## Phylogeny

Orthologues are present from invertebrates to vertebrates (Homo sapiens, Mus musculus, Rattus norvegicus, Danio rerio, Drosophila melanogaster “Asator”, Caenorhabditis elegans H05L14.1/dkf-2), underscoring conservation of the catalytic domain (Taylor et al., 2019; Liachko et al., 2014). Kinome‐wide analyses place tau-tubulin kinase 1 (TTBK1) in the Casein Kinase 1 group, TTBK family (Manning et al., 2002). The closest human paralogue is TTBK2, which shares 88 % identity (residues 23-280 of the kinase domain) (Bao et al., 2021).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr/Tyr → ADP + protein-L-Ser/Thr/Tyr-phosphate (Ikezu & Ikezu, 2014).

## Cofactor Requirements

Catalysis requires a divalent cation; crystal structures were solved with Mg²⁺ coordinated to ATP/ADP in the active site (Xue et al., 2013).

## Substrate Specificity

Verified cellular substrates include MAPT/tau (Ser198, Ser199, Ser202, Thr231, Ser396, Ser422, Tyr197), α/β-tubulin, TDP-43 (Ser409/Ser410), SV2A, Cep164, MAP1B and additional MAP family members (Dillon et al., 2020; Bao et al., 2021). A definitive TTBK1 consensus motif has not been defined; in contrast, paralogous TTBK2 prefers S/T-X-pY(+2) or primed CK1-type pS/pT-X-X-S/T sequences (Johnson et al., 2023; Ikezu & Ikezu, 2014).

## Structure

Domain organisation: N-terminal kinase domain (34-297) harbouring VAIK (Lys63), HRD (Asp164) and DFG (176-178) motifs; central regulatory region (297-770) with multiple autophosphorylation sites; poly-glutamate tract (733-771) causing anomalous SDS-PAGE mobility; C-terminal region containing SxIP and PEST motifs (Xue et al., 2013; Bao et al., 2021; Nozal & Martínez, 2019; Kiefer et al., 2014).

3-D structures: Five crystal structures of the kinase domain (PDB 4BTJ, 4BTK, 4BTM, 4NFN, 4NFM) reveal a canonical bilobed kinase fold with ordered activation segment and intact hydrophobic spines (Xue et al., 2013; Kiefer et al., 2014). AlphaFold models predict full-length architecture, positioning acidic insertions near the activation loop (Bao et al., 2021).

Unique features: A flexible insertion N-terminal to the activation loop and an extended poly-glutamate stretch are absent from other CK1 family members and may influence substrate engagement (Bao et al., 2021).

## Regulation

Autophosphorylation at thirteen identified sites (S320, T321, S322, T323, S324, T325, T344, S529, S540, S821, S942, S943, S1061) modulates activity; phospho-silencing abolishes tau-Ser422 phosphorylation, whereas phospho-mimicry reduces overall activity by ~65 % (Bao et al., 2021). TTBK1 can trans-phosphorylate TTBK2 (Bao et al., 2021). Proteolytic processing yields shorter fragments detected in neurodegenerative brain tissue (Taylor et al., 2019). No ubiquitination or allosteric regulators have been reported.

## Function

Expression pattern: mRNA and protein are highly enriched in adult human and mouse cortex, hippocampus and cerebellar granule layer, with negligible expression in peripheral tissues (Bao et al., 2021; Nozal & Martínez, 2019).

Molecular roles:  
• Cytoskeletal regulation—interacts with MAPT, MAP1A/B, MAP2/6, MAP7D2 and MAPRE1, influencing microtubule plus-end dynamics (Bao et al., 2021).  
• Vesicular trafficking—associates with DVL1-3 and AAK1, linking to WNT-regulated endocytic sorting (Bao et al., 2021).  
• Pathological phosphorylation—modifies tau, reducing microtubule affinity and promoting aggregation; phosphorylates TDP-43 at Ser409/410, facilitating cytoplasmic inclusion formation (Dillon et al., 2020). Upstream regulators remain undefined; downstream effects converge on cytoskeletal organisation and synaptic function (Bao et al., 2021).

## Inhibitors

BIIB-TTBKi-284: ATP-competitive; IC₅₀ ≈ 15 nM (TTBK1) and 8 nM (TTBK2); inhibits 13/150 kinases > 50 % at 3 µM (Bao et al., 2021).  
BIIB-TTBK1i: brain-penetrant probe; IC₅₀ ≈ 9.8 nM for tau-Ser422 reduction; 82 % in vivo target occupancy at 75 mg kg⁻¹ with high kinome selectivity (Dillon et al., 2020).  
AZ-1 and AZ-2: anilinoquinazoline derivatives; IC₅₀ = 4.4 µM (AZ-1) and 2.6 µM (AZ-2); confer neuroprotection in phospho-TDP-43 cellular assays (Baier & Szyszka, 2022).

## Other Comments

Elevated TTBK1 expression and activity correlate with Alzheimer’s disease, frontotemporal dementia, amyotrophic lateral sclerosis, chronic traumatic encephalopathy and Down’s syndrome (Nozal & Martínez, 2019). GWAS identify TTBK1 SNPs that modulate Alzheimer’s risk, although specific pathogenic coding variants have not been reported (Nozal & Martínez, 2019). Selective inhibition lowers pathological tau and TDP-43 phosphorylation in preclinical models, highlighting therapeutic potential (Dillon et al., 2020; Bao et al., 2021).

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