1. Introduction. Drugs typically bind to concave “pockets” or “clefts” on the surface of their macromolecular targets. A favorable free energy of binding results from formation of an array of weak bonds such as ionic bonds, hydrogen bonds, and van der Waals forces between functional groups on the surface of the drug and functional groups on the surface of the drug target (e.g. the protein). Effective drug-target binding, characterized by a large $K_B$ or a small $K_D$, requires shape and functional group complementarity between the drug and the drug target. A successful drug-target interaction is something like a hand-in-glove fit.

A deep understanding of the organic chemistry of drug design and drug action can be facilitated by consideration of protein structure and the molecular interactions underlying the hand-in-glove fit of small molecules into protein pockets. Perhaps the best way to visualize these structures is using computer graphics. In this assignment, we will use a computer program called Pymol to visualize a protein-ligand structure that was determined using X-ray crystallography. Before we get to the mechanics of how to access and use Pymol, let’s consider the biochemistry, biology, and medicinal chemistry of the protein that we’re going to examine in this assignment, protein tyrosine phosphatase 1B (PTP1B). The enzyme PTP1B is an interesting “emerging target” in medicinal chemistry.

2. Protein tyrosine phosphatases: biological function and catalytic mechanism. Reversible phosphorylation of tyrosine residues serves as a biochemical “switch” that alters the functional properties of proteins in many critical mammalian signal transduction pathways.\textsuperscript{1-3} For example, tyrosine phosphorylation plays a central role in regulating cell metabolism, growth, proliferation, differentiation, immune response, motility, tissue homeostasis, and apoptosis. The phosphorylation status of tyrosine residues on proteins involved in these signal transduction pathways is controlled by the opposing actions of protein tyrosine kinases that catalyze the addition of phosphoryl groups and protein tyrosine phosphatases (PTPs) that catalyze their removal (Scheme 1).\textsuperscript{1,3,4} In many cases, PTP-catalyzed removal of phosphoryl groups from target proteins serves as an “off switch” that terminates the cellular responses to an extracellular stimulus.\textsuperscript{1,3,4}

![Scheme 1. Phosphorylation status of tyrosine residues is controlled by the coordinated action of PTKs and PTPs.](image)

For many years, PTPs were viewed as “housekeeping” enzymes charged with the routine task of removing phosphoryl groups from phosphorylated protein tyrosine residues in a nonselective and unregulated manner. Research over the last 10-15 years has rendered this view obsolete. We now know that the human genome codes for about 100 different PTPs each with distinct substrate specificities.\textsuperscript{5} Recent work has led to the general suggestion that kinases control the amplitude of the signaling response, while phosphatases control the rate of onset and duration of the response.\textsuperscript{3} Thus, inhibition of PTPs can yield profound biological effects by potentiating cellular responses to a given stimulus. For this reason, PTP inhibitors represent an emerging class of therapeutic agents.\textsuperscript{4,6-9}
The catalytic mechanism by which PTPs remove the phosphoryl group from phosphotyrosine residues is well established. The numbering used here is for the enzyme PTP1B, which is the focus of this assignment. PTPs share an active site sequence motif [I/V]HCXXGXXR[T/S], referred to as the P-loop (or phosphate-binding loop) comprised of residues 213-222. The cysteine residue within this P-loop (Cys215) has an abnormally low pK_a value of about 5.7 (versus about 8.5 for a normal cysteine thiol group) and, therefore, exists almost exclusively in the nucleophilic thiolate form (RS^-) at physiological pH. The altered pK_a of this group is due to the fact that the thiolate anion is stabilized by a network of hydrogen bonding interactions with amide NH groups in the P-loop and by the helix dipole of α-4, which points into the active site pocket.

Substrate phosphotyrosine residues bind at the active site via an array of interactions with backbone amide residues and the guanidinium side chain of Arg221 in the P-loop (Scheme 2). These interactions position the substrate’s phosphoryl residue for nucleophilic attack by the thiolate residue of Cys215. Substrate binding also stabilizes the closed conformation of the so-called WPD loop (residues 79-189), which brings Asp181 into position to assist catalysis. PTP1B provides a great demonstration of how substrate (or inhibitor) binding can induce substantial changes in protein structure. Following substrate binding to the catalytic pocket of the enzyme, in-line attack of the active site thiolate residue on the phosphorus atom of the phosphotyrosine substrate yields a phosphoryl cysteine intermediate. This step proceeds via a dissociative mechanism in which the metaphosphate group is stabilized “in flight” by Arg221 while Asp181 serves as a general acid catalyst to protonate the departing hydroxyl group of the substrate tyrosine residue. In the final step of catalysis, Asp181 acts as a general base to catalyze attack of water on the phosphoryl cysteine intermediate leading to release of inorganic phosphate and regenerate the native enzyme. We will view many of these catalytic residues as part of this assignment. In the mechanism of PTP1B, we see catalysis by proximity, nucleophilic catalysis, electrostatic stabilization of a transition state, and general acid-base catalysis.

The role of protein tyrosine phosphatase 1B (PTP1B) in insulin signaling and glucose homeostasis. Insulin is an important peptide hormone that regulates glucose homeostasis in the human body. Binding of insulin to the extracellular domains of the insulin receptor stimulates the intrinsic receptor tyrosine kinase activity of the intracellular domain of the protein. This results in autophosphorylation of tyrosine residues (Y1158, Y1162, and Y1163) on the cytoplasmic β-subunit of the insulin receptor. A number of adapter proteins such as insulin receptor substrates (IRS) bind to these phosphotyrosine residues and, in turn, are phosphorylated on their own tyrosine residues. The multisubunit enzyme phosphatidyl inositol-3-kinase assembles on the phosphorylated IRS platform and converts phosphatidyl inositol 4,5-diphosphate into phosphatidyl inositol 3,4,5-triphosphate (Figure 1). This lipid product embedded in the cytoplasmic membrane activates phosphatidyl inositol-dependent protein.
kinase-1. Phosphatidylinositol-dependent protein kinase-1, in turn, activates Akt kinase. Activation of these kinases is required for translocation of the glucose transporter GLUT4 from intracellular vesicles to the cell surface, thus initiating glucose uptake and utilization.

Clearly, activation of the insulin signaling cascade relies upon several tyrosine phosphorylation events. Accordingly, termination of the insulin signal involves enzymatic removal of these tyrosine phosphoryl groups. The enzyme PTP1B is the primary negative regulator of the insulin signaling pathway, catalyzing removal of tyrosine phosphoryl groups from both the insulin receptor and IRS. Again, PTP1B is the primary enzyme responsible for "shutting off" cellular responses to insulin. Thus, small molecule enzyme inhibitors that block the catalytic activity of PTP1B are expected to potentiate (increase the duration) cellular responses to insulin. For this reason, many pharmaceutical companies have sought to develop inhibitors of PTP1B as potential therapeutic agents in the treatment of type 2 diabetes, a disease that is characterized by decreased insulin sensitivity and compromised glucose utilization.

Figure 2. The enzyme PTP1B is the major negative regulator of the insulin signaling cascade... that is, removal of phosphoryl groups (the green “P’s” in the Scheme) by PTP1B "shuts down" cellular responses to insulin.

Interest in PTP1B as a potential therapeutic target for the treatment of type 2 diabetes was galvanized in the year 2000 by the development of two strains of knockout mice in which the gene coding for PTP1B was deleted. Importantly, these mice are completely normal in fertility, lifespan, and appearance, yet, have low levels of body fat and are resistant to weight gain when placed on a high fat diet. In addition, the animals show increased ability to dispose of glucose. These effects can be traced to the role of PTP1B in the insulin signaling pathway. For example, cells from these mice show prolonged phosphorylation of the insulin receptor in response to insulin treatment. (Some of the anti-obesity effects observed in the knockout mice stem from the beneficial role of PTP1B deletion on the leptin signaling pathway which we will not discuss here.) With these findings, PTP1B was "validated" as a therapeutic target for the treatment of type 2 diabetes. In the ensuing years many PTP1B inhibitors have been reported.

The active site of PTPs contains a well-defined phosphotyrosine binding pocket comprised of backbone amide residues in the P-loop (residues 213-222) and the guanidinium side chain of Arg 221 (Scheme 2, above). Accordingly, almost all PTP inhibitors contain a phosphotyrosine isostere of some sort. For example, difluoromethylene phosphonates, squaric acids, oxalylamino benzoic acids, and carboxemethoxy aromatic acids have been employed in this role with good success (Figure 3).
High affinity binding to PTP1B (defined here as low micromolar $K_d$ or better) typically requires contacts beyond the catalytic pocket of the enzyme. Indeed, many potent PTP1B inhibitors make contacts with a secondary phosphotyrosine binding pocket that resides near the catalytic active site of the enzyme. This secondary phosphotyrosine binding site is not catalytically active and, likely, serves to bind endogenous substrates of PTP1B, such as the insulin receptor and IRS (Figure 2), that contain contiguous (adjacent, neighboring) phosphotyrosine residues. The secondary phosphotyrosine binding site takes advantage of ion pairing interactions with residues Arg24 and Arg254, weakly polar interactions with Met258 and Gln262, and van der Waals contacts with Ile219, Asp48, and Val49. Thus, an effective strategy for the design of potent PTP1B inhibitors involves linking two phosphotyrosine isosteres via a 9-14 atom linker (Figure 4). Addition of a secondary-site-binding unit to an active site-binding phosphotyrosine isostere can improve affinity for PTP1B by a factor of 15-800 (Figure 4). Several other phosphatases including YopH, TCPTP and PTPL1 have similar secondary phosphate binding pockets outside the active site. For other phosphatase targets, viable recognition points outside the active site remain to be identified.

**Figure 3.** Phosphotyrosine isosteres serve as the “core” of most PTP inhibitors.

**Figure 4.** Potent PTP1B inhibitors gain significant enzyme affinity through contacts with the “secondary phosphotyrosine” binding site. **Left side:** a selection of PTP1B inhibitors that incorporate a “secondary-site-binder”. **Right side top:** Crystal structure of an active-site-binder. **Right side bottom:** Crystal structure of a two-site-binding compound complexed with PTP1B (Note: if you wish to access these crystal structures, use PBD codes 2H4K top and 2BQR, bottom).
The images in Figure 4 nicely illustrate that the active site of PTP1B is composed of both a deep catalytic pocket and a long valley or cleft where substrate peptides bind. Inhibitors of the enzyme mimic the interactions that the endogenous phosphotyrosine-containing peptide substrates normally share with the enzyme. Inhibitors such as those shown in Figure 4 are able to block the catalytic action of PTP1B in cells, causing hyperphosphorylation of the insulin receptor and increased glucose uptake and utilization. A number of PTP1B inhibitors show utility in animal models for type 2 diabetes and at least one candidate advanced to phase II clinical trials for the treatment of type 2 diabetes. Overall, though, it is important to recognize that the development of phosphatase inhibitors is at an early stage and requires further research.

**Literature Citations.**


