1. Phylogeny  
   Tyrosine‐protein kinase BTK is a member of the Tec family of non‐receptor tyrosine kinases, a group that also includes BMX, ITK, TEC, and TXK. BTK is highly conserved among mammals and is found in all species possessing a functional adaptive immune system, suggesting its emergence early in vertebrate evolution. It traces its origin to a premetazoan ancestor, and the Tec family kinases form an evolutionary core set that is related to other central signaling enzymes within the kinome (ortutay2008phylogenyoftec pages 7-10, burger2019brutontyrosinekinase pages 1-3).
2. Reaction Catalyzed  
   BTK catalyzes the transfer of a phosphate group from ATP to tyrosine residues on protein substrates. The chemical reaction can be represented as: ATP + protein–tyrosine → ADP + protein–phosphotyrosine + H⁺. This phosphorylation event is a critical post‐translational modification that regulates downstream signal transduction cascades in B cells and other immune cells (alu2022btkinhibitorsin pages 3-5, qiu2000signalingnetworkof pages 1-2).
3. Cofactor Requirements  
   The kinase activity of BTK depends on ATP as a phosphate donor and requires divalent metal ions, predominantly Mg²⁺, as an essential cofactor to facilitate the proper alignment of ATP and the protein substrate in the active site (alu2022btkinhibitorsin pages 3-5, mao2001crystalstructureof pages 5-5).
4. Substrate Specificity  
   BTK exhibits substrate specificity typical of protein tyrosine kinases. It preferentially phosphorylates tyrosine residues on substrate proteins that are key components of the B-cell receptor (BCR) signaling cascade. A well‐characterized substrate of BTK is phospholipase C-γ2 (PLCγ2), which, upon phosphorylation, triggers calcium mobilization and activates downstream effectors such as members of the protein kinase C (PKC) family. The substrate specificity is also defined by the context of the surrounding amino acid residues near the target tyrosine, although a strict consensus motif has not been universally established. Instead, the functional substrates are mainly components of the BCR and Toll-like receptor (TLR) pathways (alu2022btkinhibitorsin pages 3-5, burger2019brutontyrosinekinase pages 1-3, xing2014brutonstkinhibitors pages 1-2).
5. Structure  
   BTK is a protein of 659 amino acids that is organized into five distinct domains. At its N-terminus, the pleckstrin homology (PH) domain binds phosphatidylinositol 3,4,5-trisphosphate (PIP3), mediating the recruitment of BTK to the plasma membrane upon activation of PI3-kinase. Adjacent to the PH domain, the Tec homology (TH) region often contains a zinc-binding motif, which is important for protein stability. BTK further contains Src homology domains SH3 and SH2 that facilitate protein–protein interactions and proper positioning within signaling complexes. The C-terminal kinase domain, also known as the SH1 domain, is responsible for catalytic activity and contains critical regulatory residues, including Tyr551 in the activation loop—phosphorylation at this residue by upstream kinases (such as LYN) is required for full enzymatic activation—and Tyr223 in the SH3 domain, which undergoes autophosphorylation. In addition, the kinase domain houses a conserved cysteine residue (Cys481), which serves as the binding site for covalent inhibitors such as ibrutinib. Structural studies, including crystallographic analyses, have revealed both active and inactive conformations of the BTK kinase domain, highlighting a flexible αC-helix and an activation loop that adopts a conformation conducive to substrate binding once phosphorylated. These features, including the hydrophobic spines and the arrangement of the N- and C-terminal lobes, are essential for catalysis and regulatory control (alu2022btkinhibitorsin pages 3-5, burger2019brutontyrosinekinase pages 1-3, lin2023structureofbtk pages 9-11, mao2001crystalstructureof pages 3-3, tasso2021thedevelopmentof pages 1-2).
6. Regulation  
   The activity of BTK is primarily regulated through phosphorylation and conformational changes. Activation is initiated when membrane recruitment occurs via the PH domain binding to PIP3, facilitating proximity to upstream Src-family kinases. These kinases phosphorylate BTK at Tyr551 in the activation loop, which is a prerequisite for the enzyme’s full activation; subsequently, BTK autophosphorylates at Tyr223, solidifying its active configuration. In addition to these phosphorylation events, BTK is subject to negative regulation by phosphatases such as SHP1 and the lipid phosphatase SHIP1, which dephosphorylate key residues or reduce the availability of PIP3, respectively. Beyond phosphorylation, BTK activity is modulated through conformational shifts within its kinase domain, including movements of the αC-helix and repositioning of the activation loop—mechanisms that have been elucidated through crystallographic studies. These regulatory modifications are crucial to ensuring that BTK activation is tightly coupled to receptor engagement, thereby preventing aberrant signal transduction (alu2022btkinhibitorsin pages 3-5, satterthwaite1998btkfunctionin pages 1-2, mao2001crystalstructureof pages 5-5, mcdonald2021theroleof pages 2-5).
7. Function  
   BTK plays a pivotal role in mediating both adaptive and innate immune responses. It is indispensable for normal B-cell development, differentiation, and signaling. Upon antigen binding to the B-cell receptor, BTK becomes activated and phosphorylates substrates such as PLCγ2, which in turn leads to the generation of second messengers that trigger calcium mobilization and activation of downstream kinases including members of the PKC family. In addition to its well-known role in BCR signaling, BTK also functions in Toll-like receptor (TLR) pathways—particularly TLR8 and TLR9—to induce the activity of transcription factors such as NF-κB, which controls the expression of numerous genes involved in inflammation and cell survival. BTK additionally phosphorylates adapter proteins like TIRAP, thereby modulating the TLR pathway and facilitating processes such as the degradation of TIRAP. Further extending its functional repertoire, BTK is involved in transcriptional regulation through the transient phosphorylation of factors like GTF2I, which then translocate to the nucleus to influence gene expression. BTK also serves as an activator of the NLRP3 inflammasome by mediating the phosphorylation of NLRP3, thereby linking its activity to innate immune responses and cytokine production. Moreover, BTK plays a dual role in the regulation of apoptosis, acting as both a promoter and inhibitor of cell death under different circumstances. Expression of BTK is primarily restricted to hematopoietic cells such as B lymphocytes, but it is also present in other cell types including macrophages, neutrophils, mast cells, and certain myeloid cells, highlighting its multifaceted role in both innate and adaptive immunity (alu2022btkinhibitorsin pages 1-3, burger2019brutontyrosinekinase pages 1-3, mcdonald2021theroleof pages 25-28, qiu2000signalingnetworkof pages 1-2).
8. Other Comments  
   BTK is a well‐validated therapeutic target in a range of conditions. Inhibition of BTK has revolutionized the treatment of B-cell malignancies such as chronic lymphocytic leukemia, mantle cell lymphoma, Waldenström’s macroglobulinemia, and marginal zone lymphoma. First-generation inhibitors like ibrutinib covalently bind to Cys481 in the kinase domain, thereby irreversibly inhibiting enzyme activity; however, resistance mutations—most notably the substitution of Cys481 with serine—can reduce inhibitor efficacy. Second-generation inhibitors such as acalabrutinib and zanubrutinib have been developed with improved selectivity and safety profiles to address these resistance mechanisms. Beyond cancer, BTK inhibitors are also under investigation for their potential in autoimmune diseases and inflammatory disorders, as BTK plays a central role in TLR-mediated cytokine production and inflammasome activation. Disease-associated mutations in BTK are responsible for X-linked agammaglobulinemia (XLA), a condition characterized by defective B-cell maturation and severe immunodeficiency. Additionally, BTK is involved in the regulation of transcription factors such as NF-κB, ARID3A, and NFAT, contributing to its role in cell survival and apoptosis. Notable inhibitors include ibrutinib, acalabrutinib, zanubrutinib, tirabrutinib, and third-generation reversible inhibitors like pirtobrutinib, all of which target BTK’s catalytic function through covalent or noncovalent mechanisms (alu2022btkinhibitorsin pages 25-26, burger2019brutontyrosinekinase pages 1-3, tasso2021thedevelopmentof pages 26-28, vargas2013inhibitorsofbtk pages 1-3, xing2014brutonstkinhibitors pages 1-2).
9. References  
   alu2022btkinhibitorsin pages 1-3; alu2022btkinhibitorsin pages 3-5; alu2022btkinhibitorsin pages 25-26; burger2019brutontyrosinekinase pages 1-3; lin2023structureofbtk pages 9-11; mao2001crystalstructureof pages 3-3; mcdonald2021theroleof pages 2-5; mcdonald2021theroleof pages 25-28; ortutay2008phylogenyoftec pages 7-10; qiu2000signalingnetworkof pages 1-2; satterthwaite1998btkfunctionin pages 1-2; tasso2021thedevelopmentof pages 1-2, 24-26, 26-28; vargas2013inhibitorsofbtk pages 1-3; xing2014brutonstkinhibitors pages 1-2, 18-19; hantschel2007thebtktyrosine pages 1-1; kokabee2022palmitoylationofthe pages 4-5, 10-11, 12-13.

References

1. (alu2022btkinhibitorsin pages 3-5): Aqu Alu, Hong Lei, Xuejiao Han, Yuquan Wei, and Xiawei Wei. Btk inhibitors in the treatment of hematological malignancies and inflammatory diseases: mechanisms and clinical studies. Journal of Hematology & Oncology, Oct 2022. URL: https://doi.org/10.1186/s13045-022-01353-w, doi:10.1186/s13045-022-01353-w. This article has 114 citations.
2. (burger2019brutontyrosinekinase pages 1-3): JA Burger. Bruton tyrosine kinase inhibitors: present and future. Unknown journal, 2019.
3. (lin2023structureofbtk pages 9-11): David Y. Lin and Amy H. Andreotti. Structure of btk kinase domain with the second-generation inhibitors acalabrutinib and tirabrutinib. PLOS ONE, 18:e0290872, Aug 2023. URL: https://doi.org/10.1371/journal.pone.0290872, doi:10.1371/journal.pone.0290872. This article has 13 citations and is from a peer-reviewed journal.
4. (mao2001crystalstructureof pages 3-3): Chen Mao, Min Zhou, and Fatih M. Uckun. Crystal structure of bruton’s tyrosine kinase domain suggests a novel pathway for activation and provides insights into the molecular basis of x-linked agammaglobulinemia. Journal of Biological Chemistry, 276:41435-41443, Nov 2001. URL: https://doi.org/10.1074/jbc.m104828200, doi:10.1074/jbc.m104828200. This article has 138 citations and is from a domain leading peer-reviewed journal.
5. (mcdonald2021theroleof pages 2-5): Charlotte McDonald, Charalampos Xanthopoulos, and Efterpi Kostareli. The role of bruton’s tyrosine kinase in the immune system and disease. Immunology, 164:722-736, Oct 2021. URL: https://doi.org/10.1111/imm.13416, doi:10.1111/imm.13416. This article has 100 citations and is from a peer-reviewed journal.
6. (mcdonald2021theroleof pages 25-28): Charlotte McDonald, Charalampos Xanthopoulos, and Efterpi Kostareli. The role of bruton’s tyrosine kinase in the immune system and disease. Immunology, 164:722-736, Oct 2021. URL: https://doi.org/10.1111/imm.13416, doi:10.1111/imm.13416. This article has 100 citations and is from a peer-reviewed journal.
7. (ortutay2008phylogenyoftec pages 7-10): Csaba Ortutay, Beston F. Nore, Mauno Vihinen, and C.I. Edvard Smith. Phylogeny of tec family kinases: identification of a premetazoan origin of btk, bmx, itk, tec, txk, and the btk regulator sh3bp5. Advances in Genetics, 64:51-80, Jan 2008. URL: https://doi.org/10.1016/s0065-2660(08)00803-1, doi:10.1016/s0065-2660(08)00803-1. This article has 38 citations and is from a peer-reviewed journal.
8. (satterthwaite1998btkfunctionin pages 1-2): Anne B Satterthwaite, Zuomei Li, and Owen N Witte. Btk function in b cell development and response. Seminars in Immunology, 10:309-316, Aug 1998. URL: https://doi.org/10.1006/smim.1998.0123, doi:10.1006/smim.1998.0123. This article has 229 citations and is from a peer-reviewed journal.
9. (tasso2021thedevelopmentof pages 1-2): Bruno Tasso, Andrea Spallarossa, Eleonora Russo, and Chiara Brullo. The development of btk inhibitors: a five-year update. Molecules, 26:7411, Dec 2021. URL: https://doi.org/10.3390/molecules26237411, doi:10.3390/molecules26237411. This article has 59 citations and is from a peer-reviewed journal.
10. (tasso2021thedevelopmentof pages 26-28): Bruno Tasso, Andrea Spallarossa, Eleonora Russo, and Chiara Brullo. The development of btk inhibitors: a five-year update. Molecules, 26:7411, Dec 2021. URL: https://doi.org/10.3390/molecules26237411, doi:10.3390/molecules26237411. This article has 59 citations and is from a peer-reviewed journal.
11. (vargas2013inhibitorsofbtk pages 1-3): Leonardo Vargas, A. Hamasy, A. Hamasy, B. Nore, B. Nore, and C. I. E. Smith. Inhibitors of btk and itk: state of the new drugs for cancer, autoimmunity and inflammatory diseases. Scandinavian Journal of Immunology, Aug 2013. URL: https://doi.org/10.1111/sji.12069, doi:10.1111/sji.12069. This article has 91 citations and is from a peer-reviewed journal.
12. (xing2014brutonstkinhibitors pages 1-2): Li Xing and Adrian Huang. Bruton’s tk inhibitors: structural insights and evolution of clinical candidates. Future medicinal chemistry, 6 6:675-95, Jun 2014. URL: https://doi.org/10.4155/fmc.14.24, doi:10.4155/fmc.14.24. This article has 13 citations and is from a peer-reviewed journal.
13. (alu2022btkinhibitorsin pages 1-3): Aqu Alu, Hong Lei, Xuejiao Han, Yuquan Wei, and Xiawei Wei. Btk inhibitors in the treatment of hematological malignancies and inflammatory diseases: mechanisms and clinical studies. Journal of Hematology & Oncology, Oct 2022. URL: https://doi.org/10.1186/s13045-022-01353-w, doi:10.1186/s13045-022-01353-w. This article has 114 citations.
14. (alu2022btkinhibitorsin pages 25-26): Aqu Alu, Hong Lei, Xuejiao Han, Yuquan Wei, and Xiawei Wei. Btk inhibitors in the treatment of hematological malignancies and inflammatory diseases: mechanisms and clinical studies. Journal of Hematology & Oncology, Oct 2022. URL: https://doi.org/10.1186/s13045-022-01353-w, doi:10.1186/s13045-022-01353-w. This article has 114 citations.
15. (hantschel2007thebtktyrosine pages 1-1): Oliver Hantschel, Uwe Rix, Uwe Schmidt, Tilmann Bürckstümmer, Michael Kneidinger, Gregor Schütze, Jacques Colinge, Keiryn L. Bennett, Wilfried Ellmeier, Peter Valent, and Giulio Superti-Furga. The btk tyrosine kinase is a major target of the bcr-abl inhibitor dasatinib. Proceedings of the National Academy of Sciences, 104:13283-13288, Aug 2007. URL: https://doi.org/10.1073/pnas.0702654104, doi:10.1073/pnas.0702654104. This article has 362 citations.
16. (kokabee2022palmitoylationofthe pages 4-5): MOSTAFA KOKABEE, XIANHUI WANG, ELENA VOORAND, EDEN ALIN, LEILA KOKABEE, FAIZA KHAN, SOPHIA DESROSIERS, and DOUGLAS S. CONKLIN. Palmitoylation of the alternative amino terminus of the btk-c isoform controls subcellular distribution and signaling. Cancer Genomics - Proteomics, 19:415-427, Jan 2022. URL: https://doi.org/10.21873/cgp.20329, doi:10.21873/cgp.20329. This article has 5 citations.
17. (mao2001crystalstructureof pages 5-5): Chen Mao, Min Zhou, and Fatih M. Uckun. Crystal structure of bruton’s tyrosine kinase domain suggests a novel pathway for activation and provides insights into the molecular basis of x-linked agammaglobulinemia. Journal of Biological Chemistry, 276:41435-41443, Nov 2001. URL: https://doi.org/10.1074/jbc.m104828200, doi:10.1074/jbc.m104828200. This article has 138 citations and is from a domain leading peer-reviewed journal.
18. (qiu2000signalingnetworkof pages 1-2): Yun Qiu and Hsing-Jien Kung. Signaling network of the btk family kinases. Oncogene, 19:5651-5661, Nov 2000. URL: https://doi.org/10.1038/sj.onc.1203958, doi:10.1038/sj.onc.1203958. This article has 345 citations and is from a domain leading peer-reviewed journal.