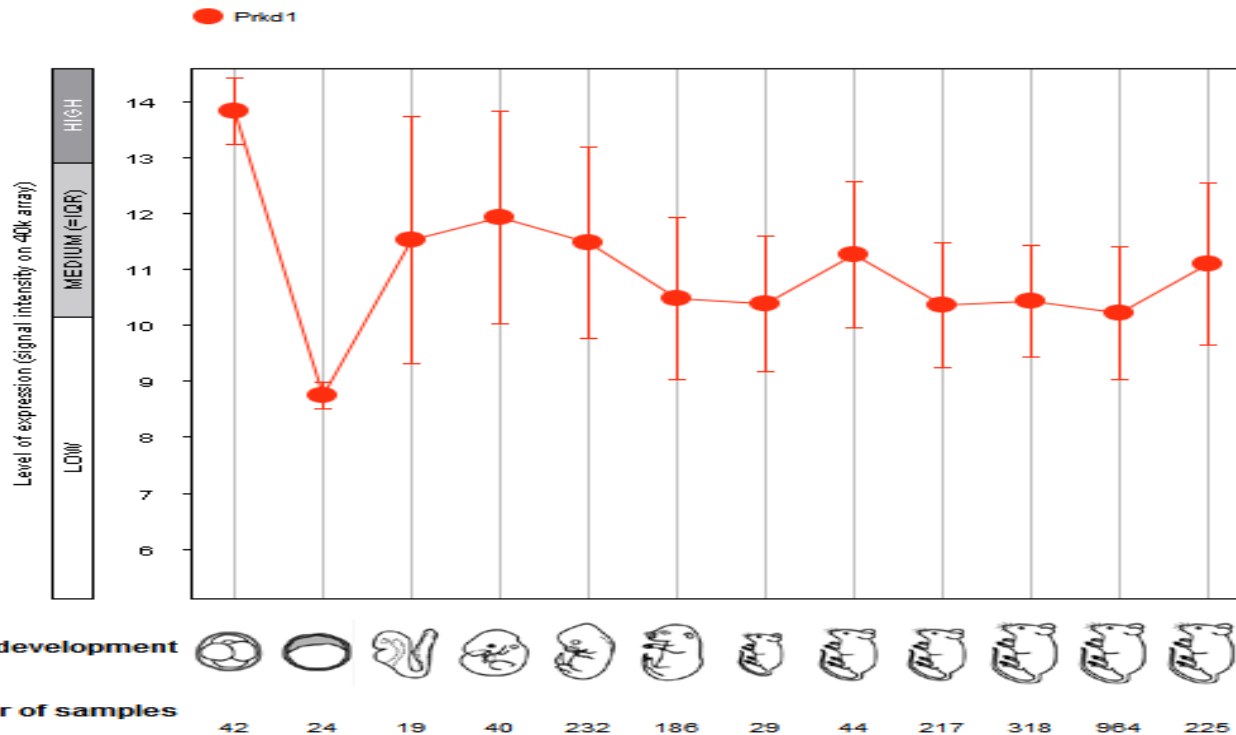
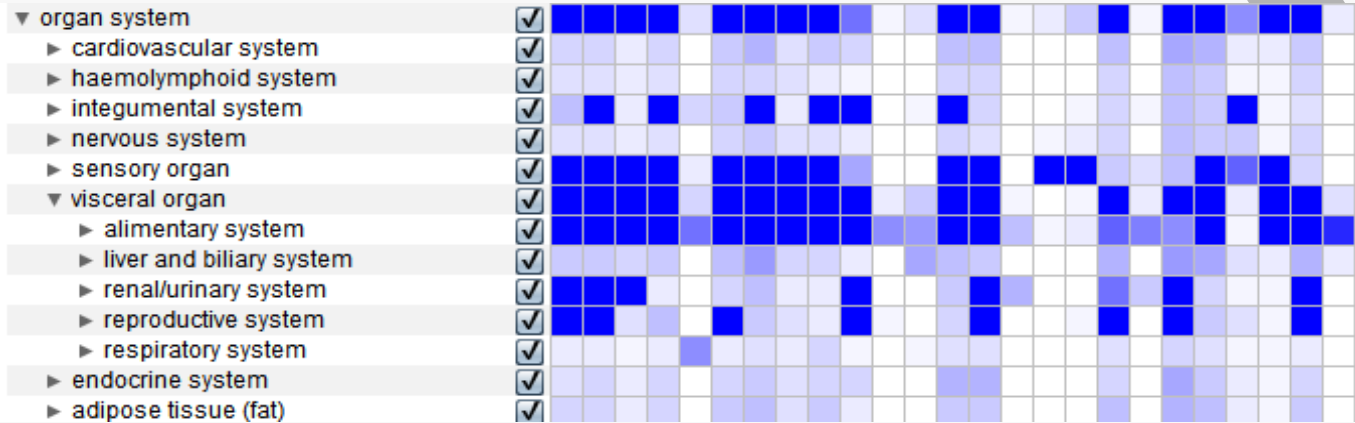


NCBI Reference Sequence	NM_008858.3
Gene name	Prkd1
Gene ID	18760
Location	Chromosome 12, 12C1
CDS	251..3007
Homology	Conserved in human, chimpanzee, Rhesus monkey, dog, cow, rat, chicken, zebrafish, and frog

PKD1 Animo Acid Sequence

UniProt accession	KPCD1_MOUSE Q62101
Sequence length	918AA
Enzyme regulation	Activated by DAG and phorbol esters
Subcellular location	Cytoplasm, Cell membrane, Golgi apparatus
Biological process	Angiogenesis, Apoptosis, Differentiation, Immunity, Inflammatory response
PTM	Phosphoprotein

PKD1 Expression



PKD Family Phylogeny Tree

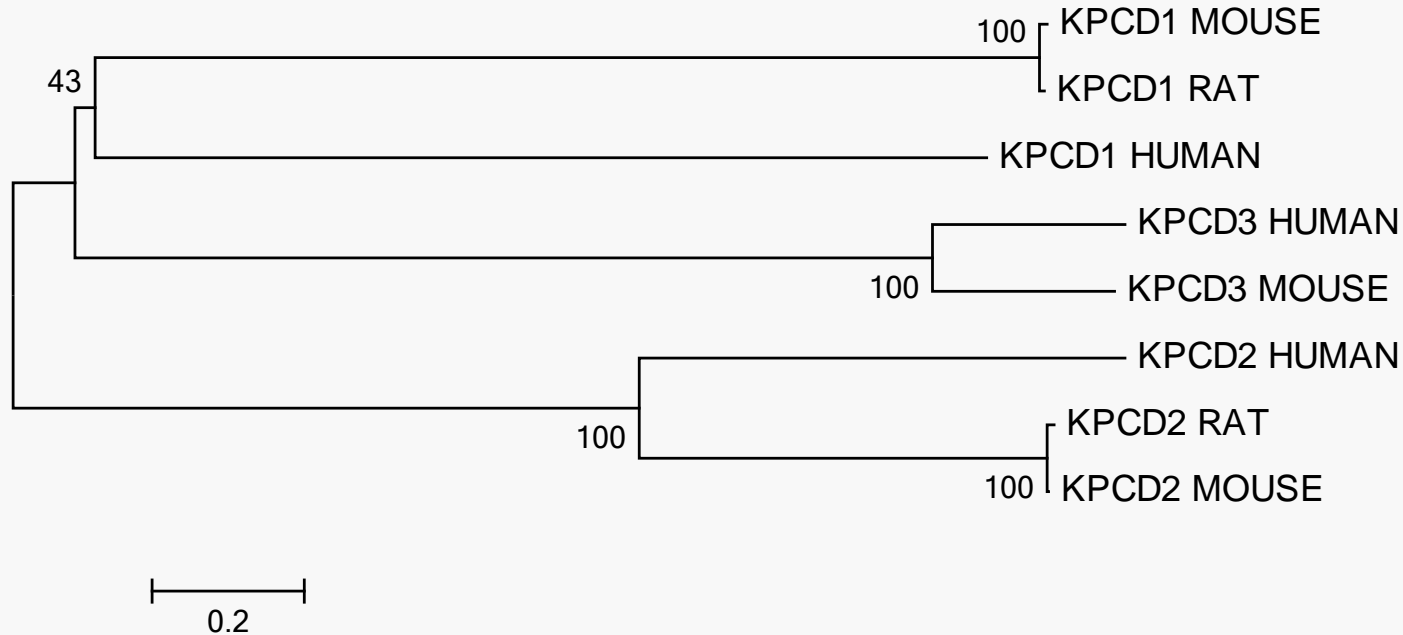
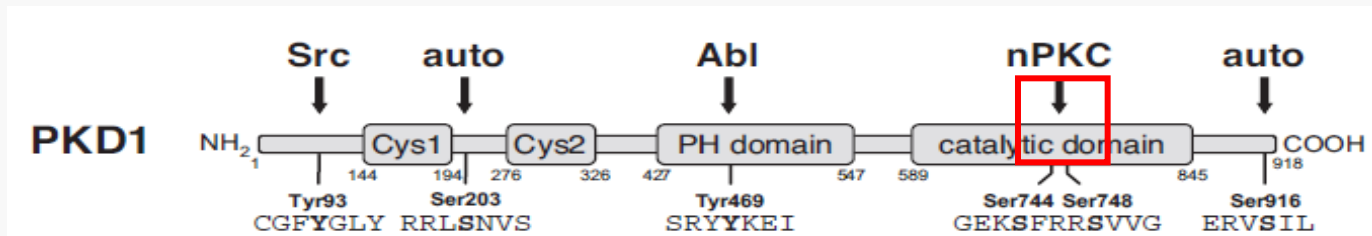


Table. Different species PKD1 protein sequences alignment

Species	Accession	Identity	Similarity
Human/Mouse	Q15139/Q62101	851/920(92.5%)	874/920(95.0%)
Human/Rat	Q15139/Q9WTQ1	855/920(92.9%)	876/920(95.2%)
Mouse/Rat	Q62101/Q9WTQ1	902/918(98.3%)	906/918(98.7%)

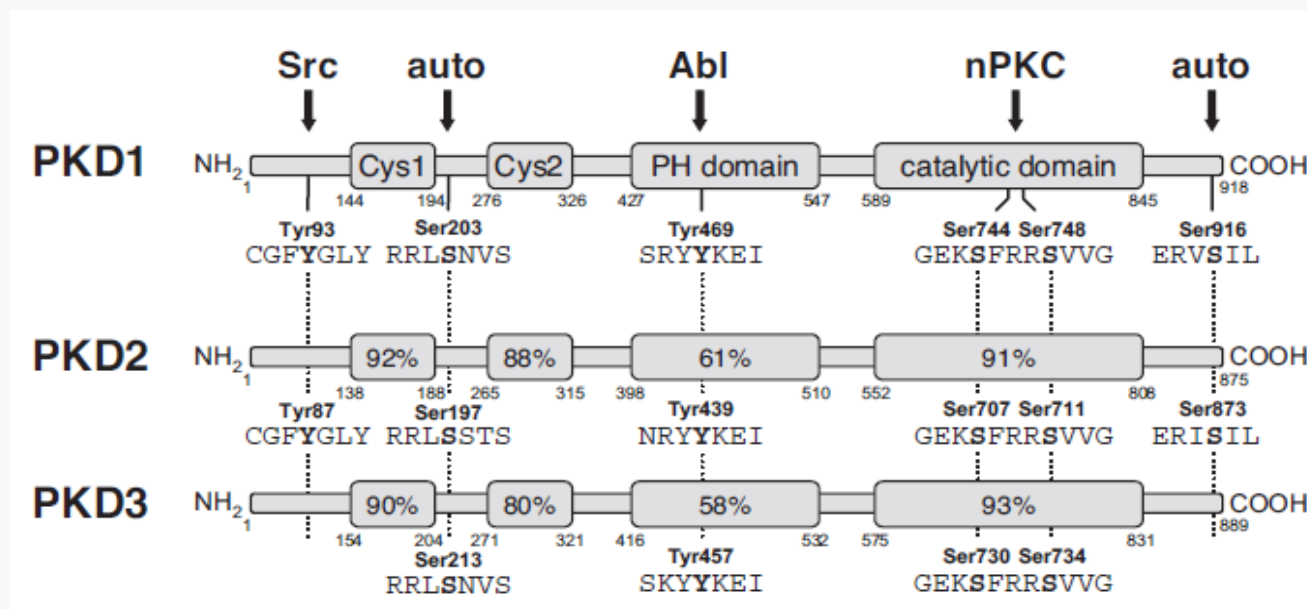
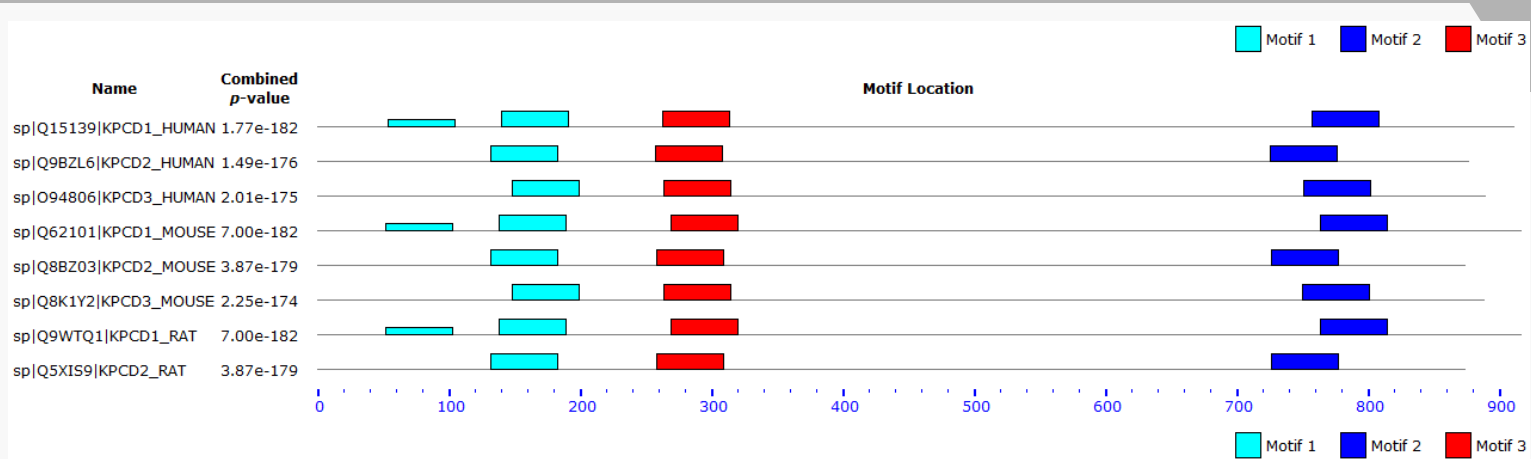
Comparison of PKD family activation loop sequences



	723	744	748	759
KPCD2_HUMAN	ADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD3_MOUSE	AEPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD1_MOUSE	ADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD3_HUMAN	AEPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD1_HUMAN	ADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD2_RAT	ADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			
KPCD2_MOUSE	ADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPE			

The activation loop segment is 100% conserved among all members of the novel PKD protein kinase subfamily.

Conserved Motif Prediction



Homology: catalytic domain > Cys1 > Cys2 > PH domain

Conserved Motif Prediction

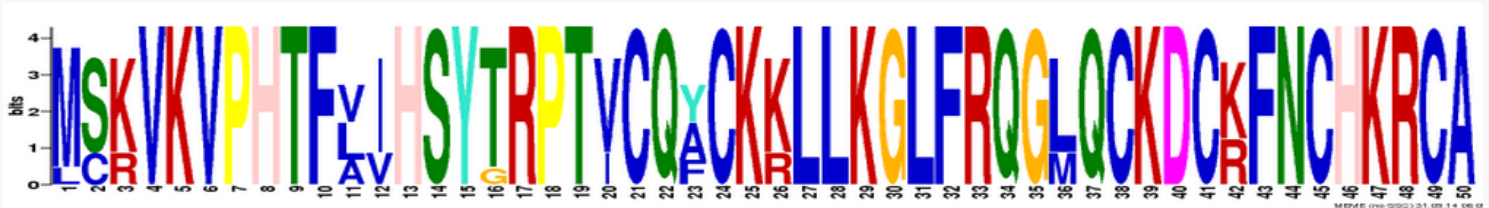
Motif 1



Motif 2



Motif 3



PKDs are highly conserved enzymes, which revealed the important functions of PKD in cells and their involvement in disease.

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PKD1 Structure Prediction

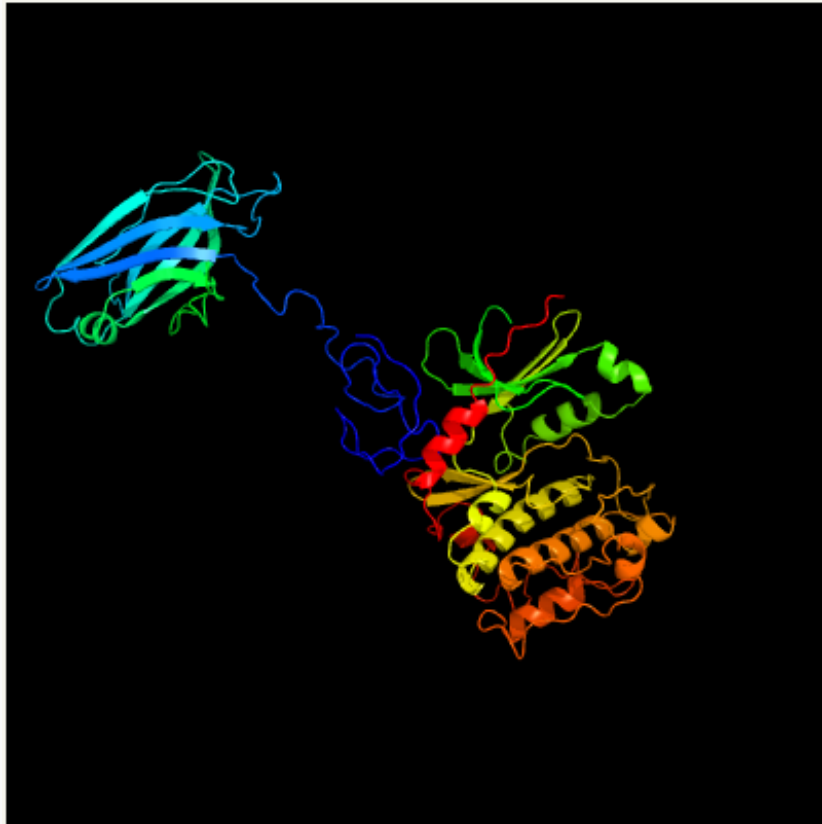


Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:82.802 **Y**:106.454 **Z**:61.307

Model (left) based on template [c3pfqA](#)

Top template information

PDB header:transferase

Chain: A: **PDB Molecule:**protein kinase c beta type;

PDBTitle: crystal structure and allosteric activation of protein kinase c beta2 ii

Confidence and coverage

Confidence:

100.0%

Coverage:

53%

485 residues (53% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.






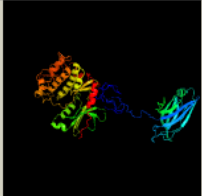




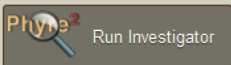


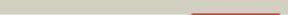

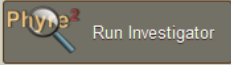
You may wish to submit your sequence to [Phyrealarm](#). This will automatically scan your sequence every week for new potential templates as they appear in the Phyre2 library.

3D viewing

[Interactive 3D view in JSmol](#)

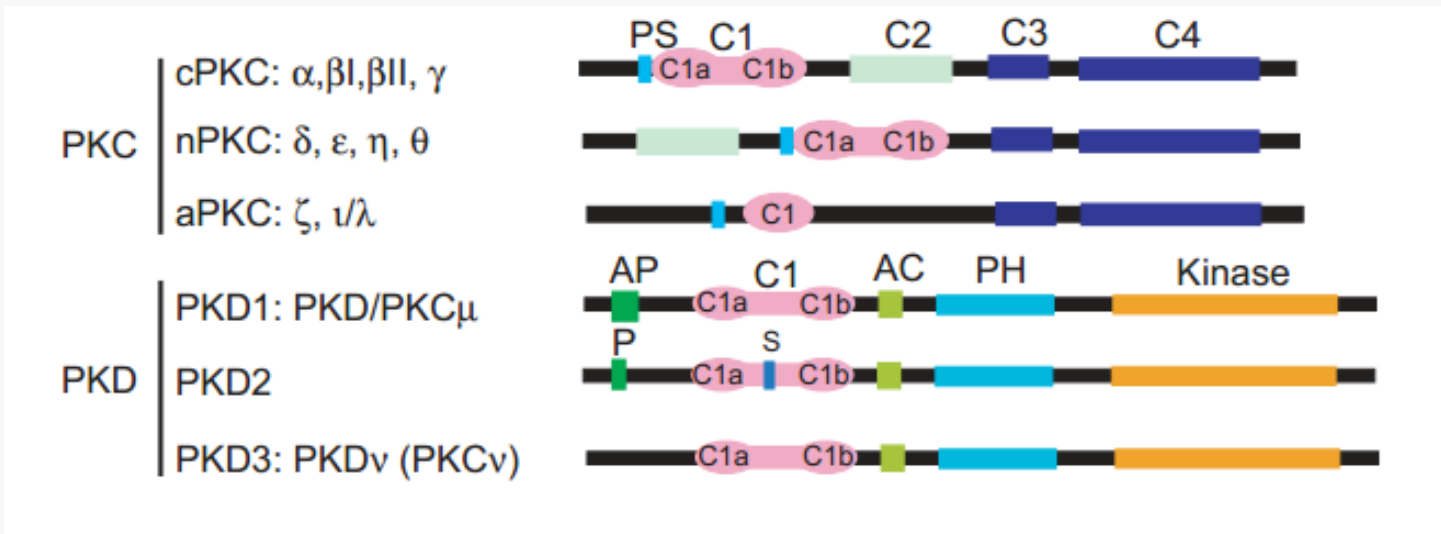
For other options to view your downloaded structure offline see the [FAQ](#)

PKD1 Structure Prediction

#	Template	Alignment Coverage	3D Model	Confidence	% i.d.	Template Information
1	c3pfqA  	 <input type="button" value="Alignment"/>		100.0	25	<p>PDB header:transferase Chain: A: PDB Molecule:protein kinase c beta type; PDBtitle: crystal structure and allosteric activation of protein kinase c beta2 ii</p> <p>View investigator results</p>
2	c3nyoB  	 <input type="button" value="Alignment"/>		100.0	29	<p>PDB header:transferase Chain: B: PDB Molecule:g protein-coupled receptor kinase 6; PDBtitle: crystal structure of g protein-coupled receptor kinase 6 in complex2 with amp</p> <p></p>
3	c2acxB  	 <input type="button" value="Alignment"/>		100.0	29	<p>PDB header:transferase Chain: B: PDB Molecule:g protein-coupled receptor kinase 6; PDBtitle: crystal structure of g protein coupled receptor kinase 6 bound to2 amppnp</p> <p></p>

Template: Protein kinase C beta

The PKC and PKD families



AC, acidic domain;

AP, apolar region;

C1, DAG-binding domain that contains C1a and C1b domains;

C2, Ca²⁺-binding domain;

C3, ATP-binding domain in PKC;

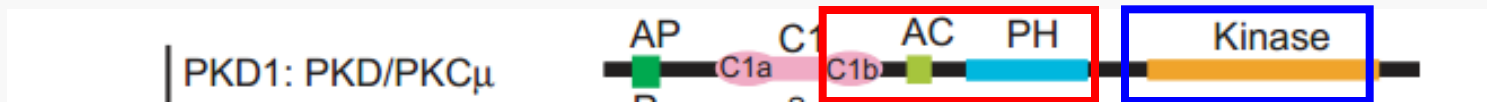
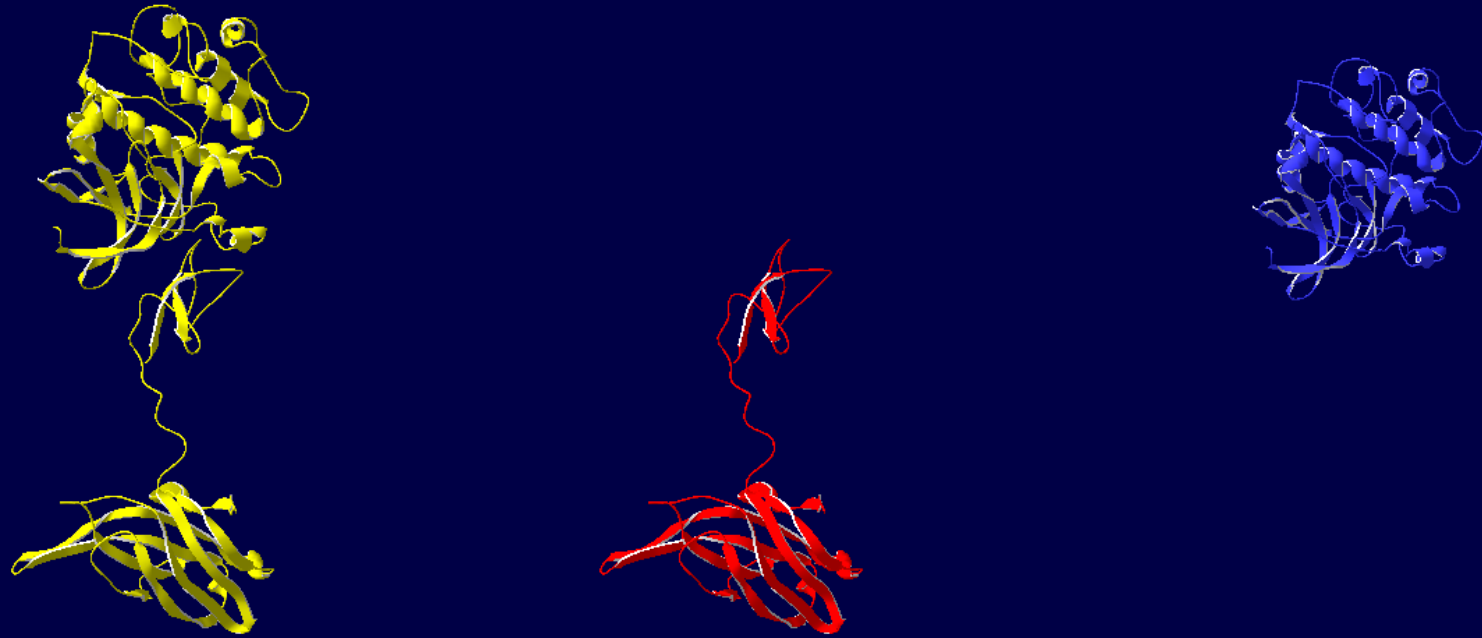
C4, substrate-binding domain in PKC;

Kinase, catalytic domain;

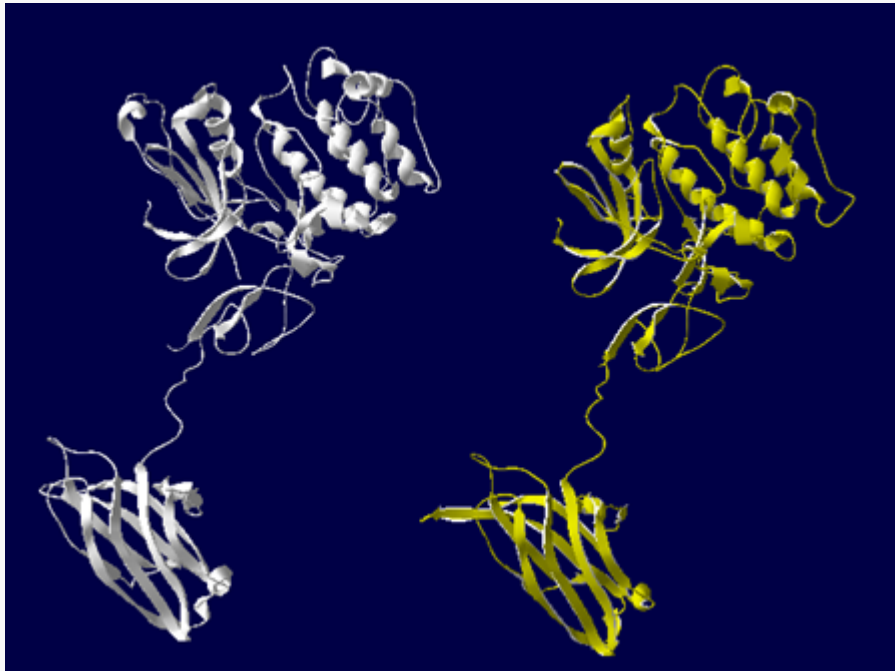
P, proline-rich region;

PS, pseudosubstrate domain.

PKD1 Structure Prediction

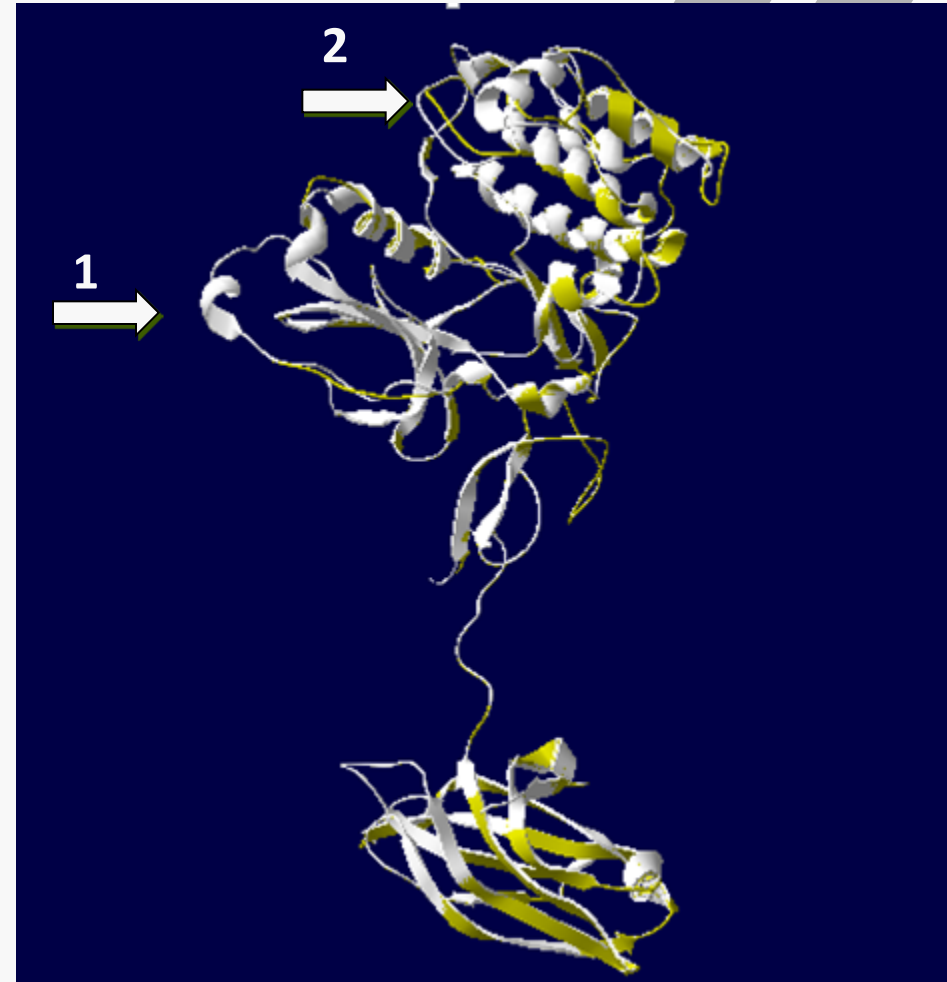


PKD1 Structure Prediction



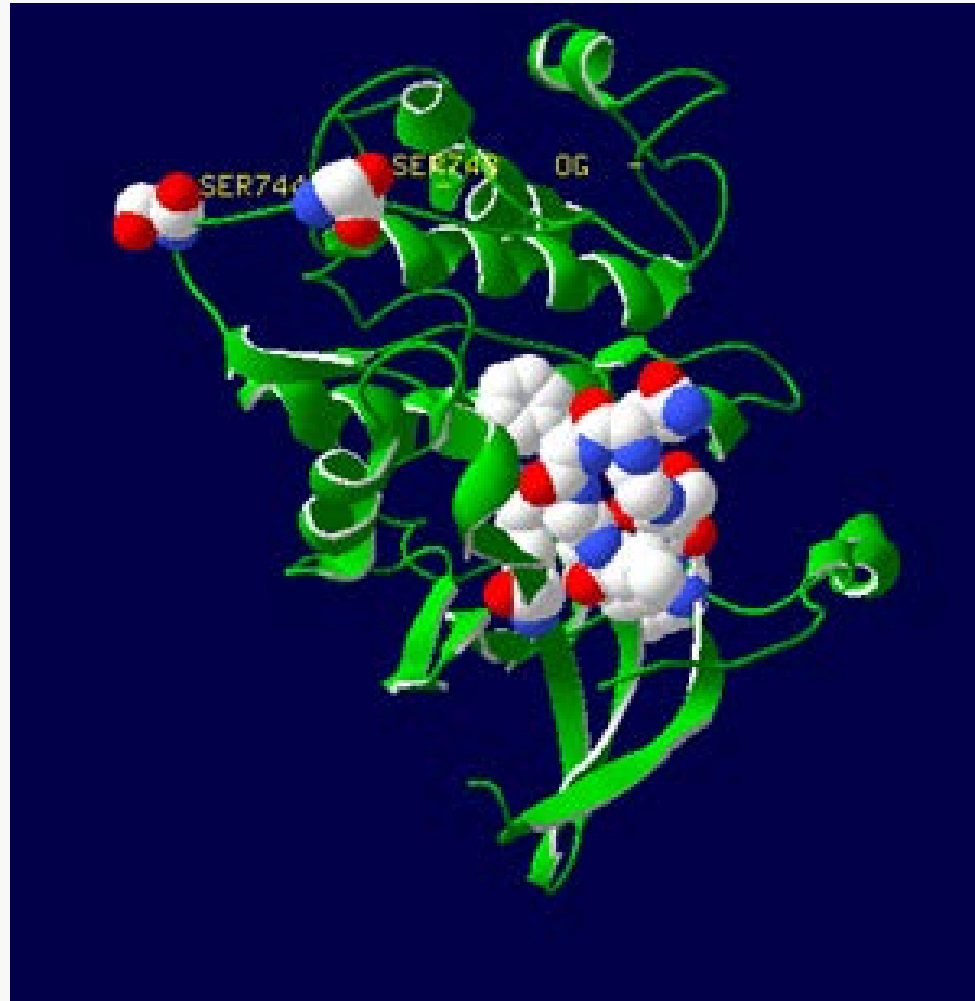
PKC beta

PKD



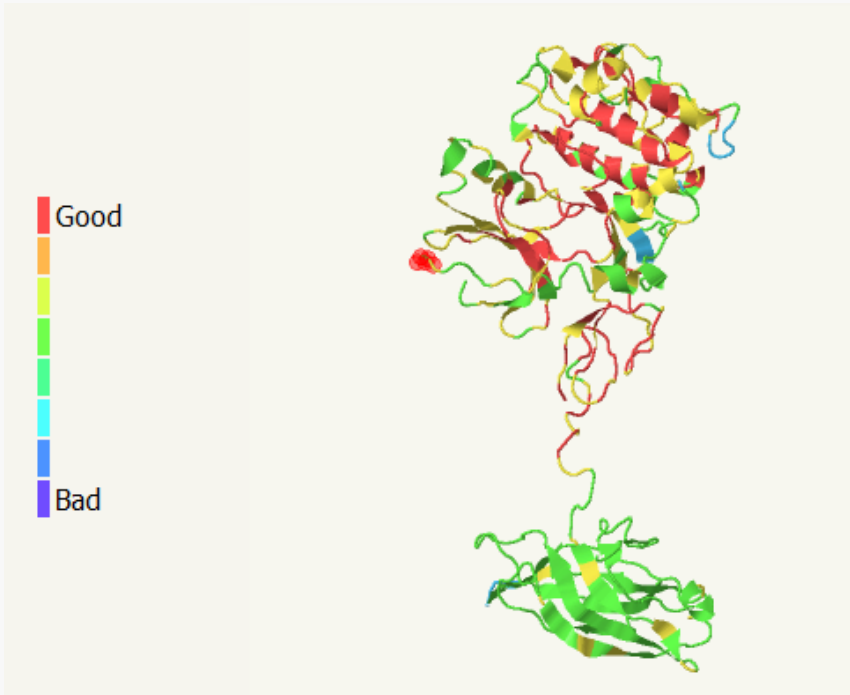
nb atoms involved: 471 RMS: 0.41 ?

PKD1 Structure Prediction

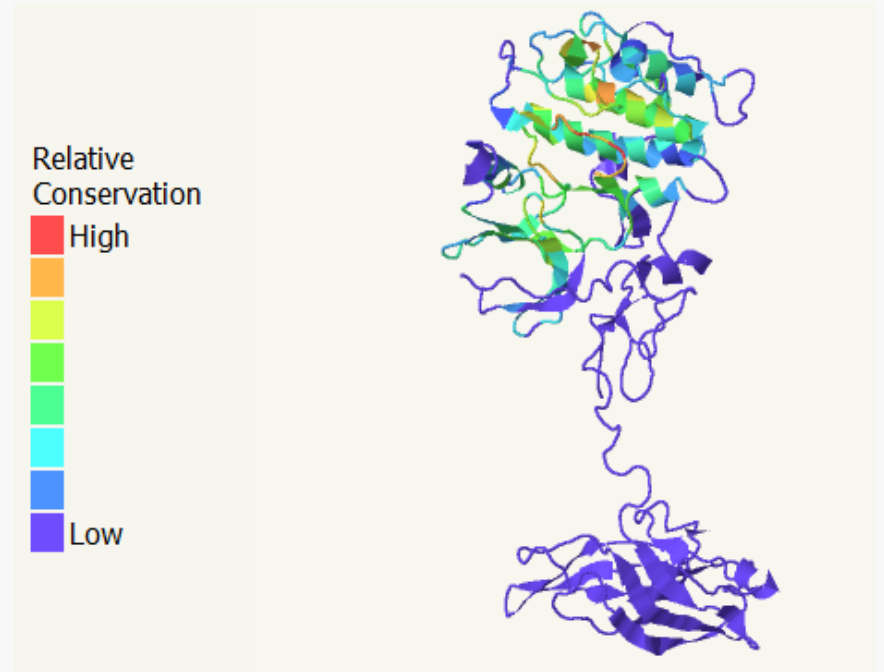


Phosphorylation sites: Ser-744 Ser-748

PKD1 Structure Prediction

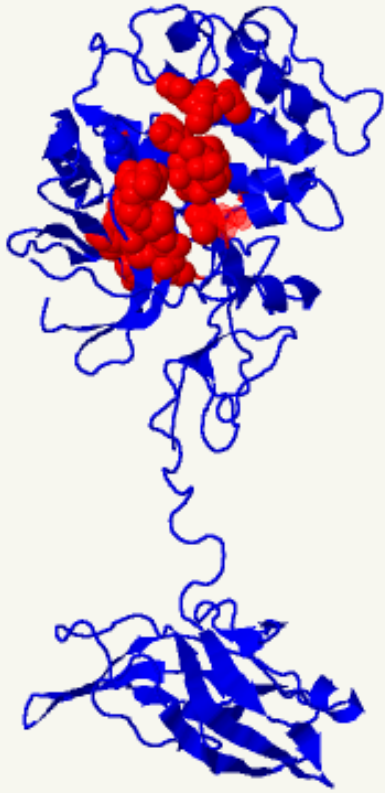


Alignment confidence

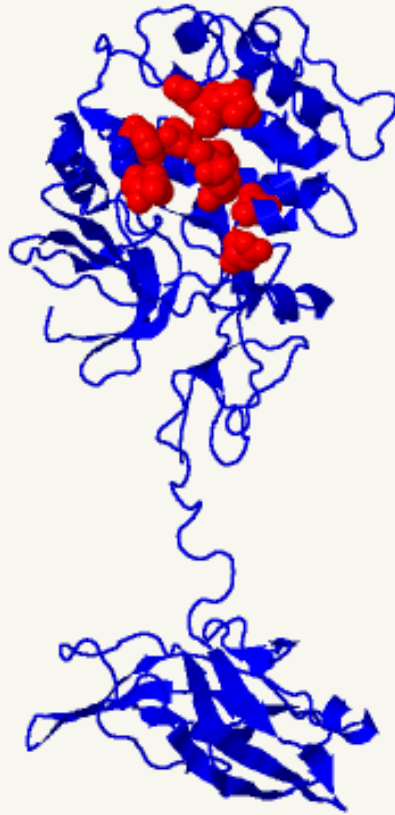


Conservation

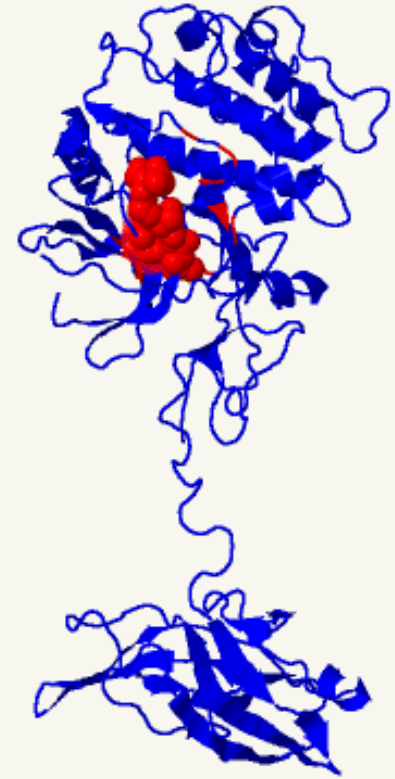
PKD1 Structure Prediction



Active site



Substrate-binding site



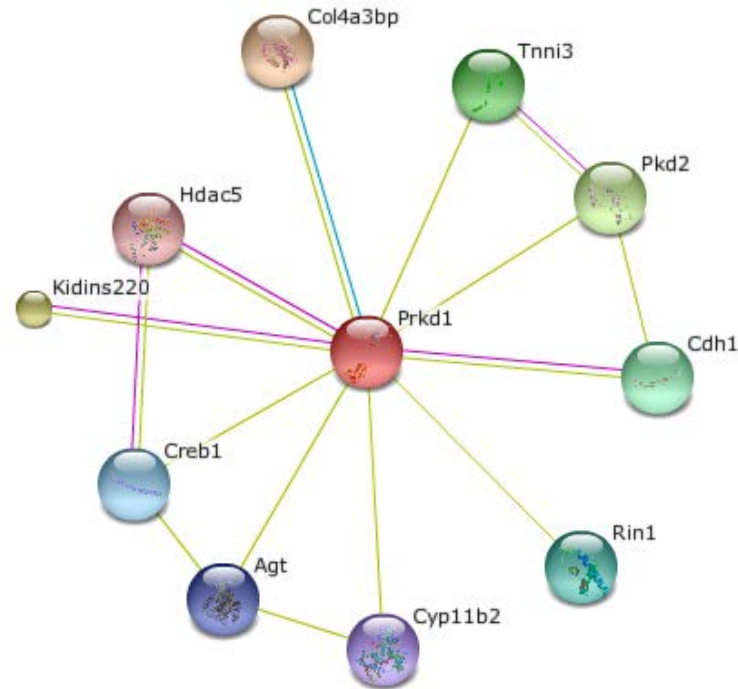
ATP-binding site

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PKD Signaling



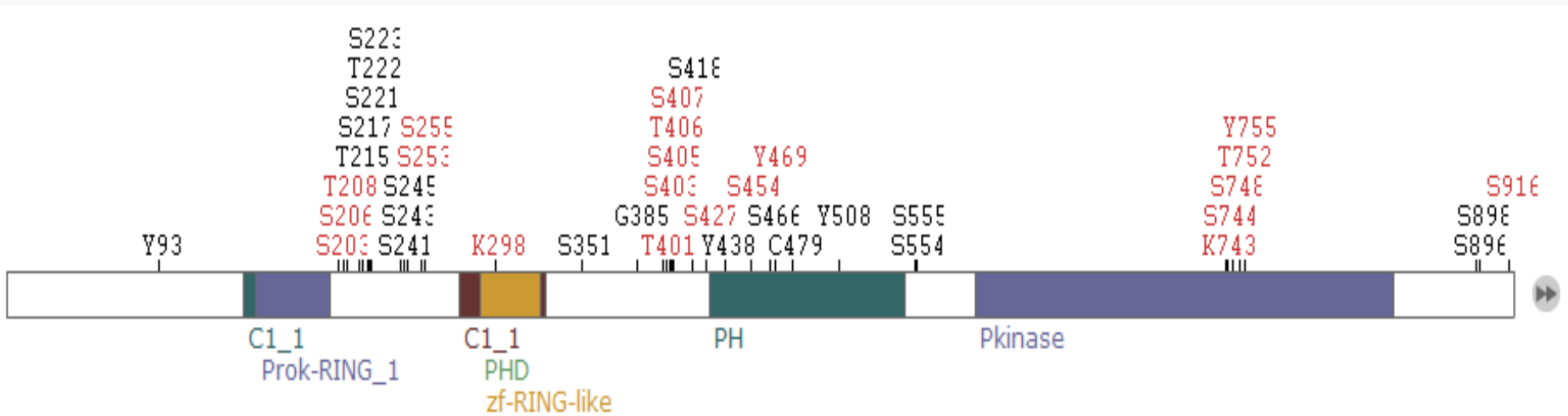
Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
								0.989
				•				0.954
								0.915
								0.884
								0.879
								0.856
								0.845
								0.843
								0.836
				•				0.831

- Col4a3bp collagen, type IV, alpha 3 (Goodpasture antigen) binding protein Gene; May mediate the intracel [...] (624 aa)
- Kidins220 kinase D-interacting substrate 220 Gene (1793 aa)
- Pkd2 polycystic kidney disease 2 Gene; Functions as a calcium permeable cation channel. PKD1 and PKD [...] (966 aa)
- Tnni3 troponin I, cardiac 3 Gene; Troponin I is the inhibitory subunit of troponin, the thin filament [...] (211 aa)
- Cdh1 cadherin 1 Gene; Cadherins are calcium-dependent cell adhesion proteins. They preferentially in [...] (884 aa)
- Rin1 Ras and Rab interactor 1 Gene; Ras effector protein, which may serve as an inhibitory modulator [...] (763 aa)
- Creb1 cAMP responsive element binding protein 1 Gene; This protein binds the cAMP response element (C [...] (341 aa)
- Agt angiotensinogen (serpin peptidase inhibitor, clade A, member 8) Gene; Essential component of th [...] (482 aa)
- Cyp11b2 cytochrome P450, family 11, subfamily b, polypeptide 2 Gene; Forms corticosterone from 11-deoxy [...] (500 aa)
- Hdac5 histone deacetylase 5 Gene; Responsible for the deacetylation of lysine residues on the N-termi [...] (1121 aa)

PKD Signaling

Substrate Protein	PKD Target Motif(s)	Functional Consequence	Ref
Kidins220	⁹¹⁴ I <u>T</u> R <u>Q</u> M <u>S</u> F <u>D</u> L <u>T</u> K ⁹²⁴ (Ser919)	Polarized plasma membrane localization in neural cells	41
RIN1	³⁴⁶ L <u>L</u> R <u>S</u> M <u>S</u> A <u>A</u> F <u>C</u> S ³⁵⁶ (Ser351)	Binding to 14-3-3 protein and altered subcellular localization	80
PI ₄ KIII-β	²⁸⁹ L <u>L</u> R <u>T</u> A <u>S</u> N <u>P</u> K <u>V</u> E ²⁹⁹ (Ser294)	Increased lipid kinase activity and enhanced vesicular transport to plasma membrane	27
Hsp27	⁷⁷ L <u>S</u> R <u>Q</u> L <u>S</u> S <u>G</u> V <u>S</u> E ⁸⁷ (Ser82)	Unknown	34
cTnl	¹⁷ P <u>I</u> R <u>R</u> R <u>S</u> S <u>N</u> Y <u>R</u> A ²⁷ (Ser22)	Decreased myofibrillar calcium sensitivity	57
	¹⁸ I <u>R</u> R <u>R</u> S <u>S</u> N <u>Y</u> R <u>A</u> Y ²⁸ (Ser23)		
HDAC5	²⁵⁴ L <u>R</u> K <u>T</u> A <u>S</u> E <u>P</u> N <u>L</u> K ²⁶⁴ (Ser259)	Binding to 14-3-3 protein and nuclear export	36,64
	⁴⁹³ L <u>S</u> R <u>T</u> Q <u>S</u> S <u>P</u> L <u>P</u> Q ⁵⁰³ (Ser498)		
HDAC7	¹⁵⁰ L <u>R</u> K <u>T</u> V <u>S</u> E <u>P</u> N <u>L</u> K ¹⁶⁰ (Ser155)	Binding to 14-3-3 protein and nuclear export	81
	¹⁷⁶ L <u>L</u> R <u>K</u> E <u>S</u> A <u>P</u> P <u>S</u> L ¹⁸⁶ (Ser181)		
	³¹⁶ L <u>S</u> R <u>T</u> R <u>S</u> E <u>P</u> L <u>P</u> P ³²⁶ (Ser321)		
	⁴⁴⁴ L <u>S</u> R <u>A</u> Q <u>S</u> S <u>P</u> A <u>A</u> P ⁴⁵⁴ (Ser449)		
CREB	¹²⁸ L <u>S</u> R <u>R</u> P <u>S</u> Y <u>R</u> K <u>I</u> L ¹³⁸ (Ser133)	Transcriptional activation of CREB-responsive genes via recruitment of co-activators CBP/p300	30
CERT	¹²⁷ L <u>R</u> R <u>H</u> G <u>S</u> M <u>V</u> S <u>L</u> V ¹³⁷ (Ser132)	Decreased affinity for PI ₄ P and Golgi membrane dissociation	82
VR1	¹¹¹ L <u>Y</u> D <u>R</u> R <u>S</u> I <u>F</u> E <u>A</u> V ¹²¹ (Ser116)	Enhanced expression and activity of VR1 in response to capsaicin and low pH	42
HPK1	¹⁶⁶ L <u>A</u> R <u>R</u> L <u>S</u> F <u>I</u> G <u>T</u> P ¹⁷⁶ (Ser171)	Activation of JNK/SAPK and NF-κB pathways in the adaptive immune response	43
TLR5	⁸⁰⁰ L <u>M</u> K <u>H</u> Q <u>S</u> I <u>R</u> G <u>F</u> V ⁸¹⁰ (Ser805)	p38 MAPK activation and increased production and release of inflammatory cytokines e.g. IL-8	83
c-Jun	⁵³ L <u>R</u> A <u>K</u> N <u>S</u> D <u>L</u> L <u>T</u> S ⁶³ (Ser58)	Suppression of JNK signaling pathway, possibly by modulation of c-Jun phosphorylation by JNK	84,85
SPHK2	⁴¹⁶ L <u>H</u> R <u>S</u> V <u>S</u> D <u>L</u> PLP ⁴²⁶ (Ser421)	Nuclear export	46

PKD1 Modification Sites and Domains



PKD is responsive to physiologically important stimuli and may regulate fundamental processes such as cell proliferation, motility, death, cardiac hypertrophy and inflammation.

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Summary



- The multitude of PKD domains and the wide variety of proteins that interact with them point to multiple and diverse biological roles for the enzyme.
- PKD emerges as a valuable target for development of novel therapeutic approaches in common diseases, including cardiac hypertrophy and cancer.
- Information regarding the structure of this kinase, which is still absent from the field, will greatly facilitate the design of specific drugs that modulate PKD signaling.



Thank you