In a phosphate transfer reaction, a phosphate group is transferred from a phosphate group donor molecule to a phosphate group acceptor molecule:

\[
\text{OH} + \text{PO}_3^- \rightarrow \text{PO}_4^3^- + \text{HO}_2
\]

A very important aspect of biological phosphate transfer reactions is that the electrophilicity of the phosphorus atom is usually enhanced by the Lewis acid (electron-accepting) effect of one or more magnesium ions. Phosphate transfer enzymes generally contain a $\text{Mg}^{2+}$ ion bound in the active site in a position where it can interact with non-bridging phosphate oxygens on the substrate. The magnesium ion pulls electron density away from the phosphorus atom, making it more electrophilic.

\[
= \text{Mg}^{2+}
\]

Without this metal ion interaction, a phosphate is actually a poor electrophile, as the negatively-charged oxygens shield the phosphorus center from attack by a nucleophile.

**Note**

For the sake of simplicity and clarity, we will not draw the magnesium ion or other active site groups interacting with phosphate oxygens in most figures in this chapter - but it is important to keep in mind that these interactions play an integral role in phosphate transfer reactions.

Mechanistically speaking, a phosphate transfer reaction at a phosphorus center can be thought of as much like a $\text{(S}_2\text{N)}$ reaction at a carbon center. Just like in an $\text{(S}_2\text{N)}$ reaction, the nucleophile in a phosphoryl transfer approaches the electrophilic center from the backside, opposite the leaving group:
As the nucleophile gets closer and the leaving group begins its departure, the bonding geometry at the phosphorus atom changes from tetrahedral to trigonal bipyramidal at the pentavalent (5-bond) transition state. As the phosphorus-nucleophile bond gets shorter and the phosphorus-leaving group bond grows longer, the bonding picture around the phosphorus atom returns to its original tetrahedral state, but the stereochemical configuration has been 'flipped', or inverted.

In the trigonal bipyramidal transition state, the five substituents are not equivalent: the three non-bridging oxygens are said to be equatorial (forming the base of a trigonal bipyramid), while the nucleophile and the leaving group are said to be apical (occupying the tips of the two pyramids).

Although stereochemical inversion in phosphoryl transfer reactions is predicted by theory, the fact that phosphoryl groups are achiral made it impossible to observe the phenomenon directly until 1978, when a group of researchers was able to synthesize organic phosphate esters in which stable oxygen isotopes $^{17}O$ and $^{18}O$ were specifically incorporated. This created a chiral phosphate center.
Subsequent experiments with phosphoryl transfer-catalyzing enzymes confirmed that these reactions proceed with stereochemical inversion. (Nature 1978 275, 564; Ann Rev Biochem 1980 49, 877).

The concerted (\(S_{N2}\)-like) is not the only mechanism that has been proposed for these reactions - in fact, two other possible mechanisms have been suggested. In an alternative two-step mechanistic model, the nucleophile could attack first, forming a pentavalent, trigonal bipyramidal intermediate (as opposed to a pentavalent transition state). The reaction is completed when the leaving group is expelled. The intermediate species would occupy an energy valley between the two transition states.

**Addition-elimination model:**

This is often referred to as an 'addition-elimination' mechanism - the nucleophile adds to the phosphate first, forming a pentavalent intermediate, and then the leaving group is eliminated.

An addition-elimination mechanism with a pentavalent intermediate is not possible for an \(S_{N2}\) reaction at a carbon center, because carbon, as a second-row element, does not have any d orbitals and cannot form five bonds. Phosphorus, on the other hand, is a third-row element and is quite capable of forming more than four bonds. Phosphorus pentachloride, after all, is a stable compound that has five bonds to chlorine arranged in trigonal bipyramidal geometry around the central phosphorus.
The phosphorus atom in $\text{PCl}_5$ (and in the hypothetical pentavalent intermediate pictured above) is considered to be $\text{sp}^3\text{d}$ hybridized:

There is a third possibility: the reaction could proceed in an $\text{S_N1}$-like manner: in other words, elimination-addition. In this model, the phosphorus-leaving group bond breaks first, resulting in a metaphosphate intermediate. This intermediate, which corresponds to the carbocation intermediate in an $\text{S_N1}$ reaction and like a carbocation has trigonal planar geometry, is then attacked by the nucleophile to form the reaction product.

Elimination-addition model:

So which mechanistic model - concerted ($\text{SN}_2$)-like), addition-elimination, or elimination-addition - best describes enzymatic phosphate transfer reactions? Chemists love to investigate and debate questions like this! Just like with the $\text{S_N1}/\text{S_N2}$ argument discussed in chapter 8, it really boils down to one question: which happens first, bond-forming or bond-breaking - or do these two events occur at the same time? From the evidence accumulated to date, it appears that many enzymatic phosphate transfer reactions can best be described by the concerted model, although there is still argument about this, and still many unanswered questions about other details of how these reactions are catalyzed.
active sites. Considering the importance of phosphate transfer reactions in metabolic pathways, this area is clearly a very promising one for further investigation. If you are interesting in learning more about this research, a great place to start is a review article written by Professor Daniel Herschlag at Stanford University (Annu. Rev. Biochem. 2011, 80, 669).

For the sake of simplicity and clarity, phosphoryl transfers in this text will be depicted using the concerted model.

**Exercise 9.3.1**

Predict the approximate angles between the two bonds indicated in a phosphate transfer transition state. $\langle O_a \rangle$ refers to an oxygen at the apical position, and $\langle O_e \rangle$ to an oxygen in the equatorial position.

a. $\langle O_a-P-O_a \rangle$

b. $\langle O_a-P-O_e \rangle$

c. $\langle O_e-P-O_e \rangle$