## Phylogeny

NEK6 is a member of the NIMA-related kinase (Nek) family (11 human paralogues). Within the Manning kinome it belongs to the CMGC group. Full-length sequence trees cluster NEK6 with Nek4/7/8/9/10, whereas kinase-domain analysis places it with Nek7 and Nek10. NEK6 and its closest paralogue NEK7 share > 85 % identity in their kinase domains. Orthologues are present throughout eukaryotes, including fungi, yeast and metazoans (Bachus et al., 2022; Fry et al., 2012; Li et al., 2025; Moniz et al., 2011; Yin et al., 2003).

## Reaction Catalyzed

Protein-Ser/Thr + ATP ⇌ phospho-protein-Ser/Thr + ADP (Bachus et al., 2022; Moniz et al., 2011; Yin et al., 2003).

## Cofactor Requirements

Requires divalent Mg²⁺ or Mn²⁺ ions for activity (Bachus et al., 2022; Moniz et al., 2011; Yin et al., 2003).

## Substrate Specificity

A kinome-wide peptide screen defined a consensus motif spanning positions –5 to +4, with preference for hydrophobic residues flanking the target Ser/Thr (Johnson et al., 2023; Moniz et al., 2011). Reports of p70 S6K phosphorylation at Thr412 are conflicting—initially positive (Belham et al., 2001) but not confirmed by later studies (Yin et al., 2003; Fry et al., 2012).

## Structure

A compact 302–313-residue protein composed mainly of an N-terminal catalytic kinase domain plus a short disordered N-terminal tail; it lacks the extended C-terminal/coiled-coil regions common to several Nek kinases (Bachus et al., 2022; Moniz et al., 2011; Li et al., 2025). Conserved elements include the C-helix, HRD catalytic motif and an activation loop bearing Ser206. A tyrosine-down sequence in the N-lobe provides autoinhibition. Mutating Lys74 (K74M) in the ATP pocket abolishes activity (Fry et al., 2012; Belham et al., 2001).

## Regulation

Activity peaks in mitosis and depends on phosphorylation of Ser206 by the upstream kinase NEK9; NEK9 itself is activated by CDK1 and Plk1. Thr201/202 within the activation loop can also be phosphorylated. The NEK9–NEK6 interaction is attenuated by dynein light-chain LC8-type 1. DNA-damage kinases Chk1/Chk2 suppress NEK6, and the S206A mutant is catalytically compromised. Owing to the absence of a coiled-coil, NEK6 relies on NEK9 rather than trans-autophosphorylation for activation (Bachus et al., 2022; Yin et al., 2003; Moniz et al., 2011; Meirelles et al., 2010).

## Function

Highest mRNA levels occur in liver, with notable expression in brain and kidney (Belham et al., 2001). NEK6 functions as a hub kinase (≈ 91 partners) essential for spindle assembly, centrosome separation, chromosome segregation and cytokinesis; depletion causes G2/M arrest and apoptosis (Moniz et al., 2011; Yin et al., 2003; Fry et al., 2012). Upstream activators: CDK1 → Plk1 → NEK9. Reported substrates: Kif11 (Ser1033), Eg5, Hsp72 (Thr66), TPP1, Oct1, CIR, PTN, RAD26L, RBBP6 and TRIP4. Interactors include Kif11, Kif20A, Hsp72, Pin1 and UNC-45 (Bachus et al., 2022; Meirelles et al., 2010). Additional roles: telomere length control, recovery from DNA-damage checkpoints, suppression of p53-induced senescence and modulation of NF-κB signalling (Bachus et al., 2022).

## Inhibitors

Isogranulatimide binds the ATP site and inhibits NEK6, showing greater potency toward the unphosphorylated S206A variant (Moraes et al., 2015).

## Other Comments

NEK6 is frequently over-expressed in tumours; high levels correlate with poor prognosis, metastasis and drug resistance. Oncogenic mutations (e.g., Y295C, Y291\*) and aneuploidy induction have been reported. A cancer-specific circRNA (circNek6) influences Wnt signalling, and miR-141-3p targets NEK6 mRNA (Moniz et al., 2011; Bachus et al., 2022; Li et al., 2025).

## 9. References

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