## Phylogeny

LIM kinase 2 (LIMK2) belongs to the Tyrosine-Kinase-Like (TKL) group, LISK family, LIMK sub-family in the canonical kinome classification (Manning et al., 2002; Shah & Cook, 2023). Some studies have alternatively placed the enzyme within the STE, CaMK or AGC groups (Smolich et al., 1997; Vallée et al., 2018; Shah & Cook, 2023). The LIMK family is restricted to vertebrates and a few insects, absent from C. elegans and yeast, and comprises only two paralogues, LIMK1 and LIMK2, which likely arose from an early vertebrate gene-duplication event (Scott & Olson, 2007; Shah & Cook, 2023). Orthologues of LIMK2 have been reported in human, mouse, rat, chicken, Xenopus and Drosophila species (Scott & Olson, 2007; Ribba et al., 2022).

## Reaction Catalyzed

ATP + protein ⇌ ADP + phosphoprotein (Hanke et al., 2022; Scott & Olson, 2007; Chatterjee et al., 2022).

## Cofactor Requirements

Catalytic activity requires a divalent metal ion; Mg²⁺ is the physiologically preferred cofactor, while Mn²⁺ can substitute in vitro (Knape et al., 2017; Lovitt et al., 2010).

## Substrate Specificity

• Dual-specificity kinase that phosphorylates Ser, Thr and Tyr residues (Hanke et al., 2022; Chatterjee et al., 2022).  
• Kinome-wide profiling groups LIMK2 with CAMK-like enzymes, suggesting preference for basic or hydrophobic residues near the phospho-acceptor (Johnson et al., 2023).  
• Phosphotyrosine consensus is defined but the exact sequence was not provided (Yaron-Barir et al., 2024).  
• Substrate recognition involves a “rock-and-poke” mechanism in which a distal anchor helix docks the substrate before the catalytic step (Chatterjee et al., 2022).  
• LIMK2-1 isoform uses an R/K-V/I-X-F motif to engage protein phosphatase 1 (Vallée et al., 2018).

## Structure

The protein comprises two N-terminal LIM zinc-finger domains, a central PDZ domain, a Pro/Ser-rich segment, and a C-terminal protein-kinase domain (Scott & Olson, 2007; Manetti, 2012).  
Key features of the kinase domain (Chatterjee et al., 2022; Villalonga et al., 2023):  
– canonical two-lobe fold with ATP-binding cleft between an antiparallel β-sheet N-lobe and an α-helical C-lobe;  
– glycine-rich loop (ATP cap), VAIK motif (lysine engages ATP adenine), catalytic HRD X K XX N loop, and DFG motif governing ATP access;  
– active state stabilized by a Lys-Glu salt bridge (K368–E384 in LIMK1 numbering);  
– atypical catalytic loop sequence (DLNSHN) and Asn at HRD + 2 may influence ATP affinity;  
– pronounced conformational flexibility in the G-loop, αC helix, DFG switch and activation loop.  
The LIM and PDZ modules mediate intra- and intermolecular interactions and contribute to subcellular localisation (Manetti, 2012).

## Regulation

• Activation-loop phosphorylation: Thr505 (LIMK2a) or Thr484 (LIMK2b/2-1) is phosphorylated by ROCK1/2, PAK1/2/4, MRCKα and Aurora A, generating a Thr–Arg (DFG + 3) salt bridge that locks the kinase in the active DFG-in state (Hanke et al., 2022; Chatterjee et al., 2022; Shah & Cook, 2023).  
• N-terminal LIM/PDZ domains provide auto-inhibitory control (Manetti, 2012).  
• Homodimerisation, Hsp90 binding and trans-phosphorylation enhance stability and activity (Scott & Olson, 2007; Manetti, 2012).  
• PKC-mediated phosphorylation at Ser283 and Thr494 limits nuclear import (Vallée et al., 2018).  
• Slingshot phosphatases dephosphorylate and inactivate LIMK2 (Manetti, 2012).

## Function

LIMK2 is ubiquitously expressed, with higher levels in testis and brain, and localises to both cytoplasm and nucleus (Ribba et al., 2022; Chatterjee et al., 2022; Shah & Cook, 2023). Acting downstream of Rho-family GTPases (RhoA, Rac, Cdc42), activated LIMK2 phosphorylates ADF/cofilin on Ser3, suppressing its actin-severing activity and thereby stabilising filamentous actin (Chatterjee et al., 2022; Villalonga et al., 2023). Through this pathway, LIMK2 regulates cell motility, morphology, invasion, neurite outgrowth, cell-cycle progression, spermatogenesis and platelet function (Scott & Olson, 2007; Manetti, 2012; Chatterjee et al., 2022). Reported interactors include Hsp90, Neurofibromin (NF1) and ROCK1 (Scott & Olson, 2007; Shah & Cook, 2023; Vallée et al., 2018).

## Inhibitors

Selective small-molecule probes have been developed: LIMKi3 (type I), TH470 (type II) and TH257 (type III). TH257 binds the inactive DFG-out conformation. All show low-nanomolar potency and good kinome selectivity (Hanke et al., 2022; Chatterjee et al., 2022).

## Other Comments

Aberrant LIMK2 signalling is linked to cancer invasion/metastasis, fragile X syndrome, cardiovascular disorders and urogenital defects (Scott & Olson, 2007; Manetti, 2012; Hanke et al., 2022; Ribba et al., 2022). Three human isoforms generated by alternative splicing—LIMK2a, LIMK2b and LIMK2-1—differ in length, localisation and mechanism; LIMK2-1 contains a C-terminal PP1-inhibitory module and modulates actin dynamics by blocking cofilin dephosphorylation rather than by direct phosphorylation (Vallée et al., 2018; Shah & Cook, 2023).

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