## Phylogeny

PRKD3 is one of three vertebrate Protein Kinase D paralogs. It branches earlier than PKD1 and PKD2, forming a distinct lineage within the family (Reinhardt et al., 2020, pp. 1-3). In the Manning kinome, all PKDs, including PRKD3, are allocated to the CAMK group, PKD subfamily (Reinhardt et al., 2020, pp. 1-3). A large-scale multi-omics study instead assigns PRKD3 to the AGC kinase group, underscoring a classification discrepancy (Unknown Authors, 2021, pp. 19-25). No curated list of non-mammalian orthologs is provided (Reinhardt et al., 2020, pp. 1-3).

## Reaction Catalyzed

protein-Ser/Thr + ATP ⇌ protein-Ser/Thr-phosphate + ADP (Unknown Authors, 2021, pp. 19-25).

## Cofactor Requirements

Catalytic turnover requires ATP and a divalent metal ion, typically Mg²⁺ (Zhang et al., 2021, pp. 1-2).

## Substrate Specificity

PRKD3 preferentially phosphorylates serine/threonine residues located in the consensus sequence LXRXXpS/pT (Unknown Authors, 2024a, pp. 37-41).

## Structure

• Modular organization: N-terminal ubiquitin-like dimerization domain (ULD) → tandem C1 domains (C1a, C1b) that bind diacylglycerol (DAG) → pleckstrin-homology (PH) domain → C-terminal bilobal kinase domain (Reinhardt et al., 2020, pp. 3-4).  
• The ULD mediates homodimerization via a conserved phenylalanine essential for activation-loop trans-autophosphorylation (Reinhardt et al., 2020, pp. 3-4).  
• C1a supplies high-affinity DAG sensing and membrane recruitment; lipid binding is modulated by surrounding phosphatidylserine (Reinhardt et al., 2020, pp. 8-10).  
• The PH domain resembles DAPP1/TAPP1 PH domains but binds lipids weakly on its own, suggesting additional partners are required for stable membrane residence (Reinhardt et al., 2020, pp. 3-4).  
• The kinase domain contains the catalytic Lys, HRD and DFG motifs, a conserved regulatory spine, and a Chk2-like activation loop with Ser731, Ser738 and Ser742 (Unknown Authors, 2024a, pp. 37-41).  
• PRKD3 lacks the C-terminal PDZ-binding motif found in PKD1/2, indicating divergent scaffold interactions (Reinhardt et al., 2020, pp. 5-7).

## Regulation

• Activation-loop phosphorylation on Ser731, Ser738 and Ser742 is introduced by trans-autophosphorylation within the ULD-mediated dimer and/or by upstream PKC isoforms, switching the kinase to an active conformation (Unknown Authors, 2024a, pp. 37-41; Reinhardt et al., 2020, pp. 3-4).  
• Additional sites include Ser910 (autophosphorylation) and Tyr749; both influence catalytic output, though their full physiological roles remain unresolved (Unknown Authors, 2024c, pp. 37-41).  
• DAG binding to the C1 domains relieves autoinhibition and promotes membrane localization required for activation-loop phosphorylation (Reinhardt et al., 2020, pp. 1-3).  
• Certain ATP-competitive inhibitors paradoxically enhance membrane translocation and phosphorylation, illustrating tight allosteric coupling between the active site and regulatory modules (Reinhardt et al., 2020, pp. 5-7).

## Function

PRKD3 is broadly expressed but markedly enriched in triple-negative breast cancer (TNBC) cells, where it localizes to endolysosomal compartments (Unknown Authors, 2024a, pp. 37-41). Upstream activation is driven by DAG generated downstream of GPCR- or RTK-stimulated phospholipase C, frequently involving PKC family kinases (Reinhardt et al., 2020, pp. 1-3). Verified substrates include PI4KIIIβ and CERT (lipid transport at the trans-Golgi network), cortactin and SSH1L (actin dynamics), and class II HDACs (gene transcription) (Unknown Authors, 2024b, pp. 37-41). PRKD3 supports NF-κB activation under oxidative stress, regulates vesicle trafficking, cell migration and lysosomal homeostasis, and interacts with Rabaptin-5 to couple activity to endocytic pathways (Unknown Authors, 2024a, pp. 37-41).

## Inhibitors

• First-generation ATP-competitive scaffold: bisindolylmaleimide Ro 31-8220 inhibits all PKDs with limited selectivity (Reinhardt et al., 2020, pp. 5-7).  
• Second-generation compounds CRT0066101, CRT0066051 and pyrazolopyrimidine 3-IN-PP1 show nanomolar IC₅₀ values against PRKD3 but still inhibit other PKD isoforms (Wang & Wipf, 2022, pp. 6-8).  
• Amidobipyridyl analogues and CID755673 derivatives are under development to improve potency and pharmacokinetics, though comprehensive PRKD3 selectivity data remain scarce (Wang & Wipf, 2022, pp. 14-17).  
• Phorbol-ester DAG mimetics bind the C1 domains and may acutely activate or induce prolonged desensitization of PRKD3 depending on exposure (Gilles et al., 2021, pp. 1-3).

## Other Comments

Over-expression and altered localization of PRKD3 drive TNBC progression by sustaining lysosomal function and invasive migration (Unknown Authors, 2024a, pp. 37-41). Mutation of the conserved ULD phenylalanine disrupts dimerization and abolishes activation, explaining potential loss-of-function variants (Reinhardt et al., 2020, pp. 3-4). Dysregulated PRKD3 activity also contributes to oxidative-stress resistance and broader oncogenic signaling (Zhang et al., 2021, pp. 1-2).

## 9. References

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