

**PROSTHETIC GROUP AS NON POLYPEPTIDE BIOCATALYST ESSENTIAL FOR
BIOCHEMICAL CHEMISTRY**

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ABSTRACT

A tightly bound non-polypeptide structure required for the activity of an enzyme or other protein, for example the haem of haemoglobin. A characteristic non amino acid substance that is strongly bound to a protein and necessary for the protein portion of an enzyme to function; often used to describe the function, as in hemeprotein for hemoglobin. An organic and non-protein compound that forms a part of conjugated proteins. In enzymatic catalysis the prosthetic group is usually referred to as a coenzyme. Coenzymes are firmly bonded to the protein part of a biocatalyst by an apoenzyme and remain affixed to the protein molecule for the duration of the catalysis; examples of coenzymes include lipoic acid, riboflavin, biotin and hemes. Carrier coenzymes differ from prosthetic groups. The activity of carrier coenzymes is associated with their transfer from one enzyme molecule to another. However, this differentiation is often arbitrary, since the same compound (for example, FAD: flavine-adenine di-nucleotide) can sometimes act like a typical dissociating coenzyme, while at other times it remains firmly bonded to the protein.

KEYWORDS: Organic units, Inorganic units, Metal complex, Cofactors, Coenzymes, Proteins, Vitamins, Sugars, Lipids, Enzyme partners, NAD, FAD.

INTRODUCTION

The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids. Prosthetic groups are bound tightly to proteins and may even be attached through a covalent bond, as opposed to coenzymes,

which are loosely bound. Prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. In enzymes, prosthetic groups are often involved in the active site, playing an important role in the functions of enzymes.^[1-3]

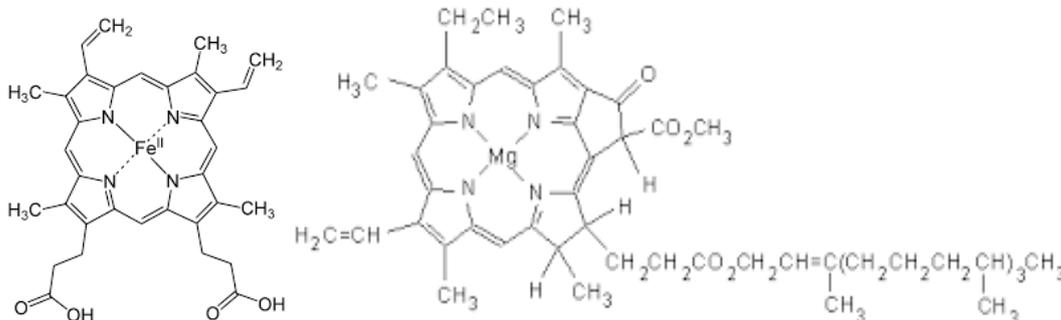


Figure-1: Haemoglobin and Chlorophyll

Vitamins are another common prosthetic group. This is one of the reasons why vitamins are required in the human diet. Inorganic prosthetic groups, however, are usually transition metal ions such as iron. The haem group in haemoglobin is a prosthetic group located in the porphyrin, which is a tetramer of cyclic carbon groups. It contains an organic component called a protoporphyrin made up of four pyrrole rings and an iron atom in the ferrous state (Fe^{2+}).^[4] The red color of blood and muscles

is attributed to the haem groups. The difference between a prosthetic group and a cofactor depends on how tightly or loosely bound to the enzyme they are. If tightly connected, the cofactor is referred to as a prosthetic group. Heme group is a prosthetic group consisting of a protoporphyrin ring and a central iron (Fe) atom. A protoporphyrin ring is made up of four pyrrole rings linked by methine bridges. Four methyl, two vinyl and two propionate side chains are attached. Heme of

hemoglobin protein is a prosthetic group of heterocyclic ring of porphyrin of an iron atom; the biological function of the group is for delivering oxygen to body tissues, such that bonding of ligand of gas molecules to the iron atom of the protein group changes the structure of the protein by amino acid group of histidine residue around the heme molecule.^[5,6]

The iron lies in the center is an organic component called protoporphyrin, which is bound to four pyrrole nitrogen atom linked by a methine bridge that forms a tetra pyrrole ring. The iron can either be in the ferrous (Fe^{2+}) or the ferric (Fe^{3+}) oxidation state. However, it is only able to bind to oxygen when in the ferrous state. The iron can form two additional.

Table-1: Metal prosthetics

Ion	Examples of enzymes containing this ion
Cupric	Cytochrome oxidase
Ferrous or Ferric	Catalase, Cytochrome (via Heme), Nitrogenase, Hydrogenase
Magnesium	Glucose 6-phosphatase, Hexokinase, DNA polymerase
Manganese	Arginase
Molybdenum	Nitrate reductase, Nitrogenase
Nickel	Urease
Zinc	Alcohol dehydrogenase, Carbonic anhydrase, DNA polymerase
Iron	Examples of enzymes containing this ion

bonds in fifth and in sixth coordination which on both side of heme plane. The fifth coordination sites are linked to a distal histidine while the sixth coordination site can, not always, bind to oxygen.

Upon binding, the haem group will actually shrink in size and descend further into the plane of the porphyrin ring. Along with it, the distal histidine will follow and this histidine is attached to the α - β interface thus resulting in local to complete conformational change.^[7,8]

Chlorophyll is a chlorin pigment, which is structurally similar to and produced through the same metabolic pathway as other porphyrin pigments such as heme. At the center of the chlorin ring is a magnesium ion. This was discovered in 1906 and was the first time that magnesium had been detected in living tissue.

For the structures depicted in this article, some of the ligands attached to the Mg^{2+} center are omitted for clarity. The chlorin ring can have several different side chains, usually including a long phytol chain. There are a few different forms that occur naturally, but the most widely distributed form in terrestrial plants is chlorophyll a.

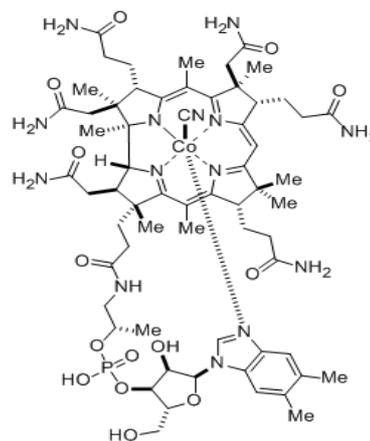
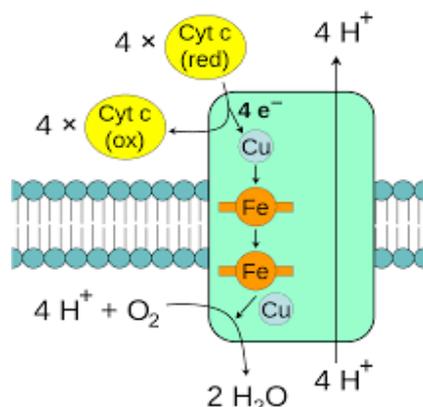


Figure-2: Cytochrome oxidase and Cyanocobalamin

A conjugate protein combined with its specific prosthetic group is termed a holoprotein, while a protein in its absence is called an apoprotein. Prosthetic groups have varying functions, such as oxidizing-reducing reactions (redox), methylation reactions, oxygenation reactions and so forth. The haem groups give muscles and blood their distinctive red color. Some enzymes require helpers to recognize a substrate or complete a reaction. These helpers include cofactors, coenzymes and prosthetic groups, which are required for some enzymes' functions.^[9]

Enzyme Partners: Sometimes we get by with a little help from our friends. There are some tasks, like going to the store for groceries, that are perfectly easy to do by yourself. Some tasks, like moving a large piece of furniture into your house and up a few flights of stairs - well, friends are good to have in situations like this, particularly strong ones who will lift anything for some free pizza.^[10]

We all rely on other people at certain times in our lives, so we shouldn't be surprised that the same thing can happen in a biological process. For example, enzymes



benefit from a few close friends, too. Remember that **enzymes** are proteins that catalyze chemical reactions.

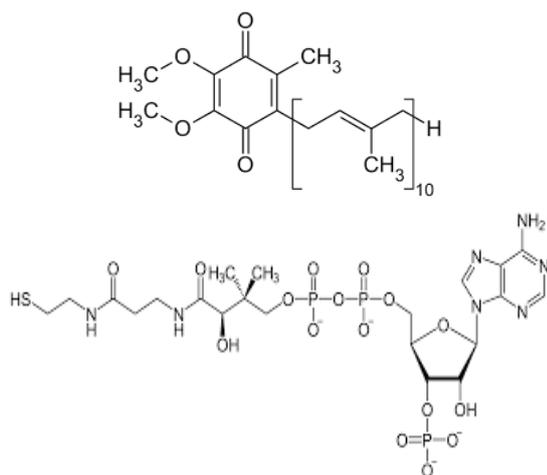


Figure-3: Q10 enzyme and Coenzyme A

In other lessons, we've learned how they functioned. In this lesson, we'll learn about who functions with them, or rather, who helps them catalyze these reactions. Some enzymes need helpers or partners, and some don't. There are different types of enzyme helpers, too, with different enzymes requiring different helpers or different kinds of friends. There are examples of cofactors, coenzymes and prosthetic groups in many biological processes. For example, **cellular respiration** occurs in all of your cells, which is a process that converts food into energy. The details of this process will be saved for other lessons, but you can imagine it as a long series of events that need to occur, like steps in a relay triathlon, except with way more than three events! Enzymes are the proteins that perform many of these steps in cellular respiration, passing the biological baton from the cold-water swim to the bicycle race.

Cofactors: The first type of enzyme partner is a group called **cofactors**, or molecules that increase the rate of reaction or are required for enzyme function. Cofactors are not proteins but rather help proteins, such as enzymes, although they can also help non-enzyme proteins as well. Examples of cofactors include metal ions like iron and zinc.^[11]

Coenzymes: A specific type of cofactor, **coenzymes**, is organic molecules that bind to enzymes and help them function. The key here is that they're organic. 'Organic' does not mean you'll find them in a special aisle in the grocery store. Rather, organic molecules are simply molecules that contain carbon. Don't let the name 'coenzymes' fool you, either; coenzymes are not really enzymes. As the prefix 'co-' suggests, they work with enzymes. Many coenzymes are derived from vitamins. These molecules often sit at the active site of an enzyme and aid in recognizing, attracting, or repulsing a substrate and product. Remember that a **substrate** is the molecule

upon which an enzyme catalyzes a reaction. Coenzymes can also shuttle chemical groups from one enzyme to another enzyme. Coenzymes bind loosely to enzymes, while another group of cofactors do not.^[12]

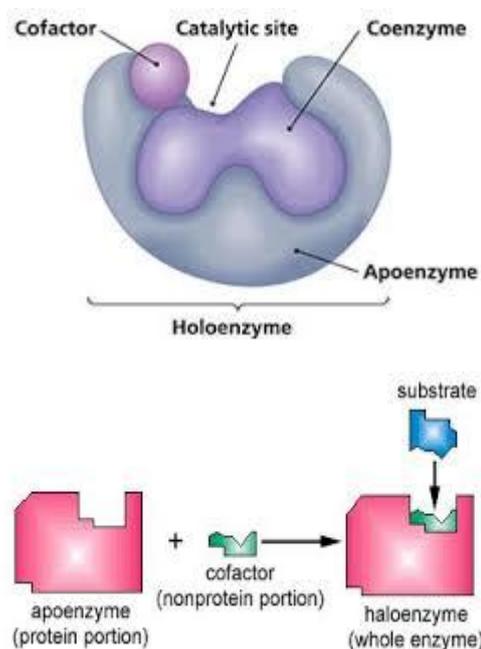


Figure-4: Cofactor and Coenzyme

Prosthetic Groups: **Prosthetic groups** are cofactors that bind tightly to proteins or enzymes. As if holding on for dear life, they are not easily removed. They can be organic or metal ions and are often attached to proteins by a covalent bond. The same cofactors can bind multiple different types of enzymes and may bind some enzymes loosely, as a coenzyme and others tightly, as a prosthetic group. Some cofactors may always tightly bind their enzymes. It's important to note, though, that these prosthetic groups can also bind to proteins other than enzymes. An enzyme that requires a helper group really isn't complete without it. Once attached, it's called a holoenzyme. A **holoenzyme** is an enzyme with any metal ions or coenzymes attached to it that is now ready to catalyze a reaction.

A cofactor is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" that assist in biochemical transformations.^[13]

Cofactors can be subdivided into either one or more inorganic ions or a complex organic or metalloorganic molecule called a coenzyme; most of which are derived from vitamins and from required organic nutrients in small amounts. A cofactor that is tightly or even covalently bound is termed a prosthetic group. Additionally, some sources also limit the use of the term "cofactor" to inorganic substances.

Vitamins and derivatives

Table-2: Vitamin prosthetics

Cofactor	Vitamin	Additional component	Chemical group(s) transferred	Distribution
Thiamine pyrophosphate	Thiamine (B ₁)	None	2-carbon groups, α cleavage	Bacteria, archaea and eukaryotes
NAD ⁺ and NADP ⁺	Niacin (B ₃)	ADP	Electrons	Bacteria, archaea and eukaryotes
Pyridoxal phosphate	Pyridoxine (B ₆)	None	Amino and carboxyl groups	Bacteria, archaea and eukaryotes
Methylcobalamin	Vitamin B ₁₂	CH ₃ group	Acyl groups	Bacteria, archaea and eukaryotes
Cobalamine	Cobalamine (B ₁₂)	None	hydrogen, alkyl groups	Bacteria, archaea and eukaryotes
Biotin	Biotin (H)	None	CO ₂	Bacteria, archaea and eukaryotes
Coenzyme A	Pantothenic acid (B ₅)	ADP	Acetyl group and other acyl groups	Bacteria, archaea and eukaryotes
Tetrahydrofolic acid	Folic acid (B ₉)	Glutamate residues	Methyl, formyl, methylene and form-imino groups	Bacteria, archaea and eukaryotes
Menaquinone	Vitamin K	None	Carbonyl group and electrons	Bacteria, archaea and eukaryotes
Ascorbic acid	Vitamin C	None	Electrons	Bacteria, archaea and eukaryotes
Flavin mononucleotide	Riboflavin (B ₂)	None	Electrons	Bacteria, archaea and eukaryotes
Flavin adenine dinucleotide	Riboflavin (B ₂)	ADP	Electrons	Bacteria, archaea and eukaryotes
Coenzyme F420	Riboflavin (B ₂)	Amino acids	Electrons	Methanogens and some bacteria

An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme. Some enzymes or enzyme complexes require several cofactors.^[14] For example, the multienzyme complex pyruvate dehydrogenase at the junction of glycolysis and the citric acid cycle requires five organic cofactors and one metal ion: loosely bound thiamine pyrophosphate (TPP), covalently bound lipoamide and flavin adenine dinucleotide (FAD) and the co substrates nicotinamide adenine dinucleotide (NAD⁺) and coenzyme A (CoA) and a metal ion (Mg²⁺). Organic cofactors are often vitamins or are made from vitamins.

Many contain the nucleotide adenosine monophosphate (AMP) as part of their structures, such as ATP, coenzyme A, FAD and NAD⁺. This common structure may reflect a common evolutionary origin as part of ribozymes in an ancient RNA world. It has been suggested that the AMP part of the molecule can be

considered a kind of "handle" by which the enzyme can "grasp" the coenzyme to switch it between different catalytic centers.^[15]

Cofactors can be divided into two broad groups: **organic cofactors**, such as flavin or heme and **inorganic cofactors**, such as the metal ions Mg²⁺, Cu²⁺, Mn²⁺, or iron-sulfur clusters. Organic cofactors are sometimes further divided into *coenzymes* and *prosthetic groups*. The term coenzyme refers specifically to enzymes and as such, to the functional properties of a protein.

On the other hand, "prosthetic group" emphasizes the nature of the binding of a cofactor to a protein (tight or covalent) and thus, refers to a structural property. Different sources give slightly different definitions of coenzymes, cofactors and prosthetic groups. Some consider tightly bound organic molecules as prosthetic groups and not as coenzymes, while others define all non-protein organic molecules needed for enzyme activity as coenzymes, and classify those that are tightly bound as coenzyme prosthetic groups. It should be noted that these terms are often used loosely.^[16]

Non-vitamins**Table-3: Non-vitamins prosthetics**

Cofactor	Chemical group(s) transferred	Distribution
Adenosine triphosphate	Phosphate group	Bacteria, archaea and eukaryotes
S-Adenosyl methionine	Methyl group	Bacteria, archaea and eukaryotes
Coenzyme B	Electrons	Methanogens
Coenzyme M	Methyl group	Methanogens
Coenzyme Q	Electrons	Bacteria, archaea and eukaryotes
Cytidine triphosphate	Diacylglycerols and lipid head groups	Bacteria, archaea and eukaryotes
Glutathione	Electrons	Some bacteria and most eukaryotes
Heme	Electrons	Bacteria, archaea and eukaryotes
Lipoamide	Electrons, acyl groups	Bacteria, archaea and eukaryotes
Methanofuran	Formyl group	Methanogens
Molybdopterin	Oxygen atoms	Bacteria, archaea and eukaryotes
Nucleotide sugars	Monosaccharides	Bacteria, archaea and eukaryotes
3'-Phosphoadenosine-5'-phosphosulfate	Sulfate group	Bacteria, archaea and eukaryotes
Pyrroloquinoline quinone	Electrons	Bacteria
Tetrahydrobiopterin	Oxygen atom and electrons	Bacteria, archaea and eukaryotes
Tetrahydromethanopterin	Methyl group	Methanogens

Classification

A 1979 letter in *Trends in Biochemical Sciences* noted the confusion in the literature and the essentially arbitrary distinction made between prosthetic groups and coenzymes and proposed the following scheme. Here, cofactors were defined as an additional substance apart from protein and substrate that is required for enzyme activity and a prosthetic group as a substance that undergoes its whole catalytic cycle attached to a single enzyme molecule. However, the author could not arrive at a single all-encompassing definition of a "coenzyme" and proposed that this term be dropped from use in the literature.^[17]

Metal ions

Metal ions are common cofactors. The study of these cofactors falls under the area of bioinorganic chemistry. In nutrition, the list of essential trace elements reflects their role as cofactors. In humans this list commonly includes iron, magnesium, manganese, cobalt, copper, zinc and molybdenum. Although chromium deficiency causes impaired glucose tolerance, no human enzyme that uses this metal as a cofactor has been identified. Iodine is also an essential trace element, but this element is used as part of the structure of thyroid hormones rather than as an enzyme cofactor. Calcium is another special case, in that it is required as a component of the human diet and it is needed for the full activity of many enzymes, such as nitric oxide synthase, protein phosphatases and adenylate kinase, but calcium activates these enzymes in allosteric regulation, often binding to these enzymes in a complex with calmodulin.^[18] Calcium is, therefore, a cell signaling molecule and not usually considered a cofactor of the enzymes it regulates. Other

organisms require additional metals as enzyme cofactors, such as vanadium in the nitrogenase of the nitrogen-fixing bacteria of the genus *Azotobacter*, tungsten in the aldehyde ferredoxin oxidoreductase of the thermophilic archaean *Pyrococcus furiosus* and even cadmium in the carbonic anhydrase from the marine diatom *Thalassiosira weissflogii*. In many cases, the cofactor includes both an inorganic and organic component. One diverse set of examples is the hemoproteins, which consist of a porphyrin ring coordinated to iron. A simple [Fe₂S₂] cluster containing two iron atoms and two sulfur atoms, coordinated by four protein cysteine residues.^[19]

Iron-sulfur clusters: Iron-sulfur clusters are complexes of iron and sulfur atoms held within proteins by cysteinyl residues. They play both structural and functional roles, including electron transfer, redox sensing, and as structural modules. Organic cofactors are small organic molecules (typically a molecular mass less than 1000 Da) that can be either loosely or tightly bound to the enzyme and directly participate in the reaction. In the latter case, when it is difficult to remove without denaturing the enzyme, it can be called a prosthetic group. It is important to emphasize that there is no sharp division between loosely and tightly bound cofactors. Indeed, many such as NAD⁺ can be tightly bound in some enzymes, while it is loosely bound in others. Another example is thiamine pyrophosphate (TPP), which is tightly bound in transketolase or pyruvate decarboxylase, while it is less tightly bound in pyruvate dehydrogenase. Other coenzymes, flavin adenine dinucleotide (FAD), biotin and lipoamide, for instance, are covalently bound. Tightly bound cofactors are, in

general, regenerated during the same reaction cycle, while loosely bound cofactors can be regenerated in a subsequent reaction catalyzed by a different enzyme. In the latter case, the cofactor can also be considered a substrate or cosubstrate.^[20]

Vitamins can serve as precursors to many organic cofactors (e.g., vitamins B₁, B₂, B₆, B₁₂, niacin, folic acid) or as coenzymes themselves (e.g., vitamin C). However, vitamins do have other functions in the body. Many organic cofactors also contain a nucleotide, such as the electron carriers NAD and FAD and coenzyme A, which carry acyl groups. Most of these cofactors are found in a huge variety of species, and some are universal to all forms of life. An exception to this wide distribution is a group of unique cofactors that evolved in methanogens, which are restricted to this group of archaea.^[21]

Cofactors as metabolic intermediates: Metabolism involves a vast array of chemical reactions, but most fall under a few basic types of reactions that involve the transfer of functional groups. This common chemistry allows cells to use a small set of metabolic intermediates to carry chemical groups between different reactions. These group-transfer intermediates are the loosely bound organic cofactors, often called coenzymes. Each class of group-transfer reaction is carried out by a particular cofactor, which is the substrate for a set of enzymes that produce it, and a set of enzymes that consume it. An example of this are the dehydrogenases that use nicotinamide adenine dinucleotide (NAD⁺) as a cofactor.^[22] Here, hundreds of separate types of enzymes remove electrons from their substrates and reduce NAD⁺ to NADH. This reduced cofactor is then a substrate for any of the reductases in the cell that require electrons to reduce their substrates. Therefore, these cofactors are continuously recycled as part of metabolism. As an example, the total quantity of ATP in the human body is about 0.1 mole. This ATP is constantly being broken down into ADP, and then converted back into ATP. Thus, at any given time, the total amount of ATP + ADP remains fairly constant. The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily, which is around 50 to 75 kg. In typical situations, humans use up their body weight of ATP over the course of the day. This means that each ATP molecule is recycled 1000 to 1500 times daily.^[23]

Abiogenesis: Organic cofactors, such as ATP and NADH, are present in all known forms of life and form a core part of metabolism. Such universal conservation indicates that these molecules evolved very early in the development of living things. At least some of the current set of cofactors may, therefore, have been present in the last universal ancestor, which lived about 4 billion years ago. Organic cofactors may have been present even earlier in the history of life on Earth. It is interesting to note that the nucleotide adenosine is present in cofactors that catalyze many basic metabolic reactions such as

methyl, acyl and phosphoryl group transfer, as well as redox reactions. This ubiquitous chemical scaffold has, therefore, been proposed to be a remnant of the RNA world, with early ribozymes evolving to bind a restricted set of nucleotides and related compounds. Adenosine-based cofactors are thought to have acted as interchangeable adaptors that allowed enzymes and ribozymes to bind new cofactors through small modifications in existing adenosine-binding domains, which had originally evolved to bind a different cofactor. This process of adapting a pre-evolved structure for a novel use is referred to as exaptation. A computational method, IPRO, recently predicted mutations that experimentally switched the cofactor specificity of *Candida boidinii* xylose reductase from NADPH to NADH.^[24, 25]

CONCLUSION

Prosthetic groups are cofactors that bind tightly to proteins or enzymes. As if holding on for dear life, they are not easily removed. They can be organic or metal ions and are often attached to proteins by a covalent bond. The same cofactors can bind multiple different types of enzymes and may bind some enzymes loosely, as a coenzyme, and others tightly, as a prosthetic group. Some cofactors may always tightly bind their enzymes. It's important to note, though, that these prosthetic groups can also bind to proteins other than enzymes. Conjugated proteins function in association with non-protein chemical structures attached either covalently or non-covalently. The non-peptide section of a conjugated protein is called its prosthetic group. While glycoproteins are typically the most abundant conjugated proteins, those in which the prosthetic group contains a coordinated metal atom constitute an important class. Coordination chemistry is an important topic for understanding protein chemistry. Prosthetic groups often contain metal atoms such as zinc or magnesium, or organic molecular structures containing metal atoms, such as in heme.

The first type of enzyme partner is a group called cofactors, or molecules that increase the rate of reaction or are required for enzyme function. Cofactors are not proteins but rather help proteins, such as enzymes, although they can also help non-enzyme proteins as well. Examples of cofactors include metal ions like iron and zinc.

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There are examples of cofactors, coenzymes, and prosthetic groups in many biological processes. For example, cellular respiration occurs in all of your cells, which is a process that converts food into energy. The details of this process will be saved for other lessons, but you can imagine it as a long series of events that need to occur, like steps in a relay triathlon, except with way more than three events! Enzymes are the proteins that perform many of these steps in cellular respiration, passing the biological baton from the cold-water swim to the bicycle race.

In this lesson, we've learned about some of the help an enzyme can get to perform its reactions. 'Cofactors' is a term broadly given to molecules that increase the rate of reaction or are required for enzyme function. Metal ions are usually cofactors. Coenzymes are a specific type of helper or partner that are organic molecules required for enzyme function that bind loosely to an enzyme. They are often, though not always, derived from vitamins. Prosthetic groups are enzyme partner molecules that bind tightly to an enzyme.

REFERENCES

1. Nelson David. Lehninger Principles of Biochemistry. New York: W.H. Freeman and Company, 2008; 184.
2. Sauke David J., Metzler David E., Metzler Carol M. Biochemistry: the chemical reactions of living cells (2nd ed.). San Diego: Harcourt/Academic Press, 2001.
3. Jordan Frank, Patel Mulchand S. Thiamine: catalytic mechanisms in normal and disease states. New York, N.Y: Marcel Dekker, 2004; 588.
4. Denessiouk KA, Rantanen VV, Johnson MS. Adenine recognition: a motif present in ATP-, CoA-, NAD-, NADP-, and FAD-dependent proteins". *Proteins.*, 2001; 44(3): 282–91.
5. Bryce CFA. SAM – semantics and misunderstandings". *Trends Biochem. Sci.*, 1979; 4(3): N62.
6. Aggett PJ. Physiology and metabolism of essential trace elements: an outline. *Clin Endocrinol Metab.*, 1985; 14(3): 513–3.
7. Stearns DM. Is chromium a trace essential metal? *BioFactors.*, 2000; 11(3): 149–62.
8. Vincent JB. The biochemistry of chromium. *J. Nutr.*, 2000; 130(4): 715–8.
9. Cavalieri RR. Iodine metabolism and thyroid physiology: current concepts. *Thyroid* 7(2): 177–81, 1997.
10. Clapham DE. Calcium signaling. *Cell.*, 2007; 131(6): 1047–58.
11. Niki I, Yokokura H, Sudo T, Kato M, Hidaka H. Ca²⁺ signaling and intracellular Ca²⁺ binding proteins. *J. Biochem.*, 1996; 120(4): 685–98.
12. Eady RR. The vanadium-containing nitrogenase of *Azotobacter*. *BioFactors.*, 1988; 1(2): 111–6.
13. Chan MK, Mukund S, Kletzin A, Adams MW, Rees DC. Structure of a hyperthermophilic tungstopterin enzyme, aldehyde ferredoxin oxidoreductase. *Science.*, 1995; 267(5203): 1463–9.
14. Lane TW, Morel FM. A biological function for cadmium in marine diatoms. *Proc. Natl. Acad. Sci. U.S.A.*, 2000; 97(9): 4627–31.
15. Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FM. Biochemistry: a cadmium enzyme from a marine diatom. *Nature.*, 2005; 435(7038): 42.
16. Meyer J. Iron-sulfur protein folds, iron-sulfur chemistry, and evolution. *J. Biol. Inorg. Chem.*, 2008; 13(2): 157–70.
17. Bolander FF. Vitamins: not just for enzymes. *Curr Opin Investig Drugs.*, 2006; 7(10): 912–5.
18. Frank RA, Leeper FJ, Luisi BF. Structure, mechanism and catalytic duality of thiamine-dependent enzymes. *Cell. Mol. Life Sci.*, 2007; 64(7–8): 892–905.
19. Banerjee R, Ragsdale SW. The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes". *Annu. Rev. Biochem.*, 2003; 72: 209–47.
20. Jitrapakdee S, Wallace JC. The biotin enzyme family: conserved structural motifs and domain rearrangements. *Curr. Protein Pept. Sci.*, 2003; 4(3): 217–29.
21. Leonardi R, Zhang YM, Rock CO, Jackowski S. Coenzyme A: back in action. *Prog. Lipid Res.*, 2005; 44(2–3): 125–53.
22. Søballe B, Poole RK. Microbial ubiquinones: multiple roles in respiration, gene regulation and oxidative stress management. *Microbiology.*, 1999; 145(8): 1817–30.
23. Joosten V, van Berkel WJ. Flavoenzymes. *Curr Opin Chem Biol.*, 2007; 11(2): 195–202.
24. Taylor CD, Wolfe RS. Structure and methylation of coenzyme M(HSCH₂CH₂SO₃). *J. Biol. Chem.*, 1974; 249(15): 4879–85.
25. Balch WE, Wolfe RS. Specificity and biological distribution of coenzyme M (2-mercaptoethanesulfonic acid). *J. Bacteriol.*, 1979; 137(1): 256–63.