Crystal structures of the RLPH2 protein phosphatase from *Arabidopsis thaliana* reveal a novel mechanism for recognizing dually phosphorylated substrates

Anne-Marie Labandera[1], R. Glen Uhrig[2], Keaton Colville[1], Greg B. Moorhead[1*] and Kenneth K.S. Ng[1*]

Affiliations:
[1]Department of Biological Sciences, University of Calgary, 2500 University Dr. NW Calgary, Alberta, Canada T2N 1N4
[2]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2R3.

[*]Corresponding authors jointly supervised this work. Email: moorhead@ucalgary.ca (G.B.M.) and ngk@ucalgary.ca (K.K.S.N.)

Abstract: Despite belonging to the phosphoserine- and phosphothreonine-specific phosphoprotein phosphatase (PPP) family, *Arabidopsis thaliana Rhizobiales*-like phosphatase 2 (RLPH2) strongly prefers substrates bearing phosphorylated tyrosine residues. We used the anomalous scattering signal from sulfur atoms in the native protein to calculate phases for modest-quality diffraction patterns measured from needle-like crystals (1.0 x 0.01 x 0.01 mm) of RLPH2 ($P6_1$; d_{min} = 2.2 Å; R_{sym} = 0.092; redundancy = 30.9; λ = 1.853 Å). As expected for a PPP-family enzyme, the structure of RLPH2 contains a central domain that forms a binding site for two divalent metal ions. Distinctive structural elements from two flanking domains contribute structural elements that suggest a novel mechanism for the selective dephosphorylation of phosphotyrosine residues. Co-crystallization with the phosphate mimetic tungstate also suggests how positively charged residues that are highly conserved in the RLPH2 class form an additional pocket that is specific for a phosphothreonine residue located near the phosphotyrosine residue that is bound to the active site. Site-directed mutagenesis confirmed that this auxiliary recognition element facilitates the recruitment of dual-phosphorylated substrates containing a pTxpY motif. The combination of an auxiliary site specific for phosphothreonine and the active-site specific for phosphotyrosine reveals a novel mechanism for the recognition of dually phosphorylated substrates that appears to be conserved in the RLPH family of plant protein phosphatases.