cellular signals into intracellular responses. Upon nucleotide exchange catalyzed by activated receptors, heterotrimers dissociate into Gα·GTP subunits and $G_{\beta\gamma}$ dimers, either of which can modulate numerous downstream effectors. The crystal structures of the trimeric ($G_{t\alpha\beta\gamma}$), dimeric ($G_{t\beta\gamma}$), and three monomeric forms ($G_{t\alpha}$·GDP, $G_{t\alpha}$·GTPγS, $G_{t\alpha}$·GDP·$AlF_4^-$) of transducin, the heterotrimeric G protein involved in the visual pathway, have been solved and refined to high resolution.

The heterotrimeric form of transducin, $G_{t\alpha\beta\gamma}$, reveals the mechanism of the nucleotide dependent engagement/disengagement between the α and βγ subunits that regulates their interaction with receptor and effector molecules. The interaction involves two distinct interfaces and dramatically alters the conformation of the α but not the βγ subunits. The location of the known sites for post-translational modification and receptor coupling suggest a plausible orientation for the heterotrimer with respect to both the membrane surface and the activated heptahelical receptor.

Multiwavelength anomalous diffraction data were used to solve the crystal structure of $G_{t\beta\gamma}$. The β subunit is primarily a seven-bladed β-propeller that is partially encircled by an extended γ subunit. The β-propeller, which contains seven structurally similar WD-repeats, defines the stereochemistry of the WD-repeat and the probable architecture of all WD-repeat containing domains. The structure details interactions between G protein β and γ subunits and highlights regions implicated in effector modulation for the conserved family of G protein βγ dimers.

**MS04.10.04 STRUCTURAL ANALYSIS OF A DIMERIC STATE OF THE FIRST INTRACELLULAR DOMAIN OF PTPa, A PROTEIN RECEPTOR-LIKE TYROSINE PHOSPHATASE.** A. M. Bilwes*, J. den Hertog°, T, Hunter £. J. P. Noel*, *Structural Biology Laboratory, £Molecular Biology and Virology Laboratory, Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037, °Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Like most of the members of the receptor-like protein tyrosine phosphatase (RPTP) family, the protein tyrosine phosphatase alpha (PTPa) is involved in key cellular functions such as cell growth division and differentiation. All exhibit common features: an extracellular N-terminal domain, a membrane-spanning helix and one or two intracellular domains (D1 and D2) displaying tyrosine phosphatase activity at different levels. Extracellular domains vary drastically in structure and are likely to bind ligands. Here we describe the X-ray structure of PTPa's domain D1. An unexpected tight, symmetric and apparently inactive dimer was present in the crystal. The amino-terminal segment of one monomer which adopts a helix-loop-helix conformation is wedged into the other monomer's active site preventing the swing of its catalytically essential loop. This amino-terminal motif resembles an elbow and the encompassed sequence is conserved amongst D1 domains of all the receptor-like PTPs (except PTPx) and not in D2 domains or non-receptor PTPs. We propose that dimerization of D1 domains is a physiological event inducing a reversible inactive state of the enzyme as a consequence of colocalization of the intracellular domains by extracellular mediated events.

**MS04.10.05 PH DOMAINS: DIVERSE SEQUENCES WITH A COMMON MEMBRANE ASSOCIATION FUNCTION.** Kathryn M. Ferguson#, Mark A. Lemmon†, Joseph Schlessinger†, Paul B. Sigler#, #Dept. of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University; †Dept. of Pharmacology, New York University Medical Center

In efforts to understand intermolecular interactions that control signal transduction, we have studied the structure and function of pleckstrin homology (PH) domains. These domains of ≈120 aa are found in some 90 different signal transduction proteins. Initially we studied the dynamin PH domain (DynPH). DynPH is a β-sandwich of two orthogonal β-sheets closed at one corner by a C-terminal α-helix. The X-ray crystal structure of DynPH confirmed that regions of sequence homology coincide with secondary structure elements, and that conserved hydrophobic residues, including the highly conserved W, form a well packed core. DynPH is electrostatically polarized, with three particularly variable loops coinciding with the positive face. These characteristics, common to all PH domains of known structure, suggest that PH domain ligands may be negatively charged, consistent with the previous suggestion that PH domains interact with anionic membrane components. The N-terminal region of phospholipase C-$\delta_1$, (PLC$\delta_1$) which contains a PH domain, has been implicated in binding to phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)$P_2$). We have shown that the isolated PH domain binds with high affinity and specificity to inositol-(1,4,5)-trisphosphate (Ins(1,4,5)$P_3$), and to PtdIns(4,5)$P_2$ in lipid vesicles. The X-ray crystal structure of PLCδPH complexed with Ins(1,4,5)$P_3$ shows that the ligand binds to the positive face, interacting with the three variable loops. No high affinity ligands have been identified for other PH domains. Recently, the phosphotyrosine binding (PTB) domain of the adapter protein Shc was shown to have a PH domain fold. The NPXpY binding motif, recognized by the Shc PTB domain, therefore represents a second high affinity PH domain ligand. Like PtdIns(4,5)$P_2$ binding to PLCδ-PH, this interaction also targets the host protein (Shc) to the cell membrane.

**MS04.10.06 ARCHITECTURE AND INTRASTERIC REGULATION OF GIANT PROTEIN KINASES.** B. Kobe, J. Heierhorst, S.C. Feil, M.W. Parker, G.M. Benian* and B.E. Kemp. St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia; *Dept. of Pathology, Emory University, Atlanta, Georgia 30322, USA.

Protein kinases are key regulatory enzymes in most cellular processes and must themselves be tightly regulated. Such regulation is often achieved by an intrasteric mechanism, where a part of the enzyme binds to the active site and autoinhibits its activity. The giant myosin associated proteins twitchin, projectin and titin are the largest members of the protein kinase family (600-3000 kDa) and have important roles in muscle contraction and cytoskeletal structure. Their sequences consist of numerous fibronectin type III-like and immunoglobulin (Ig)-like motifs, and a single autoinhibited protein Ser/Thr kinase structurally related to vertebrate myosin light chain kinases. We have recently shown that twitchin can be activated by the $Ca^{2+}$/S100$A1_2$ protein complex. To study the regulatory mechanisms of giant protein kinases, we determined the crystal structures of two recombinant fragments of twitchin. The structure of the autoinhibited twitchin kinase from *Aplysia* (A) reveals the conservation and flexibility of the autoinhibitory mechanism in giant protein kinases. The autoinhibitory sequence is positioned in the cleft between the two protein kinase lobes and inhibits the kinase by extensive contacts with residues implicated in substrate binding, ATP binding and catalysis. The structure of an extended fragment of *C. elegans*