1. Phylogeny  
   Tyrosine‐protein kinase BTK belongs to the Tec family of non‐receptor tyrosine kinases, a subgroup that also includes related kinases such as ITK, TEC, TXK (also known as Rlk or BPK) and BMX. In vertebrates, BTK orthologs are widely conserved in species possessing a complex immune system, and its evolutionary history can be traced from lower metazoans to mammals. BTK’s inclusion in the Tec family is supported by its unique domain organization, which is distinct from other tyrosine kinase groups such as the Src or receptor tyrosine kinases. Its phylogenetic relationship with other members of the human kinome has been systematically analyzed and it is grouped together with kinases that emerged early during the evolution of eukaryotic signaling pathways (darragh2024comprehensivecharacterizationof pages 1-4, yu2009tecfamilykinases pages 23-26).
2. Reaction Catalyzed  
   BTK catalyzes a phosphorylation reaction in which the terminal phosphate group of ATP is transferred to a tyrosine residue within substrate proteins. The chemical reaction can be summarized as follows:  
   ATP + protein –OH → ADP + protein –O–P + H⁺.  
   In this reaction, ATP provides the phosphate that is covalently attached to the hydroxyl group of a tyrosine residue in the target protein, thereby modifying its activity. (dinh2007activationmechanismand pages 1-2, darragh2024comprehensivecharacterizationof pages 1-4)
3. Cofactor Requirements  
   The kinase activity of BTK is dependent on the presence of ATP as the phosphate donor and requires magnesium ions (Mg²⁺) as an essential cofactor. Mg²⁺ is generally required for proper coordination of ATP within the catalytic pocket, facilitating the transfer reaction. (dinh2007activationmechanismand pages 1-2, darragh2024comprehensivecharacterizationof pages 1-4)
4. Substrate Specificity  
   BTK displays substrate specificity characteristic of tyrosine kinases. It preferentially phosphorylates tyrosine residues on substrates that participate in the B-cell receptor (BCR) signaling cascade. Notably, BTK phosphorylates phospholipase Cγ2 (PLCγ2) at multiple sites and acts in cooperation with the adapter protein BLNK, thus ensuring efficient propagation of downstream signals. In addition, substrates such as TIRAP are known targets in pathways triggered by Toll-like receptors, which underscores BTK’s critical role in both adaptive and innate immune responses. While the intrinsic substrate specificity in terms of consensus amino acid motifs has been the subject of extensive studies for tyrosine kinases, recent advances have identified that BTK substrates typically include flanking acidic residues and structural contexts that facilitate recognition by the kinase domain. (darragh2024comprehensivecharacterizationof pages 1-4, sonowal2023luxeptinibinterfereswith pages 14-15, vargas2013inhibitorsofbtk pages 3-6)
5. Structure  
   The structural organization of BTK is defined by a modular architecture consisting of five principal domains. At the N-terminus, BTK contains a Pleckstrin Homology (PH) domain that mediates membrane localization via interaction with phosphatidylinositol 3,4,5-trisphosphate (PIP3) produced by PI3K. Adjacent to the PH domain is the Tec Homology (TH) domain, which is unique to Tec family kinases and includes regions such as a zinc-binding motif and a proline-rich region that contribute to protein stability and intra-molecular regulation. Following the TH domain are the SH3 and SH2 domains, which facilitate protein-protein interactions by binding proline-rich sequences and phosphotyrosine motifs, respectively. These domains also participate in maintaining an autoinhibitory conformation in the resting state. At the C-terminus, the catalytic (kinase) domain is responsible for the enzymatic transfer of phosphate from ATP to a substrate tyrosine. Critical residues within this domain include the activation loop tyrosine (Tyr551), whose phosphorylation is necessary for full activation, and Tyr223 within the SH3 domain that, through autophosphorylation, modulates the overall catalytic efficiency. Importantly, the kinase domain also features a conserved cysteine residue (Cys481) that is targeted by covalent inhibitors such as ibrutinib, providing a structural basis for therapeutic intervention. High-resolution crystallographic studies and available structural models have detailed the positioning of the catalytic cleft, the hydrophobic spine, and the alignment of the C-helix, all of which are essential for proper kinase function. (darragh2024comprehensivecharacterizationof pages 1-4, lopezherrera2015bruton’styrosinekinase pages 1-4, marcotte2010structuresofhuman pages 1-3, lou2012bruton’styrosinekinase pages 1-2, kueffer2021reininginbtk pages 12-13)
6. Regulation  
   BTK activity is regulated by multiple mechanisms that include post-translational modifications, interdomain interactions, and membrane recruitment. One primary mechanism of activation involves phosphorylation: initial phosphorylation of Tyr551 within the activation loop, often by Src family kinases such as Lyn, is followed by autophosphorylation at Tyr223 (located in the SH3 region), which contributes to a substantial increase in catalytic activity. These phosphorylation events induce conformational changes that shift BTK from an autoinhibited state to an active conformation. In addition, the binding of the PH domain to PIP3 at the plasma membrane is vital for recruiting BTK to the site of receptor signaling, thereby positioning it in proximity to its substrates. Furthermore, interactions with adaptor proteins such as BLNK reinforce the activation process by providing docking platforms that facilitate substrate recognition and subsequent phosphorylation. Allosteric regulation also plays a role; the interdomain contacts among the PH, TH, SH3, and SH2 domains help maintain the basal inactive state of BTK and are released upon appropriate stimuli. (darragh2024comprehensivecharacterizationof pages 1-4, dinh2007activationmechanismand pages 8-8, kueffer2021reininginbtk pages 11-12, kueffer2021reininginbtk pages 13-14)
7. Function  
   BTK is indispensable for normal B lymphocyte development, differentiation, and signaling. In B cells, engagement of the B-cell receptor (BCR) triggers a signaling cascade in which BTK plays several roles. Following antigen binding and receptor clustering at the plasma membrane, BTK becomes activated by membrane recruitment through its PH domain binding to PIP3. Active BTK then phosphorylates key downstream effectors such as PLCγ2, which leads to the formation of inositol trisphosphate (IP3) and subsequent calcium mobilization. This calcium flux is critical for further activation of protein kinase C (PKC) family members and the induction of transcription factors such as NF-κB, which ultimately regulate gene expression related to cell proliferation, survival, and differentiation. Beyond its central role in BCR signaling, BTK is also involved in cytokine receptor signaling and Toll-like receptor (TLR) pathways, highlighting its importance in both adaptive and innate immune responses. In the context of TLR signaling, BTK phosphorylates TIRAP, leading to its degradation and modulating the inflammatory response. BTK additionally contributes to the activation of transcription factors like GTF2I, ARID3A, and NFAT, affecting broader transcriptional programs. The dual roles of BTK in promoting survival signals and regulating apoptotic pathways underscore its functional complexity in immune cell biology. (darragh2024comprehensivecharacterizationof pages 1-4, dinh2007activationmechanismand pages 8-8, joseph2020differentialimpactof pages 1-2, kueffer2021reininginbtk pages 12-13)
8. Other Comments  
   BTK is a well‐validated therapeutic target in B cell malignancies such as chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and a variety of autoimmune conditions. The clinical success of BTK inhibitors, notably the irreversible inhibitor ibrutinib, has highlighted the importance of Cys481 as a key binding site; however, resistance mutations such as C481S have been identified, prompting the development of next‐generation inhibitors that include reversible molecules (e.g., pirtobrutinib) and dual-mechanism agents designed to overcome such resistance. In addition, BTK inhibitors are now being explored in inflammatory and autoimmune disorders, as the enzyme plays an integral role in TLR signaling and cytokine production. Disease mutations in BTK, including loss-of-function mutations that cause X-linked agammaglobulinemia (XLA), further exemplify the critical role of this kinase in immune homeostasis. Besides small-molecule inhibitors, combination therapeutic approaches and emerging modalities such as PROTAC degraders have been proposed to target BTK more effectively in cases where resistance may limit the efficacy of standard inhibitors. (darragh2024comprehensivecharacterizationof pages 26-28, lopezherrera2015bruton’styrosinekinase pages 4-7, akinleye2013ibrutinibandnovel pages 1-2, alu2022btkinhibitorsin pages 25-26, ringheim2021bruton’styrosinekinase pages 17-17)
9. References  
   darragh2024comprehensivecharacterizationof pages 1-4; darragh2024comprehensivecharacterizationof pages 26-28; dinh2007activationmechanismand pages 1-2; dinh2007activationmechanismand pages 8-8; lopezherrera2015bruton’styrosinekinase pages 4-7; lopezherrera2015bruton’styrosinekinase pages 7-10; marcotte2010structuresofhuman pages 1-3; ringheim2021bruton’styrosinekinase pages 1-2; sonowal2023luxeptinibinterfereswith pages 14-15; vargas2013inhibitorsofbtk pages 3-6; yu2009tecfamilykinases pages 23-26; akinleye2013ibrutinibandnovel pages 1-2; alu2022btkinhibitorsin pages 25-26.

References

1. (darragh2024comprehensivecharacterizationof pages 1-4): Antonia C. Darragh, Andrew M. Hanna, Justin H. Lipner, Nicole B. Servant, Alastair J. King, and Mirza Jahic. Comprehensive characterization of btk inhibitor specificity, potency, and biological effects: insights into covalent and non-covalent mechanistic signatures. BioRxiv, Sep 2024. URL: https://doi.org/10.1101/2024.09.06.611550, doi:10.1101/2024.09.06.611550. This article has 0 citations.
2. (darragh2024comprehensivecharacterizationof pages 26-28): Antonia C. Darragh, Andrew M. Hanna, Justin H. Lipner, Nicole B. Servant, Alastair J. King, and Mirza Jahic. Comprehensive characterization of btk inhibitor specificity, potency, and biological effects: insights into covalent and non-covalent mechanistic signatures. BioRxiv, Sep 2024. URL: https://doi.org/10.1101/2024.09.06.611550, doi:10.1101/2024.09.06.611550. This article has 0 citations.
3. (dinh2007activationmechanismand pages 1-2): Marie Dinh, Dorit Grunberger, Hoangdung Ho, Stan Y. Tsing, David Shaw, Simon Lee, Jim Barnett, Ronald J. Hill, David C. Swinney, and J. Michael Bradshaw. Activation mechanism and steady state kinetics of bruton’s tyrosine kinase. Journal of Biological Chemistry, 282:8768-8776, Mar 2007. URL: https://doi.org/10.1074/jbc.m609920200, doi:10.1074/jbc.m609920200. This article has 61 citations and is from a domain leading peer-reviewed journal.
4. (dinh2007activationmechanismand pages 8-8): Marie Dinh, Dorit Grunberger, Hoangdung Ho, Stan Y. Tsing, David Shaw, Simon Lee, Jim Barnett, Ronald J. Hill, David C. Swinney, and J. Michael Bradshaw. Activation mechanism and steady state kinetics of bruton’s tyrosine kinase. Journal of Biological Chemistry, 282:8768-8776, Mar 2007. URL: https://doi.org/10.1074/jbc.m609920200, doi:10.1074/jbc.m609920200. This article has 61 citations and is from a domain leading peer-reviewed journal.
5. (kueffer2021reininginbtk pages 12-13): Lauren E. Kueffer, Raji E. Joseph, and Amy H. Andreotti. Reining in btk: interdomain interactions and their importance in the regulatory control of btk. Frontiers in Cell and Developmental Biology, Jun 2021. URL: https://doi.org/10.3389/fcell.2021.655489, doi:10.3389/fcell.2021.655489. This article has 14 citations and is from a peer-reviewed journal.
6. (kueffer2021reininginbtk pages 13-14): Lauren E. Kueffer, Raji E. Joseph, and Amy H. Andreotti. Reining in btk: interdomain interactions and their importance in the regulatory control of btk. Frontiers in Cell and Developmental Biology, Jun 2021. URL: https://doi.org/10.3389/fcell.2021.655489, doi:10.3389/fcell.2021.655489. This article has 14 citations and is from a peer-reviewed journal.
7. (lopezherrera2015bruton’styrosinekinase pages 4-7): G. Lopez-Herrera, J. L. Maravillas-Montero, J. C. Rodríguez-Alba, and L. Santos-Argumedo. Bruton’s tyrosine kinase (btk) beyond b lymphocytes: a protein kinase with relevance in innate immunity. Rare Diseases of the Immune System, pages 99-115, Jan 2015. URL: https://doi.org/10.1007/978-3-319-22714-6\_7, doi:10.1007/978-3-319-22714-6\_7. This article has 1 citations.
8. (lopezherrera2015bruton’styrosinekinase pages 7-10): G. Lopez-Herrera, J. L. Maravillas-Montero, J. C. Rodríguez-Alba, and L. Santos-Argumedo. Bruton’s tyrosine kinase (btk) beyond b lymphocytes: a protein kinase with relevance in innate immunity. Rare Diseases of the Immune System, pages 99-115, Jan 2015. URL: https://doi.org/10.1007/978-3-319-22714-6\_7, doi:10.1007/978-3-319-22714-6\_7. This article has 1 citations.
9. (marcotte2010structuresofhuman pages 1-3): Douglas J. Marcotte, Yu‐Ting Liu, Robert M. Arduini, Catherine A. Hession, Konrad Miatkowski, Craig P. Wildes, Patrick F. Cullen, Victor Hong, Brian T. Hopkins, Elisabeth Mertsching, Tracy J. Jenkins, Michael J. Romanowski, Darren P. Baker, and Laura F. Silvian. Structures of human bruton’s tyrosine kinase in active and inactive conformations suggest a mechanism of activation for tec family kinases. Protein Science, Mar 2010. URL: https://doi.org/10.1002/pro.321, doi:10.1002/pro.321. This article has 170 citations and is from a peer-reviewed journal.
10. (ringheim2021bruton’styrosinekinase pages 1-2): Garth E. Ringheim, Matthew Wampole, and Kinsi Oberoi. Bruton’s tyrosine kinase (btk) inhibitors and autoimmune diseases: making sense of btk inhibitor specificity profiles and recent clinical trial successes and failures. Frontiers in Immunology, Nov 2021. URL: https://doi.org/10.3389/fimmu.2021.662223, doi:10.3389/fimmu.2021.662223. This article has 103 citations and is from a peer-reviewed journal.
11. (sonowal2023luxeptinibinterfereswith pages 14-15): Himangshu Sonowal, William G. Rice, and Stephen B. Howell. Luxeptinib interferes with lyn-mediated activation of syk and modulates bcr signaling in lymphoma. PLOS ONE, 18:e0277003, Mar 2023. URL: https://doi.org/10.1371/journal.pone.0277003, doi:10.1371/journal.pone.0277003. This article has 4 citations and is from a peer-reviewed journal.
12. (vargas2013inhibitorsofbtk pages 3-6): 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.
13. (yu2009tecfamilykinases pages 23-26): L Yu. Tec family kinases: transcriptional and posttranslational regulation. Unknown journal, 2009.
14. (akinleye2013ibrutinibandnovel pages 1-2): Akintunde Akinleye, Yamei Chen, Nikhil Mukhi, Yongping Song, and Delong Liu. Ibrutinib and novel btk inhibitors in clinical development. Journal of Hematology & Oncology, 6:59-59, Aug 2013. URL: https://doi.org/10.1186/1756-8722-6-59, doi:10.1186/1756-8722-6-59. This article has 301 citations.
15. (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.
16. (joseph2020differentialimpactof pages 1-2): Raji E Joseph, Neha Amatya, D Bruce Fulton, John R Engen, Thomas E Wales, and Amy Andreotti. Differential impact of btk active site inhibitors on the conformational state of full-length btk. eLife, Nov 2020. URL: https://doi.org/10.7554/elife.60470, doi:10.7554/elife.60470. This article has 32 citations and is from a domain leading peer-reviewed journal.
17. (kueffer2021reininginbtk pages 11-12): Lauren E. Kueffer, Raji E. Joseph, and Amy H. Andreotti. Reining in btk: interdomain interactions and their importance in the regulatory control of btk. Frontiers in Cell and Developmental Biology, Jun 2021. URL: https://doi.org/10.3389/fcell.2021.655489, doi:10.3389/fcell.2021.655489. This article has 14 citations and is from a peer-reviewed journal.
18. (lopezherrera2015bruton’styrosinekinase pages 1-4): G. Lopez-Herrera, J. L. Maravillas-Montero, J. C. Rodríguez-Alba, and L. Santos-Argumedo. Bruton’s tyrosine kinase (btk) beyond b lymphocytes: a protein kinase with relevance in innate immunity. Rare Diseases of the Immune System, pages 99-115, Jan 2015. URL: https://doi.org/10.1007/978-3-319-22714-6\_7, doi:10.1007/978-3-319-22714-6\_7. This article has 1 citations.
19. (lou2012bruton’styrosinekinase pages 1-2): Yan Lou, Timothy D. Owens, Andreas Kuglstatter, Rama K. Kondru, and David M. Goldstein. Bruton’s tyrosine kinase inhibitors: approaches to potent and selective inhibition, preclinical and clinical evaluation for inflammatory diseases and b cell malignancies. Journal of Medicinal Chemistry, 55:4539-4550, Mar 2012. URL: https://doi.org/10.1021/jm300035p, doi:10.1021/jm300035p. This article has 148 citations and is from a highest quality peer-reviewed journal.
20. (ringheim2021bruton’styrosinekinase pages 17-17): Garth E. Ringheim, Matthew Wampole, and Kinsi Oberoi. Bruton’s tyrosine kinase (btk) inhibitors and autoimmune diseases: making sense of btk inhibitor specificity profiles and recent clinical trial successes and failures. Frontiers in Immunology, Nov 2021. URL: https://doi.org/10.3389/fimmu.2021.662223, doi:10.3389/fimmu.2021.662223. This article has 103 citations and is from a peer-reviewed journal.