## Phylogeny

• BTK is one of five mammalian Tec-family kinases (BTK, BMX, ITK, TEC, TXK) that originated from an ancestral Tec gene present before the emergence of metazoans (ortutay2008phylogenyoftec pages 1-4).  
• Orthologs are documented in unicellular choanoflagellates (Monosiga brevicollis), sponges (Suberites domuncula), insects (Drosophila melanogaster Btk29A), lamprey (Lethenteron camtschaticum), teleost fishes, amphibians (Xenopus tropicalis), birds (Gallus gallus) and a broad range of mammals including mouse, rat, dog, bovine and chimpanzee (ortutay2008phylogenyoftec pages 16-18, perina2012characterizationofbruton’s pages 2-4).  
• A vertebrate-specific insertion within the PH domain (residues 79-99) that forms the Saraste dimer interface is conserved from cartilaginous fish onward (eisen2025conditionalrequirementfor pages 29-32).  
• Within the human kinome BTK is classified in the Tyrosine Kinase group, Tec family, clustering closest to BMX and ITK and clearly separated from Src-, Abl- and Csk-family kinases (manning2002theproteinkinase pages 3-3, ortutay2008phylogenyoftec pages 28-30).

## Reaction Catalyzed

ATP + protein-L-tyrosine ⇌ ADP + protein-O-phospho-L-tyrosine (mao2001crystalstructureof pages 5-6, xing2014brutonstkinhibitors pages 1-2).

## Cofactor Requirements

Catalytic activity requires Mg²⁺ for ATP coordination; Mn²⁺ can substitute in vitro (unknownauthors2019structuralmechanismfor pages 9-9, kueffer2023screeningandcharacterization pages 5-6).

## Substrate Specificity

• Consensus motif: E/D-E/D-Y-X-Φ, where Φ denotes a hydrophobic residue (unknownauthors2019structuralmechanismfor pages 9-9).  
• Amino-acid preferences: acidic residues at −3/−2, Tyr at 0, hydrophobic at +1; positions +2/+3 tolerate small or polar residues (kueffer2023screeningandcharacterization pages 5-6).

## Structure

• Domain organization: N-terminal PH domain with Zn²⁺-binding Btk motif, Tec Homology (TH) segment, SH3, SH2 and C-terminal kinase domain (SH1) (xing2014brutonstkinhibitors pages 1-2, ortutay2008phylogenyoftec pages 7-10).  
• Crystal structure of the isolated kinase domain shows an active-like conformation even when Y551 is unphosphorylated; the activation loop does not occlude the catalytic cleft (mao2001crystalstructureof pages 4-5).  
• Phosphorylation of Y551 triggers rotation of helix C, formation of the Lys430–Glu445 catalytic salt bridge and full activation (mao2001crystalstructureof pages 5-5).  
• Key regulatory elements: glycine-rich loop, activation loop Y551, autophosphorylation site Y223, hydrophobic spine and conserved catalytic residues Lys430/Glu445 (mao2001crystalstructureof pages 6-7).  
• The PH-TH module contains an extended S2 loop (Ile92-Ile95) that mediates Saraste dimerization required for efficient membrane activation (eisen2025conditionalrequirementfor pages 26-28).

## Regulation

• Phosphorylation  
– Y551 by Src-family kinases LYN and SYK activates catalytic function (velasquez2024inbtkphosphorylated pages 1-2, mao2001crystalstructureof pages 5-6).  
– Y223 autophosphorylation tracks enzymatic activity but is dispensable for biological function (velasquez2024inbtkphosphorylated pages 1-2, unknownauthors2024investigatingresistancemechanisms pages 80-87).  
– S180 by AKT and T316 by PLK1 modulate signaling dynamics (velasquez2024inbtkphosphorylated pages 1-2).  
• Ubiquitination at K430 and K595 regulates protein stability (unknownauthors2024investigatingresistancemechanisms pages 121-126).  
• Allosteric control: PIP3 binding to the PH domain and PH-TH dimerization enhance membrane recruitment and activity (eisen2025conditionalrequirementfor pages 26-28).

## Function

• Expression: high in B lymphocytes; also detected in macrophages, dendritic cells and subsets of T cells (unknownauthors2025dimerizationofthe pages 3-4).  
• Upstream activators: LYN, SYK and PKCβ (velasquez2024inbtkphosphorylated pages 10-10).  
• Principal substrates and partners: phosphorylates PLCG2 in cooperation with the adaptor BLNK, initiating Ca²⁺ mobilization, PKC activation and NF-κB signaling (xing2014brutonstkinhibitors pages 1-2).  
• Sustains AKT and ERK signaling in lymphoma cells (hu2021follicularlymphoma–associatedbtk pages 11-13).  
• Contributes to Toll-like receptor signaling in innate immune cells (ortutay2008phylogenyoftec pages 4-7).

## Inhibitors

• Ibrutinib – covalent, targets C481, IC₅₀ ≈ 78 nM for BTK (ahn2021targetingbruton’styrosine pages 3-3).  
• Acalabrutinib – covalent, C481-directed, IC₅₀ ≈ 9.2 nM (ahn2021targetingbruton’styrosine pages 3-3).  
• Zanubrutinib – second-generation covalent C481 inhibitor with improved selectivity (patel2017comparisonofacalabrutinib pages 10-11).  
• Fenebrutinib – non-covalent, engages K430/M477/D539, active against C481 mutants (gu2021targetingbrutontyrosine pages 3-5).  
• Resistance mutations: C481S disrupts covalent binding; T474I and L528W reduce binding of several covalent and non-covalent agents (gu2021targetingbrutontyrosine pages 5-7, joseph2024impactofthe pages 22-26).

## Other Comments

• Germline missense mutations including K430E/R, R544K/G and Y551F impair catalytic mechanisms and cause X-linked agammaglobulinemia (mao2001crystalstructureof pages 6-7).  
• Kinase-dead mutations in follicular lymphoma lower Y223 autophosphorylation yet permit augmented AKT signaling (hu2021follicularlymphoma–associatedbtk pages 11-13).  
• Somatic resistance mutations in treated malignancies frequently occur at C481 (C481S/F/Y/R/G/T) and at positions T474 and L528, compromising inhibitor efficacy (unknownauthors2024investigatingresistancemechanisms pages 29-34).

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