## Phylogeny

NEK2 is one of eleven human NIMA-related kinases (NEK1–NEK11) (Dana et al., 2022, pp. 1-2). Its catalytic domain shares ~42–44 % identity with the founding Aspergillus nidulans kinase NIMA, making it the closest human ortholog (Fry, 2002, pp. 1-3; Dana et al., 2022, pp. 1-2). Documented orthologs such as Pfnek-1 in Plasmodium falciparum illustrate conservation of NEK-type cell-cycle kinases across apicomplexan parasites (Dana et al., 2022, pp. 13-14). Across eukaryotes, NEK kinases localise to microtubule-organising centres, supporting an evolutionarily conserved role in centrosome regulation (Fry et al., 2017, pp. 1-2).

## Reaction Catalyzed

ATP + protein-Ser/Thr ⇌ ADP + protein-O-phospho-Ser/Thr (Dana et al., 2022, pp. 1-2).

## Cofactor Requirements

Mg²⁺ or Mn²⁺ ions are required for catalytic activity (Dana et al., 2022, pp. 28-29).

## Substrate Specificity

NEK2 preferentially phosphorylates Ser/Thr residues within basic sequence contexts (Fry, 2002, pp. 1-3). Verified cellular substrates include CEP250/C-Nap1, CROCC/Rootletin, CEP68, NDC80/Hec1, CDC20, MAD2L1, PP1 catalytic subunit (Thr320), NPM1 (Ser70/Ser88), β-catenin, Dishevelled, TRF1, SGO1 and NECAB3 (Dana et al., 2022, pp. 2-9, 13-14, 28-29).

## Structure

The 445-residue protein contains an N-terminal bilobal kinase domain and a C-terminal regulatory region with two coiled-coils; the first forms a leucine-zipper that mediates homodimerisation and trans-autophosphorylation (Fry, 2002, pp. 1-3). Crystal structures reveal canonical Lys-Glu, HRD and DFG motifs, and an activation loop phosphorylated at Thr175/Thr179 that stabilises the active state (Rellos et al., 2007, pp. 10-11; Fry et al., 2017, pp. 6-8). A non-conserved Cys22 in the Gly-rich loop enables reversible covalent inhibitor binding (Dana et al., 2022, pp. 26-28). Unphosphorylated NEK2 can adopt a DFG-out inactive conformation, captured in complexes with aminopyrazine or benzimidazole inhibitors (Byrne et al., 2020, p. 14).

## Regulation

Post-translational modifications  
• Activating autophosphorylation at Thr170/Ser171 and Thr175; Thr179 and Ser241 are inhibitory (Fang & Zhang, 2016, pp. 1-2; Rellos et al., 2007, pp. 10-11).  
• In interphase, a PP1–MST2 complex suppresses NEK2; Plk1 phosphorylation of MST2 at mitotic entry releases PP1 and activates NEK2 (Dana et al., 2022, pp. 2-4).  
• CDK1 and Plk1 provide additional G2/M-phase activating phosphorylations (Fry et al., 2017, pp. 6-8).  
• PP1 dephosphorylates and inhibits NEK2; ATM signalling enhances PP1 after DNA damage, down-regulating NEK2 (Dana et al., 2022, pp. 6-7).  
• APC/C recognises KEN- and D-box motifs in NEK2A for degradation at mitotic exit (Fang & Zhang, 2016, pp. 1-2; Dana et al., 2022, pp. 7-9).

Allosteric and signalling inputs  
EGFR–PI3K–Akt signalling activates NEK2 via MST2 phosphorylation (Fry et al., 2017, pp. 4-5). CIP2A binding augments activity independently of PP1 (Dana et al., 2022, pp. 2-4).

Transcriptional control  
Expression is up-regulated by FoxM1 and repressed by E2F4, p53 and microRNA-128, resulting in S/G2 accumulation (Fang & Zhang, 2016, pp. 1-2).

## Function

NEK2 localises to centrosomes and kinetochores, with maximal expression in late S/G2 (Dana et al., 2022, pp. 1-2; Fry, 2002, pp. 1-3). It drives centrosome separation by phosphorylating linker proteins (CEP250, CROCC, CEP68) (Dana et al., 2022, pp. 2-4, 28-29) and stabilises kinetochore–microtubule attachments via NDC80 phosphorylation while modulating the spindle-assembly checkpoint through CDC20 and MAD2L1 (Dana et al., 2022, pp. 13-14, 28-29). After DNA damage, NEK2 prevents premature centrosome splitting; this restraint is relieved by ATM-PP1 signalling (Dana et al., 2022, pp. 2-4, 6-7). Additional signalling roles include PP1 Thr320 phosphorylation linking to Akt, β-catenin/Dishevelled phosphorylation interfacing with Wnt, and MST2 interaction situating NEK2 in the Hippo pathway (Dana et al., 2022, pp. 6-9; Fry et al., 2017, pp. 4-5). NEK2 also promotes osteoclast differentiation and bone resorption via heparanase up-regulation (Dana et al., 2022, pp. 6-7).

## Inhibitors

Reported inhibitors include ATP-competitive indolinone SU11652 and related purines (Fry et al., 2017, pp. 6-8); aminopyrazine and benzimidazole scaffolds that lock a DFG-out conformation (Byrne et al., 2020, p. 14); reversible covalent inhibitors targeting Cys22 (Dana et al., 2022, pp. 26-28); the Hec1/NEK2 disruptor T-1101 tosylate with oral bioavailability (Dana et al., 2022, pp. 26-28); and emerging PROTAC degraders and activity-based probes (Dana et al., 2022, pp. 26-28).

## Other Comments

NEK2 is frequently over-expressed in breast, ovarian, colon, uterine endometrial and lung adenocarcinomas, correlating with aneuploidy, tumour aggressiveness and therapy resistance (Dana et al., 2022, pp. 1-2, 13-14). Dysregulated signalling is also implicated in bone disorders, ciliopathies, immune and kidney diseases, and NEK2 orthologs in Plasmodium spp. are prospective anti-malarial targets (Dana et al., 2022, pp. 1-2, 13-14).

## 9. References

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