## Phylogeny

Member of the NIMA-related kinase (NEK) family (NEK1–NEK11) that originated from the Aspergillus nidulans NimA kinase (Bachus et al., 2022; “Stop Ne(c)king around”, 2014).  
Full-length sequence analysis groups NEK3 with NEK1, NEK2 and NEK5, whereas kinase-domain trees place it with NEK1, NEK2, NEK4, NEK5 and NEK11 (Bachus et al., 2022).  
NEK3, NEK5 and NEK11 constitute a sub-clade that lacks the canonical autoinhibitory tyrosine present in most other NEKs (Bachus et al., 2022).  
Orthology is well conserved: human NEK3 shares 56 % amino-acid identity with mouse Nek3, and rodent as well as wider vertebrate orthologues are documented (Harrington & Clevenger, 2016; “Stop Ne(c)king around”, 2014).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + L-O-phosphoseryl/threonyl-[protein] (Bachus et al., 2022)

## Cofactor Requirements

Catalytic activity requires divalent cations; both Mg²⁺ and Mn²⁺ support phosphorylation in vitro (“Cell Cycle Studies…”, 2011).

## Substrate Specificity

Validated cellular substrates are paxillin (PXN) and VAV2 during prolactin signalling (Bachus et al., 2022).  
NEK3 autophosphorylates and phosphorylates β-casein in vitro (“Cell Cycle Studies…”, 2011).  
Phosphoproteomic / Scansite analyses suggest a preferred motif that was further refined by Johnson (2023); a definitive consensus sequence is still unresolved (Harrington & Clevenger, 2016).

## Structure

Single-polypeptide, 506-residue protein comprising an N-terminal kinase domain and a C-terminal regulatory tail that lacks coiled-coil motifs (“Stop Ne(c)king around”, 2014).  
AlphaFold modelling predicts the canonical bilobal kinase fold with a distinct regulatory tail (Bachus et al., 2022).  
Conserved catalytic motifs: VAIK (Lys33), HRD (Asp127) and DFG; mutation of Lys33 or Asp127 abolishes activity (“Cell Cycle Studies…”, 2011).  
Activation loop contains regulatory Thr165 (Harrington & Clevenger, 2016).  
Regulatory tail harbours a PEST motif with phospho-Thr475 (“Stop Ne(c)king around”, 2014).  
Lacks the “tyrosine-down” autoinhibitory motif characteristic of several other NEKs (Bachus et al., 2022).  
No experimental crystal structure is currently available (Bachus et al., 2022).

## Regulation

• Autophosphorylation enhances catalytic output (“Cell Cycle Studies…”, 2011).  
• JAK2 phosphorylates Thr165 within the prolactin receptor (PRLR) complex, promoting focal-adhesion remodelling and cell migration (Harrington & Clevenger, 2016).  
• Phosphorylation of Thr475 in the PEST motif influences cell morphology and polarity (“Stop Ne(c)king around”, 2014).  
• Kinase activity decreases as NaCl concentration rises, indicating ionic-strength sensitivity (“Cell Cycle Studies…”, 2011).  
• Hormone-induced association with PRLR and VAV2 positions NEK3 for substrate engagement (Bachus et al., 2022).

## Function

Expression: high in testis, prostate, ovary and brain; moderate-to-low in lung and liver (“Stop Ne(c)king around”, 2014).  
Localization: cytoplasmic during interphase and associated with spindle-like structures in mitosis (“Cell Cycle Studies…”, 2011).  
Neuronal roles include regulation of microtubule acetylation, neurite morphogenesis and polarity (Moniz et al., 2011).  
In prolactin signalling, PRLR/JAK2 activation enables NEK3 to phosphorylate VAV2 and PXN, activating RAC1/RhoA, driving actin remodelling and increasing motility in breast-cancer cells (Bachus et al., 2022).  
Knock-down results in spindle defects, implicating NEK3 in mitotic spindle organisation (“Cell Cycle Studies…”, 2011).  
Reported to participate in DNA-damage repair pathways (Nguyen et al., 2023).

## Other Comments

Aberrant NEK3 activity promotes breast-cancer cell migration and invasion (Bachus et al., 2022).  
Cancer-associated mutations include D413Y in ovarian tumours and Y398\* truncation in stomach cancer cell lines (Moniz et al., 2011).  
A truncating polymorphism at chromosome 13q14 lies within a recognised cancer hotspot (“Stop Ne(c)king around”, 2014).  
COSMIC records the highest NEK3 mutation frequency in pancreatic tissue (3.23 %) (Nguyen et al., 2023).  
Prognostic impact is tumour-type dependent; elevated NEK3 expression correlates with either favourable or unfavourable survival depending on the cancer (Nguyen et al., 2023).

## References

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