

Title: Sculpting the GAS6 LG1 domain to inhibit/enhance AXL activity

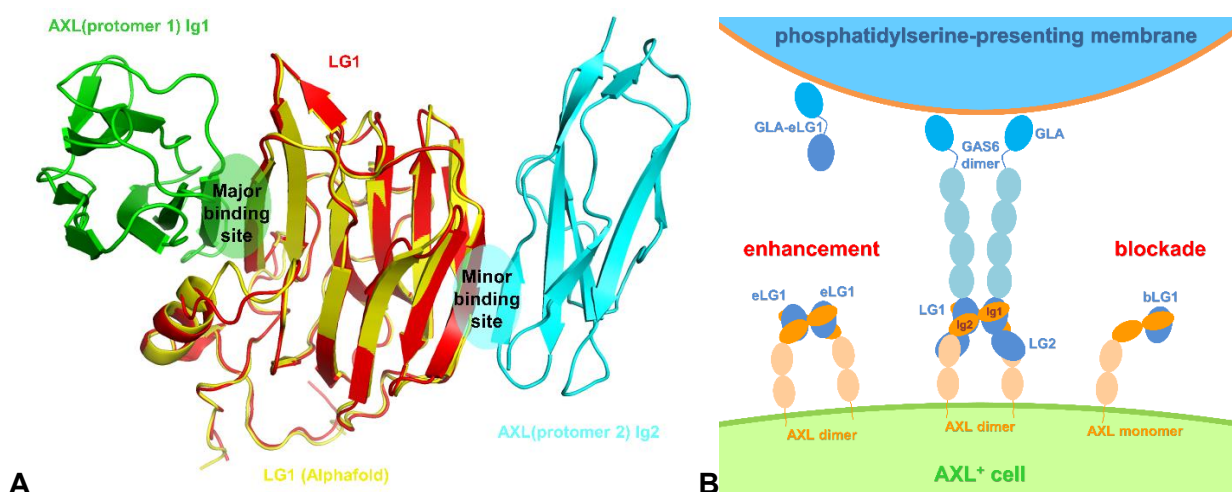
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Research theme/topic: Structural Biology and Protein Engineering

Background: AXL belongs to the TAM (TYRO3, AXL, MER) family of receptor tyrosine kinases (RTKs). Natural ligand PROS1 binds only TYRO3 and MER, while GAS6 interacts with all three TAM proteins with the highest binding affinity to AXL. Interaction with the ligands leads to the homo or heterodimerization of TAM receptors and subsequent autophosphorylation. This results in the full activation of the intrinsic kinases, and generation of docking sites for recruitment of various adaptor molecules inducing different signaling pathways. Under normal conditions, TAM receptors are expressed in endothelial cells, cells of the innate immune and nervous systems, platelets and others, reflecting their diverse functions in various physiological processes. AXL is involved in efferocytosis, drug tolerance and epithelial mesenchymal transition, and has an important role in several pathologies including a wealth of cancer types but also autoimmune, neurological and infectious diseases. TAM ectodomains contain two immunoglobulin (Ig) domains followed by two fibronectin type III (FNIII) domains. Ig1 and Ig2 are responsible for the interactions with GAS6 and PROS1. At their N-terminus, GAS6 and PROS1 possess a γ -carboxyglutamate-rich (GLA) domain that recognizes phosphatidylserine on membranes that expose it. GAS6 presents also four epidermal growth factor-like (EGF) domains, and two C-terminal Laminin G-like domains (LG1 and LG2). The GAS6 binding site for AXL is contained within LG1.

Aims: Developing recombinant GAS6 LG1 variants capable to block/enhance the GAS6:AXL interaction. The preliminary project started in Astana (Kazakhstan) and is continuing in Pavia.



Representation of the LG1 sculpting approach. **A)** Isolated GAS6 LG1 3D model generated using AlphaFold (yellow) overlapped to the experimental 3D structure of LG1 (red) complexed with AXL(protoomer 1) Ig1 (green) and AXL(protoomer 2) Ig2 (cyan) (from PDB entry 2C5D). **B)** Generation of blocking LG1 (bLG1) and enhancing LG1 (eLG1), and a mini GAS6 (GLA-eLG1).

Techniques: Protein design (*in silico*), engineering and production (wet lab). Structure-guided design of isolated soluble GAS6 LG1 domain (for optimal expression yield and folding) and its AXL affinity disruptive/enhancing mutants by analyzing the GAS6:AXL complex. LG1 molecular refinements validation by AlphaFold agent interrogation of predicted 3D structure and its overlap with experimental coordinates. Recombinant LG1 variants cloning in dedicated plasmids for expression in bacteria (*E. coli* and lactobacilli), yeast and mammalian cells. Purification and characterization of activity by a competitive ELISA. Biochemical/cellular set ups to test several disease conditions. 3D structure characterization of final bLG1 and eLG1 and AXL complexes.