Phylogeny  
CaMKK2 is a member of the Ca2+/calmodulin-dependent protein kinase (CAMK) group, CaMKK family, and is most closely related to CaMKK1 (O’Byrne et al., 2020). Orthologues are found in Homo sapiens (Q96RR4), Mus musculus (Q3UFV3), Rattus norvegicus (Q63537), Danio rerio (Q7SXW4) and Drosophila melanogaster (CG1491) (Najar et al., 2021; Racioppi & Means, 2012). Mammalian sequences share >90 % identity across the kinase core, whereas invertebrate homologues retain catalytic motifs but diverge in regulatory regions (Santiago et al., 2018).

Reaction Catalyzed  
ATP + protein-Ser/Thr ⇌ ADP + protein-O-phospho-Ser/Thr (Najar et al., 2021; Marcelo et al., 2016).

Cofactor Requirements  
• Ca2+/calmodulin binding is essential for activation (Najar et al., 2021).  
• Catalysis requires divalent Mg2+ or Mn2+ (Langendorf et al., 2020; Profeta et al., 2019).

Substrate Specificity  
Preferred consensus: [R/K]-X-[R/K]-X-S/T\*-Φ, favouring basic residues at −3/−2 and a hydrophobic residue at +1 (Langendorf et al., 2020). Verified phosphorylation targets include CaMKI Thr177, CaMKIV Thr200, AMPKα Thr172 and CaMK1D Thr180 (Racioppi & Means, 2012; Fujiwara et al., 2016).

Structure  
The protein comprises an N-terminal regulatory segment (~1–125), a bilobal kinase domain (~125–400) and a C-terminal autoinhibitory/CaM-binding region (~400–505) (Najar et al., 2021). Crystal structures 6BKS and 6BYH show the active kinase core bound to ATP-competitive inhibitors with an ordered activation loop (Profeta et al., 2019; Marcelo et al., 2016). Key catalytic motifs are VAIK Lys157 (ATP anchoring), HRD His301-Asp303 (catalysis) and DFG Asp319 (Mg2+ coordination) (Racioppi & Means, 2012). Activation depends on a Lys157–Glu175 salt bridge and a completed hydrophobic spine; the C-terminal tail blocks the substrate groove until displaced by Ca2+/CaM (Santiago et al., 2018).

Regulation  
Post-translational modifications  
– Thr85 autophosphorylation confers Ca2+-independent activity (Langendorf et al., 2020).  
– Thr200 autophosphorylation in the activation loop is required for full activity (Santiago et al., 2018).  
– PKA phosphorylates Ser495 and Ser511, generating 14-3-3 docking sites and suppressing activity (Langendorf et al., 2020).  
– Ser129 (by CDK5) and Thr287 (by GSK3) phosphorylation are inhibitory (Racioppi & Means, 2012; Santiago et al., 2018).

Allosteric control  
Ca2+/calmodulin removes the autoinhibitory tail and aligns αC for catalysis (Najar et al., 2021). Phospho-Ser495/Ser511 recruit 14-3-3 proteins, stabilising an inactive state; fusicoccins further tighten this complex (Santo et al., 2020). cAMP-PKA signalling enhances 14-3-3 binding, linking Ca2+ and cAMP pathways (Langendorf et al., 2020).

Function  
Expression is enriched in brain (arcuate nucleus, hippocampus), heart, liver and osteoblast lineage cells (Najar et al., 2021; Beghi et al., 2022). Activation follows intracellular Ca2+ elevation that enables Ca2+/CaM binding (Marcelo et al., 2016). Downstream:  
– AMPK activation regulates glucose uptake, fatty-acid oxidation, autophagy and cardioprotection (Beghi et al., 2022; Fujiwara et al., 2016).  
– CaMKI/IV phosphorylation promotes CREB-dependent transcription supporting neurite outgrowth and synaptic plasticity (Marcelo et al., 2016; Racioppi & Means, 2012).  
– Facilitates GLUT4 translocation during cardiac ischaemic stress (Beghi et al., 2022).

Inhibitors  
STO-609 is an ATP-competitive inhibitor (IC50 ≈ 80 nM) but is limited by poor solubility and CYP1A2 metabolism (Langendorf et al., 2020; York et al., 2017). Kinase-focused library screens yielded additional nanomolar scaffolds with supporting structural data (Profeta et al., 2019). SGC-CaMKK2-1 has been released as a selective cellular probe (Wells et al., 2023).

Other Comments  
CaMKK2 promotes prostate cancer growth and metastasis via AMPK-dependent metabolic reprogramming (Marcelo et al., 2016; O’Byrne et al., 2020). Pharmacological or genetic inhibition alleviates non-alcoholic fatty liver disease in mouse models (York et al., 2017). Cardiac-specific knockout exacerbates pressure-induced hypertrophy and mortality, highlighting a cardioprotective role (Beghi et al., 2022).

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