## Phylogeny

Orthologous LIMK1 proteins are documented in Mus musculus, Rattus norvegicus, Danio rerio, Xenopus laevis and Drosophila melanogaster, whereas no homologues are detected in Caenorhabditis elegans or Saccharomyces cerevisiae, indicating acquisition in metazoans after divergence from these lineages (scott2007limkinasesfunction pages 1-3, shah2023limk2amultifaceted pages 13-16).  
Within the human kinome, LIMK1 together with LIMK2 forms the LIMK subfamily inside the LISK family of the Tyrosine Kinase-Like (TKL) group; the closest paralogues are the TESK1/2 kinases that branch on the same TKL clade (shah2023limk2amultifaceted pages 1-3, scott2007limkinasesfunction pages 3-4).

## Reaction Catalyzed

ATP + protein-L-Ser/Tyr ⇌ ADP + protein-L-Ser/Tyr-phosphate (salah2019lessonsfromlimk1 pages 6-7).

## Cofactor Requirements

Catalytic turnover requires divalent cations; all enzymatic assays and crystal structures were performed in the presence of 2–2.5 mM MgCl₂ (salah2019lessonsfromlimk1 pages 4-5, salah2019lessonsfromlimk1 pages 10-11).

## Substrate Specificity

Physiological substrates are the actin-binding proteins cofilin-1 (CFL1), cofilin-2 (CFL2) and destrin (DSTN), each phosphorylated at their N-terminal Ser3 (salah2019lessonsfromlimk1 pages 1-2, scott2007limkinasesfunction pages 1-3).  
LIMK1 accepts a Ser3→Tyr mutant of CFL1 but not a Ser3→Thr variant, establishing dual Ser/Tyr specificity and threonine exclusion; structural analysis attributes threonine rejection to steric shielding by activation-loop Leu481 (salah2019lessonsfromlimk1 pages 6-7).  
Substrate recognition is governed by a distal anchor-helix interface on CFL1 that docks into a pocket formed by the αF–αG loop and activation segment of LIMK1; no linear consensus motif has been identified (chatterjee2022structuralaspectsof pages 4-6).

## Structure

Full-length LIMK1 consists of two N-terminal LIM zinc-finger domains, a PDZ domain, a proline/serine-rich linker and a C-terminal kinase domain spanning residues 329–638 (manetti2012limkinasesare pages 1-3).  
Nine X-ray structures of the isolated kinase domain are available, including substrate complexes 5L6W and 5HVK and inhibitor complexes 3S95 and 5NXC (chatterjee2022structuralaspectsof pages 1-3, unknownauthors2018humanlimdomain pages 1-4).  
The kinase domain displays the canonical bilobal fold with a GKGCFG P-loop, VAIK Lys368, catalytic HRDLNSHN motif and DFG motif; the active conformation shows the DFG-in position, an intact Lys368–Glu384 salt bridge and fully assembled catalytic and regulatory hydrophobic spines (chatterjee2022structuralaspectsof pages 1-3).  
A 13-residue C-terminal shift of the αG helix, together with an enlarged αF–αG loop, forms the unique substrate-docking cradle required for the “rock-and-poke” mechanism (chatterjee2022structuralaspectsof pages 4-6).  
Activation-loop Thr508 phosphorylation locks the DFG-in state by a Thr508–Arg483 salt bridge (chatterjee2022structuralaspectsof pages 3-4).  
Binding of PF-477736 (5NXC) induces an αC-out, R-spine-broken conformation, whereas staurosporine (3S95) captures an active DFG-in/αC-in state; both structures illustrate substantial P-loop and αC mobility (salah2019lessonsfromlimk1 pages 8-9).  
Cys349 within the P-loop is unique to LIMK1 and provides a covalent attachment point exploited for isoform-selective inhibitor design (mandel2025covalenttargetingleads pages 1-4).

## Regulation

• Phosphorylation  
– Thr508: activated by PAK1, PAK4 and ROCK1, increasing catalytic efficiency >10-fold (salah2019lessonsfromlimk1 pages 10-11, manetti2012limkinasesare pages 3-6).  
– Ser323: phosphorylated by MK2 downstream of p38 MAPK, relieving N-terminal autoinhibition (manetti2012limkinasesare pages 9-11).  
– Autophosphorylation occurs on serine and tyrosine residues but not on Thr508 (scott2007limkinasesfunction pages 4-5).  
– SSH1 phosphatase dephosphorylates Thr508, attenuating activity (scott2007limkinasesfunction pages 4-5).  
• Protein–protein interactions  
– Hsp90 binds residues 387–402, stabilising the kinase and promoting dimer-mediated trans-autophosphorylation (scott2007limkinasesfunction pages 3-4).  
• Ubiquitin/Proteolysis  
– RNF6 and parkin ubiquitinate LIMK1, targeting it for proteasomal degradation (manetti2012limkinasesare pages 9-11, scott2007limkinasesfunction pages 7-8).  
– Caspase-3 cleavage at Asp240 removes the inhibitory N-terminal segment, generating a constitutively active fragment (manetti2012limkinasesare pages 3-6).  
• miRNA  
– miR-134 represses LIMK1 translation (manetti2012limkinasesare pages 9-11).  
• Conformational control  
– Type-2 and type-3 inhibitors bind preferentially to the non-phosphorylated DFG-out conformation; Thr508 phosphorylation diminishes their affinity (chatterjee2022structuralaspectsof pages 4-6).

## Function

LIMK1 is enriched in brain and skeletal muscle, whereas LIMK2 is more broadly expressed (chatterjee2022structuralaspectsof pages 10-11).  
In adult mouse hippocampus, LIMK1 accounts for ~70 % of phosphorylated cofilin, underscoring its dominant neuronal role (salah2019lessonsfromlimk1 pages 1-2).  
Upstream regulators include ROCK1/2, PAK1/4 and BMPR2; signals from RhoA, Rac1 and Cdc42 converge on these kinases to activate LIMK1 (salah2019lessonsfromlimk1 pages 2-2, manetti2012limkinasesare pages 9-11).  
Downstream, LIMK1 phosphorylates CFL1, CFL2 and DSTN at Ser3, inhibiting their actin-severing activity and thereby stabilising F-actin to control cell migration, neurite extension, cytokinesis and differentiation (salah2019lessonsfromlimk1 pages 1-2, scott2007limkinasesfunction pages 1-3).  
Direct interaction with the BMPR2 C-terminal tail links BMP receptor signalling to actin remodelling (unknownauthors2018humanlimdomain pages 1-4).

## Inhibitors

Staurosporine binds the ATP site in an active DFG-in conformation (PDB 3S95) (unknownauthors2018humanlimdomain pages 1-4).  
PF-477736 engages the ATP pocket but enforces an αC-out inactive state (PDB 5NXC) (salah2019lessonsfromlimk1 pages 8-9).  
LIMKi3 is a nanomolar type-I inhibitor, though limited by tubulin off-target activity (salah2019lessonsfromlimk1 pages 2-2).  
LX-7101 is a dual LIMK/ROCK inhibitor that progressed to phase I clinical testing for glaucoma (salah2019lessonsfromlimk1 pages 2-2).  
Type-III ligands exemplified by “Ligand 22” target the DFG-out allosteric pocket with high kinome selectivity (chatterjee2022structuralaspectsof pages 8-10).  
Kinase profiling identified the approved BRAF inhibitor dabrafenib as a LIMK1 binder (salah2019lessonsfromlimk1 pages 7-7).  
Covalent inhibitors that alkylate Cys349 achieve LIMK1 isoform selectivity (mandel2025covalenttargetingleads pages 1-4).

## Other Comments

Haploinsufficiency of LIMK1 within the 7q11.23 microdeletion contributes to the cognitive phenotype of Williams–Beuren syndrome (scott2007limkinasesfunction pages 1-3).  
Hyperactive LIMK1 signalling is implicated in Fragile X syndrome and C9ORF72-linked amyotrophic lateral sclerosis (salah2019lessonsfromlimk1 pages 1-2).  
Altered LIMK1 activity is associated with Alzheimer’s disease, Parkinson’s disease and invasive behaviour in breast and colon cancers (mandel2025covalenttargetingleads pages 1-4).  
Systemic LIMK inhibition disrupts platelet activation and reduces osteoblast numbers, underscoring the therapeutic need for isoform-specific targeting (chatterjee2022structuralaspectsof pages 10-11).

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