## Phylogeny

• Kinase classification: Tyrosine kinase (TK) group → Receptor tyrosine kinase (RTK) family → Eph receptor sub-family → EphB subclass (berrou2018amutationof pages 1-5, overman2013biochemicalandbiophysical pages 1-2).  
• The catalytic domains of EphB receptors display ~88 % sequence identity; EphB2 shares 83 % identity with EphB4, indicating recent divergence within the EphB branch (overman2013biochemicalandbiophysical pages 11-14).  
• The kinase domain is highly conserved across vertebrate species, demonstrating strong evolutionary constraint and the presence of orthologs throughout vertebrates (berrou2018amutationof pages 5-9).

## Reaction Catalyzed

ATP + protein-L-tyrosine ⇌ ADP + protein-L-tyrosine-phosphate (unknownauthors2014studyingtheoligomerization pages 5-9, berrou2018amutationof pages 5-9).

## Cofactor Requirements

Catalytic activity requires divalent cations; enzymatic assays are performed in the presence of Mg²⁺ together with ATP (unknownauthors2023designandsynthesis pages 94-100).

## Substrate Specificity

Published studies do not define a consensus linear phosphorylation motif for EphB2; substrate preferences remain undetermined in the available literature (overman2013biochemicalandbiophysical pages 9-10).

## Structure

• Domain organization: ligand-binding domain → cysteine-rich region → two fibronectin type III domains → single transmembrane helix → juxtamembrane segment (JMS) → kinase domain (KD) → sterile-alpha motif (SAM) → C-terminal PDZ-binding motif (unknownauthors2014studyingtheoligomerization pages 5-9).  
• Autoinhibited state: the JMS contacts αC and the β4 strand of the KD, disordering the activation segment and suppressing catalysis; ephrin-induced clustering relieves this autoinhibition (unknownauthors2014studyingtheoligomerization pages 5-9).  
• Catalytic core features: Lys721 (β3 strand) pairs with Glu739 (αC) for ATP positioning, Asp746 acts as the catalytic base, and Arg745 contributes to nucleotide coordination—mutation R745C disrupts this network (berrou2018amutationof pages 5-9).  
• Regulatory tyrosines: Y594/Y604 in the juxtamembrane region (alternative numbering Y605/Y611) serve as the primary activation switch; phosphorylation orders the activation loop and creates SH2 docking sites (berrou2018amutationof pages 16-20, zisch2000replacingtwoconserved pages 1-2).  
• Crystal structures: isolated EphB2 KD has been solved in complex with ADP (PDB 2HEN), confirming a canonical bilobal kinase fold (unknownauthors2023designandsynthesis pages 94-100).  
• Comparative structural analysis shows EphB2 possesses Ser706 (EphB4 numbering) within the hinge, a residue differing from most EphB kinases and exploitable for isozyme-selective inhibition (overman2014completingthestructural pages 7-9).

## Regulation

• Ligand-driven receptor clustering promotes trans-autophosphorylation of Y594/Y604, relieving JMS autoinhibition and activating the kinase (unknownauthors2014studyingtheoligomerization pages 5-9, berrou2018amutationof pages 16-20).  
• Phosphorylated Y594/Y604 recruit SH2-domain proteins such as Src, Fyn, RasGAP, Nck and Crk, linking EphB2 to downstream signalling pathways (zisch2000replacingtwoconserved pages 6-8).  
• Phenylalanine substitution of Y605/Y611 abolishes kinase activity, whereas glutamate substitution preserves catalysis but prevents SH2 binding, separating catalytic activation from adaptor recruitment (zisch2000replacingtwoconserved pages 1-2).  
• The p.R745C missense variant in the KD abolishes autophosphorylation, dampens Src activation and impairs phosphorylation of Syk and other effectors in platelets (berrou2018amutationof pages 16-20).  
• Recombinant expression requires co-expression of phosphatase to maintain an unphosphorylated KD, underscoring sensitivity to cellular phosphatases (overman2013biochemicalandbiophysical pages 7-8).

## Function

• Platelet biology: EphB2 amplifies GPVI and G-protein-coupled receptor signalling, enhances αIIbβ3 activation, dense-granule secretion, aggregation and thrombus stability (berrou2018amutationof pages 1-5).  
• Neural development: regulates commissural axon guidance, midline targeting of inner-ear efferents, retinal ganglion cell routing, dendritic spine maturation and excitatory synapse formation (lisabeth2013ephreceptorsignaling pages 1-2).  
• Additional physiological roles include vascular development, tissue boundary formation, bone homeostasis and pancreatic β-cell communication (berrou2018amutationof pages 16-20).  
• Expression: high in platelets and broadly expressed in neural and developmental tissues (berrou2018amutationof pages 1-5, lisabeth2013ephreceptorsignaling pages 1-2).  
• Signalling network: upstream ligands are ephrin-B family members; downstream effectors include Src family kinases (Src, Lyn), Syk, PLCγ2, PI3K/Akt and cytoskeletal regulators such as RasGAP and Nck (berrou2018amutationof pages 16-20, zisch2000replacingtwoconserved pages 6-8).

## Inhibitors

• ATP-competitive inhibitors: CMPD1 shows high potency toward EphB2, whereas potency drops against EphB3 due to hinge residue variation (overman2013biochemicalandbiophysical pages 7-8).  
• Broad-spectrum tyrosine kinase inhibitors such as dasatinib and prototypes like NVP-BHG712 display family-wide activity with limited isoform selectivity (overman2013biochemicalandbiophysical pages 9-10).  
• Structure–activity studies identify Gly699 (EphB4 numbering) conserved in EphB2 as a critical determinant of inhibitor binding, explaining the challenge of isozyme-specific design (overman2013biochemicalandbiophysical pages 7-8).

## Other Comments

• Disease mutation: homozygous p.R745C causes a recessive platelet function defect with severe bleeding due to loss of kinase activity (berrou2018amutationof pages 1-5, berrou2018amutationof pages 5-9).  
• Oncology: inactivating mutations and reduced activity of EphB2 are reported in prostate and colorectal cancers, consistent with a tumour-suppressor role (lisabeth2013ephreceptorsignaling pages 12-13, overman2013biochemicalandbiophysical pages 1-2).  
• Platelets lack compensatory Eph receptors, accounting for the isolated haemostatic phenotype in EPHB2-deficient individuals (berrou2018amutationof pages 16-20).

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